Supplemental material to

Loss of cellular senescence improves regenerative capacity and allograft survival

Heidi Braun[1*], Bernhard M.W. Schmidt[2*], Mirja Raiß[1], Arpita Baisantry[1,2], Dan Mircea-Constantin[1], Shijun Wang[3], Marie-Luise Groß[4], Manuel Serrano[5], Roland Schmitt[2], and Anette Melk[1]

[1]Division of Pediatric Nephrology, Children's Hospital, Hannover Medical School, Hannover, Germany, Germany

[2]Department of Nephrology, Hannover Medical School, Hannover, Germany, Germany

[3]Department of Cellular Pathology, German Cancer Research Centre, Heidelberg, Germany

[4]Department of Pathology, University of Heidelberg, Heidelberg, Germany

[5]Tumor Suppression Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain

*These authors contributed equally

Corresponding author:

Anette Melk, MD, PhD

Division of Pediatric Nephrology, Children's Hospital, Hannover Medical School

Carl-Neuberg-Strasse 1

D-30625 Hannover

Germany

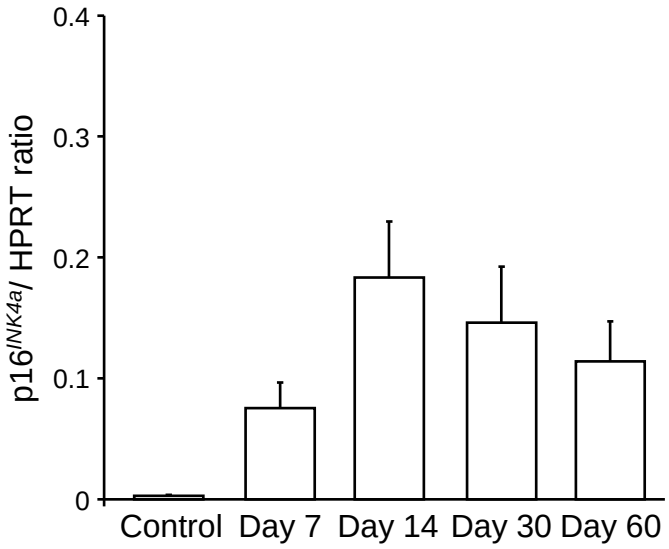
Tel.: +49-511-532 5597

Fax: +49-511-532 3911

Email: melk.anette@mh-hannover.de

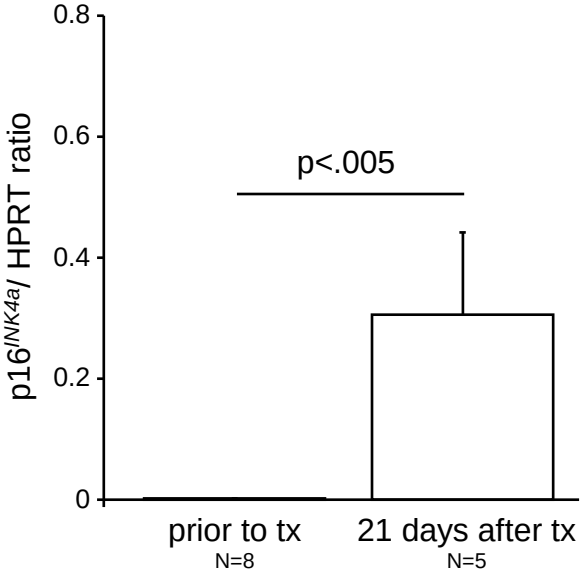
Suppl. figure 1

a



Suppl. Fig. 1a: p16^{INK4a} mRNA expression following renal IRI. Control kidneys from wildtype mice had very low p16^{INK4a} expression. Following renal IRI p16^{INK4a} expression increased significantly by day 14 (p<.05).
Data is shown as mean±SEM.

b

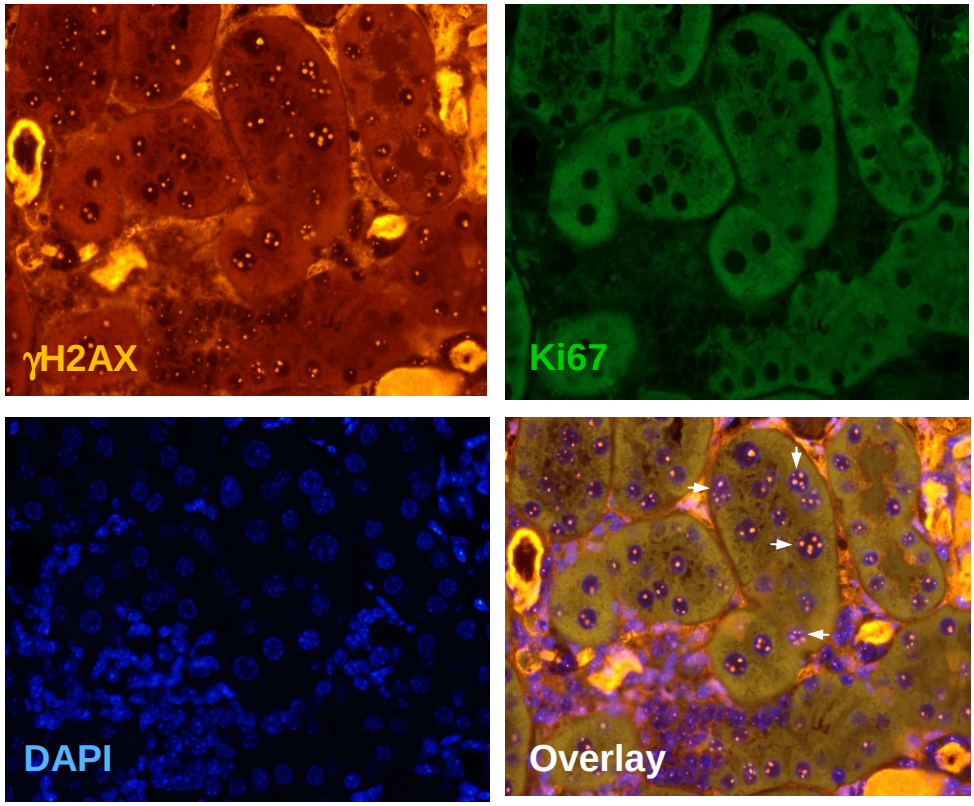


Suppl. Fig. 1b: p16^{INK4a} mRNA expression following renal transplantation. Control kidneys from wildtype mice had very low p16^{INK4a} expression. Following renal transplantation p16^{INK4a} expression measured in transplants from surviving mice increased significantly (p<.005).
Data is shown as mean±SEM.

Suppl. Figure 2

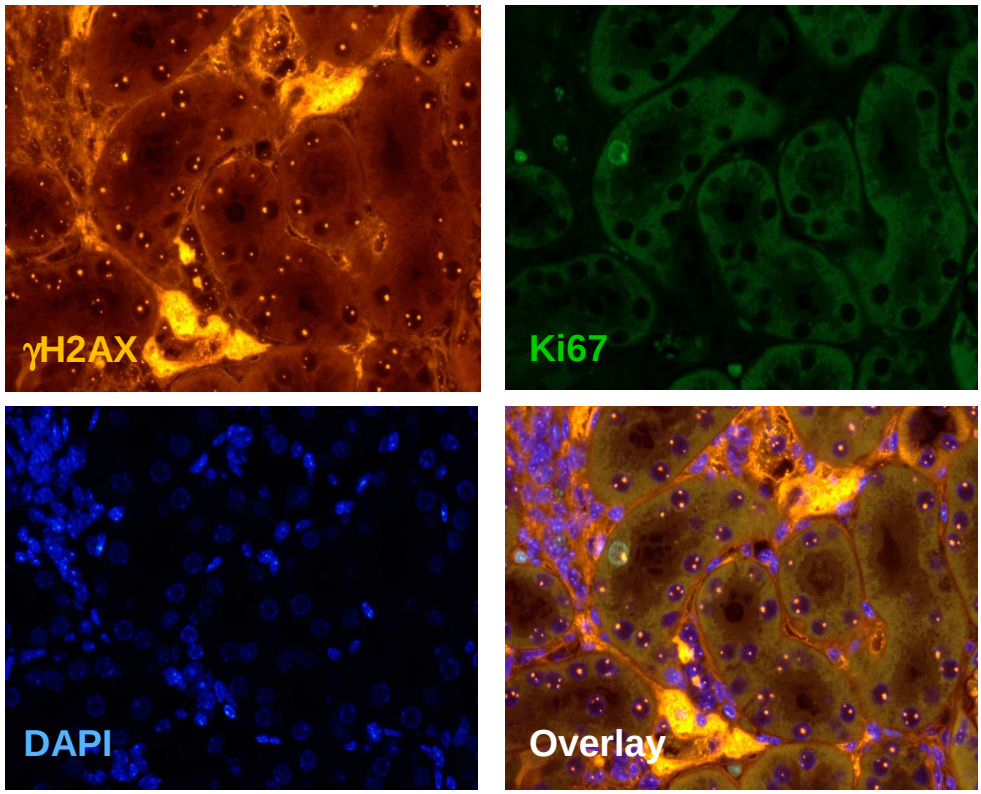
a

WT



b

INK4a$^{-/-}$

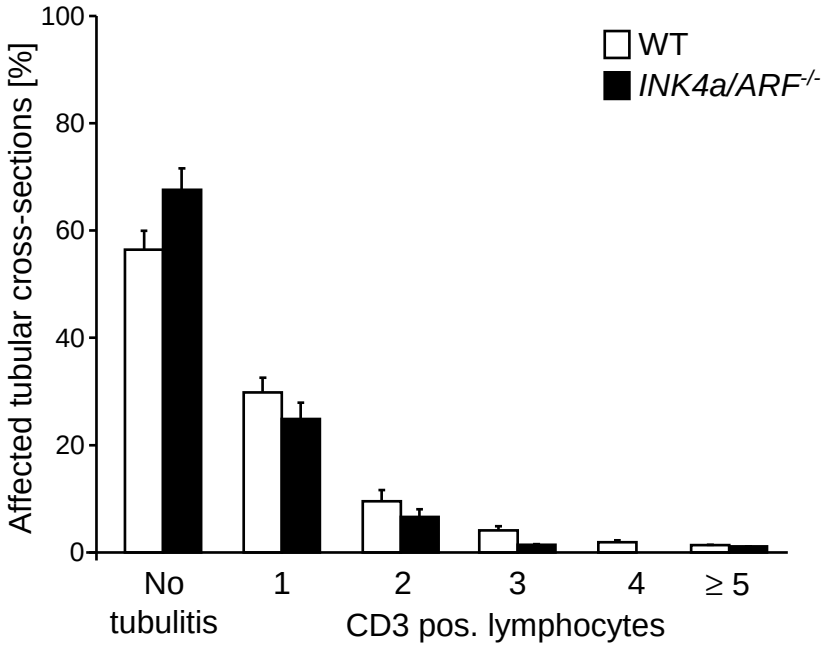


Suppl. Figure 2, cont./

Suppl. Fig. 2: Representative immunofluorescence co-staining for phosphorylated γH2A.X and Ki67 in **(A)** wildtype (WT) and **(B)** *INK4a*$^{-/-}$ kidneys 60 days after ischemia-reperfusion injury (original magnification 400x).

Paraffin-embedded sections of wildtype and *INK4a*$^{-/-}$ kidneys were co-stained for γH2A.X and Ki67. The images were merged to determine the nuclear co-localization of γH2A.X foci (red) with Ki67 (green) and overlayed with DAPI (blue) to visualize DNA. Tubular epithelial cells that were negative for the proliferation marker Ki67 and contained high frequency γH2A.X foci (≥4 γH2A.X foci per nucleus) were designated as senescent cells. The frequency of senescent cells, as indicated by white arrows in the overlay was found to be significantly higher in wildtype kidneys relative to *INK4a*$^{-/-}$ kidneys.

Suppl. Figure 3



Suppl. Fig. 3: Tubulitis following renal transplantation.

Tubulitis occurred only in 44% and 32% of tubular cross-sections from either wildtype (WT) or *INK4a/ARF*$^{-/-}$ transplants (difference not significant). Predominantly, lesions with only one CD3-positive lymphocyte per tubular cross-section were found, whereas lesions with more lymphocytes were rare.

Data is shown as mean±SEM.

Suppl. Table 1

CD3-positive lymphocytes [number of cells/high power field]	WT/ KO	MW ± SD	P-value
Interstitial compartment	WT	1916 ± 388	n.s.
	KO	1600 ± 332	
Tubular compartment	WT	213 ± 57	n.s.
	KO	200 ± 58	
Glomerular compartment	WT	48 ± 21	n.s.
	KO	84 ± 45	

Suppl. Table 1: Infiltrating CD3-positive lymphocytes following renal transplantation.

CD3-postive lymphocytes were mainly found in the interstitium and less frequently in the tubular and glomerular compartment. No significant differences were seen between wildtype (WT) or *INK4a/ARF*$^{-/-}$ (KO) transplants

Data is shown as mean±SD.