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Supplemental Information

CtlP-Specific Roles during Cell Reprogramming Have Long-Term Consequences in the Survival and Fitness of Induced Pluripotent Stem Cells

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FACS DNA-end resection

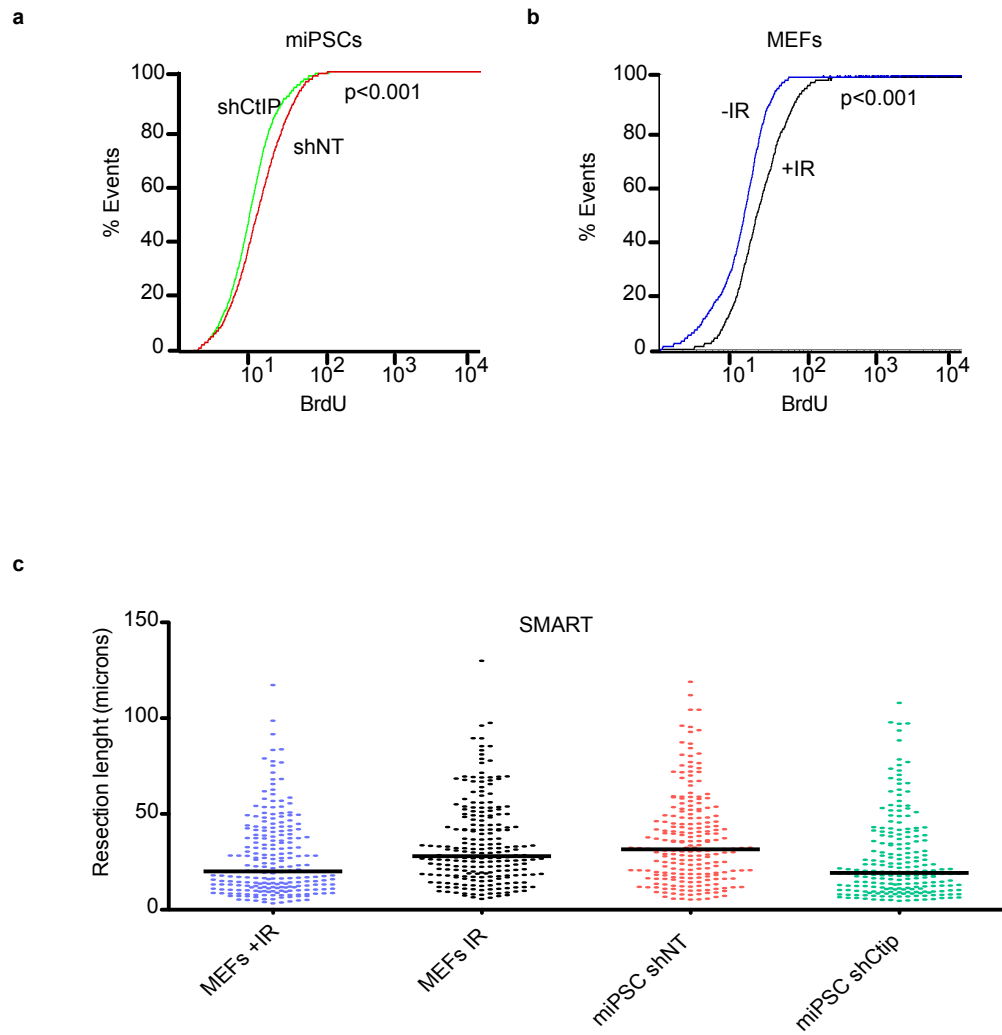


Fig. S1 Gómez-Cabello et al.

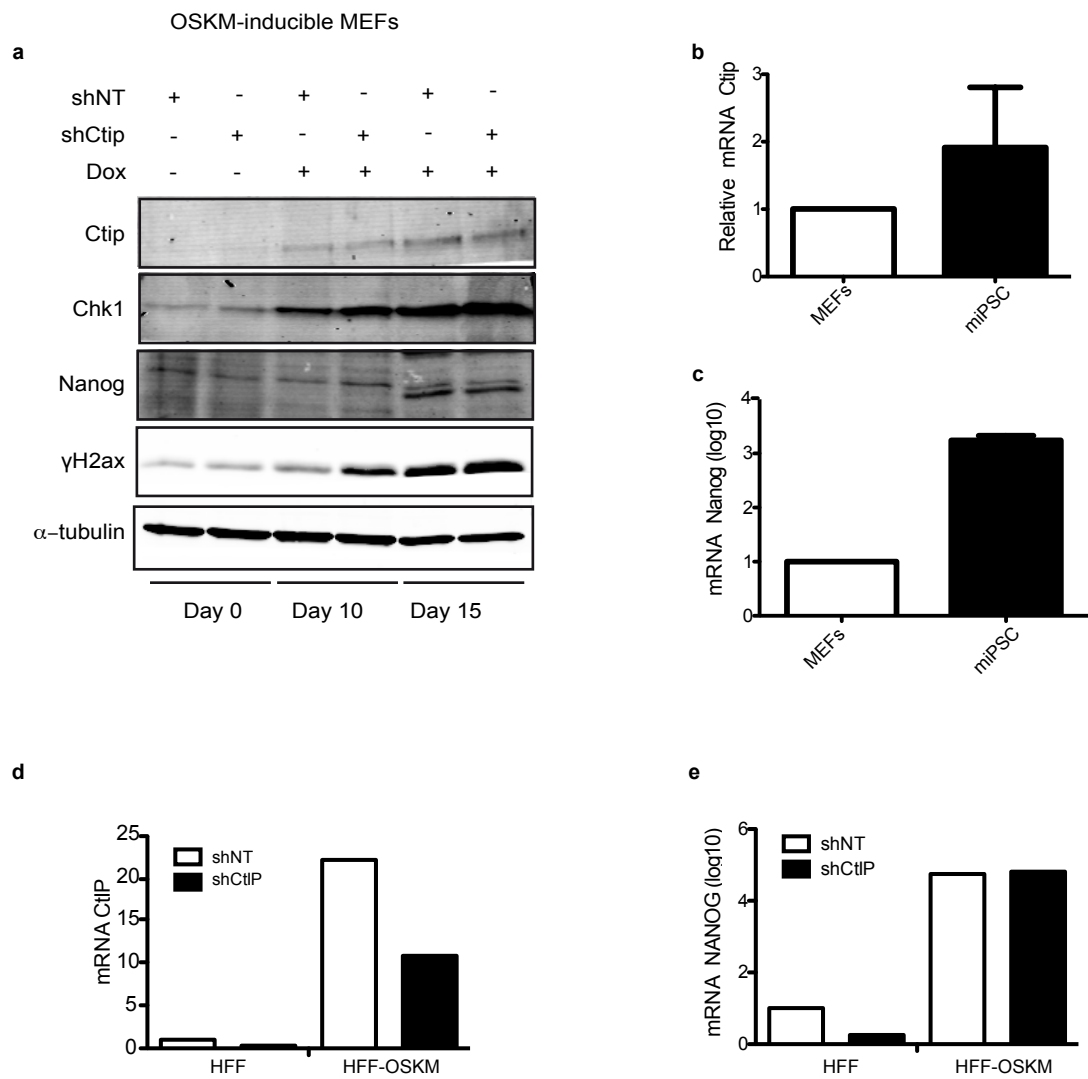
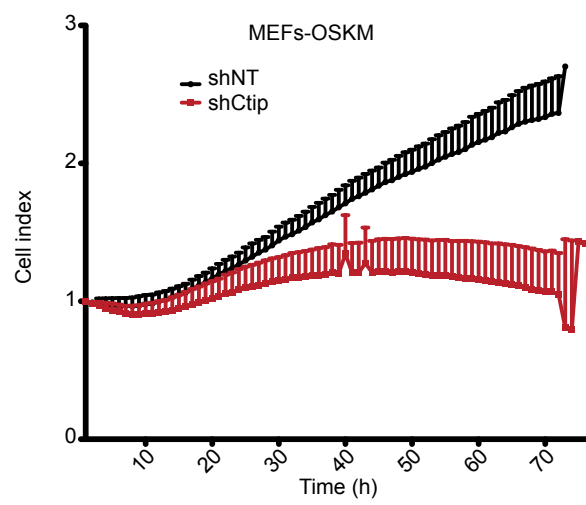


Fig. S2 Gómez-Cabello et al.

a



b

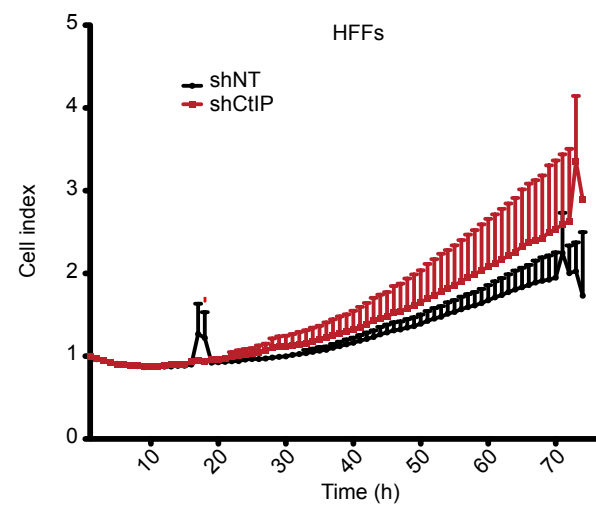


Fig. S3 Gómez-Cabello et al.

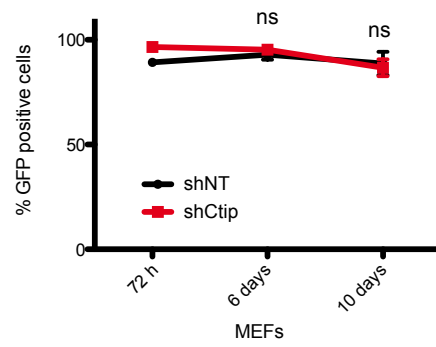


Fig. S4 Gómez-Cabello et al.

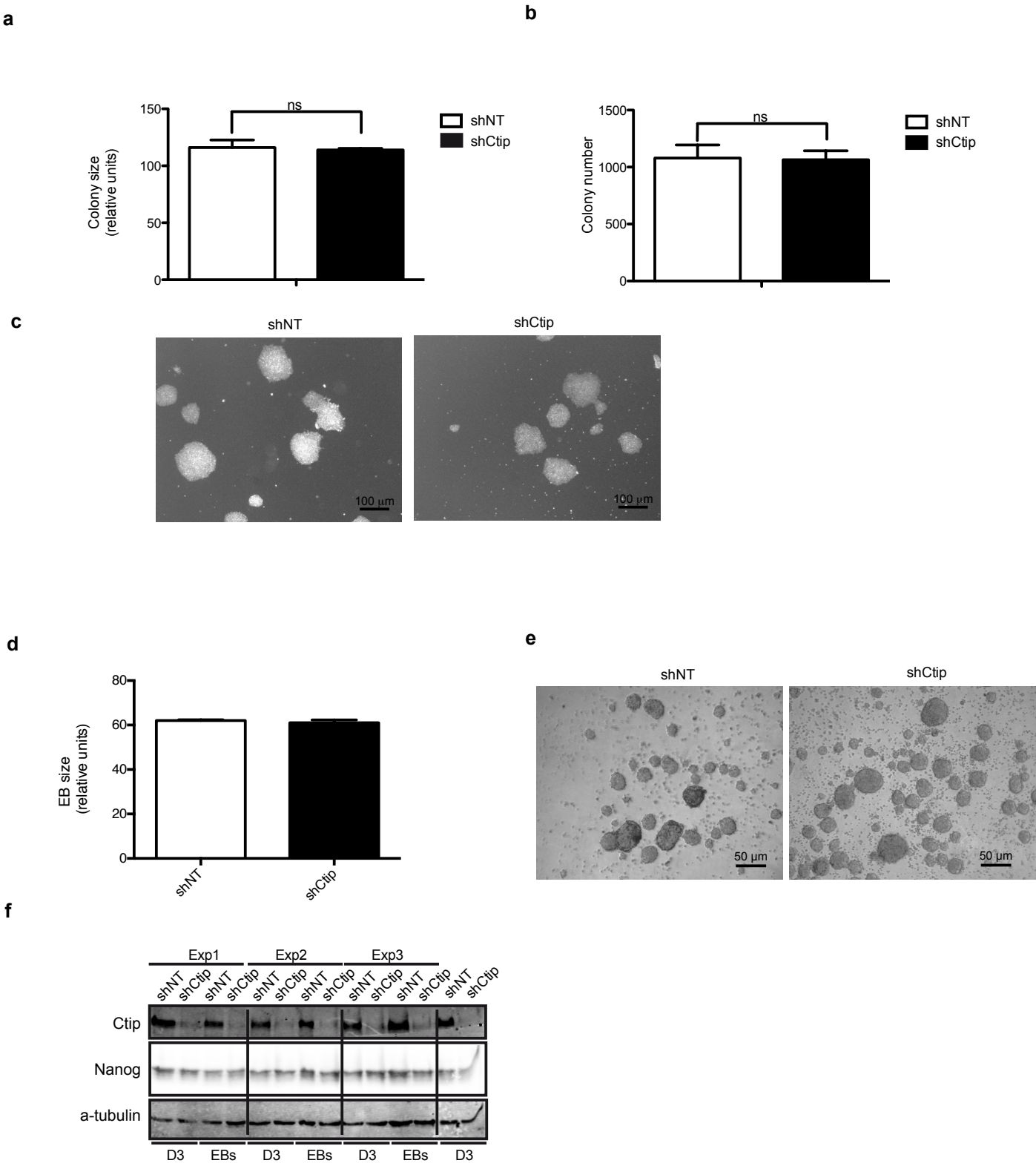


Fig. S5 Gómez-Cabello et al.

Supplemental Figure Legends

Figure S1. DNA end resection in MEFs and mouse iPS cells. Related to Figure 1

(A) FACS analysis of BrdU exposure in ssDNA. iPS cells reprogrammed upon depletion of CtIP or expressing shRNA control were grown in the presence of BrdU (10 μ M) during 24 hours and BrdU exposure was analyzed by FACS. At least three independent experiments were performed. Representative histogram is shown.

(B) Mouse embryonic fibroblasts were treated or not with 10 Gy of ionizing radiation and treated as (a). p value was calculated using Kolmogorov-Smirnov test by CellQuest Pro software. At least three independent experiments were performed. Representative histogram is shown.

(C) SMART assay in MEFs with or without irradiation (10 Gy) and iPS cells reprogrammed in the presence or absence of CtIP protein. At least three independent experiment using different biological samples were performed. Representative histogram is shown.

Figure S2. CtIP expression level increases during mouse and human reprogramming. Related to figures 1, 2 and 3

(A) Immunoblot using antibodies against CtIP, Chk1, γ H2ax and Nanog from whole protein extract of OSKM-inducible MEFs 0, 10 and 15 days after reprogramming induction with doxycycline (1 μ g/ml). At least three independent experiments were performed. Representative western blot is shown.

(B) Relative quantification of mRNA levels of CtIP using qRT-PCR in MEFs and their respective iPS cells. At least three independent experiments with technical triplicate were performed.

(C) Same as (b) for Nanog mRNA.

(D) Same as (b) using human foreskin fibroblast (HFFs) and human iPS cells. (e) same as (c) but in human cells.

Figure S3. Role of CtIP in fibroblast proliferation. Related to figures 1 and 2.

(A) Cell growth in MEFs depleted for CtIP (red) or expressing a control shRNA (black). At least three independent experiments using technical duplicate were performed. Representative growth curve is shown.

(B) Same as (a) but in human primary fibroblasts.

Figure S4. Stability of GFP-shRNA in primary MEFs. Related to figure 4.

Primary MEFs bearing a GFP-shRNA against CtIP (red) or a control sequence (black) were enriched for GFP expression and the remaining percentage of GFP positive cells was measured at the indicated times by FACS. At least three independent experiments were performed.

Figure S5. Role of CtIP in the self-renewal and pluripotency of mouse embryonic stem cells. Related to figures 4 and 5.

(A) Relative colony size formed by D3 embryonic stem cells depleted (black bars) or not (white bars) of CtIP. At least 300 colonies for condition were measured. The average and standard deviation of three independent experiments are plotted. A t-student test was performed to compare both conditions.

(B) The same amount of D3 embryonic stem cells transduced with an shRNA control (white bars) or shCtIP (black bars) were seeded at low density and the number of colonies were measured. The relative number of colonies formed from three independent experiments is plotted. A t-student test was performed to compare both conditions.

(C) Representative image of typical D3 colonies formed in the presence or absence of CtIP.

(D) Average size of embrionic bodies derived from D3 cells expressing a control shRNA or an shRNA against CtIP. Other details as (a).

(E) Representative images of embryonic bodies derived from D3 cells expressing the indicated shRNAs.

(F) Depletion level of CtIP in D3 cells and embryonic bodies derived from D3 in different experiments. At least three independent experiments were performed.

Supplemental experimental procedures.

RNA isolation and quantitative RT-PCR

Total RNA was isolated using RNeasy mini Kit (Quiagen) according to manufacturer's instructions. cDNA was synthesized using QuantiTect Reverse Transcription Kit (Quiagen). Quantitative RT-PCR was carried out using the Universal SYBR Green Supermix (Bio-Rad) in the ViiA 7 Real-Time PCR System. At least three independent experiments in triplicate were performed and the values of gene expression were normalized to the actin housekeeping gene. Primer sequences are shown in table S3.

Proliferation assay

Primary mouse and human cells were plated in 16-well plates to measure proliferation after transduction with shCtIP and shNT particles in The xCELLigence® RTCA DP (ACEA Biosciences). All experiments were performed using technical duplicate for each sample in three independent experiments.

D3 ES

D3 mouse embryonic stem cells were cultured in DMEM supplemented with 15% FBS, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1 mM 2-mercaptoethanol, and 1,000 U/ml mouse leukemia inhibitory factor (LIF, Millipore) (ES media). D3 cells were passaged every 2–3 days to maintain a state of self-renewal. Self-renewal assays were performed plating 2000 D3 cells on 6 well plate by triplicate and counting colonies after 5 days. Several pictures were done in order to measure colony diameter using Adobe Photoshop CS6 (Adobe Systems Incorporated).

Mouse D3 ES growing in 6-well plates were detached with trypsin, counted and replated onto ultra-low attachment 6-well plate with iPES media without LIF for 3-4 days. Embryonic bodies were analysed for size and number through microscopy images using Adobe Photoshop CS6 (Adobe Systems Incorporated).

Table S1

	Plasmid	References
Retrovirus	pCL-Ampho	1
	pMXs-OCT4	2
	pMXs-SOX2	2
	pMXs-KLF4	2
	pMXs-cMYC	2
Lentivirus	p8.91	3
	pCMV-VSVg	3
	pLKO-shNT	Sigma SHC016
	pLKO-shNT-GFP	Sigma SHC005
	pLKO-shCtip-GFP (mouse)	Sigma TRCN0000088050
	pLKO-shCtIP (human)	Sigma TRCN0000005403
	pLKO-IPTG3-xLacO-shNT	Sigma SHC332
	pLKO-IPTG-3xLacO-shCtIP(human)	Sigma TRCN0000005403

Table S2

Antibodies	References	Dilution
BrdU	Amersham-RPN202	1:500
CtIP	R. Baer ⁴	1:500
γH2AX	Millipore- 05-636	1:1000
NANOG	Bethyl Lab –A300-397A	1:500
Tubulin	Sigma T9020	1:40000
OCT4	Santa Cruz Biotech SC-9081	1:500
CHK1	Santa Cruz Biotech SC-8408	1:500
Caspase-3	Novus 31A1067	1:50
Alexa Fluor 647 goat anti-mouse	Invitrogen	1:1000
Alexa Fluor 488 goat anti-rabbit	Invitrogen	1:1000
IR-Dye 680RD goat antimouse IgG (H+L)	Li-Cor	1:5000
IR-Dye 800CW goat antirabbit IgG (H+L)	Li-Cor	1:5000

Table S3

Genes	Forward (5'-3')	Reverse (5'-3')
NanoG mouse	AGCAGATGCAAGAACTCTCCTC CA	CCGCTTGCACTTCATCCTTTGGTT
NANOG human	ACAACTGGCGCAAGAATAGCA	GGTTTCCAGTCGGGTTCAC
Ctip mouse	TTCCTGCTCAAGACACCGATT	CGTCTGAGTAGAAGGAAAACCAACT
CtIP human	AGAAATTTGCTTCCTGCTCAAG	GAAAACCAACTTCCCCAAAATTCTC
ACTIN	ACGAGGCCCAAGAGCAAGA	GACGATGCCGTGCTCTGAT

Supplemental References

- 1 Gomez-Cabello, D. *et al.* Regulation of the microRNA processor DGCR8 by the tumor suppressor ING1. *Cancer Res* **70**, 1866-1874, doi:10.1158/0008-5472.CAN-09-2088 (2010).
- 2 Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872, doi:10.1016/j.cell.2007.11.019 (2007).
- 3 Cruz-Garcia, A., Lopez-Saavedra, A. & Huertas, P. BRCA1 accelerates CtIP-mediated DNA-end resection. *Cell Rep* **9**, 451-459, doi:10.1016/j.celrep.2014.08.076 (2014).
- 4 Sartori, A. A. *et al.* Human CtIP promotes DNA end resection. *Nature* **450**, 509-514, doi:10.1038/nature06337 (2007).