## Figure S4 A





**NTU3 hESC line** 

B



## Figure S4. Most neural lineage markers were expressed at lower levels in *MEG3*-OFF hESCs- derived cells throughout differentiation.

(A) Significantly higher expression levels of PAX6, RTN1 and MAP2 were detected

In NTU1 and NTU3 MEG3-ON hESC-derived cells compared with the MEG3-OFF groups throughout the process of neural lineage differentiation. beta-III TUBULIN was also significantly upregulated in NTU1 cells derived from MEG3-ON hESCs compared with the MEG3-OFF groups at the EB stage, at both 3 days and 18 days after attachment; however, beta-III TUBULIN was upregulated in the MEG3-OFF group at the NES stage. beta-III TUBULIN was also detected in NTU3 lines and was upregulated in MEG3-ON groups at 18 days after attachment but not at the NES stage or at 3 days after attachment. The quantitation of mRNA expression was performed using the  $2^{-\Delta\Delta C_P}$  method (using the housekeeping gene *GAPDH* for normalization). Error bars represent the SEM generated from 2 biological samples with 3 technical repeats each. \*P<0.05, \*\* P<0.01 compared with the corresponding MEG3-ON groups by Student's *t*-test.

(B) The melting curves of *PAX6* gene that was significantly expressed at lower but detectable levels in *MEG3*-OFF hESC-differentiated cells at the EB and NES stages.