



Supplemental figure 1

Supplemental figure 1. (A) Culture conditions for motor neuron differentiation. (B) Top: UMAP of all high-quality single-cell RNA-seq profiles, clusters colored based on identity. Bottom: UMAP of all high-quality single-cell RNA-seq profiles after removal of interneuron-lineage/fibroblast-like cells, colored based on replicate. (C) Numbers of single-cell profiles (successfully time-stamped and not) from second batch of 10X single-cell gene expression profiling. Note, the first batch of human cells were not time-stamped, leading to overall lower proportion of time-stamped human cells. (D) Timestamp distribution (day 4-6) in mouse single-cell gene expression datasets across the two replicates. (Day 7 was not collected for first replicate.) (E) UMAP of combined human and mouse single-cell gene expression profiles following CCA-mediated integration, colored by normalized expression level of key marker genes. (F) Chi-square distance between human and mouse clusters. Smaller distances indicate greater similarity in constituent cells' distribution across common clusters. (G) Classification of human (top) and mouse (bottom) cells according to human-based random-forest classifier shows that all mouse clusters had the most of their cells classified as H0-2 (pMN), H6 (OPC-like), or H7-9 (MN), leaving the H3-5 (vpMN) category void of classified mouse cells. (H) Macaque iPSC cultures display NKX2-2 and OLIG2 co-expressing cells at day 10, when ISL1/2⁺ MNs begin to appear, mimicking human cultures. (I) UMAP of scRNA-seq data from Carnegie Stage 12 human embryonic spinal cords, colored based on cluster identity or NKX2-2/OLIG2 expression. (J) Left: Alignment of human in vitro (top) and human CS12 embryonic (bottom) single-cell RNA-seq data shows that human-specific clusters map onto a distinct OLIG2-high, NKX2-2-low clusters found in vivo. Right: Alignment of human in vitro (top) and mouse E9.5-10.5 spinal cord shows that H4 cells show poor overlap with all embryonic mouse clusters, suggesting that H4-like cells are found in human (but not mouse) embryonic spinal cords.