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Supplemental material

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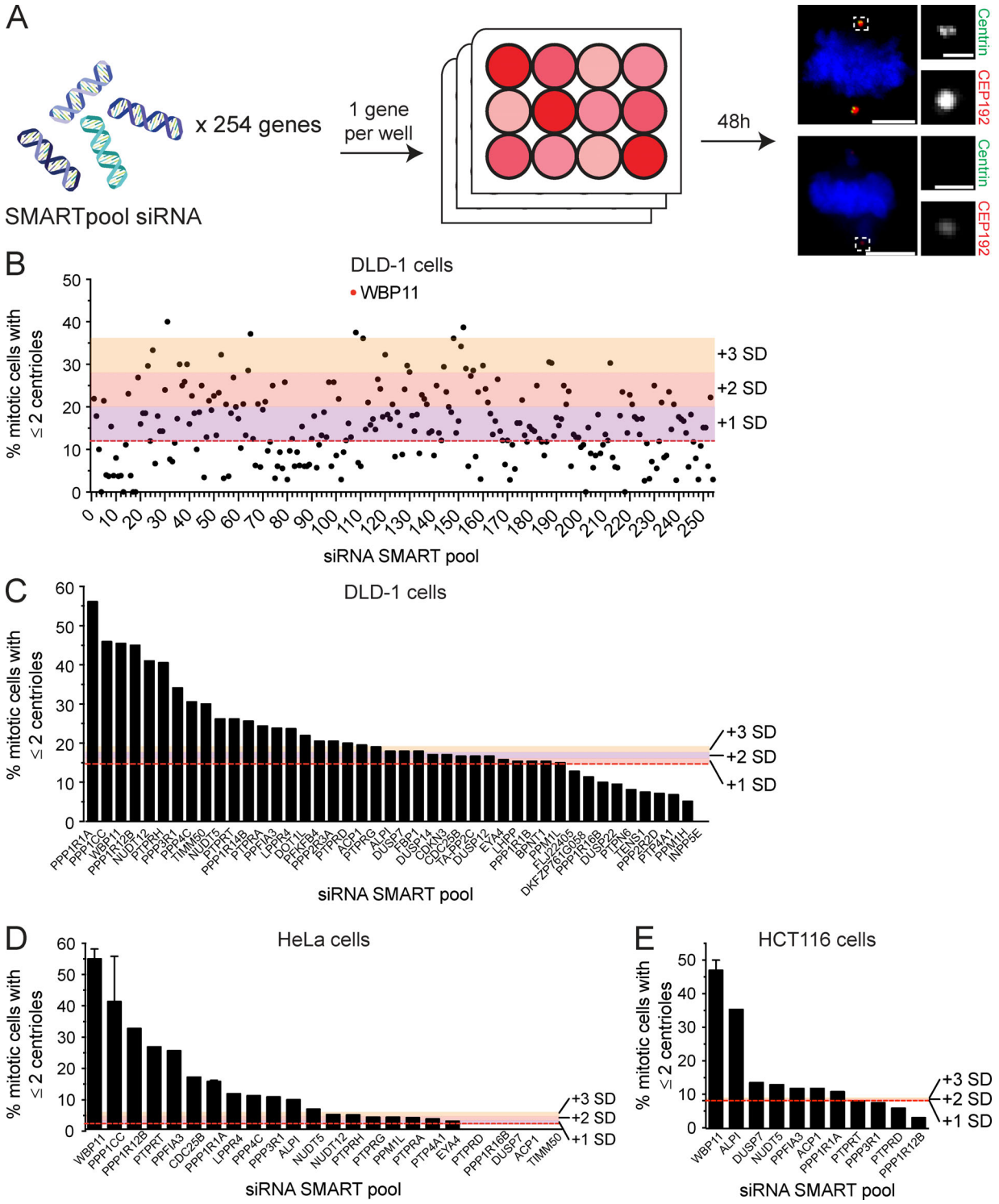


Figure S1. **siRNA screen to identify novel phosphatase or phosphatase-interacting proteins required for centriole biogenesis. (A)** Schematic representation of the screen design. A SMARTpool of four siRNAs targeting each gene was transfected into DLD-1 cells. 48 h after siRNA transfection, centriole number was analyzed in mitotic cells. A total of 254 genes were screened. Scale bars represent 5 µm; 1 µm in inset. **(B)** Graph shows the fraction of cells with two or fewer centrioles in mitosis. Each point represents a single gene. Raw data are displayed in Table S1. Colors indicate one, two, or three SDs above the level of centriole underduplication observed in untransfected DLD-1 cells (red line). Hits were considered as genes that are more than two SDs above the control. More than 25 mitotic cells per siRNA were analyzed. **(C)** Secondary validation of the top hits from the initial screen in DLD-1 cells. Colors indicate one, two, or three SDs above the level of centriole underduplication observed in untransfected DLD-1 cells (red line). n = 1, ≥25 mitotic cells per siRNA were analyzed. **(D)** Validation of the top hits from the initial screen in HeLa cells. Colors indicate one, two, or three SDs above the level of centriole underduplication observed in untransfected DLD-1 cells (red line). $n \geq 1$, ≥25 mitotic cells per siRNA were analyzed. Error bars represent SD. **(E)** Validation of the top hits from the initial screen in HCT116 cells. Colors indicate one, two, or three SDs above the level of centriole underduplication observed in untransfected DLD-1 cells (red line). $n \geq 1$, ≥25 mitotic cells per siRNA were analyzed. Error bars represent SD.

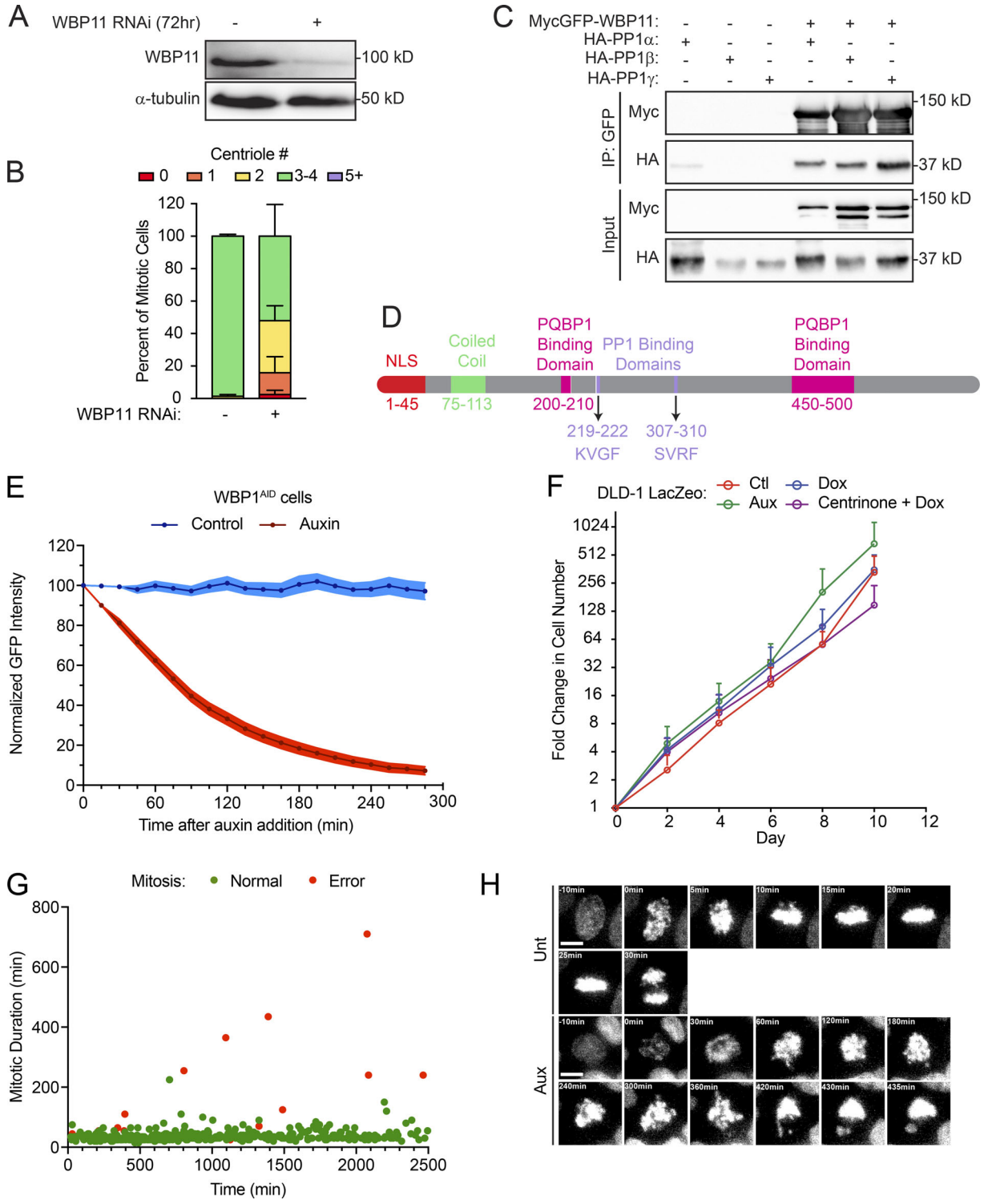


Figure S2. **Cells lacking WBP11 show major growth defects. (A)** Immunoblot showing expression levels of WBP11 72 h after siRNA transfection in RPE-1 cells. **(B)** Quantification of centriole number in mitotic RPE-1 cells 72 h after depletion of WBP11 with SMARTpool siRNA. *n* = 3, ≥50 cells per experiment. Error bars represent SD. **(C)** Immunoblot showing coimmunoprecipitation (IP) of HA-PP1α, β, and γ with MycGFP-WBP11. **(D)** Schematic of WBP11 showing its functional domains and the two PP1 binding sites. **(E)** Quantification of the intensity of the WBP11-mAID-EGFP transgene measured from time-lapse videos of WBP11^{AID} cells after auxin addition. *n* = 3, 20 cells analyzed per point per replicate. Error bars represent SEM. **(F)** Growth assay showing the fold increase in cell number of DLD-1 LacZeo cells treated with tetracycline, auxin, or centrinone. Data are means ± SEM, *n* = 3 (untreated *n* = 2), performed in triplicate. **(G)** Quantification of mitotic duration from time-lapse videos of untreated WBP11^{AID} cells expressing H2B-iRFP. The x axis shows how long after the beginning of filming WBP11^{AID} cells entered into mitosis. Green dots mark cells that completed mitosis normally and red dots mark cells that underwent mitotic errors. *n* = 3, ≥100 cells per experiment. **(H)** Representative frames from videos of WBP11^{AID} cells stably expressing H2B-iRFP. Cells were either untreated or treated with auxin to induce WBP11^{AID} destruction. Scale bars represent 10 µm.

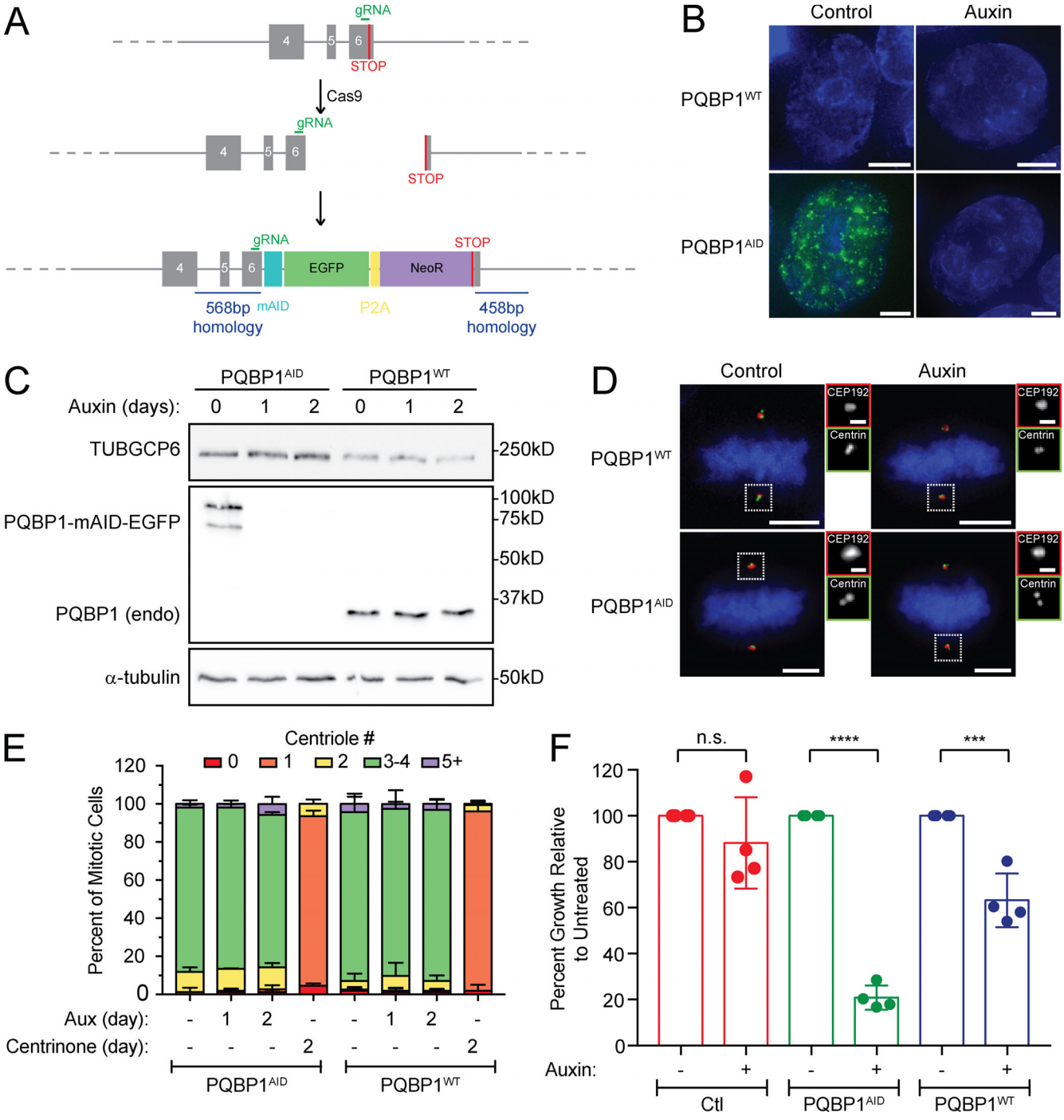


Figure S3. **PQBP1 is not required for centriole biogenesis. (A)** Schematic depicting the strategy for endogenous tagging of the PQBP1 gene. Cells were cotransfected with a plasmid encoding the repair template and a plasmid that expresses Cas9 and an sgRNA. Homozygous PQBP1-AID-EGFP clones were identified by immunoblot. **(B)** Representative images of a control cell line in which PQBP1 was not tagged ($PQBP1^{WT}$) and a cell line in which both PQBP1 alleles were endogenously tagged with mAID-EGFP ($PQBP1^{AID}$). Scale bars represent 5 µm. **(C)** Immunoblot showing expression of endogenously tagged PQBP1-mAID-EGFP in $PQBP1^{AID}$ cells and its degradation after auxin addition. $PQBP1^{WT}$ cells are shown as a control. **(D)** Representative images of control cells ($PQBP1^{WT}$) and cells with endogenously tagged PQBP1-mAID-EGFP ($PQBP1^{AID}$). Cells were either untreated or treated with auxin to induce $PQBP1^{AID}$ destruction. Scale bars represent 5 µm; 1 µm in zoomed-in regions. **(E)** Quantification of centriole number in mitotic cells after 48 h of PQBP1-mAID-EGFP degradation by auxin or PLK4 inhibition by centrinone. $n = 3$, ≥50 cells per experiment. Error bars represent SD. **(F)** Clonogenic growth assay of the $PQBP1^{AID}$ cell line compared with a nontransduced cell line (Ctl) and to a transduced but not tagged cell line ($PQBP1^{WT}$). $n = 4$. Unpaired parametric t test: ***, $P = 0.0007$; ****, $P < 0.0001$. Error bars represent SD.

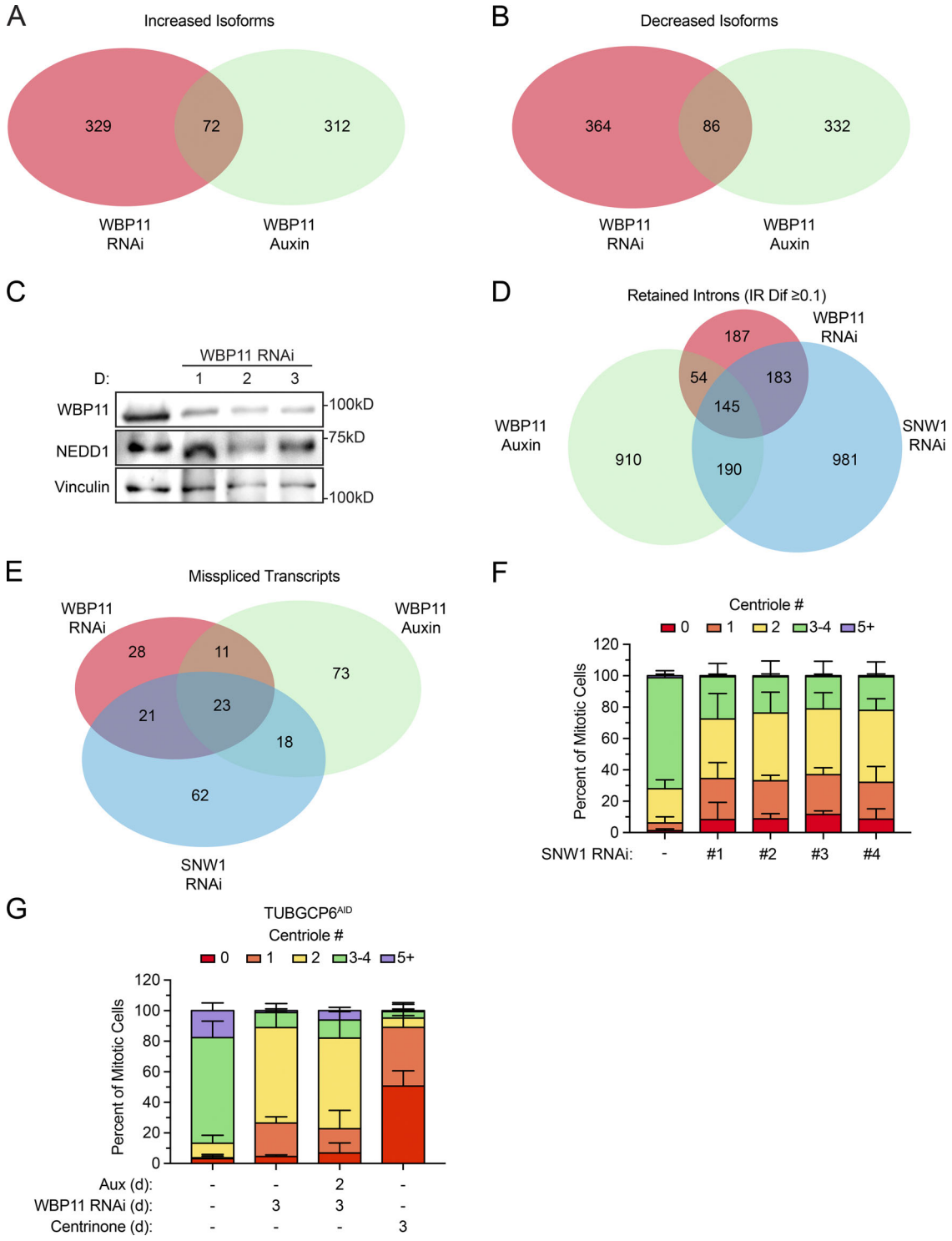


Figure S4. **WBP11 and SNW1 are required for the correct splicing of a common set of pre-mRNAs. (A)** Overlapping hits for isoforms with expression increased more than two SDs following WBP11 or SNW1 RNAi or WBP11 degradation with auxin. A set of 72 isoforms that depend on both WBP11 and SNW1 for appropriate expression were identified. **(B)** Overlapping hits for isoforms with expression decreased more than two SDs following WBP11 or SNW1 RNAi or WBP11 degradation with auxin. A set of 86 isoforms that depend on both WBP11 and SNW1 for appropriate expression were identified. **(C)** Immunoblot showing NEDD1 protein levels after siRNA-mediated depletion of WBP11. **(D)** Overlapping hits for retained introns with an IRDif score of ≥0.1 following WBP11 or SNW1 RNAi or WBP11 degradation with auxin. A set of 145 introns that depend on WBP11 and SNW1 for efficient splicing were identified. **(E)** Overlapping hits for the genes that experienced a greater than three SD decrease of correctly spliced mRNA after WBP11 or SNW1 RNAi or WBP11 degradation with auxin. A set of 23 genes that depend on WBP11 and SNW1 for efficient splicing were identified. **(F)** Quantification of centriole number in mitotic cells 72 h after depletion of SNW1 with one of four independent siRNAs. *n* = 3, ≥50 cells per experiment. Error bars represent SD. **(G)** Quantification of centriole number in mitotic cells 72 h after depletion of WBP11 by siRNA in TUBGCP6^{AID} cells. TUBGCP6^{AID} was degraded by auxin where indicated. *n* = 3, ≥50 cells per experiment. Error bars represent SD.

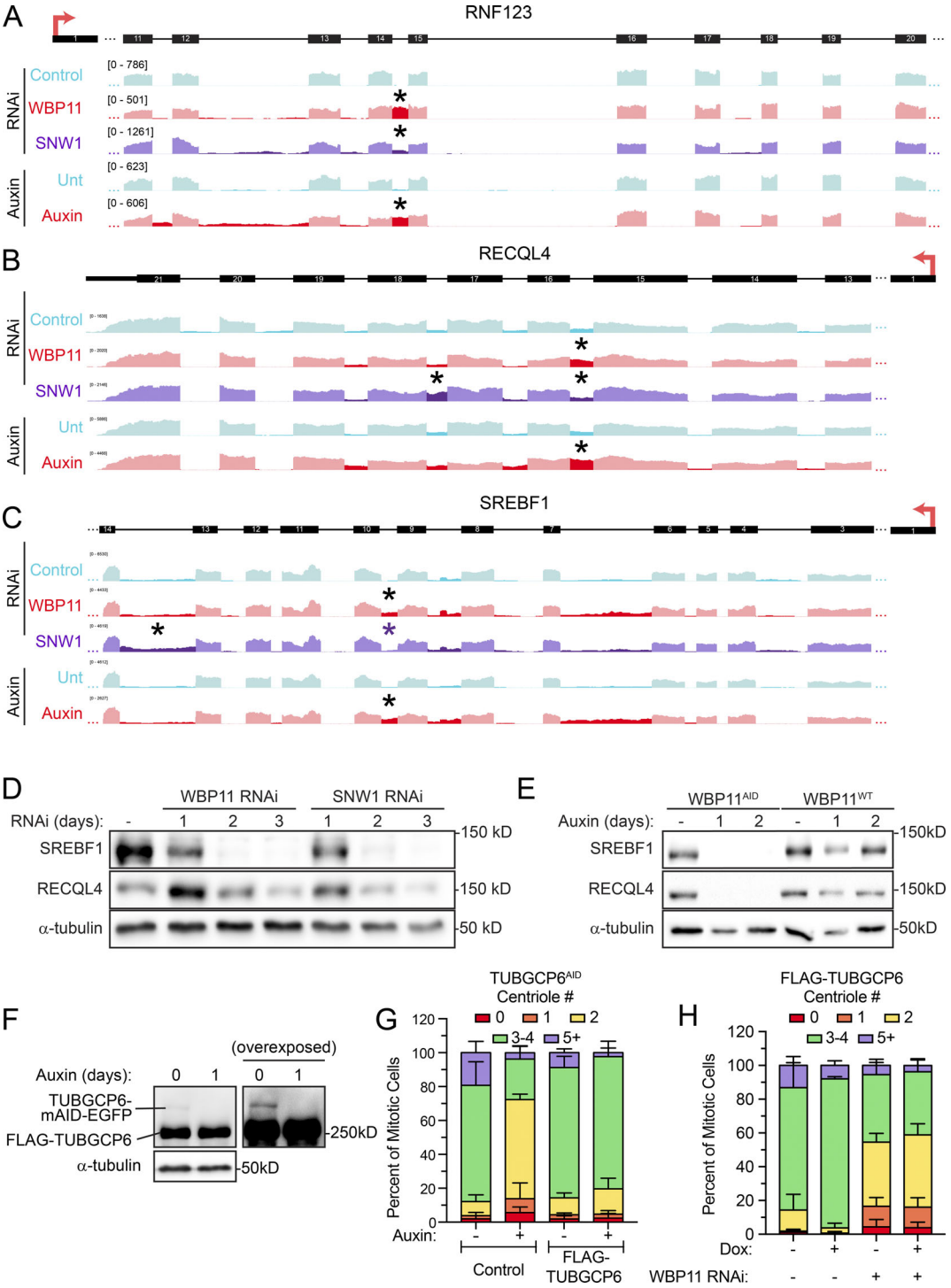
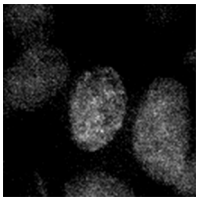
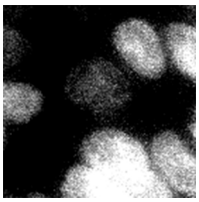


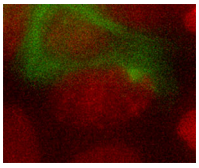
Figure S5. **WBP11 and SNW1 are required for the correct splicing of a subset of introns. (A)** A region of the *RNF123* gene is aligned to IGV reads to highlight retained introns (*) observed after loss of WBP11 or SNW1. *RNF123* requires WBP11 and, to a lesser extent, SNW1 for efficient splicing of one of its introns (*). **(B)** A region of the *RECQL4* gene is aligned to IGV reads to highlight retained introns (*) observed after loss of WBP11 or SNW1. *RECQL4* requires WBP11 and SNW1 for efficient splicing of some introns (*). Note that some introns require SNW1, but not WBP11, for efficient splicing. **(C)** A region of the *SREBF1* gene is aligned to IGV reads to highlight retained introns (*) observed after loss of WBP11 or SNW1. *SREBF1* requires WBP11 and SNW1 for efficient splicing of some introns (*). Note that some introns require SNW1, but not WBP11, for efficient splicing. **(D)** Immunoblot showing expression levels of RECQL4 and SREBF1 after 72 h of SNW1 or WBP11 siRNA knockdown. **(E)** Immunoblot showing expression levels of RECQL4 and SREBF1 in WBP11^{WT} and WBP11^{AID} cells at 48 h after auxin addition. **(F)** Immunoblot showing TUBGCP6 expression in TUBGCP6^{AID} cells expressing a FLAG-TUBGCP6 transgene. **(G)** Quantification of centriole number in mitotic TUBGCP6^{AID} cells 24 h after auxin addition. TUBGCP6^{AID} cells with or without expression of a FLAG-TUBGCP6 transgene were used. Data are shown alongside the TUBGCP6^{AID} data from Fig. 7 C for comparison. *n* = 3, 50 cells per experiment. Error bars represent SD. **(H)** Graph showing the percentage of mitotic cells with three to four centrioles after 72 h of WBP11 depletion by siRNA. Expression of the FLAG-TUBGCP6 was induced by addition of doxycycline where indicated. *n* = 3, 50 cells/experiment. Error bars represent SD.



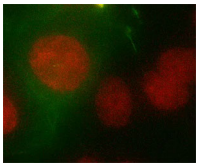
Video 1. **Untreated WBP11^{AID} cell expressing H2B-iRFP.** Still images are represented in Fig. S2 H. One frame captured every 5 min; displayed at 3 frames/s.



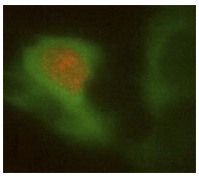
Video 2. **Auxin-treated WBP11^{AID} cell expressing H2B-iRFP.** Still images are represented in Fig. S2 H. One frame captured every 5 min; displayed at 3 frames/s.



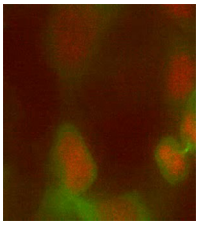
Video 3. **Untreated WBP11^{AID} cell co-expressing H2B-iRFP (red) and RFP-tubulin (green).** Still images are represented in Fig. 2 H. One frame captured every 5 min; displayed at 3 frames/s.



Video 4. **Auxin-treated WBP11^{AID} cell co-expressing H2B-iRFP (red) and RFP-tubulin (green).** Still images are represented in Fig. 2 H. One frame captured every 5 min; displayed at 3 frames/s.



Video 5. **Untreated TUBGCP6^{AID} cell co-expressing H2B-iRFP (red) and RFP-tubulin (green).** Still images are represented in Fig. 7 D. One frame captured every 5 min; displayed at 3 frames/s.



Video 6. **Auxin-treated TUBGCP6^{AID} cell co-expressing H2B-iRFP (red) and RFP-tubulin (green).** Still images are represented in Fig. 7 D. One frame captured every 5 min; displayed at 3 frames/s.

Table S1 lists phosphatases and their associated proteins that were knocked down in the siRNA screen. The first tab shows the siRNA sequences used to knockdown each protein. A SMARTpool of four siRNAs per gene was used for each knockdown. The second tab shows the percentage of mitotic cells with the indicated number of centrioles for each gene that was knocked down in the primary screen. Tabs three to five show the secondary screen of the top hits from the primary data, performed in DLD-1, HeLa, and HCT116 cells.

Table S2 shows proteins identified in the WBP11 proximity interactome. The first four tabs show four independent experiments of the WBP11-BirA proximity-dependent biotinylation. Proteins found to be biotinylated in the WBP11-BirA sample and control FLAG-BirA sample are indicated in the table, along with the number of peptides identified. Proteins with and an enrichment ratio greater than five are highlighted in yellow. The final tab lists proteins that were found in at least three of the four biological replicates with an enrichment ratio greater than five.

Table S3 provides the differential expression analysis used to determine the degree to which a transcript is downregulated following protein depletion. Each tab of the table relates to cells depleted of WBP11 by RNAi for 72 h, cells depleted of WBP11 by auxin for 48 h, or cells depleted of SNW1 by RNAi for 72 h. The fold change in mRNA expression level was calculated using EBSeq PostFC, which takes into consideration both the raw fold change and the total number of reads. Proteins highlighted in red and blue are upregulated or downregulated by more than two SDs, respectively. The final tab shows those genes in which there was a greater than two SD decrease in expression in all three data sets.

Table S4 provides the differential expression analysis used to determine the degree to which isoform expression is altered following protein depletion. The first three tabs of the table relates to cells depleted of WBP11 by RNAi for 72 h, cells depleted of WBP11 by auxin for 48 h, or cells depleted of SNW1 by RNAi for 72 h. The accession number correlating to each isoform is shown next to the gene name. The expected counts and fold change in mRNA expression level were calculated using EBSeq PostFC. The total reads columns (H and I) are a summation of the reads for all isoforms of each gene. The proportion (J and K) was calculated by dividing the expected read counts of the indicated isoform (C and D) by the total number of read counts for that gene (H and I), and corresponds to the proportion of total reads for the gene that are accounted for by the indicated isoform. ΔIsoform is the difference between experimental and control proportions. Gene change is the total number of reads in the experimental condition over the control condition (H over I). Isoforms highlighted in red and blue have a ΔIsoform value that is upregulated or downregulated by more than two SDs, respectively. The final tab shows those isoforms in which there was a more than two SD change of mRNA expression after WBP11 RNAi or WBP11 degradation with auxin.

Table S5 shows RNA-seq analyses using IRFinder identified introns that are selectively retained following WBP11 or SNW1 depletion. The first three tabs show RNA-seq analysis for cells depleted of WBP11 by RNAi for 72 h, cells depleted of WBP11 by auxin for 48 h, or cells depleted of SNW1 by RNAi for 72 h. The fourth tab shows introns retained in all three samples. The introns are sorted by increasing amount retained in the experimental condition, as calculated by IRDif. Condition A is the depleted sample, and condition B is the control. The IRratio refers to the percentage of transcripts in each condition that contains the indicated intron. IRDif was calculated as described in the text by subtracting the IRratio of the control (B-IRratio) from the IRratio of the depleted sample (A-IRratio). We then used this difference in the percentage of unspliced transcripts to calculate the probability that a particular intron will be correctly spliced under the indicated experimental conditions (Probability_Correct), which is equal to one minus the IRDif for that intron. Finally, MAXENT refers to the splice site strength calculated using MaxEntScan (Christopher Burge Laboratory). Retained introns with an IRDif of ≥0.1 are highlighted in red, while introns that show increased splicing with an IRDif score of −0.1 or less are highlighted in blue.

Table S6 shows RNA-seq analyses used to determine the degree to which a transcript is misspliced following protein depletion. Each tab of the table relates to cells depleted of WBP11 by RNAi for 72 h, cells depleted of WBP11 by auxin for 48 h, or cells depleted of SNW1 by RNAi for 72 h. The percentage change in fraction of spliced mRNA was calculated using the IRDif for each intron within a transcript, as described in Materials and methods. The final tab shows those genes in which there was a greater than three SD decrease of correctly spliced mRNA after WBP11 or SNW1 RNAi or WBP11 degradation with auxin.

Two data files are provided online. The first data file contains the source data for the figures. The second data file contains information about the statistical tests that were run and their results.

Reference

Christopher Burge Laboratory. MaxEntScan:score5ss for human 5′ splice sites. Available at: http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html (accessed November 14, 2018).