Behavioral tests

One week prior to behavioural tests, animals were brought to the institute and were daily handled for 10 minutes by the experimenter for acclimatization. Open field, grip test, rotarod and pole test were performed to evaluate the therapeutic effects of acute CSF-1R inhibition on motor function recovery. The four behavioural tests were carried out the week prior to surgery and at days 14 and 35 post ischemia.

Open field

The open field test is used to assess general locomotor activity level by recording free movements. A squared arena (40 x 40 x 25 cm^3) was placed in a quiet environment. Before the test, litter coming for the animal cage was spread in the arena to hide possible contaminating odours and let for 5 minutes. Once the litter has been removed, the mouse was placed in the middle of the maze and was free to move for 10 minutes. Locomotion was recorded using EthoVision XT15 (Noldus, Wageningen, The Netherlands) video tracking system attached to a pole placed above the arena. Distance travelled (cm), velocity (cm/s) and laterality (clockwise and counter-clockwise rotations of the center-nose axis) were measured.

Grip strength test

The grip test uses a grip strength meter (Grip-Strength Meter, 47200, Ugo Basile, Italy) to determine forelimbs muscle strength. The mouse was able to grasp the grip trapeze with the forepaws and was gently pulled away until the grasp was released. Each mouse was tested five times. The average of the peak force (in gf) was calculated from the last three trials.

Pole test

The pole test is a general test to assess motor functions. The mouse was placed head upward just below the top of a vertical pole (diameter: 2.5 cm; height: 60 cm) and then allowed to descend into their home

cage. The time needed to reach the floor was manually measured. Each mouse was tested five consecutive times, but only the last three trials are used for calculation.

Rotarod

The rotarod test (ITC LifeScience Inc., Woodland Hills, CA, USA) assesses motor coordination and balance. In this test, a mouse was placed on a rotating cylinder (3 cm in diameter) suspended 30 cm above the protected apparatus floor. The mouse was placed on the rod and left to acclimatise for 30 s. Then, the rotarod was turned on to accelerate in 300 s from 4 to 40 rpm. The trial is complete when the mouse fell down. The test was repeated five consecutive times. The time spent on the cylinder (in seconds) and the covered distance (in cm) were automatically recorded for each trial and reported as the latency to fall (in seconds). The averaged values were calculated from the last three trials.

Supplemental Table 1. Study design and animal number. Longitudinal PET imaging and behavioural tests were performed on n = 8 stroke mice per group (groups A & C). Those animal were sacrificed at the end of the study (day 35 post ischemia) for further *ex vivo* characterization. N= 4 mice/ group were used for immunoreactivity and n = 4 for gene expression analysis. Another n = 8 stroke mice per group were used to characterise the 14-day time point (groups B & D). Similarly, n = 4 mice/ group were used for immunoreactivity and n = 4 for gene expression analysis.

		In vivo			***Ex vivo***	
Group		**T_2w-MRI Day 1**	**18F-DPA-714 PET-CT**	**Behaviour**	**Day 14**	**Day 35**
Control	**A (n = 8)**	X	X	X		X
	B (n = 8)	X			X	
PLX5622	**C (n = 8)**	X	X	X		X
	D (n = 8)	X			X	

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

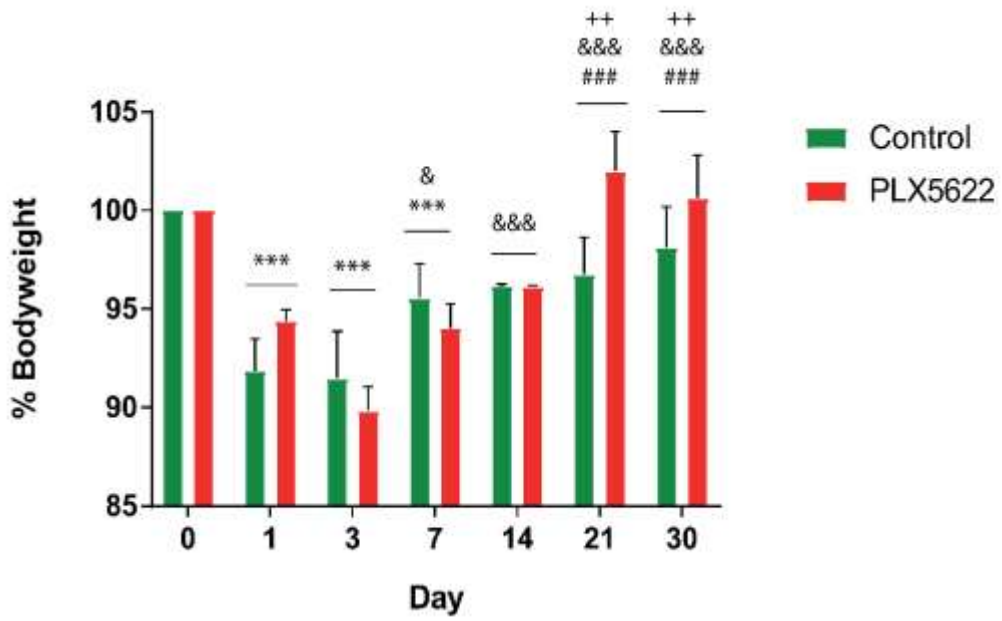
Target	**Dilution**	**Species**	**ID**	**Provider**
Anti-TSPO	1:250	Rabbit	ab109497	Abcam
Recombinant Alexa Fluor 488 anti-TSPO	1:250	Rabbit	ab199779	Abcam
Anti-Iba-1	1:500	Rabbit	019-19741	Wako
Red fluorochrome (635)-conjugated Iba-1	1:500	Rabbit	013-26471	Wako
Anti-CSF-1R	1:250	Rabbit	SAB4500498	Merck
Anti-GFAP	1:500	Chicken	ab4675	Abcam
Anti-CD68	1:250	Rabbit	ab125212	Abcam
Biotin Anti-rabbit	1:800	Goat	B21078	Life Technologies
Biotin Anti-chicken	1:800	Goat	ab6876	Abcam
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*; TSPO: *translocator protein*; CD68: *cluster of differentiation 68*.

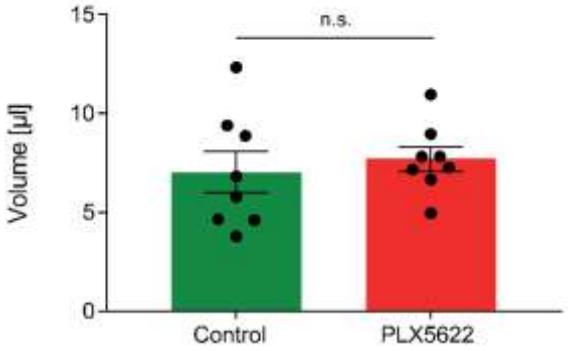
Supplemental Table 3. Primers for real-time qPCR.

Primer	Forward (FW)	Reverse (RV)
Csf-1r	gccatatacaggtacacattc	gtgccattaagaagtactgg
Cx3cr1	aacaccatgctgtcatattc	gtaagctactatgcttgctg
Ccr2	accacatgtgctaagaattg	ctggttttatgacaaggctc
P2ry12	taccctacagaaacactcaag	gctgaatctgaaggatatgag
Aif1	ttcatcctctctcttccatc	tcagcttttgaaatctcctc
Mrc1	aaatgatgagctgtggattg	ccatccttgcctttcataac
Trem2	tcatctcttttctgcacttc	tcataagtacatgacaccctc
Gapdh	ctggagaaacctgccaagta	tgttgctgtagccgtattca

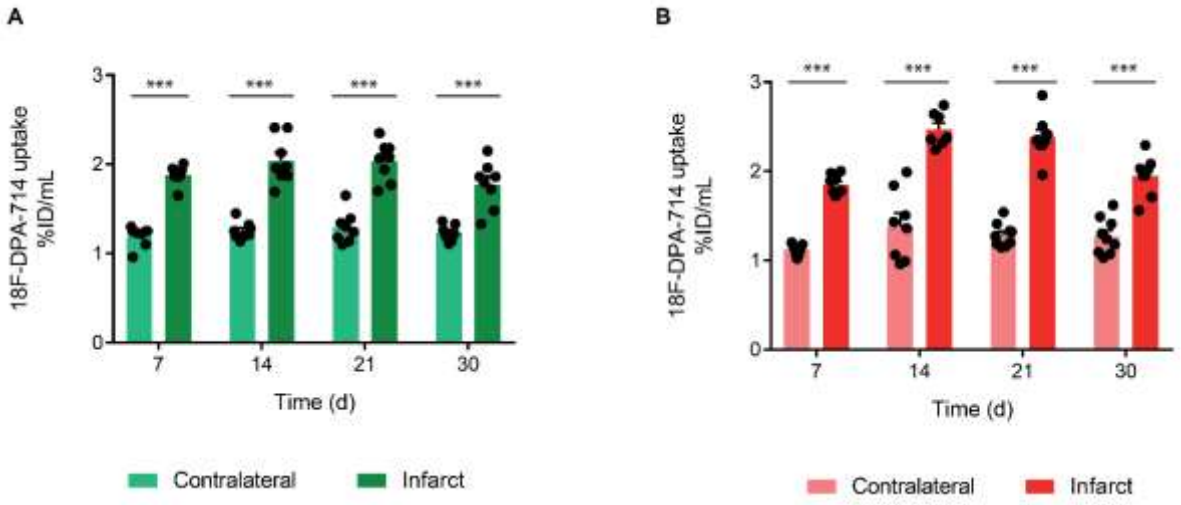
Abbreviations: *Csf-1r*: Colony stimulating factor-1 receptor; *Cx3cr1*: C-X3-C chemokine receptor 1; *Ccr2*: C-C chemokine receptor 2; *P2ry12*: purinergic receptor P2Y12; *Aif1*: allograft inflammatory factor 1; *Mrc1*: mannose receptor C-type 1; *trem2*: Triggering receptor expressed on myeloid cells 2; *gapdh*: Glyceraldehyde 3-phosphate dehydrogenase.



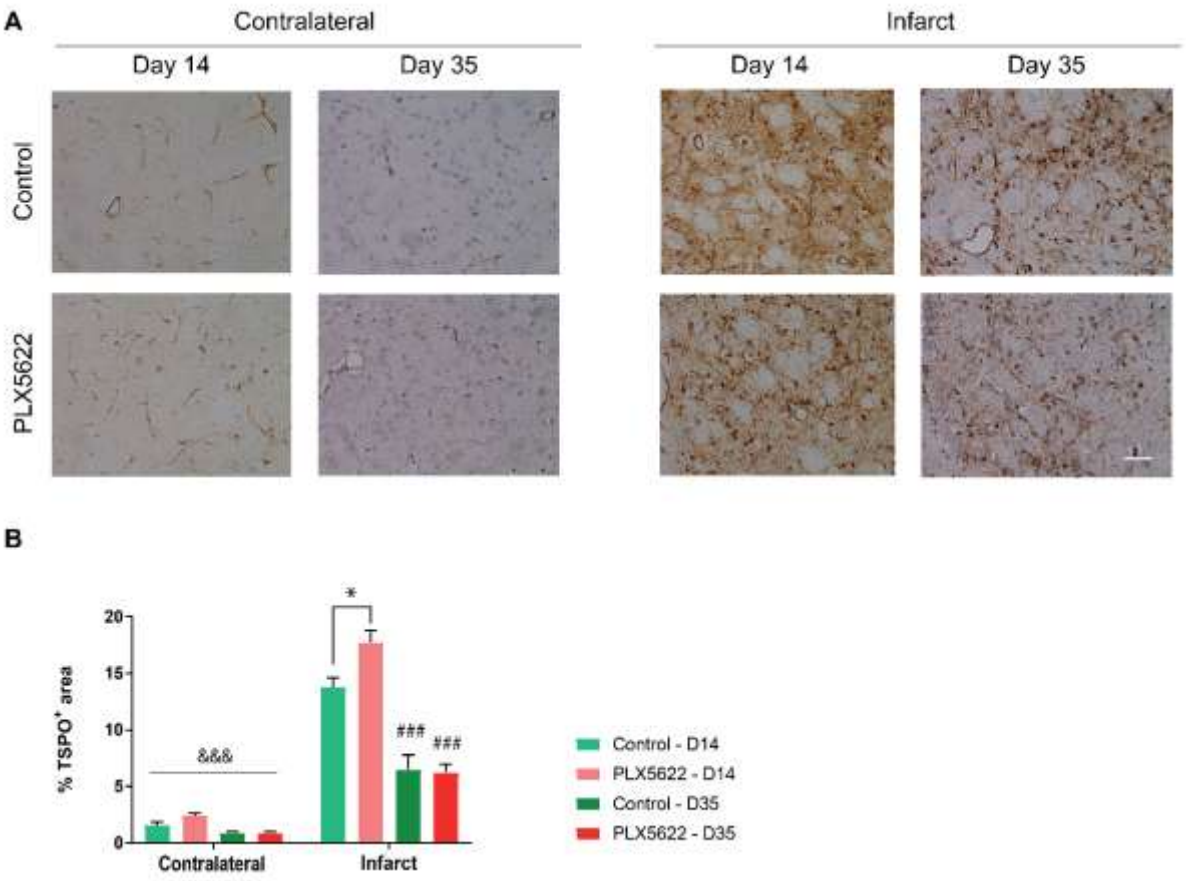
Supplemental Figure 1. Bodyweight. Two-way RM ANOVA indicated time effect ($p < 0.001$) but not treatment ($p = 0.57$) or time*treatment ($p = 0.084$) effect. In both groups, bodyweight significantly decreased within the first 3 days and then increased again (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, * *vs.* day 0, # *vs.* day 1, & *vs.* day 3, + *vs.* day 7).



Supplemental Figure 2. T_2w-MRI-derived lesion on day 1 post ischemia. T-test indicated no significant difference between both experimental groups (control: 7.03 ± 2.76 µl, PLX5622: 7.70 ± 1.75 µl, $p = 0.22$).

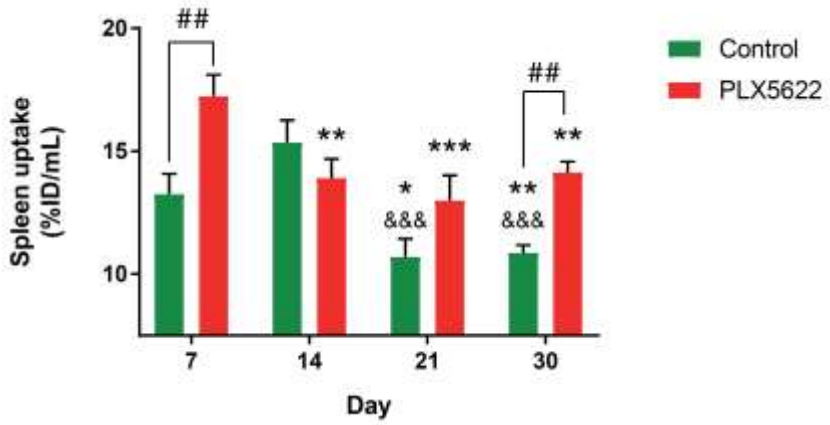


Supplemental Figure 3. 18F-DPA-714 tracer uptake (%ID/mL) of both (a) control and (b) PLX5622-treated mice within the infarct and the contralateral striatum. Two-way RM ANOVA indicated a significant effect of brain region in both groups ($p < 0.001$). Data are reported as mean ± SEM (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$).

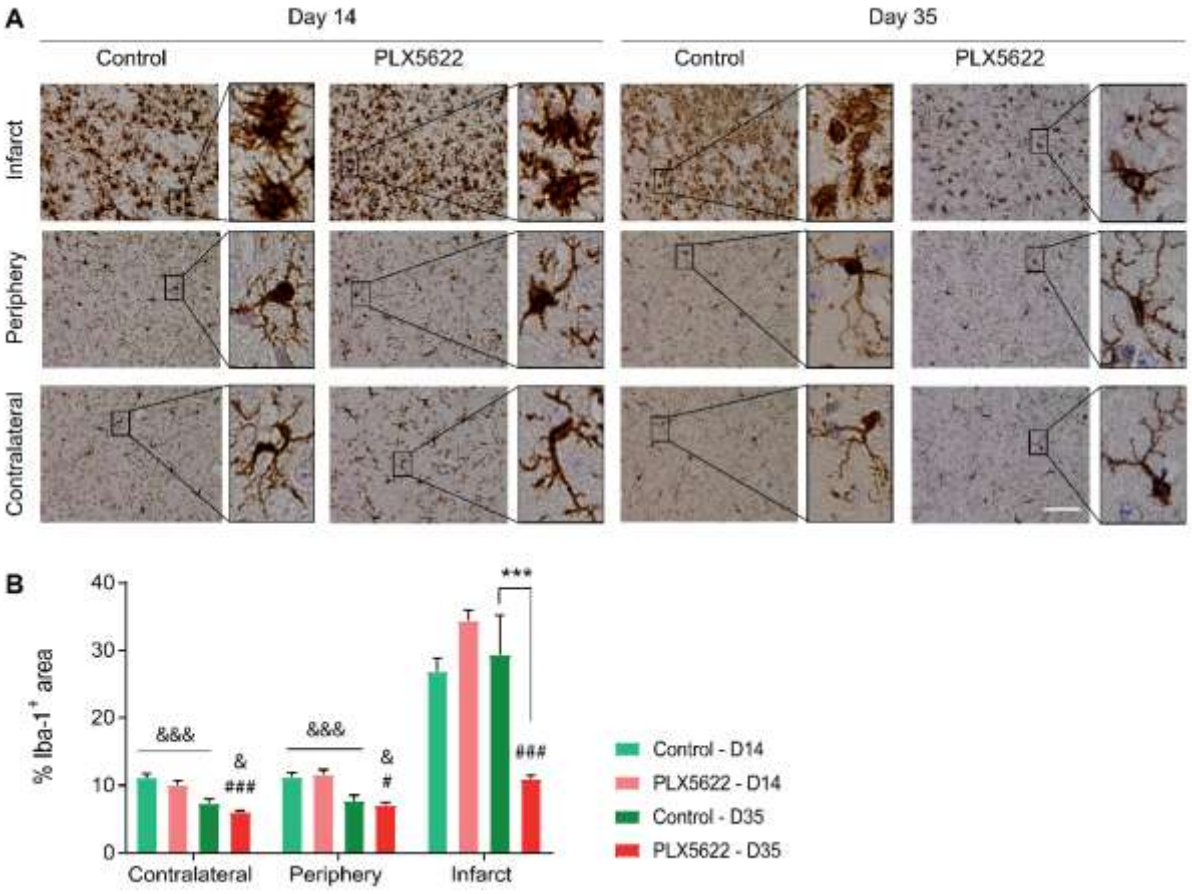


Supplemental Figure 4. 18F-DPA-714 PET imaging data were cross-validated by immunohistochemistry.

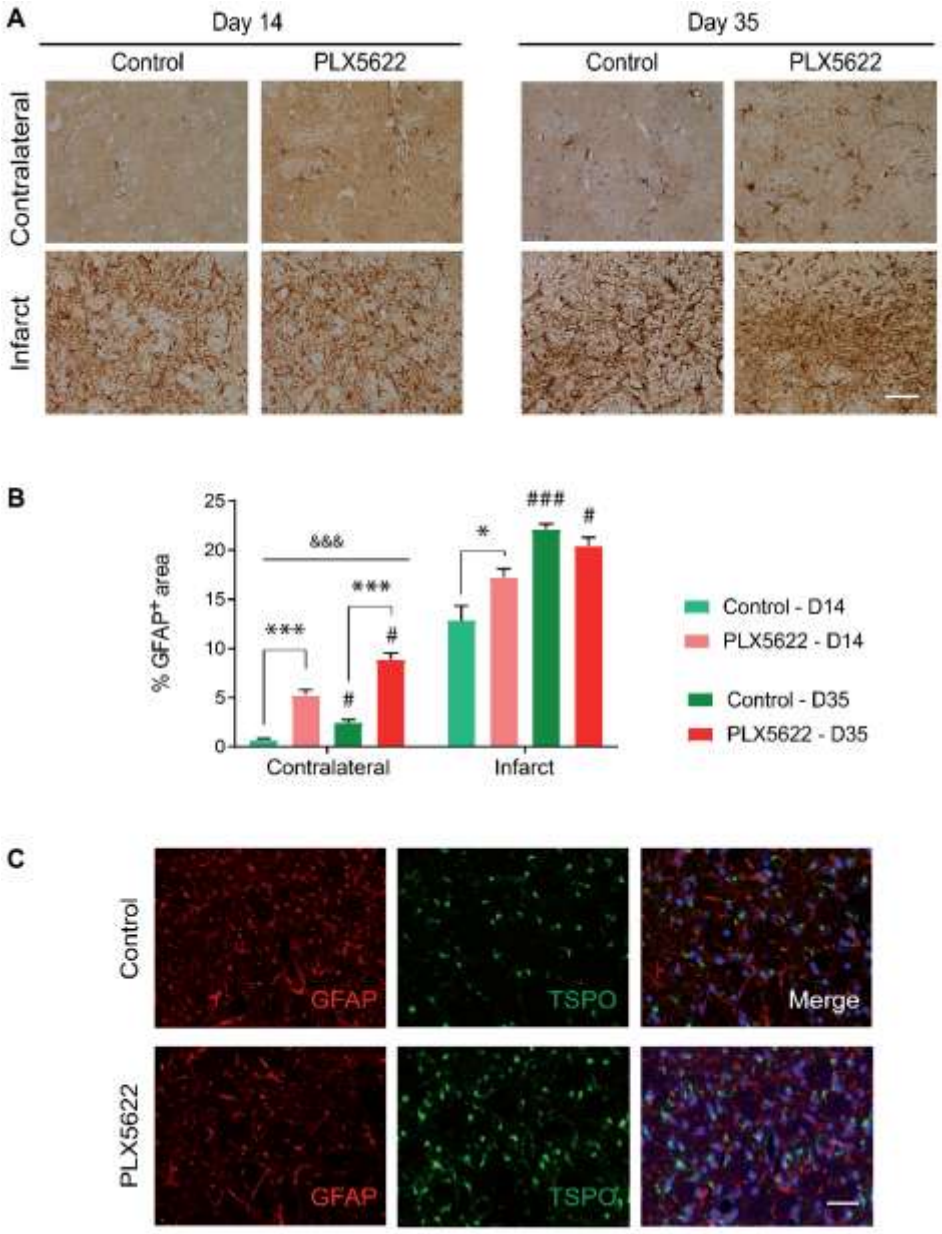
(A) Representative images of the anti-TSPO immunoreactivity showing TSPO-positive cells and vessels within the infarct and the contralateral tissues at both days 14 and 35 post ischemia for both control and PLX5622-treated mice. (B) Quantification of the images showed significant higher percentage of TSPO-positive area in PLX5622-treated mice compared to control mice within the infarct at day 14 ($p = 0.023$) while no difference was observed at day 35 post ischemia, cross-validating 18F-DPA-714 PET imaging data at both time points (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, * *vs.* treatment, # *vs.* time, & *vs.* infarct).



Supplemental Figure 5. 18F-DPA-714 tracer uptake within the spleen. The spleen was manually delineated on PET images co-registered with CT scans. The dataset passed the normality ($p = 0.68$) and equal variance ($p = 0.42$) tests. Statistical analysis indicated time ($p < 0.001$, power: 0.99), treatment ($p = 0.027$, power: 0.54) and time*treatment ($p < 0.001$) effects on spleen tracer uptake. Treatment effect was observed at days 7 and 30 post ischemia, where PLX5622-treated mice showed higher tracer uptake compared to control mice (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, * *vs.* day 7, & *vs.* day 14, # *vs.* treatment).

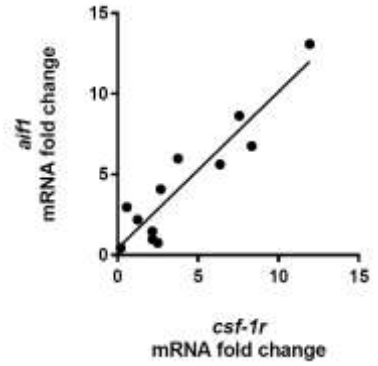


Supplemental Figure 6. Iba-1$^+$ (microglia/macrophages) immunoreactivity. (A) Representative images of the anti-Iba-1 immunoreactivity showing Iba-1$^+$ cells within the infarct, at the periphery of the infarct and in the contralateral striatum at both days 14 (n = 4/group) and 35 (n = 4/group) post ischemia for both control and PLX5622-treated mice. (B) A significantly decreased percentage of Iba-1$^+$ area within the infarct of PLX5622-treated mice compared to control mice at day 35 post ischemia was observed. Values are depicted as mean ± SEM (3 fields of view per region per mouse) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; * vs. treatment, # vs. time, & vs. infarct).

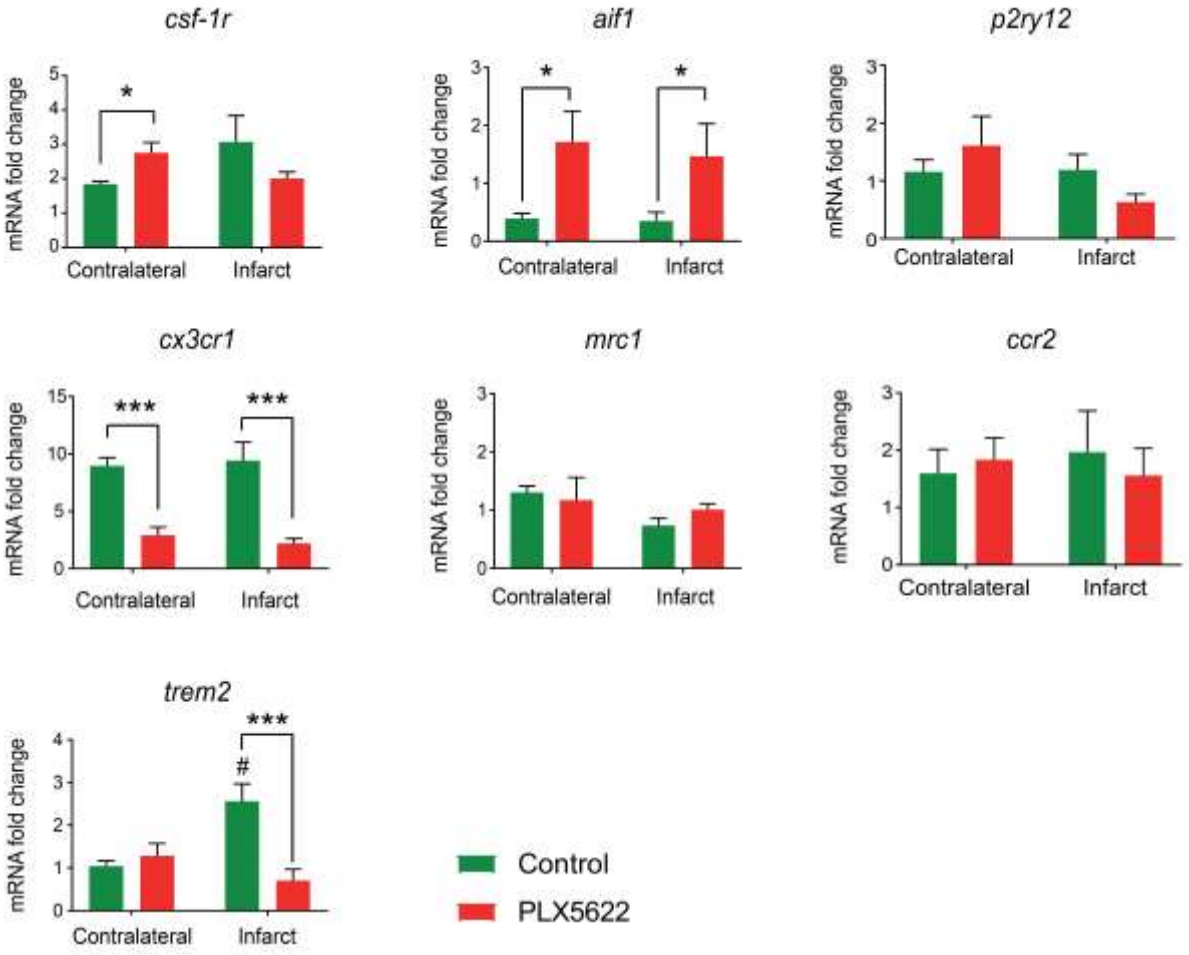


Supplemental Figure 7. Number of GFAP$^+$ increased over time after acute PLX5622 treatment. (A) Representative GFAP staining within the infarct and contralateral striatum for both experimental groups at days 14 and 35 post ischemia. (B) Treatment effect was observed in both regions at day 14: PLX5622-treated mice showed a higher percentage of GFAP$^+$ area compared to control mice in both infarct ($p = 0.014$) and contralateral striatum ($p < 0.001$). At day 35 post ischemia, treatment effect was still observed in the contralateral striatum while no difference was observed within the infarct. (C) No colocalization

between GFAP and TSPO was observed at day 14 post ischemia, indicating that astrocytes were not contributing to the increased 18F-DPA-714 PET signal in PLX5622-treated mice at day 14 post ischemia. Values are depicted as mean ± SEM (control: n = 4 and PLX5622: n = 4 for both time points, 3 fields of view per region per mouse, scale bar: 20 μm) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; * vs. treatment, # vs. time, & vs. infarct).



Supplemental Figure 8. Positive correlation between *aif1* and *csf-1r* gene expression during repopulation ($R^2 = 0.86$).



Supplemental Figure 9. Gene expression of microglia/macrophages-related markers at day 35 post ischemia (n = 4/group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, # vs contralateral).