Supplemental Figure Captions

Table S1. Data collection and refinement statistics for the inosine and m^6A antibody crystal structures.

Table S2. Complete table of λ-dynamics results for inosine antibody screening with the RNA library. RNA chemical structures available in **Fig S3**. Relative binding free energy, $\Delta\Delta G_{bind}$. Standard deviation, $\pm\sigma$. Unbound, u.b. Not specified due to bad sampling, n.s. Entries corresponding to favorable modifications ($\Delta\Delta G_{bind} \leq$ -0.7 kcal/mol) are emphasized in bold italics. Patch name from Xu et al., 2016.

Table S3. Complete table of λ-dynamics results for m^6A antibody screening with the RNA library. RNA chemical structures available in **Fig S3**. Relative binding free energy, $\Delta\Delta G_{bind}$. Standard deviation, $\pm\sigma$. Unbound, u.b. Not specified due to bad sampling, n.s. Entries corresponding to favorable modifications ($\Delta\Delta G_{bind} \leq$ -0.7 kcal/mol) are emphasized in bold italics. Patch name from Xu et al., 2016.

Fig S1. A previously published poly-inosine antibody has a large binding pocket that may accommodate multiple nucleobases. Overview (left) and magnified image (right) of the poly-inosine antibody fragment (PDB ID: 1MRD) binding pocket. An inosine mononucleotide (orange) was modeled into the missing ligand density (green). Heavy chain residues (H) in dark blue and light chain residues (L) in light blue. Water molecules substituting for the potential second mononucleotide are depicted as red spheres, indicating the potential space to bind a second nucleobase. Interacting amino acids include heavy chain residue Arg96 and light chain residues Asn28, Asn30, Tyr32, Lys50, and Ser91. The extended binding pocket (red arrow) includes light chain residue Arg96 and heavy chain residues Gln35, Trp47, Glu50, and Asn58.

Fig S2. Crystal structure of the m^6A Fab apo- form to 2.05 Å (PDB ID: 8TCA). Critical binding pocket amino acids discussed in the main text are labeled. Heavy chain residues (H) are represented in dark blue, light chain residues (L) in light blue, and waters as red spheres. Depicted binding pocket amino acids match those of the m^6A Fab holo- form (**Fig 1A**).

Fig S3. Chemical library of ribonucleoside bases. The library includes the 4 canonical ribonucleobases (A, C, G, and U) and 44 naturally occurring modified derivatives (12 As, 6 Cs, 8 Gs, and 18 Us). Differences between each modification and its respective canonical base are highlighted in green.

Fig S4. Molecular dynamics simulation movie example of the m^6A antibody with a bound nucleoside target. The m^6A Fab binds tightly to $m^6{}_2A$. Movie made in Pymol (Schrödinger, Inc.).

Fig S5. Molecular dynamics simulation movie example of the m^6A antibody with an unbinding nucleoside target. The m^6A Fab unbinds from uridine. Movie made in Pymol (Schrödinger, Inc.).

Fig S6. Structural models of inosine and m^6A antibodies bound to representative off-target RNAs. (**A**) Magnified binding site of the inosine antibody fragment in complex with uridine. (**B-C**) Magnified binding site of the m^6A antibody fragment in complex with (**B**) $m^6{}_2A$ or (**C**) adenosine

611 (A). Heavy chain residues (H) are represented in dark blue, light chain residues (L) in light blue,
612 and the off-target nucleoside in orange. Critical amino acid contacts labeled. (**D-E**) Table of t-test
613 p-value statistics for (**D**) inosine and (**E**) m^6A antibody ELISA binding assay results reported in
614 **Fig 5**. p-values < 0.01 in bold.

Table S1. Data collection and refinement statistics.

	Inosine Fab w/ Inosine (PDB ID: 8SIP)	m6A Fab only (PDB ID: 8TCA)	m6A Fab w ligand (PDB ID: 8VEV)
Wavelength	0.9793	0.9793	0.8731
Resolution range	57.04 - 1.94 (2.009 - 1.94)	70 - 2.02 (2.092 - 2.02)	48.23 - 3.06 (3.18 - 3.06)
Space group	P 1	P 43 21 2	P 21 21 21
Unit cell	39.8174 49.0903 57.3853 83.8419 88.8169 89.6813	79.906 79.906 145.127 90 90 90	83.64 128.377 150.476 90 90 90
Total reflections	218254 (14586)	230543 (22581)	203373 (23011)
Unique reflections	30888 (2931)	31198 (2999)	31244 (3399)
Multiplicity	7.1 (4.9)	7.4 (7.3)	6.5 (6.8)
Completeness (%)	96.20 (91.25)	98.24 (96.71)	99.90 (100.00)
Mean I/sigma(I)	6.33 (3.17)	9.71 (1.08)	16.80 (4.00)
Wilson B-factor	26.61	27.17	78.19
R-merge	0.1901 (1.506)	0.2183 (2.290)	0.078 (0.416)
R-meas	0.2027 (1.642)	0.2341 (2.466)	0.085 (0.451)
R-pim	0.06938 (0.6427)	0.08188 (0.8885)	0.0330 (0.1720)
CC1/2	0.991 (0.586)	0.996 (0.546)	0.998 (0.963)
CC*	0.998 (0.86)	0.999 (0.84)	1.000 (0.990)
Reflections used in refinement	30804 (2931)	31042 (2999)	31207 (3391)
Reflections used for R-free	1554 (154)	1543 (162)	1307 (142)
R-work	0.2043 (0.2460)	0.1807 (0.3043)	0.2238 (0.3156)
R-free	0.2454 (0.2914)	0.2238 (0.3201)	0.2531 (0.3100)

CC(work)	0.947 (0.722)	0.968 (0.727)	0.928 (0.846)
CC(free)	0.929 (0.494)	0.952 (0.735)	0.896 (0.865)
Number of non-hydrogen atoms	3531	3562	9807
macromolecules	3277	3239	9649
ligands	19	36	108
solvent	235	287	50
Protein residues	426	425	1267
RMS(bonds)	0.008	0.009	0.002
RMS(angles)	1.10	1.18	0.56
Ramachandran favored (%)	97.62	97.61	95.34
Ramachandran allowed (%)	2.38	2.39	4.58
Ramachandran outliers (%)	0.00	0.00	0.08
Rotamer outliers (%)	0.80	0.54	0.09
Clashscore	6.46	1.70	2.09
Average B-factor	32.66	33.80	91.07
macromolecules	32.19	33.22	91.26
ligands	32.70	53.08	81.28
solvent	39.15	37.94	75.58
Number of TLS groups	1	9	6

Statistics for the highest-resolution shell are shown in parentheses.

Table S2: Relative binding free energies for inosine Fab screening.

Modified Base	Patch Name	$\Delta\Delta G_{bind}$	± σ
A	ADE	-0.112	0.172
m^2A	2MA	u.b.	u.b.
m^6A	6MA	1.726	0.186
$m^6{}_2A$	M6A	0.605	0.231
m^8A	8MA	2.721	0.389
m^1I	1MI	0.783	0.138
I	INO	0.089	0.024
ms^2m^6A	SMA	-0.037	0.335
ac^6A	6AA	u.b.	u.b.
i^6A	6IA	n.s.	n.s.
ms^2i^6A	MIA	0.598	0.352
ms^2io^6A	SIA	u.b.	u.b.
io^6A	HIA	1.868	0.389
G	GUA	-0.642	0.134
m^1G	1MG	u.b.	u.b.
m^2G	2MG	0.286	0.168
$m^2{}_2G$	M2G	1.057	0.262
preQ0	**DCG**	***-1.737***	***0.187***
imG-14	DWG	-0.005	0.319
imG	IMG	u.b.	u.b.
imG2	**IWG**	***-1.164***	**0.331**
mimG	MWG	u.b.	u.b.
U	**URA**	***-0.926***	***0.145***
D	H2U	u.b.	u.b.
mo^5U	**MOU**	***-1.656***	***0.199***
m^5s^2U	52U	u.b.	u.b.
m^5D	MDU	0.707	0.161
ψ	**PSU**	***-1.985***	***0.490***
$m^3ψ$	**3MP**	***-1.174***	***0.446***
m^3U	3MU	-0.146	0.320
s^4U	**4SU**	***-1.702***	***0.181***
m^5U	**5MU**	***-1.340***	***0.159***
ho^5U	**5HU**	***-1.613***	***0.162***
s^2U	2SU	-0.644	0.233
$m^1ψ$	**1MP**	***-0.896***	***0.112***
cnm^5U	**CYU**	***-2.147***	***0.233***
mcm^5s^2U	**70U**	***-1.379***	***0.330***
$mchm^5U$	CMU	-0.612	0.381
ncm^5U	**BCU**	***-1.683***	***0.393***
mcm^5U	**OCU**	***-1.307***	***0.312***
$mcmo^5U$	OEU	-0.491	0.396
C	CYT	u.b.	u.b.
m^5C	5MC	u.b.	u.b.
ac^4C	4AC	u.b.	u.b.
m^4C	4MC	u.b.	u.b.
f^5C	5FC	0.596	0.265
hm^5C	HMC	u.b.	u.b.
s^2C	2SC	u.b.	u.b.

"Patch Name" = 3-letter name assigned by Xu et al. (2016)

Modifications with ΔΔG ≤ -0.7 kcal/mol in bold italics.

Table S3: Relative binding free energies for m^6A Fab screening.

Modified Base	Patch Name	$\Delta\Delta G_{bind}$	± σ
A	ADE	0.300	0.165
m^2A	2MA	3.084	0.156
m^6A	6MA	-0.034	0.032
$m^6{}_2A$	**M6A**	***-2.091***	**0.052**
m^8A	8MA	4.432	0.337
m^1I	1MI	n.s.	n.s.
I	INO	6.247	0.301
ms^2m^6A	SMA	2.634	0.209
ac^6A	**6AA**	***-1.156***	**0.197**
i^6A	6IA	1.624	0.491
ms^2i^6A	MIA	3.587	0.434
ms^2io^6A	SIA	n.s.	n.s.
io^6A	HIA	n.s.	n.s.
G	GUA	4.881	0.471
m^1G	1MG	3.029	0.521
m^2G	2MG	u.b.	u.b.
$m^2{}_2G$	M2G	n.s.	n.s.
preQ0	DCG	4.229	0.283
imG-14	DWG	u.b.	u.b.
imG	IMG	n.s.	n.s.
imG2	IWG	u.b.	u.b.
mimG	**MWG**	***-2.437***	**0.441**
U	URA	u.b.	u.b.
D	H2U	2.841	0.242
mo^5U	MOU	1.861	0.459
m^5s^2U	52U	3.817	0.504
m^5D	MDU	1.520	0.25
ψ	PSU	3.564	0.531
$m^3ψ$	3MP	n.s.	n.s.
m^3U	3MU	n.s.	n.s.
s^4U	4SU	n.s.	n.s.
m^5U	5MU	1.270	0.519
ho^5U	5HU	0.965	0.305
s^2U	2SU	2.830	0.705
$m^1ψ$	1MP	3.044	0.663
cnm^5U	CYU	2.396	0.282
mcm^5s^2U	70U	n.s.	n.s.
$mchm^5U$	CMU	2.686	0.235
ncm^5U	BCU	n.s.	n.s.
mcm^5U	OCU	1.113	0.402
$mcmo^5U$	OEU	3.469	0.527
C	CYT	5.033	0.708
m^5C	5MC	n.s.	n.s.
ac^4C	**4AC**	***-1.199***	**0.325**
m^4C	4MC	2.159	0.287
f^5C	5FC	2.275	0.422
hm^5C	HMC	u.b.	u.b.
s^2C	2SC	u.b.	u.b.

"Patch Name" = 3-letter name assigned by Xu et al. (2016)

Modifications with ΔΔG ≤ -0.7 kcal/mol in bold italics.

Figure S1

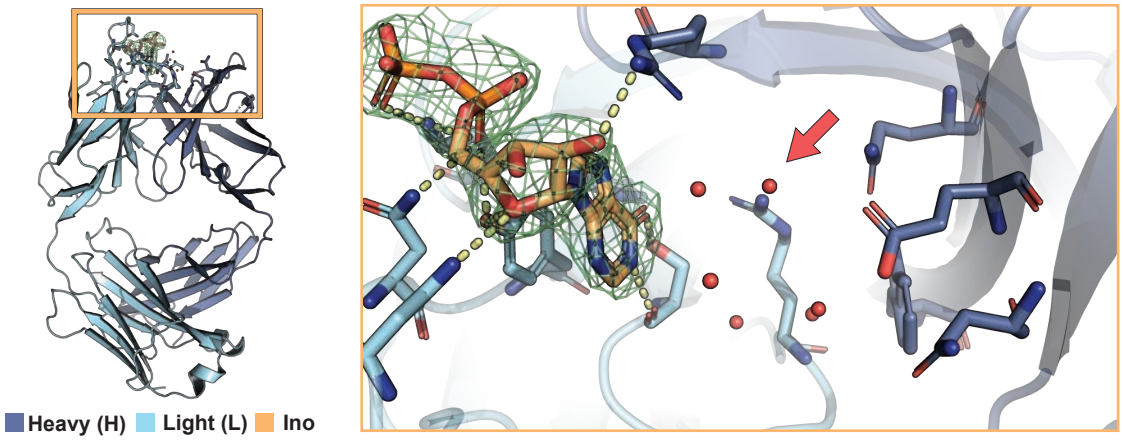


Figure S1

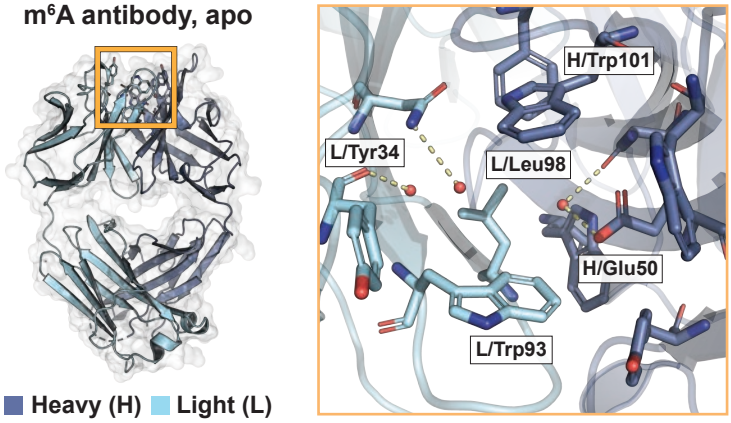


Figure S3

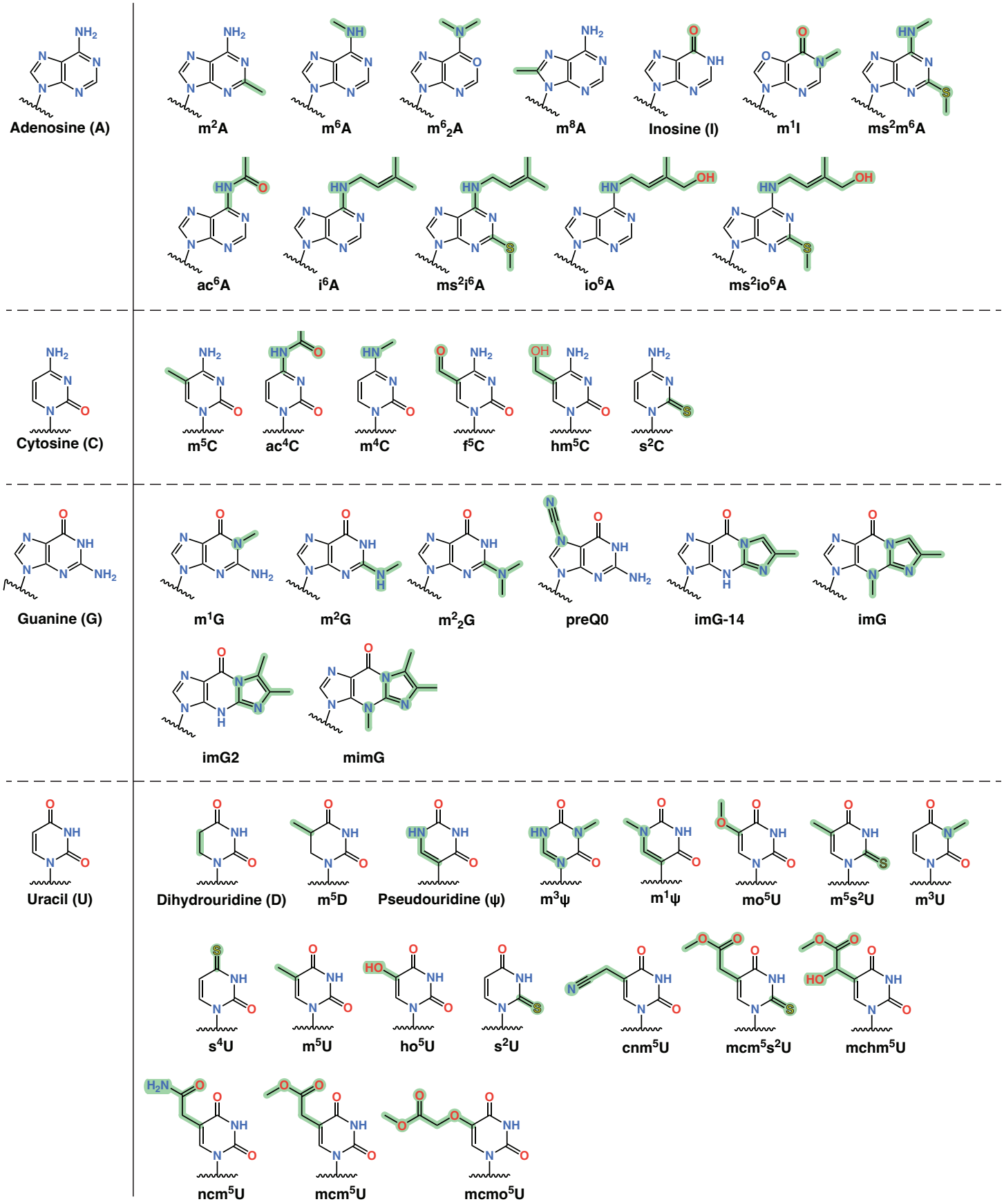
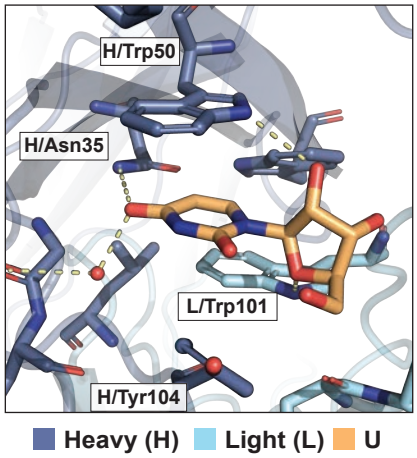


Figure S6

A



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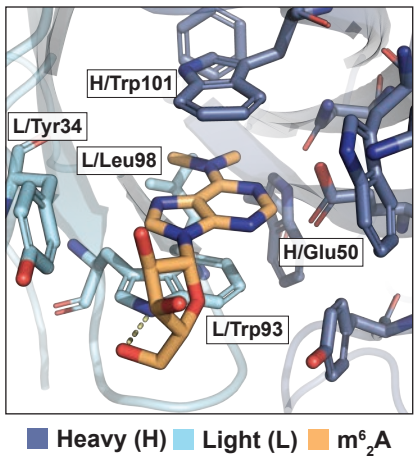

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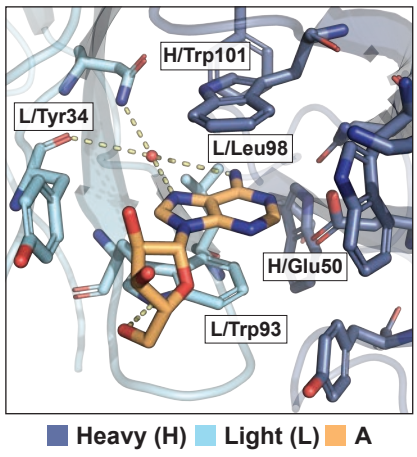

D Inosine antibody ELISA results, t-test p values. p < 0.01 in bold italics.

Concentration (ng/ml)	I vs. C	U vs. C	A vs. C	I vs. U
1000	***0.008176***	***0.000068***	0.231657	0.022235
100	***0.005824***	***0.000199***	0.371234	***0.006713***
10	***0.000112***	0.079051	0.703358	***0.000115***
1	***0.000376***	0.828319	0.872158	***0.000401***
0.1000	0.030556	0.608653	0.650652	0.032961
0.0100	0.289681	0.795255	>0.999999	0.284906
0.0010	0.390739	0.366411	0.421648	0.507010
0	0.593139	0.350393	0.507644	0.440630

E m^6A antibody ELISA results, t-test p values. p < 0.01 in bold italics.

Concentration (ng/ml)	m^6A vs. C	A vs. C	$m^6{}_2A$ vs. C	m^6A vs. A
100	***0.00007***	***0.001121***	***0.000043***	***0.004438***
33.300	***0.000017***	***0.000294***	***0.000005***	***0.001808***
11.100	***0.000034***	***0.000209***	***0.000024***	***0.005227***
3.700	***0.000009***	***0.000072***	***0.000017***	***0.002905***
1.230	***0.000055***	***0.001183***	***0.001946***	***0.000026***
0.412	0.086944	0.342808	0.080748	0.239781
0.137	0.723367	0.72624	0.506021	0.974438
0	0.799706	0.959434	0.884815	0.781924