SUPPLEMENTARY INFORMATION

Table S1. Yeast strain genotypes, plasmids and qPCR primers.

In addition to the indicated genotype all strains are homozygous for *ura3 trp1 lys2 and ho::LYS2 mutations.* All strains are from the SK1 genetic background (35). All yeast strains and plasmids were constructed for this work, except ORD7304 (gift from V. Borde), ORD8175, pAP1, pAP11 and p_gRNA_handle plasmids (21,25,34).

Table S2. Genomic sites targeted by CRISPR-dCas9-Spo11.

To clone the guide sequence into the p_gRNA_handle vector, the following pair of oligonucleotides were PCR amplified:

5'-gactagccttattttaacttgctatttctagctctaaaac$\underline{(N)_{20}}$-3' and

5'-ctgggaacgaaactctgggagctgcgattggcagaagctt$(N)_{20}$-3', where $\underline{N_{20}}$ is the target site sequence indicated in the table. $\underline{N_{20}}$ and N_{20} are complementary. Lowercase texts correspond to sequences identical to the regions flanking the *Hind*III site of the p_gRNA_handle vector.

Table S3. Features of the DNA binding modules used in this study to build the Spo11 fusion constructs.

[a] corresponds to the number of binding sites identified in the yeast genome for each DNA binding module.

[b] Y represent any pyrimidine.

Table S4. Genetic assays of recombination at TSF-targeted *GAL2, PUT4, MSG5, HHF2* and *MSC1* regions. After sporulation, four spore tetrads were dissected and genotyped for the segregation of the antibiotic resistance cassettes (see details in Methods). The number of parental ditype (PD) tetrads was compared to that of tetratypes (T) and non-parental ditypes (NPD). The genetic distance was determined according to the formula cM = 100(T+6NPD)/2(PD+T+NPD). Recombination rates were compared using a two-tailed Fischer's exact test.

[a] Data from (25).

Table S5. Targeting of DSB formation by the various TSFs.

Figure S1. Nucleotide sequence of the TSF gene constructs used in this study.

Sequence of the *GAL4-SPO11* construct *(*pRSM046 plasmid).

ATGGGAAAACCTATTCCTAATCCTCTGCTGGGCCTGGATTCTACCGGAGGCATGGCCCCTAAGAAAAAGCGGAAG
GTGGACGGCGGAATGAAGCTACTGTCTTCTATCGAACAAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGC
TCCAAAGAAAAACCGAAGTGCGCCAAGTGTCTGAAGAACAACTGGGAGTGTCGCTACTCTCCCAAAACCAAAAGG
TCTCCGCTGACTAGGGCACATCTGACAGAAGTGGAATCAAGGCTAGAAAGACTGGAACAGCTATTTCTACTGATT
TTTCCTCGAGAAGACCTTGACATGATTTTGAAAATGGATTCTTTACAGGATATAAAAGCATTGTTAACAGGATTA
TTTGTACAAGATAATGTGAATAAAGATGCCGTCACAGATAGATTGGCTTCAGTGGAGACTGATATGCCTCTAACA
TTGAGACAGCATAGAATAAGTGCGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTA
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TTTGATTGGTCTGAAGAGGATGACATGTCGGATGGCTTGCCCTTCCTGAAAACGGACCCCAACAATAATGGGTTC
TTTGGCGACGGTTCTCTCTTATGTATTCTTCGATCTATTGGCTTTAAACCGGAAAATTACACGAACTCTAACGTT
AACAGGCTCCCGACCATGATTACGGATAGATACACGTTGGCTTCTAGATCCACAACATCCCGTTTACTTCAAAGT
TATCTCAATAATTTTCACCCCTACTGCCCTATCGTGCACTCACCGACGCTAATGATGTTGTATAATAACCAGATT
GAAATCGCGTCGAAGGATCAATGGCAAATCCTTTTTAACTGCATATTAGCCATTGGAGCCTGGTGTATAGAGGGG
GAATCTACTGATATAGATGTTTTTTACTATCAAAATGCTAAATCTCATTTGACGAGCAAGGTCTTCGAGTCAGGT
TCCATAATTTTGGTGACAGCCCTACATCTTCTGTCGCGATATACACAGTGGAGGCAGAAAACAAATACTAGCTAT
AATTTTCACAGCTTTTCCATAAGAATGGCCATATCATTGGGCTTGAATAGGGACCTCCCCTCGTCCTTCAGTGAT
AGCAGCATTCTGGAACAAAGACGCCGAATTTGGTGGTCTGTCTACTCTTGGGAGATCCAATTGTCCCTGCTTTAT
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GGTCCCACCATATATCATGGCATCATTGAAACAGCAAGGCTCTTACAAGTTTTCACAAAAATCTATGAACTAGAC
AAAACAGTAACTGCAGAAAAAAGTCCTATATGTGCAAAAAAATGCTTGATGATTTGTAATGAGATTGAGGAGGTT
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TCCATCATGTTAAGCGATGCAGCACAAAGAACTGTTATGTCTGTAAGTAGCTATATGGACAATCATAATGTCACC
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AAACTGGCCACTTTTAAAATCCAGACTTGTGAAAAATACATTCAAGTACTGGAAGAGGTATGTGCGCCGTTTCTG
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CTCATTACAGGTAAGGGATTTCCAGATTTCTTGACAAGGTTATTCCTAAAAAAACTAGAACAATATTGCTCCAAA
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AATGAACGCAACGCTTATATTTGCACGATGGCAAACTATAAAGGAATTCGTATTACGCAAGTTTTGGCACAAAAT
AATGAAGTGCATAACAAATCCATTCAATTATTGAGTTTGAATCAGCGCGACTACTCCTTAGCCAAGAATTTGATA
GCATCTCTGACTGCCAACAGCTGGGATATTGCAACTTCACCATTAAAGAACGTCATCATAGAATGTCAGCGGGAA
ATTTTTTTCCAAAAGAAAGCTGAAATGAACGAGATTGATGCCAGAATTTTTGAATACAAATGA

Sequence of the *TEC1-SPO11* construct *(*pRSM026 plasmid).

ATGAGTCTTAAAGAAGACGACTTTGGCAAGGATAATTCTAGAAATATAGAATCATATACTGGTAGAATTTTTGAC
GTATATATACAAAAAGATTCGTATTCACAGTCGGCCTTGGATGATATGTTTCCAGAAGCCGTAGTTTCAACCGCC
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GGACTGGGTGCTAAATCGGACGATTTGAAATCTCCATCAGCAAAGGCTACGTTCACTGACAAGCAGAGGAAGAAT
GAAGTACCAAATATATCTGTGAGCAACTACTTTCCCGGACAAAGTAGCGAAACGTCGTCAACAACGGAATCTTGG
ACTATCGGTTGTGATAAGTGGTCAGAAAAGGTAGAAGAGGCATTCCTTGAGGCACTTAGACTGATAATGAAAAAT
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TCGCCTTTCCCCATTGATATTTATGACAATATTCTGACATGTGAAAATGAACCAAAGATGCAAAAGCAAACAATT
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GAAATTTTTTTCCAAAAGAAAGCTGAAATGAACGAGATTGATGCCAGAATTTTTGAATACAAATGA

Sequence of the *RSC3-SPO11* construct *(*pRSM070 plasmid).

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CAAATATTAAAGTTTCCTTCACAATCGATAACACAGACCCTTATCAACAAATACTTATCCACGATAACGGAAACG
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CTCATTACAGGTAAGGGATTTCCAGATTTCTTGACAAGGTTATTCCTAAAAAAACTAGAACAATATTGCTCCAAA
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AATGAAGTGCATAACAAATCCATTCAATTATTGAGTTTGAATCAGCGCGACTACTCCTTAGCCAAGAATTTGATA
GCATCTCTGACTGCCAACAGCTGGGATATTGCAACTTCACCATTAAAGAACGTCATCATAGAATGTCAGCGGGAA
ATTTTTTTCCAAAAGAAAGCTGAAATGAACGAGATTGATGCCAGAATTTTTGAATACAAATGA

Sequence of the *MTW1-SPO11* construct *(*pMLM023 plasmid).

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CGTGTTAAGCCGATTTGTGGGAACTGTATGAAGCATAATAAAATGGACTGCTTTTATCCGGACGTTCCCGGACAA
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CACGAGTTGAGTAAAATTTCAGAATCTCTGATAAAAACGGATTTTTATGAACAGAGGAAAAATAGCACAGTTTCT
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GCATCTCTGACTGCCAACAGCTGGGATATTGCAACTTCACCATTAAAGAACGTCATCATAGAATGTCAGCGGGAA
ATTTTTTTCCAAAAGAAAGCTGAAATGAACGAGATTGATGCCAGAATTTTTGAATACAAATGA

Sequence of the *NLS-TALE-SPO11* construct *(*pML060 plasmid).

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CACCCTGCCGCCCTGGGAACAGTGGCCGTGAAATATCAGGATATGATTGCCGCCCTGCCTGAGGCCACACACGAA
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GGCCTGACACCTGAACAGGTGGTGGCTATCGCCTCTAACAACGGAGGAAAACAGGCTCTGGAAACAGTGCAGCGG
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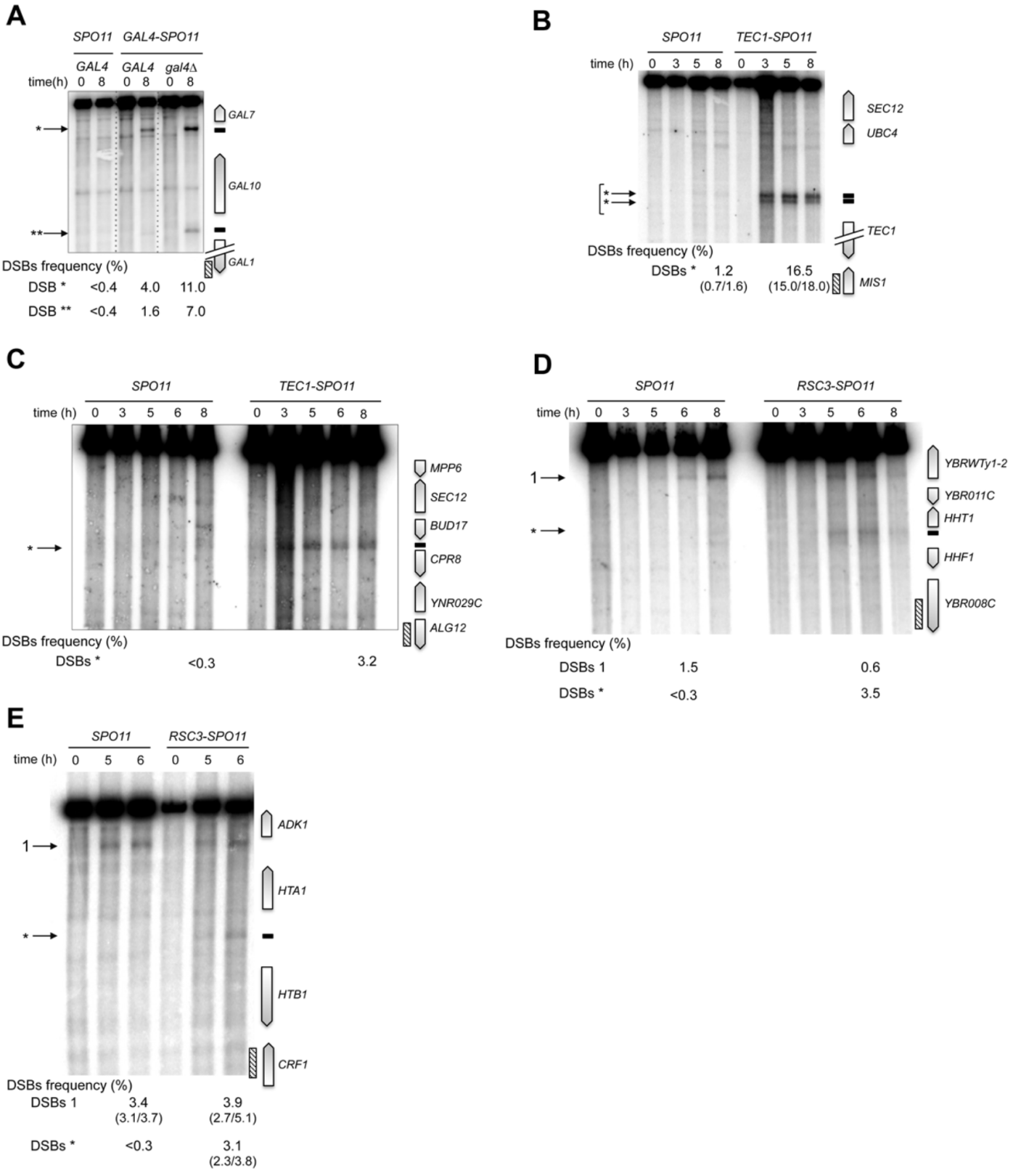

Figure S2. Gal4-, Tec1- and Rsc3-Spo11 fusions promote DSB formation in targeted DSB-cold promoter-containing regions.

(**A-E**) DSB formation was analyzed by Southern Blot at the *GAL7* and *GAL1/GAL10*

promoters targeted by Gal4-Spo11 (**A**), at the *TEC1* (**B**) and *CPR8* (**C**) promoters targeted by Tec1-Spo11, and at the *HHF1/HHT1* (**D**) and *HTA1/HTB1* (**E**) promoters targeted by Rsc3-Spo11. Cells were collected at the indicated time after transfer into sporulation medium (t=0h). TSF target sites and probes are shown as black bars in gene promoter maps and as hatched rectangle in ORF maps, respectively. At the left of the blot, arrowheads indicate wild-type (numbered) and targeted (asterisks) DSBs. DSB values are from single experiments or the mean of two independent experiments with minimum and maximum values indicated into parentheses.

(**A**) genomic DNA was prepared from *SPO11/SPO11 GAL4*/*GAL4* (ORD7304), *GAL4-SPO11/GAL4-SPO11 spo11*Δ*/spo11*Δ *GAL4*/*GAL4* (AND2096) and *GAL4-SPO11/GAL4-SPO11 spo11*Δ*/spo11*Δ *gal4*Δ*/gal4*Δ (AND1969) diploids, digested with *Cla*I and *Aat*II and probed with an internal *GAL1* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304) and *TEC1-SPO11/TEC1-SPO11 spo11*Δ*/spo11*Δ (AND1926) diploids, digested with *EcoR*V and probed with an internal *MIS1* fragment.

(**C**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304) and *TEC1-SPO11/TEC1-SPO11 spo11*Δ*/spo11*Δ (AND1926) diploids, digested with *Hpa*I and *Xba*I and probed with an internal *ALG12* fragment.

(**D**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304) and *RSC3-SPO11/RSC3-SPO11 spo11*Δ*/spo11*Δ (AND2006) diploids, digested with *EcoR*I and *Hpa*I and probed with an internal *YBR008C* fragment.

(**E**) genomic DNA was prepared *SPO11/SPO11* (ORD7304) and *RSC3-SPO11/RSC3-SPO11 spo11*Δ*/spo11*Δ (AND2006) diploids, digested with *Aat*II and probed with an internal *CRF1* fragment.

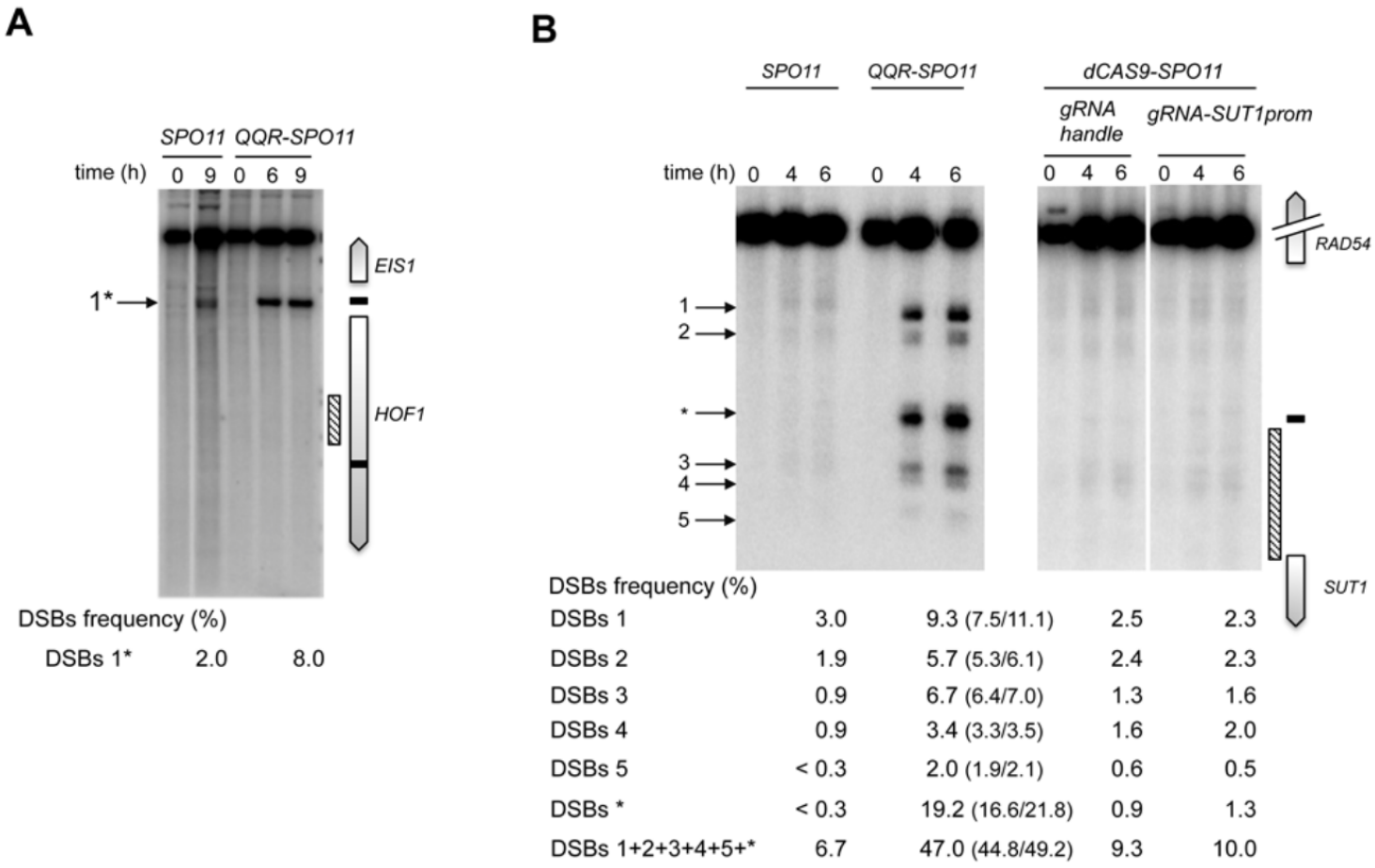

Figure S3. QQR-Spo11 triggers DSB formation in DSB-cold QQR recognition site-containing regions.

(**A-B**) Southern blot analysis of DSBs targeted by QQR-Spo11 at the *EIS1/HOF1* (**A**) and *RAD54/SUT1* (**B**) promoters. Cells were collected at the indicated time after transfer into sporulation medium (t=0h). QQR target sites and probes are shown as black bars in gene promoter maps and as hatched rectangle in ORF maps, respectively. At the left of the blot, arrowheads indicate wild-type (numbered) and targeted (asterisks) DSBs. DSB values are from single experiments or the mean of two independent experiments with minimum and maximum values indicated into parentheses.

(**A**) genomic DNA was prepared from *SPO11/SPO11* (ORD7239) and *QQR-SPO11/QQR-SPO11 spo11Δ/spo11Δ* (ORD8146) diploids, digested with *Xba*I and probed with an internal *HOF1* fragment.

(**B**) DNA was prepared from *SPO11/SPO11* (ORD7239), *QQR-SPO11/QQR-SPO11 spo11Δ/spo11Δ* (ORD8146), *dCAS9-SPO11/0* + gRNA handle *spo11Δ/spo11Δ*

(ANT2527) and *dCAS9-SPO11/0* + gRNA-*SUT1prom spo11Δ/spo11Δ* (ANT2709) diploids, digested with *Hinc*II and probed with an intergenic *RAD54-SUT1* fragment.

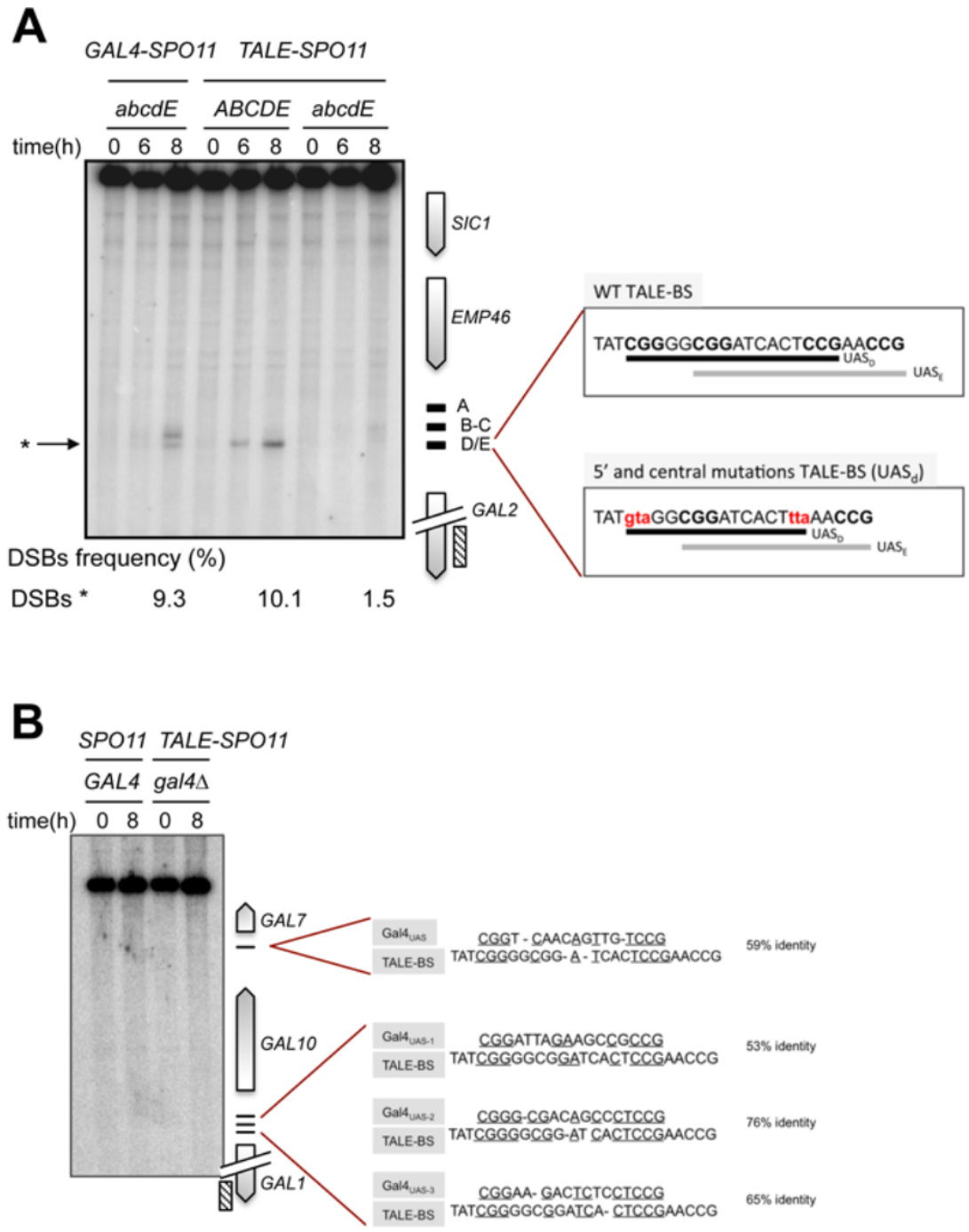

Figure S4. TALE-Spo11-mediated DSB formation is dependent on the presence of TALE binding sequence.

(**A-B**) TALE-Spo11-mediated DSB formation in *gal4*Δ cells was analyzed by Southern blot at the *GAL2* (**A**) and *GAL1-GAL10-GAL7* (**B**) regions. Mutations in the TALE binding site abolish TALE-Spo11 DSB formation at the targeted *GAL2* region (**A**). Mutations introduced in the TALE binding site are indicated in the right panel. From the top to the bottom, the wild-type sequence of TALE binding site (TALE-BS, 25 nt) overlapping Gal_{UAS-D} and Gal_{UAS-E} and the TALE binding site with 5' and

central mutations are shown. TALE-Spo11 fusion does not induce DSB formation in the untargeted $Gal4_{UAS}$-containing *GAL1-GAL10-GAL7* region (**B**). This region contains several $Gal4_{UAS}$ sites in gene promoters as indicated in the right panel. It is efficiently targeted by Gal4BD- and Gal4-Spo11 (Ref. (25) and **Supplementary Fig. 2A**). Cells were collected at the indicated time after transfer into sporulation medium (t=0h). $Gal4_{UAS}$ sites and probes are shown as black bars in gene promoter maps and as hatched rectangle in ORF maps, respectively. At the left of the blot, arrowheads indicate targeted DSBs (asterisks).

(**A**) genomic DNA was prepared from *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* UAS_{abcdE} (AND3019), *TALE-SPO11/TALE-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* (AND2540) and *TALE-SPO11/TALE-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* UAS_{abcdE} (AND3010) diploids, digested with *Xba*I and probed with an internal *GAL2* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11 GAL4*/*GAL4* (ORD7304) and *TALE-SPO11/TALE-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* (AND2540) diploids, digested with *Cla*I and *Aat*II and probed with an internal *GAL1* fragment.

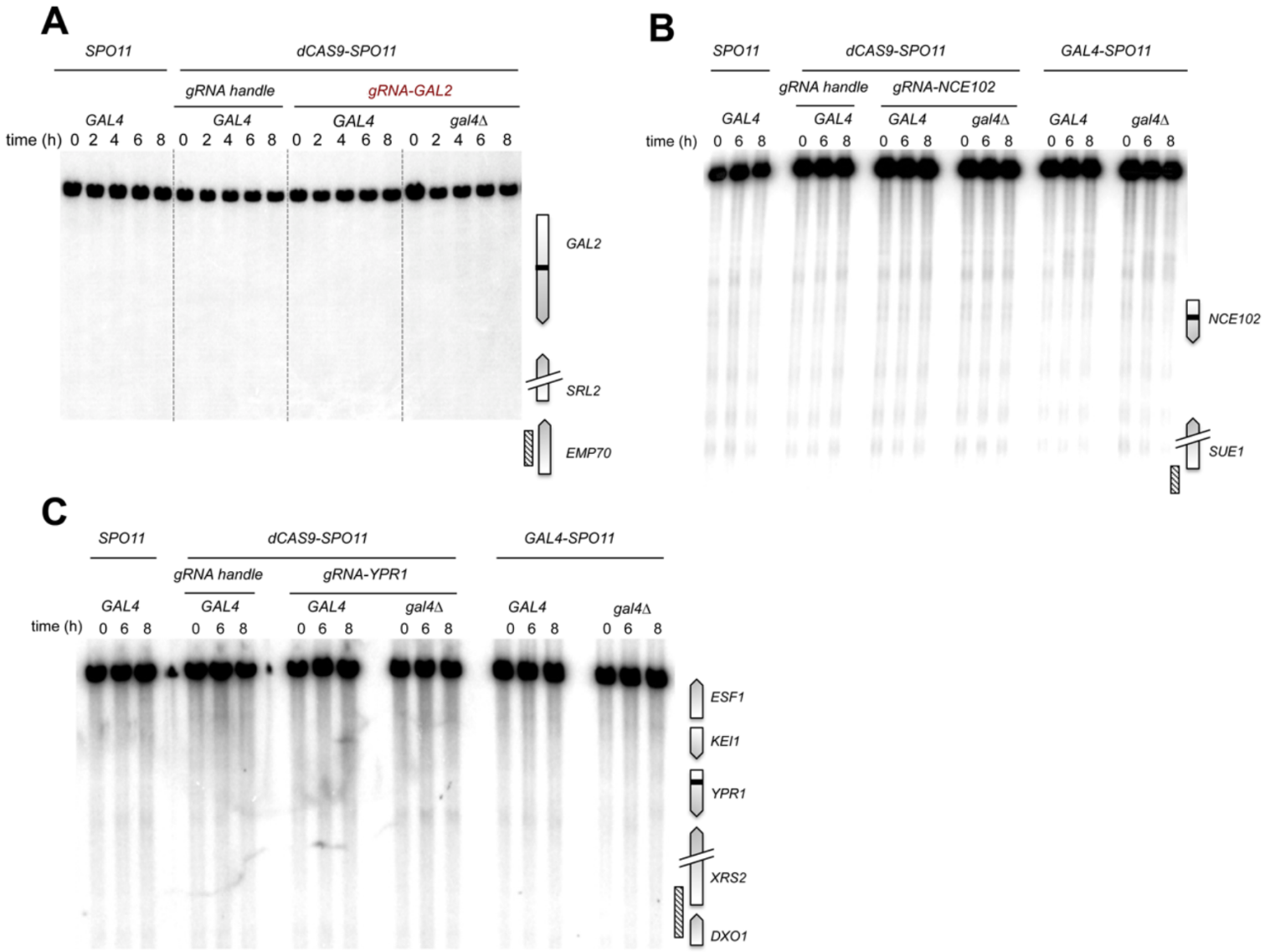

Figure S5. Targeting of DSB-cold gene coding sequences by Gal4-Spo11 and CRISPR-dCas9-Spo11.

(**A-C**) DSB formation was analyzed by Southern Blot at the targeted *GAL2* (**A**), *NCE102* (**B**) and *YPR1* (**C**) ORFs. Cells were collected at the indicated time after transfer into sporulation medium (t=0h). TSF target sites and probes are shown as black bars as hatched rectangles in gene ORF maps, respectively.

(**A**) genomic DNA was prepared from *SPO11/SPO11 GAL4*/*GAL4* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527), *dCAS9-SPO11/0* + gRNA-*GAL2 spo11Δ/spo11Δ GAL4*/*GAL4* (ANT2595) and *dCAS9-SPO11/0* + gRNA-*GAL2 spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2596) diploids, digested with *Afl*II and *Pvu*I and probed with an internal *EMP70* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11 GAL4*/*GAL4* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527), *dCAS9-SPO11/0* + gRNA-*NCE102 spo11Δ/spo11Δ GAL4*/*GAL4* (ANT2549), *dCAS9-SPO11/0* + gRNA-*NCE102 spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2555), *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ GAL4*/*GAL4* (AND2096) and *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* (AND1969) diploids, digested with *Sal*I and *Pst*I and probed with an internal *URN1* fragment.

(**C**) genomic DNA was prepared from *SPO11/SPO11 GAL4*/*GAL4* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527), *dCAS9-SPO11/0* + gRNA-*YPR1 spo11Δ/spo11Δ GAL4*/*GAL4* (ANT2548), *dCAS9-SPO11/0* + gRNA-*YPR1 spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2554), *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ GAL4/GAL4* (AND2096) and *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* (AND1969) diploids, digested with *Age*I and *Pac*I and probed with an internal *DXO1* fragment.

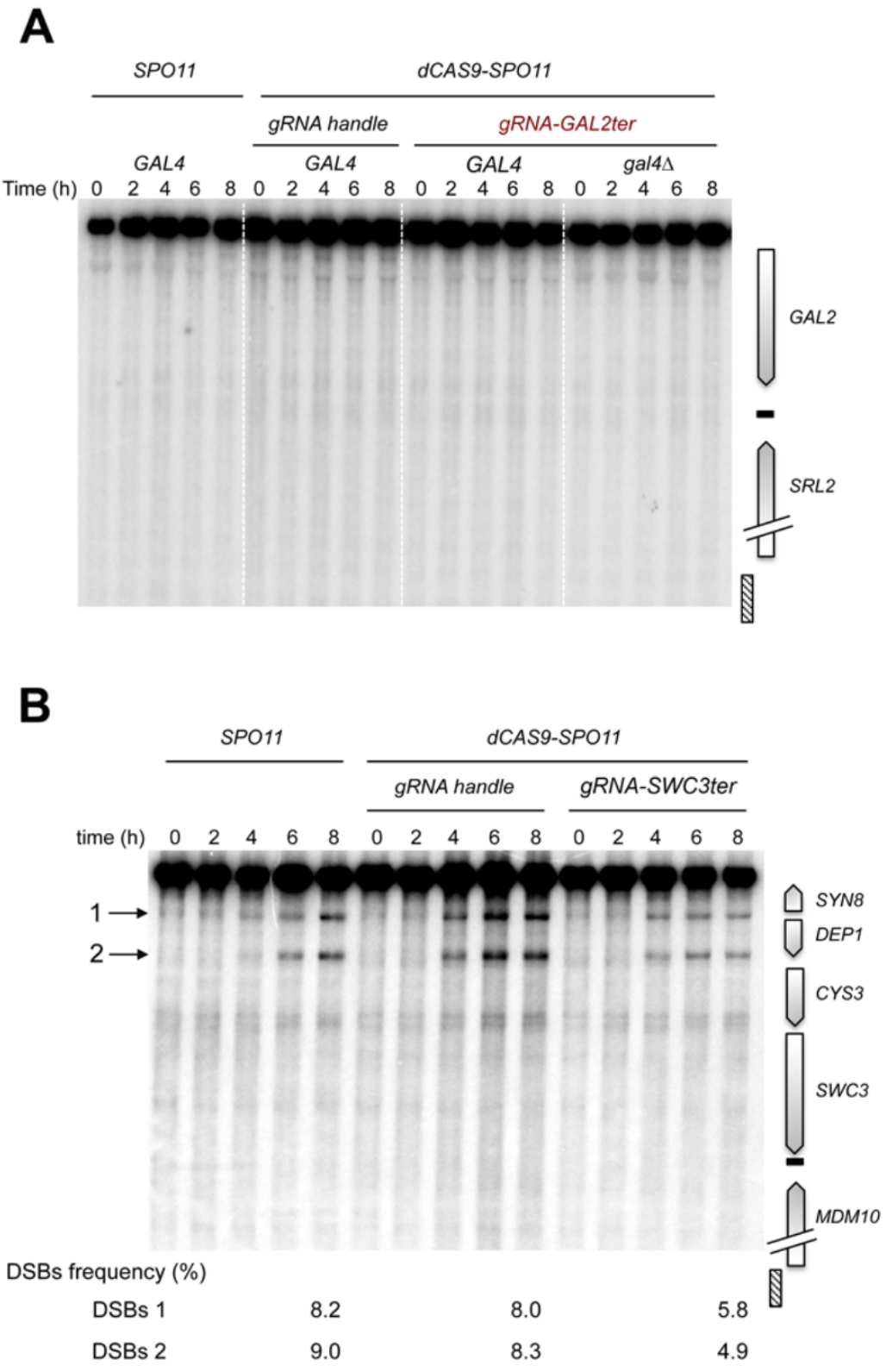

Figure S6. CRISPR-dCas9-Spo11 does not trigger DSBs in targeted gene terminator-containing regions.

Southern blot analysis of CRISPR-dCas9-Spo11 DSBs at the targeted *GAL2* (**A**) and *SWC3* terminator regions (**B**). Diploid cells were collected at the indicated time after transfer into sporulation medium (t=0h). CRISPR-dCas9-Spo11 target sites are shown as black bars. At the left of the gel, arrowheads indicate wild-type (numbered) and targeted (asterisks) DSBs.

(**A**) genomic DNA was prepared from *SPO11/SPO11 GAL4/GAL4* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527), *dCAS9-SPO11/0* + gRNA-*GAL2ter spo11Δ/spo11Δ* (ANT2567) and *dCAS9-SPO11/0* + gRNA-*GAL2ter spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2568) diploids, digested with *Afl*II and *Pvu*I and probed with an internal *EMP70* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-*SWC3ter spo11Δ/spo11Δ* (ANT2597) diploids, digested with *Pac*I and *Avr*II and probed with an internal *SPO7* fragment.

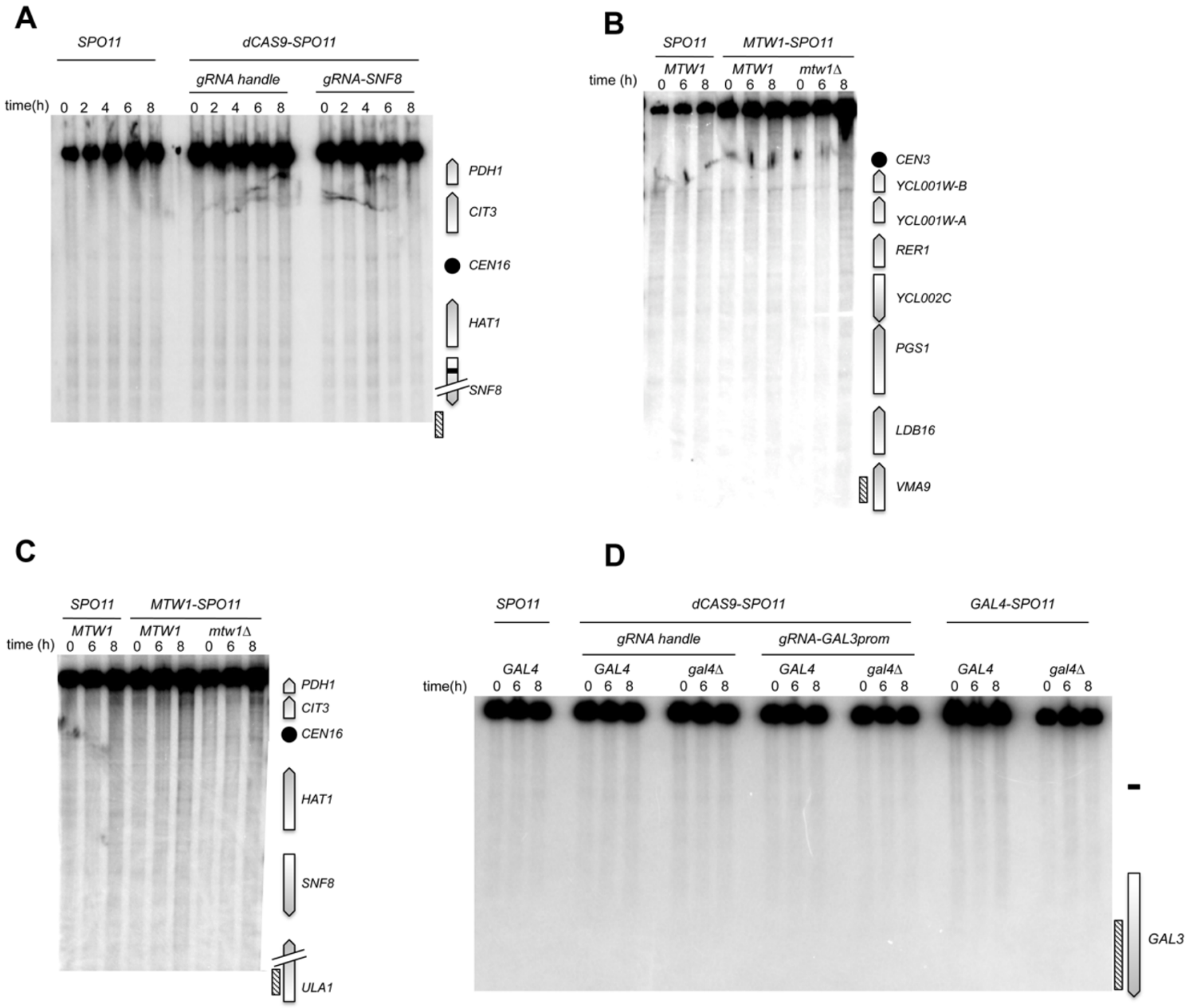

Figure S7. TSFs do not stimulate meiotic DSB formation in the targeted pericentric regions.

(**A-D**) Analysis of TSF DSB formation in pericentric regions by Southern Blot. Regions containing the centromere of chromosomes III (*CEN3*) and XVI (*CEN16*) were targeted by CRISPR-dCas9-Spo11 (**A**) and Mtw1-Spo11 (**B**-**C**). Targeting of Mtw1-Spo11 was examined in *MTW1* and *mtw1*Δ cells. The targeting of meiotic DSBs at the pericentric *GAL3* promoter (13 kb to the centromere) by Gal4-Spo11 and CRISPR-dCas9-Spo11 was analyzed in *GAL4* and *gal4*Δ cells (**D**). Mtw1-Spo11

targets are centromeres; Gal4- and CRISPR-dCas9-Spo11 target sites are shown as black bars. Probes are indicated as hatched rectangle in ORF maps.

(**A**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-*SNF8* *spo11Δ/spo11Δ* (ANT2544) diploids, digested with *Spe*I and probed with a *ULA1* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11 MTW1/MTW1* (ORD7304), *MTW1-SPO11*/*MTW1-SPO11 spo11Δ/spo11Δ* (AND2304) and *MTW1-SPO11*/*MTW1-SPO11 spo11Δ/spo11Δ mtw1Δ*/*mtw1Δ* (AND2582) diploids, digested with *Bgl*II and probed with an internal *VMA9* fragment.

(**C**) genomic DNA was prepared from *SPO11/SPO11 MTW1/MTW1* (ORD7304), *MTW1-SPO11*/*MTW1-SPO11 spo11Δ/spo11Δ* (AND2304) and *MTW1-SPO11*/*MTW1-SPO11 spo11Δ/spo11Δ mtw1Δ*/*mtw1Δ* (AND2582) diploids, digested with *Spe*I and probed with an internal *ULA1* fragment

(**D**) genomic DNA was prepared from *SPO11/SPO11 GAL4/GAL4* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2536), *dCAS9-SPO11/0* + gRNA-*GAL3prom spo11Δ/spo11Δ GAL4/GAL4* (ANT2538), *dCAS9-SPO11/0* + gRNA-*GAL3prom spo11Δ/spo11Δ gal4Δ/gal4Δ* (ANT2537), *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ GAL4/GAL4* (AND2096) and *GAL4-SPO11/GAL4-SPO11 spo11Δ/spo11Δ gal4Δ/gal4Δ* (AND1969) diploids, digested with *Pst*I and *Xho*I and probed with an internal *GAL3* fragment.

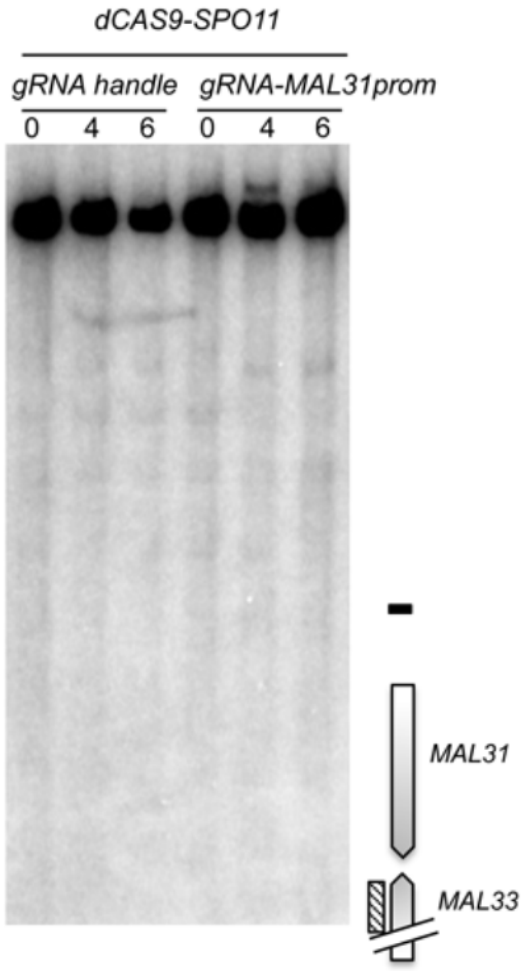

Figure S8. CRISPR-dCas9-Spo11 does not induce DSB formation at the targeted subtelomeric region.

DSB formation at the subtelomeric region containing the target *MAL31* gene was analyzed by Southern blot. CRISPR-dCas9-Spo11 target site is shown as a black bar in the *MAL31* promoter. Diploid cells were collected at the indicated time after transfer into sporulation medium (t=0h).

Genomic DNA was prepared from *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-*MAL31prom spo11Δ/spo11Δ* (ANT2569) diploids, digested with *Avr*II and *Sph*I and probed with a *MAL33* fragment.

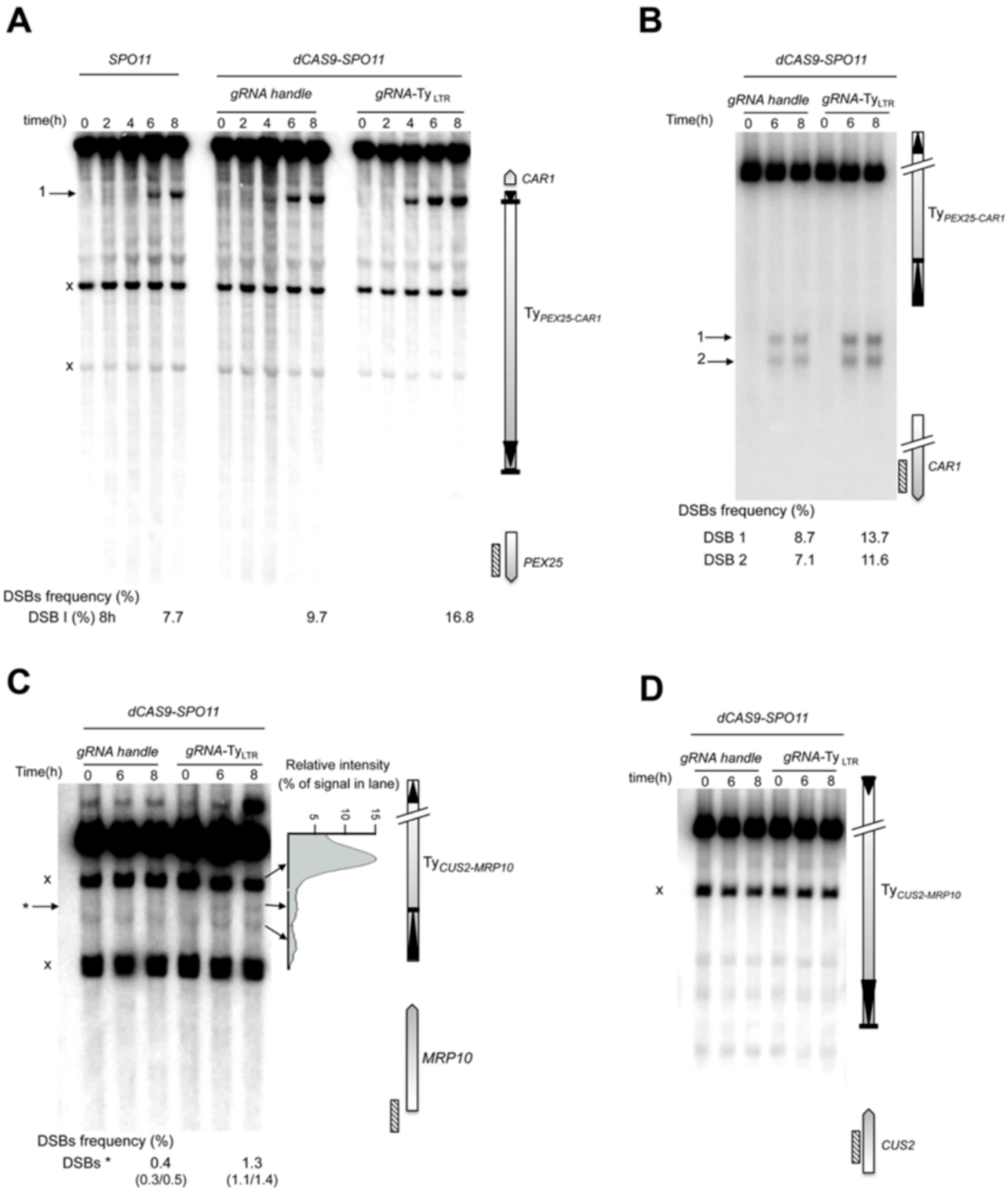

Figure S9. CRISPR-dCas9-Spo11-mediated targeting of Ty LTR sequences.

Natural and targeted DSB formation at the 5' and 3' extremities of the $Ty_{PEX25\text{-}CAR1}$ (**A-B**) and $Ty_{CUS2\text{-}MRP10}$ (**C-D**) elements was analyzed by Southern Blot. To examine precisely natural and targeted DSB formation at the 5' end of the $Ty_{PEX25\text{-}CAR1}$ a

higher resolution of DSB mapping was performed (**B**). The profile of DSB quantification in *dCAS9-SPO11* diploids expressing the gRNA-Ty_{LTR} at the $Ty_{CUS2\text{-}MRP10}$ locus (t=8h) is shown to the right of **Supplementary Fig. 9C**. Cells were collected at the indicated time after transfer into sporulation medium (t=0h). CRISPR-dCas9-Spo11 target sites and probes are shown as black bars in Ty maps and as hatched rectangle in ORF maps, respectively; crosses indicate cross hybridizing bands. DSB values are from single experiments or the mean of two independent experiments with minimum and maximum values indicated into parentheses.

(**A**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-Ty_{LTR} *spo11Δ/spo11Δ* (ANT2562), diploids, digested with *Nco*I and probed with an internal *PEX25* fragment.

(**B**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-Ty_{LTR} *spo11Δ/spo11Δ* (ANT2562), diploids, digested with *Sph*I and *Xba*I and probed with an internal *CAR1* fragment.

(**C**) DNA was prepared from *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-Ty_{LTR} *spo11Δ/spo11Δ* (ANT2562) diploids, digested with *Nae*I and *Sph*I and probed with an intergenic *MRP10-WSC2* fragment.

(**D**) genomic DNA was prepared from *SPO11/SPO11* (ORD7304), *dCAS9-SPO11/0* + gRNA-handle *spo11Δ/spo11Δ* (ANT2527) and *dCAS9-SPO11/0* + gRNA-Ty_{LTR} *spo11Δ/spo11Δ* (ANT2562), diploids, digested with *Sph*I and probed with an internal *CUS2* fragment.

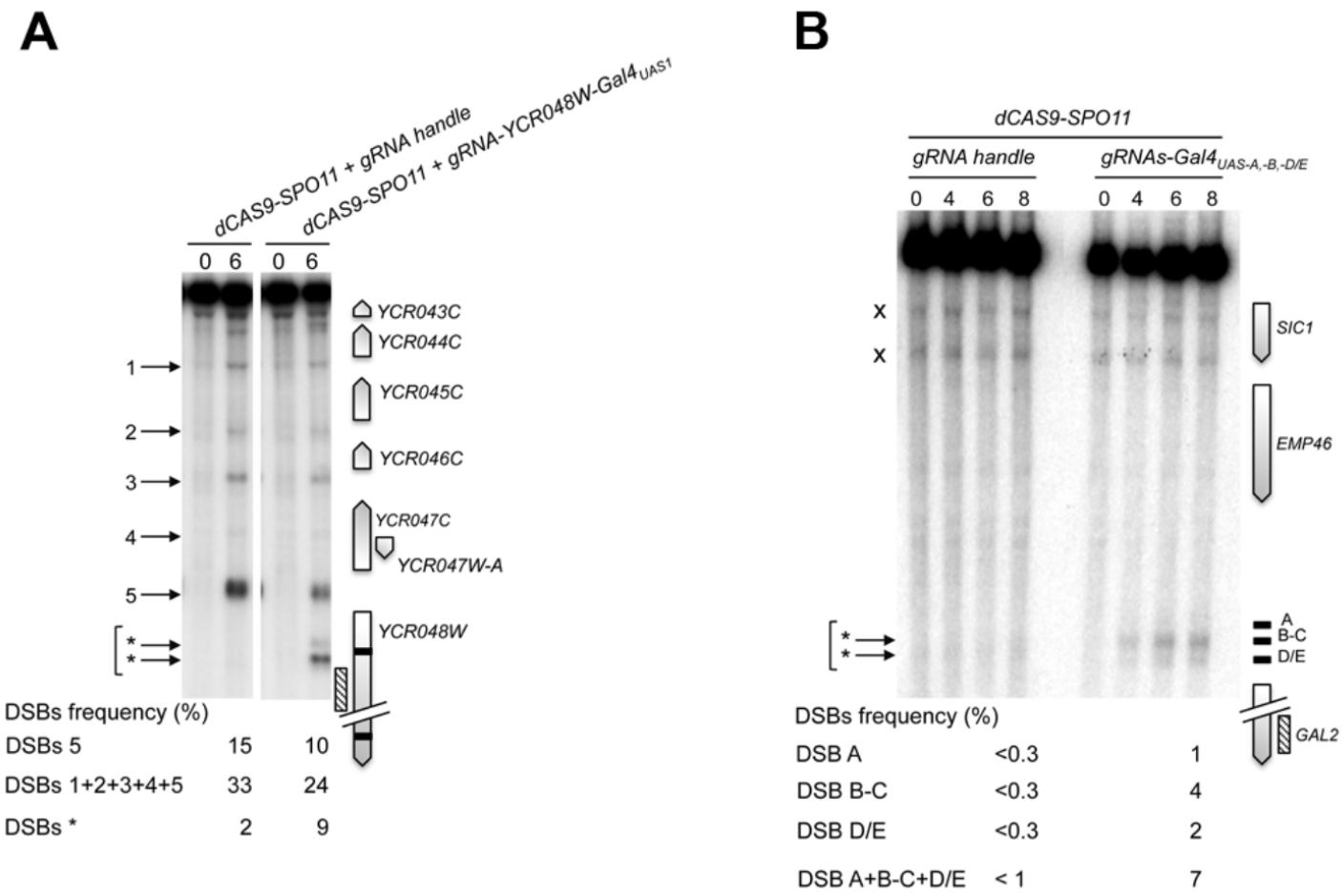

Figure S10. CRISPR-dCas9-Spo11-targets meiotic DSBs in hot and cold regions in *SPO11* cells.

Southern blot analysis of dCas9-Spo11 DSBs at the targeted *YCR048W* natural hotspot (**A**) and at the cold *GAL2* promoter regions (**B**) upon expression of a sgRNA or multiple gRNAs. Diploid *GAL4/GAL4* cells were collected at the indicated time after transfer into sporulation medium (t=0h). CRISPR-dCas9-Spo11 target sites are shown as black bars. At the left of the gel, arrowheads indicate wild-type (numbered) and targeted (asterisks) DSBs. Frequency of DSBs* corresponds to the sum of frequencies detected in the *YCR048W* ORF (indicated by *arrows).

(**A**) genomic DNA was prepared from *dCAS9-SPO11/0* + gRNA-handle (ANT2518) and *dCAS9-SPO11/0* + gRNA-*YCR048W*-Gal4$_{UAS1}$ (ANT2519) diploids, digested with *Ase*I and probed with an internal *YCR048W* fragment.

(**B**) genomic DNA was prepared from *dCAS9-SPO11/0* + gRNA-handle (ANT2518) and *dCAS9-SPO11/0* + gRNAs targeting *GAL2prom*-Gal4$_{UAS-A,-B,-D/E}$ (ANT2713)

diploids, digested with *Xba*I and probed with an internal *GAL2* fragment. Crosses indicate cross hybridizing bands. DSB values are the means of two independent cultures when the standard deviation is indicated.