

Rational Development and Characterization of a Ubiquitin Variant with Selectivity for Ubiquitin C-Terminal Hydrolase L3

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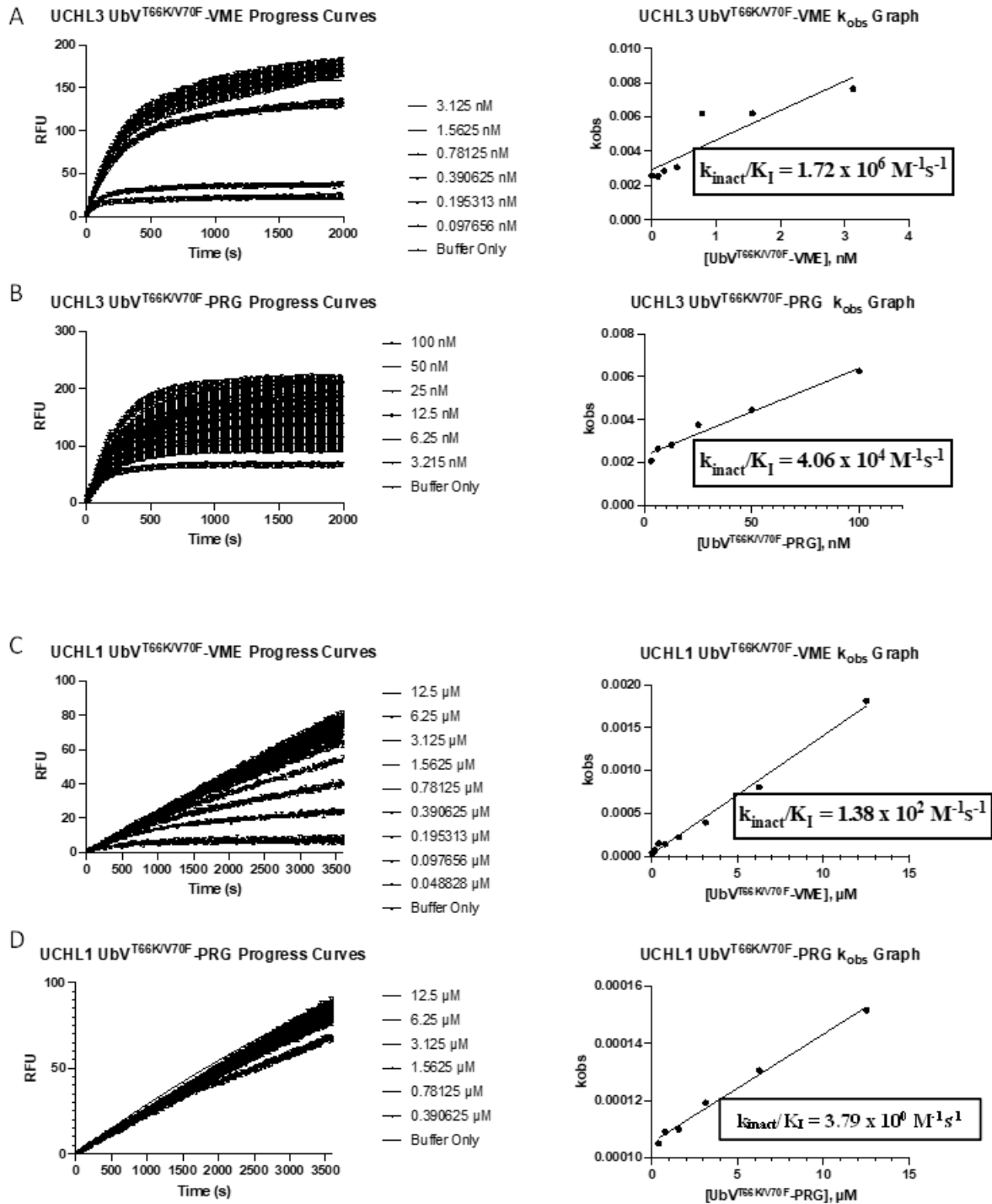


Figure S1. Progress Curves for UbV^{T66K/V70F}-ABPs and linear fits k_{obs} vs [UbV-ABP] for against UCHL3 and UCHL1. **A)** UCHL3 with HA-UbV^{T66K/V70F}-VME, **B)** UCHL3 with HA-UbV^{T66K/V70F}-PRG, **C)** UCHL1 with HA-UbV^{T66K/V70F}-VME, **D)** UCHL1 with HA-UbV^{T66K/V70F}-PRG

Table S1. BioLuminate PPI interaction analysis for Ub-UCHL3.

Ub Residue	Closest UCHL3 residue	Distance	Specific Interactions	# HB	# Salt Bridges	# Pi Stacking	# Disulfides	# vdW Clash	Surface Complementarity	Buried SASA
B:6:Lys				0	0	0	0	0	0.2	23.60%
B:7:Thr	A:220:Leu	3.7 A		0	0	0	0	0	0.62	93.30%
B:8:Leu	A:224:Ala A:34:Val A:37:Met A:223:Asn A:36:Gly A:213:Met A:222:Phe	3.4 A 3.4 A 3.5 A 3.7 A 3.8 A 3.9 A 4.0 A		0	0	0	0	0	0.78	99.00%
B:9:Thr	A:220:Leu A:222:Phe A:213:Met	3.4 A 3.5 A 3.9 A		0	0	0	0	0	0.85	75.20%
B:10:Gly				0	0	0	0	0	0	2.10%
B:11:Lys	A:220:Leu	3.7 A		0	0	0	0	0	0.89	14.70%
B:13:Ile				0	0	0	0	0	0.6	0.00%
B:18:Glu			1x salt bridge to A:108:Lys	0	1	0	0	0	0	0.00%
B:27:Lys				0	0	0	0	0	0	41.10%
B:34:Glu	A:220:Leu	3.2 A		0	0	0	0	0	0.81	21.90%
B:36:Ile				0	0	0	0	0	0.4	53.10%
B:38:Pro				0	0	0	0	0	0	0.10%
B:39:Asp	A:156:Gln A:157:Thr	2.9 A 3.3 A	1x hb to A:156:Gln	1	0	0	0	0	0.83	42.90%
B:40:Gln	A:157:Thr	3.8 A		0	0	0	0	0	0.9	27.60%
B:41:Gln	A:156:Gln	2.8 A	1x hb to A:156:Gln	1	0	0	0	0	0.65	100.00%
B:42:Arg	A:156:Gln A:14:Glu	3.1 A 3.3 A	1x hb, 1x salt bridge to A:14:Glu 1x hb to A:156:Gln	2	1	0	0	0	0.77	58.40%
B:44:Ile	A:35:Tyr	3.6 A		0	0	0	0	0	0.71	99.90%
B:46:Ala	A:44:Met	4.0 A		0	0	0	0	0	0	10.00%
B:47:Gly	A:44:Met A:35:Tyr	3.3 A 3.7 A		0	0	0	0	0	0.8	57.00%
B:49:Gln				0	0	0	0	0	0.46	21.70%
B:68:Hie	A:41:Leu A:35:Tyr	3.5 A 3.9 A		0	0	0	0	0	0.68	74.50%
B:69:Leu				0	0	0	0	0	0.07	91.70%
B:70:Val	A:223:Asn A:33:Asp A:35:Tyr	3.4 A 3.6 A 3.7 A		0	0	0	0	0	0.81	96.80%
B:71:Leu	A:223:Asn A:220:Leu	2.9 A 3.2 A	1x hb to A:223:Asn	1	0	0	0	0	0.67	98.00%
B:72:Arg	A:33:Asp A:11:Ala A:10:Glu A:12:Asn A:157:Thr A:13:Pro A:156:Gln A:223:Asn	2.8 A 3.1 A 3.4 A 3.4 A 3.6 A 3.6 A 3.8 A 4.0 A	1x hb to A:11:Ala 2x hb, 1x salt bridge, 1x clash to A:33:Asp	3	1	0	0	1	0.84	99.70%

B:73:Leu	A:11:Ala A:55:Leu A:221:Arg A:10:Glu A:222:Phe A:168:Leu	2.8 A 3.4 A 3.5 A 3.5 A 3.7 A 3.9 A	1x hb to A:11:Ala	1	0	0	0	0	0.87	98.10%
B:74:Arg	A:152:Ala A:157:Thr A:8:Pro A:154:Glu A:158:Glu A:9:Leu A:160:Pro A:168:Leu A:10:Glu A:166:Val A:159:Ala A:55:Leu	2.8 A 2.9 A 2.9 A 3.1 A 3.2 A 3.4 A 3.5 A 3.6 A 3.6 A 3.6 A 3.8 A 3.9 A	1x hb to A:8:Pro 1x salt bridge to A:10:Glu 1x hb to A:152:Ala 1x hb to A:154:Glu 2x hb to A:157:Thr	5	1	0	0	0	0.87	91.30%
B:75:Gly	A:9:Leu A:8:Pro A:55:Leu A:170:Phe A:168:Leu A:95:Cys	3.1 A 3.3 A 3.4 A 3.5 A 3.7 A 3.7 A	1x hb to A:9:Leu	1	0	0	0	0	0.9	100.00%
B:75A:Nma	A:168:Leu A:166:Val A:95:Cys A:93:Asn A:8:Pro	3.0 A 3.4 A 3.4 A 3.5 A 3.8 A	1x hb to A:168:Leu	1	0	0	0	0	0.84	95.60%

Table S2. BioLuminate PPI interaction analysis for Ub-UCHL1.

Residue	Closest	Distance	Specific Interactions	# HB	# Salt Bridges	# Pi Stacking	# Disulfides	# vdW Clash	Surface Complementarity	Buried SASA
B:6:Lys	A:35:Glu	3.9 A	1x salt bridge to A:35:Glu	0	1	0	0	0	0.13	34.10%
B:7:Thr	A:212:Val A:32:Leu	3.8 A 4.0 A		0	0	0	0	0	0.5	98.50%
B:8:Leu	A:31:Val A:34:Leu A:215:Ser A:33:Gly A:216:Ala A:32:Leu A:214:Phe	3.4 A 3.4 A 3.5 A 3.6 A 3.6 A 3.7 A 3.9 A		0	0	0	0	0	0.84	98.60%
B:9:Thr	A:214:Phe A:205:Thr	3.4 A 3.6 A		0	0	0	0	0	0.82	83.50%
B:10:Gly				0	0	0	0	0	0.04	16.10%
B:11:Lys	A:212:Val	3.8 A		0	0	0	0	0	0.72	24.80%
B:13:Ile				0	0	0	0	0	0.77	1.40%
B:34:Glu	A:213:Arg A:212:Val	3.2 A 3.4 A	1x hb to A:213:Arg	1	0	0	0	0	0.85	45.40%
B:35:Gly	A:213:Arg	3.1 A	1x hb to A:213:Arg	1	0	0	0	0	0.86	16.60%
B:36:Ile	A:213:Arg A:212:Val	3.5 A 3.8 A		0	0	0	0	0	0.65	100.00%
B:37:Pro	A:154:Val A:213:Arg	3.7 A 3.9 A		0	0	0	0	0	0.5	79.40%
B:39:Asp	A:153:Arg	3.5 A		0	0	0	0	0	0.73	28.30%
B:40:Gln	A:153:Arg A:155:Asp	3.4 A 3.6 A		0	0	0	0	0	0.77	97.50%
B:42:Arg				0	0	0	0	0	0	20.20%
B:44:Ile				0	0	0	0	0	0.75	60.10%
B:47:Gly				0	0	0	0	0	0	9.90%
B:49:Gln				0	0	0	0	0	0	3.10%
B:68:His	A:32:Leu A:38:Ser	3.7 A 3.8 A		0	0	0	0	0	0.58	64.20%
B:69:Leu				0	0	0	0	0	0.09	93.10%
B:70:Val	A:32:Leu A:30:Asp	3.5 A 3.9 A		0	0	0	0	0	0.76	93.00%
B:71:Leu	A:215:Ser A:212:Val A:8:Ile A:213:Arg A:214:Phe	2.9 A 3.5 A 3.9 A 3.9 A 3.9 A		0	0	0	0	0	0.87	96.70%

B:72:Arg	A:30:Asp A:153:Arg A:9:Asn A:8:Ile A:7:Glu A:10:Pro	2.8 A 2.9 A 3.2 A 3.2 A 3.4 A 3.7 A	1x hb to A:8:Ile 1x clash to A:9:Asn 2x hb, 1x salt bridge, 1x clash to A:30:Asp 1x clash to A:153:Arg	3	1	0	0	3	0.86	97.00%
B:73:Leu	A:8:Ile A:52:Leu A:213:Arg A:7:Glu A:6:Met A:215:Ser	3.0 A 3.4 A 3.5 A 3.5 A 3.7 A 3.9 A	1x hb, 1x clash to A:8:Ile	1	0	0	0	1	0.82	100.00%
B:74:Arg	A:155:Asp A:151:Gln A:160:Phe A:156:Asp A:6:Met A:153:Arg A:5:Pro	3.0 A 3.2 A 3.4 A 3.5 A 3.5 A 3.9 A 3.9 A	1x hb to A:155:Asp	1	0	0	0	0	0.78	83.50%
B:75:Gly	A:6:Met A:5:Pro A:52:Leu A:162:Phe A:160:Phe A:90:Cys	2.9 A 3.6 A 3.6 A 3.7 A 3.8 A 3.9 A	2x hb to A:6:Met	2	0	0	0	0	0.8	100.00%
B:75A:Nma	A:160:Phe A:90:Cys A:88:Asn	2.9 A 3.4 A 3.6 A	1x clash to A:90:Cys 1x hb to A:160:Phe	1	0	0	0	1	0.65	91.60%

Table S3. BioLuminate Residue Scanning Analysis at Gln40 for both Ub-UCHL3 and Ub-UCHL1

Gln40(X); X =	Affinity^{UCHL3} (kcal/mol)	Stability^{UCHL3} (kcal/mol)	Affinity^{UCHL1} (kcal/mol)	Stability^{UCHL1} (kcal/mol)
Ala	1.0	3.6	6.9	0.2
Arg	-1.2	-2.1	-6.0	3.7
Asn	-0.3	3.1	4.1	3.0
Asp	6.8	7.8	10.2	9.3
Cys	0.0	5.7	4.4	2.4
Glu	4.9	-1.5	5.4	-2.1
Gly	1.7	11.5	7.9	8.0
Hid	-1.1	-1.5	4.6	11.9
Hie	-0.7	3.9	3.4	16.3
Hip	-5.5	0.8	8.1	11.5
Ile	-1.0	-8.7	1.5	-6.6
Leu	-1.1	-2.7	4.7	1.1
Lys	-0.9	13.4	7.6	17.6
Met	-2.0	-11.3	0.2	-11.1
Phe	-2.8	0.8	1.2	-0.1
Pro	-0.2	31.1	3.8	32.2
Ser	0.4	6.8	5.0	3.0
Thr	-0.1	1.4	5.0	-1.6
Trp	-4.2	6.8	75.0	140
Tyr	-2.3	0.8	11.3	65.5
Val	-0.6	-6.0	4.1	-3.6

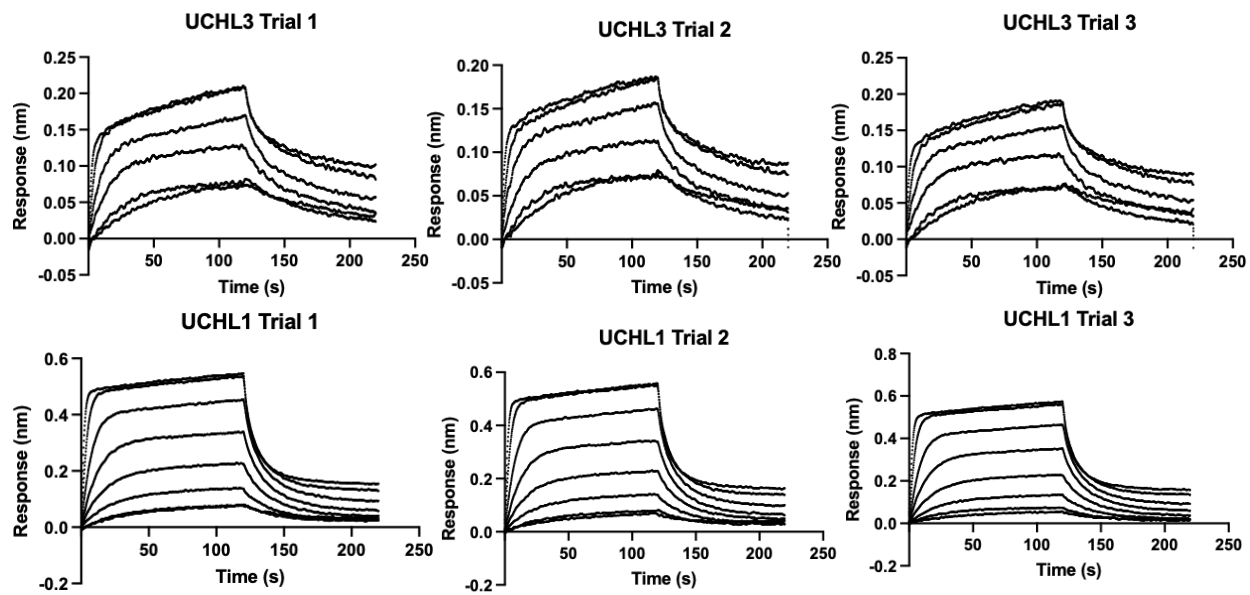


Figure S2. BLI association and dissociation curves for UbV^{Q40V/T66K/V70F} against UCHL3 (**top row**) and UCHL1 (**bottom row**). The maximum response was plotted as a function of concentration of UbV to generate steady-state binding curves in main text.

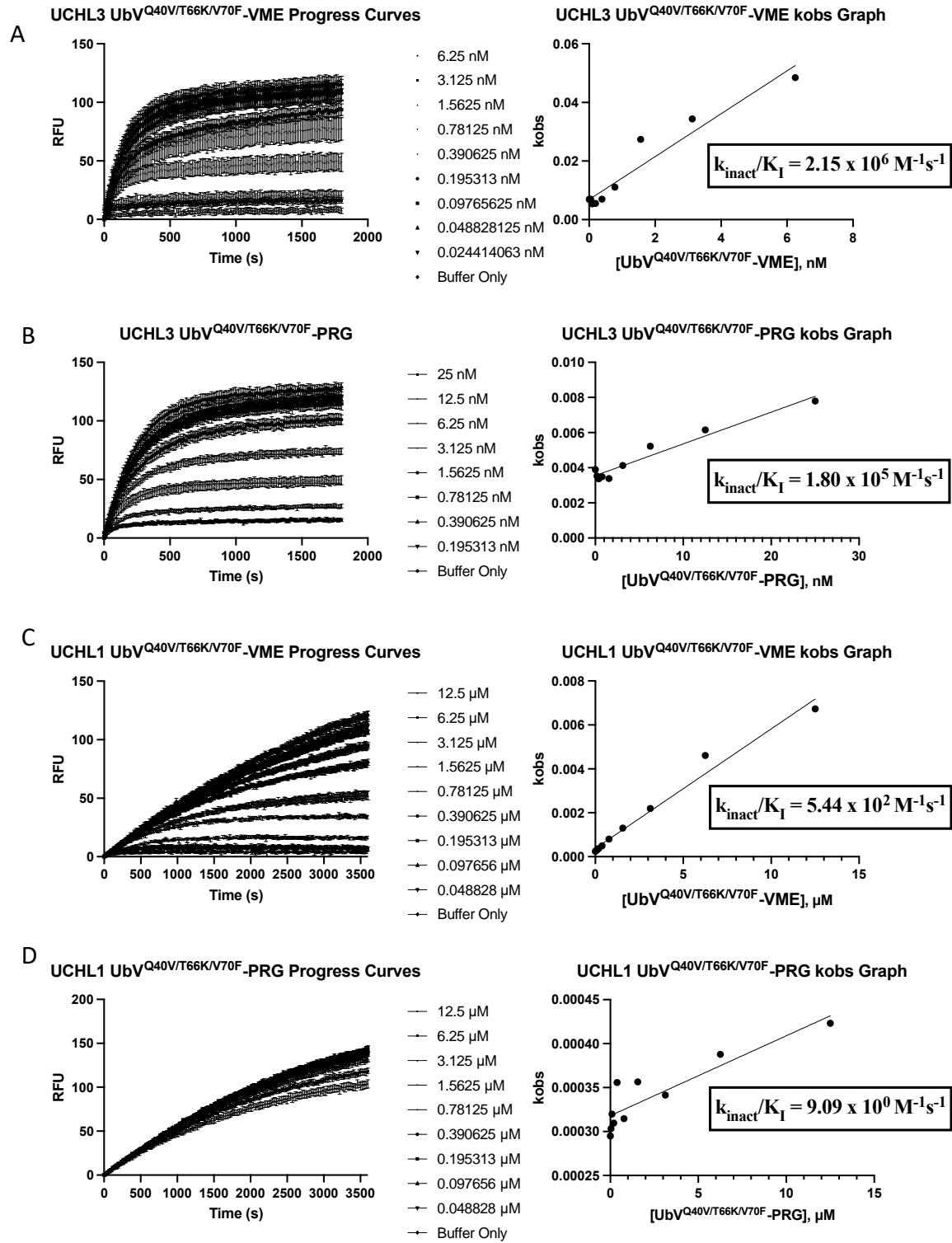


Figure S3. Progress Curves for UbV^{Q40V/T66K/V70F}-ABPs and linear fits k_{obs} vs [UbV-ABP] against UCHL3 and UCHL1 with A) UCHL3 with HA-UbV^{Q40V/T66K/V70F}-VME, B) UCHL3 with HA- UbV^{Q40V/T66K/V70F}-PRG, C) UCHL1 with HA- UbV^{Q40V/T66K/V70F}-VME, D) UCHL1 with HA- UbV^{Q40V/T66K/V70F}-PRG