Targeted homing of CCR2-overexpressing mesenchymal stromal cells to ischemic brain enhances post-stroke recovery partially through PRDX4-mediated blood-brain barrier preservation

- SUPPLEMENTARY FIGURES 1-11

- SUPPLEMENTARY TABLES 1-3

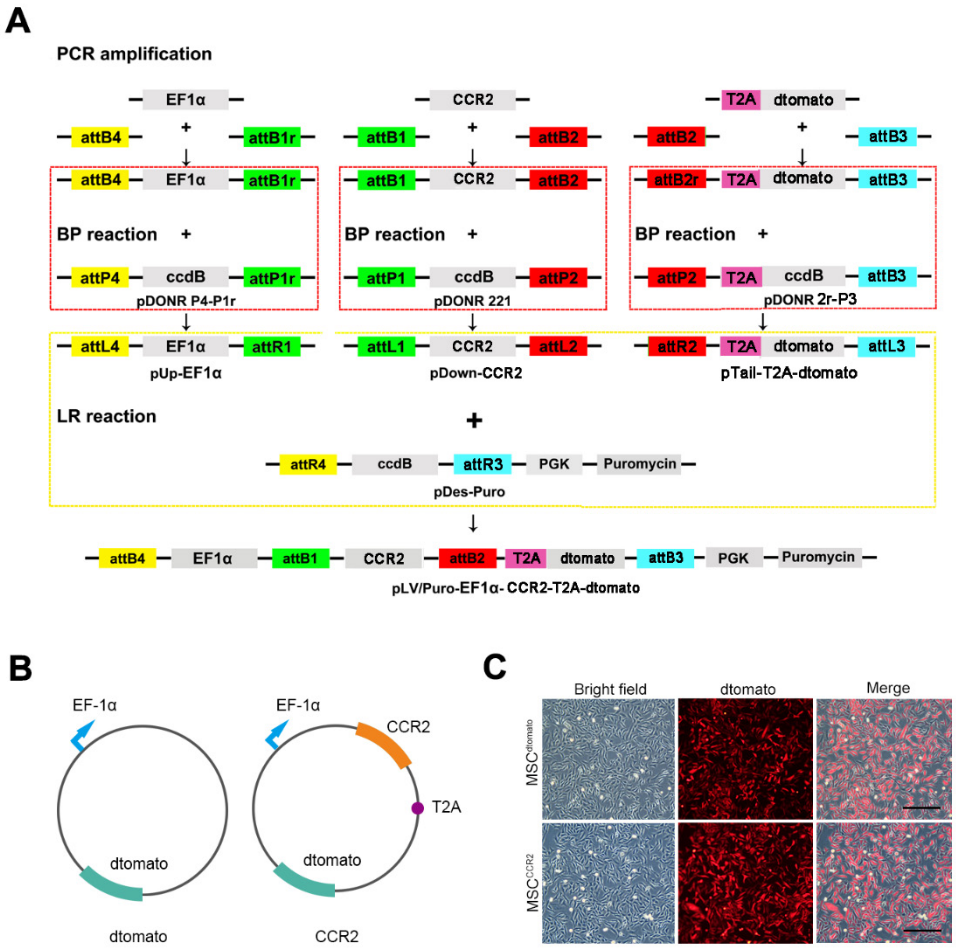

Figure S1. Construction of pLV/Puro-EF1α-CCR2-T2A-dtomato.

(A) Construction of pLV/Puro-EF1α-CCR2-T2A-dtomato. The CCR2-encoding vector was constructed using the multisite gateway method previously described.

(B) Schematic diagram of the dtomato and CCR2 plasmid.

(C) The morphologies of $MSC^{dtomato}$ and MSC^{CCR2} were not found abnormal under the bright field microscopy and the red fluorescence was observed using the fluorescence microscopy. Scale bar: 150μm.

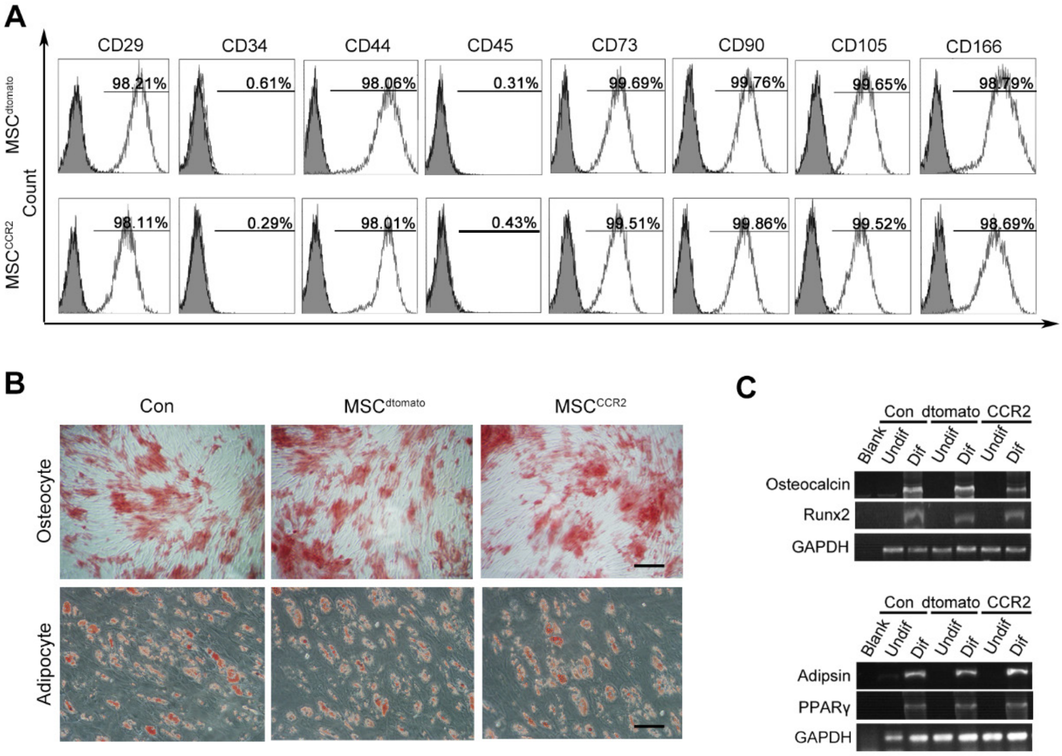

Figure S2. Characteristics of the transfected MSC^{dtomato} and MSC^{CCR2}.

(A) The expression of surface markers including CD29, CD34, CD44, CD45, CD73, CD90, CD105 and CD166 were detected by flow cytometry in both of MSC^{dtomato} and MSC^{CCR2}.

(B) FACS-sorted dtomato^{+} MSCs exhibited osteogenic and adipogenic differentiation capacity. Scale bar: 150μm.

(C) Osteogenic and adipogenic markers of differentiated MSCs were analyzed by PCR.

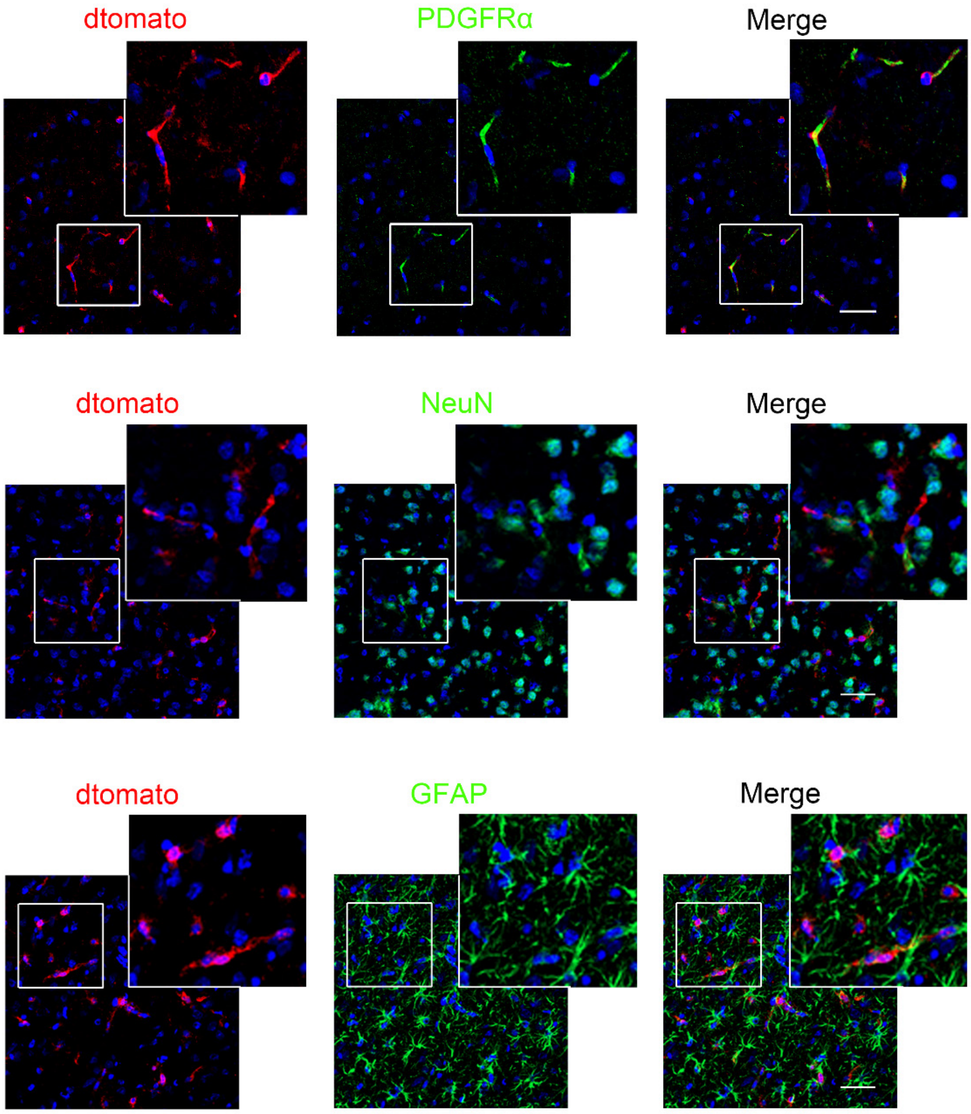

Figure S3. Fluorescent staining of brain slices with stem cell or differentiated cell markers to detect dtomato^{+} cell identity. Scale bar: 50μm.

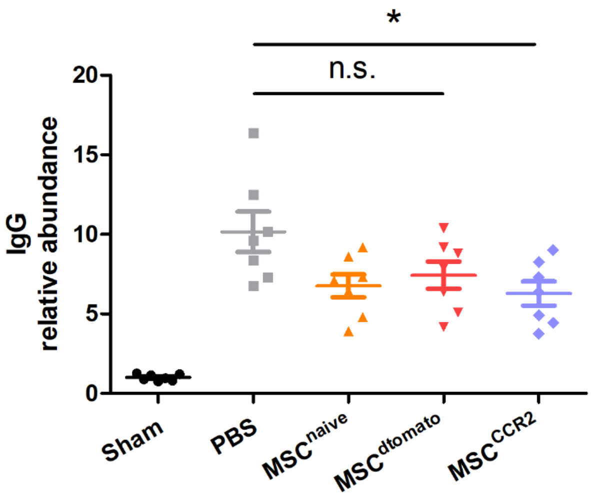

Figure S4. Quantification of relative abundance of extravascular IgG. All data are expressed as means ± SEM; *p<0.05 and n.s. is non-significant.

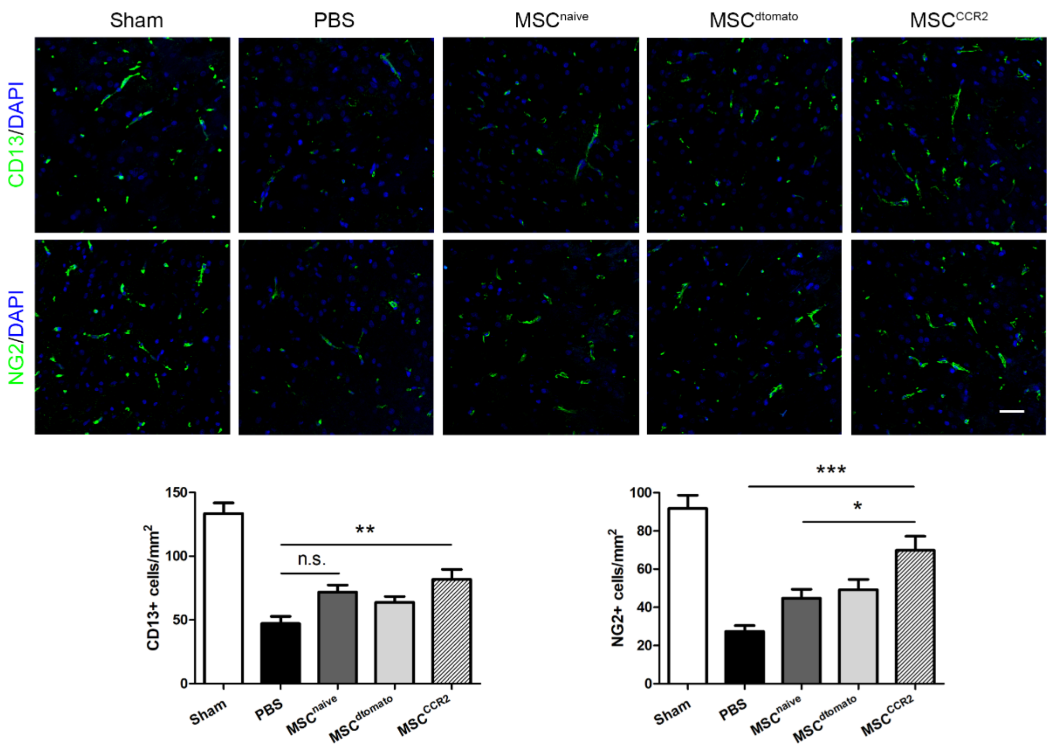

Figure S5: Confocal microscopy analysis of CD13 and NG2-expressing pericytes (green).

Scale bar: 50µm.

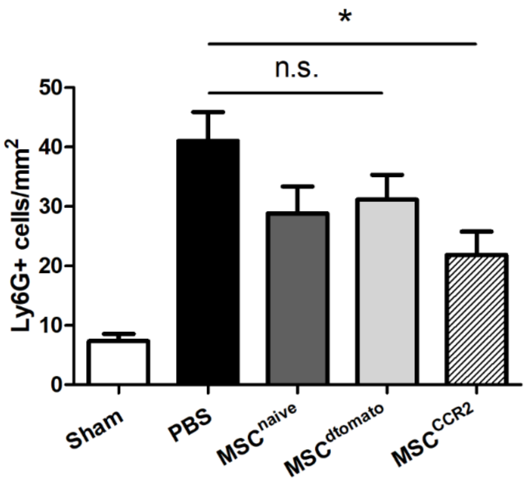

Figure S6: Quantification of the Ly6G+ cells in the ipsilateral hemisphere after MSCCCR2 administration.

Six randomized fields were measured, and the experiments were performed in four replicates. All data are expressed as means ± SEM; *p<0.05 and n.s. is non-significant.

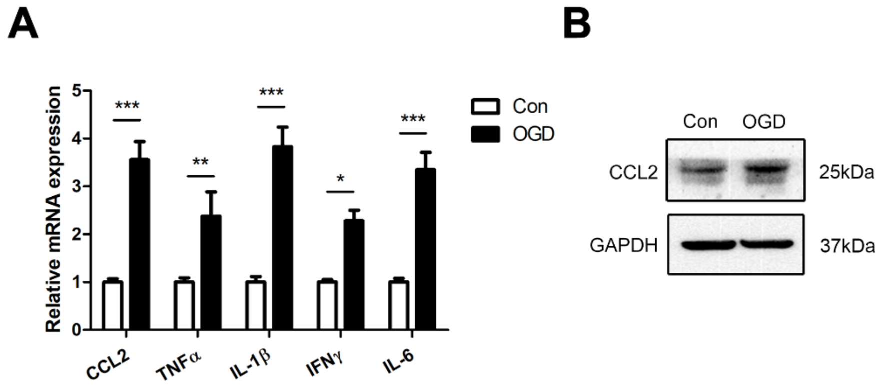

Figure S7. OGD treatment increased CCL2 expression in b.End3 cells.

(A) QRT-PCR for mRNA of CCL2, TNFα, IL-1β, IFNγ, IL-6 in ODD-treated b.End3 cells. n=4.

(B) Western blotting analysis of CCL2 in endothelial cells after 4h OGD treatment. All data are expressed as means ± SEM; *p<0.05, **p<0.01 and ***p<0.001.

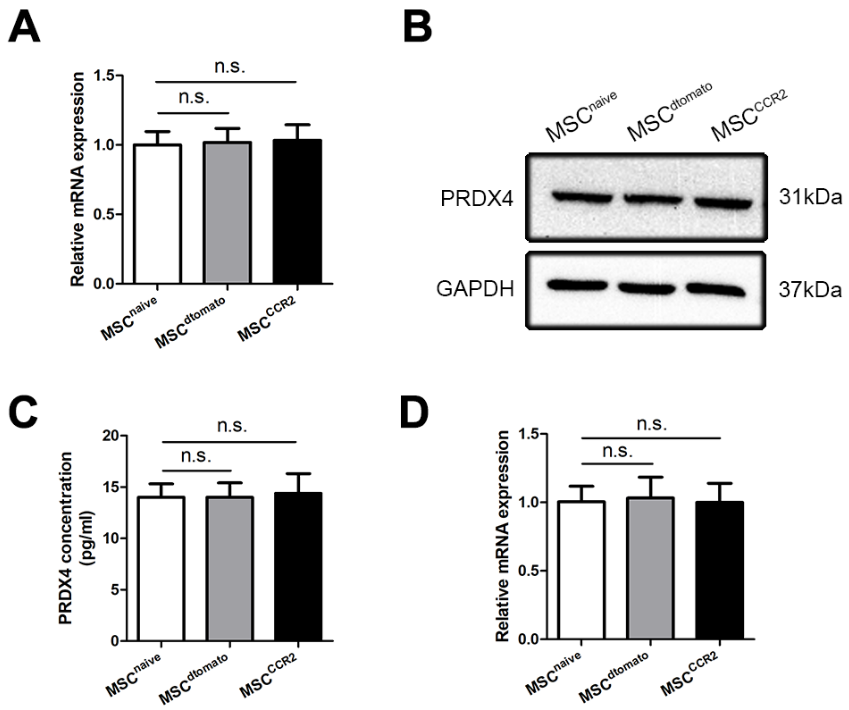

Figure S8: Genetic manipulation do not alter PRDX4 expression in MSCs.

(A-B) The expression levels of PRDX4 were analyzed by both qRT-PCR (A) and western blotting (B). n=4. (C) Overexpression of PRDX4 did not alter *in vitro* PRDX4 secretion by MSCs. n=5. (D) *In vivo* PRDX4 expression of transplanted naive MSCs and genetic modified MSCs. All data are expressed as means ± SEM; n.s. is non-significant.

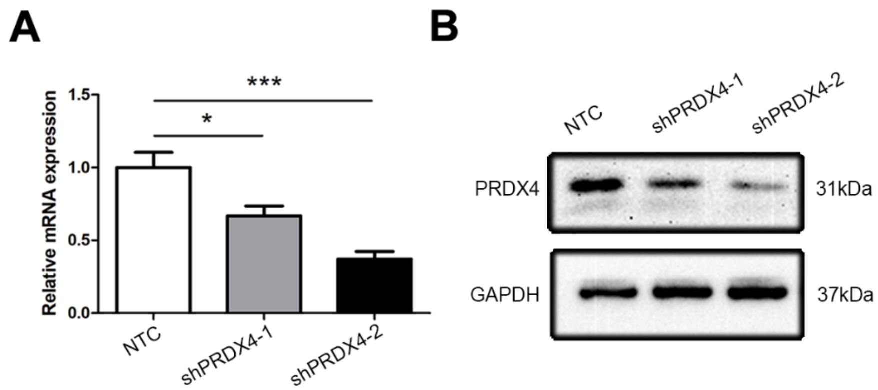

Figure S9. RNA interference efficiency of shRNAs against PRDX4.

(A-B) The interference efficiencies of shPRDX4-1 and shPRDX4-2 were determined by qRT-PCR (A) and western blotting (B). ShPRDX4-2 appeared to be more efficient than shPRDX4-1. n=4. All data are expressed as means ± SEM; *p<0.05 and ***p<0.001.

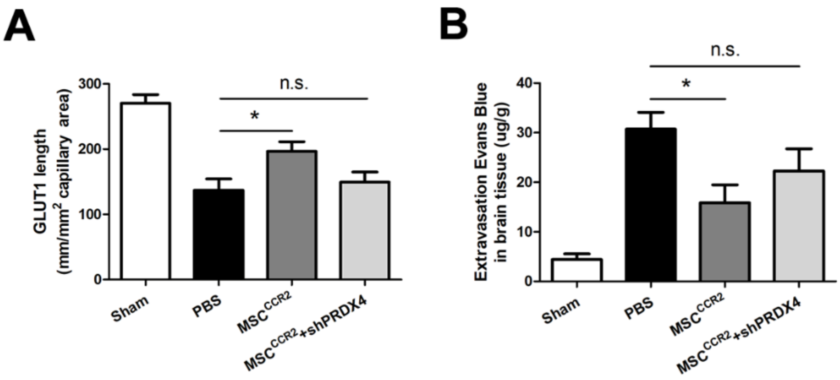

Figure S10. ShPRDX4 treatment suppresses the protective impacts of MSC^{CCR2} on BBB integrity.

(A) GLUT1 length was quantified using Neuron J. Six fields were randomly selected in the cortex per animal and three animals per group were measured. (B) Quantification of EBD extravasation. n=7. All data are expressed as means ± SEM; *p<0.05 and n.s. is non-significant.

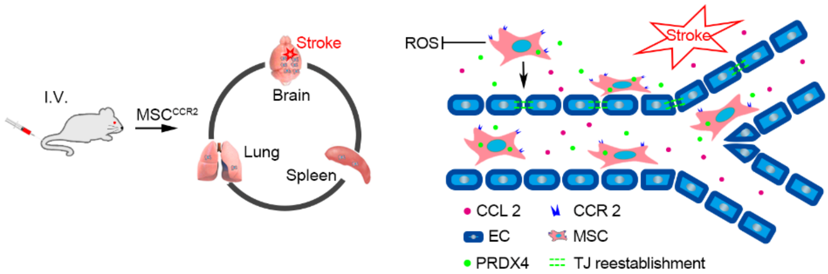

Figure S11. A schematic diagram illustrating how MSC^{CCR2} improve post-stroke recovery. Overexpression of CCR2 on MSCs surface promotes cell recruitment to the ischemic hemisphere after the intravenous (I.V.) transplantation, with less cells sequestered by lung and spleen (left panel). Increased number of MSCs secrete antioxidant molecule PRDX4 and exhibit enhanced antioxidant protection against BBB disruption (right panel).

Supplementary Table1. Primer used to amplify the rat transcripts during real-time quantitative PCR.

Gene	Sequence (5′ to 3′)	Application
CCL2 (Rat)	**Upper: TGATCCCAATGAGTCGGCTG** **Lower: GGTGCTGAAGTCCTTAGGGTT**	**qRT-PCR**
CCL3 (Rat)	**Upper: GCTTCTCCTATGGACGGCAA** **Lower: TCTGCCGGTTTCTCTTGGTC**	**qRT-PCR**
CCL5 (Rat)	**Upper: TGCTGCTTTGCCTACCTCTC** **Lower: TCTTCTCTGGGTTGGCACAC**	**qRT-PCR**
CCL11 (Rat)	**Upper: GCACGCTGAAAGCCATAGTC** **Lower: CTTTGTGGCATCCTGGACCC**	**qRT-PCR**
CX3CL1 (Rat)	**Upper: GCCATCATCCTGGAGACGAG** **Lower: CTGCTGCACCTCTAAGCGA**	**qRT-PCR**
CXCL1 (Rat)	**Upper: GCCACCAGCCGCCAA** **Lower: TTCTGAACCATGGGGGCTTC**	**qRT-PCR**
CXCL2 (Rat)	**Upper: CCAACCATCAGGGTACAGGG** **Lower: ACGATCCTCTGAACCAAGGG**	**qRT-PCR**
CXCL10 (Rat)	**Upper: TCTGAGTGGGACTCAAGGGA** **Lower: TCTCAACATGCGGACAGGAT**	**qRT-PCR**
CXCL11 (Rat)	**Upper: CCCTGGCTATGATCATCTGGG** **Lower: TCTGCATTATGAGGCGAGCTT**	**qRT-PCR**
CXCL12 (Rat)	**Upper: CCCCTGCCGATTCTTTGAGA** **Lower: TGCACACTTGTCTGTTGTTGC**	**qRT-PCR**
CXCL13 (Rat)	**Upper: CTCCAGGCCACGGTATTCTG** **Lower: GCCATTCCCAGGGCGTATAA**	**qRT-PCR**
TNFα (Rat)	**Upper: ATGGGCTCCCTCTCATCAGT** **Lower: ACCACCAGTTGGTTGTCTTTG**	**qRT-PCR**
IFNγ (Rat)	**Upper: GGAACTGGCAAAAGGACGGT** **Lower: AGGTGCGATTCGATGACACT**	**qRT-PCR**
IL-1β (Rat)	**Upper: TCTCACAGCAGCATCTCGAC** **Lower: GGTCGTCATCATCCCACGAG**	**qRT-PCR**
IL-6 (Rat)	**Upper: CACTTCACAAGTCGGAGGCTTA** **Lower: GAACTCCAGAAGACCAGAGCAG**	**qRT-PCR**
β-actin (Rat)	**Upper: CCATCATGAAGTGTGACGTTG** **Lower: CAATGATCTTGATCTTCATGGTG**	**qRT-PCR**
CCR1 (Human)	**Upper:** TGCATCCCCATAGTCAAACTC **Lower:** CAGAAAGCCCCAGAAACAAA	**qRT-PCR**
CCR2 (Human)	**Upper:** TACGGTGCTCCCTGTCATAAA **Lower:** TAAGATGAGGACGACCAGCAT	**qRT-PCR**

CCR3 (Human)	**Upper:** CAACTCAGCAGTGAAATGTGC **Lower:** TCTTCTTGTGCTTATCCGGG	**qRT-PCR**
CCR4 (Human)	**Upper:** CTTTCATCGAGGGTGGTGTC **Lower:** CACAGACCTTCCTCAGAGCC	**qRT-PCR**
CCR5 (Human)	**Upper:** CTGCGATTTGCTTCACATTG **Lower:** TGAGACATCCGTTCCCCTAC	**qRT-PCR**
CCR6 (Human)	**Upper:** AAATTCATTGATTCCCCGCT **Lower:** TGAAGGGAGTGGATCAGAGC	**qRT-PCR**
CCR7 (Human)	**Upper:** TCTCCGATGTAATCGTCCGT **Lower:** CAGCCTTCCTGTGTGGTTTT	**qRT-PCR**
CCR8 (Human)	**Upper:** TCACAGGGGCTTGAGAAGAT **Lower:** CCTCCAGAACAAAGGCTGTC	**qRT-PCR**
CCR9 (Human)	**Upper:** AGGGCTTGTGAAGTCTGTGG **Lower:** CAGAGAGCAACCCAGCTCTT	**qRT-PCR**
CCR10 (Human)	**Upper:** GTCAGGGAGACACTGGGTTG **Lower:** GACGGAGGCCACAGAGC	**qRT-PCR**
CXCR1 (Human)	**Upper:** GGCATGCCAGTGAAATTTAG **Lower:** TACTGTTGGACACACCTGGC	**qRT-PCR**
CXCR2 (Human)	**Upper:** TCTTCAAAGCTGTCACTCTCCA **Lower:** AGCAGGTCACAGCTGCTCTT	**qRT-PCR**
CXCR3 (Human)	**Upper:** CTCGGCGTCATTTAGCACTT **Lower:** AACCACAAGCACCAAAGCAG	**qRT-PCR**
CXCR4 (Human)	**Upper:** CTTGTCCGTCATGCTTCTCA **Lower:** GAACCCTGTTTCCGTGAAGA	**qRT-PCR**
CXCR5 (Human)	**Upper:** CCTTGAAGGAGGCCATGAG **Lower:** TAACGCTGGAAATGGACCTC	**qRT-PCR**
CXCR6 (Human)	**Upper:** GCAGGAAGTCTTGATGCTCC **Lower:** TGAGCAAGCTCATCTCTGGA	**qRT-PCR**
CXCR7 (Human)	**Upper:** CAGATCCATCGTTCTGAGGC **Lower:** GCAGAGCTCACAGTTGTTGC	**qRT-PCR**
CX3CR1 (Human)	**Upper:** ACTTTGAGTACGATGATTTGGCT **Lower:** GGTAAATGTCGGTGACACTCTT	**qRT-PCR**
Prdx4 (Human)	**Upper:** AGAGGAGTGCCACTTCTACG **Lower:** GGAAATCTTCGCTTTGCTTAGGT	**qRT-PCR**
β-actin (Human)	**Upper:** GGCTGTATTCCCCTCCATCG **Lower:** CCAGTTGGTAACAATGCCATGT	**qRT-PCR**

Supplementary Table2. Primary and secondary antibodies

Product	Catalogue Number	Supplier
Primary antibody:		
WB:		
rabbit anti-Claudin-5	Ab15106	Abcam
rabbit anti-ZO-1	61-7300	Invitrogen
rabbit anti-Occludin	PA5-20755	Invitrogen
mouse anti-CCR2	sc-74490	Santa Cruz
mouse anti-CCL2	ab25124	Abcam
rabbit anti-Prdx4	ab59542	Abcam
rabbit anti-GAPDH	14c10	Cell Signaling Technology
ICC:		
mouse anti-CCL2	ab25124	Abcam
mouse anti-GFAP	ab4648	Abcam
rabbit anti-Claudin-5	ab15106	Abcam
rabbit anti-ZO-1	61-7300	Invitrogen
mouse anti-CD31	ab64543	Abcam
rabbit anti-GLUT1	ab115730	Abcam
rabbit anti-Fibrinogen	ab92572	Abcam
IHC:		
mouse anti-CD68	ab955	Abcam
rat anti-Ly6g	ab25377	Abcam
Secondary antibody:		
WB:		
anti-mouse IgG HRP-linked Ab	7076	Cell Signaling Technology
anti-rabbit IgG HRP-linked Ab	7074	Cell Signaling Technology
ICC:		
goat anti-mouse IgG Alexa 488	A11054	Invitrogen
goat anti-mouse IgG Alexa 594	A11005	Invitrogen
goat anti-rabbit IgG Alexa 488	A11008	Invitrogen
goat anti-rabbit IgG Alexa 594	A11037	Invitrogen

Supplementary Table3. Antibodies for Flow Cytometry

Product	Catalogue Number	Supplier
Anti-human CCR2 (Alexa Fluor® 647)	561744	BD biosciences
Anti-human CD29 (APC)	559883	BD biosciences
Anti-human CD34 (PE)	550761	BD biosciences
Anti-human CD44 (APC)	559942	BD biosciences
Anti-human CD45 (PE)	560975	BD biosciences
Anti-human CD73 (PE)	550257	BD biosciences
Anti-human CD90 (PE-Cy7)	561558	BD biosciences
Anti-human CD105 (PerCP-Cy5.5)	560819	BD biosciences
Anti-human CD166 (PE)	559263	BD biosciences