

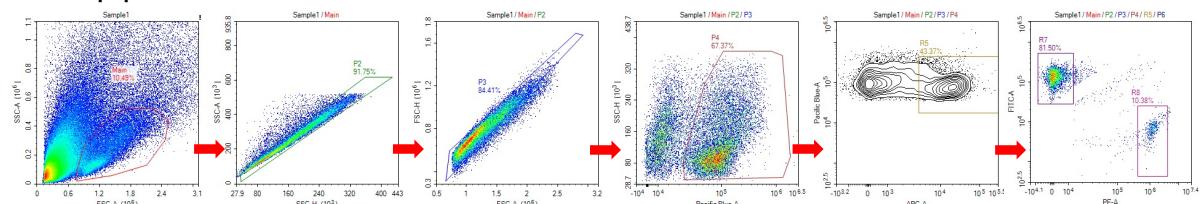
# **Transient inhibition of the Sodium-Glucose Cotransporter 2 early after ischemia/reperfusion injury ameliorates Chronic Kidney Disease in rats .**

Miguel Ángel Martínez-Rojas<sup>1,2</sup>, Hiram Balcázar<sup>1,2</sup>, Isaac González-Soria<sup>1,2</sup>, Jesús Manuel González-Rivera<sup>1,2</sup>, Mauricio E. Rodríguez-Vergara<sup>1,2</sup>, Laura A. Velazquez-Villegas<sup>3</sup>, Juan Carlos León-Contreras<sup>4</sup>, Rosalba Pérez-Villalva<sup>1,2</sup>, Francisco Correa<sup>5</sup>, Florencia Rosetti<sup>6</sup>, and Norma A. Bobadilla<sup>1,2</sup>

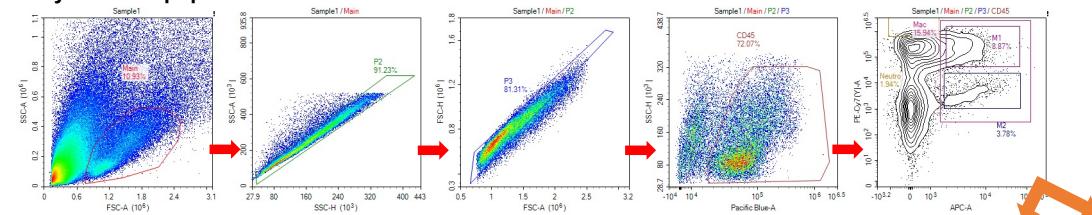
## **Supplemental Figures and Tables:**

1. Supplemental Figure 1: Gating strategy used in flow cytometry experiments.
2. Supplemental Figure 2: SGLT2 inhibition with dapagliflozin reduced myeloid infiltration 10 days after AKI.
3. Supplemental Figure 3: Cytokine and inflammatory profile in kidney cortex after 10 days of reperfusion and dapagliflozin administration.
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5. Supplemental Table 2. Probes and Antibodies used in the experiments

### A. T cell population

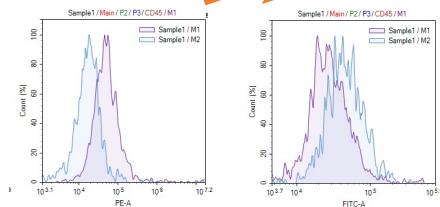


### B. Myeloid cell population

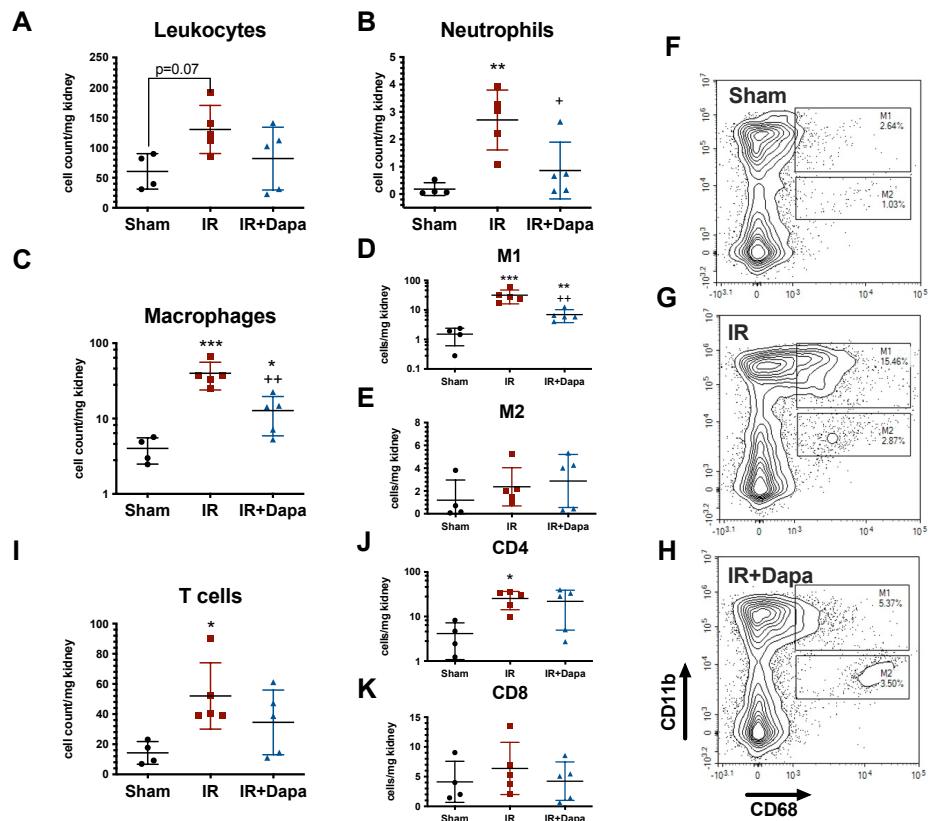


### C. Specified fluorophores and antibodies

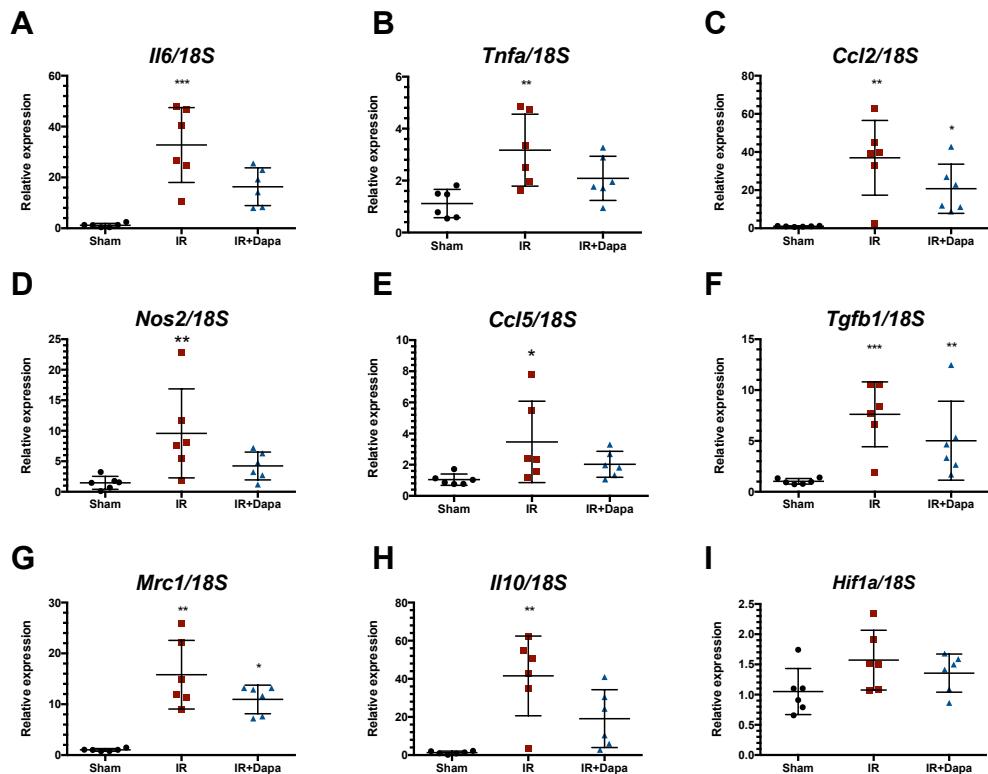
Stain	T cell-mix	Myeloid-mix
Pacific Blue	CD45	CD45
APC	CD3	CD68
PECy7	-	CD11b
FITC	CD4	CD206
PE	CD8	CD86



**Supplemental Figure 1. Gating strategy used in flow cytometry experiments.** All panels show representative dot plots from one IR sample. A. After singlet selection, T cells were identified as CD45+CD3+ cells, and the T helper (CD4+CD8<sup>neg</sup>) and cytotoxic CD8+CD4<sup>neg</sup> subpopulations are shown. B. After selection, myeloid cells were identified as CD45+CD11b+ cells, neutrophils were considered as the CD11b<sup>high</sup>CD68<sup>neg</sup> population, while macrophages as CD11b+CD68+ cells. From the macrophage populations, two subpopulations were identified: CD11b<sup>high</sup> and CD11b<sup>low</sup>, respectively; these cells showed different expression of CD86 and CD206, suggestive of M1- and M2-like phenotypes, respectively. C. The table indicates the antibodies and fluorophore used in the staining.



**Supplemental Figure 2. SGLT2 inhibition with dapagliflozin reduced myeloid infiltration 10 days after AKI.** Flow cytometry analysis of cell infiltrates from renal cortex in the three studied groups: Sham (black circles), IR (red squares), IR+Dapa (blue triangles). A. Total leukocyte number per mg of tissue is shown, B. Total neutrophils per mg, C-E. Total macrophages and their subpopulations, M1-like and M2-like cells, as indicated. F-H. Representative density plots of macrophages populations are shown (gated on live CD45<sup>+</sup> cells as indicated in Supp Fig 1). I-K. Total T lymphocytes and their subtypes per mg of tissue are presented. Each symbol represents a rat, and the results presented are cumulative from three independent experiments. Statistical differences were analyzed by ANOVA F-test for all panels (Mean ± SD). Data in panels C, D, and J were log-transformed for analysis. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs Sham; +p<0.05, ++p<0.01 vs IR.



**Supplemental Figure 3. Cytokine and inflammatory profile in kidney cortex after 10 days of reperfusion and dapagliflozin administration.** Relative mRNA expression of acute-phase molecules A) *Il6*, B) *Tnfa*, C) *Ccl2*, D) *Nos2*, and E) *Ccl5*. Relative expression of profibrotic repair-phase cytokines F) *Tgfb*, G) *Mrc1*, H) *Il10*. I) Also, the expression of *Hif1a* follows a similar trend than inflammatory genes. n= 6 per group. Mean±SD. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs Sham.

**Supplemental Table 1.** Post hoc multiple comparisons of UprotV values between long-term groups.

Month	.y.	group1	group2	n1	n2	p	p.signif	p.adj	p.adj.signif
1	logProt	IR+Dapa	IR	9	7	0.0113	*	0.0338	*
1	logProt	IR+Dapa	Sham	9	7	0.464	ns	1	ns
1	logProt	IR	Sham	7	7	0.0681	ns	0.204	ns
2	logProt	IR+Dapa	IR	9	7	0.0053	**	0.0159	*
2	logProt	IR+Dapa	Sham	9	7	0.231	ns	0.693	ns
2	logProt	IR	Sham	7	7	0.00054	***	0.00162	**
3	logProt	IR+Dapa	IR	9	7	0.0277	*	0.0832	ns
3	logProt	IR+Dapa	Sham	9	7	0.0866	ns	0.26	ns
3	logProt	IR	Sham	7	7	0.000815	***	0.00244	**
4	logProt	IR+Dapa	IR	9	7	0.0541	ns	0.162	ns
4	logProt	IR+Dapa	Sham	9	7	0.00432	**	0.013	*
4	logProt	IR	Sham	7	7	0.000075	****	0.000225	***
5	logProt	IR+Dapa	IR	9	7	0.00724	**	0.0217	*
5	logProt	IR+Dapa	Sham	9	7	0.0421	*	0.126	ns
5	logProt	IR	Sham	7	7	0.0000934	****	0.00028	***

**Supplemental Table 2.** Probes and Antibodies used in the experiments

Target Gene	Reagent	Catalog Number
Eukaryotic 18S rRNA	Probe	Rn03928990_g1
Interleukin-6 ( <i>Il6</i> )	Probe	Rn01410330_m1
Tumor Necrosis Factor $\alpha$ ( <i>Tnf</i> )	Probe	Rn99999017_m1
Chemokine CC motif Ligand 2 ( <i>Ccl2</i> )	Probe	Rn00580555
Interleukin-10 ( <i>Il10</i> )	Probe	Rn99999012_m1
Transforming Growth Factor $\beta$ -1 ( <i>Tgfb1</i> )	Probe	Rn00572010_m1
Mannose Receptor C Type 1 ( <i>Mrc1</i> )	Probe	Rn01487342_m1
Kidney Injury Molecule 1 ( <i>Havcr1</i> )	Probe	Rn00597703_m1
Serpin A3c ( <i>Serpina3c</i> )	Probe	Rn04289570_m1
Hypoxia-inducible factor 1a ( <i>Hif1a</i> )	Probe	Rn00577560_m1
Nitric oxide synthase 2 ( <i>Nos2</i> )	Probe	Rn00561646_m1
Nitric oxide synthase 3 ( <i>Nos3</i> )	Probe	Rn02132634_s1
Sirtuin 3 (SIRT3)	Mouse Antibody	sc-365175
Mitofusin 1 and 2 (MFN1, MFN2)	Mouse Antibody	ab57602
Dynamin-related protein 1 (DRP1)	Mouse Antibody	sc-271583
Optic Atrophy 1 (OPA1)	Mouse Antibody	sc-393296
OXPHOS	Mouse Antibody	ab110413
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Rabbit Antibody	ab181602
Angiotensinogen (AGT)	Rabbit Antibody	ab213705
PTEN-induced kinase 1 (PINK1)	Rabbit Antibody	P0076
Parkin	Mouse Antibody	P6248
B cell lymphoma 2 (BCL2)	Rabbit Antibody	SAB5701336
Bcl2-Associated X (BAX)	Rabbit Antibody	SAB5701333
Bcl2-induced protein 3 (BNIP3)	Mouse Antibody	sc-56167
NLR family pyrin domain containing	Rabbit Antibody	15101S

3 (NLRP3)		
Antimouse IgG-peroxidase	Goat Antibody	ab6789
Antirabbit IgG-peroxidase	Goat Antibody	A0545
Antirabbit IgG-peroxidase	Mouse Antibody	211-032-171
Antimouse IgG-peroxidase	Goat Antibody	115-035-174
Pacific blue-CD45	F-Antibody*	202225
PE/Cy7-CD11b	F-Antibody	201817
APC-CD68	F-Antibody	130-103-364
PE-CD86	F-Antibody	200307
FITC-CD206	F-Antibody	GTX43682
APC-CD3	F-Antibody	201413
FITC-CD4	F-Antibody	201505
PE-CD8a	F-Antibody	200607

\*F-Antibody: Fluorophore-coupled antibody