Expanded View Figures

Filtering steps that were used to make the GR activity gene-set

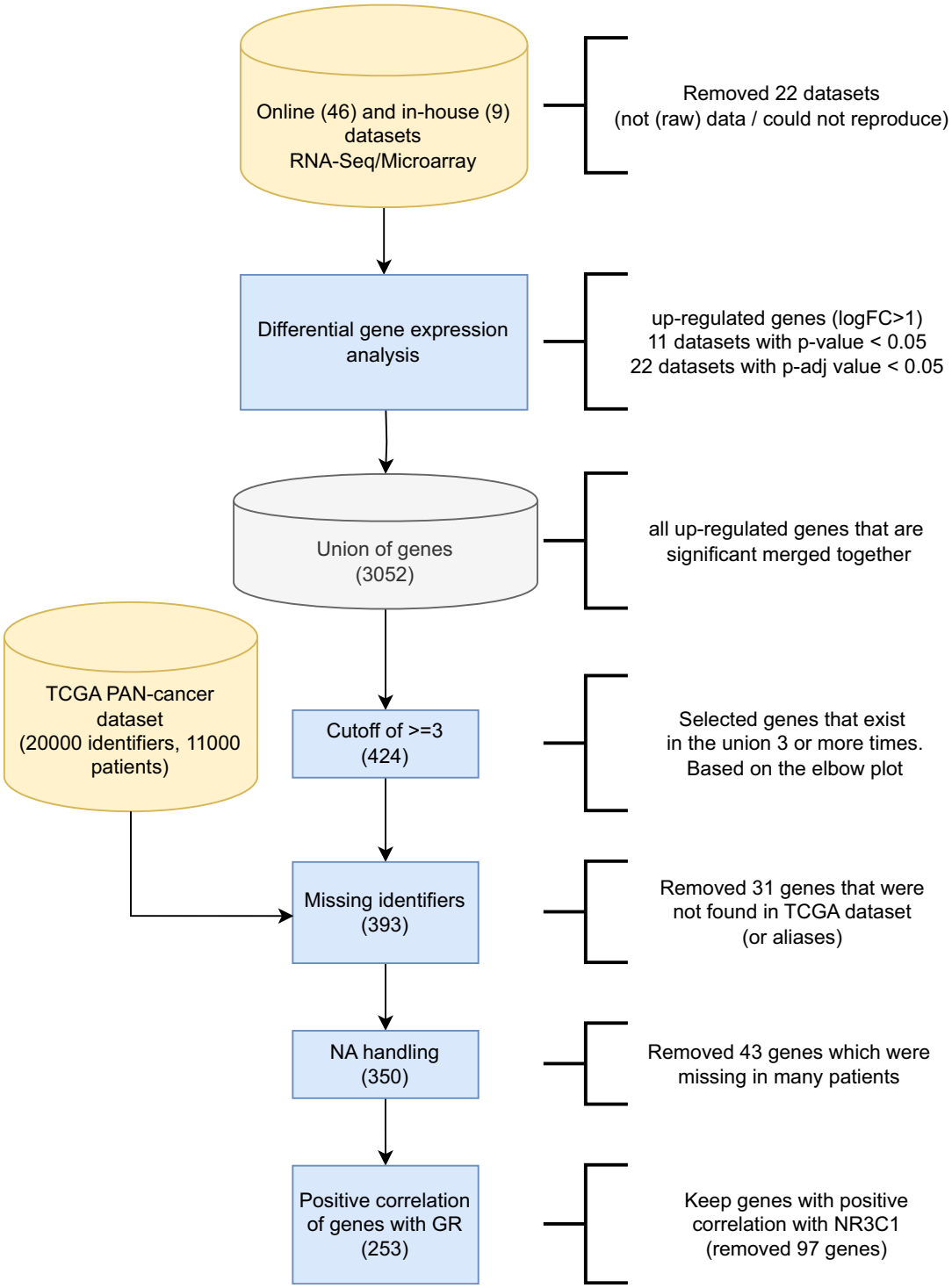

Figure EV1.

Figure EV1. The scheme representing the process and filtering steps taken to make the GR activity signature.

Figure EV2. Optimization of the GR activity signature.

A Elbow plot depicting overlap between models. This analysis aided refinement of the signature to a consensus list of number of genes shared among at least three independent models, yielding a total list of 424 genes.

B Expression correlation analyses of NR3C1 mRNA levels with expression levels of genes represented in the signature in cancer samples. Only positively correlating genes were included for further analyses, yielding a final set of 253 genes.

C Enrichment of GR binding in vicinity of GRa (right, full line) or random (left, dashed line; $n = 1{,}000$ iterations; error bars represent mean $\pm$ SD) genes in ChIP-sequencing experiments from various cell lines (full lines, A549, BEAS-2B, HeLa, LNCaP, MCF-7, THP1, U2OS, and ZR-75-1).

D Overlap of GR activity signature, with ER (gene-set reference: M5906 and M5907) and AR (gene-set reference: M5908) gene signatures.

E Correlation of NR3C1 mRNA levels with GR activity across samples represented in the TCGA dataset. Color indicates the number of samples in each bin.

F Correlation of GR mRNA levels with random gene set of equivalent size to GR activity signature among samples represented in the TCGA dataset. Color indicates the number of samples in each bin.

G Histogram depicting correlation of random gene set of equivalent size to GR activity signature with NR3C1 mRNA levels in TCGA cancers; black line depicts the correlation of GR activity with NR3C1 mRNA levels in the same cohort.

H Overrepresentation analysis of various gene sets within the GR activity signature. Each point represents a different pathway.

I Pearson correlation analysis of single-sample gene-set enrichment analysis (ssGSEA) scores for each of the TCGA samples ($n = 9{,}829$) and GR activity. Representation of the different tumor types is depicted below.

J Normalized (phospho)protein expression in human breast cancer samples of the TCGA ($n = 747$) grouped based on GR activity. The central mark indicates the median, and the bottom and top edges of the box indicate the 25^{th} and 75^{th} percentiles, respectively. The maximum whisker lengths are specified as 1.5 times the interquartile range.

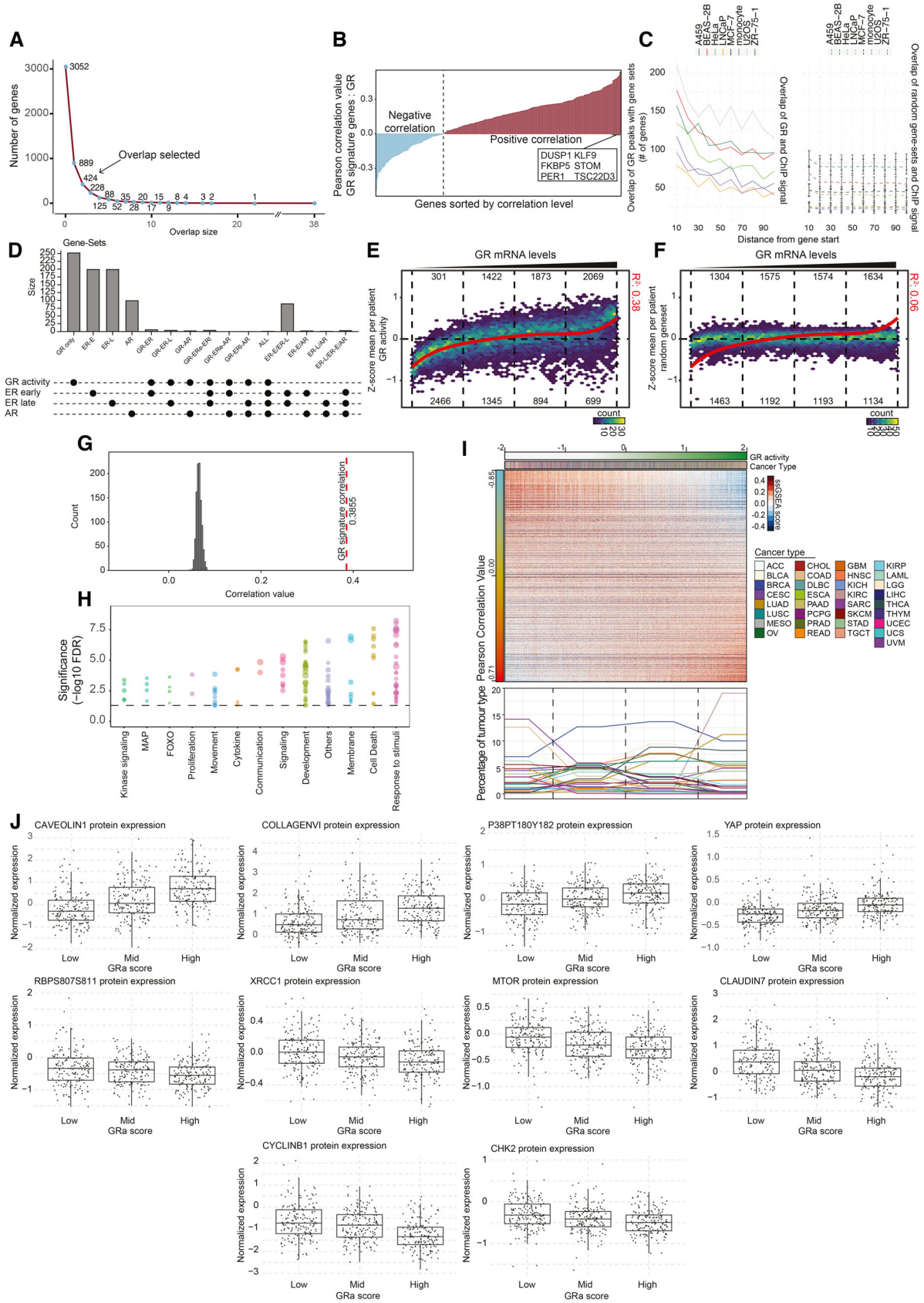

Figure EV2.

Figure EV3. GR activity in breast cancer is linked to luminal subtype status and survival probabilities.

A Boxplots depicting GR activity signature in Luminal A and B cancers of the METABRIC cohort (n = 1,134 luminal samples). The box begins in the first quartile (25%) and ends in the third (75%), while the line represents the median value. The lines represent segments to furthest data without accounting for outliers. P-values were determined by the Wilcoxon t-test.

B Boxplots depicting GR activity signature in Luminal A and B cancers of the MATADOR trial (n = 415 luminal samples). P-values were determined by the Wilcoxon t-test. The box begins in the first quartile (25%) and ends in the third (75%), while the line represents the median value. The lines represent segments to furthest data without accounting for outliers.

C The UMAP analysis of time-course experiments performed with T47D cell line utilizing the GRa genes. Each cell is colored according to the treatment time points—0, 1, 2, 4, 8, and 18 h.

D Boxplot depicting the value of GR activity per cell per time point of the time course (400 cells). The central mark indicates the median, and the bottom and top edges of the box indicate the 25^{th} and 75^{th} percentiles, respectively. The lines represent segments to furthest data without accounting for outliers.

E Average gene expression of genes corresponding to clusters identified by k-means clustering of PAM50 genes in luminal breast cancer patient samples. The box begins in the first quartile (25%) and ends in the third (75%), while the line represents the median value. The lines represent segments to furthest data without accounting for outliers.

F Overall survival probabilities of breast cancer patients (n = 1,310) grouped by transcriptomics-based 8-gene classifier (Luminal A high expression = purple, Luminal A low expression = gray, and Luminal B = blue). Overall survival probabilities in months are plotted for each group, and censored patients are shown as vertical tick marks.

G Prognostic power as determined by SigCheck of the 8-gene classifier (red dotted line) with 1,000 random gene sets of the same size (P-value < 0.05 is indicated by the blue dotted line) for overall survival parameter in KMplotter breast cancer cohort (n = 1,310).

H Overall survival probabilities of breast cancer patients (TCGA cohort n = 280) grouped by transcriptomics-based 8-gene classifier (Luminal A high expression = purple, Luminal A low expression = gray, and Luminal B = blue). Overall survival probabilities in months are plotted for each group, and censored patients are shown as vertical tick marks. First 300 months were included in the analysis.

I Overall survival probabilities of breast cancer patients (KMplotter cohort n = 3,772; TCGA cohort n = 280) grouped by GR activity signature (Luminal A high expression = purple, Luminal A low expression = gray, and Luminal B = blue). Overall survival probabilities in months are plotted for each group, and censored patients are shown as vertical tick marks. First 300 months were included in the analysis.

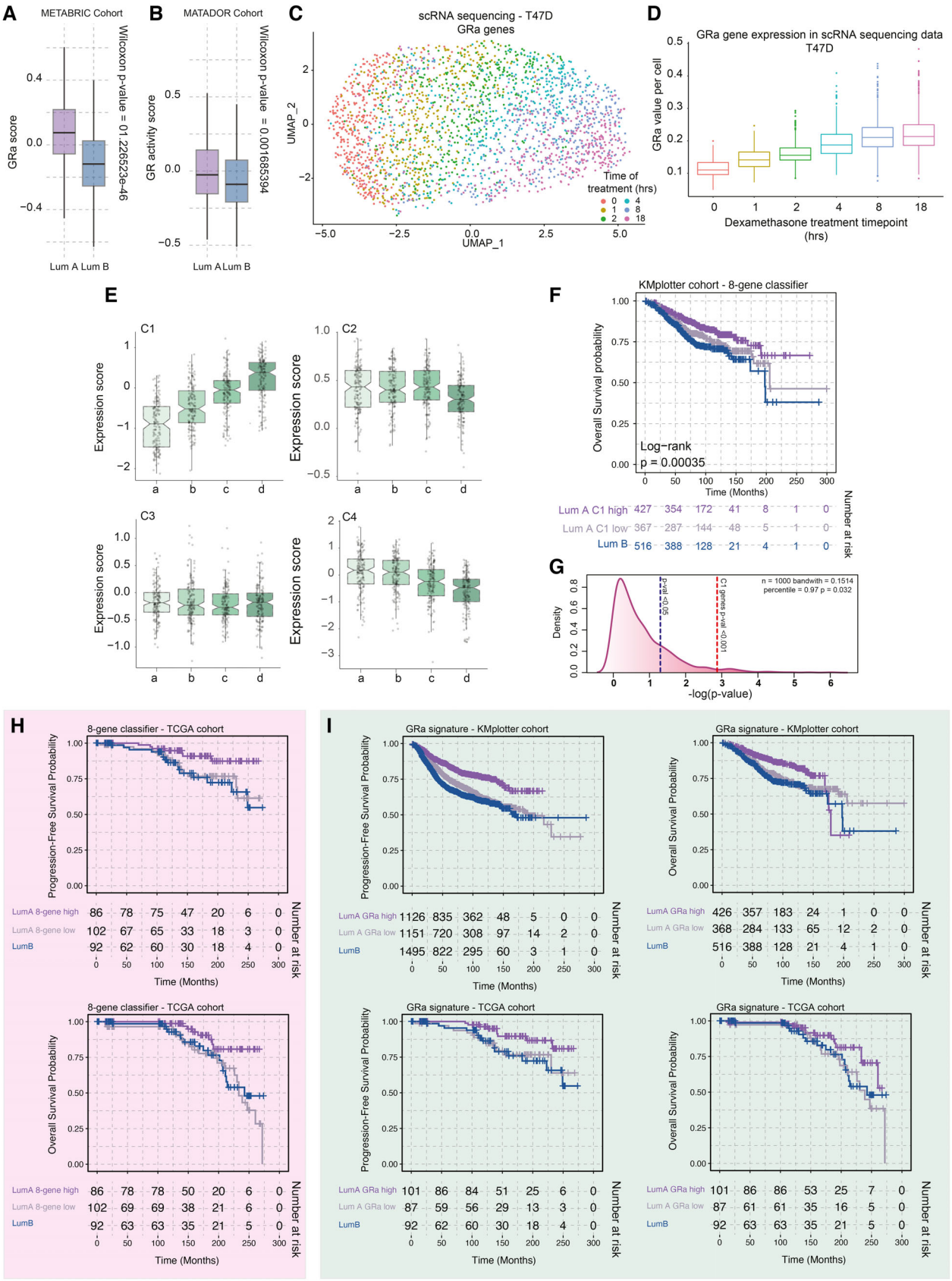

Figure EV3.

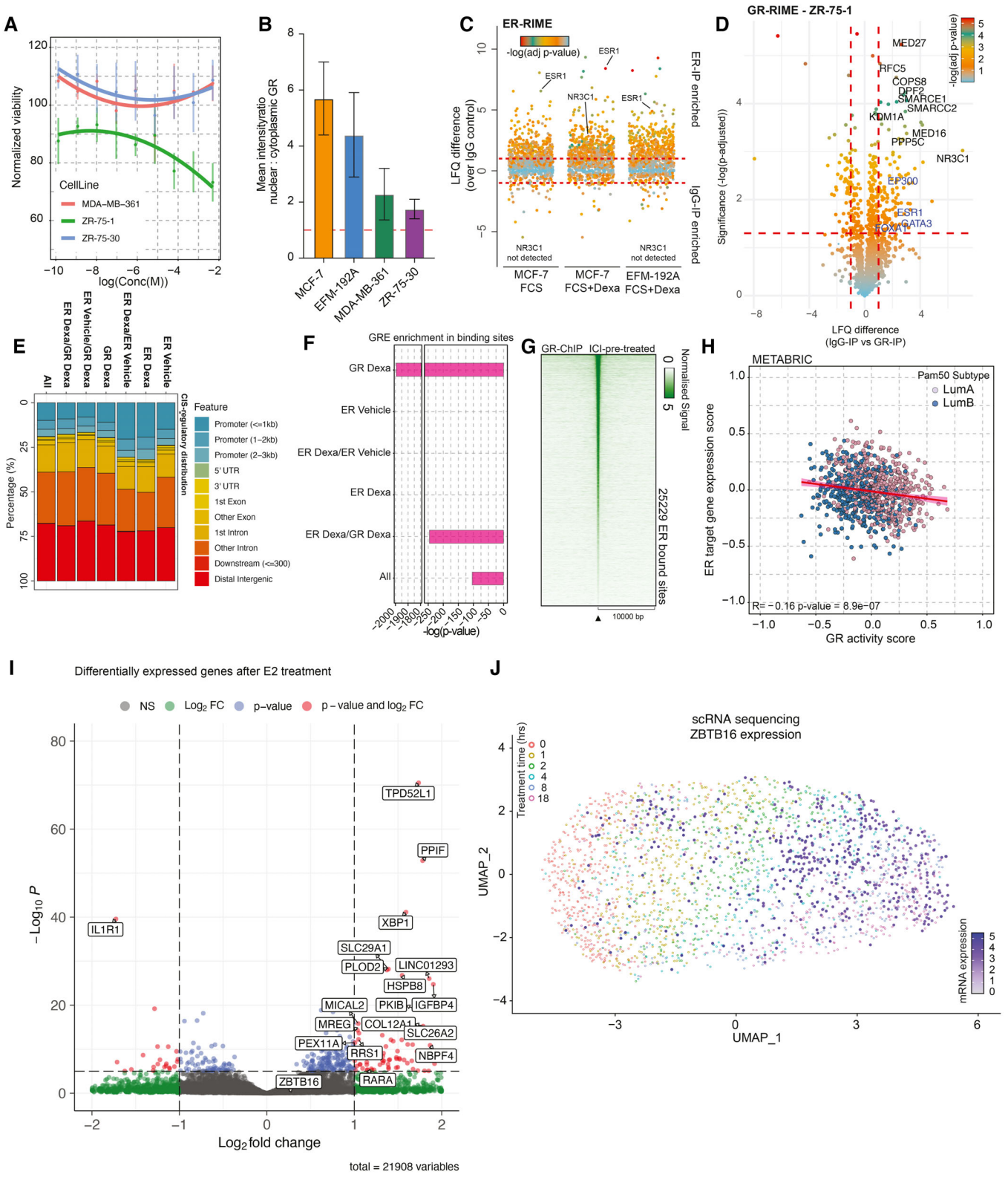

Figure EV4.

Figure EV4. Molecular aspect of GR signaling in luminal breast cancer.

A Normalized cancer cell viability (lines depict polynomial fit) in response to GR agonist (Dexa) treatment. $n = 4$. Mean values ± SEM depicted.
B Bar plot depicting nuclear-to-cytoplasmic ratio of GR in various cell lines. $n = 3$. Mean values ± SEM depicted.
C Jitter plot depicting differentially enriched interactors in ER-RIME experiments between Veh-treated MCF-7 and Dexa-treated MCF-7 and EFM-192A cell lines. $n = 4$. *P*-values were determined by two-sided *t*-test.
D Volcano plot depicting enriched (in comparison to IgG-IP control) interactors in GR-RIME experiments in ZR-75-1 cell line. $n = 3$. *P*-values were determined by two-sided *t*-test.
E Genomic distribution analyses for all the sites detected in ChIP-sequencing experiments. $n = 3$.
F HOMER motif analysis of *P*-value for GREs across different categories of sites.
G Heatmap of ChIP-sequencing signal for GR around peak midpoint for all sites detected across the genome after 24 h pre-treatment with ICI and subsequent 2 h treatment with Dexa. $n = 3$.
H Scatterplot depicting the absence of correlation of GR activity with ER target gene expression in luminal breast cancers.
I Volcano plot depicting transcriptomic differences between DCC-treated and DCC+E2-treated MCF-7 ($n = 4$). Adjusted *P*-values were determined by DESeq2 (Wald test *P*-values corrected for multiple testing using Benjamini and Hochberg method).
J The UMAP analysis of time-course experiments performed with T47D cell line utilizing the GRa genes. Each cell is colored according to the treatment time points—0, 1, 2, 4, 8, and 18 h, and the values of *ZBTB16* gene expression are projected on top (white-to-blue gradient).

Data information: All experiments were performed in biological replicate and the number (*n*) of replicates indicated.

Figure EV5. *ZBTB16* action in ER-positive breast cancer.

A Boxplot depicting *ZBTB16* mRNA expression in normal breast tissues, Luminal A, as well as Luminal B cancer samples (TCGA cohort $n = 702$). The box begins in the first quartile (25%) and ends in the third (75%), while the line represents the median value. The lines represent segments to furthest data without accounting for outliers.
B Overall survival probabilities of breast cancer patients (KMplotter meta dataset; $n = 1{,}310$) grouped by *ZBTB16* mRNA expression (Luminal A high expression = purple, Luminal A low expression = gray, and Luminal B = blue). Overall survival probabilities in months are plotted for each group, and censored patients are shown as vertical tick marks.
C Progression-free and overall survival probabilities of breast cancer patients (METABRIC dataset) grouped by *ZBTB16* mRNA expression (Luminal A high expression = purple, Luminal A low expression = gray, and Luminal B = blue). Progression-free and overall survival probabilities in months are plotted for each group, censored patients are shown as vertical tick marks.
D Western blot showing expression of V-5-tagged GFP and ZBTB16. $n = 3$.
E Normalized cancer cell viability for MCF-7 GFP (green) and ZBTB16 (orange) overexpression models. $n = 4$.
F Representative crystal violet assay image for MCF-7 GFP and ZBTB16 overexpression models. $n = 3$.
G Upper panel, volcano plot depicting differences in protein expression between GFP-OE and ZBTB16-OE models in MCF-7 cells. Significance is depicted as a color gradient; lower panel, intensity (LFQ) values per category (decrease in expression, same levels, and increase in expression upon ZBTB16 overexpression). $n = 4$. The box begins in the first quartile (25%) and ends in the third (75%), while the line represents the median value. The lines represent segments to furthest data without accounting for outliers.
H Volcano plot depicting differences in protein expression between GFP-OE and ZBTB16-OE models in EFM-192A cells. $n = 4$.
I Reactome cell cycle (gene-set reference: R-HSA-1640170) GSEA enrichment profiles based on whole-proteome data comparison between GFP-OE and ZBTB16-PE in MCF-7 cell line.
J Hallmark E2F targets (gene-set reference: M5925) GSEA enrichment profiles based on whole-proteome data comparison between GFP-OE and ZBTB16-PE in EFM-192A cell line.
K Volcano plot depicting differentially enriched (over IgG control) interactors in ZBTB16-RIME experiments in MCF-7 cells. $n = 4$. *P*-values were determined by two-sided *t*-test.
L GSEA enrichment profiles for "Reactome Signaling by nuclear receptors" gene sets based on IgG versus ZBTB-16 RIME comparison ($n = 4$).

Data information: All experiments were performed in biological replicates and the number (*n*) of replicates indicated.

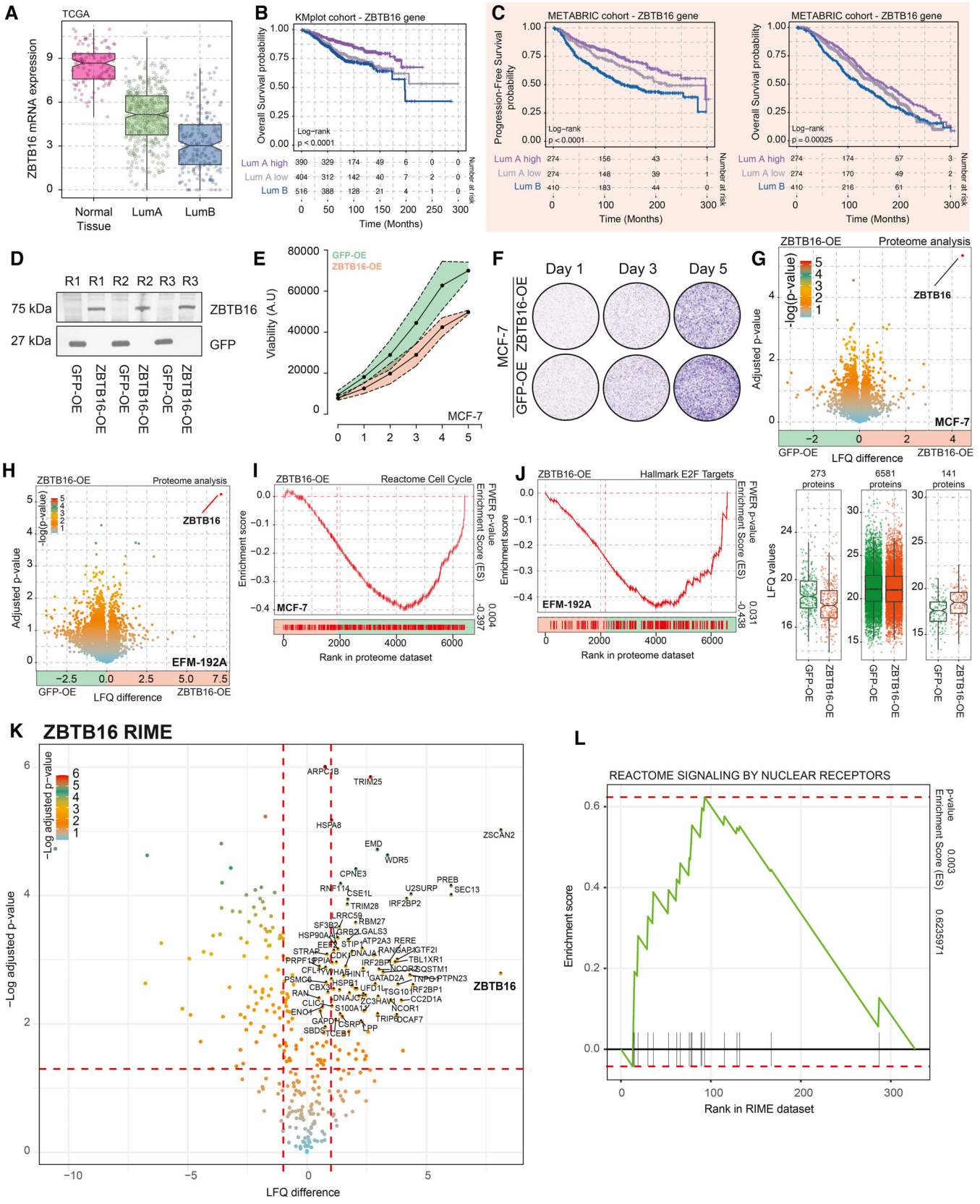
A
TCGA
ZBTB16 mRNA expression
Normal Tissue
LumA
LumB
B
KMplot cohort - ZBTB16 gene
Overall Survival probability
Log−rank p < 0.0001
Lum A high 390 329 174 49 6 0 0
Lum A low 404 312 142 40 7 2 0
Lum B 516 388 128 21 4 1 0
Number at risk
Time (Months)
C
METABRIC cohort - ZBTB16 gene
Progression-Free Survival probability
Log−rank p < 0.0001
Lum A high 274 156 43 1
Lum A low 274 148 39 1
Lum B 410 183 44 0
Overall Survival probability
Log−rank p = 0.00025
Lum A high 274 174 57 3
Lum A low 274 170 49 2
Lum B 410 216 61 1
Number at risk
Time (Months)
D
R1 R1 R2 R2 R3 R3
75 kDa
ZBTB16
27 kDa
GFP
GFP-OE
ZBTB16-OE
E
GFP-OE
ZBTB16-OE
Viability (A.U)
MCF-7
F
Day 1
Day 3
Day 5
MCF-7
ZBTB16-OE
GFP-OE
G
ZBTB16-OE
Proteome analysis
-log(p-value)
ZBTB16
Adjusted p-value
MCF-7
GFP-OE
LFQ difference
ZBTB16-OE
H
ZBTB16-OE
Proteome analysis
ZBTB16
EFM-192A
I
ZBTB16-OE
Reactome Cell Cycle
Enrichment score
FWER p-value
Enrichment Score (ES)
MCF-7
Rank in proteome dataset
J
ZBTB16-OE
Hallmark E2F Targets
EFM-192A
273 proteins
6581 proteins
141 proteins
LFQ values
K
ZBTB16 RIME
−Log adjusted p-value
ZBTB16
LFQ difference
L
REACTOME SIGNALING BY NUCLEAR RECEPTORS
Enrichment score
p-value
Enrichment Score (ES)
Rank in RIME dataset

Figure EV5.

Figure EV6. Inhibition of epigenetic pathways in additional models of breast cancer.

A Comparison of Dexa-induced proteomic changes in proteomics experiments across different time points (2- vs. 5- vs. 7-day treatment). $n = 4$. Adjusted *P*-values (*P*adj) were determined by *t*-test (*P*-values corrected for multiple testing using Benjamini and Hochberg method). Pearson correlation value is reported.

B Snake plot depicting the gene knockout fitness effects (Chronos) for top genes potentially regulated by vehicle unique H3K27Ac sites.

C Western blot showing expression of GR and ER in all the breast cancer cell line models used in the manuscript, with actin as a loading control ($n = 2$).

D Normalized cancer cell viability in response to BRD inhibitor (Molibresib) treatment. $n = 4$.

E Normalized cancer cell viability in response to BRD inhibitor (Birabresib) treatment. $n = 4$.

F Normalized cancer cell viability in response to HDAC inhibitor (Mocetinostat) treatment. $n = 4$.

G Normalized cancer cell viability in response to HDAC inhibitor (Vorinostat) treatment. $n = 4$.

H Bar chart showing response to various inhibitors for each of the organoid lines alongside key tumor characteristics. Drug concentrations (dexamethasone 100 nM, ribociclib 1,000 nM, alobresib 100 nM, birabresib 500 nM, vorinostat 500 nM, panobinostat 10 nM, and molibresib 500 nM). Error bars represent mean ± SEM. $n = 6$.

Data information: All experiments were performed in biological replicates and the number (*n*) of replicates indicated.

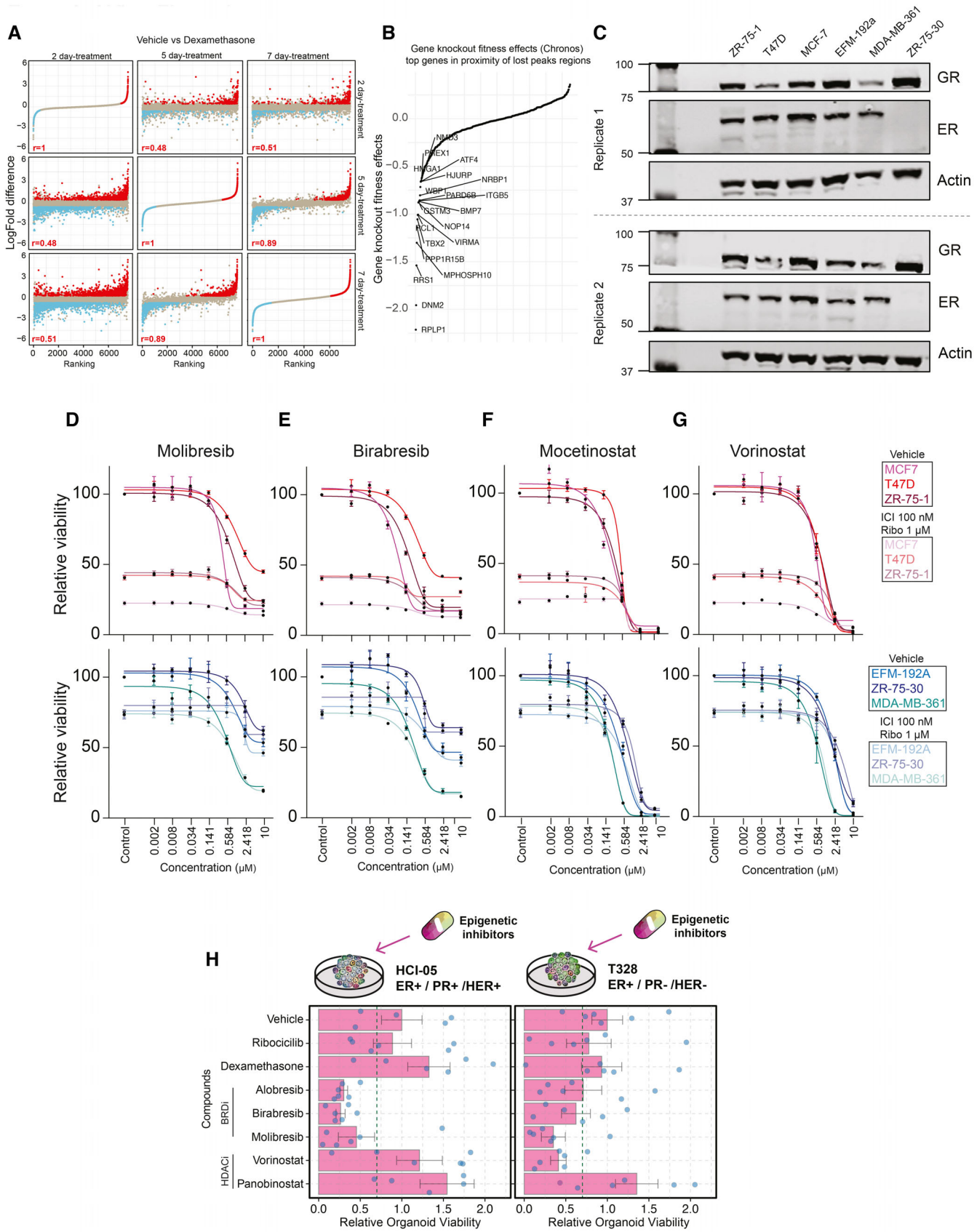
A
Vehicle vs Dexamethasone
2 day-treatment
5 day-treatment
7 day-treatment
LogFold difference
Ranking
r=1
r=0.48
r=0.51
r=0.89
B
Gene knockout fitness effects (Chronos)
top genes in proximity of lost peaks regions
Gene knockout fitness effects
NMD3
PHEX1
ATF4
HMGA1
HJURP
NRBP1
WRP1
PABD6B
ITGB5
GSTM3
BMP7
NCL1
NOP14
TBX2
VIRMA
PPP1R15B
RRS1
MPHOSPH10
DNM2
RPLP1
C
ZR-75-1
T47D
MCF-7
EFM-192a
MDA-MB-361
ZR-75-30
Replicate 1
Replicate 2
GR
ER
Actin
D
Molibresib
E
Birabresib
F
Mocetinostat
G
Vorinostat
Relative viability
Concentration (μM)
Control
Vehicle
MCF7
T47D
ZR-75-1
ICI 100 nM
Ribo 1 μM
EFM-192A
ZR-75-30
MDA-MB-361
H
Epigenetic inhibitors
HCI-05
ER+ / PR+ /HER+
T328
ER+ / PR- /HER-
Compounds
Vehicle
Ribocicilib
Dexamethasone
BRDi
Alobresib
Birabresib
Molibresib
HDACi
Vorinostat
Panobinostat
Relative Organoid Viability

Figure EV6.