



**Figure 2-figure supplement 3. qPCR validation that MN gene expression in DP follows the expected dynamics and does not involve Olig2 induction.** a) Measurement of the bulk population gene expression over time helps to confirm that the trajectory inferred from single-cell analysis matches the true dynamical events of our system. These results confirm that key MN differentiation markers are induced on a real timescale that matches their ordering in our inferred trajectory. Terminal genes such as *Mnx1* and *Tubb3* are upregulated immediately after induction of early progenitor genes such as *Sox1*. The values plotted are shown in units of fold change relative to their expression in day 0 mESC cultures. b) Plot shows that *Olig2* is expressed  $\geq 10^6$  times lower than *Gapdh* during DP. Red line indicates the expected ratio if 0.1% of the population expressed 1 molecule per cell; the observed ratios are below even this low bound.