Supplementary Information

Supplemental Table 1. Gene targeting efficiency in mutants of nonessential genes. This table is presented as a separate Excel file.

Supplemental Table 2. Genes with altered expression patterns in *fun30*Δ cells based on microarray analysis.

genes upregulated in *fun30*Δ	genes downregulated in *fun30*Δ
YNR071C,YDR259C and YLR303W	YAL019W (FUN30, internal control), YHL048W, YDL227C, YIR042C, YNL289W, YOR367W, YDR123C, YHR216W, YPR200C, YPR201W, YJL157C

Supplemental Table 3. Yeast strains used in this study.

Strain name	Parental strain	Genotype	Source
JKM139		*MATa hoΔ hml::ADE1 hmr::ADE1 ade1-100 leu2-3,112 trp1::hisG lys5 ura3-52 ade3::GAL::HO*	[1]
JKM179		*MATalpha hoΔ hml::ADE1 hmr::ADE1 ade1-100 leu2-3,112 trp1::hisG lys5 ura3-52 ade3::GAL::HO*	[1]
yGI198	JKM139	*exo1::TRP1*	[2]
yGI200	JKM139	*sgs1::KanMX*	[2]
yXC690	JKM139	*fun30::NatMX*	This study
yXC720	JKM139	*fun30-K603R* (ATPase dead)	This study
yXC721	JKM139	*fun30-helicase*Δ	This study
yXC722	JKM139	*fun30-cueE*Δ	This study
yXC920	JKM139	*degron-Myc-Fun30 +pGAL-UBR1*	This study
yXC798	JKM139	*fun30::NatMX exo1::TRP1*	This study
yXC777	JKM139	*fun30::NATMX sgs1::KanMX*	This study
yXC902	JKM139	*sgs1::KanMX exo1::TRP1 fun30::NatMX*	This study
yXC656	JKM139	*rsc2::KanMX*	This study
yXC633	JKM139	*rsc2::KanMX fun30::NatMX*	This study
yXC664	JKM139	*chd1::KanMX*	This study
yXC668	JKM139	*chd1::KanMX fun30::NatMX*	This study
yXC665	JKM139	*isw1::KanMX*	This study
yXC669	JKM139	*isw1::KanMX fun30::NatMX*	This study
yXC666	JKM139	*swr1::KanMX*	This study
yXC670	JKM139	*swr1::KanMX fun30::NatMX*	This study
yXC663	JKM139	*arp8::KanMX*	This study
yXC667	JKM139	*arp8::KanMX fun30::NatMX*	This study

yXC745	JKM139	*rad54::KanMX*	This study
yXC746	JKM139	*rad54::KanMX fun30::NatMX*	This study
yXC780	JKM139	*dot1::KanMX*	This study
yXC782	JKM139	*dot1::KanMX fun30::NatMX*	This study
KHT34	JKM179	*hta1/2-S129A*	[3]
yXC686	JKM179	*hta1/2-S129A fun30::NatMX*	This study
yXC108	JKM139	*rad9::NatMX*	This study
yXC110	JKM139	*rad9::NatMX sgs1::KanMX*	This study
yXC111	JKM139	*rad9::NatMX exo1-TRP1*	This study
yXC112	JKM139	*rad9::NatMX sgs1::KanMX exo1::TRP1*	This study
yXC632	JKM139	*fun30::KanMX rad9::NatMX*	This study
yXC778	JKM139	*rad9::NatMX fun30::URA3 rsc2::KanMX*	This study
yXC779	JKM139	*rad9::NatMX fun30::URA3 arp8::KanMX*	This study
yXC628	JKM139	*cdc28-as1 Fun30-13xMyc-NatMX*	This study
yXC909	JKM139	*fun30-cueEΔ-13xMyc-NatMX*	This study
yXC910	JKM139	*fun30-helicaseΔ-13xMyc-NatMX*	This study
yXC911	JKM139	*fun30-K603R-13xMyc-NatMX*	This study
yXC906	JKM139	*Fun30-3xFLAG-KanMX Exo1-9xMyc-TRP1*	This study
yXC907	JKM139	*Fun30-13xMyc-NatMX Rfa1-3xFLAG-KanMX*	This study
yXC923	JKM139	*Fun30-3xFLAG-KanMX Dna2-9xMyc-TRP1*	This study
yXC715	JKM139	*mec1::KanMX sml1::TRP1 Fun30-13xMyc-KanMX*	This study
yXC699	yZZ357	*sgs1::URA3 exo1::TRP1 Fun30-13xMyc-KanMX*	This study
yXC735	JKM139	*cdc28-as1 mre11::KanMX Fun30-13xMyc-NatMX*	This study
yZZ203	JKM139	*Sgs1-9xMyc-TRP1*	This study
yXC650	JKM139	*fun30::NatMX Sgs1-9xMyc-TRP1*	This study
yZZ042	JKM139	*Exo1-9xMyc-TRP1*	This study
yXC649	JKM139	*fun30::NatMX Exo1-9xMyc-TRP1*	This study
yWH526	JKM139	*cdc28-as1 Dna2-9xMyc*	This study
yXC635	JKM139	*cdk1-as1 fun30::NatMX DNA2-9Myc-TRP1*	Ths study
yXC640	JKM139	*cdc28-as1 Mre11-13xMyc-KanMX*	This study
yXC692	JKM139	*cdc28-as1fun30::NatMX Mre11-13xMyc-KanMX*	This study
yXC843A	JKM139	*Rad9-HA-KanMX*	
yXC850A	JKM139	*Rad9-HA-KanMX fun30::NatMX*	
yTT035	JKM179	*FLAG-HHT1::LEU2*	Tsukuda *et al*., 2005
yXC710	JKM179	*fun30::NatMX FLAG-HHT1::LEU2*	This study
yXC742	JKM179	*sgs1::NatMX exo1::TRP1 FLAG-HHT1::LEU2*	This study
tGI354		*MATa-inc arg5,6::MATa-HPH ade3::GAL::HO hmr::ADE1 hml::ADE1*	Ira *et al*., 2003

		ura3-52	
yXC631	tGI354	*fun30::NatMX*	This study
yXC728	tGI354	*fun30-K603R*	This study
yXC729	tGI354	*fun30-helicase*Δ	This study
yXC730	tGI354	*fun30-cue*Δ	This study
yXC748	tGI354	*arp8::KanMX*	This study
yXC750	tGI354	*rsc2::KanMX*	Ths study
yXC671	tGI354	*swr1::NatMX*	This study
yXC749	tGI354	*chd1::KanMX*	Ths study
YMV80		*ho hml::ADE1 mata::hisG hmr::ADE1 his4::NatMX-leu2 (XhoI to Asp718) leu2::MATa ade3::GAL::HO ade1 lys5 ura3-52 trp1*	[4]
yXC714	YMV80	*arp8::KanMX rad51::URA3*	This study
yXC597	YMV80	*fun30::NatMX rad51::URA3*	This study
yXC754	YMV80	*swr1::NatMX rad51::URA3*	This study
yXC753	YMV80	*rsc2::KanMX rad51::URA3*	This study
yWH378	YMV80	*rad51::URA3*	[2]

Supplemental references

[1] Lee, S. E. et al., Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94** (3), 399 (1998).

[2] Zhu, Z. et al., Sgs1 helicase and two nucleases dna2 and exo1 resect DNA double-strand break ends. *Cell* **134** (6), 981 (2008).

[3] Lee, K., Zhang, Y., and Lee, S. E., Saccharomyces cerevisiae ATM orthologue suppresses break-induced chromosome translocations. *Nature* **454** (7203), 543 (2008).

[4] Vaze, M. et al., Recovery from checkpoint-mediated arrest after repair of a double- strand break requires srs2 helicase. *Mol Cell* **10** (2), 373 (2002).

Supplemental Figure Legends

Supplemental Figure 1. Fun30 suppresses the crossover pathway during ectopic gene conversion.

Analysis of crossover frequency during ectopic recombination in wild-type cells and indicated mutants. **a**. Schematic representation of ectopic recombination assay; **b.** Southern blot analysis of DSB repair by ectopic recombination in wild type, *fun30*Δ cells

and *fun30* mutants deficient in the ATPase or helicase domain and a plot showing crossover frequencies at the 8 h timepoint; **c.** Southern blot analysis and quantification of crossover frequencies for wild-type and indicated mutants cells; **d.** The efficiency of repair by ectopic recombination in wild-type cells and indicated mutants. Plotted values are the mean values ± SD from three independent experiments

Supplemental Figure 2. Fun30 plays an important role in both Sgs1- and Exo1-dependent resection pathways.

a. Southern blot analysis and quantification of 5' strand resection at 5 kb from a DSB in wild-type cells and indicated mutants. Plotted values are the mean values ± SD from three independent experiments. **b.** Southern blot analysis of 5' strand resection at the *MAT***a** locus in *sgs1 exo1 fun30* triple mutant cells. **c.** Analysis of DNA damage sensitivity. Cells of the indicated genotypes were 1:10 serially diluted and spotted onto YEPD or YEPD with camptothecin.

Supplemental Figure 3. Deletion of the *FUN30* gene does not change the cellular level of resection proteins nor the general chromatin structure.

a. Western blot analysis of the indicated proteins in wild-type and *fun30*Δ cells.

b. Comparison of general chromatin structure in wild-type and *fun30*Δ cells. Ethidium bromide and Southern blot analysis of DNA isolated from nuclei treated with Micrococcal nuclease for 0, 1, 4, 8, or 16 minutes. DNA was separated on a 1.4% agarose gel.

Supplemental Figure 4. Fun30 co-immunoprecipitates with multiple resection enzymes upon DNA damage.

Endogenous Dna2, Exo1, RFA1 and Fun30 were tagged with either a multi Myc or a multi FLAG tag at their C-terminus. Cell extracts with or without Benzonase digestion were subjected to immunoprecipitation either with anti-Myc or anti-FLAG antibodies or with an appropriate antibody mock IgG as described in Supplemental methods. a-c. Western blot showing co-immunoprecipitation of Fun30 with RPA, Exo1 and Dna2, respectively. Bound proteins and input proteins were analyzed by immunoblotting with indicated antibodies. "*" indicates full-length Fun30 protein. d. An agarose gel stained with ethidium bromide shows an effective digestion of genomic DNA by Benzonase treatment.

Supplemental Figure 5. Analysis of expression and recruitment of *fun30* mutant proteins to a DSB.

a. Western blot showing protein levels from whole cell extracts for wild-type Fun30 and indicated mutant *fun30* proteins. **b.** ChIP analysis of the recruitment of wild-type Fun30 and indicated *fun30* mutant proteins to DSBs. Error bars represent SD from three independent experiments.

Supplemental Figure 6. Comparison of resection kinetics and histone H3 occupancy at DSBs.

Comparison of resection kinetics and histone occupancy at 1, 5 and 10 kb from the DSB in (**a**) wild-type cells, (**b**) *fun30*Δ cells, and (**c**) *sgs1*Δ *fun30*Δ cells. Resection was measured by Southern blotting and histone occupancy was followed by histone H3 ChIP at indicated time points. Error bars represent SD from three independent experiments. Because resection eliminates one of the two DNA strands at DSBs, histone H3 loss as measured by ChIP-qPCR corresponds to a decrease of H3 signal below 0.5 of the original value indicated with blue dashed line.

Supplemental Figure 7. INO80 and RSC chromatin remodeling complexes play redundant roles with Fun30 in resection close to DSBs.

a. Southern blot analysis and quantification of resection kinetics in wild-type and indicated mutant cells. Plotted values are the mean values ± SD from three independent experiments. **b.** Southern blot analysis and quantification of resection kinetics in cells deficient in Arp8 or Arp8 and Sth1. Checkpoint activation was monitored by immunoblotting using an antibody against Rad53.

Supplemental Figure 8. SWR1, CHD1 and ISW1 remodeling factors do not play significant roles in resection.

a. Southern blot analysis and quantification of resection kinetics in wild-type cells and indicated mutants. Plotted values are the mean values ± SD from three independent experiments. **b-c.** Analysis of DNA damage sensitivity. Cells of the indicated genotypes were 1:10 serially diluted and spotted onto YEPD or YEPD with camptothecin or phleomycin.

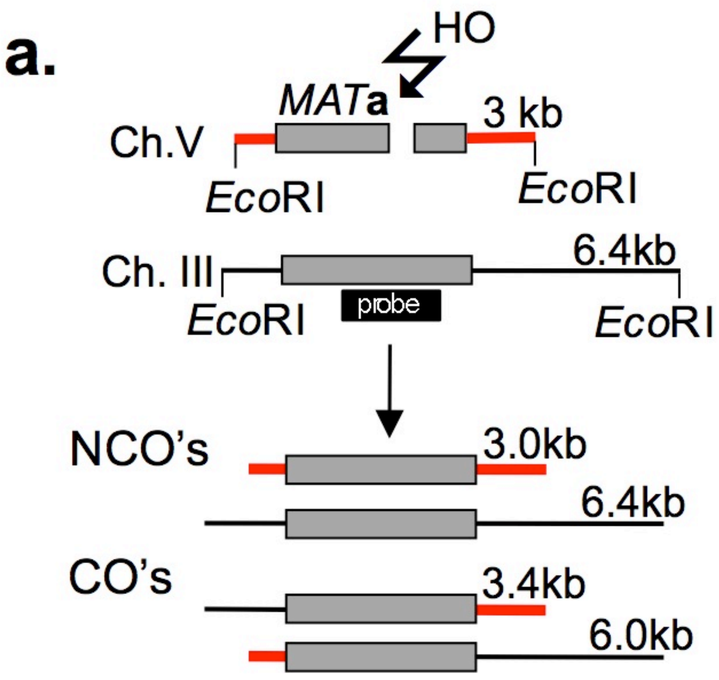
Supplemental Figure 9. Rad54 is epistatic to Fun30 with respect to DNA damage response.

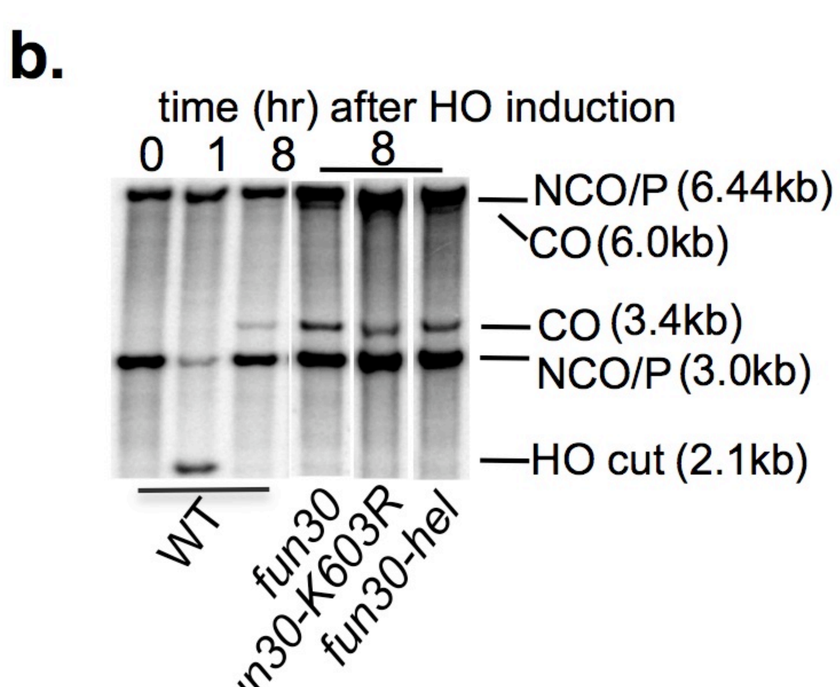
a. Southern blot analysis and quantification of resection kinetics in wild-type cells and indicated mutants. Plotted values are the mean values ± SD from three independent experiments. Analysis of DNA damage sensitivity. Cells of the indicated genotypes were 1:10 serially diluted and spotted onto YEPD or YEPD with camptothecin or phleomycin. **b.** A summary of resection defects for the tested single or double ATP-dependent nucleosome remodeling factor mutants.

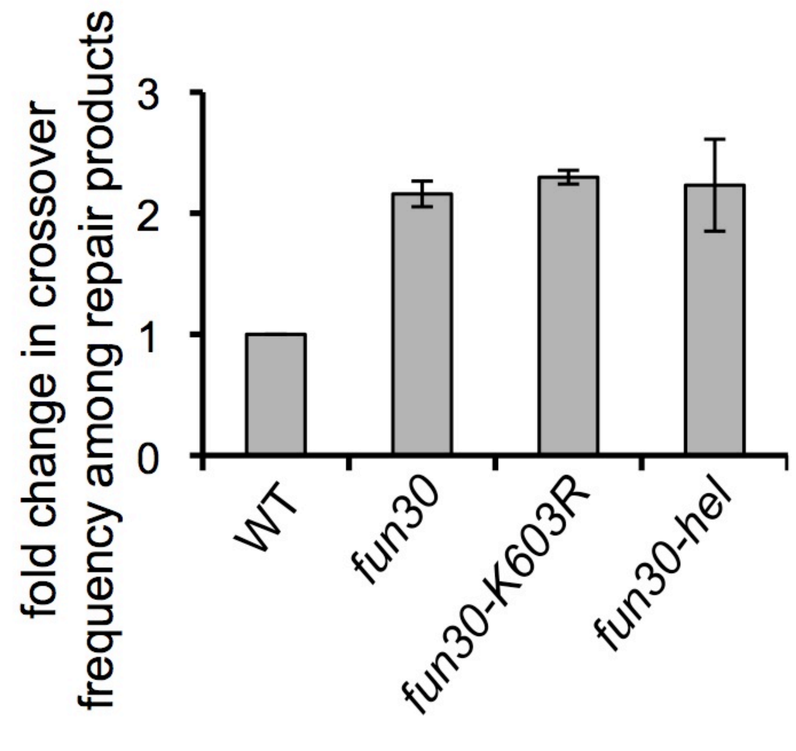
Supplemental Figure 10. Fun30 promotes resection within chromatin with methylated H3K79 and phosphorylated H2A S129.

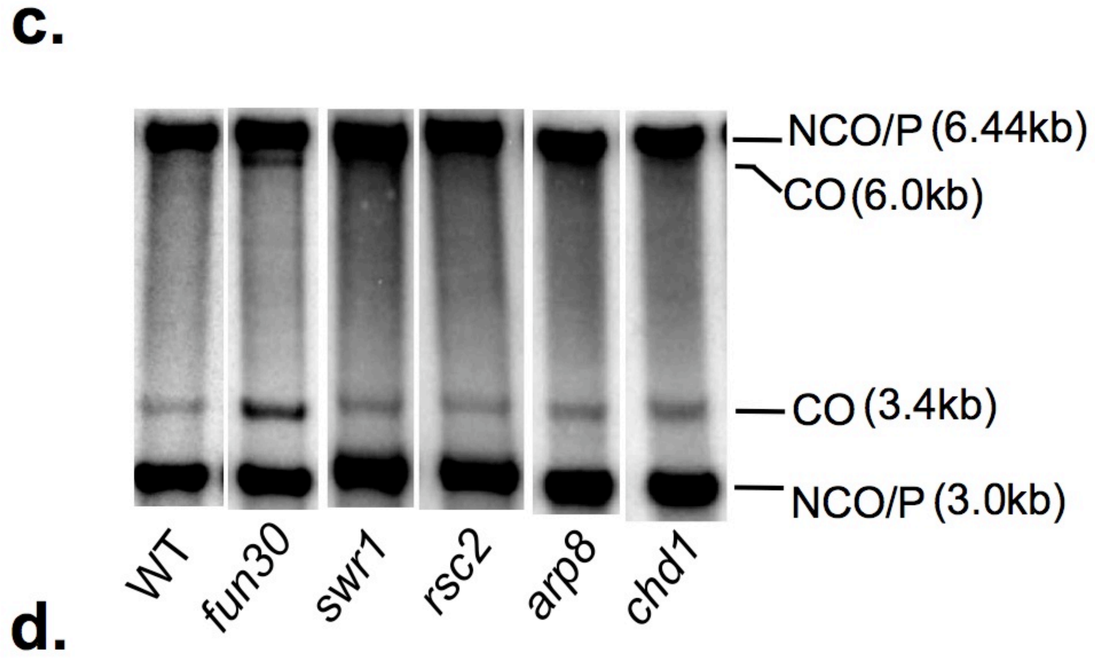
a. Southern blot analysis in indicated mutants. Quantification is shown in Figure 4b-c. **b.** Southern blot analysis and quantification of resection kinetics in wild-type cells and indicated mutants. Plotted values are the mean values ± SD from three independent experiments. **c.** Analysis of DNA damage sensitivity. Cells of the indicated genotypes were 1:10 serially diluted and spotted onto YEPD or YEPD with camptothecin.

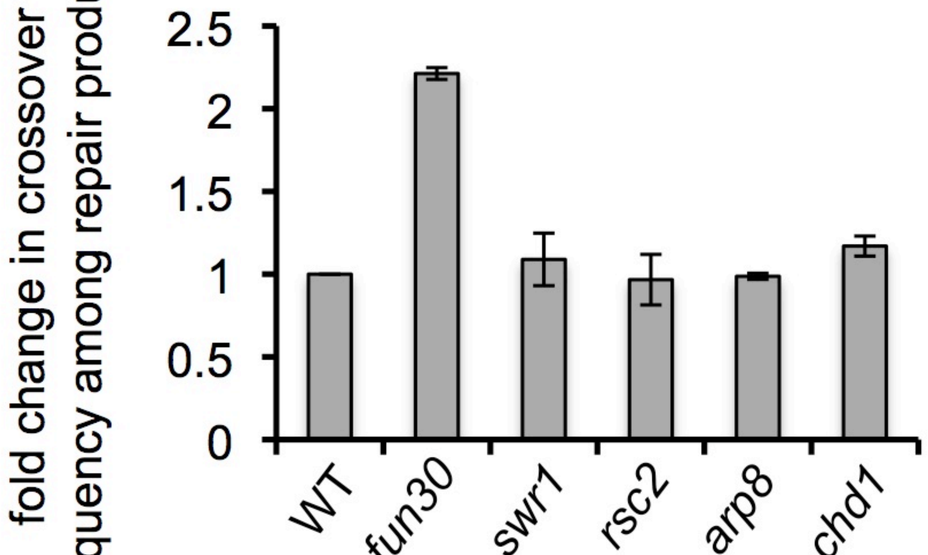
Figure S1, Chen et al.











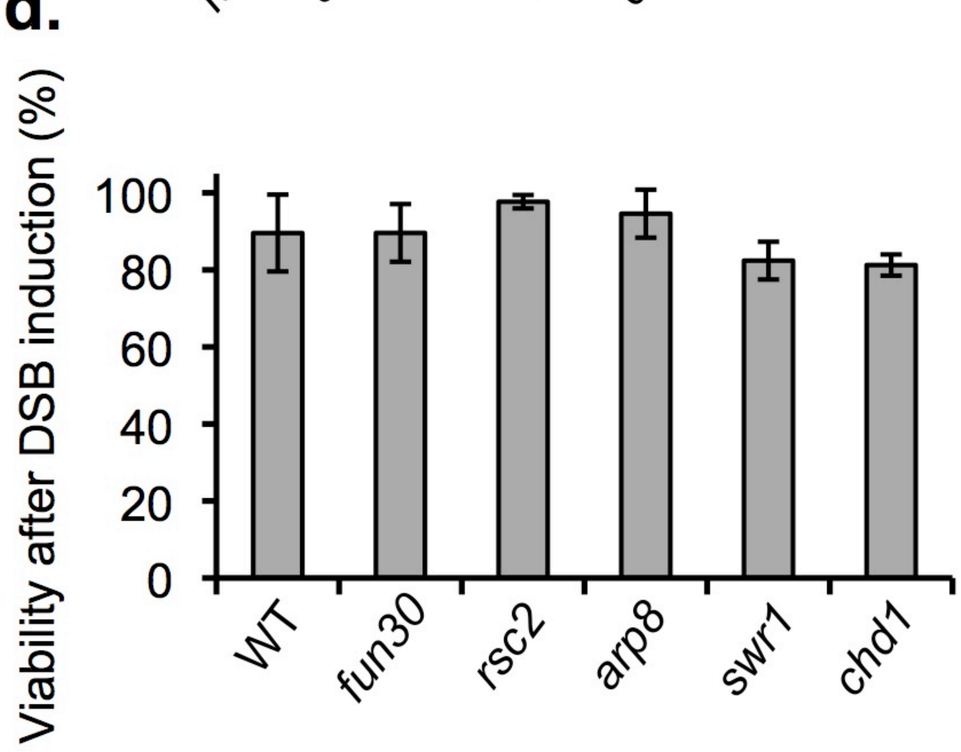


Figure S2, Chen et al.

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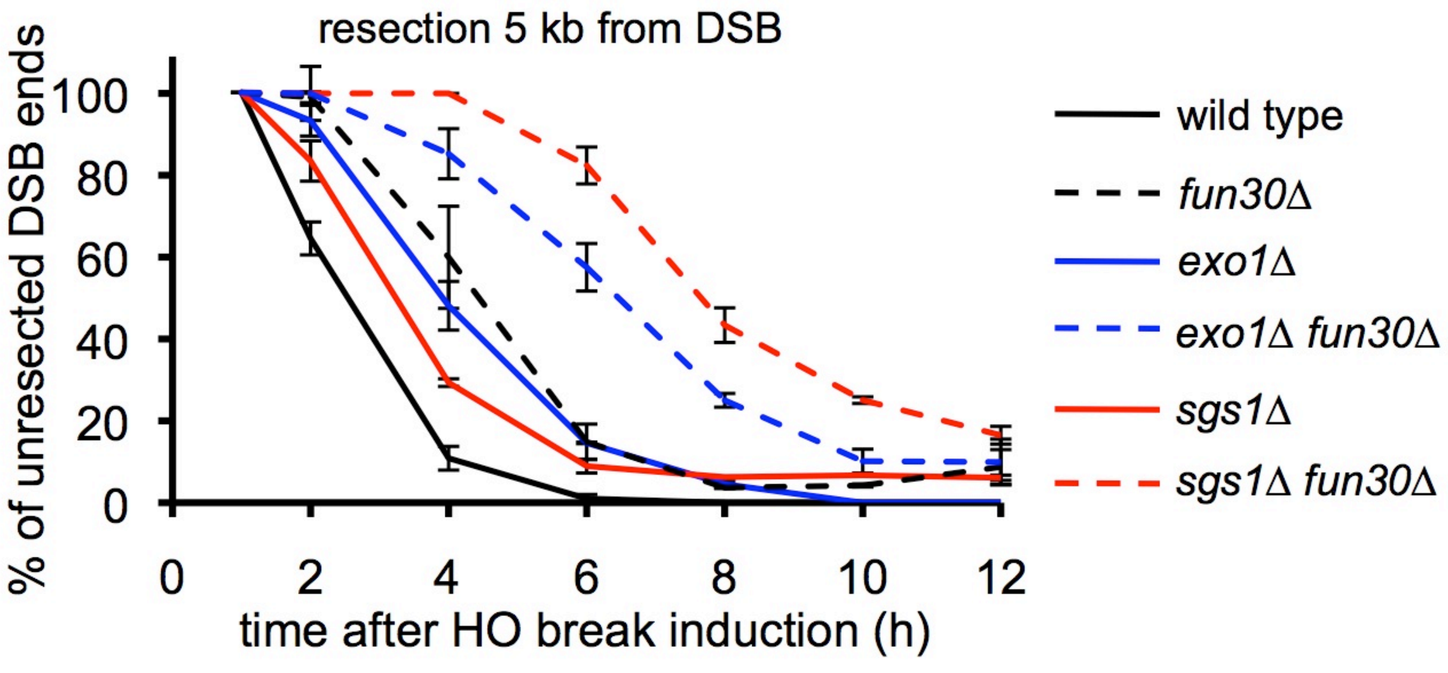

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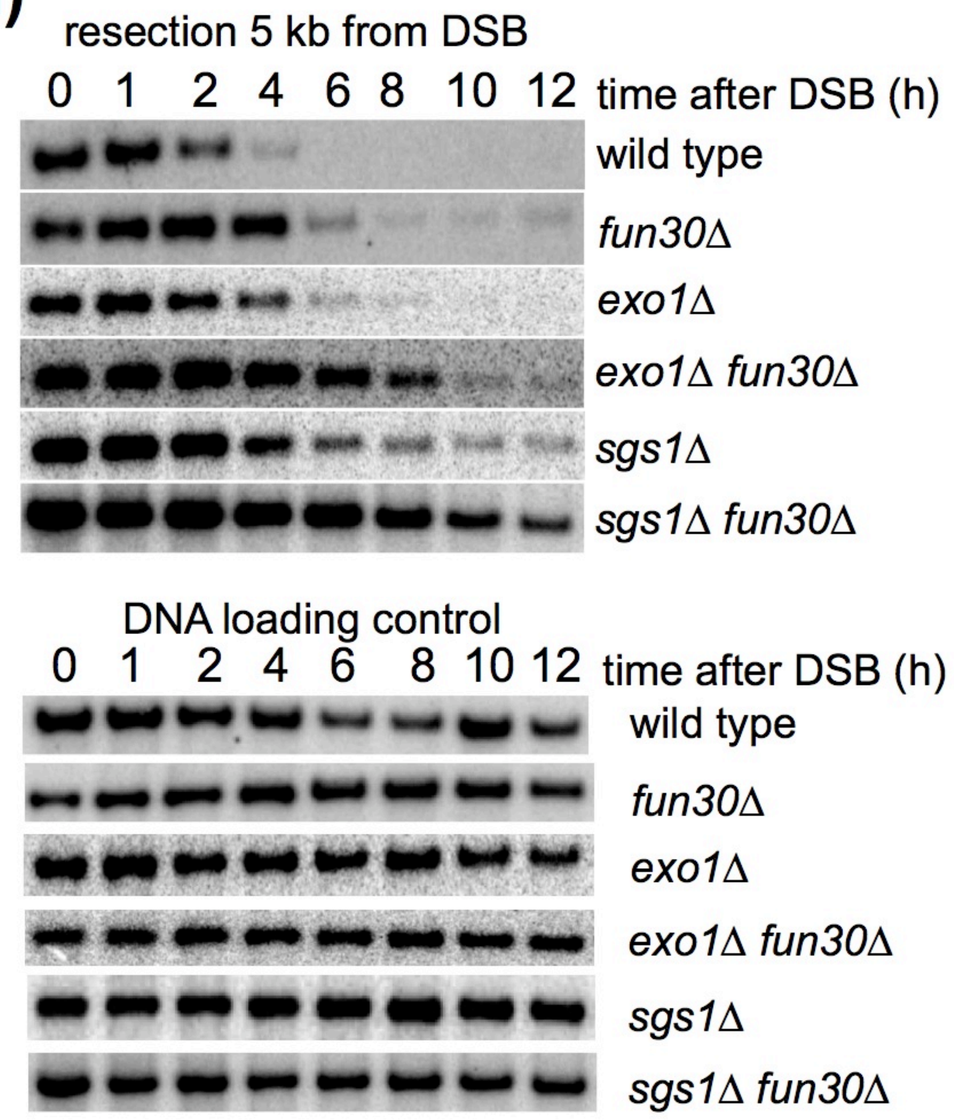

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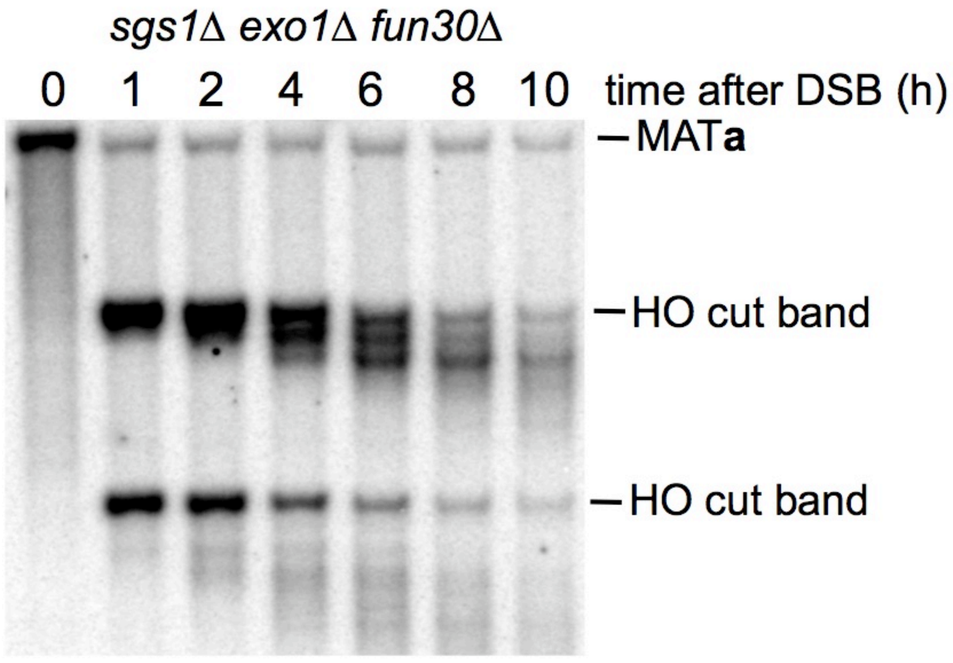

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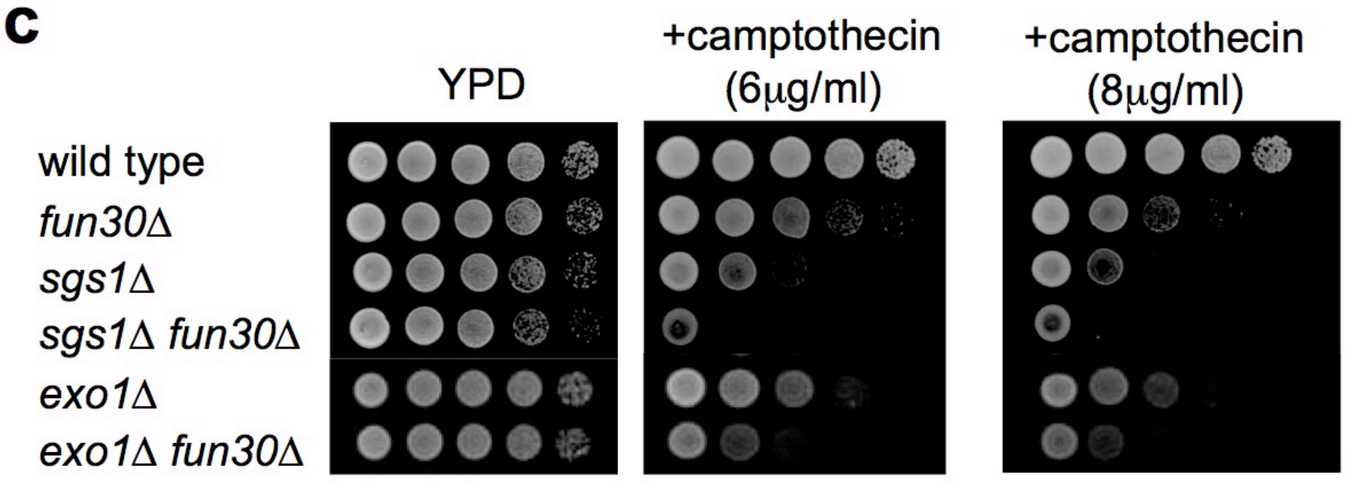

Figure S3, Chen et al.

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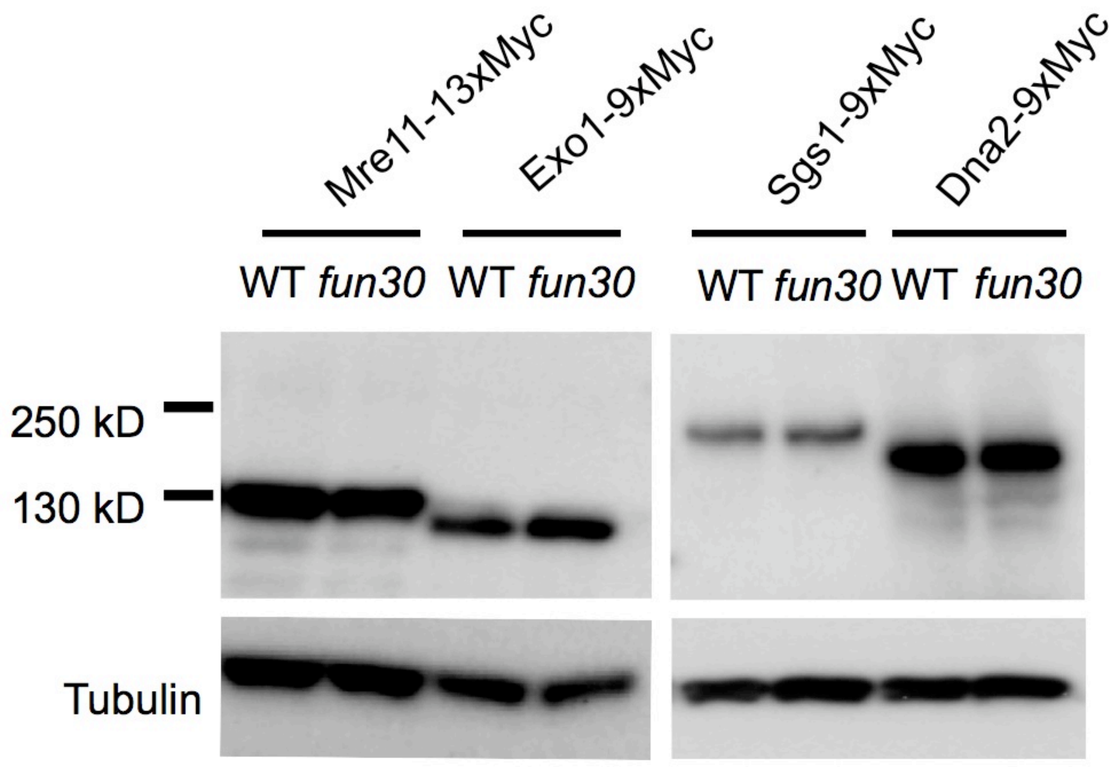

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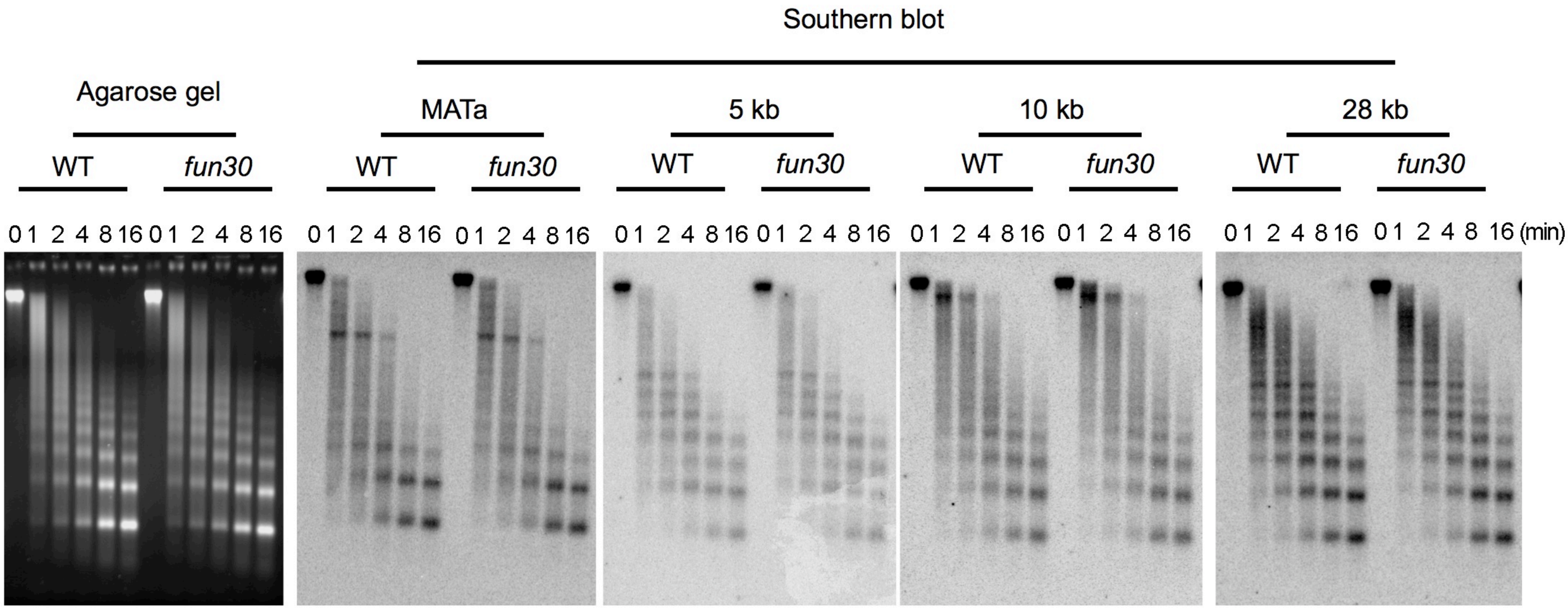

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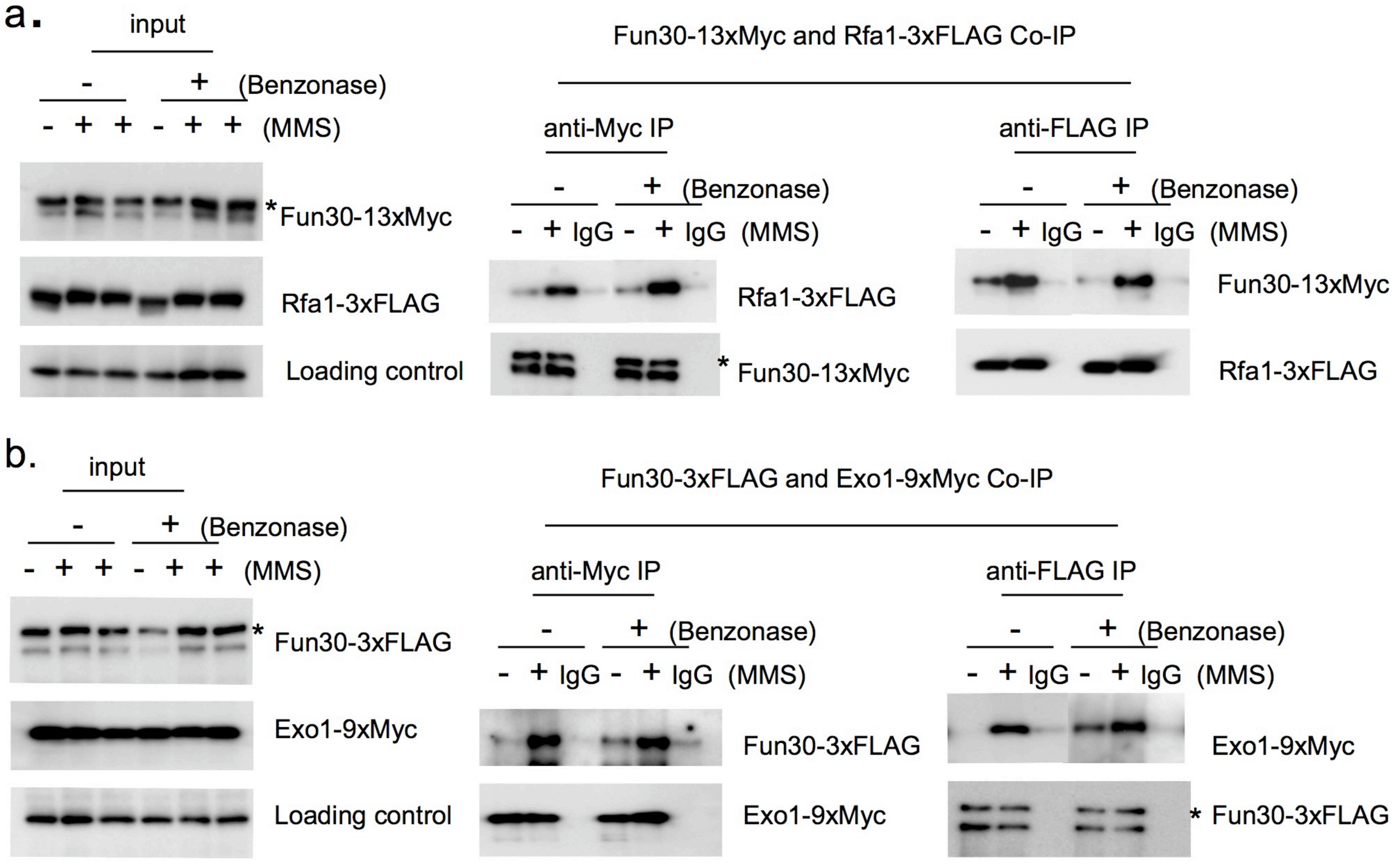

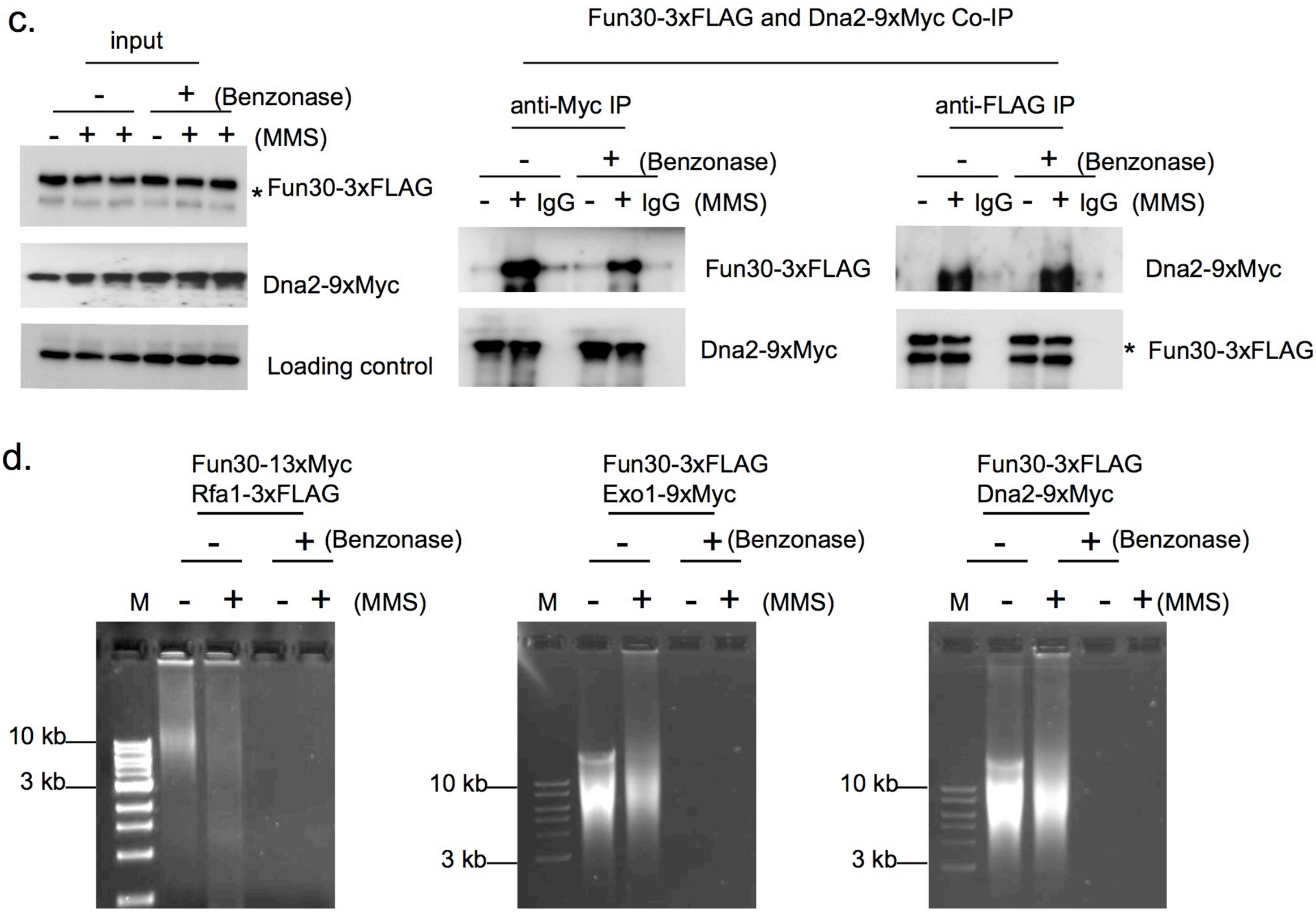

Figure S5, Chen et al.

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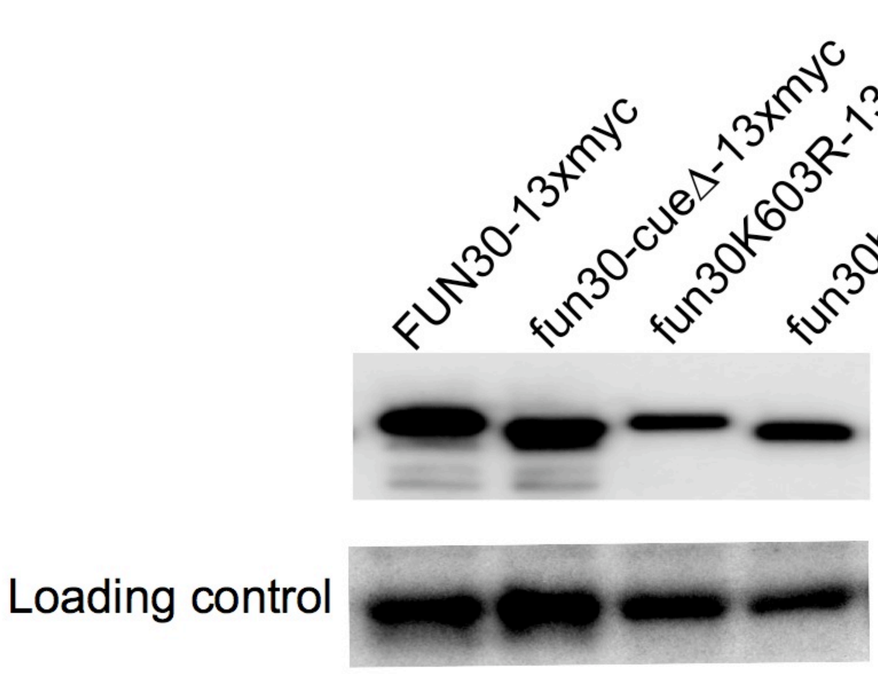

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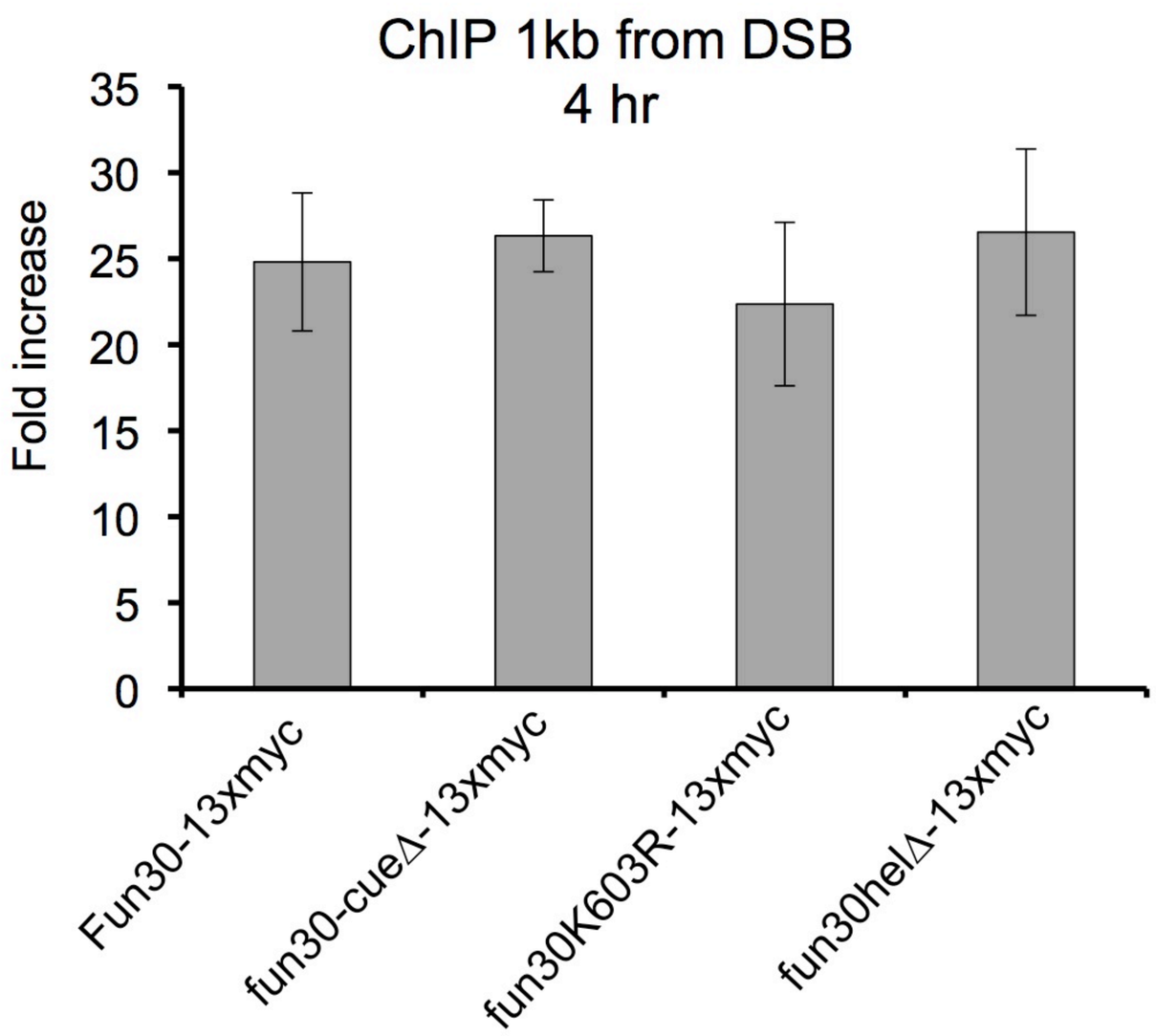

Figure S6, Chen et al.

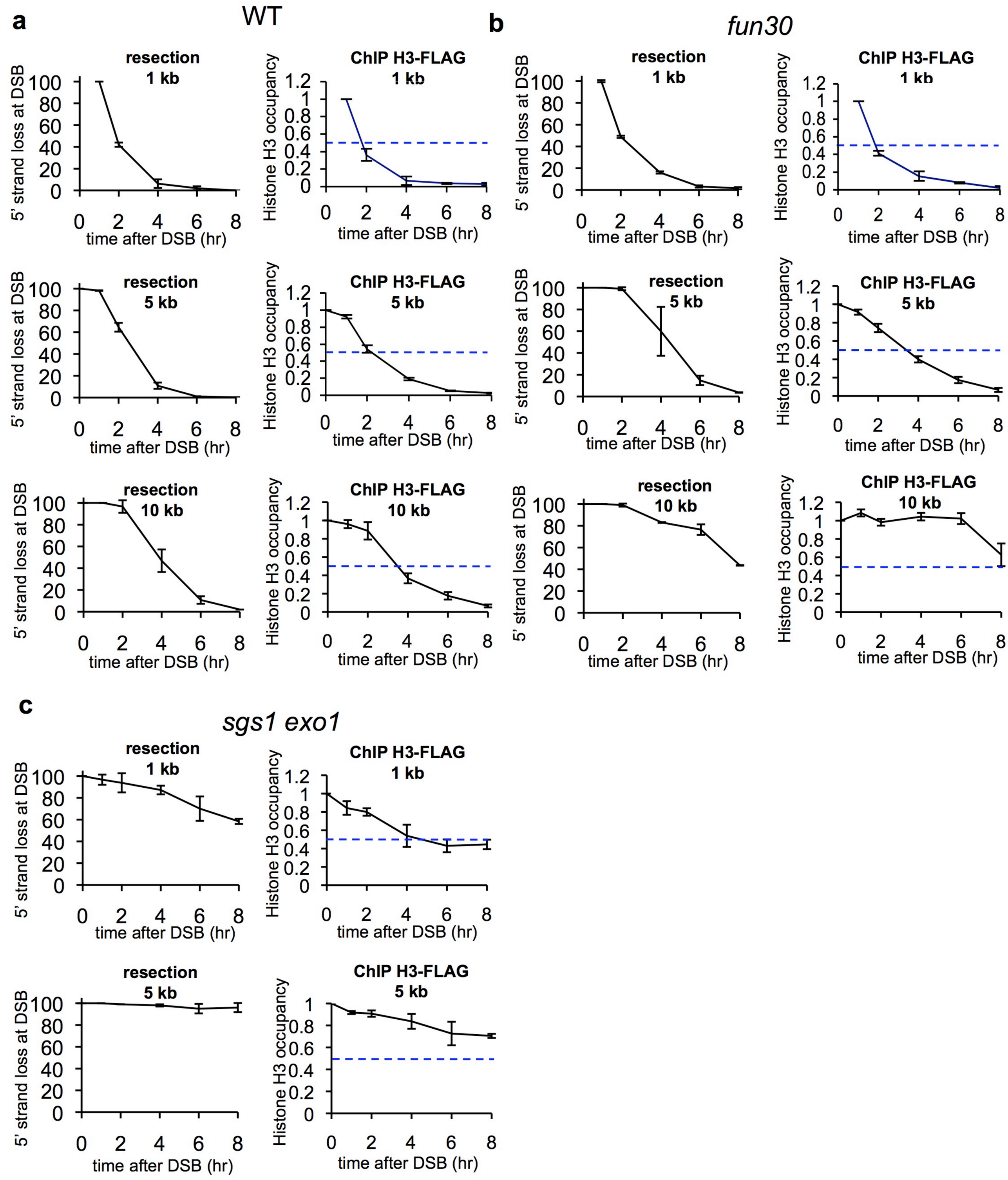

Figure S7, Chen et al.

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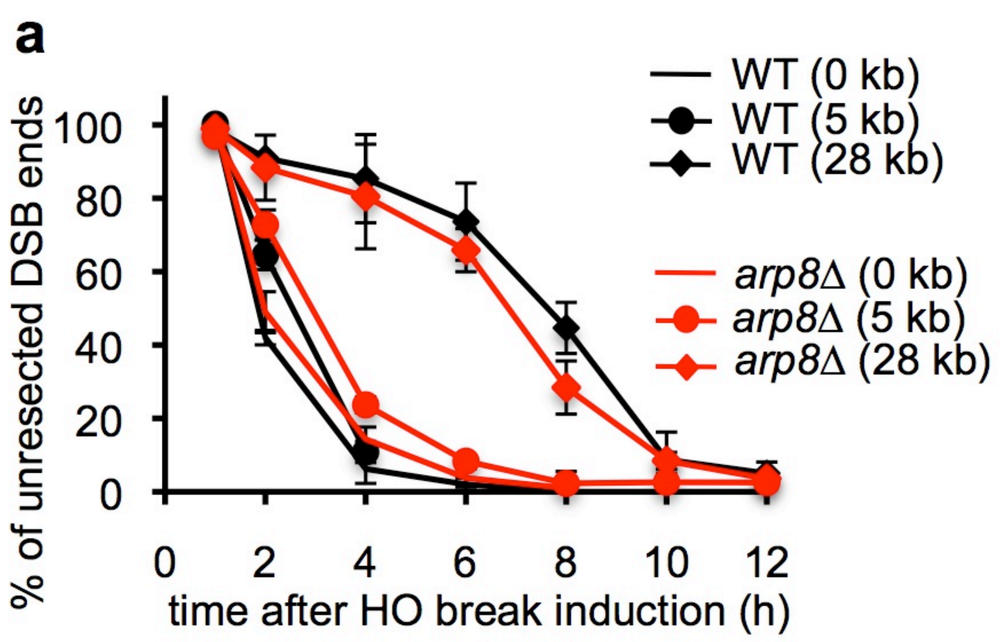

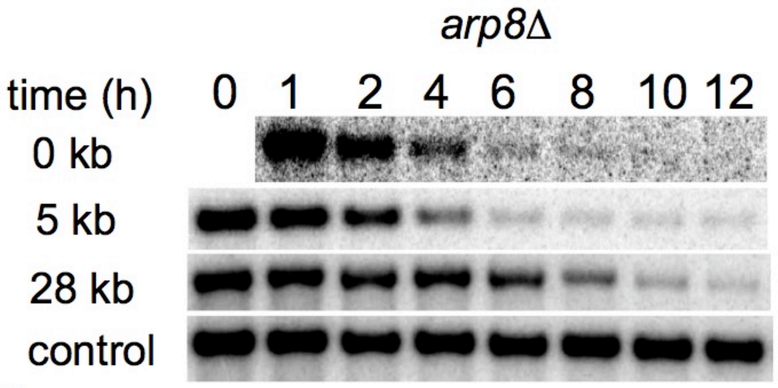

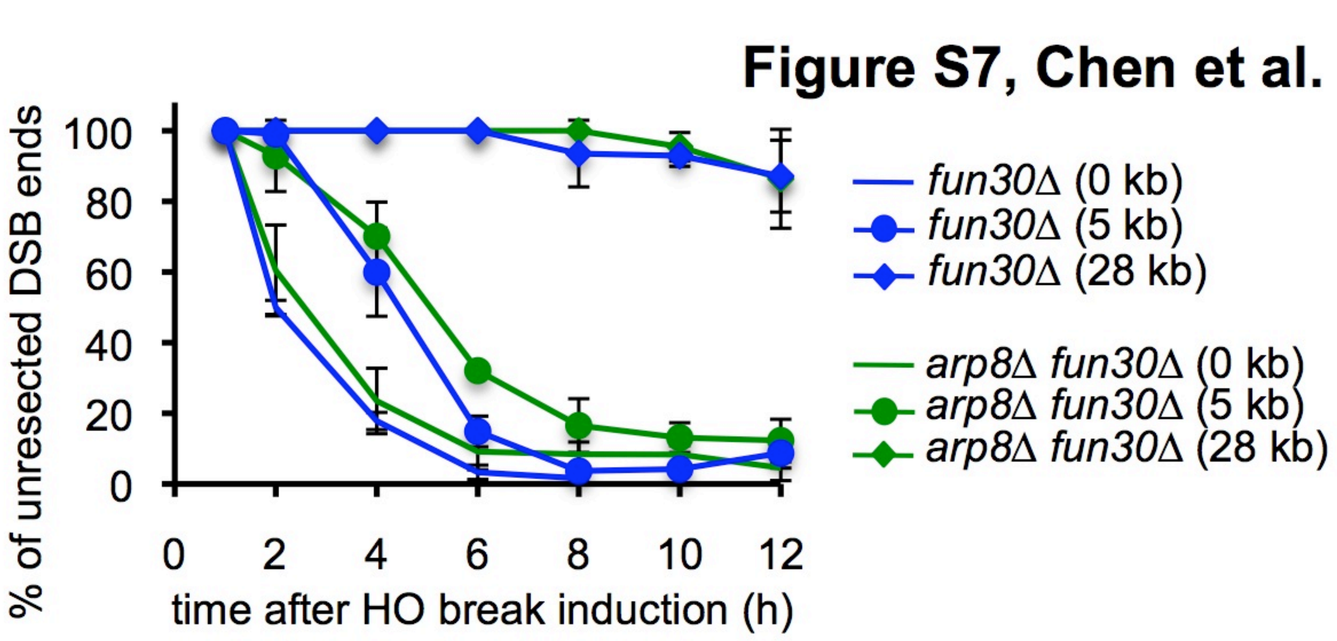

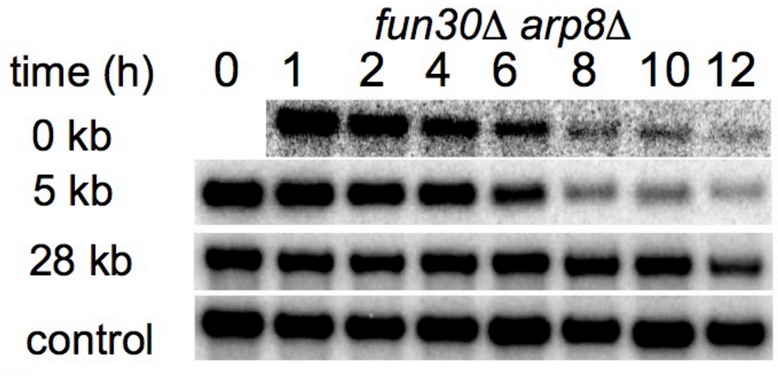

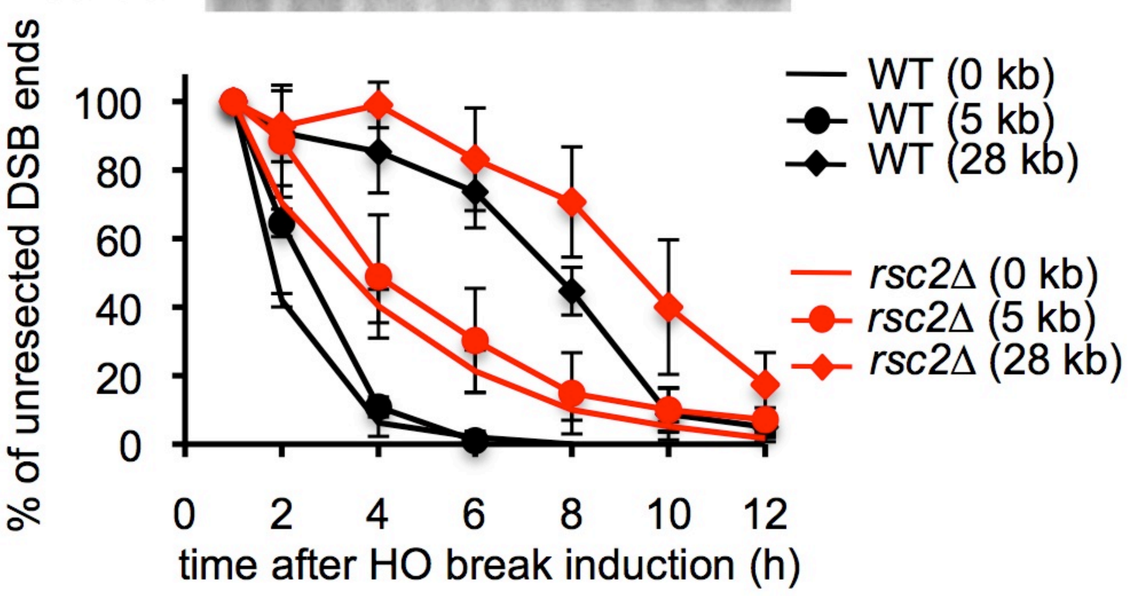

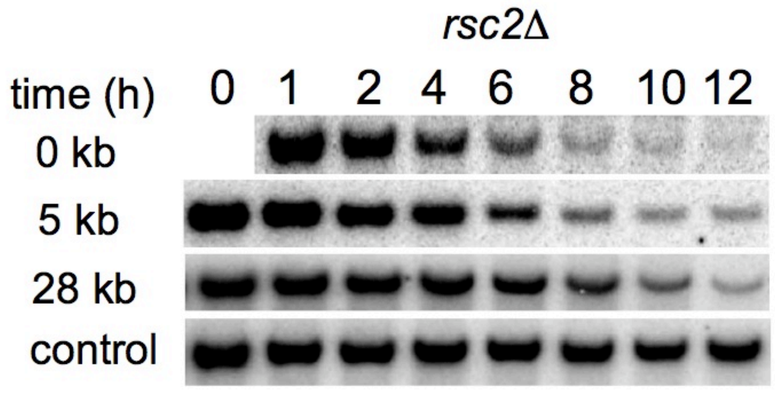

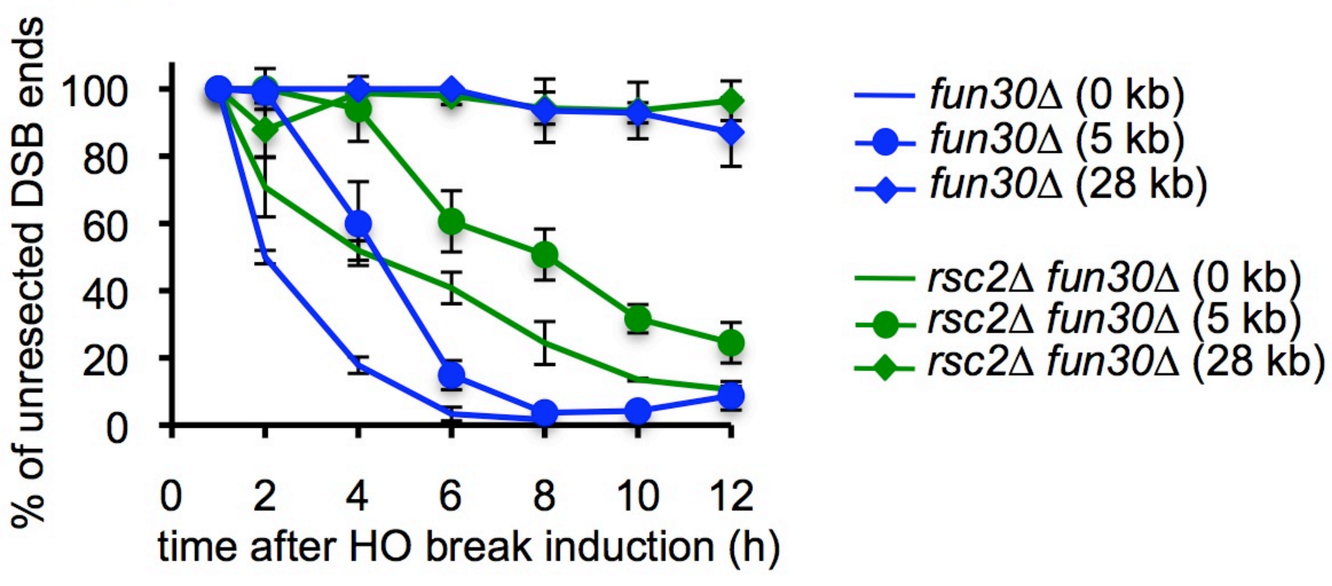

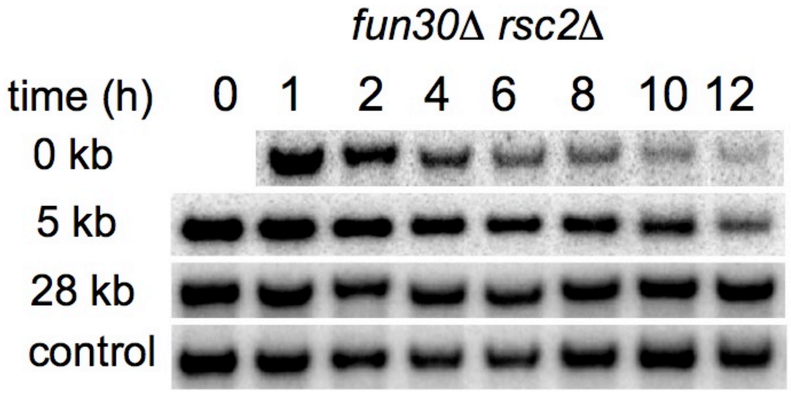

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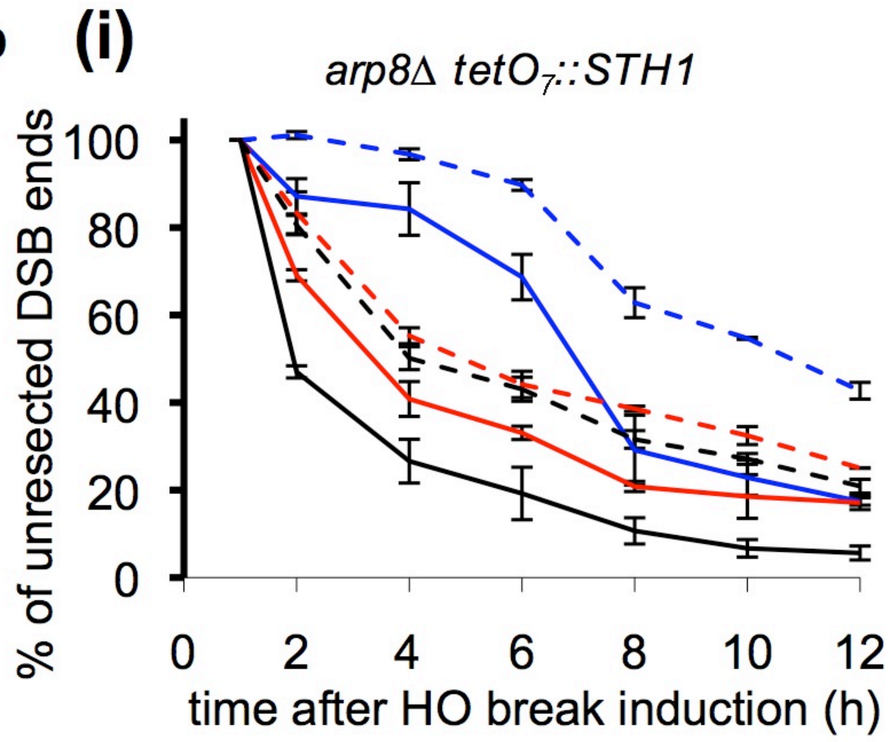

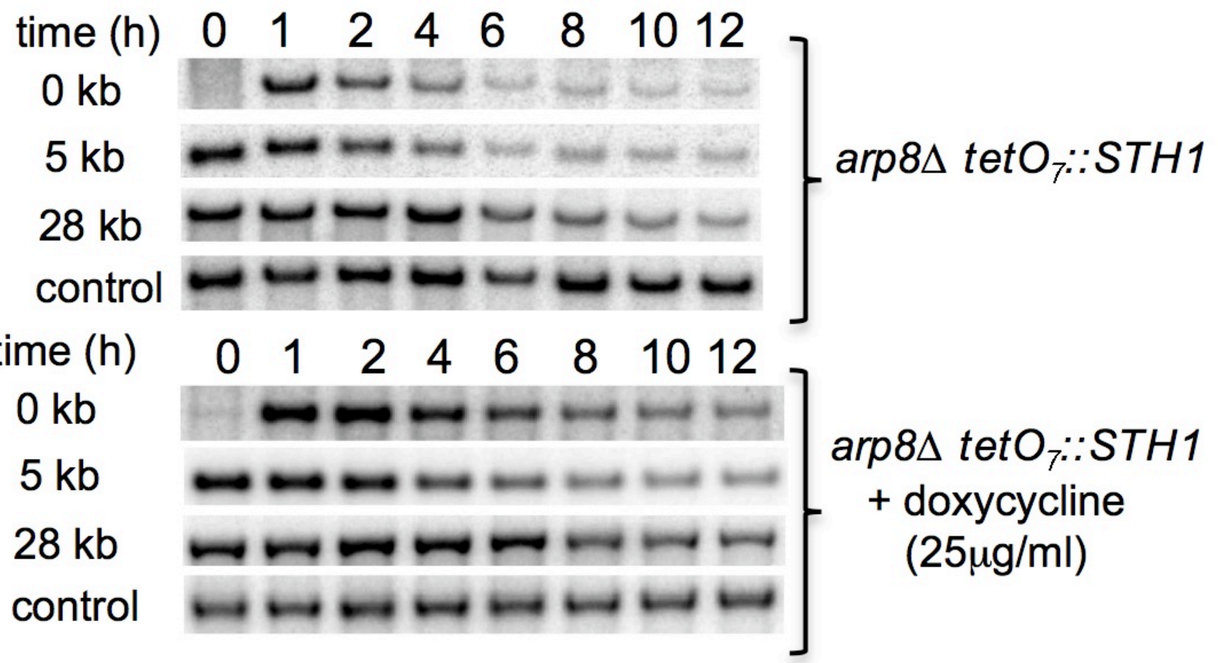

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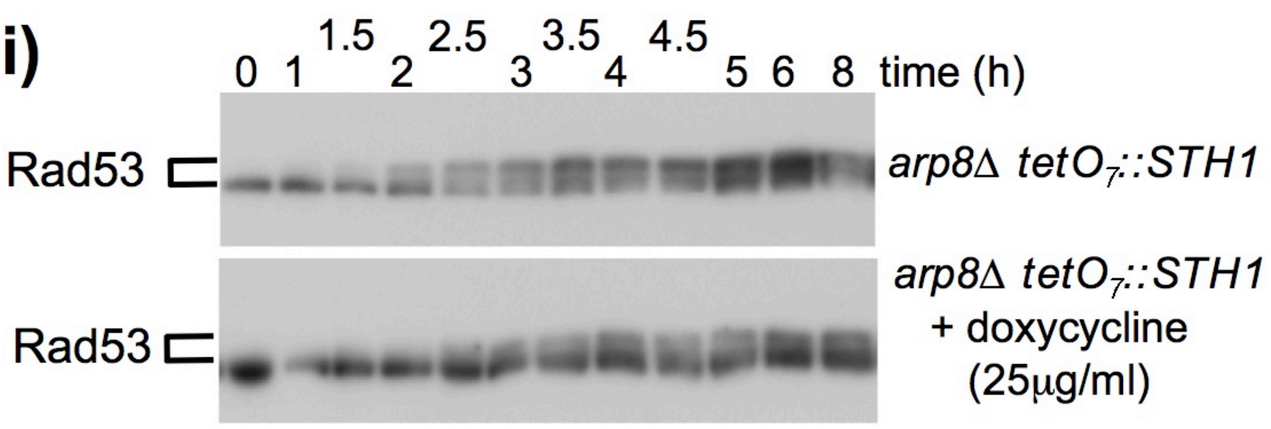

Figure S8, Chen et al.

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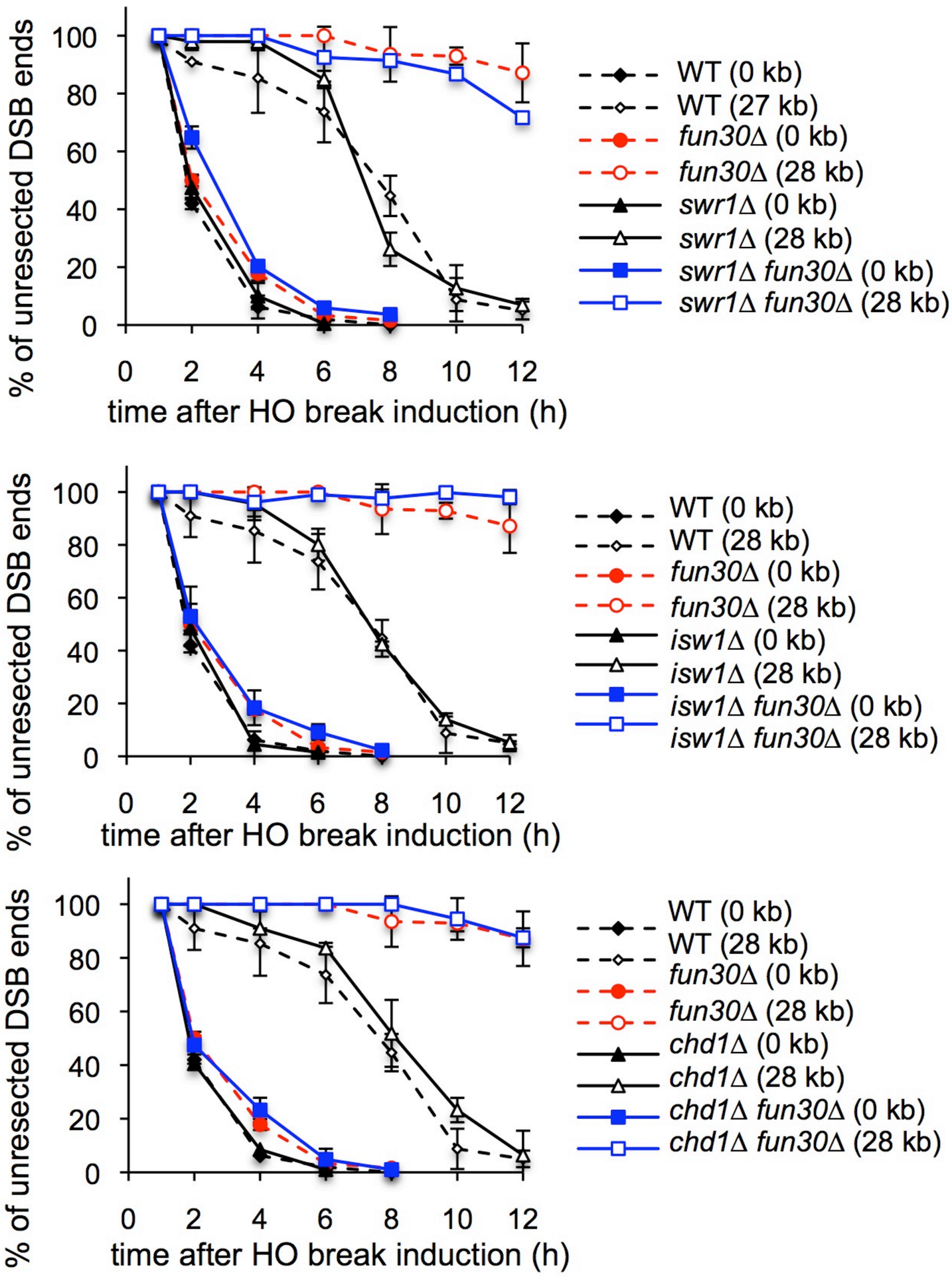
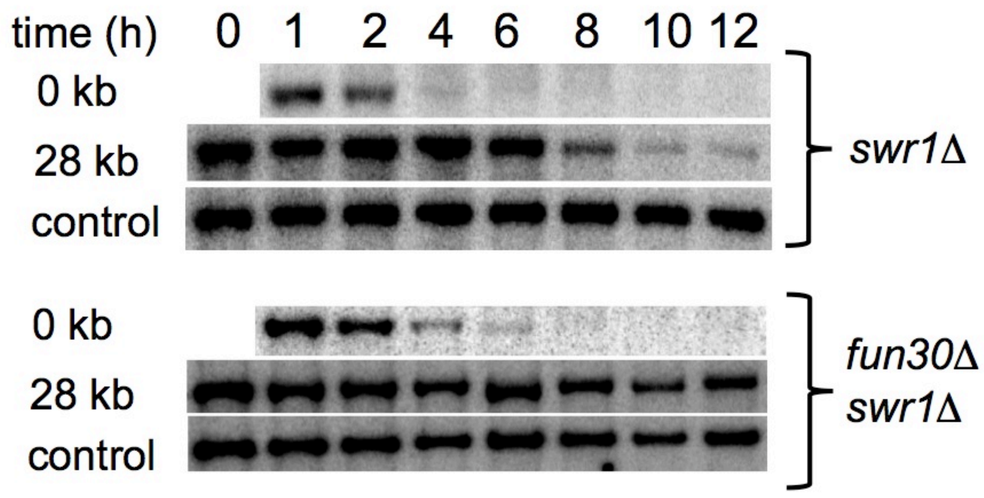
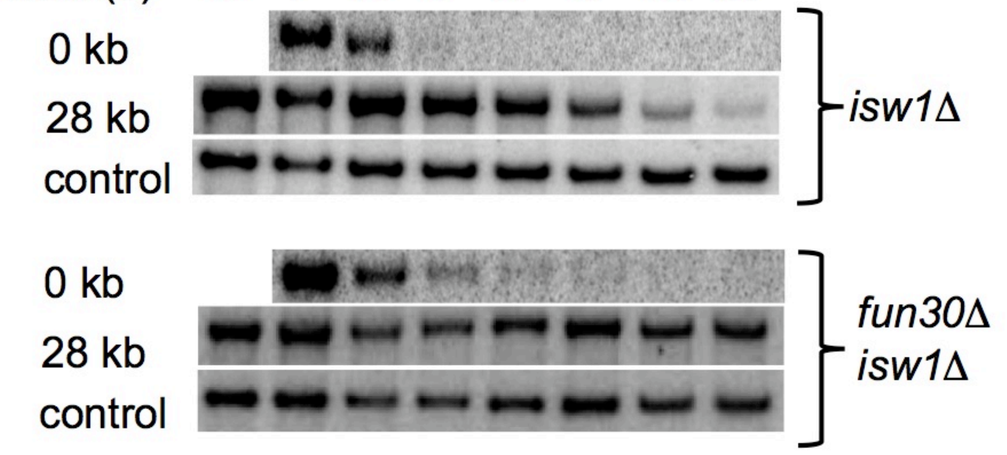
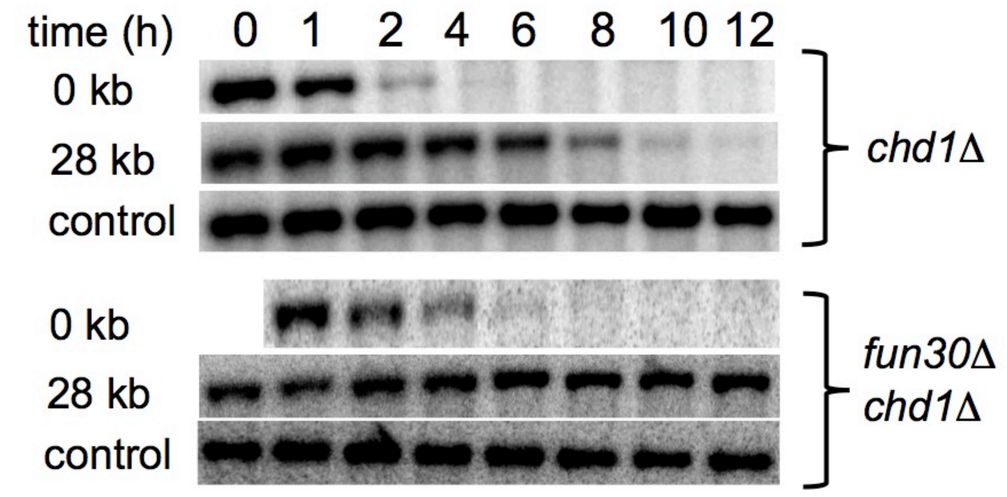
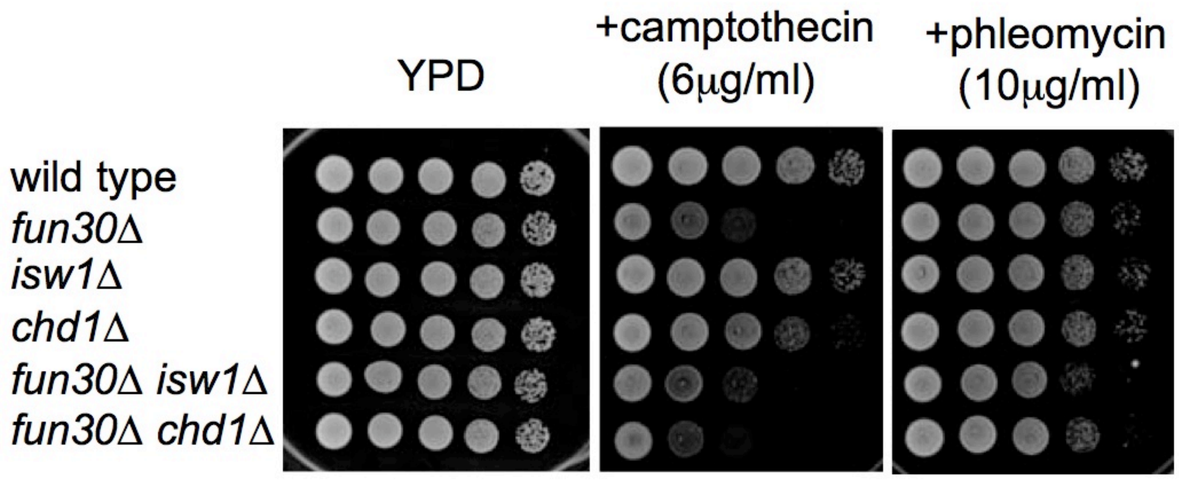








b



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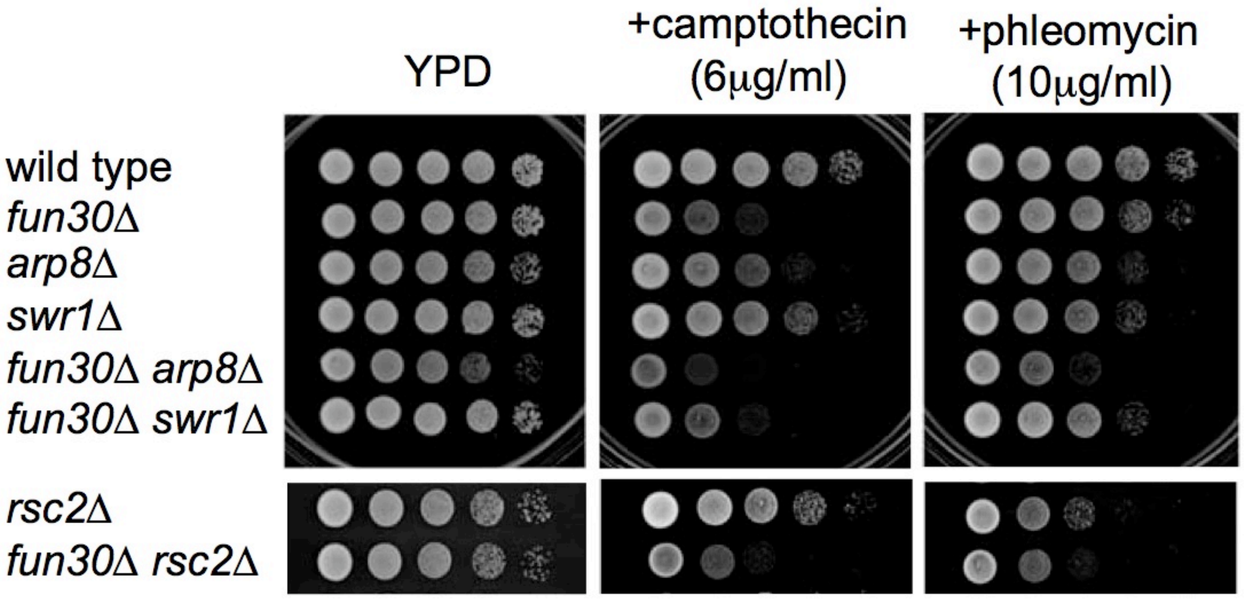

Figure S9, Chen et al.

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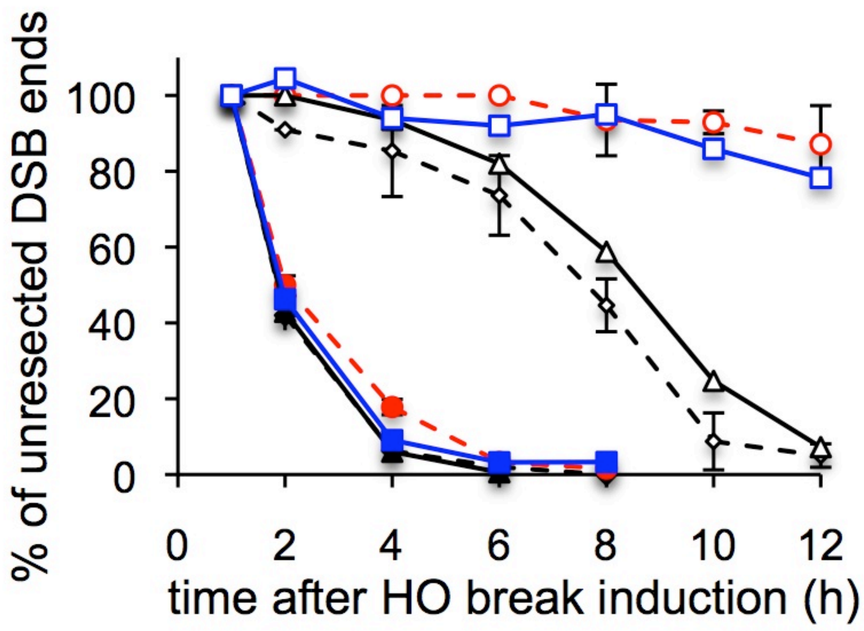

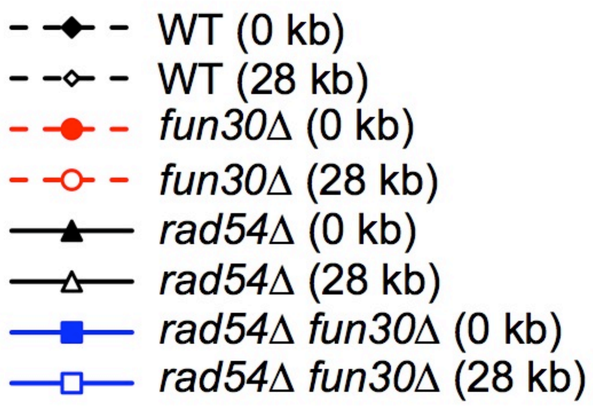

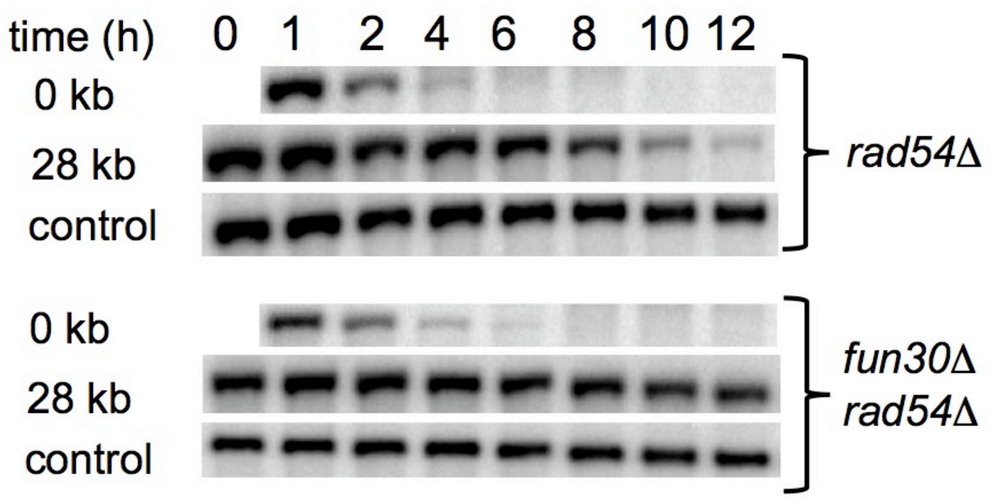

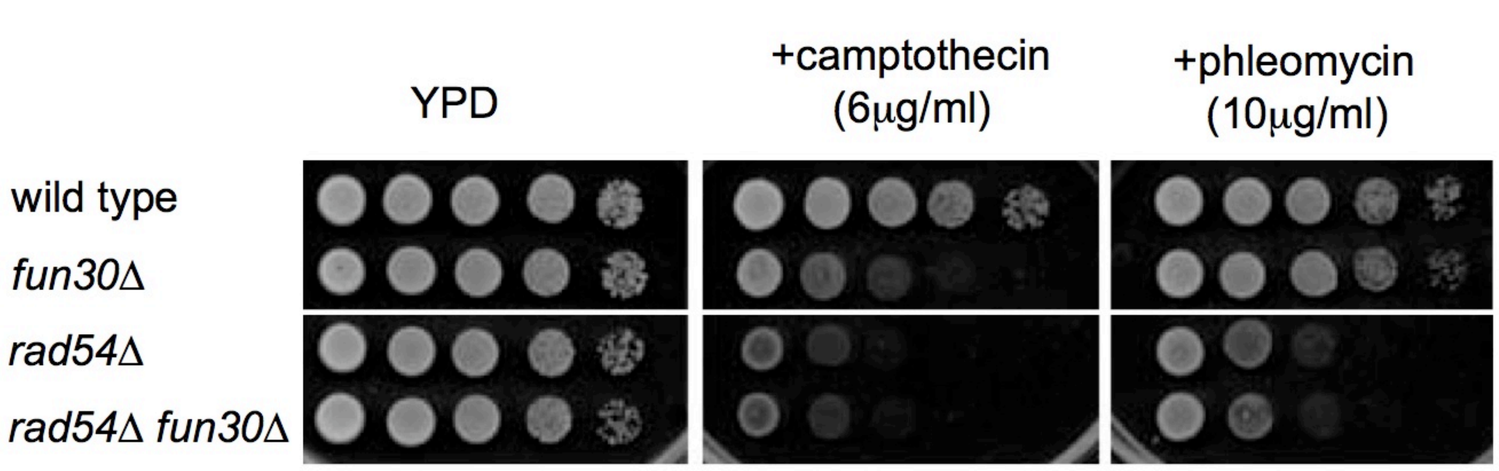

b

ATP-dependent nucleosome remodeling factor	mutant tested	resection phenotype
INO80	*arp8, nhp10**	very minor defect
SWR1	*swr1, htz1***	comparable to wild type
RSC	*rsc2, tetO7::STH1****	defective initial resection
ISW1	*isw1*	comparable to wild type
CHD1	*chd1*	comparable to wild type
Fun30	*fun30*	defective extensive resection
Rad54	*rad54*	comparable to wild type
	*arp8 fun30, nhp10 fun30**	extensive resection slightly more defective than in *fun30* cells
	swr1 fun30	as in *fun30* cells
	rsc2 fun30	defective initial and extensive resection
	isw1 fun30	as in *fun30* cells
	chd1 fun30	as in *fun30* cells
	rad54 fun30	as in *fun30* cells
	*arp8 fun30 tetO7::STH1****	very severe defect in resection

* data not shown
** Htz1 is an H2AZ histone variant, exchanged for histone H2A by the SWR1, data not shown
*** resection tested in the presence of doxycycline that shuts down *STH1* expression

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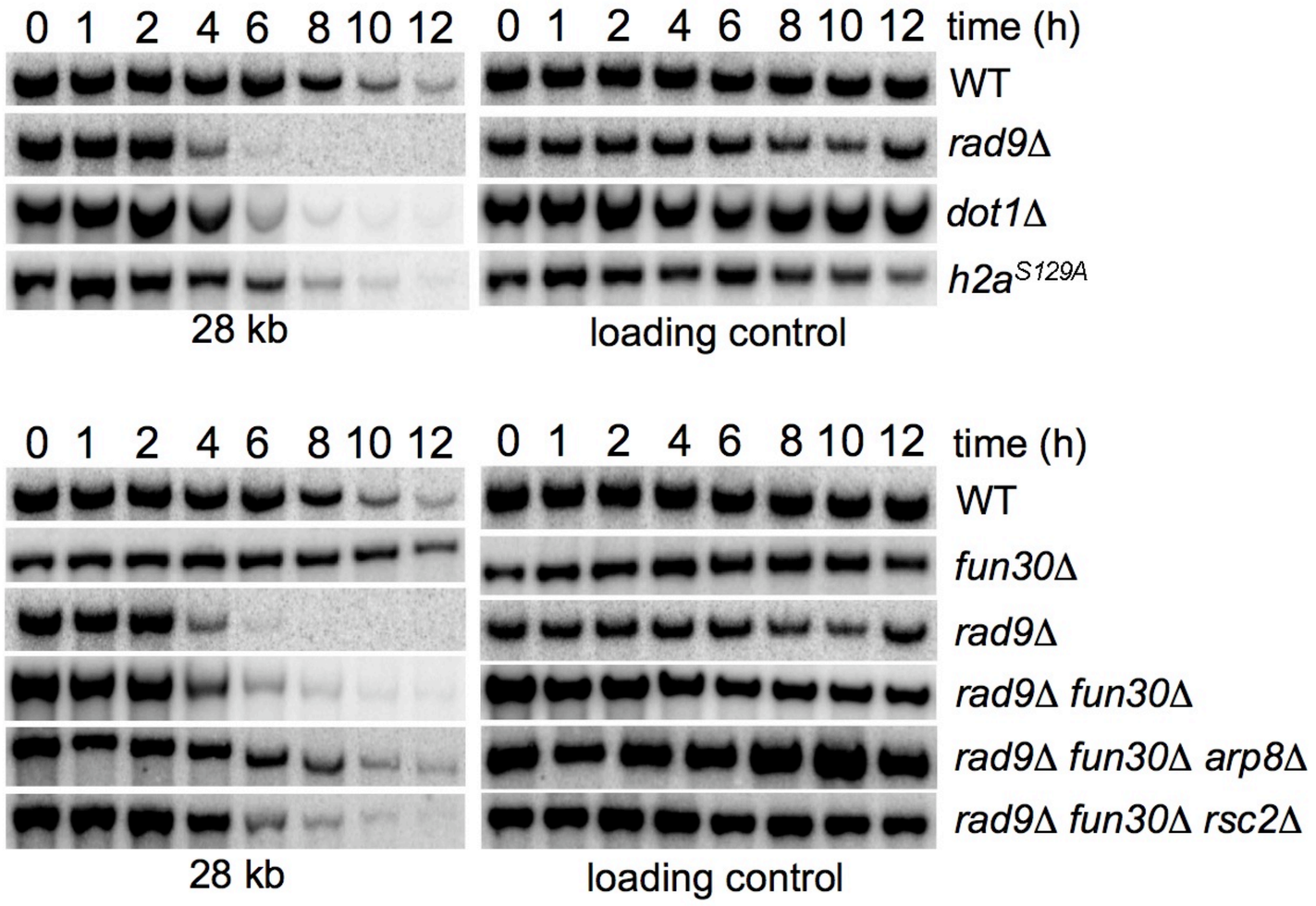
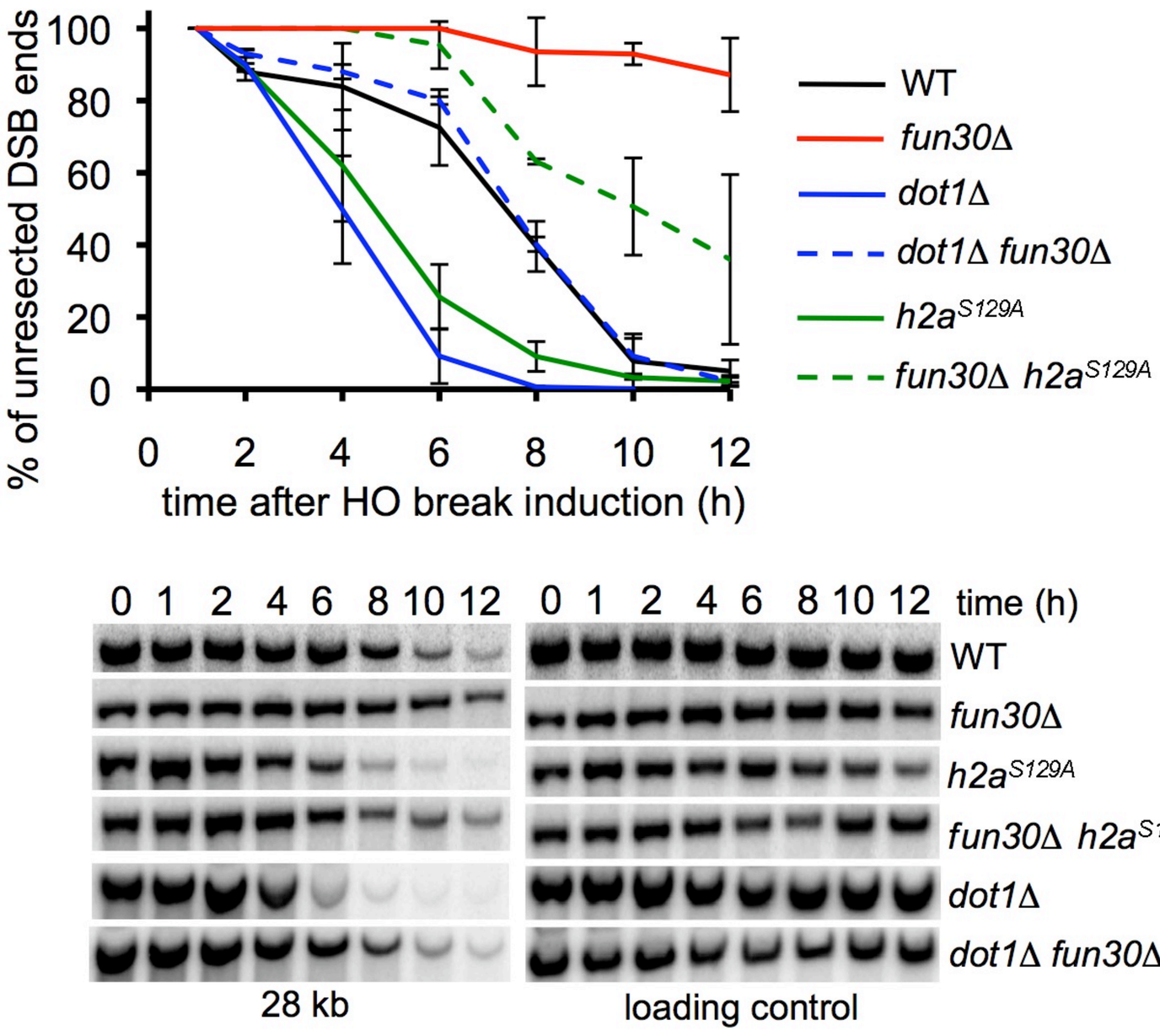
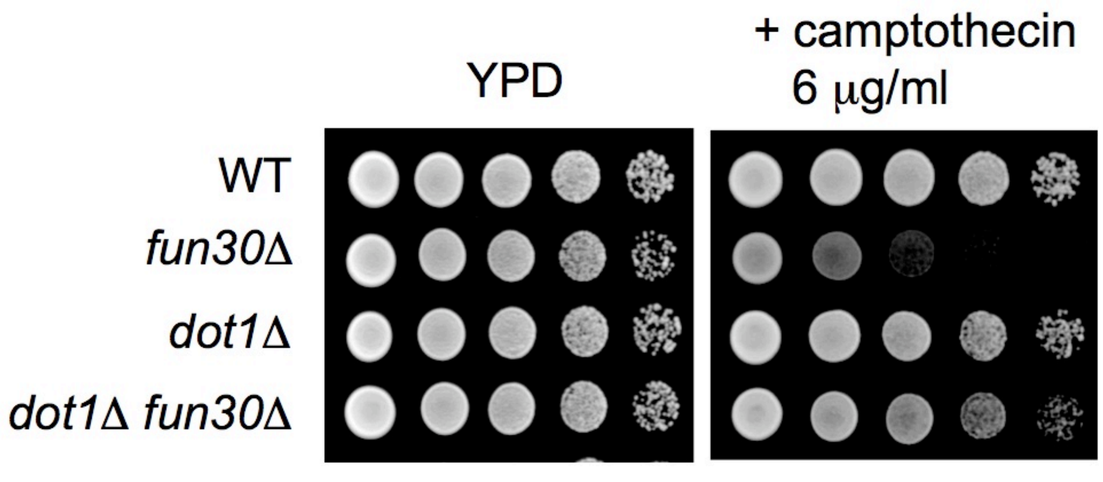

0 1 2 4 6 8 10 12
0 1 2 4 6 8 10 12
time (h)
WT
rad9Δ
dot1Δ
h2a^S129A
28 kb
loading control
0 1 2 4 6 8 10 12
0 1 2 4 6 8 10 12
time (h)
WT
fun30Δ
rad9Δ
rad9Δ fun30Δ
rad9Δ fun30Δ arp8Δ
rad9Δ fun30Δ rsc2Δ
28 kb
loading control

b


% of unresected DSB ends
100
80
60
40
20
0
0 2 4 6 8 10 12
time after HO break induction (h)
WT
fun30Δ
dot1Δ
dot1Δ fun30Δ
h2a^S129A
fun30Δ h2a^S129A
0 1 2 4 6 8 10 12
0 1 2 4 6 8 10 12
time (h)
WT
fun30Δ
h2a^S129A
fun30Δ h2a^S129A
dot1Δ
dot1Δ fun30Δ
28 kb
loading control

c


YPD
+ camptothecin
6 μg/ml
WT
fun30Δ
dot1Δ
dot1Δ fun30Δ