Supplemental methods

Laser speckle contrast imaging

Cerebral blood flow (CBF) was monitored using a MoorFLPI-2 system (Moor Instruments). The CBF in both hemispheres of naïve C57BL/6 and *IRG1*$^{-/-}$ mice was measured for 10 minutes using a 256-color palette of perfusion units (PU).

Evans blue extravasation assay

Naïve C57BL/6, naïve *IRG*$^{-/-}$ mice, and C57BL/6 mice subjected to 3h MCAO followed by 2.5h reperfusion (positive control) were i.v. administered with 4ml/kg 2% (w/v) Evans blue dye/0.9% saline solution through lateral tail vein. After 1h of circulation, mice were anesthetized and perfused with PBS to remove intravascular Evans blue. The brains were then harvested, sliced and scanned. The hemispheres of brain were separated and homogenized with 50% TCA solution. Following centrifugation, the supernatants were collected and diluted with 95% ethanol in a ratio of 1:3. The level of extravascular Evans blue in the supernatant was then calculated by measuring the fluorescence with excitation at 540/25nm and emission at 645/40nm (BioTek Synergy HT microplate reader).

Table S1:

Physiological parameters of mice during MCAO

Strain	Group	Weight (g)	During MCAO		
			Temperature (°C)	Oxygen Saturation (%)	Cerebral Blood Flow (% reduction)
WT	**Control (n=10)**	**27.4 ± 1.2**	**36.7 ± 0.2**	**96.6 ± 1.5**	**89.3 ± 3.3**
IRG1 $^{-/-}$	**Control (n=10)**	**27.4 ± 1.2**	**36.8 ± 0.2**	**96.2 ± 1.5**	**87.8 ± 3.3**
	p-value	***0.99***	***0.11***	***0.24***	***0.36***

Data were shown as mean ± SD. Comparisons between two groups were done by Mann-Whitney *U* test. A *p-value* less than 0.05 is statistically significant.

Table S2:

Physiological parameters of mice during MCAO

Strain	Group	Weight (g)	During MCAO		
			Temperature (°C)	Oxygen Saturation (%)	Cerebral Blood Flow (% reduction)
IRG1 $^{-/-}$	**Vehicle (n=10)**	**28.1 ± 1.9**	**36.9 ± 0.2**	**96.6 ± 1.8**	**88.6 ± 3.9**
IRG1 $^{-/-}$	**D3T (n=10)**	**28.4 ± 2.2**	**36.7 ± 0.2**	**97.2 ± 1.2**	**89.7 ± 3.0**
	p-value	***0.96***	***0.10***	***0.38***	***0.49***

Data were shown as mean ± SD. Comparisons between two groups were done by Mann-Whitney *U* test. A *p-value* less than 0.05 is statistically significant.

Figure S1

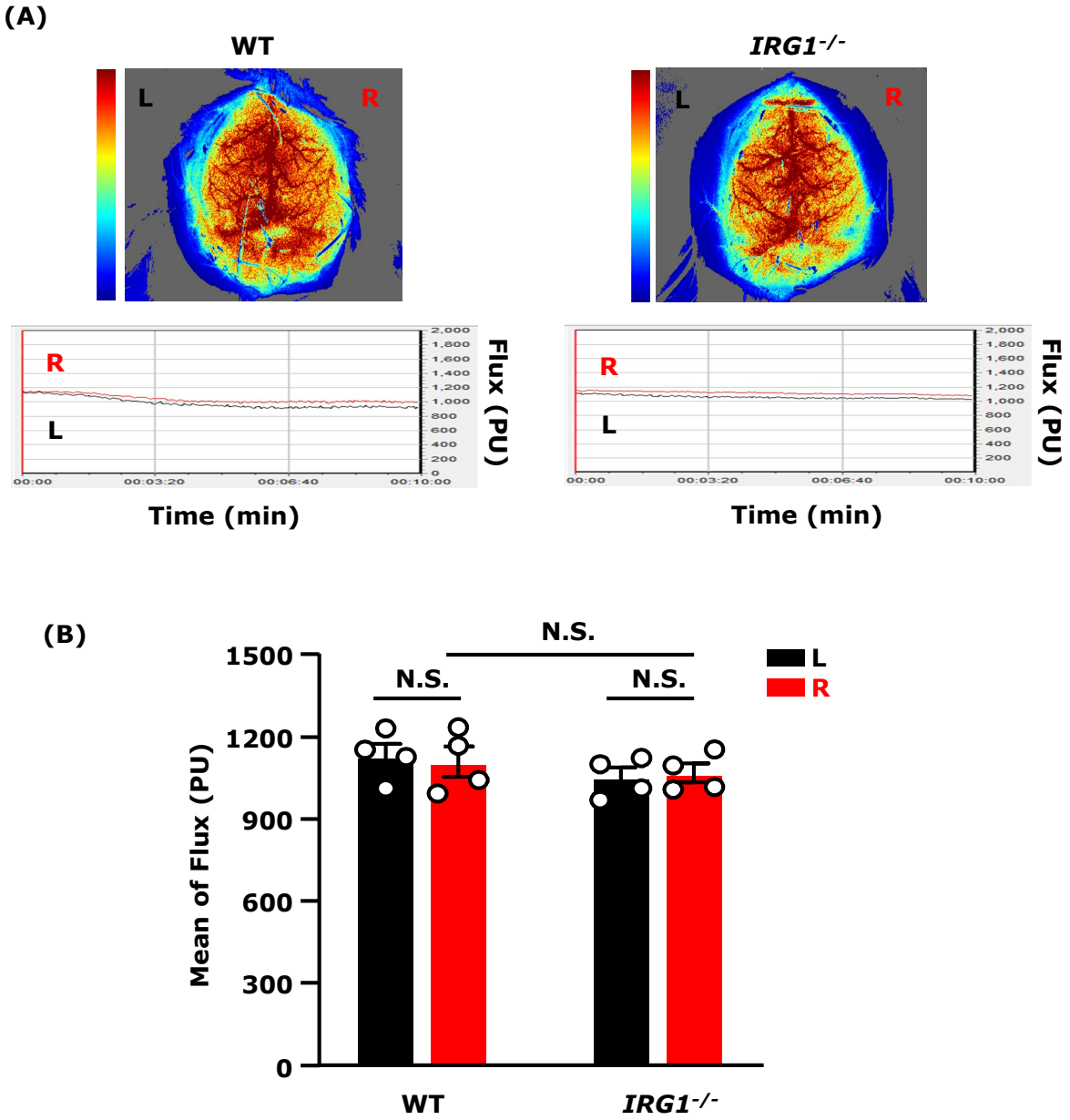

Figure S1 IRG1 deficient mice and its corresponding wild type (WT) controls exhibit a similar level of cerebral blood flow (CBF). (A) WT and *IRG1*$^{-/-}$ mice were subjected to the measurement of CBF in both hemispheres. The representative CBF images and perfusion units (PU) of WT and *IRG1*$^{-/-}$ mice were shown. (B) The level of CBF in both hemispheres of WT and *IRG1*$^{-/-}$ mice (n=4/group) was also quantified. N.S., no significant differences by Kruskal-Wallis test followed by Dunn *post hoc* test.

Figure S2

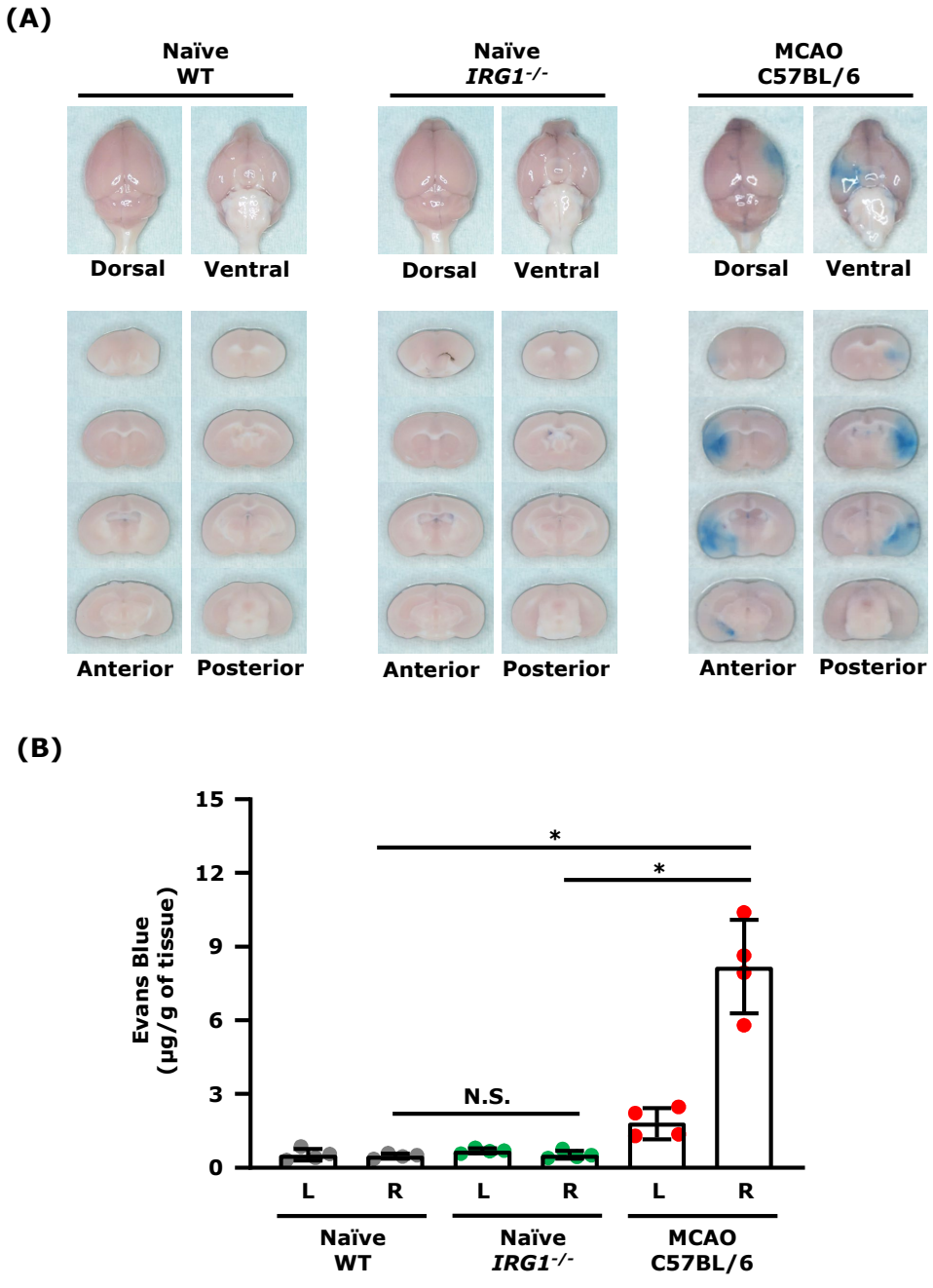

Figure S2 IRG1 deficiency does not alter blood-brain barrier integrity. (A) Naïve WT, naïve *IRG1*$^{-/-}$ or C57BL/6 MCAO mice (positive control) were i.v. administrated with Evans blue. One hour post-administration, brains were harvested followed by imaging and sectioning. The representative images of naïve WT, naïve *IRG1*$^{-/-}$ and C57BL/6 MCAO mice were shown. (B) The Evans blue leakage in the left (L) and right (R) hemispheres was measured and quantified (n=4/group). *$p<0.05$; N.S., no significant differences by Kruskal-Wallis test followed by Dunn *post hoc* test.

Figure S3

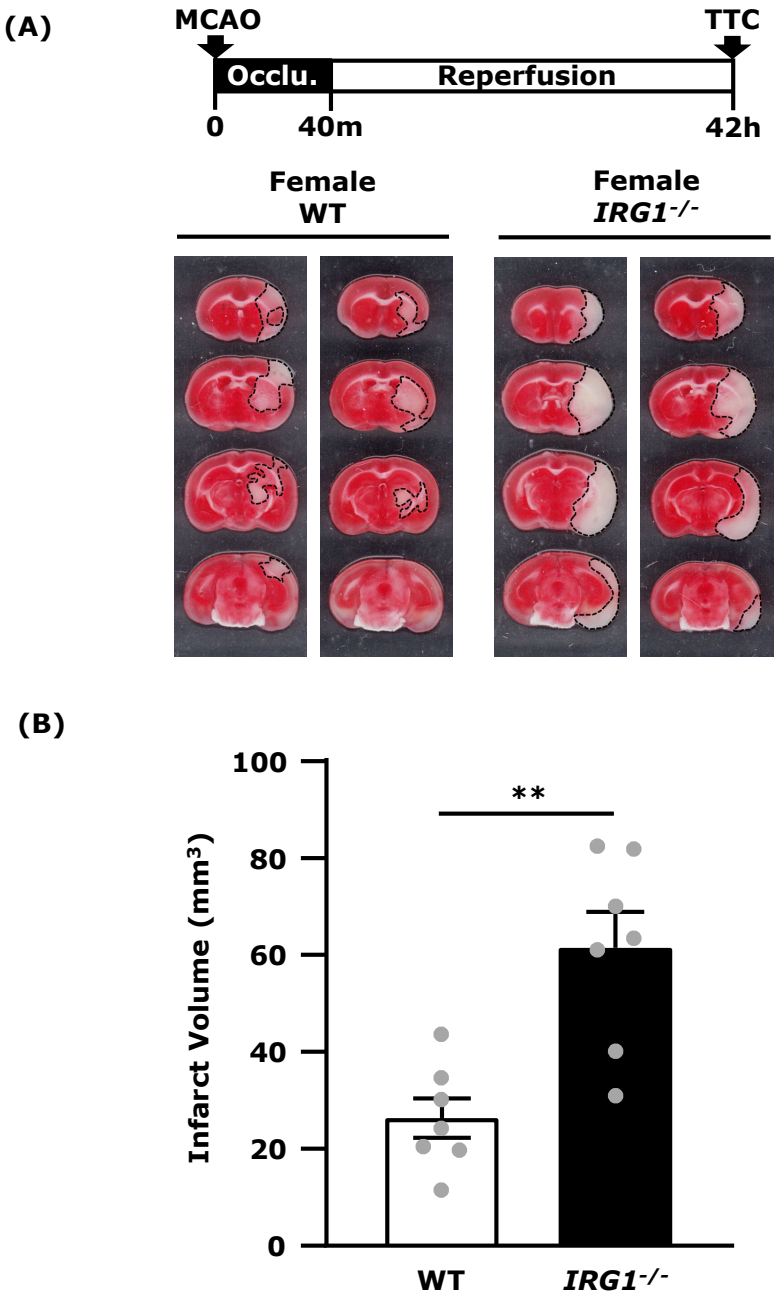

Figure S3 IRG1 deficiency exacerbates ischemic brain injury following ischemic stroke. (A) Middle-aged (12 months old) female WT and *IRG1*$^{-/-}$ mice were subjected to MCAO (n=7/group). 42 hours post-injury, mice were sacrificed, and the ischemic brains were harvested and sliced (2 mm) followed by TTC staining. Two representative TTC-stained samples of WT and *IRG1*$^{-/-}$ MCAO mice were shown. (B) The infarct volumes were also quantified. ***p<0.01* by unpaired *t* test.