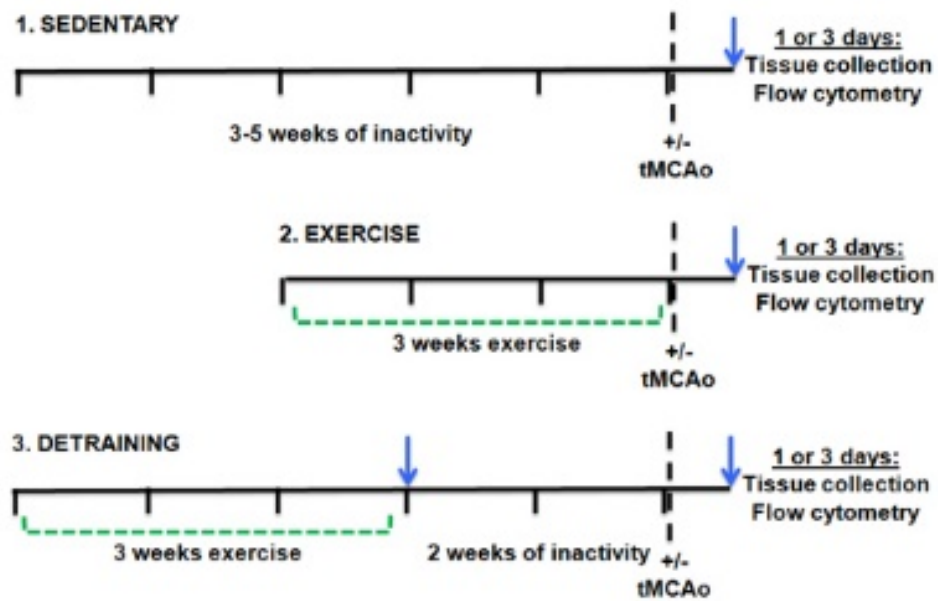


Detraining ablates exercise-induced modulation of the immune system after stroke

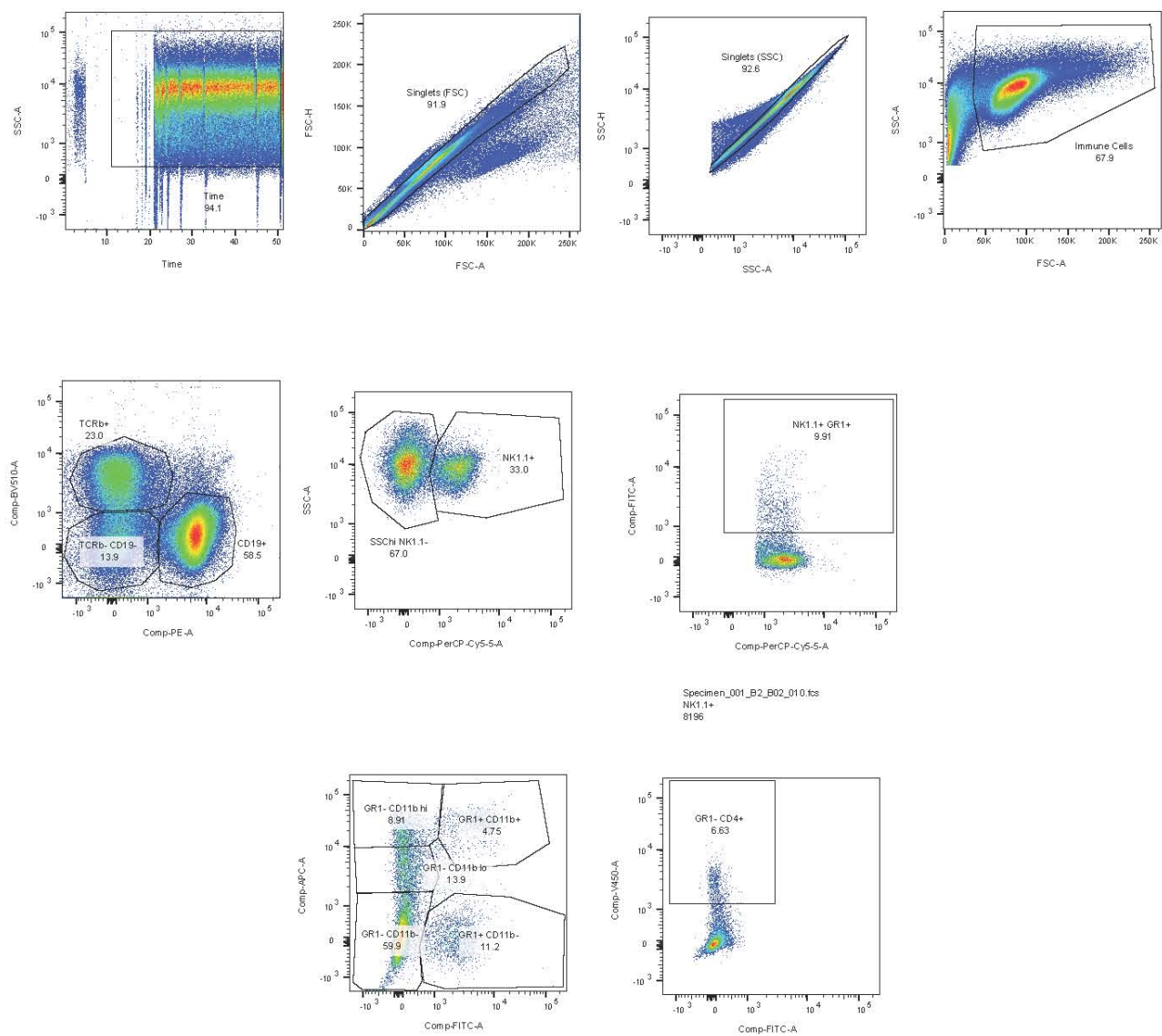
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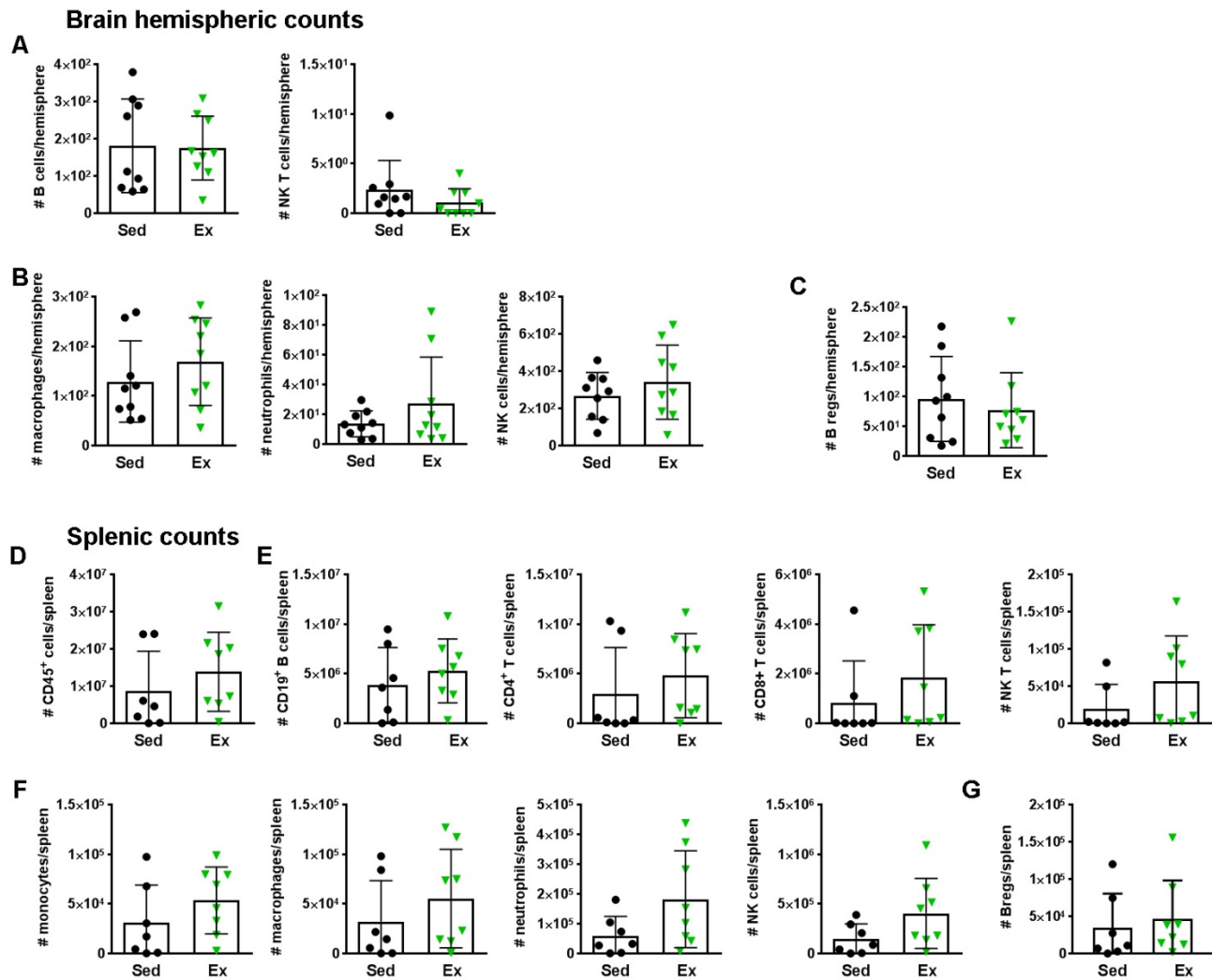
Supplemental Figures



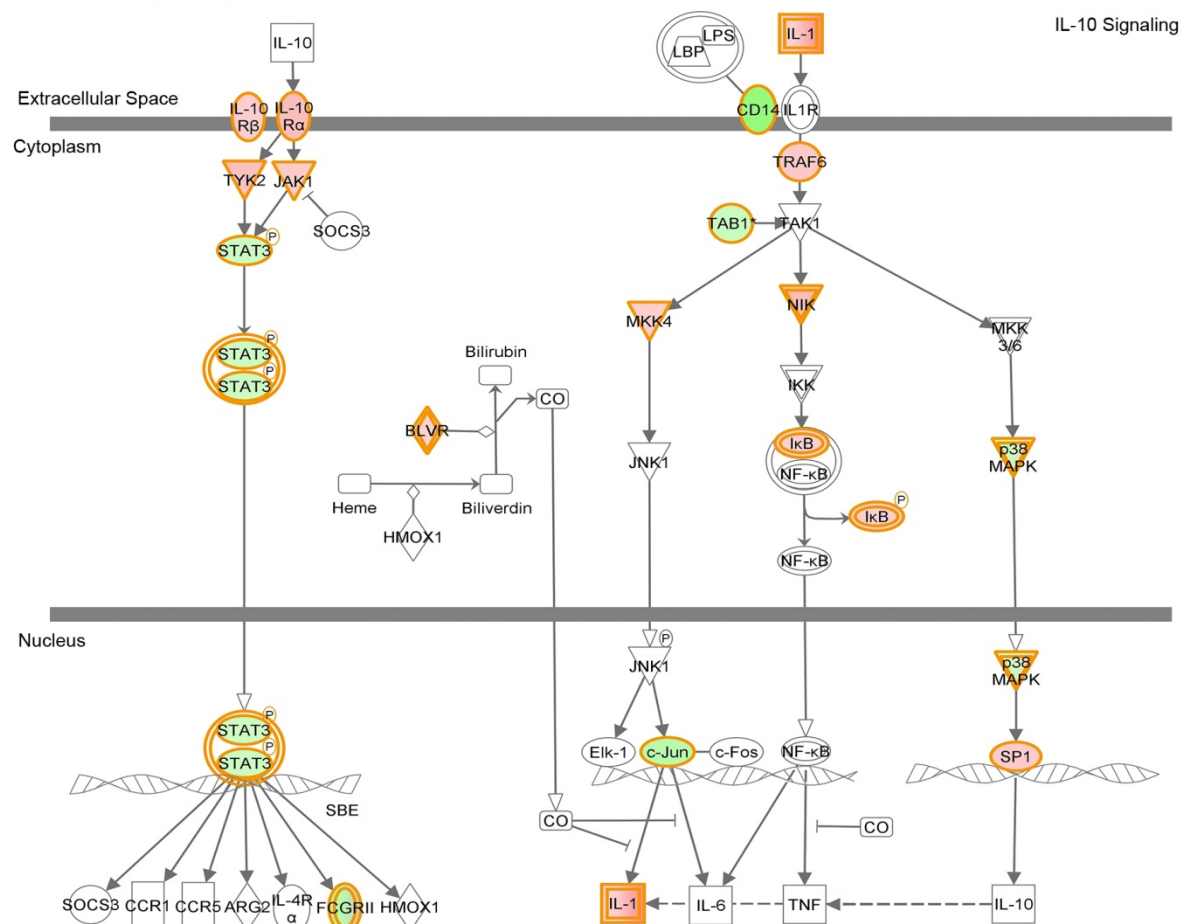
Supplemental Fig. 1. Experimental Timelines. Experimental timeline for sedentary, voluntary exercise, and detraining training regimens prior to transient middle cerebral artery occlusion (tMCAo).



Supplemental Fig. 2 Gating strategy for innate and adaptive immune cells.

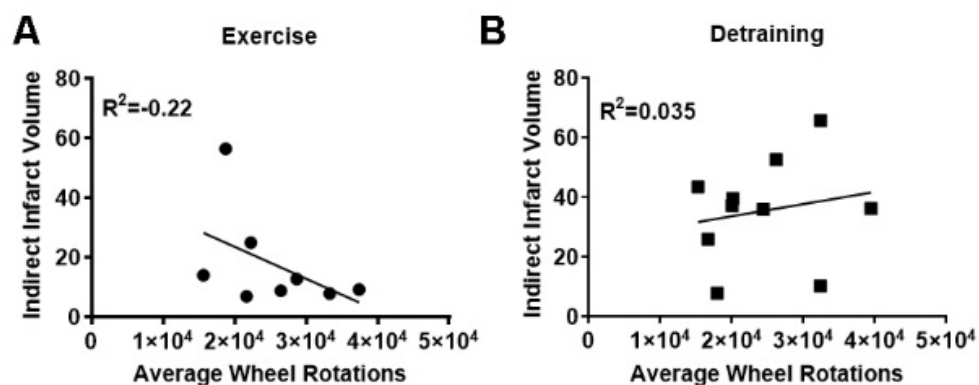


Supplemental Fig. 3 Exercise minimally affects leukocyte populations in the brain or spleen of uninjured mice. Lymphocyte subsets are identified for individual sedentary (Sed; black circles; n=9) and exercise (Ex; green triangles; n=9). Exercise exhibits minimal effects on the numbers (identified in Y axis) of (A) adaptive, (B) innate, and (C) B regulatory (Breg) immune cells in the brain, or (D-G) corresponding populations in the spleen, shown as mean \pm standard deviation.

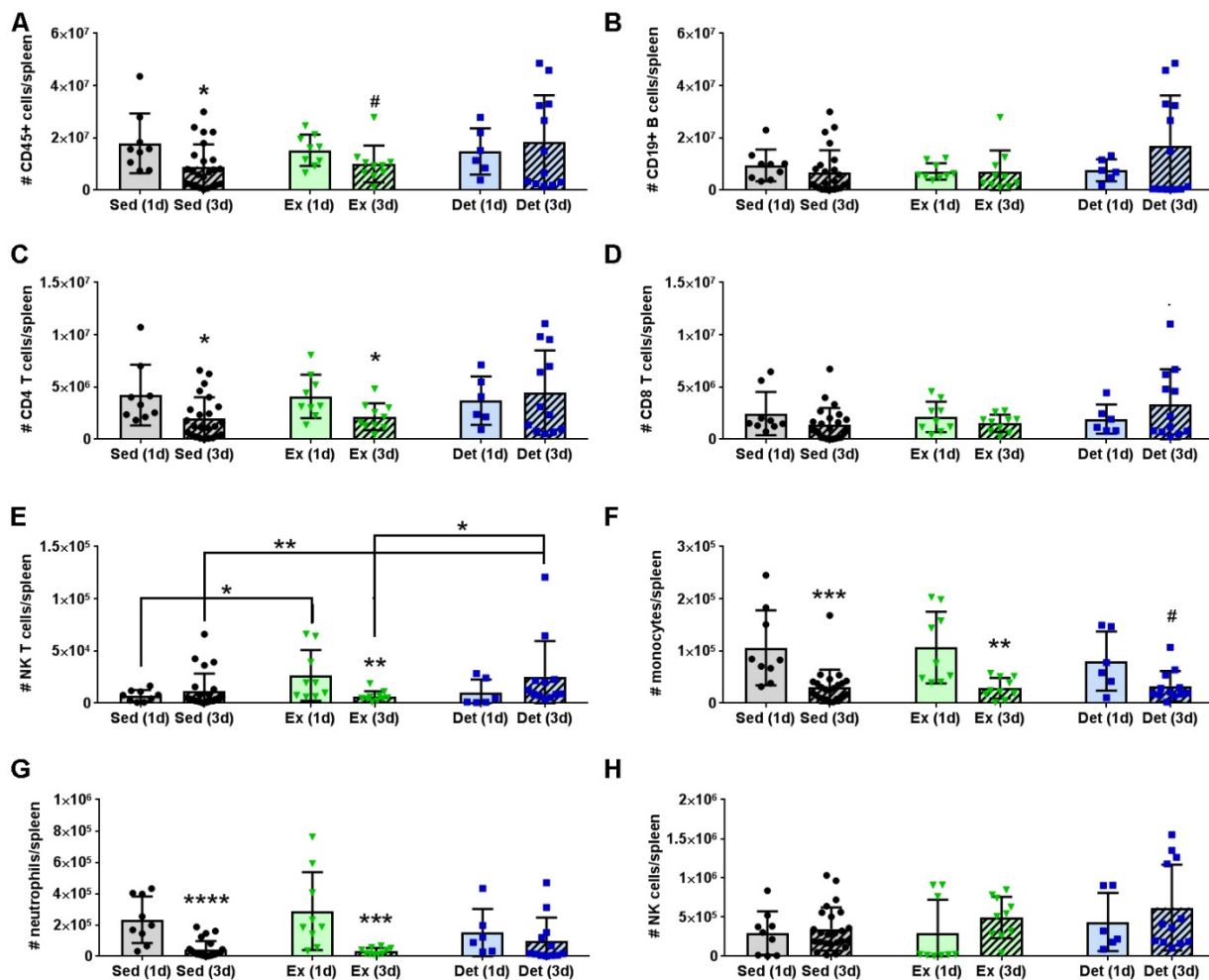


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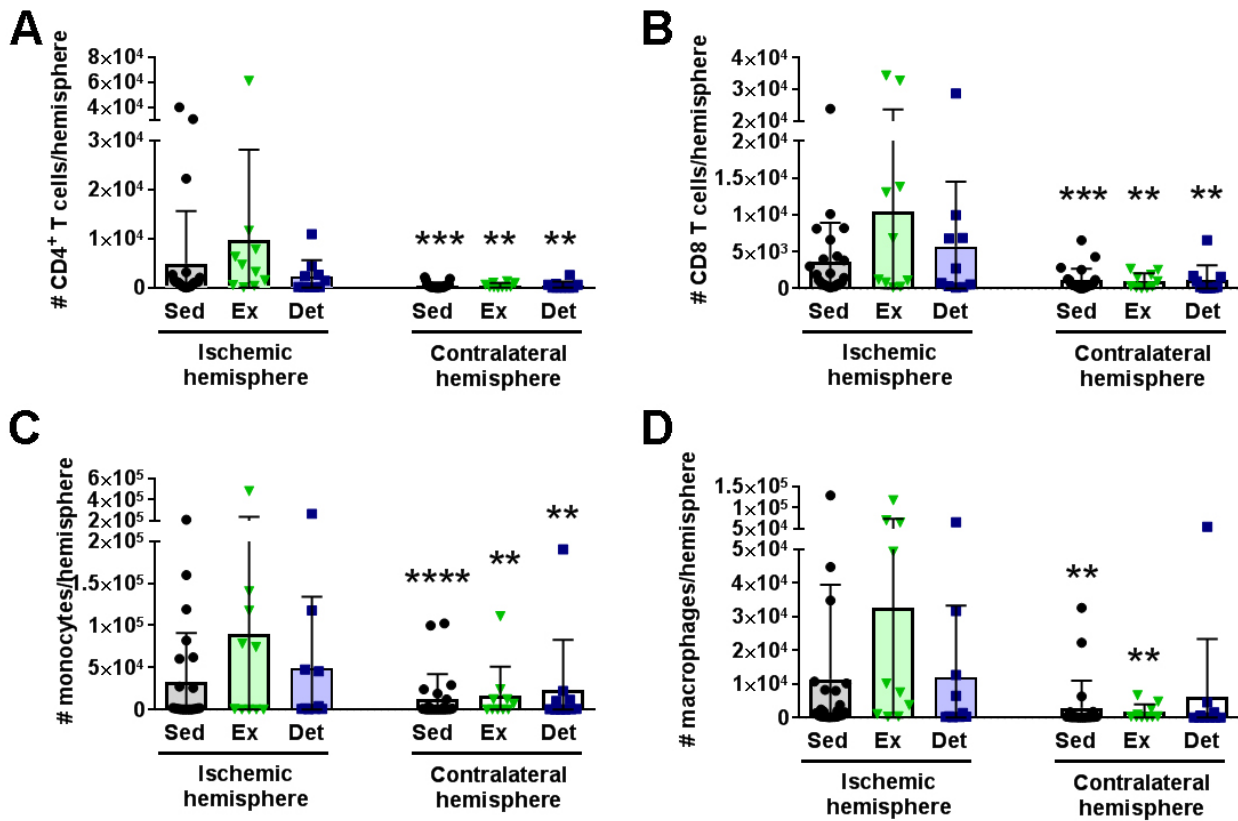
Supplemental Fig. 4 The effect of voluntary exercise on IL-10 signaling in B cells. Gene upregulation (red) and downregulation (green) for IL-10 signaling pathways are shown. Figure generated by IPA.



Supplemental Fig. 5. Exercise volume does not affect infarct volume. Average wheel rotations (x axis) and infarct volumes (y axis) show no correlation on injury for either (A) exercise (black circles) or (B) detrained (black squares) mice.



Supplemental Fig. 6 Detraining elevates splenic populations 3 days after stroke. Data are shown for individual leukocyte subsets (Y axis) for sedentary (Sed; black circles; n=9-23), exercise (Ex; green triangles; n=9-12) and detraining (Det; blue squares; n=6-12) cohorts at 1 day (d) and 3 days after stroke. In Sed and Ex mice, stroke induces a decrease in (A) general leukocytes, (C) CD4⁺ T cells, (F) monocytes, and (G) neutrophil populations that is lost with detraining. At 3d after stroke, Det mice exhibit elevations in (D) CD8 T cell, and (E) NK T cell populations. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, vs. day 1 unless otherwise indicated by brackets.



Supplemental Fig. 7 Exercise or detraining does not influence T cell and monocyte/macrophage egress into the brain. Data graphed for sedentary (Sed; black circles; n = 23), exercise (Ex; green triangles; n = 9) detraining (Det; blue squares; n = 13) populations in the ischemic hemisphere (left bars) and the uninjured contralateral hemisphere (right bars). In general, there was significant leukocyte egress in the ischemic hemisphere compared to the contralateral hemisphere for (A) CD4⁺ T cells, (B) CD8 T cells, (C) monocytes, and (D) macrophages into the ischemic brain over contralateral hemisphere. ** p<0.01; *** p<0.001; **** p<0.0001, #p<0.06 vs. ischemic hemisphere.

Table 1. Exercise intensity vs. cell population

	Spleen		CNS	
	R ²	Slope	R ²	Slope
CD45+ Leukocytes	0.17	–	0.06	–
B cells	0.26	–	0.05	–
CD4 T cells	0.12	–	0.58*	\
CD8 T cells	0.12	–	0.52*	\
NK T cells	0.14	–	0.03	–
NK cells	0.16	–	0.16	–
Neutrophils	0.001	–	0.1	–
Monocytes	0.18	–	0.01	–
Macrophages	0.04	–	0.28	–
Regulatory B cells	0.19	–	0.33	–