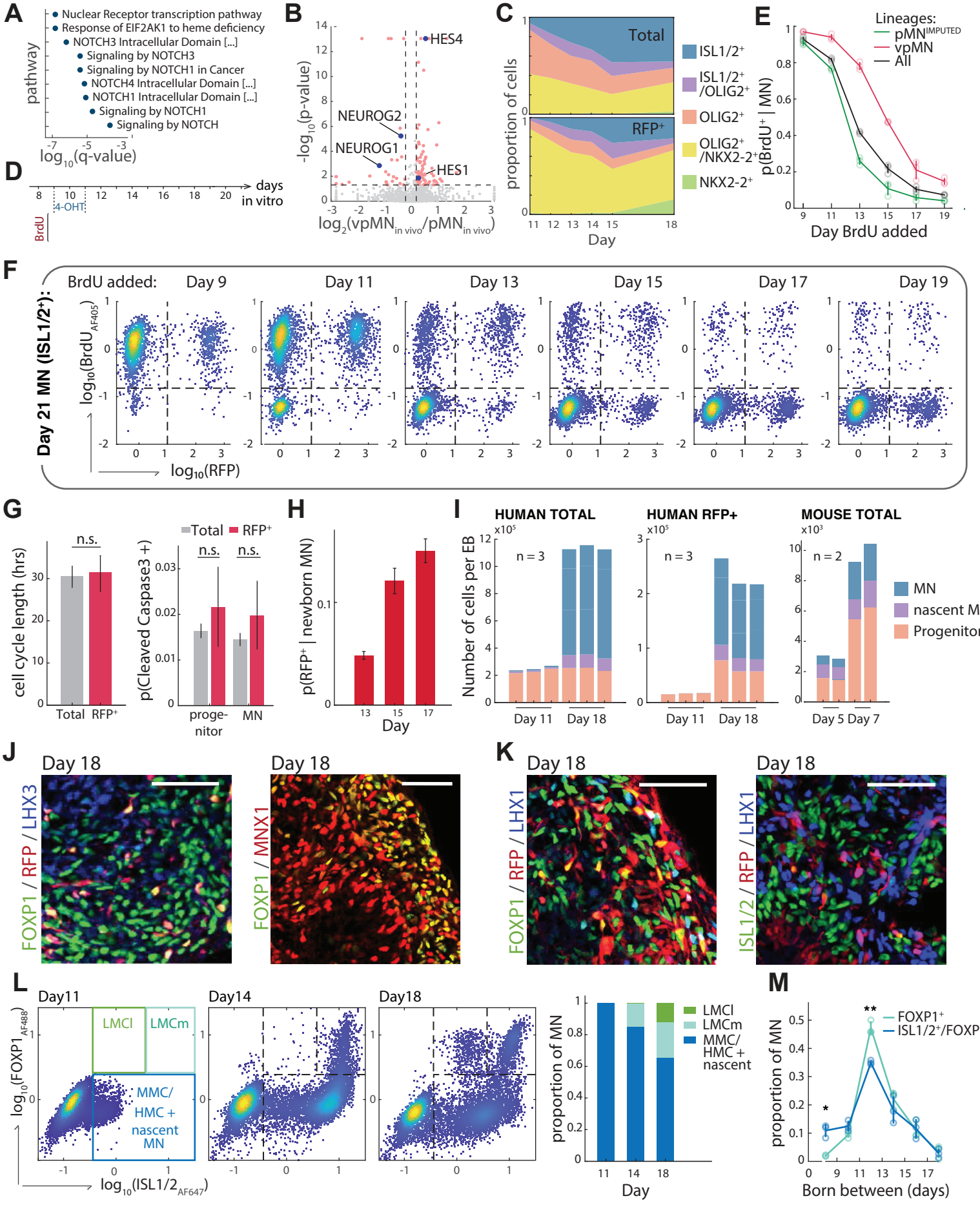

Supplemental figure 3

Supplemental figure 3. (A) Top enriched pathways in Reactome analysis of genes upregulated in vpMN relative to pMN (q-value ≤ 0.001). **(B)** Differential gene expression analysis between in vivo vpMN (H_E4) and pMN (H_E1) clusters shows that, similar to in vitro, vpMNs display characteristics of higher Notch activity relative to pMNs. **(C)** Changes in the proportion of progenitor and MN populations over time in total and RFP^+ populations (replicate of Figure 3B). **(D)** Schematic of cumulative BrdU labeling assay to derive MN birthcurve for vpMN and pMN lineages. **(E)** Changes in the proportion of $BrdU^+$, day 21 MNs for vpMN (RFP^+), total and $pMN^{IMPUTED}$ lineages following cumulative BrdU labeling at progressively later timepoints (n = 3; all scale bars in figure indicate S.D.). Despite all three curves starting at close to 100% (indicating that most vpMNs and pMNs are mitotic at day 9), the vpMN curve is right-shifted, indicating that RFP^+ vpMNs remain mitotic for longer. **(F)** Flow cytometry plots for day 21 human cultures (only $ISL1/2^+$ MNs shown) treated with BrdU starting at progressively later timepoints show slower decrease in $BrdU^+$ MNs in RFP^+ compared to RFP^- populations. **(G)** Left: Estimation of cell cycle length based on EdU pulse-labeling shows that RFP^+ have similar cell cycle length compared to the total cells. Right: RFP^+ and RFP^- cells display similar proportions of cleaved Caspase-3-positive progenitors and motor neurons, indicating that cell death rates are similarly low for vpMN and pMN lineages. **(H)** Proportion of RFP^+ cells within newborn motor neuron population (as determined by BrdU labeling) increases over time, indicating that vpMNs undergo delayed neurogenesis (n = 3). **(I)** Numbers of motor neuron progenitors and motor neurons at the onset of and tail-end of neurogenesis in both mouse and human, numbers for each replicate shown separately. **(J)** Day 18 human cultures immunostained for FOXP1, LHX3 and MNX1 show that $FOXP1^+$ cells are $LHX3^-$ and $MNX1^+$, indicating that they are LMC-like MNs (Scale bars = 50µm). **(K)** Day 18 human cultures immunostained for FOXP1, LHX1 and ISL1/2 show that LHX1 is expressed in a subset of FOXP1 cells but is not co-expressed with ISL1/2, suggesting that $FOXP1^+/LHX1^+$ cells are LMC-l-like MNs (Scale bars = 50µm). **(L)** Flow cytometry plots for human cultures at days 11-18 show sequential appearance of MMC/HMC-, LMCm- and LMCl-like MNs (n = 1). **(M)** Cumulative BrdU labeling, followed by immunolabeling for FOXP1 and ISL1/2 reveal that $FOXP^+$ and $FOXP1^-$ MNs have overlapping birth curves, with $FOXP1^+$ MNs being born slightly later (n = 3).