



**Figure 1—figure supplement 1. Single-muscle-cell sequencing and analysis.** (A) Schematic of the 10 regions dissected and macerated to isolate single cells. (B) Representative FACS plot of Hoechst-stained cells from a single region indicating the gate used to isolate non-dividing cells. (C) Representative qRT-PCR plot for the muscle marker *troponin* used to screen single-cell cDNA libraries. The libraries from cells circled in red were sequenced. (D) Principal component (PC) analysis and *troponin* expression identified 115 muscle cells. Cells separated along two significant principal components: PC1 (29.9% of variance explained,  $p=1.4\text{E-}120$ ) separated muscle from epidermal lineage and PC2 (8.3% of variance explained,  $p=4.4\text{E-}44$ ) separated neoblasts from differentiated cells (Supplementary file 1B). Cells to the left of the dashed line that expressed *troponin* were retained for further analysis as muscle cells. (E) Distribution of contigs with two or more reads in the 177 single-cell libraries used for PC analysis. Nearly all cells with high number of expressed contigs that could signal a doublet event from FACS were categorized as non-muscle cells and excluded from differential expression analysis. (F) Muscle cells from all regions were evenly distributed throughout PC-space indicating that AP region of origin did not explain a significant proportion of the variance. Inset includes number of muscle cells analyzed per region. (G) Different differential expression analysis methods were tested for the ability to identify known mRNAs. The rank order by p-value is shown on the y-axis in  $\log_{10}$  scale for several canonical mRNAs. Arrows mark the rank separating significant (filled circle) and not significant (n.s., unfilled circle) genes at  $p<0.01$  for each method. (H) Three differential expression analyses (left) using SCDE were performed between the indicated regions. Ranking of genes by a differential expression score was used to generate a receiver-operator curve (right) to evaluate whether the SCDE analysis correctly classified genes as mRNAs compared to ISH validation (Methods).