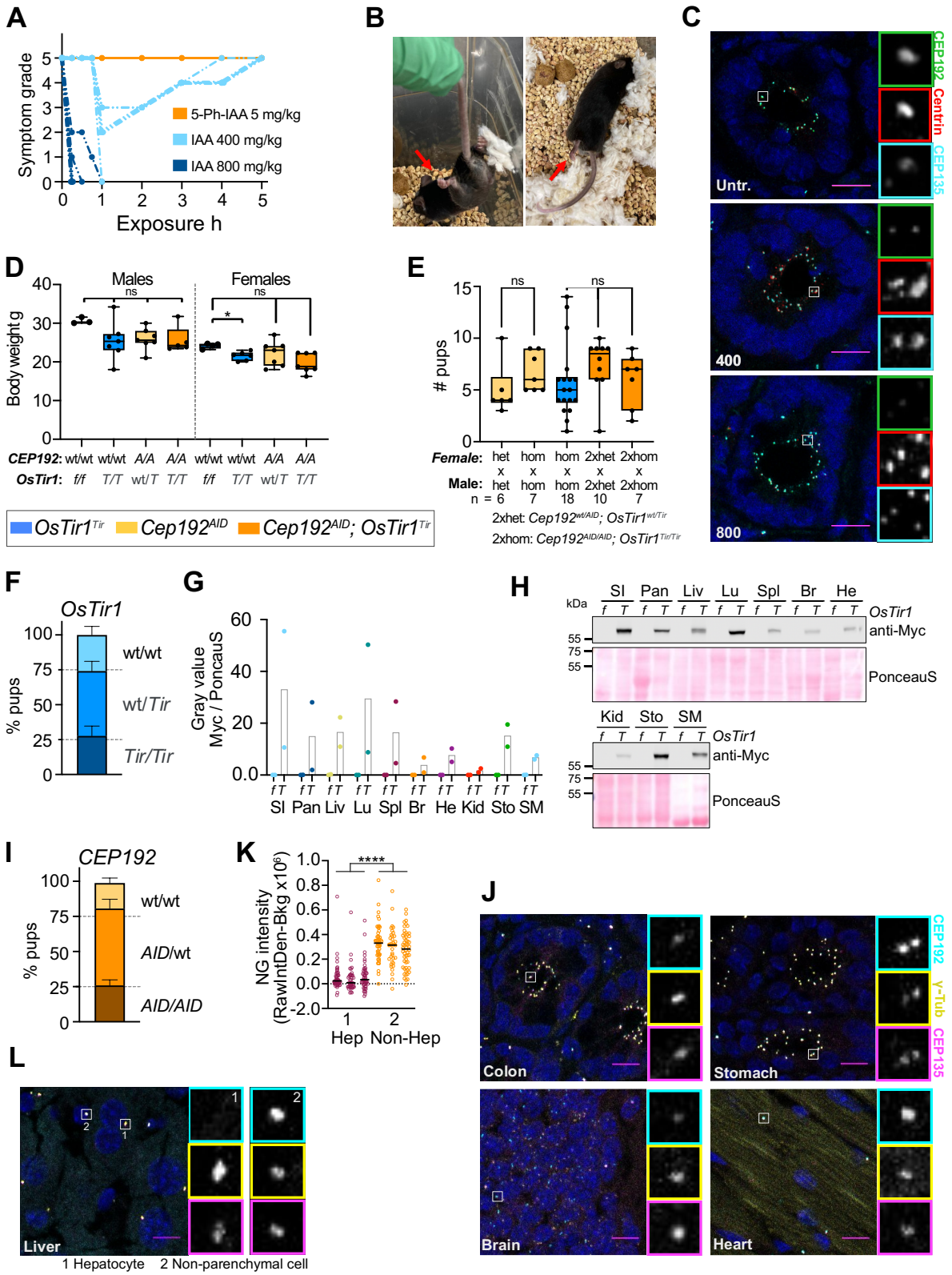
Supplemental Figures



Supplemental Figure 1: Characterization of the novel *OsTir1*^{Tir} and *Cep192*^{AID} mice.

(A) Symptom grades of the individual *OsTir1*$^{Tir/Tir}$ mice injected either with 5mg/kg 5-Ph-IAA (N = 3), 400mg/kg IAA (N = 5), or 800mg/kg IAA (N = 5) as in Fig. 1B. Symptoms including

spasms and paralysis were graded by severity over time; 0 = symptom-free, 5 = near complete paralysis, humane endpoint.

(B) Pictures of *OsTir1*$^{Tir/Tir}$ mice 30min after injection of 800 mg/kg IAA. Red arrows mark cramping paws and limbs.

(C) Representative confocal Immunofluorescence images showing CEP192^{AID} signal (NeonGreen; green), and immunostained Centrin (red) and CEP135 (cyan) in the SI of OSTIR1 expressing mice that were left untreated or injected with 400mg/kg for 5h or 800mg/kg IAA for 1h. Scale bar 10 µm.

(D) Graph showing the body weight of 9-30 weeks old male and female mice of the indicated genotype combinations. *Cep192*: A/A – AID/AID, *OsTir1*: f/f – floxed/floxed, T/T – Tir/Tir. N = 3-8 mice per genotype.

(E) Litter sizes of breedings with the indicated genotype combinations of homozygous (hom) or heterozygous (het) *Cep192*^{AID} and *OsTir1*^{Tir}alleles. n = 6-18 litters as noted in the figure. 2xhet: *Cep192*$^{wt/AID}$; *OsTir1*$^{wt/Tir}$. 2xhom: *Cep192*$^{AID/AID}$; *OsTir1*$^{Tir/Tir}$.

(F) Genotype distribution of the offspring of *OsTir1*^{Tir} heterozygous breedings. Dashed lines mark the expected Mendelian distribution. N = 9 litters.

(G) Immunoblot probed with an antibody detecting OSTIR1-F74G-Myc in the indicated organs of an *OsTir1*$^{f/f}$ *(f)* and an *OsTir1*$^{Tir/Tir}$ *(T)* mouse. This experiment is a biological replicate of the immunoblot shown in Fig. 1D. SI – small intestine, Pan – Pancreas, Liv – Liver, Lu – Lung, Spl – Spleen, Br – Brain, He – Heart, Kid – Kidney, Sto – Stomach, SM – Skeletal Muscle. Ponceau-S-staining is shown as a reference for the amount of protein loaded.

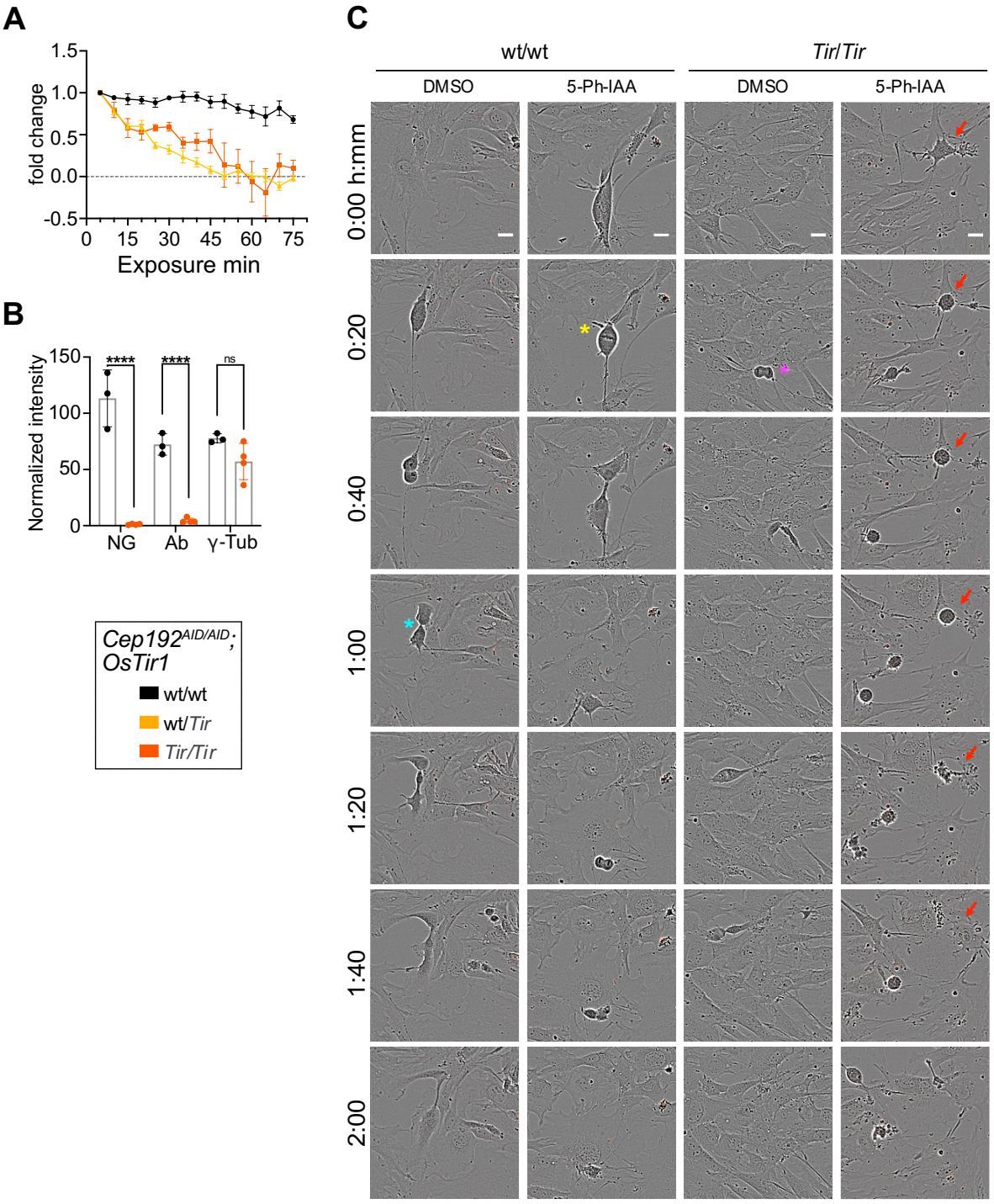
(H) Graph showing the genotype distribution of the offspring of heterozygous *Cep192*^{AID} breedings. Dashed lines mark the expected Mendelian distribution. N = 11 litters.

(I) Representative confocal immunofluorescence microscopy images of the indicated tissues as in (H) showing CEP192^{AID} signal (NeonGreeen, cyan), and immunostained γ-tubulin (yellow) and CEP135 (magenta). Scale bar 10 µm.

(J) Graph showing the individual data points of the quantification in Fig. 1E. The CEP192^{AID} NeonGreen (NG) signal was measured as raw integrated density with background subtraction of N = 3 mice across tissues. SI – Small intestine, Col – Colon, Sto – Stomach, Pan – Pancreas, Liv – Liver, Lu – Lung, He – Heart, Spl – Spleen, Br – Brain.

(K) Quantification and representative confocal immunofluorescence image of the CEP192^{AID} NeonGreen (NG) signal in hepatocytes (1) and non-parenchymal cells (2, non-Hep) in the liver as in (I-J). Scale bar 10 µm.

Data is displayed as mean ± SEM. Statistical significance was determined by one-way ANOVA with Sidak's multiple comparisons test. In (A), male and female groups were analyzed separately. ns $p \geq 0.05$, * $p < 0.05$, **** $p < 0.0001$.



Supplemental Figure 2: Complete CEP192^{AID} in MEFs impairs mitosis.

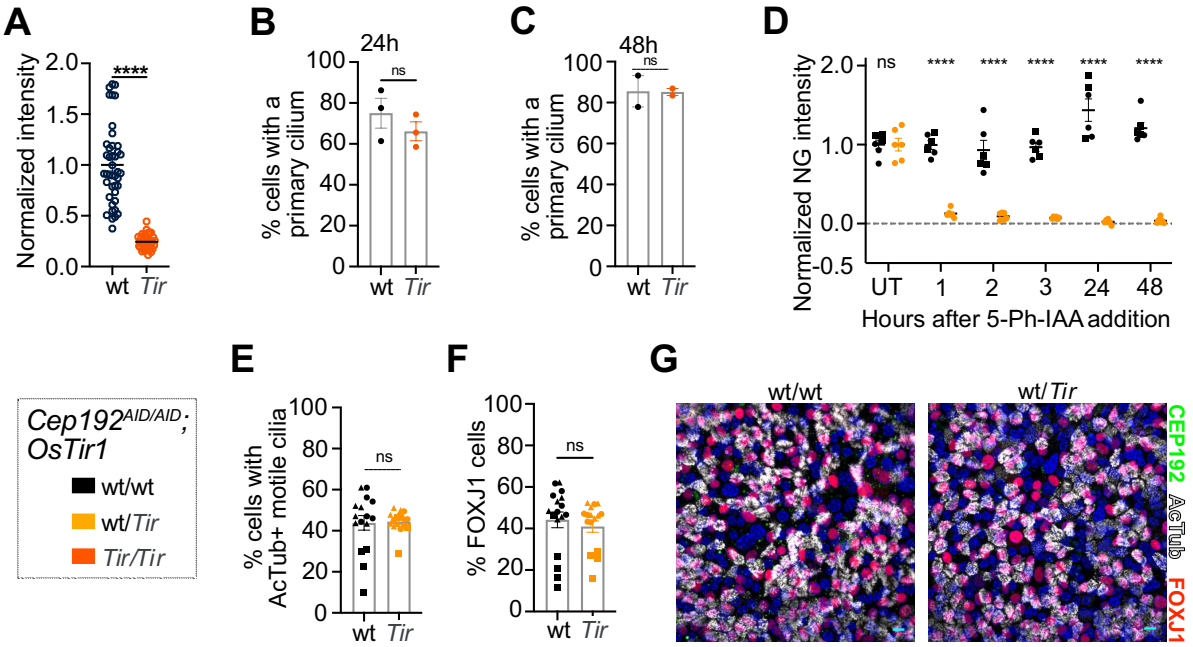
(A) Graph showing the CEP192^{AID} NeonGreen signal intensity measured relative to timepoint t = 0 min in MEF lines of the indicated genotypes. 5-Ph-IAA was added at timepoint t = 0 min. *Cep192*$^{wt/AID}$; *OsTir1*$^{wt/wt}$ N = 1 MEF lines, n = 4 cells; *Cep192*$^{wt/AID}$; *OsTir1*$^{wt/Tir}$ N = 2, n = 3-8; *Cep192*$^{wt/AID}$; *OsTir1*$^{Tir/Tir}$ N = 3, n = 3-11.

(B) *Cep192*$^{AID/AID}$; *OsTir1*$^{Tir/Tir}$ and *Cep192*$^{AID/AID}$; *OsTir1*$^{wt/wt}$ MEF lines treated with 5-Ph-IAA for 5h were immunostained with an antibody raised against mouse CEP192 and an antibody recognizing γ-tubulin. Signal intensities of CEP192^{AID} (NeonGreen), and immunostained

CEP192 and γ-tubulin were quantified relative to the DMSO condition. N = 3-4 MEF lines per genotype.

(C) Representative live imaging brightfield stills of mitotic *Cep192*$^{AID/AID}$ MEFs with or without OSTIR1 treated with 5-Ph-IAA or DMSO. MEFs without OSTIR1 or 5-Ph-IAA undergo complete mitosis with visible metaphase plates (yellow *), anaphase (magenta *), and cytokinesis (cyan *). MEFs expressing OSTIR1 and treated with 5-Ph-IAA round-up and re-adhere to the plate without undergoing cell division (red arrow).

All data is shown as mean ± SEM. Statistical significance was measured by two-way ANOVA ANOVA with Sidak's multiple comparisons test (B). ns $p \geq 0.05$, **** $p < 0.0001$.



Supplemental Figure 3: CEP192 is not needed for primary or motile cilia maintenance

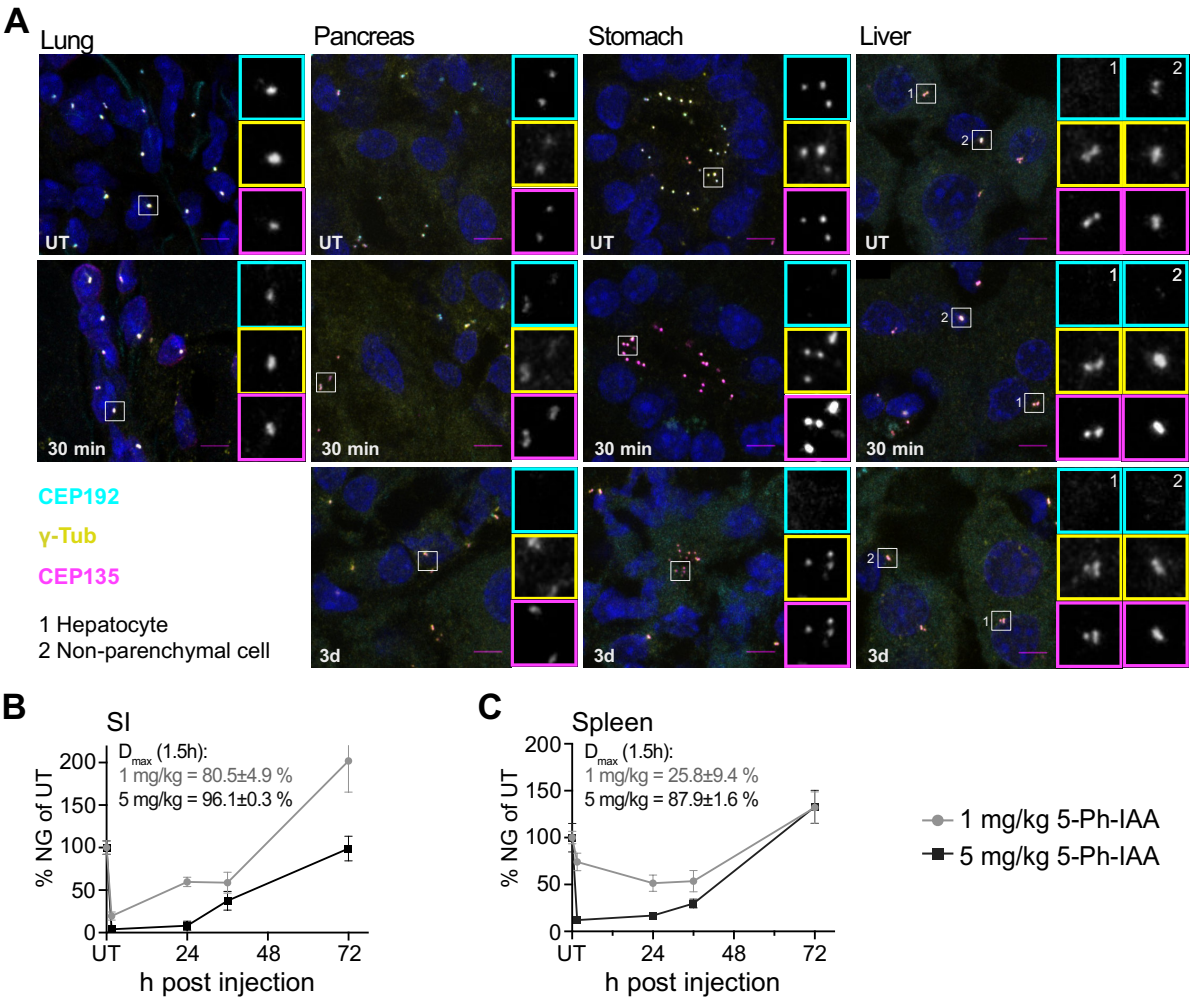
(A) Quantification of the NeonGreen signal of serum-starved *Cep192*$^{AID/AID}$ MEFs with or without OSTIR1 normalized to the mean of *Cep192*$^{AID/AID}$; *OsTir1*$^{wt/wt}$ MEFs. n = 39-42 cells per genotype.

(B-C) Quantification of *Cep192*$^{AID/AID}$ MEF cells wt/wt or wt/Tir for *OsTir1* with a primary cilium. Cells were serum starved for 24 h before 5-Ph-IAA treatment for (B) 24h or (C) 48h. N = 2 MEF lines per genotype from 1 or 2 separate passages.

(D) Time course of 5-Ph-IAA treatment of mTEC cultures showing CEP192^{AID} (NeonGreen) intensity at the centrioles of non-multiciliated cells without or with OSTIR1 (wt/*Tir*) relative to untreated control. N = 2 mice per genotype indicated by symbol shape, n = 6 fields of view.

(E-G) mTECs were cultured at an air-liquid-interphase (ALI) for 7 days to allow motile cilia formation. 5-Ph-IAA was added to the basal media for ALI days 7-9. (E) Quantification of the percentage of cells with motile cilia marked by acetylated tubulin (AcTub) and (F) the fraction of cells positive for the differentiation marker FOXJ1. N = 3 mice per genotype indicated by symbol shape, n = 6 fields of view. (G) Representative confocal images of mTEC cultures of the indicated genotypes expressing CEP192^{AID} (green) and immunostained for AcTub (gray) and FOXJ1 (red). Scale bars 10 µm.

All data is shown as mean ± SEM. Statistical significance was measured by two -way ANOVA with Sidak's multiple comparisons test (D), and two-tailed, unpaired Student's t-test (A-C, E-F). ns $p \geq 0.05$, **** $p < 0.0001$.

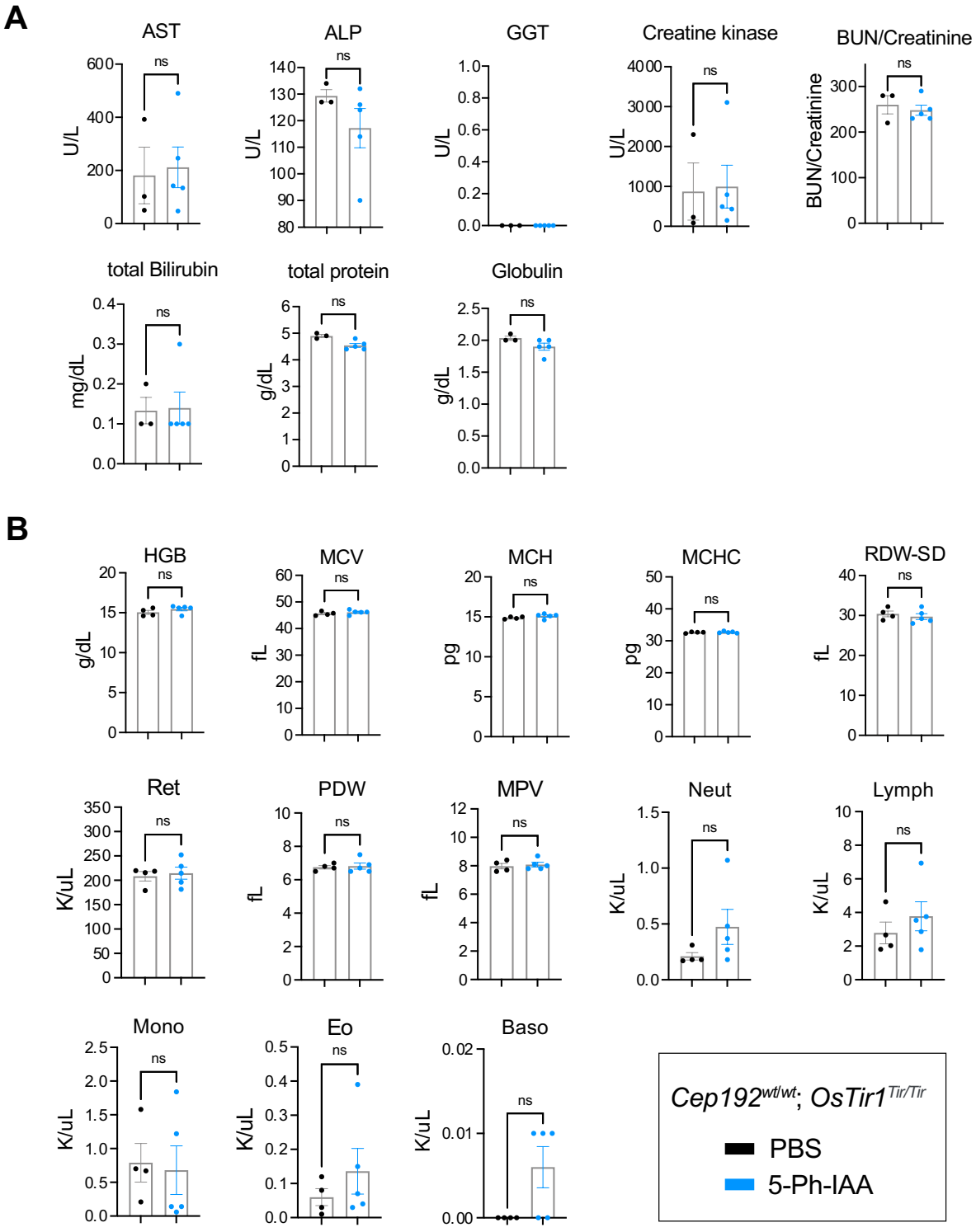


Supplemental Figure 4: Different doses of 5-Ph-IAA alter the degradation dynamics.

(A) Representative immunofluorescence images of organs from *Cep192*$^{AID/AID}$ mice expressing OSTIR1 that were untreated (UT) or injected 5-Ph-IAA. Analysis was performed 30 min after 5-Ph-IAA administration or following repeat 5-Ph-IAA administration every 12 h for 3 days. CEP192^{AID} signal (cyan), and immunostained γ-tubulin (yellow) and CEP135 (magenta); scale bar 5 µm.

(B-C) Quantification of the CEP192^{AID} NeonGreen (NG) signal relative to the untreated control (UT) of (B) the small intestine (SI) and (C) the spleen of mice injected with 1 mg/kg or 5 mg/kg 5-Ph-IAA at the indicated timepoints.

All data is shown as mean ± SEM.



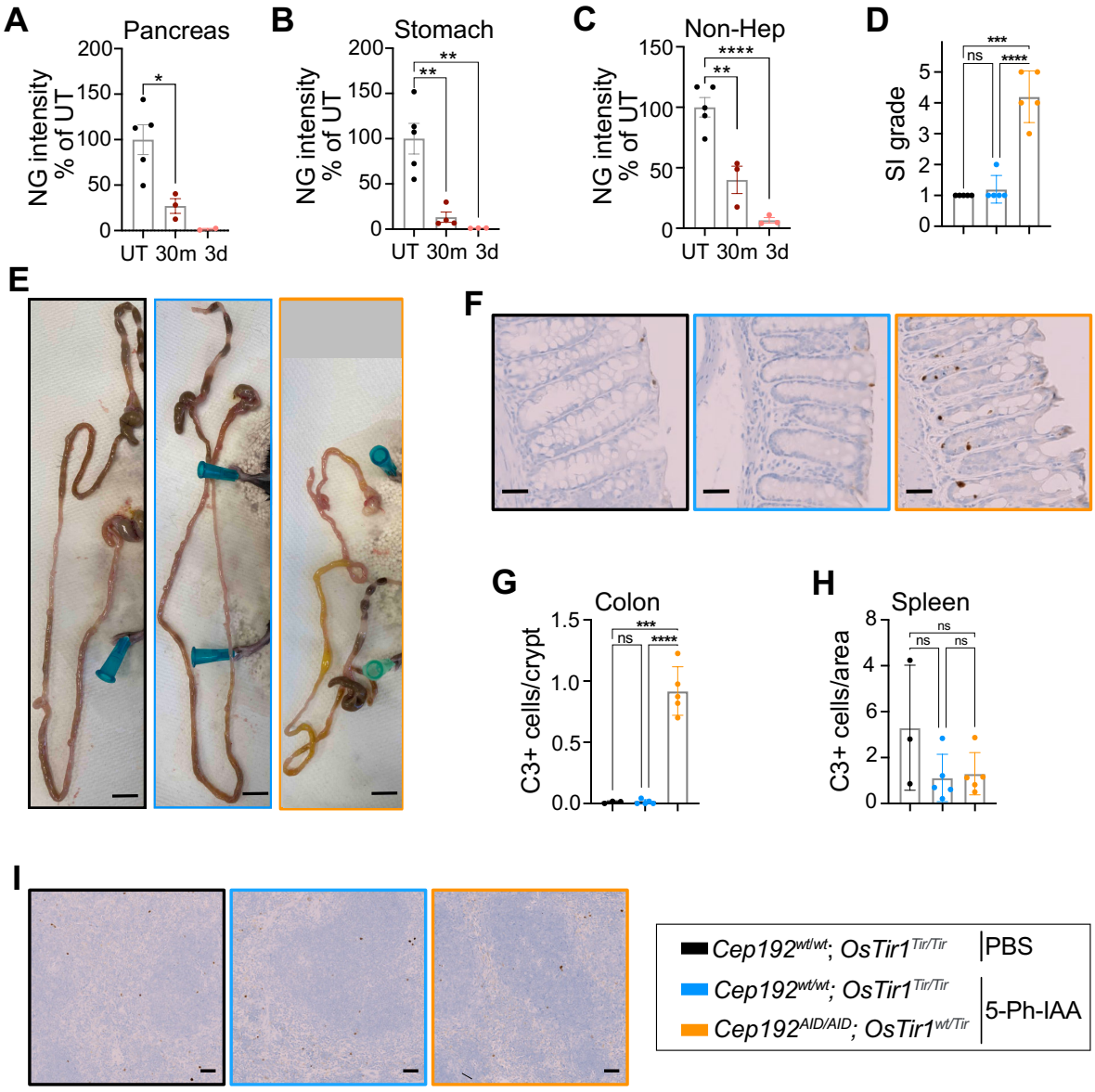
Supplemental Figure 5: Repeated dosing with 5-Ph-IAA has no impact on blood and serum parameters.

(A-B) Analysis of serum and blood parameters of *OsTir1*$^{Tir/Tir}$ mice treated with 5-Ph-IAA or PBS every 24h for 14 days.

(A) Graphs showing the serum parameters. AST – Aspartate aminotransferase, ALP – Alkaline phosphatase, GGT – Gamma-glutamyltransferase, BUN – Blood urea nitrogen.

(B) Graphs showing blood cell characterization. HGB – Hemoglobin, MCV – Mean corpuscular volume, MCH – Mean corpuscular hemoglobin, MCHC – Mean corpuscular hemoglobin concentration, RDW-SD – Red cell distribution width, Ret – Reticulocytes, PDW – Platelet distribution width, MPV – Mean platelet volume, Neut – Neutrophils, Lymph – Lymphocytes, Mono – Monocytes, Eo – Eosinophils, Baso – Basophils.

Data is displayed as mean ± SEM. Statistical significance was assessed by two-tailed, unpaired Student's t-test. ns $p \geq 0.05$.



Supplemental Figure 6: Sustained degradation of CEP192^{AID} causes gastrointestinal symptoms and cell death.

(A-C) *Cep192$^{AID/AID}$* mice expressing OSTIR1 were injected with 5 mg/kg 5-Ph-IAA. Analysis performed 30 min after 5-Ph-IAA administration or following repeat 5-Ph-IAA administration every 12 h for 3 days. CEP192^{AID} signal intensity (NeonGreen, NG) was quantified in fluorescence microscopy images relative to the untreated (UT) control in (A) the pancreas (N = 2-5), (B) the stomach (N = 3-5) and (C) in non-parenchymal liver cells (N = 3-5) at the indicated timepoints.

(D) Graph showing the severity of the gastrointestinal symptoms in mice after 8 days of repeated 5-Ph-IAA or PBS injections. Symptoms were graded 1-5 based on stool consistency and color in the small intestine. 5 = normal stool; 1 = watery liquid, yellow stool.

(E) Example images for (D) showing SI grade 1 and 5 symptoms. Images from *Cep192$^{wt/wt}$*; *OsTir1$^{Tir/Tir}$* mice treated with PBS (black box) or 5-Ph-IAA (blue box) and *Cep192$^{AID/AID}$*; *OsTir1$^{wt/Tir}$* exposed to 5-Ph-IAA (orange box). Scale bar 1cm.

(F) Cell death in the crypts of the colon was measured using immunohistochemistry for cleaved caspase-3 (C3). Representative images of the colon of *Cep192*$^{wt/wt}$; *OsTir1*$^{Tir/Tir}$ mice treated with PBS (black box) or 5-Ph-IAA (blue box), and *Cep192*$^{AID/AID}$; *OsTir1*$^{wt/Tir}$ mice injected with 5-Ph-IAA (orange box) for 8 days. Scale bars 100 µm. (G)

(G) Graph showing the number of cleaved caspase-3 positive cells per crypt.

(H) Quantification of cleaved caspase-3 positive cells in the spleen per area. Cell death was measured by immunohistochemistry for cleaved caspase-3 (C3).

(I) Representative images of the spleen of *Cep192*$^{wt/wt}$; *OsTir1*$^{Tir/Tir}$ mice injected with PBS (black box) or 5-Ph-IAA (blue box), and *Cep192*$^{AID/AID}$; *OsTir1*$^{wt/Tir}$ exposed to 5-Ph-IAA (orange box) for 8 days. Scale bars 100 µm.

All data is shown as mean ± SEM. Statistical significance was measured using one-way ANOVA with Sidak's multiple comparisons test. Of note, no statistical analysis was performed for (A) 3d, since this timepoint includes only N = 2 mice. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.