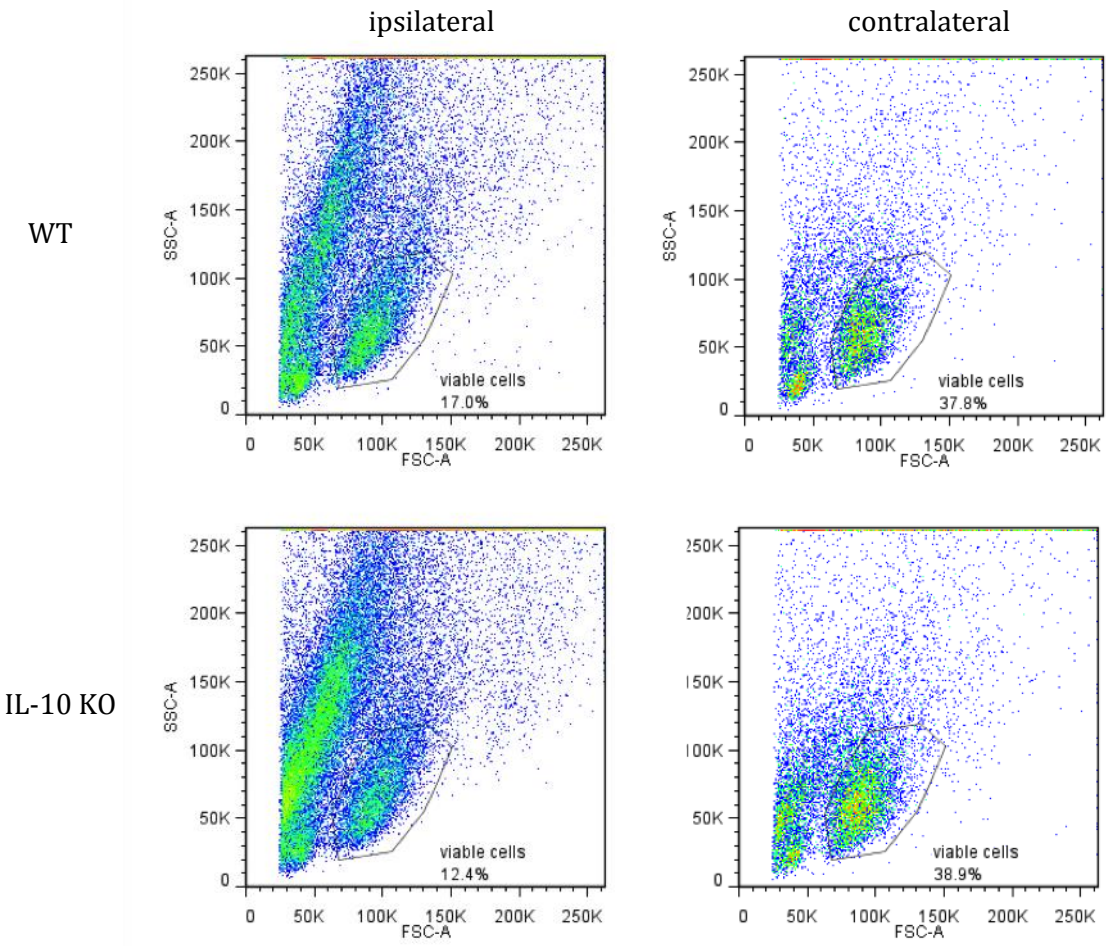
SUPPLEMENTARY MATERIALS

Supplementary TABLE 1: List of primer sequences for PCR.

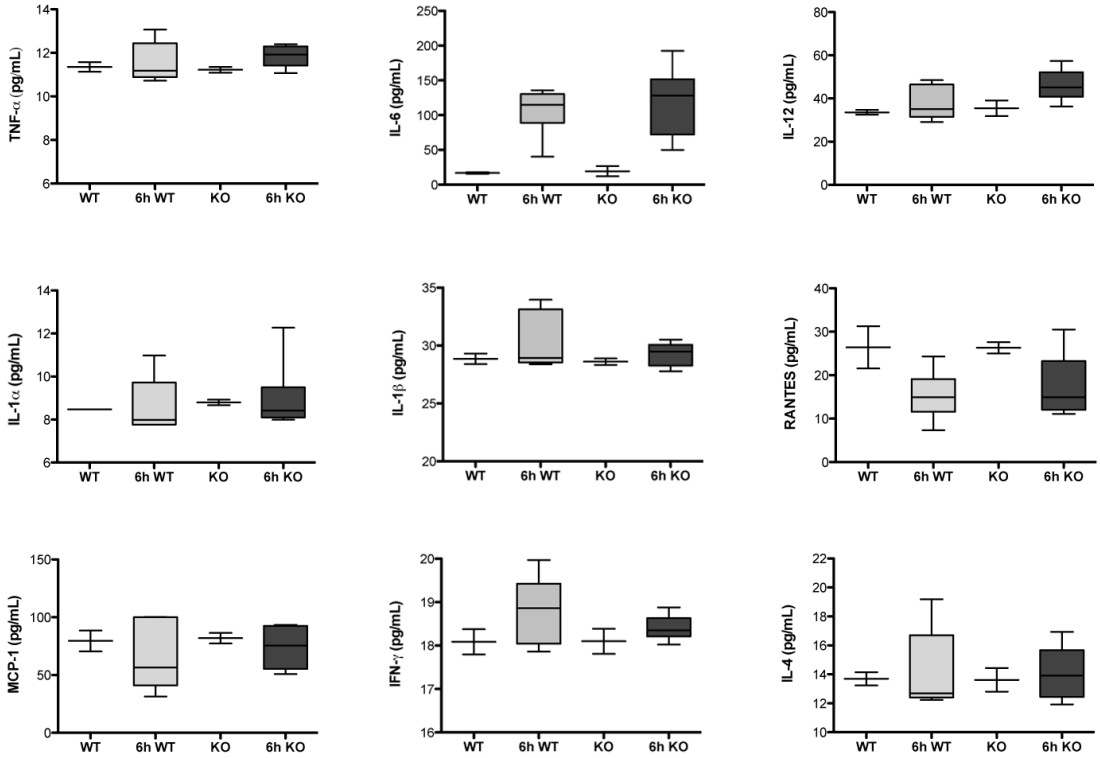
MOUSE	Primer Sequence 5'®3'	Accession number	Amplicon length (bp)	Region
Arg-1	F: TTGCGAGACGTAGACCCTGG	NM_007482.3	160	Exon 5
	R: CAAAGCTCAGGTGAATCGGC			Exon 7
COX2	F: CCACTTCAAGGGAGTCTGGA	NM_011198.3	187	Exon 3
	R: AGTCATCTGCTACGGGAGGA			Exon 4
CXCL1	F: CCGAAGTCATAGCCACACTCAA	NM_008176.3	128	Exon 2/3
	R: GCAGTCTGTCTTCTTTCTCCGTTAC			Exon 4
CX3CL1	F: TGGCTTTGCTCATCCGCTATCAG	NM_009142.3	82	Exon 2
	R: CGTCTGTGCTGTGTCGTCTCC			Exon 3
ICAM-1	F: CCGTGCCAAGCCCACGCTAC	NM_010493.2	300	Exon 1
	R: GCGGAAGCGGACGACTGCAC			Exon 2
IGF-1	F: GTGGACCGAGGGGCTTTTACTTC	NM_010512.4	246	Exon 1 or 2
	R: TTTGCAGCTTCGTTTTCTTGTTTG			Exon2 or 3
IL-1β	F: GAAGAGCCCATCCTCTGTGA	NM_008361.3	96	Exon 4-5
	R: TTCATCTCGGAGCCTGTAGTG			Exon 5
IL1RA	F: AACCACCAGGGCATCACATA	NM_001159562.1	150	Exon 5
	R: CCTCTTGCCGACATGGAATA			Exon 5
IL-6	F: GATGGATGCTACCAAACTGGA	NM_031168.1	142	Exon 3
	R: TCTGAAGGACTCTGGCTTTG			Exon 4
IL-10R	F: CCTTCCTATGTGTGGTTTGAAGCC	NM_008348.2	290	Exon 1
	R: TCATCCACTGTGAAGCGAGTCTC			Exon 2
iNOS	F: CAGCTGGGCTGTACAAACCTT	NM_010927.3	95	Exon 17
	R: CATTGGAAGTGAAGCGTTTCG			Exon 18
MCP-1	F: AGGTGTCCCAAAGAAGCTGTAG	NM_011333.3	90	Exon 2
	R: AATGTATGTCTGGACCCATTCC			Exon 3
MIP-1α	F: TTCTGCTGACAAGCTCACCCT	NM_011337.2	116	Exon 1
	R: ATGGCGCTGAGAAGACTTGGT			Exon 1
MIP-2a	F: GAACAAAGGCAAGGCTAACTGA	NM_009140.2	204	Exon 3/4
	R: AACATAACAACATCTGGGCAAT			Exon 4
MMP-9	F:CTTCTCTGGACGTCAAATGTG	NM_013599.2	276	Exon 10-11
	R: AGAAGAATTTGCCATGGCAG			Exon 13
TGF-β	F: TGCTTCAGCTCCACAGAGAA	NM_011577.1	155	Exon 5-6
	R: TACTGTGTGTCCAGGCTCCA			Exon 6
TLR-4	F: TGTTCTTCTCCTGCCTGACA	NM_021297.2	108	Exon 1
	R: TGTCATCAGGGACTTTGCTG			Exon 2
TNF-α	F: GGGGCCACCACGCTCTTCTGTC	NM_013693	155	Exon 1
	R: TGGGCTACGGGCTTGTCACTCG			Exon 3
SDHA	F: TGGGGAGTGCCGTGGTGTCA	NM_023281.1	154	Exon 6
	R: CATGGCTGTGCCGTCCCCTG			Exon 7
YM-1	F: CAGGTCTGGCAATTCTTCTGAA	NM_009892.2	197	Exon 1-2
	R: GTCTTGCTCATGTGTGTAAGTGA			Exon 3

Supplementary Fig. 1



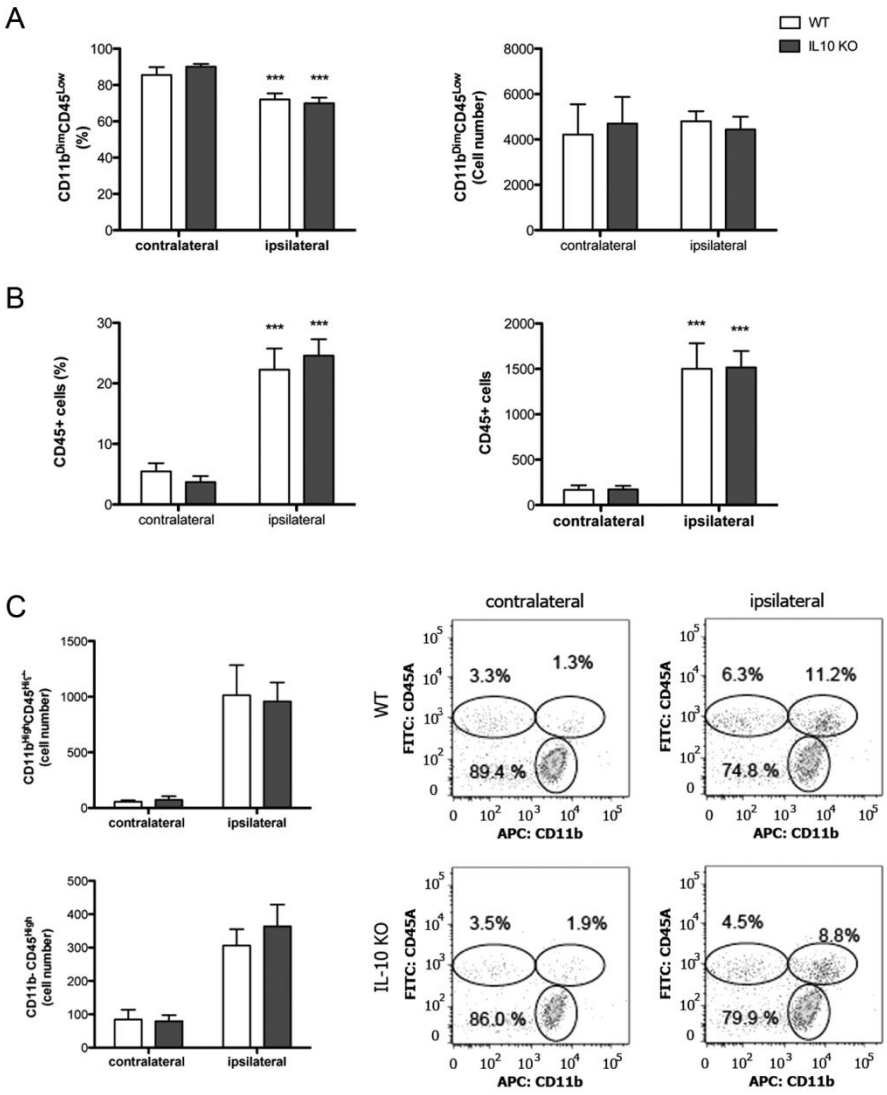
Legend to Supplementary Fig. 1: Flow cytometry of brain tissue cells: Cells were morphologically identified by forward and side scatter (FSC-A vs SSC-A) parameters. Viable cells were further analyzed by exclusion of doublets and by antigen positivity on CD11b, CD45, F4/80 and Ly6G. Images illustrate representative samples of the ipsilateral and contralateral hemispheres 4 days after pMCAO in WT mice and IL-10 KO mice.

Supplementary Fig. 2



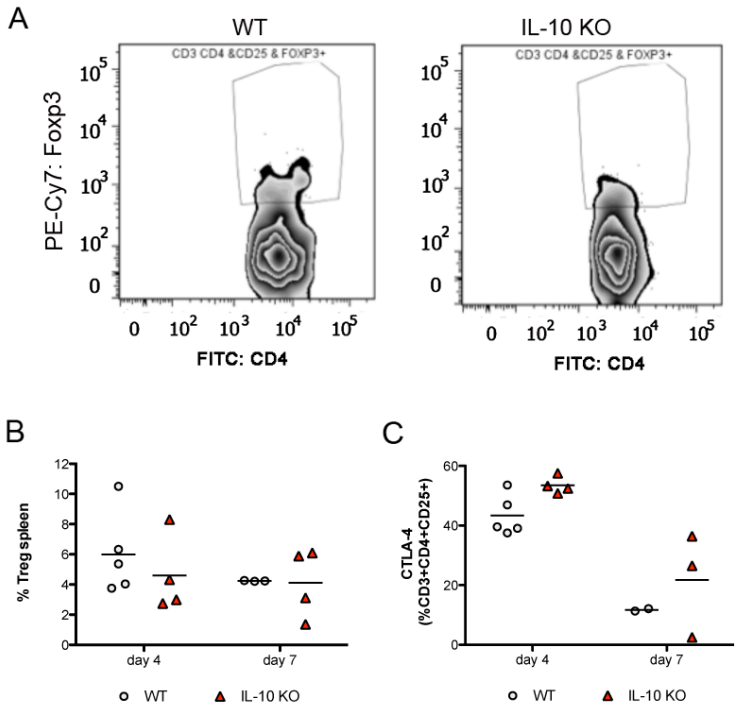
Legend to Supplementary Fig. 2: Plasma cytokines and chemokines in WT and IL-10 KO mice housed under SPF conditions. Plasma was obtained from naïf non-ischemic mice (WT and KO, n=2 per group) and from ischemic mice at 6 hours after pMCAO (6h WT and 6h KO, n=6 per group). Values are expressed as pg/mL of plasma. Although a trend for higher IL-12 concentrations is observed in IL-10 KO mice after pMCAO, group differences for all the molecules shown in the graphs are not statistically significant.

Supplementary Fig. 3



Legend to Supplementary Fig. 3: Myeloid cells in the ischemic tissue 4 days after pMCAO. Flow cytometry of brain tissue showed that the percentage of microglia cells ($CD11b^{Dim}CD45^{Low}$) decrease in the ipsilateral cortex 4 days after pMCAO (A), due to increases in the numbers of CD45+ cells demonstrating the presence of leukocytes in the ischemic tissue (B). However, no differences are observed between WT and IL-10 KO mice. (C) Ischemia increases the numbers of infiltrated ($CD45^{High}$) leukocytes, both $CD11b^{High}CD45^{High}$ myeloid cells and $CD11b\text{-}CD45^{High}$ lymphocytes, but the effects are similar in both genotypes. Flow cytometry diagrams shown in the right panels correspond to one representative sample per group, out of n=5 per group,*** $p<0.001$ versus the corresponding contralateral non-ischemic cortex.

Supplementary Fig. 4



Legend to Supplementary Fig. 4: Flow cytometry of Treg from the spleen of WT and IL-10 KO mice. A) Representative dot plots of Treg expression in splenocytes from WT and IL-10 KO mice 7 days after pMCAO. Single viable cells were plotted by FoxP3 versus CD4 expression. B) Frequency of Foxp3+ splenocytes in WT and IL-10 KO mice analyzed by flow cytometry 4 and 7 days after pMCAO. C) Frequency of CTLA-4+ (CD152) splenocytes in WT and IL-10 KO mice analyzed by flow cytometry 4 and 7 days after stroke. Points in (B) and (C) represent values from individual subject. No significant differences between groups were found.

SUPPLEMENTARY METHODS

Microglia cultures: The cerebral cortices of 1-day-old neonatal mice (C57BL/6, Charles-River, Lyon, France) (n=10-12) were dissected out. After mechanical dissociation the tissue was chemically dissociated by incubation with trypsin-EDTA (#25200-05625, Gibco-BRL, Invitrogen, Paisley, UK) for 25 min at 37 ºC in a shaker. Then samples were placed in 20 mL DMEM/F12 (#31330-038, GIBCO-BRL) (1:1) containing 10 % fetal bovine serum (FBS) (#10500-064, GIBCO-BRL) and DNase I (6,000 Units) (D-5025-150KU, Sigma-Aldrich, St. Louis, MO, USA). Cells were suspended to a final density of 350,000 cells/mL with supplemented DMEM/F12 (1:1) medium containing 20 % FBS and antibiotics (0.4 % penicillin/streptomycin #15140-122, GIBCO-BRL) and were seeded on 6-well poly-lysine coated plates. Cells were placed in an incubator at 37 ºC in an atmosphere containing 95 % atmospheric air and 5% CO_2 until they reached confluence (approximately after 16 days in vitro (DIV)). The culture medium was changed and cells were kept in culture for 3 days more. At this stage the culture medium was removed and it was kept at 37 ºC (this is the conditioned medium to be used in a later step) and cells were mildly trypsinized to induce detachment of the astroglial layer. Cells remaining attached to the wells are highly enriched in microglia (98 %). Microglial cells were then kept in fresh medium containing 50 % of the astroglia-conditioned medium obtained above and were used for treatments the following day. Cells were subjected to ischemic conditions for 3 hours by placing them in an anoxia incubator (GalaxyR/RS Biotech, New Brunswick, Eppendorf, Enfield, CT, USA) containing an atmosphere of 95 % N_2, and 5 % CO_2 at 37 ºC. After this time, cells were kept for 3 hours more under normoxic conditions. Other cells were exposed to 10 ng/mL lypopolysaccharide (LPS) (Escherichia coli 055:B5) (Sigma-Aldrich) for 6 hours. For IL-10 treatment, 10 ng/mL recombinant murine IL-10 (#210-10, PreproTech, Rocky Hill, NJ, USA) was added to the cell cultures 30 min before the above challenges.