Supplemental Methods

Cells and reagents. Synaptopodin knockdown (1) and dynamin knockdown (2) podocytes were cultured as described previously. Staurosporine, angiotensin II and actinomycin D were all obtained from Sigma.

Animals. Cathepsin L knockout mice on a mixed C57Bl6/129J background was described previously (3).

Apoptosis Assays. Wild type mouse (Control) and CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes were grown in 24 well dishes and allowed to differentiate for 10 days. Upon differentiation, cells were infected with 30 µl of lentiviruses to knock down dendrin or CatL for 24 hours in presence of 8 µg/µl polybrene. After 24 hours, the medium was replaced with serum-starved RPMI (0.2% FBS, 1% Penicillin/Streptomycin, all from Invitrogen). Twenty-four hours later, cells were treated with apoptotic inducers, e.g., 1, 2 and 5 ng/ml TGF-β1, 10 ng/ml actinomycin D and 100 nM angiotensin II for an additional 24 hours or 1 µM staurosporine for 1 h. When indicated, the cells were treated with 20 µM E-64 in serum-starved medium for 24 hours. Apoptosis assays were performed using the Cell Death Detection ELISA PLUS kit (Roche) as per the manufacturers protocol.

Supplemental References

1. Asanuma K, et al. Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. *J Clin Invest.* 2005;115:1188–1198.
2. Gu C, et al. Direct dynamin-actin interactions regulate the actin cytoskeleton. *EMBO J.* 2010;29:3593–3606.
3. Nakagawa T, et al. Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus. *Science.* 1998;280:450.

Supplemental Figure Legends:

Figure S1. Loss of CD2AP induces expression of cytosolic CatL.

(**A**) Dendrin localization in the cultured podocytes (green).

(**B**) Bar graphs depicting mRNA levels for CatL determined by RT-PCR in podocytes treated with TGF-β1 for 24 hours.

(C) CatL mRNA levels are upregulated only in CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes. Bar graphs depict CatL mRNA levels in wild type (WT), CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes, podocytes in which synaptopodin (syp^{KD}) and dynamin (Dyn^{KD}) were downregulated using lentivirus.

(D) mRNA for CatL has seven AUG codons. Translation initiation from the first AUG site yields pre-pro-CatL with a signal peptide that targets the protein to the endoplasmic reticulum (ER), and subsequently to the lysosome. Pre-pro-CatL is processed to become pro-CatL (~39 kD), which can either be delivered into the lysosomes, or can be secreted into the extracellular space. Alternatively, translation initiation from six downstream AUG sites results in short form of CatL (~32-34 kD) that is devoid of the signal peptide and therefore localizes in the cytoplasm, from which it can translocate into the nucleus by diffusion.

(E) Nuclear fraction from CD2AP$^{-/-(\text{Low TGF}\beta)}$ and CD2AP$^{-/-(\text{High TGF}\beta)}$ cells probed with anti-CatL antibody confirms the presence of cytosolic CatL in the nucleus.

(F) Total protein from wild type (Con) and CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes shows down-regulation of dynamin, synaptopodin (synpo) and RhoA.

(G-I) Downregulation of dynamin, synaptopodin and RhoA are not transcriptionally regulated. Bar graphs depicts mRNA levels using RT-PCR for endogenous dynamin 2 **(G),** RhoA (**H)** and synaptopodin **(I)** in wild type and CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes.

Figure S2. Cytosolic CatL regulated focal adhesion turnover in wild type podocytes.

(A) Bar graphs depicting the levels of CatL mRNA determined by RT-PCR in wild type podocytes infected with different shRNA constructs downregulating endogenous CatL (C2, C5, C6). Con, podocytes not infected with lentiviruses. Scr, cells infected with lentiviruses expressing a scrambled oligo.

(B) CatL levels in podocytes infected with lentiviruses expressing different shRNA contructs to downregulate CatL. Notice that majority of CatL is the lysosomal form (25 kD).

(C) Protein levels in podocytes infected with lentiviruses expressing different shRNA contructs to downregulate CatL.

(D) Protein levels in podocytes treated with CatL inhibitor, E64 for 48 hours.

(E) Organization of the actin cytoskeleton and FAs in podocytes in which CatL has been downregulated. FAs and F-actin were visualized with anti-paxillin and rhodamine-phalloidin, respectively.

(F) Bar graphs depicting number of FAs within the wild type podocytes and podocytes in which CatL was downregulated. Data represent measurements of >50 cells shown in (**E**).

(G) Downregulation of cytosolic CatL in podocytes shifts the size of FAs toward more mature and super mature forms. Data represent measurements of >50 cells shown in (**E**).

(H) Schematic diagram suggesting role of dynamin, synaptopodin and RhoA in regulating maturation of FAs in podocytes. Our study suggests that cytosolic CatL specifically targets regulatory proteins involved in regulating turnover of the FAs. Thus, downregulation of dynamin and synaptopodin (and thus indirectly RhoA) leads to decrease in number and size of FAs, whereas loss of CatL leads to opposite effects.

Figure S3. Downregulation of CatL or dendrin cannot rescue hypersensitivity to different pro-apoptotic signals in CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes.

(A-C) Bar graphs representing the specific enrichment of mono and oligonucleosomes released into the cytoplasm of CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes treated with different apoptotic inducers as indicated in the figure (Angio II: Angiotensin II; Stauro: Staurosporine; Actino D: Actinomycin D). CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes were treated with shRNA to downregulate CatL (C2 and C6), or dendrin (D3 and D4). CD2AP$^{-/-(\text{High TGF}\beta)}$ podocytes were also treated with 2 doses of CatL inhibitor E64 (20 μM each) for 24 hours prior to starting the assay.

Figure S4. Specificities of N- and C-terminal CD2AP antibodies detected by the immunoblots of HEK 293 cells, which were transfected with full length, N- and C-terminal CD2AP (CON: untransfected).

Figure S5. CatL cleaves CD2AP in vivo.

(A) Immunoblot for CD2AP in cultured podocytes that were exposed to lipopolysaccharides (LPS) for 24 h (CON: untreated).

(**B**) Immunoblot of soluble (Glom-S) and pelleted (Glom-P) fractions of the glomeruli from wild type (WT) mice (Dyn: Dynamin, Synpo: Synaptopodin).

(C) Immunofluorescent labeling of WT and cathepsin L knockout (CatL KO) mouse glomeruli against anti-N-CD2AP before and after LPS.

(D) Quantification of the staining intensity in **(C)** using Image J software (*P<0.05).

(E) CatL activity in soluble fractions from isolated glomeruli of control (untreated) and LPS-treated mice.

(F) Dendrin staining is unaltered in LPS treated WT mice with an exclusive extra-nuclear location.

(G) Urine albumin analysis reveals that both WT and Dendrin knockout (KO) mice develop LPS-mediated proteinuria. Lanes were loaded with urine samples taken at different time points following LPS injection (1: t=0, 2: 12 h, 3: 24 h, 4: 48 h, 5: 72 h, 6: 96 h, 7: 7 days).

(H) Effect of LPS on TGF-β1 (middle panel) and CatL levels (bottom panel) in wild type (WT) podocytes and podocytes in which dendrin was downregulated (Den^{KD}) using lentivirus (top panel). LPS induces upregulation of CatL in dendrin-independent manner.

Table S1. Lentiviral shRNA plasmids.

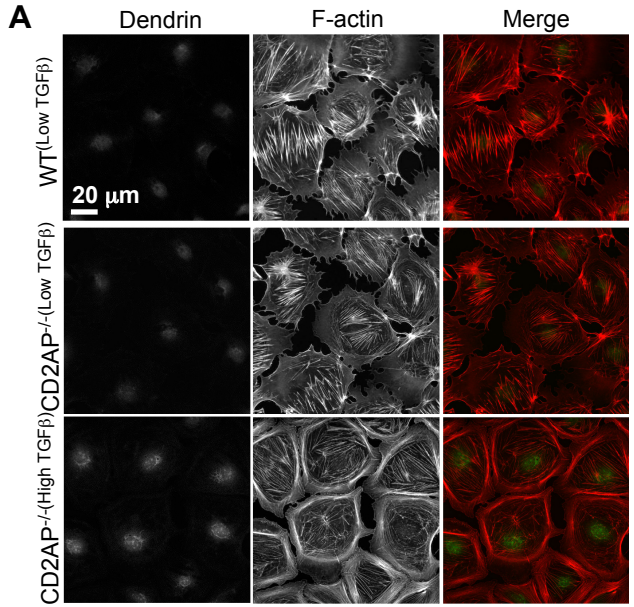
Gene	Clone	Sequence
CatL	C2	CCGGCCAGCTATCCTGTCGTGAATTCTCGAGAATTCACGACAGGATAGCTGGTTTTTG
	C5	CCGGCAGAAGACTGTATGGCACGAACTCGAGTTCGTGGCCATACAGTCTTCTGTTTTTG
	C6	CCGGAGAAGGACAGATGTTCCTTAACTCGAGTTAAGGAACATCTGTCCTTCTTTTTTG
Dendrin	D3	CCGGGATTGAAGTGAAGACTATTTCCTCGAGGAAATAGTCTTCACTTCAATCTTTTTG
	D4	CCGGGTGGACCTCAGAGTAACTATTCTCGAGAATAGTTACTCTGAGGTCCACTTTTTG

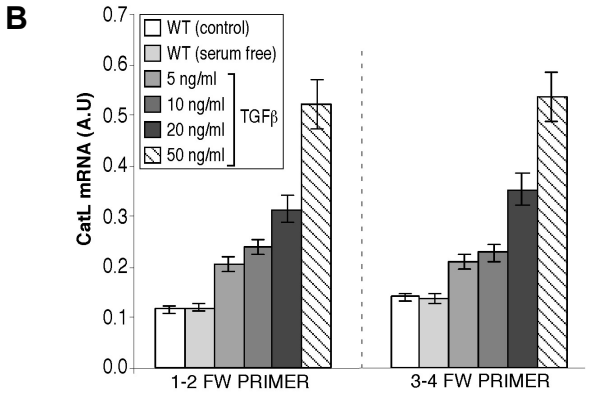
Table S2. Primers for quantitative PCR.

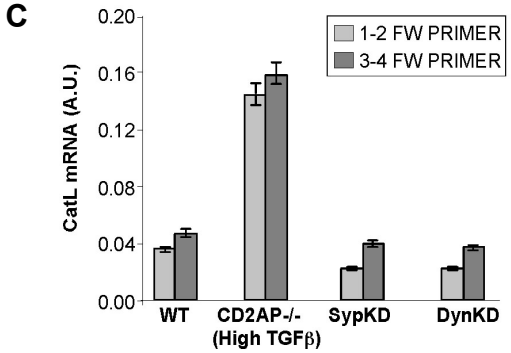
Gene	Primer	Sequence
CatL	Primer 1, exon 1, 2	F: TAGCCGCCTCAGGTGTTTGAA … R: CTTCCCCAGCTGTTCTTGACA
	Primer 2, exon 3, 4	F: TTCGGTGACATGACCAATGAG … R: CTTCCCCAGCTGTTCTTGACA
Dendrin	Primer 1, exon 2	F: AATGGAGAGGCCTTGAACCT … R: CCTGTGAAAATCCGGAAGGG
	Primer 2, exon 1, 2	F: CAGAGCCGCACGTGTAGGCTG … R: GGAAACCCTGTAGGTTGGGC
Dynamin	Primer 1, exon 1, 2	F: CGTGGGCCGGGACTTCCTTCC … R: TTCCGCATATTCTGTTTTGG
	Primer 2, exon 2, 3	F: CTTTTCCAAAACAGAATATGCGG … R: CAAGTTCAACACGTGTGGTGAG
RhoA	Primer 1, exon 1, 2	F: CTCGCCTTGAGCCTTGCATCTG … R: GGCAGCCATCACTTATAAAGG
	Primer 2, exon 3, 4	F: GATGGGAAGCAGGTAGAGTTGG … R: GGGATGTTTTCTAAACTATCAG
Synaptopodin		F: GCCTGCCTCTCTCTACCACGG … R: GAAGCAGAAGGAAGGCTTCCACAC

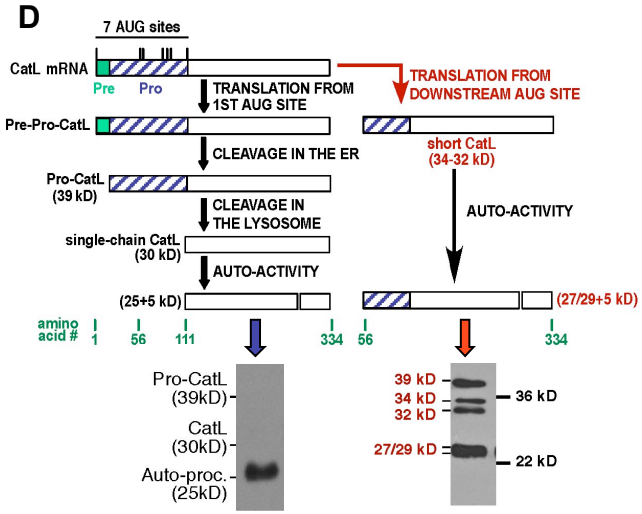
Table S3. Sixty and 24 bp oligonucleotides on CatL promoter.

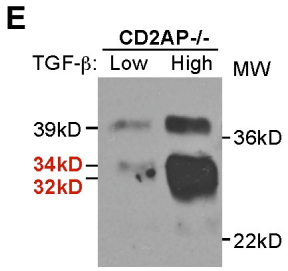
Size	Oligo	Sequence
60 bp	1	CCCAGGCTGGTCTACATAGTGAAACCCTATAATCCTATTATATATATTTCCAGGGAACTC
	2	CAGGGAACTCATGAAATTCCAGAAGAAACATTTTAAGACTGAGGAAAACAATTCATAATG
	3	ATTCATAATGCAGAGAAGAAAAAGAGCCTGCATCATTCTCAACTGCTCTTTTCTTTTCAT
	4	TTCTTTTCATTTCTTTTTTTAAGCCATCATCCTACATCCCCAATCCCGCGTCCCCCGTCT
24 bp	4-1	TTCTTTTCATTTCTTTTTTTAAGC
	4-2	TTTAAGCCATCATCCTACATCCCC
	4-3	TCCCCAATCCCGCGTCCCCCGTCT

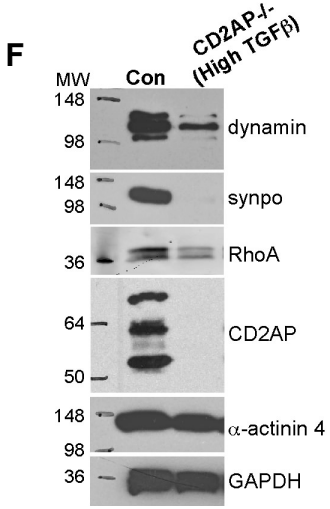


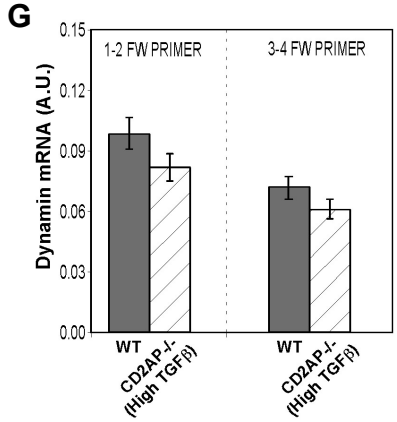


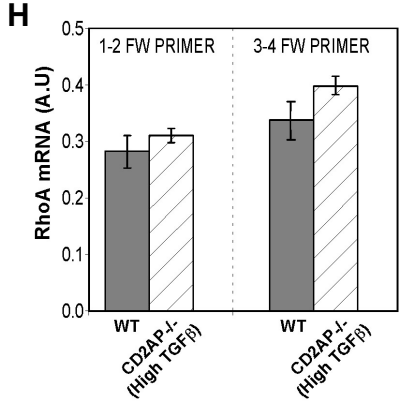


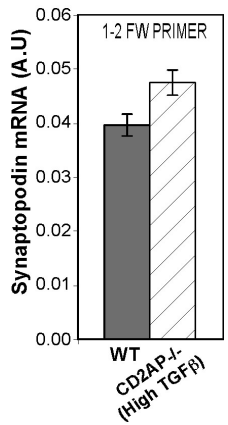












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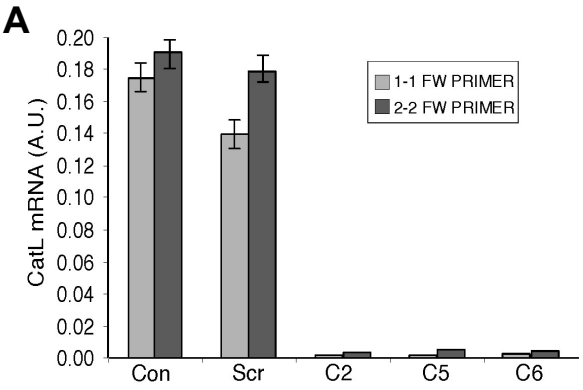

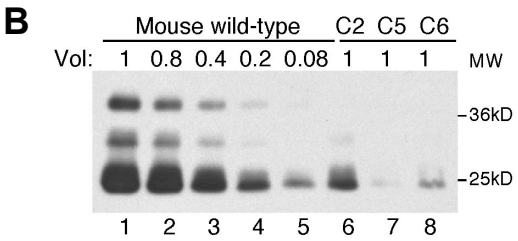

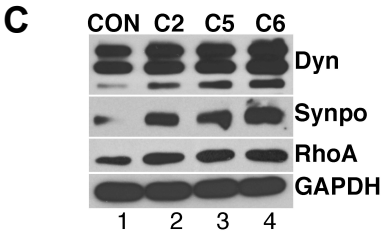

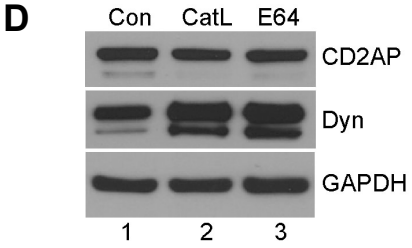

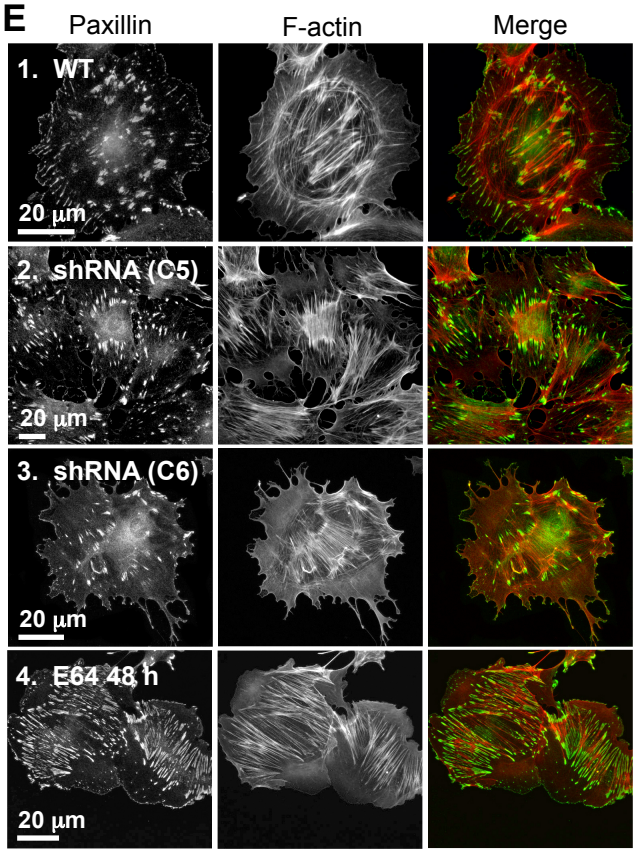

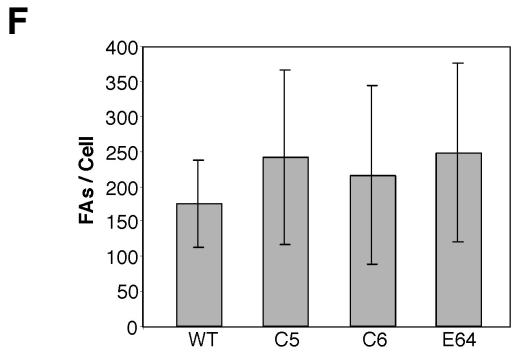

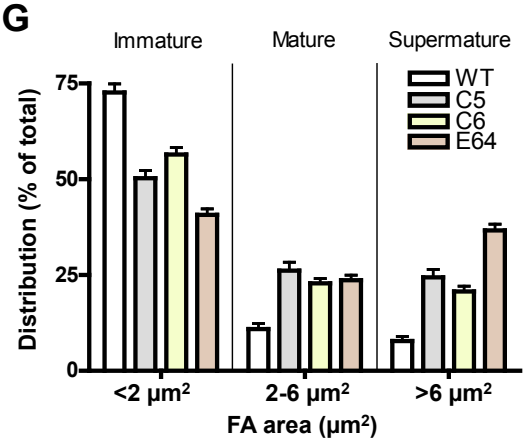

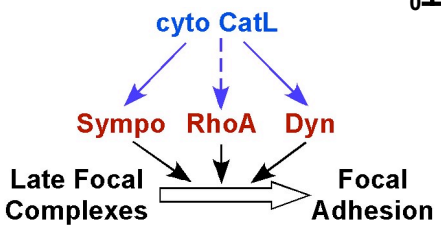

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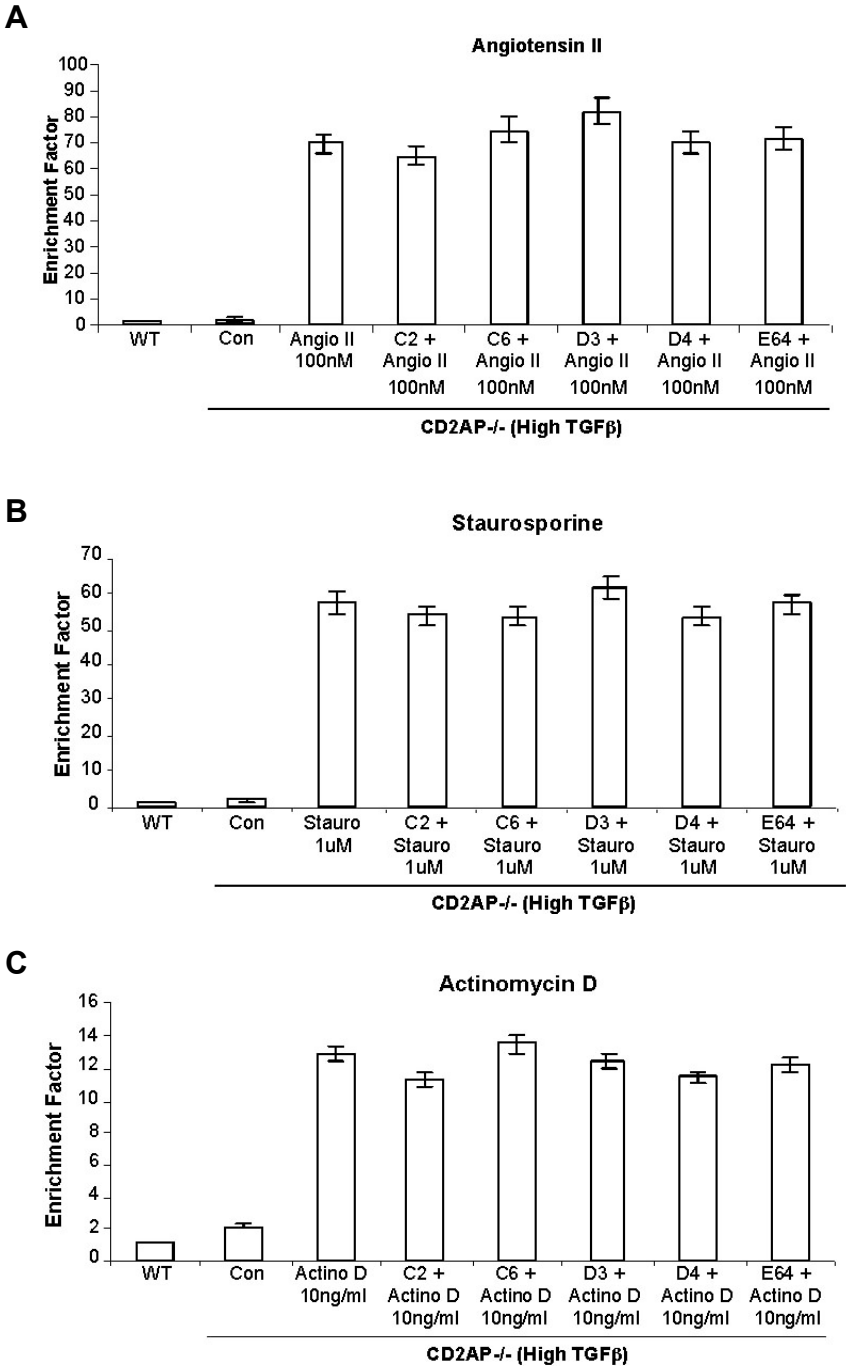

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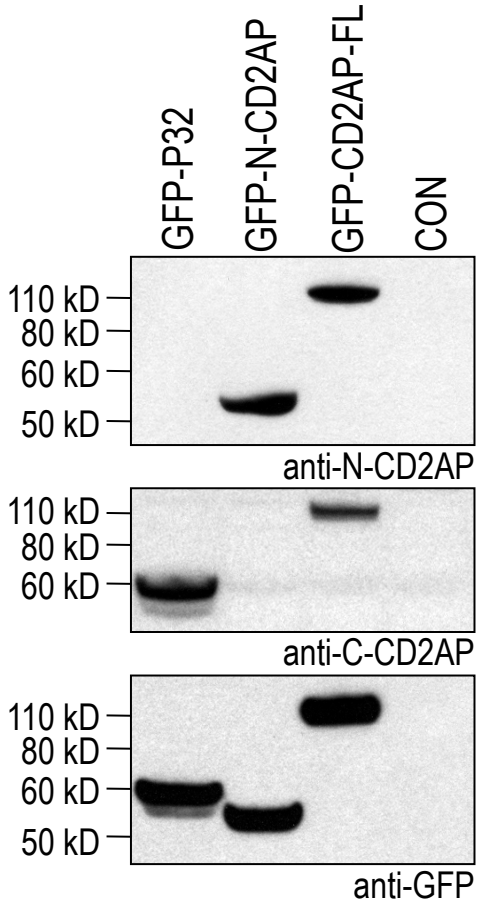

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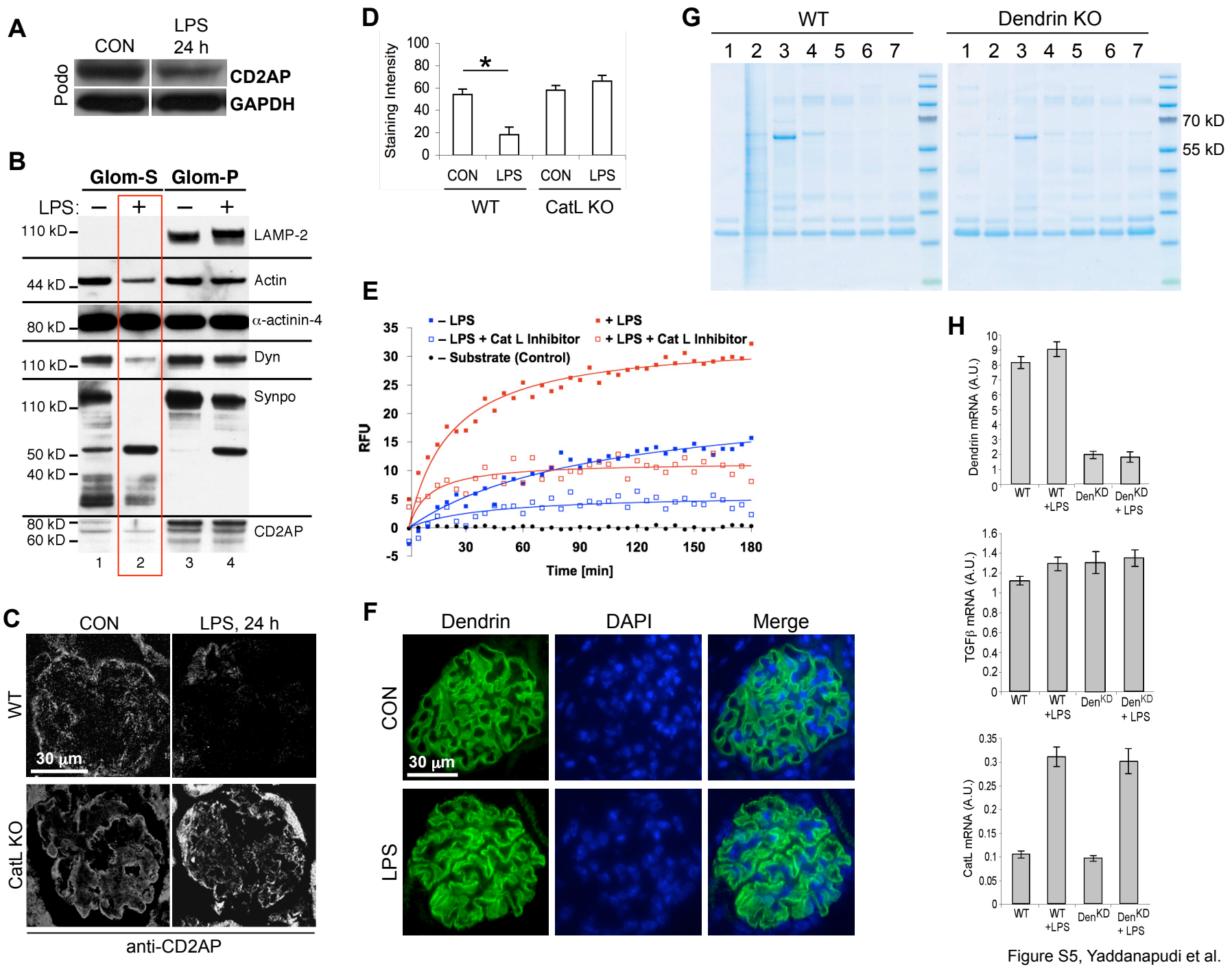

Figure S5, Yaddanapudi et al.