Supplemental Materials and Methods

BRIP1 Constructs and Site Directed Mutagenesis

pcDNA3-myc-his-BACH1 WT was a gift from Ronny Drapkin (Addgene plasmid #17642). A Not1 enzyme cut site was added to the c-terminal end of the BRIP1 WT cDNA using the primer: [CACCGGAGCGGCCGCGTTTAAACTCAATGGTGATGGTG]. The cDNA was cloned into the BamHI and NotI sites of the pCDH-EF1-MCS-(PGK-copGFP-T2A-Puro) (System Biosciences CD813A-1). Missense mutations were introduced using QuikChange II XL Site-Directed Mutagenesis kit (Agilent) and verified by Sanger sequencing (primers available upon request). All plasmid DNAs were cloned and purified from DH5α cells using the ZymoPURE II Plasmid Maxiprep Kit (Zymo Research). Plasmids were quantified using a Qubit fluorometer.

Transient Rescue Assay

HeLa *BRIP1*-/- cells were plated into 4 wells of 6 well plate (250K cells/well). After adherence, cells were transfected with 500ng of pCDH-Puro-EV or pCDH-Puro-BRIP1 missense containing vectors using Lipofectamine 2000. Sixteen hours post-transfection, cells were washed with PBS and fresh antibiotic-free media was added. Cells were allowed to recover for 32 hours. A single well of cells was collected for protein lysate to confirm transient expression by western blot [Supplemental Figure S6C]. Fresh media containing puromycin [2ug/mL] was added to the remaining three wells of cells with either 0 ng/mL, 15ng/mL or 30ng/mL MMC. After 24 hours of treatment, the MMC was removed. Cells were left in culture for 10-15 days with fresh puromycin media changes every 3-4 days. Puromycin and MMC resistant clones were counted in each well after 15 days (see Supplemental Figure S6). The number of clones from the 15 and 30ng/mL MMC wells were compared to the untreated well (0 ng/mL MMC). All wells were categorized as ≥50% of untreated growth (many clones), <50% of untreated growth (few clones) or no growth (no clones). Resistant clones were treated a second time with the same MMC dose and allowed to grow for an additional 10-15 days. Because it was not possible to detect BRIP1 protein by immunofluorescence, cells present at the end of the experiment were collected for protein and RNA to confirm expression by western blot and to authenticate the identity of the missense variant expressed. Cells in which the missense mutation and/or protein expression was not detected were excluded from the experimental results. Transfection of each allele was performed 3-5 times each. Twenty-four alleles were tested and 21 showed consistent results across all 3-5 replicates performed. These results are reported in Table 1. Results from the three alleles that showed vagaries between replicates were not reported.

As a proof of principle, the same protocol was performed on HeLa CRISPR cells lines with corresponding P47A, A745T, D791V and A349P mutations, transfected with pCDH-Puro-EV. Results from the CRISPR cell lines (endogenous protein expression) match the results from the exogenous re-expression of protein into null cell lines (see Supplemental Figure S6). With each new batch of missense mutations tested, EV, WT, P47A and A349P vectors were included as controls. Results from these controls were reproduced five times each.

Cell Line Authentication

Parental cell lines and individual clones were authenticated by the Johns Hopkins University School of Medicine (Baltimore, MD) human cell line authentication service by STR profiling using the GenePrint 10 System (Promega). STR profiles of the cell lines were compared to reference profiles (DSMZ) and found consistent with the reference profiles indicating authenticity.

Stable Clone Generation

HeLa *BRIP1*-/- clone was transfected with pCDH-BRIP1 WT and mutant constructs using Lipofectamine™ 2000 (Thermo Fisher Scientific). Puromycin selection (2ug/mL) was started at 48 hours and single colonies were isolated for expansion at day 15-21.

Western Blots

Cells were collected and lysed using using radio-immunoprecipitation (RIPA) buffer containing protease (ThermoFisher Scientific 88665) and phosphatase inhibitors (ThermoFisher Scientific A32957). 50ug of lysate was loaded onto 6% sodium dodecyl sulfate polyacrylamide gel with molecular markers and run at 100 V for 1 to 2 hours. Proteins were transferred to nitrocellulose membranes (LI-COR) using standard wet tank methods. Membranes were blocked using Odyssey® TBS blocking buffer (LI-COR) and probed overnight at 4°C with primary antibodies rabbit polyclonal anti-BACH1 (1:1000 dilution; Sigma Aldrich B1310 BRIP1 antibody) and mouse monoclonal anti-α-Tubulin (1:2000 dilution; Sigma Aldrich T6199). Membranes were washed and probed with secondary antibodies using IRDye® 800CW Donkey anti-Rabbit IgG and IRDye® 680CW Donkey anti-Mouse IgG (1:12,000 dilution; LI-COR). Membranes were imaged using the Odyssey Clx imager (LI-COR) and proteins were quantified using Image Studio™ Software (LI-COR).

qRT-PCR

RNA was prepared from HeLa and HEK293TN clones using TRIZOL (Thermo Fisher Scientific) along with Direct-zol RNA Miniprep Plus Kit (Zymo Research) and was reversed transcribed using the SuperScript™ III First-Strand Synthesis System (Thermo Fisher Scientific). Expression of the missense allele was detected using PCR and Sanger sequencing (see primer list). TaqMan® gene expression probes were used to measure BRIP1 transcript (Applied Biosystems, probes Hs00908143_m1 and Hs00908148_m1). TBP expression was used for normalization (probe Hs99999910_m1).

Assay Name	Forward Primer	Reverse Primer
P47A gDNA	CCCAGGCTGCCAAGTCTTTA	GGCTGCTGCTGTGAACTTTC
P47A cDNA	CAATTGGTGGGGTGAAGATT	AATGGCATGCACAACAACAT
A349P gDNA	ACCCATGTTAGGATATGTTTTCAGT	CCAGGTGTGGTGGCGTAG
A349P cDNA	TCTGCTAAGAAACAGGCATCCA	TTCATCCCGAGCAAACCGAA
A745T gDNA	CAGCACCATTCAGATCTCTCTAA	CCCAATTTATTTTCTTTTCACTCA
A745T cDNA	ATCCAGCTGTGGCCTTTTCA	GAATCTGCTGCCGTACCCAT
D791V gDNA	TTCCCCTTATTTACTTAAAGACATTGA	AAGCAAAGCGCAATAAAATGA
D791V cDNA	ATCCAGCTGTGGCCTTTTCA	GAATCTGCTGCCGTACCCAT

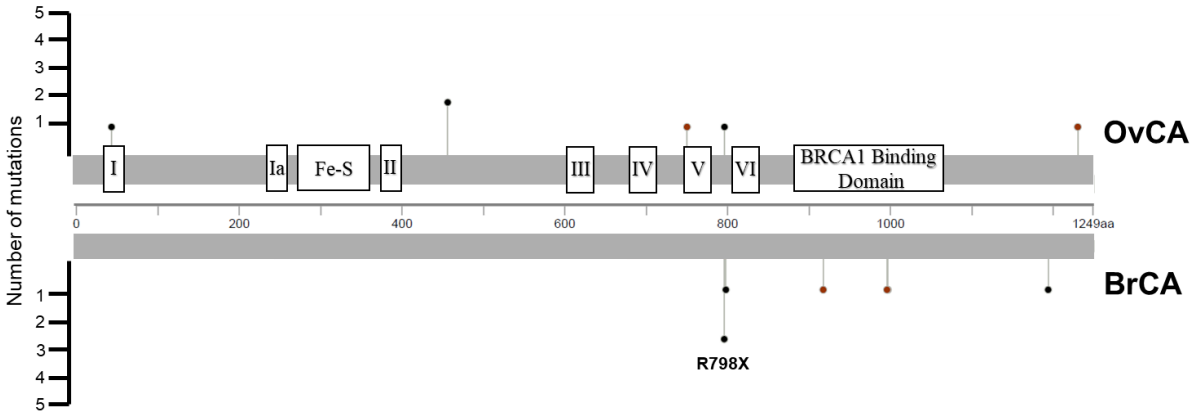

Figure S1. Graphical summary of *BRIP1* nonsense and frameshift mutations identified in ovarian cancer and early-onset breast cancer patients. Nonsense mutations are denoted by black dots and frameshifts are denoted by red. The hotspot R798X mutation was seen in one ovarian cancer patient and 3 breast cancer patients.

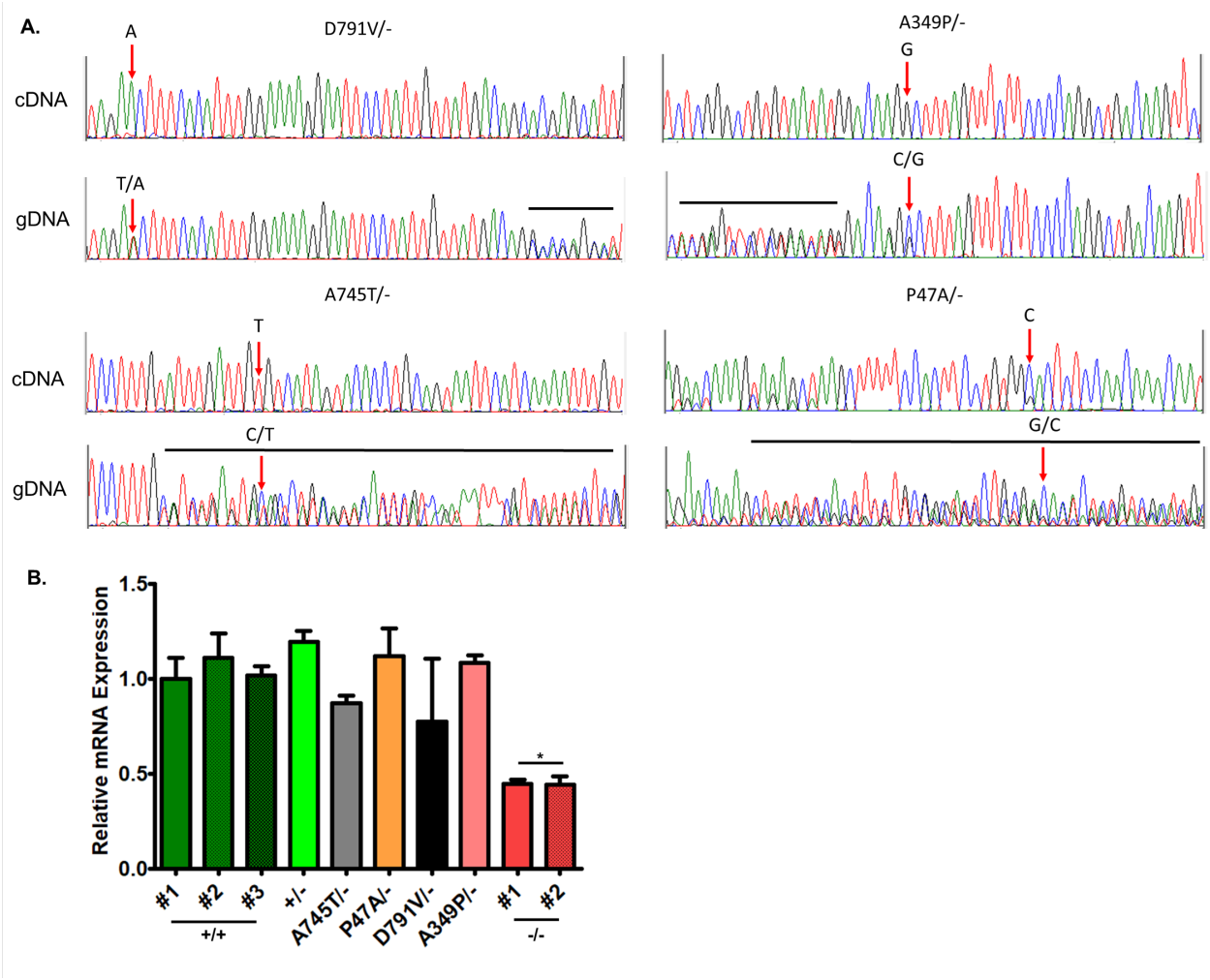

Figure S2. Expression of *BRIP1* missense alleles in isogenic HeLa cell lines. A) Chromatograms of cDNA (top) from corresponding clone with expression of candidate missense allele (red arrows) and absent/greatly reduced null allele. Chromatograms of gDNA (bottom) from a representative HeLa clone with incorporated missense allele and frameshift expected to undergo NMD. Bars mark region of sequence at which frameshift is evident in genomic DNA. B) Relative mRNA expression of three independent wildtype clones, two independent null clones and clones containing candidate missense alleles. Data presented are the average of four replicates with error bars indicating one standard deviation (* $p<0.01$ one way ANOVA with Dunnett's multiple comparison test).

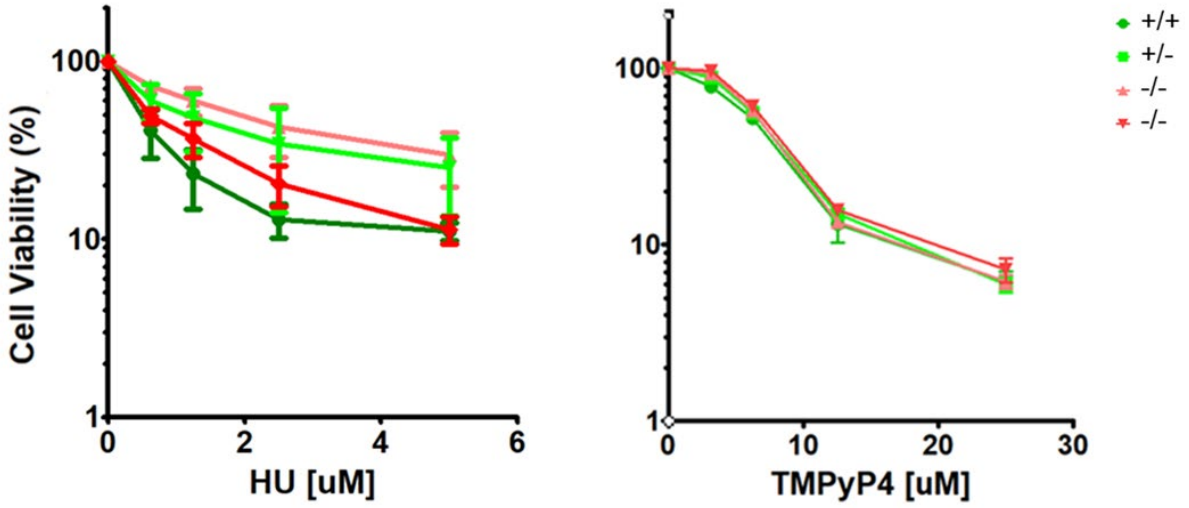

Figure S3. BRIP1 alleles and response to agents that induce replication fork stalling and G4 DNA accumulation. Clonogenic growth assays for *BRIP1*+/+, *BRIP1*+/- and two independent *BRIP1*-/- HeLa clones after exposure to hydroxyurea (left) and TMPyP4 (right). Data presented are the average of three replicates with error bars indicating one standard deviation.

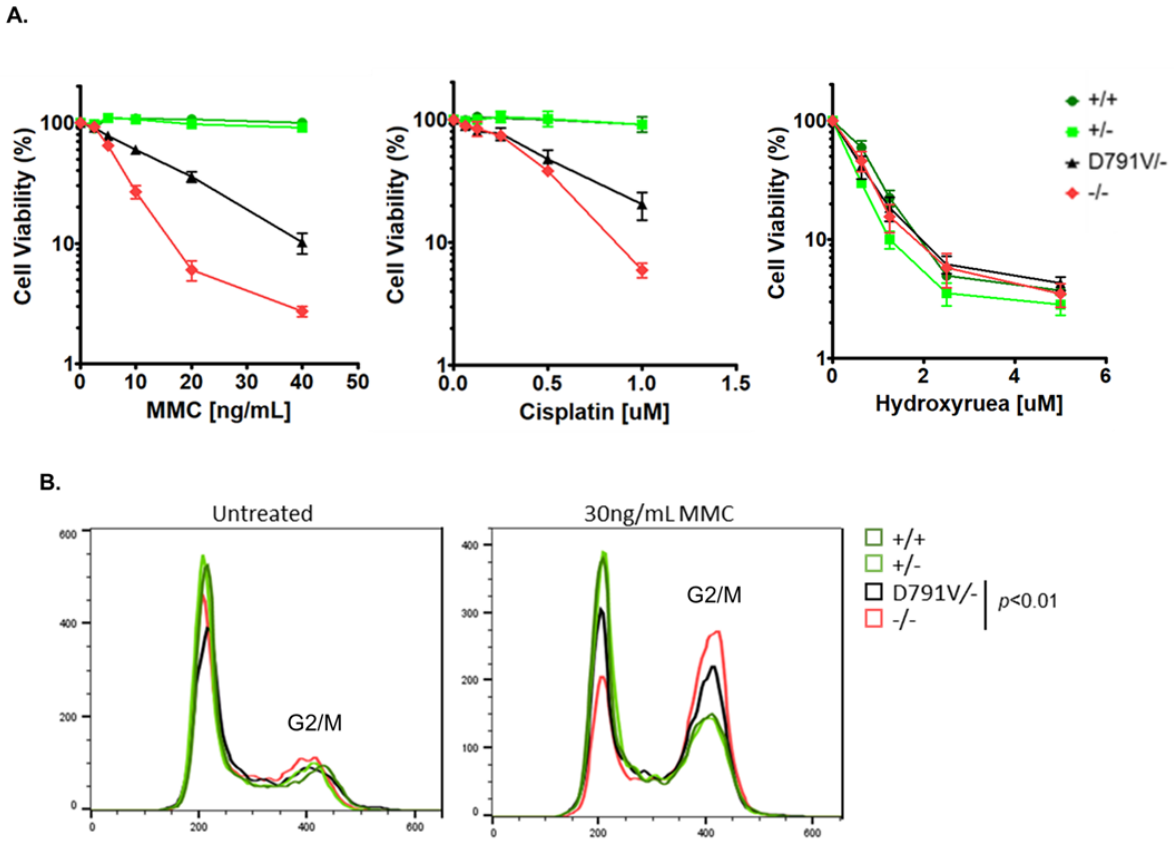

Figure S4. HEK293TN isogenic cell lines with BRIP1 deficiency are sensitive to MMC and cisplatin. A) Clonogenic growth assays of independent HEK293TN clones after exposure to MMC, cisplatin and hydroxyurea. Data presented are the average of three replicates with error bars indicating one standard deviation. B) Overlay of representative cell cycles from independent HEK293TN clones with and without MMC treatment, showing increase in G2/M fraction for *BRIP1*-/- and D791V/- clone. Statistical significance calculated by averaging percentage of cells in G2/M from 3 replicates (one-way ANOVA with Tukey's multiple comparison test).

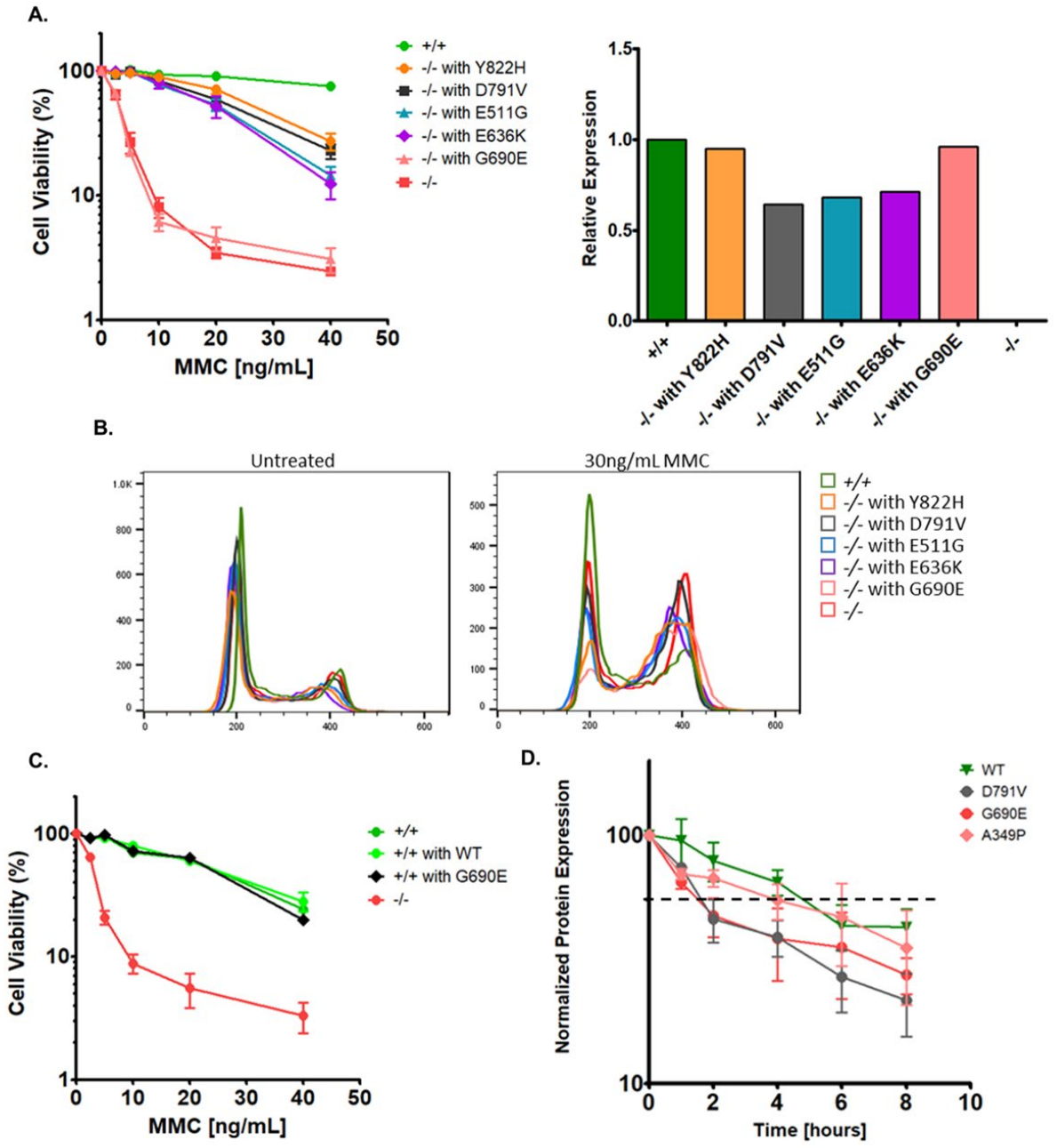

Figure S5. Stable clone rescue of HeLa *BRIP1*-/- and *BRIP1*+/+ with hypomorph and null alleles. A) Clonogenic growth assays of representative *BRIP1*-/- stable clones after exposure to increasing concentrations of MMC. Data presented are the average of three replicates with error bars indicating one standard deviation. Right panel: Quantified expression of myc-tagged BRIP1 exogenous protein in stable clones (single experiment). B) Overlay of representative flow cytometry cell cycle analyses with and without MMC treatment. C) Clonogenic growth assays of *BRIP1*+/+ stable clones after exposure to increasing concentrations of MMC. Data presented are the average of three replicates with error bars indicating one standard deviation. D) Cycloheximdie chase analysis of exogenously expressed wildtype, A349P, D791V and G690E protein. Fifty percent protein expression denoted by dotted black line.

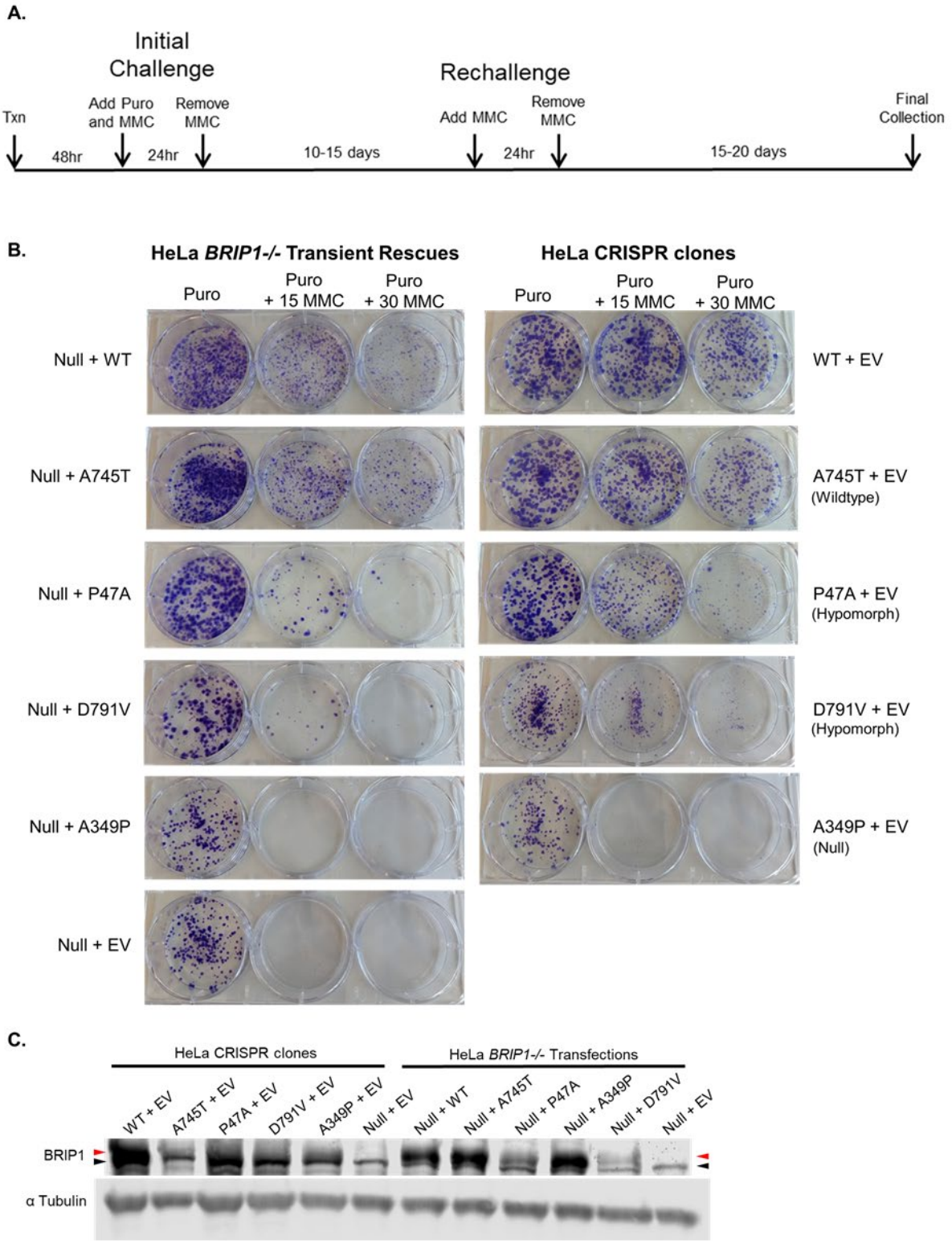

Figure S6. Transient rescue screening assay for missense variant functional testing. A) Schematic for timeline for transient rescue challenge assays. B) Representative images of

clonal outgrowth observed with and without MMC challenge in representative alleles. Left panel: HeLa *BRIP1-/-* cell line transiently transfected with BRIP1 missense variants. Right panel: HeLa CRISPR cell line expressing corresponding missense variant transfected with empty vector. C) Western blot of BRIP1 protein expression 48 hours post transfection (red arrow head denotes BRIP1 protein; black arrow head denotes non-specific band of lower mass).

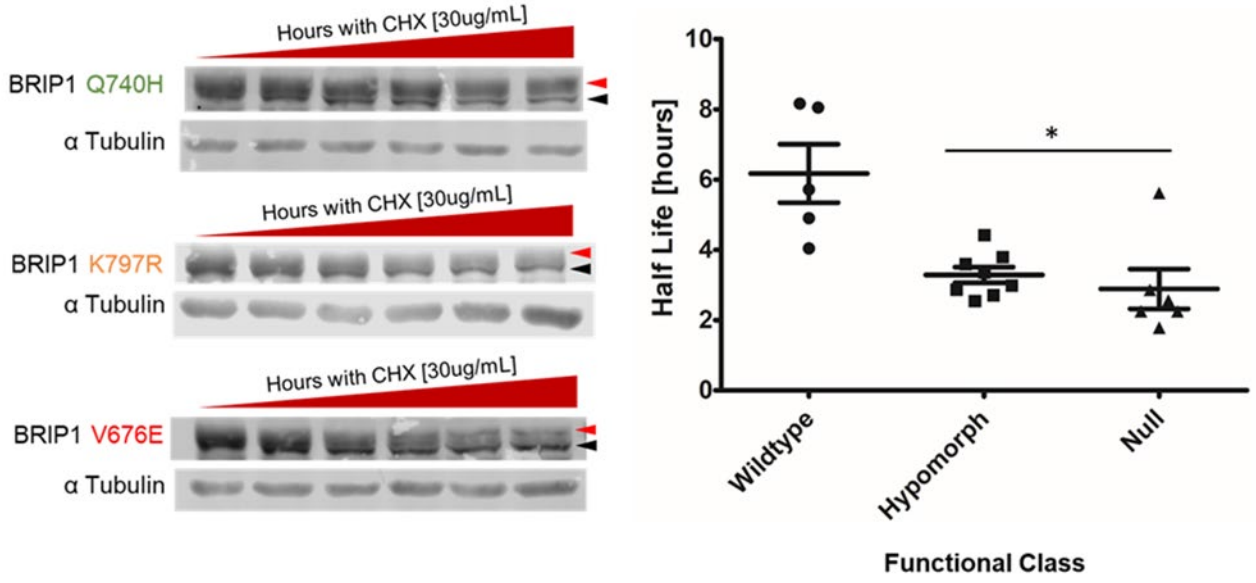

Figure S7. Protein degradation assay for missense variant functional testing. Left panel: Representative western blot of cycloheximide chase assay showing protein degradation of a functional wildtype (Q740H), hypomorphic (K797R) and null (V676E) allele (red arrow head denotes BRIP1 protein; black arrow head denotes non-specific band of lower mass). Right panel: Calculated half-lives for alleles tested, grouped by functional class. Half-life was calculated using a non-linear regression with one phase decay. Data points presented are the average of three replicates. Hypomorphic and null proteins showed decreased protein stability compared to wildtype (* $p<0.01$ one way ANOVA with Tukey's multiple comparison test).

Table S1. *BRIP1* missense variants identified in discovery cohorts

Protein_Change	dbSNP_151 (Genotype)	ESP_Frequency	CONDEL	Motif
Early-onset breast cancer cohort				
P47A	rs28903098	0.0003	Damaging	I
P47A	rs28903098	0.0003	Damaging	I
P47A	rs28903098	0.0003	Damaging	I
A60S[a]	Novel (C/A)	ND	Neutral	
S85L	rs587781830	ND	Neutral	
R106H	rs143615668	0.0001	Neutral	
S139A	rs202072866	<0.0001	Neutral	
D184Y	rs201047375	0.0002	Damaging	
P211L	rs780026145	<0.0001	Damaging	
C219R	rs730881630	ND	Damaging	
Q227E	rs45459799	<0.0001	Neutral	
Q227E	rs45459799	<0.0001	Neutral	
E262A[b]	rs876658283	ND	Damaging	Ia
D400Y	rs764711572	<0.0001	Damaging	
R403W	rs369631413	<0.0001	Damaging	
S407G	Novel (T/C)	ND	Damaging	
E511G	Novel (T/C)	ND	Damaging	
Q540L	rs4988349	<0.0001	Damaging	
A551V[a]	rs375246789	<0.0001	Damaging	
R579C	rs28997571	<0.0001	Damaging	
R579C	rs28997571	<0.0001	Damaging	
H587L[b]	rs876660646	ND	Neutral	
E636K	rs1060501769	ND	Damaging	
G690E	rs878855144	ND	Damaging	IV
I691L	rs587782356	ND	Damaging	IV
Q740H	rs45589637	0.0005	Damaging	
Q740H	rs45589637	0.0005	Damaging	
Q740H	rs45589637	0.0005	Damaging	
A745T	rs587780235	<0.0001	Damaging	
I782V	rs142806416	<0.0001	Neutral	
D791V	rs876658934	ND	Damaging	
K797R	rs730881622	ND	Damaging	
Y822H	rs760887592	<0.0001	Damaging	VI
R855H	rs200894063	<0.0001	Damaging	
R865W	rs578022079	<0.0001	Damaging	
I952V	rs200239986	<0.0001	Neutral	
I962T	rs786201632	ND	Neutral	
K979E	rs730881627	ND	Neutral	
K979E	rs730881627	ND	Neutral	
Y1011H	Novel (A/G)	ND	No Data	
T1050N	rs373040333	0.0001	Neutral	

	I1176V[a]	Novel (T/C)	ND	Neutral	
	G1243D	rs765545033	ND	Neutral	
Ovarian cohort					
	P47A	rs28903098	0.0003	Damaging	I
	K52R[a]	Novel (T/C)	ND	Damaging	I
	N119S	rs889877039	ND	Neutral	
	L138S	rs587780251	<0.0001	Neutral	
	D184Y	rs201047375	0.0002	Damaging	
	C217F	rs587782156	ND	Damaging	
	I246V	rs376893571	0.0002	Neutral	Ia
	R251H	rs780834054	<0.0001	Damaging	Ia
	C283Y[a]	rs771096783	<0.0001	Damaging	Fe-S
	N293S	rs746599076	<0.0001	Neutral	Fe-S
	A334T	rs535414791	<0.0001	Damaging	Fe-S
	L347P	rs786201819	ND	Damaging	Fe-S
	A349P	rs149364097	<0.0001	Damaging	Fe-S
	N370S	rs777511615	<0.0001	Damaging	
	H396R	Novel (T/C)	ND	Damaging	II
	E399K	rs587782816	<0.0001	Damaging	
	R419W	rs150624408	0.0004	Damaging	
	N429T	rs587781463	ND	Damaging	
	K479E[a]	Novel (T/C)	ND	Neutral	
	A521V[a]	Novel (G/A)	ND	Damaging	
	Q540L[b]	rs4988349	<0.0001	Damaging	
	A551E[b]	rs375246789	ND	Damaging	
	I562V	rs45533636	<0.0001	Neutral	
	V676E[a]	Novel (A/T)	ND	Damaging	
	I691L[b]	rs587782356	ND	Damaging	IV
	K703I[a]	rs756412722	<0.0001	Damaging	IV
	R707C	rs764803896	<0.0001	Damaging	IV
	Q740H	rs45589637	0.0005	Damaging	
	Q740H	rs45589637	0.0005	Damaging	
	A745T	rs587780235	<0.0001	Damaging	
	R777C	rs768555161	ND	Damaging	
	R814C	rs201869624	0.0005	Damaging	
	E967G	rs1176224216	ND	Neutral	
	I983N	rs587781417	ND	Neutral	
	T1050N[b]	rs373040333	0.0001	Neutral	
	S1115C	rs1419933310	ND	Neutral	

[a]not reported in ClinVar; [b]pathogenic *BRCA1/2* mutation carrier