Supplementary Materials: Figures and Tables.

Figure Captions.

Supplemental Figure 1. Principal components of expression for DEX and Sham treated LCLs showing technical replicates. Multidimensional Scaling (MDS) was used to identify principle components. Each array is shown as either DEX or Sham treated and technical replicates are over plotted with an X and linked by lines. A short or no line indicates good experimental control. A long horizontal line suggests that treatment condition was mislabeled.

Supplemental Figure 2. Principal components of paired DEX Sham LCL expression arrays after QC. The paired DEX Sham arrays for each subject are linked by a line.

Supplemental Figure 3. Distribution of Steroid Responsiveness Endophotype (SRE) among 104 CAMP participants included in Network modeling.

Supplemental Figure 4. The seven first-degree SRE neighbor genes displayed on a typical differential expression volcano plot of LCL differential DEX expression ($\log_2$(DEX) – $\log_2$(Sham)). Typical gene selection significance (*e.g.*, corrected p-value < 0.05) and fold change (*e.g.*, +/- 2 fold) cutoffs would have identified only one of the genes identified by the interaction network.

Supplemental Figure 5. The association of LCL DEX response ($\log_2$(DEX) – $\log_2$(Sham)) with SRE for first-degree SRE neighbor genes. A simple linear regression line and its associated p-value are shown.

Supplementary Figure 6. Effects of candidates genes EDARADD and FICD on NFκB activation and anti-inflammation effect of glucocorticoid. (A) A549/NFκB-Luc cells were transfected with non-targeting (NT) siRNA or siRNA targeting candidate genes, and 88 h later mRNA levels of candidate gene were measured using qPCR and normalized to the level in control siRNA transfected cells. (B) A549/NFκB-Luc cells were transfected with siRNA as in (A). 72 h after transfection, cells were treated with 5 ng/mL IL1β for 16h. The luciferase (Luc) activity was measured and normalized with the amount of total protein. The Luc level was shown relative to that of untreated NT siRNA transfected cells. (C) In the same experiment as shown in (B), additional groups of cells were treated with 5 ng/mL IL1β+100 nM DEX and the Luc level normalized with that upon 5 ng/mL IL1β treatment to determine the percentage values shown. **, *P*<0.01; ***, *P*<0.005; n.s., no significant difference.

Supplemental Figure 7. Two methods of measuring Specific Heat vs Temperature during generation and convergence of the second ensemble of 256 networks. Temperature is a parameter of the Markov Chain Monte Carlo simulation used to converge the REFS model. Specific Heat (CV) is a measure of change in model complexity per unit Temperature. CV can be computed as variance of ensemble scores (E) divided by T^2. dE/dT refers to change in ensemble score (E) over change in temperature and represents a different method of estimating specific heat. The simulation runs from right to left, starting at high Temperature, moving to lower Temperature. As Temperature decreases, the Model Specific Heat increases, indicating more rapid convergence of the Model, until the Temperature reaches ~1.5, when Specific Heat begins to decrease and the Model makes smaller improvements toward convergence. The two graphs may (and in this case do) diverge slightly because the CV method of estimation is sensitive to appearances of local minima in the ensemble and the red curve may bend upwards once ensembles are reaching local minima. A ratio of CV to dE/dT is <= 2 indicates acceptable Model convergence.

Supplemental Figure 8. Ensemble Score vs Temperature during generation and convergence of the second ensemble of 256 networks. Temperature is a parameter of the Markov Chain Monte Carlo simulation used to converge the REFS model. Mean Cost is a measure of model complexity, which decreases as the Temperature decreases. Specific Heat (Supplemental Figure 7) tracks the derivative of Mean Cost. The simulation runs from right to left, starting at high Temperature, moving to lower Temperature. Horizontal ticks in the plot indicate individual temperature steps in the MCMC simulation (shown with the fit line). This plot shows convergence of the second REFS Ensemble reached when the MCMC simulation ends at Temperature=1.

Supplemental Figure 9. Neighborhoods of the 7 direct neighbors of SRE from the second REFS network Interaction Network. Edge width is proportional to, and edges are labeled with, the proportion of the 256 ensemble networks that contain the edge. Vertex type is indicated by a red star: SRE; circle: gene. Neighborhoods, indicated by colored outlines, were determined as described in methods. Symbol size is proportional to the number of all interaction network edges incident on the vertex, some of which may not be shown. The figures were plotted using the same layout as Figure 2.

Supplemental Tables.

Supplemental Table 1.

SRE 1st Neighbor Gene	Freq	Baseline mean	SRE effect	P-value	Correlation	Correlation P-value	Illumina Probe ID
CEP152	0.754	-0.015	0.180	0.007	0.826	<0.001	ILMN_1732311
EDARADD	0.750	0.007	0.104	0.012	0.657	<0.001	ILMN_1761820
FAF1	0.617	0.052	-0.135	0.026	0.894	<0.001	ILMN_1666634
FAM129A	0.578	-0.240	0.109	0.039	0.835	<0.001	ILMN_1810725
FAM174B	0.742	0.961	0.156	0.020	0.774	<0.001	ILMN_1652797
FICD	0.879	-0.189	0.152	0.001	0.682	<0.001	ILMN_1778064
YY1	0.949	-0.063	0.143	<0.001	0.777	<0.001	ILMN_1770892

Supplemental Table 1. Descriptive statistics for SRE first-neighbor genes in the interaction network produced from the second REFS Ensemble of 256 networks. Baseline mean, SRE effect, and p-value, were produced from REFS all-versus-all simulation that perturbs every variable and determines the effect on every other variable. Correlation and Correlation p-value are produced by simulating the maximum likelihood estimates for these genes and comparing to DEX response data. Edges were created in the interaction network for every such effect with a P-value of < 0.05 (labeled “P-value” above). Freq: the proportion of Ensemble networks that contain an edge connecting SRE with the gene. Baseline mean: Mean simulated value; average LCL DEX response (Log_2(DEX) – Log_2(sham). SRE effect: Mean simulated change from baseline due to perturbation of SRE; estimated log_2 fold change effect on LCL DEX response caused by perturbation of SRE. P-value: P-value of the SRE effect. Correlation: Spearman correlation of simulated response vs observed DEX response (Log_2(DEX) – Log_2(sham). Correlation P-value: P-Value of the spearman correlation statistic.

Supplemental Table 2.

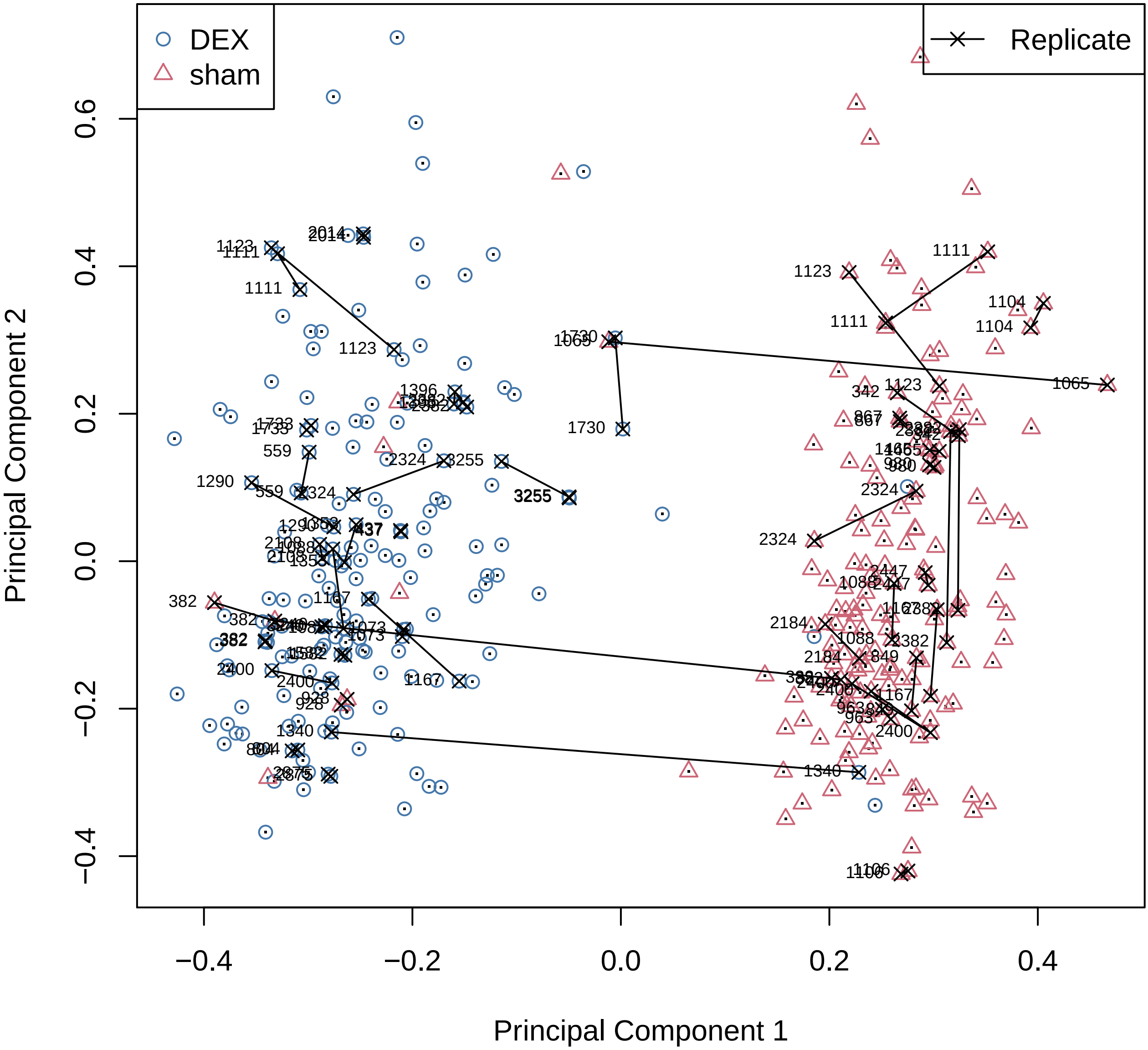
	AUC (95% conf)	**p-value vs. SRE**
SRE	0.76 (0.70-0.81)	-
AMSYMP	0.66 (0.59-0.72)	<.001
PREFEV	0.68 (0.62-074)	0.001
LNPC20	0.68 (0.61-0.74)	0.018
BDRABPCT	0.61 (0.54-0.67)	<.001
EDHOS	0.58 (0.53-0.63)	<.001
BURSTS	0.62 (0.56-0.67)	<.001

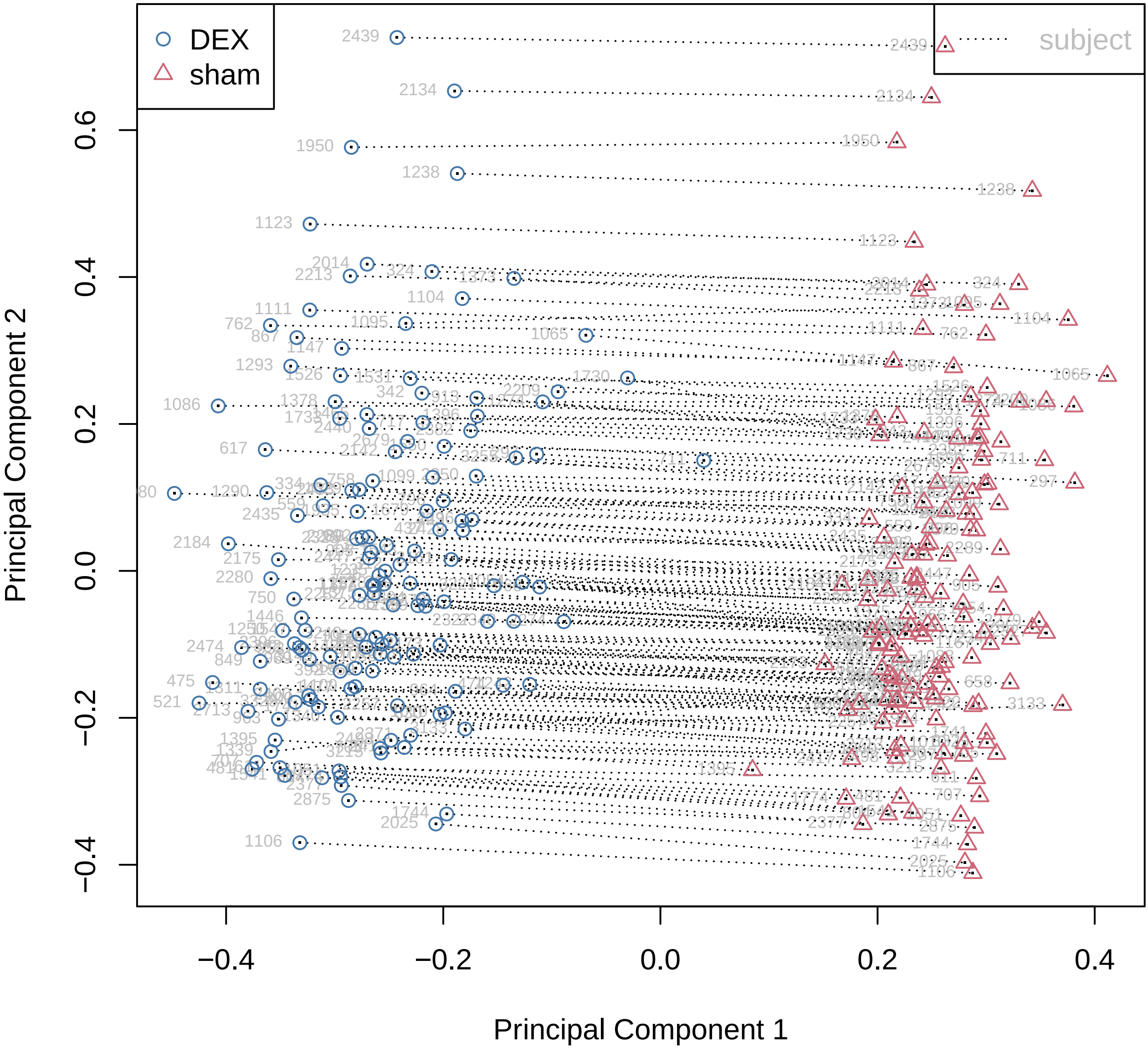
Supplemental Table 2. The accuracy (AUC and lower and upper 95% confidence interval) with which ICS responsiveness was measured in cohort subjects. SRE = Steroid Responsiveness Endophenotype; AMSYMP = asthma symptoms: PREFEV = pre-bronchodilator FEV1; LNPC20 = airway responsiveness, natural log provocative concentration of methacholine required to effect 20% reduction in FEV1; BDRABPCT = bronchodilator response, percent change; EDHOS = Emergency Department visit or Hospitalization for asthma; BURSTS = supplemental oral corticosteroid courses required for asthma symptom control. P-values are single-sided p-values showing difference in AUC from SRE.

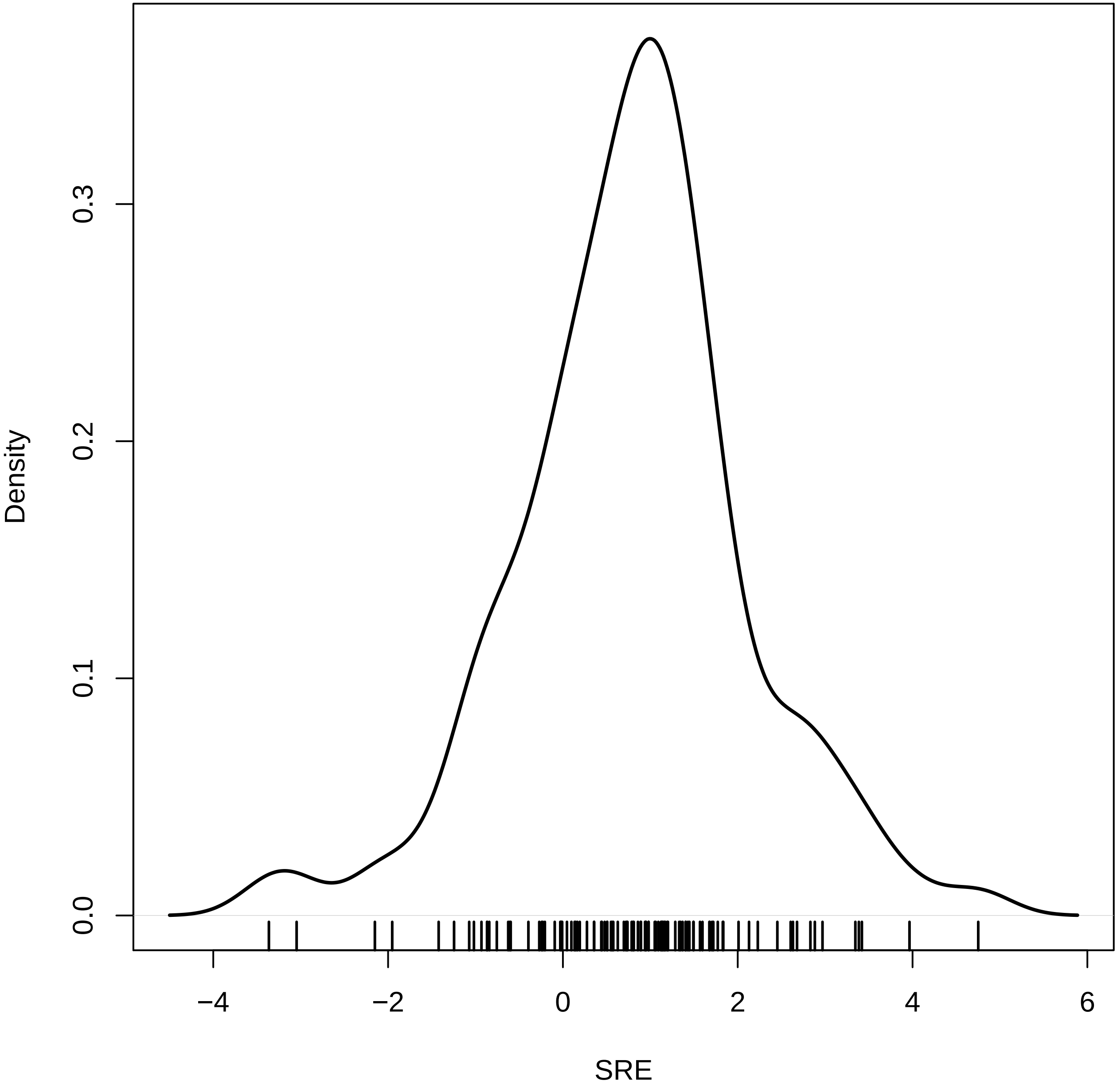
Supplemental Table 3.

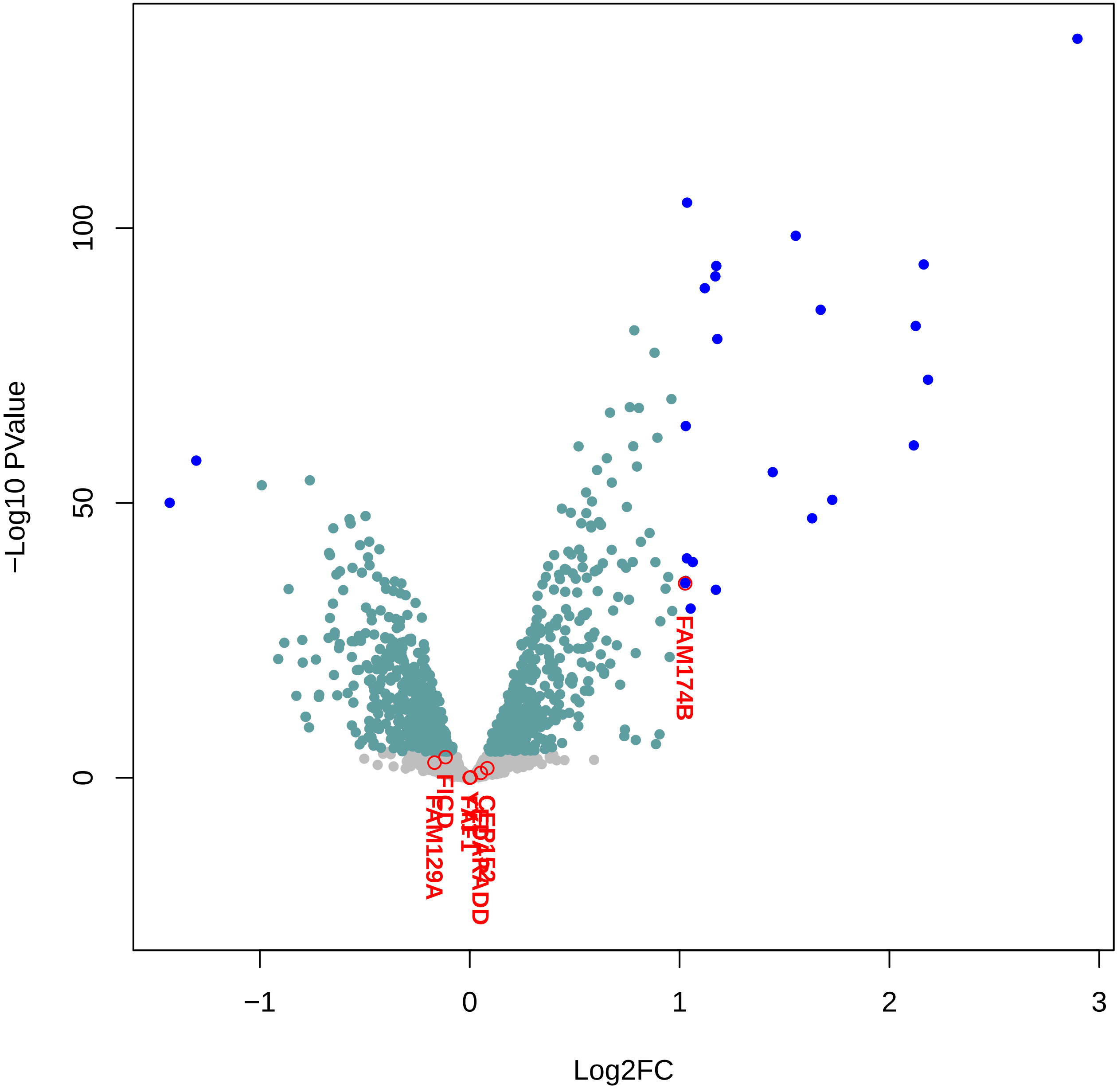
Differential Expression Cutoff	Connected to SRE	Not Connected	Total
$p >= 0.05$	2348 (87%)	334	2682
$p < 0.05$ & $\lvert Log_2(FC) \rvert <= 1$	931 (91%)	95	1026
$p < 0.05$ & $\lvert Log_2(FC) \rvert > 1$	23 (96%)	1	24
Total	3302 (88%)	430	3732

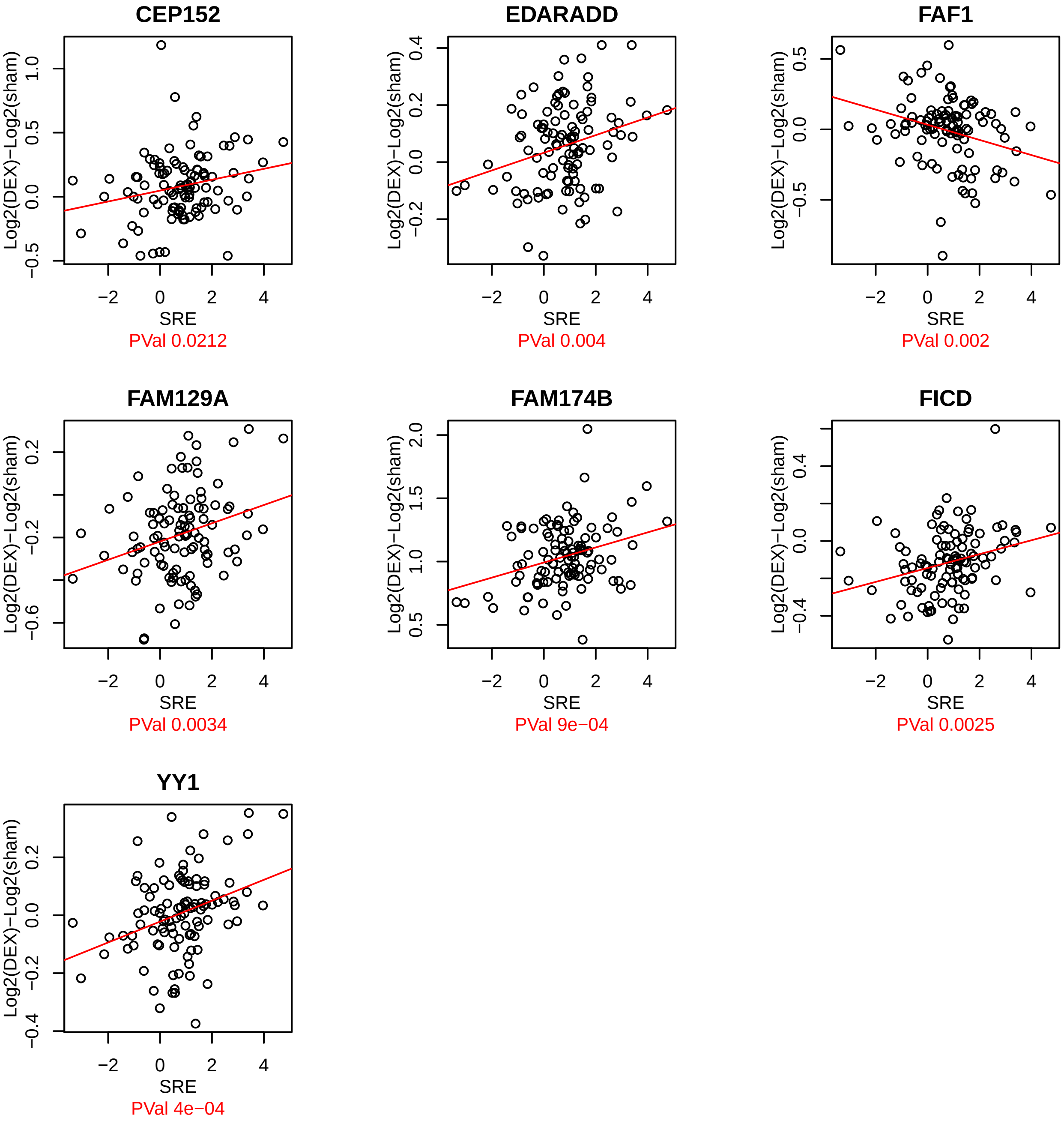
Supplemental Table 3. Occurance of training set genes from the second REFS ensemble interaction network grouped by SRE connection status and differential expression cutoff. Connected to SRE: genes that were connected directly or indirectly to SRE in the SRE subgraph of the interaction network. Not Connected: genes that were located in isolated subgraphs (Total N = 28) or not present in the interaction network (Total N = 419).

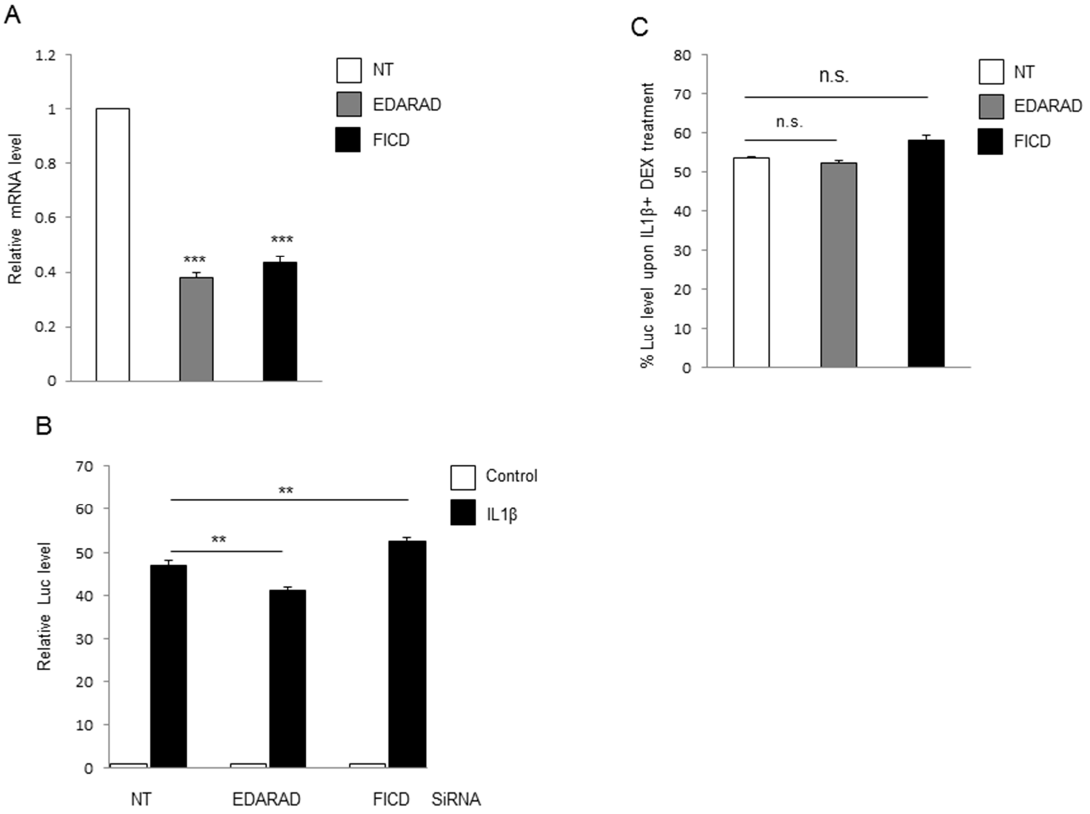

DEX
sham
Replicate
Principal Component 1
Principal Component 2
−0.4
−0.2
0.0
0.2
0.4
0.6
1123
1111
2014
1730
1065
1396
1733
559
2324
3255
1290
437
2108
1088
1353
1167
382
1073
1582
2400
928
1340
804
2875
1104
342
867
1465
980
2447
2184
849
963
1106

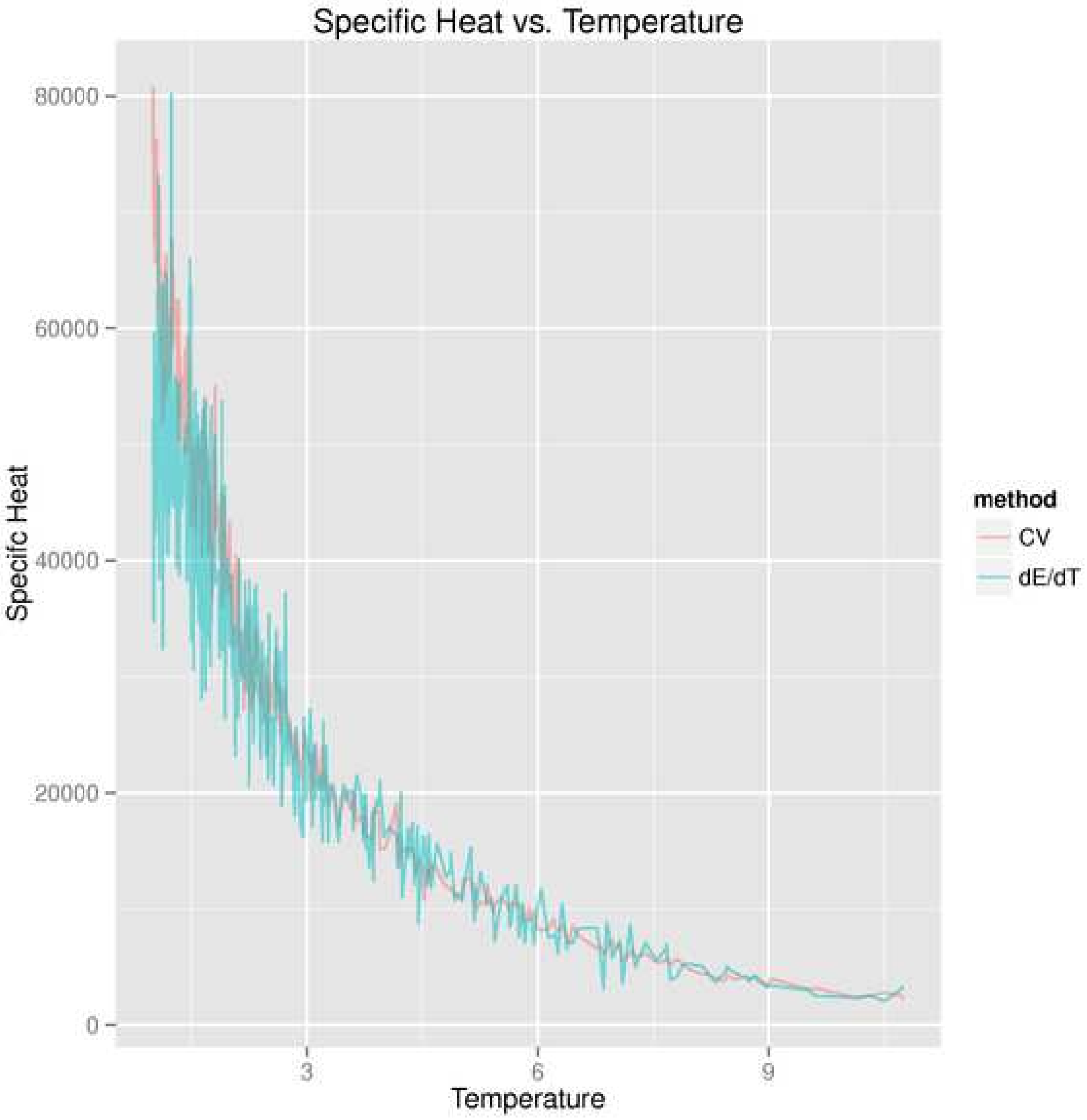

DEX
sham
subject
Principal Component 1
Principal Component 2
−0.4
−0.2
0.0
0.2
0.4
0.6
2439
2134
1950
1238
1123
2014
2213
324
1373
1104
1111
762
1095
867
1065
1147
1293
1526
1531
1730
342
2209
1086
1378
1735
2440
617
2142
711
1290
334
559
1099
2435
2184
2175
2280
750
1446
2474
849
475
1311
521
2713
963
1395
1339
1341
2377
2875
1744
2025
1106
297
3133
658
1774
2417
1167
1741
611
707
3215
2289
905
2447
2679
2382
2252

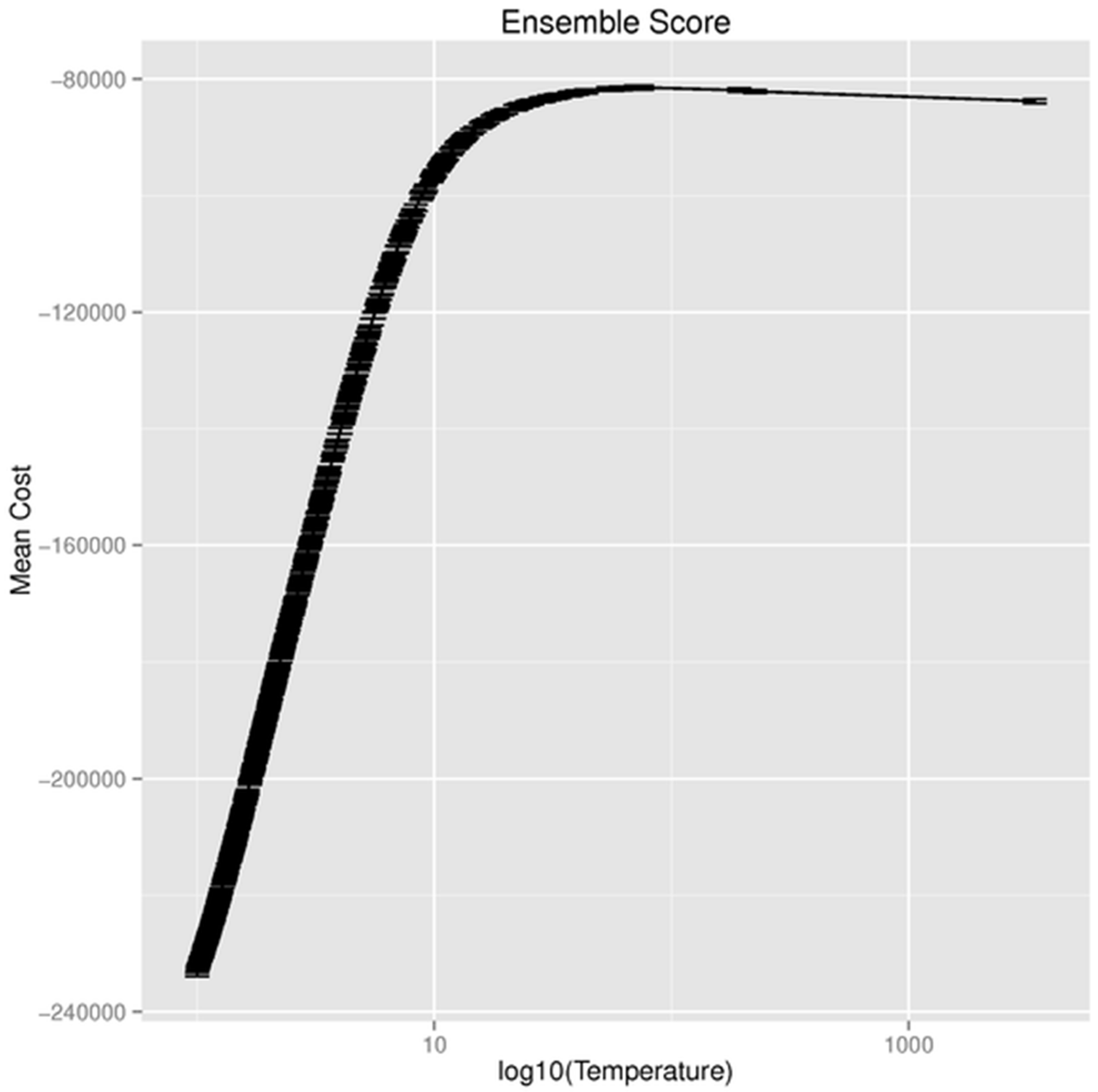

Density
0.0
0.1
0.2
0.3
−4
−2
0
2
4
6
SRE

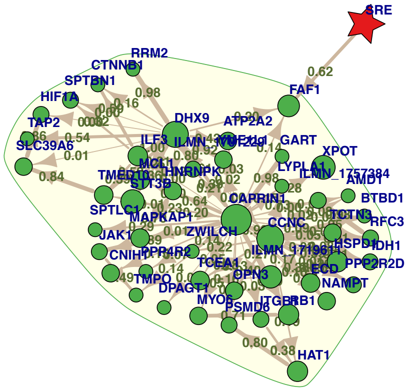

−Log10 PValue
100
50
0
Log2FC
−1
0
1
2
3
FAM174B
FICD
FAM129A
FAF1
CEP152

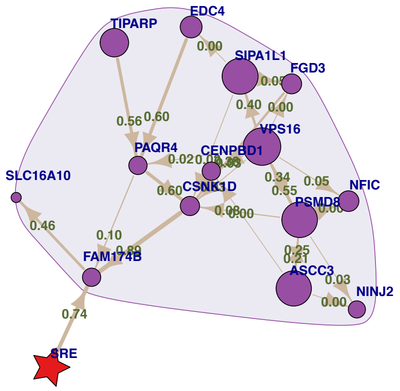

CEP152
Log2(DEX)−Log2(sham)
−0.5
0.0
0.5
1.0
−2
0
2
4
SRE
PVal 0.0212
EDARADD
Log2(DEX)−Log2(sham)
−0.2
0.0
0.2
0.4
−2
0
2
4
SRE
PVal 0.004
FAF1
Log2(DEX)−Log2(sham)
−0.5
0.0
0.5
−2
0
2
4
SRE
PVal 0.002
FAM129A
Log2(DEX)−Log2(sham)
−0.6
−0.2
0.2
−2
0
2
4
SRE
PVal 0.0034
FAM174B
Log2(DEX)−Log2(sham)
0.5
1.0
1.5
2.0
−2
0
2
4
SRE
PVal 9e−04
FICD
Log2(DEX)−Log2(sham)
−0.4
0.0
0.4
−2
0
2
4
SRE
PVal 0.0025
YY1
Log2(DEX)−Log2(sham)
−0.4
−0.2
0.0
0.2
−2
0
2
4
SRE
PVal 4e−04

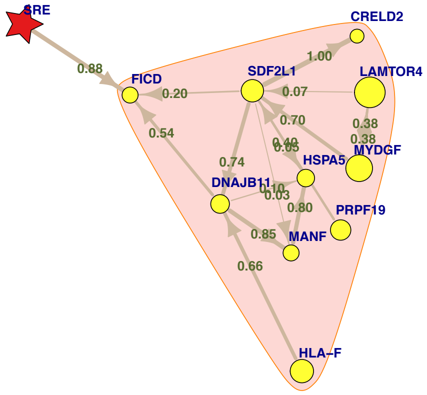

A
Relative mRNA level
1.2
1
0.8
0.6
0.4
0.2
0
***
***
NT
EDARAD
FICD
B
Relative Luc level
70
60
50
40
30
20
10
0
**
**
Control
IL1β
NT
EDARAD
FICD
SiRNA
C
% Luc level upon IL1β+ DEX treatment
80
70
60
50
40
30
20
10
0
n.s.
n.s.
NT
EDARAD
FICD

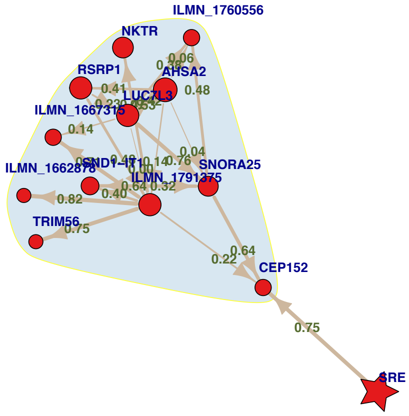

Specific Heat vs. Temperature
Specific Heat
80000
60000
40000
20000
0
3
6
9
Temperature
method
CV
dE/dT

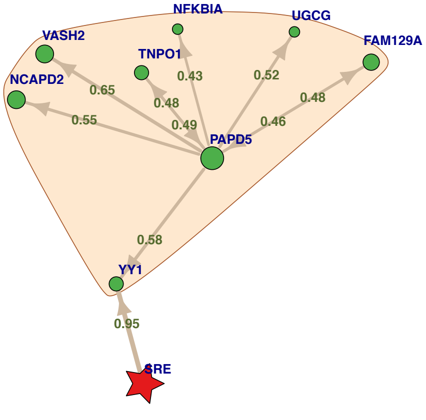

Ensemble Score
Mean Cost
-80000
-120000
-160000
-200000
-240000
10
1000
log10(Temperature)

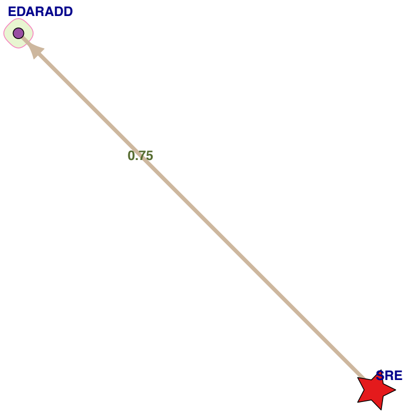

SRE
0.62
RRM2
CTNNB1
SPTBN1
HIF1A
TAP2
SLC39A6
0.98
0.16
0.54
0.01
0.84
DHX9
ATP2A2
FAF1
0.20
GART
XPOT
ILF3
MCL1
HNRNPK
TMED10
STT3B
LYPLA1
ILMN_1757384
AMD1
BTBD1
CAPRIN1
SPTLC1
MAPKAP1
TCTN3
RFC3
CCNC
ZWILCH
JAK1
HSPD1
IDH1
ILMN_1719611
CNIH1
PPP4R2
PPP2R2D
TCEA1
ECD
OPN3
TMPO
NAMPT
DPAGT1
MYO6
RB1
PSMD6
HAT1
0.80
0.38
0.71
0.64
0.98
0.14
0.22
0.29
0.23


TIPARP
EDC4
0.00
SIPA1L1
FGD3
0.05
0.40
0.00
0.56
0.60
VPS16
PAQR4
CENPBD1
0.02
SLC16A10
CSNK1D
0.60
0.34
0.05
NFIC
0.55
PSMD8
0.46
0.08
0.00
0.10
0.89
0.25
0.21
FAM174B
ASCC3
0.03
0.00
NINJ2
0.74
SRE


SRE
0.88
FICD
0.20
SDF2L1
0.07
CRELD2
1.00
LAMTOR4
0.54
0.70
0.38
0.38
MYDGF
0.74
0.49
HSPA5
DNAJB11
0.10
0.03
0.80
PRPF19
0.85
MANF
0.66
HLA-F


ILMN_1760556
NKTR
RSRP1
AHSA2
0.06
0.48
0.41
LUC7L3
ILMN_1667315
0.14
0.04
SND1-IT1
0.14
0.76
SNORA25
ILMN_1662878
ILMN_1791375
0.64
0.32
0.40
0.82
TRIM56
0.75
0.64
0.22
CEP152
0.75
SRE


NFKBIA
UGCG
VASH2
TNPO1
FAM129A
NCAPD2
0.65
0.43
0.52
0.48
0.48
0.55
0.49
0.46
PAPD5
0.58
YY1
0.95
SRE


EDARADD
0.75
SRE