Supplemental Methods:

Single-Cell RNA Sequencing (scRNA-seq) Analysis of PCRC Cell Composition
P1 PCRC were seeded at 0.5 million cells per well in 6-well plates in DMEM (Sigma, cat#D6429) supplemented with 10% FBS (Sigma, cat#12306C). 48 hrs later, PCRC were harvested with 0.05% trypsin/EDTA (Gibco cat#25300-054), viability assessed using trypan blue exclusion and submitted to the Yale Center for Genomic Analysis (YCGA) for low throughput scRNAseq analysis (target recovery of 1000 cells, 30 million reads per sample) using Chromium next GEM single cell 3' LT Reagents Kit v3.1 (10xgenomics, cat# PN-1000325) following the manufacture's protocols.

scRNA-seq Data Analysis of PCRC Cell Composition Identification
Downstream data analysis was performed using the Seurat v4.0 R package. In the quality control (QC) analysis, poor quality cells with <200 unique genes or < 5,000 UMI counts (likely cell fragments) and >8,000 unique genes or > 80,000 UMI counts (potentially cell duplets) were excluded. Cells were excluded if their mitochondrial gene percentages were over 20%. Low-complexity cells with <0.8 log10 unique genes per UMI counts were also excluded. The QC filters resulted in a total of 570 cells with a median of 5,470 reads (a mean of 5,492 reads) per cell at a sequencing depth of 45,969 unique genes across 570 cells. The gene expression for each cell was then normalized by SCTransform. Confounding sources of variation including mitochondrial gene content were removed for downstream clustering analysis.
Principle component analysis (PCA) was performed on the scaled data. The top 15 principal components were chosen for cell clustering and UMAP because minimal changes were observed beyond 15 principal components. The UMAP was used to visualize the single cells in two-dimensional space. Each cluster was screened for marker genes by differential expression analysis based on the non-parameteric Wilcoxon rank sum test for all clusters with genes expressed in at least 25% of cells. Based on the kidney cell and immune cell lineage-specific marker expression, three cell clusters were identified (Supplemental Figure 1).

Cell isolation for Single Cell RNA Sequencing of Whole Kidneys:
Eight to ten week old wild-type male mice were subjected to 27 minutes of IRI or control. Three days after IRI, mice were euthanized and perfused with chilled 3× PBS (10 mL) via the left ventricle. The injured (IRI) and control (CTRL) kidneys were harvested, minced into approximately 1 mm^3 cubes, and digested using Liberase™ (100 µg/mL) and DNase I (10 µg/mL) (Roche Diagnostics) for 25 min at 37 °C.[26] Reaction was deactivated by adding chilled DMEM with 10% FBS. The solution was then passed through a 40-µm cell strainer. After centrifugation at 300 *x g* for 10 min at 4°C, the cell pellet was resuspended in chilled DMEM with 10% FBS and passed through another 40-µm cell strainer. Cell number and viability were analyzed using trypan blue staining (Invitrogen). This method generated single cell suspensions with greater than 75% viability.

scRNA-seq Library Generation and Sequencing of Whole Kidneys:
scRNA-seq library and sequencing were performed at the Yale Center for Genome Analysis (YCGA). Briefly, single cells, reagents and a single Gel Bead containing barcoded oligonucleotides were encapsulated into nanoliter-sized Gel Bead in Emulsion (GEM) using the GemCodeTM Technology 10X Genomics. Lysis and barcoded reverse transcription of polyadenylated mRNA from single cells were performed inside each GEM. The scRNA-seq libraries were finished in single bulk reaction. The cDNA libraries were constructed using the 10x ChromiumTM Single cell 3' Library Kit. Qualitative analysis was performed using the Agilent Bioanalyzer High Sensitivity DNA assay. The final libraries from IRI and CTRL kidneys were sequenced on an Illumina HiSeq 4000 sequencer. Cell Ranger version 3.0.0 was used to

process Chromium single cell 3' RNA-seq output and align the Read to the mouse reference transcriptome (mm9), all of which were provided by the YCGA.

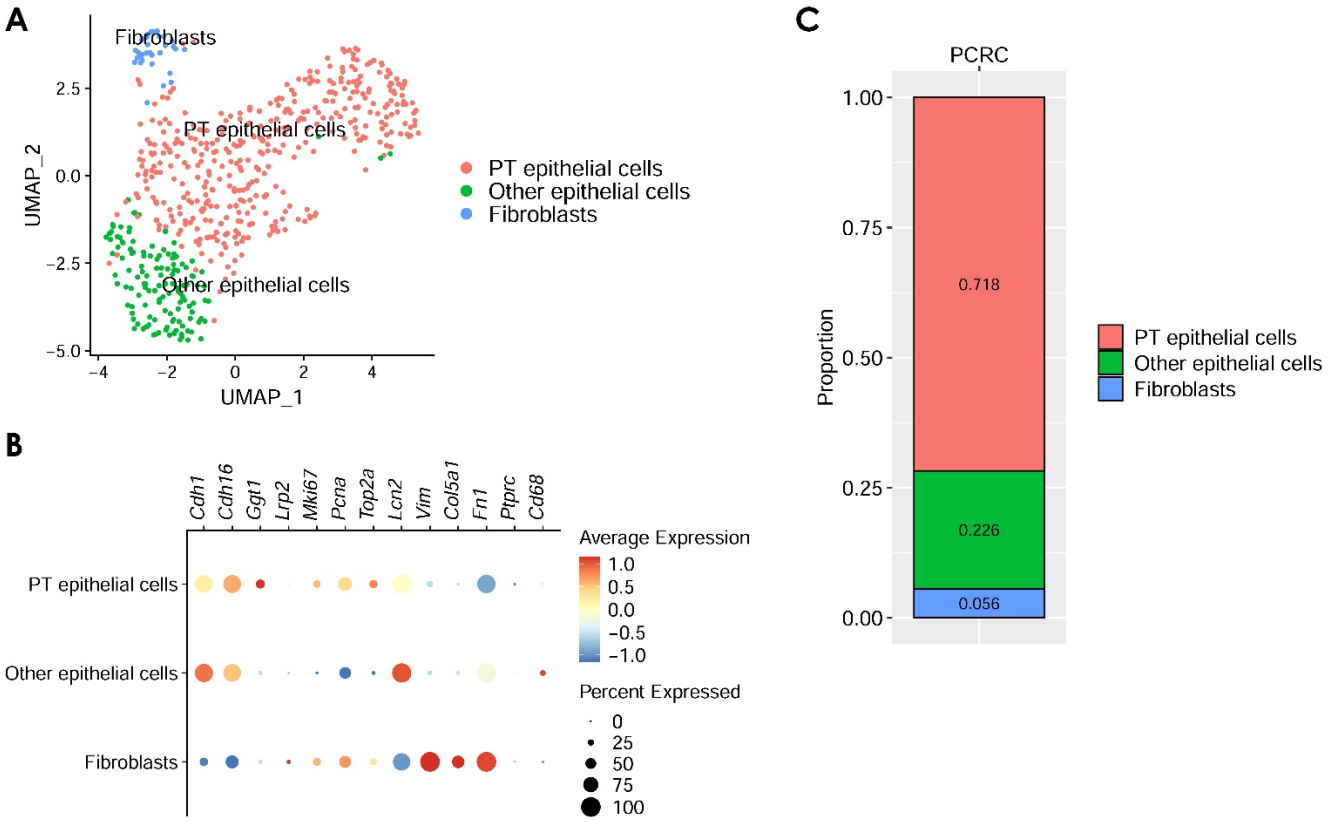
scRNA-seq Data Analysis of Whole Kidneys

Downstream data analysis was performed using the Seurat v3.2 R package. The Seurat integration strategy was performed to identify common cell types and enable comparative analyses between IRI and CTRL kidneys. [27,28] Briefly, both IRI and CTRL datasets were integrated using their identified anchors and a single integrated analysis was performed on all cells. In the quality control (QC) analysis, poor quality cells with <200 (likely cell fragment) or >3,500 (potentially cell duplet) unique expressed genes were excluded. Cells were excluded if their mitochondrial gene percentages were over 50%. Only genes expressed in 5 or more cells were used for further analysis. The QC filters resulted in a total of 11,759 cells (5,170 cells from IRI kidney and 6,589 cells from control kidney) with a median of 725 reads (a mean of 750 reads) per cell at a sequencing depth of 30,897 unique genes across 11,759 cells. The gene expression for each cell was then normalized by SCTransform. [29] Confounding sources of variation including mitochondrial gene content were removed for downstream clustering analysis.

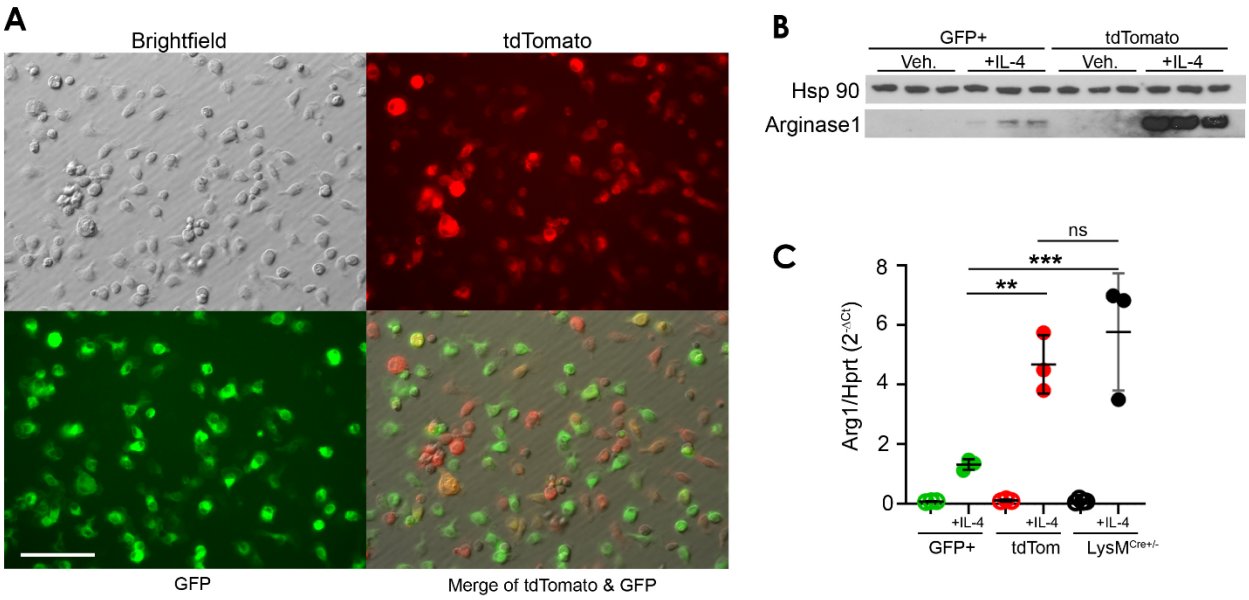
Principle component analysis (PCA) was performed on the scaled data. The top 30 principal components were chosen for cell clustering and uniform manifold approximation and projection (UMAP) because minimal changes were observed beyond 30 principal components. The UMAP was used to visualize the single cells in two-dimensional space. Each cluster was screened for marker genes by differential expression analysis based on the non-parameteric Wilcoxon rank sum test for all clusters with genes expressed in at least 25% of cells either inside or outside of a cluster. Based on the kidney cell and immune cell lineage-specific marker expression, eighteen cell clusters were identified (Supplemental Figure 4). The IRI and CTRL kidneys could be split by their anchors from the integrated analyses. The average expression of both IRI and CTRL cells was visualized in dot plots and volcano plots. The outliers in the volcano plots were used to identify the genes that were differentially expressed between IRI and CTRL kidney in each cluster. The gene set enrichment analysis was performed for the IRI-derived Ccr2+ macrophages as compared to the IRI-derived Cx3cr1+ and resident macrophages using clusterProfiler R package based on Gene Ontology (GO) term enrichment. [30] The potential ligand-receptor interaction analyses were performed using the NicheNet R package[31,32] by linking potential ligands differentially expressed by IRI-derived Ccr2+, Cx3cr1+, or resident macrophages to their target genes that were differentially expressed by IRI-derived proliferating PT and their corresponding receptors. The ligand-receptor pairings for each cell type were visualized by a chord diagram using the R package circlize. [33] The single-cell RNA-seq data has been deposited in the Gene Expression Omnibus database with the accession code GSE188966.

Isolation and Use of Urinary Casts

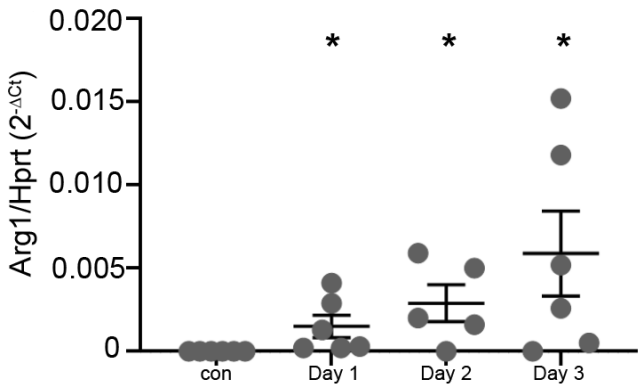
Mouse urine was manually collected from the urethral meatus into an Eppendorf tube every 3 hours starting 24 hours after IRI/CL-NX. The urine from multiple mice was pooled and cast debris collected by centrifugation at 150 *x g* for 5 minutes at 4°C and the pellet washed x1 in sterile PBS. The presence of casts was confirmed microscopically. Cast pellets were stored at -80° C until useage. Cast quantity was approximated using Bio Rad TC20TM Automated Cell Counter. Casts were reconsituted in RPMI 1640 media (Thermo Fisher Scientific) to achieve a 5:1 cast to PCRC ratio for transwell experiments. In each transwell insert (of a 12-well plate), 200μL volume of urinary casts were placed directly on cells, and the 12-well plate was centrifuged at 450 *x g* for 10 minutes at 4°C to bring the casts in contact with the apical cell surface.



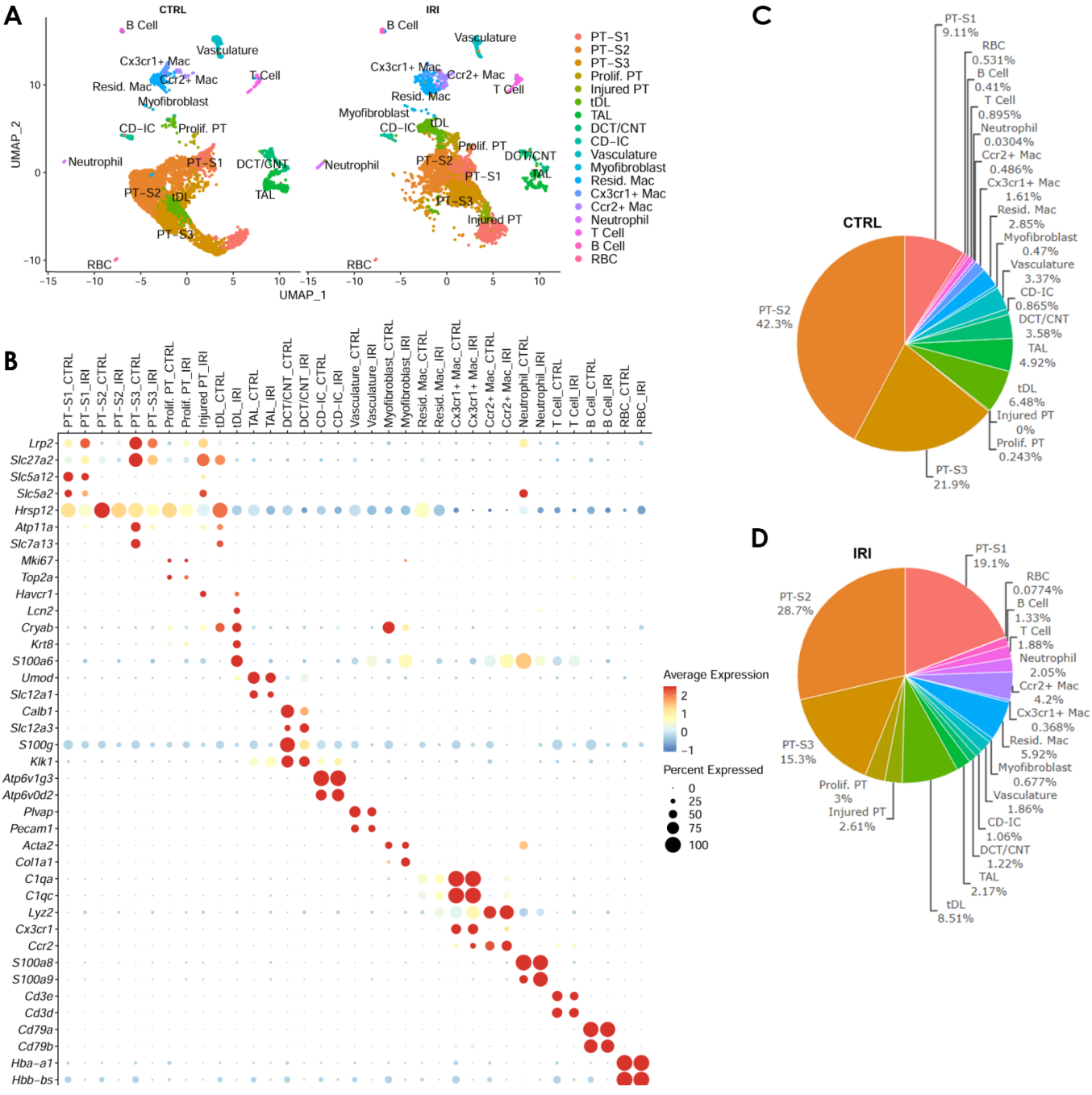
Supplemental Figure 1. **scRNA-seq analysis of PCRC composition.** (A) UMAP projection of 570 PCRC cells. (B) Cell clusters were identified by kidney cell and immune cell lineage-specific marker expression. (C) Proportion of individual cell populations is shown in a bar plot. PT, proximal tubule.



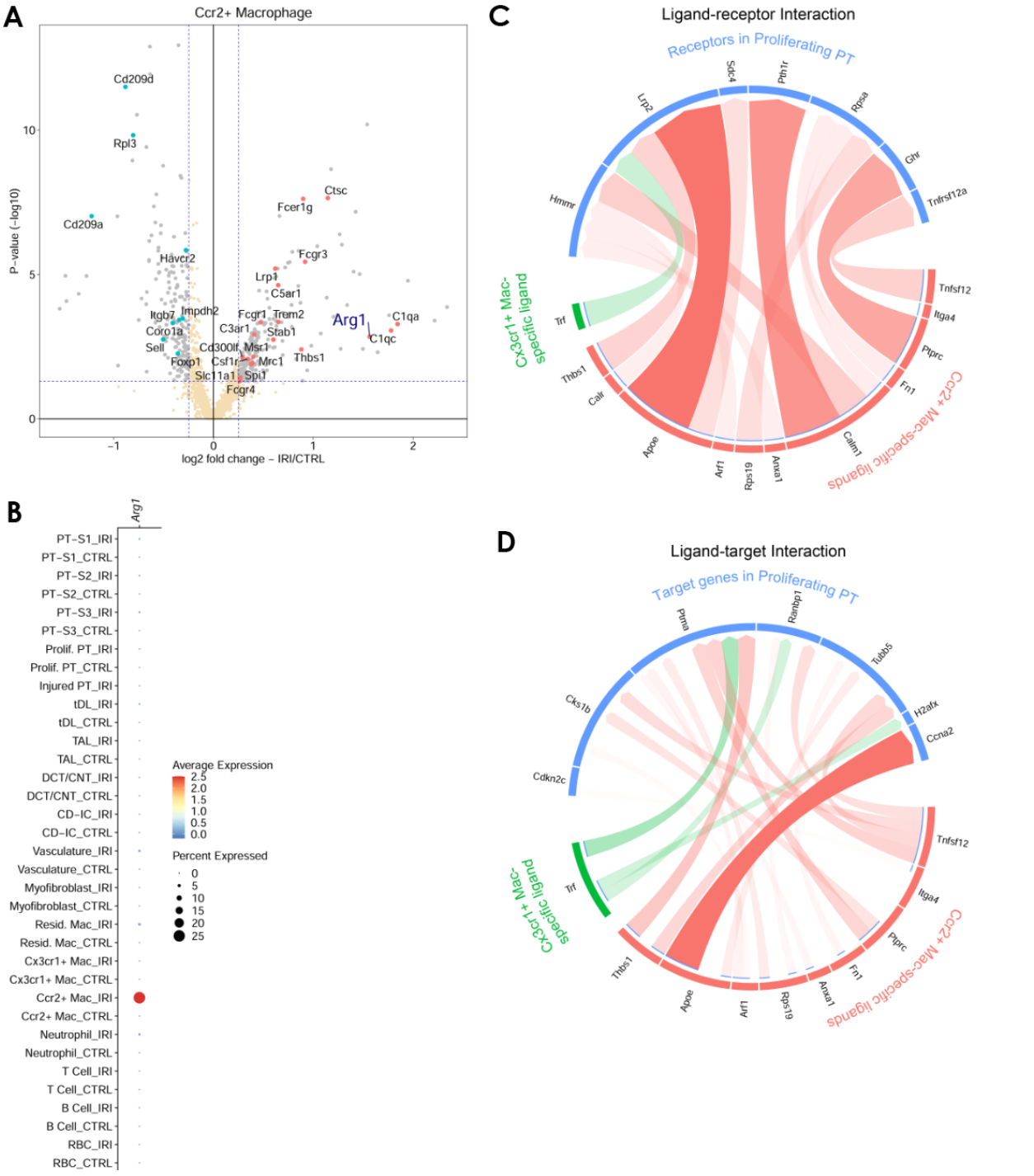
Supplemental Figure 2. **Arginase-1 in GFP+ macrophages from *Arg1$^{fl/fl}$;LysM$^{Cre/+}$;Rosa26^{mTmG}* mice. A)** Bone marrow macrophages cultured from Arg1$^{fl/fl}$;LysM$^{Cre/+}$;Rosa26^{mTmG} mice reveal a mix of GFP+ and tdTomato+ cells. **B)** Arg1$^{fl/fl}$;LysM$^{Cre/+}$;Rosa26^{mTmG} BMM were FACS sorted for GFP+ and tdTomato+ cells and cultured for 24 hours ± IL4 to induce arginase-1, revealing decreased arginase-1 expression by the Cre expressing GFP+ BMM. Scale bars 20μm. **C)** GFP+ and tdTomato+ BMM cells as in (B), as well as Arg1$^{+/+}$;LysM$^{Cre/+}$ BMM cells were cultured for 24 hours ± IL4 and RNA harvested. qPCR for *Arg1* reveals equivalent induction in tdTomato+ and control Arg1$^{+/+}$;LysM$^{Cre/+}$ BMM cells, with decreased *Arg1* expression by the Cre expressing GFP+ BMM. Each dot represents mRNA from a separate mouse (n=3 at each time point). Scale bars 20μm. (P *** < 0.001, ** P < 0.01, ANOVA).



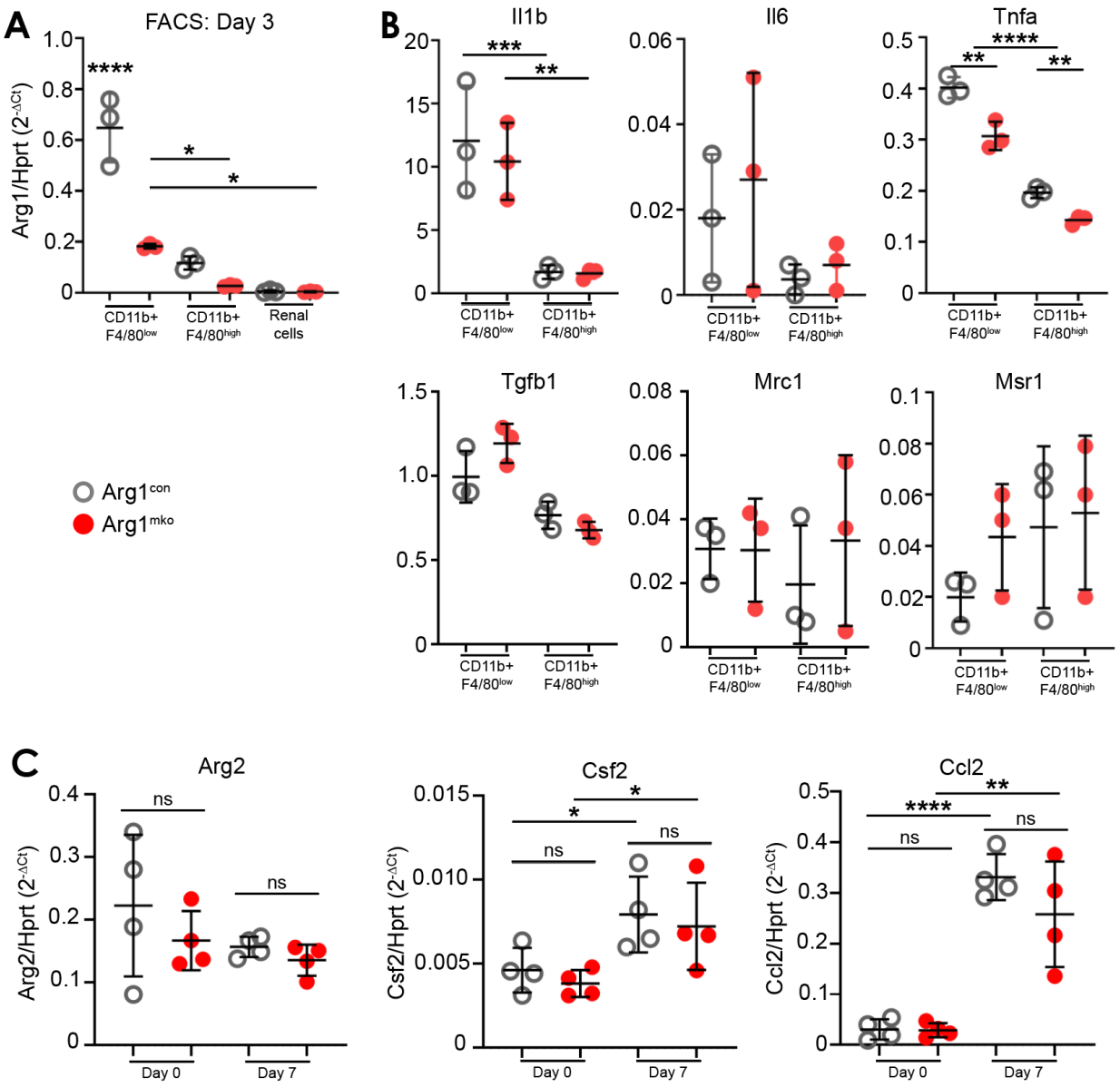
Supplemental Figure 3. **Time-course of Arginase-1 expression after kidney injury.** Total RNA was collected from microdissected outer medulla of wild-type mice at the indicated times after IRI with contralateral nephrectomy, reverse transcribed and qPCR for *Arg1* mRNA performed. Each dot represents mRNA from a separate mouse (n=5 or 6 at each time point). (* $P < 0.05$ vs time 0, Student t-tests (two-tailed))



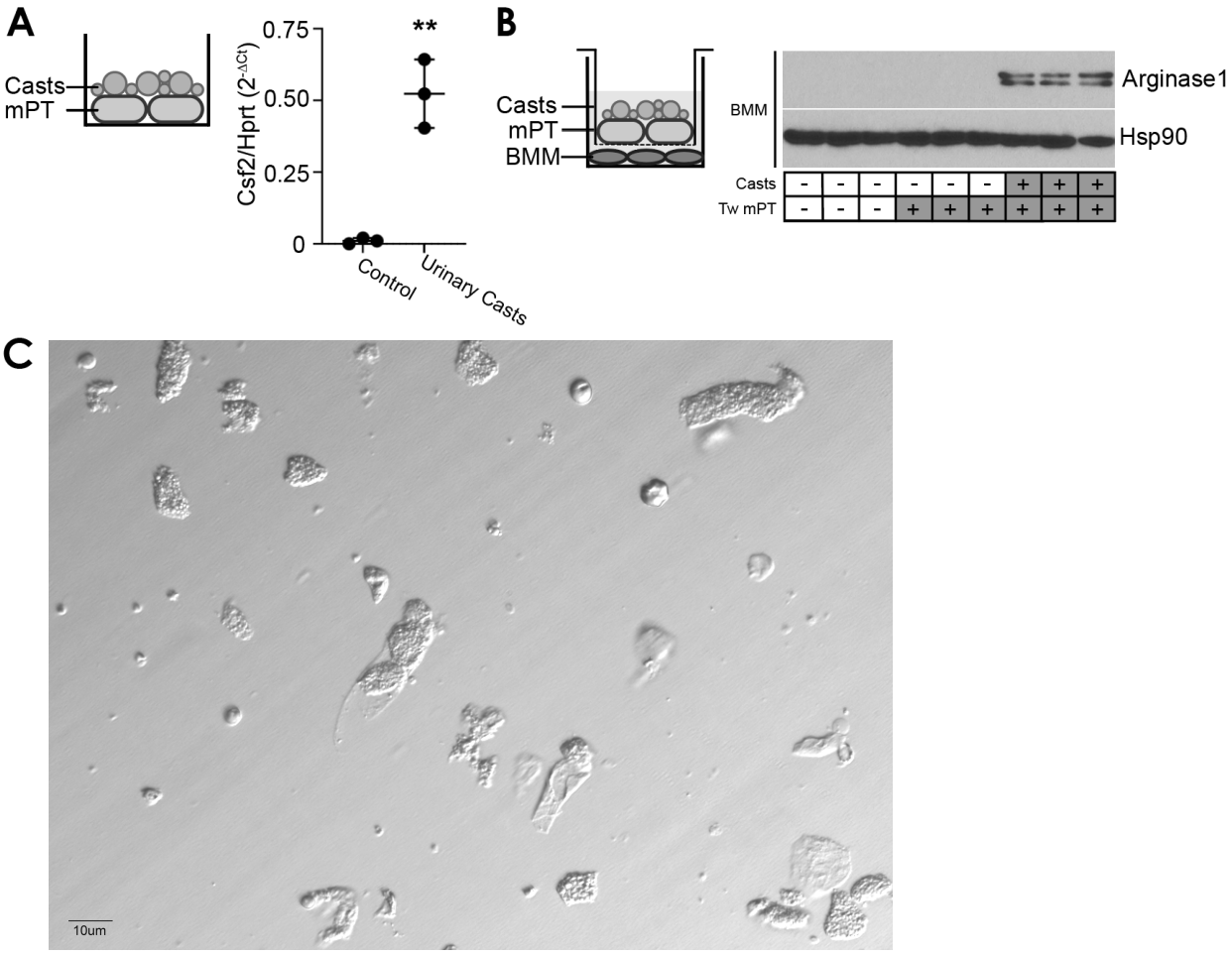
Supplemental Figure 4. **Integrated scRNA-seq analysis of differential cell populations between wild-type IRI and control kidneys. A,B**) UMAP projection of 11,759 cells (6,589 cells from normal control (CTRL) kidney and 5,170 cells from IRI kidney). Cell clusters were identified using the composite data from all cells and compared in both kidneys by kidney cell and immune cell lineage-specific marker expression as shown in (**B**). **C,D**) The percentage of individual cell populations is provided for CTRL (**C**) and IRI (**D**) kidneys. PT, proximal tubule; Prolif. PT, proliferating PT; tDL, thin descending limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD-IC, collecting duct-intercalated cell; Resid. mac, resident macrophage; Mac, macrophage; RBC, red blood cell.



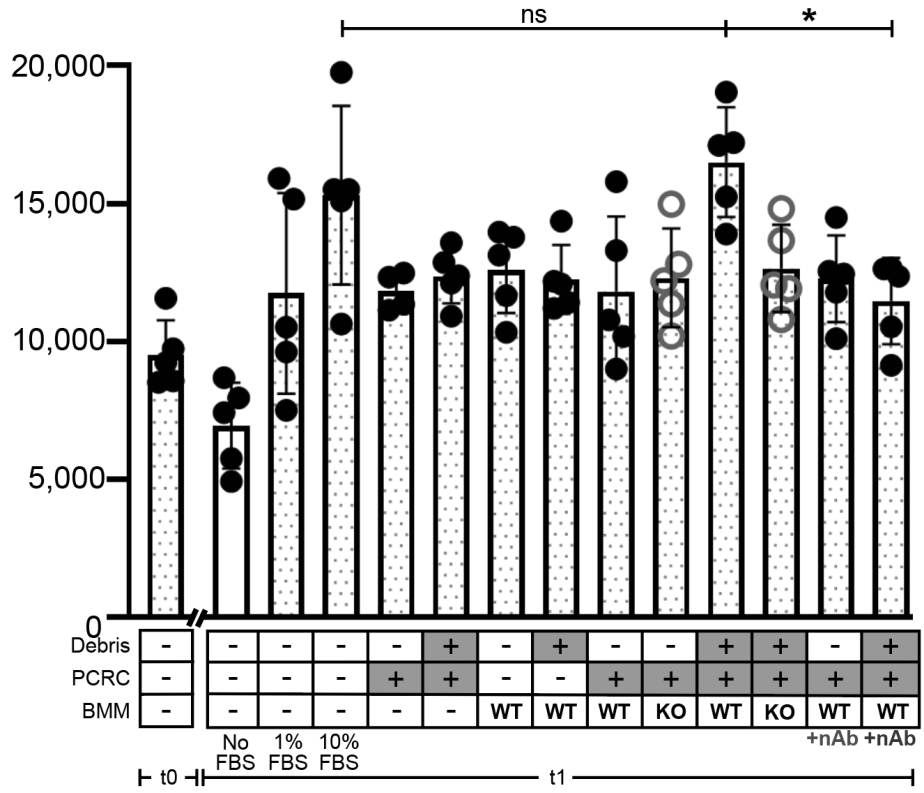
Supplemental Figure 5. ***Ccr2*+ macrophages express *Arg1* and are projected to promote proximal tubular epithelial cell proliferation. A**) Volcano plot demonstrating differential gene expression between IRI and control (CTRL) kidney derived Ccr2+ macrophages. Note the increased expression of all three GM-Csf induced alternative activation genes (*Arg1*, *Mrc1* and *Msr1*) in Ccr2+ macrophages. **B**) A dot plot of the distribution and relative expression of *Arg1* reveals that expression is almost exclusively in *Ccr2*+ trafficking macrophages. **C,D**) Cell interactions between IRI-derived macrophages and proliferating proximal tubular (PT) epithelial cells. The potential ligands specifically expressed by IRI-derived Ccr2+ macrophages (red) and Cx3cr1+ macrophages (green) were linked to their corresponding receptors (D) based on the target genes specifically expressed in the proliferating PT (E) and visualized by a chord diagram.



Supplemental Figure 6. Cytokine Expression in Renal Macrophages and Whole Kidney. A,B) Renal cells from both *Arg1*^{con} and *Arg1*^{mko} mice were FACS sorted as resident (Cd11b+, F4/80^{high}) or trafficking macrophages (Cd11b+, F4/80^{low}) or renal structural cells (*CD45-*, *Cd11b-*) on day 3 post IRI. **A**) *Arg1* mRNA expression level (relative to *Hprt1*) was significantly higher in trafficking macrophages from *Arg1*^{con} mice compared to resident macrophages of *Arg1*^{con} mice, with a significant decrease seen in both macrophage populations in the *Arg1*^{mko} mice. Renal cells, in contrast, did not express *Arg1*. (**** P < 0.0001, * P < 0.05, ANOVA). **B**) There was no change in the mRNA expression levels of pro-inflammatory cytokines *Il-1b* and *Il6* or pro-reparative markers *Mrc*, *Msr1* and *Tgfb* in trafficking or resident macrophages from *Arg1*^{con} and *Arg1*^{mko} mice. The pro-inflammatory cytokine Tnfa is higher in *Arg1*^{con} macrophages (both in trafficking and resident) despite the equivalent number of TUNEL positive cells and better outcomes in this group. (** P < 0.01, * P < 0.05, Student t-tests (two-tailed) **C**) Whole kidney samples from *Arg1*^{con} and *Arg1*^{mko} mice were analyzed on day 0 and day 7 post IRI/Nx. There was no change in Arg2 mRNA expression between *Arg1*^{con} and *Arg1*^{mko} mice either on day 0 or day 7 after IRI. The expression of both Csf2 and Ccl2 increased to an equivalent degree on day 7 post IRI/Nx (compared to day 0) in both *Arg1*^{con} and *Arg1*^{mko} mice. (**** P < 0.0001, ** P < 0.01 * P < 0.05, Student t-tests (two-tailed)).



Supplemental Figure 7. Cast-treated Mouse Proximal Tubule Cells (mPT) Express GM-CSF and Induce Arginase-1 Expression in cultured Bone-Marrow Macrophages (BMM). A) Immortalized mouse proximal tubule cells (mPT) were cultured ± urinary casts. Exposure to casts significantly upregulated *Csf2* mRNA expression compared to no cast exposure (control). Student t-tests (two-tailed) ** $P < 0.01$. **B**) mPT cells ± exposure to apical casts were cocultured on a transwell (Tw) insert with BMM in the bottom well. Western Blot demonstrates increased levels of arginase-1 in BMM cocultured with mPT exposed to casts. Each lane represents a separate transwell. **C**) Contrast brightfield image of murine urinary casts (scale bar 10 um).



Supplemental Figure 8. **Renal epithelial cell proliferation after exposure to various conditioned media**. Separate PCRC (10,000 cells/well seeded at t0) were exposed to serum-free media (no FBS), media with 1% FBS (equal to that in the conditioned medium), media with 10% FBS, or the various conditioned media (CM) generated from the transwell experiment described in Figure 4C. PCRC proliferation was greatest in media with 10% FBS and conditioned media generated from PCRC in 1% FBS exposed to debris and WT BMM. This effect disappears with the addition of GM-CSF neutralizing antibody (nAb) to the PCRC+debris+BMM coculture during generation of the conditioned medium * P = < 0.05, ANOVA.

Supplemental Table 1. Antibodies used to flow-sort cells.

Target	Provider	Clone	Cat#	Final Conc.
CD16/CD32	Biolegend	93	101302	200ng/ml
CD45-PE	Biolegend	30-F11	103106	200ng/ml
F4/80-FITC	Biolegend	BM8	123108	200ng/ml
CD11b-Cy7	Biolegend	M1/70	101226	200ng/ml

Supplemental Table 2. Primers used to perform rtPCR.
The following primers were used:
Arg1 Fw: GTGTACATTGGCTTGCGAGA, Rev: CTGAAAGGAGCCCTGTCTTG;
Cish Fw: TCGGGAATCTGGGTGGTACT, Rev: GGGTGCTGTCTCGAACTAGG;
Ccl2 Fw: AGGTCCCTGTCATGCTTCTG, Rev: TCTGGACCCATTCCTTCTTG
Csf2 Fw: TGGTCTACAGCCTCTCAGCA, Rev: CCGTAGACCCTGCTCGAATA;
Csf2ra Fw: ACGGAGGTCACAAGGTCAAG, Rev: TGAGGGTCTCAGGGTTCACT;
Hprt Fw: CAGTACAGCCCCAAAATGGT, Rev: CAAGGGCATATCCAACAACA;
Igf1 Fw:TGGATGCTCTTCAGTTCGTG, Rev: GTCTTGGGCATGTCAGTGTG;
Il1a Fw: TGTGAAATGCCACCTTTTGA, Rev: TGTCCTCATCCTGGAAGGTC;
Il1b Fw: TGTGAAATGCCACCTTTTGA, Rev: TGTCCTCATCCTGGAAGGTC;
Il-6 Fw: TCCAGTTGCCTTCTTGGGAC, Rev: GTGTAATTAAGCCTCCGACTTG
iNos Fw: CCAAGCCCTCACCTACTTCC, Rev: CTCTGAGGGCTGACACAAGG;
Mrc1 Fw: CAAGGAAGGTTGGCATTTGT, Rev: CCTTTCAGTCCTTTGCAAGC;
Msr1 Fw: AAAGGGAGAGAAGGGGAGTG, Rev: GCATGACACAGGAACCAATG;
Tgfb1 Fw: TGAGTGGCTGTCTTTTGACG, Rev: GGTTCATGTCATGGATGGTG
Tnfa Fw: GAACTGGCAGAAGAGGCACT, Rev: AGGGTCTGGGCCATAGAACT