

Expanded View Figures

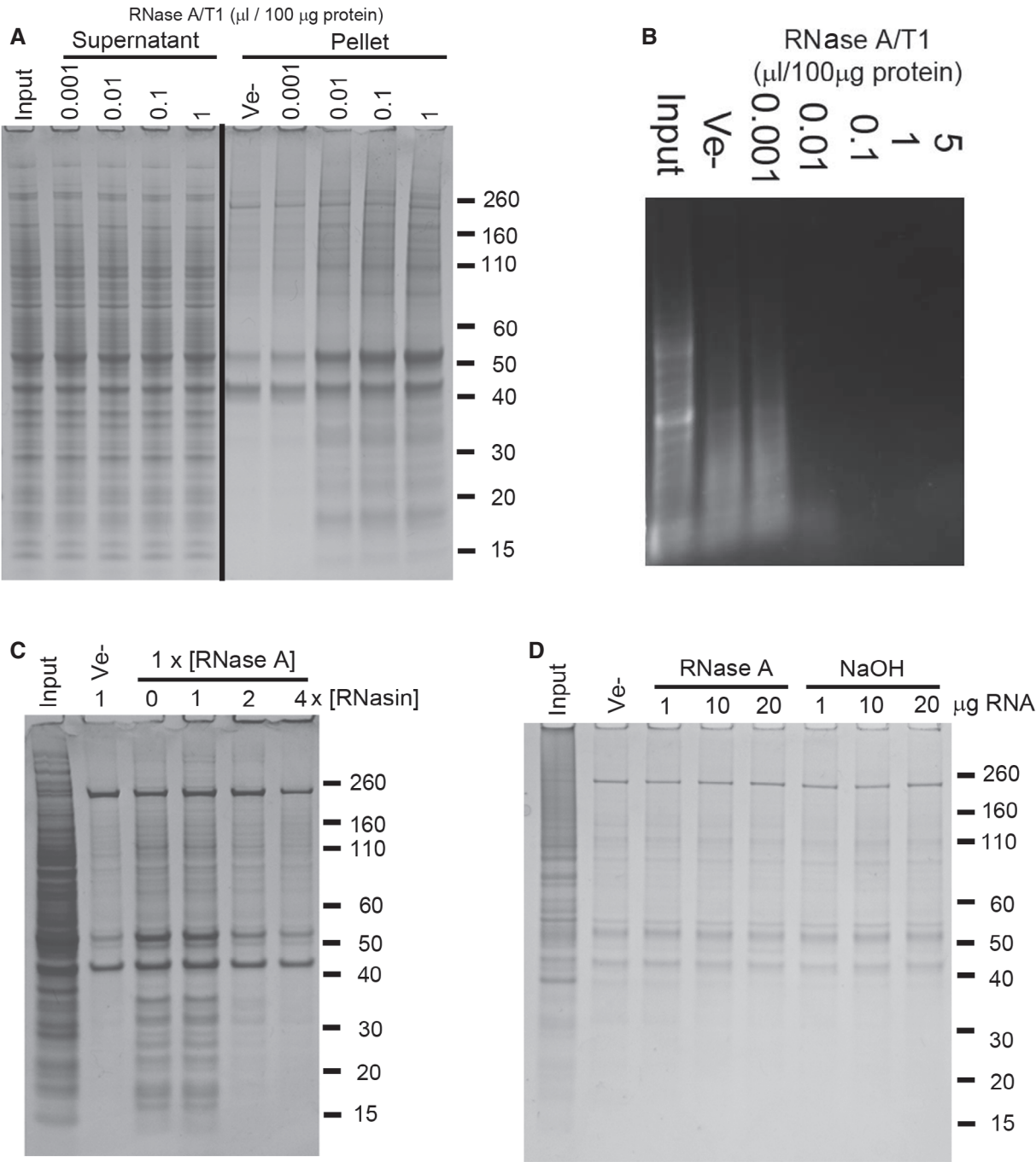


Figure EV1. Protein aggregation caused by enzymatic degradation of RNA, and effect of pre-hydrolysed RNA or RNase inhibition.

A SDS-PAGE analysis of soluble (Supernatant) and aggregated (Pellet) proteins after treatment of mouse brain tissue lysate with increasing amounts of a mixture of RNase A and T1 (A/T1) or vehicle (Ve-).

B Agarose gel electrophoresis analysis of RNA isolated from RNase-treated human neuronal cell lysate.

C, D Insoluble proteins collected by centrifugation after co-treatment of human neuronal cell lysate with RNase A and an RNase A inhibitor (RNasin, C), or following the addition of RNA pre-hydrolysed by RNase A or alkaline hydrolysis (NaOH, D).

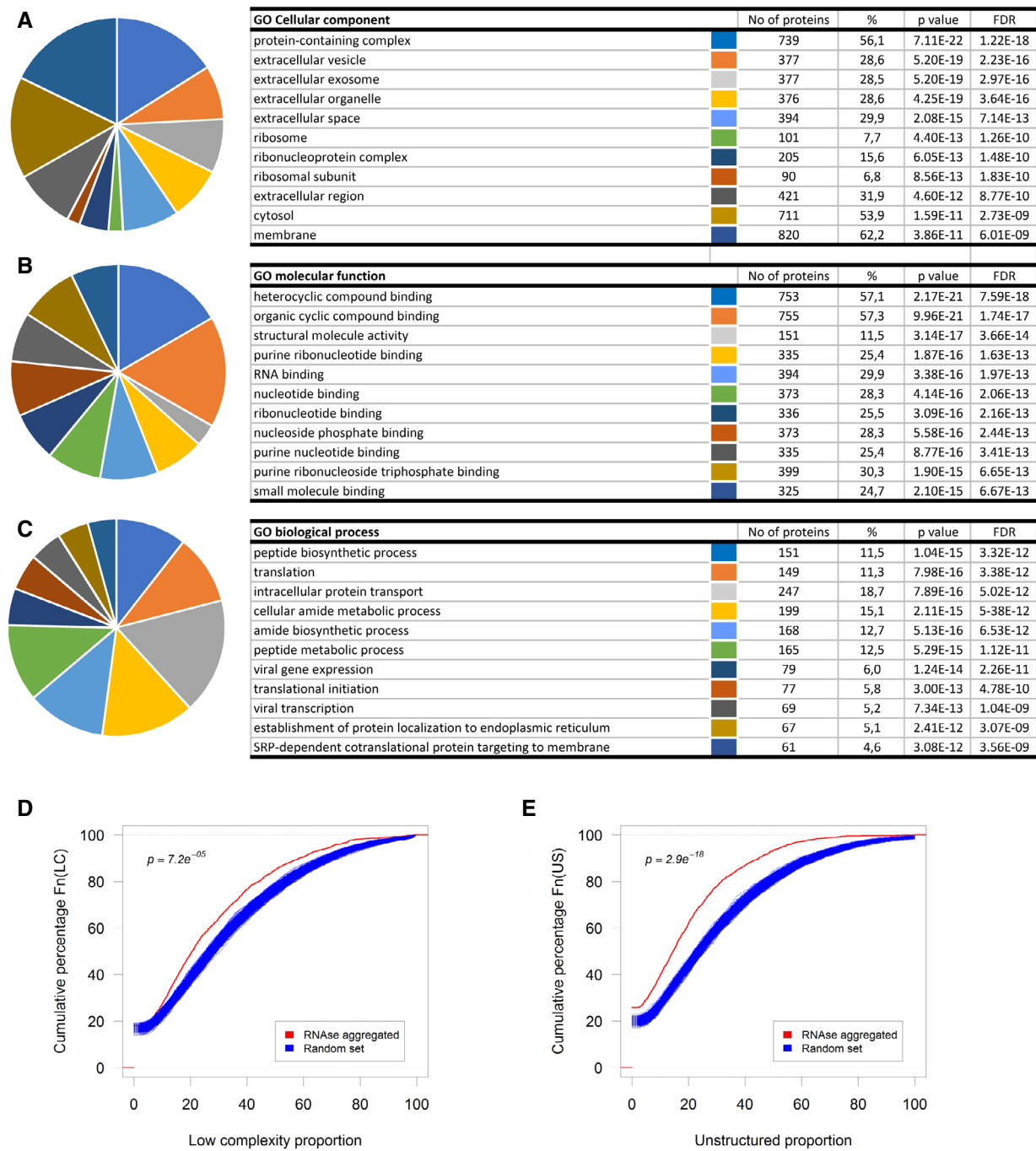


Figure EV2. Computational analysis of proteins aggregated by enzymatic degradation of RNA.

A–C Top ten gene ontology classes by Cellular component (A), Molecular function (B), or Biological process (C).
D, E Cumulative distribution of the proportion of predicted low-complexity regions (D) or unstructured regions (E) in the RNase-aggregated proteins (Red) or random sets of proteins (Blue).
Data information: P-values in (D, E) were obtained by the two-sample Kolmogorov–Smirnov test and corrected for multiple testing using Bonferroni correction.

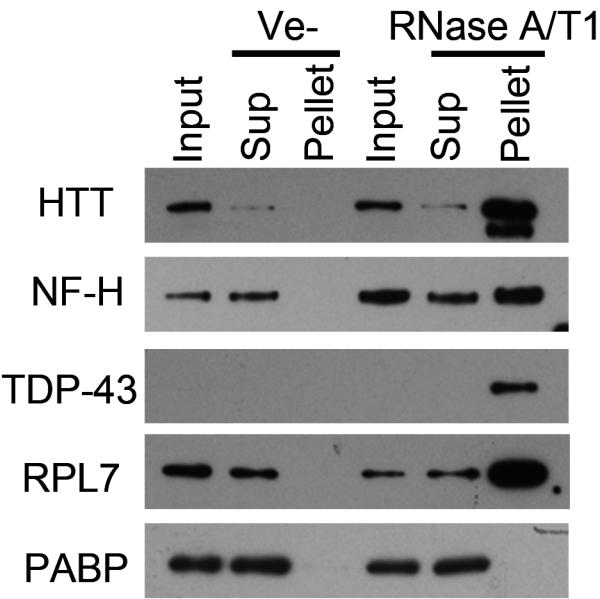


Figure EV3. Protein aggregation caused by enzymatic degradation of RNA in mouse brain tissue lysate.

Western blot analysis of aggregated proteins, collected by centrifugation, after treatment of mouse brain lysate with a mixture of RNase A and RNase T1 (RNase A/T1), or vehicle (Ve-). Input represents the starting material, Sup the supernatant (soluble fraction) and Pellet the aggregated fraction after centrifugation.

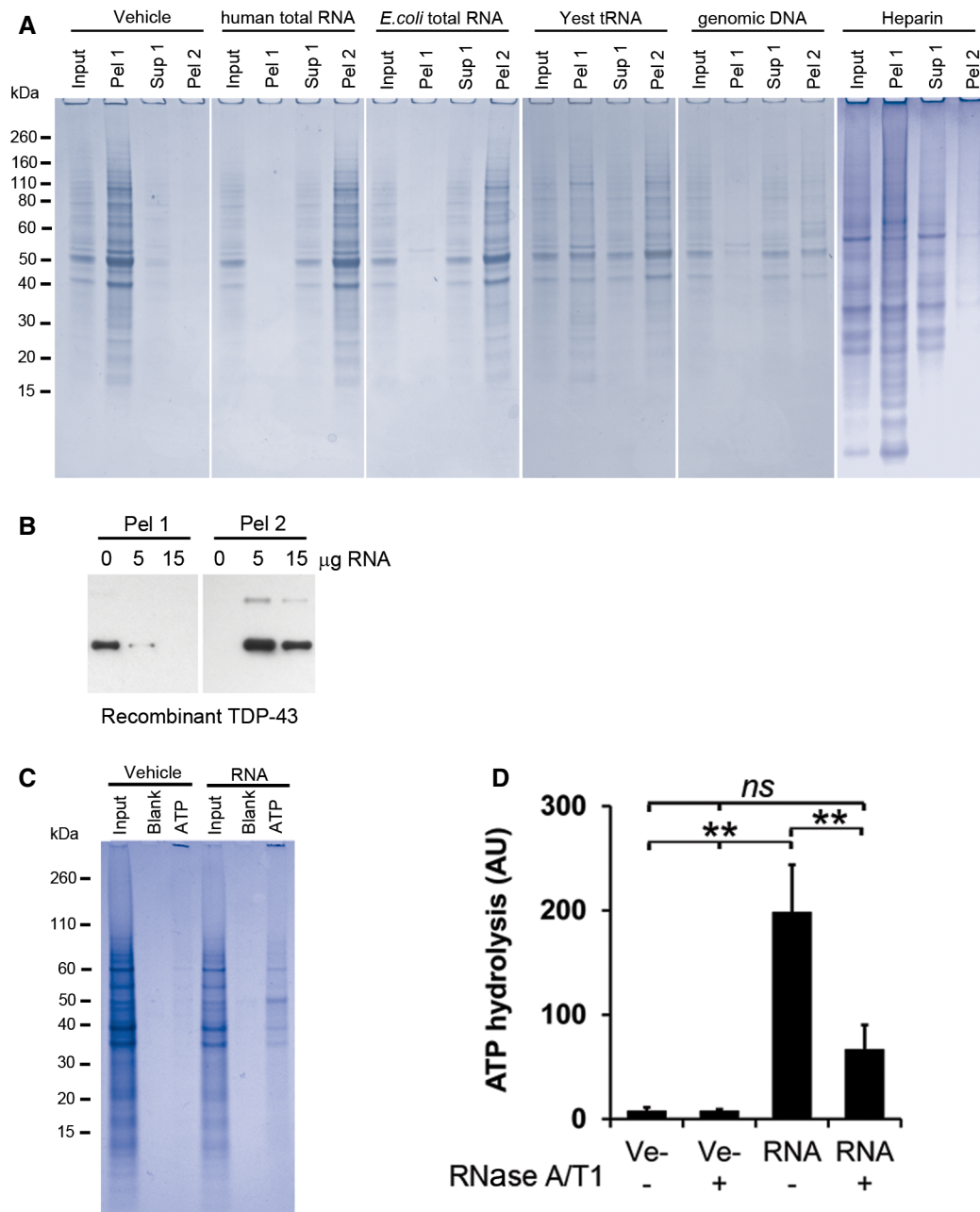


Figure EV4. Protein renaturation: effects on enzymatic activity and effects of various polyanions.

- A** Coomassie-stained SDS-PAGE gels of RNase-aggregated proteins from human neurons renatured with various polyanions. Input represents $1/10^{\text{th}}$ of each sample, taken before the first centrifugation, and Pel 1 and Sup 1 are the pellet and the supernatant, respectively, recovered after the first centrifugation. Pel 2 represents the pellet obtained after treating the Sup 1 fraction with RNase A/T1 followed by centrifugation. See experimental outline in Fig 3A for a full description.
- B** Renaturing of recombinant TDP-43 using increasing amounts of total RNA. Pel 1 represents aggregated protein after renaturing. Pel 2 is the aggregated protein after the soluble fraction (Sup 1) has been treated with RNase A/T1.
- C** Gel electrophoresis analysis of proteins from human neurons renatured with vehicle or total RNA and then captured with ATP-agarose beads (ATP). Input represents $1/10^{\text{th}}$ of each sample taken directly after renaturation, before the first centrifugation, and Blank represents the sample without any ATP-agarose beads present.
- D** ATP-hydrolysing activity of RNase-aggregated proteins from Jurkat T cells renatured in the presence (RNA) or absence (Ve-) of RNA.

Data information: Data in (C) are expressed in arbitrary units (AU) and represent the mean $\pm$ s.d of three independent experiments. $^{**}P < 0.01$ by *post hoc* ANOVA using Bonferroni correction for multiple testing.

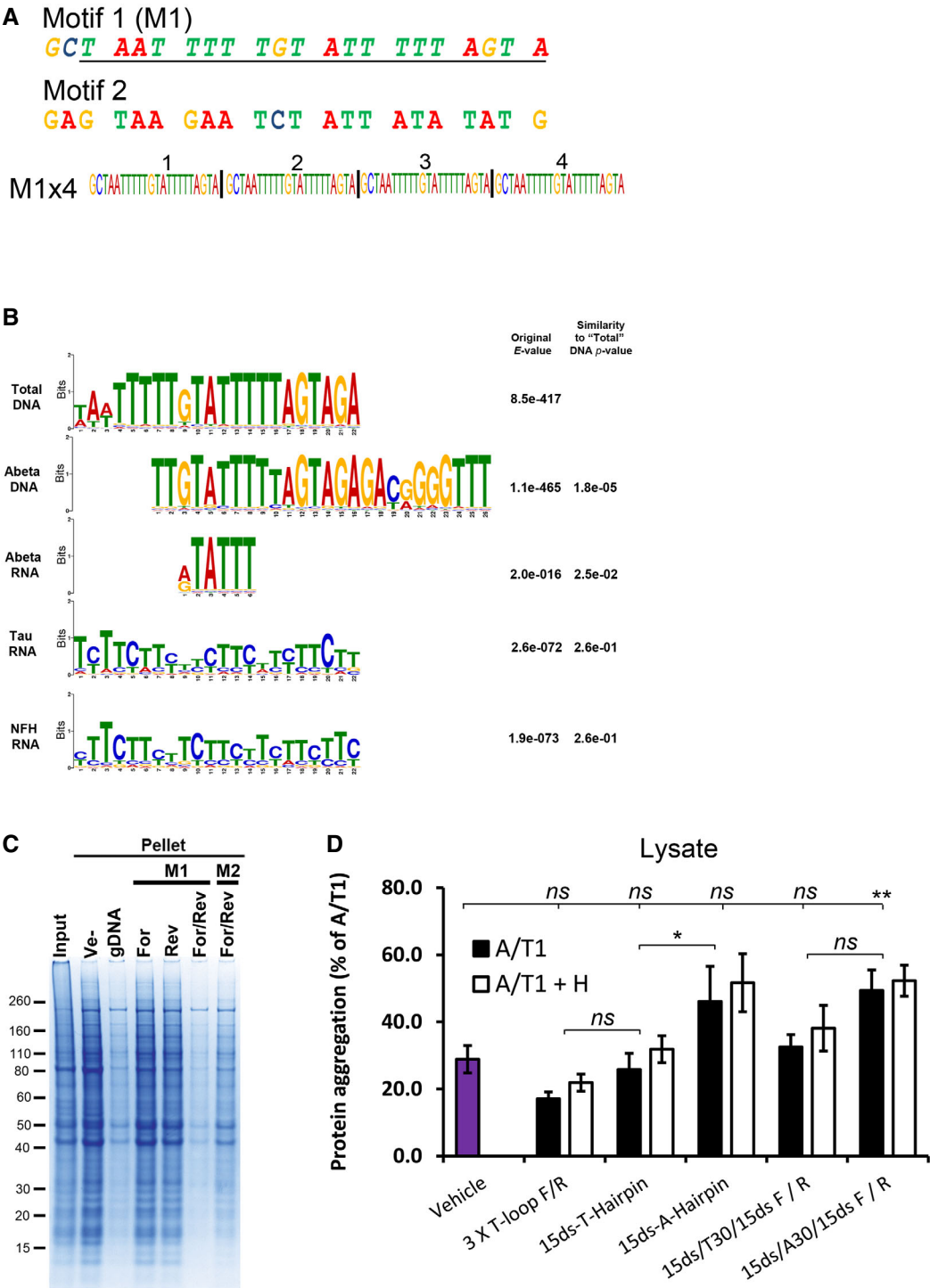


Figure EV5.

Figure EV5. Computational and experimental comparison of selected motifs and structural oligonucleotides.

- A Sequence of the selected M1 motif and a random control oligo (Motif 2, M2) investigated for their renaturing capacity. Both motifs were assembled into four consecutive repeats of the same motif (e.g. M1x4). The underlined region of M1 represents the computationally identified motif while the two extra 5' nucleotides (GC) were added for cloning purposes.
- B Alignment of motifs identified in the nucleic acids associated with soluble proteins after renaturation. All motifs are compared with the M1 motif identified in the Total DNA samples. The quoted original E-value represents the value obtained in the MEME-Chip analysis and the Similarity *P*-value is the *P*-value given by the TomTom motif comparison software package, compared with the Total DNA motif.
- C Coomassie-stained SDS-PAGE gel of aggregated proteins (Pellet) following renaturation with either single-stranded (For or Rev) or complementary (For/Rev) M1x4 or M2x4 DNA oligonucleotides. gDNA represents genomic DNA, and Ve- represents vehicle.
- D Amount of protein aggregation observed after treating cell lysate from Jurkat T cells with various oligonucleotides and RNase A/T1 (A/T1) and, if indicated, together with RNase H (A/T1 + H).

Data information: Data in (D) are expressed as a percentage of the aggregation observed in samples treated with RNase A/T1 only and represent the mean $\pm$ s.d of three independent experiments. ***P* < 0.01, **P* < 0.05 by *post hoc* ANOVA using Bonferroni correction for multiple testing.