



Fig. S2. Evaluation of lineage tracing fidelity in *Lin28a-T2A-CreER* mice and IF for Pax3, Pax7 and their matched isotype controls in the muscle cryosections.

(a) Western blot analysis for Cre protein (33 kD) in conventional (Con) Pax7+ Lin28a-tdTO- MuSCs and Lin28a-tdTO+ MuSCs shortly after culture *in vitro*. LTS-Cre cells are positive control cells which overexpressed Cre protein. Lin28a expression is extinguished shortly after Con MuSCs and tdTO+ MuSCs were cultured *in vitro* (Fig. S4a). Unprocessed original scans of blots are shown in Fig. S7.

(b) Western blot analysis for Lin28a protein *in vivo* of *wildtype* (WT), *LSL-tdTO*, *Lin28a-T2A-CreER* and *Lin28a-T2A-CreER;LSL-tdTO* mice tissues respectively. *Lin28a-T2A-CreER* mice manifested two bands: the lower band of endogenous Lin28a (26 kD) and the higher band of Lin28a-T2A (32 kD). Both bands' intensities were quantified for the Lin28a/Gapdh ratio. Gapdh protein was used as the loading control.

(c) Cryosections of the TA muscle of *Lin28a-T2A-CreER; LSL-tdTO* mice at 14 days after cryoinjury. Muscle

sections were co-stained for Pax3 (grey), DAPI (blue) and Pax7 (green). Green arrow indicates Pax7+ cells, and white arrow indicates Lin28a+ Pax3+ cells in uninjured TA. Red arrow indicates Lin28a+ Pax7+ Pax3+ cells (~3%) in injured TA. Scale bar: 100µm.

(d) Cryosections of the TA muscle of *Lin28a-T2A-CreER; LSL-tdTO* mice at 14 days after cryoinjury. Muscle sections were co-stained for mouse IgG2a (green) or mouse IgG1 (green) (isotype control for Pax3 or Pax7) antibody and DAPI (blue). Scale bar: 100µm.