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Supplemental information

Molecular profiling of individual FDA-approved clinical drugs identifies modulators of nonsense-mediated mRNA decay

Jingrong Zhao, Zhelin Li, Ruchira Puri, Kelvin Liu, Israel Nunez, Liang Chen, and Sika Zheng

Supplementary Information

Supplementary Table Captions

Table S1: NCATS Clinical Collection Drug.

Table S2: Raw data of all drug treatment.

Table S3: Primer sequence and location information.

Table S4: P_Fisher and P_permuatation of all drug.

Figure S1

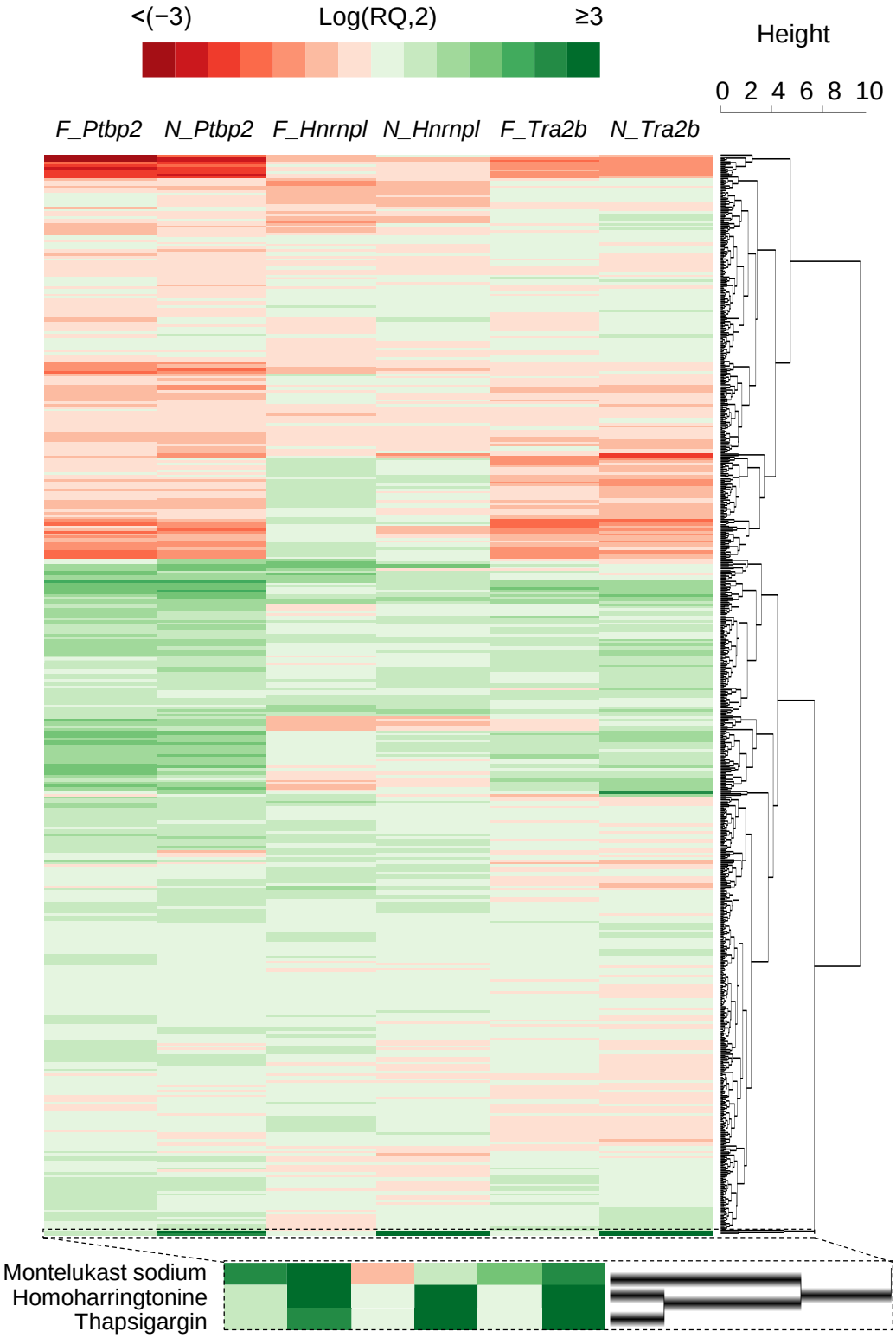

Figure S1. Visualized hierarchical clustering of reporter expression level after FDA-approved drugs administration.

Normalized expression level changes (log_2) from all drugs were clustered and visualized in a heatmap. Three drugs, thapsigargin, homoharringtonine, and montelukast sodium, with distinct responses and placed at the bottom, were shown as a subset.

Figure S2

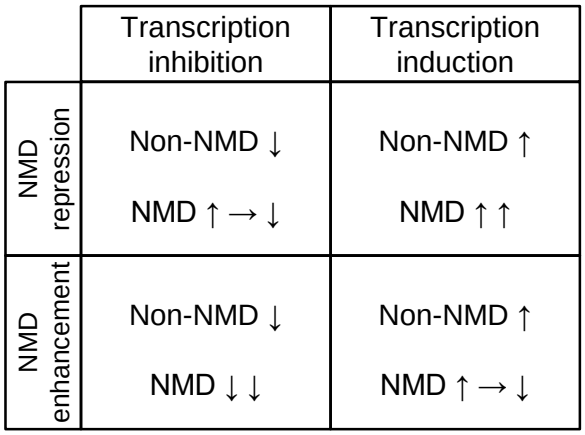

Figure S2. Combined transcriptional and NMD regulation lead to diverse scenarios of reporter gene expression changes.

Figure S3

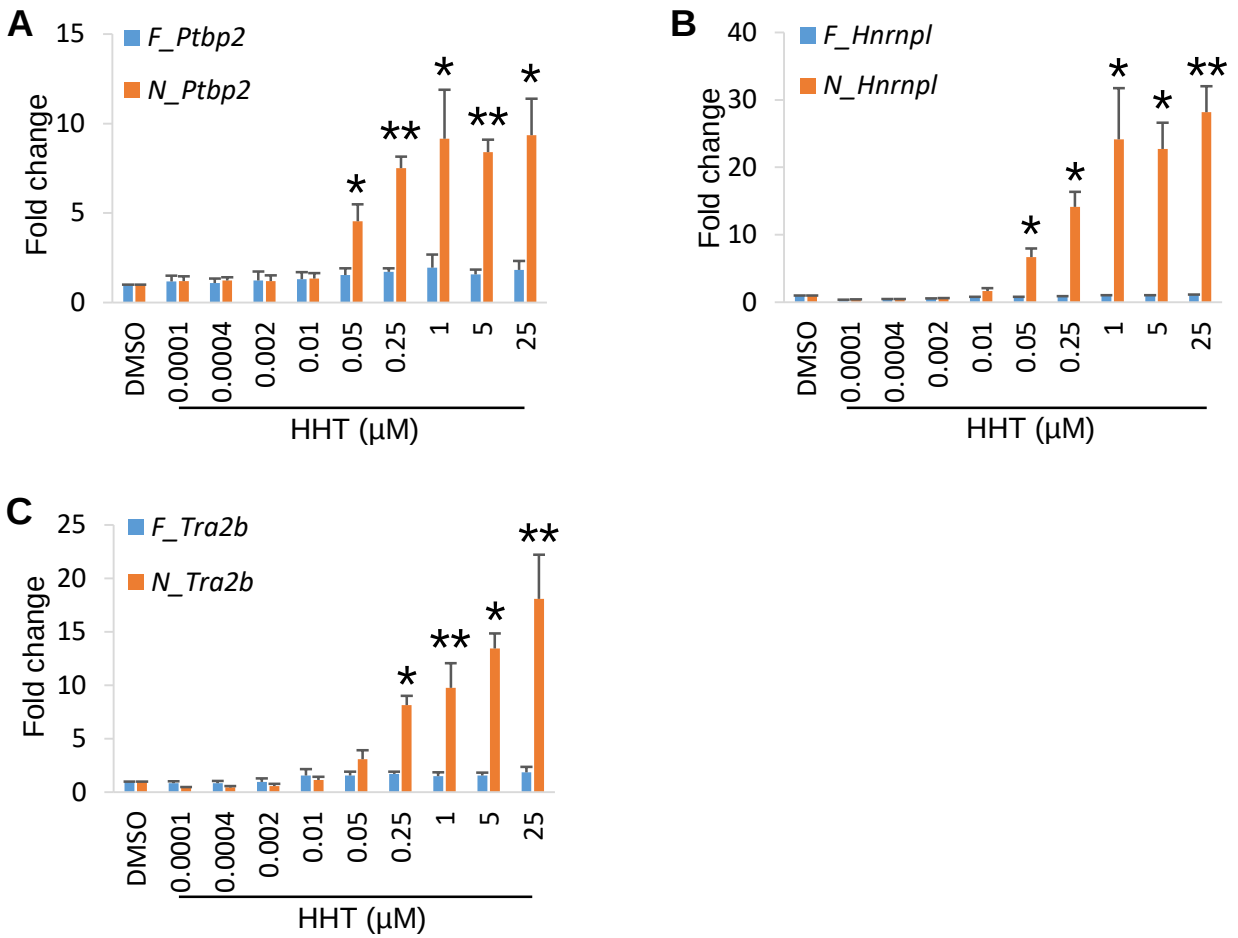

Figure S3. HHT inhibits NMD in mouse NPCs.

(**A-C**) HHT treatment in NPCs induced dosage dependent expression changes in NMD isoforms of *Ptbp2* (**A**), *Hnrnpl* (**B**), and *Tra2b* (**C**) from 0.05 μM, while Non-NMD isoforms remained largely unchanged. Data were shown as mean ± SEM of three biological replicates. *, $p<0.05$, **, $p<0.01$, Student's t test.

Figure S4

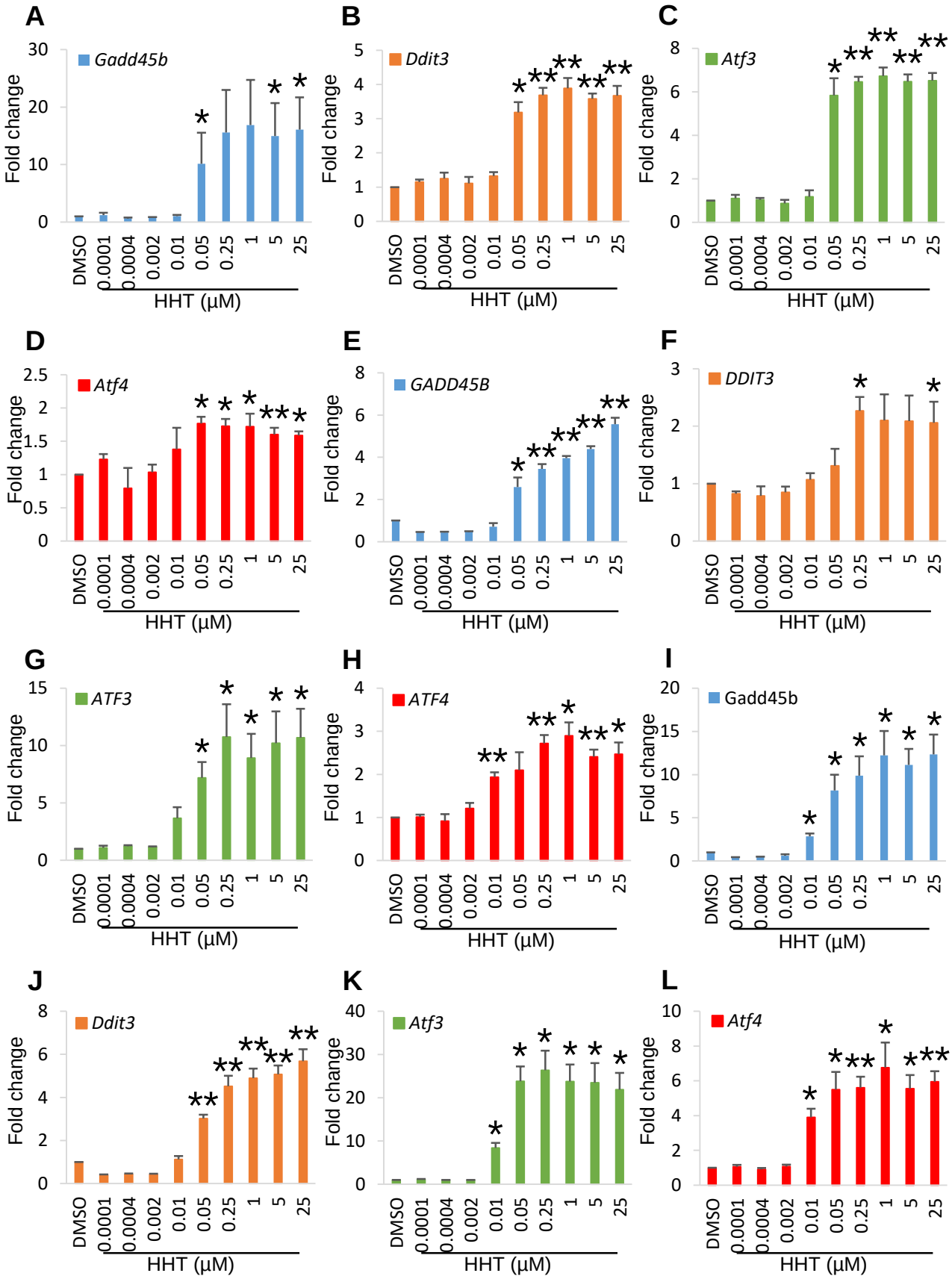

Figure S4. HHT upregulated additional NMD targets.

(**A-D**) HHT treatment in N2a cells induced a dosage dependent upregulation in the expression level of *Gadd45b* (**A**), *Ddit3* (**B**), *Atf3* (**C**), and *Atf4* (**D**) from 0.05 μM. (**E-H**) In 293T cells, HHT triggered

gene expression upregulation of *GADD45B* (**E**), *DDIT3* (**F**), *ATF3* (**G**), and *ATF4* (**H**). (**I-L**) From as low as 0.01 μM, expression level of *Gadd45b* (**I**), *Ddit3* (**J**), *Atf3* (**K**), and *Atf4* (**L**) in mouse NPCs showed sensitive responses to HHT treatment. Data were shown as mean ± SEM of three biological replicates. *, $p<0.05$, **, $p<0.01$, Student's t test.

Figure S5

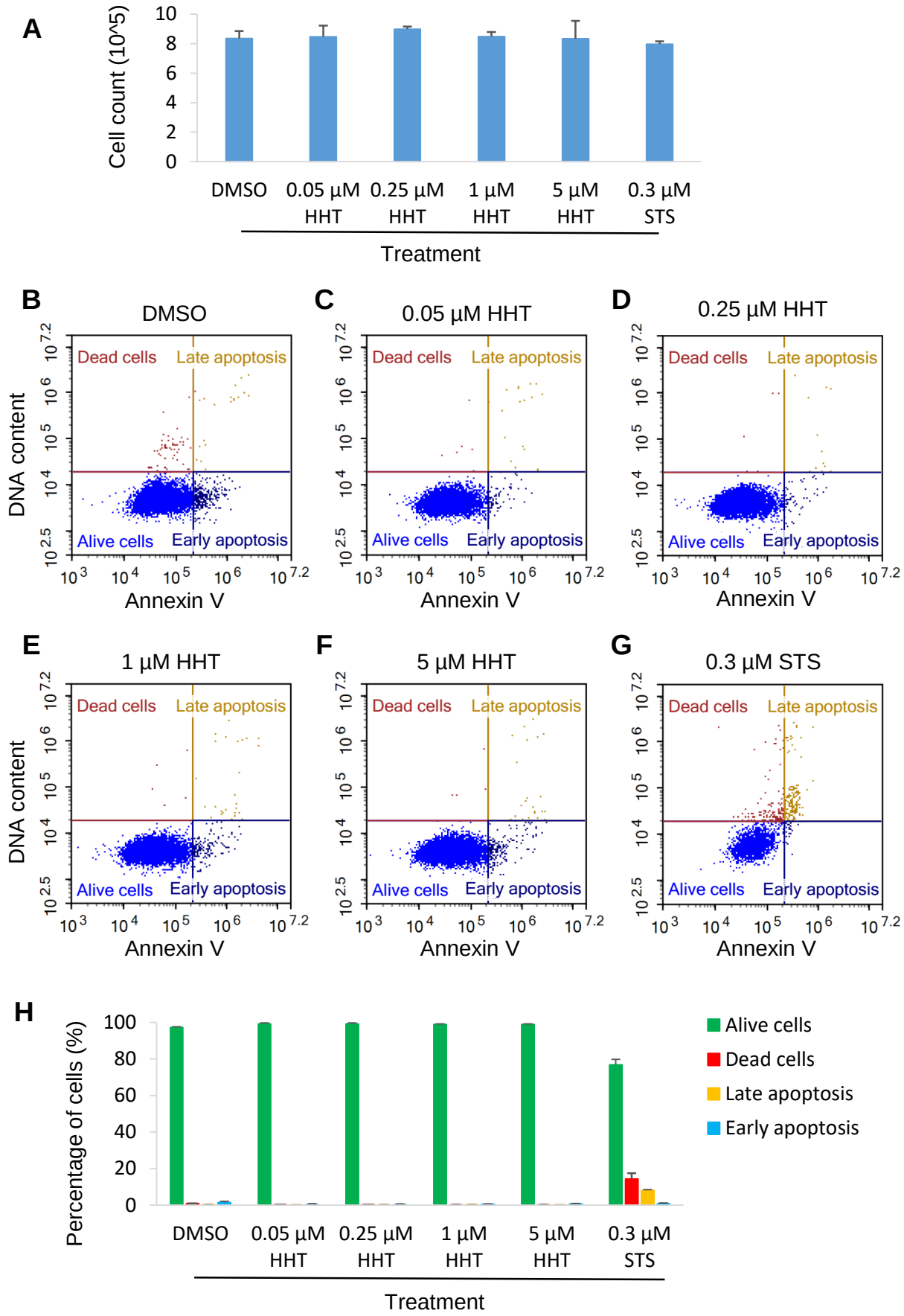

Figure S5. HHT does not induce cytotoxicity while inhibiting NMD.

(A) Cell counts of groups treated with DMSO, different dosage of HHT, and STS. No differences

were observed. **(B-H)** Annexin V apoptosis labeling showed all HHT treated groups had a similar amount of dead and apoptotic cells as the DMSO treated group, while the STS treated group had a higher percentage of cell death and apoptosis. Data were shown as mean ± SEM of three biological replicates.

Figure S6

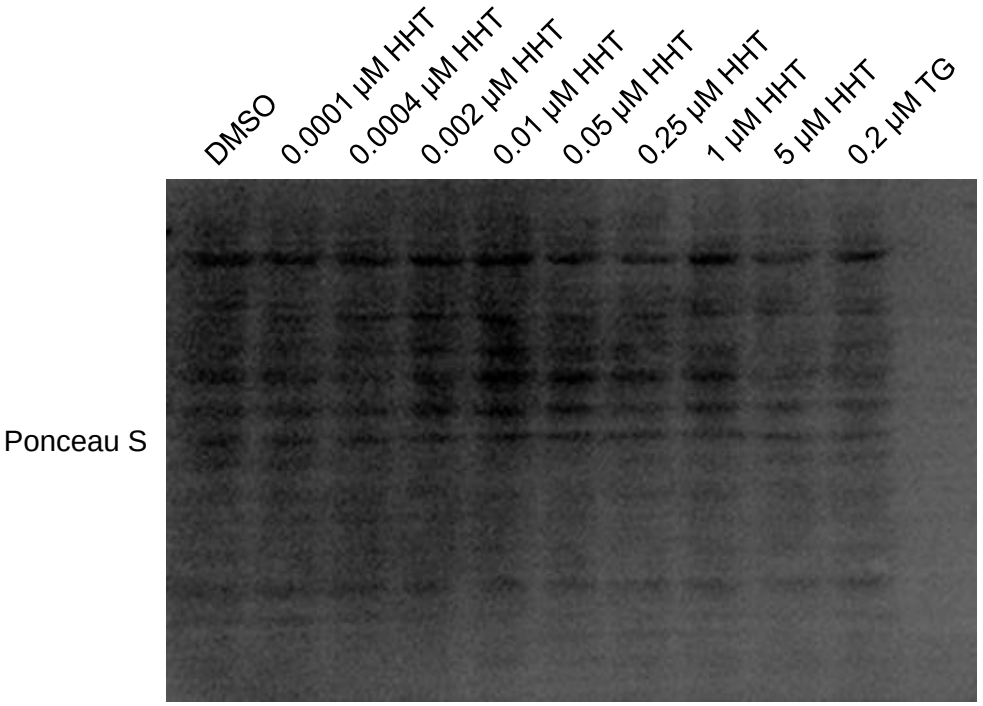

Figure S6. Total protein loading in puromycin experiments.

Ponceau S staining showed all samples had a similar amount of total protein loaded to the SDS-PAGE gel for Western blot analysis.