**Supplementary Data**

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

Lisa-Marie Kuhl 1, Vasso Makrantoni 2, Sarah Recknagel 1, Animish N. Vaze 1, Adele L. Marston 2 and Gerben Vader 1,3,\*

**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-D3C, E4B, C, F, G5C, 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, C3C, E4B, 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* | 3B4D, 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, H2D |
| yGV3308 | *MATa/MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, MCM21-6HA::TRP1, ura3::sgRNA VIII chromosome arm8::URA3* | 3B4B, 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)* | 1H2D |
| 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* | S3AS4A, 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**

Zhu X, Keeney S (2015) High-Resolution Global Analysis of the Influences of Bas1 and Ino4 Transcription Factors on Meiotic DNA Break Distributions in Saccharomyces cerevisiae. *Genetics* 201: 525-42