

# **SUPPLEMENTAL MATERIAL**

## **Data S1.**

## **Methods**

### **Body weight, brain weight and blood pressure**

Body weight and brain weight were measured at 16-week-old. Blood pressure was measured by the tail-cuff method with a blood pressure monitor (MK-2000ST NP-NIBP MONITOR, Muromachi Kikai Co., Ltd., Tokyo, Japan), before entering the cognitive tasks. Values were expressed as the mean of at least five measurements in each group.

### **Severity of the white matter lesion**

Severity of the white matter lesion was evaluated with Kluver-Barrera (KB) staining. Brain samples were embedded in paraffin and sliced into 5- $\mu$ m-thick coronal sections. We evaluated the white matter changes in corpus callosum area adjacent to the lateral ventricle with KB staining. Samples were examined with an upright microscope (Axioskop 2, Carl Zeiss, Oberkochen, Germany) at x200 magnification.

### **Time-course analysis of CBF changes after BCAS surgery**

Time-course of changes in cerebral blood flow (CBF) after BCAS surgery was measured by laser speckle flowmetry (Omegazone laser speckle blood flow imager; Omegawave,

Tokyo, Japan). CBFs were measured at the point of 0, 1, 2, 4 and 6 weeks after the BCAS and the percentage to the baseline was indicated in all mouse groups.

### **Statistical Analysis**

All values are expressed as mean  $\pm$  SEM. Comparisons between two groups were analyzed using Levene's test for equal variances then Students (equal variances) or Welch's (unequal variances) t-test as appropriate. Comparisons between three or more groups were analyzed using ANOVA then, when significant differences were indicated, were followed by post-hoc pairwise comparisons using Tukey-Kramer method to adjust for multiple hypothesis testing. For each experiment the overall significance threshold was set to 0.05. Statcel 3 (OMS Inc., Japan), add-in software for Microsoft Excel was used for statistical analysis.

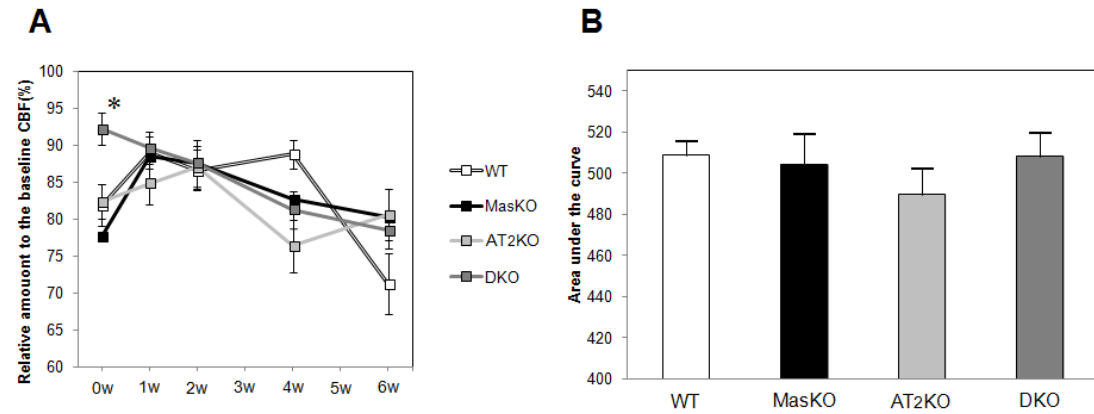
**Table S1. Body weight, brain weight and systolic blood pressure in each group.**

	BCAS (+ / -)	Body weight (g)	Brain weight (g)	Brain/Body weight ratio(%)	Systolic blood pressure(mmHg)
WT	-	26.9±0.4	0.45±0.008	1.62±0.03	105.3±4.1
	+	27.6±0.4	0.44±0.008	1.62±0.03	110.7±2.8
MasKO	-	29.3±0.7 <sup>*</sup>	0.48±0.004	1.63±0.03	102.6±3.4
	+	28.3±0.5	0.47±0.008	1.67±0.04	112.0±5.9
DKO	-	28.2±0.5	0.46±0.007	1.61±0.03	109.8±5.9
	+	28.7±0.4	0.45±0.011	1.61±0.03	124.8±2.1 <sup>†</sup>

<sup>\*</sup>p<0.05 vs. WT-sham. <sup>†</sup>p<0.05 vs. DKO-sham.

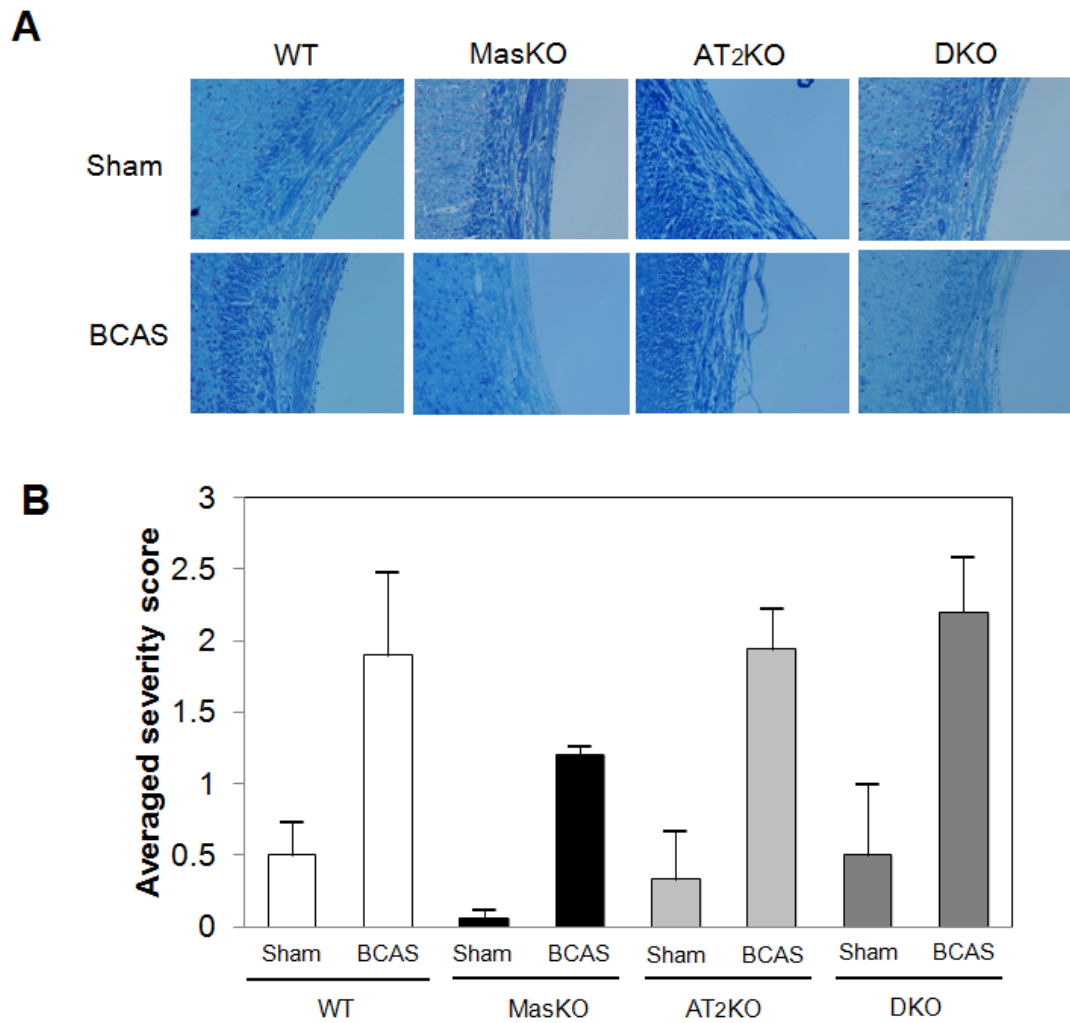
**Table S2. Body weight, brain weight and systolic blood pressure according to Ang-(1-7) treatment.**

	Ang-(1-7) (0.5 mg/kg/day)	Body weight (g)	Brain weight (g)	Brain/Body weight ratio(%)	Systolic blood pressure(mmHg)
WT	-	26.7±1.2	0.43±0.009	1.64±0.06	113.8±7.2
	+	26.8±0.8	0.42±0.005	1.60±0.05	121.0±5.1
MasKO	-	28.3±0.7	0.44±0.011	1.57±0.04	107.8±5.2
	+	28.9±0.6	0.46±0.005	1.60±0.03	115.1±2.5
AT <sub>2</sub> KO	-	27.1±1.4	0.43±0.009	1.61±0.06	103.6±3.8
	+	26.5±1.0	0.42±0.010	1.61±0.04	97.0±4.7
DKO	-	28.4±0.5	0.45±0.007	1.61±0.03	107.5±1.9
	+	28.1±0.5	0.45±0.002	1.61±0.03	104.8±3.1



**Figure S1.**

**Time-course analysis of CBF changes after BCAS surgery. A,** The percentage to the baseline CBF at the point of 0, 1, 2, 4 and 6 weeks after the BCAS surgery is indicated (n=6-11 for each group). \*p<0.01 vs. WT and MasKO. **B,** The area under the curve in the time-course graph is calculated with trapezoidal method in each group. WT, wild type mice; MasKO; Mas receptor knockout mice; AT<sub>2</sub>KO; AT<sub>2</sub> receptor knockout mice; DKO, Mas/AT<sub>2</sub> receptor double-knockout mice.



**Figure S2.**

**Severity of the white matter lesion.** The demyelinating changes in the white matter lesion are determined with Kluver-Barrera staining. The severity of the WM lesions was graded as normal (grade 0), disarrangement of the nerve fibers (grade 1), the formation of marked vacuoles (grade 2), and the disappearance of myelinated fibers (grade 3). **A**, Representative brain images with Kluver-Barrera staining in the corpus callosum level. **B**, Averaged severity scores are indicated. The score is significantly higher in BCAS

treated groups compared to the sham groups in each group, but there is no significant inter-group difference (n=3-6 for each group). WT, wild type mice; MasKO; Mas receptor knockout mice; AT<sub>2</sub>KO; AT<sub>2</sub> receptor knockout mice; DKO, Mas/AT<sub>2</sub> receptor double-knockout mice.