Supplement Information

Calcineurin inhibitors cyclosporin A and tacrolimus protect against podocyte injury induced by puromycin aminonucleoside in rodent models

Xiujin Shen[1#], Hong Jiang[1#,*], Meike Ying[1], Zhoutao Xie[1], Xiayu Li[1], Haibing Wang[2], Jie Zhao[1], Chuan Lin[1], Yucheng Wang[1], Shi Feng[1], Jia Shen[1], Chunhua Weng[1], Weiqiang Lin[1], Huiping Wang[1], Qin Zhou[1], Yan Bi[1], Meng Li[1], Lingyan Wang[3], Tongyu Zhu[4], Xiaoru Huang[5], Hui-Yao Lan[5], Jing Zhou[6],Jianghua Chen[1*]

[1]Kidney Disease Center, First Affiliated Hospital, Zhejiang University, School of Medicine; Key Laboratory of Nephropathy, Zhejiang Province, Hangzhou, Zhejiang, China, [2]National Clinical Research Base of Traditional Chinese Medicine, Zhejiang Hospital of Traditional Chinese Medicine, Zhejiang Chinese Medical University, Hangzhou, China, [3]Biomedical Research Center, Zhongshan Hospital, Fudan University, Shanghai, China, [4]Department of Urology, Zhongshan Hospital, Fudan University, Shanghai, China, [5]Li Ka Shing Institute of Health Sciences, and Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China, [6]Harvard Center for Polycystic Kidney Disease Research and Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA.

[#]These authors contributed equally to this work.

[*]Corresponding author email: annie.jh@163.com, chenjianghua@zju.edu.cn

Figure legends

Fig S1. Effects of CsA and FK506 on podocyte viability in cultured mouse podocytes *in vitro*. (**A**) MTT assays of podocytes treated with CsA or FK506 alone for 24 h. (**B)** Immunofluorescence of synaptopodin and podocin in cultured mouse podocytes after CsA and FK506 treatment.

Fig S2. PAN disrupts the podocyte cytoskeleton in cultured mouse podocytes *in vitro*. (**A**) Immunofluorescence of F-actin showed that PAN disrupted the podocyte cytoskeleton. The green fluorochrome was FITC-phalloidin. Original magnification, ×200. (**B**) Western blot analysis of synaptopodin and podocin levels in PAN-treated podocytes.

Fig S3. PAN increases apoptosis-related proteins in cultured mouse podocytes *in vitro*. Western blot analysis of Bax, Bcl-XL, BCL-2 (**A**), and caspase-family protein (**B**) expression after stimulation with 25 μg/ml and 50 μg/ml PAN for 24 h.

Fig S4. Effects of PAN, CsA, and FK506 on p38, ERK, and JNK signaling pathways in cultured mouse podocytes *in vitro*. Western blot analysis of p38, ERK, and JNK signaling pathways in PAN, CsA, and FK506-treated podocytes.

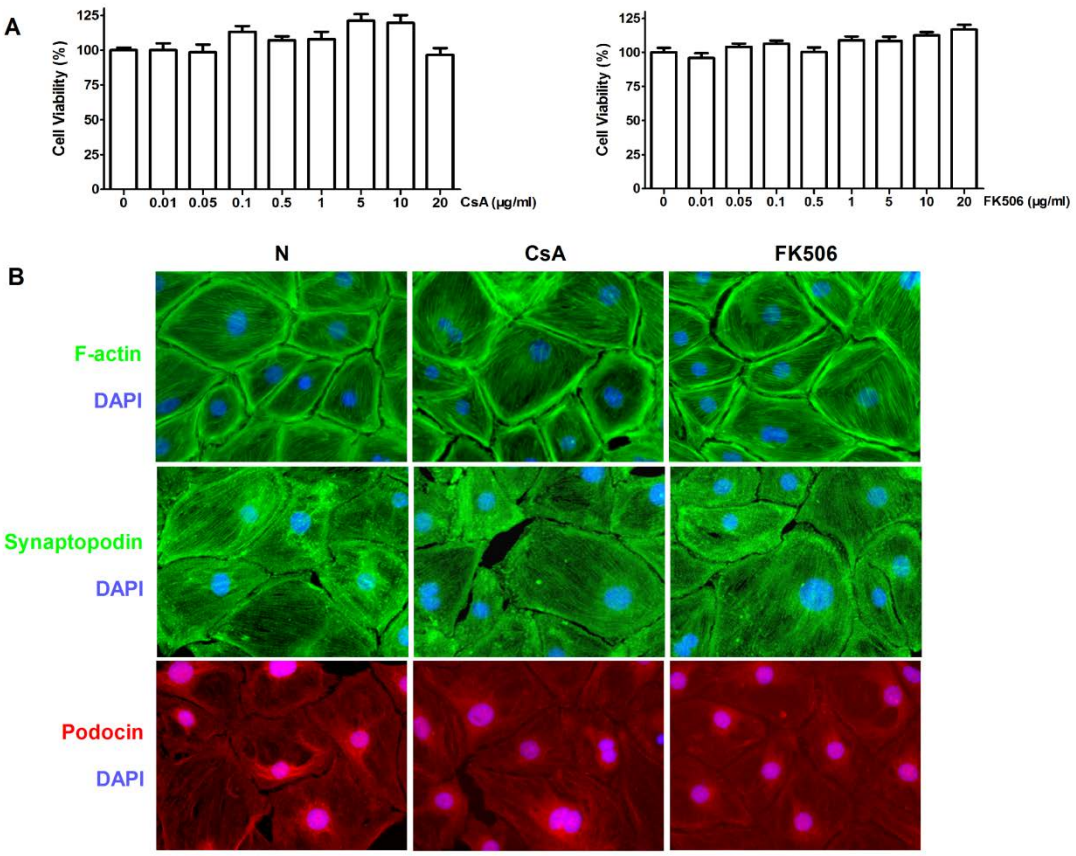

Fig S1. Effects of CsA and FK506 on podocyte viability in cultured mouse podocytes *in vitro*. (**A**) MTT assays of podocytes treated with CsA or FK506 alone for 24 h. (**B)** Immunofluorescence of synaptopodin and podocin in cultured mouse podocytes after CsA and FK506 treatment.

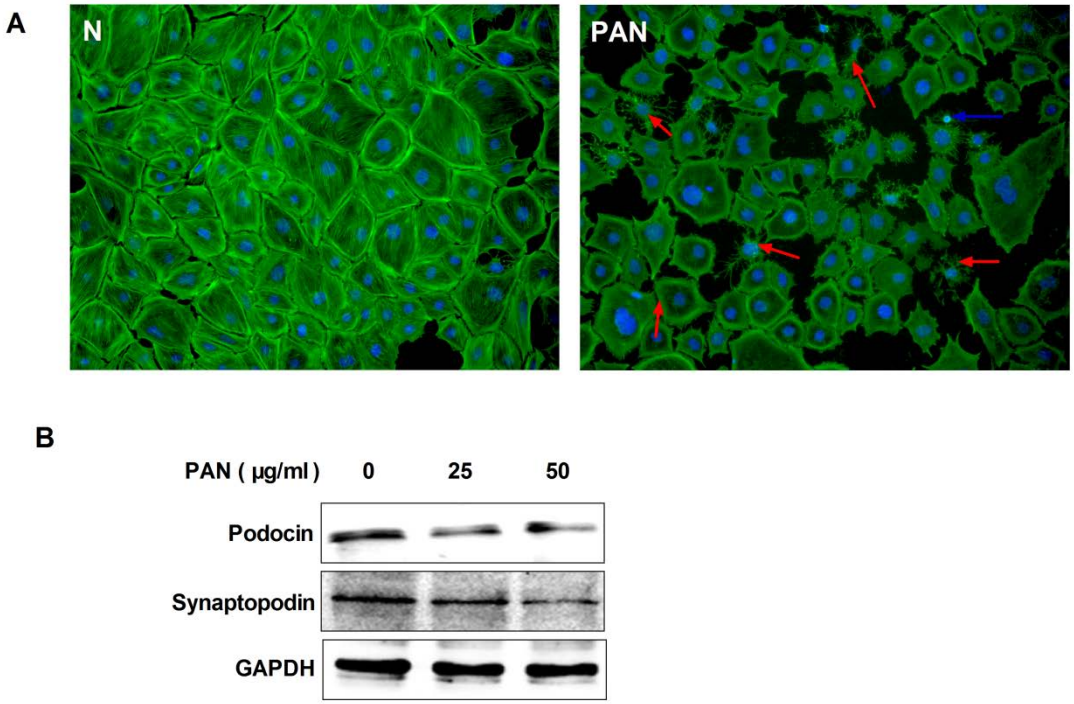

Fig S2. PAN disrupts the podocyte cytoskeleton in cultured mouse podocytes *in vitro*. (**A**) Immunofluorescence of F-actin showed that PAN disrupted the podocyte cytoskeleton. The green fluorochrome was FITC-phalloidin. Original magnification, ×200. (**B**) Western blot analysis of synaptopodin and podocin levels in PAN-treated podocytes.

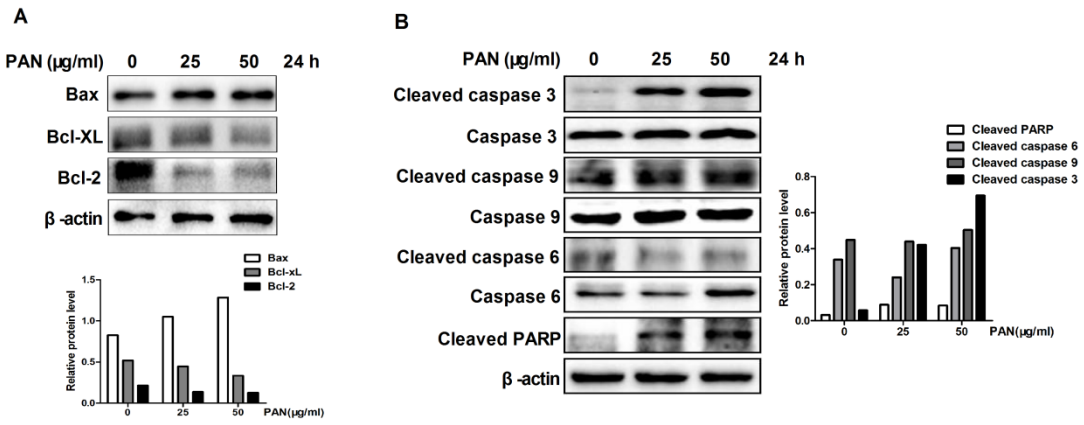

Fig S3. PAN increases apoptosis-related proteins in cultured mouse podocytes *in vitro*. Western blot analysis of Bax, Bcl-XL, BCL-2 (**A**), and caspase-family protein (**B**) expression after stimulation with 25 μg/ml and 50 μg/ml PAN for 24 h.

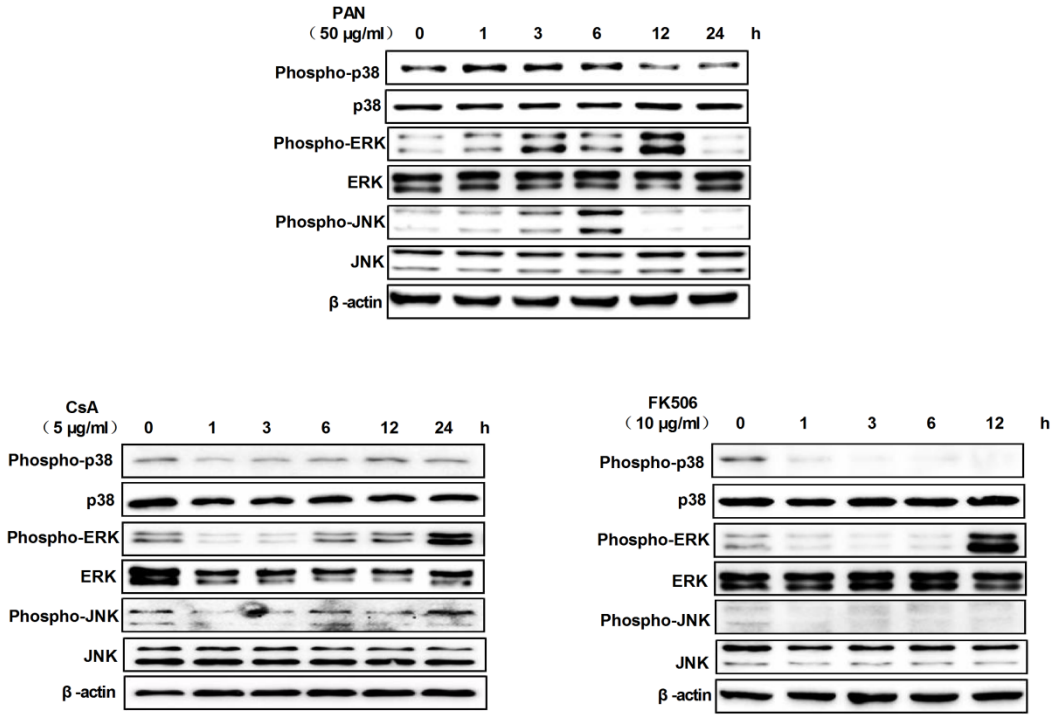

Fig S4. Effects of PAN, CsA, and FK506 on p38, ERK, and JNK signaling pathways in cultured mouse podocytes *in vitro*. Western blot analysis of p38, ERK, and JNK signaling pathways in PAN, CsA, and FK506-treated podocytes.

Table S1

Primary antibodies	Host	Dilution and supplier	Product ID	Application
ERK	Rabbit	1:1000; Cell Signaling, Danvers, MA	4695	WB
phospho-ERK	Rabbit	1:1000; Cell Signaling, Danvers, MA	4370	WB
JNK	Rabbit	1:1000; Cell Signaling, Danvers, MA	9258	WB
phospho-JNK	Rabbit	1:1000; Cell Signaling, Danvers, MA	4668	WB
p38	Rabbit	1:1000; Cell Signaling, Danvers, MA	8690	WB
phospho-p38	Rabbit	1:1000; Cell Signaling, Danvers, MA	4511	WB
Bcl-2	Rabbit	1:1000; Cell Signaling, Danvers, MA	2870	WB
Bcl-xl	Rabbit	1:1000; Cell Signaling, Danvers, MA	2764	WB
Bax	Rabbit	1:1000; Cell Signaling, Danvers, MA	2772	WB
caspase-3	Rabbit	1:1000; Cell Signaling, Danvers, MA	9662	WB
cleaved caspase-3	Rabbit	1:1000; Cell Signaling, Danvers, MA	9664	WB
caspase-6	Rabbit	1:1000; Cell Signaling, Danvers, MA	9762	WB
cleaved caspase-6	Rabbit	1:1000; Cell Signaling, Danvers, MA	9761	WB
caspase-9	Rabbit	1:1000; Cell Signaling, Danvers, MA	9504	WB
cleaved caspase-9	Rabbit	1:1000; Cell Signaling, Danvers, MA	9509	WB
cleaved PARP	Rabbit	1:1000; Cell Signaling, Danvers, MA	9544	WB
desmin	Mouse	1:60; Zhongshan Golden Bridge Biotechnology, Beijing, China	ZM0091	IHC
WT-1	Mouse	1:80; Zhongshan Golden Bridge Biotechnology, Beijing, China	ZM0269	IHC
Synaptopodin	Goat/Rabbit	1:100/1:1000; Santa Cruz, Dallas, TX	SC-21537/SC50459	IF/WB
podocin	Rabbit	1:100/1:1000; Sigma, St Louis, MO, USA	P0372	IF/WB
CaN	Rabbit	1:1000; Santa Cruz, Dallas, TX	SC-9070	WB
β-actin	Mouse	1:5000; Abcam, Cambridge, MA	Ab6276	WB