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sample ID	CIS time	CD4						CD8					
		glomerular	peri-glomerular	periarterial	interstitial	tubular	CD4 total	glomerular	peri-glomerular	periarterial	interstitial	tubular	CD8 total
VG 207-24	0.5 h	2*	0	0.5	0	0	0.5	2*	0	0.5	1	1	2.5
VG 207-32	0.5 h	0.5	1	1	0.5	0	3	1	1	1	1	0.5	4.5
VG 207-31	0.5 h	0.5	0	0.5	0.5	0	1.5	1	1	1	1	1	5
VG 207-10	0.5 h	0.5	0.5	0.5	0.5	0.5	2.5	1	1	1	1	1	5
VG 207-9	0.5 h	1	1	1	0.5	0.5	4	2	1	2	2	0.5	7.5
VG 207-28	0.5 h	1	2	3	0.5	0.5	7	2	2	3	2	2	11
VG 207-36	6 h	1	1	0.5	0.5	0.5	3.5	2	1	1	1	1	6
VG 247-10	6 h	0.5	0.5	2	0.5	0.5	4	1	1	2	1	2	7
VG 207-25	6 h	0.5	0.5	1	0.5	0.5	3	1	1	2	1	1	6
VG 207-12	6 h	1	2	2	0.5	0	5.5	2	2	2	1	0.5	7.5
VG 207-33	6 h	0.5	1	1	0.5	0.5	3.5	3	1	1	2	1	8
VG 207-14	6 h	0.5	1	3	0.5	0.5	5.5	2	2	3	1	0.5	8.5
VG 207-30	6 h	1	3	2	0.5	0	6.5	2	3	2	1	0.5	8.5
VG 247-5	6 h	0.5	1	0.5	1	1	4	2	2	1	2	2	9
VG 207-29	6 h	1	1	1	0.5	0.5	4	2	2	2	2	1	9
VG 247-1	6 h	0.5	1	1	0	0.5	3	3	2	2	2	1	10
VG 207-35	6 h	1	1	2	0.5	0.5	5	3	2	2	1	2	10
VG 247-3	6 h	1	1	2	0.5	0.5	5	3	2	2	1	2	10
VG 207-21	6 h	2	1	2	1	0.5	6.5	3	2	2	2	2	11
VG 207-22	6 h	0	0.5	1	0.5	0	2	1	1	1	1	0.5	4.5

Table S1. Scores of glomerular, periglomerular, periarterial, interstitial and tubular CD4 and CD8 T cell infiltrate were determined at d. 14 posttransplant as illustrated in Figure S1. Scores of tubular infiltration in 0.5 h and 6 h CIS allografts are highlighted in light blue and light pink, respectively. Total T cell infiltration scores in 0.5 h and 6 h CIS allografts are blue and pink. The scoring was performed by a pathologist (WMB) with no knowledge of experimental groups. Stars indicate fragmented cells.

sample ID	mAb transfer	CD4						CD8					
		glomerular	peri-glomerular	periarterial	interstitial	tubular	CD4 total	glomerular	peri-glomerular	periarterial	interstitial	tubular	CD8 total
VG 273-7	IgG2a	0	2	2	1	0	5	0	0	2	1	0	3
VG 273-8	IgG2a	1	1	1	1	0	4	2	0	2	1	0	5
VG 273-1	anti-K^d	0	2	2	0,5	0	4,5	0	1	1	1	0,5	3,5
VG 273-2	anti-K^d	0	0	2	0,5	0	2,5	1 (Large)	1	1	1	0	3
VG 273-3	anti-K^d	0	2	2	0,5	0	4,5	1	1	2	1	0,5	5,5
VG 273-14	anti-K^d	0	1	2	1	0	4	0	1	2	1	0	4
VG 273-15	anti-K^d	0	1	1	1	0	3	1	2	2	1	0	6
VG 273-4	anti-I-A^d	0	1	1	0,5	0	2,5	3	0	1	1	0,5	5,5
VG 273-6	anti-I-A^d	0	1	1	1	0	3	1	1	2	1	0	5
VG 273-10	anti-I-A^d	1	1	2	0,5	0	4,5	1	1	1	1	0	4
VG 273-12	anti-I-A^d	0	2	1	1	0	4	1	0	2	1	0,5	4,5
VG 273-13	anti-I-A^d	1	0	1	1	0	3	1	0	2	1	0,5	4,5
VG 273-16	anti-I-A^d	0	1	2	1	0	4	0	1	1	1	0,5	3,5

Table S2. B6 recipients of BALB/c kidney allografts subjected to 6 h CIS were treated with FTY720 on days -3-14 relative to transplantation and additionally injected with either mouse IgG2a, anti-K^d or anti-I-A^d on days 3, 7 and 11 after transplantation. Scores of glomerular, periglomerular, periarterial, interstitial and tubular CD4 and CD8 T cell infiltrate were determined at d. 14 posttransplant as illustrated in Figure S1. The scoring was performed by a pathologist (WMB) with no knowledge of experimental groups. Stars indicate fragmented cells.

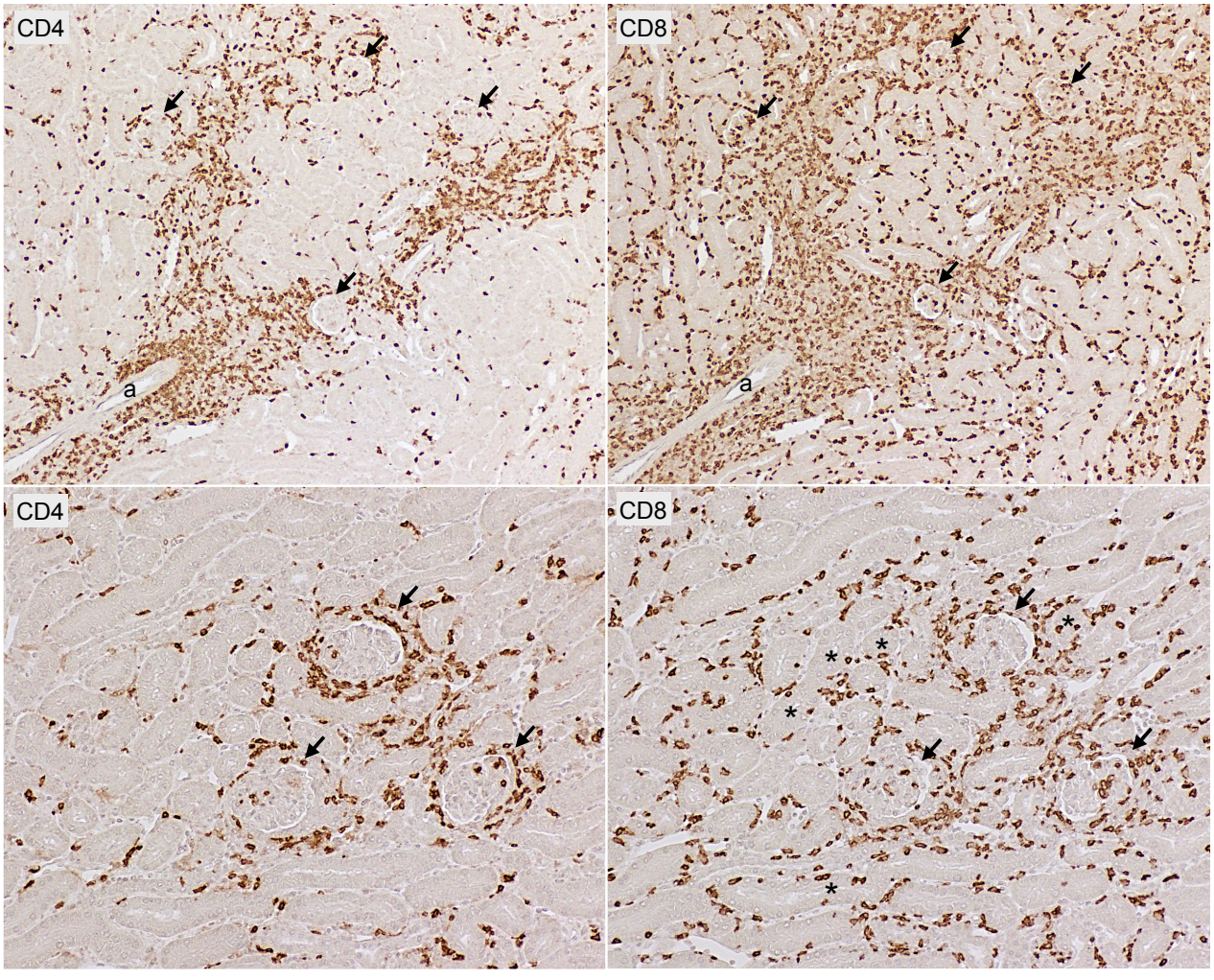

Figure S1. Examples of CD4 and CD8 infiltrates. Upper panels demonstrate periarterial, periglomerular, glomerular and interstitial infiltrates in serial sections ("a" indicates artery and arrows indicate glomeruli). Lower panels demonstrate periglomerular, glomerular and interstitial infiltrates together with tubulitis in serial sections (arrows indicate glomeruli and stars indicate tubulitis). CD8 cells were more numerous than CD4 cells in all compartments. Each compartment was scored as mild 1+, moderate 2+ and severe 3+.

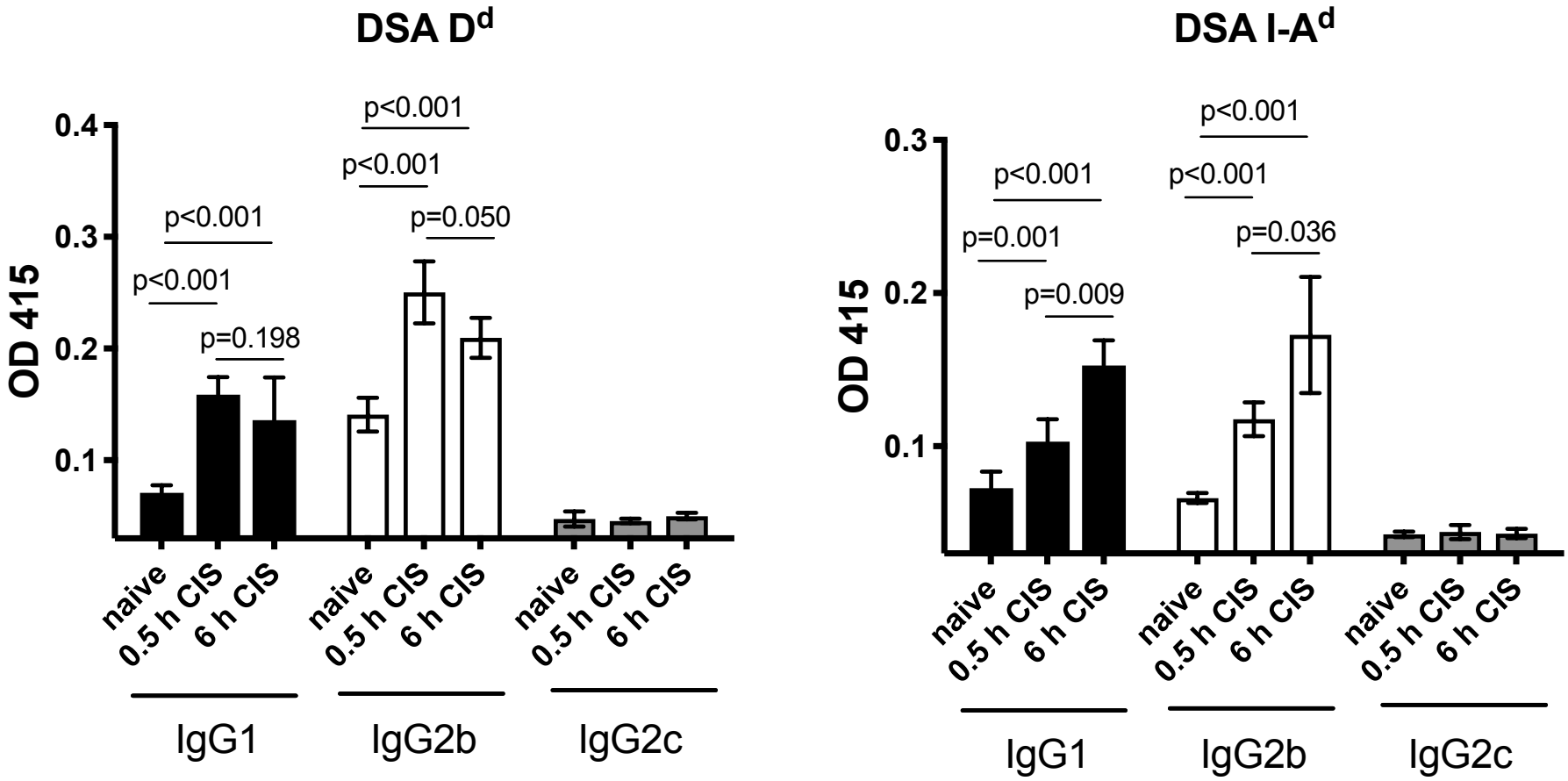

Figure S2. Prolonged CIS of renal allografts augments generation of anti-class II DSA. B6 mice were transplanted with BALB/c renal allografts subjected to 0.5 h or 6 h cold ischemia storage in UW solution. IgG1, IgG2b and IgG2c isotype antibodies were measured in pooled serum samples by ELISA ad d. 14 posttransplant.

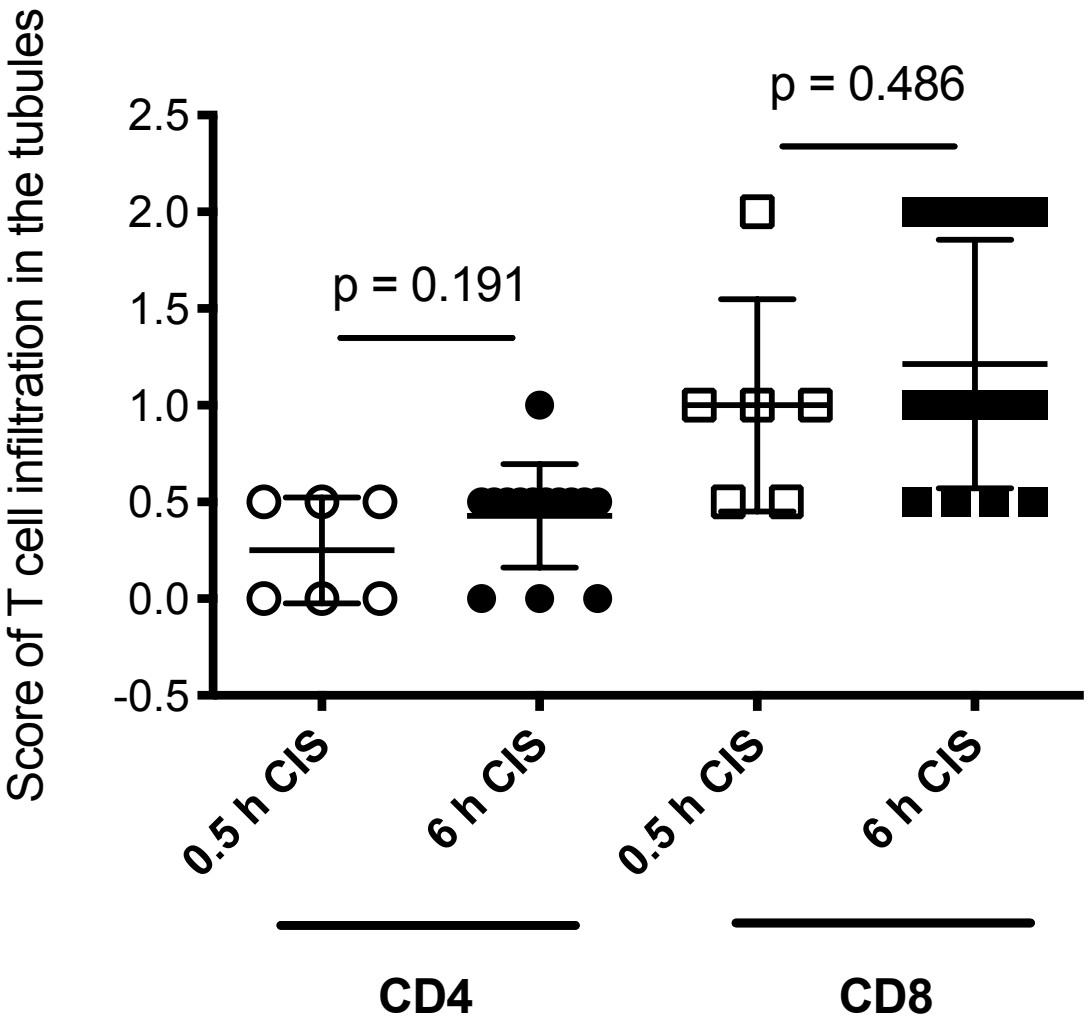

Figure S3. Scores of tubular T cell infiltrate at d. 14 posttransplant. Sections of kidney allografts were stained for CD4 and CD8 T cells. T cell infiltration in tubules was scored as described in Figure S1 and Table S1. Symbols represent individual allografts. N = 6 for 0.5 CIS group and 14 for 6 h CIS group.

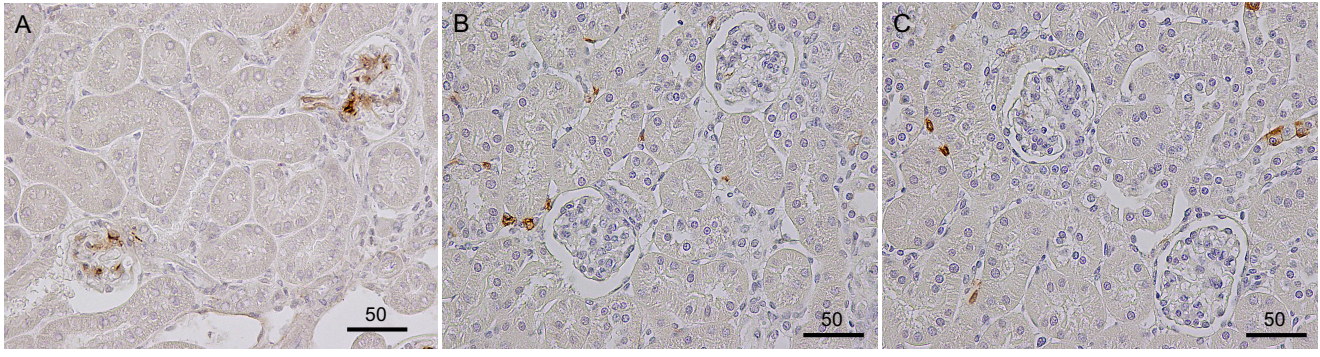

Figure S4. Immunohistology of renal isografts at 14 days subjected to 6 hours of cold ischemia before transplantation. **A**. No C4d deposits were detected in peritubular or glomerular capillaries, and minimal staining was localized in the mesangium. Only scattered CD4 (**B**) and CD8 (**C**)T cells were present in the interstitium. The images are representative of 6 isografts analyzed at d. 14.

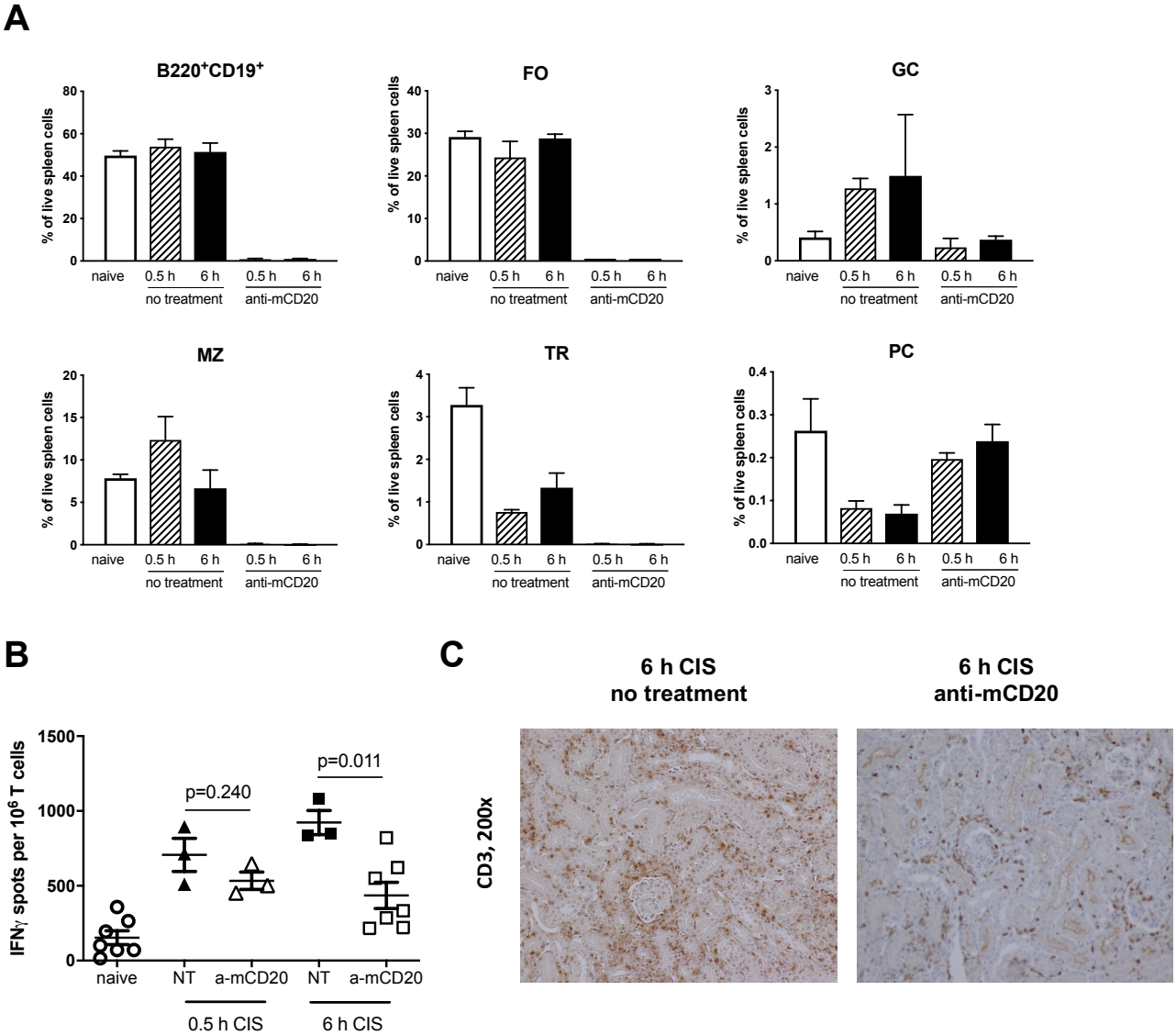

Figure S5. Effect of anti-mCD20 mAb treatment on spleen B cell subsets and anti-donor T cell responses. A. Percentages of total (B220+CD19+), follicular (FO), germinal ceneter (GC), marginal zone (MZ), and transitional (TR) B cells and plasma cells (PC) in the recipient spleen at d. 14 posttranpslant. The data are presented as mean ± SEM, N = 4-8 animals per group. **B.** IFNγ production by recipient spleen T cells on d. 14 posttransplant determined by ELISPOT assay. Graph symbols represent individual animals. **C.** Immunoperoxidase staining for CD3 performed on renal allografts on d. 14 posttransplant. The photographs are representative of 7-8 recipients per group.

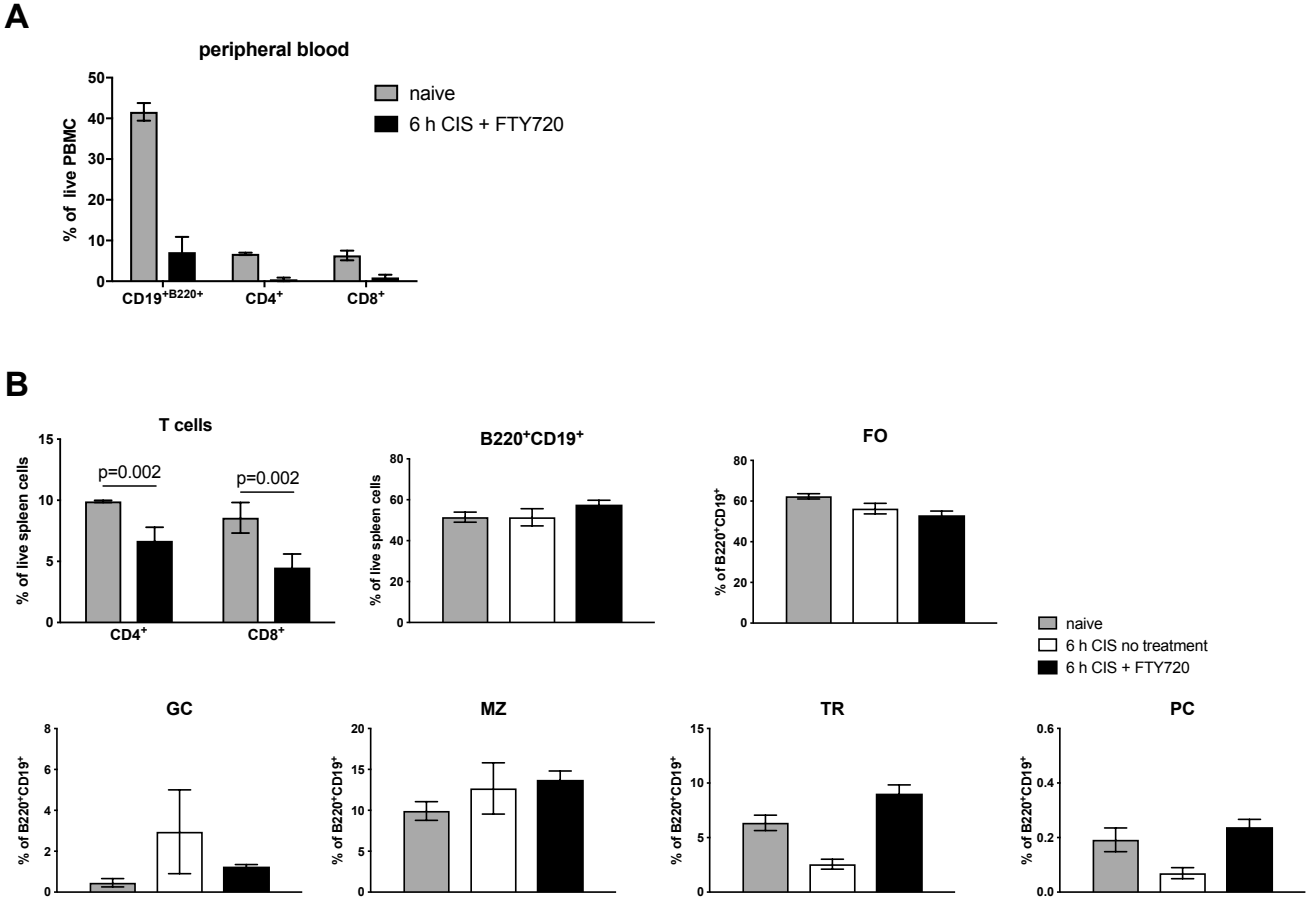

Figure S6. The effect of FTY720 treatment on peripheral lymphocyte subsets. B6 recipients of 6 h CIS BALB/c allografts were continually treated with FTY720 (3 mg/ml in drinking water) on days - 4 to 14 relative to transplantation. **A.** Percentage of $B220^+$, $CD4^+$ and $CD8^+$ subsets among live cells in peripheral blood at 14 days posstransplant. **B.** Percentages of T and B cell subsets in the spleen at d. 14 posstransplant. The data are represented as mean ± SEM, N = 3–6 animals per group.

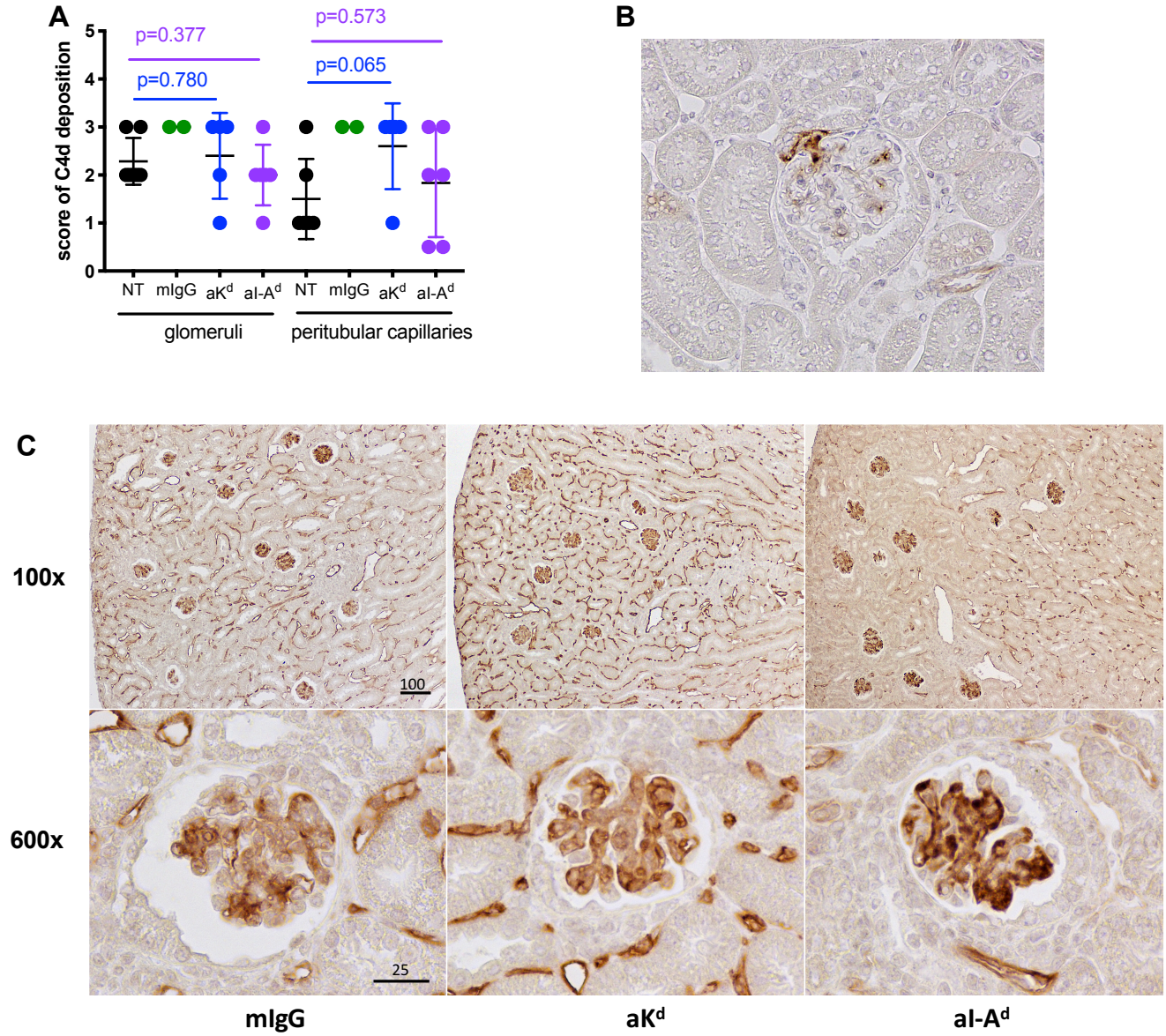

Figure S7. Histological evaluation of C4d deposition after monoclonal antibody transfer. B6 recipients of BALB/c kidney allografts subjected to 6 h CIS were treated with FTY720 on days -3-14 relative to transplantation and additionally injected with either mouse IgG2a, anti-K^d or anti-I-A^d on days 3, 7 and 11 after transplantation. **A.** Quantification of complement C4d deposition within glomeruli and peritubular capillaries at d. 14 posttransplant. **B.** C4d staining on renal isografts subjected to 6 hours of cold ischemia at 14 days after transplantation. No C4d deposits are present in the peritubular capillaries. Small amounts of C4d are localized to the mesangium and arterioles of glomeruli. **C.** Representative histology of glomerular C4d deposition. Passive transfer of isotype control (left) or antibodies to MHC class I antigen (middle) resulted in diffuse linear deposits of C4d on peritubular and glomerular capillaries. Passive transfer of antibodies to MHC class II antigen (right) also resulted in C4d deposits in the on peritubular and glomerular capillaries, but the deposits were much denser focally in many glomeruli. Many peritubular capillaries were dilated with swollen endothelial cells. N = 5-8 grafts for all groups except IgG2a (2 per group).