Supplementary Information

Vertebrate CTF18 and DDX11 essential function in cohesion is bypassed by preventing WAPL-mediated cohesin release

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The authors declare no conflicts of interest
Supplemental Table S1. DT40 Cell lines used in this study.

| No. | Strain Name | Genotype | Marker | Reference |
|-----|-------------|----------|--------|-----------|
| I008 | WT          | WT CL18  | -      | (Buerstedde et al., 1991) |
| I500 | WT          | WT + TIR1-9Myc | Bsr | (Kawasumi et al., 2017) |
| R382 | ctf18       | CTF18<sup>+</sup> + TIR1-9Myc | His/His Neo | This study |
| I546 | ctf18-aid   | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> + TIR1-9Myc | His/Bleo Neo | This study |
| I525 | esc01 esco2-aid | ESCO1<sup>-/-</sup> ESCO2<sup>-/-3mAID6FLAG</sup> + TIR1-9Myc | -/-/- Puro/His Bsr | (Kawasumi et al., 2017) |
| I558 | ctf18-aid ddx11 | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> DDX11<sup>+/+</sup> + TIR1-9Myc | Bleo/His Hyg/Puro Bsr | This study |
| R420 | ctf18-aid ddx11 +GFP-DDX11 | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> DDX11<sup>+/+</sup> + TIR1-9Myc +GFP-DDX11 | Bleo/His Hyg/Puro Bsr Neo | This study |
| R422 | ctf18-aid ddx11 +GFP-DDX11-K87R | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> DDX11<sup>+/+</sup> + TIR1-9Myc +GFP-DDX11-K87R | Bleo/His Hyg/Puro Bsr Neo | This study |
| R424 | ctf18-aid ddx11 +GFP-DDX11-KAE | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> DDX11<sup>+/+</sup> + TIR1-9Myc +GFP-DDX11-KAE | Bleo/His Hyg/Puro Bsr Neo | This study |
| I162 | tet-tipin   | TIPIN<sup>+/+</sup> +TetR +tet-TIPIN-FLAG | Puro/Bsr - Hyg | (Hosono et al., 2014) |
| R438 | tet-tipin ctf18 | TIPIN<sup>+/+</sup> CTF18<sup>+/+</sup> +TetR +tet-TIPIN-FLAG | Puro/Bsr His/His - Hyg | This study |
| R245 | ctf18-aid esco2-W615G | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> ESCO2<sup>-/-W615G</sup> + TIR1-9Myc +Cre | His/Bleo Puro/- Bsr Neo | This study |
| R305 | ctf18-aid esco1 | CTF18<sup>2mAIMD6FLAG/3mAID6HA</sup> ESCO1<sup>-/-</sup> + TIR1-9Myc | His/Bleo Puro/Puro/Puro Bsr | This study |
|   | Crosses | Genotypes | Selections |
|---|---------|------------|------------|
| R406 | ctf18-aid ddx11 +ESCO1-FLAG | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> DDX11<sup>-/-</sup> +TIR1-9Myc +ESCO1-FLAG | Bleo/His Puro/Neo |
| R407 | | | |
| R404 | ctf18-aid ddx11 +HA-ESCO2 | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> DDX11<sup>-/-</sup> +TIR1-9Myc +HA-ESCO2 | Bleo/His Puro/Neo |
| R405 | | | |
| I563 | esco2 +ESCO1-FLAG | ESCO2<sup>-/-</sup> +Cre +ESCO1-FLAG | Puro/- Neo/His |
| I122 | ddx11 | DDX11<sup>-/-</sup> | Puro/Bsr |
| I590 | smc3-aid | SMC3<sup>3mAID6FLAG</sup> +TIR1-9Myc | His/Bsr Bsr |
| R011 | smc3-aid +SMC3-3A | SMC3<sup>3mAID6FLAG</sup> +TIR1-9Myc +SMC3-3A | His/Bsr Bsr Neo |
| R029 | smc3-aid +SMC3-HA-AA | SMC3<sup>3mAID6FLAG</sup> +TIR1-9Myc +SMC3-HA-AA | His/Bsr Bsr Neo |
| R442 | smc3-aid ddx11 +SMC3-HA | SMC3<sup>3mAID6FLAG</sup> DDX11<sup>-/-</sup> +TIR1-9Myc +SMC3-HA | His/Bsr Puro/Puro Bsr Neo |
| R443 | | | |
| R444 | smc3-aid ddx11 +SMC3-HA-AA | SMC3<sup>3mAID6FLAG</sup> DDX11<sup>-/-</sup> +TIR1-9Myc +SMC3-HA-AA | His/Bsr Puro/Puro Bsr Neo |
| R445 | | | |
| R269 | ctf18-aid ddx11 wapl-aid | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> DDX11<sup>-/-</sup> WAPL<sup>3mAID6FLAG</sup> +TIR1-9Myc | Bleo/His Puro/Neo Eco/Bsr |
| R270 | | | |
| I548 | wapl-aid | WAPL<sup>3mAID6FLAG</sup> +TIR1-9Myc | Bleo/His Bsr |
| R103 | smc3-aid wapl-aid | SMC3<sup>3mAID6FLAG</sup> WAPL<sup>3mAID6FLAG</sup> +TIR1-9Myc | His/Bsr Eco/Bleo Bsr |
| R149 | smc3-aid wapl-aid +SMC3-HA | SMC3<sup>3mAID6FLAG</sup> WAPL<sup>3mAID6FLAG</sup> +TIR1-9Myc +SMC3-HA | His/Bsr Eco/Bleo Puro |
| Line | Genotypes | Genotypes | Selections | Source |
|------|------------|------------|------------|--------|
| R153 | smc3-aid   | SMC3<sup>3mAID6FLAG</sup> | His/Bsr   | This study |
|      | wapl-aid   | WAPL<sup>3mAID6FLAG</sup> | Eco/Bleo  |        |
|      | +SMC3-HA-QQ| +TIR1-9Myc | Bsr       |        |
|      |            | +SMC3-HA-QQ | Puro      |        |
| R154 | wapl-aid   | SMC3<sup>3mAID6FLAG</sup> | His/Bsr   | This study |
|      | +SMC3-HA-RR| WAPL<sup>3mAID6FLAG</sup> | Eco/Bleo  |        |
|      |            | +TIR1-9Myc | Bsr       |        |
|      |            | +SMC3-HA-RR | Puro      |        |
| R155 | smc3-aid   | SMC3<sup>3mAID6FLAG</sup> | His/Bsr   | This study |
|      | wapl-aid   | WAPL<sup>3mAID6FLAG</sup> | Eco/Bleo  |        |
|      | +SMC3-HA-AA| +TIR1-9Myc | Bsr       |        |
|      |            | +SMC3-HA-AA | Puro      |        |
| R298 | ctf18-aid  | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> | Bleo/His | This study |
|      | ddx11      | DDX11<sup>3mAID6HA</sup> | -/Puro    |        |
|      | wapl-aid   | WAPL<sup>3mAID6FLAG</sup> | -/Neo     |        |
|      | +WAPL-HA   | +TIR1-9Myc | Bsr       |        |
|      |            | +WAPL-HA   | Eco       |        |
| R300 | SMC3-EGFP  | SMC3<sup>EGFP</sup> | Eco       | This study |
| R323 | wapl-aid   | WAPL<sup>3mAID6FLAG</sup> | Bleo/His  | This study |
|      | SMC3-EGFP  | SMC3<sup>EGFP</sup> | Eco       |        |
|      |            | +TIR1-9Myc | Bsr       |        |
| R518 | ctf18-aid  | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> | Bleo/His | This study |
|      | ddx11      | DDX11<sup>-/-</sup> | Hyg/Puro  |        |
|      | SMC3-EGFP  | SMC3<sup>EGFP</sup> | Eco       |        |
|      |            | +TIR1-9Myc | Bsr       |        |
| R519 | ctf18-aid  | CTF18<sup>3mAID6FLAG/3mAID6HA</sup> | Bleo/His | This study |
|      | ddx11      | DDX11<sup>-/-</sup> | -/Puro    |        |
|      | wapl-aid   | WAPL<sup>3mAID6FLAG</sup> | -/Neo     |        |
|      | SMC3-EGFP  | SMC3<sup>EGFP</sup> | Eco       |        |
|      |            | +TIR1-9Myc | Bsr       |        |
### Supplemental Table S2. *Saccharomyces cerevisiae* strains used in this study.

| Strain name | Genotype | Reference |
|-------------|----------|----------|
| HY11043     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ WPL1/wpl1::His3MX6 CHL1/chl1delta::KanMX4 ELG1/elg1delta::HPHMX CTF18/ctf18delta::TRP1 | This study |
| HY11860     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ CTF18/ctf18::KANMX4 CHL1/chl1-K48R-3HA::LEU2 | This study |
| HY11861     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ CTF18/ctf18::TRP1 CHL1/chl1-K48R-3HA::LEU2 | This study |
| HY11044     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ CHL1/chl1delta::KanMX4 CTF18/ctf18delta::TRP1 | This study |
| HY11045     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ WPL1/wpl1::His3MX6 CHL1/chl1delta::KanMX4 CTF18/ctf18delta::TRP1 | This study |
| HY11097     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ wpl1::His3MX6/wpl1::His3MX6 elg1delta::HPHMX/elg1delta::HPHMX CHL1/chl1::KanMX4 CTF18/ctf18::TRP1 | This study |
| HY11935     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ (NatNT2)pADH1-3HA-Scc2/(NatNT2)pADH1-3HA-Scc2 CHL1/chl1::HPHMX CTF18/ctf18::KANMX4 | This study |
| HY11936     | MAT A/alpha ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100 RAD5+ (NatNT2)pADH1-3HA-Scc2/(NatNT2)pADH1-3HA-Scc2 wp1::TRP1/wp1::TRP1 CHL1/chl1::HPHMX CTF18/ctf18::KANMX4 | This study |

### Supplemental Material and Methods

**Growth curves and drug sensitivity assays**

To plot growth curves, each cell line was cultured in three different wells of 24
well-plates at the concentration of $1 \times 10^5$ cells/mL and passaged every 12 h. Cell number was determined by flow cytometry using plastic microbeads (Polysciences, 07313-5). Cell solutions were mixed with the plastic microbead suspension at a ratio of 10:1, and viable cells determined by forward scatter and side scatter were counted when a given number of microbeads were detected by flow cytometry.

For drug sensitivity assay, cells at the density of $1 \times 10^4$ cells/mL were dispensed to 24-well plate 1 mL each, prior to the drug exposure. Subsequently, 2 μL of various drugs diluted 500x by their solvent were added to the plates. After 48 h of incubation at 39.5 °C, 100 μL of cells were transferred to 96-well plates, and mixed with 15 μL of CellTiter-Glo reagent (Promega, G7571). Cells were then mixed at 800 rpm for 30 sec, then analyzed by Victor 3 plate reader (PerkinElmer).

**Plasmid construction and transfection**

To add 3xmAID-6xFLAG tag or 3xmAID-6xHA tag to endogenous CTF18 by FLP-In system, we used p3xmAID-6xFLAG and p3xmAID-6xHA vectors (Kawasumi et al. 2017; Abe et al. 2018). 2-3 kb upstream DNA sequences of stop codons of CTF18 was amplified with the primers 5'-
ATGTGTCGACGTGCTGCAGGCACCGGAACCTCAATG-3' (SalI) and 5'-
ATGGCGGCGCAGCTAGGCCAGGTCCTCGATGTAGAGGTTC-3' (NheI).
The amplified DNA fragment was cloned into p3xmAID-6xFLAG or p3xmAID-
6xHA vectors at SalI and NheI restriction enzyme sites. The CTF18 FLP-In
vectors were then linearized by SalI in the middle of the homology region and
transfected to DT40 cells.

CTF18 KO-His was generated from genomic PCR products combined with L-
hisitidinol selection marker cassette. Genomic DNA sequences were amplified
using primers 5’- aaagttacccgtggcgtgtaagtggagccag (KpnI) -3’ and 5’-
aaagtgcacaagatgcctcactcactcactgc (SalI)
-3’ (for the right arm of the KO construct); and 5’-
aaaactagttcggtgctgggtgcttgaggagaaac (SpeI) -3’ and 5’-
aaagcggccgacaactcactcctcttgaccctc (NotI) -3’ (for the left arm of the KO
construct). Amplified PCR products were purified by gel extraction and cloned
into pLoxP vectors by digesting with SalI-HF and KpnI for the right arm, SpeI and
NotI for the left arm. Briefly, 154 bp of the coding sequence of CTF18 was
replaced by drug resistance marker cassettes, causing deletion of 353-403 amino
acids which include walker A motif and also a frameshift mutation. A gRNA to
introduce a DSB in *CTF18* locus was designed by CRISPR direct 
([https://crispr.dbcls.jp/](https://crispr.dbcls.jp/)). Two phosphorylated oligo DNAs 5’-caccgacgacgcggtacaatg-3’ and 5’-aaaccatttgccggtggtggtc-3’ were annealed, and ligated with px330 cut by BbsI (Cong et al. 2013). *CTF18* knockout vector was then co-transfected with the gRNA expression vector to DT40 cells.

To introduce KAE mutation on chicken DDX11 cDNA for its expression, 5’-TTGCTAAAGCTGAGAGTGACGAGGAAAAGAAAGTGG-3’ and 5’-TCTCAGCTTTTAGCAAGAATCAGCTCCTCCTCG-3’ were used for PCR using pEGFP-GgDDX11-HA (Abe et al. 2016) as template, then PCR product was digested by DpnI. After the digestion, PCR product was purified using QIAquick PCR Purification Kit (QIAGEN, 28104), and used for transformation of competent cells. The construct was digested by AseI prior to the transfection.

To disrupt three alleles of *ESCO1* gene by single round of transfection, we designed a gRNA to introduce a DSB in *ESCO1* locus using CRISPR direct ([https://crispr.dbcls.jp/](https://crispr.dbcls.jp/)). Two phosphorylated oligo DNAs 5’-caccgACTGGCCATTGAGCCGATAT-3’ and 5’-aaacATATCGGCTCAATGGCCAGTc-3’ were annealed, and ligated with px330
cut by BbsI (Cong et al. 2013). *ESCO1* knock out vector was then co-transfected with the gRNA expression vector to DT40 cells.

For *ESCO2* cDNA cloning, we used primers 5’-gactgctagccaccATGTATCCATATGACGTTCCAGATTACGCTGCAGCTGTGAGC TCGCAGAA-3’ (NheI + kozack + HA) and 5’-gactggcgcgccTCAGTTGCCATAAACAAGTTGTAGACG-3’ (Ascl). After digestion by NheI and Ascl, the fragment was cloned into p3xmAID-6xFLAG in which *ESCO2* is placed under the RSV promoter. The plasmid was linearized by ClaI prior to the transfection.

To disrupt two alleles of the *DDX11* gene by single round of transfection, we designed a gRNA to introduce a DSB in *DDX11* locus using CRISPR direct (https://crispr.dbcls.jp/). Two phosphorylated oligo DNAs 5’-caccgAGGCTGAGAAACGATAGTAC-3’ and 5’-aaacGTACTATCGTTTTCTCAGCCTc-3’ were annealed, and ligated with px330 cut by BbsI (Cong et al. 2013). *DDX11* knock out vector was then co-transfected with the gRNA expression vector to DT40 cells.

For WAPL cDNA cloning, we used primers 5’- GCAGATATCGCCAAAATGACATCCCGATTGGAAAG-3’ (EcoRV) and 5’-
AGCCATATGTTAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGTGTTCCAA
GTATTCAATCACCCTAG-3' (Ndel + HA). HA tag was added to WAPL cDNA by PCR.

DDX11 KO-Hyg and DDX11 KO-Puro (Abe et al. 2016), WAPL KO-Eco and WAPL 3xmAID-6xFLAG (Kawasumi et al. 2017) were previously reported. These vectors were transfected to DT40 cells as previously described (Buerstedde and Takeda 1991).

To introduce EGFP-tag at C-terminus of SMC3, SMC3-EGFP FLP-In construct was generated. 3xmAID-6xFLAG + polyA sequence on p3xmAID-6xFLAG-Eco vector (Kawasumi et al. 2017) was replaced with EGFP + polyA sequence by digesting with NheI and BglII to generate pEGFP-Eco vector. SMC3 homology arm was cut out from pSMC3-3xmAID-6xFLAG-Bsr (Kawasumi et al. 2017) and cloned into pEGFP-cFLP-Eco vector by digesting with Nhel and Sall. pSMC3-EGFP-Eco vectors was linearized by AflIII prior to transfection.

**FRAP assay**
Cells were imaged on a confocal Spinning Disk CSU-X system (Olympus) based on Olympus IX83 inverted microscope equipped with Andor iXon 897 Ultra camera and driven by CellSens Dimension 1.18 software (Olympus). The images were acquired with a UPlanSApo 60x/1.35NA oil immersion objective using 488 nm laser line. Photobleaching was performed twice on square-shaped ROIs (2.81 um-wide) with the 405 nm laser line (60 mW diode) at maximum power. Ten images were acquired with 1 s intervals before bleaching, while post bleaching the acquisitions were done every 1 s for 1 min and successively every 2 s for 2 min, for a total duration of 3 min. The cells were maintained at 39.5 C in humified atmosphere and 5% CO₂ during the imaging using an Okolab incubator system.

Signal intensities were measured using ImageJ at bleached, unbleached and background regions. All signals were subjected to background correction. Fluorescence intensity of unbleached and bleached areas was normalized to that of initial pre-bleaching images. Obtained intensities at bleached regions were subtracted from that of unbleached regions, and normalized to the signals from unbleached regions to correct auto-bleaching of GFP signal. Finally, intensity differences were normalized to starting time point (bleaching, t = 0), and set as
100% of normalized intensity difference. More than 40 cells per condition were analyzed and average values were plotted. Error bars represent SEM.

Supplementary References

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Supplementary figures

Figure S1. Establishment of *CTF18* inactivation and its effects on SCC and SMC3 acetylation

(A) Schematic representation of the *CTF18* gene locus and flip-in constructs. Black boxes indicate exons, blue boxes indicate drug resistance markers, and green boxes indicate either 3miniAID-6FLAG-tag or 3miniAID-6HA-tag. (B) Schematic representation of the *CTF18* gene locus and knock out construct. Black boxes indicate exons, blue boxes indicate drug resistance markers, and
orange box indicates locus encoding ATPase site of CTF18. Purple line indicates a guide RNA that targets ATPase site of CTF18. (C) Replication fork velocity was determined by DNA fiber assay for WT and ctf18 cells. More than 50 molecules were analyzed for each condition. Middle line = median; box = 25th and 75th percentiles; bars = 10th and 90th percentiles. P value was calculated by Student’s t-test, and indicated. (D) Sister chromatid cohesion status was assessed for the cells of indicated genotypes. Metaphase chromosomes were classified in three groups depending on the level of cohesion, and more than 100 metaphase cells were analyzed for each genotype with or without Auxin treatment. The results of two independent experiments are plotted. (E) Relative level of acetylated SMC3 in WT and ctf18-aid cells with or without auxin addition measured in whole cell lysates in 3 independent experiments. Mean and SE of the derived ratios are plotted. P values, calculated by Student’s t-test indicate that the difference is not significant.
Figure S2. TIPIN is essential for viability in CTF18 depleted cells.

(A) Relative level of acetylated SMC3 in WT and *ctf18-aid ddx11* cells with or without auxin addition measured in whole cell lysates in 3 independent experiments. Mean and SD of the derived ratios are plotted. (B) Depletion of TIPIN-FLAG was monitored by Western blotting as in Figure 1A. Cells were treated with Dox for 72 h before sampling when indicated. (C) Growth curves of *tet-tipin* and *tet-tipin ctf18* cells as in Figure 1B, with or without Dox treatment. (D) Frequency of lagging chromosomes was scored for the cells of indicated genotypes as in Figure 3C. Cells were treated with Dox for 72 h when indicated.
Figure S3. Genetic interactions between ctf18-aid ddx11 with ESCO1/2 overexpression and smc3-AA allele mutated in acetylated sites.

(A) Frequency of lagging chromosomes at anaphase for the cells of indicated
genotypes was measured as in Figure 3C. (B-C) mRNA level of ESCO2 (B) and ESCO1 (C) were determined by RT-qPCR. esco2+ESCO1 was used as a reference for ESCO1 level enough for covering lack of ESCO2. Error bars represent the SD from three technical replicates. (D) Analysis of acetylated SMC3 versus SMC3 ratios in whole cell lysates, chromatin and nucleoplasmic fractions from WT and ctf18-aid ddx11 cells treated with auxin for 6 hours as in Figure 4G, with or without ESCO1/2 overexpression. Mean and SE derived from 3 independent experiments, along P values calculated by Student’s t-test, are plotted. (E) WT and SMC3-AA variants ectopically expressed in smc3-aid and ddx11 smc3-aid cells. Protein levels of complemented SMC3 variants were monitored by Western blotting using anti-SMC3 or anti-HA antibodies for the cells of indicated genotypes treated with or without auxin. (F) Growth curves of cells of indicated genotypes with or without auxin treatment as in Figure 4B.
Figure S4. Increased DNA damage of mitotic ctf18 ddx11 cells and lack of suppression of ctf18Δ chl1Δ lethality in budding yeast by higher SCC2 expression and/or WPL1 deletion.

(A) Mitotic marker (pH3-S10) and DNA damage marker (γH2AX) were monitored by Western blotting. Wild-type cells treated with methyl methanesulfonate (MMS) were kept as a positive control for DNA damage markers. (B-C) Genetic interactions between ctf18Δ, chl1Δ, wp1Δ and pADH1-3HA-SCC2 were assessed by tetrad dissection analysis of the indicated diploid budding yeast strains.