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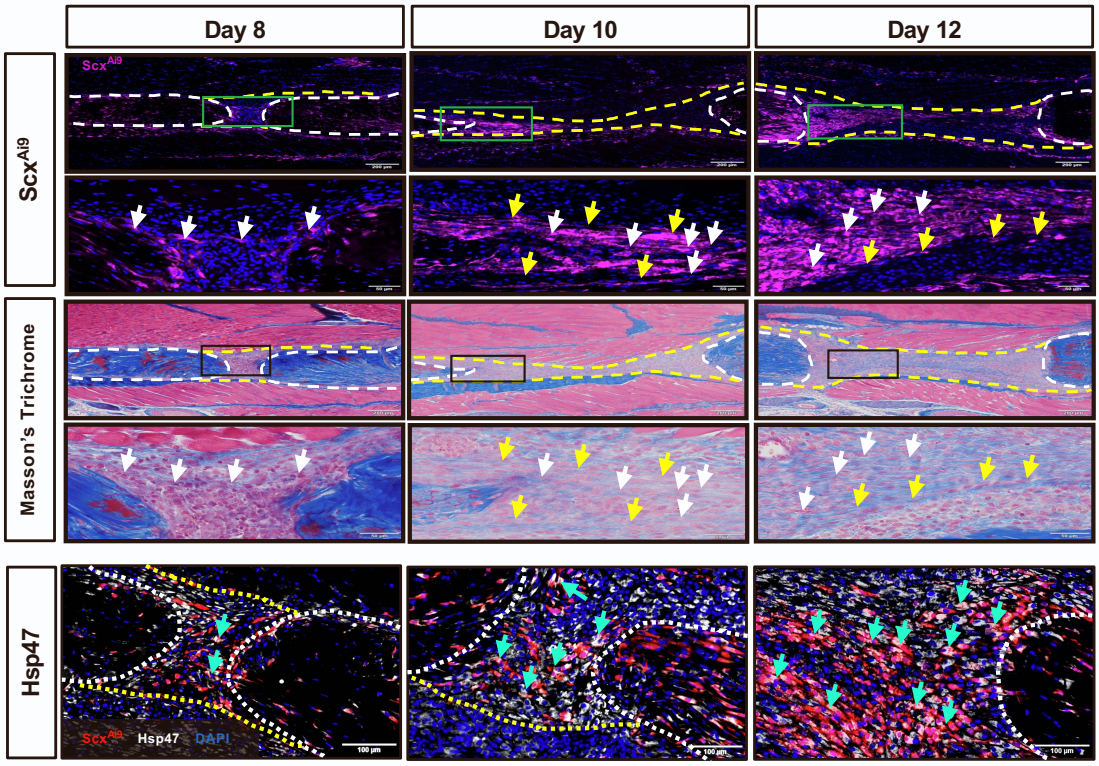
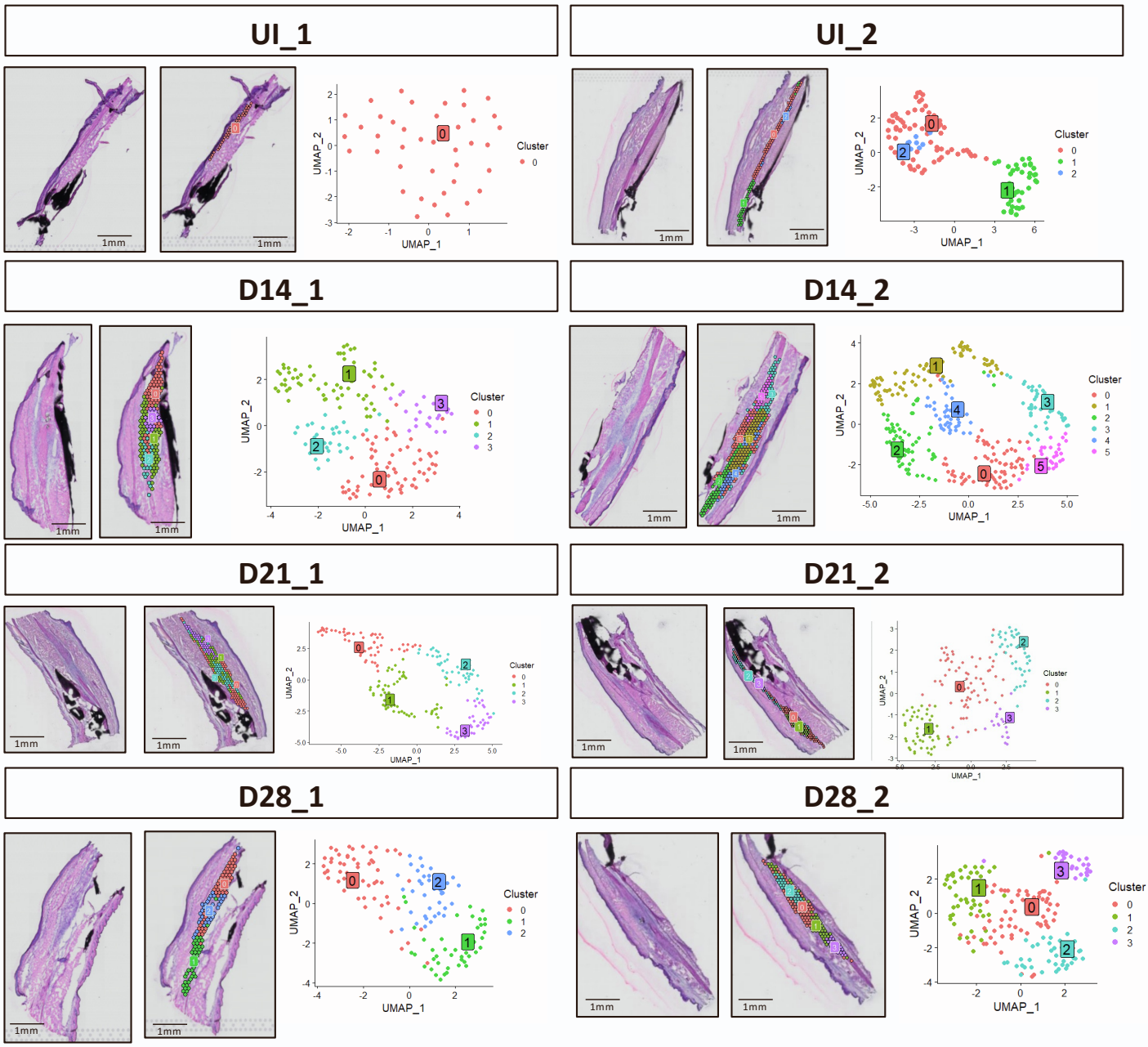
Supplemental information

Defining the spatial-molecular map of fibrotic tendon healing and the drivers of Scleraxis-lineage cell fate and function

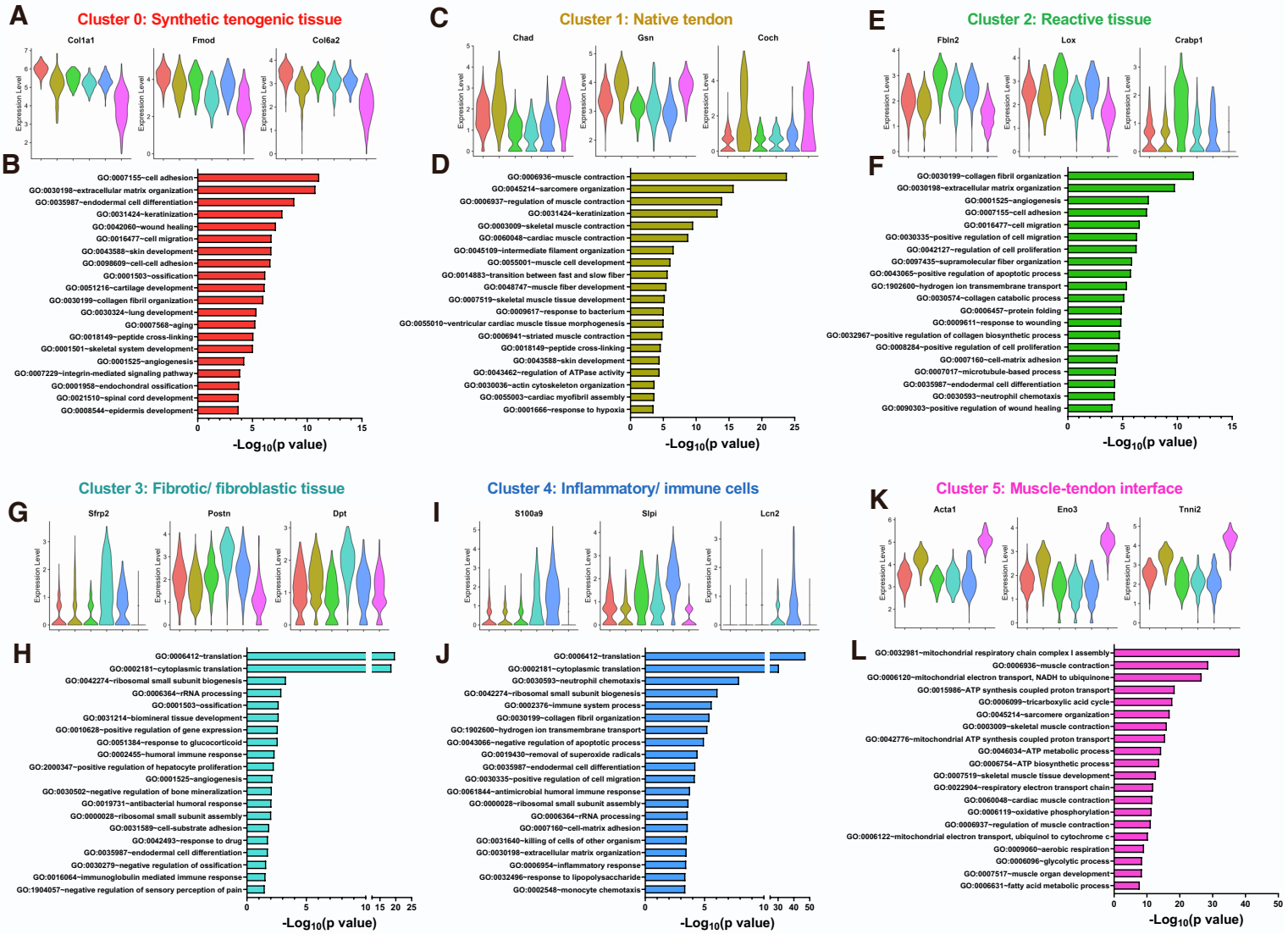
Jessica E. Ackerman, Katherine T. Best, Samantha N. Muscat, Elizabeth M. Pritchett, Anne E.C. Nichols, Chia-Lung Wu, and Alayna E. Loiselle



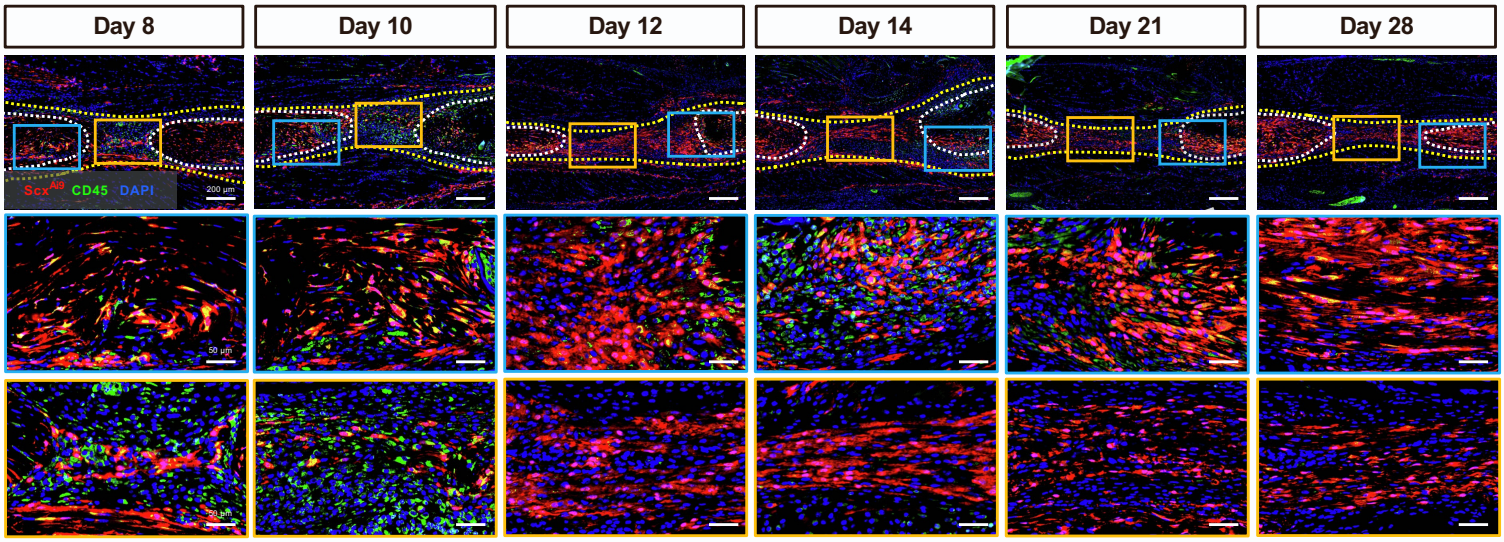
Supplemental Figure 1. Matrix elaboration by Scx^{Ai9} cells, Related to Figure 1.
Immunofluorescence of Scx^{Ai9} (pink) at days 8, 10, and 12 post-repair. Nuclei stained with DAPI. Coverslips were removed and samples were then stained with Masson's Trichrome to analyze localization of organized collagen deposition at the repair site. White arrows used to indicate Scx^{Ai9} cells and yellow arrows used to indicate non-Scx^{Ai9} cells between fluorescent and Masson's trichrome images. Scale bars represent 200μm for low power images and 50μm for higher power images. Co-immunofluorescence for Scx^{Ai9} (red) and Hsp47 (white) between D8-12 post-repair. Blue arrows indicate examples of co-localization. N=3-5 per time-point. Scale bars represent 100μm.



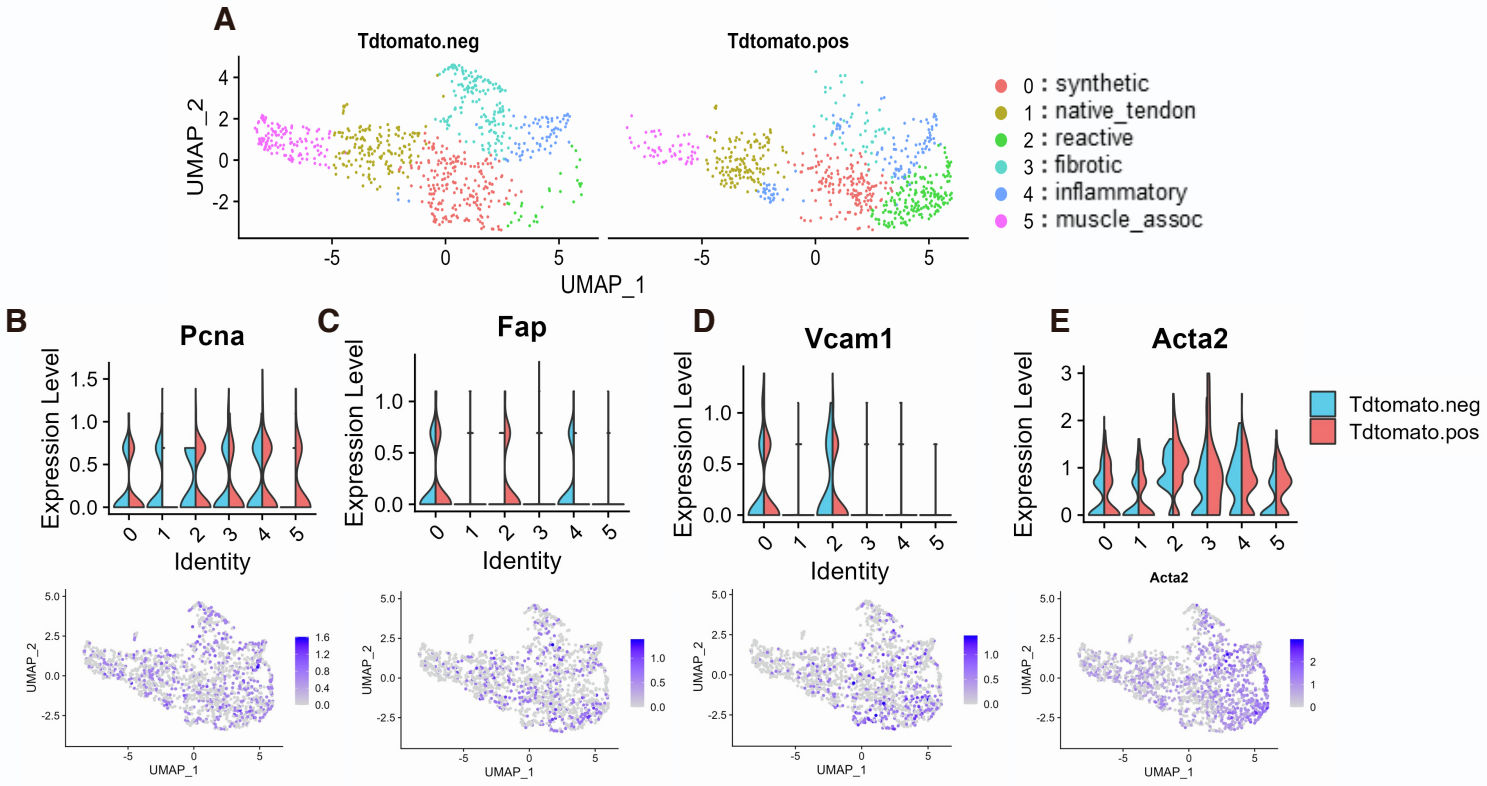
Supplemental Figure 2. Selection of tendon/scar ROI for downstream integrative analysis, Related to Figure 2. Each timepoint is labeled with the harvest day post-injury, along with the technical replicate identifier. For each sample the original H&E-stained image (left), the spots encompassing the tendon/scar using coordinates exported from selection in Loupe (middle), and UMAP of the unsupervised clustering captured from the ROI in (right) is shown. Scale bars represent 1mm.



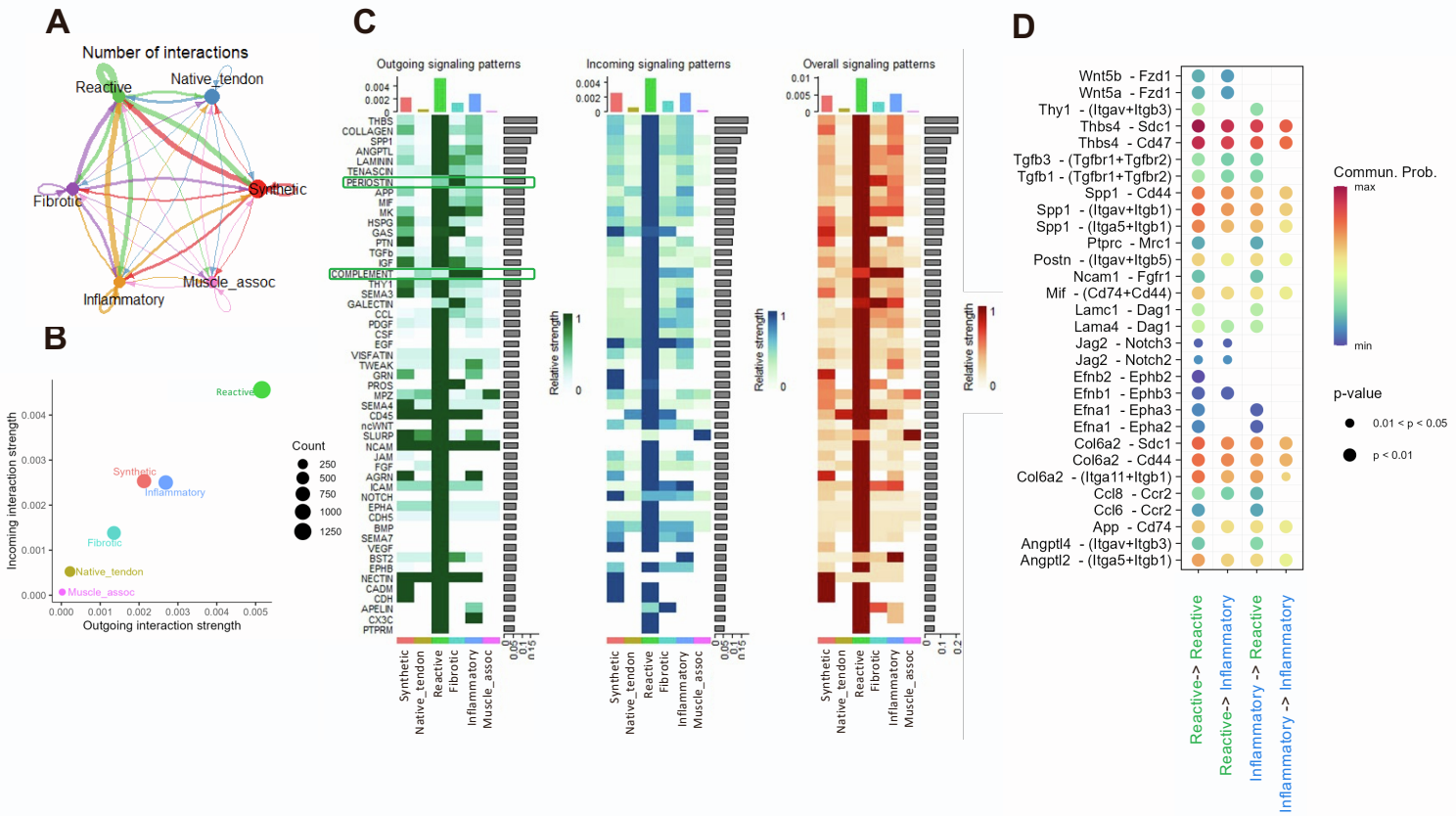
Supplemental Figure 3. Integrated cluster annotation confirmation, Related to Figure 2. The top 3 distinguishing marker genes for each cluster in the integrated dataset were mapped with violin plots, and gene ontology lists generated for each to confirm each annotation: (A,B) $C0^{synthetic}$, (C,D) $C1^{native\_tendon}$, (D,E) $C2^{reactive}$, (G,H) $C3^{fibrotic}$, (I,J) $C4^{inflammatory}$, and (K,L) $C5^{muscle\_assoc}$.



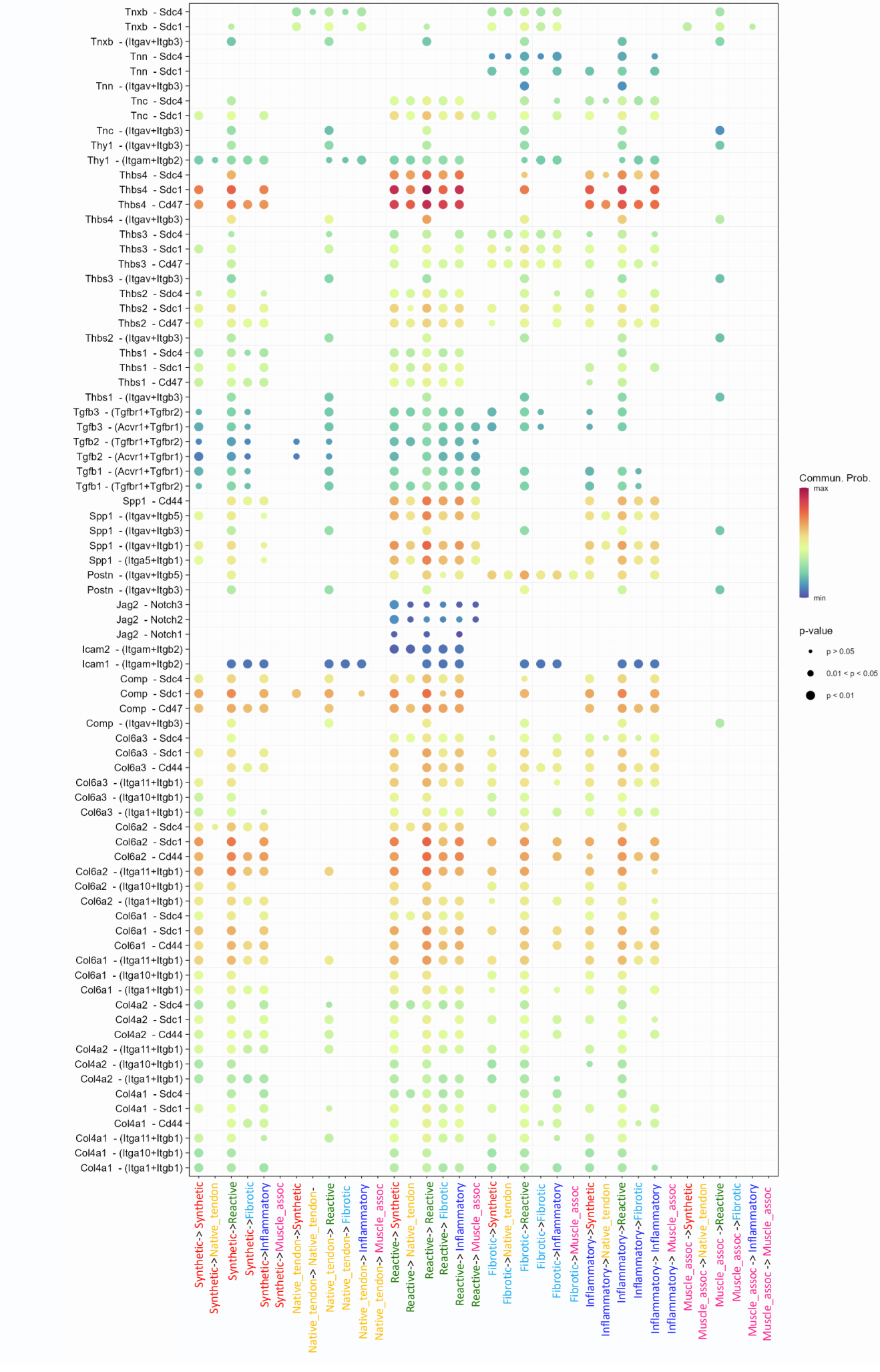
Supplemental Figure 4. Scx^{Ai9} cells co-express CD45 throughout tendon healing, Related to Figure 3. Co-immunofluorescence of Scx^{Ai9} (red) and CD45 (green), between 8-28 days post-repair. Nuclei are stained with DAPI. Tendon is outlined by white dotted lines and scar tissue by yellow dotted lines. Blue and yellow boxes represent higher magnification images taken from the tendon stub and bridging scar tissue, respectively. Scale bars of low magnification images are 200μm and 50μm for higher magnification images.



Supplemental Figure 5. Differential expression of fibroblast activation markers in tdTomato.neg vs tdTomato.pos populations, Related to Figures 1 & 3-5. (A) Integrated UMAPs for tdTomato.neg (tdTomato <1) and tdTomato.pos (tdTomato > 1) subsets. Violin plots split by tdTomato.neg (blue) and tdTomato.pos (red) depicting expression of (B) *Pcna*, (C) *Fap*, (D) *Vcam1*, and (E) *Acta2*. Bottom row depicts corresponding feature plot for each activation marker.



Supplemental Figure 6. Identification of the interactome between molecular clusters, Related to Figure 6. (A) Circle plot of integrated clusters shows relative number of interactions between each cluster (line color corresponds to originating source of signal), while (B) quantifies overall interaction strength of each cluster. (C) Outgoing, incoming, and overall signaling pattern strength are quantified and shown for each cluster. Green boxes highlight Periostin and Complement signaling. (D) Dot plot of ligand-receptor interactions within and between $C2^{reactive}$ and $C4^{inflammatory}$. Dot size and color indicate the significance of the interaction (given as a range of p values), and the probably of communication, respectively.


Tnxb - Sdc4
Tnxb - Sdc1
Tnxb - (Itgav+Itgb3)
Tnn - Sdc4
Tnn - Sdc1
Tnn - (Itgav+Itgb3)
Tnc - Sdc4
Tnc - Sdc1
Tnc - (Itgav+Itgb3)
Thy1 - (Itgav+Itgb3)
Thy1 - (Itgam+Itgb2)
Thbs4 - Sdc4
Thbs4 - Sdc1
Thbs4 - Cd47
Thbs4 - (Itgav+Itgb3)
Thbs3 - Sdc4
Thbs3 - Sdc1
Thbs3 - Cd47
Thbs3 - (Itgav+Itgb3)
Thbs2 - Sdc4
Thbs2 - Sdc1
Thbs2 - Cd47
Thbs2 - (Itgav+Itgb3)
Thbs1 - Sdc4
Thbs1 - Sdc1
Thbs1 - Cd47
Thbs1 - (Itgav+Itgb3)
Tgfb3 - (Tgfbr1+Tgfbr2)
Tgfb3 - (Acvr1+Tgfbr1)
Tgfb2 - (Tgfbr1+Tgfbr2)
Tgfb2 - (Acvr1+Tgfbr1)
Tgfb1 - (Acvr1+Tgfbr1)
Tgfb1 - (Tgfbr1+Tgfbr2)
Spp1 - Cd44
Spp1 - (Itgav+Itgb5)
Spp1 - (Itgav+Itgb3)
Spp1 - (Itgav+Itgb1)
Spp1 - (Itga5+Itgb1)
Postn - (Itgav+Itgb5)
Postn - (Itgav+Itgb3)
Jag2 - Notch3
Jag2 - Notch2
Jag2 - Notch1
Icam2 - (Itgam+Itgb2)
Icam1 - (Itgam+Itgb2)
Comp - Sdc4
Comp - Sdc1
Comp - Cd47
Comp - (Itgav+Itgb3)
Col6a3 - Sdc4
Col6a3 - Sdc1
Col6a3 - Cd44
Col6a3 - (Itga11+Itgb1)
Col6a3 - (Itga10+Itgb1)
Col6a3 - (Itga1+Itgb1)
Col6a2 - Sdc4
Col6a2 - Sdc1
Col6a2 - Cd44
Col6a2 - (Itga11+Itgb1)
Col6a2 - (Itga10+Itgb1)
Col6a2 - (Itga1+Itgb1)
Col6a1 - Sdc4
Col6a1 - Sdc1
Col6a1 - Cd44
Col6a1 - (Itga11+Itgb1)
Col6a1 - (Itga10+Itgb1)
Col6a1 - (Itga1+Itgb1)
Col4a2 - Sdc4
Col4a2 - Sdc1
Col4a2 - Cd44
Col4a2 - (Itga11+Itgb1)
Col4a2 - (Itga10+Itgb1)
Col4a2 - (Itga1+Itgb1)
Col4a1 - Sdc4
Col4a1 - Sdc1
Col4a1 - Cd44
Col4a1 - (Itga11+Itgb1)
Col4a1 - (Itga10+Itgb1)
Col4a1 - (Itga1+Itgb1)
Synthetic-> Synthetic
Synthetic-> Native_tendon
Synthetic->Reactive
Synthetic->Fibrotic
Synthetic->Inflammatory
Synthetic->Muscle_assoc
Native_tendon->Synthetic
Native_tendon-> Native_tendon-
Native_tendon-> Reactive
Native_tendon-> Fibrotic
Native_tendon-> Inflammatory
Native_tendon-> Muscle_assoc
Reactive-> Synthetic
Reactive-> Native_tendon
Reactive-> Reactive
Reactive-> Fibrotic
Reactive-> Inflammatory
Reactive-> Muscle_assoc
Fibrotic->Synthetic
Fibrotic->Native_tendon
Fibrotic->Reactive
Fibrotic->Fibrotic
Fibrotic->Inflammatory
Fibrotic->Muscle_assoc
Inflammatory->Synthetic
Inflammatory->Native_tendon
Inflammatory->Reactive
Inflammatory->Fibrotic
Inflammatory-> Inflammatory
Inflammatory-> Muscle_assoc
Muscle_assoc ->Synthetic
Muscle_assoc ->Native_tendon
Muscle_assoc ->Reactive
Muscle_assoc ->Fibrotic
Muscle_assoc -> Inflammatory
Muscle_assoc -> Muscle_assoc
Commun. Prob.
max
min
p-value
p > 0.05
0.01 < p < 0.05
p < 0.01

Supplemental Figure 7. Interactome of top ten pathways present during tendon healing, Related to Figures 2 & 6. Dot plot of ligand-receptor interactions within and between each cluster of the integrated dataset. Dot size and color indicate the significance of the interaction (given as a range of p values), and the probably of communication, respectively.