**Distinct HAND2/HAND2-AS1 Expression Levels Finetune Mesenchymal and Epithelial Cell Plasticity in Human Mesenchymal Stem Cells**

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**Supplementary Information Online**

### **Supplementary Table S1: Gene array related to endothelial cell phenotypes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Position** | **Unigene** | **GeneBank** | **Symbol** | **Description** | **Gene Name** |
| A01 | Hs.654434 | NM\_000789 | ACE | Angiotensin I converting enzyme | ACE1, CD143, DCP, DCP1, MGC26566, MVCD3 |
| A02 | Hs.404914 | NM\_003183 | ADAM17 | ADAM metallopeptidase domain 17 | ADAM18, CD156B, CSVP, MGC71942, TACE |
| A03 | Hs.19383 | NM\_000029 | AGT | Angiotensinogen | ANHU, FLJ92595, FLJ97926, SERPINA8 |
| A04 | Hs.728754 | NM\_031850 | AGTR1 | Angiotensin II receptor, type 1 | AG2S, AGTR1A, AGTR1B, AT1, AT1B, AT1R, AT2R1 |
| A05 | Hs.89499 | NM\_000698 | ALOX5 | Arachidonate 5-lipoxygenase | 5-LO, 5-LOX, 5LPG, LOG5, MGC163204 |
| A06 | Hs.369675 | NM\_001146 | ANGPT1 | Angiopoietin 1 | AGP1, AGPT, ANG1 |
| A07 | Hs.480653 | NM\_001154 | ANXA5 | Annexin A5 | ANX5, ENX2, PP4 |
| A08 | Hs.654439 | NM\_000041 | APOE | Apolipoprotein E | AD2, LDLCQ5, LPG, MGC1571 |
| A09 | Hs.624291 | NM\_004324 | BAX | BCL2-associated X protein | BCL2L4 |
| A10 | Hs.150749 | NM\_000633 | BCL2 | B-cell CLL/lymphoma 2 | Bcl-2 |
| A11 | Hs.516966 | NM\_138578 | BCL2L1 | BCL2-like 1 | BCL-XL, S, BCL2L, BCLX, BCLXL, BCLXS, Bcl-X |
| A12 | Hs.37058 | NM\_001741 | CALCA | Calcitonin-related polypeptide alpha | CALC1, CGRP, CGRP-I, CGRP1, CT, KC, MGC126648 |
| B01 | Hs.2490 | NM\_033292 | CASP1 | Caspase 1, apoptosis-related cysteine peptidase | ICE, IL1BC, P45 |
| B02 | Hs.141125 | NM\_004346 | CASP3 | Caspase 3, apoptosis-related cysteine peptidase | CPP32, CPP32B, SCA-1 |
| B03 | Hs.74034 | NM\_001753 | CAV1 | Caveolin 1, caveolae protein, 22kDa | BSCL3, CGL3, MSTP085, VIP21 |
| B04 | Hs.303649 | NM\_002982 | CCL2 | Chemokine (C-C motif) ligand 2 | GDCF-2, HC11, HSMCR30, MCAF, MCP-1, MCP1, |
| B05 | Hs.514821 | NM\_002985 | CCL5 | Chemokine (C-C motif) ligand 5 | D17S136E, MGC17164, RANTES, SCYA5, SISd, TCP228 |
| B06 | Hs.76206 | NM\_001795 | CDH5 | Cadherin 5, type 2 (vascular endothelium) | 7B4, CD144, FLJ17376 |
| B07 | Hs.390736 | NM\_003879 | CFLAR | CASP8 and FADD-like apoptosis regulator | CASH, CASP8AP1, CLARP, Casper, FLAME, FLAME-1, FLAME1, FLIP, |
| B08 | Hs.517356 | NM\_030582 | COL18A1 | Collagen, type XVIII, alpha 1 | FLJ27325, FLJ34914, KNO, KNO1, KS, MGC74745 |
| B09 | Hs.531668 | NM\_002996 | CX3CL1 | Chemokine (C-X3-C motif) ligand 1 | ABCD-3, C3Xkine, CXC3, CXC3C, NTN, NTT, |
| B10 | Hs.511899 | NM\_001955 | EDN1 | Endothelin 1 | ET1, HDLCQ7, PPET1 |
| B11 | Hs.1407 | NM\_001956 | EDN2 | Endothelin 2 | ET2, PPET2 |
| B12 | Hs.183713 | NM\_001957 | EDNRA | Endothelin receptor type A | ETA, ETAR, ETRA |
| C01 | Hs.76753 | NM\_000118 | ENG | Endoglin | CD105, END, FLJ41744, HHT1, ORW, ORW1 |
| C02 | Hs.482562 | NM\_001992 | F2R | Coagulation factor II (thrombin) receptor | CF2R, HTR, PAR-1, PAR1, TR |
| C03 | Hs.62192 | NM\_001993 | F3 | Coagulation factor III (thromboplastin, tissue factor) | CD142, FLJ17960, TF, TFA |
| C04 | Hs.244139 | NM\_000043 | FAS | Fas (TNF receptor superfamily, member 6) | ALPS1A, APO-1, APT1, CD95, FAS1, FASTM, TNFRSF6 |
| C05 | Hs.2007 | NM\_000639 | FASLG | Fas ligand (TNF superfamily, member 6) | APT1LG1, CD178, CD95-L, CD95L, FASL, TNFSF6 |
| C06 | Hs.483635 | NM\_000800 | FGF1 | Fibroblast growth factor 1 (acidic) | AFGF, ECGF, ECGF-beta, ECGFA, ECGFB, FGF-alpha |
| C07 | Hs.284244 | NM\_002006 | FGF2 | Fibroblast growth factor 2 (basic) | BFGF, FGFB, HBGF-2 |
| C08 | Hs.654360 | NM\_002019 | FLT1 | Fms-related tyrosine kinase 1 | FLT, VEGFR1 |
| C09 | Hs.203717 | NM\_002026 | FN1 | Fibronectin 1 | CIG, DKFZp686F10164, DKFZp686H0342, DKFZp686I1370 |
| C10 | Hs.597216 | NM\_001530 | HIF1A | Hypoxia inducible factor 1, alpha subunit | HIF-1alpha, HIF1, HIF1-ALPHA, MOP1, PASD8, bHLHe78 |
| C11 | Hs.517581 | NM\_002133 | HMOX1 | Heme oxygenase (decycling) 1 | HO-1, HSP32, bK286B10 |
| C12 | Hs.643447 | NM\_000201 | ICAM1 | Intercellular adhesion molecule 1 | BB2, CD54, P3.58 |
| D01 | Hs.467304 | NM\_000641 | IL11 | Interleukin 11 | AGIF, IL-11 |
| D02 | Hs.126256 | NM\_000576 | IL1B | Interleukin 1, beta | IL-1, IL1-BETA, IL1F2 |
| D03 | Hs.694 | NM\_000588 | IL3 | Interleukin 3 (colony-stimulating factor, multiple) | IL-3, MCGF, MGC79398, MGC79399, MULTI-CSF |
| D04 | Hs.654458 | NM\_000600 | IL6 | Interleukin 6 (interferon, beta 2) | BSF2, HGF, HSF, IFNB2, IL-6 |
| D05 | Hs.591873 | NM\_000880 | IL7 | Interleukin 7 | IL-7 |
| D06 | Hs.505654 | NM\_002205 | ITGA5 | Integrin, alpha 5 (fibronectin receptor, alpha polypeptide) | CD49e, FNRA, VLA5A |
| D07 | Hs.436873 | NM\_002210 | ITGAV | Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51) | CD51, DKFZp686A08142, MSK8, VNRA |
| D08 | Hs.643813 | NM\_002211 | ITGB1 | Integrin, beta 1 | CD29, FNRB, GPIIA, MDF2, MSK12, VLA-BETA, VLAB |
| D09 | Hs.218040 | NM\_000212 | ITGB3 | Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) | CD61, GP3A, GPIIIa |
| D10 | Hs.479756 | NM\_002253 | KDR | Kinase insert domain receptor | CD309, FLK1, VEGFR, VEGFR2 |
| D11 | Hs.479754 | NM\_000222 | KIT | V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog | C-Kit, CD117, PBT, SCFR |
| D12 | Hs.171995 | NM\_001648 | KLK3 | Kallikrein-related peptidase 3 | APS, KLK2A1, PSA, hK3 |
| E01 | Hs.83169 | NM\_002421 | MMP1 | Matrix metallopeptidase 1 | CLG, CLGN |
| E02 | Hs.513617 | NM\_004530 | MMP2 | Matrix metallopeptidase 2 | CLG4, CLG4A, MMP-II, MONA, TBE-1 |
| E03 | Hs.297413 | NM\_004994 | MMP9 | Matrix metallopeptidase 9 | CLG4B, GELB, MANDP2, MMP-9 |
| E04 | Hs.707978 | NM\_000603 | NOS3 | Nitric oxide synthase 3 (endothelial cell) | ECNOS, eNOS |
| E05 | Hs.219140 | NM\_002521 | NPPB | Natriuretic peptide B | BNP |
| E06 | Hs.490330 | NM\_000906 | NPR1 | Natriuretic peptide receptor A/guanylate cyclase A | ANPRA, ANPa, GUC2A, GUCY2A, NPRA |
| E07 | Hs.592605 | NM\_002538 | OCLN | Occludin | BLCPMG, FLJ08163, FLJ18079, FLJ77961, FLJ94056, MGC34277 |
| E08 | Hs.74615 | NM\_006206 | PDGFRA | Platelet-derived growth factor receptor, alpha polypeptide | CD140A, MGC74795, PDGFR2, RHEPDGFRA |
| E09 | Hs.514412 | NM\_000442 | PECAM1 | Platelet/endothelial cell adhesion molecule | CD31, FLJ34100, FLJ58394, PECAM-1 |
| E10 | Hs.81564 | NM\_002619 | PF4 | Platelet factor 4 | CXCL4, MGC138298, SCYB4 |
| E11 | Hs.252820 | NM\_002632 | PGF | Placental growth factor | D12S1900, PGFL, PLGF, PlGF-2, SHGC-10760 |
| E12 | Hs.491582 | NM\_000930 | PLAT | Plasminogen activator, tissue | DKFZp686I03148, T-PA, TPA |
| F01 | Hs.77274 | NM\_002658 | PLAU | Plasminogen activator, urokinase | ATF, UPA, URK, u-PA |
| F02 | Hs.143436 | NM\_000301 | PLG | Plasminogen | DKFZp779M0222 |
| F03 | Hs.647450 | NM\_006404 | PROCR | Protein C receptor, endothelial | CCCA, CCD41, CD201, EPCR, MGC23024, bA42O4.2 |
| F04 | Hs.302085 | NM\_000961 | PTGIS | Prostaglandin I2 (prostacyclin) synthase | CYP8, CYP8A1, MGC126858, MGC126860, PGIS, PTGI |
| F05 | Hs.196384 | NM\_000963 | PTGS2 | Prostaglandin-endoperoxide synthase 2 | COX-2, COX2, GRIPGHS, PGG, HS, PGHS-2, PHS-2, hCox-2 |
| F06 | Hs.395482 | NM\_005607 | PTK2 | PTK2 protein tyrosine kinase 2 | FADK, FAK, FAK1, FRNK, pp125FAK |
| F07 | Hs.89546 | NM\_000450 | SELE | Selectin E | CD62E, ELAM, ELAM1, ESEL, LECAM2 |
| F08 | Hs.728756 | NM\_000655 | SELL | Selectin L | CD62L, LAM1, LECAM1, LEU8, LNHR, LSEL, LYAM1, PLNHR, TQ1 |
| F09 | Hs.591014 | NM\_003006 | SELPLG | Selectin P ligand | CD162, CLA, PSGL-1, PSGL1 |
| F10 | Hs.414795 | NM\_000602 | SERPINE1 | Serpin peptidase inhibitor, clade E | PAI, PAI-1, PAI1, PLANH1 |
| F11 | Hs.443914 | NM\_000454 | SOD1 | Superoxide dismutase 1, soluble | ALS, ALS1, IPOA, SOD, hSod1, homodimer |
| F12 | Hs.68061 | NM\_021972 | SPHK1 | Sphingosine kinase 1 | SPHK |
| G01 | Hs.89640 | NM\_000459 | TEK | TEK tyrosine kinase, endothelial | CD202B, TIE-2, TIE2, VMCM, VMCM1 |
| G02 | Hs.516578 | NM\_006287 | TFPI | Tissue factor pathway inhibitor | EPI, LACI, TFI, TFPI1 |
| G03 | Hs.645227 | NM\_000660 | TGFB1 | Transforming growth factor, beta 1 | CED, DPD1, LAP, TGFB, TGFbeta |
| G04 | Hs.2030 | NM\_000361 | THBD | Thrombomodulin | AHUS6, BDCA3, CD141, THRM, TM |
| G05 | Hs.164226 | NM\_003246 | THBS1 | Thrombospondin 1 | THBS, THBS-1, TSP, TSP-1, TSP1 |
| G06 | Hs.522632 | NM\_003254 | TIMP1 | TIMP metallopeptidase inhibitor 1 | CLGI, EPA, EPO, FLJ90373, HCI, TIMP |
| G07 | Hs.241570 | NM\_000594 | TNF | Tumor necrosis factor | DIF, TNF-alpha, TNFA, TNFSF2 |
| G08 | Hs.478275 | NM\_003810 | TNFSF10 | Tumor necrosis factor (ligand) superfamily, member 10 | APO2L, CD253, TL2, TRAIL |
| G09 | Hs.592212 | NM\_001953 | TYMP | Thymidine phosphorylase | ECGF, ECGF1, MEDPS1, MNGIE, MTDPS1, PDECGF, TP, hPD-ECGF |
| G10 | Hs.109225 | NM\_001078 | VCAM1 | Vascular cell adhesion molecule 1 | CD106, DKFZp779G2333, INCAM-100, MGC99561 |
| G11 | Hs.73793 | NM\_003376 | VEGFA | Vascular endothelial growth factor A | MGC70609, MVCD1, VEGF, VPF |
| G12 | Hs.440848 | NM\_000552 | VWF | Von Willebrand factor | F8VWF, VWD |

### **Supplementary Table S2: Gene array related to cardiomyocyte phenotypes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Unigene** | **GeneBank** | **Symbol** | **Description** | **Gene Name** |
| Hs.498178 | NM\_001103 | ACTN2 | Actinin, alpha 2 | CMD1AA |
| Hs.99913 | NM\_000684 | ADRB1 | Adrenergic, beta-1-, receptor | ADRB1R, B1AR, BETA1AR, RHR |
| Hs.334347 | NM\_001824 | CKM | Creatine kinase, muscle | CKMM, M-CK |
| Hs.594952 | NM\_001927 | DES | Desmin | CMD1I, CSM1, CSM2, FLJ12025, FLJ39719, FLJ41013, FLJ41793 |
| Hs.243987 | NM\_002052 | GATA4 | GATA binding protein 4 | MGC126629 |
| Hs.388245 | NM\_021973 | HAND2 | Heart and neural crest derivatives expressed 2 | DHAND2, FLJ16260, Hed, MGC125303, MGC125304, Thing2, bHLHa26, dHand |
| Hs.95162 | NM\_000218 | KCNQ1 | Potassium voltage-gated channel, KQT-like subfamily, member 1 | ATFB1, ATFB3, FLJ26167, JLNS1, KCNA8, KCNA9, KVLQT1, Kv1.9, Kv7.1, LQT, LQT1, RWS, SQT2, WRS |
| Hs.517586 | NM\_005368 | MB | Myoglobin | MGC13548, PVALB |
| Hs.929 | NM\_000257 | MYH7 | Myosin, heavy chain 7, cardiac muscle, beta | CMD1S, CMH1, DKFZp451F047, MGC138376, MGC138378, MPD1, MYHCB, SPMD, SPMM |
| Hs.75535 | NM\_000432 | MYL2 | Myosin, light chain 2, regulatory, cardiac, slow | CMH10, DKFZp779C0562, MLC2 |
| Hs.517939 | NM\_000258 | MYL3 | Myosin, light chain 3, alkali; ventricular, skeletal, slow | CMH8, MLC1SB, MLC1V, VLC1 |
| Hs.75636 | NM\_021223 | MYL7 | Myosin, light chain 7, regulatory | MYL2A, MYLC2A |
| Hs.54473 | NM\_004387 | NKX2-5 | NK2 homeobox 5 | CHNG5, CSX, CSX1, FLJ52202, FLJ97166, FLJ97195, FLJ97197, FLJ99536, NKX2.5, NKX2E, NKX4-1 |
| Hs.75640 | NM\_006172 | NPPA | Natriuretic peptide A | ANF, ANP, ATFB6, CDD-ANF, PND |
| Hs.170839 | NM\_002667 | PLN | Phospholamban | CMD1P, PLB |
| Hs.109514 | NM\_001035 | RYR2 | Ryanodine receptor 2 (cardiac) | ARVC2, ARVD2, RYR-2, VTSIP |
| Hs.728911 | NM\_021097 | SLC8A1 | Solute carrier family 8 (sodium/calcium exchanger), member 1 | DKFZp779F0871, FLJ37694, FLJ43417, MGC119581, NCX1 |
| Hs.644596 | NM\_000363 | TNNI3 | Troponin I type 3 (cardiac) | CMD1FF, CMD2A, CMH7, MGC116817, RCM1, TNNC1, cTnI |
| Hs.533613 | NM\_000364 | TNNT2 | Troponin T type 2 (cardiac) | CMH2, CMPD2, LVNC6, MGC3889, RCM3, TnTC, cTnT |
| Hs.591847 | NM\_000662 | NAT1 | N-acetyltransferase 1 (arylamine N-acetyltransferase) | AAC1, MNAT, NAT-1, NATI |
| Hs.592355 | NM\_002046 | GAPDH | Glyceraldehyde-3-phosphate dehydrogenase | G3PD, GAPD, MGC88685 |

All gene arrays were performed in a 96-well plate format, with at least n=3 biological sample repetitions. Fold regulation (2-ΔΔ(Ct)) expression values (following interpolate calibration and normalization to house-keeping genes and plate controls) were obtained using Qiagen web-based software based on their recommended analyses pipeline. The results (**Supplementary Table S3**) indicated an initially high HAND2 spike in the cardiomyocyte arrays (day 4, 2409-fold regulation compared to plate controls) followed by gradually increased expression of endothelial cell markers at longer time points (for clarity, non-significant results are not reported).

**Supplementary Table S3: mRNA RT-qPCR fold regulation of hMSC expression for significantly up-regulated endothelial and cardiomyocyte genes**

|  |  |  |
| --- | --- | --- |
|  |  | **Fold Regulation** |
|  | **Gene** | **Day 4** | **Day 23** | **Day 30** |
| ENDOTHELIAL | APOE | 75.24 | 157.1 | 288.44 |
| CASP1 | 3.05 | 2.55 | 8.97 |
| CCL2 | 21.32 | 6.03 | 16.62 |
| CCL5 | 2.24 | 6.08 | -1.52 |
| COL18A1 | 15.97 | 1.69 | 2.68 |
| ICAM1 | 22.86 | 11.81 | 17.87 |
| IL11 | 11.3 | 1.65 | 3.59 |
| IL1b | 125.27 | 8.55 | 34.36 |
| IL6 | 14.51 | 16.98 | 17.56 |
| IL7 | 7.37 | 7.69 | 11.69 |
| KDR | 13.72 | 10.28 | 5.51 |
| MMP9 | 12.43 | 93.29 | 12.22 |
| PECAM1\* | 16.68 | 17.87 | 22.05 |
| PF4 | 6.03 | -1.3 | 4.81 |
| PTGS2 | 21.6 | 1.42 | 1.51 |
| SLC8A1 | -5.84 | -3.58 | -5.39 |
| THBD | 6.34 | 15.62 | 3.39 |
| TIMP1 | 5.68 | 2.7 | 6.93 |
| TNSF10 | -1.09 | 8.1 | 6.43 |
| TYMP | 6.38 | 2.78 | 3.88 |
| VCAM1 | 42.79 | 96.45 | 177.09 |
| VEGFA | 5.53 | 5.24 | 2.93 |
| vFW | 5.44 | 3.63 | 3.97 |
| CARDIAC | DES | 29.18 | 2.92 | 4.83 |
| HAND2\* | 2409.29 | 9.51 | 1.64 |
| MYL3 | 1.31 | 1.84 | 10.64 |

\*Red highlighted gene expression was also verified at the protein level in Figure 1.

**CRISPR-Cas9 editing**

CRISPR-Cas9 gene editing was outsourced to the Animal Gene Editing Laboratory (AGEL) at the Biological Resource Center (BRC) at the agency for Science Technology & Research (A\*Star), Singapore. Briefly, prior to gene editing, hMSCs were screened for diploidy and were found to be suitable for transfection. Cells were transfected (electroporation without any liposomal aids) with a pCEP4-PACdTKmchy-HAND2/HAND2AS1\_1&2\_2 episomal vector (**Supplementary Figure S1**) along with two flanking guide RNA (gRNA) vectors (under a human U6 promoter) and a Cas9 vector under a CMV constitutive promoter. Successful Cas9-based editing of the flanked region activated a downstream puromycin antibiotic resistance selection gene that, following editing, became close enough to the upstream CMV promoter. Following electro-nucleofection, cells were allowed to recuperate for two days prior to antibiotic selection.

**Characterization of CRISPR-Cas9 edited cells**

Following selection, two types of cells appeared on the plate:

1. Type 1 cells were non-dividing, hypertrophic, had a non-hMSC characteristic, and many seemed to have a neurological-like phenotype, although this was not further verified.
2. Type 2 cells – approximately 2-3 colonies in total appeared to have a wild type-like phenotype.

Given the non-dividing nature of the type I cells, we could not subculture these plates. On day 20, we decided to separately pick up most of the colony-forming cells while maintaining clonal identity using very short and low-volume localized trypsinization. These colonies (clones 1-3) were further sub-cultured and subsequently PCR amplified and analyzed using DNA gel electrophoresis (**Figure 1g**, clone 1, clone 2 and clone 3) compared to PCR amplifications of the original wild type (MIX WT) and the mixture of the remaining cells on the plate containing some of the colony-forming cells but mostly senescent type 2 cells (Mix MUT).

Sanger sequencing performed on the amplified PCR products confirmed that the colony clones (type I cells) were of the WT phenotype (data not shown), while MIX MUT cells comprising mostly the senescent-like cell population were positive for HAND2-HAND2-AS1 deletion (**Supplementary Fig. S2**).

**Supplementary Fig. S1: CRISPR-Cas9 HAND2-HAND2-AS1 knockout plasmid map**

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**Supplementary Fig. S2: Sanger sequencing and alignment results**. PCR-amplified Mix-MUT cDNA was Sanger sequenced and aligned to the original locus sequence (top). Four independent Sanger sequencing results are shown in subsequent rows. Given the length of the sequence, it appears in separate images in the next four pages.

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