**Supplementary Data**

**A dCas9-based system identifies a central role for Ctf19 in kinetochore-derived suppression of meiotic recombination**

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**Yeast strains**

All strains are of the SK1 background.

| **Strain name** | **Genotype** | | **Used in figure** | |
| --- | --- | --- | --- | --- |
| yGV3129 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::p11\_pHOP1\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, SGD. coord. 150521-151070::pYKL050c-RFP::LEU*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::p11\_pHOP1\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA* | | 1C, G, H, 2B-D  3C, E  4B, C, F, G  5C, D, H, I | |
| yGV3166 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::p11\_pHOP1\_CTF19\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA*  *MATα ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::p11\_pHOP1\_CTF19\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, SGD. coord. 150521-151070::pYKL050c-RFP::LEU* | | 2B, C  3C, E  4B, C | |
| yGV3179 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::p11\_pHOP1\_IML3\_3xFLAG-dCas9::TRP,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::p11\_pHOP1\_IML3\_3xFLAG-dCas9::TRP,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA* | | 2B, C | |
| yGV3272 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG4, MCM21-6HA::TRP1, trp::p11\_pHOP1\_CTF19\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3* | | 3B  4D, H | |
| yGV3303 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::p11\_pHOP1\_3xFLAG-dCas9::TRP*  *ura3:: sgRNA mock::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::p11\_pHOP1\_3xFLAG-dCas9::TRP*  *ura3:: sgRNA mock::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 1G, H  2D | |
| yGV3308 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, MCM21-6HA::TRP1, ura3::sgRNA VIII chromosome arm8::URA3* | | 3B  4B, H | |
| yGV3311 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::p11\_pHOP1\_CTF19\_3xFLAG-dCas9::*  *ura3:: sgRNA mock::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::p11\_pHOP1\_CTF19\_3xFLAG-dCas9::TRP*  *ura3:: sgRNA mock ::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 2D | |
| yGV3433 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,CHL4-6HA::k1TRP1, trp1::pHOP1\_CTF19\_3xFLAG-dCAS9::TRP1, ura3:: sgRNA VIII chromosome arm8::URA3* | | 3D | |
| yGV3450 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA III YCR047C::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA III YCR047C::URA3,*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 1H  2D | |
| yGV3451 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA III YCR047C::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TR1P*  *ura3:: sgRNA III YCR047C::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 2D | |
| yGV3522 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::pHOP1\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA, iml3::KANMX*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::pHOP1\_3xFLAG-dCAS9::TRP, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP1, SGD. coord. 150521-151070::pYKL050c-RFP::LEU, iml3::KANMX* | | 3F, G | |
| yGV3523 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::pHOP1\_CTF19\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, SGD. coord. 150521-151070::pYKL050c-RFP::LEU, iml3Δ::KANMX*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp::pHOP1\_CTF19\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA, iml3Δ::KANMX* | | 3F, G | |
| yGV3527 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1, ura3::sgRNA VIII chromosome arm8::URA3* | | 3D, E | |
| yGV3535 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *CHL4-6HA::k1TRP1*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *CHL4-6HA::k1TRP1* | | 3E | |
| yGV3543 | *MATα,, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *Mcm21-6HA::TRP1*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *Mcm21-6HA::TRP1* | | 3C | |
| yGV3983 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA* | | 4F, G | |
| yGV4013 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 4B, C | |
| yGV4015 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 4F, G | |
| yGV4079 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, SGD. coord. 150521-151070::pYKL050c-RFP::LEU*  *MATα*, *ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA* | | 5C-F | |
| yGV4081 | *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA* | | 2B, C | |
| yGV4083 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA* | | 2B, C | |
| yGV4117 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF19\_3xFLAG-dCas9\_6xGly\_DBF4::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF19\_3xFLAG-dCas9\_6xGly\_DBF4::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA* | | 5H, I | |
| yGV4125 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_NDC10\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_NDC10\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 2B, C | |
| yGV4216 | *MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *sae2Δ::LEU2*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA III YCR047C::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *sae2Δ::LEU2*  *trp::pHOP1\_3xFLAG-dCas9::TRP1,*  *ura3:: sgRNA III YCR047C::URA3* | | 6E, F | |
| yGV4218 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA III YCR047C::URA3 sae2Δ::LEU2*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA III YCR047C::URA3 sae2Δ::LEU2* | | 6E, F | |
| yGV4230 | *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCas9::TRP1, sae2Δ::LEU2 ura3:: sgRNA III YCR047C::URA3*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCas9::TRP1, sae2Δ::LEU2 ura3:: sgRNA III YCR047C::URA3* | | 6E, F | |
| yGV4255 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF19\_3xFLAG-dCas9\_6xGly\_DBF4::TRP1, sae2Δ::LEU2, ura3:: sgRNA III YCR047C::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF19\_3xFLAG-dCas9\_6xGly\_DBF4::TRP1, sae2Δ::LEU2, ura3:: sgRNA III YCR047C::URA3* | | 6E, F | |
| yGV4348 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9\_6Gly-DBF4-p11::TRP1*  *THR1::pYKL050c-CFP::TRP*  *SGD. coord. 150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9\_6Gly-DBF4-p11::TRP1*  *THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP\*::URA* | | 5H | |
| yGV4686 | *MATa*, *ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, mcm21Δ::KANMX, THR1::pYKL050c-CFP::TRP, SGD. coord. 150521-151070::pYKL050c-RFP::LEU*  *MATα*, *ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, trp1::pHOP1\_ctf19-1-30(aa)2x\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3, mcm21Δ::KANMX, THR1::pYKL050c-CFP::TRP, ARG4::pYKL050c-GFP::URA* | | 5E, F | |
| yGV4709 | *MATa*, *ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, MCM21-6HA::TRP1, trp1::pHOP1\_ctf19-1-30(aa)\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, MCM21-6HA::TRP1, trp1::pHOP1\_ctf19-1-30(aa)\_3xFLAG-dCAS9::TRP1* | | 4H | |
| yGV4710 | *MATa/MATα*, *ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, MCM21-6HA::TRP1, trp1::pHOP1\_ctf19-9A\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3* | | 4D | |
| yGV4808 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::p11\_pHOP1\_3xFLAG-dCas9::TRP, ura3:: sgRNA III YCR047C::URA3*  *MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG4,*  *ARE1::HIS3MX6, trp::p11\_pHOP1\_3xFLAG-dCas9::TRP,*  *ura3:: sgRNA III YCR047C::URA3* | | 6B, C | |
| yGV4809 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::p11\_pHOP1\_CTF19-1-30(aa) DIMER\_3xFlag-*  *dCas9::TRP1, ura3:: sgRNA III YCR047C::URA3*  *MATalpha, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG4,*  *ARE1::HIS3MX6,* *trp1::p11\_pHOP1\_CTF19-1-30(aa) DIMER\_3xFlag-*  *dCas9::TRP1, ura3:: sgRNA III YCR047C::URA3, cyh2, ARG4* | | 6B,C | |
| yAM14087 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *SGD.150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)* | | 7A |
| yAM14240 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *ctf19Δ::KanMX6*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *SGD.150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *ctf19Δ::KanMX6* | | 7A |
| yAM26969 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *ARG4::pYKL050c-GFP\*::URA*  *ctf19-9A::LEU2*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP*  *SGD.150521-151070::pYKL050c-RFP::LEU (~10kb to right of ARG4)*  *ctf19-9A::LEU2* | | 7A |
| yAM13149 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *CEN8::pYKL050c-RFP::LEU*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *SGD.115024-115572::pYKL050c-GFP\*::URA (~10kb to right of CEN8)* | | 7A |
| yAM13411 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *CEN8::pYKL050c-RFP::LEU*  *ctf19Δ::KanMX6*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *SGD.115024-115572::pYKL050c-GFP\*::URA (~10kb to right of CEN8)*  *ctf19Δ::KanMX6* | | 7A |
| yAM28733 | | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *CEN8::pYKL050c-RFP::LEU*  *ctf19-9A::LEU2*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *THR1::pYKL050c-CFP::TRP (~50kb to right of CEN8)*  *SGD.115024-115572::pYKL050c-GFP\*::URA (~10kb to right of CEN8)*  *ctf19-9A::LEU2* | | 7A |

**Supplementary Figure Legends**

**Supplementary Figure 1. A.** Schematic of the genomic region within the chromosome arm interval on Chromosome *VIII*. SGD coordinates for binding of sgRNA ‘*VIII*’ are indicated. Representative genome browser profile of meiotic hotspots for Spo11-oligo mapping (Zhu & Keeney, 2015). Normalized Spo11 oligo counts (RPM) is shown.

**Supplementary Figure 2. A.** Spore viability analysis of indicated strains. N indicates total number of analyzed tetrads. Error bars indicates standard deviation from two independent experiments. **B.** Spore viability analysis of indicated strains. N indicates total number of analyzed tetrads. Error bars indicates standard deviation from two independent experiments. **C.** Spore viability analysis of indicated strains. N indicates total number of analyzed tetrads. **D.** Spore viability analysis of indicated strains. N indicates total number of analyzed tetrads. Cells were grown (including for sporulation) at 230 C.

**Supplementary Figure 3. A**. Co-immunoprecipitation of Iml3-3xFlag-dCas9 and Chl4-6HA (via α-Flag IP) during meiotic prophase (5 hours into meiotic program). Pgk1 is used as loading control. **B**. Co-immunoprecipitation of Ctf19-3xFlag-dCas9 and Mtw1-GFP (via α-Flag IP) during meiotic prophase (5 hours into meiotic program). Pgk1 is used as loading control.

**Supplementary Figure 4. A**. Co-immunoprecipitation of Ctf19-3xFlag-dCas9, Ctf19-9A-3xFlag-dCas9 and Chl4-6HA (via α-Flag IP) during meiotic prophase (5 hours into meiotic program). Pgk1 is used as loading control. **B**. Co-immunoprecipitation of Ctf19-3xFlag-dCas9 Ctf191-30-3xFlag-dCas9 and Chl4-6HA (via α-Flag IP) during meiotic prophase (5 hours into meiotic program). Pgk1 is used as loading control.

**Supplementary Table 1.** Raw recombination frequencies, as used throughout the manuscript.

**Supplementary Table 2.** Spore viability data, as used in **Supplementary Figure 2**.

**Yeast strains (Supplementary Figures)**

All strains are of the SK1 background.

| **Strain name** | **Genotype** | **Used in figure** |
| --- | --- | --- |
| yGV8 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG* | S2A, B, D |
| yGV49 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his4B::LEU2, arg4-Bgl II*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his4X::LEU2 (Bam)-URA3, arg4-Nsp* | S2C |
| yGV1820 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, TRP1, ARG4,*  *his4X::LEU2-URA3, ndc10-1::KanMX*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *his3::hisG, ura3, arg4-nsp, TRP1, dmc1Δ::ARG4, ndc10-1::KanMX* | S2D |
| yGV2953 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP* | S2B |
| yGV2960 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG*  *trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP* | S2A |
| yGV3049 | *MATa, ho::LYS2, lys2, leu2::hisG, his4X::LEU2-URA3, ura3, arg4,*  *TRP1, ctf19Δ::KanMX6*  *MATα, ho::LYS2, lys2, leu2::hisG, his4X::LEU2-URA3, ura3, arg4,*  *TRP1, ctf19Δ::KanMX6* | S2A |
| yGV3086 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, arg4, iml3 Δ::KANMX*  *MATα, ho::LYS2, lys2, leu2::hisG, his4X::LEU2-URA3, ura3, arg4-nsp, TRP1, iml3 Δ::KANMX* | S2B |
| yGV3095 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, ARG*  *trp1::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1, ctf19Δ::KanMX6,*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, his4X::LEU2-URA3,trp1::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1, ctf19Δ::KanMX6* | S2A |
| yGV3128 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP1, iml3 Δ::KANMX, arg4-nsp/ARG4*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *his4X::LEU2-URA3, trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP1,iml3 Δ::KANMX, ARG4* | S2B |
| yGV3433 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *CHL4-6HA::k1TRP1,trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP, ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *CHL4-6HA::k1TRP1, trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1 ura3:: sgRNA VIII chromosome arm8::URA3* | S4A, B |
| yGV3527 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1,ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1,ura3:: sgRNA VIII chromosome arm8::URA3* | S3A  S4A, B |
| yGV4044 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1, ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1* | S2C |
| yGV4073 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1,ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1, ura3:: sgRNA VIII chromosome arm8::URA3* | S2C |
| yGV4124 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::p11\_pHOP1\_NDC10\_3xFlag-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *trp1::p11\_pHOP1\_NDC10\_3xFlag-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3* | S2D |
| yGV4341 | *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *ctf3Δ::KanMX6, trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *ctf3Δ::KanMX6, trp1::pHOP1\_CTF3\_3xFLAG-dCas9::TRP1* | S2C |
| yGV4392 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *ctf3Δ::KanMX6*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG*  *ctf3Δ::KanMX6* | S2C |
| yGV4406 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *ndc10-1::KanMX*  *trp1::p11\_pHOP1\_NDC10\_3xFlag-dCas9::TRP1 ura3:: sgRNA VIII*  *chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *ndc10-1::KanMX, trp1::p11\_pHOP1\_NDC10\_3xFlag-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3* | S2D |
| yGV4407 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, wip1Δ::NatMX*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, wip1Δ::NatMX* | S2C |
| yGV4616 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, wip1Δ::NatMX, trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, wip1Δ::NatMX, trp1::pHOP1\_WIP1\_3xFLAG-dCas9::TRP1* | S2C |
| yGV4667 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *Mtw1-yEGFP::KanMX*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG,*  *Mtw1-yEGFP::KanMX* | S3B |
| yGV4669 | *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG4*  *Mtw1-yEGFP::KanMX,trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1*  *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, ARG4*  *Mtw1-yEGFP::KanMX,trp::pHOP1\_CTF19\_3xFLAG-dCas9::TRP1* | S3B |
| yGV4680 | *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1,trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII chromosome arm8::URA3*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1,trp1::pHOP1\_ctf19-9A\_3xFLAG-dCas9::TRP1*  *ura3:: sgRNA VIII::URA3* | S4A |
| yGV4687 | *MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1, trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP1*  *MATa, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1, trp::pHOP1\_IML3\_3xFLAG-dCas9::TRP1* | S3A |
| yGV4716 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, CHL4-6HA::k1TRP1, trp1::hisG, trp1::pHOP1\_ctf19-1-30(aa)\_3xFLAG-dCAS9::TRP1, ura3::sgRNA VIII chromosome arm8::URA3* | S4B |

**References**

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