

Supplementary data

LIM-only protein FHL2 is a Positive Regulator of Liver X Receptors in Smooth Muscle Cells involved in Lipid Homeostasis

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Fig. S1. The top 30 canonical pathways associated with differentially expressed genes comparing FHL2-KO with WT SMCs as identified by IPA. A. The line graph represents the ratio of the number of differentially expressed genes from the dataset over the number of genes present in each canonical pathway. The pathways are ranked from highest to lowest degree of association between genes from the dataset with the pathways by the P-value, calculated by a right tailed Fisher Exact Test. The bars represent the $-\log(P\text{-value})$ for each pathway and the threshold represents $P=0.01$. Pathways concerning cholesterol metabolism are underlined and LXR-RXR pathways are indicated in bold. **B.** Generation of networks of the top canonical pathways with at least 4 shared dataset molecules, resulted in two networks; cholesterol synthesis and metabolism and signalling

Fig. S2. Network analysis for the differential gene expression of WT and FHL2-KO SMCs revealed that lipid metabolism is affected by FHL2 deficiency. Network #15 (Table 1 and Figure 2B) is shown including its correlation with the IPA canonical pathways ‘metabolism of cholesterol’ and ‘efflux of cholesterol’ (A), ‘LPS/IL-1 mediated inhibition RXR function and LXR/RXR activation’ (B) and with the IPA function ‘synthesis of cholesterol’ (C) is indicated by the blue lines. Interactions between molecules are shown as explained in the legend. Red symbols indicate that genes are up-regulated in FHL2-KO SMCs as compared to WT cells, green symbols indicate down regulation of the genes and genes with unchanged expression are depicted in white.

Figure S2

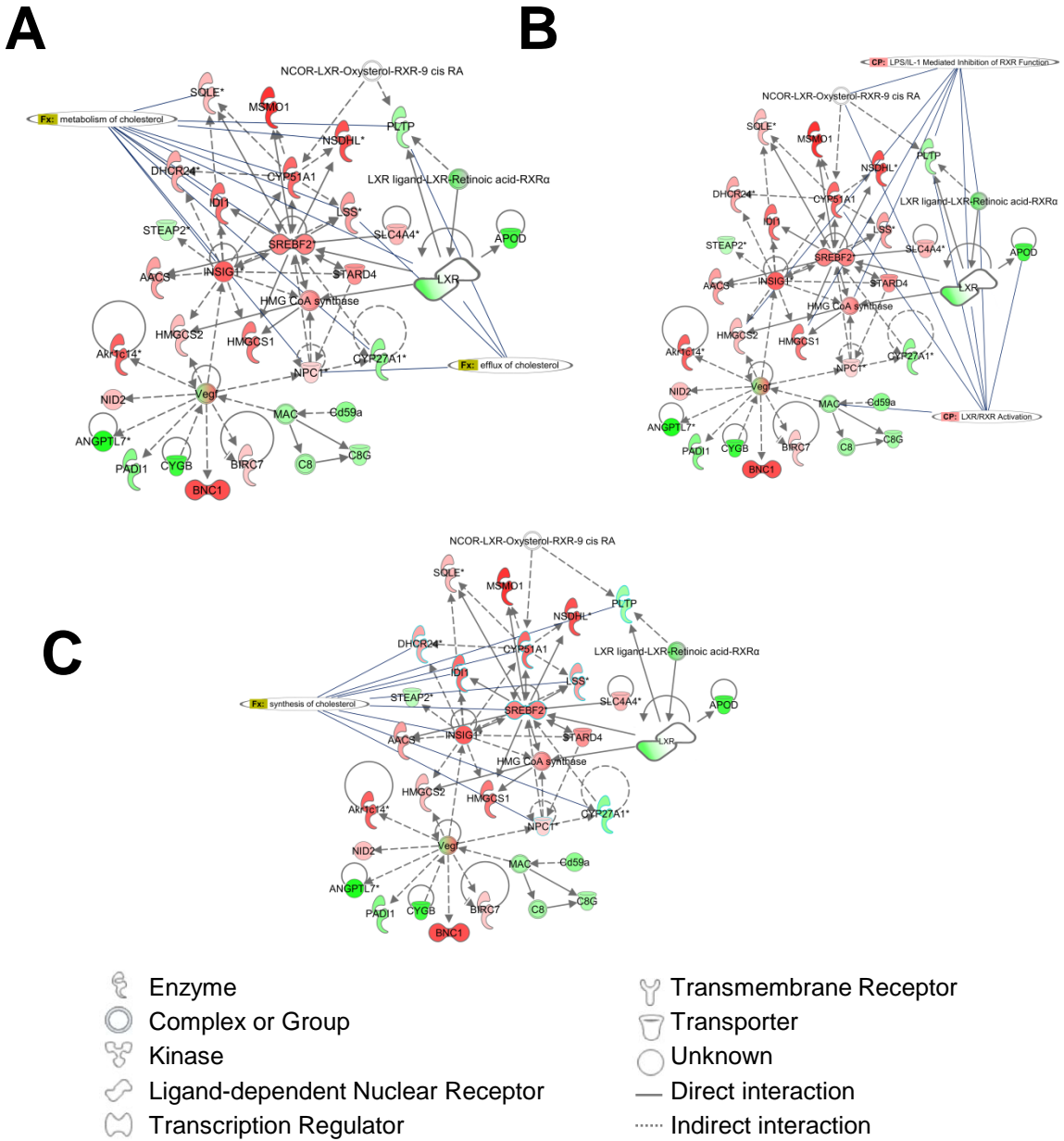


Table S1. Top 30 up- and down-regulated genes in FHL2-KO vs WT SMCs

Gene Symbol	Log2 Fold Change (KO/WT)	P-value	Adjusted P-value	Entrez Gene ID
Up-regulated genes				
<i>Upk1b</i>	6.6832	1.17E-13	2.88E-09	22268
<i>Moxd1</i>	5.4093	7.26E-12	4.46E-08	59012
<i>Atp1b1</i>	4.9782	1.02E-05	1.01E-03	11931
<i>Cib3</i>	4.8205	3.08E-09	2.44E-06	234421
<i>Ppl</i>	4.7222	1.09E-10	1.77E-07	19041
<i>O3far1</i>	4.5867	9.28E-11	1.63E-07	107221
<i>Upk3b</i>	4.5017	1.65E-06	2.64E-04	100647
<i>Gjb3</i>	4.4790	1.92E-08	8.56E-06	14620
<i>Pbp2</i>	4.4706	8.64E-07	1.62E-04	76400
<i>Il33</i>	4.4289	4.49E-09	3.24E-06	77125
<i>Akap5</i>	4.3442	5.75E-10	6.14E-07	238276
<i>Ezr</i>	4.1641	8.30E-09	4.74E-06	22350
<i>Akr1c18</i>	4.1503	1.58E-07	4.75E-05	105349
<i>Actg2</i>	4.1066	5.01E-06	5.86E-04	11468
<i>Aldh3a1</i>	4.0791	2.64E-11	9.26E-08	11670
<i>Fam101a</i>	4.0710	7.25E-09	4.57E-06	73121
<i>Msln</i>	4.0281	5.99E-11	1.34E-07	56047
<i>Hopx</i>	3.9277	1.30E-06	2.18E-04	74318
<i>Mtap2</i>	3.8370	1.50E-09	1.36E-06	17756
<i>Cxadr</i>	3.8150	3.71E-09	2.76E-06	13052
<i>Pkhd11l</i>	3.5932	2.13E-06	3.16E-04	192190
<i>Kif23</i>	3.5642	1.70E-06	2.68E-04	71819
<i>Igf1</i>	3.5228	9.09E-09	4.96E-06	16000

<i>Smpd3</i>	3.5108	5.98E-09	3.97E-06	58994
<i>Mmp17</i>	3.4393	1.62E-07	4.79E-05	23948
<i>Stc2</i>	3.3409	1.46E-05	1.32E-03	3.3409
<i>Adam23</i>	3.3187	6.20E-09	4.01E-06	3.3187
<i>Foxa1</i>	3.3083	3.68E-06	4.58E-04	3.3083
<i>Podxl</i>	3.2622	5.52E-04	2.10E-02	3.2622
<i>Pbk</i>	3.2555	6.25E-07	1.31E-04	3.2555

Down-regulated genes

<i>Gpr39</i>	-3.6863	2.63E-09	2.22E-06	71111
<i>Egfl7</i>	-3.7688	1.08E-08	5.44E-06	353156
<i>Gpnm3</i>	-3.8035	4.88E-10	5.45E-07	93695
<i>Col28a1</i>	-3.8379	1.81E-07	5.22E-05	213945
<i>ApoE</i>	-3.8533	8.58E-08	3.05E-05	11816
<i>Klhl6</i>	-3.8820	2.77E-10	3.78E-07	11816
<i>Unc45b</i>	-3.9217	5.48E-07	1.17E-04	239743
<i>Serpina3n</i>	-3.9307	1.16E-10	1.77E-07	217012
<i>Tcfap2b</i>	-3.9672	3.27E-06	4.27E-04	20716
<i>Isl1</i>	-3.9676	3.54E-06	4.46E-04	21419
<i>Meg3</i>	-4.0516	1.74E-08	8.21E-06	16392
<i>Megf10</i>	-4.2217	1.42E-09	1.34E-06	70417
<i>Ephx2</i>	-4.2252	7.24E-11	1.44E-07	13850
<i>Bmx</i>	-4.3916	6.58E-07	1.37E-04	12169
<i>Stk32b</i>	-4.4717	1.17E-09	1.19E-06	64293
<i>Ramp2</i>	-4.6254	2.89E-08	1.20E-05	54409
<i>Comp</i>	-4.6268	3.62E-09	2.76E-06	12845
<i>Fhl2</i>	-4.6325	1.42E-11	5.81E-08	14200
<i>Dpysl4</i>	-4.6587	2.77E-08	1.19E-05	26757
<i>Lpl</i>	-4.6739	1.40E-09	1.34E-06	16956
<i>Xist</i>	-4.8129	5.90E-09	3.97E-06	213742

<i>Esm1</i>	-4.9070	2.68E-10	3.78E-07	71690
<i>Xlr3b</i>	-4.9737	1.27E-11	5.81E-08	574437
<i>Gdf10</i>	-5.2468	1.00E-08	5.36E-06	14560
<i>Mia1</i>	-5.2740	3.95E-11	1.21E-07	12587
<i>Dlk1</i>	-5.5971	4.12E-10	5.12E-07	13386
<i>Spp1</i>	-5.6046	9.93E-08	3.48E-05	20750
<i>Emcn</i>	-5.6530	7.65E-11	1.44E-07	59308
<i>Scg2</i>	-5.8287	5.47E-11	1.34E-07	20254
<i>Gpc3</i>	-8.5022	3.38E-12	2.86E-08	14734

Table S2. Upstream Regulators

Transcription Factor	* Activation Z-Score	** P-Value of Overlap
SNAI1	-2.745	5.61E-02
SOX9	-2.737	4.02E-02
ETV5	-2.607	9.63E-04
KMT2A	-2.566	7.33E-05
TCF3	-2.518	1.62E-03
CDKN2A	-2.329	6.80E-05
NUPR1	-2.323	3.45E-06
ATF3	-2.279	5.12E-02
TP53	-2.253	3.07E-26
SIX5	-2.219	8.89E-02
CITED2	-2.209	5.55E-04
LXR	-2.189	5.74E-03
BRCA1	-2.068	2.29E-05
MYOD1	-2.044	2.18E-02
MTPN	2.011	2.92E-03
MKL1	2.037	3.44E-02
GATA1	2.102	2.60E-02
TRIM24	2.183	9.59E-03
MAX	2.213	7.61E-02
HDAC4	2.216	4.88E-01
KLF4	2.293	8.90E-03

NKX2-3	2.340	2.50E-09
NFE2L2	2.353	1.51E-03
SRF	2.495	2.96E-04
CREB	2.611	6.15E-02
MYC	2.892	7.31E-14
TBX2	3.081	2.85E-06
SREBF2	3.336	2.20E-04

*The bias-corrected z-score is used to infer the activation states of transcriptional regulators. It is calculated from the proportions of genes which are differentially regulated in an expected direction based on the known interactions between the regulator and the genes present in the Ingenuity database. Those genes with a z-score greater or less than two are considered to be either activated or inhibited, respectively

**The P-value of overlap is the calculated statistical significance of overlap between genes from the dataset and genes that are known to be regulated by the upstream regulator using a right tailed Fisher's Exact Test.

Table S3. Primers used for qRT-PCR

Gene	Forward	Reverse
mABCA1	GGTTTGGAGATGGTTATACAATAGTTGT	TTCCCGGAAACGCAAGTC
mABCG1	CCTTCCTCAGCATCATGCG	CCGATCCCAATGTGCGA
mIDOL	ATCTGCAGACCGGACAGG	AGGAGATCAACTCCACCTT
mLPL	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
mLXR α	TACAACCGGGAAGACTTTGC	TGCAGAGAAGATGCTGATGG
mLXR β	CAGGAGATTGTGGACTTTGC	TTGTAGCGTCTGGCTGTTTC
hABCA1	ATGAGGACAACAACACTACAAAGCC	GGGAAAGAGGACTAGACTCCAAA
hABCG1	ATTCAGGGACCTTTCCTATTCGG	CTCACCACTATTGAACTTCCCG