

Supplemental Data

Pentoxifylline Attenuates Proteinuria in Anti-Thy1 Glomerulonephritis via Downregulation of Nuclear Factor- κ B and Smad2/3 Signaling

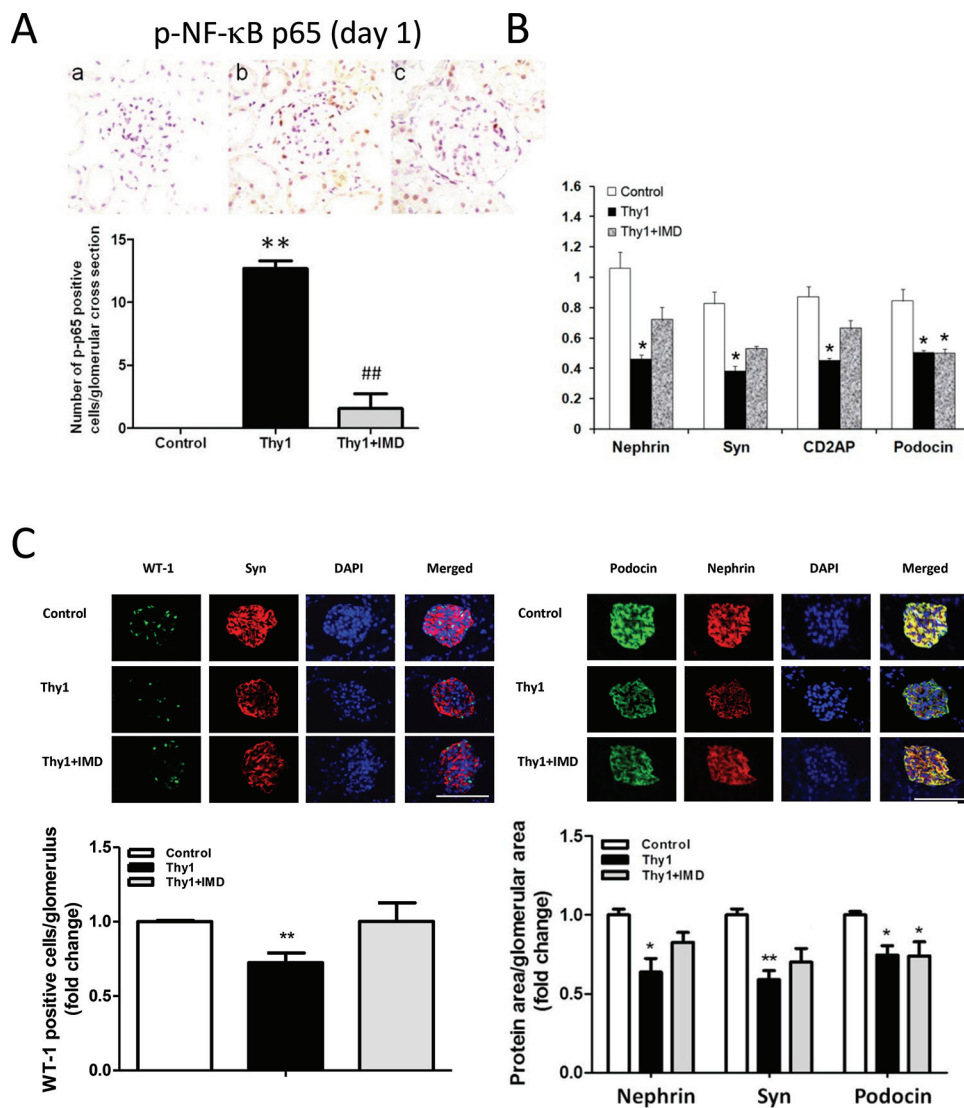
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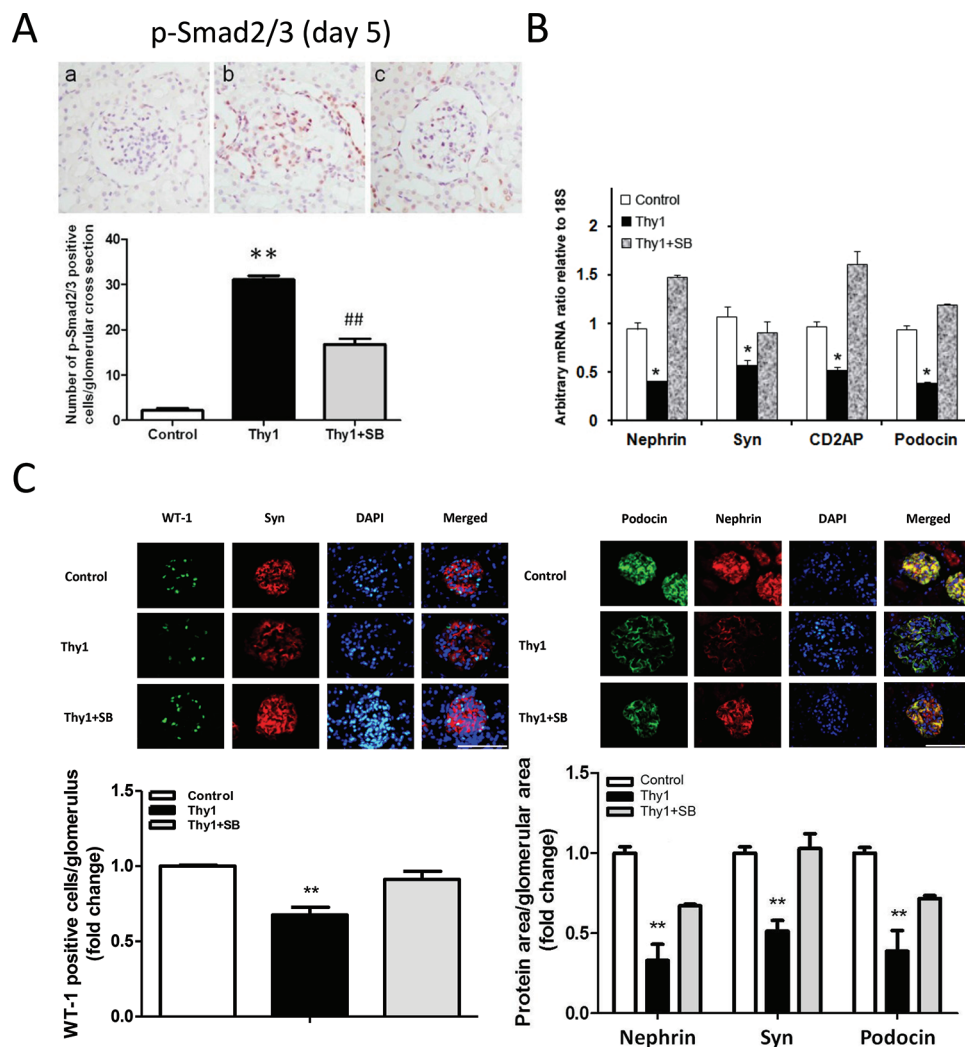
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Supplementary Table S1. Primer pairs used for quantitative PCR.

Genes		Sequences
Rat TNF- α	<i>upper</i>	5' CGATTGGCCACTTCATAC
	<i>lower</i>	5' GACTCCGTGATGTCTAAG
Rat IL-1 β	<i>upper</i>	5' AATCTCACAGCAGCATCTC
	<i>lower</i>	5' AGCAGGTCGTCATCATCC
Rat MCP-1	<i>upper</i>	5' GCATCCACTCTCTTTCCAC
	<i>lower</i>	5' AGGCATCACATTCCAAATCAC
Rat TGF- β 1	<i>upper</i>	5' TATATGTTCTTCAATACG
	<i>lower</i>	5' TATATTTCTGGTAGAGTT
Rat TGF- β 2	<i>upper</i>	5' TCATACCGTCTAATAATTACATCA
	<i>lower</i>	5' TAGGAGGGCAACAACATT
Rat TGF- β 3	<i>upper</i>	5' GAACATTTCTTTCTGCTA
	<i>lower</i>	5' TCATTATCTTCTCTACC
Rat activin- β A	<i>upper</i>	5' GCACITGAAGAAGAGACC
	<i>lower</i>	5' TCCTCTATCTCCACATACC
Rat activin- β B	<i>upper</i>	5' AACTCATCTCCAACAACCT
	<i>lower</i>	5' TTATCTTTCTTCTCACTTC
Rat nephrin	<i>upper</i>	5' ATGACGCAGAGTATGAAT
	<i>lower</i>	5' ACTAGAATGGAGAGGATTAC
Rat podocin	<i>upper</i>	5' GGTCGTCTCATCAITCATA
	<i>lower</i>	5' GAGCAGTCAGTGTTCATAA
Rat synaptopodin	<i>upper</i>	5' CTC GCT GCC CTT CAC CTA CTA TG
	<i>lower</i>	5' ATC TGG TAT GGC TGC TGC TTG G
Rat CD2AP	<i>upper</i>	5' GTATGACTACGGTGCTGTA
	<i>lower</i>	5' GCCATCCTTCTTCTGTA
Rat agrin	<i>upper</i>	5' CCTACCTGGAAGTAAAG
	<i>lower</i>	5' CCATTGTAGAGGAGTAAGC
Rat perlecan	<i>upper</i>	5' ATT CGA GGT TCG GAC CAG
	<i>lower</i>	5' CTG TAG TCC AAG GCT AAT G
Rat glypican-1	<i>upper</i>	5' GTATCCAGGAAGACAGTAG
	<i>lower</i>	5' CTCTAACCCAGTGACAAA
Rat syndecan-4	<i>upper</i>	5' TGTGTCTGTCTGTCTGAG
	<i>lower</i>	5' ACTGGCTCTACATTCTATTCT
Rat heparanase	<i>upper</i>	5' CACTGGCTGTAAGAAGAC
	<i>lower</i>	5' AGCAATCAGAGGAAGAAGA
Rat hyaluronidase	<i>upper</i>	5' GCTGGTGTATCTTCTGG
	<i>lower</i>	5' TAGGTACTTCTTGAGGTCT



Supplementary Figure S1. (A) Representative immunohistochemical staining showing glomerular p-NF-κB p65 signals on d 1 (a, b, and c denote control rats, vehicle-treated nephritic rats, and IMD-0354-treated nephritic rats, respectively; original magnification, 400X). Bar graphs show semi-quantitative results of p-p65-positive cells per glomerular cross section. Values are the mean ± s.e.m. of 25-30 glomeruli. ** $p < 0.01$ vs. control rats; ## $p < 0.01$ vs. vehicle-treated nephritic rats. (B) IMD-0354 partially reversed downregulated mRNAs for nephrin, synaptopodin, and CD2AP on d 1. Each bar represents the mean ± s.e.m. of three experiments. * $p < 0.05$ vs. control rats. (C) Upper panels show representative immunofluorescent staining of WT-1, synaptopodin, podocin, and nephrin within the glomeruli on d 1. Bar size, 100 μm. Lower graphs show semi-quantitatively IMD-0354 partially reversed the reduction of WT-1-positive cells per glomerulus and the area positively stained for podocyte-related proteins per glomerular area. Values are the mean ± s.e.m. of 25-30 glomeruli. * $p < 0.05$, ** $p < 0.01$ vs. control rats. Abbreviations: Syn, synaptopodin; Thy1, vehicle-treated nephritic rats; Thy1+IMD, IMD-0354-treated nephritic rats.



Supplementary Figure S2. (A) Representative immunohistochemical staining showing glomerular p-Smad2/3 signals on d 5 (a, b, and c denote control rats, vehicle-treated nephritic rats, and SB431542-treated nephritic rats, respectively; original magnification, 400X). Bar graphs show semi-quantitative results of p-Smad2/3-positive cells per glomerular cross section. Values are the mean \pm s.e.m. of 25-30 glomeruli. ** p <0.01 vs. control rats; ## p <0.01 vs. vehicle-treated nephritic rats. (B) SB431542 partially restored the downregulated podocyte-related gene expression on d 5. Each bar represents the mean \pm s.e.m. of three experiments. * p <0.05 vs. control rats. (C) Upper panels show representative immunofluorescent staining of WT-1, synaptopodin, podocin, and nephrin within the glomeruli on d 5. Bar size, 100 μ m. Lower graphs show semi-quantitatively SB431542 partially reversed the reduction of WT-1-positive cells per glomerulus and the area positively stained for podocyte-related proteins per glomerular area. Values are the mean \pm s.e.m. of 25-30 glomeruli. ** p <0.01 vs. control rats. Abbreviations: Syn, synaptopodin; Thy1, vehicle-treated nephritic rats; Thy1+SB, SB431542-treated nephritic rats.