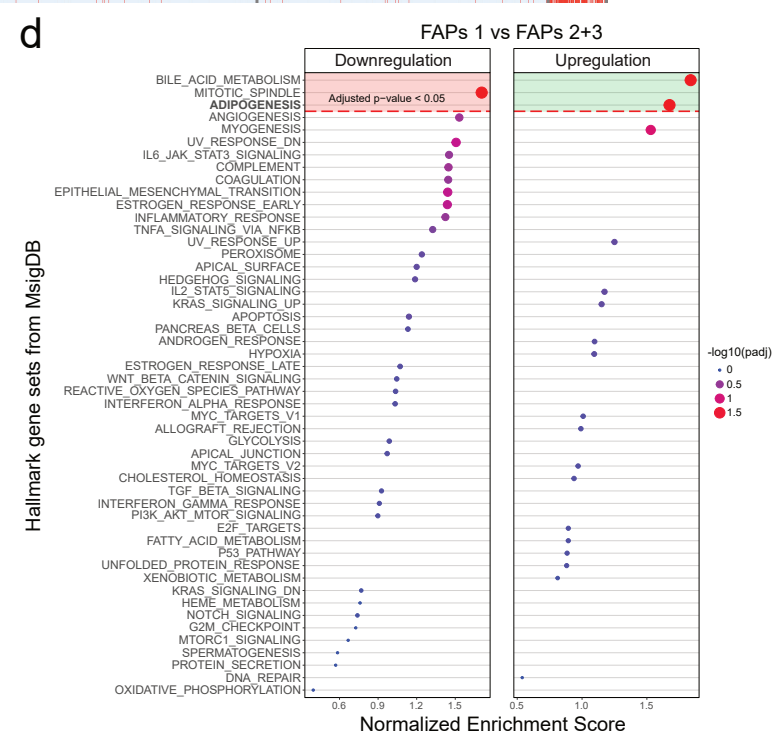
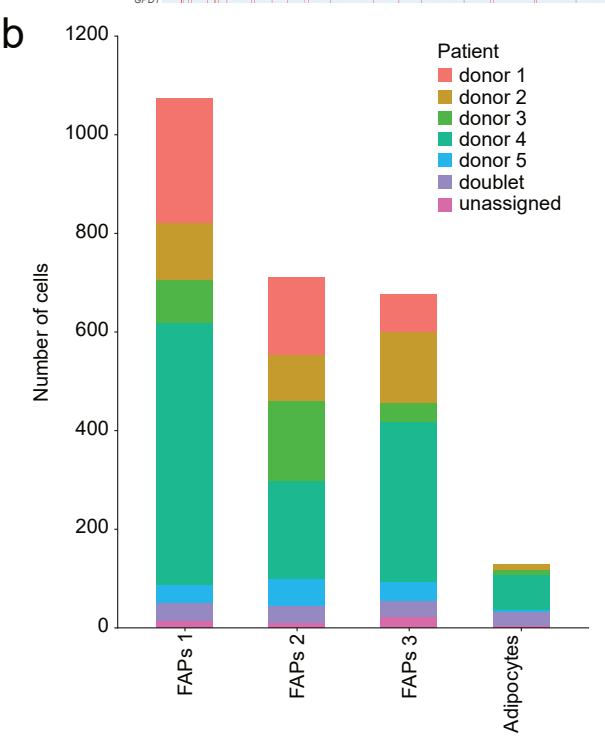
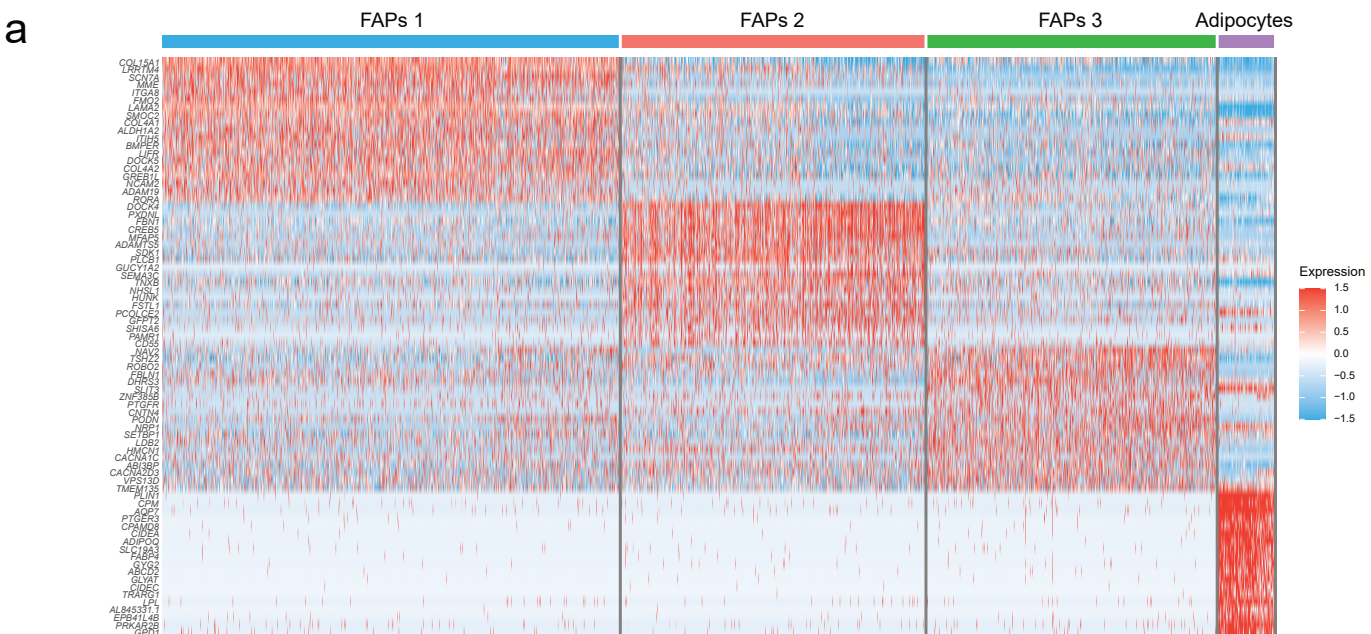


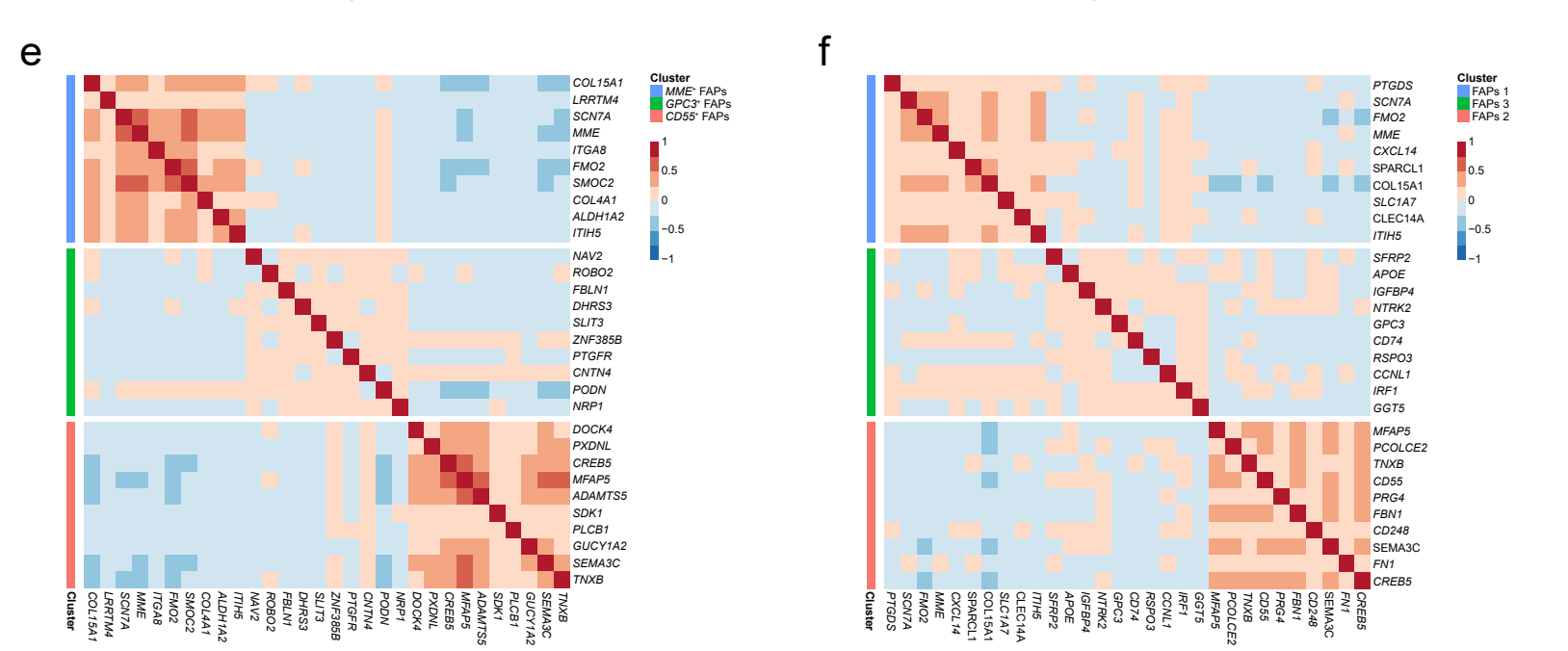
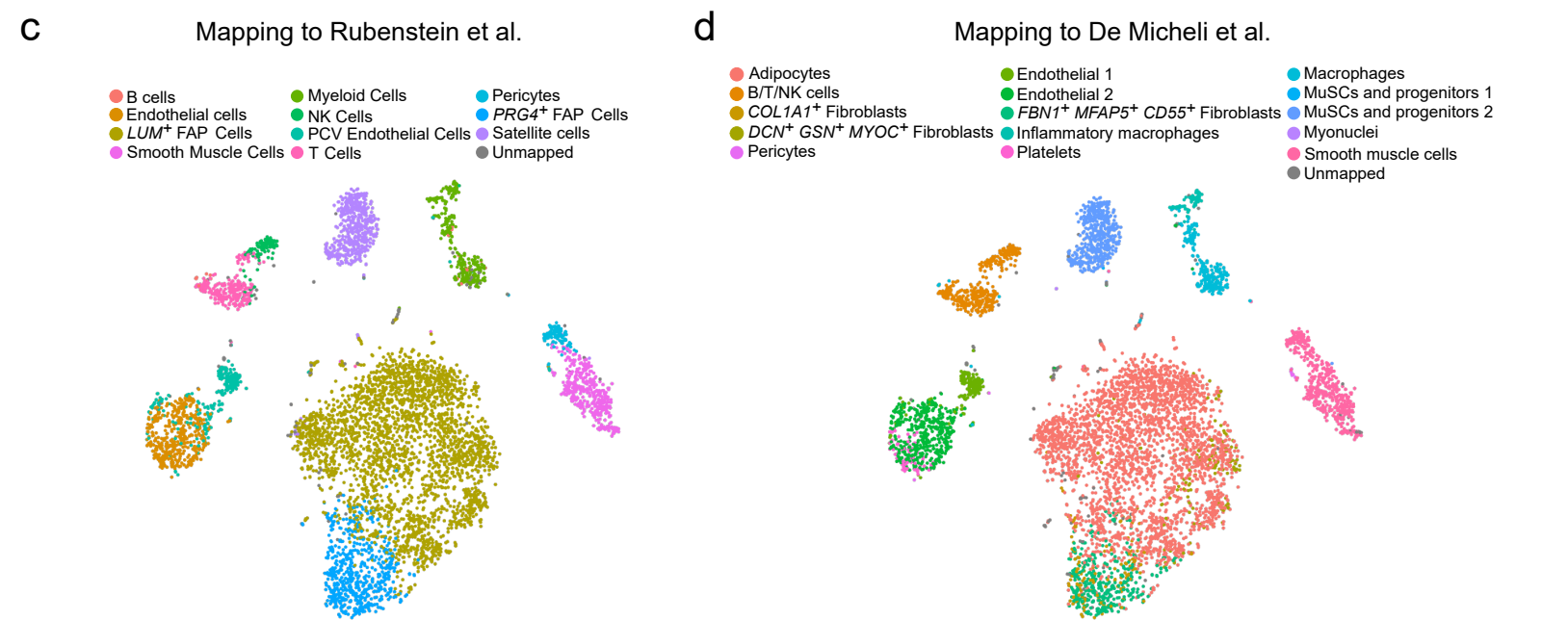
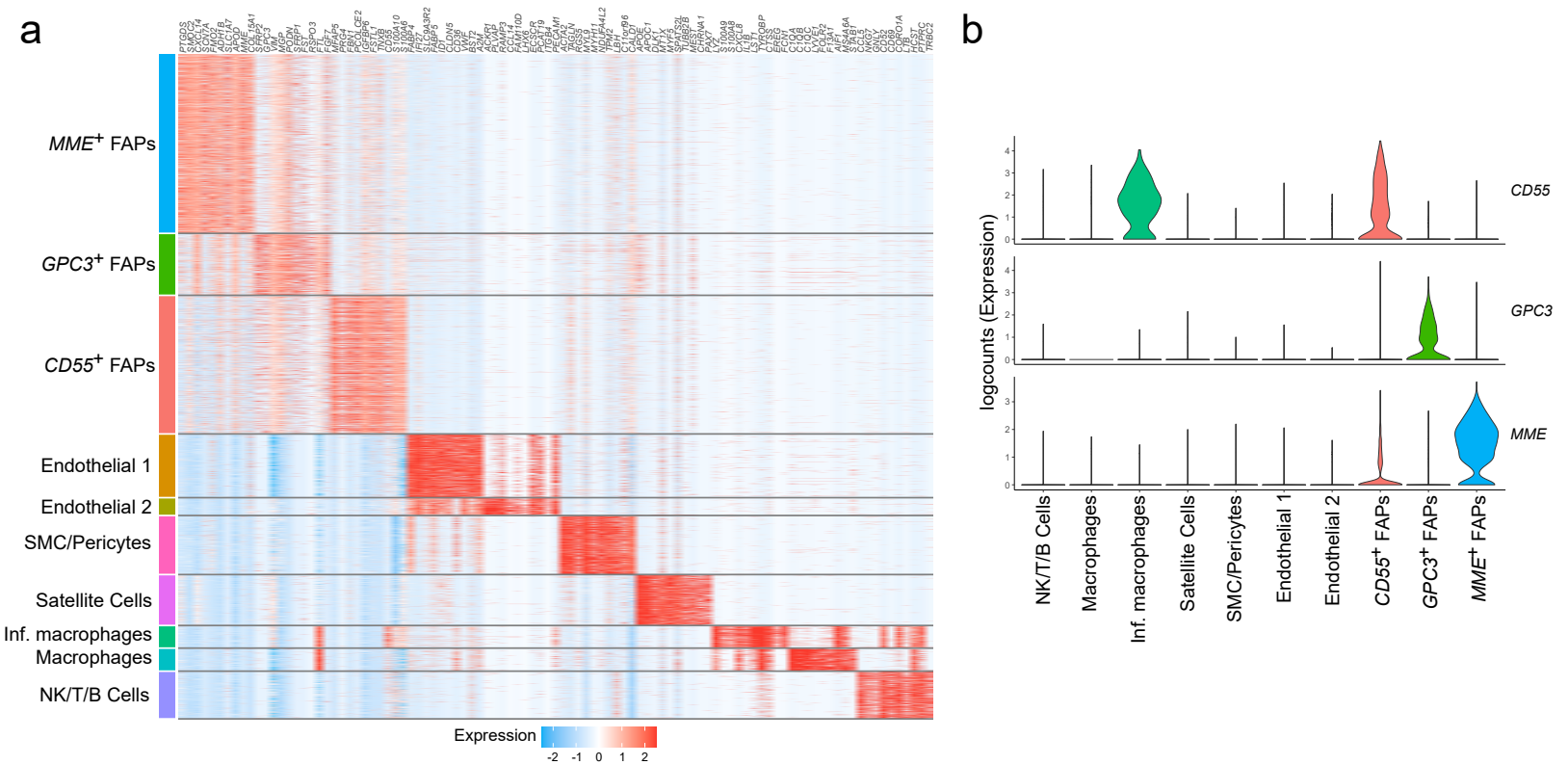
Supplementary Fig. 1: snRNA-seq analysis from human muscle identifies main cell populations. Related to Fig. 1.

a TSNE plot of main cell types from human muscle, color-coded by the identified populations. **b** Dot plot of centered logcounts values from cell type-specific marker genes. Color and size of the dots indicate the centered logcount value and the proportion of cells that express the gene, respectively. **c** TSNE plots of full (upper-left) and per-patient (upper-middle, upper-right and bottom panels) snRNA-seq datasets, color-coded by the patient of origin. **d** Stacked bar plots showing the contribution of each human patient to each cluster. Color-coded by the patient of origin.



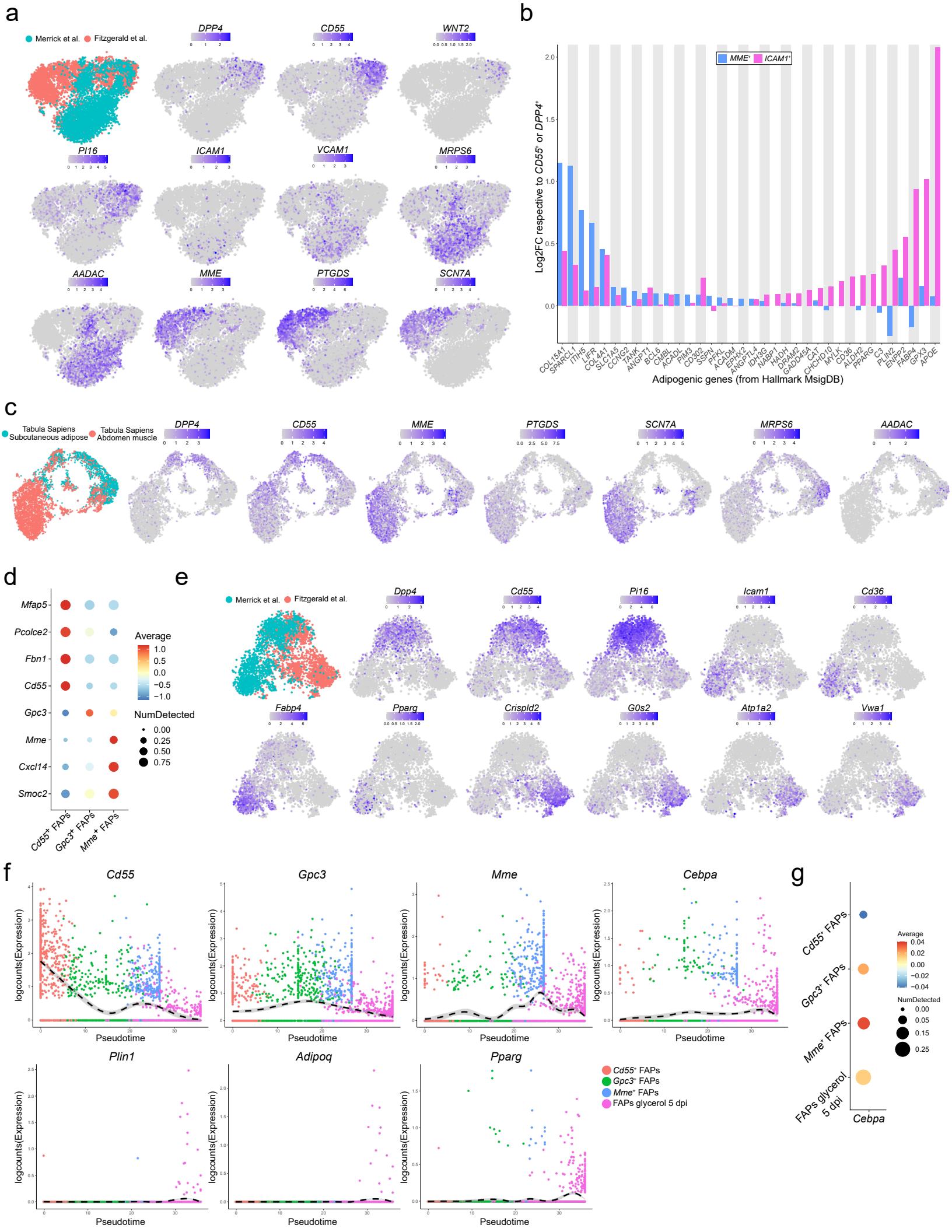
Supplementary Fig. 2: snRNA-seq analysis from human muscle identifies 3 FAPs populations. Related to Fig. 1.

a Heatmap with Z-scores showing top enriched marker genes per cell type. Each row represents a gene and each column a cell. Columns are ordered and color-coded based on the cluster of origin. **b** Stacked bar plots showing the contribution of each human patient to each cluster. Color-coded by the patient of origin. **c** TSNE plots of full (upper-left) and per-patient (upper-middle, upper-right and bottom panels) snRNA-seq datasets, color-coded by the patient of origin. **d** Dot plots showing downregulated (left panel) or upregulated (right panel) pathways from GSEA of FAPs 1 compared to FAPs 2+3. X-axis indicates the normalized enrichment score (NES) for each pathway. Color and size of the dots indicate adjusted p-values (FDR). Significant values are delimited by the red dashed line (Adjusted p-value < 0.05 or $-\log_{10}(\text{adjusted p-value}) > 1.3$).



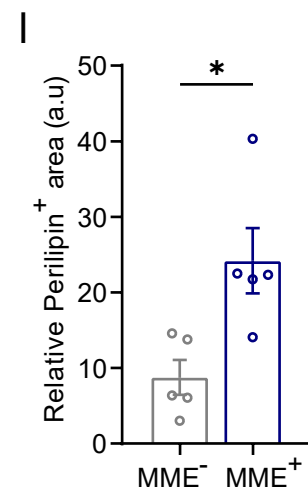
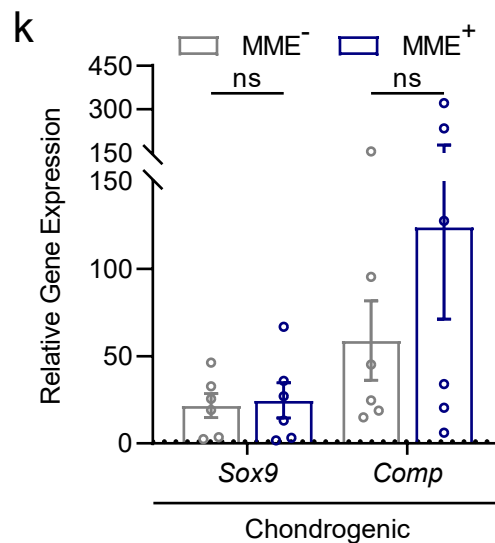
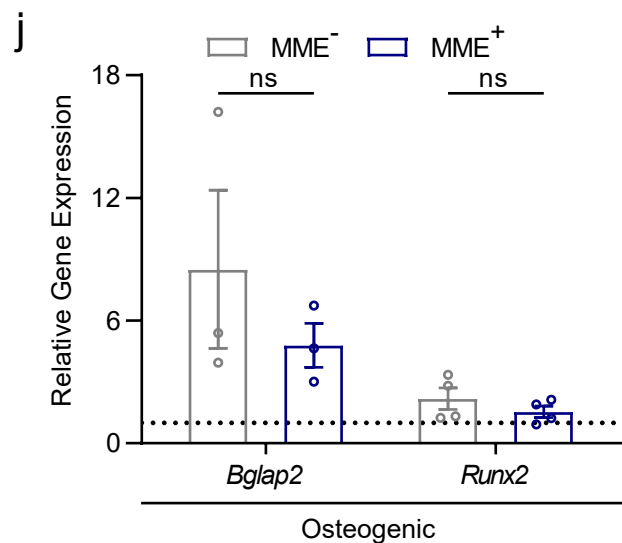
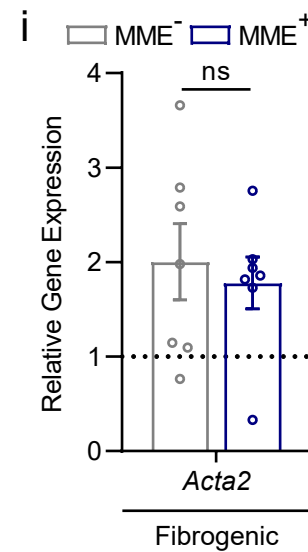
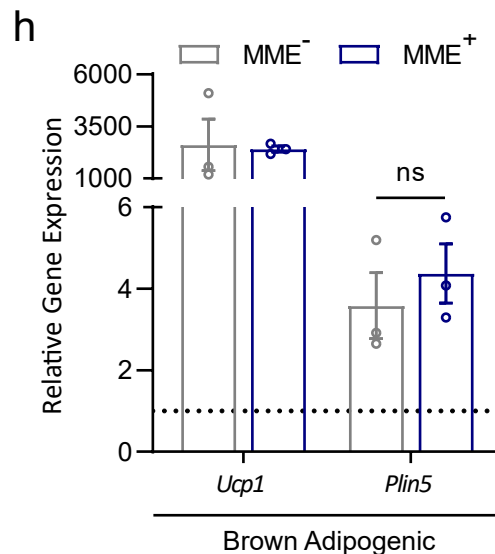
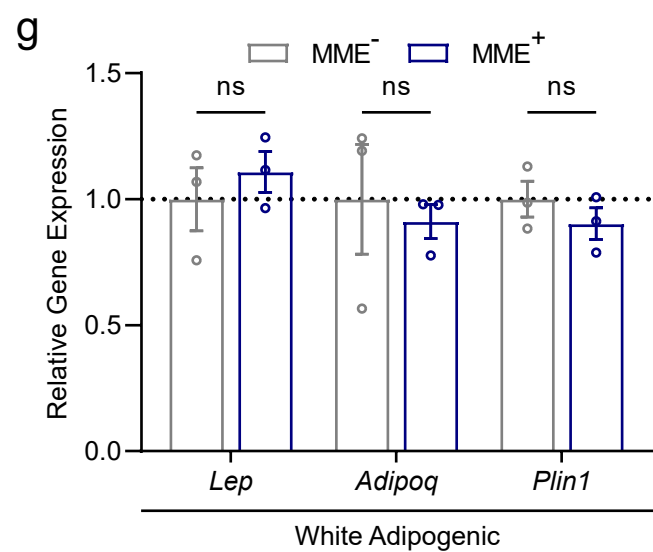
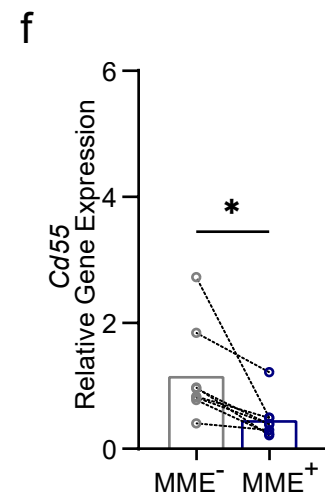
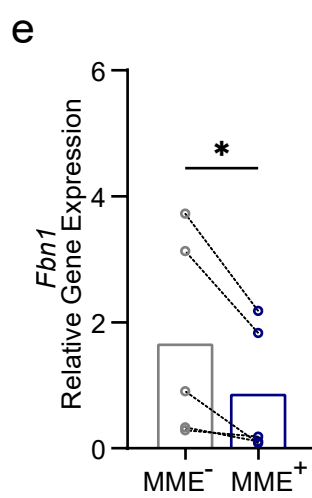
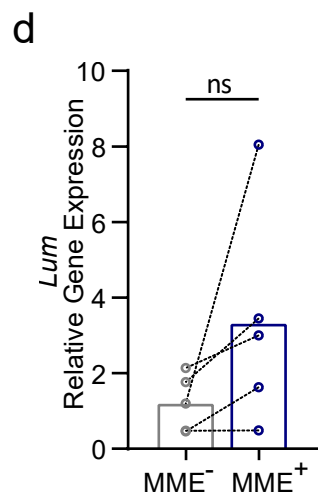
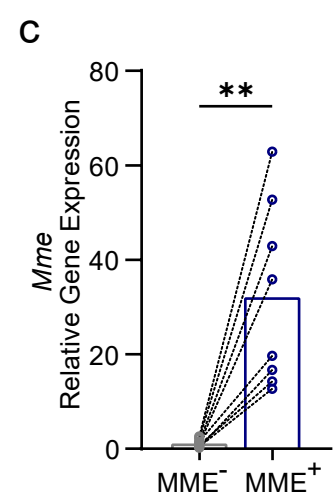
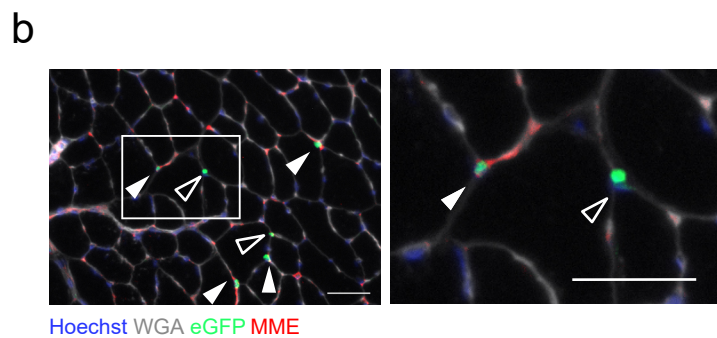
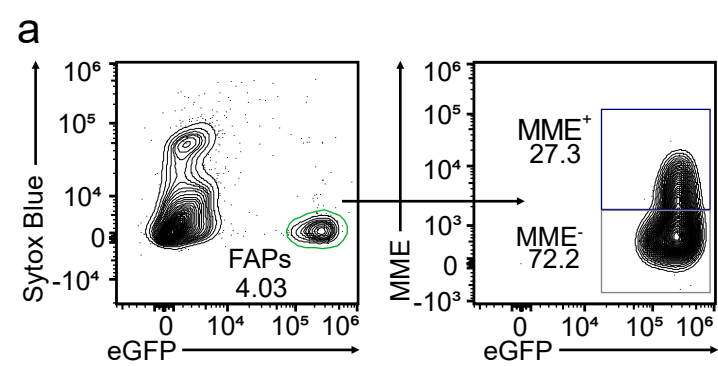
Supplementary Fig. 3: scRNA-seq analysis from human muscle identifies main FAPs and mononuclear cell populations. Related to Fig. 2.

a Heatmap with Z-scores showing top enriched marker genes per cell type. Each column represents a gene and each row a cell. Rows are ordered and color-coded based on the cluster of origin. **b** Violin plots of logcounts values from marker genes naming each FAP subpopulation, color-coded by the identified subpopulations. **c** TSNE plot of main cell populations identified in human muscle by the automatic mapping to Rubenstein et al. dataset, color-coded by the identified populations. **d** Same as **c** but mapping to De Micheli et al. dataset. **e** Heatmap representing Pearson's correlation values of human FAP markers from the snRNA-seq dataset in the human FAPs scRNA-seq dataset. Horizontal color-coded separation indicates the set of marker genes from each FAP subpopulation. **f** Heatmap representing Pearson's correlation values of human FAP markers from the scRNA-seq dataset in the human FAPs snRNA-seq dataset. Horizontal color-coded separation indicates the set of marker genes from each FAP subpopulation.



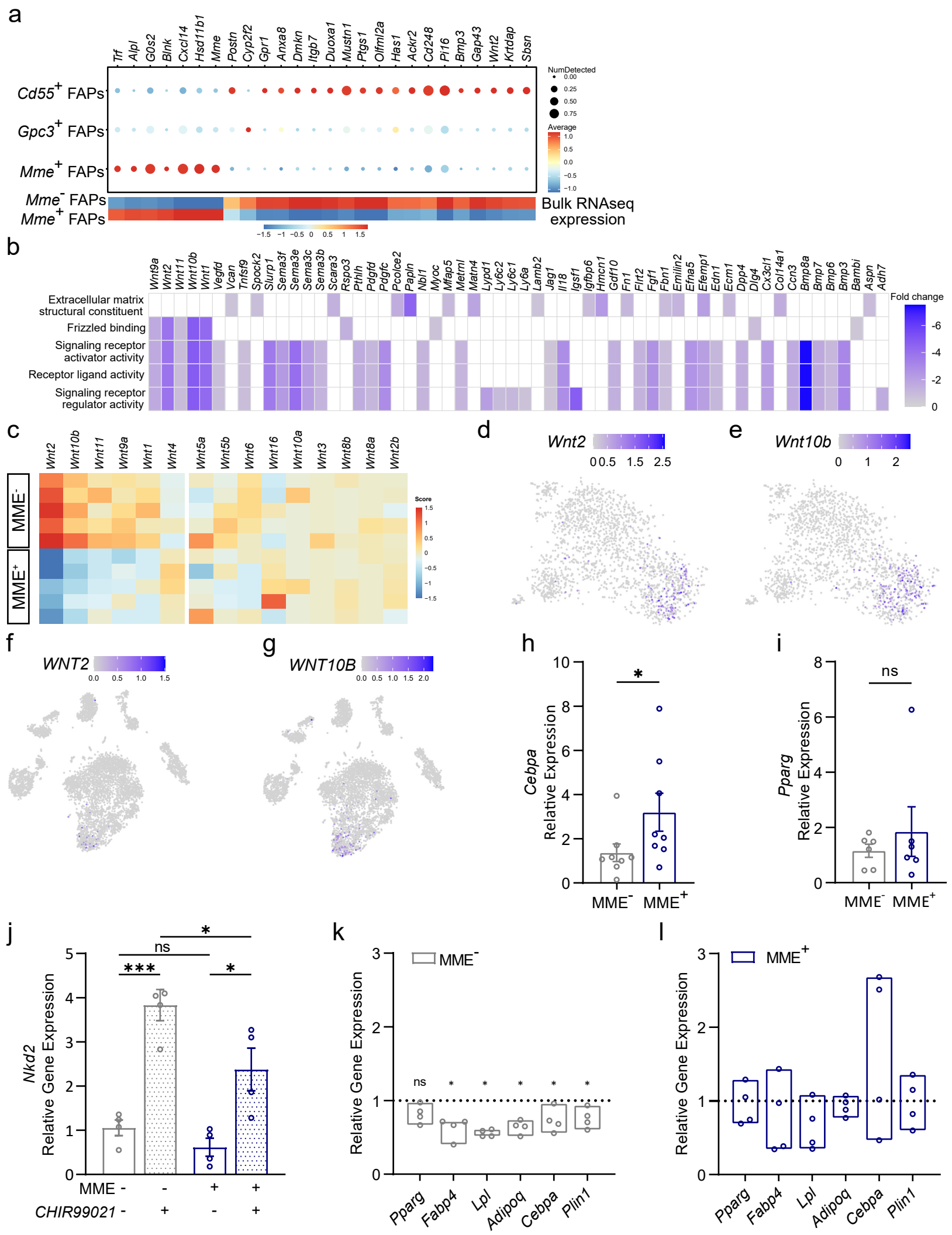
Supplementary Fig. 4: *MME*⁺ and *GPC3*⁺ muscle FAPs are not found in adipose tissue. Related to Fig. 3.

a TSNEplots of FAPs marker genes identified in the integration of human muscle FAPs and human adipose progenitor cells from Merrick et al., color-coded by logcounts values. **b** Log₂-FoldChange of adipogenic genes (from Hallmark MsigDB) found to be upregulated in *MME*⁺ or *ICAM1*⁺ cells. Each log₂-FoldChange is relative to *CD55*⁺ or *DPP4*⁺ for *MME*⁺ and *ICAM1*⁺ cells respectively. **c** Same as **a** but UMAP plots showing FAPs marker genes identified in the integration of human abdomen muscle and human subcutaneous adipose tissue datasets from Tabula Sapiens, color-coded by logcounts values. **d** Dot plot of centered logcounts values from identified marker genes in each mouse FAP subpopulations. Color and size of the dots indicate the centered logcount value and the proportion of cells that express the gene, respectively. **e** Same as **a** but showing FAPs marker genes identified in the integration of mouse muscle FAPs and adult mouse (10 weeks) adipose progenitor cells from Merrick et al., color-coded by logcounts values. **f** Scatter plots showing logcounts values (Y-axis) of genes along the pseudotime trajectory (X-axis) from the integration of basal mouse muscle FAPs with FAPs 5 days after glycerol injection from Xu *et al.*, color-coded by the different FAPs populations. Dashed line indicates the smoothed conditional mean of logcount values along the pseudotime axis. **g** Dot plot of centered logcounts values from *Cebpa* in each mouse FAP subpopulations from the integration between basal mouse muscle FAPs and FAPs 5 days after glycerol injection from Xu *et al.* Color and size of the dots indicate the centered logcount value and the proportion of cells that express the gene.



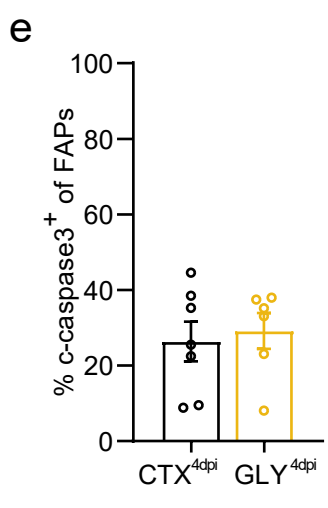
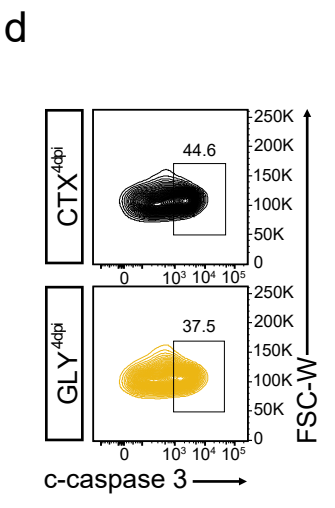
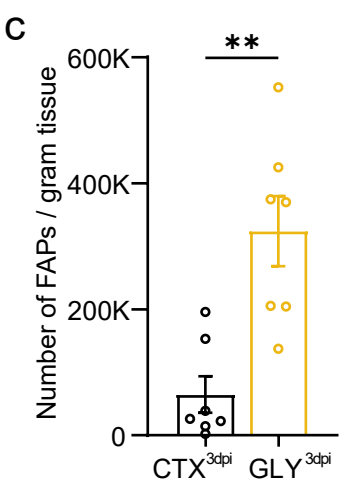
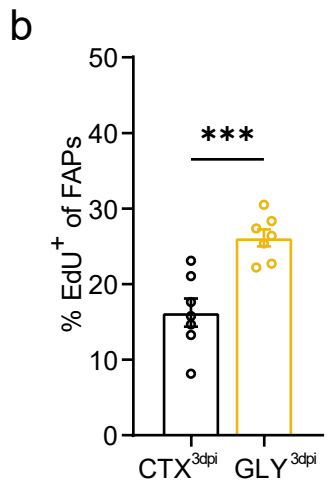
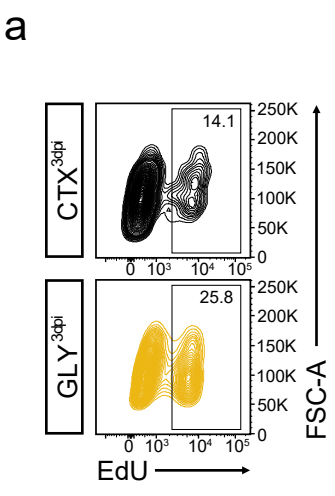
Supplementary Fig 5: MME⁺ are a highly adipogenic fraction of PDGFR α ⁺ FAPs. Related to Fig. 4.

a Representative FACS plot outlining the sorting strategy to isolate PDGFR α -eGFP⁺MME^{-/+} FAPs. **b** Immunofluorescent staining of MME on muscle sections from PDGFR α -eGFP mice demonstrating the presence of both MME⁺ (full white arrows) and MME⁻ (white outlined arrows) FAPs. (green, eGFP⁺; grey, WGA⁺, red, MME⁺, blue, Hoechst⁺ Scale Bar: 50um). **c** Relative expression of *Mme* in freshly sorted MME^{-/+} FAPs. **d** Relative expression of *Lum* in freshly sorted MME^{-/+} FAPs. **e** Relative expression of *Fbn1* in freshly sorted MME^{-/+} FAPs. **f** Relative expression of *Cd55* in freshly sorted MME^{-/+} FAPs. **g** Relative gene expression of *Lep*, *Adipoq*, and *Plin1* in MME^{-/+} FAP derived adipocytes differentiated in full white adipogenic medium. Related to Fig. 4e. **h** Relative gene expression of *Ucp1* and *Plin5* in MME^{-/+} FAPs derived brown adipocytes. **i** Relative gene expression of *Acta2* in MME^{-/+} FAP derived fibroblasts differentiated in fibrogenic medium. **j** Relative gene expression of *Bglap2* and *Runx2* in MME^{-/+} FAP derived osteocytes. **k** Relative gene expression of *Sox9* and *Comp* in MME^{-/+} FAP derived chondrocytes. **l** Quantification of adipocytes 14 days after transplantation of mT⁺MME^{-/+} FAPs into glycerol-injected tibialis anterior muscle at the mid belly of the muscle. Related to Fig. 4m. The expression of all genes in **g-k** was normalized to their expression in MME⁻ FAPs derived white adipocytes (dotted line). Each dot represents a single mouse. Bar graphs represent the mean in **c-f** and represent the mean \pm SEM in **g-l**. Student's t-test (two tailed, paired, *p<0.05, **p<0.01, ns>0.05) was used in **c-f**, **i**, and **l**. Two-way ANOVA with Šidák's multiple comparison test (ns>0.05) was used in **g**, **h**, **j**, and **k**.



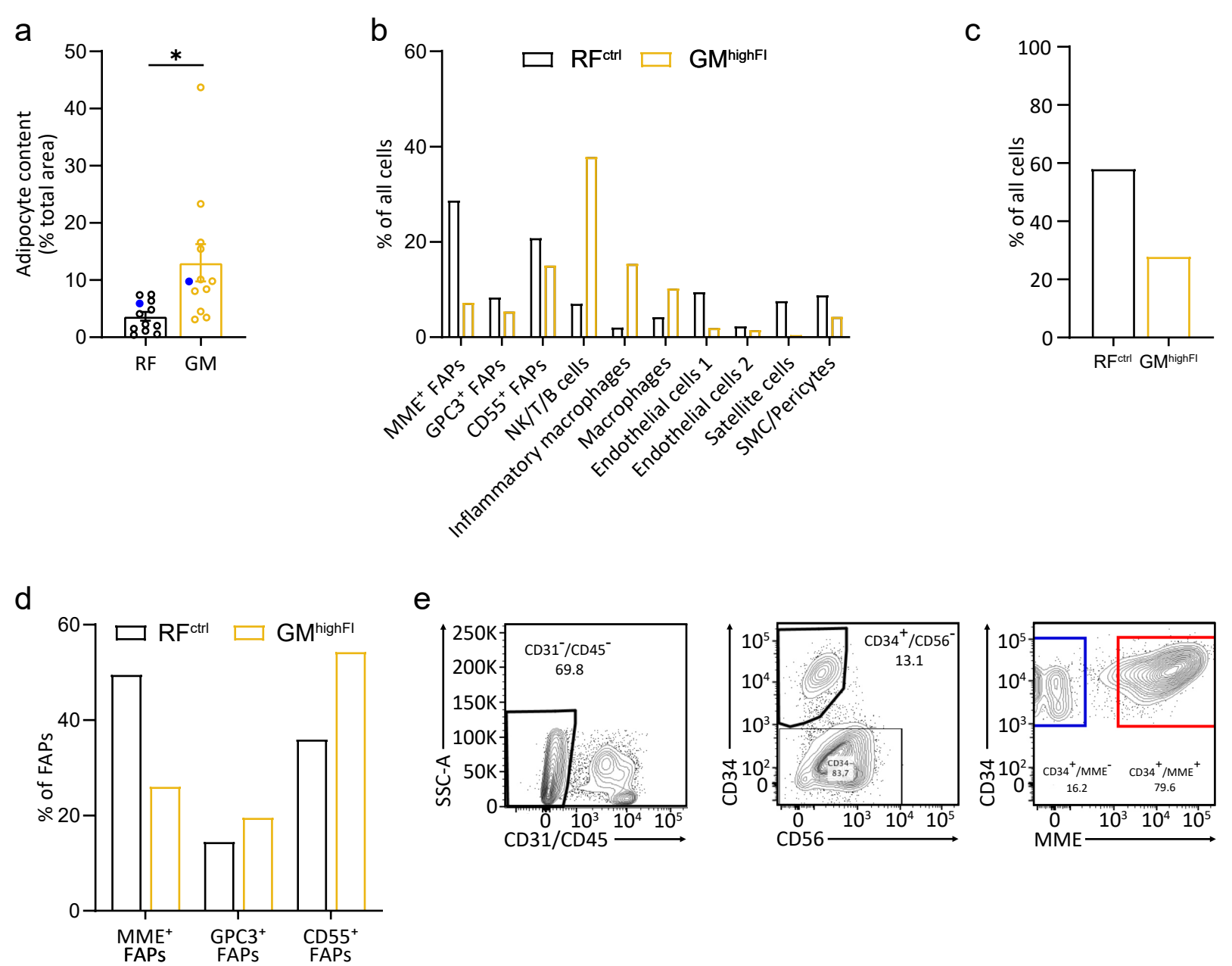
Supplementary Fig 6: MME⁺ FAPs are characterized by reduced WNT signaling and refractory to WNT-mediated inhibition of adipogenesis. Related to Fig. 5.

a Dot plot from mouse scRNA-seq of high variable genes identified in the mouse bulk RNA-seq (see Fig. 5b). Each row represents a FAP subpopulation and each column a gene. Color and size of the dots indicate the level of expression and the percentage of cells that express the gene respectively. The simplified heatmap below displays Z-scores averaged per cell type from the bulk RNA-seq data set (see Fig. 5b). **b** Heatmap showing the top 5 significantly downregulated *molecular function* processes (adjusted p-value < 0.05) in a Gene Ontology (GO) enrichment analysis in MME⁺ FAPs from the mouse bulk RNA-seq. Color-coded by the log₂-FoldChange respective to MME⁻ FAPs. Each row represents a *molecular function* process and each column a gene which is significantly regulated within our dataset that belongs to that *molecular function* process (log₂-FoldChange > 0.5 and FDR < 0.05). Blank tiles indicate that a gene does not belong to that *molecular function* process. **c** Heatmap with Z scores showing WNT ligands in MME^{-/+} FAPs. Genes displayed left of the line break are differentially expressed (log₂- FoldChange > 0.5 and FDR < 0.05). **d** TSNE plot of *Wnt2* color-coded by logcounts values in mouse FAPs. **e** TSNE plot of *Wnt10b* color-coded by logcounts values in mouse FAPs. **f** TSNE plot of *WNT2* color-coded by logcounts values in FAPs in human muscle. **g** TSNE plot of *WNT10B* color-coded by logcounts values in FAPs in human muscle. **h-i** Relative expression of *Cebpa* (**h**) and *Pparg* (**i**) in freshly sorted MME^{-/+} FAPs. **j** Relative gene expression of *Nkd2* in MME⁻ FAP derived and MME⁺ FAP derived adipocytes after differentiation in low insulin medium with (CHIR99021) or without (Vehicle, DMSO) GSK-3 inhibition. **k** Gene expression of *Pparg*, *Fabp4*, *Lpl*, *Adipoq*, *Cebpa*, and *Plin1* in MME⁻ FAP derived adipocytes after differentiation in low insulin medium with (CHIR99021) GSK-3 inhibition as compared to their expression in MME⁻ FAP-derived adipocytes without (Vehicle, DMSO) GSK-3 inhibition (dotted line). **l** Gene expression of *Pparg*, *Fabp4*, *Lpl*, *Adipoq*, *Cebpa*, and *Plin1* in MME⁺ FAP derived adipocytes after differentiation in low insulin medium with (CHIR99021) GSK-3 inhibition as compared to their expression in MME⁺ FAP-derived adipocytes without (Vehicle, DMSO) GSK-3 inhibition (dotted line). Each dot represents a single mouse. Bar graphs represent the mean ± SEM. Student's t-test (two tailed, paired, ns>0.05, *p<0.05) was used in **h** and **i**.



Supplementary Fig. 7: MME⁺ FAPS are more prone to apoptosis after injury and deplete upon glycerol injury. Related to Fig. 6.

a-b Representative flow cytometric analysis (**a**) and quantification (**b**) of EdU⁺ FAPs as a percentage of all FAPs at 3 dpi in cardiotoxin (CTX) injected or glycerol (GLY) injected muscle. **c** Quantification of the number of FAPs in CTX and GLY injected muscle at 3 dpi. **d-e** Representative flow cytometric analysis (**d**) and quantification (**e**) of c-caspase3⁺ FAPs as a percentage of eGFP⁺ FAPs at 4 dpi in CTX injected and GLY injected muscle. Each dot represents a single mouse. Bar graphs represent the mean \pm SEM. Student's t-test (two tailed, unpaired, *p < 0.05, **p < 0.01, ***p < 0.001) was used in **b,c**, and **e**.



Supplementary Fig. 8: Human MME⁺ FAPs are highly adipogenic and are exhausted in fatty infiltrated human muscle. Related to Fig. 7.

a Relative adipocyte area as quantified by adipocyte area relative to total biopsy area on H&E-stained sections. Biopsies from the patient used for scRNA-seq are marked with blue dots. **b** Bar plots showing the percentage of the main cell types within each dataset from either control muscle (RF^{ctrl}) or highly fatty infiltrated (GM^{highFI}) human muscle. **c** Bar plots showing the percentage of the combined FAP populations within each dataset from either control muscle (RF^{ctrl}) or highly fatty infiltrated (GM^{highFI}) human muscle. **d** Bar plots showing the percentage of FAPs relative to the total FAP fraction within each dataset from either control muscle (RF^{ctrl}) or highly fatty infiltrated (GM^{highFI}) human muscle. **e** Representative FACs plots outlining the sorting strategy to isolate MME^{-/+} human FAPs. Each dot represents an individual patient. Bar graphs represent mean \pm SEM in **a**. Student's t test (two tailed, paired, *p<0.05) was used in **a**. Bar plots in **b**, **c**, and **d** represent the percentage of all cells or the percentage of FAPs in the RF^{ctrl} and GM^{highFI} single cell datasets.

Supplemental table

Supplementary Table 1. Primers used for RT-PCR. Related to Fig. 5 and Supplementary Fig. 5,6.

Gene Name	Forward Primer	Reverse Primer
<i>Rna18s5</i>	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA
<i>Wnt2</i>	ACAACAGAGCTGGAAGGAAGGCTGT	AGTGAAGCCAGTGCCATCCTGG
<i>Wnt10b</i>	GCTGACTGACTCGCCACCG	AAGCACACGGTGTTGGCCGT
<i>Mme</i>	GGGAGGCTTTATGTGGAAGC	CCGGATTTGTGCAATCAAGT
<i>Lum</i>	CCCACCCTGACAGAGTTCAC	ATTGGCCACTGACACTACCG
<i>Fbn1</i>	TGAGAGTCCGAGCCGCTAGT	ACAGCTTTCTTCTCCAGGGAC
<i>Cd55</i>	GGAGAGCCTAACACAGGTGG	TCTTCGTA ACTCTTCGTTGGCT
<i>Lep</i>	TCAAGACCATTGTCACCAGG	TGAAGCCCAGGAATGAAGTC
<i>Adipoq</i>	TGTTCCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCTT
<i>Ucp1</i>	GGACGACCCCTAATCTAATGAG	GCAAAACCCGGCAACAAG
<i>Plin5</i>	CCATCTCGCCTATGAACACTCTT	CAGCTGGGCCAGCATCTC
<i>Plin1</i>	CTGTGTGCAATGCCTATGAGA	CTGGAGGGTATTGAAGAGCCG
<i>Pparg</i>	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
<i>Acta2</i>	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
<i>Bglap2</i>	CTGACCTCACAGATCCCAAGC	TGGTCTGATAGCTCGTCACAAG
<i>Runx2</i>	CCTGAACTCTGCACCAAGTCCT	TCATCTGGCTCAGATAGGAGGG
<i>Sox9</i>	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT
<i>Comp</i>	ACTGCCTGCGTTCTAGTGC	CGCCGCATTAGTCTCCTGAA
<i>Cebpa</i>	GCCGAGATAAAGCCAAACAAC	GACCCGAAACCATCCTCTG
<i>Nkd2</i>	GCTCTCAGCTCGGGATTACAC	CCCCTTCGGGACTCTCTCTC
<i>Axin2</i>	GGCTGCGCTTTGATAAGGTC	GCTCATGTGAGCCTCCTCTC