SUPPLEMENTARY MATERIALS

Supplementary methods

Isolation of KSP$^+$ cells

KSP$^+$ positive cells were isolated from EBs culture using magnetic activated cells separation (MACS) (miltenyi Biotec. 1989). The cell suspension was incubated in buffer (phosphate buffered saline supplemented with 0.5% BSA, Sigma) containing KSP primary antibody (SantaCruz, diluted 1:50), 1 µl of diluted antibody/10^6 cells for 25 minutes at 4°C, then washed in buffer. The cell pellet was resuspended in buffer containing secondary biotinylated anti-goat (DAKO, diluted 1:200) for 25 minutes at 4°C, then washed in buffer. Finally, the cell pellet was resuspended in buffer with streptavidin microbeads (diluted 1:10), then washed. The supernatant was discarded and the cell pellet resuspended in buffer and loaded onto the magnetic separation (MS) column which was placed in a magnetic field. Unlabeled cells were washed though the column (3x with buffer). The column was then removed from the magnetic field and washed with buffer to elute the labelled cells. Two cell suspensions were obtained: the labelled cells (KSP$^+$ cells) and the non-labelled cells (KSP$^-$ cells).

GPSCs and KSP$^+$ cells injection after renal IRI

Studies were conducted in accordance with National Institute of Health Guide for Care and Use of Laboratory Animals. All experiments were performed with 8-week-old female 129sv/C57; mice were allowed free access to water and standard mouse chow. The experimental protocol was the same of the previous experiments and the mice were sacrificed 48h after IRI. We injected immediately after IRI, $2.5x10^5$ undifferentiated GPSCs (N=6) or KSP$^+$ cells (N=6) recovered from MACS sorting 4 days after isolation, through tail vein. In both experiments, control mice were only given vehicle (N=4).

Tubular Dilation(TD)/cystic index

Ten cortical grid fields (original magnification x200) for 2 serial sections of each kidney were evaluated. The TD/cystic quantification was performed using a screen with dots distant one from another by 13.625 micron. The measures were obtained by counting the number of dots located in the tubular lumen. The degree of TD was defined by the number of dots in the lumen, following previously suggested criteria: (*1*) TDI, including only one dot; (*2*) TDII (Tubular Dilation type II), two dots; (*3*) MCs, three to nine dots; and (*4*) cysts, ten dots or more.

Proliferation of GTCs Y^+ cells

To evaluate the proliferative potential of GTCs-injected Y^+ cells, we performed BrdU staining after *in situ* analysis of Y chromosome on renal sections. The primary antibody (DAKO, clone Bu20a) was diluted in 1% Bovine Serum Albumin (BSA, Sigma) and incubated for 1h at room temperature.

Supplementary Figures

Supplementary figure 1

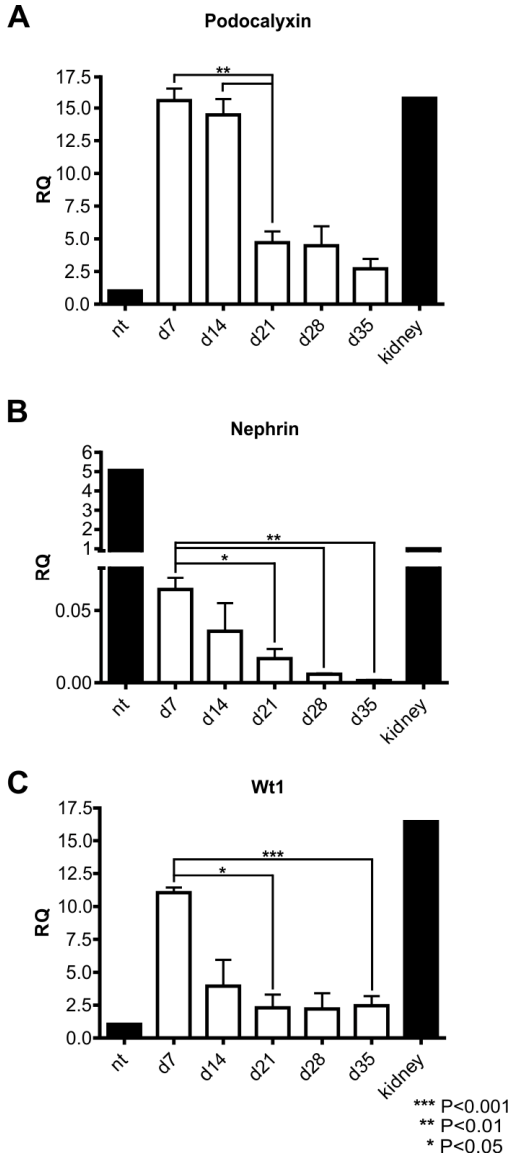

Figure S1. Characterization of GPSCs derived tubular-like cells. RNA was extracted at different time points and RealTime-PCR analysis was performed. Podocalyxin is a marker of the podocytes, a type of cell that compose the glomerolus, and its expression drops after day 14 (A). The same trend is showed by nephrin (B) and Wt1 (C), another two markers of glomerular cells. Each column refers to 3 independent samples (N=3).

Supplementary figure 2

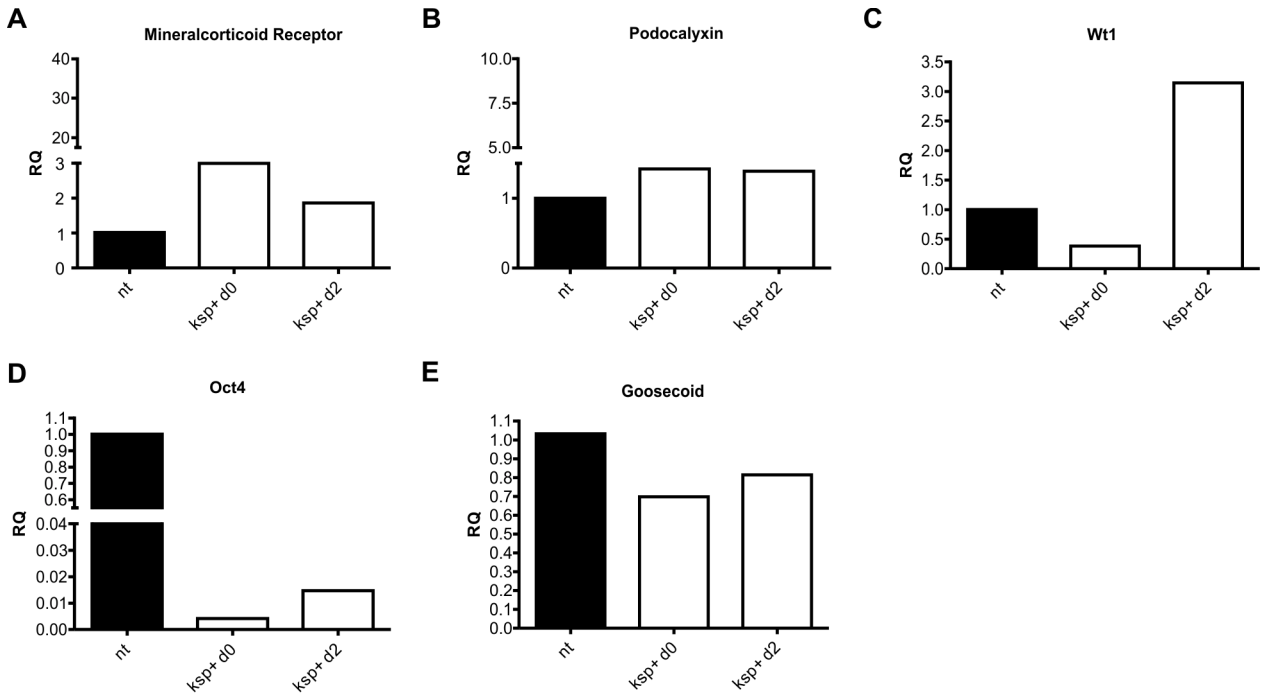

Figure S2. KSP$^+$ cells fraction collected from MACS were tested for the expression of various differentiation markers. KSP$^+$ cells express mineralcorticoid receptor (A) that start to decrease two days from MACS separation. This fraction of cells do not express podocalyxin (B), Wt-1 (C), oct4 (D) and goosecoid (E). (KSP$^+$ d0: RNA was extracted immediatly after isolation, KSP$^+$ d2: RNA was extracted 2 days after cells isolation).

Supplementary figure 3

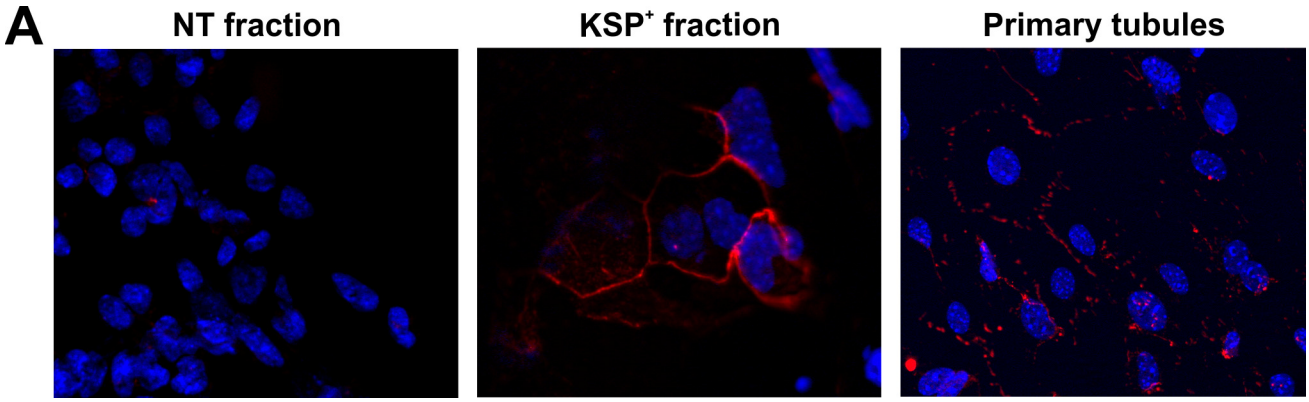

Figure S3. The pictures refer to immunofluorescence staining for ZO-1 of NT fraction, KSP^+ fraction and primary tubules. DNA is counterstained with DAPI. Original magnification x630. NT: undifferentiated GSPCs, KSP^+: cells recovered from MACS sorting. (ZO-1: Zonula Occludens protein-1; DAPI: 4,6 diamidino-2-phenylindole).

Supplementary figure 4

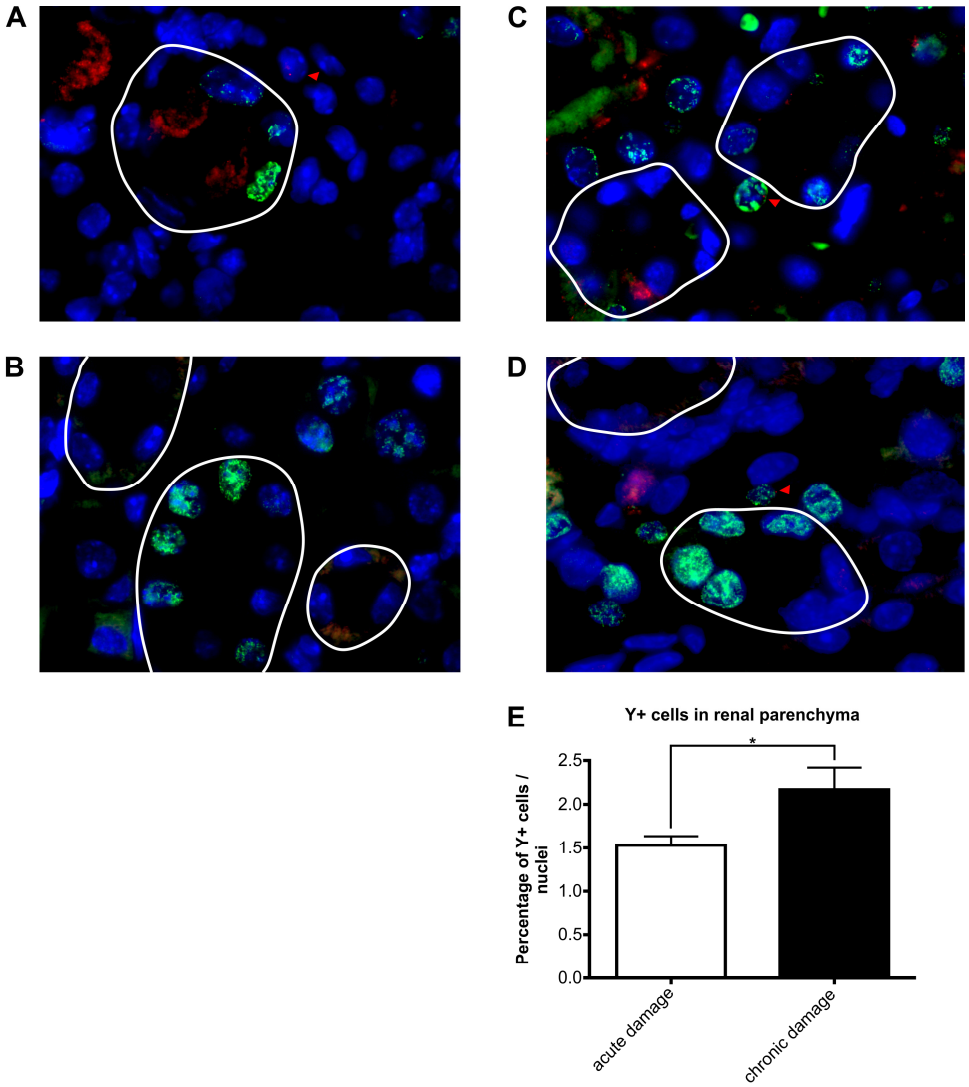

Figure S4. Proliferation of Y^+ cells 48h post IRI. Picture (A) show a $Y^+/BrdU^-$ cell in the tubular interstitium, picture (B) represents a negative control of the same mouse stained for BrdU alone. Pictures (C) and (D) display two areas characterized by the presence of $Y^+/BrdU^+$ cells in the renal parenchyma. Pictures C and D highlight that the number of Y^+ proliferating cells is lower compared to the endogenous proliferating ones. Original magnification x1000. $BrdU^+$ cells are revealed with a secondary antibody alexa 565 (green signal), red dots represent the Y chromosome, nuclei are counterstained with DAPI. The chart below (E) shows the quantification of Y^+ cells in renal parenchyma 48h and six weeks after IRI. (acute damage, N=8; chronic damage, N=10).

Supplementary figure 5

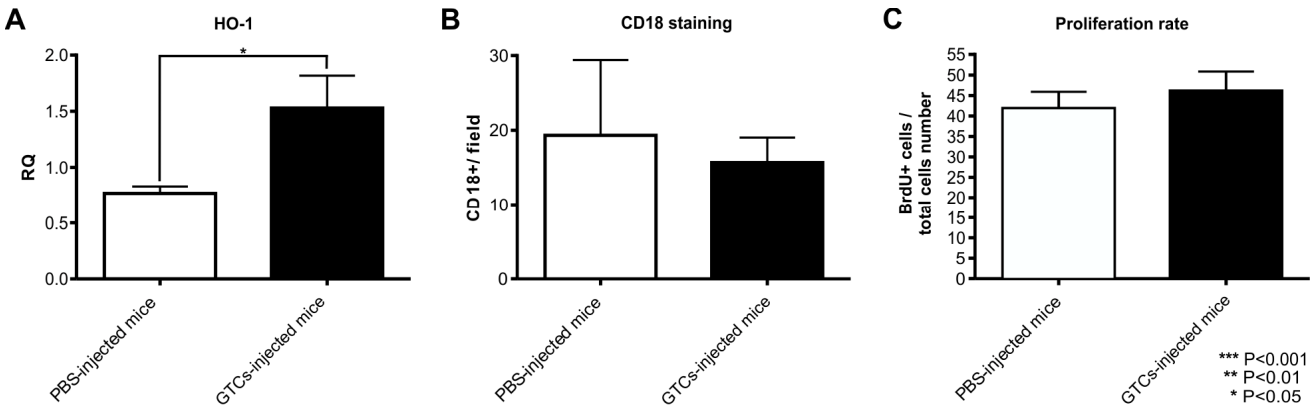

Figure S5. (A) RealTime-PCR analysis 48h post IRI that show up-regulation of HO-1 in mice injected with GTCs compared to mice injected with PBS. No difference is detected between ischemized mice injected or not with GTCs 48h post ischemia concerning the CD18+ cells (B) and the proliferation rate (C). (GTCs-injceted mice, N=8; PBS-injected mice, n=8).

Supplementary figure 6

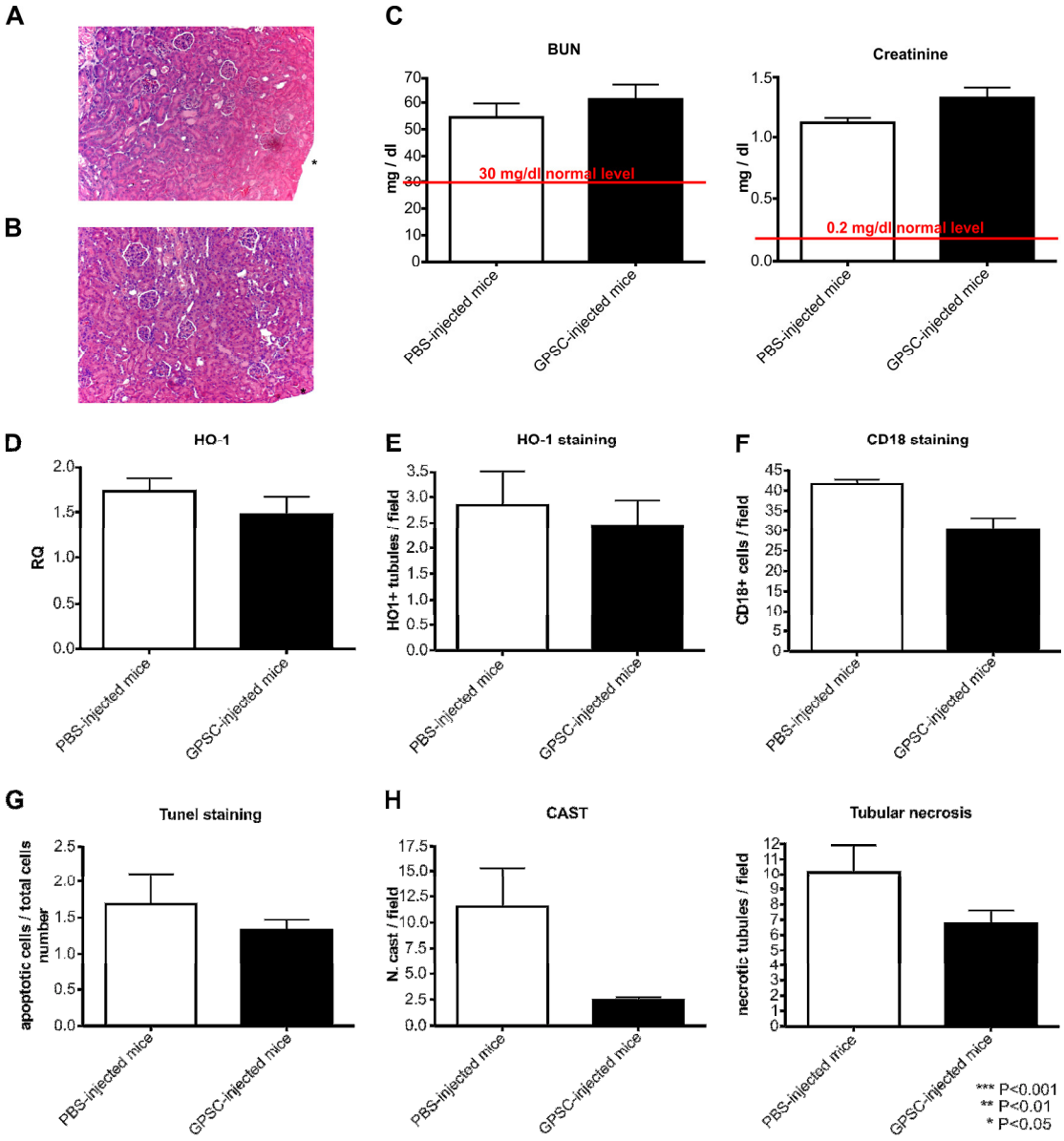

Figure S6. GPSCs injection after AKI. Representative H&E of PBS-injected mice (A) and GPSCs-injected mice (B) (Original magnification x100). The level of BUN and creatinine (C) in the blood do not differ significantly between the two groups of mice. Real-Time PCR analysis shows that GPSCs are not able to up-regulate HO-1 (D), as confirmed by the counting of HO-1^{+} tubules (E). No statistically relevant difference is detected in the number of inflammatory infiltrates (F), CAST and tubular necrosis (H). The incidence of apoptosis (G) is not affected by GPSCs injection. (GPSC-injected mice, N=6; PBS-injected mice, N=4).

Supplementary figure 7

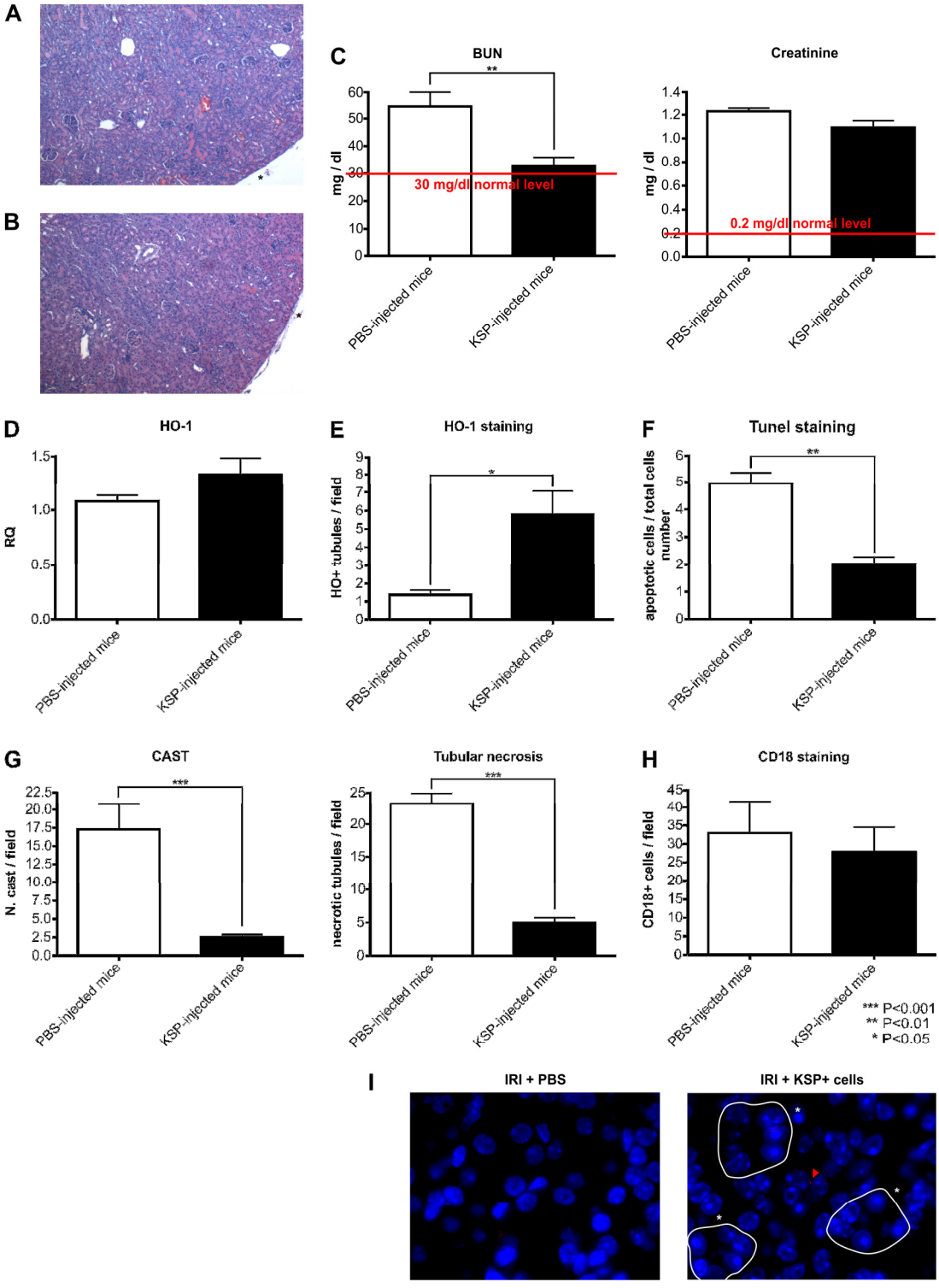

Figure S7. Protection of kidney 48h post ischemia by KSP^+ cells. Representative H&E staining of IRI-PBS mice (A) and IRI-KSP^+ cells mice (B) (Original magnification x100). BUN but not creatinine (C) in the blood of mice injected with KSP^+ cells is significantly lower compared to mice injected with PBS. RealTime-PCR analysis (D) shows up-regulation of HO-1, confirmed by HO-1 staining (E), in renal parenchyma of mice injected with KSP^+ cells compared to control mice. Number of apoptotic cells (F), CAST and tubular necrosis (G), is significantly down-regulated in mice injected with KSP^+ cells compared to PBS-injected mice. No difference is found in the number of inflammatory infiltrates (H). Pictures (I) show a representative *in situ* analysis for Y chromosome. (*) Indicate the tubules. (Original magnification x1000, nuclei are counterstained with DAPI). (KSP-injected mice, N=6; PBS injected mice, N=4).