Supplemental Figures and Legends

Enwerem et al.

Figure S1. Effect of depletion of CNOT2, 3, 9, 10, and 11 subunits on repression activity of PUM1 and PUM2. Plotted data, listed in **Table S1**, are graphed as mean log2 fold change values +/- SEM. The '*' symbols denote a p-value < 0.05 relative to the negative control (above x-axis) or between the indicated RNAi conditions (below the x-axis). n=9.

- **A.** Western blot of PUM1&2, CNOT2, or CNOT3 confirming RNAi depletion in HCT116 cells. GAPDH served as loading control. NTC: non-targeting control siRNA.
- **B.** Effect of depletion of CNOT2, CNOT3, or PUM1&2 on repression of Nluc 3xPRE, relative to the Nluc 3xPRE mt reporter. NTC RNAi serves as a control.
- **C.** Western blot of PUM1&2 or CNOT9 confirming RNAi depletion in HCT116 cells.
- **D.** Effect of depletion of CNOT9 or PUM1&2 on repression of Nluc 3xPRE, relative to the Nluc 3xPRE mt reporter. NTC RNAi serves as a control. n=9.
- **E.** Western blot of PUM1&2, CNOT10, or CNOT11 confirming RNAi depletion in HCT116 cells.
- **F.** Effect of depletion of CNOT10, CNOT11, or PUM1&2 on repression of Nluc 3xPRE, relative to the Nluc 3xPRE mt reporter. NTC RNAi serves as a control. n=9.

Supplemental Figure S1. Enwerem et al.

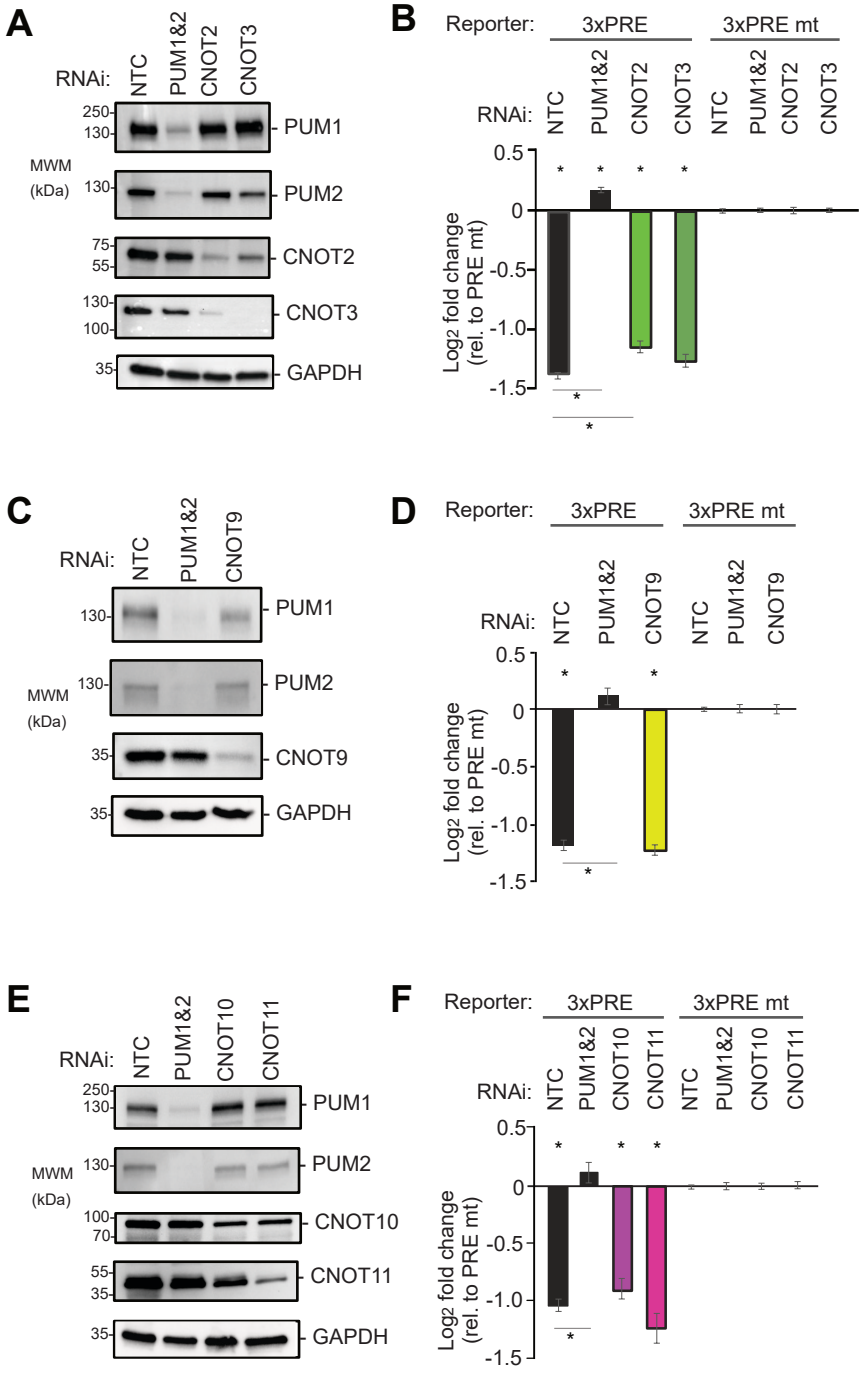

Figure S2. Comparisons of PUM1 and PUM2 effects on the transcriptome.

Panels A-C. Volcano plots of statistical significance (adjusted p-value) versus mean log2 fold change of gene expression measured by PAC-Seq in response to RNAi of PUM1, PUM2, or both proteins. Fold change values were calculated relative to the NTC condition. The number of up- or down-regulated genes (≥1.3 fold or greater with adjusted p-value of 0.05) are reported in the inset box. Complete PAC-Seq datasets are reported in **Table S2**. **A**. PUM1 RNAi; **B.** PUM2 RNAi; **C.** PUM1&2 RNAi.

Panels E-G. Venn diagrams of overlapping gene sets that were significantly regulated in the indicated RNAi conditions. For significance calling, the '*' symbol for each comparison denotes a p-value of less than 0.000001 (hypergeometric test for multi-set overlap). The '**' symbol denotes a p-value of less than 0.00000025. Gene sets and the statistics of the overlapping datasets are reported in **Table S3**.

E. Venn diagram comparing genes that were upregulated by depletion of PUM1&2 to those that contain a PRE sequence (PRE) and to genes whose mRNAs were shown to be bound by PUM1 or PUM2 (Bound).

F. Venn diagram comparing genes that were downregulated in response to depletion of PUM1&2 to PRE and Bound gene sets.

G. Venn diagram comparing genes that were mutually upregulated by depletion of PUM1&2 and CNOT1 and CNOT8 to PRE and Bound gene sets.

Supplemental Figure S2. Enwerem et al.

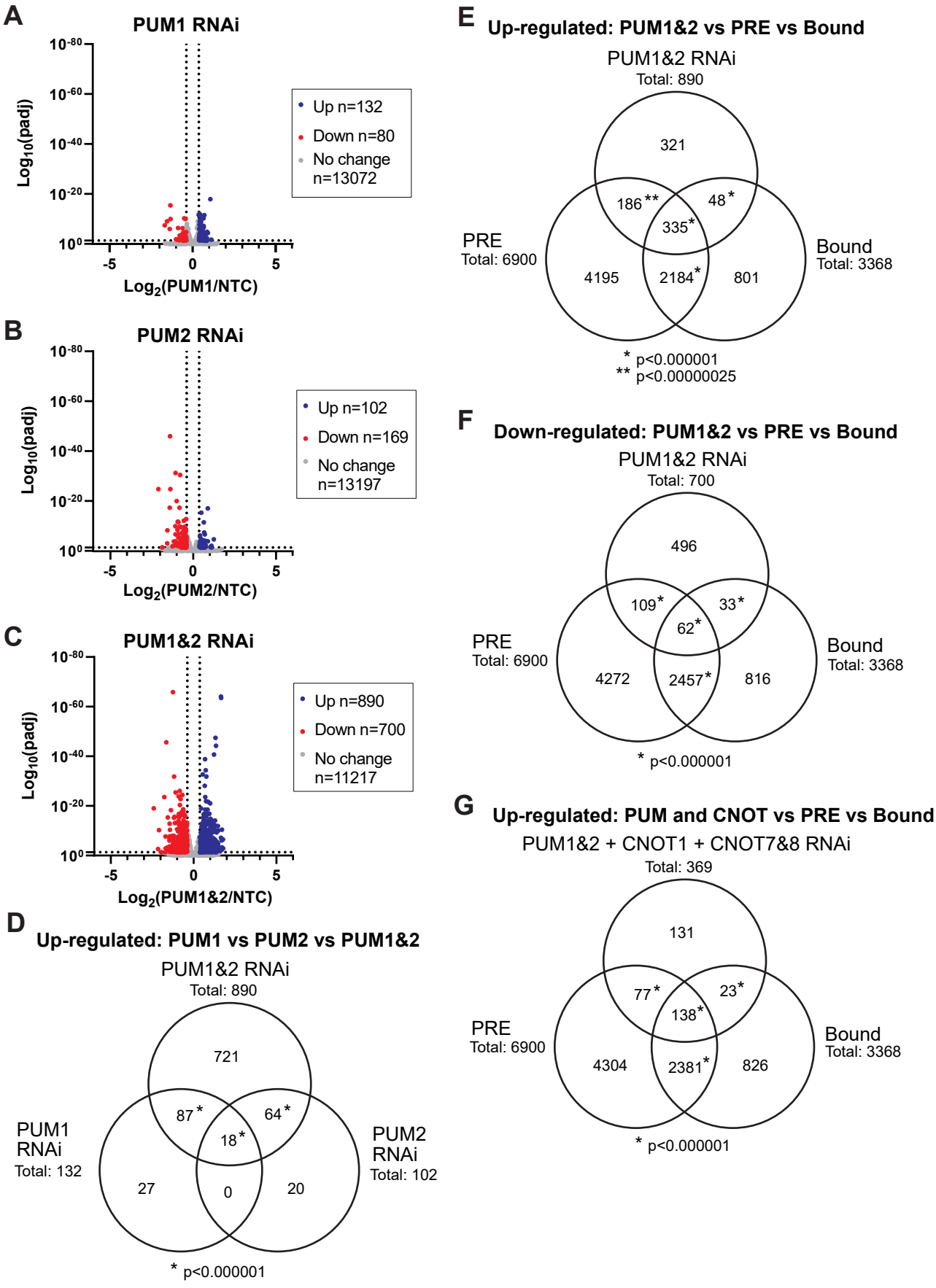

Figure S3. The putative cap-binding motif is not necessary for repression activity of human PUM1 and PUM2. Plotted data, listed in **Table S1**, are graphed as mean log2 fold change values +/- SEM relative to HT negative control. The '*' symbols denote a p-value < 0.05 relative to the negative control (above x-axis) or between the indicated proteins (below the x-axis). n=9.

A. The altered specificity reporter, Nluc 3xPRE UGG, was used to measure the repressive activity of wild type or mutant PUM proteins, wherein the putative cap binding residue was mutated (PUM1 R6as W466G and PUM2 R6as W350G). n=9.

B. Western blot of HT-tagged effector proteins from a representative experimental replicate from panel A. Tubulin was detected as a loading control.

Supplemental Figure S3. Enwerem et al.

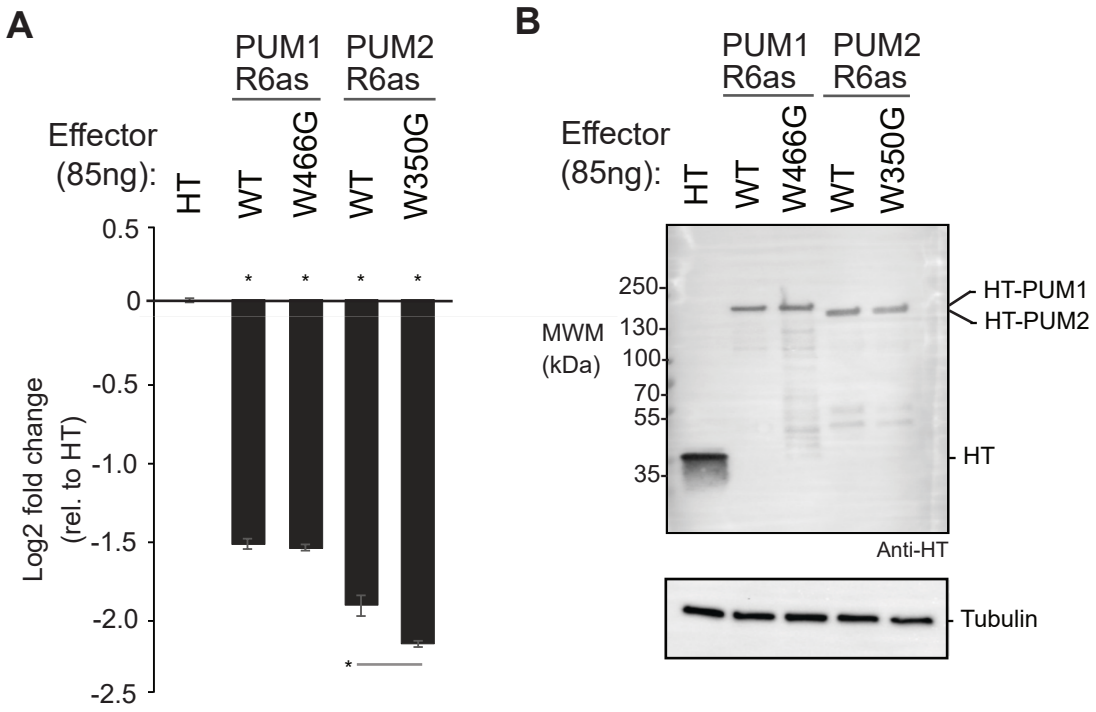

Figure S4. Confirmation of RNA digestion in coimmuniprecipitation analysis. For Figure 6A, digestion of cellular RNA by RNAses A and 1 in cellular extract was confirmed by ethidium bromide-stained, denaturing formaldehyde agarose gel electrophoresis of RNA purified from samples with (+) or without (-) RNases. RNA molecular mass markers (ladder) and 28S and 18S rRNA are indicated.

Supplemental Figure S4. Enwerem et al.

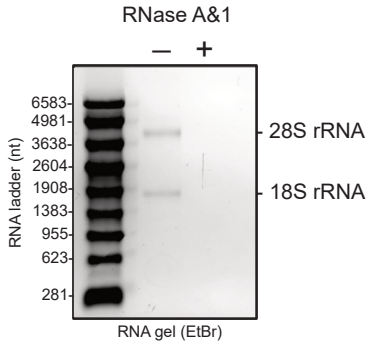

Figure S5. PUM N-terminal regions reduce mRNA level in the tethered function assay. Northern blot of Nluc 4xMS2 MALAT1 mRNA measured the effect of tethered MS2 fusions of PUM1 N, PUM2 N, or HT. Ethidium Bromide (EtBr) and northern blot detection of rRNA assessed RNA integrity and loading. n=3.

Supplemental Figure S5. Enwerem et al.

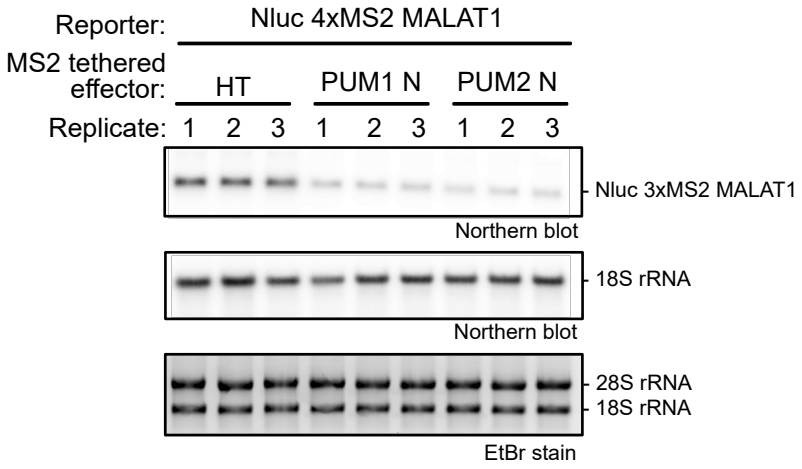

Figure S6. Effect of disease-associated PUM1 mutations on repression activity. Plotted data, listed in **Table S1**, are graphed as mean log2 fold change values +/- SEM calculated relative to HT negative control. The '*' symbols denote a p-value < 0.05 relative to the negative control (above x-axis) or between the indicated proteins (below the x-axis). n=9.

A. The altered specificity assay measured the repressive activity of PUM1 R6as in comparison to PUM1 bearing the PADDAS, R1139W and R1147W, and PRCA, T1035S, associated mutations.

B. Western blot of HT-tagged effector proteins from a representative experimental replicate from panel A. GAPDH served as a loading control.

Supplemental Figure S6. Enwerem et al.