

Supplemental Materials and Methods

Table S1. Cell treatment details. For cellular stimulation with ligands, cells were serum-starved overnight and challenged with Heregulin (20 ng/ml), EGF (100 ng/ml) or CXCL12 (10nM) for the indicated times. To determine the influence of cellular components on the signalling dynamics of these ligands, cells were pre-treated with the following inhibitors prior to stimulation.

Treatment	Specifications
AG1478	500nM for 2h
AMD3100	10µM 2h
CPMPD101	30µM, 2h
PP2	5µM for 2h
PTX	100µg/mL for 2h
VUN400 (C2)	10µg/mL
VUN700 (2C5)	10µg/mL

Table S2. Sources and experimental conditions of antibodies used

Primary Antibodies

Antigen	Reference	Supplier	Species	Dilution
AKT	9272	Cell Signalling	Rabbit	WB (1/1000)
CXCR4-PE	306505	Biologend	Mouse	FC (2.5µl/10 ⁶ cells)
ACKR3-APC	331113	Biologend	Mouse	FC(2.5µl/10 ⁶ cells)
ERK1	(C-16): sc-93	Santa Cruz	Rabbit	WB (0.75 mg/ml)
ERK2	(c-14): sc-154	Santa Cruz	Rabbit	WB (0.75 mg/ml)
FAK	3285	Cell Signalling	Rabbit	WB (1/1000)
GRK2	C15 (sc-562)	Santa Cruz	Rabbit	WB (1/1000)
p-AKT (Ser473)	9271	Cell Signalling	Rabbit	WB (1/1000)
PARK7/DJ1	Ab18257	Abcam	Rabbit	WB (1/5000)
p-Erk1-2 (Thr202/204)	9101	Cell Signalling	Rabbit	WB (1/1000)
p-FAK (Tyr397)	3283	Cell Signalling	Rabbit	WB (1/500)
p-p38 MAPK (Thr180/Tyr182)	4631	Cell Signalling	Rabbit	WB (1/500)
p-S6 ribosomal protein (Ser240/244)	2215	Cell Signalling	Rabbit	WB (1/1000)
p-Src Family (Tyr416)	2101	Cell Signalling	Rabbit	WB (1/1000)
p38α MAPK	9218	Cell Signalling	Rabbit	WB (1/1000)
S6 ribosomal protein (5G10)	2217	Cell Signalling	Rabbit	WB (1/000)
Src	2108	Cell Signalling	Rabbit	WB (1/000)

Secondary antibodies

Name, antigen	Company	Dilution
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Biotin-conjugated donkey anti-mouse IgG	Jackson Immunoresearch	WB (1/50000)/ IHC (1/100)
Biotin-conjugated donkey anti-rabbit IgG	Jackson Immunoresearch	WB (1/50000)/ IHC (1/100)
Soluble rabbit peroxidase-anti-mouse IgG	Nordic immunology	WB (1/50000)
Soluble mouse peroxidase-anti-rabbit IgG	Nordic immunology	WB (1/50000)
Soluble mouse peroxidase-anti-goat IgG	Nordic immunology N1	WB (1/50000)
Streptavidin POD-conjugated	Roche	WB (1/50000)

Table S3. Primers used for mRNA analysis

Target	Primer	Sequence 5'- 3'
<i>ACKR3</i>	Fw	AAGAAGATGGTACGCCGTGT
	Rv	AGGCAGAGACACGCAGAAG
<i>CXCL12</i>	Fw	GCTGGTCCTCGTGCTGAC
	Rv	GCATGGGCATCTGTAGCTC
<i>CXCR4</i>	Fw	GGTGGTCTATGTTGGCGTCT
	Rv	ACTGACGTTGGCAAAGATGA
<i>EGFR</i>	Fw	TCCCCGTAATTATGTGGTGAC
	Rv	AGGCCCTTCGCACTTCTTAC
<i>ERBB2</i>	Fw	CAGAATGGCTCAGTGACCTG
	Rv	GCAGAAGGGAGGGTCCTTAT
<i>ERBB3</i>	Fw	ACAGCCCCAGATCTGCAC
	Rv	GTTGGGCGAATGTTCTCATC
<i>GRK2</i>	Fw	CATGCACAATCGCTTTGTAGTC
	Rv	GGTCCGAGATTCTCACATGG
<i>GRK3</i>	Fw	AACTTGGCCACGAAGAAGATT
	Rv	CTCAGGCCGTACCTCTGGT
<i>GRK5</i>	Fw	GGTGGGCACTGTTGGCTA
	Rv	CTCAGGCCGTACCTCTGGT
<i>GRK6</i>	Fw	ATCAGCCAGTGCGAAGA
	Rv	CGTGGCACAGAACTCTCG