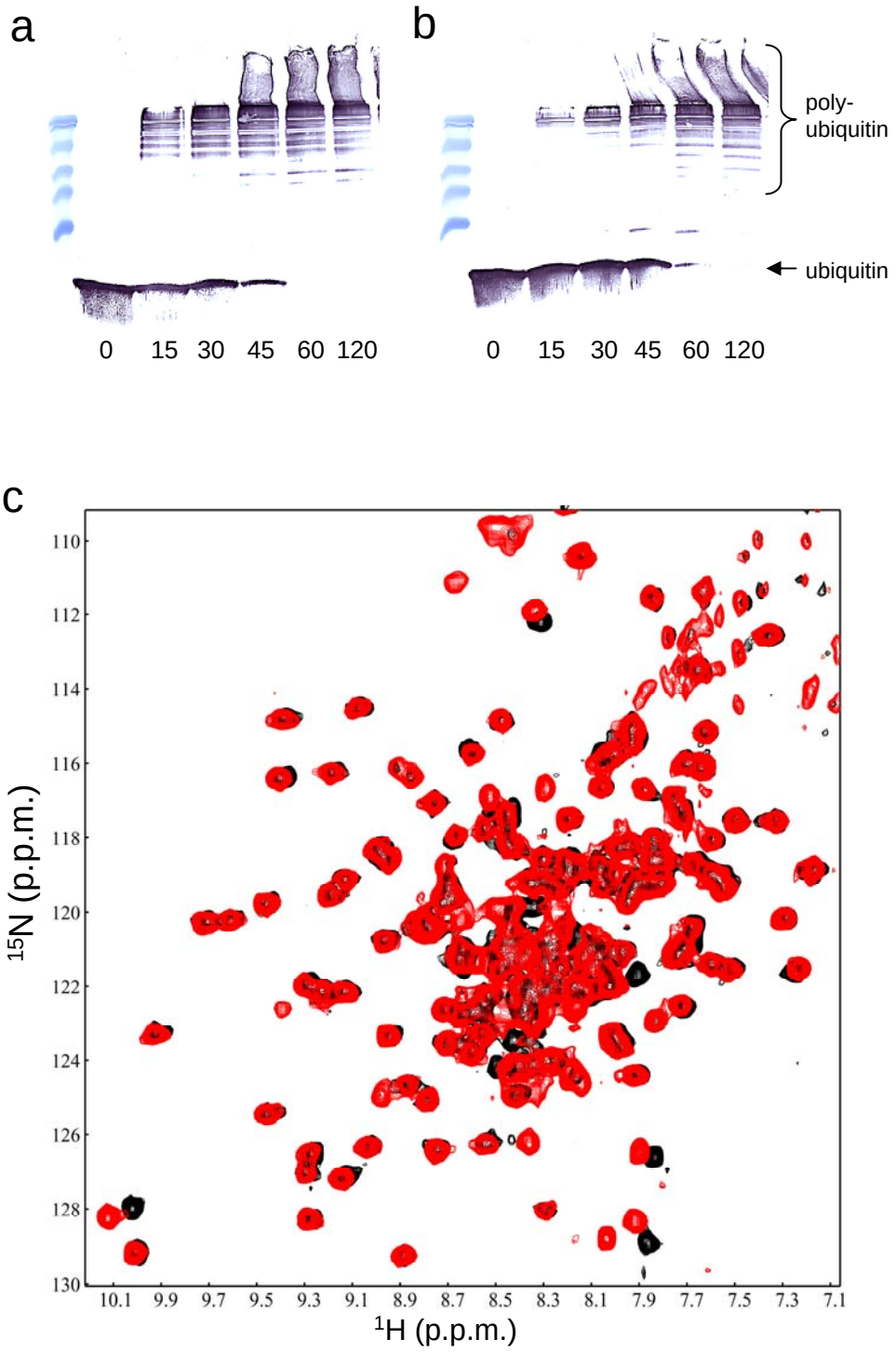
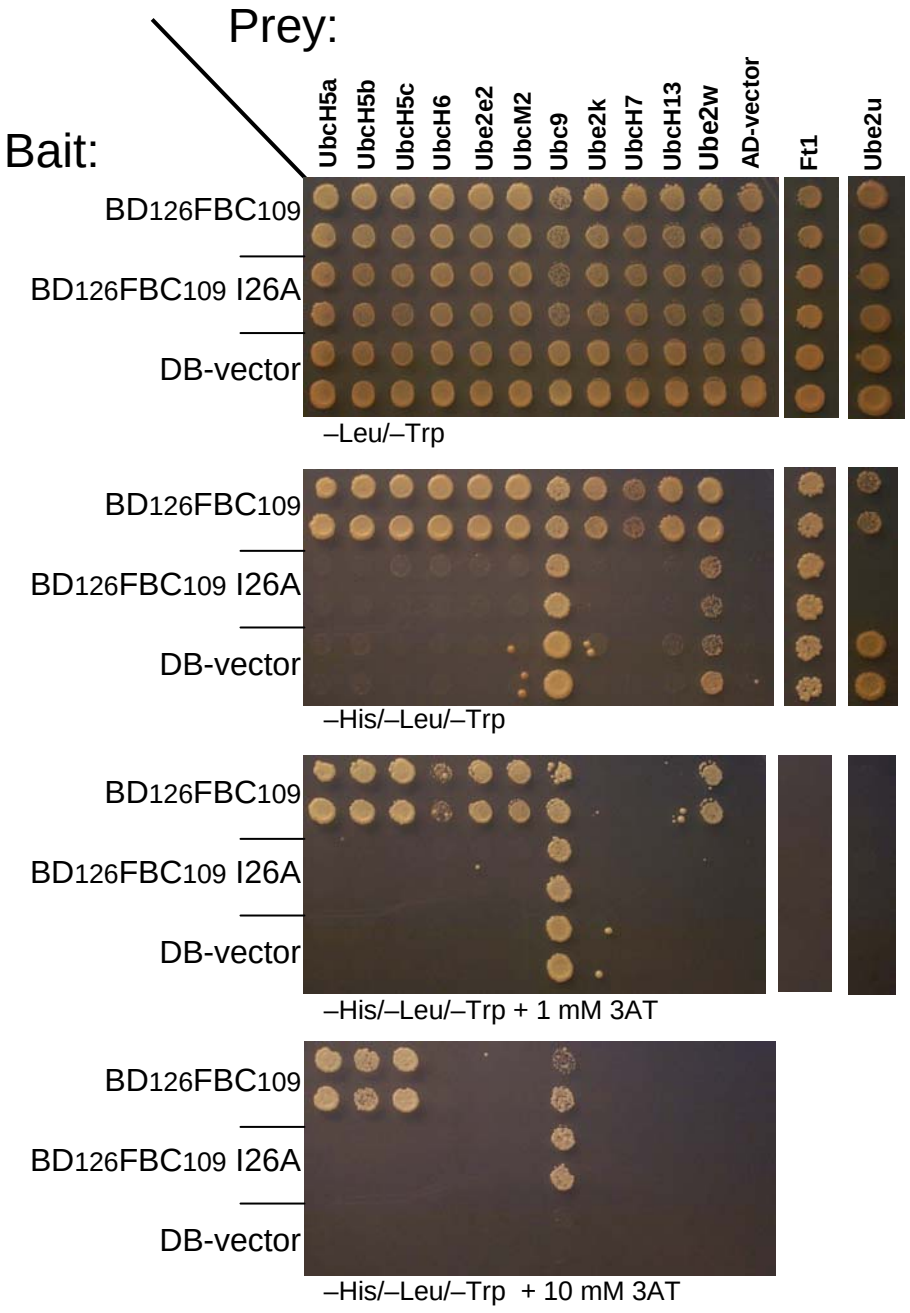
Supplementary Figure 1



Supplementary Figure 1. $BD_{126}FBC_{109}$ and BC_{112}/BD_{140} Display Identical Poly-Ubiquitination Activity.

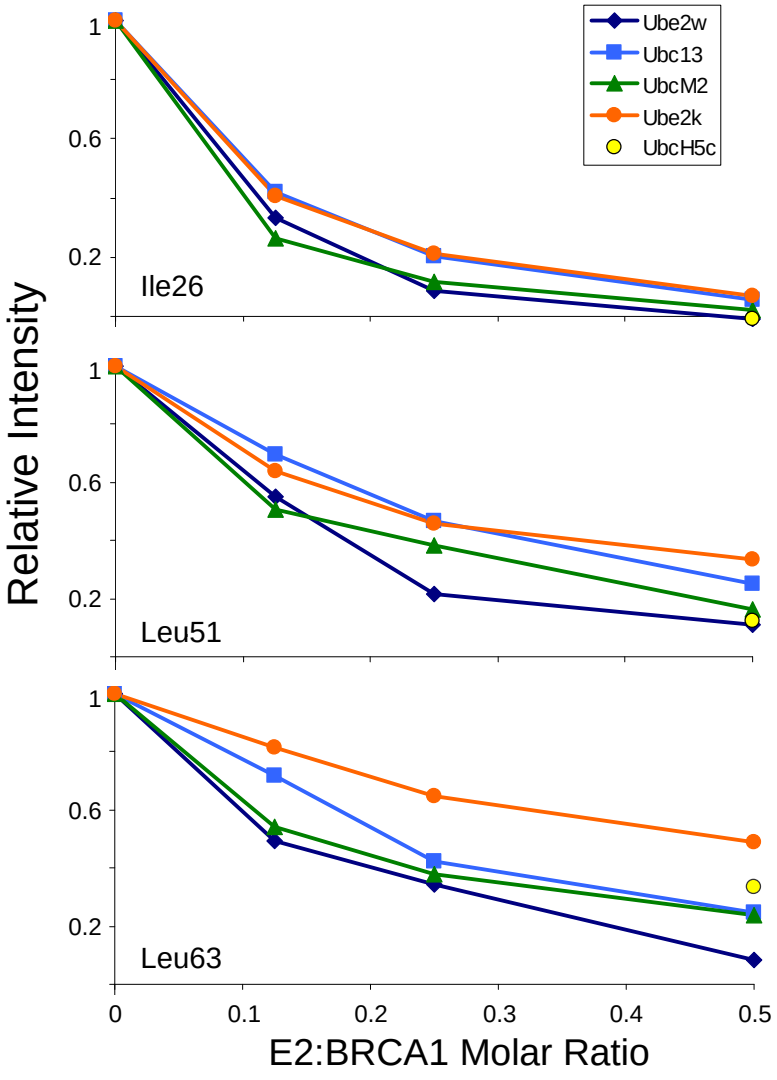
α-ubiquitin western blot showing poly-ubiquitin chain formation during ubiquitination reactions using $BD_{126}FBC_{109}$ (a) and BC_{112}/BD_{140} (b) as the E3 with UbcH5c as the E2. Numbers below each lane indicate minutes after the addition of ATP. (c) The major structural features of $BD_{126}FBC_{109}$ are the same as BC_{112}/BD_{140} as indicated by ^{1}H, ^{15}N-TROSY-HSQC spectra. ^{1}H, ^{15}N-TROSY-HSQC spectrum of ^{15}N-labeled $BD_{126}FBC_{109}$ (red spectrum) overlaid on ^{15}N-labeled BC_{112}/BD_{140} (black spectrum). Differences between the spectra of the fused and non-fused proteins arise from changes in amino acid composition at the N- and C-terminal regions of BARD1 and BRCA1, respectively. Data were collected at 37°C and with a protein concentration of 300 μM.

Supplementary Figure 2



Supplementary Figure 2. Structure-Based E2 BRCA1/BARD1 Screen.
Directed yeast two-hybrid screens with E2s found to interact with BD126FBC109. Plasmids for each "bait" and "prey" pair were co-transformed together and with GAL4 DNA binding (DB) and GAL4 activation domain (AD) empty vector controls into the yeast strain AH109. Positive transformants were selected on minimal synthetic media lacking leucine and tryptophan (–Leu/–Trp). Two colonies for each "bait" and "prey" were replica plated on selective (–His/–Leu/–Trp ± 3AT) and non-selective media (–Leu/–Trp). Each E2 was also screened against BD126FBC109-I26A to test for interaction with the BRCA1 RING.

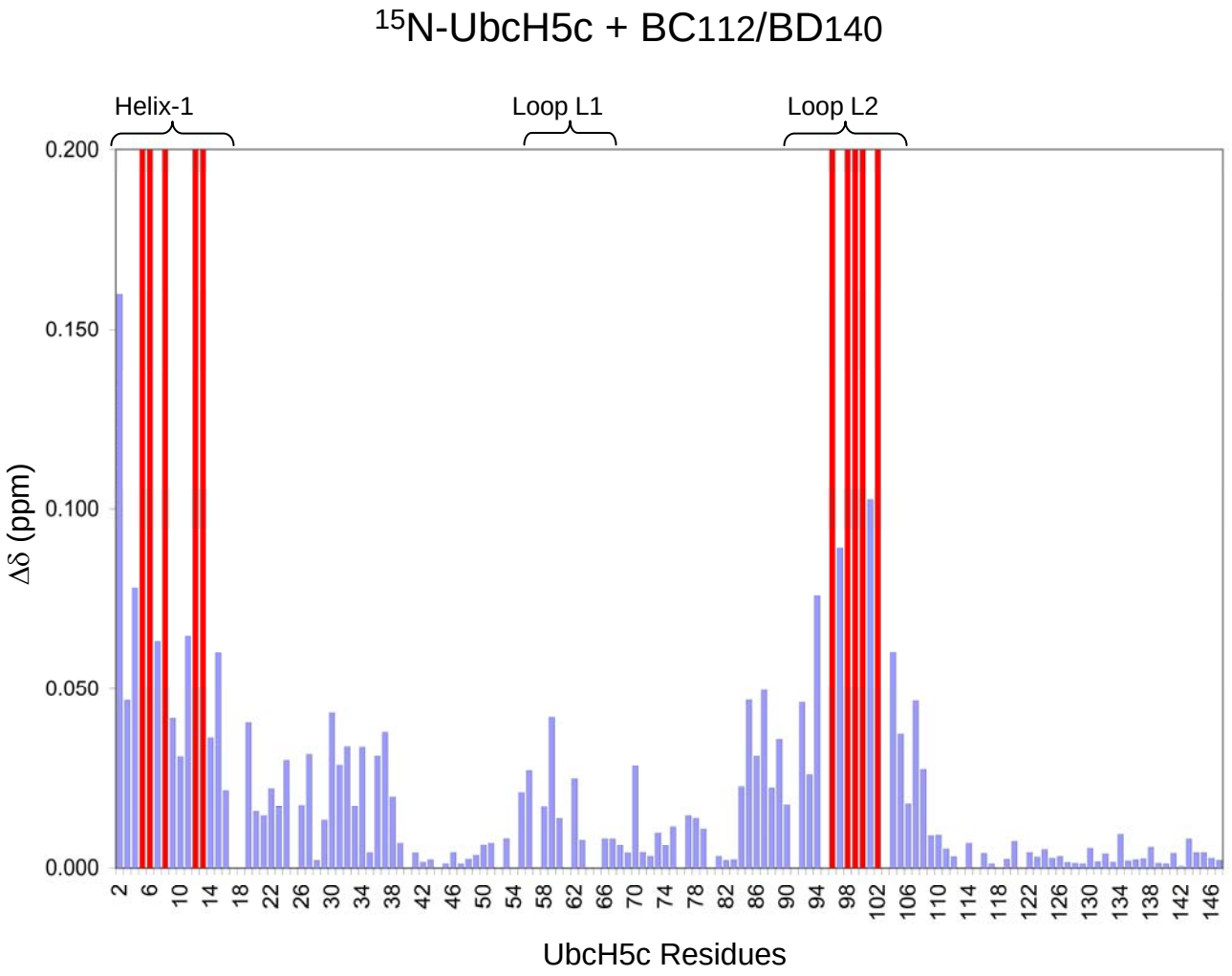
Supplementary Figure 3



Supplementary Figure 3. E2-Induced Intensity Changes in Representative BRCA1 Amide Resonances.

Addition of (unlabeled) E2 to ^{15}N-BC112/BD140 causes selective broadening of NMR resonances for residues in the E2-binding site of the BRCA1 RING. Peak intensities for strongly affected peaks were measured at each point in a titration with E2 and normalized to the BC112/BD140 alone spectrum. Normalized resonance intensity changes in Ile26 in Zn^{2+}-loop I (top), Leu51 in the central helix (middle), and Leu63 in Zn^{2+}-loop II (bottom) of the BRCA1 RING domain are plotted. For comparison, intensity changes induced by UbcH5c at 0.5 molar ratio to BRCA1 are also shown (yellow points). These results suggest only modest differences in the interaction between BRCA1 and select E2 proteins.

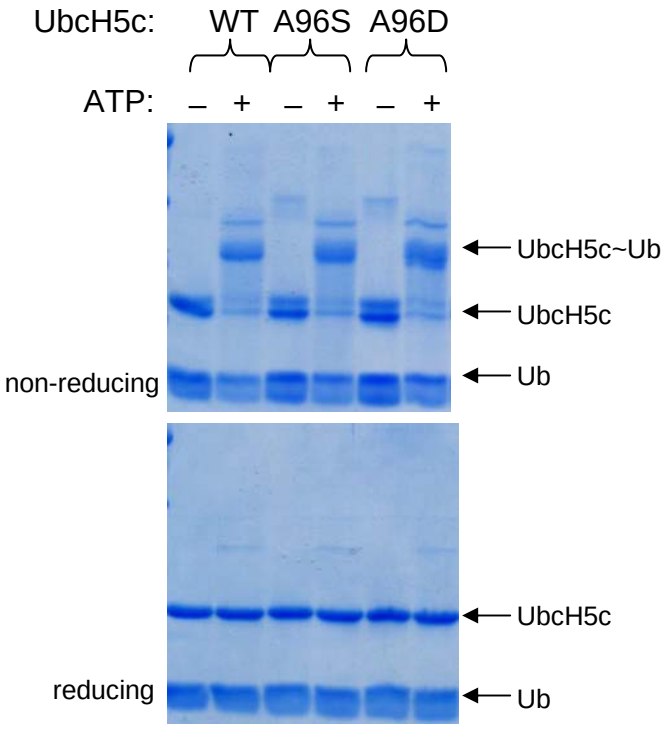
Supplementary Figure 4



Supplementary Figure 4. Spectral Perturbations on ^{15}N-UbcH5c due to binding of BC112/BD140.

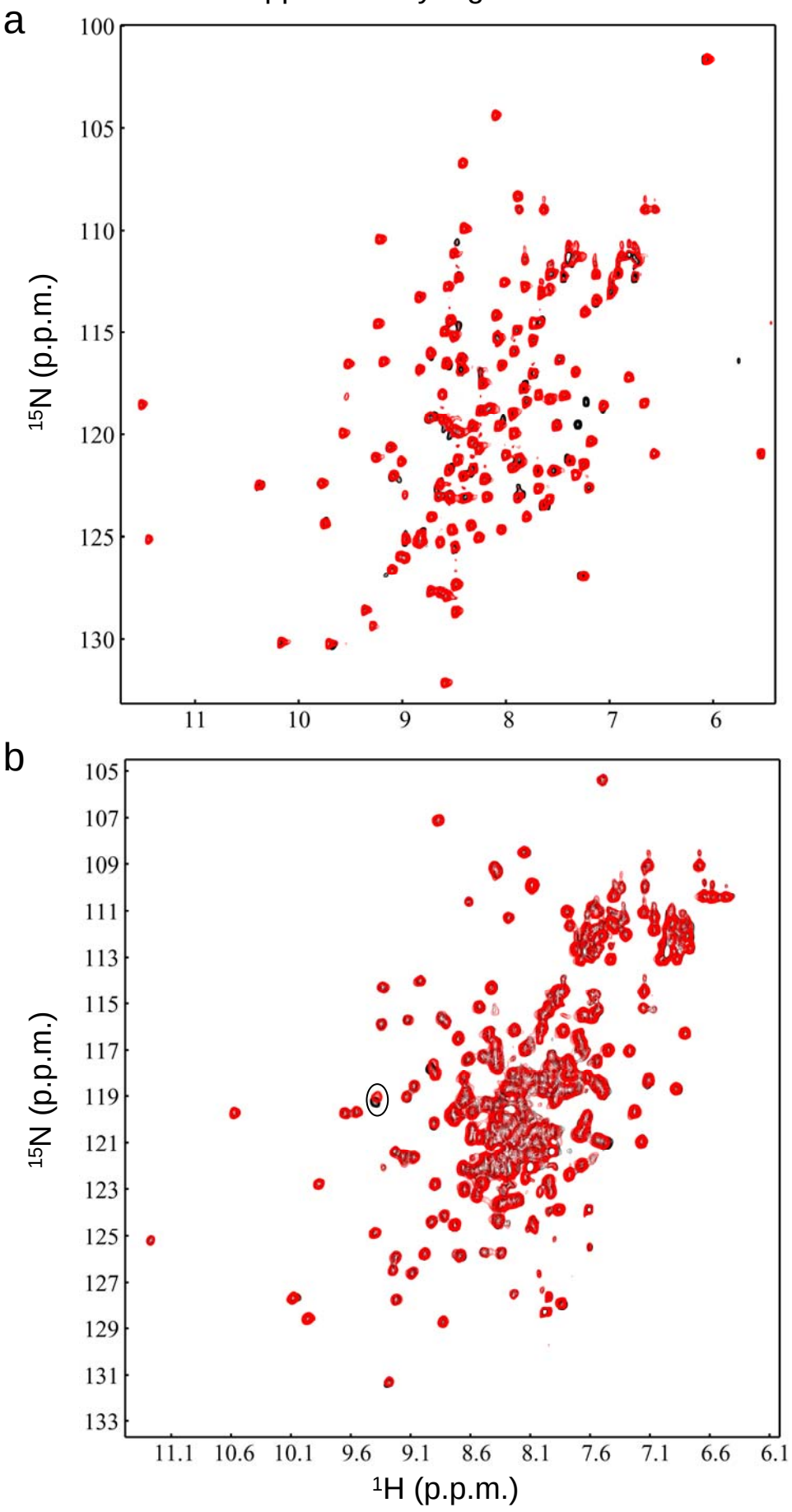
Chemical shift perturbations of ^{15}N-UbcH5c residues (350 μM) upon addition of BC112/BD140 (1.0 eq). Combined chemical shift differences were calculated using the equation $\Delta\delta=[(\delta_{HN})^2 + (\delta_N/6.51)^2]^{1/2}$. Red bars correspond to resonances of UbcH5c that are completely broadened upon addition of BC112/BD140. The average $\Delta\delta$ of all UbcH5c resonances observed in the presence of BC112/BD140 is 0.03 ppm. E2 structural elements involved in other reported E2-E3 interactions are noted.

Supplementary Figure 5



Supplementary Figure 5. UbcH5c Ala96 Mutants Form a Thiol Ester with Ubiquitin as Efficiently as Wild-Type E2.
Coomassie stained SDS-PAGE gels showing E2~ubiquitin thiol ester formation with mutant and wild-type UbcH5c. Assays contained 10 μM E2, 0.5 μM E1, 20 μM Ubiquitin (Ub), and either 0 (–) or 5 mM ATP with 10 mM $MgCl_2$ (+). Reactions were incubated 30 minutes at 37°C. Duplicate samples were analyzed in the absence (top) or presence (bottom) of reducing agent to identify species containing a thiol ester.

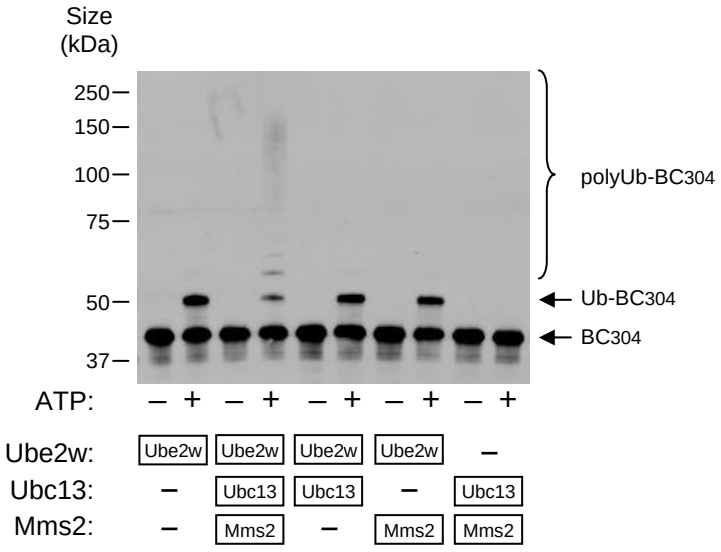
Supplementary Figure 6



Supplementary Figure 6. Mutation of Ala96 to Asp does not Affect the Structure of UbcH5c, but Prevents Binding to BRCA1.

a) ^{1}H, ^{15}N-TROSY-HSQC of ^{15}N-UbcH5-A96D (red spectrum) overlaid on the spectrum of ^{15}N-UbcH5 (black spectrum). Two resonances in the black spectrum without overlaid red peaks correspond to Ala96 and Leu97. No other major differences are observed between the two spectra. b) Overlaid ^{1}H, ^{15}N-TROSY-HSQC spectra of ^{15}N-BC_{112}/BD_{140} in the absence (black) and presence (red) of 1 equivalent of UbcH5c-A96D. Only a small shift in the resonance peak arising from Ile26 of BRCA1 (circled) is observed after addition of UbcH5c-A96D. No other major differences are observed between the two spectra. Spectra were collected at 37°C and at 300 μM each protein.

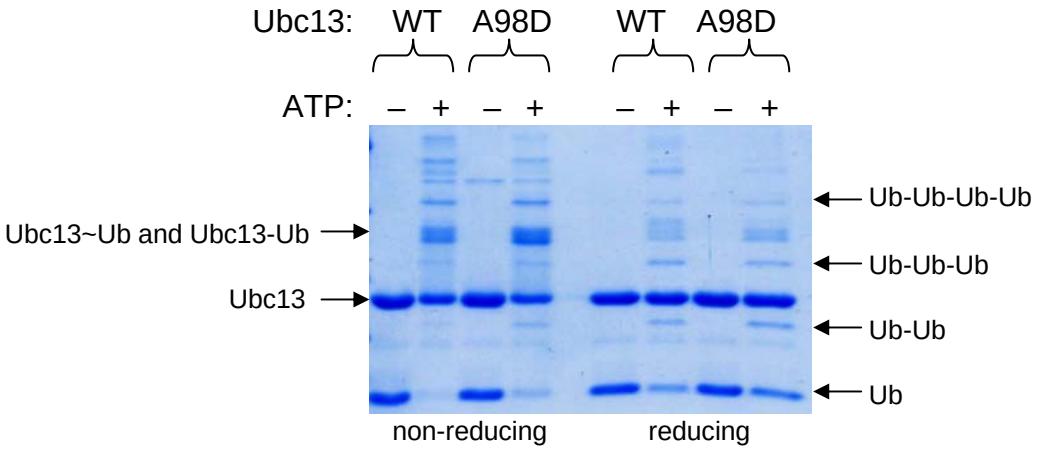
Supplementary Figure 7



Supplementary Figure 7. Poly-Ubiquitination of BRCA1 by Ubc13/Mms2 and Ube2w.

α-Flag western blot showing auto-ubiquitination of Flag-BC304/BD327 with equal concentration of each indicated E2. Only the combination of Ubc13, Mms2 and Ube2w results in poly-ubiquitinated BRCA1. Omission of any one of the E2s results in either mono-ubiquitination or no activity. Ubc13/Mms2 can also poly-ubiquitinate BRCA1 in combination with the other mono-ubiquitinating E2s, UbcM2 and UbcH5c-S22R (data not shown).

Supplementary Figure 8



Supplementary Figure 8. Ubc13-A98D is Charged with Ubiquitin and Synthesizes Poly-Ubiquitin Chains as Efficiently as Wild-Type.
Coomassie stained SDS-PAGE gels showing E2~ubiquitin thiol ester formation with mutant and wild-type Ubc13 and poly-ubiquitin chain formation. Assays contained 10 μM E2, 10 μM Mms2, 0.5 μM E1, 20 μM Ubiquitin (Ub), and either 0 (–) or 5 mM ATP with 10 mM $MgCl_2$ (+) and were incubated at 37°C for 30 minutes. Thiol ester bonds are sensitive to reducing agents and are indicated by (~) and isopeptide bonds are not sensitive to reducing agents and are indicated by (-).