Nitric oxide synthase mediates cerebellar dysfunction in mice exposed to repetitive blast-induced mild traumatic brain injury

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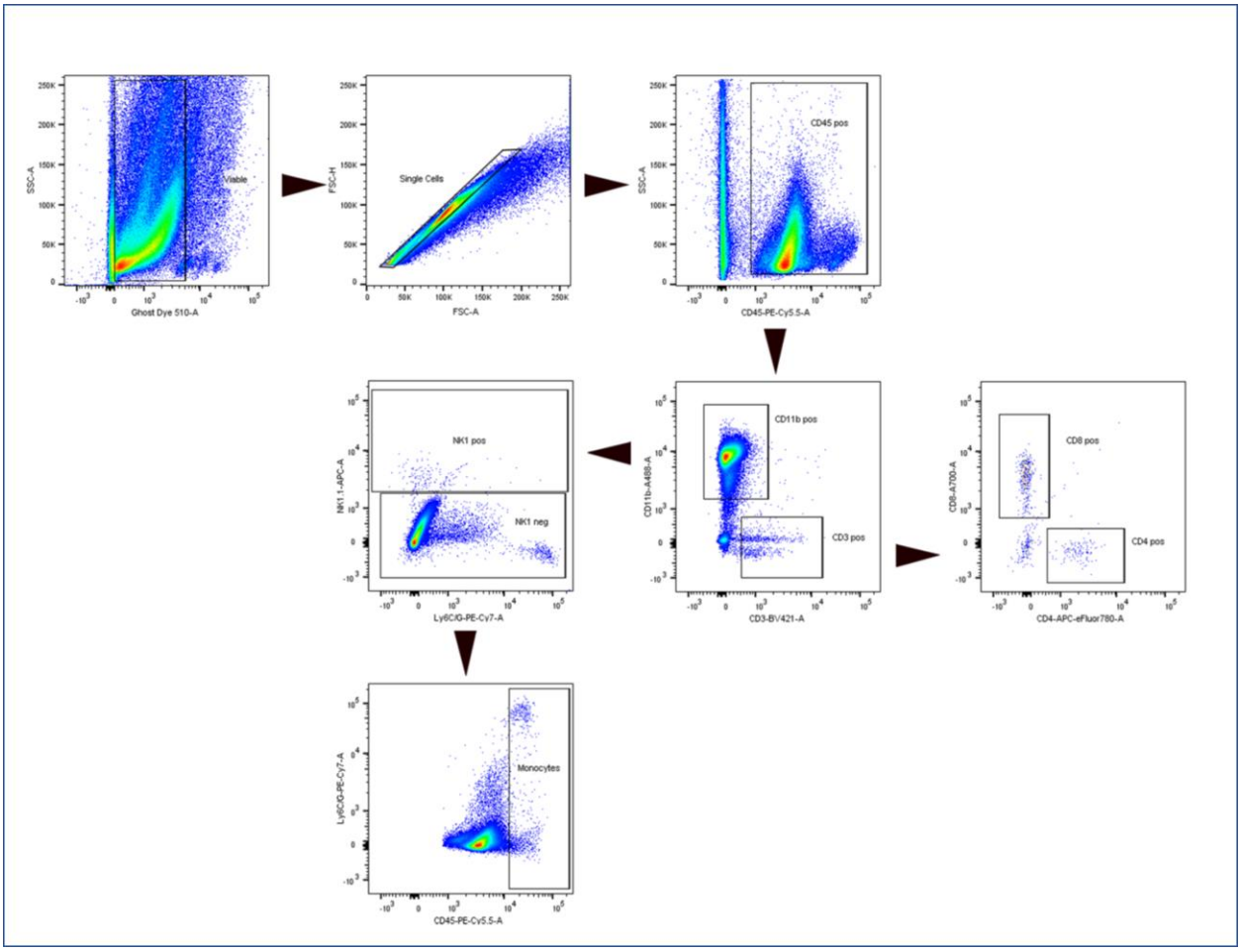
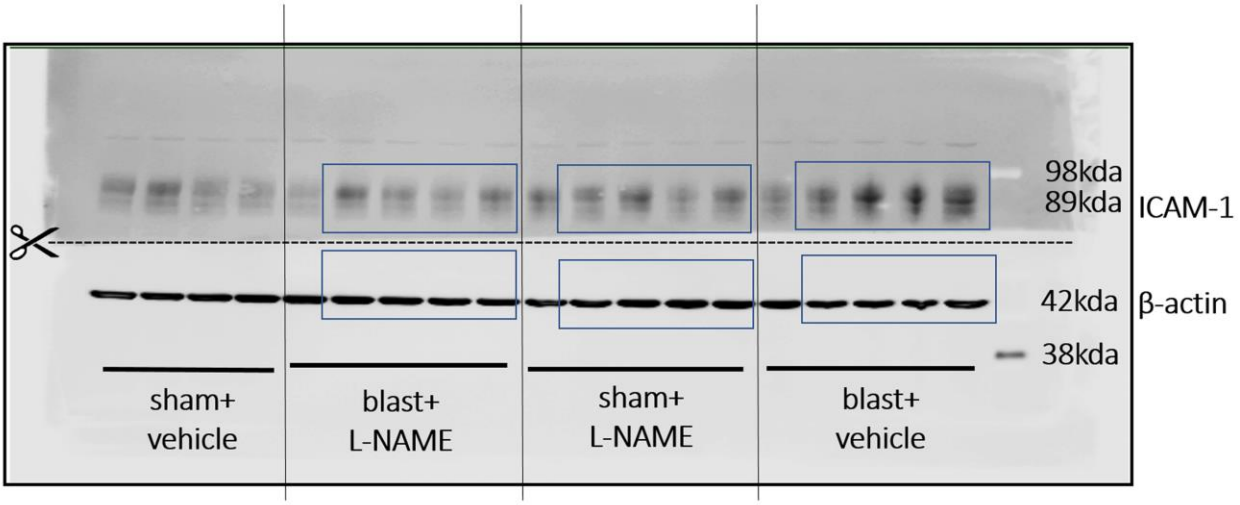
Supplementary Figures

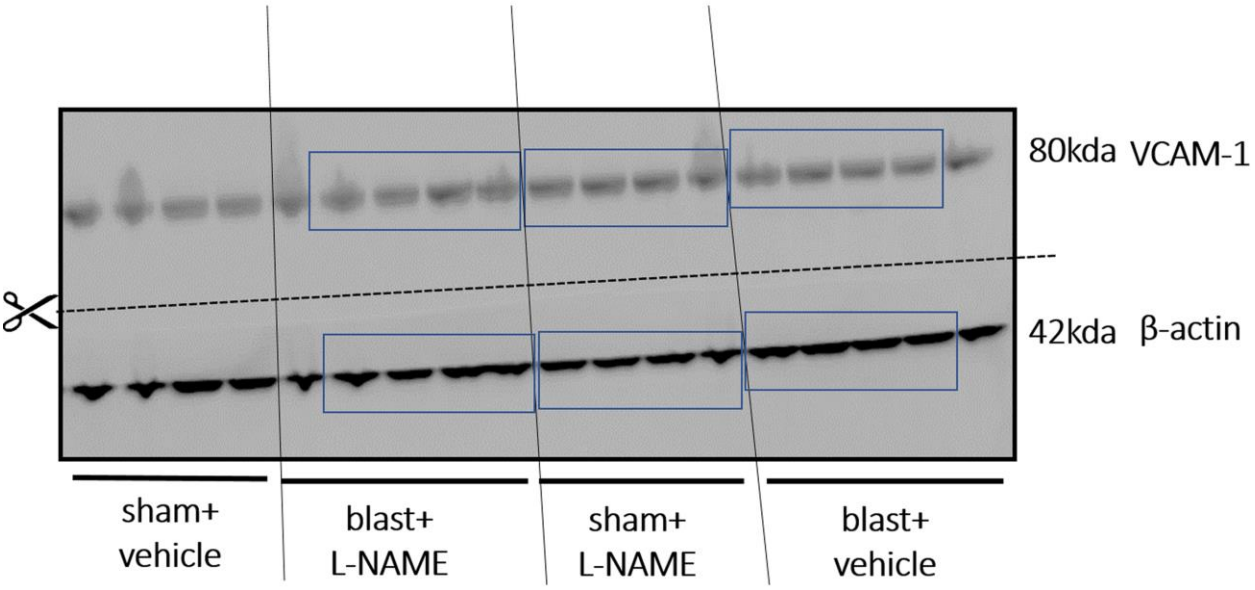


Supplementary Figure 1. Flow cytometry gating strategy. Viable cells were identified and dead cells and debris eliminated by side scatter and low binding of a viability dye. Single cells were identified based on FSC-Area vs FSC-Height. Microglia and leukocytes are characterized by CD45 positivity, and divided by the binding of CD11b (microglia and myeloid cells) or CD3 (T cells). The T cells were classified as CD4+ or CD8+. NK1-negative CD11b-positive cells were identified as microglia (CD45-low) or monocyte/macrophage (CD45-high) based on CD45 expression. FSC-A/SSC-A

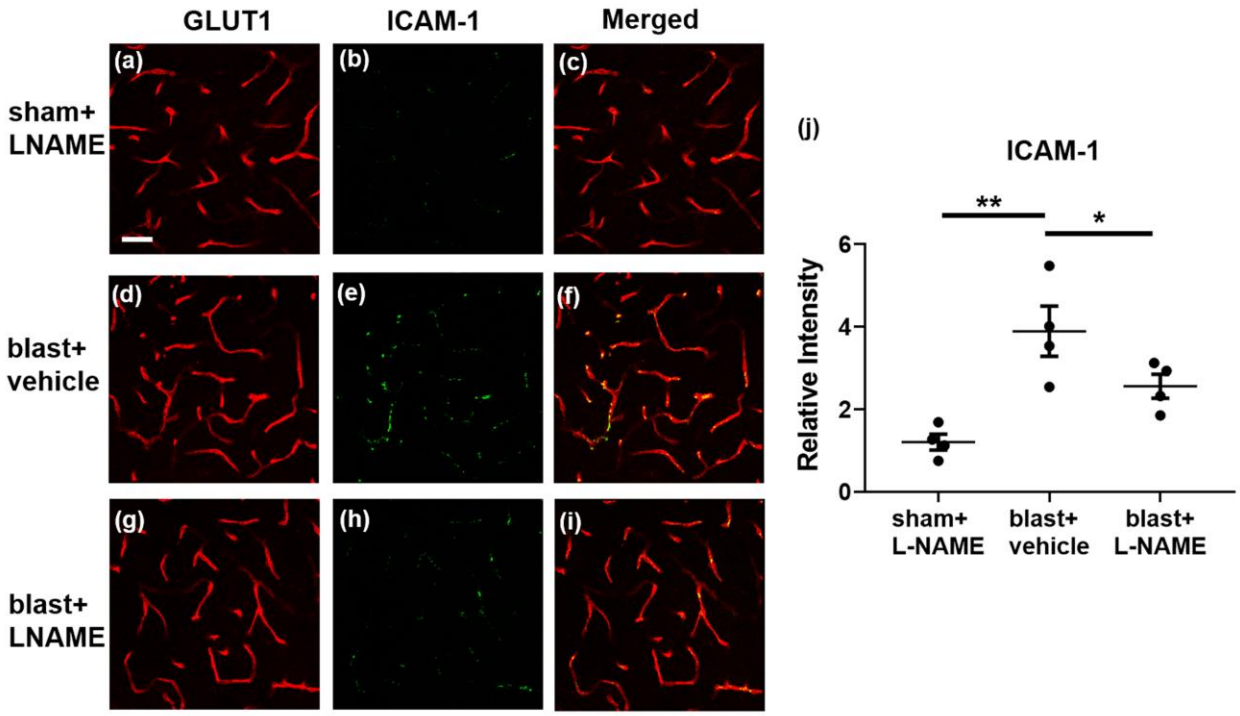
analysis of the CD45-high population (not shown) indicated that granulocytes (high SSC-A) were a minority of this population at the time of these analyses.



Supplementary Figure 2. Full length immunoblots. Western blot analysis of ICAM-1 (89kDa) and β-Actin (42kDa) from mouse cerebellum. Inset blue boxes demarcate representative immunoblots for ICAM-1 and β-actin in **Figure 5A**.



Supplementary Figure 3. Full length immunoblots. Western blot analysis of VCAM-1 (80kDa) and β-Actin (42kDa) from mouse cerebellum. Inset blue boxes demarcate representative immunoblots for VCAM-1 and β-actin in **Figure 5B**.



Supplementary Figure 4. Repetitive blast significantly increased ICAM-1 on GLUT1^{+} blood vessels in the cerebellum. **(a)** Shows representative immunofluorescent images of GLUT1 (red), **(b)** ICAM-1 (green), and **(c)** merged within lobule IX of the cerebellum in control mice at 72 h after 3X Sham and L-NAME administration. **(d)** Shows representative images of GLUT1, **(e)** ICAM-1, and **(f)** merged at 72 h after repetitive mTBI. **(g)** Shows representative images of GLUT1, **(h)** ICAM-1, and **(i)** merged at 72 h after repetitive mTBI with L-NAME (10 mg/kg, ip injected 48, 54, and 71 h after the last blast). (**j**) A significant increase in ICAM-1 ($F_{(2,9)}$=10.93; **p≤0.01) was observed in lobule IX of the cerebellum of mice exposed to repetitive mTBI plus vehicle (**p≤0.01), and L-NAME significantly attenuated this effect (*p≤0.05). One-way ANOVA *post hoc* Newman-Keuls. Values represent means±SEM. Scale bars=30 µm.