

Figure S1. **Meiotic polySUMO chains derived from SUMOylated Ecm11 and SUMO E3 ligases Siz1 and Siz2 are required for efficient SUMOylation of Ecm11 and Zip1 assembly.** (A) SK1 *ndt80* diploid cells that produce Ecm11 fused to various sizes of extra amino acid sequences as indicated were introduced into meiosis, and the whole-cell extract prepared from cells at the 7-h time point was subjected to Western blotting with anti-Smt3 (SUMO) and anti-myc (Ecm11-myc) antibodies. (*) For unknown reasons, Ecm11 plus 3 kD (1xmyc) did not show detectable signals in the Western blotting with anti-myc antibodies, whereas 4xmyc and 7xmyc versions did. The presence of extra in-frame DNA sequences encoding additional 3-, 7-, and 12-kD polypeptides was confirmed by DNA sequencing. (B) SUMOylation of Ecm11 in the indicated strains was analyzed as in Fig. 1. SPM, sporulation media. (C) Quantitative analysis of chromosomal Zip1 localization. See Materials and methods for more detail. (D) Meiotic chromosome spreads were stained for Zip1, and chromosomal localization pattern of Zip1 was examined as described in C and Materials and methods. *, $P < 0.001$ (Mann-Whitney test). (E) Meiotic chromosome spreads of the indicated strains were examined for the size of PCs (top; *, $P < 0.001$ [Mann-Whitney test]) and the ratio of chromosome spreads carrying a PC (bottom; 100 chromosome spreads examined; *, $P < 0.001$ [χ^2 test]).

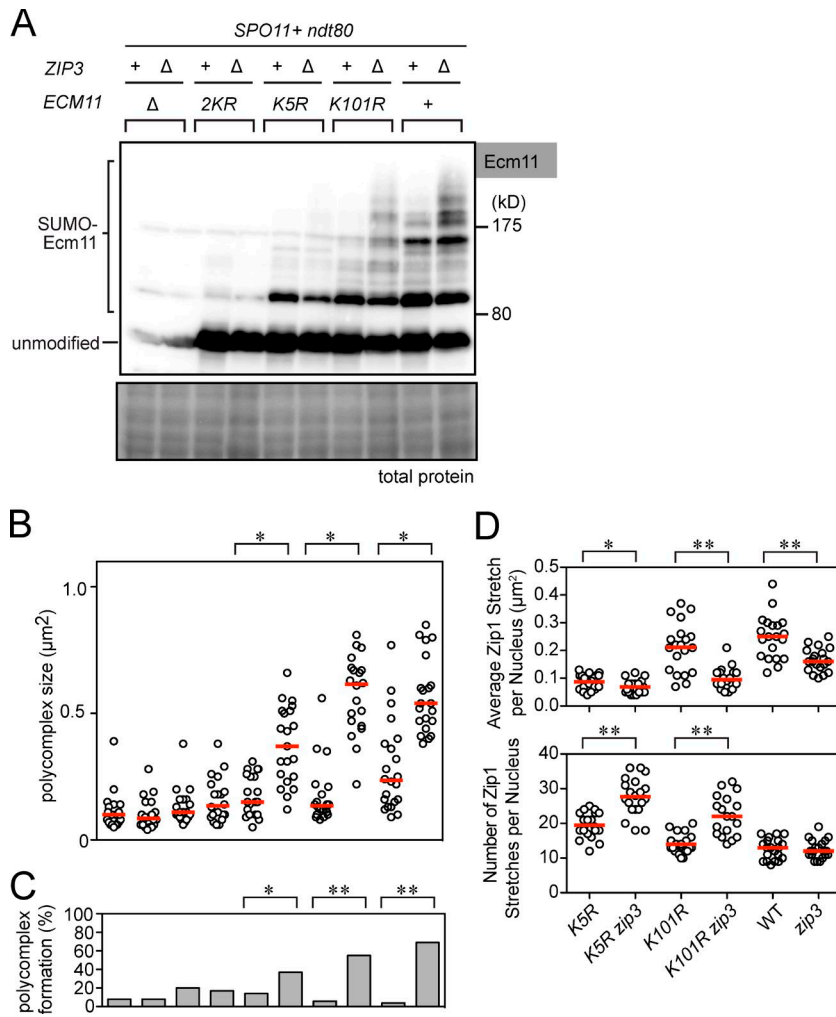


Figure S2. **The relationship between the level of Ecm11 SUMOylation and Zip1 assembly in the presence of Spo11.** (A) SUMOylation of Ecm11 at the 22-h time point in indicated strains was analyzed as in Fig. 1. Ponceau S staining was used as a loading control. (B) The size of PCs was quantitatively analyzed at the 22-h time point as described in Materials and methods. Red bars indicate median values. *, $P < 0.001$ (Mann-Whitney test). (C) The ratio of chromosome spreads carrying a polycplex was measured with the sample used in B. ≥ 50 spreads were examined per strain. *, $P < 0.01$; **, $P < 0.001$ (χ^2 test). (D) Chromosomal localization of Zip1 was quantitatively examined at 22 h as explained in Fig. S1 C and Materials and methods. *, $P < 0.05$; **, $P < 0.001$ (Mann-Whitney test).

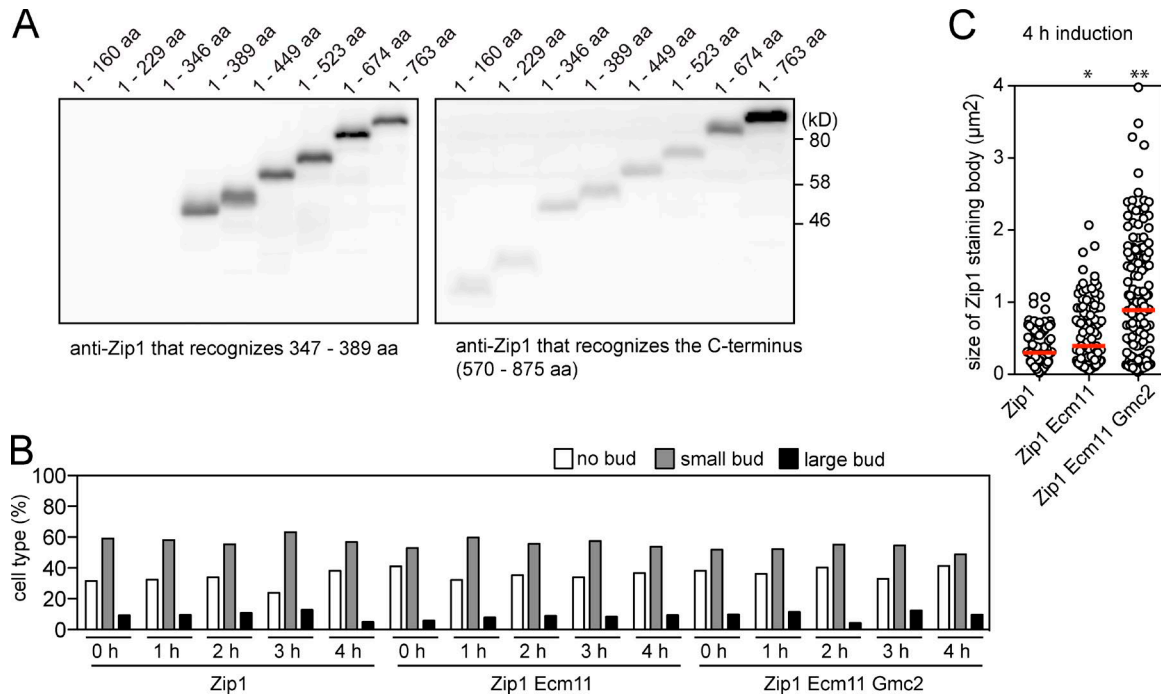


Figure S3. **Induction levels of various truncated Zip1 proteins and the effect of mitotically inducing Zip1, Ecm11, and Gmc2 on the cell cycle.** (A) Various Zip1 proteins were produced in vegetative cells using the galactose-inducible promoter and detected by Western blotting using two different antibodies as indicated. Note that all of the Zip1 truncation proteins also carry a C-terminal fragment (825–875 aa; see Fig. 4), thus are reactive to the C-terminal antibody. The results show that truncated Zip1 proteins are produced at a similar level in vegetative cells. (B) The mitotic cell cycle of strains producing indicated proteins was monitored over the course of protein induction experiments. Cells were examined for their cell-cycle stage based on their budding status at each time point. ≥ 100 cells were examined per time point. The difference in the distribution of cell types (no bud, small bud, and large bud) at a time point between three strains was not statistically significant (χ^2 test). (C) Chromosome spreads prepared at the 4-h time point, shown in B, were immunostained for Zip1, and the size of Zip1 assembly bodies was examined. *, $P < 0.01$; **, $P < 0.001$ (Mann-Whitney test).

Table S1. Strains used in this study

Strain	Genotype	Background
TBR2065	<i>MATα/MATα leu2-3, 112/leu2-3, 112 his4-260/his4-260 ura3-1/ura3-1 ade2-1/ade2-1 thr1-4/thr1-4 trp1-289/trp1-289 lys2/lys2</i>	BR1919
TBR6621	<i>MATα/MATα ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his3::hisG/his3::hisG trp1::hisG/trp1::hisG</i>	SK1
TBR1996	<i>MATα RAD5 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	W303
TBR9892	<i>MATα/MATα RAD5/RAD5 ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 KAN-P_{GAL}:FLAG-ECM11/+ KAN-P_{GAL}:myc-GMC2/+</i>	W303
TBR309	TBR2065 but <i>spo11::ADE2</i>	BR1919
TBR5296	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR5636	TBR2065 but <i>ecm11-K5,101R-13myc::KAN ndt80::LEU</i>	BR1919
TBR5641	TBR2065 but <i>ecm11-K5R-13myc::KAN ndt80::LEU</i>	BR1919
TBR5649	TBR2065 but <i>ecm11-K101R-13myc::KAN ndt80::LEU</i>	BR1919
TBR5896	TBR2065 but <i>ndt80::LEU2</i>	BR1919
TBR5921	TBR2065 but <i>ndt80::LEU2 ecm11::KAN</i>	BR1919
TBR6088	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2 zip3::URA3</i>	BR1919
TBR6090	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2 zip1::LEU2</i>	BR1919
TBR6458	TBR1996 but <i>KAN-P_{GAL}:myc-GMC2</i>	W303
TBR7039	TBR2065 but <i>ecm11::KAN ndt80::LEU2</i>	BR1919
TBR7060	TBR2065 but <i>ecm11::KAN zip3::LEU2 ndt80::LEU2</i>	BR1919
TBR7348	TBR2065 but <i>ndt80::LEU2 ecm11-K5R-13myc::KAN zip3::URA3</i>	BR1919
TBR7349	TBR2065 but <i>ndt80::LEU2 ecm11-K101R-13myc::KAN zip3::URA3</i>	BR1919
TBR7350	TBR2065 but <i>ndt80::LEU2 ecm11-K5,101R-13myc::KAN zip3::URA3</i>	BR1919
TBR7496	TBR1996 but <i>KAN-P_{GAL}:ZIP1</i>	W303
TBR7507	TBR2065 but <i>ndt80::LEU2 ecm11::KAN zip3::HYG</i>	BR1919
TBR7556	TBR6621 but <i>ndt80::LEU2</i>	SK1
TBR7567	TBR2065 but <i>siz2::NAT</i>	BR1919
TBR7569	TBR2065 but <i>siz1::HYG</i>	BR1919
TBR7611	TBR2065 but <i>siz2::NAT ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR7613	<i>siz1::HYG ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR7609	<i>siz2::NAT siz1::HYG</i>	BR1919
TBR7706	<i>siz2::NAT siz1::HYG ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR7783	<i>KAN-P_{CLB2}:HA-ULP1</i>	BR1919
TBR7785	<i>KAN-P_{CLB2}:HA-ULP2</i>	BR1919
TBR7789	TBR2065 but <i>KAN-P_{CLB2}:HA-ULP2 ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR7829	TBR2065 but <i>KAN-P_{CLB2}:HA-ULP1 ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR7885	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2 zip3::URA3 siz1::HYG siz2::NAT</i>	BR1919
TBR8904	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2 zip3::URA3 zip1::LEU2</i>	BR1919
TBR9286	TBR1996 but <i>KAN-P_{GAL}:FLAG-ECM11 KAN-P_{GAL}:ZIP1</i>	W303
TBR9293	TBR1996 but <i>KAN-P_{GAL}:FLAG-ECM11 KAN-P_{GAL}:ZIP1 KAN-P_{GAL}:myc-GMC2</i>	W303
TBR9446	TBR6621 but <i>ECM11-13myc-KAN</i>	SK1
TBR9450	TBR6621 but <i>ECM11-13myc-KAN zip3::NAT</i>	SK1
TBR9451	TBR6621 but <i>zip3::NAT</i>	SK1
TBR9455	TBR1996 but <i>KAN-P_{GAL}:ZIP1 KAN-P_{GAL}:FLAG-ECM11-9MYC-HYG KAN-P_{GAL}:myc-GMC2</i>	W303
TBR9467	TBR2065 but <i>ECM11-13myc-KAN ndt80::LEU2 zip1-NM2 zip3::HYG</i>	BR1919
TBR9468	TBR2065 but <i>zip1-M1 ECM11-13myc-KAN ndt80::LEU2 zip3::HYG</i>	BR1919
TBR9469	TBR2065 but <i>zip1-C1 ndt80::LEU2 ECM11-13myc-KAN zip3::HYG</i>	BR1919
TBR9884	TBR9892 but <i>+KAN-P_{GAL}:zip1-N160-TRP1</i>	W303
TBR9885	TBR9892 but <i>+KAN-P_{GAL}:zip1-N229-TRP1</i>	W303
TBR9887	TBR9892 but <i>+KAN-P_{GAL}:zip1-N389-TRP1</i>	W303
TBR9888	TBR9892 but <i>+KAN-P_{GAL}:zip1-N449-TRP1</i>	W303
TBR9889	TBR9892 but <i>+KAN-P_{GAL}:zip1-N523-TRP1</i>	W303
TBR9890	TBR9892 but <i>+KAN-P_{GAL}:zip1-N674-TRP1</i>	W303
TBR9891	TBR9892 but <i>+KAN-P_{GAL}:zip1-N763-TRP1</i>	W303
TBR9916	TBR9892 but <i>+KAN-P_{GAL}:ZIP1</i>	W303
TBR9917	TBR9892 but <i>+KAN-P_{GAL}:zip1-N346-TRP1</i>	W303
TBR10638	TBR2065 but <i>spo11-Y135F-HYG ndt80::LEU2 zip3::URA3 ecm11::NAT</i>	BR1919
TBR10639	TBR2065 but <i>ecm11::NAT spo11-Y135F-HYG ndt80::LEU2</i>	BR1919
TBR10640	TBR2065 but <i>ECM11-13myc-KAN spo11-Y135F-HYG ndt80::LEU2 zip3::URA3</i>	BR1919
TBR10641	TBR2065 but <i>ECM11-13myc-KAN spo11-Y135F-HYG ndt80::LEU2</i>	BR1919
TBR10642	TBR2065 but <i>ecm11-K5R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2 zip3::URA3</i>	BR1919
TBR10643	TBR2065 but <i>ecm11-K5R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2</i>	BR1919

Table S1. **Strains used in this study** (Continued)

Strain	Genotype	Background
TBR10644	TBR2065 but <i>ecm11-K101R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2 zip3::URA3</i>	BR1919
TBR10645	TBR2065 but <i>ecm11-K101R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2</i>	BR1919
TBR10646	TBR2065 but <i>ecm11-K5,101R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2 zip3::URA3</i>	BR1919
TBR10647	TBR2065 but <i>ecm11-K5,101R-13myc::KAN spo11-Y135F-HYG ndt80::LEU2</i>	BR1919
TBR11045	TBR2065 but <i>ecm11-K5,101R ndt80::LEU2</i>	BR1919
TBR11099	TBR6621 but <i>ndt80::LEU2 ECM11-1xmyc-KAN</i>	SK1
TBR11101	TBR6621 but <i>ndt80::LEU2 ECM11-4xmyc-KAN</i>	SK1
TBR11103	TBR6621 but <i>ndt80::LEU2 ECM11-7xmyc-KAN</i>	SK1
TBR11113	TBR2065 but <i>siz1::HYG siz2::NAT spo11::ADE2 ECM11-13myc-KAN ndt80::LEU2</i>	BR1919
TBR11114	TBR2065 but <i>spo11::ADE2 ECM11-13myc-KAN ndt80::LEU2</i>	BR1919