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Title	The impact of fingolimod on Treg function in brain ischaemia
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Supplemental Table 1: Flow cytometry antibody reagents

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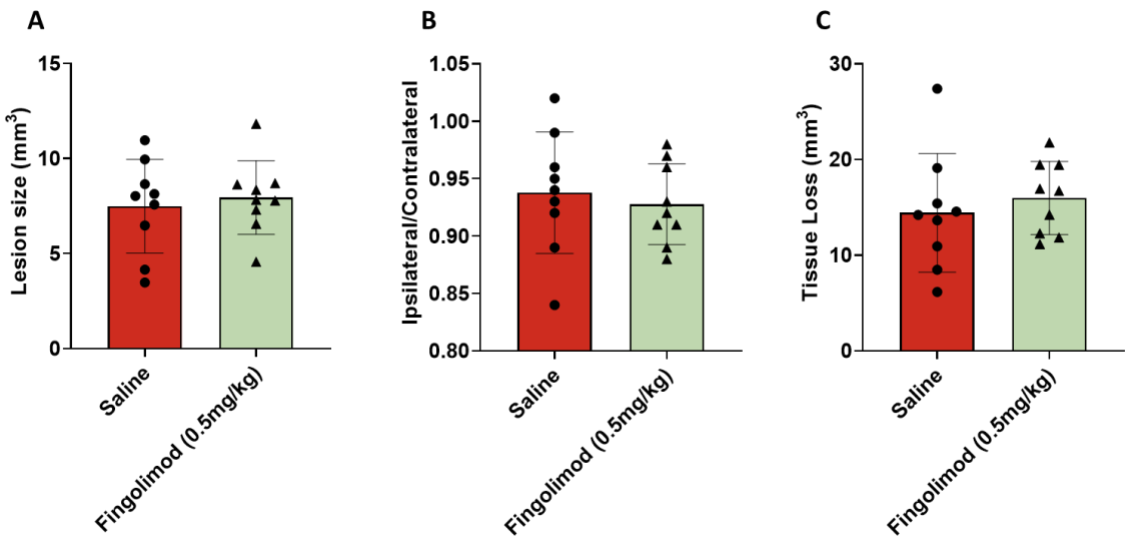
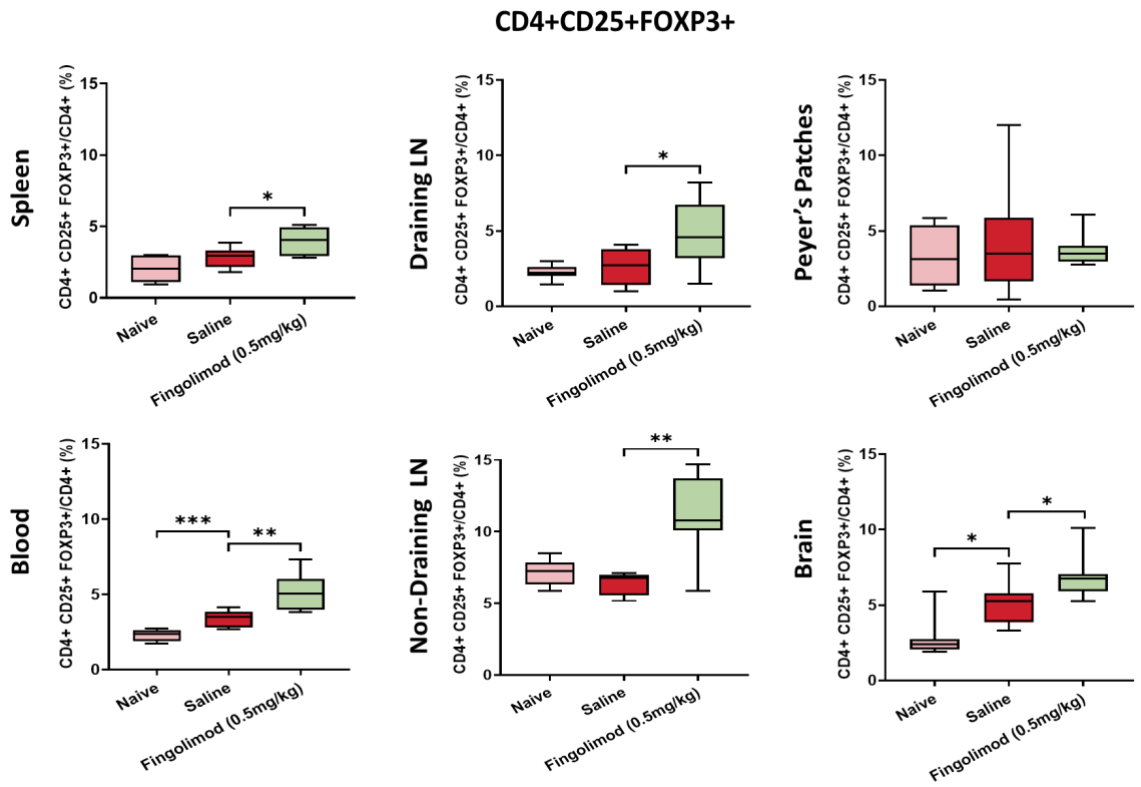
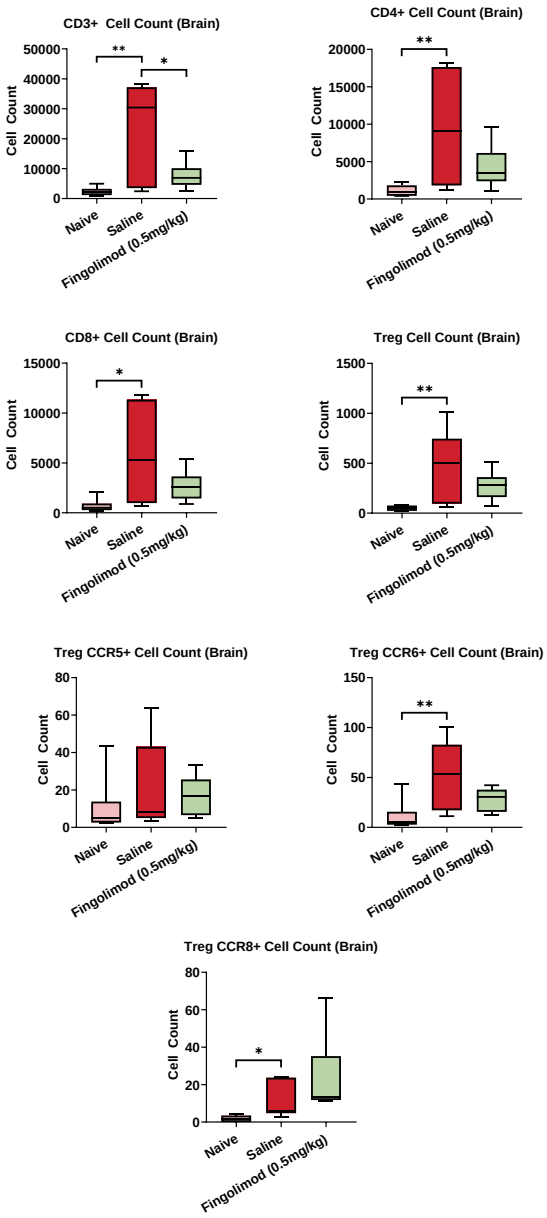
Panel 1: Chemokine Expression (Target, fluorochrome, clone)	**Cat #**	**Titration**	**Supplier**
Anti-Mouse CD45 PerCP-CY5.5 (30-F11)	#45045182	1:100	Thermo Fisher
Anti-Mouse CD3e PE-Cy7 (145-2C11)	#14003182	1:100	Thermo Fisher
Anti-Mouse CD4 FITC (RM4-5)	#11004282	1:800	Thermo Fisher
Anti-Mouse CD8a Pacific Blue (5H10)	#MCD0828	1:100	Thermo Fisher
Anti-Mouse CD25 APC (PC61.5)	#17025182	1:100	Thermo Fisher
CCR5: Anti-Mouse CD195 PerCP eFluor710 (HM-CCR5 (7A4))	#46195182	1:100	Thermo Fisher
CCR6: Anti-Mouse CD196 PE-Vio615 (REA277)	#130108396	1:20	Miltenyi Biotec
CCR8: Anti-Mouse CD198 PE-Vio615 (REA921)	#130119922	1:50	Miltenyi Biotec
Anti-mouse FoxP3 PE (FJK-16s)	#12577382	1:100	Thermo Fisher
Fixable Viability Dye eFluor 780	#65086514	1:10000	Thermo Fisher
Panel 2: Cytokine Expression (Target, fluorochrome, clone)	**Cat #**	**Titration**	**Supplier**
Anti-Mouse CD3e PE-Cy7 (145-2C11)	#14003182	1:100	Thermo Fisher
Anti-Mouse CD4 FITC (RM4-5)	#11004282	1:800	Thermo Fisher
Anti-Mouse CD8a Pacific Blue (5H10)	#MCD0828	1:100	Thermo Fisher
Anti-Mouse IFN-γ eFluor610 (XMG1.2)	#61731182	1:100	Thermo Fisher
Anti-Mouse IL-17 PE (TC11-18H10)	#559502	1:100	BD
Anti-Mouse IL-10 PerCP-Cy5.5 (JES5-16E3)	#45710182	1:50	Thermo Fisher
Fixable Viability Dye eFluor 780	#65086514	1:10000	Thermo Fisher



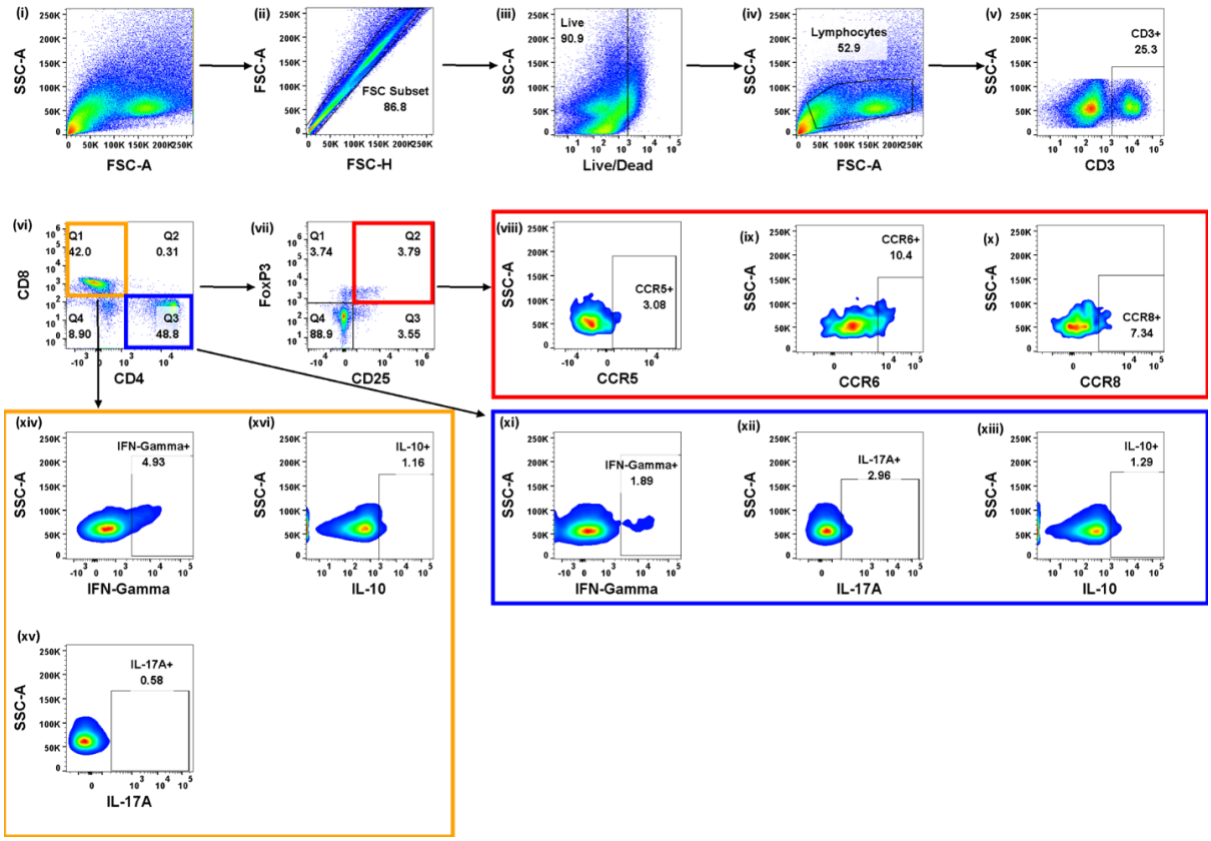
Supplemental Figure 1: Comparison of A) lesion size (mm^3), B) ipsilateral/contralateral hemispheric volume ratio, and C) tissue loss (mm^3) between saline (n = 9) and fingolimod-treated (n = 9) mice as quantified by H & E staining (t = 10 days). Two-sided, independent-samples t-tests was used to investigate differences between groups. Scatter plots depict mean ± standard deviation.



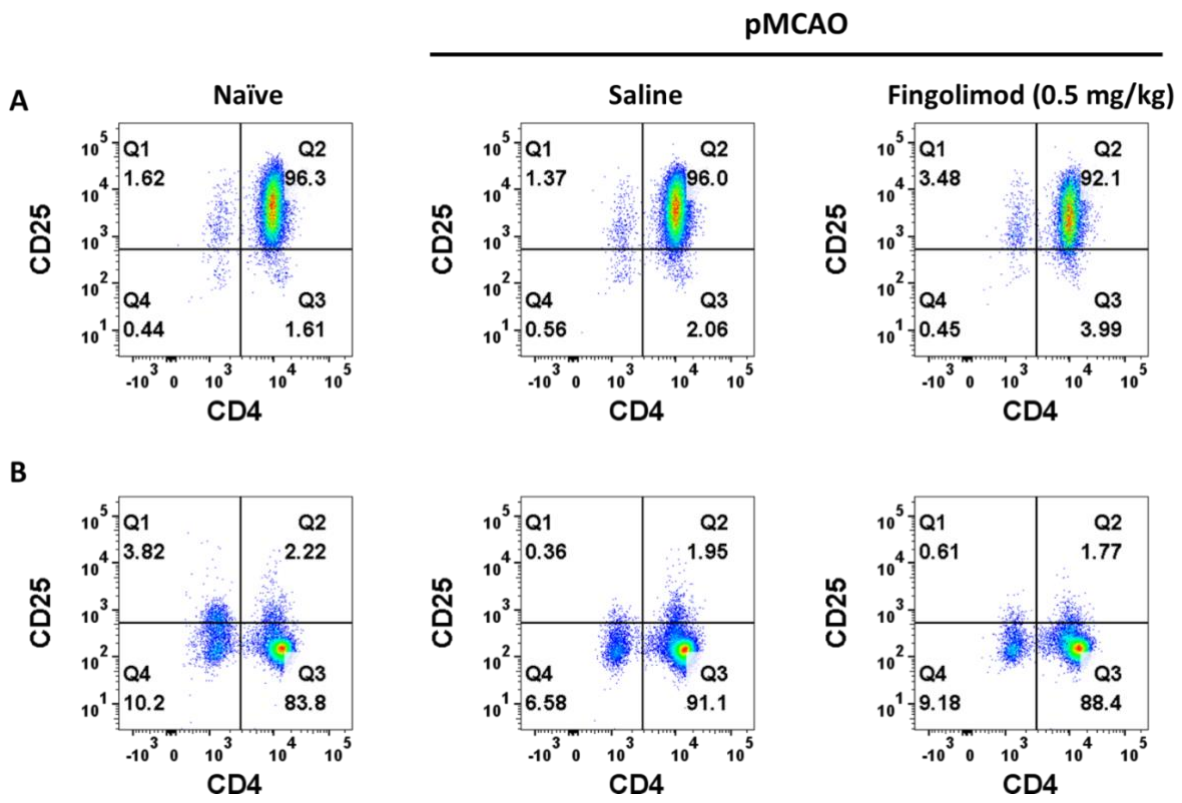
Supplemental Figure 2: Frequency of CD4+ CD25+ FoxP3+ cells in blood, brain, and secondary lymphoid tissue in response to saline or fingolimod (0.5 mg/kg)) treatment post-brain ischaemia in young mice (t = 10 days). Two-sided, independent-samples T tests investigated differences between two groups (* = p<0.05, ** = p<0.01, *** = p<0.001). Box and whisker plots display the 90/10 percentile at the whiskers, the 75/25 percentiles at the boxes, and the median in the centre line. Notably, once daily fingolimod 0.5 mg/kg treatment caused an increased Treg frequency in spleen, draining lymph nodes, non-draining lymph nodes, blood, and brain. N = 7 per group.



Supplemental Figure 3: Absolute cell counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, Tregs, CCR5+ Tregs, CCR6+ Tregs, and CCR8+ Tregs in brain tissue in mice treated with saline or fingolimod (0.5 mg/kg)) post-brain ischaemia in young mice (t = 10 days) or in untreated, naïve, mice (n = 7 mice per group). Two-sided, independent-samples t-tests investigated differences between two groups (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ as compared to saline). Box and whisker plots display the 90/10 percentile at the whiskers, the 75/25 percentiles at the boxes, and the median in the centre line.



Supplemental Figure 4: Gating strategy for determination of chemokine expression among Tregs (red), cytokine expression among CD4+ cells (blue), and cytokine expression among CD8+ cells (yellow) in representative mouse splenocytes post-brain ischaemia (t = 10 days). (i) = Initial population, (ii) = singlets, (iii) = Live cells, (iv) = lymphocytes, (v) = CD3+ cells (T cells), (vi) = CD4+ vs. CD8+ cells, (vii) = CD4+ CD25+ FoxP3+ cells (quadrant in red) designated Tregs, (viii) = CCR5+ Tregs, (ix) = CCR6+ Tregs, (x) = CCR8+ Tregs, (xi) = CD4+ IFNγ+ cells, (xii) CD4+ IL-17A+ cells, (xiii) CD4+ IL-10+ cells, (xiv) CD8+ IFNγ+ cells, (xv) CD8+ IL-17A+ cells, (xvi) CD8+ IL-10+ cells. All gates were determined by both negative cells and fluorescence minus one controls.



Supplemental Figure 5: Treg and Tconv cells were isolated from spleens of naïve, saline-treated, and fingolimod-treated mice. Pseudocolour plots depict representative image from 1 replicate (n = 3 replicates total). A) Frequency of CD4 and CD25 +/- cells among enriched CD4+ CD25+ "Treg" cells. B) Frequency of CD4 and CD25 +/- cells among remaining cells in the CD25- fraction, termed "Tconv" cells.