

Supplementary Information

Dynamic profiling of double-stranded RNA binding proteins

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Supplementary Figure 1

Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4

Supplementary Figure 5

Supplementary Table 1

Supplementary Figure S1

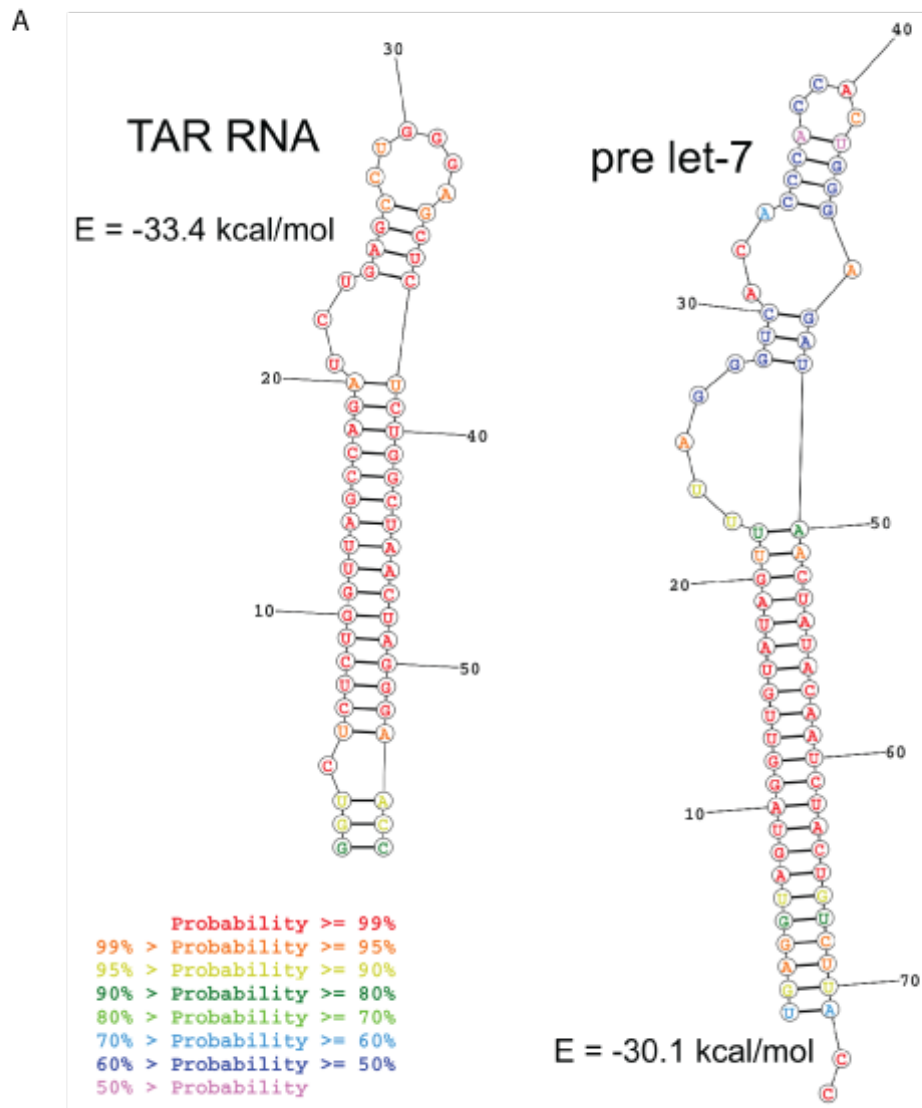


Figure S1. Predicted structure of TAR RNA and pre let-7. A) TAR RNA and pre let-7 secondary structure predictions with the lowest free energies, obtained with the RNAstructure webserver ⁶. 3D structures of TAR RNA and prelet-7, shown in Figure 2B, are based on the predictions shown here. 3D structures were obtained through the 3dRNA web server ⁷.

Supplementary Figure S2

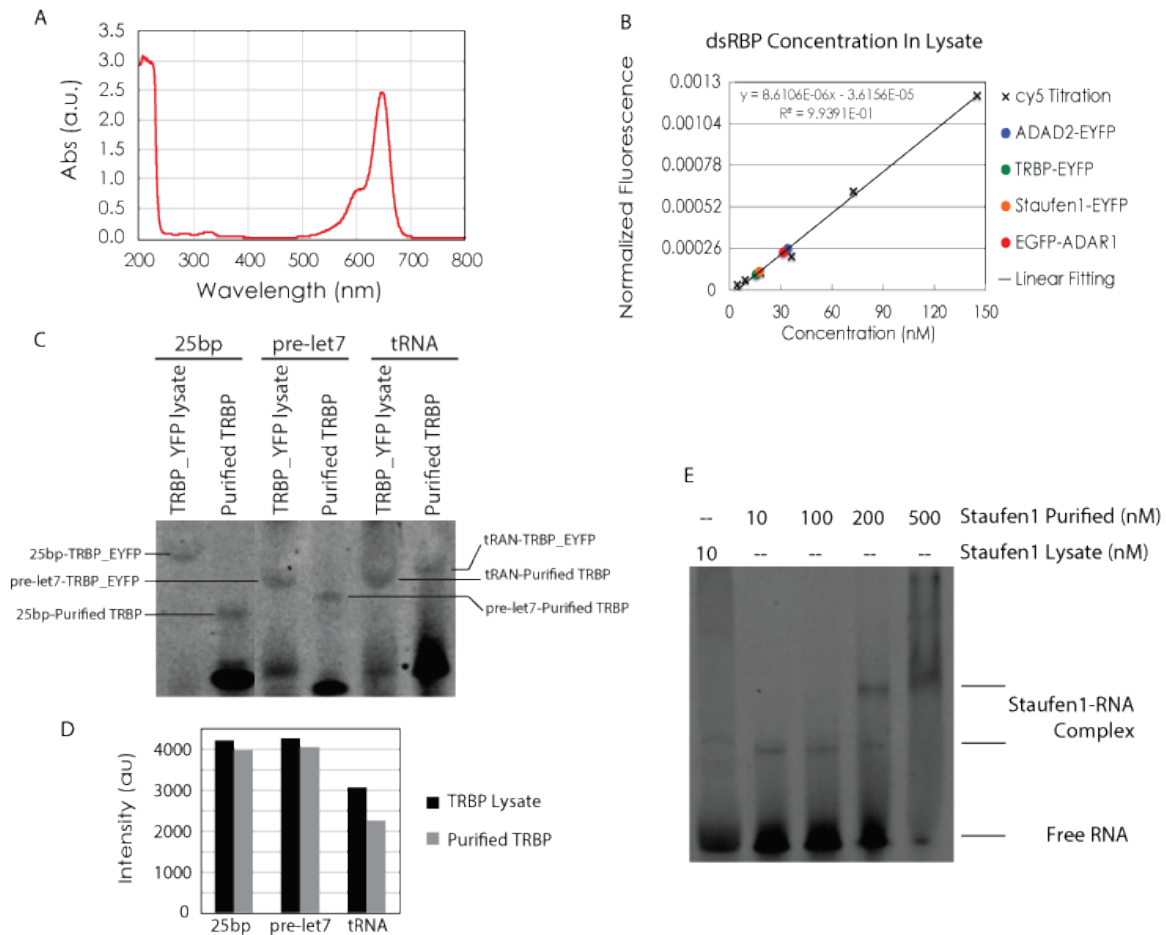


Figure S2. Characterization of protein concentration, purity and dimer/monomer status of dsRBPs from lysate. **A)** Spectrophotometry absorption measurement of free Cy5 dye sample. **B)** Determination of dsRBP concentration based on the calibration curve generated from Cy5 dye. Cy5 dye sample was serially diluted to generate a standard fluorescence-to-concentration line. Plotting normalized fluorescence intensity of four dsRBPs from lysate onto the calibration line enables estimation of concentration for each dsRBPs in lysate. **C)** EMSA assay to test the affinity of TRBP_YFP from lysate vs. purified TRBP to three representative RNA substrates labeled with Cy3. **D)** Quantification of shifted protein-RNA complex bands in C). **E)** EMSA assay to test concentration dependent oligomerization of purified Staufen1 interacting with Cy3-tRNA in parallel with Staufen1 lysate.

Supplementary Figure S3

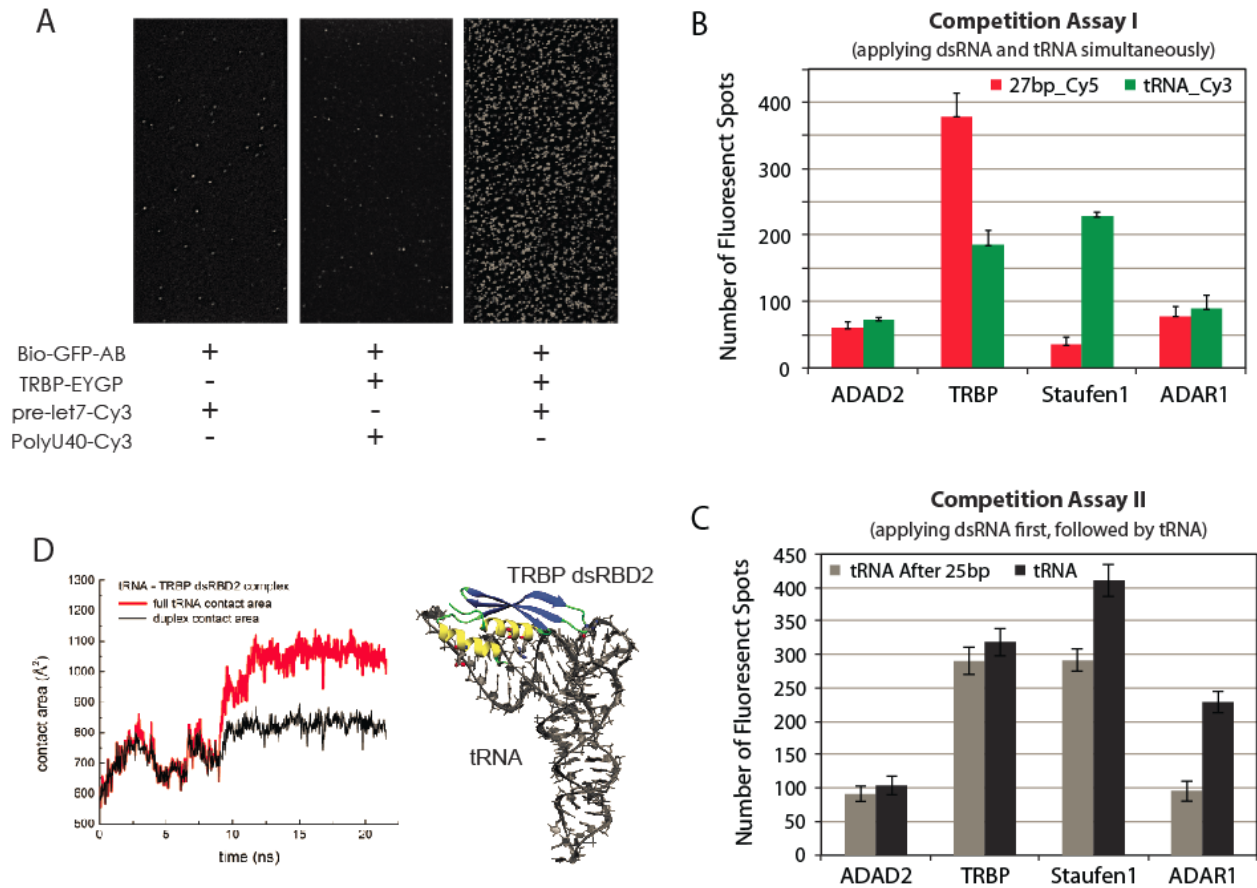


Figure S3. Nonspecific binding test and competitive binding affinity of dsRBPs towards tRNAs vs. dsRNA **A)** Testing nonspecific binding by omitting cell lysate, pre-let7 RNA and adding U40 ssRNA. **B)** Competitive binding assay between duplex Cy3-tRNA and Cy5-dsRNA (27 bp) on single molecule platform by applying the same concentration (500pM each) of the two RNAs at each of the four dsRBPs immobilized surface. **C)** Competitive binding assay in which dsRNA pre-bound to dsRBP was competed away by labeled tRNA (gray bar). The tRNA binding result is put in as a comparison (black bar). The tRNA was able to compete against dsRNA to sufficient degree for all four proteins tested. **D)** MD simulation of dsRBD2 of TRBP to binding to dsRNA vs. tRNA. The interaction to tRNA is comparable to dsRNA.

Supplementary Figure S4

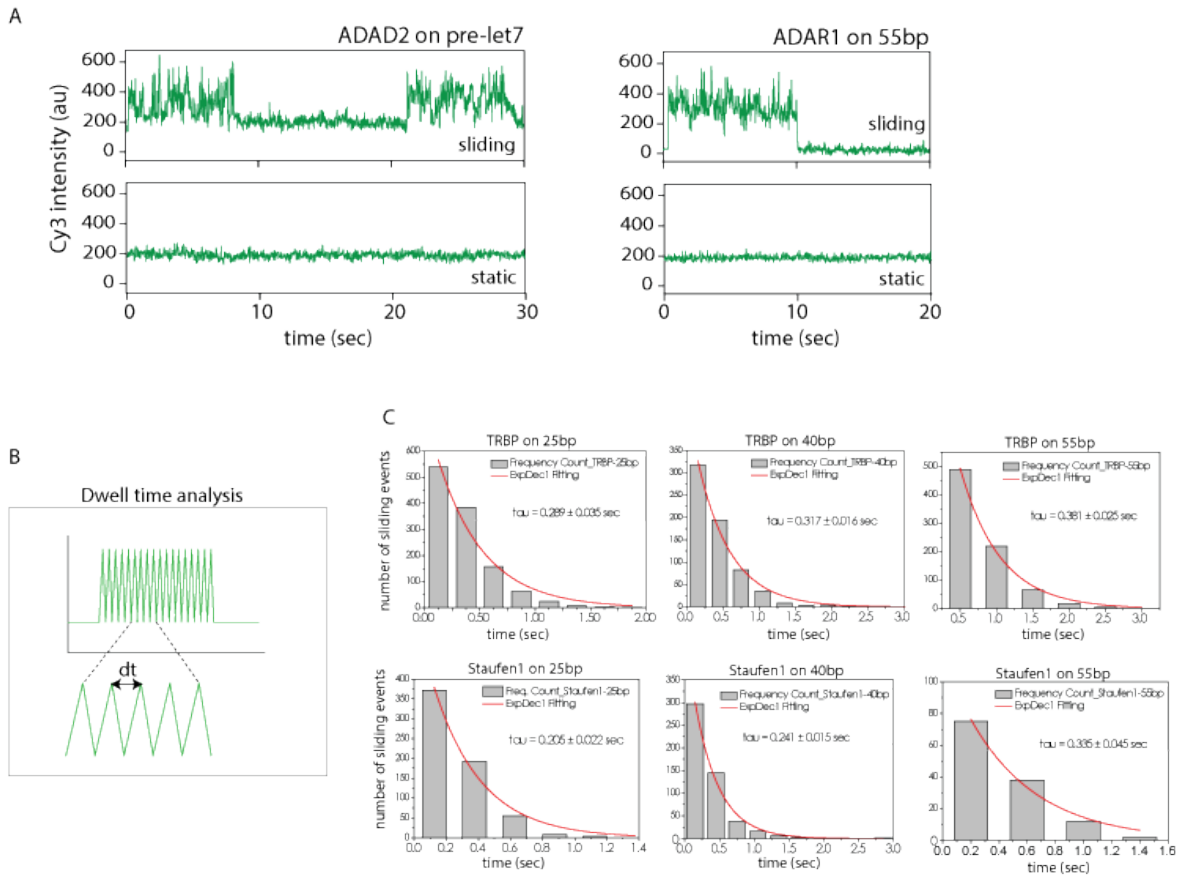


Figure S4 Dwell time analysis of TRBP and Staufen1 on length variant dsRNA **A)** Two different types (sliding vs. static) of smPIFE traces observed in ADAD2-pre-let7 and ADAR1-55bp dsRNA. **B)** Schematic of dwell time (δt) collection from smPIFE data. The time intervals between PIFE peak to peak were collected from over 500 sliding events for dwell time analysis. **C)** Dwell time distribution of TRBP and Staufen1 sliding on 25, 40 and 55 bp of dsRNA.

Supplementary Figure S5

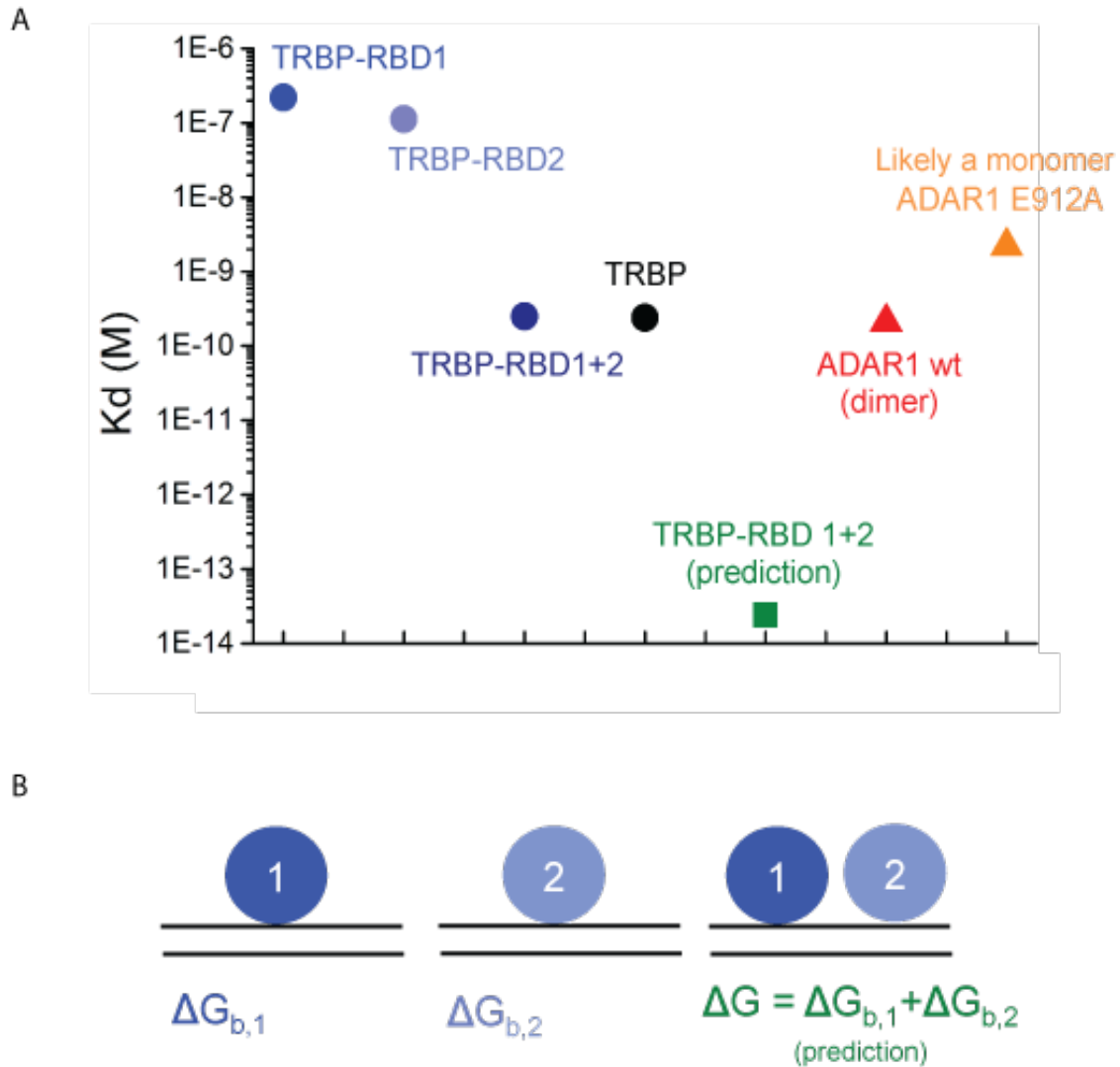


Figure S5. Dissociation constants (K_d) of dsRNA and several dsRBPs or their parts. A) K_d values of several individual dsRBDs (TRBP-RBD1, TRBP-RBD2¹⁵), dsRBD constructs (TRBP-RBD1+2¹⁵), and dsRBPs (TRBP¹⁵, ADAR1¹⁶, ADAR1 E912A¹⁶; K_d values for ADAR1 are reported for siRNA substrate. The predicted K_d value for dsRNA and TRBP-RBD1+2 construct, based on the assumption that dsRBDs in this construct act independently, is also shown in green. **B)** A scheme showing the relationship between binding free energies of two TRBP dsRBDs binding independently to dsRNA.

Supplementary Table 1

Name	Sequence
25bp RNA	5'- rGrCrUrUrGrUrCrGrGrGrArGrCrGrCrCrArCrCrCrUrCrUrGrC-3' (up) 5'- rGrCrArGrArGrGrGrUrGrGrCrGrCrUrCrCrCrGrArCrArArGrC-DY547-3' (down)
40bp RNA	5'- rGrCdTrUrArArCrArArCrArGrArUrCrArArArGrArArArArArCrArGrArCrArUrU rGrUrCrA-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrCrUrUrUrGrArUrCrUrGrGrUrUrGrU rUrArArGrCrGrU-DY547-3' (down)
55bp RNA	5'- rArCrGrCrUrUrArArCrArArCrCrArGrArUrCrArArArGrArArArArArCrArGrArCrA rUrUrGrUrCrArArUrUrGrCrArArArGrCrArArArA-3' (up) 5'- rUrUrUrUrUrGrCrUrUrUrGrCrArArUrUrGrArCrArArUrGrUrCrUrGrUrUrUrUrU rCrUrUrUrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-DY547-3' (down)
Pre-let7	5'-cy3- rUrGrArGrGrUrArGrUrArGrGrUrUrGrUrArUrArGrUrUrUrUrArGrGrGrUrCrArCrA rCrCrCrArCrCrArCrUrGrGrGrArGrArUrArArCrUrArUrArCrArArUrCrUrArCrUrGrU rCrUrUrArCrC-3'
TAR	5'-cy3- rGrGrUrCrUrCrUrCrUrGrGrUrUrArGrCrCrArGrArUrCrUrGrArGrCrCrUrGrGrGrA rGrCrUrCrUrCrUrGrGrCrUrArArCrUrArGrGrGrArArCrC-3'
tRNA	5'-cy3- rGrGrGrArArGrCrCrCrGrGrArUrArGrCrUrCrArGrUrCrGrGrUrArGrArGrCrArUrC rArGrArCrUrUrUrArArUrCrUrGrArGrGrGrUrCrCrArGrGrGrUrUrCrArArGrUrCrC rCrUrGrUrUrCrGrGrGrCrCrA-3'
PolyU40	5' - rU rUrUrUrUrUrU-cy3-3'

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