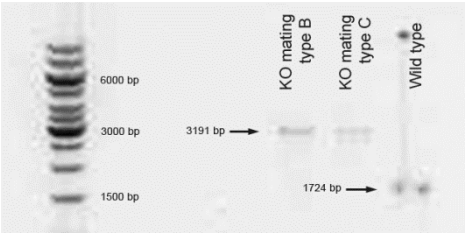
File S1 Evidence for the dispensability of the *Tetrahymena MLH3* homolog, TTHERM_01044360, for meiotic bivalent formation and separation

The protein sequence of *Tetrahymena* Mlh3 shows the conserved metalh binding motif DQHA(2X)E(4X)E that is found in a subset of MLH proteins predicted to have endonuclease activities (Kadyrov F.A., Dzantiev L., Constantin N., Modrich P. 2006. Endonucleolytic function of MutLα in human mismatch repair. *Cell* 126:297h 30).

MISDYVFNQTYATIRSSKNSDNQKKRTSYIQTLSLKNDFKQKILENDYQDISLADRNQNFNQQIQIFSKQSNEE
VQQISQVSKLDRTIFEDIEIFGNCNNKVIICFNQQKGMLFGLDQHAIHERIRYEYFCNQFKASAFCLQYKQSRNE
IDSKCINLSQRNKDKQFPSILWFDQTRTNCLELDAYIFQRLQKNVDKLSQFKIQVVKIQNINQNKFEVYLWPQL
YILNKPITYDLKFIDSILNSELGHIPNTIDEIIMSKACKGAIKFNEELNQNQMDMLIKNIKLCEFPFYCVHGRSSIHP
FFSLEIQDVCQIQKNYQI

Knockout lines were produced by deleting bp h 37 from the translation start to bp 526 from the open reading frame of the 960 bph gene. PCR with primers flanking the TTHERM_01044360 gene showed that knockout in both mating types was complete.



Of the 50 diakinesish metaphase I nuclei scored, 48 had only 5 bivalents or 4 bivalents plus a single univalent (bivalents: red arrows, univalent: blue arrow). The latter is due to the fact that one of the two parental strains from which knockout strains were derived is monosomic for one chromosome. Only two nuclei displayed an unclear situation with possibly one chromosome (univalent) too much. Hovever, all bivalents in all nuclei had both arms bound, thus it is unlikely that chiasmata are reduced in *mlh3*. Moreover, of 100 telophases I/interkineses/prophases II, 0 showed anaphase bridges or lagging chromosomes, or any other sign of a chromosome segregation defect. Likewise, all of 50 anaphases II scored looked normal.