

Supplemental Material legends:

Suppl Figure 1: High variation in podometrics from control adults and adults with FSGS. (a) Podocyte density; (b) Total podocyte volume per unit of podocyte density (TPV:PD ratio); (c) Quintile analysis of podocyte density (PD); and (d) Quintile analysis of TPV:PD. Red error bars represent medians and IQRs.

Suppl Figure 2: Mouse podometrics during body growth. (a) Body weight; (b) Glomerular volume; (c) Podocyte density; and (d) Total podocyte volume per unit of podocyte density (TPV:PD ratio). **** $P < 0.0001$, ** $P < 0.01$; in (A) bars represent means and error bars SEMs, each data point (red) represents one mouse; in violin plots, red lines represent medians, blue lines IQRs and every grey dot represents one glomerulus

Suppl Figure 3: Functional glomerular recovery is associated with sustained podocyte hypertrophy and podocyte injury recovery in a mouse model of FSGS. (a) Functional glomerular recovery based on urinary albumin to creatinine ratio (UACR); (b) Changes in total podocyte volume per unit of podocyte density (TPV:PD ratio) between baseline, week 1 (W1) and week 5 (W5) post DT injection; (c) Changes in podocyte number between baseline, week 1 and week 5 post DT injection; (d) Representative confocal image from indirect immunofluorescence showing a healthy podocyte (on the right) that expresses both synaptopodin (SNP, green) and p57 (red) and two injured podocytes that express SNP, but only have low expression levels of p57 – identified as p57-; (e) Quantification of the percentage of p57- podocytes per group – 5-10 randomly sampled SNP⁺ podocytes from 10 systematically sampled glomeruli per mouse (n=4 mice per group); **** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, NS: not statistically significant. Scale bar represents 10 μ m

Suppl Figure 4: Podocyte hypertrophy in diabetic mice. (a) Schematic representation of experimental design for a hyperglycaemic model via streptozotocin (STZ) injection; (b) Fasting plasma glucose; (c) Urinary albumin to creatinine ratio (UACR); (d) Glomerular volume; (E) Podocyte number; (f) Podocyte density; (g) Total podocyte volume per unit of podocyte density (TPV:PD ratio); **** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$; in (B and C) bars represent means, and error bars SEMs – each dot represents average data for one mouse; in violin plots, red lines represent medians, blue lines IQRs and every grey dot represents one glomerulus.

Suppl Figure 5: Additional potential drivers of PEC activation. (a) Podocyte number; and (b) Glomerular volume; (c) Association between percentage (%) of glomeruli with CD44+ PECs and glomerular volume; (d) Association between % of CD44+ PECs and total podocyte volume per unit of podocyte density (TPV:PD ratio);. * $P < 0.05$. Solid lines represent the regression line and dotted lines the 95% CI; bars represent means and error bars SEMs – each dot represents one mouse.

Suppl Figure 6: Pharmacological mTOR inhibition is not harmful in control mice without active podocyte loss. (a) Urinary albumin to creatinine ratio; (b) Podocyte number; (c) Representative confocal images after indirect immunofluorescence showing absence of PEC activation in control mice treated with mTOR inhibitors; and (d) Representative histological stainings showing normal glomeruli in control mice treated with mTOR inhibitors. ** $P < 0.01$, NS: not statistically significant; in (a and b) bars represent means and error bars SEMs; scale bars represent 40 μ m

Suppl Figure 7: mTOR-signaling modulation in primary podocytes and PECs. (a) Representative confocal images after indirect immunofluorescence for F-actin, showing remodelling of actin cytoskeleton of primary podocytes upon serum starvation/stimulation cycles; (b) Quantification of serum/stimulation cycles in primary podocytes showing modulation of cell size via mTOR inhibitors (RAD001 and INK128); (c) Migration of primary parietal epithelial cells (PECs) is not enhanced by mTOR inhibitors; (d) mRNA levels of PEC activation signals are not enhanced by mTOR inhibitors; in (D) bars represent means and error bars SEMs – each dot represents one biological replicate; in violin plots, red lines represent medians, blue lines IQRs and every grey dot represents one measured field from three biological replicates; scale bars represent 40um.

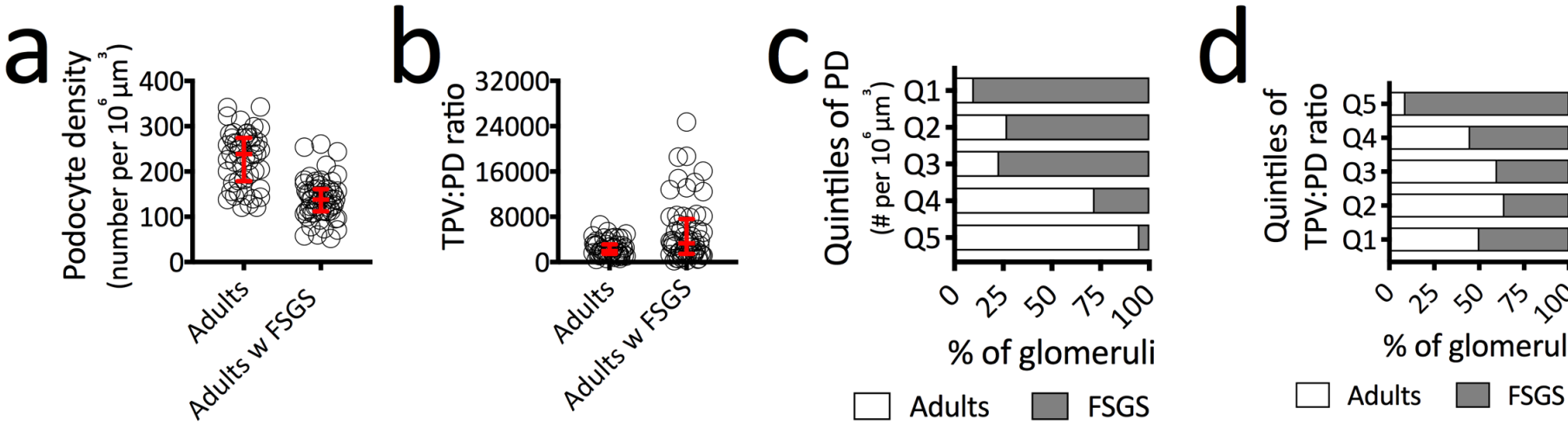
Suppl Table 1: Demographics of autopsy samples (children) AA: African American and CA: Caucasian American, F: female, M: male, SIDS: sudden infant death syndrome, Mixed: haematological, infectious and/or neoplastic

Suppl Table 2: Demographics of adults without kidney disease (63). BMI: body mass index, BSA: body surface area, T2D: type 2 diabetes, IHD: ischaemic heart disease, HTN: hypertension, RA: rheumatoid arthritis

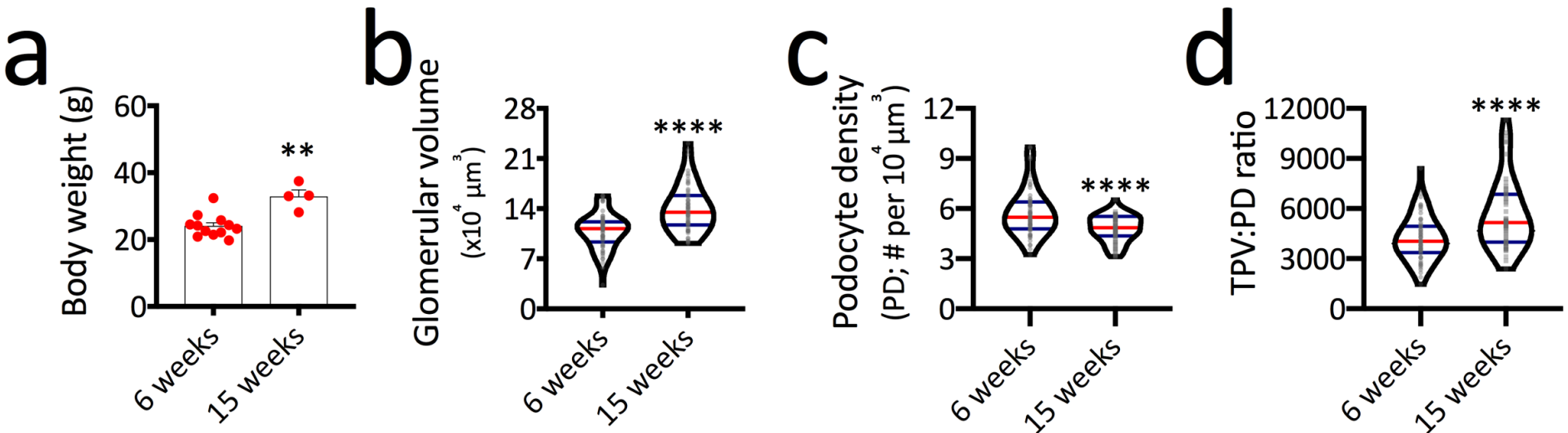
Suppl Table 3: Demographics of patients with primary FSGS. Pred: Prednisone, Tac: Tacrolimus, CNI: Calcineurin inhibitor, Ritux: Rituximab, MMF: Mycophenolate Mofetil, CyA: Cyclosporine A, POB: place of birth, COPD: chronic obstructive pulmonary disease, TB: tuberculosis, HTN: hypertension, IHD: ischemic heart disease, Neo: neoplasma, TD: thyroid disease, GI: gastrointestinal, and DVT: deep vein thrombosis

Suppl Table 4: Summary of antibodies used in this study.

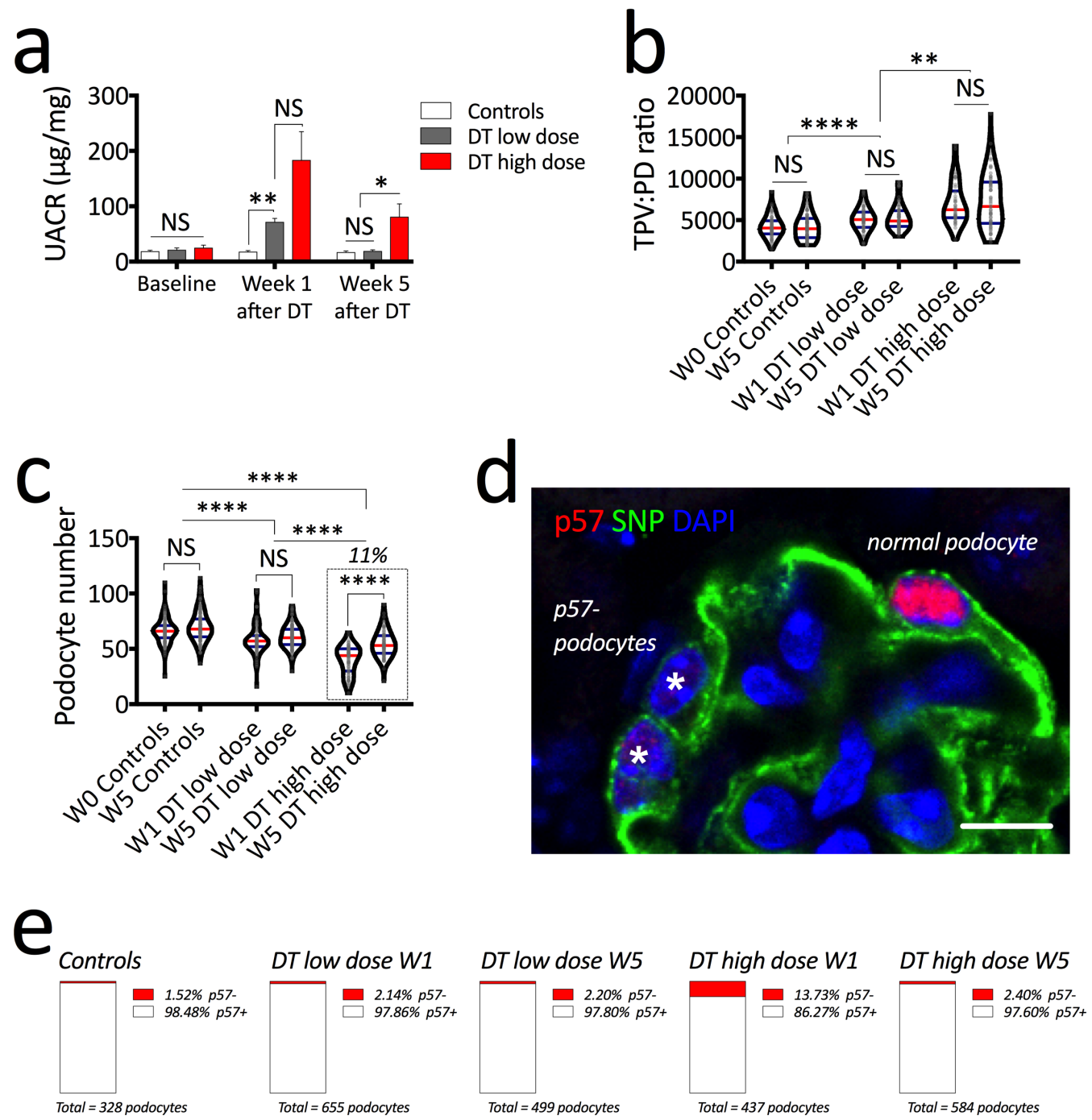
Suppl Figure 1



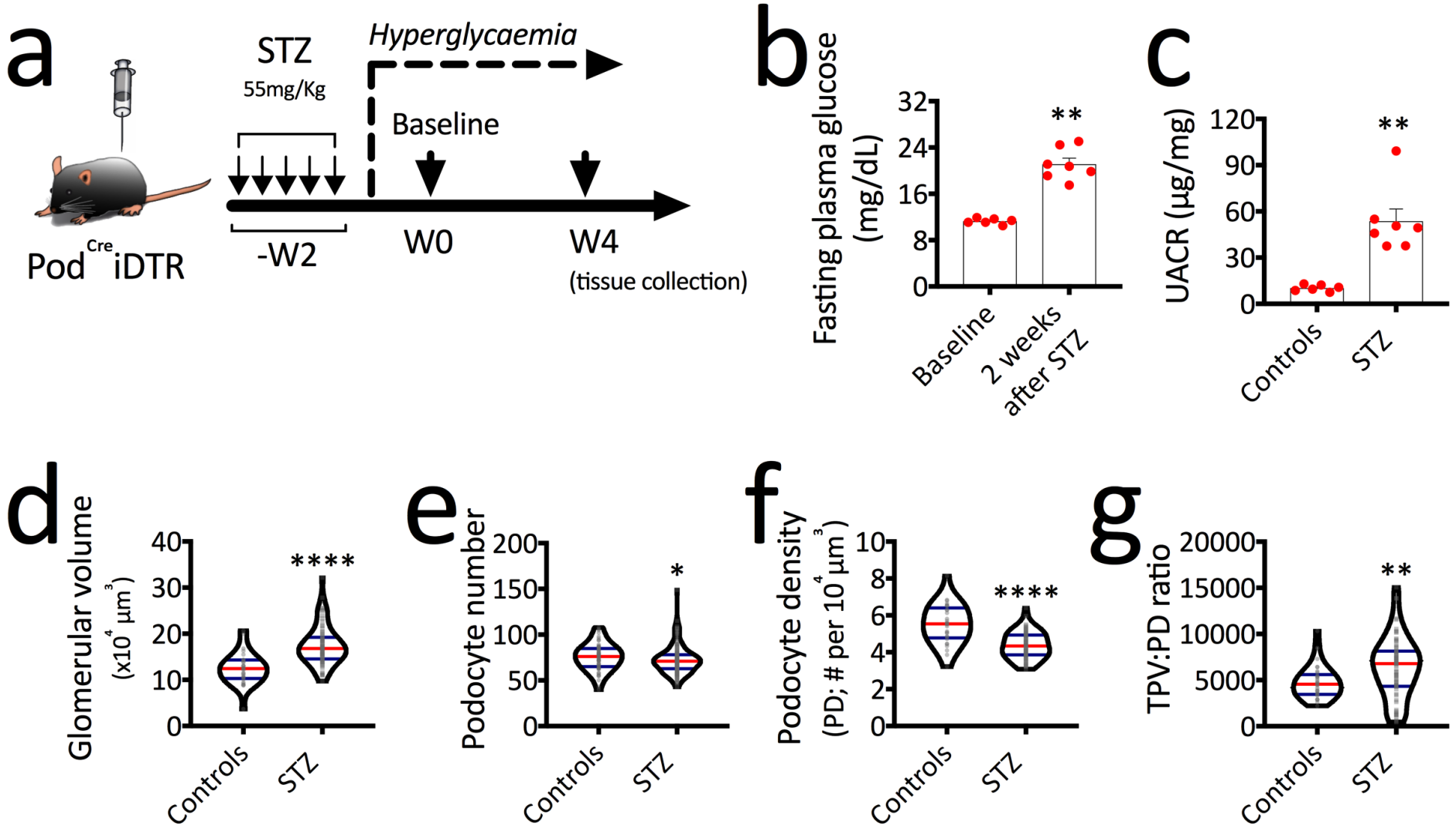
Suppl Figure 2



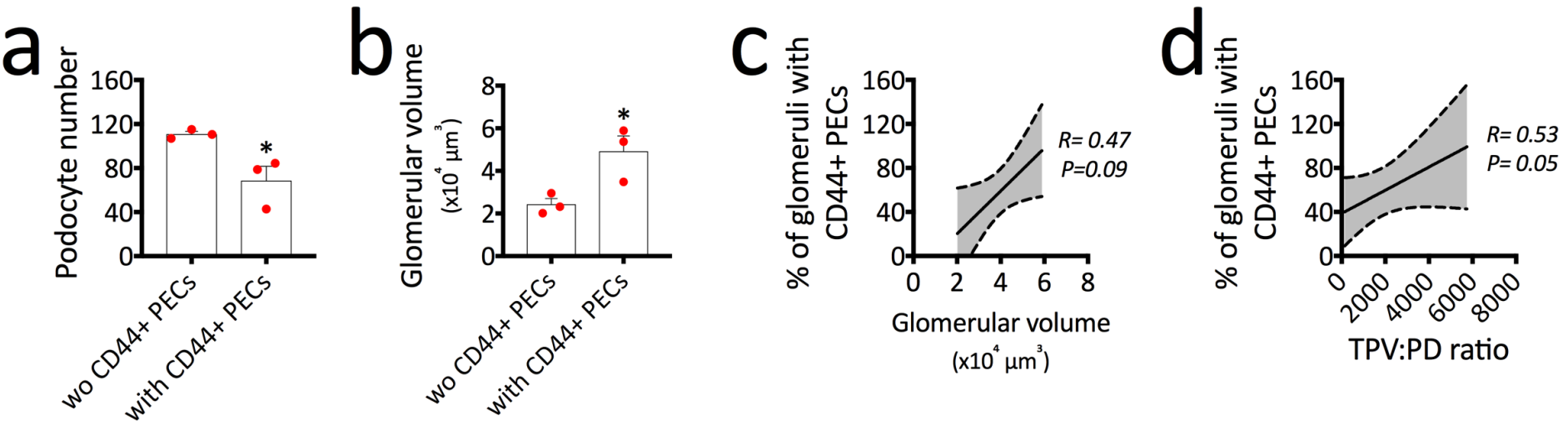
Suppl Figure 3



Suppl Figure 4

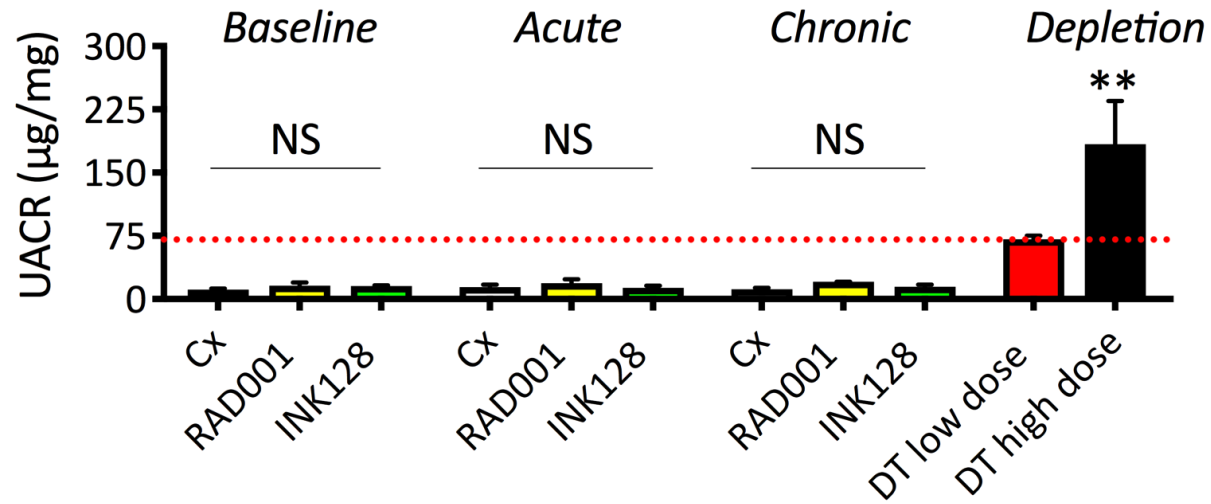


Suppl Figure 5

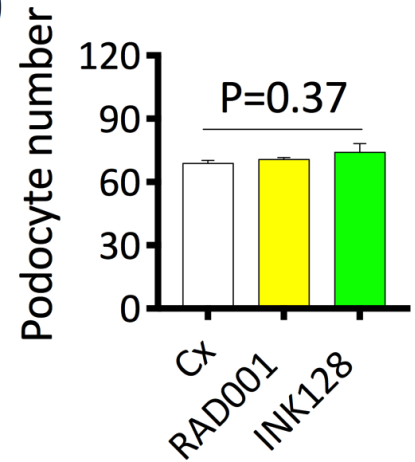


Suppl Figure 6

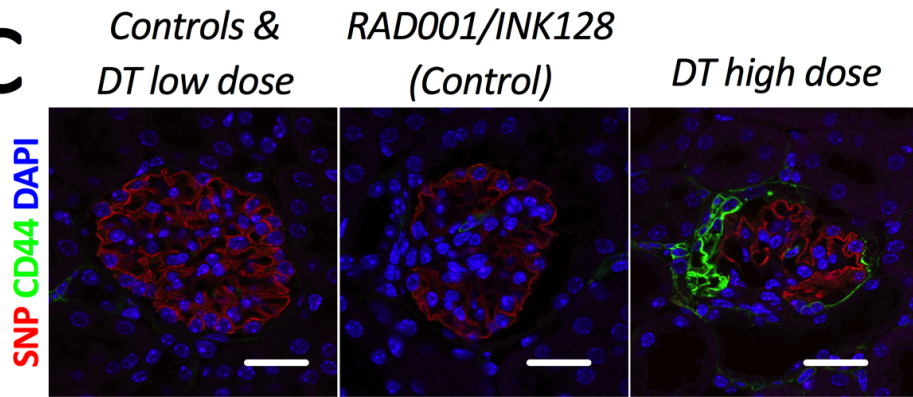
a



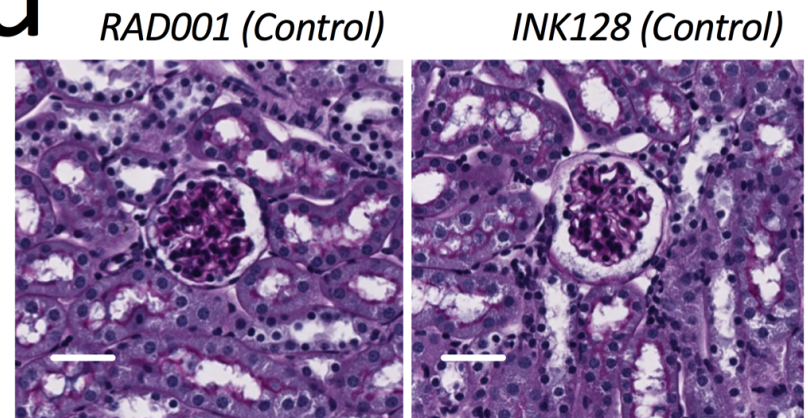
b



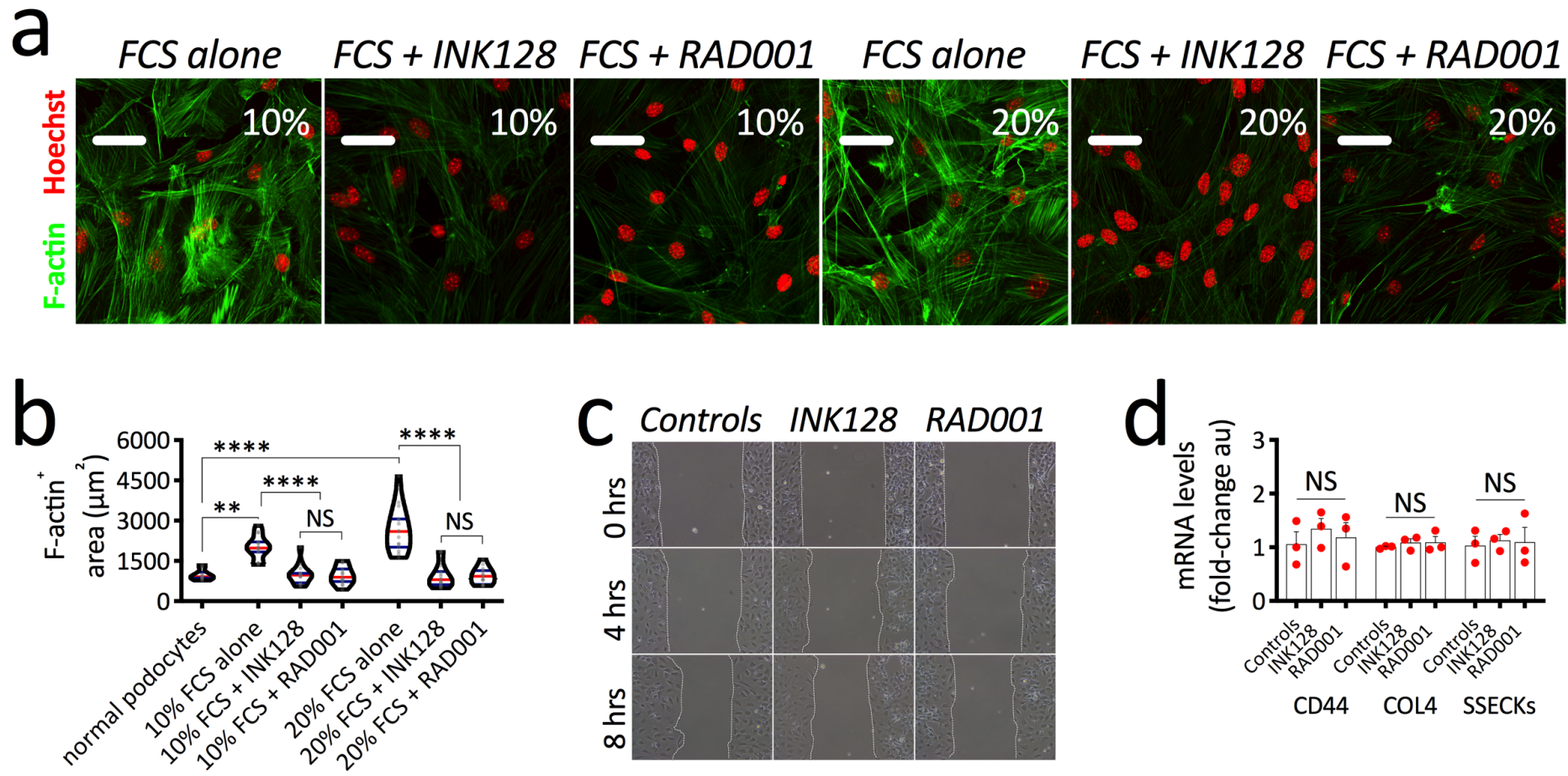
c



d



Suppl Figure 7



Suppl Table 1

Subjects	Race	Sex	Age (years)	BSA (m ²)	Birth Weight (Kg)	Nephron Number (million)	COD
1	AA	F	0.25	0.35	3.07	0.66	SIDS
2	CA	M	0.25	0.32	3.09	0.69	Cardiac
3	CA	M	0.25	0.36	2.92	1.11	SIDS
4	AA	M	0.33	0.38	2.60	1.04	SIDS
5	CA	M	0.50	0.32	3.54	0.84	Mixed
6	AA	M	0.50	0.41	3.39	0.79	SIDS
7	AA	F	1.00	0.46	3.53	0.71	Mixed
8	AA	F	1.08	0.44	3.06	1.30	Mixed
9	CA	F	2.92	0.84	3.40	1.19	Accident
10	AA	F	3.00	0.62	3.63	0.81	Accident
11	CA	M	3.00	0.68	3.63	0.90	Mixed

Suppl Table 2

Controls	Age	Height	Weight	BMI	BSA	Co-morbidities	eGFR	Report	Proteinuria
1	48	1.62	67.00	25.53	1.74	None	83.71	Normal	Absent
2	65	1.74	86.50	28.57	2.04	T2D, IHD, gout, HTN	93.47	Normal	Absent
3	56	1.69	73.00	25.56	1.85	HTN	91.80	Normal	Absent
4	60	1.85	90.00	26.30	2.15	IHD	75.47	Normal	Absent
5	51	1.64	62.00	23.05	1.68	RA	85.21	Normal	Absent

Suppl Table 3

Patients	Treatment	Age	POB	Weight	Co-morbidities	eGFR
1	Pred	24	Sri Lanka	63	none	>90
2	Pred/MMF	75	France	82	HTN, IHD, Neo	60
3	Pred/CNI/MMF	35	Poland	72	none	64
4	CyA	56	Netherlands	89	HTN, TD	>90
5	Pred	82	Australia	102	HTN, GI	19
6	Pred/CyA	52	China	59	none	50
7	Pred/CyA/Tac	57	Australia	80	HTN	48
8	CyA, Pred	67	England	74	HTN, GI, DVT	32

Suppl Table 4

Antibody	Company	Catalogue #
Rabbit anti-mouse p57	Santa Cruz	sc-8298
Goat anti-mouse SNP	Santa Cruz	sc-21537
Rabbit anti-mouse Claudin-1	Abcam	ab15098
Rat anti-mouse CD44	BD Pharmingen	553131
Rabbit anti-mouse p-rps6	Cell signalling technology	#5364
Chicken anti-human HB-EGF	R&D Systems	AF-259-NA
Goat anti-rabbit Alexa Flour® 647	Invitrogen	A21247
Donkey anti-rabbit Alexa Flour® 555	Invitrogen	A31572
Chicken anti-goat Alexa Flour® 488	Invitrogen	A21467