SUPPLEMENTARY FIGURES

These figures show the results of the inhibition studies to investigate the regulatory interactions between sentinel proteins. They show how the phosphorylation of a sentinel is affected by the inhibition of the other studied sentinel proteins, i.e., how the inhibition of a protein (regulator) impacts the phosphorylation of another protein (target). The x-axis in the figures shows the phosphorylation level of the target protein when the cells were stimulated with growth factors and no inhibitor was added. The y-axis shows the phosphorylation level of the target protein when the cells were first treated with an inhibitor and then stimulated with the ligands. Hollow points show the control results when the target was itself inhibited, i.e., these points should have low y-values. Solid points show the results when the regulator protein was inhibited. If the regulator and target protein is positively correlated (i.e., if regulator is activating its target), inhibition of the regulator protein would lower the phosphorylation of its target protein as well. Thus, those points would lie below the diagonal line. When the correlation is negative, the points would lie above the diagonal line because inhibition of the regulator protein would remove the suppression of its target and the phosphorylation of the target protein would go up. When there is no effect, the points would line along the diagonal. Results of all cell lines and ligand treatment cases are shown all together. Data points are marked with circles for R2−3− cells, with triangles for R2+3− cells, with diamonds for R2−3+ cells, and with squares for R2+3+ cells.

Supplementary Figure 1. Inhibition studies to investigate the interaction between sentinel proteins. This figure shows how the phosphorylation of ERK was affected by the inhibition of studied sentinel proteins. The x-axis shows the phosphorylation level of ERK when the cells were stimulated with growth factors and no inhibition was added. The y-axis shows the phosphorylation level of ERK when the cells were first treated with an inhibitor and then stimulated with the ligands. Hollow points show the control results when ERK was inhibited itself, i.e., these points should have low y-values. Solid points show the results when the regulator protein was inhibited. Data points are marked with circles for R2−3− cells, with triangles for R2+3− cells, with diamonds for R2−3+ cells, and with squares for R2+3+ cells. Regulator protein is: (A) p38, (B) AKT, (C) JNK, and (D) STAT3.

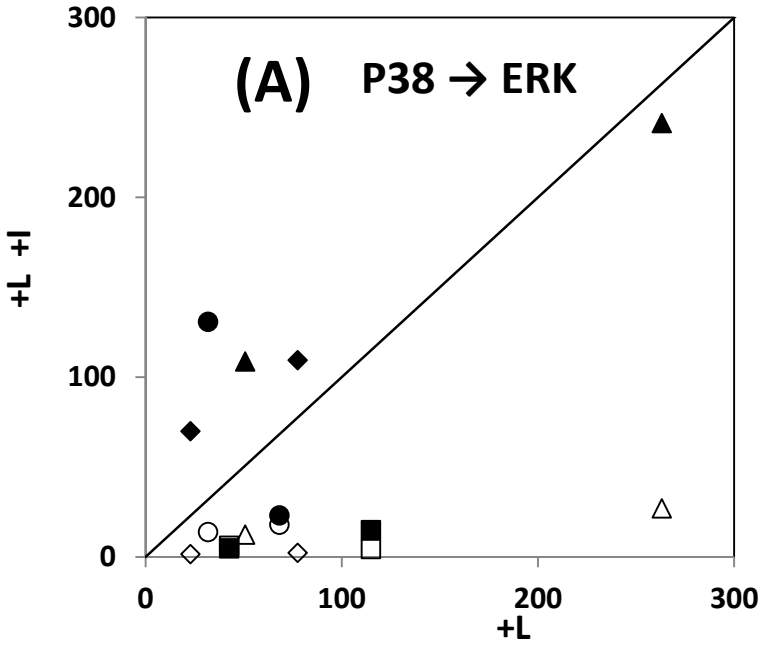
Supplementary Figure 2. Regulation of AKT by the studied sentinel proteins, Regulator → AKT (target). Details are as in Supplementary Figure 1. Regulator protein is: (A) ERK, (B) p38, (C) JNK, and (D) STAT3.

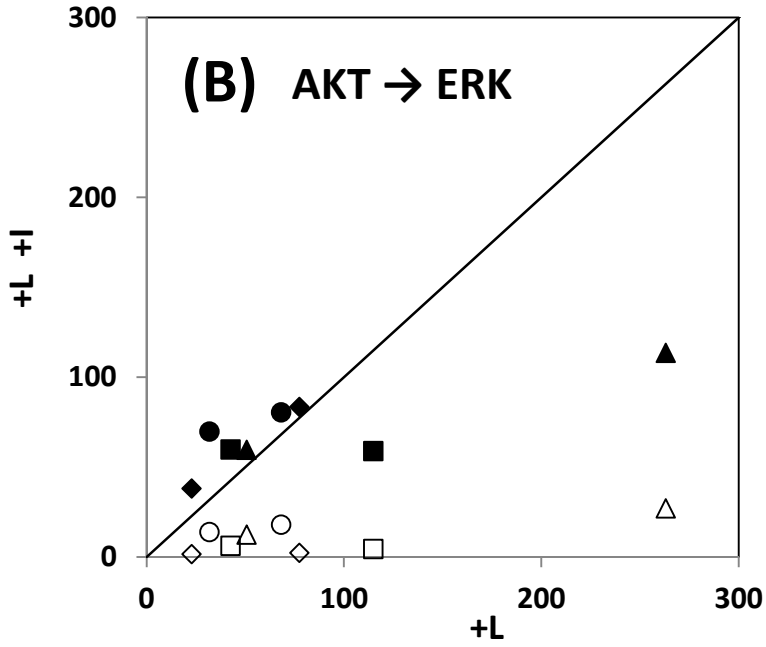
Supplementary Figure 3. Regulation of JNK by the studied sentinel proteins, Regulator → JNK (target). Details are as in Supplementary Figure 1. Regulator protein is: (A) ERK, (B) AKT, (C) p38, and (D) STAT3.

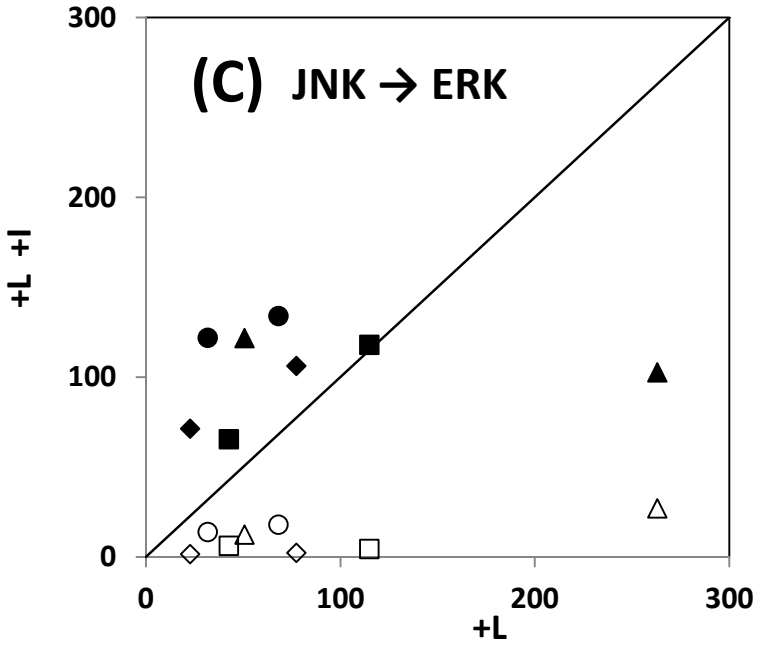
Supplementary Figure 4. Regulation of p38 by the studied sentinel proteins, Regulator → p38 (target). Details are as in Supplementary Figure 1. Regulator protein is: (A) ERK, (B) AKT, (C) JNK, and (D) STAT3.

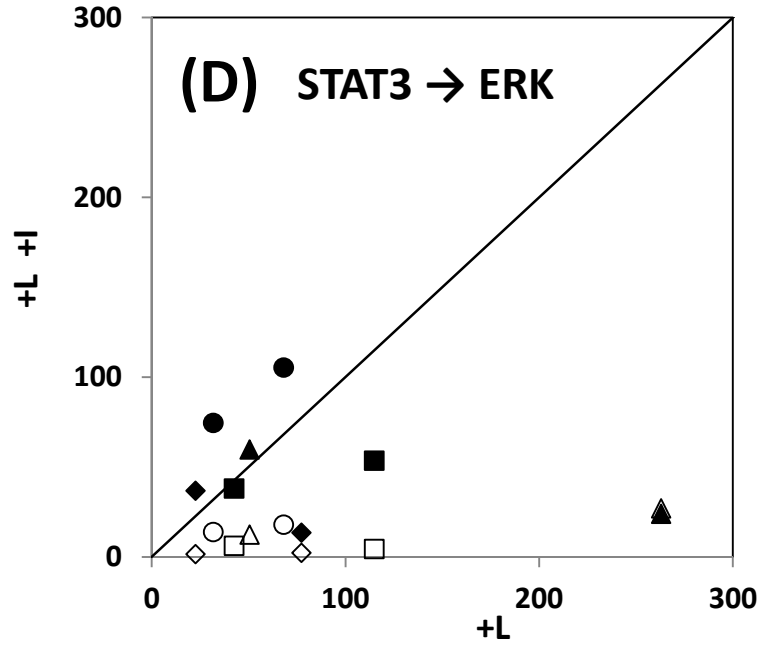
Supplementary Figure 5. Regulation of STAT3 by the studied sentinel proteins, Regulator → STAT3 (target). Details are as in Supplementary Figure 1. Regulator protein is: (A) ERK, (B) AKT, (C) JNK, and (D) p38.

Supplementary Figure 6. Regulation of AKT by PI3K. Details are as in Supplementary Figure 1.

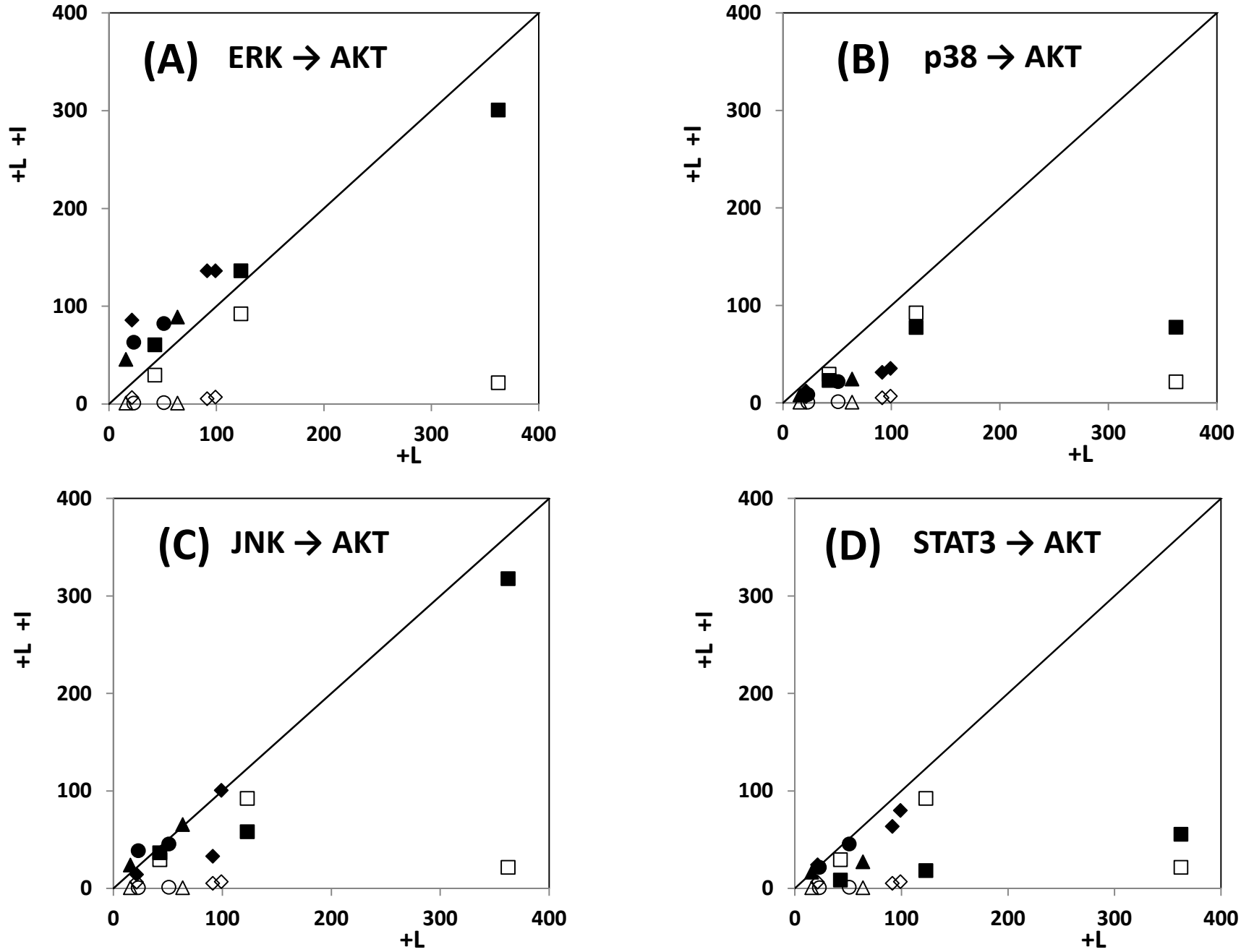








SUPPLEMENTARY FIGURE 1



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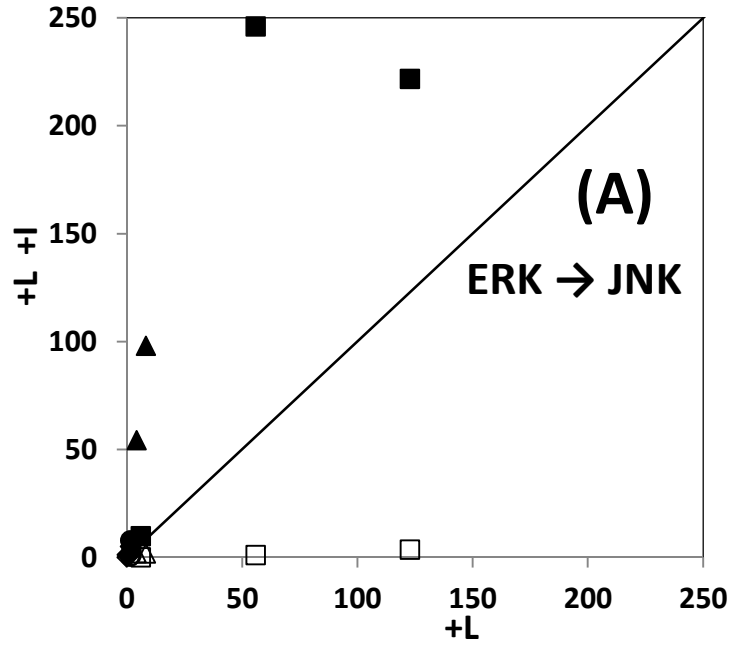
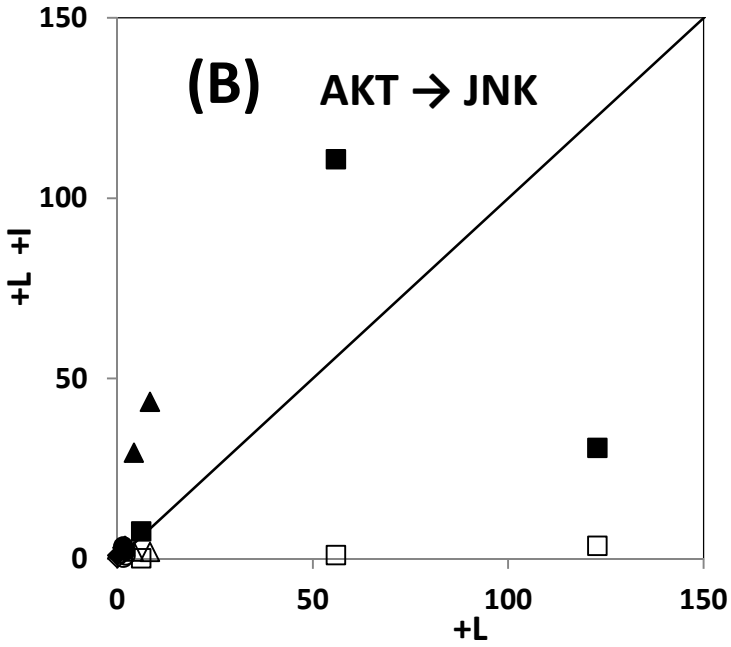
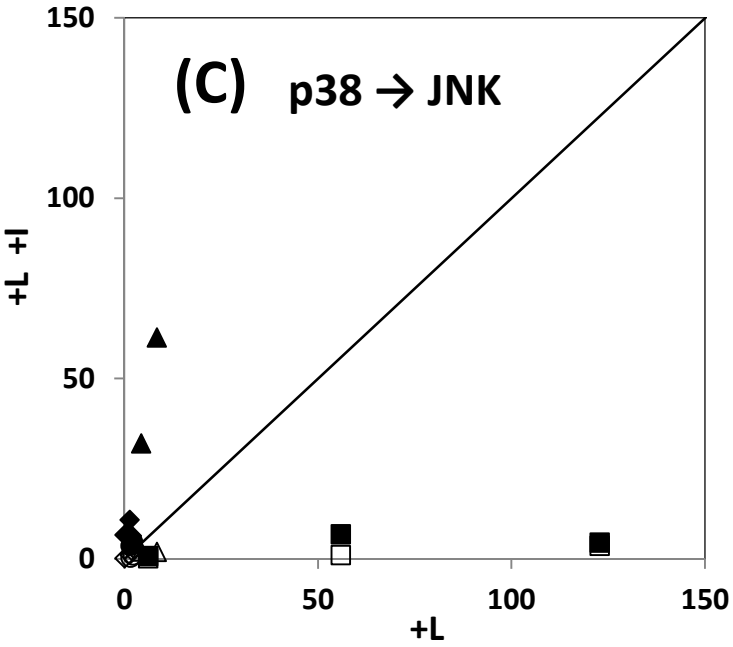
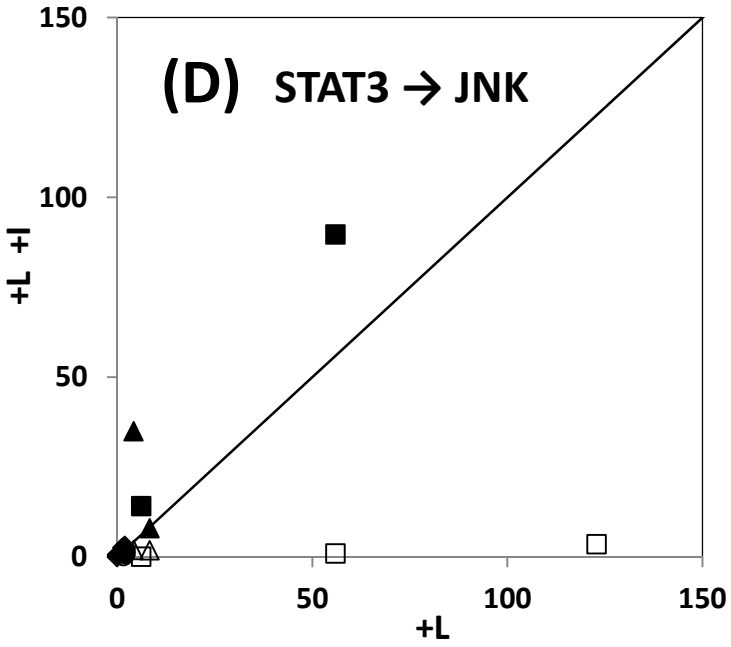
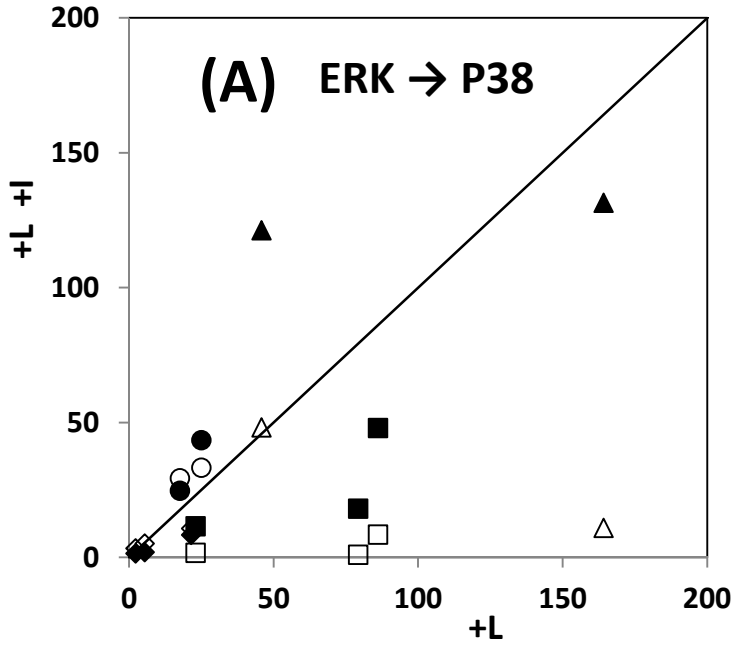


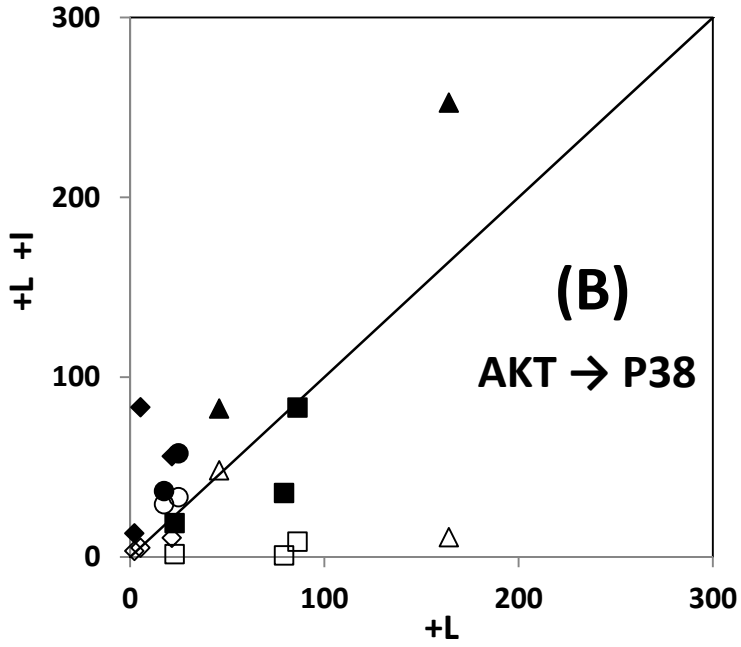


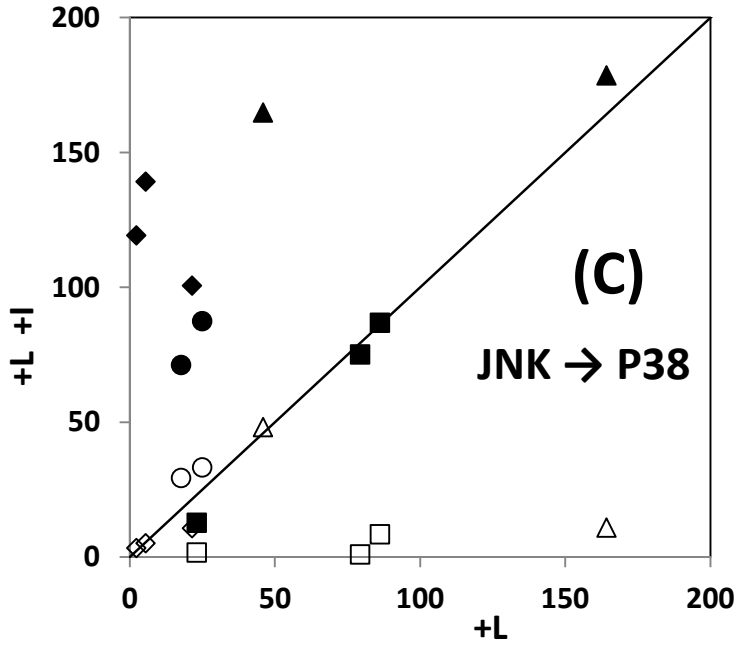


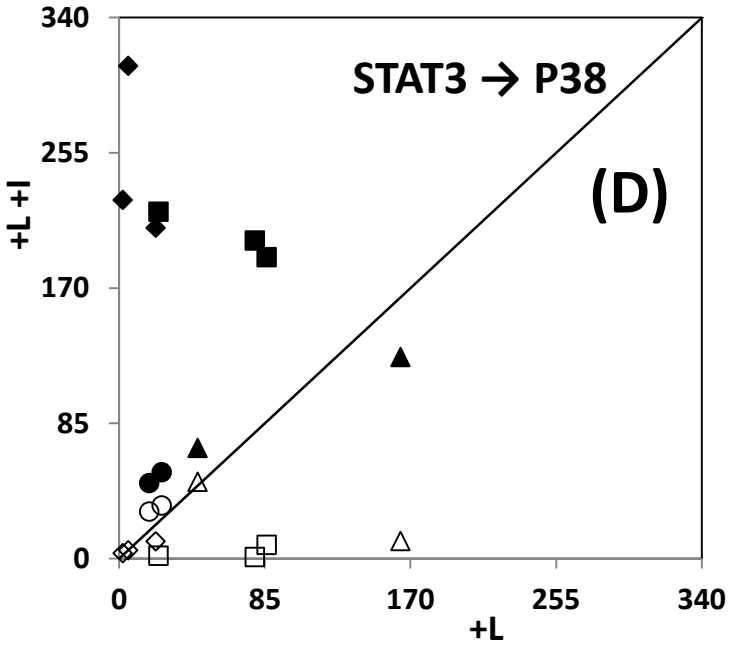


SUPPLEMENTARY FIGURE 3









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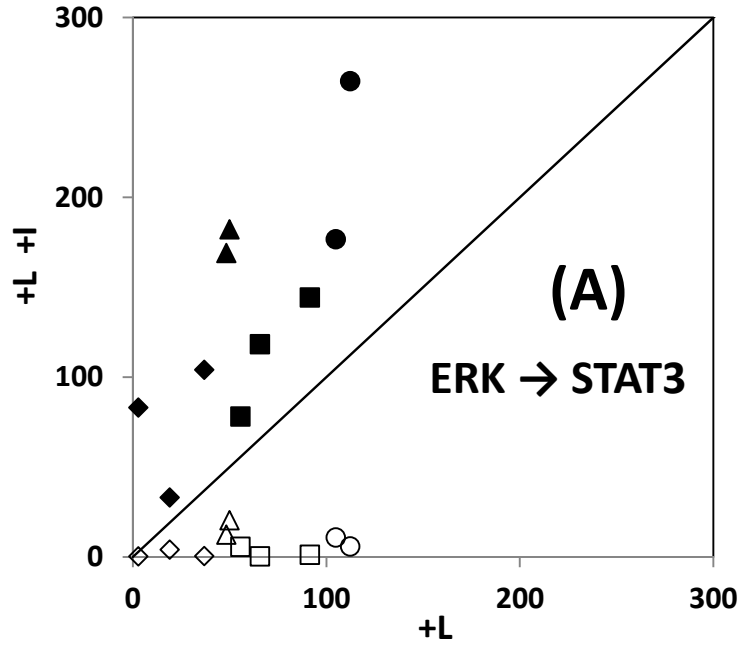
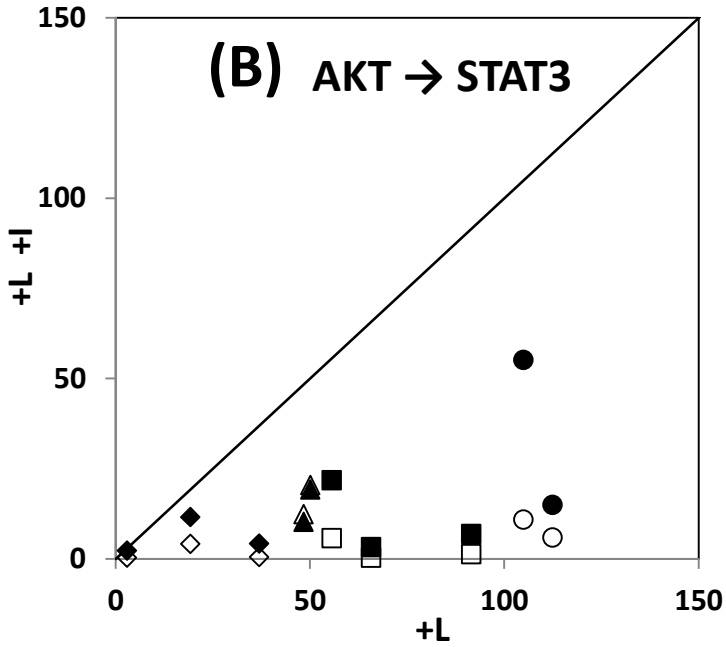
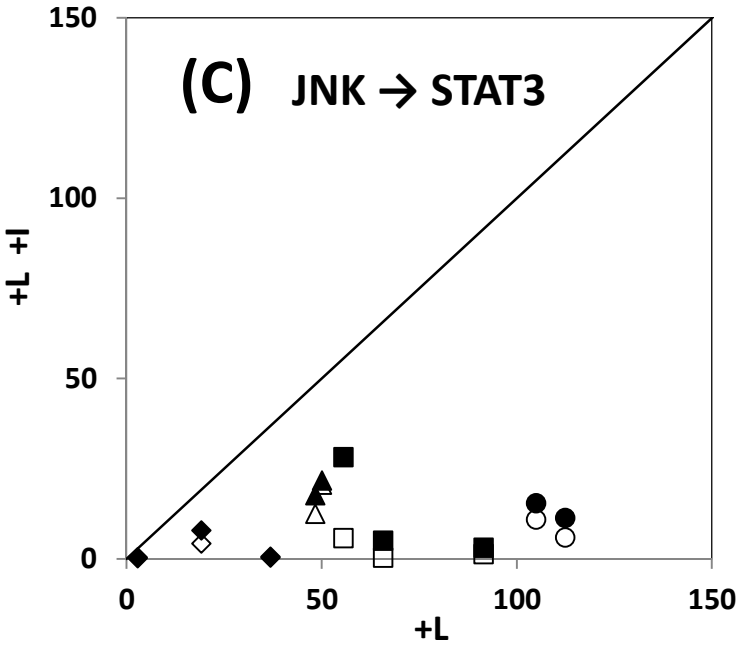
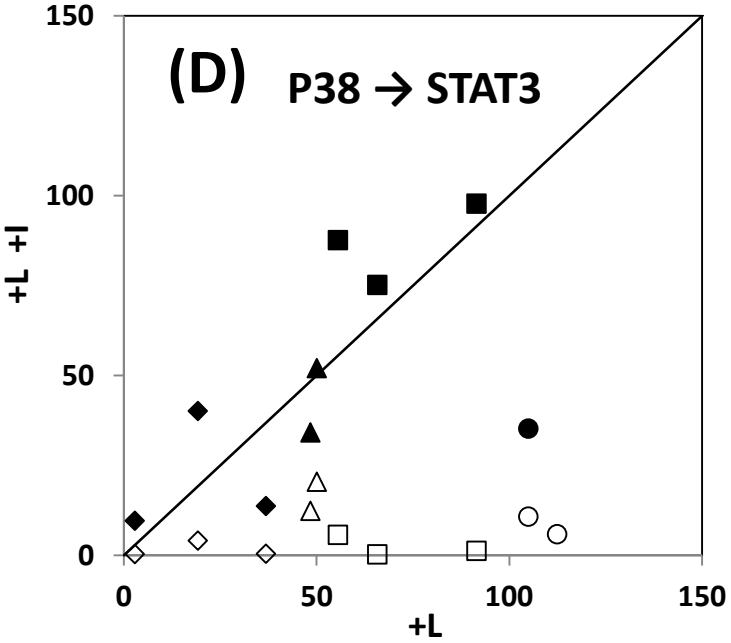
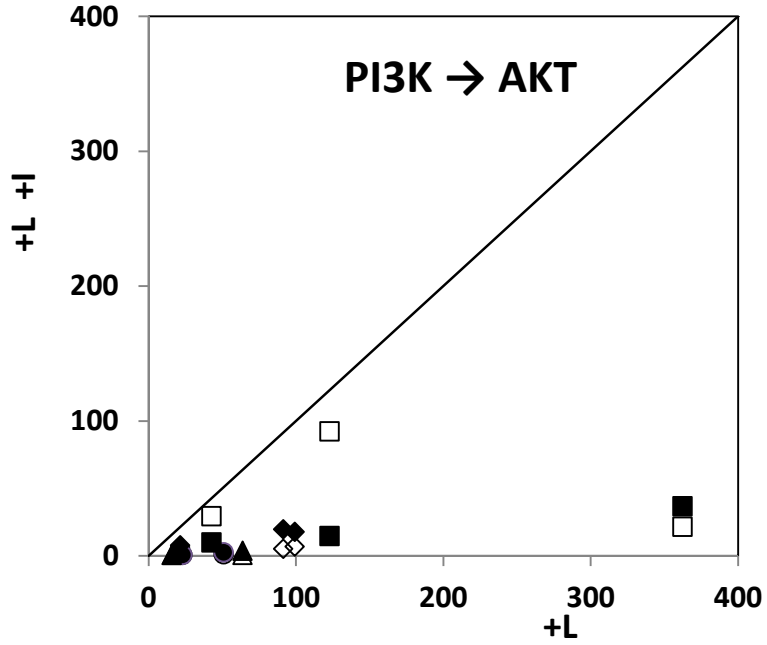








SUPPLEMENTARY FIGURE 5



SUPPLEMENTARY FIGURE 6

SUPPLEMENTARY TABLES

Integrated analysis reveals that STAT3 is central to the crosstalk between HER/ErbB receptor signaling pathways in human mammary epithelial cells

Chunhong Gong, Yi Zhang, Harish Shankaran and Haluk Resat

These tables tabulate the results of the Bayesian Variable Selection Algorithm (BVSA, Supplementary Table 1) and the Modular Response Analysis (MRA, Supplementary Table 2), which are described in the main text.

SUPPLEMENTARY TABLE 1: Bayesian Variable Selection Algorithm Results *

Regulation of AKT by				
Dataset	**ERK**	**P38**	**STAT3**	**JNK**
All	0.09	0.23	**0.88**	0.18
HER2 based	0.13	0.13	0.13	0.14
HER3 based	0.11	0.11	**0.93**	0.15
Regulation of ERK by				
Dataset	**AKT**	**P38**	**STAT3**	**JNK**
All	0.09	0.19	0.16	0.10
HER2 based	0.12	0.16	**0.70**	**0.68**
HER3 based	0.12	*0.34*	0.24	*0.53*
Regulation of p38 by				
Dataset	**AKT**	**ERK**	**STAT3**	**JNK**
All	0.08	0.09	**0.99**	*0.49*
HER2 based	0.13	0.17	0.15	**0.57**
HER3 based	0.14	0.11	0.17	**0.68**
Regulation of STAT3 by				
Dataset	**AKT**	**ERK**	**P38**	**JNK**
All	**0.98**	0.34	*0.61*	**0.97**

HER2 based	0.15	*0.47*	0.16	*0.44*
HER3 based	0.12	*0.37*	0.14	*0.41*
Regulation of JNK by				
Dataset	**AKT**	**ERK**	**P38**	**STAT3**
All	0.33	0.23	*0.48*	0.14
HER2 based	0.12	**0.73**	0.24	**0.73**
HER3 based	0.19	*0.39*	**0.62**	*0.32*

* Response matrix elements are classified into 3 classes: Elements with interaction probabilities r that are i) $r < \mu_r$, ii) $\mu_r < r < \mu_r+\sigma_r$, and iii) $r > \mu_r+\sigma_r$ where μ_r and σ_r are the mean and standard deviation of the derived probabilities, respectively. In our analysis, they were $\mu_r=0.34$ and $\sigma_r=0.27$. Interactions in the first category are unlikely to be present. The ones in the last category are most likely to be present in our system and they are marked in **bold**. Interactions with r probabilities in the intermediate category are probable but they are hard to estimate with our data and their prediction is uncertain. These are marked in *italics*.

SUPPLEMENTARY TABLE 2. Modular Response Analysis Results *

Regulation of AKT by				
Dataset	**ERK**	**P38**	**STAT3**	**JNK**
All	–0.09 ± 0.15	0.48 ± 0.31	**0.61 ± 0.29**	–0.28 ± 0.14
	N/I	N/I	0.31 ± 0.06	N/I
HER2 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
HER3 based	0.18 ± 0.14	0.11 ± 0.15	**1.10 ± 0.30**	–0.20 ± 0.14
	N/I	N/I	**0.94 ± 0.21**	N/I
Regulation of ERK by				
Dataset	**AKT**	**P38**	**STAT3**	**JNK**
All	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	0.31 ± 0.06	N/I
HER2 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	**–2.46 ± 2.90**	**0.76 ± 0.99**
HER3 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	n. r. d.	N/I	n. r. d.
Regulation of p38 by				
Dataset	**AKT**	**ERK**	**STAT3**	**JNK**
All	n. r. d.	n. r. d.	n. r. d.	n. r. d.

	N/I	N/I	**–0.67 ± 0.10**	**0.43 ± 0.12**
HER2 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	N/I	n. r. d.
HER3 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	N/I	**0.88 ± 0.25**
Regulation of STAT3 by				
Dataset	**AKT**	**ERK**	**P38**	**JNK**
All	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	1.02 ± 0.23	N/I	**–0.48 ± 0.19**	**0.62 ± 0.10**
	1.39 ± 0.23	N/I	N/I	**0.64 ± 0.11**
HER2 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	n. r. d.	n. r. d.	N/I	n. r. d.
	N/I	**–0.65 ± 0.23**	N/I	**0.45 ± 0.13**
HER3 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	n. r. d.	n. r. d.	N/I	n. r. d.
	N/I	**–0.76 ± 0.25**	N/I	**0.45 ± 0.12**
Regulation of JNK by				
Dataset	**AKT**	**ERK**	**P38**	**STAT3**
All	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	**1.83 ± 0.63**	N/I

HER2 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	**1.34 ± 0.51**	N/I	**2.68 ± 1.22**
HER3 based	n. r. d.	n. r. d.	n. r. d.	n. r. d.
	N/I	n. r. d.	n. r. d.	n. r. d.
	N/I	N/I	**2.30 ± 0.66**	N/I

* Interaction strengths, r_{ij}, are expressed as mean prediction +/- standard deviation.

N/I: Not Included in the analysis. These interactions were assumed to have $r_{ij}=0$.

n.r.d.: not reliably derived. These interactions had a coefficient of variation ($CV=\sigma/\mu$) larger than 1.5, and therefore, were not reliably predicted from the available data.