Supplementary Information

Monitoring and manipulating cellular crosstalk during kidney fibrosis inside a 3D in vitro co-culture

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Drugs compound	Pathological results	Working concentration
Cyclosporine A	Tubular atrophy, collagen matrix deposition in interstitial space	1.5 µM for 24 h incubation
Gentamicin	Tubular necrosis, proximal tubule desquamation	No response
Diclofenac	Proximal tubule apoptosis, damaged and hyperthrophied glomerulus	No response
Aristolochic acid	Acute tubular necrosis, tubulointerstitial damage	125 µM for 24 h incubation
Lithium	Tubular distrophy, interstitial fibrosis	No response

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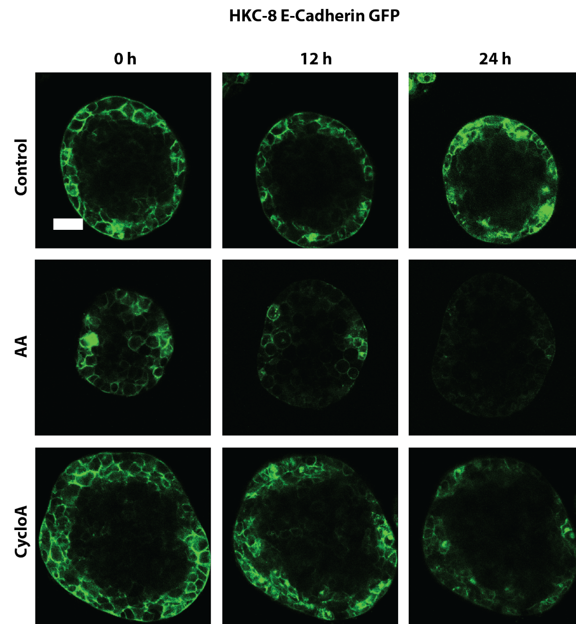
25 **Supplementary Figure S1. List of clinically relevant nephrotoxins cytotoxicity study in**
26 **HKC-8 spheroids (24 hours)**
27 HKC-8 spheroids' responses to various clinically relevant nephrotoxins were examined by the
28 cell viability assay (MTT). Of the five tested nephrotoxins, HKC-8 spheroids showed a dose
29 dependency to cyclosporine A and aristolochic acid. Gentamicin, diclofenac, and lithium did
30 not induce an appreciable cytotoxic effect on the HKC-8 3D model. This discrepancy is
31 probably due to the fact that we employed immortalized, stably-transfected fluorescent cell
32 lines for the 3D model, which might have changed genetically under the subjected
33 experimental conditions, leading to the phenotypic differences.
34

a)



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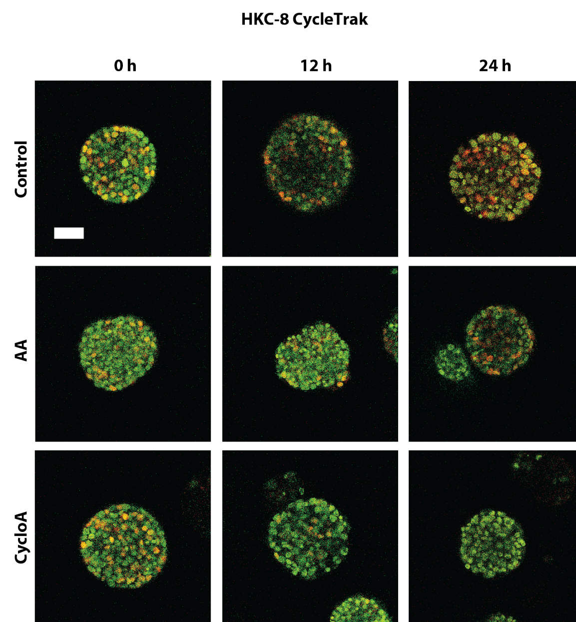

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36 **Supplementary Figure S2. Representative HKC-8 spheroid reporter images during**
37 **nephrotoxin treatment**

38 (**a**) HKC-8 E-cadherin GFP and (**b**) HKC-8 CycleTrak spheroids during nephrotoxins
39 treatment. (**a**), green signal represents E-cadherin. (**b**), green signal represents G2/M phase,
40 red signal represents G1 phase, and yellow signal represents S phase. Scale bar, 50 µm.

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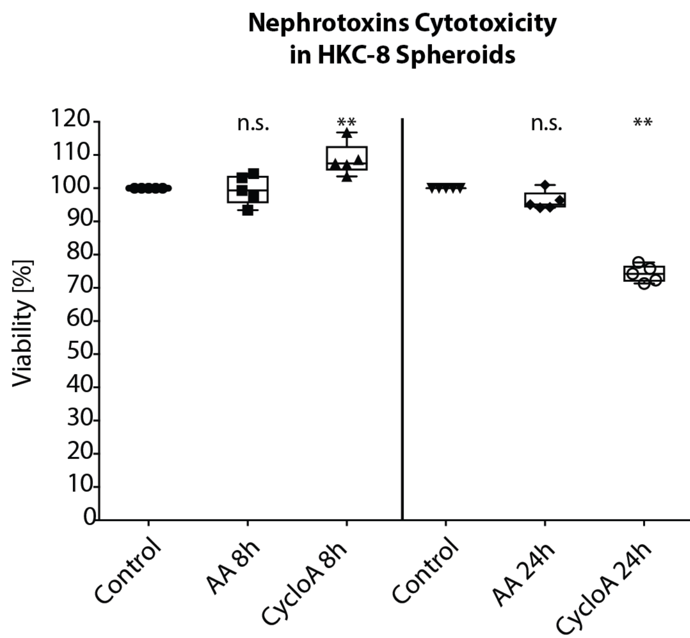

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43 **Supplementary Figure S3. Toxicity assessments of aristolochic acid and cyclosporine A**

44 Cytotoxicity of aristolochic acid and cyclosporine A at the working concentration in HKC-8

45 spheroids were examined by the cell viability assay (MTT) after 8 and 24 hours. After 24

46 hours of drug treatment, only cyclosporine A treatment induced noticeable viability reduction

47 (n=5). **P<0.01, n.s., not significant (Mann Whitney *U* test).

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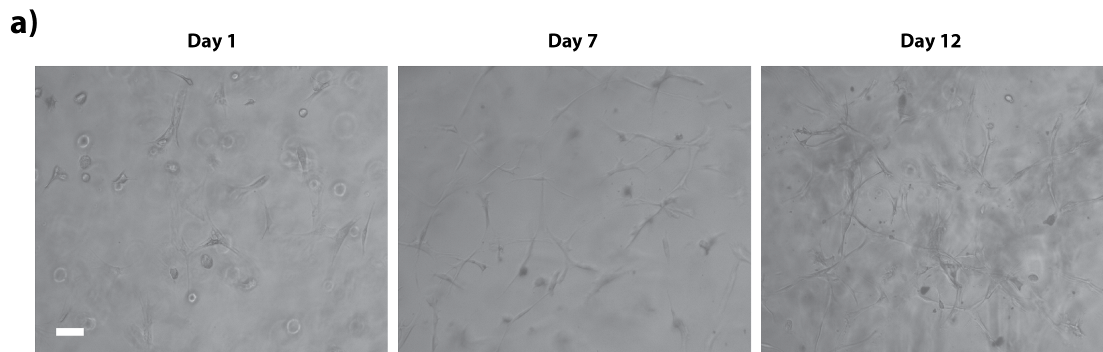

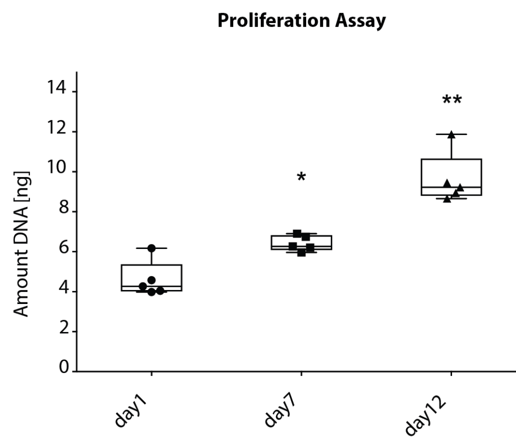

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50 **Supplementary Figure S4. Human renal fibroblasts 3D culture in dextran hydrogel**

51 Cell migration and proliferation were investigated using (**a**) bright field images and (**b**) the

52 PicoGreen Assay, respectively. Proliferating renal fibroblasts migrated in the hydrogel over

53 the culture forming sprouting constructs (n=5). *P<0.1; **P<0.01 (Mann Whitney *U* test).

54 Scale bar, 20 μm (**a**).

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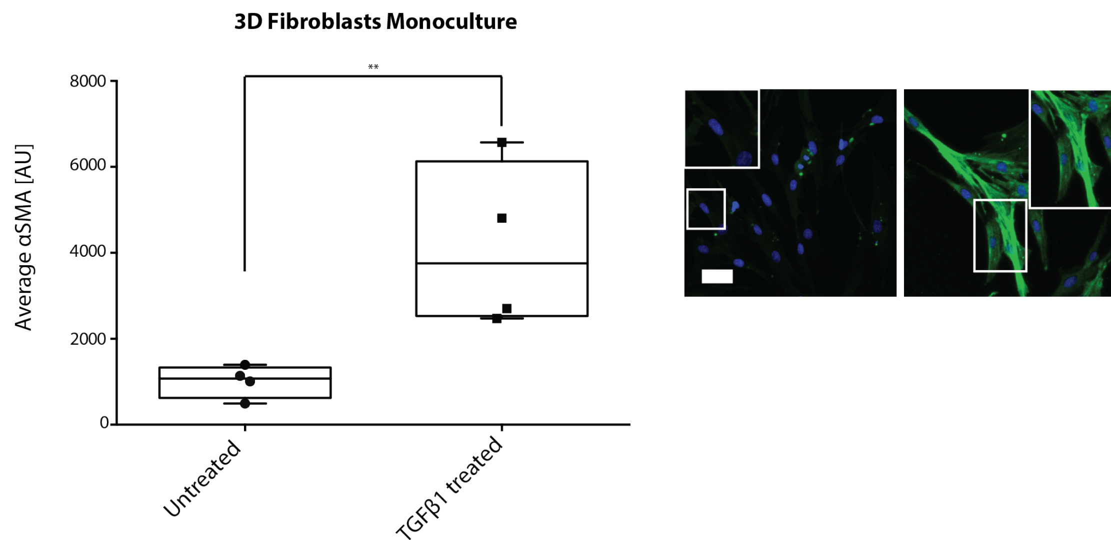

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57 **Supplementary Figure S5. TGFβ1-treated fibroblasts monoculture identification using**

58 **αSMA-staining**

59 To confirm that the human renal fibroblasts used in this work respond to profibrogenic

60 cytokine TGFβ1, a 3D monoculture of human renal fibroblasts in the dextran hydrogel

61 containing MMP-sensitive crosslinker was treated with 10 ng/mL TGFβ1 for 3 days. The cells

62 were then fixed and stained for αSMA (n=4). **P<0.01. Scale bar, 5 μm.

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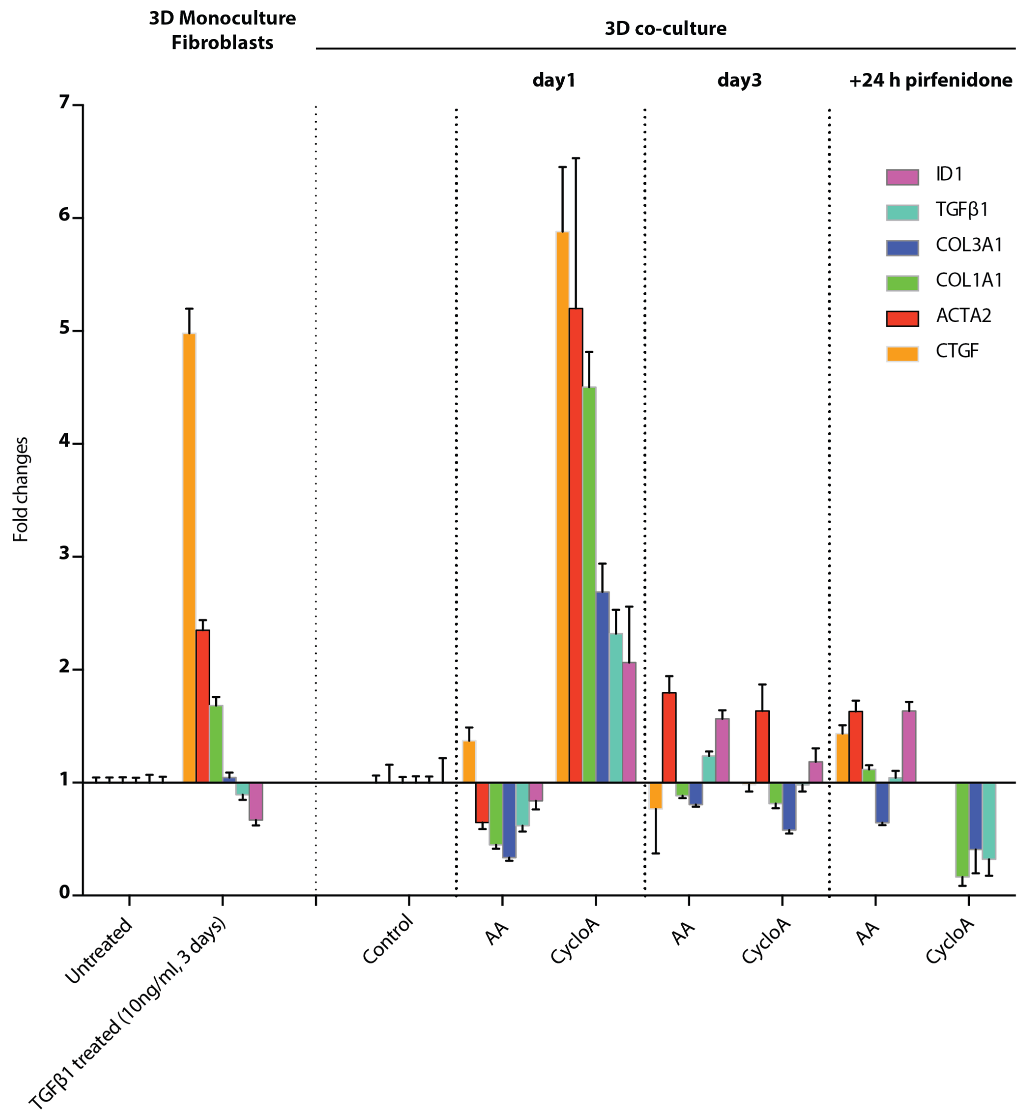

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65 **Supplementary Figure S6. Myofibroblasts gene expression analysis using the Taqman**

66 **gene expression assay**

67 The signature genes of myofibroblasts were analyzed using the Taqman assay, in comparison

68 to the human renal fibroblasts treated with TGFβ1 as control. Myofibroblasts activation is

69 inhibited upon addition of pirfenidone, a known inhibitor of TGFβ1 production (n=4).

70

71 **Supplementary Table T1. Kidney molecular toxicity pathway analysis of the HKC-8**
72 **spheroids' response to nephrotoxins**
73 At 8 and 24 hours after treatment of 125 μM aristolochic acid and 1.5 μM cyclosporine A, the
74 RNA was extracted from HKC-8 spheroids and analyzed with Mol Tox panel genes (~1600
75 genes) in Ampliseq. The count readouts from these genes were classified into relevant
76 pathways/mechanisms. The readouts were then normalized to the readouts of control samples.