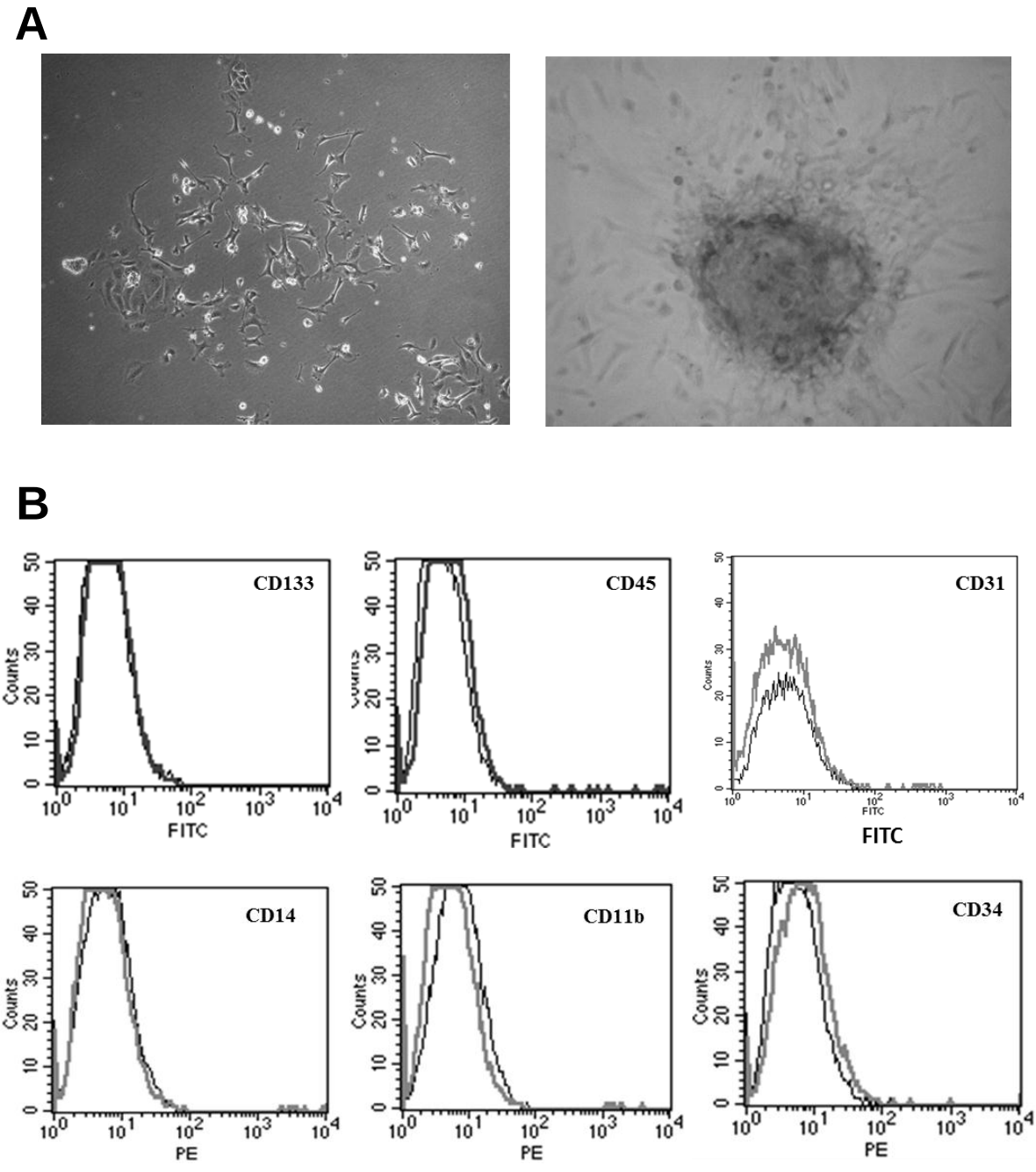
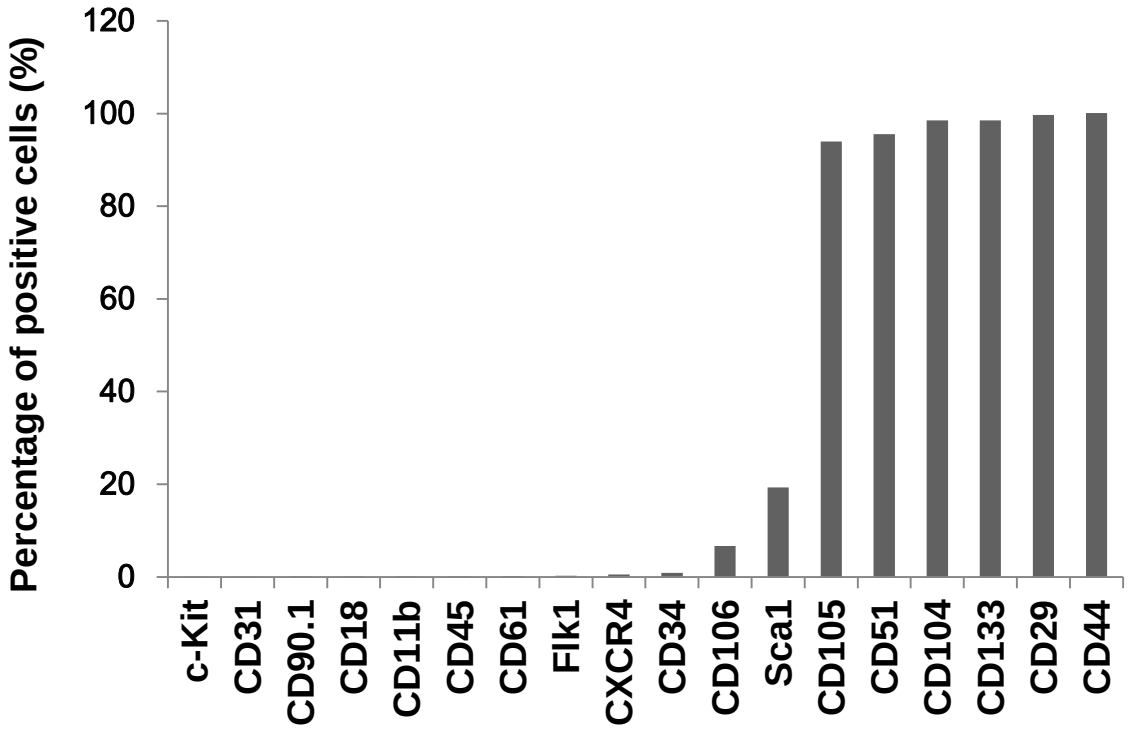
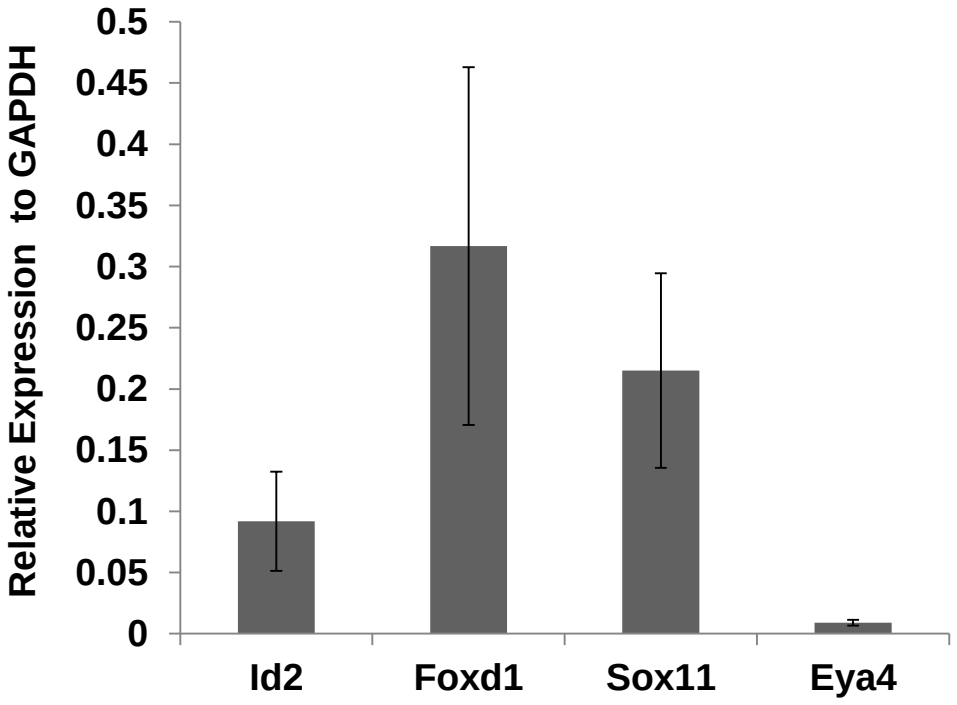


Supplemental Figure 1. Isolation and characterization of renal MSC-like cells**. (A)** Representative light microscopy images of CD44+ cells in culture during passage 1. Magnification at 10X and 40X respectively. **(B)** FACS analysis of MSC, hematopoietic and endothelial markers in CD44+ cells after 3-5 passages in culture.

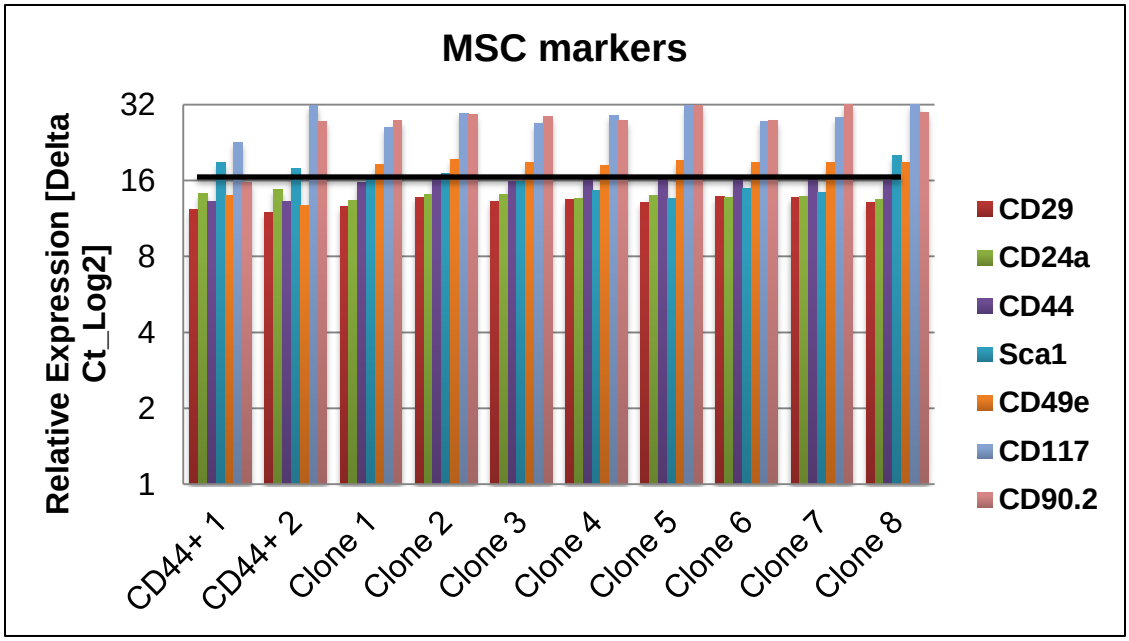
A

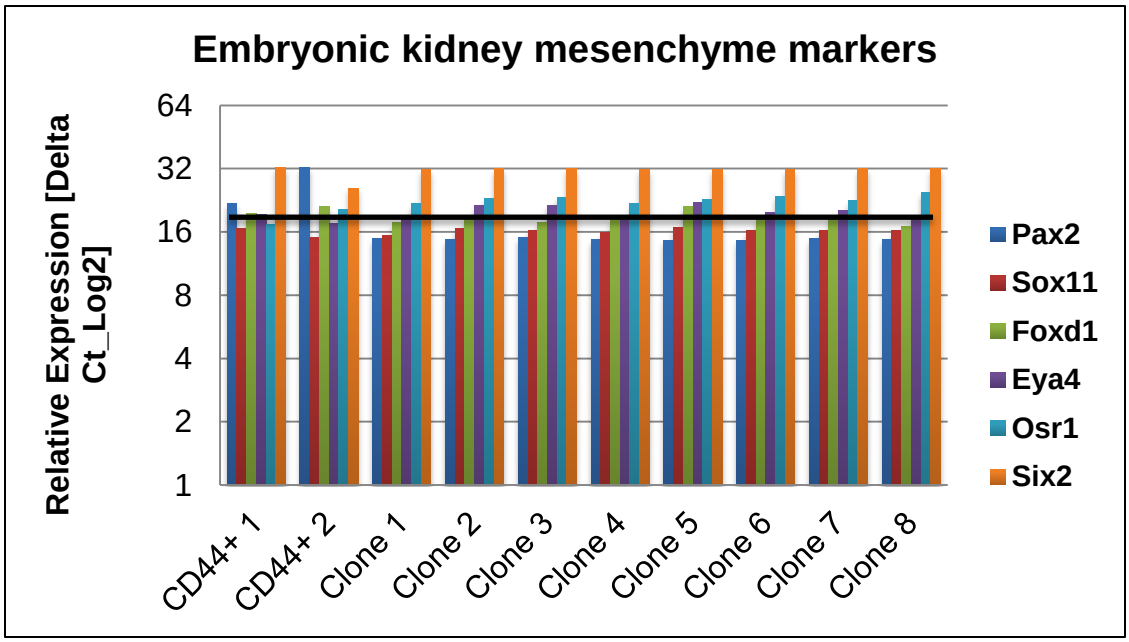

Percentage of positive cells (%)
120
100
80
60
40
20
0
c-Kit
CD31
CD90.1
CD18
CD11b
CD45
CD61
Flk1
CXCR4
CD34
CD106
Sca1
CD105
CD51
CD104
CD133
CD29
CD44

B

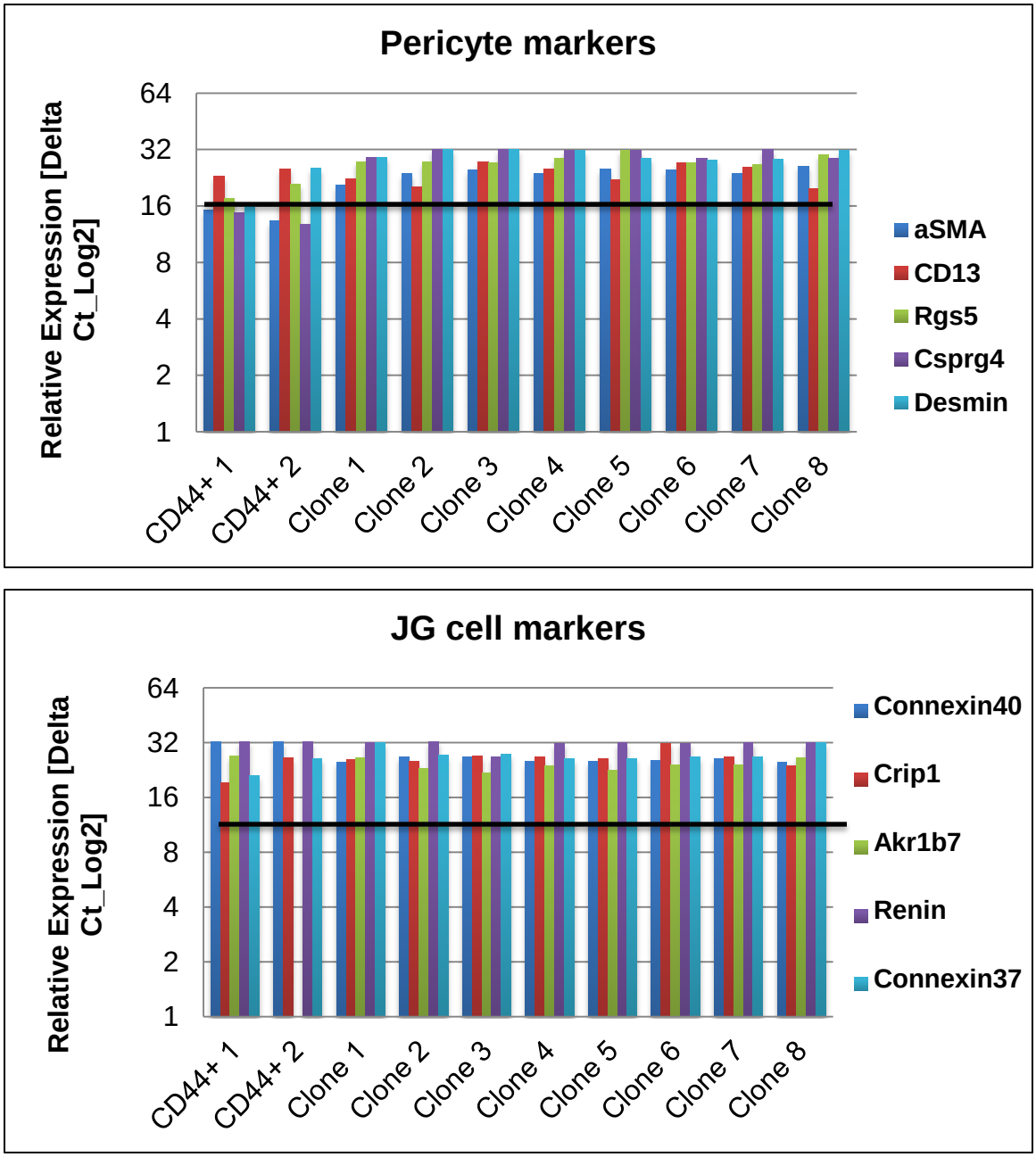

Relative Expression to GAPDH
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Id2
Foxd1
Sox11
Eya4

C

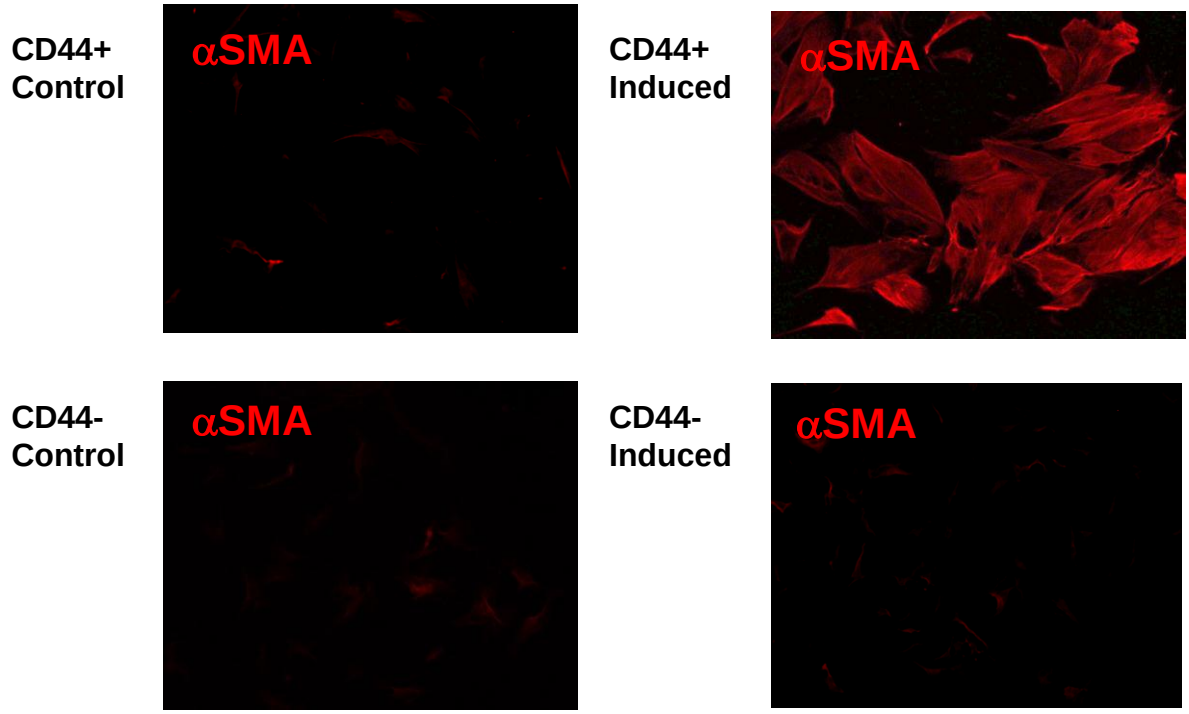

MSC markers
Relative Expression [Delta Ct_Log2]
32
16
8
4
2
1
CD44+ 1
CD44+ 2
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
CD29
CD24a
CD44
Sca1
CD49e
CD117
CD90.2


Embryonic kidney mesenchyme markers
Relative Expression [Delta Ct_Log2]
64
32
16
8
4
2
1
CD44+ 1
CD44+ 2
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Pax2
Sox11
Foxd1
Eya4
Osr1
Six2

C continued

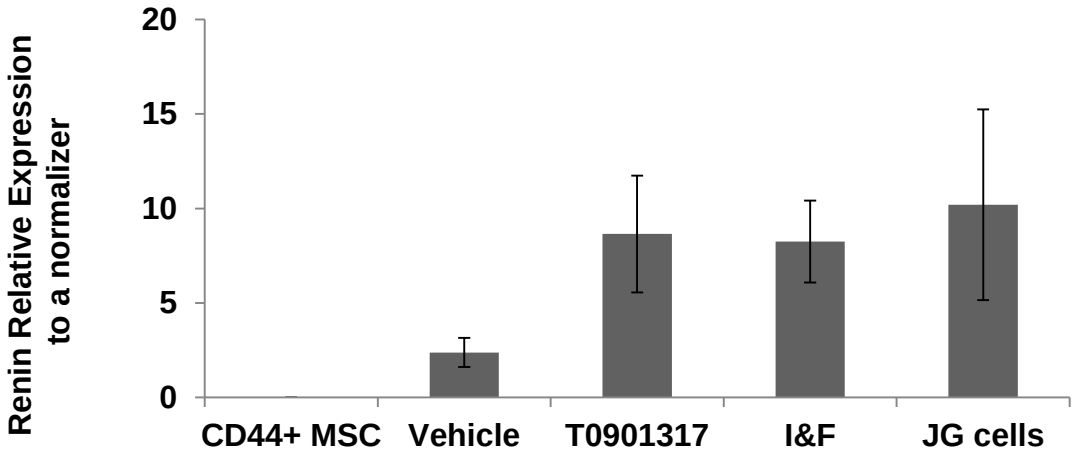


Supplemental Figure 2. (A) Flow cytometry analysis for the expression of MSC surface markers in CD44+ cultured cells (passage 5). **(B)** Real time PCR data showing the expression of different metanephric mesenchyme markers in CD44+ cultured cells. Data are presented as the mean ± SEM, n=7 in each group. **(C)** Representative real time PCR data showing the expression of different markers in CD44+ cultured cells (1 and 2) and clones derived from the CD44+ cells (Clones 1-8). The data are presented as Delta Ct=Ctgene-Ct18s to ensure that values for all data sets can be visualized. Everything with a Delta Ct >18 is considered borderline or not expressed. The black line on the graphs depicts this threshold. As shown the clones showed consistent expression of MSC markers as well expression of the embryonic mesenchyme markers Pax2, Sox11 and Foxd1. Pericytes or JG cell markers were not detectable.

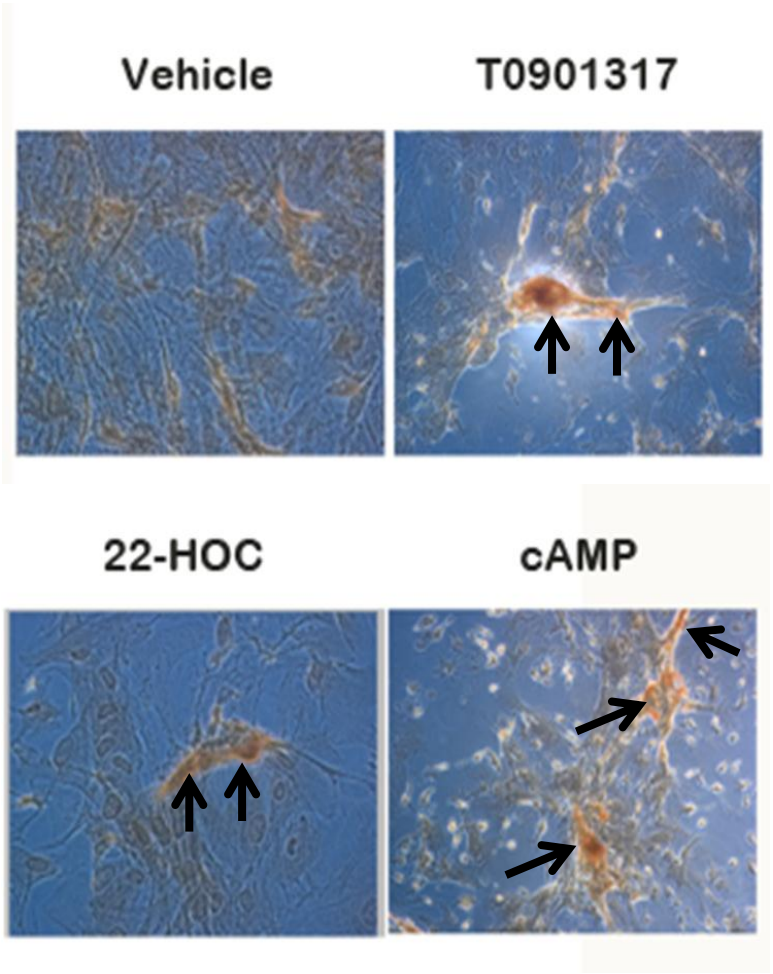


Supplemental Figure 3. Representative images of renal CD44+ and CD44- cells at baseline and after induction of the smooth muscle lineage. Images were taken 6 days post treatment. Expression of the SMC specific marker αSMA is shown in red.

A


Renin Relative Expression to a normalizer
20
15
10
5
0
CD44+ MSC
Vehicle
T0901317
I&F
JG cells

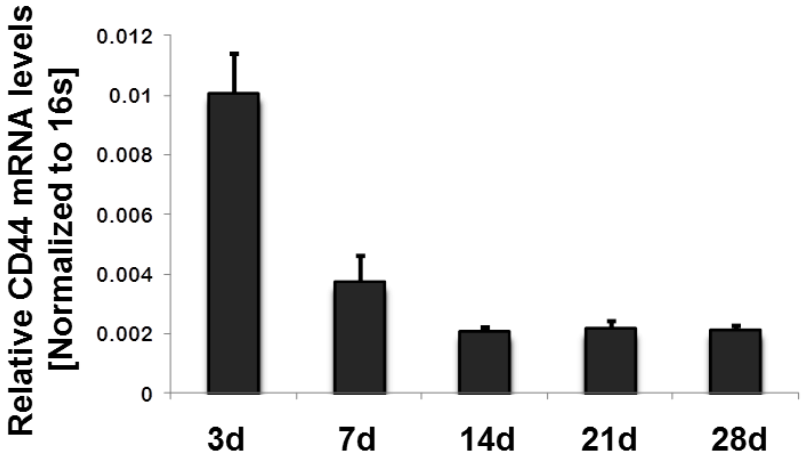
B


Vehicle
T0901317
22-HOC
cAMP

C

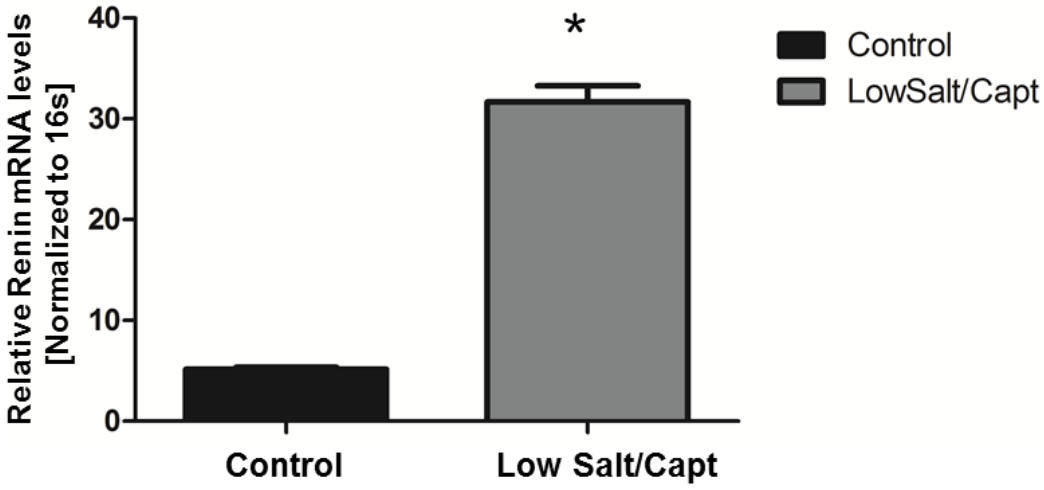
Sample	Renin	GAPDH
	CT	CT
Renal CD44+ MSC 1	Und	16.98
Renal CD44+ MSC 2	Und	19.55
Renal CD44+ MSC 3	Und	17.97
Renal CD44+ MSC 4	Und	19.47
Renal CD44+ MSC 5	Und	15.96
Renal CD44+ MSC 6	Und	15.63
Renal CD44+ MSC 7	Und	15.95
Renal CD44+ MSC 8	Und	17.77
JG YFP+ cells 1	19.97	24.16
JG YFP+ cells 2	16.96	18.91
JG YFP+ cells 3	17.18	22.38
JG YFP+ cells 4	19.77	21.68
JG YFP+ cells 5	18.97	22.08

D



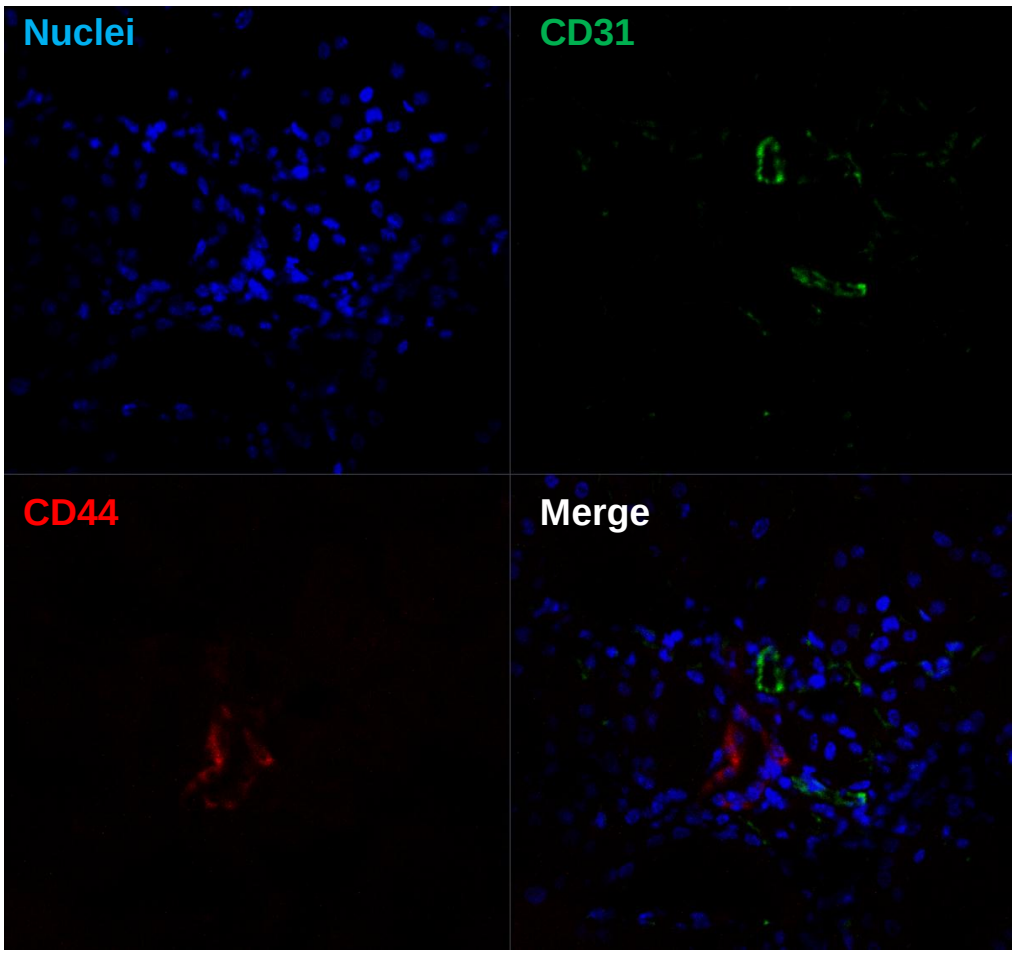
Supplemental Figure 4. **(A)** Real time PCR of renin expression in JG cells and renal CD44+ MSC cells after treatment with T0901317 or IBMX. JG sample normalized to GAPDH and other samples to 16S. **(B)** Immunocytochemistry using DAB staining and renin-specific antibody (brown) in CD44+ cells treated for two weeks with cAMP, T0901317, 22-HOC, or DMSO as vehicle. Arrows point at renin positive cells. **(C)** Expression of Renin in CD44+ cells at baseline and JG cells isolated from normal kidneys. **(D)** CD44+ expression in CD44+ cells treated with T0901317 (1 μM) for different lengths of time (3, 7, 14, 21 or 28 days) as determined by real time PCR analysis. The expression of CD44 mRNA was normalized to the expression of 16s. Data are presented as the mean + SEM, n=3 in each group.

A

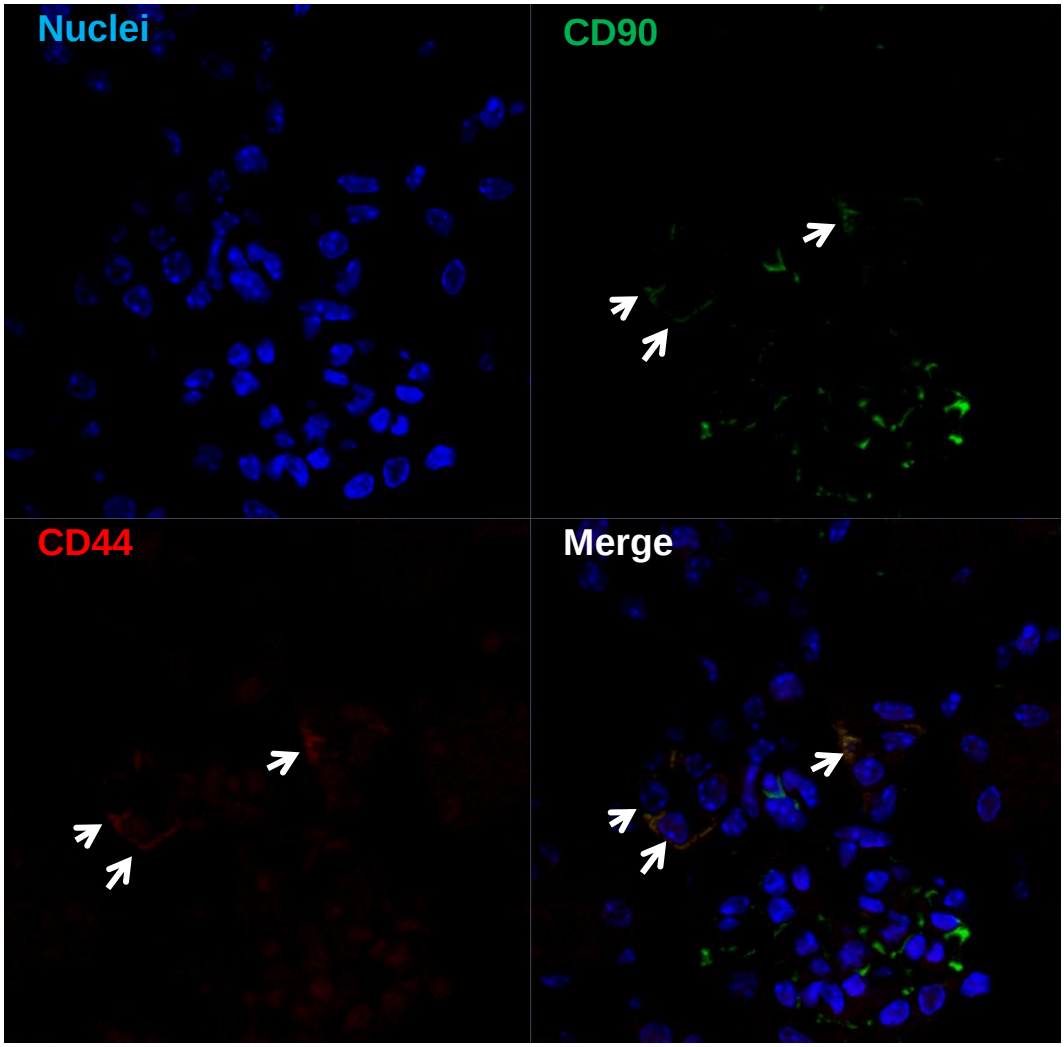

Relative Renin mRNA levels
[Normalized to 16s]
40
30
20
10
0
*
Control
LowSalt/Capt
Control
Low Salt/Capt

B

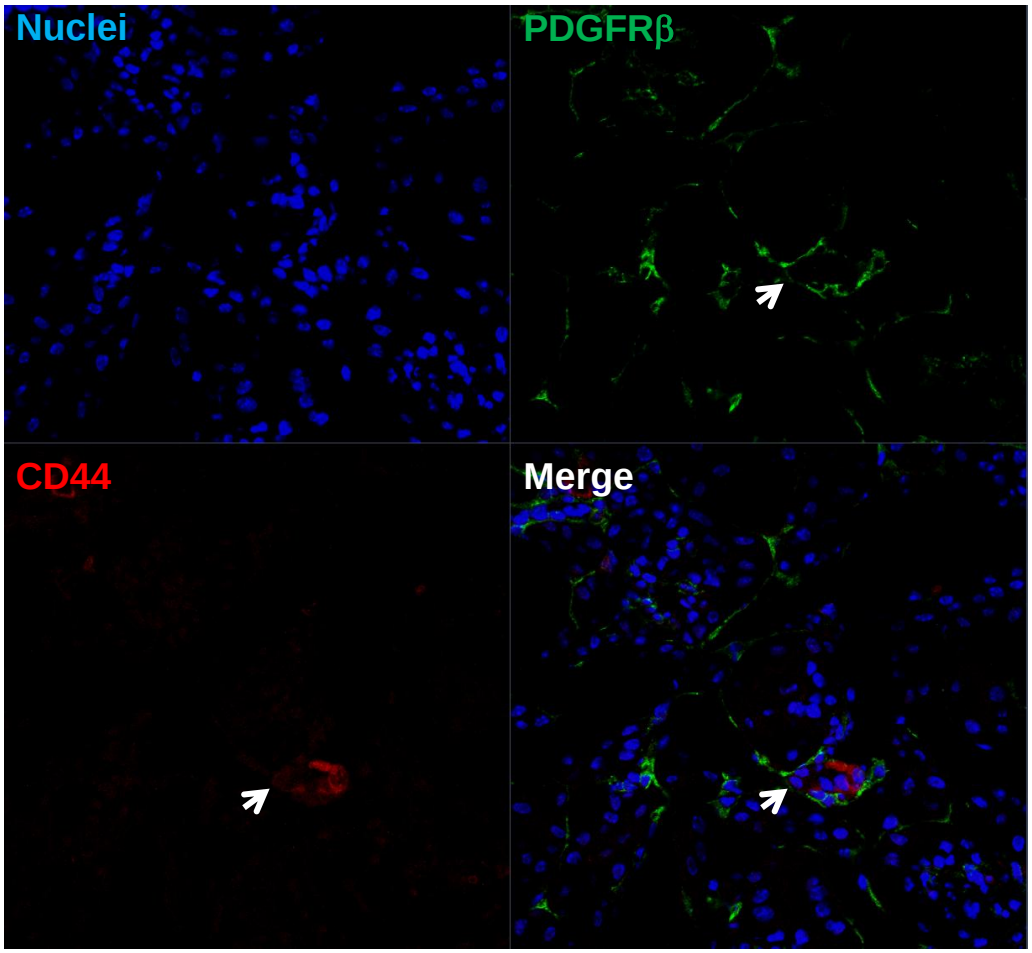
i


Nuclei
CD31
CD44
Merge

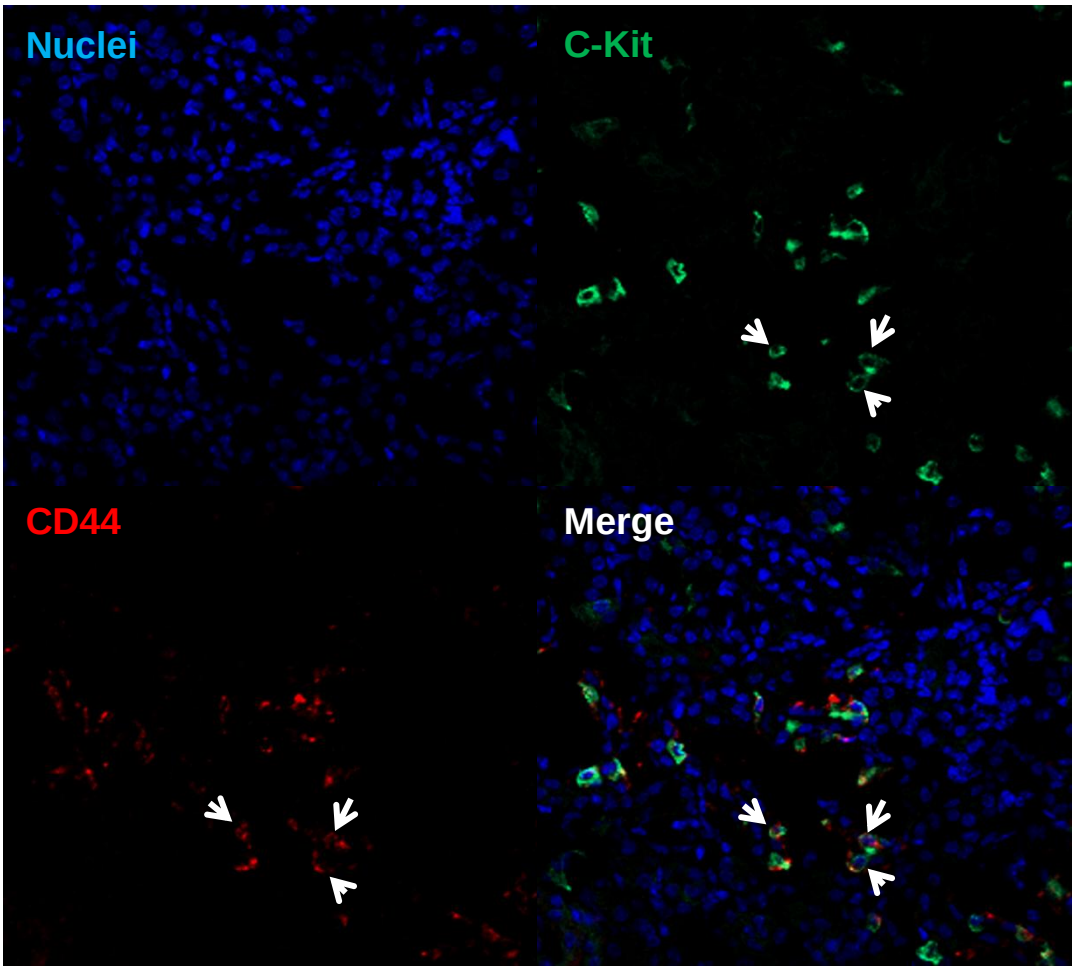
ii



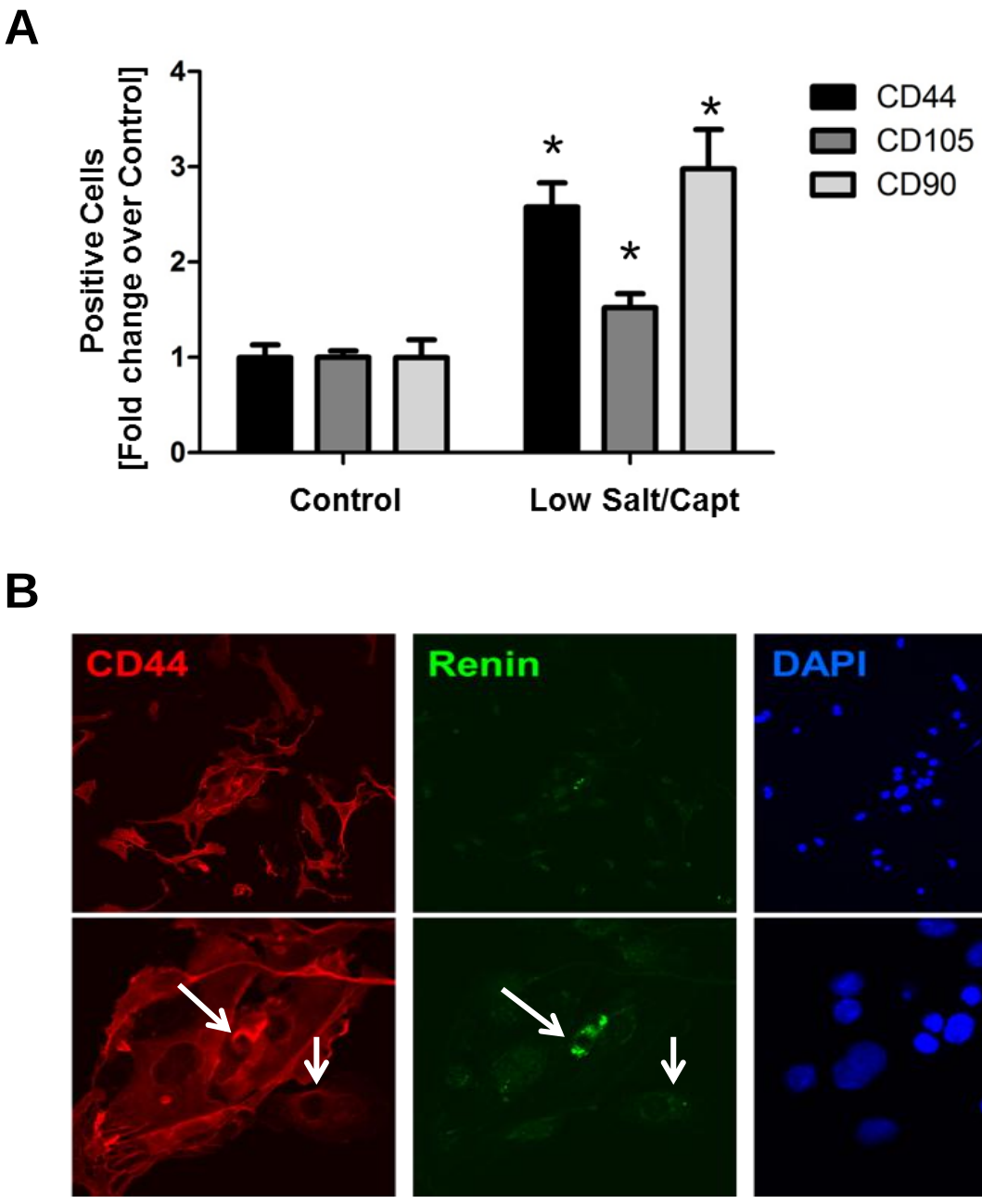
iii



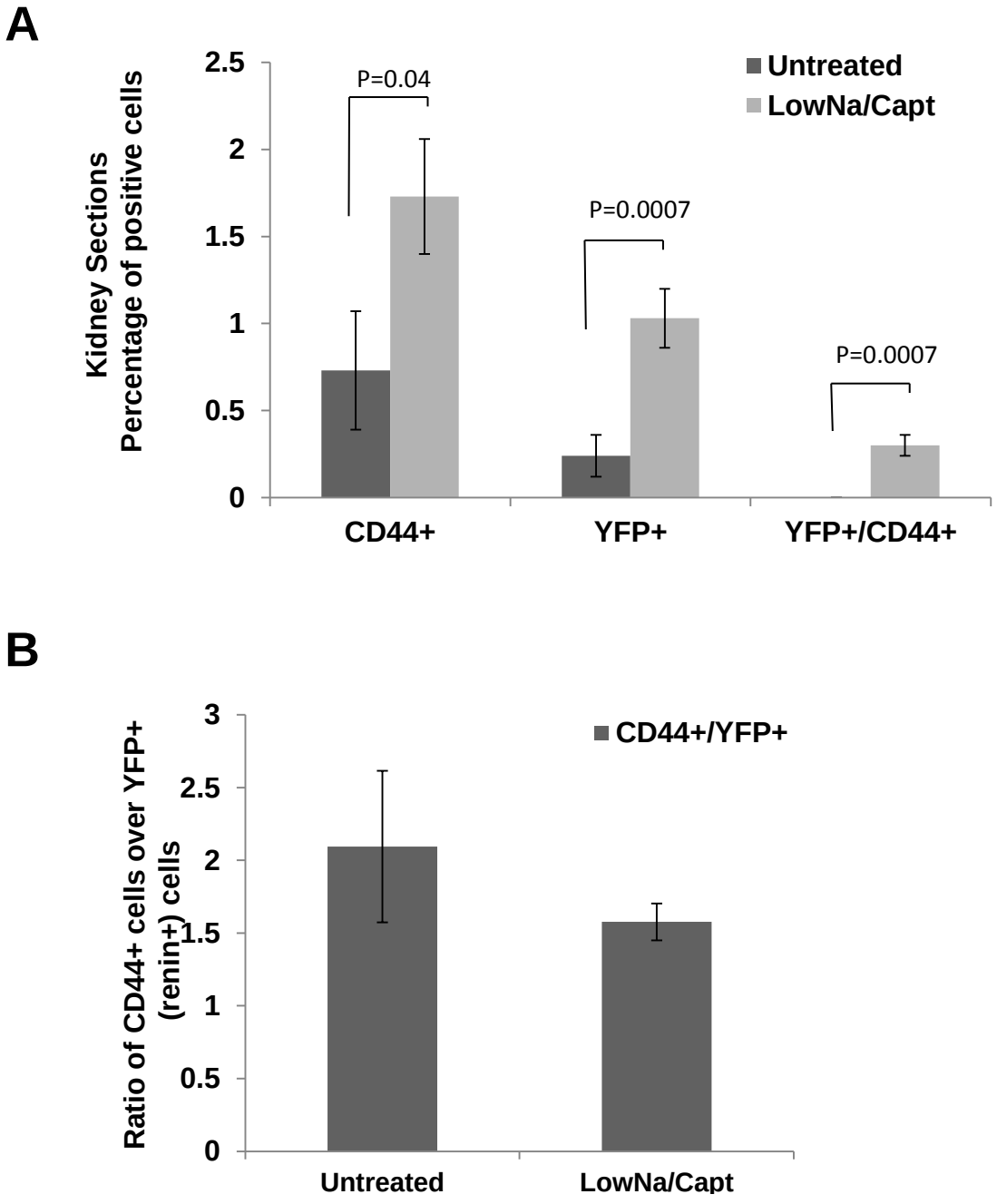
iv



Supplemental Figure 5. C57BL/6 wild type male mice (6-8 week old) were administered a LowSalt/Capt or control vehicle diet for 10 days. **(A)** After treatments kidneys were perfused, digested with collagenase, the cells collected, and the expression of renin mRNA was analyzed by real-time PCR. LowSalt/Capt treatment notably increased renin mRNA expression in mouse kidney compared control mice without treatment. The expression of renin mRNA was normalized to 16s expression levels. Data are presented as the mean + SEM, n=10 in each group; * :P<0.05 treated vs. control. **(B)** Characterization of endogenous renal CD44+ cells. Representative 40X confocal images (n=3) of kidney from LowSalt/Capt treated animals; From top to bottom: i) Staining for the vascular cell marker CD31 (PECAM-1).; ii) staining for the MSC/pericyte marker CD90 iii) staining for the pericyte marker PDGFRβ; iv) staining for the MSC marker c-Kit. For all images; arrows show double positive cells.

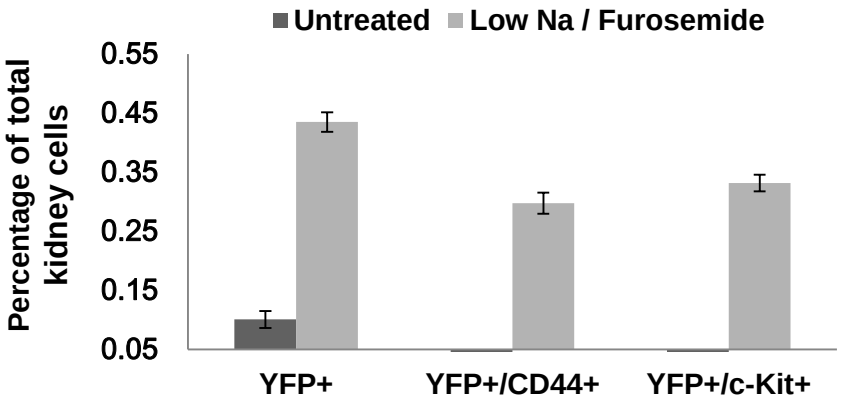


Supplemental Figure 6. C57BL/6 wild type male mice (6-8 week old) were administered a LowSalt/Capt or control vehicle diet for 10 days. **(A)** FACS analysis of renal cells isolated from the kidneys of control and mice under LowSalt/Capt treatment using anti-CD44, anti-CD90 and anti-CD105 specific antibodies. Data are presented as the mean + SEM, n=8-9 per group; *: $P<0.05$ treated vs. control. **(B)** After treatment kidneys were perfused, digested with collagenase and the CD44+ cells collected, re-plated on glass slides for staining with Dapi (blue) and antibodies specific for CD44 (red) and renin (green). Representative images are shown (20X magnification). Arrows highlight double positive CD44+ and Renin+ cells

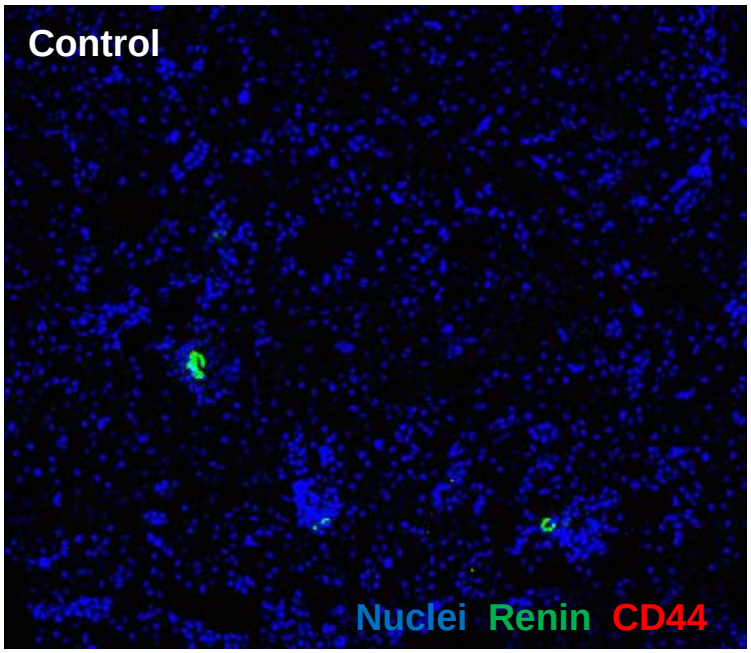


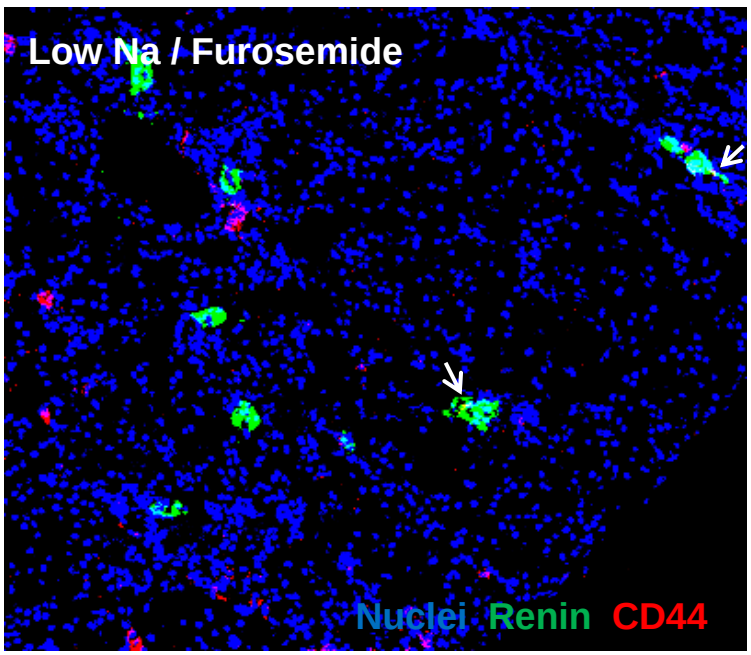
Supplemental Figure 7. C57BL6 Ren1c YFP mice low sodium diet and captopril treatment or normal diet and vehicle as control. Quantification of IHC images using Imaris. Quantification data of image analysis from three independent experiments using 3 mice per group with 5 section per kidney and 3 images per section. P values determined using un-paired student T test. Data are presented as the men ± SEM.

A

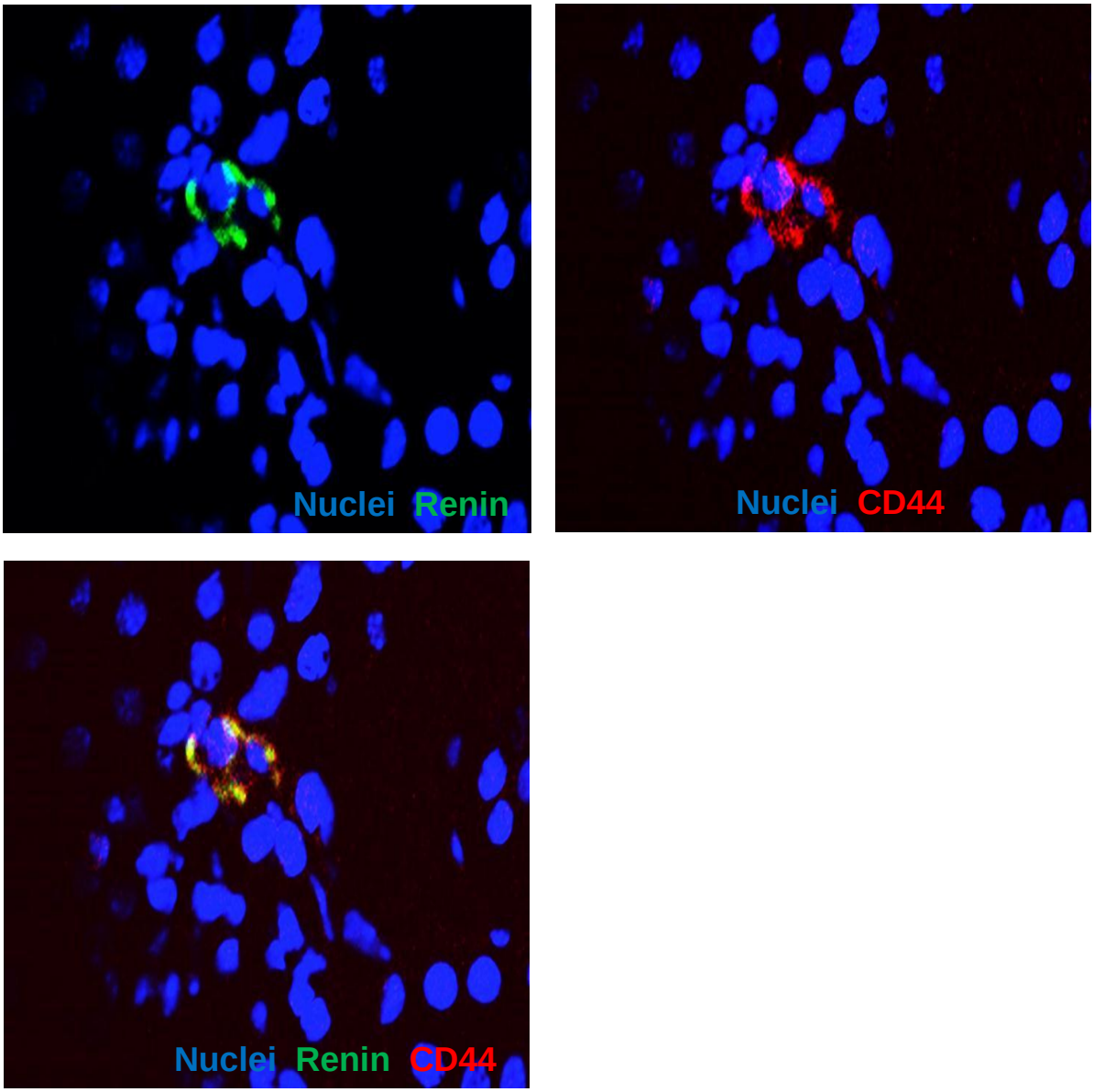

Untreated
Low Na / Furosemide
Percentage of total kidney cells
0.55
0.45
0.35
0.25
0.15
0.05
YFP+
YFP+/CD44+
YFP+/c-Kit+

B


Control
Nuclei Renin CD44


Low Na / Furosemide
Nuclei Renin CD44

C

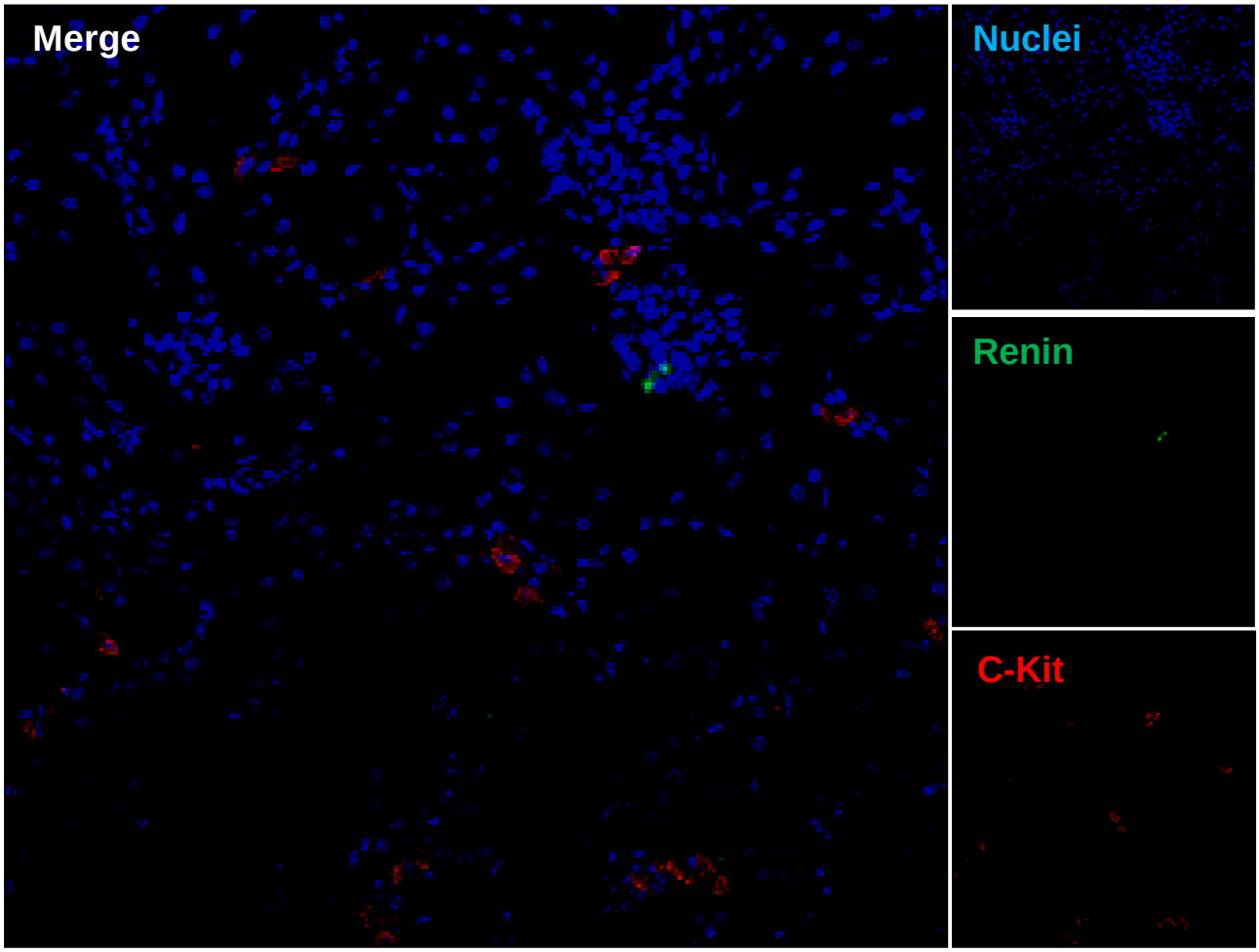


Supplemental Figure 8. C57BL/6 Ren1c YFP male mice (6-8 week old) were administered a Low Salt + furosemide or control vehicle diet for 10 days as presented in material and methods section. **(A)** FACS analysis of isolated kidney cells using anti-CD44, anti-c-Kit antibodies and YFP expression from mice treated with Low Salt + Furosemide and control . Data are presented as the mean and SEM. **(B)** Immunohistochemistry using Dapi stain and specific antibodies for CD44 (red) or renin (green) in kidney section of 10 days low sodium diet / furosemide treated and control un treated mice. Images are 10X magnification. Arrows point at c-Kit positive, renin positive and double positive cells. **(C)** Immunohistochemistry using an specific antibody for CD44 (red), renin (green) and nuclei (blue) in kidney section of 10 days low sodium diet / furosemide treated and control un treated mice. Images are 20X magnification with 6X zooms.

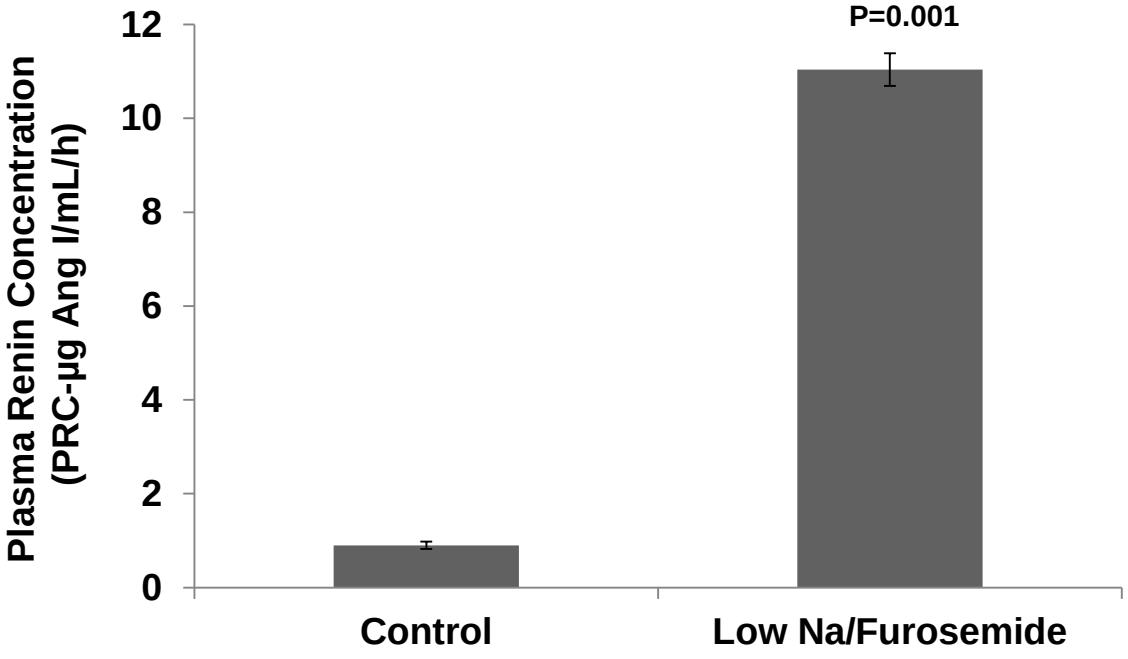
A

ng RNA	Sample	CT c-Kit	CT GAPDH
150	**CD44+ MSC 1**	29.406281	18.093214
150	**CD44+ MSC 2**	27.368956	20.538872
200	**CD44+ MSC 1**	28.692442	17.68063
200	**CD44+ MSC 2**	27.160828	20.20038
150	**JG cells 1**	Undetermined	16.250963
150	**JG cells 2**	Undetermined	20.068624
200	**JG cells 1**	Undetermined	15.940108
200	**JG cells 2**	Undetermined	19.618931

B



Supplemental Figure 9. (A) Quantitative real time PCR data (CT values) for c-Kit and GAPDH in freshly isolated mouse renal CD44+ MSCs and juxtaglomerular cells from C57BL6 Ren1c YFP mice. **(B)** Representative image from kidney section of normal kidney. Immunohistochemistry using an specific antibody for c-Kit (red) and renin (green) in kidney section of male mice. 20X magnification.



Supplemental Figure 10. C57BL6 wild type mice were treated for 10 days with low sodium diet and furosemide. After the treatments blood was acquired and plasma separated to determine plasma renin concentration (PRC). Data presented from 4 mice per group and bar graphs show PRC as µg Ang I/mL/h ± SEM. P values calculated using t student un paired test.

Table 1. Glossary of terms.

Term	Definition
Progenitor cell	Any cell that has the ability to proliferate
Adult tissue stem cell	A relatively undifferentiated cell that can a) self-renew for an extended period of time and b) has the potential to differentiate into any of the tissues surrounding it
Facultative progenitor cell	A differentiated cell type that retains its proliferation capacity but still possesses all the specialized functions of a differentiated cell type.
Multipotent	Ability of an adult stem cell to generate multiple cell types of one lineage.
Embryonic Stem Cell	Pluripotent stem cells isolated from the inner cell mass of early developing blastocysts. These cells are capable of giving rise to all embryonic lineages and all adult cell types.
Mesenchymal Stromal Cells (MSC)	MSCs are multi-lineage cells which can self-renew, express specific surface markers and adhere to plastic in culture. MSC can differentiate *in vitro* to adipogenic, chondrogenic and osteogenic lineages. Although no unique marker exists, MSCs typically express CD44, CD34, CD106 and CD105 and CD73. MSCs lack the expression of the hematopoietic markers. The presence of MSCs have been reported in different adult tissues including heart, liver and kidney. In vivo the terminology used is usually MSC-like cells.

Modified from Stripp B.R. Proc Am Thorac Soc. 2008 Aug 15;5(6):695-8

Table 2: Power Analysis sample size.

Figure	Samples	Power Analysis N=	We have N=
1C	CFU CD44+ cells vs CD44- cells	**4**	**6**
2E	Renin Activity Control vs IBMX Control vs BAPTA IBMX vs IBMX + Thapsi BAPTA vs BAPTA + Thapsi	**3**	**8**
4C	YFP+ cells Control vs LowNa/Capt YFP+/CD44+ Control vs LowNa/Capt	 **2** **4**	 **3** **4**
6B	CD44+GFP-	**4**	**5**
Sup F 4A	T0901317 IBMX (cAMP)	**4** **2**	**3** **3**
Sup F 7A	CD44+ YFP+ CD44+/YFP+ (border line)	**7** **3** **3**	**14** **3** **3**
Sup F 11A	Relative Renin mRNA levels Control vs LowNa/Capt	**2**	**10**
Sup F 12A	CD44+ CD90 CD105	**5** **5** **13**	**8** **8** **8**
Sup F 14A	LowNa/Furosemide YFP+ YFP+/CD44+ YFP+/c-Kit+	 **2** **3** **3**	 **3** **3** **3**