Expanded View Figures

Figure EV1. Single-cell atlas of the proximal wing imaginal disc. Related to Fig 1.

A, B   UMAP plot of the wild-type reference single-cell atlas generated from wandering third instar larval wing discs coloured by replicates (A) and split by replicates (B). Four replicates *1151-GAL4* are indicated as 1151 and 4 replicates *1151>mCherry-RNAi* as Cherry.

C, D   Number of genes with at least 1 UMI count and number of UMI across all cells (left and right panel, respectively) grouped by replicates (C) and cell type (D).

E   Total number of cells per sample in the reference atlas after applying filters and removing clusters that were unevenly represented across replicates.

F   Dimensions of the data were reduced by PCA. Genes (rows) and cells (columns) were ordered by their PCA scores. Heatmap showing distribution of the 500 most extreme cells and 30 most extreme genes. PC1 separates epithelial cells from myoblast cells as indicated by the muscle-specific genes *Him* and *zfh1*, and the epithelial genes *Fas3* and *grh*.

G   Confocal single plane images of wandering third instar larval wing discs and orthogonal view of *twi-lacZ* (β-gal, in red) and *grh::GFP* (green). Scale bars: 50 μm. Full genotype: *w-; twi-lacZ; grh[VK00033]::GFP.*

H   Dot plot showing the expression of the main markers distinguishing Trachea_1 from Trachea_2 across all clusters of the reference atlas.
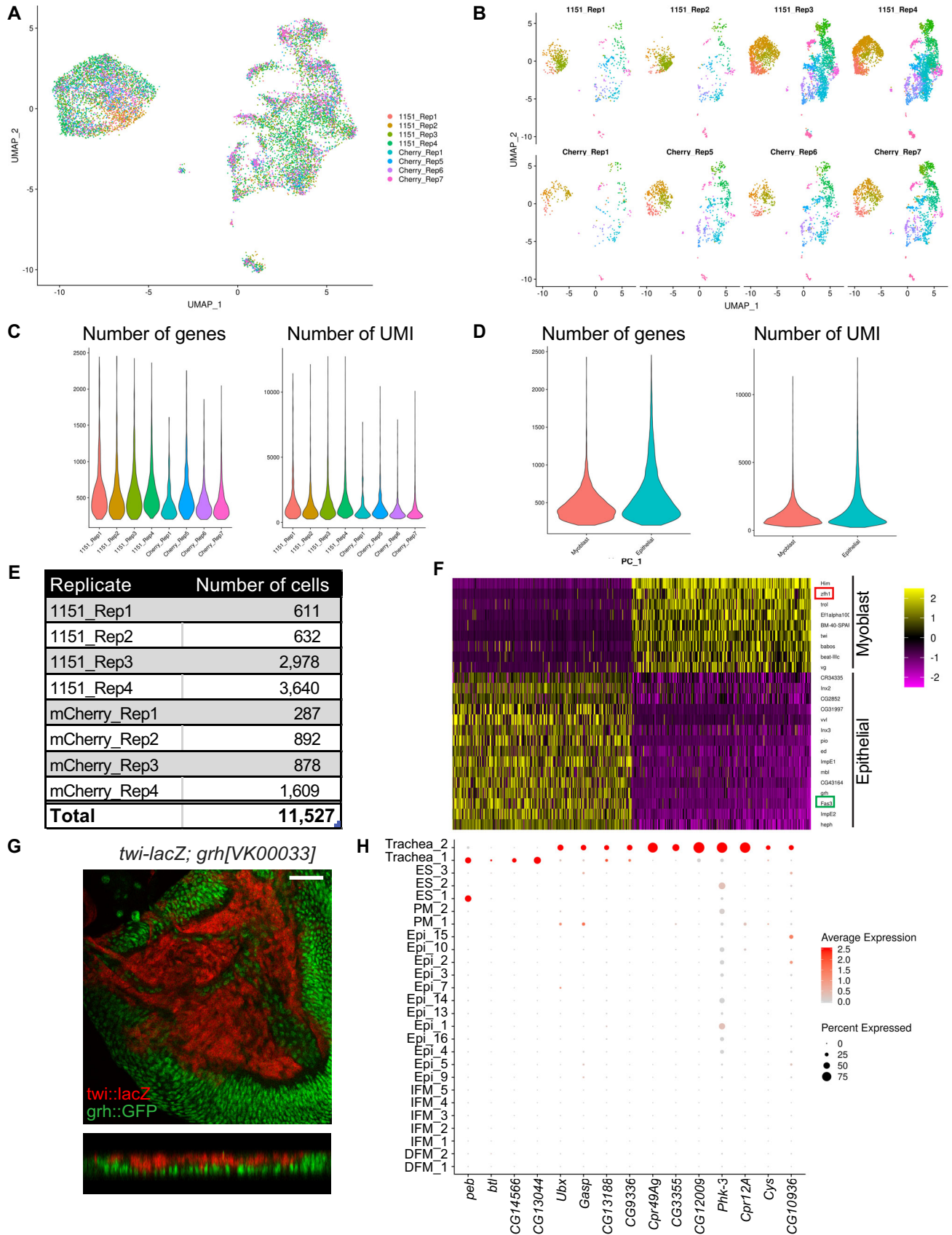
Figure EV1.

Figure EV2. Markers for the epithelial clusters. Related to Fig 2.

A   Average expression of the genes used as known markers to map localization of epithelial clusters to the cell fate map of the wing discs in the reference atlas dataset. Marker gene for Epi_5 and 9 is *CG17278*, Epi_13 is *caup*, *ara*, *mirr* and *un*, Epi_2 and 3 is *toe*, Epi_10 is *knrl*, Epi_15 is *tup*, and PM_2 is *Antp*.

B   Violin plot representing the distribution of expression levels of *scabrous* (*sca*) across the epithelial clusters assigned as in the reference atlas dataset.
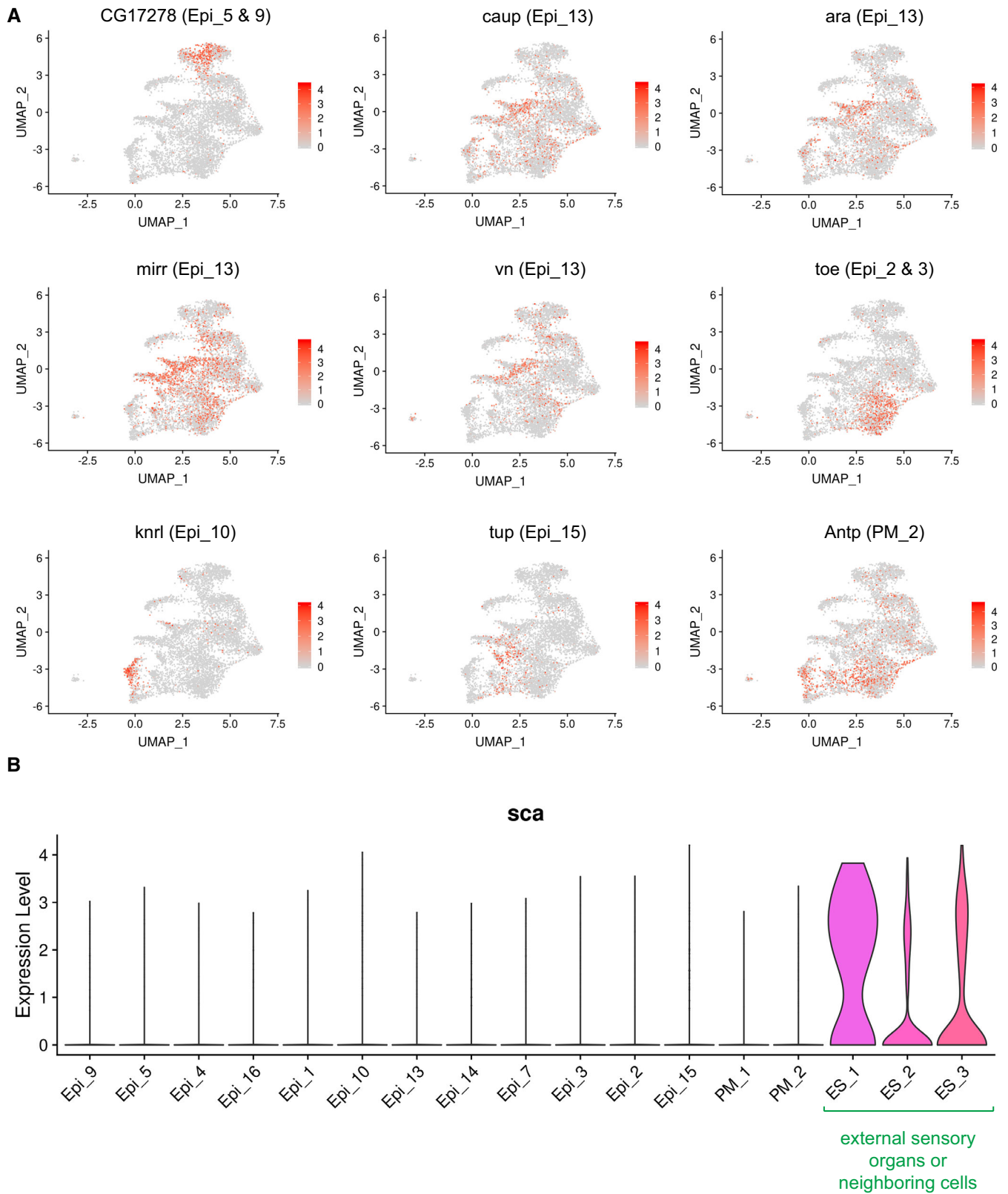
▶

A

B

Figure EV2.

Figure EV3. Markers for DFM and IFM myoblast clusters. Related to Fig 3.

A   Heatmap showing the 10 tops differentially expressed gene markers for each myoblast cluster of the reference single-cell atlas dataset. Cells (columns) are
          clustered by the expression of the main marker genes (rows).
B   Confocal single plane images of third instar larval wing discs stained with anti-Nrt (white) in DFM myoblast area of *Ama[NP1297]>ChFP* and *Argk[CB3789]::GFP*, top
          and bottom panel, respectively.
C–E   Confocal single plane images of *Ama[NP1297]>gTRACE* showing the lineage of *Ama-GAL4* (green) and the active *GAL4* (red) in: (C) third instar larval wing disc
          stained with anti-Ct (white). Dashed yellow line: DFM myoblast area. Yellow arrowhead: cell lineage expression of *Ama* in few IFM myoblasts; (D) forming DFM at
          28 h APF stained with anti-Zfh1 (white). White arrows: wing hinge; (E) adult IFM (specifically, DLM) stained with DAPI (white), anterior up.

Data information: Scale bars: 50 μm. Full genotypes: (B) *w-; +; Ama[NP1297]-GAL4/UAS-mCD8. ChRFP* and *y-, w-; +; Argk[CB03789]::GFP*. (C) *w-; UAS-gTRACE/+; Ama
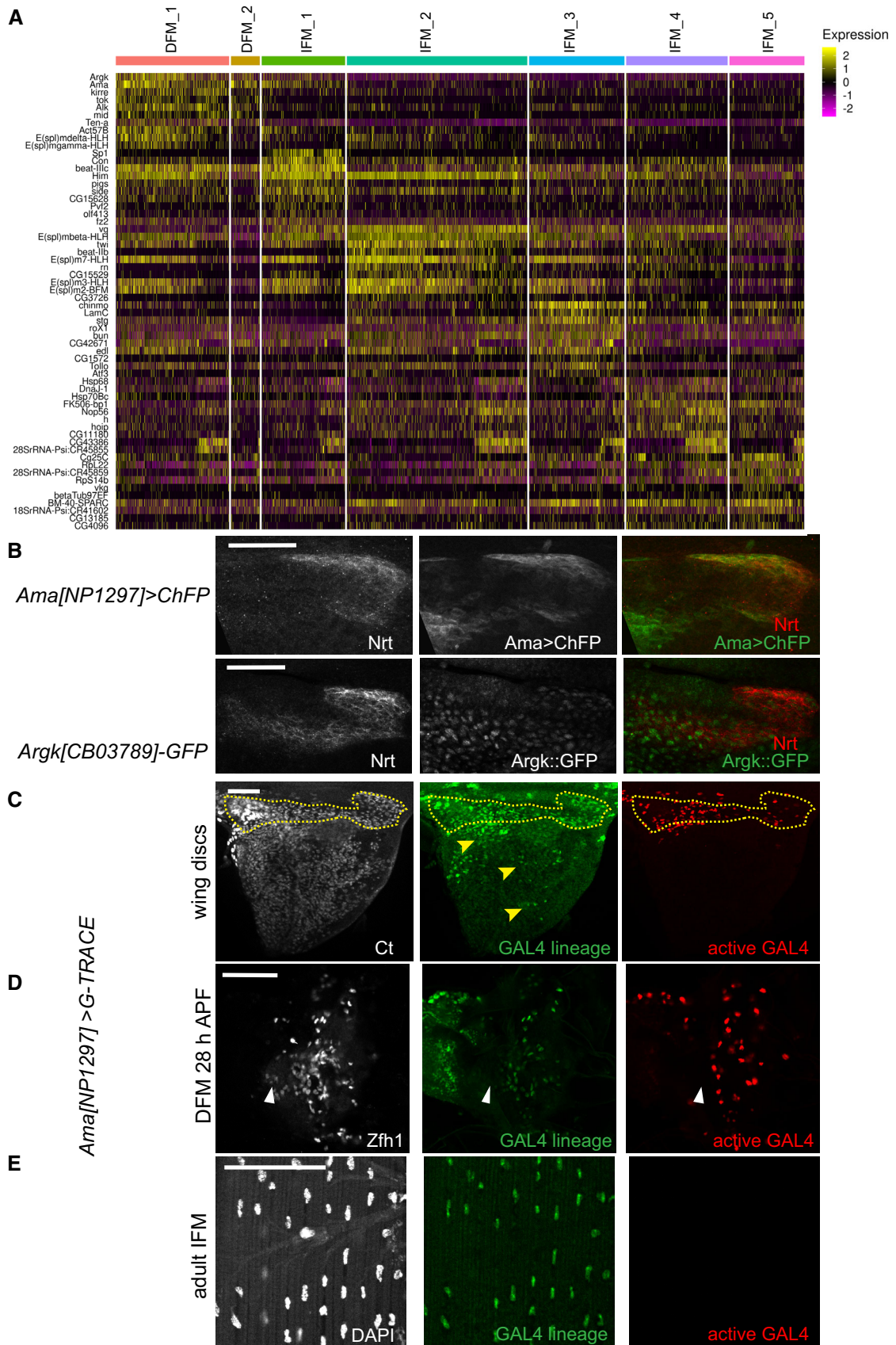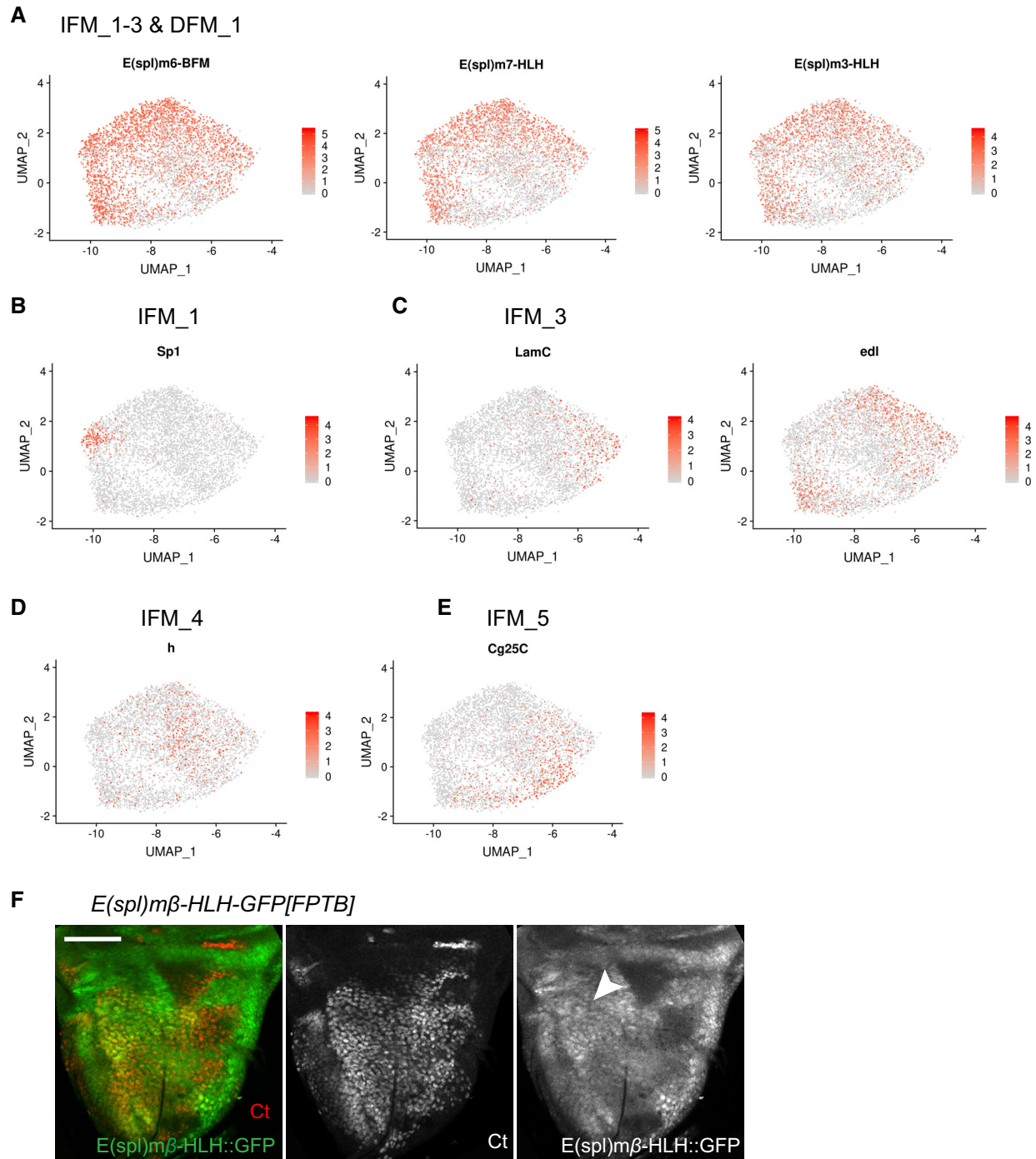[NP1297]-GAL4/+*.

Figure EV3.

Figure EV4. Markers for different state of differentiation. Related to Fig 4.

A–E   Average expression level of the genes identified as markers for the myoblast clusters assigned as in the reference atlas dataset. Marker gene for (A) IFM_1-3 and DFM_1 are *E(spl)-m6-BFM*, *E(spl)-m7-HLH*, *E(spl)-m3-HLH*, (B) IFM_1 is *Sp1*, (C) IFM_3 are *LamC* and *edl*., (D) IFM_4 is *h* and (E) IFM_5 is *Cg25C*.

F   Confocal single plane image of third instar larval wing discs *E(spl)-mbeta-HLH-GFP[FPTB]* stained with anti-Ct (red). Scale bar: 50 μm. Full genotype: *y- w-*; P{*E(spl) mbeta-HLH-GFP.FPTB}attP40*.

Data information: White arrowheads point to location of IFM_1-2 cells.

Figure EV5. Loss of Amalgam in myoblast of larval wing disc. Related to Fig 7.

A   UMAP plot of *1151>Ama-RNAi* from third instar larval wing discs coloured by cell type (myoblast, epithelial and tracheal cells) and split by replicate (Rep1, Rep4 and Rep5). The total number of cells after applying filters was 1,232, 706 and 1,400 for each replicate, respectively.

B   The cells from *1151>Ama-RNAi* dataset were projected into the reference single-cell atlas. Number of cells in each cluster normalized to the total number of epithelial cells in each genotype is plotted across epithelial cells.

C   Violin plot representing the distribution of expression levels of the epithelial marker *grh* across all the clusters, split by *1151>Ama-RNAi* (pink) and reference cell atlas dataset (blue).

D, E Confocal single plane images of third instar larval wing discs from *1151>mCherry-RNAi* and *1151>Ama-RNAi* stained with anti-Ct (green), DAPI (red) and (D) anti-pH3 (magenta) or (E) anti-Dcp1 (magenta).

F   Dot plots showing the expression levels of the known differentiation genes across the cluster of myoblast cells in *1151>Ama-RNAi* and the reference cell atlas.

Data information: Scale bars: 50 μm. Full genotypes: *1151-GAL4; +; UAS-mCherry-RNAi* and *1151-GAL4; +; UAS-Ama[HMS00297]-RNAi.*
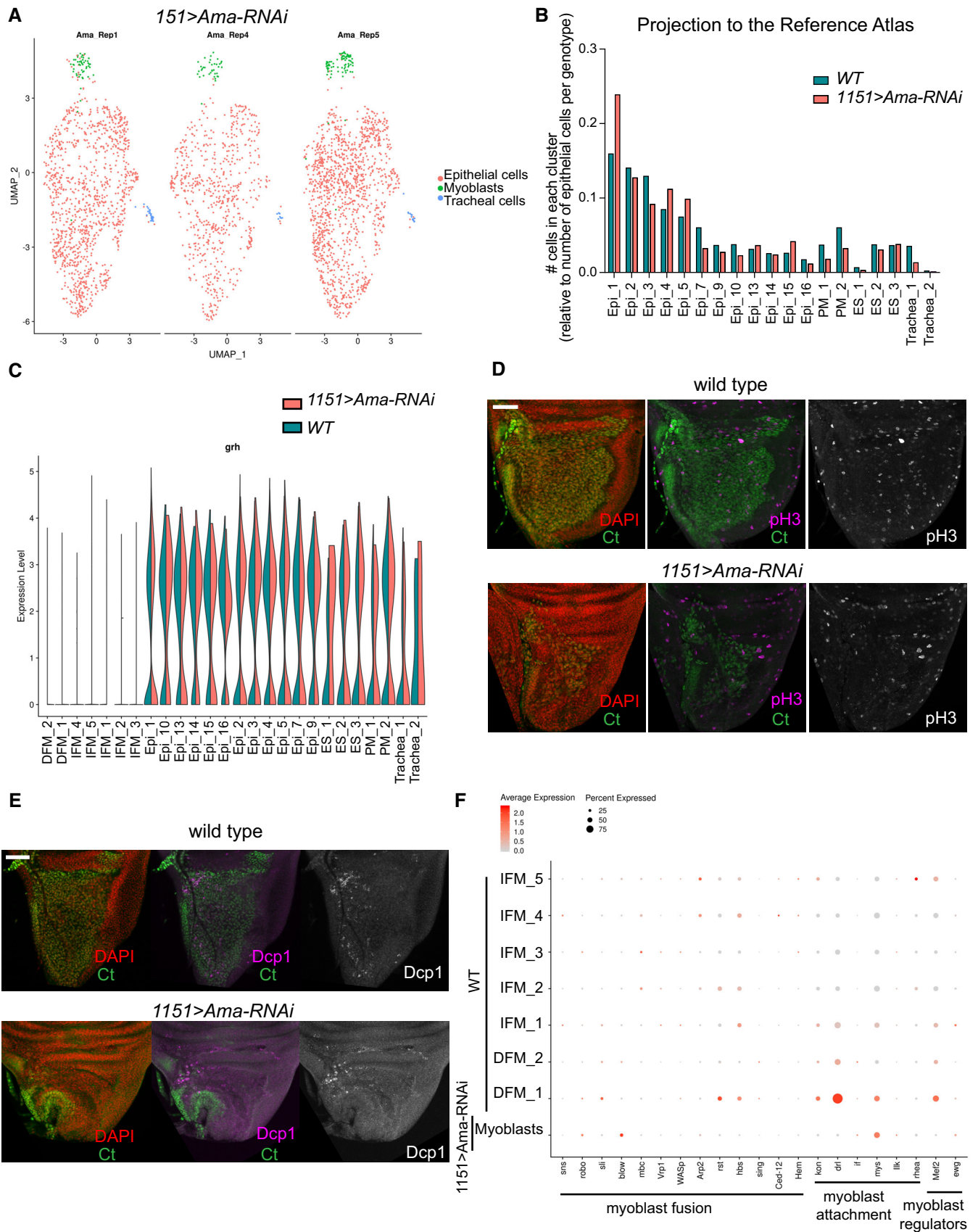
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Figure EV5.