**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 coefficient scores of the average Nanostring counts versus RNA-seq.

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

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

**Dataset S2.** Customized Nanostring nCounter code sets

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

**Dataset S4.** Genes regulated by single- and combinatorial pathway stimulations
Dataset S5. Reference genes selected per cell line to normalize the Nanostring nCounter data

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

Comparison of Nanostring versus RPKM RNA seq data

Cell line | Pearson correlation score
--- | ---
Kc | 0.89
S2R+ | 0.90
S2 DRSC | 0.81

B

Log10 normalized Nanostring counts

Log10 RNA seq RPKM
Supplementary Figure 2

![Supplementary Figure 2](image-url)
JNK

-log10 p-value

log2 fold change

Rel
upd2
rho
Socs36E
upd3
pyr
AttA

CycE
Pvr
E(spl)m3
E(spl)mbeta
ptc
Ser
dpp
Act5C
aos

Thor
Pvf2
Pvf3
Sulf1
shg
fz
ths
vn

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

-log10 p-value

log2 fold change
JAK/STAT

-log10 p-value

log2 fold change

Ptp61F

Socs36E

pyr

Ser

upd

aos

ths

Thor

upd3
EGFR

-log10 p-value

-log2 fold change

spi

upd3

Socs36E

upd2

aos

ths
Notch

-log10 p-value

log2 fold change

E(spl)mbeta

E(spl)m3

trk

fz

Socs36E

rho

ptc

hh

pyr
Insulin + JNK

-log10 p-value

log2 fold change
Insulin + JAK/STAT

-log10 p-value

log2 fold change