Supplementary Material:

Gli1 pericyte loss induces capillary rarefaction and proximal tubular injury

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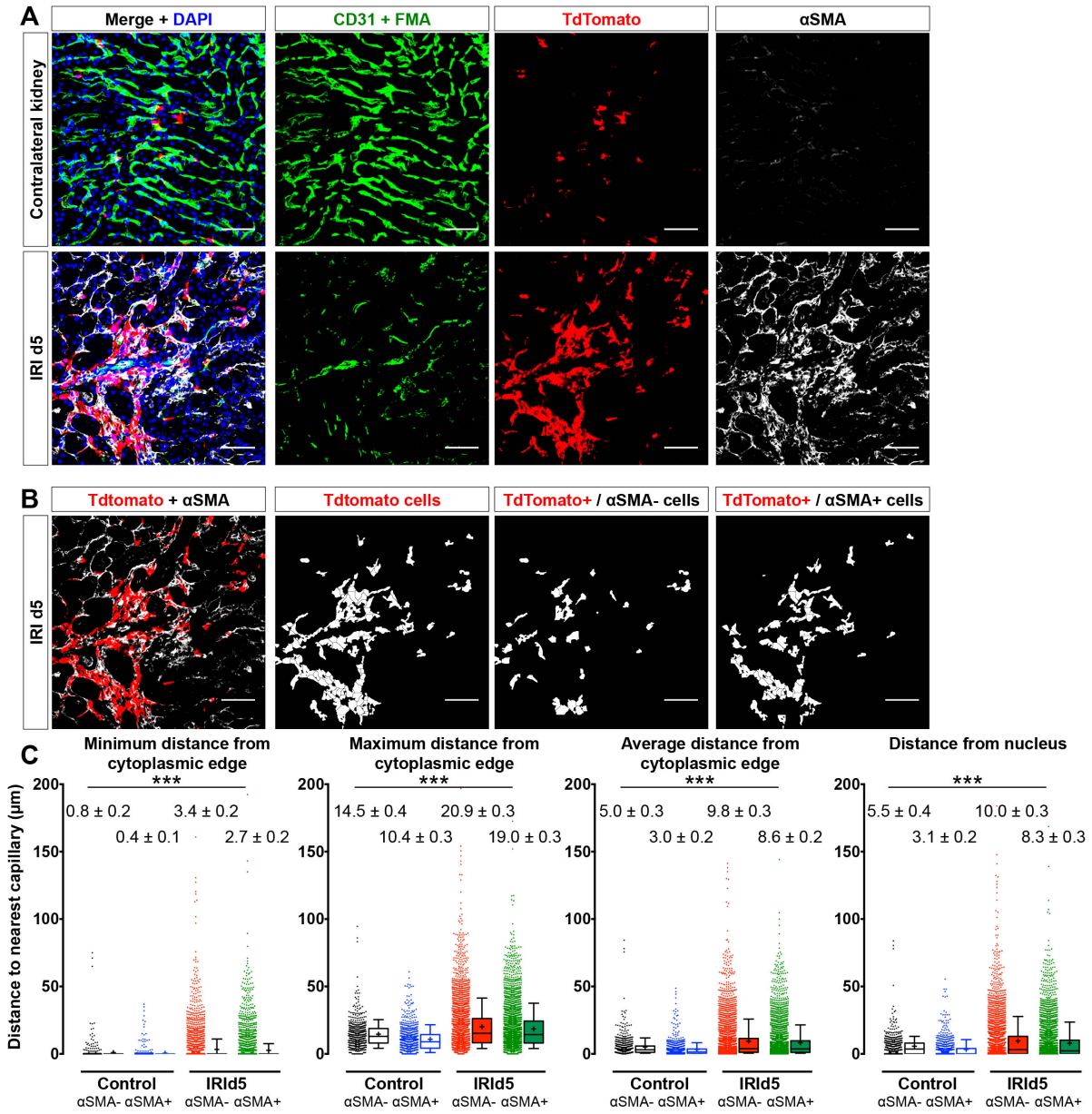
Supplementary Material 1 : ImageJ script for distance analysis

```
Open the selected image
run("8-bit");
run("Split Channels"); //split image into different channels
selectWindow("C2-pic");
selectWindow("C3-pic");
selectWindow("C4-pic");
run("Duplicate...", "title=cy5");
selectWindow("C3-pic");
run("Duplicate...", "title=tdt");
selectWindow("C2-pic");
run("Duplicate...", "title=fitc");
selectWindow("C1-pic");
run("Duplicate...", "title=dapi");
run("Merge Channels...", "c1=tdt c2=fitc c3=dapi c4=cy5 create keep");
selectWindow("cy5");
run("Gaussian Blur...", "sigma=2"); //using blur function to clear up noise
setAutoThreshold("Huang"); //Huang autothreshold is the best for our cy5 staining
//run("Threshold...");
setAutoThreshold("Huang dark");
//setThreshold(38, 255);
setOption("BlackBackground", true);
run("Convert to Mask");
run("Invert");
run("Distance Map"); //Create a distance map from cy5 channel
selectWindow("fitc");
run("Gaussian Blur...", "sigma=2");
setAutoThreshold("Huang dark"); //Huang autothreshold is the best for our FITC
staining
//run("Threshold...");
//setThreshold(21, 255);
run("Convert to Mask");
run("Invert");
run("Distance Map"); //Create a distance map from FITC channel
selectWindow("tdt");
run("Gaussian Blur...", "sigma=2");
setAutoThreshold("Moments dark"); //Moments autothreshold is the best for our
TRITC color
//run("Threshold...");
//setThreshold(5, 255);
run("Convert to Mask");
run("Duplicate...", "title=redarea");
selectWindow("redarea");
selectWindow("dapi");
run("Gaussian Blur...", "sigma=2");
run("Find Maxima...", "noise=20 output=[Single Points]"); //use the maximal point of
intensity of each nucleus as representative point
imageCalculator("AND create", "dapi Maxima","redarea"); //select nuclei that are
within tdTomato+ area
selectWindow("Result of dapi Maxima");
```

```
selectWindow("dapi Maxima");
run("Find Connected Regions", "allow_diagonal display_one_image display_results
regions_for_values_over=100 minimum_number_of_points=1 stop_after=-1");
//Assign each nucleus as different data point
run("Marker-controlled Watershed", "input=redarea marker=All mask=redarea
calculate use"); //Use nuclei to divide large red area into equal pieces base on nuclei
within the area
setAutoThreshold("Huang dark"); //convert divided red area into binary data
//run("Threshold...");
setAutoThreshold("Huang");
run("Convert to Mask");
run("Invert");
run("Options...", "iterations=2 count=5 black edm=32-bit do=Erode");
run("Tile");
selectWindow("EDM of cy5");
selectWindow("EDM of fitc");
selectWindow("redarea");
selectWindow("dapi Maxima");
selectWindow("Result of dapi Maxima");
selectWindow("All connected regions");
selectWindow("redarea-watershed");
```

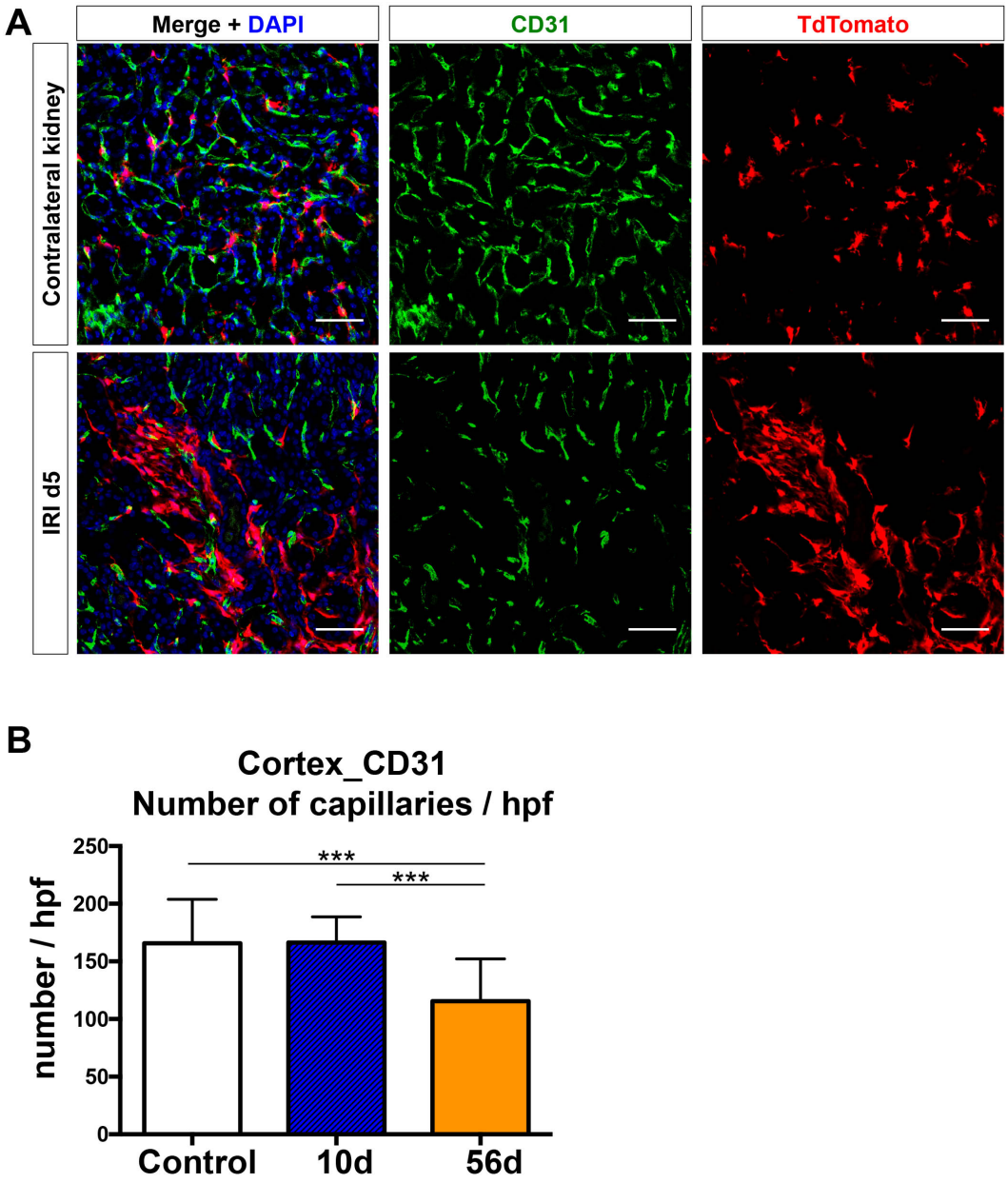


Supplementary figure S1: Undifferentiated Gli1$^+$ pericytes and Gli1 derived myofibroblasts both detach from the microvasculature.

(A) Representative images of kidneys from bigenic Gli1CreER;tdTomato mice at day 5 after ischemia reperfusion injury versus sham with CD31 staining, fluorescence microangiography (FMA) and αSMA co-staining. Scale bars 50µm, DAPI, 4',6-diamidino-2-phenylindole

(B) To dissect activated Gli1 derived myofibroblasts and undifferentiated Gli1$^+$ pericytes the tdTomato$^+$ cells were subdivided based on their expression of αSMA using further image processing algorighm. Scale bars 50µm

(C) Measured distances of tdTomato cells to the closest capillary in kidneys of bigenic Gli1CreER;tdTomato mice at day 5 following ischemia reperfusion injury (IRI) versus sham (control) stratified for αSMA expression. Of note, data represents n=11 mice, 6 female and 5 male, in the CLK group and n=10 mice, 5 female and 5 male, in the severe IRI group; mean ± SEM; box and whiskers with 10th-90th percentiles; + indicates mean; ***p<0.001, by one way ANOVA with posthoc Bonferroni.



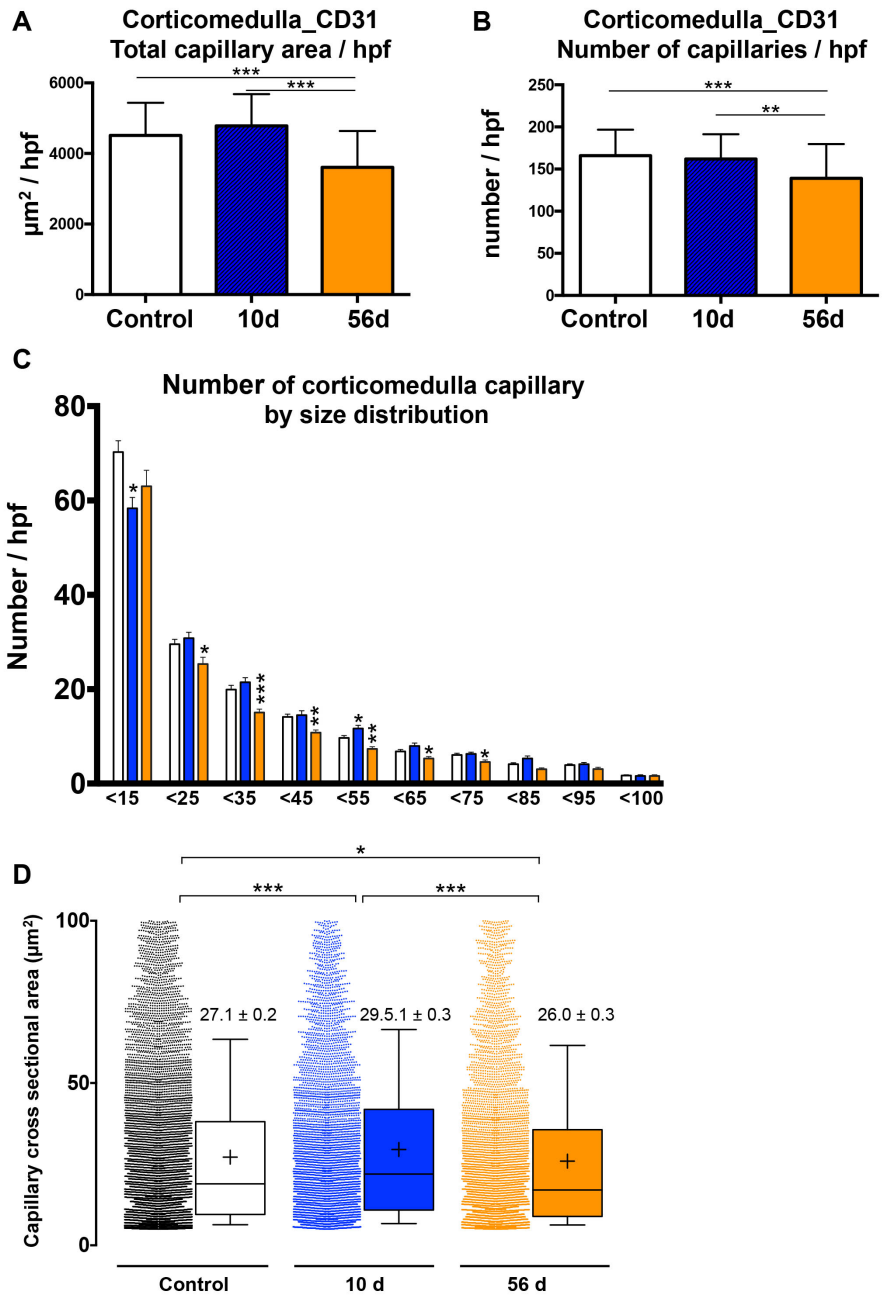
Supplementary Figure S2: Ischemia reperfusion injury (IRI) triggers capillary rarefaction

(A) Representative pictures of kidney outer-medullary microvasculature stained by CD31 at day 5 after severe IRI. Note the decreased numbers of stained capillaries and increased number of tdTomato^{+} cells after IRI (scale bars are 50 µm).

(B) Total number of capillaries after pericyte ablation was decreased from 165.7 ± 6.5 in control to 115.6 ± 6.6 capillaries/hpf at 56 days. Total number of capillaries at day 10 was 166.4 ± 5.0 capillaries/hpf. Data represent n = 7 mice in control, n = 4 mice in 10 days group and n = 6 in 56 days group; ***p<0.001.

Supplementary Material 2 : Software-based high throughput automated analysis of fluorescence microangiography

```
folder_name = uigetdir; %Prompts user to select folder
filename = uigetfile; %Prompts user to select file to be analyzed
uiimport = (filename); %Imports selected file name
I = imread(filename); %Reads imported file
background = imopen(I,strel('disk', 15)); %Standardizes background and threshold
figure, surf(double(background(1:8:end,1:8:end))),zlim([0 255]);
set(gca,'ydir','reverse');
I2 = I - background; %Removes excess noise
imshow(I2);
level = graythresh(I2);
bw = im2bw(I2, level);
bw = bwareaopen(bw,50); %States capillary area
cc = bwconncomp(bw,4);
cc.NumObjects;
labeled = labelmatrix(cc);
whos labeled;
RGB_label = label2rgb(labeled, @spring, 'c', 'shuffle');%colors individual capillaries
with pretty colors
figure, imshow(RGB_label);
capillarydata = regionprops(cc,'all'); %reads all perimeter data of the capillaries
capillary_peri = [capillarydata.Perimeter];
capillary_area = [capillarydata.Area];
[min_perim, idx] = min(capillary_peri);
capillary = false(size(bw));
capillary(cc.PixelIdxList{idx}) = true;
%Converts perimeter data to micrometers
PDataInMicrons =capillary_peri*0.30120';
%Converts Area data to Micrometers
ADataInMicrons =capillary_area*0.0907';
nbins = 50;
figure, hist(ADataInMicrons, nbins) %Generates capillary Area histogram
title('Histogram of Capillary Area Data')
figure, hist(PDataInMicrons, nbins) %Generates capillary Perimeter histogram
title('Histogram of Capillary Perimeter Data')
SA = ADataInMicrons';
SP = PDataInMicrons';
csvwrite('AreaQuant1.csv', SA) %Writes data to area excel sheet
csvwrite('PerimQuant1.csv', SP) %Writes data to perimeter excel sheet
```



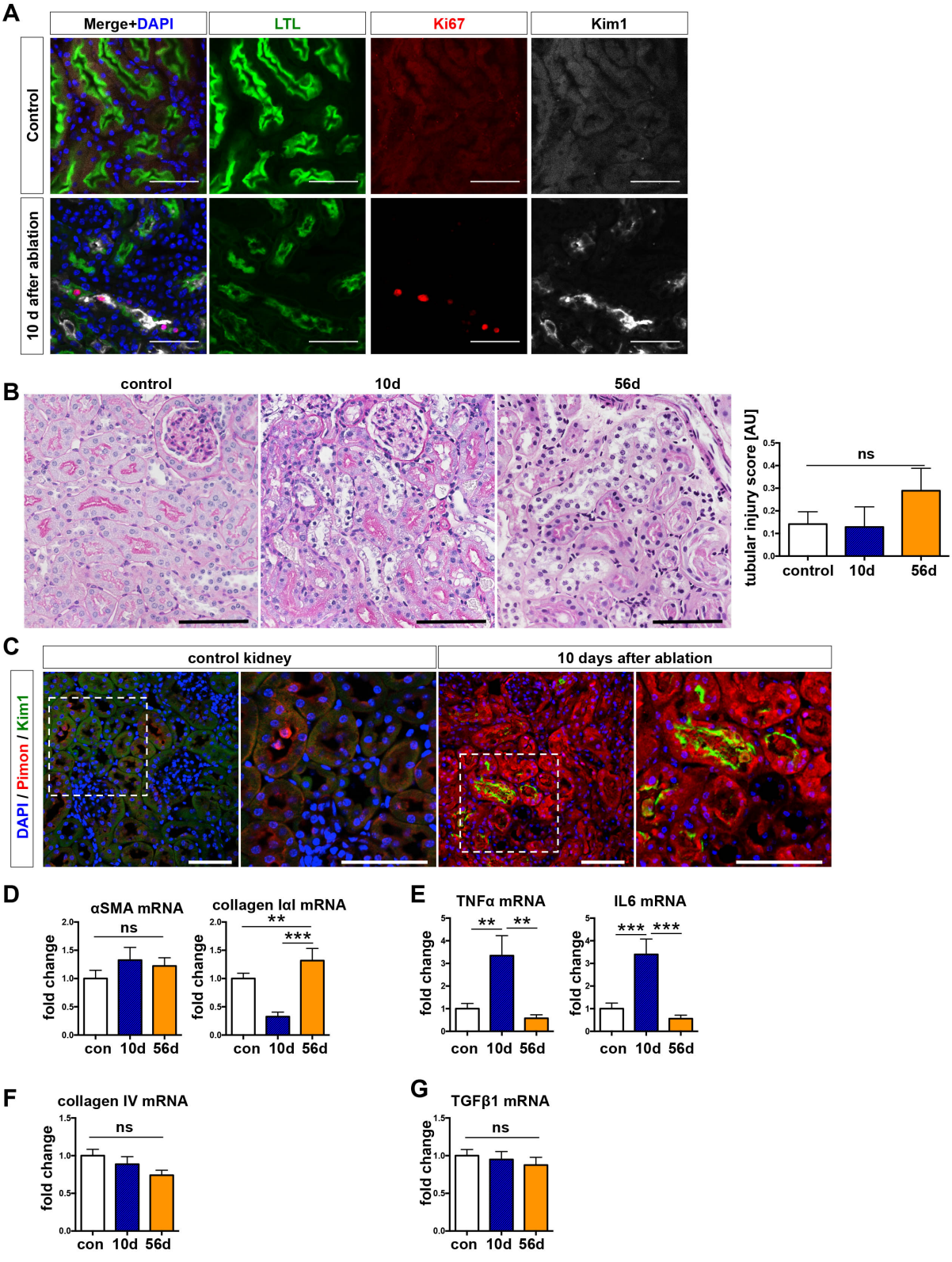
Supplementary Figure S3 : Capillary rarefaction in the outer medulla following pericyte ablation.

(A-B) Ablation of Gli1^{+} cells resulted in reduction of total capillary cross-sectional area (control 4514±124.4, 10 days 4782±152.2 and 56 days 3608±153.4 µm2) and total number of capillaries in the outer medulla (control 166±4.15, 10 days 162±4.96 and 56 days 139±6.07 capillaries/high-power field (hpf, 400x)) (mean±SEM, **p<0.01, ***p<0.001 by one way ANOVA with posthoc Bonferroni).

(C) Gli1^{+} cell ablation resulted in loss of small and large capillaries in the outer medulla. (mean±SEM, *p<0.05,**p<0.01, ***p<0.001 by one way ANOVA with posthoc Bonferroni).

(D) The individual outermedullary capillary cross-sectional area slightly decreased after pericyte ablation (mean±SEM; box and whiskers with 10th-90th percentiles; + indicates mean; one-way ANOVA with post hoc Bonferroni)

(Of note, data represents n = 12 mice in control, n = 7 mice in 10 days group and n = 9 in 56 days group)



Supplementary Figure S4 : Gli1 pericyte ablation induces hypoxic tubular injury, inflammation and mild tubulointerstitial fibrosis.

(A) We detected increased proliferation (Ki67 positive nuclei) in tubules that strongly expressed Kim1. Scale bars 50µm

(B) Representative images of Periodic acid-Schiff (PAS) stained images of kidneys from mice at 10 days and 56 days after Gli1 ablation versus control. Images very blindly scored for the severity of tubular injury (0-5% - 0; 5-10% - 1, 11-25% - 2, 26-

45% - 3, 46-75% - 4, 76-100% - 5 for tubular atrophy, dilatation, protein casts, necrotic cells and brush border loss). Scale bars 100μm
(C) Representative images of pimonidazole (Pimon) stained kidney from control mice and mice at 10days after Gli1 cell ablation costatined for kidney injury molecule 1 (Kim1) indicating hypoxia in areas of tubular injury (Kim1). Scale bars 50μm
(D-G) Relative mRNA expression for the fibrotic readouts alpha smooth muscle actin (α-SMA) and collagenIαI, the inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6), the most prominent collagen of the basement membrane (collagen IVaI) and the profibrotic growth factors transforming growth factor beta 1 (TGFβ1). **$p<0.01$, ***$p<0.001$ by one way ANOVA with posthoc Bonferroni.