Reactivity of N-Acyl Hydrazone Probes with the Mammalian Proteome

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SUPPLEMENTARY INFORMATION

Supplemental Tables

Table S1. MS identification of putative targets of hydrazone probes A, B and C by pull-down of Huh7 lysates and on-bead digest

Accession #	Protein ID	Mascot Score	MW (Da)	pI	Sequence Coverage (%)	# of Peptides
Hydrazone A						
IPI00021439	ACTB Actin, cytoplasmic 1	209	41710	5.29	20.8	6
IPI00166768	TUBA1C Uncharacterized protein	176	36719	8.76	10.7	2
IPI00065051	CCDC13 Coiled-coil domain-containing protein 13	48	80834	8.83	1.3	1
IPI00013876	APOBEC3A Isoform 1 of Probable DNA dC->dU-editing enzyme APOBEC-3A	42	22997	6.34	4.5	1
IPI00218429	PREX1 Isoform 2 of Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein	39	106026	5.32	2.1	1
Hydrazone B						
IPI00021439	ACTB Actin, cytoplasmic 1	548	41710	5.29	29.1	9
IPI00008603	ACTA2 Actin, aortic smooth muscle	438	41982	5.23	27.1	8
IPI00166768	TUBA1C Uncharacterized protein	385	36719	8.76	22.1	5
IPI00414676	HSP90AB1 Heat shock protein HSP 90-beta	344	83212	4.97	20.4	11
IPI00382470	HSP90AA1 Isoform 2 of Heat shock protein HSP 90-alpha	333	98099	5.07	9	6
IPI00007752	TUBB2C Tubulin beta-2C chain	194	49799	4.79	9.9	2
IPI00645452	TUBB Uncharacterized protein	162	47736	4.7	13.1	3
IPI00784154	HSPD1 60 kDa heat shock protein, mitochondrial	162	61016	5.7	4.4	1
IPI00219757	GSTP1 Glutathione S-transferase P	130	23341	5.43	9	1
IPI00005719	RAB1A Isoform 1 of Ras-related protein Rab-1A	121	22663	5.93	18.5	3
IPI00396485	EEF1A1 Elongation factor 1-alpha 1	115	50109	9.1	15.8	5
IPI00030131	TMPO Isoform Beta of Lamina-associated polypeptide 2, isoforms beta/gamma	113	50639	9.39	6.2	2
IPI00027223	IDH1 Isocitrate dehydrogenase [NADP] cytoplasmic	105	46630	6.53	6.3	2
IPI00978298	RPS3 Uncharacterized protein	97	25355	9.73	12.6	2
IPI00009235	SSR3 Translocon-associated protein subunit gamma	93	21067	9.61	7.6	1
IPI00218914	ALDH1A1 Retinal dehydrogenase 1	83	54827	6.3	2.6	1
IPI00031697	TMEM109 Transmembrane protein 109	71	26194	10.48	4.9	1
IPI00022202	SLC25A3 Isoform A of Phosphate carrier protein, mitochondrial	66	40069	9.45	3.3	1
IPI00186290	EEF2 Elongation factor 2	64	95277	6.41	4.7	1
IPI00023001	FAM162A Protein FAM162A	58	17331	9.81	7.1	1
IPI00329596	TMX2 Uncharacterized protein	58	42444	8.86	2.7	1
IPI00011893	- mRNA clone with similarity to L-glycerol-3-phosphate:NAD oxidoreductase and albumin gene sequences	54	12560	6.41	14.7	1
IPI00216587	RPS8;SNORD55 40S ribosomal protein S8	52	24190	10.32	5.3	1
IPI00013296	RPS18;RPS18P9 40S ribosomal protein S18	52	17708	10.99	7.2	1
IPI00000875	EEF1G cDNA FLJ56389, highly similar to Elongation factor 1-gamma	51	56114	7.6	4.7	1
IPI00292056	PIK3C2B Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing subunit beta	49	184651	6.95	0.5	1

IPI00440493	ATP5A1 ATP synthase subunit alpha, mitochondrial	47	59714	9.16	2.4	1
IPI00012493	SNORD54;RPS20 40S ribosomal protein S20	46	13364	9.95	12.6	1
IPI00160775	SIDT1 Isoform 2 of SID1 transmembrane family member 1	46	94295	6.6	1	1
IPI00872541	SCN10A Sodium channel protein type 10 subunit alpha	45	220484	5.67	0.4	1
IPI00215884	SRSF1 Isoform ASF-1 of Serine/arginine-rich splicing factor 1	45	27728	10.37	4.8	1
IPI00383879	AADAC Arylacetamide deacetylase	45	45705	8.75	3	1
IPI00976298	ASCC1 Protein	44	20583	6.27	8	1
IPI00735270	KIAA1109 185 kDa protein	43	185104	6.52	0.5	1
IPI00011399	IL24 Interleukin-24	41	23809	8.94	6.3	1
IPI00218429	PREX1 Isoform 2 of Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein	40	106026	5.32	2.1	1

Accession #	**Protein ID**	**Mascot Score**	**MW (Da)**	**pI**	**Sequence Coverage (%)**	**# of Peptides**
Hydrazone C						
IPI00021439	ACTB Actin, cytoplasmic 1	243	41710	5.29	24	6
IPI00166768	TUBA1C Uncharacterized protein	176	36719	8.76	10.7	2
IPI00218914	ALDH1A1 Retinal dehydrogenase 1	139	54827	6.3	8.6	3
IPI00014424	EEF1A2 Elongation factor 1-alpha 2	84	50438	9.11	4.3	1
IPI00878429	PRR14L Protein	42	23555	9.59	10.6	1
IPI00041320	MDM2 p53-binding protein	42	27289	5.22	3.3	1
IPI00386085	MAFK Putative uncharacterized protein DKFZp547K209 (Fragment)	40	20112	9.02	7.8	1
IPI00218429	PREX1 Isoform 2 of Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 protein	38	106026	5.32	2.1	1

Table S2. MS identification of interacting residues of APOBEC3A with probe A

	Trial 1		Trial 2		Trial 3	
Residue	Confidence	Xcorr	Confidence	Xcorr	Confidence	Xcorr
S4	High	1.392324	Medium	1.22949	High	0.720779
S7	High	0.698057	Medium	1.22949	Medium	1.0996
S20	High	1.654854	nd	nd	nd	nd
C34	High	2.57	Medium	0.597153	Medium	0.796544
C34	High	1.39	High	2.336112	High	1.012486
C34	High	1.01	High	1.098587	High	2.366712
S45	High	1.258144	Medium	0.587737	Medium	0.856634
S45	Medium	0.876965	High	0.894275	Medium	0.796544
S81	nd	nd	High	1.183304	High	1.638683
S97	nd	nd	High	1.183304	High	1.461768
S99	nd	nd	High	0.822397	High	1.461768
S103	nd	nd	High	0.822397	High	1.638683
S151	nd	nd	High	1.134772	nd	nd
C161	nd	nd	High	1.355711	nd	nd
C171	nd	nd	High	1.355711	nd	nd
S183	nd	nd	High	1.355711	nd	nd
S187	nd	nd	Medium	0.738073	nd	nd

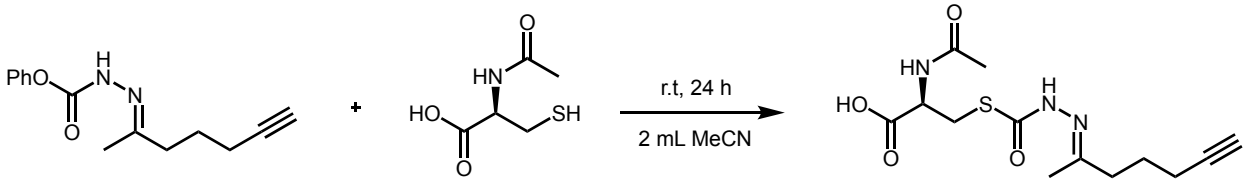
Table S3. MS identification of interacting residues of APOBEC3A with probe A

	Confidence	Annotated Sequence	Modification	Charge	m/z [Da]	MH+ [Da]	DeltaM [ppm]	Deltam/z [Da]	XCorr	Area
Trial 1	High	[K].TYLcYEVER.[L]	C34 (+150)	2	663.3096	1325.612	1.831705	0.001214	2.574408	640610.2
Trial 2	High	[K].TYLcYEVER.[L]	C34 (+150)	2	663.3063	1325.605	-3.14095	-0.00208	2.336112	719621
Trial 3	High	[K].TYLcYEVER.[L]	C34 (+150)	2	663.3102	1325.613	2.844653	0.001885	2.366712	478769.8

General

IR spectra were collected on all samples in the solid state on a Varian 640 FT-IR spectrometer in the 525-4000 cm^{-1} range.

Procedure for the reaction shown in equation 1



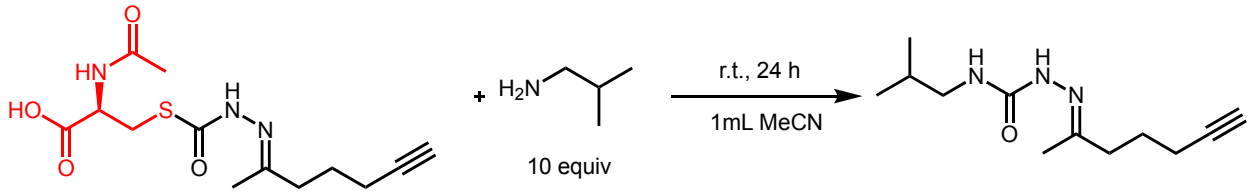
0.2 mmol

0.2 mmol

w/ 0.1 mmol Et_3N: 22% product, starting materials remaining
w/ 0.4 mmol Et_3N: 60% product, no starting material left

The reaction was performed according to the procedure described in the literature,[i] on the reaction scale described above, and isolated by silica gel column chromatography to afford a white solid, m.p. 160-161 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 8.15 (d, J = 7.9 Hz, 1H), 4.29 (td, J = 8.4, 4.7 Hz, 1H), 3.28 (dd, J = 13.5, 4.8 Hz, 1H), 2.89 (dd, J = 13.6, 8.7 Hz, 1H), 2.77 (t, J = 2.6 Hz, 1H), 2.27 (dd, J = 8.1, 7.0 Hz, 2H), 2.20 (td, J = 7.1, 2.7 Hz, 2H), 1.83 (s, 3H), 1.83 (s, 3H), 1.65 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.6, 170.5, 169.6, 153.8, 84.7, 71.9, 52.7, 37.3, 30.1, 24.9, 22.8, 17.7, 17.1. IR (FT-IR): 3277, 3176, 3056, 2919, 2850, 1703, 1652, 1539, 1419, 1271, 1227, 1177, 1112, 1041, 986, 817, 719, 647, 599, 563, 535 cm^{-1}. HRMS (ESI): Exact mass calculated for $C_{13}H_{19}N_3O_4SNa$ $[M+Na]^+$ 336.0994, found 336.0992. Similarly, a reaction was performed in D_2O at a concentration of 0.01 M. While the probe was not fully soluble at the beginning of the reaction, most of the probe dissolved as the reaction proceeded. After 24 hours, the reaction was analyzed by ^{1}H NMR spectroscopy, after adding a known amount of *N,N*-dimethylformamide to serve as internal standard. This revealed that 86% of the starting material was converted to the product shown above.

Procedure for the reaction shown in equation 2



10 equiv

The reaction was performed according to the procedure described in the literature,[i] but using a 10-fold excess of isobutylamine but on half the reaction scale described above, and isolated by silica gel column chromatography to afford a yellow oil (18% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 6.20 (s, 1H), 3.14 – 3.06 (m, 2H), 2.41 – 2.28 (m, 2H), 2.22 (td, J = 7.0, 2.6 Hz, 2H), 1.95 (t, J = 2.6 Hz, 1H), 1.85 – 1.79 (m, 1H), 1.78 (s, 3H), 1.75 (dd, J = 6.9, 3.6 Hz, 2H), 0.92 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.5, 148.1, 83.8, 68.8, 47.0, 37.4, 29.0, 24.9, 20.0, 17.9, 15.3. IR(FT-IR): 3417, 3310, 3208, 2956, 2870, 1671, 1532, 1467, 1386, 1369, 1341, 1291, 1240, 1163, 1107, 1057, 863, 821, 761, 625 cm^{-1}. HRMS (ESI): Exact mass calculated for $C_{12}H_{21}N_3ONa$ $[M+Na]^+$ 246.1592, found 246.1582. For comparison, the same reaction was performed with the probe and conditions shown in equation 1, leading to a 58% yield.

Supplemental Figures

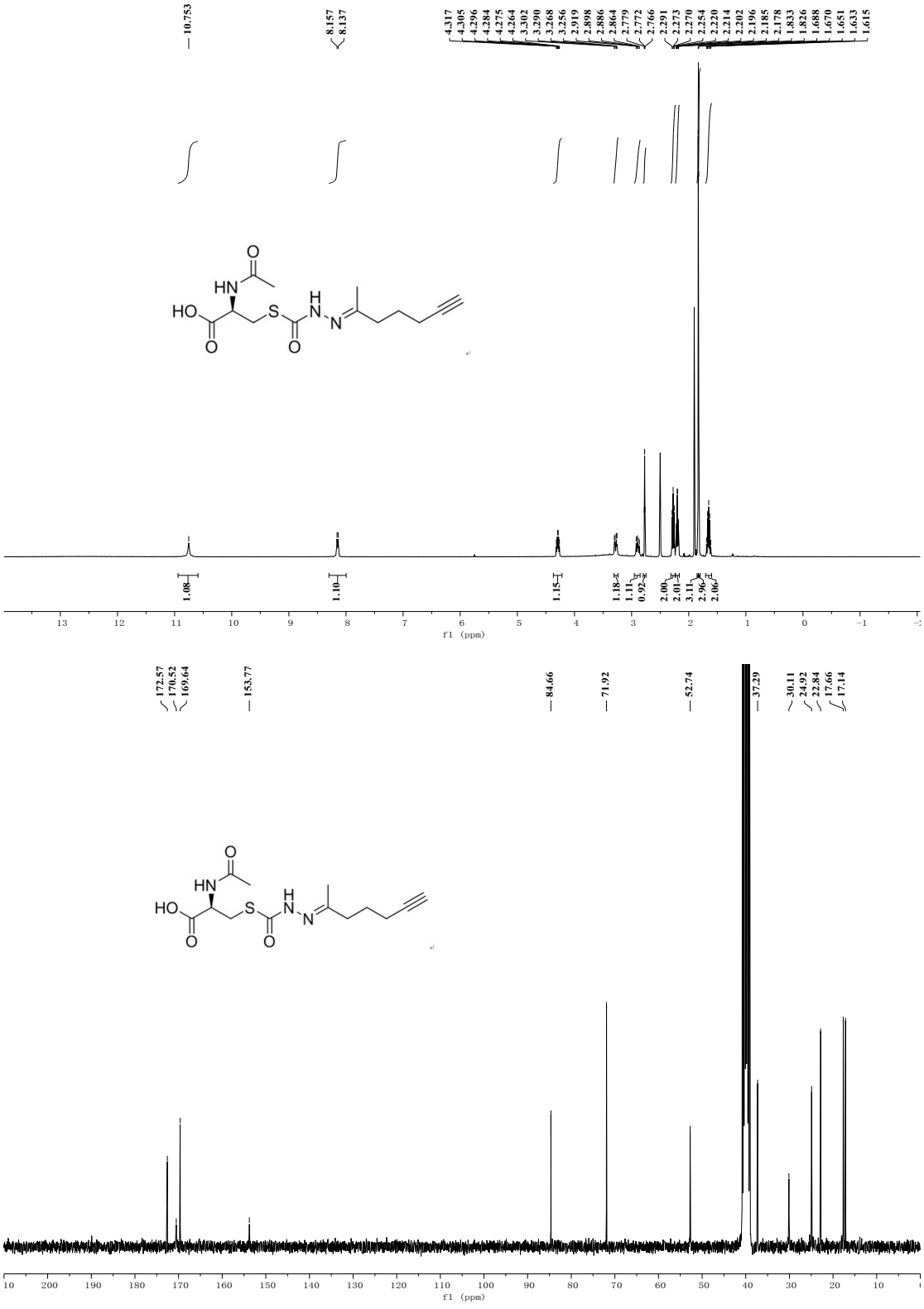


Figure S1. ^{1}H and ^{13}C NMR spectra of the N-AcCysteine - probe addition product (equation 1)

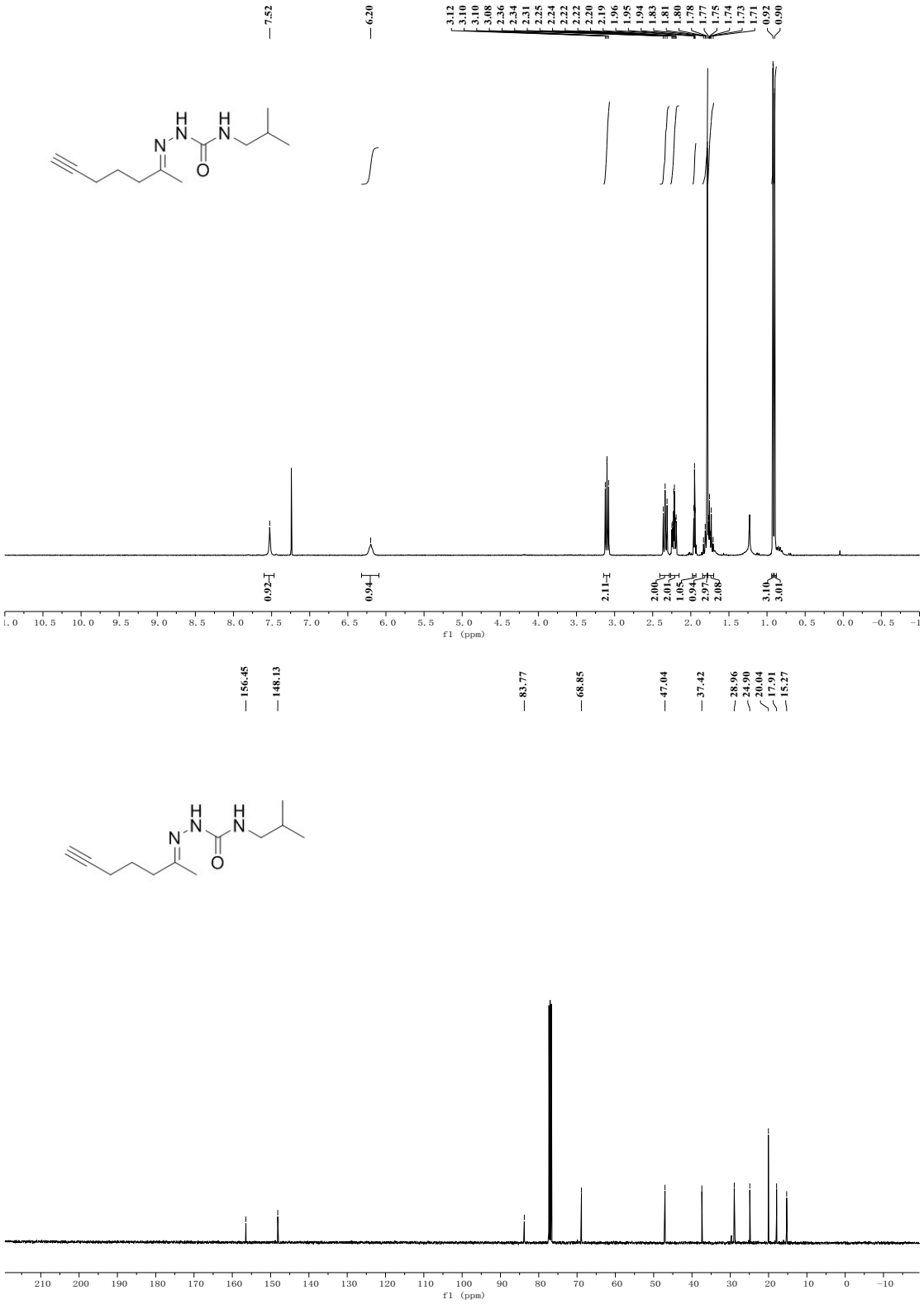


Figure S2. ^{1}H and ^{13}C NMR spectra of the substitution product of the reaction with isobutylamine (equation 2)

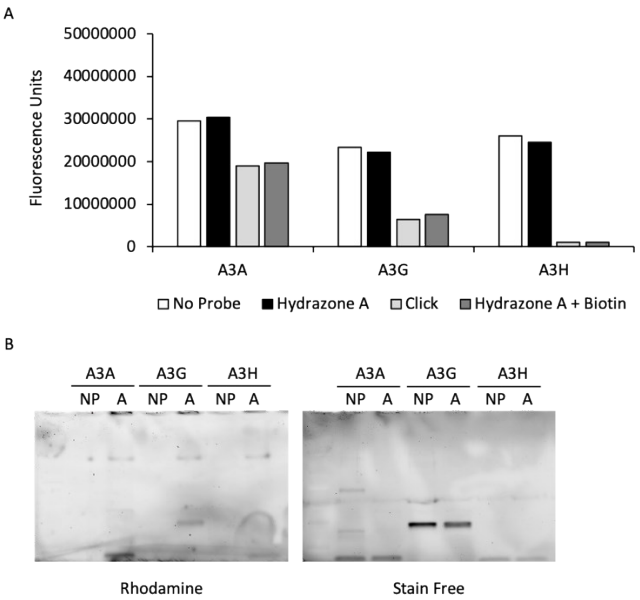


Figure S3. Activity of APOBEC proteins incubated with probe A. Purified APOBEC proteins were incubated with probe A for 1 hour before being reacted with biotin azide, rhodamine azide or control. The proteins were then used for A) deamination assay or B) In-gel fluorescence showing rhodamine fluorescence from probe-labeled purified proteins, used as a control to confirm click reaction. On the right is corresponding stain free gel showing presence of proteins. APOBEC3A, 3G, and 3H are abbreviated A3A, A3G, and A3H. NP is no probe control.

REFERENCES

(1) Garland, K.; Gan, W.; Depatie-Sicard, C.; Beauchemin, A. M. A Practical Approach to Semicarbazone and Hydrazone Derivatives via Imino-Isocyanates. Org. Lett. 2013, 15 (16), 4074–4077. https://doi.org/10.1021/ol4016089.