Fig. S1. **Interactions within the RAP80 complex allow for BRCC36 DUB activity.** *A* Coomassie-stained gel representing purified proteins used for *in vitro* DUB assays. Recombinant Abraxas, BRCC36, BRCC45, MERIT40 and RAP80 (all of which are FLAG-HA-tagged) were purified from baculovirus infected Sf9 cells by FLAG-IP and FLAG peptide elution and are estimated to be ~95% pure. Cp. = Complex. *B* BRCC36 mutant (ΔN13) does not localize to DSBs. (top panel) Schematic of wildtype and ΔN13 BRCC36. BRCC36 ΔN13 lacks the first 13 N-terminal residues. (bottom panel) IF for FLAG-HA-BRCC36 and 53BP1 at 4 hrs after 10 Gy IR in HeLa S3 cells demonstrates loss of colocalization with 53BP1 for BRCC36 ΔN13. Bars, 10 μm. *C* A BRCC36 mutant (ΔN13) does not interact with components of the RAP80 complex. IB of wildtype tagged BRCC36 and mutant BRCC36 ΔN13. FLAG-HA-tagged BRCC36s were FLAG IP'd and blotted as indicated. *D* Coomassie-stained gel representing purified proteins used for in vitro DUB assays in Fig. 1. Recombinant wildtype BRCC36 and wildtype BRCC36 complex containing Abraxas, BRCC45, MERIT40 and RAP80; and mutant BRCC36 ΔN13 and mutant BRCC36 ΔN13 complex containing Abraxas, BRCC45, MERIT40 and RAP80 were purified from baculovirus infected Sf9 cells and FLAG-HA-tagged and are estimated to be ~95% pure.

Fig S2. **Abraxas and KIAA0157 interact with BRCC36 through MPN- domains.** *A* Interaction profile of an Abraxas and KIAA0157 MPN domain mutants. FLAG-HA-tagged plasmids encoding wildtype Abraxas and Abraxas W99E, and wildtype KIAA1057 and KIAA0157 W98E (corresponding mutation) were transiently transfected into 293T cells and IP performed 48 hours later. Both the Abraxas W99E and KIAA0157 W98E mutants demonstrated loss of interaction with RAP80, MERIT40 and BRCC36. *B* Full BRCC36 DUB activity requires protein-protein interactions and Wt Abraxas or KIAA0157 MPN domains. Ectopic Abraxas or KIAA0157 were FLAG-purified from transiently transfected 293T cells 48 hrs post-transfection. FLAG-purified complexes were incubated with Hexa K63-Ub for 1 hour and the products detected by IB with the P4D1 antibody to ubiquitin.

Fig. S3. **KIAA0157 but not Abraxas is sufficient to activate BRCC36 DUB activity in vitro.** *A* Coomassie-stained gel representing FLAG-purified complexes purified proteins used for in vitro DUB assays. Recombinant KIAA0157 (left lane), or the entire BRISC complex including KIAA0157, BRCC36, BRCC45 and MERIT40 were Flag-purified from baculovirus infected Sf9 cells and estimated to be ~95% pure. *B* The BRISC complex displays K63Ub specific DUB activity. FLAG-HA-tagged BRCC36 expressed by itself or together with the other BRISC components was purified from baculovirus infected Sf9 cells 48 hrs post-infection. FLAG-purified complexes were incubated with either Hexa K48-Ub or Hexa K63-Ub for 1 hour and the products detected by IB with the P4D1 antibody to ubiquitin. The figure is representative of 2 independent experiments. *C* The BRISC complex demonstrates greater K63Ub DUB activity than the RAP80 complex in vitro. FLAG-HA-tagged BRISC or RAP80 complexes were purified from baculovirus infected Sf9 cells 48 hrs post-infection. Equivalent amounts of FLAG-purified complexes were incubated with increasing concentrations of Hexa K63-Ub for 1 hour and the products detected by IB with the P4D1 antibody. Appearance of di-ubiquitin was quantified using NIH Image J software and DUB activity was calculated as a function of input Hexa K63-Ub. The figure is an average of 2 independent experiments. Enzyme kinetics were determined using nonlinear regression (Michaelis-Menten enzyme kinetics). The representative graph is displayed alongside the calculated V_{max} and K_m values.

Fig S4. **KIAA0157 localization.** *A* Abraxas and KIAA0157 share similar protein associations with MERIT40 and BRCC36, but differ with respect to interaction with RAP80. IB of ectopic Abraxas and ectopic KIAA0157 complexes after FLAG IP from HeLa S3 cells. Abraxas interacts with RAP80, MERIT40 and BRCC36. KIAA0157 interacts with MERIT40 and BRCC36 but not RAP80. *B* IB of KIAA0157 following fractionation of HeLa S3 cells into cytoplasmic (Cyto) and nuclear extracts (NE). *C* Full length IB of ectopic BRCC36 and BRCC36-QSQ complexes after FLAG IP from HeLa S3 cells.

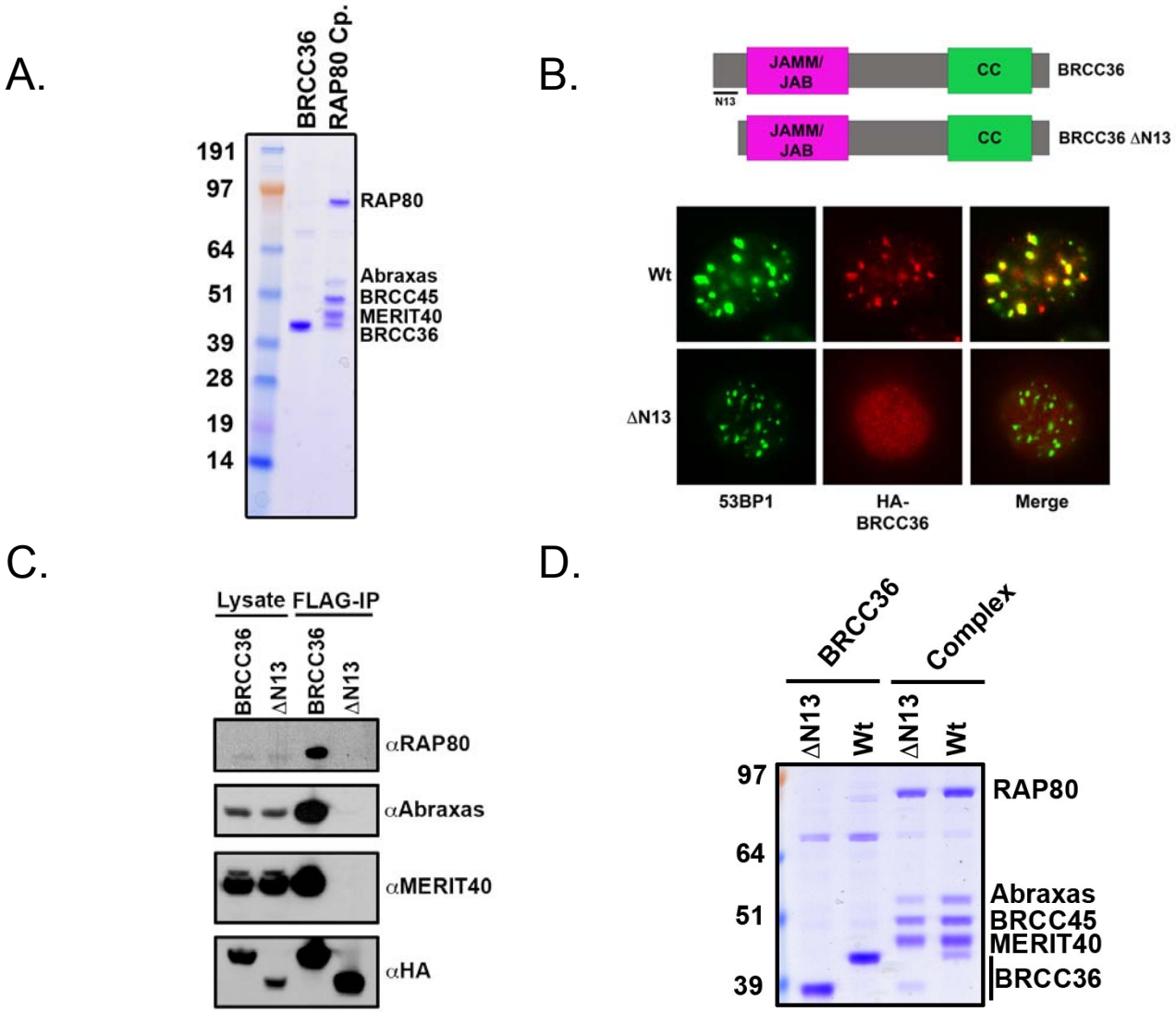
IB was probed for both RAP80 and K63-linked Ubiquitin demonstrating increased K63-linked polyubiquitination of RAP80 is associated with the BRCC36-QSQ complex.

Fig. S5. **Differential roles of Abraxas and KIAA0157 in the response to ionizing radiation.** *A* Ectopic Abraxas and KIAA0157 were detected by IF using an anti-HA antibody in HeLa S3 cells at 4 hrs following 10 Gy of ionizing radiation (IR). Antibody to endogenous 53BP1 was used to examine localization of each protein to IR induced foci. Bars, 10 μm. *B* KIAA0157 deficiency does not cause cellular hypersensitivity to ionizing radiation. Cells were seeded at low density following transfection of siRNA targeted to control (Ct), Abraxas, or KIAA0157 and then treated with the indicated doses of IR. Colonies were counted at 12 days after IR and normalized as a percentage of colonies formed at 0 Gy. Each point represents the average of 3 independent experiments. Error bars indicate SD. *C* IF to detect colocalization of ectopic BRCC36 and endogenous 53BP1 at 4 hrs after 10 Gy IR in U2OS cells following knockdown of Ct (left panel), Abraxas (middle) or KIAA0157 (right). Abraxas deficiency reduced BRCC36 IRIF, KIAA0157 deficiency did not diminish BRCC36 IRIF. Bars, 10 μm. *D* IF for BRCA1 and 53BP1 at 4 hrs after 10 Gy IR in HeLa cells following Ct (left panel), Abraxas (middle) or KIAA0157 (right) siRNA treatment demonstrates reduced BRCA1 DSB localization with Abraxas depletion but not with KIAA0157 depletion. Bars, 10 μm.

Fig. S6. **Influence of RAP80 and BRISC complexes on K63-Ub IR induced foci.** *A* K63-Ub forms foci IR induced foci. Prior to IR, K63-Ub is pan-cellular. Following treatment with 10 Gy of IR, K63-Ub forms foci and K63-Ub cytostolic staining is reduced. IF was performed for K63-Ub before and after IR in U2OS cells. Bars, 10 μm. *B* BRCC36 DUB activity is in equilibrium between the RAP80 and KIAA0157 complexes. IF was performed for γH2AX and K63-Ub following Ct, Abraxas, BRCC45, MERIT40, KIAA0157 and RAP80 siRNA treatment in U2OS cells exposed to 10 Gy after 4 hrs. Knockdown of Abraxas, BRCC45, MERIT40 and RAP80 increased K63-Ub foci. Bars, 10 μm. *C* Quantification of K63-Ub foci formation displayed graphically from *B*. At least 100 cells were counted in triplicate for the analysis. Error bars represent SD and p values were calculated by the Student's t-test.

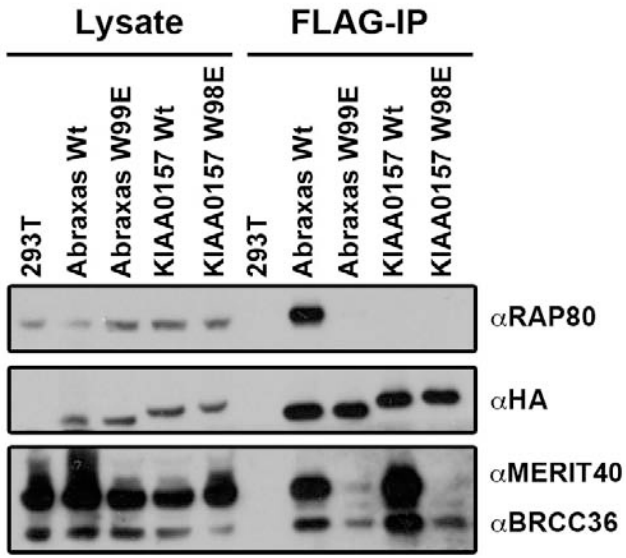
Fig. S7. **Sequence comparisons of BRCC36, Poh1, and AMSH-LP JAMM domains.** Sequence alignment of BRCC36, Poh1 and AMSH-LP. Regions of high conservation are boxed. Insertions in BRCC36 are highlighted in yellow. Ins-1 and Ins-2 are underlined and residues in the active site or which coordinate zinc are highlighted in cyan. Pairwise comparison of the sequences indicates that BRCC36 is 14% identical and 39% similar with Poh1 and 12% identical 22% similar with AMSH-LP.

Fig. S8. **Experimental controls.** *A* IB of K48-linked or K63-linked Ub using antibodies directed against all ubiquitin (P4D1) or specifically against K63-linked Ub (K63) demonstrating specificity of K63 antibody. *B* IB of siRNA-mediated knockdowns used in Figure 6A. *C* IB of siRNA-mediated knockdowns used in Figure 6D.


A.
BRCC36
RAP80 Cp.
191
97
64
51
39
28
19
14
RAP80
Abraxas
BRCC45
MERIT40
BRCC36
B.
JAMM/
JAB
CC
BRCC36
N13
JAMM/
JAB
CC
BRCC36 ΔN13
Wt
ΔN13
53BP1
HA-
BRCC36
Merge
C.
Lysate
FLAG-IP
BRCC36
ΔN13
BRCC36
ΔN13
αRAP80
αAbraxas
αMERIT40
αHA
D.
BRCC36
Complex
ΔN13
Wt
ΔN13
Wt
97
64
51
39
RAP80
Abraxas
BRCC45
MERIT40
BRCC36

Patterson-Fortin et al. Figure S2

A.



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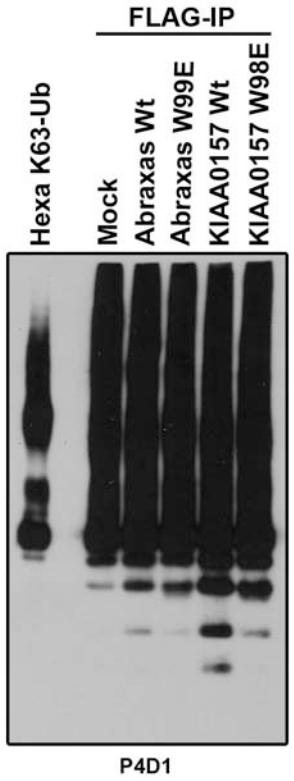
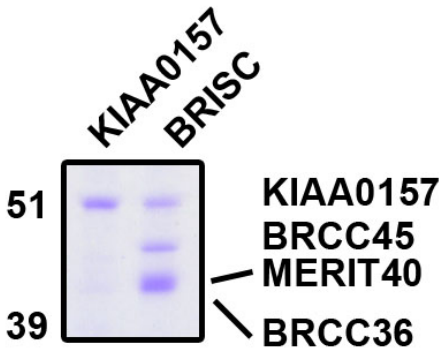


A.



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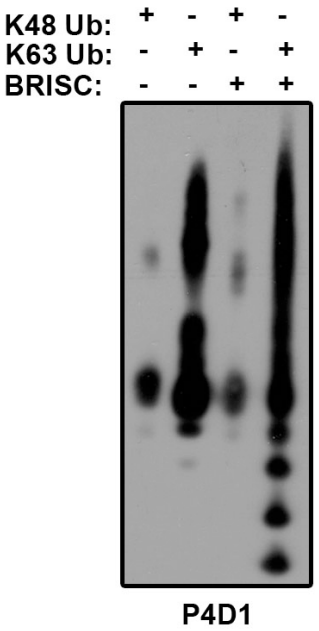
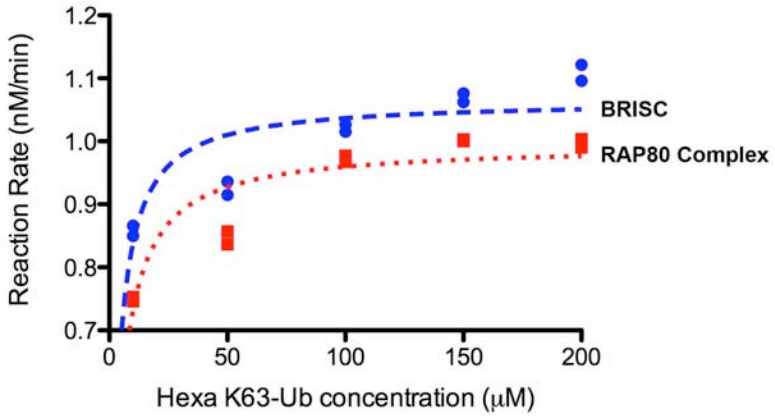
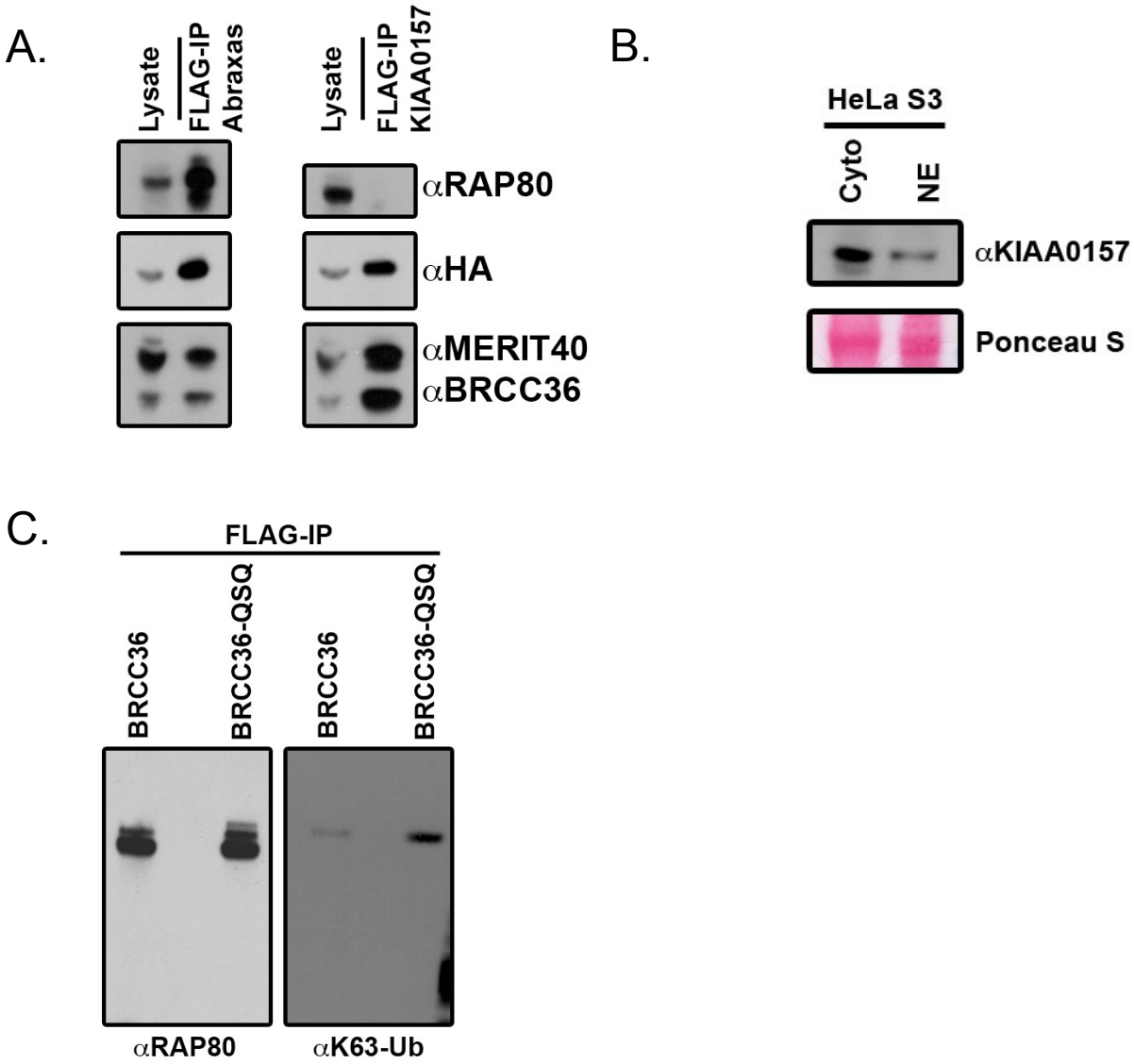


C.

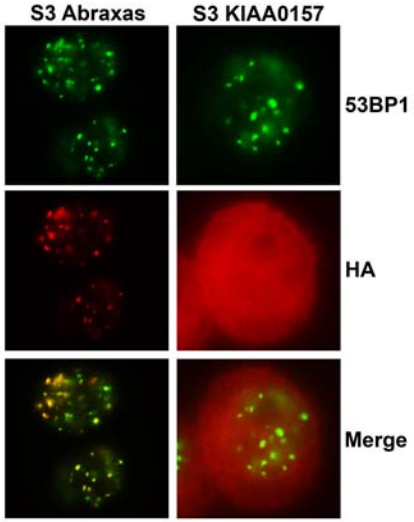


	BRISC	RAP80 Complex
Vmax (nM min^{-1})	1.065 +/- 0.02	0.9942 +/- 0.02
Km (μM)	2.708 +/- 0.71	3.597 +/- 0.73


A.
Lysate
FLAG-IP
Abraxas
Lysate
FLAG-IP
KIAA0157
αRAP80
αHA
αMERIT40
αBRCC36
B.
HeLa S3
Cyto
NE
αKIAA0157
Ponceau S
C.
FLAG-IP
BRCC36
BRCC36-QSQ
BRCC36
BRCC36-QSQ
αRAP80
αK63-Ub

Patterson-Fortin et al. Figure S5

A.



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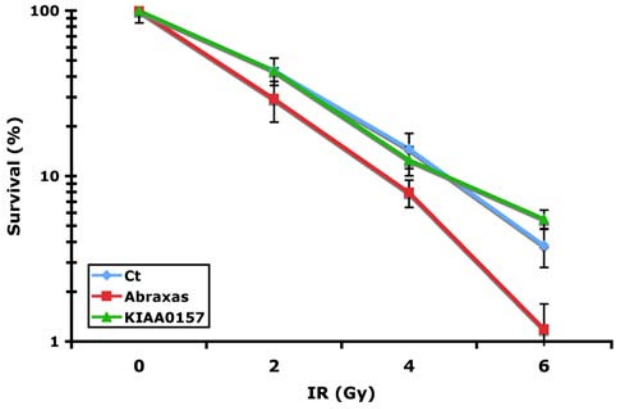

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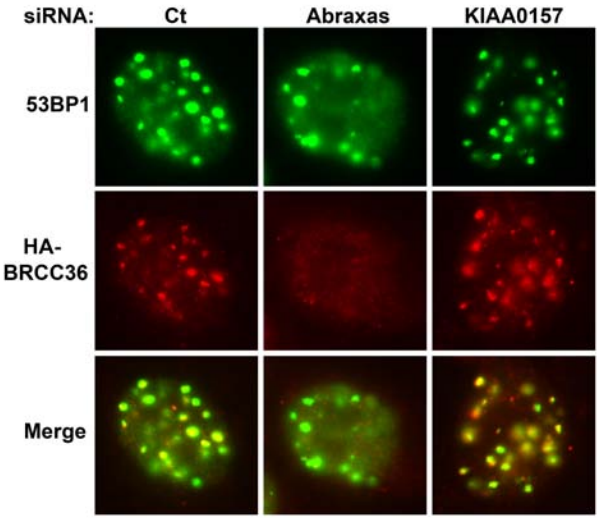

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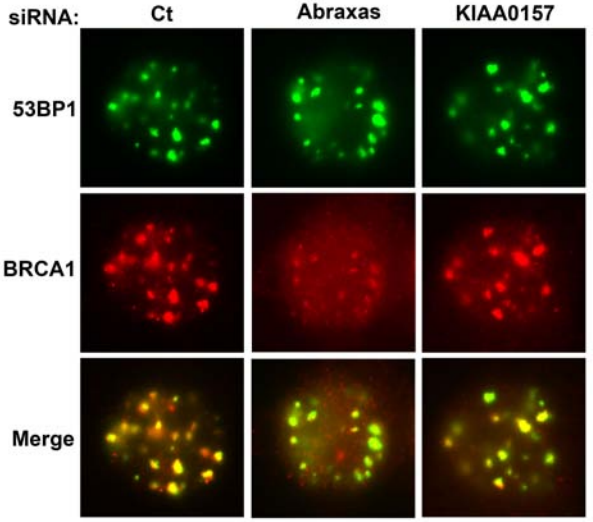
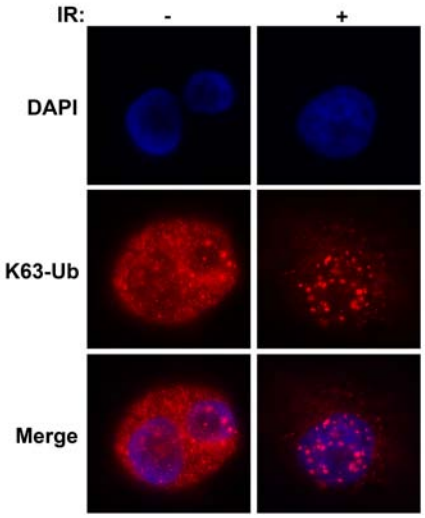


Patterson-Fortin et al. Figure S6

A.



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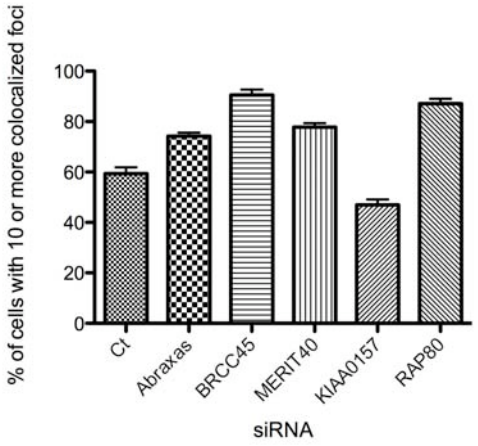

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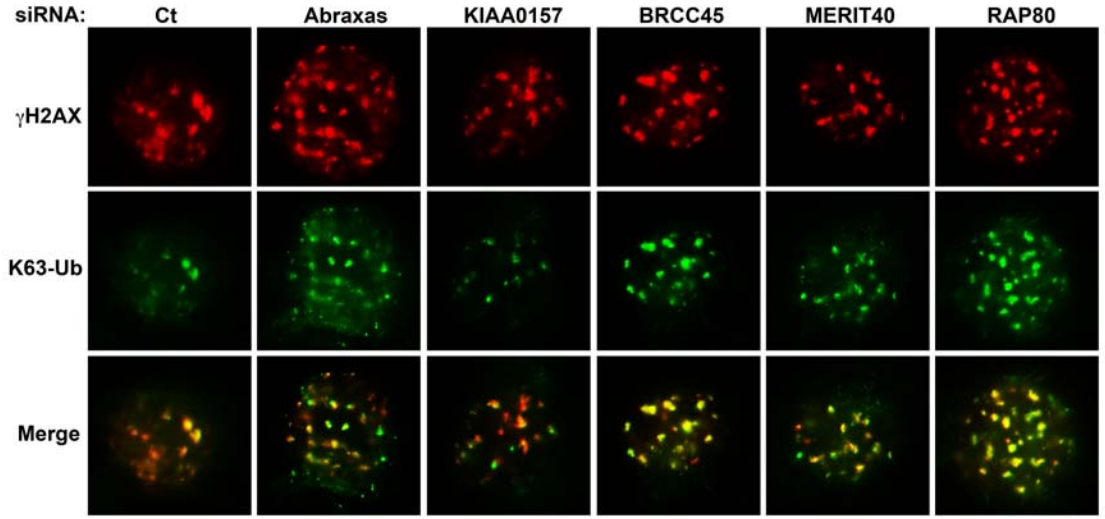
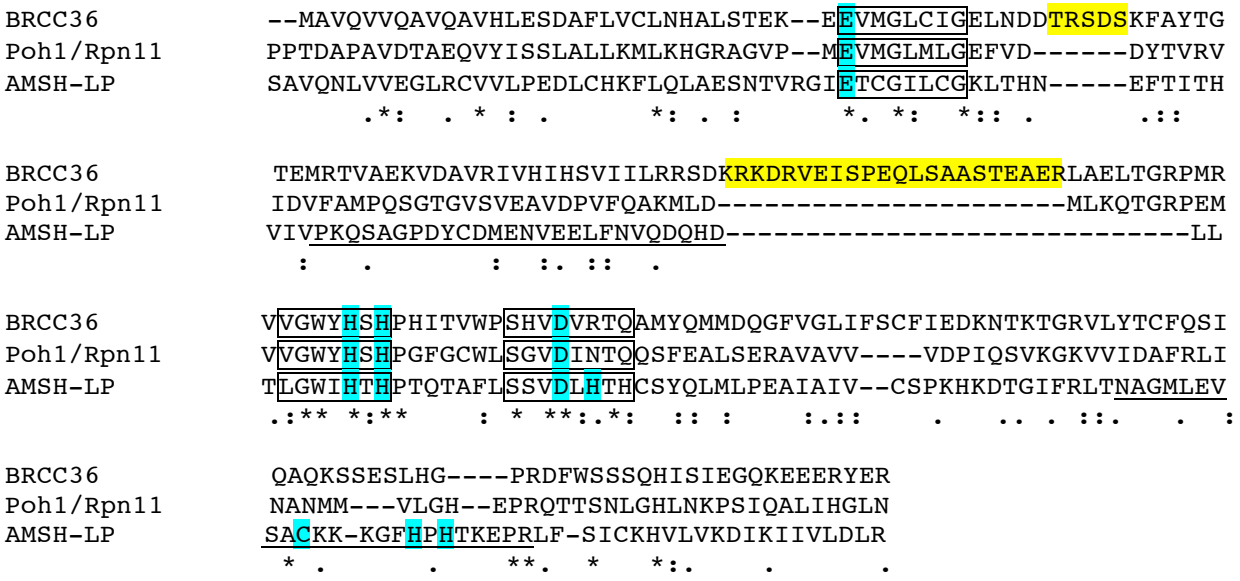
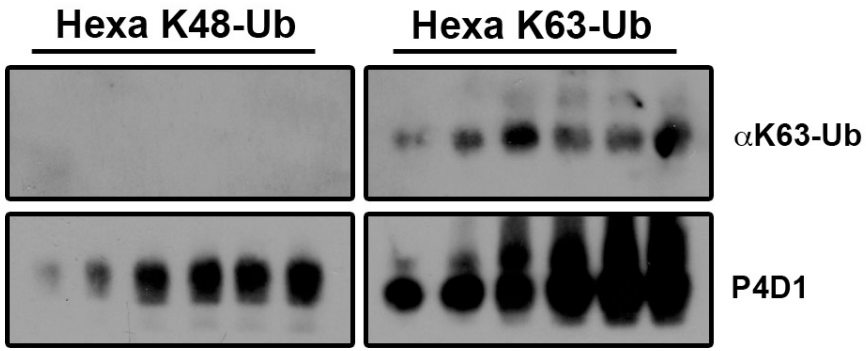


Patterson-Fortin et al. Figure S7



Patterson-Fortin et al. Figure S8

A.



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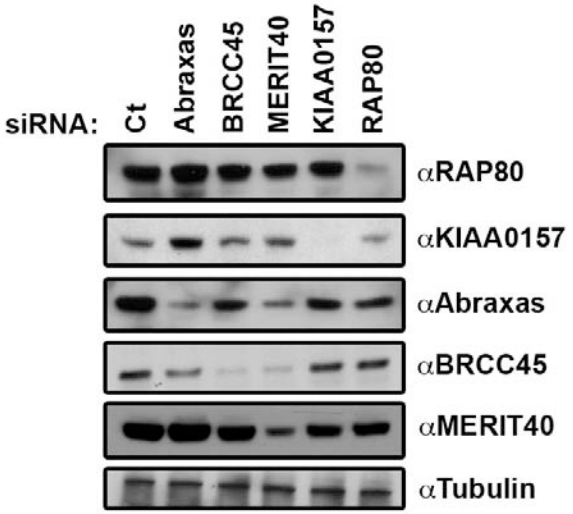

C.