Supplementary Information

A Biomimetic Renal Fibrosis Progression Model On-chip Evaluates

Anti-Fibrotic Effects Longitudinally in a Dynamic Fibrogenic Niche

Di Wu^{1,2,7,#}, Jianguo Wu^{1,#}, Hui Liu^{1,3,4,#}, Shengyu Shi¹, Liangwen Wang⁵, Yixiao Huang^{3,4}, Xiaorui Yu^{3,4}, Zhuoyue Lei^{3,4}, Tanliang Ouyang^{3,4}, Jia Shen⁶, Guohua Wu^{2,3,7,*}, Shuqi Wang^{1,3,4,7,*}

¹ Institute for Translational Medicine, School of Medicine, Zhejiang University, Hangzhou, 310029, China

² Henan Key Laboratory of Rare Diseases, Endocrinology and Metabolism Center, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, 471003, China.

³ National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, 610065, China

⁴ Clinical Research Center for Respiratory Disease, West China Hospital, Sichuan University, Chengdu, 610065, China

⁵ Department of Interventional Radiology, Zhongshan Hospital, Fudan University, Shanghai, 200032, China

⁶ Kidney Disease Center, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310029, China

⁷ Tianfu Jincheng Laboratory, City of Future Medicine, Chengdu, 641400, China

#: These authors contributed equally.

*Corresponding author: Guohua Wu (wuguohua@zju.edu.cn), Shuqi Wang (shuqi@scu.edu.cn)



Fig. S1 The percentage of NRK-49F cells with nuclear-localized YAP (nYAP⁺ cells) indicates the extent of myofibroblast activation. (A) NRK-49F cells were cultured in Soft, Medium, and Stiff DKM-GelMA hydrogels for 3 days and then transferred to Stiff-to-Soft DKM-GelMA for 2 days. (B) NRK-49F cells were cultured in Soft, Medium, and Stiff DKM-GelMA hydrogels for 7 days and then transferred to Stiff-to-

Soft DKM-GelMA for 2 days. YAP was immunostained in red and nuclei were stained in blue. NRK-49F cells with nuclear-localized YAP (nYAP⁺ cells) were identified in merged fluorescence images with white arrows indicated (I). The percentage of nYAP+ cells cultured in different matrix stiffness was calculated from 4 individual fluorescence images (n = 4) with a minimum of 8 cells in each image. (Avg \pm SD, * stands for p <0.05, NS indicates p > 0.05). Scale bar: 100 µm.



Fig. S2 Effect of drug treatment on ECM stiffness evaluated by measuring Young's modulus. The control group did not receive drug treatment, and the experimental group was treated with 0.5 mg/mL PFD for 48 hours. (n = 3). (Avg \pm SD, * stands for p < 0.05, NS indicates p > 0.05).



Fig. S3 Characterization of tissue fibrosis phenotype in rat UUO model. (A) Masson's trichrome staining representative images indicate fibrotic activation in UUO models. (B) and (C) Immunohistochemical representative images and immunofluorescence representative images of fibronectin (Green) protein in left kidney (n = 4). Scale bar: 100 µm.