Supporting Information

Chemical Modulation of Protein O-GlcNAcylation *via* OGT Inhibition Promotes Human Neural Cell Differentiation

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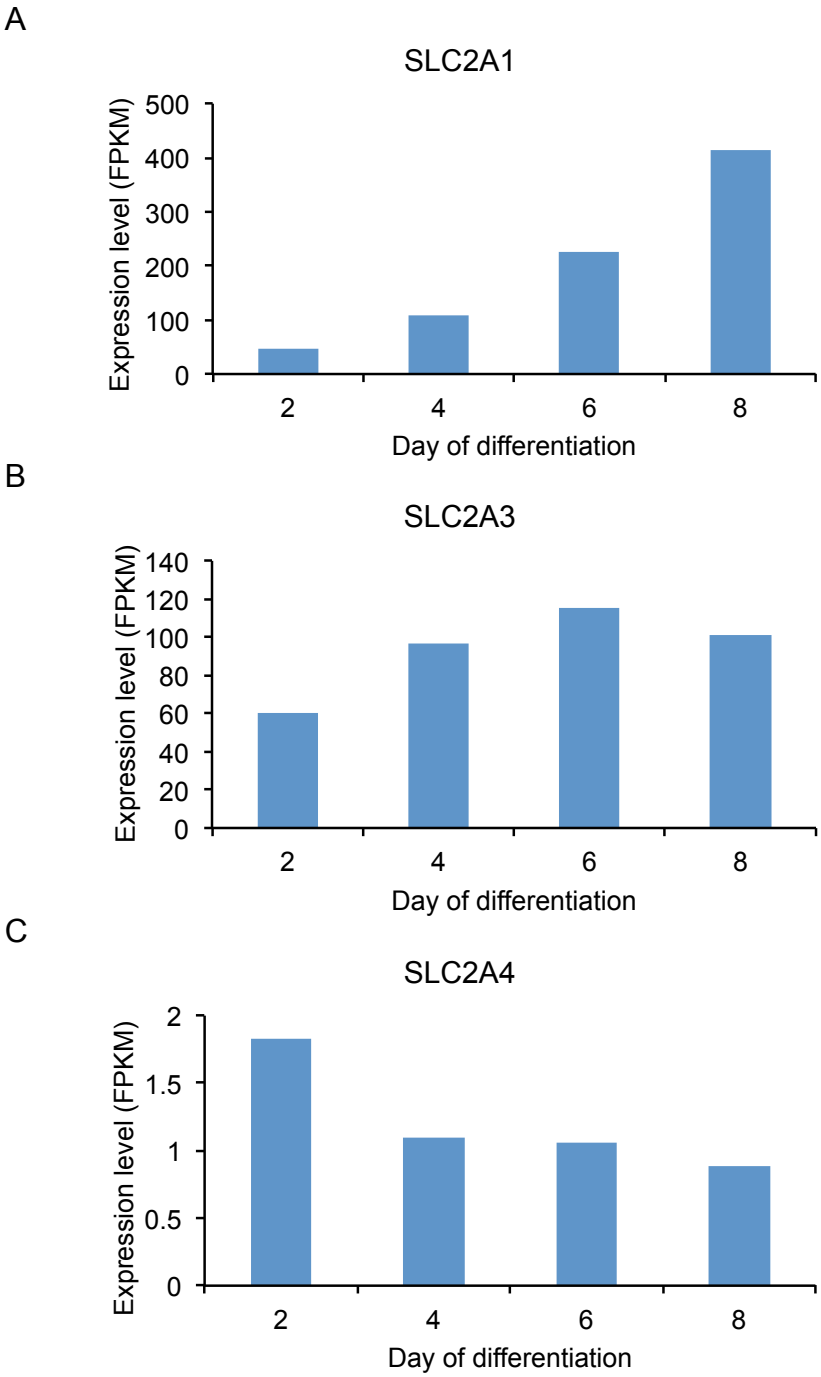

Figure S1. Glucose transporters transcript levels change during neural induction of hESCs. RNA-seq analysis of hESCs undergoing neural differentiation revealed changes in transcript levels of the glucose transporters GLUT1 (SLC2A1) (A), GLUT3 (SLC2A3) (B), and GLUT4 (SLC2A4) (C). Bars show Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values of glucose transporters (n = 2).

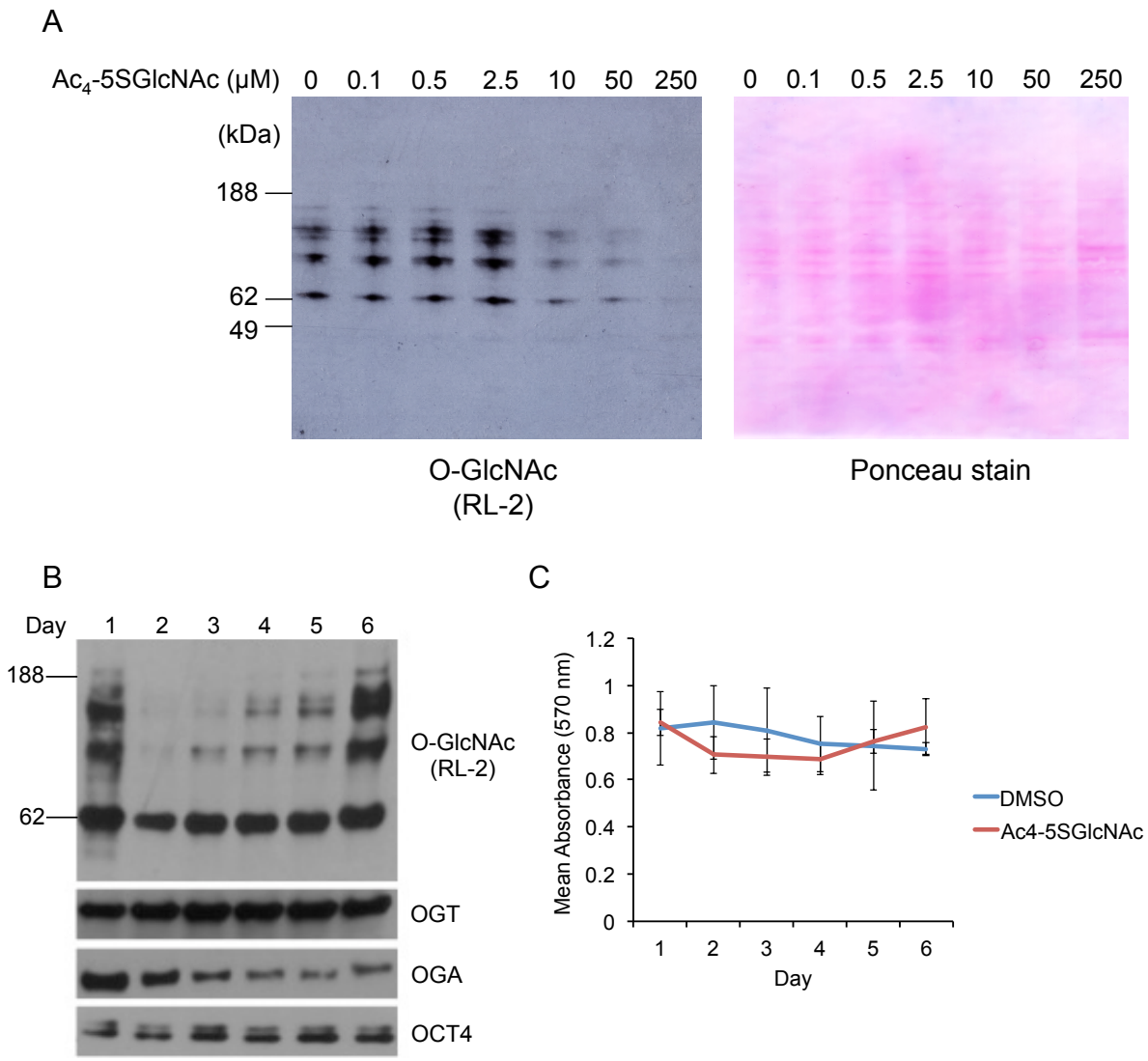

Figure S2. Ac_4-5SGlcNAc lowers global O-GlcNAc levels. (A) Dose-dependent decrease in protein O-GlcNAcylation after treating undifferentiated hESCs with Ac_4-5SGlcNAc for 24 h, as measured by Western blot. Ponceau stain of Western blot membrane shows equal loading. (B) Treatment of undifferentiated hESCs with 50 μM Ac_4-5SGlcNAc for 6 days caused an increase in protein O-GlcNAc levels, coinciding with a decrease in OGA protein expression. No detectable change in protein levels of OCT4 and OGT occurred during the 6 days of treatment with Ac_4-5SGlcNAc. (C) MTT colorimetric assay was used to determine the proliferation of hESCs treated with DMSO or with 50 μM Ac_4-5SGlcNAc for 6 days in culture (n = 2; mean ± SEM).

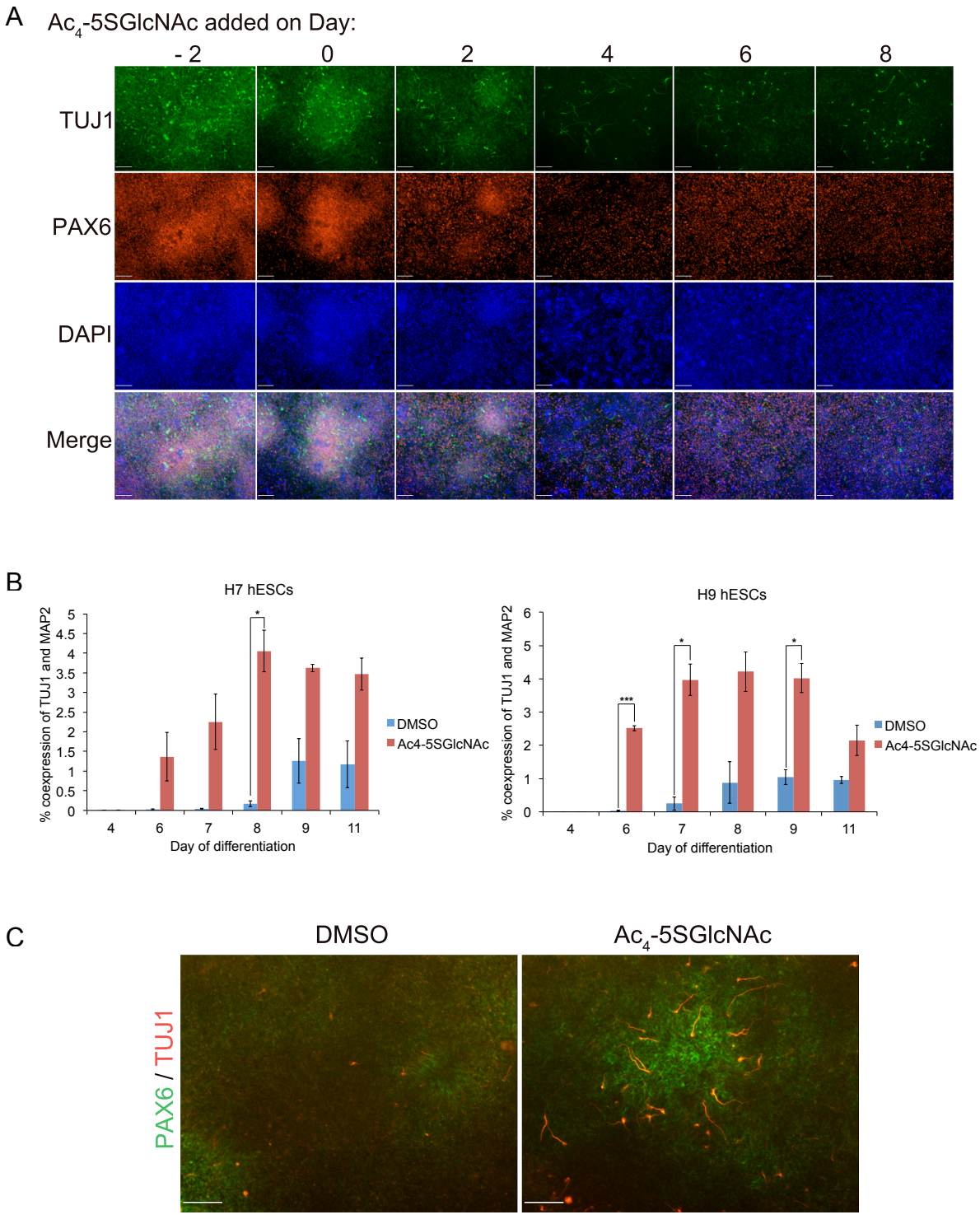

Figure S3. Ac_4-5SGlcNAc-treated hESCs prematurely acquire a neuronal phenotype within 11 days of differentiation. (A) Ac_4-5SGlcNAc (50 μM) was added on different days during the dual-SMAD inhibition neural induction of hESCs. Cells were fixed on day 11 and examined for the acquisition of TUJ1 and PAX6 by immunofluorescence. Day –2 was determined to be the optimal day for addition of Ac_4-5SGlcNAc. (B) Flow cytometry analysis shows an increase in TUJ1 and MAP2

coexpression when H7 and H9 hESCs were differentiated in the presence of Ac_4-5SGlcNAc for 11 days, as compared with DMSO-treated cells (n = 2; mean ± SEM; **P* < 0.05, ****P* < 0.001). (C) H1 hESCs differentiated with a lower concentration of Ac_4-5SGlcNAc (10 μM) from day –2 also acquire a premature neuronal phenotype as compared to DMSO. Cells were fixed on day 8 of neural differentiation and examined for the acquisition of TUJ1 and PAX6 by immunofluorescence.

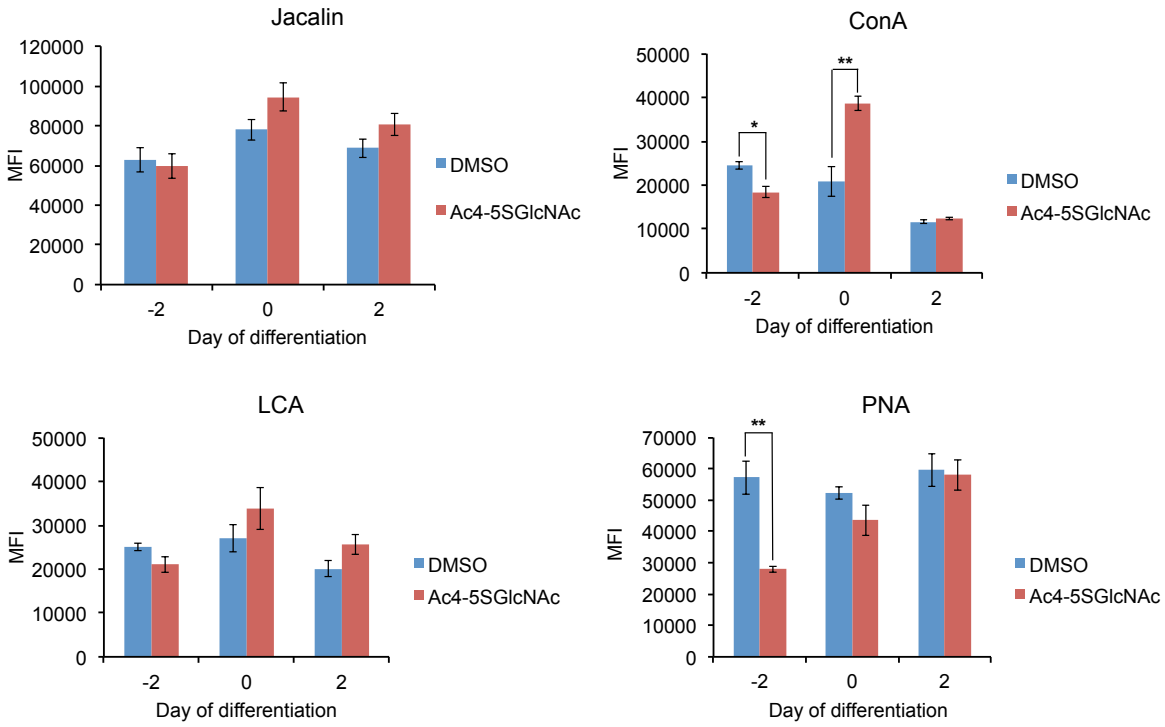

Figure S4. N-linked and mucin type O-linked glycosylation is perturbed in hESCs differentiated in the presence of Ac_4-5SGlcNAc. Bars represent mean (± SEM) fluorescence intensity of four lectins (PNA, ConA, LCA and Jacalin) in differentiated hESC samples treated with 50 μM Ac_4-5SGlcNAc or DMSO. Asterisks indicate statistically significant differences between treatments (n = 3; **P* < 0.05, ***P* < 0.01).

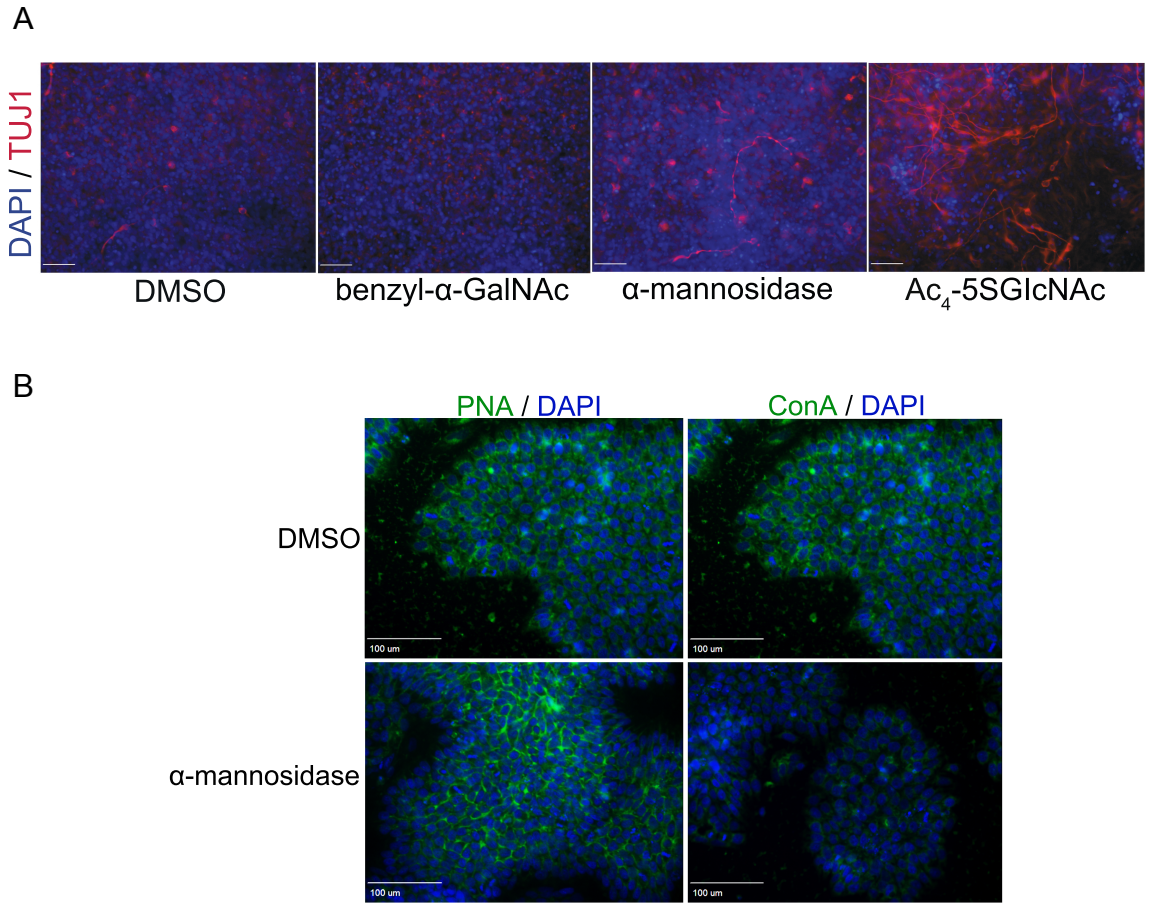

Figure S5. Perturbation of cell surface glycan does not reproduce the same TUJ1 staining and phenotype as observed in Ac_4-5SGlcNAc-treated hESCs. (A) hESCs were treated with DMSO, benzyl-α-GalNAc, α-mannosidase, or Ac_4-5SGlcNAc from day –2 to 8 of neural differentiation. Cells were consequently fixed on day 8 of neural differentiation and examined for the coexpression of TUJ1 and DAPI by immunofluorescence. Treatment of differentiating hESCs with benzyl-α-GalNAc and α-mannosidase did not reproduce the same neuronal phenotype observed in the presence of Ac_4-5SGlcNAc. (B) Differentiated hESCs treated with α-mannosidase were fixed on day 8 and probed against the lectins *Arachis hypogaea agglutinin* (PNA) and *Canavalia ensiformis agglutinin* (ConA) to test the efficacy of α-mannosidase treatment on cells.

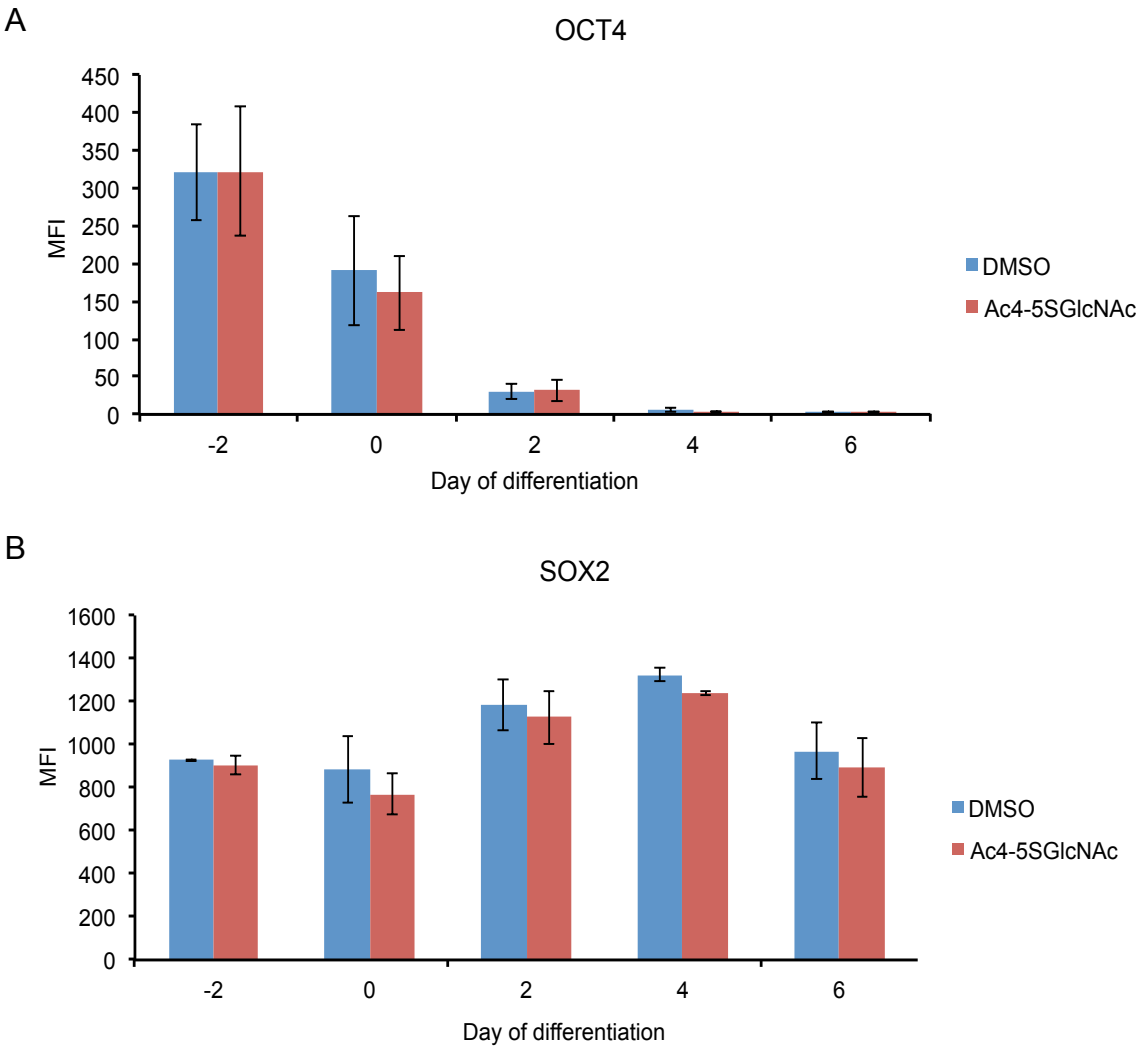

Figure S6. Flow cytometry analysis of pluripotency markers during the neural induction of Ac_4-5SGlcNAc-treated hESCs. (A) OCT4 and (B) SOX2 expression levels were similar between Ac_4-5SGlcNAc-treated (50 μM) and DMSO-treated cells as observed at different days of neural induction (n = 2; mean ± SEM).