# Synthetic RNA Recognition Motifs that Selectively Recognize HIV-1 Trans-Activation Response Element Hairpin RNA

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# Materials and Instrumentation

## Materials

LB Miller's Broth – Fisher
5-alpha chemically competent E.coli – New England Biolabs
BL21(DE3) Competent E.coli – New England Biolabs
Bacteriological agar - Fisher
Carbenicillin Disodium Salt – GoldBio Technology
L(+) arabinose – GoldBio Technology
Kanamycin – GoldBio Technology
IPTG, dioxane free – GoldBio Technology
Innova 42/42R incubator shakers – New Brunswick Scientific
Molecular imager gel doc XR+ system – Biorad
Q5 DNA Polymerase – New England Biolabs
15% Ready Gel precast gels - Biorad
Black 384-well polystyrene plates (Corning)

All water was obtained from a Milli-Q water purification system.

## Instrumentation

Sonifier W-350 cell disruptor
J2-21 centrifuges – Beckman
Perkin-Elmer Victor V multimode microplate reader
MJ mini gradient thermal cycler – Biorad

# Experimental Data

## Cloning, expression, and purification of U1A variants

Plasmids containing U1A and U1A ΔK50 ΔM51 were generously provided by Professor Laird-Offringa (University of Southern California). Point mutations were introduced by site directed mutagenesis using standard molecular biology techniques. A general protocol is as follows: To generate the U1A E19S mutation, dNTPs, the template pET3d plasmid containing wtU1A, the forward primer FP E19S (5′-CCT CAA TTC GAA GAT CAA GAA GGA TGA GCT CAA AAA GTC CC-3′), the reverse primer RP E19S (5′-CCT TCT TGA TCT TCG AAT TGA GGT TGT TGA TAT AAA TAG TGT GG-3′), and Pfu Turbo DNA polymerase were mixed in the appropriate reaction buffer. PCR was carried out using the following cycle: 94°C for 30 seconds, 58°C for 2 minutes, 64°C for 8 minutes, repeat 15 times and finally 64°C for ten minutes. At the conclusion of the PCR, 20 units of DpnI (New England Biolabs) was added to the reaction followed by incubation at 37°C for 1 hour. Amplification of the mutated plasmid was verified by running 20 μL of the reaction on a 1% agarose gel with ethidium bromide and then 1 μL of the reaction was transformed into 5-α chemically competent *E.coli* (New England Biolabs) according to the manufacturers instructions. All other mutations were prepared in a similar manner using the appropriate primers from the table below. In some cases where two codons in close proximity were mutated, a double mutant primer was designed to incorporate both mutants. Mutants were expressed and purified as previously described.[S1]

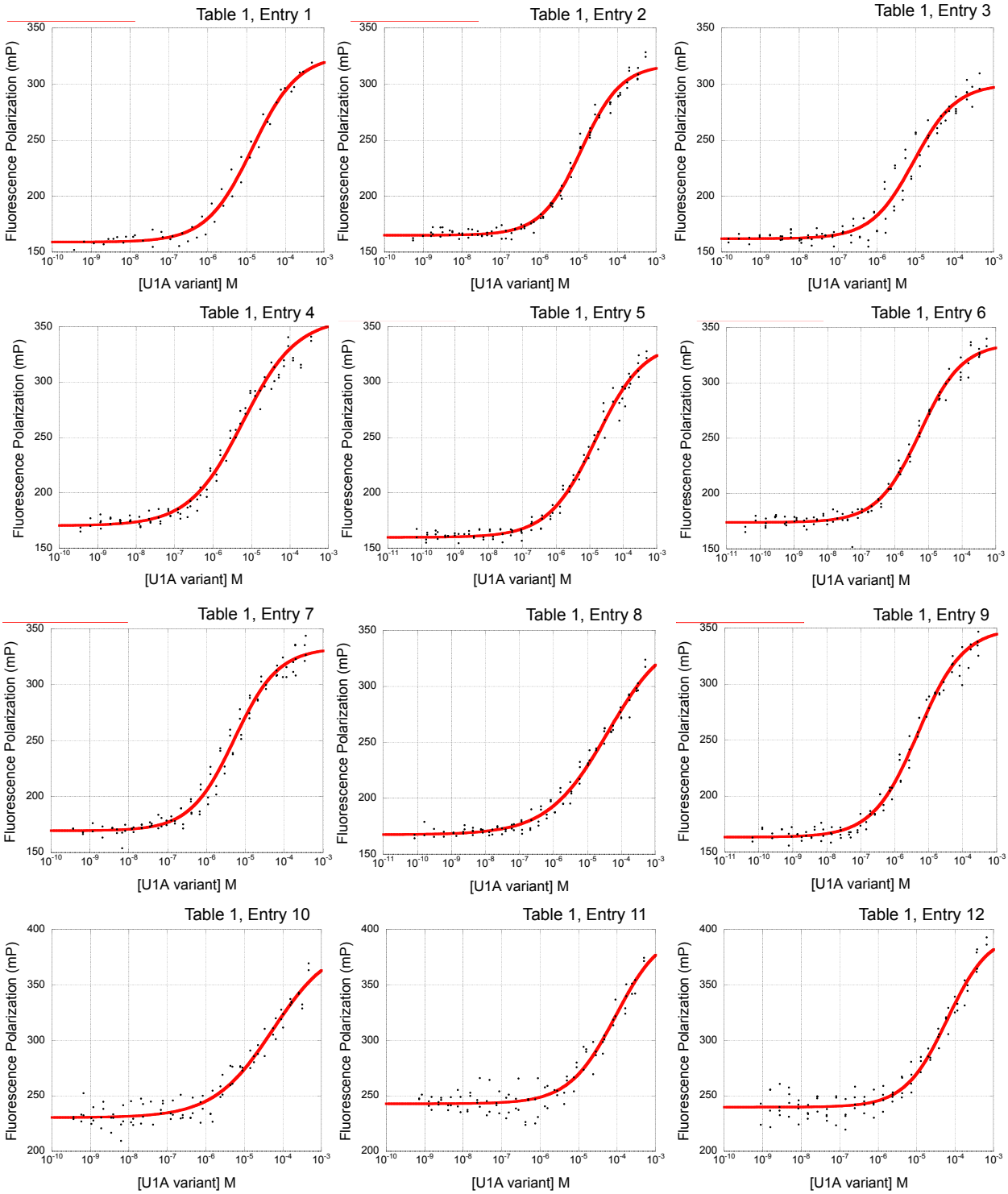
## Table S1. Nucleic acids used in this work

| Nucleic Acid | Sequence |
|---|---|
| TAR RNA | 5′-GGCAGAUCUGAGCCUGGGAGCUCUCUGCC-3′ |
| U1hpII RNA | 5′-AGCTTATCCATTGCACCGGATAAGCT-3′ |
| ESS-3 RNA | 5′-GGAUCCAUUCGAUUAGUGAACGGAUCC-3′ |
| TAR DNA | 5′-GGCAGATCTGAGCCTGGGAGCTCTCTGCC-3′ |
| ESS-3 DNA | 5′-GGATCCATTCGATTAGTGAACGGATCC-3′ |

# Table S2. Primers used in this work

| Primer Name | Sequence |
|---|---|
| FP U1A N15A | 5′-CCTAACCACACTATTTATATCGCCAACCTCAATGAGAAGATCAAG-3′ |
| RP U1A N15A | 5′-CTTGATCTTCTCATTGAGGTTGGCGATATAAATAGTGTGGTTAGG-3′ |
| FP U1A N16A | 5′-CCTAACCACACTATTTATATCAACGCCCTCAATGAGAAGATCAAGAAGG-3′ |
| RP U1A N16A | 5′-CCTTCTTGATCTTCTCATTGAGGGCGTTGATATAAATAGTGTGGTTAGG-3′ |
| FP U1A E19A | 5′-CCACACTATTTATATCAACAACCTCAATGCGAAGATCAAGAAGGATGAGCTC-3′ |
| RP U1A E19A | 5′-GAGCTCATCCTTCTTGATCTTCGCATTGAGGTTGTTGATATAAATAGTGTGG-3′ |
| FP U1A S46A | 5′-GATATCCTGGTAGCACGGAGCCTGAAG-3′ |
| RP U1A S46A | 5′-CTCCGTGCTACCAGGATATCCAGGATC-3′ |
| FP U1A S48A | 5′-ACGGGCCCTGAAGATGAGGGGC-3′ |
| RP U1A S48A | 5′-CATCTTCAGGGCCCGTGATACCAGGATATCCAGG-3′ |
| FPU1A L49A | 5′-GAGCGCGAAGATGAGGGGCCAAG-3′ |
| RP U1A L49A | 5′-CCTCATCTTCGCGCTCCGTGATACCAGG-3′ |
| FP U1A K50A | 5′-GCCTGGCGATGAGGGGCC-3′ |
| RP U1A K50A | 5′-CCTCATCGCCAGGCTCCGTGATACC-3′ |
| FP U1A M51A | 5′-AGCCTGAAGGCGAGGGGCC-3′ |
| RP U1A M51A | 5′-CCTCGCCTTCAGGCTCCGTGATACCAG-3′ |
| FP ΔKΔM S46A | 5′-GATATCCTGGTAGCACGGAGCCTGAGG-3′ |
| RP ΔKΔM S46A | 5′-CTCCGTGCTACCAGGATATCCAGGATC-3′ |
| FP ΔKΔM S48A | 5′-GGTATCACGGGCCCTGAGGGGCC-3′ |
| RP ΔKΔM S48A | 5′-CTCAGGGCCCGTGATACCAGGATATCCAGGC-3′ |
| FP ΔKΔM L49A | 5′-CGGAGCGCGAGGGGC-3′ |
| RP ΔKΔM L49A | 5′-CCTCGCGCTCCGTGATACCAGG-3′ |
| FP U1A E19S | 5′-CCTCAATTCGAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A E19S | 5′-CCTTCTTGATCTTCGAATTGAGGTTGTTGATATAAATAGTGTGG-3′ |
| FP U1A E19F | 5′-CCTCAATTTCAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A E19F | 5′-CCTTCTTGATCTTGAAATTGAGGTTGTTGATATAAATAGTGTGG-3′ |
| FP U1A E19K | 5′-CCTCAATAAGAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A E19K | 5′-CCTTCTTGATCTTCTTATTGAGGTTGTTGATATAAATAGTGTGG-3′ |
| FP U1A E19Q | 5′-CCTCAATCAGAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A E19Q | 5′-CCTTCTTGATCTTCTGATTGAGGTTGTTGATATAAATAGTGTGG-3′ |
| FP U1A L49E | 5′-GAGCGAGAAGATGAGGGGCCAAG-3′ |
| RP U1A L49E | 5′-CCTCATCTTCTCGCTCCGTGATACCAGG-3′ |
| FP U1A L49F | 5′-GAGCTTCAAGATGAGGGGCCAAG-3′ |
| RP U1A L49F | 5′-CCTCATCTTGAAGCTCCGTGATACCAGG-3′ |
| FP U1A L49K | 5′-GAGCAAGAAGATGAGGGGCCAAG-3′ |
| RP U1A L49K | 5′-CCTCATCTTCTTGCTCCGTGATACCAGG-3′ |
| FP U1A L49N | 5′-GAGCAACAAGATGAGGGGCCAAG-3′ |
| RP U1A L49N | 5′-CCTCATCTTGTTGCTCCGTGATACCAGG-3′ |
| FP U1A L49S | 5′-GAGCAGCAAGATGAGGGGCCAAG-3′ |
| RP U1A L49S | 5′-CCTCATCTTGCTGCTCCGTGATACCAGG-3′ |
| FP U1A M51E | 5′-AGCCTGAAGGAGAGGGGCC-3′ |
| RP U1A M51E | 5′-CCTCTCCTTCAGGCTCCGTGATACCAG-3′ |
| FP U1A M51F | 5′-AGCCTGAAGTTCAGGGGCC-3′ |
| RP U1A M51F | 5′-CCTGAACTTCAGGCTCCGTGATACCAG-3′ |
| FP U1A M51K | 5′-AGCCTGAAGAAGAGGGGCC-3′ |
| RP U1A M51K | 5′-CCTCTTCTTCAGGCTCCGTGATACCAG-3′ |
| FP U1A M51N | 5′-AGCCTGAAGAACAGGGGCC-3′ |
| RP U1A M51N | 5′-CCTGTTCTTCAGGCTCCGTGATACCAG-3′ |
| FP U1A M51S | 5′-AGCCTGAAGAGCAGGGGCC-3′ |
| RP U1A M51S | 5′-CCTGCTCTTCAGGCTCCGTGATACCAG-3′ |
| FP U1A Y13Q | 5′-CGCGTCCTAACCACACTATTGGTATCAACAACCTCAATGAGAAGATC-3′ |
| RP U1A Y13Q | 5′-GGTTGTTGATCTGAATAGTGTGGTTAGGACGCGTCTCGG-3′ |
| FP U1A F56A | 5′-CGGAGCCTGAGGGGCCAAGCCGCGGTCATCTTCAAGGAGG-3′ |
| RP U1A F56A | 5′-CCTCCTTGAAGATGACCGCGGCTTGGCCCCTCAGGCTCCG-3′ |
| FP U1A N15V | 5′-CCACACTATTTATATCGTCAACCTCAATGAGAAGATCAAGAAGGATGAGCTCAAAAAGTC-3′ |
| RP U1A N15V | 5′-GAGGTTGACGATATAAATAGTGTGGTTAGGACGCGTCTCGGG-3′ |
| FP U1A L49A M51A | 5′-GAGCGCGAAGGCGAGGGGCCAAGCTTTTGTC-3′ |
| RP U1A L49A M51A | 5′-CCCTCGCCTTCGCGCTCCGTGATACCAGG-3′ |
| FP U1A S48A L49A | 5′-ACGGGCCGCGAAGATGAGGGGCCAAGC-3′ |
| RP U1A S48A L49A | 5′-CATCTTCGCGGCCCGTGATACCAGGATATCCAGG-3′ |
| FP U1A S48A M51A | 5′-ACGGGCCCTGAAGGCGAGGGGCCAAGCTTTTGTC-3′ |
| RP U1A S48A M51A | 5′-CCCTCGCCTTCAGGGCCCGTGATACCAGG-3′ |
| FP U1A S48A L49A M51A | 5′-ACGGGCCGCGAAGGCGAGGGGCCAAGCTTTTGTC-3′ |
| RP U1A S48A L49A M51A | 5′-CCCTCGCCTTCGCGGCCCGTGATACCAGGATATCCAGG-3′ |
| FP U1A N15A N16A | 5′-CTATTTATATCGCCGCCCTCAATGAGAAGATCAAGAAGGATGAGCTCAAAAAGTCC-3′ |
| RP U1A N15A N16A | 5′-GAGGGCGGCGATATAAATAGTGTGGTTAGGACGCGTCTCGGG-3′ |
| FP U1A N15A E19A | 5′-GCCAACCTCAATGCGAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A N15A E19A | 5′-CGCATTGAGGTTGGCGATATAAATAGTGTGGTTAGGACGCGTCTCGG-3′ |
| FP U1A N16A E19A | 5′-ACGCCCTCAATGCGAAGATCAAGAAGGATGAGCTCAAAAAGTCCC-3′ |
| RP U1A N16A E19A | 5′-CGCATTGAGGGCGTTGATATAAATAGTGTGGTTAGGACGCGTCTCG-3′ |
| FP U1A Y13Q E19S | 5′-CCACACTATTCAGATCAACAACCTCAATTCGAAGATCAAGAAGGATGAGC-3′ |
| FP U1A Y13Q E19A | 5′-CCACACTATTCAGATCAACAACCTCAATGCGAAGATCAAGAAGGATGAGC-3′ |
| FP U1A Y13Q E19F | 5′-CCACACTATTCAGATCAACAACCTCAATTTCAAGATCAAGAAGGATGAGC-3′ |
| FP U1A N15V E19S | 5′-CCACACTATTTATATCGTCAACCTCAATTCGAAGATCAAGAAGGATGAGCTCAAAAAGTC-3′ |
| FP U1A N15V E19A | 5′-CCACACTATTTATATCGTCAACCTCAATGCGAAGATCAAGAAGGATGAGCTCAAAAAGTC-3′ |
| FP U1A N15V E19F | 5′-CCACACTATTTATATCGTCAACCTCAATTTCAAGATCAAGAAGGATGAGCTCAAAAAGTC-3′ |

**Figure S1. Fluorescence Polarization Data for Table 1.** *Binding affinities for complexes involving TAR RNA and U1A or ΔK51ΔM51, and specific alanine mutants thereof.*



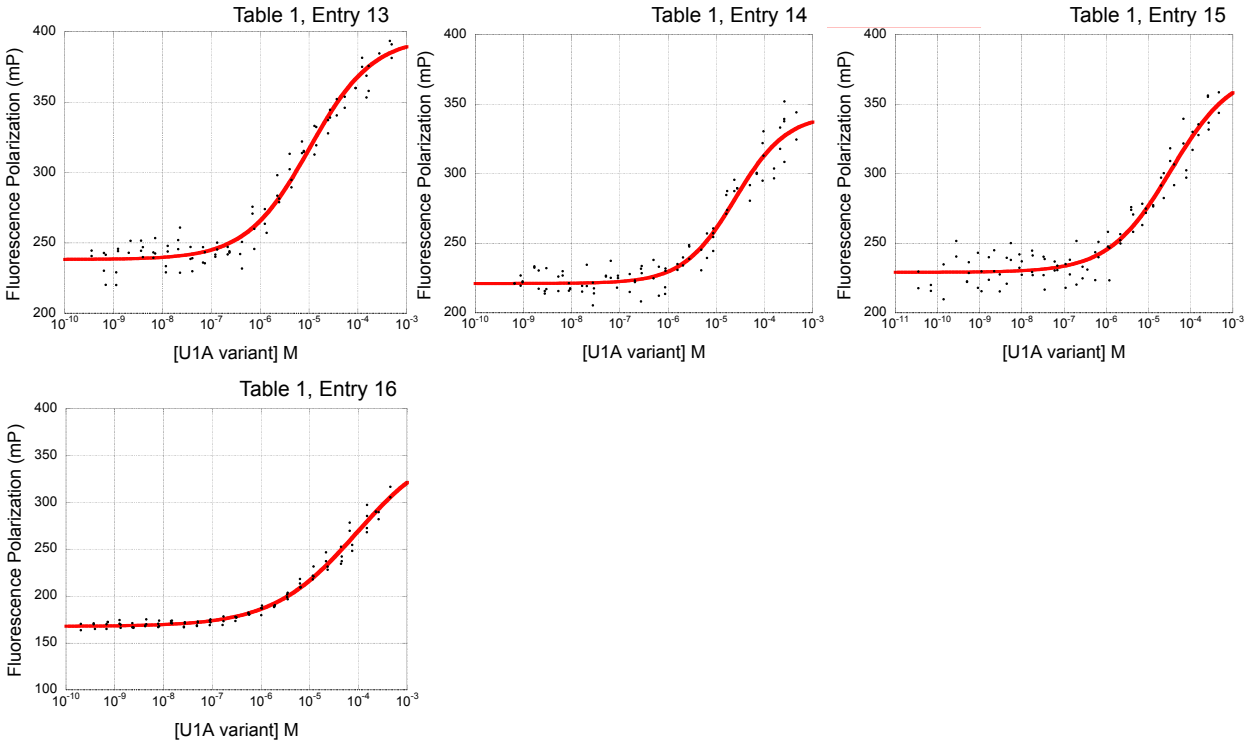
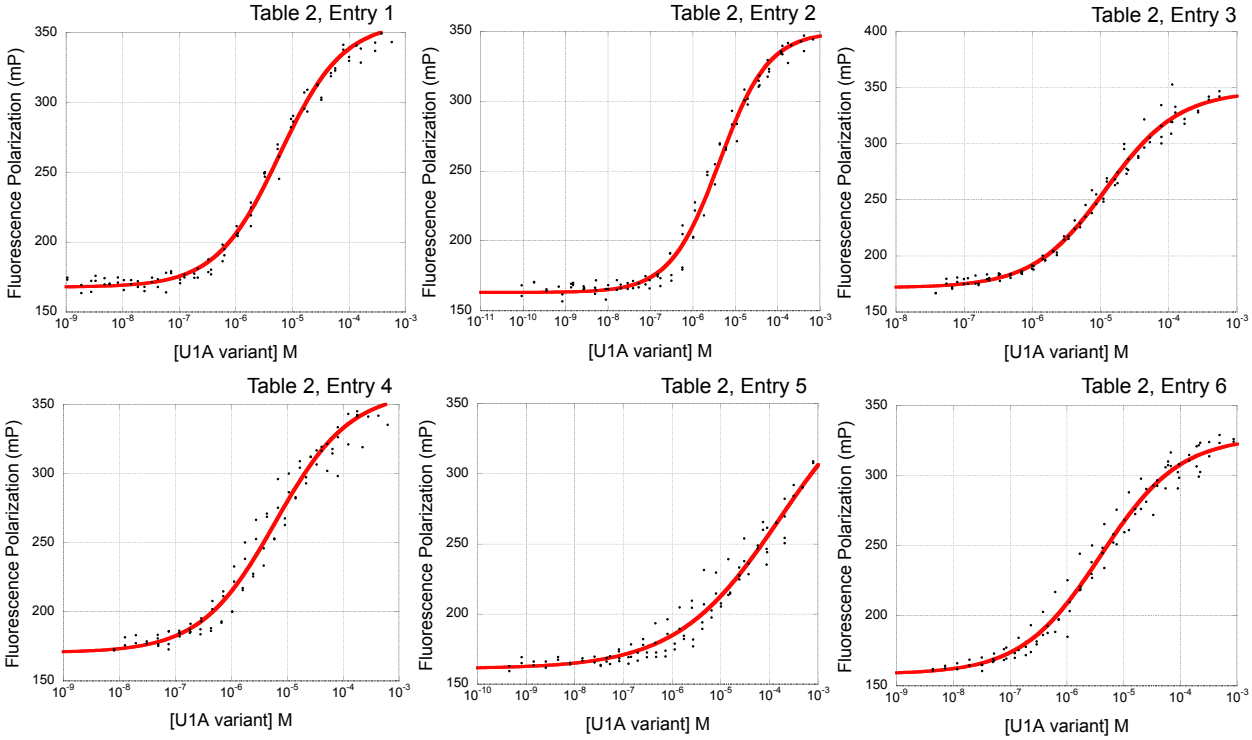



**Figure S2. Fluorescence Polarization Raw Data for *Table 2.*** *Binding affinities for complexes involving TAR RNA and specific U1A- and ΔK50ΔM51-derived mutants.*



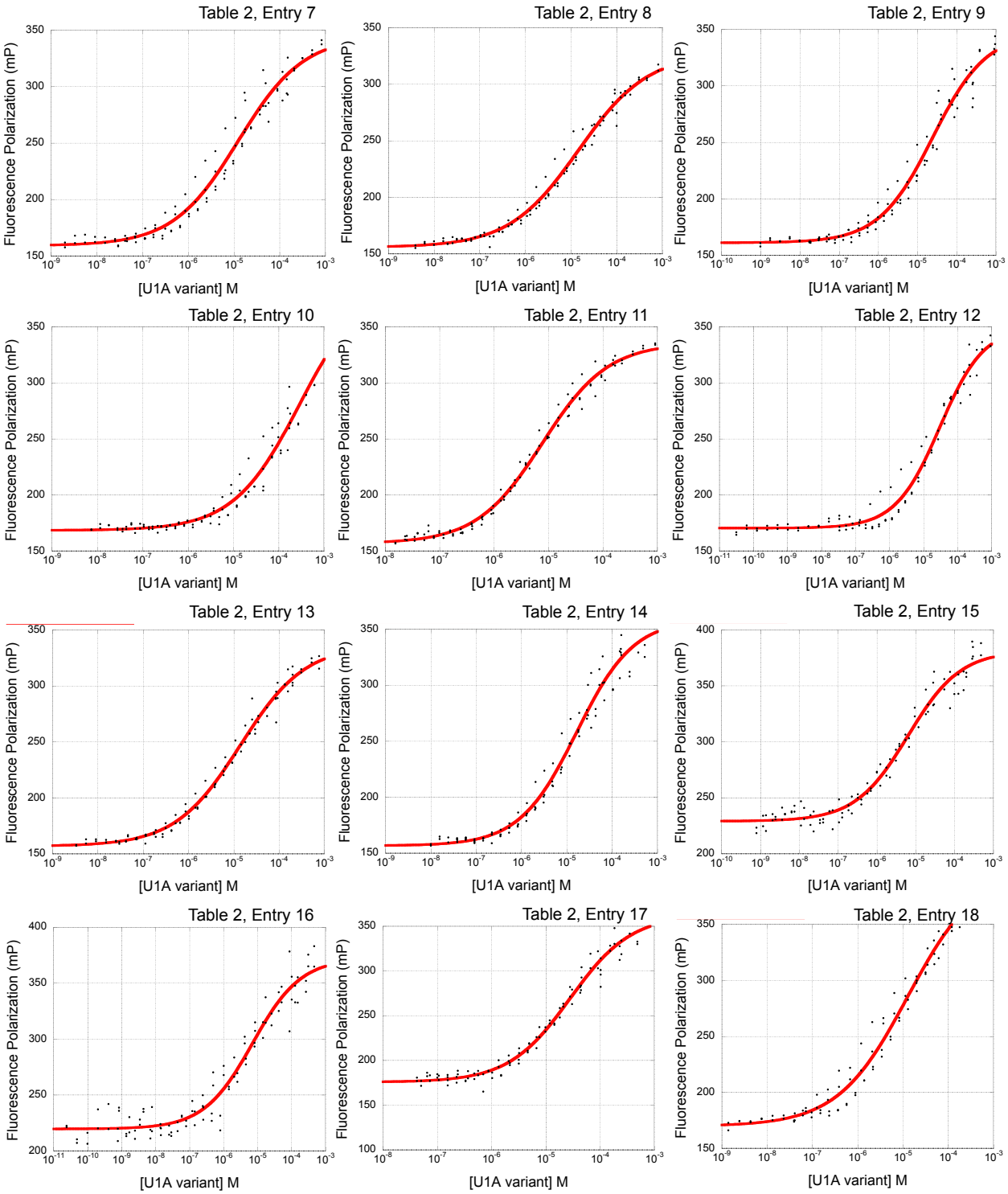
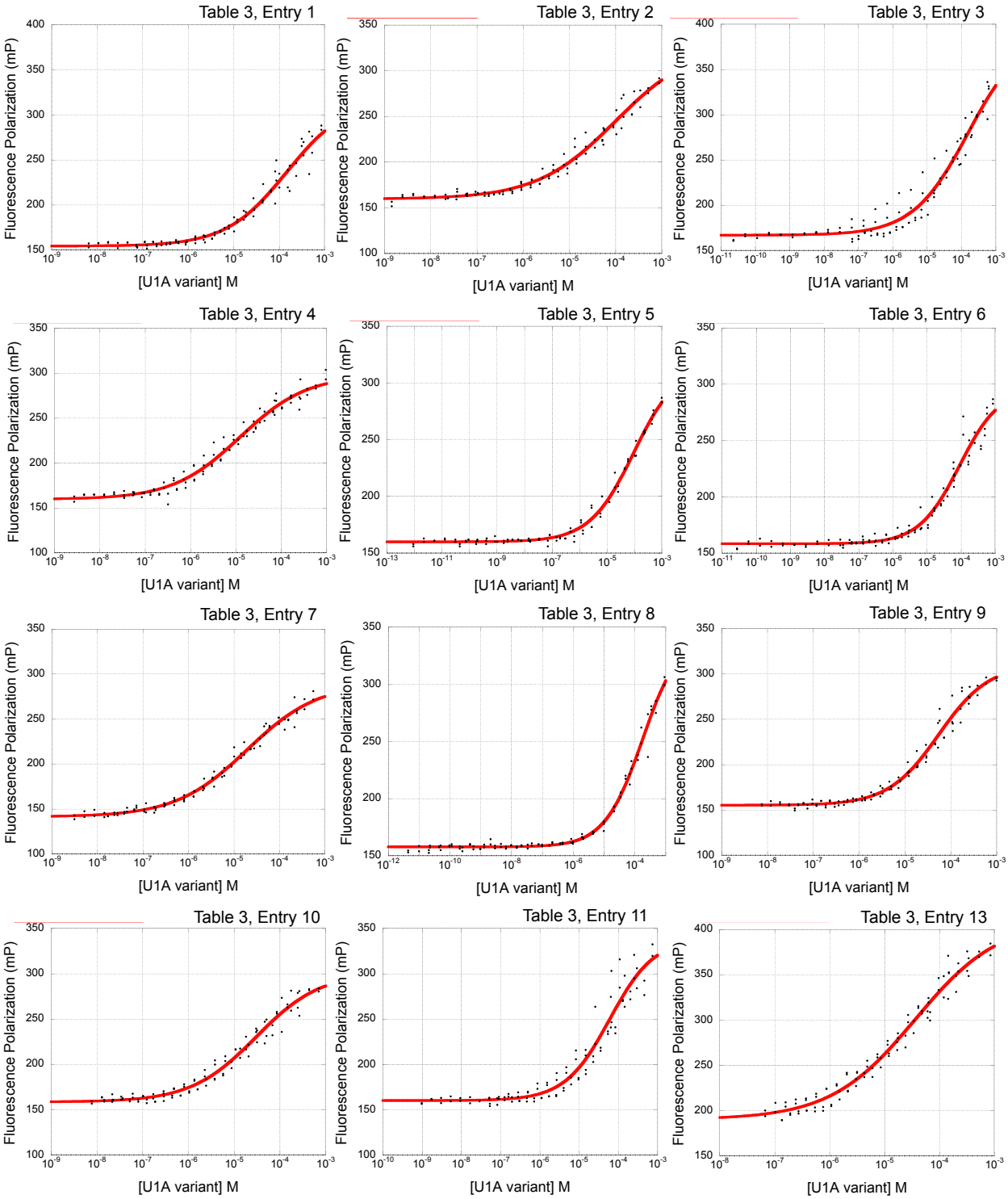


Table 2, Entry 7
Table 2, Entry 8
Table 2, Entry 9
Table 2, Entry 10
Table 2, Entry 11
Table 2, Entry 12
Table 2, Entry 13
Table 2, Entry 14
Table 2, Entry 15
Table 2, Entry 16
Table 2, Entry 17
Table 2, Entry 18
Fluorescence Polarization (mP)
[U1A variant] M

**Figure S3. Fluorescence Polarization Raw Data for Table 3.** *Binding selectivity of specific U1A- and ΔK50ΔM51-derived mutants for TAR RNA over TAR DNA or U1hpII RNA.*



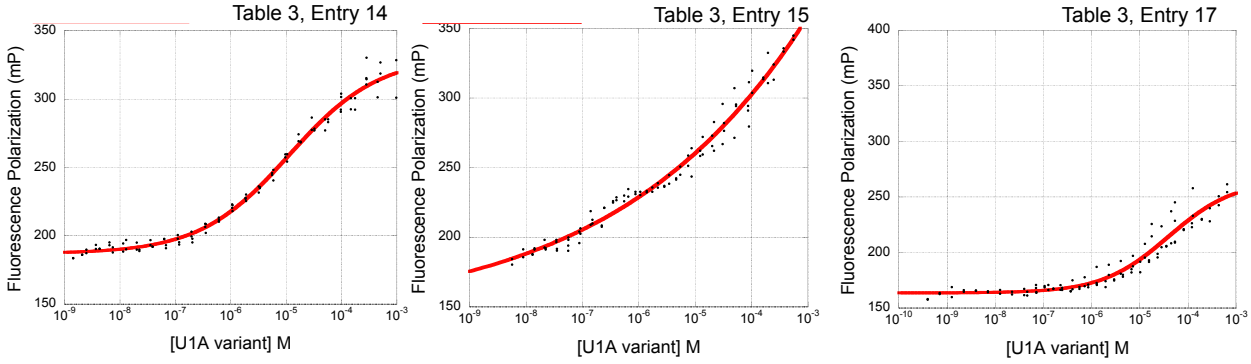
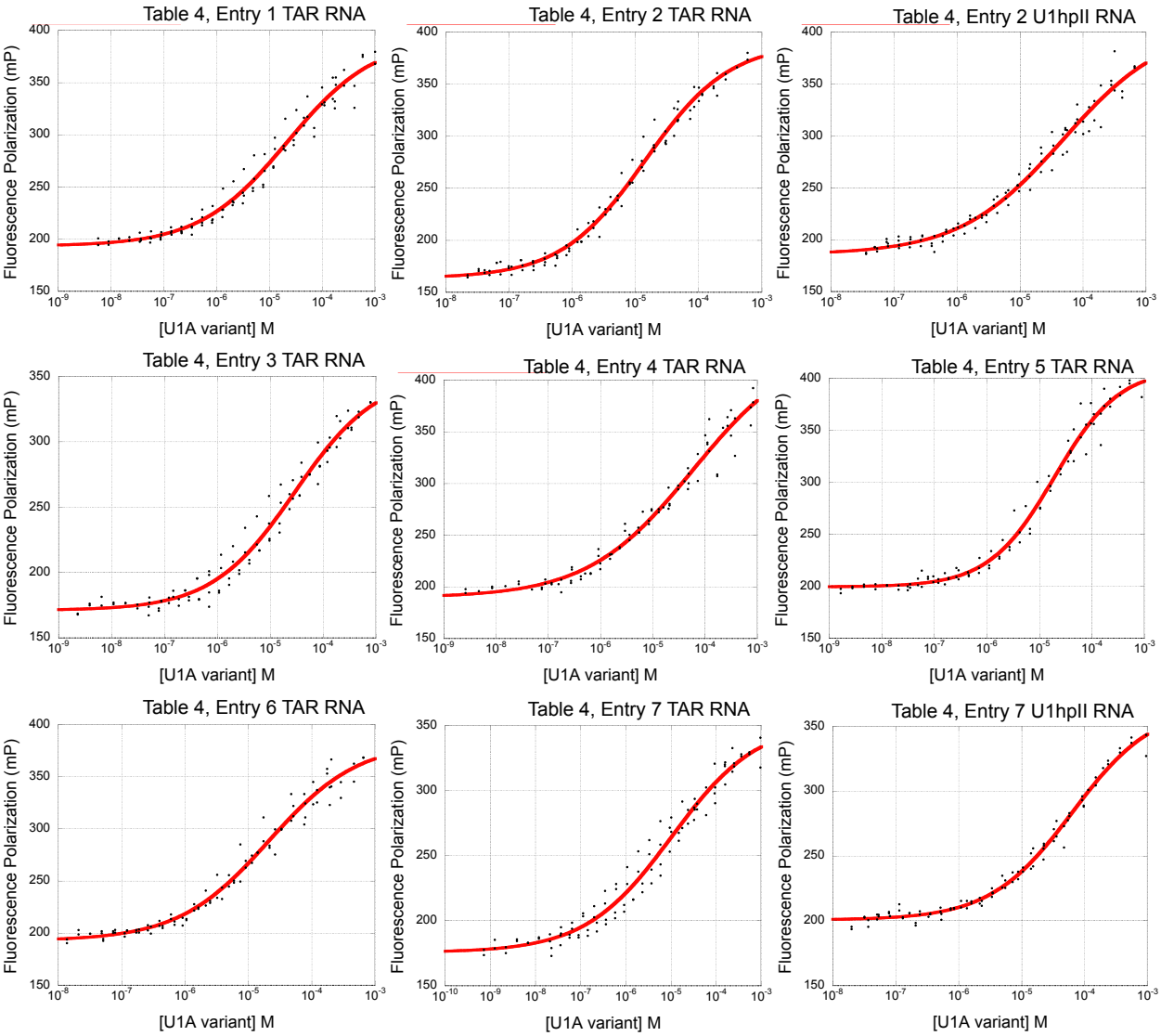



**Figure S4. Fluorescence Polarization Raw Data for *Table 4.*** *Binding selectivity of U1A-derived double mutants for TAR RNA over U1hpII RNA or TAR DNA*.



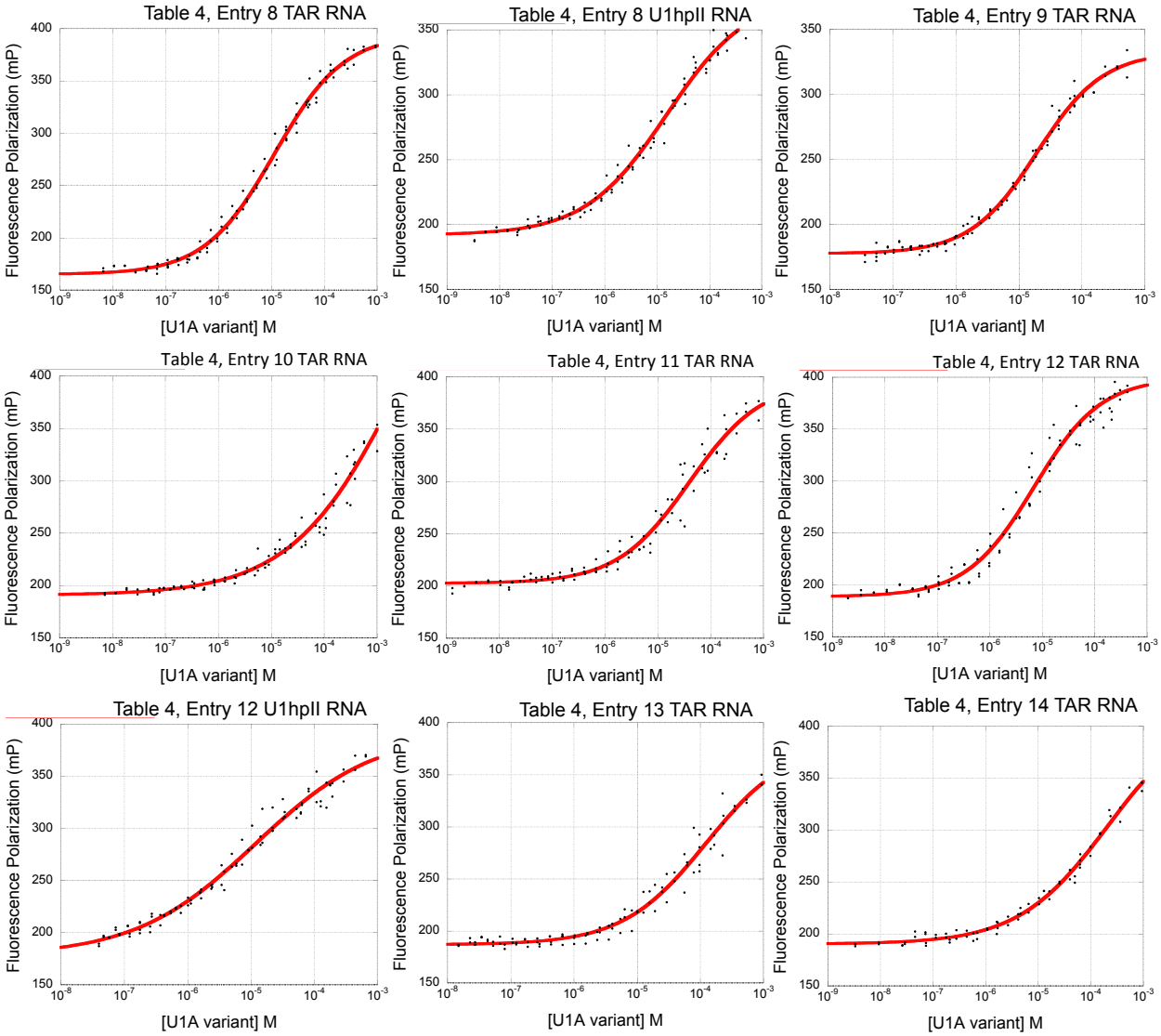
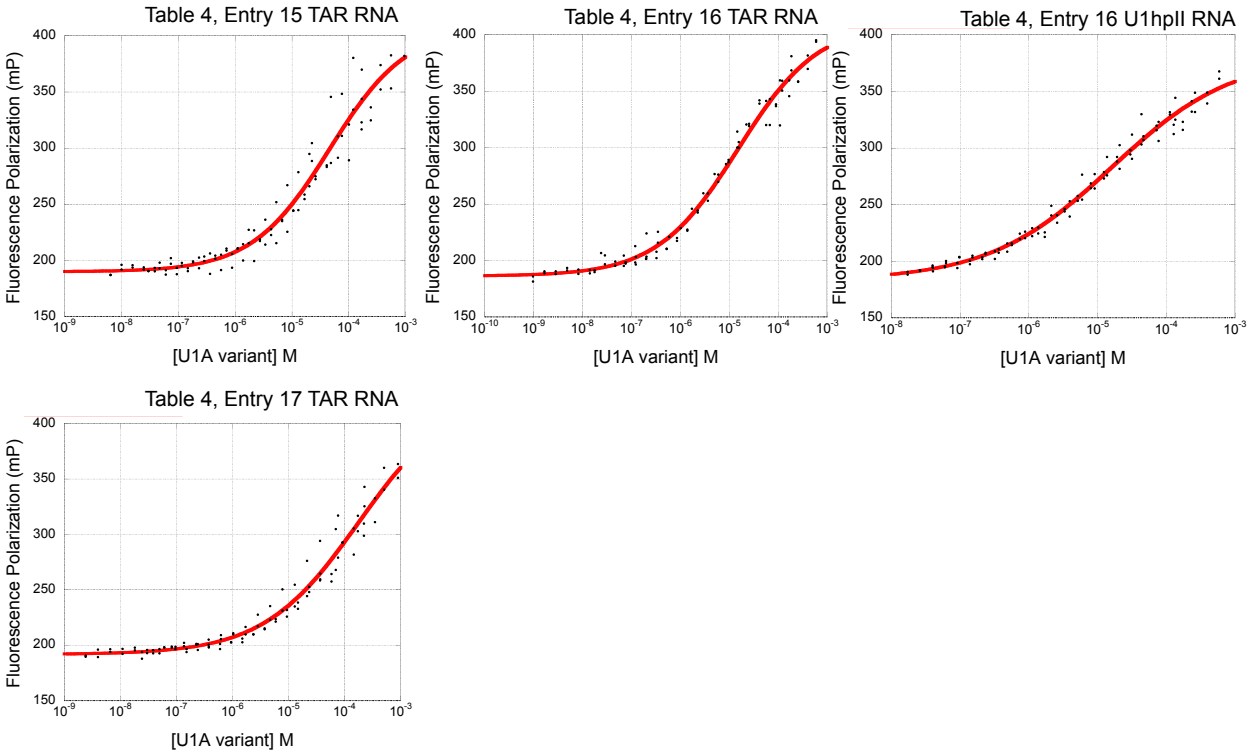


Table 4, Entry 8 TAR RNA
Table 4, Entry 8 U1hpII RNA
Table 4, Entry 9 TAR RNA
Table 4, Entry 10 TAR RNA
Table 4, Entry 11 TAR RNA
Table 4, Entry 12 TAR RNA
Table 4, Entry 12 U1hpII RNA
Table 4, Entry 13 TAR RNA
Table 4, Entry 14 TAR RNA
Fluorescence Polarization (mP)
[U1A variant] M




# Table S3. Selected Variants of U1A Binding to TAR RNA in the Presence or Absence of 10 Molar Equivalents of tRNA

| Entry | RRM scaffold | Mutation | Off Mutation | TAR RNA $K_D$ (μM)[a] | TAR RNA + tRNA $K_D$ (μM)[a] | Fold-change in presence of tRNA[b] |
|---|---|---|---|---|---|---|
| 1 | U1A | Glu19Ser | --- | 4.1 (±0.3) | 6.4 (±0.5) | 1.6 |
| 2 | U1A | Glu19Phe | --- | 6.2 (±0.8) | 19.9 (±1.8) | 3.2 |
| 3 | U1A | Glu19Ser | Tyr13Gln | 10.3 (±1.4) | 10.2 (±0.7) | 1 |

[a]All double mutants are derived from native U1A. [b,c]The error for each reported dissociation constant ($K_D$) is the standard deviation of three separate experiments. [d]Fold-change was calculated by ($K_D$ for TAR RNA + 10 M eq tRNA / $K_D$ TAR RNA).

**Figure S5. Fluorescence Polarization Raw Data for *Table S3.*** *Selected Variants of U1A in Complex with TAR RNA in the Presence of 10 Molar Equivalents of tRNA*.

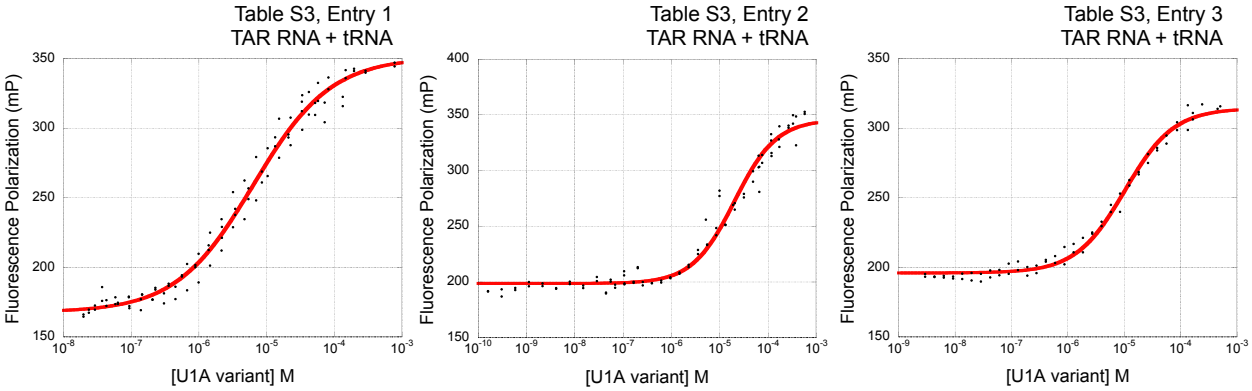
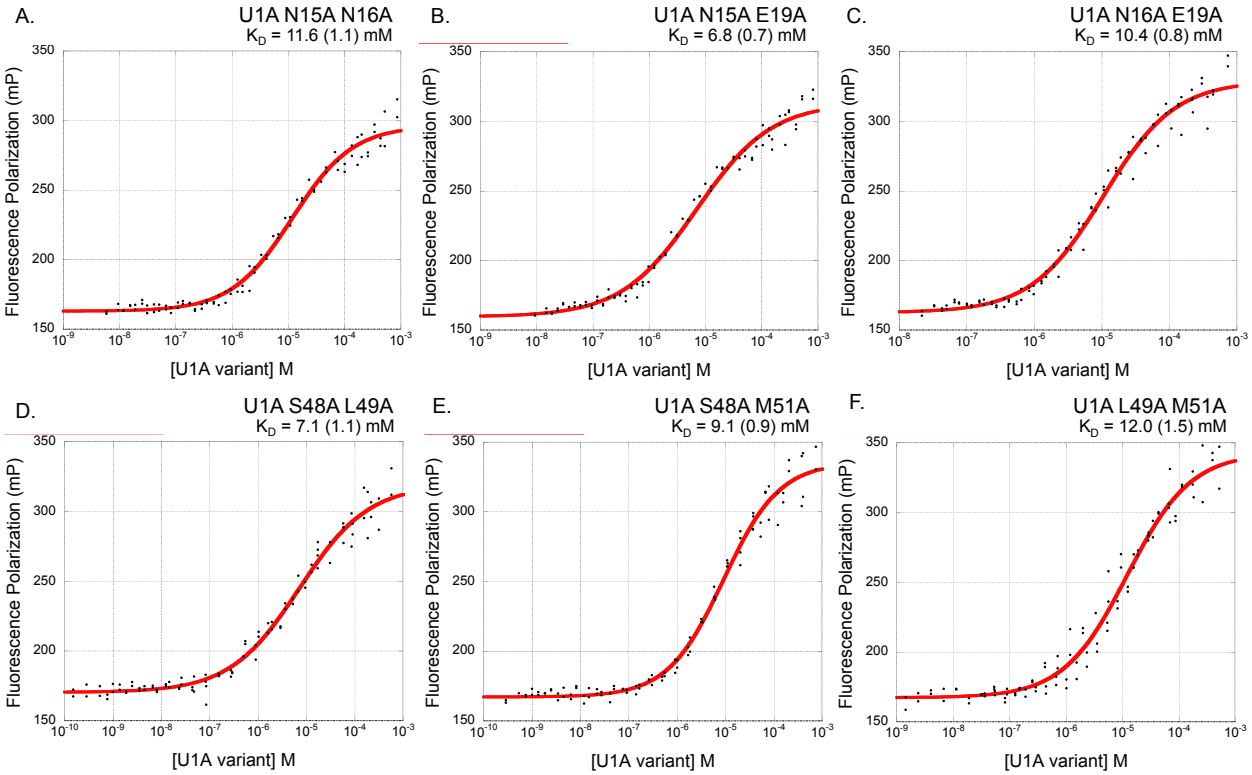
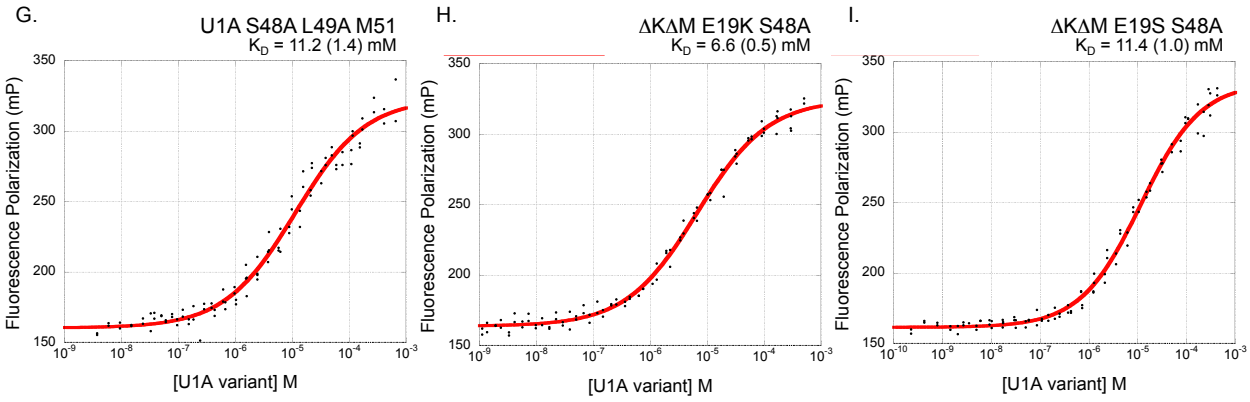


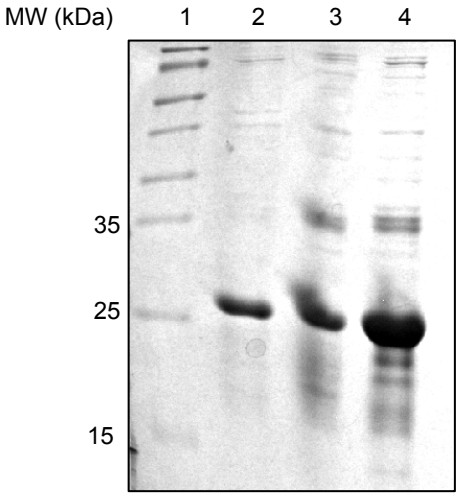

**Figure S6. Combinations of Beneficial Single Mutations into the U1A or ΔK50ΔM51 Scaffold and Tested for Affinity to TAR RNA by Fluorescence Polarization.**






## Purity of Select Proteins Used in this Study as Analyzed by PAGE

## Figure S7. Selected Variants of U1A Featured in Table S3




Lane 1: ladder
Lane 2: U1A Glu19Ser
Lane 3: U1A Glu19Phe
Lane 4: U1A Glu19Ser:Tyr13Gln

[S1] Blakeley, B.D.; Shattuck, J.; Coates, M.B.; Tran, E.; Laird-Offringa, I.A.; McNaughton, B.R. *Biochemistry* **2013**, *52*, 4745.