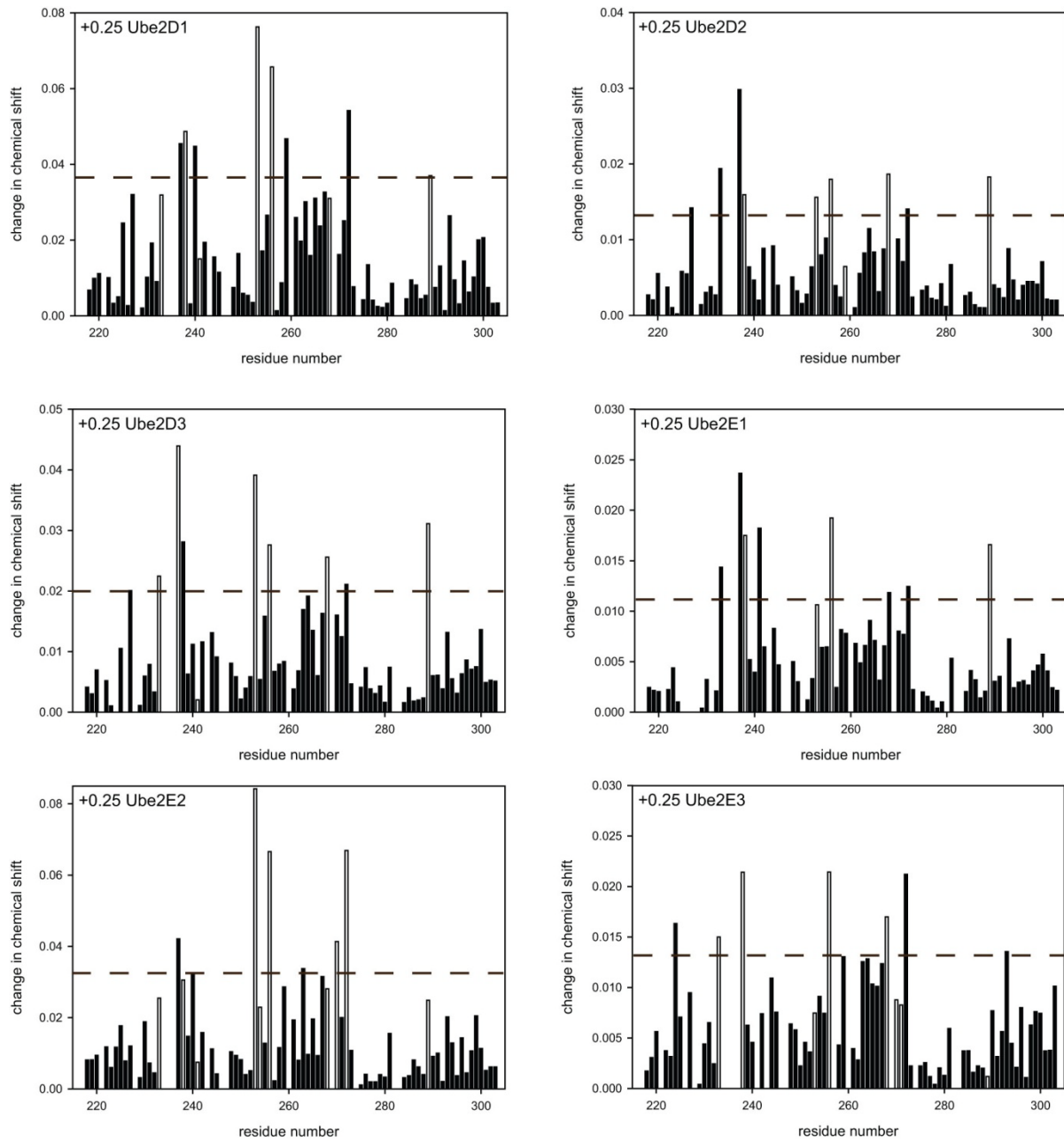
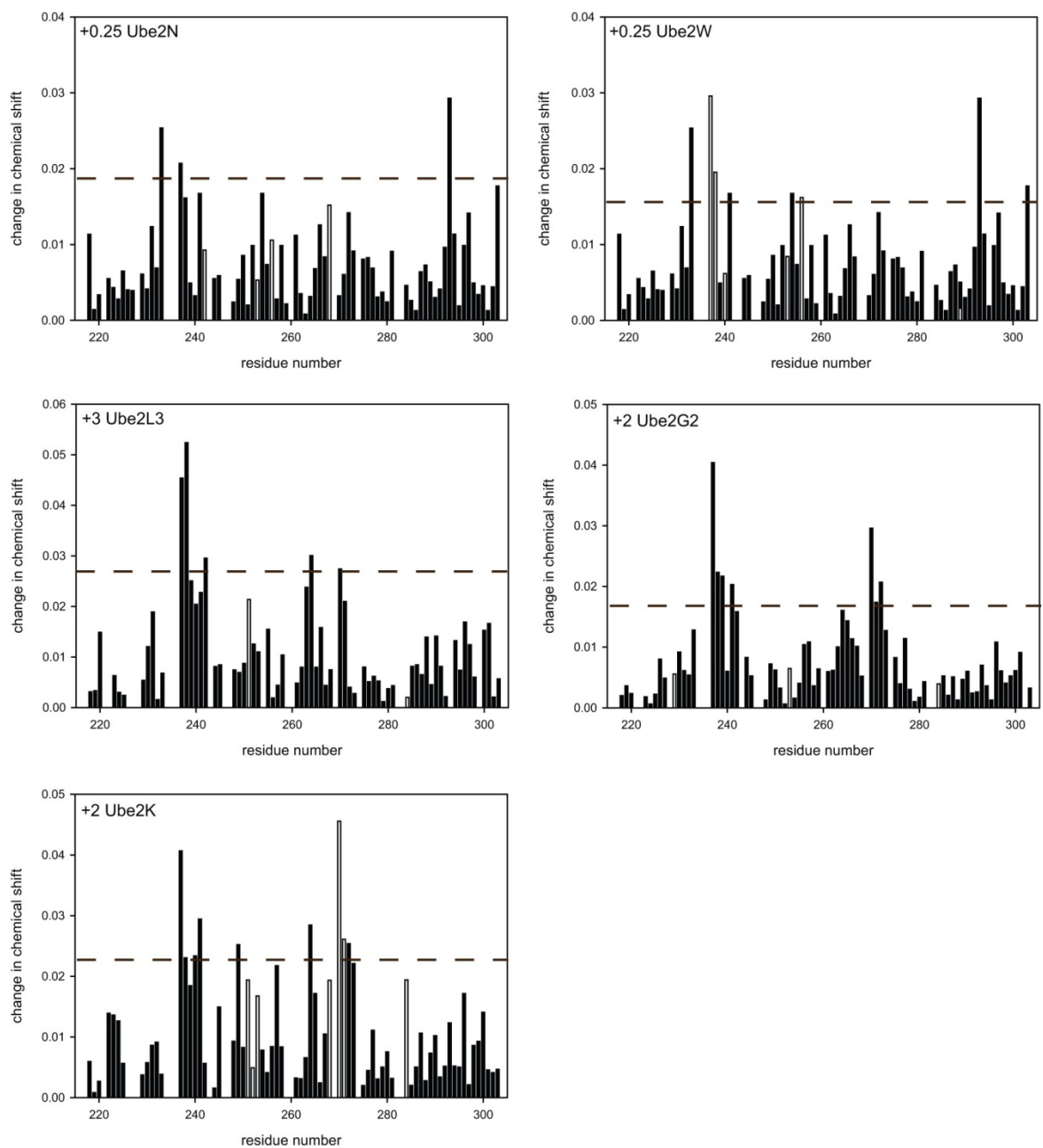


Supplementary information for

# E2 selectivity and the requirements for function of the E3 ubiquitin ligase CHIP

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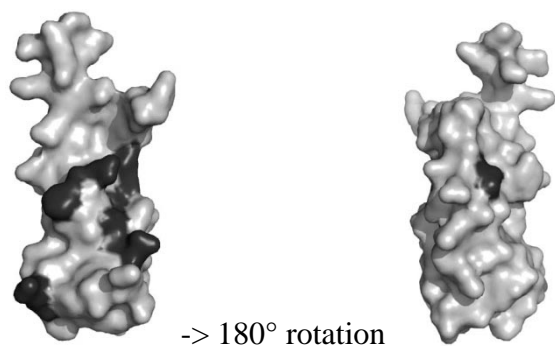


Supplementary Figure 1. CHIPU-E2 titrations. The change in chemical shift is plotted for each observed residue, the dashed line showing the cutoff used for determining a significant change in chemical shift. The open bars represent resonances that were significantly broadened following addition of the specific E2 at the molar ratio labeled.

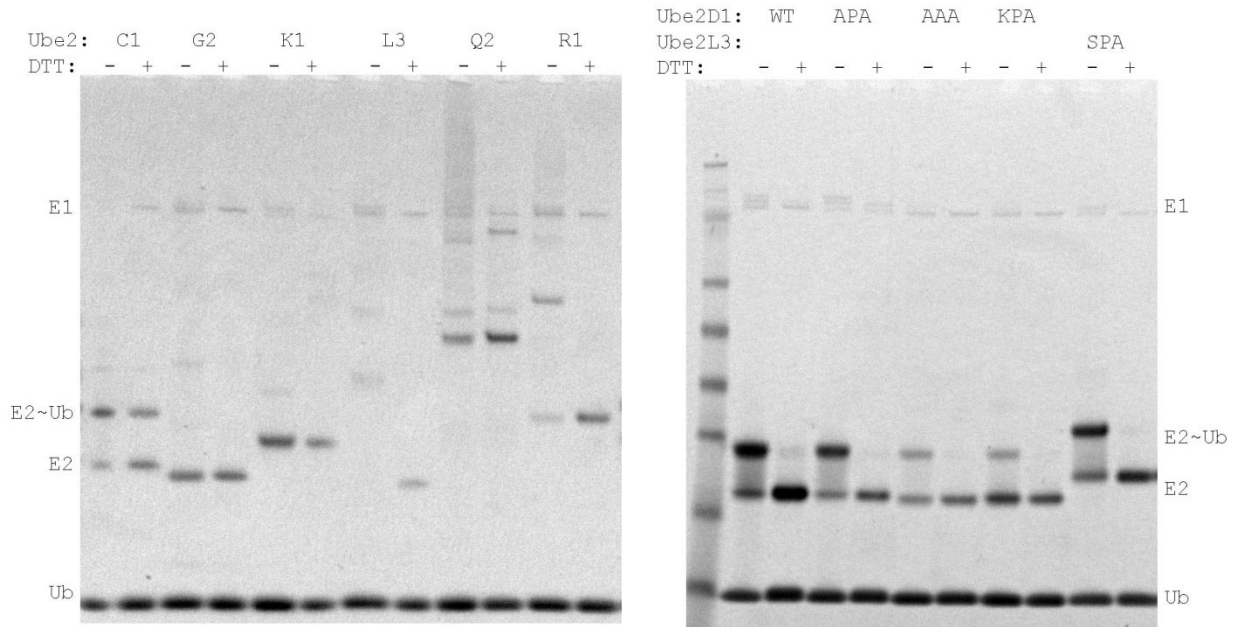
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          *   **  *           *   *   *           *   *           *
Ube2D1  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2D2  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2D3  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2E1  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2E2  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2E3  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2N   GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2W   GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2L3  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2G2  GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY
Ube2K   GPGSVDEKRKKRDIPDYLCGKISFELMREPCITPSGITYDRKDIEEHLQRVGHFDPVTRSPLTQEQLIPNLAMKEVIDAFISENGWVEDY

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Supplementary Figure 2. The CHIP U-box domain has a common binding site for all E2 enzymes. Top: The results for NMR titration data for  $^{15}\text{N}$ -CHIPU are summarized on the CHIPU sequence. Residues perturbed by the addition of the indicated E2 are marked as white on black, while residues whose resonances could not be monitored are in grey. Specific binding interactions are listed first, followed by the three E2s found to have very weak non-specific interactions. Below: E2 perturbations are mapped onto the CHIPU structure showing one major surface and the isolated V289 on the opposite face.



Supplementary Figure 3. *In vitro* Ub charging assays demonstrate the formation of the DTT-sensitive E2~Ub conjugate for (A) E2 enzymes showing no activity with CHIP and (B) all mutant E2 enzymes in this study.

Supplementary Table 1. Summary of selected data for the E2 enzymes tested in this study. Data for CHIP is compared to previous reports and data for BRCA1-BARD1 (BCBD).

E2	Alternate names	Loop 7	BCBD bind (1)	BCBD function (1)	CHIP bind	CHIP function	Previous reports for CHIP
Ube2C1	UbcH10	SAL	N			N	
Ube2D1	UbcH5a	SPA	Y	Poly-Ub	Y	Poly-Ub	Functional in assays and crystallized complex observed (2)
Ube2D2	UbcH5b	SPA	Y	Poly-Ub	Y	Poly-Ub	Functional in assays and bound by NMR (3)
Ube2D3	UbcH5c	SPA	Y	Poly-Ub	Y	Poly-Ub	Functional in assays with and without substrates (4)
Ube2E1	UbcH6, UbcH6a	SPA	Y	Mono-Ub	Y	Mono-Ub	No ubiquitination of luciferase substrate (4)
Ube2E2	UbcH6b, UbcH8	SPA	Y	Mono-Ub	Y	Mono-Ub	May produce CHIP-Ub <i>in vitro</i> (2)
Ube2E3	UbcH6c, UbcH9, UbcM2	SPA	Y	Mono-Ub	Y	Mono-Ub	
Ube2G2		SPV	N		N	N	
Ube2K	Ubc1, E2-25K	AAA	Y	Ub chains	N	N	No autoubiquitination <i>in vitro</i> (5)
Ube2L3	UbcH7	KPA	Y	N	N	N	No autoubiquitination <i>in vitro</i> (4, 5)
Ube2N/Uev1	Ubc13/Uev1a	SPA	Y	Ub chains	Y	Ub chains	Production of free poly-Ub, and complex with CHIP observed (6)
Ube2Q2		SSA	N			N	
Ube2R1	Ubc3, CDC34	NPT	N			N	No autoubiquitination <i>in vitro</i> (4, 5)
Ube2W		SPA	Y	Mono-Ub	Y	Mono-Ub	

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