

Supplemental figures

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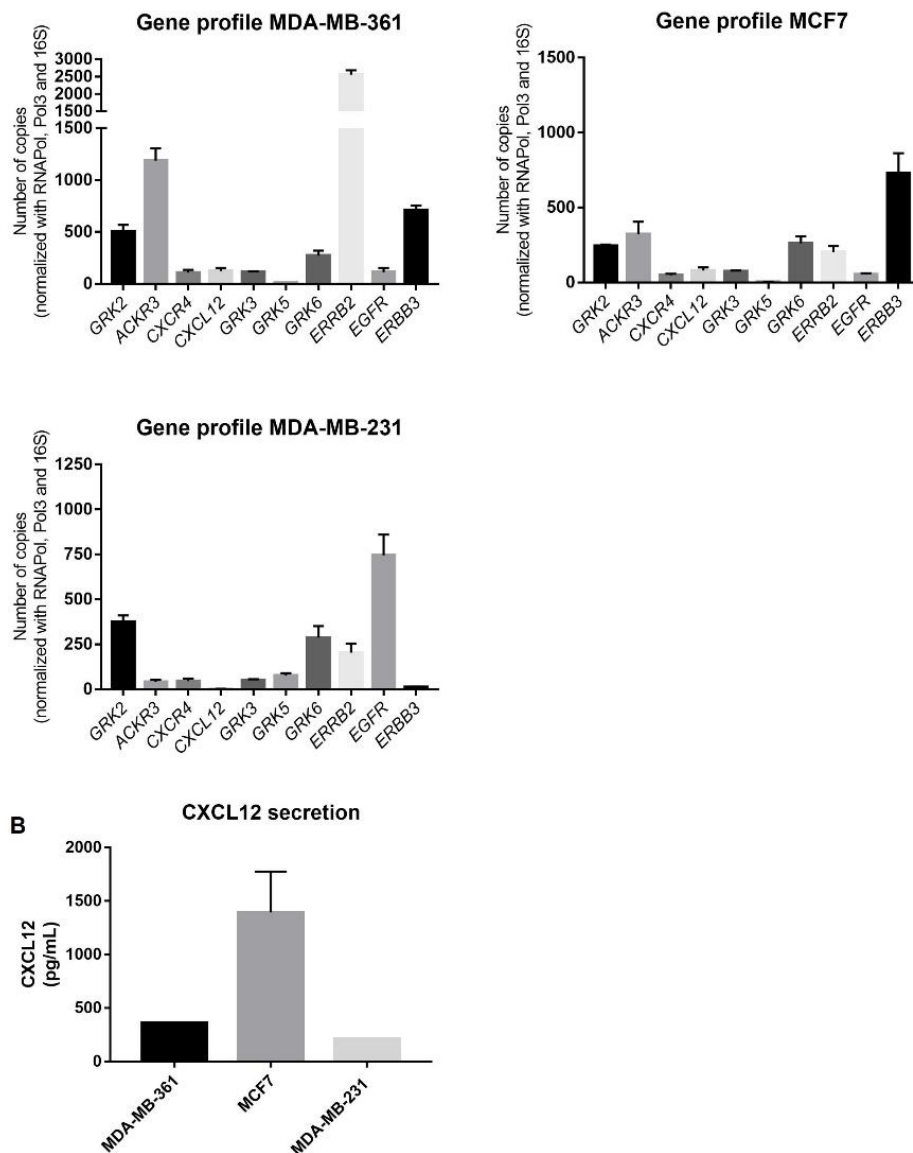
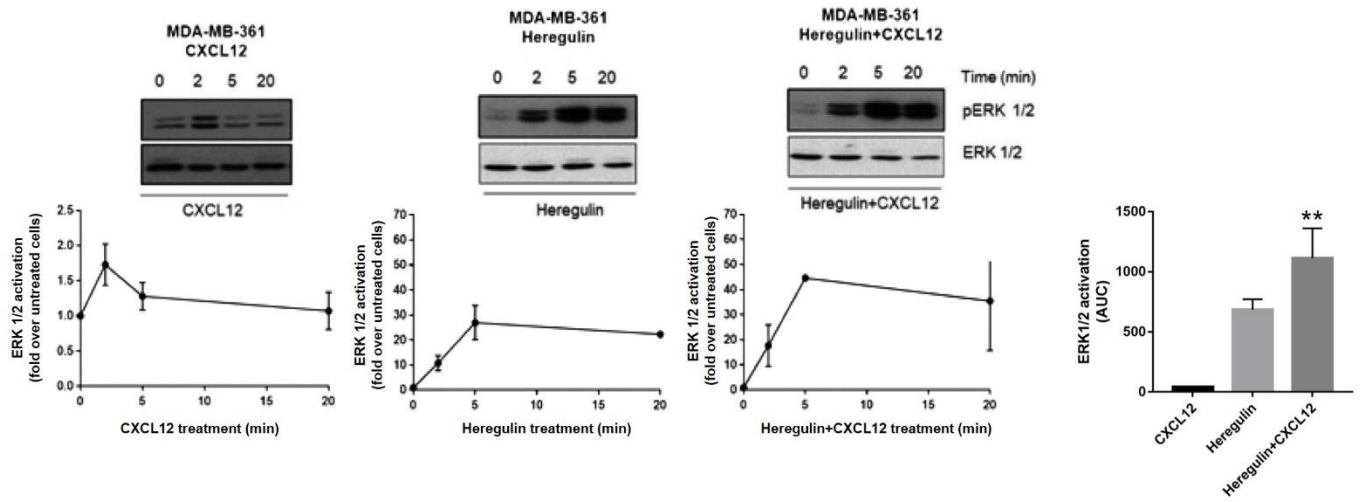


Figure S1. Expression profile of GRK and EGFR (ERBB) family members and of the CXCL12/CXCR4/ACKR3 signaling module in model breast cancer cell lines. **(A)** qRT-PCR analysis of mRNA expression of the indicated genes. mRNA levels were normalized by a geometric mean of RNAPol, Pol3 and 16S. Data are mean \pm SEM of 4 independent experiments. **(B).** CXCL12 secretion levels in conditioned media of MCF7, MDA-MB 361 and MDA-MB 231 cell lines determined as described in Materials and Methods. Data are mean \pm SEM from 2-3 experiments.

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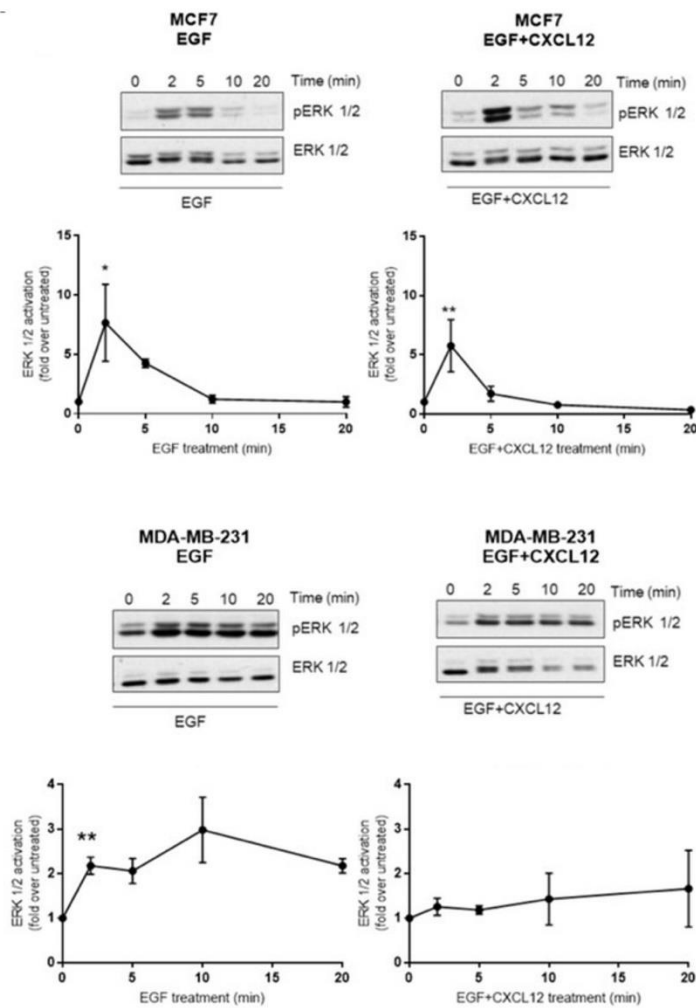


Figure S2. (A) Simultaneous activation of the CXCL12/CXCR4/ACKR3 and HER2 pathways fosters the ERK cascade stimulation pattern in MDA-MB-361 cells (ER+ and HER2+). Serum-starved cells were stimulated with 10nM CXCL12, 20ng/mL heregulin or the combination of both ligands for the indicated times. ERK1/2 activation was assessed and plotted over time. Data of the area under the curve (AUC) are also represented for comparative statistical analysis. Representative blots are shown. Data are mean \pm SEM of 3 independent experiments. ** $p < 0.01$, comparing to non-stimulated cells. **(B)** Simultaneous activation of the CXCL12/CXCR4/ACKR3 and EGFR cascades attenuates ERK pathway stimulation pattern in MCF7 (ER+) and MDA-MB-231 (triple negative) breast cancer cells. MCF7 and MDA-MB-231 cells were serum-starved (1%FBS) overnight and stimulated with 10nM CXCL12, 100ng/mL EGF or the combination of both for the indicated times. ERK1/2 activation was assessed in cell lysates by western blot with specific antibodies and data normalized by total ERK levels. No additive but rather antagonistic effects were observed between CXCR4/ACKR3-EGFR pathways for ERK activation. Representative blots are shown. Data are mean \pm SEM of 2-4 independent experiments. * $p < 0.05$ or ** $p < 0.01$, comparing to non-stimulated cells.

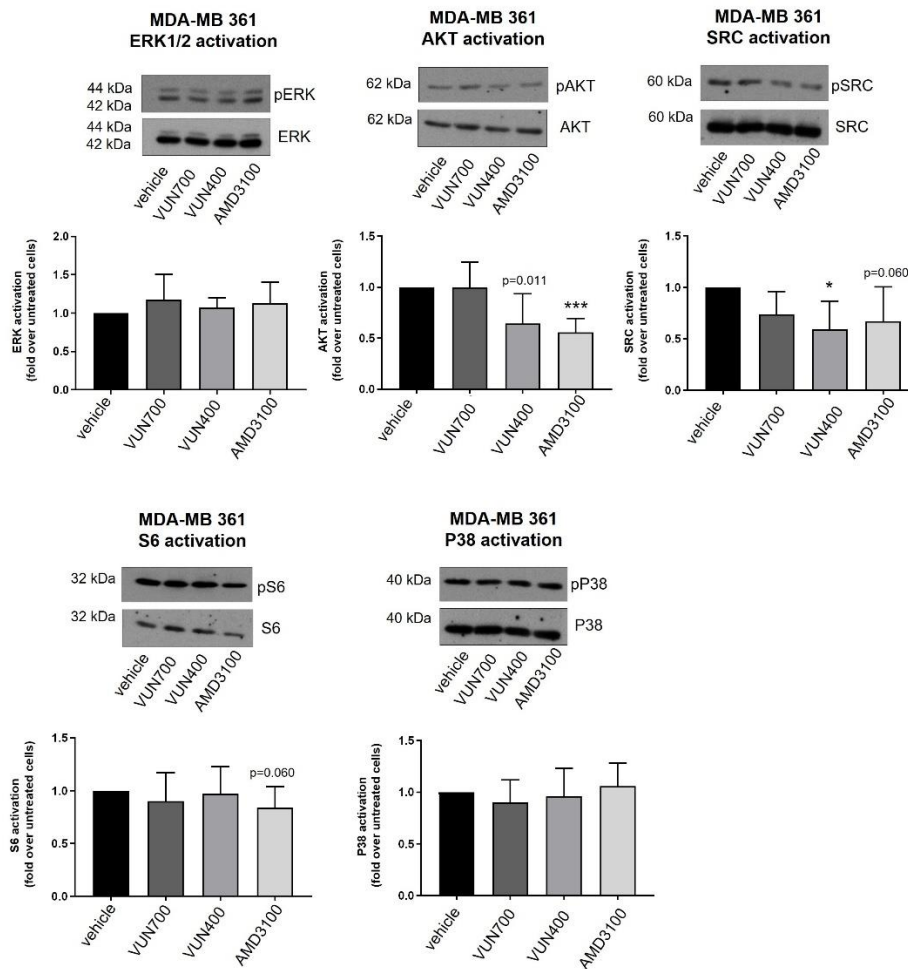


Figure S3. The presence of CXCR4 modulators decreases basal AKT and SRC activation in MDA-MB-361 cells (ER+, Her2+). Cells were treated with the indicated inhibitors for 24h. ERK1/2, AKT, SRC, SRC and p38 activation was assessed in cell lysates by western blot with specific antibodies as detailed in Methods. Representative blots are shown. Data are mean \pm SEM of 5 independent experiments. * $p < 0.05$ or *** $p < 0.001$, comparing to non-stimulated cells.

