Development of an RNA-protein crosslinker to capture protein interactions with diverse RNA structures in cells

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Supplementary Materials

Figure S1.

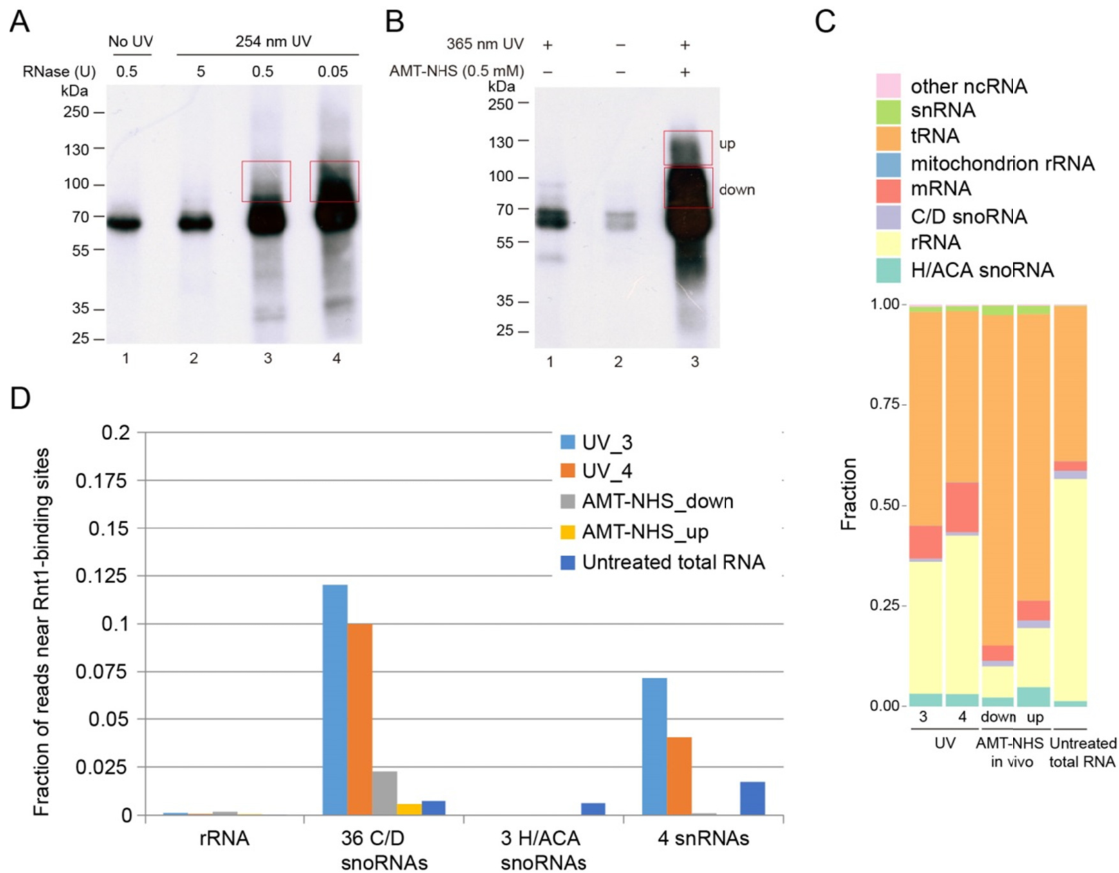

Figure S1. Crosslinking of RNase III Rnt1-HTP by UV and AMT-NHS.
(A-B) Autoradiogram of crosslinked Rnt1-RNA complex resolved in SDS-PAGE. RNA and protein were crosslinked by 254-nm UV light (A) or by AMT-NHS in vivo (B). RNA was digested by increasing amounts of RNase A/T1 in UV crosslinking and by 0.5 units of RNase A/T1 in AMT-NHS crosslinking. The boxed regions were excised for library construction. (C) RNA class distribution of reads. The two UV crosslinking samples (UV_3, UV_4) were digested by 0.5 and 0.05 U of RNase A/T1, respectively. The two AMT-NHS crosslinking samples (down and up) were excised from two regions of a single lane. The last sample was the sequencing data of total RNA from untreated cells. (D) Fraction of reads near Rnt1-binding sites over all reads on substrate RNAs. Rnt1 cleaves two opposite staggered sites at a duplex structure located on the 5' or 3' spacers of precursor RNAs. The hairpin between two cleavage sites and its flanking 25 nt on both sides, but excluding any nucleotide from mature RNA, are considered as Rnt1-binding sites. The analyzed Rnt1 substrates were compiled from literature and include the 35S pre-rRNA (cleavage sites at the 3' ETS), 36 C/D snoRNAs (snR4, snR13, snR17A, snR17B, snR39B, snR40, snR41, snR47, snR48, snR50, snR51, snR52, snR53, snR55, snR56, snR57, snR58, snR59, snR60, snR61, snR62, snR63, snR64, snR65, snR66, snR67, snR68, snR69, snR71, snR73, snR75, snR76, snR78, snR79, snR128, snR190), 3 H/ACA snoRNAs (snR36, snR43, snR46) and 4 snRNAs (Lsr1, snR14, snR7-L, snR19).