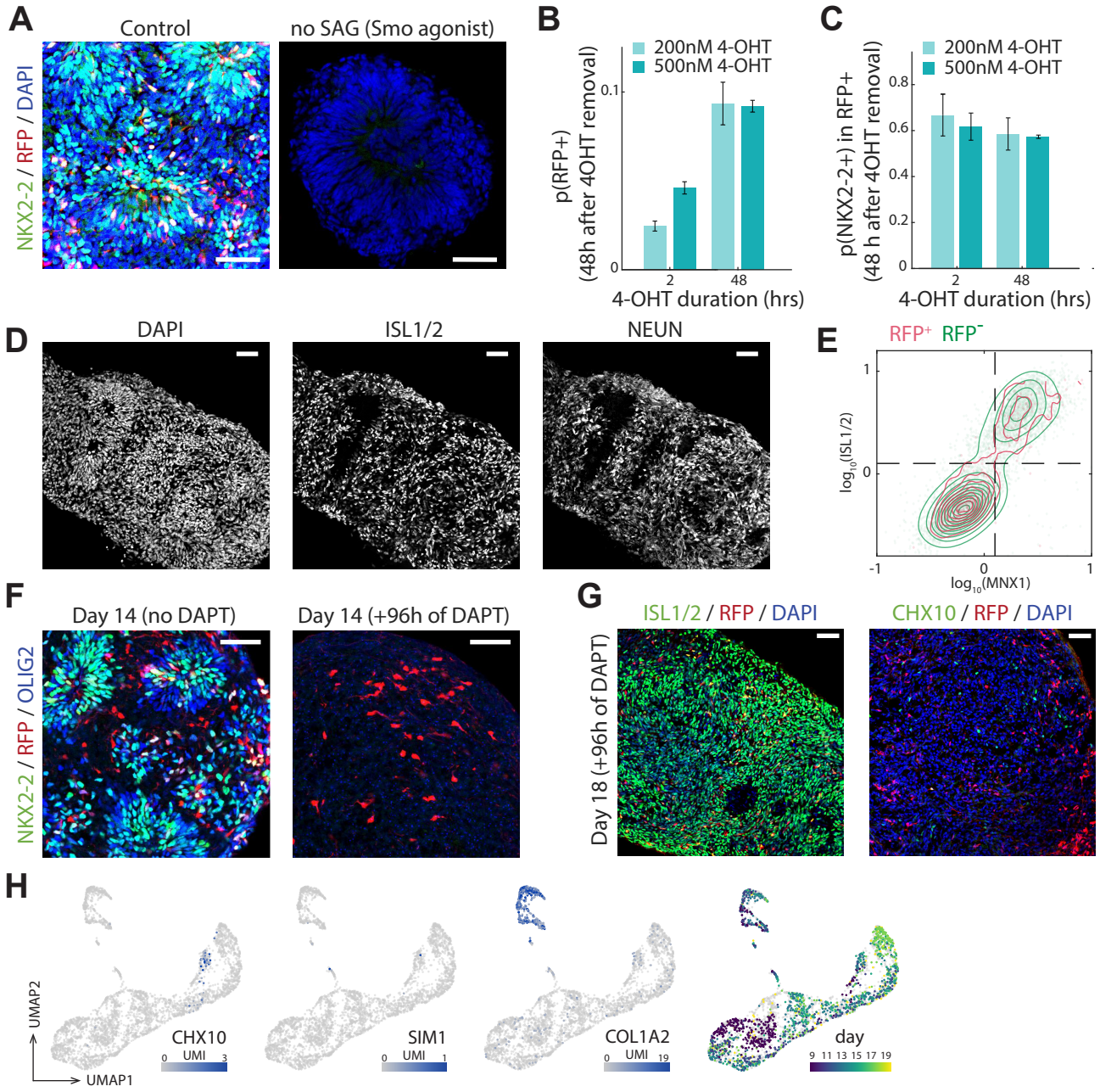

Supplemental figure 2. (A) Day 12 human embryoid bodies differentiated with and without SAG (Smoothened agonist) with 4OHT pulse between days 9-11 show that in the absence of SAG, NKX2-2 expression as well as Cre-dependent recombination is lost (All scale bars in figure = 50µm). **(B)** Varying lengths and concentrations of 4OHT pulse affect the efficiency of recombination-based RFP expression (n = 3; all scale bars in figure indicate S.D.). **(C)** Proportions of NKX2-2-positive cells within RFP-positive populations on day 13, 48 hours post 4OHT removal (n = 3). **(D)** Immunostaining day 16 human cultures with pan-neuronal marker NEUN and ISL1/2 shows that the vast majority of neurons produced are motor neurons. **(E)** Flow cytometry analysis of day 16 human cultures shows that the vast majority of ISL1/2-positive cells are MNX1-positive and vice versa in both RFP^+ and RFP^- populations (representative differentiation, n = 1, sample pooled across 50+ EBs). **(F)** Human cultures following 4OHT treatment on days 9-11 show that many RFP^+ cells retain progenitor identity (OLIG2+ or $OLIG2^+/NKX2\text{-}2^+$) on day 14; however, with DAPT treatment, virtually no cells express OLIG2 or NKX2-2. **(G)** Majority of cells in human cultures express ISL1/2 in response to DAPT, with a small fraction (<1%) of cells expressing V2 interneuron marker CHX10. **(H)** UMAP of all high-quality human scRNA-seq profiles, colored based on expression of V2 and V3 interneuron markers (CHX10 and SIM1), fibroblast marker (COL1A2), and timestamp identity.

Supplemental figure 2