



**Fig. S3. Differentiation potential of freshly sorted Lin28a+ MuSCs.**

(a) Phase contrast and fluorescence microscopy (each channel and merge) to assess Lin28a+ cells after *in vitro* differentiation in adipogenic, endothelial or osteogenic conditions. Alkaline phosphatase (ALP) staining revealed that, after differentiation in osteogenic conditions for 10 days, tdTO+ cells still expressed MyoD. The yellow arrows indicate some tdTO+ cells that are lightly stained by ALP and co-stain for MyoD. Scale bar: 200  $\mu$ m.

(b) Proportion of Oil Red-positive cells and MyoD-positive cells per field after culture in adipogenic conditions. Data are mean  $\pm$  SEM. N =3 independent experiments. For each experiment, a total of 5 fields were counted and averaged.

(c) Proportion of MyoD-positive cells per field after culture in endothelial conditions. Data are mean  $\pm$  SEM. N =3 independent experiments. For each experiment, a total of 5 fields were counted and averaged.

(d) Proportion of ALP-positive cells and MyoD-positive cells per field after culture in osteogenic conditions. Data are mean  $\pm$  SEM. N =3 independent experiments. For each experiment, a total of 5 fields were counted and averaged.

(e) Immunofluorescence staining for osteocalcin in tdTO+ cells. Proportion of osteocalcin-positive cells per field. Data are mean  $\pm$  SEM. N =3 independent experiments. For each experiment, a total of 5 fields were counted and averaged. Scale bar: 100 $\mu$ m.