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**Figure S1. ERRα silencing reduces mitochondrial proton gradient in wild-type H9c2 cardiomyocytes.** (A) Left panel, representative Western blots of ERRα protein in in wild-type H9c2 (untransfected for LANCL overexpression) silenced for ERRα, compared with control cells, transfected with the scrambled sequences (SCR); upper right panel, ERRα mRNA levels in SHERRα cells relative to SCR; lower right panel, densitometric quantitation of the ERRα protein in SHERRα cells relative to SCR. Values are normalized against vinculin, as housekeeping protein. \*\*p<0.01 relative to SCR control cells by unpaired t-test. Data shown are the mean ± SD of 3 experiments per group, with each value calculated in triplicate. (B) SHERRα cells were loaded with the ∆Ψ-sensitive ratiometric fluorescent dye JC-1, a higher ΔΨ resulting in a higher red/green fluorescence ratio. Left panel, representative confocal microscopy images; right panel, red/green fluorescence ratio calculated in at least 3 microscopic fields (scale bar: 20µm) for each experiment. \*\*p<0.01 relative to SCR cells by unpaired t-test.



**Figure S2.** **What triggers ERRα and LANCL1/2 activation in the “stressed” heart?** Nitric oxide (NO), locally produced in the heart not only by cardiomyocytes but also by endothelial cells and erythrocytes, plays a central role in allowing adaptation of cardiomyocyte metabolism, contraction and electrical conduction to changing physiological conditions [54-56]. Here we observed that LANCL1/2 and ERRα are linked in a feed-forward mechanism of reciprocal transcriptional activation (Figure 5A). In addition, both ERRα [57] and LANCL1/2 [5] activate NO-generating eNOS. Here, we also show that NO is required for LANCL1/2-induced stimulation of ERRα transcription in H9c2 cells (Figure 5A). Finally, ABA release is stimulated by hypoxia in H9c2 and ABA in turn stimulates NO production and LANCL1/2-expression [5]. Does NO activate ABA release from H9c2 (dotted line)? Is NO necessary for ERRa-mediated LANCL1/2 transcriptional activation (dotted line)? These questions are still awaiting an answer. In any case, NO and ABA, via LANCL1/2 and ERRα mediate all transcriptional and functional responses of H9c2 described in this study.

Table S1. Primer sequences used to amplify rat target genes.

|  |  |  |  |
| --- | --- | --- | --- |
| Rat genes | Accession N. | Forward Primer 5’-3’ | Reverse Primer 5’-3’ |
| Hprt1 | NM\_012583 | TTGGTCAAGCAGTACAGCCC | TGGCCTGTATCCAACACTTCG |
| Lancl1 | NM\_053723 | TCTTGCTCCTCATCCTGCTCATC | CACTGTACTCGCCGAAGGTCTC |
| Lancl2 | NM\_001014187 | GGTGCCACGGTGCTCCAG | CCTCGCTGCCAAATCACATCAC |
| Slc2a1 | NM\_138827 | GACCCTGCACCTCATTGGT | CTCAGATAGGACATCCAGGGC |
| Slc2a4 | NM\_012751 | CCAGCCTACCGCCACCATAG | TTCCAGCAGCAGCAGAGC |
| Pfk1 | NM\_031715 | AGTTGGTATCTTCACGGGCG | CATAGACACGCTCTCCCACG |
| PK | M24359 | CCAAGAGAACGAGCTACCCC | TGGAGCCCCACTTAAAGCAG |
| Gapdh | AF106860 | ATGACTCTACCCACGGCAAG | CTGGAAGATGGTGATGGGTT |
| Pdha1 | NM\_001004072 | GATGGAGCTAAAGGCGGATCA | TCCGTAGGGTTTATGCCAGC |
| Ucp3 | NM\_013167 | CCCCCTACACTGTATGCTGA | TTCCAGGATCCCAGACGCA |
| Mt-nd1 | KJ530565 | CCACGCTTCCGTTACGATCA | GTATGGTGGTACTCCCGCTG |
| ANT1 | D12770 | TGGATGATTGCGCAGAGTGT | AATATCAGCCCCTTTCCGGC |
| Cpt1b | NM\_013200 | TGTCTACCTCCGAAGCAGGA | TGAACGGCATTGCCTAGACG |
| Acads | NM\_022512 | GAGAAGGAGTTGGTCCCCATT | CCGAGCTCACCCATCTTCTTA |
| Esrra | NM\_001008511 | CCCTGACAGTCCAAAGGGTT | CATCCTCCTCCTCCTTGTGC |
| KCNK2 | AF385402 | CAGGTGGGTCGGACATTGAA | CCCGTAGCCAGTCTCCAATC |
| Cacna1c | NM\_012517 | CTGCCCTATGTGGCCCTTTT | TCTGTGGTGTCATTCAGGGC |
| Scn1b | NM\_001271045 | CTGCTGGCTCTCGTGGTG | CCATACACTGCCTCGGTCTC |
| Ccnd1 | NM\_171992 | CTACCGCACAACGCACTTTC | CAGGCTTGACTCCAGAAGGG |
| Ccnd2 | NM\_022267 | CCAAGATCACCCACACCGAT | TTGTGCTGCTCTTGACGGAA |
| Ccnd3 | NM\_012766 | AACCACGCCCCTGACTATTG | CACTTGAGCTTCCCCAGGAC |
| Ccne1 | NM\_001100821 | GACAAGACTGTGAAAAGCCAGG | GATGAAAGAGCAGGGGTCCA |
| Ccna2 | NM\_053702 | CTCTTTACCCGGAGCCAGAAA | ACATTCACTGGCTTTTCGTCTT |
| Cdk2 | NM\_199501 | GGCTGCATCTTTGCCGAAAT | CTGGCCAAACCACCTCATCT |
| cdk4 | L11007 | GTACAAAGCCCGAGATCCCC | ACCTCACGAACTGTGCTGAC |
| E2f4 | NM\_001271345 | TTGAGCCCATCAAGGCAGAC | CGGAGCTCATGCACTCTCTT |
| Actc1 | NM\_019183 | GAGCTGTCTTCCCGTCCATC | TTGCTCTGGGCTTCATCACC |
| Tubb2a | NM\_001109119 | ACTTGCAGCTGGAGAGGATCA | CACTAGGATGGCCCGAGGTA |
| Ctnnb1 | NM\_053357 | TACGAGCACATCAGGACACC | TGGAGAGCTCCAGTACACCC |
| Myh7 | NM\_017240 | CAGCAGTTGGATGAGCGACT | GCTCATCCTCAATCCTGGCAT |
| Gja1 | NM\_012567 | TTACAACAAGCAAGCCAGCG | GGGAGTTGGAGATGGTGCTT |
| Prkaa2 | NM\_019142 | AGAAGCAGAAGCACGACGG | GAAGGTGCCGACGCCC |
| Ppargc1a | NM\_031347 | GCACACATCGCAATTCTCCC | CTCTGCGGTATTCGTCCCTC |
| Sirt1 | NM\_001372090 | CAGTGTCATGGTTCCTTTGC | CACCGAGGAACTACCTGAT |
| Nampt | NM\_177928 | TCGGTTCTGGTGGAGGTTTGCTAC | TCCCTGCTGGCGTCCTATGTAAAG |
| Nos3 | NM\_021838 | AGGCCTTGGTATTGGTGGTG | TAGGGGCCCGACATTTCCAT |
| Fgf21 | NM\_130752 | CACACCGCAGTCCAGAAAGT | CCTAGAGGCTTTGACACCCA |

Table S2. Primary and secondary antibodies used for Western blot.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primary Antibody** | **Host** | **Concentrations** | **Manufacturer** |
| Anti-LANCL1 | Rabbit | 1:250 | Novus Biologicals |
| Anti-LANCL2 | Mouse | 1:1000 | Reference [58] |
| Anti-ERRα | Mouse | 1:200 | Santa Cruz Biotechnology Inc., California |
| Anti-vinculin | Rabbit | 1:1000 | Cell Signaling Technology, Danvers, MA |
| **Secondary Antibody** | **Concentrations** | **Manufacturer** |
| Anti-Mouse | 1:2000 | Santa Cruz Biotechnology Inc., California |
| Anti-Rabbit | 1:1000 | Santa Cruz Biotechnology Inc., California |