SUPPLEMENTAL METHODS

Cell culture

Human pre-adipocytes were propagated in MesenPRO RS™ Media (Invitrogen). For differentiation, cells were plated on collagen-coated plates, basic FGF was added (PeproTech; 4ng/ml) and two days after confluence induction media was added (DMEM/F12 supplemented with 10% FBS plus 1 μM dexamethasone, 1.7 mM insulin, 0.5mM IBMX, 10 μM rosiglitazone, 20ng/ml BMP4, 17 mM pantothenic acid, and 33 mM biotin). After three days induction media was replaced with maintenance media (DMEM/F12 supplemented with 10% FBS plus 1μM dexamethasone, 1.7 mM insulin, 17 mM pantothenic acid, and 33 mM biotin). Immortalized wild-type MEFs overexpressing PPARγ were cultured in DMEM high glucose with 10% FBS, 1 mM sodium pyruvate, and 2 mM L-glutamine (GIBCO). Differentiation was induced with 1 μM dexamethasone, 800 nM insulin, 0.5 mM IBMX, and 1 μM rosiglitazone for 3 days followed by 2 days with 1 μM dexamethasone and 800 nM insulin. HeLa cells were grown in DMEM high glucose with 10% FBS, 1 mM sodium pyruvate, and 2 mM L-glutamine (GIBCO).

Generation of adipocyte-specific knockout and osteoblast precursor-specific knockout mouse models

The adipocyte-specific *Fosl2* knockout mice (Fosl2-adKO) were generated by crossing Fosl2$^{fl/fl}$ mice with mice carrying the aP2-Cre^{ERT} allele (1). At the age of 6 weeks the animals were injected with tamoxifen i.p. (Sigma) 1mg / mouse for 5 consecutive days and sacrificed 19 days later. Osteoblast precursor specific *Fosl2* knockout mice were generated by crossing Fosl2$^{fl/fl}$ mice with Osx-tTA-Cre mice (2). All mice were maintained on a mixed C57BL6/129 background.

Reporter Assays

pLep and the enhancer regions were PCR amplified from genomic DNA from hASCs. pLEP was cloned into 5' position of pGL4.10 (Promega) using Bgl II and HindIII.

Potential enhancer amplicons were cloned into the 3' position using BamHI and SalI. Ligation was performed using the Rapid Ligation Kit (Roche) and constructs were verified by sequencing. Undifferentiated and differentiated hASCs were transfected with 4µg of luciferase (pGL4.10 construct) and 250ng renilla *Rluc* normalization plasmids (pGL4.75) using the Amaxa™ nucleofection system (Lonza). Cells were harvested after 48hrs and analyzed using the Dual-Luciferase® Reporter Assay System (Promega) in a FluoStar Optima plate reader (BMG Labtech). HeLa cells were transfected with 150 ng of luciferase (pGL4.10 construct) and 2.5 ng renilla *Rluc* normalization plasmids (pGL4.75) and either FlexiTube pooled siRNA against *FOSL2* (10nM) or AllStars Negative Control siRNA (10nM) (Qiagen) using HiPerFect transfection reagent (Qiagen). Cells were harvested after 36 hrs.

Stable Isotope Labeling by Amino acids In Cell culture (SILAC)

Other than the growth media used, cell culture growth conditions and procedures for growing SILAC labeled cells were identical to cells cultured in standard media. For the hASC: cells were induced with DMEM:F12 (1:1) media for SILAC (medium lacking arginine and lysine, Thermo Scientific) supplemented with 10 % dialyzed FBS (Caisson Laboratories, Inc.) plus 1 µM dexamethasone, 1.7 mM insulin, 0.5 mM IBMX, 10 µM rosiglitazone, 40 ng/ml BMP4, 17 mM pantothenic acid, and 33 mM. After three days the induction media was replaced with maintenance media (DMEM:F12 (1:1) Media for SILAC supplemented with 10 % dialyzed FBS plus 1µM dexamethasone, 1.7 mM insulin, 17 mM pantothenic acid, and 33 mM). SILAC amino acids $^{13}C_6$ $^{15}N_4$ L-arginine (157 mg/L) and $^{13}C_6$ $^{15}N_2$ L-lysine (97 mg/L) (Sigma-Aldrich) were added to heavy SILAC labeling media. Natural isotopic abundance amino acids L-arginine (147.5 mg/L) and L-lysine (91.25 mg/L) were added to light SILAC media. L-Proline (200 mg/L) was added to both light and heavy SILAC media to minimize arginine-to-proline conversion. Nuclear extracts were harvested at d11 after induction. The following procedures were performed

on light and heavy cells in parallel to generate separate SILAC labeled light and heavy nuclear lysates: cells were scraped from tissue culture dishes in hypotonic buffer containing 10 mM Hepes, pH 7.9, 10mM KCl, 1.5mM MgCl2, 0.5mM DTT, EDTA-free Complete Protease Inhibitor tablet (Roche) and incubated on ice for 10 minutes. Cells were then lysed in lysis buffer containing 10 mM Hepes pH 7.9, 10mM KCl, 1.5mM MgCl2, 0.5mM DTT, 0.5% Triton X-100, Complete protease inhibitors. The cell lysate was spun down at 300 x g to pellet nuclei, the cytoplasmic fraction was discarded. Lysates from the enriched nuclear fraction were generated by lysing nuclei in modified RIPA buffer containing 50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, and Complete Protease Inhibitors.

For MEFs: cells were induced with DMEM Media for SILAC (Thermo Scientific) supplemented with 10% FBS plus 1 μM dexamethasone, 800 mM insulin, 0.5 mM IBMX, and 1 μM rosiglitazone for three days followed by two days with SILAC media supplemented with 10% FBS plus 1μM dexamethasone and 800 mM insulin. Afterwards cells were maintained in SILAC media supplemented with 10% FBS. Nuclear extracts were harvested at d11 after induction and further processed as described for the hASCs.

DNA-protein interaction screen with SILAC labeled nuclei extracts

Nuclear extracts from light and heavy SILAC labeled cells (1.37 mg protein from hASCs, or 2.2 mg protein from MEFs) were incubated with 1500 pmoles of biotinylated oligonucleotide sequences of the mutant and wild-type sequence respectively, together with 200 μL of Dynal MyOne streptavidin bead slurry (Invitrogen) per tube, rotating end-over-end for 4 hours at 4 deg C. The replicate, label-swap, experiment with the reversed lysate-bait combination was performed at the same time.

Beads were collected with a magnet (Invitrogen) and washed twice by gently by inverting beads in 2 x 1 mL of modified RIPA buffer. Cysteine residues were reduced by incubating with 1 mM dithiothreitol at 60 deg C and mixing (Eppendorf thermomixer) for

20 minutes and subsequently alkylated with 10 mM iodoacetamide for 20 minutes in the dark. Proteins from each pull-down were prepared for one-dimensional gel electrophoresis by heating at 70 deg C in 4x LDS buffer (Invitrogen) for 10 minutes and loaded onto a NuPage 4-12% Bis-Tris gel (Invitrogen) with MES running buffer. The resulting gel lanes were stained with colloidal Coomassie and divided into ten slices for GeLC-MS analyses. Proteins were digested with trypsin using an in-gel digestion protocol and peptides desalted using StageTips before LC-MS analyses. The peptides were then resuspended by vortexing in 7 μL of 0.1% TFA and analyzed by nanoflow-LCMS with an Agilent 1100 with autosampler and a LTQ-Orbitrap-Velos (Thermo). Peptides were resolved on a 10 cm column, made in-house by packing a self-pulled 75 μm I.D. capillary, 15 μm tip (P-2000 laser based puller, Sutter Instruments) column with 3 μm Reprosil-C18-AQ beads (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) with an analytical flowrate of 200 nL/min and a 100 min linear gradient (~ 0.57 %B/min) from 0.1% formic acid in water to 0.1% formic acid/90% acetonitrile. The run time was 155 min for a single sample, including sample loading and column reconditioning.

We used a MS method with a master Orbitrap full scan (60,000 resolution) and data dependent LTQ MS/MS scans for the top twelve precursors (excluding z=1) from the Orbitrap scan. Each cycle was approximately 2.5 secs long. We used MaxQuant (v.1.0.13.13) with Mascot (v.2.2.06, Matrix Science Ltd) for MS peak identification and quantification. We searched the IPI human or mouse v3.70 (http://ebi.ac.uk) with a decoy database search strategy including common contaminants like BSA and proteolytic enzymes, with +/- 7 ppm and +/- 0.5 Da mass tolerance at the precursor and fragment levels, respectively. The protein and peptide false discovery rate for protein identification was set at 0.01. Protein ratios are reported only when two or more peptides were quantitated. Search variables for SILAC were Arg-$^{12}C_6$ and Lys-$^{12}C_6{}^{14}N_2$ (light) or Arg-$^{13}C_6$ and Lys-$^{13}C_6{}^{15}N_2$ (heavy), variable modifications were oxidized methionine and

acetylation of protein N-termini while only carbamidomethylated cysteines were considered. Protein ratios were reported only when two or more peptides were quantitated. Quantification of SILAC peptides by MaxQuant (3) gave ratios and significance scores for proteins in forward and reverse experiments. Combined significance scores for each protein were then calculated as $10^{\sum \log(significance\ score\ for\ each\ expt)}$. Combined significance scores are not calculated for proteins with log2 ratios that do not invert in label-swap experiments.

SUPPLEMENTAL REFERENCES

1. Imai, T., Jiang, M., Chambon, P., and Metzger, D. 2001. Impaired adipogenesis and lipolysis in the mouse upon selective ablation of the retinoid X receptor alpha mediated by a tamoxifen-inducible chimeric Cre recombinase (Cre-ERT2) in adipocytes. *Proc Natl Acad Sci U S A* 98:224-228.
2. Rodda, S.J., and McMahon, A.P. 2006. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. *Development* 133:3231-3244.
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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Computational motif finding in the 30 bp region of UpE.

Motif finding in the 30 bp region of UpE region was employed using the MULAN algorithm and the TRANSFAC database. The "Motif ID" refers to the annotation provided by the database, "Position" refers to the base pair number in the 958 bp UpE region, and "Sequence" refers to the relevant stretch of DNA with the core binding site in upper case.

Supplemental Figure 2: *Lep* mRNA expression in MEFs.

Immortalized mouse embryonic fibroblasts (MEFs) overexpressing PPARγ were differentiated into mature adipocytes *in vitro*. mRNA was harvested at day -2 (pre-ads) and day 7 (ads) after induction of differentiation. Gene expression was assessed by RT-qPCR. Data are shown as mRNA levels relative to cyclophilin expression, expressed as mean ± SEM. *$p<0.05$.

Supplemental Figure 3: Experimental set-up for the proteome-wide screening for adipocyte nucleoproteins that specifically bind enhancer element UpE using SILAC.

Experimental set-up for the Stable Isotope Labeling by Amino acids In Cell culture (SILAC) protein-DNA interaction screen. In the SILAC-based interaction screen, relative enrichment of proteins bound to sequence-specific DNA baits are measured by their SILAC ratios; proteins that are specifically enriched by the wild-type bait (WT) have increased H/L ratios while proteins bound equally to WT and mutant (MT) baits have a ratio close to one.

Supplemental Figure 4: Proteome-wide screening for MEF adipocyte nucleoproteins that specifically bind enhancer element UpE using SILAC.

Scatter plot of the results from the SILAC experiments in mouse embryonic fibroblasts over-expressing *Pparg* (MEFs). The 'nucleic acid binding' term includes all proteins bearing the following GO terms: nucleic acid binding, nucleosome binding, nucleotide binding, nucleoside binding, or DNA binding.

Supplemental Figure 5: Increased levels of *LEP* gene expression correlate with increased levels of *FOSL2* gene expression in the context of glucocorticoid stimulation.

Mature human adipocytes were stimulated with dexamethasone (10nM) or vehicle for 16 hours and gene expression was assessed with RT-qPCR. Data shown are mRNA levels relative to cyclophilin expression, expressed as mean ± SEM. *p<0.05

Supplemental Figure 6: Knockdown of Fosl2 decreases UpE activity and leptin expression.

(A) Luciferase assay with HeLa cells, transfected with either the LEP promoter (pLEP) construct or the UpE enhancer construct and either negative control siRNA or pooled siRNA against *FOSL2*. *p<0.05 compared to pLEP of the corresponding control group. $^{\$}$p<0.05 compared to UpE treated with negative control siRNA.

(B) Seven days after differentiation MEF adipocytes were transduced with different lentiviral knockdown constructs against *Fosl2* or luciferase as control. Seven days later mRNA was prepared and gene expression was assessed by RT-qPCR. Data shown are mRNA levels relative to cyclophilin expression, expressed as mean ± SEM. *, $ p<0.05 as compared to corresponding control.

Supplemental Figure 7: Overexpression of AP-1 members in mature adipocytes is not sufficient to increase *LEP* expression.

Seven days after differentiation, human adipocytes were transduced with lentiviral overexpression constructs carrying the indicated cDNA. Five days later mRNA was prepared and gene expression was assessed by RT-qPCR. Data shown are mRNA levels relative to cyclophilin expression, expressed as mean ± SEM.

Supplemental Figure 8: Overexpression of Fosl2 in MEFs during adipogenesis can drive increased *Lep* expression.

MEFs were transduced with lentiviruses to overexpress PPARγ along with either Fosl2 or GFP and subsequently differentiated into adipocytes. Secreted Lep levels were assed with ELISA (A). Markers of adipogenesis such as PPARg (B), aP2 (C), and adiponectin (D) were measured by RT-qPCR and were equivalent in cells that received Fosl2 or EGFP, and both cell types accumulated similar amounts of lipid by oil Red O macroscopically (E) and microscopically (200x) (F). Data are shown as mean ± SEM. *$p<0.05$. n.s. = non significant.

Wrann_Supplemental Figure 1

MOTIF ID	POSITION	SEQUENCE
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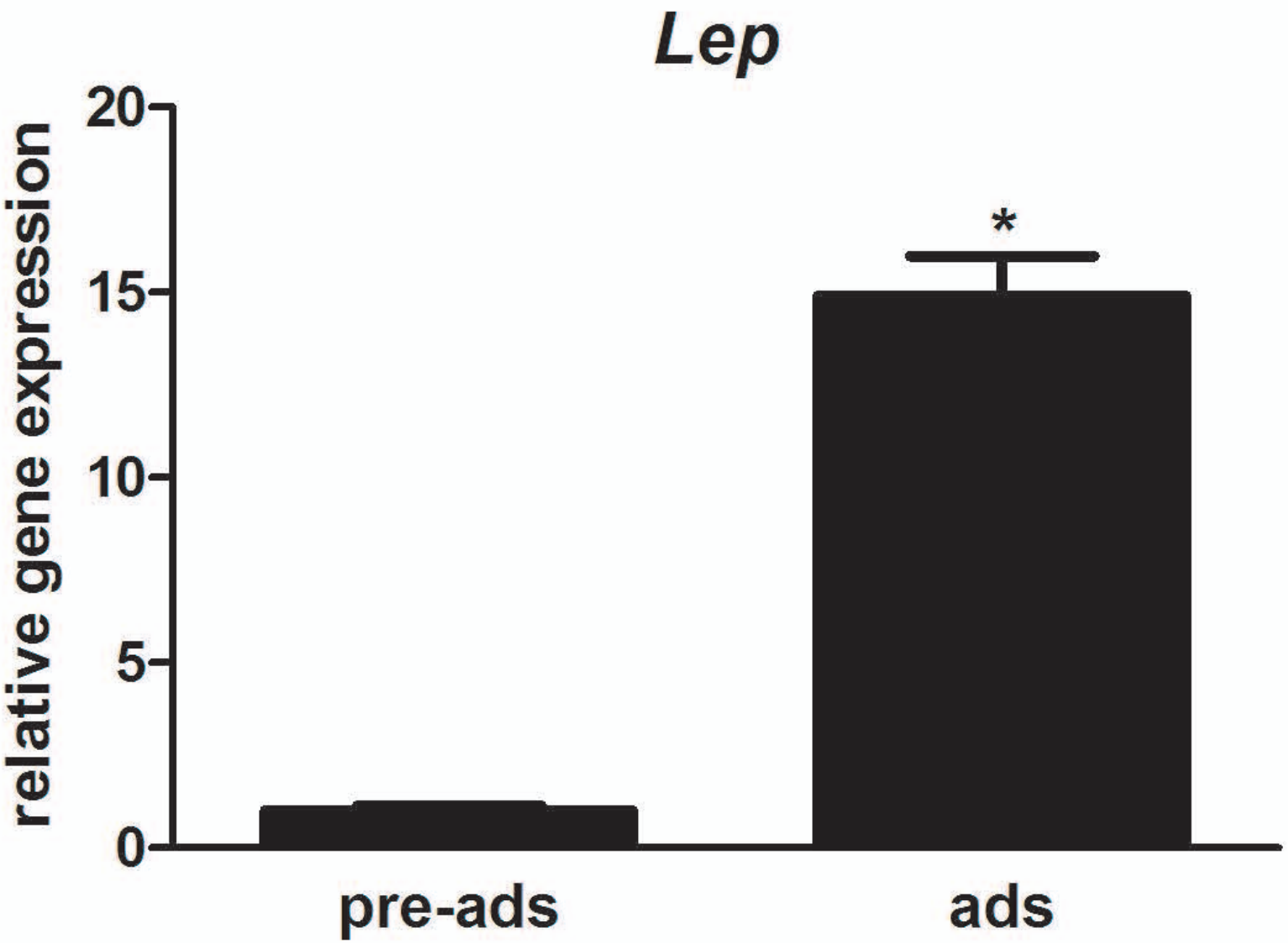
V$TCF11MAFG_01	429 (-)	gaaaagTGCTTGCTCAttgggg
V$BEL1_B	431 (+)	aAAGTGCTTGCTCATTGGGGTTTGCCat
V$AR_Q6	436 (-)	gctTGCTCa
V$PPARA_02	437 (+)	cttGCTCATTGGGGTttgc
V$ALPHACP1_01	441 (-)	CTCATTGGGGT
V$TAACC_B	443 (-)	caTTGGGGTTTGCCATCTTTTGc
V$NFKAPPAB50_01	447 (+)	GGGGTTTGCC
V$YY1_Q6_02	451 (+)	tttgCCATctt
V$YY1_Q6	454 (+)	GCCATCTtt

Wrann_Supplemental Figure 2



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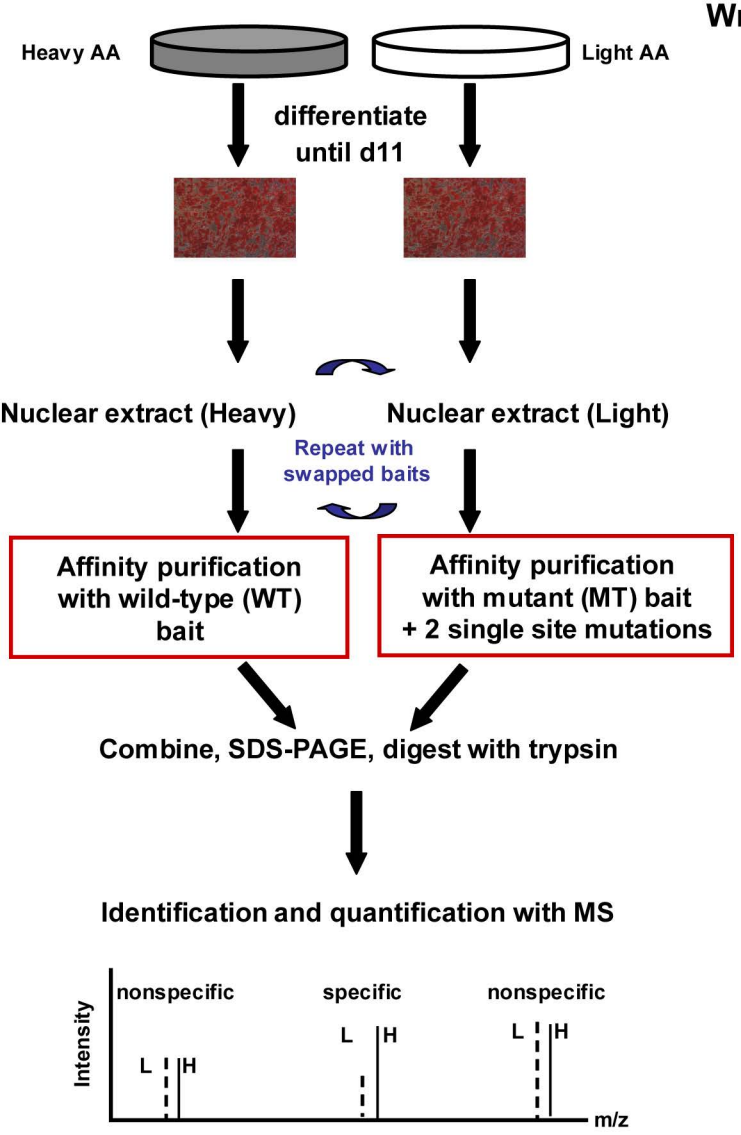

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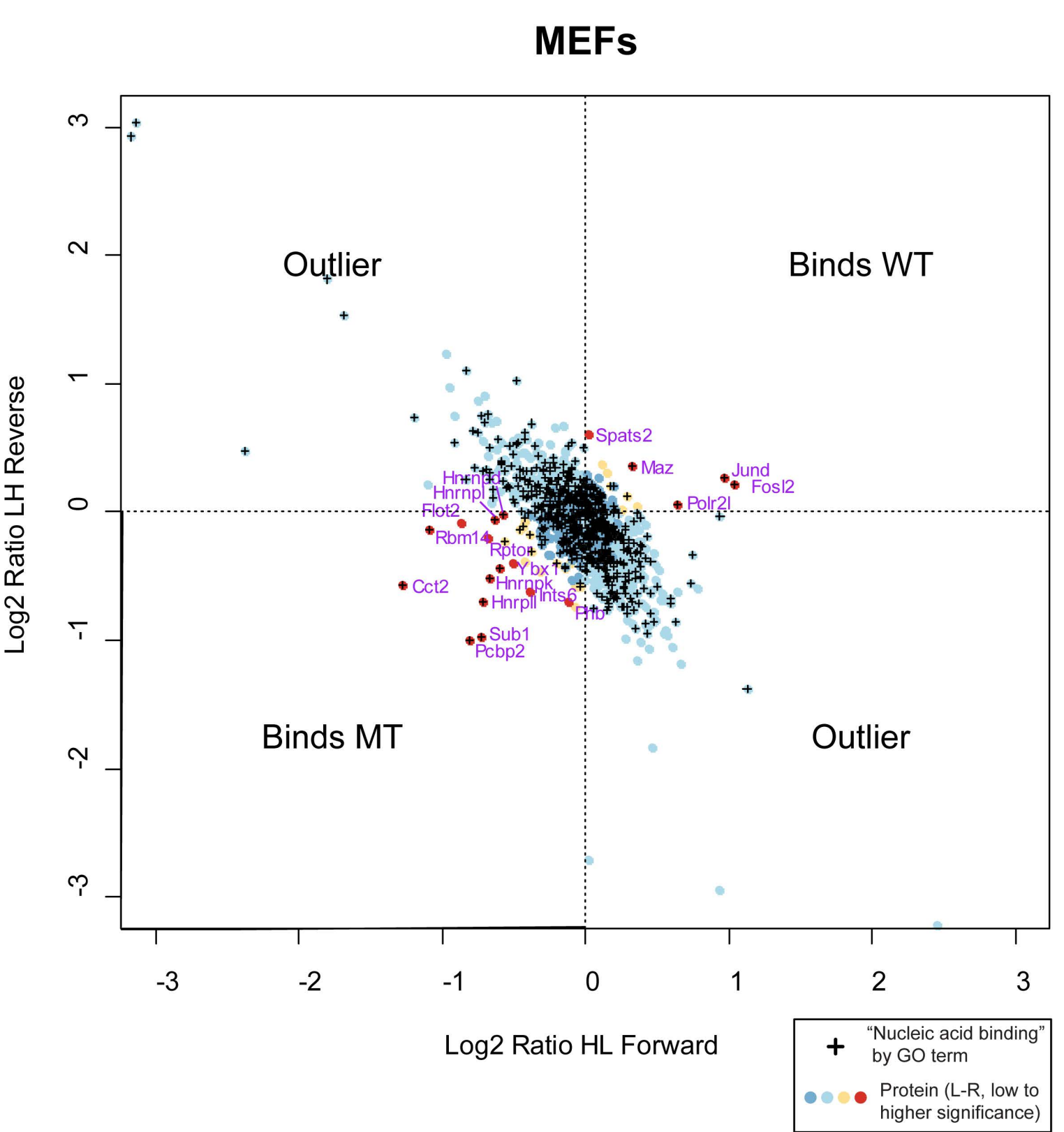

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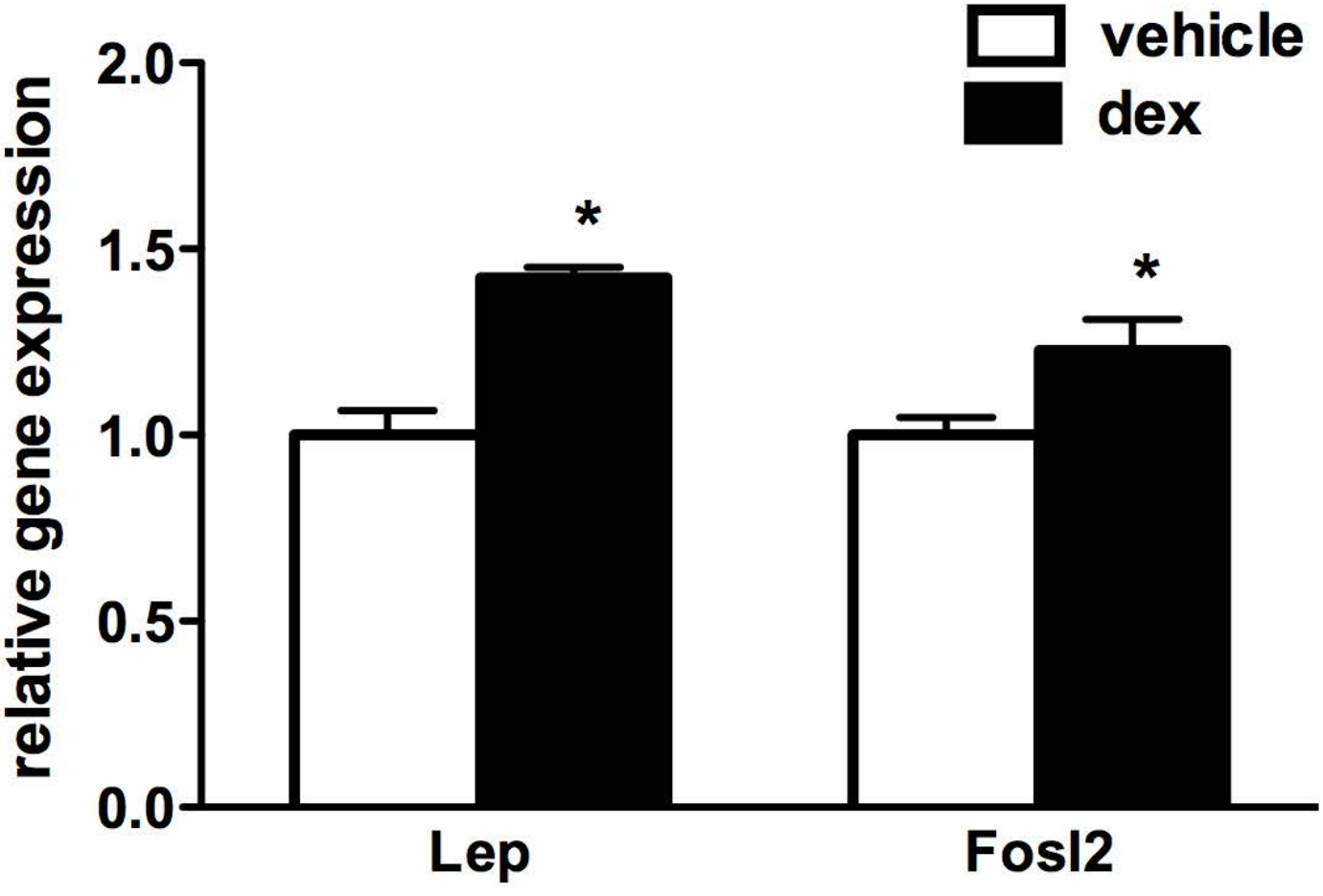

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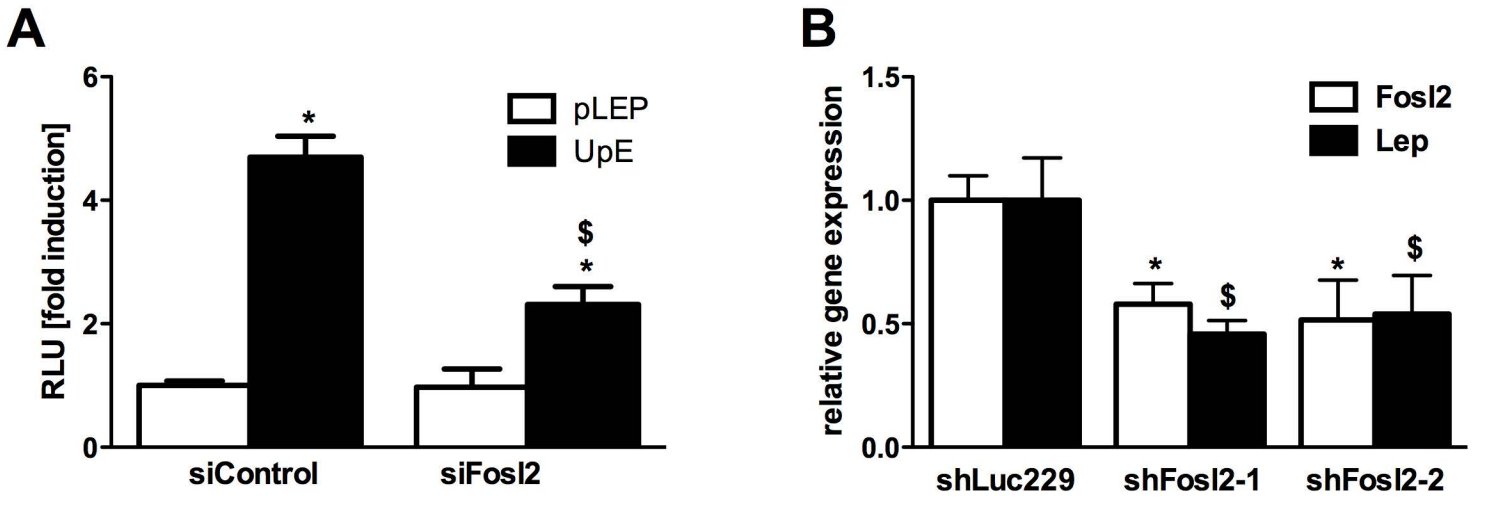
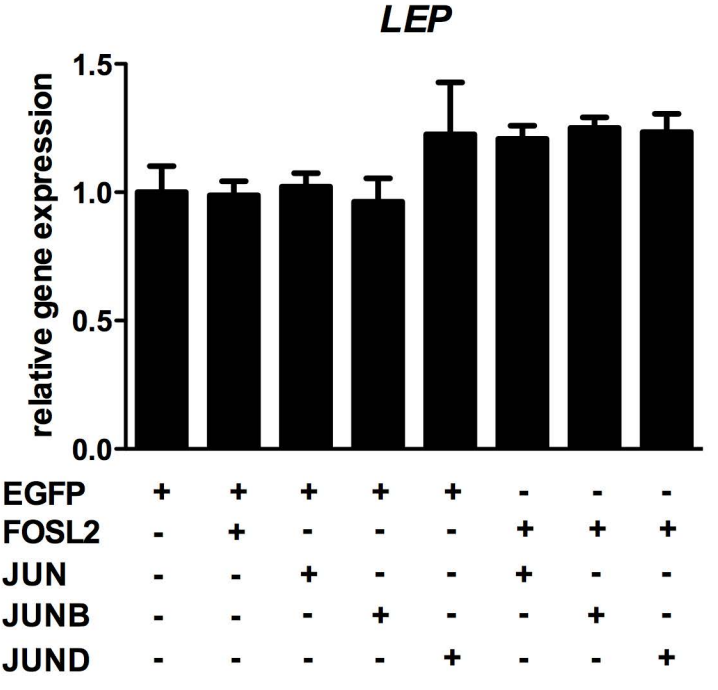


Wrann_Supplemental Figure 7



Wrann_Supplemental Figure 8