APCN X TSN 2025, Dec.5(Fri.) 14:51-15:00
Oral Presentation YIA_010

The Involvement of Interstitial Palladin Expression in Patients with Chronic Kidney Disease

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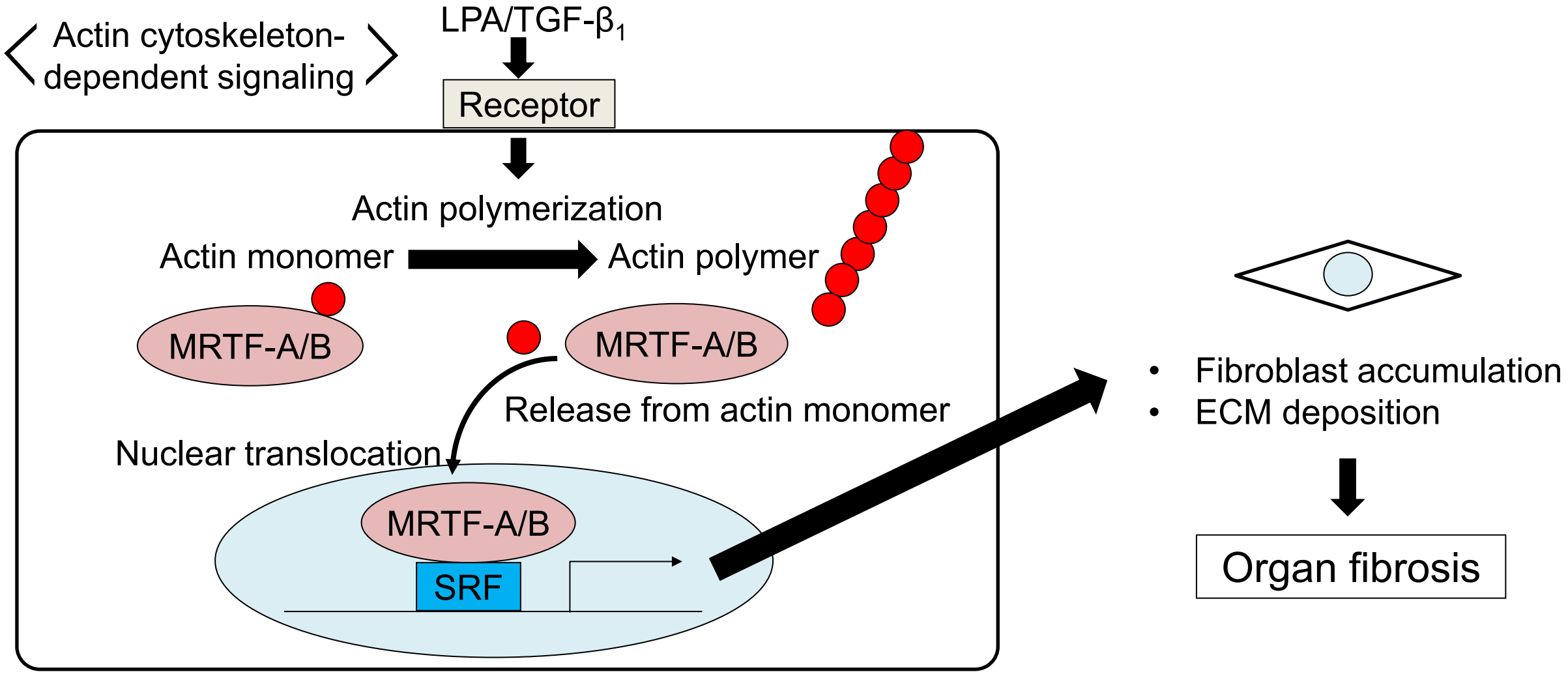
Actin polymerization drives fibroblast activation via MRTF-SRF signaling



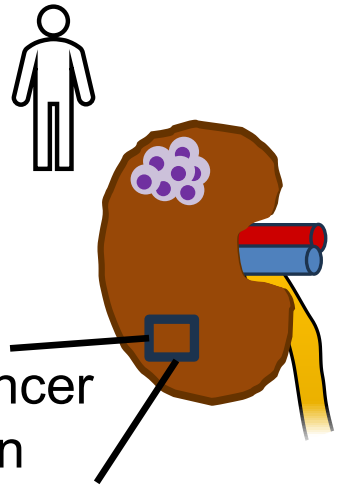
LPA：lysophosphatidic acid
TGF-β_1 : Transforming growth factor-β
MRTF：myocardin-related transcription factor
SRF：serum response factor

Sakai N, et al. FASEB J 2013
Sakai N, et al. Kidney Int 2017
Yamamura Y, Sakai N, et al. FASEB J 2023

Molecular pathway that regulates actin cytoskeleton was significantly correlated with eGFR and COL1A1 expression

Method

Patients with renal cell carcinoma (n=16)

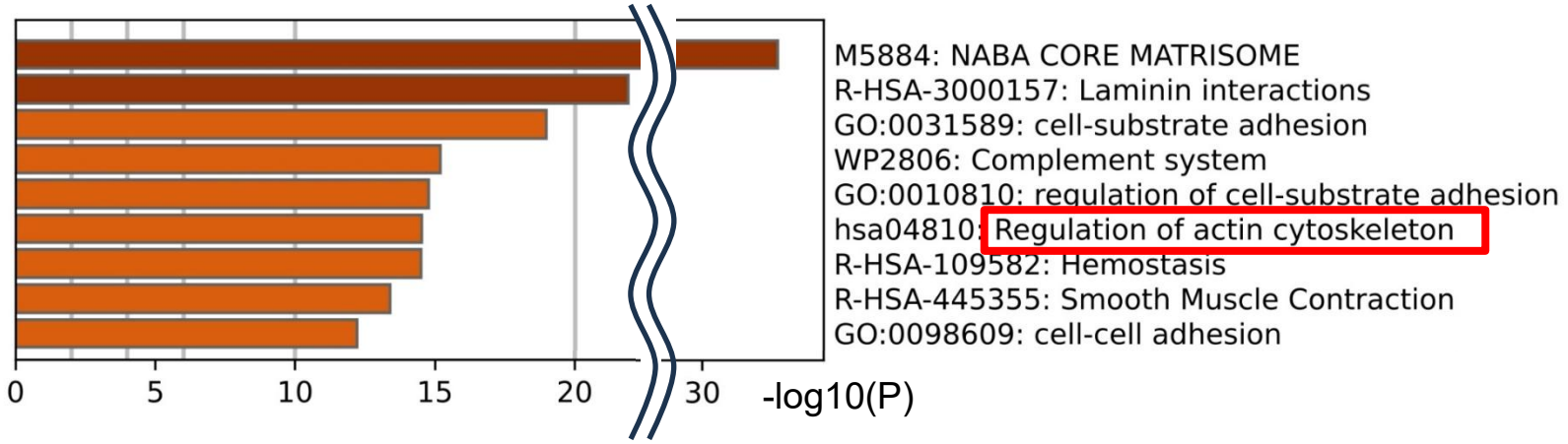


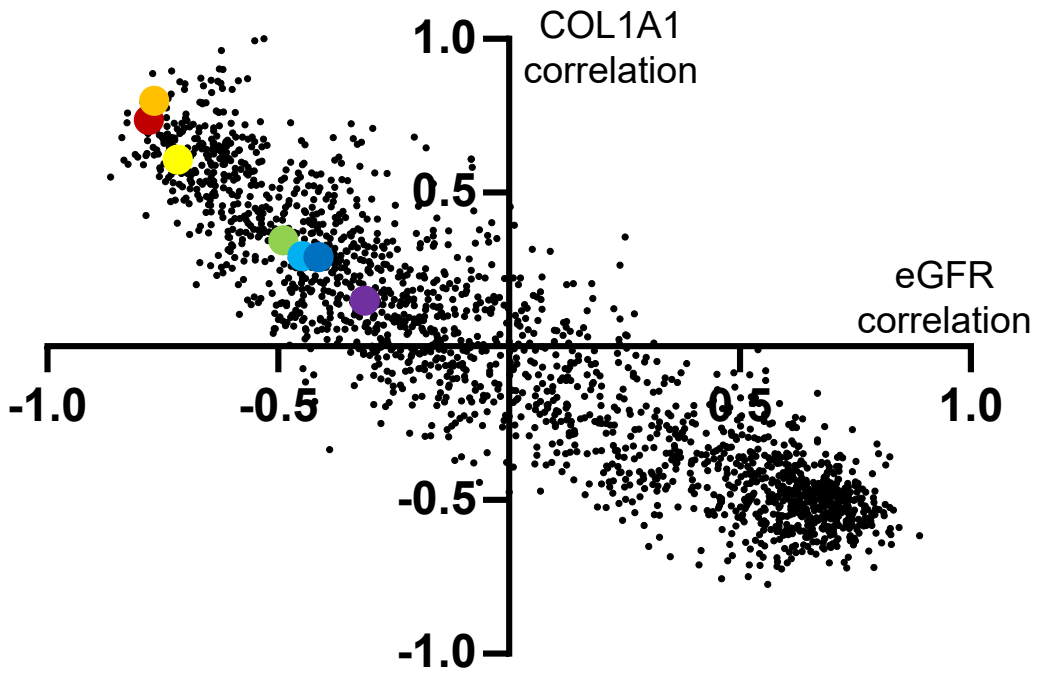
Non-cancer lesion

Proteome analysis

- Enrichment analysis in Metascape (eGFR correlation < -0.7)
- Correlation analysis

<Enrichment analysis>

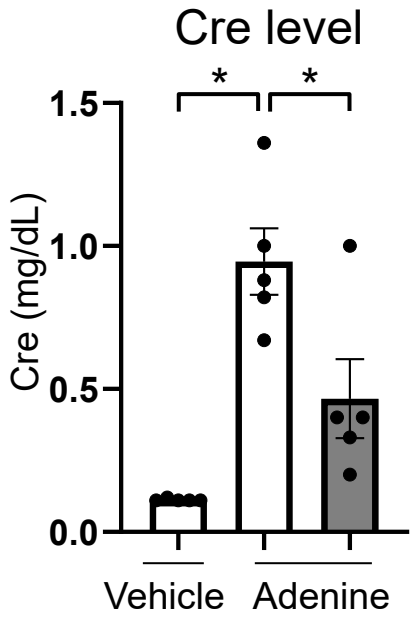


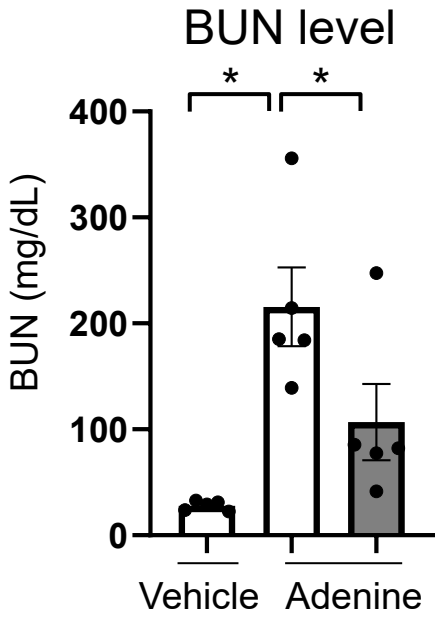


Actin-associated proteins

- Vinculin
- Palladin
- Actinin-α1
- Profilin 1
- Cofilin 1
- Zyxin
- VASP

Serum creatinine level, BUN level, hydroxyproline, ECM expression were suppressed in fibroblast-specific palladin deficient mice





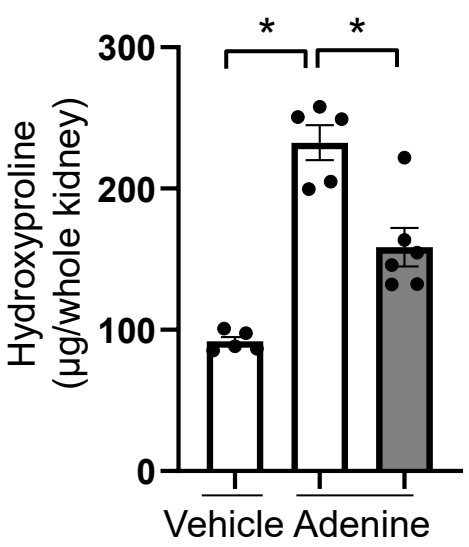
Palladin^{WT}

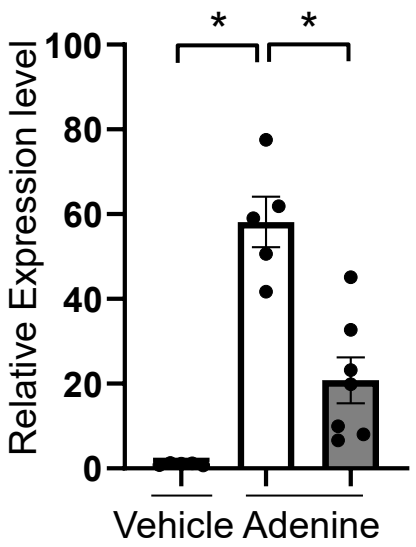
Palladin^{iFBKO}

Fibroblast-specific palladin deficient mice

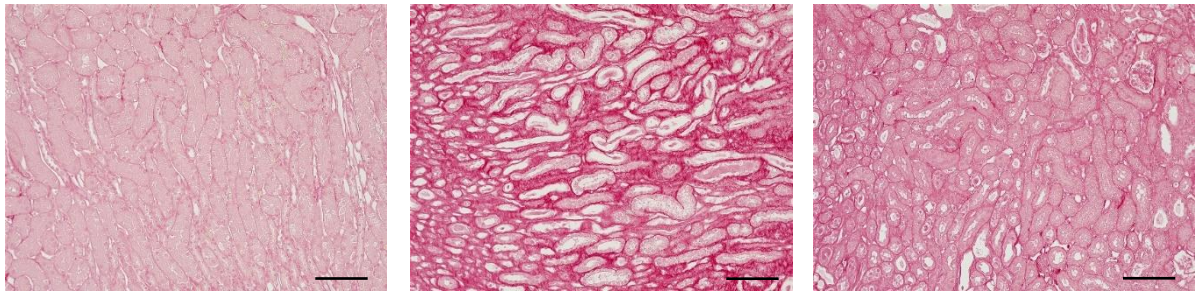
N=5~7

* $p < 0.05$





Sirius red staining



Palladin^{WT} Vehicle

Palladin^{WT} Adenine

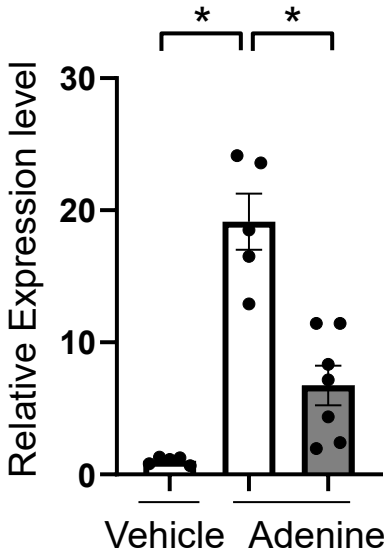
Palladin^{iFBKO} Adenine

× 200

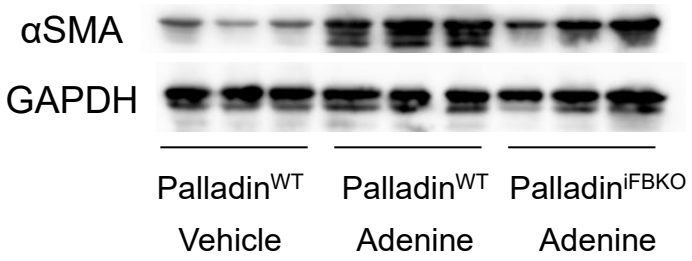
Bars, 100 μm

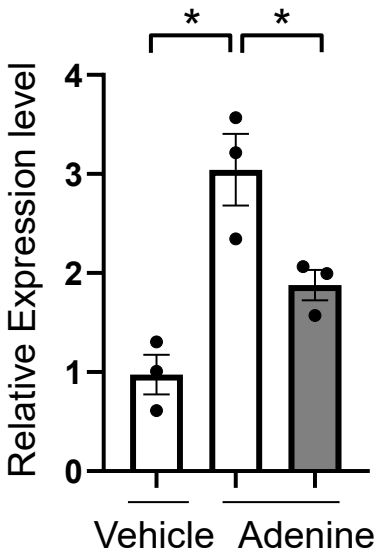
αSMA expression was suppressed in palladin^{iFBKO}

αSMA / GAPDH mRNA



αSMA / GAPDH protein





Palladin^{WT}

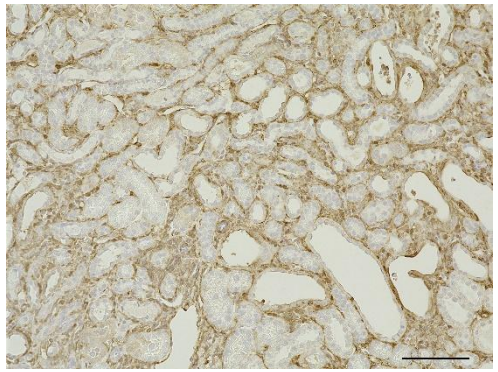
Palladin^{iFBKO}

N=5~7

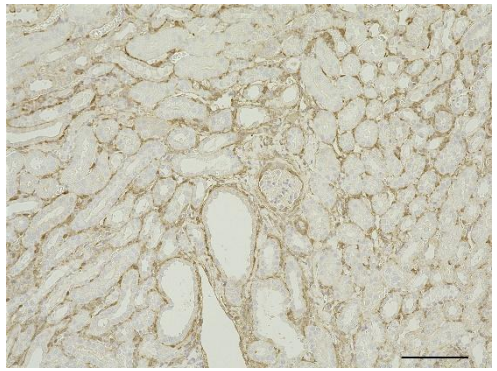
* p < 0.05

αSMA staining

Palladin^{WT}
Vehicle



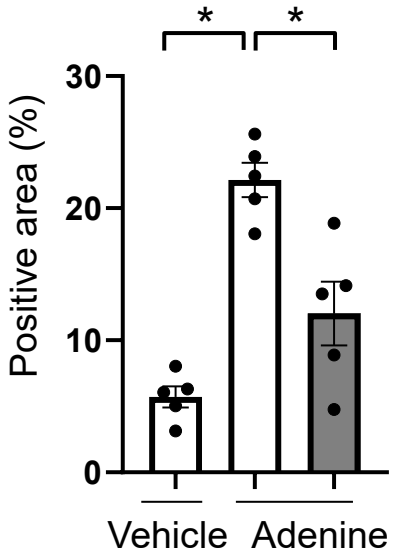
Palladin^{WT}
Adenine



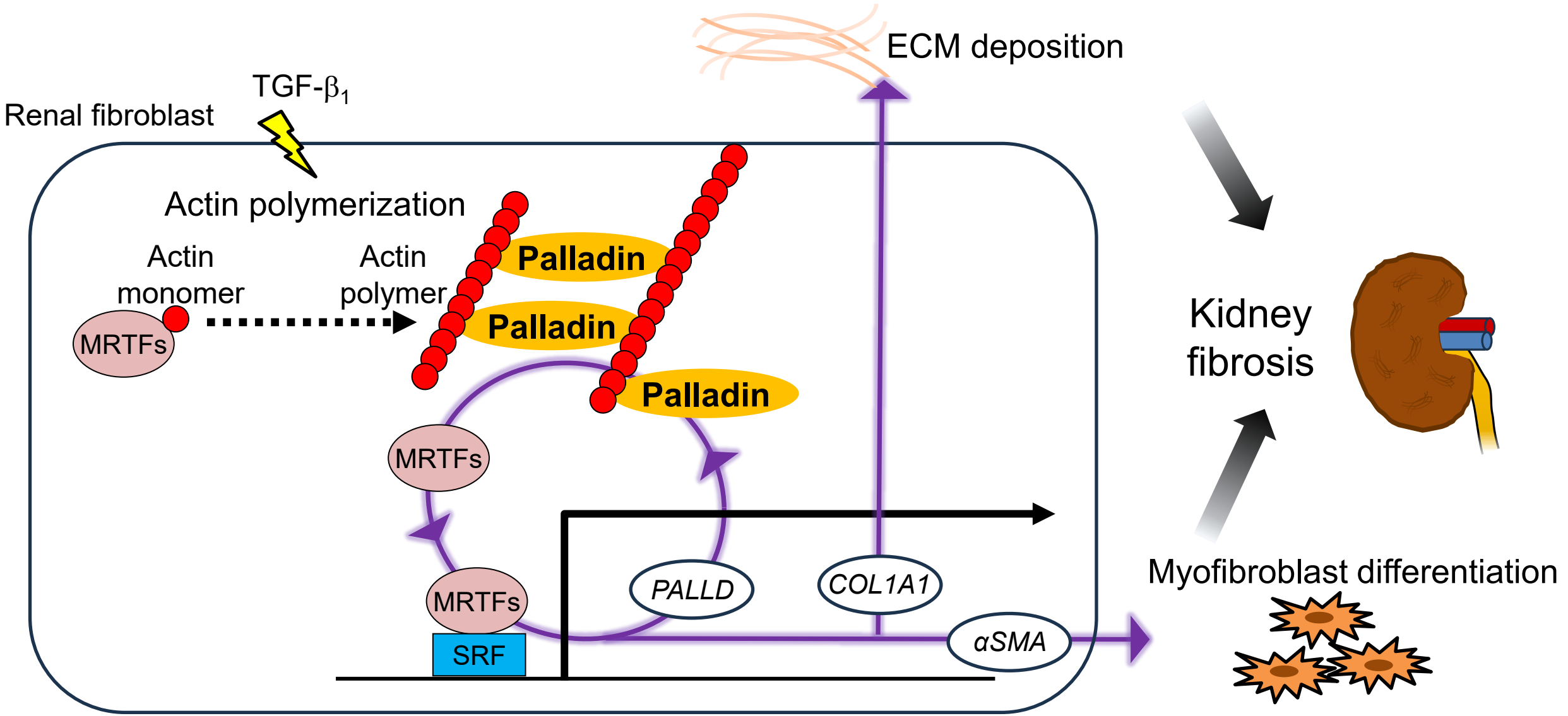
Palladin^{iFBKO}
Adenine

×200
Bars: 100 μm

αSMA positive area



Palladin promotes kidney fibrosis through ECM deposition and myofibroblast differentiation



Yamamoto N, Sakai N, et al. J Pathol in press

Hypothesis

Interstitial palladin is involved in kidney dysfunction and fibrosis in patients with chronic kidney disease

Palladin expression is highly enriched in fibroblasts of the human kidney

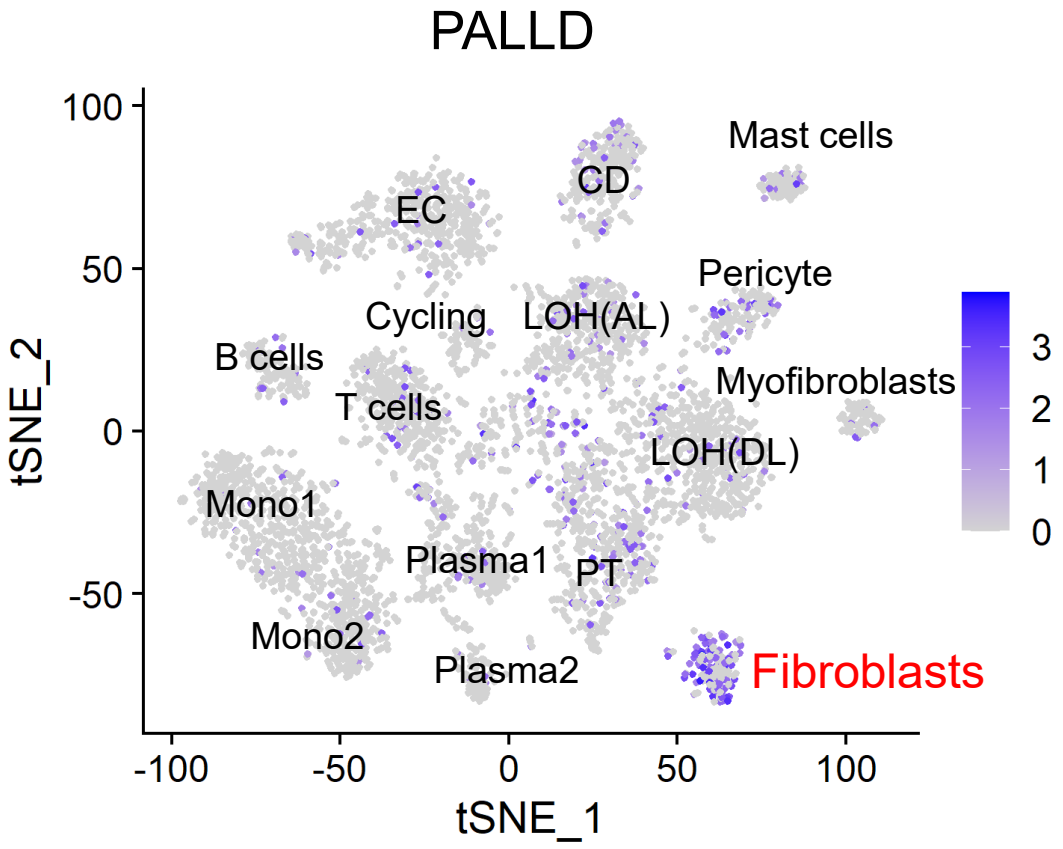
Method

- Public database
(GSE 109564 and GSE 114156)
Human healthy adult kidney and rejecting kidney allograft biopsy

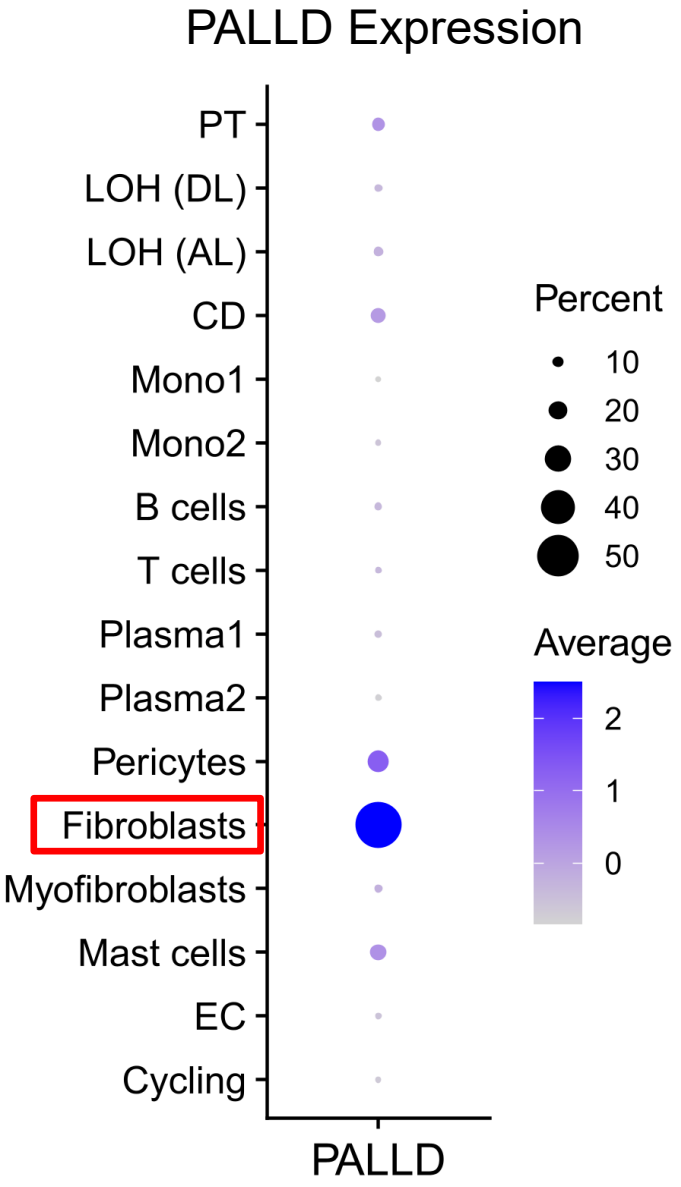
↓ Using R version 4.5.2

<Single cell analysis >

- Clustering analysis
- Expression analysis



CD, collecting duct; EC, endothelial cell;
LOH (AL), loop of Henle, ascending limb;
LOH (DL), loop of Henle, distal limb;
Mono, monocyte; PT, proximal tubule



Methods

<Participants>

Japanese patients who underwent kidney biopsy for CKD at Kanazawa University Hospital between March 2022 and December 2023

CKD was defined by either persistent urinary abnormalities or eGFR < 60 mL/min/1.73m^2 for at least three months.

<Exclusion criteria>

- Patients with acute kidney injury
- Insufficient tissue
- Patients who did not provide consent

<Outcome measures>

- Immunofluorescence (palladin, αSMA)
- Azan-Mallory (AZAN) staining (fibrotic area)
- eGFR, UPCR at the time of biopsy
- Urinary β2MG and NAG

<Study design>

- Cross-sectional study

αSMA: α-smooth muscle actin

eGFR: estimated glomerular filtration ratio

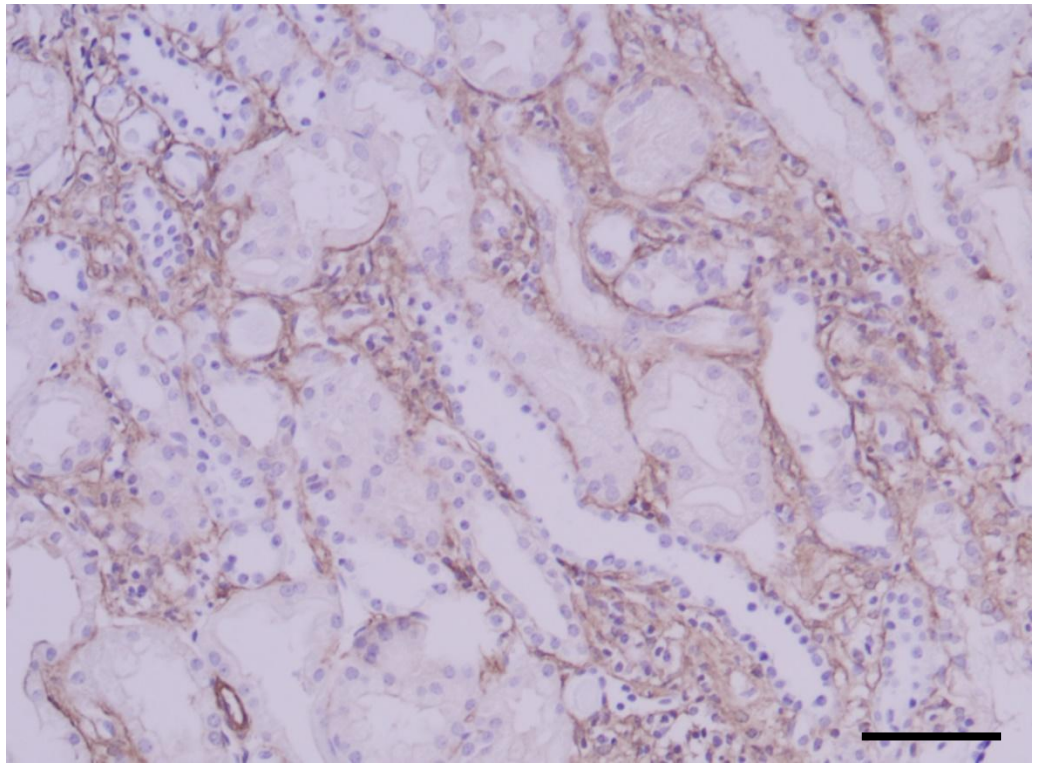
Baseline clinical characteristics of patients included in the study

Number of patients		57
Age (years)		56.4 ± 17.1
Men, n(%)		26(45.6)
Primary disease n(%)	IgA nephropathy	11(19.3)
	Tubulointerstitial nephritis	10(17.5)
	Benign nephroscrelosis	6(10.5)
	ANCA-associated vasculitis	5(8.8)
	Diabetic kidney disease	5(8.8)
	Lupus nephritis	4(7.0)
	Membranous nephropathy	4(7.0)
	Minimal change disease	3(5.3)
	Focal segmental glomerulosclerosis	2(3.5)
	Thrombotic microangiopathy	2(3.5)
	Others	5(8.8)
eGFR (mL/min/1.73 m^2)		49.0 ± 23.78
UPCR (g/gCr)		2.6 ± 3.2
β_2MG (μg/L)		2851 ± 4763
NAG (IU/L)		13.4 ± 12.3

UPCR, urine protein-creatinine ratio

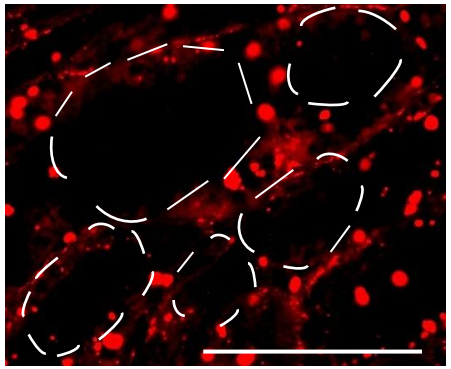
Palladin was colocalized with αSMA-expressing cells in kidney interstitium

Immunohistochemistry
(DAB staining)

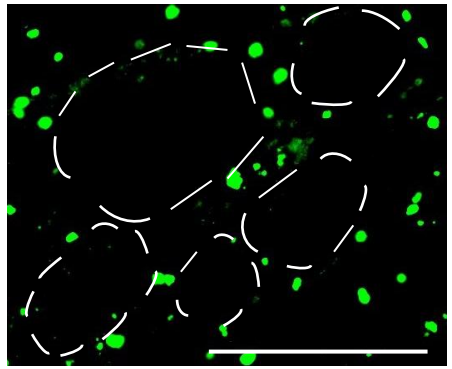


Immunofluorescence

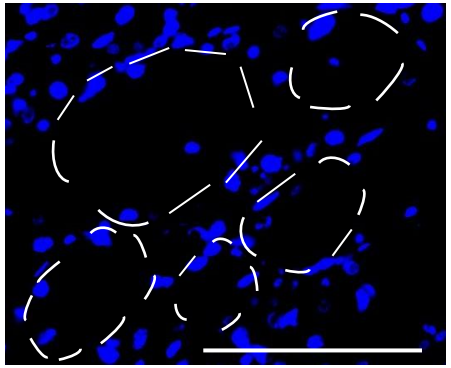
Palladin



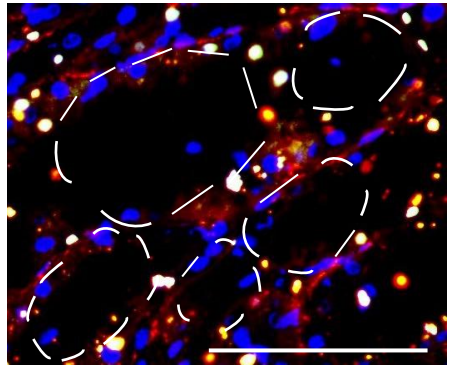
αSMA



DAPI

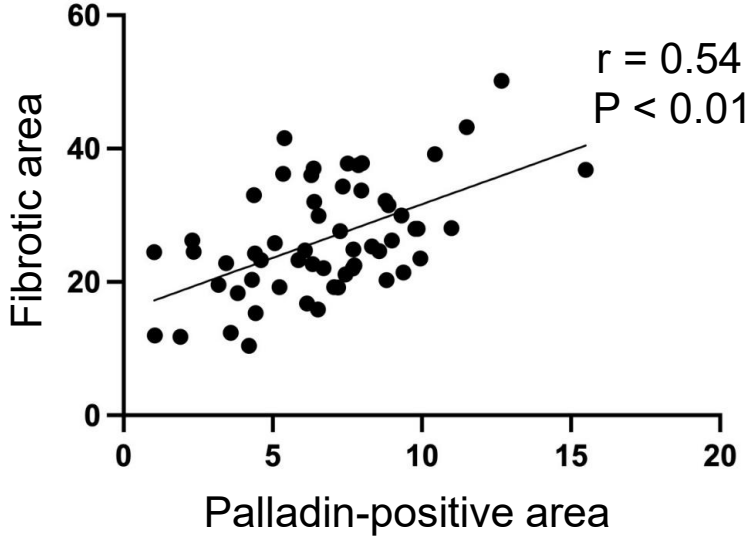


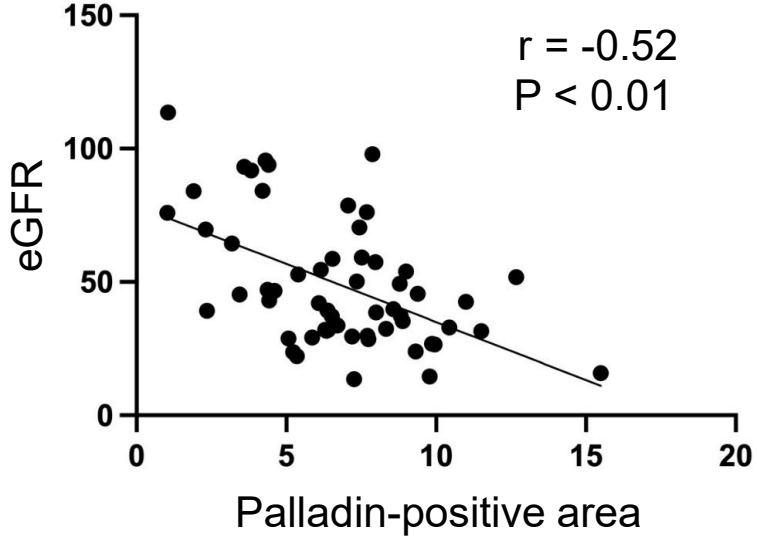
Merged

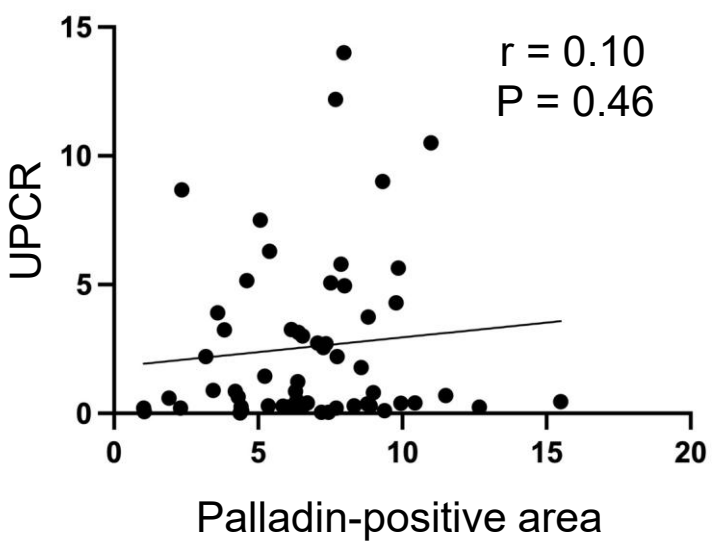


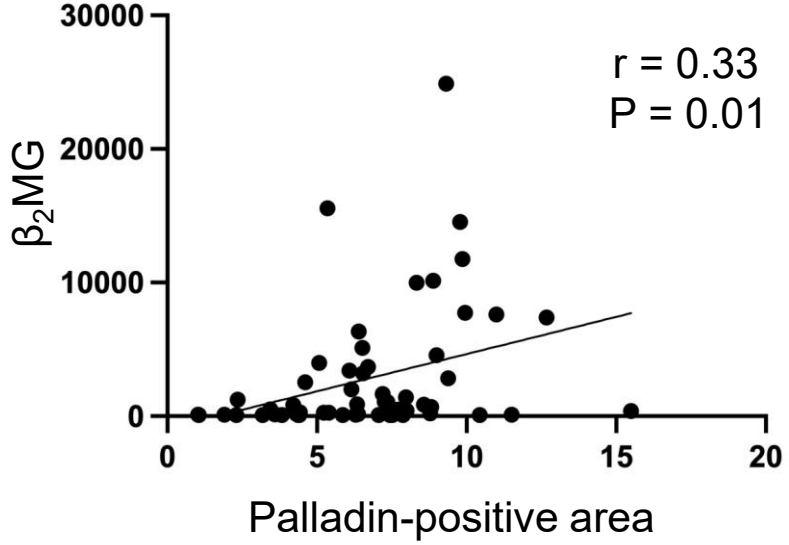
Bars, 100 μm

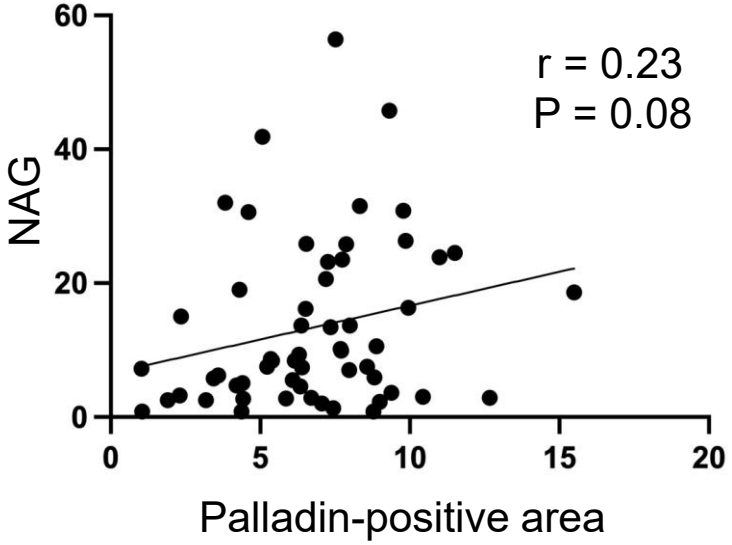
Interstitial palladin-positive area was positively correlated with fibrotic area and inversely correlated with eGFR











Interstitial palladin-positive area was independently associated with fibrotic area and eGFR after adjustment for age, sex, and UPCR

Multiple regression analysis

Fibrotic area model

Variable	β(SE)	p value
Palladin-positive area (per 1% increase)	1.4 (0.4)	<0.001
Age (per 1-year increase)	0.1 (0.1)	0.10
Men (vs women)	0.2 (1.9)	0.91
UPCR (per 1 g/gCr increase)	0.2 (0.3)	0.54

eGFR model

Variable	β(SE)	p value
Palladin-positive area (per 1% increase)	-2.8 (0.7)	<0.001
Age (per 1-year increase)	-0.8 (0.1)	<0.001
Men (vs women)	-6.7 (4.0)	0.10
UPCR (per 1 g/gCr increase)	1.0 (0.6)	0.10

SE, standard error

Conclusion

Interstitial palladin is associated with kidney dysfunction and fibrosis in patients with CKD