Supplementary figure legends

S1. BRIT1 interacts with SWI/SNF. (**a**) Endogenous BRIT1 are co-immunoprecipitated with SWI/SNF subunits. The whole cell extracts were subjected to immunoprecipitation with control, anti-BRM, anti-BRG1 or BAF170 antibodies and then subjected to Western blot analysis with the indicated antibodies. (**b-e**) BRG1, BRM, and SNF5 subunits are not required for BRIT1-SWI/SNF interaction. (**b**) Western blot analysis for BRG1 and BRM knockdown in U2OS cells. (**c**) **and** (**d**) Cell extracts were prepared from BRG1/BRM knockdown cells and immunoprecipitated with Flag affinity beads. The BRIT1-SWI/SNF interaction was analyzed by Western blotting with the indicated antibodies. (**e**) SNF5 was knocked down in U2OS cells. Cell extracts were subjected to immunoprecipitation with Flag affinity beads, and BRIT1-SWI/SNF interaction was analyzed by Western blotting with the indicated antibodies. (**f**) Knockdown of BAF155 or BAF150 did not disrupt the SWI/SNF complex. Cells were depleted with BAF155 or BAF170 by siRNAs and immunoprecipitation were conducted using anti-SNF5 antibody. (**g-h**) Specific regions on BAF155 and BAF170 are required for their interactions with BRIT1. (**g**) The middle region (595-839aa) of BAF155 is required for BRIT1-SWI/SNF interaction. Flag-BRIT1 and a series of deletion mutants of BAF155 tagged with c-Myc were expressed in cells to assess their ability of interaction. Cell lysates were immunoprecipitated with Flag affinity beads and blotted with the indicated antibodies. (Top) Schematic diagram of deletions in BAF155. Conserved domains are indicated. (Bottom) Co-immunoprecipitation of BAF155-Myc with Flag-BRIT1. BRIT1 bound effectively to full-length BAF155 as well as the mutants with deletions (Δ2, Δ3, and Δ5), whereas it failed to associate with the mutants ((Δ1 and Δ4) with the deletion of middle region containing a conserved SANT

domain. (**h**) N-terminal of BAF170 is implicated in BRIT1-SWI/SNF interaction. Flag-BRIT1 and deletion mutants of BAF170 tagged with c-Myc were expressed in cells to assess their ability of interaction. Cell lysates were immunoprecipitated with Flag affinity beads and blotted with the indicated antibodies. (Top) Schematic diagram of deletions in BAF170. Conserved domains are indicated. (Bottom) Co-immunoprecipitation of BAF170-Myc with Flag-BRIT1. BRIT1 bound effectively to full-length BAF170 as well as the mutants with deletions (Δ1-Δ4 and Δ6), whereas it failed to associate with the mutants (Δ5) lack of the N-terminal region.

S2. ATM/ATR-dependent phosphorylation of BAF170 mediates DNA damage-enhanced BRIT1-SWI/SNF interaction. (**a**) Depletion of either ATM or ATR does not affect BRIT1-SWI/SNF interaction. (Left) Cell lysates for immunoprecipiations. ATM or ATR was depleted by transient siRNA transfection. (Right) Immunoprecipitations by anti-Flag antibody and blotted with BAF170. Twenty-four hours after siRNA transfection, cells were transfected with empty Flag vector or Flag-BRIT1. Forty-eight hours later, cell lysates were prepared for immunoprecipitations. (**b**) Depletions of ATM, ATR or ATM+ATR in cells affected activation of their downstream effectors upon DNA damage signaling. 48 hrs after transfection, cells were exposed to UV (50 J) or IR (10 Gy), and 2 hrs later, cell lysates were extracted for Western blotting analysis to detect phosphorylation of Chk1, Chk2 or p53. (**c**) Recognition of BAF170 (S969) after IR by p-BRCA1 (S1423) antibody. (Top) Protein sequences around BAF170 (S969) are evolutionarily conserved and very similar to the sequences around BRCA1 (S1423). (Bottom) 293T cells were transfected with wild-type or S969A mutated (M7) BAF170.

48 hrs after transfection, cells were exposed to IR (10 Gy). 1 hr later, cell lysates were harvested and incubated with anti-p-SQ/TQ or anti-p-BRCA1 (S1423) antibody. Similar condition was also used for Fig. 2c and Fig. 2d. (**d**) The binding between BAF170 mutant M7 (S969A) and BRIT1 did not increase after IR. 293T cells were transfected with indicated plasmids. Immunoprecipitation was performed by anti-Flag M2 antibody and detected by anti-Myc-tag antibody on Western blot. (**e**) In BAF170 depleted cells, reconstitution of BAF170 mutant M7 (S969A) was unable to rescue IR-enhanced BRIT1-SWI/SNF interaction. (Left) The expression levels of endogenous and ectopic BAF-170 in the cell lysates. (Right) Endogenous SWI/SNF subunits associated with BRIT1 were detected by the indicated antibodies in immunoprecipitation assay. Cells were transfected with indicated plasmids and 5 hrs later, BAF170 was depleted by siRNA transfection. 48 hrs after the transfection, cells were harvested and immunoprecipiation was performed as above.

S3. BRIT1 depletion impairs DNA DSB repair. (a) Comet analyses at the indicated time points after exposure of U2OS cells transfected with control siRNA (C) or BRIT1 siRNA#1 (#1) to ionizing radiation (IR). (Left) Representative images. Scale bar is 40 μM. (Right) Quantitative analysis of three independent experiments. Percentage of cells with intact DNA (tail moment less than 2) in cells without IR exposure was set as 1. At least 100 cells were scored in each sample and each value represents the mean ± SEM of three independent experiments; Student *t*-test. (**b**) (Left) Representative images of Comet analyses at the indicated time points after exposure of U2OS cells transfected with control or BRIT1 siRNA#1 to IR (10 Gy). Scale bar is 40 μM. (Right) Quantitative

analysis of cells with intact DNA (defined as tail moment less than 2) in each sample. Western blotting analysis showed the effective BRIT1 knockdown in the cells (next to the graph). Student *t*-test. (**c-i**) HR repair analysis in BRIT1-depleted cells. (**c**) **and** (**d**) Generation of stable cell line for HR repair assay. (**c**) Schematic diagram of HR assay. The DR-GFP reporter substrate was integrated into cellular genomic DNA. SceGFP contains an I-SceI endonuclease site within the coding region, which abolishes GFP expression. iGFP is a truncated GFP, which contains homologous sequence for the SceGFP. Expression of I-SceI induces a single DSB in the genome. When this DSB is repaired by HR, the expression of GFP can be restored and analyzed by flow cytometry to indicate the efficiency of HR repair. A Southern probe was used to verify-single copy integration of the reporter. (**d**) Representative Southern blot of positive clone integrated with a single copy of reporter, which exhibited two specific bands after digestion with HindIII and probed with indicated Southern probe. (**e-i**) Impaired HR specifically results from the loss of BRIT1. (**e**) Cell-cycle profiles of cells that were analyzed for HR repair efficiency. (**f**) Transfection efficiency was monitored by using a control GFP-expression vector (pEGFP-C1) in indicated cells. (**g**) Cutting efficiency of I-SceI was detected by semi-quantitative PCR using a primer pair flanking the DSB site. BRIT1 knockdown cells exhibited a reduction in PCR products compared to control cells at 48 hs after I-SceI transfection owing to the lack of efficient DNA repair, but no differences in PCR products were apparent between BRIT1 knockdown and control cells at early time-points, suggesting similar cutting efficiency of I-SceI in BRIT1 knockdown and control cells. (**h and i**) Ectopic expression of siRNA-resistant BRIT1 but not BRCA1 rescued the impaired HR in BRIT1 knockdown cells. (**h**) Effect of ectopic expression of siRNA-

resistant BRIT1 on HR repair in BRIT1-depleted cells. (Left) Quantitative summary of multiple experiments. Each value is relative to the percentage of GFP+ cells transfected with control siRNA, which was set as 1, and represents the mean ± SD of three independent experiments. (Right) Western blot analysis of lysates from the BRIT1 knockdown cells ectopically expressing siRNA-resistant BRIT1 mutant. (**i**) Effect of ectopic expression of BRCA1 on HR repair in BRIT1-depleted cells. (Left) Quantitative summary of multiple experiments. Each value is relative to the percentage of GFP+ cells transfected with control siRNA, which was set as 1, and represents the mean ± SD of three independent experiments. (Right) Western blot analysis of lysates from BRIT1 knockdown cells with overexpressing BRCA1. (**j-k**) NHEJ repair is impaired in BRIT1-depleted cells. (**j**) PCR strategy to analyze NHEJ repair at the DR-GFP locus. PCR products around the I-SceI-induced DSB site were amplified by the indicated primers and subjected to enzyme digestion with I-SceI or I-SceI and BcgI. The fragment resistant to I-SceI digestion consisted of repaired DSB products, which cause loss of the I-SceI restriction enzyme site. As HR repair replaces the I-SceI site with the BcgI site, the fragment resistant to both I-SceI and BcgI digestion represents DSB products repaired by NHEJ. NHEJ repair efficiency can be quantitated by the percentage of uncut DNA present in PCR products after I-SceI and BcgI digestion. (**k**) Analysis of NHEJ repair in BRIT1 knockdown cells with ectopically expressed BRIT1 resistant to siRNA#1 and BRCA1 (The efficiency of knockdown and ectopic expression was represented as in **h** and **i**.) The NHEJ repair deficiency was not reversed by ectopic expression of BRCA1, but was reversed by ectopic expression of siRNA-resistant BRIT1, confirming the specific role of BRIT1 in NHEJ repair.

S4. Recruitment of SWI/SNF and DNA repair proteins to chromatin is impaired in BRIT1-depleted cells. (**a**) Binding of BRG1 and Rad51 to chromatin was reduced in BRIT1-deficient cells. Cells transfected with the control or BRIT1 siRNA #1 were exposed to IR (10 Gy), and cell lysates were then prepared at indicated time points (**b**) Densitometry analyses (Western blots were shown in Fig. 3c) of indicated protein values normalized against ORC2. Each value represents the mean ± SD of three independent experiments (* P≤ 0.05; ** P≤ 0.01). Student *t*-test. (**c**) BRIT1 knockdown does not affect the expression of SWI/SNF and DNA repair proteins. U2OS cells were transfected with control or BRIT1 siRNA. Cell lysates were prepared 48 h after the transfection and subjected to Western blot analysis with the indicated antibodies. (**d**) Recruitments of BRIT1 to chromatin and DNA damage sites are SWI/SNF independent. (Top) Association of BRIT1 with chromatin in SWI/SNF knockdown cells. U2OS cells were transfected with siRNA or shRNA vectors to knockdown individual SWI/SNF subunit as indicated. Association of BRIT1 with chromatin was analyzed by chromatin fractionation assay 48 hr after transfection. (Bottom) BRIT1 Foci formation in SWI/SNF knockdown cells. Cells were treated with 10 Gy of IR after knockdown of each SWI/SNF subunit. One hour later, cells were fixed and stained with antibody to BRIT1. Nuclei were visualized by DAPI staining. Scale bar is 10 μM.

S5. Impaired chromatin relaxation and HR repair in cells with dysfunction of SWI/SNF. (**a**) (Top) A small deletion on BRIT1 N-terminal region abolishes BRIT1-SWI/SNF interaction. Cells were transfected with indicated plasmids and treated with or

without IR (10 Gy). 15 minutes after IR, immunoprecipitation was performed by anti-Flag M-2 beads. (Bottom) The function of BRIT1 in chromatin relaxation is dependent on its interaction with SWI/SNF. BRIT1 with N-terminal deletion (BRIT1-ND), which lacks its SWI/SNF interaction domain, was unable to rescue the defect of chromatin relaxation. (**b**) Foci formation of indicated Flag-tagged BRIT1 proteins detected by immunostaining with anti-Flag M2 antibody 1 hrs after IR (10 Gy). Scale bar is 10 μM. (**c**) BRIT1-ND-reconstituted cells were sensitive to IR. Indicated plasmids were transfected into U2OS cells and then treated with control or BRIT1 siRNAs. 2 days later, cells were plated at low density and exposed to IR. Colonies were counted 2 weeks later. The graph represents the mean ± SD of three independent experiments (**d**) Western blots for BRM and BAF155 knockdown efficiency. (**e**) Impaired chromatin relaxation analyzed by MNase sensitivity assay in BRM or BAF155 knockdown cells. (**f**) Impaired HR repair in BRM or BAF155 knockdown cells analyzed 48 hours after I-SceI transfection. Each value is relative to the percentage of GFP+ cells in I-SceI-transfected cells with control siRNA expression, which was set to 1 and represents the mean±SD of three independent experiments. Student *t*-test. (**g**) (Top) sequences indicating conserved hydrophobic sequences on BAF155 and BAF170. Mutations were made at corresponding sites to replace leucines to arginines. (Bottom) L4R mutants of BAF155 and BAF170 can be incorporated into endogenous SWI/SNF complex. 293T cells were transfected with indicated plasmids. 48 hrs later, immunoprecipitation was performed using anti-SNF5 antibody. (**h**) L4R mutants lack their binding activities to BRIT1 and can exert dominant-negative effects toward BRIT1-SWI/SNF interaction. Cells were co-transfected with Flag-BRIT1 and indicated plasmids. 48 hrs later, immunoprecipitation was performed

using anti-Flag M2 beads. (**i**) Impaired HR repair in cells transfected with L4R mutants of BAF155 or BAF170 (Left) Western blots that showed ectopic expression of indicated plasmids. (Right) GFP analyses were performed 48 hours after I-SceI transfection. Each value is relative to the percentage of GFP+ cells in I-SceI-transfected cells with empty vector transfected, which was set to 1 and represents the mean±SD of three independent experiments.

S6. Chromatin relaxation agents improve activation of DNA repair protein RPA. Western blotting analysis showed the effective BRIT1 knockdown in the cells (shown above the graph in each panel). (**a**) RPA34 foci formation improved by sodium butyrate (Na but)-induced chromatin relaxation. (Left) Representative immunostaining images. Scale bar is 10 μM. (Right) Quantitative analysis of RPA34 foci induced in BRIT1 depleted-cells from multiple experiments. At least 50 cells were scored in each sample. The number of foci per cell without treatment was set as 1. (**b**) (Left) Representative Western blot for p-RPA after trichostatin A treatment. BRIT1 knockdown ells were exposed to 30 J/m^2 of UV irradiation in the presence or absence of trichostatin A (200 ng/mL). Cell lysates were prepared and subjected to Western blotting analysis 8 hr after irradiation. (Right) Densitometry analysis of p-RPA induction (mean ± SD) by trichostatin A in BRIT1 knockdown cells after irradiation from three independent experiments. (**c**) (Left) Representative Western blot for p-RPA after sodium butyrate treatment (5 mM). (Right) Densitometry analysis of p-RPA induction (mean ± SD) by sodium butyrate in BRIT1 knockdown cells after irradiation from three independent experiments. (**d**) (Left) Representative Western blot for chromatin-associated RPA after

sodium butyrate treatment (5 mM). BRIT1 knockdown cells were treated with 50 J/m^2 of UV irradiation in the presence or absence of sodium butyrate (5 mM). Two hours after treatment, chromatin-enriched fractions were subjected to Western blot analysis. (Right) Densitometry analysis of induction of RPA binding to chromatin (mean ± SD) by sodium butyrate in BRIT1 knockdown cells after irradiation (from three independent experiments).

S7. Impaired DNA repair in MCPH cells. (**a**) Deficiency of BRIT1 expression in human MCPH lymphoblastoid cell lines. Cell lysates were prepared from MCPH cells with BRIT1 mutations and subjected to Western blotting analysis for analysis of BRIT1 expression. (**b**) Representative images of Comet analyses for Fig. 5a. Scale bar is 40 μM. (**c**) Increased sensitivity to IR-induced DNA damage in G1-arrested BRIT1 LCLs. The percentage of cells in G1 phase was analyzed by flow cytometry and shown at the bottom of the graph. Arrested cells and non-synchronized cells were both exposed to IR (10 Gy). Cell survival was then measured 72 h after IR. Each value is relative to the percentage of survival cells in the control groups without IR exposure, which was set to 1 and represents the mean±SD of three independent experiments. (**d**) (Top) Impaired association of Rad51 and Ku70 with chromatin. MCPH cells were treated with 10 Gy IR. After 5 h, chromatin-enriched fractions were isolated. (Bottom) Densitometry analyses of Rad51 and Ku70 values normalized against ORC2. Each value represents the mean± SD of three independent experiments; Student *t*-test. (**e**) Deficiency of BRIT1 in MCPH cells does not affect expression of SWI/SNF and DNA repair proteins. Cell lysates were

prepared from MCPH cells and subjected to Western blot analysis with the indicated antibodies.

(**f-h**) Impaired RPA activation and chromatin relaxation in MCPH cells. (**f**) (Left) Impaired association of phosphorylated PRA with chromatin. MCPH cells were treated with 50 J/m^2 of UV irradiation. After 2 h, chromatin-enriched fractions were subjected to Western blotting with antibodies against p-RPA34. (Right) Densitometry analyses of p-RPA34 values normalized against ORC2. Each value represents the mean± SD of three independent experiments. (**g**) Chromatin relaxation improves phosphorylation of RPA in MCPH cells. (Left) Representative Western blot for p-RPA after trichostatin A treatment. Cells were treated as described in Supplementary Fig. 6b. (Right) Densitometry analysis of p-RPA induction (mean ± SD of three independent experiments) by trichostatin A in MCPH cells after irradiation from three independent experiments. (**h**) Time-course study of partial MNase digestion of chromatin obtained from U2OS cells (Left) or MCPH #1 cells (Right). (**i-l**) Wild-type BRIT1 but not BRCT-Δ1 rescues the defects of cell viability, DNA repair and chromatin relaxation in BRIT1 LCLs. (**i**) Western blots for the ectopic expression of indicated plasmids in LCLs. 24 h after first transfection, cells were retransfected with the plasmids and incubated for additional 48 h before processing. (**j**) Increased cell survival following the transfection with Flag-BRIT1. The percentage of cell survival was measured after exposure to 1 μM Etoposide to induce DNA damage. The bar graph represents mean ± SD of three independent experiments. (**k**) Recovered DNA repair efficiency following the transfection of Flag-BRIT1. Cells were exposed to IR (10 Gy). The repair efficiency was analyzed in each group by the percentage of cells

with intact DNA (tail moment less than 2) 6 h after IR exposure. (**I**) Rescued the defect of chromatin relaxation following the transfection with Flag-BRIT1. Chromatin relaxation was measured by MNase sensitivity assay.

S8. Full scans of key Western blots presented in the manuscript.

Supplementary Information Methods:

Comet assay

Double-strand break (DSB) repair was analyzed by neutral comet assay using the Trevigen's Comet Assay kit (4250-050-K) according to the manufacturer's instruction. Cells were exposed to 10 Gy γ-irradiation (IR) and subjected to comet analysis at indicated time points. After staining with SYBR green, comet images were captured by fluorescence microscopy. Tail moments (percentage of DNA in tail x tail length) were quantitated for 100 cells/slide by using CometScore software (available through www.autocomet.com). The calculation method was previously described1. In cells not treated with IR, more than 95% of cells contained tail moments less than 2, which was set as the parameter for cells with intact DNA. In each sample, the percentage of cells with tail moment less than 2 at the different time points after IR exposure was calculated and represented the cells with intact DNA. In the bar graph, the percentage of cells with intact DNA in the cells without IR exposure was set as "1". The percentage of cells with intact DNA from different time points after IR exposure was then drawn relative to this control.

HR repair analysis

DR-GFP, pCAGGS and pCBASce plasmids were kindly provided by Dr. Maria Jasin from Memorial Sloan-Kettering Cancer Center (New York, NY). U2OS cells containing a single copy of the HR repair reporter substrate DR-GFP in a random locus were generated as previously described[2]. Positive clone integrated with a single copy of reporter exhibits two specific bands after digestion with HindIII and probed with indicated Southern probe. GFP expressing plasmid (pEGFP-C1) was used for transfection efficiency control. pCAGGS was used in mock transfection. Twenty-four hours after

BRIT1 siRNA transfection, cells were re-seeded and then the next day they were transfected with mock or pCBASce plasmids. Forty-eight hours later, flow cytometry analysis was performed to detect GFP positive cells using a FACScalibur apparatus with Cellquest software (Becton Dickinson, San Jose, CA) at the M. D. Anderson Cancer Center Flow Cytometry Facility. To induce chromatin relaxation, cells were incubated for 16 hr in sodium butyrate (5 mM) or trichostatin A (200 ng/mL) before analysis by flow cytometry.

NHEJ repair analysis and PCR analysis of I-SceI-induced DNA cutting

The genomic DNA of mock- or I-SceI-transfected cells was extracted. PCR using primers around the DSB site and enzyme digestion with I-SceI or I-SceI+BcgI were performed as previously described[3]. I-SceI and BcgI restriction enzymes were purchased from New England Biolabs. After gel electrophoresis, the intensity for the enzyme-resistant or enzyme-cleaved fragments was quantified using NIH IMAGE software. To assay the cutting efficiency of I-SceI in cells transfected with control or BRIT1 siRNA, genomic DNA was extracted at different time points after I-SceI transfection and adjusted to equal concentrations. Semi-quantitative PCR was carried out using the same primers for NHEJ analysis (30 cycles). Beta-actin primers (Applied biosystems) were used as an internal control (25 cycles). Cycle number was determined to generate PCR products in the range of linear amplification.

Cell cycle analysis

Cells from HR repair analysis were fixed in 70% cold ethanol (-20^{o}C) overnight. After washing with cold phosphate-buffered saline (PBS), cells were incubated in staining solution (10 μg/mL propidium iodide, 20 μg/mL RNAase A and 0.05% Triton X-100)).

Cell cycle analysis was performed at the M. D. Anderson Cancer Center Flow Cytometry Facility. For fluorescence-activated cell sorter (FACS) analysis, the percentage of cells in each phase of the cell cycle was quantitated with Cellquest software (Becton-Dickinson) and ModFit software (Verity Software House Inc.)

Micrococcal nuclease (MNase) sensitivity assay

To induce DNA damage, U2OS cells were treated with 4 Gy IR. MCPH cell lines were treated with the DSB inducing agent neocarzinostatin (400 ng/mL) (Sigma) for 2 hr. Cells (2×10^6 for U2OS cells and 8×10^6 for MCPH cells) were harvested by scrapping cells into lysis buffer (10 mM Tris/HCl pH8.0, 10 mM MgCl2, 1 mM DTT and 0.5% NP-40). Nuclei were pelleted and then digested with MNase at a concentration of 0.2 U/200 ul digestion buffer (15 mM Tris-HCl pH 7.4, 60 mM KCl, 15 mM NaCl, 0.25 M sucrose, 1 mM $CaCl_2$ and 0.5 mM DTT). Aliquots (50 μl) were prepared at 1 min, 2 min, and 5 min. Genomic DNA was purified and separated by electrophoresis in 1.2% agarose gel. Band intensity was determined using NIH IMAGE software. The average nucleosome size was calculated as described previously[4]. Briefly, intensity of each lane and intensity of individual band in each lane were obtained. Oligonucleosome average size was calculated as $\sum_{N1\text{-}Ni}(NiP_{Ni})$, where Ni is the oligonucleosome size (expressed as number of nucleosomes) and P_{Ni} is the fraction of that oligonucleosome out of the total scan calculated as the ratio of specific band intensity to the intensity of the whole lane. Statistical significance of the difference in average oligonucleosome size between the control group and BRIT1 deficient groups were analyzed by Student's t-test.

Colony-forming assay

Transfection and colony-forming assay were performed as previously described[5]. Briefly, U2OS cells were transfected with indicated plasmids, and 5 h later were transfected with BRIT1 siRNA. Cells were exposed to IR two days after transfection, seeded at low density and left for 2 weeks to allow colonies to form. Colonies were stained with 2% methylene blue/50% ethanol and counted. Colonies containing 50 or more cells were counted.

Cell viability assay

The sensitivity of MCPH cells to DSB-inducing agents was analyzed using CellTiter 96 Aq_{ueous} One Solution Cell Proliferation Assay (MTS assay, Promega). Briefly, $1x10^4$ cells/well were seeded in a 96-well plate in a total volume of 100 μl in triplicate in each experiment. The next day, cells were treated with the DSB-inducing agent etoposide (Sigma) or camptothecin (Sigma) at indicated concentrations. To inhibit DNA replication, cells were pre-incubated with aphidicolin (Sigma) for 90 min before treatment with DSB-inducing agents. Seventy-two hours later, the number of surviving cells was assayed using MTS solution. G1 arrest in patient cells was induced as described previously[6]. Cell cycle arrest was confirmed by flow cytometry. Arrested cells and non-synchronized cells were exposed to IR (10 Gy). Cell viability assay was performed 72 h after IR exposure. Plates were read at 490 nm and 650 nm (background) in a microplate reader (Molecular Devices). After subtraction of background, the cell viability was calculated as fold change relative to control cells.

Immunofluorescent staining for foci formation

For detection of DNA damage induced foci of p-RPA34 and RPA34, immunofluorescent staining was carried out essentially as described previously[7]. To detect Rad51 foci, cells

were exposed to IR (10 Gy) and the staining was performed 5 h after IR as in the previous publication[32]. For MCPH cell lines, cells were subjected to the same procedures as cells grown on coverslips including cytoskeleton buffer, stripping buffer and fixing buffer. Then cells were spread onto the slides for the staining. Rabbit anti-Rad51 antibody (1:500) was used to detect Rad51 foci. Primary antibodies were incubated for 2 h at room temperature (RT) and secondary antibody rhodamine-conjugated goat anti-rabbit IgG was incubated for 1 h at RT. Slides were mounted in medium containing DAPI (Vector laboratories, Burlingame, CA) and analyzed under a fluorescence microscope. The number of foci per cell was scored for at least 50 cells per sample. For chromatin relaxation, cells were incubated in the presence of sodium butyrate (5 mM) after ultraviolet (UV) exposure before staining for p-RPA foci was performed.

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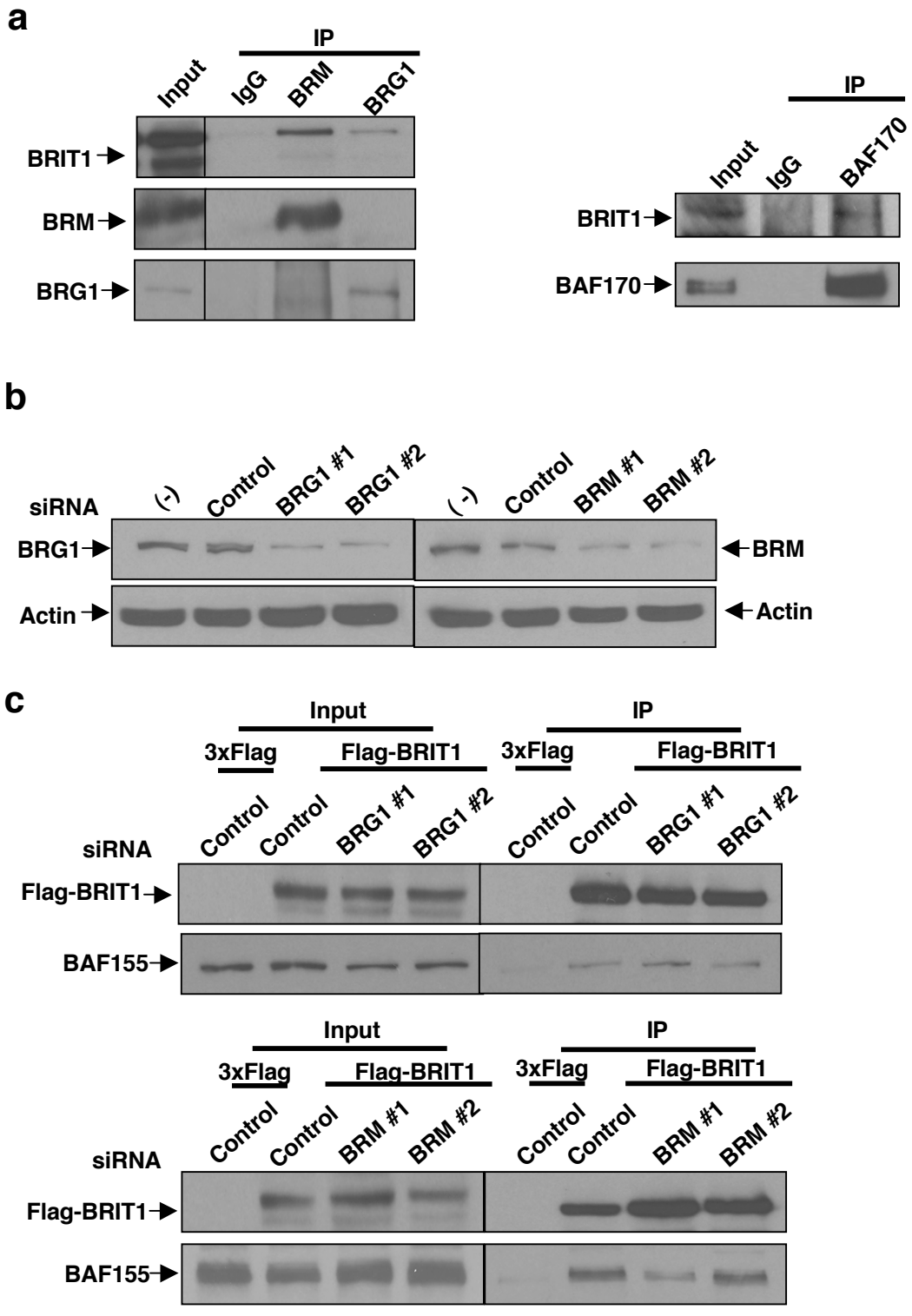
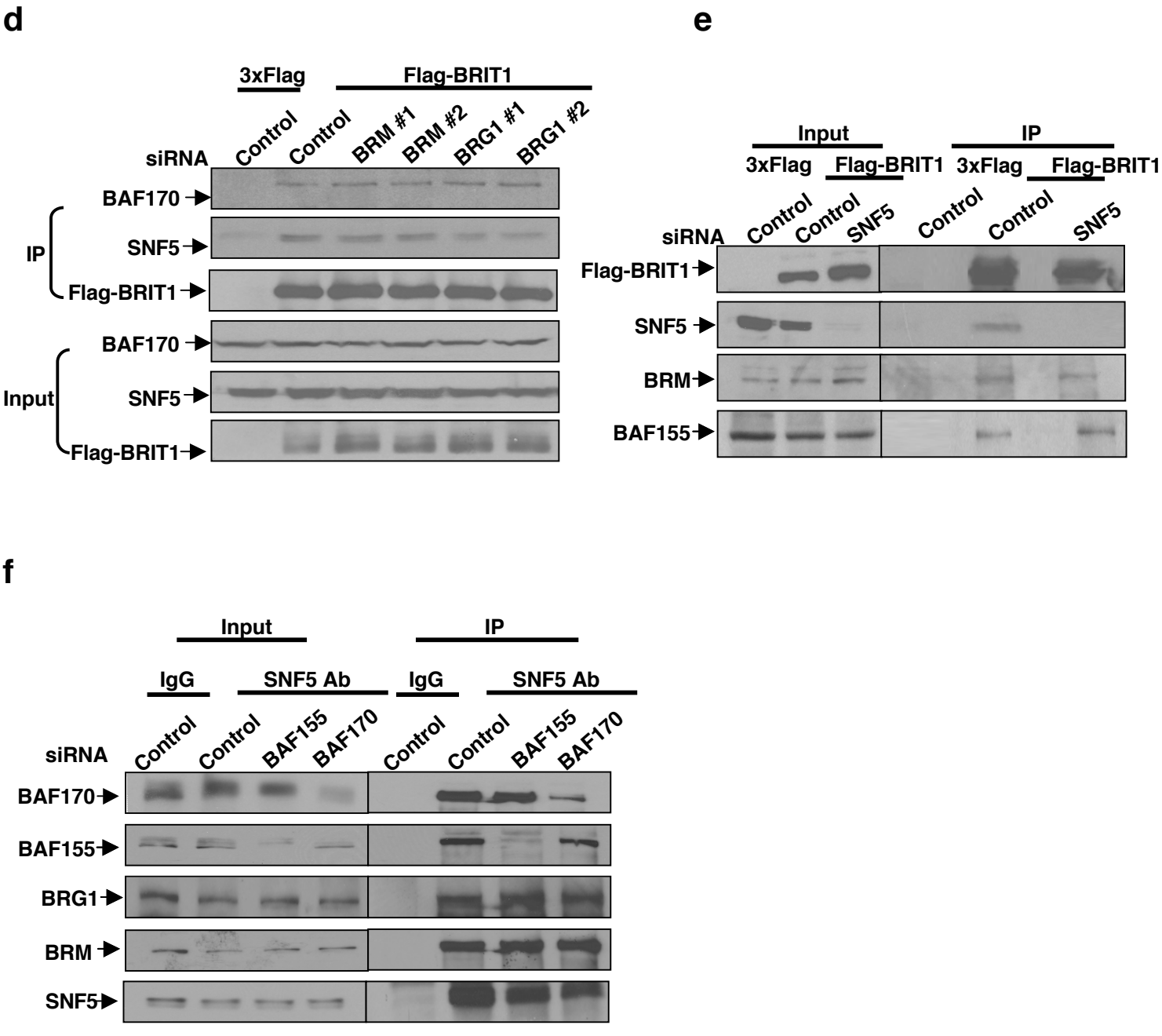


Supplementary Fig. 1



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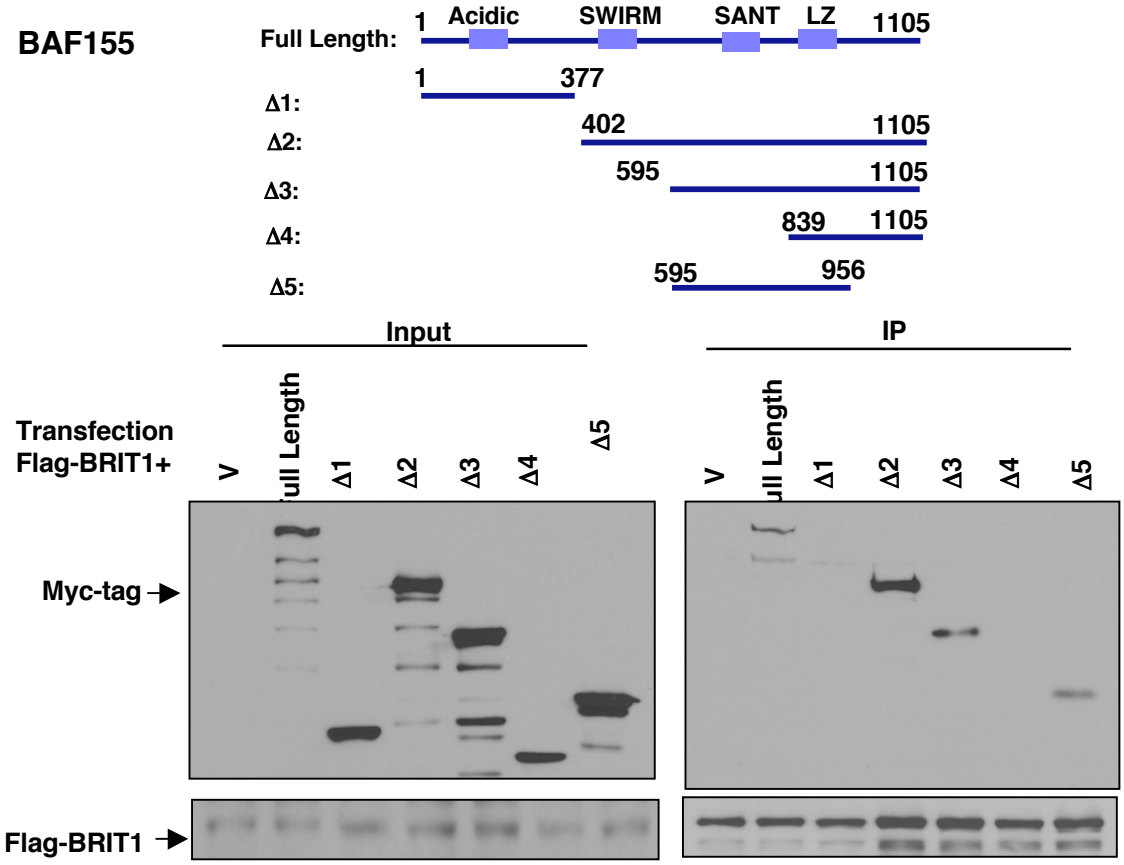

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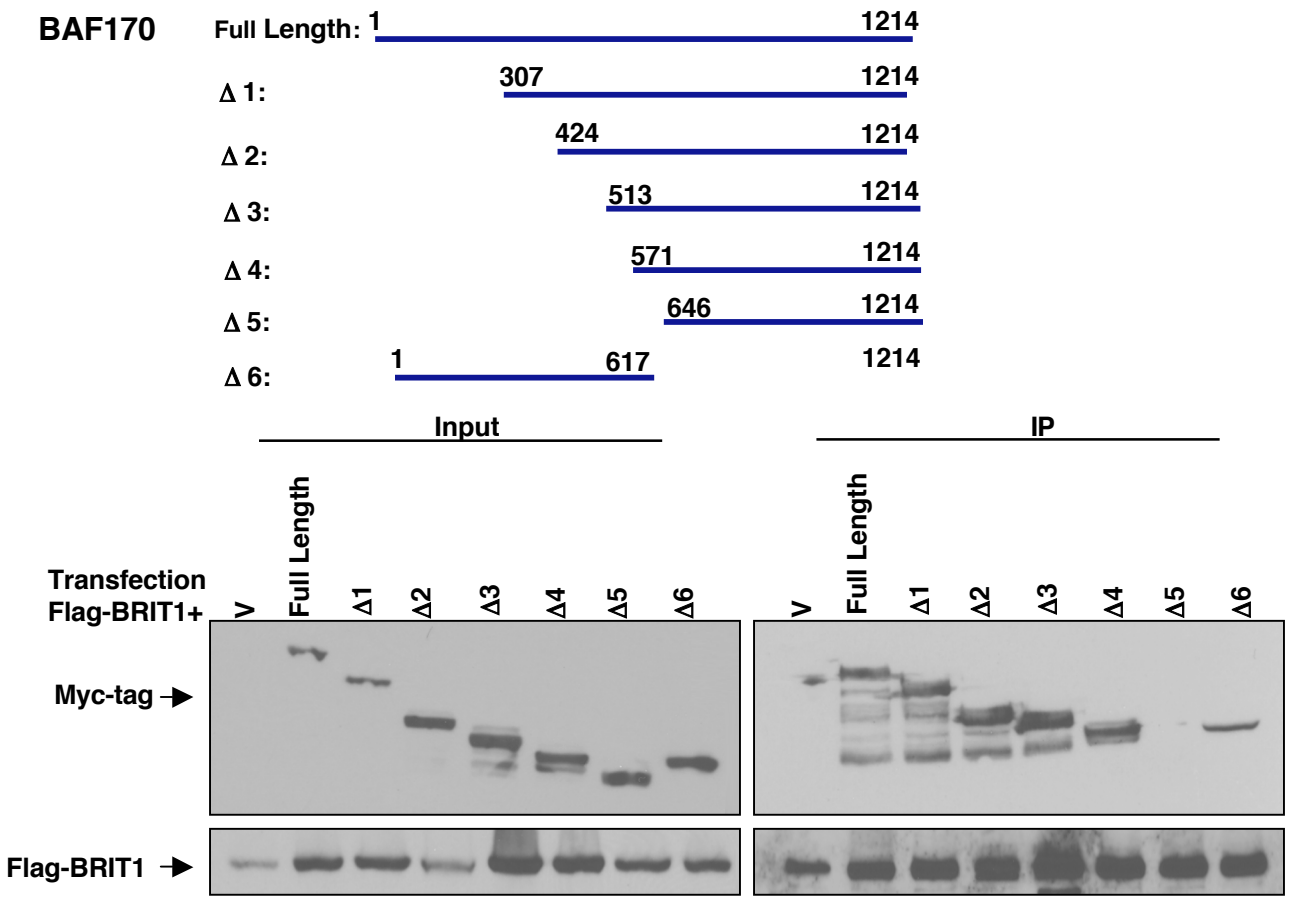

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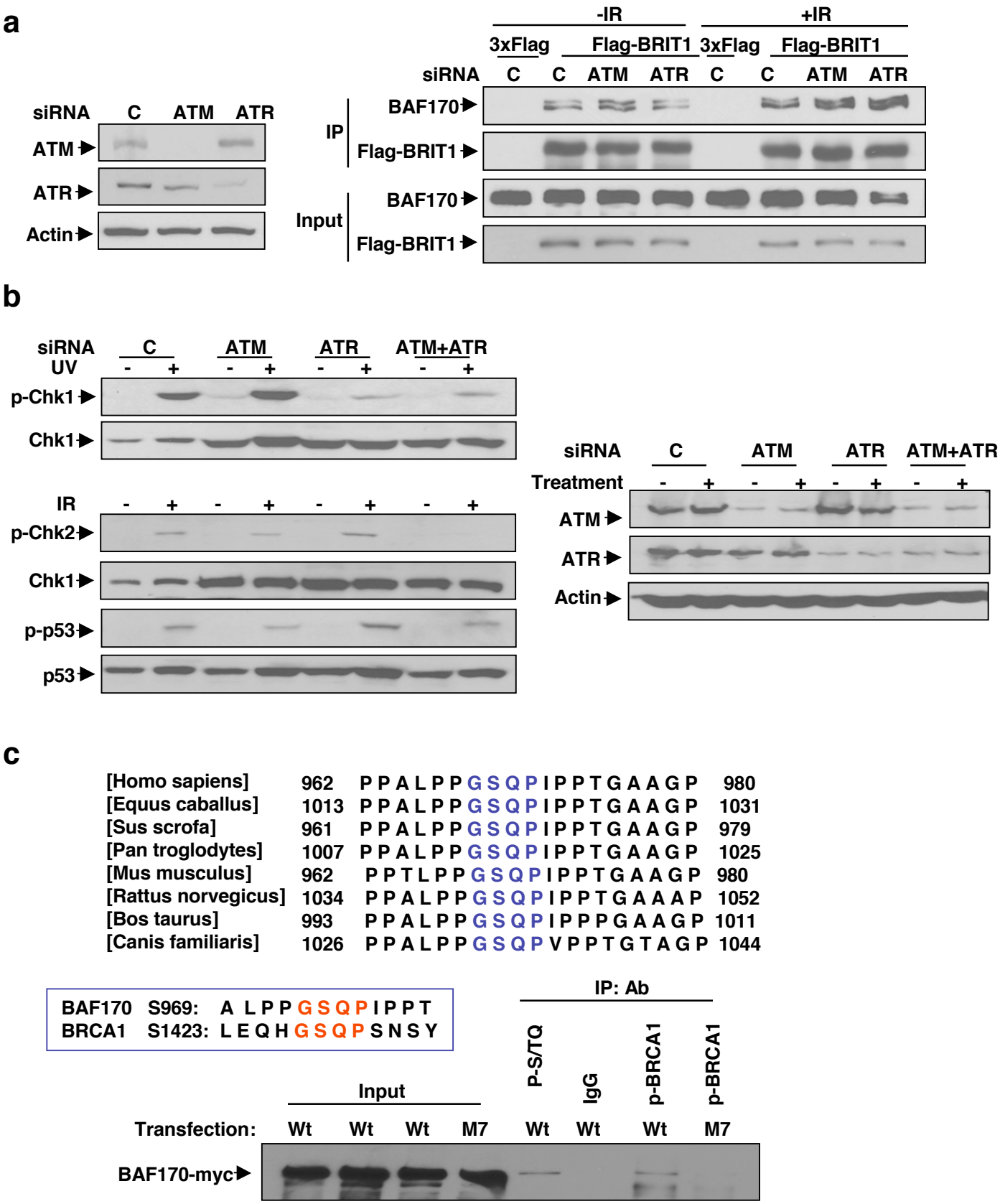

Supplementary Fig. 2

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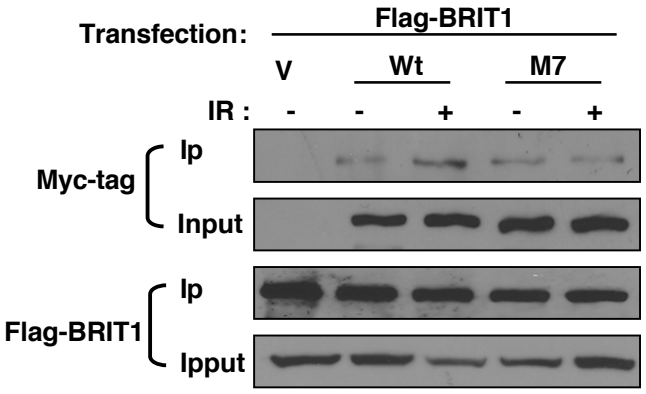

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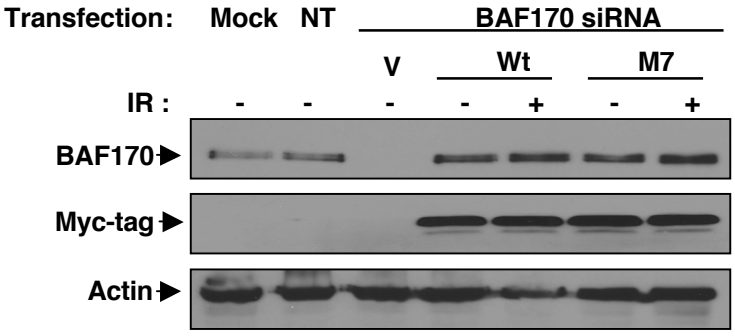
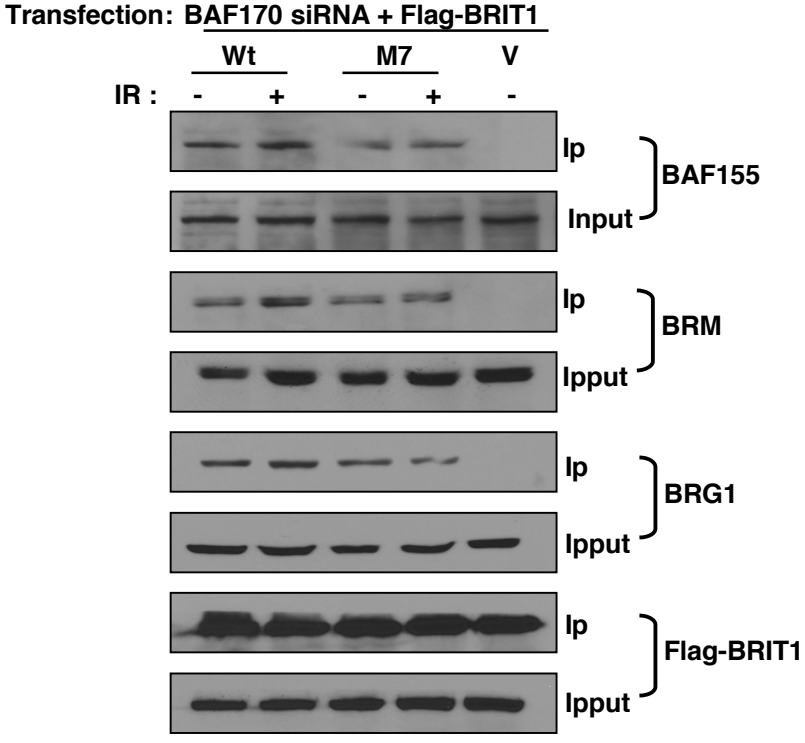
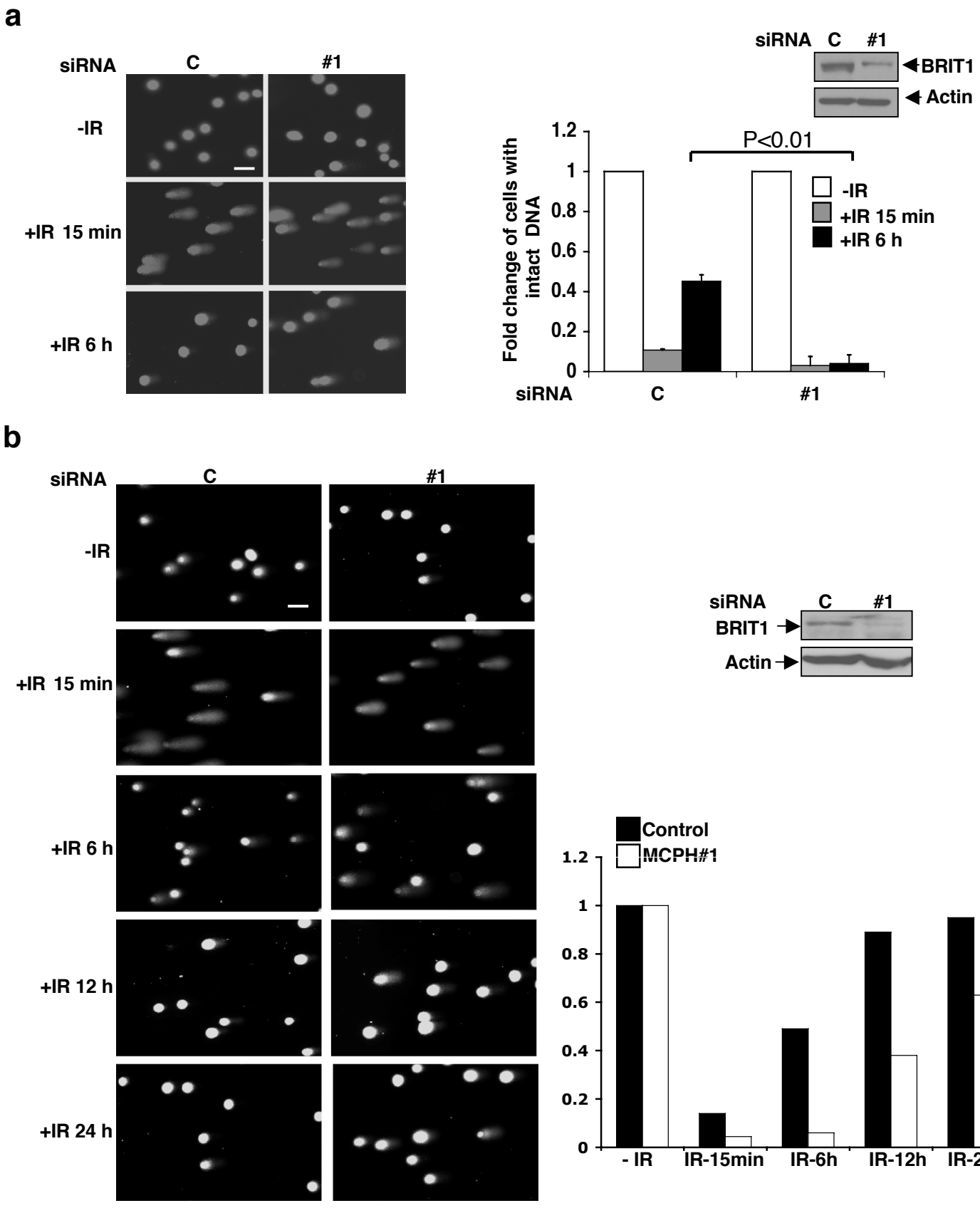
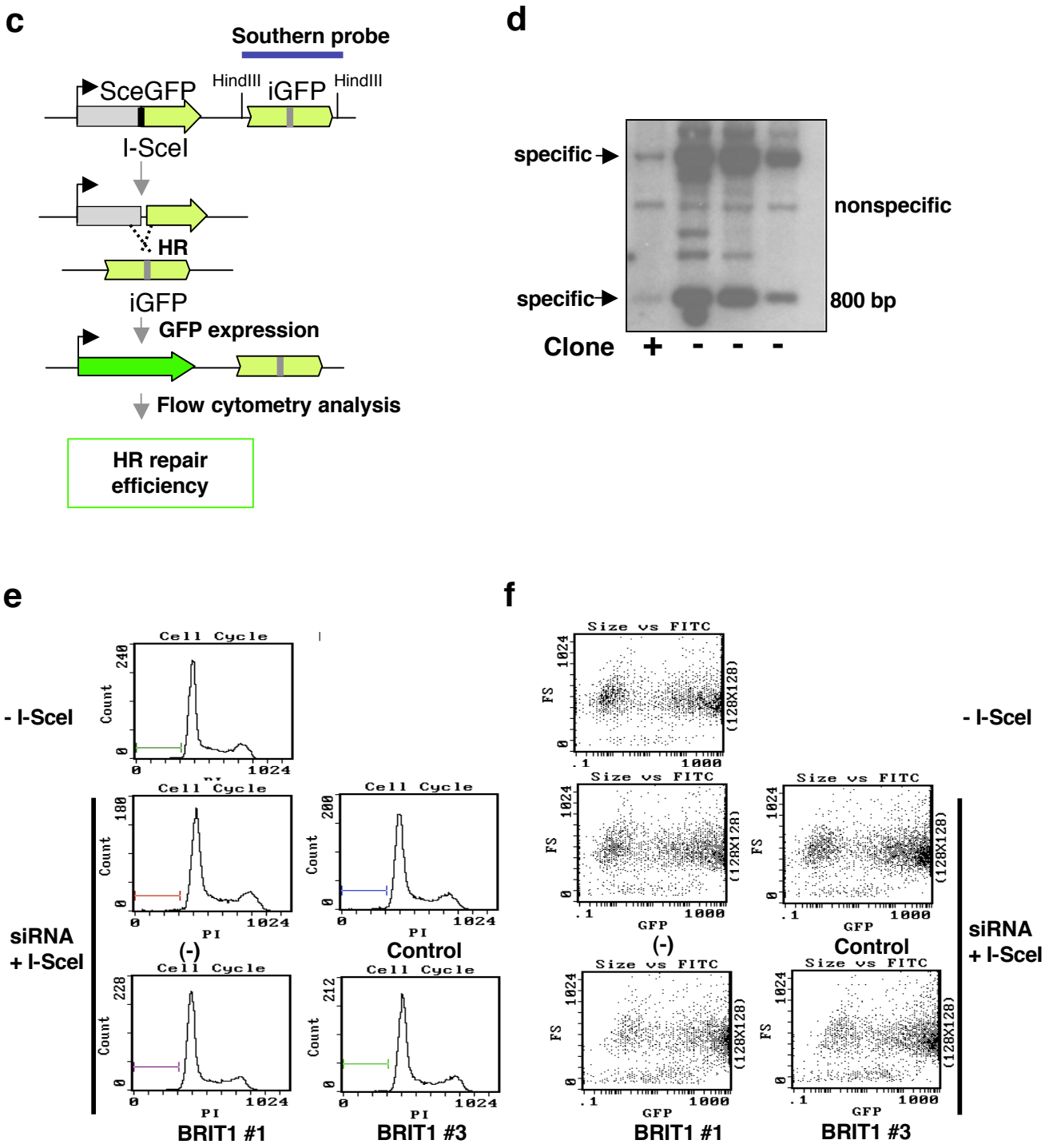




Supplementary Fig. 2

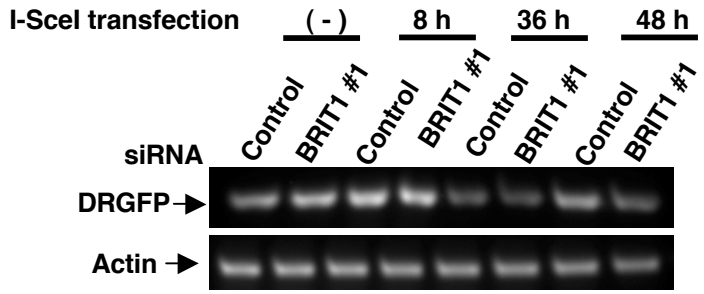


Supplementary Fig. 3



Supplementary Fig. 3

g



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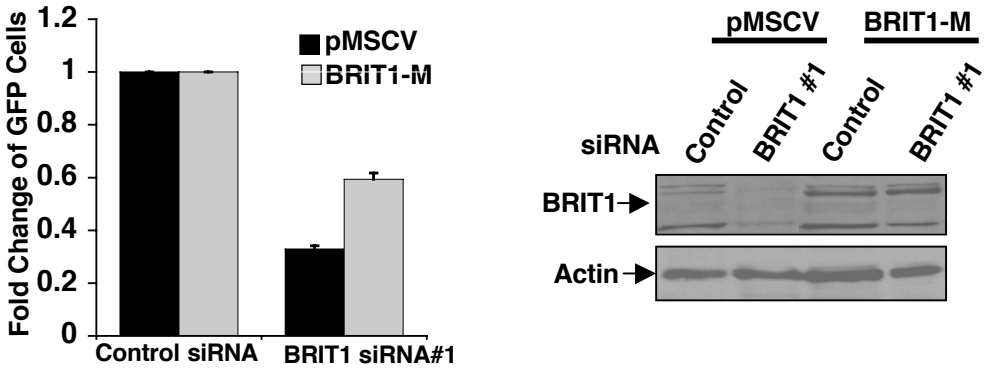

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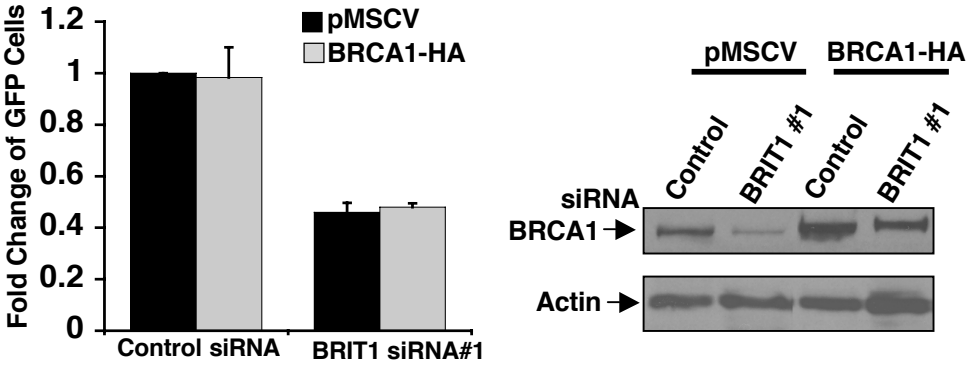

Supplementary Fig. 3

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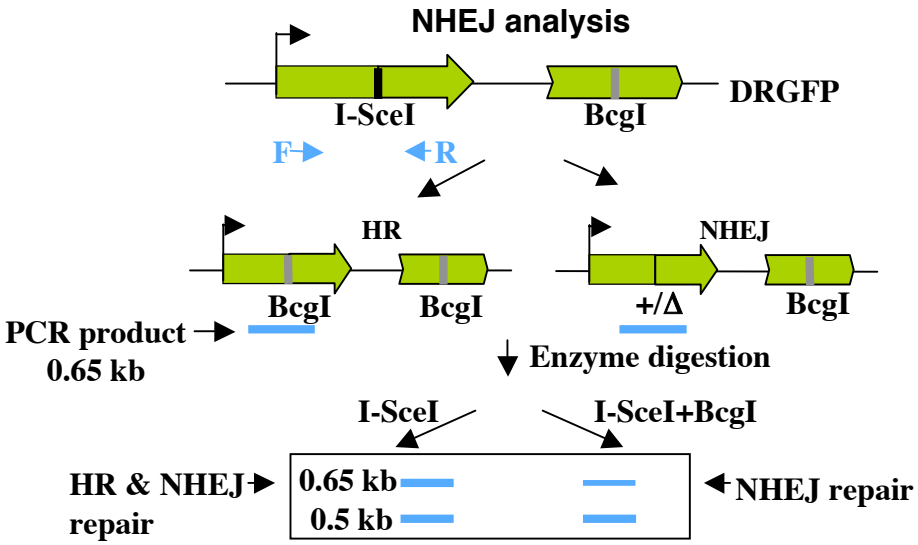

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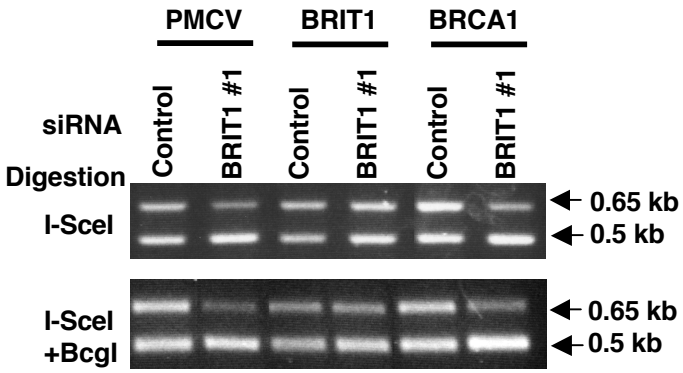
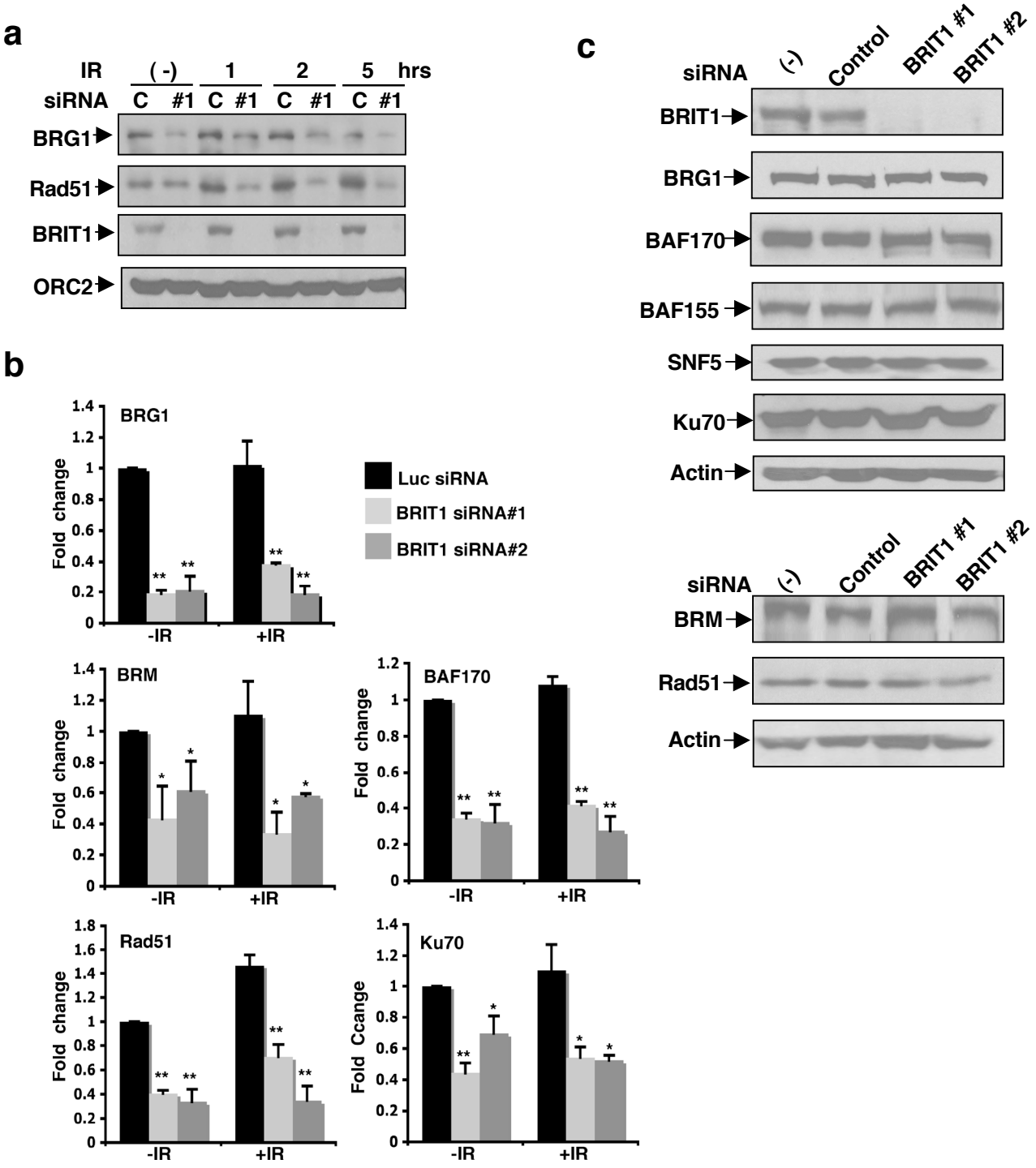
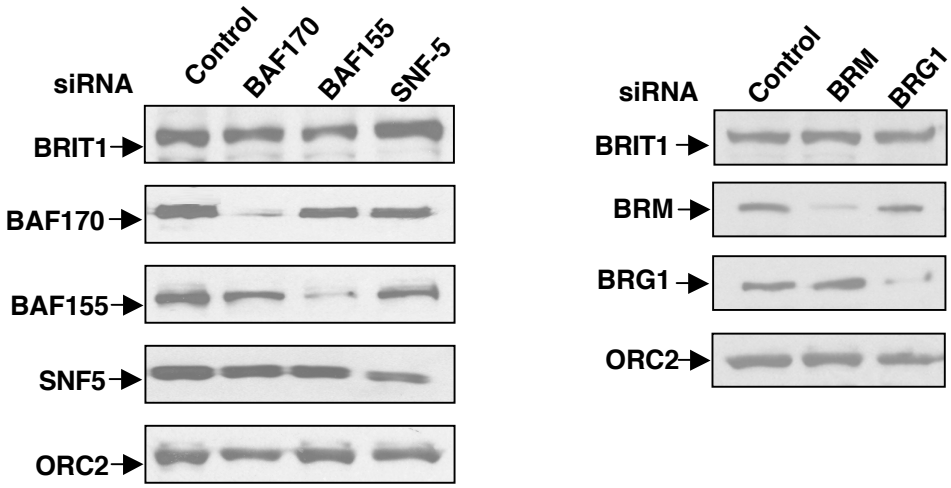


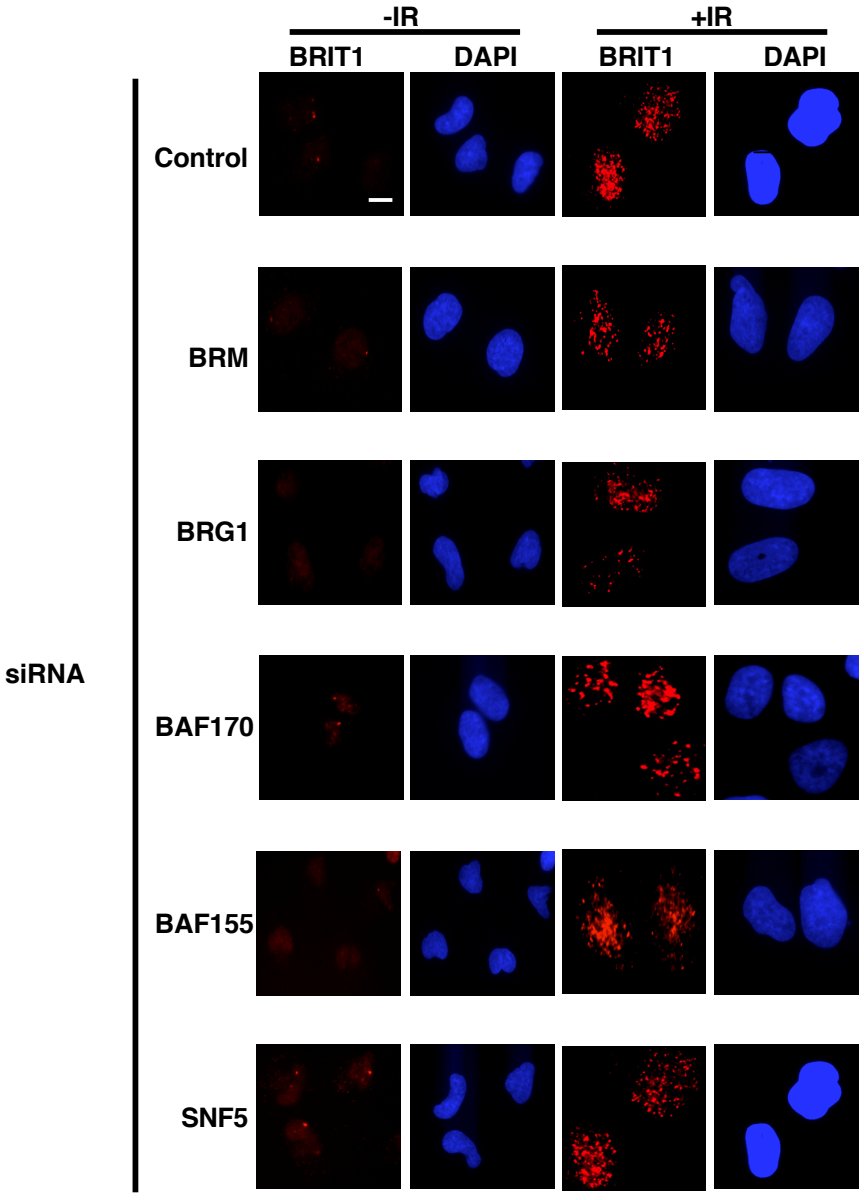
Supplementary Fig. 3



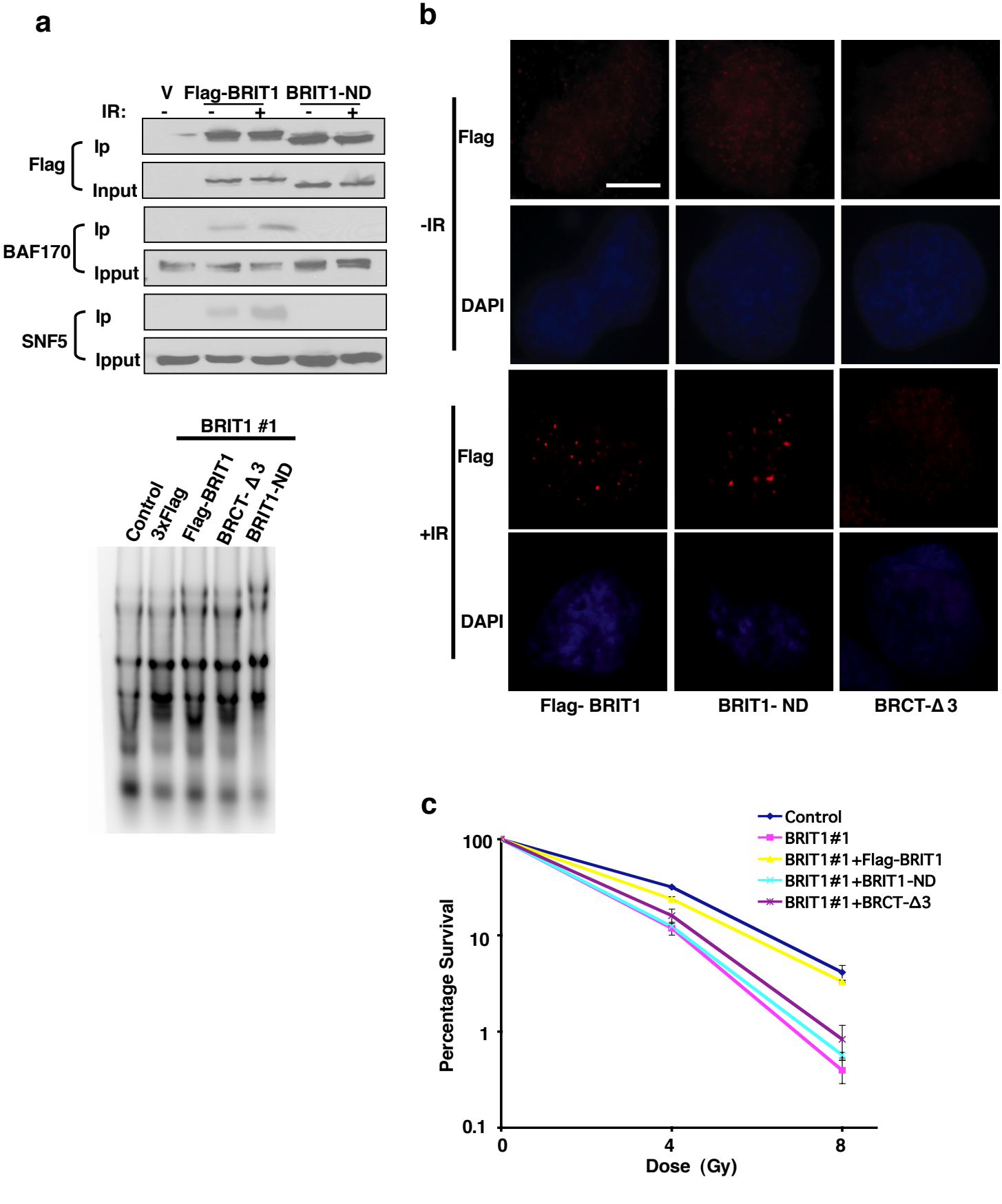
Supplementary Fig. 4

d

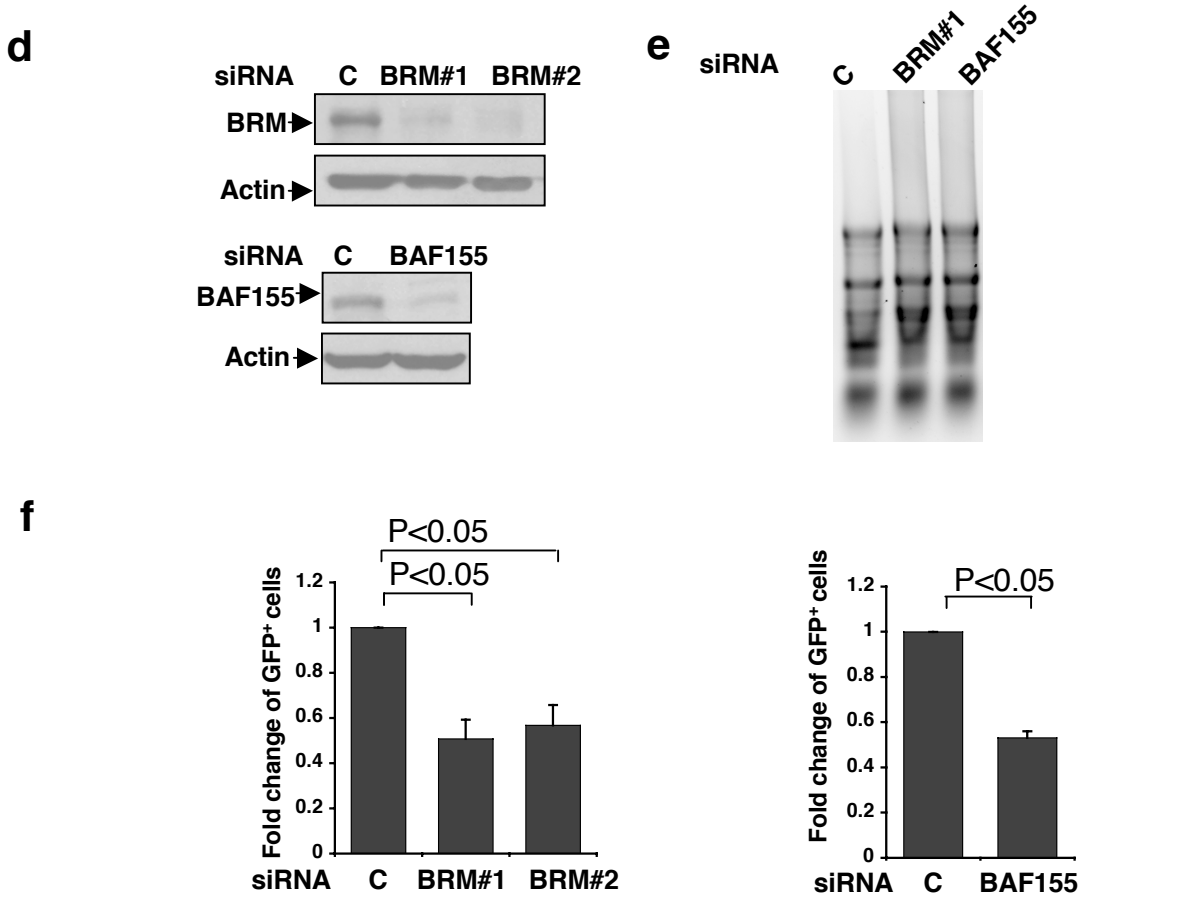




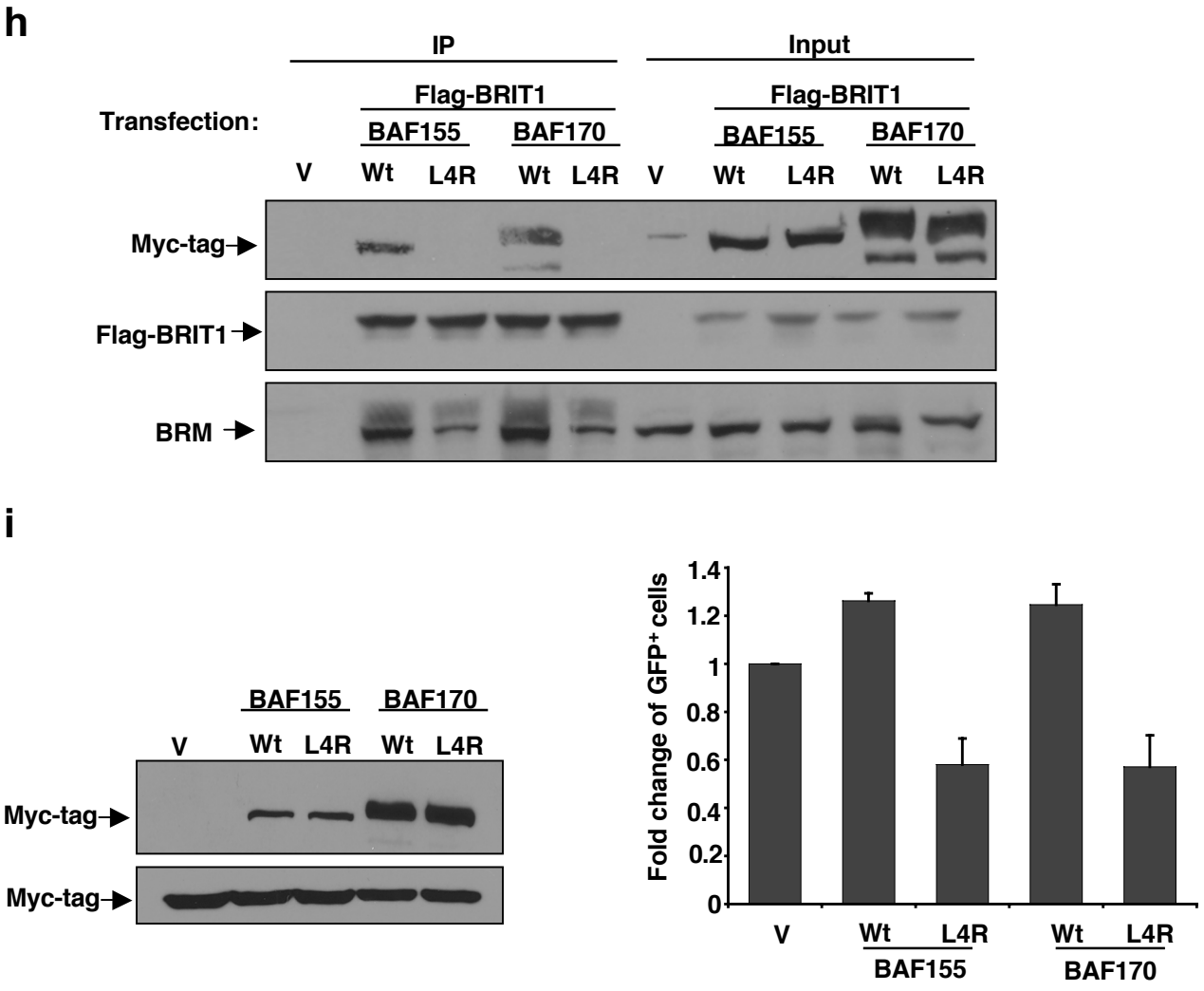
Supplementary Fig. 4



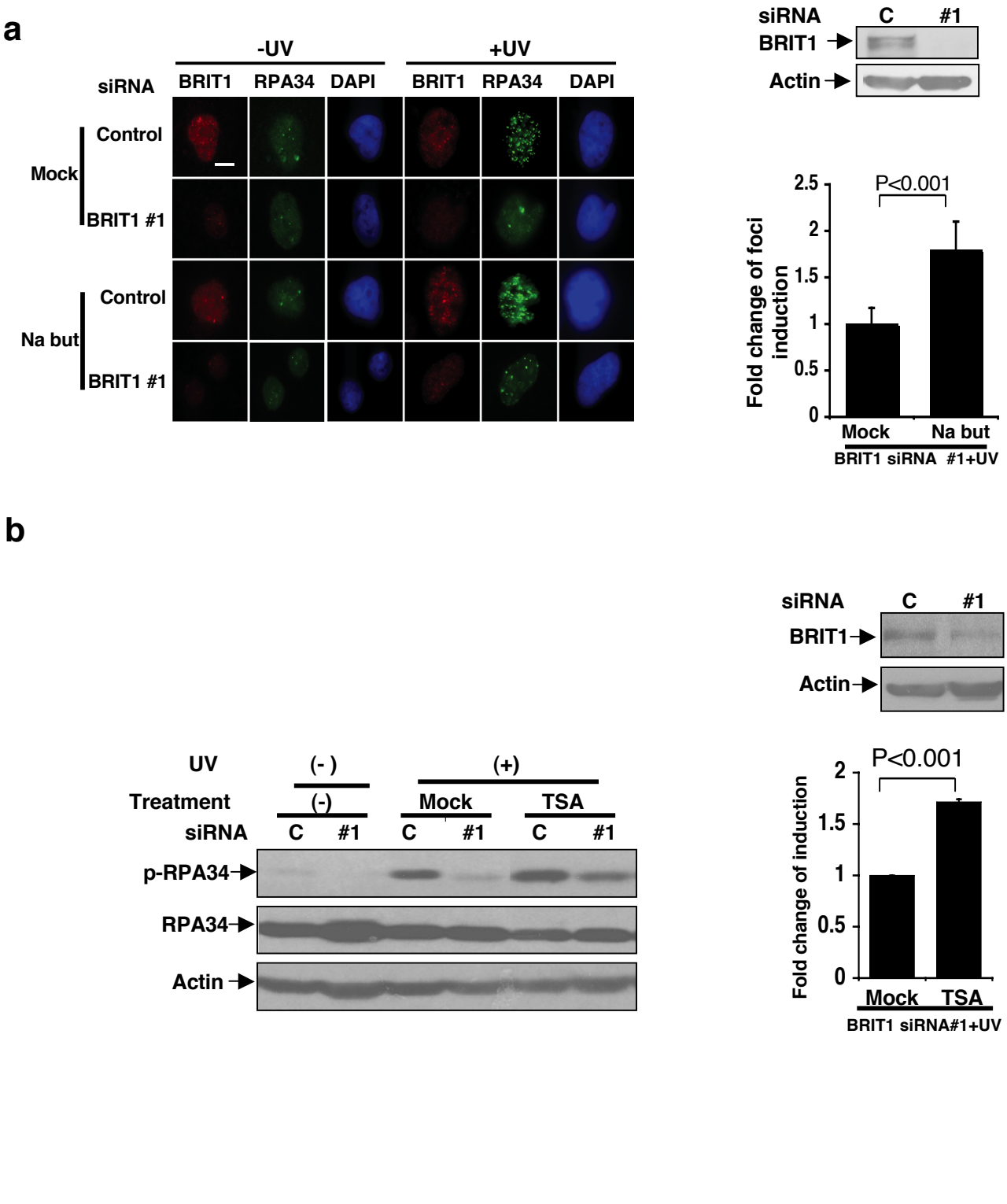
Supplementary Fig. 5



Supplementary Fig. 5

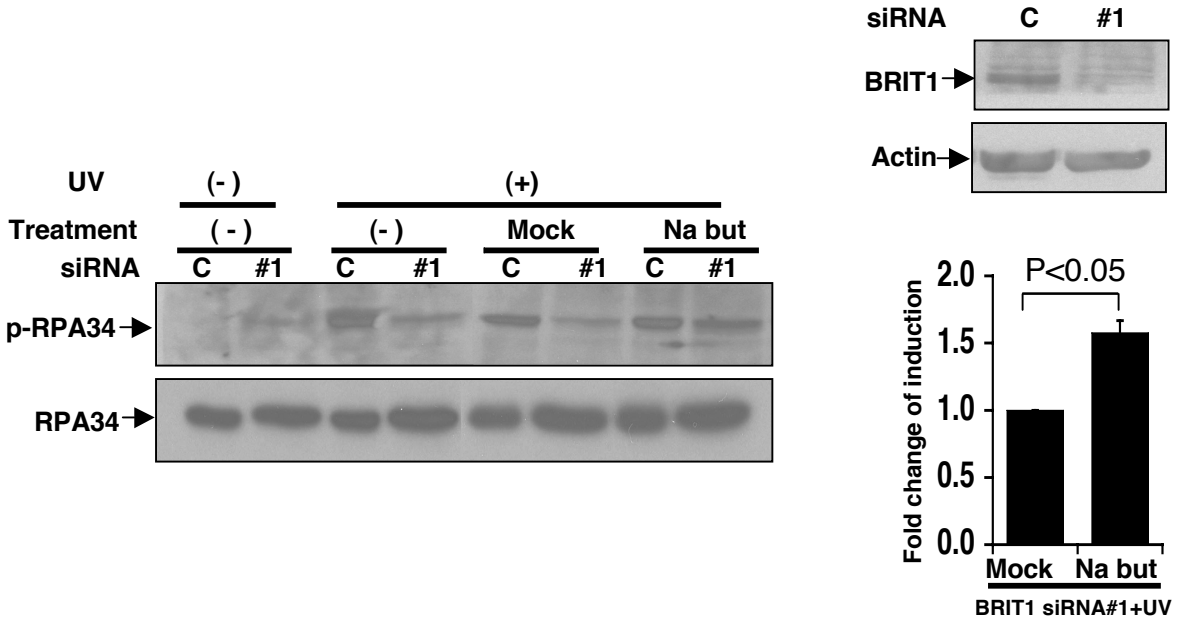


Supplementary Fig. 5



Supplementary Fig. 6

c



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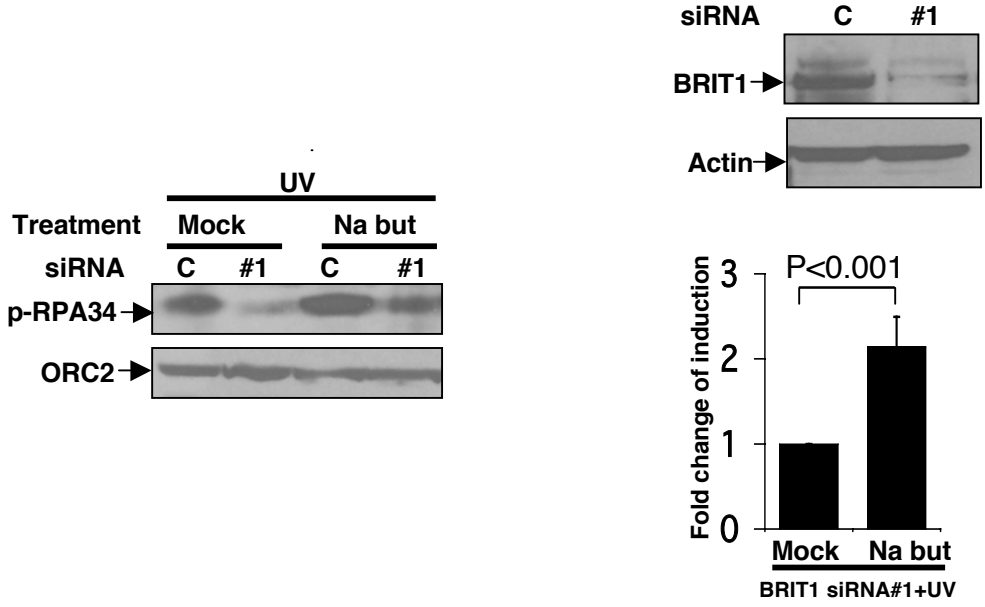
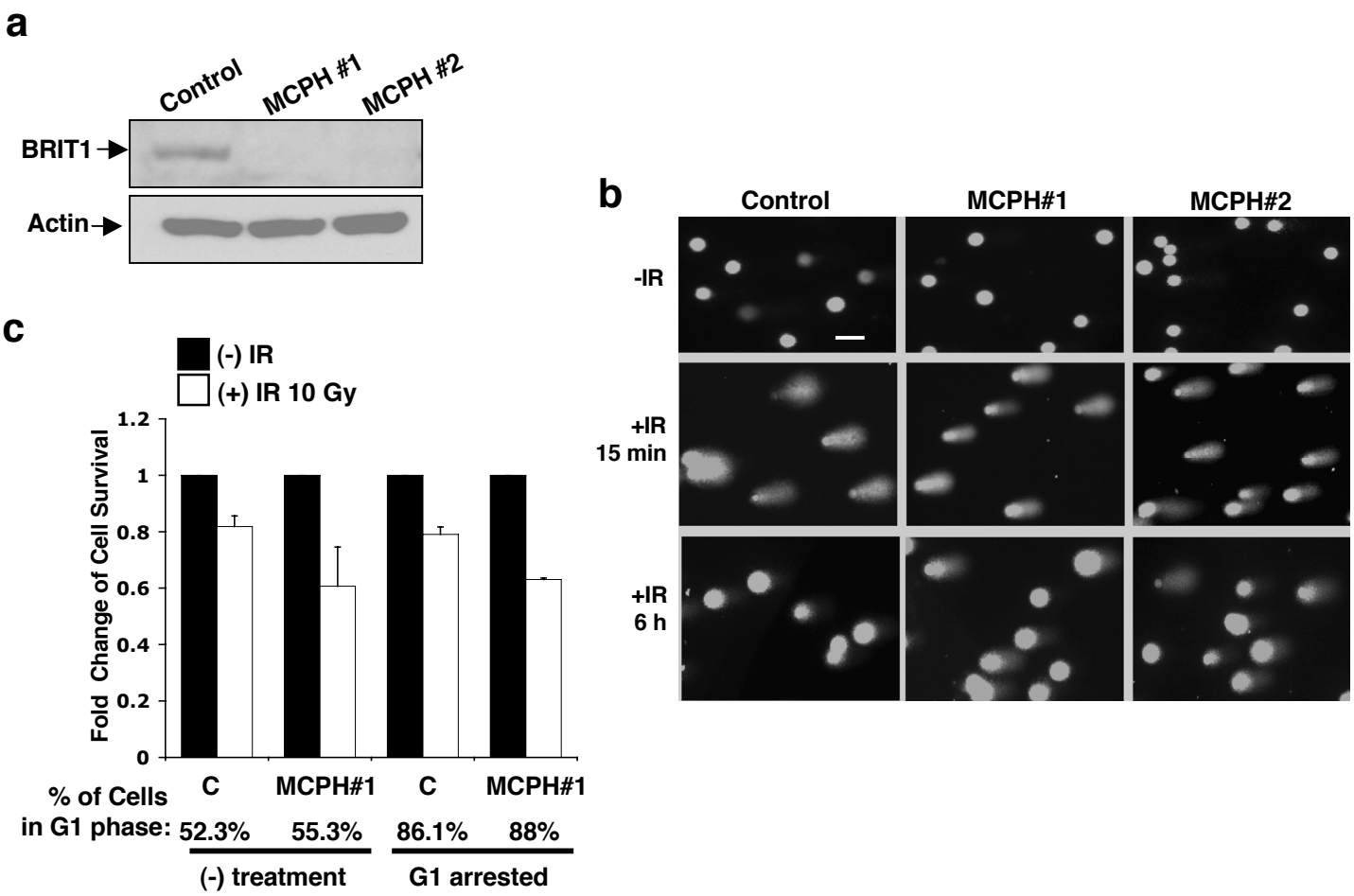
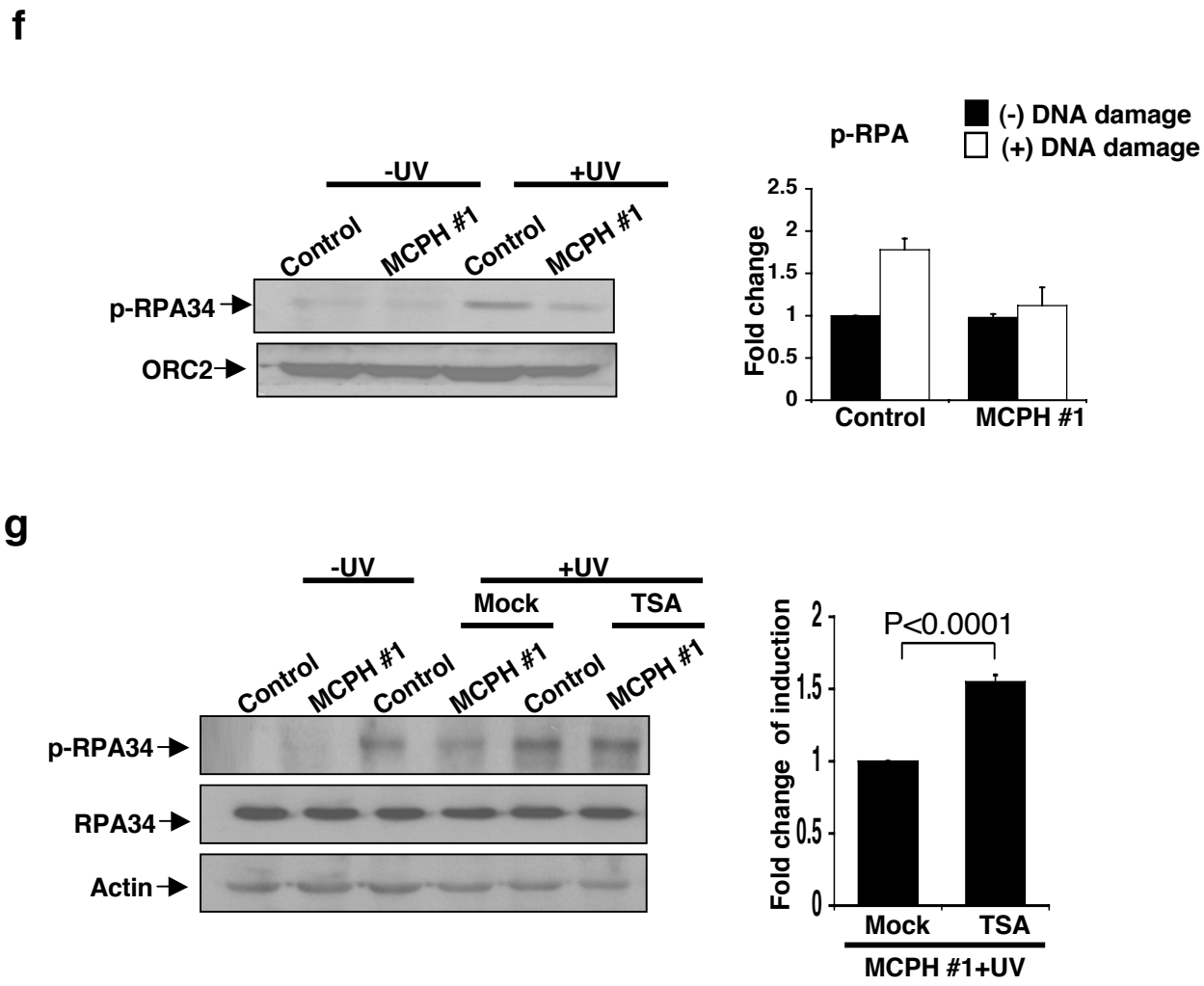


Supplementary Fig. 6

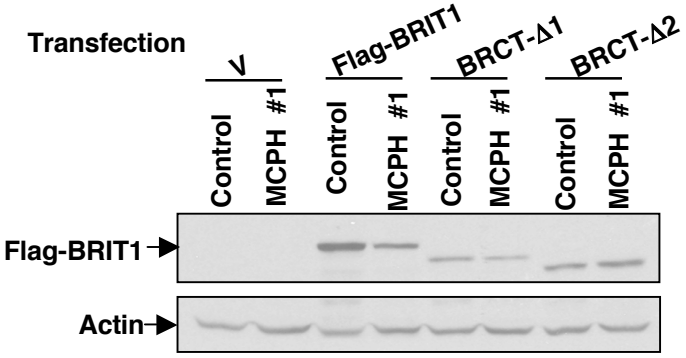


Supplementary Fig. 7



Supplementary Fig. 7

i



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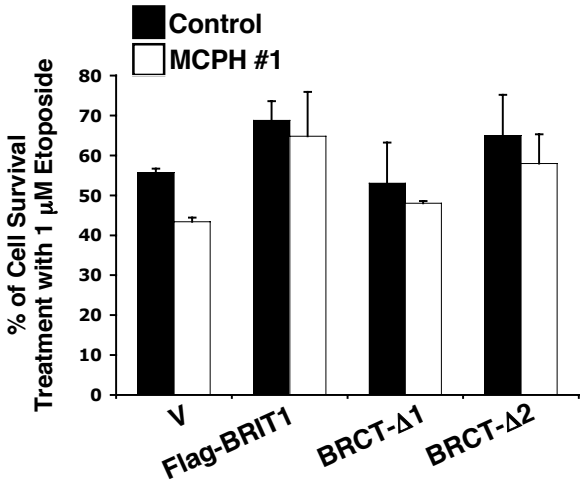

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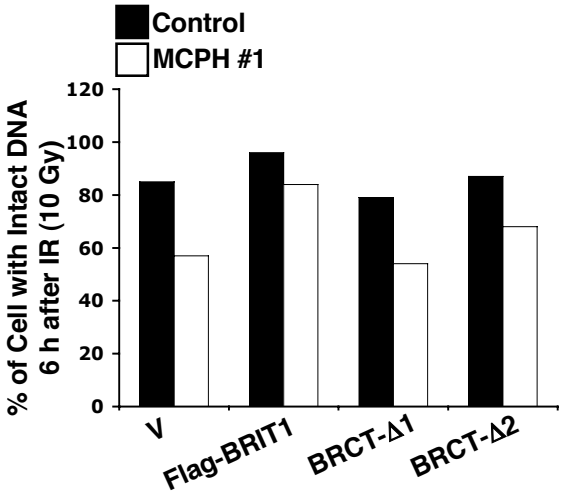

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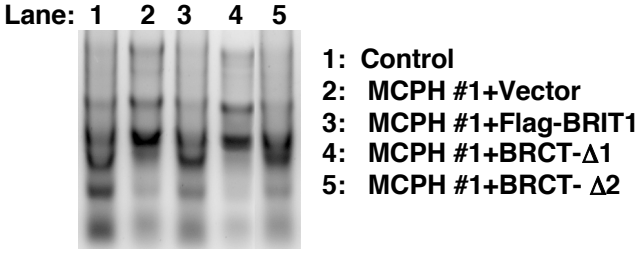


Supplementary Fig. 7

Fig. 1b

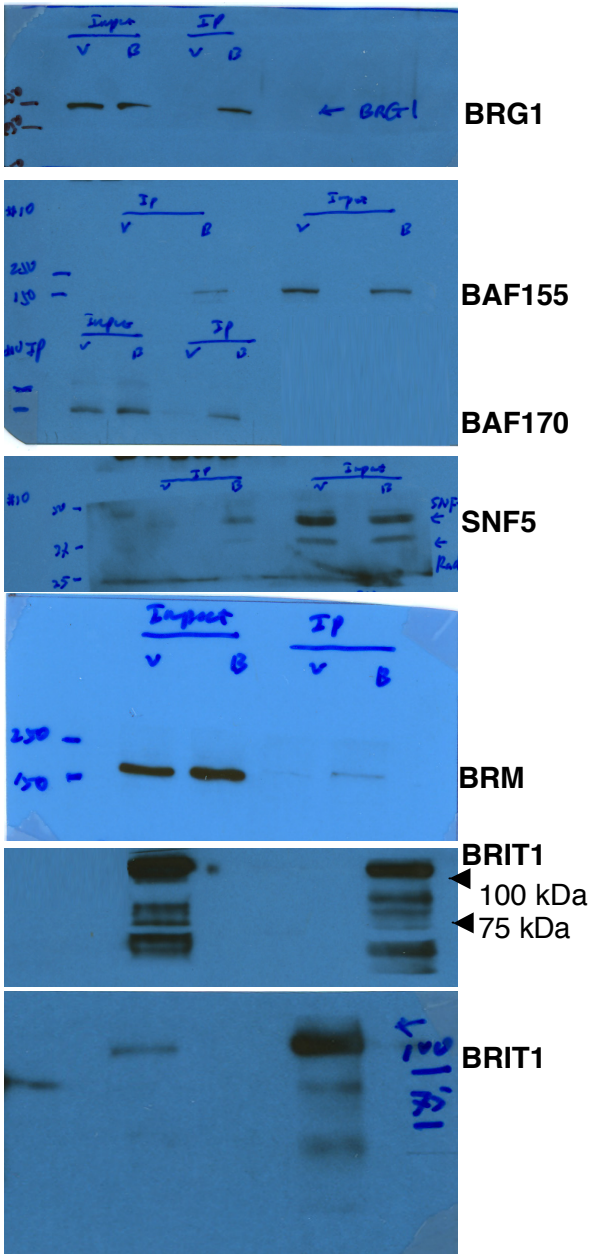


Fig. 1c

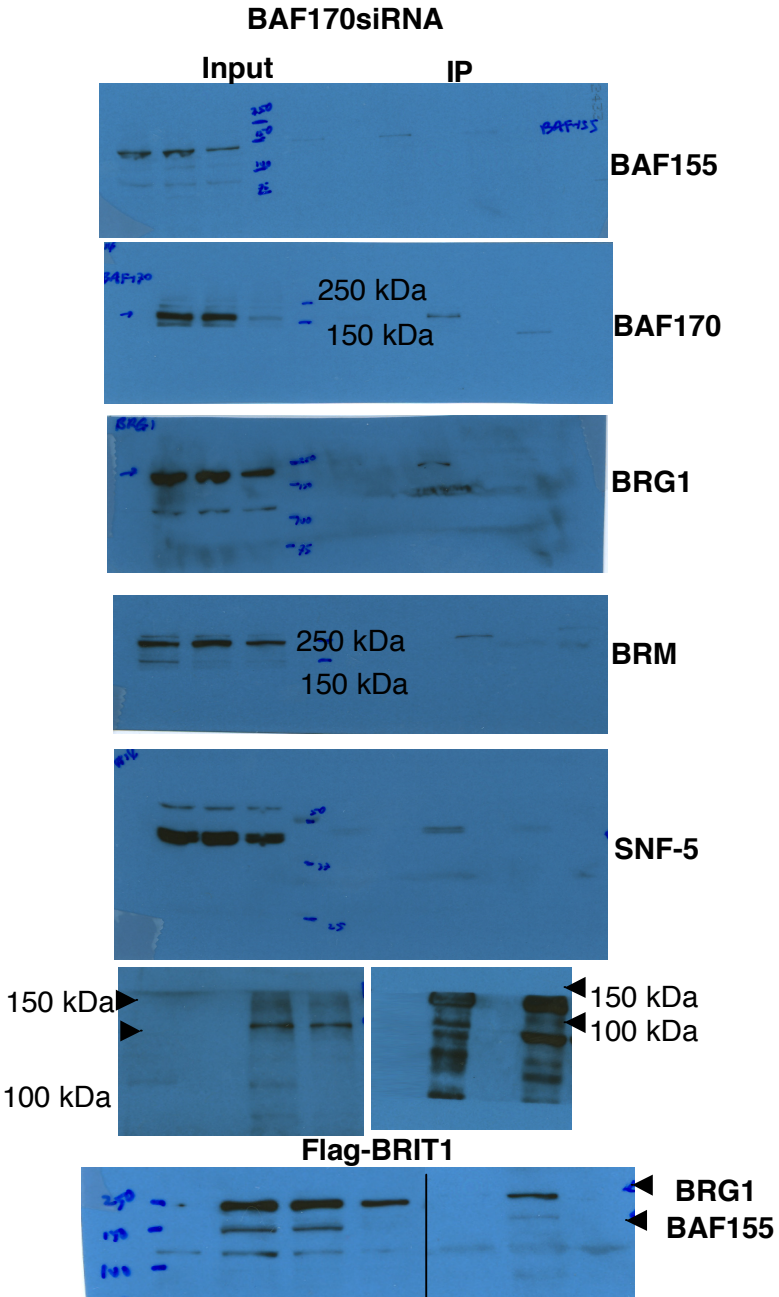
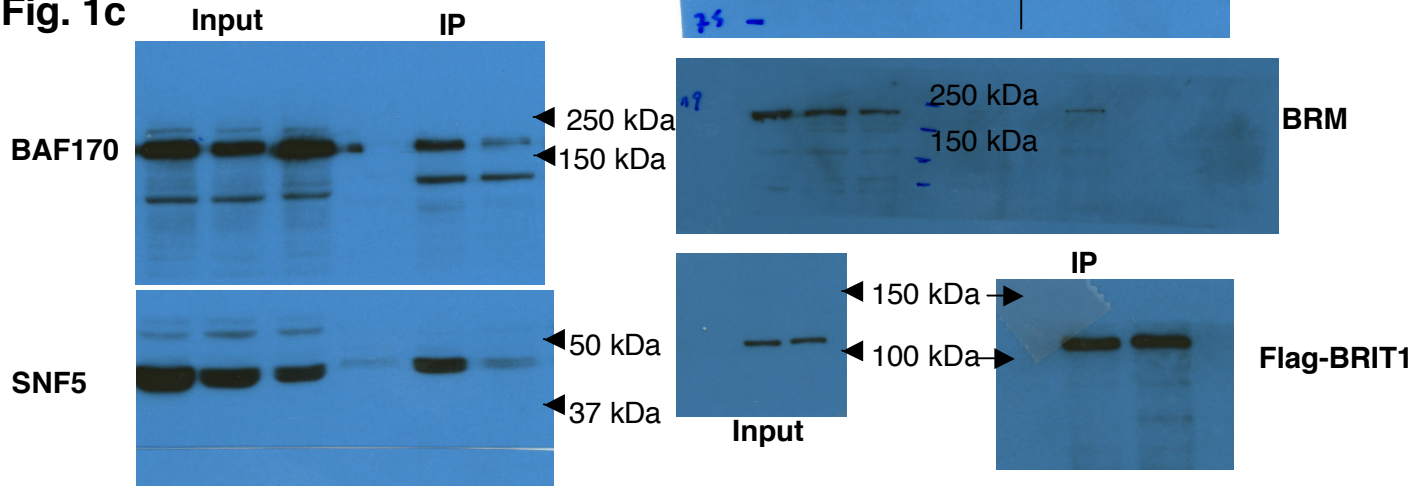


Fig. 1c



Supplementary Fig. 8

Fig.1d

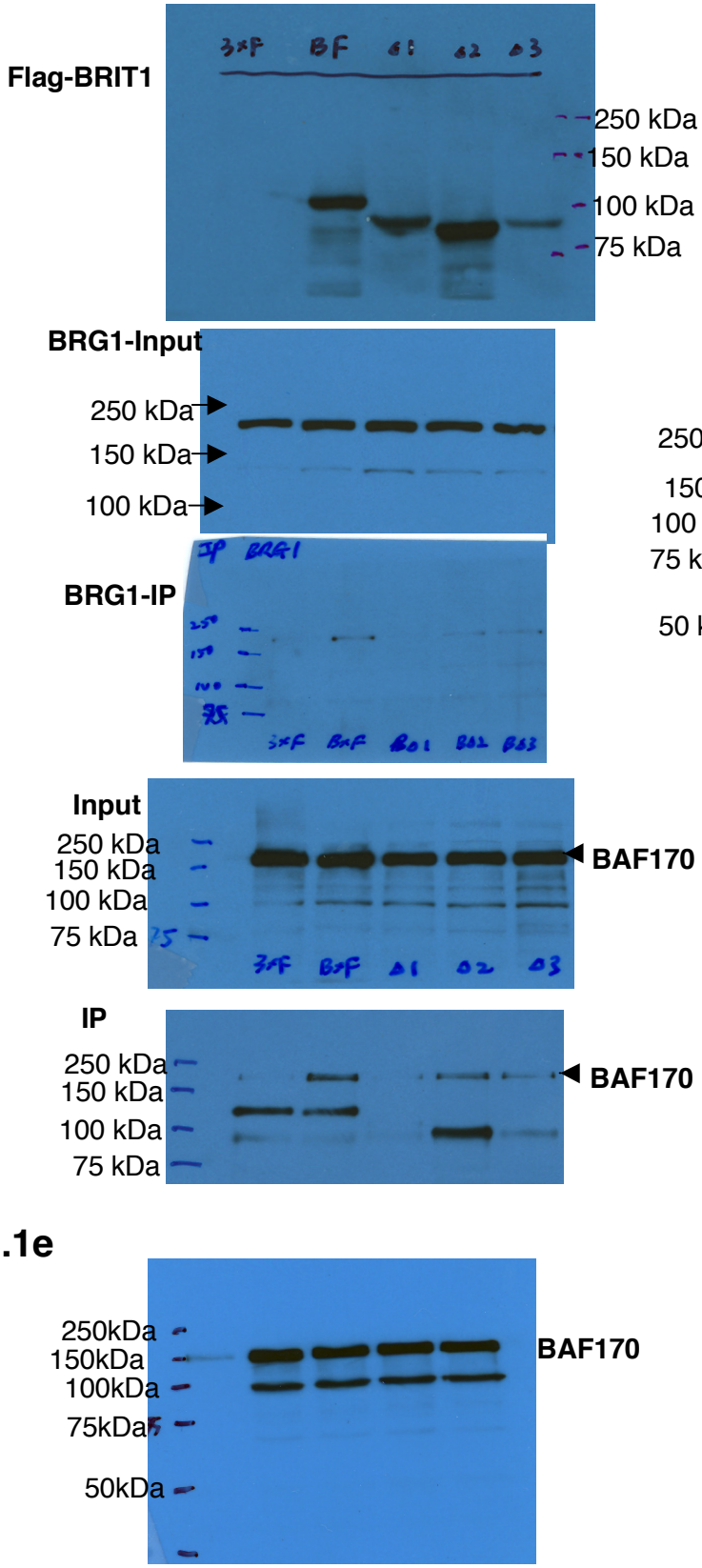


Fig.1e

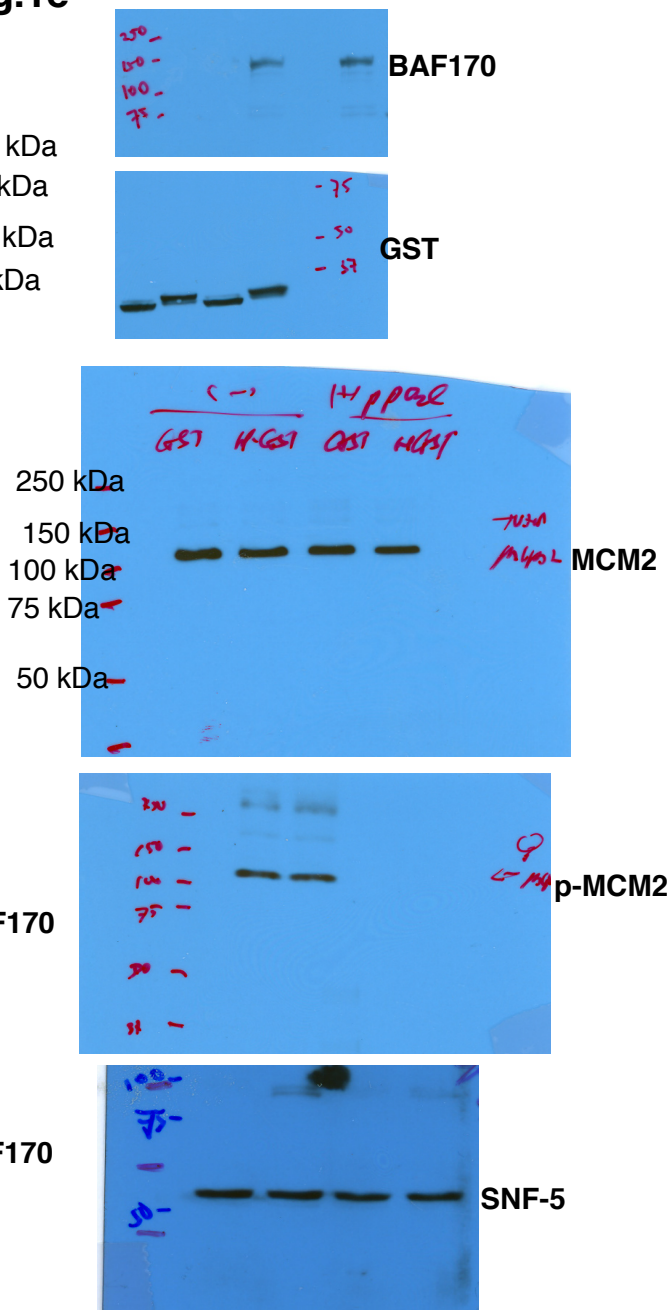
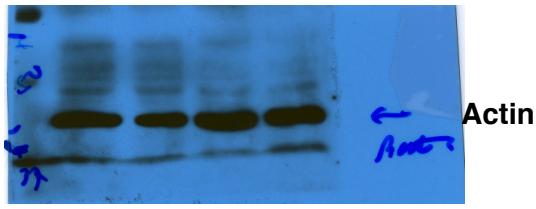


Fig.1e



Supplementary Fig. 8

Fig. 2a

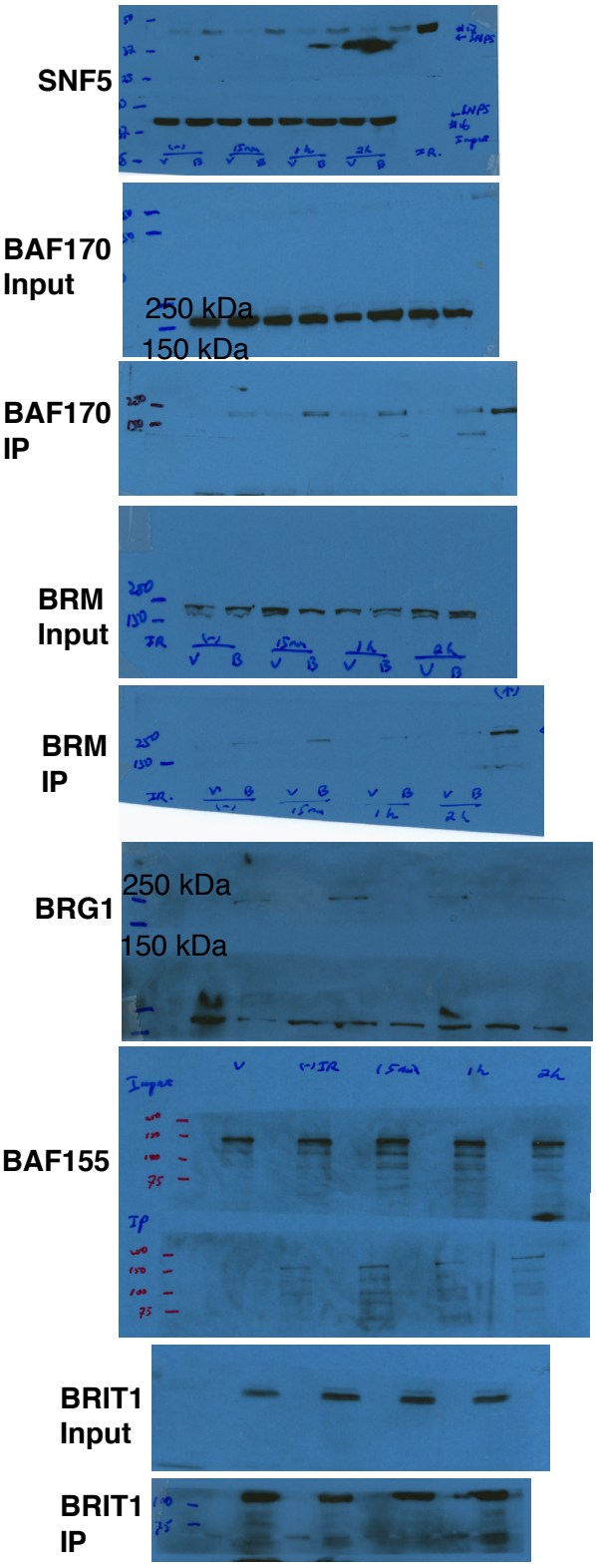
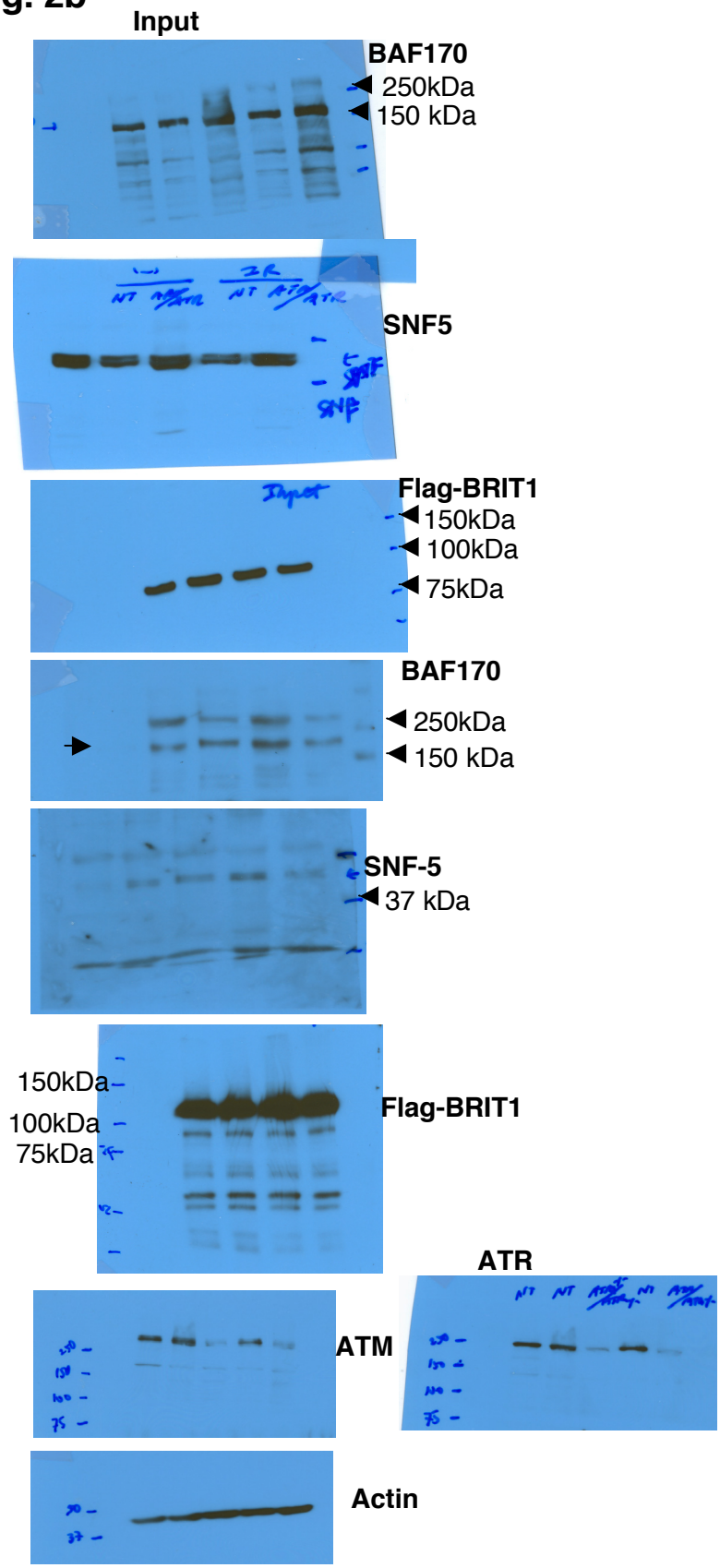


Fig. 2b



Supplementary Fig. 8

Fig. 2c

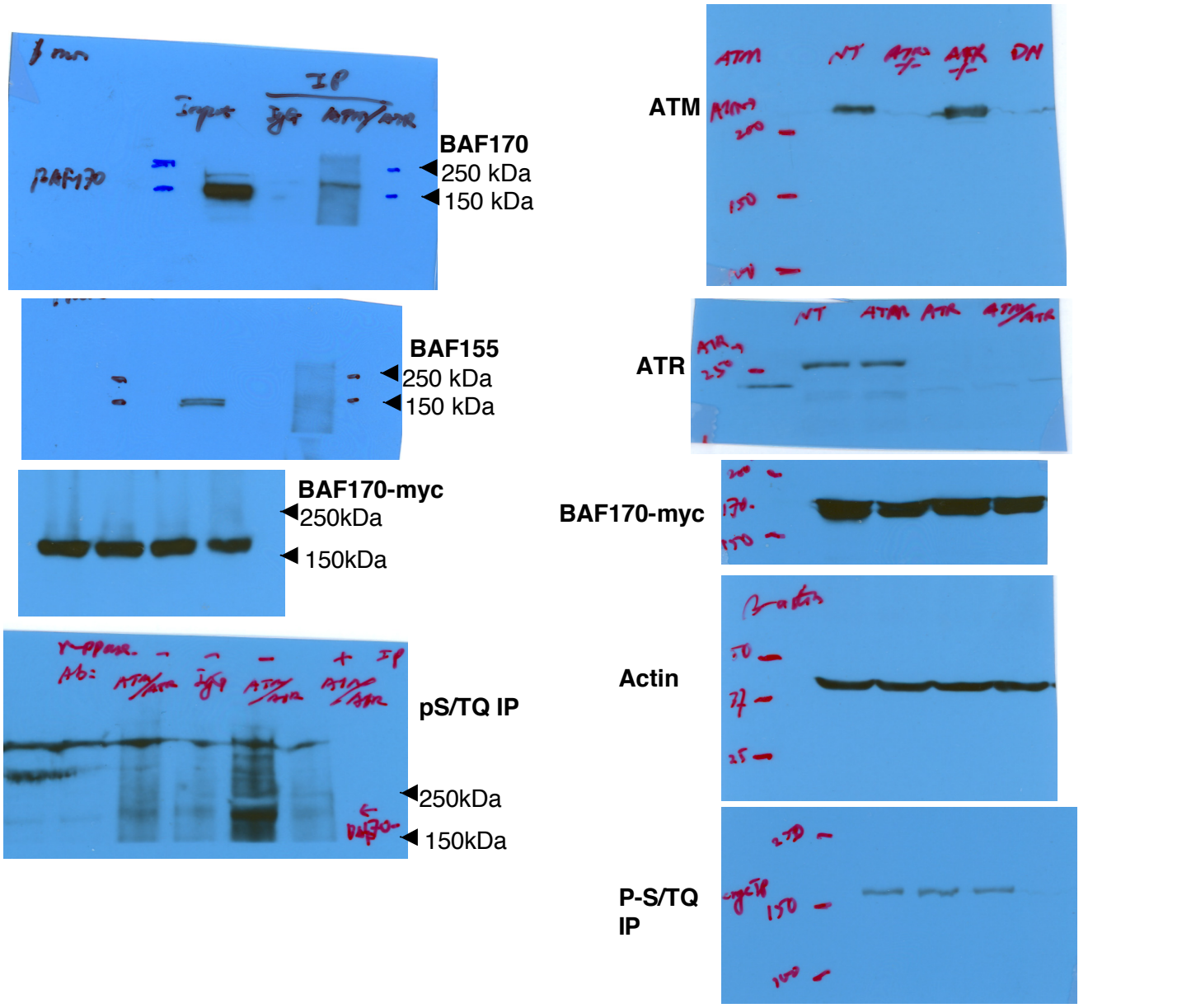
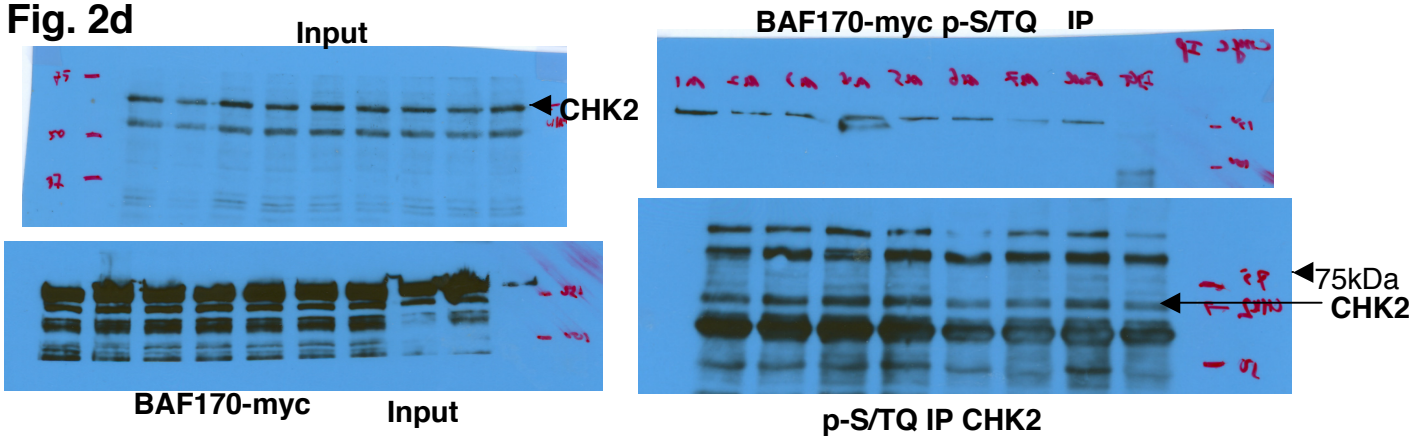


Fig. 2d



Supplementary Fig. 8

Fig. 3c

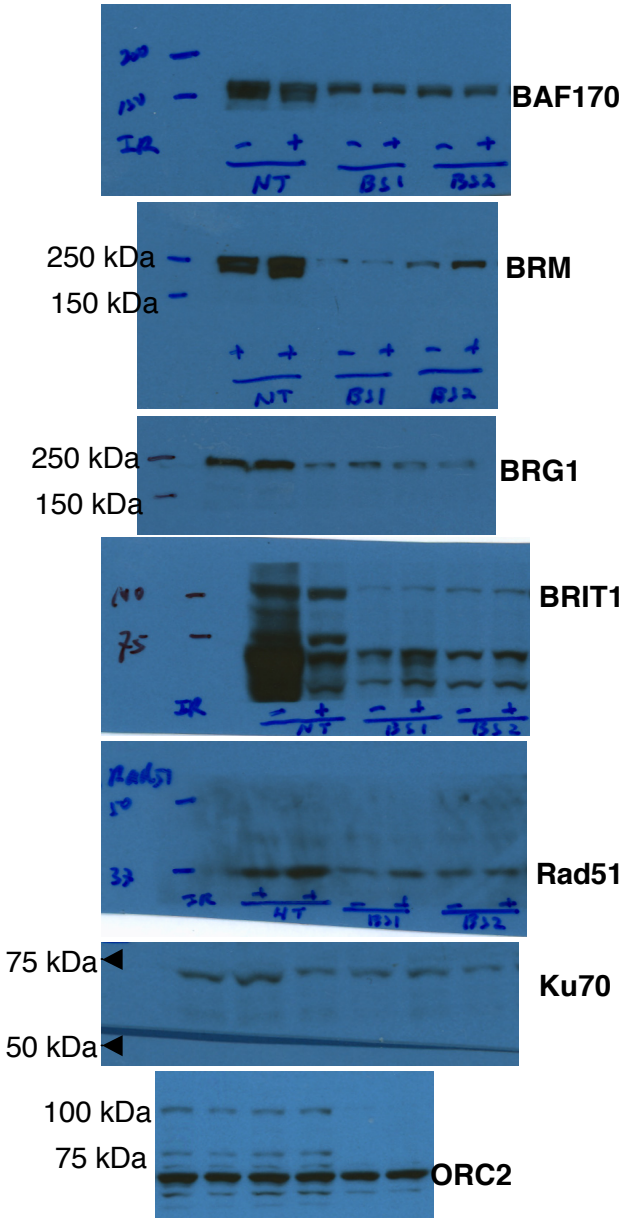


Fig. 4b

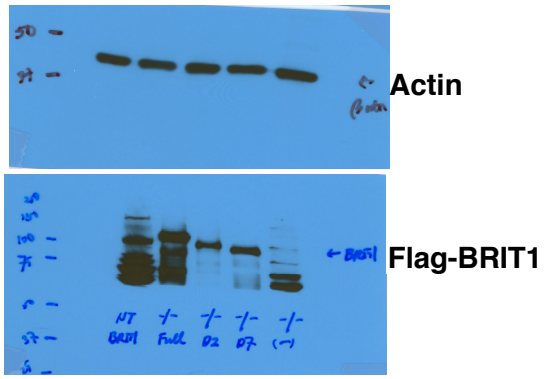
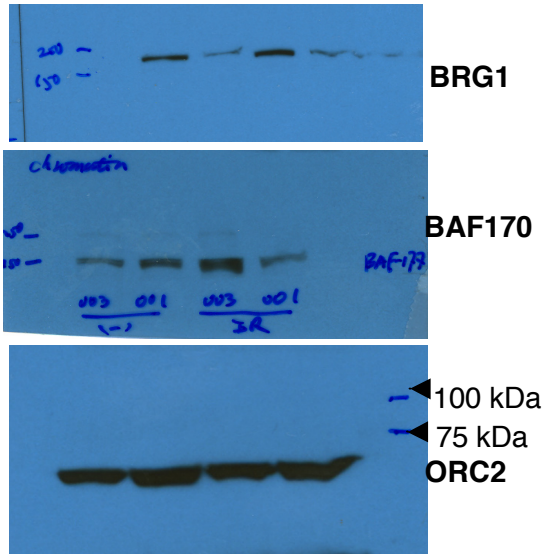


Fig. 5d



Supplementary Fig. 8