Supplementary Table 1. List of TFrs used in the study

TF Reporter	Sequence	Reference
AP1	TGACTAA x8	Panomics
CMYC	CACGTG x5	Transfac ID R02207
CRE	GCACCAGACAGTGACGTCATGGCCGTCATGACGTCACCCCATTGACGTCAATGGGAGAAC	Panomics
E2F1	TTTGGCGCGTAA x5	Panomics
ELK1	TTTGCAAAATGCAGGAATTGTTTTCACAGT x2	Panomics
GATA	CACTTGATAACAGAAAGTGATAAT GATAACAGAAAGTGATAACTCT	Panomics
HIF1	GTGACTACGTGCTGCCTAG x4	Panomics
HSE	CTGGAATTTTCTAGA x3	Panomics
IRF1	GGAAGCGAAAATGAAATTGACT x6	Panomics
NFAT	GGAGGAAAAACTGTTTCATACAGAAGGCGT x3	Panomics
NFkB	GGGAATTTCC x6	Panomics
NOTCH1	TTTCCCACGx3	Transfac ID R27998
P53	TACAGAACATGTCTAAGCATGCTGTGCCTTGCCTGGACTTGCCTGGCCTTGCCTTGGG	Panomics
PTTG	TTATCTAACTCGCTGTAGTAATTC x3	*Pei 2001
RAR	AGGTCACCAGG x5	Panomics
SMAD	AGTATGTCTAGACTGA x3	Panomics
SMAD3/4	TCGAGAGCCAGACAAAAAGCCAGACATTTGCCAGACAC	Panomics
SP1	GTATTTCCCAGAAAAGGAAC x3	Panomics
SRF	GGATGTCCATATTAGGACATC	Panomics
STAT1	GTATTTCCCAGAAAAGGAAC x3	Panomics
STAT3	TGCTTCCCGAATTCCCGAATTCCCGAATTCCCGAATTCCCGAATTCCCGAACGT	Panomics
STAT4	CTAGAGCCTGATTTCCCCGAAATGATGAGCTAG x2	Panomics
STAT5	ATTTCCAGGAAAT x3	Transfac ID M00457
TCF/LEF	AAGATCAAAGGGGGTAAGATCAAAGGGGGTAAAATCAAAGGGGGCCCCCTTTGATCTTACCCCCTTTGATCTTACCCCCTTTGATCTTA	*Veeman et al. 2003
YY1	CGCTCCCCGGCCATCTTGGCGGGTGGT x2	Panomics

Supplementary Table 2. P-values for constitutive ErbB2 activity experiments. Average TFr activity for the control has been subtracted for EGF and DA conditions. The p-values correspond to the differences between the corresponding time points versus the initial time (day 0). No false discovery rate correction has been applied to these p-values. Contrasts with p-values ≤0.15 are considered significant. The values for the discretization of the normalized TFr needed for NTRACER are obtained using the *decideTests* function from limma with no p-value adjustment at 0.15. We note that p-values of 0.15 were chosen largely as a result of the variability that was observed during the last 3 days of culture (Pearson correlation values dropped from 0.9 to 0.7 in the last three days of culture)

TFr	DA					EGF				
	Time (day)									
	1	3	5	7	10	1	3	5	7	10
AP1	6.E-02	1.E-01	7.E-02	3.E-01	2.E-01	5.E-01	6.E-01	4.E-01	1.E-01	3.E-02
CMYC	2.E-01	8.E-01	6.E-01	6.E-01	3.E-01	7.E-01	8.E-02	2.E-01	6.E-01	9.E-01
CRE	3.E-01	7.E-01	7.E-01	1.E-01	8.E-01	7.E-01	4.E-01	3.E-01	6.E-01	5.E-03
E2F1	5.E-01	4.E-01	1.E-01	6.E-02	3.E-02	8.E-01	2.E-01	4.E-01	3.E-01	2.E-01
ELK1	5.E-01	5.E-01	4.E-01	7.E-03	1.E-01	4.E-01	1.E-01	3.E-01	5.E-01	7.E-02
GATA	9.E-01	1.E+00	5.E-01	3.E-01	7.E-01	9.E-01	6.E-01	9.E-01	7.E-01	3.E-01
HIF1	8.E-01	6.E-01	4.E-01	2.E-02	2.E-01	7.E-01	1.E-01	1.E-01	6.E-02	1.E+00
HSE	4.E-01	6.E-01	8.E-02	5.E-01	1.E+00	6.E-01	2.E-01	6.E-01	1.E+00	5.E-01
IRF1	5.E-01	2.E-01	3.E-01	5.E-01	8.E-01	8.E-01	2.E-02	1.E-01	9.E-01	5.E-01
NFAT	9.E-01	5.E-01	2.E-01	6.E-02	1.E-02	6.E-01	2.E-01	3.E-01	6.E-01	6.E-01
NFKB	3.E-01	4.E-01	1.E-01	1.E-02	3.E-01	1.E+00	6.E-03	3.E-01	1.E-01	1.E+00
NOTCH1	5.E-01	3.E-01	4.E-01	6.E-01	4.E-01	7.E-01	5.E-01	2.E-01	1.E-01	4.E-03
P53	8.E-01	4.E-01	3.E-01	4.E-01	1.E-02	3.E-01	6.E-01	9.E-01	5.E-01	6.E-01
PTTG	7.E-01	8.E-01	6.E-01	6.E-02	3.E-02	5.E-01	9.E-01	9.E-01	4.E-01	1.E+00
RAR	3.E-01	3.E-01	1.E-01	9.E-03	2.E-02	1.E+00	8.E-01	9.E-01	7.E-01	5.E-01
SMAD3/4	1.E+00	9.E-01	6.E-01	2.E-01	1.E-01	5.E-01	5.E-01	1.E+00	9.E-01	7.E-01
SP1	6.E-01	8.E-01	6.E-01	1.E-01	4.E-02	5.E-01	1.E-01	5.E-01	4.E-01	1.E-01
SRE	7.E-01	1.E-02	8.E-04	3.E-05	7.E-06	7.E-01	9.E-02	1.E-01	7.E-01	6.E-01
STAT1(2)	7.E-01	3.E-02	1.E-02	3.E-04	7.E-03	4.E-01	1.E-02	2.E-01	6.E-01	3.E-02
STAT3(1)	5.E-01	4.E-01	7.E-01	3.E-03	5.E-04	9.E-01	2.E-01	4.E-01	5.E-02	1.E-02
STAT4	6.E-01	9.E-01	3.E-01	2.E-02	2.E-02	4.E-01	4.E-02	5.E-02	3.E-02	2.E-01
STAT5	9.E-01	9.E-01	2.E-01	2.E-02	3.E-01	4.E-01	3.E-01	5.E-01	9.E-01	3.E-01
YY1	1.E+00	4.E-01	4.E-02	6.E-04	5.E-03	7.E-01	4.E-01	4.E-01	4.E-01	6.E-01

Supplementary Table 3. P-values under lapatinib treatment. Average TFr activity for the control has been subtracted for lapatinib, pertuzumab and trastuzumab conditions. The p-values correspond to the differences between the corresponding time points versus the initial time (day 0). False discovery rate correction has been applied to these p-values. Contrasts with p-values ≤0.05 are considered significant. Note that the 10A/ErbB2 cells lack the extracellular domain of ErbB2, and thus the limited efficacy of trastuzumab and pertuzumab was expected and served as a control for the array. Trastuzumab and pertuzumab did not stimulate any of the key TFrs found using lapatinib. The values for the discretization of the normalized TFr needed for NTRACER are obtained using the *decideTests* function from limma with fdr p-value adjustment at 0.05.

	Lapatinib			Pertuzumab			Trastuzumab		
	Time (day)								
TFr	1	2	3	1	2	3	1	2	3
AP1	2.E-01	2.E-01	9.E-01	8.E-01	6.E-01	9.E-01	9.E-01	3.E-01	9.E-01
CMYC	5.E-02	3.E-02	1.E+00	6.E-01	2.E-01	8.E-01	9.E-01	4.E-01	7.E-01
CRE	5.E-02	5.E-02	1.E-02	8.E-01	2.E-01	9.E-01	1.E+00	5.E-01	9.E-01
E2F1	4.E-04	2.E-01	2.E-03	6.E-01	8.E-01	9.E-01	9.E-01	2.E-01	9.E-01
ELK1	5.E-03	4.E-04	9.E-01	1.E+00	9.E-01	3.E-01	9.E-01	3.E-01	8.E-01
GATA	5.E-02	2.E-01	5.E-01	1.E+00	9.E-01	3.E-01	9.E-01	7.E-01	5.E-01
HIF1	6.E-01	2.E-01	8.E-01	6.E-01	5.E-02	5.E-01	9.E-01	2.E-01	6.E-01
HSE	6.E-01	9.E-01	2.E-01	7.E-01	2.E-01	9.E-01	9.E-01	3.E-01	9.E-01
IRF1	7.E-02	3.E-02	5.E-01	8.E-01	5.E-01	8.E-01	9.E-01	4.E-01	9.E-01
NFAT	5.E-02	2.E-01	1.E-02	7.E-01	4.E-01	3.E-01	9.E-01	5.E-01	6.E-01
NFKB	5.E-01	1.E+00	1.E-02	6.E-01	2.E-01	9.E-01	9.E-01	4.E-01	1.E+00
NOTCH1	6.E-01	1.E+00	8.E-03	8.E-01	9.E-01	4.E-01	9.E-01	6.E-01	8.E-01
P53	2.E-02	4.E-07	2.E-03	6.E-01	2.E-01	8.E-01	9.E-01	3.E-01	1.E+00
PTTG	5.E-01	3.E-01	3.E-01	8.E-01	5.E-01	9.E-01	9.E-01	4.E-01	1.E+00
RAR	1.E-01	1.E-01	8.E-03	6.E-01	4.E-01	8.E-01	9.E-01	8.E-01	6.E-01
SMAD3/4	7.E-01	3.E-01	2.E-03	8.E-01	8.E-01	3.E-01	9.E-01	2.E-01	9.E-01
SP1	2.E-01	3.E-01	1.E-02	6.E-01	3.E-01	9.E-01	9.E-01	4.E-01	8.E-01
SRE	1.E-01	2.E-01	9.E-01	6.E-01	2.E-01	5.E-01	9.E-01	4.E-01	1.E+00
STAT1(2)	5.E-01	6.E-03	9.E-01	7.E-01	2.E-01	8.E-01	9.E-01	2.E-01	1.E+00
STAT3(1)	1.E-01	1.E-01	2.E-01	6.E-01	2.E-01	9.E-01	9.E-01	4.E-01	1.E+00
STAT4	6.E-04	7.E-04	9.E-01	1.E+00	9.E-01	3.E-01	9.E-01	6.E-01	8.E-01
STAT5	1.E-01	1.E-02	9.E-01	8.E-01	9.E-01	3.E-01	9.E-01	3.E-01	9.E-01
YY1	4.E-01	3.E-01	1.E-01	1.E+00	9.E-01	3.E-01	9.E-01	2.E-01	1.E+00

Supplementary Table 4. Truth tables for the Boolean model. Examples for activating and inhibiting gates for the gate outcomes of the modified CellNOptR algorithm

Gate Type	Input state	Input prior change	Output Prior level	Output change rate	Gate outcome
A->B	1	1	-1	0	1
	1	1	-1	1	0
	1	1	-1	2	1
	1	1	0	-1	1
	1	1	0	0	1
	1	1	0	1	1
	1	1	1	-2	1
	1	1	1	-1	1
	1	1	1	0	1
	REST OF CONDITIONS				No change
A-|B	1	1	-1	0	-1
	1	1	-1	1	-1
	1	1	-1	2	-1
	1	1	0	-1	-1
	1	1	0	0	-1
	1	1	0	1	-1
	1	1	1	-2	-1
	1	1	1	-1	0
	1	1	1	0	-1
	REST OF CONDITIONS				No change

Supplementary Table 5. Parameters used for the genetic algorithm for the modified CellNOptR

Parameters	DA	Lapatinib
Population size	50	50
Elitism	5	1
randedgeper	0.1	random (between 0 and 1)
Selective Pressure	2	2
Maximum generations	1500	1500
Maximum stall generations	1000	1000
Maximum iteration time (s)	3600000	3600000
$size_{Pen}$	0.001	0.001
$Stim_{Pen}$	0.003	0.003
$InhM_{Pen}$	10	8
SP	2	2
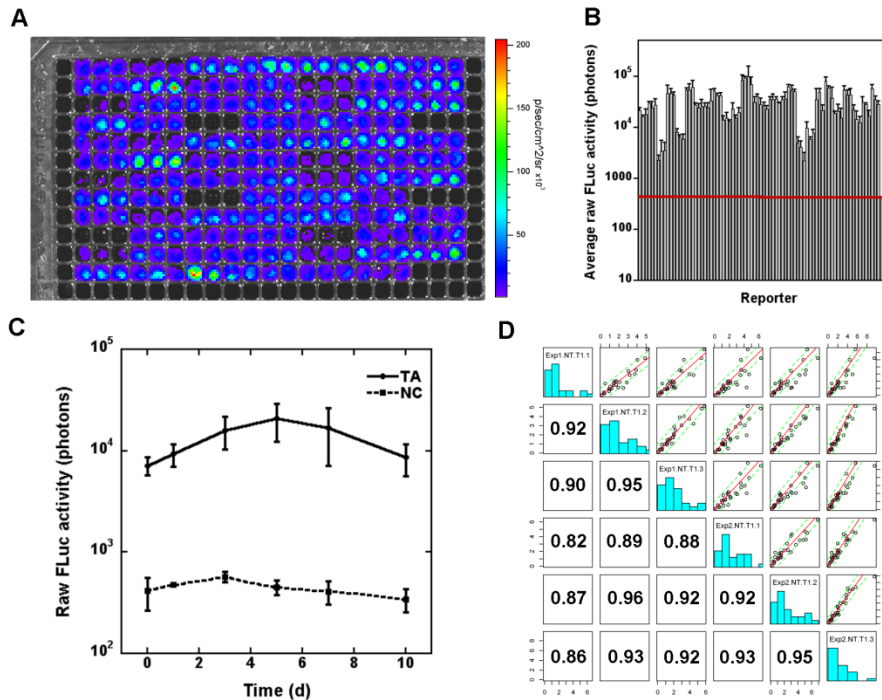
Supplementary Figure 1. Transduced cell array to monitor large-scale TFr activities. 10A/ErbB2 cells growing in BME were assessed for large-scale TFr activity using transduced cell arrays. A) Sample pseudo-color image for FLuc light production from 25 individual reporters in triplicate, in addition to TA and blank hydrogel conditions (NC), representing cells stimulated with no stimulation, EGF, or DA. B) Average FLuc activity ± s.d. from reporters was consistently above background levels from NC (red line). C) Time course imaging of average FLuc activity ± s.d. from TA reporter and NC over a period of 10 d. D) Correlation matrix between replicates of normalized TFr activity, comparing replicates for all reporter genes pairwise. Panels on the diagonal are histograms, panels on the right are pairwise correlation plots, and panels on the left are values of the correlations.
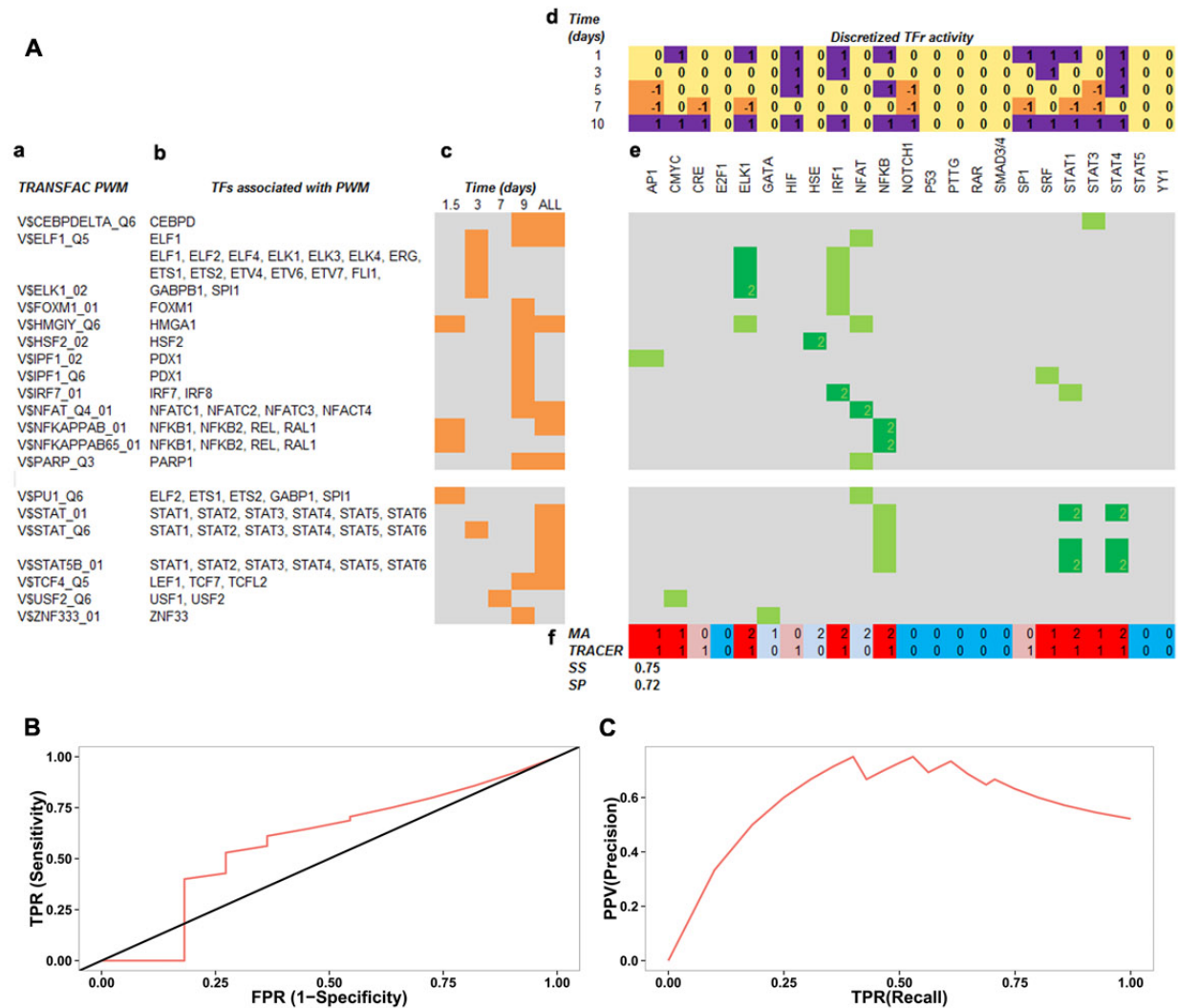
Supplemental Figure 2. Comparison between TF activity determined by transcriptomics and transcriptional activity in the cell array for EGF treated MCF10A cells. A) Panels include: a) most likely active PWMs for the significantly changed genes according to the mRNA microarray results; b) the TFs associated with each of the most likely active PWMs; c) in orange, the times at which those PWMs are active; d) top panel indicates the discretized TFr activity according to the TRACER measurements (in purple, upregulated TFr activity, 1; in yellow, no change, 0; and in orange, down-regulated TFr activity, -1); e) the middle center panel represents whether the given PWM from the microarray analysis is also associated with each of the TF reporters. Dark green indicates reporters that are associated with the TF for which they were originally designed; light green indicates those TFr that are associated with TFs different from that which they were originally designed; grey indicates no association between the microarray data and the TRACER results; f) bottom panel reflects the results from the microarray study (MA) and the transcriptional activity cell array results (TRACER). The MA results are coded as 0, the TF is not computationally predicted to be active; 1 the reporter is computationally predicted to be active due to a different TF; 2 the reporter is indeed predicted as active due to the TF for which it was designed. TRACER results indicate whether the TF was altered during the experiment, 1, or not, 0. Sensitivity, SS, and specificity, SP, are reported assuming the MA results as reference. A p-value of 0.15 for the TRACER results has been employed to generate panel A. B) the receiving operating characteristic (ROC) curve shows the variation of sensitivity (true positive rate, TPR) as a function of the false positive rate (FPR), which is in turned related with the specificity of the method (in red); in black, it is the diagonal line (TPR=FPR). C) Precision-recall plots where the positive predicted value (PPV) is represented as a function of the TPR.
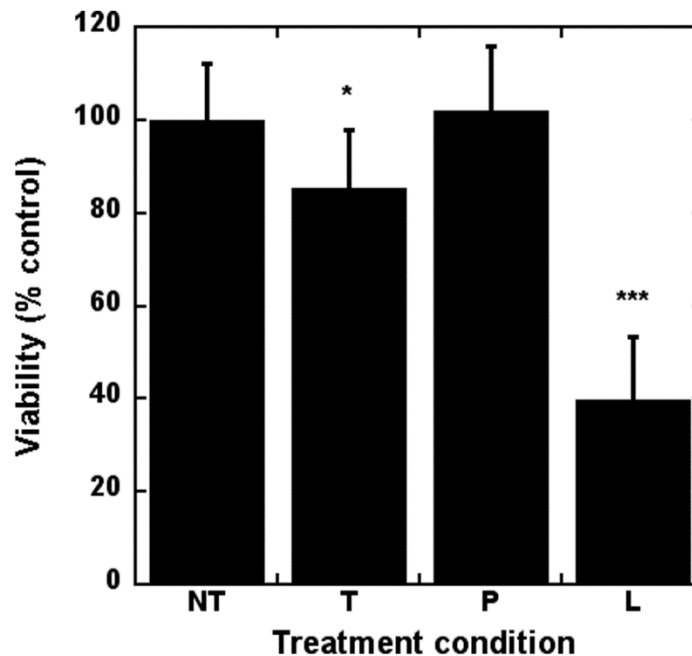
Supplementary Figure 3. Treatment of cells with ErbB2-targeting therapeutics. 10A/ErbB2 cells growing in BME were stimulated with DA subsequently treated with no therapeutic (NT), trastuzumab (T), pertuzumab (P), or lapatinib (L), and cell viability was assessed after 3 d. Relative to NT, T and L both decreased viability. Values represent average ± s.d. from triplicate wells and experiment was carried out three times. (* p<0.05, *** p<0.001). Note that the 10A/ErbB2 cells lack the extracelluar domain of ErbB2, and thus the limited efficacy of trastuzumab and pertuzumab was expected and served as a control for the array. Trastuzumab and pertuzumab did not stimulate any of the key TFrs found using lapatinib.
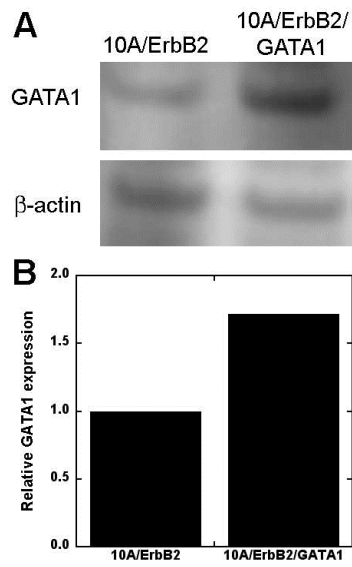
Supplementary Figure 4. GATA1 overexpression in 10A/ErbB2 cells. A) Sample Western blot depicting bands for GATA1 and β-actin from 10A/ErbB2 and 10A/ErbB2/GATA1 cells to confirm overexpression. B) Sample quantification of a Western blot for relative GATA1 expression.
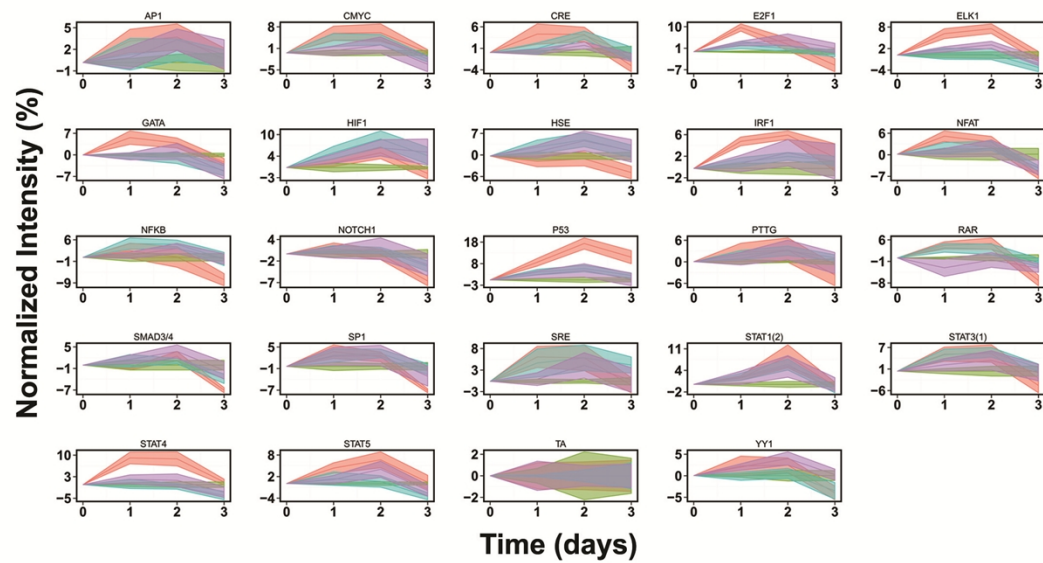
Supplementary Figure 5. Treatment of cells with ErbB2-targeting therapeutic. Transduced cell arrays measured activities of TF reporters in DA activated 10A/ErbB2 cells growing in BME following no treatment (blue), lapatinib treatment (red), pertuzumab (green) and trastuzumab (pink). Normalized transcription factor intensities for TFr above the background are represented (TCF/LEF, SMAD and NC are excluded). Shaded areas around the average lines represent ± standard error. For more details about TF reporter significance see methods and supplemental table 4. Note that the 10A/ErbB2 cells lack the extracellular domain of ErbB2, and thus the limited efficacy of trastuzumab and pertuzumab was expected and served as a control for the array. Trastuzumab and pertuzumab did not activate any of the key TFr associated with lapatinib treatment.
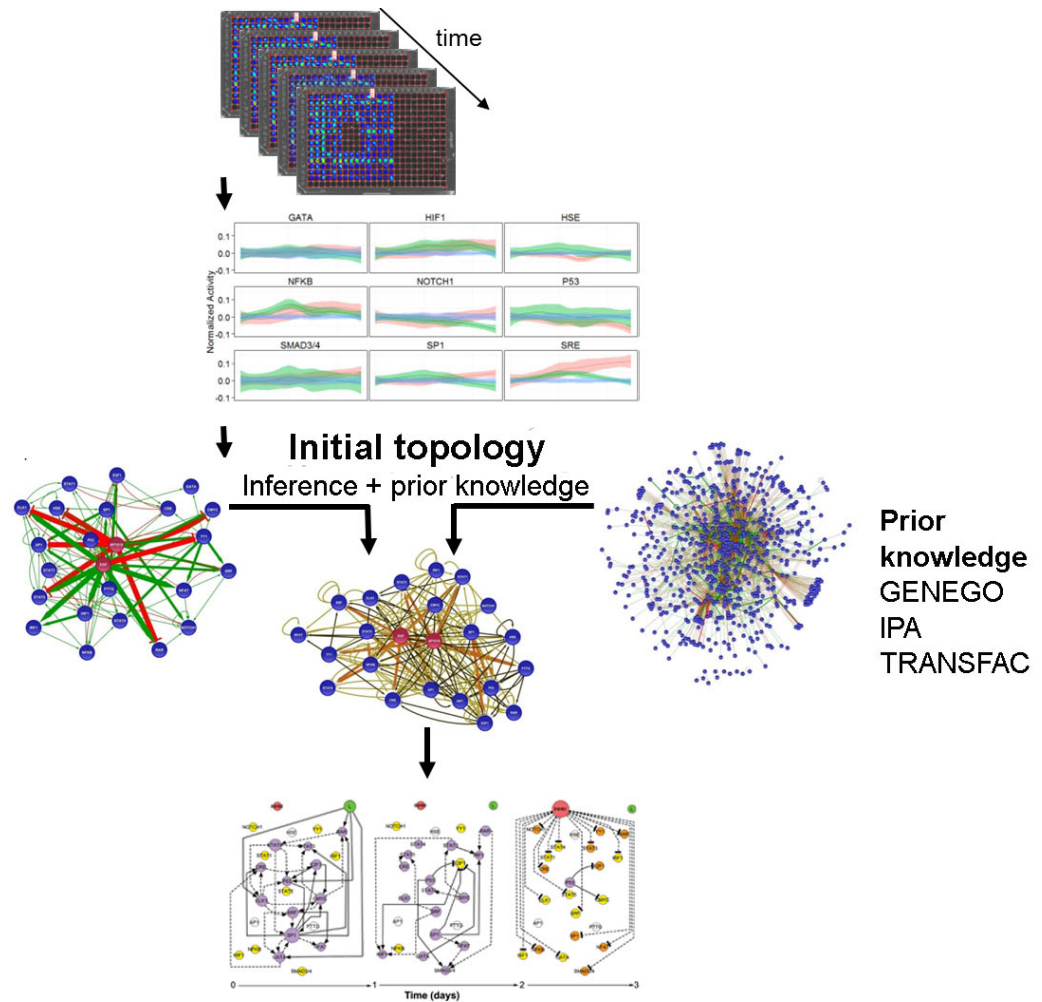
TRACER
(Transcriptional Activity
Cell Arrays)

ATRACER
(Statistical analysis
to identify significant
reporters)

NTRACER

Inference
PLSR
MI
BN

Dynamic networks

Initial topology
Inference + prior knowledge

**Prior
knowledge**
GENEGO
IPA
TRANSFAC

Supplementary Figure 6. **Overview of the pipeline to analyze transcriptional activities arrays (TRACER).** Biolumiscencent results from TRACER are analyzed to identify the key significant TFrs that are modulated by the experimental treatments. Normalized activity of these key significant TFrs is employed to infer connection between them using the union of diverse linear and non-linear inference methods. Inferred connections are combined with a prior knowledge network obtained from different databases to generate an initial topology for the genetic algorithm used in the modified version of CellNOptR. Optimization results yield to the dynamic networks.
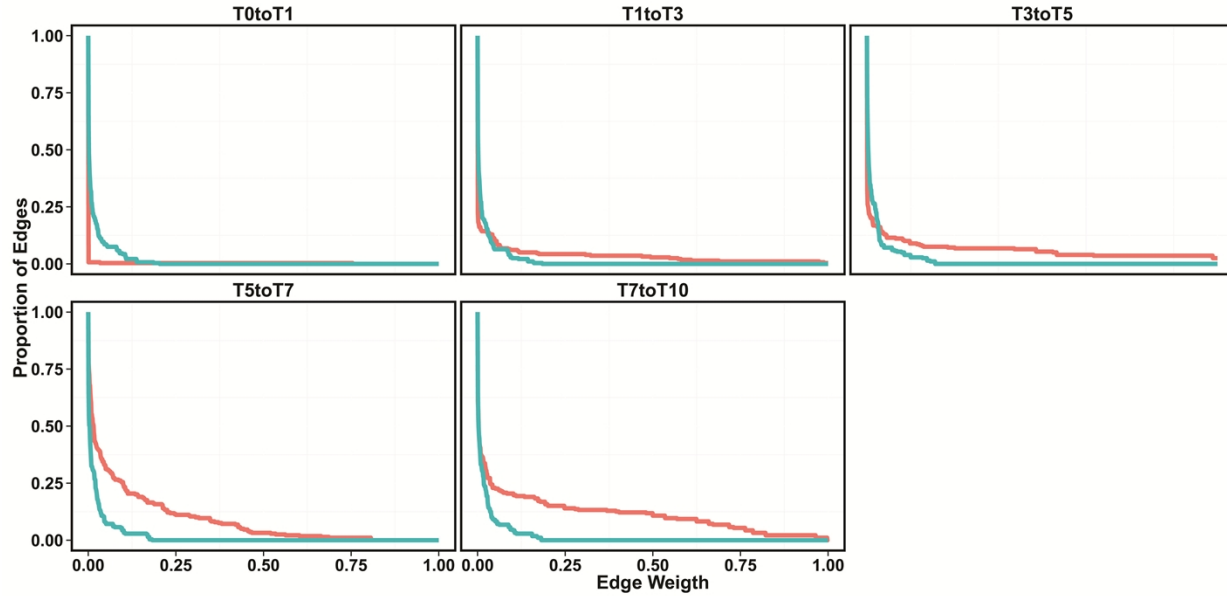
Supplementary Figure 7. **Significant edges in the ErbB2 activation cells as a function of their probability of appearance (edge weight) in the bootstrapping and permutation simulations.** In red, the results for the bootstrapping analysis; and in blue, the permutation results. Edges identified during the bootstrapping simulation have high probabilities or edge weights compared with the null model as measured by the permutation simulations and are time interval dependent. P-values for the significant edges with respect to the null model are summarized in Supplementary File 2.
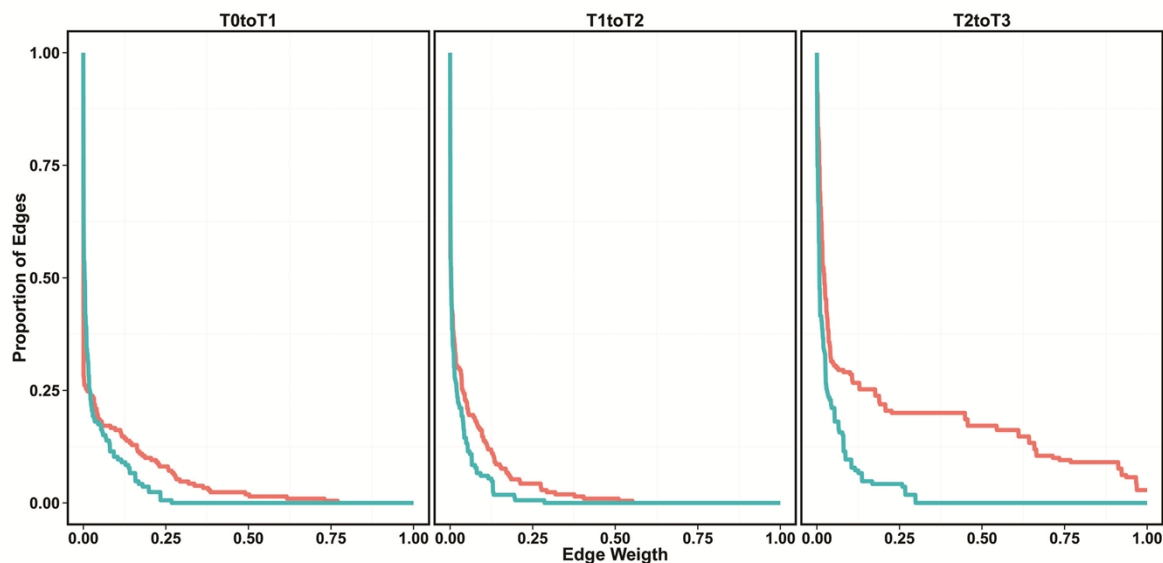
Supplementary Figure 8. **Significant edges in the after lapatinib of ErbB2 activation cells as a function of their probability of appearance (edge weight) in the bootstrapping and permutation simulations.** In red, the results for the bootstrapping analysis; and in blue, the permutation results. Edges identified during the bootstrapping simulation have high probabilities or edge weights compared with the null model as measured by the permutation simulations and are time interval dependent. P-values for the significant edges with respect to the null model are summarized in Supplementary File 2.
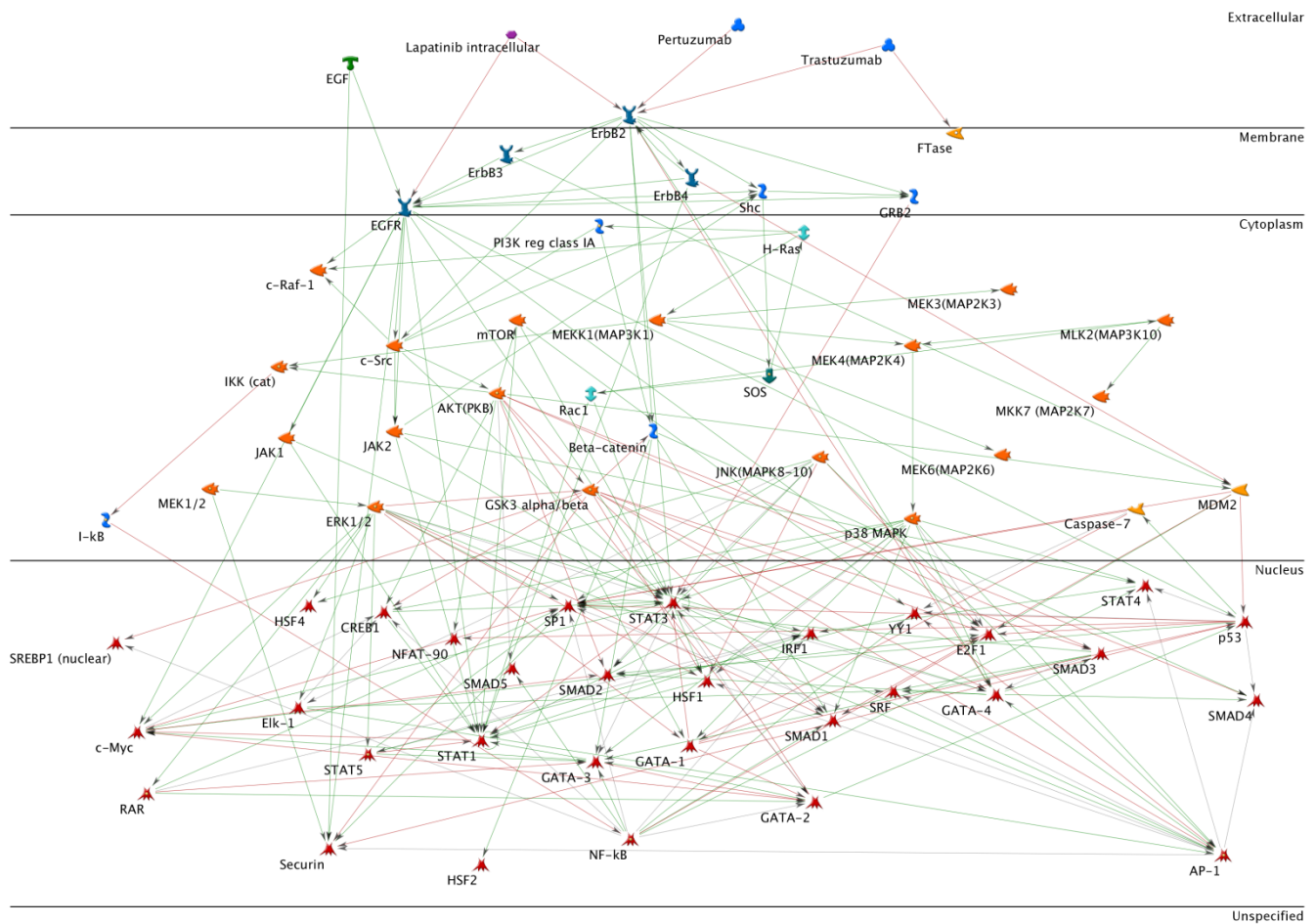
Supplementary Figure 9. **Signaling pathways for the ErbB receptor family.** Signaling pathways link the different treatments given to the cells (i.e., EGF, lapatinib) to TFrs measured by TRACER. Signaling networks are obtained from GENEGO.