

## Supplemental Information

### The Differential Modulation of USP Activity by Internal Regulatory Domains, Interactors and Eight Ubiquitin Chain Types

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#### Inventory of Supplemental Information

**Table S1.**     *Related to Figure 1.*

Comparison with published UbAMC kinetics.

**Figure S1**     *Related to Figure 2.*

Exemplary raw data UbAMC assays and relative activity with EDTA.

**Figure S2**     *Related to Figure 3.*

Di-Ub assay for inactive USPs and comparison USP7CD vs USP7CD-HUBL.

**Figure S3**     *Related to Figure 4.*

Synthesis, LC-MS of FP reagents and exemplary raw data of hydrolysis.

**Figure S4**     *Related to Figure 6.*

Curves of the Michaelis-Menten analysis of the UAF1 and GMPS modulation.

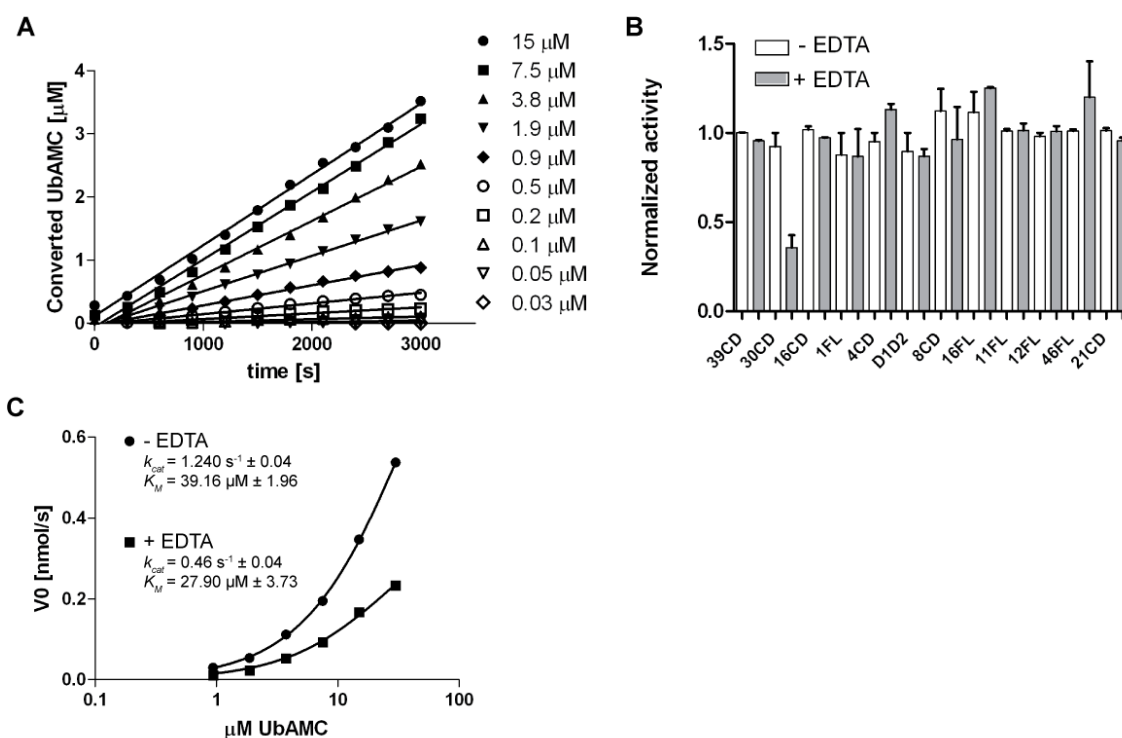
## Supplemental Table and Figures

### Supplemental Table S1

	$k_{cat}$ (s <sup>-1</sup> )	$K_M$ ( $\mu$ M)	$k_{cat}/K_M$ (x10 <sup>3</sup> M <sup>-1</sup> s <sup>-1</sup> )	Reference
USP1	0.014 0.08	1.4 9.71	10 9	Cohn et al. (2007)
USP1+UAF1	0.26 0.69	0.7 18.71	371 37	Cohn et al. (2007)
Ubp8/SAGA	0.17	1.5	110	Samara et al. (2011)
USP2	0.62 0.14	2.5 0.55	250 252	Zhang et al. (2011) Renatus et al. (2006)
USP4-D1D2	0.01	0.2	50	Luna-Vargas et al. (2011b)
USP4	0.01	12.80	1	Luna-Vargas et al. (2011b)
USP7FL	3.56 1.37	17.5 2.89	203 483	Fernandez-Montalvan et al. (2007) Faesen et al. (2011)
USP7CD	0.077 0.06	44.2 15.13	2 4	Fernandez-Montalvan et al. (2007) Faesen et al. (2011)
USP7CD-HUBL	0.805 1.37	22.8 2.73	35 519	Fernandez-Montalvan et al. (2007) Faesen et al. (2011)
USP7CD-HUBL+GMPS	7.70	8.90	865	
USP8	2.4 7.90	10.2 17.30	235 465	Avvakumov et al. (2006)
USP11	0.07	0.77	96	
USP12	0.0002	12.00	0	
USP12+UAF1	0.0017	12.60	0	
USP16CD	0.01	0.53	17	
USP16FL	0.07	1.40	49	
USP21	0.041 0.10	0.26 2.60	158 39	Ye et al. (2011)
USP25	0.12 0.74	5 13.10	24 56	Meulmeester et al. (2008)
USP30	0.46	62.70	7	
USP39	not fitted	not fitted		van Leuken et al. (2008)
USP46	0.01	30.00	0	
USP46+UAF1	0.37	18.24	21	

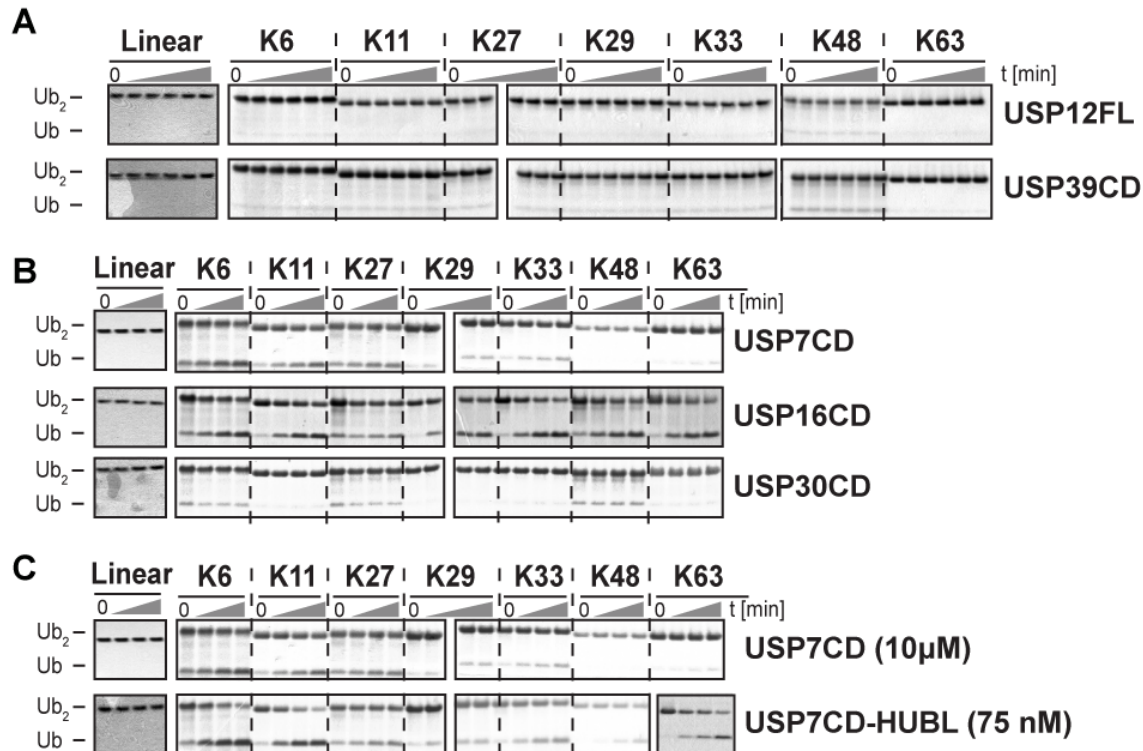
**Supplemental Table S1. Related to Figure 1. Comparison with published UbAMC kinetics.**

## Supplemental Fig. S1



**Supplemental Figure S1. Related to Figure 2.** **A.** Exemplary raw data of UbAMC hydrolysis by USP7CD-HUBL. Measurements were done using a five minute interval. The signal was stable for at least one hour. Timepoint 0 corresponds to the start of the reaction. **B.** Relative activity in presence and absence of 1 mM EDTA of the (predicted) zinc containing USPs. Activity is normalized against the activity without EDTA. **C.** Michealis-Menten analysis of USP30 in presence and absence of 1 mM EDTA.

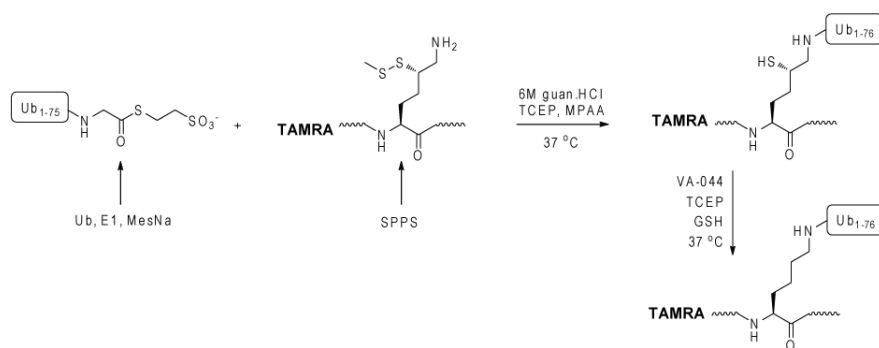
## Supplemental Fig. S2



**Supplemental Figure S2. Related to Figure 3. Di-Ub assay for inactive USPs and comparison USP7CD versus USP7CD-HUBL. A. and B.** Di-Ub assay for USP12FL, USP39CD, USP46FL, USP7CD, USP16CD and USP30CD. Time-course using all di-Ub topoisomers (5 μM) (Linear, K6, K11, K27, K29, K33, K48 and K63) for USPs (75 nM). Samples from each time-point (0, 5, 10, 30, 60, 180 min) (A) and (0, 10, 30, 60 min) (B) were analyzed on coomassie stained SDS-PAGE gels. **C.** Di-Ub assay USP7CD versus USP7CD-HUBL. Time-course using all di-Ub topoisomers (5 μM) for USP7 constructs (10 μM and 75 nM). Samples from each time-point (0, 10, 30, 60 min) were analyzed as in B.

## Supplemental Fig. S3A

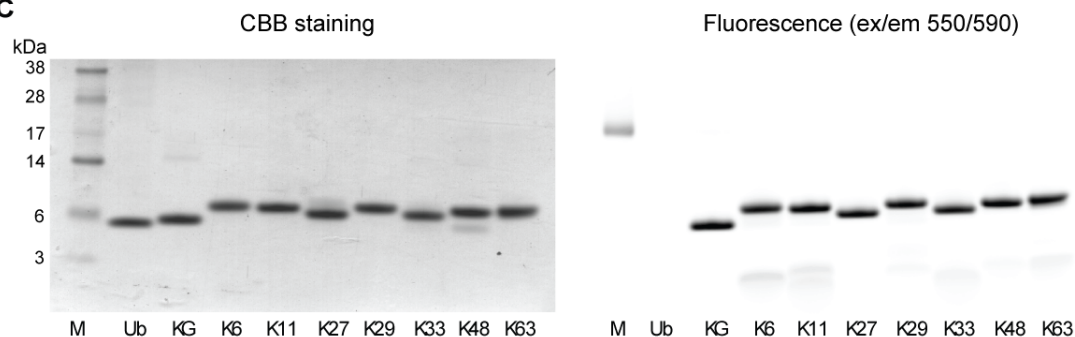
**A**



**B**

Linkage type	Peptide sequence	MW (Da)	Yield
KG	TAMRA-K(Ub)G	9162	1.3 mg (25%)
K6 (Ub 1-14)	TAMRA-MQIFVK(Ub)TLTGKTIT	10540	0.8 mg (16%)
K11 (Ub 4-17)	TAMRA-FVKTLTGK(Ub)TITLEV	10509	1.0 mg (18%)
K27 (Ub 20-33)	TAMRA-SDTIENVK(Ub)AKIQDK	10548	0.5 mg (10%)
K29 (Ub 22-35)	TAMRA-TIENVKAK(Ub)IQDKEG	10532	1.8 mg (30%)
K33 (Ub 26-39)	TAMRA-VKAKIQDK(Ub)EGIPPD	10497	1.7 mg (28%)
K48 (Ub 41-54)	TAMRA-QRLIFAGK(Ub)QLEDGR	10590	0.6 mg (12%)
K63 (Ub 56-69)	TAMRA-LSDYNIQK(Ub)ESTLHL	10620	1.6 mg (32%)

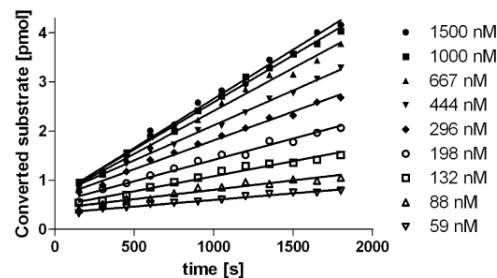
**C**



**D**

	$k_{cat}$ [s <sup>-1</sup> ]	$K_M$ [nM]	$k_{cat}/K_M$ [M <sup>-1</sup> s <sup>-1</sup> ]
KG	0.068 ± 0.006	271.8 ± 43.2	2.51 × 10 <sup>5</sup>
K6	0.157 ± 0.015	1532 ± 256	1.03 × 10 <sup>5</sup>
K11	0.060 ± 0.001	229.2 ± 16.0	2.61 × 10 <sup>5</sup>
K27	0.038 ± 0.001	150.1 ± 11.4	2.56 × 10 <sup>5</sup>
K29	0.060 ± 0.002	299.7 ± 20.5	1.99 × 10 <sup>5</sup>
K33	0.048 ± 0.002	330.5 ± 31.6	1.46 × 10 <sup>5</sup>
K48	0.044 ± 0.001	165.5 ± 11.7	2.65 × 10 <sup>5</sup>
K63	0.047 ± 0.001	220.7 ± 12.2	2.12 × 10 <sup>5</sup>

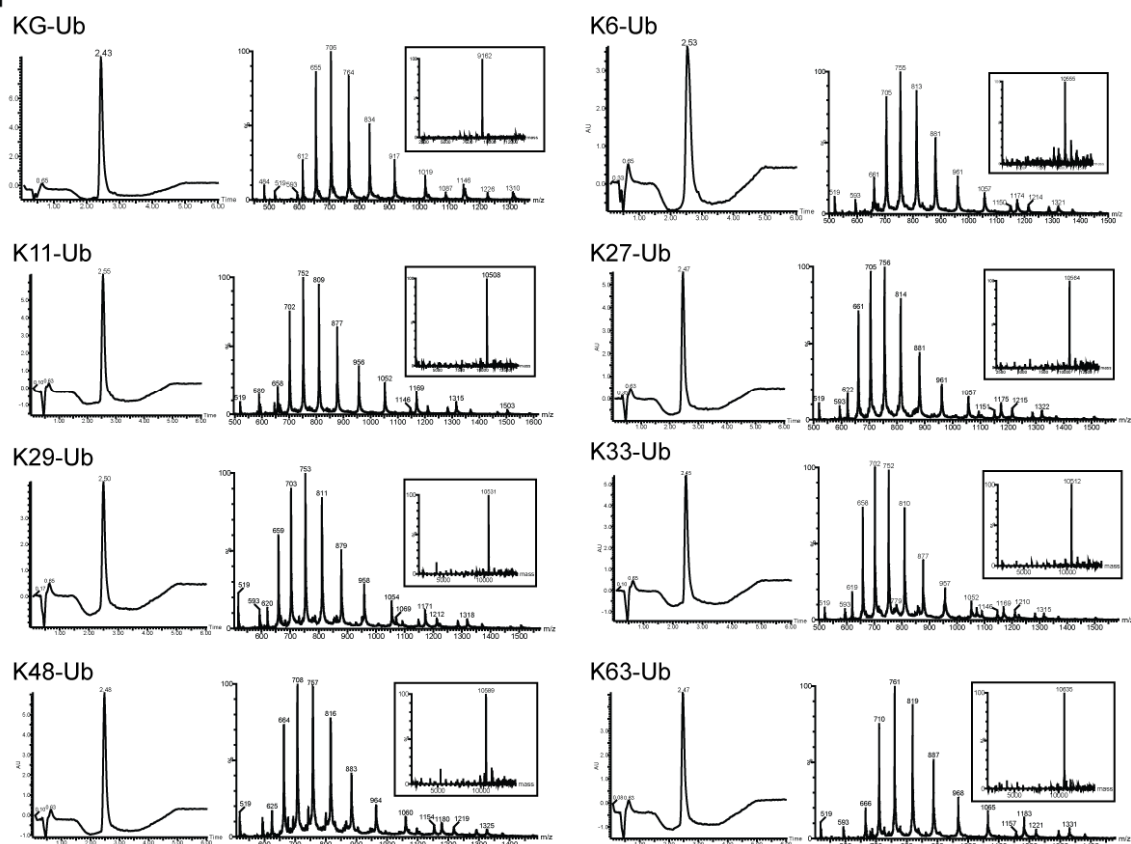
**E**



**Supplemental Figure S3. Related to Figure 4. Synthesis, LC-MS of FP reagents and exemplary raw data of hydrolysis.** **A.** Ligation of Ub to the peptides. **B.** Peptide sequence, molecular weight and typical yield of the reaction. **C.** Coomassie staining and fluorescence scan of SDS electrophoresis analysis of the FP-reagent. **D.** Kinetic parameters of USP4-D1D2 using the FP-reagents. **E.** Exemplary data of hydrolysis of FP reagents (K29 with USP4-D1-D2). Timepoint 0 corresponds to the start of the reaction.

## Supplemental Fig. S3B

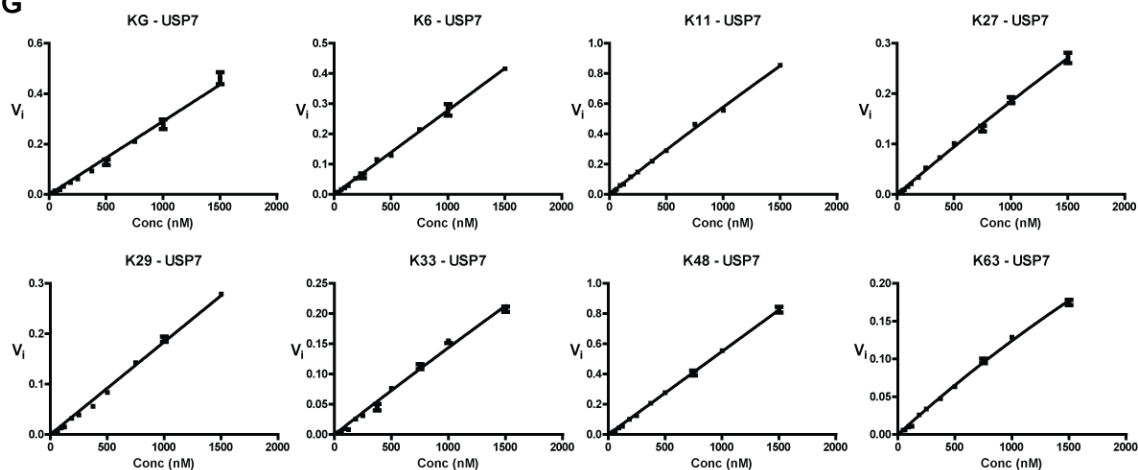
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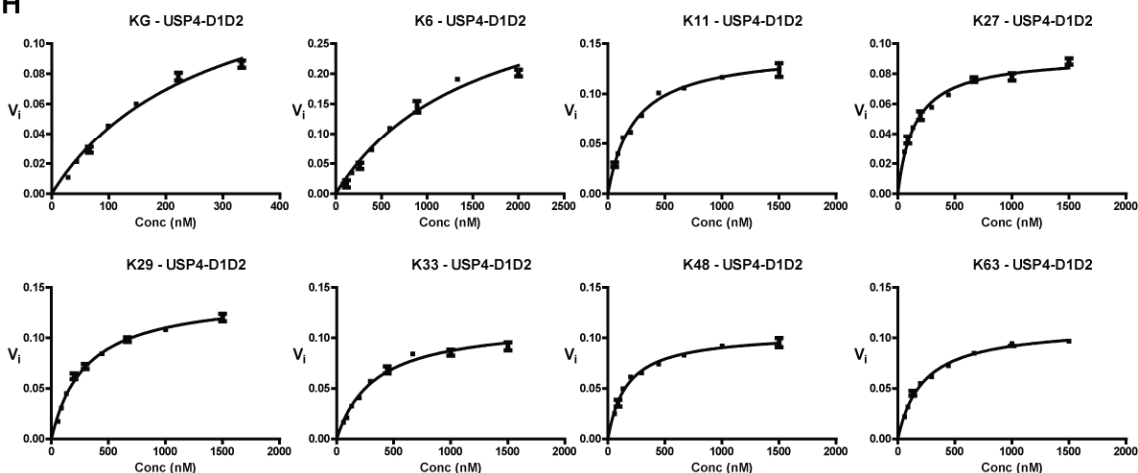
**Supplemental Figure S3. Related to Figure 4. Synthesis and LC-MS of FP reagents and exemplary raw data of hydrolysis. F. LC-MS spectra of the FP-reagents.**

## Supplemental Fig. S3C

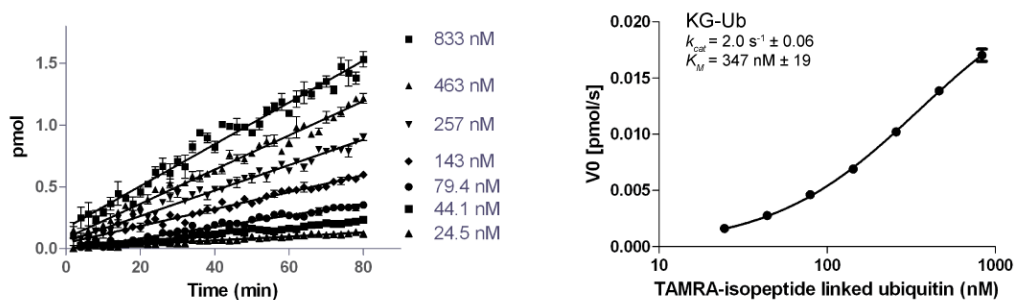
G



H

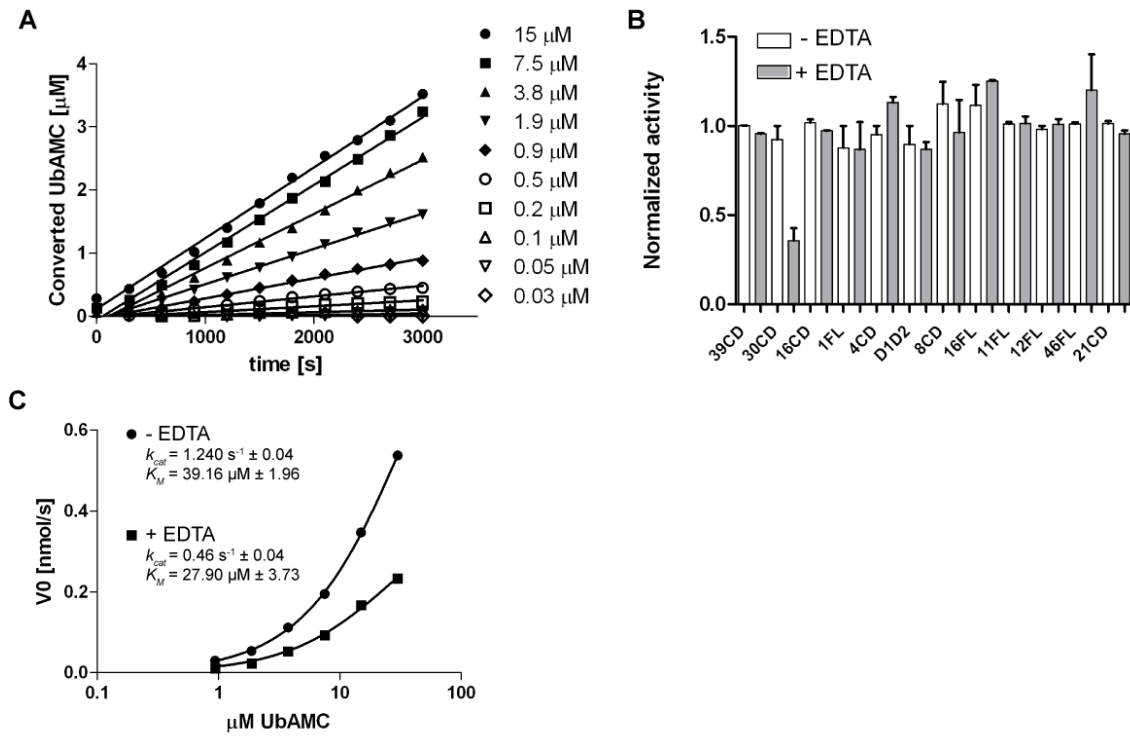


I

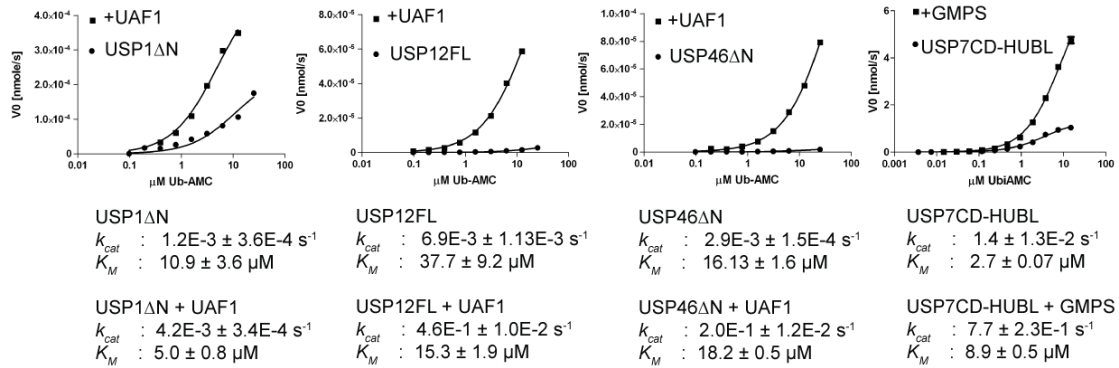


**Supplemental Figure S3. Related to Figure 4. Synthesis and LC-MS of FP reagents and exemplary raw data of hydrolysis. G.** Michaelis-Menten analysis of USP7 hydrolysis of the FP reagents. Data could not be fitted with an exponential Michaelis-Menten curve. **H.** Michaelis-Menten analysis of USP4 hydrolysis of the FP reagents. Kinetic parameters are in (D). **I.** Raw data and Michaelis-Menten analysis of USP21CD hydrolysis of the minimal 'KG' FP reagent.

## Supplemental Fig. S1



## Supplemental Fig. S4



**Supplemental Figure S4. Related to Figure 6. Curves of the Michaelis-Menten analysis of the UAF1 and GMPS modulation.** The Michaelis-Menten curves for the different USPs obtained by determining the initial rates ( $V_0$ ) at 2-fold serial dilutions of UbAMC.