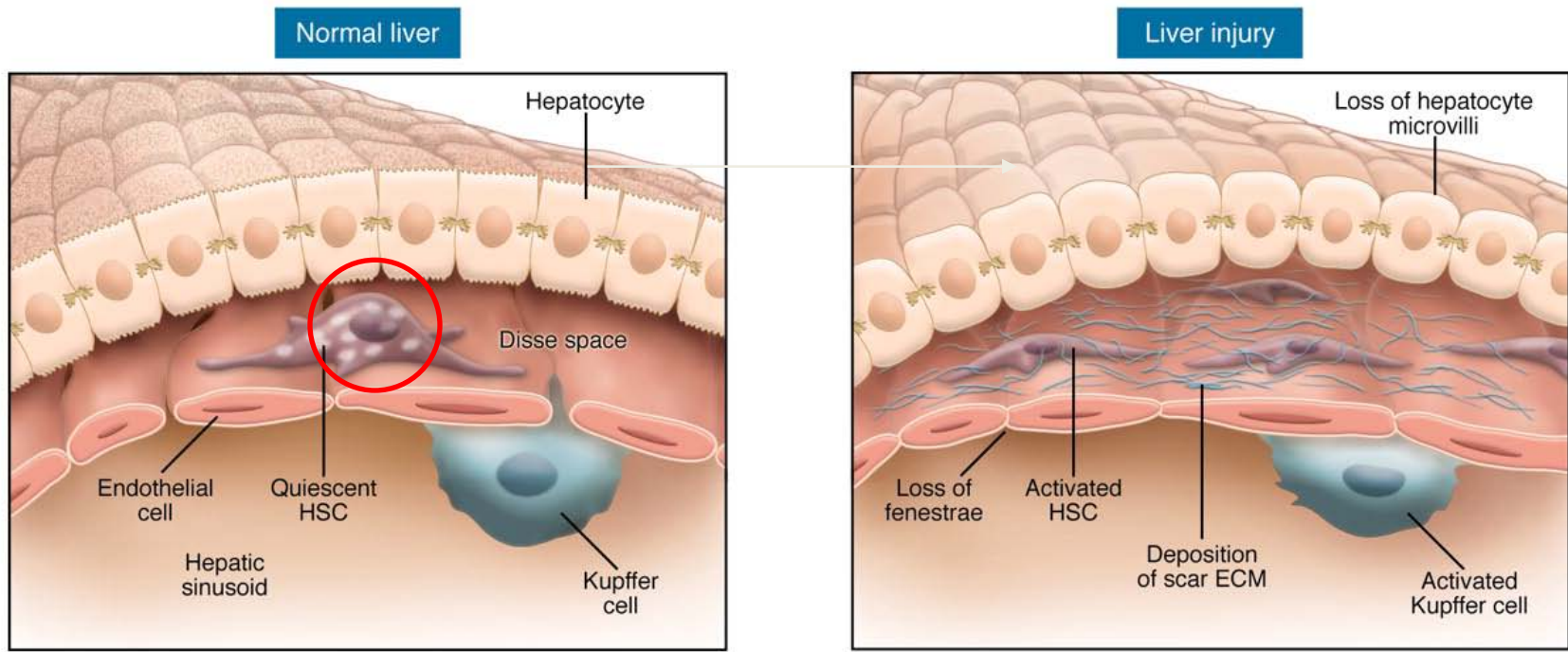


Selective α_v integrin deletion identifies a core, targetable molecular pathway that regulates fibrosis across solid organs

Neil Henderson
Wellcome Trust Clinician Scientist
and Consultant Hepatologist,
MRC Centre for Inflammation Research
University of Edinburgh



Development of a system to allow gene manipulation in hepatic stellate cells (liver specific pericytes)



- Paucity of tools to specifically inactivate genes in liver myofibroblasts
- Limited progress in understanding the underlying biology of liver fibrogenesis
- Hindered the development of new, mechanistically targeted therapies

Pericytes



Platelet derived growth factor receptor beta (PDGFR β)

Early induction of PDGFR β during HSC activation

Hepatic stellate cells previously termed liver specific pericytes

PDGFR β is a widely used pericyte marker throughout vascular beds

PDGF-BB is the most potent hepatic stellate cell (HSC) mitogen

PDGFR β Cre mice

Express Cre recombinase under the control of a fragment of the gene encoding platelet derived growth factor receptor beta

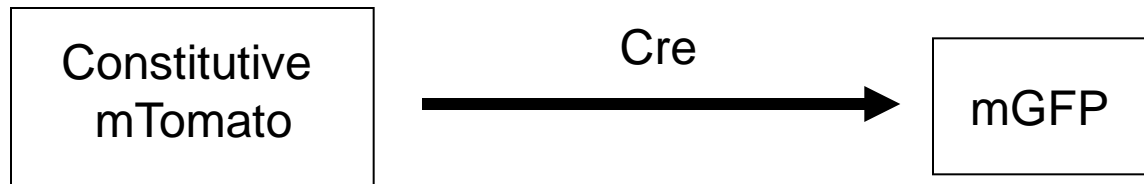
PDGFR β Cre line originally developed by Ralf Adams to specifically target pericytes (Foo SS et al. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell*, 2006)

mTmG; *PDGFR* β Cre reporter mice

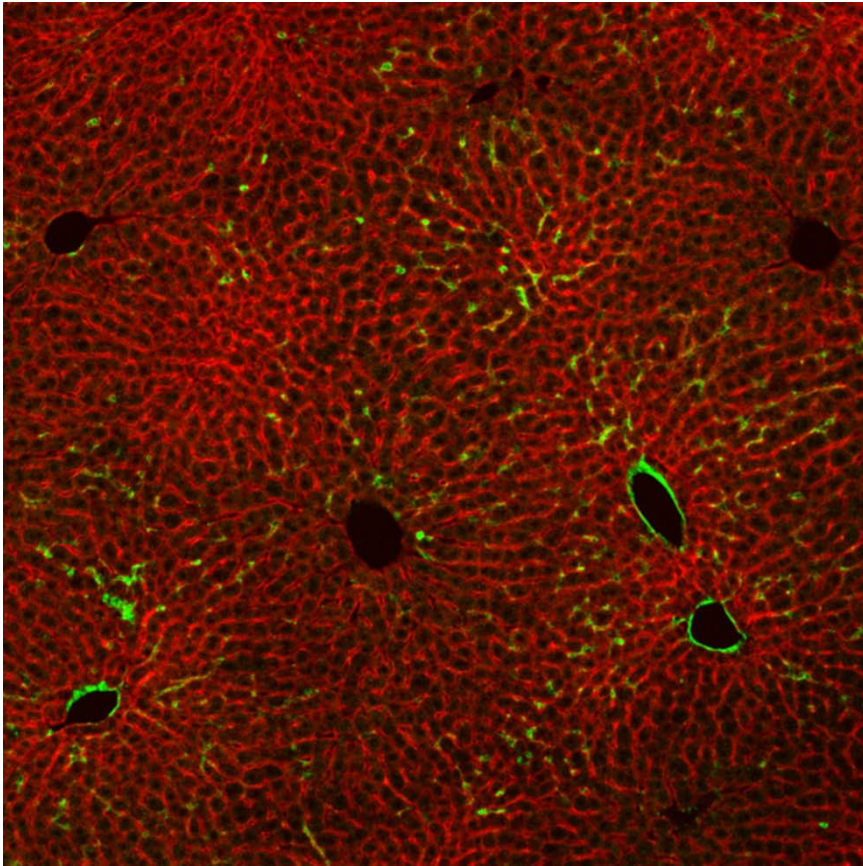
B6.129(Cg)-*Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J* x *PDGFR* β Cre^{+/-}

Express red fluorescence prior to, and green fluorescence following, Cre mediated recombination

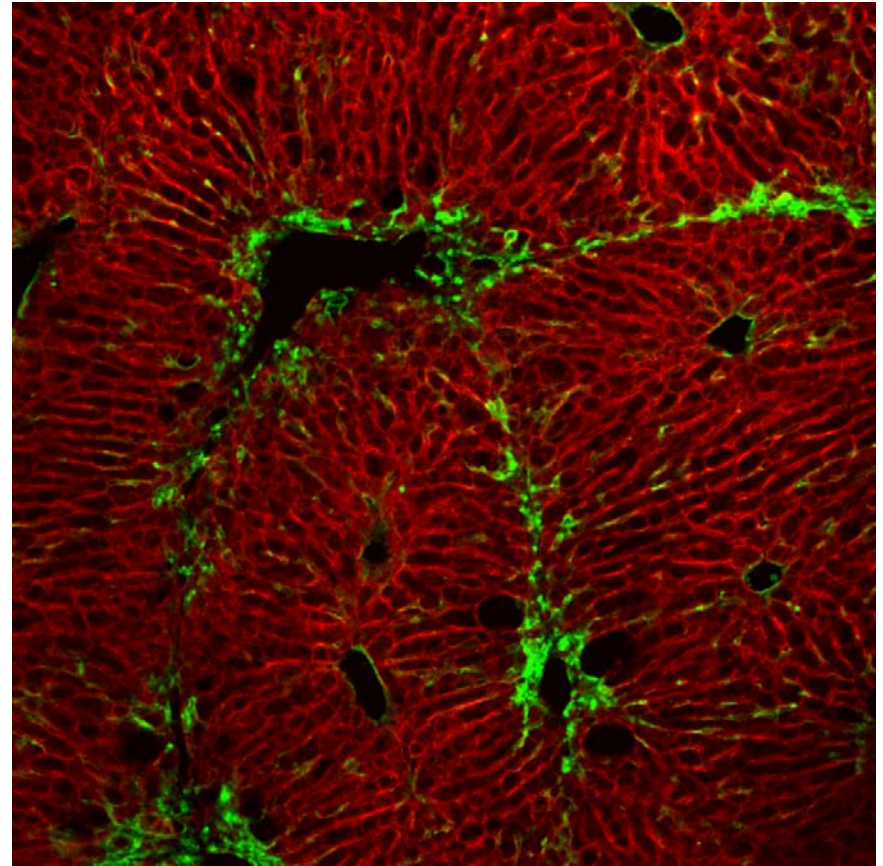
Membranous fluorophore expression



mTmG; *PDGFR* β Cre



Uninjured liver

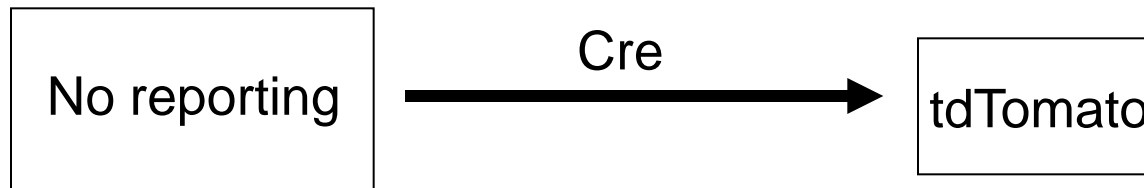


Fibrotic liver
(6 weeks CCL₄ twice weekly)

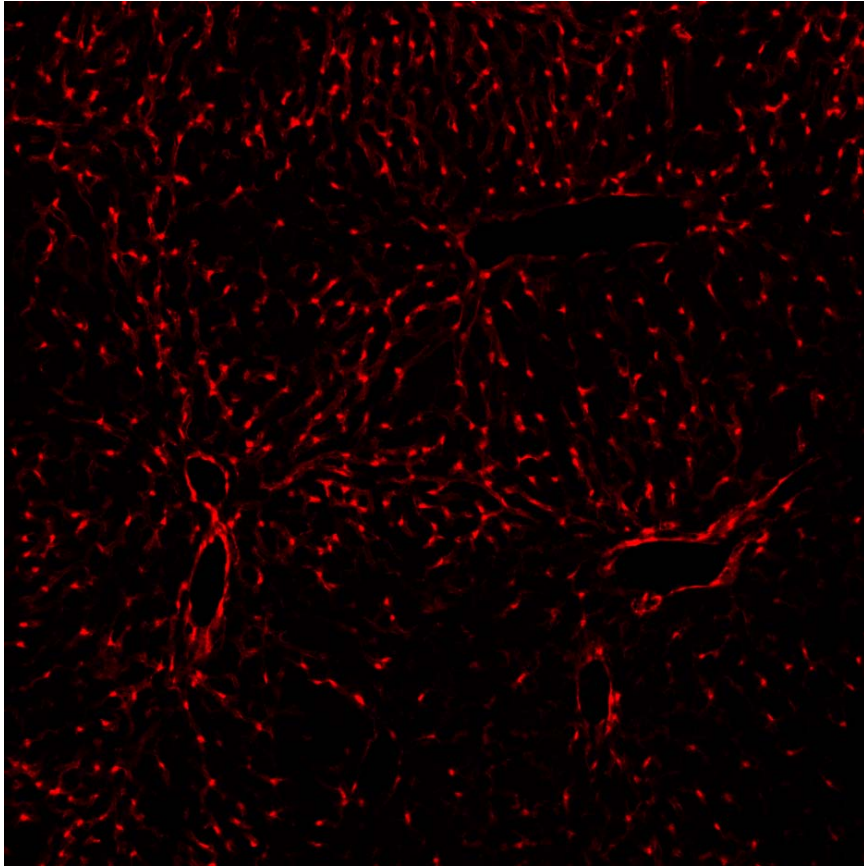
Ai14;*PDGFR* β Cre reporter mice

B6.Cg-*Gt(ROSA)26Sor*^{tm14(CAG-tdTomato)Hze/J} \times *PDGFR* β Cre^{+/-}

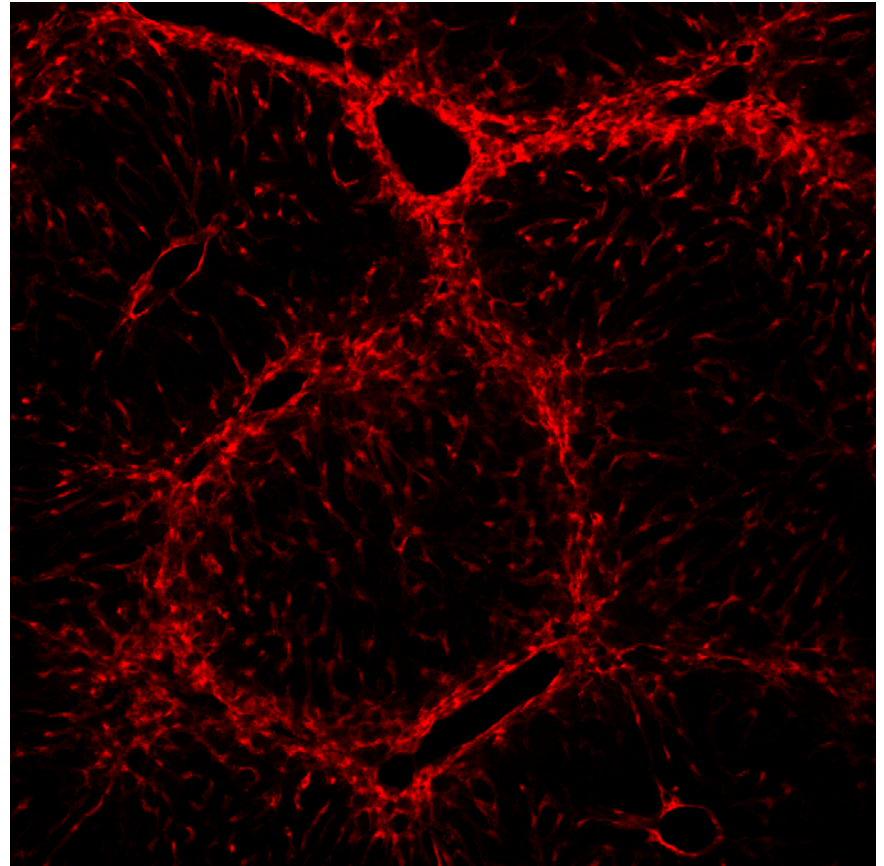
These Ai14 mice harbor a targeted mutation of the *Gt(ROSA)26Sor* locus with a *loxP*-flanked STOP cassette preventing transcription of a CAG promoter-driven red fluorescent protein variant (tdTomato)



Ai14;*PDGFR* β Cre



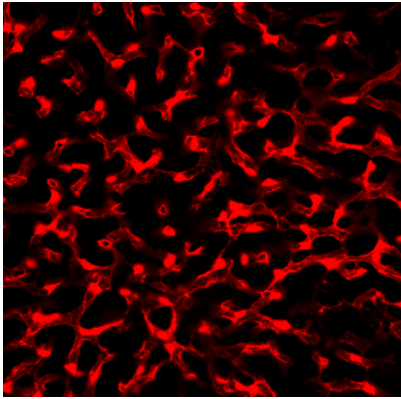
Uninjured liver



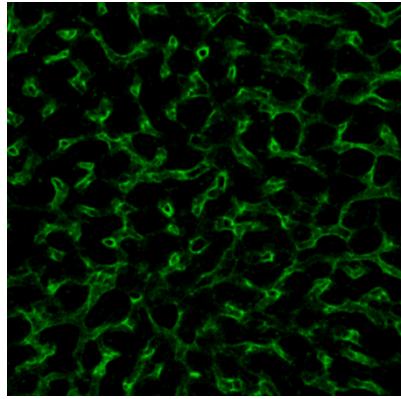
Fibrotic liver

Ai14; *PDGFR β* Cre: Uninjured liver

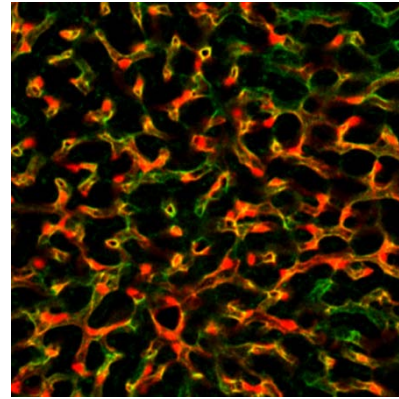
Reporter



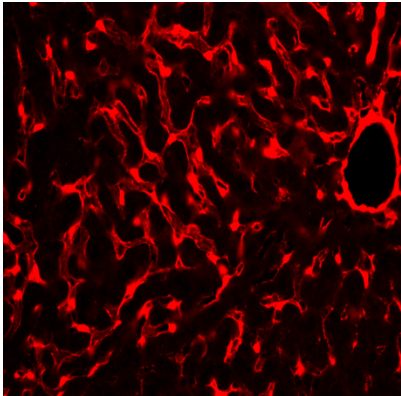
Desmin



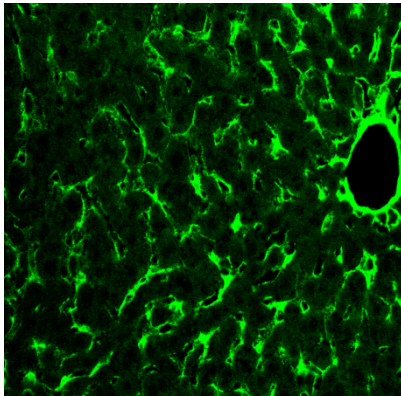
Reporter / Desmin



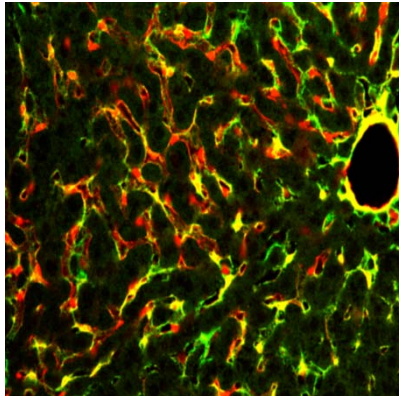
Reporter



PDGFR β

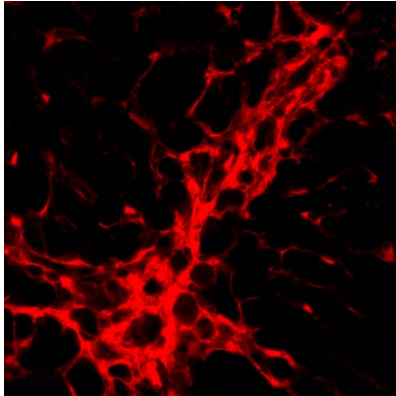


Reporter / *PDGFR β*

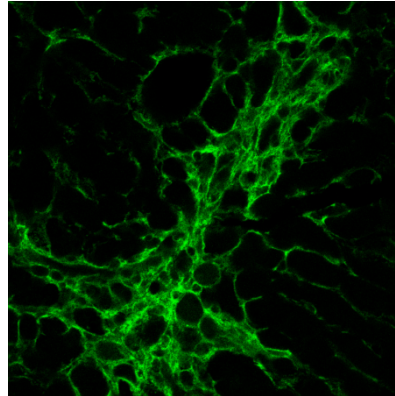


Ai14; *PDGFR* β Cre: Fibrotic liver

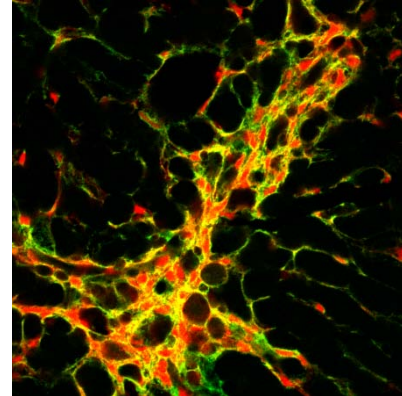
Reporter



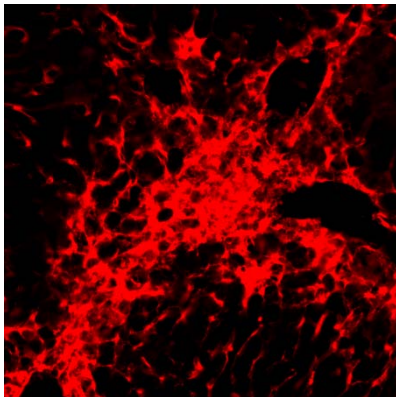
PDGFR β



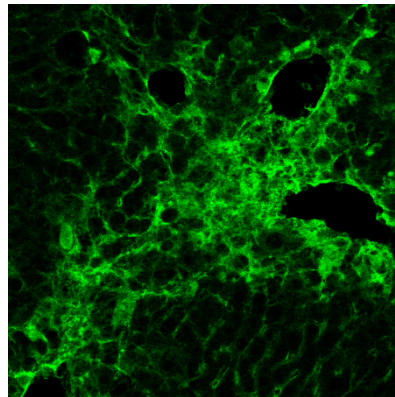
Reporter / *PDGFR* β



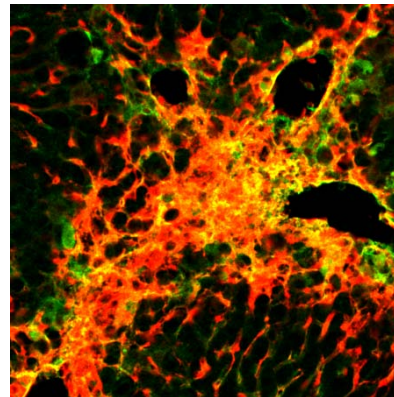
Reporter



α SMA



Reporter / α SMA



Cell sorting from Ai14; *PDGFR β* Cre^{+/-} mice

Uninjured liver (6 weeks olive oil):



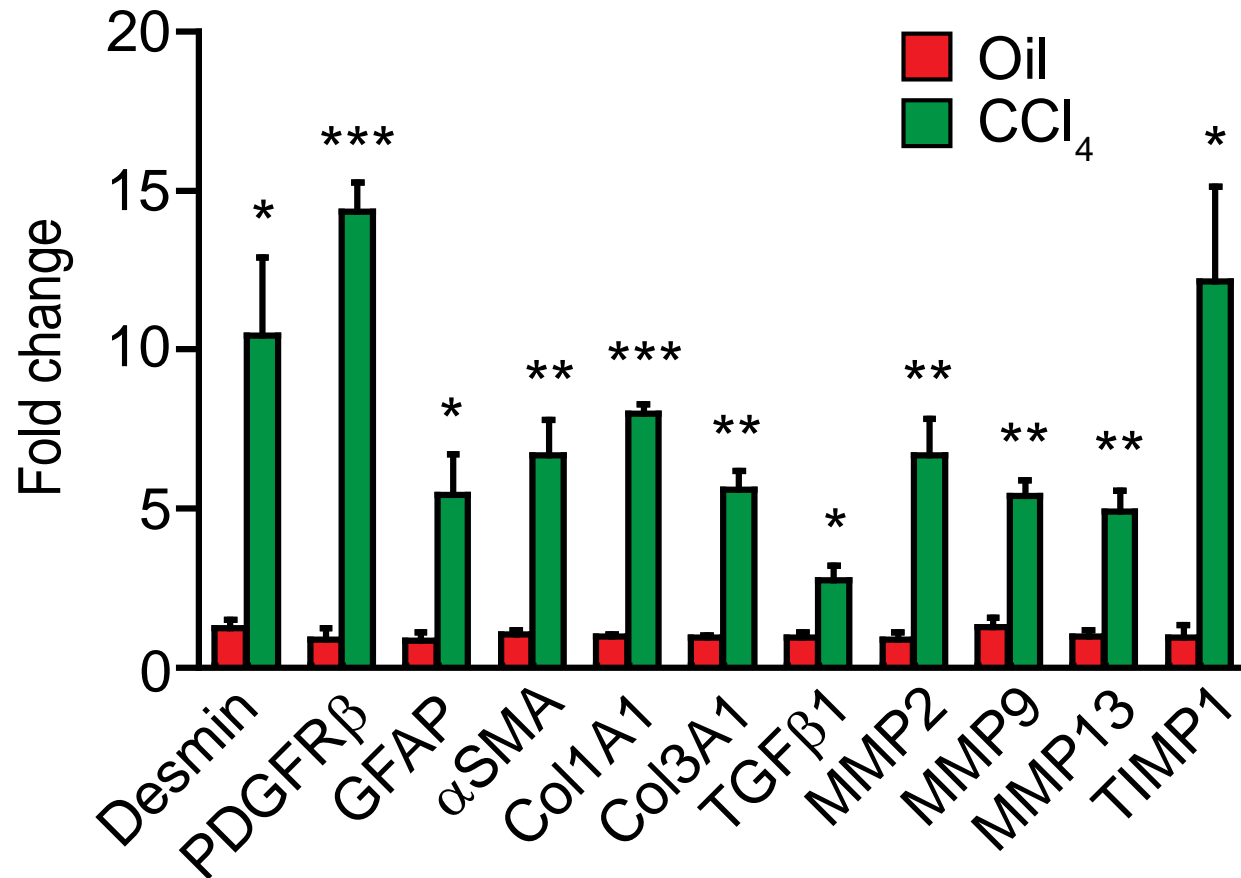
Single cell suspension
Cell sort of TdTomato +ve cells
mRNA extraction

Fibrotic liver (6 weeks CCl₄):



Single cell suspension
Cell sort of TdTomato +ve cells
mRNA extraction

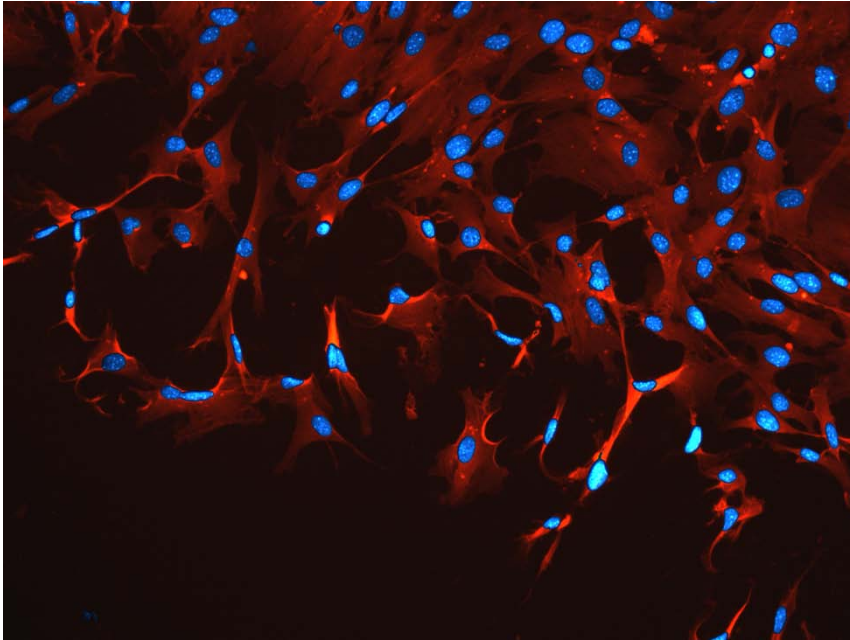
Gene expression profiling of sorted TdTomato+ve cells



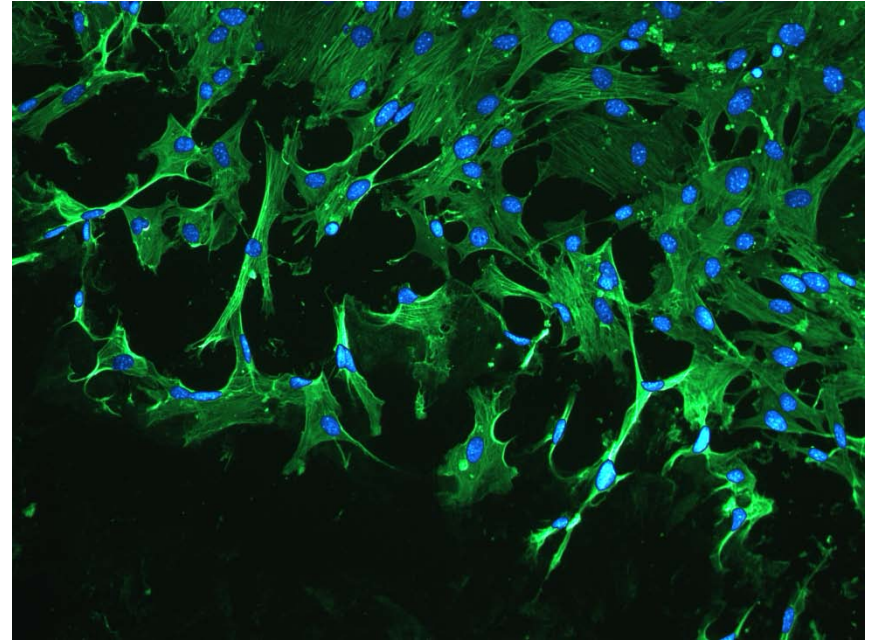
Ai14;PDGFR β Cre mice

Cell sorted TdTomato+ve cells after 7 days in culture

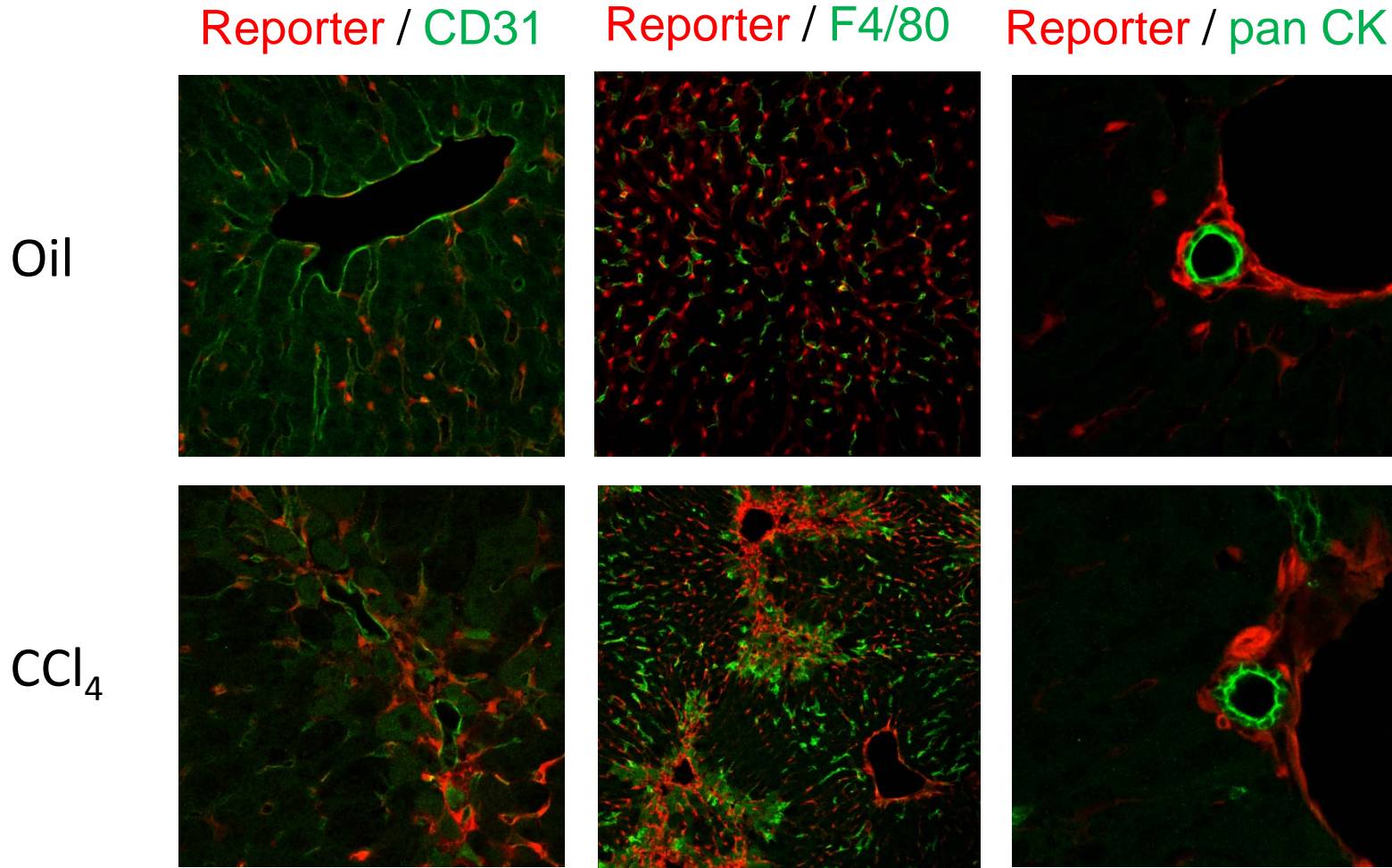
Reporter / DAPI



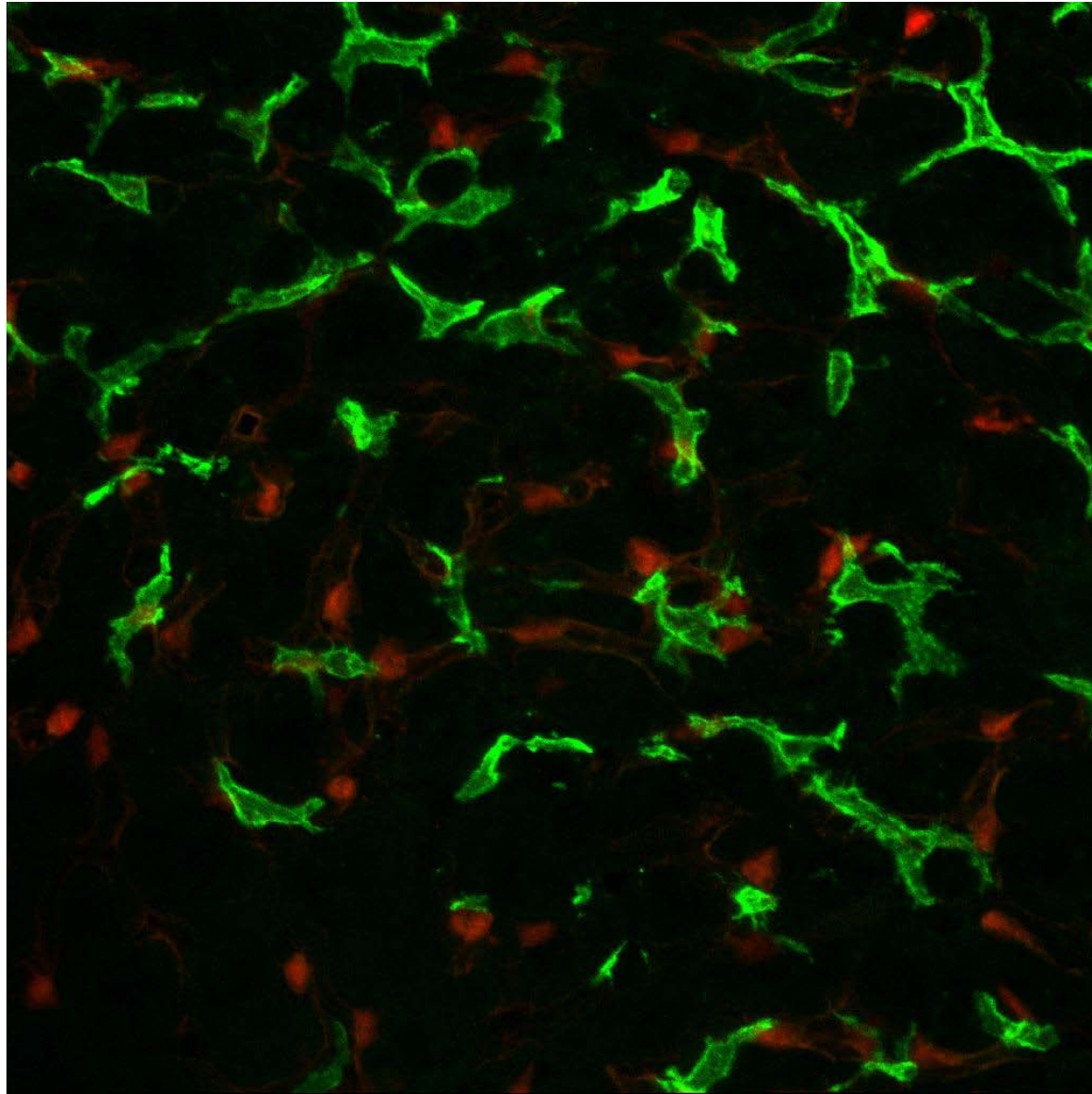
α SMA / DAPI



PDGFR β Cre mediates specific recombination in hepatic stellate cells

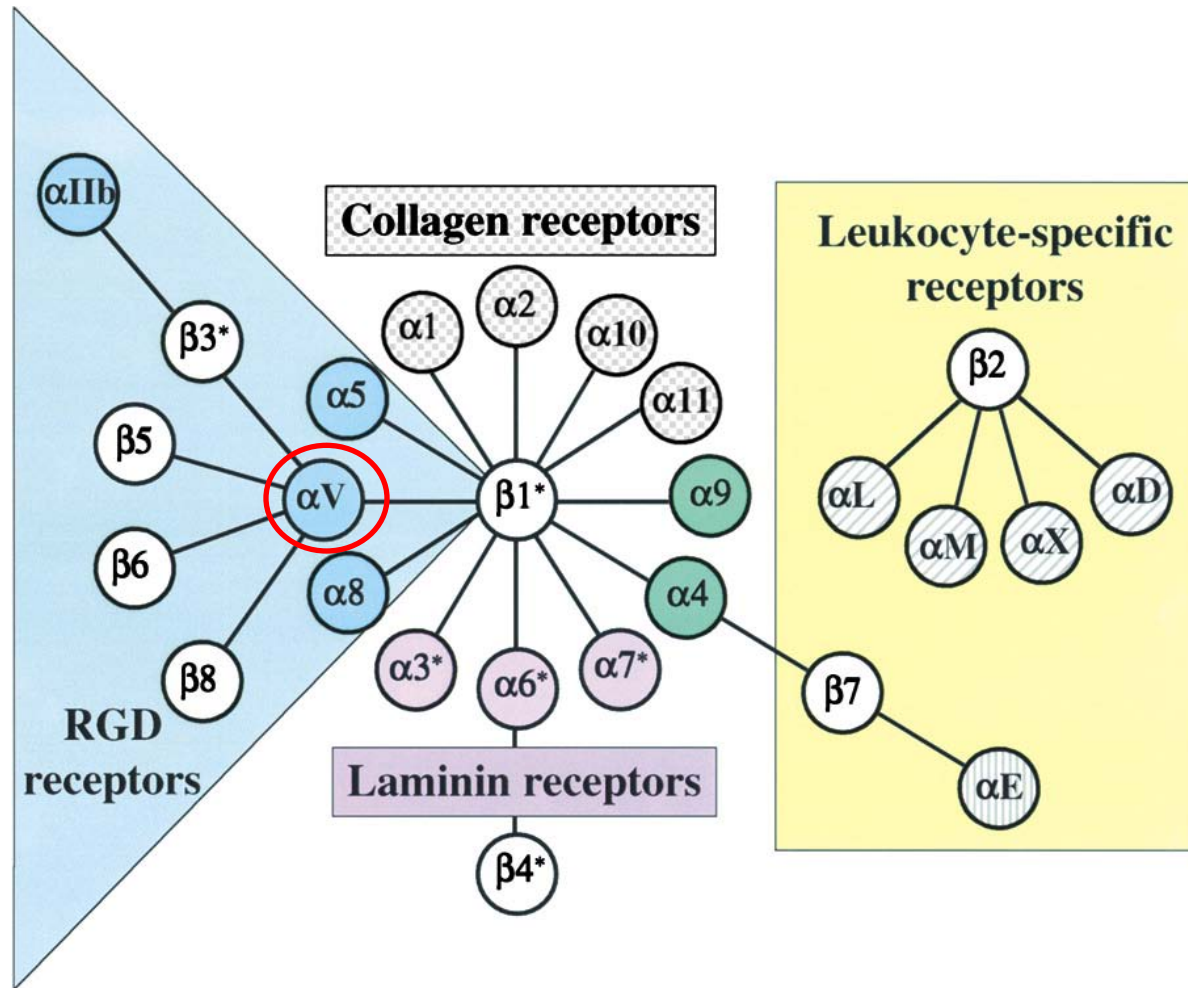


Stellate cells / Macrophages

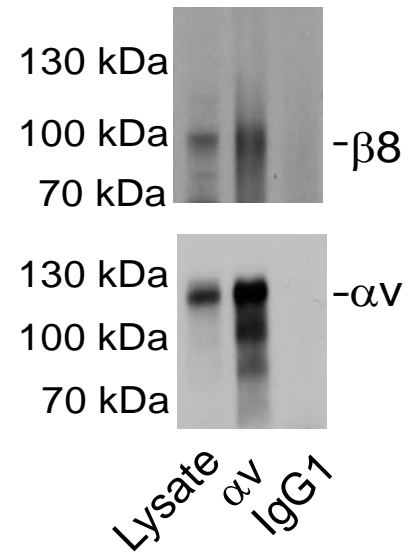
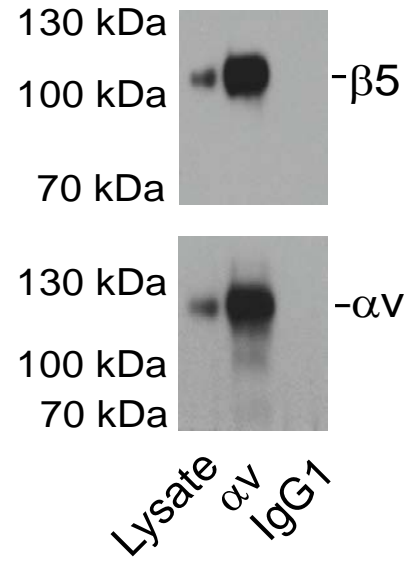
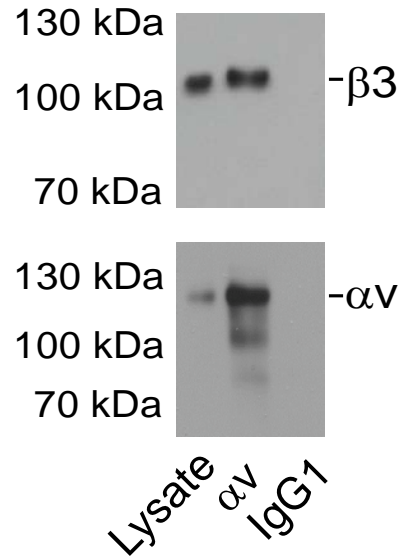
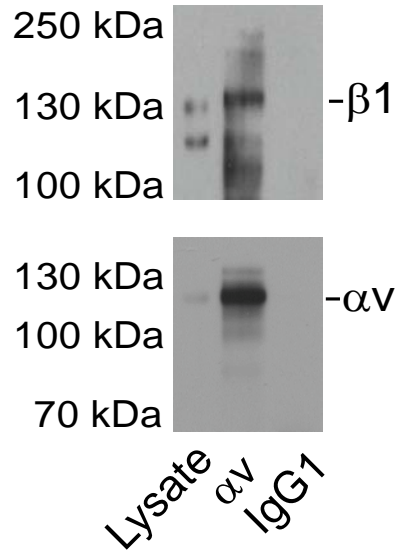


Can we use this system to investigate the biological function of genes in hepatic stellate cells?

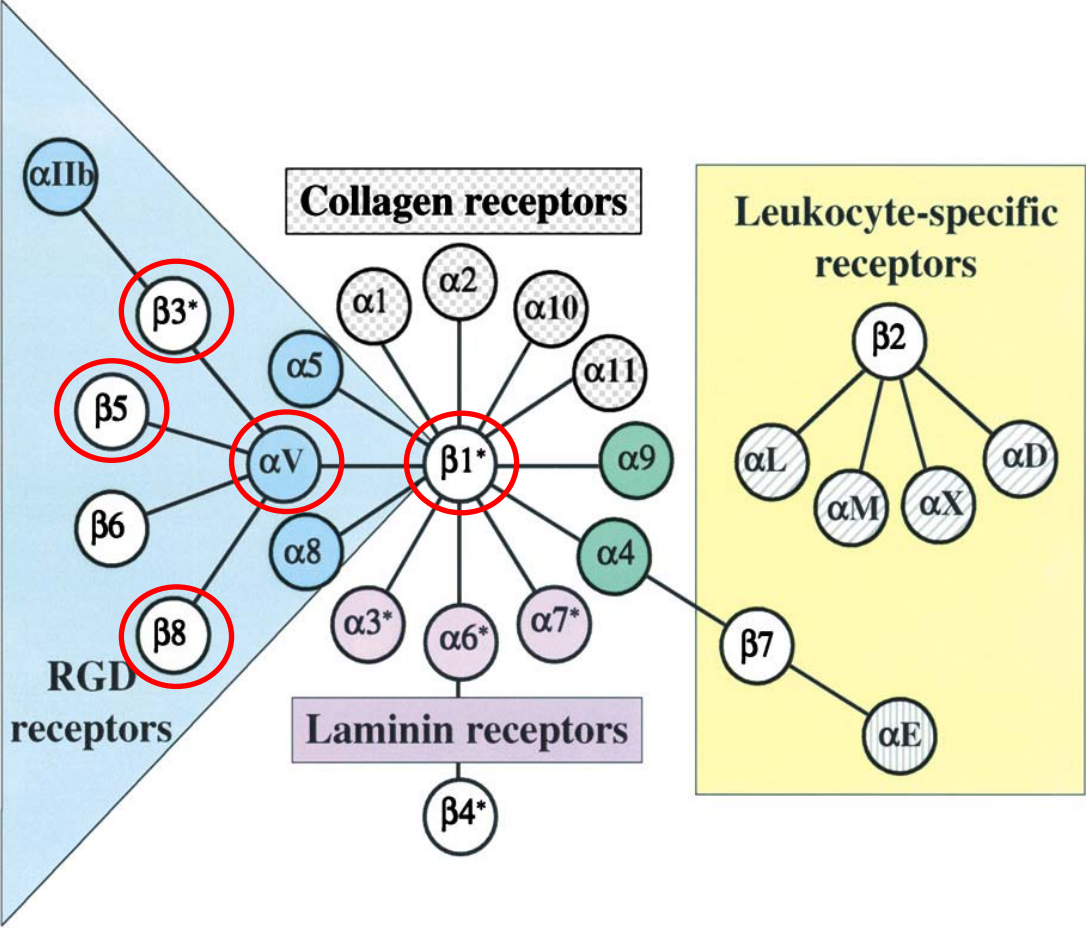
Integrins



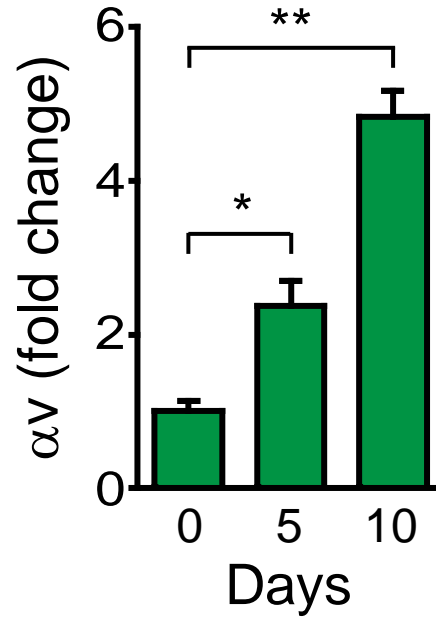
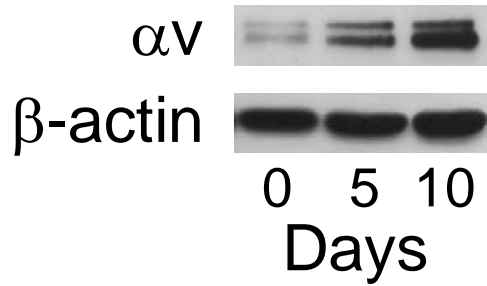
β subunit binding partner expression on sorted Td Tomato reporter cells



β subunit binding partner expression on sorted Td Tomato reporter cells

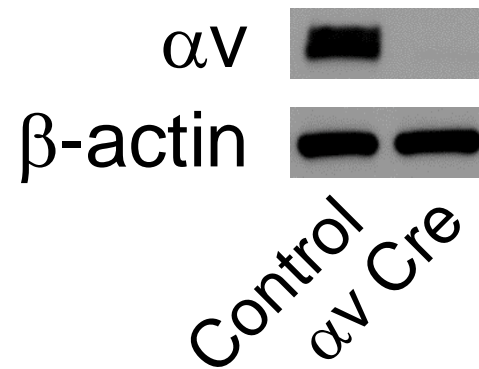


HSC α_v integrin expression increases with activation *ex-vivo*



Effective gene deletion in *itgav^{flox/flox}; PDGFR β* Cre mice

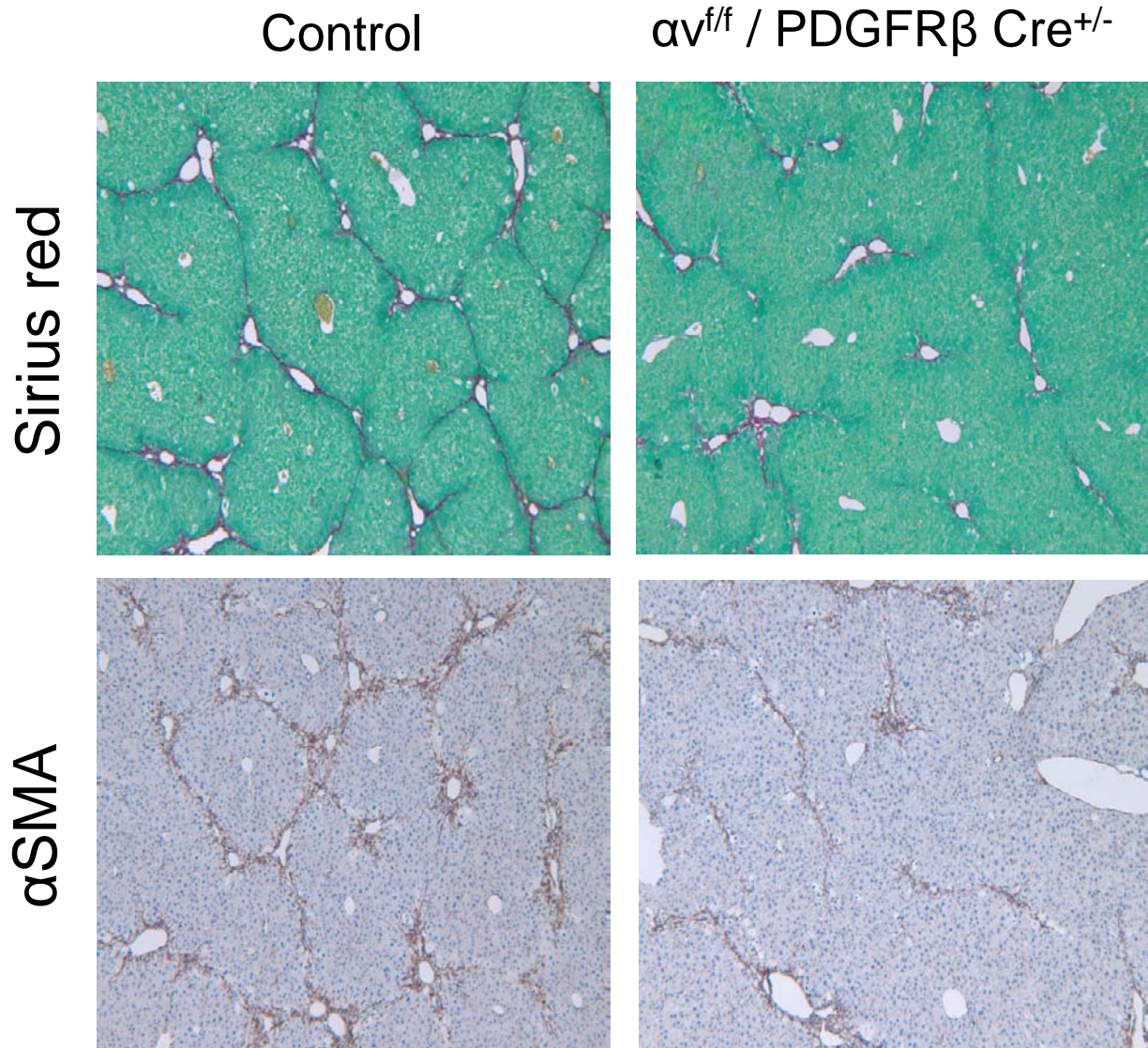
Hepatic stellate cells:



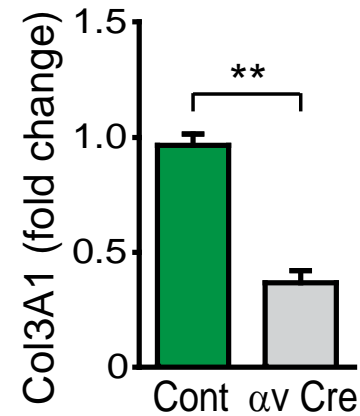
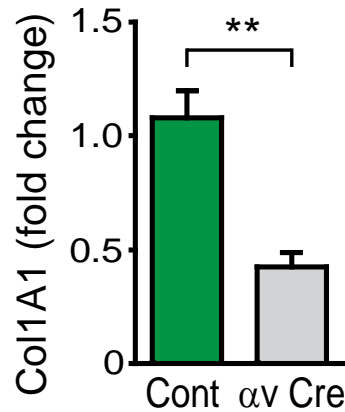
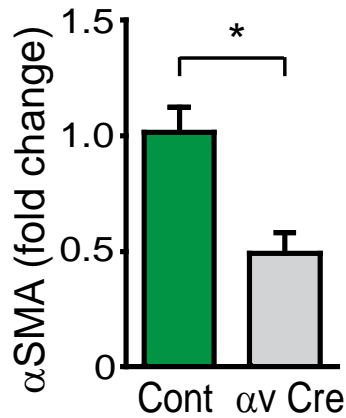
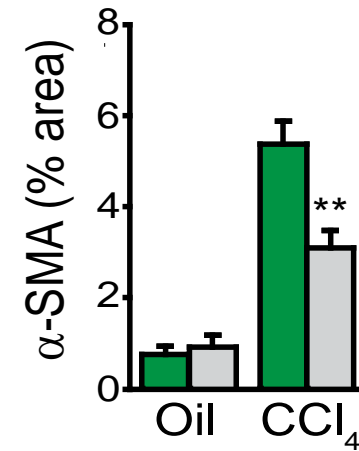
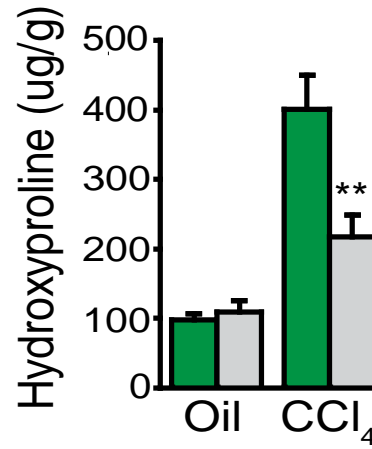
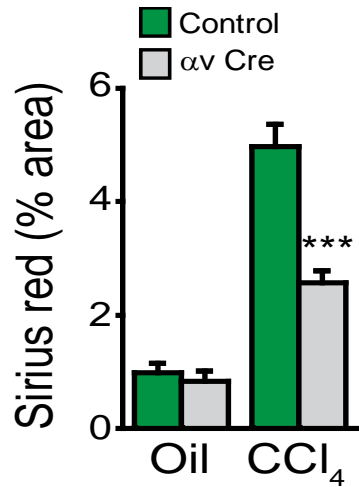
itgav^{flox/flox}; PDGFR β Cre^{-/-} (Control)

itgav^{flox/flox}; PDGFR β Cre^{+/-} (αV Cre)

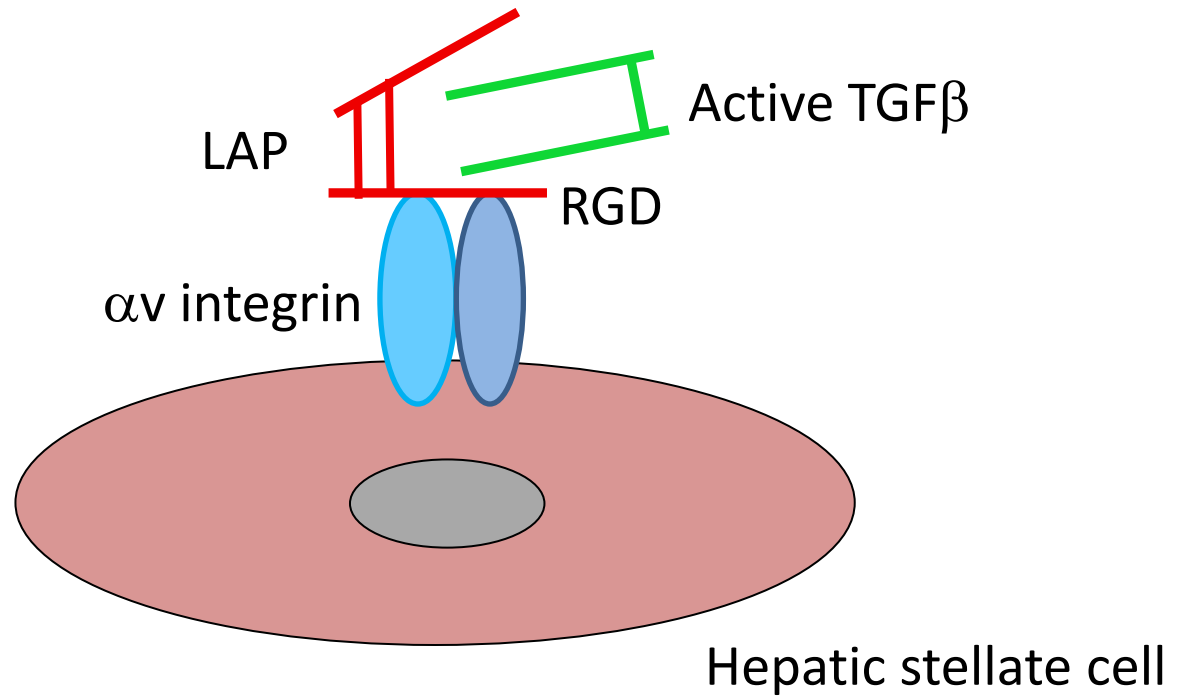
Deletion of the α_v integrin on HSC protects mice from CCl₄-induced hepatic fibrosis



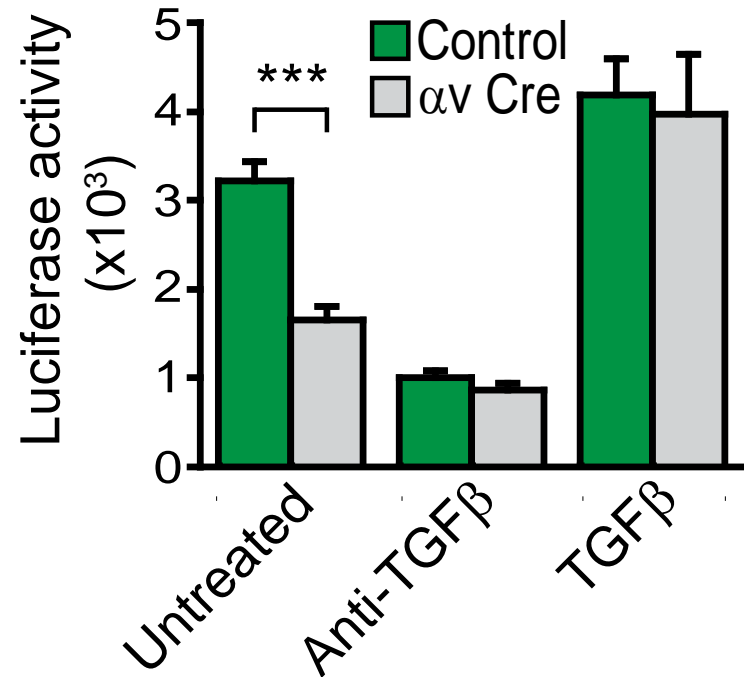
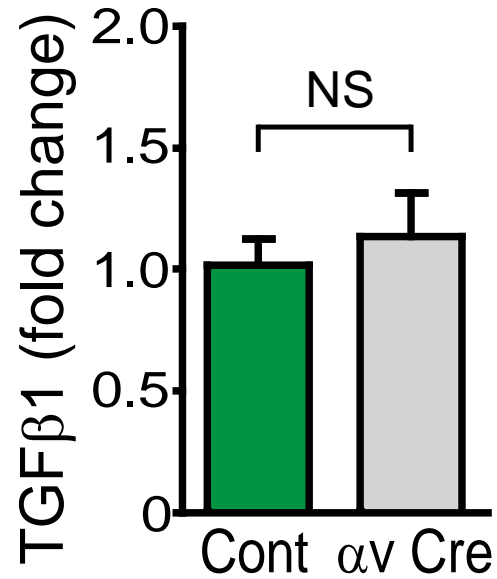
itgav^{flx/flx}; PDGFR β Cre mice are protected from liver fibrosis



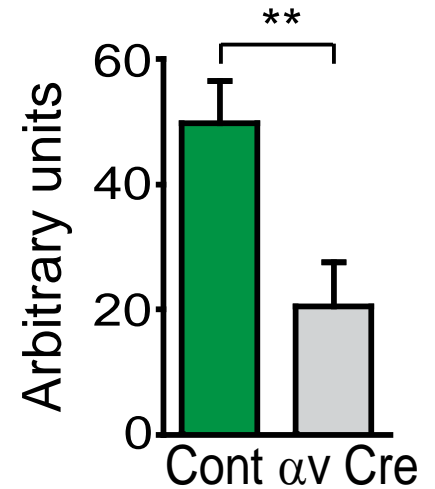
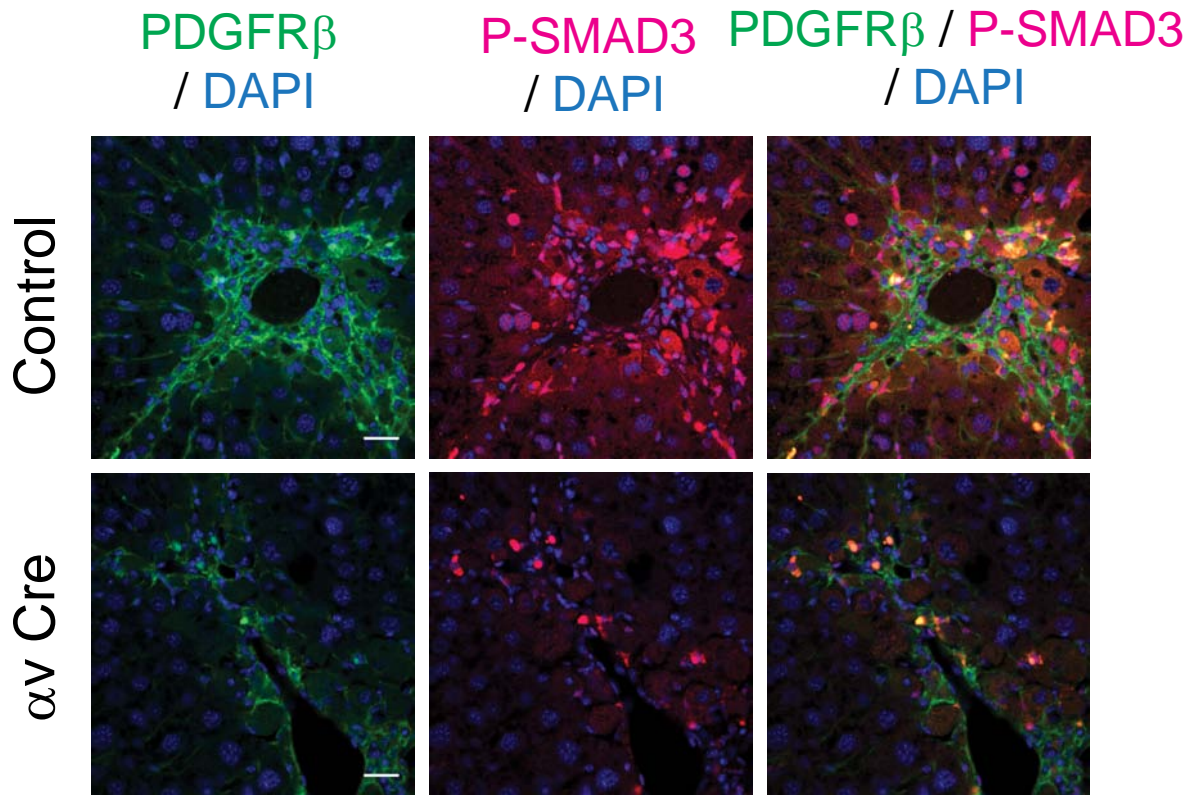
TGF β activation by α v integrins



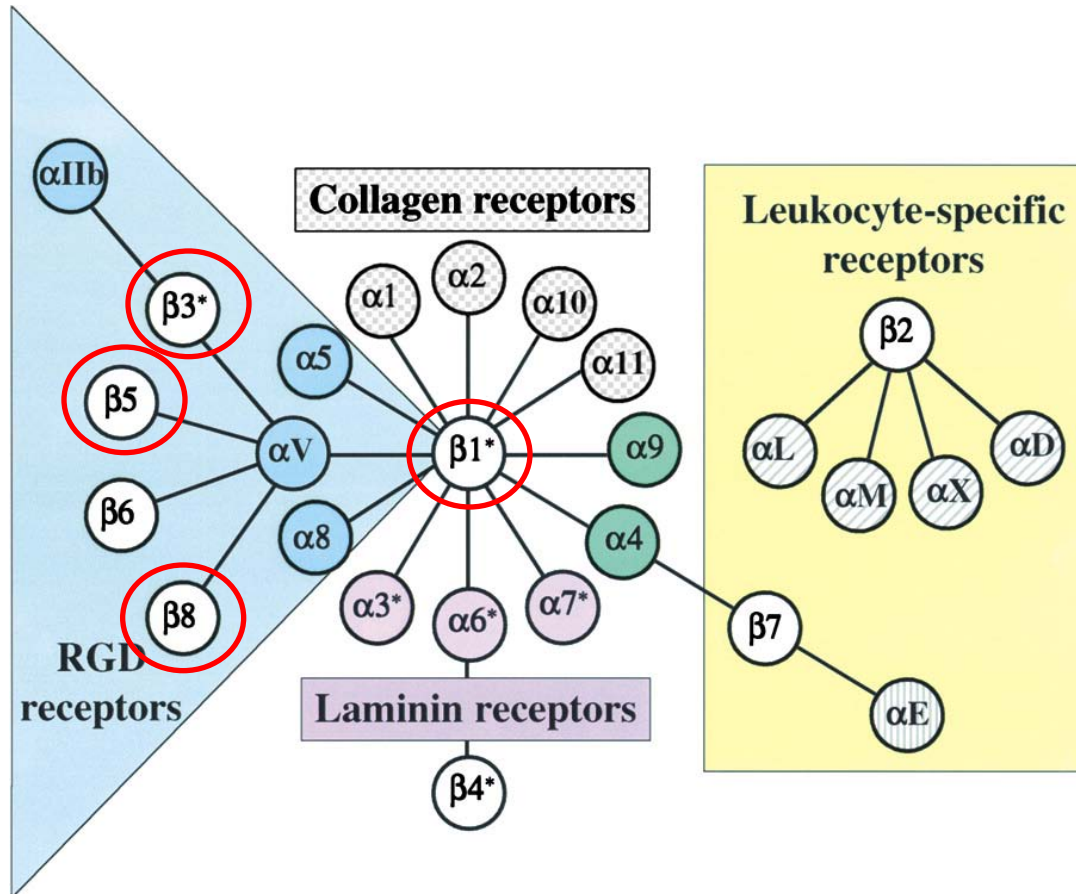
α v integrin deletion on hepatic stellate cells inhibits pro-fibrotic gene expression via a reduction in TGF β activation



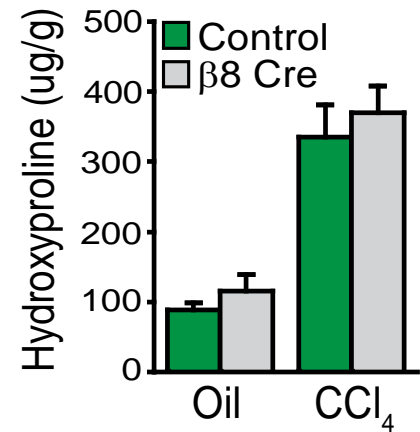
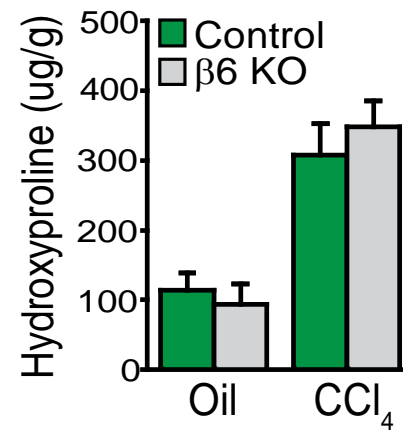
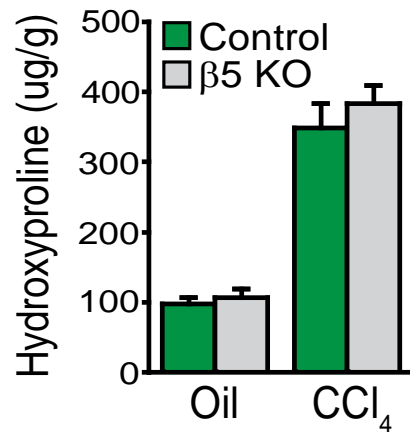
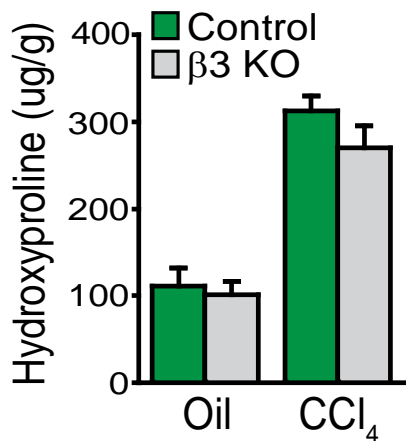
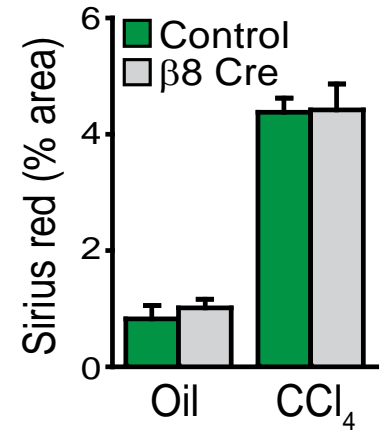
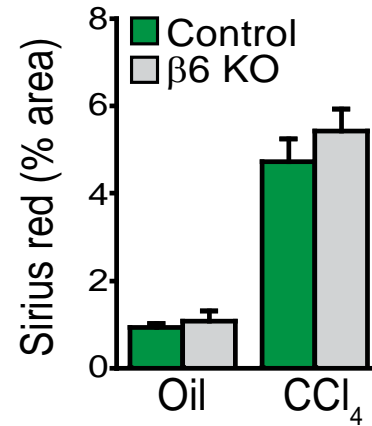
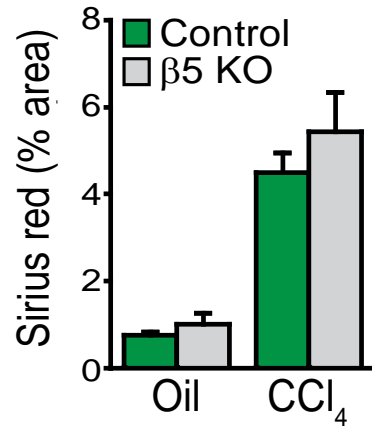
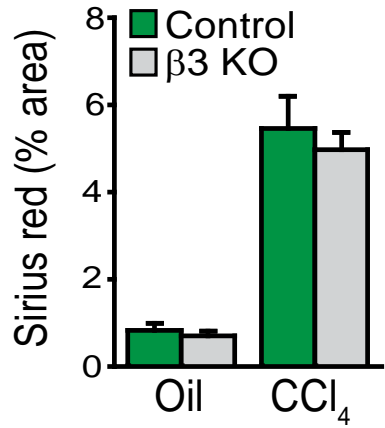
α v integrin deletion on hepatic stellate cells inhibits pro-fibrotic gene expression via a reduction in TGF β activation



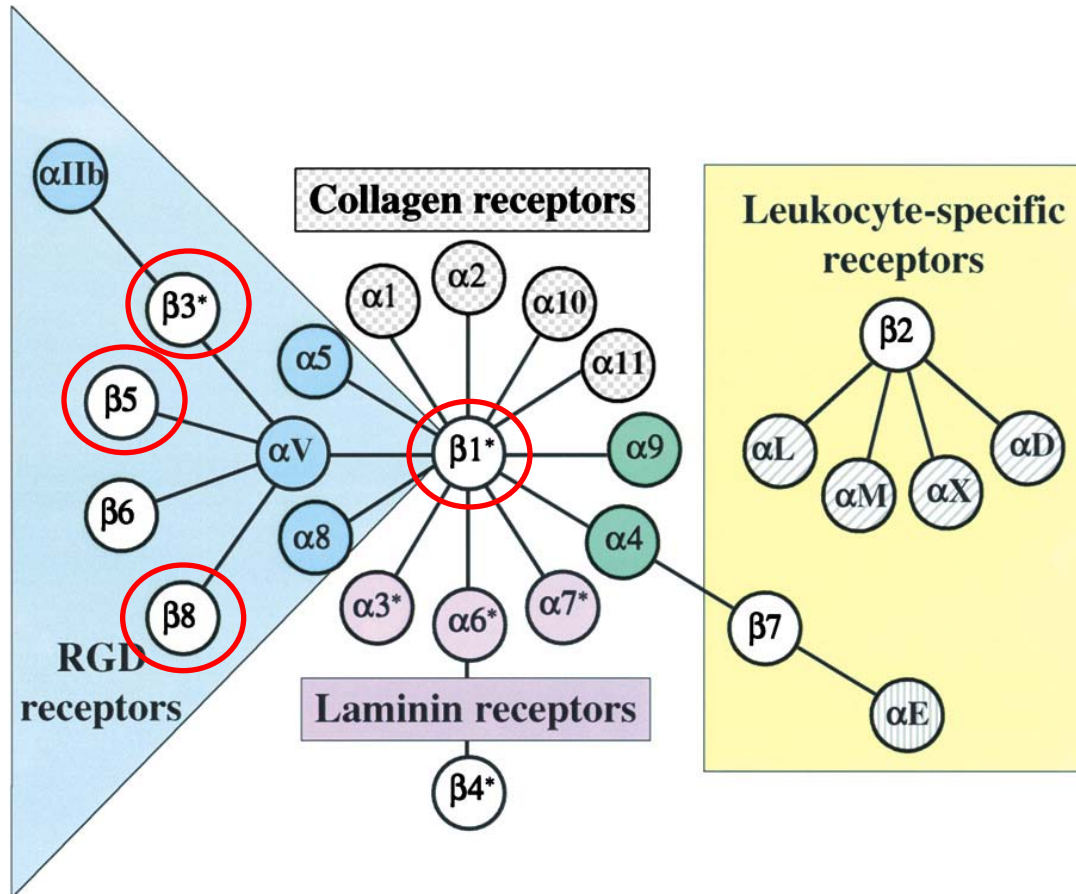
Integrins



Global loss of $\alpha\beta 3$, $\alpha\beta 5$ or $\alpha\beta 6$ or conditional loss of $\alpha\beta 8$ on HSCs does not protect mice from CCl_4 -induced hepatic fibrosis

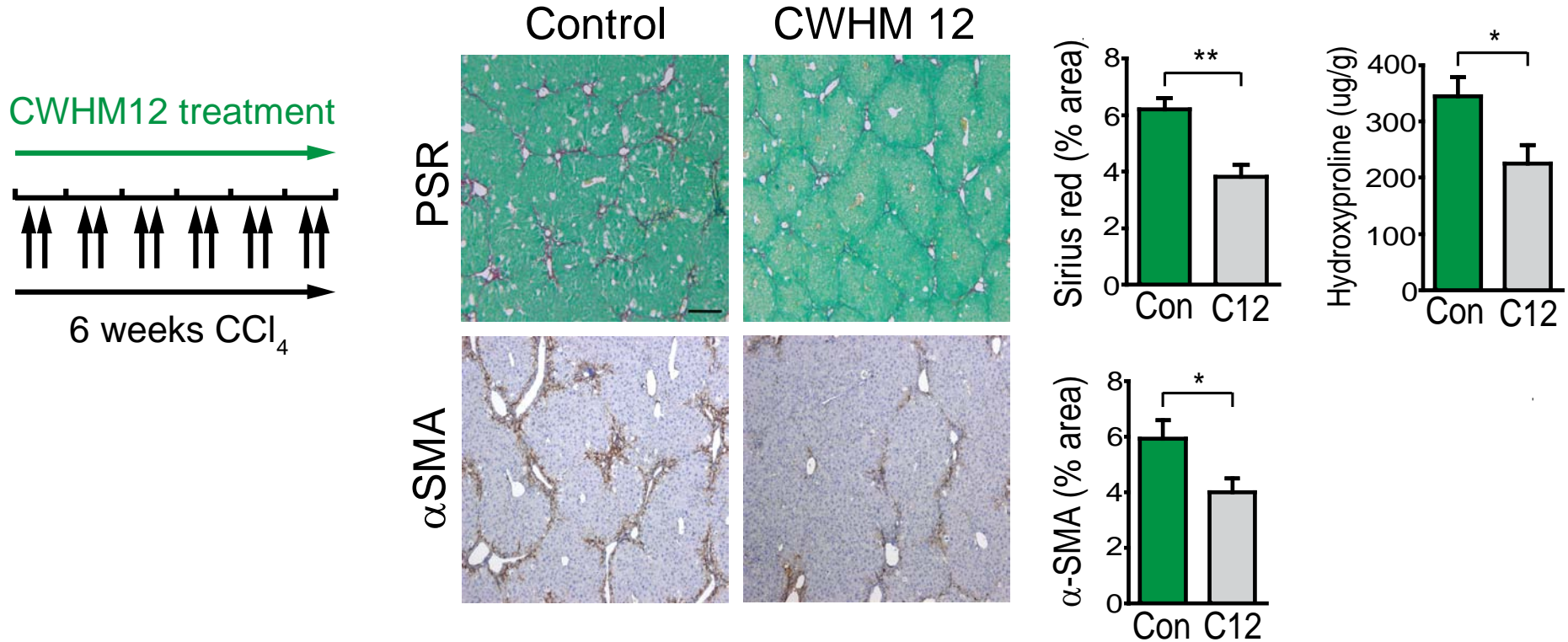


Integrins

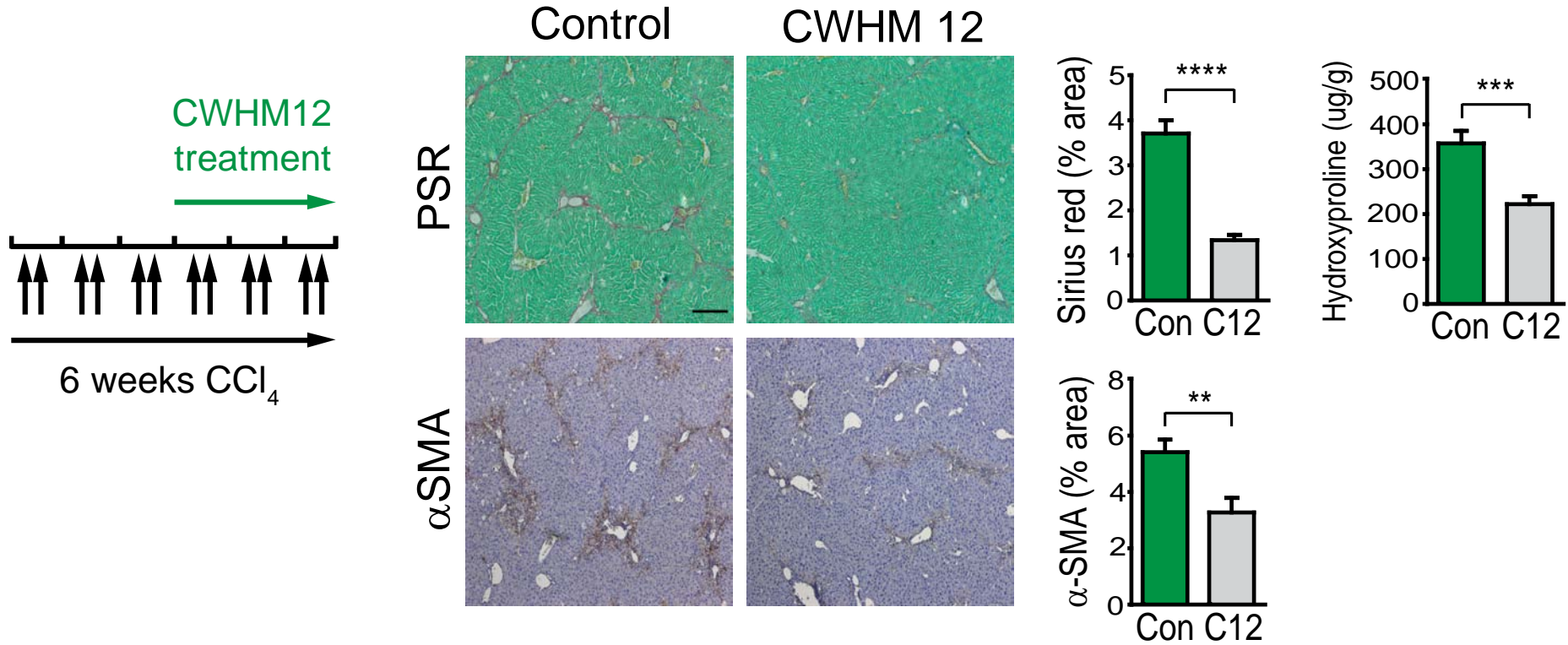


Can we target α_v integrins using small molecule inhibitors?

Blockade of α_v integrins by a novel small molecule (CWHM 12) attenuates liver fibrosis



Blockade of α_v integrins by a novel small molecule (CWHM 12) attenuates liver fibrosis



Can we use this system to manipulate genes in pericytes / myofibroblasts in other organs?

PDGFR β Cre in the lung

The capillary network of normal and emphysematous human lungs studied by injections of Indian ink

J. ALEXANDER REID¹ AND BRIAN E. HEARD

From the Postgraduate Medical School of London, Du Cane Road, London, W.12

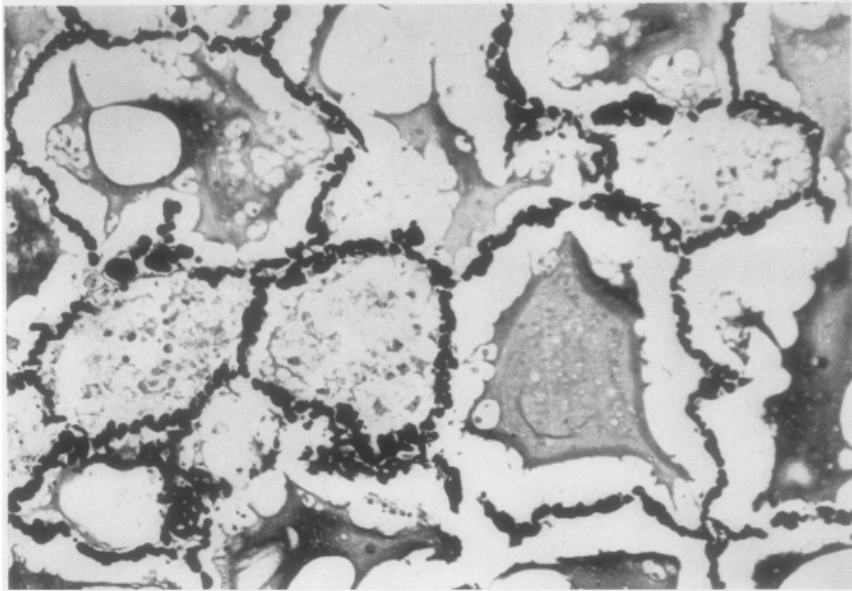


FIG. 5. Paraffin section of normal alveoli after Indian ink injection. Note remarkable number of capillaries in alveolar walls and how they appear to bulge in the lumina ($\times 100$).

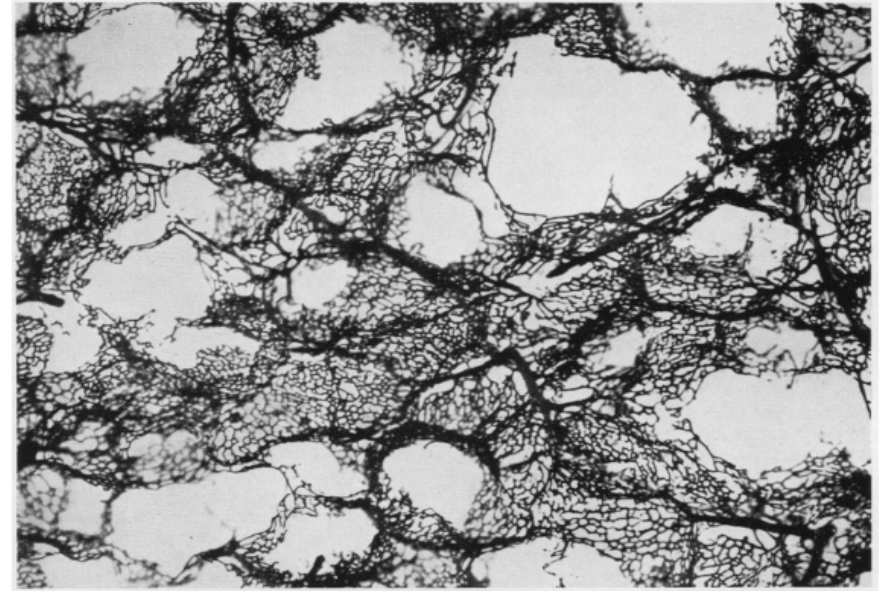
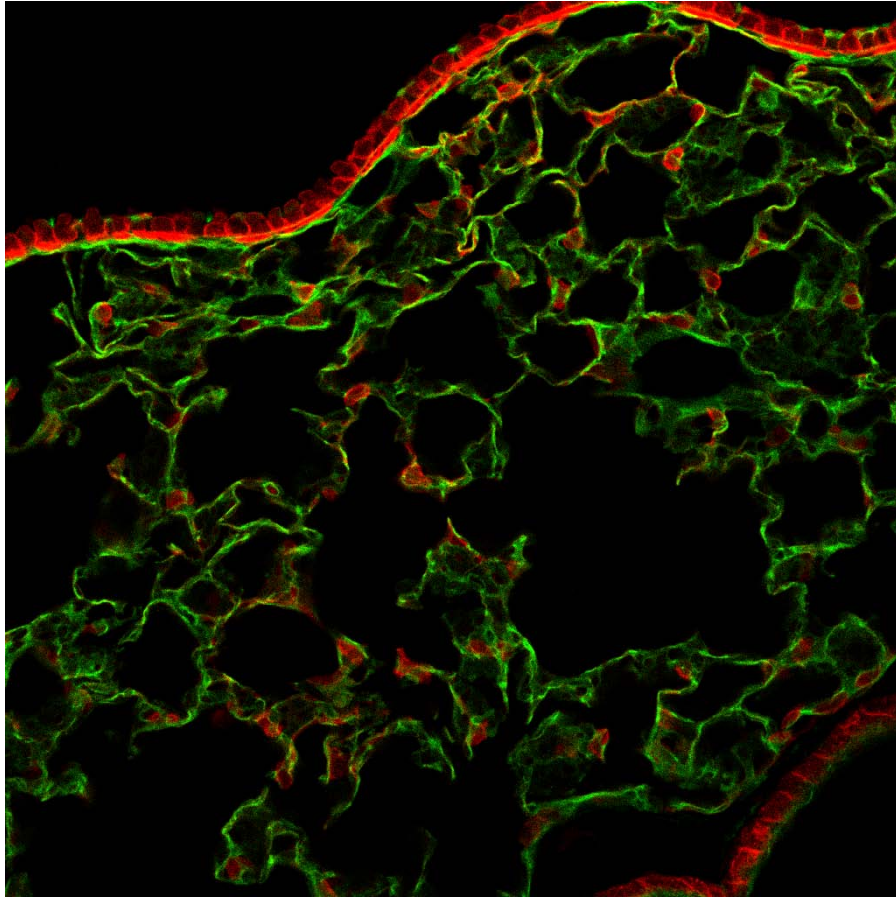


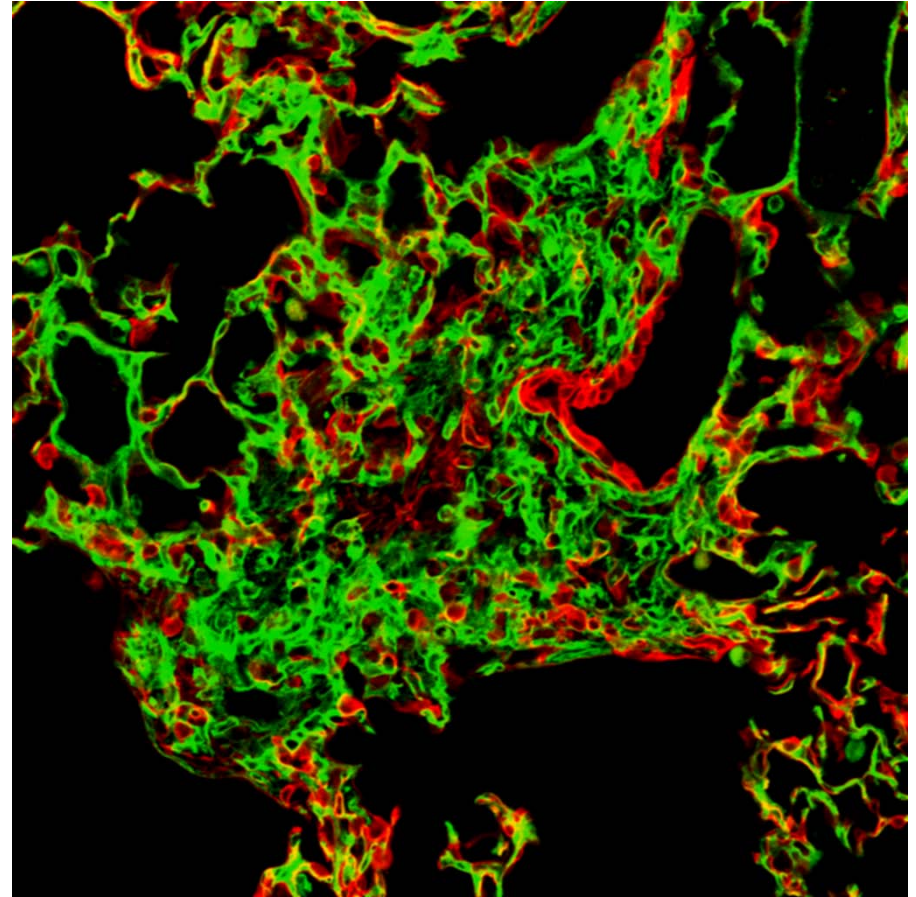
FIG. 12. Normal upper lobe showing similar pattern to normal lower lobe in Fig. 13. Both from same lung ($\times 80$).

PDGFR β Cre induced recombination in the lung

Saline

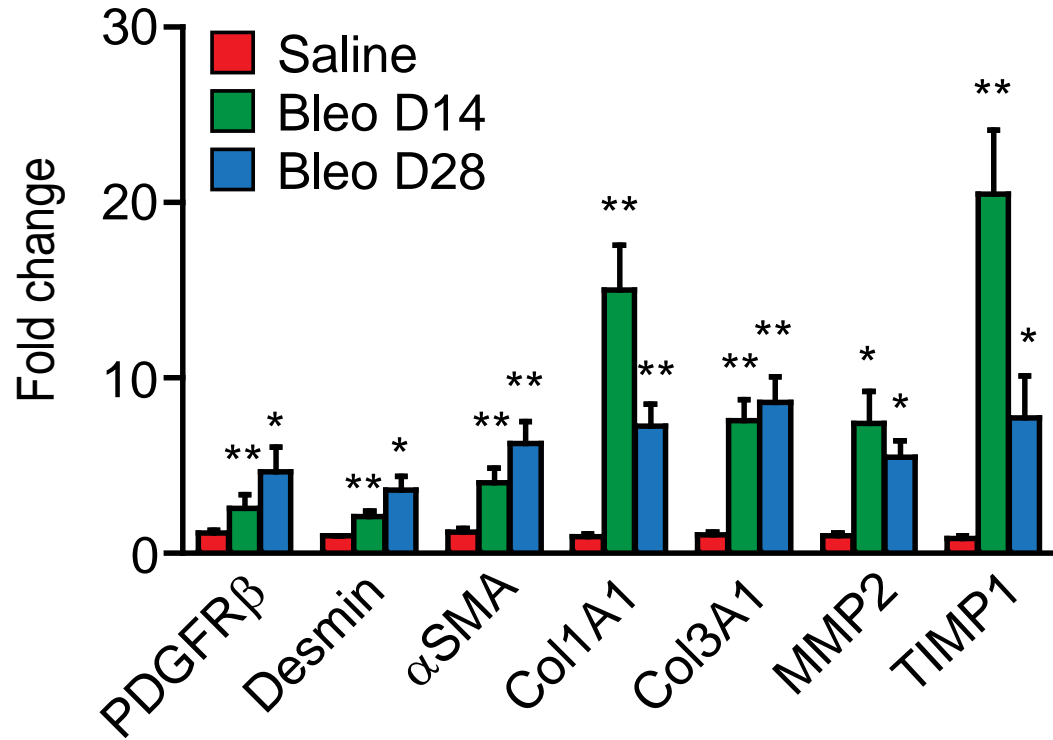


Bleomycin

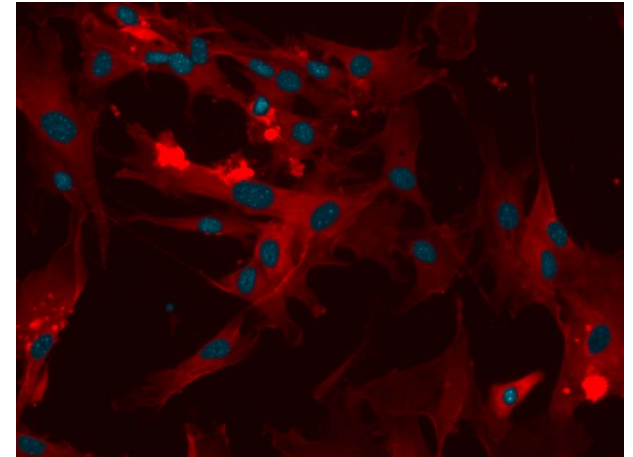


mTmG;PDGFR β Cre mice

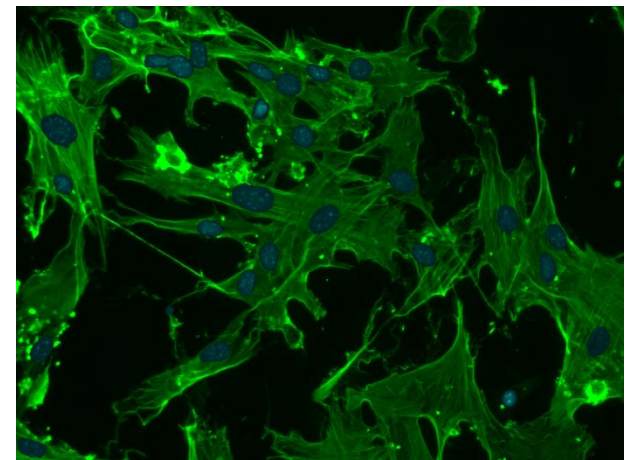
Cell sorting from bleomycin injured lungs



Reporter / DAPI



α SMA / DAPI

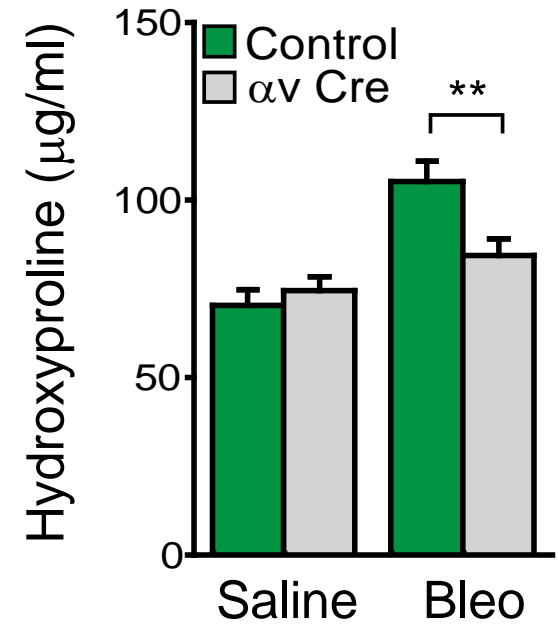
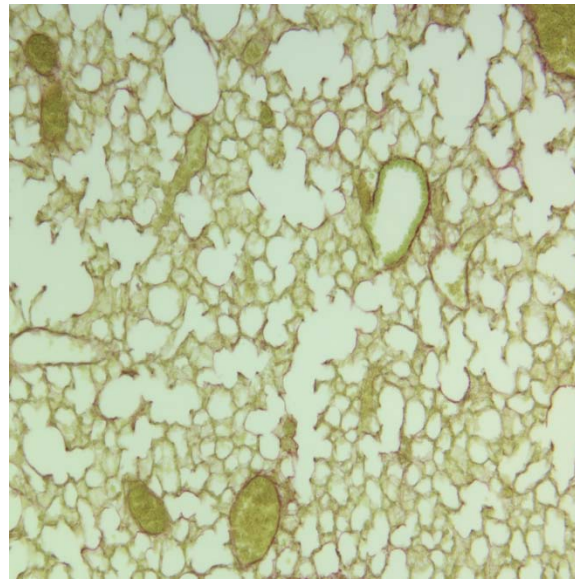
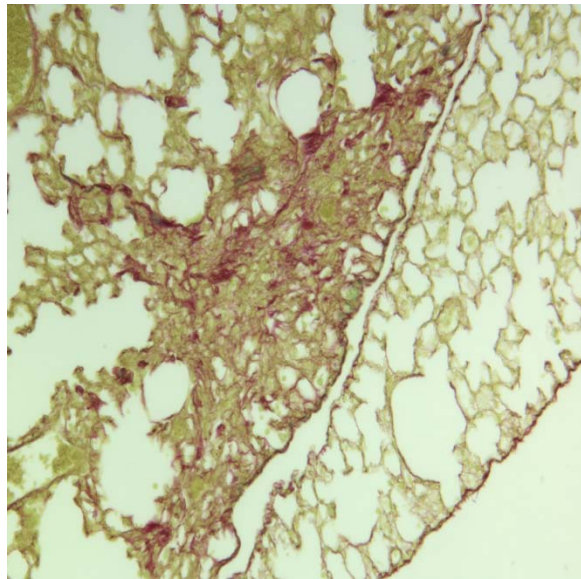


Ai14;PDGFR β Cre mice

itgαv^{flox/flox}; PDGFRβ Cre mice are protected from lung fibrosis

Control

αv Cre

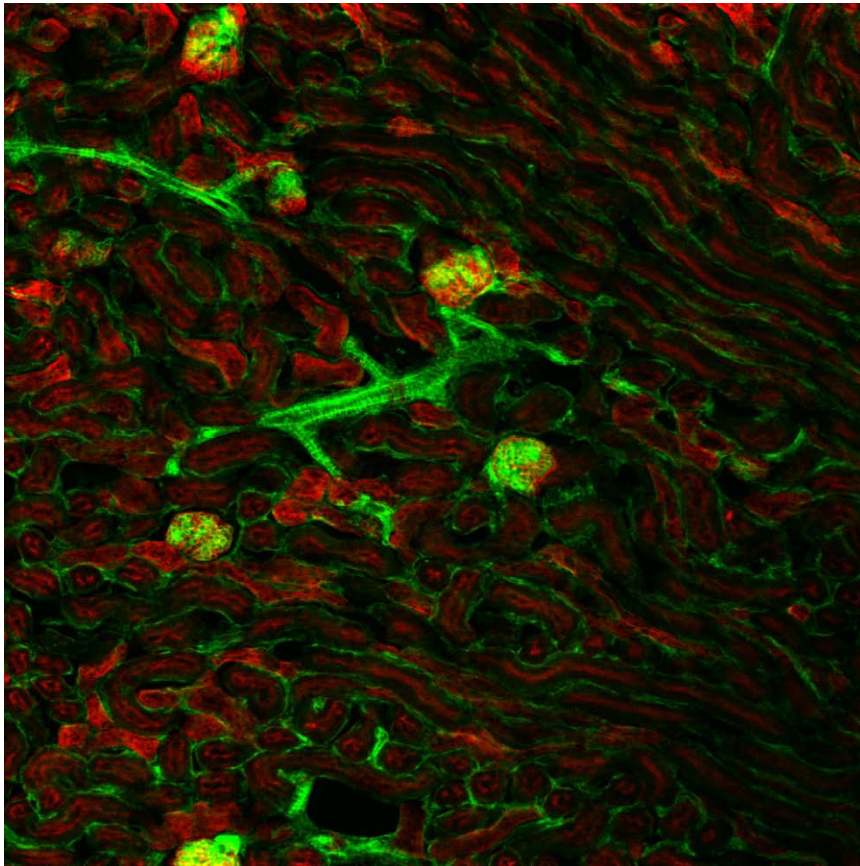


Day 28 post bleomycin (1.5U/Kg)

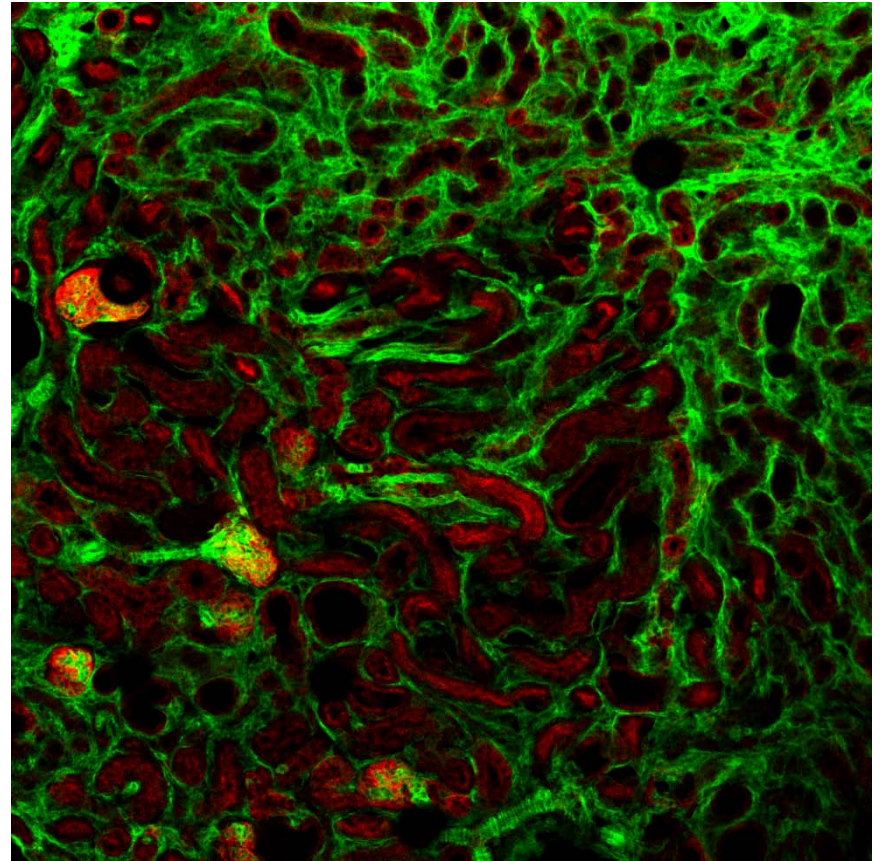
Kidney fibrosis – unilateral ureteric
obstruction model (UUO)

Renal fibrosis – unilateral ureteric obstruction model (UUO)

Sham



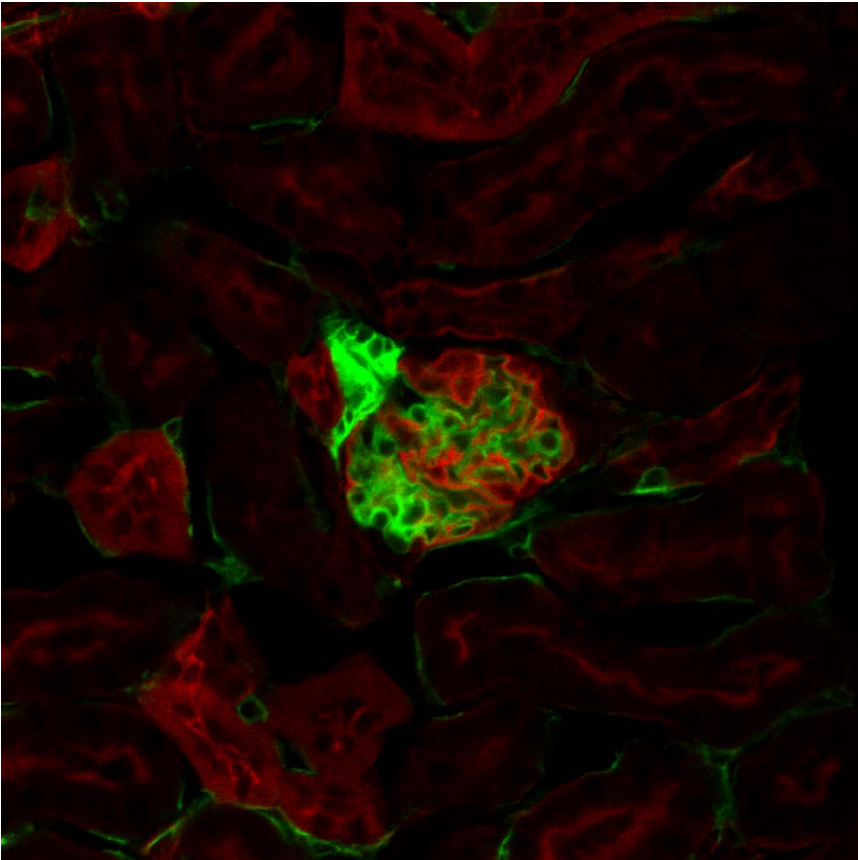
UUO



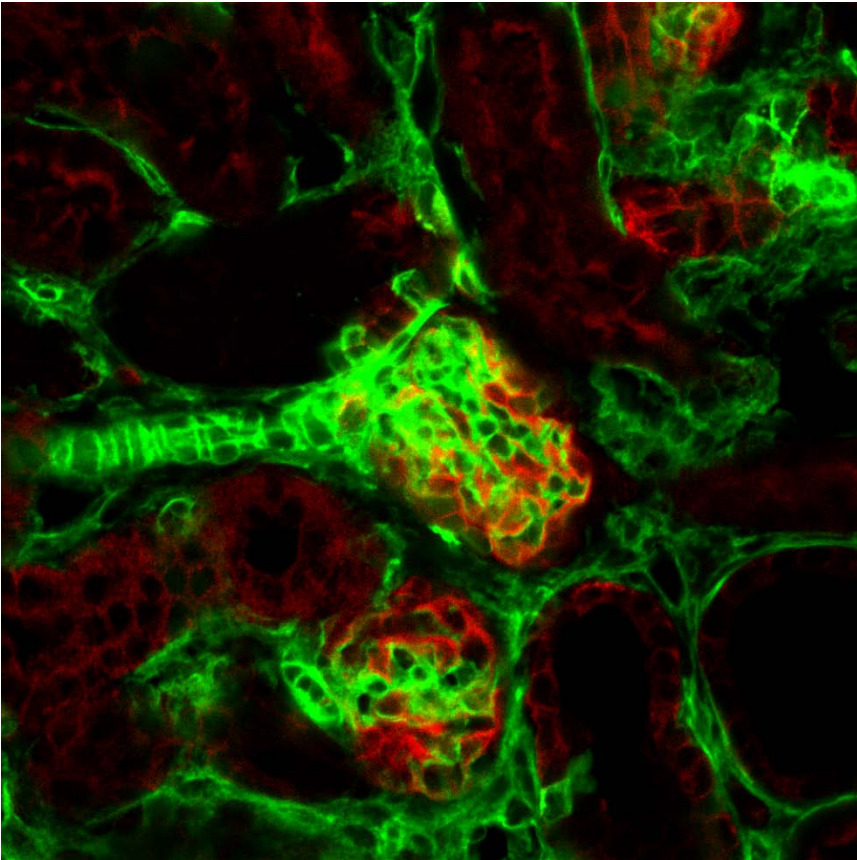
mTmG; *PDGFR* β Cre mice

Renal fibrosis – unilateral ureteric obstruction model

Sham

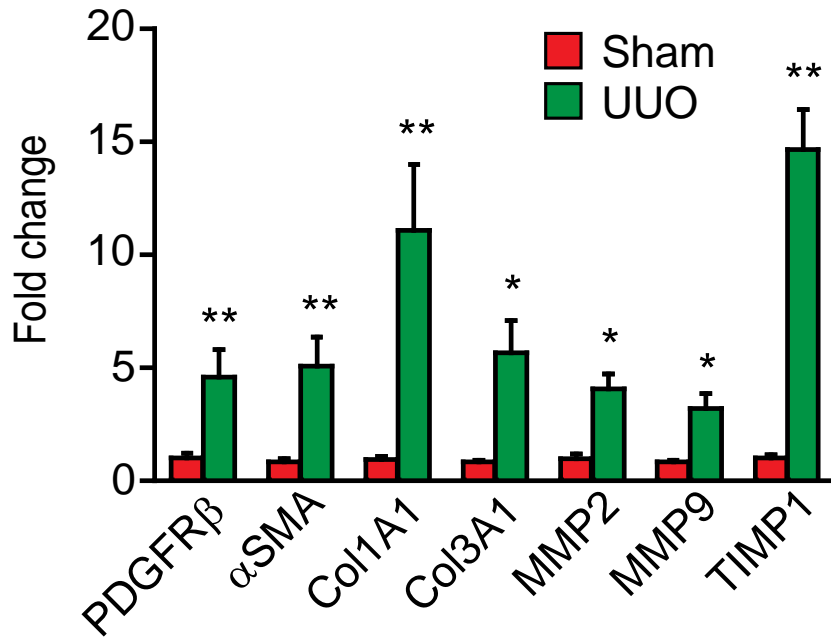


UUO



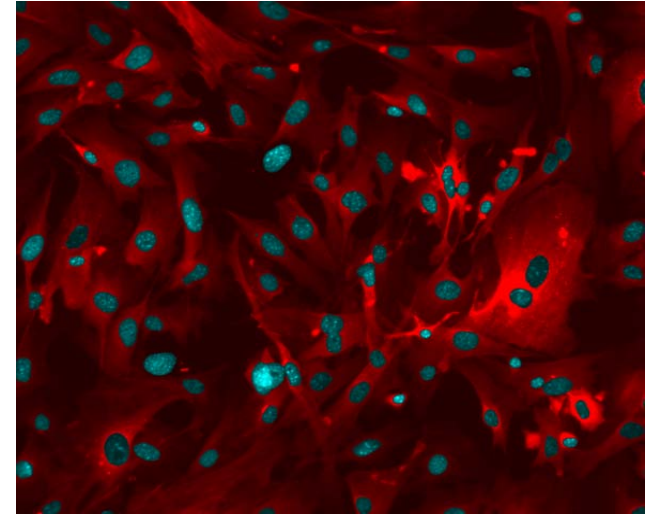
mTmG; *PDGFR* β Cre mice

Cell sorted TdTomato+ve cells from kidneys

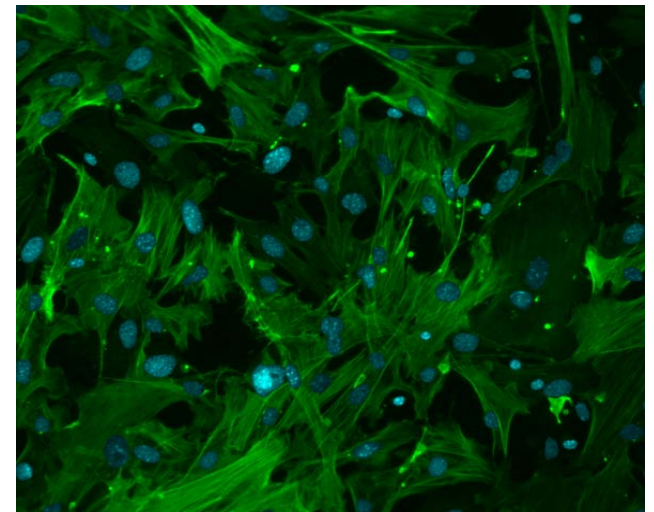


Day 7 post op

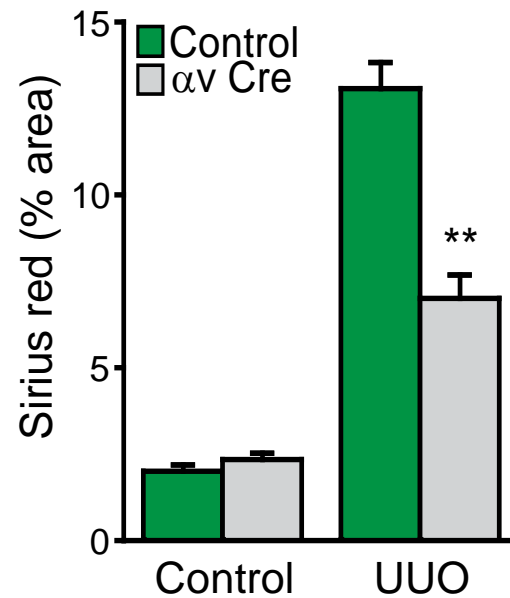
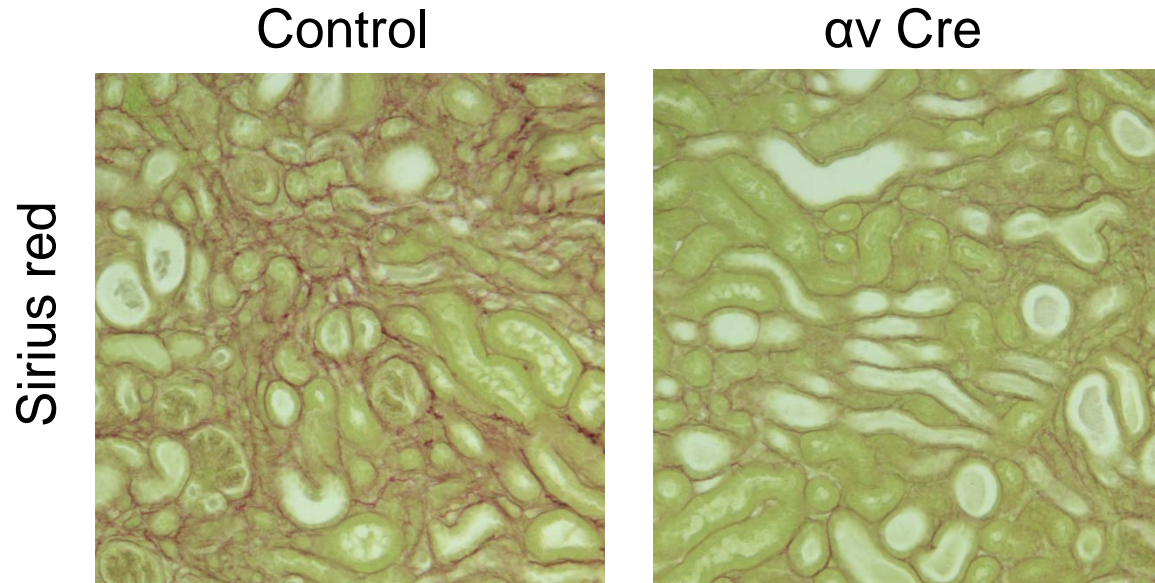
Reporter / DAPI



α SMA / DAPI



itgav^{flox/flox}; *PDGFRβ* Cre mice are protected from kidney fibrosis



Summary

Novel system which allows gene manipulation in pericytes and tissue myofibroblasts during organ fibrogenesis

PDGFR β Cre driven α v integrin deletion identifies a core, targetable molecular pathway that regulates fibrosis across solid organs

This system will hopefully accelerate progress in understanding the molecular mechanisms underlying a wide range of fibrotic diseases, leading to the development of new, mechanistically targeted therapies

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