

Figure 7. Partial knockdown of FBL promoted neural differentiation of ES cells in a p53-dependent manner. (A) Neural differentiation of ES cells in monolayer culture. Cells were dissociated, attached to poly-L-lysine/laminin/fibronectin (PLL/LN/FN)-coated dishes, and cultured for 7 days. The concentration of Tc was changed to the indicated concentration from the beginning of differentiation. (Left panels) Tuj1⁺ cells were significantly increased only under FBL-reduced conditions. The graph shows the percentage of Tuj1⁺-neurons for each culture condition. (B) Neural differentiation of ES cells by embryoid body-based culture. Embryoid bodies were cultured in a low cell attachment culture dish for 5 days, dissociated, attached to PLL/LN/FN-coated dishes, and cultured for 4 days. The concentration of Tc was changed on day 3 to the indicated concentration. (C) The percentage of Tuj1⁺-neurons. The ES cells were differentiated as in (B) under the indicated culture conditions. (D) The percentage of NF200⁺-neurons. The ES cells were differentiated as in (B) under the indicated culture conditions. (E, F) Overexpression of FBL slightly inhibited neural differentiation. (E) Neural differentiation of Tc-inducible FBL-expressing ES cells under monolayer culture. ES cells were cultured for 6 days as described in (A). (F) The percentage of Tuj1⁺ neurons in (E) was counted. (G, H) Inhibition of p53 suppressed neural differentiation of ES cells. (G) Cells were cultured as in (A) with or without p53 and apoptosis inhibitors. All of the inhibitors were added from the beginning of differentiation. (H) The number of Tuj1⁺ neurons in (G) was counted. **p* < 0.01 in (A, C, D, and H). Scale bars: (A) 200 μm; (B, G, H) 100 μm.



