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Supplemental information

A Tead1-Apelin axis directs paracrine communication from myogenic to endothelial cells in skeletal muscle

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SUPPLEMENTARY MATERIALS

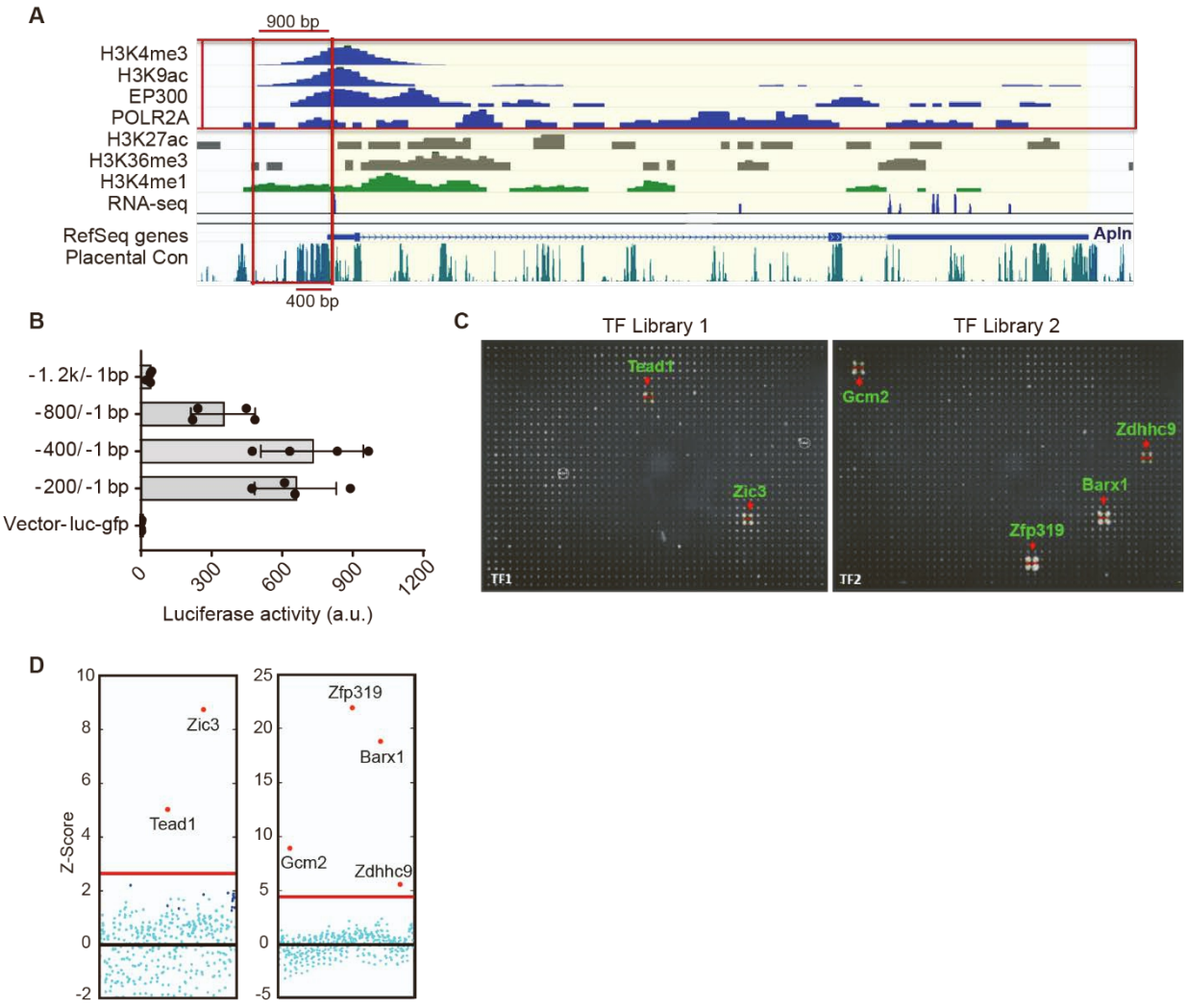
Supplementary Figure 1.
Characterization of the *Apln* promoter and identification of transcription factors in Yeast 1 Hybrid screen, Related to Figure 1.

Supplementary Figure 2.
Effects of Zfp319 and Zdhhc9 on *Apln* expression in C2C12 cells, Related to Figure 1.

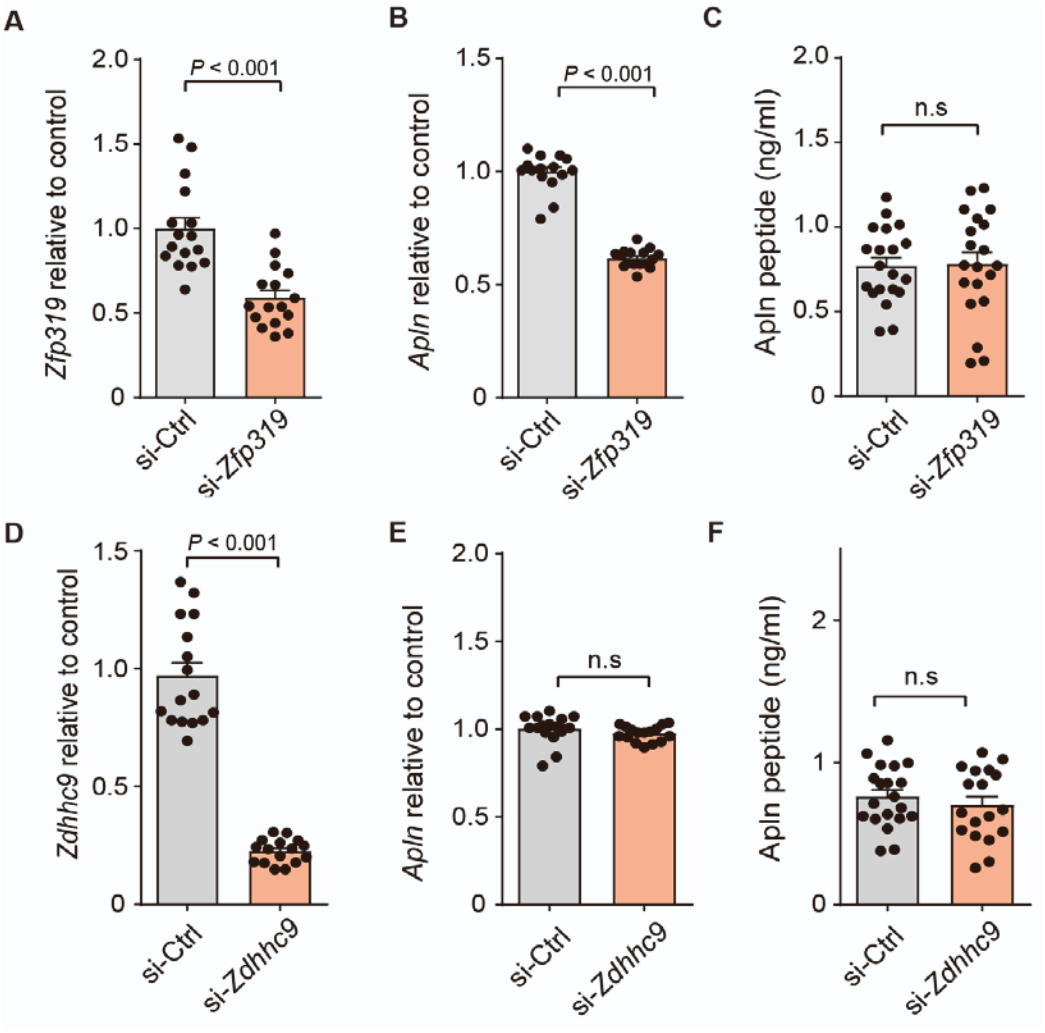
Supplementary Figure 3.
Expression profiles of single-cell RNA-sequencing clusters, Related to Figure 3.

Supplementary Figure 4.
Expression profiles of six transcription factors in Y1H from single-cell and nucleus RNA-sequencing datasets, Related to Figure 3.

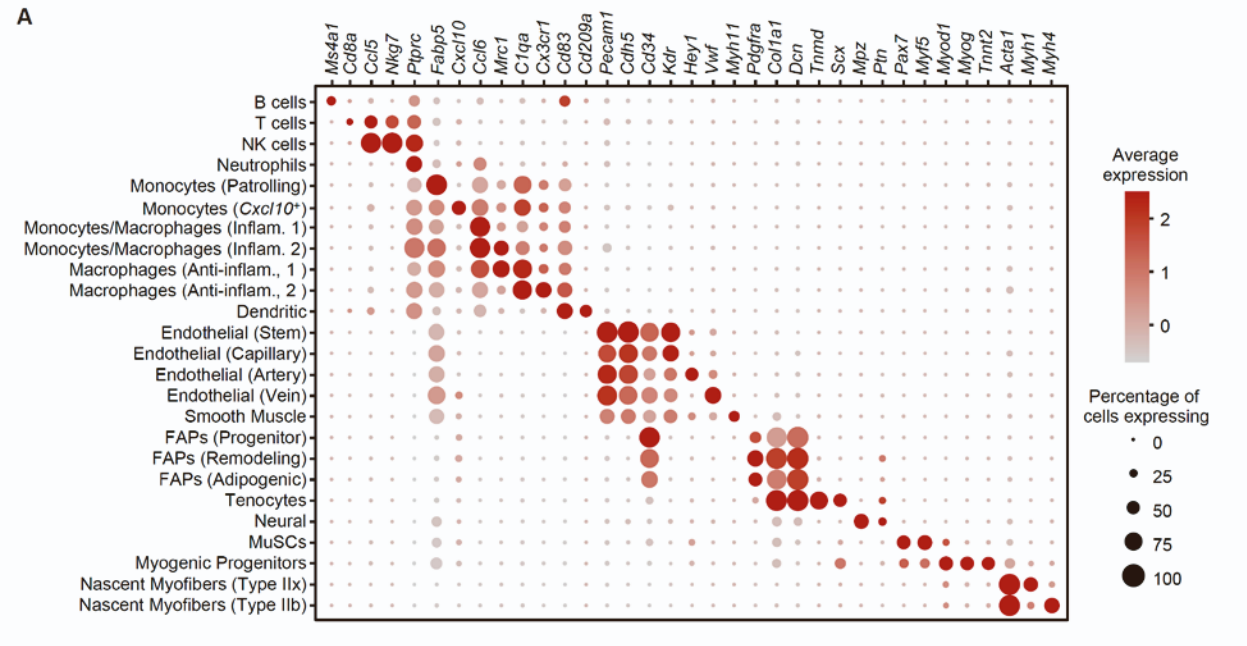
Supplementary Table 1. Primers used for Chromatin Immunoprecipitation (ChIP) qPCR Assay, Related to Figure 1.



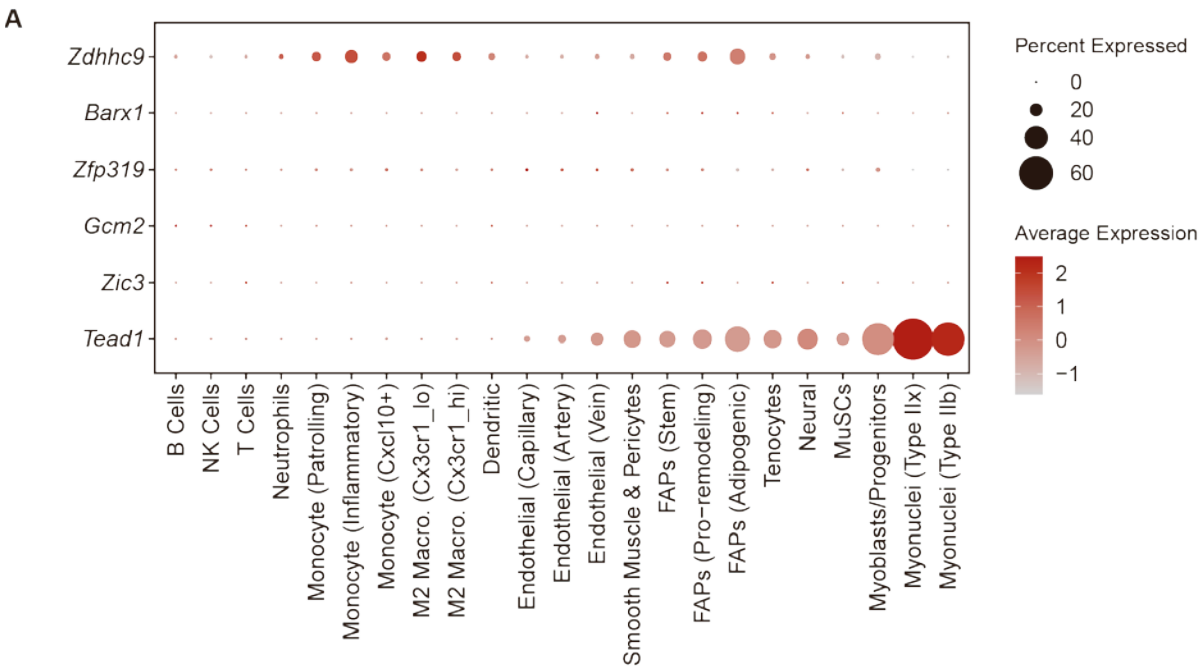
Supplementary Figure 1. Characterization of the *Apln* promoter and identification of transcription factors in Yeast 1 Hybrid screen, Related to Figure 1. (A) Epigenomic annotation near the TSS of apelin precursor gene (*Apln*) in mouse ES-Bruce4 cells from the WashU Epigenome Browser. Tag densities (blue) of proximal promoter markers H3K4me3, H3K9ac, EP300, and POLR2A by ChIP-seq are highlighted (top). Placental Con represents the conservation score between 20 mammalian species available from WashU Epigenome Browser (Miller et al., 2007) **(B)** Nano luciferase activity of four putative *Apln* promoter regions and no-promoter vector transfected into C2C12 cells, normalized by co-transfected firefly luciferase activity. Mean ± s.e.m. of n = 4 cell culture replicates per group. **(C)** Y1H screen growth cluster results using -400/-1bp fragment of *Apln* promoter mated with two TF libraries. **(D)** Z-score-normalized Y1H spot intensities for all 745 TF-promoter interactions using -400/-1bp promoter fragments.



Supplementary Figure 2. Effects of Zfp319 and Zdhhc9 on *Apln* expression in C2C12 cells, Related to Figure 1. (A-B) *Zfp319* (**A**) and *Apln* (**B**) mRNA expression measured by RT-qPCR in C2C12 myoblasts transfected with scrambled control or *Zfp319* targeted siRNA for 3 d,. n = 16 cell culture replicates per condition. **(C)** Apln peptide concentration in culture medium measured by enzyme immunoassay (EIA) after 3 d culture from the same experiment. n = 20 cell culture supernatants per condition. **(D-E)** *Zdhhc9* (**D**) and *Apln* (**E**) mRNA expression in C2C12 myoblasts transfected with scrambled control or *Zdhhc9* targeted siRNA for 3 d, n = 16 cell culture replicates per condition. **(F)** Apln peptide concentration in culture medium measured by enzyme immunoassay (EIA) after 3 d culture from the same experiment. n = 20 from one experiment. For all panels, data are presented as mean ± s.e.m., and *P* values are reported from two-tailed, unpaired t-tests between conditions.



Supplementary Figure 3. Expression profiles of single-cell RNA-sequencing clusters, Related to Figure 3. (A) Dot plots representing marker gene expression used to characterize cell clusters in the single-cell RNA-sequencing muscle regeneration data set from **Figure 3C-E**. Cell clustering was performed using SNN and annotations were manually determined based on these gene expression patterns. For each gene/cluster combination, the average expression level is shown in red color and the fraction of cells with non-zero expression is shown as the dot size. Some clusters shown here are not plotted in **Figure 3E** if not consistently observed at different time-points in the dataset.



Supplementary Figure 4. Expression profiles of six transcription factors in Y1H from single-cell and nucleus RNA-sequencing datasets, Related to Figure 3. (A) Dot plots representing cell-specific TF gene expression from integrated 102 publically-available single-cell or nucleus RNA-sequencing datasets by harmony batch-correction (McKellar et al., 2020).

Gene	Forward Primer	Reverse Primer	Genomic Coordinates
Ankrd	GAGGGGAGGACAAGCTAACC	CGATGTGATCACCACCAAAG	Chr10:92681001–92681083
Ctgf	GCCAATGAGCTGAATGGAGT	CAATCCGGTGTGAGTTGATG	Chr6:132272566-132272653
Apln	CTCTCCCTCTTCCTGCCTC	TTTTGTAGCTTGTGGTTTGGC	Chr X:48034975 - 48034875
Neg- control	ACCAACACTCTTCCCTCAGC	TTATTTTGGTTCAGGTGGTTGA	Chr10:60902566-60902665

Supplementary Table 1. Primers used for Chromatin Immunoprecipitation (ChIP) qPCR Assay, Related to Figure 1. Forward and reverse primers to detect *Ankrd1, Ctgf, Apln, Neg* promoters in ChIP-qPCR detection.