Genome-scale regression analysis reveals a linear relationship for promoters and enhancers after combinatorial drug treatment

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Supplementary notes

RNA sample preparation

Human MCF-7 breast cancer cells were purchased from the American Type Culture Collection and maintained in DMEM (Invitrogen) supplemented with 10% FBS containing Penicillin-Streptomycin mixed solution (Nacalai Tesque, Japan). One day before drug treatment, medium was switched to DMEM supplemented with 2% FBS. Cells (approx. 2 x 10^6 cells/ sample) were treated separately or in pair-wise combinations with Gefitinib (ZD1839) (1 μM, a generous gift from AstraZeneca), U0126 (500 nM, Calbiochem), and Wortmannin (10 nM, Nacalai Tesque) for six hours. All drugs were dissolved in DMSO and DMSO was used as the treatment control. After the treatment, the cells were washed with PBS twice followed by RNA purification using the miRNeasy Mini kit (QIAGEN) and quality check using Bioanalyzer (Agilent technology).

Single molecule CAGE data production

Triplicate samples were analyzed for each drug treatment. CAGE libraries for single molecule sequencing were constructed using 5 μg of total RNA as described previously (Kanamori-Katayama *et al.*, 2011), and sequenced on HeliScope. CAGE tags were processed and mapped to genomic positions as described in detail in (Kajiyama *et al.*, 2013). Briefly, tags remaining after filtering of low quality and ribosomal tags were mapped to the human genome (hg19) using Delve.

CAGE promoter and enhancer expression normalization and differential expression analysis

Mapped CAGE tags were projected onto FANTOM 5 pre-defined robust decomposition peak identification (DPI) cluster regions of promoters and enhancers (Forrest *et al.*, 2014; Andersson *et al.*, 2014; Arner *et al.*, 2015) by the intersectBed function of bedtools (Quinlan, 2014) with default parameters. Expression tables were normalized by the "Relative Log Expression" (RLE) method

as implemented in edgeR (Robinson *et al.*, 2010). Lowly expressed tag clusters were subsequently removed and we kept only tag clusters expressed more than five counts per million (CPM) in at least one sample from the RLE-normalized expression for promoters and more than five counts in at least twelve samples from the RLE-normalized expression for enhancers. In addition, the voom transformation was performed to adjust CPM values to fit a normal distribution by limma (Diboun *et al.*, 2006). We used negative binomial generalized linear models (GLM) for differential analysis of promoters (McCarthy *et al.*, 2012). A GLM likelihood ratio test was applied to identify the significantly altered promoters of treatment conditions against the control condition. The p-values for differential expression were adjusted for multiple testing using false discovery rate (FDR) < 0.05 as a threshold for statistical significance.

Alternative regression models

Quantile Regression: Ordinary least-squares linear regression models the relationship between predictor variables and the conditional mean of the response variable for given levels of the predictor variables. Quantile regression models the relationship between predictor variables and the conditional quantiles of the response variable for given levels of the predictor variables(Koenker, Roger, 1978). All the models were evaluated using ten-fold cross validation. We used 'quanteg' R package for fitting the models(Koenker). We firstly applied quantile regression using the median (Quantile regression=0.5). We also performed internal cross-validation (we kept one fold for validation) to find the quantile that maximizes the Pearson correlation coefficient in the validation set. The results shown in Supplementary Tables 5-7 are the mean performance after ten-fold cross-validation in the test set.

Regression Tree: Linear regression is a global model, where there is an only one predictive function holding over the whole data-space. However if the independent variables interact in nonlinear ways, one simple approach would be to partition the space into smaller regions and fit a different model for each partition. Regression trees are used to represent the recursive partitioning. We used 'tree' R package for fitting the regression tree(Ripley). We optimized the within-node deviance parameter (mindev) using internal cross-validation. The results shown in Supplementary Tables 5-7 are the mean performance after ten-fold cross-validation in the test set.

Linear regression using one predictor variable: We further tried to fit the linear regression model using only the dominant drug profile as input variable. The results shown in Supplementary Tables 5-7 are the mean performance after ten-fold cross-validation in the test set.

Multivariable linear regression with interaction term: We also fit the full model with the two drug profiles as input variables plus a term for their pairwise interaction. The results shown in Supplementary Tables 5-7 are the mean performance after ten-fold cross-validation in the test set.

We further tested whether the performance of the alternative regression models is significantly different than the performance of multivariable linear regression (two explanatory variables). After we performed an F-test to compare whether the variances of samples are equal (p-value>0.05 in all

comparisons), we performed two-sample t-tests for equal means and confidence level 95%, contrasting the 10-fold Pearson correlation coefficients of multivariable linear regression with the 10-fold Pearson correlation coefficients achieved by the alternative models. The exact p-values of the tests are illustrated in Supplementary Tables 5-7 for all drug pairs. As we can notice there is no statically significant difference between multivariable linear regression, quantile regression, and multivariable linear regression with interaction term and moreover, the multivariable linear regression outperforms simple linear regression and regression tree.

Evaluation Metrics

To evaluate the robustness and prediction ability of the regression models on new unseen values of the response variable, we performed 10-fold cross validation. We used three different metrics to assess the performance of the regression models on the test set.

Mean Absolute Error (MAE): measures the average magnitude of the errors in a set of predictions.

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{n}|y(i) - \hat{y}(i)|$$

where $y(i)$ is the observed value, $\hat{y}(i)$ is the predicted value, and n equals the number of observations in the test set.

Pearson Correlation Coefficient: measures the linear correlation between paired variables.

$$r=\frac{n(\sum y_i\hat{y}_i)-\sum y_i\sum \hat{y}_i}{\sqrt{n[\sum {y_i}^2-(\sum y_i)^2][n\sum {\hat{y}_i}^2-(\sum \hat{y}_i)^2]}}$$

where y_i and $\hat{y}_i$ are the observed and the predicted value respectively for i=1, 2, …n, and n is the total number of observations in the test set.

Spearman correlation coefficient: measures the strength of a monotonic relationship between paired variables. It is defined as the Pearson's correlation on the ranked variables.

$$r_s= 1-\frac{6\sum d_i}{n(n^2-1)}$$

where d_i=rg(y_i)- rg($\hat{y}_i$) is the difference between the two ranks of each observation and n is the total number of observations in the test set.

Action of the Gefitinib, Wortmannin, and U0126

Gefitinib inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme (Wakeling *et al.*, 2002). EGFR lies upstream of the Ras-ERK and PI3K-Akt pathways. U0126 is a highly selective inhibitor of MEK1 and MEK2 kinases (Duncia *et al.*, 1998), while Wortmannin is a fungal metabolite that acts as a potent, irreversible inhibitor of phosphatidylinositol 3-kinase (PI3K) (Powis *et al.*, 1994). The composite effects of the three compounds have been previously reported in the literature. Gefitinib and U0126 mixture inhibits the growth of RAS-active cancer cells (El-Chaar *et al.*, 2014). Gefitinib in combination with Wortmannin suspends proliferation of U87 cells, compared to the individual drugs alone (Yelskaya *et al.*, 2013) while U0126 and Wortmannin jointly lead to synergistic induction of apoptosis in LN215 and LN229 cell lines (Failly *et al.*, 2007).

Permutation tests

To validate the statistical significance of the results and test whether both drug conditions contribute to the model, we performed the same regression analysis 100,000 times with random permutations of one of the single drug treatment profiles. The Pearson correlation between the observed and predicted values after the permutations was significantly lower for all combinations (p-value < 2.2e-16) compared to the regression model based on the non-permuted individual drug profiles (Supplementary Figure 3 and 6 for promoters and enhancers respectively).

Promoters that do not follow the global linear trend

To identify the promoters that were not described efficiently by the linear regression function, we examined the distribution of residuals. We selected all the promoters, which fell further than two standard deviations away from what would have been expected based on the regression model and compared to the set of differentially expressed ones (Supplementary Figure 9).

Gene Ontology enrichment of promoters not described by the linear model

To gain further insight into this group of promoters and investigate whether there is any distinct pattern that characterizes them, we performed a multiple step analysis. The non-linearly described promoters were subjected to Gene Ontology (Ashburner *et al.*, 2000) enrichment analysis (Supplementary Tables 10-17). In the Molecular Function ontology, out of the model promoters in all drug combinations were highly enriched with "RNA polymerase II transcription factor activity"

and "nucleic acid binding transcription factor activity" terms, suggesting that transcription factors are abundant among the promoters that do not behave linearly (Supplementary Figure 10).

We used only promoters which have been associated with Entrez Gene IDs. We ran the topGO R package (topGO: Enrichment Analysis for Gene Ontology. R package version 2.24.0.) for gene ontology enrichment analysis (algorithm = "classic", statistic = "fisher", genes of interest: genes associated with the non-linearly behaved promoters, background genes: genes associated with all the promoters in the study). We applied the Benjamini-Hochberg (BH) adjustment of the p-values. The analysis yielded no significantly enriched GO terms for the Cellular Component domain for the Gefitinib-Wortmannin drug combination. We then used REVIGO software(Supek *et al.*, 2011) to summarize and cluster the enriched Gene Ontology terms based on semantic similarity (Allowed similarity=0.7).

The ratio of transcription factors in the non-well explained promoters (11.3% for Gefitinib-U0126, 11.4% for Gefitinib-Wortmannin, 13.2% for U0126-Wortmannin) was higher than the ratio of the transcription factors in the well-described promoters (9.7% for Gefitinib-U0126, 9.7% for Gefitinib-Wortmannin, 9.6% for U0126-Wortmannin) for all drug combinations, with statistically significant difference (Fisher's exact test, conf.level = 0.95) for the U0126-Wortmannin pair (p-value=0.005695). We also checked whether the promoters of the transcription factors were lowly expressed compared to all the promoters of our analysis. The distribution of the expression (median log2cpm values across all the samples) in these two sets suggests that our findings are not due to low expression of transcription factors. However the transcription factors in the linearly described promoters have higher expression than the transcription factors in the non-linearly described promoters (Supplementary Figure 11).

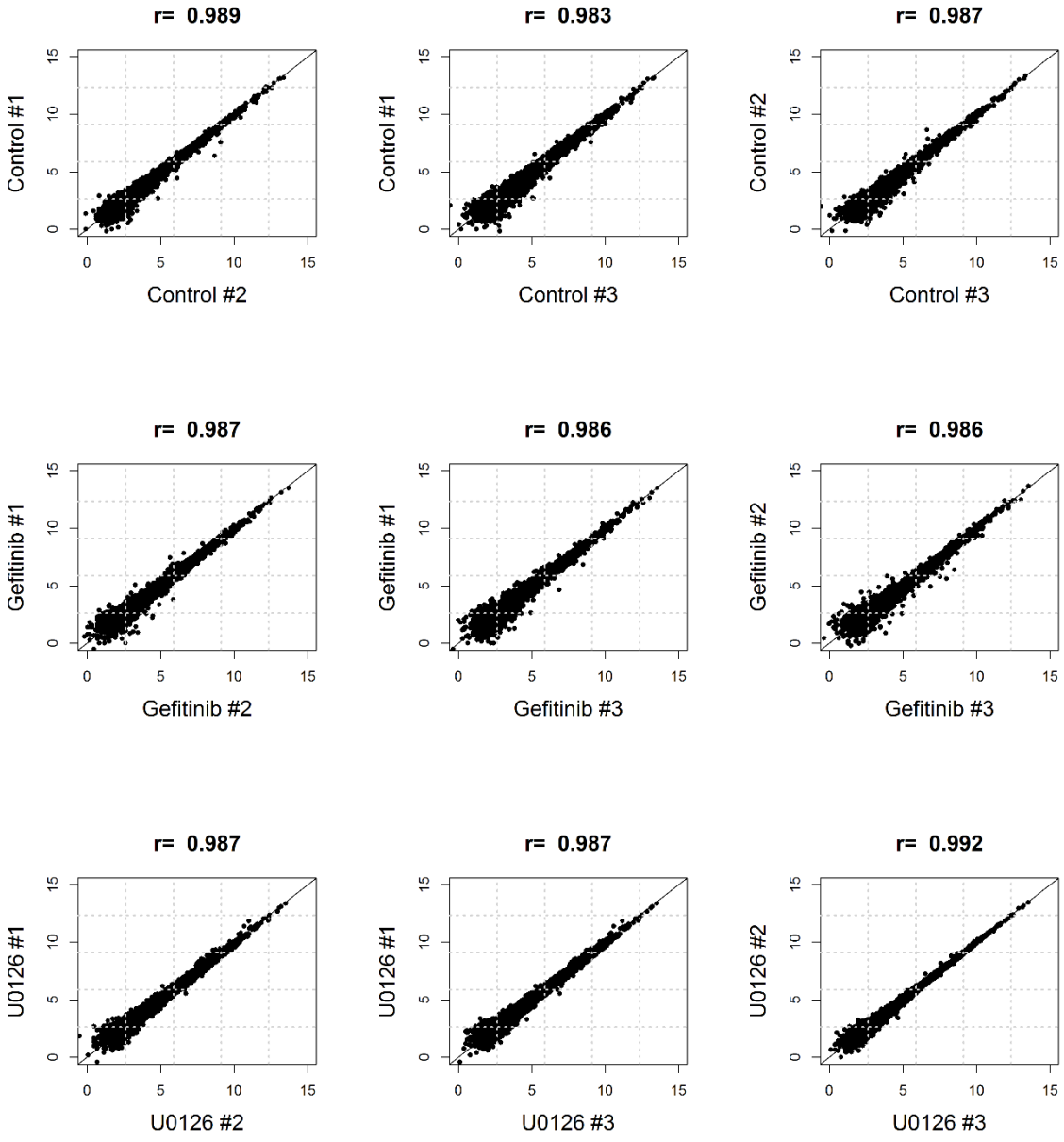
Transcription factor binding site analysis of promoters not described by the linear model

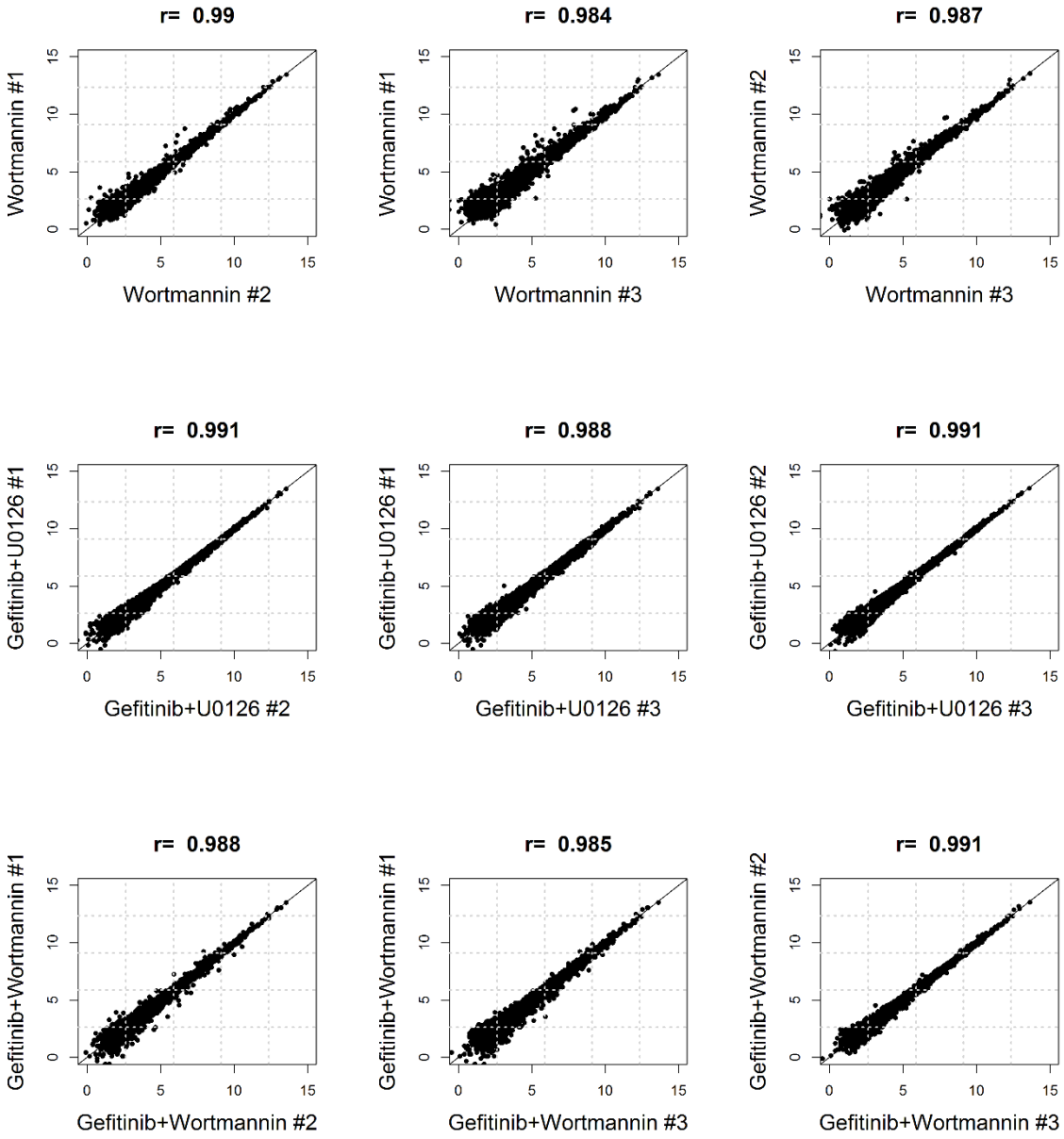
We further identified conserved transcription factor DNA binding sites (TFBSs) in the promoter regions. Overrepresented motifs in non-linearly described promoters for every combinatorial treatment were identified using the Binomial test (Supplementary Tables 18-20). Several motifs overrepresented in promoters not described by the linear model were common to all drug combinations, and are listed in Supplementary Table 21. Redoing the analysis excluding promoters overlapping between drug combinations suggested that these common motifs were due to the common promoter regions in the three drug combinations.

Potential transcription factor binding sites (TFBSs) for motifs known to be recognized by transcription factors (TFs) were identified as described in detail in (Arner *et al.*, 2015). Briefly, MotEvo (Arnold *et al.*, 2012) was used to identify conserved instances of motifs from the SwissRegulon database (Pachkov *et al.*, 2007) within a 400 base region (-300 to +100 base pairs with respect to the representative position) of each promoter. Overrepresented motifs in non-linearly behaved promoters for every combinatorial treatment were realized using one-tailed Binomial test (alternative: "greater", number of successes: number of occurrences of a motif in the

non-linearly described promoters, number of trials: number of the non-linearly described promoters, background probability: number of occurrences of a motif in all the promoters divided by the number of all the promoters). Then we applied the Benjamini-Hochberg (BH) adjustment of the p-values.

Supplementary Figures

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Wortmannin #2
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Wortmannin #1
Wortmannin #3
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Wortmannin #3
r= 0.991
Gefitinib+U0126 #1
Gefitinib+U0126 #2
r= 0.988
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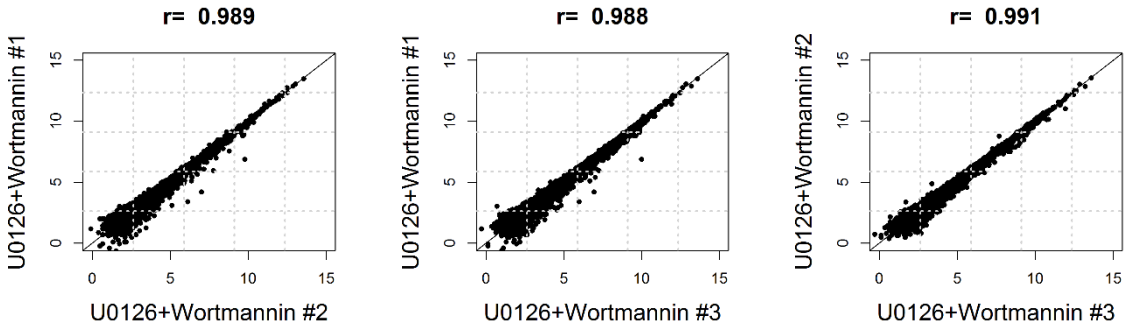
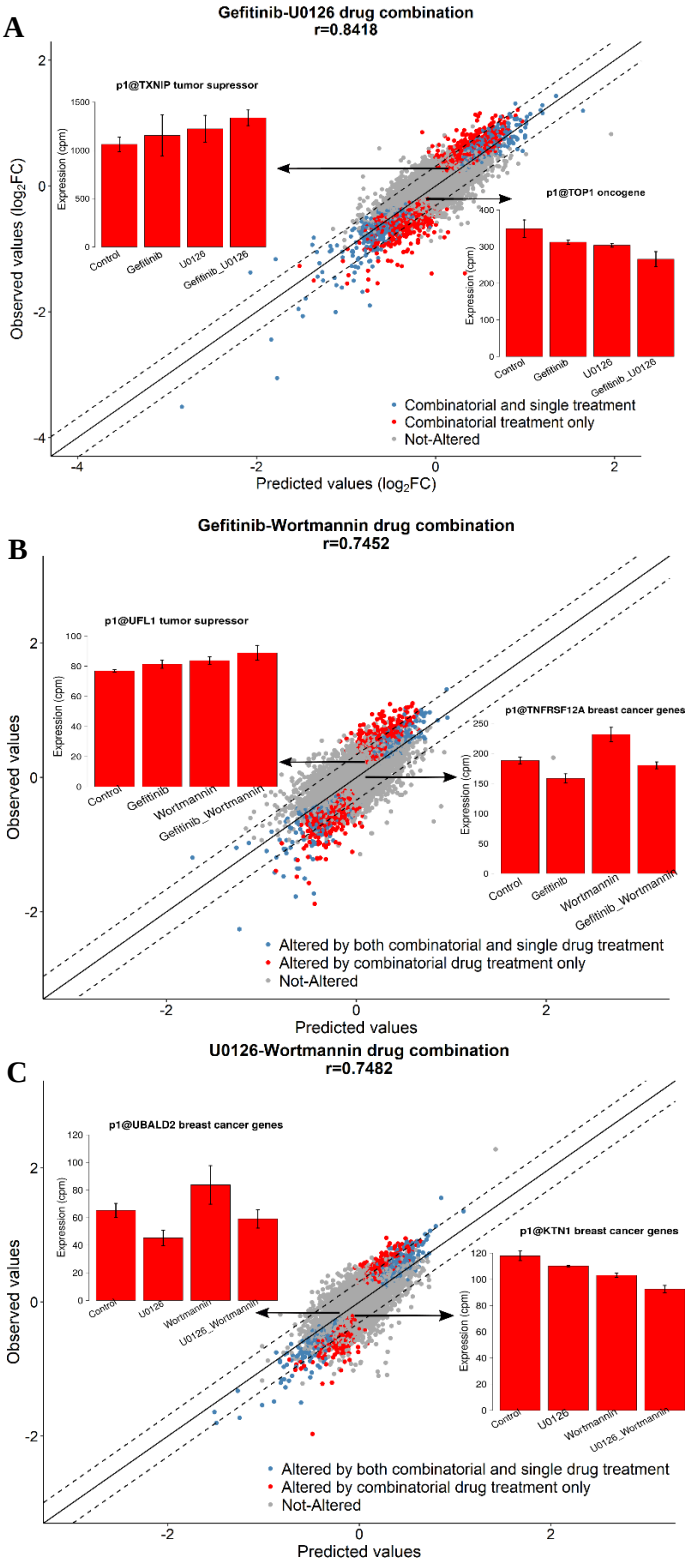
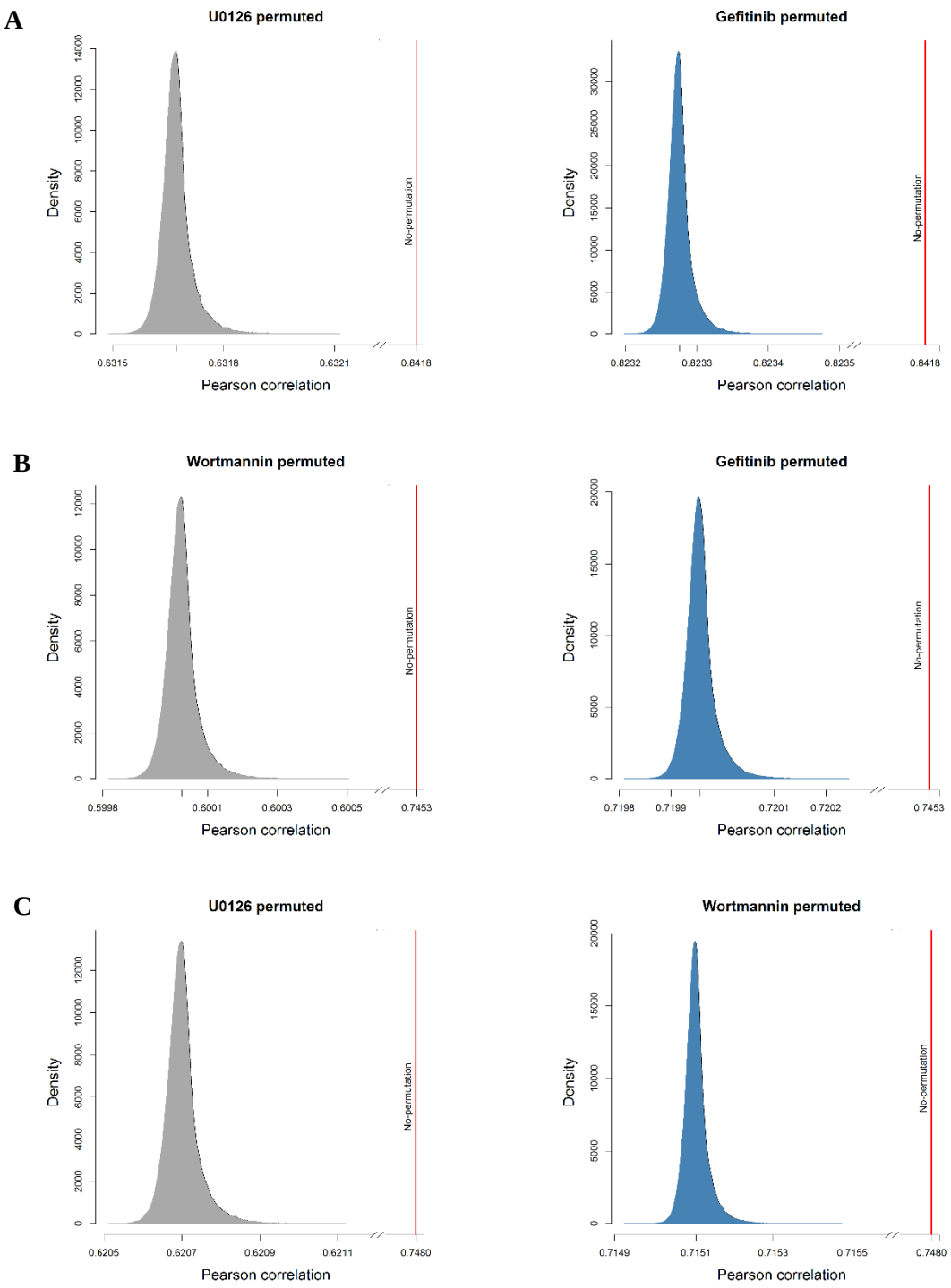
5
10
15



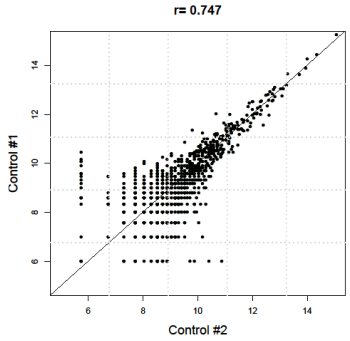
Supplementary Figure 1: Scatter plots of promoter expression (log_2cpm) among triplicates. The high Pearson correlation denotes that the quantification of the transcriptome by the HeliScopeCAGE method is reproducible even in a low-dosage drug experiment.



Supplementary Figure 2: Scatter plots of observed versus predicted log2FC values for A) Gefitinib_U0126, B) Gefitinib-Wortmannin and C) U0126-Wortmannin drug combination. Blue dots indicate promoters differentially expressed both in single and combinatorial treatment, red dots denote promoters differentially expressed only in combinatorial treatment and gray dots represent the non-significantly altered promoters. The dashed lines define the bounds for the two standard deviations of the residual error. Barplots show the expression of key genes important for the phenotypic outcome after single and combinatorial treatment. The linear regression model is able to effectively capture both amplifications and cancellations of the combinatorial transcriptome response.



Supplementary Figure 3: Density plots of the Pearson correlation coefficients between observed and predicted values after the permutations of individual profiles for (A) Gefitinib_U0126, (B) Gefitinib_Wortmannin, and (C) U0126_Wortmannin drug combinations in promoters. The Pearson correlation coefficient achieved without permutation is also reported.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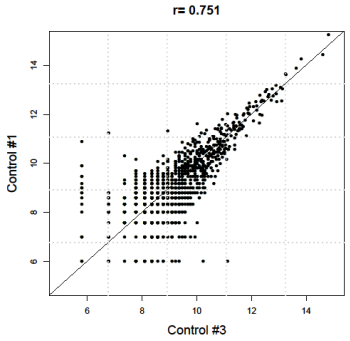
Control #2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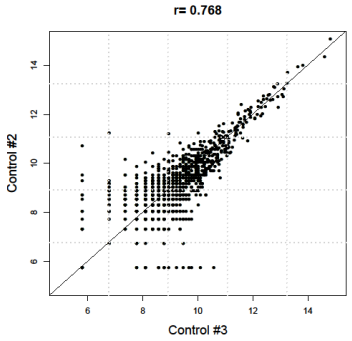
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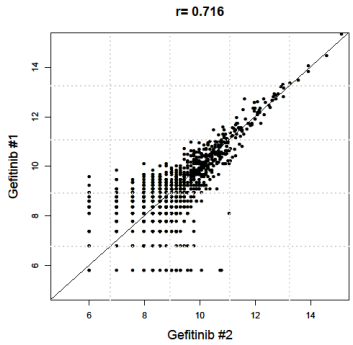
Control #3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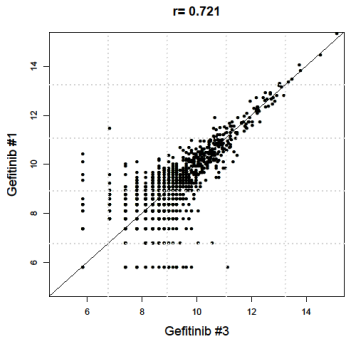
Gefitinib #2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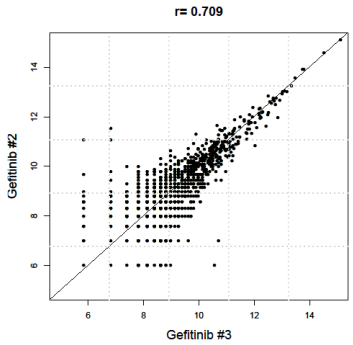
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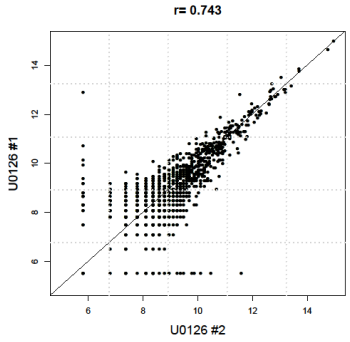
Gefitinib #2
Gefitinib #3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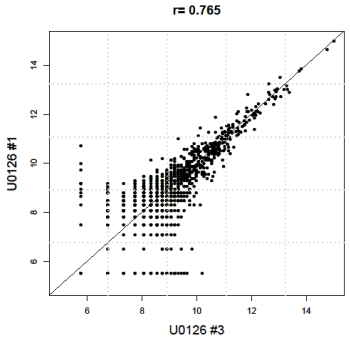
U0126 #1
U0126 #2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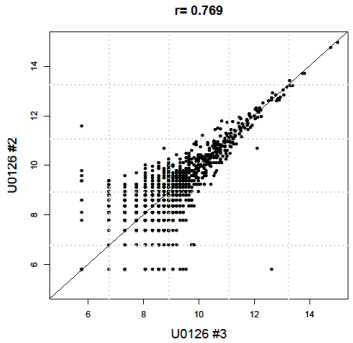
U0126 #3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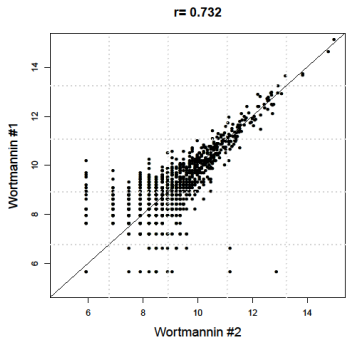
U0126 #3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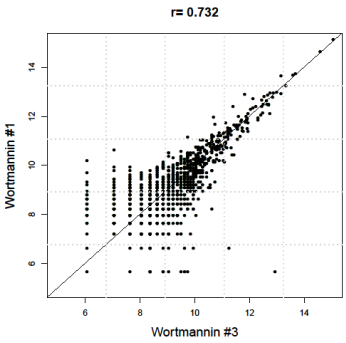
Wortmannin #1
Wortmannin #2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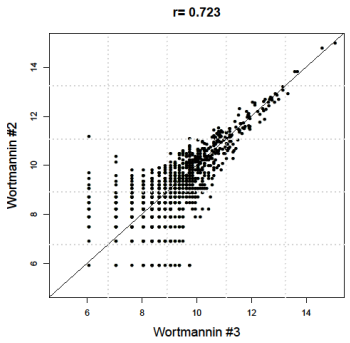
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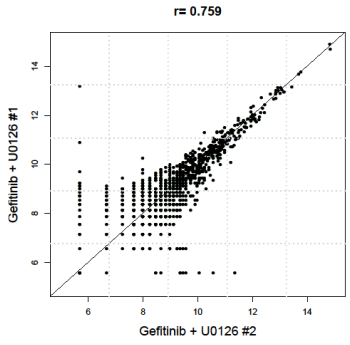
Wortmannin #3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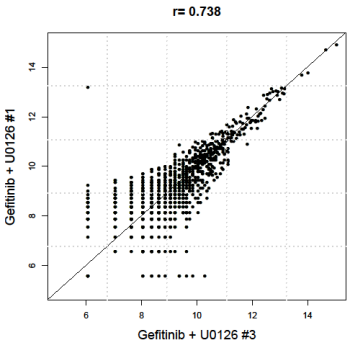
Gefitinib + U0126 #1
Gefitinib + U0126 #2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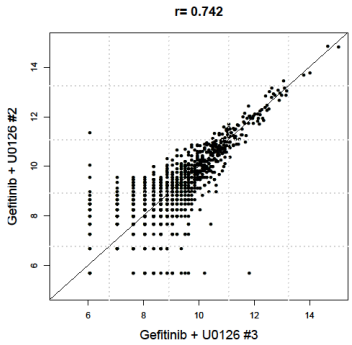
Gefitinib + U0126 #3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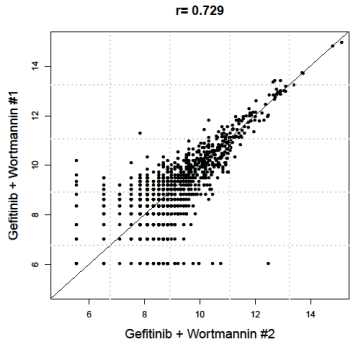
Gefitinib + U0126 #2
Gefitinib + U0126 #3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Gefitinib + Wortmannin #1
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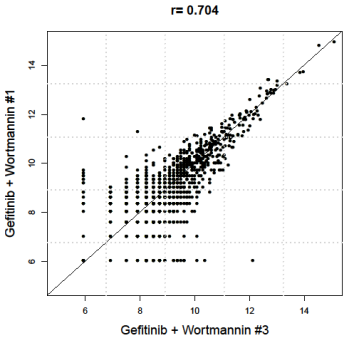
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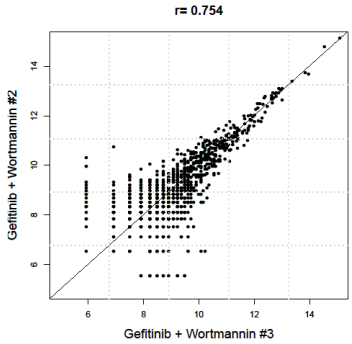
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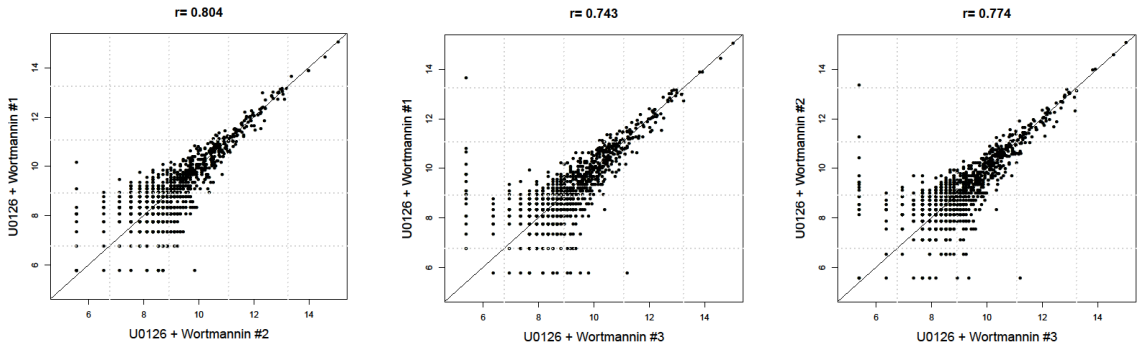
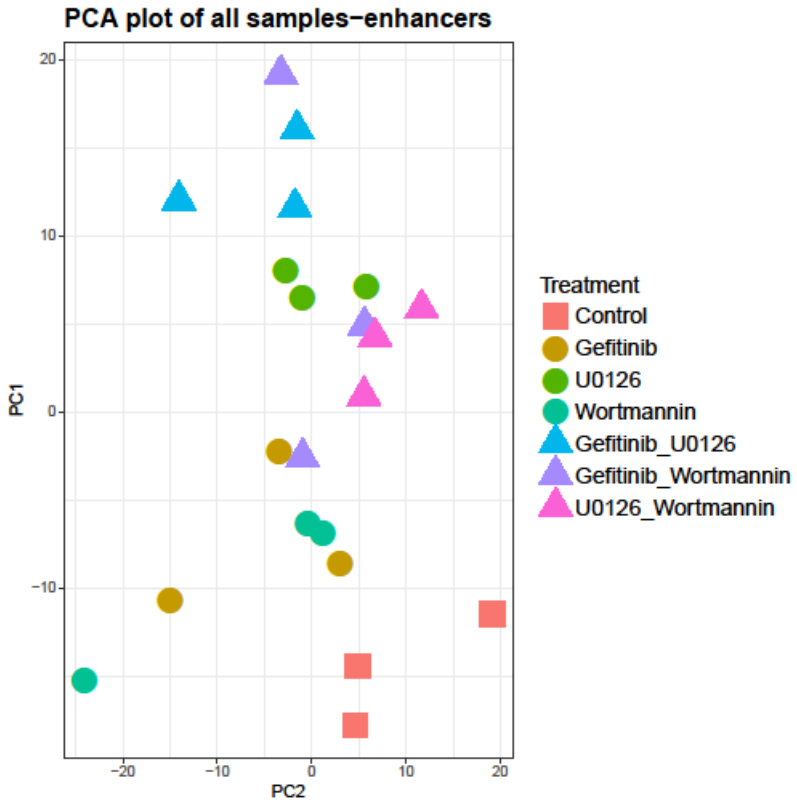
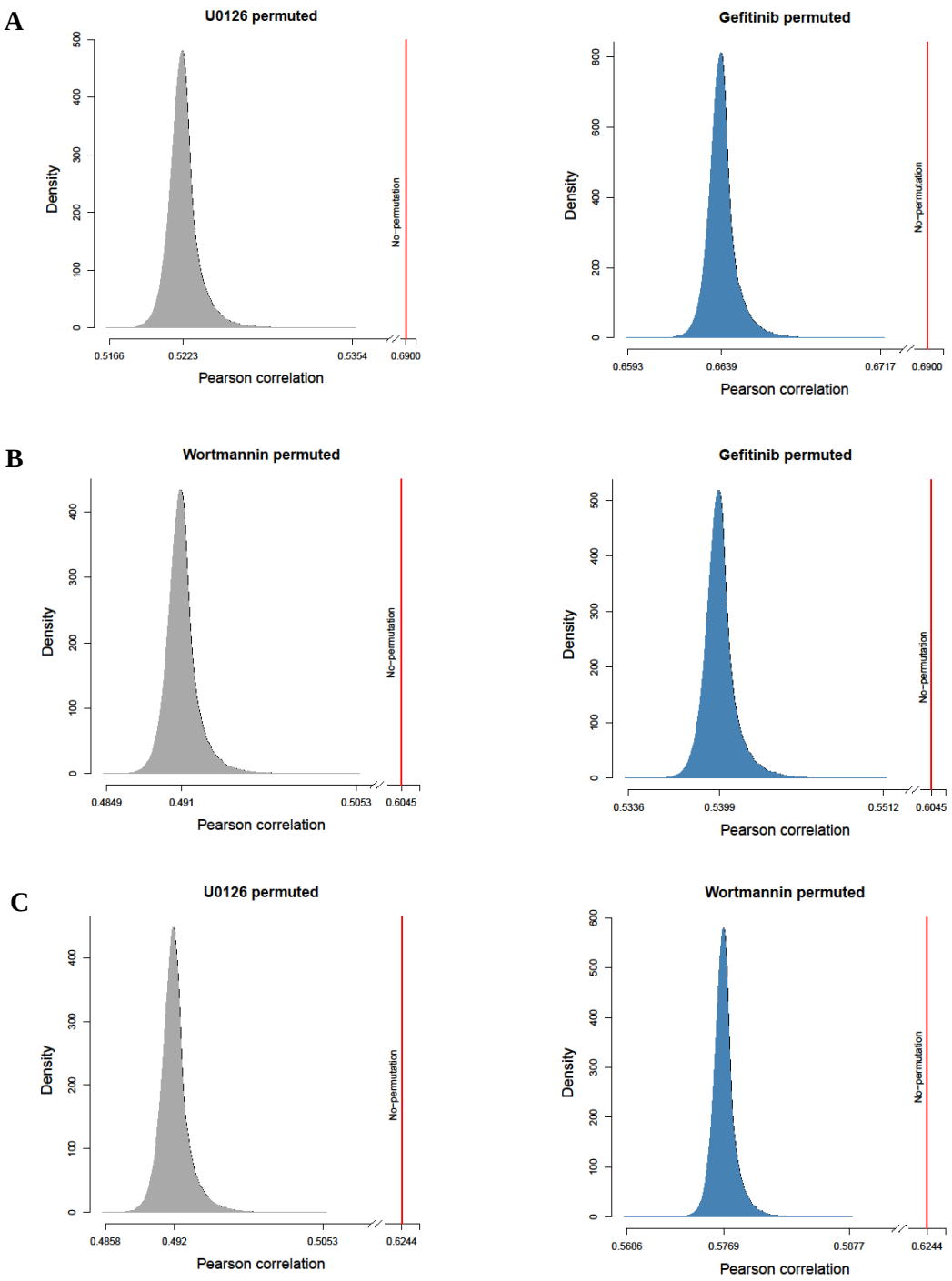


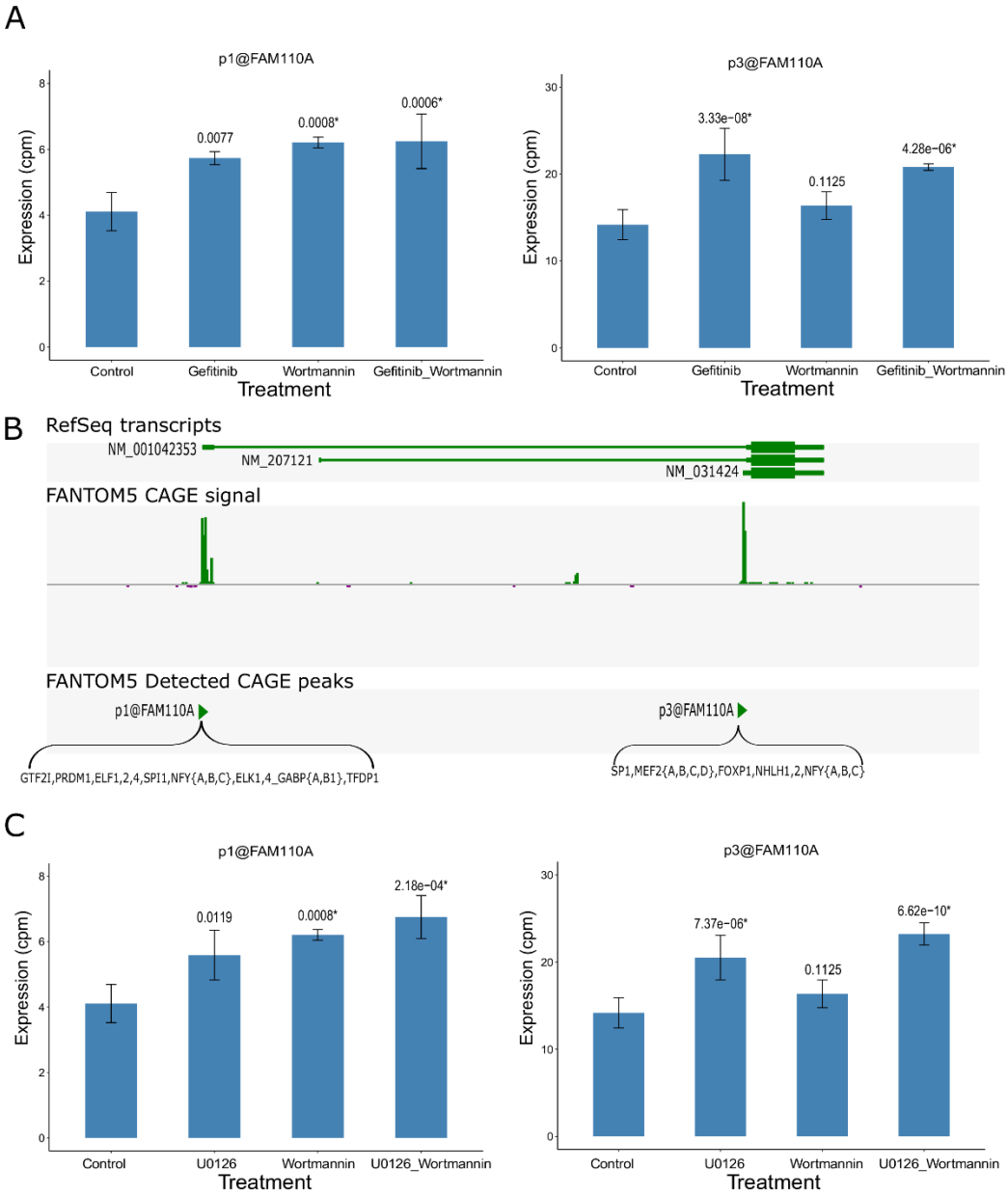
Supplementary Figure 4: Scatter plots of enhancers' expression (log_2cpm) among triplicates after filtering. The mean Pearson among triplicates is 0.74.



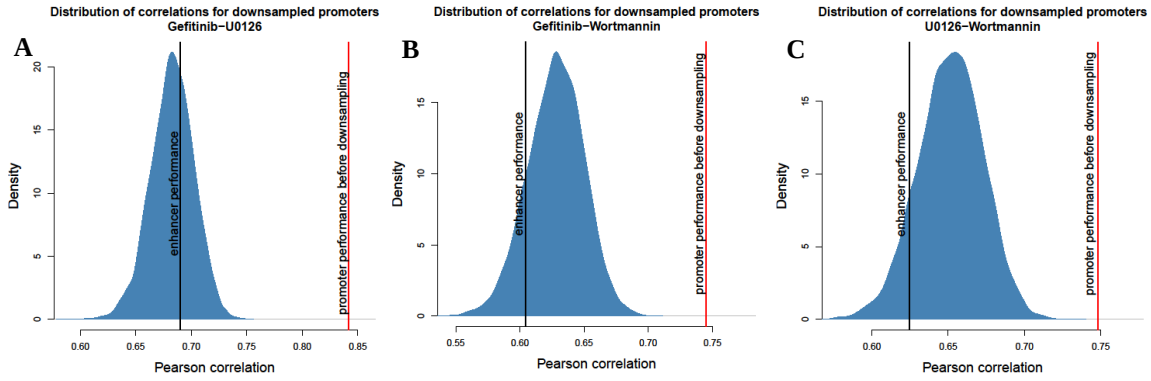
Supplementary Figure 5: PCA plot of enhancer activities. Colors represent different treatments individually or in combination, with squares indicating control (DMSO) treatment, circles indicating individual drug treatment and triangles indicating combinatorial treatment.



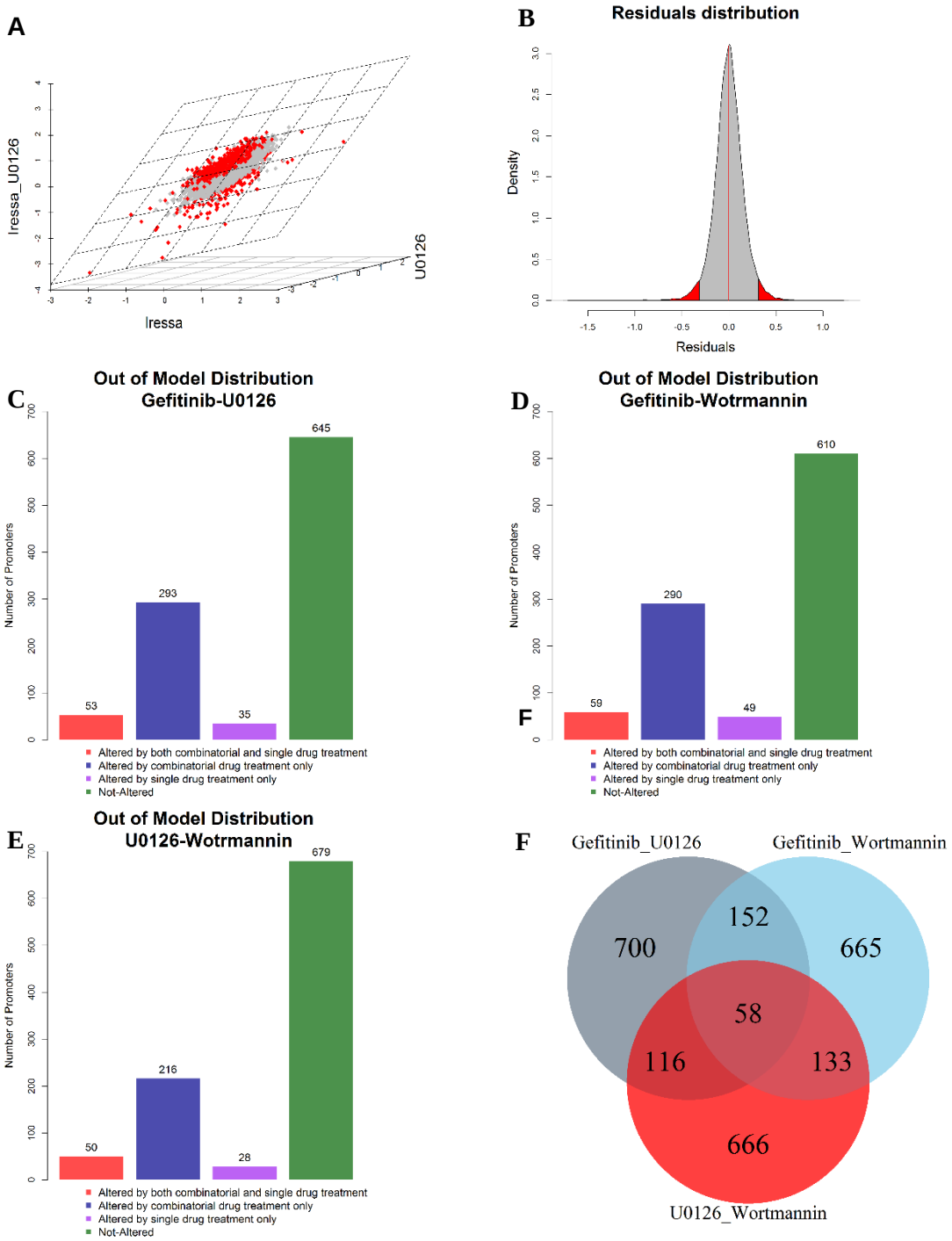
Supplementary Figure 6: Density plots of the Pearson correlation coefficients between observed and predicted values after the permutations of individual profiles for (**a**) Gefitinib_U0126, **(b)** Gefitinib_Wortmannin, and **(c)** U0126_Wortmannin drug combinations in enhancers. The Pearson correlation coefficient achieved without permutation is also reported.



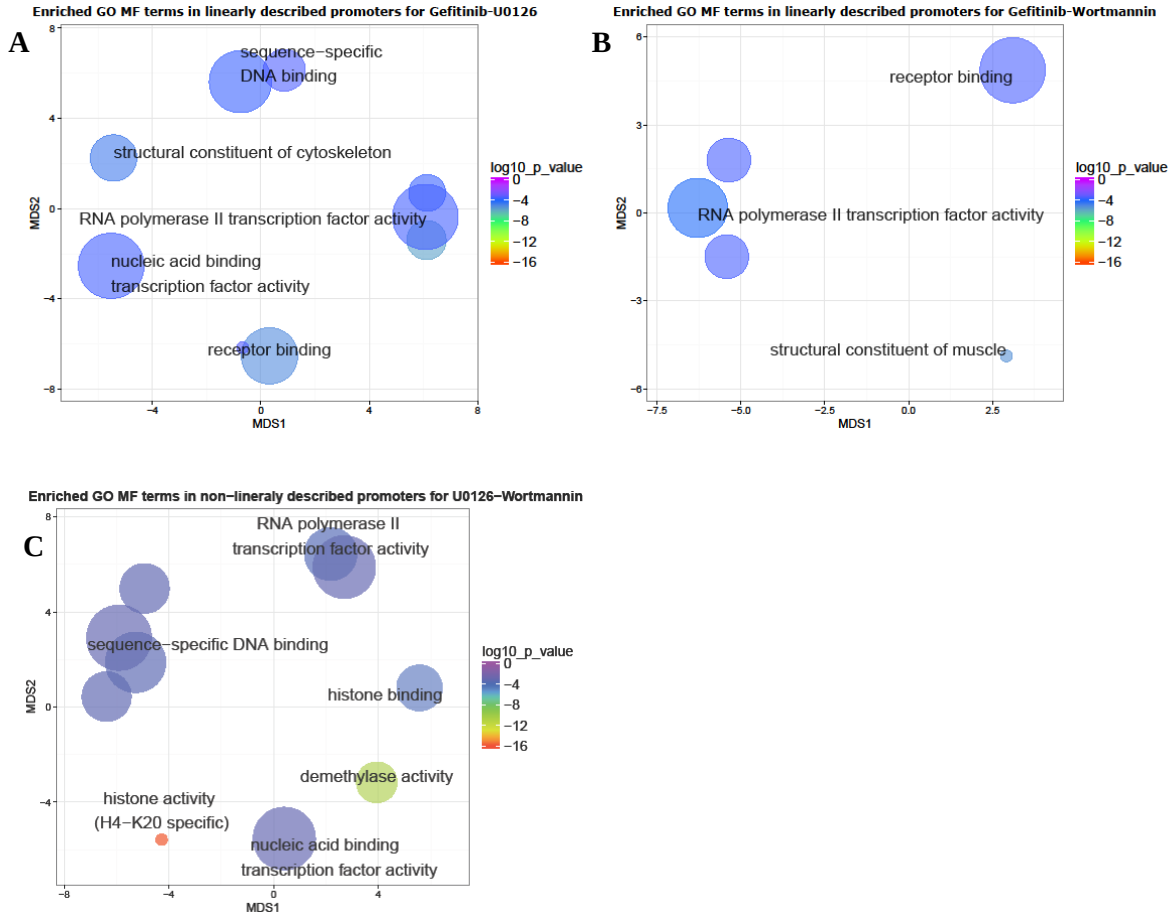
Supplementary Figure 7: Distinct effects of single and combinatorial therapy on different promoter elements of the same gene. A) FAM110A gene, an uncharacterized protein-coding gene mainly expressed in blood cells, has two different promoters upregulated by Gefitinib-Wortmannin combinatorial treatment. The first promoter (p1@FAM110A) is upregulated mainly because of the effect of Wortmannin and to a lesser extent because of Gefitinib. On the other hand, Wortmannin did not change the expression of the other promoter (p3@FAM110A), which was upregulated by Gefitinib treatment only. B) Zenbu Genome browser view of CAGE promoters p1 and p3. These two promoters are widely separated (10875 bp) and have a completely different set of transcription factor binding sites (noted in brackets). C) The same pattern is observed in U0126-Wortmannin combinatorial treatment. Wortmannin again plays the dominant role in the upregulation of p1@FAM110A but has no effect on the expression of p3@FAM110A. In contrast, U0126 clearly upregulates the p3@FAM110A promoter in the U0126-Wortmannin combination. Bar plots show the expression of promoters p1 (right panel) and p3 (left panel) of FAM110A gene, in the control condition, after single and combinatorial drug treatments. Above each bar, the p-value of the differential expression analysis of the corresponding treatment is reported (McCarthy *et al.*, 2012). Asterisks indicate the statistical significant upregulated therapies after FDR correction.



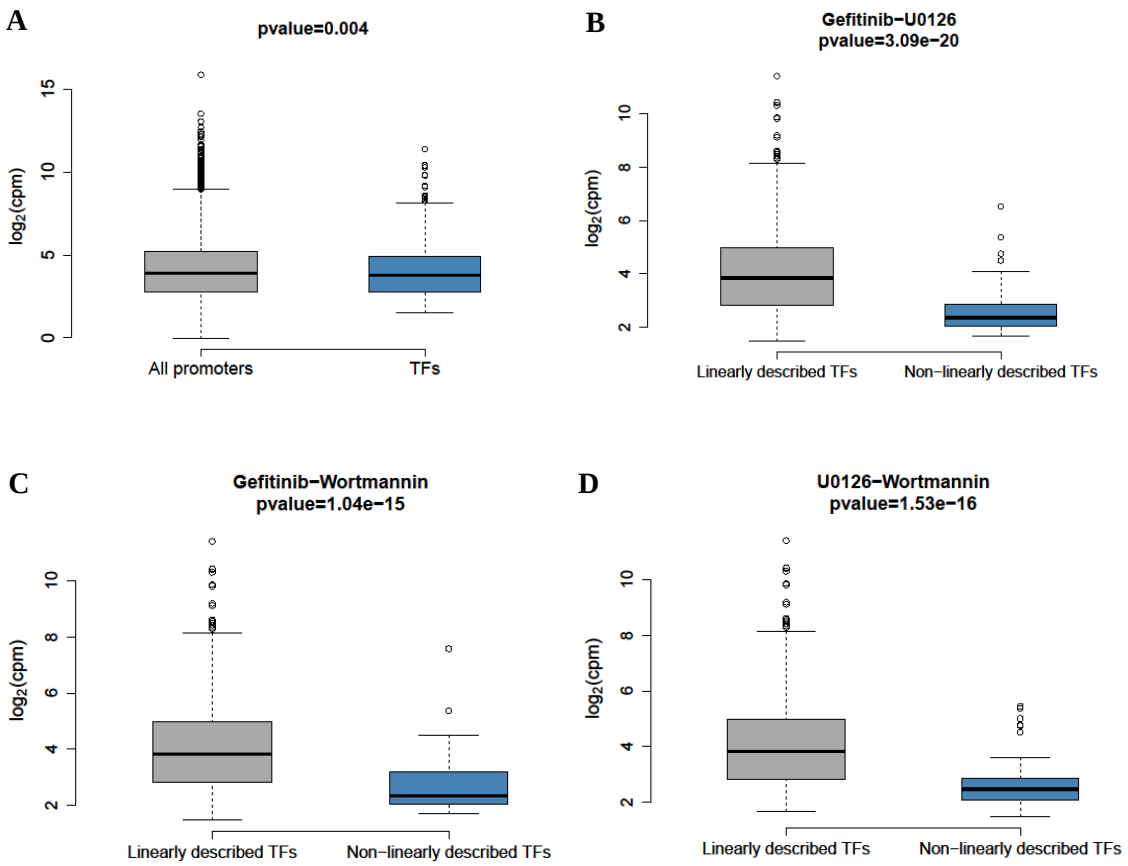
Supplementary Figure 8: We sampled 1028 low expressed promoter 10000 times, and applied linear regression for the three drug pairs. The density plots of the Pearson correlation between observed and estimated combinatorial response which we obtained after down sampling of promoters are presented in A) for Gefitinib-U0126, B) Gefitinib-Wortmannin and C) U0126-Wortmannin drug pairs. The red line denotes the Pearson correlation coefficient achieved for all the promoters while the black line indicates the Pearson correlation coefficient achieved for enhancers. The results suggest that the lower performance of the linear regression in enhancers compared to promoters can be attributed to the appreciably lower expression of eRNAs since the performance of the low expressed promoters decreased to almost the same levels as the performance in enhancers. The correlation between estimated and observed transcriptional response of all the promoters is higher than the mean of correlations obtained after down sampling, by 8.15, 5.38 and 4.55 standard deviations for Gefitinib-U0126, Gefitinib-Wortmannin, and U0126-Wortmannin combinations respectively.



Supplementary Figure 9: A) Multivariable regression model with two explanatory variables fits a regression plane in 3-dimensional space. Red dots represent promoters which fall further than two standard deviations away from what would have been expected based on the regression surface. **B**) Distribution of the residuals for Gefitinib_U0126 regression model. Composition of the "non-linearly described" promoters for the **C**) Gefitinib_U0126, **D**) Gefitinib_Wortmannin and **E**) U0126_Wortmannin combinatorial treatment. Using linear regression, it is possible to explain the response of 2963 out of 3256 (91%) promoters significantly differentially expressed by combinatorial treatment only for the Gefitinib-U0126 drug pair (85% and 80% respectively for the Gefitinib-Wortmannin and U0126-Wortmannin combinations). **F**) Overlap of the non-linearly explained promoters among the three different drug combinations. The overlap of the "out of the model" promoters among the three different combinatorial treatments is small, with only 58 promoters in common.



Supplementary Figure 10: Gene Ontology enrichment analysis for the promoters that do not follow the global linear trend. Molecular Function enrichment analysis visualized as an MDS plot, using REVIGO software(Supek *et al.*, 2011) (Allowed similarity=0.7) in (**A**) Gefitinib-U0126 (**B**) Gefitinib-Wortmannin and (**C**) U0126-Wortmannin combinatorial drug treatments. Clusters of circles represent terms that are closely related. Circle color indicates the log10 p-value of the enrichment test while circle size indicates the frequency of the GO term in the Uniprot database.



Supplementary Figure 11: Box plots of the expression (median $\log_2$cpm values across all the samples) in A) all the promoters of our analysis and only in the promoters of transcription factors (TFs). TF promoters as a whole do not have substantially lower expression than the whole promoter set. However the TFs in the linearly described promoters have higher expression than the TFs in the non-linearly described promoters for the B) Gefitinib-U0126, C) Gefitinib-Wortmannin and D) U0126-Wortmannin drug combination. The p-values of two sample Wilcoxon test comparing the expression of TF promoters which are well explained by the linear model versus the expression of TF promoters not well explained by the linear model are also reported.

Supplementary Tables 1-21

Supplementary Table 1: Promoters of phenotypically important genes (obtained from UniProtKB and (Sjoblom *et al.*, 2006)) which are well described by the linear regression model (within two standard deviations of the residual error) and are strongly regulated in Gefitinib-U0126 combinatorial treatment. For every promoter, the log_2FC expression values of combinatorial and single drug treatments compared to control, the residual error and the phenotype category are given.

Promoters	Gefitinib_U0126 (log_2FC)	Gefitinib (log_2FC)	U0126 (log_2FC)	Residuals	Category
p1@NCOA4	0.334646	0.101528	0.16021	0.14927	oncogene
p2@NCOA4	0.375721	0.179023	0.262725	0.06864	oncogene
p2@CCDC6	-0.39564	0.067342	-0.19835	-0.22878	oncogene
p3@CCDC6	-0.48386	-0.30512	-0.4398	0.028544	oncogene
p2@CCND1	-0.60879	-0.55169	-0.234	-0.21603	oncogene
p1@PICALM	-0.26796	-0.09458	-0.16705	-0.08072	oncogene
p1@DDIT3	0.283415	0.158984	0.046417	0.18865	oncogene
p2@MDM2	0.206962	0.110179	0.175325	0.004503	oncogene
p1@CDT1	-0.30058	-0.00455	-0.27401	-0.03933	oncogene
p1@ELAC2	0.230876	0.009271	0.114724	0.117443	oncogene
p2@YES1	-0.43229	-0.37158	-0.36677	0.031132	oncogene
p1@TPM3	-0.39032	0.039099	-0.18468	-0.22772	oncogene
p1@PBX1	-0.35289	-0.08869	-0.14964	-0.18406	oncogene
p1@TOP1	-0.39188	-0.15936	-0.19931	-0.15382	oncogene
p2@TOP1	-0.31106	-0.07869	-0.21251	-0.08542	oncogene
p1@AURKA	0.261269	0.17893	0.112259	0.097584	oncogene
p1@RUNX1	-0.48457	-0.17984	-0.1768	-0.26161	oncogene
p5@RUNX1	-0.53647	-0.07012	-0.29899	-0.23109	oncogene
p1@EWSR1	-0.19071	-0.13414	-0.12518	-0.0311	oncogene
p1@MKL1	-0.31867	-0.24674	-0.05564	-0.1904	oncogene
p1@PIM3	0.323689	0.108056	0.120917	0.173728	oncogene
p1@TFG	0.276067	-0.02285	0.124536	0.163243	oncogene
p1@WWTR1	0.261255	0.209425	0.102207	0.097693	oncogene
p1@RHOA	0.199587	0.093437	0.041945	0.129405	oncogene
p4@AFF1	-0.54389	-0.20105	-0.40415	-0.09772	oncogene
p3@AFF4	-0.21215	-0.01076	-0.07255	-0.14093	oncogene

p1@FOXO3	0.380783	0.407646	0.108792	0.149492	oncogene
p1@MYB	-0.34534	-0.09502	-0.22305	-0.1046	oncogene
p1@FAM83B	0.217532	0.121525	0.140818	0.044433	oncogene
p1@CREB3L2	0.288149	0.114077	0.198462	0.062434	oncogene
p1@MYC	-0.41929	-0.1079	-0.21317	-0.18397	oncogene
p1@PCM1	-0.36932	-0.17367	-0.241	-0.08709	oncogene
p15@SET	-0.34378	-0.03091	-0.22448	-0.12156	oncogene
p1@JAK2	0.351242	0.274533	0.269381	0.008207	oncogene
p1@MTCP1	-0.38249	-0.15481	-0.25555	-0.09225	oncogene
p1@ZMYND11	-0.36573	-0.04537	-0.06103	-0.29475	tumor supressor
p2@HTATIP2	0.607389	0.414589	0.284422	0.206601	tumor supressor
p2@EXT2	0.943464	0.54377	0.630427	0.172942	tumor supressor
p1@EXT2	0.300974	-0.06026	0.207477	0.120722	tumor supressor
p1@BRMS1	-0.31984	-0.13116	-0.11841	-0.1676	tumor supressor
p1@TCHP	-0.36608	-0.14912	-0.24758	-0.08519	tumor supressor
p1@CDKN1B	0.316064	0.180056	0.090772	0.172503	tumor supressor
p1@GPRC5A	0.270881	0.007319	0.140064	0.133908	tumor supressor
p1@BRCA2	-0.36526	-0.11501	-0.21805	-0.12309	tumor supressor
p4@STARD13	-0.20186	-0.20114	-0.06211	-0.08157	tumor supressor
p1@PNN	-0.23177	-0.12075	-0.07594	-0.12322	tumor supressor
p1@BUB1B	-0.52247	-0.19674	-0.23672	-0.23716	tumor supressor
p1@PALB2	-0.2788	-0.0749	-0.16455	-0.10005	tumor supressor
p1@PYCARD	0.252434	0.12689	0.180992	0.039393	tumor supressor
p1@BRD7	-0.3299	-0.06879	-0.13783	-0.17849	tumor supressor
p1@CYLD	0.369011	0.002177	0.189655	0.186381	tumor supressor
p2@RBL2	0.559558	0.289622	0.373774	0.112377	tumor supressor
p1@CTCF	-0.22722	-0.08612	-0.0513	-0.15288	tumor supressor
p2@CTCF	-0.56382	-0.23981	-0.32931	-0.17694	tumor supressor
p1@PHLPP2	-0.44917	-0.21659	-0.23259	-0.16166	tumor supressor
p3@NF1	-0.59506	-0.19513	-0.26022	-0.28787	tumor supressor
p1@TXNIP	0.330836	0.11937	0.202587	0.09955	tumor supressor

p1@CDC73	-0.27483	-0.00609	-0.15283	-0.12857	tumor supressor
p2@CDC73	-0.29258	-0.1642	-0.19271	-0.05931	tumor supressor
p1@RBL1	-0.45326	-0.19584	-0.17399	-0.22802	tumor supressor
p1@CHEK2	-0.24367	-0.14443	-0.15533	-0.05213	tumor supressor
p1@TMEM127	0.377517	0.178951	0.149488	0.178353	tumor supressor
p1@TET2	-0.5506	-0.2626	-0.32493	-0.16083	tumor supressor
p1@SDHA	0.184283	-0.065	0.051995	0.153645	tumor supressor
p2@TRIM24	0.372905	0.401095	0.145055	0.109092	tumor supressor
p1@RBMX	0.458237	0.335962	0.185055	0.176505	tumor supressor
p1@SBNO1	-0.21919	-0.08869	-0.10892	-0.08916	breast cancer gene
p3@THBS3	-0.49564	-0.1421	-0.14739	-0.31239	breast cancer gene
p1@MACF1	-0.47446	-0.24776	-0.27212	-0.13961	breast cancer gene
p1@SULF2	-0.33701	-0.28864	-0.2358	-0.02409	breast cancer gene
p2@SULF2	-0.29708	-0.14802	-0.15592	-0.10388	breast cancer gene
p3@SULF2	-0.37175	-0.41333	-0.25783	0.000812	breast cancer gene
p4@SULF2	-0.26054	-0.29724	-0.29449	0.110961	breast cancer gene
p1@ZFP64	-0.32782	-0.12554	-0.1973	-0.10216	breast cancer gene
p1@SLC9A2	0.577064	0.229123	0.270715	0.246838	breast cancer gene
p2@RAPH1	-0.38208	-0.18156	-0.15832	-0.17619	breast cancer gene
p3@RAPH1	-0.43375	-0.14494	-0.18692	-0.21196	breast cancer gene
p3@LRRFIP1	-0.3202	-0.08647	-0.27991	-0.02793	breast cancer gene
p1@GAB1	-0.56814	0.039318	-0.49332	-0.11153	breast cancer gene
p1@DBN1	0.243235	0.052843	0.128279	0.103377	breast cancer gene
p1@PRPF4B	-0.25406	-0.16341	-0.07098	-0.13701	breast cancer gene
p1@GSN	0.393441	0.180389	0.174745	0.169766	breast cancer gene
p2@GSN	0.460701	0.228113	0.185942	0.21156	breast cancer gene

Supplementary Table 2: Promoters of phenotypically important genes (obtained from UniProtKB and (Sjoblom *et al.*, 2006)) which are well described by the linear regression model (within two standard deviations of the residual error) and are strongly regulated in Gefitinib-Wortmannin combinatorial treatment. For every promoter, the log_2FC expression values of combinatorial and single drug treatments compared to control, the residual error and the phenotype category are given.

Genes	Gefitinib_Wortmannin (log_2FC)	Gefitinib (log_2FC)	Wortmannin (log_2FC)	Residuals	Category
p2@CCDC6	-0.42939	0.067342	-0.17399	-0.33732	oncogene
p2@PICALM	-0.4352	-0.02888	-0.36805	-0.18404	oncogene
p1@DDIT3	0.335786	0.158984	0.232598	0.12793	oncogene
p1@MDM2	0.217063	0.028296	0.07757	0.15264	oncogene
p1@AKT1	-0.24623	-0.08105	-0.09499	-0.16197	oncogene
p3@CBFB	-0.70006	-0.54108	-0.52787	-0.18641	oncogene
p1@SPECC1	-0.23934	-0.02203	-0.07321	-0.18757	oncogene
p2@YES1	-0.41219	-0.37158	-0.30901	-0.09647	oncogene
p1@PRKACA	-0.21538	-0.03295	-0.19493	-0.07885	oncogene
p3@TPM3	-0.40849	-0.02588	-0.19222	-0.27591	oncogene
p1@TPM3	-0.35494	0.039099	-0.22779	-0.21829	oncogene
p1@TOP1	-0.33918	-0.15936	-0.22083	-0.14692	oncogene
p2@TOP1	-0.35755	-0.07869	-0.19373	-0.20793	oncogene
p53@GNAS	0.527622	0.216335	0.408416	0.184689	oncogene
p1@RUNX1	-0.35714	-0.17984	-0.03632	-0.28214	oncogene
p1@TFG	0.215109	-0.02285	0.128822	0.131916	oncogene
p1@AFF1	-0.53711	-0.23853	-0.2673	-0.28972	oncogene
p4@AFF1	-0.54127	-0.20105	-0.51945	-0.13651	oncogene
p1@FER	-0.3699	-0.16825	-0.22881	-0.1696	oncogene
p1@DEK	-0.32006	-0.08593	-0.16852	-0.18511	oncogene
p7@SET	-0.63292	-0.42666	-0.46163	-0.19835	oncogene
p1@JAK2	0.333681	0.274533	0.175511	0.128943	oncogene
p1@BRMS1	-0.26168	-0.13116	-0.15457	-0.12233	tumor supressor
p1@ING4	0.337535	0.167189	0.037327	0.257869	tumor supressor
p1@BRCA2	-0.4	-0.11501	-0.23323	-0.21291	tumor supressor
p1@BUB1B	-0.53497	-0.19674	-0.26058	-0.30476	tumor supressor
p1@PYCARD	0.253342	0.12689	0.173073	0.095067	tumor supressor
p1@PHLPP2	-0.40329	-0.21659	-0.07728	-0.28973	tumor supressor
p3@NF1	-0.45166	-0.19513	-0.2436	-0.2333	tumor supressor
p1@PAF1	-0.257	0.035183	-0.14255	-0.1762	tumor supressor
p2@EFNA1	0.642197	0.220102	0.363328	0.328294	tumor supressor
p1@FH	-0.24949	-0.19239	-0.1462	-0.09714	tumor supressor
p1@TMEM127	0.300896	0.178951	0.197182	0.110679	tumor supressor
p1@PRKCD	0.406829	0.270667	0.107057	0.249077	tumor supressor
p1@TET2	-0.4004	-0.2626	-0.11642	-0.24666	tumor supressor
p1@PLK2	-0.24721	-0.07303	0.087122	-0.28726	tumor supressor
p1@RASA1	0.245191	0.098157	0.044698	0.181554	tumor supressor
p1@UFL1	0.208436	0.08148	0.126871	0.094871	tumor supressor

p1@AMFR	0.259775	0.109864	0.117867	0.143617	breast cancer gene
p1@SULF2	-0.35762	-0.28864	-0.28104	-0.08581	breast cancer gene
p2@SULF2	-0.23624	-0.14802	-0.18114	-0.07399	breast cancer gene
p3@RAPH1	-0.31157	-0.14494	-0.151	-0.17042	breast cancer gene
p1@LRRFIP1	-0.24921	-0.00237	-0.08696	-0.19421	breast cancer gene
p3@LRRFIP1	-0.35546	-0.08647	-0.13657	-0.24173	breast cancer gene
p1@HDLBP	0.209796	0.182969	0.156101	0.045852	breast cancer gene
p2@HDLBP	0.205334	0.055827	0.199532	0.050931	breast cancer gene

Supplementary Table 3: Promoters of phenotypically important genes (obtained from UniProtKB and (Sjoblom *et al.*, 2006)) which are well described by the linear regression model (within two standard deviations of the residual error) and are strongly regulated in U0126-Wortmannin combinatorial treatment. For every promoter, the log_2FC expression values of combinatorial and single drug treatments compared to control, the residual error and the phenotype category are given.

Genes	U0126_Wortmannin (log_2FC)	U0126 (log_2FC)	Wortmannin (log_2FC)	Residuals	Category
p2@MXI1	0.369284	0.155083	0.242662	0.203165	oncogene
p3@CCDC6	-0.42874	-0.4398	-0.23299	-0.09896	oncogene
p1@LETMD1	0.189006	0.072418	0.070623	0.124101	oncogene
p3@BCAS4	0.461749	0.172736	0.29642	0.268826	oncogene
p2@BCAS4	0.298246	0.131069	-0.01283	0.224194	oncogene
p1@PIM3	0.380252	0.120917	0.219632	0.241306	oncogene
p1@WWTR1	0.268747	0.102207	0.224637	0.139311	oncogene
p1@DCUN1D1	-0.34976	-0.14128	0.058468	-0.28492	oncogene
p1@TCTA	0.262496	0.106377	0.182333	0.1435	oncogene
p1@CREB3L2	0.359336	0.198462	0.218126	0.175113	oncogene
p1@AGR2	-0.23613	-0.00382	0.089691	-0.26189	oncogene
p1@JAK2	0.299522	0.269381	0.175511	0.086465	oncogene
p1@BRCA2	-0.42488	-0.21805	-0.23323	-0.22581	tumor supressor
p1@BUB1B	-0.39667	-0.23672	-0.26058	-0.17824	tumor supressor
p3@NF1	-0.48958	-0.26022	-0.2436	-0.26248	tumor supressor
p2@NBL1	-0.24321	-0.10373	-0.07815	-0.15886	tumor supressor
p1@CHEK2	-0.22753	-0.15533	-0.11751	-0.10074	tumor supressor
p1@TET2	-0.46096	-0.32493	-0.11642	-0.23447	tumor supressor
p1@DLC1	0.308235	0.205354	0.168449	0.135094	tumor supressor
p2@EPB49	0.394629	0.267418	0.219693	0.169257	tumor supressor
p1@KTN1	-0.34987	-0.09855	-0.19245	-0.23372	breast cancer gene
p10@SULF2	-0.34297	-0.17647	-0.27568	-0.15548	breast cancer gene
p1@KPNA5	-0.36321	-0.14653	-0.27798	-0.19268	breast cancer gene

Supplementary Table 4: For every combinatorial treatment in promoters, we report the permuted drug profile, the coefficient of this profile in the linear prediction function, the p-value of one sample t-test comparing the Pearson correlation of the regression model with the Pearson coefficients obtained after permutations of drug profile (assuming approximately Gaussian distributions, null hypothesis: mean of correlation coefficients of the permutated profile is equal to the correlation of the regression model, confidence level: 95%), and the number of the standard deviations which the correlation of the regression model based on the non-permuted drug expression is higher than the mean of correlation coefficients obtained using the permutated drug profile.

	Permuted drug	**Coefficient**	**p-value**	**No.of.sd**	**Permuted drug**	**Coefficient**	**p-value**	**No.of.sd**
Gefitinib-U0126	Gefitinib	0.310036	<2.2e-16	1073.51	U0126	0.952823	<2.2e-16	5020.19
Gefitinib-Wortmannin	Gefitinib	0.303648	<2.2e-16	858.32	Wortmannin	0.669232	<2.2e-16	3103.47
U0126-Wortmannin	U0126	0.589808	<2.2e-16	2943.02	Wortmannin	0.304918	<2.2e-16	1096.52

Supplementary Table 5: Performance of the different regression models for the Gefitinib-U0126 drug combination. The p-values of two sample t-test comparing the performance of each model with multivariable linear regression (2 explanatory variables) are also reported.

	Mean Absolute Error	Pearson correlation	Spearman correlation	P-Value
Linear regression (2 explanatory variables)	0.1160	0.8418	0.8284	-
Linear regression (1 explanatory variables)	0.1216	0.8233	0.8071	0.0003197
Linear regression (2 explanatory variables plus interaction term)	0.1160	0.8415	0.8284	0.9449
Quantile regression (0.5 quantile)	0.1158	0.8418	0.8281	0.9867
Quantile regression (0.35 quantile)	0.1234	0.8418	0.8283	0.9963
Regression tree	0.1190	0.8294	0.8214	0.01836

Supplementary Table 6: Performance of the different regression models for the Gefitinib-Wortmannin drug combination. The p-values of two sample t-test comparing the performance of each model with multivariable linear regression (2 explanatory variables) are also reported.

	Mean Absolute Error	Pearson correlation	Spearman correlation	P-Value
Linear regression **(2 explanatory variables)**	0.1238	0.7453	0.7474	-
Linear regression **(1 explanatory variable)**	0.1285	0.7202	0.7173	0.0002241
Linear regression **(2 explanatory variables plus interaction term)**	0.1236	0.7447	0.7475	0.9308
Quantile regression **(0.5 quantile)**	0.1237	0.7453	0.7473	0.994
Quantile regression **(0.4 quantile)**	0.1272	0.7453	0.7475	0.9973
Regression tree	0.1283	0.7212	0.7305	0.0003259

Supplementary Table 7: Performance of the different regression models for the U0126-Wortmannin drug combination. The p-values of two sample t-test comparing the performance of each model with multivariable linear regression (2 explanatory variables) are also reported.

	Mean Absolute Error	Pearson correlation	Spearman correlation	P-value
Linear regression (2 explanatory variables)	0.1152	0.7480	0.7182	-
Linear regression (1 explanatory variables)	0.1208	0.7152	0.6840	1.018e-12
Linear regression (2 explanatory variables plus interaction term)	0.1152	0.7476	0.7182	0.9484
Quantile regression (0.5 quantile)	0.1152	0.7480	0.7182	0.9988
Quantile regression (0.3 quantile)	0.1289	0.7480	0.7182	0.9933
Regression tree	0.1205	0.7167	0.6964	9.93e-05

Supplementary Table 8: For every combinatorial treatment in enhancers, we report the permuted drug profile, the coefficient of this profile in the linear prediction function, the p-value of one sample t-test comparing the Pearson correlation of the regression model with the Pearson coefficients obtained after permutations of drug profile (assuming approximately Gaussian distributions, null hypothesis: mean of correlation coefficients of the permutated profile is equal to the correlation of the regression model, confidence level: 95%), and the number of the standard deviations which the correlation of the regression model based on the non-permuted drug expression is higher than the mean of correlation coefficients obtained using the permutated drug profile.

	Permuted drug	**Coefficient**	**p-value**	**No.of.sd**	**Permuted drug**	**Coefficient**	**p-value**	**No.of.sd**
Gefitinib-U0126	Gefitinib	0.262787	<2.2e-16	36.55	U0126	0.642445	<2.2e-16	138.91
Gefitinib-Wortmannin	Gefitinib	0.297348	<2.2e-16	57.47	Wortmannin	0.420427	<2.2e-16	86.37
U0126-Wortmannin	U0126	0.443403	<2.2e-16	100.67	Wortmannin	0.29523	<2.2e-16	47.91

Supplementary Table 9: Enhancer and promoter pairs which are located within 500 kb of each other and have Pearson correlation (log_2cpm values) greater than 0.5 across the samples of our study (21 different samples). These eleven pairs also have Pearson correlation greater than 0.5 across all the FANTOM5 phase 1 samples.

Enhancer	Promoter	Correlation in our samples	Correlation in f5 samples
chr1:151485676-151485841	p1@CGN	0.530208	0.590527
chr10:98032542-98032692	p1@BLNK	0.701042	0.598473
chr11:85463912-85464270	p1@SYTL2	0.593274	0.516982
chr12:69036661-69037118	p1@ENST00000414313	0.903059	0.849396
chr12:7592182-7592797	p1@ENST00000538078	0.790416	0.948344
chr14:61969529-61970113	p1@PRKCH	0.595925	0.71889
chr19:13262107-13262928	p1@S69623	0.665645	0.622801
chr20:5589880-5590681	p1@GPCPD1	0.596857	0.627143
chr5:172192263-172193766	p1@DUSP1	0.572354	0.773816
chr8:126525260-126525748	p1@TRIB1	0.514582	0.677793
chr9:68455261-68455556	p2@ENST00000376334	0.852421	0.789771

Supplementary Table 10: Enriched GO BP terms in non-linearly described promoters for Gefitinib-U0126 combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0048518	positive regulation of biological process...	2968	230	180.34	3.00E-06
GO:0050794	regulation of cellular process	5415	378	329.03	5.40E-06
GO:0043627	response to estrogen	99	19	6.02	6.30E-06
GO:0050789	regulation of biological process	5705	392	346.65	1.70E-05
GO:0022414	reproductive process	555	58	33.72	2.80E-05
GO:0065007	biological regulation	5956	404	361.91	4.40E-05
GO:0032501	multicellular organismal process	3091	231	187.82	4.70E-05
GO:0097305	response to alcohol	164	24	9.97	5.00E-05
GO:0048522	positive regulation of cellular process	2584	198	157.01	5.50E-05
GO:0048545	response to steroid hormone	197	27	11.97	5.60E-05
GO:0071495	cellular response to endogenous stimulus	602	60	36.58	8.00E-05
GO:0044707	single-multicellular organism process	2990	223	181.68	8.40E-05
GO:0009719	response to endogenous stimulus	803	75	48.79	8.80E-05
GO:0030509	BMP signaling pathway	56	12	3.4	0.00011
GO:0048731	system development	2107	165	128.03	0.00011
GO:0009725	response to hormone	482	50	29.29	0.00012
GO:0007165	signal transduction	2712	204	164.79	0.00013
GO:0048513	organ development	1491	123	90.6	0.00013
GO:0032355	response to estradiol	66	13	4.01	0.00014
GO:0007275	multicellular organismal development	2393	183	145.41	0.00015
GO:0071772	response to BMP	58	12	3.52	0.00015
GO:0071773	cellular response to BMP stimulus	58	12	3.52	0.00015
GO:0014070	response to organic cyclic compound	386	42	23.45	0.00015
GO:0044700	single organism signaling	2901	215	176.27	0.00019
GO:0023052	signaling	2907	215	176.64	0.00022
GO:2000026	regulation of multicellular organismal d...	752	69	45.69	0.00029
GO:0007154	cell communication	3037	222	184.54	0.00034
GO:0009966	regulation of signal transduction	1422	116	86.41	0.00034
GO:0009888	tissue development	894	79	54.32	0.00034
GO:0030198	extracellular matrix organization	153	21	9.3	0.00036
GO:0051171	regulation of nitrogen compound metabolism...	2680	199	162.85	0.00036
GO:0051239	regulation of multicellular organismal p...	1164	98	70.73	0.00036
GO:0044767	single-organism developmental process	2854	210	173.42	0.00038
GO:0033993	response to lipid	378	40	22.97	0.00039
GO:0043062	extracellular structure organization	154	21	9.36	0.00039
GO:0009891	positive regulation of biosynthetic proc...	1039	89	63.13	0.0004
GO:0044702	single organism reproductive process	507	50	30.81	0.00041
GO:0071407	cellular response to organic cyclic comp...	188	24	11.42	0.00042

Supplementary Table 11: Enriched GO MF terms in non-linearly described promoters for Gefitinib-U0126 combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0001228	RNA polymerase II transcription regulatory...	156	26	9.43	2.00E-06
GO:0005102	receptor binding	609	64	36.81	7.40E-06
GO:0005200	structural constituent of cytoskeleton	48	12	2.9	2.00E-05
GO:0001077	RNA polymerase II core promoter proximal...	114	19	6.89	4.70E-05
GO:0004879	ligand-activated sequence-specific DNA b...	28	8	1.69	0.00018
GO:0098531	direct ligand regulated sequence-specific...	28	8	1.69	0.00018
GO:0043565	sequence-specific DNA binding	518	52	31.31	0.00018
GO:0001012	RNA polymerase II regulatory region DNA ...	331	37	20	0.0002
GO:0001071	nucleic acid binding transcription facto...	657	62	39.71	0.00025
GO:0003700	sequence-specific DNA binding transcript...	657	62	39.71	0.00025
GO:0000977	RNA polymerase II regulatory region sequence...	329	36	19.88	0.00037
GO:0046875	ephrin receptor binding	18	6	1.09	0.00047
GO:0000976	transcription regulatory region sequence...	372	39	22.48	0.0005
GO:0000982	RNA polymerase II core promoter proximal...	170	22	10.27	0.00056
GO:0000978	RNA polymerase II core promoter proximal...	194	24	11.72	0.00063
GO:0000987	core promoter proximal region sequence-s...	201	24	12.15	0.00104
GO:0001159	core promoter proximal region DNA binding...	201	24	12.15	0.00104

Supplementary Table 12: Enriched GO CC terms in non-linearly described promoters for Gefitinib-U0126 combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0071944	cell periphery	1869	155	112.98	7.10E-06
GO:0005886	plasma membrane	1815	150	109.72	1.30E-05
GO:0031012	extracellular matrix	110	18	6.65	9.60E-05
GO:0045111	intermediate filament cytoskeleton	81	14	4.9	0.00031

Supplementary Table 13: Enriched GO BP terms in non-linearly described promoters for Gefitinib-Wortmannin combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0048731	system development	2107	162	102.93	6.10E-11
GO:0048513	organ development	1491	122	72.84	1.10E-09
GO:0007275	multicellular organismal	2393	173	116.91	1.60E-09
GO:0044707	Single multicellular org..	2990	203	146.07	5.60E-09
GO:0048856	anatomical structure d...	2546	178	124.38	1.20E-08
GO:0008283	cell proliferation	1007	88	49.2	2.20E-08
GO:0032501	multicellular organismal	3091	205	151	3.60E-08
GO:0044767	single-organism develop…	2854	192	139.43	4.90E-08
GO:0032502	developmental process	2898	194	141.58	5.90E-08
GO:0014070	response to organic cyclic	386	44	18.86	1.00E-07
GO:0042127	regulation of cell prolife..	752	68	36.74	3.50E-07
GO:0009887	organ morphogenesis	439	46	21.45	6.30E-07
GO:0030154	cell differentiation	1832	131	89.5	9.50E-07
GO:0040011	locomotion	791	69	38.64	1.10E-06
GO:0097305	response to alcohol	164	24	8.01	1.20E-06
GO:0009653	anatomical structure mo..	1354	103	66.15	1.30E-06
GO:0001944	vasculature development	275	33	13.43	1.40E-06
GO:0072358	cardiovascular system dev	450	45	21.98	3.10E-06
GO:0072359	circulatory system de..	450	45	21.98	3.10E-06
GO:0048545	response to steroid horm	197	26	9.62	3.30E-06
GO:0001568	blood vessel development	261	31	12.75	3.70E-06
GO:2000026	regulation of multicellular	752	64	36.74	6.40E-06
GO:0048869	cellular developmental pr	1989	136	97.17	6.70E-06
GO:0007166	cell surface receptor sign	1354	100	66.15	7.60E-06
GO:0006928	movement of cell or sub	887	72	43.33	8.40E-06
GO:0009888	tissue development	894	72	43.67	1.10E-05
GO:0001501	skeletal system develop	223	27	10.89	1.10E-05
GO:0006935	chemotaxis	304	33	14.85	1.30E-05
GO:0042330	taxis	304	33	14.85	1.30E-05
GO:0050896	response to stimulus	4132	244	201.86	2.40E-05
GO:0048514	blood vessel morphog	221	26	10.8	2.70E-05
GO:0008285	negative regulation of cell	344	35	16.81	2.80E-05
GO:0048646	anatomical structure form	583	51	28.48	3.00E-05
GO:0061035	regulation of cartilage dev	27	8	1.32	3.00E-05
GO:0001525	angiogenesis	184	23	8.99	3.00E-05
GO:0006915	apoptotic process	1124	84	54.91	3.20E-05
GO:0044700	single organism signaling	2901	181	141.72	3.50E-05
GO:0051216	cartilage development	82	14	4.01	3.70E-05
GO:0033993	response to lipid	378	37	18.47	3.80E-05

GO:0023052	signaling	2907	181	142.02	4.00E-05
GO:0048705	skeletal system morphog	94	15	4.59	4.50E-05
GO:0007435	salivary gland morphog	15	6	0.73	4.50E-05
GO:0046683	response to organophos	54	11	2.64	4.70E-05
GO:0012501	programmed cell death	1140	84	55.69	5.40E-05
GO:0048519	negative regulation of biol	2581	163	126.09	6.00E-05
GO:0043408	regulation of MAPK cas	329	33	16.07	6.30E-05
GO:0051239	regulation of multicellular	1164	85	56.87	6.60E-05
GO:0048704	embryonic skeletal syst	38	9	1.86	6.70E-05
GO:0007154	cell communication	3037	186	148.37	8.00E-05
GO:0014706	striated muscle tissue dev	158	20	7.72	8.20E-05
GO:0061061	muscle structure dev	277	29	13.53	8.20E-05
GO:0009725	response to hormone	482	43	23.55	8.30E-05
GO:0043627	response to estrogen	99	15	4.84	8.30E-05
GO:0001775	cell activation	439	40	21.45	9.30E-05
GO:0009891	positive regulation of bios	1039	77	50.76	9.70E-05
GO:0061448	connective tissue develop	112	16	5.47	0.0001
GO:0050794	regulation of cellular proc	5415	302	264.54	0.0001
GO:0048568	embryonic organ develop	212	24	10.36	0.0001
GO:0007431	salivary gland develop	17	6	0.83	0.0001
GO:0090257	regulation of muscle syst	79	13	3.86	0.0001
GO:0048523	negative regulation of cell	2398	152	117.15	0.00011
GO:0071407	cellular response to organ	188	22	9.18	0.00012
GO:0008544	epidermis development	150	19	7.33	0.00012
GO:0007411	axon guidance	215	24	10.5	0.00013
GO:0097485	neuron projection guid	215	24	10.5	0.00013
GO:0060537	muscle tissue develop	163	20	7.96	0.00013
GO:0016337	single organismal cell-cell	327	32	15.97	0.00013
GO:0014074	response to purine-contai	60	11	2.93	0.00013
GO:1901654	response to ketone	71	12	3.47	0.00015
GO:0031328	positive regulation of cell	1023	75	49.98	0.00017
GO:0008219	cell death	1196	85	58.43	0.00017
GO:0016265	death	1196	85	58.43	0.00017
GO:0044763	single-organism cellular p	6518	351	318.42	0.00018
GO:0042221	response to chemical	1919	125	93.75	0.00019
GO:0007165	signal transduction	2712	167	132.49	0.00019
GO:0048706	embryonic skeletal sys	53	10	2.59	0.00021
GO:0009605	response to external stim	1152	82	56.28	0.00022
GO:0008284	positive regulation of cell	381	35	18.61	0.00022
GO:0000165	MAPK cascade	369	34	18.03	0.00026
GO:0023014	signal transduction by pro	385	35	18.81	0.00027
GO:0002040	sprouting angiogenesis	28	7	1.37	0.00031
GO:0032355	response to estradiol	66	11	3.22	0.00031
GO:0098609	cell-cell adhesion	375	34	18.32	0.00035

GO:0007399	nervous system develop	1151	81	56.23	0.00035
GO:1901700	response to oxygen-cont	742	57	36.25	0.00036
GO:1901342	regulation of vasculature	78	12	3.81	0.00037
GO:0051385	response to mineralocort	14	5	0.68	0.00038
GO:0050673	epithelial cell prolifer	177	20	8.65	0.00039
GO:0098602	single organism cell adh	363	33	17.73	0.0004
GO:0016477	cell migration	567	46	27.7	0.00043
GO:0043066	negative regulation of ap	504	42	24.62	0.00044
GO:0007517	muscle organ develop	153	18	7.47	0.00046
GO:0030198	extracellular matrix organ	153	18	7.47	0.00046
GO:0060429	epithelium development	570	46	27.85	0.00048
GO:0034109	homotypic cell-cell adh	235	24	11.48	0.00048
GO:0010557	positive regulation of mac	989	71	48.32	0.00049
GO:0010628	positive regulation of gen	1024	73	50.03	0.00049
GO:0050793	regulation of develop	1059	75	51.74	0.00049
GO:0043062	extracellular structure org	154	18	7.52	0.0005
GO:0035272	exocrine system develop	22	6	1.07	0.0005
GO:0022612	gland morphogenesis	70	11	3.42	0.00053
GO:0050678	regulation of epithelial	142	17	6.94	0.00054
GO:0040012	regulation of locomotion	356	32	17.39	0.0006
GO:0048468	cell development	1031	73	50.37	0.0006
GO:0043401	steroid hormone mediat	31	7	1.51	0.0006
GO:0043069	negative regulation of pro	512	42	25.01	0.00061
GO:0006937	regulation of muscle cont	50	9	2.44	0.00061
GO:0048732	gland development	225	23	10.99	0.00062
GO:0048701	embryonic cranial skelet	23	6	1.12	0.00065
GO:0051591	response to cAMP	41	8	2	0.0007
GO:0045165	cell fate commitment	96	13	4.69	0.00075
GO:0043410	positive regulation of MA	214	22	10.45	0.00075
GO:0002042	cell migration involved in	16	5	0.78	0.00076
GO:0002521	leukocyte differentiation	215	22	10.5	0.0008
GO:1903708	positive regulation of hem	74	11	3.62	0.00086
GO:0009719	response to endogenous	803	59	39.23	0.00087
GO:0048870	cell motility	603	47	29.46	0.00089
GO:0051674	localization of cell	603	47	29.46	0.00089
GO:0048522	positive regulation of cel	2584	156	126.24	0.00096
GO:1903522	regulation of blood circul	75	11	3.66	0.00096
GO:0001936	regulation of endothelial	43	8	2.1	0.00098
GO:0031348	negative regulation of def	64	10	3.13	0.00099
GO:0048608	reproductive structure	219	22	10.7	0.00102
GO:0008347	glial cell migration	17	5	0.83	0.00103
GO:0035924	cellular response to vasc	17	5	0.83	0.00103
GO:0065007	biological regulation	5956	321	290.97	0.00105
GO:0051240	positive regulation of mul	625	48	30.53	0.00107

GO:0031325	positive regulation of cell	1629	105	79.58	0.00108
GO:0006357	regulation of transcription	1088	75	53.15	0.00108
GO:0010033	response to organic subs	1486	97	72.6	0.00117
GO:0043010	camera-type eye devel	139	16	6.79	0.00119
GO:0022008	neurogenesis	797	58	38.94	0.00121
GO:0051270	regulation of cellular com	356	31	17.39	0.00122
GO:0061458	reproductive system dev	222	22	10.85	0.00123
GO:0051173	positive regulation of nitr	1058	73	51.69	0.00124
GO:0060548	negative regulation of cell	547	43	26.72	0.00124
GO:0048518	positive regulation of biol	2968	175	145	0.00125
GO:2000145	regulation of cell motility	326	29	15.93	0.00126
GO:0010171	body morphogenesis	35	7	1.71	0.00129
GO:0045785	positive regulation of cell	167	18	8.16	0.0013
GO:0051716	cellular response to stim	3544	204	173.14	0.00131
GO:0002062	chondrocyte differentiat	45	8	2.2	0.00133
GO:0045778	positive regulation of ossi	45	8	2.2	0.00133
GO:0044057	regulation of system proc	154	17	7.52	0.00135
GO:0045595	regulation of cell differen	734	54	35.86	0.00141
GO:0007155	cell adhesion	617	47	30.14	0.00144
GO:0010562	positive regulation of pho	470	38	22.96	0.00144
GO:0045937	positive regulation of pho	470	38	22.96	0.00144
GO:1901698	response to nitrogen com	470	38	22.96	0.00144
GO:0048562	embryonic organ morp	129	15	6.3	0.00152
GO:0022610	biological adhesion	619	47	30.24	0.00153
GO:0032846	positive regulation of ho	68	10	3.32	0.00159
GO:0050789	regulation of biological pr	5705	308	278.71	0.00165
GO:0019220	regulation of phosphate	842	60	41.13	0.00166
GO:0001503	ossification	185	19	9.04	0.00171

Supplementary Table 14: Enriched GO MF terms in non-linearly described promoters for Gefitinib-Wortmannin combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0008307	structural constituent of muscle	11	6	0.54	4.80E-06
GO:0000981	sequence-specific DNA binding RNA polyme...	314	32	15.27	5.60E-05
GO:0004879	ligand-activated sequence-specific DNA b...	28	7	1.36	0.0003
GO:0098531	direct ligand regulated sequence-specific	28	7	1.36	0.0003
GO:0005102	receptor binding	609	49	29.63	0.00031

Supplementary Table 15: Enriched GO BP terms in non-linearly described promoters for U0126-Wortmannin combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0045653	negative regulation of megakaryocyte dif...	17	15	0.85	2.90E-18
GO:1901533	negative regulation of hematopoietic pro...	21	16	1.05	1.80E-17
GO:0035574	histone H4-K20 demethylation	16	14	0.8	5.30E-17
GO:1901532	regulation of hematopoietic progenitor c...	34	18	1.69	2.70E-15
GO:0045652	regulation of megakaryocyte differentiat...	22	15	1.1	2.90E-15
GO:0030219	megakaryocyte differentiation	38	16	1.89	9.00E-12
GO:0070076	histone lysine demethylation	29	14	1.45	1.90E-11
GO:0016577	histone demethylation	31	14	1.55	5.80E-11
GO:0051290	protein heterotetramerization	31	14	1.55	5.80E-11
GO:0006335	DNA replication-dependent nucleosome ass...	32	14	1.6	9.90E-11
GO:0032776	DNA methylation on cytosine	32	14	1.6	9.90E-11
GO:0034723	DNA replication-dependent nucleosome org...	32	14	1.6	9.90E-11
GO:0006482	protein demethylation	33	14	1.64	1.60E-10
GO:0008214	protein dealkylation	33	14	1.64	1.60E-10
GO:0000183	chromatin silencing at rDNA	39	15	1.94	1.90E-10
GO:0070988	demethylation	44	15	2.19	1.40E-09
GO:0045638	negative regulation of myeloid cell diff...	53	16	2.64	3.00E-09
GO:0034080	CENP-A containing nucleosome assembly	42	14	2.09	7.00E-09
GO:0061641	CENP-A containing chromatin organization	42	14	2.09	7.00E-09
GO:1903707	negative regulation of hemopoiesis	72	18	3.59	8.80E-09
GO:0031055	chromatin remodeling at centromere	43	14	2.14	9.90E-09
GO:0002244	hematopoietic progenitor cell differenti...	107	22	5.33	1.00E-08
GO:0034508	centromere complex assembly	50	15	2.49	1.00E-08
GO:0006305	DNA alkylation	67	17	3.34	1.80E-08
GO:0006306	DNA methylation	67	17	3.34	1.80E-08
GO:1903706	regulation of hemopoiesis	164	27	8.17	3.30E-08
GO:0043486	histone exchange	47	14	2.34	3.60E-08
GO:0044728	DNA methylation or demethylation	74	17	3.69	8.80E-08
GO:0043044	ATP-dependent chromatin remodeling	66	16	3.29	9.50E-08
GO:0006352	DNA-templated transcription, initiation	259	34	12.91	1.80E-07
GO:0006336	DNA replication-independent nucleosome a...	53	14	2.64	1.90E-07
GO:0034724	DNA replication-independent nucleosome o...	53	14	2.64	1.90E-07
GO:0030154	cell differentiation	1832	135	91.32	3.50E-07
GO:0006304	DNA modification	90	18	4.49	3.50E-07
GO:0016458	gene silencing	121	21	6.03	4.60E-07
GO:0006342	chromatin silencing	65	15	3.24	4.80E-07
GO:0006338	chromatin remodeling	133	22	6.63	5.80E-07
GO:0051291	protein heterooligomerization	77	16	3.84	9.10E-07
GO:0051262	protein tetramerization	96	18	4.79	9.50E-07
GO:0045637	regulation of myeloid cell differentiati...	109	19	5.43	1.50E-06

GO:0032502	developmental process	2898	190	144.45	2.50E-06
GO:0044767	single-organism developmental process	2854	186	142.26	5.40E-06
GO:0007275	multicellular organismal development	2393	161	119.28	5.70E-06
GO:0048513	organ development	1491	109	74.32	1.10E-05
GO:2000026	regulation of multicellular organismal d...	752	64	37.48	1.20E-05
GO:0048869	cellular developmental process	1989	137	99.14	1.30E-05
GO:0044707	single-multicellular organism process	2990	191	149.04	1.40E-05
GO:0001944	vasculature development	275	31	13.71	1.60E-05
GO:0065004	protein-DNA complex assembly	151	21	7.53	1.80E-05
GO:0002683	negative regulation of immune system pro...	175	23	8.72	1.80E-05
GO:0045596	negative regulation of cell differentiat...	292	32	14.55	2.10E-05
GO:0032501	multicellular organismal process	3091	195	154.07	2.50E-05
GO:1901342	regulation of vasculature development	78	14	3.89	2.60E-05
GO:0000723	telomere maintenance	80	14	3.99	3.50E-05
GO:2000113	negative regulation of cellular macromol...	859	69	42.82	3.70E-05
GO:0031327	negative regulation of cellular biosynth...	925	73	46.11	3.90E-05
GO:0032200	telomere organization	82	14	4.09	4.60E-05
GO:0051172	negative regulation of nitrogen compound...	967	75	48.2	5.40E-05
GO:0009890	negative regulation of biosynthetic proc...	937	73	46.71	5.90E-05
GO:0010558	negative regulation of macromolecule bio...	905	71	45.11	6.10E-05
GO:0048856	anatomical structure development	2546	164	126.91	6.10E-05
GO:0050793	regulation of developmental process	1059	80	52.79	7.20E-05
GO:0048731	system development	2107	139	105.02	0.0001
GO:0043414	macromolecule methylation	209	24	10.42	0.00011
GO:0048534	hematopoietic or lymphoid organ developm...	420	39	20.94	0.00012
GO:0071824	protein-DNA complex subunit organization	172	21	8.57	0.00012
GO:0045595	regulation of cell differentiation	734	59	36.59	0.00014
GO:0030099	myeloid cell differentiation	213	24	10.62	0.00015
GO:0010629	negative regulation of gene expression	969	73	48.3	0.00017
GO:1903507	negative regulation of nucleic acid-temp...	778	61	38.78	0.00022
GO:0030097	hemopoiesis	403	37	20.09	0.00022
GO:0051239	regulation of multicellular organismal p...	1164	84	58.02	0.00023
GO:0002520	immune system development	449	40	22.38	0.00023
GO:0001568	blood vessel development	261	27	13.01	0.00024
GO:0045892	negative regulation of transcription, DN...	753	59	37.53	0.00028
GO:1902679	negative regulation of RNA biosynthetic ...	786	61	39.18	0.00029
GO:0051253	negative regulation of RNA metabolic pro...	821	63	40.92	0.00031
GO:0040029	regulation of gene expression, epigeneti...	211	23	10.52	0.00033
GO:0002682	regulation of immune system process	725	57	36.14	0.00033
GO:0051259	protein oligomerization	281	28	14.01	0.00035
GO:0001763	morphogenesis of a branching structure	99	14	4.93	0.00037
GO:0045746	negative regulation of Notch signaling p...	14	5	0.7	0.00041
GO:0006334	nucleosome assembly	112	15	5.58	0.00042
GO:0045814	negative regulation of gene expression, ...	112	15	5.58	0.00042

GO:0031497	chromatin assembly	125	16	6.23	0.00046
GO:0097191	extrinsic apoptotic signaling pathway	150	18	7.48	0.00046
GO:0045765	regulation of angiogenesis	70	11	3.49	0.00063
GO:0051093	negative regulation of developmental pro...	388	34	19.34	0.00092
GO:0060249	anatomical structure homeostasis	173	19	8.62	0.00098
GO:0045934	negative regulation of nucleobase-contai...	892	65	44.46	0.001

Supplementary Table 16: Enriched GO MF terms in non-linearly described promoters for U0126-Wortmannin combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0035575	histone demethylase activity (H4-K20 spe...	16	14	0.78	3.90E-17
GO:0032452	histone demethylase activity	29	14	1.41	1.40E-11
GO:0032451	demethylase activity	35	15	1.71	2.20E-11
GO:0042393	histone binding	142	20	6.92	1.70E-05
GO:0000981	RNA polymerase II transcription factor a...	314	32	15.31	5.80E-05
GO:0043565	sequence-specific DNA binding	518	44	25.25	0.0002
GO:0044212	transcription regulatory region DNA bind...	465	40	22.67	0.00031
GO:0000975	regulatory region DNA binding	467	40	22.77	0.00034
GO:0001067	regulatory region nucleic acid binding	467	40	22.77	0.00034
GO:0003677	DNA binding	1562	103	76.15	0.00053
GO:0001071	nucleic acid binding transcription facto...	657	51	32.03	0.00057
GO:0003700	transcription factor activity, sequence-...	657	51	32.03	0.00057
GO:0000977	RNA polymerase II regulatory region sequ...	329	30	16.04	0.00068
GO:0001012	RNA polymerase II regulatory region DNA ...	331	30	16.14	0.00075

Supplementary Table 17: Enriched GO CC terms in non-linearly described promoters for U0126-Wortmannin combinatorial treatment. GO.ID: Gene Ontology ID. Term: Description string. Annotated: number of genes annotated with this GO id in the background dataset. Significant: the actual number of genes annotated with this GO id in the non-linearly described promoter set. Expected: the expected number of genes annotated with this GO id in the non-linearly described promoter set. P-value: the p-value of the test.

GO.ID	Term	Annotated	Significant	Expected	P-value
GO:0000786	nucleosome	77	14	3.79	2.00E-05
GO:0044815	DNA packaging complex	83	14	4.09	4.70E-05

Supplementary Table 18: Overrepresented motifs in non-linearly described promoters for Gefitinib-U0126 combinatorial treatment. In the first column the names of the overrepresented motifs are listed. The p-value after BH adjustment is reported. The observed ratio denotes the number of occurrences of the motif in the non-linearly described promoters divided by number of the non-linearly described promoters. The background ratio denotes the number of occurrences of the motif in the under study promoters divided by the number of the under study promoters. Odds ratio is the observed ratio divided by the background ratio.

	p-value	obs_ratio	back_ratio	odds_ratio
PITX1..3	0.001029	36/1001	313/19414	2.230689
FOXP1	1.41E-09	113/1001	1105/19414	1.98334
ZBTB16	0.001029	27/1001	206/19414	2.542011
HMGA1,2	0.029173	18/1001	150/19414	2.327353
NKX6-1,2	0.007534	19/1001	141/19414	2.613457
EVI1	0.006915	21/1001	161/19414	2.529731
PRRX1,2	0.015723	18/1001	140/19414	2.493592

Supplementary Table 19: Overrepresented motifs in non-linearly described for Gefitinib-Wortmannin combinatorial treatment. In the first column the names of the overrepresented motifs are listed. The p-value after BH adjustment is reported. The observed ratio denotes the number of occurrences of the motif in the non-linearly described promoters divided by number of the non-linearly described promoters. The background ratio denotes the number of occurrences of the motif in the under study promoters divided by the number of the under study promoters. Odds ratio is the observed ratio divided by the background ratio.

	p-value	obs_ratio	back_ratio	odds_ratio
GATA4	0.0234	36/953	423/19414	1.733741
NR1H4	0.008592	20/953	170/19414	2.396642
TOPORS	0.002426	38/953	386/19414	2.00548
PAX4	0.039172	15/953	136/19414	2.246852
TEF	0.007379	12/953	72/19414	3.395243
FOXL1	0.004492	19/953	146/19414	2.65108
LHX3,4	0.049624	8/953	54/19414	3.017994
PITX1..3	0.041923	27/953	313/19414	1.757282
FOXP1	6.76E-10	110/953	1105/19414	2.027928
ZBTB16	0.001206	26/953	206/19414	2.571155
POU1F1	0.041923	9/953	63/19414	2.910208
STAT2,4,6	0.024437	42/953	523/19414	1.635949
DBP	0.028931	20/953	199/19414	2.047383
FOXD3	0.044731	16/953	155/19414	2.10286
IRF1,2	0.024543	36/953	434/19414	1.689798
CDX1,2,4	0.002056	23/953	179/19414	2.617562
HMGA1,2	0.024437	17/953	150/19414	2.308765
NKX6-1,2	0.015666	17/953	141/19414	2.456133
ONECUT1,2	0.002962	15/953	94/19414	3.250765
EVI1	0.000259	24/953	161/19414	3.036739
CRX	0.024543	16/953	140/19414	2.328167
PRRX1,2	0.024543	16/953	140/19414	2.328167
HBP1_HMGB_SSRP1_UBTF	0.041923	22/953	239/19414	1.875197

Supplementary Table 20: Overrepresented motifs in non-linearly described promoters for U0126-Wortmannin combinatorial treatment. In the first column the names of the overrepresented motifs are listed. The p-value after BH adjustment is reported. The observed ratio denotes the number of occurrences of the motif in the non-linearly described promoters divided by number of the non-linearly described promoters. The background ratio denotes the number of occurrences of the motif in the under study promoters divided by the number of the under study promoters. Odds ratio is the observed ratio divided by the background ratio.

	p-value	obs_ratio	back_ratio	odds_ratio
TOPORS	0.003924	37/923	386/19414	2.016173
FOXL1	0.010522	18/923	146/19414	2.593182
PITX1..3	0.010522	30/923	313/19414	2.015999
FOXP1	1.02E-08	104/923	1105/19414	1.979632
ZBTB16	0.003924	24/923	206/19414	2.450515
NKX3-1	0.018264	10/923	60/19414	3.505598
OCT4_SOX2{dimer}	0.041242	16/923	146/19414	2.305051
FOXD3	0.003924	20/923	155/19414	2.714011
FOXO1,3,4	0.018264	23/923	227/19414	2.131156
EVI1	0.020158	18/923	161/19414	2.351581

Supplementary Table 21: Common motifs overrepresented in not linearly described promoters. For every drug combination the odds ratios and p-values after BH adjustment are reported. Odds ratio is the observed ratio divided by the background ratio. Performing the enrichment analysis excluding the common promoters among the non-linearly described ones for every combinatorial treatment revealed that the common overrepresented motifs are due to the promoter regions shared between the treatments. Specifically, the enrichment analysis after BH adjustment yielded no enriched motifs for the Gefitinib-U0126 pair, two enriched motifs (FOXP1, EVI1) for the Gefitinib Wortmannin pair and two enriched motifs the (FOXP1, FOXD3) for U0126-Wortmannin pair.

Motifs	**Gefitinib_U0126**		**Gefitinib_Wortmannin**		**U0126_Wortmannin**	
	Odds ratio	P-value	Odds ratio	P-value	Odds ratio	P-value
EVI1	2.53	6.91e-03	3.04	2.59e-04	2.35	2.02e-02
FOXP1	1.98	1.41e-09	2.03	6.76e-10	1.98	1.02e-08
PITX1..3	2.23	1.03e-03	1.76	4.19e-02	2.02	1.05e-02
ZBTB16	2.54	1.03e-03	2.57	1.21e-03	2.45	3.92e-03

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