**Topical administration of a soluble TNF inhibitor reduces infarct volume after focal cerebral ischemia in mice**

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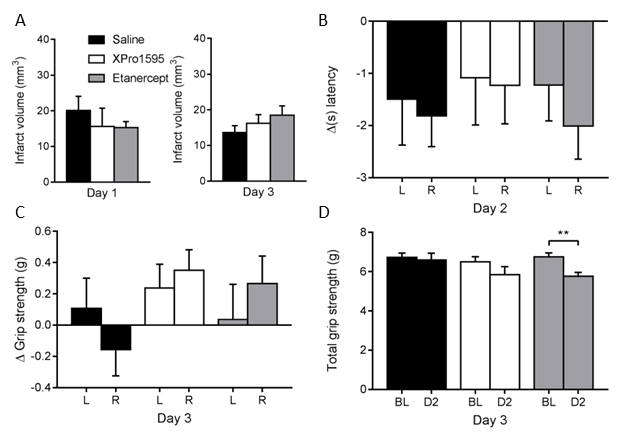
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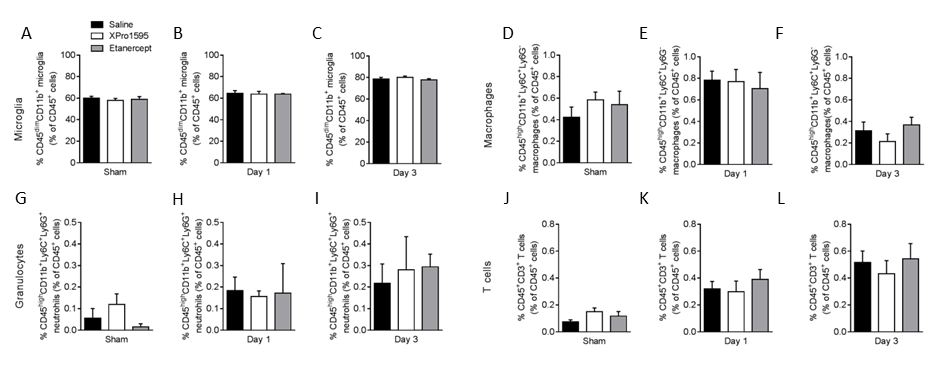
5Department of Health Science and Technology, University of Aalborg, Aalborg, Denmark

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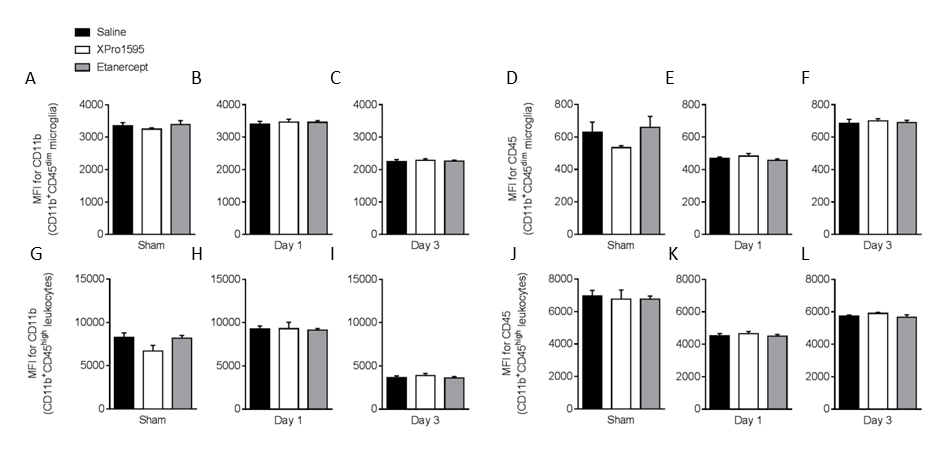
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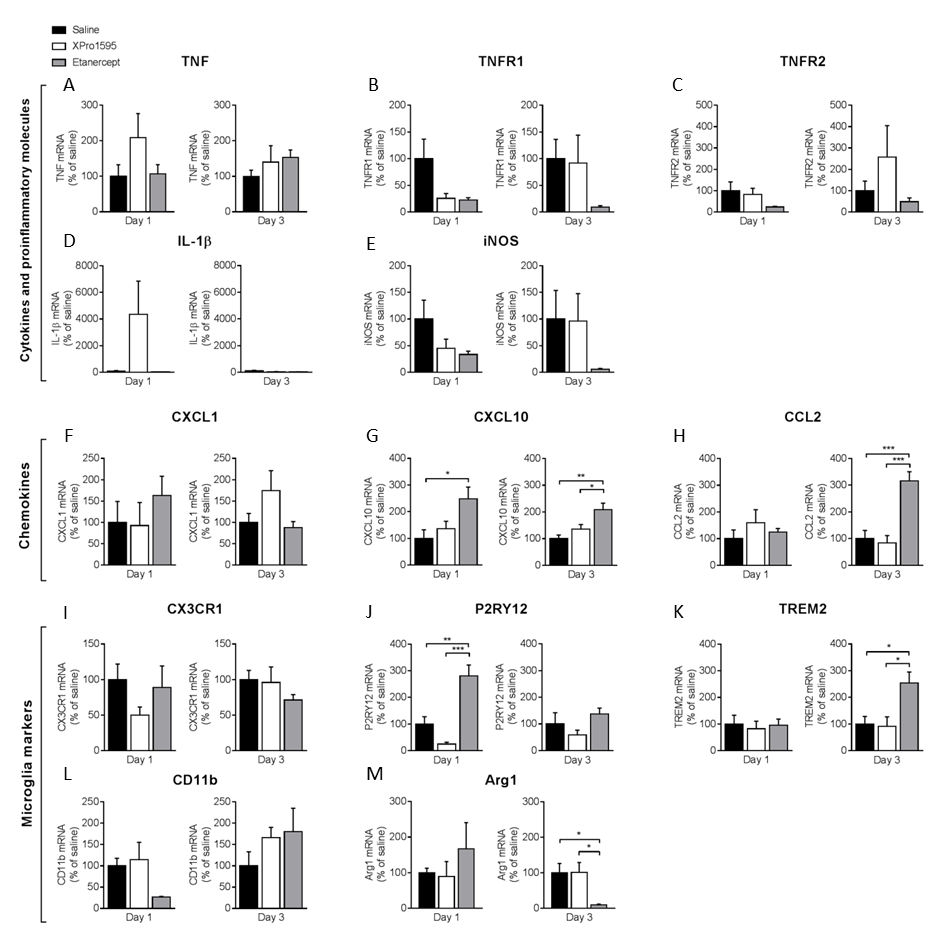
**Supplementary Figure 1. Infarct volume and functional outcome after i.c.v. administration**. **(A)** Infarct volume 1 and 3 days after i.c.v administration of TNF inhibitors n(day 1)= 3-5/group, n(day 3)=15-17/group. **(B)** Withdrawal latency after nociceptive stimuli measured by Hargreaves test two days after pMCAO; n=13-17/group. **(C)** Grip strength test of neuromuscular function measured as asymmetry between the right and left side three days after pMCAO; n=16-18/group. **(D)** Grip strength test of neuromuscular function measured as total grip strength of both paws three days after pMCAO; n=16-17/group. \*\*p≤0.01, Student’s paired t-test.



**Supplementary Figure 2. Flow cytometric analysis of contralateral hemispheres in sham and pMCAO mice after topical saline, XPro1595, or etanercept treatment. (A-C)** Changes in CD45dimCD11b+ microglia presented as % of CD45+ cells after sham surgery or 1 or 3 days after pMCAO. **(D-F)** Changes in CD45highCD11b+Ly6C+Ly6G- macrophages presented as % of CD45+ cells after sham surgery or 1 or 3 days after pMCAO. **(G-I)** Changes in CD45highCD11b+Ly6C+Ly6G+ granulocytes presented as % of CD45+ cells after sham surgery or 1 or 3 days after pMCAO. **(J-L)** Changes in CD3+ T cells presented as % of CD45+ cells after sham surgery or 1 or 3 days after pMCAO. n(sham)=4-5/group; n(day 1)=4-5/group; n(day 3)=5/group.



**Supplementary Figure 3. Mean fluorescence intensity (MFI) of CD11b and CD45 in the ipsilateral cortex of mice treated topically with saline, XPro1595, or etanercept. (A-C)** MFI for CD11b of CD11b+CD45dim microglia after sham surgery or 1 or 3 days after pMCAO. **(D-F)** MFI for CD45 of CD11b+CD45dim microglia after sham surgery or 1 or 3 days after pMCAO. **(G-I)** MFI for CD11b of CD11b+CD45high leukocytes after sham surgery or 1 or 3 days after pMCAO. **(J-L)** MFI for CD45 of CD11b+CD45high leukocytes after sham surgery or 1 or 3 days after pMCAO. N(sham)=4-5/group; n(day 1)=4-5/group; n(day 3)=5/group.



**Supplementary Figure 4. Inflammatory gene profile 1 and 3 days after pMCAO in mice treated i.c.v. with saline, XPro1595, or saline.** mRNA expression of cytokines, pro-inflammatory molecules, chemokines, and microglial markers presented as % of gene expression in saline-treated animals **(A-M). (A)** Expression of *Tnf* mRNA. **(B)** Expression of *Tnfrsf1a* (TNFR1) mRNA. **(C)** Expression of *Tnfrsf1b* (TNFR2) mRNA. **(D)** Expression of *Il1β* mRNA. **(E)** Expression of *iNOS* mRNA. **(F)** Expression of *Cxcl1* mRNA. **(G)** Expression of *Cxcl10* mRNA. **(H)** Expression of *Ccl2* mRNA. **(I)** Expression of *Cx3cr1* mRNA. **(J)** Expression of *P2ry12* mRNA. **(K)** Expression of *Trem2* mRNA. **(L)** Expression of *Cd11b* mRNA. **(M)** Expression of *Arg1* mRNA. N(day 1)=3-5/group, n(day 3)=4-6/group;\*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001; one-way ANOVA followed by Tukey’s *post hoc* test.



**Supplementary Figure 5. Cytokine and receptor protein expression in brain tissue 1 and 3 days after pMCAO in mice treated i.c.v. with saline, XPro1595, or etanercept. (A)** Expression of TNF. **(B)** Expression of TNFR1. **(C)** Expression of TNFR2. **(D)** Expression of IL-1β. **(E)** Expression of Cxcl1. **(F)** Expression of IL-6. **(G)** Expression of IL-10. **(H)** Expression of IL-4. **(I)** Expression of IL-5. **(J)** Expression of IL-12p70. N=3-6/group, \*\*p≤0.01; one-way ANOVA with Tukey’s *post hoc* test.

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**Supplementary Figure 6. Proteomics of neurons from tmTNFΔ/Δ and tmTNFwt/wt mice.** Up- and downregulated proteins in neurons from tmTNFΔ/Δ and tmTNFwt/wt mice. N(tmTNFwt/wt)= 4, n(tmTNFΔ/Δ)= 6.