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Supplementary Materials for

Therapeutically Targeting ErbB3: A Key Node in Ligand-Induced Activation of the ErbB Receptor–PI3K Axis

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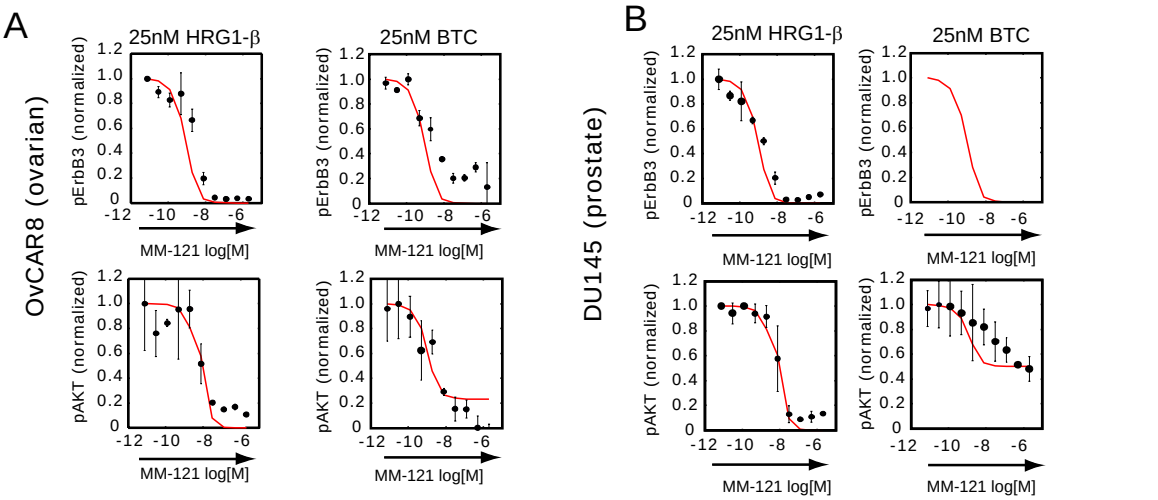

	HRG1-β		BTC	
	pErbB3	pAkt	pErbB3	pAkt
IC50 [nM]	3.1	9.7	1.7	3.7
95 % Confidence Interval	1.7 - 6.1	3.0 - 32.7	0.7 - 3.9	1.2 - 11.6
simulated IC50 [nM]	1.9	7.1	2.1	2.0

	HRG1-β		BTC	
	pErbB3	pAkt	pErbB3	pAkt
IC50 [nM]	1.8	8.2	N/A	20.4
95 % Confidence Interval [nM]	1.0 - 3.1	4.1 - 16.1		8.4 -49.6
simulated IC50 [nM]	2.1	5.7	2.4	2.5

Fig. S1. Characterization of MM-121 in OvCar8 and DU145 cells. Serum-starved OvCar8 (ovarian) cells (A) and DU145 (prostate) cells (B) were pre-incubated for 30 minutes with a 10-point, 4-fold serial dilution of MM-121 starting at 2 μM and then stimulated for 10 minutes with 25 nM of HRG1-β or BTC. Dose-response curves include ELISA measurements (solid circles) of pErbB3 and pAKT and simulations of MM-121 under equivalent conditions (red solid lines). The data is the mean of two experiments ± SD. Mean values were background-subtracted and normalized to the stimulated ligand control for phosphorylated AKT and ErbB3. Tables include the experimentally determined IC_{50} values and corresponding 95% confidence intervals, as well as simulated IC_{50} values.

Supplemental Text: Model Development

Model Structure

The ErbB receptor signaling network model consists of three receptors (ErbB1, ErbB2, ErbB3), a receptor phosphatase, phosphatidylinositol 3-kinase (PI3K, which binds to the receptors) and components of the PI3K-AKT cascade (phosphatidylinositol bisphosphate, PIP2; phosphoinositide-dependent protein kinase, PDK1; phosphatase and tensin

homolog deleted from chromosome 10, PTEN; serine, threonine protein kinase also known as protein kinase B, AKT; Protein phosphatase 2A, PP2A) (Table S3). Two ErbB receptor ligands were included: Heregulin1-beta (HRG1-β), which binds to ErbB3 and ErbB4 (not included in model because experimentally ErbB4 expression levels were very difficult to detect in cell lines (Table S1) and Betacellulin (BTC), which binds primarily to ErbB1 [1] [2]. The mass-action kinetic reactions are graphically summarized in Figure 2A and listed in full in Table S6 were converted to ordinary differential equations using Matlab Simbiology 2.3 (Mathworks, MA).

We included the ligand-induced dimerization, internalization, recycling, and degradation as described in the literature for all homo- and heterodimers [3, 4]. We implemented receptor dimer stabilities using the relative scale as published by Shankaran *et al*. [5] where the coexpression of ErbB1 with ErbB2 or ErbB3 biases signaling to the cell surface and retards signal downregulation and where the simultaneous coexpression of ErbB1-3 leads to an abundance of ErbB2-ErbB3 heterodimers. Therefore, ErbB3-containing dimers internalize and degrade slower than do ErbB1 homodimers or ErbB1-ErbB2 heterodimers. Constitutive dimerization is also included, although ligand-free dimers are assumed not to trigger any downstream signaling [6], and to remain at the cell surface. ErbB ligands bind with different affinities to ErbB homo- and heterodimers [7], but to reduce the number of kinetic parameters to be estimated and to describe the experimental data with the simplest model possible we constrained the binding affinity of the ligands to ErbB homo- and heterodimers to be the same.

Dimerized receptors undergo rapid phosphorylation and activate downstream pathways through binding of signaling adaptors to phosphotyrosine sites on the receptor cytoplasmic tail [8]. Here, we implemented a simplified PI3K-AKT cascade, whereby PI3K binds directly to ligand-bound heterodimers, activates PIP2, which forms a complex with PDK1 and AKT, leading to a two-step double phosphorylation of AKT. ErbB3 has 6 sites for PI3K binding, whereas the other ErbB receptors only have one [9, 10]; although it is not known whether six PI3K molecules can bind simultaneously to ErbB3, PI3K is activated 10-20 times more strongly by ErbB3 [11] than by the other

receptors. There is also evidence that PI3K binds to ErbB3 with greater affinity than it does to the other ErbB receptors [12]. We incorporated these phenomena into the model and avoided the combinatorial complexity of including six binding sites, by imposing the following stipulations on ErbB3-containing dimers: An enhanced PI3K-binding rate; enhanced PIP2-binding rate to PI3K, and enhanced activation rate of PIP3. PIP2 is not present in endosomes [13], thus, only plasma membrane-bound receptor dimers are capable of activating PIP2 in the model. Initial simulations accelerated AKT activation too slowly, making it necessary to almost completely deactivate the AKT phosphatase prior to simulation and incorporate a negative feedback loop whereby AKT activates its own phosphatase, a phenomenon described elsewhere [14]. The MAPK cascade was neglected both for simplicity and because of our recent findings that AKT signaling is relatively insensitive to species and parameters in the MAPK cascade [15]; therefore, MAPK-PI3K crosstalk is not required to describe AKT signaling dynamics. Initial species values were directly measured (ErbB receptors, PDK1, AKT), inferred from the literature [16, 17] or set to non-rate-limiting values.

Model Calibration

Many of the reaction rate parameters are unknown in the cell system we studied and therefore must be estimated (Table S5). To avoid biasing our starting point, we set all parameters to default values [18]. We limited the number of parameters estimated by performing the estimation in two stages: first the model was calibrated against the experimentally observed ErbB receptor phosphorylation data; second, the parameters sensitive to AKT phosphorylation were optimized against the experimental data. Estimated values were only accepted if the parameters were constrained under multiple parameter runs using a genetic algorithm with a population size of 50 and 25 generations (Mathworks, MA). The ligand binding rate constants of HRG1-β and BTC were estimated separately using known K_ds [19] [20] [2] and initial dose responses curves to approximate a forward binding rate of $1 \times 10^5 M^{-1}s^{-1}$ for both ligands. We performed sensitivity analysis (Mathworks, MA) to identify parameters that strongly influenced the activities of ErbB1, ErbB2, ErbB3, and AKT phosphorylation with HRG1-β or BTC stimulation (Table S6). Each parameter was permitted to vary separately while the system

was stimulated with either 1nM of HRG1-β or BTC. The normalized sensitivities of each species were integrated over the two hour stimulation, and parameters with normalized, integrated sensitivities greater than an arbitrary threshold of 1000 are listed in Table S6.

The ErbB receptor phosphorylation profiles were sensitive to dimerization, internalization, recycling, and degradation parameters (enzymatic reactions, dimer dissociation, and phosphatase rate constants were not sensitive). These parameters were fit using a high-density dataset (Fig. 2B: 2 ligands, 3 readouts (phosphorylated ErbB1, ErbB2, and ErbB3), 8 doses, 12 time points). Each readout was normalized to the maximum activation achieved by either ligand, preserving the relative potency of each ligand. ErbB receptor dimerization rates were tightly constrained and parameter relationships were observed that paralleled literature findings: ErbB2 is the preferred dimerization partner for ligand-bound ErbB1 or ErbB3 (more noticeably with HRG1-β stimulation). From the available data, it was not possible to constrain both the recycling and internalization rates; therefore recycling rates were set to $0.005s^{-1}$ and only internalization rates were fit: The resulting observations therefore apply equally to recycling rates (in inverse). Internalization rates varied based on ligand stimulus, with HRG1-β-bound dimers exhibiting much slower internalization than BTC-bound homo- and heterodimers, a view supported elsewhere [21]. Degradation rates were also well-constrained, but were similar for all dimers, suggesting that individual dimer degradation rates are not required to explain the observed data. Phosphorylated AKT is sensitive to many parameters within the PI3K cascade and at the receptor level (Table S6). Therefore, we restricted the calibration to model parameters that were in the PI3K-AKT cascade and trained against the time courses of AKT phosphorylation shown in Fig. 2B while we locked the parameter values already trained on the ErbB receptor profiles.

Following model training, local, manual adjustment of parameters was performed to decipher the impact of each parameter, to determine if further improvement was possible, and to restrict parameters to biologically plausible values. For standardization, parameters were rounded and condensed to similar values when conserved across a parameter type (as explained for degradation rates).

Inhibitor implementation

ErbB network inhibitors were included in the model using the simplest interpretation of known mechanisms of action (Table S7): MM-121 sequesters ErbB3 by preventing ligand binding and induces internalization and degradation; cetuximab sequesters ErbB1 and prevents ligand binding; lapatinib inhibits activation of ErbB1 and ErbB2 receptors but not dimerization or ligand binding; and pertuzumab blocks ErbB2 dimerization. For MM-121 the rate constants measured by Kinexa were used and for the other inhibitors the rate constants reported in the literature [22, 23] [24] and listed in Table S8 were used; cetuximab parameters were experimentally confirmed by Kinexa.

Summary of Information presented in the tables:

Table S1: Measured ErbB receptor expression and mutation status for investigated cell lines

Table S2A: Characterization of ErbB1 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β

Table S2B: Characterization of ErbB2 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β

Table S2C: Characterization of ErbB3 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β

Table S2D: Characterization of AKT phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β

Table S3: Initial amounts of non-zero species in the computational model

Table S4: Summary of biochemical reactions implemented into the computational model using mass action kinetics with corresponding parameters

Table S5: Description of parameters with values. Parameter number corresponds to first reaction in which that parameter appears

Table S6: Sensitive parameters controlling ErbB3, ErbB2, ErbB1 and AKT phosphorylation

Table S7: Summary of biochemical reactions describing the implementation of the inhibitors: MM-121, cetuximab, pertuzumab, lapatinib

Table S8: Inhibitor parameter values

Table S1: Measured ErbB receptor expression and mutation status for investigated cell lines.

		ErbB abundance (molecules/cell)#				Mutation status*		
cell line	Tumor Type	ErbB1	ErbB2	ErbB3	ErbB4	Kras	PI3K	PTEN
ADRr	ovarian	177818	40792	33205	2094	none	none	none
OvCAR8	ovarian	236157	53272	31813	ND	none	none	none
ACHN	renal	448284	45456	15200	2086	none	none	none
MALME-3M	melanoma	2914	56422	67367	584	none	none	none
DU145	prostate	437841	69068	19422	ND	none	none	none

ND – Non-Detectable
#-as measured by qFACS

* Web site used to determine the mutation status of the cell lines investigated - http://www.sanger.ac.uk/perl/genetics/CGP/core_line_viewer?action=nci60_list

Table S2A: Characterization of ErbB1 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β.

pErbB1		NCI-ADRr	% stimulation at 5min relative to maximum
EGF	EC_{50} [nM]	6.0	100.0
	95% CI	2.5-14	
HB-EGF	EC_{50} [nM]	21.0	87.0
	95% CI	12-38	
Epigen	EC_{50} [nM]	N/A	12.0
	95% CI		
AR	EC_{50} [nM]	37.0	95.0
	95% CI	21-68	
BTC	EC_{50} [nM]	5.7	100.0
	95% CI	2.3-14	
TGFα	EC_{50} [nM]	30.0	100.0
	95% CI	10-87	
Epiregulin	EC_{50} [nM]	N/A	9.0
	95% CI		
HRG1-β	EC_{50} [nM]	N/A	0.0

N/A: Not Applicable
CI: 95% Confidence Interval

Table S2B: Characterization of ErbB2 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β.

pErbB2		NCI-ADRr	% stimulation at 5min relative to maximum
EGF	EC_{50} [nM]	27.0	100.0
	95% CI	15-51	
HB-EGF	EC_{50} [nM]	230.0	94.0
	95% CI	74-730	
Epigen	EC_{50} [nM]	N/A	25.0
	95% CI		
AR	EC_{50} [nM]	N/A	54.0
	95% CI		
BTC	EC_{50} [nM]	27.0	94.0
	95% CI	4-190	
TGFα	EC_{50} [nM]	41.0	36.0
	95% CI	20-86	
Epiregulin	EC_{50} [nM]	N/A	18.0
	95% CI		
HRG1-β	EC_{50} [nM]	7.0	43.0
	95% CI	2.1-23	

N/A: Not Applicable
CI: 95% Confidence Interval

Table S2C: Characterization of ErbB3 phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β.

pErbB3		NCI-ADRr	% stimulation at 5min relative to maximum
EGF	EC50 [nM] 95% CI	N/A	7.0
HB-EGF	EC50 [nM] 95% CI	N/A	32.0
Epigen	EC50 [nM] 95% CI	N/A	3.0
AR	EC50 [nM] 95% CI	380.0 240-600	17.0
BTC	EC50 [nM] 95% CI	100.0 47-220	35.0
TGFa	EC50 [nM] 95% CI	N/A	9.0
Epiregulin	EC50 [nM] 95% CI	N/A	22.0
HRG1-beta	EC50 [nM] 95% CI	9.9 7.9-12	100.0

N/A: Not Applicable
CI: 95% Confidence Interval

Table S2D: Characterization of AKT phosphorylation dose response curves for ErbB1 binding ligands and HRG1-β.

pAKT		NCI-ADRr	% stimulation at 5min relative to maximum
EGF	EC_{50} [nM] 95% CI	N/A	26.0
HB-EGF	EC_{50} [nM] 95% CI	N/A	20.0
Epigen	EC_{50} [nM] 95% CI	N/A	11.0
AR	EC_{50} [nM] 95% CI	0.0 0.064-27	28.0
BTC	EC_{50} [nM] 95% CI	N/A	22.0
TGFα	EC_{50} [nM] 95% CI	N/A	27.0
Epiregulin	EC_{50} [nM] 95% CI	N/A	11.0
HRG1–β	EC_{50} [nM] 95% CI	1.9 0.23-17.1	100.0

N/A: Not Applicable
CI: 95% Confidence Interval

Table S3: Initial amounts of non-zero species in the computational model.

Species name	Initial Amount	Additional description
HRG	variable	HRG1-β
BTC	variable	BTC
E1	178000	ErbB1 (EGFR)
E2	41000	ErbB2
E3	33000	ErbB3
RTKpase	500000	Receptor tyrosine kinase phosphatase
PI3K	800000	phosphatidylinositol 3-kinase
PIP2	700000	phosphatidylinositol bisphosphate
PTEN	350000	phosphatase and tensin homolog deleted from chromosome 10
PDK1	9500000	phosphoinositide-dependent protein kinase
AKT	900000	Serine, threonine protein kinase also known as protein kinase B
PP2A	4000	Protein phosphatase 2A (AKT phosphatase)
PP2Aoff	64000	Inactive AKT phosphatase

Table S4: Summary of biochemical reactions implemented into the computational model using mass action kinetics with corresponding parameters

Definition of abbreviations used:

:	indicates a protein complex e.g. ligand bound to receptor
_p	indicates that a protein is phosphorylated
i	indicates that a species is internalized
d	indicates that a species is degraded
<->	indicates a reversible reaction
->	indicates an irreversible reaction

Reaction Number	Reaction	Forward parameter	Reverse parameter
Ligand Binding			
v1	HRG + E3 <-> [E3:HRG]	kf1	kr1
v2	HRG + [E2:E3] <-> [E2:E3:HRG]	kf2	kr2
v3	BTC + E1 <-> [BTC:E1]	kf3	kr3
v4	BTC + [E1:E1] <-> [BTC:E1:E1]	kf4	kr3
v5	BTC + [E1:E2] <-> [BTC:E1:E2]	kf5	kr3
v6	BTC + [E1:E3] <-> [BTC:E1:E3]	kf5	kr3
Dimerization			
v7	[E3:HRG] + E2 <-> [E2:E3:HRG]	kf7	kr7
v8	[E3:HRG_p] + E2_p <-> [E2:E3:HRG_p]	kf7	kr7
v9	[E3:HRG_p] + E2 -> [E2:E3:HRG_p]	kf7	
v10	[E3:HRG] + E1 <-> [E1:E3:HRG]	kf10	kr7
v11	[E3:HRG_p] + E1 <-> [E1:E3:HRG_p]	kf10	kr7
v12	E2_p + E2 -> [E2:E2_p]	kf12	

v13	E2_p + E2_p <-> [E2:E2_p]	kf12	kr12
v14	[BTC:E1] + E1 <-> [BTC:E1:E1]	kf14	kr14
v15	[BTC:E1_p] + E1 <-> [BTC:E1:E1_p]	kf14	kr14
v16	[BTC:E1] + [BTC:E1] <-> [BTC:E1:E1:BTC]	kf16	kr16
v17	[BTC:E1_p] + [BTC:E1] <-> [BTC:E1:E1:BTC_p]	kf16	kr16
v18	[BTC:E1_p] + [BTC:E1_p] <-> [BTC:E1:E1:BTC_p]	kf16	kr16
v19	[BTC:E1] + E2 <-> [BTC:E1:E2]	kf19	kr19
v20	[BTC:E1] + E2_p -> [BTC:E1:E2_p]	kf19	
v21	[BTC:E1_p] + E2_p <-> [BTC:E1:E2_p]	kf19	kr19
v22	[BTC:E1_p] + E2 -> [BTC:E1:E2_p]	kf19	
v23	[BTC:E1] + E3 <-> [BTC:E1:E3]	kf23	kr19
v24	[BTC:E1_p] + E3 <-> [BTC:E1:E3_p]	kf23	kr19
v25	E3 + E2 <-> [E2:E3]	kf12	kr12
v26	E3 + E1 <-> [E1:E3]	kf12	kr12
v27	E1 + E2 <-> [E1:E2]	kf12	kr12
v28	E1 + E1 <-> [E1:E1]	kf12	kr12
v29	E2 + E2 <-> [E2:E2]	kf12	kr12
Phosphorylation and dephosphorylation			
v30	[E2:E3:HRG] -> [E2:E3:HRG_p]	kf30	
v31	[E1:E3:HRG] -> [E1:E3:HRG_p]	kf30	
v32	[BTC:E1:E1] -> [BTC:E1:E1_p]	kf30	
v33	[BTC:E1:E1:BTC] -> [BTC:E1:E1:BTC_p]	kf30	
v34	[BTC:E1:E2] -> [BTC:E1:E2_p]	kf30	
v35	[BTC:E1:E3] -> [BTC:E1:E3_p]	kf30	
v36	[E3:HRG_p] -> HRG + E3	kr1	
v37	[BTC:E1_p] -> BTC + E1	kr3	
v38	[E2:E3:HRG_p] + RTKpase <-> [E2:E3:HRG_p:RTKpase]	kf38	kr38
v39	[E1:E3:HRG_p] + RTKpase <-> [E1:E3:HRG_p:RTKpase]	kf38	kr38
v40	[BTC:E1:E1_p] + RTKpase <-> [BTC:E1:E1_p:RTKpase]	kf38	kr38
v41	[BTC:E1:E1:BTC_p] + RTKpase <-> [BTC:E1:E1:BTC_p:RTKpase]	kf38	kr38
v42	[BTC:E1:E2_p] + RTKpase <-> [BTC:E1:E2_p:RTKpase]	kf38	kr38
v43	[BTC:E1:E3_p] + RTKpase <-> [BTC:E1:E3_p:RTKpase]	kf38	kr38
v44	[E2:E2_p] + RTKpase <-> [E2:E2_p:RTKpase]	kf38	kr38
v45	[E2:E3:HRG_p:RTKpase] -> [E2:E3:HRG] + RTKpase	kf45	
v46	[E1:E3:HRG_p:RTKpase] -> [E1:E3:HRG] + RTKpase	kf45	
v47	[BTC:E1:E1_p:RTKpase] -> [BTC:E1:E1] + RTKpase	kf45	
v48	[BTC:E1:E1:BTC_p:RTKpase] -> [BTC:E1:E1:BTC] + RTKpase	kf45	
v49	[BTC:E1:E2_p:RTKpase] -> [BTC:E1:E2] + RTKpase	kf45	

v50	[BTC:E1:E3_p:RTKpase] -> [BTC:E1:E3] + RTKpase	kf45	
v51	[E2:E2_p:RTKpase] -> [E2:E2] + RTKpase	kf45	
PI3K binding and PIP2 activation			
v52	[E2:E3:HRG_p] + PI3K <-> [E2:E3:HRG_p:PI3K]	kf52	kr52
v53	[E1:E3:HRG_p] + PI3K <-> [E1:E3:HRG_p:PI3K]	kf52	kr52
v54	[E2:E2_p] + PI3K <-> [E2:E2_p:PI3K]	kf54	kr54
v55	[BTC:E1:E1_p] + PI3K <-> [BTC:E1:E1_p:PI3K]	kf54	kr54
v56	[BTC:E1:E1:BTC_p] + PI3K <-> [BTC:E1:E1:BTC_p:PI3K]	kf54	kr54
v57	[BTC:E1:E2_p] + PI3K <-> [BTC:E1:E2_p:PI3K]	kf54	kr54
v58	[BTC:E1:E3_p] + PI3K <-> [BTC:E1:E3_p:PI3K]	kf52	kr52
v59	[E2:E3:HRG_p:PI3K] + PIP2 <-> [E2:E3:HRG_p:PI3K:PIP2]	kf59	kr59
v60	[E1:E3:HRG_p:PI3K] + PIP2 <-> [E1:E3:HRG_p:PI3K:PIP2]	kf59	kr59
v61	[E2:E2_p:PI3K] + PIP2 <-> [E2:E2_p:PI3K:PIP2]	kf61	kr61
v62	[BTC:E1:E1_p:PI3K] + PIP2 <-> [BTC:E1:E1_p:PI3K:PIP2]	kf61	kr61
v63	[BTC:E1:E1:BTC_p:PI3K] + PIP2 <-> [BTC:E1:E1:BTC_p:PI3K:PIP2]	kf61	kr61
v64	[BTC:E1:E2_p:PI3K] + PIP2 <-> [BTC:E1:E2_p:PI3K:PIP2]	kf61	kr61
v65	[BTC:E1:E3_p:PI3K] + PIP2 <-> [BTC:E1:E3_p:PI3K:PIP2]	kf59	kr59
v66	[E2:E3:HRG_p:PI3K:PIP2] -> [E2:E3:HRG_p:PI3K] + PIP3	kf66	
v67	[E1:E3:HRG_p:PI3K:PIP2] -> [E1:E3:HRG_p:PI3K] + PIP3	kf66	
v68	[E2:E2_p:PI3K:PIP2] -> [E2:E2_p:PI3K] + PIP3	kf68	
v69	[BTC:E1:E1_p:PI3K:PIP2] -> [BTC:E1:E1_p:PI3K] + PIP3	kf68	
v70	[BTC:E1:E1:BTC_p:PI3K:PIP2] -> [BTC:E1:E1:BTC_p:PI3K] + PIP3	kf68	
v71	[BTC:E1:E2_p:PI3K:PIP2] -> [BTC:E1:E2_p:PI3K] + PIP3	kf68	
v72	[BTC:E1:E3_p:PI3K:PIP2] -> [BTC:E1:E3_p:PI3K] + PIP3	kf66	
AKT activation cascade			
v73	PIP3 + PTEN <-> [PIP3:PTEN]	kf73	kr73
v74	[PIP3:PTEN] -> PIP2 + PTEN	kf74	
v75	PIP3 + AKT <-> [PIP3:AKT]	kf75	kr75
v76	[PIP3:AKT] + PDK1 <-> [PIP3:AKT:PDK1]	kf76	kr76
v77	[PIP3:AKT:PDK1] -> AKT_p + [PIP3:PDK1]	kf77	
v78	[PIP3:PDK1] -> PIP3 + PDK1	kf78	
v79	PIP3 + AKT_p <-> [PIP3:AKT_p]	kf75	kr75
v80	[PIP3:AKT_p] + PDK1 <-> [PIP3:AKT_p:PDK1]	kf76	kr76
v81	[PIP3:AKT_p:PDK1] -> AKT_p_p + [PIP3:PDK1]	kf81	
v82	AKT_p_p + PP2A <-> [AKT_p_p:PP2A]	kf82	kr82
v83	[AKT_p_p:PP2A] -> AKT_p + PP2A	kf83	
v84	AKT_p + PP2A <-> [AKT_p:PP2A]	kf82	kr82
v85	[AKT_p:PP2A] -> AKT + PP2A	kf83	

v86	AKT_p_p + PP2Aoff <-> [AKT_p_p:PP2Aoff]	kf86	kr86
v87	[AKT_p_p:PP2Aoff] -> AKT_p_p + PP2A	kf87	
Internalizaton			
v88	[E3:HRG] <-> [iE3:HRG]	kf88	kr88
v89	[E3:HRG_p] <-> [iE3:HRG_p]	kf88	kr88
v90	E2_p <-> iE2_p	kf88	kr88
v91	[BTC:E1] <-> [iBTC:E1]	kf88	kr88
v92	[BTC:E1_p] <-> [iBTC:E1_p]	kf88	kr88
v93	[E2:E3:HRG] <-> [iE2:E3:HRG]	kf93	kr93
v94	[E2:E3:HRG_p] <-> [iE2:E3:HRG_p]	kf93	kr93
v95	[E2:E3:HRG_p:RTKpase] <-> [iE2:E3:HRG_p:RTKpase]	kf93	kr93
v96	[E2:E3:HRG_p:PI3K] <-> [iE2:E3:HRG_p:PI3K]	kf93	kr93
v97	[E2:E3:HRG_p:PI3K:PIP2] <-> [iE2:E3:HRG_p:PI3K:PIP2]	kf93	kr93
v98	[E1:E3:HRG] <-> [iE1:E3:HRG]	kf98	kr98
v99	[E1:E3:HRG_p] <-> [iE1:E3:HRG_p]	kf98	kr98
v100	[E1:E3:HRG_p:RTKpase] <-> [iE1:E3:HRG_p:RTKpase]	kf98	kr98
v101	[E1:E3:HRG_p:PI3K] <-> [iE1:E3:HRG_p:PI3K]	kf98	kr98
v102	[E1:E3:HRG_p:PI3K:PIP2] <-> [iE1:E3:HRG_p:PI3K:PIP2]	kf98	kr98
v103	[E2:E2_p] <-> [iE2:E2_p]	kf93	kr93
v104	[E2:E2_p:RTKpase] <-> [iE2:E2_p:RTKpase]	kf93	kr93
v105	[E2:E2_p:PI3K] <-> [iE2:E2_p:PI3K]	kf93	kr93
v106	[E2:E2_p:PI3K:PIP2] <-> [iE2:E2_p:PI3K:PIP2]	kf93	kr93
v107	[BTC:E1:E1] <-> [iBTC:E1:E1]	kf107	kr107
v108	[BTC:E1:E1:BTC] <-> [iBTC:E1:E1:BTC]	kf107	kr107
v109	[BTC:E1:E1_p] <-> [iBTC:E1:E1_p]	kf107	kr107
v110	[BTC:E1:E1:BTC_p] <-> [iBTC:E1:E1:BTC_p]	kf107	kr107
v111	[BTC:E1:E1_p:RTKpase] <-> [iBTC:E1:E1_p:RTKpase]	kf107	kr107
v112	[BTC:E1:E1:BTC_p:RTKpase] <-> [iBTC:E1:E1:BTC_p:RTKpase]	kf107	kr107
v113	[BTC:E1:E1_p:PI3K] <-> [iBTC:E1:E1_p:PI3K]	kf107	kr107
v114	[BTC:E1:E1:BTC_p:PI3K] <-> [iBTC:E1:E1:BTC_p:PI3K]	kf107	kr107
v115	[BTC:E1:E1_p:PI3K:PIP2] <-> [iBTC:E1:E1_p:PI3K:PIP2]	kf107	kr107
v116	[BTC:E1:E1:BTC_p:PI3K:PIP2] <-> [iBTC:E1:E1:BTC_p:PI3K:PIP2]	kf107	kr107
v117	[BTC:E1:E2] <-> [iBTC:E1:E2]	kf117	kr117
v118	[BTC:E1:E2_p] <-> [iBTC:E1:E2_p]	kf117	kr117
v119	[BTC:E1:E2_p:RTKpase] <-> [iBTC:E1:E2_p:RTKpase]	kf117	kr117
v120	[BTC:E1:E2_p:PI3K] <-> [iBTC:E1:E2_p:PI3K]	kf117	kr117
v121	[BTC:E1:E2_p:PI3K:PIP2] <-> [iBTC:E1:E2_p:PI3K:PIP2]	kf117	kr117
v122	[BTC:E1:E3] <-> [iBTC:E1:E3]	kf117	kr117

v123	[BTC:E1:E3_p] <-> [iBTC:E1:E3_p]	kf117	kr117
v124	[BTC:E1:E3_p:RTKpase] <-> [iBTC:E1:E3_p:RTKpase]	kf117	kr117
v125	[BTC:E1:E3_p:PI3K] <-> [iBTC:E1:E3_p:PI3K]	kf117	kr117
v126	[BTC:E1:E3_p:PI3K:PIP2] <-> [iBTC:E1:E3_p:PI3K:PIP2]	kf117	kr117
Endosomal ligand binding			
v127	iHRG + iE3 <-> [iE3:HRG]	kf127	kr1
v128	iHRG + [iE2:E3] <-> [iE2:E3:HRG]	kf127	kr1
v129	iBTC + iE1 <-> [iBTC:E1]	kf129	kr3
v130	iBTC + [iE1:E1] <-> [iBTC:E1:E1]	kf129	kr3
v131	iBTC + [iE1:E2] <-> [iBTC:E1:E2]	kf129	kr3
v132	iBTC + [iE1:E3] <-> [iBTC:E1:E3]	kf129	kr3
Endosomal dimerization			
v133	[iE3:HRG] + iE2 <-> [iE2:E3:HRG]	kf7	kr7
v134	[iE3:HRG_p] + iE2_p <-> [iE2:E3:HRG_p]	kf7	kr7
v135	[iE3:HRG_p] + iE2 -> [iE2:E3:HRG_p]	kf7	
v136	[iE3:HRG] + iE1 <-> [iE1:E3:HRG]	kf10	kr7
v137	[iE3:HRG_p] + iE1 <-> [iE1:E3:HRG_p]	kf10	kr7
v138	iE2_p + iE2 -> [iE2:E2_p]	kf12	
v139	iE2_p + iE2_p <-> [iE2:E2_p]	kf12	kr12
v140	[iBTC:E1] + iE1 <-> [iBTC:E1:E1]	kf14	kr14
v141	[iBTC:E1_p] + iE1 <-> [iBTC:E1:E1_p]	kf14	kr14
v142	[iBTC:E1] + [iBTC:E1] <-> [iBTC:E1:E1:BTC]	kf16	kr16
v143	[iBTC:E1_p] + [iBTC:E1] <-> [iBTC:E1:E1:BTC_p]	kf16	kr16
v144	[iBTC:E1_p] + [iBTC:E1_p] <-> [iBTC:E1:E1:BTC_p]	kf16	kr16
v145	[iBTC:E1] + iE2 <-> [iBTC:E1:E2]	kf19	kr19
v146	[iBTC:E1_p] + iE2 -> [iBTC:E1:E2_p]	kf19	
v147	[iBTC:E1] + iE2_p -> [iBTC:E1:E2_p]	kf19	
v148	[iBTC:E1_p] + iE2_p <-> [iBTC:E1:E2_p]	kf19	kr19
v149	[iBTC:E1] + iE3 <-> [iBTC:E1:E3]	kf23	kr19
v150	[iBTC:E1_p] + iE3 <-> [iBTC:E1:E3_p]	kf23	kr19
v151	iE3 + iE2 <-> [iE2:E3]	kf12	kr12
v152	iE3 + iE1 <-> [iE1:E3]	kf12	kr12
v153	iE1 + iE2 <-> [iE1:E2]	kf12	kr12
v154	iE1 + iE1 <-> [iE1:E1]	kf12	kr12
v155	iE2 + iE2 <-> [iE2:E2]	kf12	kr12
Endosomal phosphorylation and dephosphorylation			
v156	[iE2:E3:HRG] -> [iE2:E3:HRG_p]	kf30	
v157	[iE1:E3:HRG] -> [iE1:E3:HRG_p]	kf30	

v158	[iBTC:E1:E1] -> [iBTC:E1:E1_p]	kf30	
v159	[iBTC:E1:E1:BTC] -> [iBTC:E1:E1:BTC_p]	kf30	
v160	[iBTC:E1:E2] -> [iBTC:E1:E2_p]	kf30	
v161	[iBTC:E1:E3] -> [iBTC:E1:E3_p]	kf30	
v162	[iE3:HRG_p] -> iHRG + iE3	kr1	
v163	[iBTC:E1_p] -> iBTC + iE1	kr3	
v164	[iE2:E3:HRG_p] + RTKpase <-> [iE2:E3:HRG_p:RTKpase]	kf38	kr38
v165	[iE1:E3:HRG_p] + RTKpase <-> [iE1:E3:HRG_p:RTKpase]	kf38	kr38
v166	[iE2:E2_p] + RTKpase <-> [iE2:E2_p:RTKpase]	kf38	kr38
v167	[iBTC:E1:E1_p] + RTKpase <-> [iBTC:E1:E1_p:RTKpase]	kf38	kr38
v168	[iBTC:E1:E1:BTC_p] + RTKpase <-> [iBTC:E1:E1:BTC_p:RTKpase]	kf38	kr38
v169	[iBTC:E1:E2_p] + RTKpase <-> [iBTC:E1:E2_p:RTKpase]	kf38	kr38
v170	[iBTC:E1:E3_p] + RTKpase <-> [iBTC:E1:E3_p:RTKpase]	kf38	kr38
v171	[iE2:E3:HRG_p:RTKpase] -> [iE2:E3:HRG] + RTKpase	kf45	
v172	[iE1:E3:HRG_p:RTKpase] -> [iE1:E3:HRG] + RTKpase	kf45	
v173	[iE2:E2_p:RTKpase] -> [iE2:E2] + RTKpase	kf45	
v174	[iBTC:E1:E1_p:RTKpase] -> [iBTC:E1:E1] + RTKpase	kf45	
v175	[iBTC:E1:E1:BTC_p:RTKpase] -> [iBTC:E1:E1:BTC] + RTKpase	kf45	
v176	[iBTC:E1:E2_p:RTKpase] -> [iBTC:E1:E2] + RTKpase	kf45	
v177	[iBTC:E1:E3_p:RTKpase] -> [iBTC:E1:E3] + RTKpase	kf45	
Endosomal PI3K binding			
v178	[iE2:E3:HRG_p] + PI3K <-> [iE2:E3:HRG_p:PI3K]	kf52	kr52
v179	[iE1:E3:HRG_p] + PI3K <-> [iE1:E3:HRG_p:PI3K]	kf52	kr52
v180	[iE2:E2_p] + PI3K <-> [iE2:E2_p:PI3K]	kf54	kr54
v181	[iBTC:E1:E1_p] + PI3K <-> [iBTC:E1:E1_p:PI3K]	kf54	kr54
v182	[iBTC:E1:E1:BTC_p] + PI3K <-> [iBTC:E1:E1:BTC_p:PI3K]	kf54	kr54
v183	[iBTC:E1:E2_p] + PI3K <-> [iBTC:E1:E2_p:PI3K]	kf54	kr54
v184	[iBTC:E1:E3_p] + PI3K <-> [iBTC:E1:E3_p:PI3K]	kf52	kr52
Degradation			
v185	iHRG -> dHRG	kf185	
v186	iBTC -> dBTC	kf185	
v187	[iE3:HRG] -> [dE3:HRG]	kf187	
v188	[iBTC:E1] -> [dBTC:E1]	kf187	
v189	[iE3:HRG_p] -> [dE3:HRG_p]	kf187	
v190	[iBTC:E1_p] -> [dBTC:E1_p]	kf187	
v191	iE2_p -> dE2_p	kf187	
v192	[iE2:E3:HRG] -> [dE2:E3:HRG]	kf192	
v193	[iE2:E3:HRG_p] -> [dE2:E3:HRG_p]	kf192	

v194	[iE2:E3:HRG_p:RTKpase] -> [dE2:E3:HRG_p:RTKpase]	kf192
v195	[iE2:E3:HRG_p:PI3K] -> [dE2:E3:HRG_p:PI3K]	kf192
v196	[iE2:E3:HRG_p:PI3K:PIP2] -> [dE2:E3:HRG_p:PI3K:PIP2]	kf192
v197	[iE2:E2_p] -> [dE2:E2_p]	kf192
v198	[iE2:E2_p:RTKpase] -> [dE2:E2_p:RTKpase]	kf192
v199	[iE2:E2_p:PI3K] -> [dE2:E2_p:PI3K]	kf192
v200	[iE2:E2_p:PI3K:PIP2] -> [dE2:E2_p:PI3K:PIP2]	kf192
v201	[iE1:E3:HRG] -> [dE1:E3:HRG]	kf201
v202	[iE1:E3:HRG_p] -> [dE1:E3:HRG_p]	kf201
v203	[iE1:E3:HRG_p:RTKpase] -> [dE1:E3:HRG_p:RTKpase]	kf201
v204	[iE1:E3:HRG_p:PI3K] -> [dE1:E3:HRG_p:PI3K]	kf201
v205	[iE1:E3:HRG_p:PI3K:PIP2] -> [dE1:E3:HRG_p:PI3K:PIP2]	kf201
v206	[iBTC:E1:E1] -> [dBTC:E1:E1]	kf206
v207	[iBTC:E1:E1_p] -> [dBTC:E1:E1_p]	kf206
v208	[iBTC:E1:E1_p:RTKpase] -> [dBTC:E1:E1_p:RTKpase]	kf206
v209	[iBTC:E1:E1_p:PI3K] -> [dBTC:E1:E1_p:PI3K]	kf206
v210	[iBTC:E1:E1_p:PI3K:PIP2] -> [dBTC:E1:E1_p:PI3K:PIP2]	kf206
v211	[iBTC:E1:E1:BTC] -> [dBTC:E1:E1:BTC]	kf206
v212	[iBTC:E1:E1:BTC_p] -> [dBTC:E1:E1:BTC_p]	kf206
v213	[iBTC:E1:E1:BTC_p:RTKpase] -> [dBTC:E1:E1:BTC_p:RTKpase]	kf206
v214	[iBTC:E1:E1:BTC_p:PI3K] -> [dBTC:E1:E1:BTC_p:PI3K]	kf206
v215	[iBTC:E1:E1:BTC_p:PI3K:PIP2] -> [dBTC:E1:E1:BTC_p:PI3K:PIP2]	kf206
v216	[iBTC:E1:E2] -> [dBTC:E1:E2]	kf201
v217	[iBTC:E1:E2_p] -> [dBTC:E1:E2_p]	kf201
v218	[iBTC:E1:E2_p:RTKpase] -> [dBTC:E1:E2_p:RTKpase]	kf201
v219	[iBTC:E1:E2_p:PI3K] -> [dBTC:E1:E2_p:PI3K]	kf201
v220	[iBTC:E1:E2_p:PI3K:PIP2] -> [dBTC:E1:E2_p:PI3K:PIP2]	kf201
v221	[iBTC:E1:E3] -> [dBTC:E1:E3]	kf201
v222	[iBTC:E1:E3_p] -> [dBTC:E1:E3_p]	kf201
v223	[iBTC:E1:E3_p:RTKpase] -> [dBTC:E1:E3_p:RTKpase]	kf201
v224	[iBTC:E1:E3_p:PI3K] -> [dBTC:E1:E3_p:PI3K]	kf201
v225	[iBTC:E1:E3_p:PI3K:PIP2] -> [dBTC:E1:E3_p:PI3K:PIP2]	kf201

Table S5: Description of parameters with values. Parameter number corresponds to first reaction in which that parameter appears.

Name	Value	Units	Description
Av	6.0×10^{23}		Avogadro's number
Vmedia	1.00×10^{-04}	Liters	Media volume per well
Vcell	1.00×10^{-12}	Liters	Cell volume

Num_cells	30000		Number of cells per well.
kf1	5.00×10^{-11}	molecules-1 sec-1	HRG binding to E3. Unit conversion calculated as 1×10^{5}M-1s-1 /(Vmedia*Av/Num_cells).
kr1	0.001	sec-1	HRG dissociation from E3
kf2	5.00×10^{-11}	molecules-1 sec-1	HRG binding to E3:E2 dimers
kr2	0.001	sec-1	HRG dissociation from E3:E2 dimers
kf3	5.00×10^{-11}	molecules-1 sec-1	BTC binding to E1
kr3	0.001	sec-1	BTC dissociation from E1
kf4	5.00×10^{-11}	molecules-1 sec-1	BTC binding to E1 homodimers
kf5	5.00×10^{-11}	molecules-1 sec-1	BTC binding to E1 heterodimers
kf7	3.00×10^{-06}	molecules-1 sec-1	Dimerization of E2 to HRG:E3
kr7	0.001	sec-1	Dissociation of E2 to HRG:E3
kf10	3.00×10^{-08}	molecules-1 sec-1	Dimerization of E1 to HRG:E3
kf12	4.20×10^{-09}	molecules-1 sec-1	Dimerization - constitutive and ligand free
kr12	0.001	sec-1	Dissociation - constitutive and ligand free
kf14	1.70×10^{-05}	molecules-1 sec-1	Dimerization of E1 to BTC:E1
kr14	0.001	sec-1	Dissociation of E1 to BTC:E1
kf16	1.70×10^{-05}	molecules-1 sec-1	Dimerization of BTC:E1 to BTC:E1
kr16	0.001	sec-1	Dissociation of BTC:E1 to BTC:E1
kf19	3.30×10^{-05}	molecules-1 sec-1	Dimerization of E2 to BTC:E1
kr19	0.001	sec-1	Dissociation of E2 or E3 to BTC:E1
kf23	4.70×10^{-06}	molecules-1 sec-1	Dimerization of E3 to BTC:E1
kf30	1	sec-1	Enzymatic auto-phosphorylation rate of ligand-bound dimers
kf38	5.00×10^{-06}	molecules-1 sec-1	Receptor phosphatase binding
kr38	0.1	sec-1	Receptor phosphatase dissociation
kf45	1	sec-1	Enzymatic rate for receptor dephosphorylation
kf52	3.00×10^{-06}	molecules-1 sec-1	PI3K binding to E3 containing dimers
kr52	0.1	sec-1	PI3K dissociation from E3 containing dimers
kf54	7.50×10^{-07}	molecules-1 sec-1	PI3K binding to non-E3 containing dimers
kr54	0.1	sec-1	PI3K dissociation from non-E3 dimers
kf59	5.00×10^{-06}	molecules-1 sec-1	PIP2 binding to E3 containing heterodimers
kr59	0.1	sec-1	PIP2 dissociation from E3 containing heterodimer
kf61	5.00×10^{-07}	molecules-1 sec-1	PIP2 binding to non-E3 containing heterodimers
kr61	0.1	sec-1	PIP2 dissociation from non-E3 containing heterodimer
kf66	0.2	sec-1	PIP3 activation by E3 containing dimers
kf68	0.013	sec-1	PIP3 activation rate by non E3 containing dimers
kf73	5.00×10^{-06}	molecules-1 sec-1	PIP3 binding to PTEN
kr73	0.1	sec-1	PIP3 dissociating from PTEN
kf74	0.1	sec-1	PIP3 inactivation by PTEN
kf75	2.60×10^{-04}	molecules-1 sec-1	PIP3 binding to Akt or Akt_p
kr75	0.1	sec-1	PIP3 dissociation from Akt or Akt_p
kf76	6.70×10^{-05}	molecules-1 sec-1	PDK1 binding to PIP3:Akt
kr76	0.1	sec-1	PDK1 dissociation from PIP3:Akt
kf77	1	sec-1	Enzymatic phosphorylation rate for Akt
kf78	0.2	sec-1	PDK1 dissociating from PIP3
kf81	1	sec-1	Enzymatic phosphorylation rate for Akt_p
kf82	1.70×10^{-06}	molecules-1 sec-1	PP2A binding to phosphorylated Akt
kr82	0.1	sec-1	PP2A dissociation from phosphorylated Akt
kf83	1.5	sec-1	Akt dephosphorylation and dissociation
kf86	8.30×10^{-09}	molecules-1 sec-1	binding of AKT_p_p to PP2Aoff

kr86	0.5	sec-1	Dissociation of AKT_p_p from PP2Aoff
kf87	0.1	sec-1	Activation of PP2Aoff by AKT_p_p
kf88	0.1	sec-1	Internalization rate for ligand-bound or active monomers
kr88	0.005	sec-1	Recycling rate ligand-bound or active monomers
kf93	0.005	sec-1	Internalization rate for HRG-bound E2:E3 heterodimers and E2 homodimers
kr93	0.005	sec-1	Recycling rate for HRG-bound E2:E3 heterodimers and E2 homodimers
kf98	0.005	sec-1	Internalization rate for HRG-bound E1:E3 heterodimers
kr98	0.005	sec-1	Recycling rate for HRG-bound E1:E3 heterodimers
kf107	0.1	sec-1	Internalization rate for BTC-bound ErbB1 homodimers
kr107	0.005	sec-1	Recycling rate for BTC-bound ErbB1 homodimers
kf117	0.1	sec-1	Internalization rate for BTC-bound ErbB1 heterodimers
kr117	0.005	sec-1	Recycling rate for BTC-bound E1 containing heterodimers
kf127	0.038	molecules-1 sec-1	HRG binding in the endosome. Volume of an endosome was 4.5x 10^{-18}, as in [25], therefore kf127 = kf1*Vmedia/Num_cells/4.5 x $10^{-18.}$
kf129	0.038	molecules-1 sec-1	BTC binding in the endosome. Volume of an endosome was 4.5x 10^{-18}, as in [25], therefore kf127 = kf3*Vmedia/Num_cells/4.5 x $10^{-18.}$
kf185	0.002	sec-1	Degradation rate for ligand
kf187	0.002	sec-1	Degradation rate for ligand bound monomers
kf192	0.002	sec-1	Degradation rate for ligand-bound E2 containing homo or heterodimers
kf201	0.002	sec-1	Degradation rate for ligand-bound E1 containing heterodimers
kf206	0.002	sec-1	Degradation of ligand-bound E1 homodimers

Table S6: ErbB3, ErbB2, ErbB1. and AKT sensitive parameters.

pErbB3 sensitivity during HRG1-β stimulation		**pErbB3 sensitivity during BTC stimulation**	
kf1	4799	kf5	3824
kr93	2163	kf23	2353
kf7	1902	kf3	1821
kr88	1277	kf12	1389
kr12	1192	kr12	-1343
kf12	-1111	kf14	-1742
kf187	-1136	kf14	-6462
kf88	-1907		
kf93	-3122		
kf192	-4452		

pErbB2 sensitivity during HRG1-β stimulation		**pErbB2 sensitivity during BTC stimulation**	
kf1	4747	kf3	3878
kr93	2245	kf19	3124
kf7	2136	kf5	1060
kr88	1267	kf14	-2768
kr12	1155	kf14	-6515
kf12	-1071		
kf187	-1137		
kf88	-1892		
kf93	-3239		
kf192	-4617		

pErbB1 sensitivity during HRG1-β stimulation		**pErbB1 sensitivity during BTC stimulation**	
kf10	6990	kf3	2847
kf1	6222	kf4	1044
kr12	2224	kf14	-1665
kr98	2174	kf206	-4983

kr88	1616
kf187	-1423
kf12	-2189
kf88	-2414
kf98	-3155
kf14	-4457
kf7	-4469

pAKT sensitivity during HRG1-β stimulation		pAKT sensitivity during BTC stimulation	
kf66	6657	kf75	5462
kf75	5418	kf66	5395
kf1	4799	kr117	4420
kr93	3937	kf5	3250
kr73	2705	kr86	2854
kf7	2022	kr73	2726
kr86	1918	kf3	2351
kf78	1584	kf68	1815
kr88	1209	kf23	1804
kr12	1127	kf78	1750
kf12	-1044	kr107	1024
kf187	-1073	kf83	-1019
kf87	-1929	kf14	-1338
kf88	-1967	kf107	-1552
kf86	-2302	kf74	-2720
kf83	-2520	kf87	-2861
kf74	-2719	kf86	-3425
kf192	-3667	kf14	-3890
kf82	-4458	kf73	-5459
kf73	-5416	kf82	-5921
kf93	-5748	kf117	-6849

Table S7: Implementation scheme for MM-121, Cetuximab, Pertuzumab, and Lapatinib.

Reaction Number	Reaction	Forward parameter	Reverse parameter
MM-121 implementation			
MM121_v1	MM121 + E3 <-> [E3:MM121]	kfMM121_1	krMM121_1
MM121_v2	[E3:MM121] + E3 <-> [E3:MM121:E3]	kfMM121_2	krMM121_2
MM121_v3	[E3:MM121:E3] <-> [iE3:MM121:E3]	kfMM121_3	krMM121_3
MM121_v4	[iE3:MM121:E3] <-> [dE3:MM121:E3]	kfMM121_4	
Cetuximab implementation			
Cetuximab_v1	Cetuximab + E1 <-> [E1:Cetuximab]	kfCetuximab_1	krCetuximab_1
Cetuximab_v2	[E1:Cetuximab] + E1 <-> [E1:Cetuximab:E1]	kfCetuximab_2	krCetuximab_2
Pertuzumab implementation			
Pertuzumab_v1	[Pertuzumab] + E2 <-> [E2:Pertuzumab]	kfPertuzumab_1	krPertuzumab_1
Pertuzumab_v2	[E2:Pertuzumab] + E2 <-> [E2:Pertuzumab:E2]	kfPertuzumab_2	krPertuzumab_2
Lapatinib implementation			
Lapatinib_v1	Lapatinib + E1 <-> [Lapatinib:E1]	kfLapatinib_1	krLapatinib_1
Lapatinib_v2	Lapatinib + E2 <-> [Lapatinib:E2]	kfLapatinib_2	krLapatinib_2
Lapatinib_v3	Lapatinib + [E1:E1] <-> [Lapatinib:E1:E1]	kfLapatinib_3	krLapatinib_1
Lapatinib_v4	Lapatinib + [E1:E2] <-> [Lapatinib:E1:E2]	kfLapatinib_3	krLapatinib_1
Lapatinib_v5	Lapatinib + [E1:E3] <-> [Lapatinib:E1:E3]	kfLapatinib_1	krLapatinib_1
Lapatinib_v6	Lapatinib + [E2:E2] <-> [Lapatinib:E2:E2]	kfLapatinib_4	krLapatinib_2

Lapatinib_v7	Lapatinib + [E2:E3] <-> [Lapatinib:E2:E3]	kfLapatinib_2	krLapatinib_2
Lapatinib_v8	Lapatinib + [Lapatinib:E1:E1] <-> [Lapatinib:E1:E1:Lapatinib]	kfLapatinib_1	krLapatinib_1
Lapatinib_v9	Lapatinib + [Lapatinib:E1:E2] <-> [Lapatinib:E1:E2:Lapatinib]	kfLapatinib_1	krLapatinib_1
Lapatinib_v10	Lapatinib + [Lapatinib:E2:E2] <-> [Lapatinib:E2:E2:Lapatinib]	kfLapatinib_2	krLapatinib_2
Lapatinib_v11	Lapatinib + [BTC:E1] <-> [Lapatinib:BTC:E1]	kfLapatinib_1	krLapatinib_1
Lapatinib_v12	Lapatinib + [BTC:E1:E1] <-> [Lapatinib:BTC:E1:E1]	kfLapatinib_3	krLapatinib_1
Lapatinib_v13	Lapatinib + [BTC:E1:E1:BTC] <-> [Lapatinib:BTC:E1:E1:BTC]	kfLapatinib_3	krLapatinib_1
Lapatinib_v14	Lapatinib + [BTC:E1:E2] <-> [Lapatinib:BTC:E1:E2]	kfLapatinib_3	krLapatinib_1
Lapatinib_v15	Lapatinib + [BTC:E1:E3] <-> [Lapatinib:BTC:E1:E3]	kfLapatinib_1	krLapatinib_1
Lapatinib_v16	Lapatinib + [E2:E3:HRG] <-> [Lapatinib:E2:E3:HRG]	kfLapatinib_2	krLapatinib_2
Lapatinib_v17	Lapatinib + [E1:E3:HRG] <-> [Lapatinib:E1:E3:HRG]	kfLapatinib_1	krLapatinib_1
Lapatinib_v18	Lapatinib + [Lapatinib:BTC:E1:E1] <-> [Lapatinib:BTC:E1:E1:Lapatinib]	kfLapatinib_1	krLapatinib_1
Lapatinib_v19	Lapatinib + [Lapatinib:BTC:E1:E1:BTC] <-> [Lapatinib:BTC:E1:E1:BTC:Lapatinib]	kfLapatinib_1	krLapatinib_1
Lapatinib_v20	Lapatinib + [Lapatinib:BTC:E1:E2] <-> [Lapatinib:BTC:E1:E2:Lapatinib]	kfLapatinib_1	krLapatinib_1
Lapatinib_v21	[Lapatinib:E1] + E1 <-> [Lapatinib:E1:E1]	kf12	kr12
Lapatinib_v22	[Lapatinib:E1] + E2 <-> [Lapatinib:E1:E2]	kf12	kr12
Lapatinib_v23	[Lapatinib:E1] + E3 <-> [Lapatinib:E1:E3]	kf12	kr12
Lapatinib_v24	[Lapatinib:E2] + E1 <-> [Lapatinib:E1:E2]	kf12	kr12
Lapatinib_v25	[Lapatinib:E2] + E3 <-> [Lapatinib:E2:E3]	kf12	kr12
Lapatinib_v26	[Lapatinib:E1] + [Lapatinib:E1] <-> [Lapatinib:E1:E1:Lapatinib]	kf12	kr12
Lapatinib_v27	[Lapatinib:E1] + [Lapatinib:E2] <-> [Lapatinib:E1:E2:Lapatinib]	kf12	kr12
Lapatinib_v28	[Lapatinib:E2] + [Lapatinib:E2] <-> [Lapatinib:E2:E2:Lapatinib]	kf12	kr12
Lapatinib_v29	[Lapatinib:BTC:E1] + E1 <-> [Lapatinib:BTC:E1:E1]	kf14	kr14
Lapatinib_v30	[Lapatinib:BTC:E1] + E2 <-> [Lapatinib:BTC:E1:E2]	kf19	kr19
Lapatinib_v31	[Lapatinib:BTC:E1] + E3 <-> [Lapatinib:BTC:E1:E3]	kf23	kr19
Lapatinib_v32	[Lapatinib:BTC:E1] + [BTC:E1] <-> [Lapatinib:BTC:E1:E1:BTC]	kf16	kr16
Lapatinib_v33	[Lapatinib:BTC:E1] + [Lapatinib:BTC:E1] <-> [Lapatinib:BTC:E1:E1:BTC:Lapatinib]	kf16	kr16
Lapatinib_v34	[Lapatinib:BTC:E1] + [Lapatinib:E2] <-> [Lapatinib:BTC:E1:E2:Lapatinib]	kf19	kr19
Lapatinib_v35	[Lapatinib:E2] + [HRG:E3] <-> [Lapatinib:E2:E3:HRG]	kf7	kr7
Lapatinib_v36	[Lapatinib:E1] + [HRG:E3] <-> [Lapatinib:E1:E3:HRG]	kf10	kr7
Lapatinib_v37	[Lapatinib:E1] + BTC <-> [Lapatinib:BTC:E1]	kf3	kr3
Lapatinib_v38	[Lapatinib:E1:E1] + BTC <-> [Lapatinib:BTC:E1:E1]	kf4	kr3
Lapatinib_v39	[Lapatinib:E1:E2] + BTC <-> [Lapatinib:BTC:E1:E2]	kf5	kr3
Lapatinib_v40	[Lapatinib:E1:E3] + BTC <-> [Lapatinib:BTC:E1:E3]	kf5	kr3
Lapatinib_v41	[Lapatinib:BTC:E1:E1] + BTC <-> [Lapatinib:BTC:E1:E1:BTC]	kf4	kr4
Lapatinib_v42	[Lapatinib:E2:E3] + HRG <-> [Lapatinib:E2:E3:HRG]	kf2	kr1

Table S8: Inhibitor parameter values.

Name	Value	Units	Description
Vcell	1.0E-12	Liters	Cell volume.
Vshell	4.8×10^{-15}	Liters	Reduced volume Vshell for 2nd arm of IgG binding. Vshell = 4/3*pi*((Cell_radius + Cell_shellheight)^3- cell_radius) Cell radius (Vcell*3/(4*pi()))^(1/3) in decimeters. Cell_shellheight = 1×10^{-7} decimeters. Cell_shellhight represents the average distance between antibody binding sites. [26] [27]
MM-121 parameters			
kfMM121_1	7.15×10^{-11}	$molecules^{-1}\ sec^{-1}$	MM121 inhibitor binding to ErbB3. Unit conversion calculated as $1.43 \times 10^{5}\ M^{-1}s^{-1}$ /(Vmedia*Av/Num_cells). Num_cells = 30,000 per well.

krMM121_1	1.10×10^{-04}	sec^{-1}	MM121 inhibitor dissociation from ErbB3
kfMM121_2	4.96×10^{-05}	$molecules^{-1}\ sec^{-1}$	MM121 inhibitor binding to a second ErbB3. Avidity due to volume reduction =krMM121_1*(Vmedia/Numcells)/Vshell.
krMM121_2	2.20×10^{-04}	sec^{-1}	MM121 inhibitor dissociation from a second ErbB3 (2*krmm121_1)
kfMM121_3	5.56×10^{-04}	sec^{-1}	Internalization rate for MM121 bound to two ErbB3 molecules
krMM121_3	5.00×10^{-03}	sec^{-1}	Recycling rate for MM121 bound to two ErbB3 molecules
kfMM121_4	2.00×10^{-04}	sec^{-1}	Degradation of MM121 bound species
Cetuximab parameters			
kfCetuximab_1	1.10×10^{-10}	$molecules^{-1}\ sec^{-1}$	Cetuximab inhibitor binding to ErbB1. Unit conversion calculated as $2.2 \times 10^{5}\ M^{-1}s^{-1}$ /(Vmedia*Av/Num_cells). [23]
krCetuximab_1	1.10×10^{-03}	sec^{-1}	Cetuximab inhibitor dissociation from ErbB1
kfCetuximab_2	7.64×10^{-05}	$molecules^{-1}\ sec^{-1}$	Cetuximab inhibitor binding to a second ErbB1. Avidity due to volume reduction =krCetuximab_1*(Vmedia/Numcells)/Vshell.
krCetuximab_2	2.20×10^{-03}	sec^{-1}	Cetuximab inhibitor dissociation when bound to two ErbB1 molecules (2*krcetuximab_1)
Pertuzumab parameters			
kfPertuzumab_1	5.60×10^{-11}	$molecules^{-1}\ sec^{-1}$	Pertuzumab inhibitor binding to ErbB2. Unit conversion calculated as $1.12 \times 10^{5}\ M^{-1}s^{-1}$ /(Vmedia*Av/Num_cells). [24]
krPertuzumab_1	9.50×10^{-04}	sec^{-1}	Pertuzumab inhibitor dissociation from ErbB2 [24]
kfPertuzumab_2	3.90×10^{-05}	$molecules^{-1}\ sec^{-1}$	Pertuzumab inhibitor binding to a second ErbB2. Avidity due to volume reduction =krPertuzumab_1*(Vmedia/Numcells)/Vshell.
krPertuzumab_2	1.90×10^{-03}	sec^{-1}	Pertuzumab inhibitor dissociation from a second ErbB2 (2*krpertuzumab_1).
Lapatinib parameters			
kfLapatinib_1	6.40×10^{-12}	$molecules^{-1}\ sec^{-1}$	Lapatinib inhibitor binding to ErbB1 or 1-3 dimers. Unit conversion calculated as $1.28 \times 10^{4}\ M^{-1}s^{-1}$ /(Vmedia*Av/Num_cells). [22].
krLapatinib_1	3.83×10^{-05}	sec^{-1}	Lapatinib inhibitor dissociation from ErbB1 or 1-3 dimers. [22].
kfLapatinib_2	1.50×10^{-12}	$molecules^{-1}\ sec^{-1}$	Lapatinib inhibitor binding from ErbB2 or 2-3 dimers. Unit conversion calculated as $2.95 \times 10^{3}\ M^{-1}s^{-1}$ /(Vmedia*Av/Num_cells). [22]
krLapatinib_2	3.83×10^{-05}	sec^{-1}	Lapatinib inhibitor dissociation to ErbB2 or 2-3 dimers.
kfLapatinib_3	1.28×10^{-11}	$molecules^{-1}\ sec^{-1}$	Lapatinib inhibitor binding to 1-1 or 1-2 dimers
kfLapatinib_4	3.00×10^{-12}	$molecules^{-1}\ sec^{-1}$	Lapatinib inhibitor binding to 2-2 dimers

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