SUPPLEMENTARY DATA

The Pch2 AAA+ ATPase promotes phosphorylation of the Hop1 meiotic checkpoint adaptor in response to synaptonemal complex defects

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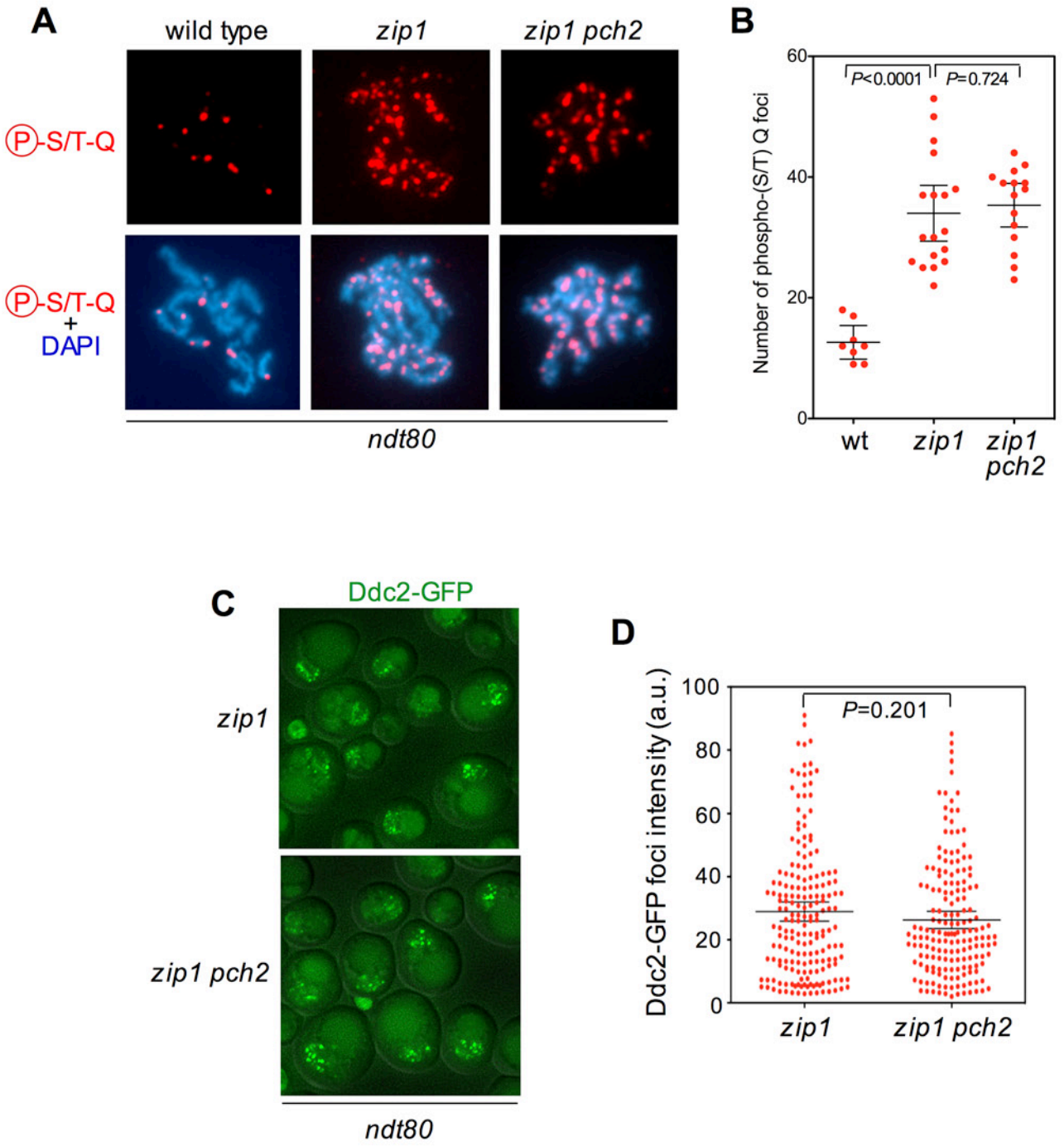

Figure S1. Pch2 does not affect widespread Mec1 signaling. (A) Immunofluorescence of meiotic chromosomes stained with anti-phospho-S/T-Q antibodies (red) and DAPI (blue). Representative nuclei are shown. (B) Quantification on the number of phospho-S/T-Q foci per nucleus. (C) Representative images of Ddc2-GFP foci formation in live cells. Maximum projection images of 10-plane stacks are shown. (D) Quantification of the Ddc2-GFP signal. Strains are: DP424 (wild type), DP460 (*zip1*) and DP1251 (*zip1 pch2*). Prophase-arrested *ndt80* cells were analyzed 24 h after meiotic induction.

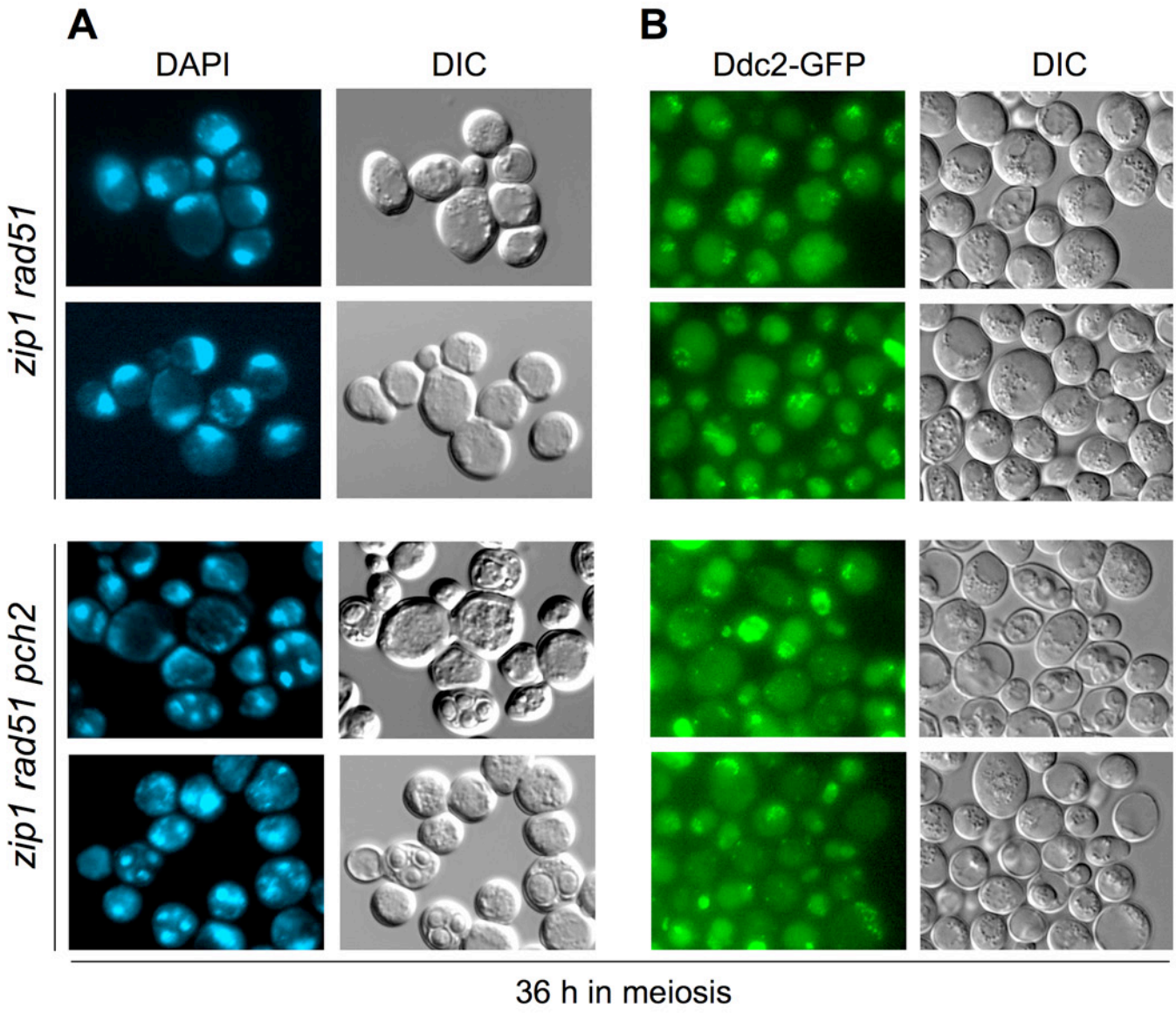

Figure S2. Representative images of meiotic nuclear divisions and Ddc2 foci detection for the analyses presented in Figure 7B and 7C.

(A) Aliquots from meiotic cultures were taken a different time points, fixed with ethanol and stained with DAPI to monitor meiotic nuclear divisions. (B) Independent aliquots from the same cultures were directly analyzed by live-cell fluorescence microscopy to detect Ddc2-GFP. Two different representative fields from the 36 h time point of the *zip1 rad51* (DP1381) and *zip1 rad51 pch2* (DP1382) strains are shown for DAPI and other two representative fields for Ddc2-GFP. The corresponding differential interference contrast (DIC) images are also presented.

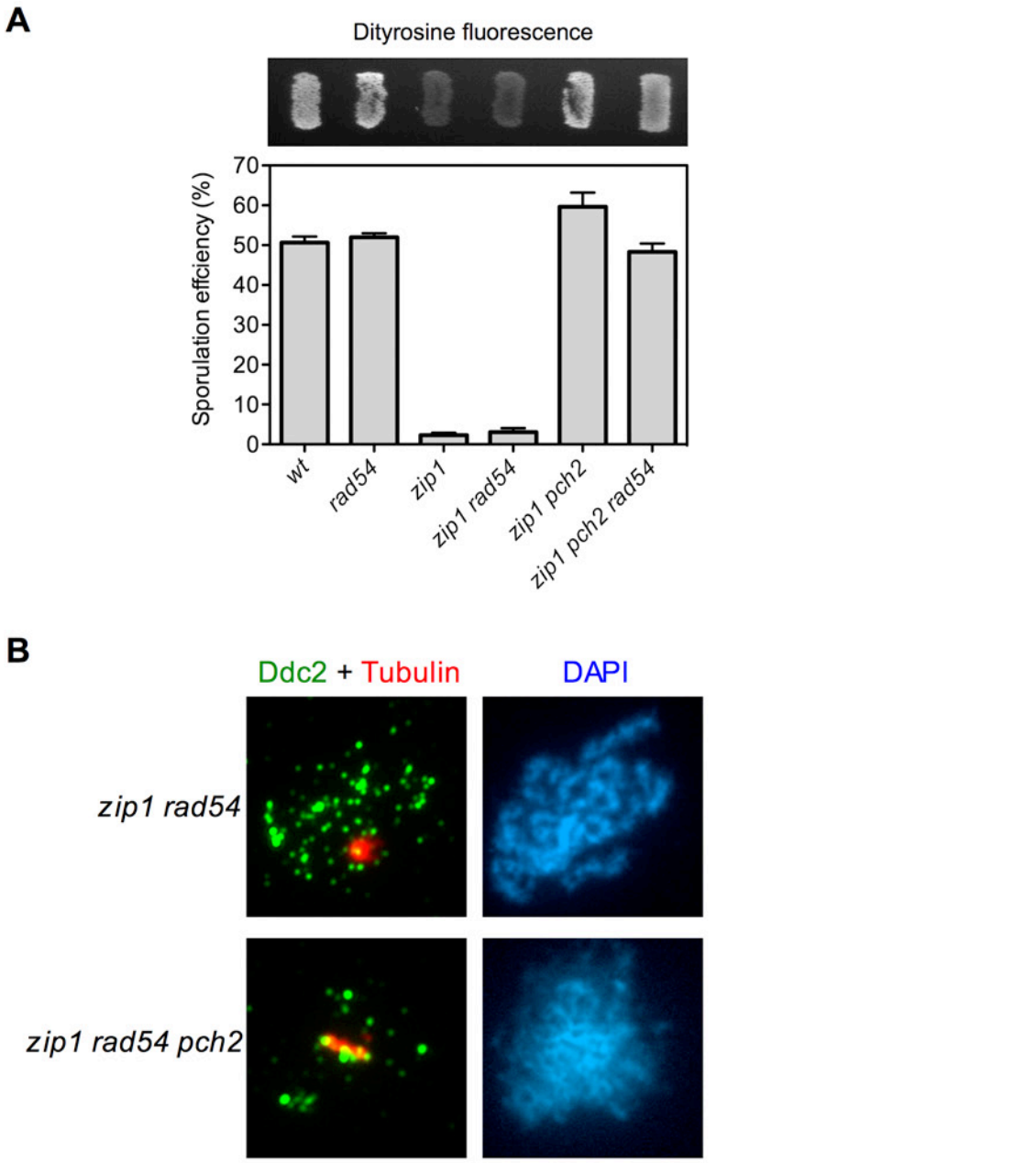

Figure S3. Deletion of *RAD54* does not prevent meiotic progression in *zip1 pch2*. (A) Dityrosine fluorescence and sporulation efficiency after three days on sporulation plates. Strains are: DP421 (wild type), DP1168 (*rad54*), DP422 (*zip1*), DP1169 (*zip1 rad54*), DP1161 (*zip1 pch2*) and DP1171 (*zip1 pch2 rad54*). (B) Immunofluorescence of spread meiotic chromosomes stained with anti-tubulin (red), anti-GFP antibodies to detect Ddc2 (green) and DAPI (blue). A representative prophase nucleus from *zip1 rad54* (DP1378) and a representative meiosis I nucleus from *zip1 rad54 pch2* (DP1387) are shown. Spreads were prepared 24 h after meiosis induction.

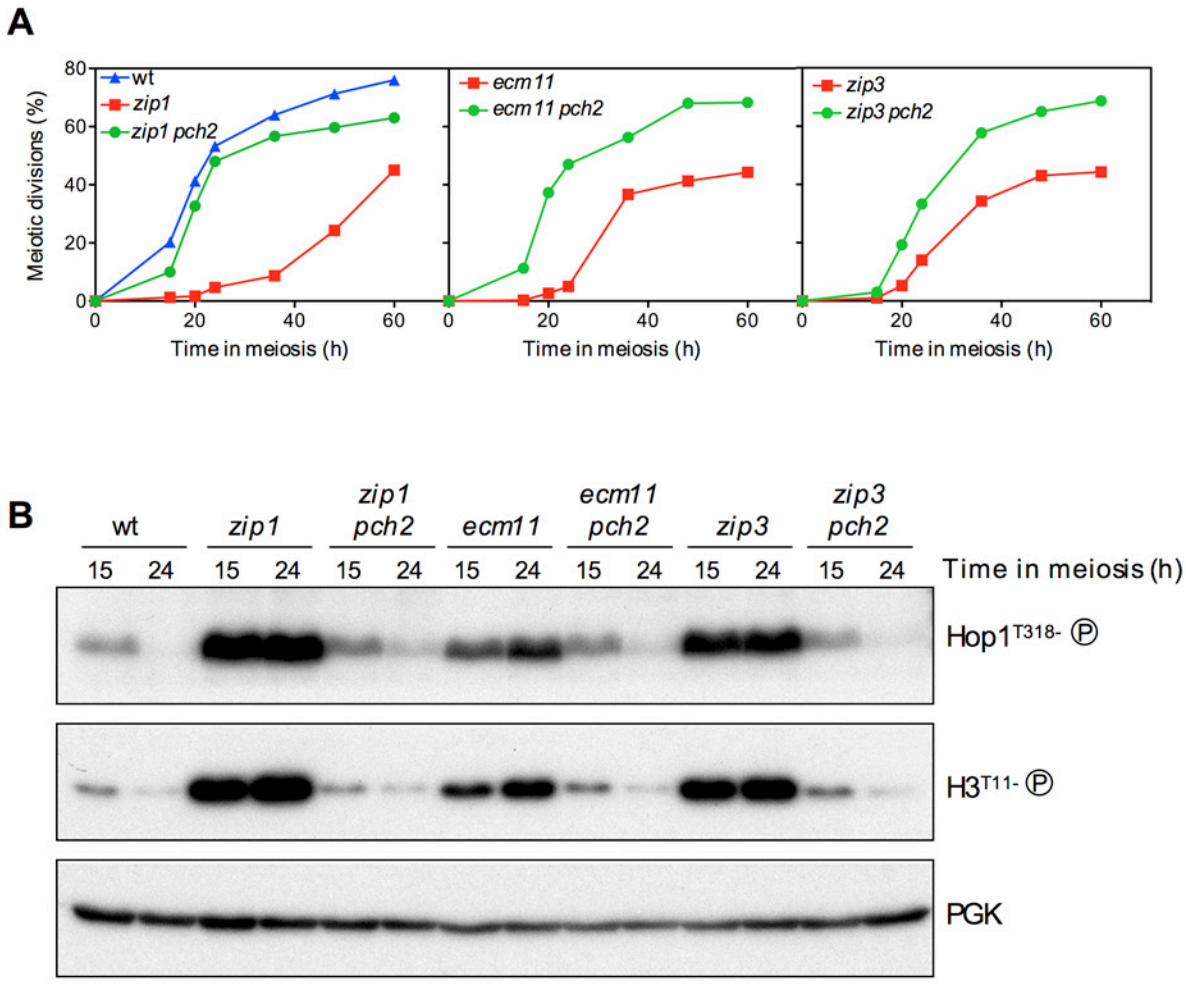

Figure S4. Pch2 is required for checkpoint activation in the SC-deficient *ecm11* and *zip3* mutants. (A) Time course of meiotic nuclear divisions; the percentage of cells containing more than two nuclei is represented. (B) Western blot analysis of phospho-Hop1^{T318}, phospho-H3^{T11} (as indicator of Mek1 activity) and PGK (as loading control). Extracts were prepared at 15 h and 24 h after meiotic induction. Strains are: DP421 (wild type), DP422 (*zip1*), DP1029 (*zip1 pch2*), DP1388 (*ecm11*), DP1390 (*ecm11 pch2*), DP1384 (*zip3*) and DP1386 *(zip3 pch2*).

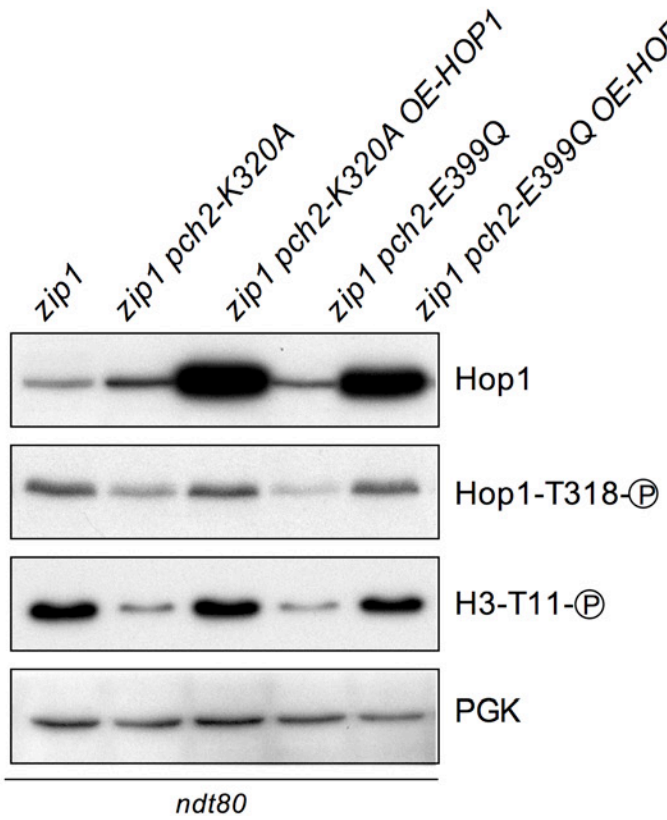

Figure S5. Hop1 overproduction restores *zip1*-induced checkpoint function in *pch2* ATPase mutants. Western blot analysis of total Hop1, phospho-Hop1^{T318}, phospho-H3^{T11} (as indicator of Mek1 activity) and PGK (as loading control). Extracts were prepared after 24 h of meiotic induction in *ndt80* strains. Strains are: DP1190 (*zip1*), DP1192 *(zip1 pch2-K320A*) and DP1302 (*zip1 pch2-E399Q*), transformed with empty vector or with R1692 (*OE-HOP1*).

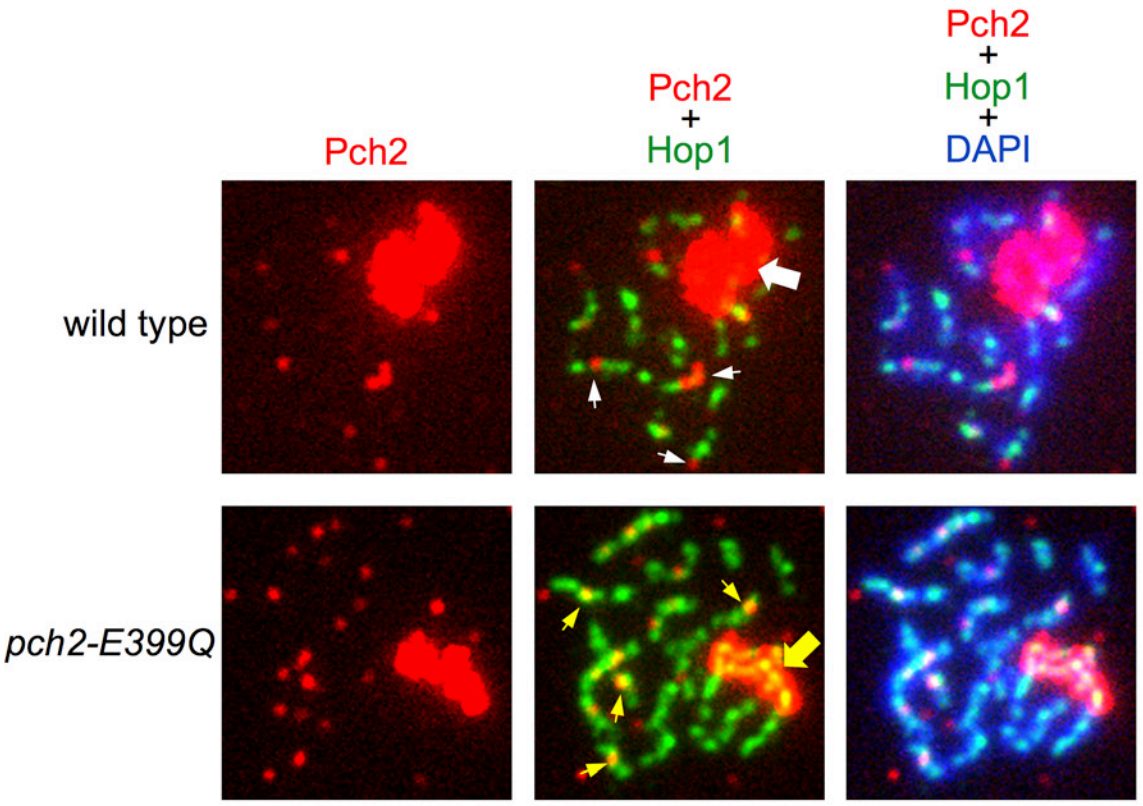

Figure S6. In contrast with wild-type Pch2, the ATPase-deficient Pch2-E399Q version colocalizes with Hop1 at the rDNA and chromosome axes.
Overexposed images of the corresponding panels shown in Figure 9A to reveal Pch2 chromosomal localization. Thick arrows point to the rDNA. Thin arrows point to interstitial Pch2 chromosomal foci. Strains are: DP1243 (wild type) and DP1262 (*pch2-E399Q*).

Table S1. *Saccharomyces cerevisiae* strains

Strain	Genotype*	Source
BR2495	*MAT***a**/*MATα leu2-27/leu2-3,112 his4-280/his4-260 arg4-8/ARG4 thr1-1/thr1-4 trp1-1/trp1-289 cyh10/CYH10 ura3-1 ade2-1*	Roeder Lab
MY63	BR2495 *zip1::LEU2*	Roeder Lab
S3483	BR2495 *ndt80::LEU2*	Roeder Lab
S4278	BR2495 *zip1::LEU2 ddc1::ADE2*	Roeder Lab
S4286	BR2495 *zip1::LYS2 rad17::LEU2 lys2*	Roeder Lab
S4295	BR2495 *zip1::LEU2 rad24::TRP1*	Roeder Lab
DP174	BR2495 *zip1::LEU2 dot1::TRP1*	PSS Lab
DP221	BR2495 *zip1::LEU2 pch2Δ$^{90-564}$-lacZ::TRP1*	This work
DP223	BR2495 *zip1::LEU2 pch2::TRP1*	PSS Lab
DP228	BR2495 *zip1::LEU2 pch2Δ$^{90-564}$-lacZ::TRP1/PCH2*	This work
DP267	BR2495 *zip1::LYS2 sir2::LEU2 lys2*	PSS Lab
BR1919-2N	*MAT***a**/*MATα leu2-3,112 his4-260 thr1-4 trp1-289 ura3-1 ade2-1*	Roeder Lab
DP421	BR1919-2N *lys2ΔNheI*	PSS Lab
DP422	DP421 *zip1::LYS2*	PSS Lab
DP424	DP421 *ndt80::LEU2*	PSS Lab
DP428	DP421 *zip1::LYS2 ndt80::LEU2*	PSS Lab
DP448	DP421 *DDC2-GFP::TRP1*	PSS Lab
DP449	DP421 *zip1::LYS2 DDC2-GFP::TRP1*	PSS Lab
DP460	DP421 *zip1::LYS2 ndt80::LEU2 DDC2-GFP::TRP1*	PSS Lab
DP582	DP421 *zip1::LYS2 ndt80::LEU2 MEK1-GFP::kanMX6*	PSS Lab
DP700	DP421 *hop1::hphMX4*	This work
DP881	DP421 *zip1::LYS2 pch2::TRP1 ndt80::LEU2*	This work
DP1029	DP421 *zip1::LYS2 pch2::TRP1*	This work
DP1058	DP421 *pch2::TRP1 ndt80::LEU2*	This work
DP1111	DP421 *zip1::LYS2 pch2::TRP1 ndt80::LEU2 MEK1-GFP::kanMX6*	This work
DP1151	BR1919-2N *PCH2-3HA*	This work
DP1152	BR1919-2N *zip1::LEU2 PCH2-3HA*	This work
DP1161	BR1919-2N *zip1::LEU2 pch2::TRP1*	This work

DP1162	BR1919-2N *zip1::LEU2 pch2-3HA-K320A*	This work
DP1163	BR1919-2N *pch2-3HA-K320A*	This work
DP1164	BR1919-2N *pch2::TRP1*	This work
DP1168	BR1919-2N *rad54::LEU2*	This work
DP1169	BR1919-2N *zip1::URA3 rad54::LEU2*	This work
DP1171	BR1919-2N *zip1::URA3 pch2::TRP1 rad54::LEU2*	This work
DP1190	BR1919-2N *zip1::LEU2 ndt80::kanMX3 PCH2-3HA*	This work
DP1192	BR1919-2N *zip1::LEU2 ndt80::kanMX3 pch2-3HA-K320A lys2/LYS2*	This work
DP1193	BR1919-2N *ndt80::kanMX3 pch2-3HA-K320A lys2/LYS2*	This work
DP1243	BR1919-2N *PCH2-3MYC*	This work
DP1244	BR1919-2N *zip1::LEU2 PCH2-3MYC*	This work
DP1245	DP421 *zip1::LYS2 pch2::TRP1 pph3::kanMX6*	This work
DP1247	DP421 *pph3::kanMX6*	This work
DP1249	DP421 *zip1::LYS2 pph3::kanMX6*	This work
DP1251	DP421 *zip1::LYS2 pch2::URA3 ndt80::LEU2 DDC2-GFP::TRP1*	This work
DP1262	BR1919-2N *pch2-3MYC-E399Q*	This work
DP1263	BR1919-2N *zip1::LEU2 pch2-3MYC-E399Q*	This work
DP1287	BR1919-2N *pch2-3HA-E399Q*	This work
DP1288	BR1919-2N *zip1::LEU2 pch2-3HA-E399Q*	This work
DP1302	BR1919-2N *zip1::LEU2 pch2-3HA-E399Q ndt80::kanMX3*	This work
DP1325	BR1919-2N *PCH2-3HA / PCH2-3MYC*	This work
DP1329	BR1919-2N *PCH2 / PCH2-3MYC*	This work
DP1337	BR1919-2N *pch2-3HA-K320A / pch2-3MYC-K320A*	This work
DP1378	DP421 *zip1::LYS2 rad54::LEU2 DDC2-GFP::TRP1*	This work
DP1379	DP421 *zip1::LYS2 pch2::URA3 DDC2-GFP::TRP1*	This work
DP1381	DP421 *zip1::LYS2 rad51::natMX4 DDC2-GFP::TRP1*	This work
DP1382	DP421 *zip1::LYS2 rad51::natMX4 pch2::URA3 DDC2-GFP::TRP1*	This work
DP1384	BR1919-2N *zip3::URA3*	This work
DP1386	BR1919-2N *zip3::URA3 pch2::TRP1*	This work
DP1387	DP421 *zip1::LYS2 rad54::LEU2 pch2::URA3 DDC2-GFP::TRP1*	This work

DP1388	BR1919-2N *ecm11::kanMX6*	This work
DP1390	BR1919-2N *ecm11::kanMX6 pch2::TRP1*	This work

* All strains are diploids isogenic to BR2495 or BR1919 and, unless specified, homozygous for the indicated markers. DP421 is a *lys2* version of the original BR1919-2N.

Table S2. Plasmids

Plasmid name	Vector	Relevant parts	Source
R1566	YCp50	*URA3 CEN4 lacZ*	Roeder Lab
pSS51	YCp50	*URA3 CEN4 pch2-lacZ*	This work
pSS67	pUC18	*pch2-lacZ::TRP1*	This work
pSS54	YEp352	*URA3 2μ PCH2*	This work
R1692	YEp24	*URA3 2μ HOP1*	Hollingsworth Lab
pSS314	pJET1.2	*HOP1*	This work
pSS315	pJET1.2	*hop1-T318A*	This work
pSS316	pRS426	*URA3 2μ HOP1*	This work
pSS317	pRS426	*URA3 2μ hop1-T318A*	This work

Table S3. Primary antibodies

Antibody	Host and type	Application* (Dilution)	Source / Reference
Mek1	Rabbit polyclonal	WB (1:2000)	(1)
Cdc5	Goat polyclonal	WB (1:1000)	Santa Cruz Biotechnology sc-6733
Hop1	Rabbit polyclonal	WB (1:2000) IF (1:400)	(2)
Hop1-T318-P	Rabbit polyclonal	WB (1:1000) IF (1:150)	Jesús Carballo (3)
H3-T11-P	Rabbit polyclonal	WB (1:2000)	Abcam ab5168
Rad51	Rabbit polyclonal	IF (1:300)	Santa Cruz Biotechnology sc-33626
GFP	Rabbit polyclonal	IF (1:400)	Molecular Probes A-6455
Phospho-(S/T)Q	Rabbit polyclonal	IF (1:400)	Cell Signaling Technology #2851
HA (12CA5)	Mouse monoclonal	WB (1:2000) IF (1:200)	Roche 11 666 606 001
Myc (4A6)	Mouse monoclonal	IF (1:200) WB (1:1000)	Millipore 05-724
GFP (JL-8)	Mouse monoclonal	IF (1:200)	Clontech 632381
PGK (22C5)	Mouse monoclonal	WB (1:10000)	Molecular Probes A-6457
Tubulin (TAT1)	Mouse monoclonal	IF (1:400)	(4)

*WB, western blot; IF, immunofluorescence

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2. Smith, A.V. and Roeder, G.S. (1997) The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J Cell Biol*, **136**, 957-967.

3. Carballo, J.A., Johnson, A.L., Sedgwick, S.G. and Cha, R.S. (2008) Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. *Cell*, **132**, 758-770.

4. Refolio, E., Cavero, S., Marcon, E., Freire, R. and San-Segundo, P.A. (2011) The Ddc2/ATRIP checkpoint protein monitors meiotic recombination intermediates *J. Cell Sci.*, **124**, 2488-2500.