Supplemental Table 1. Study design and animal number. All *in vivo* PET imaging and behavioural tests were performed on n = 8 stroke mice per group (groups A & E). Another n = 8 stroke mice per group were used for MR imaging (groups B & F). At day 35 post ischemia, those animals were sacrificed and used for histological characterization of the inflammatory.

Besides, four groups of n=4 mice per group without imaging were added for gene expression analysis of tissues harvested at day 35 post ischemia (group C: wild type with control diet, group G: wild type with PLX5622 diet, group D: stroke with control diet and group H: stroke with PLX5622 diet).

Diet	**Group**	**Tmcao**	**PET & Behaviour**	**MRI**	***Ex vivo* only**
Control (n = 24)	A	Yes	8	-	-
	B	Yes	-	8	-
	C	No	-	-	4
	D	Yes	-	-	4
PLX5622 (n = 24)	E	Yes	8	-	-
	F	Yes	-	8	-
	G	No	-	-	4
	H	Yes	-	-	4

Supplemental Table 2. List of antibodies used for immunohistochemistry and immunofluorescence.

Primary antibodies

Target	Dilution	Species	ID	Provider
Anti-PBR	1:250	Rabbit	ab109497	Abcam
Recombinant Alexa Fluor 488 anti-PBR	1:250	Rabbit	ab199779	Abcam
Anti-Iba-1	1:500	Rabbit	019-19741	Wako
Red fluorochrome (635)-conjugated Iba-1	1:500	Rabbit	013-26471	Wako
Anti-CSF-1R	1:250	Rabbit	SAB4500498	Sigma-Aldrich
Anti-GFAP	1:500	Chicken	ab4675	Abcam
Anti-TMEM119	1:200	Rabbit	ab209064	Abcam

Secondary antibodies

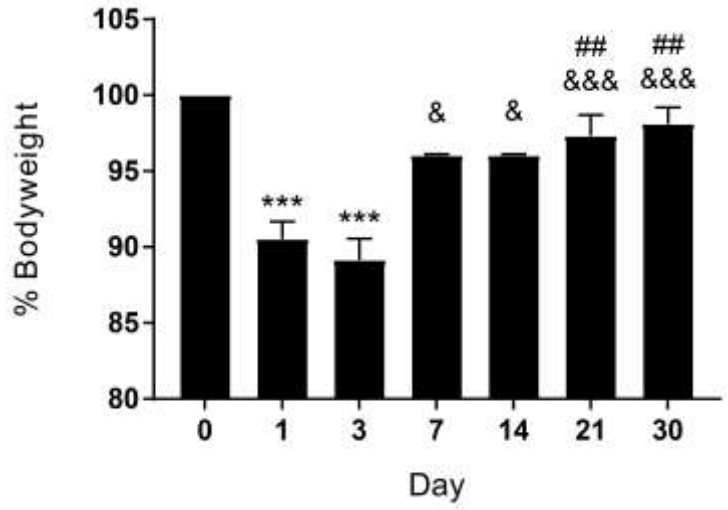
Target	Dilution	Species	ID	Provider
Biotin Anti-rabbit	1:800	Goat	B21078	Life Technologies
Biotin Anti-chicken	1:800	Goat	D20701	Life Technologies
Alexa Fluor 488 anti-rabbit	1:1000	Goat	A-21206	Life Technologies
Alexa Fluor 555 anti-rabbit	1:1000	Goat	A-21432	Life Technologies
Alexa Fluor 488 anti-chicken	1:1000	Goat	A-11039	Life Technologies

Abbreviations: CSF-1R: *colony stimulating factor-1 receptor*; GFAP: *glial fibrillary acidic protein*; Iba-1: *ionized calcium binding adapter molecule-1*; PBR: *peripheral benzodiazepine receptor*; TMEM119: *transmembrane protein 119*.

Supplemental Table 3. Primers for real time qPCR.

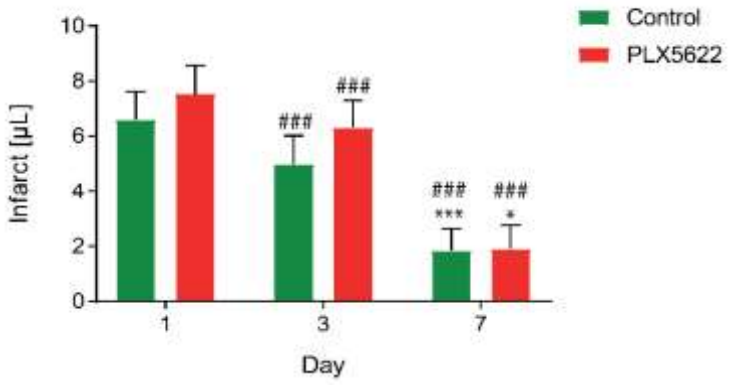
Primer	Forward	Reverse
Csf-1r	gccatatacaggtacacattc	gtgccattaagaagtactgg
Tspo	ggatctttccagaacatcag	acgtacaaagtaggctcc
Cd4	tagcaactctaaggtctctaac	gatagctgtgctctgaaaac
Il1b	ggatgatgatgataacctgc	catggagaatatcacttgttgg
Il6	aagaaatgatggatgctacc	gagtttctgtatctctctgaag
Arg1	ctgacctatgtgtcatttgg	catctgggaactttcctttc
Tmem119	ccagagctggttccatag	gggagtgacacagagtag
Cx3cr1	aacaccatgctgtcatattc	gtaagctactatgcttgctg
Ccl2	caagatgatcccaatgagtag	ttggttgacaaaaactacagc
Nos2	catcaaccagtattatggctc	tttcctttgttacagcttcc
Tnf	ctatgtctcagcctcttctc	catttgggaacttctcatcc
Trem2	tcatctcttttctgcacttc	tcataagtacatgacaccctc
Gfap	ggaagatctatgaggaggaag	ctgcaaacttagaccgatac
Gapdh	ctggagaaacctgccaagta	tgttgctgtagccgtattca

Abbreviations: CSF-1R: *Colony stimulating factor-1 receptor*; Tspo: *Translocator protein*, Cd4: *Cluster of differentiation 4*; Il1b: *Interleukin-1β*; Il6: *Interleukin-6*; Arg1: *Arginase 1*; Tmem119: *Transmembrane protein 119*; Cx3cr1: *C-X3-C chemokine receptor* 1; Ccl2: *Chemokine C-C ligand 2*; Nos2: *Nitric oxide synthase 2*; Tnf: *Tumor necrosis factor*; Trem2: *Triggering receptor expressed on myeloid cells 2*; Gfap: *Glial fibrillary acidic protein*; Gapdh: *Glyceraldehyde 3-phosphate dehydrogenas*e.



Supplemental Figure 1. Bodyweight. Two-way RM ANOVA indicated a significate effect of time ($p < 0.01$) but not of treatment ($p = 0.20$) on bodyweight. Friedman repeated measures ANOVA on ranks indicated that bodyweight significantly decreased after surgery within the first days after stroke, indicative of reduced food intake.

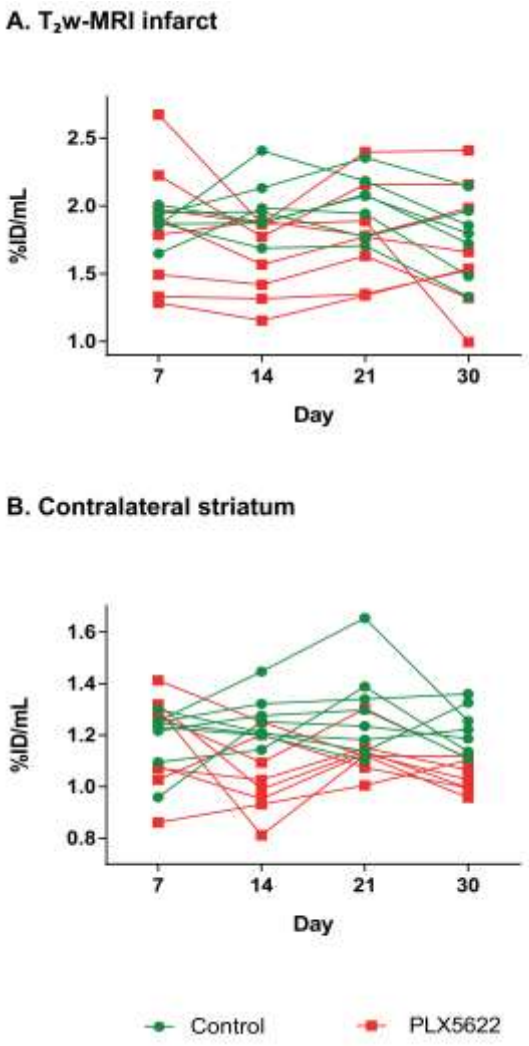
Statistical analysis was carried out with Friedman RM ANOVA followed by Tukey's post hoc test for multiple comparisons (n = 16). Data are expressed as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; * vs day 0 (baseline), # vs day 1 and & vs day 3).



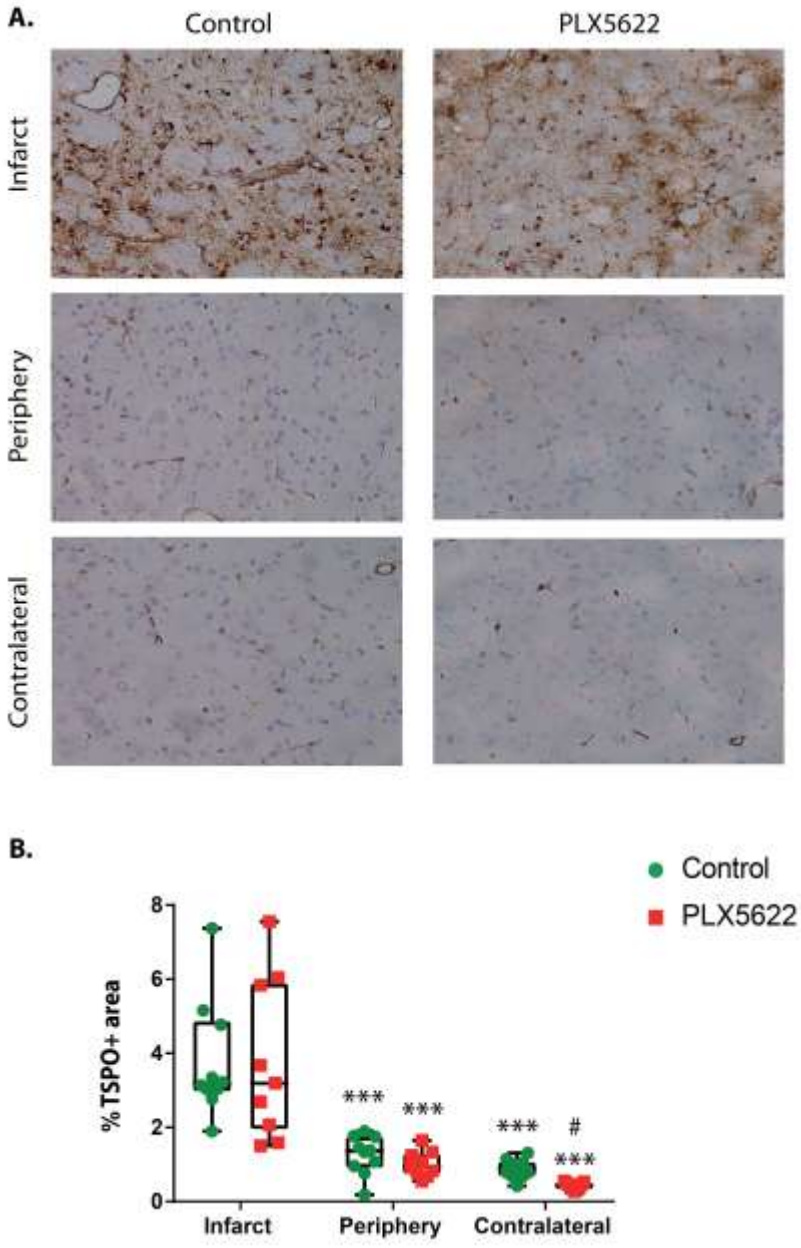
Supplemental Figure 2. PLX5622 treatment does not affect T_2w-MRI infarct volume.

Quantification of T_2w-MRI infarct volume at days 1, 3 and 7 after tMCAo for both experimental groups (green: control, red: PLX5622) showed decreased infarct volume over time. No treatment effect was observed ($p = 0.54$).

Data were analysed with a two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons. Data are depicted as mean ± SEM (n = 8/group) (* $p < 0.05$, ** $p < 0.01$, ***$p < 0.005$; # vs day 1, * vs day 3).

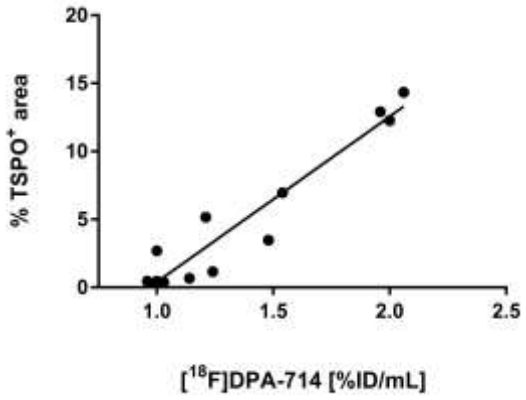


Supplemental Figure 3. **Individual 18F-DPA-714 radiotracer uptake over time.** Two-way RM ANOVA indicated a treatment (p = 0.014) and treatment*time (p = 0.011) effect but no time effect (p = 0.412) on tracer uptake within the infarct. Data was analysed with a two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons. Data are depicted as %ID/mL (n = 8/group, green line: control, red line: PLX5622-treated mice).

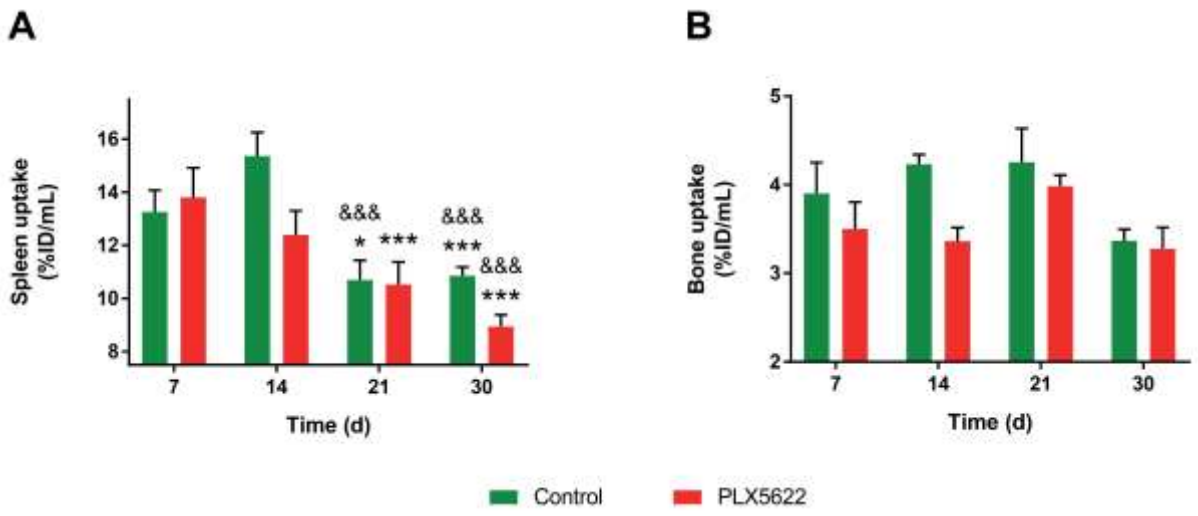


Supplemental Figure 4. Quantification of TSPO$^+$ area validated 18F-DPA-714 imaging at day 35 post ischemia. (A) The representative images of the anti-TSPO immunoreactivity showing TSPO$^+$ cells and vessels within the infarct, at the periphery of the infarct and in contralateral striatum at day 35 post ischemia of both control (n = 4) and PLX5622-treated mice (n = 3). (B) Quantification of the percentage of TSPO$^+$ area in the 3 regions of interest showed significant decreased of TSPO$^+$

area in PLX5622 mice compared to control mice only in the contralateral striatum, confirming 18F-DPA-714 PET images acquired at day 30 post ischemia. Data was analysed by two-way RM ANOVA followed by Sidak's post hoc test (*p<0.05, **p<0.01, ***p<0.005;* vs infarct, # vs diet).

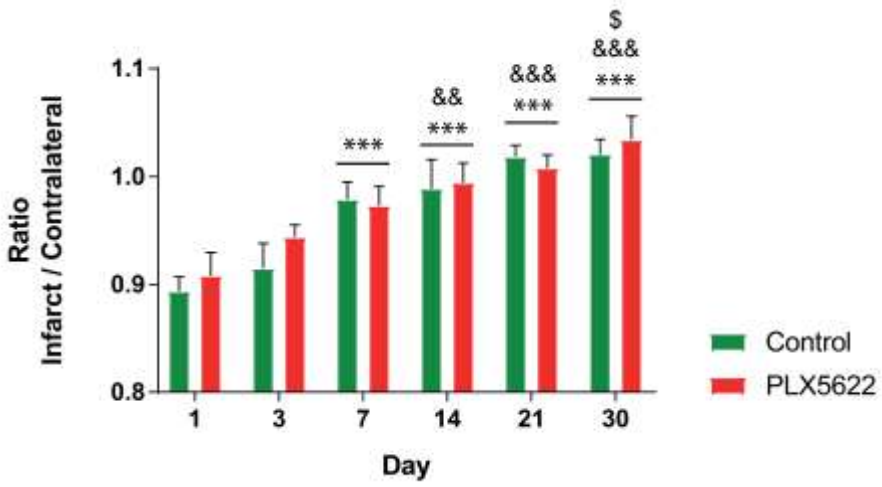


Supplemental Figure 5. Cross-validation of 18F-DPA-714 PET images by TSPO immunoreactivity at day 35 post ischemia (R^2 = 0.91)**.**

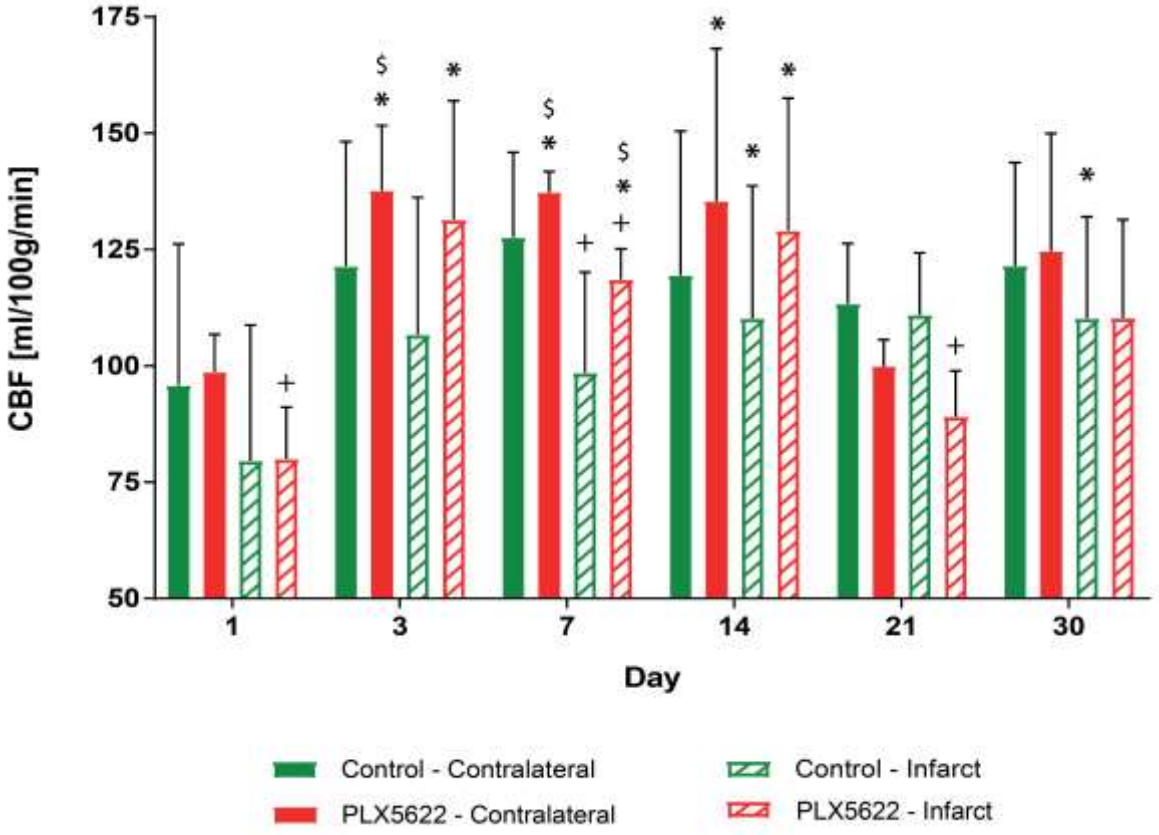


Supplemental Figure 6. Spleen and bone 18F-DPA-714 tracer uptake. (A) The dataset passed the normality (p = 0.25) and equal variance (p = 0.32) tests. Two-way RM ANOVA indicated a significant effect of time (p < 0.001, power = 0.98) but not of treatment (p = 0.20, power = 0.13) or time*treatment (p = 0.09) on spleen tracer uptake (n = 8/group). Spleen uptake was significantly decreased from days 7/14 to days 21/30 post ischemia. (B) Two-way RM ANOVA indicated neither effect of time (p = 0.56) or treatment (p = 0.16) on bone tracer uptake. An indicative treatment effect could be observed at day 14 on both spleen and bone tracer uptake while not significant.

The volumes-of-interest were manually delineated on the CT scans and co-registered PET images. The bone region included the manubrium and the first connecting sternebra). Data are expressed as mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.005; * vs day 7, & vs day 14, # vs treatment).



Supplemental Figure 7. Temporal dynamics of the infarct-to-contralateral ADC ratio within the infarct and contralateral striatum in control and PLX5622-treated mice. Two-way RM ANOVA analysis indicated significant effect of time ($p < 0.001$) but not of treatment ($p = 0.589$) or time*treatment ($p = 0.899$) on the infarct-to-contralateral ADC ratio. Data was analysed with a two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons. Data are depicted as mean ± SEM (n = 8/group) (*p<0.05, **p<0.01, ***p<0.005, * vs day 1, & vs day 3, $ vs day 7).



Supplemental Figure 8. Temporal dynamics of CBF mean values within the infarct and contralateral striatum in control and PLX5622 treated mice. In control mice, no significant difference in CBF values was observed in the contralateral side over time ($p > 0.05$).

Mean CBF values within the infarct at day 14 (110.2 ± 27.1 mL/100g/min, $p = 0.044$) and 30 (110.2 ± 20.8mL/100g/min, $p = 0.039$) were significantly higher compared to day 1 (79.7 ± 27.6 mL/100g/min) post ischemia (Sidak's post hoc test).

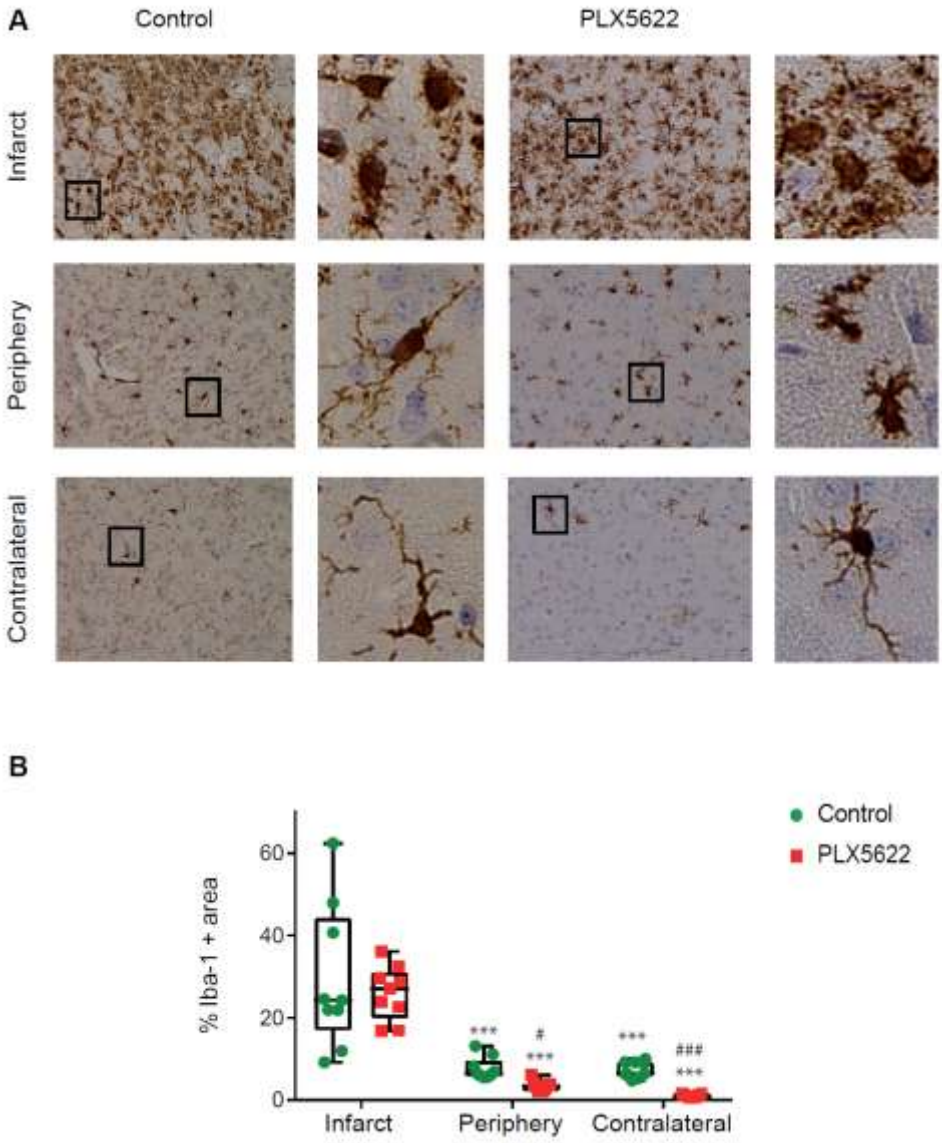
A significant difference between infarct and contralateral striatum was observed at day 7 ($p = 0.042$) post ischemia. No significant difference between both hemispheres was observed at days 21 and 30 post ischemia.

In PLX5622-treated mice, mean CBF values in the contralateral side significantly increased from day 1 (98.6 ± 7.4 mL/100g/min) to days 7 (137.7 ± 4.08 mL/100g/min, $p = 0.026$) and 14 (135.3 ± 25.45 mL/100g/min, $p = 0.044$), followed by a significant decrease at day 21 post ischemia. Mean CBF values at day 21 (99.9 ± 5.19 mL/100g/min) were significantly decreased compared to day 3 (137.7 ± 12.1 mL/100g/min, $p = 0.030$) and 7 ($p = 0.028$) post ischemia (Sidak's post hoc test).

Mean CBF values within the infarct were increased at day 3 (131.4 ± 20.8 mL/100g/min, $p = 0.047$), 7 (118.6 ± 9.6 mL/100g/min, $p = 0.01$) and 14 (129.1 ± 26.0 mL/100g/min, $p = 0.037$) compared to day 1 (80.0 ± 10.2 mL/100g/min) post ischemia (Sidak's post hoc). Mean CBF values within the infarct at day 21 (89.1 ± 10.4 mL/100g/min) were decreased compared to day 7 ($p = 0.02$) post ischemia.

In PLX5622-treated mice, mean CBF within the infarct was significantly lower than in the contralateral side at day 1 ($p = 0.034$), 7 ($p = 0.012$) and 21 ($p = 0.046$) post.

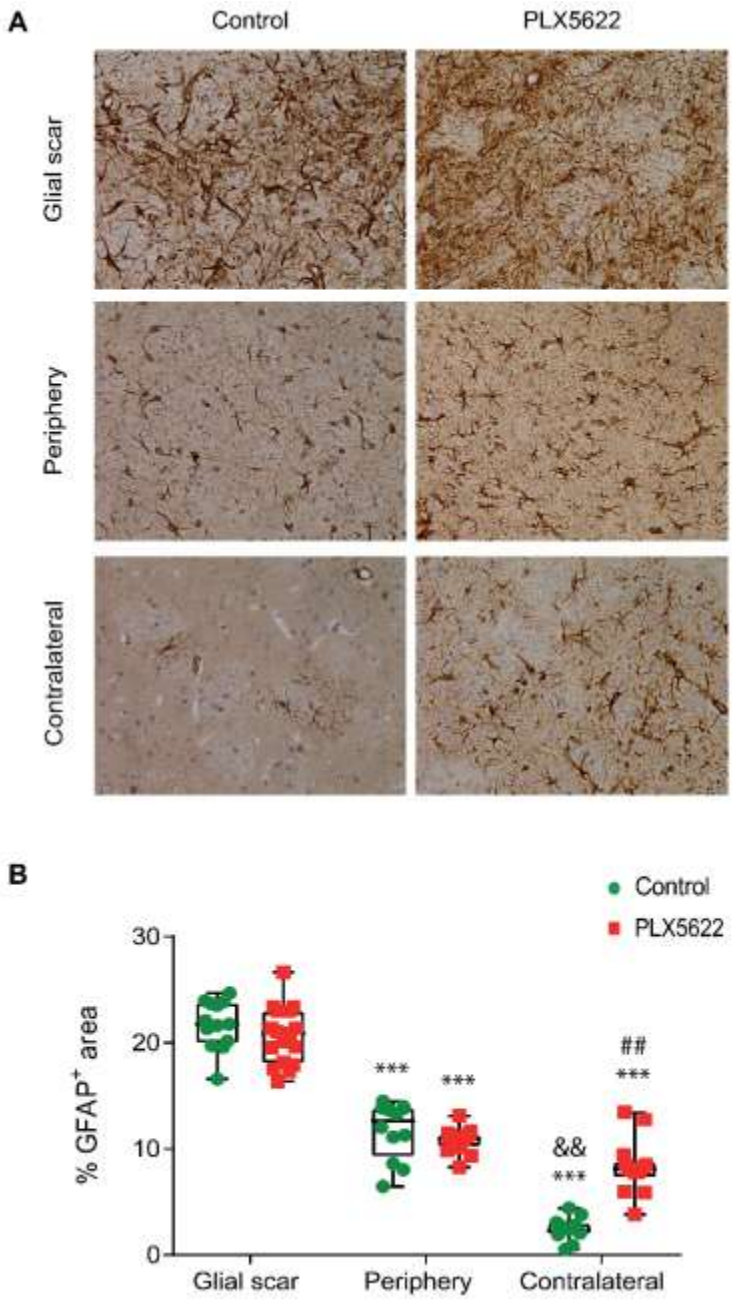
Data are depicted as mean ± SD (n = 8/group) (*$p<0.05$, **$p<0.01$, ***$p<0.005$, * vs day 1, # vs treatment, + vs contralateral, $ vs day 21).



Supplemental Figure 9. PLX5622 treatment modulated Iba-1 immunoreactivity in PLX5622-treated mice brains at day 35 post ischemia. (A) Representative images of Iba-1 immunoreactivity within the infarct, at the periphery of the infarct and contralateral side in control and PLX5622-treated mice at day 35 post ischemia. PLX5622 treatment may modulate microglia reactivity, as indicated by the difference in morphology. At the periphery of the infarct, microglial cells have elongated thin processes in control mice while they showed shorter thicker processes in PLX5622 treated mice. (B) Quantification of Iba-1 immunoreactivity within the

infarct, at the periphery of the infarct and contralateral side expressed in percentage of stained area. Therapy effect was observed at the periphery and contralateral striatum, where PLX5622-treated mice showed a lower percentage of Iba-1^+ area (n = 3/group, 3 fields of view per mouse and region).

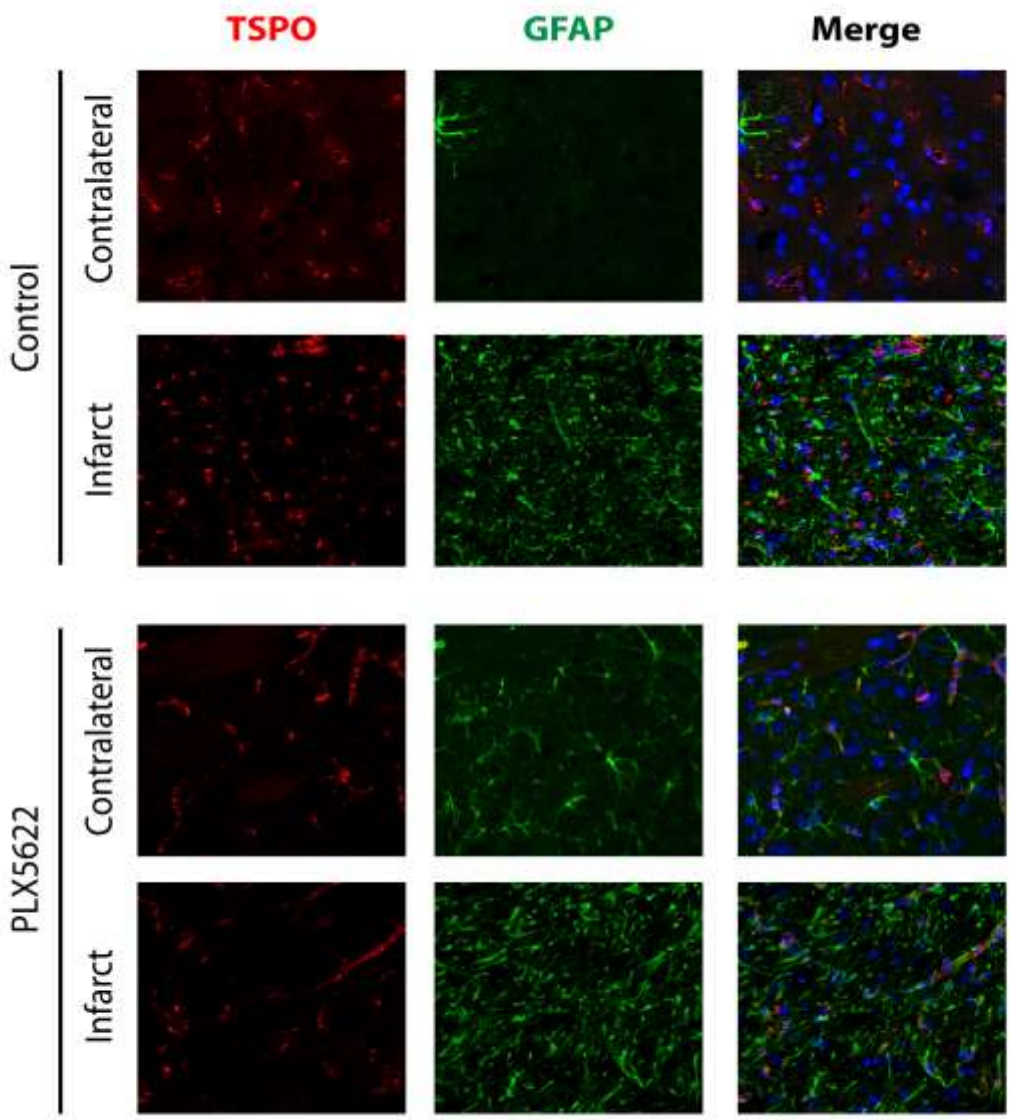
Data were analysed by two-way RM ANOVA followed by Sidak's post hoc test for multiple comparisons (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; * vs infarct, # vs treatment).



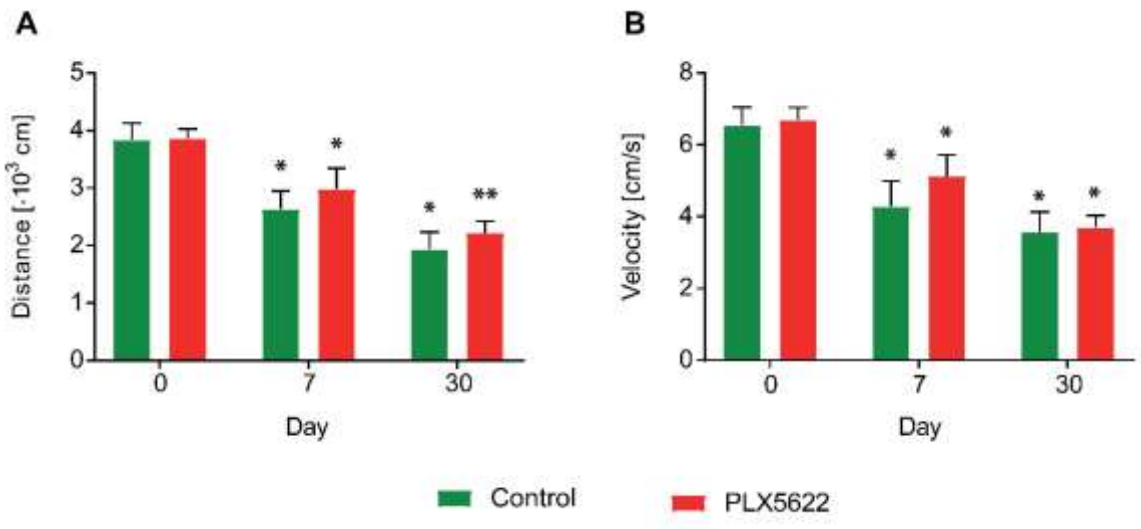
Supplemental Figure 10. PLX5622 treatment increased the number of GFAP$^+$ cells in the contralateral side after long-term CSF-1R inhibition. (A) Representative GFAP staining in three regions (glial scar, periphery and contralateral) for both experimental groups. (B) A treatment effect was observed in the contralateral side: PLX5622-treated mice showed a higher percentage of GFAP$^+$ area compared to control mice (p = 0.006). The percentage of GFAP$^+$ area at the glial

scar and periphery was unaffected by PLX5622 treatment. (Control: n = 4, PLX5622: n = 5, 3 fields of view per region per mouse).

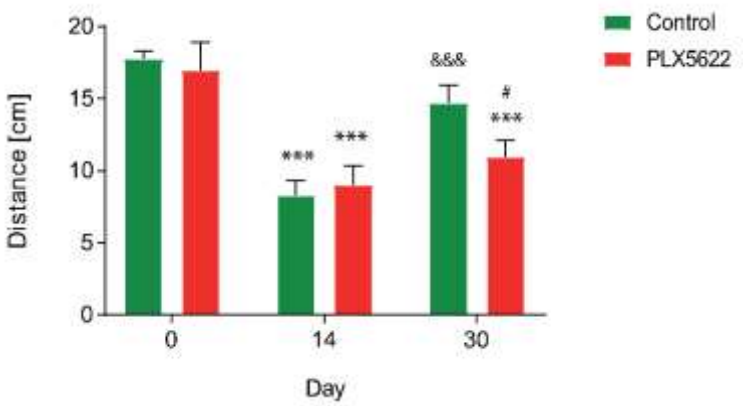
Data were analysed by two-way RM ANOVA followed by Sidak's post hoc test for multiple comparisons (**$p < 0.01$, ***$p < 0.005$; * vs glial scar, & vs periphery, # vs treatment).



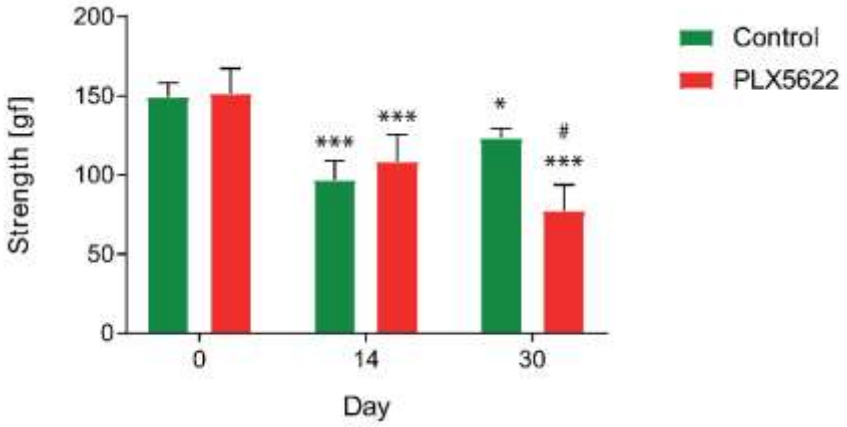
Supplemental Figure 11. GFAP-positive cells are mostly TSPO-negative at day 35 post ischemia. Representative co-staining of TSPO (red) and GFAP (green) within the infarct and contralateral side and the corresponding merged image for both experimental groups. No or few $GFAP^+$ cells were co-localizing with TSPO within the infarct and contralateral striatum for both experimental group. PLX5622 did not affect the percentage of $GFAP^+$ area within the infarct but increased GFAP expression in the contralateral side of PLX5622-treated mice compared to control.



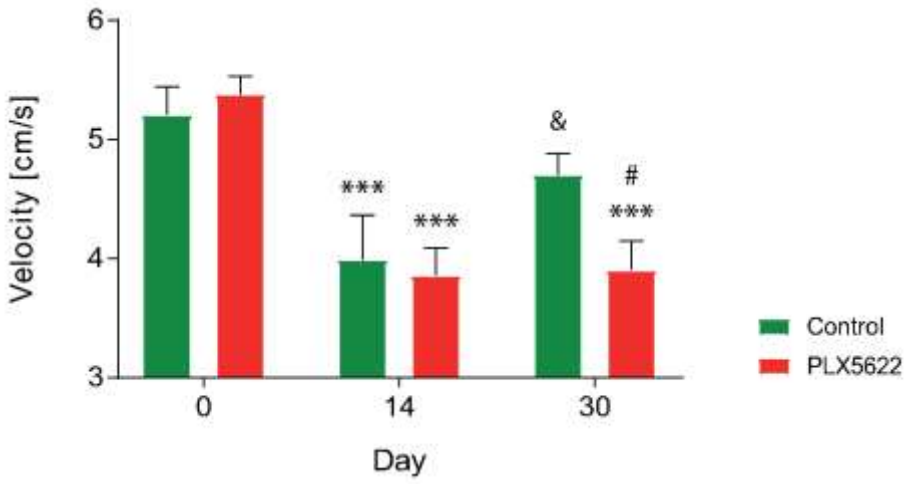
Supplemental Figure 12. **Long-term PLX5622 treatment did not improve general locomotion.** (A) Travelled distance and (B) velocity were assessed as indexes of global locomotion using the open field test. Overall, no treatment effect was observed between both experimental groups. Walking distance (R^2 = 0.25) and velocity (R^2 = 0.30) did not correlate with 18F-DPA-714 tracer uptake within the infarct at day 14 post ischemia. Statistical analysis was carried out with two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons (n = 8/group). Data are expressed as mean ± SEM (*p < 0.05, **p < 0.01, * vs day 1).



Supplemental Figure 13. Long-term PLX5622 treatment impaired coordination. PLX5622-treated mice showed impaired coordination/balance compared to control mice at day 30 post ischemia. The run distance on the rotarod did not correlate with 18F-DPA-714 tracer uptake within the infarct at day 14 post ischemia (R^2 = 0.11). Statistical analysis was carried out with two-way RM ANOVA s followed by Holm Sidak's post hoc test for multiple comparisons (n = 8/group). Data are expressed as mean ± SEM (***p < 0.005; * vs day 1, & vs day 14, # vs treatment).



Supplemental Figure 14. Long-term PLX5622 treatment impaired forelimbs strength recovery after ischemia. Treatment effect on forelimbs strength was observed at day 30 post ischemia. PLX5622-treated mice showed less strength in the forelimbs compared to control mice. Forelimbs strength did not correlate with 18F-DPA-714 tracer uptake within the infarct at day 14 post ischemia (R^2 = 0.21). Statistical analysis was carried out with two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons (n = 8/group). Data are expressed as mean ± SEM (*p < 0.05, ***p < 0.005; * vs day 1, & vs day 14, # vs treatment).



Supplemental Figure 15. PLX5622 treatment impaired long-term recovery of general motor functions. A treatment effect was observed at day 30 post ischemia: PLX5622-treated mice walked slower than control mice over the pole, indicative of impaired motor recovery. Velocity did not correlate with 18F-DPA-714 tracer uptake within the infarct at day 14 post ischemia (R^2 = 0.34). Statistical analysis was carried out with two-way RM ANOVA followed by Holm Sidak's post hoc test for multiple comparisons (n = 8/group). Data are expressed as mean ± SEM (***$p < 0.005$; * vs day 1, & vs day 14, # vs treatment).