Supplement

Supplement Figure 1.

A) Schematic depiction of the kidney filtration barrier that consists of podocyte foot processes (FP), slit diaphragm (SD), glomerular basement membrane (GBM) and endothelial cells. Filtration occurs from the capillary lumen into the urinary space. Upper panel, healthy foot processes. Lower panel, effaced foot processes.

B) Labeling of secretory lysosomes (arrow) in cultured differentiated podocytes using anti-CatL antibody (red) and anti-Lamp-2 antibody (green). Staining was carried out in cells treated with 50 μg/ml of LPS for 24 hours.

C) Cultured podocytes were stained with anti-mannosidase alpha antibody (red) and anti-Lamp-2 antibody to specifically label lysosomes (green). Staining was carried out in untreated cells (left panels), or cells treated with 50 μg/ml of LPS for 24 hours (right panels).

D) Lysosomal leakage assay using Lucifer Yellow. Lysosomes of untreated control cells, cells after LPS treatment, and cells after treatment with sphingosine (lysosomal leakage) were analyzed for the presence of Lucifer Yellow. Note that lysosomal integrity was not affected by LPS.

E) Detection of endogenous WT-1 transcription factor from fractionated podocyte lysates. Fractions containing soluble (S), membranes (P) and nuclei (N) were obtained. Note that soluble fractions are not contaminated by nuclear proteins.

F) Double immunofluorescence of cultured CatL -/- fibroblasts after transfection with a control vector (left panels), HA-tagged Pre-Pro-CatL (middle panels) and HA-tagged short CatL (right panels). The expression of short CatL yields cytoplasmic distribution

G) Subcellular fractionation of podocytes after transfection with HA-tagged Pre-Pro-CatL, and HA-tagged short CatL and Western Blot using anti-HA antibodies.

Supplement Figure 2.

A) Immunocytochemistry of dynamin in rat kidney using Hudy-1 antibody during PAN-nephrosis. The results are similar to the findings during LPS nephrosis.

B) Quantitative PCR analysis of CatL in wt and CatL knockdown podocytes.

C) Immuoblot of CatL in control podocytes and after stable knockdown of CatL using siRNA.

Supplement Figure 3.

A) RT-PCR of dynamin in podocytes. For total RNA isolation, differentiated podocytes were harvested and lysed in Trizol reagent. RT was performed using oligo-dT primer and Superscript TM II reverse transcriptase (Invitrogen, Carlsbad, CA). PCR was done with DNA Taq polymerase (Qiagen, Germantown, MD). The primer pairs used for amplifying dynamin isoforms were as follows:

Dyn1, 5' CGACATTGAGCTGGCTTACA 3' (For), 5' CATCGAGTGCATGAAGCTGT 3' (Rev)

Dyn2, 5' ACCCCACACTTGCAGAAAAC 3' (For), 5' GGCTCTTTCAGCTTGACCAC 3' (Rev)

Dyn3, 5' CACTCTTCAACACCGAGCAA 3' (For), 5' GGTTGCGTATGGTCTCCACT 3' (Rev)

B) Different amounts of recombinant dyn1 and dyn2 were probed in a Western Blot using hudy1 or MAB 5402 antibodies.

C) Immunogold electron micrographs of gene transferred CMV-driven and podocine driven expression of dyn^{K44A}.

D) Western blot analysis of CatL -/- kidney extracts after gene delivery of dyn^{WT} before and after LPS.

Supplement Figure 4.

A) Urinary protein profiles in relation to podocyte FP morphology over time following gene delivery of podocin-driven dyn^{K44A} in mice. Please note that proteinuria correlated with podocyte FP effacement. Resolution of podocyte structure and proteinuria was obvious after termination of dyn^{K44A} expression.

Supplement Figure 5.

Cultured podocytes were infected with adenoviruses expressing indicated dynamin constructs. 18 h after infection, cells were stained using hudy1 (green) to stain for dynamin and rhodamine phalloidin (red) to visualize F-actin.

Supplement Figure 5.

A) Effects of dynamin mutants on rhodamine transferrin (R-Tfn) internalization in podocytes. Internalization of rhodamine- transferrin (R-Tfn) was performed using 20 μg/ml R-Tfn in PBS containing 1 mM $CaCl_2$, 1mM $MgCl_2$, 5 mM glucose, and 0.2% BSA for 10 min at 37˚C.

B) Cultured podocytes were infected with adenoviruses expressing auxilin mutant, aux^{H875Q}, and 18 h after infection, cells were stained with anti-auxilin or anti-dynamin (for non-infected undifferentiated control cells) antibody, and rhodamine phalloidin (red) to visualize F-actin.

C) Cultured podocytes were transiently transfected with DNA encoding p40 under control of the podocine promoter. Efficiency of transfection was ~5%. Cells were stained using anti-dynamin GTPase antibody that recognizes N-terminal GTPase domain and thus p40 fragment (green), and rhodamine phalloidin (red) to visualize F-actin. Note the background staining of nuclei in untransfected cells.

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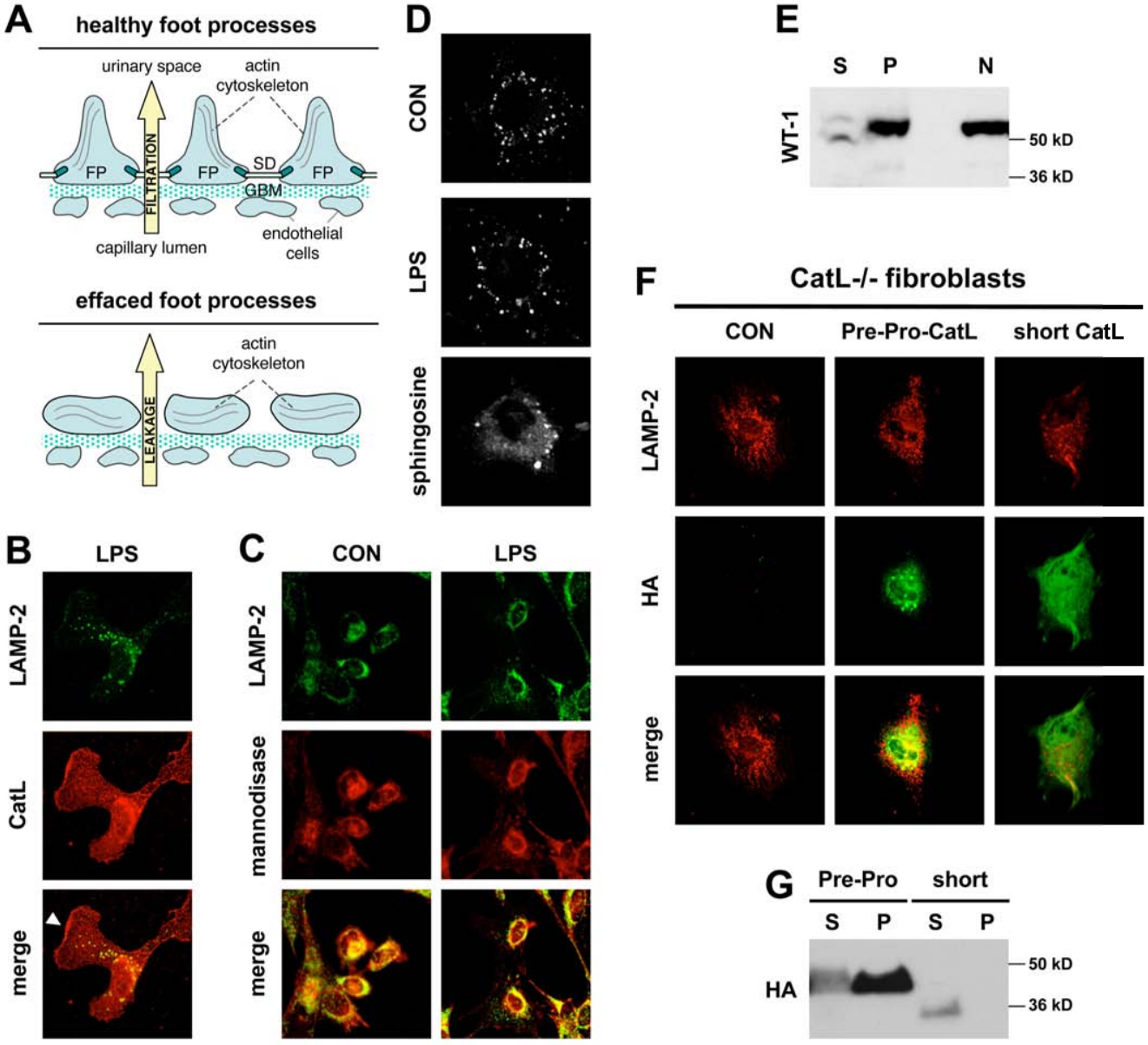
SUPPLEMENTARY FIGURE S1



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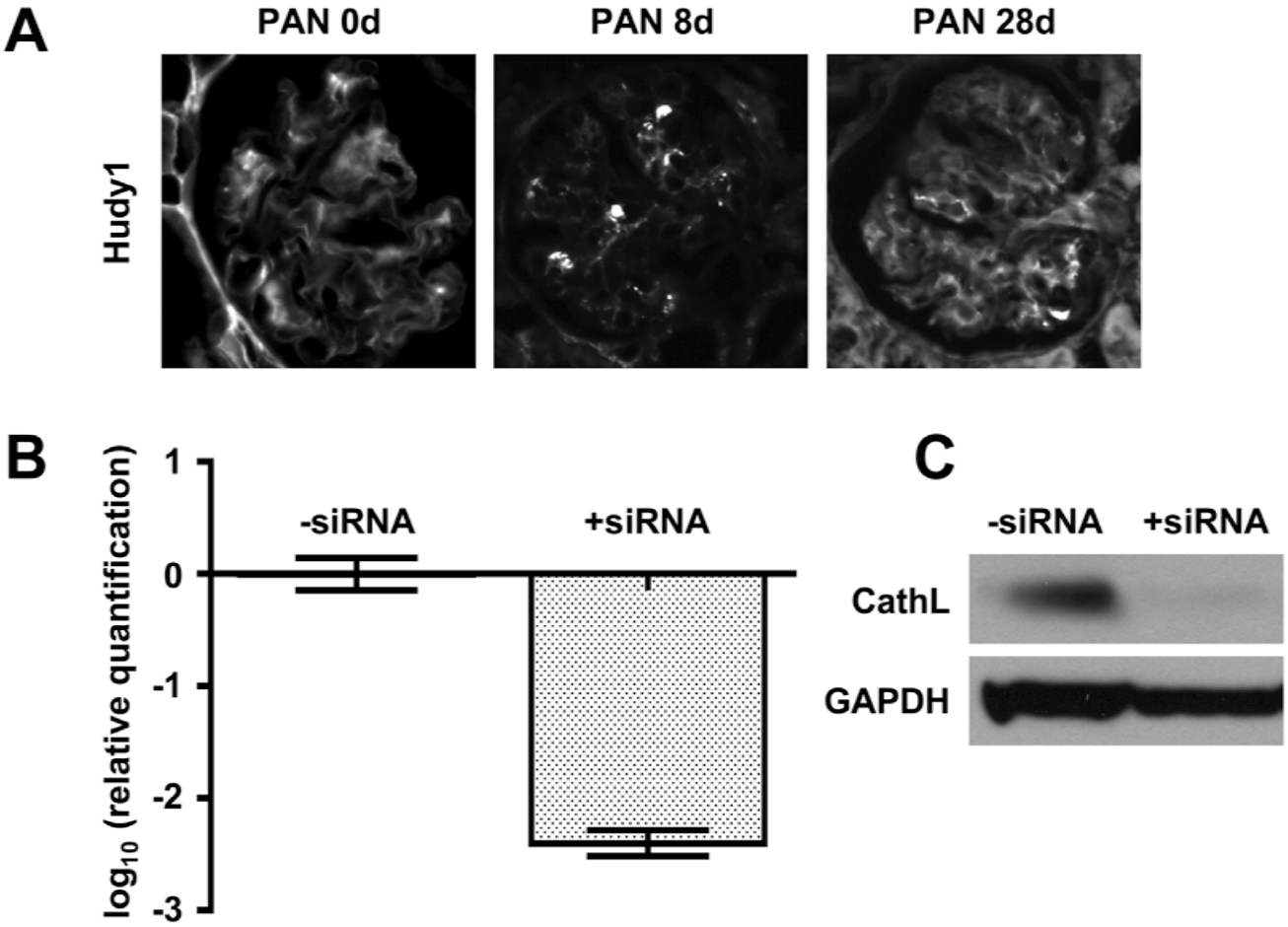
SUPPLEMENTARY FIGURE S2



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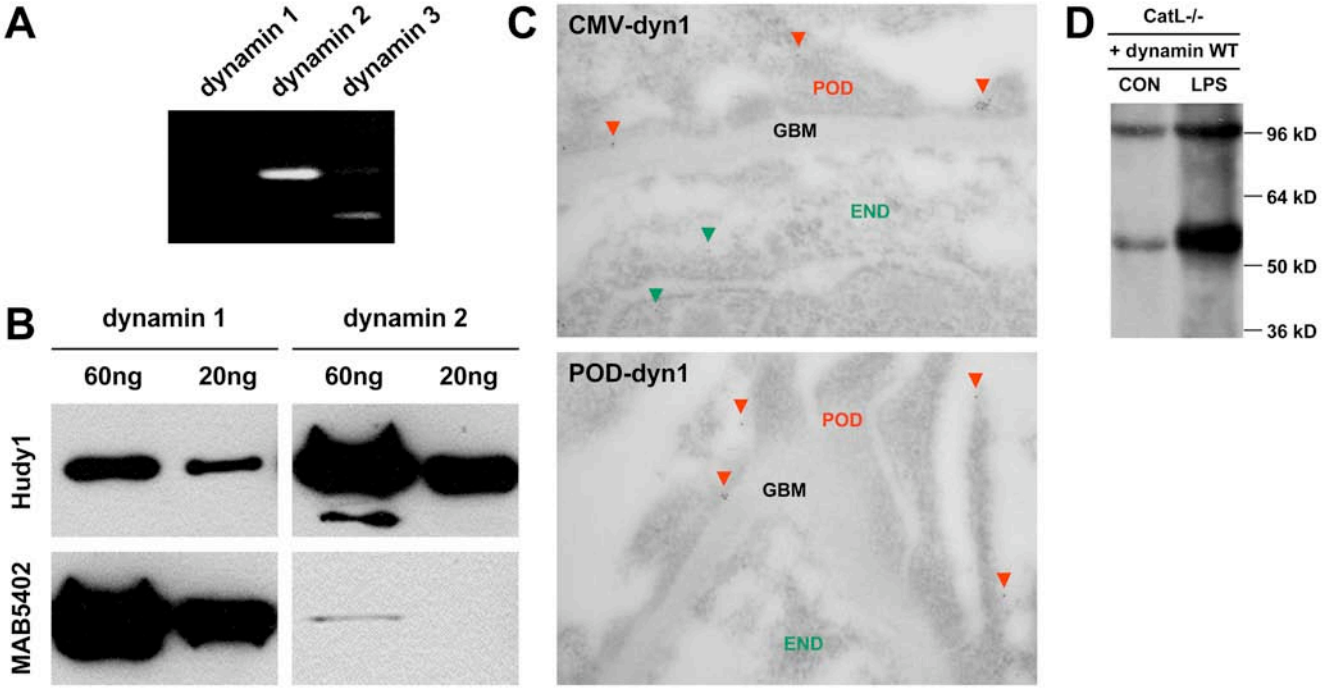
SUPPLEMENTARY FIGURE S3



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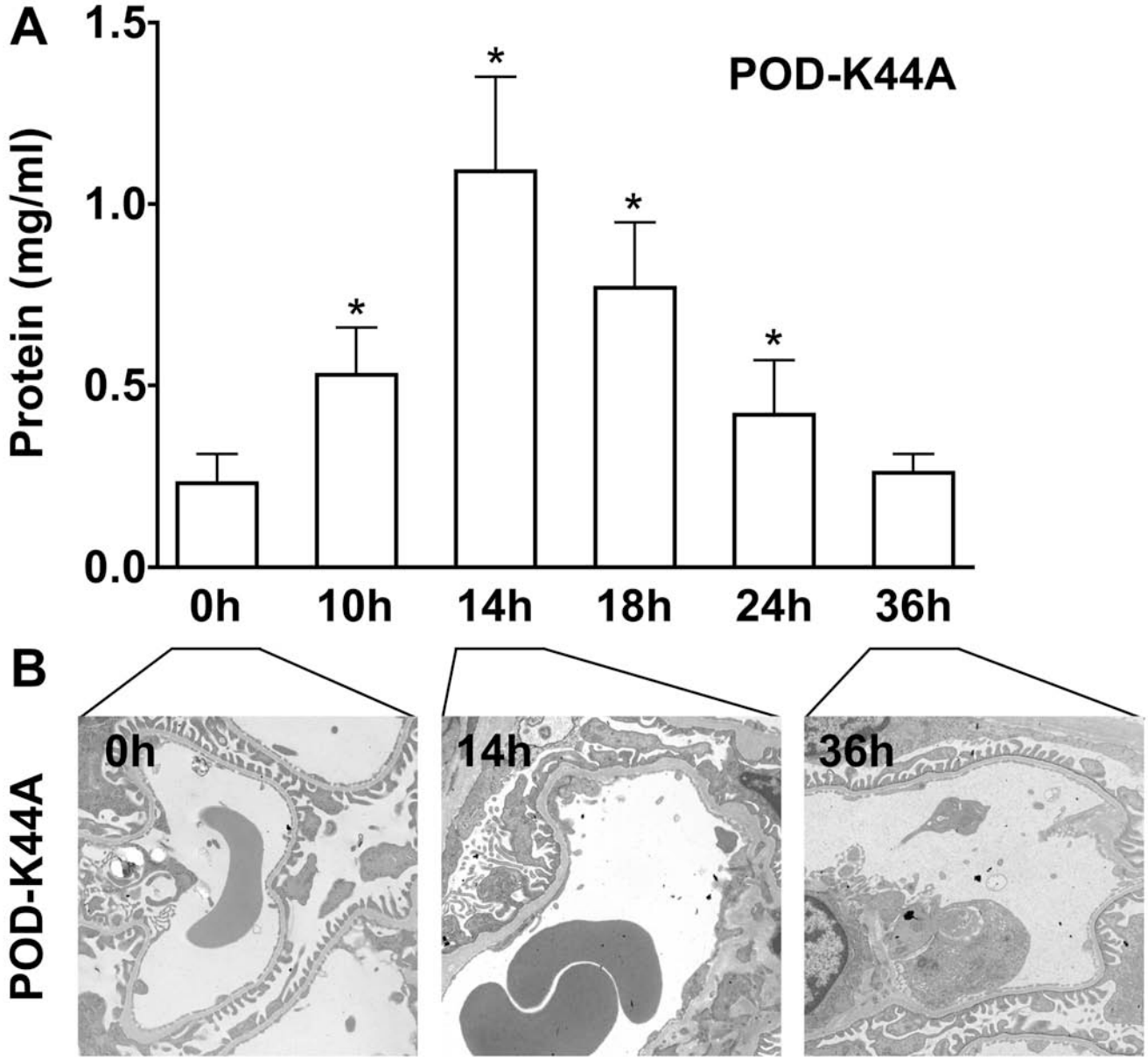
SUPPLEMENTARY FIGURE S4



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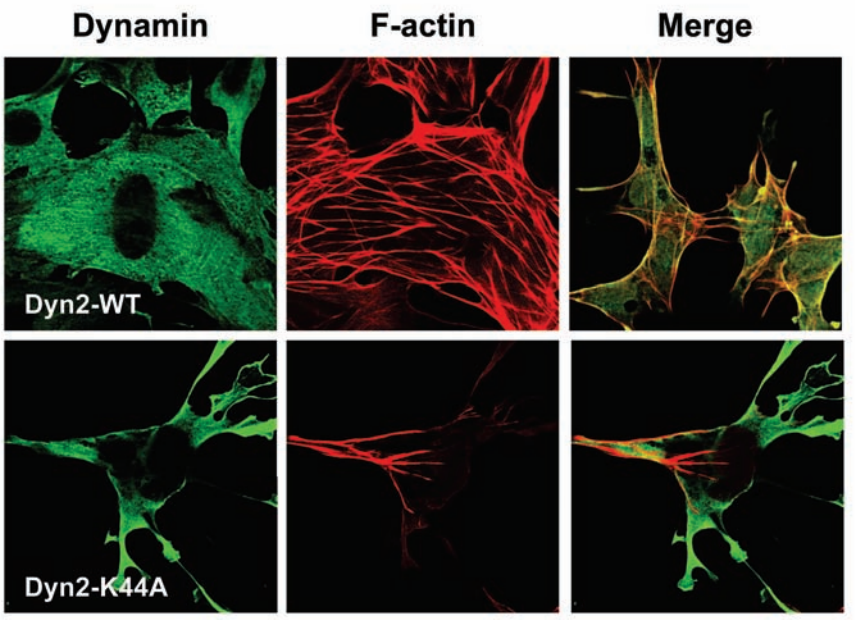
SUPPLEMENTARY FIGURE S5



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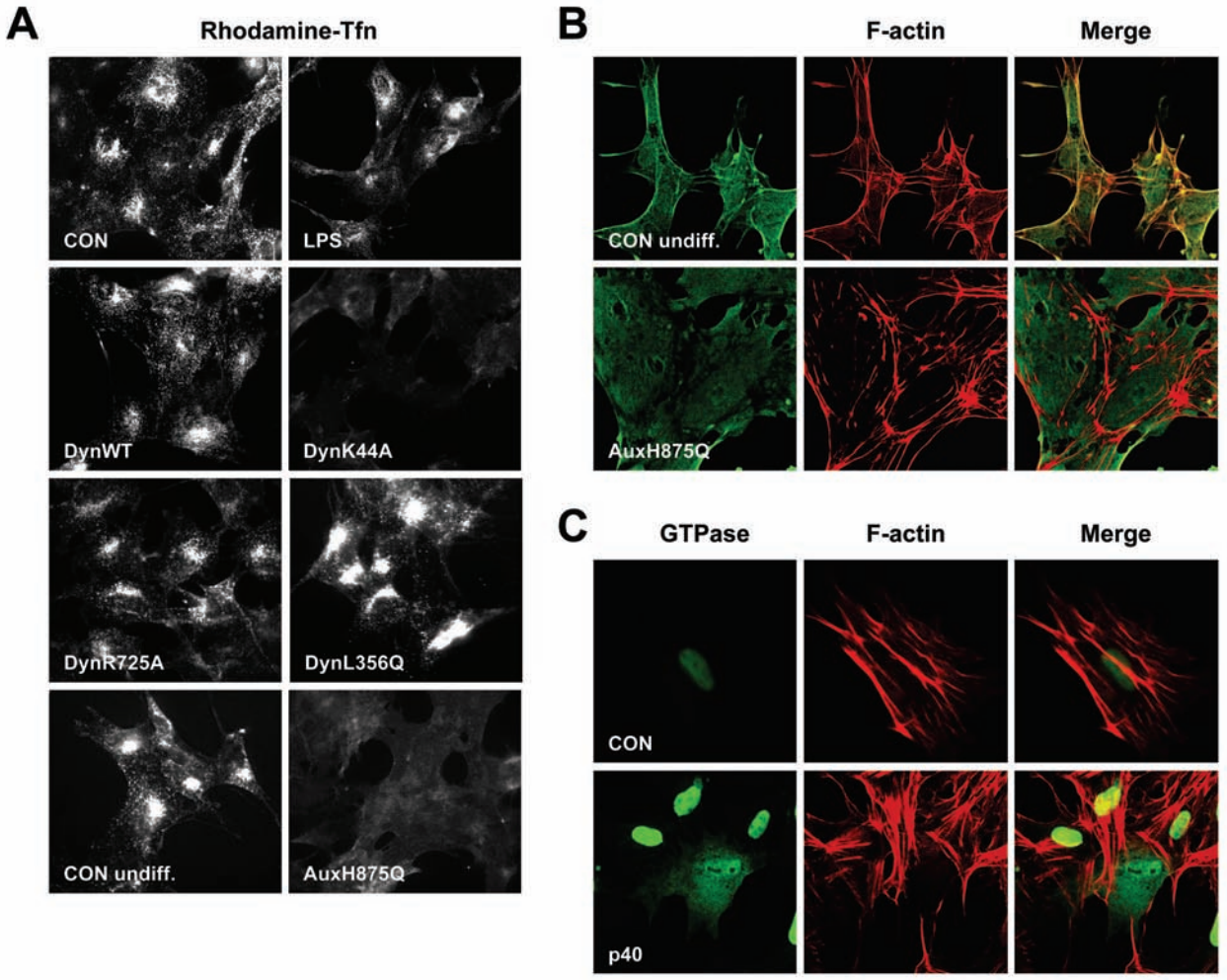
SUPPLEMENTARY FIGURE S6



Supplement Table 1. Protein (mg/ml) in mice urine determined by standard Bradford assay.

Experiment					
FIGURE 6A	**t=0 h**	**t=14 h**	**p-value**	**t=24 h**	**p-value**
CON	0.26±0.09	0.40±0.10	-	0.37±0.20	-
WT	0.28±0.06	0.54±0.13	0.25	0.46±0.16	0.19
K44A	0.24±0.08	1.09±0.27	0.001	0.42±0.16	0.20
p40	0.33±0.14	0.87±0.18	0.001	0.52±0.27	0.22
FIGURE 6C	**t=0 h**	**t=48 h**	**p-value**	**t=60 h**	**p-value**
CON	0.24±0.11	1.26±0.39	-	1.32±0.37	-
WT	0.30±0.11	1.17±0.20	-	1.10±0.18	0.53
L356Q	0.18±0.08	1.20±0.28	-	0.71±0.09	0.002
R725A	0.28±0.10	1.15±0.17	-	0.38±0.23	0.002
FIGURE 6D	**t=0 h**	**t=48 h**	**p-value**	**t=60 h**	**p-value**
CON	0.29±0.09	1.49±0.37	-	1.38±0.16	-
WT	0.25±0.10	1.40±0.45	-	0.71±0.39	0.002
L356Q	0.29±0.05	1.36±0.49	-	0.73±0.29	0.002
R725A	0.23±0.07	1.26±0.34	-	0.49±0.23	0.002

P values were generated by two-tailed t-test where the changes were relative to the control (empty vector). In the reversal experiments, p values were calculated relative to the control values (CON) at 60 h time point. The statistically significant values are $p<0.05$, shown in red. Number of mice per each experimental data set was at least 10.