Transplantation-Induced Ischemia/Reperfusion Injury modulates Antigen Presentation by Donor Renal CD11c^{+}F4/80^{+} Macrophages through IL-1R8 Regulation.

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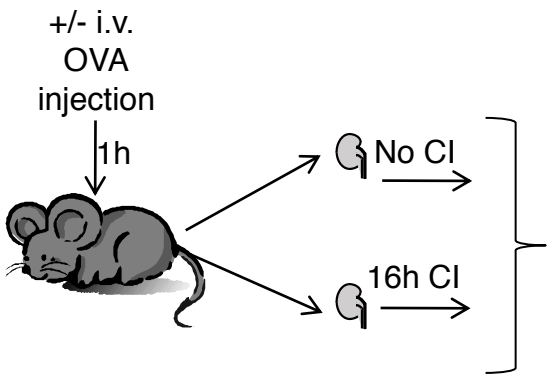
Supplemental Material:
Supplementary Figures 1-9

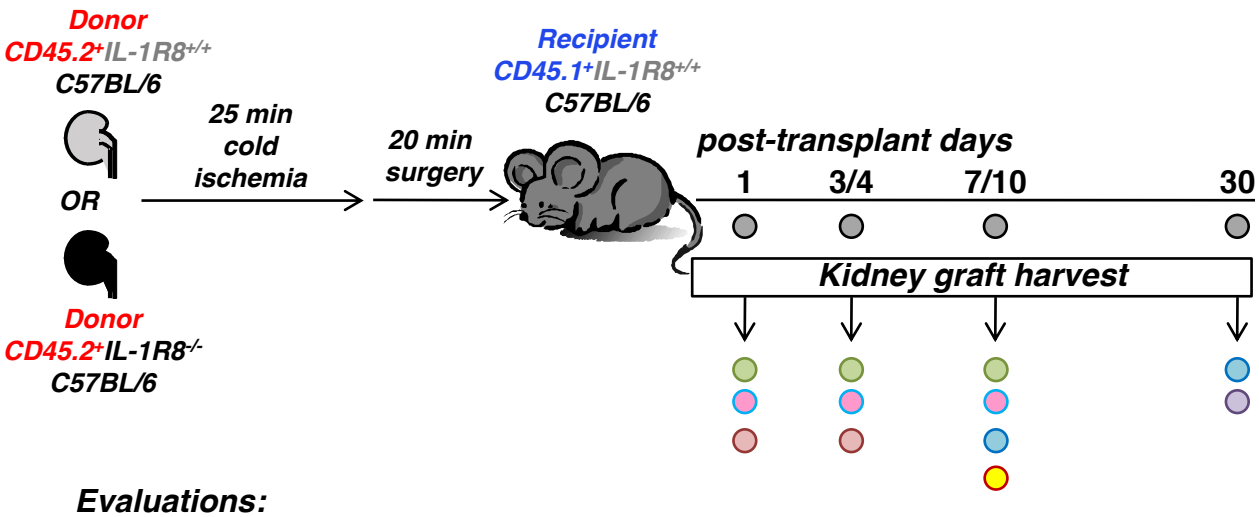
A **Experimental Design 1: *in vitro* cold ischemia (CI) experiments**



On renal CD11c$^+$F4/80$^+$ macrophages, evaluation of:

- Cell number
- Phenotype (expression of MHCII, TLR4, EpoR, CX3CR1, IRF4, IRF8, IL-1R8)
- APC function: ability to generate either alloreactive or OVA-specific T-cell clones (+/- LPS stimulation).

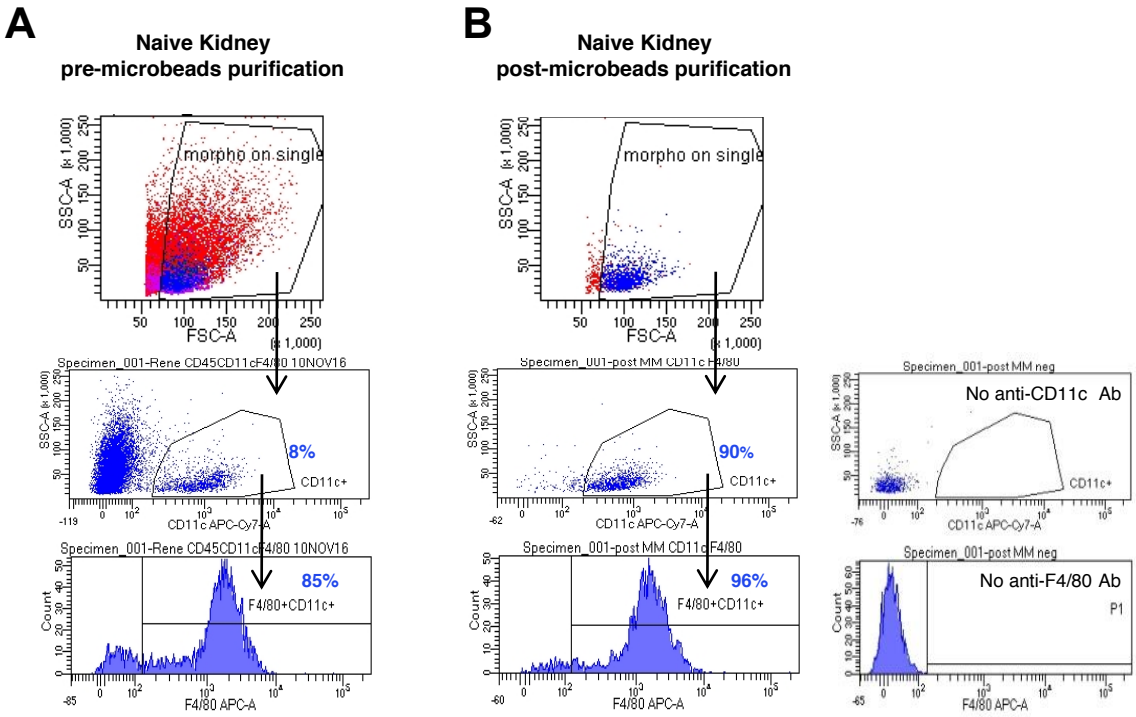
B **Experimental Design 2: *post-transplant* ischemia/reperfusion injury experiments**



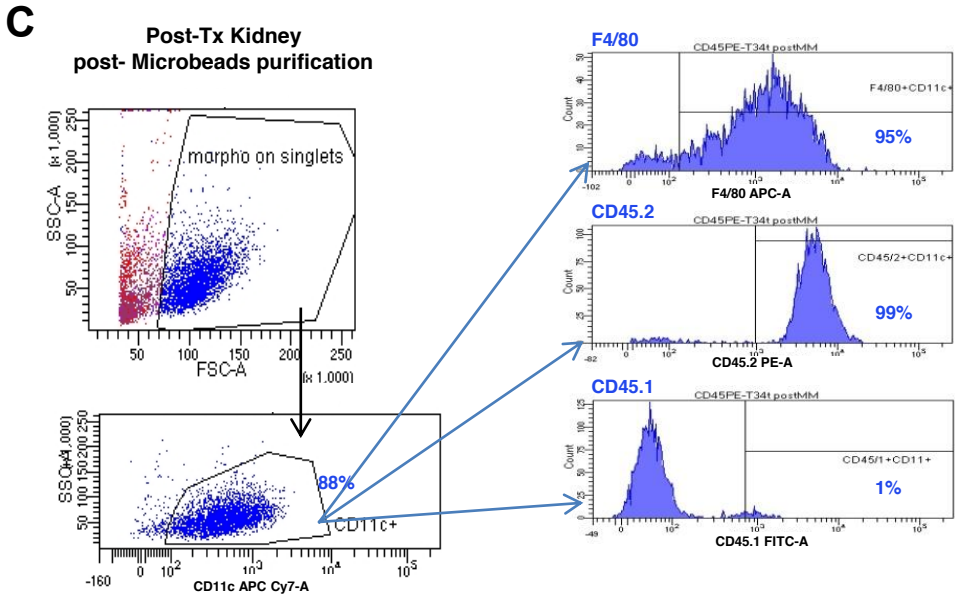
Evaluations:

- BUN
- Cell count of donor CD45.2$^+$ and recipient CD45.1$^+$ cell subsets
- Phenotype of donor CD45.2$^+$CD11c$^+$F4/80$^+$ macrophages
- Antigen presentation function of donor CD45.2$^+$CD11c$^+$F4/80$^+$ macrophages (ability to generate either alloreactive or OVA-specific T-cell clones +/- LPS stimulation)
- Tissue tubular damage
- Tissue oxidative stress and M1/M2 markers expression
- Collagen III deposition, collagen-1 mRNA expression, interstitial fibrosis grade, number of infiltrating B cells, tissue IgG deposition.

Supplementary Figure 1

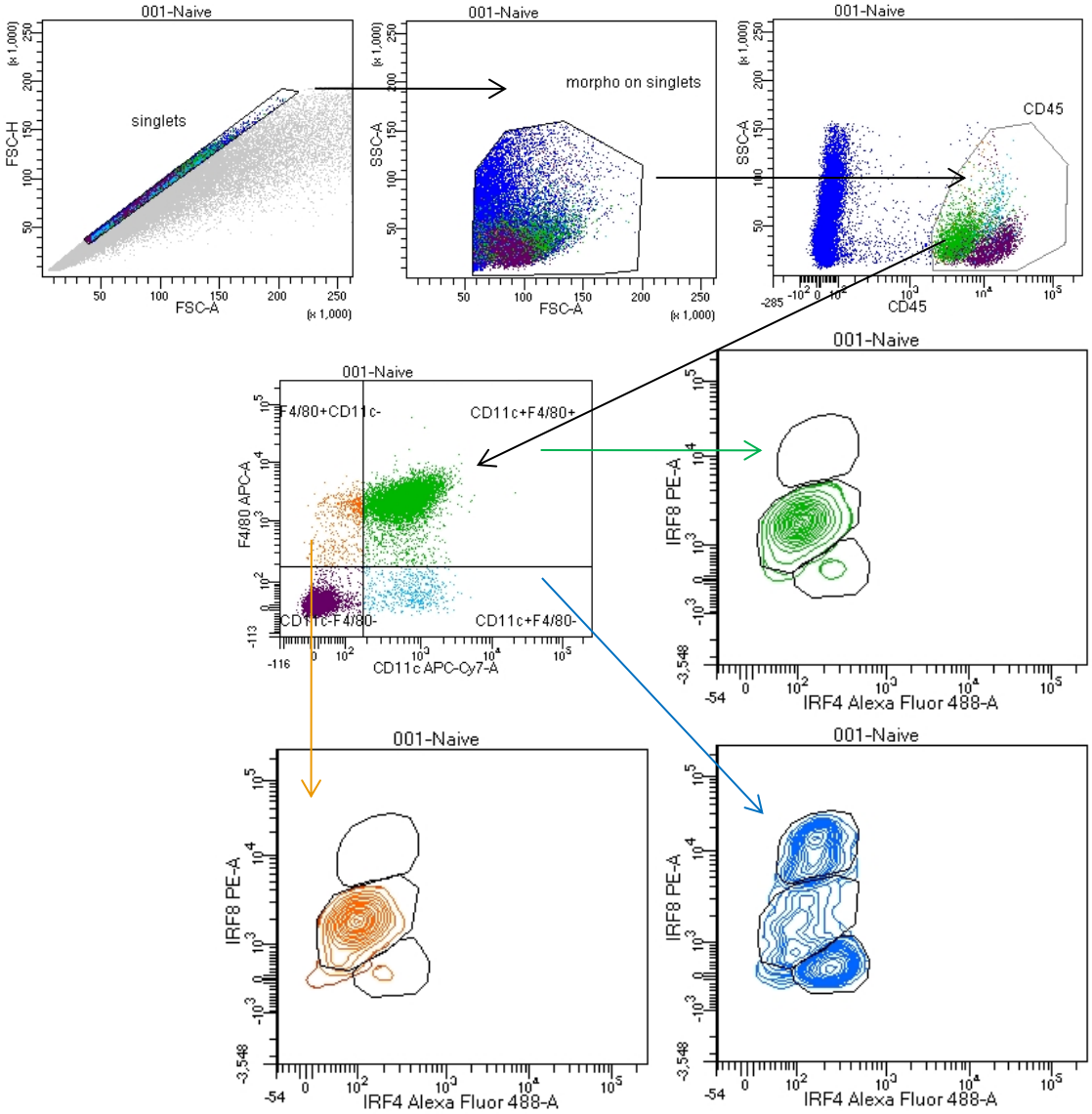


Dot plots and histograms showing FACS analysis for CD11c and F4/80 expression on renal cells pre-microbeads or post-microbeads enrichment.



Dot plots and histograms showing FACS analysis for CD11c, F4/80, CD45.2 and CD45.1 expression on renal cells obtained from kidney grafts by microbeads enrichment.

Supplementary Figure 2

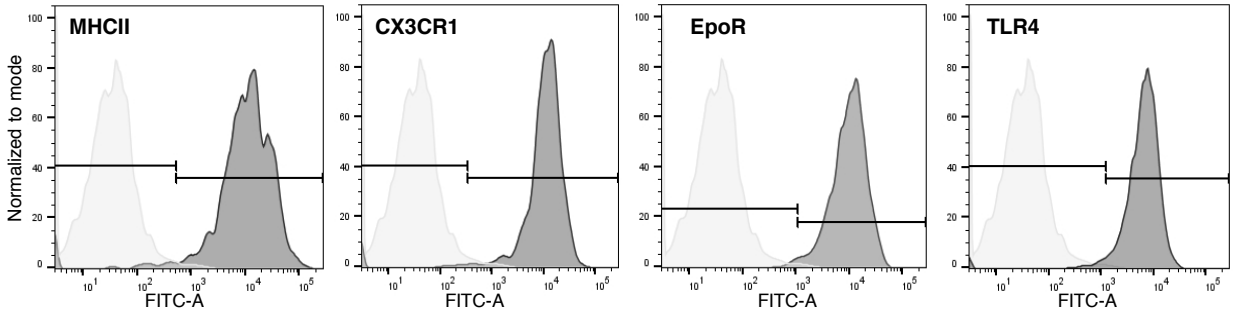


Representative dot plots showing the gating strategy for FACS analysis of IRF4 and IRF8 expression in $CD11c^{-}F4/80^{+}CD45^{+}$, $CD11c^{+}F4/80^{+}CD45^{+}$ and $CD11c^{+}F4/80^{-}CD45^{+}$ renal cells.

Supplementary Figure 3

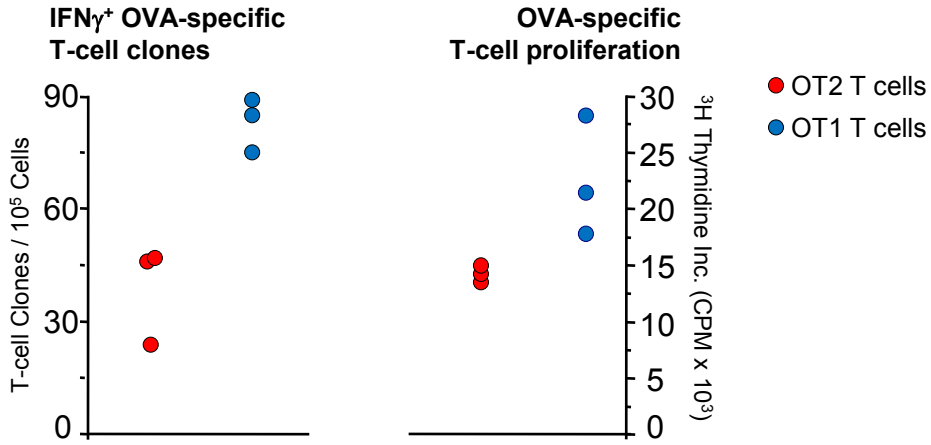
A

CD11c⁺F4/80⁺CD45⁺ single cells



FACS histograms showing staining for MHCII, CX3CR1, EpoR and TLR4 on pre-gated $CD11c^+F4/80^+CD45^+$ renal single cells (95±2%, 76±21%, 87±10%, 90±10%, representative figure of n=5 independent experiments).

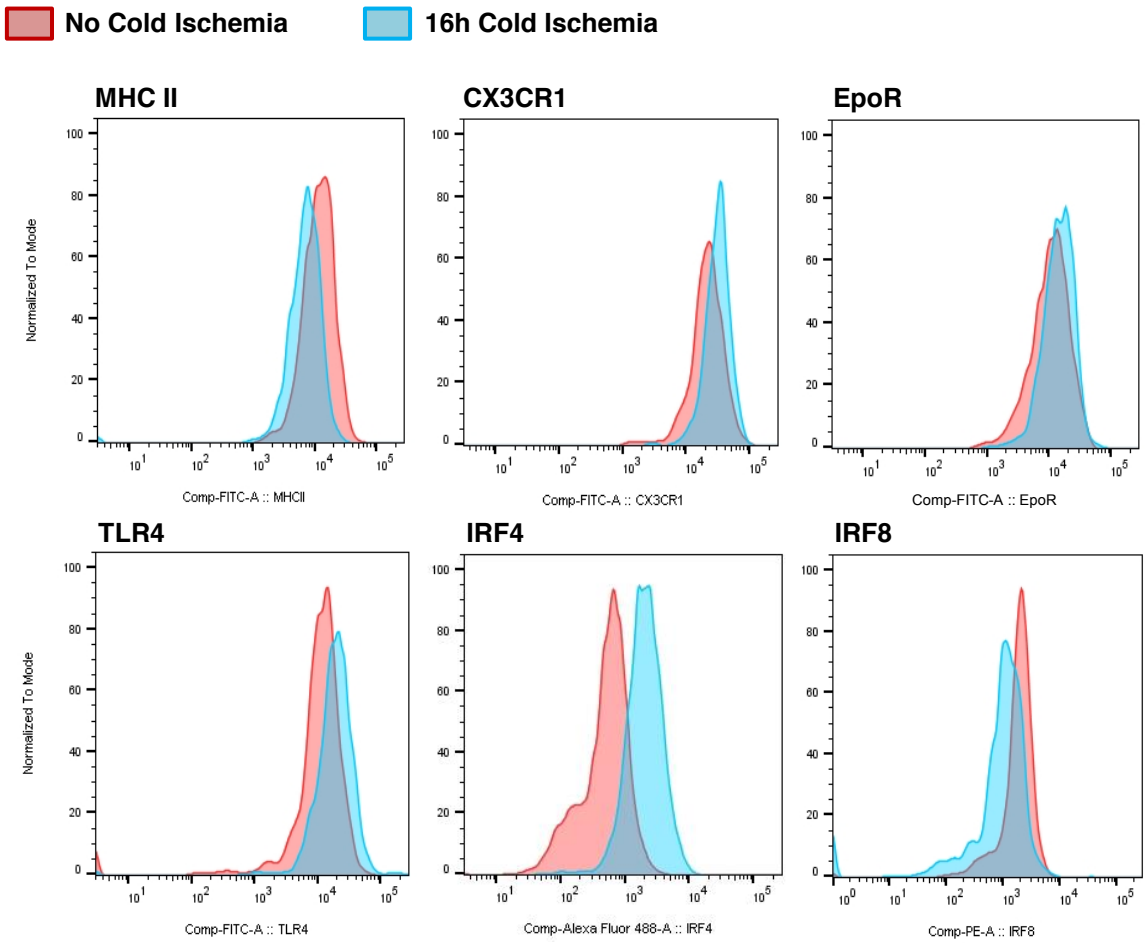
B



OVA-specific T-cell clone formation and T-cell proliferation. After 2-day exposure to renal $CD11c^+F4/80^+$ macrophages in the presence of OVA, the frequency of IFN-γ^+ OVA-specific OT2 ($CD4^+$ Th1) and OT1 ($CD8^+$ Tc1) T-cell clones and OVA-specific T-cell proliferation were evaluated by ELISpot and ^3H Thymidine incorporation assays, respectively. The number of T-cell clones counted in the absence of OVA was 0-2 both for OT2 and OT1 T cells (data not shown). CPM measured in the absence of OVA were <300 both for OT2 and OT1 T cells (data not shown). N = 3 replicates of a representative experiment.

Supplementary Figure 4

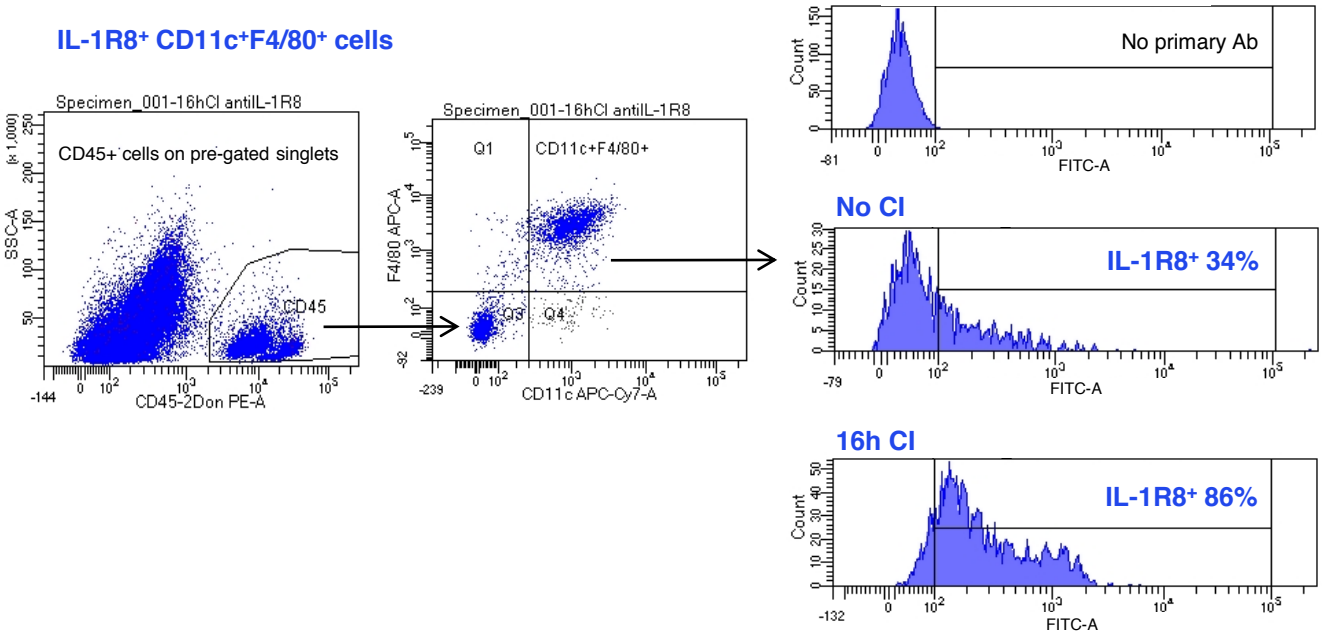
CD11c⁺F4/80⁺CD45⁺ single cells



Representative histograms of MHCII, CX3CR1, EpoR, TLR4, IRF4 and IRF8 expression on pre-gated CD11c⁺F4/80⁺CD45⁺ renal single cells in kidneys subjected or not to 16h cold ischemia.

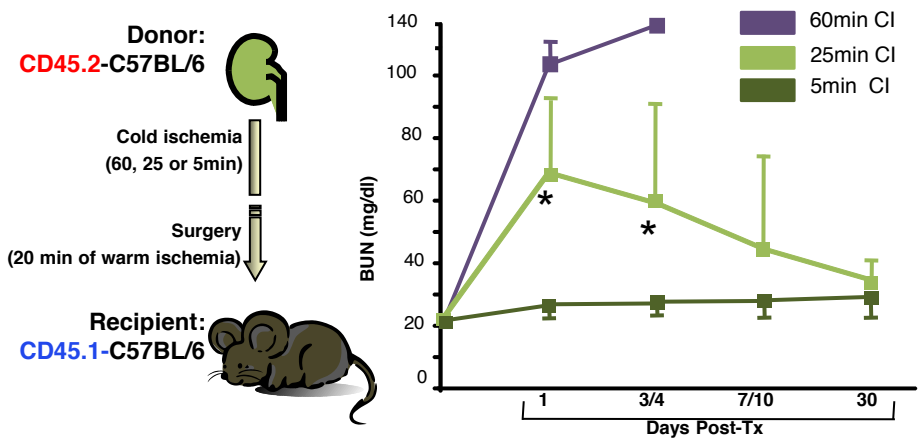
Supplementary Figure 5

A



Representative dot plots and histograms showing IL-1R8 expression on CD11c$^+$F4/80$^+$CD45$^+$ cells obtained from kidneys subjected (16h CI) or not (No CI) to 16h cold ischemia.

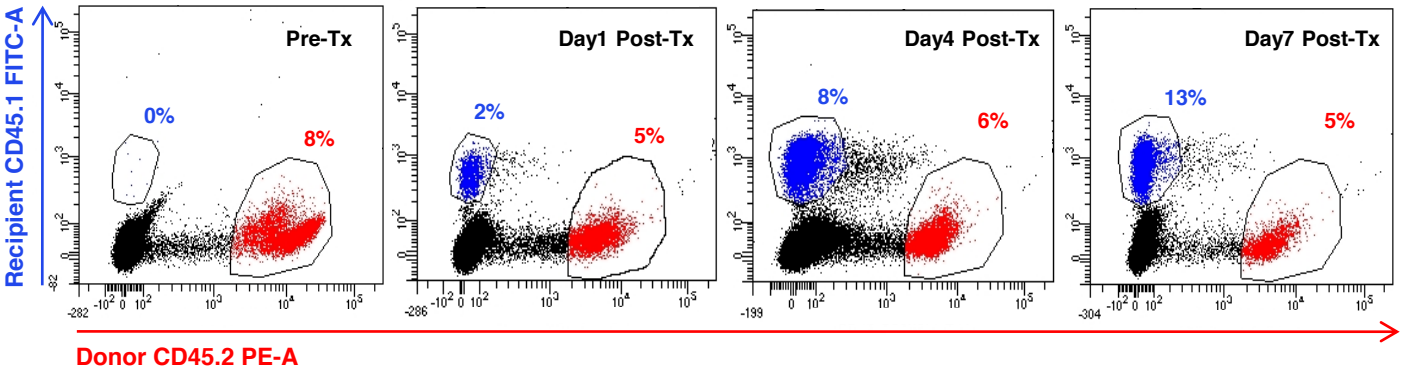
B



Blood urea nitrogen (BUN) concentration in CD45.1$^+$-C57Bl6 mice receiving a CD45.2$^+$-C57Bl6 kidney graft subjected to 60min cold ischemia (CI) (n=4 mice), or 5min CI (n=5 mice) or 25min CI (n=10 mice). *$p<0.05$ vs 60min and 5min.

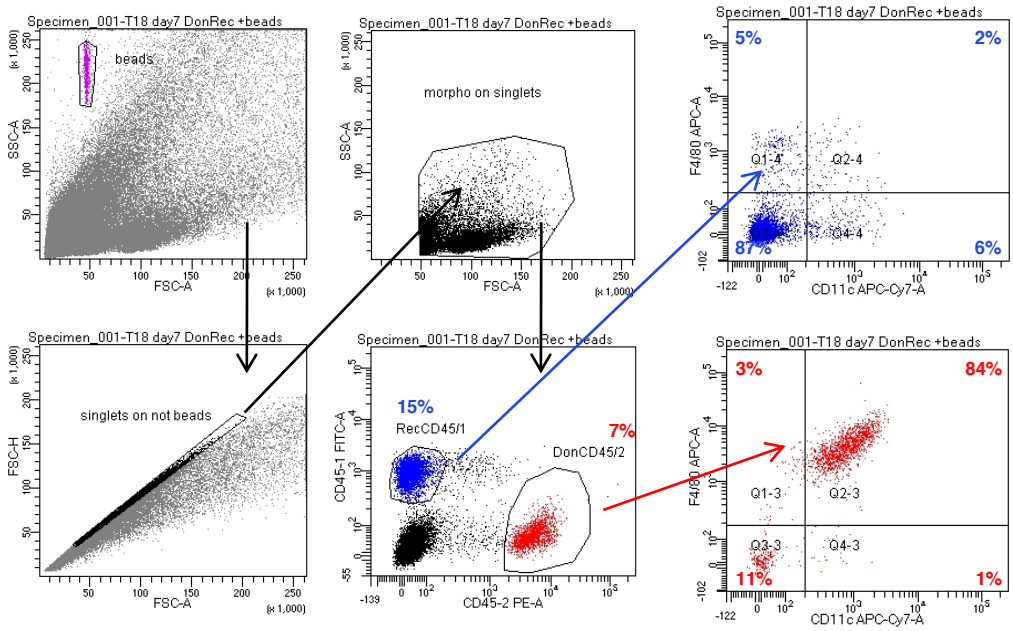
Supplementary Figure 6

A CD45.2+ and CD45.1+ cells in pre- or post-transplant kidney grafts



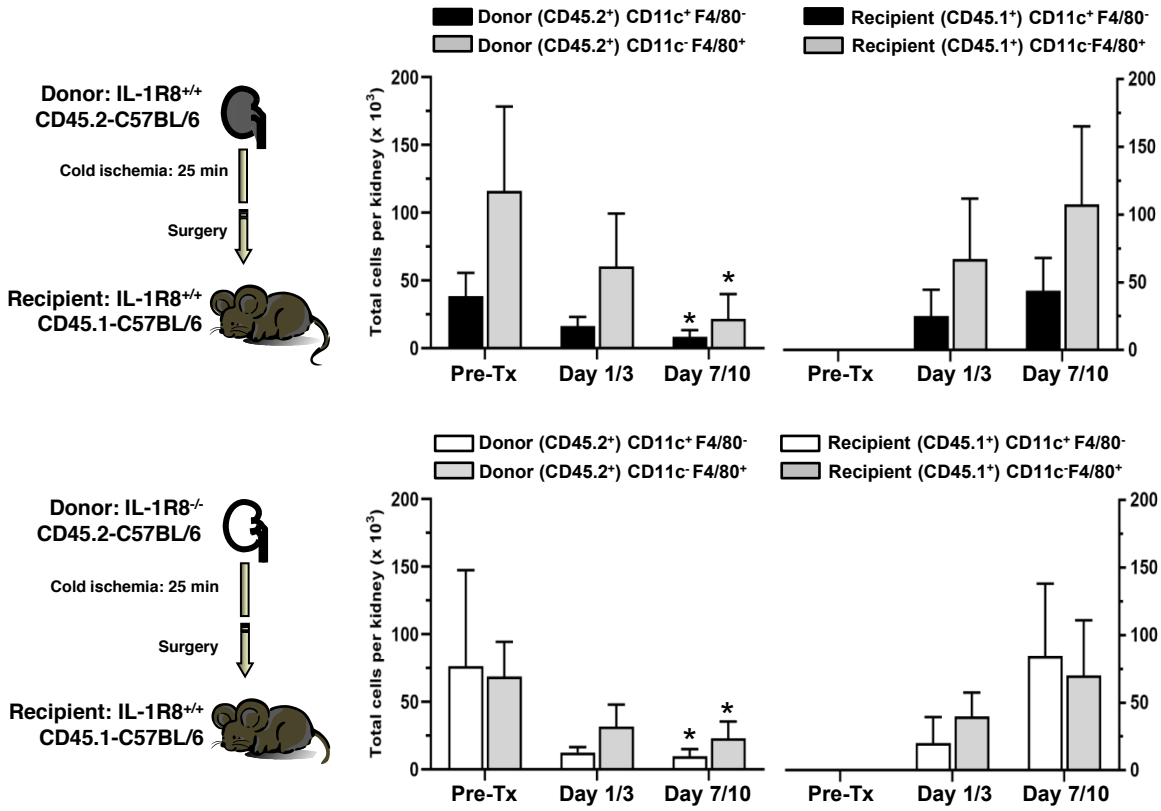
Representative dot plots of donor CD45.2+ (PE) and recipient CD45.1+ (FITC) cells in IL-1R8+/+ CD45.2+ kidneys transplanted into CD45.1+ recipient mice. Kidneys were harvested at pre-transplant or post-transplant days 1, 4 and 7.

B FACS analysis on post-transplant day 7 renal cells

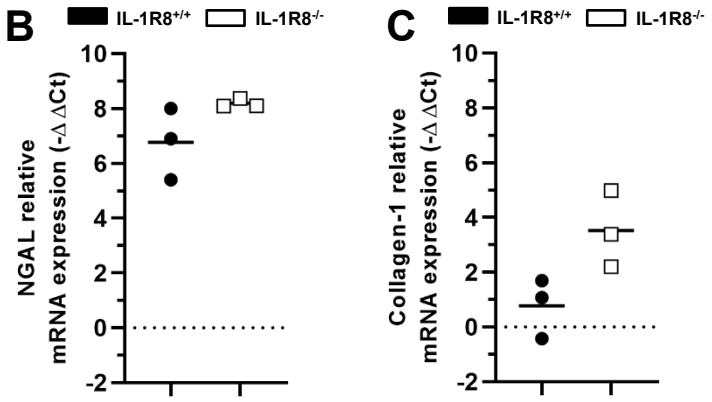


Dot plots from FACS analysis performed to count both donor CD45.2+ (PE) and recipient CD45.1+ (FITC) cells in kidney grafts (a representative FACS analysis of cells from IL-1R8-/-CD45.2+ kidney graft at day7 post-transplant is shown).

Supplementary Figure 7

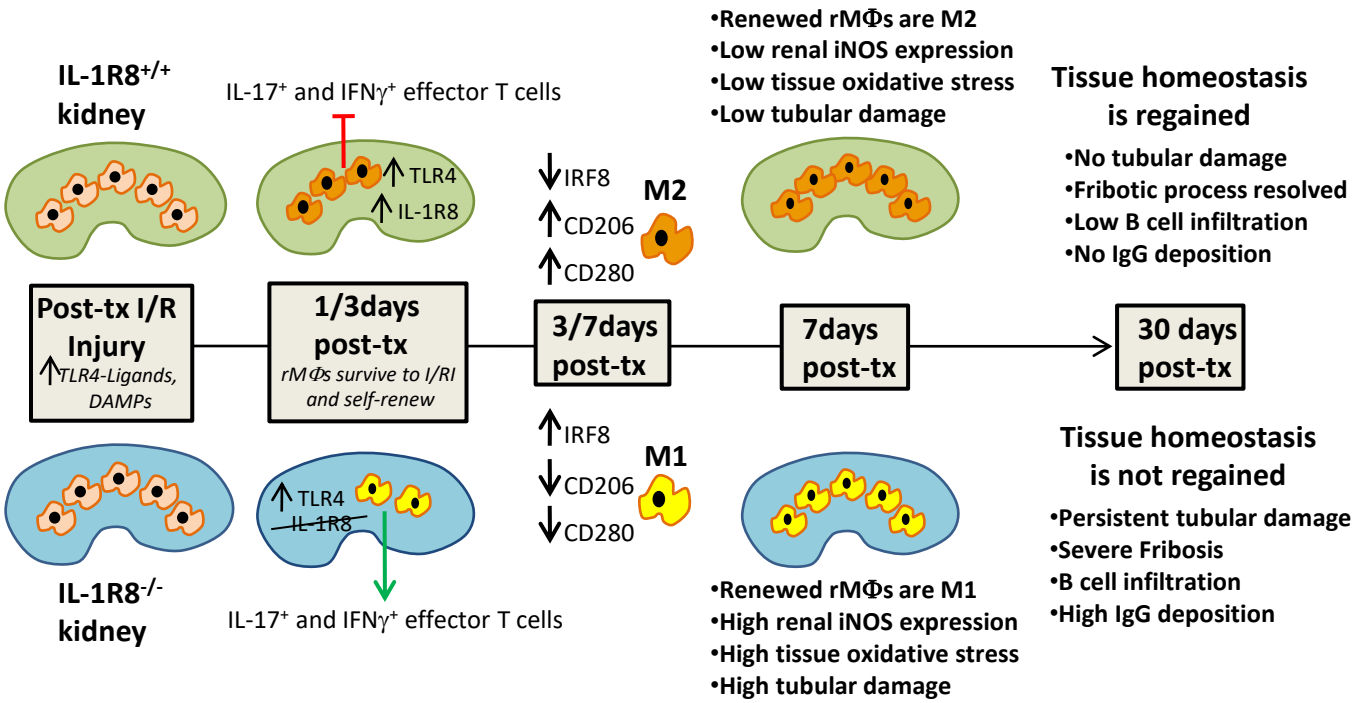


Cell counts, by using true-count beads and FACS analysis, of total donor CD45.2$^+$CD11c$^-$F4/80$^+$ and CD45.2$^+$CD11c$^+$F4/80$^-$ cells, and total recipient CD45.1$^+$CD11c$^-$F4/80$^+$ and CD45.1$^+$CD11c$^+$F4/80$^-$ cells in wt (IL-1R8$^{+/+}$) and IL-1R8$^{-/-}$ kidney grafts pre-transplant and at day1/3 and 7/10 post-transplant (values are mean ± SD, n=3-6 mice at each time point). *P<0.05 vs pre-Tx.



Neutrophil gelatinase-associated lipocalin (NGAL, B), and Collagen-1 (C) mRNA expression in wild-type (IL-1R8$^{+/+}$) and IL-1R8$^{-/-}$ kidney grafts at day 3 (NGAL) or 30 (Collagen-1) post-transplant. The cDNA content was calculated by ΔΔCt technique, using the cDNA expression in pre-transplant kidneys as calibrator. n=3 mice.

Supplementary Figure 8



Supplementary Figure 9