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Figure legends

FIGURE 1:

Time-dependent translocation of HMGB1 in neurons in the TBI site and the the effects of anti-HMGB1 mAb. (**A**) After fluid percussion injury on the right temporal cortex (top panel), the rats received intravenous injection of anti-HMGB1 (1 mg/kg) or control mAb twice at 5 minutes and 6 hours thereafter and the brains were fixed at different time points. The brain sections were double immunostained with anti-HMGB1 and anti-MAP-2 antibodies, followed by AlexaFluor 555-labelled and AlexaFluor 488-labelled secondary antibodies, respectively. Scale bars; 50 μm (yellow) and 5 μm (white). (**B**) Decrease in HMGB1 levels in the TBI region. cerebral cortex from both sides (3 mm square) were collected 24 hours after injury for western blotting. The lane on the left side represents recombinant HMGB1. β-actin was used as the internal control. (**C**) Quantitative analyses were performed using NIH Image J software. Results are expressed as mean ± SEM of 5 rats. $^{**}P < 0.01$ compared with contralateral side. $^{\#\#}P < 0.01$ compared with control rats.

FIGURE 2:

Effect of anti-HMGB1 mAb on brain lesion and BBB permeability in rats with TBI induced by fluid percussion. (**A**) Histological examination of brain sections from TBI rats. The brains were fixed at 6 hours after injury and sections were stained with

hematoxylin-eosin (left) or cresyl violet (right). There were few intact neurons in the injured site in control rats whereas the numerous intact neurons remained in anti-HMGB1-treated rats. (**B**) The permeability of brain capillary vessels was examined by intravenously injecting Evans blue (40 mg/kg) at 6 hours after injury and then measuring the leakage of Evans blue-albumin into the brain parenchyma at 3 hours post-injury. (**C**) Results are expressed as the mean ± SEM of 7 rats. *P < 0.05 compared with the control group. (**D**) Albumin extravasation 6 hours after injury detected by anti-rat albumin Ab.

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Effect of anti-HMGB1 mAb on T2-weighted MRI. (**A**) Three representative MRIs from each group (n=5) are shown. (**B**) The quantitative evaluation was performed. Results are expressed as mean ± SEM. of 5 rats. **P < 0.01 compared with the control group.

FIGURE 4:

Effects of anti-HMGB1 mAb on the impairment of motor functions. (**A**) The results of rotarod test were expressed as mean ± SEM of 6 rats. *P < 0.05 and **P <0.01 compared with the sham control at each time point. $^{\#}$P < 0.05 compared with the corresponding control at the same time point. (**B**) The results of limb-use asymmetry

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FIGURE 5:

Transmission electron microscopic observation of capillary vessels and determination of MMP activity by zymography. **(A)** Rat brains were fixed at 6 hour after percussion injury by transcardial perfusion with 4% paraformaldehyde and 2.5% glutalaldehyde in 0.1 M cacodylic acid buffer (pH 7.3) under deep pentobarbital anesthesia. Ultrathin sections of cerebral cortex at 3 mm anterior to the injury site were prepared. Representative images from both groups are shown. Swelling of astrocyte endfeet was indicated by asterisks. The inset shows the deformed vascular endothelial cell. (**B**) Swelling of astrocyte endfeet was evaluated as described previously (19). Results are expressed as mean ± SEM of 12 samples from 4 rats. *P < 0.05 compared with the control. (**C**) Zymographic determination of MMP-9 and MMP-2 activity in rats with TBI induced by fluid percussion. Brain samples were prepared by homogenization of the hemisphere (lesion site) with PBS containing proteinase inhibitor cocktail and 20 μg protein was loaded on SDS-PAGE gels containing 1 mg/ml gelatin without heating and reducing reagents. The reaction was developed for 36 hours at 37 °C and the gel was stained with Coomassie brilliant blue. (**D**) The results of zymography were quantified by NIH Image J and are expressed as mean ± SEM of 4 rats. *P < 0.05 compared with the control.

FIGURE 6:

Expression of inflammation-related molecules in rats with TBI. Real-time quantitative PCR was performed for the determination of inflammation-related molecule expression. The results were normalized to the expression of GAPDH. Results are expressed as mean ± SEM of 6 rats. *P < 0.05, **P < 0.01 compared with contralateral side. #P < 0.05, ##P < 0.01 compared with control rats.

FIGURE 7:

Effects of exogenous HMGB1 in rats with TBI. **(A)** Exacerbation of brain lesions by intravenous injection of recombinant HMGB1. Rats received moderate intensity of TBI (2.0-2.2 atm injury). Recombinant HMGB1 (19) (0.04, 0.2 and 0.4 mg/kg) were administered intravenously to rats 10 min after percussion injury. BBB permeability was determined by Evans blue leakage during a 3-hour period beginning 6 hours after the induction of TBI as described in Figure 2B. (**B**) Results are expressed as mean ± SEM of 5 rats. *P < 0.05 compared with the albumin control. (**C**) The rotarod test was performed and the results are expressed as mean ± SEM of 5 rats. *P < 0.05 compared with the albumin control. (**D**) Determination of plasma levels of HMGB1 by ELISA in rats with TBI. Blood samples were collected 6 hours after induction of injury. Results are expressed as mean ± SEM of 6 rats. *P <0.05 compared with

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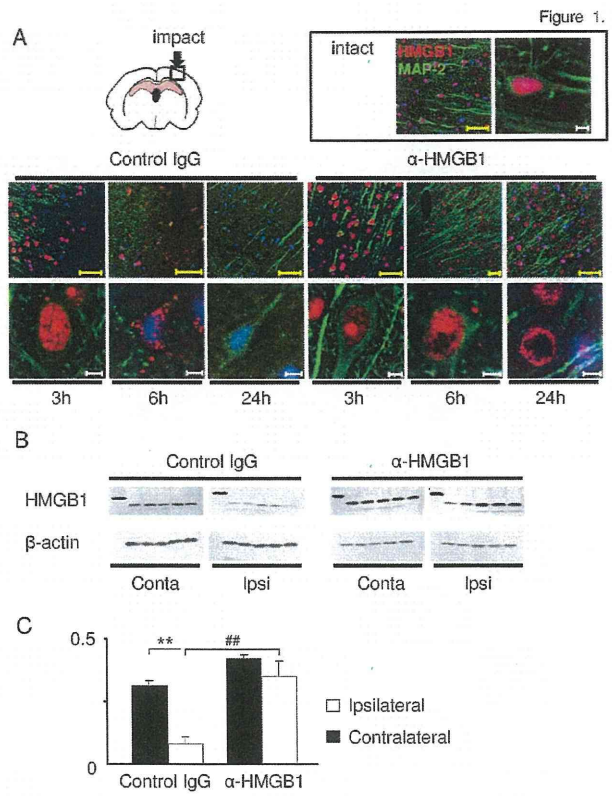
FIGURE 8:

TBI in mice deficient in RAGE, TLR-4 or TLR-2. (**A**) TBI was induced in RAGE-/-, TLR-4-/- , and TLR-2-/- mice and their wild-type as described for the TBI model. Anti-HMGB1 mAb or control IgG was injected 5 minutes postinjury. BBB permeability was assessed by measurement of Evans blue leakage. Results are expressed as mean ± s.e.m. of 5 mice. $^{**}P < 0.01$ compared with control IgG-treated group. $^{\#\#}P < 0.01$ compared with wild-type (WT) mice. (**B**) Coordinated motor function was evaluated using the rotarod test as shown in Figure 2d. Results are expressed as mean ± s.e.m. of 5 mice. $^{*}P < 0.05$ compared with the respective sham control. $^{\#\#}P < 0.01$ compared with the respective control. $^{\$}P < 0.05$ compared with wild-type (WT) mice.

Table1 Percussion injury and physiological parameters in rats

Percussion (atm)			Control IgG	(n=10)	αHMGB1	(n=10)
			2.38	±0.03	2.41	±0.02
	Pre	(n=5)	Control IgG	(n=4)	αHMGB1	(n=4)
Glucose	117	±29	277	±29	223	±10
Na	137.8	±1.5	129.0	±1.7	127.0	±3.1
K	3.06	±0.34	3.93	±0.08	3.88	±0.15
tCO2	24.2	±2.7	33.3	±1.0	32.8	±1.0
iCa	0.99	±0.15	1.16	±0.05	1.06	±0.08
Hct	36.8	±5.0	38.8	±2.3	38.3	±2.6
Hb	12.5	±1.7	13.2	±0.8	13.0	±0.9
pH	7.32	±0.04	7.47	±0.02	7.47	±0.02
pCO2	44.3	±4.2	43.3	±0.7	42.6	±2.4
pO2	283	±60	276	±13	221	±51
HCO3	22.8	±2.7	31.8	±0.9	31.2	±1.0
BE	-3.0	±3.0	8.0	±1.1	7.5	±1.0
sO2	99.4	±0.6	100	±0	99	±1



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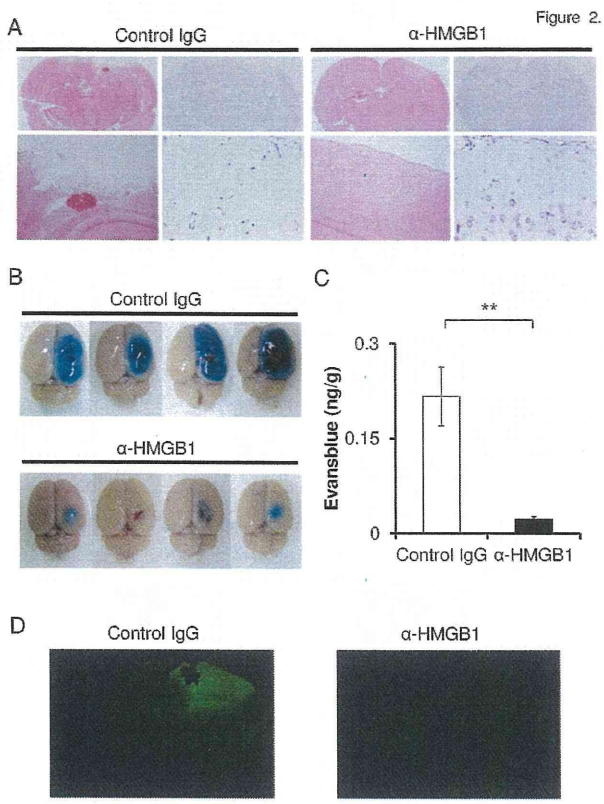

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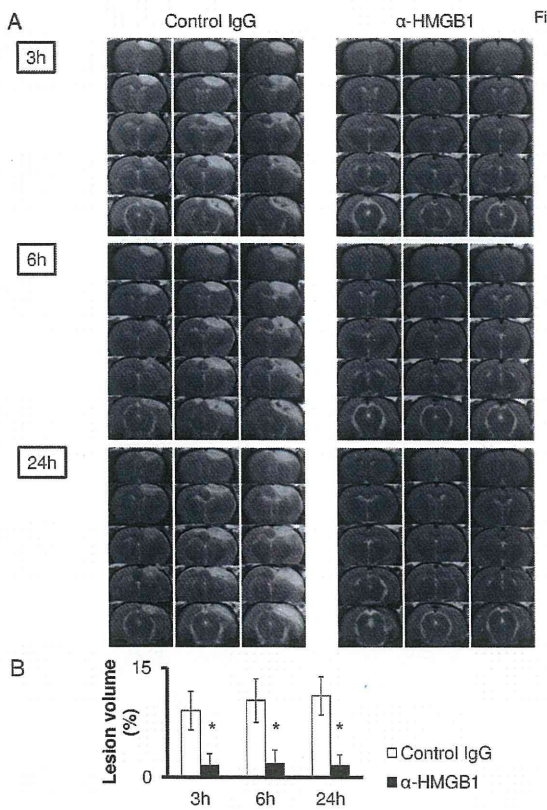

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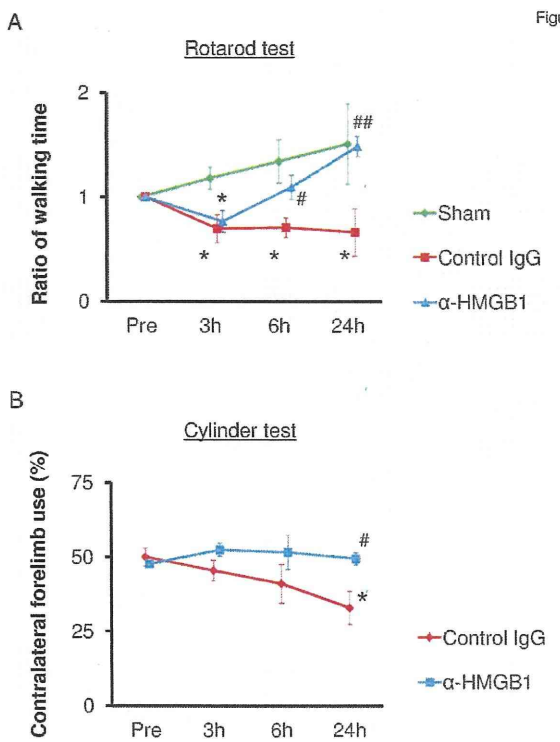

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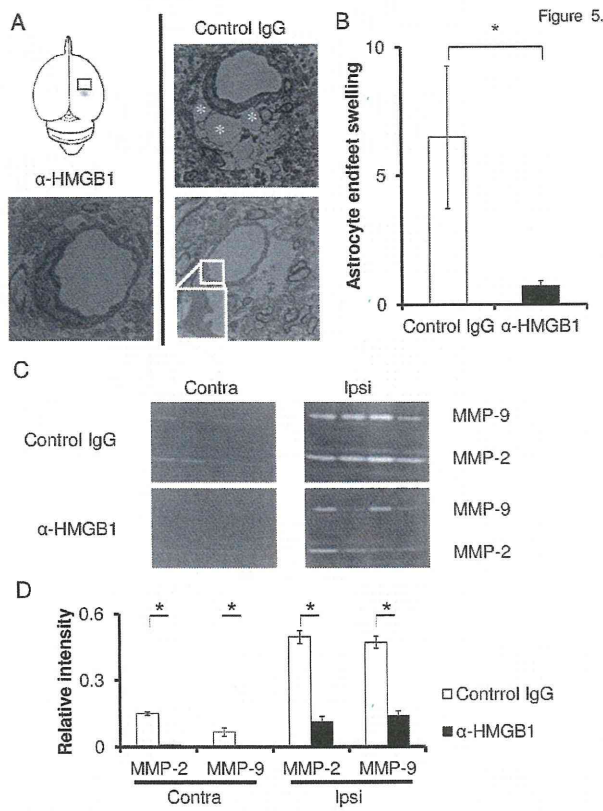

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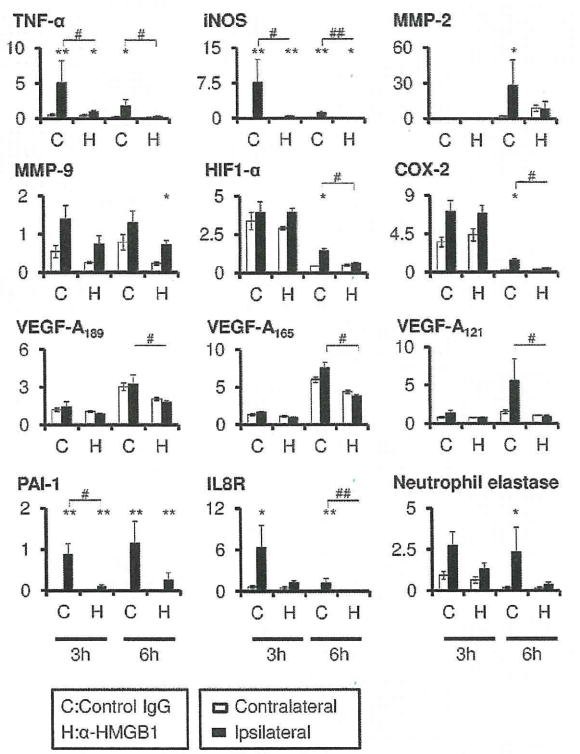

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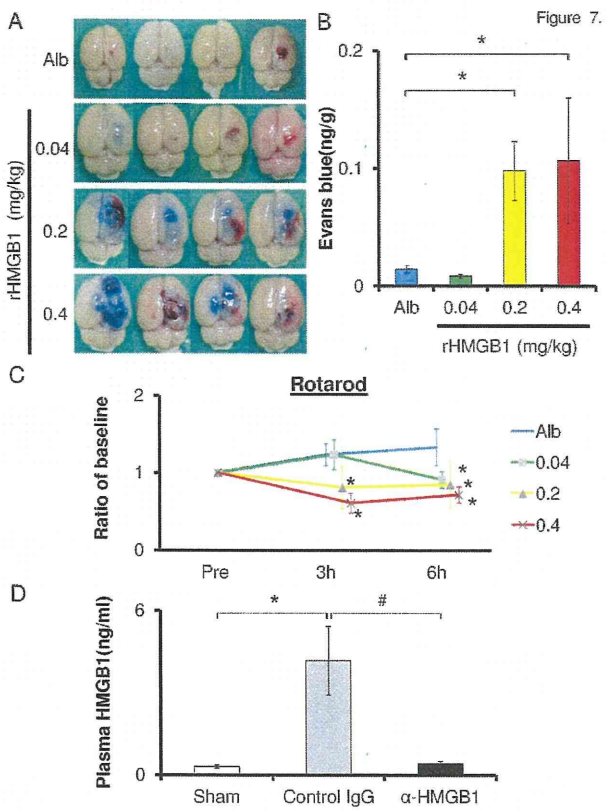

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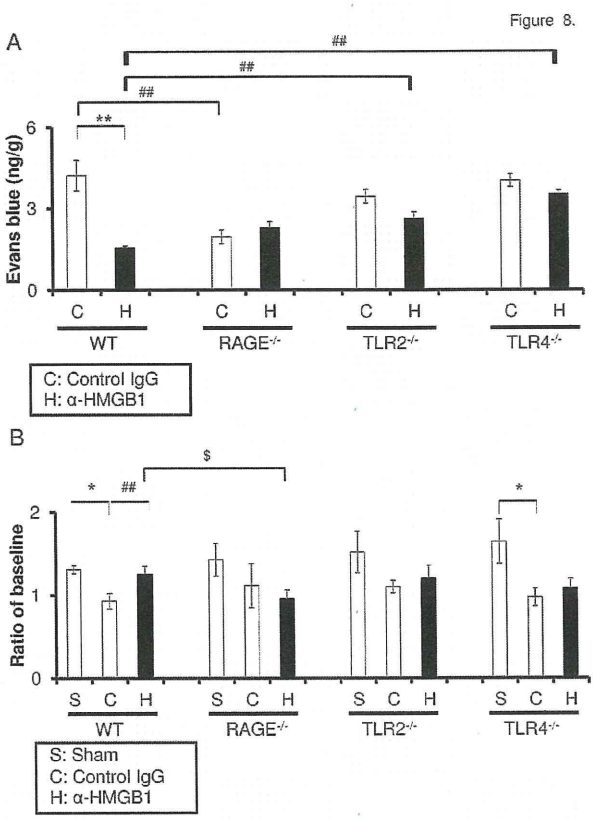

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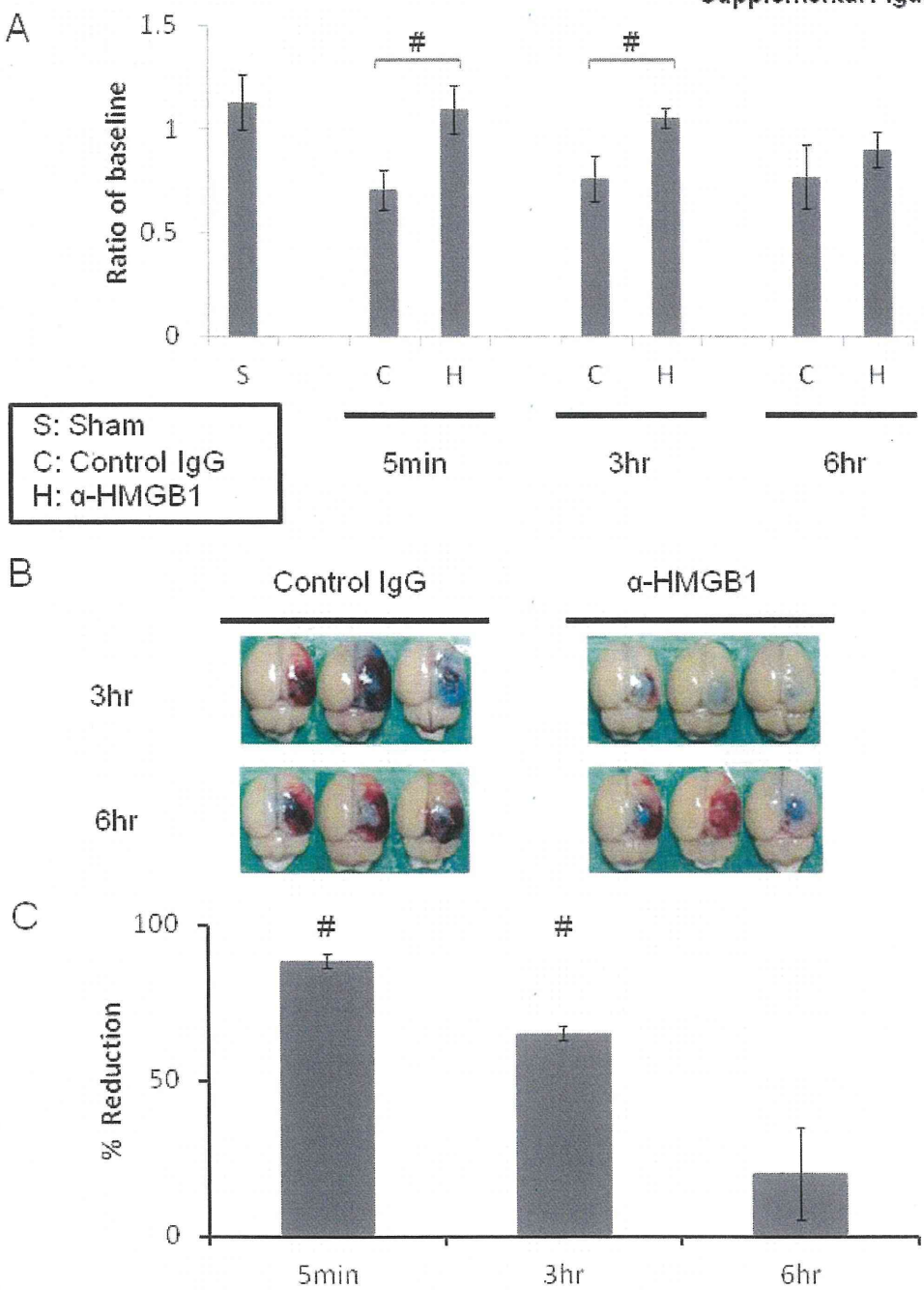
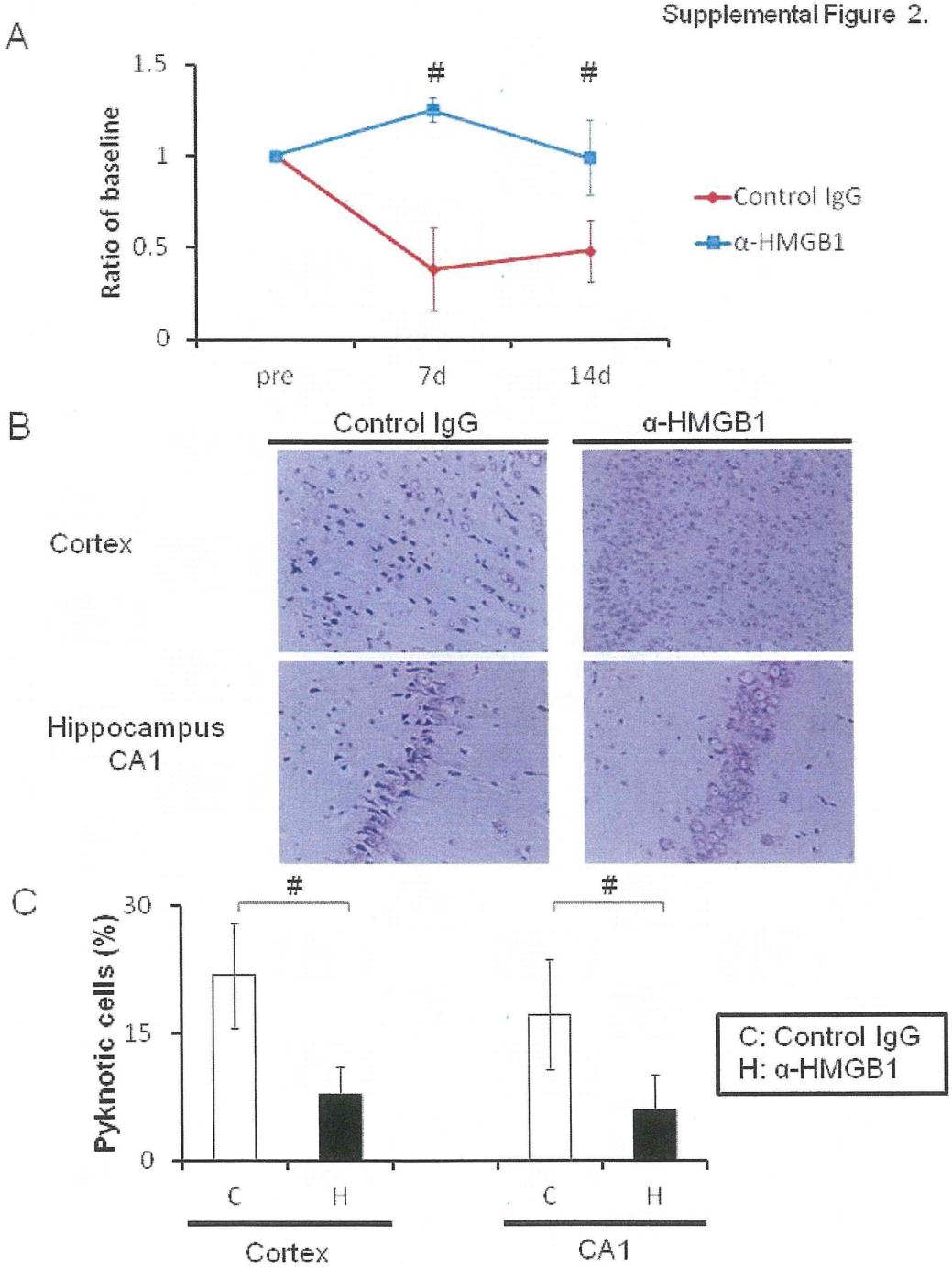


Supplemental Figure 2.



Supplemental Figure 3.