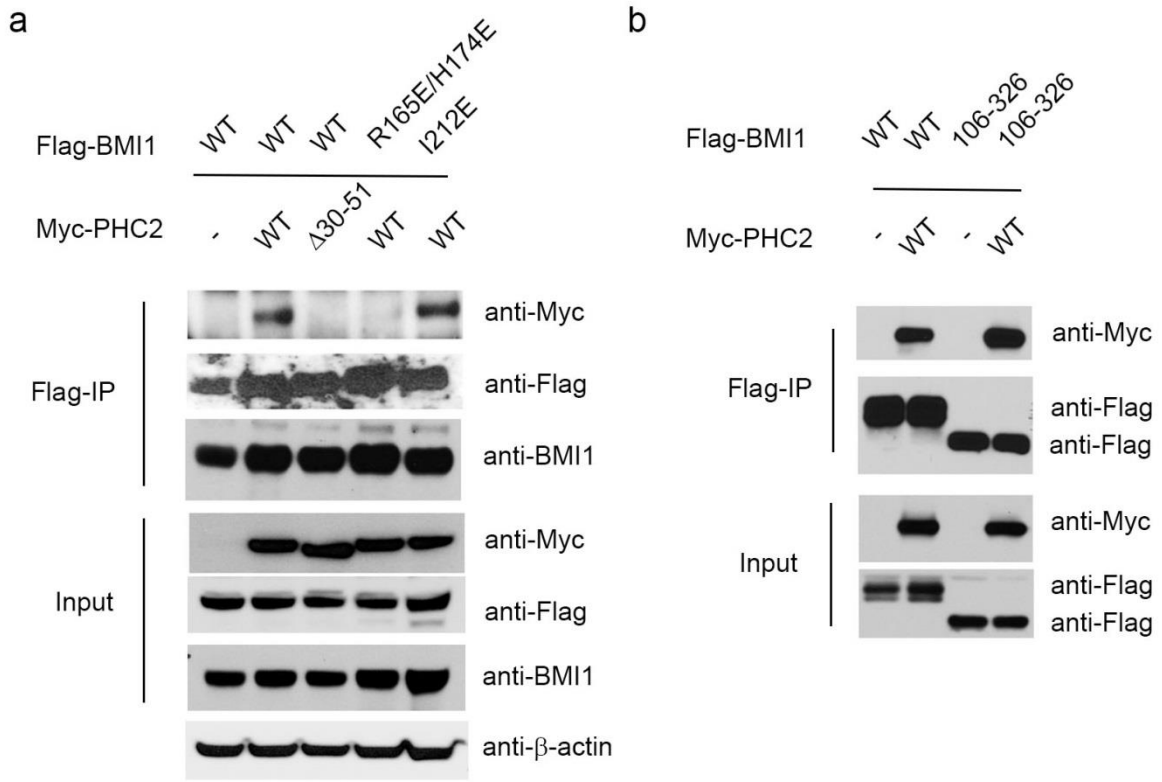
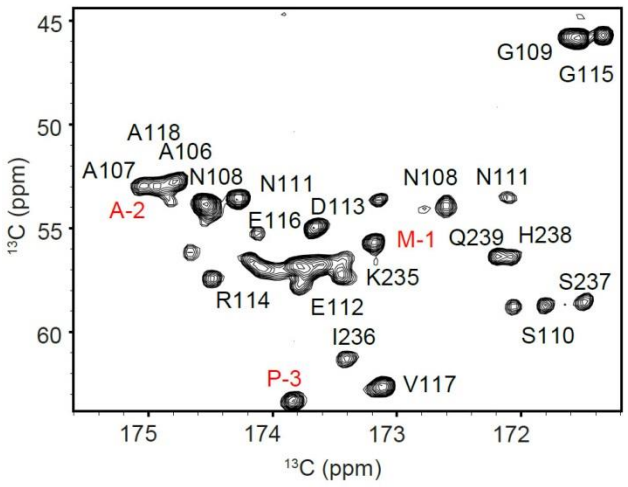
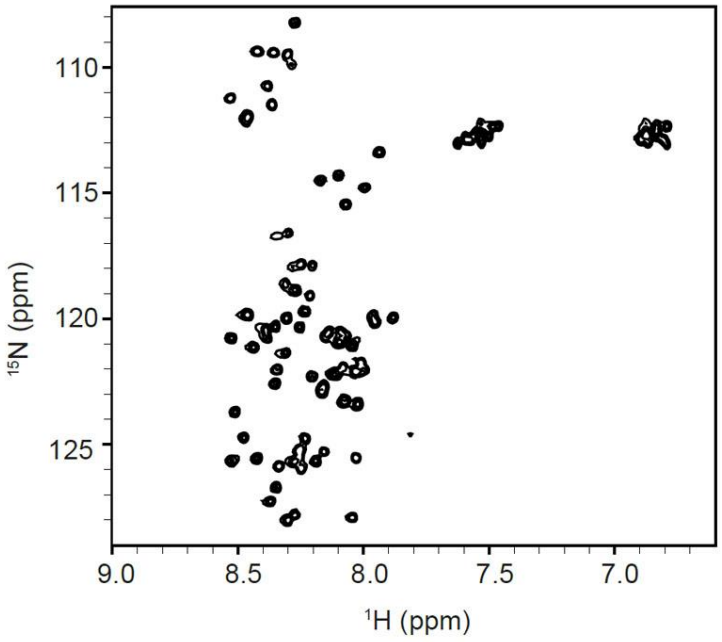


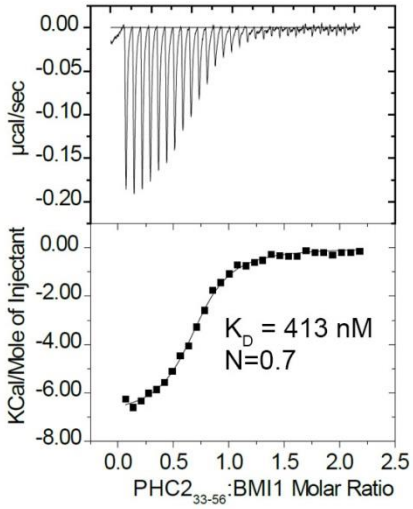
Supplementary Figure 1. (**a**) Flag-tag co-immunoprecipitations from HEK293 cells transfected with Flag-tagged full length BMI1 wild-type or mutants (R165E/H174E or I212E) and Myc-tagged PHC2_B or Myc-PHC2_B with deleted residues 30-51 (PHC2Δ30-51). Western blots are probed as indicated. (**b**) Flag-tag co-immunoprecipitation from HEK293 cells transfected with Flag-tagged full length BMI1 wild-type or BMI1 106-326. 500µg whole cell lysates were used for immunoprecipitation experiments. Western blots are probed as indicated.



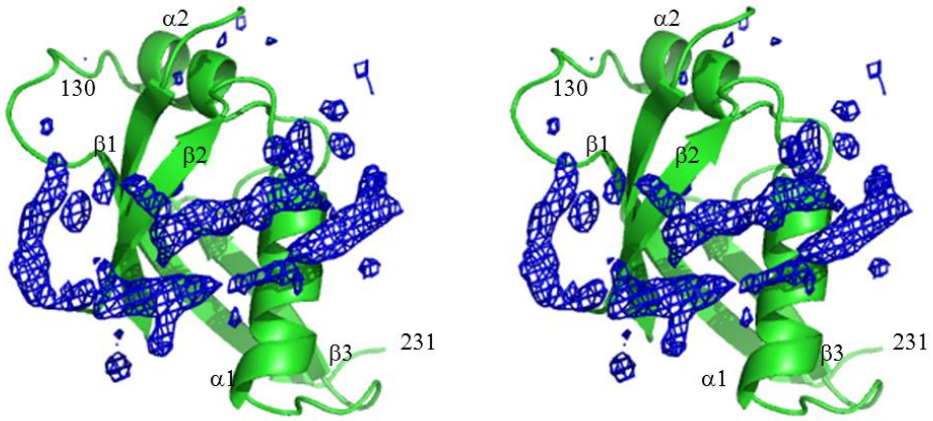
Supplementary Figure 2: ^{13}C-detected 2D CBCACO spectrum for BMI1$_{106-240}$ with assignment for flexible regions. Residues remaining from tag cleavage during purification are colored in red.



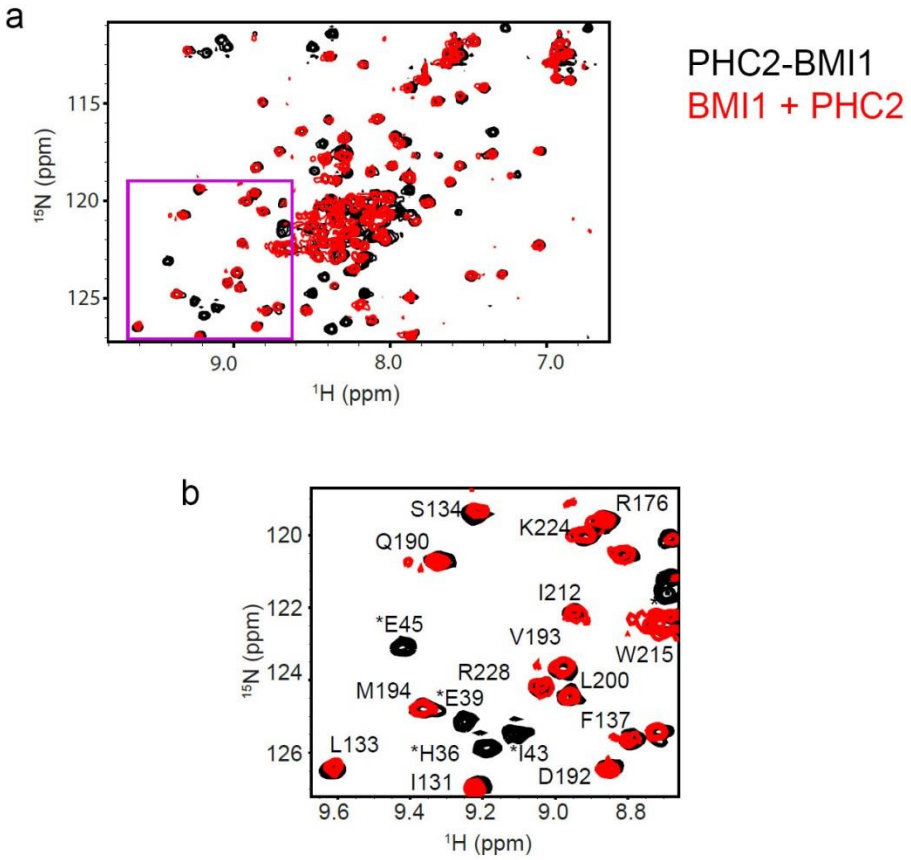
Supplementary Figure 3. ^{1}H-^{15}N HSQC spectrum of 50 μM $PHC2_{1-79}$.



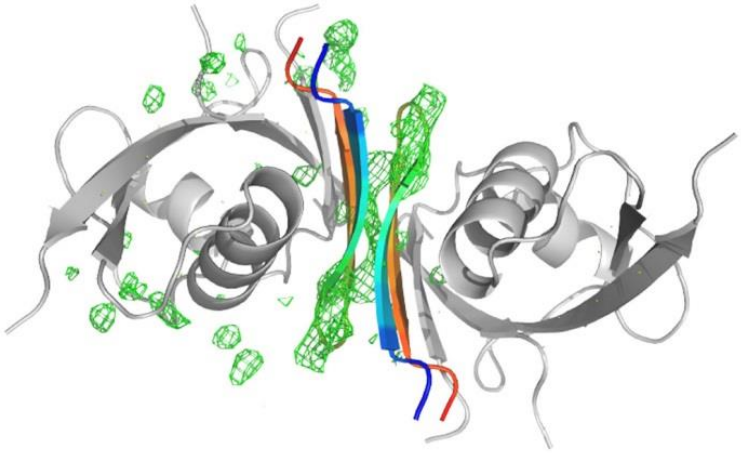
Supplementary Figure 4. Isothermal titration calorimetry experiments for BMI1 UBL titrated with $PHC2_{33-56}$ peptide.



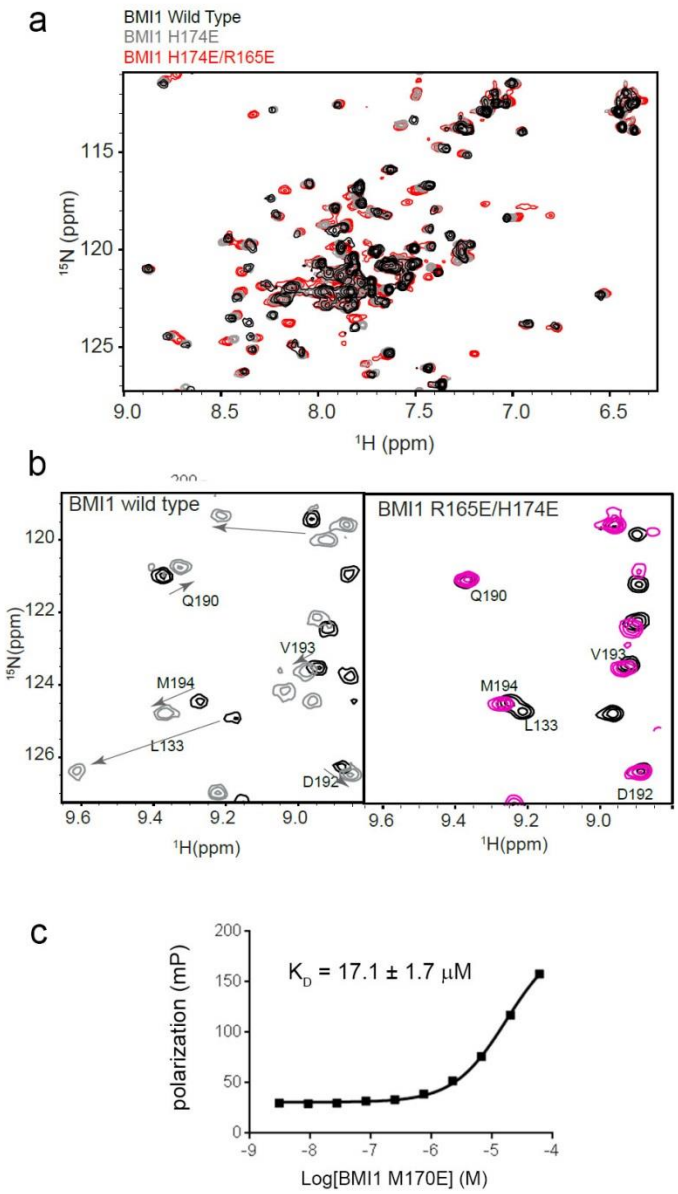
Supplementary Fig 5. Stereo view of the Fo - Fc omit electron density maps contoured at 3σ level.



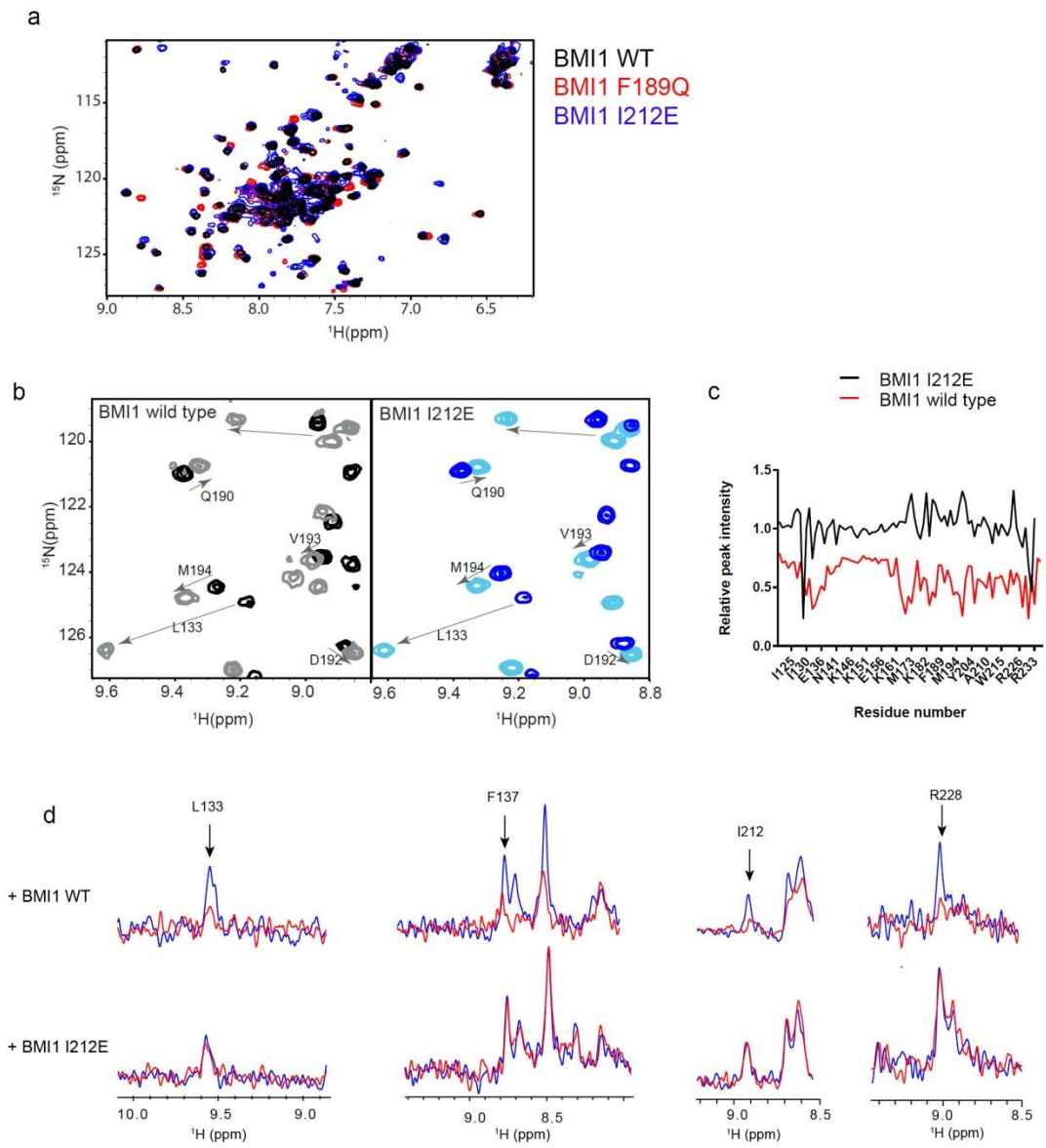
Supplementary Figure 6. (**a**) Superposition of ^{1}H-^{15}N HSQC spectra for 50 μM ^{15}N $PHC2_{30-64}$-BMI1 fusion protein (black) and 60 μM ^{15}N BMI1 UBL saturated with 60 μM $PHC2_{32-61}$ (red). (**b**) Fragment of same spectra outlined in purple box with assignment. Residues from PHC2 fragment of fusion protein are indicated with asterisk.



Supplementary Figure 7. Superposition of the structure of PHC2-BMI1 with the Fo-Fc difference omit map at 3 σ level (green) for the crystal structure of BMI1 UBL obtained in the presence of PHC2 peptide. PHC2 is colored from blue at the N-terminus to red at the C-terminus.

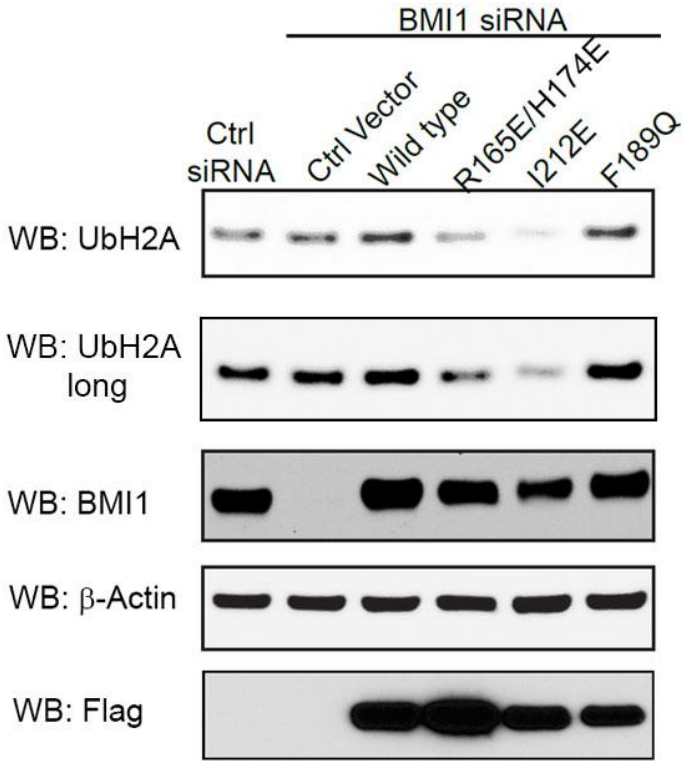


Supplementary Figure 8. (**a**) Comparison of ^{1}H-^{15}N HSQC spectra for 80 μM BMI1 UBL wild-type (black), 50 μM BMI1 UBL H174E (gray) and 100 μM BMI1 UBL R165E/H174E (red). (**b**) Fragment of ^{1}H-^{15}N HSQC spectra for 60 μM BMI1 UBL-domain wild-type (left) or 100 μM R165E/H174E (right) in the absence (black) or presence of $PHC2_{33-56}$ at 1-1 molar ratio (gray and magenta, respectively). (**c**) Binding affinity of PHC2 with BMI1 M170E mutant determined using fluorescence polarization (FP) experiments.

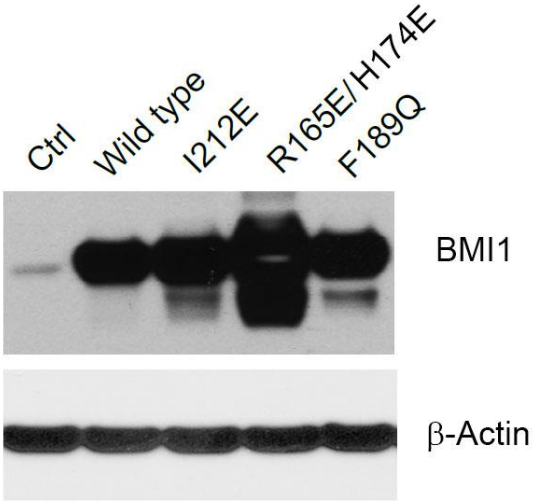


Supplementary Figure 9. (**a**) Comparison of ^{1}H-^{15}N HSQC spectra for 80 µM BMI1 UBL wild-type (black), 100 µM BMI1 UBL F189Q (red) and 100 µM BMI1 UBL I212E (blue). (**b**) Fragments of ^{1}H-^{15}N HSQC spectra for 60 µM wild-type BMI1 UBL (black, left) and 100 µM BMI1 UBL I212E (dark blue, right) superimposed with the spectra for BMI1 UBL with PHC2$_{33-56}$ peptide at 1-1 molar ratio (gray and light blue, respectively). Resonances perturbed by PHC2 binding are labeled and arrows indicate direction of chemical shift perturbation. (**c**) Quantification of peak intensities from ^{1}H-^{15}N HSQC spectra for 50 µM ^{15}N BMI1 UBL-PHC2$_{33-56}$ complex mixed with 100 µM unlabeled BMI1 UBL I212E-PHC2$_{33-56}$ complex (black) or 100 µM unlabeled BMI1 UBL wild-type-PHC2$_{33-56}$ complex (red). Intensities are normalized to 50 µM ^{15}N BMI1 UBL -PHC2$_{33-56}$ complex. (**d**) Cross-sections for selected peaks from spectra in Figures 3E and 3F. Top row: selected peaks from ^{1}H-^{15}N HSQC spectrum for 50 µM ^{15}N BMI1 UBL-PHC2$_{33-56}$ complex (blue) mixed with 100 µM unlabeled wild-type-PHC2$_{33-56}$ complex (red).

Bottom row: selected peaks from ^{1}H-^{15}N HSQC spectrum for 50 µM ^{15}N BMI1 UBL-PHC2$_{33-56}$ complex (blue) mixed with 100 µM BMI1 UBL I212E-PHC2$_{33-56}$ complex (red).

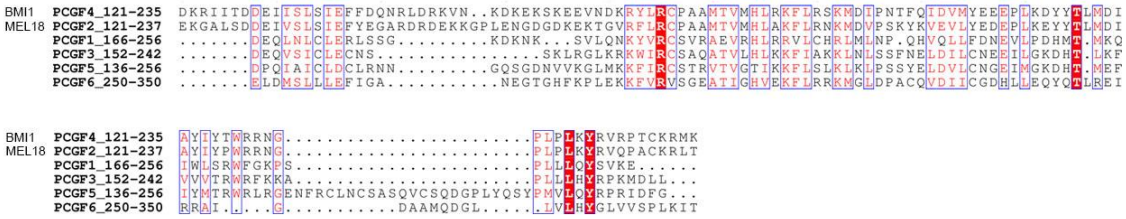


Supplementary Figure 10. Characterization of Ub-H2A levels upon overexpression of BMI1 mutants in HeLa cells. HeLa cells transfected with control or BMI1 3' UTR siRNA for 48 hours were transfected with plasmids encoding Flag-tagged full length wild-type BMI1 or mutants and analyzed by immunoblotting after 48 hours. UbH2A long represents longer exposure.



Supplementary Figure 11. Analysis of BMI1 expression in whole cell lysate of U2OS cells used for clonogenic survival assays expressing BMI1 shRNA and transfected with BMI1 wild-type or mutants. Western blot probed as indicated.

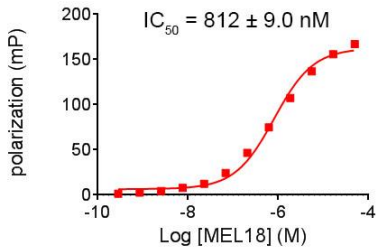
a



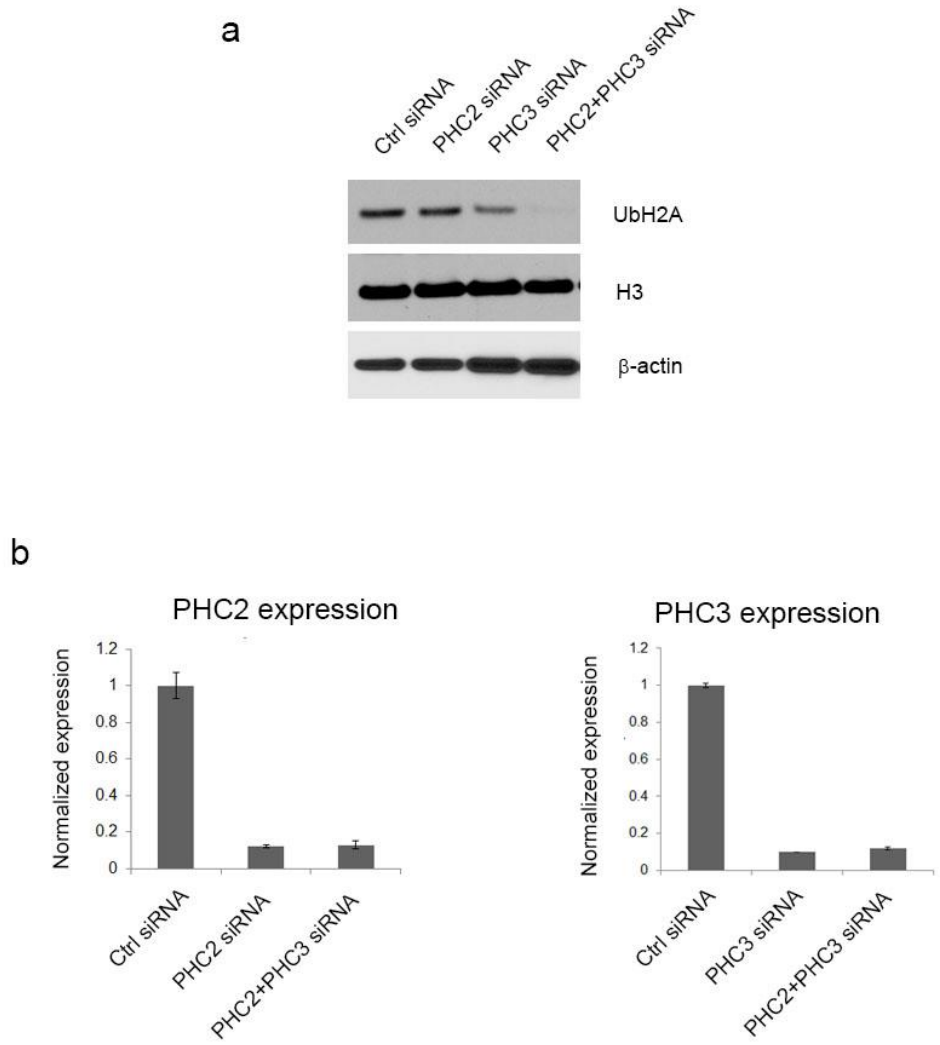
b

Sequence identity	PCGF2 (MEL18)	PCGF3	PCGF4 (BMI1)	PCGF5	PCGF6
PCGF1	24.44	38.10	31.11	28.57	23.17
PCGF2		30.00	60.00	28.57	30.11
PCGF3			31.11	50.55	28.57
PCGF4				27.84	26.88
PCGF5					27.84

c

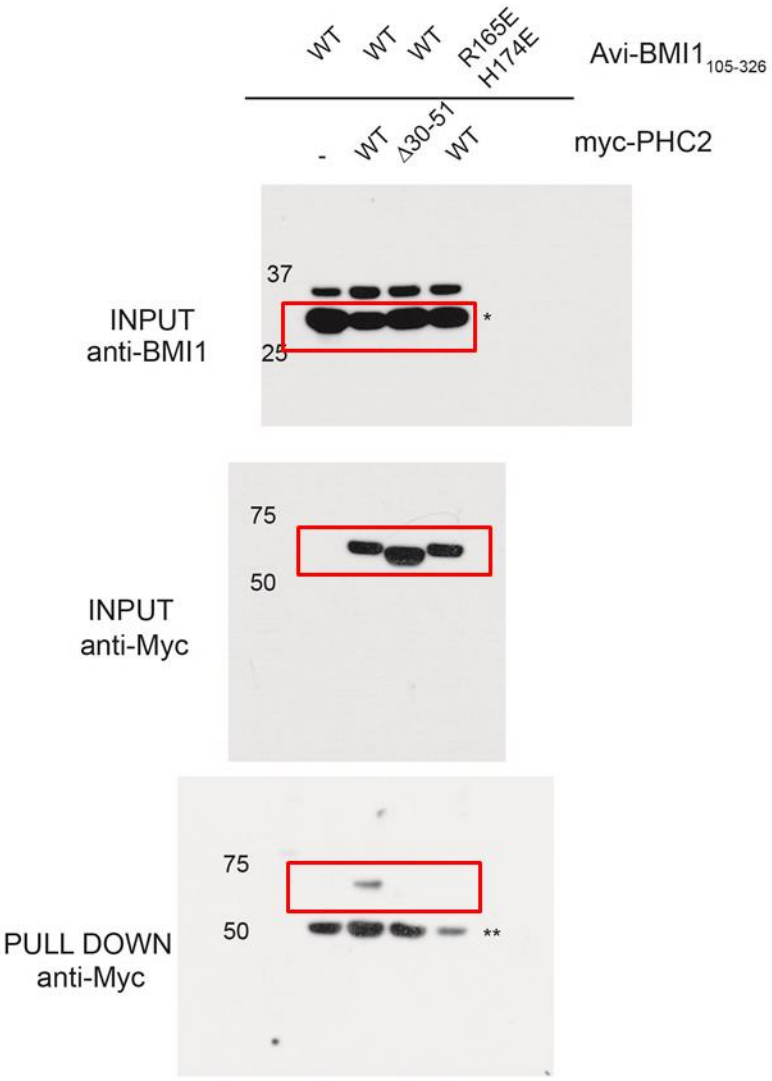


Supplementary Figure 12. (**a**) Sequence alignment of UBL domains for PCGF family members. (**b**) Matrix showing sequence identity for UBL domains among PCGF family members. UBL domain of BMI1 (PCGF4) shares 60% sequence identity with MEL18 (PCGF2). (**c**) Fluorescence polarization experiment showing the affinity of $MEL18_{121-237}$ binding with $PHC2_{32-61}$.



Supplementary Figure 13. (**a**) Analysis of the Ub-H2A levels upon knockdown of PHC2 and PHC3 in HeLa cells. (**b**) Expression of PHC2 and PHC3 in HeLa cells upon siRNA knockdown assessed by RT-qPCR.

Figure 1b



* Endogenous BMI
** myc non-specific

Figure 4a

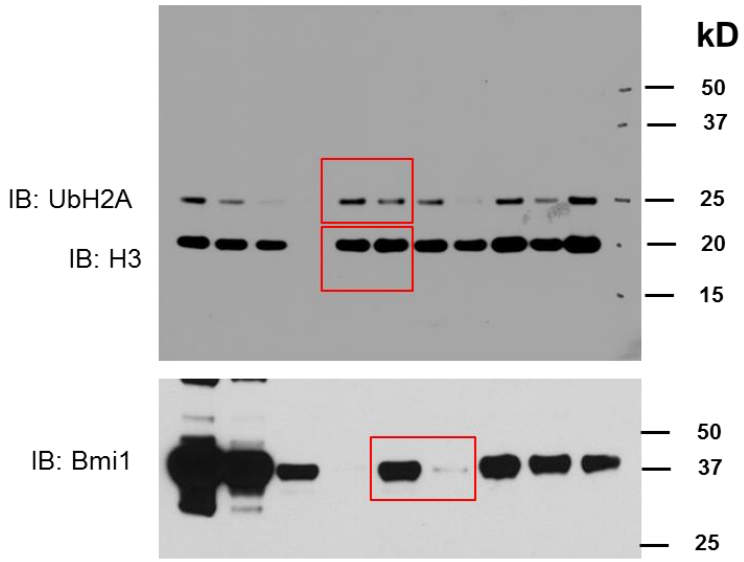


Figure 4b and Supplementary Fig 10

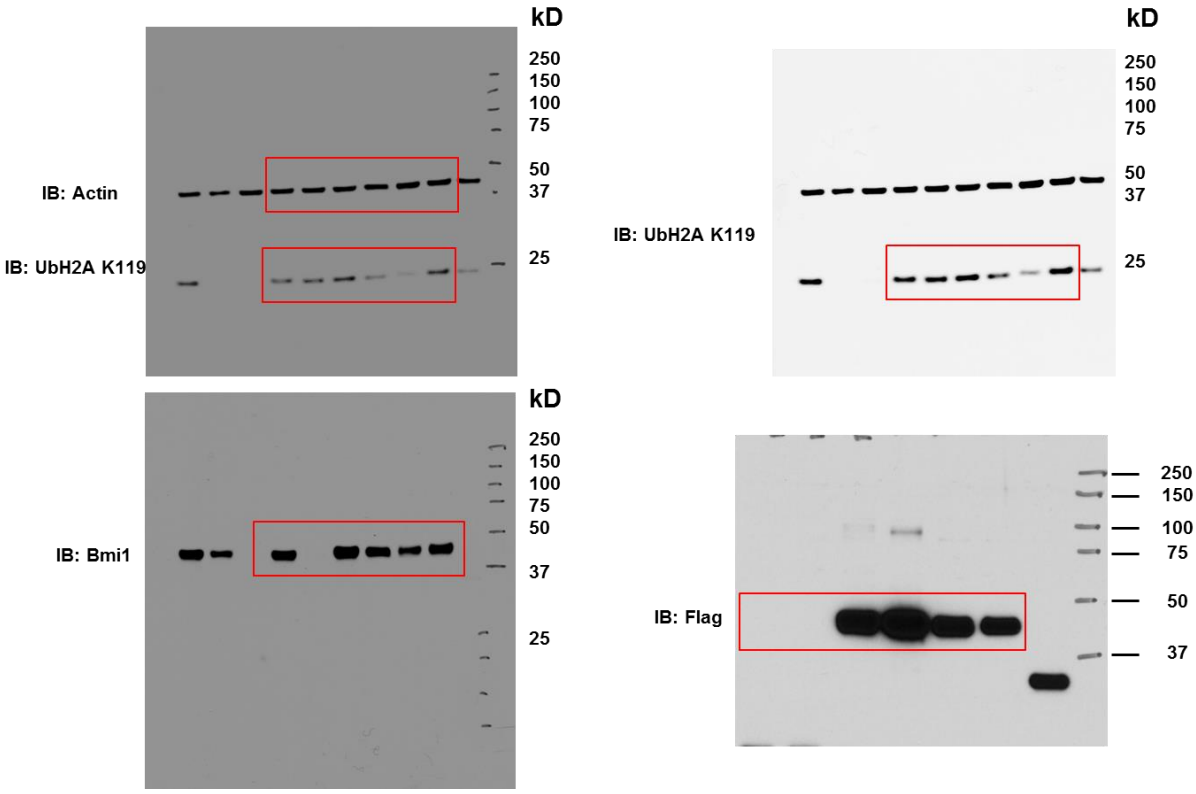
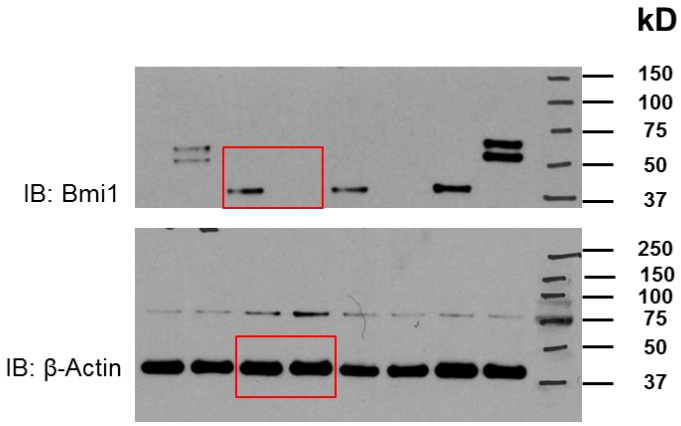
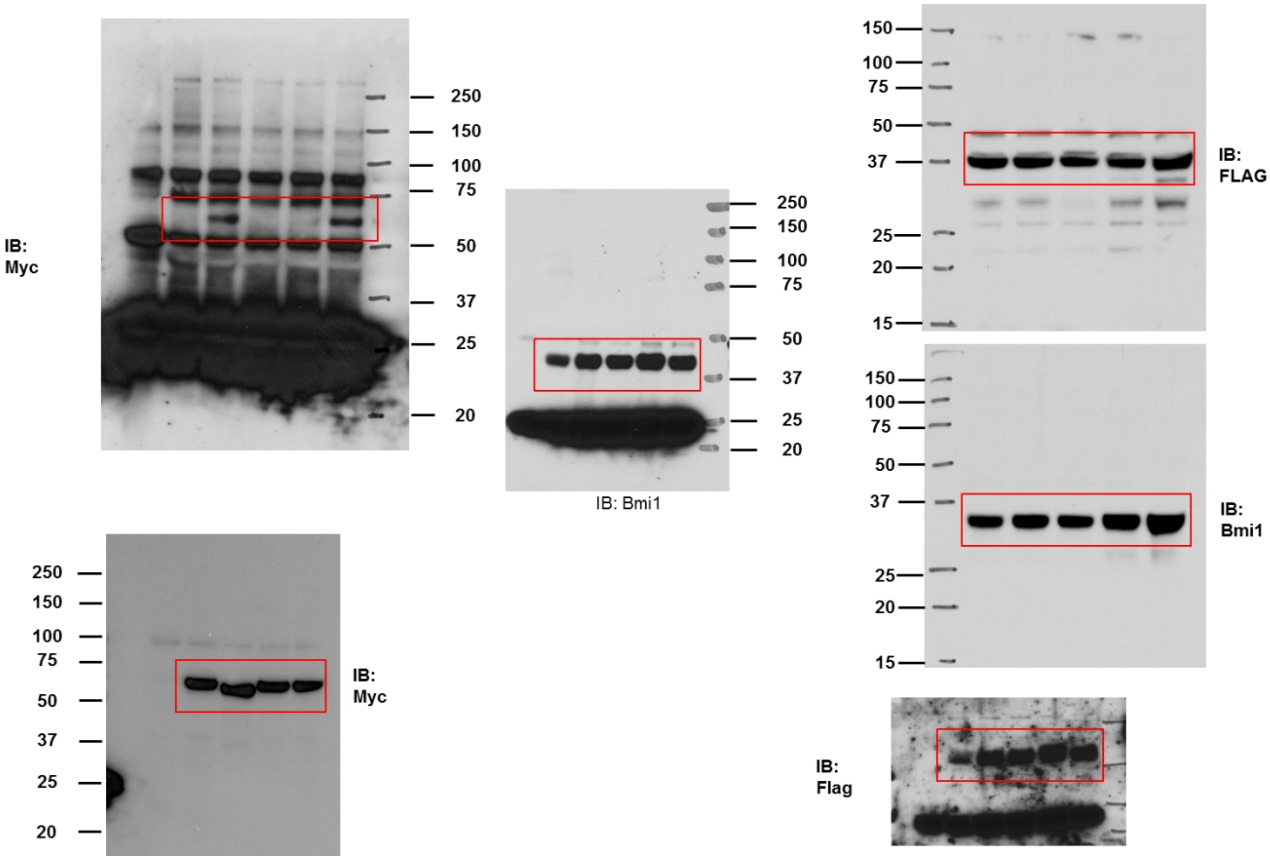


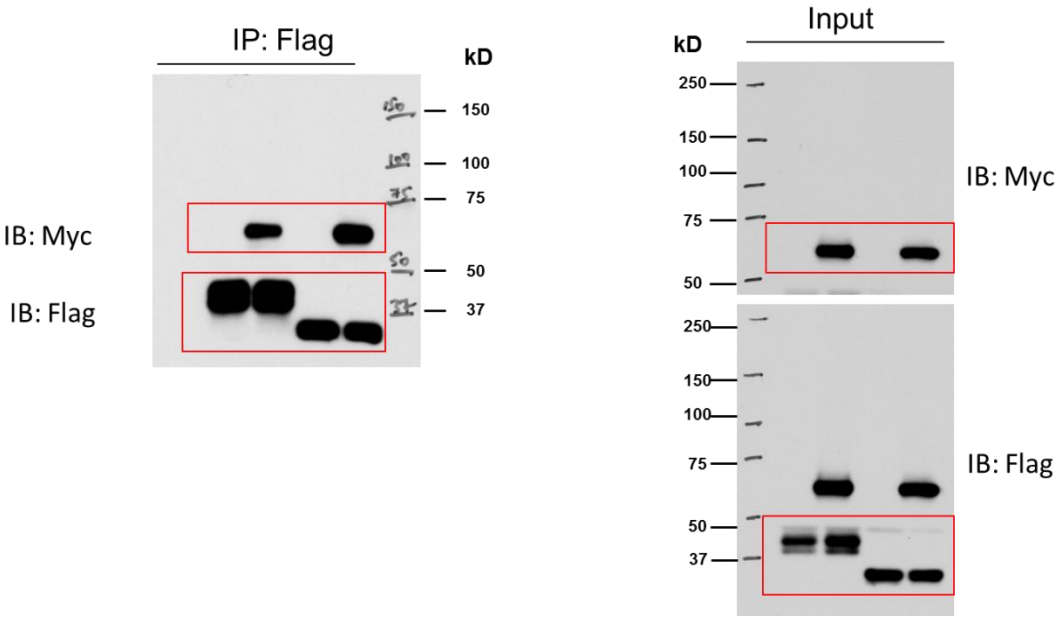
Figure 4c



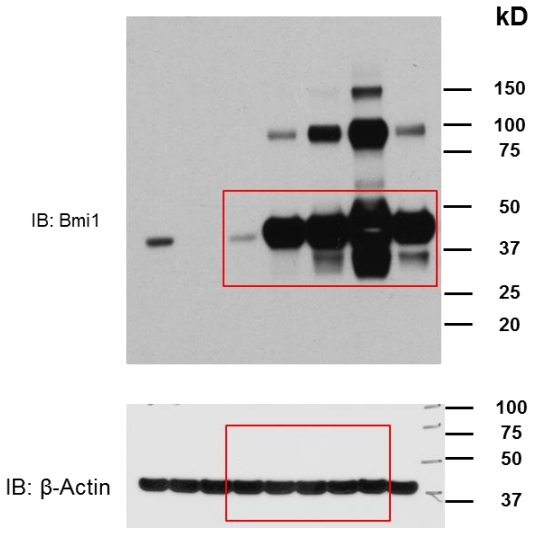
Supplementary Fig 1a



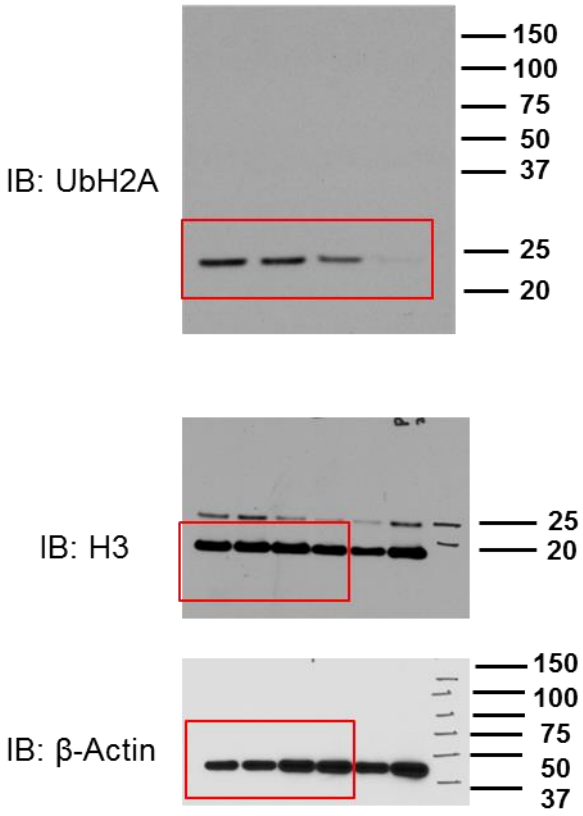
Supplementary Fig 1b



Supplementary Fig 11



Supplementary Fig 13



Supplementary Figure 14. Uncropped scans of all western blots with reference to original figures. Molecular weight ladder is shown.