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Figure 1. Expression of HMGN family proteins in mouse brains.
(A): HMGN1, 2 and 3 (red) and Sox9 (green) expression in the coronal sections of the E18.5 mouse forebrains. Cell nuclei were visualized with Hoechst in blue. The bottom-most panels are higher magnification views of the boxed areas. Scale bar = 100 μm. Scale bar in the higher magnification views = 50 μm. (B-D): HMGN1, 2 and 3 (red) and Sox9 (green) expression in the P3, P7 and adult mouse cortices. Cell nuclei were visualized with Hoechst in blue. The bottom-most panels are higher magnification views of the boxed areas. Arrowheads indicate double-positive cells (yellow). Scale bars = 50 μm. Scale bars in the higher magnification views = 25 μm. (E): Double staining with HMGN1, 2 and 3 (red) and GFAP (green) in the P7 and adult mouse cortices. Arrowheads indicate double-positive cells (yellow). Scale bar = 20 μm. (F): HMGN1, 2 and 3 (red) and Sox2 (green) expression in the coronal sections of the E12.5 mouse forebrains. Cell nuclei were visualized with Hoechst in blue. The bottom-most panels are higher magnification views of the boxed areas. Scale bar = 100 μm. Scale bar in the higher magnification views = 50 μm. Abbreviations: VZ, ventricular zone; SVZ, subventricular zone.

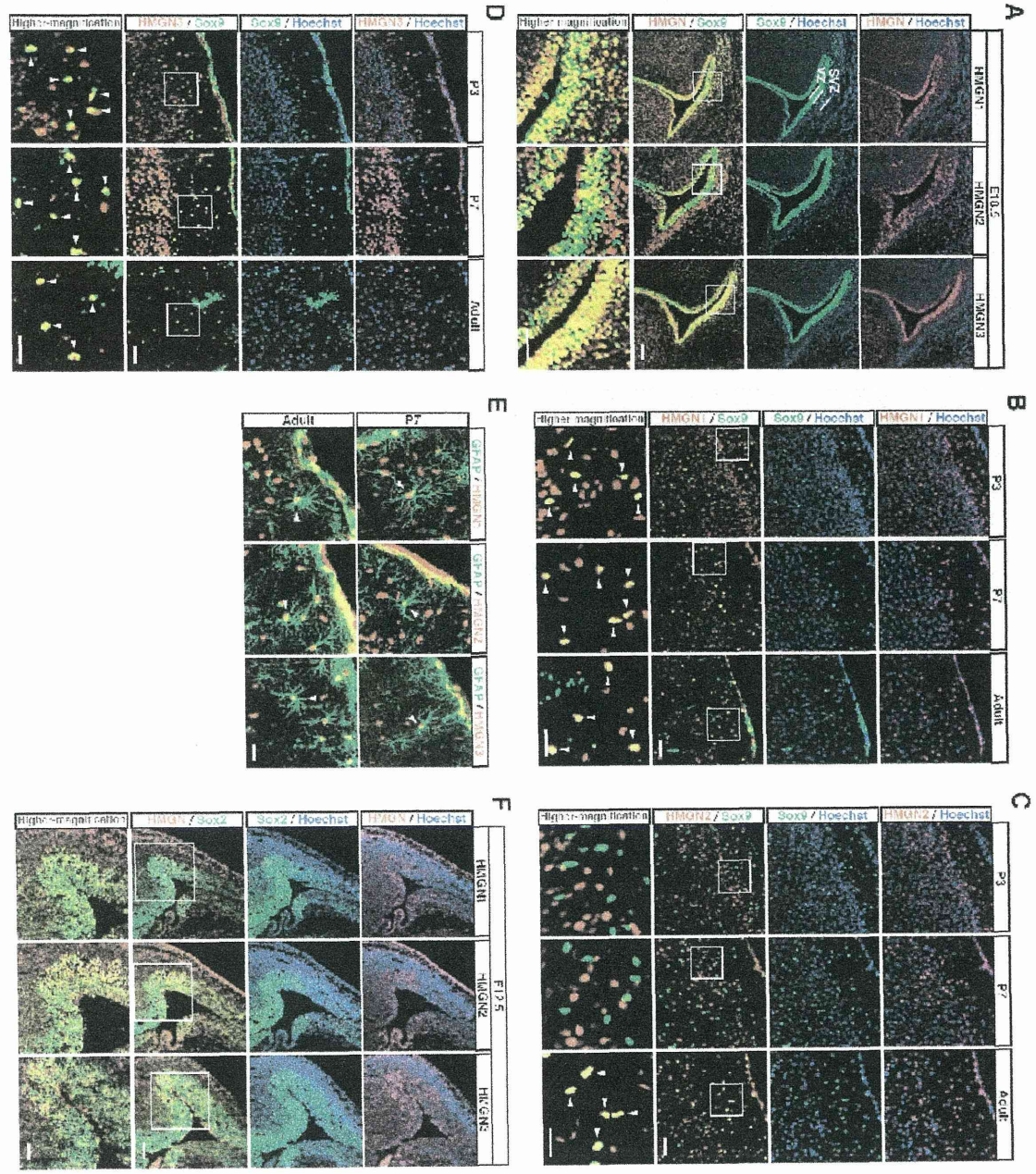

Figure 2. Forced expression of HMGN family proteins promotes differentiation of NPCs into astrocyte *in vitro*.
(A, B): NPCs derived from E16.5 mouse forebrains were infected with control (GFP alone) or HMGN1, 2, 3a or 3b viruses. Two days after infection, the cells were subjected to the differentiation assay. Six days after induction of differentiation, the cells were stained with TuJ1 (neuron marker), GFAP (astrocyte marker), Sox9 (glial progenitor marker), O4 (oligodendrocyte marker) and GFP antibodies. Arrows indicate marker$^+$/GFP$^+$ cells. Scale bar = 50 μm. The percentages of marker$^+$ cells among total GFP$^+$ cells are quantified. All values represent the mean ± SD from at least three separate experiments. *, $P < 0.01$ compared with control. (C-E): The effect of HMGN family proteins on proliferation and survival of NPCs. E16.5 NPCs infected with control or HMGN1, 2, 3a or 3b viruses were labeled with EdU for 2 h in the presence of FGF2 and EGF. The cells were subjected to EdU and cleaved Caspase3 (cl-Casp3) staining 1 day after plating. Arrows indicate EdU$^+$/GFP$^+$ cells or cleaved Caspase3$^+$/GFP$^+$ cells. Scale bar = 50 μm. The percentages of EdU$^+$ or cleaved Caspase3$^+$ cells among GFP$^+$ cells are shown. All values represent the mean ± SD from three experiments.

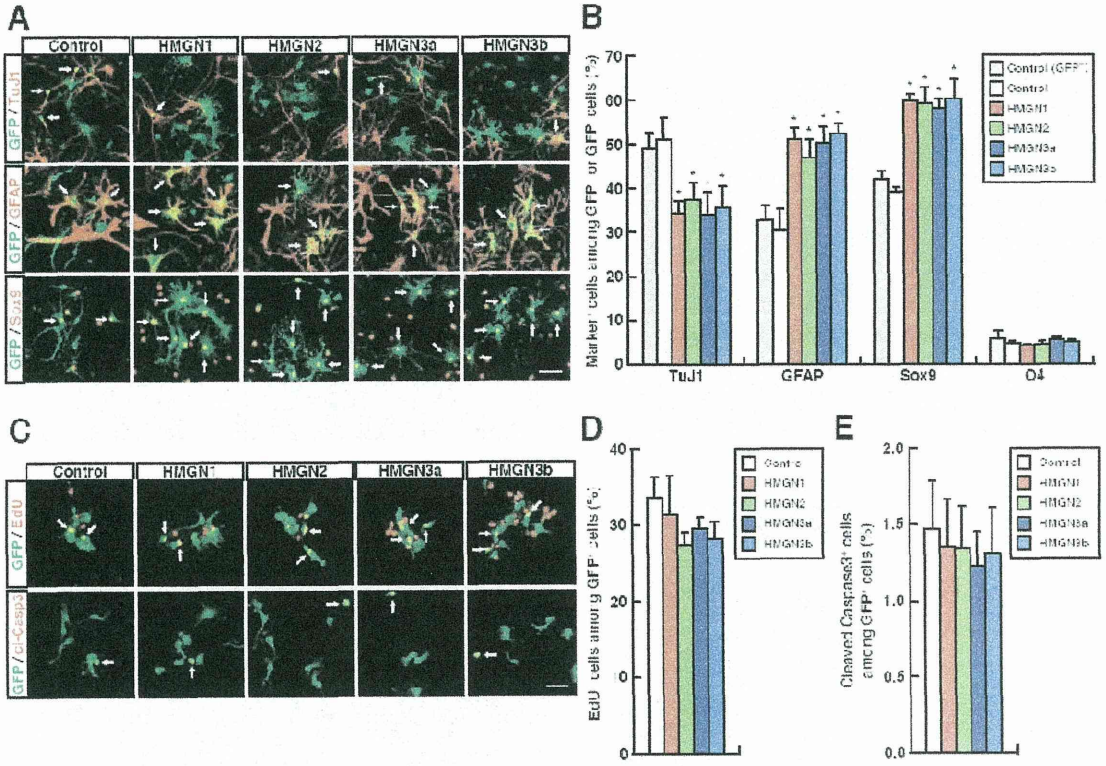

Figure 3. Knockdown of HMGN family proteins suppresses differentiation of NPCs into astrocyte *in vitro*.
(A-C): E16.5 NPCs were infected with control (sh-Luc), or HMGN1, 2 or 3 shRNA viruses. sh-HMGN1, 2 and 3 are retroviruses expressing shRNAs for HMGN1, 2 and 3, and sh-Luc expresses shRNA for luciferase. The cells were harvested at day 3 after infection and the expression level of HMGN1, 2 and 3 mRNA was measured by quantitative RT-PCR. Data are the mean ± SD (n=3, Control is designated as 1.0). *, $P < 0.01$ compared with control. Two different shRNA viruses for each HMGN (#1 and #2) were used. (D): E16.5 NPCs were infected with control (sh-Luc), or HMGN1, 2 or 3 shRNA viruses. Two days after infection, the cells were subjected to the differentiation assay. Six days after induction of differentiation, the cells were stained with TuJ1, GFAP, Sox9, O4 and GFP antibodies. Arrows indicate marker$^+$/GFP$^+$ cells. Scale bar = 50 □m. (E): The percentages of marker$^+$ cells among total GFP$^+$ cells are quantified. All values represent the mean ± SD from at least three separate experiments. *, $P < 0.01$ compared with control. Two different shRNA viruses for each HMGN (#1 and #2) were used. (F-H): E16.5 NPCs were infected with control (sh-Luc), or HMGN1, 2 or 3 shRNA and HMGN1, 2 or 3a viruses. The cells were harvested at day 3 after infection and the expression level of HMGN1, 2 and 3 mRNA was measured by quantitative RT-PCR. Data are the mean ± SD (n=3, Control is designated as 1.0). *, $P < 0.01$ compared with control. #, $P < 0.01$ compared with only sh-HMGN. (I): E16.5 NPCs were infected with control (sh-Luc), or HMGN1, 2 or 3 shRNA and HMGN1, 2 or 3a viruses. Two days after infection, the cells were subjected to the differentiation assay. Six days after induction of differentiation, the cells were stained with TuJ1, GFAP, O4 and GFP antibodies. Arrows indicate marker$^+$/GFP$^+$ cells. Scale bar = 50 □m. (J): The percentages of marker$^+$ cells among total GFP$^+$ cells are quantified. All values represent the mean ± SD from at least three separate experiments. *, $P < 0.01$ compared with control. #, $P < 0.01$; ##, $P < 0.05$ compared with only sh-HMGN.

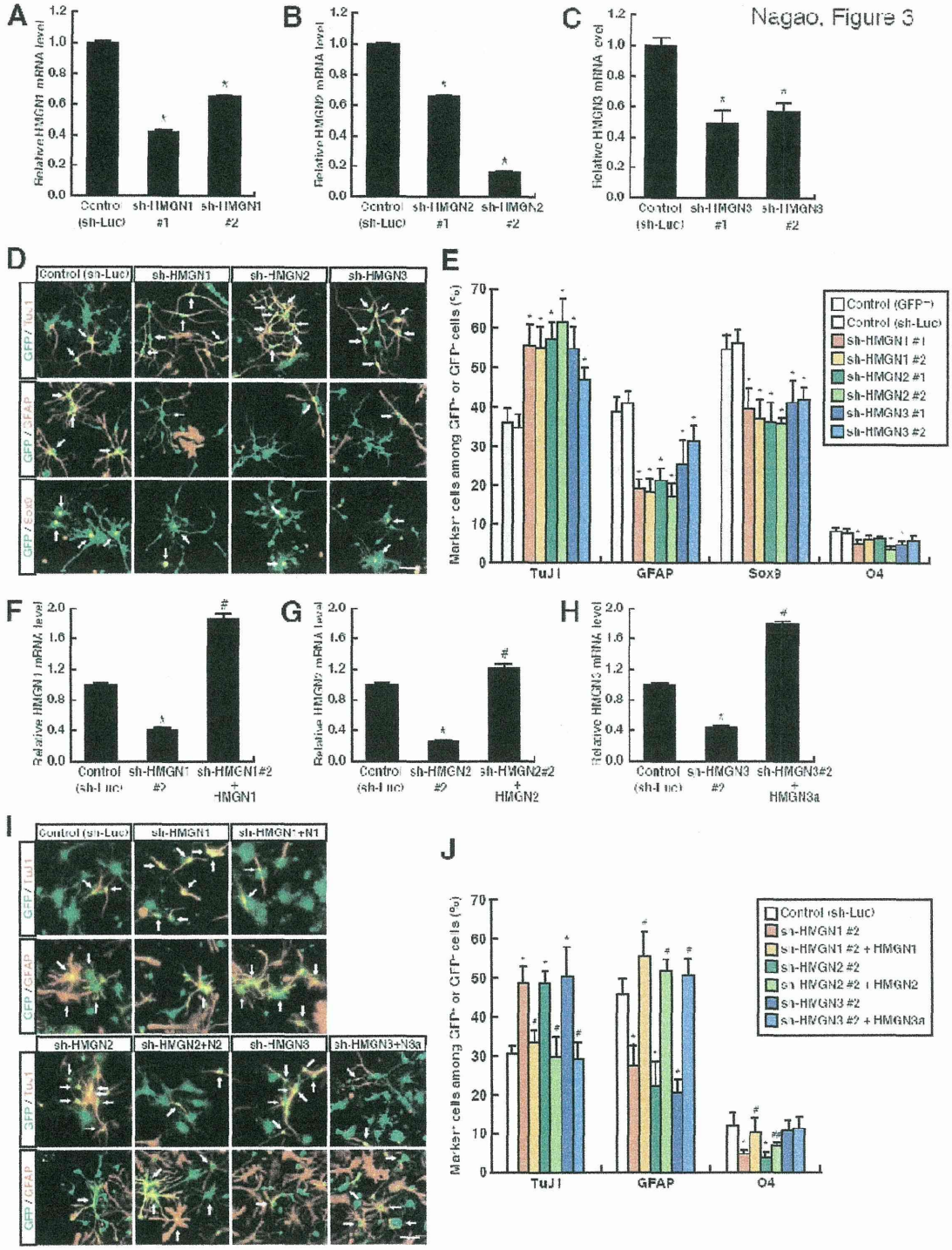

Figure 4. Forced expression of HMGN family proteins increases the generation of astrocytes *in vivo*.
(A): Expression plasmids for control (GFP alone) or HMGN1, 2, 3a or 3b were injected into the lateral ventricle of E16.5 mouse forebrains *in utero* and electroporated into the dorsolateral region of the neocortex. The brains were isolated at P7 and subjected to immunohistochemistry with antibodies for GFAP, S100β (red, astrocyte markers), Sox9 (red, glial progenitor marker), Cux1 (red, neuron marker) and GFP (green). Arrows indicate marker$^+$/GFP$^+$ cells. The right-most panels are higher magnification views of the boxed areas. Scale bar = 75 μm. Scale bar in the higher magnification views = 25 μm. (B, C): The numbers of marker$^+$/GFP$^+$ cells in the defined area are shown. All values represent the mean ± SD (n=3-5). *, P < 0.01 compared with control. Abbreviations: Cx, cortex; LV, lateral ventricle.

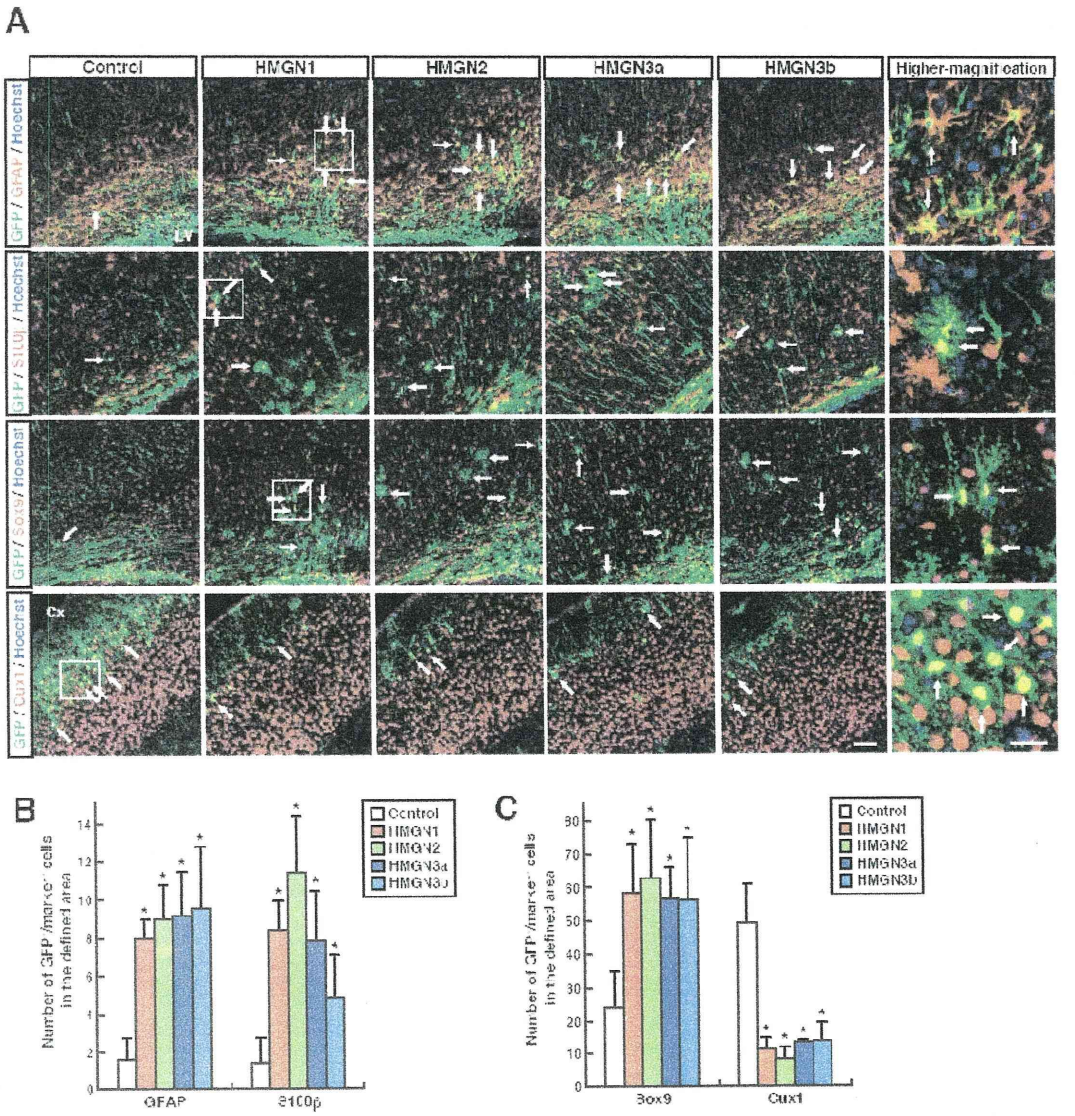

Figure 5. Knockdown of HMGN family proteins suppresses differentiation of NPCs into astrocyte *in vivo*.
(A): Knockdown plasmids for control (sh-Luc) or HMGN1, 2 or 3 were injected into the lateral ventricle of P0 mouse pups and electroporated into the dorsolateral region of the neocortex. The brains were isolated at P3 and P7 and subjected to immunohistochemistry with antibodies for GFAP (red, astrocyte marker) at P7, Tbr2 (red, immature neuron and neuronal progenitor marker) at P3 and GFP (green). Arrows indicate marker$^+$/GFP$^+$ cells. The right-most panels are higher magnification views of the boxed areas. Scale bar = 50 □m. Scale bar in the higher magnification views = 25 □m. (B): The percentages of marker$^+$ cells among total GFP$^+$ cells are quantified. All values represent the mean ± SD (n=4-6). *, $P < 0.01$ compared with control. Two different shRNA viruses for each HMGN (#1 and #2) were used. (C): Knockdown plasmids for control (sh-Luc) or HMGN1, 2 or 3 and expression plasmids for HMGN1, 2 or 3a were injected into the lateral ventricle of P0 mouse pups and electroporated into the dorsolateral region of the neocortex. The brains were isolated at P7 and subjected to immunohistochemistry with antibodies for GFAP (red) and GFP (green). Arrows indicate GFAP$^+$/GFP$^+$ cells. Higher magnification views of the boxed areas are shown to the right. Scale bar = 50 □m. Scale bar in the higher magnification views = 25 □m. (D): The percentages of GFAP$^+$ cells among total GFP$^+$ cells are quantified. All values represent the mean ± SD (n=9-11). *, $P < 0.01$ compared with control. #, $P < 0.01$ compared with only sh-HMGN. Abbreviations: LV, lateral ventricle.

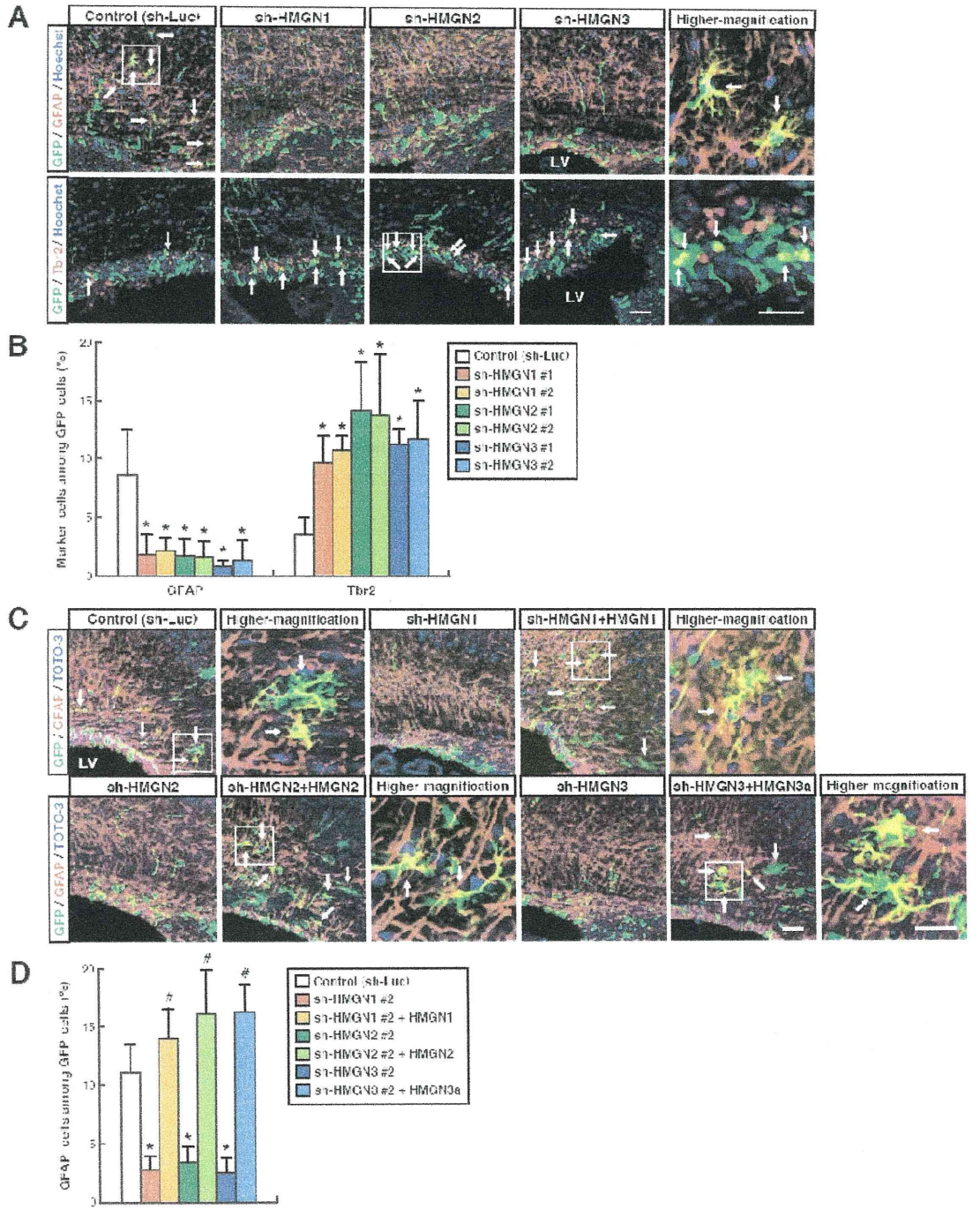

Figure 6. HMGN family proteins do not regulate the STAT pathway and the GFAP promoter in early steps of NPC differentiation.

(A, B): NPCs derived from the E11.5 mouse forebrain were infected with control (GFP alone) or HMGN1, 2, 3a or 3b viruses. The infected cells were cultured without FGF2 and EGF and with CNTF for 2 days and were analyzed by immunoblotting with antibodies for HMGN1, 2 and 3, STAT3 pY705, STAT3 and GAPDH. (C): E11.5 NPCs were transfected with STAT-dependent or *gfap* promoter-Luc plasmid, expression plasmids for HMGN family proteins or STAT3-C, and the pRL-SV40 plasmid encoding Renilla luciferase. After the cells were cultured for 24 h with and then 24 h without FGF2 and EGF, they were subjected to the luciferase reporter assay. Data are normalized by the Renilla luciferase activity and are the mean ± SD from at least three separate experiments (Control is designated as 1.0). *, $P < 0.01$ compared with control. (D): E11.5 NPCs were cultured without (No treatment) and with CNTF. The cells were harvested 1 day after CNTF stimulation and the expression levels of HMGN1, 2 and 3 were measured by quantitative RT-PCR. Data are the mean ± SD (n=3, No treatment is designated as 1.0). (E): E11.5 NPCs were infected with viruses for GFP alone control, HMGN1 or an activated mutant of Notch1 (Notch intracellular domain, NICD), and were cultured with FGF2 and EGF for 4 days. The methylation status of the STAT3 binding site and other CpG sites around this sequence in the *gfap* promoter was examined by bisulfite sequencing. Closed and open circles indicate methylated and unmethylated CpG sites, respectively. (F): E11.5 NPCs were infected with control (GFP alone) or HMGN1, 2, 3a or 3b viruses. The infected cells were cultured without FGF2 and EGF for 2 days, and they were subjected to ChIP analysis with antibodies for H3K9/K14ac and control IgG and with PCR primers specific for the *gfap* promoter, the *gapdh* promoter and the *MageA2* locus. The *gapdh* promoter and *MageA2* locus were used as positive and negative controls, respectively. Data are expressed as percentage of the input and are the mean ± SD (n=3).