Extended Experimental Procedures

Cell culture and differentiation

3T3-L1 was grown and differentiated to mature adipocyte as described (MacIsaac, Lo et al. 2010). In brief, 3T3-L1 cells (ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum (Invitrogen), 100 units/ml penicillin, and 100µg/ml streptomycin. Two days after confluence (Day 0), cells were induced to differentiate with DMEM containing 10% fetal bovine serum (FBS), 1µM dexamethasone, 10 µg/ml insulin and 0.5mM 3-isobutyl-1-methyxanthine for 2 days. Cells were then incubated in DMEM containing 10% FBS and 10 µg/ml insulin for 2 more days. After Day 4, cells were maintained in DMEM containing 10% FBS, with medium change every other day. Experiments were performed using Day 8 to Day12 mature adipocytes.

Western blot analysis

3T3-L1 adipocytes were treated with the different insulin resistance-inducing agents for 24h, except in the case of high insulin where the medium was changed to a low-glucose, insulin-free medium during the last hour to recover from chronic insulin treatment. Cells were then stimulated with 20nM of insulin for 10min. They were then lysed in RIPA buffer with protease and phosphatase inhibitors. Proteins were extracted and quantified using Bradford reagent. 30ug of soluble protein was boiled with sample loading buffer for 10min and loaded onto a Criterion TGX precast gel (Bio-Rad) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins on the SDS-PAGE gel were then immunoblotted onto nitrocellular membranes. Primary antibodies were then used to probe for the protein of interest: pAkt (Cell Signaling 4051), C/EBPβ (Santa Cruz Biotechnology sc-150), and loading control Akt (Cell Signaling 9272) or actin (Millipore MAB1501). Membranes were then washed and incubated with either horseradish peroxidase-conjugated secondary antibody or IRDye secondary

antibodies from Licor Biosciences. Signals were detected either using chemiluminescence or the Licor Odyssey Classic Infrared Imaging System. Signals were quantified using the Syngene GeneTools software or the Licor Image Studio software package.

2-deoxyglucose (2-DOG) uptake assay

Glucose uptake in 3T3-L1 was performed as described (Lakshmanan, Elmendorf et al. 2003). Fully differentiated 3T3-L1, untreated or after 24h of insulin resistance treatment (except in the case of high insulin where the medium was changed to a low-glucose, insulin-free medium during the last hour to recover from chronic insulin treatment), was washed with warm KRPH buffer and incubated in KRPH buffer at 37°C for 15 min. For each insulin resistance model or the control model, cells were divided into 2 groups: one for determining basal glucose uptake (no insulin treatment) and the other for insulin-stimulated glucose uptake (20nM insulin incubation for 30min). [^{3}H]-2-deoxy-D-glucose (PerkinElmer, 20µCi/ml) was added after the 30min insulin incubation, and glucose uptake was measured by scintillation counting 10 min later. Data presented are mean uptake ± SEM from 5 biological replicates. Counts per minute were converted to pmol of 2-deoxyglucose uptake per well.

RNA isolation and reverse transcription-PCR

Total RNA was extracted using Qiagen kit. 1ug of RNA was reverse-transcribed to cDNA and used for quantitative PCR using SYBR Green I master mix in the Light Cycler 480 instrument (Roche). At least three biological replicates were performed for each experiment. Primers sequences are in Table S8.

Microarray gene expression analyses

Expression data of DIO mouse fat pad (GSE14363, GSM498652 and GSE48220) and adipogenesis (GSE80696, GSE14004 and GSE29899) were downloaded from GEO database.

GSE29899 is a RNA-Seq data set, and it was analyzed as described above in the main text. Microarray expression data sets were analyzed using R and Bioconductor (http://www.bioconductor.org/). Gene expression data were normalized and summarized with the RMA algorithm (Irizarry, Hobbs et al. 2003) with an updated Entrez gene probeset definitions (Dai, Wang et al. 2005). Differentially expressed genes were identified using the LIMMA package (Smyth 2004). For the adipogenesis data sets, the 1,319 differentially expressed genes are those that have a log2 fold change of > 1 or < -1 and a q-value < 0.05 between preadipocytes and adipocytes.

Gene set enrichment analysis (GSEA)

Gene set enrichment analysis was performed as described (Subramanian, Tamayo et al. 2005). For each insulin resistance model, rank-ordered gene list according to gene expression fold changes with respect to the control model (untreated 3T3-L1 for the *in vitro* models and normal chow diet-fed mouse for the DIO mouse models) was generated. These ranked lists were used as input to GSEA, which mapped genes from members of the publicly curated KEGG (Kanehisa and Goto 2000) and Reactome (Croft, O'Kelly et al. 2010) pathways to the ranked-ordered lists. For each insulin resistance model and for each pathway, a p-value was calculated to indicate the significance of upregulation or downregulation of the particular pathway. The p-values were log transformed ($-\log_{10}$ pval) to an Enrichment Significance Score: upregulated pathways have positive Enrichment Significance Scores (shown as red in heatmap), and downregulated pathways have negative Enrichment Significance Score (shown in blue in heatmap), with the color intensity representing the significance of upregulation/downregulation. White represents no upregulation/downregulation, and grey represents uncalculated Enrichment Significance Score as the number of mapped genes from a pathway to the rank-ordered gene list is less than

15. Rows (pathways) in the heatmaps were ranked according to the *in vivo* combined Enrichment Significance Scores, which were calculated by summing the Enrichment Significance Scores of the 3 mouse models.

Hierarchical clustering

Clustering was done in Cluster (de Hoon, Imoto et al. 2004) and visualized in Treeview (Saldanha 2004). For each insulin resistance model, the gene expression fold changes (log2) with respect to the control model (untreated 3T3-L1 for the *in vitro* models and normal chow diet-fed mouse for the DIO mouse models) were calculated, median centered and normalized to have unit variance before clustering.

Principal component analysis

Gene expression fold change values with respect to the control model for each of the seven insulin resistance models were normalized to have a mean of zero and unit variance within each data set. Principal component analysis was then applied to the resulting values. Projections of each model against the first two principal components were computed and used to infer similarity between models.

Pathway visualization

Expression fold changes in the different pathways across the different *in vitro* or *in vivo* models were visualized using PathVisio 2 (van Iersel, Kelder et al. 2008). Genes that are upregulated after the insulin resistance treatments (or in DIO mouse adipose tissue) are shown as red, those that are downregulated are shown as blue. Missing expression values are shown as green or grey.

Chromatin Immunoprecipitation (ChIP) and ChIP-Seq

ChIP assays in 3T3-L1 were carried out as previously described (Lo, Bauchmann et al. 2011). Antibodies used for ChIP: p65 (abcam 7970) and C/EBPβ (sc-150x). Primers sequences used for

ChIP-PCR analysis are in Table S8. ChIP-Seq was done essentially as described (Lo, Bauchmann et al. 2011). ChIP-Seq libraries were prepared and sequenced using the Illumina GAII protocol and platform. Single-end, 36-bp reads were aligned to the mouse reference genome mm9. Peaks calling were done using MACS (Zhang, Liu et al. 2008).

DNA sequence motif discovery

Over-represented transcription factor motifs were identified in each experiment using THEME (MacIsaac, Gordon et al. 2006). THEME uses principled statistical methods to test hypotheses about the binding specificity of the immunoprecipitated protein. Hypotheses are position weight matrix representations of DNA sequence motifs in the TRANSFAC and JASPAR database. They were evaluated based on their cross-validation error, which represents their ability to predict which sequences from a held-out test set were bound by the protein and which were not. Each hypothesis was either accepted or rejected based on a p-value, which was derived from comparing its predictive value to those of motifs derived by applying the same algorithm to randomly selected sequences for 25 times.

Mouse studies

Mice on the C57BL/6 background were kept at the University of Massachusetts Medical School animal faculties. Mice were fed with a normal chow diet or a high-fat diet for 16 weeks starting from 8-week of age. All mice were sacrificed at 24 week. Glucose tolerance test and insulin tolerance test were performed at week 23 to ensure that the high fat diet-fed mice were insulin resistant.

Adipocytes and stromal vascular fraction isolation

Adipocytes and the stromal vascular cell fraction of mouse fat pads were isolated as described (Herrero, Shapiro et al. 2010). Briefly, epididymal adipose tissue was excised and minced in 10

ml of KRB solution containing 12.5 mM HEPES (pH 7.4), 120 mM NaCl, 6 mM KCl, 1.2 mM MgSO4, 1 mM CaCl2, 2% BSA, and 2.5 mM glucose. Collagenase II (Sigma C6885, 1 mg/ml) and DNase I (Sigma DN-25, 0.2 mg/ml) were added and the tissue was incubated at 37 °C for 40min with shaking. Larger particles were removed using a 100 mm nylon sieve and the filtrates were centrifuged at 1,000 rpm for 3 min. The floating adipocytes were collected. The pelleted SVF was further centrifuged for 5 min at 3,000 rpm, followed by and a wash with PBS supplemented with 2% FBS. The SVF was treated with 500 µl ACK lysing buffer (Biowhittaker) and washed. RNA was extracted from both the adipocytes and the SVF. RNA using a Qiagen RNeasy kit.

3T3-L1 knockdown experiment and analysis of gene expression after knock-down

Day 8 differentiated 3T3-L1 cells were treated for 72 h with 0.5 nmol of either of two separate siRNAs against *Cebpb* or with 0.5 nmol of a non-targeting siRNA using the Endo-Porter carrier for intracellular delivery as described by the manufacturer (GeneTools, LLC). The siRNAs were purchased from Thermo Scientific (ON-TARGETplus product line, Catalogue numbers: J-043110-10, J-043110-11 and D-001810-0). TNFα treatment (2.5 nM) was performed during the final 24 hours of this 72 h period. Following ±TNFα treatment, RNA was isolated (RNeasy, Qiagen) and converted to cDNA (High Capacity cDNA RT Kit, Applied Biosystems). Quantitative PCR analysis of mRNA expression was performed using TaqMan® probes and a 7500 Fast Real-Time PCR machine (Applied Biosystems). The following probes from ABI were used: *Cebpb* (Mm00843434); *Irf2* (Mm00515206); *Il15* (Mm00434210); *Lcn2* (Mm01324470) and *18s* (432809). A duplex PCR of the target mRNA and *18s* mRNA was performed in a single well. The data are presented as relative mRNA expression normalized to *18s* mRNA.

Software

The site (http://fraenkel.mit.edu/adipo_sight/) accepts as input a list of mouse gene in the form of MGI official gene symbols and calculates DNA sequence motif enrichment from four different sets of condition-specific DNase hypersensitivity regions (dexamethasone-induced, high insulin-induced, hypoxia-induced, and TNFα-induced) in 3T3-L1 adipocytes and a set of non-conditional specific DNase hypersensitive regions from untreated mature 3T3-L1 adipocytes (general adipocyte DHS). The condition-specific DNase hypersensitive regions are called using the MACS software (Zhang, Liu et al. 2008) with the treated sample as the foreground and the untreated 3T3-L1 adipocytes as the background. The general adipocyte DNase hypersensitive regions are called with the untreated 3T3-L1 adipocytes as the foreground and total genomic DNA treated with the same amount of DNase I as background (Sabo, Kuehn et al. 2006). Hypersensitive peaks are mapped to genes if any portion falls within 10kb of a gene's transcription start site. Motifs with high information content from the TRANSFAC database (Matys, Fricke et al. 2003) are used to find enrichment. Enrichment is calculated using the hypergeometric distribution as the likelihood that a query with k out of n sites containing a motif are drawn from all sites N with m motif matches. A site is considered to contain a motif if the maximum score is above a precomputed threshold. To calculate these thresholds, an empirical probability mass function (epmf) for all scores in the general adipocyte DHS set was built for each motif. The motif score that contains the top percentages of the probability mass (equivalent to mass above 1,2, or 3 standard deviation of a normal distribution) was chosen as the thresholds. For each motif, a p-value is calculated from the hypergeometric distribution and it is corrected for multiple hypotheses with Benjamini-Hochberg correction. Only p-values less than 0.05 were displayed, and the output for each condition can be sorted by p-value by clicking on the

condition name.

Supplemental References

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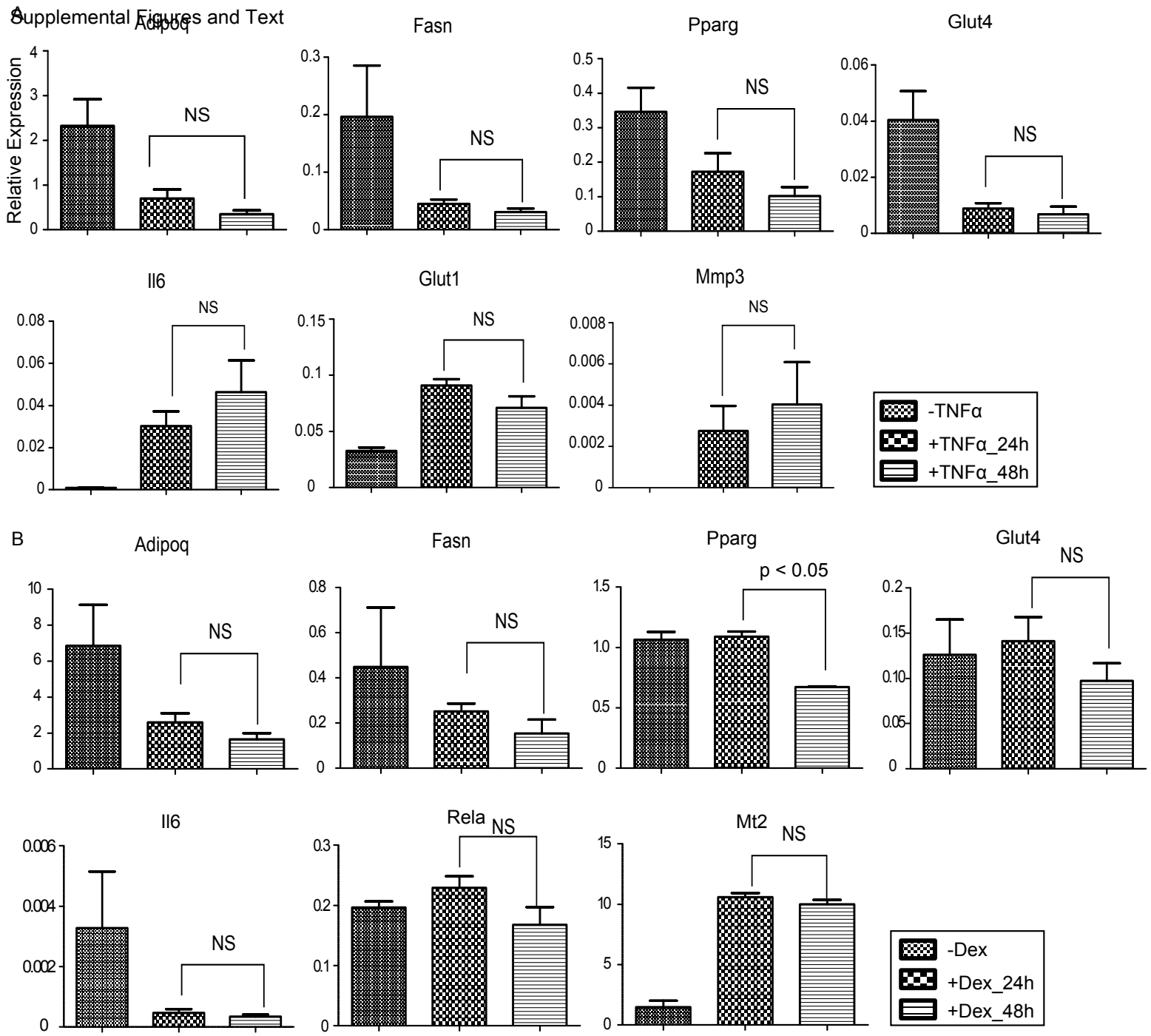
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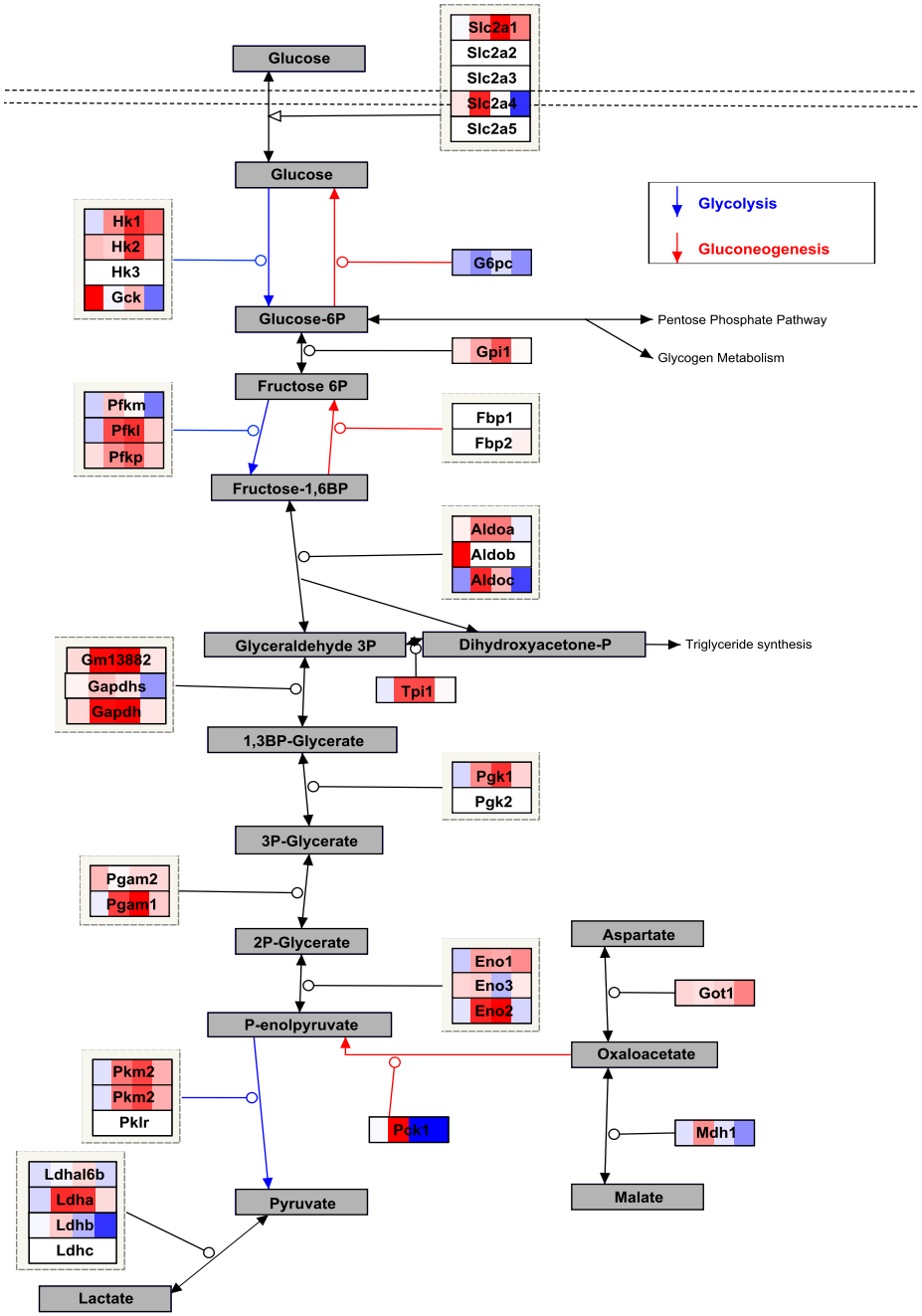
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Glucose
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Hk3
Gck
G6pc
Glucose-6P
Pentose Phosphate Pathway
Glycogen Metabolism
Gpi1
Fructose 6P
Pfkm
Pfkl
Pfkp
Fbp1
Fbp2
Fructose-1,6BP
Aldoa
Aldob
Aldoc
Glyceraldehyde 3P
Dihydroxyacetone-P
Triglyceride synthesis
Gm13882
Gapdhs
Gapdh
Tpi1
1,3BP-Glycerate
Pgk1
Pgk2
3P-Glycerate
Pgam2
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2P-Glycerate
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Eno3
Eno2
Got1
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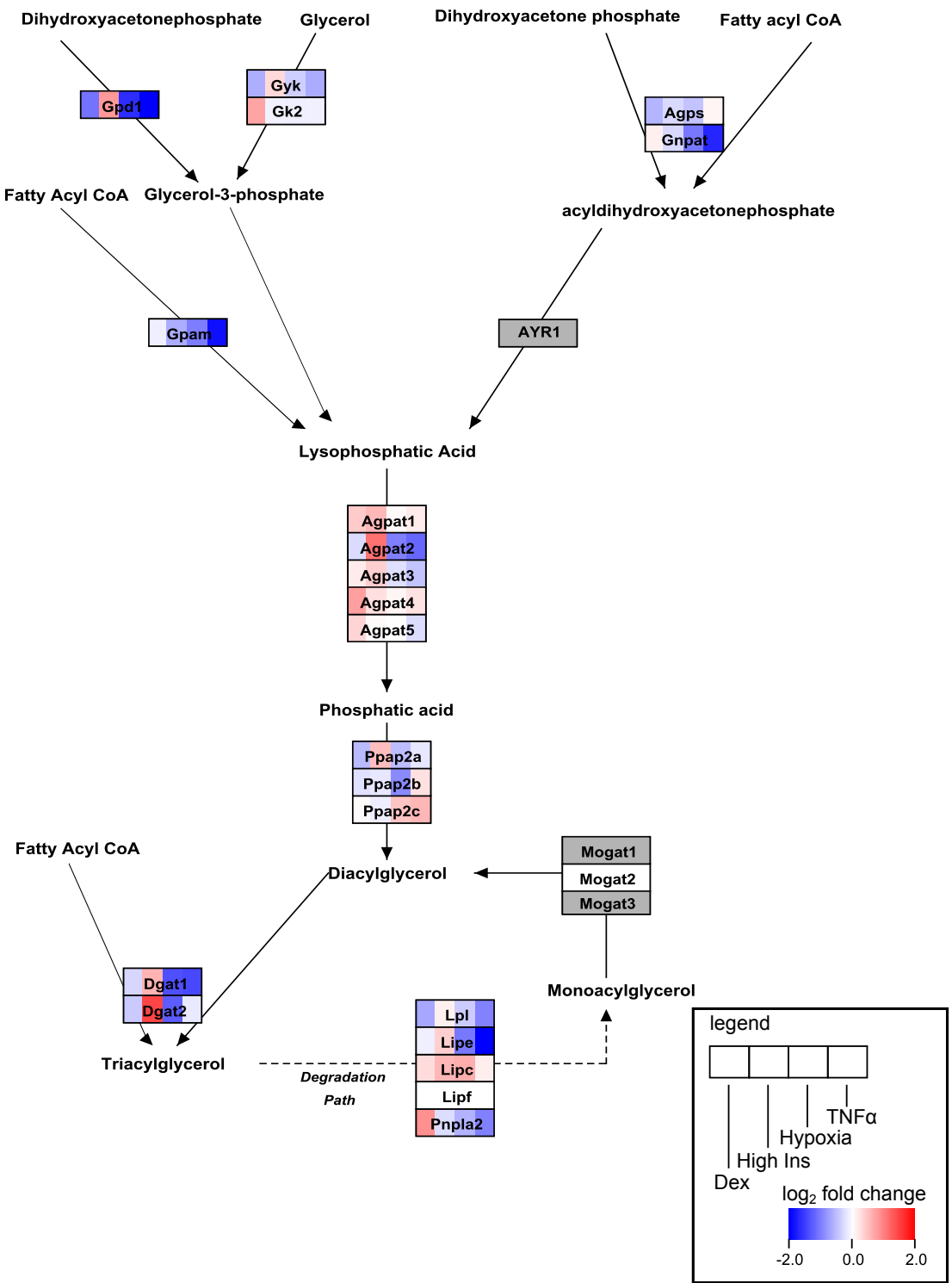
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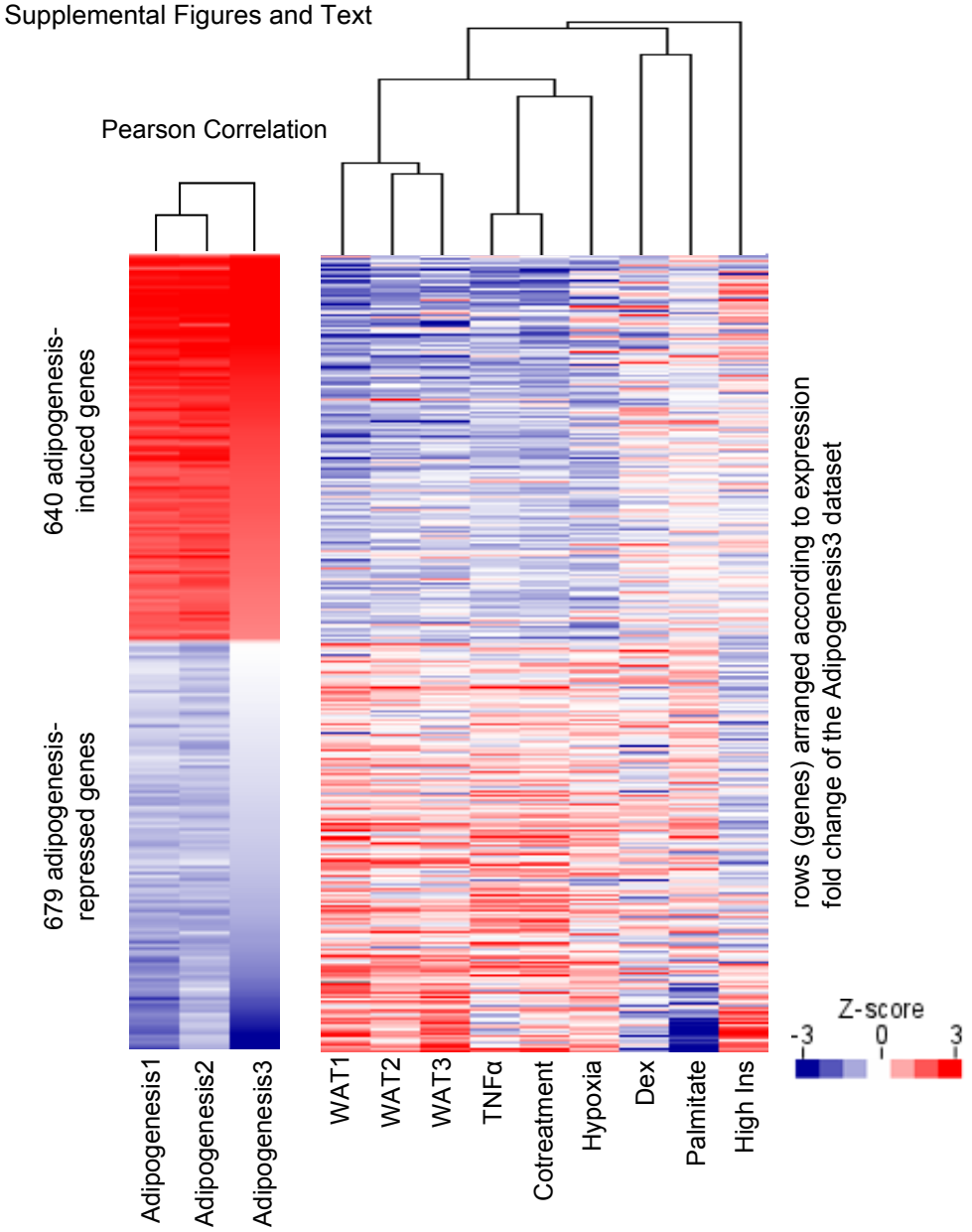
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679 adipogenesis-repressed genes
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0
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Adipogenesis2
Adipogenesis3
WAT1
WAT2
WAT3
TNFα
Cotreatment
Hypoxia
Dex
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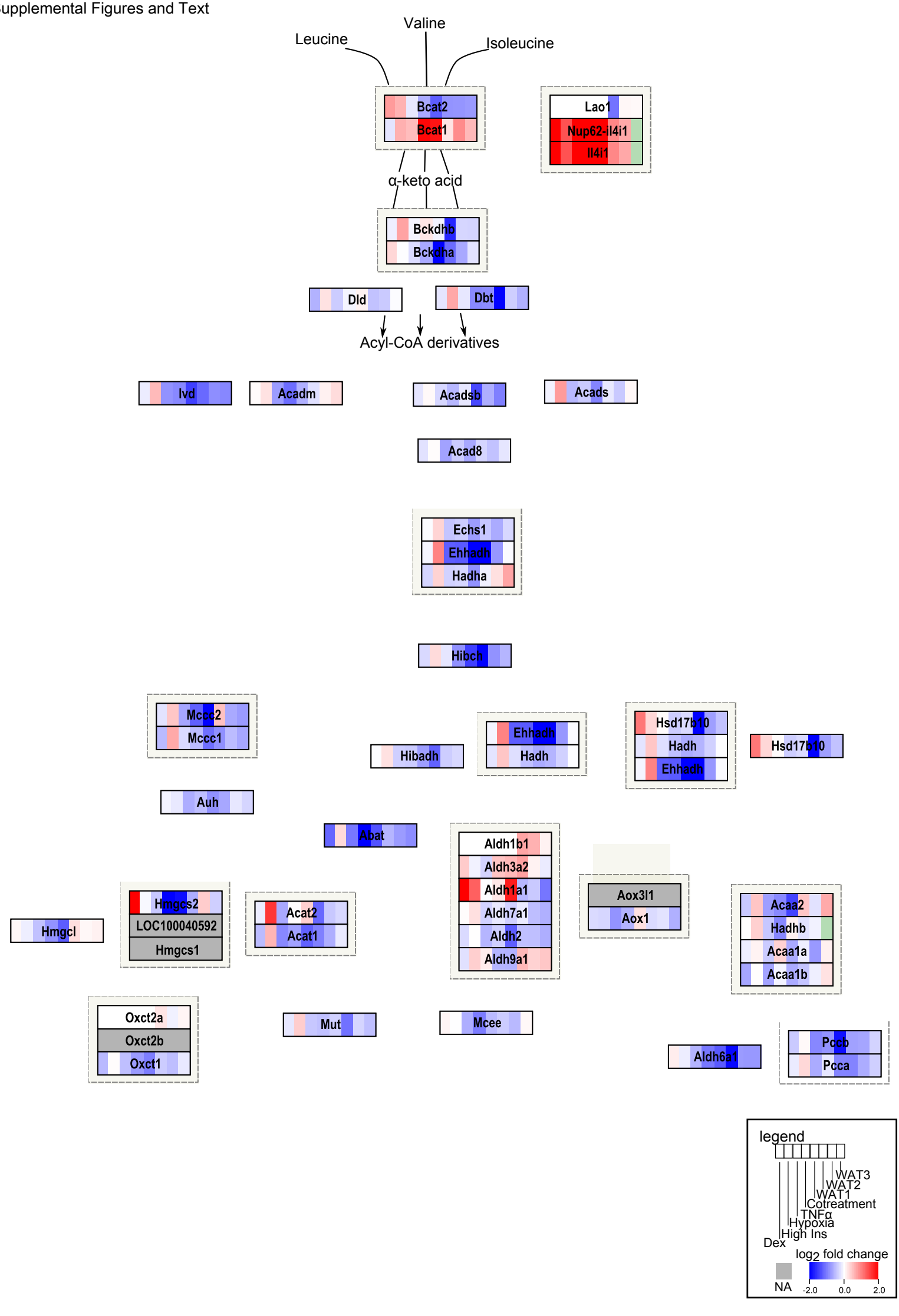
High Ins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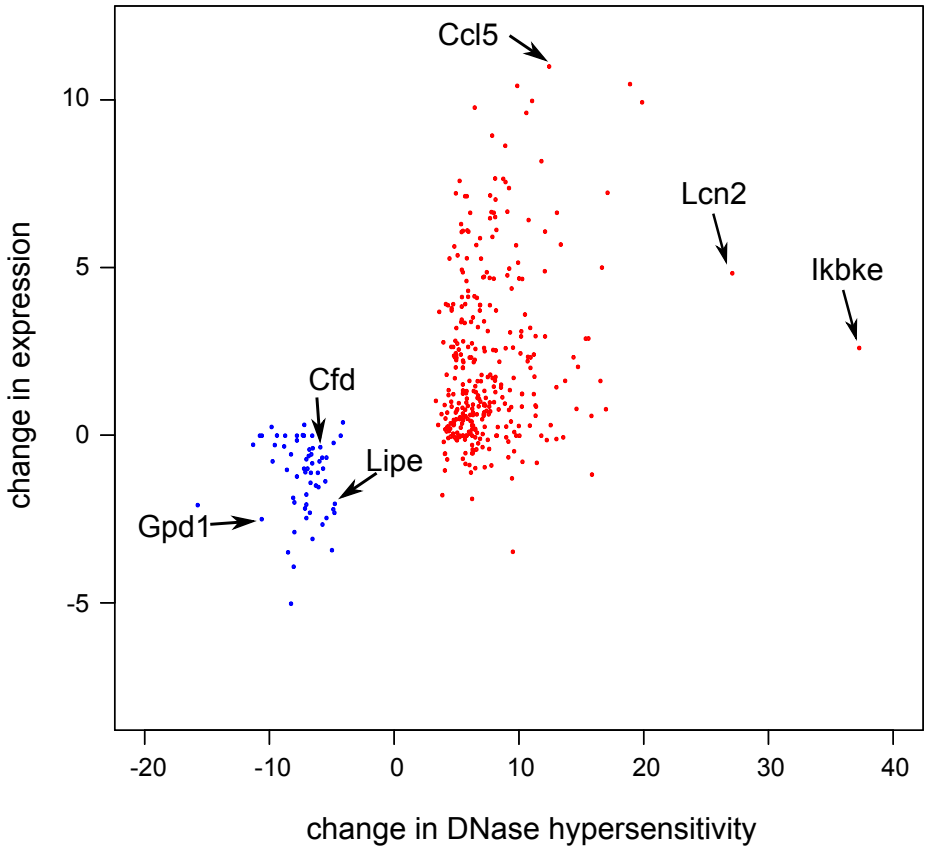
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change in expression
change in DNase hypersensitivity