SUPPLEMENTARY FIGURE LEGENDS

Figure S1. USP44 loss leads to chromosome missegregation. A. Genotypes obtained from the indicated numbers of pups at day of life (DOL) 10, or embryonic day (ED) 13.5. **B.** Male mice of the indicated genotypes (n = 5-9 each) were monitored from 2-8 weeks of life. The graph represents the mean +/– SEM at each time point. **C.** Representative Southern blot from targeted ES cell clones showing the expected pattern for *Usp44*$^{+/+}$ and *Usp44*$^{+/H}$. **D.** PCR-based genotyping was performed on tail DNA showing results for the wild-type, hypomorphic and null alleles. **E.** RT-PCR was performed using *Usp44* exon 1-specific, or control (Tata Binding Protein; TBP) primers on RNA extracted from MEFs of the indicated genotype. **F.** DNA sequencing of tail DNA from a *Usp44* null mouse showing continuous sequence from the region 5' to exon 1 directly connected to the expected sequence 3' of exon 1. The *lox*P sites are shown as red triangles, the FRT sites as blue triangles. The 5' *lox*P sequence is shown in italics.

Figure S2. Potential impact of a truncated USP44. A. (Upper) Nucleotide sequence of the potential cDNA encoded by exon 2-5 of the *Usp44* gene. Potential ATG codons are in italics, with the first in-frame ATG in bold-italics and the following open reading frame (ORF) underlined. (Lower) translation of the ORF with an engineered N-terminal HA tag underlined. **B.** Expression levels of the various constructs used in this study. Exp = exposure. **C.** Live-cell microscopy of wild-type MEFs trandsduced or not with USP44^{C25}HA, the potential C-terminal truncated protein in (A). Graph represents the mean +/- SEM of three independent experiments for each condition.

Figure S3. Reduction in MAD2 bound to APC^{CDC20} in *Usp44* null MEFs. A. Representative experiment in which MEFs of the indicated genotypes were subjected to immunoprecipitation with the indicated antibodies. Precipitates were then immunoblotted for the indicated proteins. **B.** Quantitation of MAD2 levels (normalized to CDC27) from four independent Cdc20 immunoprecipitations as in A. *$p<0.05$ using unpaired t-test.

Figure S4. Stability of mitotic regulators in *Usp44* null MEFs. A. MEFs of the indicated genotype were arrested in G0/1 by serum starvation, followed by release into complete medium. Nocodazole was added to cultures 23 hours after release. Cells were harvested at the indicated time-points after release and immunoblotted for the indicated antibodies. Results are representative of at least three independent MEF lines per genotype. PH3 = phospho-histone H3 (Ser10). **B.** Extracts from mitotic MEFs (shake-off) of the indicated genotypes were immunoblotted using the indicated antibodies. **C.** Extracts from asynchronous MEFs (n = 2 each) of the indicated genotype were immunoblotted with the indicated antibodies. **D.** MEFs of the indicated genotypes were transduced to express H2B-YFP and were then monitored by live cell imaging. The graph represents the mean +/– SEM of three lines per genotype. N = 72 – 91 total cells per condition from three lines. **E.** Chromosome counts were performed on MEFs of the indicated genotypes. The graph represents the mean +/– SEM for three lines per genotype, n = 50 cells per line.

Figure S5. Cyclin B1 is degraded normally in the absence of USP44. A. Measurement of cyclin B1 was performed in MEFs of the indicated genotypes by confocal immunofluoresescent microscopy. Fluorescent images were quantitated using imageJ. The graph represents the mean

+/- SEM at each phase from three lines per genotype. N = 10 cells per phase from each line (total = 30 cells per phase, per genotype). **B.** Live-cell microscopy was performed on MEFs of the indicated genotype. MEFs were transduced with H2B-YFP to monitor chromosomes, and were nucleofected with plasmid DNA encoding cyclin B1venus. Cells in G2 were marked and imaged every 3 minutes through anaphase. The intensity of cyclin-B1^{venus} was quantitated using imageJ. The graph represents the mean +/– SEM. N = 17-25 cells from two lines per genotype.

Figure S6. Aurora B is not perturbed in *Usp44* null MEFs. A. MEFs of the indicated genotype were arrested in G0/1 by serum starvation and were released into complete medium. Nocodazole was added at 23 hours following release. Cells were harvested at the indicated time-points and samples were immunoblotted for the indicated proteins. Results are representative of at least three independent MEF lines per genotype. PH3 = phospho-histone H3 (Ser10). **B.** MEFs of the indicated genotype were imaged using confocal immunofluorescence using the indicated antibodies. Results are representative of at least three independent MEF lines per genotype. Scale bar = 5 μm.

Figure S7. Characterization of spindle defects seen in *Usp44* null MEFs. A. Spindle angles (the angle formed at the intersection of a line between the centrosomes and one parallel to the metaphase plate) were determined on images captured by confocal immunofluorescence on MEFs of the indicated genotypes stained for γ-tubulin and Hoechst to visualize DNA. N = 33-34 per genotype from three independent MEF lines each. P-value calculated with the unpaired t test. **B.** Incidence of spindle angles < 82 degrees in cells from (A). P-value calculated with the Fisher's exact test. **C.** Incidence of normal or abnormal chromosome segregation events in MEFs

(N = 58 wild-type and 60 *Usp44* null) expressing H2B-RFP and α-tubulin-GFP as viewed by live-cell microscopy. **D.** Example of a cell over-expressing $USP44^{Cherry}$ with excessive and disordered centrin 2/3 signals at the centrosome. The scale Bar in the main images = 5 μm. Scale bar in inset image = 1 μm.

Figure S8. USP44 interacts with centrin through a highly conserved binding domain. A. (upper) Previously published consensus centrin binding domains (CBDs) from XPC of various species, and from the 33 CBDs of human SFI1. (lower) Domain map of mouse Usp44 showing the zinc-finger (ZnF-Ubp), ubiquitin carboxy-terminal hydrolase (UCH), and two putative centrin binding domains (CBD). The CBD sequences from the indicated vertebrate genomes are shown. The residues in red are the consensus residues from the XPC and SFI1 CBDs, with the asterisk representing the essential tryptophan residue. **B.** Sequence logo (weblogo.berkeley.edu/) including all 33 CBDs of human SFI1, XPC from the 10 species listed in (A) (in reverse sequence direction to align with Sfi1 and USP44). **C.** As in (B) but with USP44 CBD1 sequences from the 10 species in (A).

Figure S9. USP44 activity towards di-ubiquitin chains of various lysine-linkages. A, B. $USP44^{WT}$HA, $USP44^{C281A}$HA (catalytic mutant), or $USP44^{W162A}$HA (centrin binding mutant) were immunoprecipitated from stably transduced MEFs and incubated with the indicated di-Ub chains. The reactions were terminated after 60 minutes at room temperature, and samples were immunoblotted for Ub or HA. The image represents typical results from three independent experiments.

Figure S10. Aneuploidy is common in human and mouse lung tumors. A. Interphase FISH was performed on the paraffin-embedded formalin-fixed slides from the indicated mouse tissues using probes for chromosome 4 and 7. The number of cells with the indicated number of signals for each chromosome is shown. **B.** Interphase FISH probing for chromosomes 7 and 8 was performed on the indicated tumors with normal (NL) or reduced (L) *USP44* mRNA compared with adjacent normal tissue. FISH was also performed on the adjacent normal lung tissue as an internal control. Where indicated with an "X", normal tissue was not evaluable due to insufficient amounts normal tissue on slides.

Figure S1. Zhang et al.

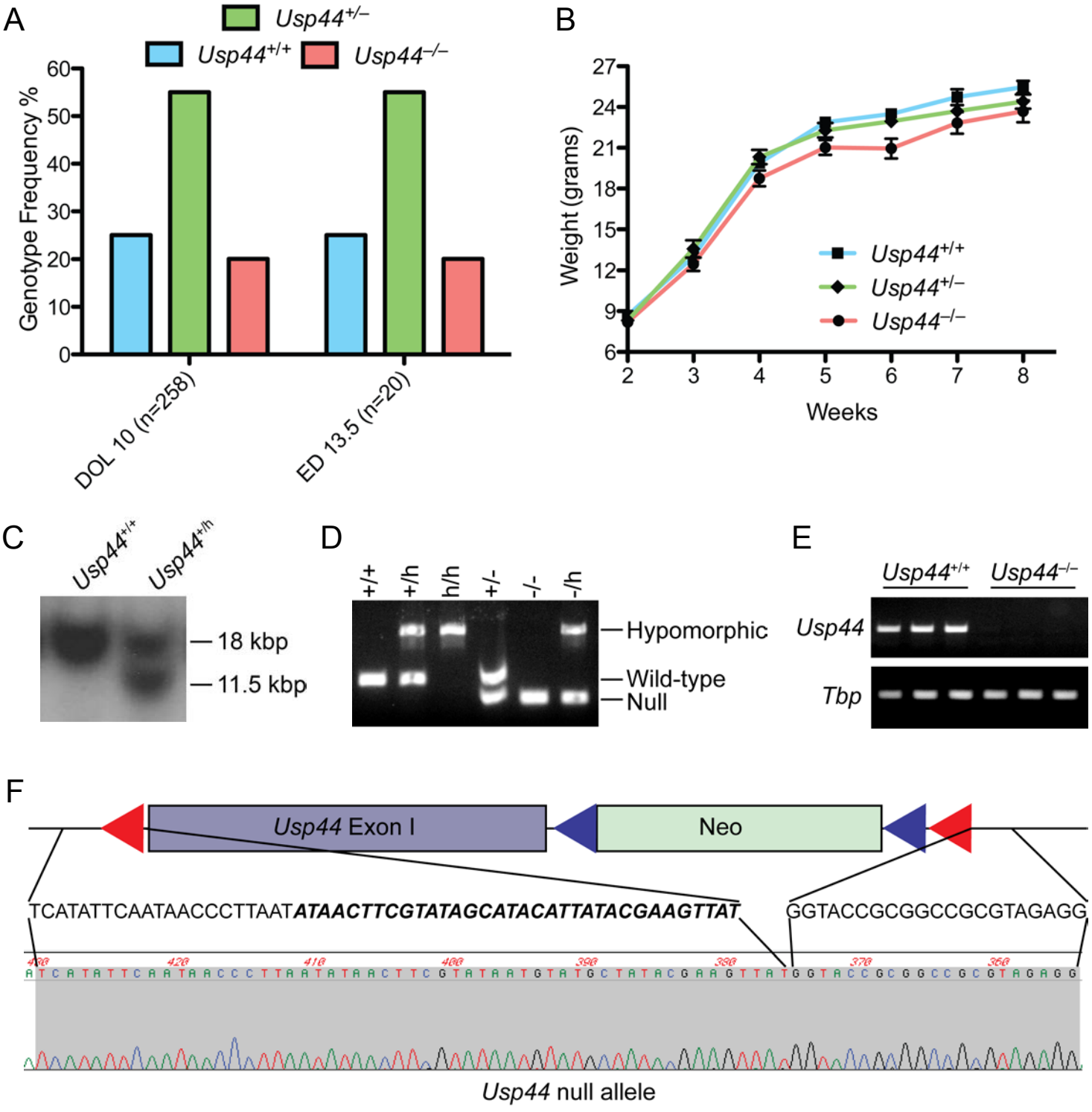

Figure S2. Zhang et al.

A

EXON 2-5

GTTAC*ATG*TCTTGCTTGTGACAACAAATCAGATACCATAGAATCCTTCTGGGACTTGTCCCTGGAGTTTCCAGAGAGATACC
AGTGCAGTGGGAAAG*ATG*CTGCCTCCCAGCCGTGCCTGGTTACTGAC***ATG***TTGGACAAATTTACAGAAACAGAAGCTTTAGA
AGGGAAAATCTACATGTGTGACCATTGTAACCGTAGAAAGTTTTCGTCAAAATCAGTTGTATTCACAGAAGCCCAGAAGCAG
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CTTATATCTTATTTTATACTCAGCGAGTTACTGAGAATGGACATTCGAAACTCTTGCCTCCCGAGCTCCTGTCCAATAGCCA
GCACCCCAGTAAGGAGACTGATGCCTCTTCTAATGAAGTCCTTAGCTGA

TRANSLATION

M*YPYDVPDYA*LDKFTETEALEGKIYMCDHCNRRKFSSKSVVFTEAQKQLMICHLPQVLRLHLKRFRWSGRNNREKIGVHVVF
EETLNMEPYCCRETLNALRPECFLYNLSAVVIHHGKGFGSGHYTAYCYNSEGGFWVHCNDSKLSMCTMEEVRKAQAYILFYT
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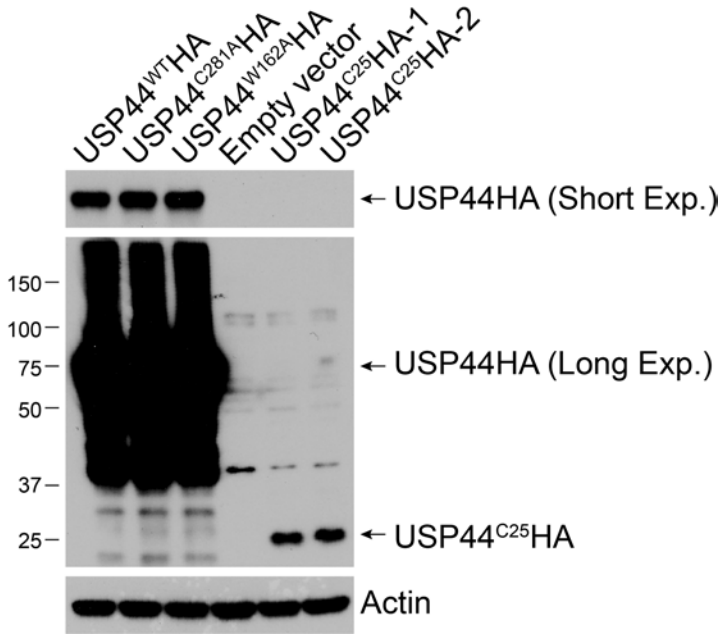
B



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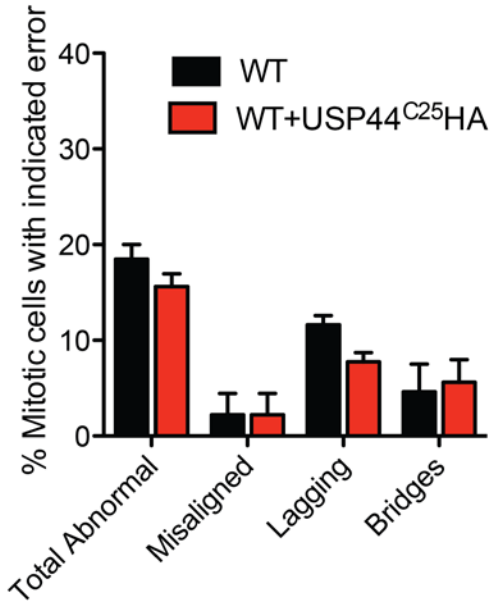

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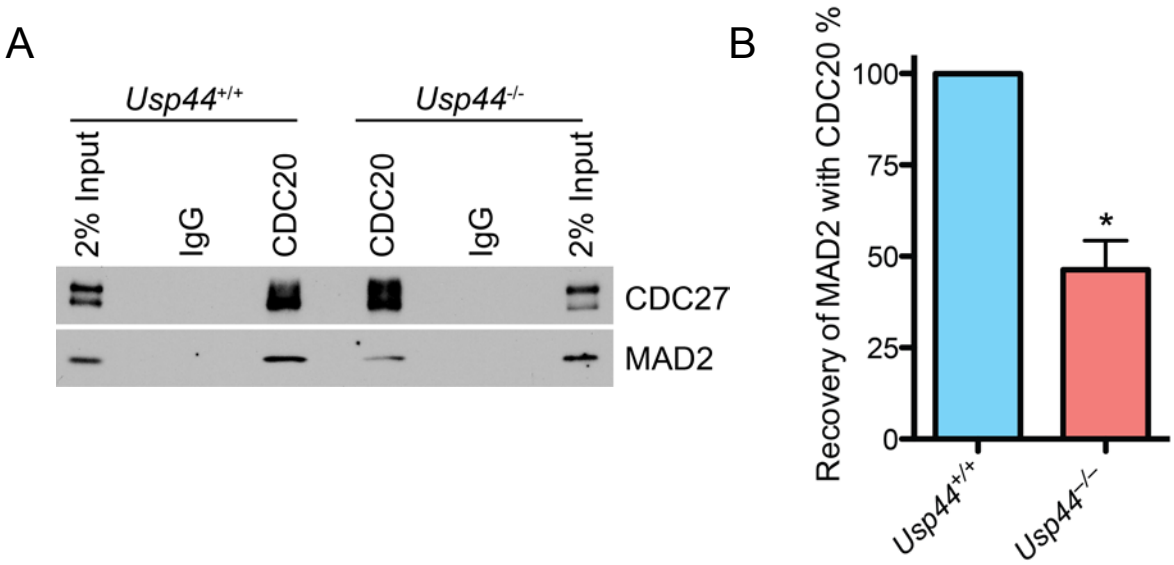
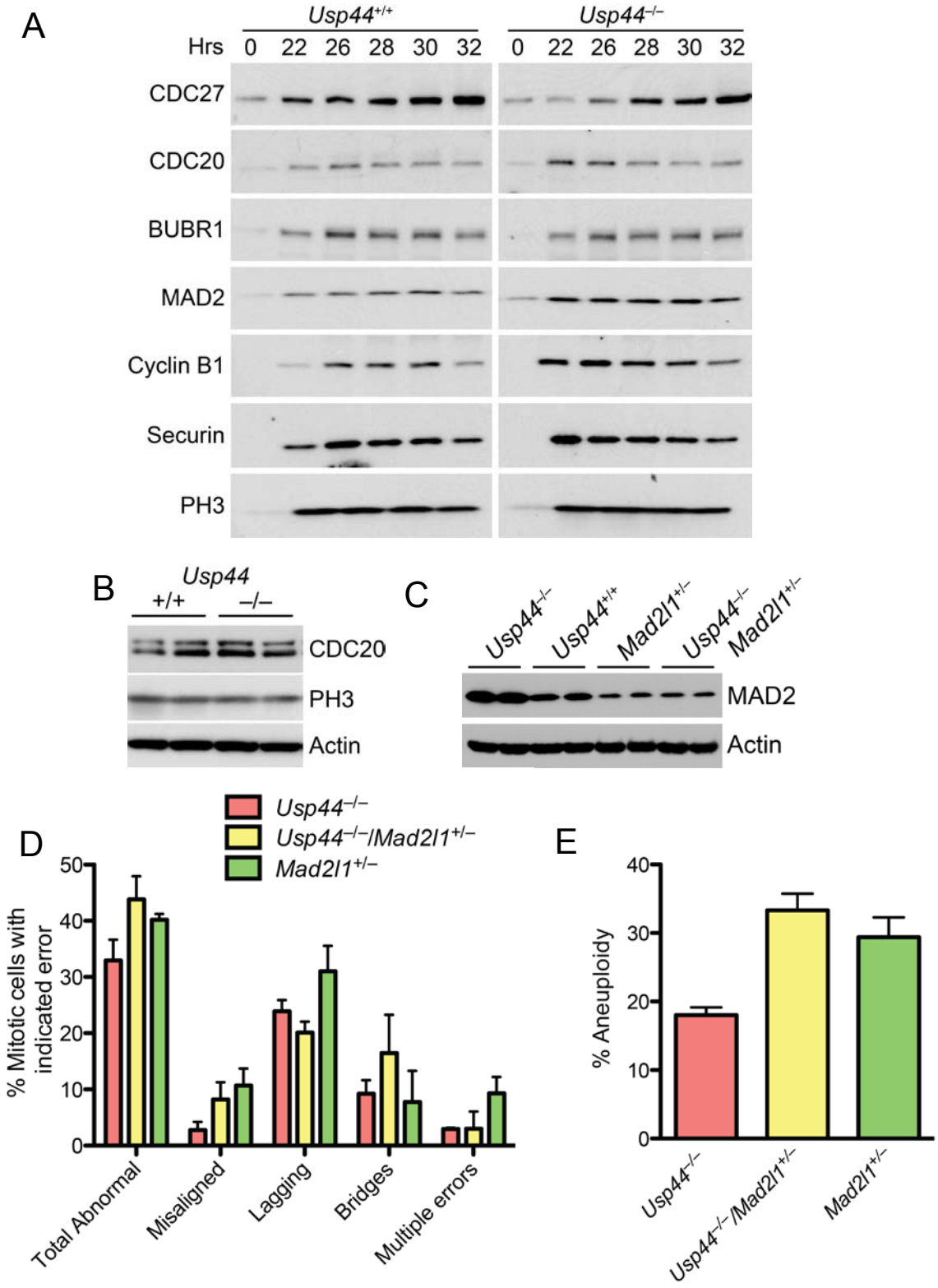


Figure S4. Zhang et al.



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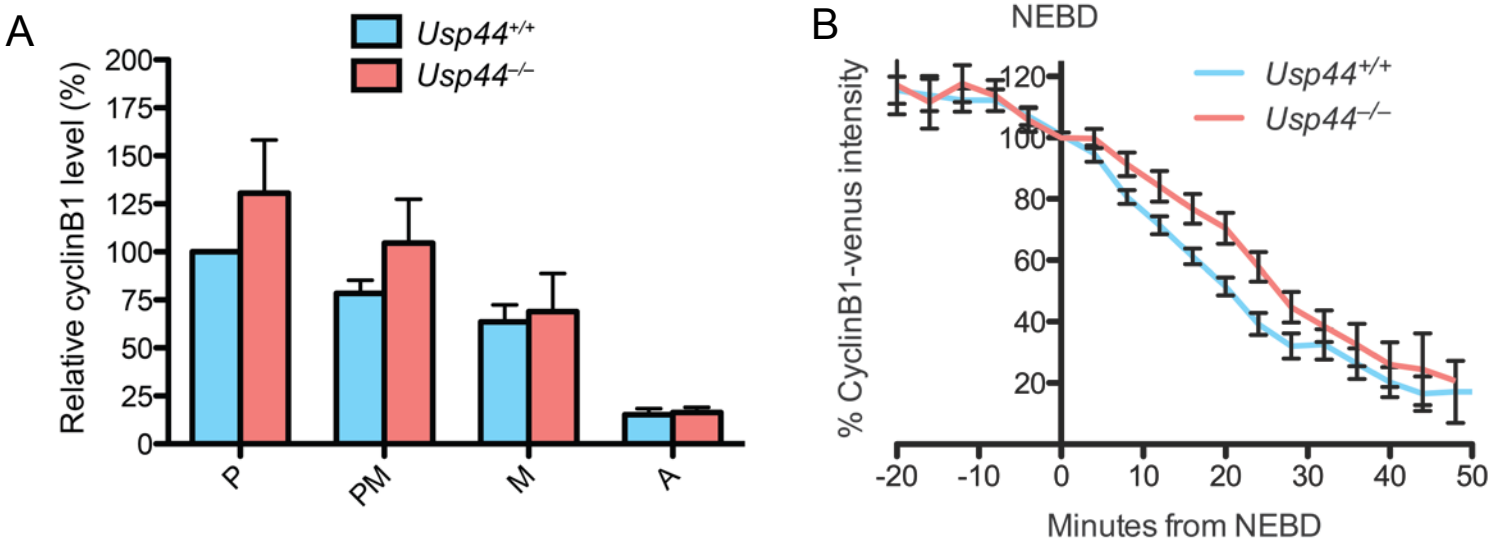
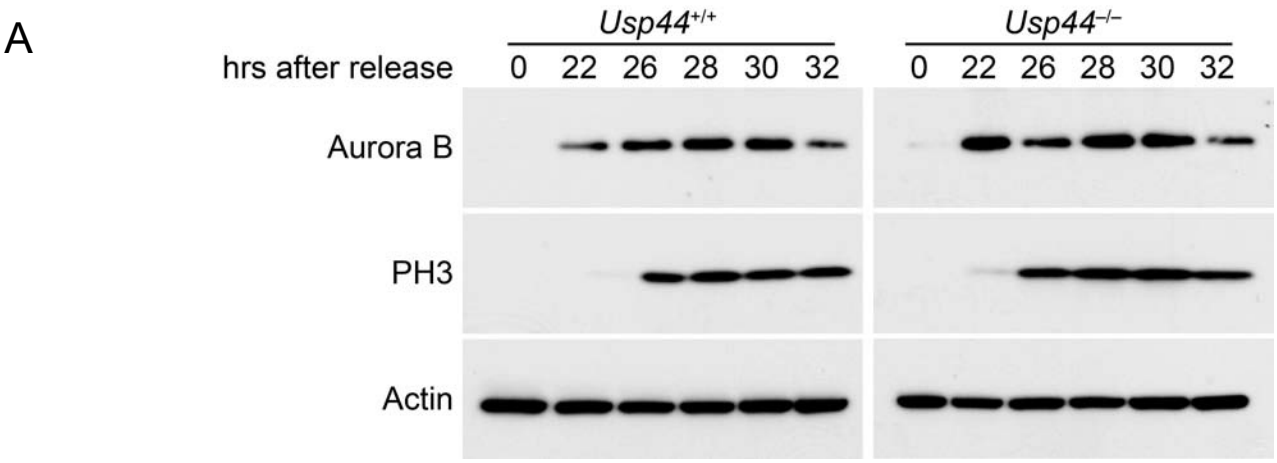


Figure S6. Zhang et al.



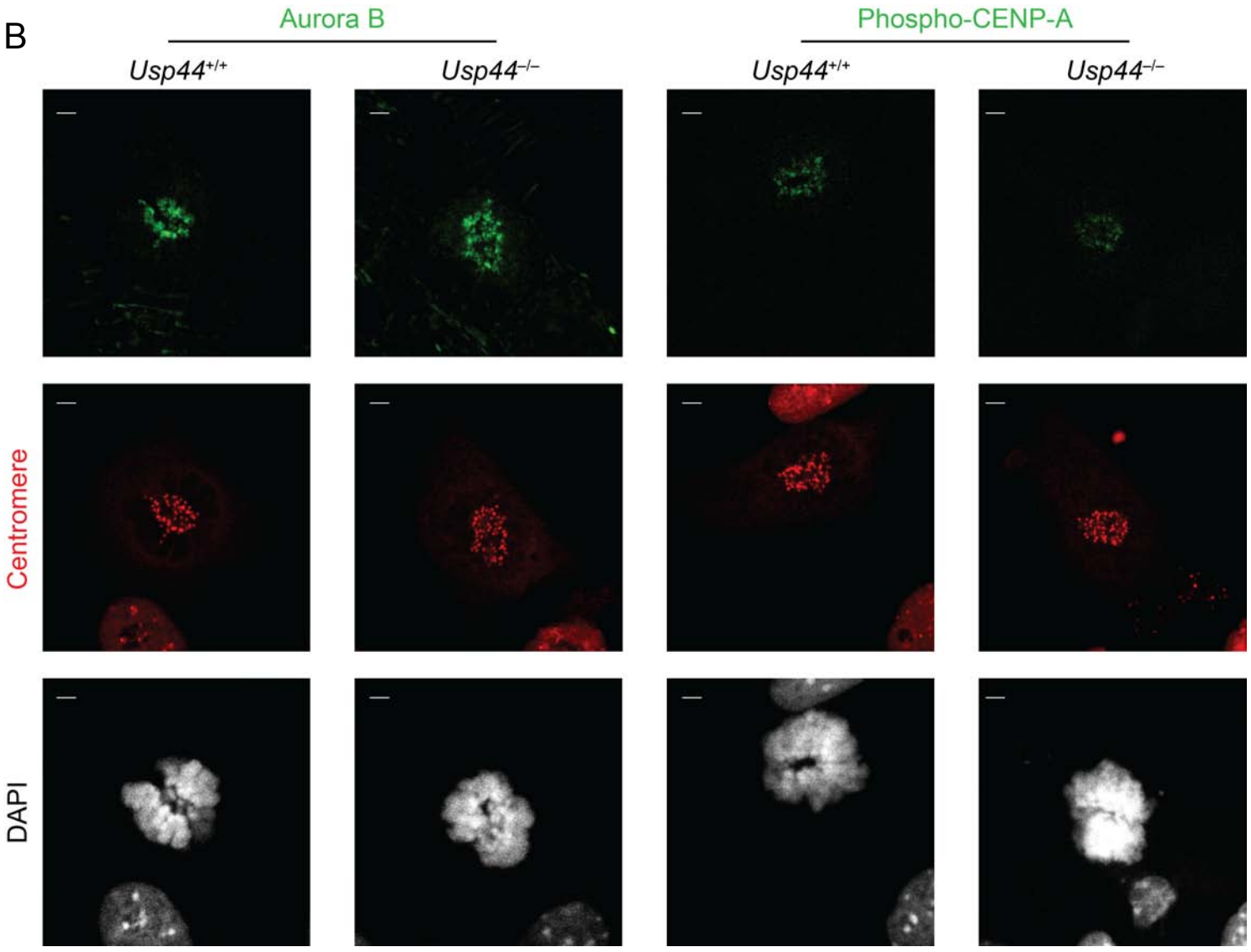


Figure S7. Zhang et al.

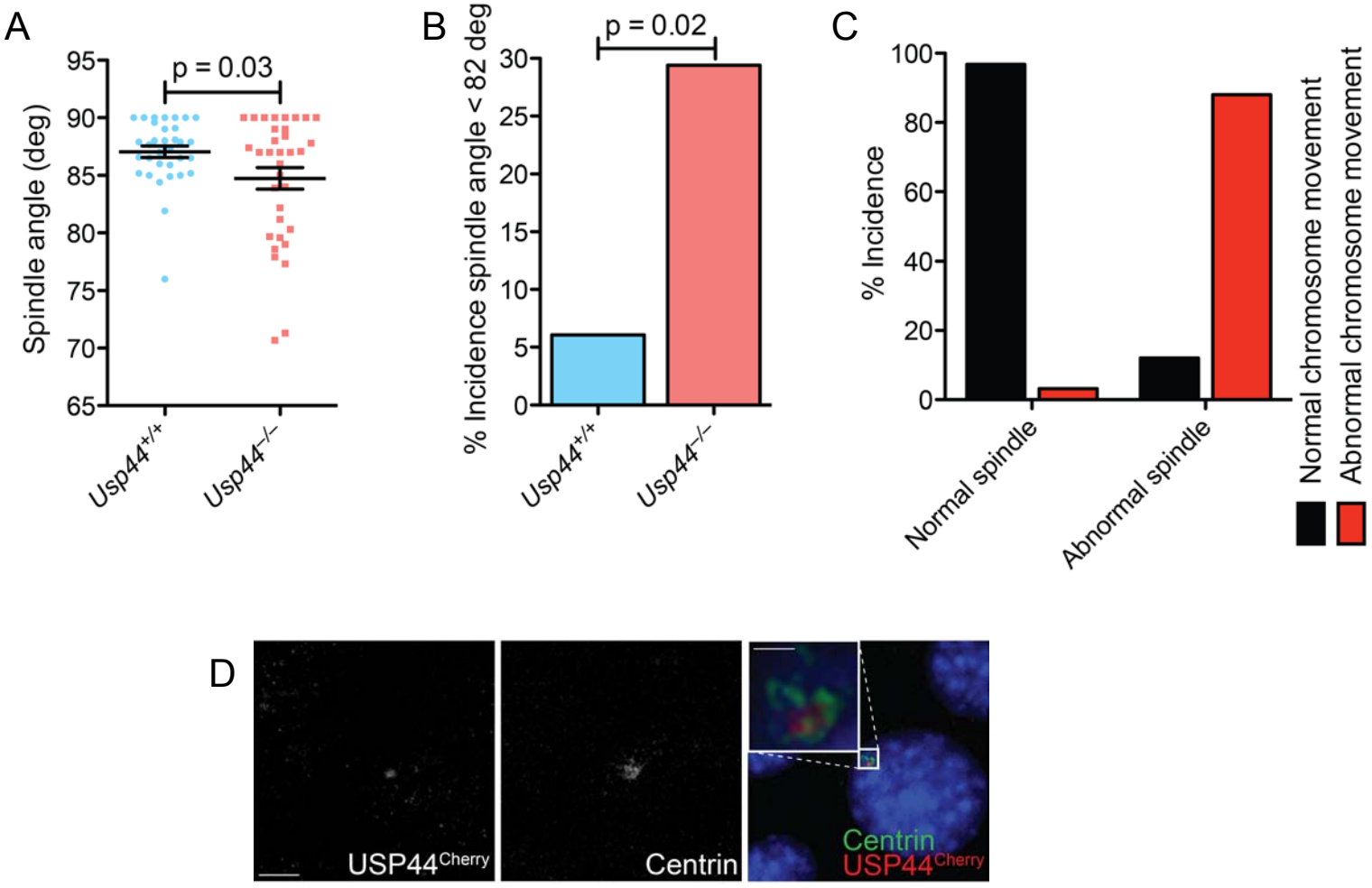


Figure S8 Zhang et al.

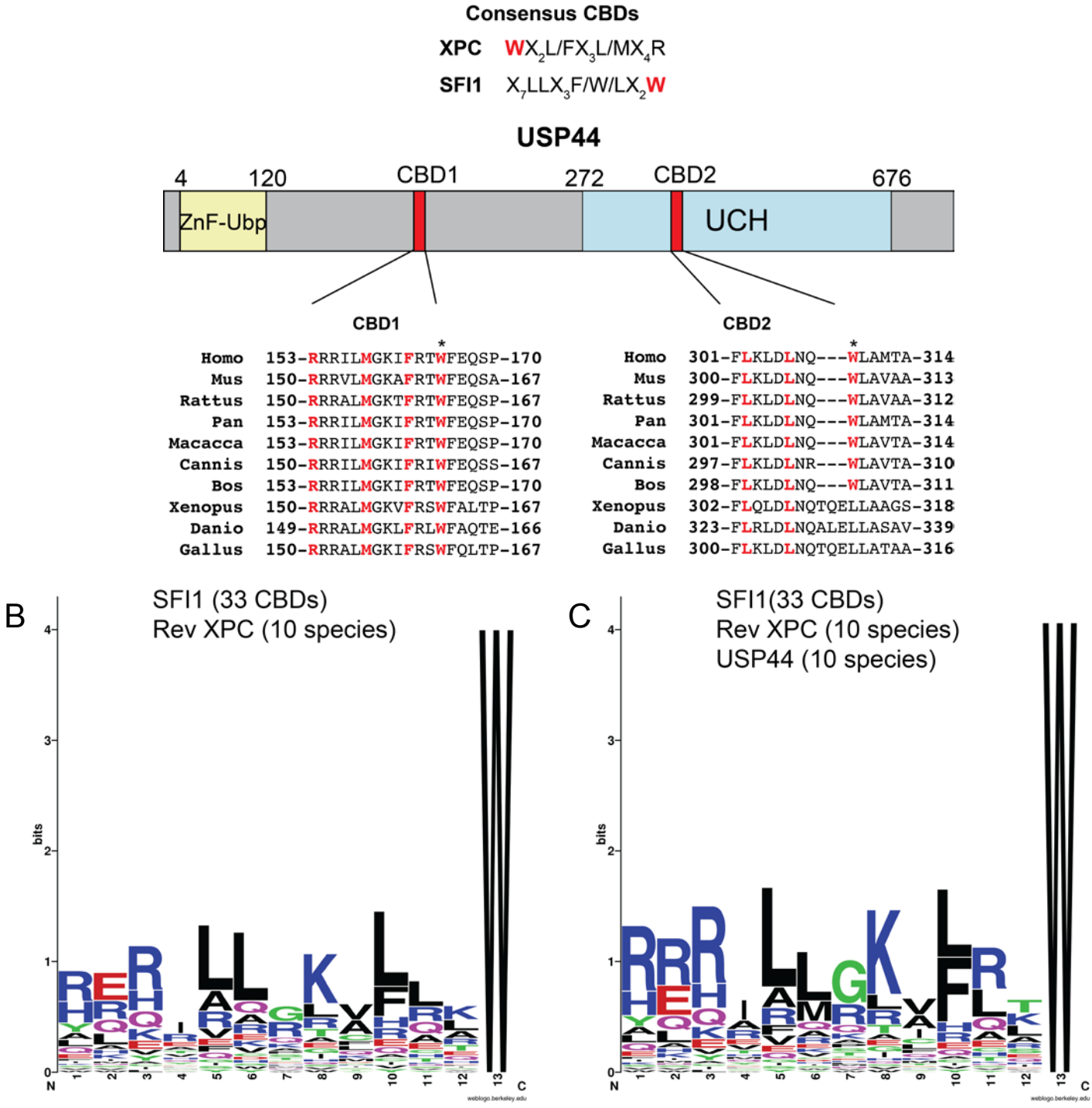


Figure S9. Zhang et al.

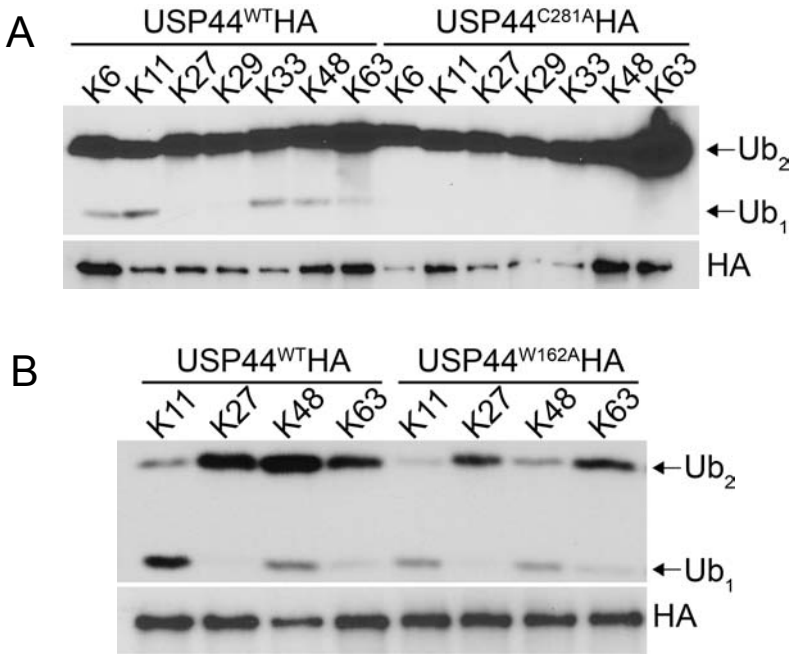


Figure S10. Zhang et al.

A

Mouse Genotype	Tissue	Number of cells with indicated chromosome chromosome 4 copies					Number of cells with indicated chromosome chromosome 7 copies				
		1	2	3	4	5	1	2	3	4	5
Usp44 +/+	Normal lung 1	11	87	2	0	0	13	86	1	0	0
Usp44 +/+	Normal lung 2	13	85	2	0	0	8	90	2	0	0
Usp44 +/+	Normal lung 3	7	85	5	3	0	6	85	6	3	0
Usp44 −/−	Lung tumor 1	13	84	3	0	0	12	86	2	0	0
Usp44 −/−	Lung tumor 2	23	74	3	0	0	15	83	2	0	0
Usp44 −/−	Lung tumor 3	28	67	5	0	0	2	29	29	35	5
Usp44 −/−	Lung tumor 4	10	90	0	0	0	5	94	1	0	0
Usp44 −/−	Lung tumor 5	50	49	1	0	0	7	83	10	0	0
Usp44 −/−	Lung tumor 6	42	58	0	0	0	22	73	5	0	0
Usp44 −/−	Lung tumor 7	5	91	2	2	0	3	52	40	5	0
Usp44 −/−	Lung tumor 8	12	88	0	0	0	15	84	1	0	0
Usp44 −/−	Lung tumor 9	10	87	3	0	0	11	89	0	0	0
Usp44 −/−	Lung tumor 10	9	89	2	0	0	1	98	1	0	0

B

Tissue	Tumor Usp44 Status	Number of cells with indicated chromosome chromosome 7 copies							Number of cells with indicated chromosome chromosome 8 copies									
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10
Adenocarcinoma 1	NL	14	37	36	11	2	0	0	0	10	6	18	21	17	15	11	1	1
Adenocarcinoma 2	NL	14	38	37	11	0	0	0	33	61	6	0	0	0	0	0	0	0
Adenocarcinoma 3	NL	11	53	17	17	2	0	0	7	54	22	14	3	0	0	0	0	0
Adenocarcinoma 4	NL	3	28	23	18	17	9	2	2	26	22	21	16	12	0	0	1	0
Adenocarcinoma 5	NL	5	39	29	25	1	1	0	3	47	25	20	5	0	0	0	0	0
Adenocarcinoma 6	NL	35	63	2	0	0	0	0	10	39	35	14	2	0	0	0	0	0
Adenocarcinoma 7	NL	3	47	19	17	7	6	1	4	48	27	19	2	0	0	0	0	0
Adenocarcinoma 8	NL	4	34	45	14	3	0	0	0	21	52	16	10	1	0	0	0	0
Adenocarcinoma 9	NL	8	24	35	30	3	0	0	1	12	26	40	14	6	1	0	0	0
Adenocarcinoma 10	NL	5	24	34	34	3	0	0	1	20	29	32	14	4	0	0	0	0
Adenocarcinoma 11	LO	19	56	22	3	0	0	0	11	61	24	4	0	0	0	0	0	0
Adenocarcinoma 12	LO	6	42	38	14	0	0	0	24	73	3	0	0	0	0	0	0	0
Adenocarcinoma 13	LO	8	13	36	30	9	2	2	2	12	21	37	14	7	5	0	2	0
Adenocarcinoma 14	LO	4	18	34	38	6	0	0	3	19	32	32	11	1	1	1	0	0
Adenocarcinoma 15	LO	10	39	33	14	2	0	2	0	34	13	14	17	11	7	3	1	0
Adenocarcinoma 16	LO	19	55	21	5	0	0	0	3	6	37	33	16	4	1	0	0	0
Adenocarcinoma 17	LO	25	72	3	0	0	0	0	25	73	2	0	0	0	0	0	0	0
Adenocarcinoma 18	LO	7	33	30	19	9	1	1	1	30	16	26	20	1	5	0	1	0
Adenocarcinoma 19	LO	13	28	44	11	4	0	0	8	31	27	27	6	1	0	0	0	0
Adjacent lung 1	NL	28	71	1	0	0	0	0	23	77	0	0	0	0	0	0	0	0
Adjacent lung 2	NL	28	72	0	0	0	0	0	27	73	0	0	0	0	0	0	0	0
Adjacent lung 3	NL	24	76	0	0	0	0	0	22	78	0	0	0	0	0	0	0	0
Adjacent lung 4	NL	22	77	1	0	0	0	0	19	80	1	0	0	0	0	0	0	0
Adjacent lung 5	NL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	0	0
Adjacent lung 6	NL	25	75	0	0	0	0	0	25	74	1	0	0	0	0	0	0	0
Adjacent lung 7	NL	20	76	4	0	0	0	0	18	80	2	0	0	0	0	0	0	0
Adjacent lung 8	NL	14	85	1	0	0	0	0	10	88	2	0	0	0	0	0	0	0
Adjacent lung 9	NL	24	76	0	0	0	0	0	22	77	1	0	0	0	0	0	0	0
Adjacent lung 10	NL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	0	0
Adjacent lung 11	LO	26	73	1	0	0	0	0	17	82	1	0	0	0	0	0	0	0
Adjacent lung 12	LO	25	75	0	0	0	0	0	28	72	0	0	0	0	0	0	0	0
Adjacent lung 13	LO	20	80	0	0	0	0	0	17	83	0	0	0	0	0	0	0	0
Adjacent lung 14	LO	29	70	1	0	0	0	0	22	78	0	0	0	0	0	0	0	0
Adjacent lung 15	LO	24	76	0	0	0	0	0	17	83	0	0	0	0	0	0	0	0
Adjacent lung 16	LO	28	72	0	0	0	0	0	25	75	0	0	0	0	0	0	0	0
Adjacent lung 17	LO	26	74	0	0	0	0	0	23	76	1	0	0	0	0	0	0	0
Adjacent lung 18	LO	29	70	1	0	0	0	0	26	72	2	0	0	0	0	0	0	0
Adjacent lung 19	LO	27	73	0	0	0	0	0	18	81	1	0	0	0	0	0	0	0