Supplemental Material to:

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Combinatorial regulation of lipoprotein lipase by microRNAs during mouse adipogenesis

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SUPPLEMENTARY MATERIAL

Mathematical model (Systems Biology Toolbox 2 format)

********** MODEL NAME

Combinatorial regulation of lipoprotein lipase by microRNAs during mouse adipogenesis

********** MODEL NOTES

Systems Biology Toolbox 2 model by:
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Parameter set: Best fit of time course 2
Initial conditions: Steady state values for these parameters with Xc=Rosi=0 and Xm27=Xm29=1

********** MODEL STATES

d/dt(Cebpa) = r_1 + r_3 + r_10 - r_15
\[
\begin{align*}
\frac{d}{dt}(\text{CEBPA}) &= r_7 - r_{16} \\
\frac{d}{dt}(\text{Lpl27}) &= \text{ratio} \cdot r_{14} - r_{17} \\
\frac{d}{dt}(\text{Lpl29}) &= \text{ratio} \cdot r_{13} - r_{18} \\
\frac{d}{dt}(\text{Lpl}) &= r_{2} + r_{11} - r_{13} - r_{14} - r_{21} \\
\frac{d}{dt}(\text{MIR27a}) &= r_{5} - \text{ratio} \cdot r_{12} - \text{ratio} \cdot r_{14} - r_{19} \\
\frac{d}{dt}(\text{MIR29a}) &= r_{6} - \text{ratio} \cdot r_{13} - r_{20} \\
\frac{d}{dt}(\text{Pparg27}) &= \text{ratio} \cdot r_{12} - r_{24} \\
\frac{d}{dt}(\text{Pparg}) &= r_{4} + r_{8} - r_{12} - r_{22} \\
\frac{d}{dt}(\text{PAR}) &= r_{9} - r_{23}
\end{align*}
\]
acbasal = 0.17165
apbasal = 0.059056
ratioPPAR27 = 0.5434
cLPL27 = 0.0035711
ratioLPL29 = 0.84611
eCEBPA = 3.8376
eCEBPAP = 8.0636
ePPAR = 4.9943
eCPPARP = 40.529
eLPPARP = 21.702
dCEBPA = 11.94
dPPAR = 3.9995
dCEBPAP = 16.248
dM27 = 0.38249
dM29 = 0.16052
dLPL = 1.37
dPPARP = 9.9901
kPPARligint= 0.0024599
Xm27_D0 = 0.50092
ratio= 0.009562
Xc = 0
Xm27 = 1
Xm29 = 1
Rosi = 0
ratio2729=0.543
**MODEL VARIABLES**

meas27 = MIR27a + Lpl27 + Pparg27
meas29 = MIR29a + Lpl29

**MODEL REACTIONS**

r_1 = ac*Xc
r_2 = al
r_3 = acbasal
r_4 = apbasal
r_5 = dM27*Xm27*ratio2729
r_6 = dM29*Xm29
r_7 = eCEBPA*Cebpa
r_8 = eCEBPAP*CEBPA
r_9 = ePPAR*Pparg
r_10 = eCPPARP*PPAR*(kPPARligint+Rosi)
r_11 = eLPPARP*PPAR*(kPPARligint+Rosi)

r_12 = ratioPPAR27*cLPL27*MIR27a*Pparg
r_13 = ratioLPL29*cLPL27*MIR29a*Lpl
r_14 = cLPL27*MIR27a*Lpl
r_15 = dCEBPA*Cebpa
r_16 = dCEBPAP*CEBPA
r_17 = dM27*Lpl27
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\[ r_{18} = dM29*Lpl29 \]
\[ r_{19} = dM27*MIR27a \]
\[ r_{20} = dM29*MIR29a \]
\[ r_{21} = dLPL*Lpl \]
\[ r_{22} = dPPAR*Pparg \]
\[ r_{23} = dPPARP*PPAR \]
\[ r_{24} = dM27*Pparg27 \]

********** MODEL FUNCTIONS

********** MODEL EVENTS

********** MODEL MATLAB FUNCTIONS
**SUPPLEMENTARY TABLES**

**Table S1: siRNA sequences.** The sequences of siRNAs used in this study are provided.

<table>
<thead>
<tr>
<th>siRNA</th>
<th>Sequence (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>siPparg-1</td>
<td>CCAUCCGAUUGAAGCUUAU</td>
</tr>
<tr>
<td>siPparg-2</td>
<td>CAACAGGCUUCAUGAAGAA</td>
</tr>
<tr>
<td>siPparg-3</td>
<td>GUUGAUUUCUCCAGCAUUU</td>
</tr>
<tr>
<td>siControl</td>
<td>UGCGCUACGAUCGACGAUG</td>
</tr>
</tbody>
</table>

**Table S2: Primer pairs for RT-qPCR.** The sequences of primer pairs used are provided.

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Sequence (5’ - 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cebpα</td>
<td>GAGCTGAGTGAGGCTTCATTCT TGGGAGGCAGACGAAAAAAC</td>
</tr>
<tr>
<td>Lpl</td>
<td>GACTCTGTGTCTAATGGCCACTTCA CCCGTACCCTCCATCCAT</td>
</tr>
<tr>
<td>Pparg</td>
<td>CACAAGAGCTGACCCAATGTT GATCGCACTTTGGATATCTTGGA</td>
</tr>
<tr>
<td>Rpl13a</td>
<td>TGGTCCCTGCTGCTCTCTCA CCCAGGTAAGCAAACTTTCT</td>
</tr>
</tbody>
</table>
Table S3: Details on parameters of the mathematical model. Parameter names, the corresponding reactions and a short description are given in columns A-C. Lower and upper bounds (columns D-E) were applied during the parameter estimation (see Materials and Methods). The model was fitted to the three differentiation time courses separately. Mean and relative standard deviation of the selected good fits per differentiation are given in columns G-N. The parameter values of the best obtained fit per differentiation are given in columns P-R. The simulation and prediction figures were obtained by simulating all obtained good parameter sets and taking the median +/- 68% range (see Material and Methods).
**Supplementary Figure S1.** Changes in the levels of histone modifications H3K4me3 and H3K27ac in the vicinity of miR-27 and miR-29 family clusters. Enrichment of histone H3K4 trimethylation and H3K27 acetylation have been shown to correlate closely with proximal and/or distal active promoter regions, respectively. Presented dataset (GEO GSE20752) shows the dynamic development of modification patterns over 7 days of 3T3-L1 cell line differentiation for (A) miR-27a (miR-23a~27a~24-2 cluster), (B) miR-27b (miR-23b~27b~24-1 cluster), (C) miR-29a (miR-29a/b-1 cluster) and (D) miR-29b (miR-29c/b-2 cluster). Track height is set identically to 100 on all panels. Modified from Integrated Genomics Viewer.
Supplementary Figure S2. Oil Red O staining of lipids in D0 (A) and D8 (B) differentiated 3T3-L1 cells was used to control the differentiation of the adipocytes. Representative images after Oil Red O stainings showing that D0 cells contain no or very little lipids while D8 cells have high accumulation of lipids in most cells, indicating efficient differentiation. Presented images correspond to the experiments in Supplementary Figure S5.
Supplementary Figure S3. In silico model predictions for target mRNA level changes in response to miRNA perturbations during Differentiation 2. The fitted model (A) predicts for the target mRNAs, especially Lpl, a stronger and faster upregulation when miRNA-target complexes are not forming (B); weaker and delayed mRNA upregulation when miRNA levels remain at Day 0 levels (C); up to 80% reduction in Lpl upregulation when the miRNAs are two-fold over-expressed at differentiation start (D). Black dotted line represents measured mRNA levels and the red dashed line represents the median of all iterations of the model fit within an optimal cost threshold of 1.33-fold of the best obtained fit, respectively the median of the predictions obtained by using these selected model fits, with red fading up to +/-68% of confidence levels. Measured mRNA expression values are normalized to highest mRNA data point and measured miRNA expression values are normalized to highest miRNA data point. All axes and data points correspond directly to measured cDNA ratios. Confidence intervals are 68% for shown fits.
**Cebpa**

**Lpl**

**Pparg**

**miR-27a**

**miR-29a**

**Supplementary Figure S4.** In silico model predictions for target mRNA level changes in response to miRNA perturbations during Differentiation 3. See figure legend S3 for more details.
Supplementary Figure S5. Impact of transient overexpression of individual miRNAs in pre-adipocytes on Lpl expression during 3T3-L1 adipocyte differentiation. The level of Lpl mRNA was quantified with gene specific primers during induced adipogenesis of 8 days in the mouse 3T3-L1 cell line following either a transient transfection of 25 nM siControl, miR-27a mimic, miR-29a mimic or no transfection. Measured expression values were normalized to Rpl13a mRNA and presented as relative to D0 that is set to 1. The data indicate the mean expression values of three independent experiments and the error bars represent SEM.