

Supplemental Material to:

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**Combinatorial regulation of lipoprotein lipase by
microRNAs during mouse adipogenesis**

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TEXT

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 $X_c=Rosi=0$ and $X_{m27}=X_{m29}=1$

***** MODEL STATES

$d/dt(Cebpa) = r_1 + r_3 + r_{10} - r_{15}$

$$d/dt(\text{CEBPA}) = r_7 - r_{16}$$

$$d/dt(\text{Lpl27}) = \text{ratio} * r_{14} - r_{17}$$

$$d/dt(\text{Lpl29}) = \text{ratio} * r_{13} - r_{18}$$

$$d/dt(\text{Lpl}) = r_2 + r_{11} - r_{13} - r_{14} - r_{21}$$

$$d/dt(\text{MIR27a}) = r_5 - \text{ratio} * r_{12} - \text{ratio} * r_{14} - r_{19}$$

$$d/dt(\text{MIR29a}) = r_6 - \text{ratio} * r_{13} - r_{20}$$

$$d/dt(\text{Pparg27}) = \text{ratio} * r_{12} - r_{24}$$

$$d/dt(\text{Pparg}) = r_4 + r_8 - r_{12} - r_{22}$$

$$d/dt(\text{PPAR}) = r_9 - r_{23}$$

$$\text{Cebpa}(0) = 0.0145$$

$$\text{CEBPA}(0) = 0.0034$$

$$\text{Lpl27}(0) = 0$$

$$\text{Lpl29}(0) = 0$$

$$\text{Lpl}(0) = 0.0657$$

$$\text{MIR27a}(0) = 0.543$$

$$\text{MIR29a}(0) = 1$$

$$\text{Pparg27}(0) = 0$$

$$\text{Pparg}(0) = 0.0216$$

$$\text{PPAR}(0) = 0.0108$$

***** MODEL PARAMETERS

$$\text{ac} = 0.00032746$$

$$\text{al} = 0.089728$$

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

$$\text{meas27} = \text{MIR27a} + \text{Lpl27} + \text{Pparg27}$$

$$\text{meas29} = \text{MIR29a} + \text{Lpl29}$$

***** MODEL REACTIONS

$$r_1 = ac * Xc$$

$$r_2 = al$$

$$r_3 = ac_{\text{basal}}$$

$$r_4 = ap_{\text{basal}}$$

$$r_5 = dM27 * X_{m27} * \text{ratio2729}$$

$$r_6 = dM29 * X_{m29}$$

$$r_7 = eCEBPA * Cebpa$$

$$r_8 = eCEBPAP * CEBPA$$

$$r_9 = ePPAR * Pparg$$

$$r_{10} = eCPPARP * PPAR * (kPPAR_{\text{ligint}} + \text{Rosi})$$

$$r_{11} = eLPPARP * PPAR * (kPPAR_{\text{ligint}} + \text{Rosi})$$

$$r_{12} = \text{ratioPPAR27} * cLPL27 * \text{MIR27a} * \text{Pparg}$$

$$r_{13} = \text{ratioLPL29} * cLPL27 * \text{MIR29a} * \text{Lpl}$$

$$r_{14} = cLPL27 * \text{MIR27a} * \text{Lpl}$$

$$r_{15} = dCEBPA * Cebpa$$

$$r_{16} = dCEBPAP * CEBPA$$

$$r_{17} = dM27 * \text{Lpl27}$$

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.

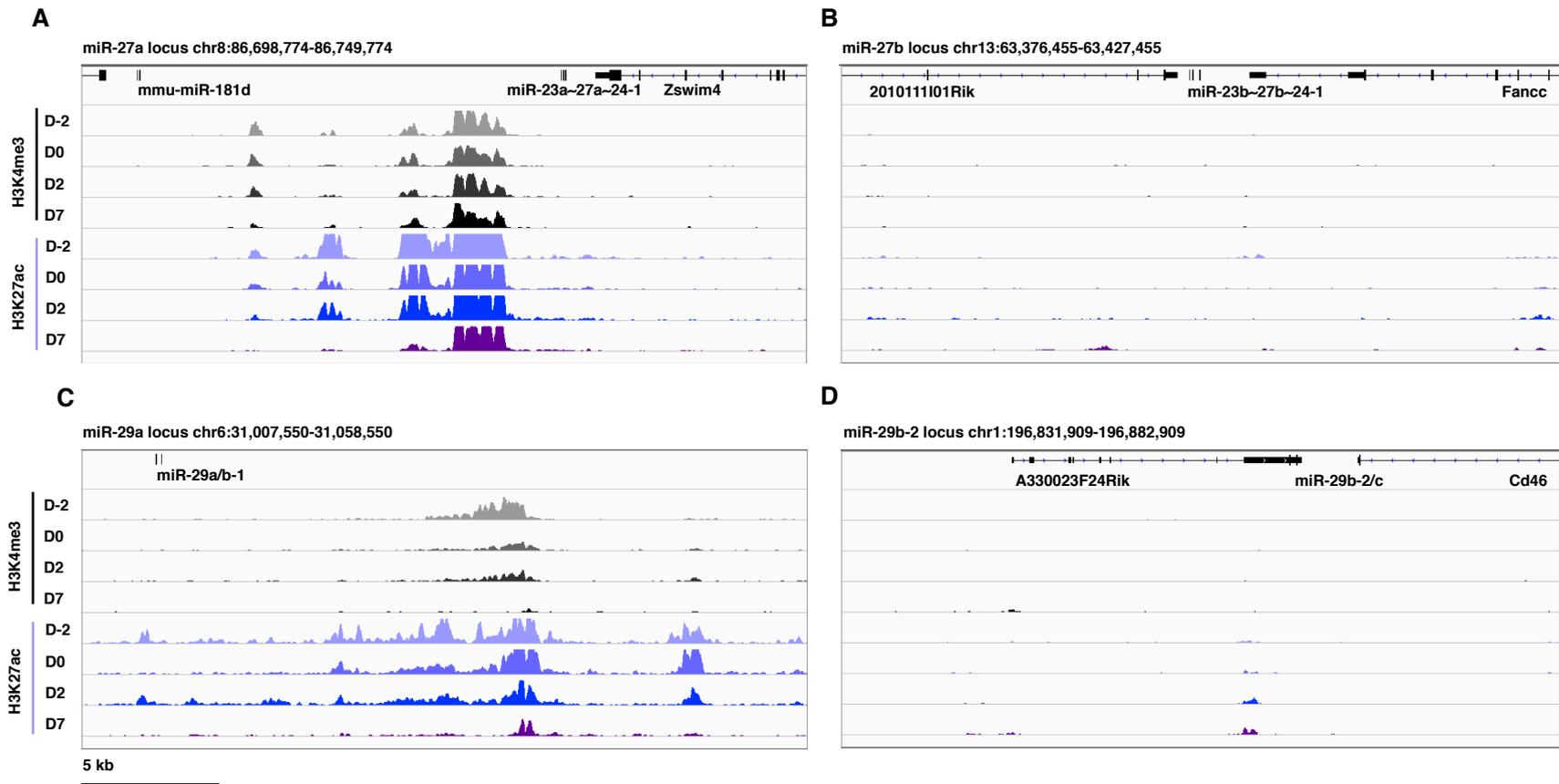
| siRNA | Sequence (5' – 3') |
|------------------|----------------------|
| <i>siPparg-1</i> | CCAUCCGAUUGAAGCUUUAU |
| <i>siPparg-2</i> | CAACAGGCCUCAUGAAGAA |
| <i>siPparg-3</i> | GUUGAUUUCUCCAGCAUUU |
| <i>siControl</i> | UGCVCUACGAUCGACGAUG |

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

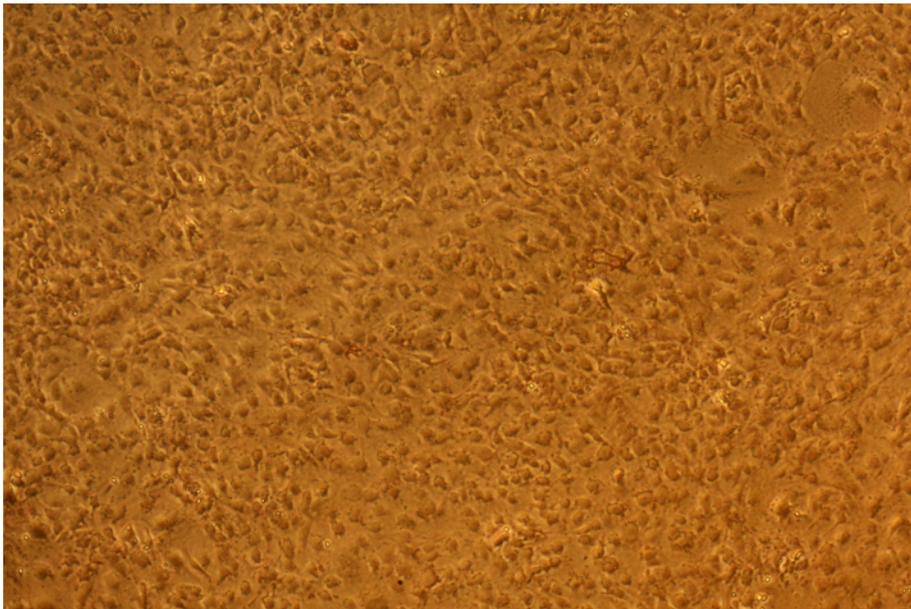
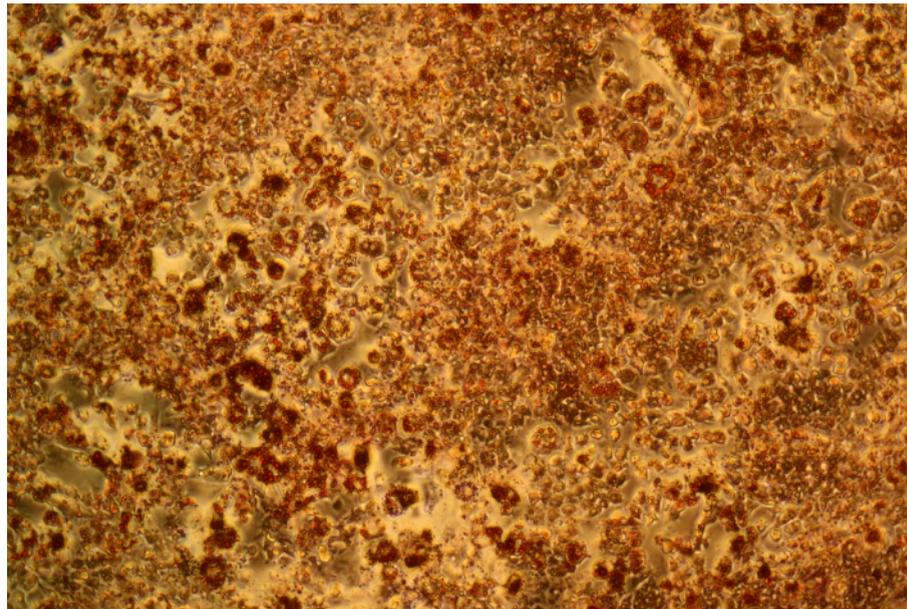
| Primer pair | Sequence (5' - 3') |
|---------------|--|
| <i>Cebpa</i> | GAGCTGAGTGAGGCTCTCATTCT TGGGAGGCAGACGAAAAAAC |
| <i>Lpl</i> | GACTCTGTGTCTAACTGCCACTTCA CCCGTTACCGTCCATCCAT |
| <i>Pparg</i> | CACAAGAGCTGACCCAATGGT GATCGCACTTTGGTATTCTTGGA |
| <i>Rpl13a</i> | TGGTCCCTGCTGCTCTCA CCCCAGGTAAGCAAACCTTCT |

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)

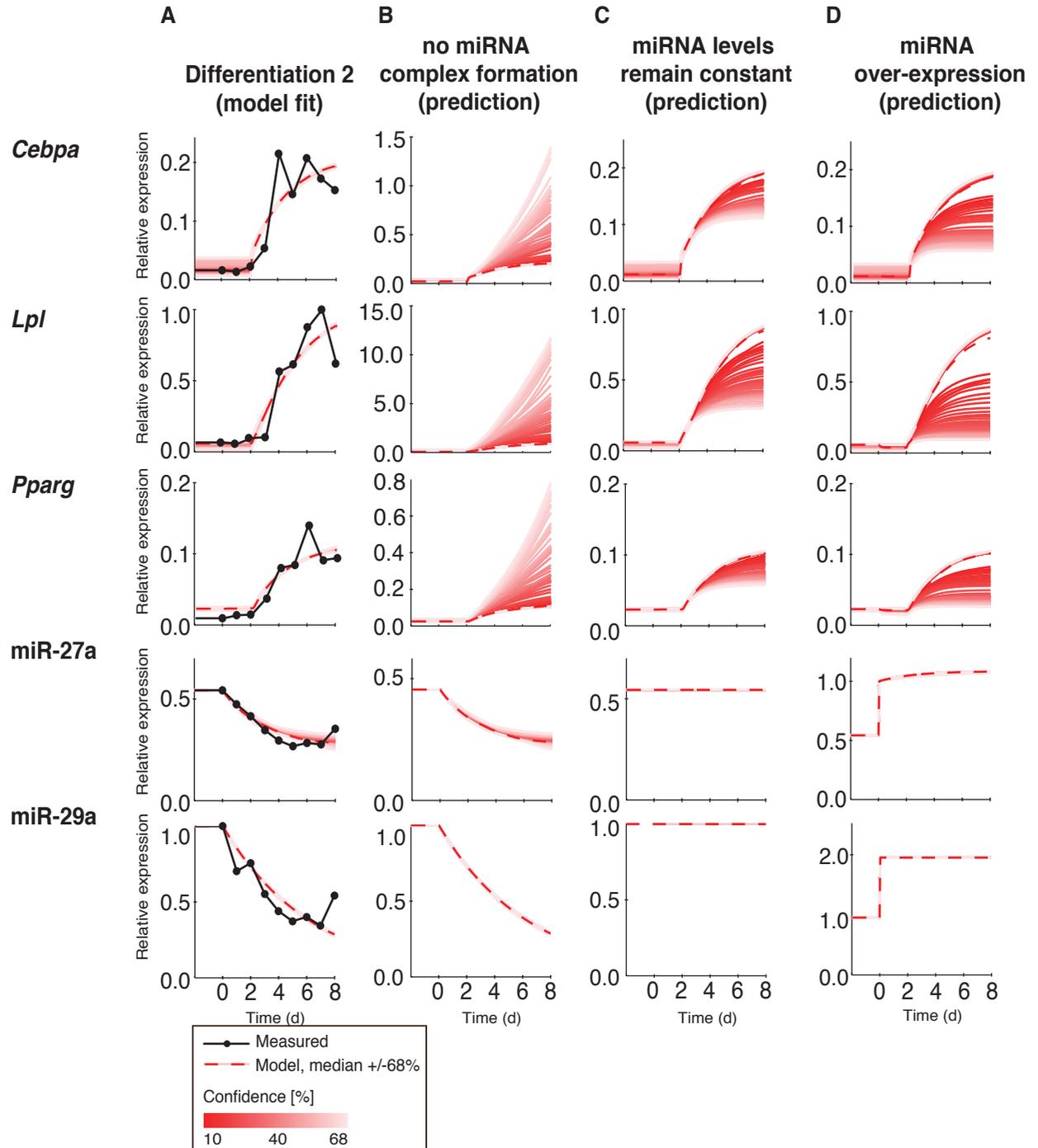
| Parameter unit: [1/d] | in reaction: | description: | lower bound | upper bound | Differentiation 1 | | Differentiation 2 | | Differentiation 3 | | Best fits: | | |
|----------------------------|-----------------------|---|-------------|-------------|-------------------|---------|-------------------|---------|-------------------|---------|---------------|---------------|---------------|
| | | | | | Mean | rel Std | Mean | rel Std | Mean | rel Std | Time course 1 | Time course 2 | Time course 3 |
| ac | r 1 | input (Xc)-dependent transcription of Cebpa | 0 | 1000 | 0.0748 | 1.9933 | 0.0026 | 2.9421 | 0.004 | 3.0207 | 0.23951 | 0.00032746 | 0.0021402 |
| al | r 2 | basal transcription of Lpl | 0 | 1000 | 0.9182 | 0.9378 | 0.147 | 1.3805 | 0.2326 | 2.2173 | 0.86591 | 0.089728 | 0.064742 |
| acbasal | r 3 | basal transcription of Cebpa | 0 | 1000 | 0.3057 | 0.8217 | 0.1362 | 1.0578 | 0.1103 | 1.2617 | 0.34772 | 0.17165 | 0.095665 |
| apbasal | r 4 | basal transcription of Pparg | 0 | 1000 | 0.161 | 0.5913 | 0.0787 | 0.7116 | 0.0835 | 0.6299 | 0.17548 | 0.059056 | 0.073514 |
| ratioPPAR27 | r 12 | ratio of miR27a Pparg complex formation to miR27a Lpl complex formation | 0.5 | 2 | 1.5551 | 0.3123 | 1.2493 | 0.4276 | 1.2758 | 0.4592 | 1.9986 | 0.5434 | 1.4212 |
| cLPL27 | r 14, (r 12, r 13) | miR27a Lpl complex formation | 0 | 10 | 2.7275 | 0.9121 | 1.5912 | 1.527 | 1.462 | 1.7212 | 2.4017 | 0.0035711 | 0.001273 |
| ratioLPL29 | r 13 | ratio of miR29a Lpl complex formation to miR27a Lpl complex formation | 0.5 | 2 | 0.6808 | 0.5564 | 0.8526 | 0.5616 | 1.0139 | 0.5218 | 0.51242 | 0.84611 | 0.50309 |
| eCEBPA | r 7 | translation of CEBPA | 0 | 1000 | 197.6492 | 1.6836 | 163.5129 | 1.857 | 154.2769 | 2.0026 | 81.606 | 3.8376 | 24.138 |
| eCEBPAP | r 8 | CEBPA-dependent transcription of Pparg | 0 | 1000 | 28.6422 | 4.1834 | 35.2276 | 4.1192 | 41.3223 | 3.9706 | 0.3885 | 8.0636 | 1.0396 |
| ePPAR | r 9 | translation of PPARG | 0 | 1000 | 123.9134 | 1.9842 | 72.898 | 2.6335 | 128.895 | 1.9603 | 383.15 | 4.9943 | 39.388 |
| eCPPARP | r 10 | partially input (Rosi)-dependent PPARG-dependent transcription of Cebpa | 0 | 1000 | 114.1534 | 1.8361 | 92.6377 | 2.0574 | 137.9765 | 1.8825 | 0.60585 | 40.529 | 6.1873 |
| eLPPARP | r 11 | partially input (Rosi)-dependent PPARG-dependent transcription of Lpl | 0 | 1000 | 98.865 | 1.9117 | 79.1107 | 2.004 | 93.96 | 1.8784 | 0.45469 | 21.702 | 3.2646 |
| dCEBPA | r 15 | degradation of Cebpa | 9 | 12 | 11.3062 | 0.0865 | 10.9618 | 0.1035 | 10.9933 | 0.1071 | 11.985 | 11.94 | 11.959 |
| dPPAR | r 22 | degradation of Pparg | 3 | 4 | 3.8021 | 0.0886 | 3.8447 | 0.0743 | 3.8149 | 0.0842 | 3.9938 | 3.9995 | 3.9996 |
| dCEBPAP | r 16 | degradation of CEBPA | 9 | 17 | 14.1345 | 0.2116 | 13.8116 | 0.2182 | 14.3909 | 0.2062 | 16.964 | 16.248 | 16.862 |
| dM27 | r 5, r 17, r 19, r 24 | degradation and input (Xm27)-dependent transcription of miR27a | 0.11 | 0.4 | 0.4006 | 0.1449 | 0.3486 | 0.2975 | 0.2511 | 0.4923 | 0.42 | 0.38249 | 0.15686 |
| dM29 | r 6, r 18, r 20 | degradation and input (Xm29)-dependent transcription of miR29a | 0.11 | 0.4 | 0.1109 | 0.0177 | 0.157 | 0.0541 | 0.25 | 0.2004 | 0.11037 | 0.16052 | 0.23098 |
| dLPL | r 21 | degradation of Lpl | 0.83 | 1.66 | 1.5581 | 0.1244 | 1.3439 | 0.1771 | 1.5469 | 0.1496 | 1.641 | 1.37 | 1.6586 |
| dPPARP | r 23 | degradation of PPARG | 3 | 10 | 8.6729 | 0.2194 | 8.083 | 0.2633 | 8.1331 | 0.2718 | 9.9867 | 9.9901 | 9.8421 |
| kPPARligint | r 10, r 11 | relative internal ligand concentration activating PPARG | 0 | 0.5 | 0.0851 | 1.6058 | 0.0491 | 1.9462 | 0.0355 | 2.7559 | 0.0012564 | 0.0024599 | 0.00065546 |
| Xm27_D0 | r 5 | miR27a transcription activating input level from D0 on | 0 | 1 | 0.6684 | 0.1769 | 0.4563 | 0.3989 | 0.1637 | 1.2332 | 0.70824 | 0.50092 | 0.0014839 |
| ratio | r_12, r_13, r_14 | ratio of mRNA to miRNA molecules | 0.001 | 0.01 | 0.0023 | 1.042 | 0.0028 | 0.9296 | 0.0035 | 0.8845 | 0.0016473 | 0.009562 | 0.0041764 |
| Fixed experiment specific: | | | | | | | | | | | | | |
| ratio2729 | r 5 | experiment specific ratio of miR27a to miR29a | --- | --- | --- | --- | --- | --- | --- | --- | 0.509 | 0.543 | 0.5308 |



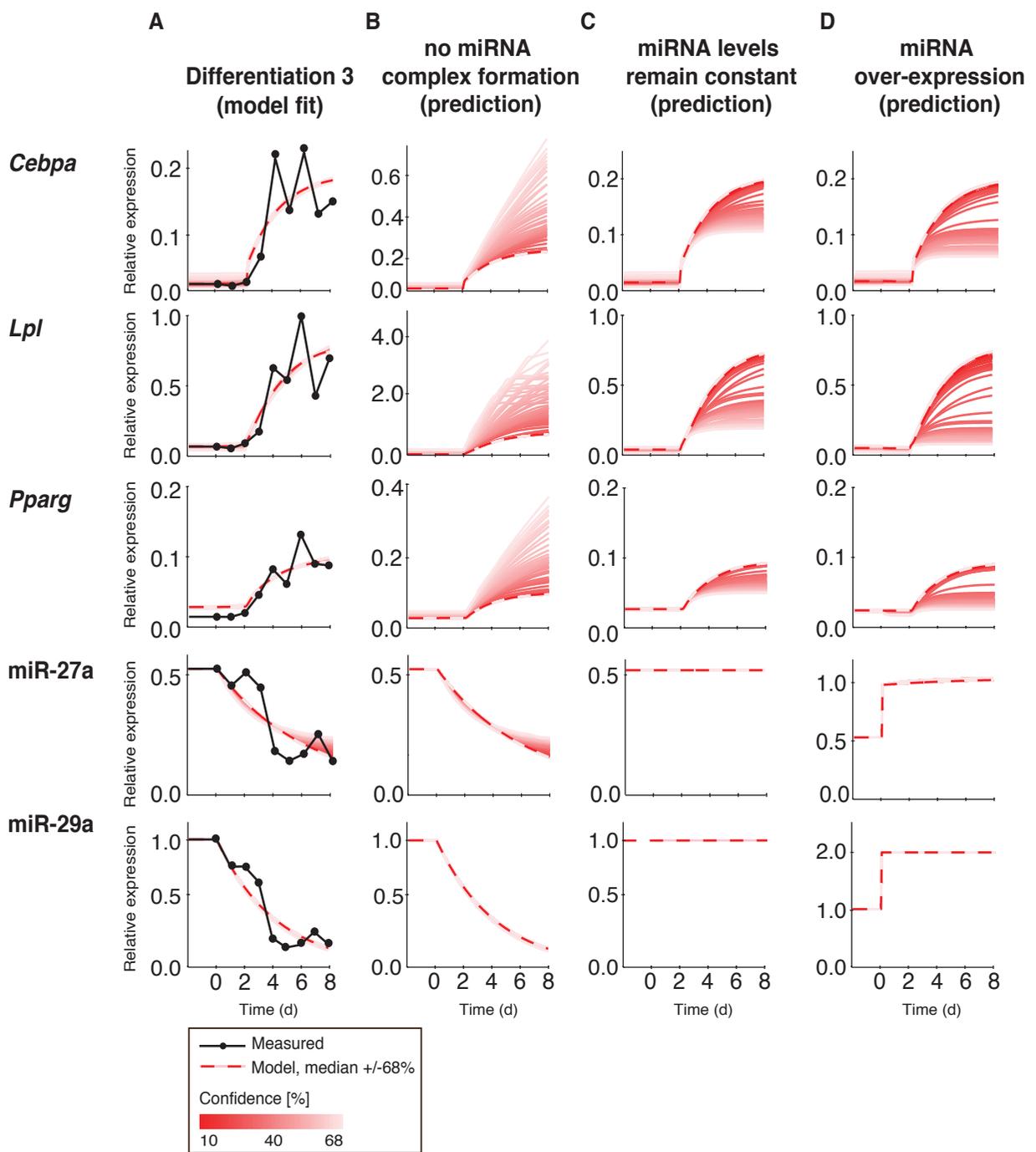
Supplementary Figure S1. Changes in the levels of histone modifications H3K4me3 and H3K27ac in the vicinity of miR-27 and miR-29 family clusters. Enrichment of histone H3K4 trimethylation and H3K27 acetylation have been shown to correlate closely with proximal and/or distal active promoter regions, respectively. 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.

A**B**

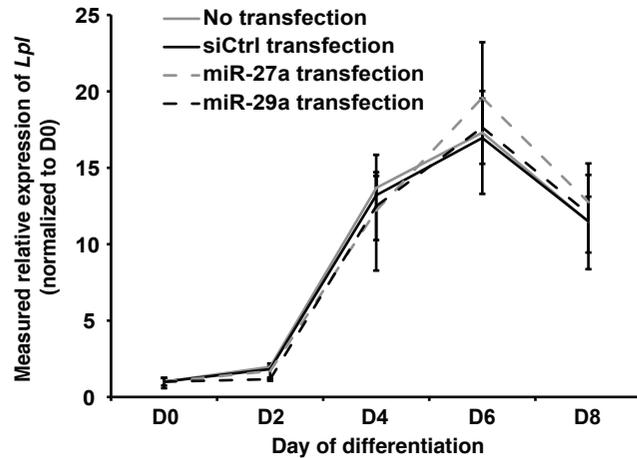
Supplementary Figure S2. Oil Red O staining of lipids in D0 (A) and D8 (B) differentiated 3T3-L1 cells was used to control the differentiation of the adipocytes. Representative images after Oil Red O stainings showing that D0 cells contain no or very little lipids while D8 cells have high accumulation 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.



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.