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Integrative Network Meta-Analysis Reveals Estrogen-Mediated RUNX2–PDLIM3–microRNA Crosstalk via Erg Signaling: Implications for Bone and Tissue Regeneration

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Integrative Network Meta-Analysis Reveals Estrogen-Mediated RUNX2–PDLIM3–microRNA Crosstalk via ERG Signaling: Implications for Bone and Tissue Regeneration

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Abstract

Estrogens govern the female reproductive cycle indefinitely. Estrogens, including estrone (E1), estradiol (E2), estriol (E3), and estetrol (E4), regulate the female life cycle since early embryonic stages and play a crucial role in development, metabolism, and cell function. Throughout evolution, estrogen has regulated reproduction by affecting reproductive organ development and behavior. Estrogen impacts all vertebrates, including fish, and has a role in physiological and pathological states in both genders. The RUNX-2 gene is a member of the RUNX family of transcription factors and encodes a nuclear protein with a Runt DNA-binding domain. This protein is essential for osteoblastic differentiation and skeletal morphogenesis and acts as a scaffold for nucleic acids and regulatory factors involved in skeletal gene expression. The protein can bind DNA both as a monomer or, with more affinity, as a subunit of a heterodimeric complex. In 2022, a study was conducted to characterize novel genes that are regulated by estrogen binding to its receptors (α or β). The PDLIM3 gene, with a coefficient of variation (CV) of 0.083, received the most stable CV score among other genes. Our integrative research uncovers a unique regulatory cascade in which estrogen binding to ERα/β enhances *PDLIM3* expression, then modulating the expression of miR-9, miR-10, and the newly identified miR-6769b, finally activating RUNX2 transcription.

Keywords: osteogenesis; bone regeneration; estrogen; estrogen receptor; estrogen signaling pathway; RUNX-2; PDLIM3; IPA tool; regulatory microRNA

I. Introduction

Estrogen, a steroid hormone playing a fundamental role in the female reproductive tract, is an essential regulator of reproductive physiology. Besides its traditional roles, estrogen possesses neuroprotective activity in preventing neurodegenerative disorders such as dementia and reducing the severity of traumatic brain injury. It is also widely utilized in hormone replacement therapy (HRT) for the treatment of symptoms of menstrual irregularities and menopause [1,2]. Among all of the endogenous estrogens, 17β-estradiol (E2) is the most potent and biologically active form found in systemic circulation. E2 regulates a wide array of physiological events in diverse tissues and organs by diffusing through the plasma membrane of target cells and binding to intracellular estrogen receptors (ERs), i.e., ERα and ERβ [2,3]. These bindings trigger cascades of signals that can be divided into genomic and non-genomic mechanisms. In genomic mechanisms, the estradiol-ER complex undergoes a hormone-binding conformational change, translocates into the nucleus, and binds to estrogen response elements (EREs) in enhancer regions, promoters, or untranslated regions of estrogen-responsive genes [4,5]. This receptor-DNA interaction controls gene transcription and subsequent protein synthesis. Otherwise, estrogen may act with membrane-bound receptors such as GPER1 or cytoplasmic ERs in non-genomic signaling, triggering rapid activation of intracellular signaling cascades independent of genomic interaction [6,7]. Both modes of action point to the

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sophistication and flexibility of the hormone's function in human physiology. The complex moves to the nucleus and attaches to chromatin at ERE sequences, enhancer regions near promoters, and 30-untranslated regions of target genes. (Figure 1). The RUNX2 gene in humans encodes the transcription factor known as Runt-related transcription factor 2 (RUNX2) or core-binding factor subunit alpha-1 (CBFα1). RUNX2 is recognized as an early marker of osteogenic differentiation and plays a pivotal role in initiating osteoblast-specific extracellular matrix (ECM) synthesis by regulating the expression of critical matrix proteins such as collagen type I and osteopontin (OPN) [8]. It functions as a key transcriptional regulator of osteoblast lineage commitment. RUNX2 encodes a nuclear-localized transcription factor containing a conserved Runt homology domain, which is essential for osteoblast differentiation and skeletal morphogenesis. It operates as a molecular scaffold for nucleic acids and transcriptional co-regulators involved in the control of skeletal gene expression. [9].

Also, RUNX2 can bind DNA either as a monomer or with greater affinity when part of a heterodimeric complex. The N-terminal domain of the protein includes two potential trinucleotide repeat expansions, which, along with other mutations in the gene, have been implicated in the skeletal disorder cleidocranial dysplasia (CCD) [10].More recently, somatic mutations in the RUNX2 gene, along with its distinct expression signatures in both healthy and neoplastic tissues, have highlighted its prognostic and diagnostic value in multiple human malignancies, supporting its consideration as a cancer biomarker. Studies have demonstrated that RUNX2 contributes to the regulation of essential oncogenic processes, including tumor cell proliferation, angiogenesis, metastasis, cancer stem cell maintenance, and resistance to chemotherapy. These findings underscore the need for deeper investigation into RUNX2-mediated mechanisms as a foundation for novel therapeutic strategies [11].The actin-associated LIM protein (ALP), a product of the PDLIM3 gene also called PDZ and LIM domain protein 3, is a structural and signaling protein found primarily in Z-discs and intercalated discs of cardiac and skeletal muscle tissue. ALP has a key role in the structural integrity of muscle, as it has a role in crosslinking actin filaments via alpha-actinin-2 and is also involved in right ventricle development and functional contractility [12]. Its dysfunction has been implicated in the development of dilated cardiomyopathy (DCM), muscular dystrophy, and tumor growth, highlighting its function in biological and clinical conditions. Despite mounting evidence for the involvement of PDLIM3 in muscle and cardiac physiology, its direct prognostic significance and immunological role within the tumor microenvironment, as in gastric cancer, remain ill-defined [13]. Unexpectedly, in 2022, PDLIM3 was reported as part of a panel of estrogen-responsive genes (ERGs). Estrogen has a considerable effect on gene expression by its interaction with nuclear receptors ERα and ERβ, thus either promoting or suppressing transcriptional activity. Notably, PDLIM3 has been recognized as one of the most sensitive targets under this regulatory mechanism, with a coefficient of variation (CV) of 0.083, which reflects tight regulation by estrogenic signaling [14]. Meanwhile, microRNAs (miRNAs), small non-coding RNAs approximately 19 to 25 nucleotides long, have emerged as key regulators of post-transcriptional gene expression in a variety of developmental and disease settings. Previously disregarded as genomic "noise," miRNAs are now known to inhibit gene expression, regulate cellular homeostasis, and coordinate responses in diseases from autoimmune disorders to cancer development and viral infection [15].

In this regulatory framework, miR-9 and miR-10 are interesting because of their roles in osteogenic differentiation, whereas mechanistic pathways are still incompletely understood. Western blot studies have shown both miRNAs to influence the expression of Runt-related transcription factor-2 (RUNX2) and the extracellular signal-regulated kinase (ERK) pathway, hypothesizing an intimate interaction between miRNA signaling and osteogenesis [16]. In addition, downregulation of miR-9 in postmitotic neurons is linked to neurodegenerative disorders, emphasizing its role in neuronal survival and maintenance [17]. miR-10 has been demonstrated to suppress T-cell proliferation, induce apoptosis, and facilitate tumor development through multiple models [17]. Interestingly, miR-9 has also been shown to promote differentiation and immunosuppressive activity

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of myeloid-derived suppressor cells (MDSCs) through targeting Runx1, with possible implications in immunomodulation and tumor immunity [18].

Moreover, miR-10a, a strongly conserved microRNA, has also been involved in various pathological processes, such as rheumatoid arthritis [19], juvenile dermatomyositis [20], and a range of cancers [21], underlining its therapeutic and diagnostic utility in a variety of clinical settings.Previous functional studies have also corroborated the idea that miR-10a-3p actively suppresses the production of Inhibitors of Differentiation (ID) genes ID3, boosting the activity of the ossification core factor RUNX2. [22] Another microRNA we would like to highlight is microRNA6769B (mammalian), which was discovered to have an indirect regulatory influence on the expression of the RUNX-2 gene via miR-1896 (and other miRNAs w/seed GGUGGGU) (mammalian) activation, leading to upregulation of downstream signaling pathways.

The purpose of our research is to shed light on current mechanistic findings and the modulatory role of estrogen via both direct and indirect effects on the signaling pathways that regulate RUNX-2 expression. Depending on the data analyzed, our primary goal is to link the expression and regulation of microRNA9, microRNA10, miR-1896, and microRNA6769B to RUNX-2 expression by modulating the new estrogen receptor gene, ERG-PDLIM3. QIAGEN's bioinformatics tool, Ingenuity Pathway Analysis (IPA), was used to design molecular networks and analyze their biological roles. The molecular networks were compared to QIAGEN Knowledge Base (QKB) findings using canonical and signaling pathway analysis, as well as other statistical approaches.

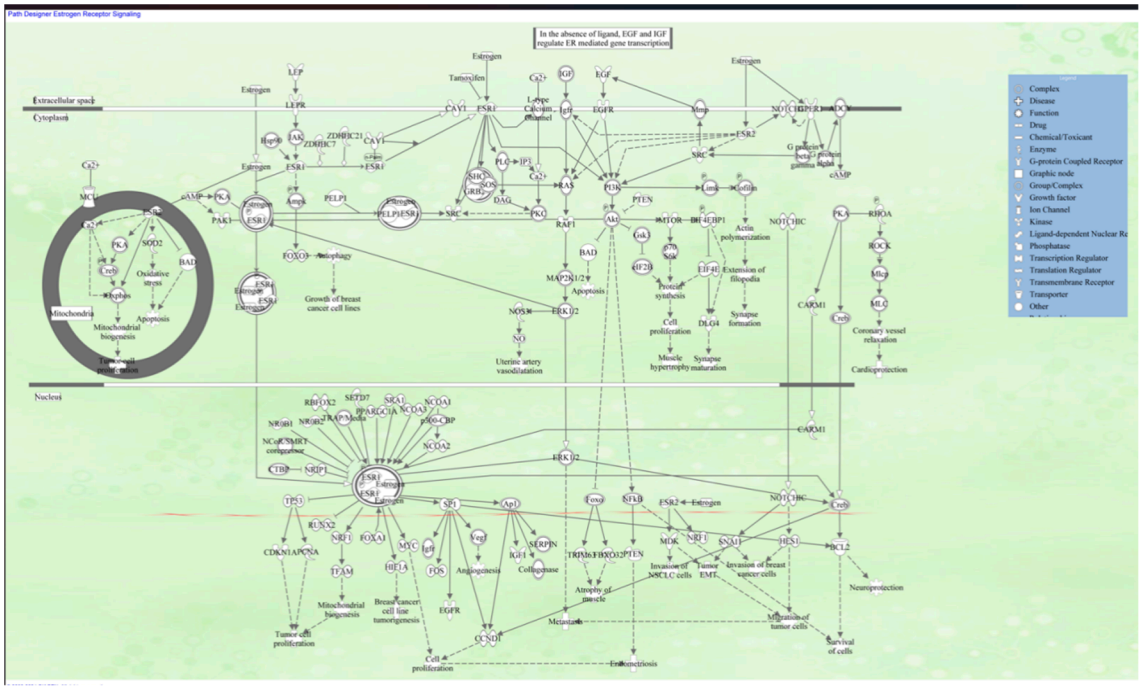

Figure 1. Estrogen receptor signaling, Schematic created using QIAGEN's Ingenuity Pathway Analysis (IPA). Estrogen receptor signaling pathway, illustrating the key interactions and regulatory mechanisms involved. It includes elements like estrogen receptors (ERα and ERβ), heat shock proteins (HSPs), and various signaling molecules, highlighting processes such as gene expression, cell proliferation, and apoptosis. This pathway plays a crucial role in physiological functions and is particularly important in understanding conditions like breast cancer and hormone-related disorders.ERα, ERβ – Estrogen Receptor Alpha and Beta | E2 – Estradiol | HSP – Heat Shock Protein | SRC – Steroid Receptor Coactivator | PI3K – Phosphoinositide 3-Kinase | AKT – Protein Kinase B | MAPK – Mitogen-Activated Protein Kinase | NF-κB – Nuclear Factor Kappa B | p53 – Tumor Protein p53 | AP-1 – Activator Protein 1 | CREB – cAMP Response Element-Binding Protein | c-Myc – Cellular Myc Protein | STAT – Signal Transducer and Activator of Transcription | EGFR – Epidermal Growth Factor Receptor | GR – Glucocorticoid Receptor | IGF-1 – Insulin-Like Growth Factor 1 | Ras – Small GTPase involved in cell signaling | JNK – c-Jun N-terminal Kinase | Fos – Proto-oncogene c-Fos | Jun – Proto-oncogene c-Jun | VEGF –

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Vascular Endothelial Growth Factor | Cyclin D – Cell Cycle Regulatory Protein | CDK – Cyclin-Dependent Kinase | PTEN – Phosphatase and Tensin Homolog | GSK3β – Glycogen Synthase Kinase 3 Beta | IRS-1 – Insulin Receptor Substrate 1 | MEK – Mitogen-Activated Protein Kinase Kinase | mTOR – Mechanistic Target of Rapamycin | BCL-2 – B-Cell Lymphoma 2 | BAX – Bcl-2-Associated X Protein---designed using IPA_QIAGEN.

II. Material and Methods

Ingenuity Pathway Analysis Software.

IPA, a bioinformatics software tool for data mining, uses canonical pathways and gene regulatory networks from literature to help interpret and analyze various biological pathways. Various techniques were used to create pathways depicting the molecular networks connected with estrogen, RUNX-2, different microRNAs, and their intermediary molecules to evaluate functional hypotheses. The bioinformatics tool utilized data from the QIAGEN Knowledge Base (QKB) between February 5th, 2024, and June 14th, 2025. [23–26]. Figure 2 illustrates the workflow utilized from QIAGEN's Ingenuity Pathway Analysis (IPA) bioinformatics software for data mining.

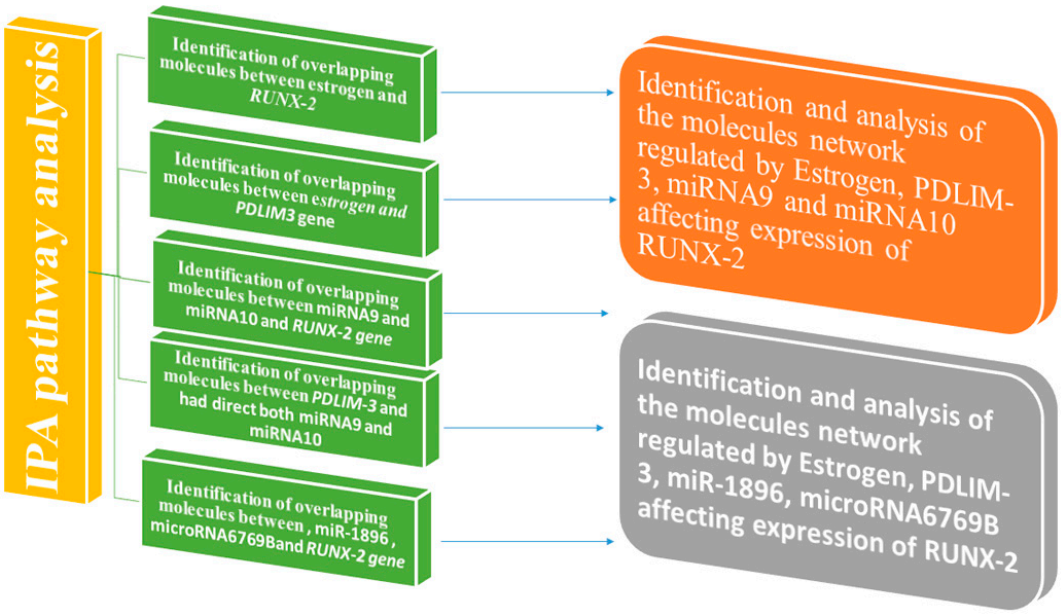

Figure 2. The data mining workflow was based on QIAGEN's Ingenuity Pathway Analysis (IPA) bioinformatics tools. The "Grow", "Connect", "Pathway Explorer", and "Molecule Activity Predictor" (MAP) tools from the "My Pathway" feature were used to develop biological networks that showed the connectivity between distinct nodes. Furthermore, the "Core Analysis: Expression Analysis" tool was utilized to compare the molecules within the produced molecular route to canonical pathways recorded within QIAGEN's knowledge base (QKB).

III. Results and Outcomes, all the figures and tables are provided as supplementary materials

1. Molecular Pathway Analysis of Molecules Mediating the Relationship Between Estrogen and *PDLIM3*

The "MAP" program was used to generate a connectivity map depicting the interaction between the 10 molecules involved with estrogen's direct influence and their relationship with *PDLIM3*. This is depicted in Figure 3 and Supplemental Tables 1–2. Our findings show that estrogen is associated with intermediates such as Proliferating Cellular Nuclear Antigen (PCNA), Cyclin-Dependent Kinase 4 (CDK4), Luteinizing Hormone (LH), Mitogen-Activated Protein Kinase-1 (MAPK-1), Estrogen Receptor 1 and 2 (ESR 1 & 2), and Follicle-Stimulating Hormone (FSH), all of which are linked to the

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PDLIM3 gene. This indicates that estrogen's modulation of PDLIM3 may be closely associated with cell cycle progression and gonadotropin signaling pathways, placing PDLIM3 at the nexus of hormonal and proliferative signals.

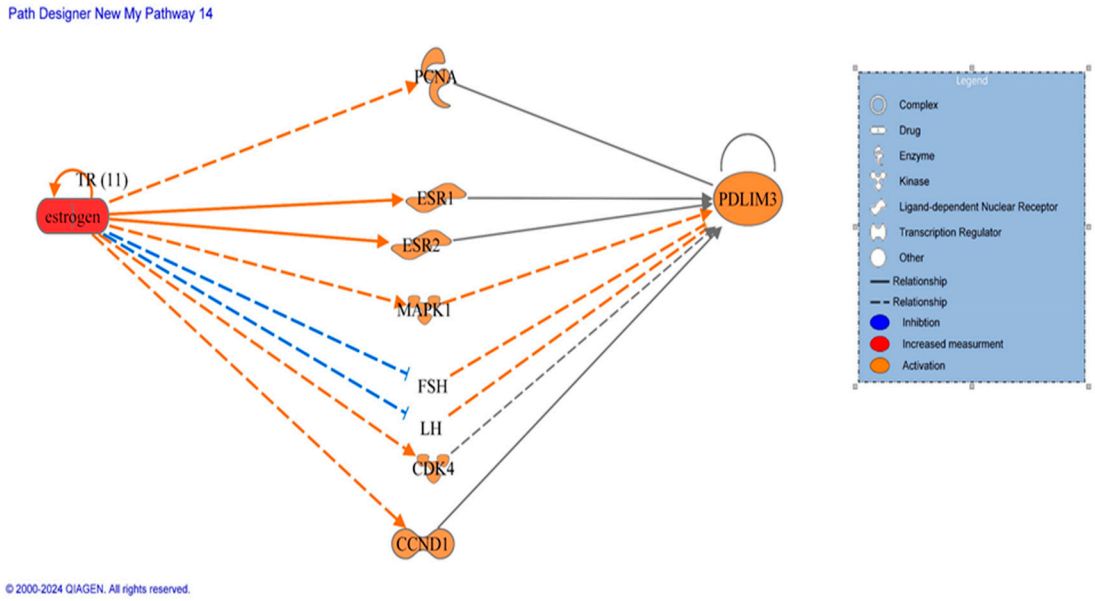

Figure 3. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of estrogen on PDLIM3 expression.

Table 1. : Estrogen-regulated compounds that impact the expression of the *PDLIM3* gene.

Symbol	Gene Name	Location	Family
CCND1	cyclin D1	Nucleus	transcription regulator
CDK4	cyclin dependent kinase 4	Nucleus	kinase
ESR1	estrogen receptor 1	Nucleus	ligand-dependent nuclear receptor
ESR2	estrogen receptor 2	Nucleus	ligand-dependent nuclear receptor
Estrogen	estrogen	Other	chemical drug
FSH	Follicle stimulating hormone	Plasma Membrane	complex
LH	Luteliizng hormone	Plasma Membrane	Complex
MAPK1	mitogen-activated protein kinase 1	Cytoplasm	Kinase
PCNA	proliferating cell nuclear antigen	Nucleus	Enzyme
PDLIM3	PDZ and LIM domain 3	Cytoplasm	Other

Table 2. Various interactions between molecules incorporated in the estrogen-*PDLIM3* pathway.

From Molecule(s)	Relationship Type	To Molecule(s)
1. CCND1	expression	PDLIM3
2. CDK4	expression	PDLIM3
3. ESR1	chemical-protein interactions	estrogen
4. ESR1	expression	PDLIM3
5. ESR1	regulation of binding	Estrogen
6. ESR2	chemical-protein interactions	Estrogen
7. ESR2	expression	PDLIM3
8. FSH	expression	PDLIM3
9. LH	expression	PDLIM3
10. MAPK1	expression	PDLIM3
11. PDLIM3	protein-protein interactions	PCNA
12. PDLIM3	protein-protein interactions	PDLIM3
13. estrogen	activation	CDK4

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14. estrogen	activation	ESR1
15. estrogen	activation	ESR2
16. estrogen	activation	MAPK1
17. estrogen	chemical-protein interactions	ESR1
18. estrogen	chemical-protein interactions	ESR2
19. estrogen	expression	CCND1
20. estrogen	expression	ESR1
21. estrogen	expression	ESR2
22. estrogen	expression	FSH
23. estrogen	expression	LH
24. estrogen	expression	PCNA
25. estrogen	localization	FSH
26. estrogen	localization	LH
27. estrogen	molecular cleavage	ESR1
28. estrogen	phosphorylation	ESR1
29. estrogen	phosphorylation	ESR2
30. estrogen	phosphorylation	MAPK1
31. estrogen	regulation of binding	CCND1
32. estrogen	regulation of binding	CDK4
33. estrogen	regulation of binding	ESR1
34. estrogen	regulation of binding	ESR2
35. estrogen	transcription	CCND1
36. estrogen	translocation	ESR1
37. estrogen	translocation	ESR2
38. estrogen	translocation	estrogen

2. Molecular Pathway Analysis of Molecules Mediating the Relationship Between Estrogen and Affect Expression of RUNX-2

The "MAP" program and QKB can identify 75 estrogen-controlled pathways that regulate RUNX-2 expression. These molecules included biological medicines, the canonical pathway, complexes, cytokines, enzymes, G-protein coupled receptors, kinases, nuclear receptors, peptidase, phosphatase, transcription and translation regulators, transmembrane receptors, and transporters, as depicted in Figure 4 and Tables 3 and 4, respectively. The magnitude and variety of this network highlight the pivotal and multifaceted role of estrogen as a principal regulator of *RUNX-2*, able to affect osteogenesis through an extensive array of signaling pathways.

Table 3. Estrogen-regulated compounds that impact the expression of the *RUNX-2* gene.

Symbol	molecule/ Gene Name	Location	Family
39. AKT	AKT Serine/Threonine Kinase 1.	Cytoplasm	group
40. APOB	apolipoprotein B	Extracellular Space	Transporter
41. AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
42. beta-estradiol	beta-estradiol	Other	chemical - endogenous mammalian
43. BMP2	bone morphogenetic protein 2	Extracellular Space	growth factor
44. CALCA	calcitonin related polypeptide alpha	Plasma Membrane	Other
45. Calcitriol	calcitriol	Other	chemical drug
46. CEBPB	CCAAT enhancer binding protein beta	Nucleus	transcription regulator
47. CXCL12	C-X-C motif chemokine ligand 12	Extracellular Space	Cytokine

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48. dinoprost	dinoprost	Other	chemical - endogenous mammalian
49. DUSP1	dual specificity phosphatase 1	Nucleus	Phosphatase
50. E2F1	E2F transcription factor 1	Nucleus	transcription regulator
51. EGF	epidermal growth factor	Extracellular Space	growth factor
52. EGFR	epidermal growth factor receptor	Plasma Membrane	Kinase
53. EP300	E1A binding protein p300	Nucleus	transcription regulator
54. ERK1/2	ERK1/2	Cytoplasm	Group
55. ESR1	estrogen receptor 1	Nucleus	ligand-dependent nuclear receptor
56. ESR2	estrogen receptor 2	Nucleus	ligand-dependent nuclear receptor
57. Estrogen	estrogen	Other	chemical drug
58. FAS	Fas cell surface death receptor	Plasma Membrane	transmembrane receptor
59. FASLG	Fas ligand	Extracellular Space	Cytokine
60. FGF7	fibroblast growth factor 7	Extracellular Space	growth factor
61. FN1	fibronectin 1	Extracellular Space	Other
62. FOS	Fos proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
63. FOXO1	forkhead box O1	Nucleus	transcription regulator
64. GATA3	GATA binding protein 3	Nucleus	transcription regulator
65. HDAC1	histone deacetylase 1	Nucleus	enzyme
66. HGF	hepatocyte growth factor	Extracellular Space	growth factor
67. HIF1A	hypoxia inducible factor 1 subunit alpha	Nucleus	transcription regulator
68. histone H3	histone H3	Nucleus	group
69. HIVEP3	HIVEP zinc finger 3	Nucleus	transcription regulator
70. HSPH1	heat shock protein family H (Hsp110) member 1	Cytoplasm	other
71. IFNG	interferon gamma	Extracellular Space	cytokine
72. IGF1	insulin like growth factor 1	Extracellular Space	growth factor
73. IGF1R	insulin like growth factor 1 receptor	Plasma Membrane	transmembrane receptor
74. IGFBP5	insulin like growth factor binding protein 5	Extracellular Space	other
75. IHH	Indian hedgehog signaling molecule	Extracellular Space	enzyme
76. IL1	IL1	Extracellular Space	group
77. IL1B	interleukin 1 beta	Extracellular Space	cytokine
78. IL6	interleukin 6	Extracellular Space	cytokine
79. JUN	Jun proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
80. JUNB	JunB proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
81. JUND	JunD proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator

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82. LCN2	lipocalin 2	Extracellular Space	transporter
83. LDLR	low density lipoprotein receptor	Plasma Membrane	Transporter
84. LH	LH	Plasma Membrane	Complex
85. MAPK	MAPK	Cytoplasm	Group
86. MSX1	msh homeobox 1	Nucleus	transcription regulator
87. OXTR	oxytocin receptor	Plasma Membrane	G-protein coupled receptor
88. PDLIM7	PDZ and LIM domain 7	Cytoplasm	Other
89. prostaglandin E2	prostaglandin E2	Other	chemical - endogenous mammalian
90. PTEN	phosphatase and tensin homolog	Cytoplasm	Phosphatase
91. PTGS2	prostaglandin-endoperoxide synthase 2	Cytoplasm	Enzyme
92. PTH	parathyroid hormone	Extracellular Space	Other
93. RAG1	recombination activating 1	Nucleus	Enzyme
94. RB1	RB transcriptional corepressor 1	Nucleus	transcription regulator
95. RELA	RELA proto-oncogene, NF-kB subunit	Nucleus	transcription regulator
96. RPTOR	regulatory associated protein of MTOR complex 1	Cytoplasm	Other
97. RUNX2	RUNX family transcription factor 2	Nucleus	transcription regulator
98. SERPINA3	serpin family A member 3	Extracellular Space	Other
99. SKP2	S-phase kinase associated protein 2	Nucleus	other
100. SMAD2	SMAD family member 2	Nucleus	transcription regulator
101. SMAD2/3	SMAD2/3	Cytoplasm	group
102. SMAD3	SMAD family member 3	Nucleus	transcription regulator
103. SOD1	superoxide dismutase 1	Cytoplasm	Enzyme
104. SPP1	secreted phosphoprotein 1	Extracellular Space	Cytokine
105. STAT1	signal transducer and activator of transcription 1	Nucleus	transcription regulator
106. STAT3	signal transducer and activator of transcription 3	Nucleus	transcription regulator
107. STAT5A	signal transducer and activator of transcription 5A	Nucleus	transcription regulator
108. TGF beta	TGF beta	Extracellular Space	Group
109. TGFB1	transforming growth factor beta 1	Extracellular Space	growth factor
110. TNF	tumor necrosis factor	Extracellular Space	Cytokine
111. TNFRSF11B	TNF receptor superfamily member 11b	Plasma Membrane	transmembrane receptor
112. TP53	tumor protein p53	Nucleus	transcription regulator
113. VDR	vitamin D receptor	Nucleus	transcription regulator
114. VEGFA	vascular endothelial growth factor A	Extracellular Space	growth factor

Table 4. Various interactions between molecules incorporated in the Estrogen-*RUNX-2* pathway.

From Molecule(s)	Relationship Type	To Molecule(s)
1. AR	protein-protein interactions	ESR2
2. AR	protein-protein interactions	FKBP4
3. AR	protein-protein interactions	HSP90 (family)

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4. ASAH1	expression	RUNX2
5. CBL	activation	RUNX2
6. CBL	expression	RUNX2
7. CBX5	expression	RUNX2
8. CEBPA	expression	RUNX2
9. CEBPB	expression	RUNX2
10. CEBPB	protein-protein interactions	ESR1
11. CEBPB	protein-protein interactions	RUNX2
12. CREBBP	expression	RUNX2
13. CREBBP	protein-protein interactions	AFP
14. CREBBP	protein-protein interactions	RUNX2
15. CTNNB1	expression	RUNX2
16. CTNNB1	inhibition	RUNX2
17. CTNNB1	protein-DNA interactions	RUNX2
18. CTNNB1	protein-protein interactions	RUNX2
19. CTNNB1	transcription	RUNX2
20. DDX5	protein-protein interactions	ESR1
21. EED	expression	RUNX2
22. EED	modification	RUNX2
23. ELK1	expression	RUNX2
24. ELK1	protein-DNA interactions	RUNX2
25. EP300	expression	RUNX2
26. EP300	protein-protein interactions	ESR1
27. EP300	protein-protein interactions	RUNX2
28. ER/estrogen	membership	ESR1
29. ERBB2	activation	CTNNB1
30. ERBB2	activation	EP300
31. ERBB2	activation	ERK1/2
32. ERBB2	activation	NOTCH1
33. ERBB2	activation	SP1
34. ERBB2	activation	STAT1
35. ERBB2	activation	STAT3
36. ERBB2	activation	histone H3
37. ERBB2	inhibition	FOXO3
38. ERBB2	inhibition	RB
39. ERBB2	inhibition	RB1
40. ERBB2	phosphorylation	CTNNB1
41. ERBB2	phosphorylation	EP300
42. ERBB2	phosphorylation	ERK1/2
43. ERBB2	phosphorylation	FOXO3
44. ERBB2	phosphorylation	RB
45. ERBB2	phosphorylation	RB1
46. ERBB2	phosphorylation	SP1
47. ERBB2	phosphorylation	STAT1
48. ERBB2	phosphorylation	STAT3
49. ERBB2	phosphorylation	histone H3
50. ERBB2	protein-protein interactions	CBL
51. ERBB2	protein-protein interactions	CCNB1
52. ERBB2	protein-protein interactions	CTNNB1
53. ERBB2	protein-protein interactions	CUL4A
54. ERBB2	protein-protein interactions	NOTCH1

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55. ERBB2	protein-protein interactions	STAT1
56. ERBB2	protein-protein interactions	STAT3
57. ERK1/2	activation	RUNX2
58. ERK1/2	protein-protein interactions	RUNX2
59. ESR1	RNA-RNA interactions: non-targeting interactions	ESR2
60. ESR1	activation	Ap1
61. ESR1	activation	ESR2
62. ESR1	activation	RELA
63. ESR1	activation	RUNX2
64. ESR1	activation	SMAD3
65. ESR1	activation	SP1
66. ESR1	activation	STAT5A
67. ESR1	activation	TP53
68. ESR1	chemical-protein interactions	estrogen
69. ESR1	expression	AR
70. ESR1	expression	CBL
71. ESR1	expression	CCNB1
72. ESR1	expression	CEBPA
73. ESR1	expression	CEBPB
74. ESR1	expression	CEBPD
75. ESR1	expression	CTNNB1
76. ESR1	expression	CUL4B
77. ESR1	expression	EHMT2
78. ESR1	expression	EP300
79. ESR1	expression	ESR2
80. ESR1	expression	FOS
81. ESR1	expression	FOSB
82. ESR1	expression	FOSL1
83. ESR1	expression	FOSL2
84. ESR1	expression	FOXO1
85. ESR1	expression	FOXO4
86. ESR1	expression	GATA3
87. ESR1	expression	GLI2
88. ESR1	expression	GSN
89. ESR1	expression	HES1
90. ESR1	expression	HEY1
91. ESR1	expression	HIF1A
92. ESR1	expression	HSPD1
93. ESR1	expression	HSPH1
94. ESR1	expression	ID1
95. ESR1	expression	IRF4
96. ESR1	expression	JUN
97. ESR1	expression	JUNB
98. ESR1	expression	JUND
99. ESR1	expression	LIMA1
100.ESR1	expression	NFYB
101.ESR1	expression	NOTCH1
102.ESR1	expression	NR0B2
103.ESR1	expression	OSTF1
104.ESR1	expression	PPARD

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105.ESR1	expression	PPARG
106.ESR1	expression	RB1
107.ESR1	expression	RBL2
108.ESR1	expression	RELA
109.ESR1	expression	RUNX2
110.ESR1	expression	SKP2
111.ESR1	expression	SMAD2
112.ESR1	expression	SMAD3
113.ESR1	expression	SMAD5
114.ESR1	expression	SMAD6
115.ESR1	expression	SMURF1
116.ESR1	expression	SMURF2
117.ESR1	expression	SNAI1
118.ESR1	expression	SNAI2
119.ESR1	expression	SOX9
120.ESR1	expression	SP1
121.ESR1	expression	STAT1
122.ESR1	expression	STAT3
123.ESR1	expression	STAT5A
124.ESR1	expression	TCF7L2
125.ESR1	expression	THRB
126.ESR1	expression	TP53
127.ESR1	expression	TRIB3
128.ESR1	expression	TSC22D3
129.ESR1	expression	VDR
130.ESR1	expression	ZBTB7B
131.ESR1	inhibition	RELA
132.ESR1	inhibition	RUNX2
133.ESR1	inhibition	TP53
134.ESR1	protein-DNA interactions	CREBBP
135.ESR1	protein-DNA interactions	ESRRA
136.ESR1	protein-DNA interactions	FOSL1
137.ESR1	protein-DNA interactions	FOXC1
138.ESR1	protein-DNA interactions	GATA3
139.ESR1	protein-DNA interactions	KAT6B
140.ESR1	protein-DNA interactions	NR0B2
141.ESR1	protein-DNA interactions	PPARGC1A
142.ESR1	protein-DNA interactions	SIRT1
143.ESR1	protein-DNA interactions	SOX9
144.ESR1	protein-DNA interactions	STAT5A
145.ESR1	protein-DNA interactions	TP53
146.ESR1	protein-DNA interactions	ZMYND8
147.ESR1	protein-protein interactions	ALYREF
148.ESR1	protein-protein interactions	AR
149.ESR1	protein-protein interactions	Ap1
150.ESR1	protein-protein interactions	CBX5
151.ESR1	protein-protein interactions	CEBPA
152.ESR1	protein-protein interactions	CEBPB
153.ESR1	protein-protein interactions	CIC
154.ESR1	protein-protein interactions	CREBBP
155.ESR1	protein-protein interactions	CTBP2

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156.ESR1	protein-protein interactions	CTNNB1
157.ESR1	protein-protein interactions	CUL4B
158.ESR1	protein-protein interactions	DDX5
159.ESR1	protein-protein interactions	EHMT2
160.ESR1	protein-protein interactions	EP300
161.ESR1	protein-protein interactions	ERBB2
162.ESR1	protein-protein interactions	ESR2
163.ESR1	protein-protein interactions	ESRRA
164.ESR1	protein-protein interactions	FOS
165.ESR1	protein-protein interactions	FOSL2
166.ESR1	protein-protein interactions	FOXO1
167.ESR1	protein-protein interactions	FOXO4
168.ESR1	protein-protein interactions	GATA3
169.ESR1	protein-protein interactions	GSN
170.ESR1	protein-protein interactions	HDAC1
171.ESR1	protein-protein interactions	HIF1A
172.ESR1	protein-protein interactions	HSPD1
173.ESR1	protein-protein interactions	HSPH1
174.ESR1	protein-protein interactions	JUN
175.ESR1	protein-protein interactions	JUNB
176.ESR1	protein-protein interactions	JUND
177.ESR1	protein-protein interactions	LIMA1
178.ESR1	protein-protein interactions	NR0B2
179.ESR1	protein-protein interactions	PPARG
180.ESR1	protein-protein interactions	PPARGC1A
181.ESR1	protein-protein interactions	RELA
182.ESR1	protein-protein interactions	RUNX2
183.ESR1	protein-protein interactions	SIRT1
184.ESR1	protein-protein interactions	SKP2
185.ESR1	protein-protein interactions	SMAD2
186.ESR1	protein-protein interactions	SMAD3
187.ESR1	protein-protein interactions	SMURF1
188.ESR1	protein-protein interactions	SP1
189.ESR1	protein-protein interactions	STAT1
190.ESR1	protein-protein interactions	STAT3
191.ESR1	protein-protein interactions	STAT5A
192.ESR1	protein-protein interactions	TCF7L2
193.ESR1	protein-protein interactions	TP53
194.ESR1	protein-protein interactions	ZBTB7B
195.ESR1	protein-protein interactions	ZMYND8
196.ESR1	transcription	ESRRA
197.ESR1	transcription	FOS
198.ESR1	transcription	PPARGC1A
199.ESR1	transcription	RUNX2
200.ESR1	transcription	SIRT1
201.ESR1	transcription	TP53
202.ESR2	RNA-RNA interactions: non-targeting interactions	ESR1
203.ESR2	activation	AR
204.ESR2	activation	ESR1
205.ESR2	activation	RELA

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206.ESR2	activation	SP1
207.ESR2	activation	STAT3
208.ESR2	activation	STAT5A
209.ESR2	chemical-protein interactions	estrogen
210.ESR2	expression	AR
211.ESR2	expression	CEBPD
212.ESR2	expression	CREBBP
213.ESR2	expression	CTNNB1
214.ESR2	expression	EHMT2
215.ESR2	expression	ELK1
216.ESR2	expression	EP300
217.ESR2	expression	ESR1
218.ESR2	expression	EZH2
219.ESR2	expression	FOS
220.ESR2	expression	FOSL2
221.ESR2	expression	FOXC2
222.ESR2	expression	FOXO1
223.ESR2	expression	FOXO3
224.ESR2	expression	GATA1
225.ESR2	expression	GATA3
226.ESR2	expression	HSPD1
227.ESR2	expression	JAG2
228.ESR2	expression	JUNB
229.ESR2	expression	KLF4
230.ESR2	expression	NOTCH1
231.ESR2	expression	RELA
232.ESR2	expression	RUNX2
233.ESR2	expression	SKP2
234.ESR2	expression	SMAD2
235.ESR2	expression	SMAD3
236.ESR2	expression	SMAD4
237.ESR2	expression	SNAI1
238.ESR2	expression	SNAI2
239.ESR2	expression	SP1
240.ESR2	expression	SRF
241.ESR2	expression	TWIST1
242.ESR2	expression	YY1
243.ESR2	expression	osteocalcin
244.ESR2	inhibition	RELA
245.ESR2	protein-DNA interactions	FOS
246.ESR2	protein-RNA interactions	CTNNB1
247.ESR2	protein-protein interactions	ALYREF
248.ESR2	protein-protein interactions	AR
249.ESR2	protein-protein interactions	ASAH1
250.ESR2	protein-protein interactions	CIC
251.ESR2	protein-protein interactions	CREBBP
252.ESR2	protein-protein interactions	CTBP2
253.ESR2	protein-protein interactions	CTNNB1
254.ESR2	protein-protein interactions	EED
255.ESR2	protein-protein interactions	EHMT2
256.ESR2	protein-protein interactions	EP300

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257.ESR2	protein-protein interactions	ESR1
258.ESR2	protein-protein interactions	FOS
259.ESR2	protein-protein interactions	FOXO3
260.ESR2	protein-protein interactions	HSPD1
261.ESR2	protein-protein interactions	SMAD3
262.ESR2	protein-protein interactions	SMAD4
263.ESR2	protein-protein interactions	SP1
264.ESR2	protein-protein interactions	STAT3
265.ESR2	protein-protein interactions	STAT5A
266.ESRRA	protein-DNA interactions	RUNX2
267.ESRRA	protein-protein interactions	ESR1
268.ESRRA	transcription	RUNX2
269.ESTG:ESR1:chaperone	membership	ESR1
270.ESTG:ESR2:chaperone	membership	ESR2
271.ESTG:Me-PalmS-ESR dimers	membership	ESR1
272.ESTG:Me-PalmS-ESR dimers	membership	ESR2
273.ETS1	protein-DNA interactions	RUNX2
274.ETS1	protein-protein interactions	ESR1
275.ETS1	protein-protein interactions	RUNX2
276.ETS1	transcription	RUNX2
277.EWSR1	protein-protein interactions	ESR1
278.EZH2	expression	RUNX2
279.EZH2	protein-protein interactions	ESR1
280.FHL2	protein-protein interactions	ESR1
281.FHL2	protein-protein interactions	ESR2
282.FKBP4	activation	AR
283.FKBP4	chemical-protein interactions	estrogen
284.FKBP4	protein-protein interactions	AR
285.FKBP4	protein-protein interactions	CTNNB1
286.FKBP4	protein-protein interactions	DET1
287.FKBP4	protein-protein interactions	ESR1
288.FKBP4	protein-protein interactions	EWSR1
289.FKBP4	protein-protein interactions	EZH2
290.FKBP4	protein-protein interactions	NR3C1
291.FOS	protein-DNA interactions	RUNX2
292.FOS	protein-protein interactions	ESR1
293.FOS	protein-protein interactions	ESR2
294.FOS	protein-protein interactions	RUNX2
295.FOS	protein-protein interactions	estrogen receptor
296.FOSB	protein-DNA interactions	RUNX2
297.FOSL1	protein-DNA interactions	RUNX2
298.FOSL2	protein-DNA interactions	RUNX2
299.FOSL2	protein-protein interactions	ESR1
300.FOXC1	expression	RUNX2
301.FOXC2	expression	RUNX2
302.FOXO1	activation	RUNX2
303.FOXO1	expression	RUNX2
304.FOXO1	protein-protein interactions	ESR1
305.FOXO1	protein-protein interactions	RUNX2
306.FOXO1	protein-protein interactions	estrogen receptor
307.FOXO3	expression	RUNX2

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308.FOXO3	protein-protein interactions	ESR1
309.FOXO3	protein-protein interactions	ESR2
310.FOXO3	protein-protein interactions	FKBP4
311.FOXO4	expression	RUNX2
312.FOXO4	protein-protein interactions	ESR1
313.GATA1	expression	RUNX2
314.GATA3	protein-DNA interactions	ESR1
315.GATA3	protein-protein interactions	ESR1
316.GLI2	protein-protein interactions	RUNX2
317.GLI2	transcription	RUNX2
318.GNB2	protein-protein interactions	ESR1
319.GNB2	protein-protein interactions	ESR2
320.GNB2	protein-protein interactions	FKBP4
321.GPER1:Heterotrimeric G-protein Gs:ESTG	membership	GNB2
322.GSN	protein-protein interactions	ERBB2
323.GSN	protein-protein interactions	ESR1
324.GSN	protein-protein interactions	ESR2
325.HDAC1	protein-protein interactions	ESR1
326.HDAC1	protein-protein interactions	ESR2
327.HDAC3	activation	RUNX2
328.HDAC3	protein-protein interactions	ESR1
329.HDAC3	protein-protein interactions	ESR2
330.HDAC3	protein-protein interactions	RUNX2
331.HDAC4	activation	RUNX2
332.HDAC4	expression	RUNX2
333.HDAC4	protein-protein interactions	ESR1
334.HDAC4	protein-protein interactions	RUNX2
335.HDAC5	expression	RUNX2
336.HDAC5	protein-protein interactions	ESR1
337.HDAC5	protein-protein interactions	RUNX2
338.HDAC6	protein-protein interactions	ERBB2
339.HDAC6	protein-protein interactions	ESR2
340.HDAC7	protein-protein interactions	ESR1
341.HES1	activation	RUNX2
342.HES1	protein-protein interactions	RUNX2
343.HIF1A	protein-protein interactions	ESR1
344.HMGB2	expression	RUNX2
345.HMGB2	protein-DNA interactions	RUNX2
346.HMGB2	protein-protein interactions	ESR1
347.HMGB2	protein-protein interactions	RUNX2
348.HMGB2	protein-protein interactions	estrogen receptor
349.HMGB2	transcription	RUNX2
350.HSP90 (family)	activation	AR
351.HSP90 (family)	activation	STAT3
352.HSP90 (family)	activation	TP53
353.HSP90 (family)	chemical-protein interactions	estrogen
354.HSP90 (family)	protein-protein interactions	AR
355.HSP90 (family)	protein-protein interactions	ESR1
356.HSP90 (family)	protein-protein interactions	EWSR1
357.HSP90 (family)	protein-protein interactions	GATA3

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358.HSP90 (family)	protein-protein interactions	HDAC1
359.HSP90 (family)	protein-protein interactions	HDAC6
360.HSP90 (family)	protein-protein interactions	HIF1A
361.HSP90 (family)	protein-protein interactions	NR3C1
362.HSP90 (family)	protein-protein interactions	STAT3
363.HSP90 (family)	protein-protein interactions	STUB1
364.HSP90 (family)	protein-protein interactions	TP53
365.HSP90 (family)	protein-protein interactions	histone H4
366.HSPA4	protein-protein interactions	ERBB2
367.HSPA4	protein-protein interactions	ESR1
368.HSPA4	protein-protein interactions	ESR2
369.HSPA4	protein-protein interactions	FKBP4
370.HSPA4L	protein-protein interactions	ERBB2
371.HSPA4L	protein-protein interactions	ESR1
372.HSPD1	protein-protein interactions	ERBB2
373.HSPD1	protein-protein interactions	ESR1
374.HSPD1	protein-protein interactions	ESR2
375.HSPH1	protein-protein interactions	ESR1
376.HSPH1	protein-protein interactions	ESR2
377.HSPH1	protein-protein interactions	HSP90 (family)
378.ID1	expression	RUNX2
379.IFI16	activation	RUNX2
380.IFI16	protein-protein interactions	FKBP4
381.IFI16	protein-protein interactions	RUNX2
382.IGF1	activation	ESR1
383.IGF1	activation	TP53
384.IGF1	chemical-protein interactions	estrogen
385.IMPDH1	protein-protein interactions	ESR1
386.IRF4	expression	RUNX2
387.IRF4	protein-protein interactions	FKBP4
388.JUN	protein-DNA interactions	RUNX2
389.JUN	protein-protein interactions	ESR1
390.JUN	protein-protein interactions	ESR2
391.JUN	protein-protein interactions	RUNX2
392.JUN	protein-protein interactions	estrogen receptor
393.JUNB	expression	RUNX2
394.JUNB	protein-protein interactions	ESR1
395.JUNB	protein-protein interactions	RUNX2
396.JUND	protein-DNA interactions	RUNX2
397.JUND	protein-protein interactions	ESR1
398.KAT2B	protein-protein interactions	ESR1
399.KAT2B	protein-protein interactions	estrogen receptor
400.KAT6A	expression	RUNX2
401.KAT6A	protein-protein interactions	ESR1
402.KAT6A	protein-protein interactions	RUNX2
403.KAT6B	protein-protein interactions	ESR2
404.KLF4	expression	RUNX2
405.KLF4	protein-DNA interactions	RUNX2
406.KLF4	protein-protein interactions	ESR1
407.KLF4	protein-protein interactions	RUNX2
408.LEF1	expression	RUNX2

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409.LEF1	inhibition	RUNX2
410.LEF1	protein-DNA interactions	RUNX2
411.LEF1	protein-protein interactions	ESR1
412.LEF1	protein-protein interactions	RUNX2
413.LIMA1	protein-protein interactions	ESR1
414.LIMA1	protein-protein interactions	HSP90 (family)
415.MAP2K1	activation	RUNX2
416.MAP2K1	phosphorylation	RUNX2
417.MAP2K1	protein-protein interactions	ERBB2
418.MAP2K1	protein-protein interactions	HSP90 (family)
419.MATK	protein-protein interactions	ERBB2
420.MEN1	expression	RUNX2
421.MEN1	protein-protein interactions	ESR1
422.MEN1	protein-protein interactions	ESR2
423.MEN1	protein-protein interactions	RUNX2
424.MEN1	protein-protein interactions	estrogen receptor
425.MSX2	activation	RUNX2
426.MSX2	protein-protein interactions	ESR1
427.MSX2	protein-protein interactions	RUNX2
428.NFYB	protein-DNA interactions	RUNX2
429.NFYB	protein-protein interactions	RUNX2
430.NOTCH1	activation	RUNX2
431.NOTCH1	expression	RUNX2
432.NOTCH1	protein-protein interactions	ERBB2
433.NOTCH1	protein-protein interactions	RUNX2
434.NR0B2	protein-protein interactions	ESR1
435.NR0B2	protein-protein interactions	ESR2
436.NR0B2	protein-protein interactions	estrogen receptor
437.NR3C1	protein-DNA interactions	RUNX2
438.NR3C1	protein-protein interactions	ESR1
439.NR3C1	protein-protein interactions	FKBP4
440.NR3C1	protein-protein interactions	HSP90 (family)
441.NR3C1	protein-protein interactions	estrogen receptor
442.OSTF1	protein-DNA interactions	RUNX2
443.PIN1	activation	RUNX2
444.PIN1	protein-protein interactions	ERBB2
445.PIN1	protein-protein interactions	ESR1
446.PIN1	protein-protein interactions	RUNX2
447.PML	protein-protein interactions	ESR2
448.PPARD	expression	RUNX2
449.PPARD	protein-protein interactions	HSP90 (family)
450.PPARG	expression	RUNX2
451.PPARG	protein-protein interactions	ESR1
452.PPARG	protein-protein interactions	HSP90 (family)
453.PPARG	protein-protein interactions	RUNX2
454.PPARGC1A	protein-protein interactions	ESR1
455.PPARGC1A	protein-protein interactions	ESR2
456.PPARGC1A	transcription	RUNX2
457.PPARGC1B	protein-protein interactions	ESR1
458.PPARGC1B	transcription	RUNX2
459.PTH	chemical-protein interactions	estrogen

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460.RB	activation	RUNX2
461.RB	protein-protein interactions	RUNX2
462.RB1	protein-protein interactions	ESR2
463.RB1	protein-protein interactions	estrogen receptor
464.RBM14	protein-protein interactions	ERBB2
465.RBM14	protein-protein interactions	ESR1
466.RBM28	protein-protein interactions	ESR1
467.RELA	expression	RUNX2
468.RELA	protein-protein interactions	ESR1
469.RUNX1	expression	RUNX2
470.RUNX1	protein-DNA interactions	RUNX2
471.RUNX1	protein-protein interactions	ESR1
472.RUNX1	protein-protein interactions	RUNX2
473.RUNX2	activation	NOTCH1
474.RUNX2	activation	RUNX2
475.RUNX2	expression	RUNX2
476.RUNX2	inhibition	RUNX2
477.RUNX2	localization	RUNX2
478.RUNX2	modification	RUNX2
479.RUNX2	molecular cleavage	RUNX2
480.RUNX2	protein-DNA interactions	RUNX2
481.RUNX2	protein-protein interactions	ALYREF
482.RUNX2	protein-protein interactions	AR
483.RUNX2	protein-protein interactions	Ap1
484.RUNX2	protein-protein interactions	CCNB1
485.RUNX2	protein-protein interactions	CEBPB
486.RUNX2	protein-protein interactions	CEBPD
487.RUNX2	protein-protein interactions	CIC
488.RUNX2	protein-protein interactions	CREBBP
489.RUNX2	protein-protein interactions	CTBP2
490.RUNX2	protein-protein interactions	CTNNB1
491.RUNX2	protein-protein interactions	CUL4A
492.RUNX2	protein-protein interactions	CUL4B
493.RUNX2	protein-protein interactions	DDX5
494.RUNX2	protein-protein interactions	DET1
495.RUNX2	protein-protein interactions	EHMT2
496.RUNX2	protein-protein interactions	EP300
497.RUNX2	protein-protein interactions	ERK1/2
498.RUNX2	protein-protein interactions	ESR1
499.RUNX2	protein-protein interactions	ETS1
500.RUNX2	protein-protein interactions	EWSR1
501.RUNX2	protein-protein interactions	FHL2
502.RUNX2	protein-protein interactions	FOS
503.RUNX2	protein-protein interactions	FOXO1
504.RUNX2	protein-protein interactions	GATA3
505.RUNX2	protein-protein interactions	GLI2
506.RUNX2	protein-protein interactions	GNB2
507.RUNX2	protein-protein interactions	GSN
508.RUNX2	protein-protein interactions	HDAC1
509.RUNX2	protein-protein interactions	HDAC3
510.RUNX2	protein-protein interactions	HDAC4

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511.RUNX2	protein-protein interactions	HDAC5
512.RUNX2	protein-protein interactions	HDAC6
513.RUNX2	protein-protein interactions	HDAC7
514.RUNX2	protein-protein interactions	HES1
515.RUNX2	protein-protein interactions	HEY1
516.RUNX2	protein-protein interactions	HIF1A
517.RUNX2	protein-protein interactions	HMGB2
518.RUNX2	protein-protein interactions	HSPA4
519.RUNX2	protein-protein interactions	HSPA4L
520.RUNX2	protein-protein interactions	HSPD1
521.RUNX2	protein-protein interactions	HSPH1
522.RUNX2	protein-protein interactions	IFI16
523.RUNX2	protein-protein interactions	IMPDH1
524.RUNX2	protein-protein interactions	JAG2
525.RUNX2	protein-protein interactions	JUN
526.RUNX2	protein-protein interactions	JUNB
527.RUNX2	protein-protein interactions	KAT2B
528.RUNX2	protein-protein interactions	KAT6A
529.RUNX2	protein-protein interactions	KAT6B
530.RUNX2	protein-protein interactions	KLF4
531.RUNX2	protein-protein interactions	LEF1
532.RUNX2	protein-protein interactions	LIMA1
533.RUNX2	protein-protein interactions	MATK
534.RUNX2	protein-protein interactions	MEN1
535.RUNX2	protein-protein interactions	MSX2
536.RUNX2	protein-protein interactions	NFYB
537.RUNX2	protein-protein interactions	NOTCH1
538.RUNX2	protein-protein interactions	NR0B2
539.RUNX2	protein-protein interactions	PIN1
540.RUNX2	protein-protein interactions	PML
541.RUNX2	protein-protein interactions	PPARG
542.RUNX2	protein-protein interactions	RB
543.RUNX2	protein-protein interactions	RB1
544.RUNX2	protein-protein interactions	RBL2
545.RUNX2	protein-protein interactions	RBM14
546.RUNX2	protein-protein interactions	RBM28
547.RUNX2	protein-protein interactions	RUNX1
548.RUNX2	protein-protein interactions	SMAD4
549.RUNX2	protein-protein interactions	SMAD5
550.RUNX2	protein-protein interactions	SNAI1
551.RUNX2	protein-protein interactions	osteocalcin
552.RUNX2	regulation of binding	RUNX2
553.RUNX2	ubiquitination	RUNX2
554.SIRT1	expression	RUNX2
555.SIRT1	protein-protein interactions	AFP
556.SIRT1	protein-protein interactions	ESR1
557.SIRT1	protein-protein interactions	RUNX2
558.SKIC2	protein-protein interactions	ESR1
559.SKIC2	protein-protein interactions	ESR2
560.SKIC2	protein-protein interactions	RUNX2
561.SKP2	protein-protein interactions	ESR1

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562.SKP2	protein-protein interactions	FKBP4
563.SKP2	protein-protein interactions	RUNX2
564.SMAD1	expression	RUNX2
565.SMAD1	protein-protein interactions	ERBB2
566.SMAD1	protein-protein interactions	ESR1
567.SMAD1	protein-protein interactions	RUNX2
568.SMAD2	expression	RUNX2
569.SMAD2	protein-protein interactions	ESR1
570.SMAD2	protein-protein interactions	RUNX2
571.SMAD2	protein-protein interactions	estrogen receptor
572.SMAD3	expression	RUNX2
573.SMAD3	protein-protein interactions	ESR1
574.SMAD3	protein-protein interactions	ESR2
575.SMAD3	protein-protein interactions	RUNX2
576.SMAD3	protein-protein interactions	estrogen receptor
577.SMAD4	expression	RUNX2
578.SMAD4	protein-DNA interactions	RUNX2
579.SMAD4	protein-protein interactions	ESR1
580.SMAD4	protein-protein interactions	ESR2
581.SMAD4	protein-protein interactions	RUNX2
582.SMAD4	protein-protein interactions	estrogen receptor
583.SMAD5	expression	RUNX2
584.SMAD5	protein-protein interactions	RUNX2
585.SMAD6	inhibition	RUNX2
586.SMAD6	protein-protein interactions	RUNX2
587.SMARCA4	expression	RUNX2
588.SMARCA4	protein-DNA interactions	RUNX2
589.SMARCA4	protein-protein interactions	ESR1
590.SMARCA4	protein-protein interactions	ESR2
591.SMARCA4	protein-protein interactions	RUNX2
592.SMARCA4	protein-protein interactions	estrogen receptor
593.SMURF1	inhibition	RUNX2
594.SMURF1	protein-protein interactions	ESR1
595.SMURF1	protein-protein interactions	RUNX2
596.SMURF1	ubiquitination	RUNX2
597.SMURF2	protein-protein interactions	ERBB2
598.SMURF2	protein-protein interactions	FKBP4
599.SMURF2	protein-protein interactions	RUNX2
600.SNAI1	protein-protein interactions	RUNX2
601.SNAI1	transcription	RUNX2
602.SNAI2	activation	RUNX2
603.SNAI2	transcription	RUNX2
604.SOX2	protein-protein interactions	FKBP4
605.SOX2	protein-protein interactions	HSP90 (family)
606.SOX2	protein-protein interactions	RUNX2
607.SOX9	expression	RUNX2
608.SOX9	protein-protein interactions	RUNX2
609.SP1	expression	ESR1
610.SP1	expression	RUNX2
611.SP1	protein-DNA interactions	RUNX2
612.SP1	protein-protein interactions	ESR1

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613.SP1	protein-protein interactions	ESR2
614.SP1	protein-protein interactions	estrogen receptor
615.SP1	transcription	RUNX2
616.SRF	activation	RUNX2
617.SRF	protein-protein interactions	RUNX2
618.STAT1	inhibition	RUNX2
619.STAT1	protein-protein interactions	ERBB2
620.STAT1	protein-protein interactions	ESR1
621.STAT1	protein-protein interactions	ESR2
622.STAT1	protein-protein interactions	RUNX2
623.STAT3	protein-protein interactions	ERBB2
624.STAT3	protein-protein interactions	ESR1
625.STAT3	protein-protein interactions	ESR2
626.STAT3	protein-protein interactions	HSP90 (family)
627.STAT3	protein-protein interactions	RUNX2
628.STAT5A	expression	RUNX2
629.STAT5A	protein-protein interactions	ESR1
630.STAT5A	protein-protein interactions	ESR2
631.STAT5A	protein-protein interactions	RUNX2
632.STUB1	protein-protein interactions	ERBB2
633.STUB1	protein-protein interactions	ESR1
634.STUB1	protein-protein interactions	ESR2
635.STUB1	protein-protein interactions	HSP90 (family)
636.STUB1	protein-protein interactions	RUNX2
637.SUV39H1	protein-protein interactions	ESR1
638.SUV39H1	protein-protein interactions	RUNX2
639.TAF1A	protein-protein interactions	ESR1
640.TAF1A	protein-protein interactions	RUNX2
641.TCF7L2	protein-protein interactions	ESR1
642.TCF7L2	protein-protein interactions	ESR2
643.TCF7L2	protein-protein interactions	RUNX2
644.TFAM	expression	RUNX2
645.TFAM	protein-protein interactions	ESR1
646.THRAP3	expression	RUNX2
647.THRAP3	protein-protein interactions	ESR1
648.THRB	expression	RUNX2
649.THRB	protein-DNA interactions	RUNX2
650.THRB	protein-protein interactions	estrogen receptor
651.THRB	transcription	RUNX2
652.TLE1	activation	RUNX2
653.TLE1	protein-protein interactions	ESR1
654.TLE1	protein-protein interactions	RUNX2
655.TP53	expression	ESR1
656.TP53	expression	RUNX2
657.TP53	protein-protein interactions	ESR1
658.TP53	protein-protein interactions	ESR2
659.TP53	protein-protein interactions	FKBP4
660.TP53	protein-protein interactions	HSP90 (family)
661.TP53	protein-protein interactions	PTH
662.TP53	protein-protein interactions	RUNX2
663.TP53	transcription	RUNX2

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664.TP73	protein-protein interactions	HSP90 (family)
665.TP73	protein-protein interactions	RUNX2
666.TRIB3	expression	RUNX2
667.TRPS1	expression	RUNX2
668.TRPS1	protein-protein interactions	ESR1
669.TSC22D3	expression	RUNX2
670.TWIST1	expression	RUNX2
671.TWIST1	protein-protein interactions	RUNX2
672.UBTF	protein-protein interactions	ESR1
673.UBTF	protein-protein interactions	RUNX2
674.VDR	expression	RUNX2
675.VDR	protein-protein interactions	HSP90 (family)
676.VDR	protein-protein interactions	RUNX2
677.WDR5	expression	RUNX2
678.WDR5	protein-DNA interactions	RUNX2
679.WDR5	protein-protein interactions	ESR1
680.WDR5	protein-protein interactions	ESR2
681.WWP1	protein-protein interactions	ESR2
682.WWP1	protein-protein interactions	RUNX2
683.WWP1	ubiquitination	RUNX2
684.WWP2	protein-protein interactions	ERBB2
685.WWP2	protein-protein interactions	RUNX2
686.XRCC5	protein-protein interactions	ESR1
687.XRCC5	protein-protein interactions	ESR2
688.XRCC5	protein-protein interactions	RUNX2
689.XRCC6	protein-protein interactions	ESR1
690.XRCC6	protein-protein interactions	RUNX2
691.YAP1	expression	RUNX2
692.YAP1	protein-protein interactions	ESR2
693.YAP1	protein-protein interactions	RUNX2
694.YY1	activation	RUNX2
695.YY1	protein-protein interactions	RUNX2
696.ZBTB16	expression	RUNX2
697.ZBTB16	protein-protein interactions	ESR1
698.ZBTB7B	protein-protein interactions	ESR1
699.ZBTB7B	protein-protein interactions	ESR2
700.ZBTB7B	protein-protein interactions	RUNX2
701.ZMYND8	expression	RUNX2
702.ZMYND8	protein-protein interactions	ERBB2
703.ZMYND8	protein-protein interactions	ESR1
704.ZMYND8	protein-protein interactions	ESR2
705.estrogen	activation	ERBB2
706.estrogen	activation	ESR1
707.estrogen	activation	ESR2
708.estrogen	activation	RUNX2
709.estrogen	activation	estrogen receptor
710.estrogen	chemical-protein interactions	AFP
711.estrogen	chemical-protein interactions	ERBB2
712.estrogen	chemical-protein interactions	ESR1
713.estrogen	chemical-protein interactions	ESR2
714.estrogen	chemical-protein interactions	estrogen receptor

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715.estrogen	inhibition	ERBB2
716.estrogen	reaction	ER/estrogen
717.estrogen	reaction	ESTG:ESR1:chaperone
718.estrogen	reaction	ESTG:ESR2:chaperone
719.estrogen	reaction	ESTG:Me-PalmS-ESR dimers
720.estrogen	translocation	estrogen
721.estrogen receptor	activation	FOS
722.estrogen receptor	activation	JUN
723.estrogen receptor	activation	TP53
724.estrogen receptor	chemical-protein interactions	estrogen
725.estrogen receptor	inhibition	DDX5
726.estrogen receptor	membership	ESR1
727.estrogen receptor	membership	ESR2
728.estrogen receptor	protein-DNA interactions	FOS
729.estrogen receptor	protein-protein interactions	AR
730.estrogen receptor	protein-protein interactions	CREBBP
731.estrogen receptor	protein-protein interactions	DDX5
732.estrogen receptor	protein-protein interactions	EP300
733.estrogen receptor	protein-protein interactions	FOS
734.estrogen receptor	protein-protein interactions	JUN
735.histone H3	protein-DNA interactions	RUNX2
736.histone H4	protein-DNA interactions	RUNX2

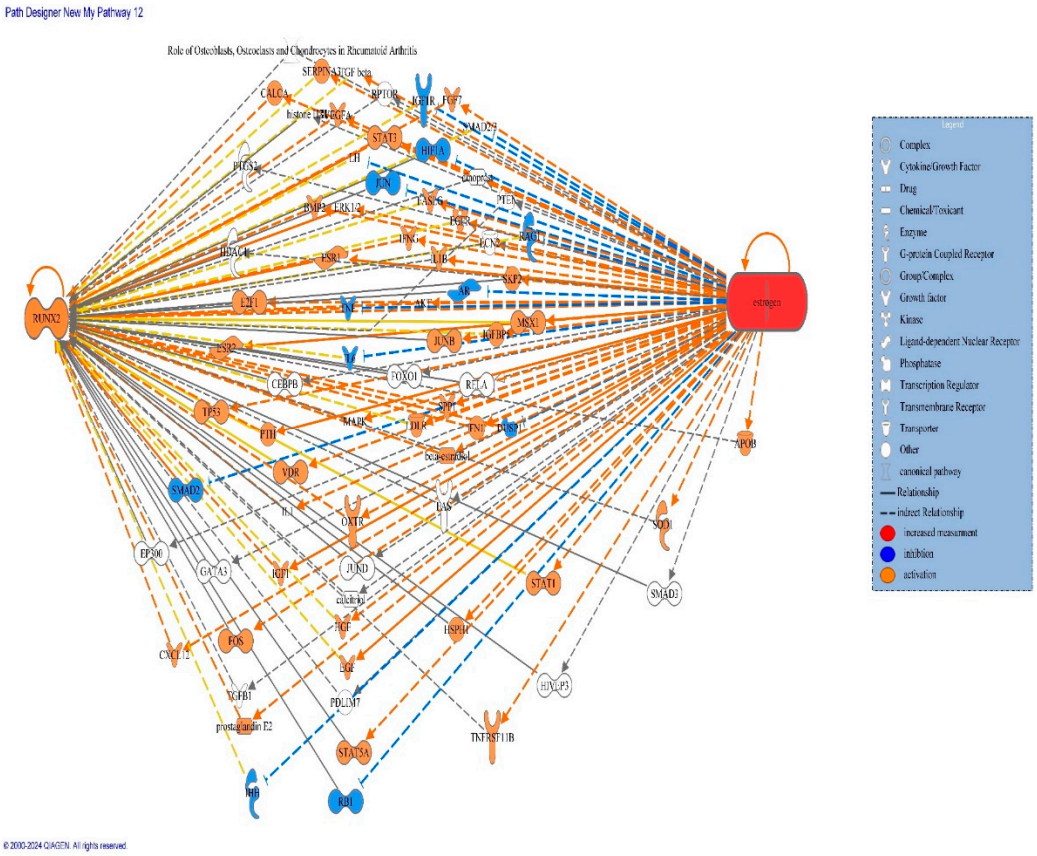

Figure 4. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of estrogen on *RUNX-2* expression.

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3. Molecular Pathway Analysis of Molecules Mediating the Relationship Between miRNA9 and Directly Affecting Expression of the RUNX-2 Gene

The "MAP" program and QKB identified 8 pathways controlled by miRNA9 that modulate RUNX-2 expression. These molecules comprised G-protein coupled receptors, kinases, nuclear receptors, transcription and translation regulators, transmembrane receptors, and transporters (Figure 5 and Tables 5 and 6, respectively). This targeted and substantial network identifies miR-9 as a distinct and powerful epigenetic modulator that precisely regulates the expression of the osteogenic master regulator *RUNX-2.*

Table 5. Various molecules incorporated in the miRNA-9 and *RUNX-2 gene* expression.

Symbol	Molecule/ Gene Name	Location	Family
1. AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
2. CTNNB1	catenin beta 1	Nucleus	transcription regulator
3. CXCR4	C-X-C motif chemokine receptor 4	Plasma Membrane	G-protein coupled receptor
4. ELAVL1	ELAV like RNA binding protein 1	Cytoplasm	other
5. ETS1	ETS proto-oncogene 1, transcription factor	Nucleus	transcription regulator
6. MEIS2	Meis homeobox 2	Nucleus	transcription regulator
7. mir-9 (includes others)	relatives of microRNA 9	Cytoplasm	microRNA
8. RUNX1	RUNX family transcription factor 1	Nucleus	transcription regulator
9. RUNX2	RUNX family transcription factor 2	Nucleus	transcription regulator
10. SP1	Sp1 transcription factor	Nucleus	transcription regulator

Table 6. Various interactions between molecules incorporated in the miRNA-9 and *RUNX-2 gene* expression.

From Molecule(s)	Relationship Type	To Molecule(s)
1. CTNNB1	expression	RUNX2
2. CTNNB1	inhibition	RUNX2
3. CTNNB1	protein-DNA interactions	RUNX2
4. CTNNB1	protein-protein interactions	RUNX2
5. CTNNB1	transcription	RUNX2
6. CXCR4	expression	RUNX2
7. ELAVL1	expression	RUNX2
8. ETS1	protein-DNA interactions	RUNX2
9. ETS1	protein-protein interactions	RUNX2
10. ETS1	transcription	RUNX2
11. MEIS2	expression	RUNX2
12. RUNX1	expression	RUNX2
13. RUNX1	protein-DNA interactions	RUNX2
14. RUNX1	protein-protein interactions	RUNX2
15. RUNX2	activation	RUNX2
16. RUNX2	expression	RUNX2
17. RUNX2	inhibition	RUNX2
18. RUNX2	localization	RUNX2
19. RUNX2	modification	RUNX2
20. RUNX2	molecular cleavage	RUNX2

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21.	RUNX2	protein-DNA interactions	RUNX2
22.	RUNX2	protein-protein interactions	AR
23.	RUNX2	protein-protein interactions	CTNNB1
24.	RUNX2	protein-protein interactions	ETS1
25.	RUNX2	protein-protein interactions	RUNX1
26.	RUNX2	regulation of binding	RUNX2
27.	RUNX2	ubiquitination	RUNX2
28.	SP1	expression	RUNX2
29.	SP1	protein-DNA interactions	RUNX2
30.	SP1	transcription	RUNX2
31.	mir-9	RNA-RNA interactions: microRNA targeting	AR
32.	mir-9	RNA-RNA interactions: microRNA targeting	CXCR4
33.	mir-9	RNA-RNA interactions: microRNA targeting	ETS1
34.	mir-9	RNA-RNA interactions: microRNA targeting	RUNX1
35.	mir-9	RNA-RNA interactions: non-targeting interactions	AR
36.	mir-9	expression	AR
37.	mir-9	expression	CTNNB1
38.	mir-9	expression	CXCR4
39.	mir-9	expression	ETS1
40.	mir-9	expression	MEIS2
41.	mir-9	expression	RUNX1
42.	mir-9	expression	SP1
43.	mir-9	inhibition	ELAVL1

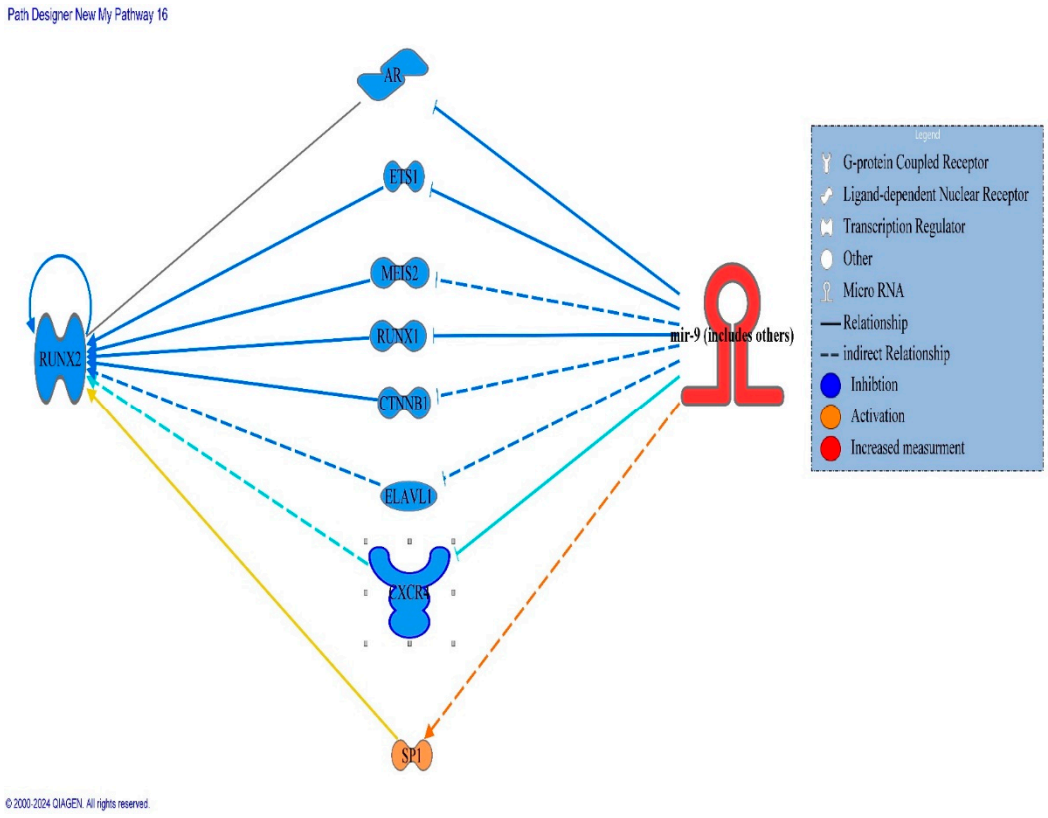

Figure 5. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of miRNA9 on RUNX-2 expression.

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4. Molecular Pathway Analysis of Molecules Mediating the Relationship Between miRNA10 and Directly Affecting Expression of the RUNX-2 Gene

The "MAP" program and QKB identified 34 pathways controlled by miRNA10 that modulate RUNX-2 expression. These molecules comprised G-protein coupled receptors, kinases, nuclear receptors, transcription and translation regulators, transmembrane receptors, and transporters, as shown in Figure 6 and Tables 7 and 8, respectively. The extensive regulatory scope of miR-10, in contrast to miR-9, implies it may function as a superior integrator, harmonizing several signals to facilitate RUNX-2 activation.

Table 7. Various molecules incorporated in the regulation of miRNA-10 and affect *RUNX-2 gene* expression.

	Symbol	Molecule/ Gene Name	Location	Family
1.	AKT		Cytoplasm	group
2.	AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
3.	BMPR2	bone morphogenetic protein receptor type 2	Plasma Membrane	kinase
4.	CASP3	caspase 3	Cytoplasm	peptidase
5.	E2F1	E2F transcription factor 1	Nucleus	transcription regulator
6.	EGFR	epidermal growth factor receptor	Plasma Membrane	kinase
7.	ERK1/2		Cytoplasm	group
8.	GLI1	GLI family zinc finger 1	Nucleus	transcription regulator
9.	HDAC4	histone deacetylase 4	Nucleus	transcription regulator
10.	HIF1A	hypoxia inducible factor 1 subunit alpha	Nucleus	transcription regulator
11.	histone H3		Nucleus	Group
12.	hydrogen peroxide		Other	chemical - endogenous mammalian
13.	IFNG	interferon gamma	Extracellular Space	Cytokine
14.	IGF1R	insulin like growth factor 1 receptor	Plasma Membrane	transmembrane receptor
15.	IL6	interleukin 6	Extracellular Space	Cytokine
16.	IRF4	interferon regulatory factor 4	Nucleus	transcription regulator
17.	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
18.	KLF4	KLF transcription factor 4	Nucleus	transcription regulator
19.	mir-10 (includes others)	relatives of microRNA 10	Cytoplasm	microRNA
20.	MTOR	mechanistic target of rapamycin kinase	Nucleus	Kinase
21.	MYD88	MYD88 innate immune signal transduction adaptor	Plasma Membrane	other
22.	NFE2L2	NFE2 like bZIP transcription factor 2	Nucleus	transcription regulator
23.	p38 MAPK		Cytoplasm	group
24.	PPP1CA	protein phosphatase 1 catalytic subunit alpha	Cytoplasm	phosphatase
25.	PTEN	phosphatase and tensin homolog	Cytoplasm	phosphatase
26.	RB1	RB transcriptional corepressor 1	Nucleus	transcription regulator
27.	RUNX2	RUNX family transcription factor 2	Nucleus	transcription regulator
28.	SMAD2	SMAD family member 2	Nucleus	transcription regulator
29.	SMAD4	SMAD family member 4	Nucleus	transcription regulator
30.	STAT3	signal transducer and activator of transcription 3	Nucleus	transcription regulator
31.	SUV39H1	SUV39H1 histone lysine methyltransferase	Nucleus	enzyme

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32. TGF beta		Extracellular Space	group
33. TGFBR2	transforming growth factor beta receptor 2	Plasma Membrane	kinase
34. TNF	tumor necrosis factor	Extracellular Space	cytokine
35. TP53	tumor protein p53	Nucleus	transcription regulator
36. VEGFA	vascular endothelial growth factor A	Extracellular Space	growth factor

Table 8. Various interactions between molecules incorporated in the miRNA-10 and RUNX-2 gene expression.

From Molecule(s)	Relationship Type	To Molecule(s)
1. AKT	activation	RUNX2
2. AKT	expression	RUNX2
3. AKT	phosphorylation	RUNX2
4. BMPR2	expression	RUNX2
5. BMPR2	regulation of binding	RUNX2
6. CASP3	expression	RUNX2
7. E2F1	regulation of binding	RUNX2
8. EGFR	expression	RUNX2
9. ERK1/2	activation	RUNX2
10. ERK1/2	expression	RUNX2
11. ERK1/2	phosphorylation	RUNX2
12. ERK1/2	protein-protein interactions	RUNX2
13. GLI1	expression	RUNX2
14. GLI1	protein-protein interactions	RUNX2
15. GLI1	transcription	RUNX2
16. HDAC4	activation	RUNX2
17. HDAC4	expression	RUNX2
18. HDAC4	modification	RUNX2
19. HDAC4	protein-protein interactions	RUNX2
20. IFNG	expression	RUNX2
21. IGF1R	expression	RUNX2
22. IL6	expression	RUNX2
23. IRF4	expression	RUNX2
24. JUN	protein-DNA interactions	RUNX2
25. JUN	protein-protein interactions	RUNX2
26. KLF4	expression	RUNX2
27. KLF4	protein-DNA interactions	RUNX2
28. KLF4	protein-protein interactions	RUNX2
29. MTOR	expression	RUNX2
30. MYD88	expression	RUNX2
31. NFE2L2	protein-protein interactions	RUNX2
32. NFE2L2	regulation of binding	RUNX2
33. PPP1CA	expression	RUNX2
34. PTEN	regulation of binding	RUNX2
35. RUNX2	activation	RUNX2
36. RUNX2	expression	RUNX2
37. RUNX2	inhibition	RUNX2
38. RUNX2	localization	RUNX2
39. RUNX2	modification	RUNX2
40. RUNX2	molecular cleavage	RUNX2
41. RUNX2	protein-DNA interactions	RUNX2
42. RUNX2	protein-protein interactions	AR
43. RUNX2	protein-protein interactions	ERK1/2
44. RUNX2	protein-protein interactions	GLI1

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45. RUNX2	protein-protein interactions	HDAC4
46. RUNX2	protein-protein interactions	HIF1A
47. RUNX2	protein-protein interactions	JUN
48. RUNX2	protein-protein interactions	KLF4
49. RUNX2	protein-protein interactions	NFE2L2
50. RUNX2	protein-protein interactions	RB1
51. RUNX2	protein-protein interactions	SMAD4
52. RUNX2	regulation of binding	RUNX2
53. RUNX2	ubiquitination	RUNX2
54. SMAD2	expression	RUNX2
55. SMAD2	protein-protein interactions	RUNX2
56. SMAD4	expression	RUNX2
57. SMAD4	protein-DNA interactions	RUNX2
58. SMAD4	protein-protein interactions	RUNX2
59. STAT3	expression	RUNX2
60. STAT3	protein-protein interactions	RUNX2
61. SUV39H1	protein-protein interactions	RUNX2
62. SUV39H1	protein-protein interactions	mir-10 (includes others)
63. TGF beta	expression	RUNX2
64. TGFBR2	expression	RUNX2
65. TNF	expression	RUNX2
66. TNF	localization	RUNX2
67. TNF	molecular cleavage	RUNX2
68. TNF	transcription	RUNX2
69. TP53	expression	RUNX2
70. TP53	protein-protein interactions	RUNX2
71. TP53	transcription	RUNX2
72. VEGFA	expression	RUNX2
73. histone H3	protein-DNA interactions	RUNX2
74. hydrogen peroxide	expression	RUNX2
75. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	AR
76. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	BMPR2
77. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	HDAC4
78. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	IGF1R
79. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	KLF4
80. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	MTOR
81. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	MYD88
82. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	PPP1CA
83. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	PTEN
84. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SMAD2
85. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SMAD4
86. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SUV39H1
87. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	TNF

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88. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	TP53
89. mir-10 (includes others)	RNA-RNA interactions: non-targeting interactions	JUN
90. mir-10 (includes others)	activation	AKT
91. mir-10 (includes others)	activation	CASP3
92. mir-10 (includes others)	activation	EGFR
93. mir-10 (includes others)	activation	ERK1/2
94. mir-10 (includes others)	activation	STAT3
95. mir-10 (includes others)	activation	TP53
96. mir-10 (includes others)	expression	AKT
97. mir-10 (includes others)	expression	AR
98. mir-10 (includes others)	expression	BMPR2
99. mir-10 (includes others)	expression	E2F1
100. mir-10 (includes others)	expression	GLI1
101. mir-10 (includes others)	expression	HDAC4
102. mir-10 (includes others)	expression	HIF1A
103. mir-10 (includes others)	expression	IGF1R
104. mir-10 (includes others)	expression	IL6
105. mir-10 (includes others)	expression	IRF4
106. mir-10 (includes others)	expression	KLF4
107. mir-10 (includes others)	expression	MTOR
108. mir-10 (includes others)	expression	MYD88
109. mir-10 (includes others)	expression	NFE2L2
110. mir-10 (includes others)	expression	PPP1CA
111. mir-10 (includes others)	expression	PTEN
112. mir-10 (includes others)	expression	RB1
113. mir-10 (includes others)	expression	SMAD2
114. mir-10 (includes others)	expression	SMAD4
115. mir-10 (includes others)	expression	SUV39H1
116. mir-10 (includes others)	expression	TGF beta

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117. mir-10 (includes others)	expression	TGFBR2
118. mir-10 (includes others)	expression	TNF
119. mir-10 (includes others)	expression	TP53
120. mir-10 (includes others)	expression	VEGFA
121. mir-10 (includes others)	expression	p38 MAPK
122. mir-10 (includes others)	localization	IFNG
123. mir-10 (includes others)	localization	hydrogen peroxide
124. mir-10 (includes others)	molecular cleavage	CASP3
125. mir-10 (includes others)	phosphorylation	AKT
126. mir-10 (includes others)	phosphorylation	EGFR
127. mir-10 (includes others)	phosphorylation	ERK1/2
128. mir-10 (includes others)	phosphorylation	STAT3
129. mir-10 (includes others)	protein-protein interactions	SUV39H1
130. mir-10 (includes others)	regulation of binding	IL6
131. mir-10 (includes others)	regulation of binding	histone H3
132. mir-10 (includes others)	translocation	STAT3
133. p38 MAPK	activation	RUNX2
134. p38 MAPK	expression	RUNX2

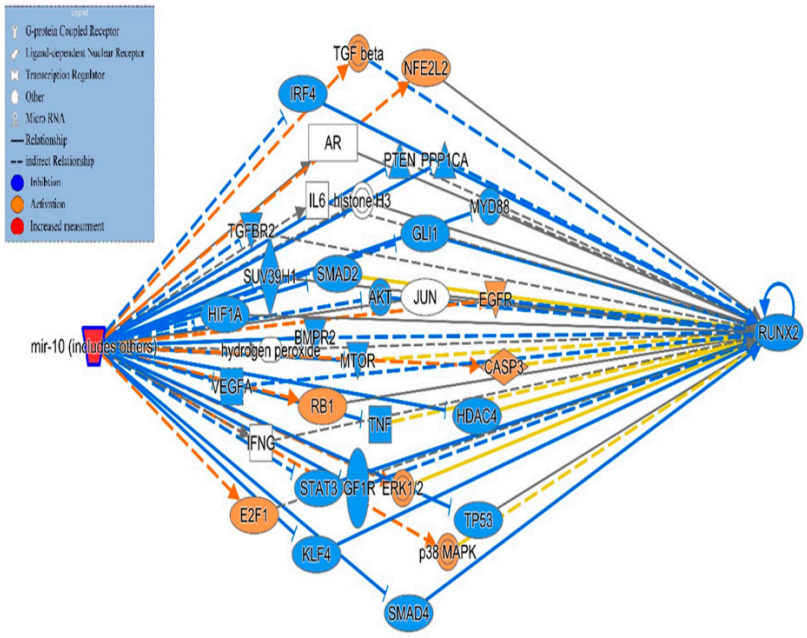

Figure 6. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of miRNA10 on *RUNX-2* expression.

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5. Molecular Pathway Analysis of Molecules Mediating the Relationship Between *PDLIM-3* and Had Direct Effects on Expression of miRNA9

The "MAP" program and QKB identified 49 pathways directly regulated by *PDLIM-3* that modulate miRNA9 expression. These molecules comprised G-protein coupled receptors, kinases, nuclear receptors, transcription and translation regulators, transmembrane receptors, and transporters, as shown in Figure 7 and Tables 9 and 10, respectively. This discovery establishes a molecular connection, demonstrating how PDLIM3, a structural protein, functions as a signaling hub to epigenetically modulate gene expression by regulating miR-9 levels.

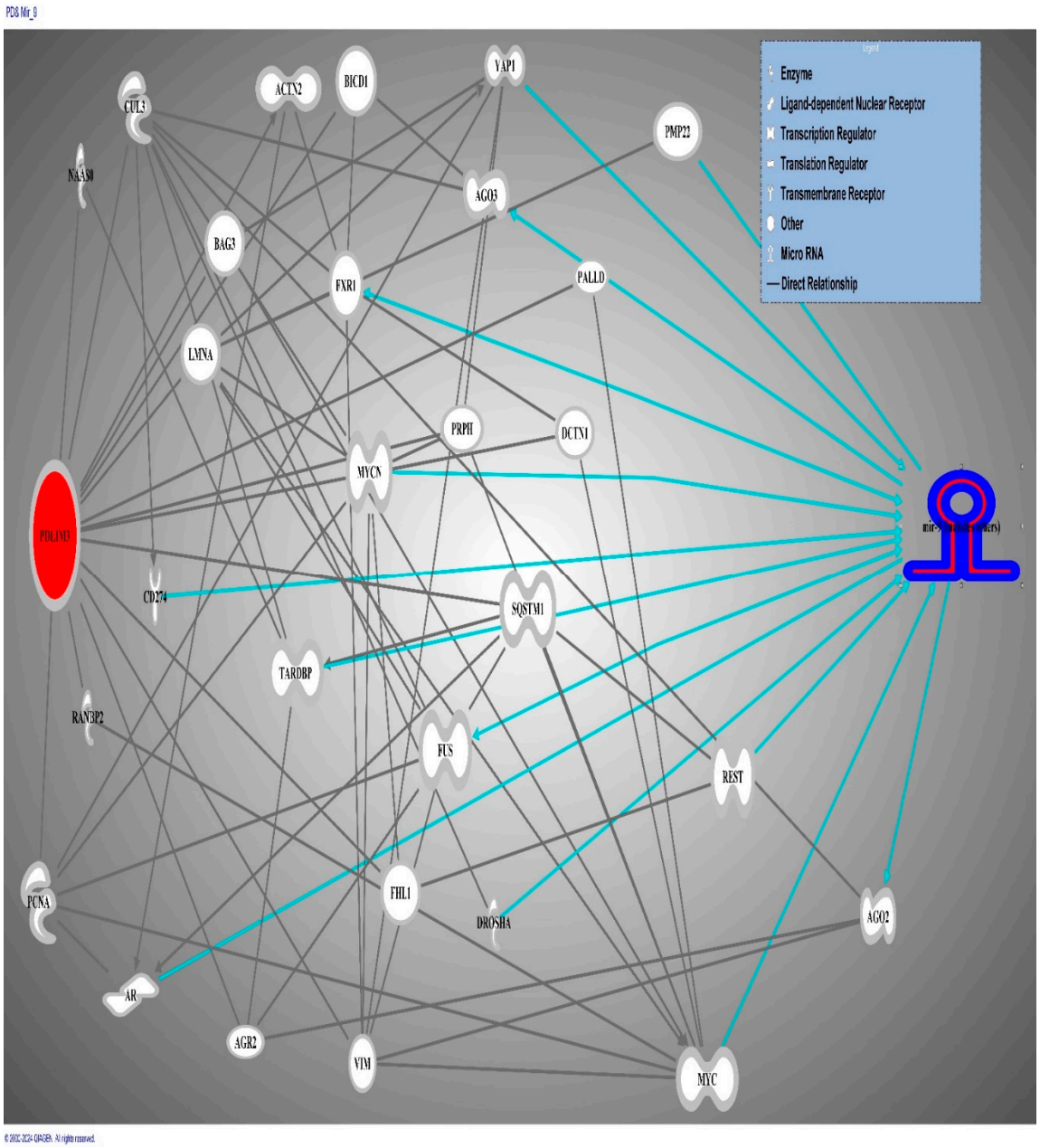

Figure 7. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of PDLIM-3 on miRNA-9 expression.

Table 9. Various molecules incorporated in the regulation of PDLIM-3 and affect *miRNA9* expression.

Symbol	Molecule/ Gene Name	Location	Family
1. ACTN2	actinin alpha 2	Nucleus	transcription regulator

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2.	AGO 2	argonaute RISC catalytic component 2	Cytoplasm	translation regulator
3.	AGO 3	argonaute RISC catalytic component 3	Cytoplasm	translation regulator
4.	AGR 2	anterior gradient 2, protein disulphide isomerase family member	Extracellular Space	other
5.	AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
6.	BAG3	BAG cochaperone 3	Cytoplasm	other
7.	BICD 1	BICD cargo adaptor 1	Cytoplasm	other
8.	CD27 4	CD274 molecule	Plasma Membrane	transmembrane receptor
9.	CUL3	cullin 3	Nucleus	enzyme
10.	DCT N1	dynactin subunit 1	Cytoplasm	other
11.	DRO SHA	drosha ribonuclease III	Nucleus	enzyme
12.	FHL1	four and a half LIM domains 1	Cytoplasm	other
13.	FUS	FUS RNA binding protein	Nucleus	transcription regulator
14.	FXR1	FMR1 autosomal homolog 1	Cytoplasm	other
15.	LMN A	lamin A/C	Nucleus	other
16.	mir-9	relatives of microRNA 9	Cytoplasm	microRNA
17.	MYC	MYC proto-oncogene, bHLH transcription factor	Nucleus	transcription regulator
18.	MYC N	MYCN proto-oncogene, bHLH transcription factor	Nucleus	transcription regulator
19.	NAA 40	N-alpha-acetyltransferase 40, NatD catalytic subunit	Cytoplasm	enzyme
20.	PALL D	palladin, cytoskeletal associated protein	Plasma Membrane	other
21.	PCN A	proliferating cell nuclear antigen	Nucleus	enzyme
22.	PDLI M3	PDZ and LIM domain 3	Cytoplasm	other
23.	PMP2 2	peripheral myelin protein 22	Plasma Membrane	other
24.	PRP H	peripherin	Plasma Membrane	other
25.	RAN BP2	RAN binding protein 2	Nucleus	enzyme
26.	REST	RE1 silencing transcription factor	Nucleus	transcription regulator
27.	SQST M1	sequestosome 1	Cytoplasm	transcription regulator
28.	TAR DBP	TAR DNA binding protein	Nucleus	transcription regulator
29.	VIM	vimentin	Cytoplasm	other
30.	YAP1	Yes1 associated transcriptional regulator	Nucleus	transcription regulator

Table 10. Various interactions between molecules incorporated in the PDLIM-3 and affect *miRNA9* expression.

From Molecule(s)	Relationship Type	To Molecule(s)
1. ACTN2	activation	AR
2. ACTN2	protein-protein interactions	AR
3. AGR2	protein-protein interactions	AGO2
4. AR	protein-protein interactions	ACTN2
5. BAG3	expression	MYC

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6. BAG3	protein-protein interactions	MYC
7. BICD1	protein-protein interactions	AGO3
8. CD274	expression	mir-9 (includes others)
9. CUL3	expression	CD274
10. CUL3	protein-protein interactions	AGO2
11. CUL3	protein-protein interactions	AGO3
12. CUL3	protein-protein interactions	CD274
13. DROSHA	protein-DNA interactions	mir-9 (includes others)
14. DROSHA	protein-protein interactions	CUL3
15. FUS	protein-RNA interactions	mir-9 (includes others)
16. FUS	protein-protein interactions	AGR2
17. FUS	protein-protein interactions	CUL3
18. FUS	regulation of binding	mir-9 (includes others)
19. FXR1	expression	mir-9 (includes others)
20. FXR1	molecular cleavage	mir-9 (includes others)
21. FXR1	protein-RNA interactions	mir-9 (includes others)
22. FXR1	protein-protein interactions	ACTN2
23. FXR1	protein-protein interactions	BICD1
24. FXR1	protein-protein interactions	CUL3
25. FXR1	protein-protein interactions	DCTN1
26. LMNA	expression	MYC
27. LMNA	inhibition	YAP1
28. LMNA	localization	YAP1
29. LMNA	phosphorylation	YAP1
30. LMNA	protein-protein interactions	FUS
31. LMNA	protein-protein interactions	FXR1
32. LMNA	protein-protein interactions	MYC
33. LMNA	protein-protein interactions	YAP1
34. LMNA	transcription	YAP1
35. MYC	expression	mir-9 (includes others)
36. MYC	protein-DNA interactions	mir-9 (includes others)
37. MYC	protein-protein interactions	BAG3
38. MYC	protein-protein interactions	DCTN1
39. MYC	protein-protein interactions	LMNA
40. MYC	transcription	mir-9 (includes others)
41. MYCN	protein-DNA interactions	mir-9 (includes others)
42. MYCN	protein-protein interactions	CUL3
43. MYCN	protein-protein interactions	FHL1
44. MYCN	protein-protein interactions	LMNA
45. PALLD	protein-protein interactions	MYC
46. PCNA	protein-protein interactions	AR

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47. PCNA	protein-protein interactions	FUS
48. PCNA	protein-protein interactions	MYC
49. PCNA	protein-protein interactions	MYCN
50. PDLIM3	protein-protein interactions	ACTN2
51. PDLIM3	protein-protein interactions	AGR2
52. PDLIM3	protein-protein interactions	BAG3
53. PDLIM3	protein-protein interactions	BICD1
54. PDLIM3	protein-protein interactions	CUL3
55. PDLIM3	protein-protein interactions	DCTN1
56. PDLIM3	protein-protein interactions	FHL1
57. PDLIM3	protein-protein interactions	LMNA
58. PDLIM3	protein-protein interactions	NAA40
59. PDLIM3	protein-protein interactions	PALLD
60. PDLIM3	protein-protein interactions	PCNA
61. PDLIM3	regulation of binding	ACTN2
62. PMP22	RNA-RNA interactions: non-targeting interactions	mir-9 (includes others)
63. PMP22	protein-protein interactions	LMNA
64. PRPH	protein-protein interactions	MYC
65. PRPH	protein-protein interactions	MYCN
66. PRPH	protein-protein interactions	PDLIM3
67. RANBP2	protein-protein interactions	MYC
68. RANBP2	protein-protein interactions	PDLIM3
69. REST	protein-DNA interactions	mir-9 (includes others)
70. REST	protein-protein interactions	FHL1
71. SQSTM1	activation	AR
72. SQSTM1	localization	AR
73. SQSTM1	molecular cleavage	TARDBP
74. SQSTM1	protein-protein interactions	AR
75. SQSTM1	protein-protein interactions	FUS
76. SQSTM1	protein-protein interactions	MYC
77. SQSTM1	protein-protein interactions	PDLIM3
78. SQSTM1	protein-protein interactions	REST
79. SQSTM1	protein-protein interactions	TARDBP
80. TARDBP	expression	mir-9 (includes others)
81. TARDBP	protein-protein interactions	AGR2
82. TARDBP	protein-protein interactions	CUL3
83. TARDBP	protein-protein interactions	NAA40
84. TARDBP	protein-protein interactions	SQSTM1
85. VIM	protein-protein interactions	AGO2
86. VIM	protein-protein interactions	FUS
87. VIM	protein-protein interactions	FXR1
88. VIM	protein-protein interactions	MYC
89. VIM	protein-protein interactions	MYCN
90. VIM	protein-protein interactions	PDLIM3
91. YAP1	protein-protein interactions	BAG3
92. YAP1	protein-protein interactions	LMNA
93. YAP1	protein-protein interactions	PCNA
94. YAP1	protein-protein interactions	PRPH

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95. YAP1	protein-protein interactions	VIM
96. YAP1	transcription	mir-9 (includes others)
97. mir-9 (includes others)	RNA-RNA interactions: non-targeting interactions	AR
98. mir-9 (includes others)	protein-RNA interactions	AGO2
99. mir-9 (includes others)	protein-RNA interactions	AGO3
100. mir-9 (includes others)	protein-RNA interactions	FUS
101. mir-9 (includes others)	protein-RNA interactions	FXR1

6. Molecular Pathway Analysis of Molecules Mediating the Relationship Between *PDLIM-3* and Had Direct Effects on Expression of miRNA10

The "MAP" program and QKB identified 98 pathways directly regulated by *PDLIM-3* that modulate miRNA9 expression. These molecules comprised G-protein coupled receptors, kinases, nuclear receptors, transcription and translation regulators, transmembrane receptors, and transporters, as shown in Figure 8 and Tables 11 and 12, respectively. This is the most comprehensive connectivity in our data, strongly implying that the regulation of miR-10 is a significant downstream function of PDLIM3, probably its principal role in the estrogen-mediated signaling pathway leading to osteogenesis.

Table 11. Various molecules incorporated in the regulation of PDLIM-3 and affect *miRNA10* expression.

Symbol	Entrez Gene Name	Location	Family
1. ACTN2	actinin alpha 2	Nucleus	transcription regulator
2. AGO1	argonaute RISC component 1	Cytoplasm	translation regulator
3. AGO2	argonaute RISC catalytic component 2	Cytoplasm	translation regulator
4. AGO3	argonaute RISC catalytic component 3	Cytoplasm	translation regulator
5. AGR2	anterior gradient 2, protein disulphide isomerase family member	Extracellular Space	other
6. APC	APC regulator of WNT signaling pathway	Nucleus	enzyme
7. AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
8. BAG3	BAG cochaperone 3	Cytoplasm	other
9. BICD1	BICD cargo adaptor 1	Cytoplasm	other
10. BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase	Cytoplasm	enzyme
11. CD274	CD274 molecule	Plasma Membrane	transmembrane receptor
12. CTNNB1	catenin beta 1	Nucleus	transcription regulator
13. CUL3	cullin 3	Nucleus	enzyme
14. DCTN1	dynactin subunit 1	Cytoplasm	other
15. DDX17	DEAD-box helicase 17	Nucleus	enzyme
16. DROSHA	drosha ribonuclease III	Nucleus	enzyme
17. E2F3	E2F transcription factor 3	Nucleus	transcription regulator

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18. EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit	Nucleus	transcription regulator
19. FHL1	four and a half LIM domains 1	Cytoplasm	other
20. FUS	FUS RNA binding protein	Nucleus	transcription regulator
21. JUN	Jun proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
22. KLC2	kinesin light chain 2	Cytoplasm	other
23. LMNA	lamin A/C	Nucleus	other
24. MAP3K7	mitogen-activated protein kinase kinase kinase 7	Cytoplasm	kinase
25. mir-10	relatives of microRNA 10	Cytoplasm	microRNA
26. MYC	MYC proto-oncogene, bHLH transcription factor	Nucleus	transcription regulator
27. NAA40	N-alpha-acetyltransferase 40, NatD catalytic subunit	Cytoplasm	enzyme
28. NPM1	nucleophosmin 1	Nucleus	transcription regulator
29. PALLD	palladin, cytoskeletal associated protein	Plasma Membrane	other
30. PCNA	proliferating cell nuclear antigen	Nucleus	enzyme
31. PDLIM3	PDZ and LIM domain 3	Cytoplasm	other
32. PPARA	peroxisome proliferator activated receptor alpha	Nucleus	ligand-dependent nuclear receptor
33. PRPH	peripherin	Plasma Membrane	other
34. RANBP2	RAN binding protein 2	Nucleus	enzyme
35. RELA	RELA proto-oncogene, NF-kB subunit	Nucleus	transcription regulator
36. RHOU	ras homolog family member U	Cytoplasm	enzyme
37. RNF207	ring finger protein 207	Other	other
38. SNAI1	snail family transcriptional repressor 1	Nucleus	transcription regulator
39. SQSTM1	sequestosome 1	Cytoplasm	transcription regulator
40. SUV39H1	SUV39H1 histone lysine methyltransferase	Nucleus	enzyme
41. TP53	tumor protein p53	Nucleus	transcription regulator
42. TRIM28	tripartite motif containing 28	Nucleus	transcription regulator
43. TWIST1	twist family bHLH transcription factor 1	Nucleus	transcription regulator
44. VIM	vimentin	Cytoplasm	other
45. YAP1	Yes1 associated transcriptional regulator	Nucleus	transcription regulator

Table 12. Various interactions between molecules incorporated in the *PDLIM-3* and affect *miRNA10* expression.

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From Molecule(s)	Relationship Type	To Molecule(s)
1. ACTN2	activation	AR
2. ACTN2	protein-protein interactions	AR
3. AGR2	inhibition	TP53
4. AGR2	protein-protein interactions	AGO2
5. AGR2	protein-protein interactions	TP53
6. AR	expression	mir-10
7. AR	protein-DNA interactions	mir-10
8. AR	protein-protein interactions	ACTN2
9. BICD1	protein-protein interactions	AGO1
10. BICD1	protein-protein interactions	AGO3
11. BTRC	protein-protein interactions	BICD1
12. CD274	expression	mir-10
13. CTNNB1	expression	mir-10
14. CUL3	protein-protein interactions	AGO1
15. CUL3	protein-protein interactions	AGO2
16. CUL3	protein-protein interactions	AGO3
17. CUL3	protein-protein interactions	CD274
18. CUL3	protein-protein interactions	CTNNB1
19. DCTN1	protein-protein interactions	APC
20. DDX17	protein-protein interactions	AGR2
21. DDX17	protein-protein interactions	CUL3
22. DROSHA	protein-DNA interactions	mir-10
23. DROSHA	protein-protein interactions	CUL3
24. E2F3	expression	mir-10
25. EZH2	expression	mir-10
26. EZH2	protein-protein interactions	BAG3
27. EZH2	protein-protein interactions	CUL3
28. EZH2	protein-protein interactions	DCTN1
29. FHL1	protein-protein interactions	CTNNB1
30. FUS	protein-protein interactions	AGR2
31. FUS	protein-protein interactions	CUL3
32. LMNA	activation	TP53
33. LMNA	protein-DNA interactions	PPARA
34. LMNA	protein-protein interactions	CTNNB1
35. LMNA	protein-protein interactions	EZH2
36. LMNA	protein-protein interactions	FUS
37. LMNA	protein-protein interactions	TP53
38. MYC	expression	mir-10 (includes others)
39. MYC	protein-protein interactions	BAG3
40. MYC	protein-protein interactions	DCTN1
41. MYC	protein-protein interactions	LMNA
42. NAA40	protein-protein interactions	DDX17
43. NAA40	protein-protein interactions	KLC2
44. NPM1	expression	mir-10 (includes others)
45. NPM1	protein-protein interactions	AGR2
46. NPM1	protein-protein interactions	BICD1
47. NPM1	protein-protein interactions	CUL3
48. NPM1	protein-protein interactions	LMNA
49. NPM1	protein-protein interactions	NAA40
50. PALLD	protein-protein interactions	BTRC
51. PALLD	protein-protein interactions	MYC
52. PCNA	protein-protein interactions	APC
53. PCNA	protein-protein interactions	AR
54. PCNA	protein-protein interactions	CTNNB1
55. PCNA	protein-protein interactions	DDX17
56. PCNA	protein-protein interactions	E2F3

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57. PCNA	protein-protein interactions	EZH2
58. PCNA	protein-protein interactions	FUS
59. PCNA	protein-protein interactions	MAP3K7
60. PCNA	protein-protein interactions	MYC
61. PDLIM3	protein-protein interactions	ACTN2
62. PDLIM3	protein-protein interactions	AGR2
63. PDLIM3	protein-protein interactions	BAG3
64. PDLIM3	protein-protein interactions	BICD1
65. PDLIM3	protein-protein interactions	CUL3
66. PDLIM3	protein-protein interactions	DCTN1
67. PDLIM3	protein-protein interactions	FHL1
68. PDLIM3	protein-protein interactions	LMNA
69. PDLIM3	protein-protein interactions	NAA40
70. PDLIM3	protein-protein interactions	PALLD
71. PDLIM3	protein-protein interactions	PCNA
72. PDLIM3	protein-protein interactions	PDLIM3
73. PPARA	expression	mir-10
74. PRPH	protein-protein interactions	MYC
75. PRPH	protein-protein interactions	PDLIM3
76. RANBP2	protein-protein interactions	APC
77. RANBP2	protein-protein interactions	CTNNB1
78. RANBP2	protein-protein interactions	EZH2
79. RANBP2	protein-protein interactions	MYC
80. RANBP2	protein-protein interactions	PDLIM3
81. RELA	expression	mir-10 (includes others)
82. RELA	protein-DNA interactions	mir-10 (includes others)
83. RELA	protein-protein interactions	PCNA
84. RHOU	protein-protein interactions	CTNNB1
85. RHOU	protein-protein interactions	PDLIM3
86. RNF207	protein-protein interactions	NPM1
87. RNF207	protein-protein interactions	PDLIM3
88. SNAI1	expression	mir-10 (includes others)
89. SNAI1	protein-protein interactions	ACTN2
90. SNAI1	protein-protein interactions	PCNA
91. SNAI1	transcription	mir-10 (includes others)
92. SQSTM1	activation	AR
93. SQSTM1	activation	TP53
94. SQSTM1	expression	RELA
95. SQSTM1	expression	SNAI1
96. SQSTM1	protein-protein interactions	AR
97. SQSTM1	protein-protein interactions	CTNNB1
98. SQSTM1	protein-protein interactions	FUS
99. SQSTM1	protein-protein interactions	JUN
100. SQSTM1	protein-protein interactions	MAP3K7
101. SQSTM1	protein-protein interactions	MYC
102. SQSTM1	protein-protein interactions	NPM1
103. SQSTM1	protein-protein interactions	PDLIM3
104. SQSTM1	protein-protein interactions	RELA
105. SQSTM1	protein-protein interactions	SNAI1
106. SQSTM1	protein-protein interactions	TP53
107. SQSTM1	transcription	JUN
108. SUV39H1	protein-protein interactions	PCNA
109. SUV39H1	protein-protein interactions	mir-10
110. TP53	expression	mir-10
111. TP53	protein-protein interactions	AGR2
112. TP53	protein-protein interactions	BICD1
113. TP53	protein-protein interactions	CUL3

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114. TP53	protein-protein interactions	LMNA
115. TP53	protein-protein interactions	NAA40
116. TP53	protein-protein interactions	PALLD
117. TP53	protein-protein interactions	PCNA
118. TP53	protein-protein interactions	RANBP2
119. TP53	protein-protein interactions	SQSTM1
120. TRIM28	expression	mir-10
121. TRIM28	protein-protein interactions	AGR2
122. TRIM28	protein-protein interactions	CUL3
123. TRIM28	protein-protein interactions	DCTN1
124. TRIM28	protein-protein interactions	LMNA
125. TRIM28	protein-protein interactions	NAA40
126. TRIM28	protein-protein interactions	PCNA
127. TRIM28	protein-protein interactions	RANBP2
128. TWIST1	expression	mir-10
129. TWIST1	protein-protein interactions	PALLD
130. VIM	protein-protein interactions	AGO1
131. VIM	protein-protein interactions	AGO2
132. VIM	protein-protein interactions	APC
133. VIM	protein-protein interactions	BTRC
134. VIM	protein-protein interactions	EZH2
135. VIM	protein-protein interactions	FUS
136. VIM	protein-protein interactions	MYC
137. VIM	protein-protein interactions	NPM1
138. VIM	protein-protein interactions	PDLIM3
139. VIM	protein-protein interactions	RELA
140. VIM	protein-protein interactions	TP53
141. VIM	protein-protein interactions	TRIM28
142. YAP1	protein-protein interactions	BAG3
143. YAP1	protein-protein interactions	LMNA
144. YAP1	protein-protein interactions	PCNA
145. YAP1	protein-protein interactions	PRPH
146. YAP1	protein-protein interactions	VIM
147. YAP1	transcription	mir-10
148. mir-10	RNA-RNA interactions: non-targeting interactions	APC
149. mir-10	RNA-RNA interactions: non-targeting interactions	BTRC
150. mir-10	RNA-RNA interactions: non-targeting interactions	JUN
151. mir-10	RNA-RNA interactions: non-targeting interactions	MAP3K7
152. mir-10	protein-RNA interactions	AGO1
153. mir-10	protein-RNA interactions	AGO2
154. mir-10	protein-RNA interactions	AGO3
155. mir-10	protein-RNA interactions	DDX17
156. mir-10	protein-RNA interactions	FUS
157. mir-10	protein-protein interactions	KLC2

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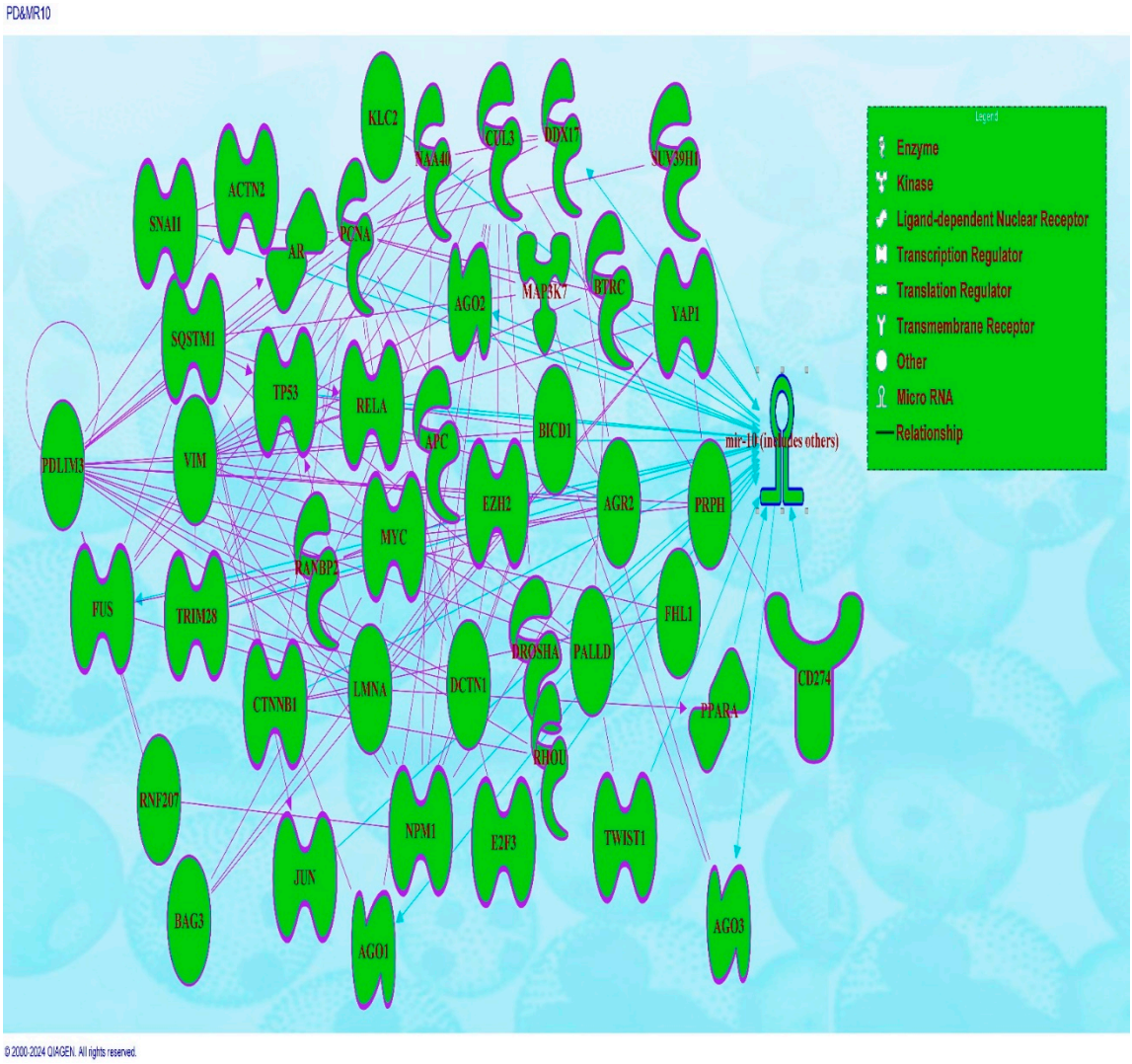

Figure 8. Molecular network depicting the connectivity and relationships among the overlapping molecules associated with direct influence of PDLIM-3 on miRNA-10 expression.

7. Identification and Analysis of the Molecule Network Regulated by Estrogen, PDLIM-3, miRNA9, and miRNA10 affects the expression of RUNX-2.

The "MAP" program and QKB identified around 39 molecules involved directly or indirectly in the expression of *RUNX-2,* regulated by estrogen, miRNA9, miRNA10, and *PDLIM-3* interaction.

The full classification of those molecules, including the gene name, its location, and its family, is shown in Figure 9 and Tables 13 and 14, respectively. This integrated map consolidates our findings, visually delineating the novel multi-layered regulatory pathway from estrogen receptor stimulation to the transcriptional regulation of RUNX-2.

Table 13. Molecules regulated by Estrogen, PDLIM-3, miRNA9 and miRNA10 affecting RUNX-2 expression.

Symbol	Gene Name	Location	Family
1. ACTN2	actinin alpha 2	Nucleus	transcription regulator
2. AGR2	anterior gradient 2, protein disulphide isomerase family member	Extracellular Space	other
3. APC	APC regulator of WNT signaling pathway	Nucleus	enzyme
4. AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
5. BAG3	BAG cochaperone 3	Cytoplasm	Other
6. CTNNB1	catenin beta 1	Nucleus	transcription regulator
7. CUL3	cullin 3	Nucleus	Enzyme
8. DCTN1	dynactin subunit 1	Cytoplasm	Other

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9. EGF	epidermal growth factor	Extracellular Space	growth factor
10.ERBB2	erb-b2 receptor tyrosine kinase 2	Plasma Membrane	Kinase
11.ESR1	estrogen receptor 1	Nucleus	ligand-dependent nuclear receptor
12.ESR2	estrogen receptor 2	Nucleus	ligand-dependent nuclear receptor
13.ETS1	ETS proto-oncogene 1, transcription factor	Nucleus	transcription regulator
14.EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit	Nucleus	transcription regulator
15.FHL1	four and a half LIM domains 1	Cytoplasm	other
16.FKBP4	FKBP prolyl isomerase 4	Nucleus	enzyme
17.HDAC4	histone deacetylase 4	Nucleus	transcription regulator
18.HSP90 (family)		Cytoplasm	group
19.JUN	Jun proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
20.KLF4	KLF transcription factor 4	Nucleus	transcription regulator
21.LMNA	lamin A/C	Nucleus	other
22.mir-10	relatives of microRNA 10	Cytoplasm	microRNA
23.mir-9	relatives of microRNA 9	Cytoplasm	microRNA
24.MYC	MYC proto-oncogene, bHLH transcription factor	Nucleus	transcription regulator
25.NAA40	N-alpha-acetyltransferase 40, NatD catalytic subunit	Cytoplasm	enzyme
26.NPM1	nucleophosmin 1	Nucleus	transcription regulator
27.PALLD	palladin, cytoskeletal associated protein	Plasma Membrane	other
28.PCNA	proliferating cell nuclear antigen	Nucleus	enzyme
29.PDLIM3	PDZ and LIM domain 3	Cytoplasm	other
30.PRPH	peripherin	Plasma Membrane	other
31.RANBP2	RAN binding protein 2	Nucleus	enzyme
32.RUNX1	RUNX family transcription factor 1	Nucleus	transcription regulator
33.RUNX2	RUNX family transcription factor 2	Nucleus	transcription regulator
34.RYR1	ryanodine receptor 1	Cytoplasm	ion channel
35.SMAD2	SMAD family member 2	Nucleus	transcription regulator
36.SMAD4	SMAD family member 4	Nucleus	transcription regulator
37.SNAI1	snail family transcriptional repressor 1	Nucleus	transcription regulator
38.SQSTM1	sequestosome 1	Cytoplasm	transcription regulator
39.SUV39H1	SUV39H1 histone lysine methyltransferase	Nucleus	enzyme
40.TP53	tumor protein p53	Nucleus	transcription regulator
41.VIM	vimentin	Cytoplasm	other

Table 14. Various interactions regulated by Estrogen, PDLIM-3, miRNA9 and miRNA10 affecting RUNX-2.

From Molecule(s)	Relationship Type	To Molecule(s)
1. AR	expression	mir-10 (includes others)
2. AR	protein-DNA interactions	mir-10 (includes others)
3. ERBB2	protein-protein interactions	CUL3
4. ESR1	activation	RUNX2
5. ESR1	chemical-protein interactions	estrogen
6. ESR1	expression	RUNX2
7. ESR1	inhibition	RUNX2
8. ESR1	protein-protein interactions	CUL3
9. ESR1	protein-protein interactions	RUNX2
10. ESR1	transcription	RUNX2
11. ESR2	chemical-protein interactions	estrogen
12. ESR2	expression	RUNX2
13. ESR2	protein-protein interactions	CUL3
14. ESR2	protein-protein interactions	DCTN1

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15. ETS1	protein-DNA interactions	RUNX2
16. ETS1	protein-protein interactions	RUNX2
17. ETS1	transcription	RUNX2
18. EZH2	expression	mir-10 (includes others)
19. FHL1	protein-protein interactions	ESR1
20. FKBP4	chemical-protein interactions	estrogen
21. FKBP4	protein-protein interactions	AGR2
22. HDAC4	activation	RUNX2
23. HDAC4	expression	RUNX2
24. HDAC4	protein-protein interactions	RUNX2
25. HSP90 (family)	chemical-protein interactions	estrogen
26. HSP90 (family)	protein-protein interactions	CUL3
27. JUN	protein-DNA interactions	RUNX2
28. JUN	protein-protein interactions	RUNX2
29. KLF4	expression	RUNX2
30. KLF4	protein-DNA interactions	RUNX2
31. KLF4	protein-protein interactions	RUNX2
32. LMNA	protein-protein interactions	CTNNB1
33. LMNA	protein-protein interactions	EGF
34. LMNA	protein-protein interactions	ESR1
35. LMNA	protein-protein interactions	HSP90 (family)
36. MYC	expression	mir-9 (includes others)
37. MYC	protein-DNA interactions	mir-9 (includes others)
38. MYC	protein-protein interactions	BAG3
39. MYC	protein-protein interactions	DCTN1
40. MYC	protein-protein interactions	LMNA
41. MYC	transcription	mir-9 (includes others)
42. NAA40	protein-protein interactions	FKBP4
43. NPM1	expression	mir-10 (includes others)
44. NPM1	protein-protein interactions	LMNA
45. PALLD	protein-protein interactions	MYC
46. PCNA	protein-protein interactions	ESR2
47. PCNA	protein-protein interactions	EZH2
48. PCNA	protein-protein interactions	MYC
49. PDLIM3	protein-protein interactions	ACTN2
50. PDLIM3	protein-protein interactions	AGR2
51. PDLIM3	protein-protein interactions	BAG3
52. PDLIM3	protein-protein interactions	CUL3
53. PDLIM3	protein-protein interactions	DCTN1
54. PDLIM3	protein-protein interactions	FHL1
55. PDLIM3	protein-protein interactions	LMNA
56. PDLIM3	protein-protein interactions	NAA40
57. PDLIM3	protein-protein interactions	PALLD
58. PDLIM3	protein-protein interactions	PCNA
59. PDLIM3	protein-protein interactions	PDLIM3
60. PRPH	protein-protein interactions	MYC
61. PRPH	protein-protein interactions	PDLIM3
62. RANBP2	protein-protein interactions	APC
63. RANBP2	protein-protein interactions	ERBB2
64. RANBP2	protein-protein interactions	ESR1
65. RANBP2	protein-protein interactions	ESR2
66. RANBP2	protein-protein interactions	PDLIM3
67. RUNX1	expression	RUNX2
68. RUNX1	protein-DNA interactions	RUNX2
69. RUNX1	protein-protein interactions	RUNX2
70. RUNX2	activation	RUNX2
71. RUNX2	expression	RUNX2

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72. RUNX2	inhibition	RUNX2
73. RUNX2	localization	RUNX2
74. RUNX2	modification	RUNX2
75. RUNX2	molecular cleavage	RUNX2
76. RUNX2	protein-DNA interactions	RUNX2
77. RUNX2	protein-protein interactions	AR
78. RUNX2	protein-protein interactions	BAG3
79. RUNX2	protein-protein interactions	ESR1
80. RUNX2	protein-protein interactions	ETS1
81. RUNX2	protein-protein interactions	HDAC4
82. RUNX2	protein-protein interactions	JUN
83. RUNX2	protein-protein interactions	KLF4
84. RUNX2	protein-protein interactions	RUNX1
85. RUNX2	protein-protein interactions	SMAD4
86. RUNX2	regulation of binding	RUNX2
87. RUNX2	ubiquitination	RUNX2
88. RYR1	protein-protein interactions	ESR2
89. RYR1	protein-protein interactions	PDLIM3
90. SMAD2	expression	RUNX2
91. SMAD2	protein-protein interactions	RUNX2
92. SMAD4	expression	RUNX2
93. SMAD4	protein-DNA interactions	RUNX2
94. SMAD4	protein-protein interactions	RUNX2
95. SNAI1	expression	mir-10 (includes others)
96. SNAI1	protein-protein interactions	ACTN2
97. SNAI1	transcription	mir-10 (includes others)
98. SQSTM1	protein-protein interactions	CTNNB1
99. SQSTM1	protein-protein interactions	ESR1
100. SQSTM1	protein-protein interactions	ESR2
101. SQSTM1	protein-protein interactions	FKBP4
102. SQSTM1	protein-protein interactions	NPM1
103. SQSTM1	protein-protein interactions	PDLIM3
104. SUV39H1	protein-protein interactions	RUNX2
105. SUV39H1	protein-protein interactions	mir-10 (includes others)
106. TP53	expression	RUNX2
107. TP53	protein-protein interactions	RUNX2
108. TP53	transcription	RUNX2
109. VIM	protein-protein interactions	ESR2
110. VIM	protein-protein interactions	MYC
111. VIM	protein-protein interactions	PDLIM3
112. estrogen	activation	ESR1
113. estrogen	activation	ESR2
114. estrogen	activation	RUNX2
115. estrogen	chemical-protein interactions	EGF
116. estrogen	chemical-protein interactions	ERBB2
117. estrogen	chemical-protein interactions	ESR1
118. estrogen	chemical-protein interactions	ESR2
119. estrogen	translocation	estrogen
120. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	AR
121. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	HDAC4
122. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	KLF4
123. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SMAD2

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124. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SMAD4
125. mir-10 (includes others)	RNA-RNA interactions: microRNA targeting	SUV39H1
126. mir-10 (includes others)	RNA-RNA interactions: non-targeting interactions	APC
127. mir-10 (includes others)	RNA-RNA interactions: non-targeting interactions	JUN
128. mir-10 (includes others)	expression	AR
129. mir-10 (includes others)	protein-protein interactions	SUV39H1
130. mir-9 (includes others)	RNA-RNA interactions: microRNA targeting	AR
131. mir-9 (includes others)	RNA-RNA interactions: microRNA targeting	ETS1
132. mir-9 (includes others)	RNA-RNA interactions: microRNA targeting	RUNX1
133. mir-9 (includes others)	RNA-RNA interactions: non-targeting interactions	AR
134. mir-9 (includes others)	expression	AR
135. mir-9 (includes others)	expression	ETS1
136. mir-9 (includes others)	expression	RUNX1

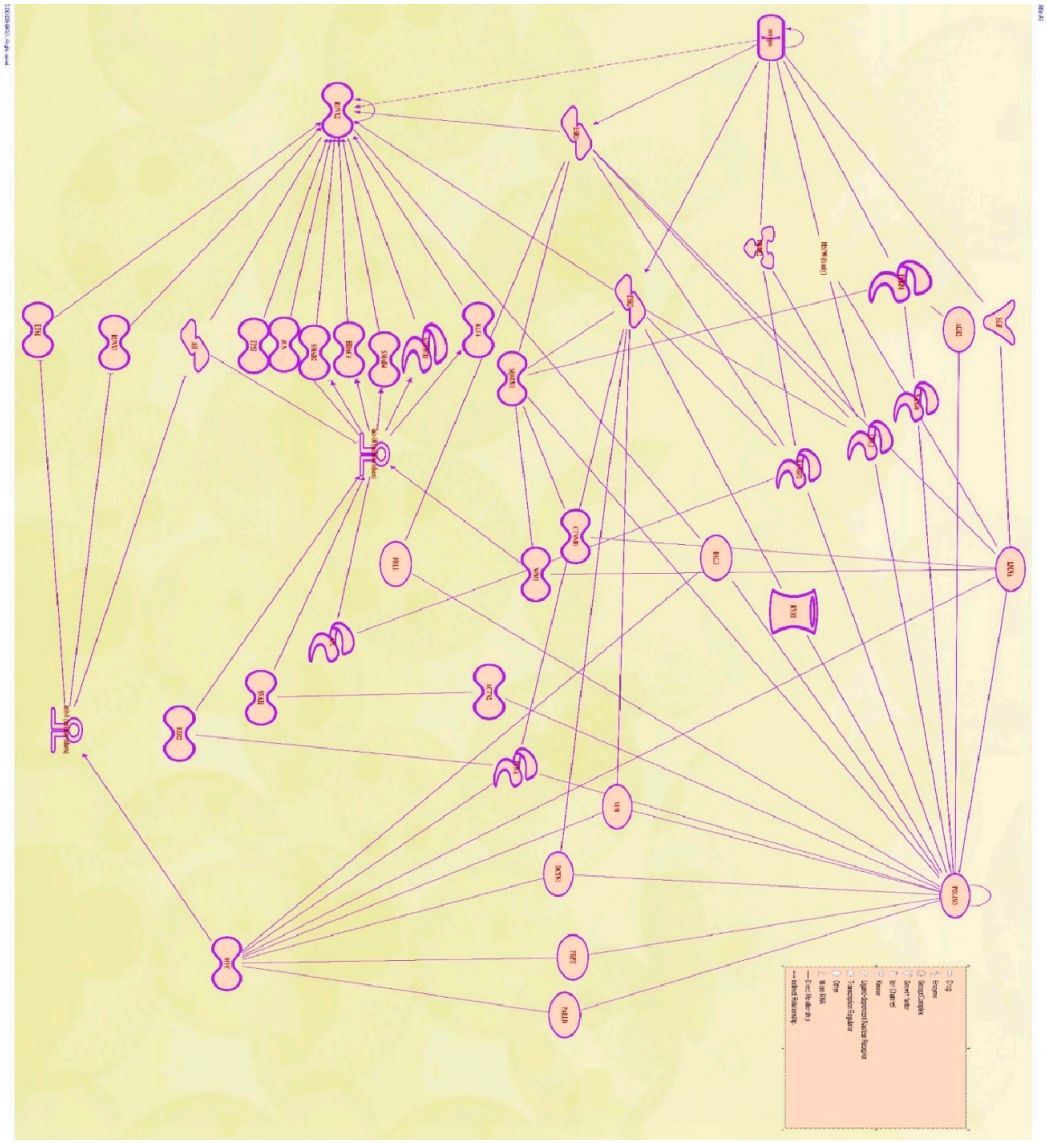

Figure 9. Molecules network influenced by estrogen, *PDLIM-3*. miRNA-10, miRNA-9 affecting RUNX-2expression.

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8. Identification and Analysis of the Molecules Network Regulated by Estrogen, PDLIM-3, miR-1896, microRNA6769B affects the expression of RUNX-2.

The "MAP" program and QKB identified around 45 molecules involved directly or indirectly in the expression of RUNX-2, regulated by estrogen, microRNA-1896, microRNA-6769B, and *PDLIM-3*. The full classification of those molecules, including the gene name, its location, and its family, is shown in Figure 10 and Tables 15 and 16, respectively. This not only confirms the function of PDLIM3 as an epigenetic regulator but also considerably broadens the suggested regulatory axis by incorporating miR-6769b as a novel and possibly crucial participant in estrogen-responsive osteogenesis.

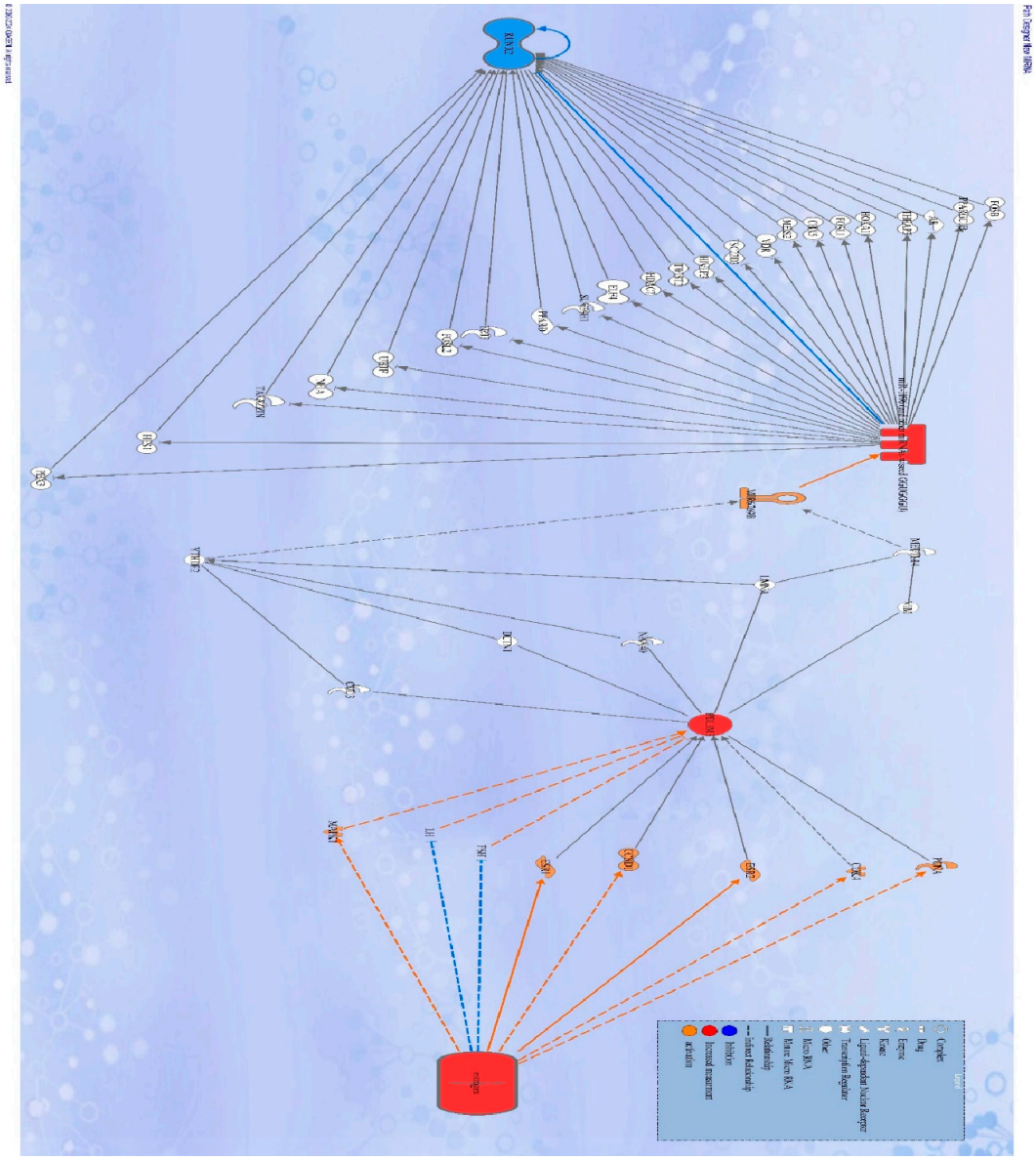

Figure 10. Molecules network regulated by estrogen, *PDLIM-3*. microRNA-1896 , microRNA6769B affecting RUNX-2expression.

Table 15. Molecules regulated by Estrogen, PDLIM-3, , microRNA-1896 , microRNA6769B, affecting RUNX-2.

Symbol	Molecule/ Gene Name	Location	Family
1. AR	androgen receptor	Nucleus	ligand-dependent nuclear receptor
2. CBX5	chromobox 5	Nucleus	transcription regulator
3. CCND1	cyclin D1	Nucleus	transcription regulator

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4. CDK4	cyclin dependent kinase 4	Nucleus	kinase
5. CUL3	cullin 3	Nucleus	Enzyme
6. DCTN1	dynactin subunit 1	Cytoplasm	Other
7. ELF4	E74 like ETS transcription factor 4	Nucleus	transcription regulator
8. ESR1	estrogen receptor 1	Nucleus	ligand-dependent nuclear receptor
9. ESR2	estrogen receptor 2	Nucleus	ligand-dependent nuclear receptor
10. estrogen		Other	chemical drug
11. FOSB	FosB proto-oncogene, AP-1 transcription factor subunit	Nucleus	transcription regulator
12. FOSL1	FOS like 1, AP-1 transcription factor subunit	Nucleus	transcription regulator
13. FOSL2	FOS like 2, AP-1 transcription factor subunit	Nucleus	transcription regulator
14. FSH		Plasma Membrane	Complex
15. HDAC7	histone deacetylase 7	Nucleus	transcription regulator
16. HES1	hes family bHLH transcription factor 1	Nucleus	transcription regulator
17. HIVEP3	HIVEP zinc finger 3	Nucleus	transcription regulator
18. HOXA11	homeobox A11	Nucleus	transcription regulator
19. LH		Plasma Membrane	Complex
20. LMNA	lamin A/C	Nucleus	Other
21. MAPK1	mitogen-activated protein kinase 1	Cytoplasm	Kinase
22. MEIS2	Meis homeobox 2	Nucleus	transcription regulator
23. METTL14	methyltransferase 14, N6-adenosine-methyltransferase subunit	Nucleus	Enzyme
24. miR-1896		Cytoplasm	mature microRNA
25. MIR6769B	microRNA 6769b	Other	microRNA
26. NAA40	N-alpha-acetyltransferase 40, NatD catalytic subunit	Cytoplasm	Enzyme
27. NFIA	nuclear factor I A	Nucleus	transcription regulator
28. PBX3	PBX homeobox 3	Nucleus	transcription regulator
29. PCNA	proliferating cell nuclear antigen	Nucleus	Enzyme
30. PDLIM3	PDZ and LIM domain 3	Cytoplasm	Other
31. PPARD	peroxisome proliferator activated receptor delta	Nucleus	ligand-dependent nuclear receptor
32. PPARGC1B	PPARG coactivator 1 beta	Nucleus	transcription regulator
33. RUNX2	RUNX family transcription factor 2	Nucleus	transcription regulator
34. SUV39H1	SUV39H1 histone lysine methyltransferase	Nucleus	Enzyme
35. TAFAZZIN	tafazzin, phospholipid-lysophospholipid transacylase	Nucleus	Enzyme
36. TET3	tet methylcytosine dioxygenase 3	Nucleus	Enzyme
37. THRAP3	thyroid hormone receptor associated protein 3	Nucleus	transcription regulator
38. TSC22D3	TSC22 domain family member 3	Nucleus	transcription regulator
39. TWIST2	twist family bHLH transcription factor 2	Nucleus	transcription regulator
40. UBTF	upstream binding transcription factor	Nucleus	transcription regulator
41. VDR	vitamin D receptor	Nucleus	transcription regulator
42. VIM	vimentin	Cytoplasm	Other
43. YTHDF2	YTH N6-methyladenosine RNA binding protein F2	Cytoplasm	Other

Table 16. Various interactions regulated by estrogen, *PDLIM-3*. microRNA-1896 , microRNA6769B affecting RUNX-2.

From Molecule(s)	Relationship Type	To Molecule(s)
1. CBX5	expression	RUNX2
2. CCND1	expression	PDLIM3

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3. CDK4	expression	PDLIM3
4. ESR1	chemical-protein interactions	estrogen
5. ESR1	expression	PDLIM3
6. ESR1	regulation of binding	estrogen
7. ESR2	chemical-protein interactions	estrogen
8. ESR2	expression	PDLIM3
9. FOSB	protein-DNA interactions	RUNX2
10. FOSL1	protein-DNA interactions	RUNX2
11. FOSL2	protein-DNA interactions	RUNX2
12. FSH	expression	PDLIM3
13. HES1	activation	RUNX2
14. HES1	protein-protein interactions	RUNX2
15. HIVEP3	inhibition	RUNX2
16. HIVEP3	protein-protein interactions	RUNX2
17. HOXA11	expression	RUNX2
18. HOXA11	protein-protein interactions	RUNX2
19. HOXA11	transcription	RUNX2
20. LH	expression	PDLIM3
21. LMNA	protein-DNA interactions	YTHDF2
22. MAPK1	expression	PDLIM3
23. MEIS2	expression	RUNX2
24. METTL14	expression	MIR6769B
25. METTL14	protein-protein interactions	LMNA
26. MIR6769B	processing yields	miR-1896
27. NFIA	expression	RUNX2
28. PBX3	expression	RUNX2
29. PDLIM3	protein-protein interactions	CUL3
30. PDLIM3	protein-protein interactions	DCTN1
31. PDLIM3	protein-protein interactions	LMNA
32. PDLIM3	protein-protein interactions	NAA40
33. PDLIM3	protein-protein interactions	PCNA
34. PPARD	expression	RUNX2
35. PPARGC1B	transcription	RUNX2
36. RUNX2	activation	RUNX2
37. RUNX2	expression	RUNX2
38. RUNX2	inhibition	HIVEP3
39. RUNX2	inhibition	RUNX2
40. RUNX2	localization	RUNX2
41. RUNX2	modification	RUNX2
42. RUNX2	molecular cleavage	RUNX2
43. RUNX2	protein-DNA interactions	RUNX2
44. RUNX2	protein-protein interactions	AR
45. RUNX2	protein-protein interactions	ELF4
46. RUNX2	protein-protein interactions	HDAC7

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47. RUNX2	protein-protein interactions	HES1
48. RUNX2	protein-protein interactions	HIVEP3
49. RUNX2	protein-protein interactions	HOXA11
50. RUNX2	protein-protein interactions	TAFAZZIN
51. RUNX2	regulation of binding	RUNX2
52. RUNX2	ubiquitination	RUNX2
53. SUV39H1	protein-protein interactions	RUNX2
54. TAFAZZIN	protein-protein interactions	RUNX2
55. TET3	protein-protein interactions	RUNX2
56. THRAP3	expression	RUNX2
57. TSC22D3	expression	RUNX2
58. TWIST2	protein-protein interactions	RUNX2
59. UBTF	protein-protein interactions	RUNX2
60. VDR	expression	RUNX2
61. VDR	protein-protein interactions	RUNX2
62. VIM	protein-protein interactions	METTL14
63. VIM	protein-protein interactions	PDLIM3
64. YTHDF2	expression	MIR6769B
65. YTHDF2	protein-protein interactions	CUL3
66. YTHDF2	protein-protein interactions	DCTN1
67. YTHDF2	protein-protein interactions	NAA40
68. estrogen	activation	CDK4
69. estrogen	activation	ESR1
70. estrogen	activation	ESR2
71. estrogen	activation	MAPK1
72. estrogen	chemical-protein interactions	ESR1
73. estrogen	chemical-protein interactions	ESR2
74. estrogen	expression	CCND1
75. estrogen	expression	ESR1
76. estrogen	expression	ESR2
77. estrogen	expression	FSH
78. estrogen	expression	LH
79. estrogen	expression	PCNA
80. estrogen	localization	FSH
81. estrogen	localization	LH
82. estrogen	molecular cleavage	ESR1
83. estrogen	phosphorylation	ESR1
84. estrogen	phosphorylation	ESR2
85. estrogen	phosphorylation	MAPK1
86. estrogen	regulation of binding	CCND1
87. estrogen	regulation of binding	CDK4
88. estrogen	regulation of binding	ESR1
89. estrogen	regulation of binding	ESR2
90. estrogen	transcription	CCND1

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91.	estrogen	translocation ESR1
92.	estrogen	translocation ESR2
93.	miR-1896	RNA-RNA interactions: microRNA targeting AR
94.	miR-1896	RNA-RNA interactions: microRNA targeting CBX5
95.	miR-1896	RNA-RNA interactions: microRNA targeting ELF4
96.	miR-1896	RNA-RNA interactions: microRNA targeting FOSB
97.	miR-1896	RNA-RNA interactions: microRNA targeting FOSL1
98.	miR-1896	RNA-RNA interactions: microRNA targeting FOSL2
99.	miR-1896	RNA-RNA interactions: microRNA targeting HDAC7
100.	miR-1896	RNA-RNA interactions: microRNA targeting HES1
101.	miR-1896	RNA-RNA interactions: microRNA targeting HIVEP3
102.	miR-1896	RNA-RNA interactions: microRNA targeting HOXA11
103.	miR-1896	RNA-RNA interactions: microRNA targeting MEIS2
104.	miR-1896	RNA-RNA interactions: microRNA targeting NFIA
105.	miR-1896	RNA-RNA interactions: microRNA targeting PBX3
106.	miR-1896	RNA-RNA interactions: microRNA targeting PPARD
107.	miR-1896	RNA-RNA interactions: microRNA targeting PPARGC1B
108.	miR-1896	RNA-RNA interactions: microRNA targeting RUNX2
109.	miR-1896	RNA-RNA interactions: microRNA targeting SUV39H1
110.	miR-1896	RNA-RNA interactions: microRNA targeting TAFAZZIN
111.	miR-1896	RNA-RNA interactions: microRNA targeting TET3
112.	miR-1896	RNA-RNA interactions: microRNA targeting THRAP3
113.	miR-1896	RNA-RNA interactions: microRNA targeting TSC22D3
114.	miR-1896	RNA-RNA interactions: microRNA targeting TWIST2
115.	miR-1896	RNA-RNA interactions: microRNA targeting UBTF
116.	miR-1896	RNA-RNA interactions: microRNA targeting VDR
117.	miR-1896	expression RUNX2

IV. Discussion and Conclusion

This study elucidates a novel, multilayered regulatory axis in which estrogen activates RUNX2 expression through both direct genomic signaling and epigenetic modulation involving PDLIM3 and specific microRNAs. The identification of *PDLIM3* as an intermediary, along with the integration of

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miR-9, miR-10, and the newly implicated miR-6769b, offers novel perspectives on estrogen-responsive osteogenesis and opens potential avenues for targeted therapeutic strategies.

Figure 11. Estrogen-driven regulatory cascade linking the ERα/β–PDLIM3–miRNAs–RUNX2 axis.

The pathway identified can be summarized as

1. Classical Estrogen Receptor Signaling and PDLIM3 Activation

Estrogen binding to ERα and ERβ activates classical genomic signaling, whereby the ligand-receptor complex translocates to the nucleus and binds estrogen response elements (EREs) in the regulatory regions of target genes [27]. Our analysis revealed a strong association between estrogen signaling and *PDLIM3* expression. Although *PDLIM3* has previously been associated primarily with muscle function [28], this is the first report linking it to estrogen-mediated osteogenic pathways and RUNX2 regulation. Its identification as a novel intermediary in this axis is supported by its low coefficient of variation (CV = 0.083), indicating tight estrogenic regulation [29].

2. PDLIM3-Mediated Regulation of miR-9 and miR-10

Downstream of *PDLIM3*, our IPA-based analysis identified significant associations with miR-9 and miR-10, two miRNAs implicated in bone formation, neurodevelopment, and immune regulation [30,31]. Both miRNAs have been shown to regulate *RUNX2* directly or via ERK signaling, and their increased expression in response to estrogen-PDLIM3 signaling suggests a crucial post-transcriptional modulatory role in osteogenesis [32].

3. Novel Discovery of miR-6769b in Osteogenic Regulation:

An especially noteworthy finding is the identification of miR-6769b as a novel epigenetic player within this regulatory framework. While limited prior data exist linking miR-6769b to bone biology, emerging literature suggests its involvement in exosome-mediated bone remodeling and cell proliferation control [33,34]. Our network data suggest that miR-6769b is induced downstream of PDLIM3, representing a new layer of epigenetic regulation for RUNX2.

4. Integrated Signaling Cascade:

Our findings support a multi-step model for estrogen-driven RUNX2 activation:

Estrogen → ERα/β → PDLIM3 ↑ → miR-9/miR-10/miR-6769b ↑ → RUNX2 ↑

This pathway expands upon the classical model of estrogen action by incorporating miRNA-mediated epigenetic regulation and identifying new targets such as PDLIM3 and miR-6769b that can serve as biomarkers or therapeutic entry points in skeletal disorders [27,29].

5. Implications and Future Directions

1) Biological Significance: This model enriches our understanding of estrogenic regulation of skeletal development and uncovers new candidate targets for bone regeneration and disease treatment.

2) Experimental Validation: Further experimental validation using ChIP-qPCR (to confirm ER binding to PDLIM3), gene knockdown (for PDLIM3 and miR-6769b), and miRNA modulation studies is critical to establish functional causality.

V. Conclusion

In this study, pathway relationships between estrogen, *PDLIM3*, microRNAs (miR-9, miR-10, and miR-6769b), and RUNX2 were primarily modeled using QIAGEN's Ingenuity Pathway Analysis (IPA) tools, in conjunction with curated biological insights from literature. While z-score-based predictions are commonly employed to infer activation or inhibition states in large-scale differential expression studies, they were not the focus of this analysis. Instead, we aimed to systematically map molecular interactions and delineate hierarchical regulatory networks rather than statistically quantify expression changes.

Consequently, the findings are presented descriptively, highlighting directionality and mechanistic connectivity as supported by canonical pathway data and peer-reviewed evidence. This approach has uncovered a novel, multilayered regulatory cascade whereby estrogen signaling through ERα/β leads to upregulation of *PDLIM3*, which in turn enhances the expression of key

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microRNAs, including miR-9, miR-10, and the newly implicated miR-6769b, ultimately driving the transcriptional activation of RUNX2.

These insights reveal new mediators within the estrogen–RUNX2 axis and offer promising implications for future research into skeletal development, osteogenic differentiation, and hormone-responsive bone pathologies such as osteoporosis and fracture repair.

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Conflicts of Interest: The authors affirm that they have no financial, commercial, or other relationships that could be perceived as potential conflicts of interest in relation to the submitted work.

Abbreviations

Abbreviation	Definition
AKT	Protein kinase B; serine/threonine kinase in the PI3K/AKT signaling pathway regulating survival and metabolism.
ALP	Actin-associated LIM protein; structural and signaling protein encoded by **PDLIM3**, localized in cardiac and skeletal muscle Z-discs.
AP-1	Activator protein 1; transcription factor complex (Fos/Jun) regulating proliferation and apoptosis.
BAX	Bcl-2–associated X protein; pro-apoptotic member of the Bcl-2 family.
BCL-2	B-cell lymphoma 2; anti-apoptotic protein that promotes cell survival.
CBFα1	Core-binding factor subunit alpha-1; alternative name for RUNX2 transcription factor.
CCD	Cleidocranial dysplasia; hereditary skeletal disorder caused by mutations in **RUNX2**.
CDK	Cyclin-dependent kinase; enzyme family that regulates cell-cycle progression.
CDK4	Cyclin-dependent kinase 4; G1 phase cell-cycle regulator.
CREB	cAMP response element-binding protein; transcription factor that regulates metabolism, survival, and plasticity.
CV	Coefficient of variation; measure of relative variability in gene expression or stability.
DCM	Dilated cardiomyopathy; disorder characterized by dilation and impaired contraction of cardiac chambers.
E1	Estrone; endogenous estrogen.
E2	17β-estradiol; the most potent and biologically active endogenous estrogen.
E3	Estriol; estrogen predominant during pregnancy.
E4	Estetrol; fetal liver–derived estrogen present during pregnancy.
ECM	Extracellular matrix; structural network of proteins and polysaccharides surrounding cells.
EGFR	Epidermal growth factor receptor; receptor tyrosine kinase regulating proliferation and survival.
ER	Estrogen receptor; ligand-activated transcription factor mediating estrogen actions.
ERα / ERβ	Estrogen receptor alpha / beta; two main nuclear estrogen receptor isoforms.
ERGs	Estrogen-responsive genes; genes whose transcription is modulated by estrogen receptor signaling.
EREs	Estrogen response elements; DNA sequences bound by ER complexes to regulate transcription.
ERK	Extracellular signal-regulated kinase; MAPK family kinase involved in proliferation and differentiation.
ESR1 / ESR2	Estrogen receptor 1 / 2; gene symbols encoding ERα and ERβ, respectively.

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Abbreviation	Definition
FSH	Follicle-stimulating hormone; gonadotropin regulating follicle development and spermatogenesis.
Fos	Proto-oncogene c-Fos; component of the AP-1 transcription factor complex.
GPER1	G protein-coupled estrogen receptor 1; membrane-bound estrogen receptor mediating rapid non-genomic signaling.
GR	Glucocorticoid receptor; nuclear receptor for glucocorticoid hormones.
GSK3β	Glycogen synthase kinase 3 beta; serine/threonine kinase involved in metabolism and Wnt/MAPK signaling.
HRT	Hormone replacement therapy; clinical use of hormones (e.g., estrogens) to treat menopausal or deficiency symptoms.
HSPs	Heat shock proteins; molecular chaperones that stabilize and refold proteins, also associated with steroid receptors.
ID	Inhibitor of differentiation; family of helix-loop-helix transcriptional regulators.
ID3	Inhibitor of DNA-binding protein 3; an ID family member downregulated by miR-10a-3p during osteogenic differentiation.
IGF-1	Insulin-like growth factor 1; peptide growth factor important for growth and metabolism.
IPA	Ingenuity Pathway Analysis; QIAGEN bioinformatics software for pathway and network modeling.
IRS-1	Insulin receptor substrate 1; adaptor protein transmitting insulin/IGF-1 receptor signaling.
JNK	c-Jun N-terminal kinase; stress-activated protein kinase within the MAPK family.
LH	Luteinizing hormone; gonadotropin regulating ovulation and gonadal steroid production.
MAP	Molecule Activity Predictor; Ingenuity Pathway Analysis (IPA) tool predicting activation/inhibition effects within networks.
MAPK / MAPK-1	Mitogen-activated protein kinase / MAPK-1; serine/threonine kinases mediating downstream signaling (ERK2 often referred to as MAPK-1).
MDSCs	Myeloid-derived suppressor cells; immune-suppressive myeloid cell population modulating tumor and inflammatory responses.
MEK	Mitogen-activated protein kinase kinase; dual-specificity kinase upstream of ERK in the MAPK cascade.
miR / miRNA	MicroRNA; small (~19–25 nt) non-coding RNA that regulates gene expression post-transcriptionally.
miR-9	MicroRNA-9; miRNA regulating neurogenesis, osteogenesis, and immune functions.
miR-10 / miRNA10 / miR-10a	MicroRNA-10; family associated with differentiation, apoptosis, and cancer; miR-10a is a specific isoform.
miR-1896	MicroRNA-1896; miRNA implicated in indirect regulation of RUNX2 via shared seed sequences.
miR-6769b / microRNA6769B	MicroRNA-6769b; newly implicated epigenetic regulator in RUNX2-related signaling.
mTOR	Mechanistic target of rapamycin; central kinase controlling growth and metabolism.
NF-κB	Nuclear factor kappa-B; transcription factor regulating inflammatory and immune responses.
OPN	Osteopontin; extracellular matrix phosphoprotein involved in bone remodeling and mineralization.
PCNA	Proliferating cell nuclear antigen; sliding clamp protein and marker of DNA replication and repair.
PDLIM3	PDZ and LIM domain protein 3; actin-associated structural and signaling protein (ALP), here identified as an estrogen-regulated intermediary upstream of RUNX2.
PI3K	Phosphoinositide 3-kinase; lipid kinase that generates PIP3 and activates AKT signaling.

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Abbreviation	Definition
PTEN	Phosphatase and tensin homolog; tumor suppressor and negative regulator of PI3K/AKT signaling.
QKB	QIAGEN Knowledge Base; curated database underpinning Ingenuity Pathway Analysis.
Ras	Rat sarcoma; small GTP-binding protein family regulating proliferation and survival pathways.
RUNX2 / RUNX-2	Runt-related transcription factor 2; master transcription factor for osteoblast differentiation and skeletal morphogenesis.
SRC	Steroid receptor coactivator; transcriptional co-regulator that enhances nuclear receptor–mediated gene expression.
STAT	Signal transducer and activator of transcription; transcription factor family that mediates cytokine and growth factor signaling.
VEGF	Vascular endothelial growth factor; central regulator of angiogenesis and vascular permeability.

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