

Supplemental figure 4. (A) Both WT and NKX2-2 KO cultures display broad OLIG2 expression and begin to show ISL1/2⁺ MNs at day 10. (**B**) NKX2-2 KO cells lack NKX2-2 immunoreativity in both RFP⁺ and RFP⁻ cells. (**C**) GFP-expressing WT cells can easily be distinguished from NKX2-2 KO cells in co-cultures. (**D**) Schematic for BrdU pulse-labeling. (**E**) Proportion of MNs born after day 17 (assessed at day 19) in WT and NKX2-2 KO MNs (RFP⁺ and total) show a \sim 2.5-fold-change between WT and KO cells (n = 3; all error bars in figure indicate S.D.). (**F**) The proportion of GFP- NKX2-2 KO cells decreases over time in co-cultures, both in total and MN populations (n = 3).