Supporting Information

The Potassium Channel Kv1.3 as a Therapeutic Target for Immunocytoprotection after Reperfusion

Supplementary Table 1

Group	**T2-weighed total infarct on day-8 after tMCAO** [% area of hemisphere]	**Neurological Score on day-8 after tMCAO** [normal mouse = 14]
16-week old WT males (n = 10)	20.46 ± 1.26	2.85 ± 0.45
16-week old Kv1.3$^{-/-}$ males (n = 14)	13.32 ± 1.26	4.58 ± 0.35
16-week old WT females (n = 11)	11.40 ± 1.42	4.94 ± 0.73
16-week old Kv1.3$^{-/-}$ females (n = 14)	7.42 ± 1.31	4.43 ± 0.48
PAP-1 treated 16-week old WT females (n = 15)	5.66 ± 0.77	6.79 ± 0.33
80-week old WT males (n = 9)	17.56 ± 1.90	2.33 ± 0.24
80-week old Kv1.3$^{-/-}$ males (n = 12)	13.29 ±3.15	7.90 ± 0.69
80-week old WT females (n = 9)	17.91 ± 2.10	3.22 ± 0.96
80-week old Kv1.3$^{-/-}$ females (n = 18)	8.16 ± 1.23	7.86 ± 0.49
PAP-1 treated 80-week old WT females (n = 9)	11.10 ± 1.50	8.00 ± 0.96

All data are mean ± S.E.M; Confidence intervals are shown in the main text figures. *P* values for various comparisons are provided in the main text and the Supplementary Figure Legends.

Supplementary Figure Legends

Supplementary Figure 1. Body weight evolution in young and old male and female C57BL/6J mice after tMCAO surgery. Shown are daily body weights before and for 8 days after 60-min of tMCAO in 16-week-old female (n = 11) and male (n = 10) mice (*Left*), and 80-week-old female (n = 9) and male (n = 9) male mice. Data are shown as whisker plots with induvial data overlay. The boxes show mean ± S.E.M, the whiskers show confidence intervals.

Supplementary Figure 2. Comparison of stroke severity in young versus old animals of the same sex. Please note that this is the same data as in Figure 1 of the main text, but here we are comparing percent of infarcted hemisphere area as determined by T2-weigthed MRI on day-8 and daily neurological deficit scoring between 16-week-old females (n = 11) and 80-week-old females (n = 9) [P =0.016 for infarct, P = 0.003 for NES], and between 16-week old males (n = 10) and 80-week old males (n = 9) [P =0.211 for infarct, P = 0.393 for NES]. Data are shown as whisker plots with individual data overlay. The boxes show mean ± S.E.M, the whiskers show confidence intervals.

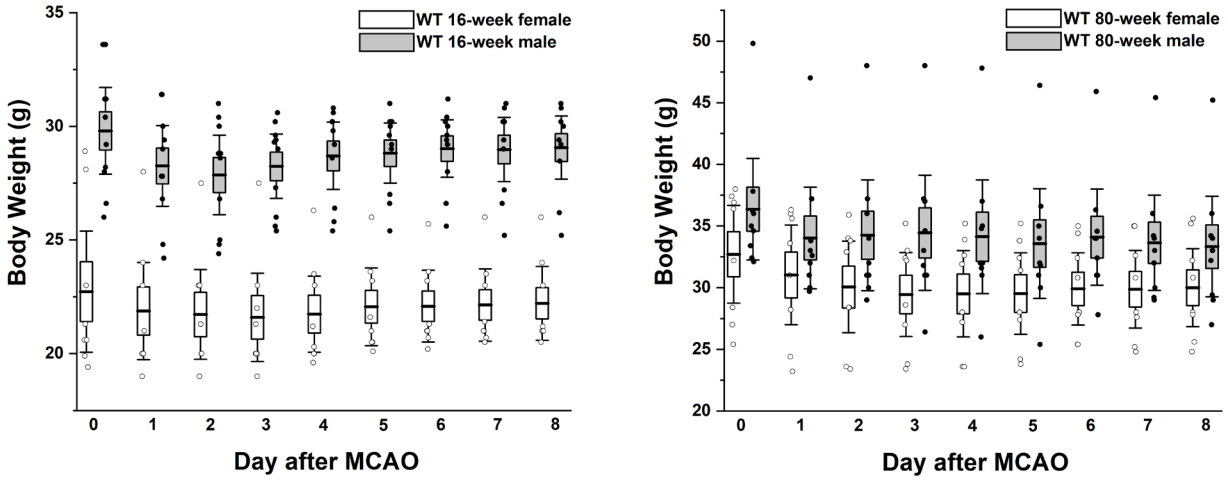
Supplementary Figure 3. Intense Kv1.3 staining is observed in the infarcted are in both sexes. Heterozygous male and female CX3CR1$^{+/GFP}$ mice, which express GFP (green fluorescent protein) instead of one copy of CX3CR1 in microglia, macrophages and dendritic cells, were used for tMCAO surgery. On day-8 animals were sacrificed and 14-µm thick cryosections were stained with a polyclonal rabbit anti-Kv1.3 antibody (Alomone, APC-101, 1:1000) followed by an Alexa Fluor®546-conjugated secondary antibody (1:1000, Life Technologies). Please note that

$CX3CR1^{+/GFP}$ mice were only used for immunohistochemistry but not for any treatment experiments because of their lack of one copy of CX3CR1.

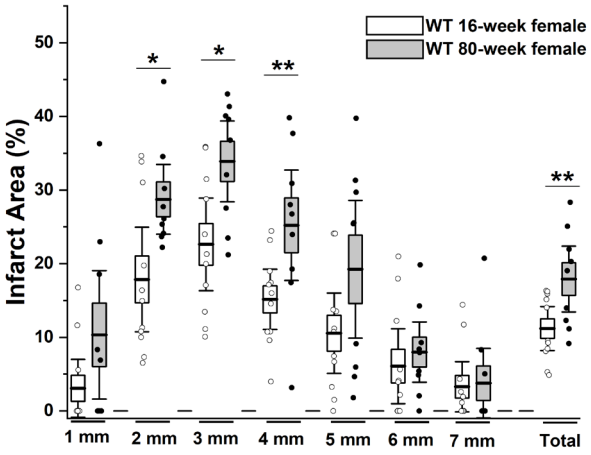
Supplementary Figure 4. Comparison of the composition of immune cell infiltrate in 16-week old male and female C57BL/6J mice on day-8 after tMCAO. Serial paraffin sections (5 μm thick) cut at 4 and 6 mm from the frontal pole from male and female mice were stained for Iba-1 and CD3 as described in the method section of the main text. Positive pixels were ratioed to the infarcted area and not to the hemisphere area to account for the smaller infarcts in young females. Data are shown as whisker plots with individual data overlay. The boxes show mean ± S.E.M, the whiskers show confidence intervals.

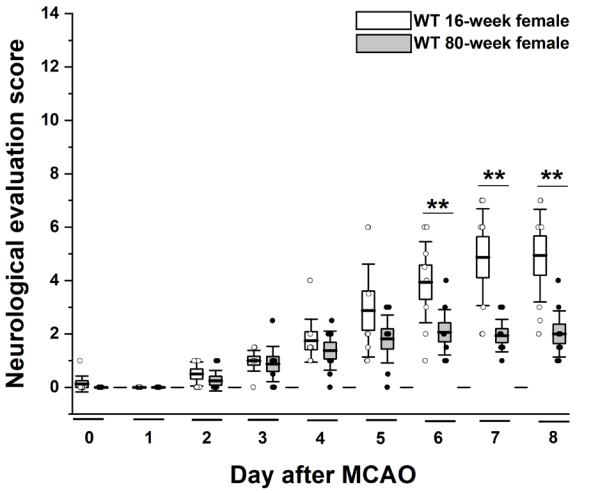
Supplementary Figure 5. Body weight evolution in WT versus $Kv1.3^{-/-}$ mice or versus PAP-1 treated mice after tMCAO surgery. Shown are daily body weights before and for 8 days after 60-min of tMCAO. (**A)** Body weights in 16-week-old male WT mice (n = 10) versus male $Kv1.3^{-/-}$ mice (n = 14), and in 16-week-old female WT (n = 11) versus female $Kv1.3^{-/-}$ mice (n = 14). (**B)** Body weights in 80-week old male WT mice (n = 9) versus male $Kv1.3^{-/-}$ mice (n = 12), and in 80-week-old female WT (n = 9) versus female $Kv1.3^{-/-}$ mice (n = 18). (**C)** Body weights in 16-week old-female WT mice (n = 11) versus female WT mice treated with 40 mg/kg of PAP1 (n = 15), and body weights in 80-week-old female WT mice (n = 9) versus female WT mice treated with 40 mg/kg PAP-1 (n = 9). Data are shown as whisker plots with individual data overlay. The boxes show mean ± S.E.M, the whiskers show confidence intervals.

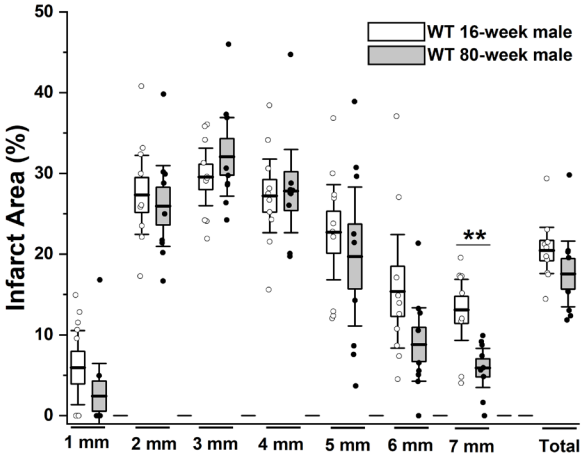
Supplementary Figure 1

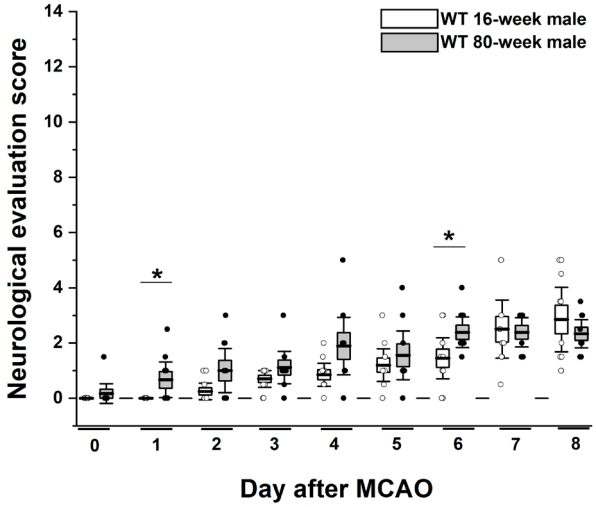


Supplementary Figure 2

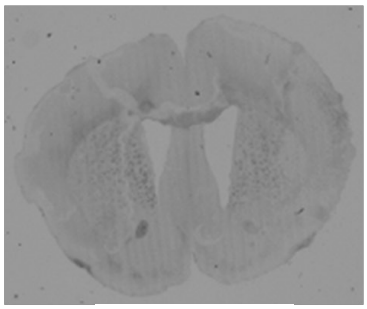








Supplementary Figure 3



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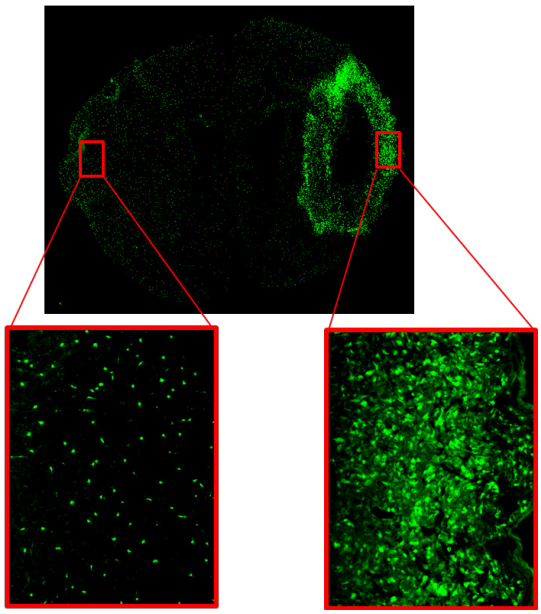


Heterozygous CX3CR1-GFP Mice

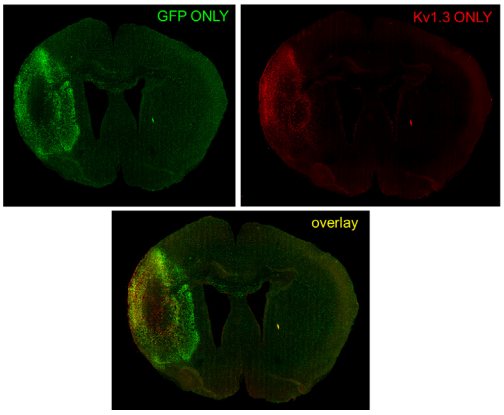
CX3CR1$^{+/GFP}$

Microglia

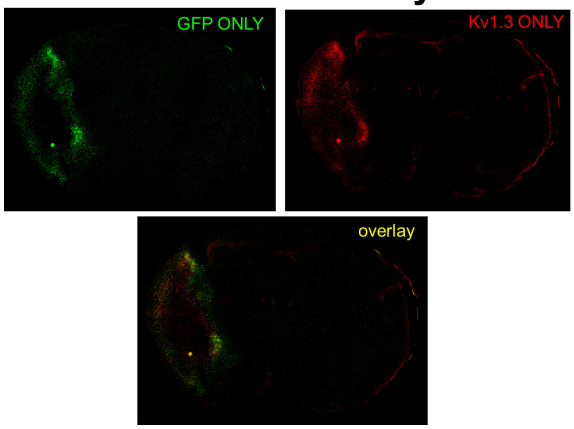
Macrophages

Dendritic cells

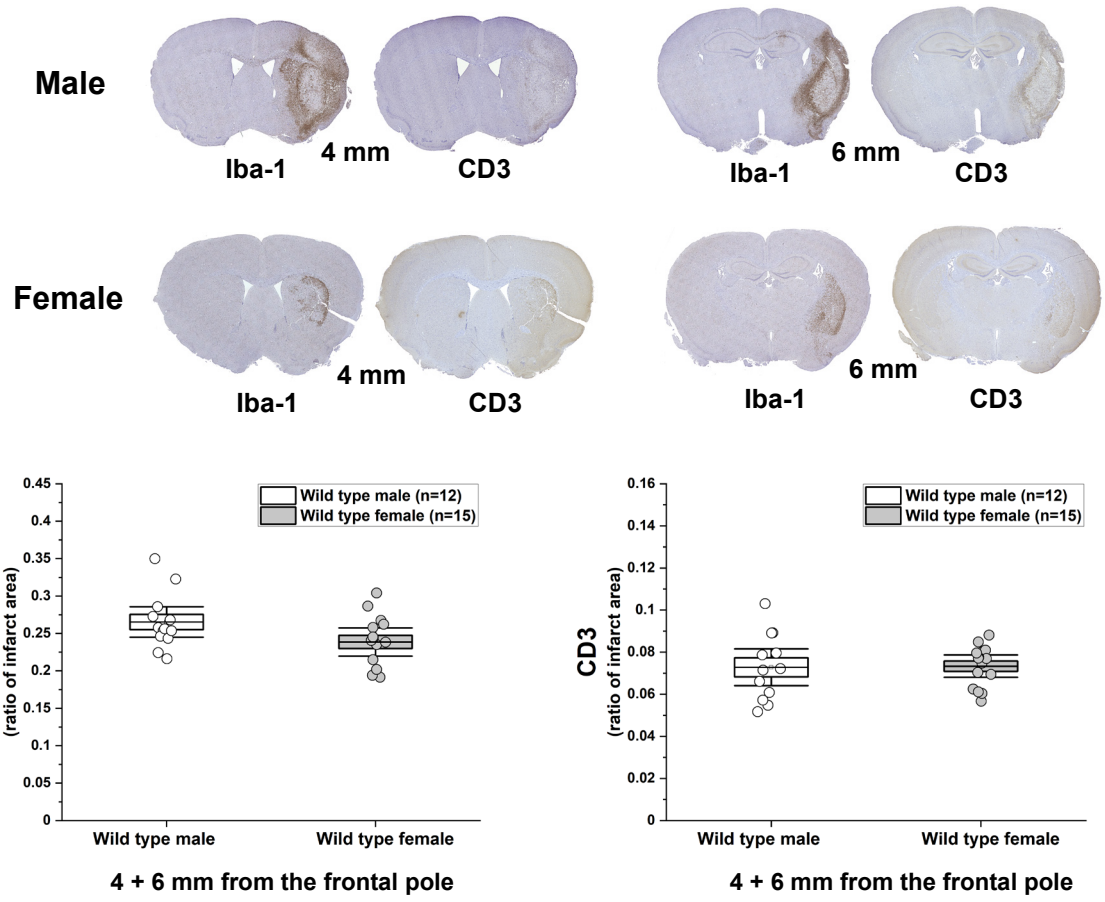
4 mm Male Day-8



6 mm Female Day-8



Supplementary Figure 4



Supplementary Figure 5