

Supplemental Materials for

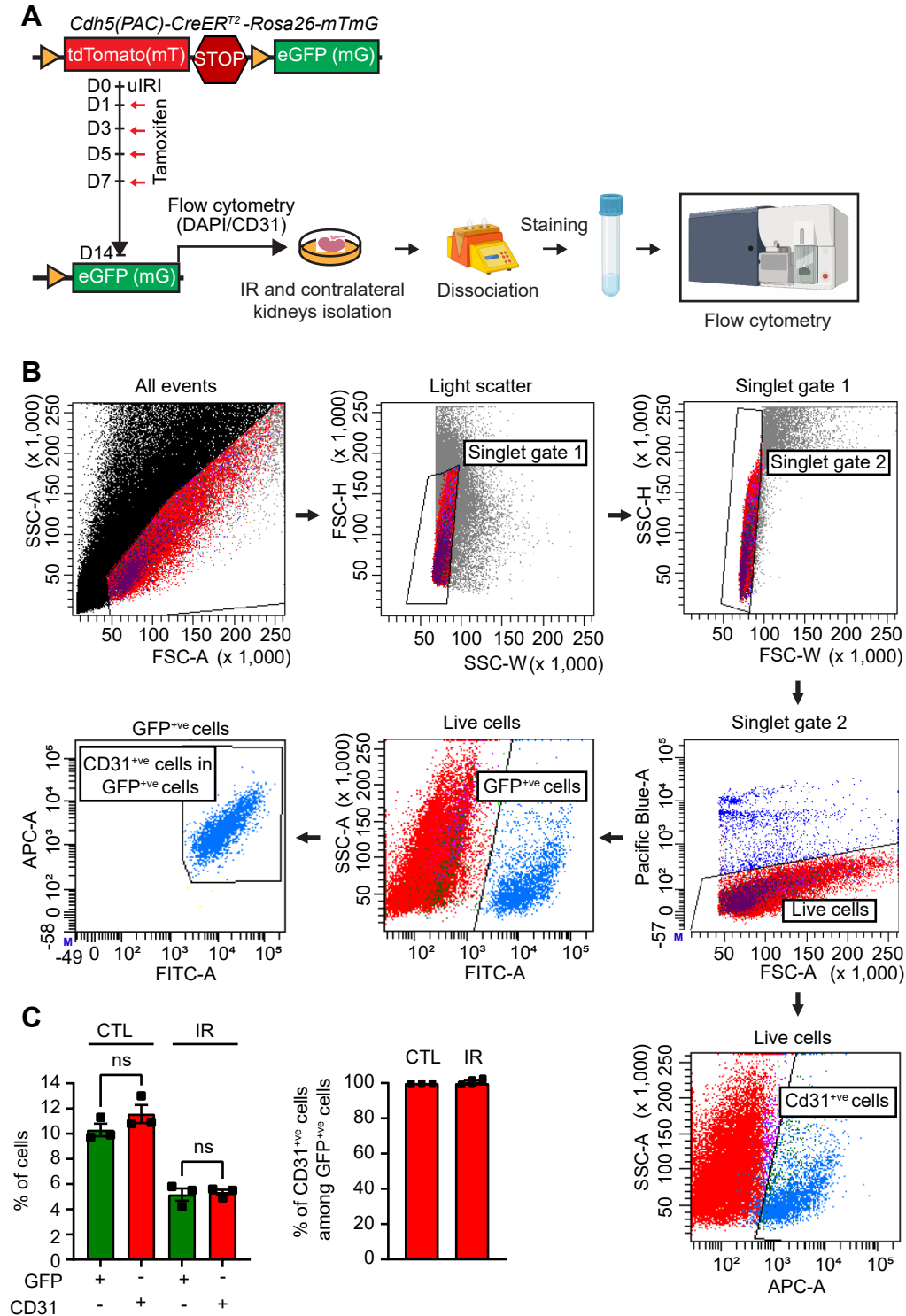
Post-ischemic inactivation of HIF prolyl hydroxylases in endothelium promotes maladaptive kidney repair by inducing glycolysis

Tiwari et al.

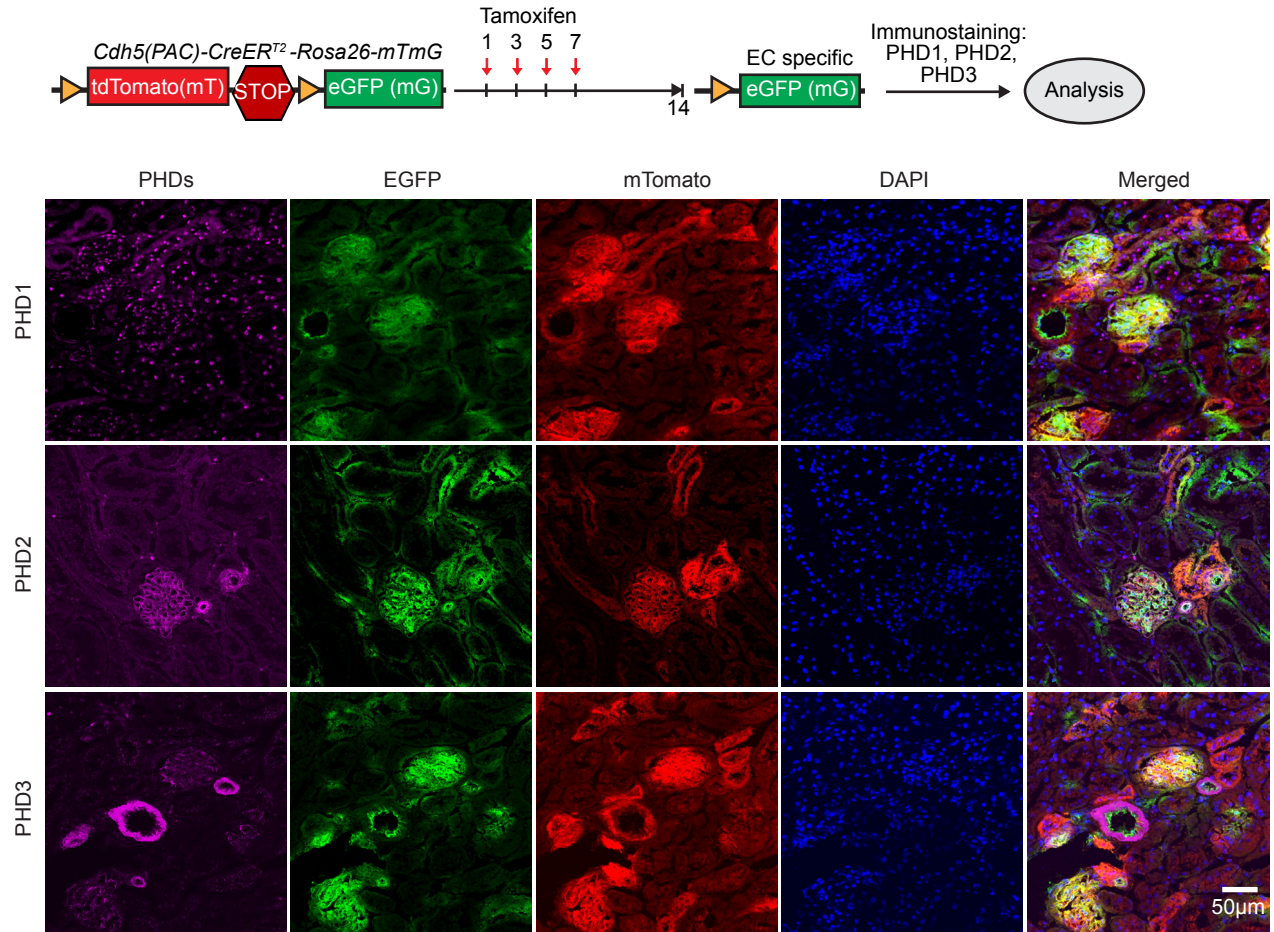
The PDF file includes:

Supplemental Figures 1 to 9

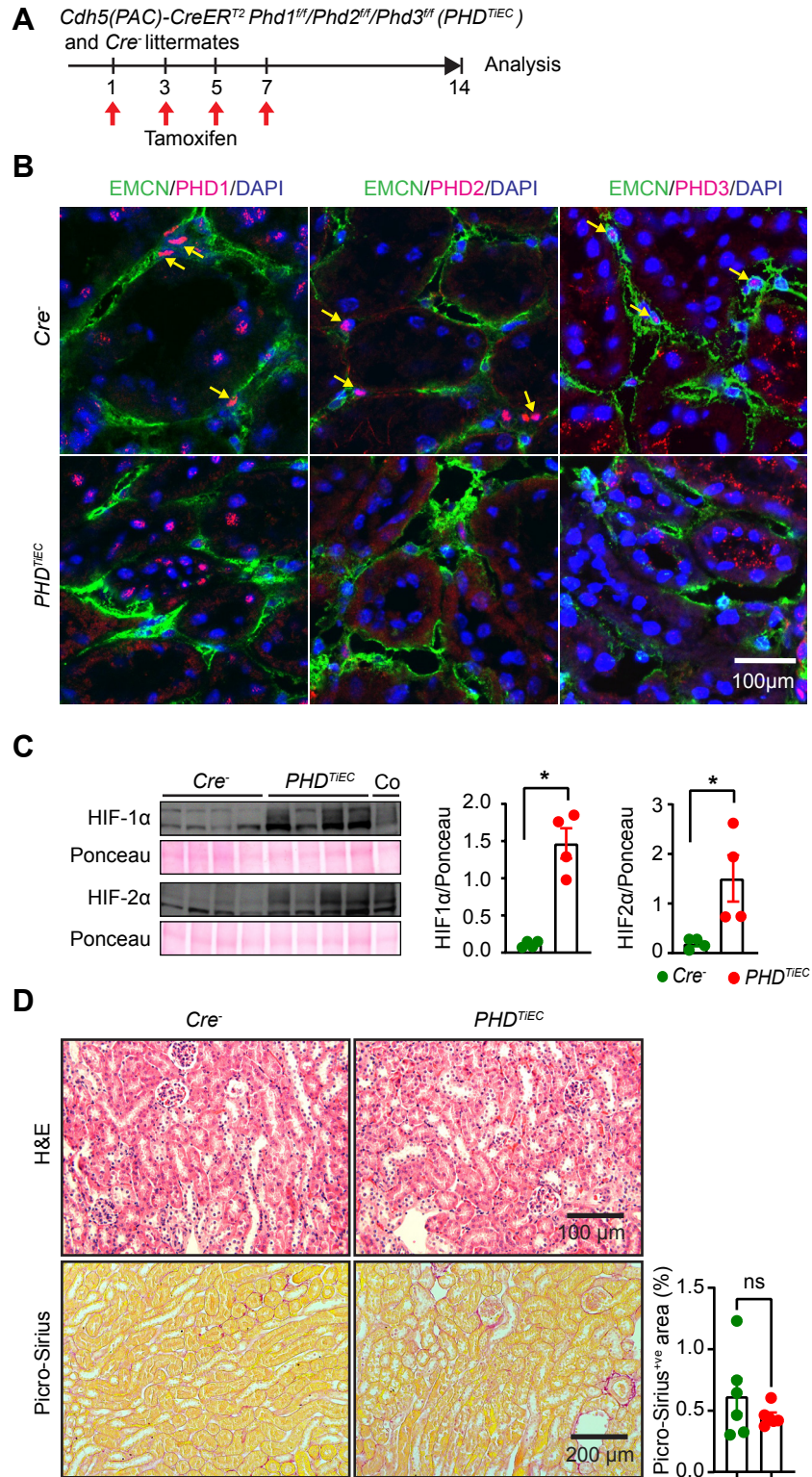
Supplemental Tables 1 to 3



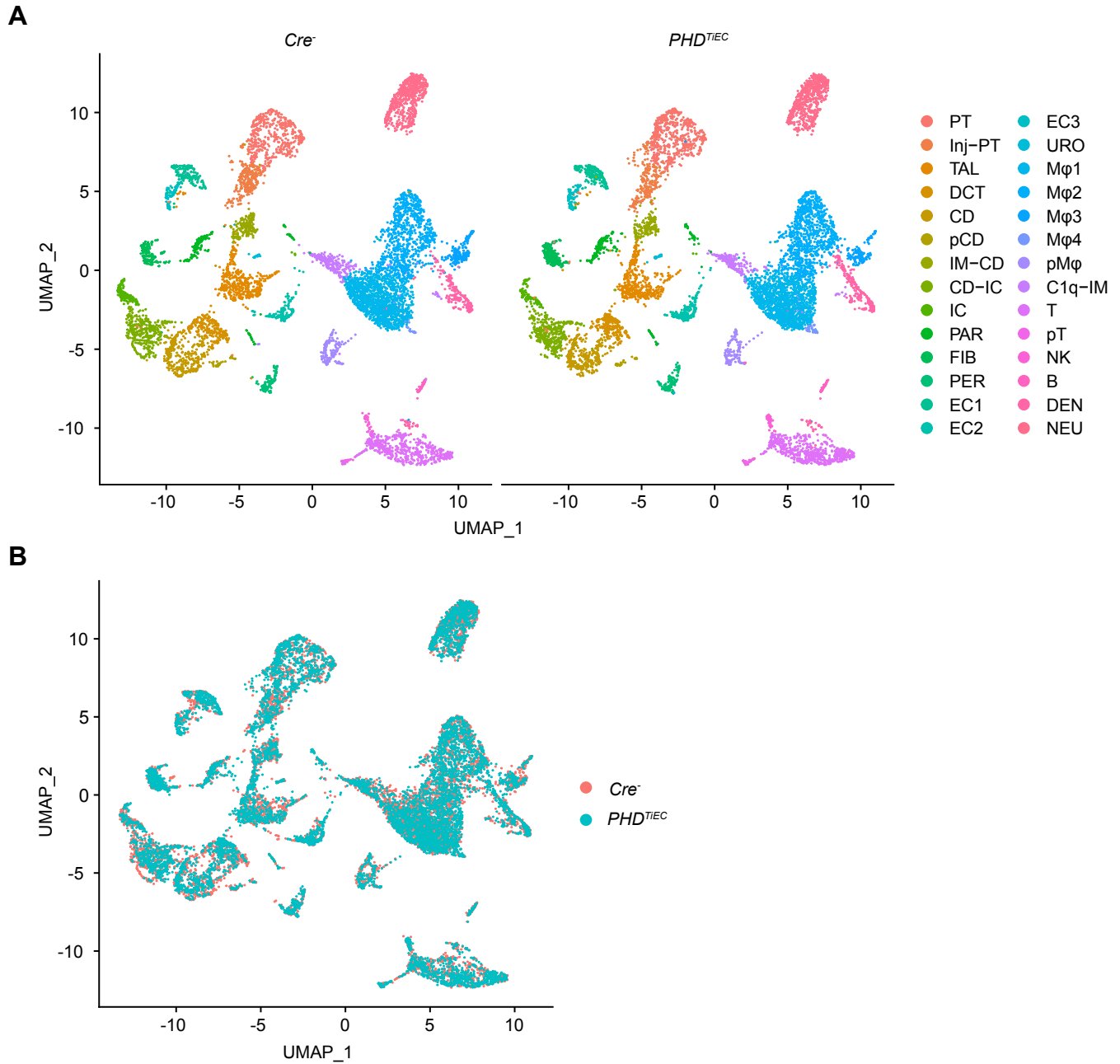
Supplemental Figure 1, associated with Figure 1. Efficiency of Cre⁺ recombination in *Cdh5(PAC)-CreER^{T2}* transgenic mice following kidney IRI. (A) By crossing *Cdh5(PAC)-CreER^{T2}* mice with *ROSA26-ACTB-tdTomato,-EGFP (mTmG)* reporter mice, we generated *Cdh5(PAC)-CreER^{T2}; Rosa26-mTmG* mice, in which successful Cre⁺ mediated excision is being indicated by GFP expression in endothelial cells (ECs). Mice were subjected to uIRI followed by tamoxifen administration starting at day 1 post uIRI to a total of 4 intraperitoneal (IP) injections given every other day. The degree of EC-specific recombination was assessed by FACs analysis in single-cell suspensions prepared by CTL and IR kidneys. (B) Flow cytometric gating strategy used to define recombined ECs. Staining for the endothelial marker CD31 was used to detect ECs, while GFP identified cells with tamoxifen induced expression of *Cdh5-CreER^{T2}*. (C) Shown are the percentages of GFP⁺ve and CD31⁺ve cells (left panel) as well as the percentage of CD31⁺ve cells among GFP⁺ve cells (right panel) in CTL and IR kidneys at day 14 post uIRI (n=3). For (C), statistics were determined by one-way ANOVA with Sidak correction for multiple comparisons. ns, not statistically significant. ECs, endothelial cells.



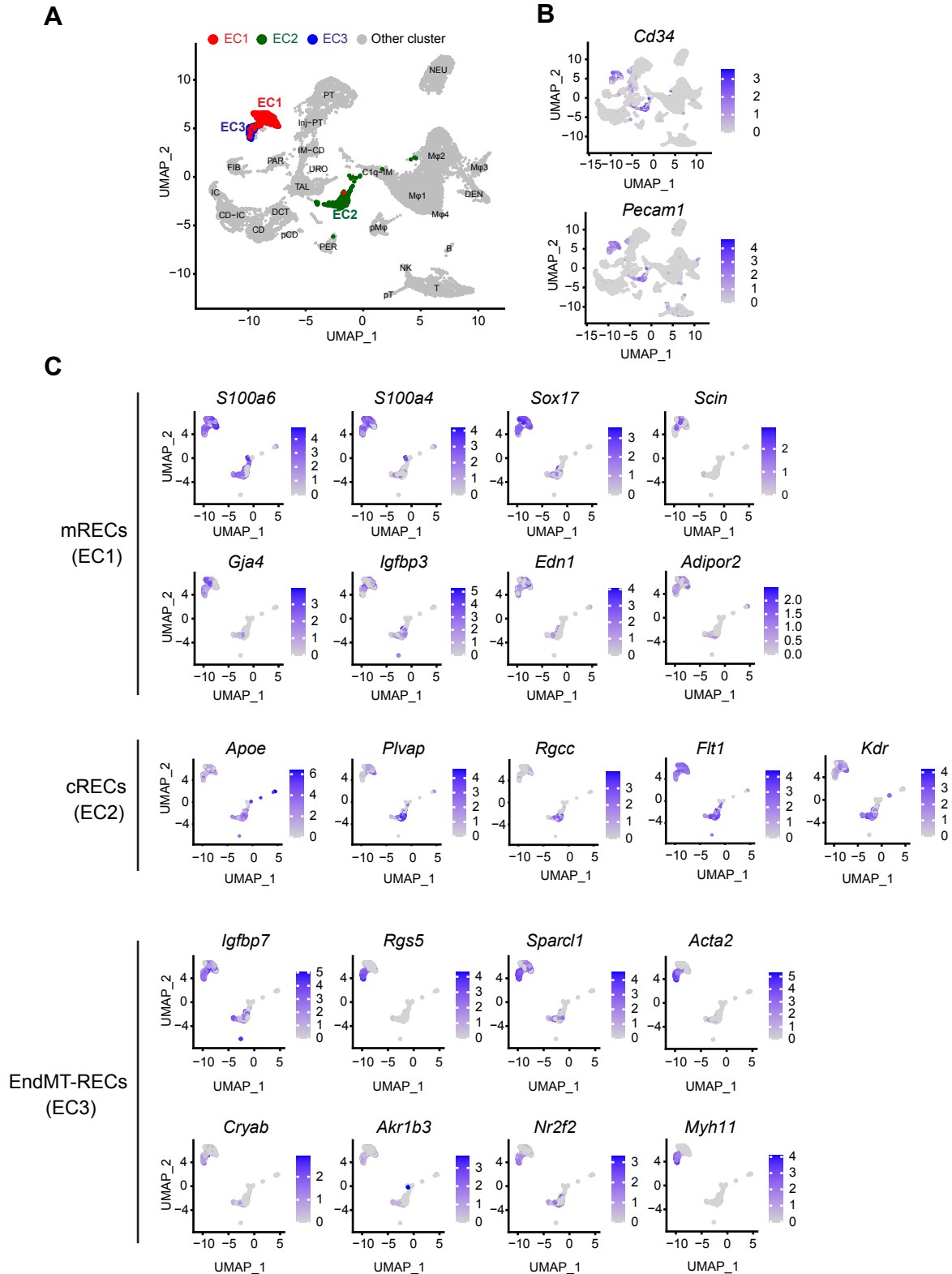
Supplemental Figure 2, associated with Figure 2. Murine kidney endothelium shows compartment-specific differences in the expression of PHD1, PHD2 and PHD3. Schematic view of experiment and representative images of immunofluorescence staining for PHD1, PHD2, PHD3 (magenta) and nuclear DAPI staining (blue) on kidney sections from *Cdh5(PAC)-CreER^{T2}-Rosa26mTomG* reporter mice after tamoxifen-induced recombination. Non recombined cells express membrane bound mTomato (red), whereas recombined cells express membrane-bound EGFP (green).



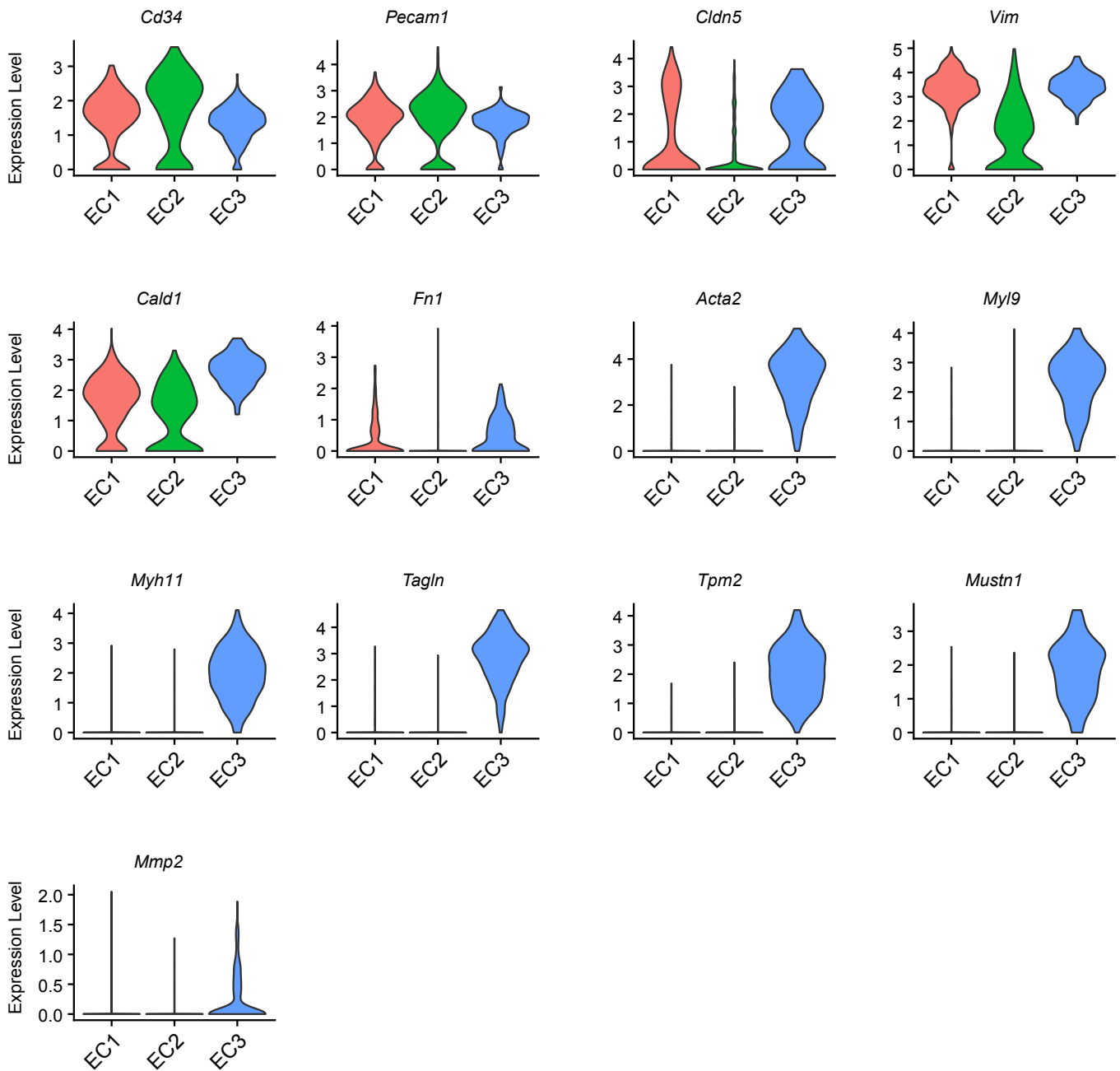
Supplemental Figure 3, associated with Figure 3. Simultaneous acute inactivation of endothelial PHD1, 2, and 3 stabilizes HIF without significantly affecting kidney morphology. (A) Schematic diagram depicting the tamoxifen administration regimen and the experimental strategy to obtain *PHD^{TIEC}* mice and *Cre⁻* control mice. (B) Immunofluorescence staining for PHD1-3 (red), EMCN (green) and nuclear DAPI (blue) using kidney sections from *PHD^{TIEC}* mice and *Cre⁻* controls collected 1 week after the last dose of tamoxifen. Scale bar, 100 μ m. (C) Immunoblot analysis of HIF-1 α and HIF-2 α in kidney nuclear extracts isolated from *PHD^{TIEC}* mice and *Cre⁻* littermates. Co, positive control. (D) Representative images of H&E and Picro-Sirius red stained kidney sections for the indicated genotypes. Right side graph shows semi-quantitative analysis of Picro-Sirius red^{+ve} area for *PHD^{TIEC}* mice and *Cre⁻* littermates 1 week after the last dose of tamoxifen (n=4-6). Scale bars indicate 100 μ m and 200 μ m for H&E and Picro-Sirius red images, respectively. Data are represented as mean \pm SEM. Statistics were determined by unpaired t-test with Welch's correction. *, $P < 0.05$; ns, not statistically significant. EMCN, endomucin.



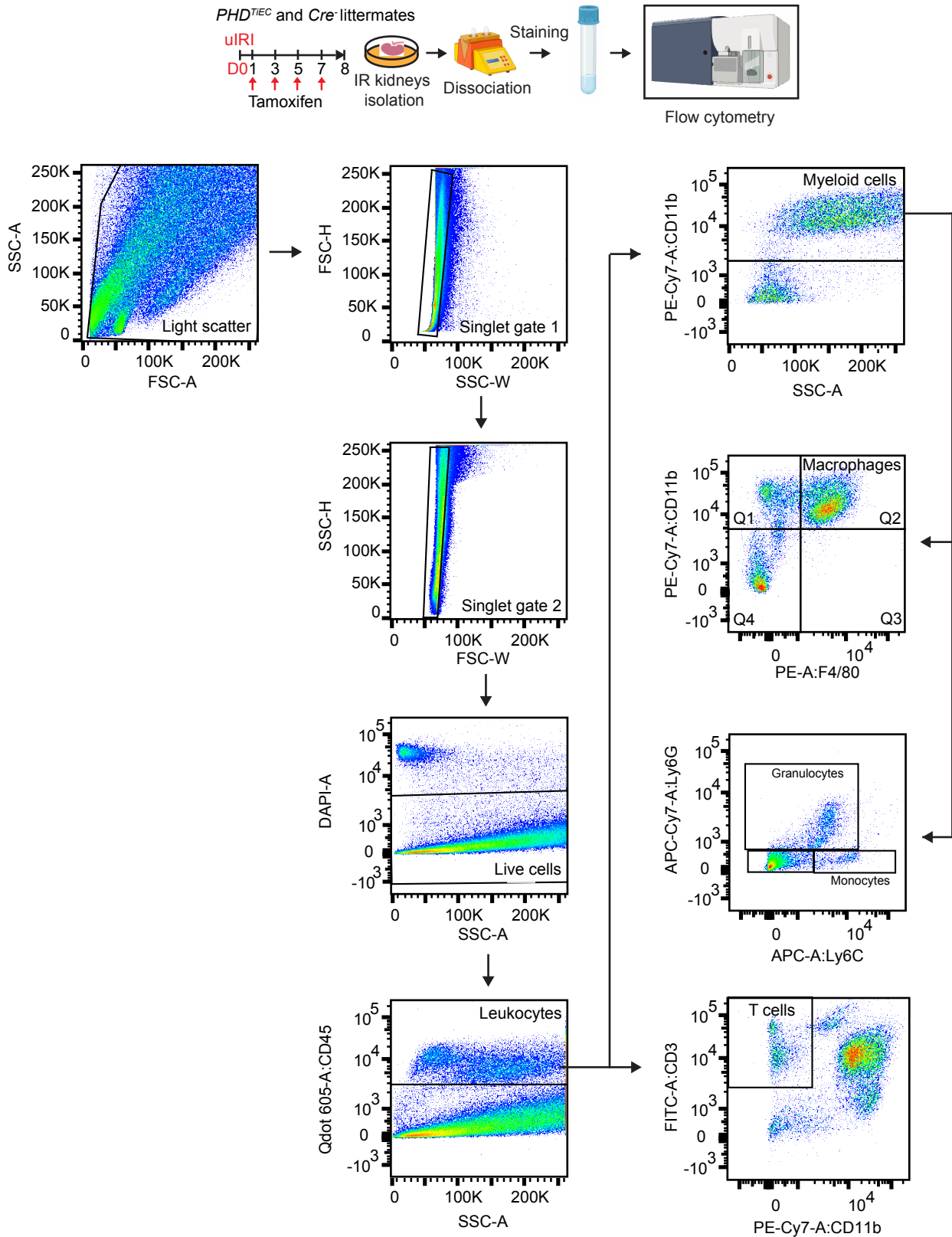
Supplemental Figure 4, associated with Figure 4. scRNA-seq analysis showing similar cell populations in day 14 post-ischemic kidneys of *PHD^{TIEC}* and *Cre* mice. (A) UMAP showing different cell clusters in day 14 post-ischemic kidneys from *PHD^{TIEC}* and *Cre* mice. (B) UMAP after overlaying samples of *Cre* and *PHD^{TIEC}* day 14 post-ischemic kidneys showing similar clustering.



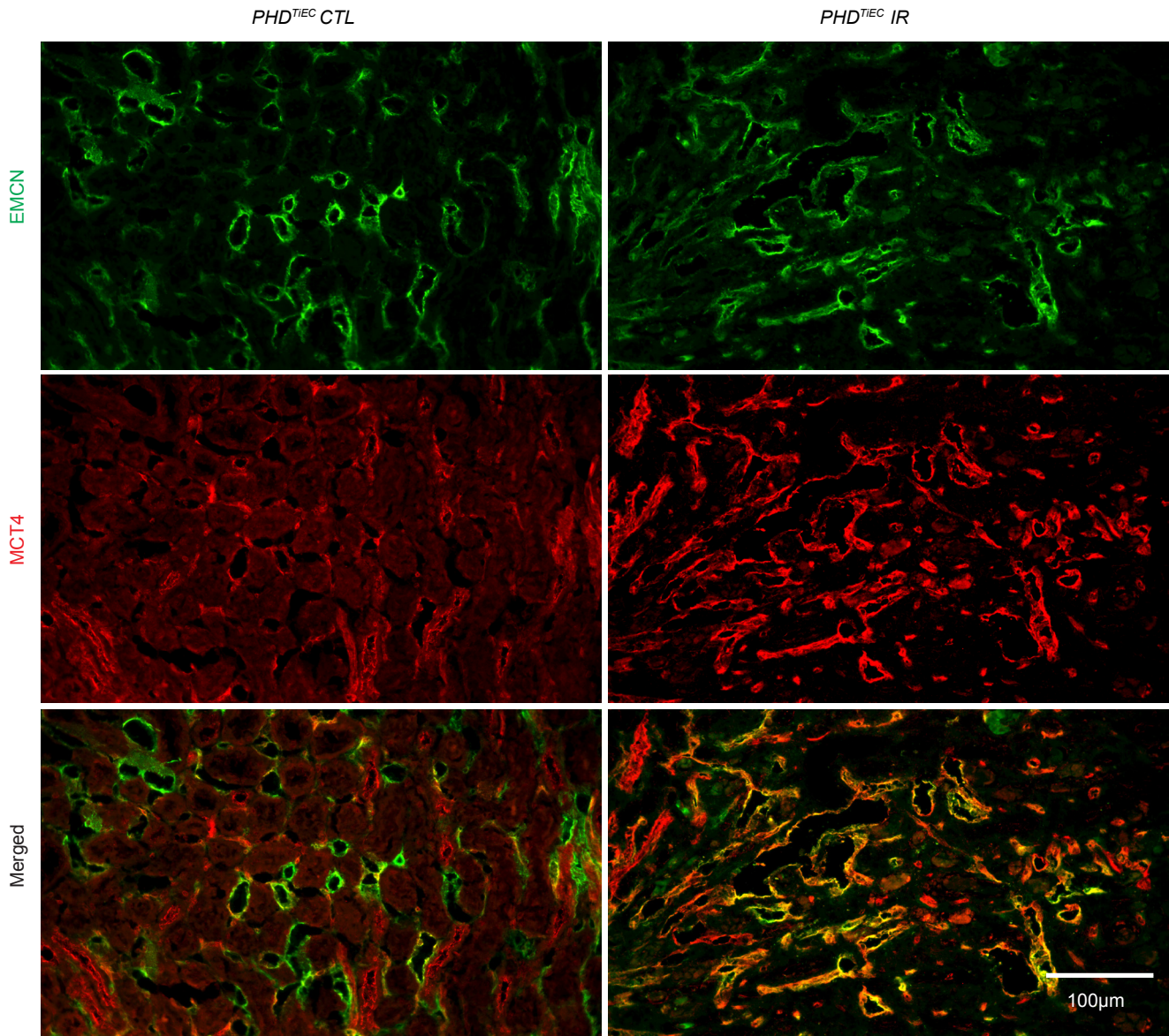
Supplemental Figure 5, associated with Figure 5. Marker genes used to identify different EC clusters. (A) UMAP plot with highlighted EC1 (red), EC2 (green) and EC3 (blue) cluster. **(B)** Feature plot showing expression of endothelial marker genes *Cd34* and *Pecam1* in EC1, EC2 and EC3 clusters. **(C)** Feature plot of EC marker genes used to identify mRECs (EC1), cRECs (EC2) and EndMT-RECs (EC3) clusters.



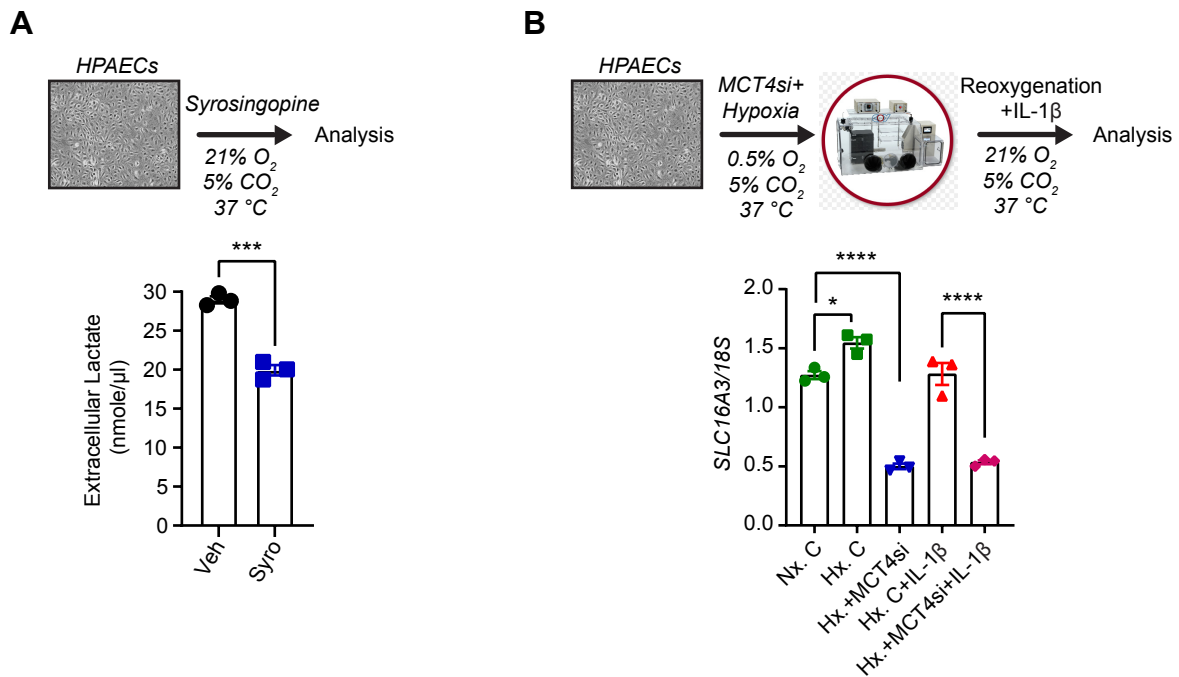
Supplemental Figure 6, associated with Figure 5. Violin plots showing the expression of endothelial and mesenchymal markers in EC1, EC2 and EC3 clusters. Violin plots show the expression of marker genes for ECs (*Cd34*, *Pecam1*, *Cldn5*) and mesenchymal cells (*Vim*, *Cald1*, *Fn1*, *Acta2*, *Myl9*, *Myh11*, *Tagln*, *Tpm2*, *Mustn1*, and *Mmp2*) in three EC clusters.



Supplemental Figure 7, associated with Figure 6. Strategy for the analysis of immune cells by flow cytometry. Schematic view of FACS experiment and gating strategy for the analysis of different immune cell populations in day 8 post-IRI kidneys from *PHD^{TIEC}* and *Cre* mice. CD45⁺ (leukocytes), CD45⁺ CD3⁺ (T cells), CD45⁺ CD11b⁺ (myeloid cells), CD45⁺ CD11b⁺ F4/80⁺ (macrophages), CD45⁺ CD11b⁺ Ly6C⁺ (monocytes), CD45⁺ CD11b⁺ Ly6G⁺ (granulocytes) cells were measured.



Supplemental Figure 8, associated with Figure 7. MCT4 expression in contralateral and day 14 post-ischemic kidney of *PHD^{TIEC}* mouse . Representative images of immunofluorescence staining for MCT4 (red) and EMCN (green) of CTL and IR kidneys on day 14 post-IRI from *PHD^{TIEC}* mice indicating increased expression of endothelial MCT4 following ischemic injury. Images were captured using Nikon a Ti2 Widefield fluorescence microscope. Scale bar,100 µm. CTL, contralateral kidney; IR, kidney subjected to uIRI.



Supplemental Figure 9, associated with Figure 8. Effect of syrosingopine on extracellular lactate levels and knockdown efficiency of MCT4 siRNA. (A) HPAECs were treated for 24 hours with syrosingopine or vehicle (DMSO) and extracellular lactate levels were measured. (B) Experimental scheme for HPAECs subjected to 0.5% O_2 for 18 hours in the presence of MCT4 siRNA followed by reoxygenation for 8 hours in the presence of IL-1 β (1 ng/ml). Graph showing *SLC16A3* (*MCT4*) mRNA levels in cells transfected with MCT4 siRNA compared to negative control siRNA transfected cells. All bars show mean \pm SEM. For (A), unpaired t-test with Welch's correction was used. For (B), statistics were determined using one-way ANOVA with Sidak correction for multiple comparisons. *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$. Veh, vehicle; Syro, syrosingopine; Nx, Normoxia; Hx, Hypoxia/Reoxygenation; C, negative control siRNA; MCT4si, MCT4 siRNA.

Supplemental Table 1. List of GEO numbers and details of samples which were used to assess the level of *Eglns* in human kidney ECs.

Samples	GEO	Source name	Organism	Subject status	Tissue
1	GSM4191941	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
2	GSM4191942	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
3	GSM4191943	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
4	GSM4191944	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
5	GSM4191945	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
6	GSM4191946	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
7	GSM4191947	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
8	GSM4191948	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
9	GSM4191949	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
10	GSM4191950	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
11	GSM4191951	Tumor-nephrectory	Homo sapiens	Normal/healthy	Adult kidney
12	GSM4191952	Living donor	Homo sapiens	Normal/healthy	Adult kidney
13	GSM4191953	Living donor	Homo sapiens	Normal/healthy	Adult kidney

14	GSM4191954	Living donor	Homo sapiens	Normal/healthy	Adult kidney
15	GSM4191955	Tumor-nephrectomy	Homo sapiens	Normal/healthy	Adult kidney
16	GSM4191956	Tumor-nephrectomy	Homo sapiens	Normal/healthy	Adult kidney
17	GSM4191957	Tumor-nephrectomy	Homo sapiens	Normal/healthy	Adult kidney
18	GSM4191958	Tumor-nephrectomy	Homo sapiens	Normal/healthy	Adult kidney
19	GSM4191959	Tumor-nephrectomy	Homo sapiens	Normal/healthy	Adult kidney
20	GSM4191960	Surveillance biopsy	Homo sapiens	Normal/healthy	Adult kidney
21	GSM4191961	Surveillance biopsy	Homo sapiens	Normal/healthy	Adult kidney
22	GSM4191962	Surveillance biopsy	Homo sapiens	Normal/healthy	Adult kidney
23	GSM4191963	Surveillance biopsy	Homo sapiens	Normal/healthy	Adult kidney
24	GSM4191964	Surveillance biopsy	Homo sapiens	Normal/healthy	Adult kidney

Supplemental Table 2. List of used marker genes to identify different cell cluster of scRNA seq data of *Cre*⁻ and *PHD*^{TEC} mice and their references.

Marker genes	Cell cluster	Reference
<i>Pck1, Lrp2, Slc5a12, Slc34a1</i>	Proximal tubule (PT)	(1, 2)
<i>Havcr1</i>	Injured PT (Inj-PT)	(1)
<i>Umod, Slc12a1</i>	Thick ascending limb (TAL)	(1)
<i>Slc12a3</i>	Distal convoluted tubule (DCT)	(3)
<i>Fxyd4, Aqp2</i>	Collecting duct (CD)	(4)
<i>Slc14a2</i>	Inner medullary collecting duct (IM-CD)	(5)
<i>Atp6v1g3, Scl26a4</i>	Intercalated cells (IC)	(1, 6)
<i>Ncam1</i>	Parietal cells (PAR)	(7)
<i>Colla2, Col1a2, Dcn</i>	Fibroblasts (FIB)	(2, 8, 9)
<i>Myh11, Acta2,</i>	Pericytes (PER)/smooth muscle cells	(9)
<i>Cd34, Pecam1, Igfbp3</i>	Endothelial cells (EC)	(1, 10)
<i>Upk1b</i>	Urothelial cells (URO)	(11)
<i>Mki67, Top2a</i>	Proliferating cells (p)	(1) (12)
<i>Ptpnc</i>	Immune cells	(6)
<i>Itgam</i>	Myeloid cells	(12)
<i>Adgre1, Cx3cr1</i>	Macrophages (Mφ)	(1, 12)
<i>C1qa, C1qb, C1qc</i>	C1qa, C1qb and C1qc expressing immune cell (C1q-IM); potentially resident macrophage/monocyte	(13, 14)
<i>Trbc2, Skap1</i>	T cells	(14, 15)
<i>GZMA, GZMB</i>	Natural killer cells (NK cells)	(16)
<i>Ms4a1, Pax5, Bcl11a</i>	B cells	(15, 17-19)
<i>Flt3</i>	Dendritic cells (DC)	(20, 21)
<i>S100a8, S100a9</i>	Neutrophils (NEU)	(22)

Supplemental Table 3. Primer sequences. Shown are sequences of primer sets used for the expression analysis of the indicated mouse and human genes by RT-PCR.

Gene	Forward Primer	Reverse Primer
Mouse		
<i>Loxl2</i>	5'-GATCTTCAGCCCCGATGGA-3'	5'-CAAGGGTTGCTCTGGCTTGT-3'
<i>Tgfb1</i>	5'-TGGCGAGCCTTAGTTTGGGA-3'	5'-TCGACATGGAGCTGGTGAAA-3'
<i>Acta2</i>	5'-CCTGACGCTGAAGTATCCGATAG-3'	5'-TTTTCCATGTCGTCGCCAGTTG-3'
<i>Kim1</i>	5'-AAACCAGAGATTCCCACACG-3'	5'-GTCGTGGGTCTTCCTGTAGC-3'
Human		
<i>SLC16A3</i>	5'-GGGTGGGAACCGTGTCATT-3'	5'-CTTGCGGCTTGGCTTCA-3'
<i>VCAM1</i>	5'-GCTTCAGGAGCTGAATACCC-3'	5'-AAGGATCACGACCATCTTCC-3'
<i>ICAM1</i>	5'-CCACAGTCACCTATGGCAAC-3'	5'-AGTGTCTCCTGGCTCTGGTT-3'

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