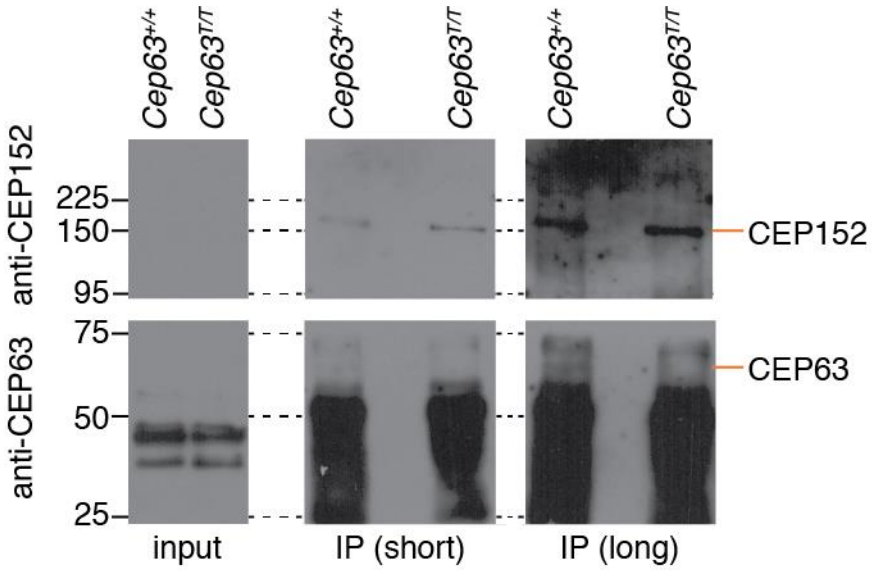
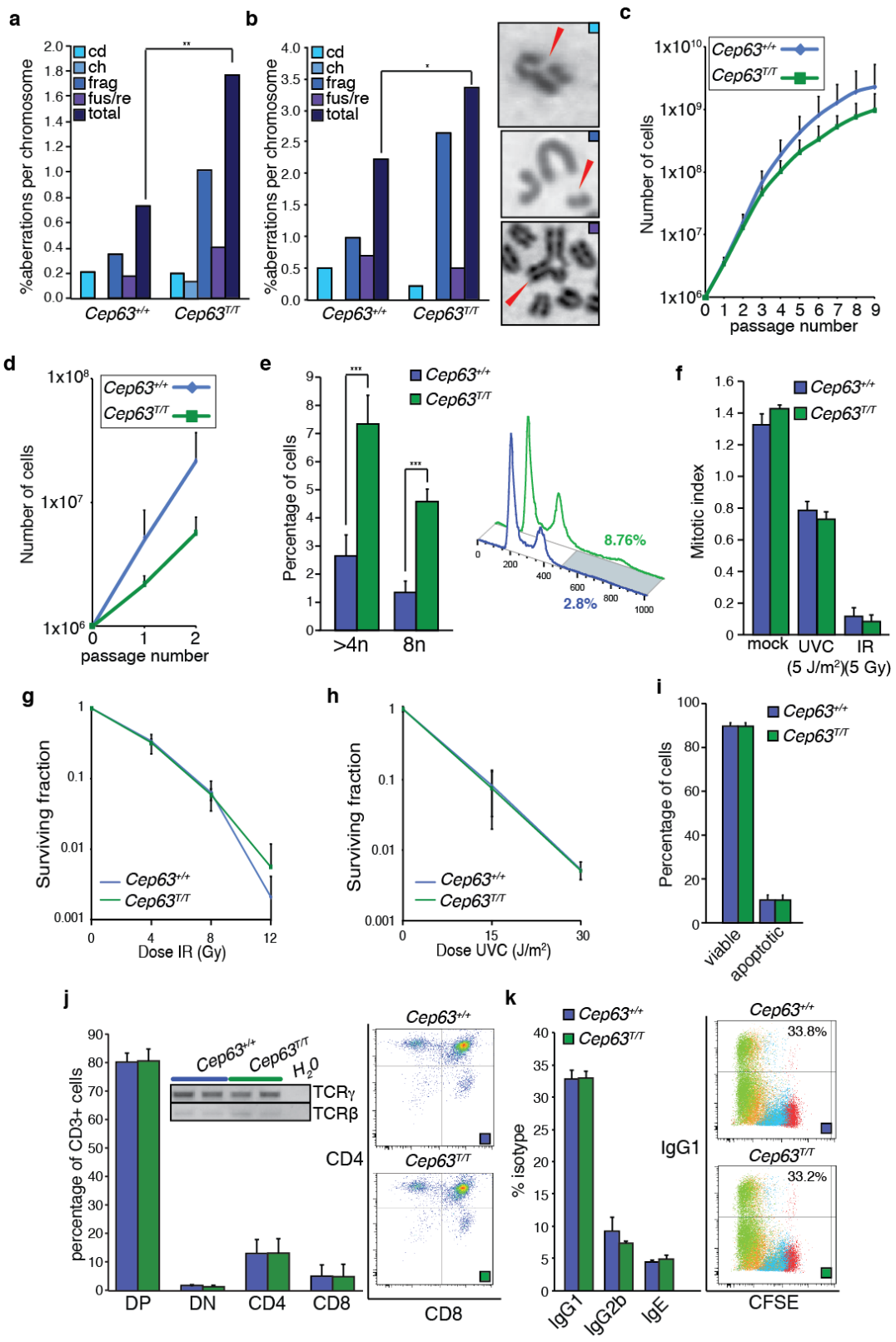
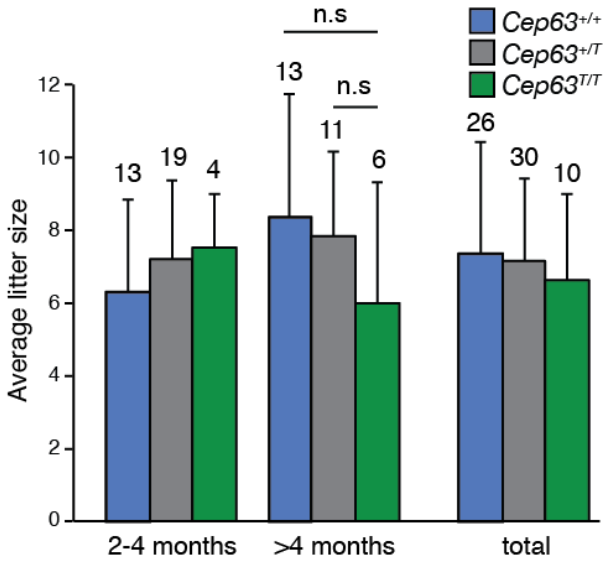


Supplementary Figure 1: CEP63 and CEP152 protein levels in mouse tissues. Protein lysates were generated from fresh liver tissue isolated from 1.5 month old *Cep63*$^{+/+}$ or *Cep63*$^{T/T}$ mice. Immunoprecipitations (IPs) were performed from 4 mg of lysate using CEP63 and CEP152 antibodies and IPs were run out on SDS-PAGE gels and transferred to PVDF membrane. 80 μg of lysate was run as input and we performed immunodetection for CEP63 or CEP152 using enhanced chemiluminescence. While CEP63 and CEP152 were not visible by standard Western blotting and ECL of the input samples (left panels), similar levels of CEP152 were observed in both genotypes (top, right panels) following IP, while a band corresponding to CEP63 was observed only in lysates from *Cep63*$^{+/+}$ mice (bottom panels) following IP. Similar results were reported previously in lysates from *Cep63*$^{+/+}$ or *Cep63*$^{T/T}$ MEFs[1]. Neither protein was detectable in IPs from 2 mg of brain lysates (whole brain tissue pooled from 2 *Cep63*$^{+/+}$ or *Cep63*$^{T/T}$ mice). Further IPs were not performed from brain tissue in order to minimize animal use.

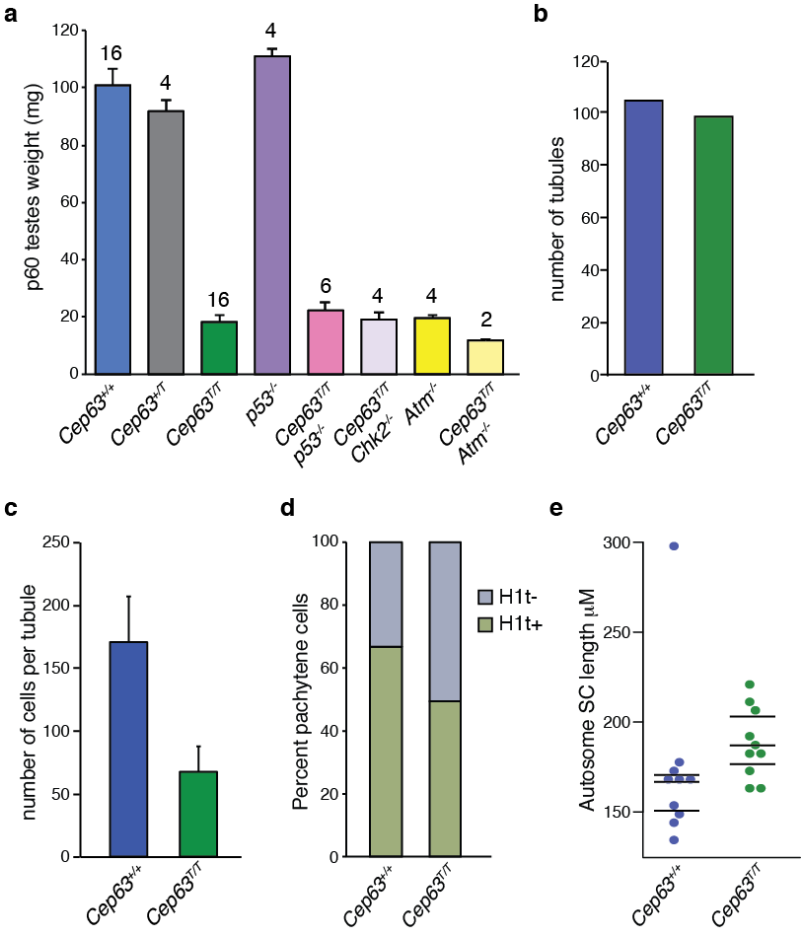


Supplementary Figure 2: Chromosome stability, DNA damage responses and immunological development in *Cep63$^{T/T}$* mice. (**a**) Metaphase aberrations in *Cep63$^{+/+}$* and *Cep63$^{T/T}$* early passage (p2) primary MEFs or (**b**) spontaneously immortalized MEFs (cd; chromatid breaks, ch; chromosome breaks, frag; fragments, fus/re; fusions and rearrangements. Examples are shown in the panels to the right of (b). 100 metaphases per genotype were scored for each plot (n = 6, 9, 2 and 3 animals

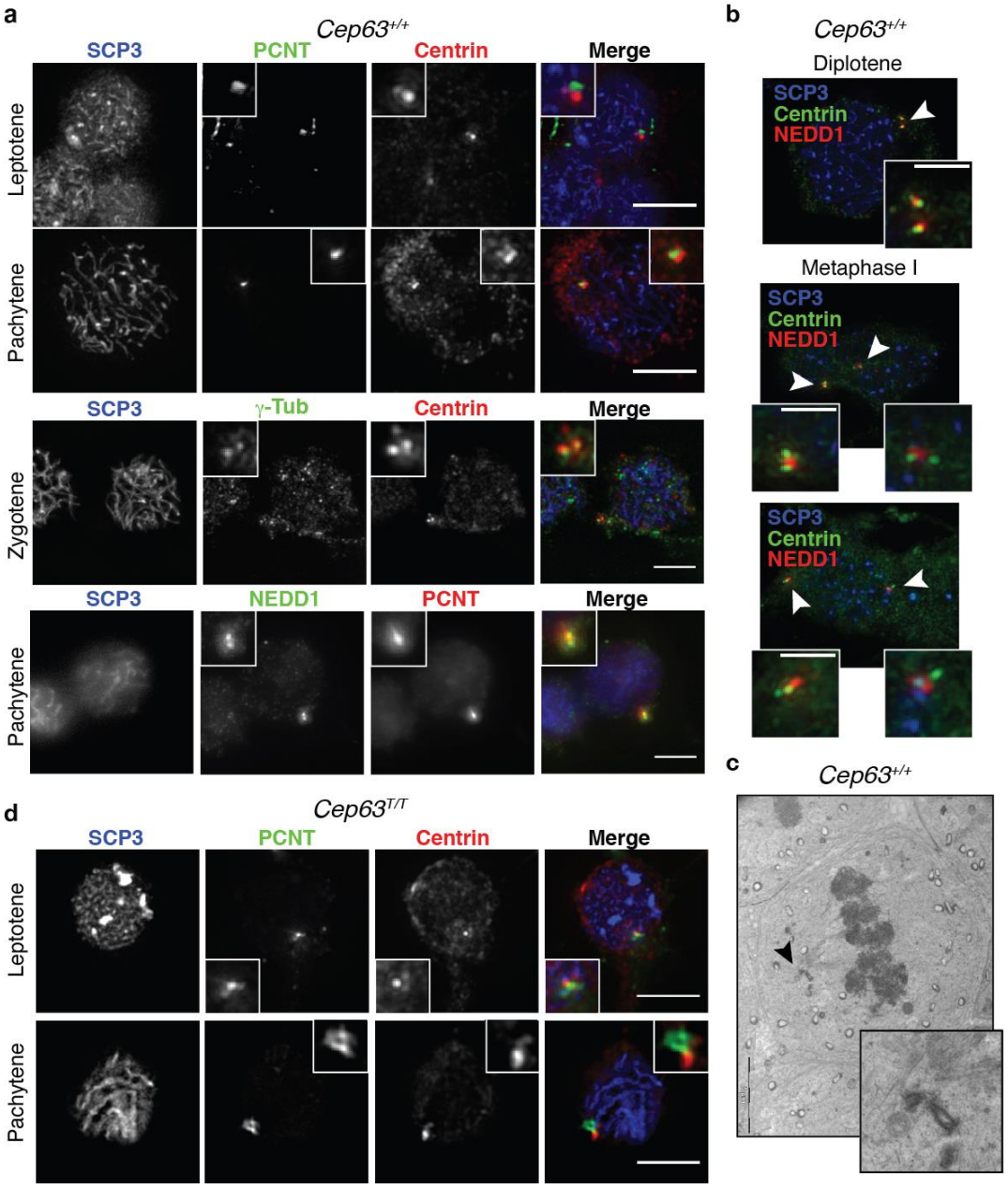
respectively). Statistical significance (p-values = 0.005 (a) and (b) 0.02) were determined using Fisher's exact test (two-sided) in MSTAT as described previously[2]. (**c**) The cumulative growth of primary MEFs over the first 9 passages. Data is combined from 6 *Cep63*$^{+/+}$ and 9 *Cep63*$^{T/T}$ cultures. (**d**) The growth rate of 3T3 transformed MEFs (>20 passages) over 2 passages is plotted from 2 *Cep63*$^{+/+}$ and 3 *Cep63*$^{T/T}$ cultures. (**e**) Quantification of aneuploidy and polyploidy in MEFs of the indicated genotype using propidium iodide staining and flow cytometry (*Cep63*$^{+/+}$ n = 4, *Cep63*$^{T/T}$ n = 5 independent experiments from 4 and 3 animals per genotype, respectively). (**f**) G2/M checkpoint responses in MEFs of the indicated genotype 1 hour following IR or UVC treatment (*Cep63*$^{+/+}$ n = 3, *Cep63*$^{T/T}$ n = 3 animals). (**g**, **h**) DNA damage sensitivity to IR and UVC in MEFs of the indicated genotype (n = 3 independent experiments, MEFs from 2 animals per genotype were used). (**i**) Apoptosis in T-cells from mice of the indicated genotype measured by Annexin-V and propidium iodide incorporation and flow cytometry. (**j**) T-cell differentiation and V(D)J recombination of TCRγ and TCRβ loci (inset showing no difference between genotypes for indicated PCR loci) occurs normally in *Cep63*$^{T/T}$ mice. Examples of flow cytometry of CD3+ cells to distinguish CD4 and CD8 positive populations are shown to the right. (**k**) Class switching and proliferation induced by treatment of primary B-cells with LPS and IL-4 occurs normally in *Cep63*$^{T/T}$ mice. Examples of flow cytometry data (right panels) showing switching to IgG1 over 4 days (day 1 in red, day 4 in green). (Fig 3i-k, n = 3 animals per genotype in three independent experiments).



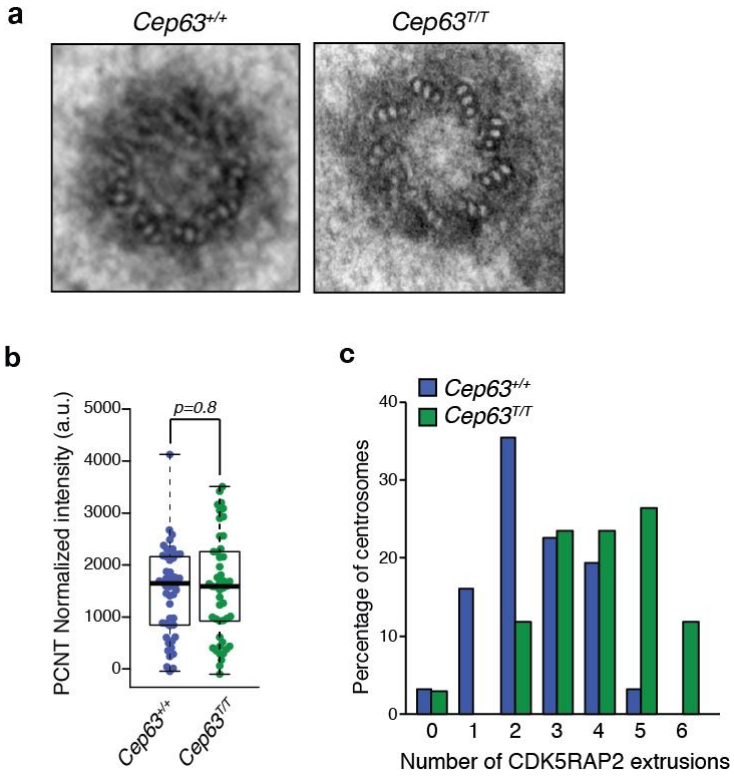
Supplementary Figure 3: Female litter sizes. Average litter sizes and standard deviations from mothers of the indicated genotype are graphed. The total number of litters from mice (n) of the indicated ages is shown above each column. The Wilcoxon rank sum test was used for statistical analysis and no significant differences were found between the genotypes. Total is a combination of the data in the 2-4 month and >4 months categories.



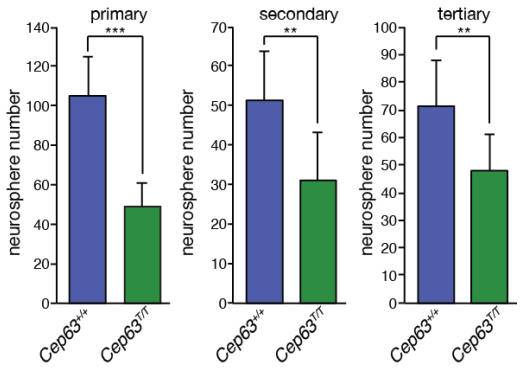
Supplement ary Figure 4: Defects in testes development in *Cep63* deficient animals. (**a**) Testicle weight of 2-month old animals. Average and standard deviation of the weight of testicles from p60 animals of the indicated genotype are plotted. n = number of testicles analyzed per genotype is shown over each bar. (**b**) The average number of tubules at p60 for the indicated genotype is plotted (n = 1 animal per genotype used). (**c**) The average number of cells per p60 tubule of the indicated genotype is plotted (right panel, n = 2 animals per genotype used, 6 tubules per genotype scored). Bars indicate standard deviation. (**d**) Scoring of pachytene cells, identified by SC configuration with SCP3, for H1t positivity in the indicated genotypes (n = 1 animal per genotype used, 75 cells per genotype scored). (**e**) Synaptonemal complex (SC, SCP3 staining) length of autosomes in MLH1 bright pachytenes was measured on spermatocyte spreads using ImageJ (2 animals per genotype used, n = 10 cells per genotype measured)



Supplementary Figure 5: Examples of centrosome configurations in prophase I spermatocytes. (**a**) Spermatocyte squashes from *Cep63*$^{+/+}$ mice were staged with staining for SCP3 and stained with the indicated antibodies. Top 2 rows correspond to centrosome examples shown in Fig. 6B (scale bar = 5 µm). (**b**) Centrosome configurations in *Cep63*$^{+/+}$ mice spermatocytes at diplotene and metaphase I. Note fully duplicated centrosomes that are visibly separated at metaphase I. (**c**) EM analysis of a metaphase I spindle pole. Metaphase I spindles were identified by EM and centrioles at spindle poles were visualized. The example image shows one of the two spindle poles with a pair of centrioles (mother with daughter). The inset is a magnification of the spindle pole region. (**d**) Spermatocyte squashes from *Cep63*$^{T/T}$ mice were staged using SCP3. Compared to the compact PCNT staining in cells, the PCM was more dispersed with higher numbers of extrusions (Fig. 6 and Supplementary Fig. 6 for quantification) and abnormal PCM structure.



Supplementary Figure 6: Centriole ultrastructure in prophase I spermatocytes. (**a**) Centrioles were identified by EM and cross sections from *WT* and *Cep63*$^{T/T}$ centrioles were compared. No structural defects were observed in spermatocytes from *Cep63*$^{T/T}$ mice. (**b**) Recruitment of PCNT to centrosomes in prophase I spermatocytes. Intensity of centrosomal PCNT staining was measured in spermatocyte squashes from *Cep63*$^{+/+}$ and *Cep63*$^{T/T}$ mice. An 11 slice-stack around the PCNT staining was used for a maximum projection and PCNT intensity was quantified in a 50 pixel circular area around centrosomes (ImageJ). Background was measured in an adjacent area of the same dimension and subtracted (2 independent experiments performed, quantification from one experiment shown where *Cep63*$^{+/+}$ n = 54 and *Cep63*$^{T/T}$ n = 45 centrosomes quantified). (**c**) Quantification of centrosome extrusions based on CDK5RAP2 staining. Spermatocytes of the indicated genotypes were stained with CDK5RAP2 antibody and extrusions were scored. SCP3 staining was used for staging cells in prophase I. Results were similar to those presented in Fig. 6g with PCNT staining.



Supplementary Figure 7: Analysis of neurosphere growth. Primary, secondary and tertiary cultures of *Cep63*$^{+/+}$ and *Cep63*$^{T/T}$ neurospheres from E14.5 embryos (3 experiments using n = 5 wild type and n = 6 *Cep63*$^{T/T}$ animals). The average and standard deviation are shown. Asterisks denote statistical significance (n.s.= not significant, * = p-value <0.05, ** = p-value < 0.01, *** = p-value < 0.001) determined by the unpaired two-way Student's T-test.

Assessment of follicle numbers

		Percentage of follicles (Mean ± SD)				Total follicles analyzed	Number of follicles per section (Mean ± SD)	Mice analyzed
Age	**Genotype**	**Primordial**	**Primary**	**Secondary**	**Antral**			
21 d.p.p	***WT***	84.4±3.0	8.1±2.9	7.3±1.7	0.2±0.2	1172	36.6±14.7	3
	Cep63$^{T/T}$	68.9±1.7	13.3±3.2	17.8±4.8	0.0±0.0	796	24.9±9.7	3
3-6 mo.	***WT***	72.4±7.3	14.6±7.2	12.3±0.3	0.7±0.2	414	20.7±6.5	2
	Cep63$^{T/T}$	51.2±6.6	32.1±10.5	16.1±3.0	0.6±0.9	120	6.0±3.7	2
9 mo.	***WT***	53.8	32.7	9.6	3.8	149	14.9±3.6	1
	Cep63$^{T/T}$	53.1±8.1	25.9±15.8	18.2±5.4	2.7±2.4	177	8.9±2.5	2

Supplementary Table 1: For follicle quantification, 10 alternated sections were examined for the presence of the following four categories of follicles. Primordial follicles containing an oocyte surrounded by a single layer of flat follicular cells. Primary follicles, which contain a single layer of cubic follicular cells surrounding the oocyte. Secondary follicles when two or more layers of follicular cells were enclosing the oocyte and antral follicles which have an internal cavity. Only follicles with oocytes with a visible nucleus were counted on each section.

Antibodies used in this study

Antigen	Species	Source	reference#	Dilution
ACA	human	Antibodies Incorporated	15-235	1:200
CDK5Rap2	rabbit	Universal Biologicals	IHC-00063	1:500
CEP63	rabbit	Millipore	06-1292	1:500
CEP152	rabbit	GeneTex	GTX128027	1:500
CEP152	rabbit	Sigma	HPA039408	1:100
Centrin 3	mouse	Abnova	H00001070-M01	1:1000
Centrin 3 (Xenopus)	rabbit	Gift from A. Groen/R. Ohi		1:2000
Cleaved Caspase-3	rabbit	BD Biosciences	559565	1:500
DMC1	rabbit	Santa Cruz	SC22768	1:50
γH2Ax (Ser139)	mouse	Millipore	05-636	1:500
γH2Ax (Ser139)	rabbit	Cell Signalling	2588	1:500
γ-tubulin	mouse	Sigma	T6557	1:500
γ-tubulin	mouse	ExBio	11-465-C100	1:500
p-H3Ser10	rabbit	Millipore	05-598	1:200
p-H3Ser10	rabbit	Cell Signalling	97015	1:1000
H1t	guinea pig	Gift from M.A. Handel[3]		1:200
MLH1	mouse	BD Biosciences	551091	1:100
NEDD1	mouse	Abnova	H00121441-M05	1:250
NEDD1	rabbit	Gift from S. Kumar		1:250
PCNA (FL-261)	mouse	Santa Cruz	sc-7907	1:500
PCNT	mouse	Abcam	ab28144	1:500
PCNT	rabbit	Covance	PRB-432C	1:500
PCNT	Rabbit	Gift from T. Stearns[4]		1:500
P53 (CM5)	rabbit	Vector	VP-P956	1:1000
RAD51	rabbit	Santa Cruz	SC8349	1:100
SCP3	mouse	Abcam	ab15093	1:750
SCP3	rabbit	Abcam	ab97672	1:500
SCP1	rabbit	Abcam	ab15087	1:750
SOX2	rabbit	Millipore	AB5603	1:500
SUN1	rabbit	Abcam	ab103021	1:50
TBR2-Eomes	rabbit	Abcam	ab23345	1:200
TRF1	rabbit	Alpha Diagnostics	TRF12-S	1:50
TUJ1 (β-tubulin III)	mouse	Covance	MMS-435P	1:500
WT1	mouse	Novus Biologicals	NB110-60011	1:100

Supplementary Table 2: The antigen, host species, source, ordering reference and concentration used are listed in the table.

Supplementary Methods

Primary cell culture and transformation of MEFs

MEFs were transformed using a modified 3T3 protocol or by transfection with a linearized, origin-less SV40 genome[2]. Neurospheres were generated essentially as described[5]. In brief, E14.5 mouse brain cortex was dissected and dissociated manually by gentle pipetting. 2×10^4 cells were seeded in a 6-well plate in a serum free medium (Dulbecco's modified Eagle medium with high glucose, sodium pyruvate, 1X glutamax, 1X penicillin-streptomycin) supplemented with B27, N2, 2 mM N-acetyl-cysteine, 10 ng/ml^{-1} EGF and 20 ng/ml^{-1} bFGF (Gibco). Neurospheres were counted after 7 days *in vitro*, gently dissociated using StemPro Accutase (Gibco) and re-plated twice for additional passages.

Immunoprecipitations and Western blotting

Fresh liver was obtained from mice of the indicated genotypes, finely minced with a scalpel and incubated in lysis buffer (50 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl, 1% TX-100, 1% NP40, 0.2% SDS with protease (Roche) and phosphatase inhibitors (Sigma)) on ice for 20 minutes following mechanical disruption. Lysates were centrifuged at full speed for 30 minutes at 4°C and the supernatant removed. Protein concentration was quantified with DC Protein Assay Kit (Bio-Rad). 80 μg of total protein was taken for input and 4 mg used for IPs. Lysates were precleared by incubation with Protein-A Dynabeads (Life Technologies) for 1 hour at 4°C. Following Dynabead removal using a magnetic stand, primary antibodies for CEP63 (Millipore 06-1292) and CEP152 (Sigma, HPA039408) were added and lysates were incubated on a rotator for 6 hours at 4°C. Protein-A Dynabeads were added for 1 hour and IPs immobilized using a magnetic stand. IPs were washed 3X in lysis buffer and separated by SDS-PAGE (3-8% *NuPAGE Tris-Acetate* gels, Life Technologies) followed by transfer to Immobilon-P PVDF membrane (Merck-Millipore). Blots were blocked in 5% milk in PBST and membranes were incubated in primary antibody at 4°C overnight and then in secondary antibody 1h at room temperature following 3 washes in PBST. ECL reagent (Amersham) with X-ray film (Fujifilm) were used to detect the signal.

Assessment of DNA damage sensitivity and checkpoint analysis

The G2/M checkpoint was analyzed by staining for histone H3 phosphorylated on serine 10 (H3Ser10) to determine the mitotic index following irradiation with the indicated doses and recovery times[2]. Briefly, cells were fixed in 70% EtOH, washed and stained with primary antibody for H3Ser10 (Millipore), stained with secondary antibody conjugated to FITC and analyzed by flow cytometry. A mitotic ratio (%mitotic cells post mock or IR treatment/%mitotic cells untreated) is presented. For the clonogenic survival assay, 3T3 immortalized MEFs were seeded and treated with the indicated doses of IR or UVC. One to two weeks later, colonies were stained with 0.5% crystal violet in 20% ethanol and manually counted. Results were normalized for plating efficiencies. For both checkpoint and survival experiments, cell cultures were treated in an X-ray cabinet (Maxishot.200, Krautkramer Forster) or UVC crosslinking oven (Stratalinker, Stratagene).

Metaphase spread preparations

MEFs at early passage were incubated with colcemid (0.1μg/ml) for 1-3 hours. MEFs were hypotonically swollen in 0.075M KCL for 15 minutes at 37 degrees and then

fixed (75% MeOH and 25% acetic acid, ice cold) and washed several times in fixative. Metaphase preparations were spread on glass slides, steam treated using an 80ºC water bath for 3-5 seconds, heat dried and stained with 5% Giemsa solution (Sigma). At least 100 metaphases were analyzed per experiment.

Immunological analysis

Analysis of thymocytes was performed as described previously[6]. Thymi were dissected from 10-12 week old animals and a single-cell suspension was obtained by pressing the tissue through a 45 μm nylon mesh (Falcon). Apoptosis of thymoctes was assessed by flow cytometry after double staining with FITC-conjugated Annexin V (BD Biosciences) and propidium iodide. Thymic subpopulations were quantified by staining with fluorescence-conjugated antibodies for CD3e, CD4 and CD8 (BD Biosciences) and analyzed by flow cytometry. The CD3 population was gated and proportion of single CD4, CD8, and double positive or double negative populations were determined in FlowJo software (Tree Star). T-cell receptor (TCR) rearrangements were analyzed by PCR of genomic DNA (PureLink genomic DNA minikit, Invitrogen). A nested PCR was performed using 100 ng of genomic DNA with the following primers (all 5'-3'): TCRγ: TCRGV3S1a (ACCATACACTGGTACCGGCA) and TCRGJ1/2a (TCATCACTGGAATAAAGCAG); TCRβ; TCRBV5S1a (TGGTATCAACAGACTCAGGGG) and TCRBJ2a (TCTACTTCCAAACTACTCCAG)[7]. For the analysis of class switching, B-cells were isolated from the spleen of 8-12 week old mice using a MACS B-cell isolation kit (Miltenyi Biotec). Isolated B-cells were stained with carboxyfluorescein succinimidyl ester (CFSE, Sigma) and stimulated in B-cell medium (RPMI-1640 (Sigma) supplemented with 10% FBS (Hyclone), 1X Penicillin/streptomycin, 1X Glutamine, 1mM Sodium Pyruvate, 53 μM β-mercaptoethanol, 10 mM HEPES and 1X non-essential amino acids (Gibco)) with 5 ng/ml interleukin-4 (IL-4) and 25 μg/ml lipopolysaccharide (LPS, Sigma) for 1-4 days. For analysis by flow cytometry, B-cells incubated in FcR Blocking reagent were stained with biotinylated primary antibodies (IgG1, Ig2b and IgE, Miltenyi Biotec) for 10 min at 4°C and washed and stained with anti-biotin-APC for 10 min at 4°C. DAPI was added at a concentration of 1 μg/ml to exclude dead cells and data was collected by flow cytometry and analyzed using FlowJo software (Treestar).

References

1. Brown, N.J., Marjanovic, M., Luders, J., Stracker, T.H. & Costanzo, V. Cep63 and cep152 cooperate to ensure centriole duplication. *PLoS One* **8**, e69986 (2013).
2. Theunissen, J.W. & Petrini, J.H. Methods for studying the cellular response to DNA damage: influence of the Mre11 complex on chromosome metabolism. *Methods Enzymol* **409**, 251-284 (2006).
3. Inselman, A., Eaker, S. & Handel, M.A. Temporal expression of cell cycle-related proteins during spermatogenesis: establishing a timeline for onset of the meiotic divisions. *Cytogenet Genome Res* **103**, 277-284 (2003).
4. Luders, J., Patel, U.K. & Stearns, T. GCP-WD is a gamma-tubulin targeting factor required for centrosomal and chromatin-mediated microtubule nucleation. *Nat Cell Biol* **8**, 137-147 (2006).
5. Ferron, S.R. *et al.* A combined ex/in vivo assay to detect effects of exogenously added factors in neural stem cells. *Nat Protoc* **2**, 849-859 (2007).
6. Knobel, P.A. *et al.* USP28 Is Recruited to Sites of DNA Damage by the Tandem BRCT Domains of 53BP1 but Plays a Minor Role in Double-Strand Break Metabolism. *Mol Cell Biol* **34**, 2062-2074 (2014).
7. Lista, F., Bertness, V., Guidos, C.J., Danska, J.S. & Kirsch, I.R. The absolute number of trans-rearrangements between the TCRG and TCRB loci is predictive of lymphoma risk: a severe combined immune deficiency (SCID) murine model. *Cancer Res* **57**, 4408-4413 (1997).