Trypanosoma brucei BRCA2 acts in a life cycle-specific genome stability process and dictates BRC repeat number-dependent RAD51 subnuclear dynamics

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Supplementary Figure legends

Fig.S1. Conservation of *T. brucei* BRCA2. Residues conserved in a global sequence alignment of BRCA2 polypeptides from a number of eukaryotes are shown in the upper diagram, relative to the domain organisation of *H. sapiens* and *T. brucei* proteins. Functional domains in human BRCA2, and their conservation in *T. brucei*, are shown in the middle diagram (DSS1-DNA binding, dark grey box; H, helical domain; T, tower domain; 1, 2, 3, OB domains; BRC repeats, black boxes; C-terminal RAD51 binding site, light grey box). Conservation of BRC repeats in trypanosomatid BRCA2 proteins relative to *H. sapiens* are shown in the bottom diagram; critical residues for RAD51 binding inferred by Lo et al (Lo *et al.*, 2003) are indicated as a BRC fingerprint (O, polar; +, positively charged; I, hydrophobic; i, small hydrophobic). BRC array indicates the ~14 conserved, N-terminal BRC repeats in *T. brucei* BRCA2, while BRC C-term denotes the sequence-diverged BRC repeat found at the C-terminus of the BRC array. In the other organisms each BRC repeat in the polypeptide is shown, numbered from the N-terminal-proximal repeat.

Fig.S2. Conservation of the DSS1-DNA binding domain of *T. brucei* BRCA2. A sequence alignment of the DSS1-DNA binding domain of BRCA2 proteins from a number of eukaryotes is shown; regions corresponding to helical (helix), OB and tower domains are overlined. Residues identical or conserved in >30% of the polypeptides are boxed in black or grey, respectively.

Fig.S3. Sequence homology in the C-termini of BRCA2 homologues. A sequence alignment of the C-termini of BRCA2 sequences from a number of eukaryotes is shown. The region shown to bind RAD51 in *H. sapiens* is indicated (overlined), within which cyclin and cyclin-dependent kinase (CDK) target sites are further indicated. Residues identical or conserved in >30% of the polypeptides are boxed in black or grey, respectively.

Fig.S4. Confirmation of *BRCA2* mutants by Southern analysis. Southern blots are shown that map *BRCA2* deletion by reverse genetics in (**A**) Lister427 *T. brucei* cells, either bloodstream form (BSF) or procyclic form (PCF), and in (**B**) PCF TREU927 cells. In each case the upper diagram shows a restriction map indicating the expected products of restriction digested genomic DNA after Southern blotting and hybridisation with the 5' UTR of the *BRCA2* ORF (black arrows indicate the primers used to PCR-amplify this region as a DNA probe). *BRCA2* deletion involved two rounds of transformation, replacing (::) the ORF with a cassette encoding resistance to blasticidin (*BSD*) and puromycin (*PUR*), whose maps are indicated. Relevant restriction sites are shown in the WT and mutated loci, with the expected restriction fragment sizes detailed (kb). Lower diagrams show Southern blots: genomic DNA is shown from WT cells, two independent *BRCA2*+/- mutants and two *brca2*-/- mutants (derived from the +/- cells) after digestion with *Sac*II and *Stu*II (Lister427), or *Sac*II and *Hin*dIII (TREU927). The bands produced from the WT alleles and the *BRCA2* mutant alleles are indicated, and size markers are shown (kbp).

Fig.S5. *BRCA2* mutation leads to DNA damage sensitivity. EC50 values are shown of wild type (WT), *BRCA2* heterozygous (+/-) mutants and *brca2* homozygous (-/-) mutants in *T. brucei* Lister427 bloodstream form (BSF) cells and in procyclic form (PCF) cells from Lister427 and TREU927 strains, all grown in the presence of methyl methanesulphonate (MMS). EC50 values are the mean from three experiments expressed as a percentage of WT; bars indicate standard error.

Fig.S6. Confirmation of BRCA2 BRC variant expresser cells by Southern analysis. A. Restriction maps showing the arrangement of the *BRCA2* locus and of *BRCA2* BRC variants (1BRC, 4BRC, 7BRC, 10BRC and full length BRCA2) integrated into *tubulin*, detailing the expected products of restriction digestion after Southern blotting and hybridisation with 1.2 kb of the *BRCA2* ORF (black arrows indicate the primers used to PCR-amplify this as a DNA probe). The restriction sites are indicated, with the expected restriction fragment sizes shown (kb). **B.** Southern blots of genomic DNA extracted from WT, *brca2-/-* mutant and BRCA2 BRC variant expresser (-/-/+) cells from Lister427 BSF, Lister427 PCF and TREU927 PCF strains of *T. brucei*. In each case the DNA was digested with *Hin*dIII before being separated by electrophoresis on an agarose gel and hybridised with the 1.2 kb *BRCA2* probe shown in A.

Fig.S7. RAD51 focus formation in procyclic form *T. brucei*. A. Wild-type PCF TREU927 (WT) cells, and *BRCA2*+/- (+/-B) and *brca2*-/- (-/-BP) mutants, were treated with 1 $\mu g.ml^{-1}$ phleomycin (BLE) for 18 hours and the number of cells with a specific number of subnuclear RAD51 foci (0, 1, 2, 3, 4, > 4) were counted; graphs represent the numbers of foci-containing cells as a percentage of the total number of cells counted (N). **B.** Images of WT, *BRCA2*+/- and *brca2*-/- mutant cells after BLE treatment are shown, and compared with cells without treatment (bottom). White arrows indicate RAD51 foci. Each cell is shown in differential interface contrast (DIC), after staining with DAPI (DAPI) and after hybridisation with anti-RAD51 antiserum (1:1000 dilution) and secondary hybridisation with Alexa Fluor 594 conjugated anti-rabbit antiserum (RAD51, 1:7000 dilution). Merged images of DAPI and RAD51 cells are also shown (Merge). **C.** Total protein extracts from WT, *BRCA2*+/- and *brca2*-/- mutant cell lines were separated by SDS PAGE and western blotted before being probed with anti-RAD51 antiserum (1:500 dilution). '-' indicates protein extracts prepared without BLE treatment and '+' indicates protein extracts prepared after BLE treatment (1 $\mu g.ml^{-1}$ for 18 hours). The blots were stripped and re-probed with anti-OPB1 antiserum (1:1000 dilution) as a loading control. Sizes of the proteins are shown (kDa).

Fig.S8. Representative images of RAD51 focus formation in bloodstream form and procyclic form *T. brucei* cells expressing BRCA2 variants with altered BRC repeat numbers. Bloodstream form Lister427 (427BSF) and procyclic form TREU927 (927PCF) cells are shown to the left and right, respectively. In each case images of WT, *BRCA2*+/- and/or *brca2*-/- mutants, and *brca2*-/- cells expressing BRCA2 variants with varying numbers of BRC repeats (1BRC, 4BRC, 7BRC, 10BRC, full length BRCA2) are shown after growth in phleomycin (1 $\mu g.ml^{-1}$ for 18 hours). Each cell is shown in differential interface contrast (DIC), after staining with DAPI (DAPI) and after hybridisation with anti-RAD51 antiserum (1:1000 dilution) and secondary hybridisation with Alexa Fluor 594 conjugated anti-rabbit antiserum (1:7000 dilution, RAD51). Merged images of DAPI and RAD51 cells are also shown (Merge). White arrows indicate RAD51 foci.

Fig.S9. RAD51 expression in procyclic form TREU927 and bloodstream form Lister427 BRCA2 BRC variant expresser cells exposed to phleomycin. Total protein extracts from WT, *BRCA2*+/- and/or *brca2*-/- mutants, and from *brca2*-/- cells expressing BRCA2 variants with varying numbers of BRC repeats (1BRC, 4BRC, 7BRC, 10BRC, full length BRCA2) were separated by SDS PAGE and western blotted before being probed with anti-RAD51 antiserum (1:500 dilution). '-' indicates protein extracts prepared without phleomycin (BLE) treatment, and '+' indicates protein extracts prepared after phleomycin treatment (1 $\mu g.ml^{-1}$ for 18 hours). The blots were stripped and re-probed with anti-OPB1 antiserum (1:1000 dilution) as a loading control. Protein sizes are shown (kDa).

Fig.S10. Growth and repair efficiency of *T. brucei* cells expressing BRCA2 variants with altered BRC repeat number. A. Growth of wild type (WT) and *brca2* -/- cells is compared with cells expressing BRCA2 variants (-/-/+) with 1, 4, 7, 10 or 12 BRC repeats (indicated by 1BRC, 4BRC, 7BRC, 10BRC or BRCA2, respectively). Analysis was conducted in *T. brucei* Lister427 bloodstream form (BSF) cells and in procyclic form (PCF) cells from Lister427 and TREU927 strains. Cell densities were measured *in vitro* at 24 hr intervals; bars indicate standard errors from 3 experiments. **B.** EC50 values are shown of WT cells, of *BRCA2*+/- and/or *brca2*-/- mutants, and of BRCA2 re-expressers with varying numbers of BRC repeats in *T. brucei* Lister427 bloodstream form (BSF) cells and in procyclic form (PCF) cells from Lister427 and TREU927 strains, all grown in the presence of methyl methanesulphonate (MMS). EC50 values are the mean from three experiments expressed as a percentage of WT; bars indicate standard error.

Fig.S11. Analysis of genomic stability in procyclic form *BRCA2* mutants by pulsed field agarose gel electrophoresis. Pulsed field agarose gel electrophoresis separation of intact genomic DNA is shown from TREU927 and from Lister427 strains in **A** and **B**, respectively. In each case, clones of wild type (WT) cells and *BRCA2*+/- and *brca2*-/- mutants are shown after 380 generations and 230 generations growth *in vitro* (TREU927 and Lister427, respectively). For both strains, the left hand image shows an ethidium bromide-stain of the total DNA; lanes containing marker DNA molecules are indicated by *H. wingei*. Southern blots of the separated intact genomic DNA were then prepared and hybridised sequentially with a number of probes. For for TREU927, DNA probes used were (moving righwards) *GPI* (Glucose-6-phosphate isomerase, B), *VSG5* and *VSG1* (both described in main text); Lister427 DNA probes were against (moving rightwards) *GPI* (Glucose-6-phosphate isomerase) and *VSG 121*. Hybridising chromosomes are indicated (black arrows) and size markers are shown (Mb).

Fig.S12.Verification of the functionality of BRCA2Myc in PCF *T. brucei* cells. A. A Southern blot is shown of *Sac*II and *Xba*I-digested *T. brucei* genomic DNA, hybridised with the 5' UTR of

the *BRCA2* locus (see Fig.S4). The *BRCA2* wild type (WT) and *PUR*-deleted (Δ*brca2*::*PUR*) *BRCA2* alleles are indicated in *BRCA2* heterozygous (-/+) mutants, from which homozygous (-/-) mutants were generated by deleting the remaining *BRCA2* ORF through targeted integration of a *BSD* construct (generating the Δ*brca2*::*BSD* allele), or from which cells expressing only Myc-tagged BRCA2 (-/+12myc) were generated by targeting the intact *BRCA2* ORF with a myc-tagging construct (generating a C-terminal fusion of a sequence encoding a 12myc epitope to the *BRCA2* ORF; *BRCA2-12myc*). Size markers are shown (kbp). **B.** The concentration of methyl methanesulphonate (MMS) that caused 50% growth inhibition (EC50) is compared for the *BRCA2*-/+, *brac2*-/- and *BRCA2-12myc* (-/+12myc) cells shown in A. Values are the means from three experiments; bars indicate standard error.

Fig.S13. Analysis of genomic instability in bloodstream form Lister427 *BRCA2* mutants using an *ingi* DNA probe. Genomic DNA from clones of BSF Lister427 wild type cells (WT 427) and *brca2-/-* mutants (-/-1 or -/-2) after growth *in vitro* for ~150 generations was digested with *Xmn*I and separated by electrophoresis on an agarose gel. The DNA was Southern blotted before being hybridised with a DNA probe against *ingi (INGI-1)*. Size markers are shown (kb).

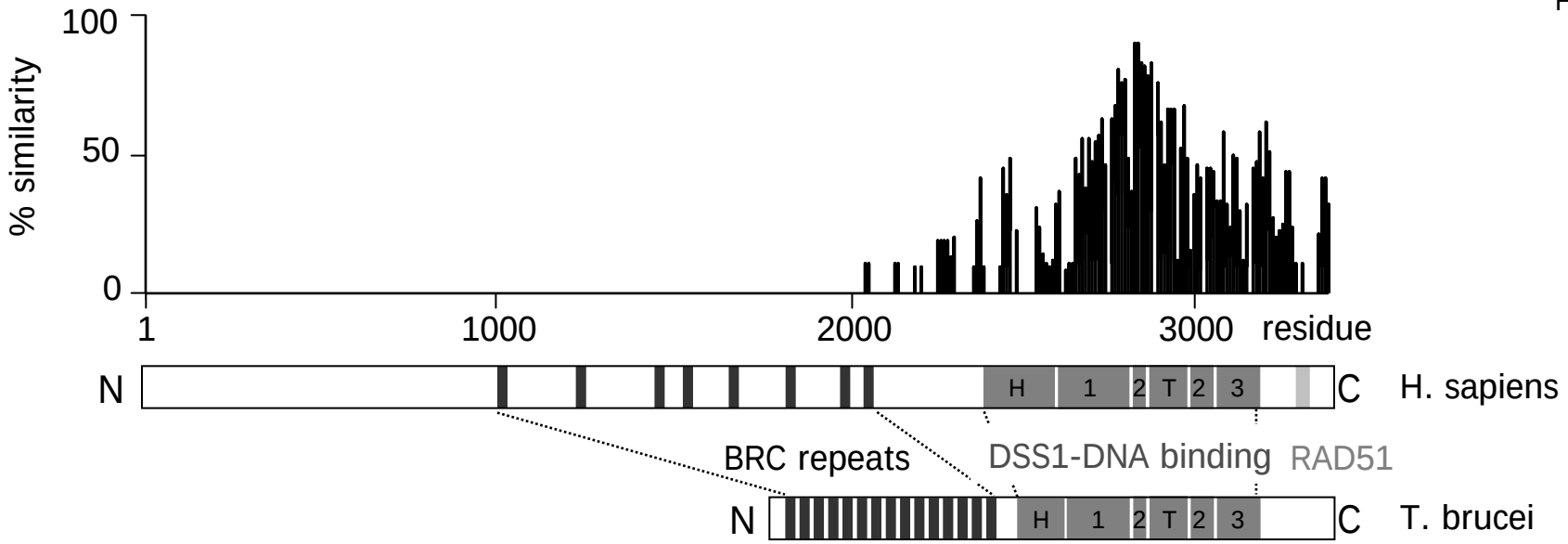


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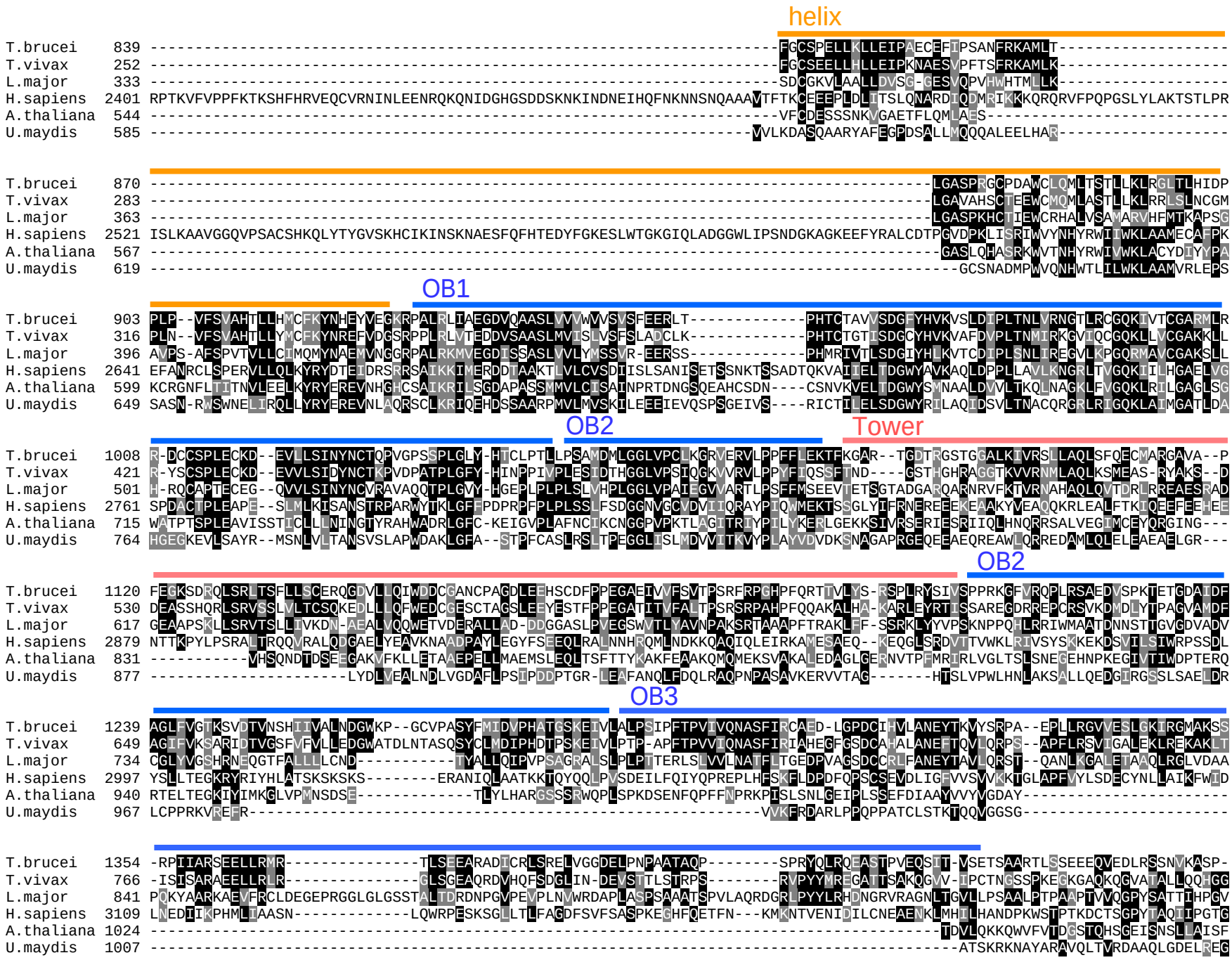
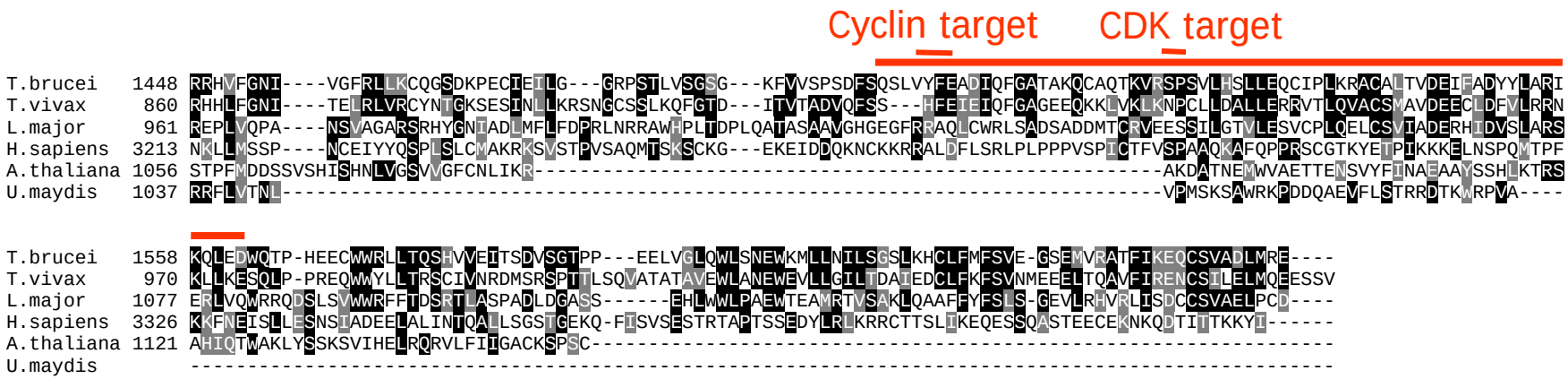
Fig.S2

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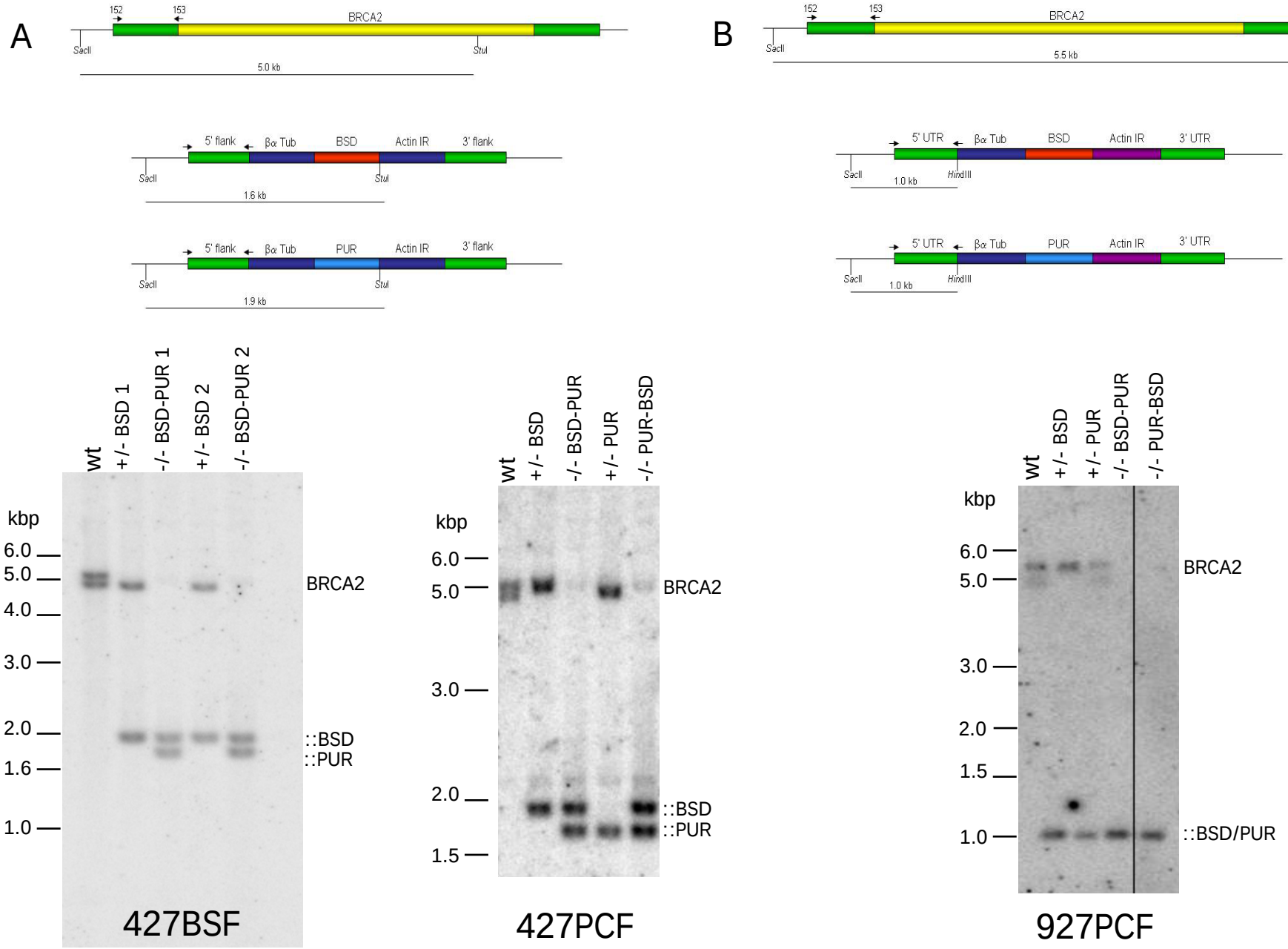
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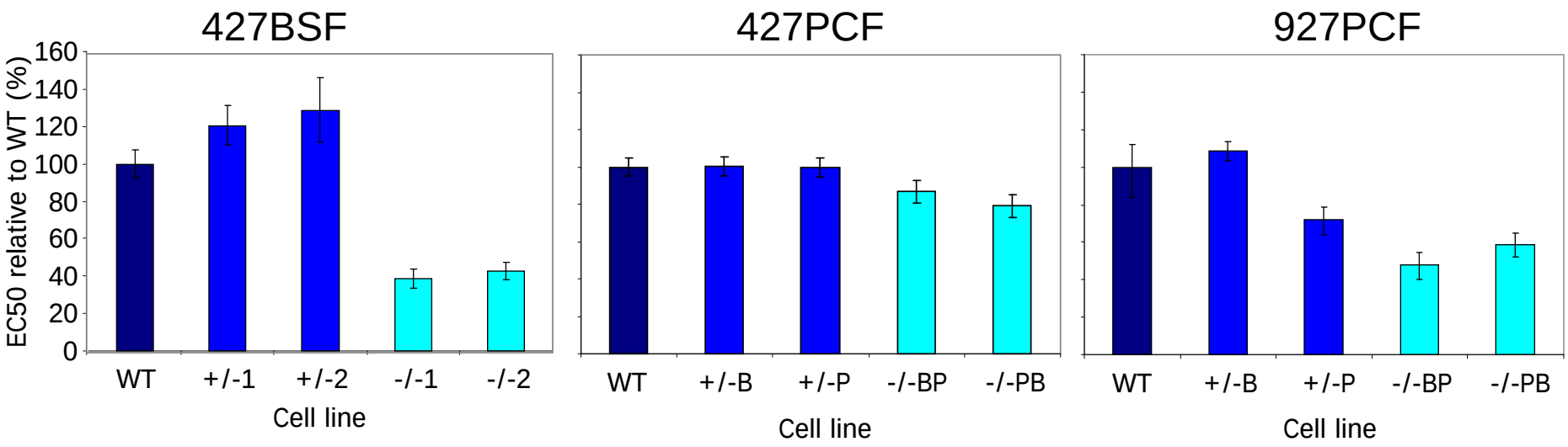
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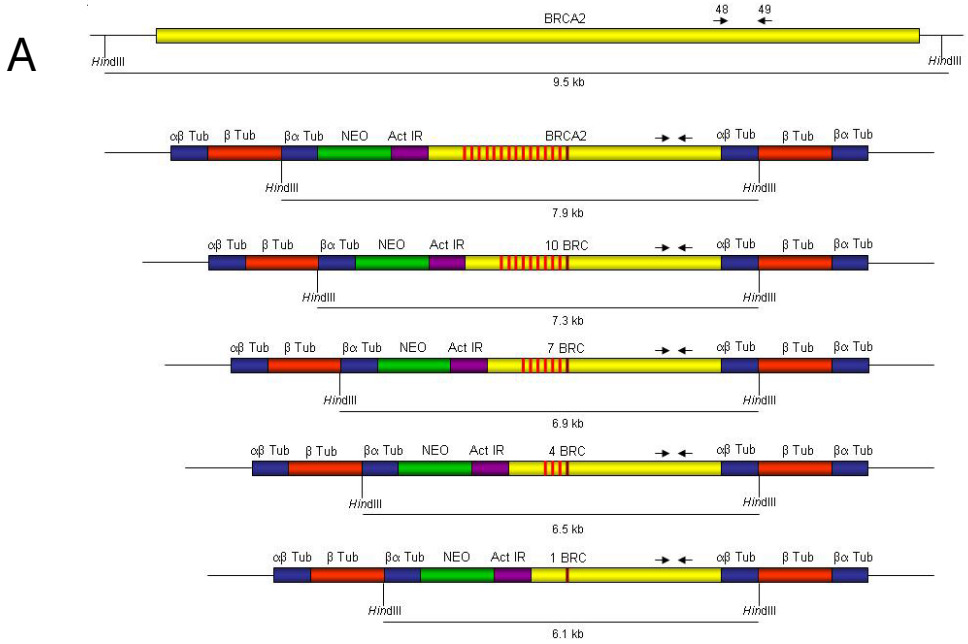
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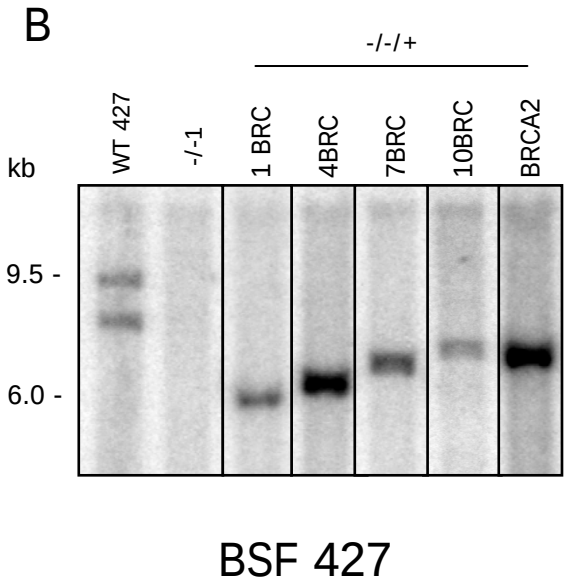
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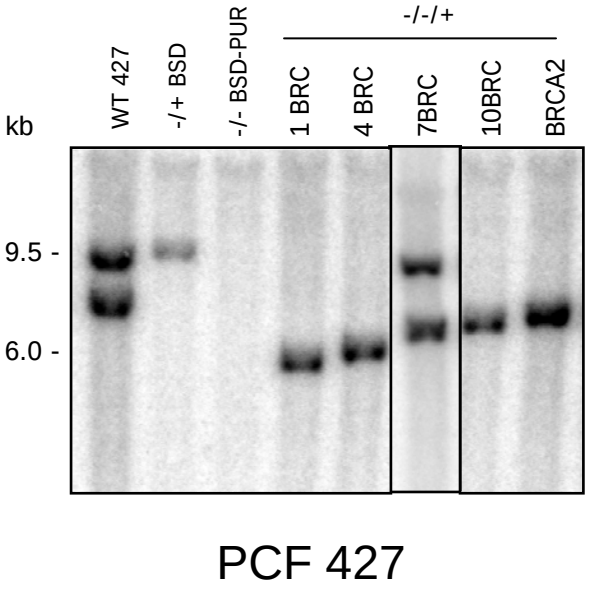
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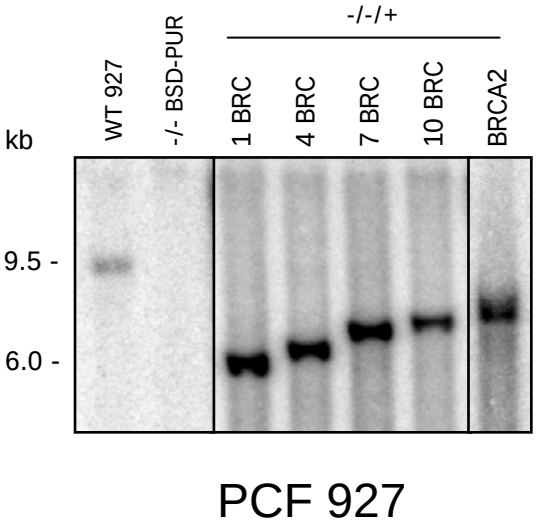
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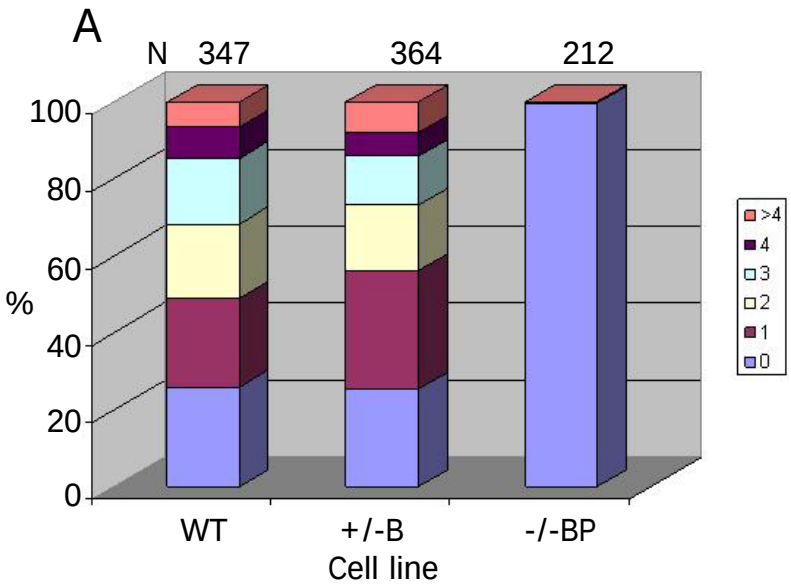
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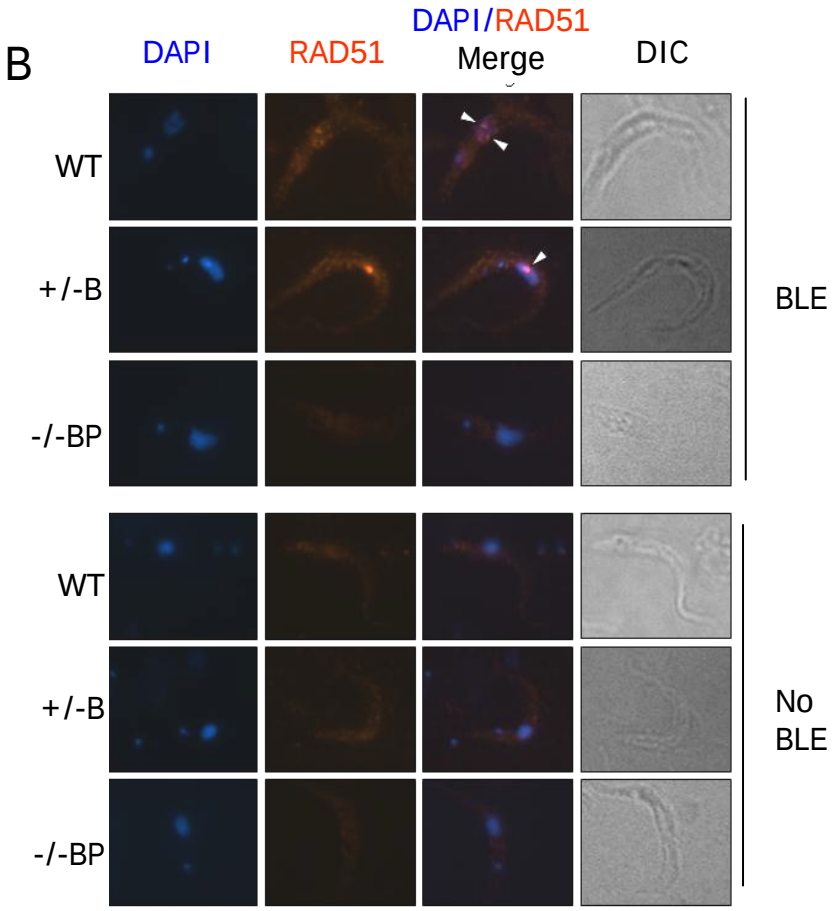
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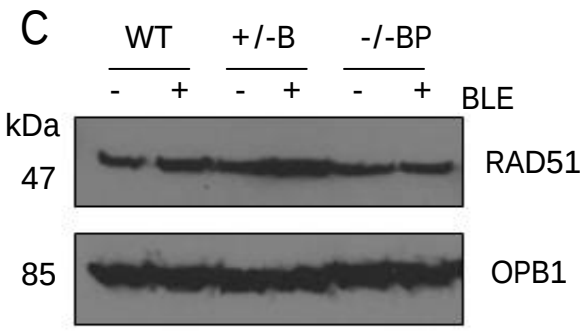
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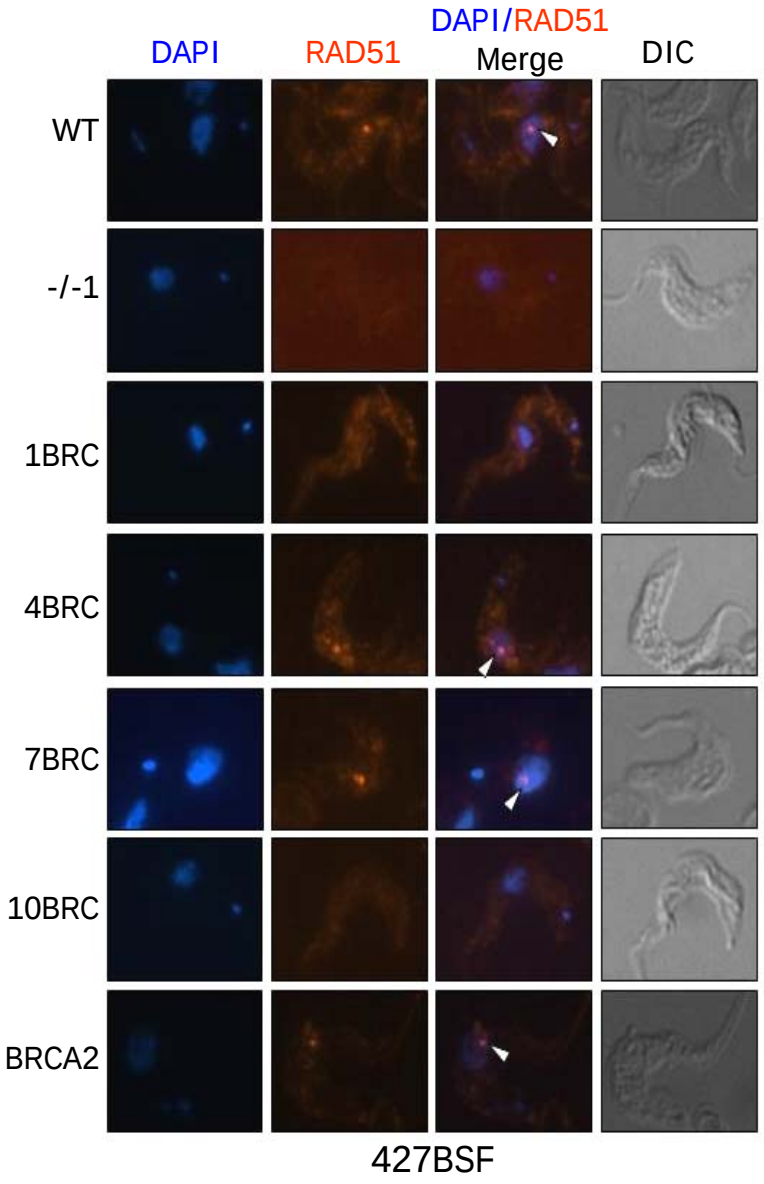
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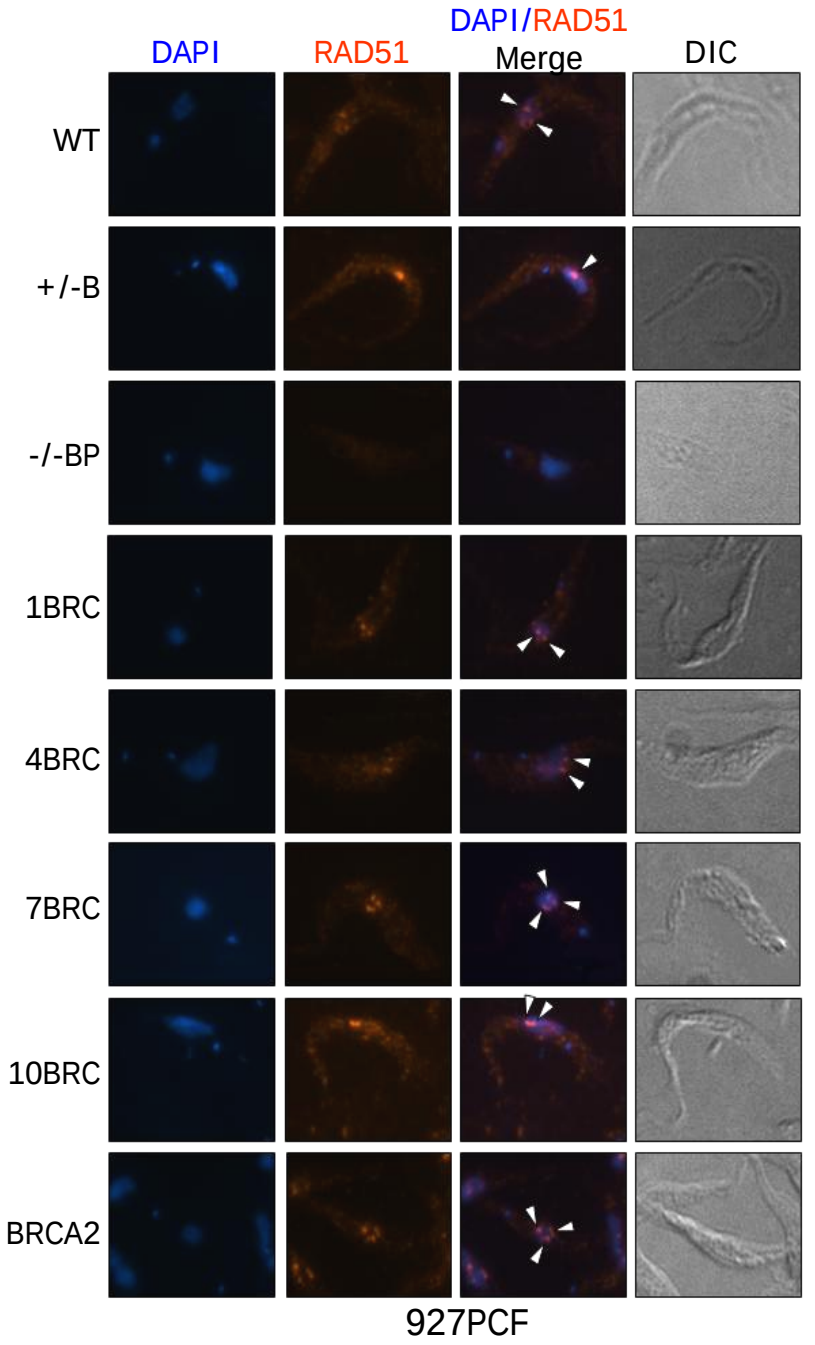
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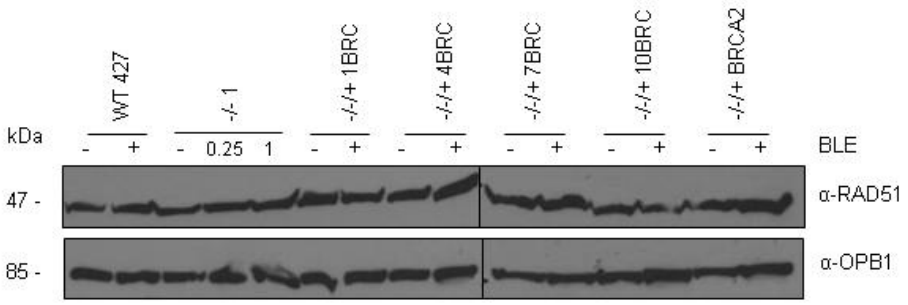
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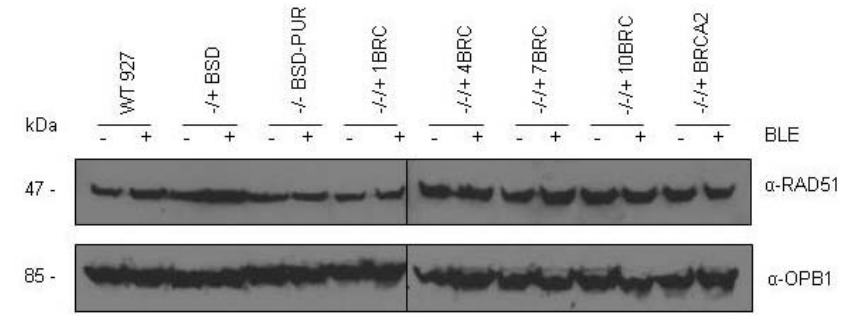
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PCF

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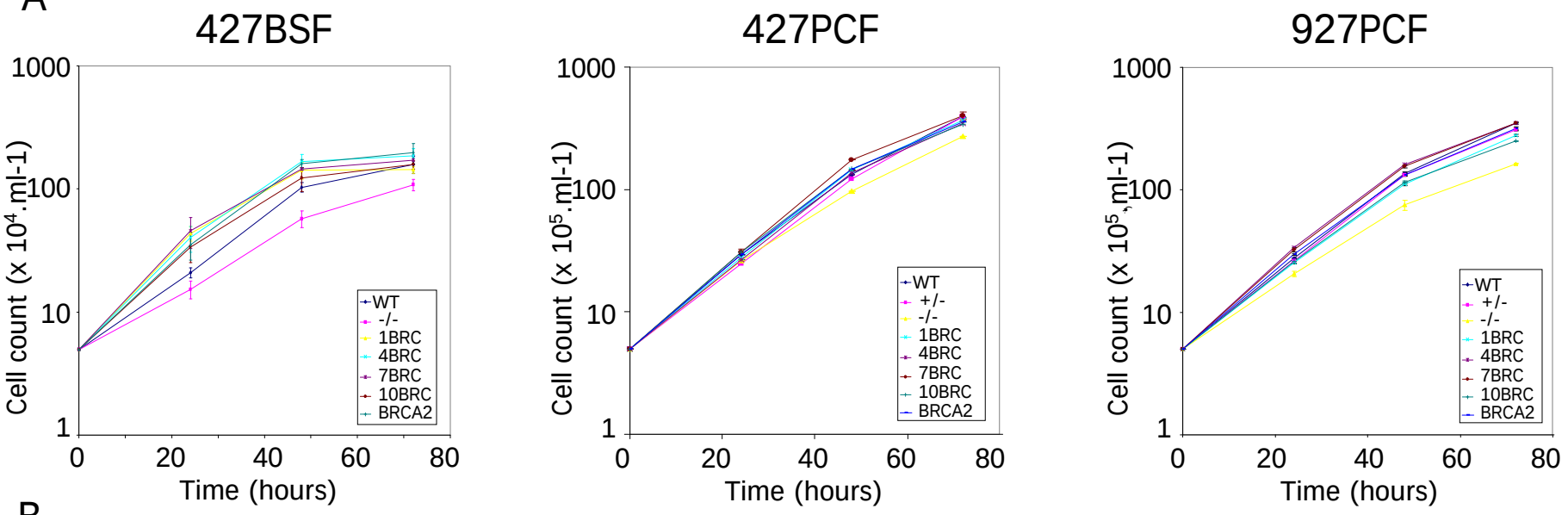
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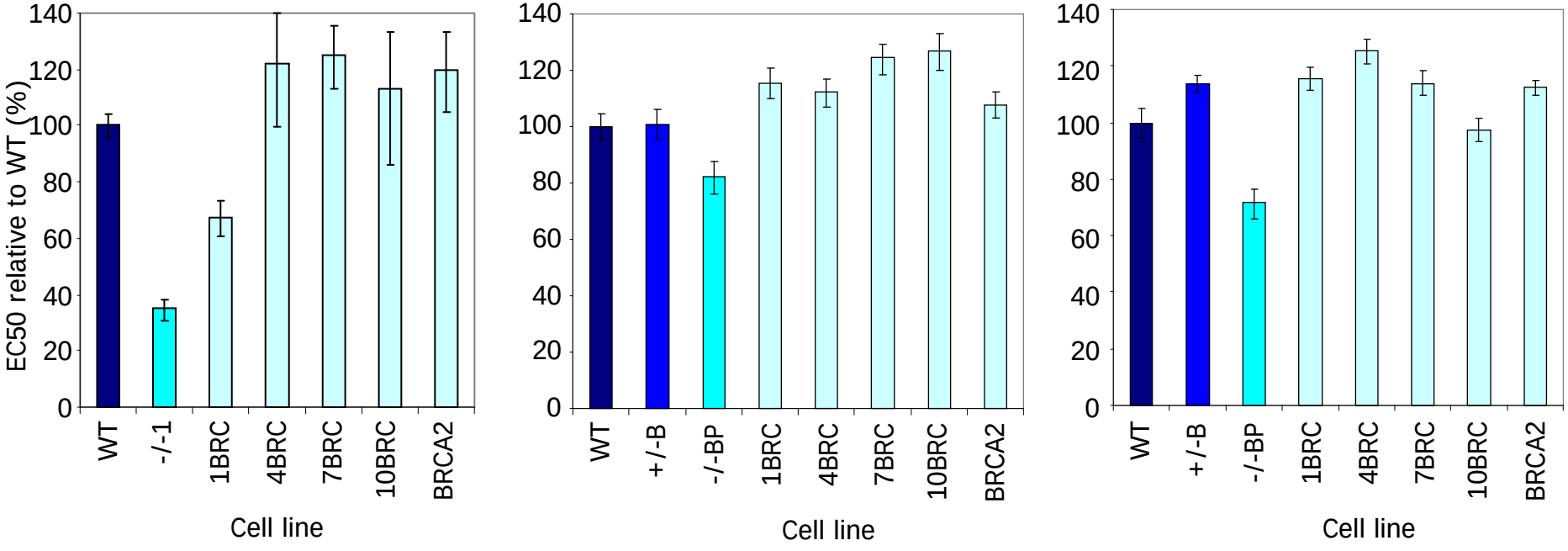
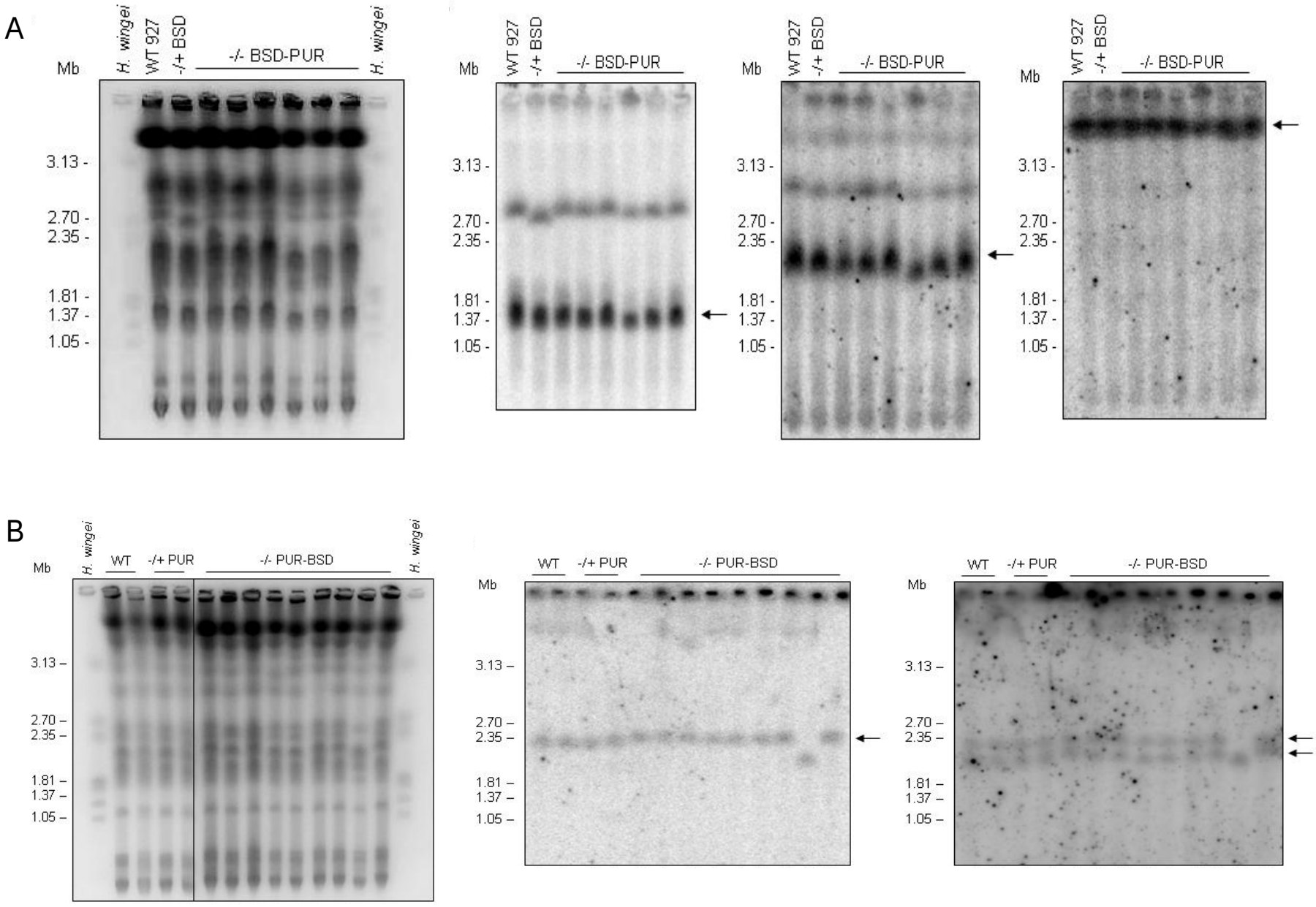
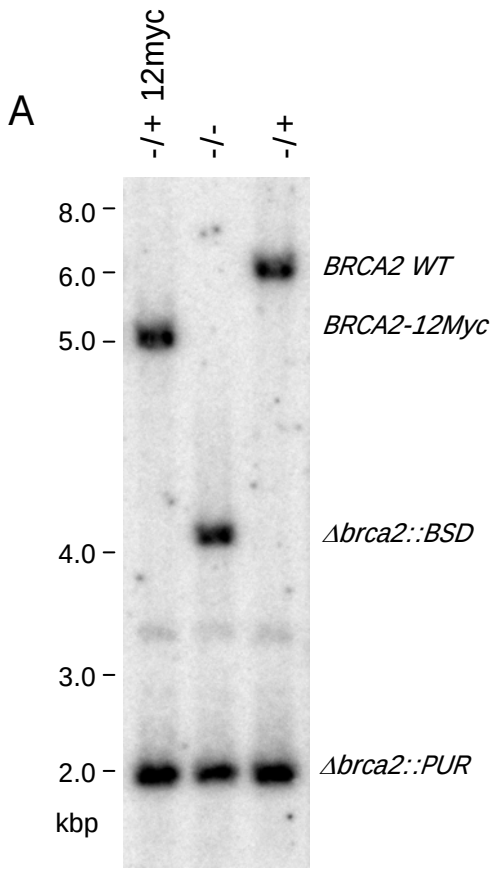
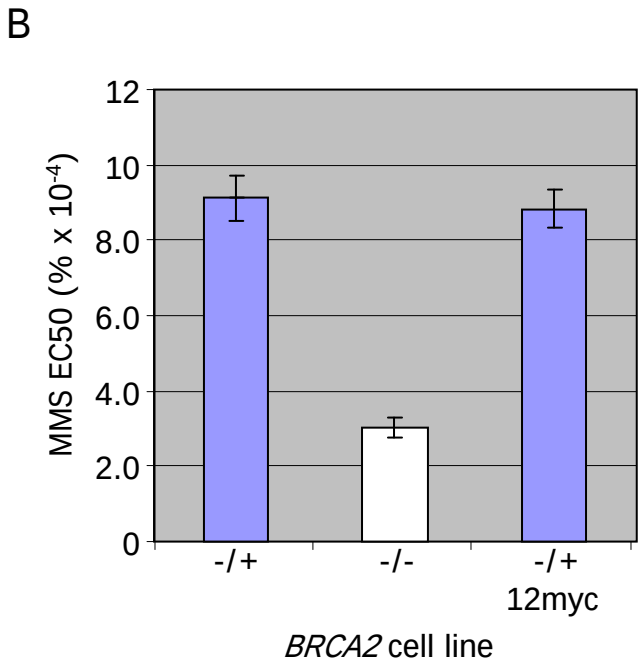
BRCA2

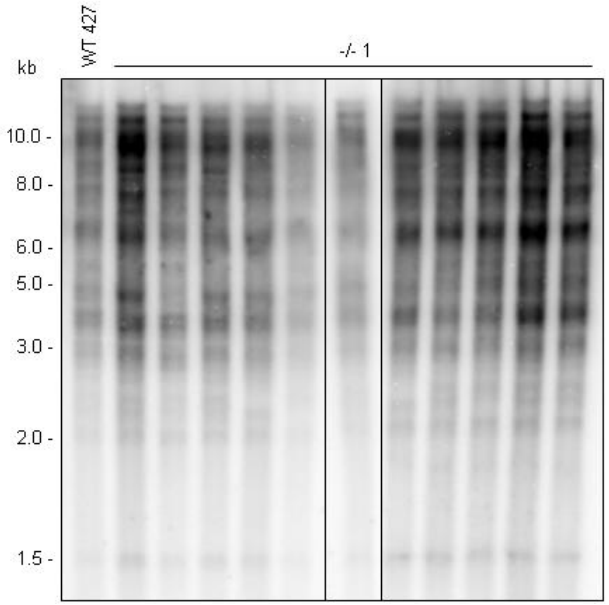
B

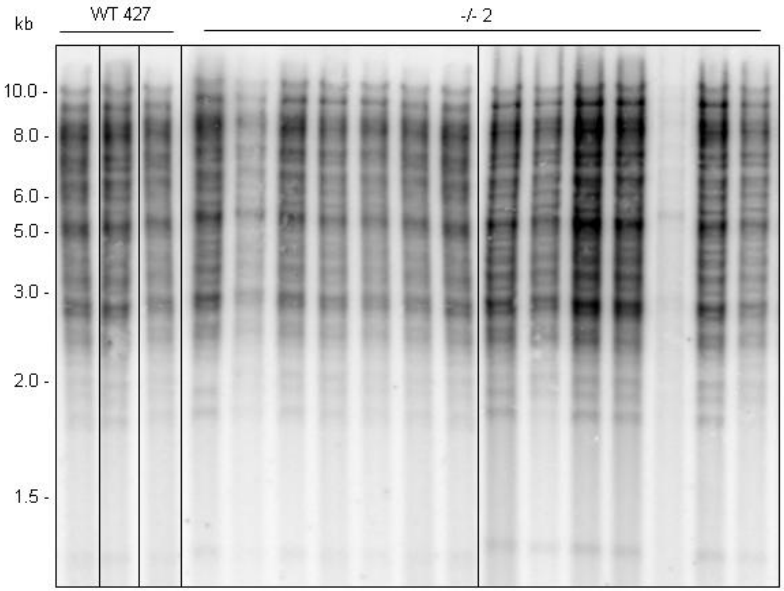

EC50 relative to WT (%)
WT
-/-1
+/-B
-/-BP
1BRC
4BRC
7BRC
10BRC
BRCA2
Cell line


A
Mb
H. wingei
WT 927
-/+ BSD
-/- BSD-PUR
H. wingei
3.13 -
2.70 -
2.35 -
1.81 -
1.37 -
1.05 -
B
Mb
H. wingei
WT
-/+ PUR
-/- PUR-BSD
H. wingei
3.13 –
2.70 –
2.35 –
1.81 –
1.37 –
1.05 –


A
-/+ 12myc
-/-
-/+
8.0
6.0
5.0
4.0
3.0
2.0
kbp
BRCA2 WT
BRCA2-12Myc
Δbrca2::BSD
Δbrca2::PUR


B
MMS EC50 (% x 10-4)
12
10
8.0
6.0
4.0
2.0
0
-/+
-/-
-/+
12myc
BRCA2 cell line


kb
WT 427
-/- 1
10.0 -
8.0 -
6.0 -
5.0 -
3.0 -
2.0 -
1.5 -


kb
WT 427
-/- 2
10.0 -
8.0 -
6.0 -
5.0 -
3.0 -
2.0 -
1.5 -