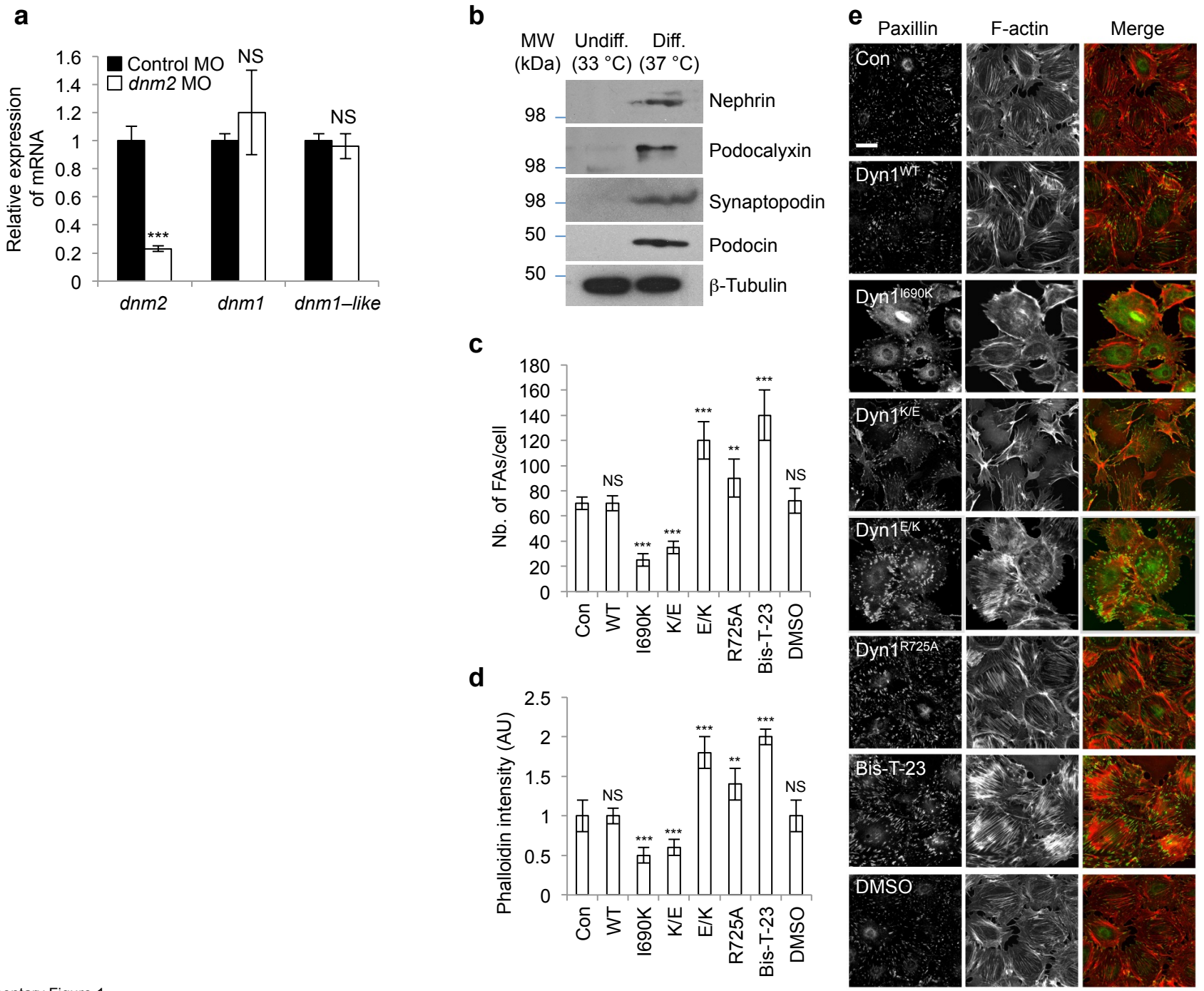
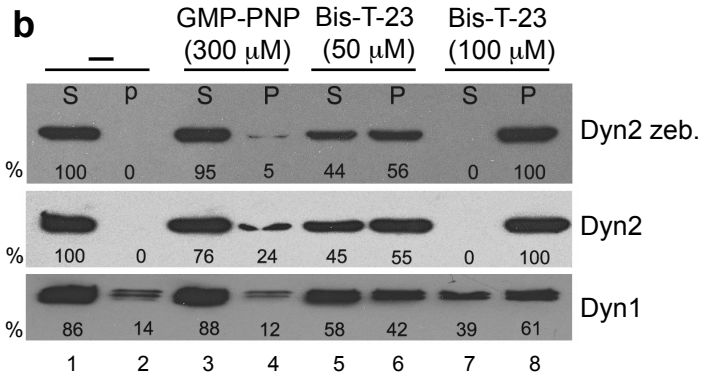


Supplementary Figure 1.

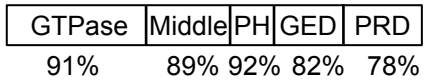
a

Protein	K_{cat} (min^{-1})
Dyn1	1.2 ± 0.2
Dyn1 + Bis-T-23	6.9 ± 0.4
Dyn2	2 ± 0.1
Dyn2 + Bis-T-23	12 ± 0.5
Dyn2 zeb.	4 ± 0.5
Dyn2 zeb. + Bis-T-23	16 ± 0.7

b



c



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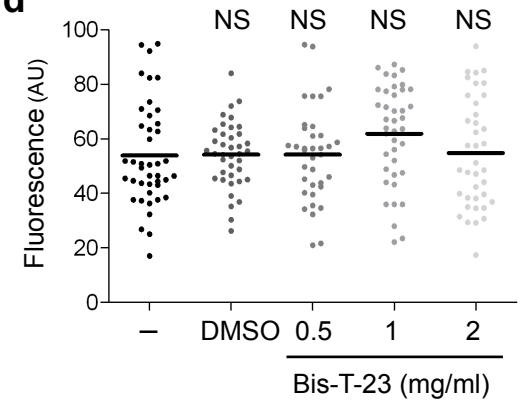

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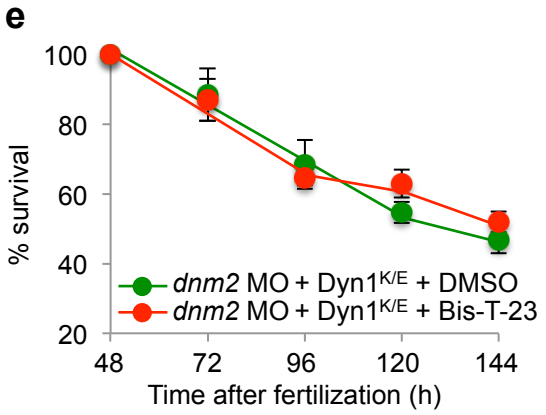

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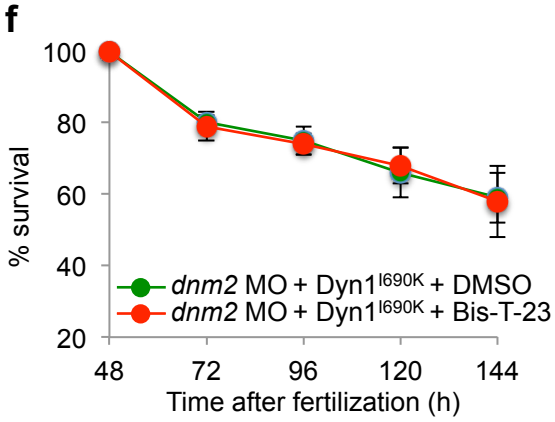

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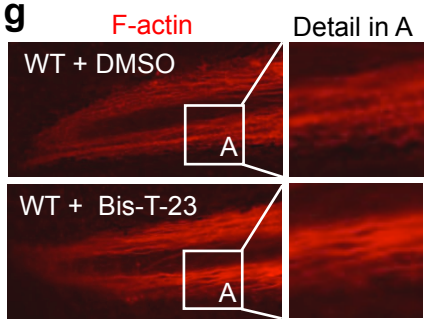
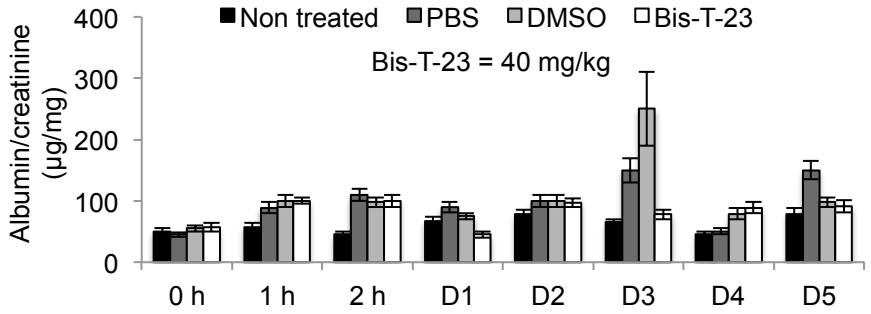


Supplementary Figure 2.

a

Time (h)	Con (μg/ml)	Con (μM)
0	–	–
0.25	14.5 ± 6	29 ± 12
0.5	11.6 ± 6	23 ± 12
1	7.7 ± 2	15 ± 4
2	1.6 ± 1	3 ± 2
4	1.1 ± 1	2 ± 2
6	0.5 ± 0.1	1 ± 0.1
8	0.3 ± 0.03	0.6 ± 0.06
24	0.04	0.08

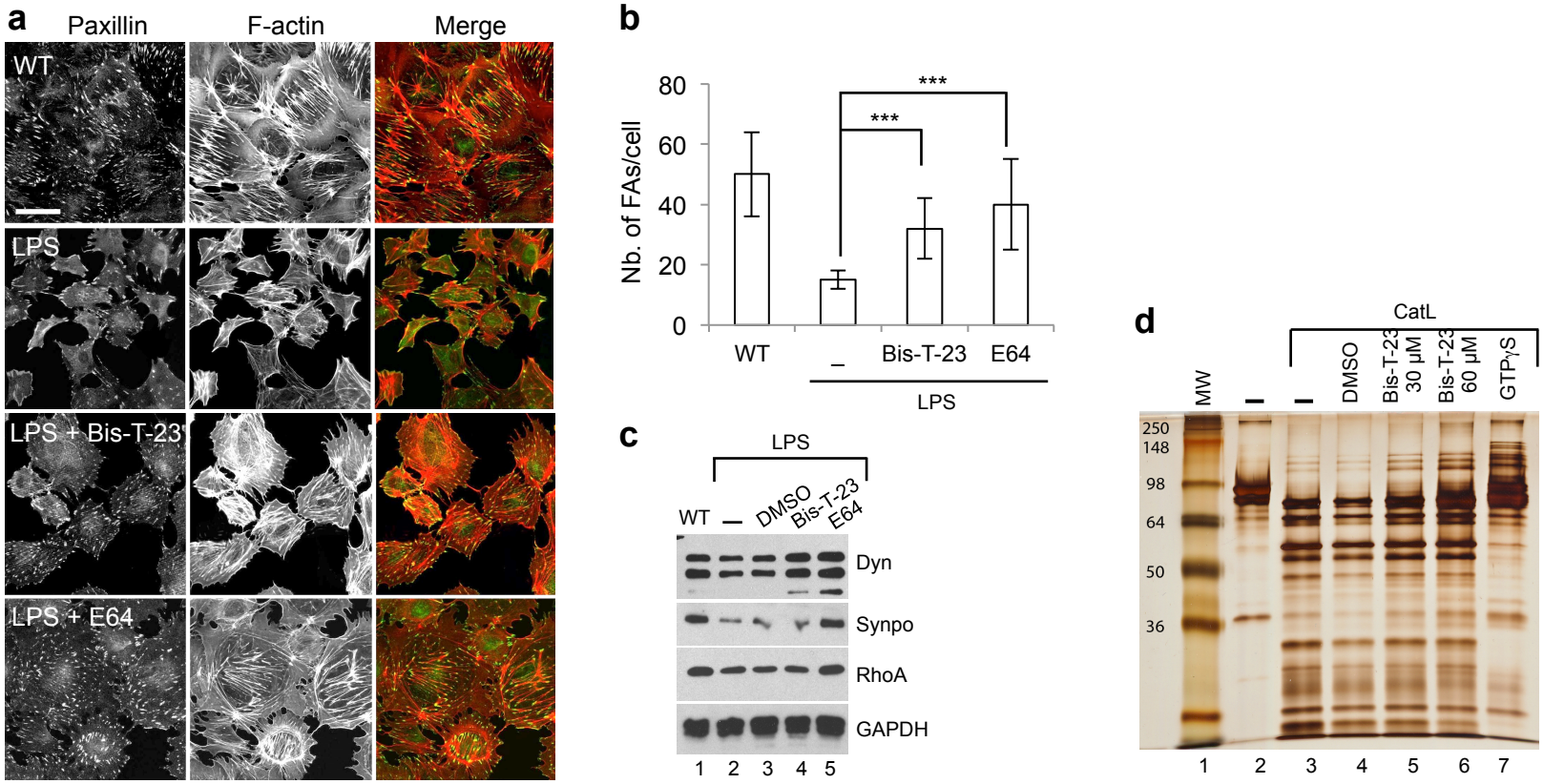
b



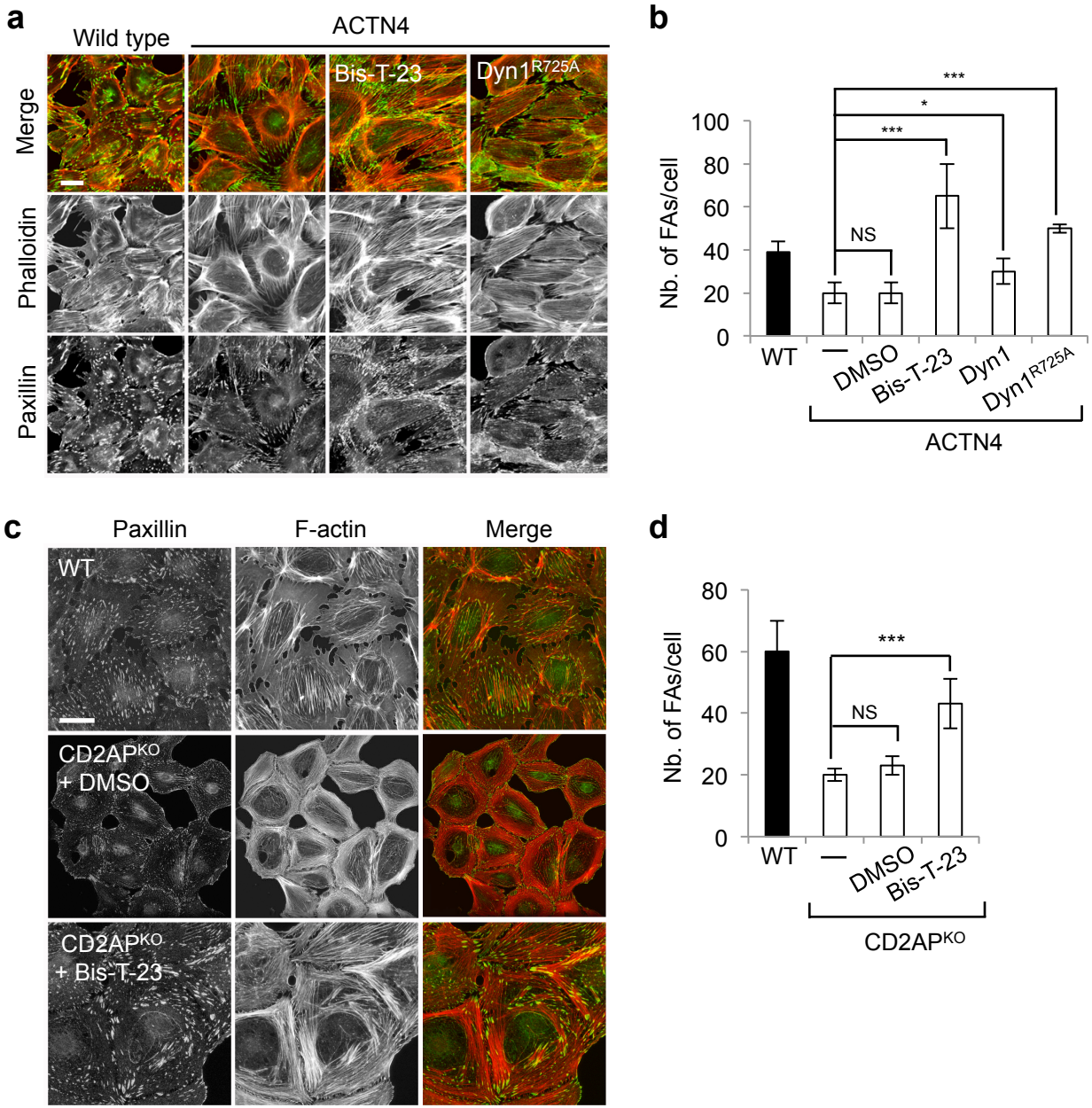
c

		BU (mg/dL)	
	CON	75 ± 11 (n = 13)	Range 57 – 89
	Bis-T-23 (2 h)	66 ± 1 (n = 3)	Range 64 – 70
	Bis-T-23 (6 h)	38 ± 1 (n = 3)	Range 37 – 39
	Bis-T-23 (24 h)	52 ± 4 (n = 3)	Range 46 – 56
2 h after treatment	LPS	68 ± 14 (n = 6)	Range 52 – 86
	LPS + Bis-T-23	52 ± 11 (n = 5)	Range 39 – 67
	LPS + DMSO	73 ± 15 (n = 3)	Range 63 – 92
24 h after treatment	LPS	59 ± 5 (n = 3)	Range 53 – 63
	LPS + Bis-T-23	68 ± 18 (n = 3)	Range 52 – 93
	LPS + DMSO	83 ± 18 (n = 3)	Range 63 – 99

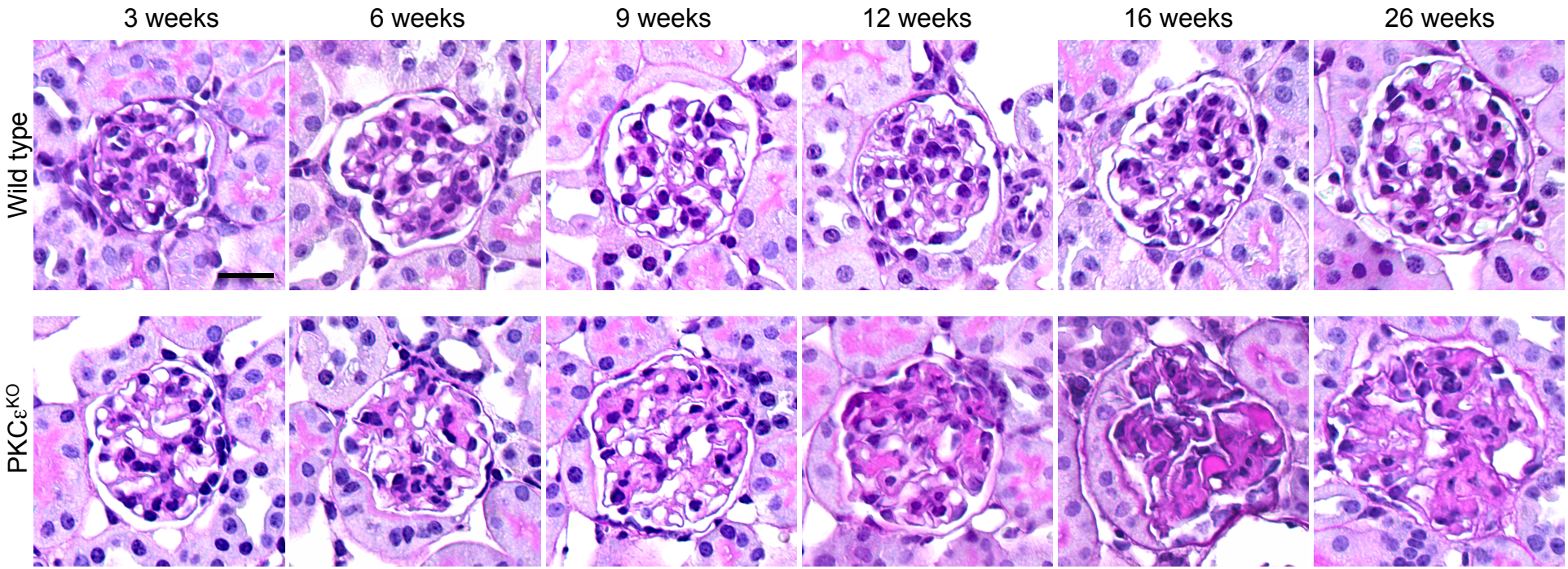
Supplementary Figure 3.



Supplementary Figure 4.

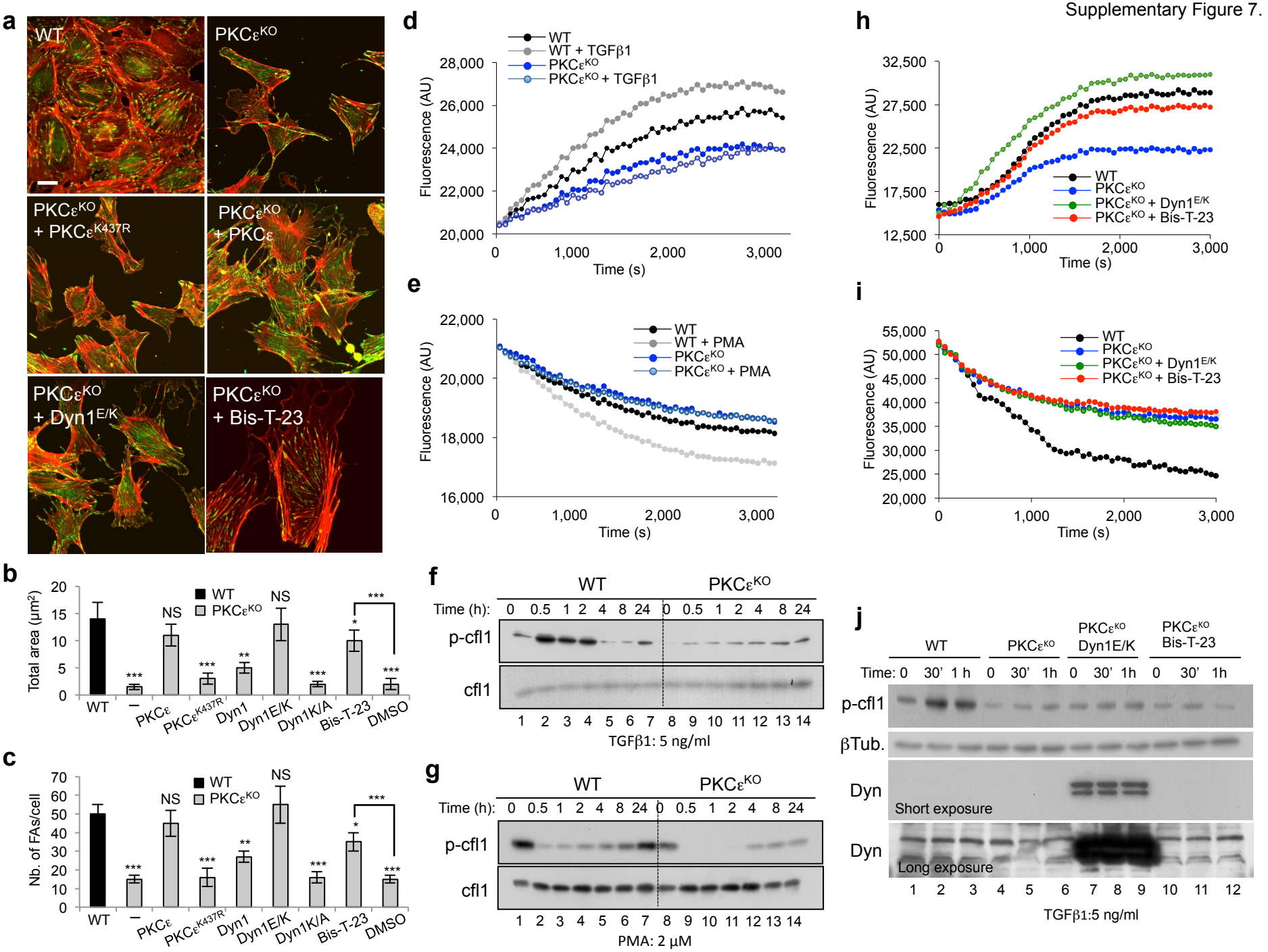


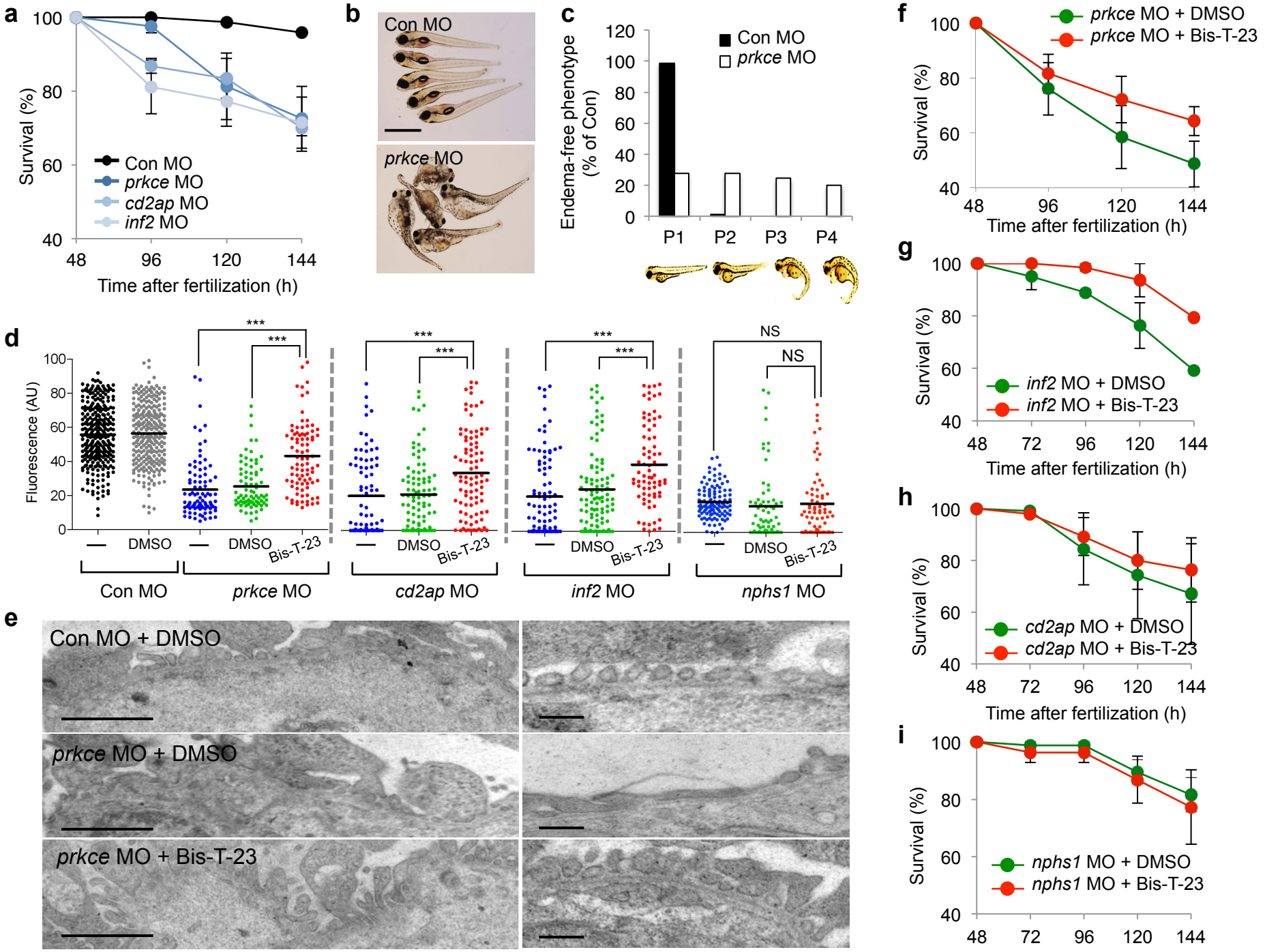
Supplementary Figure 5.



Supplementary Figure 6.

Supplementary Figure 7.





Supplementary Figure 8.

Supplementary Figure Legends

Supplementary Figure 1. Dyn^{OLIGO} regulates the actin cytoskeleton in podocytes

(**a**) Bar graph representing levels of mRNA encoding zebrafish *dnm2*, *dnm1*, and *dnm1*–like protein after larvae were injected with *dnm2*-specific morpholinos.

Error bars, mean ± SD ($P > 0.05$, not statistically significant (NS); ***$P \leq 0.001$, unpaired *t*-test).

(**b**) Western blot of cell extracts isolated from non-differentiated podocytes grown at 33˚C and differentiated podocytes grown at 37˚C. MW indicates molecular weight markers.

(**c**, **d**) Bar graphs depicting quantitative analysis of the number of focal adhesions (FAs) (**c**) and stress fibers (**d**) per cell from experiments such as those in (**e**). Each bar represents measurements of > 50 cells, from at least three independent experiments. Error bars, mean ± SD ($P > 0.05$, not statistically significant (NS); *$P \leq 0.05$; **$P \leq 0.01$, ***$P \leq 0.001$, unpaired *t*-test).

(**e**) Fluorescence microscopy of cultured podocytes expressing different dynamin mutants. When indicated, cells were treated with Bis-T-23 (30 μM) or DMSO (0.1%, vehicle control) for 30 min. F-actin and FAs were visualized with rhodamine-phalloidin and anti-paxillin antibodies respectively. Scale bar, 10 μm.

Supplementary Figure 2. Bis-T-23 promotes oligomerization of zebrafish Dyn2

(**a**) Kinetic parameters for human Dyn1, rat Dyn2, and zebrafish Dyn2 (Dyn2 zeb.). Kinetic parameters were determined by performing the GTPase assays using 0.5 μM Dyn in the presence or absence of 30 μM Bis-T-23.

(**b**) Bis-T-23 induces the formation of zebrafish Dyn2^{OLIGO}, which pellets at high speed. Western blot of zebrafish Dyn2, rat Dyn2, and human Dyn1 after high-speed

centrifugation at physiological salt in the presence of indicated concentrations of Bis-T-23 or GMP-PNP, a non-hydrolyzable analog of GTP. S indicates supernatant and P is pellet. The percentage of protein present in each sample is indicated at the bottom.

(**c**) Diagram of the domain structure of dynamin showing the N-terminal GTPase domain, the Middle domain, the PH (pleckstrin-homology) domain, the GED (GTPase effector domain), and the C-terminal PRD (proline/arginine-rich domain). The percentage of identity between rat Dyn2 and zebrafish Dyn2 calculated using the FASTA program is indicated in the figure.

(**d**) Intensity of circulating eGFP-DBP (AU: arbitrary units) in the retinal vessel plexus of the fish eye 96 hours post-fertilization. Wild-type zebrafish larvae were injected in the cardinal vein with increasing doses of Bis-T-23. Untreated, *n* = 42; DMSO, *n* = 39; Bis-T-23 (0.5 mg/ml), *n* = 36; Bis-T-23 (1 mg/ml), *n* = 40; Bis-T-23 (2mg/ml), *n* = 38.

Statistical analysis was performed using unpaired *t*-test and no statistical significance was observed between the treatments.

(**e**, **f**) Survivorship curves of *dnm2* MO morphants expressing $Dyn1^{K/E}$ or $Dyn1^{I690K}$. Morphants were treated with either Bis-T-23 (1 ng/larvae) or DMSO (20%, vehicle control). *dnm2 MO* + $Dyn1^{K/E}$ + DMSO, *n* = 200; *dnm2* MO + $Dyn1^{K/E}$ + Bis-T-23, *n* = 113; *dnm2* MO + $Dyn1^{I690K}$ + DMSO, *n* = 210; *dnm2* MO + $Dyn1^{I690K}$ + Bis-T-23, *n* = 104.

(log-rank: $P \geq 0.05$, not statistically significant).

(**g**) Wild-type zebrafish larvae were injected with Bis-T-23 (10 ng/larvae) in the cardinal vein. Animals were stained for F-actin using rhodamine-phalloidin. Bis-T-23-treated larvae displayed more pronounced actin bundles in their tails indicating a systemic effect of Bis-T-23 on F-actin.

Supplementary Figure 3. Bis-T-23 is not nephrotoxic

(**a**) Pharmacokinetics of Bis-T-23 in C57BL/6J mice (*n* = 3). Animals were injected with Bis-T-23 (40 mg/kg). Concentration of Bis-T-23 in plasma was measured using mass spectrometry. Concentration shown in µg/ml was converted to µM.

(**b**) Proteinuria of C57BL/6J mice determined by spot urine test pre-injection (0 h) and after daily injection of Bis-T-23 (40 mg/kg), DMSO (1%, vehicle), or equal volume of PBS. Error bars, mean ± SD. Statistical analysis was performed using unpaired *t*-test for animals treated with Bis-T-23, and no statistical significance was observed between the treatments.

(**c**) Blood urea (BU) levels at indicated conditions.

Supplementary Figure 4. Bis-T-23 protects dynamin from CatL proteolysis

(**a**) Bis-T-23 restores the actin cytoskeleton after LPS-induced dysregulation in cultured murine podocytes. Murine podocytes were treated with LPS (150 ng/ml) for 48 hours, followed by addition of Bis-T-23 (30 µM) for an additional 30 min. When indicated, E64 (20 µM), a CatL inhibitor, was added at the same time as LPS. FAs and F-actin were visualized with anti-paxillin antibodies and rhodamine-phalloidin, respectively. Scale bar, 10 µm.

(**b**) Bar graph depicting the number of FAs per cell under the treatments described in (**a**). Each bar represents measurements of > 50 cells, from at least three independent experiments. Error bars, mean ± SD (***$P \leq 0.001$, unpaired *t*-test).

(**c**) Bis-T-23 restores Dyn levels without affecting levels of synaptopodin (Synpo) and RhoA in podocytes upon treatment with LPS. Western blot analysis of protein levels in cell extracts isolated from murine podocytes treated with LPS (150 ng/ml) for 24 hours. Subsequently, DMSO (0.1%, vehicle) or Bis-T-23 (30 µM) was added for 12 hours. E64 (20 µM) was added at the beginning of the experiment for 24 hours. GAPDH was used as a loading control.

(**d**) Silver staining of Dyn1 (1 μM) before and after addition of CatL (1.5 μM). When indicated, the assay contained DMSO (2.5%), indicated concentrations of Bis-T-23, or GTPγS (1 mM).

Supplementary Figure 5. Bis-T-23 targets the actin cytoskeleton

(**a**) Fluorescence microscopy shows that podocytes contain a well-defined actin cytoskeleton (panel 1). Stable expression of α-actinin 4 mutant protein (*Actn4*$^{K256E/K256E}$ cells herein referred to as ACTN4 cells) results in the formation of hyper-bundled actin structures formed from thin actin fibers as well as a decrease in the number of mature FAs (panel 2). Where indicated, cells were treated with Bis-T-23 (30 μM) for 30 min. Additionally, Dyn1^{R725A} was adenovirally overexpressed in ACTN4 cells for 18 hours, prior to fixation. FAs and F-actin were visualized with anti-paxillin antibodies and rhodamine-phalloidin, respectively. Scale bar, 10 μm.

(**b**) Bar graph depicting quantitative analysis of the number of FAs per cell from experiments such as in (**a**). Each bar represents measurements of > 50 cells, from at least three independent experiments. Error bars, mean ± SD ($P > 0.05$, not statistically significant (NS); *$P \leq 0.05$; ***$P \leq 0.001$, unpaired *t*-test with respect to untreated ACTN4 cells).

(**c**) CD2AP^{KO} podocytes (*Cd2ap*$^{-/-}$ cells) in culture lose dorsal and ventral stress fibers, which are accompanied by the loss of mature FAs within the cell body. Cells also exhibit an increase in the number of arcs. When indicated, Bis-T-23 (30 μM) or DMSO (0.1%) was added for 30 min. Addition of Bis-T-23 but not DMSO resulted in the formation of FAs within the cell body as well as the formation of ventral stress fibers (stress fibers that are anchored on each side with focal adhesions). Scale bar, 10 μm.

(**d**) Bar graph depicting the number of FAs per cell from experiments such as in (**c**). Each bar represents measurements of > 50 cells, from at least three independent experiments. Error bars, mean ± SD ($P > 0.05$, not statistically significant (NS); ***$P \leq 0.001$, unpaired *t*-test*)*.

Supplementary Figure 6. PKCε^{KO} mice exhibits progressive glomerular injury

Representative images of glomeruli after Periodic acid–Schiff (PAS) staining reveal progressive mesangial expansion and glomerular injury at later stages in PKCε^{KO} mice. The animals were sacrificed at the indicated time points and compared to age-matched control mice. The mesangial expansion is detectable as early as 6 weeks of age. It progresses with detectable lesions in the majority of glomeruli at 16 to 26 weeks. Scale bar, 20 μm.

Supplementary Figure 7. PKCε^{KO} podocytes exhibit global dysregulation of actin

(**a**) Fluorescence microscopy of cultured podocytes lacking *Prkce* gene (*Prkce*$^{-/-}$) herein referred to PKCε^{KO} cells, transiently expressing wild-type PKCε, mutant PKCε in which kinase activity has been impaired (PKCε^{K437R}), or Dyn1$^{E/K}$ for 24 hours. When indicated, cells were treated with Bis-T-23 (30 μM) for 30 min. F-actin and FAs were visualized with rhodamine-phalloidin and anti-paxillin antibodies respectively. Scale bar, 10 μm.

(**b**, **c**) Bar graph depicting quantitative analysis of the cell size (**b**) and number of FAs per cell (**c**) from experiments such as in (**a**). Each bar represents measurements of > 50 cells, from at least three independent experiments. Error bars, mean ± SD ($P > 0.05$, not statistically significant (NS); *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$, unpaired *t*-test with respect to wild type cells*)*.

(**d**, **e**) Solution-based actin polymerization (**d**) and depolymerization (**e**) using cell extracts generated from wild type (WT) or PKCε^{KO} podocytes. At time zero, pyrene-labeled monomeric actin (2 μM) was added, and pyrene fluorescence was monitored. To induce actin polymerization and depolymerization, the cells were pretreated with TGF-β1 (5 ng/ml) or PMA (phorbol 12-myristate 13-acetate) (2 μM) for 30 min.

(**f**, **g**) Western blot analysis of cofilin phosphorylation in wild-type (WT) and PKCε^{KO} podocytes. When indicated, cells were stimulated with TGF-β1 (5 ng/ml) (**f**) or PMA (2 μM) (**g**) for 30 min. Total protein was isolated at indicated time points and the amount of inactive phosphorylated cofilin and total cofilin were detected using anti-cofilin antibody.

(**h**, **i**) Solution-based actin polymerization (**h**) and depolymerization (**i**) using cell extracts generated from wild-type (WT) and PKCε^{KO} podocytes transiently expressing Dyn1$^{E/K}$ for 24 hours. When indicated, Bis-T-23 (30 μM) was added to the cell extracts. At time zero, pyrene-labeled monomeric actin (2 μM) was added and pyrene fluorescence was monitored. Neither treatment had an effect on actin depolymerization (**i**).

(**j**) Western blot analysis of cofilin phosphorylation in wild-type (WT) and PKCε^{KO} podocytes transiently expressing Dyn1$^{E/K}$ or treated with Bis-T-23 (30 μM). Cells were stimulated with TGF-β1 (5 ng/ml) for 30 min. Total protein was isolated at indicated time points and the amount of inactive phosphorylated cofilin was detected using anti-phospho-cofilin antibody. β-tubulin was used as a loading control and the levels of endogenous and overexpressed Dyn1$^{E/K}$ (lanes 7–9) were examined.

Supplementary Figure 8. Bis-T-23 ameliorates proteinuria and extends survivorship in diverse genetic models of kidney injury in zebrafish

(**a**) Survivorship curves of scrambled, Con MO, *prkce* MO, *cd2ap* MO, and *inf2* MO morphants. Control MO, *n* = 81; *prkce* MO, *n* = 101; *cd2ap* MO, *n* = 77; *inf2* MO, *n* = 62.

Log-rank when compared to Con MO for all conditions: $P < 0.0001$.

(**b**) Phenotype of zebrafish larvae injected with either scrambled (Control MO) or *prkce*–specific morpholino (*prkce* MO) 120 hours post-fertilization. Scale bar, 2 mm.

(**c**) Phenotype of *prkce* MO morphants was categorized into 4 groups: P1 = no edema, P2 = mild edema, P3 = severe edema, and P4 = very severe edema.

As compared to Con MO knockdown of *prkce* induces edema in 75% of morphants.

(**d**) Intensity of circulating eGFP-DBP (AU: arbitrary units) in the retinal vessel plexus of the fish eye 96 hours post-fertilization in the Control MO, *prkce* MO, *cd2ap* MO, *inf2* MO, and *nphs1* MO morphants. Morphants were co-injected with Bis-T-23 (1 ng per larvae), or DMSO (20% per larvae, vehicle). Control MO, $n = 296$; Control MO + DMSO, $n = 265$; *prkce* MO, $n = 92$; *prkce* MO + DMSO, $n = 78$; *prkce* MO + Bis-T-23, $n = 88$; *cd2ap* MO, $n = 40$; *cd2ap* + DMSO, $n = 91$; *cd2ap* + Bis-T-23, $n = 90$; *inf2* MO, $n = 92$; *inf2* MO + DMSO, $n = 99$; *inf2* MO + Bis-T-23, $n= 74$; *nphs1* MO $n = 114$; *nphs1* MO + DMSO, $n = 64$; *nphs1* + Bis-T-23, $n = 70$. Black lines represent median intensity in each group ($P > 0.05$, not statistically significant (NS); $^{***}P \leq 0.001$, unpaired *t*-test).

(**e**) TEM of glomeruli analyzed in zebrafish larvae 120 hours post-fertilization. When indicated, animals were injected with either Control MO or *prkce* MO. Animals were injected with DMSO (20% per larvae, vehicle) or Bis-T-23 (1 ng per larvae). Micrographs reveal reversal of FP effacement in the presence of Bis-T-23. Scale bars, 2 μm and 500 nm (zoomed-in images on the right), respectively.

(**f**–i) Survivorship curves of *prkce* MO (**f**), *inf2* MO (**g**), *cd2ap* MO (**h**), and *nphs1* MO (**i**) morphants. Morphants were treated with either Bis-T-23 (1 ng/larvae) or DMSO (20%, vehicle). *prkce* MO + DMSO, $n = 96$; *prkce* MO + Bis-T-23, $n = 110$; *cd2ap* MO + DMSO, $n = 128$; *cd2ap* MO + Bis-T-23, $n = 150$; *inf2* MO + DMSO, $n = 105$; *inf2* MO + Bis-T-23, $n = 91$; *nphs1* MO + DMSO, $n = 57$; *nphs1* MO + Bis-T-23, $n = 55$.

Log-rank: *prkce* MO, $P = 0.0011$; *inf2* MO, $P = 0.0005$; *cd2ap* MO, $P = 0.012$; *nphs1* MO, $P = 0.365$ (not statistically significant).