Supplementary materials for

Renal interstitial fibroblasts coproduce erythropoietin and renin under anaemic conditions

Kenichiro Miyauchi, Taku Nakai, Sakae Saito, Tae Yamamoto, Koji Sato, Taku Nakai, Koichiro Kato, Masahiro Nezu, Mariko Miyazaki, Sadayoshi Ito, Masayuki Yamamoto, Norio Suzuki

This file includes 11 figures and 3 tables.

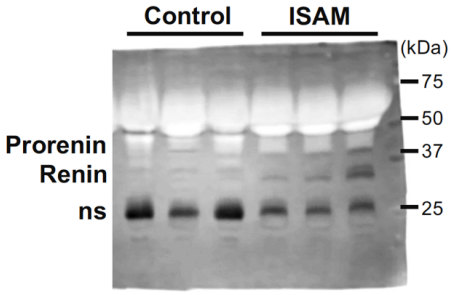

Fig. S1. Full-scan image of Figure 2b. Immunoblotting of control and ISAM plasma with an anti-renin antibody detected renin, its precursor (prorenin), and nonspecific bands (ns), which were probably immunoglobulin. Molecular size markers (kDa) are shown.

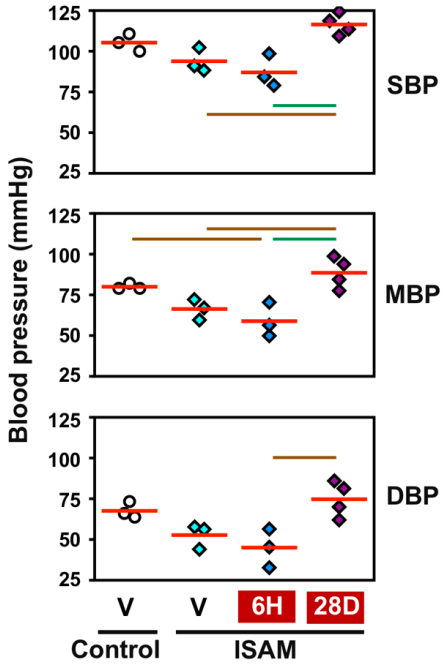

Fig. S2. Anaemic hypotension of ISAM mice is corrected after the mice recover from anaemia. SBP, MBP, and DBP were measured in ISAM mice administered C.E.R.A. for 4 weeks (28D). The littermate control mice treated with vehicle (V) were also tested at 11–14 weeks of age. n=3–4 for each group. Solid bars indicate mean values. Green and brown lines indicate $P<0.01$ and $P<0.05$, respectively. In the Tukey-Kramer HSD test

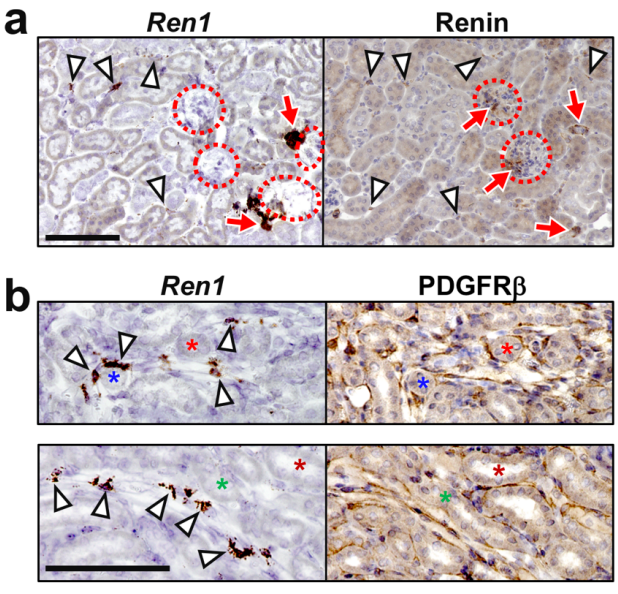

Fig. S3. Detection of renin expression in renal interstitial fibroblasts of ISAM mice. (a) Serial sections of ISAM kidney were stained with *Ren1* ISH (left) and renin IHC (right). (b) Serial sections of ISAM kidney were stained with *Ren1* ISH (left) and PDGFRβ IHC (right). The upper and lower panels are sections from different mice, and asterisks marked with the same colour indicate identical tubules in the left and right panels. The signals of each stain are brown (a and b). Dashed circles, arrows, and arrowheads indicate glomeruli, JG cells, and interstitial cells, respectively (a and b). Scale bars represent 100 μm.

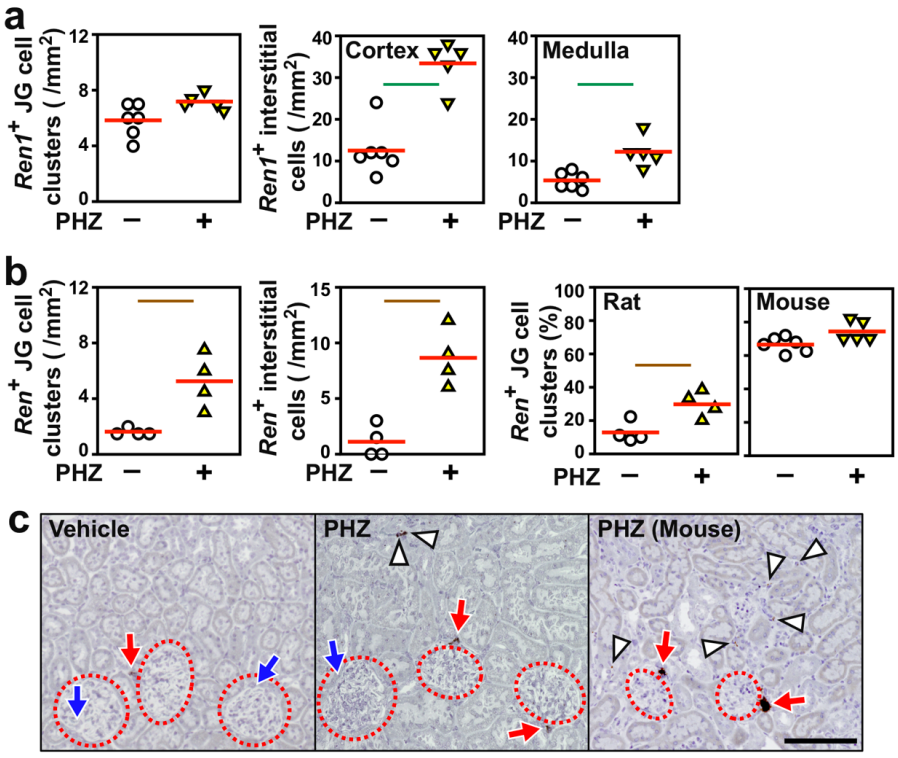

Fig. S4. Renal interstitial renin is induced by acute haemolytic anaemia in mice and rats. (a) Quantification of *Ren1* ISH data (Fig. 5d) for kidney sections from mice injected with PHZ to induce haemolytic anaemia. (b) Quantification of *Ren* ISH data (Fig. 5f) for kidney sections from rats injected with PHZ to induce haemolytic anaemia. Red bars indicate mean values. Green and brown lines indicate $P<0.01$ and $P<0.05$, respectively, in the Wilcoxon-Mann-Whitney tests. n=4–6 per group. (c) Representative images of *Ren* ISH in kidney sections of rats injected with vehicle or PHZ. An image of *Ren1* ISH from a PHZ-injected mouse kidney is also shown (right). Dotted circles and arrowheads are glomeruli and renin-expressing interstitial fibroblasts, respectively. Red and blue arrows indicate JG cells that are positive and negative for *Ren* (*Ren1* for the right panel) mRNA expression, respectively. The scale bar represents 100 μm.

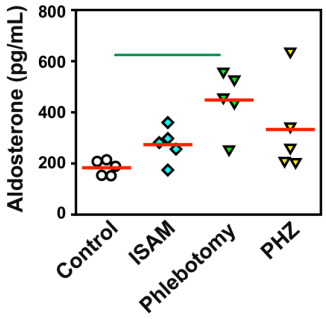

Fig. S5. Blood concentrations of aldosterone in control and ISAM mice. The data for mice subjected to phlebotomy and PHZ injection (Fig. 5a and c, respectively) are also shown. Red bars indicate mean values. The difference between the values of "Control" and "Phlebotomy" is statistically significant (green line, $P<0.01$ in the Tukey-Kramer HSD test), but the other differences are not.

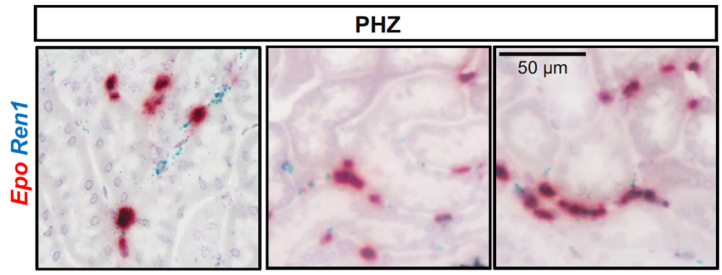

Fig. S6. Renal interstitial cells coexpressing renin and Epo. Representative images of ISH of *Epo* (red) and *Ren1* (blue) mRNA in kidney sections from anaemic mice that were injected with PHZ. The left panel is from Figure 5d.

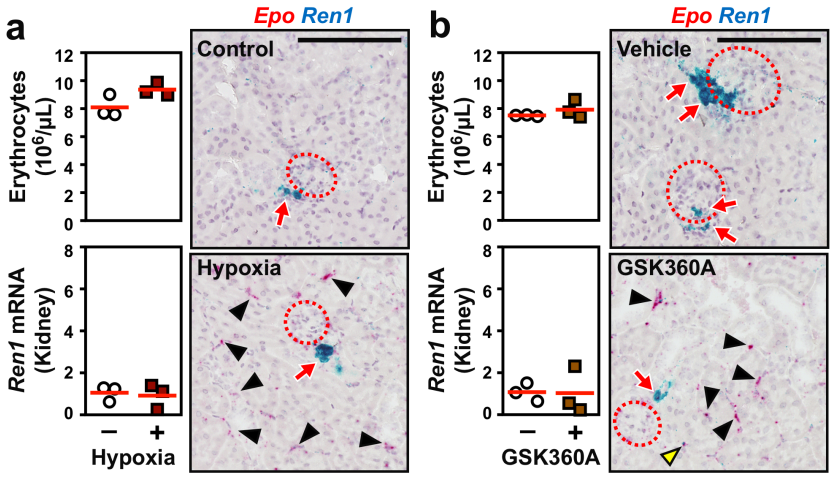

Fig. S7. Hypoxia signalling is not involved in renin induction by renal interstitial cells. (a and b) Hypoxic exposure (6% oxygen for 48 hours, a) and PHDI administration (GSK360A, b) did not alter the *Ren1* mRNA expression profiles in mouse kidneys, while *Epo* mRNA expression was activated in renal interstitial cells. Erythrocyte counts, *Ren1* mRNA levels, and representative images of ISH of *Epo* (red) and *Ren1* (blue) mRNA in kidney sections are shown. The average expression levels (red bars) in control mice were set as 1 for each gene expression analysis. n=3 for each group. Dashed circles, arrows, and arrowheads indicate glomeruli, JG cells positive for *Ren1* expression, and interstitial cells positive for *Epo* expression, respectively. Scale bars represent 100 μm.

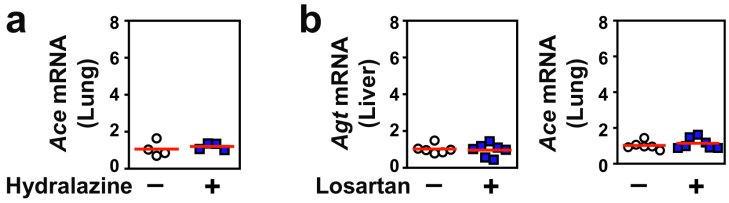

Fig. S8. Expression levels of RAS-related genes in mice administered antihypertensive agents. (a) *Ace* mRNA expression was not affected by hydralazine administration in the lungs of wild-type C57BL/6 mice. The experimental scheme is shown in Figure 6a. (b) Relative mRNA expression levels of the *Agt* gene in liver and the *Ace* gene in lung were not affected by losartan injection in wild-type DBA/2 mice. The experimental scheme is shown in Figure 6c. The average expression levels (red bars) in control mice were set as 1 for each gene expression analysis. n=4–7 per group.

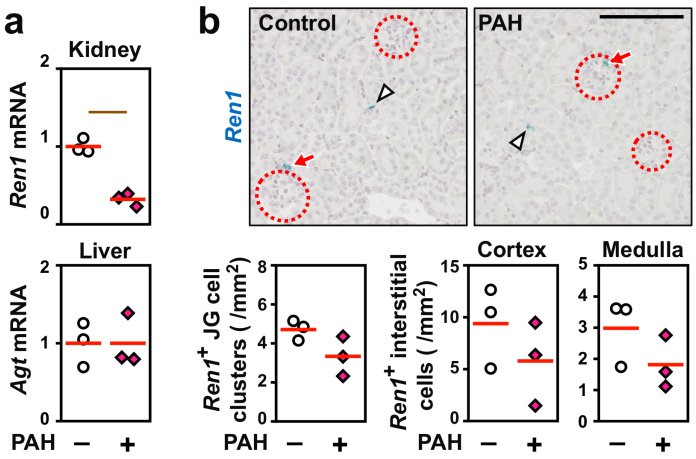

Fig. S9. The *Ren1* expression profile in the kidney of mice with genetically induced pregnancy-associated hypertension (PAH). (a) *Ren1* mRNA levels in the kidney and *Agt* mRNA levels in the liver of PAH mice at E16.5. (b) Representative images and quantification of ISH of *Ren1* (blue). Dotted circles, red arrows, and arrowheads indicate glomeruli, JG cells, and interstitial fibroblasts positive for *Ren1,* respectively. The scale bar is 100 μm. n=3 per group. The difference in *Ren1* mRNA levels (a) is statistically significant (brown line, $P<0.05$), while the other differences are not, using the Wilcoxon-Mann-Whitney test.

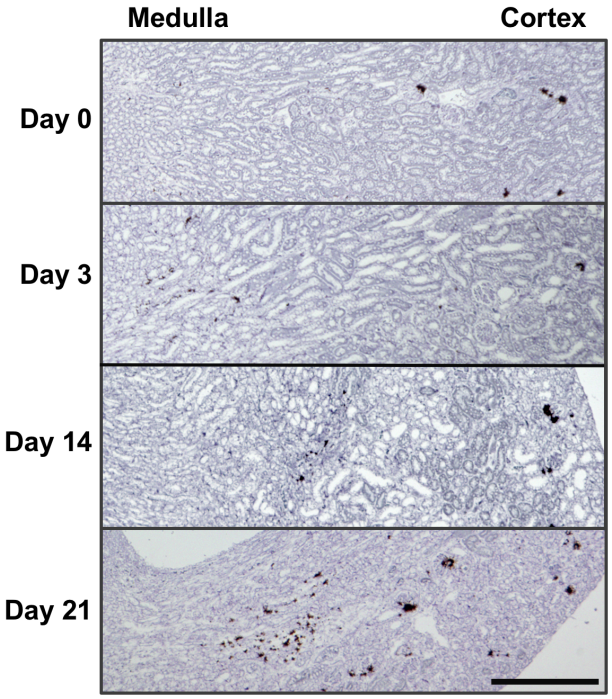

Fig. S10. ISH of *Ren1* mRNA (brown) in injured kidneys from mice subjected to UUO for 0, 3, 14, or 21 days. The scale bar is 500 μm.

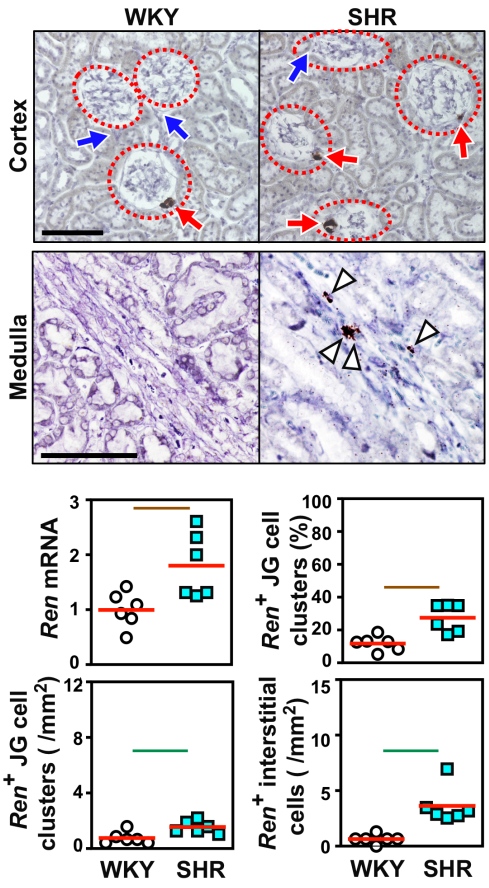

Fig. S11. Renal interstitial renin expression is activated in spontaneously hypertensive rats (SHR). Representative images and quantification of ISH of *Ren* mRNA in kidney sections of SHR and their normal control rats (WKY). Arrowheads and dotted circles are interstitial fibroblasts that are positive for *Ren* mRNA expression and glomeruli, respectively. Red and blue arrows indicate JG cells that are positive and negative for *Ren* mRNA expression, respectively. Scale bars represent 100 μm. The average expression level (red bar) in WKY were set as 1 for renal *Ren* mRNA expression analysis. Green and brown lines indicate $P<0.01$ and $P<0.05$, respectively, in the Wilcoxon-Mann-Whitney test. n=6 per group.

Table S1. Primers and probes used in this study.

Primers and probes for quantitative PCR	
Target	Sequence (5' – 3')
Ren1	CCCGACATTTCCTTTGACC
	TGTGCACAGCTTGTCTCTCC
Agt	GGCAAATCTGAACAACATTGG
	TTCCTCCTCTCCTGCTTTGA
Ace	AAAGAAGCTTCAGAACCTGGAC
	GTCTAGCAGGATCTGGTTGTACTCT
Hprt	CCGGCTCCGTTATGGC
	GGTCATAACCTGGTTCATCATCA
	(Probe) CGCAGCCCTGGCGTCGTGATTA
Epo	GAGGCAGAAAATGTCACGATG
	CTTCCACCTCCATTCTTTTCC
EpoGFP	GAAGACTTGCAGCGTGGAC
	GGTGGATCCTAAAGCAGCAG
Ren2	GCCTCAGCAAGACTGATTCC
	ATATTCATGTAGTCTCTTCTCC

Probes for ISH	
Target	Serial No. (Advanced Cell Diagnostics)
Mouse *Epo*	Mm-Epo
Mouse *Epo*	Mm-Epo-C2 (for double staining)
Mouse *Ren1*	Mm-Ren1
Rat *Ren1*	Rn-Ren
Human *REN*	Hs-REN
tdTomato	Tomato-C2 (for double staining)

Table S2. Information on the human kidney sections analysed in this study.

	Patient 1	Patient 2
Catalogue number	CS700464	CS705284
Sample ID	PA00001471	PA00005604
Case ID	CI0000000192	CI0000007856
Gender	Male	Female
Age	66	43
Race	Not Reported	White or Caucasian
Case diagnosis (Reason for Surgery)	Hydronephrosis	Chronic tubulointerstitial nephritis
Sample diagnosis	Tubulointerstitial nephritis	Tubulointerstitial nephritis
Lesional area	100% in a section	100% in a section

Provided by OriGene (https://www.origene.com/products/tissues/tissue-search).

Table S3. *Ren1*$^+$ and *tdTomato*$^+$ cell counts in the renal interstitia of ISAM-REC mice.

	Cortex	Medulla
Ren1$^+$ (/mm^2)	117.3 ± 25.7	39.5 ± 13.9
tdTomato$^+$ (/mm^2)	345.7 ± 35.7	18.4 ± 5.5
Ren1$^+$ *tdTomato*$^+$ (/ mm^2)	102.0 ± 20.6	7.0 ± 1.3
Double$^+$ / *Ren1*$^+$	0.87	0.18
Double$^+$ / *tdTomato*$^+$	0.29	0.38

Cells were counted in kidney sections from 3 independent mice that underwent ISH of *Ren1* and *tdTomato* mRNAs (Figure 4c). The data show the mean ± standard deviation.