SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 (A) qPCR analysis of PRMT1 expression in human OS infected with a non-silencing shRNA (shNS) or two independent shRNAs targeting PRMT1 (shPRMT1 #1 and #2). (B) Proliferation assessment of human OS infected with control shNS or shPRMT1 hairpins. TP53 status of the cell lines is indicated in the bracket.

Supplementary Figure 2 (A) Analysis of Prmt1 expression in Osx-GFP::Cre (OsxCre+) bone by IHC. Arrows point to OsxCre+ (GFP+) cells. (B) Representative microPET/CT images of 100-day-old $Prmt1^{wt}$, $Prmt1^{f/+}$, and $Prmt1^{f/f}$ OS mice. Arrows point to possible presence of tumors. MicroPET/CT imaging of OS was previously described in Walkley C.R. *et al.* (2008) Genes & Development. (C) PCR analysis of Prmt1 excision at loxP sites (red triangles) flanking Prmt1 exon 4 and 5 (yellow blocks) in tumors using primer set A and C (Top) and set A and B (Bottom). The expected sizes of the bands representing the wild-type, floxed, and excised alleles are indicated by the arrows. PCR analysis of the DNA samples from four independent tumors for each group (+/+, f/+, and f/f) is shown.

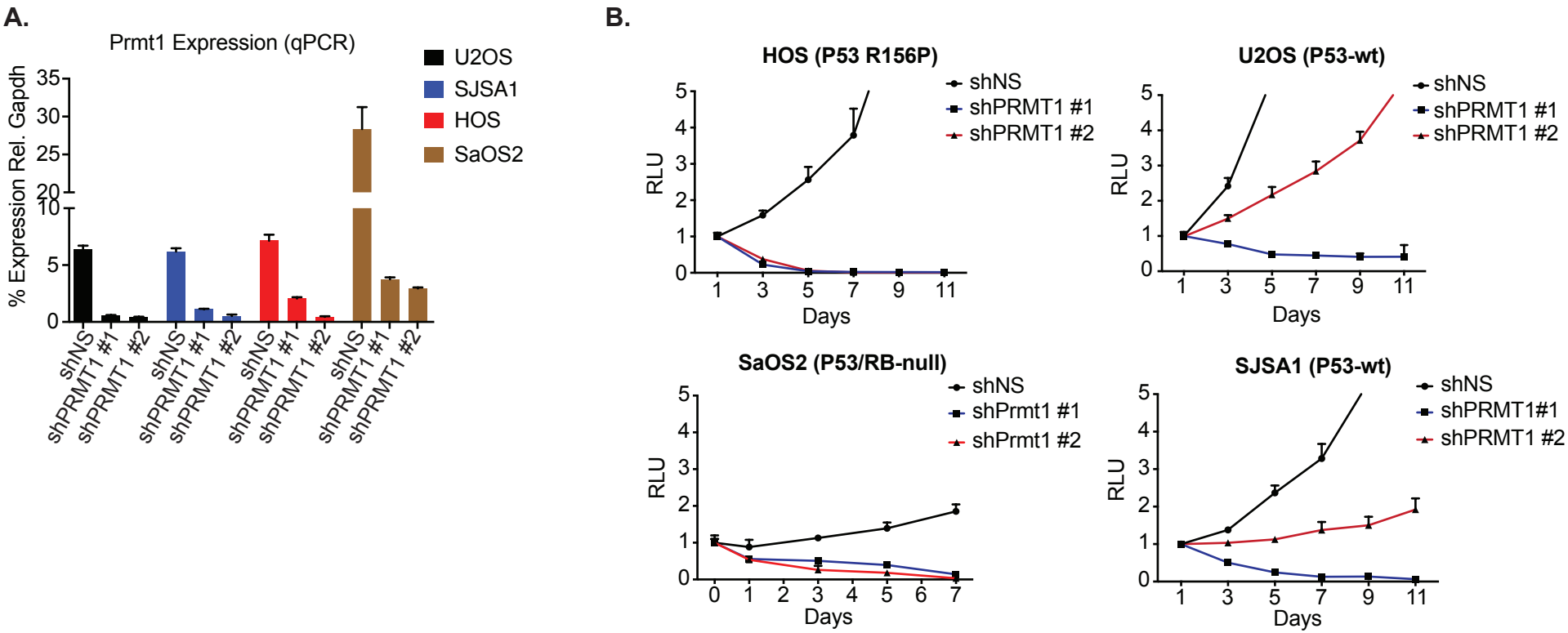
Supplementary Figure 3 (A) Quantification of several protein arginine methyltransferases following Prmt1 depletion in p53/Rb-null mOS. Log_2 ratios for six protein arginine methyltransferase detected in a SILAC-based proteomic screen. The red dotted lines indicate the location of the mean +/– 3 standard deviation measured across the entire proteome analysis. (B) GO term enrichment of putative Prmt1 substrates bearing monomethyl, asymmetric dimethyl and symmetric dimethyl arginine marks. “Regulation of translation” GO term is indicated in red.

Supplementary Figure 4 (A) Site validation for eIF4G1 singly mono-methylated on R689 and doubly mono-methylated on R689 and R698. MS2 spectrum acquired during LC-MS/MS analysis of digested eIF4G1 purified from p53/Rb-null mOS cells (top); MS2 spectrum acquired during infusion of synthetic peptide GPPR^{me}GGPGGELPR (bottom). (B) MS2 spectrum acquired during LC-MS/MS analysis of peptides enriched from p53/Rb-null mOS using anti mono-methyl antibody (top). MS2 spectrum acquired during infusion of synthetic peptide GPPR^{me}GGPGGELPR^{me}GPAGLGPR (bottom). (C) Average MS1 spectrum (m/z range: 419-429) acquired during LC-MS/MS analysis of digested eIF4G1 purified from control p53/Rb-null mOS cells ('heavy" channel, m/z 427.57) and Prmt1 depleted cells ("light" channel, m/z 420.90). (D) Assessment of *in vitro* Prmt1 methyltransferase activity toward eIF4G1 peptides (#1: GGPGGELPRGPAGLGPR; #2: GPPRGGPGGELPRGPAGLGPR; #3: GPP RGGPGGELPRGPAGLGPR) or H4 substrates (Signal Chem) using MTase-Glo^{TM} methyltransferase assay (Promega). Monomethylated R residues are underlined. (E) Western blot analysis of HA-eIF4G1 protein in p53/Rb-null cells over-expressing the wild-type or mutant (R689A) HA-eIF4G1 following cycloheximide treatment or DMSO control at the indicated time points. Gapdh serves as the loading control. "Fl" denotes full-length HA-eIF4G1, whereas "cl" indicates cleaved HA-eIF4G1 protein.

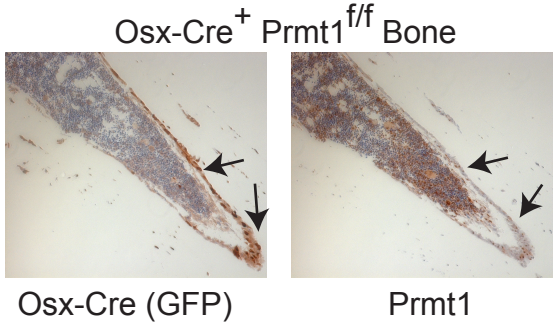
Supplementary Figure 5 (A) Volcano plot of differentially expressed transcripts in the control versus the conditional Prmt1 knockout identified by RNA-seq. Transcripts that show a statistically significant change upon Prmt1 depletion are indicated in red (fold change >1.5, $p<0.05$). (B) IGV Snapshot of the Prmt1 transcripts detected by RNA-seq mapped to the locus in the control as compared to Prmt1 conditional knockout. (C) Heatmap representation of differentially expressed transcripts associated with translation and p53 pathway in the control as compared to the conditional knockout. Three biological replicates for each condition are

shown. The dendrogram on top of the heatmap indicates that the replicates cluster with their respective group (CTRL versus KO). (D) Hierarchical clustering of “light” and “heavy” polysome associated control and Prmt1 conditional knockout samples based on whole genome RNA-seq profiles. (E) GO enrichment analysis of genes that were down regulated in the “heavy” polysomes in the Prmt1 conditional knockout as compared to the control. Translation-associated GO terms are indicated in red.

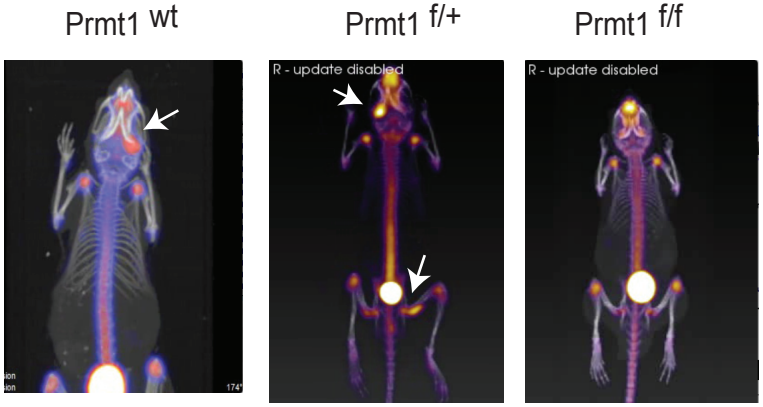
Supplementary Figure 6 Western blot analysis of Prmt1 and eIF4G1 protein expressions in p53/Rb-null mOS (482 and 493) and normal osteoblasts (MC3T3 subclones 4 and 30).


A.
Prmt1 Expression (qPCR)
% Expression Rel. Gapdh
U2OS
SJSA1
HOS
SaOS2
shNS
shPRMT1 #1
shPRMT1 #2
B.
HOS (P53 R156P)
U2OS (P53-wt)
SaOS2 (P53/RB-null)
SJSA1 (P53-wt)
RLU
Days
shNS
shPRMT1 #1
shPRMT1 #2
shPrmt1 #1
shPRMT1#1

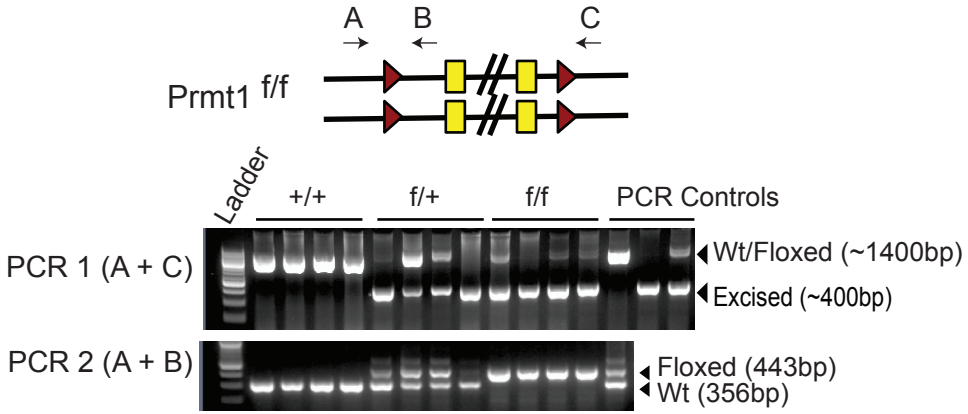
A.


Osx-Cre+ Prmt1f/f Bone
Osx-Cre (GFP)
Prmt1

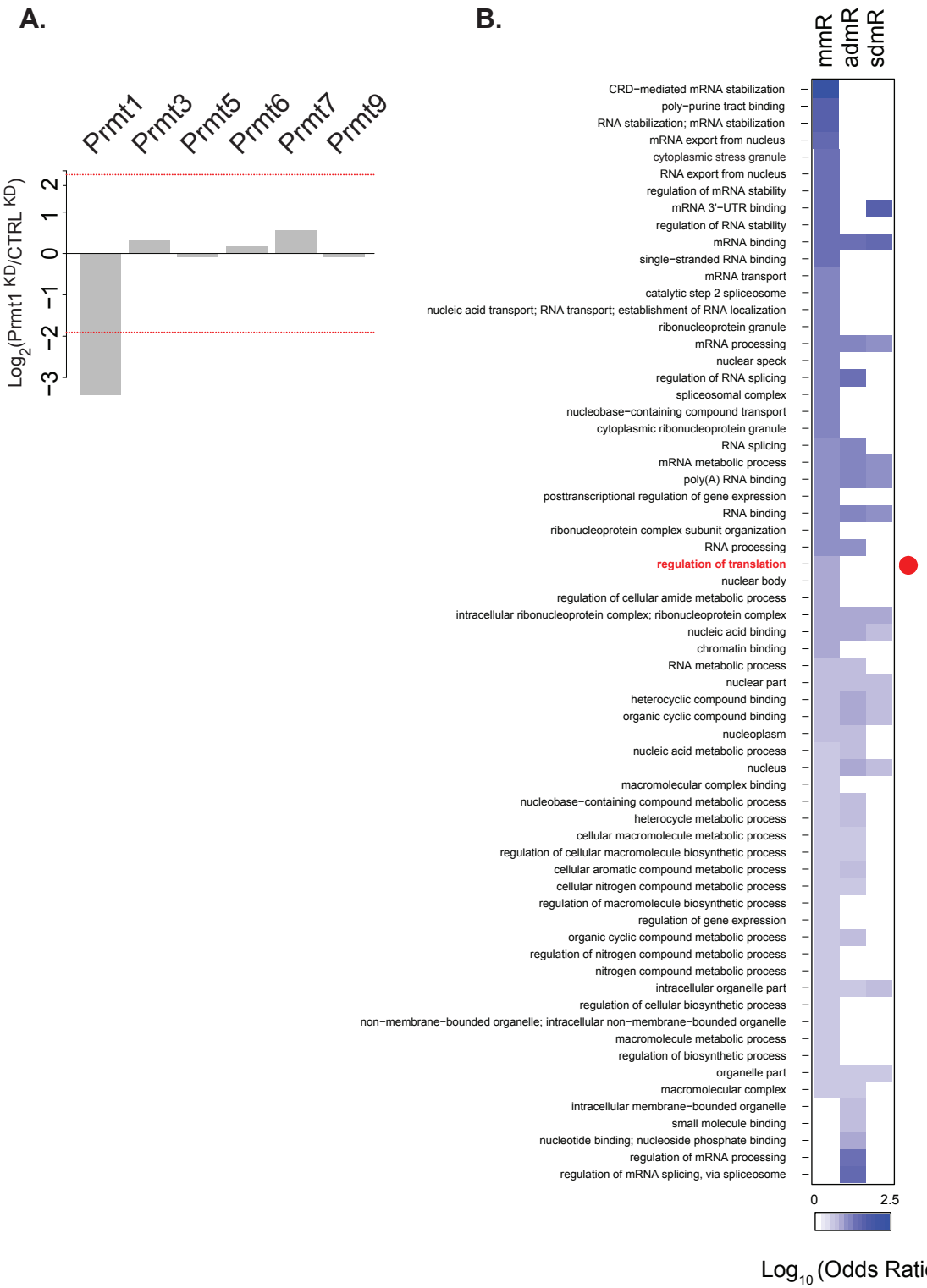
B.

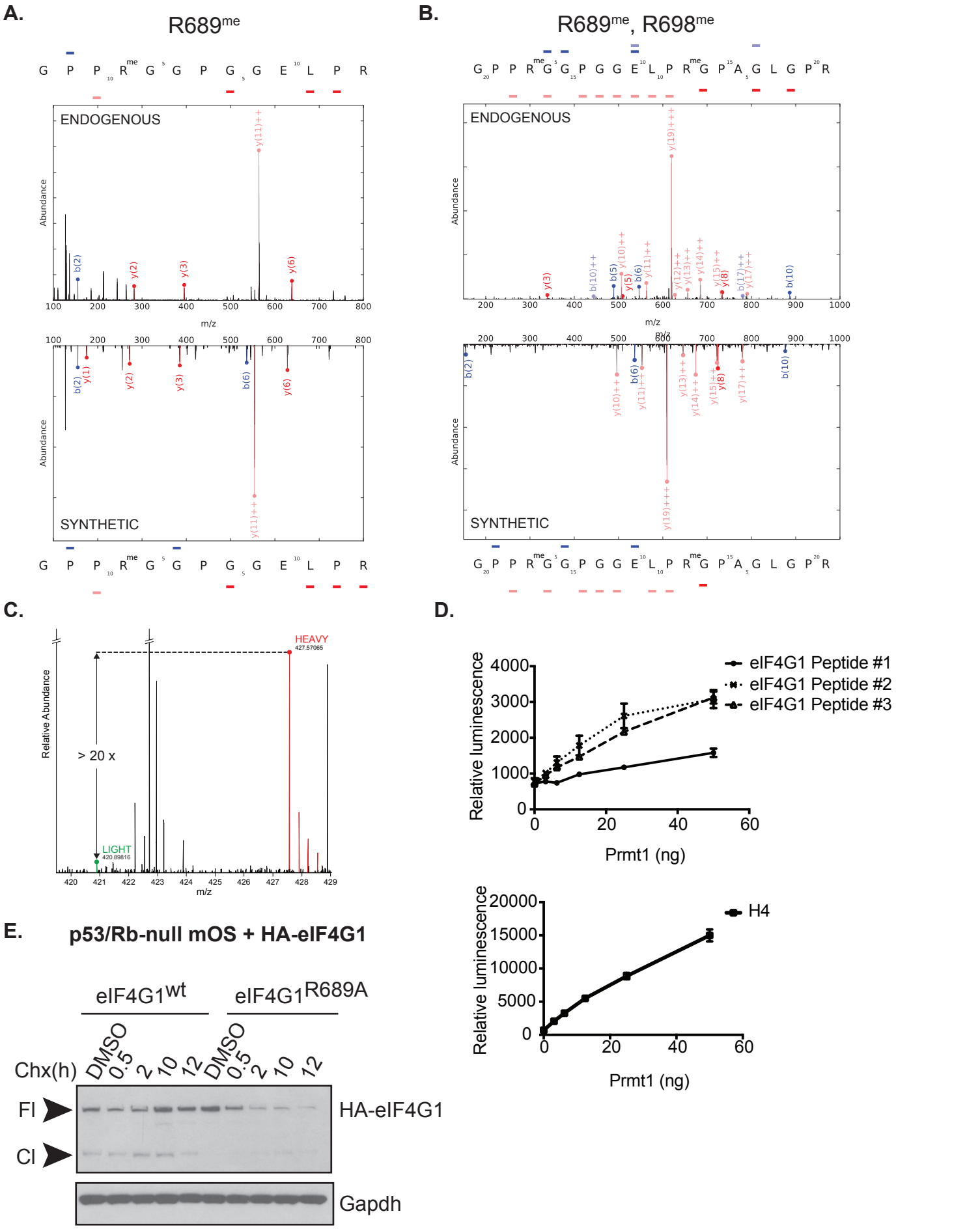

Prmt1 wt
Prmt1 f/+
Prmt1 f/f
R - update disabled
R - update disabled

C.

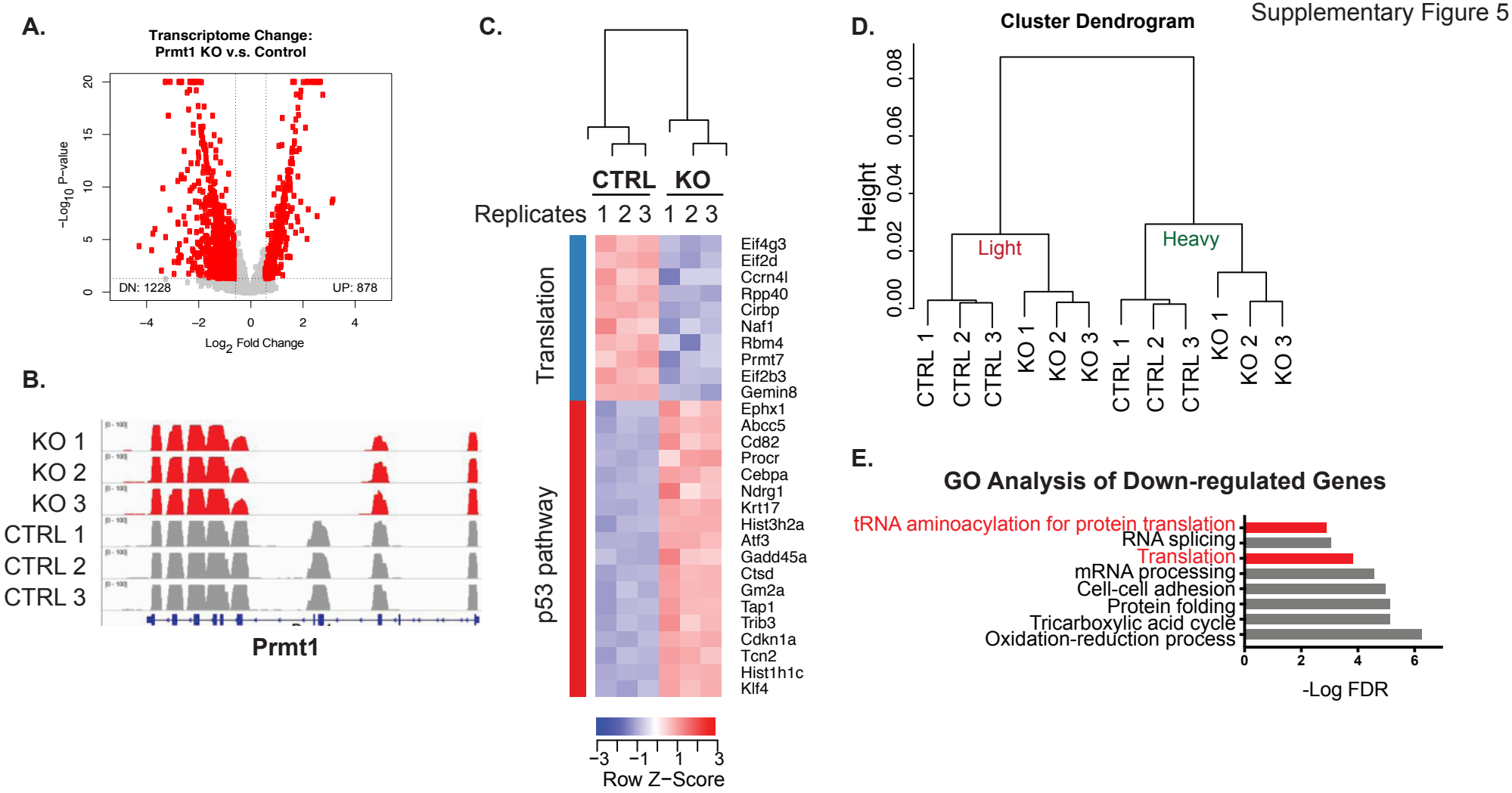

A
B
C
Prmt1 f/f
Ladder
+/+
f/+
f/f
PCR Controls
PCR 1 (A + C)
Wt/Floxed (~1400bp)
Excised (~400bp)
PCR 2 (A + B)
Floxed (443bp)
Wt (356bp)

Supplementary Figure 3




A.
R689me
ENDOGENOUS
SYNTHETIC
Abundance
m/z
B.
R689me, R698me
ENDOGENOUS
SYNTHETIC
C.
HEAVY
427.57065
LIGHT
420.89816
> 20 x
Relative Abundance
m/z
D.
eIF4G1 Peptide #1
eIF4G1 Peptide #2
eIF4G1 Peptide #3
Relative luminescence
Prmt1 (ng)
H4
E.
p53/Rb-null mOS + HA-eIF4G1
eIF4G1wt
eIF4G1R689A
Chx(h)
DMSO
0.5
2
10
12
Fl
Cl
HA-eIF4G1
Gapdh

Supplementary Figure 5



Supplementary Figure 6