Supporting Information

Ammeux et al. 10.1073/pnas.1610432113

Fig. S1. Comparison of RNA-seq and RT-qPCR and Nanostring nCounter methods. (*A*) Basal gene expression levels in S2-DRSC, Kc, and S2R+ cell lines determined using Nanostring nCounter and modENCODE RNA-seq data available from FlyBase (version 6.03). The graph represents normalized Nanostring counts ($\log_{10}$, *y* axis) as a function of RNA-seq reads per kilobase of transcript per million mapped reads (RPKM) ($\log_{10}$, *x* axis). The linear trends observed for each cell line plot indicate the strong correlation between Nanostring and modENCODE data. (*B*) Pearson correlation coefficiency scores of the average Nanostring counts versus RNA-seq.

Fig. S1

Fig. S2. Fold induction versus basal expression level. The plot represents basal gene expression level ($\log_2$ average normalized counts under control conditions, *x* axis) as a function of log2 fold change between stimulated and control conditions (*y* axis). A Pearson correlation score (−0.015) was calculated between all basal expression levels and fold inductions measured across all pathway assays and demonstrates the absence of correlation.

Fig. S2

Fig. S3. Enlarged volcano plots for each single and combinatorial assay. Data are represented for single and multicombinatorial assays (Figs. 1*D* and 2*B*). Red circles indicate high-confidence hits, purple circles represent medium-confidence hits, and blue circles represent low-confidence hits. Black dots represent nonscoring genes.

Fig. S3

Dataset S1. Basal expression level of ligand and receptor genes in *Drosophila* cell lines

Dataset S1

Summary of RNA-seq data of *Drosophila* ligand gene expression levels in S2R+, S2-DRSC, and Kc cell lines. RNA-seq data are from Fly Cell Line Expression Level (www.flyrnai.org/cellexpress) of the *Drosophila* RNAi Screening Center (DRSC) (fgr.hms.harvard.edu). The majority of ligands are either not expressed or expressed at a low level in the cell lines.

Dataset S2. Customized Nanostring nCounter code sets

Dataset S2

Tab 1 lists the Nanostring code set: version and summary of gene number and type (ligand, receptor, reporter, housekeeping gene, and others). Tabs 2 and 3 list details of the two code set versions: gene name, FlyBase identification (ID), code set probe ID, probe sequences, and lengths. Tab 4 shows the match between Nanostring code set probes and relative target gene transcript(s). Alignment was validated using version 6.03 *Drosophila* genome annotation from FlyBase.

Dataset S3. Nanostring gene expression dataset for single and combinatorial assays

Dataset S3

Expression data and statistics (counts, ratios, *t* test type and *P* values, hit selection criteria) for all genes included in the Nanostring code set (reporters, ligands, receptors, and housekeeping genes) following single- and dual-pathway assays with three biological replicates at two time points (30 min and 1 h). Hits are colored in red, blue, and purple according to confidence levels (high, medium, and low, respectively). The cell line used for each treatment is shown in the first column.

Dataset S4. Genes regulated by single- and combinatorial pathway stimulations

Dataset S4

Summary of genes regulated by either single- or combinatorial pathway stimulations.

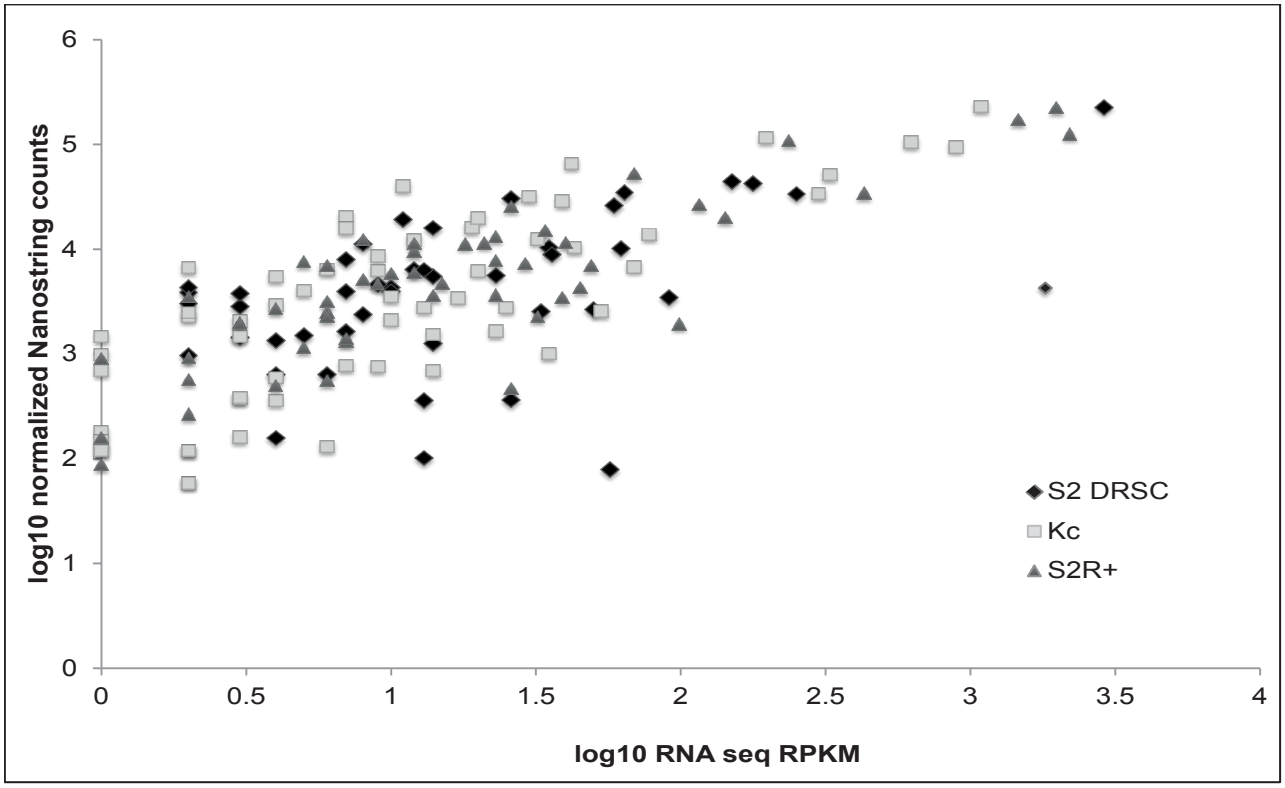
Dataset S5. Reference genes selected per cell line to normalize the Nanostring nCounter data

Dataset S5

An unbiased method was used to select reference genes across both code sets and for each cell line to normalize Nanostring nCounter data. Ten genes ranging from low to high expression and showing the lowest coefficient of variation per cell line across all assays were selected as references. The reference sets also included two housekeeping genes commonly used for *Drosophila* gene expression analysis (*alphaTub84B* and *gapdh1*).

Supplementary Figure 1

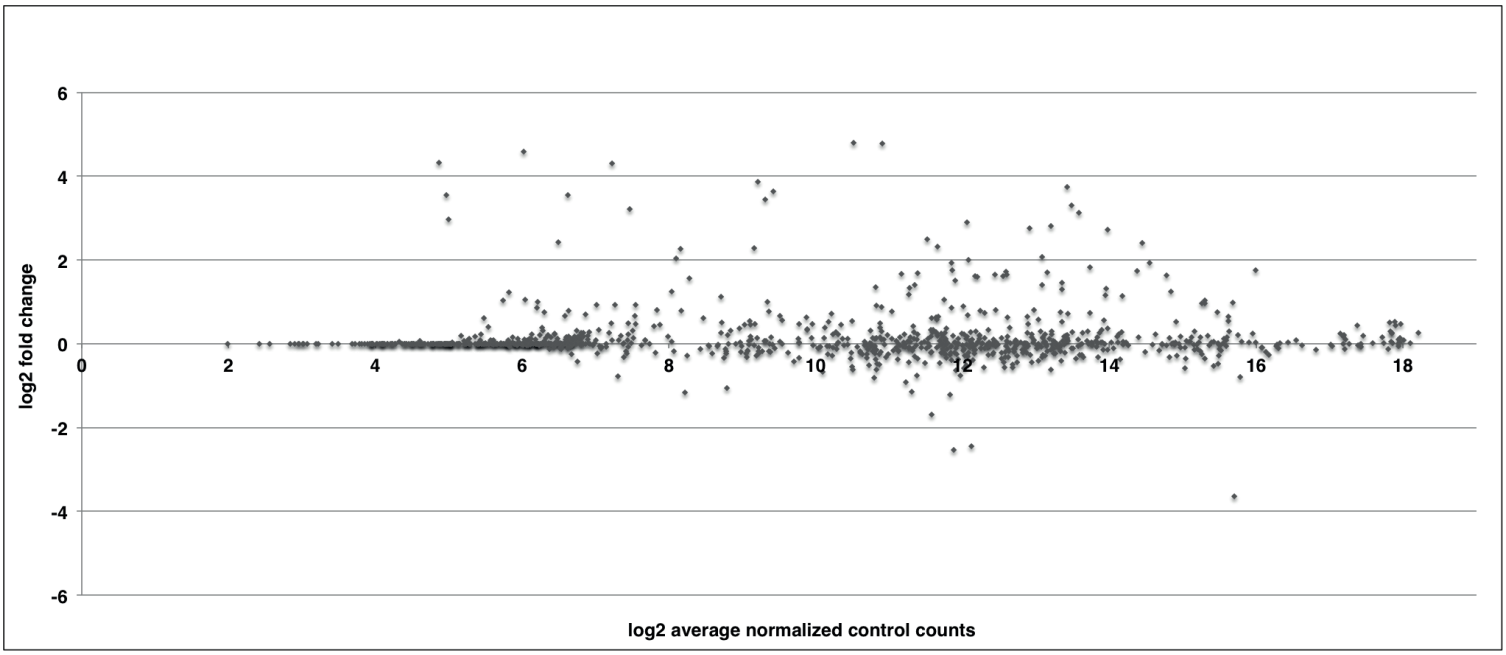
A



B

Comparison of Nanostring versus RPKM RNA seq data	
Cell line	Pearson correlation score
Kc	0.89
S2R+	0.90
S2 DRSC	0.81

Supplementary Figure 2



JNK

-log10 p-value

7
6
5
4
3
2
1
0

Rel
upd2
puc
Tollo
rho
Socs36E
upd3
AttA
pyr
Thor
Pvf2
CycE
vn
Sulf1
Pvr
ths
Pvf3
E(spl)m3
shg
dpp
E(spl)mbeta
ptc
fz
Ser
Act5C
aos

-5 -4 -3 -2 -1 0 1 2 3 4 5

log2 fold change

JAK/STAT

-log10 p-value

4

3

2

1

0

pyr

Socs36E

Ptp61F

upd

aos

ths

Ser

Thor

upd3

-2

-1

0

1

2

log2 fold change

EGFR

-log10 p-value

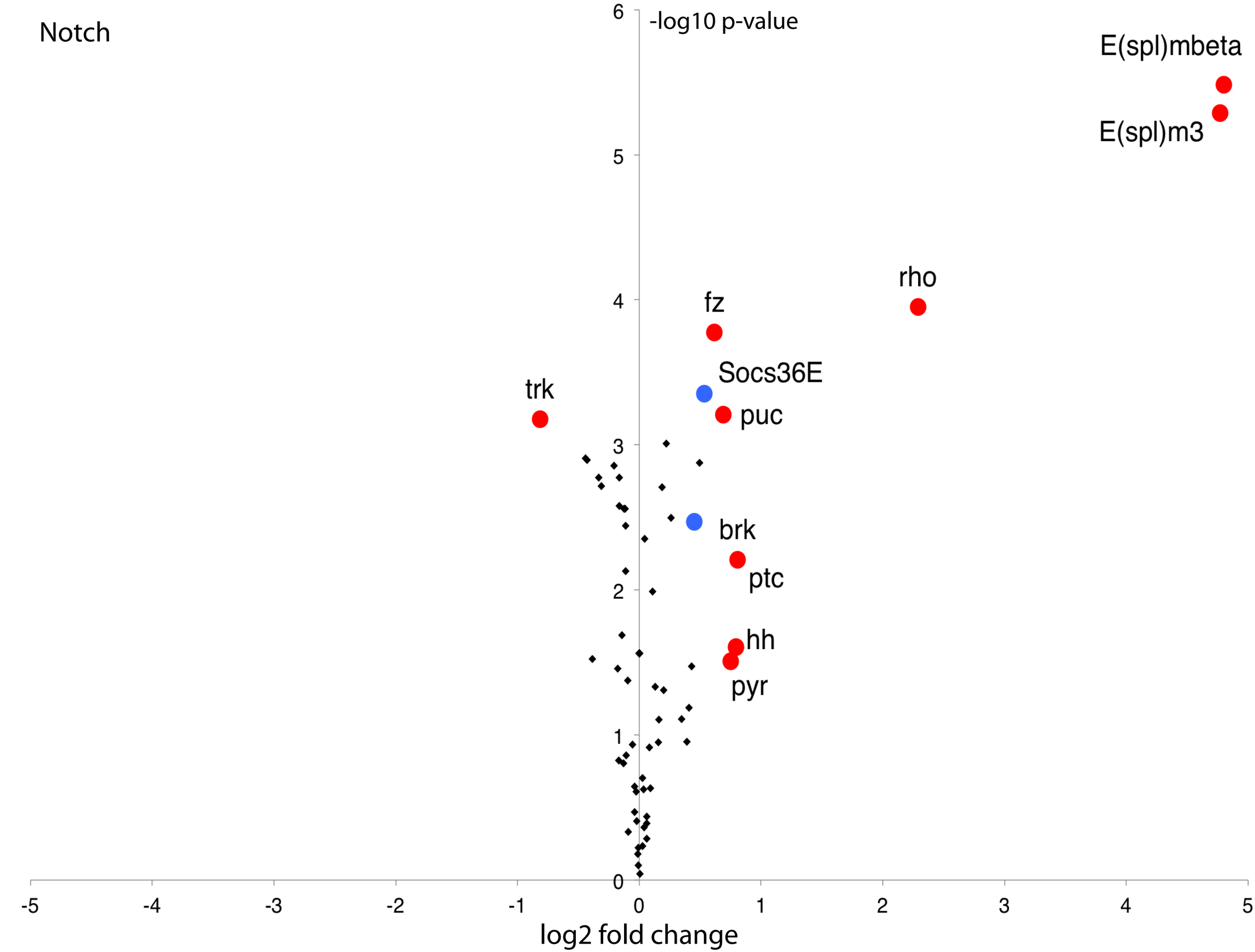
6
5
4
3
2
1
0

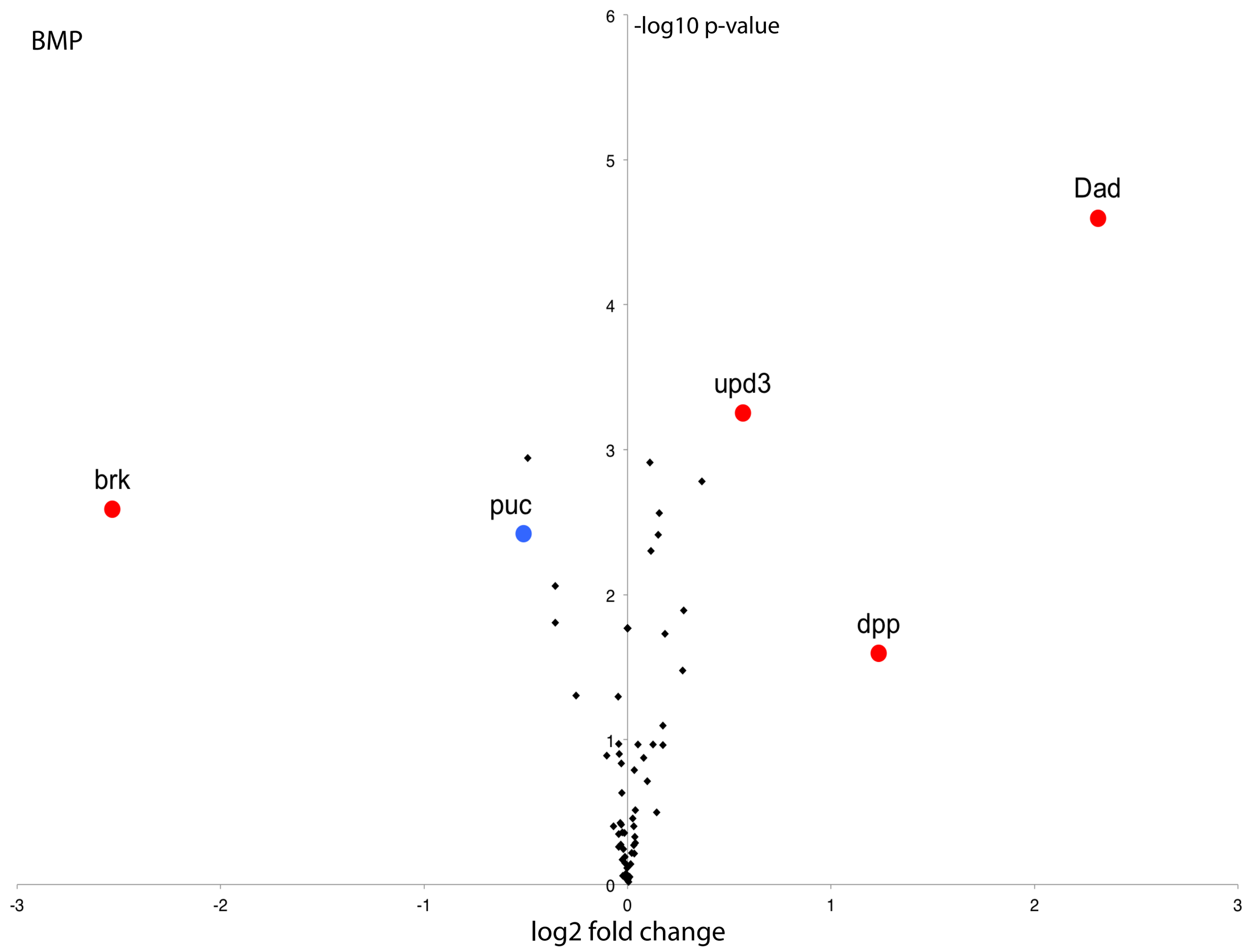
spi
upd3
Socs36E
upd2
aos
ths

-2
-1
0
1
2

log2 fold change

Notch


-log10 p-value
6
5
4
3
2
1
0
E(spl)mbeta
E(spl)m3
rho
fz
Socs36E
puc
trk
brk
ptc
hh
pyr
-5
-4
-3
-2
-1
0
1
2
3
4
5
log2 fold change


BMP
-log10 p-value
6
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2
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0
Dad
upd3
brk
puc
dpp
-3
-2
-1
0
1
2
3
log2 fold change

Insulin

-log10 p-value

4

3

2

1

0

Tollo

upd3

E(spl)mbeta

Rel

Socs36E

-2

-1

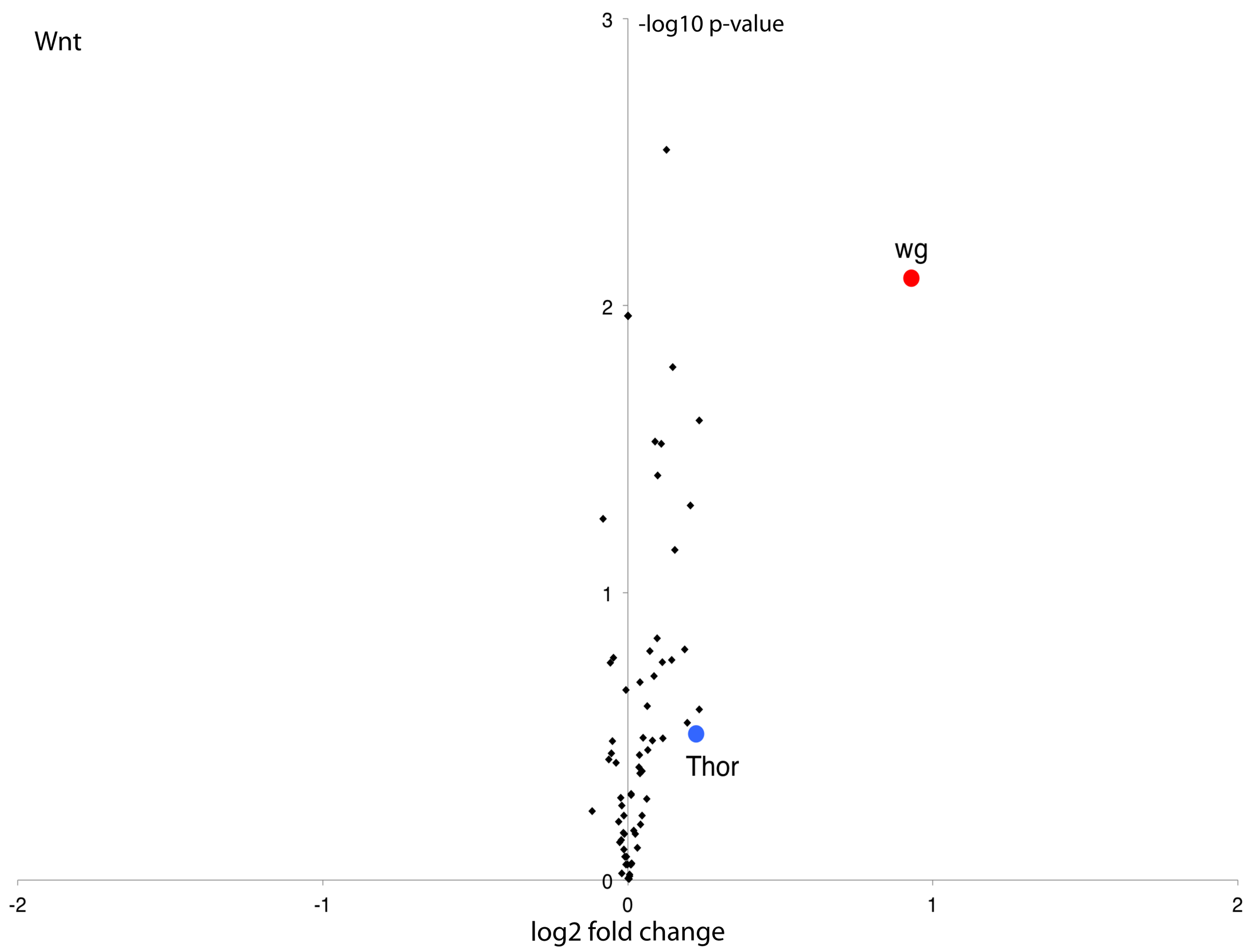
0

1

2

log2 fold change

Wnt



Insulin + JNK

-log10 p-value

3

2

1

0

N

Pvf2

fz2

E(spl)m3

Dad

Sulf1

trk

CycE

AttA

rho

InR

puc

Rel

upd3

E(spl)mbeta

shg

fz

pyr

upd2

Tollo

vn

dilp6

spi

bnl

ths

Socs36E

dally

Ptth

Thor

dpp

aos

dilp1

Egfr

Alk

gbb

sgg

Tl

grk

spz

Pvf1

numb

Ser

tkv

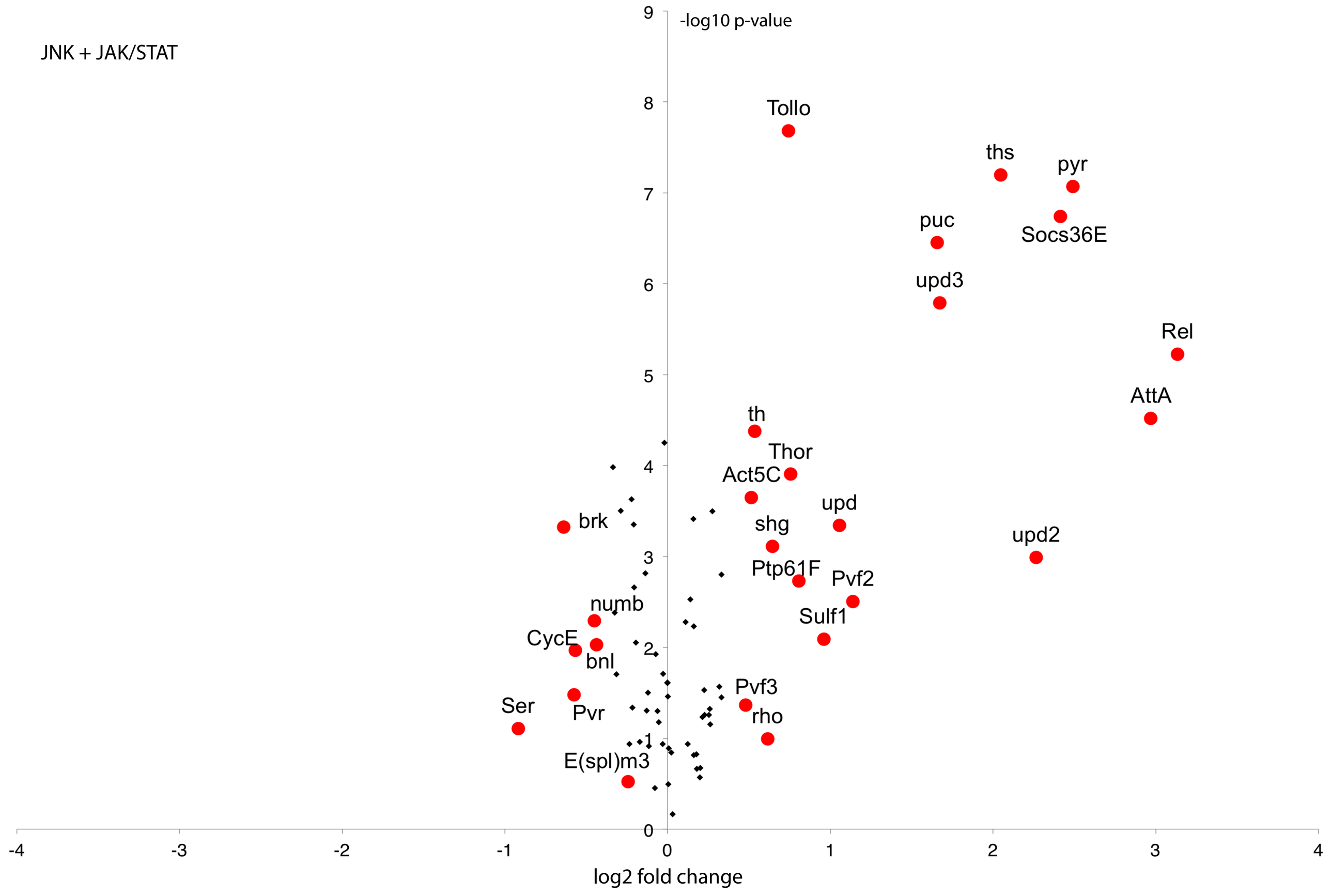
Pvf3

Wnt5

-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6

log2 fold change

JNK + JAK/STAT


-log10 p-value
log2 fold change
Tollo
ths
pyr
puc
Socs36E
upd3
Rel
AttA
th
Thor
Act5C
upd
shg
brk
upd2
Ptp61F
Pvf2
numb
Sulf1
CycE
bnl
Pvf3
Ser
Pvr
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E(spl)m3
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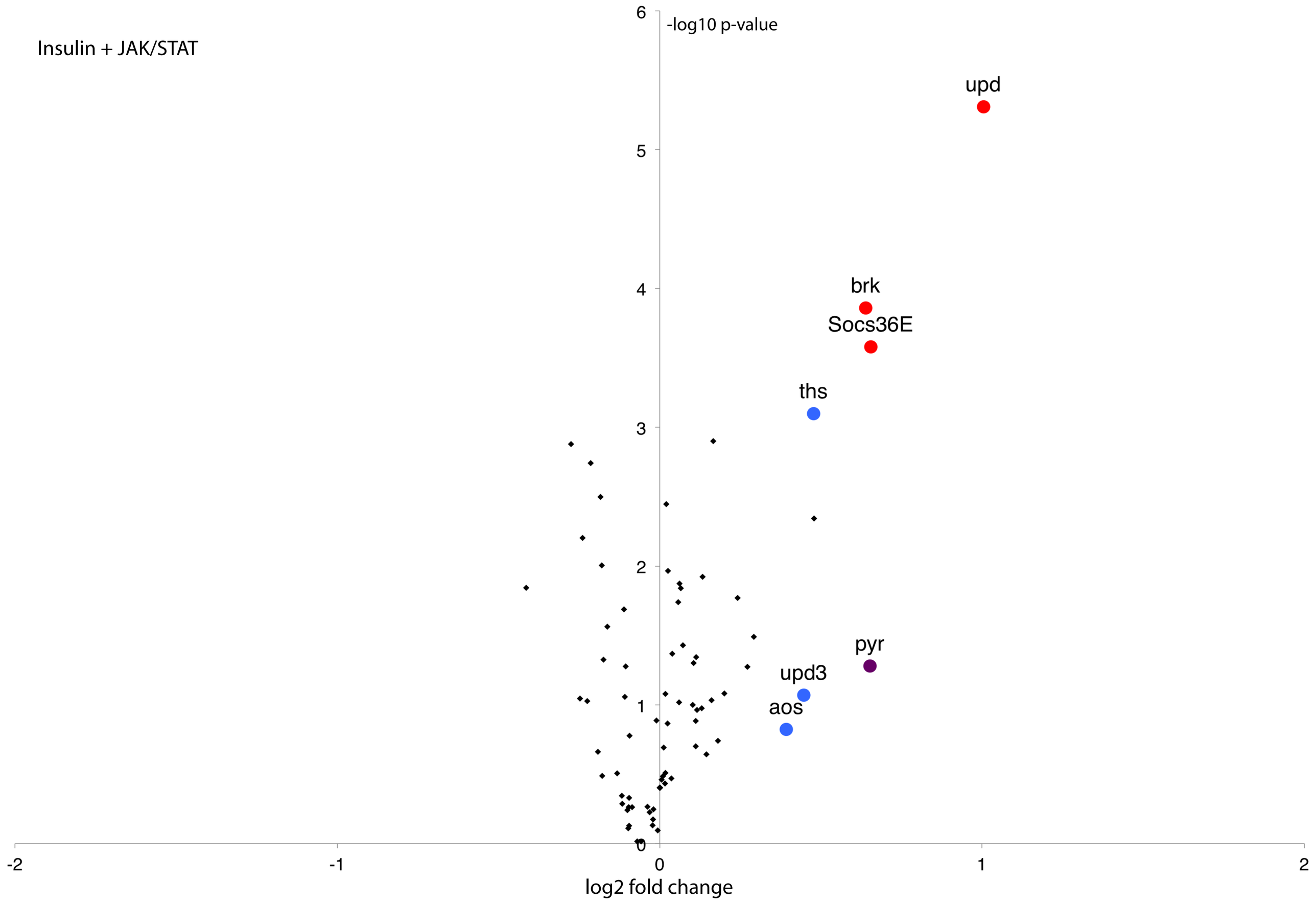
Insulin + JAK/STAT



BMP + JNK

-log10 p-value

8
7
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4
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upd2
Socs36E
Thor
upd3
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AttA
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shg
Act5C
pyr
ds
vn
Pvf2
E(spl)m3
Pvr
Pvf3
Sulf1
CycE
Ser

-5 -4 -3 -2 -1 0 1 2 3 4 5

log2 fold change

BMP + JAK/STAT

-log10 p-value

9
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Socs36E
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log2 fold change