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(71) Demandeur/Applicant:
SINAI HEALTH SYSTEM, CA

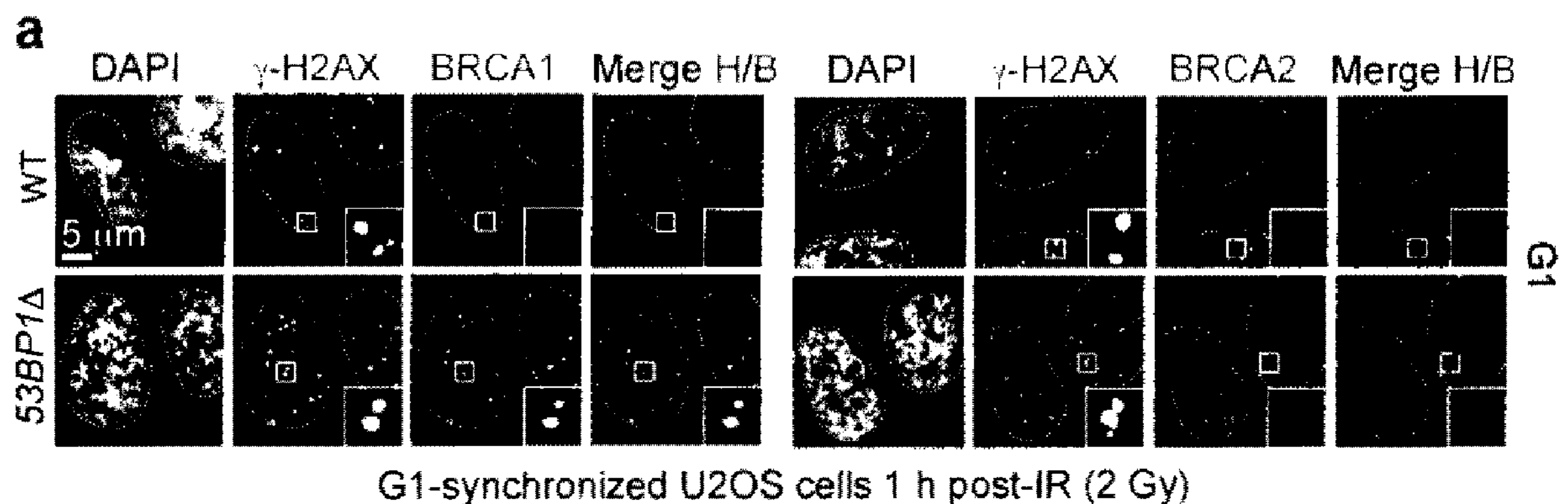
(72) Inventeurs/Inventors:
DUROCHER, DANIEL, CA;
NOORDERMEER, SYLVIE, CA;
ORTHWEIN, ALEXANDRE, CA

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

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(54) Title: HOMOLOGOUS RECOMBINATION FACTORS

FIG 1a



(57) Abrégé/Abstract:

The invention relates to factors that influence or regulate homologous recombination, methods to monitor these factors, the use of these factors to screen for agents that modulate homologous recombination, and methods to activate or modulate homologous recombination.

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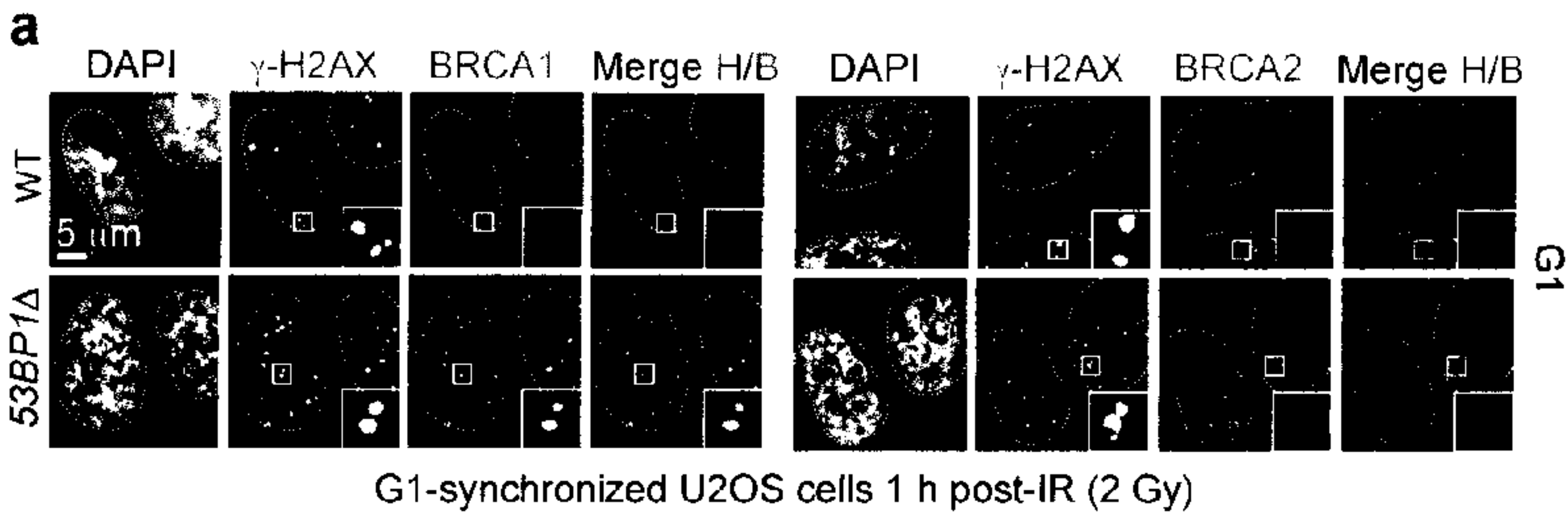
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- (71) Applicant: SINAI HEALTH SYSTEM [CA/CA]; 600 University Avenue, Toronto, Ontario M5G 1X5 (CA).
- (72) Inventors: DUROCHER, Daniel; 33 Strathcona Avenue, Toronto, Ontario, M4K 1K6 (CA). ORTHWEIN, Alexandre; 116 Gough Avenue, Toronto, Ontario, M4K 3N8 (CA). NOORDERMEER, Sylvie; 1002-80 Wellesley Street East, Toronto, Ontario, M4Y 1H3 (CA).
- (74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L., S.R.L.; Scotia Plaza, 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).
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(54) Title: HOMOLOGOUS RECOMBINATION FACTORS

FIG 1a



(57) Abstract: The invention relates to factors that influence or regulate homologous recombination, methods to monitor these factors, the use of these factors to screen for agents that modulate homologous recombination, and methods to activate or modulate homologous recombination.

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Title: HOMOLOGOUS RECOMBINATION FACTORS**FIELD OF THE INVENTION**

[0001] The invention relates to factors that influence or regulate homologous recombination, methods to monitor these factors, the use of these factors to screen for agents that modulate homologous recombination, and methods to modulate homologous recombination.

BACKGROUND OF THE INVENTION

[0002] The breast and ovarian tumour suppressors BRCA1, PALB2 and BRCA2 promote DNA double-strand break (DSB) repair by homologous recombination (HR) [8-10]. BRCA1 acts in this process minimally at two discrete steps. Firstly, it promotes DNA end resection [11, 12], the initiating step in HR that involves the nucleolytic processing of breaks to produce the single-stranded (ss) DNA necessary for homology search and strand invasion [1]. Secondly, BRCA1 interacts with PALB2 [13-15] to direct the recruitment of BRCA2 [13] and RAD51 [16, 17] to DSB sites. The accumulation of BRCA1 on the chromatin that flanks DSB sites is strikingly suppressed in G1 cells [18], reminiscent of the potent inhibition of homologous recombination in this phase of the cell cycle. The inhibition of BRCA1 recruitment in G1 is dependent on the 53BP1 and RIF1 proteins [18, 19], two inhibitors of end-resection [18-22]. BRCA1 is also involved in promoting the recruitment of BRCA2 through its interaction with PALB2 [13-15].

[0003] Tumors with compromised ability to repair double-strand DNA breaks by HR, including those with defects in BRCA1 and BRCA2, have been shown to be highly sensitive to poly ADP-ribose polymerase (PARP) inhibitors. PARP inhibitors have also been proposed for treating other conditions such as stroke, myocardial infarction, inflammatory bowel disorders, head trauma, and neurodegenerative diseases. Inhibition of ubiquitin-specific peptidase 11 (USP11) has been shown to hypersensitize cells to PARP inhibitors and it has been proposed that USP11 status or the status of other HR-proteins in tumors may provide biomarkers for use of PARP inhibitors (Wiltshire et al, JBC 285(19), 14565-14571, 2010).

[0004] Identification and assessment of factors that influence or regulate homologous recombination repair proteins and the identification of events that are both necessary and sufficient to suppress HR in G1 cells is desirable. In addition, identification and assessment of factors that influence or regulate USP11 may facilitate

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the selection and monitoring of PARP inhibitor treatments, and in particular selection of treatments that reverse or delay emergence of PARP inhibitor resistance.

SUMMARY OF THE INVENTION

[0005] The present inventors have found that the cell cycle tightly controls the interaction of BRCA1 with PALB2-BRCA2 in order to constrain BRCA2 function to the S/G2 phases. The BRCA1-interaction site on PALB2 is targeted by an E3 ubiquitin ligase composed of KEAP1, a PALB2-interacting protein [6], in complex with cullin 3(CUL3)-RBX1 [7]. PALB2 ubiquitylation suppresses its interaction with BRCA1 and is counteracted by the deubiquitylase USP11, which is itself under cell cycle control. Restoration of the BRCA1-PALB2 interaction combined with the activation of DNA end resection was sufficient to induce HR in G1-phase cells, as measured by RAD51 recruitment, unscheduled DNA synthesis and a CRISPR/Cas9-based gene targeting assay. The mechanism prohibiting HR in G1 minimally consists of the suppression of DNA end resection coupled to a multi-step block to BRCA2 recruitment to DNA damage sites that involves the inhibition of BRCA1-PALB2-BRCA2 complex assembly. The ability to induce HR in G1 cells with defined factors may be used in gene targeting applications in non-dividing cells or cells that are dormant in G1 phase. The findings also provide a basis for targeting USP11 in combination with poly(ADP-ribose) polymerase (PARP) inhibitors.

[0006] The present inventors have also found that USP11 is regulated by a cell cycle-CULLIN4-RING-ligase (CRL4) and DCAF10 acts as an adaptor for the USP11 E3 ligase.

[0007] The invention provides a method for monitoring activity of USP11 in a sample by assaying the interaction of BRCA1 and PALB2.

[0008] The invention provides a method for monitoring activity of USP11 in a sample by assaying the interaction of BRCA1, PALB2, and BRCA2.

[0009] The invention provides a method for monitoring activity of USP11 in a sample by assaying the interaction of USP11 and PALB2.

[0010] The invention provides a method for monitoring activity of USP11 in a sample by assaying DCAF10.

[0011] The invention provides a method for monitoring activity or expression of USP11 in a sample by assaying for complexes of (a) BRCA1 and PALB2; (b) BRCA1, PALB2, and BRCA2; (c) USP11 and PALB2; and/or (d) USP11 and DCAF10.

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[00012] In an aspect, the invention provides a method for monitoring activity or expression of USP11 in a sample comprising (i) isolating complexes of (a) BRCA1 and PALB2; (b) BRCA1, PALB2, and BRCA2; (c) USP11 and PALB2; and/or (d) USP11 and DCAF10 in the sample; (ii) measuring the levels of the complexes; and (iii) detecting an increase or decrease in the activity or expression of the complexes as compared to a control as an indication of the activity or expression of USP11.

[00013] In an aspect, the invention provides a method for monitoring activity or expression of USP11 in a sample comprising (i) isolating complexes of (a) BRCA1 and PALB2; (b) BRCA1, PALB2, and BRCA2; (c) USP11 and PALB2; and/or (d) USP11 and DCAF10 in the sample by immunological purification; (ii) measuring the levels of the complexes; and (iii) detecting an increase or decrease in the activity or expression of the complexes as compared to a control as an indication of the activity or expression of USP11.

[00014] In an aspect, the invention provides a method for monitoring activity or expression of USP11 in a sample comprising (i) isolating complexes of (a) BRCA1 and PALB2; (b) BRCA1, PALB2, and BRCA2; (c) USP11 and PALB2; and/or (d) USP11 and DCAF10 in the sample; (ii) preparing peptides or peptide fragments from the isolated complexes; and (iii) subjecting the peptides or peptide fragments to mass spectrometry to thereby monitor the activity or expression of USP11.

[00015] The invention provides a method for monitoring activity or expression of USP11 in a sample by assaying ubiquitylation of PALB2, in particular ubiquitylation of the N-terminus of PALB2.

[00016] In an aspect, the invention provides a method for monitoring activity or expression of USP11 in a sample by assaying ubiquitylation of PALB2 comprising measuring the amount of polyubiquitin bound to CRL3-KEAP1 E3 ligase in the sample and detecting an increase or decrease in polyubiquitin bound to CRL3-KEAP1 E3 ligase as compared to a control as an indication of the activity or expression of USP11.

[00017] In another aspect, the invention provides a method for monitoring activity or expression of USP11 in a sample by assaying ubiquitylation of PALB2 comprising measuring the activity of CRL3-KEAP1 E3 ligase, and detecting an increase or decrease in CRL3-KEAP1 E3 ligase activity as compared to a control as an indication of the activity or expression of USP11.

[00018] The methods of the invention may be performed in the presence or absence of a test compound or agent and detection of an increase or decrease in activity

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or expression of one or more of USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF complex, CRL3-KEAP1 complex, CRL3-KEAP1-PALB2 complex, and KEAP1-PALB2 complex, as compared to a control in the absence of the test compound or agent
5 indicates that the test compound or agent may be useful as a therapeutic agent, or for modulating homologous recombination.

[00019] In an aspect, the invention provides a method for identifying or evaluating an agent for its ability to sensitize or reverse or delay emergence of resistance to PARP inhibitors by determining the effect of the agent on USP11 activity
10 or expression using a method of the invention.

[00020] In an aspect, the invention relates to a method of identifying or evaluating an agent for its ability to sensitize cells or reverse or delay emergence of resistance to PARP inhibitors by determining the effect of the agent on KEAP1, CRL3-KEAP1, KEAP1-PALB2 or CRL3-KEAP1.

15 [00021] In an aspect, the present invention provides methods of detecting an anti-cancer agent comprising performing a test assay comprising contacting an immortalized cell with a test compound and assaying USP11 activity or expression using a method of the invention.

[00022] The invention also provides a method for identifying or evaluating an
20 agent for its ability to modulate homologous recombination comprising determining the effect of a test compound or agent on one or more of USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 complex and CRL3-KEAP1-PALB2 complex.

25 [00023] The invention provides a method of screening for a therapeutic agent for treatment of a disease associated with defects in HR (i.e., HR Disease), comprising identifying an agent that disrupts or modulates one or more of USP11, PALB2, PALB2 ubiquitylation, DCAF10, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 or CRL3-KEAP1-PALB2 complex.

30 [00024] The screening methods of the invention may further comprise conducting therapeutic profiling of the identified agents or further analogs thereof, for efficacy and toxicity in animals; optionally formulating a pharmaceutical composition including one or more agents identified as having an acceptable therapeutic profile; and optionally administering the agent to a subject or individual.

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[00025] The invention provides methods of treating a HR Disease in an individual comprising identifying an agent that modulates HR in accordance with a method of the invention and administering the agent to the individual.

[00026] In some embodiments, the invention provides a method for sensitizing
5 cells to PARP inhibitors in an individual comprising identifying an agent that sensitizes cells to PARP inhibitors in accordance with a method of the invention and administering the agent to the individual.

[00027] In some embodiments, the invention provides a method for reversing or delaying emergence of resistance to PARP inhibitors in an individual comprising
10 identifying an agent that reverses or delays emergence of resistance to PARP inhibitors in accordance with a method of the invention and administering the agent to the individual.

[00028] In some embodiments, the present invention provides methods of treating cancer in an individual comprising identifying an anti-cancer agent identified in
15 accordance with a method of the invention and administering the agent to the individual.

[00029] The invention also provides a method for predicting a response or categorizing a response to a PARP inhibitor in a subject comprising assaying one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1,
20 USP11-DCAF10 complex, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex and CRL3-KEAP1-PALB2 complex in a sample from the subject using a method of the invention. In an aspect, a method is provided for predicting a response or categorizing a response to a PARP inhibitor in a subject comprising assaying USP11 activity or expression in a sample from the subject using a method of the invention. In
25 an aspect, a method is provided for predicting a response or categorizing a response to a PARP inhibitor in a subject comprising assaying PALB2 activity or expression in a sample from the subject using a method of the invention.

[00030] In an aspect, a subject is categorized as responsive to a PARP inhibitor if there is a decrease in one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2,
30 KEAP1, CRL3, USP11-DCAF10, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control. In an aspect, a subject is categorized as responsive to a PARP inhibitor if there is an increase in one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1,

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CRL3, USP11-DCAF10, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control.

[00031] A method of predicting responsiveness to a PARP inhibitor may further comprise administering the PARP inhibitor to the individual.

5 [00032] The invention provides a method for treating a patient in need of treatment with a PARP inhibitor comprising (a) requesting a test providing the results of an analysis to determine if the patient is sensitive or responsive to the PARP inhibitor by detecting one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, USP11-DCAF10, CRL3, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-
10 PALB2-BRCA2, in a sample from the subject and comparing to a control to determine if the patient is sensitive or responsive to the PARP inhibitor; and (b) administering the PARP inhibitor to the patient if the patient is sensitive or responsive to the PARP inhibitor. In an aspect of this method of the invention, the patient has breast cancer. In an aspect of this method of the invention, the patient has ovarian cancer.

15 [00033] In an aspect, the invention provides a method for treating a patient in need of treatment with a PARP inhibitor comprising (a) requesting a test providing the results of an analysis to determine if the patient is sensitive to the PARP inhibitor by detecting USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1 and/or CRL3 in a sample from the subject and comparing to a control to determine if the patient is
20 sensitive to the PARP inhibitor; and (b) administering the PARP inhibitor to the patient if the patient is sensitive to the PARP inhibitor. In an aspect of this method of the invention, the patient has breast cancer. In an aspect of this method of the invention, the patient has ovarian cancer.

[00034] The invention further provides a method for assigning an individual to
25 one of a plurality of categories in a clinical trial for a PARP inhibitor comprising assaying USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, USP11-DCAF complex, KEAP1, CRL3-KEAP1 and/or CRL3-KEAP1-PALB2 complex in a sample from the subject using a method of the invention.

30 [00035] The invention also provides pharmacogenetic methods for determining suitable treatment regimens for diseases, in particular cancer, and methods for treating patients, based around selection of patients based on PARP responsiveness, in particular USP11 activity.

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[00036] The methods of the invention, in particular methods for assaying USP11 activity or CRL3-KEAP1 activity, may be used as a read out in animal model based screening methods for new therapeutic approaches and compounds. In an aspect, a method of the invention is utilized to predict the efficacy of potential new treatments in animal models for disease states.

[00037] The invention provides a method for activating or modulating (e.g., promoting) homologous recombination in a cell comprising:

- (a) promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell;
- 10 (b) activating or stimulating BRCA1 recruitment to DNA double-strand break (DSB) sites;
- (c) contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes;
- (d) inhibiting or removing KEAP1 or CRL3-KEAP1;
- 15 (e) inhibiting the degradation of USP11 or promoting USP11 activity; and/or
- (f) inhibiting or removing DCAF10.

[00038] The invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 phase of the cell cycle (G1) or G0 phase of the cell cycle, comprising administering, or stimulating assembly of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

[00039] The invention also provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0), comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

[00040] The invention also provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0), comprising administering to the cell or contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes.

30 [00041] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0), comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

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[00042] In aspects of the invention, the assembly of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes is promoted or stimulated by administering an agent that promotes or stimulates such assembly or an agent that promotes or stimulates such assembly identified using a method of the invention. In an embodiment, the agent is
5 USP11 or an agonist of USP11. In an embodiment, the agent is an inhibitor of CRL-KEAP1. In an embodiment, the agent is an inhibitor of KEAP1. In an embodiment, the agent is a PALB2 mutant. In an embodiment, the agent is a PALB2 mutant that disrupts its interaction with KEAP1. In an embodiment, the agent is a PALB2 comprising mutations of its Lys20, Lys25 and Lys30 residues.

10 [00043] A method for activating or modulating homologous recombination in a cell may be performed in a cell wherein single strand DNA (ssDNA) generation pathways are activated. In an aspect, ssDNA generation pathways in the cell are activated by DNA end resection.

[00044] The invention also provides a method for activating or modulating
15 homologous recombination in a cell, in particular a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA, comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

20 [00045] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA, comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell. In an embodiment, the assembly of the
25 complexes is promoted or stimulated by administering an agent that modulates HR. In an embodiment, the agent is an agent that modulates HR identified using a method of the invention. In an embodiment, the agent is USP11 or an agonist of USP11. In an embodiment, the agent is an inhibitor of CRL-KEAP1. In an embodiment, the agent is an inhibitor of KEAP1. In an embodiment, the agent is a PALB2 mutant. In an
30 embodiment, the agent is an inhibitor of DCAF10. In an embodiment, the agent is an inhibitor of a CULLIN4-RING-ligase.

[00046] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA,

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comprising contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes.

[00047] In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting KEAP1 or CRL3-KEAP1 or administering an inhibitor of KEAP1 or CRL3-KEAP1. In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of blocking the degradation of USP11 or promoting or stimulating USP11 activity. In an embodiment, the method comprises administering USP11 or an agonist thereof. In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting CRL-KEAP1 or administering an inhibitor of KEAP1 or CRL3-KEAP1 and blocking the degradation of USP11 or promoting or stimulating USP1 activity.

[00048] The invention also provides a method for repairing DNA double-strand breaks in a cell in G1 or G0 in which DNA end resection is or has been activated generating single-stranded DNA, the method comprising (a) inhibiting KEAP1 or CRL3-KEAP1; (b) blocking the degradation of USP11 or promoting or stimulating USP11 activity; (c) administering USP11 or an agonist thereof; (d) administering an inhibitor of KEAP1 or CRL3-KEAP1; (e) administering an inhibitor of DCAF10; and/or (e) inhibiting CRL-KEAP1 and blocking the degradation of USP11.

[00049] A method for activating or modulating homologous recombination in a cell may further comprise activating or promoting single-strand DNA (ssDNA) generation pathways. In an aspect, ssDNA generation pathways are activated by DNA end resection.

[00050] A method for activating or modulating homologous recombination in a cell may further comprise a gene editing system. In an aspect the gene editing steps comprise contacting the cell with a nuclease. In aspects of the invention the gene editing system may correct a genomic modification.

[00051] The invention also provides a method for suppressing homologous recombination in a cell, in particular a cell in G1, comprising suppressing the assembly of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell. In an embodiment, the interaction is suppressed by administering KEAP1 or CRL3-KEAP1 or an agonist thereof. In an embodiment, the interaction is suppressed by administering

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a USP11 antagonist/inhibitor (e.g., mitoxantrone). In an embodiment, the interaction is suppressed by administering an agent that inhibits or suppresses HR identified using a method of the invention.

5 [00052] The invention further provides kits for performing methods of the invention.

[00053] The invention also provides a system comprising: an assay for determining the level of USP11 activity, complexes or biomarker levels in a sample obtained from the subject; a processor for processing the results; computer coded instructions for comparing the results with a database; and a user display for providing
10 the results of the comparison. The database may comprise reference values for USP11 activity or biomarker levels.

[00054] The invention also contemplates the use of methods, kits, and systems of the invention in genome modification or editing.

[00055] In an aspect, the invention further contemplates the use of methods,
15 compositions, kits, and systems of the invention in genome modification or editing, provided that said use is not a method for treatment of the human or animal body by surgery or therapy, and provided that said use is not a process for modifying the germ line genetic identity of human beings. Genome modification may comprise modifying a target polynucleotide sequence in a cell, modifying expression of a polynucleotide
20 sequence in a cell, generating a model cell comprising a mutated disease gene, or knocking out a gene. A use of the invention may further comprise repairing or editing a cleaved target polynucleotide by inserting an exogenous template polynucleotide, wherein the repair or editing results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of the target polynucleotide.

25 [00056] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will
30 become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

[00057] The invention will now be described in relation to the drawings in which:

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[00058] Figure 1. Inhibition of the BRCA1-PALB2 interaction in G1 is CRL3-KEAP1-dependent. a, Micrographs of irradiated (2 Gy) G1-synchronized U2OS cells processed for γ -H2AX, BRCA1 and BRCA2 immunofluorescence. DAPI, 4',6-diamidino-2-phenylindole; IR, ionizing radiation; WT, wild type. b, Quantitation of the experiment shown in a and Figure 5d. ASN, asynchronously dividing cells. WT, wild type (Mean $\pm$ standard deviation (s.d.), $N=3$). c, Immunoprecipitation (IP) of PALB2 from extracts prepared from mock- or X-irradiated 293T cells synchronized in S or G1 phases. A normal immunoglobulin (Ig)G immunoprecipitation was performed as control. Cyclin A staining ascertains cell cycle synchronization. Numbers on left indicate kDa. For gel source data see Figure 5. d, Quantitation of the experiment shown in Figure 7a. *53BP1* Δ U2OS cells transfected with the indicated GFP-PALB2 vectors and short interfering (si)RNAs were irradiated (20 Gy) before being processed for microscopy. (mean $\pm$ s.d., $N=3$). e, Normal IgG and PALB2 immunoprecipitations from extracts prepared from synchronized and irradiated 293T cells of the indicated genotypes. Numbers on the left indicate kDa.

[00059] Figure 2. Ubiquitylation of PALB2 prevents BRCA1-PALB2 interaction. a, Sequence of the PALB2 N terminus and mutants. [SEQ ID NOs: 1-3] b, GFP immunoprecipitation (IP) of extracts derived from G1- or S-phase synchronized 293T cells expressing the indicated GFP-PALB2 proteins. c, *In vitro* ubiquitylation of the indicated HA-tagged PALB2 proteins by CRL3-KEAP1. d, Pulldown assay of ubiquitylated HA-PALB2 (1-103) incubated with MBP or MBP-BRCA1-CC. I, input; FT, flow-through; PD, pulldown. The asterisk denotes a fragment of HA-PALB2 competent for BRCA1 binding. b-d, Numbers on left indicate kDa.

[00060] Figure 3. USP11 opposes the activity of CRL3-KEAP1. a, Normal IgG or PALB2 immunoprecipitation (IP) of extracts derived from camptothecin (CPT)-treated 293T cells of the indicated genotypes transfected with GFP-USP11 constructs. EV, empty vector; CS, C318S; WT, wild type. b, Clonogenic survival assays of 293T cells of the indicated genotypes treated with olaparib (mean $\pm$ s.d., $N \geq 3$). c, Normal IgG or PALB2 immunoprecipitation of extracts derived from CPT-treated 293T cells of the indicated genotypes. d, Immunoblots of deubiquitylation reactions containing ubiquitylated HA-tagged PALB2 (1-103) and increasing concentrations of glutathione *S*-transferase (GST)-USP11 or its C270S (CS) mutant. USP2 was used as a control. DUB, deubiquitylase. e, Cell cycle- synchronized U2OS cells were irradiated (20 Gy dose) and processed for immunoblotting. IR, ionizing radiation. f, Immunoblots of

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extracts from irradiated U2OS cells transfected with the indicated siRNAs. CTRL, control. g, Fluorescence micrographs of G1-synchronized and irradiated (20 Gy) *53BP1Δ* U2OS cells transfected with the indicated siRNAs. The percentage of cells with more than five γ -H2AX-colocalizing BRCA2 foci is indicated (mean $\pm$ s.d., $N =$ 3). Scale bars, 5 μ m. a, c, d, f, Numbers to left or right indicate kDa.

[00061] Figure 4. Reactivation of HR in G1 phase. a, Quantitation of wild-type (WT) and *53BP1Δ* U2OS cells co-transfected with non-targeting (CTRL) or KEAP1 siRNAs and vectors expressing wild-type CtIP or the T847E (TE) mutant that were synchronized in G1, irradiated (2 Gy) and processed for γ -H2AX and RAD51 immunofluorescence (mean $\pm$ s.d., $N = 3$). b, Representative micrographs from a. IR, ionizing radiation. c, Schematic of the gene-targeting assay. d, Gene-targeting efficiency at the *LMNA* locus in asynchronously dividing (ASN) and G1-arrested U2OS cells (mean $\pm$ s.d., $N = 3$). HR, homologous recombination; sgRNA, single guide RNA. e, Gene targeting at the *LMNA* locus in G1-arrested cells transfected with the indicated siRNA or a PALB2-KR expression vector (mean $\pm$ s.d., $N = 3$). f, Model of the cell-cycle regulation of homologous recombination.

[00062] Figure 5. Suppression of PALB2-BRCA2 accumulation at DSB sites in G1 *53BP1Δ* cells. a, Schematic representation of human 53BP1 gene organization and targeting sites of sgRNAs used. Boxes indicate exons (E: yellow, coding sequence; brown, untranslated regions (UTRs)). The indels introduced by CRISPR/Cas9 and their respective frequencies are indicated. b, Wild-type (WT) and *53BP1Δ* and U2OS cells were mock- or X-irradiated (10 Gy) before being processed for 53BP1 fluorescence microscopy. DAPI was used to stain DNA and trace the outline of the nucleus. c, Wild-type (WT) and *53BP1Δ* U2OS cells were processed for 53BP1 immunoblotting. Tubulin was used as a loading control. d, Wild-type (WT) and *53BP1Δ* U2OS cells either synchronized in G1 following a double-thymidine block and release or asynchronously dividing (ASN), were irradiated (2 Gy) and processed for γ -H2AX, PALB2, BRCA2 and BRCA1 immunofluorescence. The micrographs relating to BRCA1 and BRCA2 staining in G1 are found in Figure 1a. e, Wild-type (WT) and *3BP1Δ* U2OS cells synchronized in G1 after release from a double-thymidine block were irradiated (20 Gy) and processed for γ -H2AX, BRCA1 and BRCA2 immunofluorescence. On the left are representative micrographs for the G1-arrested cells and the quantitation of the full experiment is shown on the right (mean $\pm$ s.d., $N=3$).

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[00063] Figure 6. The BRCA1–PALB2 interaction is cell cycle regulated. a, Schematic of the *LacO*/LacR chromatin-targeting system. b, U2OS 256 cells were transfected with the indicated mCherry-LacR and GFP-fusions. GFP fluorescence was measured at the site of the *lacO* array-localized mCherry focus. Each circle represents one cell analyzed and the bar is at the median. Cells were also stained with a cyclin A antibody to determine cell cycle position ($N=3$). IR, Ionizing radiation. c, Representative micrographs of U2OS 256 cells transfected with the indicated mCherry-LacR and GFP-fusions; data is quantified in d. d, Quantification of U2OS 256 cells transfected with the indicated mCherry-LacR and GFP-fusions to tether either BRCA1 or PALB2 to the *lacO* array ($N=3$). e, Schematic representation of PALB2 architecture and its major interacting proteins. f, Quantification of U2OS 256 cells transfected with the indicated GFP-PALB2 mutants and mCherry-LacR-BRCA1-CC. Cells were also stained with a cyclin A antibody to determine cell cycle position ($N=3$).

[00064] Figure 7. Inhibition of the BRCA1–PALB2 interaction in G1 depends on CRL3–KEAP1. a, Representative micrographs of the experiment shown in Figure 1d. b, Schematic representation of human *KEAP1* gene organization and targeting sites of sgRNAs used as described in Figure 5a. The indels introduced by CRISPR/Cas9 and their respective frequencies are indicated. c, Immunoprecipitation (IP) of PALB2 from extracts prepared from irradiated 293T cells. IP with normal IgG was performed as a control. d, 293T cells with the indicated genotypes were transfected with the indicated HA–KEAP1 constructs, synchronized in G1 or S phases and irradiated. Cells were processed for PALB2 immunoprecipitation (IP). EV, empty vector; WT, wild type. e, Quantification of U2OS 256 cells transfected with the indicated GFP-PALB2 mutants and mCherry-LacR-BRCA1. Cells were also stained with a cyclin A antibody to determine cell cycle position ($N=3$). f, Quantification of U2OS 256 cells transfected with GFP-PALB2 and mCherry-LacR-BRCA1-CC (wild type or K1406R mutant). Cells were also stained with a cyclin A antibody to determine cell cycle position. This panel shows that the sole lysine in the PALB2-interaction motif of BRCA1 is not involved in the cell cycle regulation of the PALB2-BRCA1 interaction. e, f, Each circle represents a cell analyzed and the bar is at the median ($N = 3$).

[00065] Figure 8. PALB2 is ubiquitinated by CRL3–KEAP1. a, HEK293 Flp-In T-REX cells expressing doxycycline (DOX)-inducible His₆-Ub were transfected with the indicated siRNAs. Cells were processed for Ni-NTA pull-down (IP). b, 293T cells transfected with an siRNA targeting USP11 and a Flag-PALB2 expression vector were

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processed for Flag immunoprecipitation followed by mass spectrometry (MS). Representative MS/MS spectra of tryptic diglycine (diG)-PALB2 peptides identified are shown (K16, top; K43, bottom), c, Schematic of the *lacO*/LacR chromatin-targeting system and the *in vivo* quantification of ubiquitylated PALB2. d, Representative
 5 micrographs of U2OS 256 cells transfected with the indicated mCherry-LacR-PALB2 vectors. Cells were processed for FK2 immunofluorescence. EV, empty vector. Scale bar, 5 μ m. e, Quantification of U2OS 256 cells transfected with the indicated mCherry-LacR-PALB2 vectors. Cells were processed for quantification of FK2 fluorescence at the LacO focus. Each circle
 10 represents a cell analyzed and the bar is at the median ($N = 3$). Cells were also stained with a cyclin A antibody to determine cell cycle position. Statistical significance was determined by a Kruskal–Wallis test ($***P < 0.001$; $**P < 0.01$).

[00066] Figure 9. Analysis of KEAP1- and USP11-dependent modulation of PALB2 and homologous recombination. a, Site-specific chemical ubiquitylation of
 15 HA-PALB2 (1–103) at residue 20 (PALB2-KC20-Ub) and 45 (PALB2-KC45-Ub) was carried out by dichloroacetone linking. The resulting ubiquitylated PALB2 polypeptides along with their unmodified counterparts were subjected to pulldown with a fusion of MBP with the coiled-coil domain of BRCA1 (MBP-BRCA1-CC). I, input; PD, pulldown. Asterisk indicates a non-specific band. b, Wild-type and KEAP1 Δ 293T cells
 20 were treated with cycloheximide (CHX) for the indicated time and then processed for NRF2 and KEAP1 immunoblotting. Actin levels were also determined as a loading control. c, Immunoprecipitation (IP) of USP11 from extracts prepared from 293T cells that were or were not treated with camptothecin (CPT; 200 nM). Immunoprecipitation with normal IgG was performed as a control. d, U2OS DR-GFP cells were transfected
 25 with the indicated siRNAs. Twenty-four hours post-transfection, cells were further transfected with the indicated siRNA-resistant USP11 expression vectors (WT, wild type; CS, C318S and CA, C318A catalytically dead mutants) or an empty vector (EV), with or without an I-SceI expression vector. The percentage of GFP-positive cells was determined 48h post-plasmid transfection for each condition and was normalized to the
 30 I-SceI plus non-targeting (siCTRL) condition (mean $\pm$ s.d., $N = 3$). e, Schematic representation of human *USP11* (top) and *KEAP1* (bottom) gene organization and targeting sites of sgRNAs (as described in Figure 5a) used to generate the *USP11* Δ and *USP11* Δ /*KEAP1* Δ 293T cells. The indels introduced by the CRISPR–Cas9 and their respective frequencies are indicated. The USP11 knockout was created first and

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subsequently used to make the *USP11Δ* /*KEAP1Δ* double mutant. f, Immunoprecipitation of PALB2 from extracts prepared from 293T cells transfected with the indicated siRNA and with or without CPT (200 nM) treatment. Immunoprecipitation with normal IgG was performed as a control.

5 [00067] Figure 10. USP11 antagonizes KEAP1 action on PALB2. a, U2OS DR-GFP cells were transfected with the indicated siRNAs or left untransfected (–). Twenty-four hours post-transfection, cells were transfected with an I-SceI expression vector (circle). The percentage of GFP-positive cells was determined 48 h post-plasmid transfection for each condition and was normalized to the I-SceI plus non-targeting
10 (CTRL) condition (mean $\pm$ range, $N = 3$). b, Parental 293T cells (wild type (WT)) or a *USP11Δ* derivative were transfected with the indicated GFP-PALB2 constructs, treated with CPT and processed for GFP immunoprecipitation (IP). c, Parental 293T cells (wild type) or a *USP11Δ* derivative were transfected with an empty vector (EV) or the indicated PALB2 expression vectors. Sensitivity of the cells to the PARP inhibitor
15 olaparib was then determined by a clonogenic survival assay (mean $\pm$ s.d., $N=3$).

[00068] Figure 11. Characterization of USP11 protein stability a, U2OS cells synchronized in G1 or S/G2 were treated with cyclohexamide (CHX) and processed at the indicated time points to monitor USP11 stability. b, Immunoprecipitation (IP) of PALB2 from extracts prepared from 293T cells that were synchronized in G1 or S
20 phase and treated or not with IR (20 Gy). c, U2OS cells were irradiated with a dose of 2 or 20 Gy and processed for USP11 immunoblotting at the indicated times post-IR. Actin was used as a loading control. d, U2OS cells, mock-treated or incubated with the ATM inhibitor KU55933 (ATMi), ATR inhibitor VE-821 (ATRi) or DNA-PKcs inhibitor NU7441 (DNAPKi), were irradiated (20 Gy) and processed for USP11 and
25 actin (loading control) immunoblotting. e, Similar experiment to d except that cells were exposed to ultraviolet (UV) radiation (50 mJ/cm²). f, U2OS cells, mock-treated or incubated with the proteasome inhibitor MG132, were irradiated (20 Gy) and processed for USP11 and actin (loading control) immunoblotting. g, U2OS cells, mock-treated or incubated with the Cullin inhibitor MLN4924, were irradiated (20 Gy) and processed
30 for USP11 and actin (loading control) immunoblotting.

[00069] Figure 12. Reactivation of RAD51 loading and unscheduled DNA synthesis in G1. a, *53BP1Δ* U2OS cells were transfected with the indicated siRNA, synchronized in G1 or S/G2 by release from a double-thymidine block and irradiated (20 Gy) before being processed for fluorescence microscopy. DAPI was used to trace

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the nuclear boundary and cyclin A staining was used to determine cell cycle position. The percentage of cells with more than five γ -H2AX-colocalizing PALB2 foci is indicated as the mean $\pm$ s.d., $N = 3$. Scale bar, 5 μ m. b, Representative micrographs of irradiated G1-synchronized wild-type (WT) and *53BP1* Δ U2OS cells transfected with the indicated siRNA and expressing wild-type CtIP. c, Representative micrographs of irradiated G1-synchronized wild-type U2OS cells transfected with the indicated siRNA and expressing CtIP(T847E). d, U2OS *53BP1* Δ cells were synchronized in G1, supplemented with BrdU, irradiated (2 Gy) and processed for γ -H2AX and BrdU immunofluorescence. The percentage of cells with more than five γ -H2AX-colocalizing BrdU foci is indicated (mean $\pm$ s.d., $N = 3$). e, Micrograph of a U2OS cell targeted with the CRISPR-mClover system showing the typical perinuclear expression pattern of lamin A. f, Micrograph of a U2OS cell targeted with the mClover system showing an expression pattern characteristic of subnuclear PML foci. g, Timeline of the gene-targeting (*LMNA*) experiment presented in Figure 4d. h, Timeline of the gene targeting (*LMNA* or *PML*) experiment presented in Figure 4e and Figure 13.

[00070] Figure 13. Analysis of homologous recombination in G1. a, Quantitation of gene targeting efficiency at the *LMNA* locus in asynchronously dividing U2OS cells transfected with increasing amount of donor template and with (grey) or without (white) sgRNAs. Gene-targeting events were detected by flow cytometry (mean $\pm$ s.d., $N \geq 3$). b, Quantitation of gene-targeting efficiency at the *LMNA* locus in asynchronously dividing cells transfected with the indicated siRNA. Gene-targeting events were detected by flow cytometry (mean $\pm$ s.d., $N = 3$). c, Gene-targeting efficiency at the *PML* locus measured by flow cytometry in G1-arrested *53BP1* Δ U2OS cells expressing the CtIP(T847E) mutant and co-transfected with the indicated siRNA or a PALB2-KR expression construct (mean $\pm$ s.d., $N = 3$). d, Gene-targeting efficiency at the *LMNA* locus measured by flow cytometry in G1-arrested parental (wild-type (WT)) and *53BP1* Δ U2OS cells transfected with KEAP1 siRNA and expressing the CtIP(T847E) mutant (mean $\pm$ s.d., $N = 3$). e, Gene-targeting efficiency at the *LMNA* locus measured by flow cytometry in G1-arrested parental (wild-type) and *53BP1* Δ U2OS cells transfected with the indicated siRNA and expressing either wild type or the CtIP(T847E) mutant (mean $\pm$ s.d., $N = 3$).

[00071] Figure 14 Identification of DCAF10 as a regulator of USP11 stability in response to DNA damage. a. siRNA screen where U2OS cells were transfected with siRNAs targeting known and predicted DCAFs along with other CUL4-interacting

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proteins. Cells were either irradiated with IR (20 Gy) or UV (50 J/m²), let to recover for 3 h and then processed for USP11 immunofluorescence. Each point plotted corresponds to the percentage of USP11 left after irradiation. The red dots correspond to the siRNA non-targeting controls (CTRL) and targeting USP11, whereas the red dots correspond to core CRL4 factors, that include CUL4 itself. b. U2OS cells were transfected with the indicated siRNAs and then irradiated with a dose of 20 Gy and processed for USP11 immunoblotting at the indicated times post-ionizing radiation. Actin was used as a loading control.

[00072] Figure 15. Validation of DCAF10 as a regulator of USP11. a. DCAF10 interacts with USP11. Immunoprecipitation (IP) of Flag-USP11 from extracts prepared from 293 Flp-IN/T-Rex cells. Cells were probed with DCAF10 and DCAF15 antibodies. b. Whole cell extracts of mouse embryo fibroblasts (MEFs) of the indicated genotypes were processed for USP11 immunoblotting. Tubulin was used as a loading control. c. U2OS DR-GFP cells were transfected with the indicated siRNAs or expression vectors. Twenty-four hours post-transfection, cells were transfected with an I-SceI expression vector. The percentage of GFP-positive cells was determined 48 h post-plasmid transfection for each condition and was normalized to the I-SceI plus non-targeting (CTRL) + empty vector (EV) condition.

[00073] Figure 16. KEAP1 inhibition can activate HR in G1 cells. Gene targeting at the LMNA locus in G1-arrested cells transfected with the indicated siRNA and vectors expressing either the R1 KEAP1 inhibitor or its FN3 scaffold control (mean $\pm$ s.d., N = 3).

DETAILED DESCRIPTION OF THE INVENTION

[00074] The preparation and use of the agents disclosed as well as the practice of the methods herein employed, unless otherwise indicated, utilize conventional techniques in molecular biology, biochemistry, chromatin structure and analysis, computational chemistry, cell culture, recombinant DNA and related fields as are within the skill of the art. The techniques are fully disclosed in the literature. [See, for example, Sambrook et al. Molecular Cloning: A Laboratory Manual, Second edition, Cold Spring Harbor Laboratory Press, 1989 and Third edition, 2001; Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1987 and periodic updates; the series Methods in Enzymology, Academic Press, San Diego; Wolffe, Chromatin Structure and Function, Third edition, Academic Press, San Diego, 1998; Methods in Enzymology, Vol. 304, "Chromatin" (P.M. Wassarman and A. P.

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Wolffe, eds.), Academic Press, San Diego, 1999; and Methods in Molecular Biology, Vol. 119, "Chromatin Protocols" (P. B. Becker, ed.) Humana Press, Totowa, 1999].

[00075] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The following definitions supplement those in the art and are directed to the present application and are not to be imputed to any related or unrelated case. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the invention, particular materials and methods are described herein.

10 [00076] As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. As used herein, the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[00077] A "gene editing system" is a system for targeting and editing genomes, including without limitation, a TALEN (Transcription Activator-Like Effector Nucleases) system, a CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) system and a Zinc-Finger Nucleases (ZFN) system. (See Nemudryi A.A. et al, Acta Naturae. 2014 Jul-Sep; 6(3): 19–40 for a review of TALEN and CRISPR systems; Gaj T. et al, Trends Biotechnol. 2013 Jul; 31(7): 397–405 for a review of TALEN, CRISPR and ZFN systems; US Published Patent Application No. 20110145940 describing a TALEN system; and Bibikova M., et al, Genetics. 2002;161(3):1169–1175; Townsend J.A., et al, Nature 2009;459(7245):442–445; Zhang F., et al, Proc. Natl. Acad. Sci. USA. 2010;107(26):12028–12033; Torikai H., et al; Blood. 2012;119(24):5697–5705; Provasi E., et al, J. Nat. Med. 2012;18(5):807–815, and Lombardo A., et al, Nat. Methods. 2011;8(10):861–869 describing ZFN systems).

[00078] A "CRISPR system" generally refers to transcripts and other elements involved in the expression of, or directing the activity of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated ("Cas") genes. A CRISPR system may include without limitation, sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence, a guide sequence, or other sequences and transcripts from a CRISPR

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locus. One or more elements of a CRISPR system may be derived from a type I, type II, or type III CRISPR system. A CRISPR system promotes the formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with one or more Cas proteins) at the site of a target sequence. A "target sequence" or

5 "target polynucleotide" refers to a sequence which is sufficiently complementary to a designed guide sequence that the target sequence hybridizes to the guide sequence promoting the formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides, and it may be located in the nucleus, cytoplasm, or an organelle, for example, mitochondria or chloroplast. In the

10 context of an endogenous CRISPR system, formation of a CRISPR complex in an endogenous CRISPR system results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence.

[00079] CRISPR systems are described in U.S. Pat. Nos. 8,697,359, 8,771,945, 8,795,965, 8,865,406, 8,871,445, 8,889,356, 8,889,418 and 8,895,308; US Patent

15 Publications US 2014-0310830, US 2014-0287938, US 2014-0273234, US2014-0273232, US 2014-0273231, US 2014-0256046, US 2014-0248702), US 2014-0242700, US 2014-0242699, US 2014-0242664, US 2014-0234972, US 2014-0227787, US 2014-0189896, US 2014-0186958, US 2014-0186919, US 2014-0186843, US 2014-0179770 and US 2014-0179006, US 2014-0170753, and US 20150232883;

20 European Patent Applications EP 2771468 (EP13818570.7), EP 2764103 (EP13824232.6), and EP 2784162 (EP14170383.5); and PCT Patent Publications WO2014/093661 (PCT/US2013/074743), WO2014/093694 (PCT/US2013/074790), WO2014/093595 (PCT/US2013/074611), WO2014/093718 (PCT/US2013/074825), WO2014/093709 (PCT/US2013/074812), WO2014/093622 (PCT/US2013/074667),

25 WO2014/093635 (PCT/US2013/074691), WO2014/093655 (PCT/US2013/074736), WO2014/093712 (PCT/US2013/074819), WO2014/093701 (PCT/US2013/074800), WO2014/018423 (PCT/US2013/051418) and WO2014/093622 (PCT/US2013/074667). General information on CRISPR-Cas Systems is also described in the following publications: Cong, L., et al., Science, February 15; 339(6121):819-23 (2013); Jiang

30 W., et al., Nat Biotechnol March; 31(3):233-9 (2013); Wang H., et al, Cell May 9; 153(4):910-8 (2013); Konermann S, et al, Nature. 2013 Aug. 22; 500(7463):472-6. doi: 10.1038/Nature12466. Epub 2013 Aug. 23; Ran, F A., et al, Cell August 28. pii: S0092-8674(13)01015-5. (2013); Hsu, P., et al, Nat Biotechnol doi:10.1038/nbt.2647 (2013); Ran, F A., et al, Nature Protocols November; 8(11):2281-308. (2013); Shalem, O., et

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al., Science December 12. (2013). [Epub ahead of print]; Nishimasu, H., et al, Cell Feb. 27. (2014). 156(5):935-49; Wu X., et al, Nat Biotechnol. (2014) Apr. 20. doi: 10.1038/nbt.2889; Platt et al., Cell 159(2): 440-455 (2014) DOI: 10.1016/j.cell.2014.09.014; Hsu et al. Cell 157, 1262-1278 (Jun. 5, 2014) (2014); Wang et al., Science. 2014 Jan. 3; 343(6166): 80-84. doi: 10.1126/science. 1246981; Doench et al., Nature Biotechnology published online 3 Sep. 2014; doi:10.1038/nbt.3026; Storrs, The Scientist, Article No. 39239, March 1, 2014; and Swiech et al, Nature Biotechnology; published online 19 Oct. 2014; doi:10.1038/nbt.3055). Several programs are available to design guide sequences, for example, MIT's CRISPR Design [http://crispr.mit.edu] and E-CRISP [www.e-crisp.org/E-CRISP] developed by the German Cancer Research Center. CRISPR systems also include the systems developed by or available from Editas Medicine (Cambridge, MA), Caribou Biosciences (Berkeley, CA), CRISPR Therapeutics (Basel, Switzerland), Addgene (Cambridge, MA) and Intellia Therapeutics (Cambridge, MA).

15 **[00080]** "DNA end resection" generally refers to nucleolytic degradation of the 5'-terminated strand of a DNA double-stranded break leading to the formation of 3'-terminated single-stranded DNA. DNA end resection in eukaryotes comprises two phases: a slow initial phase, catalyzed by the Mre11-Rad50-Nbs1 (MRN) complex in mammals, and a second and faster phase catalyzed by the exonuclease Exo1 or the

20 helicase Bloom Syndrome Protein (BLM). DNA end resection is initiated by a cell cycle activation step comprising phosphorylation of the accessory protein CtIP (also known as retinoblastoma binding protein 8). Pathways involved in DNA end resection may be activated by stimulating or activating BRCA1 recruitment to DNA double-strand breaks by inhibiting TP53BP1 (53BP1) or RIF, or blocking recruitment of

25 53BP1 or RIF to DNA double-stranded break sites. In an aspect, DNA end resection may be activated by inhibiting 53BP1 (or RIF) expression and/or activity and expressing a mutated form of CtIP that mimics constitutive phosphorylation, for example CtIP-Thr879Glu. In an aspect, DNA end resection is reconstituted or activated using inhibitors of 53BP1 and a mutated form of CtIP that mimics constitutive

30 phosphorylation, in particular CtIP-Thr879Glu. In an aspect, DNA end resection may be reconstituted or activated using purified human proteins: Bloom helicase (BLM); DNA2 helicase/nuclease; Exonuclease 1 (EXO1); the complex comprising MRE11, RAD50, and NBS1 (MRN); and Replication protein A (RPA.) (See Nimonkar A.V. et al, Genes & Development 25:350-362, 2011; Huertas, P, Nat Struct Mol Biol, 17(10:

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11-16, doi: 10.1038/nsmb.1710, 2010; Jimeno S., et al, Nucl. Acids Res doe: 101093/nar/gkui384, 2015 for descriptions of DNA end resection).

[00081] "Homologous recombination" and "HR" refer to a type of genetic recombination in which DNA strands of similar or identical nucleotide sequences are exchanged. HR can be used by cells to repair DNA double-strand breaks (DSB) by the following general steps. HR is initiated when the DSB is resected by nucleases and helicases, generating 3' single-stranded DNA (ssDNA) overhangs onto which the RAD51 recombinase assembles as a nucleoprotein filament. This structure can invade homologous duplex DNA, which is used as a template for repair DNA synthesis. The resulting intermediates can be metabolized to yield non-crossover products thereby restoring the damaged DNA molecule as it existed before the double-strand break (San Filippo et al., Annu. Rev. Biochem. 2008. 77:229–57). The terms also include recombination using single-stranded donor oligonucleotides (ssODNs), in particular recombination using single-stranded donor oligonucleotides (ssODNs) requiring resection and which may be activated by 53BP1 inhibitors.

[00082] "HR Disease" refers to any disorder, disease, condition, syndrome or combination of manifestations or symptoms recognized or diagnosed as a disorder which may be associated with or characterized by a HR defect. Exemplary diseases include, for example, cancer, cardiovascular diseases including heart failure, hypertension and atherosclerosis, respiratory diseases, renal diseases, gastrointestinal diseases including inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, hepatic, gallbladder and bile duct diseases, including hepatitis and cirrhosis, hematologic diseases, metabolic diseases, endocrine and reproductive diseases, including diabetes, bone and bone mineral metabolism diseases, immune system diseases including autoimmune diseases such as rheumatoid arthritis, lupus erythematosus, and other autoimmune diseases, musculoskeletal and connective tissue diseases, including arthritis, achondroplasia infectious diseases and neurological diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease.

[00083] Methods of the invention may be used to monitor or treat a disease caused by a defect in a gene that mediates homologous recombination, for example, BRCA1, BRCA2, PALB2, PARP-1, USP11, RAD51, and/or DCAF10.

[00084] Embodiments of the invention provide for monitoring or treatment of various cancers including but not limited to carcinomas, melanomas, lymphomas,

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sarcomas, blastomas, leukemias, myelomas, osteosarcomas, neural tumors, and cancer of organs such as the breast, ovary, and prostate.

[00085] In embodiments, the invention provides for monitoring or treatment of cancer with BRCA-1 defects, BRCA-2 defects, dual BRCA-1/BRCA-2 defects, and
5 Fanconi anemia. In embodiments of the invention, the cancer is breast cancer, in particular invasive ductal carcinoma and invasive lobular carcinoma. In embodiments of the invention, the cancer is ovarian cancer, in particular epithelial ovarian tumors, germ cell ovarian tumors, and sex cord stromal tumors.

[00086] Methods of the invention for activating or modulating homologous
10 recombination may be used to genetically modify polynucleotides associated with a genetic disorder. In some embodiments, the genetic disorder is a monogenetic disorder. In some embodiments, the genetic disorder is a multigenetic disorder. In some embodiments, the genetic disorder is associated with one or more SNPs. In particular embodiments of the invention, the genomic modification corrects a point mutation.

15 [00087] Examples of genetic disorders and polynucleotide sequences associated with the genetic disorders may be found on the World Wide Web (see for example, the National Center for Biotechnology Information, National Library of Medicine (Bethesda, MA) or the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md)), listed in published patents and applications (see,
20 for example, US Published Application No. 2015/0247150), and in publications (see for example, Turitz Cox D.B. et al, Nature Medicine 21, 121-131, 2015; and O'Connor T.P. and R.G. Crystal, Nature Reviews/Genetics Volume 7, April 2006, pages 261-276 including Supplementary Information, and publications cited therein).

[00088] In an aspect, the genetic disorder is a genetic disorder of muscle. In an
25 aspect, the genetic disorder is myotonic dystrophy type 1. In an aspect, the genetic disorder is myotonic dystrophy type 2. In an aspect, the genetic disorder is Duchenne muscular dystrophy (DMD). In an aspect, the genetic disorder is Becker muscular dystrophy.

[00089] In an aspect, the genetic disorder is a genetic disorder of the liver, for
30 example, alpha-1 antitrypsin deficiency, Wilson Disease, hereditary hemochromatosis, Type I tyrosinemia, glycogen storage disease Type IV, argininosuccinate lyase deficiency, citrin deficiency, cholesterol ester storage disease and hereditary fructose intolerance.

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[00090] In an aspect, the genetic disorder is alpha-1 antitrypsin deficiency which is an autosomal recessive (codominant) disease due to mutations in the SERPINA1 gene that encodes the serine protease inhibitor AAT.

[00091] In an aspect, the genetic disorder is Wilson disease which depends on
5 mutations in the gene encoding the ATP7B Cu translocase, a protein mainly expressed by the hepatocyte that regulates the levels of copper in the liver.

[00092] In an aspect, the genetic disorder is a genetic disorder of the lungs.

[00093] In an aspect, the genetic disorder is cystic fibrosis, an autosomal recessive disease caused by mutations of the Cystic Fibrosis Transmembrane Regulator
10 (CFTR) protein, a member of the ATP-binding cassette superfamily of transmembrane proteins.

[00094] In other aspects of the invention the genetic disorder may be hemophilia, α 1-antitrypsin deficiency, Canavan disease, Adenosine deaminase deficiency, X-linked severe combined immunodeficiency, familial amyloidotic
15 polyneuropathy, thalassemia, Tay-Sachs disease, late infantile ceroid lipofuscinosis, mucopolysaccharidosis, Niemann–Pick disease, achondroplasia, Huntington disease, spino-cerebellar ataxia, Friedreich ataxia, Amyotrophic Lateral Sclerosis, monogenic hypercholesterolemia and other monogenic disorders.

[00095] In aspects of the invention the genetic disorder is sickle cell anemia and
20 a method of the invention comprises correcting the mutated *HBB* hemoglobin gene by gene conversion with its paralog *HBD*.

[00096] An "effective amount" refers to an amount of a compound or composition, as described herein effective to achieve a particular biological result. Such results include, without limitation, the treatment of a disease or condition disclosed
25 herein as determined by any means suitable in the art.

[00097] "PARP Inhibitor" refers to an inhibitor of the nuclear enzyme poly(adenosine 5'-diphospho-ribose) polymerase ["poly(ADP-ribose) polymerase" or "PARP", which is also referred to as ADPRT (NAD:protein (ADP-ribosyl transferase (polymerising)) and PARS (poly(ADP-ribose) synthetase). PARP inhibitors have been
30 described in Banasik et al., "Specific Inhibitors of Poly(ADP-Ribose) Synthetase and Mono(ADP-Ribosyl)-Transferase", J. Biol. Chem., 267:3, 1569-75 (1992), and in Banasik et al., "Inhibitors and Activators of ADP-Ribosylation Reactions", Molec. Cell. Biochem., 138, 185-97 (1994). PARP inhibitors have been disclosed and described in the following patents and patent applications: WO 00/42040; WO 00/39070; WO

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00/39104; WO 99/11623; WO 99/11628; WO 99/11622; WO 99/59975; WO 99/11644; WO 99/11945; WO 99/11649; and WO 99/59973; US Patent No. 8,894,989, US Patent No. 8,946,221; 8,778,966; 8,669,249; 8,623,884; 8,592,416; 8,546,368; 8,541,417; 8,541,403; 8,420,650; 8,362,030; 8,236,802; 8,217,070; 8,188,103; 8,188,084; 8,183,250; 8,173,682; 8,129,382; 8,088,760; 8,080,557; 8,071,623; 8,058,275; 8,012,976; 8,008,491; 7,999,117; 7,956,064; 7,875,621; 7,820,668; 7,750,008; 7,732,491; 7,728,026; 7,652,014; 7,601,719; 7,462,724; 7,087,637; 7,041,675; 6,977,298; 6,924,284; 6,737,421; 6,635,642; 6,495,541; 6,444,676; 6,395,749; 6,380,211; 6,380,193; 6,346,536; 6,197,785; 5,756,510; and Re. 36,397.

10 **[00098]** In aspects of the invention, the PARP inhibitor is Olaparib (AstraZeneca). In aspects of the invention, the PARP inhibitor is Veliparib (AbbVie Inc, Chicago, IL). In aspects of the invention, the PARP inhibitor is Rucaparib (Clovis Oncology, Inc., Boulder, CO). In aspects of the invention, the PARP inhibitor is INO-1001 (Inotek Pharmaceuticals Corp, Lexington, MA). In aspects of the invention, the PARP inhibitor is MK-4827 (niraparib) (Tesaro, Waltham, MA, also see Montoni et al, Frontiers in Pharmacology, [4], Article 18, pages 1-7). In aspects of the invention, the PARP inhibitor is talazoparib (Medivation, Inc, San Francisco CA).

20 **[00099]** A “sample” is a sample derived from any biological source, such as tissues, extracts, or cell cultures, including cells (e.g. tumor cells), cell lines, cell lysates, and physiological fluids, such as, for example, blood or subpopulations thereof (e.g. white blood cells, erythrocytes), plasma, serum, saliva, ocular lens fluid, cerebrospinal fluid, sweat, urine, fecal matter, tears, bronchial lavage, swabbings, milk, ascites fluid, nipple aspirate, needle aspirate, synovial fluid, peritoneal fluid, lavage fluid, and the like. The sample can be obtained from animals, preferably mammals, most preferably humans. Samples can be from a single individual or pooled prior to analysis. The sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treating samples can involve filtration, distillation, extraction, centrifugation, concentration, inactivation of interfering components, the addition of reagents, and the like.

30 **[000100]** In embodiments of methods of the invention, the sample is a mammalian tissue sample. In another embodiment the sample is a cell lysate. In another embodiment the sample is a cell. In another embodiment the sample is a human physiological fluid. In a particular embodiment, the sample is human serum. In a further embodiment, the sample is white blood cells or erythrocytes.

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[000101] The terms “subject”, “individual” or “patient” refer, interchangeably, to a warm-blooded animal such as a mammal. In particular, the terms refer to a human. A subject, individual or patient may be afflicted with or suspected of having or being pre-disposed to a disease as described herein. The term also includes animals bred for food,
5 as pets, or for study including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals goats, apes (e.g. gorilla or chimpanzee), and rodents such as rats and mice.

[000102] NCBI Accession Numbers for USP11, PALB2, BRCA1, BRCA2, KEAP1, 53BP1, DCAF10, RBX1, CUL3 and CtIP are in Table 1 and the human sequences for same are in the Sequence Listing.

10 **Screening and Monitoring Assays**

[000103] The invention provides a method for monitoring activity or expression of USP11 by assaying the interaction of BRCA1 and PALB2, the interaction of BRCA1, PALB2 and BRCA2, the interaction of USP11 and DCAF10, and/or the interaction of USP11 and PALB2. Routine methods known to persons skilled in the art can be used to
15 assay protein interactions in a sample. For example, BRCA1-PALB2, BRCA1-PALB2-BRCA2, USP11-DCAF10, or USP11-PALB2 complexes may be isolated using affinity techniques such as for example immunologically-based purification (e.g. immunoaffinity chromatography), peptides may be prepared from the isolated complexes using conventional methods (e.g. gel electrophoresis, liquid
20 chromatography, capillary electrophoresis, nano-reversed phase liquid chromatography, high performance liquid chromatography, or reverse phase high performance liquid chromatography), and the peptides or peptide fragments may be subjected to mass spectrometry (e.g., quantitative mass spectrometry such as selected reaction monitoring mass spectrometry (sMRM), high resolution data independent analyses (SWATH), high
25 resolution multiple reaction monitoring (MRM^{HR}) or MS1 based quantitation).

[000104] The invention also provides a method for monitoring activity of USP11 by assaying ubiquitylation of the N-terminus of PALB2. Routine methods known to persons skilled in the art can be used to assay ubiquitination in a sample. For example, ubiquitination or PALB2 may be assayed by measuring changes in PALB2 (e.g.,
30 weight; see US Patent No. 6,413,725), the amount of poly-ubiquitin bound to CRL3-KEAP1 E3 ligase (see for example, EP 1268847), and/or the activity of CRL3-KEAP1 E3 ligase (see for example, US Publication No. 2013/0116152). Mass spectrometry techniques such as selected reaction monitoring mass spectrometry (sMRM), high resolution data independent analyses (SWATH), high resolution multiple reaction

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monitoring (MRM^{HR}) or MS1 based quantitation) can also be used to monitor ubiquitin remnants on peptides from the PALB2 N-terminus following protease digestion. In a more specific example, preparation of isotopically labeled synthetic peptides corresponding to tryptic digests of ubiquitylated PALB2, especially those that correspond to ubiquitylation on Lys14, Lys16, Lys20, Lys25, Lys30, Lys43 or Lys45 can be used as internal standards to quantitate the extent of PALB2 ubiquitylation.

5 [000105] In an aspect, the invention provides a method for assaying ubiquitylation of PALB2 polypeptides in a sample, the method comprising digesting ubiquitinated PALB2 polypeptides in the sample with a protease, thereby generating a plurality of test peptides; determining the presence of at least one isopeptide bond between ubiquitin and a lysine residue of the test peptides by mass spectrometry to determine the numbers of ubiquitination sites and thereby the amount of ubiquitination of PALB2 polypeptides in the sample. In an embodiment, the test peptides are from the PALB2 N-terminus. In an embodiment, the lysine residue corresponds to Lys14, Lys16, Lys20, Lys25, Lys30, Lys43 or Lys45. The method may utilize peptide internal standards corresponding to different peptide subsequences of PALB2 to provide for controls in a quantitative assay. In one aspect, different synthetic peptide internal standards corresponding to PALB2 are generated and differentially labeled.

10 [000106] Proximity ligation assays (PLA) may also be used to assay activity of USP11 by assaying the interaction between BRCA1 and PALB2 and/or PALB2-interacting proteins such as BRCA2, using DNA-based detection. For example, primary antibodies against binding partners of an interaction (e.g., PALB2 and BRCA1) are added to a cell lysate. A second set of antibodies, termed PLA probes or proximity probes, recognize the first set of primary antibodies. The PLA probes contain DNA strands that assemble into an assay-specific DNA molecule when in close proximity. This DNA molecule can then be amplified and detected using, for example, fluorescent probes. [See, for example, Soderberg O. et al., Nat. Methods., 2006 December; 3(12):995-1000; Jarvius M. et al., Mol. Cell. Proteomics, 2007 September; 6(9):1500-9)].

25 [000107] In an aspect, the invention provides a method for assaying BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions in a sample comprising: contacting the sample with primary antibodies to each binding partner in the interaction; contacting the sample with proximity probes comprising a secondary antibody that binds to a corresponding primary antibody, wherein each proximity probe has an oligonucleotide

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conjugated thereto; wherein when the oligonucleotides of the proximity probes are in sufficient proximity to each other, the oligonucleotides of the proximity probes interact to form circular products that are amplified by rolling circle replication producing amplification products; and, measuring the amplification products to thereby assay or
5 measure the interactions.

[000108] Assays that monitor PALB2 (or associated proteins such as BRCA2) *in situ* co-localization with BRCA1 can also provide a method for monitoring USP11 activity (see Example herein). For example, PALB2 localization at sites of DNA damage (marked by BRCA1 or other markers such as γ -H2AX) is dependent on USP11
10 activity. In such assays, cells are fixed, permeabilized and then incubated with antibodies that detect PALB2, BRCA2 or their associated proteins (e.g., BRCA1). Addition of labeled secondary antibodies enable the *in situ* visualization of protein accumulation at DNA damage sites in subnuclear structures termed foci. Addition of a genotoxic insult (such as ionizing radiation or other clastogenic treatments) increases
15 the number of “foci” and can be included to augment the dynamic range of the assay.

[000109] It will be appreciated that proximity ligation assays and *in situ* co-localization assays may be used to assay any of the interactions disclosed herein.

[000110] The methods of the invention may be performed in the presence or absence of a test compound or agent and detection of an increase or decrease in activity
20 or expression of one or more of USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 complex, CRL3-KEAP1-PALB2 complex, and KEAP1-PALB2 complex, as compared to a control in the absence of the test compound or agent indicates that the test compound or agent may be useful as a therapeutic agent, or for
25 modulating homologous recombination.

[000111] In an aspect, the invention provides a method for identifying or evaluating an agent for its ability to sensitize or reverse or delay emergence of resistance to PARP inhibitors by determining the effect of the agent on USP11 activity using a method of the invention. In an aspect, a negative effect on USP11 indicates that
30 the agent is a sensitizer of cells to PARP inhibitors or can reverse or delay emergence of resistance to PARP inhibitors. In an aspect, a positive effect on USP11 indicates that the agent is a poor sensitizer of cells to PARP inhibitors. In an aspect, the ability of an agent to sensitize or reverse or delay emergence of resistance to PARP inhibitors is determined by decreased levels of USP11 activity when compared to such levels

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obtained from a control. In an aspect, the ability of an agent to sensitize or reverse or delay emergence of resistance to PARP inhibitors is determined by increased levels of USP11 activity when compared to such levels obtained from a control.

[000112] The invention also relates to a method of identifying or evaluating an agent for its ability to sensitize cells or reverse or delay emergence of resistance to PARP inhibitors by determining the effect of the agent on KEAP1, CRL3-KEAP1, or KEAP1-PALB2. In an aspect, a negative effect on KEAP1 (loss of KEAP1 or CRL3-KEAP1 activity) indicates that the agent is a poor sensitizer of cells to PARP inhibitors. In an aspect, a positive effect on KEAP1 or CRL3-KEAP1 activity indicates that the agent is a sensitizer of cells to PARP inhibitors or can reverse or delay emergence of resistance to PARP inhibitors. In an aspect, the ability of an agent to sensitize or reverse or delay emergence of resistance to PARP inhibitors is determined by increased levels of KEAP1, CRL3-KEAP1, KEAP1-PALB2 or CRL3-KEAP1 activity or expression when compared to such levels obtained from a control. In an aspect, the ability of an agent to sensitize or reverse or delay emergence of resistance to PARP inhibitors is determined by decreased levels of KEAP1, CRL3-KEAP1, KEAP1-PALB2 or CRL3-KEAP1 activity or expression when compared to such levels obtained from a control.

[000113] Still further the present invention contemplates methods of detecting an anti-cancer agent comprising performing a test assay comprising contacting an immortalized cell with a test compound and measuring USP11 activity or CRL3-KEAP1 using a method of the invention and comparing to a control test assay in the absence of the test compound. In an aspect, USP11 activity is assayed by measuring USP11, PALB2, DCAF10, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 and/or CRL3-KEAP1-PALB2 complex in the cell. In an aspect, detecting a negative effect of the agent on USP11 activity or expression as compared with a control indicates a potential anti-cancer agent or PARP inhibitor sensitizer. In an aspect, detecting a negative effect of the agent on BRCA1-PALB2-BRCA2 complex and/or PALB2-USP11 complex activity or expression as compared with a control indicates a potential anti-cancer agent or PARP inhibitor sensitizer. In an aspect, detecting a positive effect of the agent on KEAP1, CRL3-KEAP1 and/or CRL3-KEAP1-PALB2 complex activity or expression as compared with a control indicates a potential anti-cancer agent or PARP inhibitor sensitizer. In an aspect, decreased levels of USP11 activity when

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compared to such levels obtained from a control are indicative that agent has anti-cancer activity or is a PARP inhibitor sensitizer.

[000114] The invention provides a method for identifying or evaluating an agent for its ability to modulate homologous recombination comprising determining the effect
5 of a test compound or agent on one or more of USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 complex and CRL3-KEAP1-PALB2 complex. In an aspect, the invention provides a method for identifying or evaluating an agent for its ability to modulate homologous recombination in a cell comprising (i)
10 assaying in a sample USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 and/or CRL3-KEAP1-PALB2 complex in the cell in the presence or absence of the agent, and (ii) detecting an increase or decrease in USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 complex and/or
15 CRL3-KEAP1-PALB2 complex in the sample compared to a control as an indication of the ability of the agent to modulate homologous recombination.

[000115] The invention provides a method of screening for a therapeutic agent for treatment of a disease associated with defects in HR (i.e., HR Disease), comprising
20 identifying an agent that disrupts or modulates one or more of USP11, PALB2, PALB2 ubiquitylation, DCAF10, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, KEAP1, USP11-DCAF10 complex, CRL3-KEAP1 or CRL3-KEAP1-PALB2 complex. In an aspect, detecting a positive effect of the agent on USP11, BRCA1-PALB2-BRCA2 complex and/or, PALB2-USP11 complex activity or expression as compared
25 with a control indicates a potential therapeutic agent for treatment of a HR Disease. In an aspect, detecting a negative effect of the agent on, DCFA10, KEAP1, CRL3-KEAP1 or CRL3-KEAP1-PALB2 complex activity or expression as compared with a control indicates a potential therapeutic agent for treatment of a HR Disease.

[000116] Test compounds used in the methods of the invention can be any product
30 in isolated form or in a mixture. The test compound may be defined by structure or function or it may be undefined. Examples of undefined test compounds include without limitation tissue samples, biological fluids, cell supernatants, vegetal preparations; etc. Test compounds may be peptides such as soluble peptides including Ig-tailed fusion peptides, members of random peptide libraries and combinatorial

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chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, carbohydrates, nucleic acids, antisense molecules, phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries), antibodies [e.g. polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, single
5 chain antibodies, fragments, (e.g. Fab, F(ab)₂, and Fab expression library fragments, and epitope-binding fragments thereof)], small organic or inorganic molecules, or libraries of compounds. A test compound may be an endogenous physiological compound or natural or synthetic compounds.

[000117] In embodiments, the methods of the invention for identifying agents, in
10 particular anti-cancer agents, comprise contacting more than one test compound, in parallel. In some embodiments, the methods comprises contacting 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 1000, at least 2, at least 5, at least 10, at least 50, at least 100, or at least 1000 test compounds in parallel. In some embodiments, high throughput screening of compounds and complete combinatorial libraries are assayed. Methods for
15 performing high throughput screens are well known in the art. The methods can also be automated such that a robot can perform the experiments.

[000118] In embodiments, the methods of the present invention for identifying agents, in particular anti-cancer agents, comprises the step of contacting a cell in the presence of a test compound. The cells can then be observed to determine if the test
20 compound(s) effects USP11 activity, DCAF10, PALB2, ubiquitination of PALB2, KEAP1, CRL-KEAP1 activity, the interaction of BRCA1 and PALB2, the interaction of USP11 and PALB2, the interaction of USP11 and DCAF10, and/or the interaction of BRCA1, PALB2 and BRCA2. Positive and negative controls may be performed in which known amounts of test compound and no compound, respectively, are added to
25 the assay. One skilled in the art can select and perform the appropriate controls.

[000119] The activity of a test compound(s) may be unknown, and the methods of the invention may be used to identify compounds exhibiting the selected property (e.g., PARP inhibitor sensitizer). In some embodiments, the activity or type of activity of the test compound(s) is known or expected, and the methods of the invention can be used to
30 further characterize or optimize the activity (e.g., specificity, efficacy, etc).

[000120] A method of the invention may also comprise assaying PARP activity in the presence of the test compound. PARP activity may be assayed by measuring a change of poly(ADP-ribose) polymers (PAR), and measuring NAD levels and/or ATP levels using methods routine to one of ordinary skill in the art. In some embodiments,

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the levels of NAD are depleted in the presence of the test compound. In some embodiments the levels of ATP are depleted in the presence of the test compound. In some embodiments, the levels of NAD are increased in the presence of the test compound. In some embodiments the levels of ATP are increased in the presence of the
5 test compound.

[000121] A method of the invention may comprise the step of determining if a cell has undergone necrosis following administration of a test compound. The physical characteristics of the cell can be analyzed using routine methods known to those skilled in the art to determine if a cell has undergone necrosis. For example, necrosis may be
10 determined by measuring organelle swelling, plasma membrane disintegration, intracellular vacuole formation, and nuclear degradation without condensation.

[000122] The screening methods of the invention may further comprise conducting therapeutic profiling of the identified agents or further analogs thereof, for efficacy and toxicity in animals; optionally formulating a pharmaceutical composition
15 including one or more agents identified as having an acceptable therapeutic profile; and optionally administering the agent to a subject or individual.

[000123] In an aspect, the therapeutic activity of agents and compositions identified using a method of the invention may be evaluated *in vivo* using a suitable animal model. Thus, the screening methods of the invention may further comprise
20 conducting *in vivo* studies comprising administering the agent to a suitable animal model.

[000124] The invention also provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising assaying one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1,
25 BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex and CRL3-KEAP1-PALB2 complex or PALB2 ubiquitylation in a sample from the subject using a method of the invention. Significantly different levels of one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex and CRL3-KEAP1-PALB2 complex or PALB2
30 ubiquitylation compared to a control indicate responsiveness (e.g., sensitivity) to the PARP inhibitor.

[000125] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising detecting USP11, BRCA1, BRCA2, PALB2 and KEAP1 in a sample from the subject using a method of

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the invention. In an embodiment, significantly different levels of USP11, BRCA1, BRCA2, PALB2 and KEAP1 compared to a control indicate responsiveness (e.g., sensitivity) to the PARP inhibitor.

[000126] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising detecting
5 USP11, DCAF10, BRCA1, BRCA2, PALB, KEAP1 and CRL3 in a sample from the subject using a method of the invention. In an embodiment, significantly different levels of USP11, DCAF10, BRCA1, BRCA2, PALB, KEAP1 and CRL3 compared to a control indicate responsiveness (e.g., sensitivity) to the PARP inhibitor.

10 [000127] In an aspect, a subject is categorized as responsive to a PARP inhibitor if there is a decrease in one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control. In an aspect, a subject is categorized as responsive to a PARP inhibitor if there is an increase in one or more of
15 USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control. In an embodiment, significantly different levels (e.g., lower levels) of USP11 activity compared to a control indicate sensitivity to the PARP inhibitor.

20 [000128] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising detecting one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3 and CRL3-KEAP1 activity or expression or PALB2 ubiquitylation in a sample from the subject and comparing to a control to determine if the subject will be responsive (e.g.,
25 sensitive) to the PARP inhibitor.

[000129] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising detecting USP11, BRCA1, BRCA2, PALB2 and KEAP1 activity or expression in a sample from the subject and comparing to a control to determine if the subject will be responsive (e.g.,
30 sensitive) to the PARP inhibitor.

[000130] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in a subject comprising detecting USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1 and CRL3 activity in a sample from the

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subject and comparing to a control to determine if the subject will be responsive (e.g., sensitive) to the PARP inhibitor.

[000131] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in an individual comprising assaying
5 USP11 activity or expression in a sample from the individual using a method of the invention. In an embodiment, significantly different levels (e.g., lower levels) of USP11 activity or expression compared to a control indicate sensitivity to the PARP inhibitor.

[000132] In an aspect, the invention provides a method of predicting a response or categorizing a response to a PARP inhibitor in an individual comprising detecting
10 KEAP1 in a sample from the individual and comparing to a control to determine if the individual will be sensitive to a PARP inhibitor. In an embodiment, significantly different levels (e.g., higher levels) of KEAP1 compared to a control indicate sensitivity to the PARP inhibitor.

[000133] The invention also provides a method of predicting a response or
15 categorizing a response to a PARP inhibitor in an individual comprising detecting CRL3-KEAP1 activity in a sample from the individual and comparing to a control to determine if the individual will be sensitive to a PARP inhibitor. In an embodiment, significantly different levels (e.g., higher levels) of CRL3-KEAP1 compared to a control indicate sensitivity to the PARP inhibitor.

20 [000134] The invention also provides a method of predicting a response or categorizing a response to a PARP inhibitor in an individual comprising detecting PALB2 ubiquitylation in a sample from the individual and comparing to a control to determine if the individual will be sensitive to a PARP inhibitor. In an embodiment, significantly different levels of PALB2 ubiquitylation compared to a control indicate
25 sensitivity to the PARP inhibitor.

[000135] The invention also provides a method of predicting a response or categorizing a response to a PARP inhibitor in an individual comprising detecting complexes of BRCA1, PALB2 and BRCA2 in a sample from the subject and comparing to a control to determine if the individual will be responsive (e.g., sensitive)
30 to a PARP inhibitor. In an embodiment, significantly different levels (e.g., absence or low levels) of complexes of BRCA1, PALB2 and BRCA2 indicate sensitivity to the PARP inhibitor.

[000136] The invention further provides a method for assigning an individual to one of a plurality of categories in a clinical trial for a PARP inhibitor comprising

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assaying USP11, DCAF10, PALB2, PALB2 ubiquitylation, BRCA1-PALB2-BRCA2 complex, PALB2-USP11 complex, USP11-DCAF10 complex, KEAP1, CRL3-KEAP1 or CRL3-KEAP1-PALB2 complex in a sample from the individual using a method of the invention.

5 [000137] The invention further provides a method for assigning an individual to one of a plurality of categories in a clinical trial for a PARP inhibitor comprising assaying USP11 activity in a sample from the individual using a method of the invention.

[000138] The invention further provides a method for assigning an individual to
10 one of a plurality of categories in a clinical trial for a PARP inhibitor comprising assaying CRL3-KEAP activity in a sample from the individual.

[000139] The invention further provides a method for assigning an individual to one of a plurality of categories in a clinical trial for a PARP inhibitor comprising detecting or quantitating USP11, BRCA1, BRCA2, PALB2 and KEAP1 in a sample
15 from the individual.

[000140] The invention further provides a method for assigning an individual to one of a plurality of categories in a clinical trial for a PARP inhibitor comprising detecting or quantitating USP11, DCAF10, BRCA1, BRCA2, PALB2 and KEAP1 in a sample from the individual.

20 [000141] The invention further provides a method for assigning an individual to one of a plurality of categories in a clinical trial for a PARP inhibitor comprising detecting or quantitating BRCA1-PALB2-BRCA2 complex in a sample from the individual.

[000142] The invention further provides a method for assigning an individual to
25 one of a plurality of categories in a clinical trial for a PARP inhibitor comprising detecting or quantitating PALB2 ubiquitylation in a sample from the individual.

[000143] In an aspect, an individual is assigned to a category for a clinical trial for a PARP inhibitor based on a decrease in one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control.
30 In an aspect, an individual is assigned to a category for a clinical trial for a PARP inhibitor based on an increase in one or more of USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3, CRL3-KEAP1, BRCA1-PALB2 and BRCA1-PALB2-BRCA2 activity or expression or PALB2 ubiquitylation compared to a control.

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[000144] A variety of routine methods known to a person skilled in the art can be employed for detecting or assaying the biomarkers USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1, CRL3 and/or complexes thereof in a sample. Biomarker levels present in a sample may be determined by any suitable assay, which may comprise the use of any of the group comprising or consisting of immunoassays, spectrometry, mass spectrometry, Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry, microscopy, northern blot, isoelectric focussing, SDS-PAGE, PCR, quantitative RT-PCR, gel electrophoresis, DNA microarray, and antibody microarray, or combinations thereof.

10 [000145] The invention also provides a system comprising: an assay for determining the level of USP11 activity, complexes or biomarker levels in a sample obtained from the subject; a processor for processing the results; computer coded instructions for comparing the results with a database; and a user display for providing the results of the comparison. The database may comprise reference values for USP11 activity or biomarker levels.

Treatment Methods

[000146] A method of the invention for predicting or characterizing responsiveness to a PARP inhibitor may further comprise administering the PARP inhibitor to the individual or subject. In an aspect, the invention relates to a method of treating a subject with a PARP inhibitor comprising:

- a) assaying a sample from the subject for responsiveness or sensitivity to one or more PARP inhibitors using a method of the invention;
- b) identifying a PARP inhibitor that the subject effectively responds or is sensitive to; and
- 25 c) administering the PARP inhibitor to the subject.

[000147] In an aspect, the invention provides a method for treating a patient in need of treatment with a PARP inhibitor comprising (a) requesting a test providing the results of an analysis to determine if the patient is sensitive to the PARP inhibitor by detecting USP11, DCAF10, BRCA1, BRCA2, PALB2, KEAP1 and/or CRL3 in a sample from the subject and comparing to a control to determine if the patient is sensitive to the PARP inhibitor; and (b) administering the PARP inhibitor to the patient if the patient is sensitive to the PARP inhibitor. In an aspect of this method of the invention, the patient has breast cancer. In an aspect of this method of the invention, the

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patient has ovarian cancer. In an aspect of the invention, the test detects USP11 expression or activity using a method disclosed herein.

[000148] The invention further provides a method for treating cancer in a subject the method comprising: (i) selecting a subject who is responsive to a PARP inhibitor
5 using a method of the invention, and (ii) administering to said subject the PARP inhibitor in an effective amount to treat the cancer. In an embodiment, the cancer is breast cancer. In an embodiment, the cancer is ovarian cancer.

[000149] Agents identified using the methods of the present invention have numerous therapeutic applications related to, for example, cancer, ischemia reperfusion
10 injury, inflammatory diseases, degenerative diseases, protection from adverse effects of cytotoxic compounds, and potentiation of cytotoxic cancer therapy. Agents identified using the methods of the invention may be used to potentiate radiation and chemotherapy by increasing apoptosis of cancer cells, limiting tumor growth, decreasing metastasis, and prolonging the survival of tumor-bearing subjects. In aspects
15 of the invention, the agents can be used to treat leukemia, colon cancer, glioblastomas, lymphomas, melanomas, carcinomas of the breast, ovarian cancer and cervical carcinomas.

[000150] In other aspects of the invention, the agents can be used to treat, without limitation, retroviral infection, arthritis, gout, inflammatory bowel disease, CNS
20 inflammation, multiple sclerosis, allergic encephalitis, sepsis, septic shock, hemorrhagic shock, pulmonary fibrosis, uveitis, diabetes, Parkinson's disease, myocardial infarction, stroke, other neural trauma, organ transplantation, reperfusion of the eye, reperfusion of the kidney, reperfusion of the gut, reperfusion of skeletal muscle, liver toxicity following acetaminophen overdose, cardiac and kidney toxicities
25 from doxorubicin and platinum based antineoplastic agents, and skin damage secondary to sulfur mustards.

[000151] In some embodiments, the invention provides a method for sensitizing an individual to treatment with PARP inhibitors comprising identifying an agent that sensitizes cells to PARP inhibitors in accordance with a method of the invention and
30 administering the agent to the individual.

[000152] In some embodiments, the invention provides a method for treating an individual being treated with a PARP inhibitor comprising administering to the individual an agent that sensitizes cells to the PARP inhibitor identified using a method of the invention.

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[000153] In some embodiments, the invention provides a method for reversing or delaying emergence of resistance to PARP inhibitors in an individual comprising identifying an agent that reverses or delays emergence of resistance to PARP inhibitors in accordance with a method of the invention and administering the agent to the individual.

[000154] In some embodiments, the present invention provides methods of treating cancer in an individual comprising identifying an anti-cancer agent in accordance with a method of the invention and administering the agent to the individual.

10 [000155] In another embodiment, the present invention provides a method of treating leukemia, colon cancer, glioblastomas, lymphomas, melanomas, carcinomas of the breast, ovarian cancer or cervical carcinomas in a mammal in need of such treatment comprising administering to the mammal a therapeutically acceptable amount of an agent identified using a method of the invention or a therapeutically acceptable salt thereof.

[000156] In another embodiment, the present invention provides a method of potentiation of cytotoxic cancer therapy in a mammal in need of such treatment comprising administering to the mammal a therapeutically acceptable amount of an agent identified using a method of the invention that potentiates cytotoxic cancer therapy or a therapeutically acceptable salt thereof.

20 [000157] In an aspect, the invention provides methods of treating a disease associated with defects in HR (i.e., HR Disease) in an individual comprising identifying an agent that modulates HR in accordance with a method of the invention and administering the agent to the individual.

25 [000158] In another embodiment, the present invention provides a use of an agent identified using a method of the invention to prepare a medicament for treating a HR Disease in a mammal in need of such treatment. In another embodiment, the present invention provides a use of an agent identified using a method of the invention, to prepare a medicament for inhibiting tumor growth in a mammal in need of such treatment. In another embodiment, the present invention provides a use of an agent identified using a method of the invention to prepare a medicament for treating cancer in a mammal in need of such treatment. In another embodiment, the present invention provides a use of an agent identified using a method of the invention to prepare a medicament for treating leukemia, colon cancer, glioblastomas, lymphomas,

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melanomas, carcinomas of the breast, ovarian cancer or cervical carcinomas in a mammal in need of such treatment. In another embodiment, the present invention provides a use of an agent identified using a method of the invention to prepare a medicament for potentiation of cytotoxic cancer therapy in a mammal in need of such treatment comprising administering to the mammal a therapeutically acceptable amount of the agent.

[000159] In an embodiment, the present invention provides a pharmaceutical composition comprising an agent identified using a method of the invention, or a therapeutically acceptable salt thereof, in combination with a therapeutically acceptable carrier. Pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable carriers are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the active agents in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

[000160] The pharmaceutical compositions are indicated as therapeutic agents either alone or in conjunction with other therapeutic agents or other forms of treatment. The compositions of the invention may be administered concurrently, separately, or sequentially with other therapeutic agents or therapies, for example PARP inhibitors.

Homologous Recombination Methods

[000161] The invention provides a method for activating or modulating homologous recombination in a cell comprising:

- (a) promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell;
- (b) promoting or stimulating BRCA1 recruitment to DNA double-strand break (DSB) sites;
- (c) contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes;
- (d) inhibiting KEAP1 or CRL3-KEAP1;
- (e) inhibiting the degradation of USP11 or promoting USP11 activity; and/or

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(f) inhibiting DCAF10.

5 [000162] In an aspect the cell is in the G1 phase of the cell cycle (G1). In an aspect the cell is a non-dividing cell or a dormant cell in G1. In an aspect, the cell is in the G0 phase of the cell cycle (G0). In an aspect, the methods of the invention are used *in vitro* to activate or modulate homologous recombination in cells.

[000163] In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0), comprising (a) promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2
10 complexes in the cell; and/or (b) contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes.

[000164] In embodiments, the assembly of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes is promoted or stimulated by administering an agent that promotes or stimulates BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes identified using a
15 method of the invention.

[000165] In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting KEAP1 or CRL3-KEAP1. In an aspect, the invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of blocking the degradation of USP11
20 or promoting USP11 activity. In an embodiment, the method comprises administering USP11 or an agonist thereof. In an aspect, the invention provides a method for activating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting KEAP1 and blocking the degradation of USP11. In an aspect, the invention provides a method for activating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting CRL3-KEAP1 and blocking the degradation of USP11. In an aspect, the invention provides a method for activating homologous recombination in a cell, in particular a cell in G1 or G0, comprising the step of inhibiting CRL3 and blocking the degradation of USP11.
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30 [000166] Methods of the invention may be performed in a cell, in particular a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA.

[000167] The invention provides a method for activating or modulating homologous recombination in a cell, in particular a cell in the G1 phase of the cell cycle

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(G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA, comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell. In an embodiment, the assembly of the complexes is promoted or stimulated by
5 administering an agent that promotes or stimulates assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes identified using a method of the invention.

[000168] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle
10 (G0), comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

[000169] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA,
15 comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell. In an embodiment, the assembly of the complexes is promoted or stimulated by administering an agent that promotes or stimulates the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes identified using a method of the invention.

20 [000170] The invention also provides a method for repairing DNA double-strand breaks in a cell in the G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0) in which DNA end resection is or has been activated generating single-stranded DNA, comprising contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes.

25 [000171] The invention also provides a method for repairing DNA double-strand breaks in a cell in G1 or G0 in which DNA end resection is or has been activated generating single-stranded DNA, the method comprising (a) inhibiting KEAP1 and/or CRL3-KEAP1; (b) blocking the degradation of USP11 or promoting or stimulating USP11 activity; (c) administering USP11 or an agonist thereof; and/or (d) inhibiting
30 CRL3-KEAP1 and blocking the degradation of USP11.

[000172] A method for activating homologous recombination in a cell may further comprise activating or promoting single strand DNA (ssDNA) generation pathways. In an aspect, ssDNA generation pathways are activated by DNA end resection. In an

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embodiment, a method for activating homologous recombination in a cell further comprises activating DNA end resection.

[000173] In an embodiment, DNA end resection is activated or promoted by inhibiting 53BP1 (or RIF1) expression or activity (e.g., recruitment of 53BP1 to DSB sites) and/or upregulating or expressing CtIP. In an embodiment, DNA end resection is activated or promoted by inhibiting 53BP1 (or RIF1) expression or activity (e.g., recruitment of 53BP1 to DSB sites) and upregulating or expressing CtIP. In an embodiment, the method involves inhibiting 53BP1 using antagonists, including without limitation short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs) or an inhibitor of the histone deacetylase (HDAC) family of enzymes (for example, trichostatin A), and using an analog of CtIP that mimics constitutive phosphorylation, for example CtIP-Thr879Glu.

[000174] A method for activating homologous recombination in a cell may comprise activating BRCA1 recruitment to DNA double-strand break (DSB) sites. In an embodiment, BRCA1 recruitment is activated by inhibiting expression of 53BP1 (TP53BP1) or RIF1, or inhibiting the recruitment of 53BP1 or RIF1 to DSB sites. 53BP1 or RIF1 may be inhibited using antagonists, including without limitation short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs). In an embodiment, 53BP1 is inhibited with an inhibitor of the histone deacetylase (HDAC) family of enzymes, in particular a histone deacetylase inhibitor (HDACi), preferably trichostatin (Fukuda T. et al, Cancer Sci. 2015 Aug;106(8):1050-6. doi: 10.1111/cas.12717. Epub 2015 Jul 14).

[000175] In an aspect, a method for activating or stimulating homologous recombination in a cell further comprises a gene editing system. In an aspect the gene editing system comprises contacting the cell with a nuclease. Examples of nucleases include without limitation, zinc finger nucleases (ZFNs), engineered meganucleases, transcription activator like effector nucleases (TALENs), mega or homing endonucleases, clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) protein, Ttgo nucleases, and fusions between nucleases, such as mega-TALs and compact TALENs.

[000176] In an aspect, the gene editing steps comprise a TALEN system.

[000177] In an aspect, the gene editing steps comprise a ZFN system.

[000178] In an aspect, the gene editing steps comprise a CRISPR/Cas9 system.

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[000179] In aspects of the invention the gene editing system may correct a genomic modification. A genetic modification may comprise at least one mutation in a polynucleotide sequence having a locus associated with a genetic disorder. In an aspect, the genomic modification is selected from the group consisting of insertions, deletions
5 and combinations thereof. In some embodiments, the genetic disorder is a monogenetic disorder. In some embodiments, the disorder is a multigenetic disorder. In some embodiments, the disorder is associated with one or more SNPs. In particular embodiments of the invention, the genomic modification corrects a point mutation.

[000180] In an aspect of a method of the invention to correct a genomic
10 modification, the gene editing system comprises contacting the cell with a clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of a target polynucleotide sequence associated with a genetic disorder, wherein the target polynucleotide sequence is cleaved.

15 [000181] In an aspect, the invention provides a method for altering a genetic disorder associated with a target polynucleotide sequence in a cell comprising: (1) contacting the cell with a system which activates homologous recombination in the cell wherein the system comprises BRCA1-PALB2 or BRCA1-PALB2-BRCA2 or agents that maintain the BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions throughout
20 the cell cycle; and (2) contacting the target polynucleotide sequence with a clustered regularly interspaced short palindromic repeats-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of the target polynucleotide sequence, wherein the target polynucleotide sequence is cleaved. The method may reduce expression of the target
25 polynucleotide sequence, knock out the target polynucleotide sequence, or correct the target polynucleotide sequence from an undesired sequence to a desired sequence.

[000182] In an aspect, the invention provides a method for altering a genetic disorder associated with a target polynucleotide sequence in a cell comprising: (1) contacting the cell with a system which activates homologous recombination in the cell
30 wherein the system comprises a kit, vector(s) or composition of the invention, in particular the system comprises an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an analog of CtIP that mimics constitutive phosphorylation, preferably the system comprises a KEAP1 inhibitor, an inhibitor of 53BP1 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs), and CtIP-

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Thr879Glu; and (2) contacting the target polynucleotide sequence with a clustered regularly interspaced short palindromic repeats-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of the target polynucleotide sequence, wherein the target polynucleotide sequence is cleaved. The method may reduce expression of the target polynucleotide sequence, knock out the target polynucleotide sequence, or correct the target polynucleotide sequence from an undesired sequence to a desired sequence.

[000183] The invention contemplates a method for treating or preventing a genetic disorder in a subject, the method comprising altering a target polynucleotide sequence associated with the genetic disorder in a cell by contacting the cell with a system which activates homologous recombination in the cell wherein the system comprises BRCA1-PALB2 or BRCA1-PALB2-BRCA2 or agents that maintain the BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions throughout the cell cycle; and contacting the target polynucleotide sequence with a clustered regularly interspaced short palindromic repeats-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of the target polynucleotide sequence, wherein the target polynucleotide sequence is cleaved, thereby treating or preventing the genetic disorder.

[000184] In an aspect, a method is provided for treating or preventing a genetic disorder in a subject, the method comprising (a) altering a target polynucleotide sequence associated with the genetic disorder in a cell by contacting the cell with a system which activates homologous recombination in the cell wherein the system comprises a kit, vector(s) or composition of the invention, in particular the system comprises an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an analog of CtIP that mimics constitutive phosphorylation, preferably the system comprises a KEAP1 inhibitor, an inhibitor of 53BP1 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs), and CtIP-Thr879Glu; and (b) contacting the target polynucleotide sequence with a clustered regularly interspaced short palindromic repeats-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of the target polynucleotide sequence, wherein the target polynucleotide sequence is cleaved, thereby treating or preventing the genetic disorder.

[000185] A method for treating or preventing a genetic disorder may comprise introducing the cell into the subject, thereby treating or preventing the genetic disorder

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associated with the target polynucleotide sequence. The method may comprise repairing the cleaved target polynucleotide sequence by inserting an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target
5 polynucleotide sequence.

[000186] In an aspect, the target polynucleotide sequence is associated with a genetic disorder of the lung. In an embodiment the target polynucleotide sequence is associated with cystic fibrosis, in particular the polynucleotide sequence is the cystic fibrosis transmembrane conductor receptor (CFTR) locus. Mutations in the CFTR
10 (e.g., deletion of phenylalanine at position 508 in exon 11) cause cystic fibrosis.

[000187] In an aspect, the target polynucleotide sequence is associated with a genetic disorder of muscle. In an aspect, the target polynucleotide sequence is associated with muscular dystrophies. In an aspect, the target polynucleotide sequence is associated with Duchenne muscular dystrophy (DMD) (mutations in the dystrophin
15 gene). In an aspect, the target polynucleotide sequence is associated with Becker muscular dystrophy (mutations in the dystrophin gene). In an aspect the target polynucleotide is associated with myotonic dystrophy type 1 (mutations in the *DMPK* gene) or myotonic dystrophy type 2 (mutations in the *CNBP* gene). In an aspect, the target polynucleotide sequence is associated with sickle cell anemia (mutated *HBB*
20 hemoglobin).

[000188] In aspects of the invention, the targeted polynucleotide sequence is associated with a genetic disorder of the liver. In an aspect, the target polynucleotide sequence is associated with alpha-1 antitrypsin deficiency (mutations in the *SERPINA1* gene). In an aspect, the targeted polynucleotide sequence is associated with Wilson
25 disease (mutations in the gene encoding the ATP7B Cu translocase).

[000189] In an aspect, the methods of the invention further comprise providing a functional protein with enhanced characteristics as compared to its naturally occurring counterpart, in particular a functional protein lacking or deficient in a subject, for example for treating genetic disorders. In embodiments of the invention, the methods
30 comprise integrating a sequence encoding a functional protein in a cell in a subject in need thereof by sequential administration of a gene editing system and one or more transgene encoding a non-naturally occurring protein with enhanced properties as compared to its naturally occurring counterpart. In other embodiments, the methods comprise administering to the subject a genetically modified cell expressing a

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functional version of one or more proteins aberrantly expressed in a subject. Thus, an isolated cell may be introduced into the subject (ex vivo cell therapy) or a cell may be modified when it is part of the subject (in vivo). In certain embodiments, transgene(s) are delivered using a viral vector, a non-viral vector and/or combinations thereof.

5 [000190] The invention also provides a method for suppressing homologous recombination in a cell, in particular a cell in G1, comprising suppressing the assembly of BRCA1-PALB2-BRCA2 complexes in the cell. In an embodiment, the interaction is suppressed by administering KEAP1 or CRL3-KEAP1 or an agonist thereof. In an embodiment, the interaction is suppressed by administering a USP11
10 antagonist/inhibitor (e.g., mitoxantrone). In an embodiment, the interaction is suppressed by administering an agent that suppresses homologous recombination identified using a method of the invention.

[000191] Components of the methods of the invention may be delivered by delivery systems known in the art, including without limitation viral based systems or
15 non-viral based systems. Conventional viral based systems may comprise, for example, retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. In an aspect, the expression vector is selected from the group consisting of a plasmid vector, a lentiviral vector, an adenoviral vector, and an adeno-associated virus vector. In an embodiment, the viral based system, is an adenoviral vector or
20 adeno-associated viral vector. Examples, of non-viral based systems include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions and agent-enhanced uptake of DNA.

[000192] In an aspect, the invention provides vectors comprising activators or
25 modulators of homologous recombination, and optionally activators or modulators of DNA end resection. In an aspect, the invention provides a vector (e.g. viral vector) comprising one or more of the following components encoded in the vector: 1) an activator of DNA end resection, for example, an inhibitor of 53BP1 (or RIF) expression or activity and/or a CtIP compound that mimics constitutive phosphorylation; 2) a
30 factor that activates homologous recombination, for example, a factor that maintains BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions during the cell cycle; and, optionally, 3) components of a gene editing system, in particular components of a CRISPR system, a TALEN system or a zinc finger nuclease system. In an embodiment,

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the components of the gene editing system are encoded in one or more separate expression vectors.

[000193] In another aspect, the invention provides a composition comprising activators or modulators of homologous recombination, and optionally activators or modulators of DNA end resection. In an aspect, the invention provides a composition comprising one or more of the following components: 1) an activator of DNA end resection, for example, an inhibitor of 53BP1 (or RIF) expression or activity and/or a CtIP compound that mimics constitutive phosphorylation; 2) a factor that activates homologous recombination, for example, a factor that maintains BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions during the cell cycle; and, optionally, 3) components of a gene editing system, in particular components of a CRISPR system, TALEN system or zinc finger nuclease system. In an embodiment, the components of the gene editing system are in one or more separate compositions.

[000194] Examples of activators of DNA end-resection include without limitation, the coding sequence of CtIP-Thr847Glu, a shRNA against the TP53BP1 mRNA, and a shRNA against KEAP1. The shRNA against TP53BP1 may be substituted with a shRNA against RIF1 or agents that block 53BP1 recruitment to DSB sites including a dominant-negative 53BP1 protein. The shRNA against KEAP1 may be substituted with the coding sequence of a PALB2 mutant that contains mutations of its Lys20, Lys25 and Lys30 residues or that contains a mutation that disrupts its interaction with KEAP1.

[000195] Examples of factors that maintain BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions during the cell cycle include without limitation, inhibitors of KEAP1, inhibitors of DCAF10, RNA interference agents that maintain USP11 expression in G0 and G1 cells or a mutated form of PALB2 that is insensitive to ubiquitylation by KEAP1-CUL3-RBX1 which involves the mutation of one or more of the Lys20, Lys25 or Lys30 residues. An example of a KEAP1 inhibitor is the monobody that is a potent competitive inhibitor of the KEAP1-NRF2 interaction disclosed in Guntas, G. et al, [44]. KEAP1 inhibitors are also described, for example in Canning P. et al, Acta Pharm Sin B., 2015 (4):285-99 and Wells, G., Biochem Soc Trans. 2015,43(4): 674-9.

[000196] In an embodiment, a vector of the invention comprises sequences encoding an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an analog of CtIP that mimics constitutive phosphorylation. In a particular embodiment, a vector of the invention comprises sequences encoding a KEAP1 inhibitor (e.g., R1

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KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 and CtIP-Thr879Glu. In a particular embodiment, a vector of the invention comprises sequences encoding a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs), and CtIP-Thr879Glu.

[000197] In an embodiment, a composition of the invention comprises an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an analog of CtIP that mimics constitutive phosphorylation. In a particular embodiment, a composition of the invention comprises a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 and CtIP-Thr879Glu. In a particular embodiment, a composition of the invention comprises a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs), and CtIP-Thr879Glu.

Kits

[000198] The invention further provides a kit for performing an assay or method disclosed herein or comprising compositions or vectors disclosed herein. In an embodiment, a kit of the invention comprises at least one reagent for determining USP11 activity in a sample. In another embodiment, a kit of the invention comprises at least one reagent for determining BRCA1-PALB2-BRCA2, PALB2-KEAP1, BRCA1-PALB2, USP11-DCAF10, or USP11-PALB2 complexes in a sample. In another embodiment, a kit of the invention comprises at least one reagent for determining BRCA1-PALB2-BRCA2, PALB2-KEAP1 or USP11-PALB2 complexes in a sample. In another embodiment, a kit of the invention comprises reagents for determining the levels of BRCA1, BRCA2, PALB2, USP11, DCAF10 and KEAP1 in a sample. In another embodiment, a kit of the invention comprises at least one reagent for determining ubiquitylation of PALB2, in particular ubiquitylation of the N-terminus of PALB2, in a sample. In some embodiments the reagent is an antibody or a nucleic acid or primers for use in a PCR reaction.

[000199] A kit may also comprise instructions for suitable operational parameters in the form of an insert. The instructions may inform a consumer about how to collect the sample. The kit may comprise samples, to be used as standard(s) for calibration and comparison. The kit may also comprise instructions to compare the level of activity or biomarkers detected in a sample with a calibration sample or chart. The kit may also

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include instructions indicating what level of activity or biomarkers is diagnostic of a disease disclosed herein.

[000200] In an aspect, the invention provides a kit comprising one or more of the components of a method of the invention for activating homologous recombination and optionally components of a gene editing system. A kit of the invention may also include
5 or be used in combination with a CRISPR system, a TALEN system or zinc finger nuclease system. In an embodiment, a kit of the invention includes or is used in combination with a CRISPR system. In an embodiment, a kit of the invention includes or is used in combination with a TALEN system. In an embodiment, a kit of the invention includes or is used in combination with a zinc finger nuclease system.
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[000201] In some embodiments, a kit of the invention comprises vector systems and instructions for using the kit. In an aspect, the kit comprises a vector comprising activators of DNA end resection and activators of homologous recombination discussed herein. In an aspect, the kit comprises one or more vectors (e.g. viral vectors)
15 comprising one or more of the following components: 1) activators of DNA end resection, for example, inhibitors of 53BP1 (or RIF) expression or activity and/or a CtIP compound that mimics constitutive phosphorylation; 2) factors that activate homologous recombination, for example, factors that maintain BRCA1-PALB2 interactions during the cell cycle; and, optionally, 3) components of a gene editing system, in particular components of a CRISPR system, TALEN system or zinc finger nuclease system. Examples of factors that maintain BRCA1-PALB2 interactions during the cell cycle are described herein and include without limitation, inhibitors of KEAP1, for example, RNA interference agents that maintain USP11 expression in G0 and G1
20 cells or a mutated form of PALB2 that is insensitive to ubiquitylation by KEAP1-CUL3-RBX1 which involves the mutation of one or more of the Lys20, Lys25 or Lys30 residues. Examples of activators of DNA end resection include without limitation, the coding sequence of CtIP-Thr847Glu, a shRNA against the TP53BP1 mRNA, and a shRNA against KEAP1. The shRNA against TP53BP1 may be substituted with a shRNA against RIF1 or agents that block 53BP1 recruitment to DSB
25 sites including a dominant-negative 53BP1 protein. The shRNA against KEAP1 may be substituted with a coding sequence of a PALB2 mutant that contains mutations of its Lys20, Lys25 and Lys30 residues or that contains a mutation that disrupts its interaction with KEAP1.
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[000202] In an embodiment, a kit of the invention comprises one or more vectors comprising sequences encoding an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an analog of CtIP that mimics constitutive phosphorylation. In a particular embodiment, a kit of the invention comprises one or more vectors comprising
5 sequences encoding a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 and CtIP-Thr879Glu. In a particular embodiment, a kit of the invention comprises one or more vectors comprising sequences encoding a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs),
10 and CtIP-Thr879Glu.

[000203] In some embodiments, a kit of the invention comprises a composition of the invention and instructions for using the kit. In an aspect the kit comprises a composition comprising activators or modulators of DNA end- resection and activators or modulators of homologous recombination discussed herein. In an aspect, the kit
15 comprises a composition comprising one or more of the following components: 1) an activator of DNA end resection, for example, an inhibitor of 53BP1 (or RIF) expression or activity and/or a CtIP compound that mimics constitutive phosphorylation; 2) a factor that activates homologous recombination, for example, a factor that maintains BRCA1-PALB2 or BRCA1-PALB2-BRCA2 interactions during the cell cycle; and,
20 optionally, 3) components of a gene editing system, in particular components of a CRISPR system, TALEN system or zinc finger nuclease system. In an embodiment, the components of the gene editing system are in separate kit(s).

[000204] In an embodiment, a kit of the invention comprises a composition comprising an inhibitor of 53BP1, a KEAP1 inhibitor or DCAF10 inhibitor, and an
25 analog of CtIP that mimics constitutive phosphorylation. In a particular embodiment, a kit of the invention comprises a composition comprising a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1 and CtIP-Thr879Glu. In a particular embodiment, a kit of the invention comprises a composition comprising a KEAP1 inhibitor (e.g., R1 KEAP1 inhibitor; see Example 3), an inhibitor of 53BP1
30 chosen from short interfering (si) RNA, short hairpin (sh) RNA and microRNAs (miRNAs), and CtIP-Thr879Glu.

[000205] In some aspects, a kit of the invention is used in combination with a gene editing kit, in particular a kit for a CRISPR system, a TALEN system or zinc finger nuclease system. Gene editing kits are commercially available, for example from

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Addgene (Cambridge, MA), ThermoFisher Scientific, System Biosciences Inc., and OriGene Technologies (MD), Clontech.

[000206] The following non-limiting examples are illustrative of the present invention:

5 EXAMPLE 1

[000207] The following materials and methods were used in the study described in the Example.

Plasmids

[000208] The cDNA of PALB2 was obtained from the Mammalian Gene
 10 Collection (MGC). Full length PALB2 and BRCA1 were amplified by PCR, subcloned into pDONR221 and delivered into the pDEST-GFP, pDEST-Flag and the mCherry-LacR vectors using Gateway cloning technology (Invitrogen). Similarly, the coiled-coil domain of BRCA1 (residues 1363-1437) was amplified by PCR, subcloned into the pDONR221 vector and delivered into both mCherryLacR and pDEST-GFP vectors.
 15 The N-terminal domain of PALB2 was amplified by PCR and introduced into the GST expression vector pET30-2-His-GST-TEV [31] using the EcoRI/XhoI sites. The coiled-coil domain of BRCA1 was cloned into pMAL-c2 using the BamHI/SalI sites. Truncated forms of PALB2 were obtained by introducing stop codons or deletions through site-directed mutagenesis. Full-length CtIP was amplified by PCR, subcloned
 20 into the pDONR221 and delivered into the lentiviral construct pCW57.1 (a gift of Dr. David Root; Addgene plasmid #41393) using Gateway cloning technology (Invitrogen). The USP11 cDNA was a gift of David Cortez and was amplified by PCR and cloned into the pDsRed2-C1 vector using the EcoRI/SalI sites. The bacterial codon-optimized coding sequence of pig USP11 (USP11) was subcloned into the 6 x His-GST vector
 25 pETM-30-Htb using the BamHI/EcoRI sites. siRNA-resistant versions of PALB2, BRCA1 and USP11 constructs were generated as previously described [14]. Full-length CUL3 and RBX1 were amplified by PCR from a human pancreas cDNA library (Invitrogen) as previously described [32] and cloned into the dual expression pFBDM vector using NheI/XmaI and BssHII/NotI respectively. The NEDD8 cDNA was a gift
 30 of Dmitris Xirodimas and was fused to a double StrepII tag at its C-terminus in the pET17b vector (Millipore). Human DEN1 was amplified from a vector supplied by Aude Echali er and fused to a non-cleavable N-terminal StrepII2 x tag by PCR and inserted into a pET17b vector. The pCOOL-mKEAP1 plasmid was a gift from Dr. Feng Shao. The pcDNA3-HA2-KEAP1 and pcDNA3-HA2-KEAP1 BTB were gifts from

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Dr. Yue Xiong (Addgene plasmids #21556 and 21593). gRNAs were synthesized and processed as described previously [33]. Annealed gRNAs were cloned into the Cas9-expressing vectors pSpCas9(BB)-2A-Puro (PX459) or pX330-U6-Chimeric_BB-CBh-hSpCas9, a gift from Feng Zhang (Addgene plasmids #48139 and 42230). The gRNAs targeting the *LMNA* or the *PML* locus and the mClover-tagged *LMNA* or *PML* are previously described [45]. The lentiviral packaging vector psPAX2 and the envelope vector VSV-G were a gift from Didier Trono (Addgene plasmids #12260 and 12259). His₆-Ubiquitin was cloned into the pcDNA5-FRT/TO backbone using the XhoI/HindIII sites. All mutations were introduced by site-directed mutagenesis using QuikChange (Stratagene) and all plasmids were sequence-verified.

Cell culture and plasmid transfection

[000209] All culture media were supplemented with 10% fetal bovine serum (FBS). U-2-OS (U2OS) cells were cultured in McCoy's medium (Gibco). 293T cells were cultured in DMEM (Gibco). Parental cells were tested for mycoplasma contamination and authenticated by STR DNA profiling. Plasmid transfections were carried out using Lipofectamine 2000 Transfection Reagent (Invitrogen) following the manufacturer's protocol. Lentiviral infection was carried out as previously described [18]. U2OS and 293T cells were purchased from ATCC. U2OS 256 cells were a gift from R. Greenberg.

Antibodies

[000210] The following antibodies were employed: rabbit anti-53BP1 (A300-273A, Bethyl), rabbit anti-53BP1 (sc-22760, Santa Cruz), mouse anti-53BP1 (#612523, BD Biosciences), mouse anti- γ -H2AX (clone JBW301, Millipore), rabbit anti- γ -H2AX (#2577, Cell Signaling Technologies), rabbit anti-KEAP1 (ab66620, Abcam), rabbit anti-NRF2 (ab62352, Abcam), mouse anti-Flag (clone M2, Sigma), mouse anti-tubulin (CP06, Calbiochem), mouse anti-GFP (#11814460001, Roche), mouse anti-CCNA (MONX10262, Monosan), rabbit anti-BRCA2 (ab9143, Abcam), mouse anti-BRCA2 (OP95, Calbiochem), rabbit anti-BRCA1 (#07-434, Millipore), rabbit anti-USP11 (ab109232, Abcam), rabbit anti-USP11 (A301-613A, Bethyl), rabbit anti-RAD51 (#70-001, Bioacademia), mouse anti-BrdU (RPN202, GE Healthcare), mouse anti-FK2 (BMLPW8810, Enzo), rabbit anti-PALB2 [34], rabbit anti-GST (sc-459, Santa Cruz), rabbit anti-CUL3 (A301-108A, Bethyl), mouse anti-MBP (E8032, NEB), mouse anti-HA (clone 12CA5, a gift of Dr. M. Tyers), rabbit anti-Ubiquitin (Z0458, Dako) and mouse anti-actin (CP01, Calbiochem). The following antibodies were used as

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secondary antibodies in immunofluorescence microscopy: Alexa Fluor 488 donkey anti-rabbit IgG, Alexa Fluor 488 donkey anti-goat IgG, Alexa Fluor 555 donkey anti-mouse IgG, Alexa Fluor 555 donkey anti-rabbit IgG, Alexa Fluor 647 donkey anti-mouse IgG, Alexa Fluor 647 donkey anti-human IgG, Alexa Fluor 647 donkey anti-goat IgG (Molecular Probes).

RNA interference

[000211] All siRNAs employed in this study were single duplex siRNAs purchased from ThermoFisher. RNA interference (RNAi) transfections were performed using Lipofectamine RNAiMax (Invitrogen) in a forward transfection mode. The individual siRNA duplexes used were: BRCA1 (D-003461-05), PALB2 (D-012928-04), USP11 (D-006063-01), CUL1 (M-004086-01), CUL2 (M-007277-00), CUL3 (M-010224-02), CUL4A (M-012610-01), CUL4B (M-017965-01), CUL5 (M-019553-01), KEAP1 (D-12453-02), RAD51 (M-003530-04), CtIP/RBBP8 (M-001376-00), BRCA2 (D-003462-04), 53BP1 (D-003549-01) and non-targeting control siRNA (D-001210-02). Except when stated otherwise, siRNAs were transfected 48 h prior to cell processing.

Inhibitors and fine chemicals

[000212] The following drugs were employed at the indicated concentrations: cycloheximide (CHX; Sigma) at 100 ng/mL⁻¹, camptothecin (CPT; Sigma) at 0.2 μM, ATM inhibitor (KU55933; Selleck Chemicals) at 10 μM, ATR inhibitor (VE-821; gift of Philip Reaper) at 10 μM, DNA-PKcs inhibitor (NU7441; Genetex) at 10 μM, proteasome inhibitor MG132 (Sigma) at 2 μM, Lovastatin (S2061; Selleck Chemicals) at 40 μM, Doxycycline (#8634-1; Clontech), Nedd8-activating enzyme inhibitor (MLN4929; Active Biochem) at 5 μM and olaparib (Selleck) at the indicated concentrations.

Immunofluorescence microscopy

[000213] In most cases, cells were grown on glass coverslips, fixed with 2% (w/v) paraformaldehyde in PBS for 20 min at room temperature, permeabilized with 0.3 % (v/v) Triton X-100 for 20 min at room temperature and blocked with 5% BSA in PBS for 30 min at room temperature. Alternatively, cells were fixed with 100% cold methanol for 10 min at -20°C and subsequently washed with PBS for 5 min at room temperature before PBS-BSA blocking. Cells were then incubated with the primary antibody diluted in PBS-BSA for 2 h at room temperature. Cells were next washed with PBS and then incubated with secondary antibodies diluted in PBS-BSA supplemented

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with 0.8 µg/ml of DAPI to stain DNA for 1 h at room temperature. The coverslips were mounted onto glass slides with Prolong Gold mounting agent (Invitrogen). Confocal images were taken using a Zeiss LSM780 laser-scanning microscope. For G1 vs. S/G2 analysis of the BRCA1-PALB2-BRCA2 axis, cells were first synchronized with a double-thymidine block, released to allow entry into S phase and exposed to 2 or 20 Gy of X-irradiation at 5h and 12h post-release and fixed at 1 to 5 hours post-treatment (where indicated). For the examination of DNA replication, cells were pre-incubated with 30 µM BrdU for 30 min before irradiation and processed as previously described.

CRISPR/Cas9 genome editing of USP11/KEAP1

10 [000214] 293T and U2OS cells were transiently transfected with 3 distinct sgRNAs targeting either 53BP1, USP11 or KEAP1 and expressed from the pX459 vector containing Cas9 followed by the 2A-Puromycin cassette. The next day, cells were selected with puromycin for 2 days and subcloned to form single colonies or subpopulations. Clones were screened by immunoblot and/or immunofluorescence to
15 verify the loss of 53BP1, USP11 or KEAP1 expression and subsequently characterized by PCR and sequencing. The genomic region targeted by the CRISPR/Cas9 was amplified by PCR using Turbo Pfu polymerase (Agilent) and the PCR product was cloned into the pCR2.1 TOPO vector (Invitrogen) before sequencing.

Olaparib clonogenic assay

20 [000215] 293T cells were incubated with the indicated doses of olaparib (Selleck Chemicals) for 24 h, washed once with PBS and counted by trypan blue staining. Five-hundred cells were then plated in duplicate for each condition. The cell survival assay was performed as previously described [35].

Recombinant protein production

25 [000216] GST and MBP fusions proteins were produced as previously described [36, 37]. Briefly, MBP proteins expressed in *Escherichia coli* were purified on amylose resin (New England Biolabs) according to the batch method described by the manufacturer and stored in 1X PBS, 5% glycerol. GST proteins expressed in *E. coli* were purified on glutathione sepharose 4B (GE Healthcare) resin in 50 mM Tris HCl
30 pH 7.5, 300 mM NaCl, 2 mM dithiothreitol (DTT), 1 mM EDTA, 15 µg/mL⁻¹ AEBSF and 1x Complete protease inhibitor cocktail (Roche). Upon elution from the resin using 50 mM glutathione in 50 mM Tris HCl pH 8, 2 mM DTT, the His₆-GST tag was cleaved off using His-tagged TEV protease (provided by F. Sicheri) in 50 mM Tris HCl pH 7.5, 150 mM NaCl, 10 mM glutathione, 10% glycerol, 2 mM sodium citrate and 2

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mM β -mercaptoethanol. His₆-tagged proteins were depleted using Ni-NTA-agarose beads (Qiagen) in 50 mM Tris HCl pH 7.5, 300 mM NaCl, 20 mM imidazole, 5 mM glutathione, 10% glycerol, 1mM sodium citrate and 2 mM β -mercaptoethanol followed by centrifugal concentration (Amicon centrifugal filters, Millipore). GST-mKEAP1 was

5 purified as described previously [38], with an additional anion exchange step on a HiTrap Q HP column (GE Healthcare). The GST tag was left on the protein for in vitro experiments. Purification of CUL3 and RBX1 was performed as previously described [32]. Nedd8 and Den1 were expressed in *E.coli* BL21 grown in Terrific broth media and induced overnight with 0.5 mM isopropyl- β -D-thiogalactoside (IPTG) at 16°C.

10 Cells were harvested and resuspended in wash buffer (400 mM NaCl, 50 mM Tris-HCl, pH 8, 5% glycerol, 2 mM DTT), supplemented with lysozyme, universal nuclease (Pierce), benzamidine, leupeptin, pepstatin, PMSF and Complete protease inhibitor cocktail (Roche), except for DEN1-expressing cells where the protease inhibitors were omitted. Cells were lysed by sonication and the lysate was cleared by centrifugation at

15 20,000 rpm for 50 min. The soluble supernatant was bound to a 5 ml Strep30 Tactin Superflow Cartridge with a flow rate of 3 ml/min⁻¹ using a peristaltic pump. The column was washed with 20 column volumes (CV) of washing buffer and eluted with 5 CV washing buffer, diluted 1:2 in water to reduce the final salt concentration, and supplemented with 2.5 mM desthiobiotin. The elution fractions were pooled and

20 concentrated to a total volume of 4 ml using a 3 kDa cut-off Amicon concentrator. DEN1 was further purified over a Superdex 75 size exclusion column, buffer exchanged into 150 mM NaCl, HEPES, pH 7.6, 2% glycerol and 1 mM DTT. The C-terminal pro-peptide and StrepII2x-tag were removed by incubation with StrepII2x-DEN1 in 1:20 molar ratio for 1 hour at room temperature. The DEN1 cleavage reaction

25 was buffer exchanged on a Zeba MWCO desalting column (Pierce), to remove the desthiobiotin, and passed through a Strep-Tactin Cartridge, which retains the C-terminal pro-peptide and DEN1. The GST-tagged *Sus scrofa* (pig) USP11 proteins were expressed in *E. coli* as described [39]. Cells were lysed by lysozyme treatment and sonication in 50mM Tris pH 7.5, 300mM NaCl, 1mM EDTA, 1mM AEBSF, 1 x

30 Protease Inhibitor mix (284 ng/ml leupeptin, 1.37 μ g/ml⁻¹ pepstatin A, 170 μ g/ml⁻¹ PMSF and 330 μ g/ml⁻¹ benzamidine) and 5% glycerol. Cleared lysate was applied to a column packed with glutathione sepharose 4B (GE Healthcare), washed extensively with lysis buffer before elution in 50mM Tris pH 7.5, 150 mM NaCl, 5% glycerol and 25 mM reduced glutathione. DUB activity was assayed on fluorogenic Ubiquitin-AMC

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(Enzo life sciences), measured using a Synergy Neo microplate reader (Biotek). His₆-TEV-Ubiquitin-G76C was purified on chelating HiTrap resin, following the manufacturers' instructions, followed by size exclusion chromatography on a S-75 column (GE healthcare). The protein was extensively dialysed in 1mM acetic acid and lyophilised.

In vitro ubiquitylation and deubiquitylation of PALB2

[000217] HA-tagged N-terminal fragments of PALB2 (1-103) (1 μ M) were in vitro ubiquitylated using 50 μ M wild-type (Ubi WT, Boston Biochem) or a lysine-less ubiquitin (Ubi K0, Boston Biochem), 100 nM human UBA1 (E1), 500 nM CDC34 (provided by F. Sicheri and D. Ceccarelli), 250 nM neddylated CUL3/RBX1, 375 nM GST-mKEAP1 and 1.5 mM ATP in a buffer containing 50 mM Tris HCl pH 7.5, 20 mM NaCl, 10 mM MgCl₂ and 0.5 mM DTT. Ubiquitylation reactions were carried out at 37°C for 1 hour, unless stated otherwise. For USP11-mediated deubiquitylation assays, HA-PALB2 (1-103) was first ubiquitylated using lysine-less ubiquitin with enzyme concentrations as described above in 50 μ L reactions in a buffer containing 25mM HEPES pH 8, 150 mM NaCl, 10 mM MgCl₂, 0.5 mM DTT and 1.5 mM ATP for 1.5 h at 37°C. Reactions were stopped by the addition of 1 unit Apyrase (New England Biolabs). Reaction products were mixed at a 1:1 ratio with wild-type or catalytically inactive (C270S) USP11, or USP2 (provided by Dr. F. Sicheri and E. Zeqiraj) using final concentrations of 100 nM, 500 nM and 2500 nM (USP11) and 500 nM (USP2) and incubated for 2 h at 30°C in a buffer containing 25 mM HEPES pH 8, 150 mM NaCl, 2 mM DTT, 0.1 mg/mL BSA, 0.03% Brij-35, 5 mM MgCl₂, 0.375 mM ATP.

Pulldown experiments between purified PALB2 and BRCA1

[000218] PALB2 in vitro ubiquitylation reaction products were diluted in a buffer at final concentration of 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 0.25 mM DTT and 0.1% NP-40. 20 μ g MBP or MBP-BRCA1-CC was coupled to amylose resin (New England Biolabs) in the above buffer supplemented with 0.1% BSA prior to addition of the ubiquitylation products. Pulldown reactions were performed at 4°C for 2 h, followed by extensive washing.

Co-immunoprecipitation

[000219] Cells were collected by trypsinization, washed once with PBS and lysed in 500 μ L of lysis buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% glycerol, 2 mM EDTA, 1% NP-40, Complete protease inhibitor cocktail (Roche), cocktail of

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phosphatase inhibitors (Sigma) and N-ethylmaleimide to inhibit deubiquitination) on ice. Lysates were centrifuged at 15 000 g for 10 min at 4°C and protein concentration was evaluated using absorbance at 280 nm. Equivalent amounts of proteins (~0.5-1 mg) were incubated with 2 µg of rabbit anti-PALB2, rabbit anti-USP11 antibody, rabbit
5 anti-GFP antibody or normal rabbit IgG for 5 h at 4°C. A mix of protein A/protein G-Sepharose beads (Thermo Scientific) was added for an additional hour. Beads were collected by centrifugation, washed twice with lysis buffer and once with PBS, and eluted by boiling in 2X Laemmli buffer before analysis by SDS-PAGE and immunoblotting. For MS analysis of Flag-PALB2, 150 x 10⁶ transiently transfected
10 HEK293T cells were lysed in high-salt lysis buffer (50 mM Tris-HCl pH 7.5, 300 mM NaCl, 1 mM EDTA, 1% Triton X100, 3 mM MgCl₂, 3 mM CaCl₂), supplemented with Complete protease inhibitor cocktail (Roche), 4 mM 1,10-Phenanthroline, 50 U benzonase and 50 U micrococcal nuclease. Cleared lysates were incubated with Flag-M2 agarose (Sigma), followed by extensive washing in lysis buffer and 50 mM
15 ammoniumbicarbonate.

Mass spectrometry

[000220] After immunoprecipitation of transiently transfected Flag-PALB2 from siCTRL-transfected or USP11 siRNA-depleted 293T cells, cysteine residues were reduced and alkylated on beads using 10 mM DTT (30 min. at 56 °C) and 15 mM 2-
20 chloroacetamide (1 h at room temperature), respectively. Proteins were digested using limited trypsin digestion on beads (1 µg trypsin (Worthington, NJ, USA) per sample, 20 min at 37°C), and dried to completeness. For LCMS/MS analysis, peptides were reconstituted in 5% formic acid and loaded onto a 12 cm fused silica column with pulled tip packed in-house with 3.5 µm Zorbax C18 (Agilent Technologies, CA, USA).
25 Samples were analyzed using an Orbitrap Velos (Thermo Scientific, MA, USA) coupled to an Eksigent nanoLC ultra (AB SCIEX, CA). Peptides were eluted from the column using a 90 min linear gradient from 2% to 35% acetonitrile in 0.1% formic acid. Tandem MS spectra were acquired in a data-dependent mode for the top two most abundant multiply charged peptides and included targeted scans for five specific N-
30 terminal PALB2 tryptic digest peptides (charge state 1+, 2+, 3+), either in non-modified form or including a diGly-ubiquitin trypsin digestion remnant. Tandem MS spectra were acquired using collision-induced dissociation. Spectra were searched against the human Refseq_V53 database using Mascot, allowing up to 4 missed

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cleavages and including carbamidomethyl (C), deamidation (NQ), oxidation (M), GlyGly (K) and LeuArgGlyGly (K) [SEQ ID NO: 4] as variable modifications.

[000221] In vitro ubiquitylated HA-PALB2 (1-103) (50 μ L total reaction mix) was run briefly onto an SDS-PAGE gel, followed by total lane excision, in-gel reduction using 10 mM DTT (30 min at 56 °C), alkylation using 50 mM 2-chloroacetamide and trypsin digestion for 16 h at 37 °C. Digested peptides were mixed with 20 μ L of a mix of 10 unique heavy isotope-labeled N-terminal PALB2 (AQUA) peptides (covering full or partial tryptic digests of regions surrounding Lys 16, 25, 30 or 43, either in non-modified or diG-modified form; 80–1,200 fmol μ l⁻¹ per peptide, based on individual peptide sensitivity testing) before loading 6 μ L onto a 12 cm fused silica column with pulled tip packed in-house with 3.5 μ m Zorbax C18. Samples were measured on an Orbitrap ELITE (Thermo Scientific, MA, USA) coupled to an Eksigent nanoLC ultra (AB SCIEX, CA, USA). Peptides were eluted from the column using a 180 min linear gradient from 2% to 35% acetonitrile in 0.1% formic acid. Tandem MS spectra were acquired in a data-dependent mode for the top two most abundant multiply charged ions and included targeted scans for ten specific N-terminal PALB2 tryptic digest peptides (charge states 1+, 2+, 3+), either in light or heavy isotope-labeled form. Tandem MS spectra were acquired using collision induced dissociation. Spectra were searched against the human Refseq_V53 database using Mascot, allowing up to 2 missed cleavages and including carbamidomethyl (C), deamidation (NQ), oxidation (M), GlyGly (K) and LeuArgGlyGly (K) [SEQ ID NO:4] as variable modifications, after which spectra were manually validated.

His-Ubiquitin pull-down

[000222] 293 FLIP-IN cells stably expressing His₆-Ub were transfected with the indicated siRNA and treated with doxycycline (DOX) for 24 h to induce His₆-Ub expression. Cells were pre-treated with 10 mM N-ethylmaleimide for 30 min and lysed in denaturing lysis buffer (6 M guanidinium-HCl, 0.1 M Na₂HPO₄/NaH₂PO₄, 10 mM Tris-HCl, 5 mM imidazole, 0.01 M β -mercaptoethanol, complete protease inhibitor cocktail). Lysates were sonicated on ice twice for 10 sec with 1 min break and centrifuged at 15 000 g for 10 min at 4°C. The supernatant was incubated with Ni-NTA-agarose beads (Qiagen) for 4 h at 4°C. Beads were collected by centrifugation, washed once with denaturing lysis buffer, once with wash buffer (8 M Urea, 0.1 M Na₂HPO₄/NaH₂PO₄, 10 mM Tris-HCl, 5 mM imidazole, 0.01 M β -mercaptoethanol, complete protease inhibitor cocktail), and twice with wash buffer supplemented with

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0.1% Triton X-100, and eluted in elution buffer (0.2 M imidazole, 0.15 M Tris-HCl, 30% glycerol, 0.72 M β -mercaptoethanol, 5% SDS) before analysis by SDS-PAGE and immunoblotting.

HR-based repair assays

- 5 [000223] Parental U2OS cells and U2OS cells stably expressing wild-type CtIP or CtIP(T847E) mutant were transfected with the indicated siRNA and the PALB2-KR construct, synchronized with a single thymidine block, treated with doxycycline to induce CtIP expression and subsequently blocked in G1 phase by adding 40 μ M Lovastatin. Cells were collected by trypsinization, washed once with PBS and
- 10 electroporated with 2.5 μ g of sgRNA plasmid and 2.5 μ g of donor template using the Nucleofector technology (Lonza; protocol X-001). Cells were plated in medium supplemented with 40 μ M Lovastatin and grown for 24 h before flow cytometry analysis.

PALB2 chemical ubiquitylation

- 15 [000224] PALB2 (1-103) polypeptides, engineered with only one cross-linkable cysteine, were ubiquitylated by cross-linking alkylation, as previously described [40, 41], with the following modifications. Purified PALB2 cysteine mutant (final concentration of 600 μ M) was mixed with His₆-TEV-Ubiquitin G76C (350 μ M) in 300 mM Tris pH 8.8, 120 mM NaCl and 5% glycerol. Tris(2-carboxyethyl)phosphine
- 20 (TCEP) (Sigma-Aldrich) reducing agent was added to a final concentration of 6mM to the mixture and incubated for 30 minutes at room temperature. The bi-reactive cysteine cross-linker, 1,3-dichloroacetone (Sigma-Aldrich), was dissolved in dimethylformamide and added to the protein mix to a final concentration of 5.25 mM. The reaction was allowed to proceed on ice for 1 h, before being quenched by the
- 25 addition of 5 mM β -mercaptoethanol. His₆-TEV-Ubiquitin-conjugated PALB2 was enriched by passing over Ni-NTA-agarose beads (Qiagen).

[000225] The study and the results of the study are discussed below.

- [000226] DNA repair by homologous recombination (HR) [1] is highly suppressed in G1 cells [2,3] to ensure that mitotic recombination occurs solely between
- 30 sister chromatids [4,5]. Although many HR factors are cell cycle-regulated, the identity of the events that are both necessary and sufficient to suppress recombination in G1 cells is unknown. This study has found that the cell cycle tightly controls the interaction of BRCA1 with PALB2-BRCA2 to constrain BRCA2 function to the S/G2 phases. The BRCA1-interaction site on PALB2 is targeted by an E3 ubiquitin ligase composed of

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KEAP1, a PALB2-interacting protein [6], in complex with CUL3-RBX1 [7]. PALB2 ubiquitylation suppresses its interaction with BRCA1 and is counteracted by the deubiquitylase USP11, which is itself under cell cycle control. Restoration of the BRCA1-PALB2 interaction combined with the activation of DNA-end resection is
 5 sufficient to induce HR in G1, as measured by RAD51 recruitment, unscheduled DNA synthesis and a CRISPR/Cas9-based gene targeting assay. The mechanism prohibiting HR in G1 minimally consists of the suppression of DNA-end resection coupled with a multi-step block of the recruitment of BRCA2 to DNA damage sites that involves the inhibition of BRCA1-PALB2-BRCA2 complex assembly. The ability to induce HR in
 10 G1 cells with defined factors may be used in gene targeting applications in non-dividing cells.

[000227] The breast and ovarian tumour suppressors BRCA1, PALB2 and BRCA2 promote DNA double-strand break (DSB) repair by HR [8-10]. BRCA1 promotes DNA-end resection to produce the single-stranded (ss) DNA necessary for
 15 homology search and strand invasion [1], and it interacts with PALB2 [13-15] to direct the recruitment of BRCA2 [13] and RAD51 [16, 17] to DSB sites. The accumulation of BRCA1 on the chromatin that flanks DSB sites is suppressed in G1 cells [18], reminiscent of the potent inhibition of HR in this phase of the cell cycle. Since the inhibition of BRCA1 recruitment in G1 is dependent on the 53BP1 and RIF1 proteins
 20 [18, 19], two inhibitors of end-resection [18-22], this regulation of BRCA1 was originally viewed in light of its function in stimulating DNA-end processing.

[000228] However, as BRCA1 is also involved in promoting the recruitment of BRCA2 through its interaction with PALB2 [13-15], this study investigated whether inducing BRCA1 recruitment to DSB sites in G1, through mutation of *53BP1* (also
 25 known as TP53BP1) by genome editing (*53BP1*Δ; Figure 5a-c) also resulted in BRCA2 accumulation into ionizing radiation (IR)-induced foci. In contrast with BRCA1, neither BRCA2 nor PALB2 are recruited to G1 DSB sites in U-2-OS (U2OS) cells lacking 53BP1 at IR doses ranging from 2 to 20 Gy (Figure 1ab and Figure 5d,e). Since BRCA1 and PALB2 interact directly [13,14], this result suggested that G1 cells may
 30 block BRCA2 recruitment by suppressing the BRCA1-PALB2 interaction. Indeed, while PALB2 interacts with BRCA2 irrespective of cell cycle position, it interacts efficiently with BRCA1 only during S phase (Figure 1c). The presence of DNA damage led to the loss of the residual PALB2-BRCA1 interaction in G1 whereas it had little impact on the assembly of the BRCA1-PALB2-BRCA2 complex in S phase (Figure

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1c). Since all proteins were expressed in G1 (Figure 1c), the results suggest that the assembly of the BRCA1-PALB2-BRCA2 complex is controlled during the cell cycle, possibly to restrict the accumulation of BRCA2 at DSB sites to the S/G2 phases.

[000229] These results were confirmed using a single-cell assay assessing the co-localization, at an integrated *LacO* array [23], of a mCherry-tagged LacR-BRCA1 fusion protein with GFP-tagged PALB2 (Figure 6a). This LacR/*LacO* system recapitulated the cell cycle-dependent and DNA damage-sensitive BRCA1-PALB2 interaction (Figure 6b) and enabled the finding that sequences on PALB2, located outside its amino-terminal BRCA1-interaction domain (residues 1-50) were responsible for the cell cycle-dependent regulation of its association with BRCA1 (Figure 6c,d). Further deletion mutagenesis identified a single region, encompassed within residues 46-103 in PALB2 (Figure 6e,f) responsible for the cell cycle-dependent regulation of the BRCA1-PALB2 interaction. This region corresponds to the interaction site for KEAP1 [6], identifying this protein as a candidate regulator of the BRCA1-PALB2 interaction.

[000230] KEAP1 is a substrate adaptor for a CULLIN 3-RING ubiquitin (Ub) ligase (CRL3) that targets the antioxidant regulator NRF2 for proteasomal degradation [24] and recognizes an “ETGE” motif on both PALB2 and NRF2 through its KELCH domain [6]. Depletion of KEAP1 from *53BP1Δ* cells, or deletion of the ETGE motif in full-length PALB2 (PALB2 ΔETGE) induced PALB2 IR-induced focus formation in G1 cells (Figure 1d and Figure 7a). Furthermore, in cells in which *KEAP1* was inactivated by genome editing (*KEAP1Δ*, Figure 7b) a stable BRCA1-PALB2-BRCA2 complex was detected in both G1 and S phases (Figure 1e). KEAP1 is therefore an inhibitor of the BRCA1-PALB2 interaction.

[000231] CUL3 also interacts with PALB2 (Figure 7c) and its depletion in *53BP1Δ* U2OS cells de-repressed PALB2 IR-induced foci in G1 (Figure 1d and Figure 7a). Furthermore, in G1-synchronized *KEAP1Δ* cells, expression of a CUL3-binding deficient KEAP1 protein that lacks its BTB domain (ΔBTB) failed to suppress the BRCA1-PALB2 interaction, unlike its wild type counterpart (Figure 7d). These results suggest that KEAP1 recruits CUL3 to PALB2 to suppress its interaction with BRCA1.

[000232] Using the LacR/*LacO* system and co-immunoprecipitation assays, a mutant of PALB2 lacking all 8 lysine residues in the BRCA1-interaction domain (PALB2-KR; Figure 2a) was found to interact with BRCA1 irrespective of cell cycle position (Figure 2b and Figure 7e,f). Further mutagenesis identified residues 20, 25 and

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30 in PALB2 as critical for the suppression of the BRCA1-PALB2 interaction since reintroduction of these lysines in the context of PALB2-KR (yielding PALB2-KR/K3; Figure 2a) led to the suppression of BRCA1- PALB2-BRCA2 complex assembly in G1 cells (Figure 2b and Figure 7e). Together, these results suggested a model whereby
 5 PALB2-bound KEAP1 forms an active CRL3 complex that ubiquitylates the PALB2 N-terminus to suppress its interaction with BRCA1.

[000233] While PALB2 ubiquitylation can be detected in cells (Figure 8a), the lysine-rich nature of the PALB2 N-terminus has so far precluded unambiguously mapping in vivo ubiquitylation sites on Lys 20, 25 or 30. However, ubiquitylation could
 10 be detected on Lys16 and Lys43 by mass spectrometry, indicating that the PALB2 N-terminus is ubiquitylated (Figure 8b). In a complementary set of experiments, PALB2 targeted to the *LacO* array induced immunoreactivity to conjugated ubiquitin (Figure 8c-e). Ub co-localization with PALB2 was highest in G1, and depended on the KEAP1-interaction motif and the presence of the Lys 20/25/30 residues (Figure 8d-e),
 15 consistent with a model that PALB2 is ubiquitylated on those sites in G1 cells. Indeed, ubiquitylation of the N-terminus of PALB2 (residues 1-103; fused to a haemagglutinin (HA) epitope tag) could be readily reconstituted by recombinant CRL3-KEAP1, in a manner that depended on the KEAP1-interaction domain of PALB2 (Figure 2c) and Lys25 and Lys30 were unambiguously identified as being ubiquitylated by KEAP1 *in*
 20 *vitro* by mass spectrometry.

[000234] Ubiquitylation of PALB2 by CRL3-KEAP1 inhibited its interaction with a BRCA1 fragment comprising residues 1363-1437 (BRCA1-CC), an inhibition that was more obvious with the highly modified forms of PALB2 due to the presence of ubiquitylated lysines outside the BRCA1-interaction domain (Figure 2d). In order to
 25 specifically test whether ubiquitylation of a single lysine residue of the three identified as critical inhibited the interaction with BRCA1, chemical crosslinking was used to install a single ubiquitin moiety at position 20 or 45 (yielding PALB2-K_C20-Ub and PALB2-K_C45-Ub). Ubiquitylation of PALB2 at position 20 completely suppressed its interaction with BRCA1 whereas modification of residue 45 had no impact on the
 30 interaction (Figure 9a), echoing the in vivo data (Figure 7e). Together, these results indicate that ubiquitylation of PALB2 at specific sites on its N-terminus prevents its interaction with BRCA1.

[000235] Since neither the activity of the CRL3-KEAP1 E3 ligase (Figure 9b) nor the interaction of CRL3-KEAP1 with PALB2 (Figure 7c) are regulated by the cell

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cycle, it was possible that ubiquitylation of PALB2 might be regulated in a cell cycle-dependent manner. KEAP1 physically interacts with USP11 [25], a deubiquitylase that also interacts with BRCA2 [26] and PALB2 (Figure 9c). USP11 depletion impairs gene conversion [27] (Figure 9d) and results in hypersensitivity to PARP inhibition [27]
 5 identifying it as an HR regulator of unknown function. Co-immunoprecipitation experiments confirmed that USP11 and its catalytic activity were necessary for the formation of a stable BRCA1-PALB2-BRCA2 complex, especially in the presence of DNA damage (Figure 3a and Figure 9e,f).

[000236] If USP11 antagonizes PALB2 ubiquitylation by CRL3-KEAP1, then
 10 removal of KEAP1 (or CUL3) should reverse the phenotypes imparted by loss of USP11. Indeed, deletion of KEAP1 restored resistance to PARP inhibitors (PARPi) and the BRCA1-PALB2 interaction in USP11 knockout cells prepared by genome editing (*USP11Δ*) (Figure 3b,c and Figure 9e). Likewise, depletion of CUL3 or KEAP1 reversed the gene conversion defect of USP11-depleted cells (Figure 10a). Introduction
 15 of the PALB2-KR mutant restored its interaction with BRCA1 and reversed PARPi sensitivity in *USP11Δ* cells in a manner that depended on Lys20/25/30 (Figure 10b,c). Since recombinant USP11 can directly de-ubiquitylate PALB2 (1-103) (Figure 3d), these results suggest that USP11 promotes the assembly of the BRCA1-PALB2-BRCA2 complex by reversing the inhibitory ubiquitylation on the PALB2 Lys20/25/30
 20 residues.

[000237] It was observed that USP11 turns over rapidly in G1 cells and interacts poorly with PALB2 in this phase of the cell cycle (Figure 11a,b). Furthermore, there is a rapid loss of USP11 upon DNA damage induction, specifically in G1 phase (Figure 3e and Figure 11b,c). The destabilization of USP11 following IR treatment is dependent
 25 on ATM signalling, whereas it is ATR-dependent following UV irradiation (Figure 11d,e). The drop in USP11 steady-state levels in G1 is the result of proteasomal degradation (Figure 11f). A CRL4 E3 Ub ligase is most likely responsible for controlling the stability of USP11 as treatment with MLN4924, a pan-CRL inhibitor [28] (Figure 11g), or depletion of CUL4 (Figure 3f) protected USP11 from DNA
 30 damage-induced degradation. CUL4 depletion led to BRCA2 and PALB2 IR-induced focus formation in G1 *53BP1Δ* cells (Figure 3g and Figure 12a), consistent with the regulation of USP11 by a CRL4 complex acting as the upstream signal that ultimately controls BRCA1-PALB2-BRCA2 complex assembly.

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[000238] While deletion of 53BP1 produces low levels of ssDNA in G1 cells [29], combining the *53BP1* Δ mutation with depletion of KEAP1 did not produce extraction-resistant RAD51 IR induced foci, suggesting little-to-no RAD51 nucleofilament formation (Figure 12b). ssDNA formation remained insufficient in those cells and thus
 5 took advantage of the phosphomimetic T847E mutant of CtIP that promotes resection in G1 cells [30]. Unlike wild type CtIP, introduction of CtIP-T847E into *53BP1* Δ cells depleted of KEAP1 induced RAD51 IR-induced focus formation in G1 cells (Figure 4a,b and Figure 12b,c) along with unscheduled DNA synthesis (Figure 12d). These results suggested that the steps downstream of RAD51 nucleofilament formation, i.e.
 10 strand invasion, D-loop formation and DNA synthesis, could be activated in G1.

[000239] To test whether productive HR could also be activated in G1 a CRISPR/Cas9-stimulated gene targeting assay (Pinder J. et al, Nuclei Acids Res. 43, 9379-9392, 2015) was employed in which the insertion of the coding sequence for the mClover fluorescent protein at the 5' of the lamin A (*LMNA*) or *PML* genes was
 15 monitored by microscopy or flow cytometry (Figure 4c and Figure 12e,f), with the latter method enabling the gating of cells with a defined DNA content (such as G1 cells). Synchronization protocols were also established in which G1 cells obtained after release from a thymidine block were arrested in G1 by lovastatin treatment [2] for 24 h (Figure 12g,h). Using this system, a concentration of donor template in the linear range
 20 of the assay was determined, and it was ascertained that gene targeting at the *LMNA* locus was dependent on BRCA1-PALB2-BRCA2 complex assembly (Figure 13a,b). It was also confirmed that gene targeting by HR was highly suppressed in G1 (Figure 4d).

[000240] The combined activation of resection and BRCA1 recruitment to DSB sites (i.e. in *53BP1* Δ cells expressing CtIP(T847E) was insufficient to stimulate gene
 25 targeting at either the *LMNA* or the *PML* locus in G1 cells (Figure 4e and Figure 13c). However, when the BRCA1-PALB2 interaction was restored in resection-competent G1 cells using either KEAP1 depletion or expression of the PALB2-KR mutant, a robust increase in gene targeting events at both loci was detected (Figure 4e and Figure 13c). However, the gene-targeting frequencies of G1 cells remained lower than those of
 30 asynchronously dividing cells, suggesting an incomplete activation of HR. 53BP1 inactivation and the expression of CtIP(T847E) were both necessary for G1 HR (Figure 13d,e), indicating that the simultaneous activation of end resection and BRCA2 recruitment to DSB sites were both necessary and sufficient to activate unscheduled recombination in this phase of the cell cycle.

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[000241] In conclusion, the regulation of BRCA1-PALB2-BRCA2 complex assembly is a key node in the cell cycle control of DSB repair by HR. This regulation converges on the BRCA1-interaction site on PALB2 and is enforced by the opposing activities of the E3 ligase CRL3-KEAP1 and the deubiquitylase USP11, with the latter
 5 being antagonized in G1 by a CRL4 complex (Figure 4f). In this model, the stabilization of USP11 in S phase licenses the recruitment of PALB2-BRCA2 and the subsequent loading of RAD51 at DSB sites. The studies also demonstrate that the suppression of HR in G1 cells is largely reversible and that it involves the combined suppression of end resection and BRCA2 recruitment to DSB sites (Figure 4f). As most
 10 cells in the human body are not actively cycling and are thus refractory to HR, the manipulations described herein give rise to the development of genome editing methods that enable therapeutic gene targeting in a wider variety of tissues.

EXAMPLE 2

Identification of DCAF10 as a substrate adaptor for the degradation of USP11.

15 [000242] CUL4-RING-Ligase (CRL4) complexes are composed of CULLIN4 (CUL4), RBX1, DDB1, DDA1 and a substrate adaptor called a DCAF [42]. To search for the substrate adaptor that mediates the ubiquitylation of USP11, a focused siRNA library was assembled that depletes known and predicted DCAFs along with other CUL4-interacting proteins. This library was screened in a high content microscopy
 20 assay where USP11 levels were evaluated by immunofluorescence microscopy. Cells were treated either with ultraviolet light (UV) or ionizing radiation (IR) to induce USP11 degradation. Data was normalized to the non-irradiated condition and the mean of two independent experiments was used to plot values after UV- and IR-treatment. The data, shown in Figure 14a shows that in addition to the expected stabilization of
 25 USP11 after CUL4 depletion, the depletion of DCAF10, DCA F15 and DCAF17 also led to USP11 stabilization. Since siRNA-mediated knockdown is prone to off-target effects, it was then assessed whether the knockdown of DCAF10, DCAF15 or DCAF17 by two independent siRNAs could stabilize USP11 in immunoblotting experiments. It was found that while stabilization of USP11 could only be observed with a single
 30 siRNA (Figure 14b), depletion of DCAF10 with both siRNAs led to a robust stabilization of USP11 (Figure 14b). Since CRL4 substrate adaptors bind to their substrates [42], it was next assessed whether DCAF10 or DCAF15 could interact with USP11 in co-immunoprecipitation assays. It was found that when Flag-tagged USP11 was immunoprecipitated from HEK293 cell extracts, it interacted with DCAF10 but not

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DCAF15 (Figure 15a), strongly suggesting that DCAF10 is a bona fide substrate adaptor that targets USP11 for degradation. To further assess whether DCAF10 is indeed involved in the regulation of USP11, mouse embryo fibroblasts (MEFs) were generated from congenic wild type ($Dcaf10^{+/+}$), heterozygote ($Dcaf10^{+/-}$) and $Dcaf10^{-/-}$ mice and immunoblotted for USP11. Loss of DCAF10 resulted in higher steady-state levels of USP11 in mouse cells (Figure 15b), consistent with DCAF10 being the adaptor of a CRL4 complex targeting USP11. Finally, it was assessed whether DCAF10 over-expression can suppress homologous recombination in a dominant manner using the direct repeat (DR)-GFP assay [43]. Overexpression of DCAF10, but not of DCAF15 led to a decrease in HR in the same magnitude as the depletion of USP11 and other core HR factors (Figure 15c). Collectively, these data suggest that DCAF10 regulates HR through the control of USP11.

EXAMPLE 3

A genetically-encoded inhibitor of KEAP1 can promote homologous recombination in G1 cells.

[000243] The activation of gene targeting in G1 cells requires the removal of 53BP1, the introduction of CtIP-T847E and the interaction between PALB2 and BRCA1, which can be achieved by the removal of KEAP1. To develop a system that would enable activation of HR in G1 and non-dividing cells, it was determined whether KEAP1 siRNAs could be replaced with inhibitors of KEAP1. A recently described high-affinity genetically encoded inhibitor of KEAP1, named R1, which is based on fibronectin-3 (FN3) scaffold was selected [44]. The LMNA gene targeting assay [45] was carried out in 53BP1 Δ U2OS cells synchronized in G1 phase and it was found that transfection of the R1 KEAP1 inhibitor, but not its FN3 control, led to a robust activation of gene-targeting, albeit less, and KEAP1 depletion (Figure 16). Inhibition of KEAP1 can be a propitious route for the activation of HR in non-dividing cells.

[000244] The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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[000245] All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. All publications, patents and patent
5 applications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the methodologies, reagents, etc. which are reported therein which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

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Table 1

Protein	NCBI Accession No.	SEQ ID NO.
USP11 (ubiquitin carboxyl-terminal hydrolase 11)	NP_004642.2	5
PALB2 (partner and localizer of BRCA2)	NP_078951.2	6
BRCA1 (Homo sapiens breast cancer 1)	NG_005905.2	7
BRCA2 (Homo sapiens breast cancer 2)	NG_012772.3	8
KEAP1 (kelch-like ECH-associated protein 1)	NP_036421.2	9
53BP1 (tumor suppressor p53-binding protein 1 isoform 1)	NP_001135452.1	10
53BP1 (tumor suppressor p53-binding protein 1 isoform 2)	NP_001135451.1	11
53BP1 (tumor suppressor p53-binding protein 1 isoform 3)	NP_005648.1	12
DCAF10 (DDB1- and CUL4-associated factor 10 isoform a)	NP_077321.3	13
DCAF (DDB1- and CUL4-associated factor 10 isoform b)	NP_001273739.1	14
RBX1(E3 ubiquitin-protein ligase RBX1)	NP_055063.1	15
CUL3 (Homo sapiens cullin 3)	NG_032169.1	16
CtIP/RBBP8 (Homo sapiens retinoblastoma binding protein 8)	NG_012121.1	17

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WHAT IS CLAIMED IS:

1. A method for activating or modulating homologous recombination in a cell comprising:

- (a) promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell;
- 5 (b) contacting the cell with BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes;
- (c) activating or stimulating BRCA1 recruitment to DNA double-strand break (DSB) sites;
- (d) inhibiting or removing KEAP1 or CRL3-KEAP1;
- 10 (e) inhibiting the degradation of USP11 or promoting USP11 activity; and/or
- (f) inhibiting DCAF10.

2. The method of claim 1 comprising inhibiting KEAP1 or DCAF10.

3. The method of claim 1 or 2 further comprising reconstituting or activating DNA end resection.

- 15 4. The method for activating homologous recombination in a cell in which DNA end resection is activated or reconstituted generating single-stranded DNA, comprising promoting or stimulating the assembly or occurrence of BRCA1-PALB2 or BRCA1-PALB2-BRCA2 complexes in the cell.

5. The method of claim 3 or 4 wherein DNA end resection is activated by inhibiting 53BP1 expression or activity and upregulating or expressing CtIP.

6. The method of claim 5 wherein CtIP is upregulated or expressed by administering CtIP or an analog of CtIP.

7. The method of claim 3 comprising inhibiting or removing KEAP1 and 53BP1 and upregulating or expressing CtIP or an analog of CtIP.

- 25 8. The method of claim 5, 6, or 7 wherein 53BP1 is inhibited by administering 53BP1 short interfering (si) RNA, short hairpin (sh) RNA or microRNAs (miRNAs).

9. A method of any one of claims 1 to 8 further comprising introducing into the cell a gene editing system.

- 10 10. A method of claim 9 wherein the gene editing system is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) system, a Transcription Activator-Like Effector Nucleases (TALEN) system or a zinc finger nucleases system.

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11. A method for altering a genetic disorder associated with a target polynucleotide sequence in a cell comprising: (1) activating homologous recombination in the cell in accordance with the method of any one of claims 1 to 8; and (2) contacting the target polynucleotide sequence with a CRISPR-associated (Cas) protein and from one to two ribonucleic acids, wherein the ribonucleic acids direct Cas protein to and hybridize to a selected motif of the target polynucleotide sequence, wherein the target polynucleotide sequence is cleaved whereby the expression of the target polynucleotide sequence is reduced, knocked-out, or corrected from an undesired sequence to a desired sequence
- 10 12. A method of any one of claims 1 to 11 wherein the cell is in G1 phase of the cell cycle (G1) or G0 phase of the cell cycle (G0).
13. A method of claim 12 wherein the cell is in G1 phase of the cell cycle (G1).
14. A method for monitoring activity or expression of USP11 in a sample comprising (i) isolating complexes of (a) BRCA1 and PALB2; (b) BRCA1, PALB2, and BRCA2; (c) USP11 and PALB2; and/or (d) USP11 and DCAF10 in the sample; (ii) measuring the levels of the complexes; and (iii) detecting an increase or decrease in the activity or expression of the complexes as compared to a control as an indication of the activity or expression of USP11.
- 15 15. A method of predicting a response or categorizing a response to a PARP inhibitor in an individual comprising assaying complexes of BRCA1, PALB2 and BRCA2 in a sample from the subject and comparing to a control to determine if the individual is sensitive to the PARP inhibitor.
- 20 16. A kit for stimulating or activating homologous recombination or gene editing in a cell comprising (a) an inhibitor of 53BP1; (b) an inhibitor of KEAP1; and
- 25 (c) CtIP or an analog of CtIP.

FIG 1a

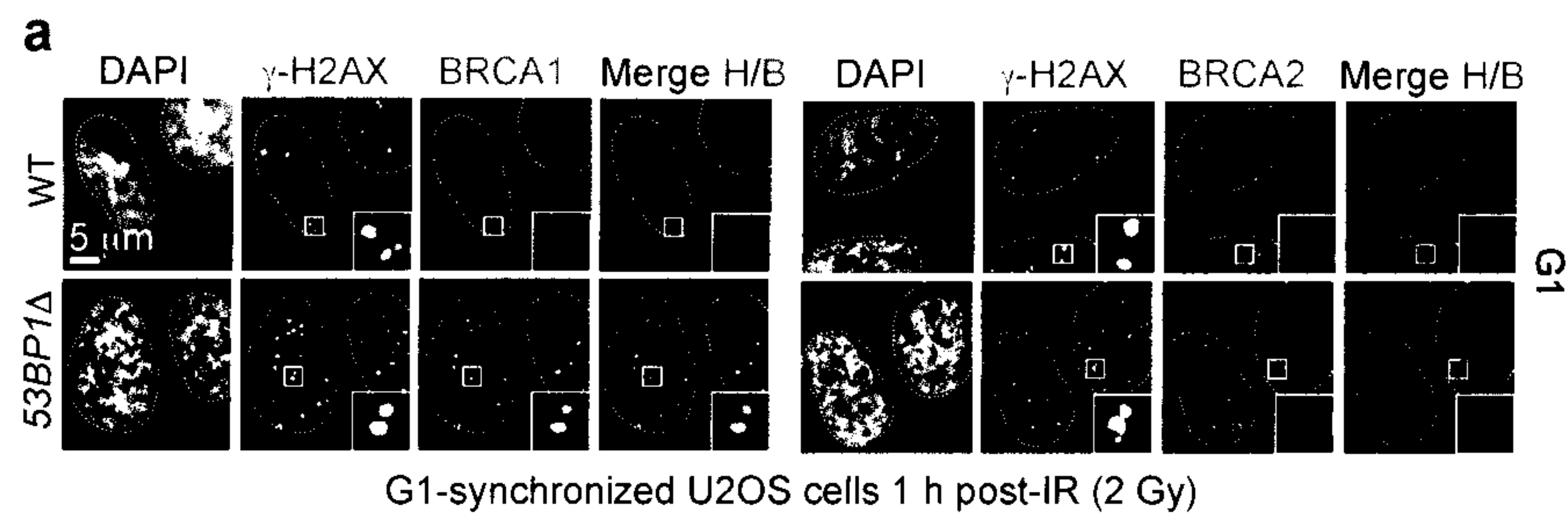


FIG 1b

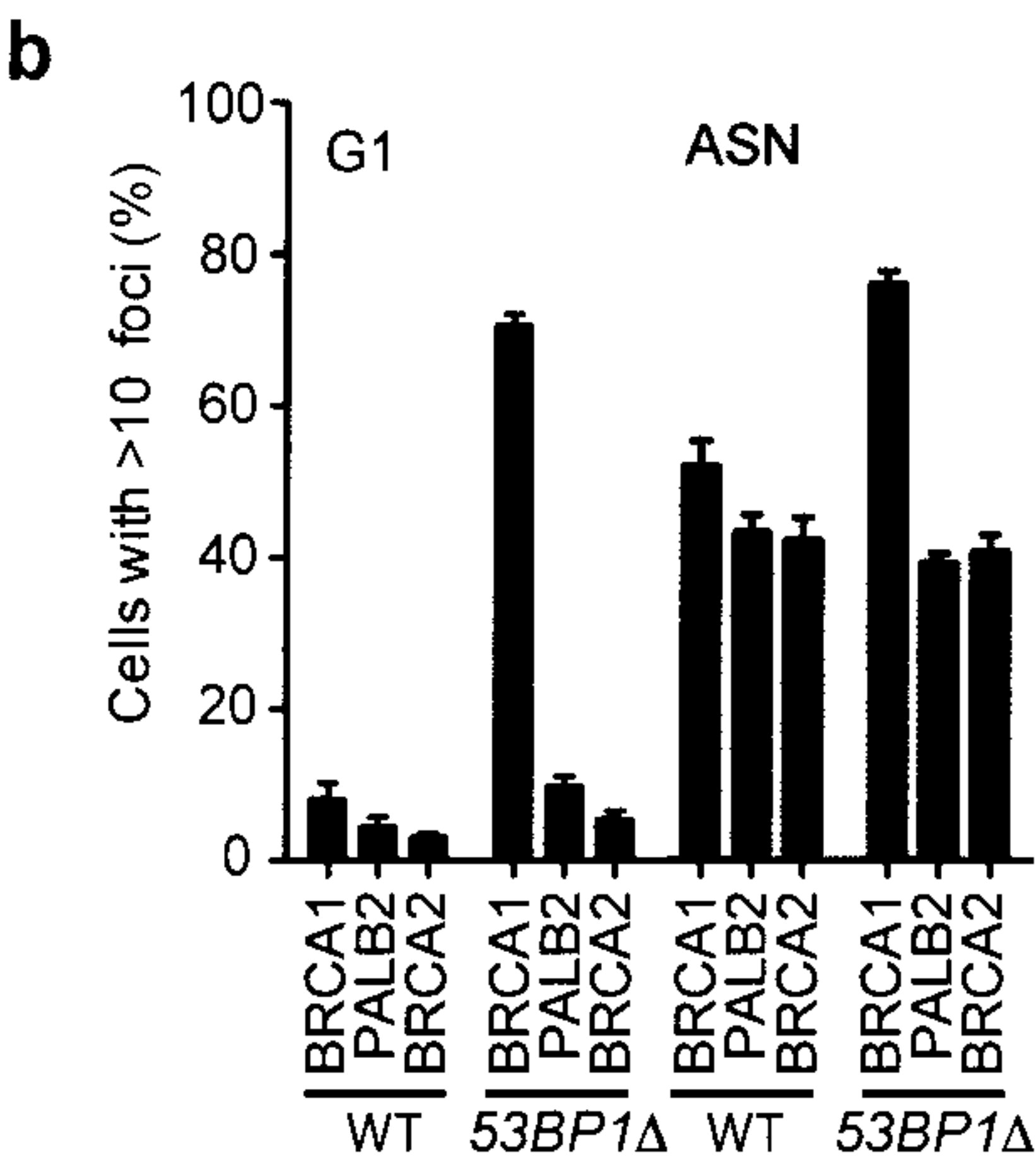


FIG 1c

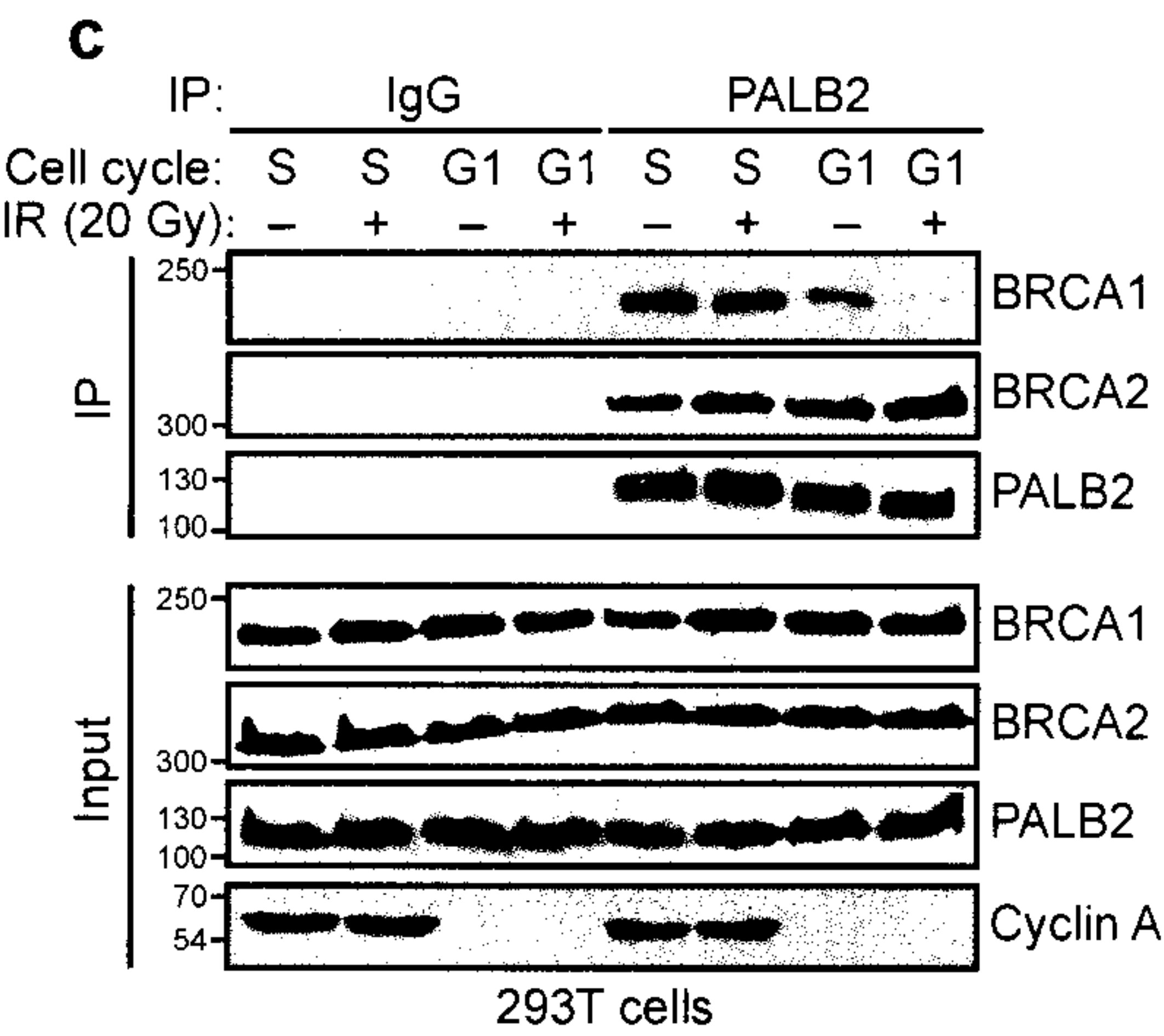


FIG 1d

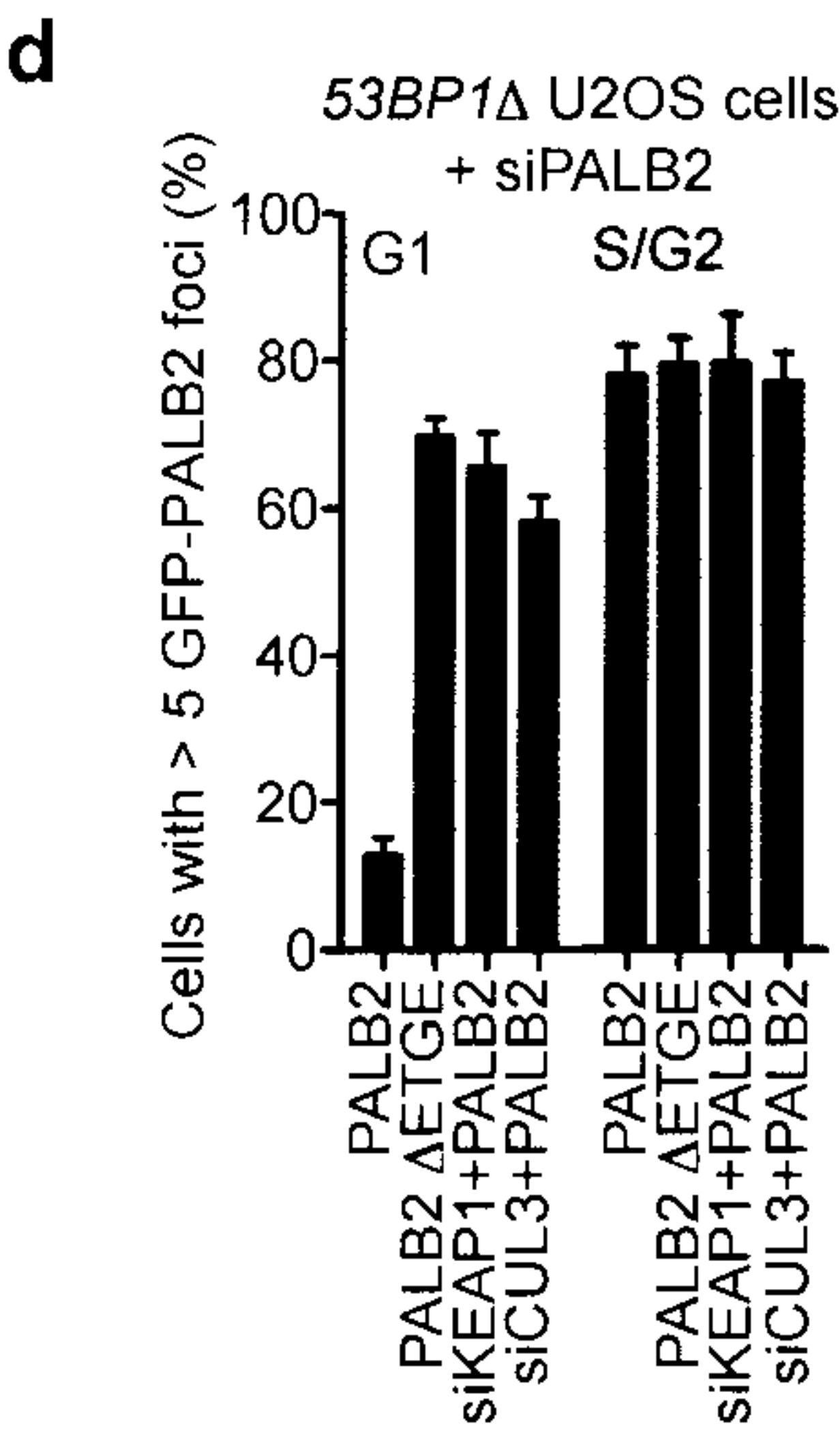
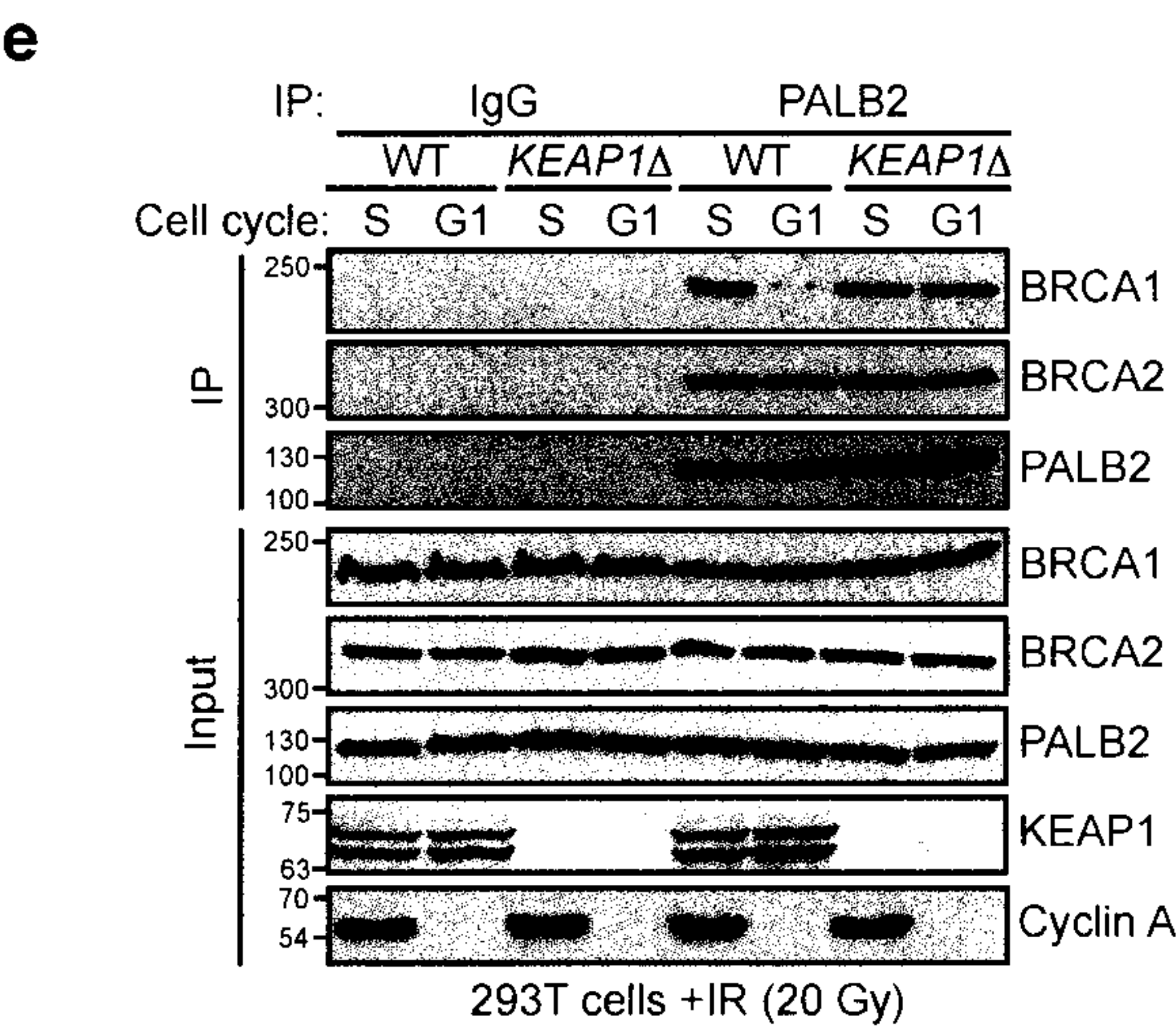


FIG 1e



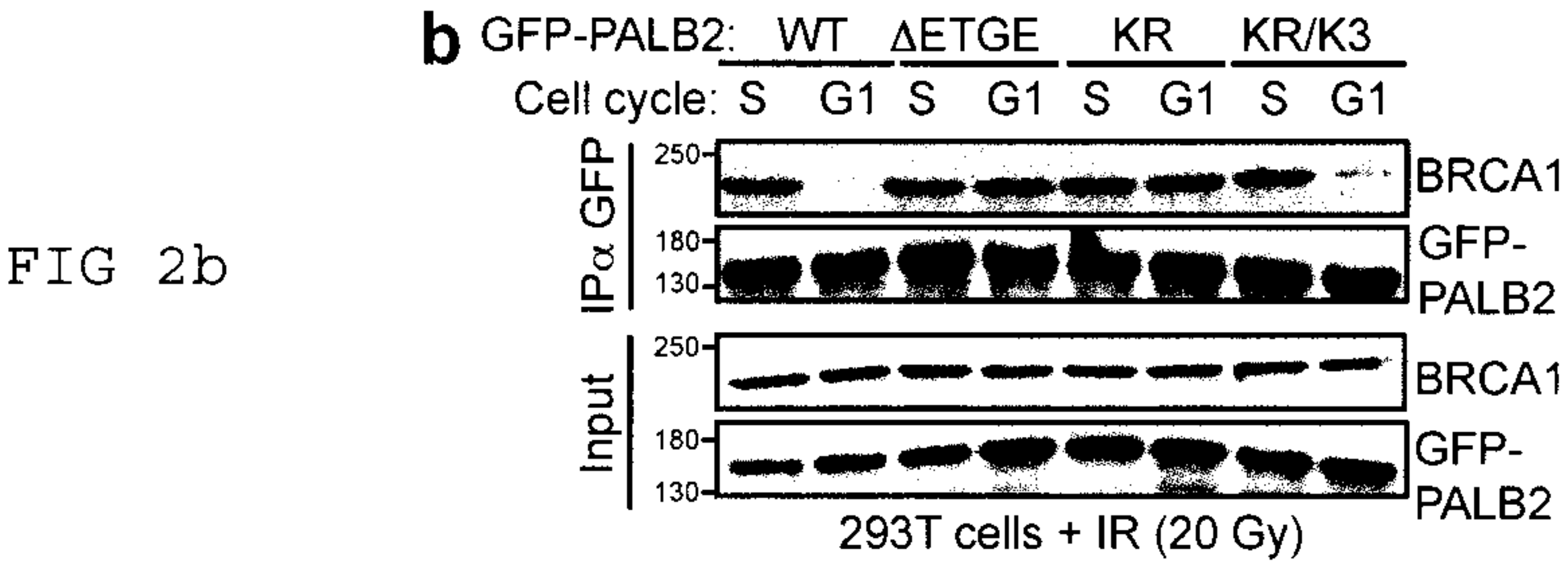
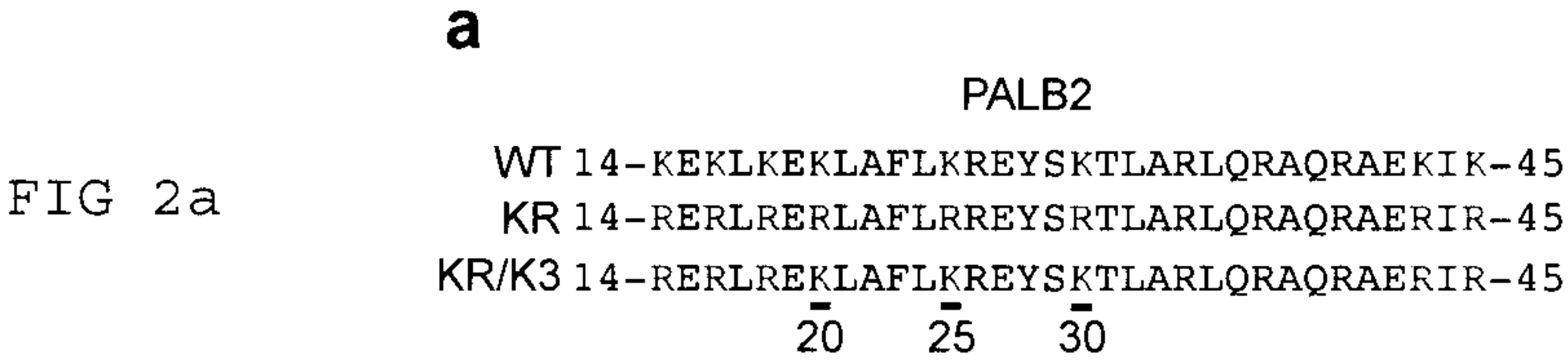


FIG 2c

FIG 2d

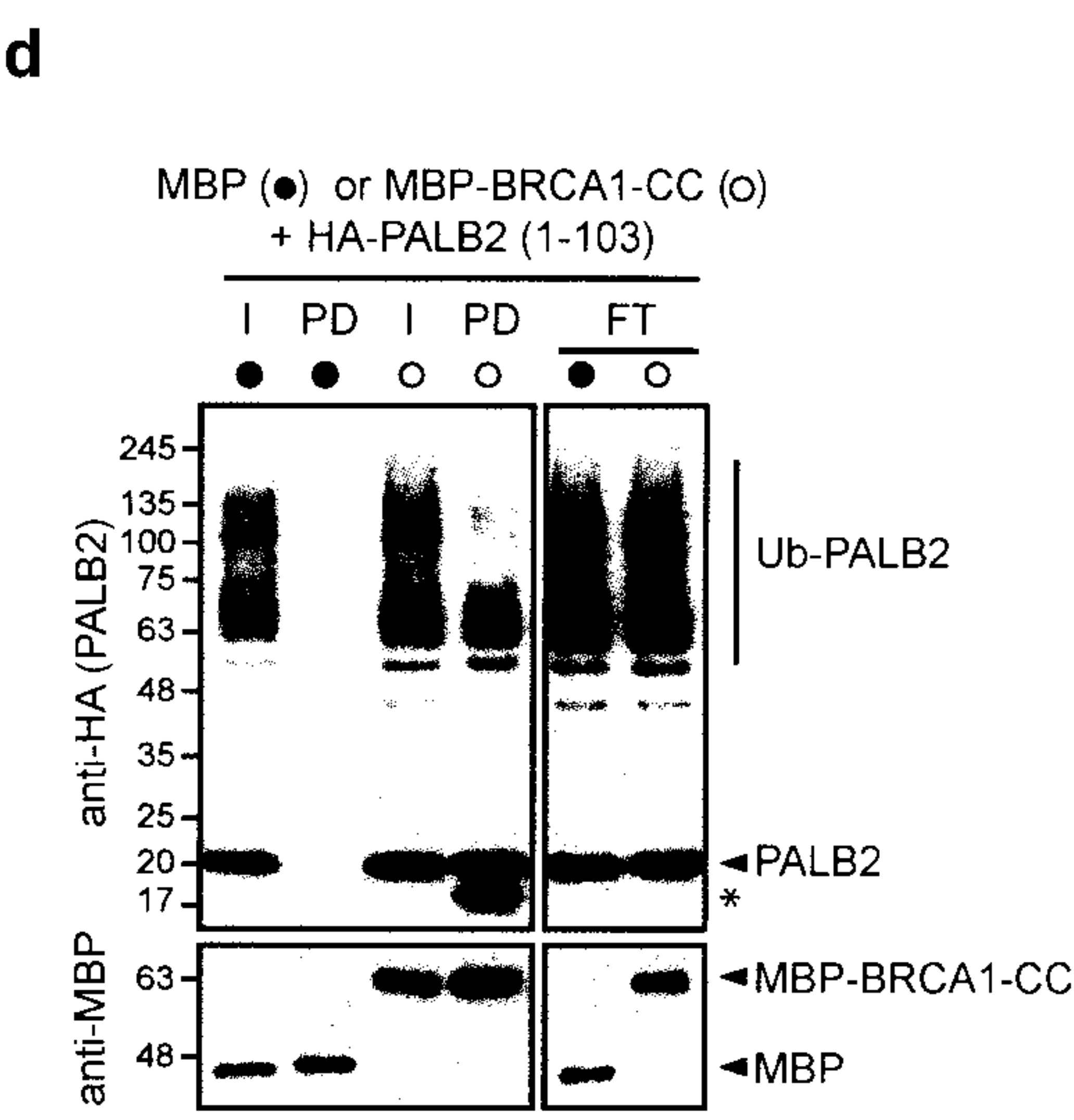
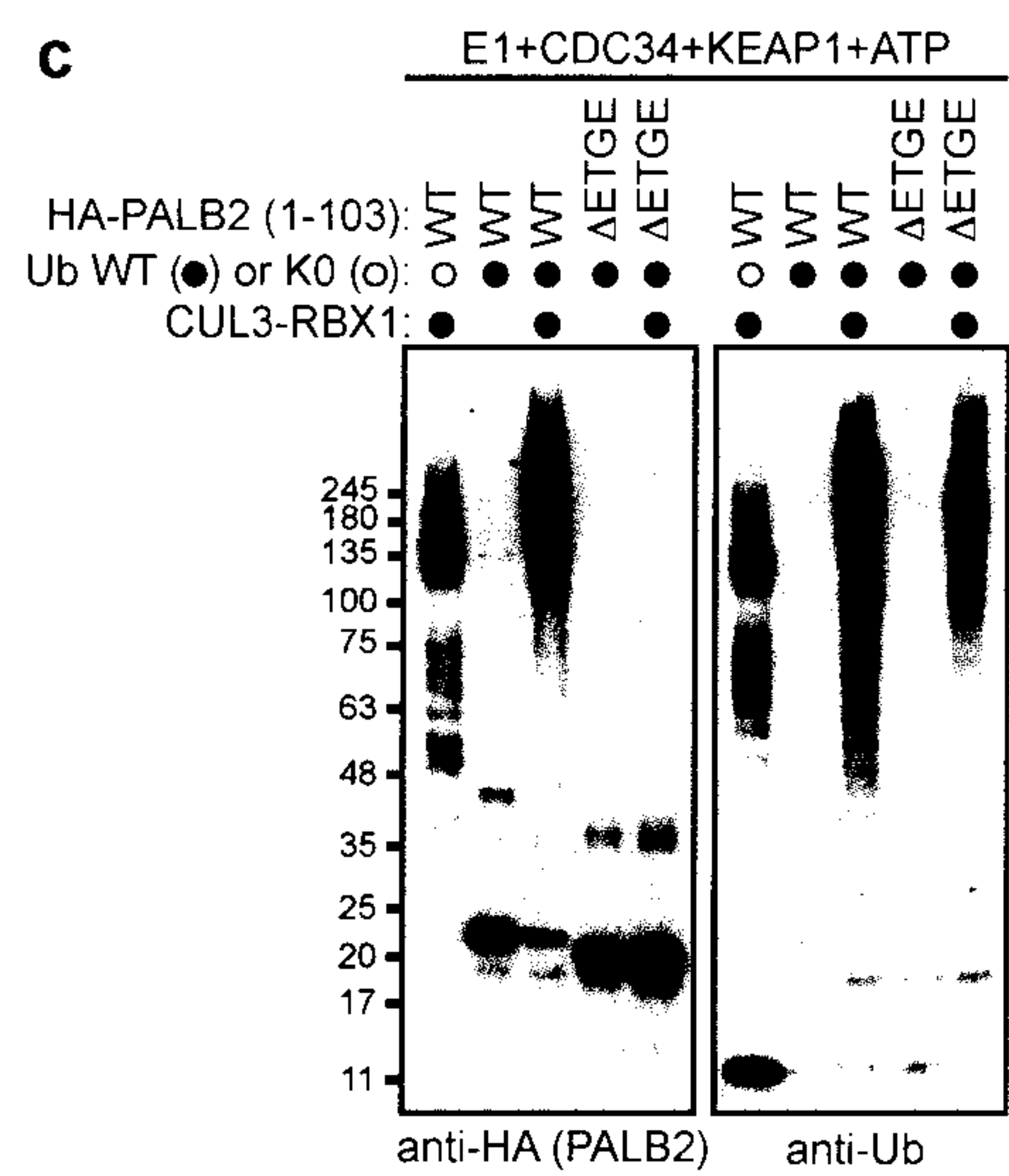


FIG 3a

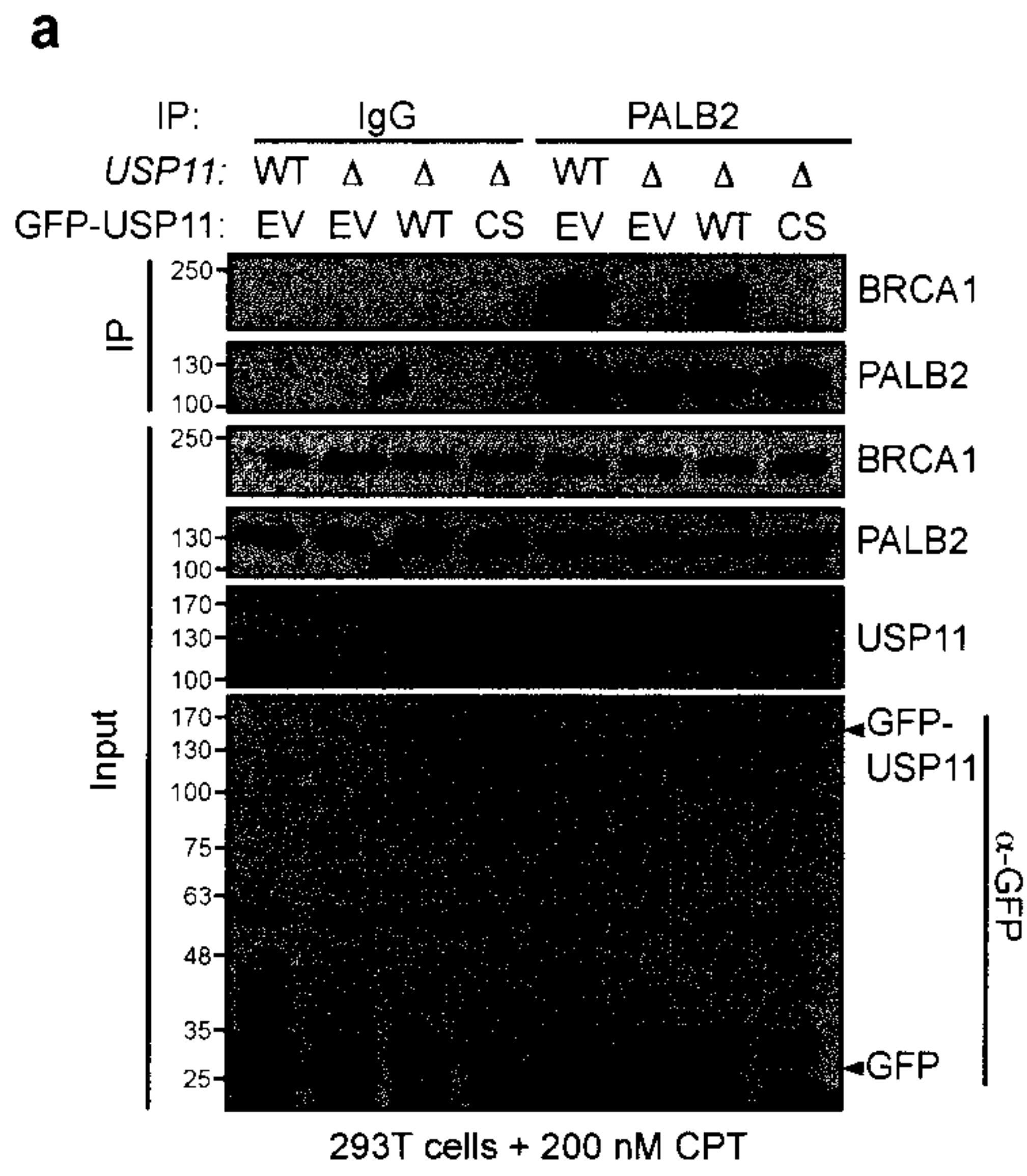


FIG 3b

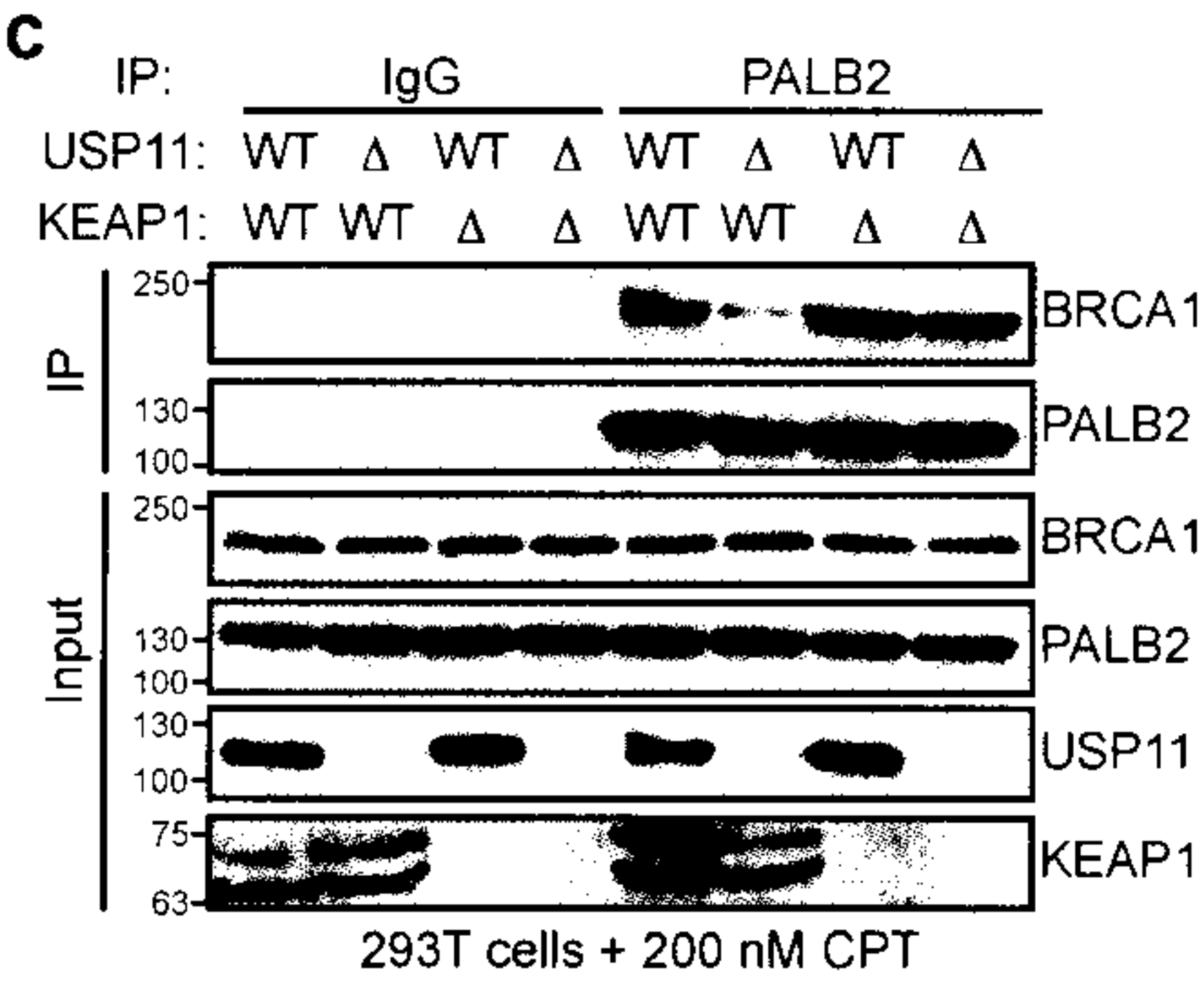
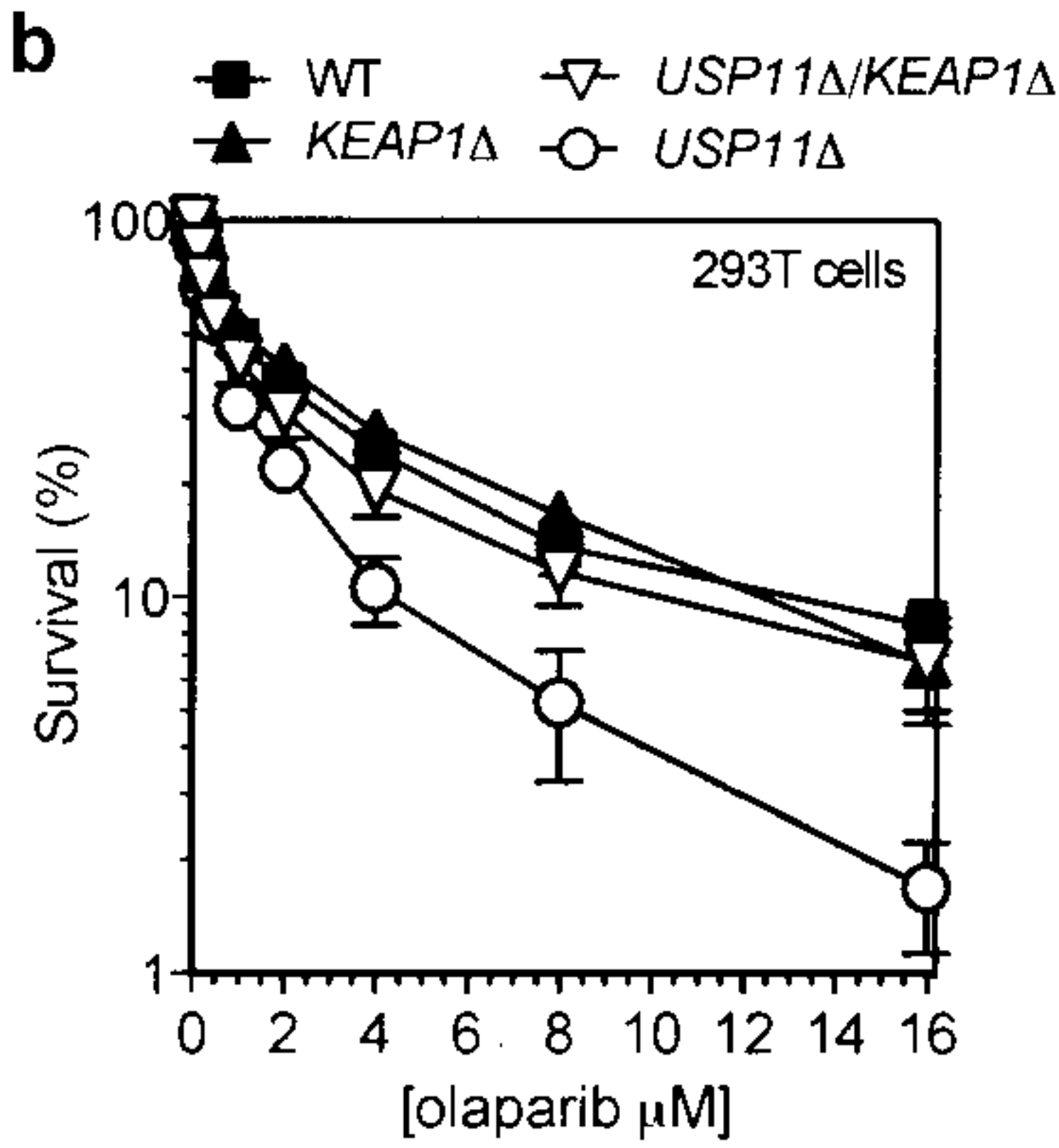


FIG 3c

FIG 3d

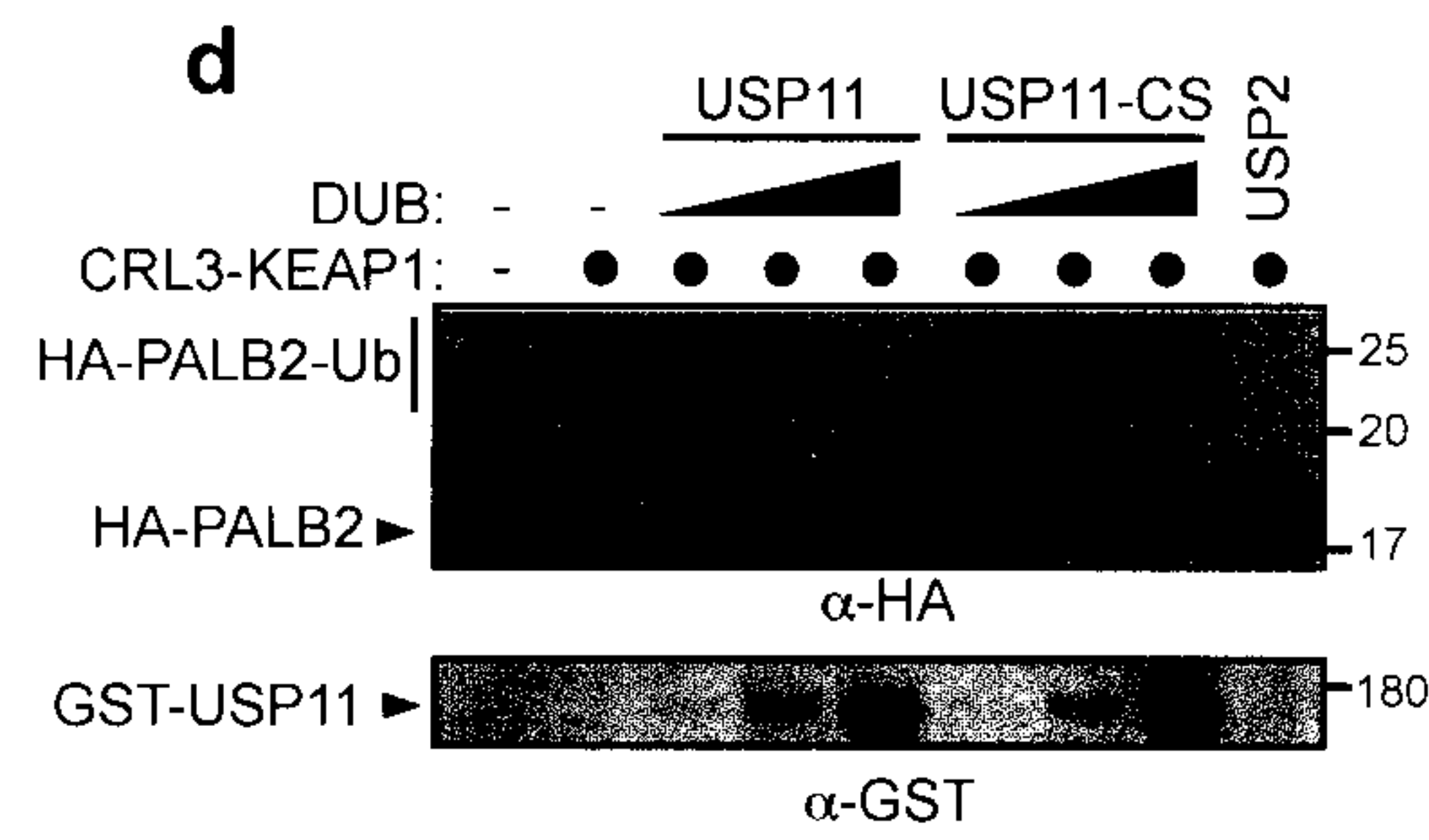


FIG 3e

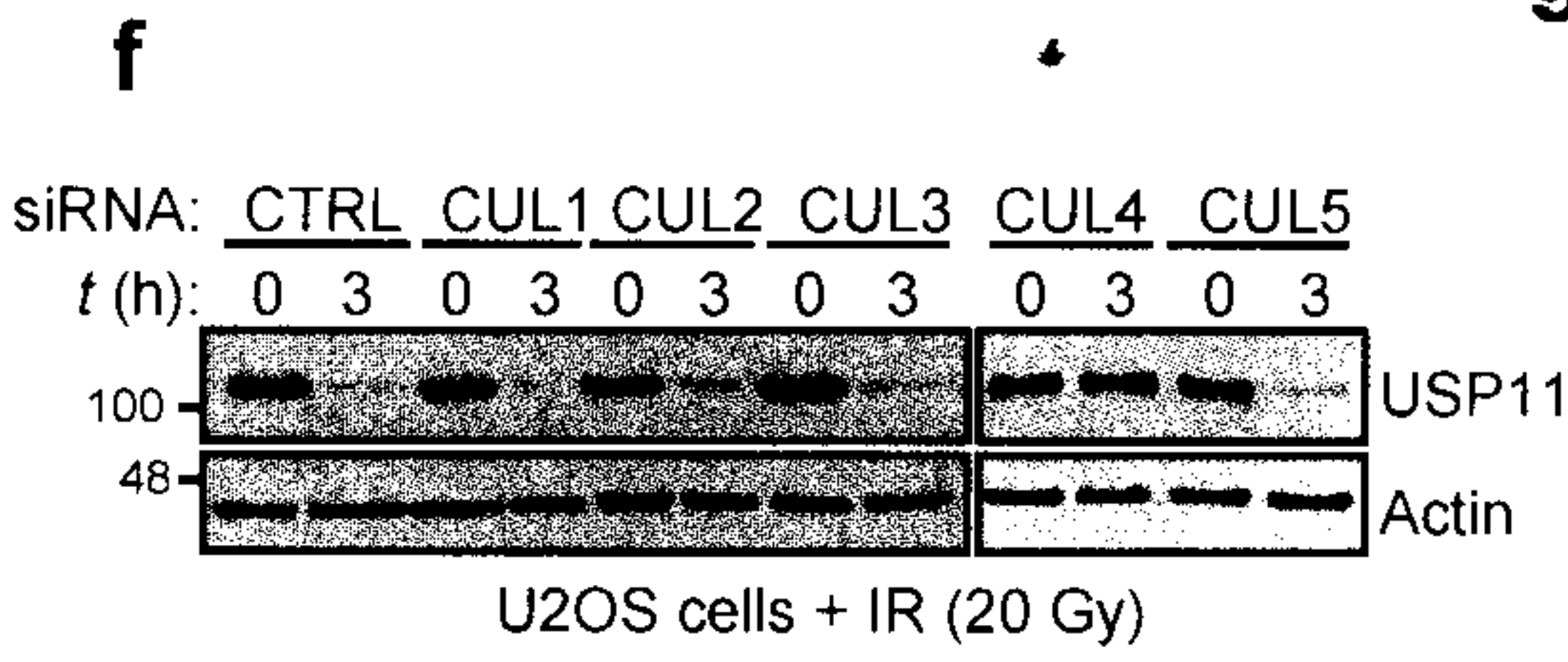
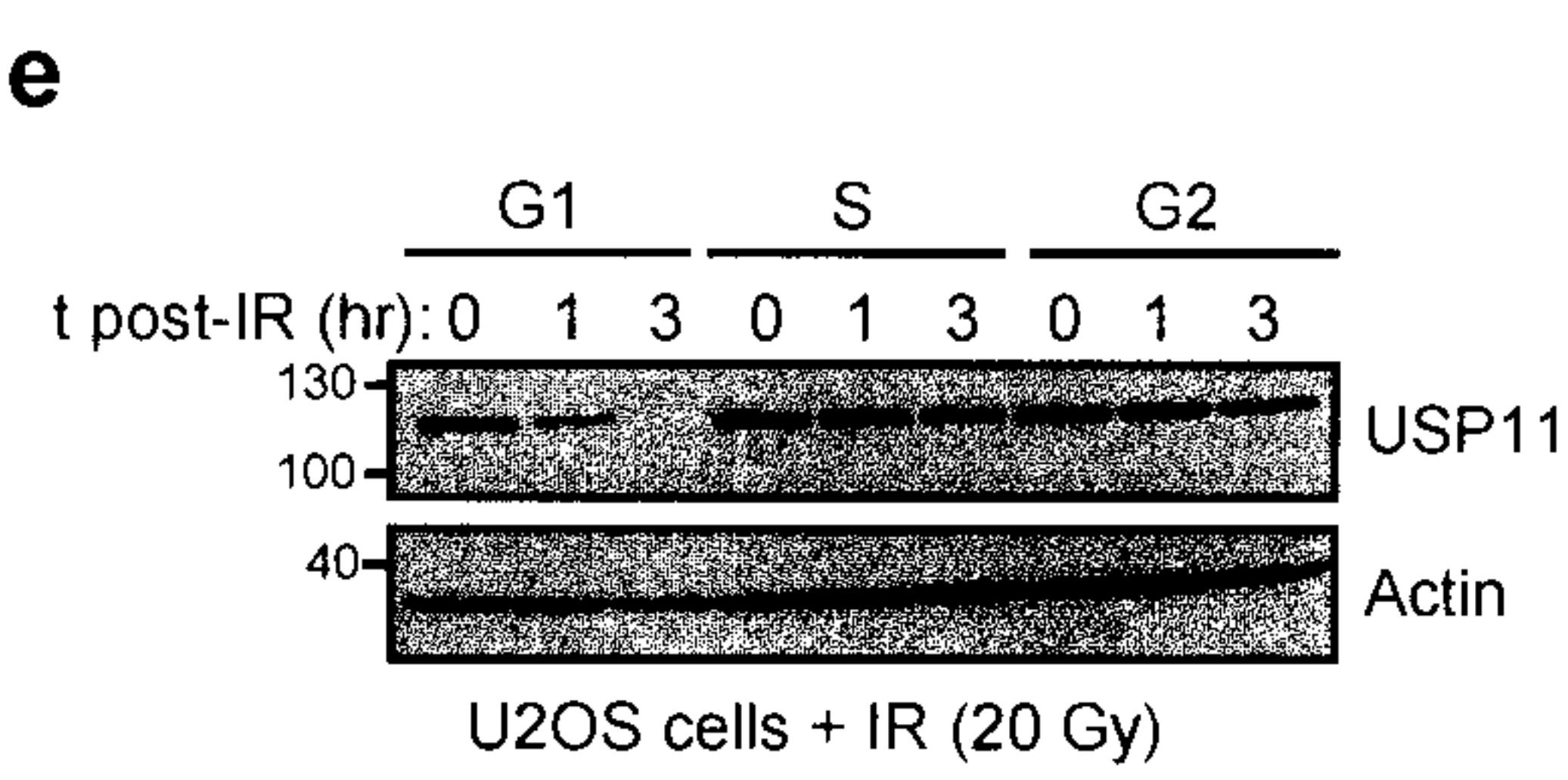


FIG 3f

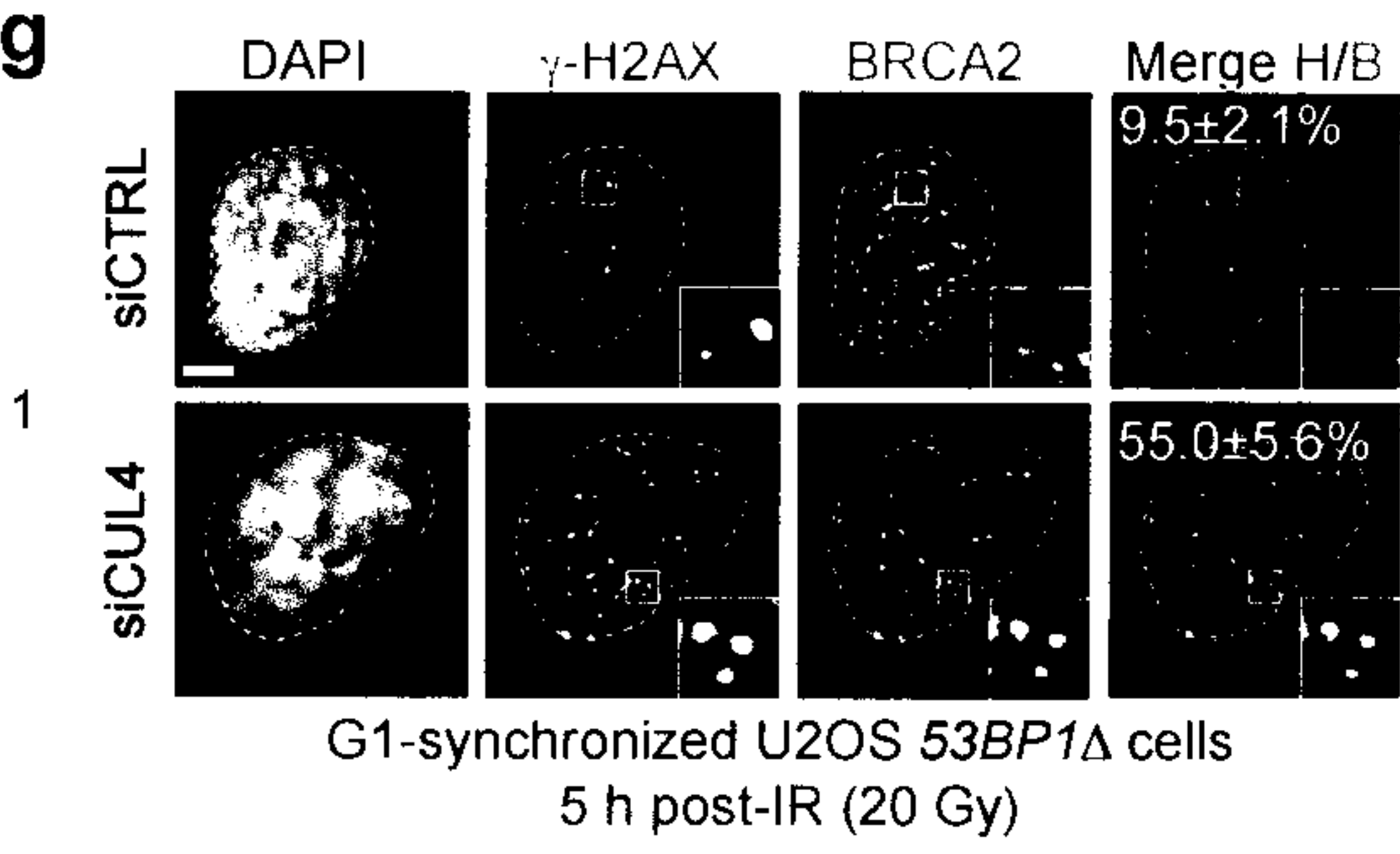


FIG 3g

FIG 4a

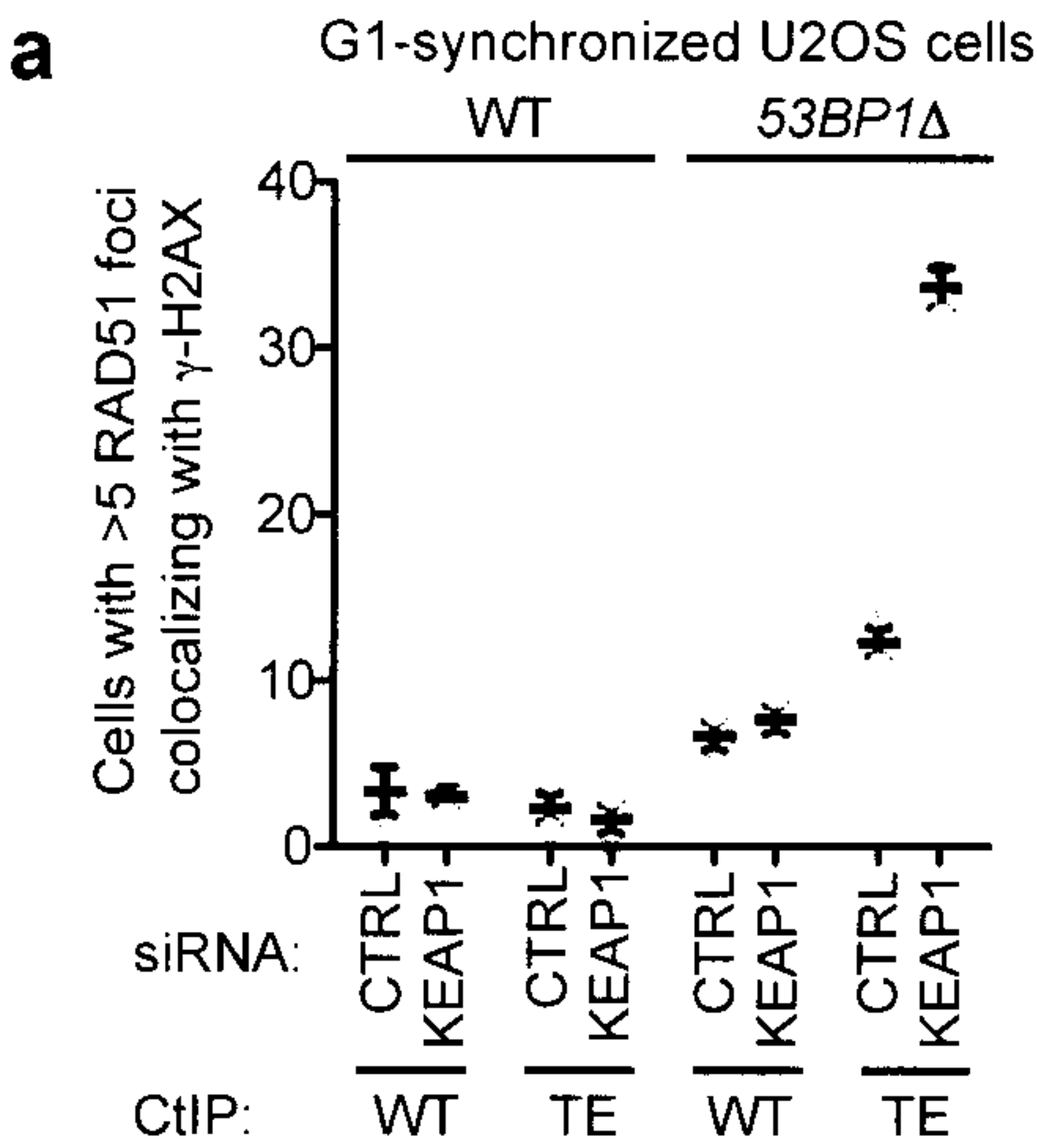


FIG 4b

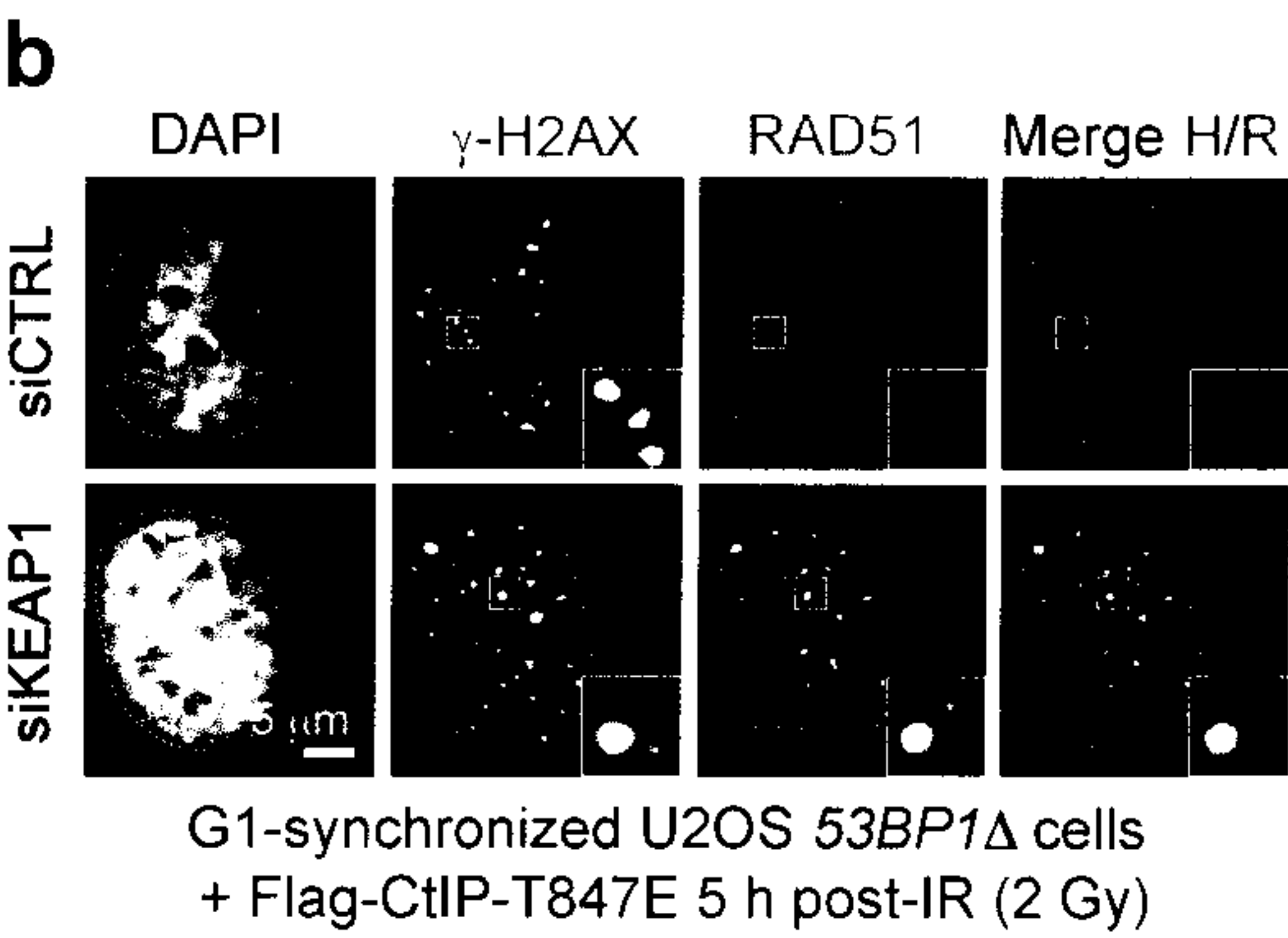


FIG 4c

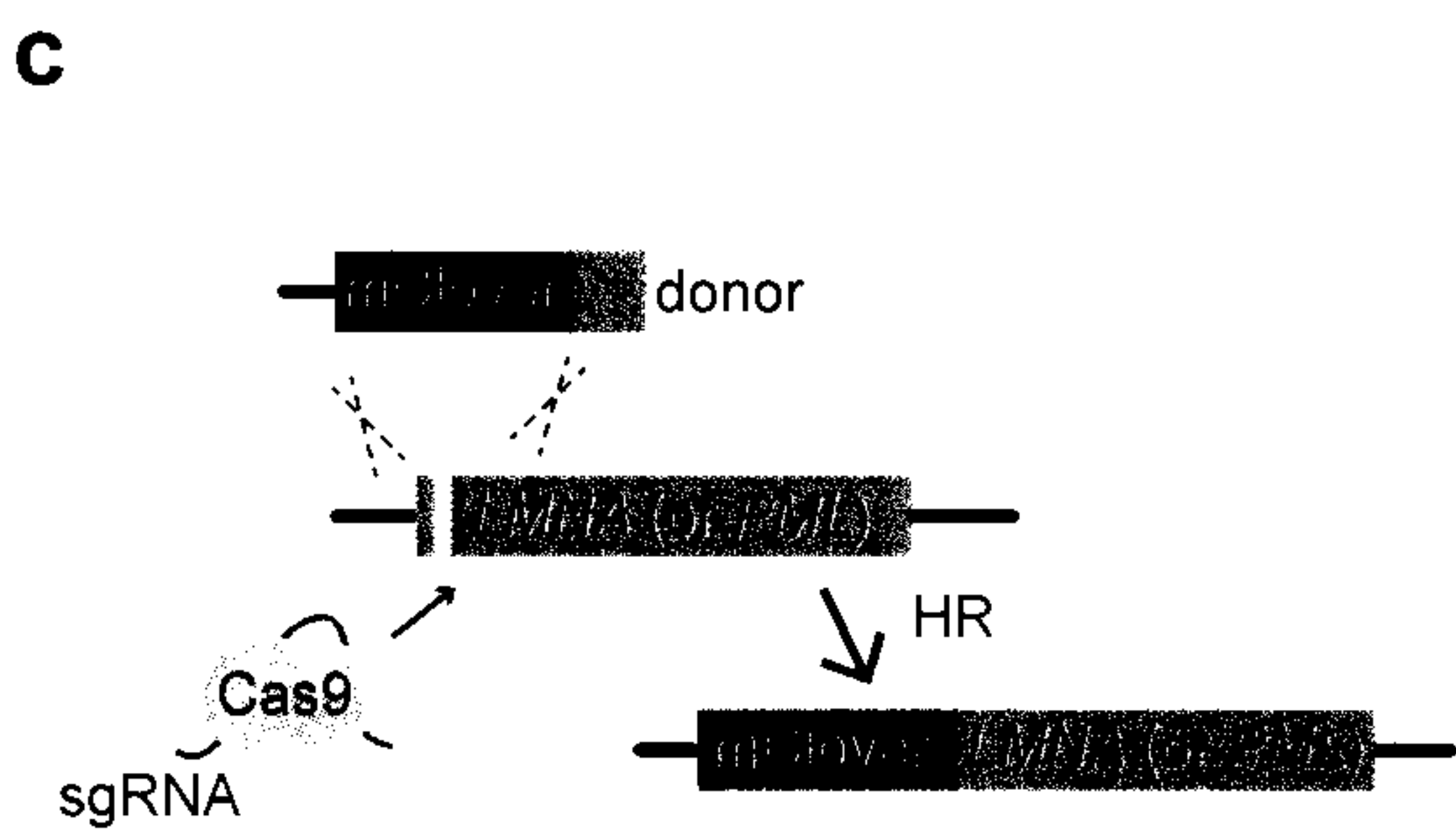


FIG 4d

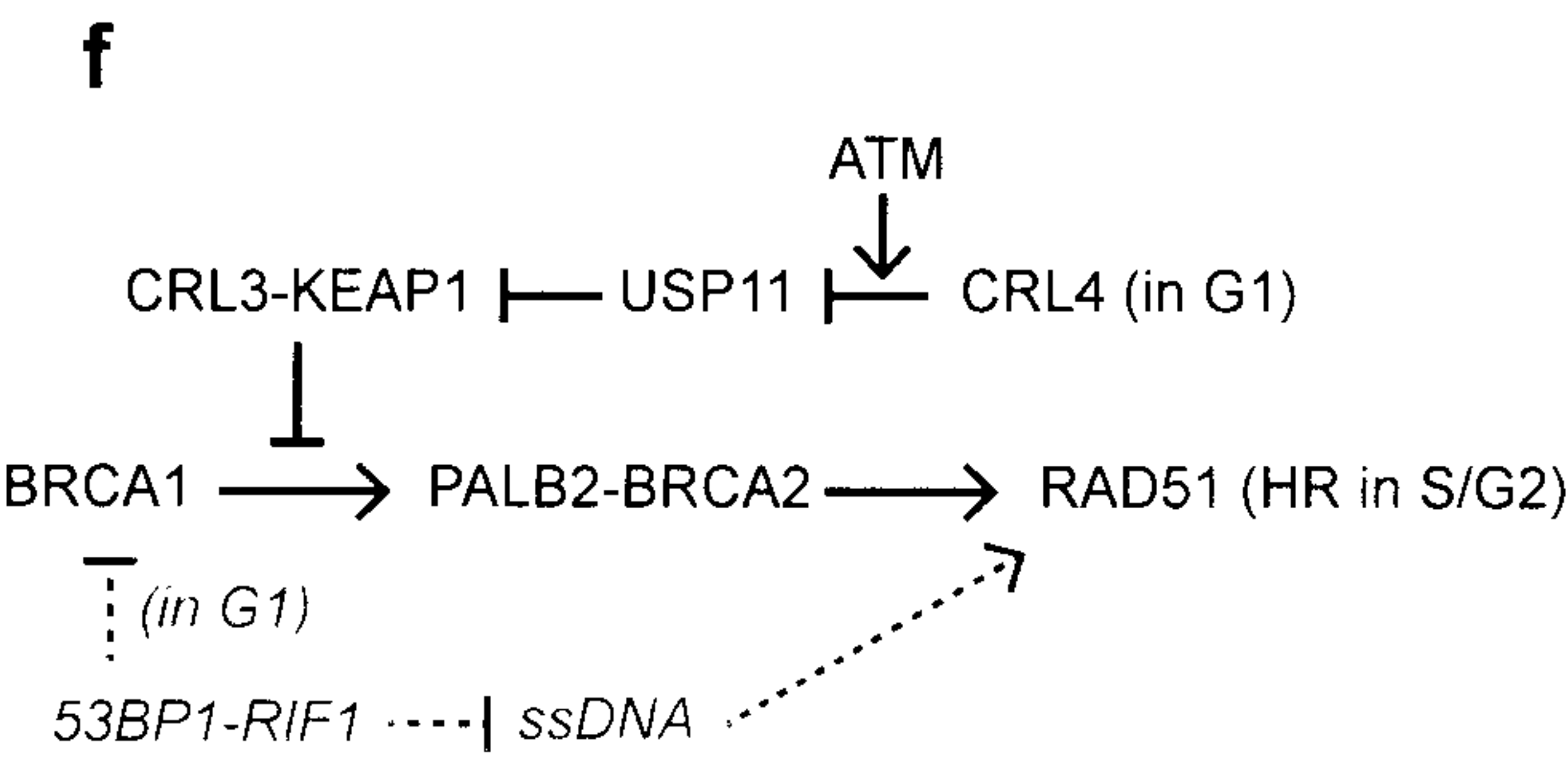
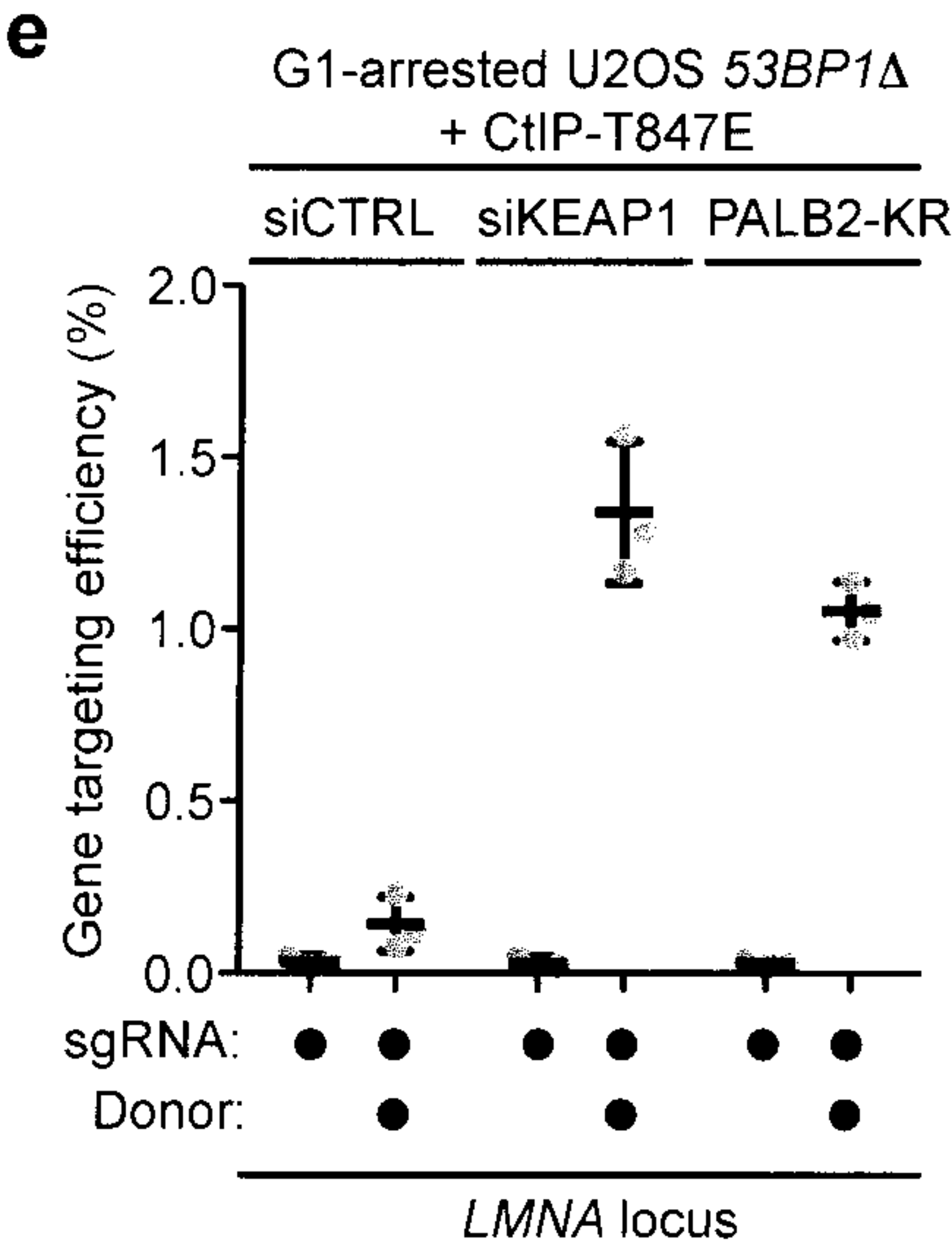
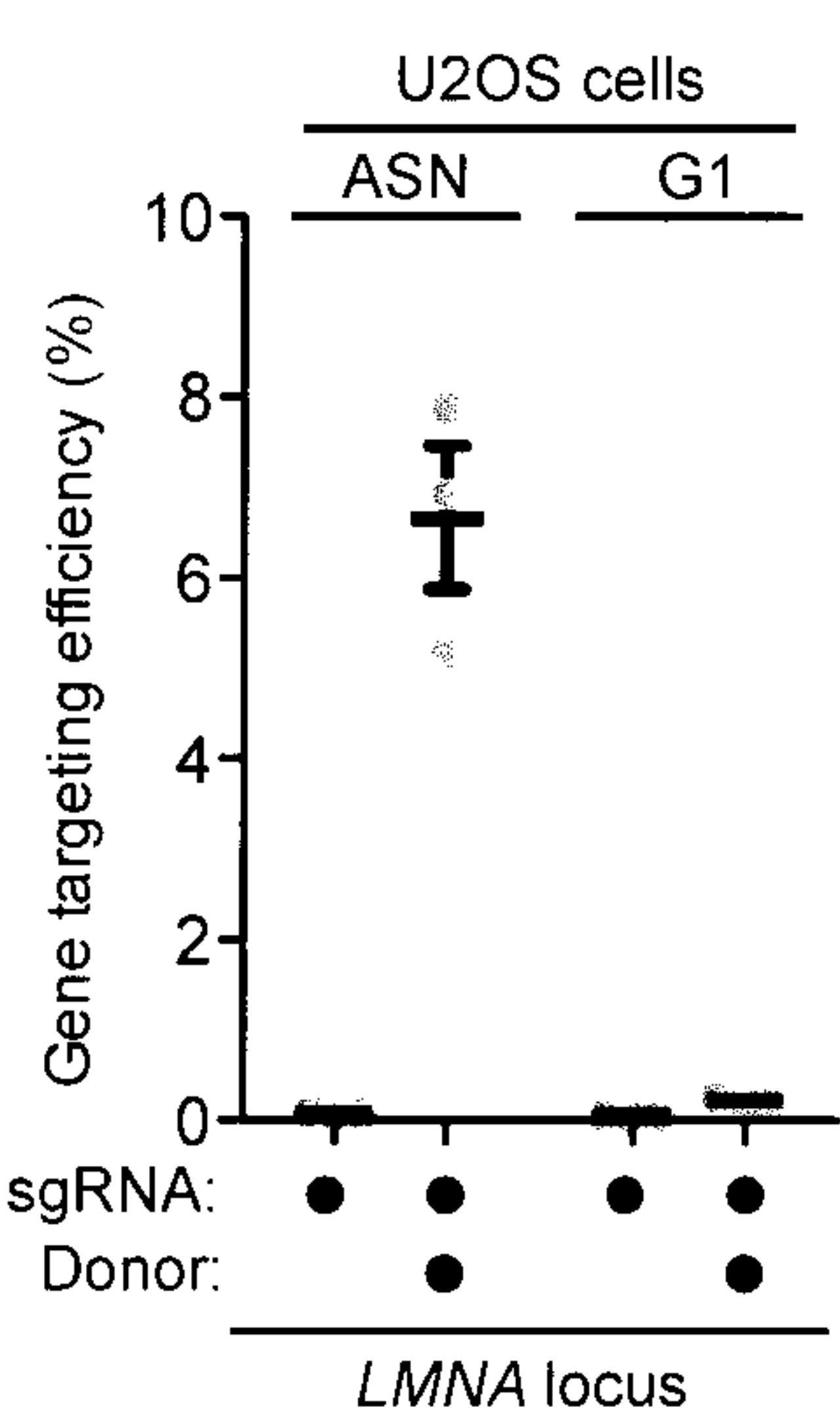


FIG 4f

FIG 4e

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FIG 5a

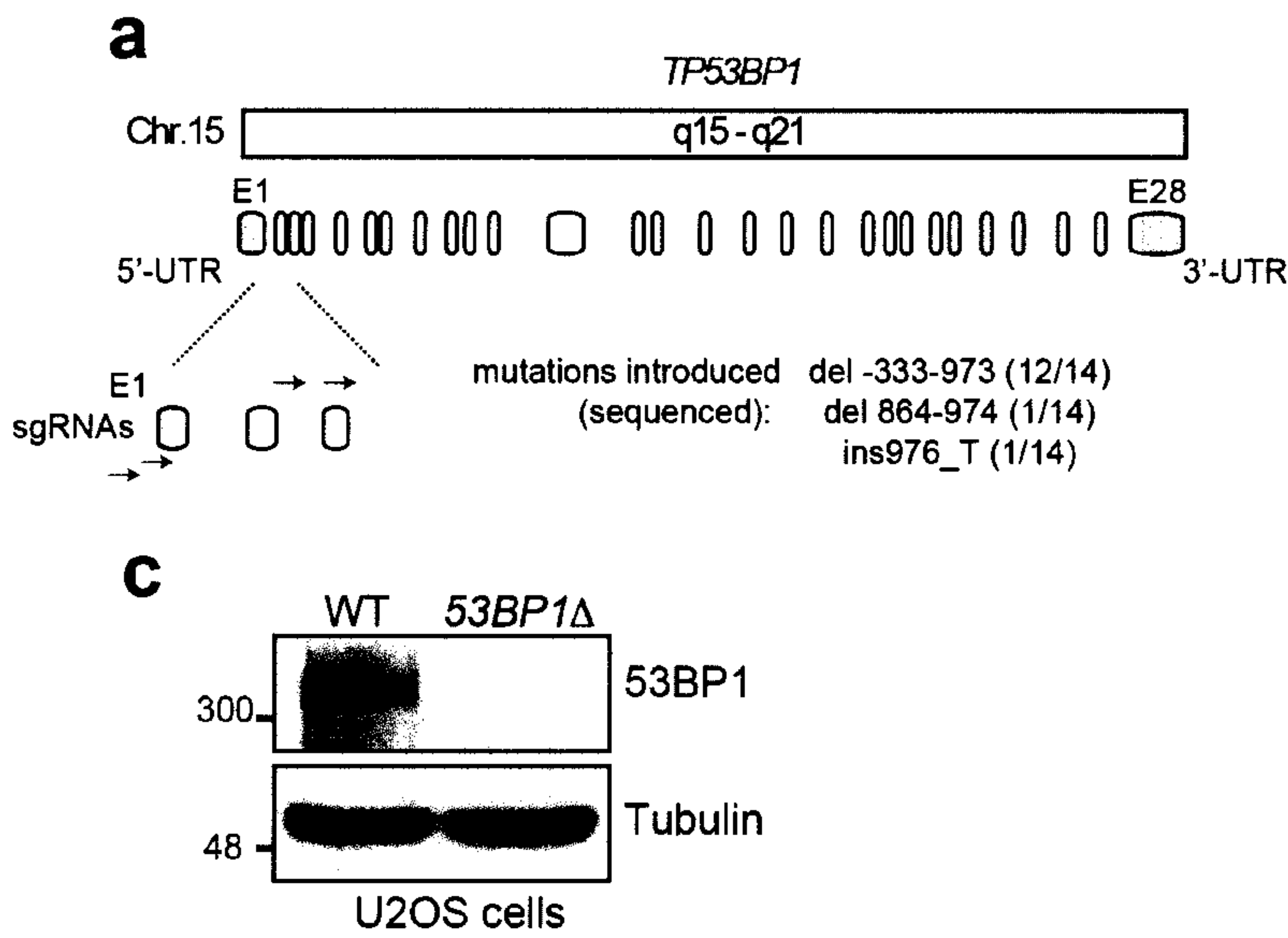
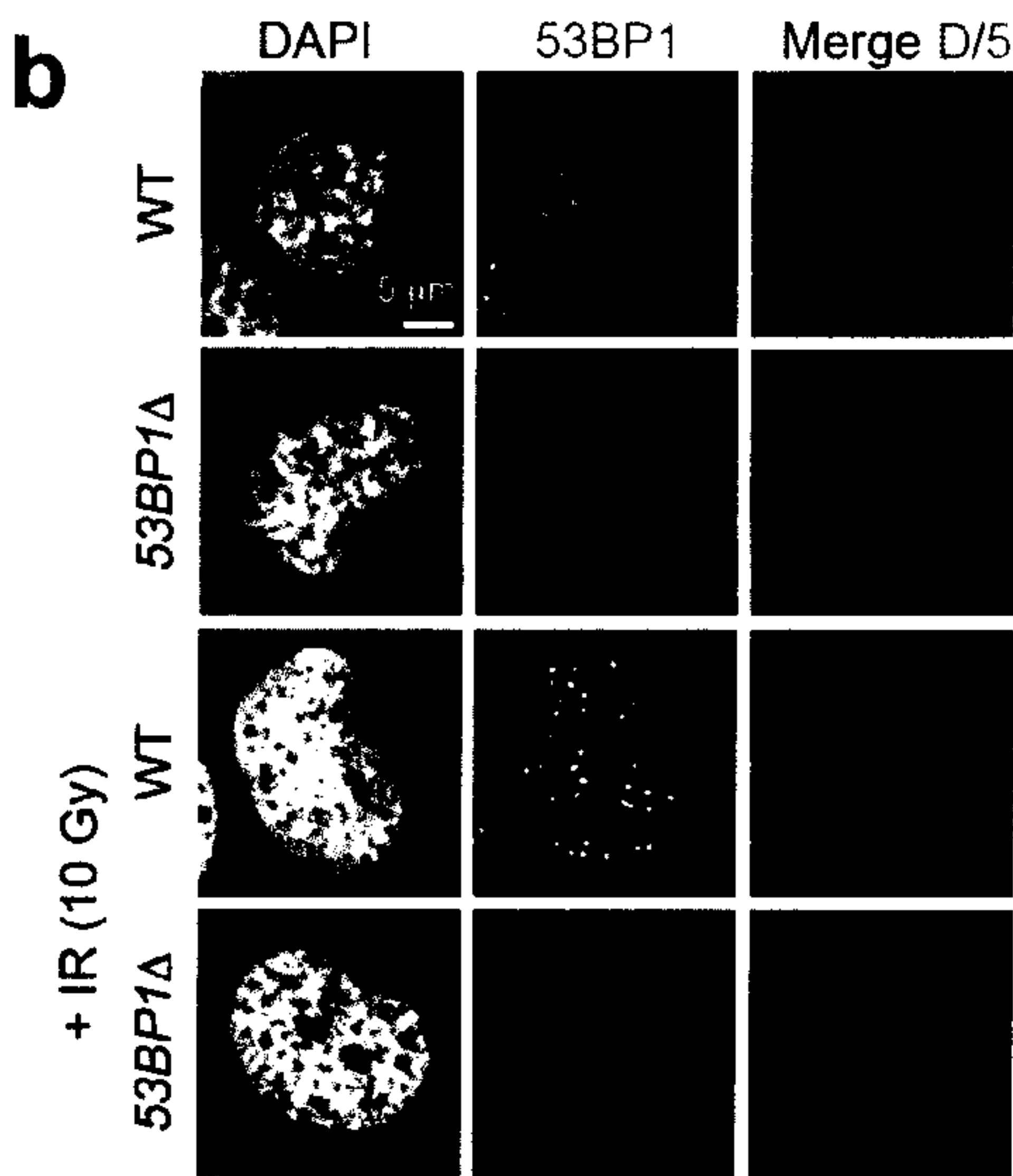


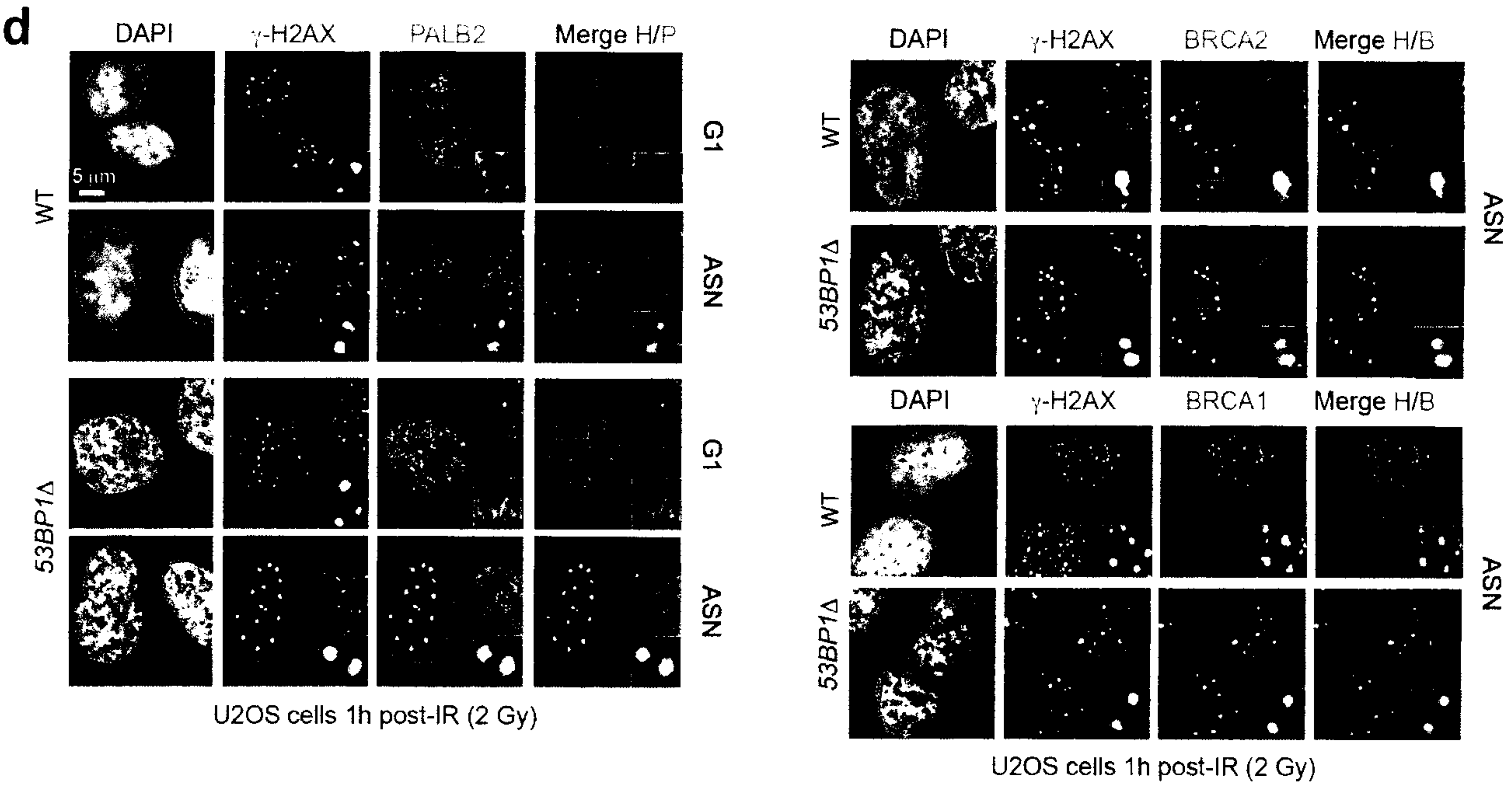
FIG 5c

FIG 5b



10/27

FIG 5d



e

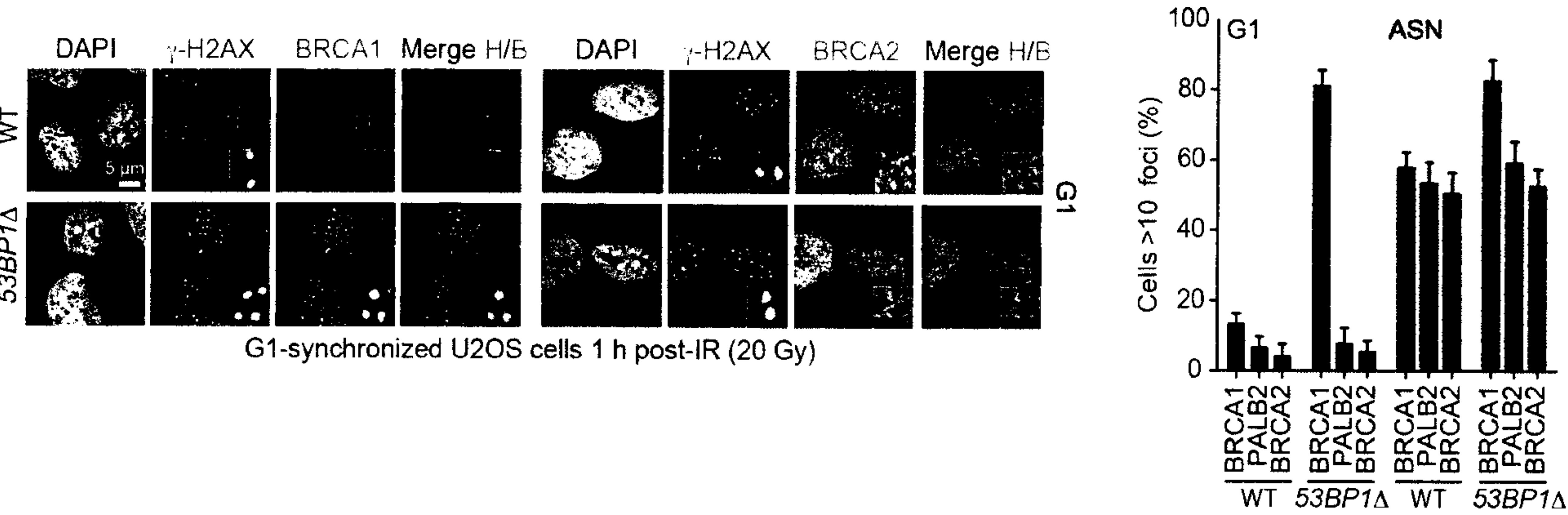


FIG 5e

11/27

FIG 6a

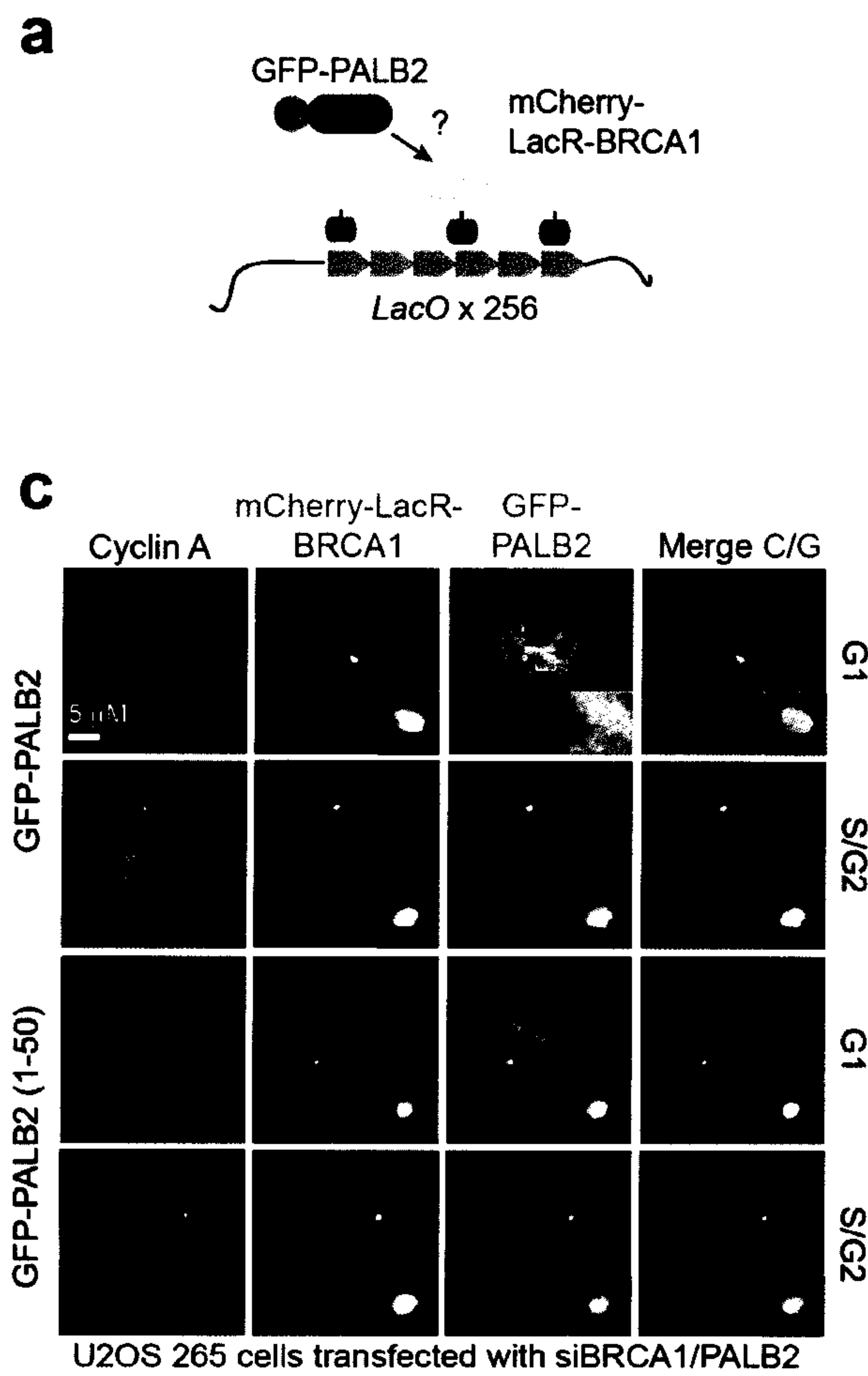


FIG 6c

FIG 6b

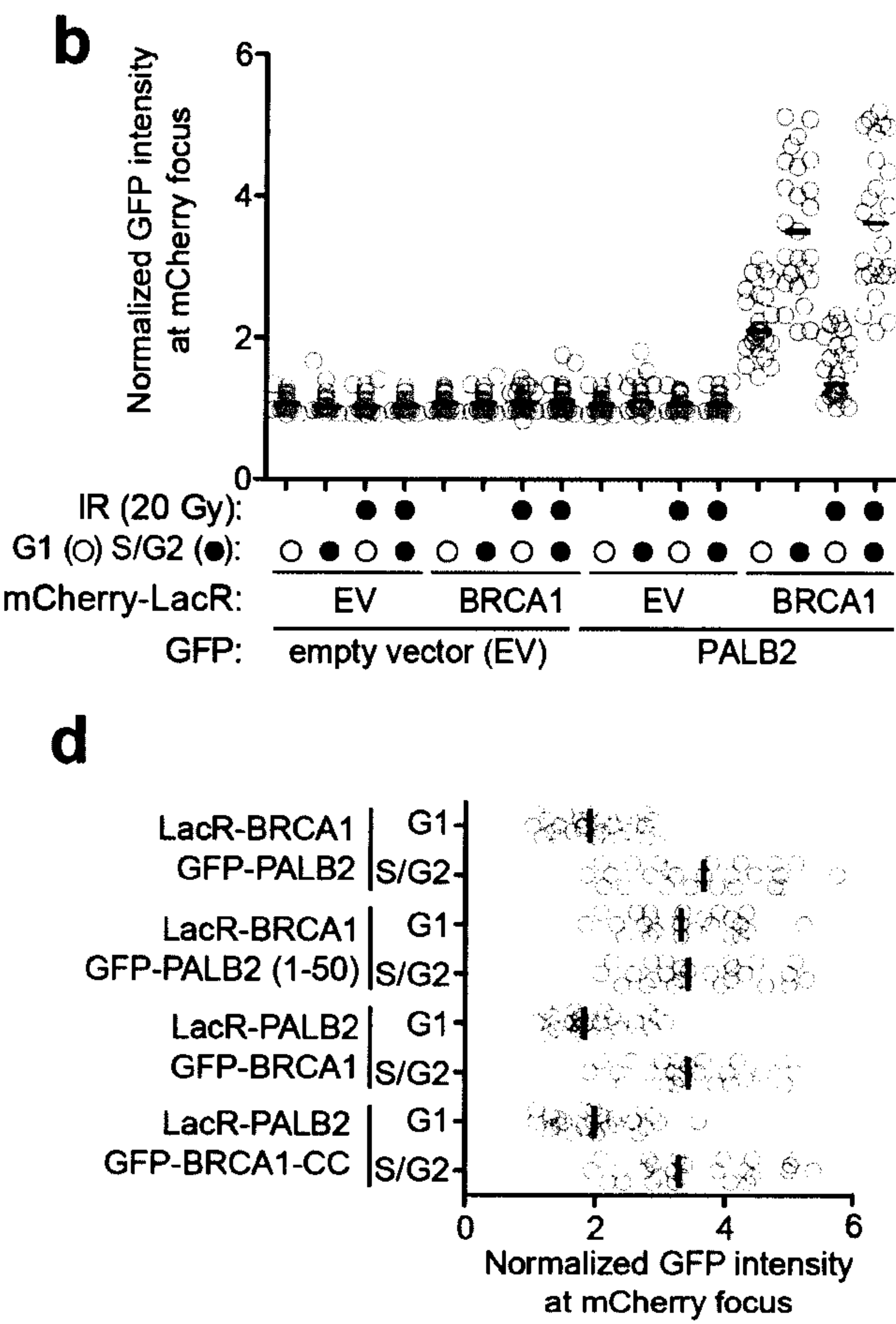


FIG 6d

12/27

FIG 6e

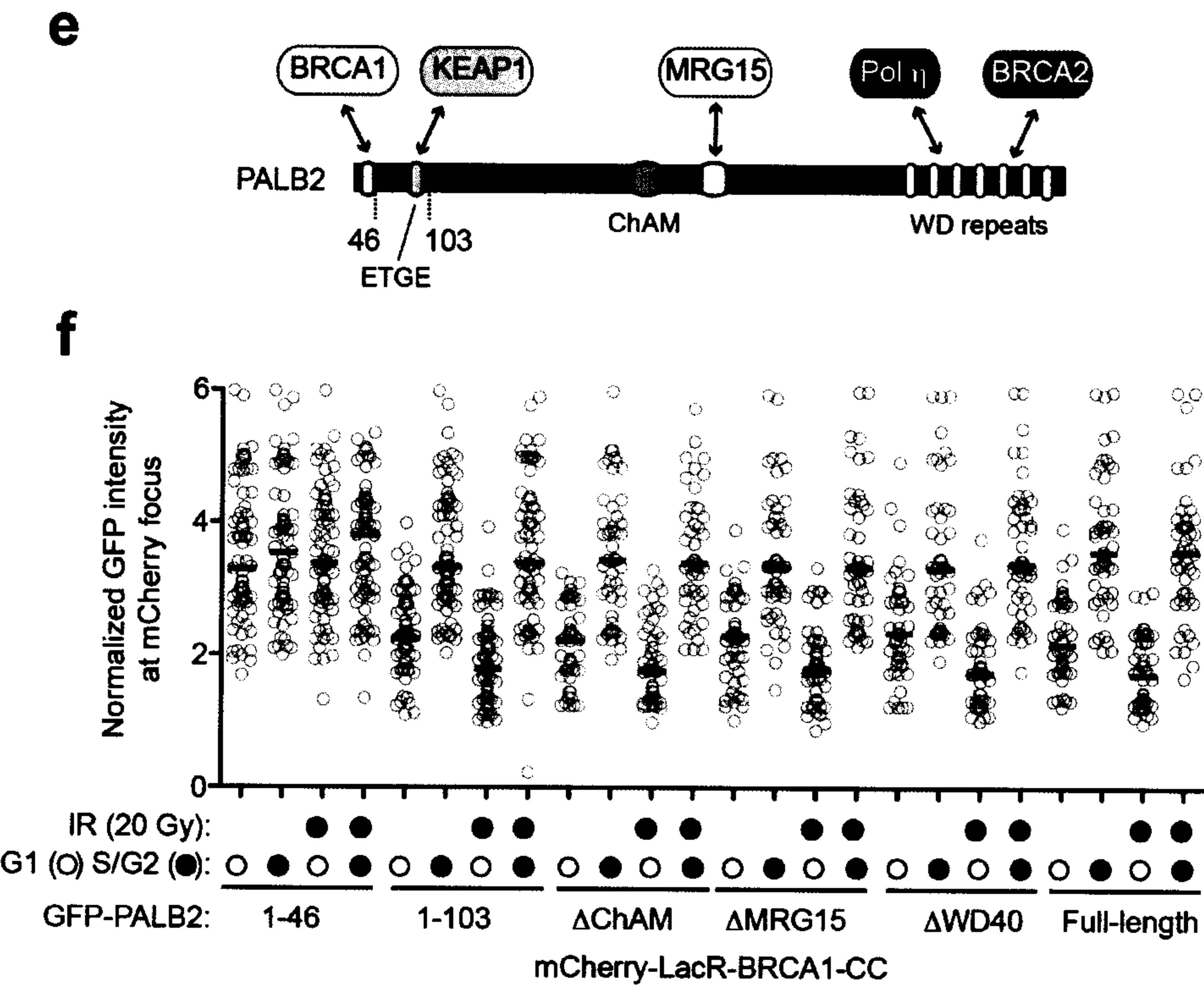


FIG 6F

FIG 7a

FIG 7c

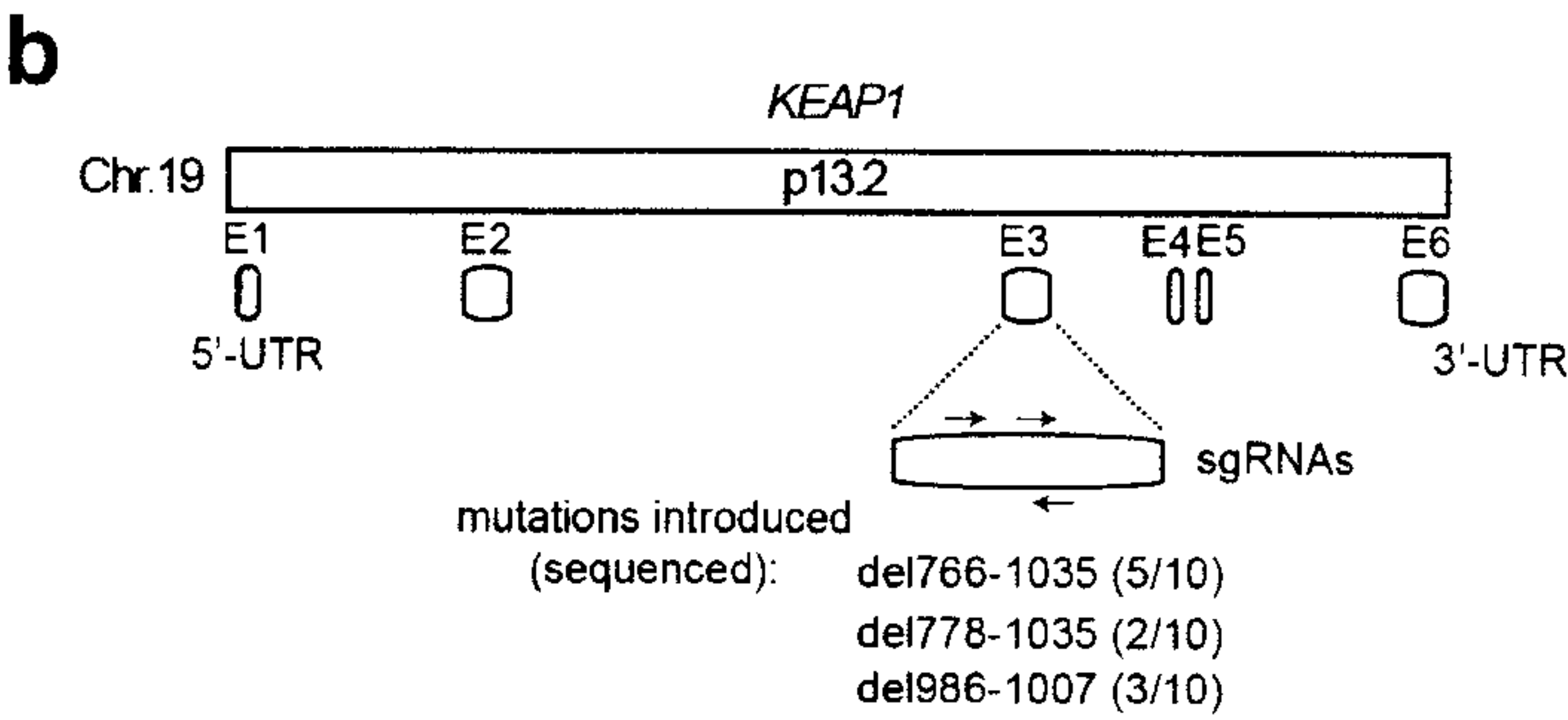
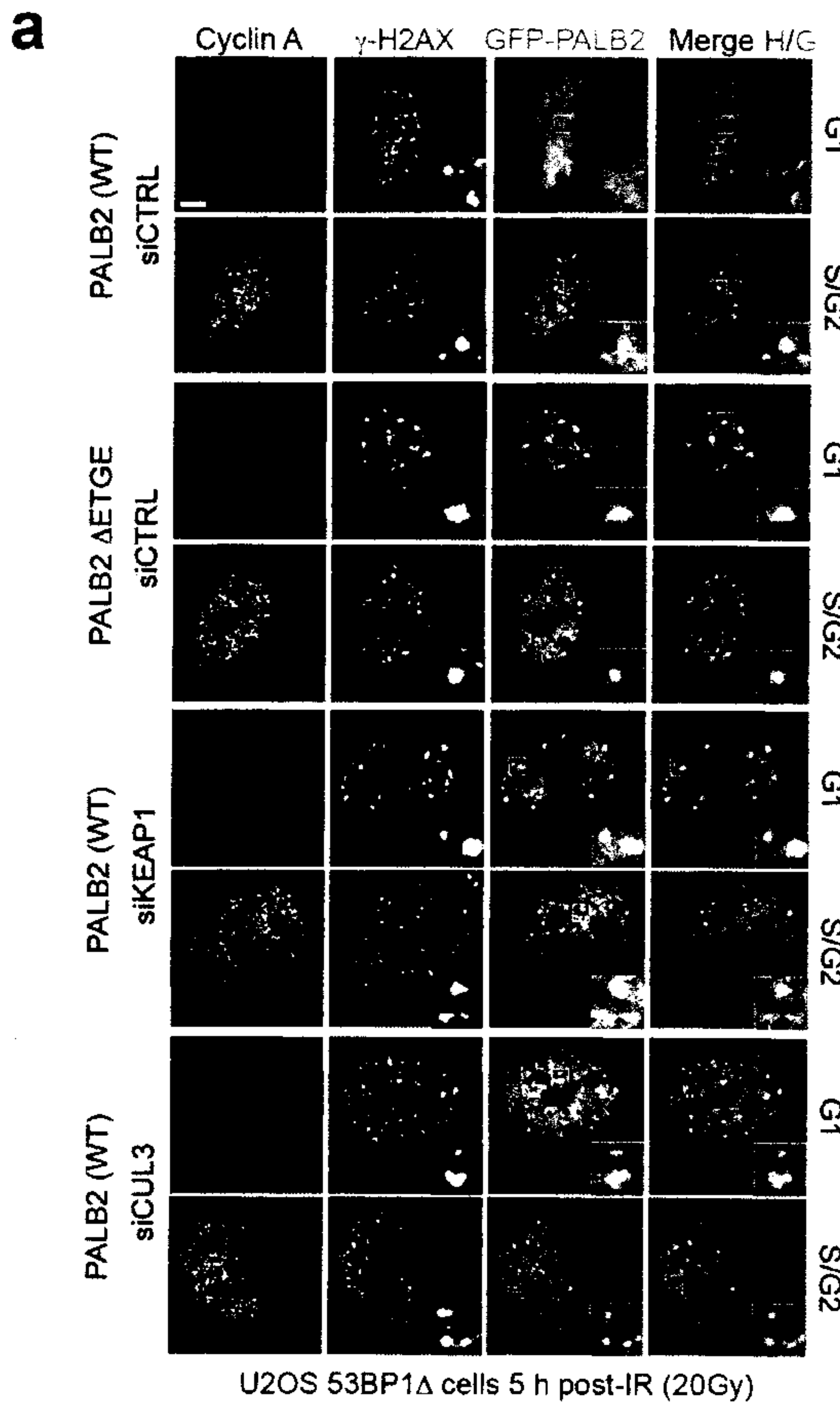


FIG 7b

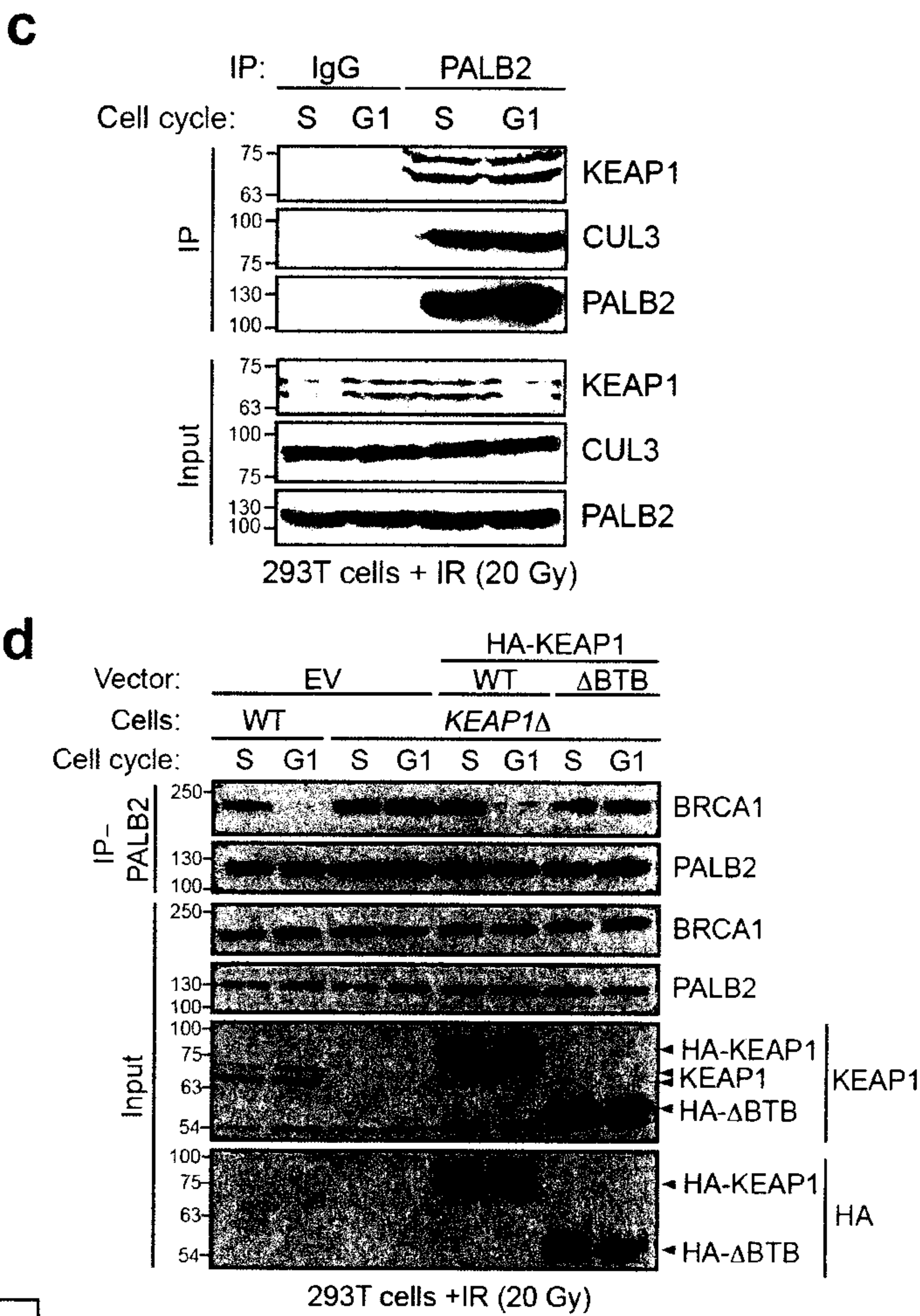


FIG 7d

FIG 7e

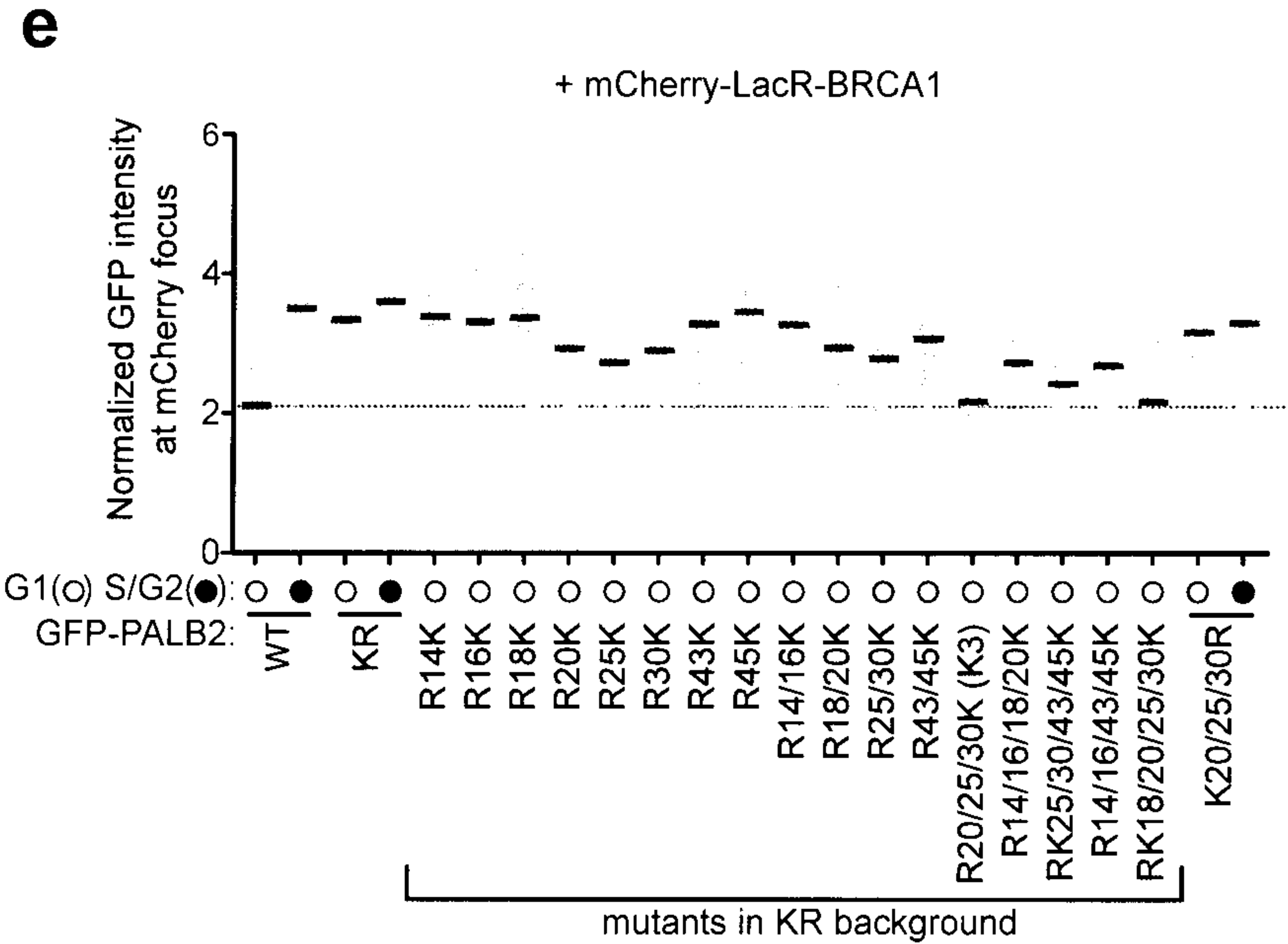


FIG 7f

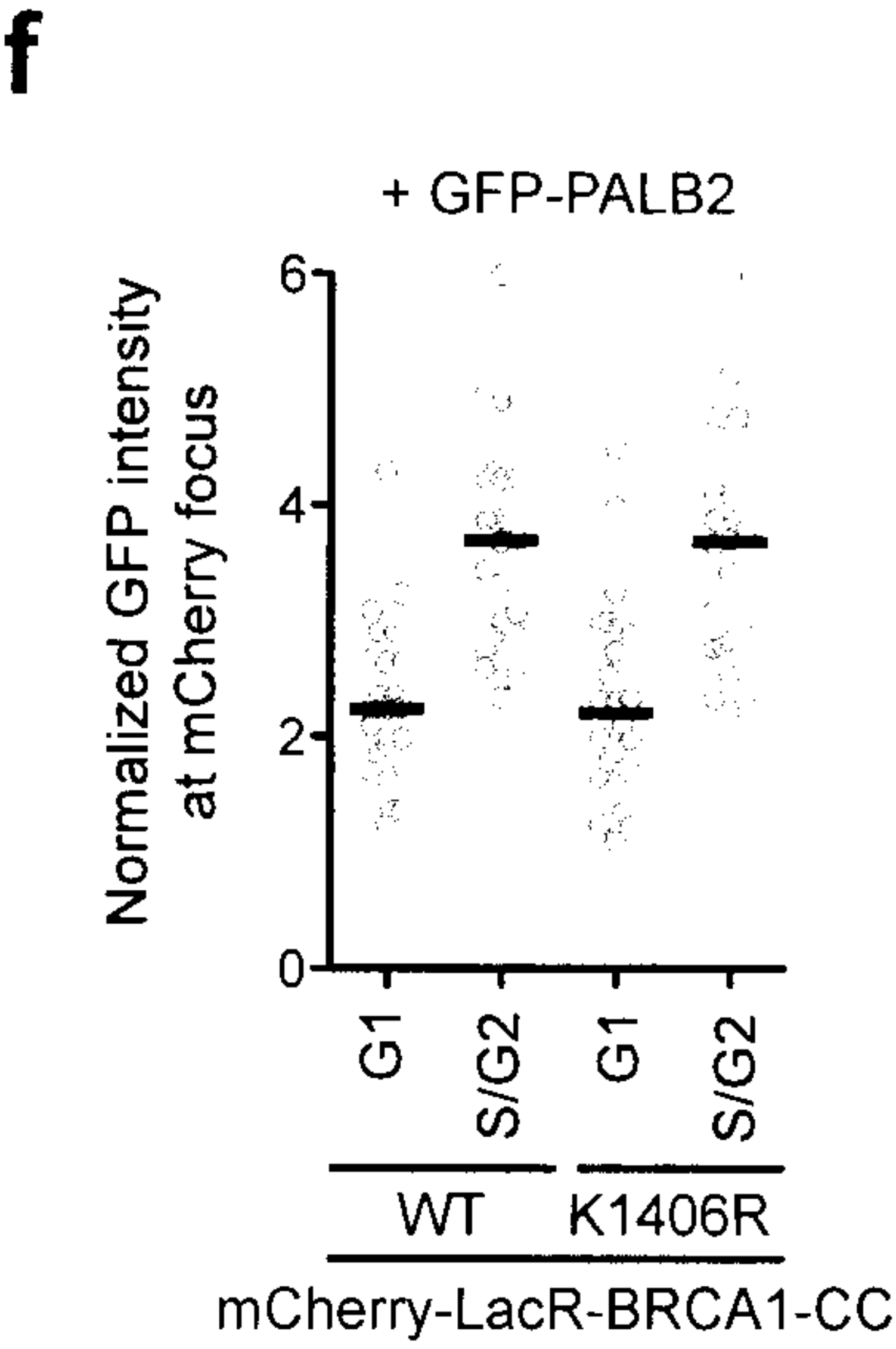


FIG 8a

FIG 8b

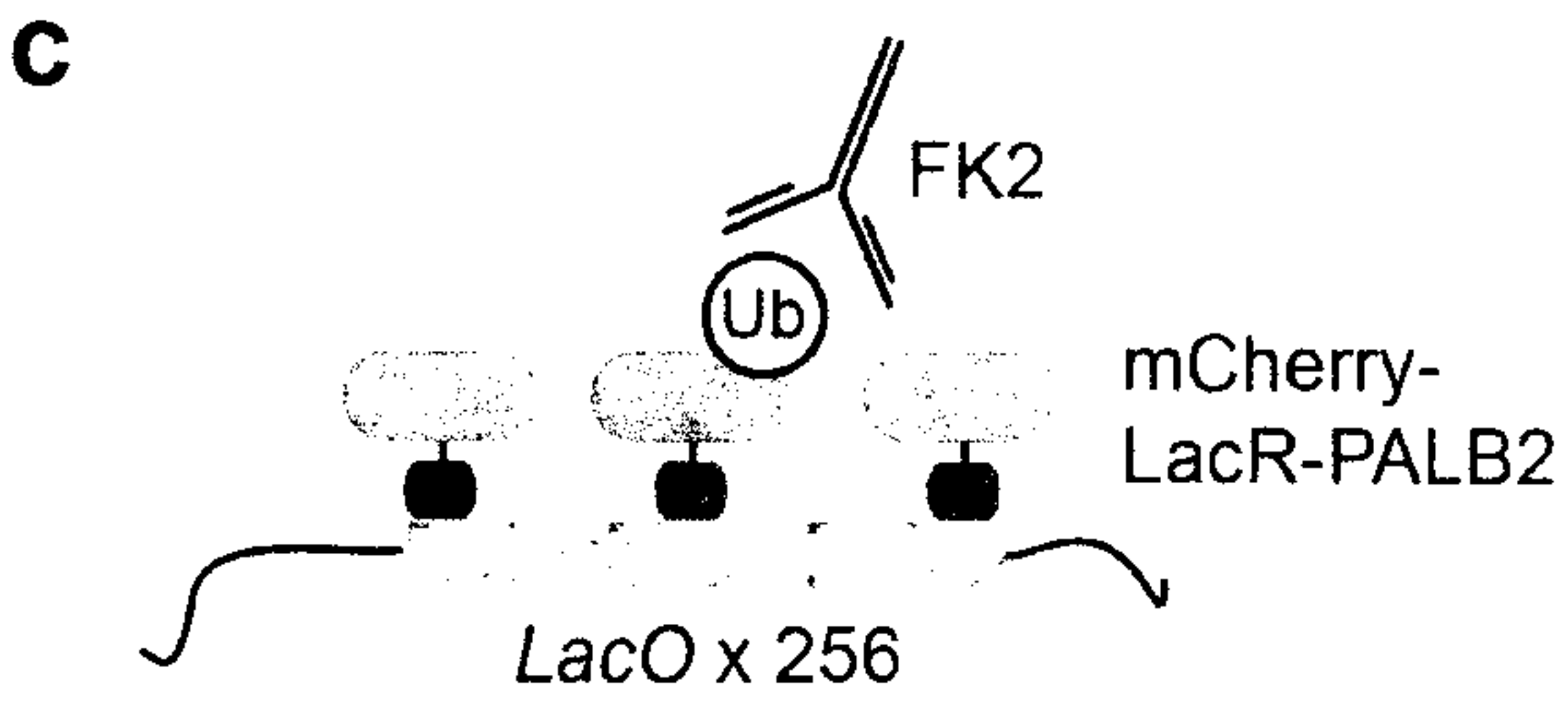
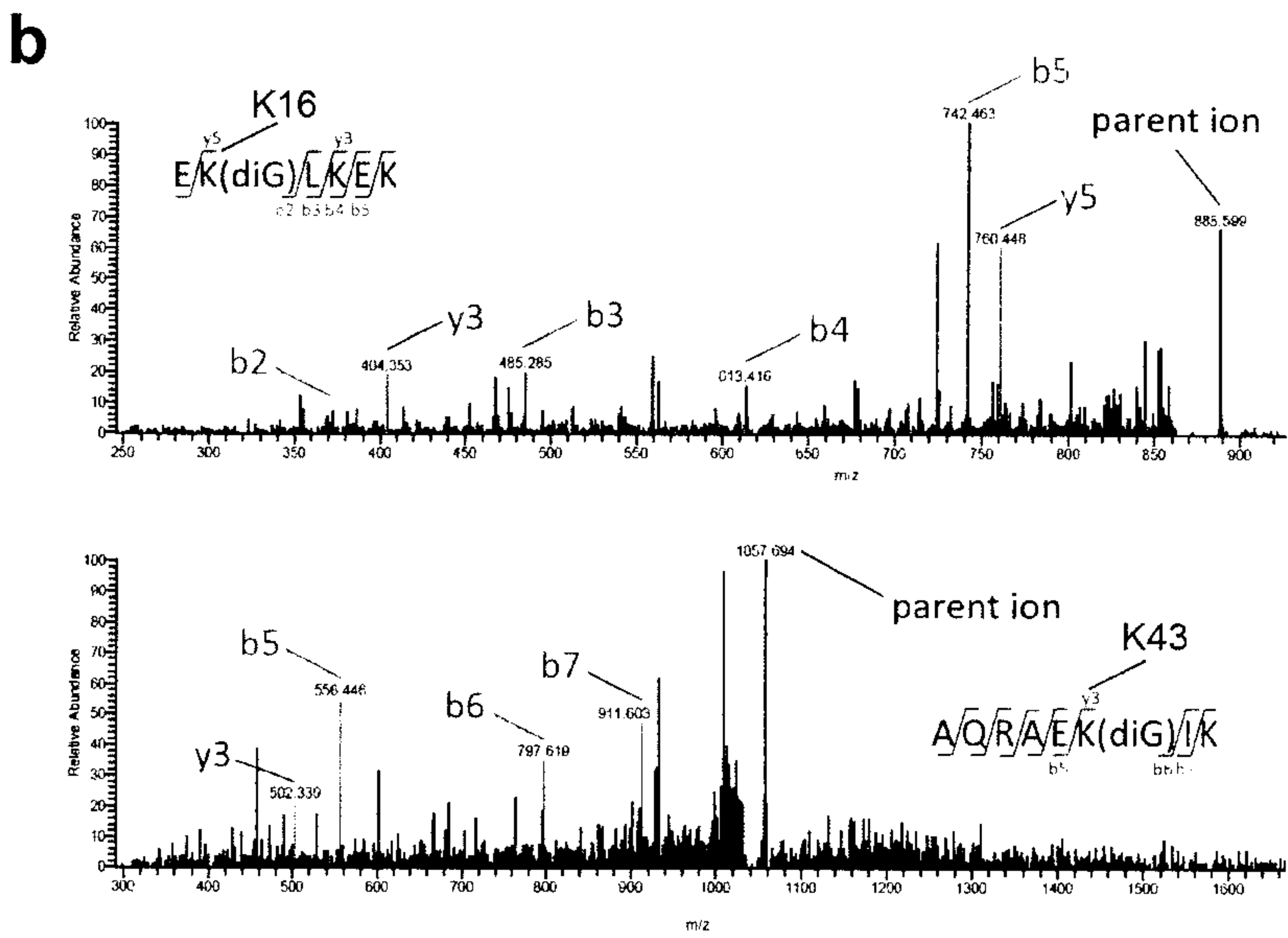
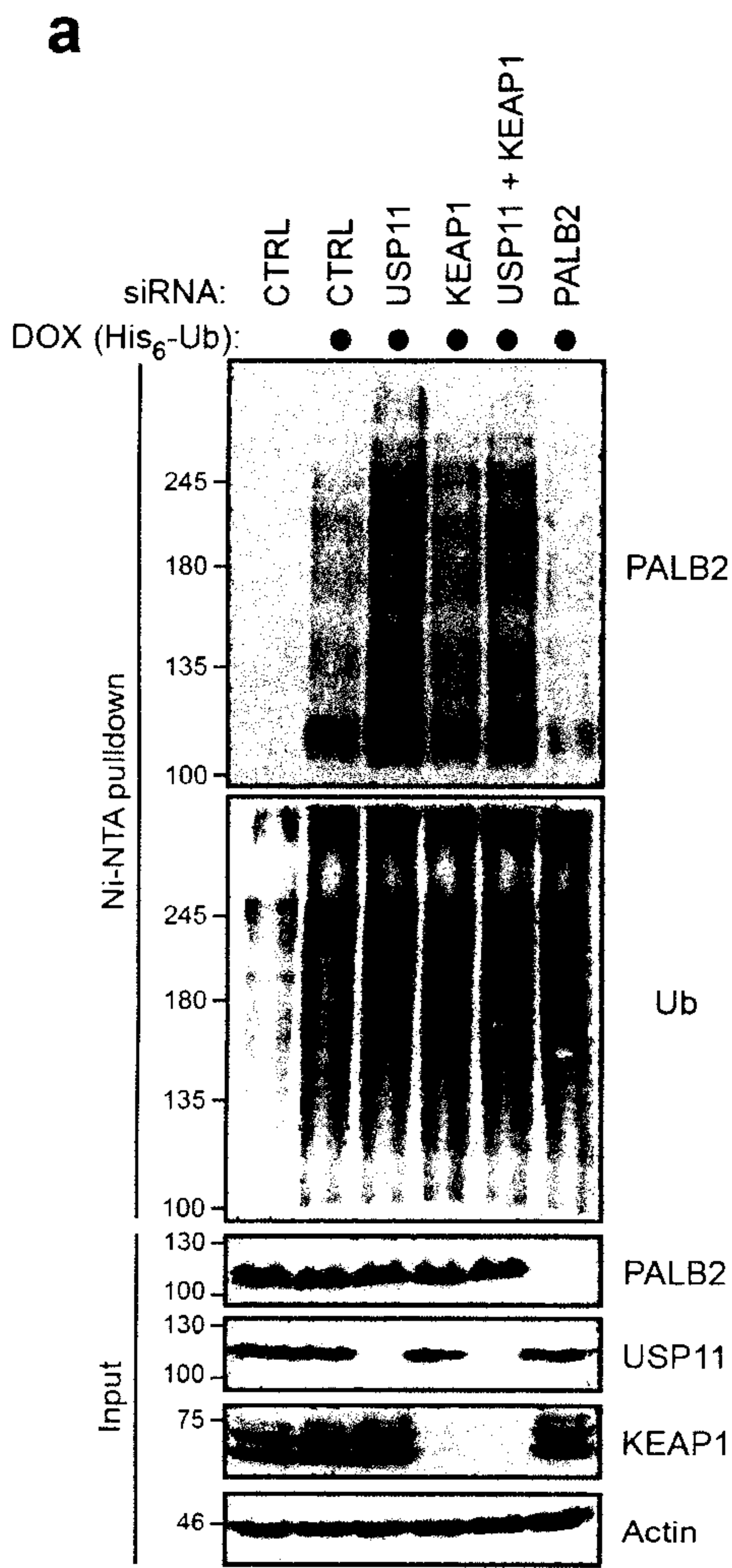


FIG 8c

FIG 8d

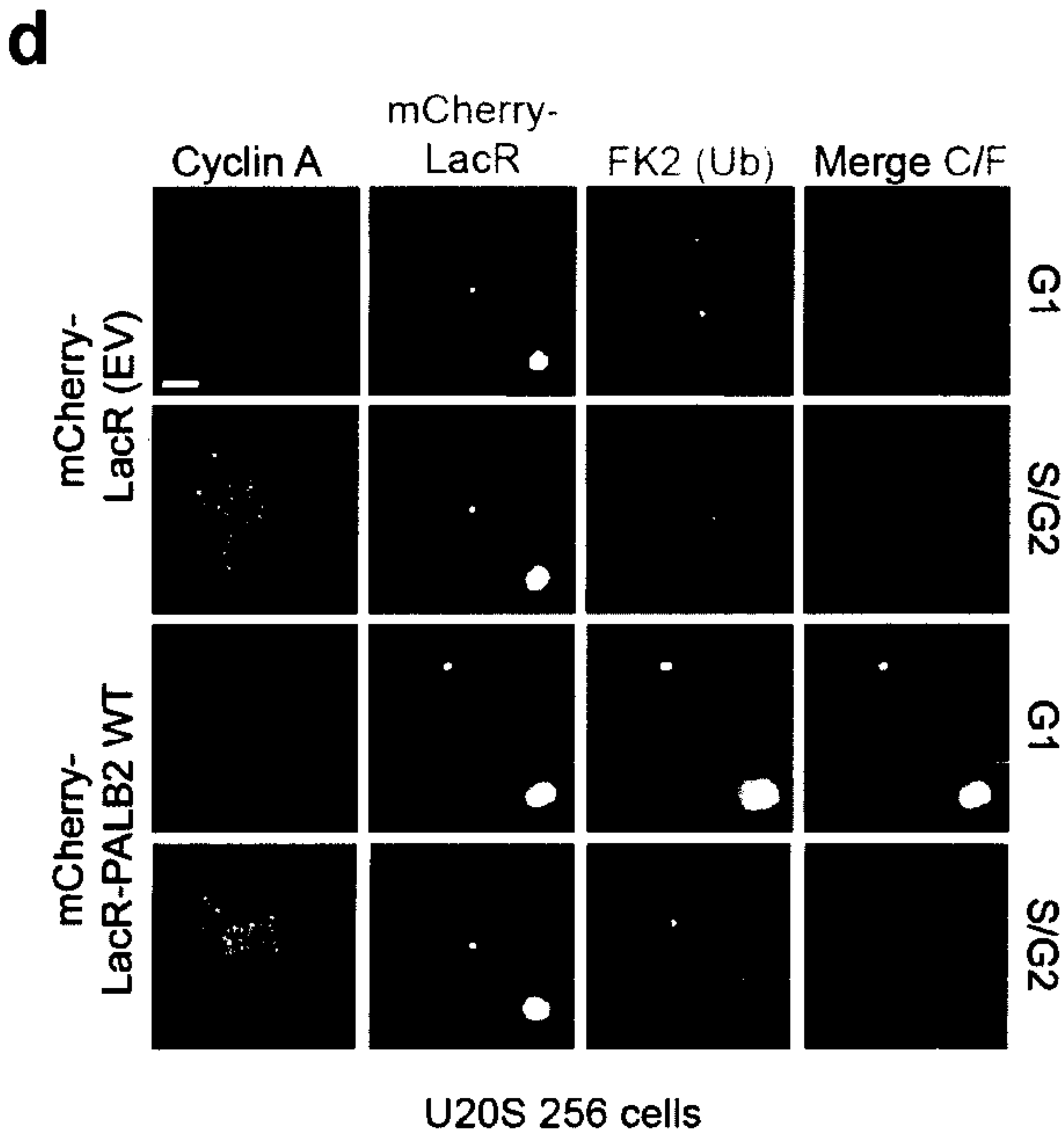


FIG 8e

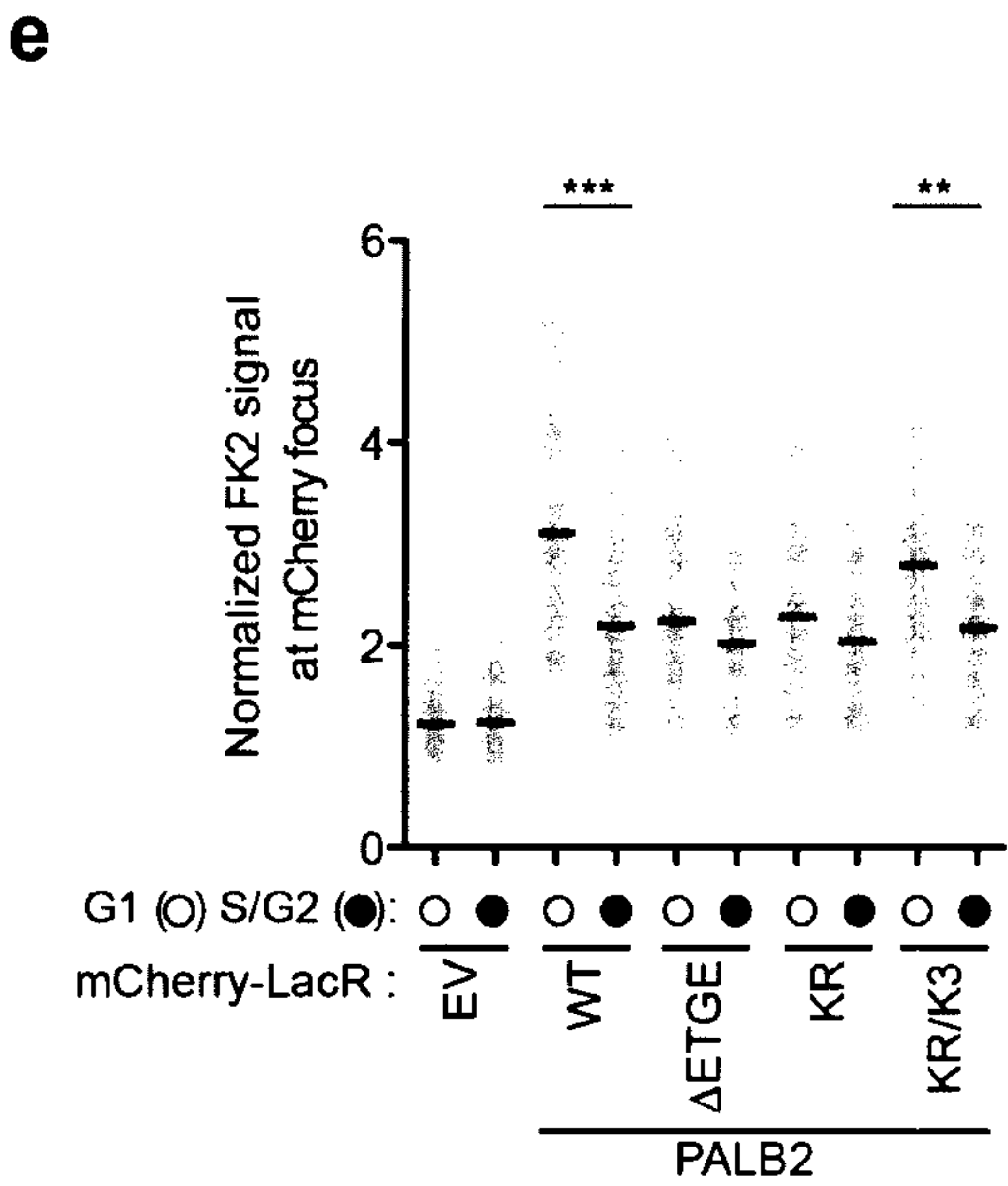


FIG 9a

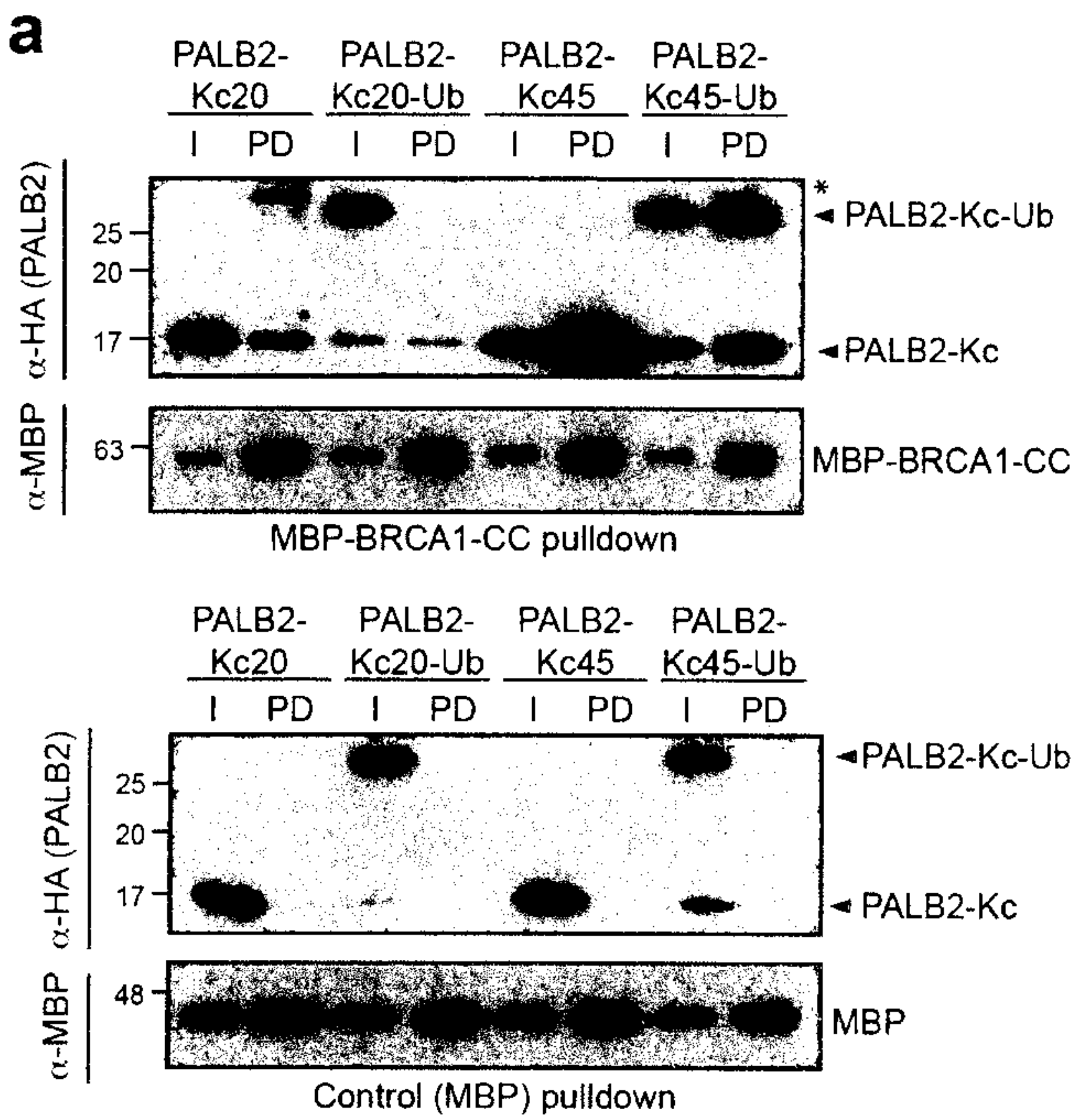


FIG 9b

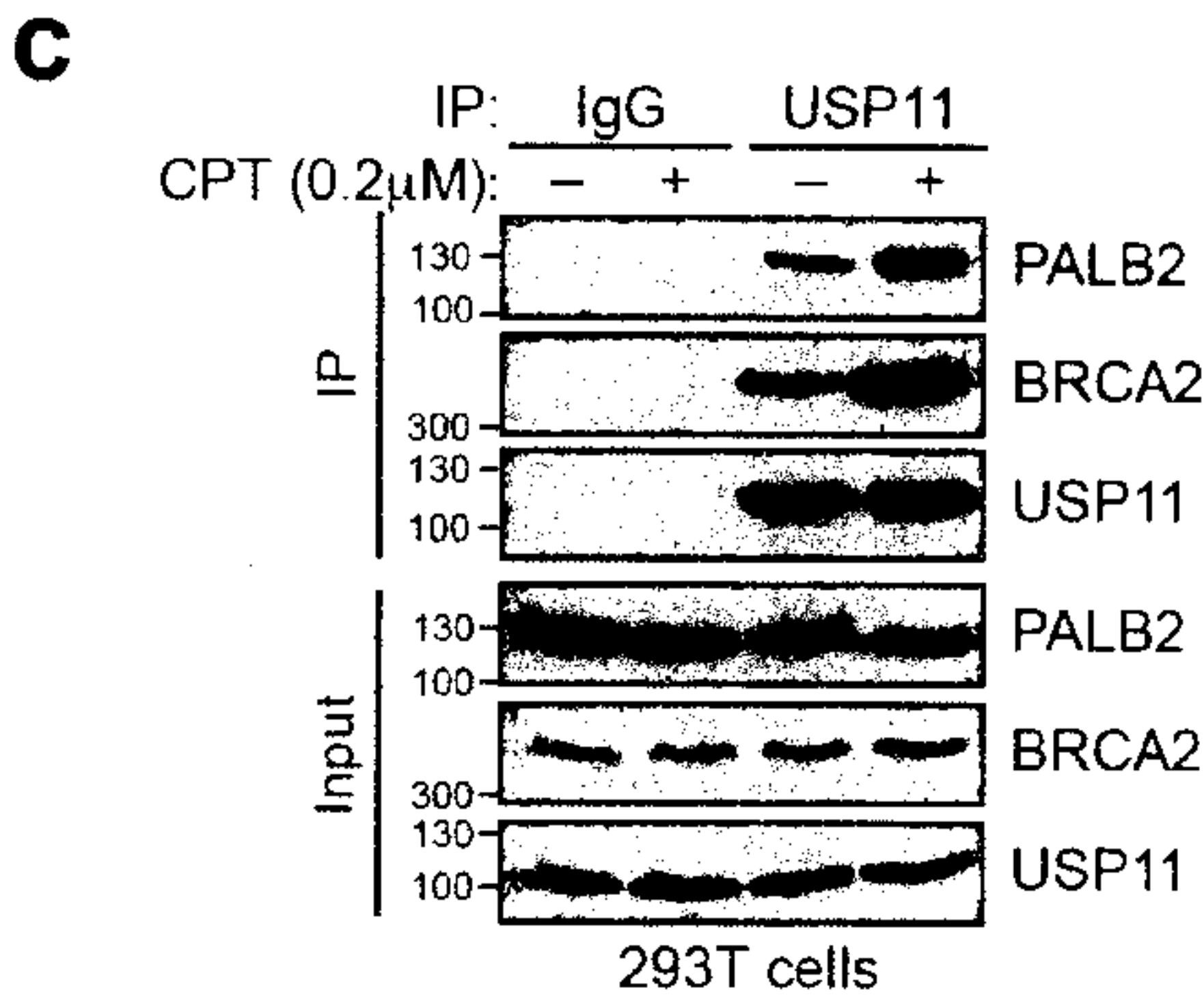
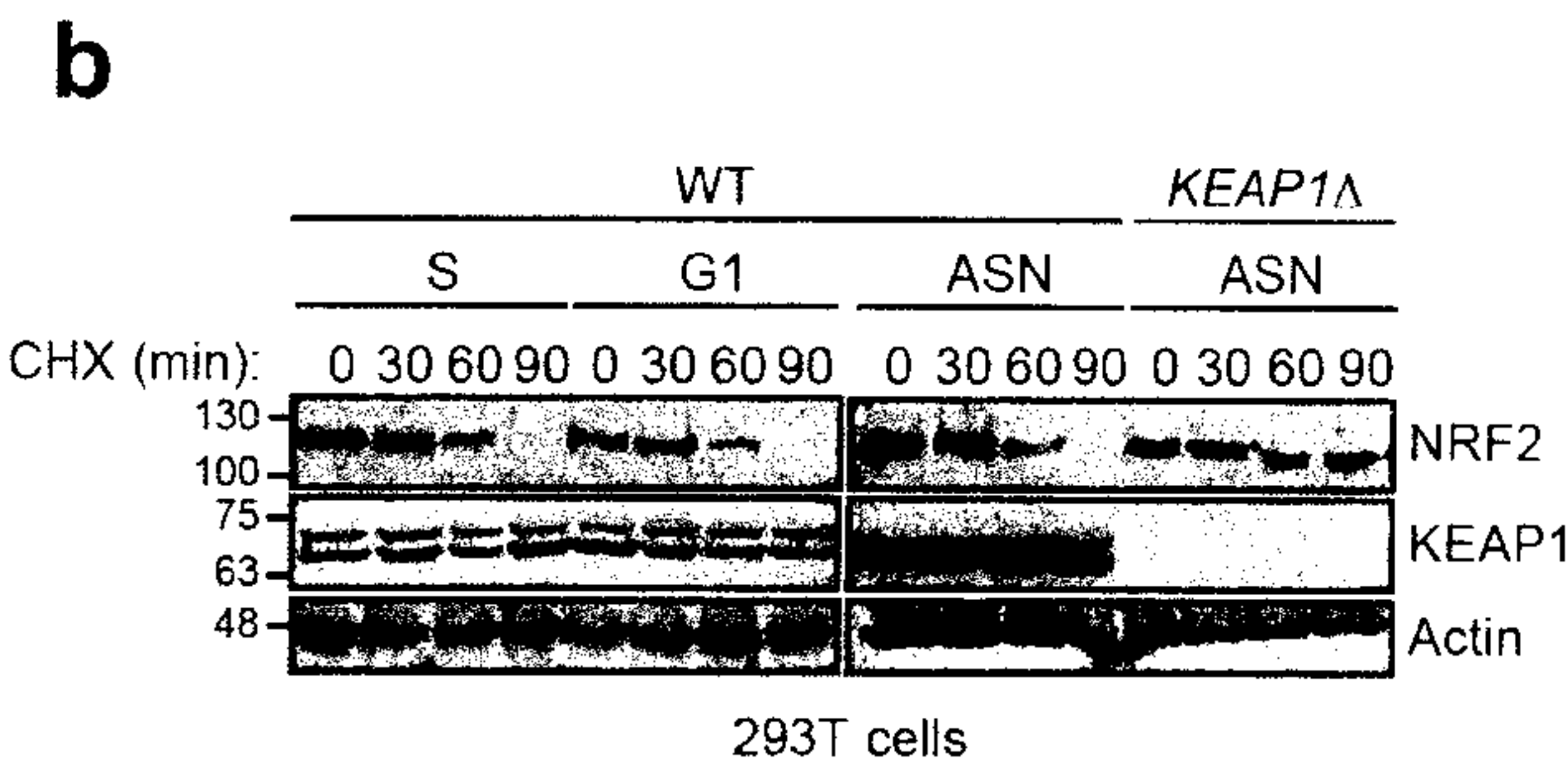


FIG 9c

FIG 9d

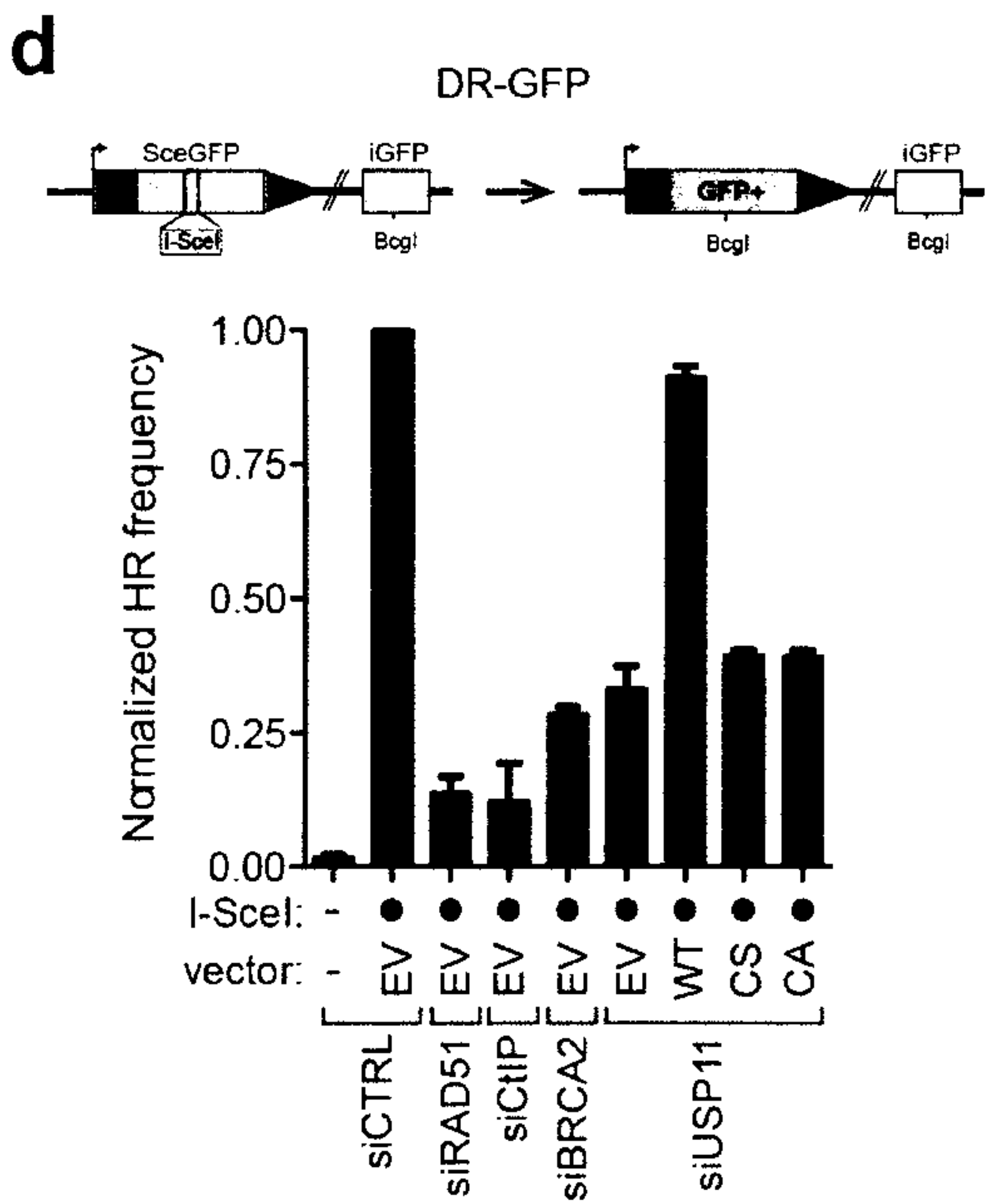


FIG 9e

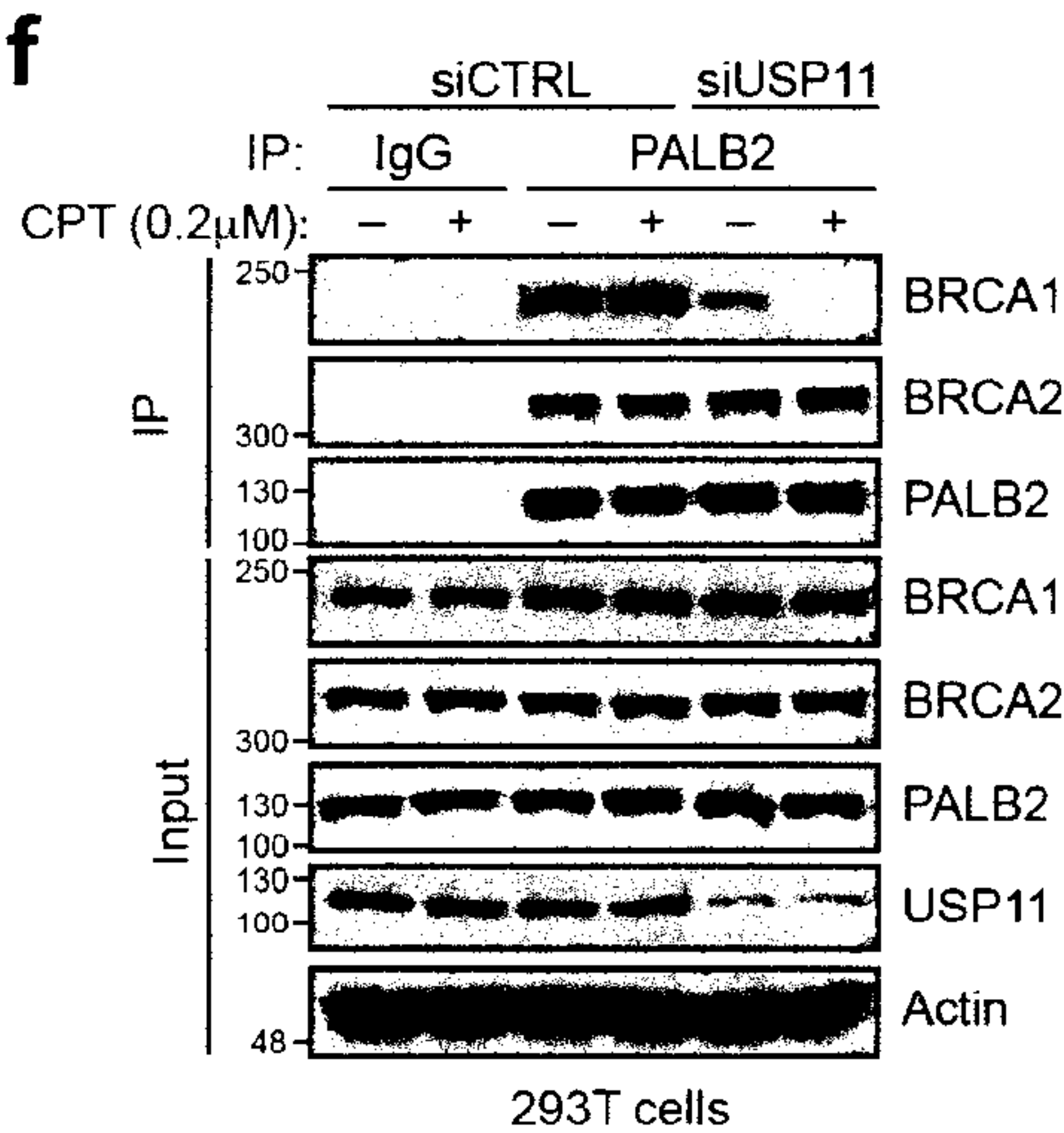
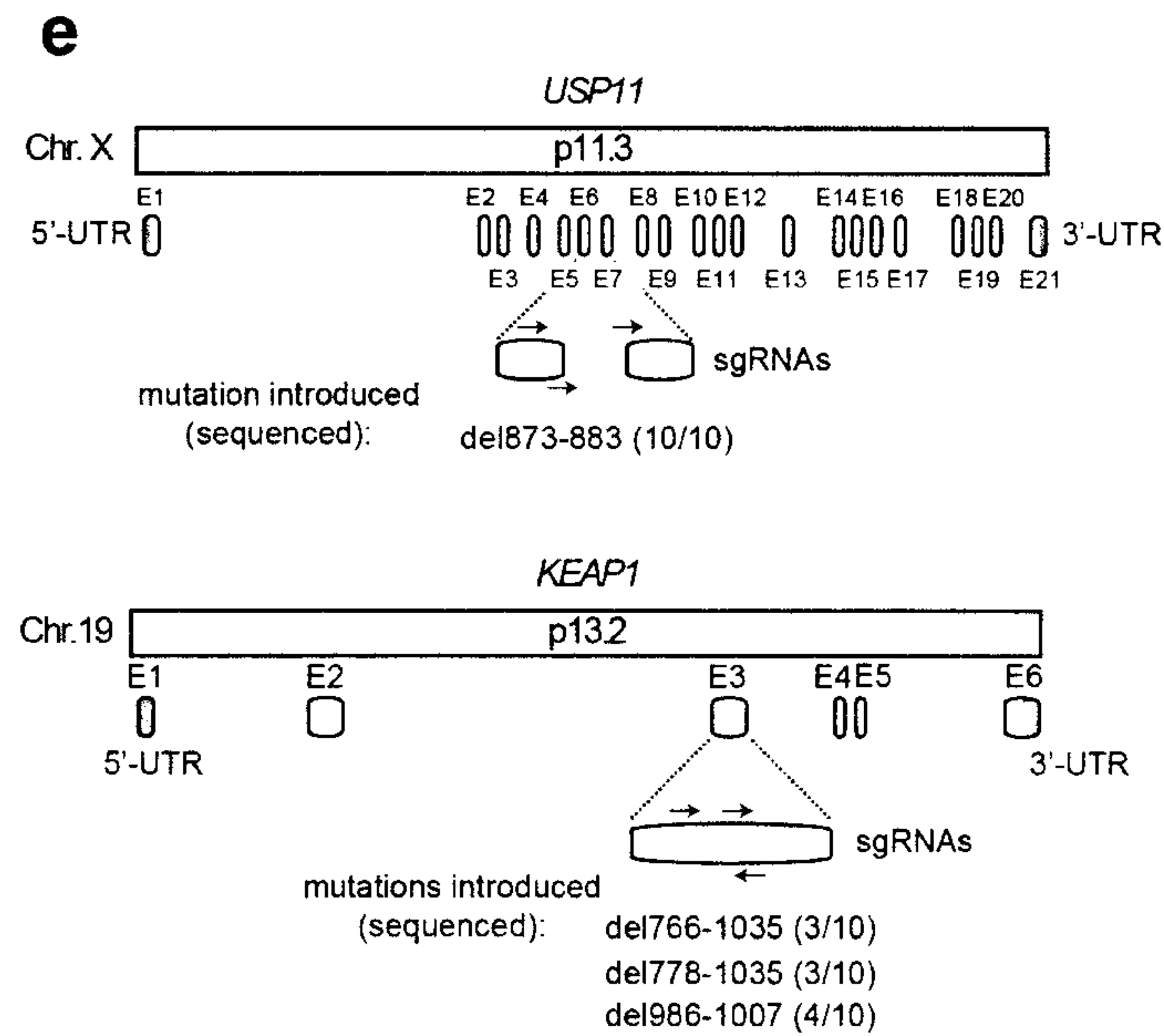


FIG 9f

FIG 10a

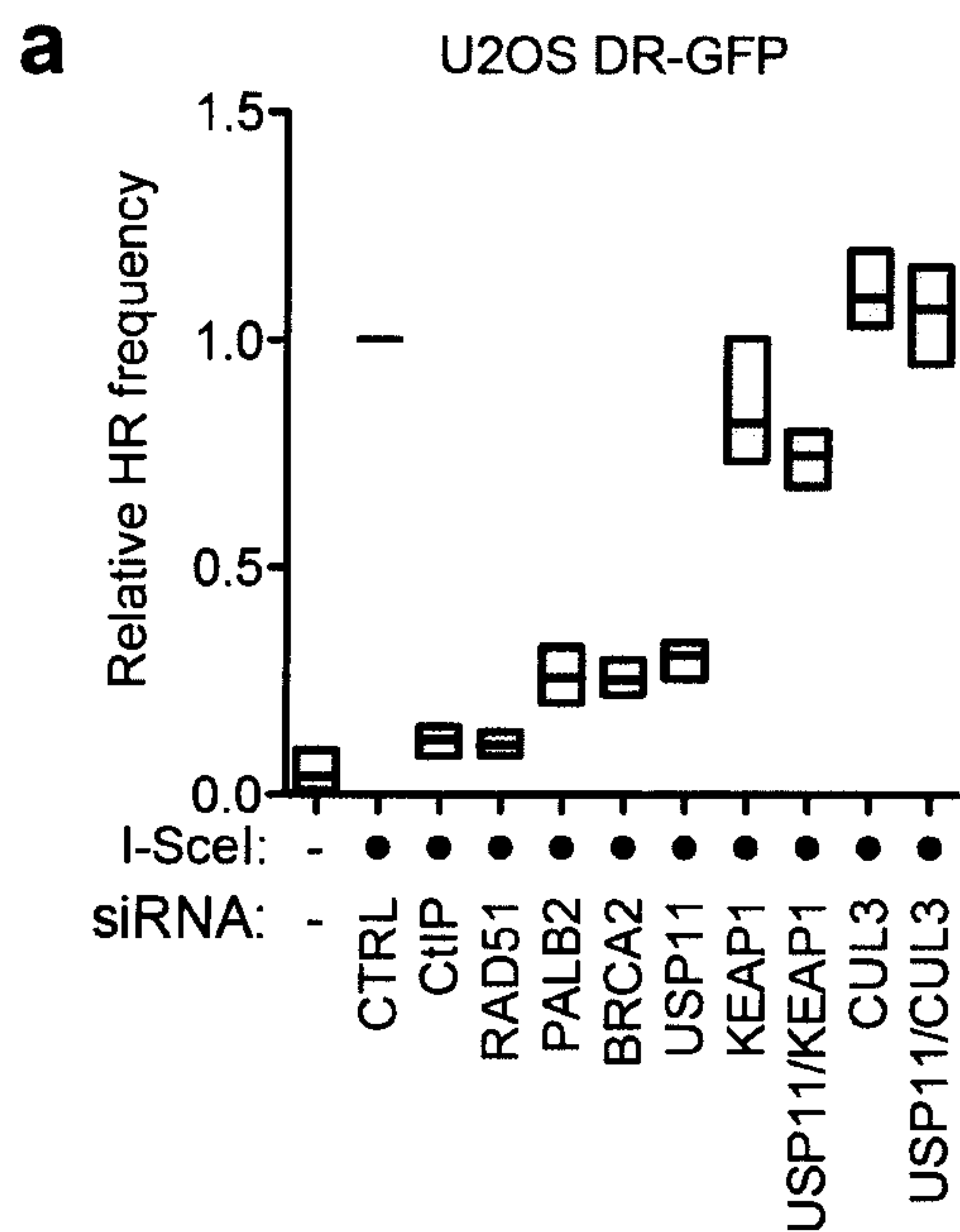


FIG 10b

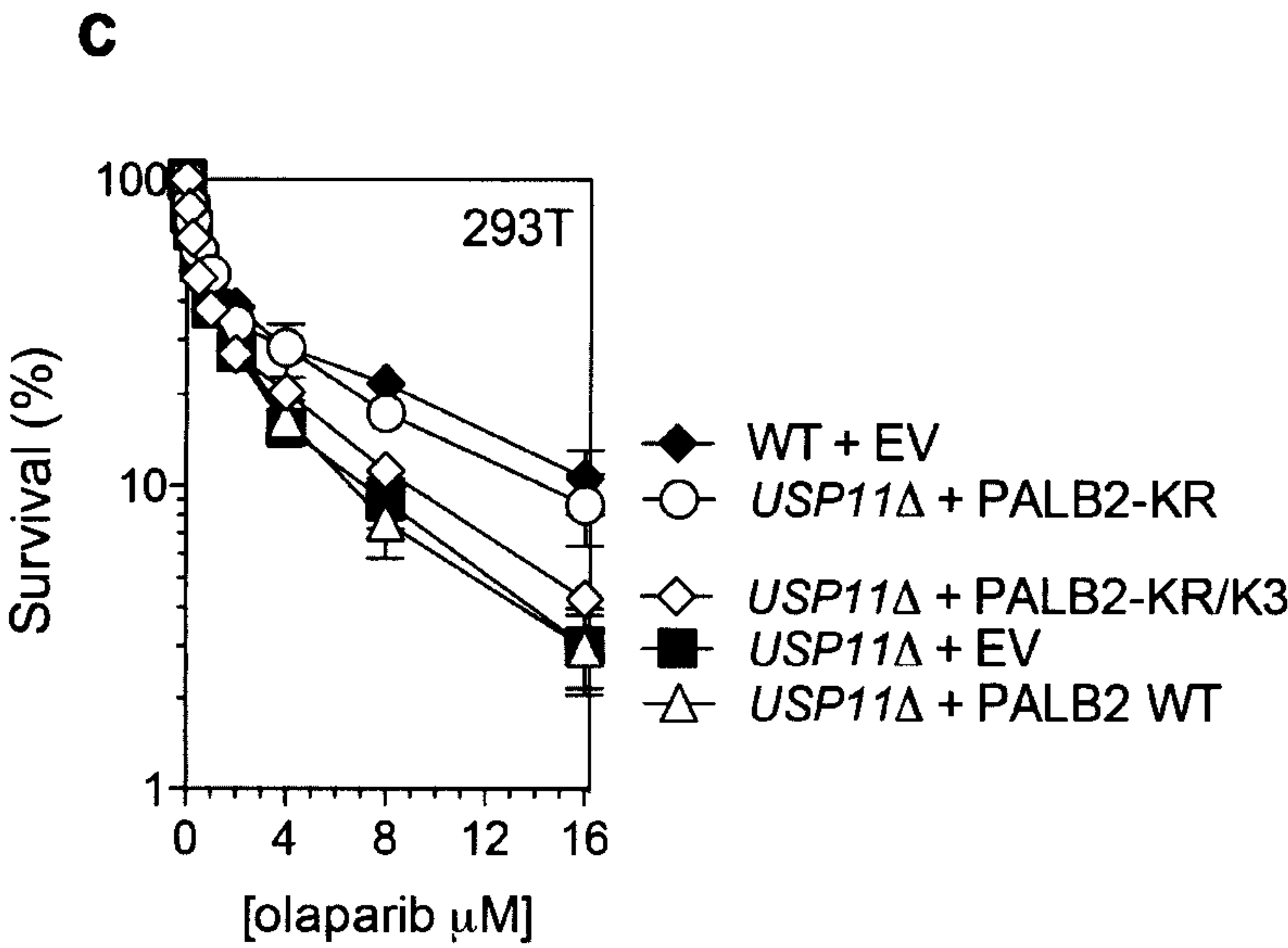
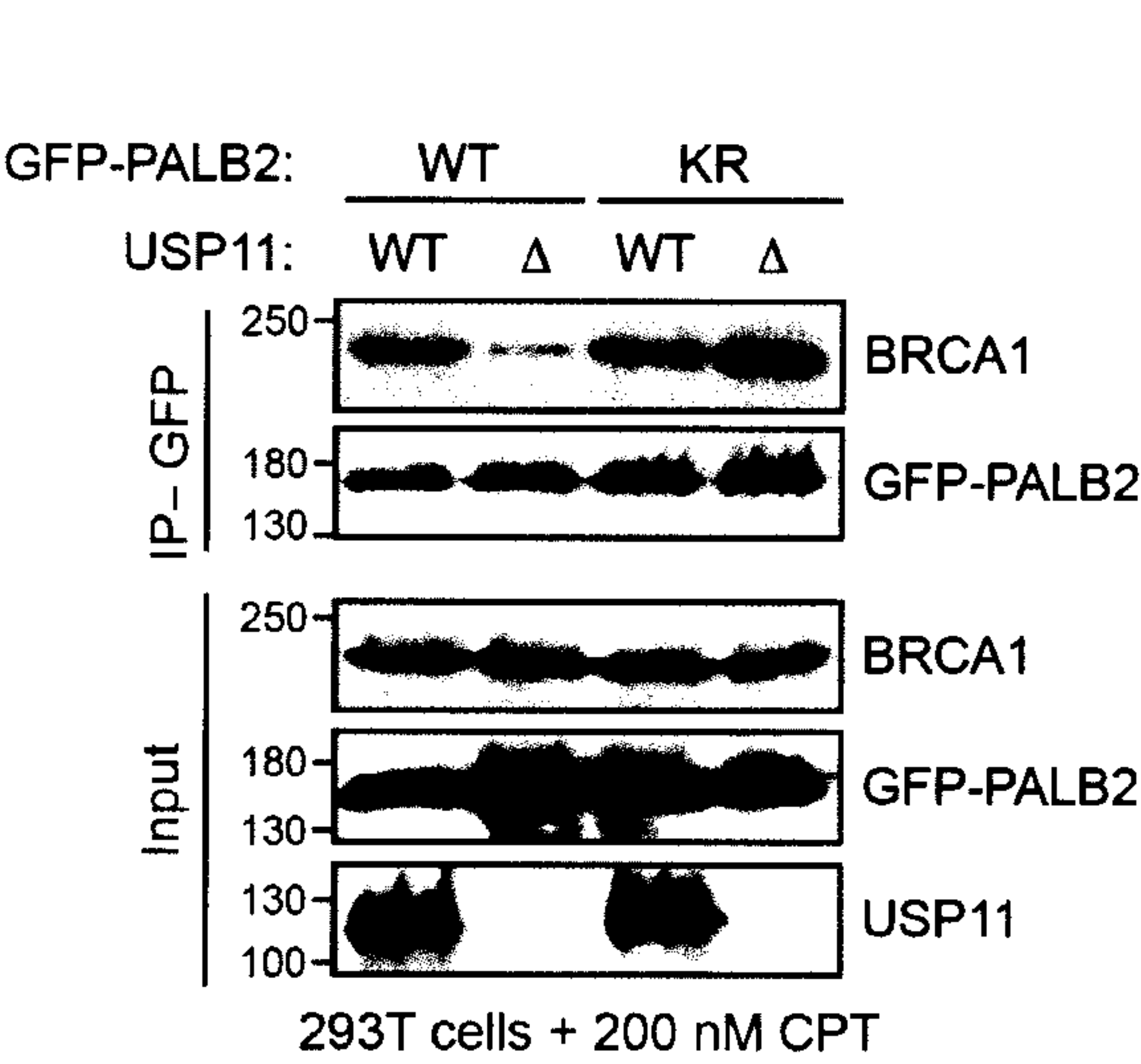


FIG 10c

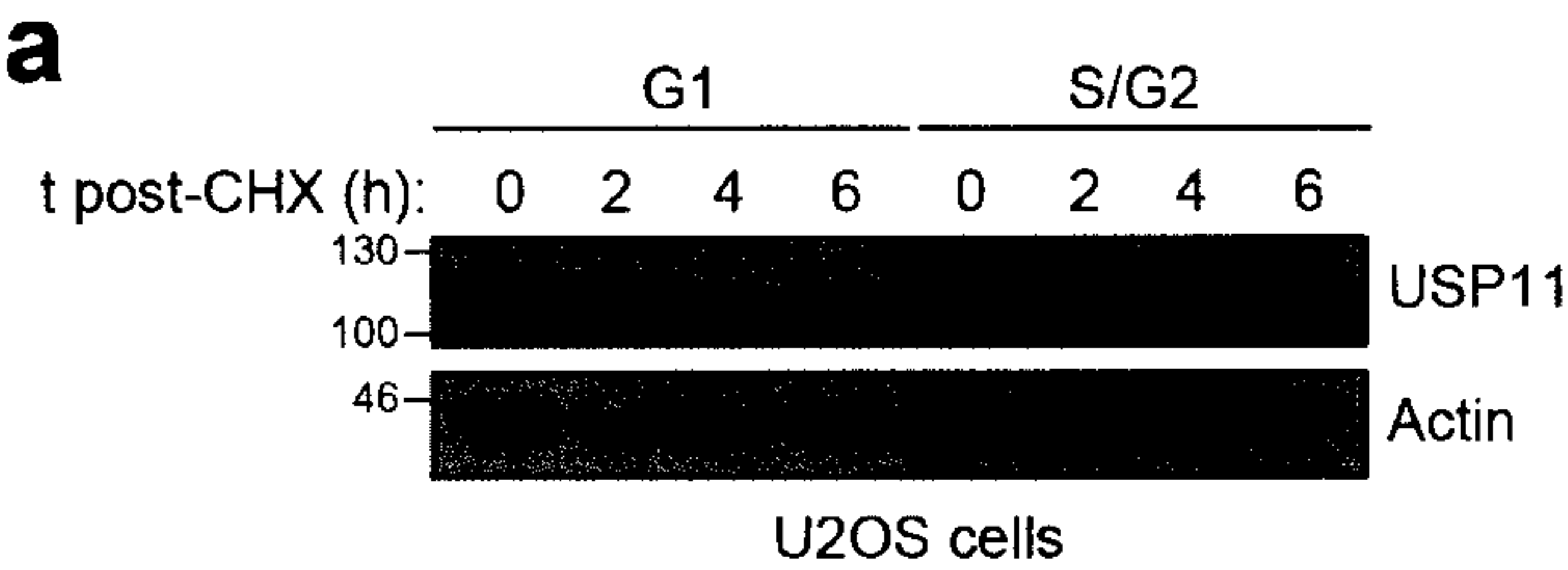


FIG 11a

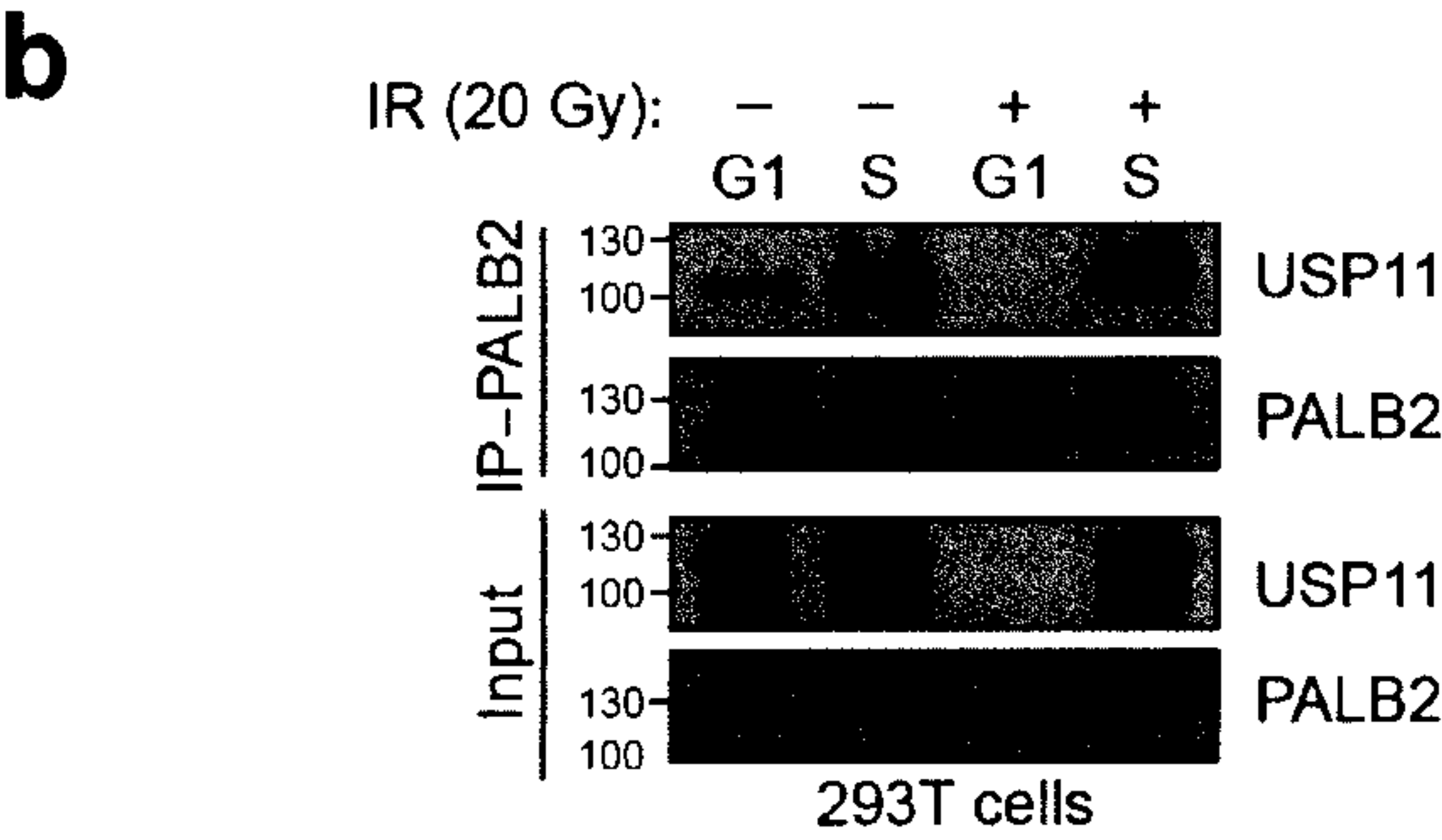


FIG 11b

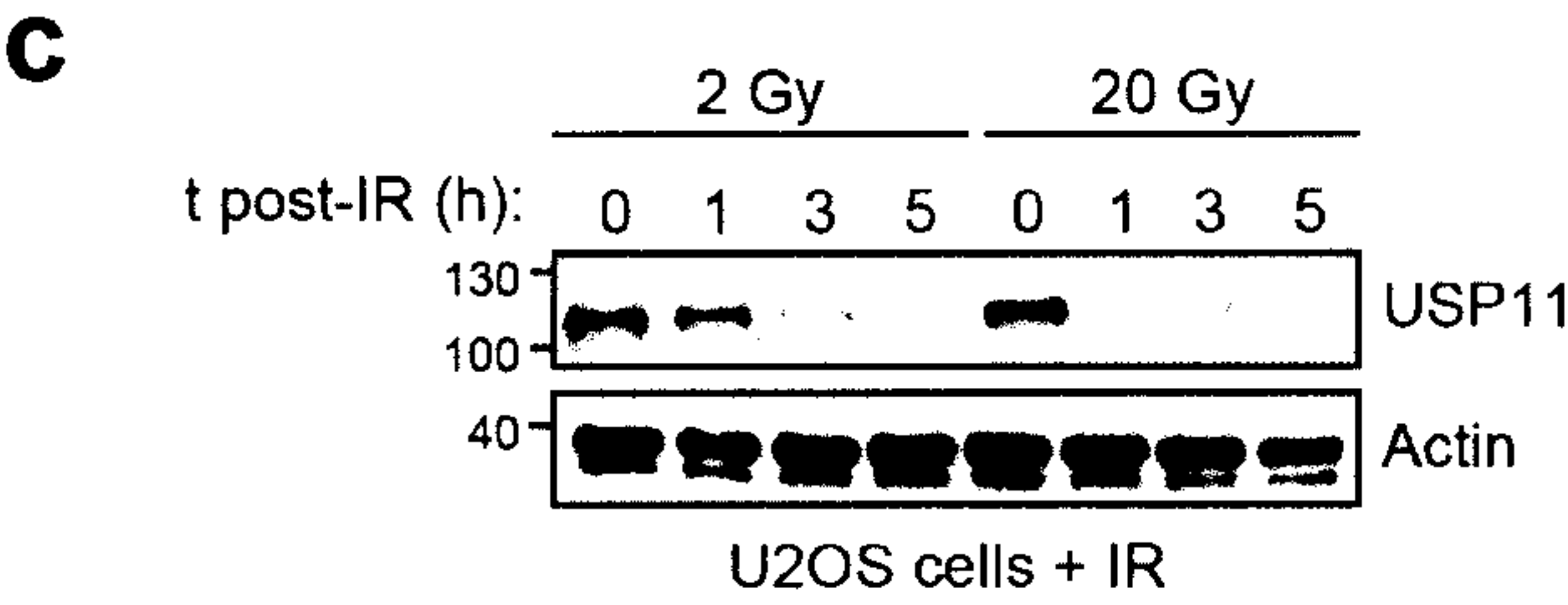


FIG 11c

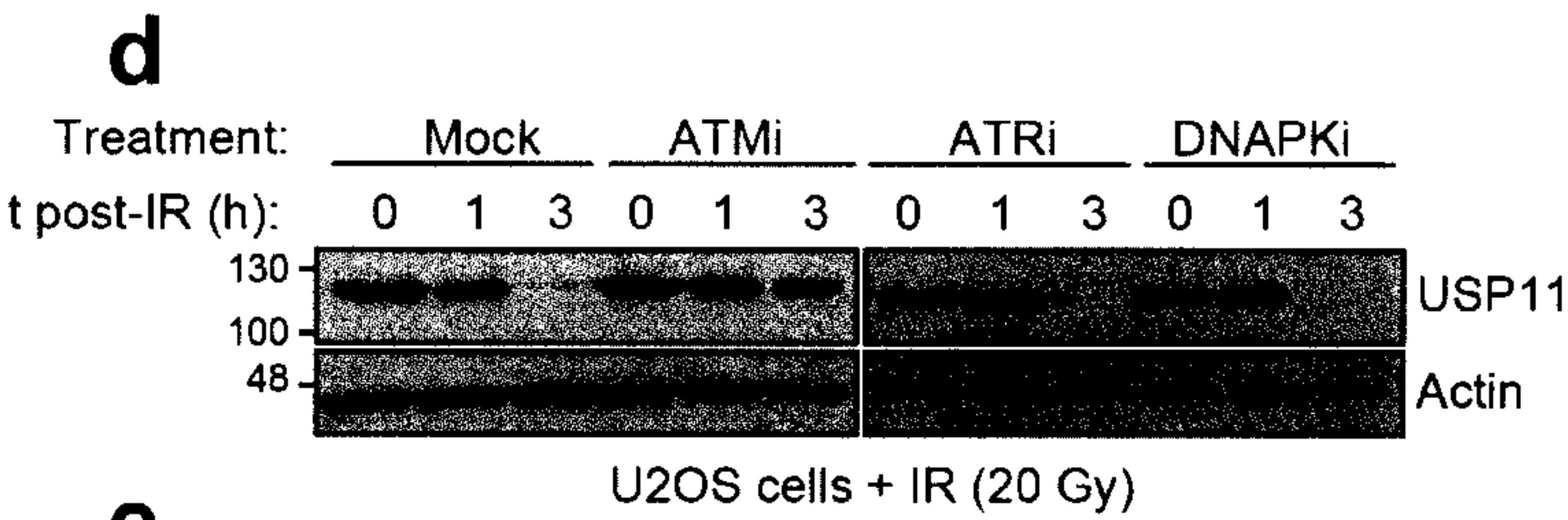


FIG 11d

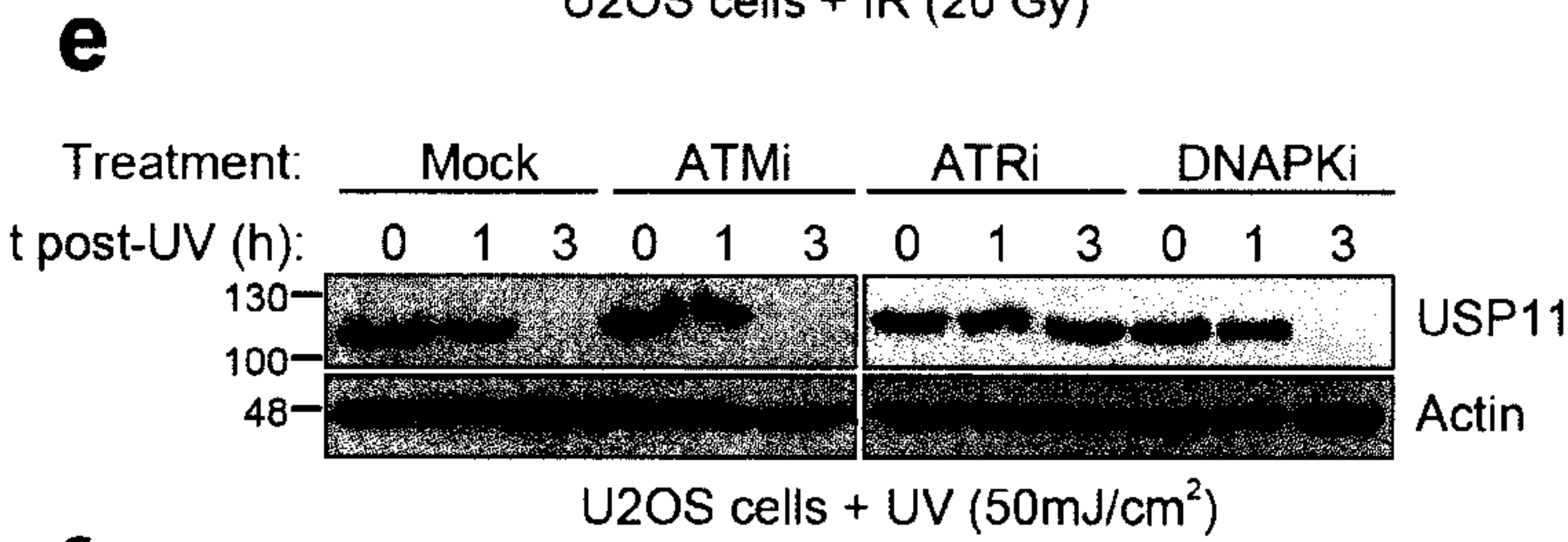


FIG 11e

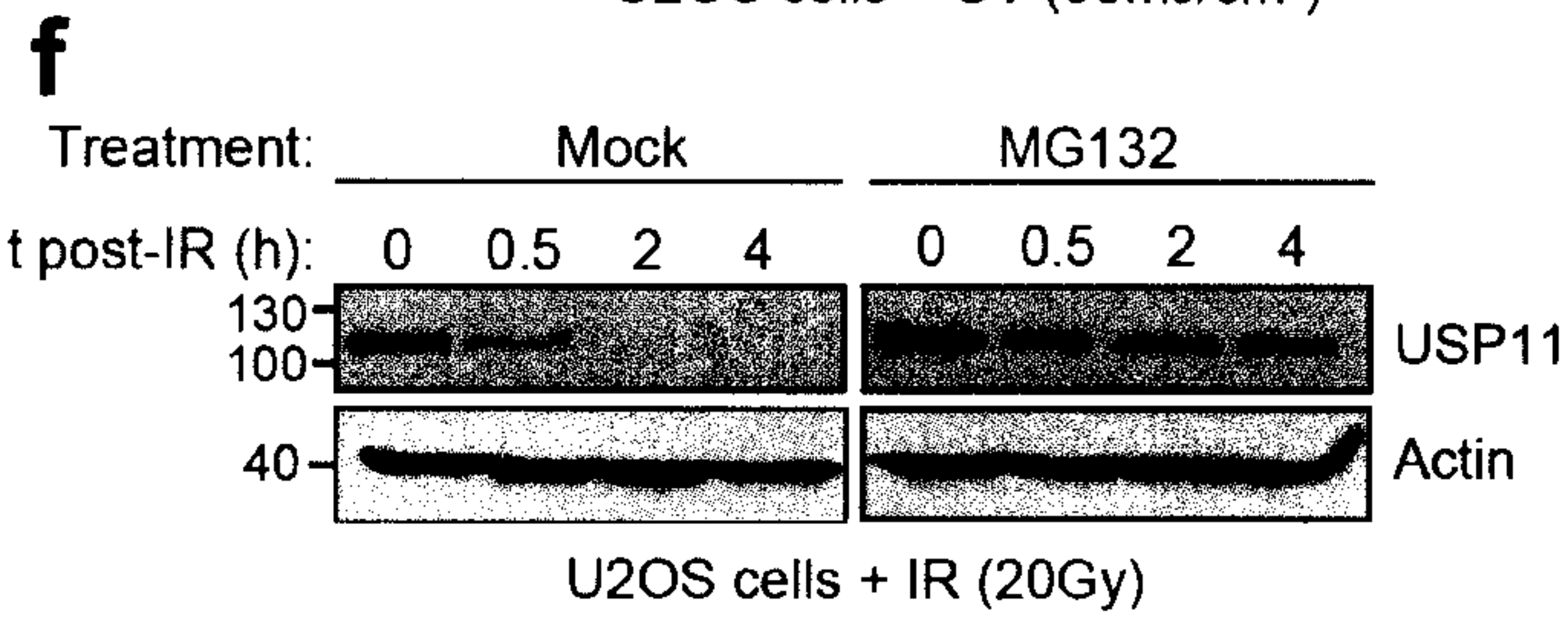


FIG 11f

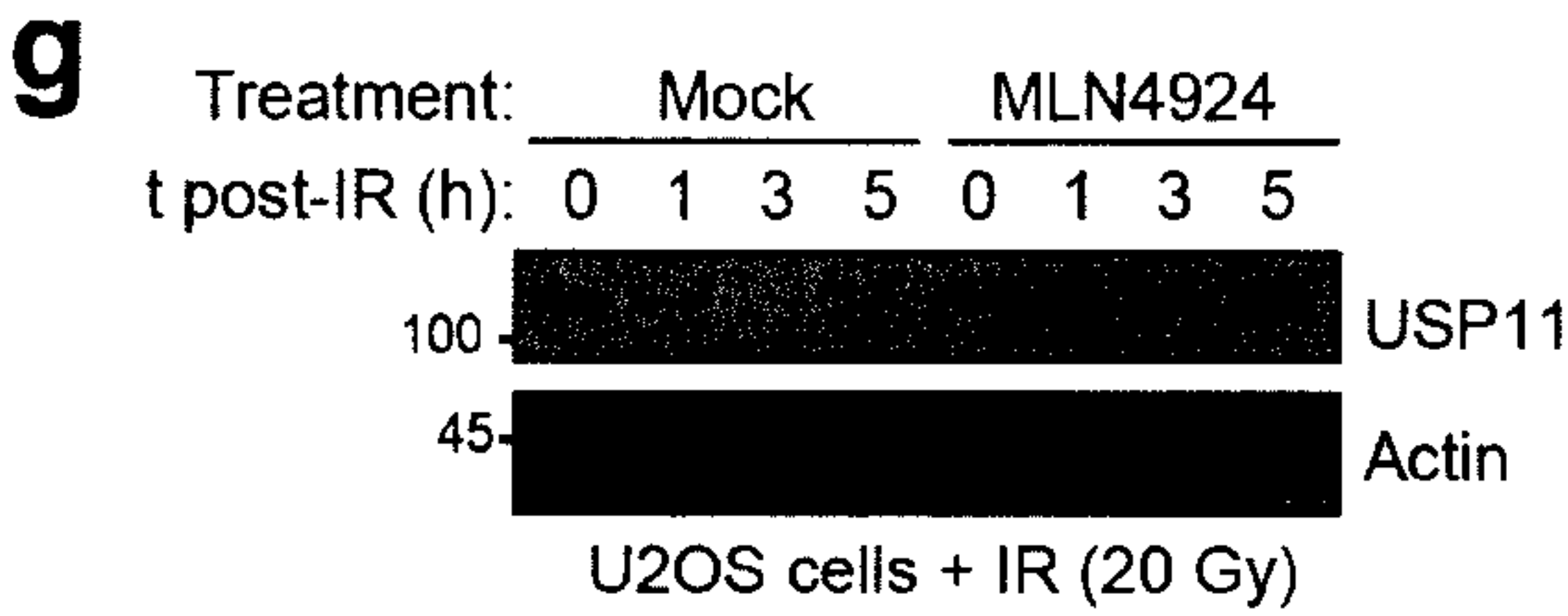


FIG 11g

FIG 12a

FIG 12b

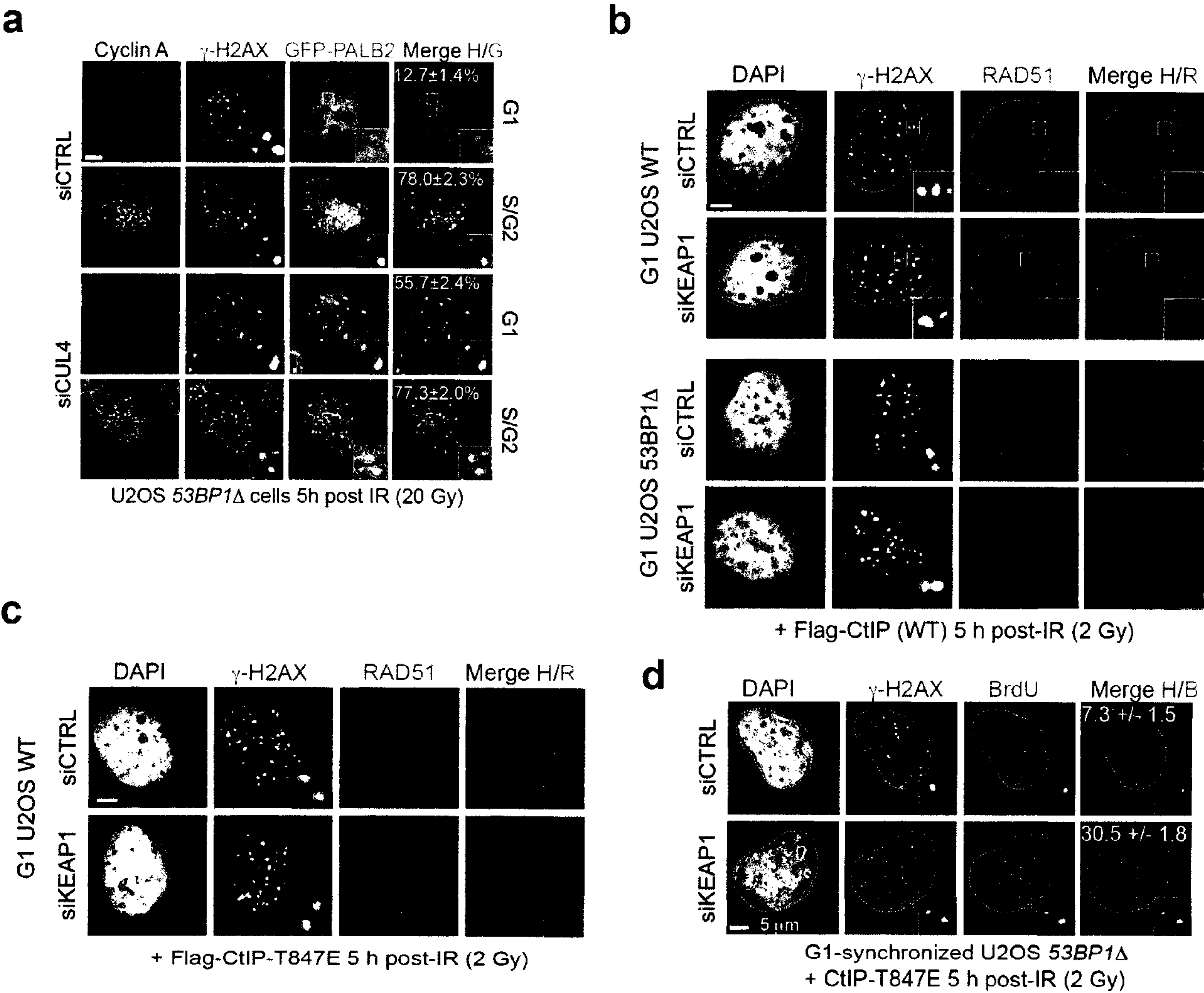


FIG 12c

FIG 12d

FIG 12e

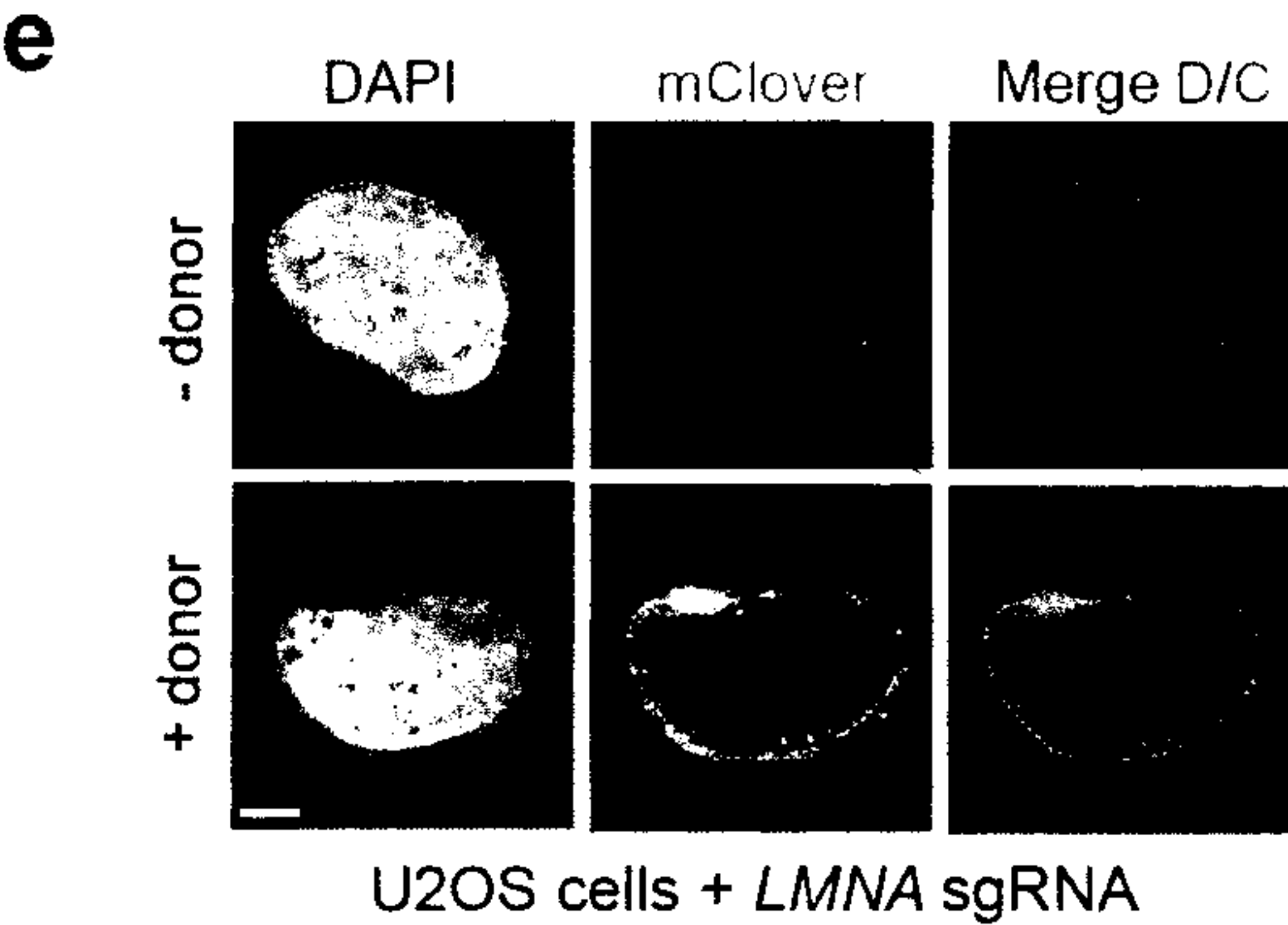


FIG 12f

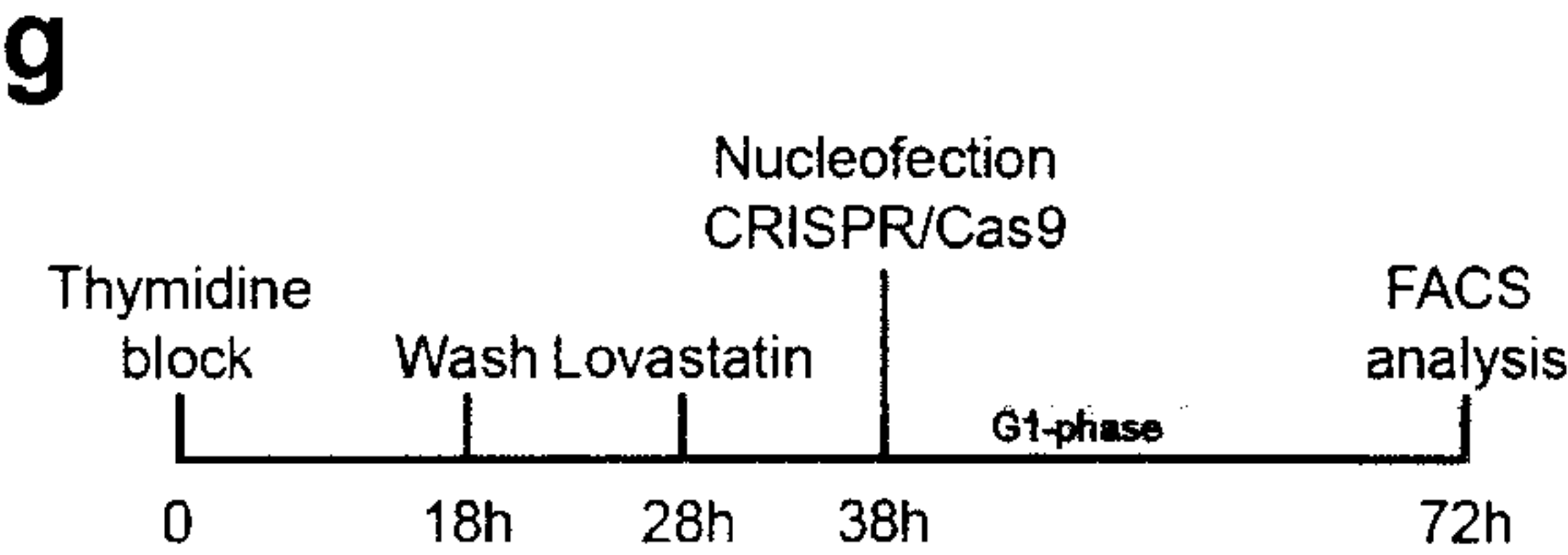
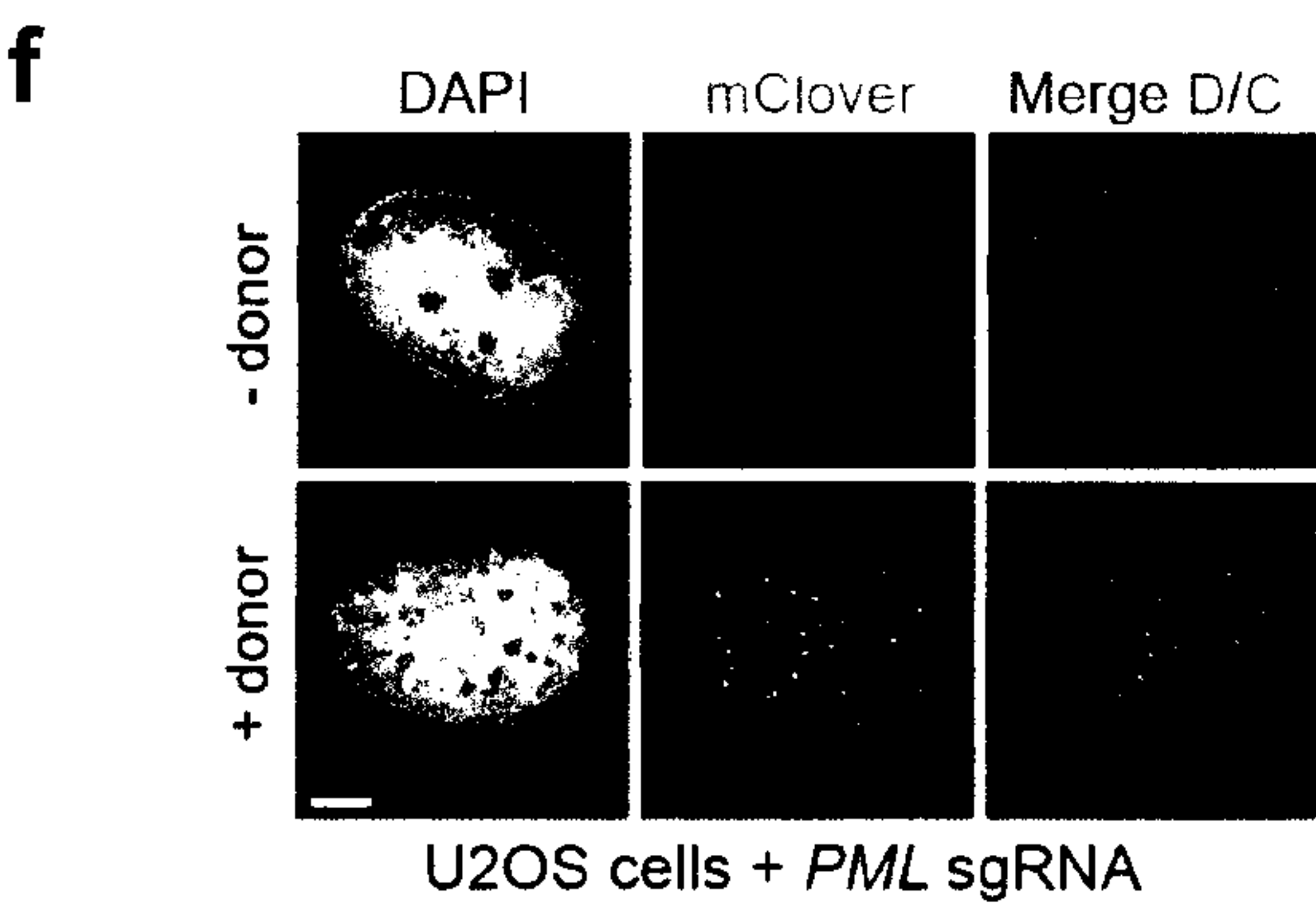


FIG 12g

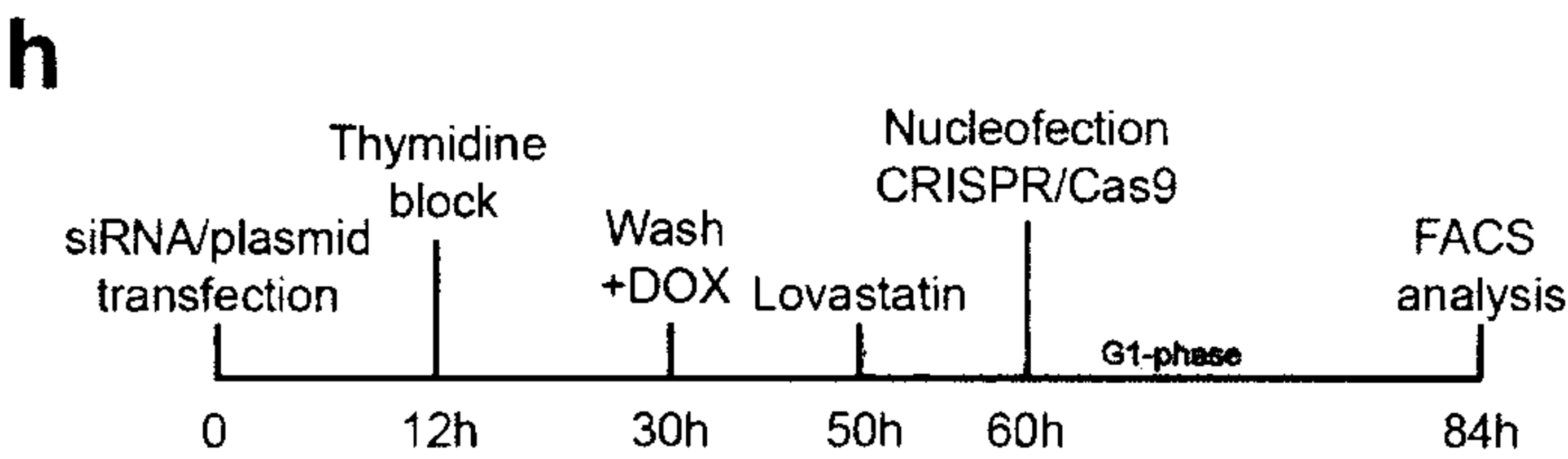


FIG 12h

FIG 13a

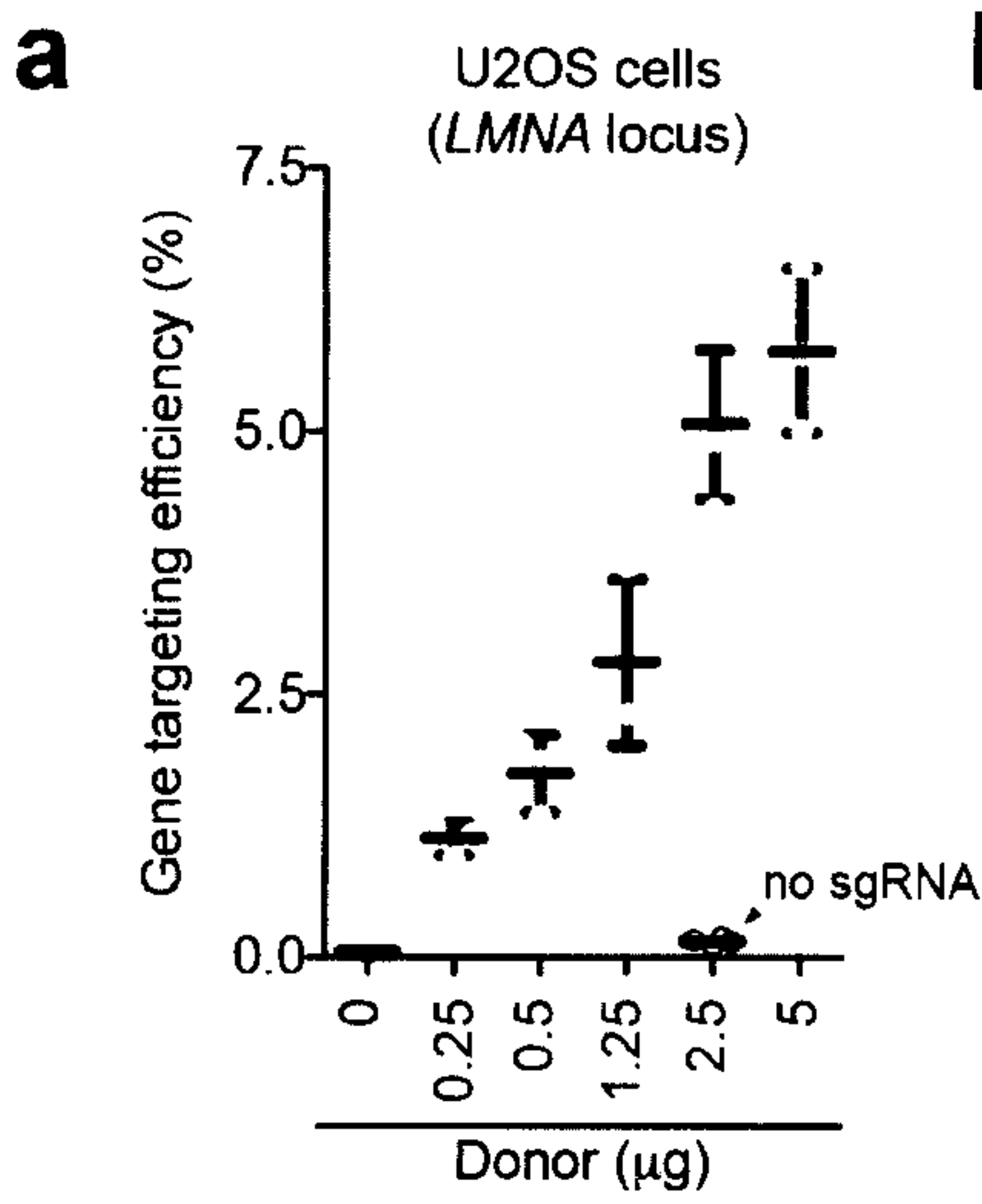


FIG 13b

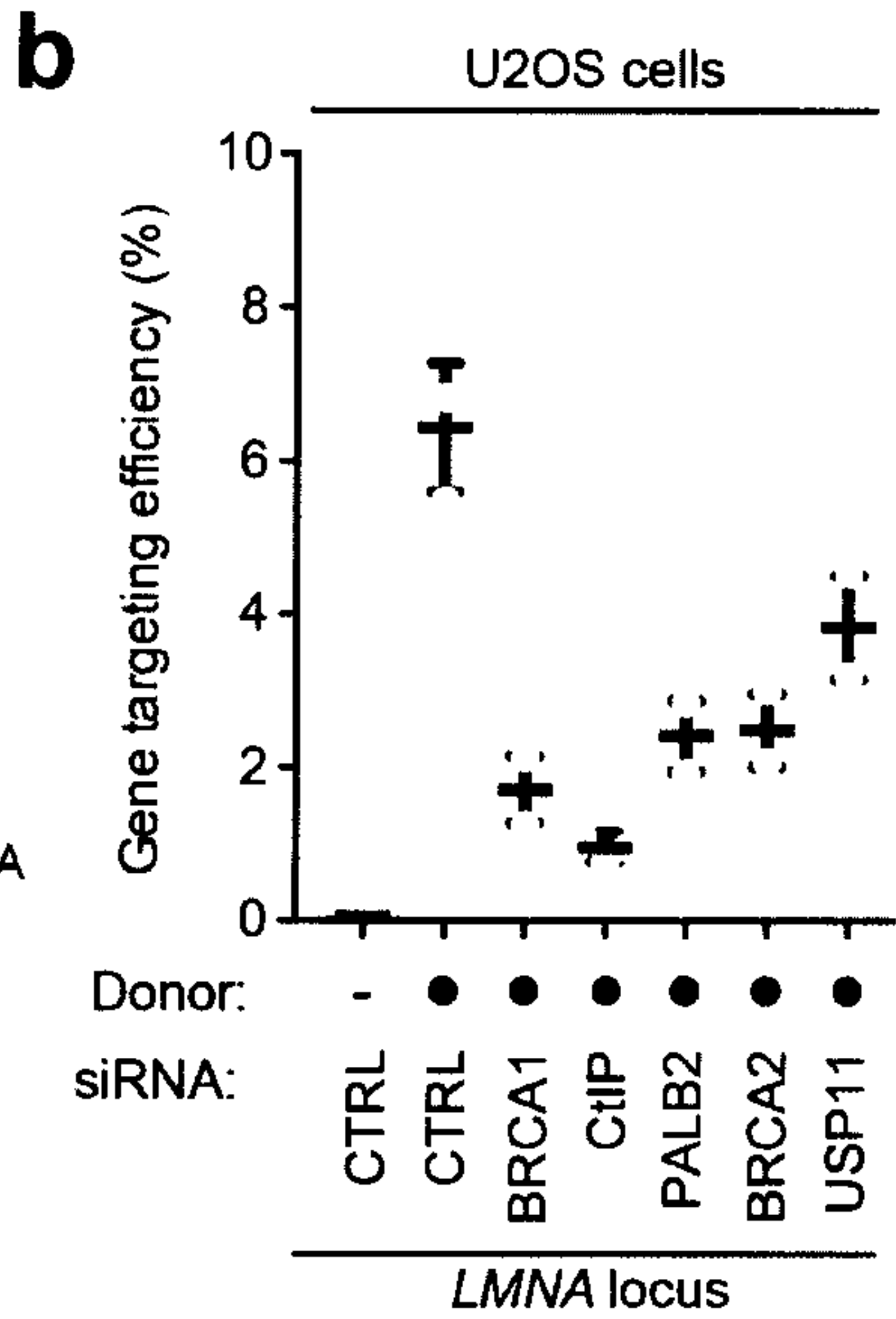


FIG 13c

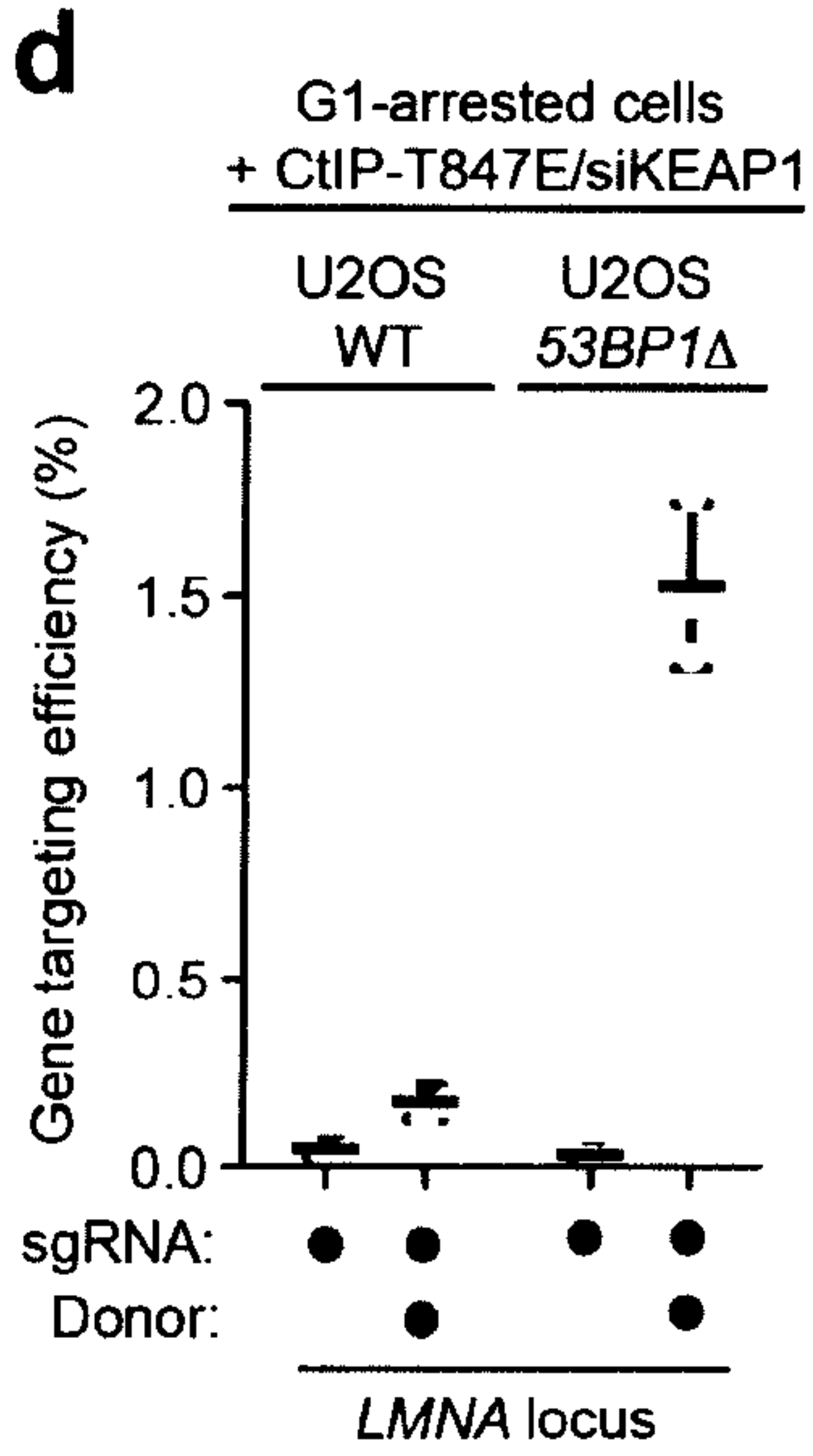
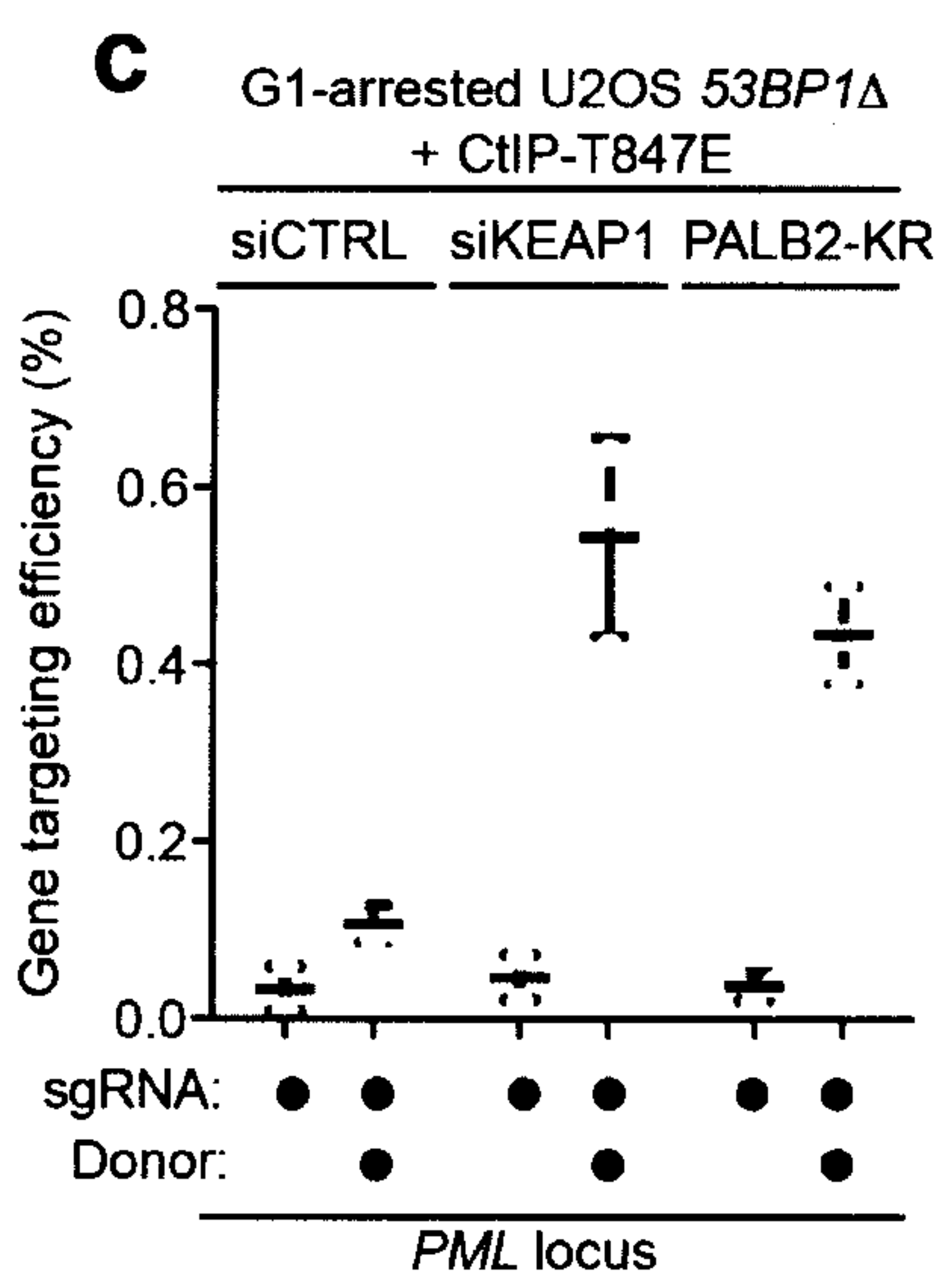


FIG 13d

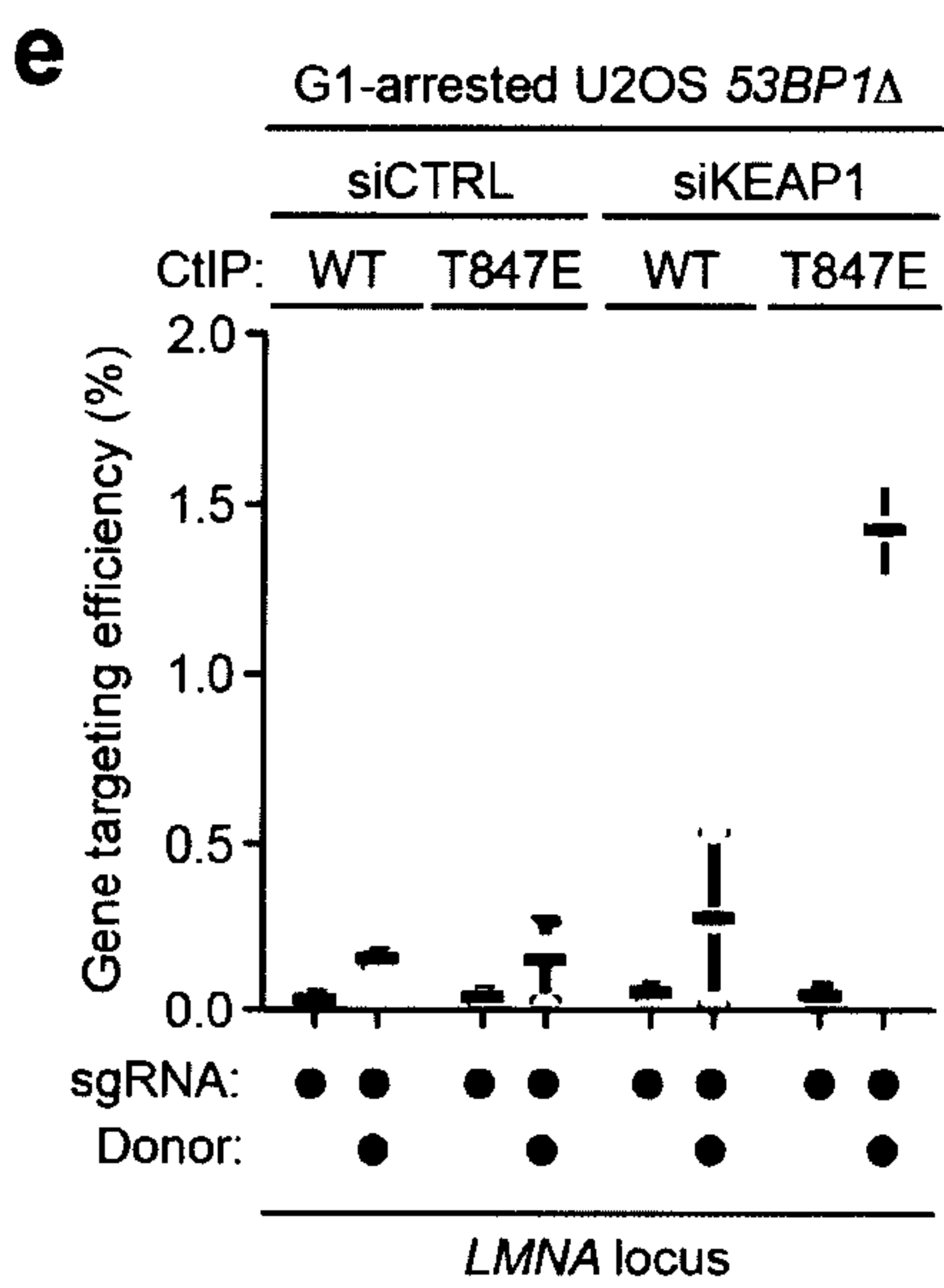


FIG 13e

FIG 14a

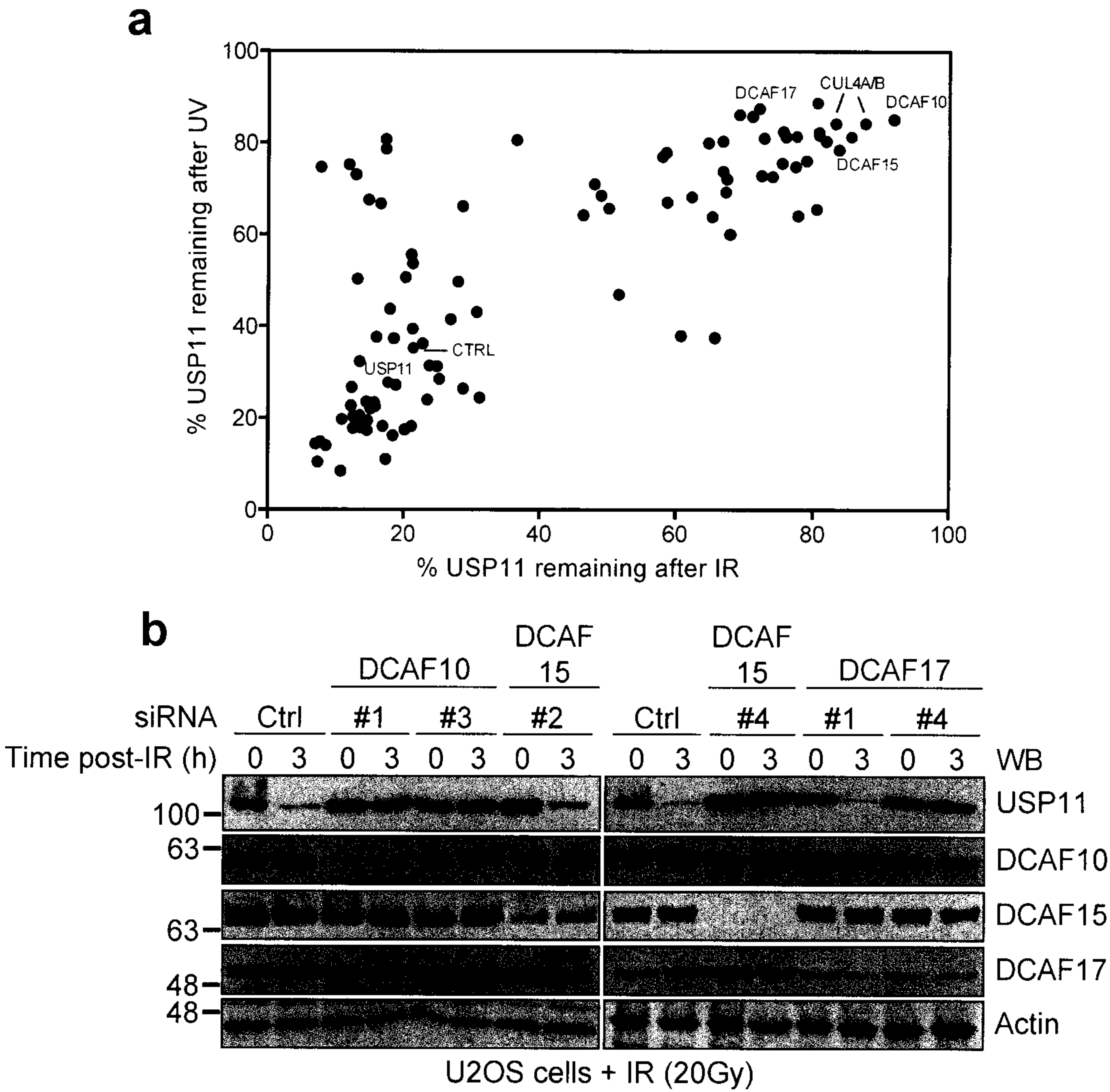
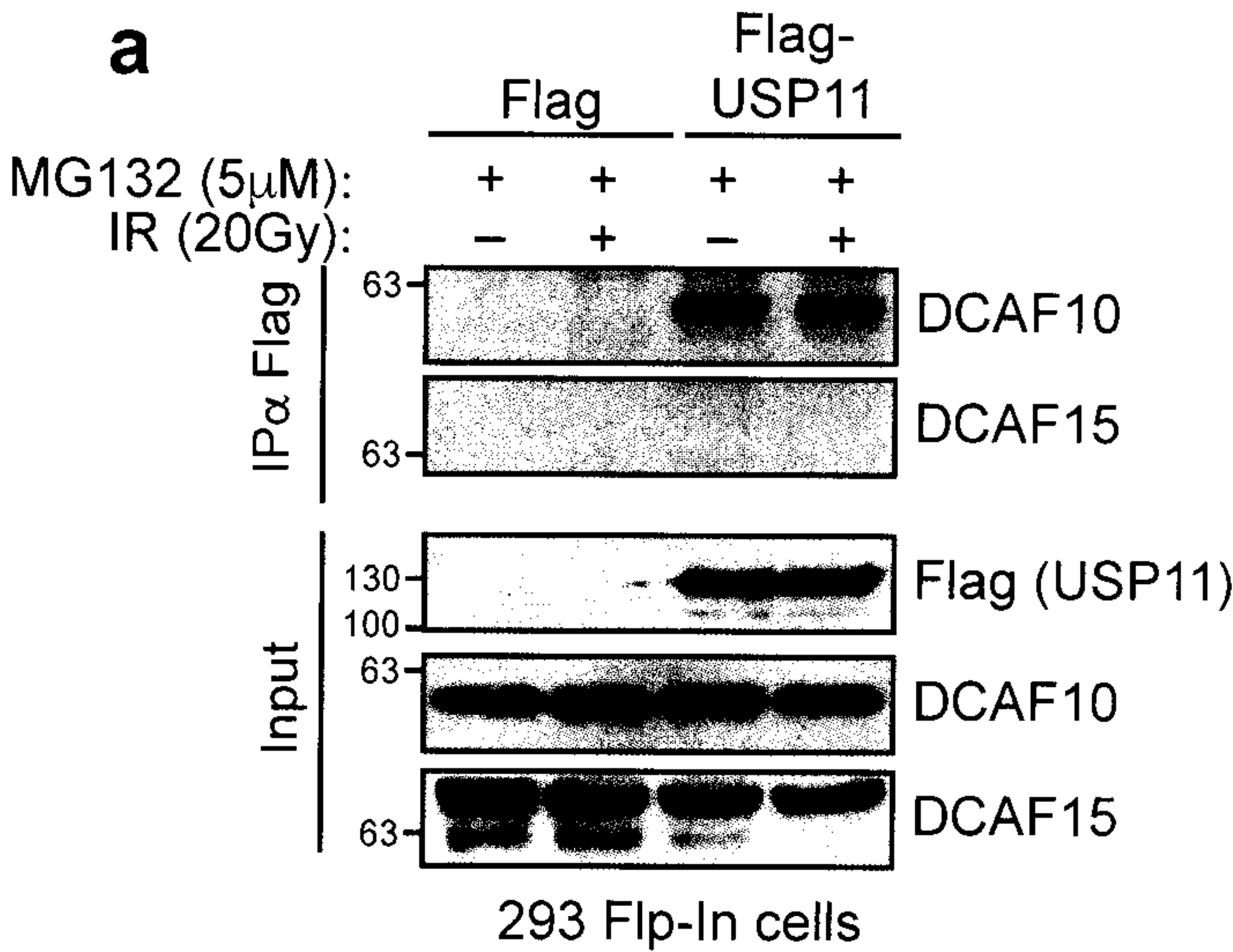


FIG 14b

FIG 15a



b

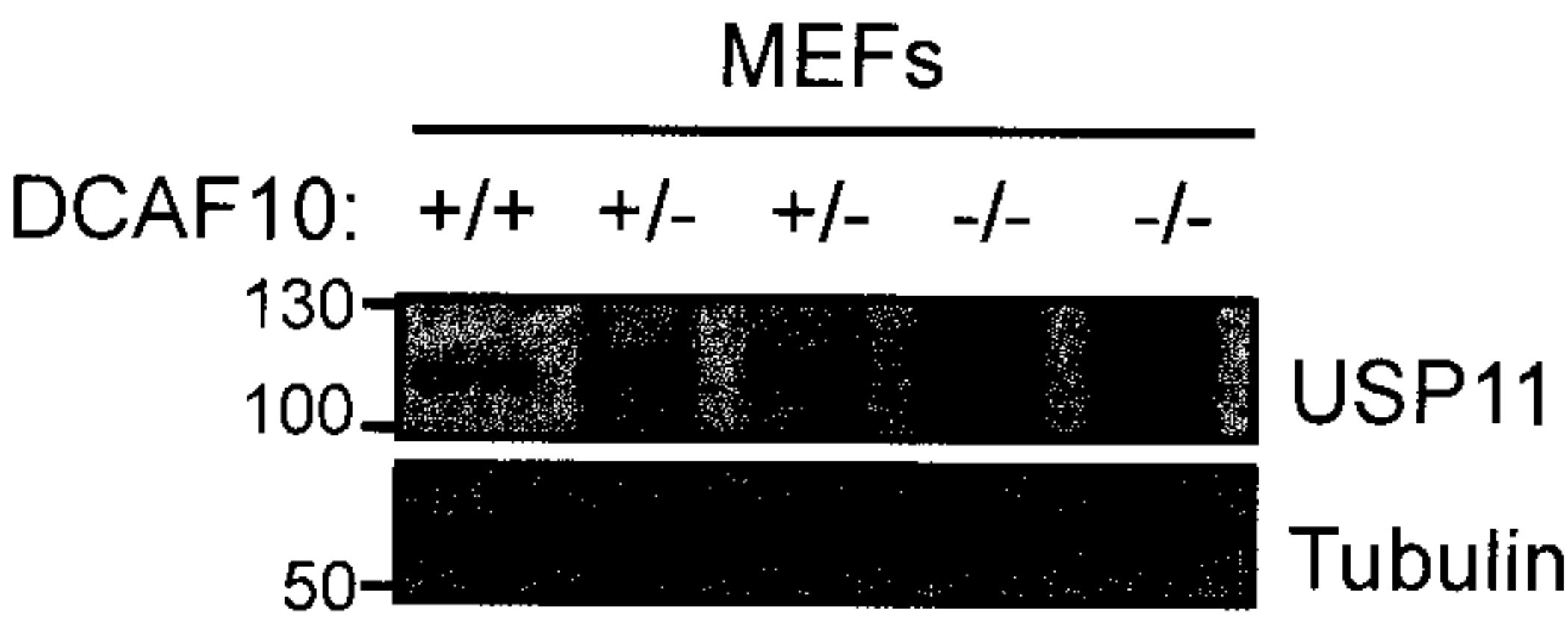


FIG 15b

FIG 15c

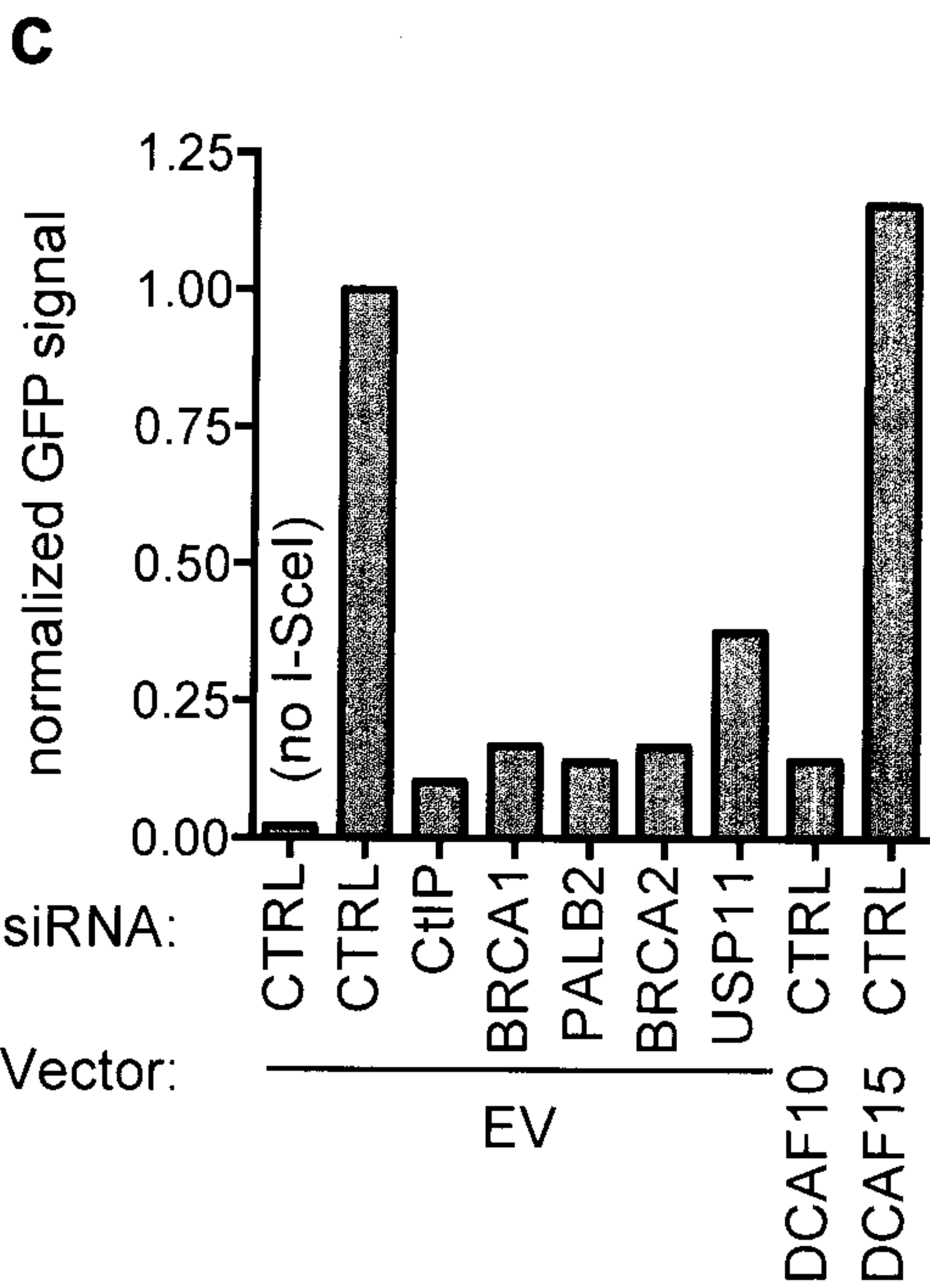


FIG 16

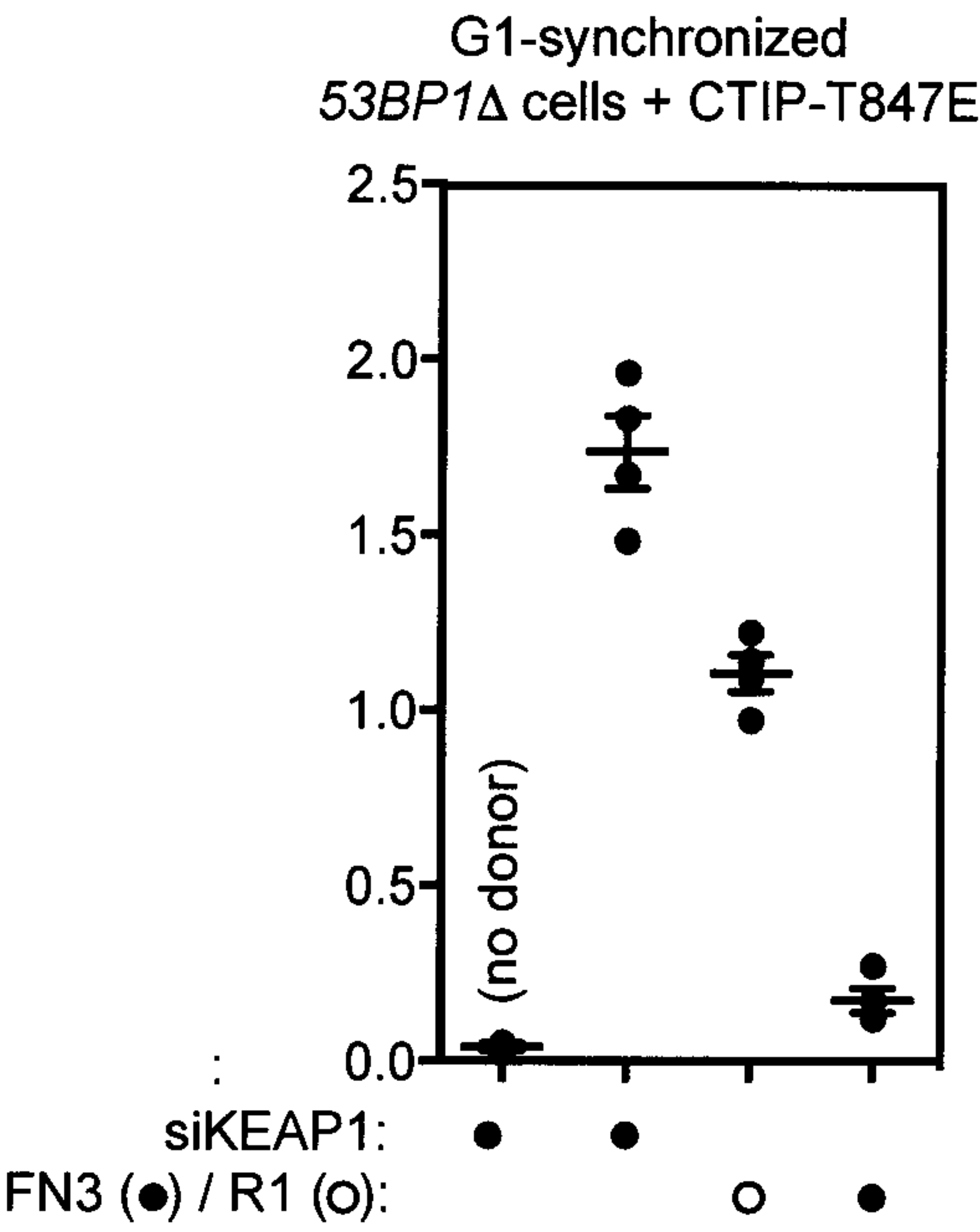


FIG 1a

