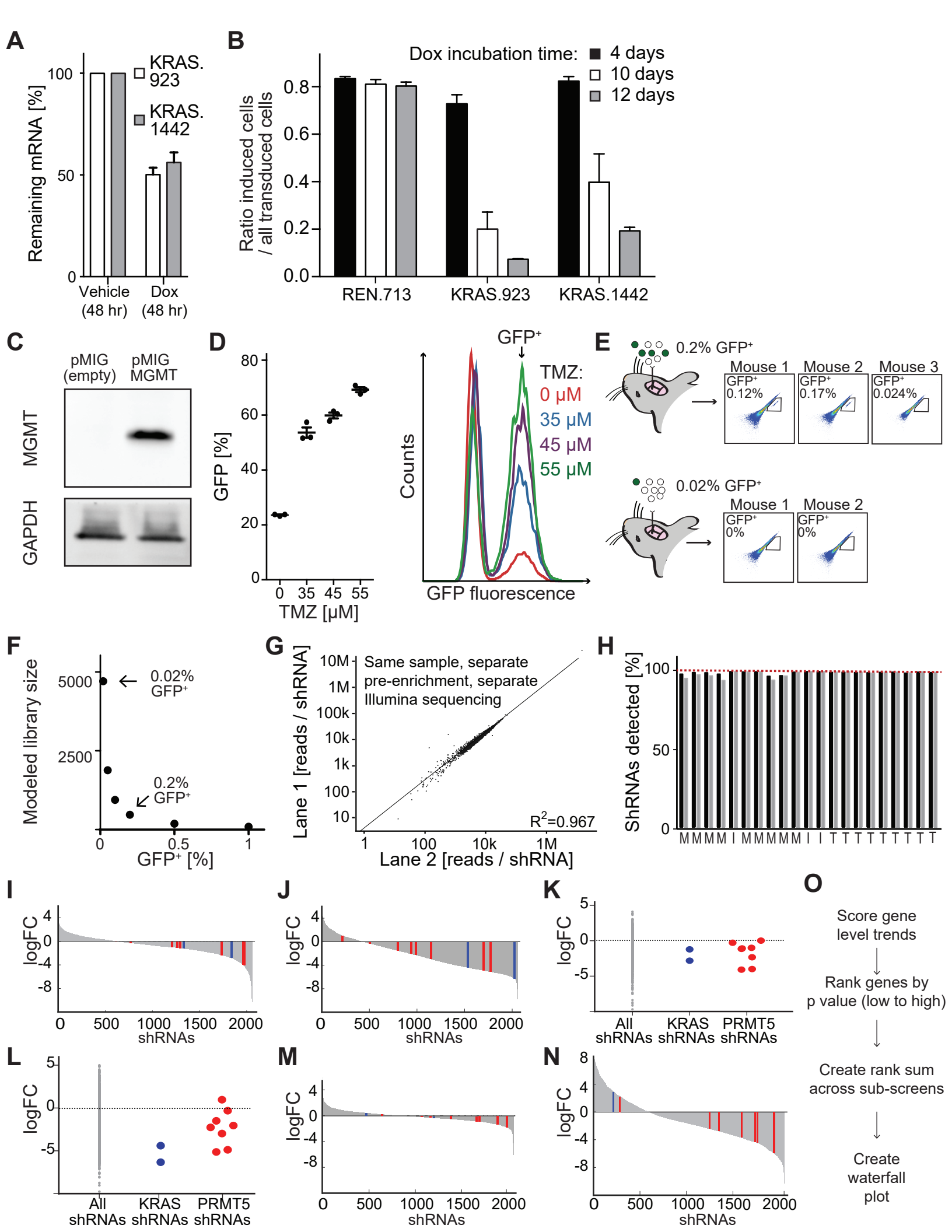
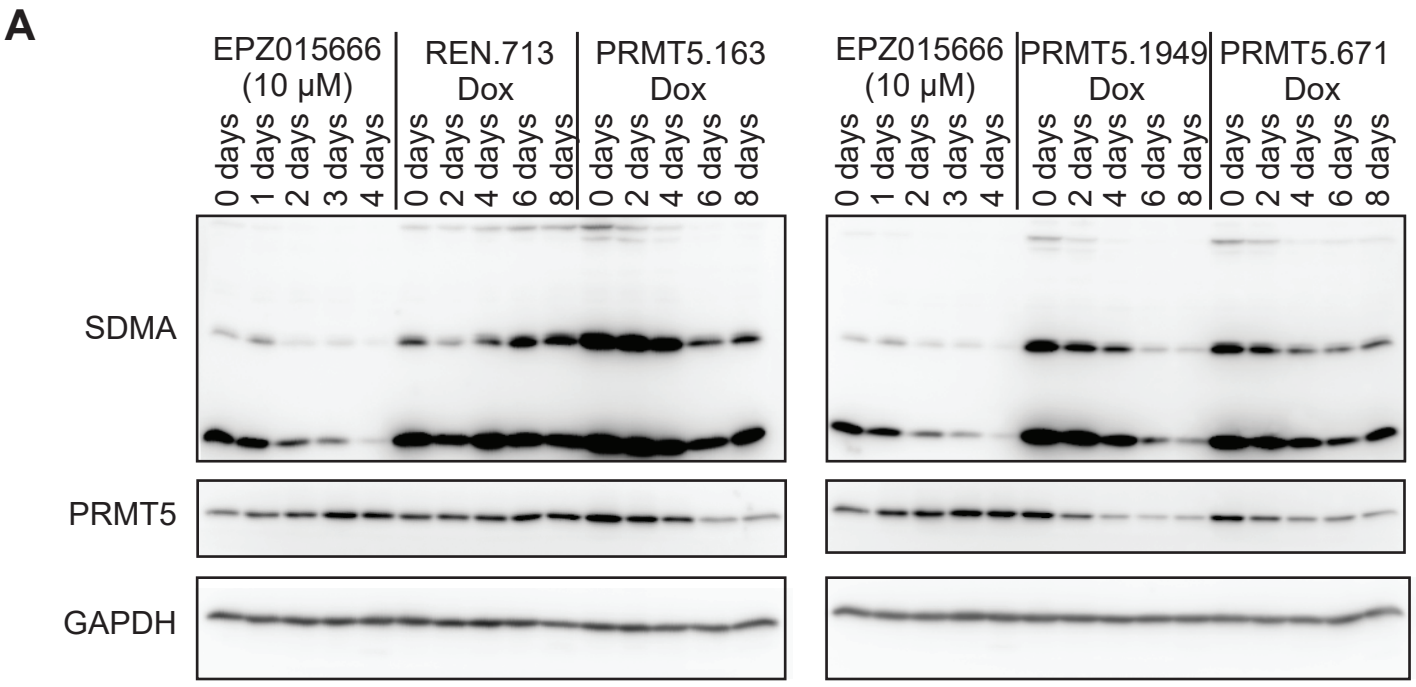
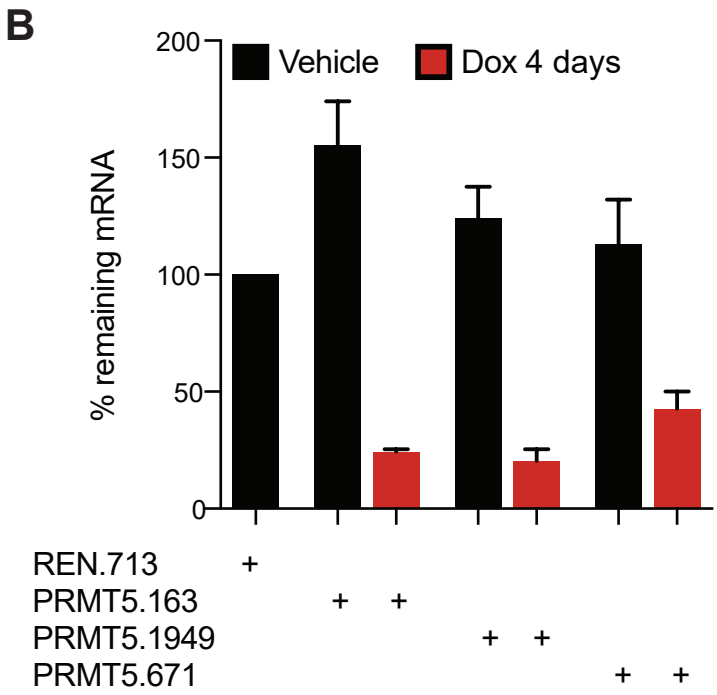
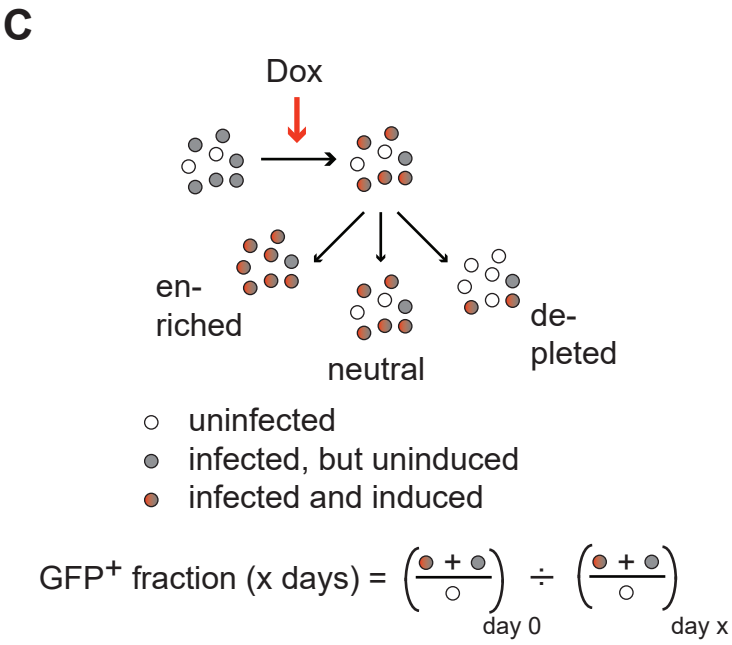

A
Remaining mRNA [%]
100
50
0
KRAS.923
KRAS.1442
Vehicle (48 hr)
Dox (48 hr)
B
Dox incubation time:
4 days
10 days
12 days
Ratio induced cells / all transduced cells
0.8
0.6
0.4
0.2
0.0
REN.713
KRAS.923
KRAS.1442
C
pMIG (empty)
pMIG MGMT
MGMT
GAPDH
D
GFP [%]
80
60
40
20
0
0 35 45 55
TMZ [μM]
GFP+
TMZ:
0 μM
35 μM
45 μM
55 μM
Counts
GFP fluorescence
E
0.2% GFP+
Mouse 1
Mouse 2
Mouse 3
GFP+ 0.12%
GFP+ 0.17%
GFP+ 0.024%
0.02% GFP+
Mouse 1
Mouse 2
GFP+ 0%
GFP+ 0%
F
Modeled library size
5000
2500
0
0.5
1
0.02% GFP+
0.2% GFP+
GFP+ [%]
G
Same sample, separate pre-enrichment, separate Illumina sequencing
Lane 1 [reads / shRNA]
10M
1M
100k
10k
1k
100
10
1
100
10k
1M
R2=0.967
Lane 2 [reads / shRNA]
H
ShRNAs detected [%]
100
50
0
M M M M I M M M M M M I I T T T T T T T T T T
I
logFC
4
0
-4
-8
0
500
1000
1500
2000
shRNAs
J
logFC
4
0
-4
-8
0
500
1000
1500
2000
shRNAs
K
logFC
5
0
-5
All shRNAs
KRAS shRNAs
PRMT5 shRNAs
O
Score gene level trends
Rank genes by p value (low to high)
Create rank sum across sub-screens
Create waterfall plot
L
logFC
5
0
-5
All shRNAs
KRAS shRNAs
PRMT5 shRNAs
M
logFC
4
0
-4
-8
0
500
1000
1500
2000
shRNAs
N
logFC
8
4
0
-4
-8
0
500
1000
1500
2000
shRNAs

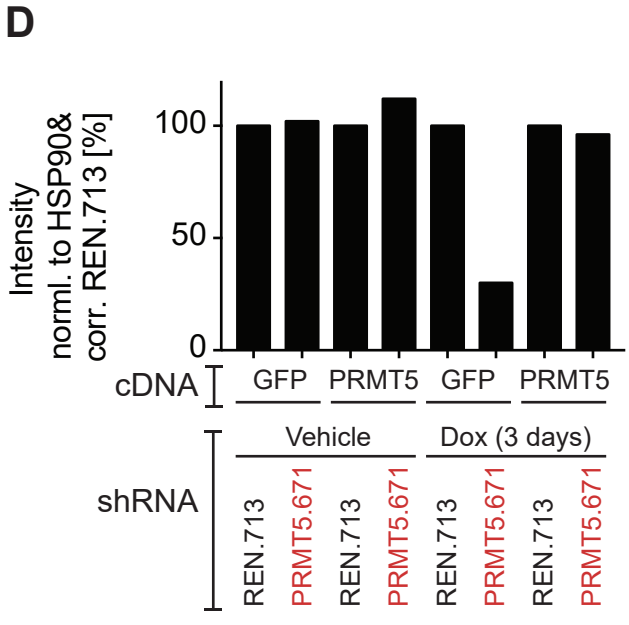
**Figure S1 (related to Figure 1). Gl261 orthotopic *in vivo* model allows for precise dissection of tumor phenotypes and for high-coverage pooled transcriptome perturbation screens**

(**A**) qRT-PCR of KRAS mRNA levels in Gl261 cells containing one of two KRAS targeting shRNAs, before and after doxycycline induction for 48 hr. n=3. Error bars represent +/- SEM. (**B**) *In vitro* competition assay, showing proportion of KRAS shRNA expressing cells compared to all shRNA transduced cells. n = 3. Error bars represent +/- SEM. (**C**) Immunoblot of Gl261 cells probed for MGMT before and after retroviral MGMT cDNA overexpression. (**D**) Dot plot of i*n vitro* competition assay using mixed populations of cells, a proportion of which are transduced with an MGMT overexpression vector that is also $GFP^+$. Doses of TMZ represent a range of IC70-IC90, and cells were analyzed by flow cytometry after 96 hr. Left graph shows percent of $GFP^+$ cells, right graph shows fluorescence counts. n=3, lines represent mean +/- SD. (**E**) Scheme outlining GFP dilution assay performed by intracranial injection of a mixed population of Gl261 cells containing a set percentage of cells expressing a retroviral GFP construct into C57BL6/J mice. The proportion of $GFP^+$ cells was determined in the final tumors by flow cytometry. (**F**) In GFP dilution assays, the percentage of $GFP^+$ cells acts as a surrogate for a certain library size (with lower GFP percentages representing larger libraries). The relationship between the percentage of $GFP^+$ cells in a mixed population and the modeled library size is: Library size = 1 / GFP%. (**G**) Plot showing correlation between different Illumina HiSeq lanes. Genomic DNA was isolated at t=0 from the same population of cells, but samples were PCR amplified separately and sequenced on different lanes. (**H**) Percentage of the library shRNAs present in the input (t=0), *in vitro*, and *in vivo* samples, with stringency > 0 or more reads (black bars) or >= 5 reads (grey bars). M = *in vivo* mouse samples, I = Tissue culture input controls, T = *in vitro* tissue culture samples. (**I**, **J**) Waterfall plots of all shRNAs across *in vitro* (**I**) and *in vivo* (**J**) samples depicting log fold change (logFC) of all shRNAs (grey), shRNAs targeting PRMT5 (red) and shRNAs targeting KRAS (blue) in comparison to t=0. (**K**,**L**) Dot plots depicting logFC of all shRNAs (grey), shRNAs targeting PRMT5 (red) and shRNAs targeting KRAS (blue) *in vitro* (**K**) and *in vivo* (**L**) in comparison to t=0. (**M**,**N**) Waterfall plots of all shRNAs across *in vitro* (**M**) and *in vivo* (**N**) samples comparing shRNAs enriched or depleted in response to TMZ versus respective vehicle treated control groups. (**O**) Hit identification pipeline across all four shRNA sub screens (*in vitro* – growth, *in vitro* – TMZ, *in vivo* – growth, *in vivo* – TMZ).


A
EPZ015666 (10 μM)
REN.713 Dox
PRMT5.163 Dox
EPZ015666 (10 μM)
PRMT5.1949 Dox
PRMT5.671 Dox
0 days
1 days
2 days
3 days
4 days
6 days
8 days
SDMA
PRMT5
GAPDH


B
Vehicle
Dox 4 days
% remaining mRNA
200
150
100
50
0
REN.713
PRMT5.163
PRMT5.1949
PRMT5.671


C
Dox
en-riched
neutral
de-pleted
uninfected
infected, but uninduced
infected and induced
GFP+ fraction (x days) =
day 0
day x


D
Intensity norml. to HSP90& corr. REN.713 [%]
100
50
0
cDNA
GFP
PRMT5
GFP
PRMT5
shRNA
Vehicle
Dox (3 days)
REN.713
PRMT5.671
REN.713
PRMT5.671
REN.713
PRMT5.671
REN.713
PRMT5.671

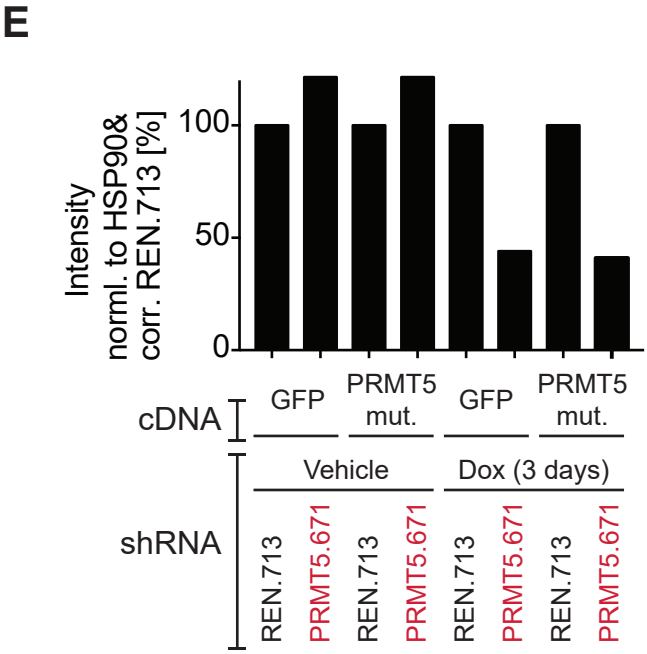

E
Intensity norml. to HSP90& corr. REN.713 [%]
100
50
0
cDNA
GFP
PRMT5 mut.
GFP
PRMT5 mut.
shRNA
Vehicle
Dox (3 days)
REN.713
PRMT5.671
REN.713
PRMT5.671
REN.713
PRMT5.671
REN.713
PRMT5.671

**Figure S2 (related to Figure 2). PRMT5 shRNAs inhibit PRMT5 function by causing a loss of PRMT5 mRNA and protein expression.**

(**A**) Immunoblot of U-87 MG cells treated either with 10 µM EPZ015666 over a period of 4 days or induced for shRNA-mediated knockdown of PRMT5 using doxycycline over 8 days. (**B**) qRT-PCR of PRMT5 knockdown in U-87 MG cells with three different inducible shRNAs or a Renilla control shRNA. n=3, error bars depict +/- SD. (**C**) Scheme outlining competition assay: starting with a mixed population of cells where approximately half contain the shRNA vector and express Venus, addition of doxycycline induces expression of shRNA and dsRed. Over time, shRNA expressing cells are either enriched, depleted, or undergo no change with respect to the starting population, depending on the identity of the shRNA. (**D**) Immunoblot quantifications of Fig. 2E. Expression levels of H4R3me2s following PRMT5 knockdown were normalized to both HSP90 and REN.713 controls. (**E**) Immunoblot quantifications of Fig. 2H. Expression levels of H4R3me2s following PRMT5 knockdown were normalized to both HSP90 and REN.713 controls.

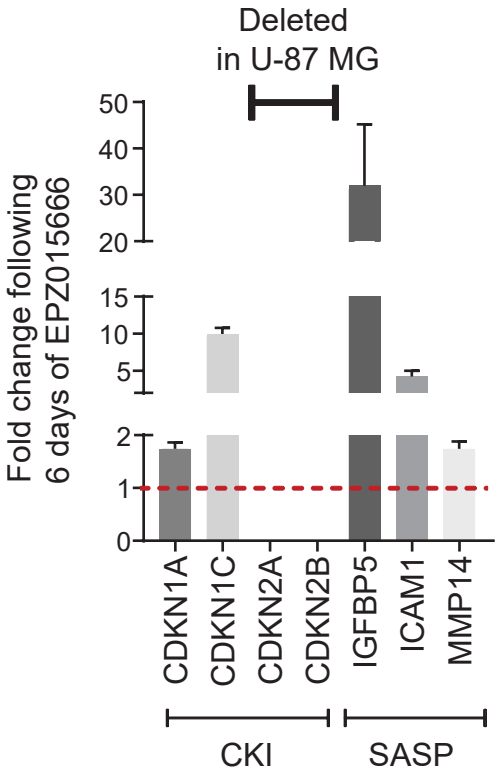
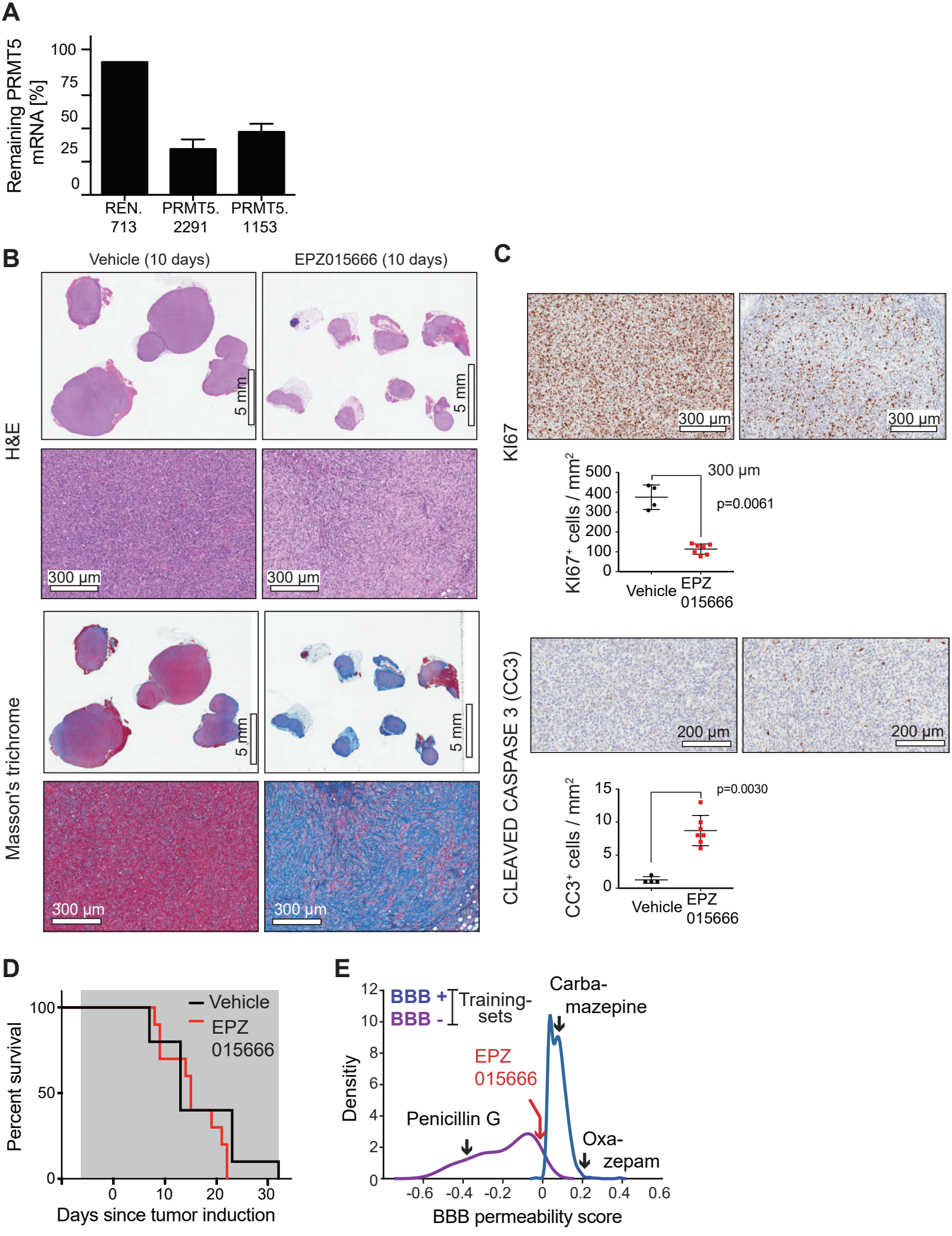
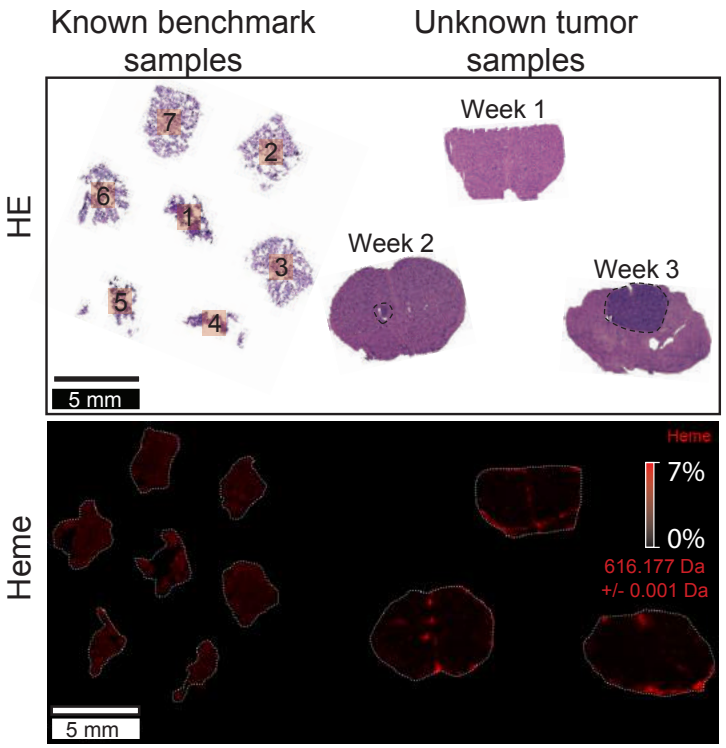

Deleted
in U-87 MG
Fold change following
6 days of EPZ015666
50
40
30
20
15
10
5
2
1
0
CDKN1A
CDKN1C
CDKN2A
CDKN2B
IGFBP5
ICAM1
MMP14
CKI
SASP

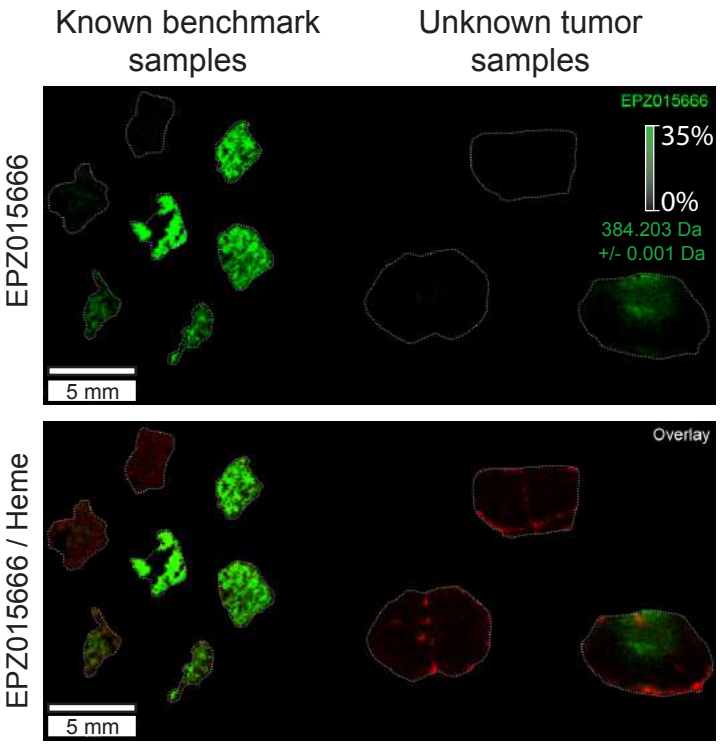
**Figure S3 (related to Figure 3). Tumor cells show increased expression levels of senescence associated transcripts following EPZ015666 treatment**

U-87 MG cells were treated with 10 µM EPZ015666 / vehicle for 96 hr followed by RNA extraction, reverse transcription and qRT-PCR. Expression levels following EPZ015666 were normalized to GAPDH as an internal control and the corresponding expression level of vehicle treated cells. n=3, error bars represent +/- SEM. CKI = Cyclin-dependent kinase inhibitor proteins. SASP = Senescence-Associated Secretory Phenotype.

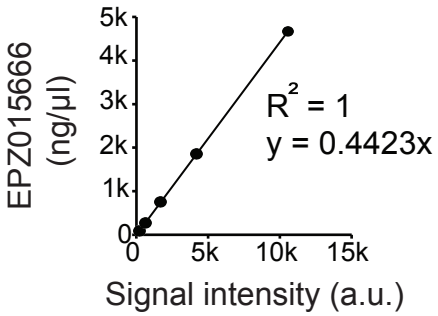

A
Remaining PRMT5 mRNA [%]
100
75
50
25
0
REN. 713
PRMT5. 2291
PRMT5. 1153
B
Vehicle (10 days)
EPZ015666 (10 days)
H&E
Masson's trichrome
5 mm
300 µm
C
KI67
CLEAVED CASPASE 3 (CC3)
300 µm
200 µm
KI67+ cells / mm2
CC3+ cells / mm2
p=0.0061
p=0.0030
Vehicle
EPZ 015666
D
Percent survival
Vehicle
EPZ 015666
Days since tumor induction
E
BBB +
BBB -
Training-sets
Carba-mazepine
EPZ 015666
Penicillin G
Oxa-zepam
Densitiy
BBB permeability score

F



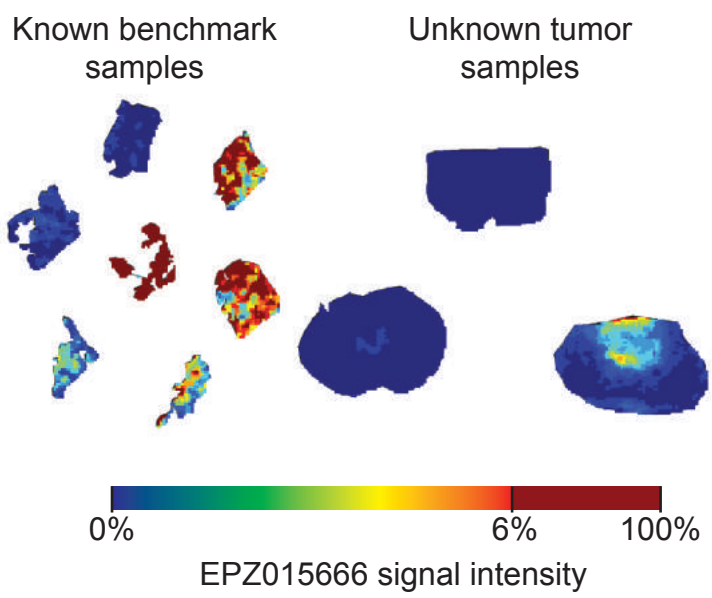

G



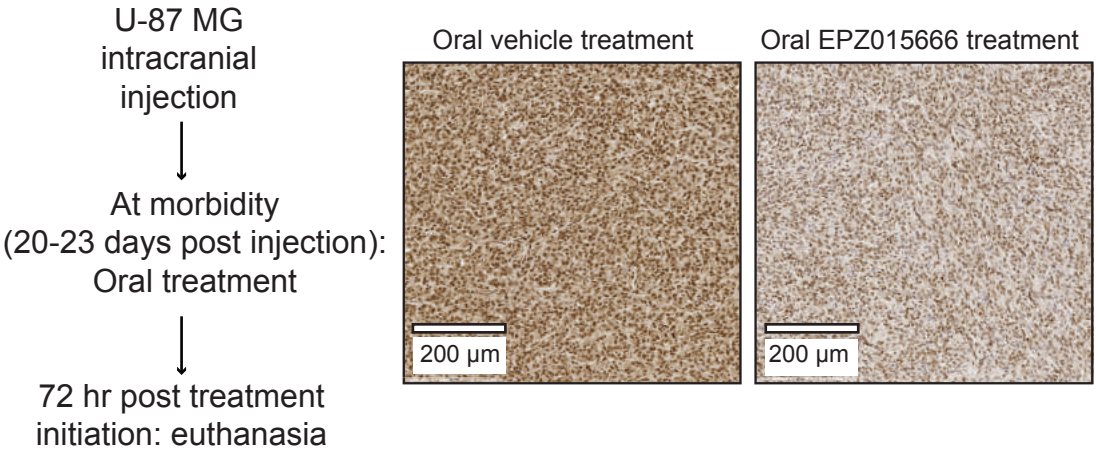

H

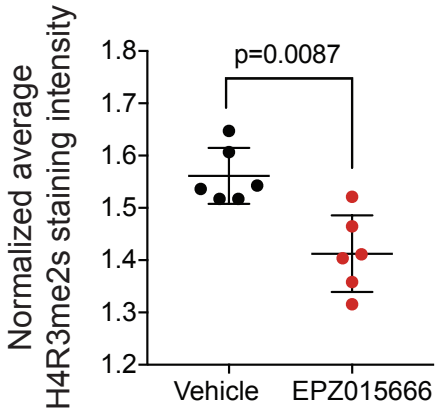
| | Signal itensity (a.u.) | EPZ015666 (ng/g) |
|---|---|---|
| Week 1 | 216.63 | 95.813 |
| Week 2 (all) | 602.83 | 266.63 |
| Week 2 (tumor only) | 1695.74 | 750.03 |
| Week 3 (all) | 4198.28 | 1856.9 |
| Week 3 (tumor only) | 10557.70 | 4669.7 |

I




J



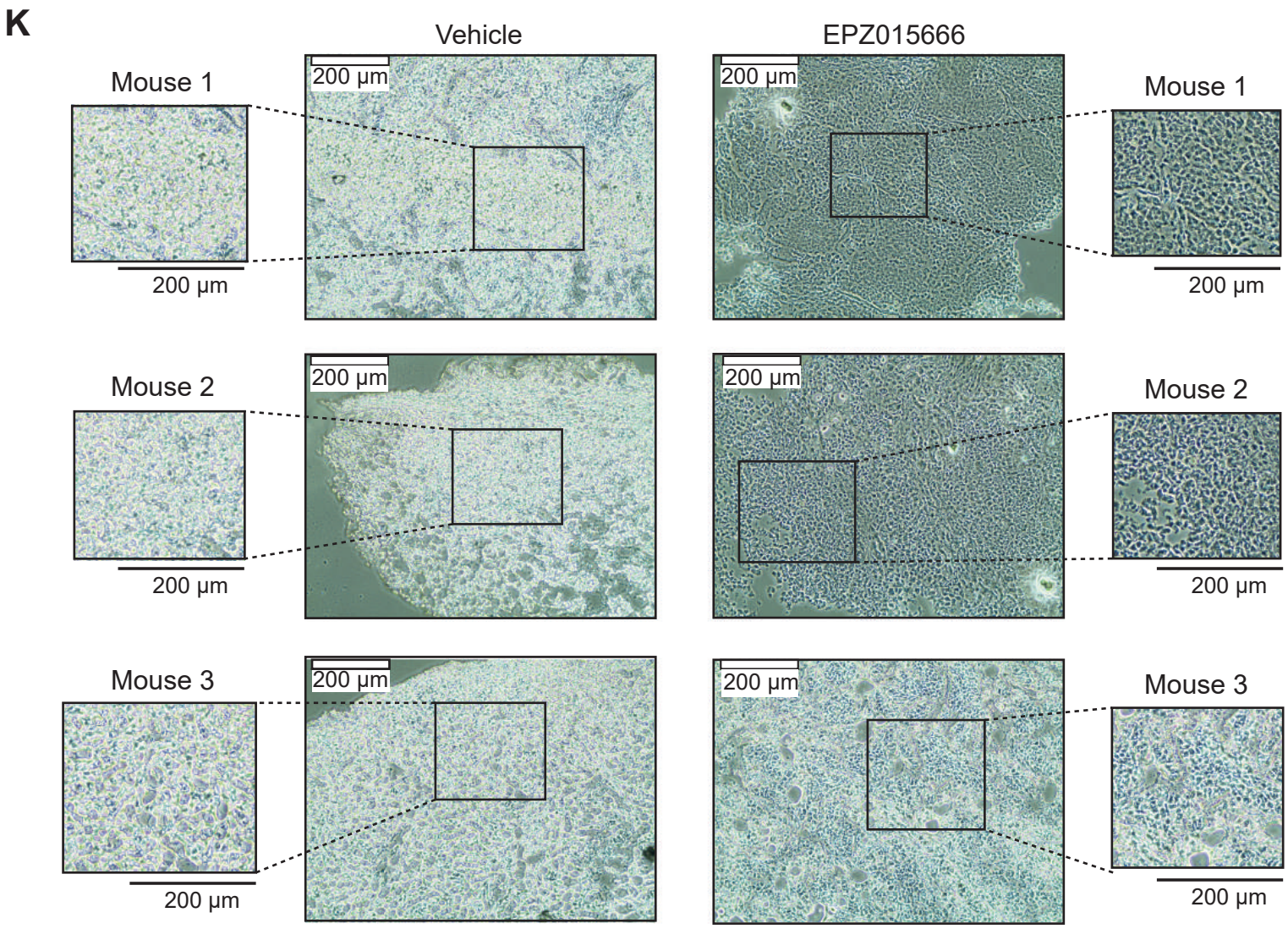

K
Vehicle
EPZ015666
Mouse 1
Mouse 2
Mouse 3
200 µm

**Figure S4 (related to Figure 4). Despite being a poor penetrator of the blood brain barrier, EPZ015666 reaches advanced brain tumors due to leaky tumor vasculature**

(**A**) qRT-PCR showing shRNA mediated knockdown of PRMT5 by two constitutive PRMT5 shRNAs and one control shRNA targeting Renilla luciferase in Gl261 cells. n=3, error bars represent +/- SEM. (**B**) H&E and Masson's trichrome staining of vehicle and EPZ015666 treated U-87 MG subcutaneous tumors. (**C**) KI67 or CC3 stains of subcutaneous U-87 MG tumors following EPZ015666 or vehicle treatment. Numbers of $KI67^+$ or $CC3^+$ cells were estimated in a blinded fashion and normalized to the counted areas. Bars represent mean and +/- SD. P values were calculated using Mann Whitney test. (**D**) Survival plot of C57BL/6J mice intracranially transplanted with Gl261 cells and treated with vehicle or EPZ015666 (100 mg/kg, twice per day, treatment period highlighted in grey) starting on day 6 and continuing until the mice were euthanized due to high tumor burden. n=10 for each group. (**E**) *In silico* assessment of blood brain barrier permeability of EPZ015666 compared to other compounds. (**F**) Tissue mimetic models ("known benchmark samples") and brains from mice 1, 2 and 3 weeks after tumor injection were subjected to MALDI-MSI. (**G**) Normalization regression of MALDI-MSI signal intensity and known EPZ015666 concentration of tissue mimetic models. (**H**) Calculation of EPZ015666 concentration in unknown samples. Signal intensity (a.u.) was determined by MALDI-MSI and EPZ015666 concentration (ng/g) was then calculated based on normalization regression of tissue mimetic models. (**I**) Pseudo-colored pictures demonstrating EPZ015666 signal intensity of both known benchmark-samples and unknown tumor samples. (**J**) U-87 MG tumor cells were transplanted intracranially into the brains of nude mice and treated with EPZ015666 or vehicle for 72 hr. Tumors were sectioned and stained for H4R3me2s. Depicted is the normalized average of H4R3me2s staining intensity. Bars represent mean and +/- SD. Mann Whitney test used for significance testing. (**K**) MGG8 PDX glioma tumor cells were transplanted subcutaneously and treated with EPZ015666 or vehicle starting at day 9 post tumor implantation. Upon euthanasia, tumors were flash frozen and stained for senescence-associated beta-galactosidase.

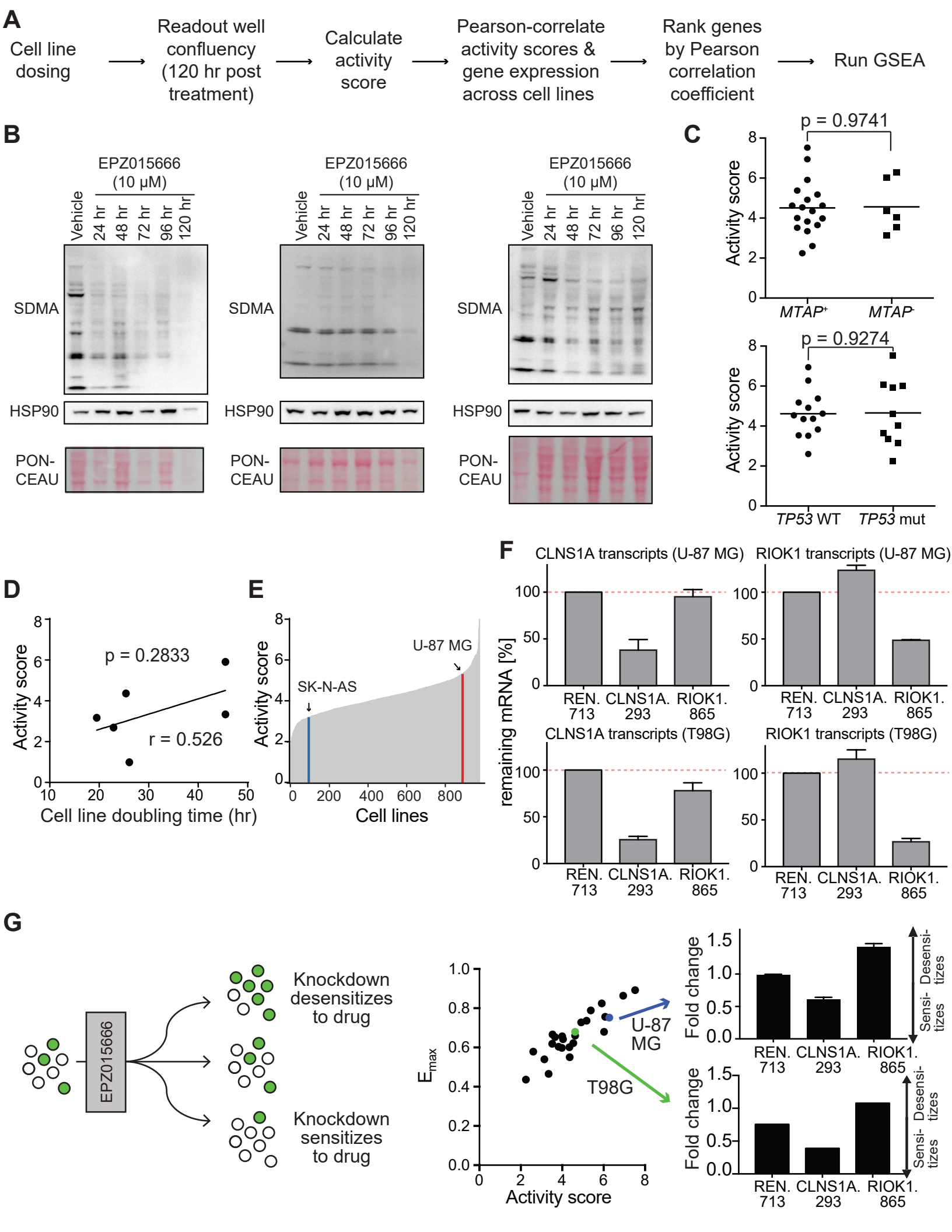
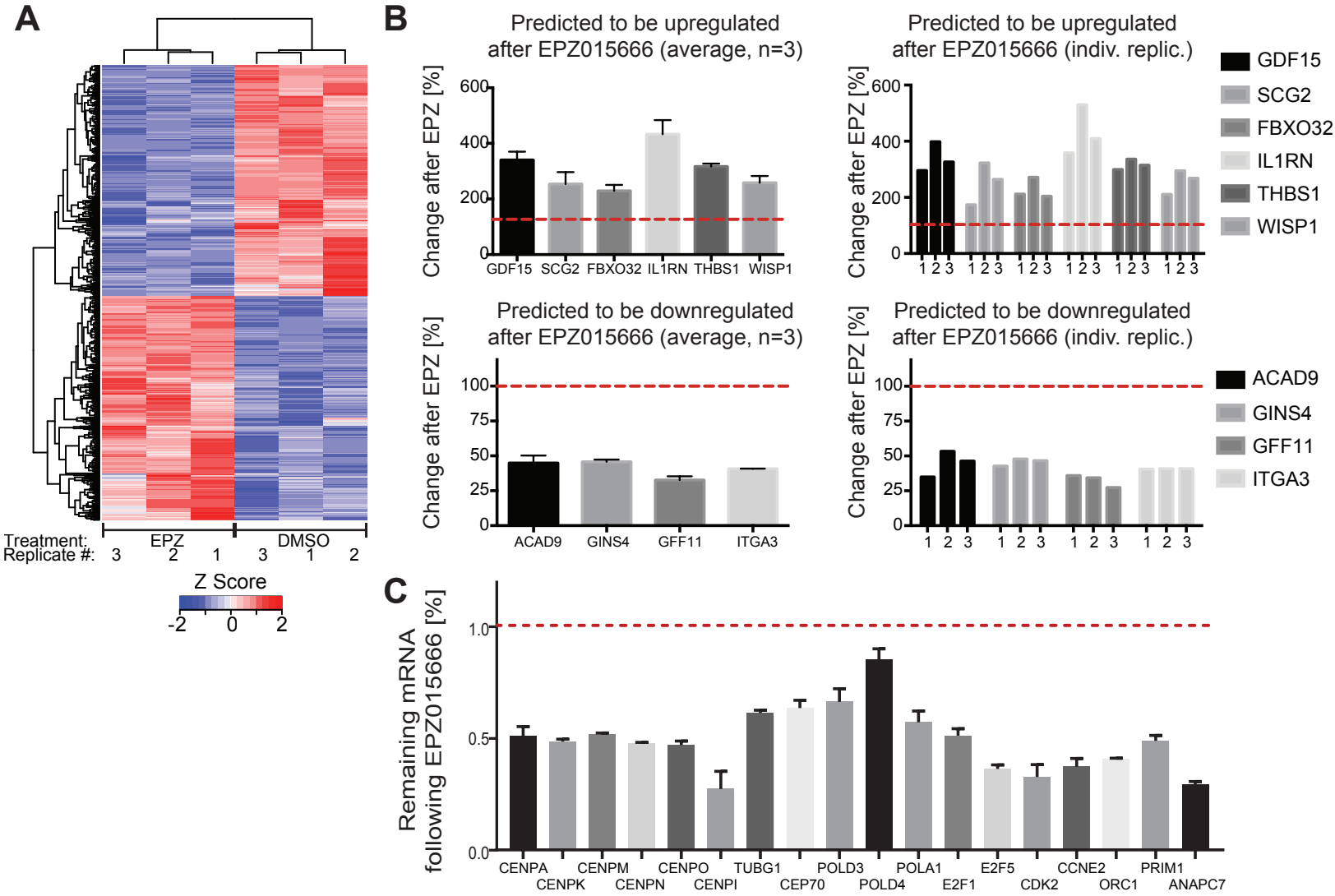

A
Cell line dosing
Readout well confluency (120 hr post treatment)
Calculate activity score
Pearson-correlate activity scores & gene expression across cell lines
Rank genes by Pearson correlation coefficient
Run GSEA
B
EPZ015666 (10 μM)
Vehicle
24 hr
48 hr
72 hr
96 hr
120 hr
SDMA
HSP90
PON-CEAU
C
p = 0.9741
Activity score
MTAP+
MTAP-
p = 0.9274
TP53 WT
TP53 mut
D
p = 0.2833
r = 0.526
Activity score
Cell line doubling time (hr)
E
SK-N-AS
U-87 MG
Activity score
Cell lines
F
CLNS1A transcripts (U-87 MG)
RIOK1 transcripts (U-87 MG)
CLNS1A transcripts (T98G)
RIOK1 transcripts (T98G)
remaining mRNA [%]
REN. 713
CLNS1A. 293
RIOK1. 865
G
EPZ015666
Knockdown desensitizes to drug
Knockdown sensitizes to drug
Emax
Activity score
U-87 MG
T98G
Fold change
Desensi-tizes
Sensi-tizes

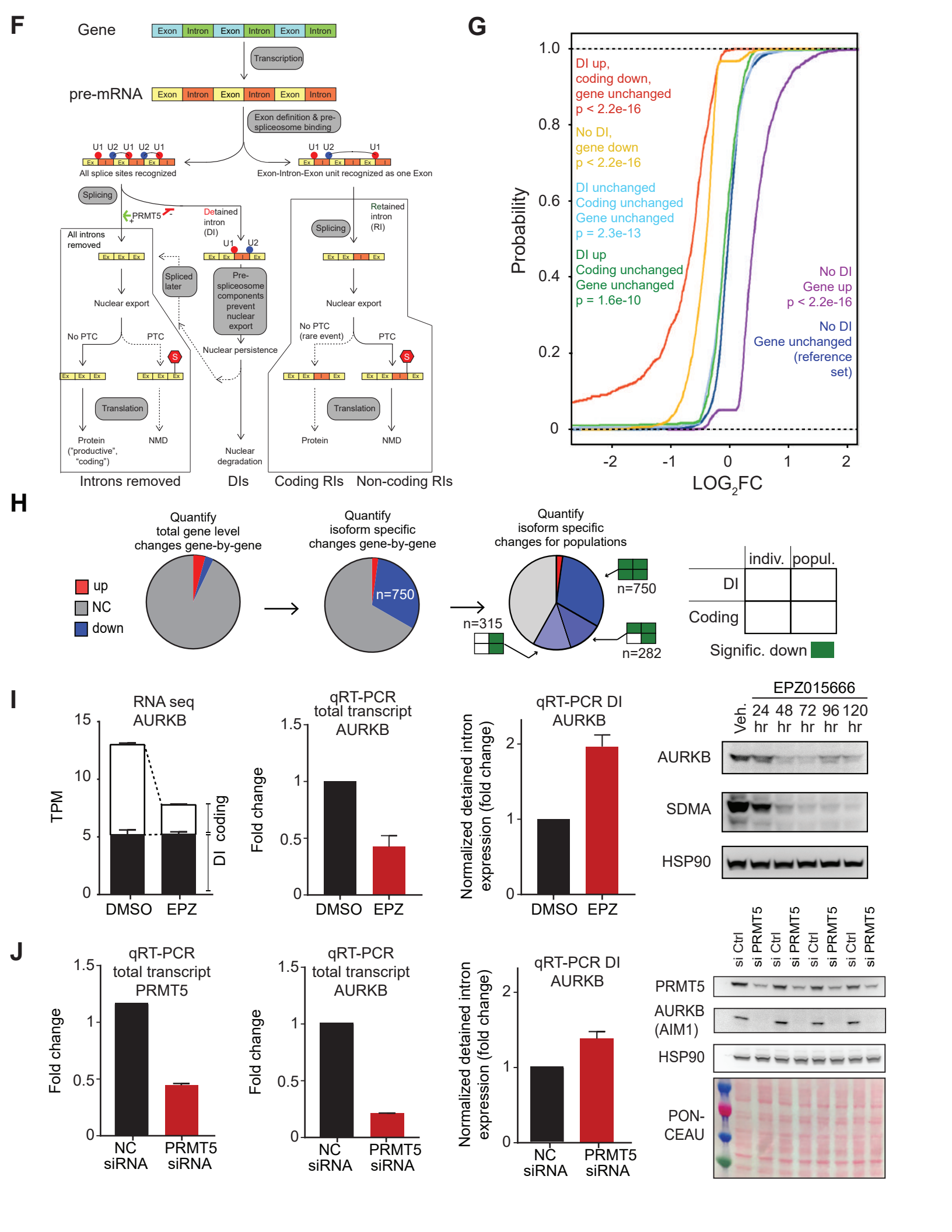
**Figure S5 (related to Figure 5). Whereas *MTAP*- and *TP53*- deletion status does not stratify cell line sensitivities to PRMT5 inhibition, the expression ratio of the two PRMT5 co-factors, CLNS1A and RIOK1, predicts EPZ015666 sensitivity of tumor cells**

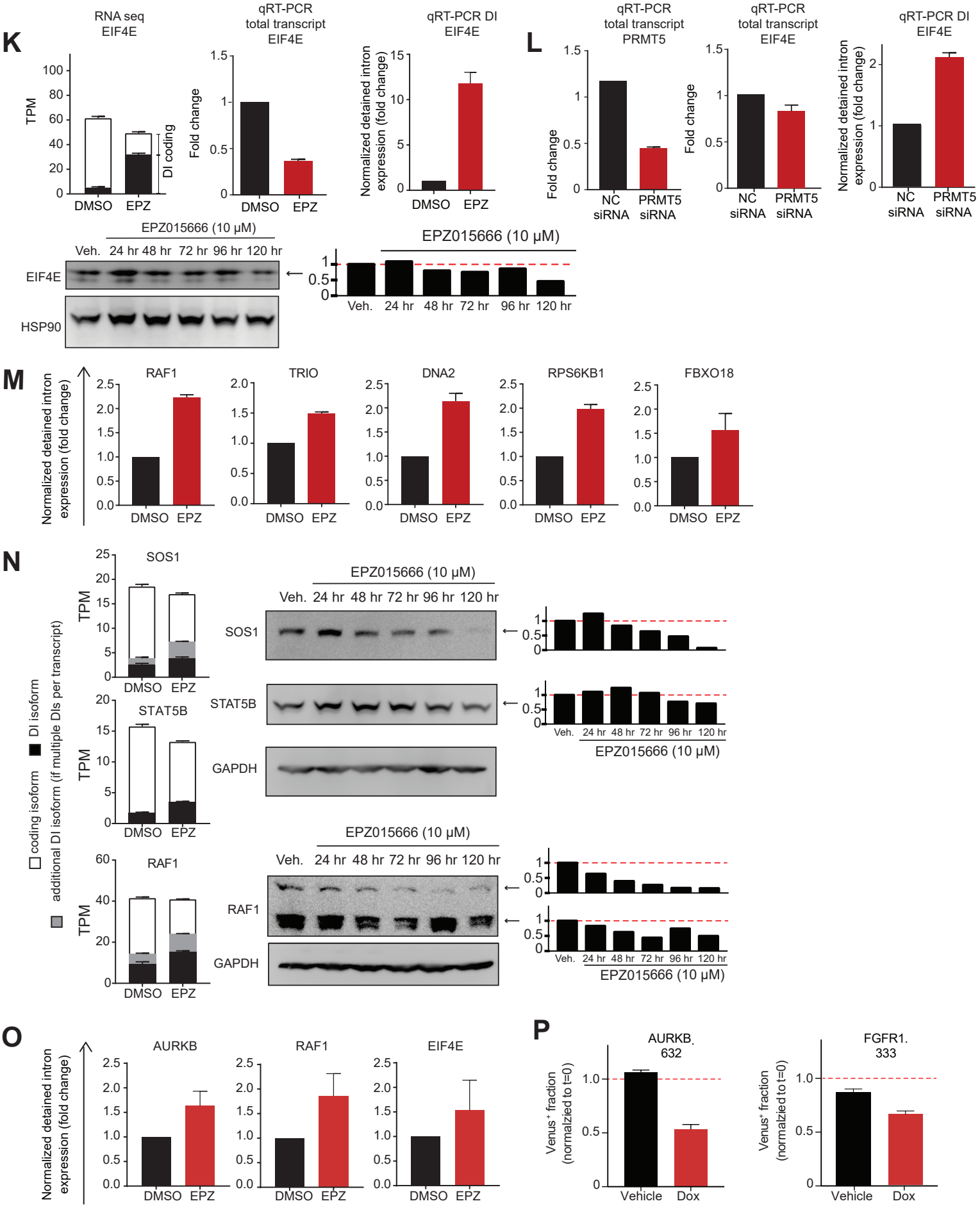
(**A**) Cell line screen schematic. (**B**) Immunoblot of SK-N-AS cells (left), SK-MEL-2 cells (middle) and PANC1 cells (right) treated with vehicle or 10 µM EPZ015666 over 120 hr. Symmetric dimethyl arginine = SDMA. (**C**) Activity scores of $MTAP^{+}$ and $MTAP^{-}$ cells (top) and activity scores of *TP53* wild-type and *TP53* mutant-cells (bottom). P value represents results of Mann-Whitney test. Horizontal lines represent mean. (**D**) EPZ015666 activity area and cell line doubling time of n=6 cell lines with linear regression analysis. (**E**) EPZ015666 activity area for >800 tumor cell lines were predicted based on publicly available CLNS1A and RIOK1 expression levels. (**F**) Confirmation of shRNA mediated knockdown of endogenous CLNS1A and RIOK1 mRNA by qRT-PCR in U-87 MG and T98G cells. n=3, error bars represent +/- SEM. (**G**) Starting with a mixed population, cells were treated with EPZ015666 (10 µM) for 6 days and analyzed by flow cytometry. Scheme outlining competition assay on the left, results of *in vitro* competition assays in U-87 MG and T98G cells on the right. n=3. Error bars represent +/- SEM.



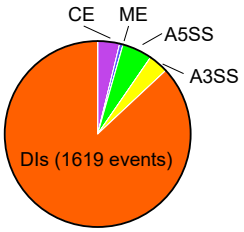
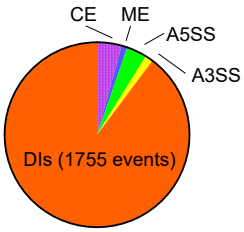
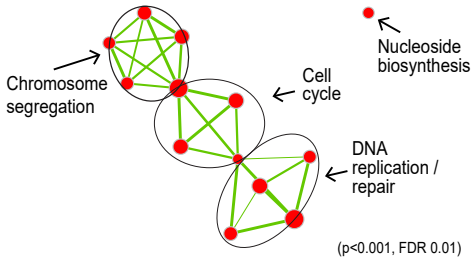
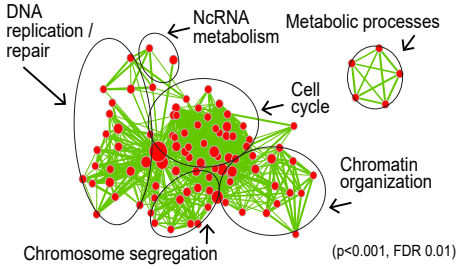
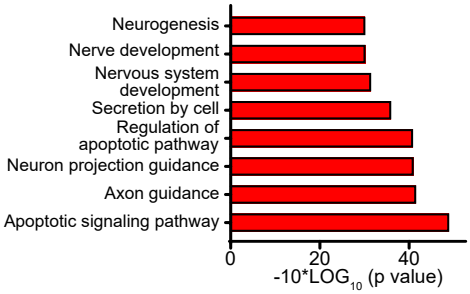
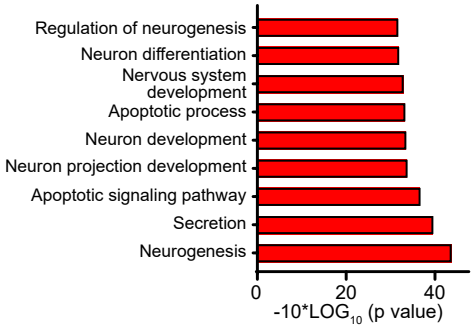
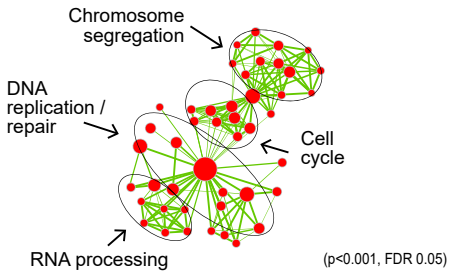
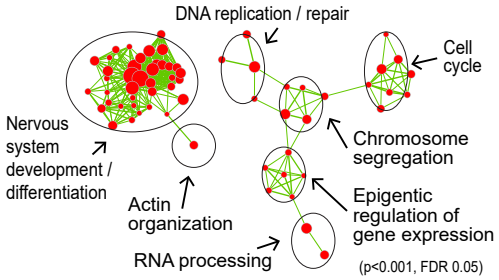

E

| | Retained Introns (RI) | Detained Introns (DI) |
|---|---|---|
| Splice site (SS) regulation | not recognized | recognized |
| Nuclear export | exported<br>translated | not exported<br>not translated |
| Nuclear degredation | not degraded | degraded |
| Translation outcome | Nonsense mediated decay (NMD) or coding sequence | not tranlated since nuclear detention<br>(if it were exported and translated, it would be NMD subtrate) |
| Bioinformatic distinction | coding RI - in frame coding sequence, no stop codon | internal intron that is predicted NMD substrate but is >=2x more abundant than expected, genome wide FDR <0.01 |

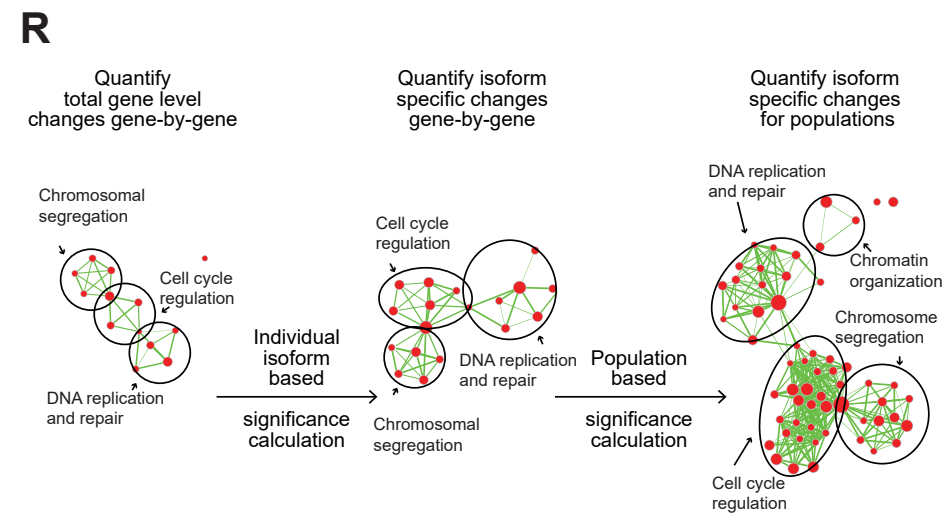

F
Gene
Exon
Intron
Transcription
pre-mRNA
Exon definition & pre-spliceosome binding
All splice sites recognized
Exon-Intron-Exon unit recognized as one Exon
Splicing
PRMT5
Detained intron (DI)
All introns removed
Spliced later
Pre-spliceosome components prevent nuclear export
Nuclear export
Nuclear persistence
No PTC
PTC
Translation
Protein ("productive", "coding")
NMD
Nuclear degradation
Retained intron (RI)
No PTC (rare event)
Introns removed
DIs
Coding RIs
Non-coding RIs
G
Probability
LOG2FC
DI up, coding down, gene unchanged p < 2.2e-16
No DI, gene down p < 2.2e-16
DI unchanged Coding unchanged Gene unchanged p = 2.3e-13
DI up Coding unchanged Gene unchanged p = 1.6e-10
No DI Gene up p < 2.2e-16
No DI Gene unchanged (reference set)
H
Quantify total gene level changes gene-by-gene
Quantify isoform specific changes gene-by-gene
Quantify isoform specific changes for populations
up
NC
down
n=750
n=315
n=282
indiv.
popul.
DI
Coding
Signific. down
I
RNA seq AURKB
TPM
DI coding
DMSO
EPZ
qRT-PCR total transcript AURKB
Fold change
qRT-PCR DI AURKB
Normalized detained intron expression (fold change)
EPZ015666
Veh.
24 hr
48 hr
72 hr
96 hr
120 hr
AURKB
SDMA
HSP90
J
qRT-PCR total transcript PRMT5
qRT-PCR total transcript AURKB
qRT-PCR DI AURKB
NC siRNA
PRMT5 siRNA
si Ctrl
si PRMT5
PRMT5
AURKB (AIM1)
HSP90
PON-CEAU


K
RNA seq
EIF4E
TPM
DI coding
DMSO EPZ
qRT-PCR
total transcript
EIF4E
Fold change
qRT-PCR DI
EIF4E
Normalized detained intron expression (fold change)
L
qRT-PCR
total transcript
PRMT5
Fold change
NC siRNA
PRMT5 siRNA
qRT-PCR
total transcript
EIF4E
qRT-PCR DI
EIF4E
EPZ015666 (10 μM)
Veh. 24 hr 48 hr 72 hr 96 hr 120 hr
EIF4E
HSP90
M
Normalized detained intron expression (fold change)
RAF1
TRIO
DNA2
RPS6KB1
FBXO18
N
SOS1
STAT5B
RAF1
GAPDH
TPM
coding isoform
DI isoform
additional DI isoform (if multiple DIs per transcript)
O
AURKB
RAF1
EIF4E
P
AURKB.
632
FGFR1.
333
Venus+ fraction
(normalzied to t=0)
Vehicle
Dox

Q

| | Pharmacological PRMT5 inhibition | Genetic *Prmt5* knockout |
|---|---|---|
| Significantly changed alternative splicing events |  <br>Total: 1862 significantly changed splicing events |  <br>Total: 1955 significantly changed splicing events |
| Directionality of DI splicing | less spliced (= "DI up"): 1557 DIs<br>more spliced (= "DI down"): 62 DIs | less spliced (= "DI up"): 1424 DIs<br>more spliced (= "DI down"): 331 DIs |
| Downregulated gene sets<br>(total transcript level, no DI correction) |  |  |
| Upregulated gene sets<br>(total transcript level, no DI correction, manual selection) |  |  |
| DI-regulated gene sets |  |  |

R




S

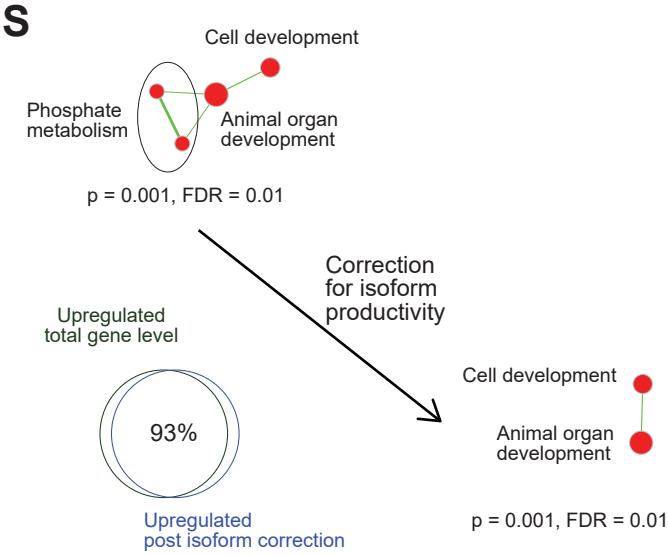
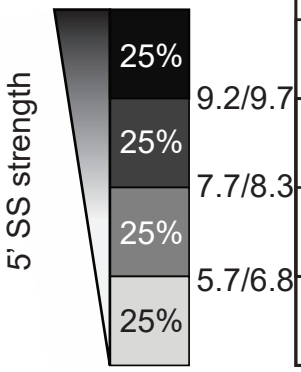


**Figure S6 (related to Figure 6). EPZ015666 treatment causes increase of intron detention and post-transcriptional inactivation of hundreds of proliferation-relevant genes**

(**A**) Unsupervised clustering of RNAseq samples. 928 genes with PPDE > 0.95 and $Log_2FC$ >= 0.5. (**B**) qRT-PCR based validation of RNAseq gene level changes, left graphs show mean and +/- SEM for three replicates, right graphs show individual replicate measurements. (**C**) qRT-PCR based validation of RNAseq gene level changes with focus on genes involved in cell cycle. n=2, error bars depict +/- SEM. (**D**) Change in percent spliced in (PSI) of the isoform shown at right between EPZ015666 treatment and control (CE, cassette exon; ME, mutually exclusive exon; A5SS and A3SS, alternative 5' and 3' splice sites, respectively; DI, detained intron). Vertical lines within boxes = median, edges of boxes = 1st and 4th quartiles, whiskers = min/max values, outliers (>/< 1.5 X interquartile range) excluded; table: number of significant events for each AS class, total number of events of each class identified, and the % of the events in each class affected by EPZ015666-treatment. (**E**) Table contrasting features of both DIs and RIs. (**F**) Model schematic contrasting detained introns (DIs) and retained introns (RIs). In brief, both DIs and RIs occur within otherwise fully spliced and polyadenylated mRNAs. Whereas DI-containing mRNAs are retained within the nucleus and either degraded there or spliced out later, RI-containing mRNAs are exported to the cytoplasm and either encode alternative peptides or are destroyed by NMD. (**G**) Cumulative distribution function (CDF) plot showing population-level shifts in expression of the coding isoform of each indicated group of genes as compared to genes with no DI that are unchanged upon EPZ treatment. Significance was determined by two-sided Kolmogorov-Smirnov (KS) tests. (**H**) Chart extending Fig 6D demonstrating the influence of an isoform-centric analysis for both high-stringency, individual isoform level significance and lower-stringency, population-based statistical analysis for gene expression profiles of 2408 $DI^+$ genes. (**I**) Estimation of AURKB gene expression changes in U-87 MG cells following EPZ015666 treatment *in vitro* using different techniques. RNAseq-based estimation of transcripts per million (TPM). Error bars represent +/- SEM (left). qRT-PCR of AURKB total transcript level. Error bars represent +/- SEM, n=2 (second to the left). qRT-PCR of AURKB DI transcripts normalized to total transcript levels, error bars represent +/- SEM, n=2 (third to the left). Immunoblot of AURKB, SDMA and HSP90 protein levels upon EPZ015666 treatment (rightmost). TPM graph is duplication of Fig. 6F, immunoblot is duplication of Fig. 6G. (**J**) Estimation of AURKB gene expression changes in U-87 MG cells following siRNA mediated knockdown of PRMT5. qRT-PCR of PRMT5 total transcript levels. Error bars represent +/- SEM, n=2 (left). qRT-PCR of AURKB total transcript level. Error bars represent +/- SEM, n=2 (second to the left). qRT-PCR of AURKB DI transcripts normalized to total transcript levels, error bars represent +/- SEM, n=2 (third to the left). Immunoblot of PRMT5, AURKB and HSP90 protein levels upon siRNAs-control or siRNA targeting PRMT5 (rightmost). (**K**) Estimation of EIF4E gene expression changes in U-87 MG cells following EPZ015666 treatment *in vitro* using different techniques. RNAseq-based estimation of transcripts per million (TPM). Error bars represent +/- SEM (left bar graph). qRT-PCR of EIF4E total transcript levels. Error bars represent +/- SEM, n=2 (middle bar graph). qRT-

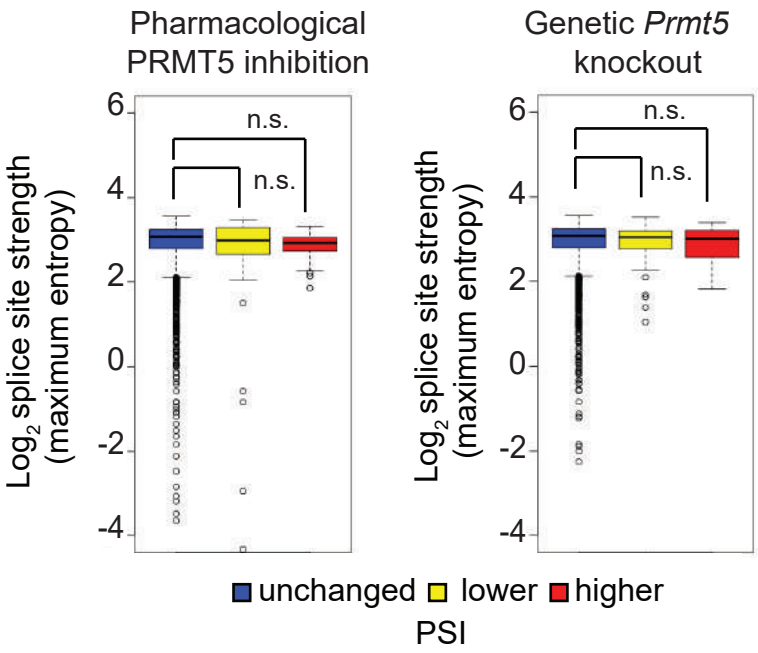
PCR of EIF4E DI transcripts normalized to total transcript levels, error bars represent +/- SEM, n=2 (right bar graph). Western blot of EIF4E and HSP90 protein levels upon EPZ015666 treatment with quantification of EIF4E protein levels normalized to HSP90 (bottom). (**L**) Estimation of EIF4E gene expression changes in U-87 MG cells following siRNA mediated knockdown of PRMT5. qRT-PCR of PRMT5 total transcript levels, n=2. Error bars represent +/- SEM (left, duplication of graph depicted in (Fig S6J)). qRT-PCR of EIF4E total transcript levels. Error bars represent +/- SEM, n=2 (middle). qRT-PCR of EIF4E DI transcripts normalized to total transcript levels, error bars represent +/- SEM, n=2 (right). (**M**) qRT-PCR of DI transcripts of different genes normalized to the corresponding total transcript levels following EPZ015666 treatment, error bars represent +/- SEM, n=2. (**N**) Transcript per million (TPM) plots and immunoblots of U-87 MG cells treated with 10 μM EPZ015666 for the indicated time points and quantification. In TPM-plots, white bar indicates coding isoform, black bar indicates isoform with one detained intron, grey bar indicates isoform with a different detained intron for genes with multiple detained introns. Error bars represent +/- SEM. Quantifications of the immunoblots normalized to control gene expressions are shown at right. (**O**) qRT-PCR of DI transcripts of different genes normalized to the corresponding total transcript levels in PDX *in vivo* tumors. Error bars represent +/- SEM. Mice had been treated with vehicle (n=2) or EPZ015666 (n=2) starting 9 days post tumor injection. Mice were euthanized upon morbidity (roughly three weeks post tumor injection). (**P**) *In vitro* competition assays of U-87 MG following knockdown of AURKB and FGFR1 (as estimated by flow cytometry 7 days following Dox-based shRNA induction, n=3, error bars represent +/- SEM). (**Q**) Detailed comparison of gene expression and splicing changes following either EPZ015666 treatment of U-87 MG cells for 72 hr (left column) or the genetic knockout of *Prmt5* in murine neuro-progenitor / stem cells (right column). Network maps display enriched gene sets in all genes down-regulated by PRMT5 inhibition / knockout versus all expressed genes (third row) and in DI-containing genes versus all expressed genes (fifth row). Each node represents a significantly enriched gene set, the size of which is proportional to the gene number and the width of the green edges indicating the degree of gene overlap between nodes. Clusters of functionally related gene sets are circled and labeled. (**R**) Network maps display enriched gene set in genes down-regulated post EPZ015666 treatment of U-87 MG cells versus all expressed in the absence of isoform correction (depicted left), following gene-by-gene isoform correction (more stringent, depicted in the middle) and following population based isoform correction (less stringent, depicted right), including both DI and non-DI containing genes. (**S**) Network maps display enriched gene set in genes up-regulated post EPZ015666 treatment of U-87 MG cells versus all expressed in the absence of isoform correction (left map) and following gene-by-gene isoform correction (right map), including both DI and non-DI containing genes. Venn diagram displays percentage of genes classified as up-regulated post EPZ015666 treatment in the absence of isoform correction (left circle) and following isoform correction (right circle).

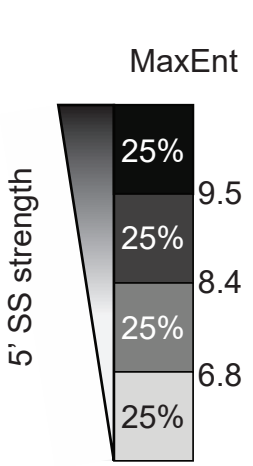
A




| Unchanged EPZ | Unchanged KO | Down EPZ | Down KO | Up EPZ | Up KO |
|---|---|---|---|---|---|
| 647 | 843 | 39 | 146 | 261 | 132 |
| 552 | 784 | 20 | 83 | 373 | 236 |
| 433 | 684 | 19 | 54 | 488 | 397 |
| 313 | 461 | 13 | 43 | 621 | 636 |

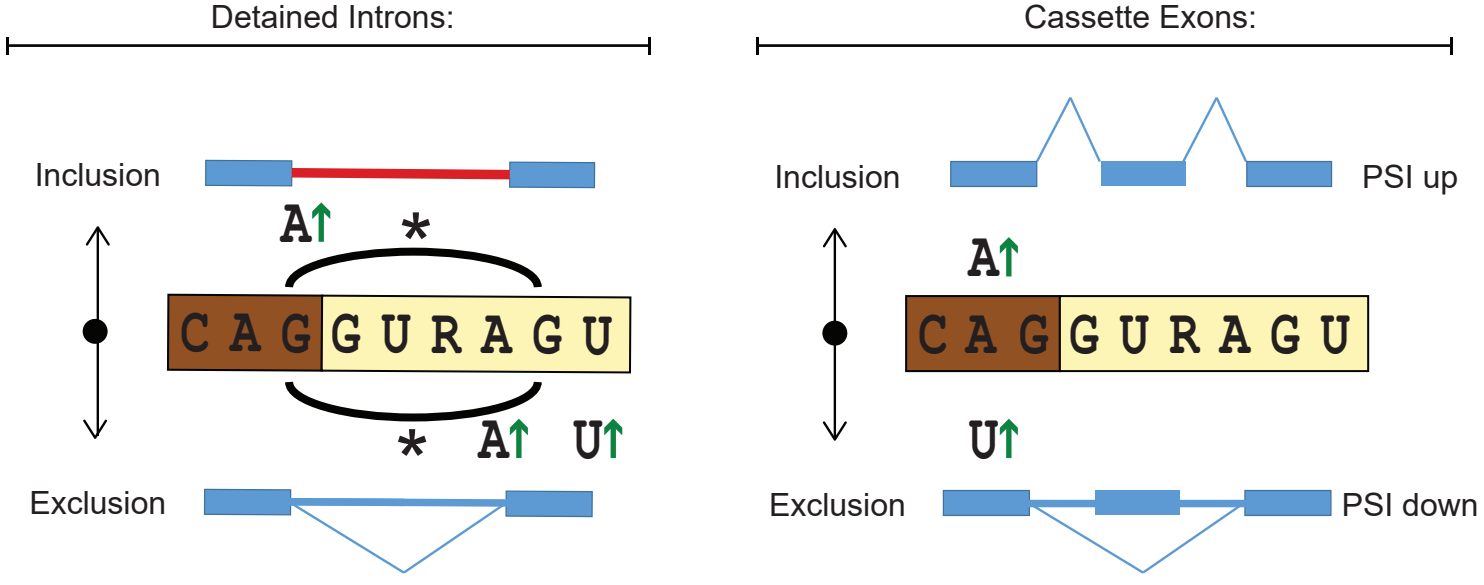
B




| Unchanged EPZ | Unchanged KO | Down EPZ | Down KO | Up EPZ | Up KO |
|---|---|---|---|---|---|
| 1369 | 1126 | 2 | 6 | 17 | 11 |
| 1330 | 1078 | 3 | 6 | 5 | 10 |
| 1362 | 1119 | 9 | 6 | 13 | 19 |
| 1357 | 1122 | 6 | 10 | 16 | 14 |

C



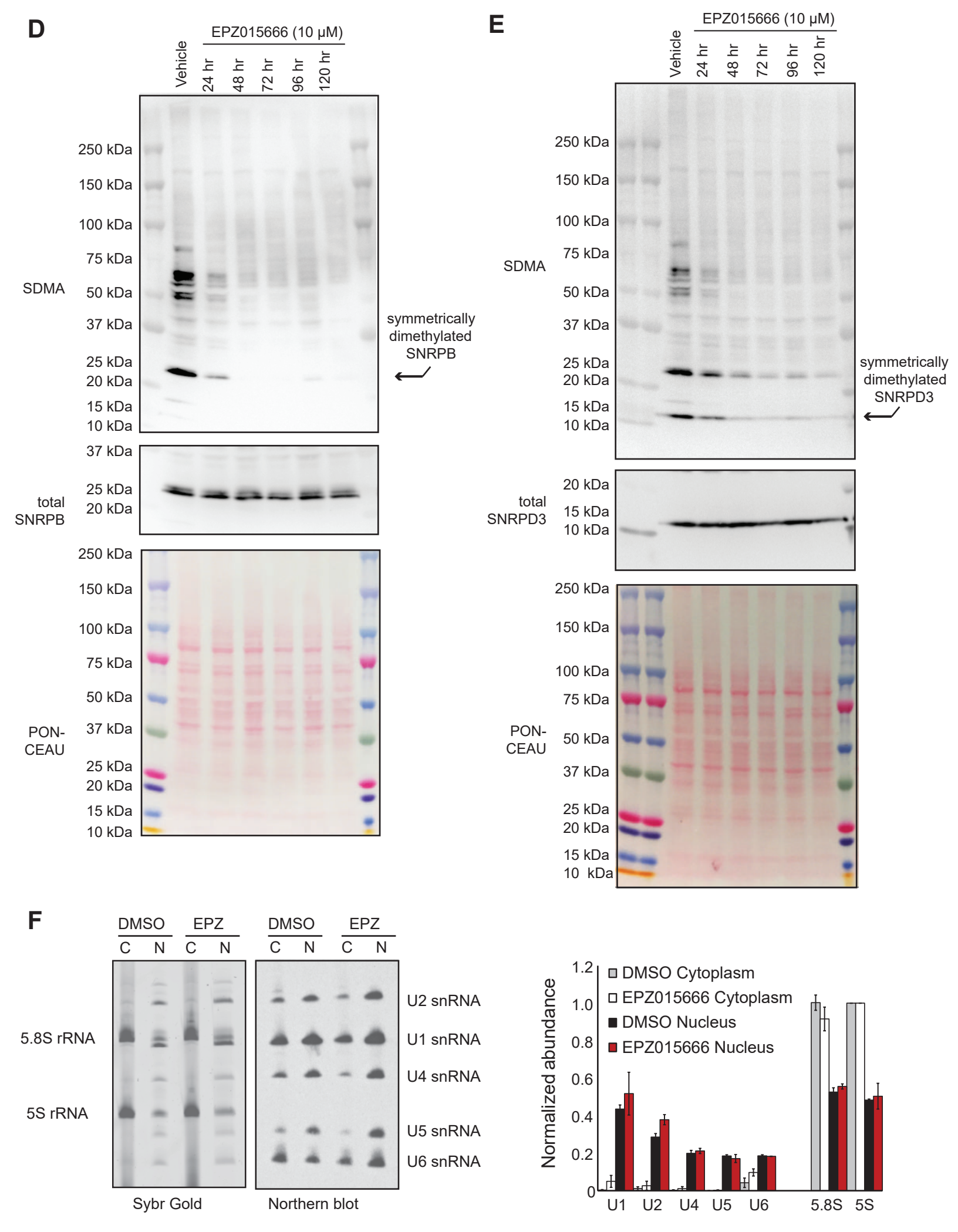

D
EPZ015666 (10 μM)
Vehicle
24 hr
48 hr
72 hr
96 hr
120 hr
250 kDa
150 kDa
100 kDa
75 kDa
SDMA
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
symmetrically dimethylated SNRPB
37 kDa
25 kDa
total SNRPB
20 kDa
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
PON-CEAU
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
E
EPZ015666 (10 μM)
Vehicle
24 hr
48 hr
72 hr
96 hr
120 hr
250 kDa
150 kDa
100 kDa
75 kDa
SDMA
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
symmetrically dimethylated SNRPD3
20 kDa
total SNRPD3
15 kDa
10 kDa
250 kDa
150 kDa
100 kDa
75 kDa
PON-CEAU
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
F
DMSO
EPZ
DMSO
EPZ
C
N
C
N
C
N
C
N
5.8S rRNA
5S rRNA
U2 snRNA
U1 snRNA
U4 snRNA
U5 snRNA
U6 snRNA
Sybr Gold
Northern blot
Normalized abundance
1.2
1.0
0.8
0.6
0.4
0.2
0
DMSO Cytoplasm
EPZ015666 Cytoplasm
DMSO Nucleus
EPZ015666 Nucleus
U1
U2
U4
U5
U6
5.8S
5S

**Figure S7 (related to Figure 7). 5' splice site strength is not the sole determinant of PRMT5-dependent splicing changes. SnRNPs lose symmetric arginine dimethylation marks upon PRMT5 inhibition but exhibit no gross changes in stability or subcellular localization.**

(**A**) The number of DIs falling within each quartile of relative 5' splice site strengths of all DIs are shown for the sets of DIs that are unchanged, downregulated or upregulated following EPZ015666 treatment of U-87 MG cells or *Prmt5* knockout in NPCs. 5' splice site strength scores were determined by MaxEnt; cutoff scores defining each quartile are shown for the EPZ015666 and *Prmt5* knockout sets. (**B**) Relative strengths of 5' and 3' splice sites of CEs (determined by MaxEnt) that are not significantly changed (blue), down-regulated (yellow), or up-regulated (red) by PRMT5 inactivation for both pharmacological PRMT5 inhibition in U-87 MG cells by EPZ015666 (left boxplot) and genetic knockout of *Prmt5* in NPCs (right boxplot). The number of cassette exons falling within each quartile of relative 5' splice site strengths of all cassette exons are shown for the sets of exons that are unchanged, down-regulated or up-regulated following EPZ015666 treatment of U-87 MG cells or *Prmt5* knockout in NPCs. 5' splice site strength scores were determined by MaxEnt; cutoff scores defining each quartile are shown and were identical between the EPZ015666 and *Prmt5* knockout sets. Horizontal lines within boxes = median, edges of boxes = 1st and 4th quartiles, whiskers = min/max values, outliers excluded; points = outliers >/< 1.5 X interquartile range (**C**) Schematic drawing illustrating the correlative impact of base exchanges in the 5' splice site on inclusion of either DIs (left) or CEs (right). Single bases below or above the consensus sequence indicate individual bases that are either correlated with increased inclusion (above the consensus splice site sequence) or exclusion (below consensus splice site sequence). * = For DIs, the presence of Gs in both positions -1 and +5 is highly correlated with DI exclusion, whereas the presence of a nucleotide other than G in either -1 or +5, or both, is highly correlated with DI inclusion. (**D**) Immunoblot of U-87 MG cells treated with EPZ015666 or vehicle for the indicated time points. Due to the lack of commercial availability of an antibody specifically binding dimethylated SNRPB, we visualized total SDMA, stripped the membrane and visualized total SNRPB allowing for size estimation. (**E**) Immunoblot of U-87 MG cells treated with EPZ015666 or vehicle for the indicated time points. Due to the lack of commercial availability of an antibody specifically binding dimethylated SNRPD3, we visualized total SDMA, stripped the membrane and visualized total SNRPD3 allowing for size estimation. (**F**) U-87 MG cells were treated with either EPZ015666 or vehicle for 72 hr followed by the separate extraction of nuclear and cytoplasmic RNA fractions. RNA from equal cell numbers was then separated by Polyacrylamide gel electrophoresis and either visualized by Sybr gold staining or transferred on a membrane for specific visualization of indicated snRNAs by northern blot. Abundance of RNA species was estimated and normalized to 5S rRNA / DMSO control across three replicates. Error bars represent +/- SEM.

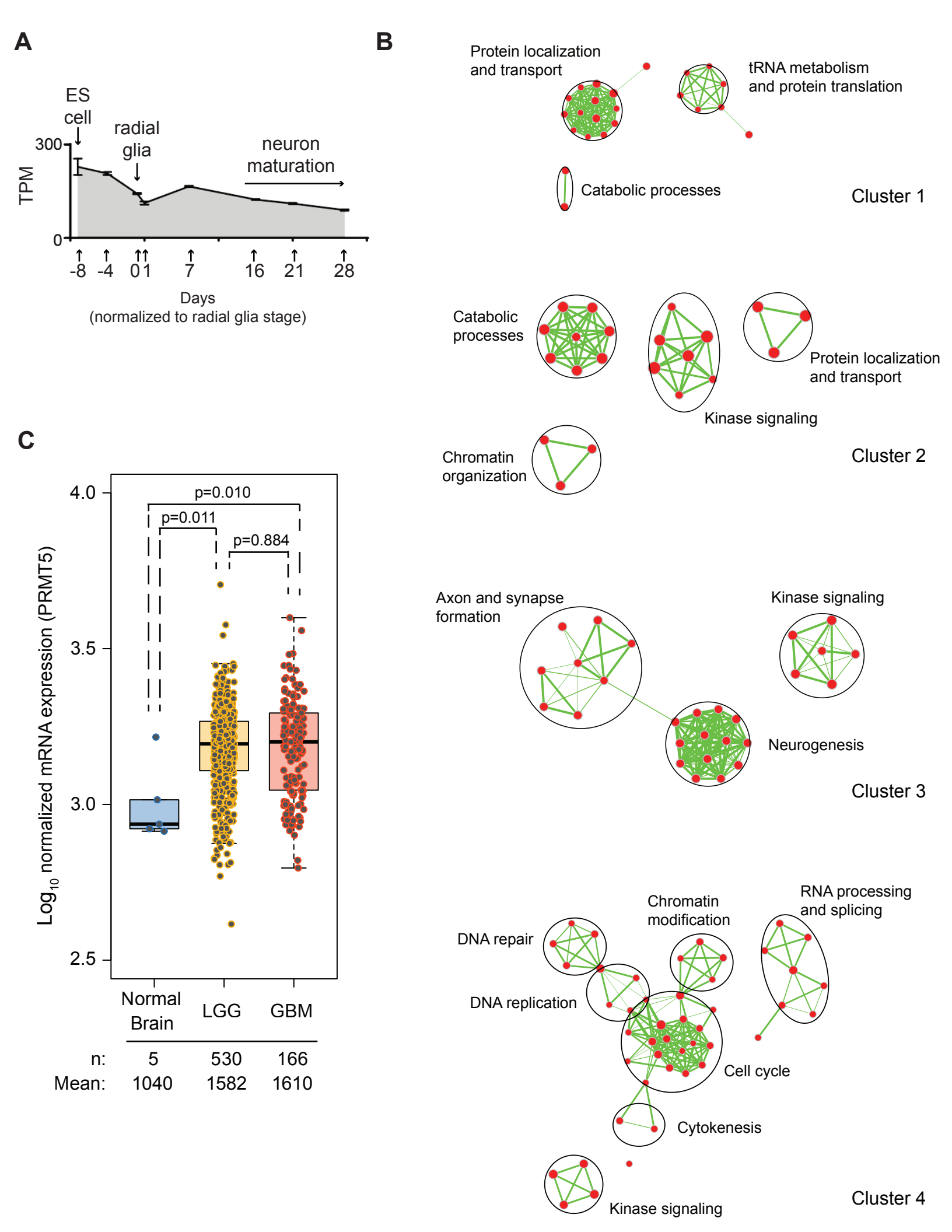

A
ES cell
radial glia
neuron maturation
300
TPM
0
-8
-4
01
7
16
21
28
Days
(normalized to radial glia stage)
B
Protein localization and transport
tRNA metabolism and protein translation
Catabolic processes
Cluster 1
Catabolic processes
Kinase signaling
Protein localization and transport
Chromatin organization
Cluster 2
Axon and synapse formation
Kinase signaling
Neurogenesis
Cluster 3
Chromatin modification
RNA processing and splicing
DNA repair
DNA replication
Cell cycle
Cytokenesis
Kinase signaling
Cluster 4
C
p=0.010
p=0.011
p=0.884
4.0
3.5
3.0
2.5
Log10 normalized mRNA expression (PRMT5)
Normal Brain
LGG
GBM
n:
5
530
166
Mean:
1040
1582
1610

**Figure S8 (related to Figure 8). Coordination of detained intron splicing contributes to gene expression regulation upon neural differentiation**

(**A**) PRMT5 Transcripts Per Kilobase Million (TPM) levels at different time points upon differentiation of ES cells into neurons. (**B**) Network maps display significantly enriched gene sets within the indicated neuro-differentiation gene cluster versus all expressed genes with functionally related gene sets circled and labeled. (**C**) PRMT5 mRNA expression levels in patients (LGG=Low grade glioma, GBM = glioblastoma multiforme). The Wilcoxon Rank-Sum test was employed to estimate significance. Horizontal lines within boxes = median, edges of boxes = 1st and 4th quartiles, whiskers = min/max values, outliers excluded; points = outliers >/< 1.5 X interquartile range.