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Spatial heterogeneity and interfacial signaling hub function of CPTCs across the ventral body wall continuum

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Abstract

Cd34+ Pdgfra+ Telocytes (CPTCs) are interstitial stromal cells implicated in long-distance intercellular communication, yet their role in bridging the distinct anatomical layers of the abdominal wall remains undefined. In this study, we combined single-cell RNA sequencing with functional assays to map the CPTC landscape across the skin, superficial fascia (SF), and deep fascia (DF), validating our findings through immunofluorescence and transmission electron microscopy. We identified a hierarchical stromal organization where CPTCs display layer-specific transcription factor networks tailored to local niches, co-existing with a conserved adhesion program. Notably, SF-CPTCs were characterized as an interfacial signaling hub with superior developmental plasticity. Ligand-receptor interaction analysis predicted that SF-CPTCs mediate cross-layer communication via the Wnt and Nectin pathways. We validated this mechanism using a sequential co-culture model, showing that SF-CPTCs integrate upstream signals to regulate target gene expression in adjacent layers. Primed SF-CPTCs significantly upregulated Lrp6 and Myc in deep fascia-resident myosatellite cells and induced Nectin2 and Ctnnb1 in cutaneous keratinocytes, whereas unprimed cells did not. Collectively, our data demonstrate that SF-CPTCs operate as a dynamic signaling gateway, integrating the skin and muscle compartments into a unified functional unit.

Introduction

The fascial system constitutes a ubiquitous, organism-wide connective tissue network that integrates the body into a functional whole[1,2]. Embedded within this interstitial matrix are Cd34 + Pdgfra+ Telocytes (CPTCs), a distinctive population of stromal cells characterized by extremely long, thin prolongations known as telopodes[3]. CPTCs have been identified across diverse organs, forming intricate three-dimensional networks through homo- and hetero-cellular contacts[4,5]. Structurally, these cells are uniquely positioned to integrate mechanical and chemical signals[6], leading to the hypothesis that TCs function as essential interstitial signaling hubs[7,8]. While their morphology suggests a role in physical connectivity, the molecular mechanisms—specifically the cell-cell communication and junctional architectures—that enable TCs to coordinate tissue homeostasis remain to be fully elucidated[9].

Anatomically, the abdominal wall represents a complex, stratified continuum rather than a series of isolated layers[10]. It transitions from the skin, which functions as the primary immune and physical barrier[11], to the deep fascia, a dense, collagen-rich sheath responsible for high-tension force transmission and muscular containment[12]. Bridging these distinct compartments is the superficial fascia, a loose, adipose-rich connective tissue layer[13]. This intermediate zone does not merely fill space; it acts as a critical "soft interface" that accommodates mechanical stimulus and houses dense neurovascular networks[14]. This structural continuity implies that physiological signals—whether mechanical forces or inflammatory mediators—must propagate across these boundaries to orchestrate a unified tissue response[15]. However, the cellular machinery that maintains this trans-tissue connectivity while respecting the specialized function of each layer is poorly understood[16].

Effective coordination across these layers requires stromal cells to communicate with highly diverse neighbors—ranging from keratinocytes in the skin to myosatellite cells in the muscle[17]. While Telocytes are known to maintain physical contacts through gap and adherens junctions[18], it remains unclear how they adapt these connections to such distinct microenvironments. Specifically, do TCs function as a uniform stromal scaffold, or do they differentiate into specialized subsets to match their local partners[19]? Furthermore, the identity of the specific stromal population responsible for bridging the superficial and deep layers—physically connecting the skin to the muscle fascia—has not yet been defined[20].

Here, we performed single-cell RNA sequencing (scRNA-seq) to delineate the transcriptional landscape and intercellular communication networks of CPTCs along the rat abdominal midline. We identify a significant layer-dependent heterogeneity among CPTCs, driven by distinct transcription factor programs that tailor their signaling output to local cell types. Despite this functional divergence, we reveal a universally conserved activation of adhesion-related pathways across all layers, underscoring the fundamental importance of cellular connectivity in CPTC function. Importantly, trajectory and communication analyses position the superficial fascia CPTCs as a high-plasticity interfacial hub that orchestrates cross-layer signal transmission. Our findings provide a high-resolution map of the stromal communication architecture, proposing that CPTCs serve as both local signaling centers and a continuous bridge integrating the skin-fascia system.

Materials and Methods

Experimental Animals and Ethical Approval

Female Sprague-Dawley (SD) rats (8 weeks old, weighing approximately 250 g) were procured from Beijing Vital River Laboratory Animal Technology Co. Ltd. Rats were housed in ventilated cages at 25°C with a natural light-dark cycle and ad libitum access to food and water. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University (ID NJAU No.20231120173) and conducted in strict compliance with the ARRIVE guidelines.

Tissue Collection and Layer-Specific Dissection

To characterize the spatial heterogeneity of the abdominal midline, subcutaneous tissues were harvested and precisely micro-dissected into three distinct anatomical layers under a stereomicroscope: (1) Skin, comprising the epidermal and dermal layers; (2) Superficial Fascia (SF), the loose, adipose-rich connective tissue layer; and (3) Deep Fascia (DF), the dense collagenous sheath overlying the abdominal muscles. Samples were collected from the abdominal midline region. For single-cell RNA sequencing, tissues were immediately processed into single-cell suspensions. For histological and ultrastructural analyses, tissues were fixed in 4% paraformaldehyde (PFA) or 2.5% glutaraldehyde, respectively.

Single-Cell Suspension Preparation

Fresh tissue samples were minced into small fragments on ice and digested with a dissociation solution containing collagenase type I and type IV dissolved in DMEM/F12 medium containing 10% Fetal Bovine Serum (FBS). Digestion was performed at 37°C with shaking for 40 min. The resulting cell suspensions were sequentially filtered through 100-μm and 40-μm nylon strainers to remove debris. Red blood cells were lysed using RBC Lysis Solution, and dead cells were removed using a Dead Cell Removal kit (Miltenyi Biotec) to ensure cell viability > 95%.

10x Genomics Library Construction and Sequencing

Single-cell libraries were constructed using the Chromium Single Cell 3' Reagent Kit v3.1 (10x Genomics) according to the manufacturer's protocol. Single cells were encapsulated into Gel Beads-in-Emulsion (GEMs), followed by reverse transcription, cDNA amplification, and library construction. Libraries were quantified using an Agilent 2100 Bioanalyzer and sequenced on an Illumina NovaSeq 6000 platform with paired-end 150 bp reads.

scRNA-seq Data Processing and Analysis

scRNA sequencing data were processed using Cell Ranger (v7.2.0) and aligned to the rat genome (mRatBN7.2). Downstream analysis was performed using Seurat (v4.3.0) in R.

Clustering and Annotation

Cells with high mitochondrial content (> 10%) or low gene counts were filtered. Data were normalized, and Harmony was employed to integrate datasets from different layers and correct for batch effects. Dimensionality reduction was performed using UMAP. Cell types were annotated based on canonical marker genes.

Regulatory Network Analysis

Transcription factor regulons were identified using pySCENIC, and Regulon Specificity Scores (RSS) were calculated to identify layer-specific regulators.

Trajectory and Velocity Analysis

Developmental trajectories were reconstructed using Monocle 3 to order cells along a pseudotime axis. Transcriptional dynamics and directionality were further inferred using RNA Velocity (scVelo). Developmental potential was scored using CytoTRACE.

Cell-Cell Communication

Intercellular communication networks were inferred using CellCall to calculate communication scores. NicheNet was employed to predict ligand-receptor links and downstream target gene activation.

Histology and Immunofluorescence (IF)

Tissues fixed in 4% PFA were embedded in paraffin and sectioned (5 μm). Sections were stained with Hematoxylin and Eosin (H&E) and Masson's trichrome to visualize tissue architecture. For immunofluorescence, sections underwent heat-induced epitope retrieval and were incubated overnight at 4°C with primary antibodies. After washing, sections were incubated with fluorophore-conjugated secondary antibodies and counterstained with DAPI. Images were captured using a confocal microscope (Carl Zeiss, LSM900).

Transmission Electron Microscopy (TEM)

Tissues fixed in 2.5% glutaraldehyde were post-fixed with 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in epoxy resin. Ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate and examined using a Hitachi HT7800 transmission electron microscope operated at 80 kV.

Extraction and culture of CPTCs

TCs were extracted from the rat AMF. Tissue samples were collected and processed following established protocols[21]. The AMF was dissected and digested with a dissociation solution, composed of collagenase type 4 and type 1, dissolved in DMEM/F12 medium containing 10% Fetal Bovine Serum (FBS). The resulting cell suspension was first filtered through a 100 μm mesh and then through a 40 μm mesh to remove tissue debris. To stop the enzymatic reaction, the suspension was washed with PBS. The resulting cell suspension was plated in T75 flasks for 2 hours. Then, slowly adherent cells left on the flask surface were transferred to new culture flasks and cultured.

Isolated CPTCs were cultured in DMEM/F12 medium, supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were incubated in a 37°C incubator with 5% CO_2 and 95% humidity. Immunofluorescence staining was performed to confirm the purity of the CPTC population. Cell slides were positioned in the wells of 12-well plates before seeding. Samples were harvested at 72 hours by adding 1 mL of 4% PFA solution to each well and then fixed at 4 ℃ for 24 hours before any assays were conducted.

Sequential Co-culture Assay

SF-CPTCs were seeded in the upper chamber of Transwell inserts (0.4 μm pore size). In the priming phase, SF-CPTCs were treated with recombinant Wnt2 or Nectin protein for 12 hours. The inserts were then washed three times with PBS to remove free ligands. In the effector phase, the primed SF-CPTC

inserts were transferred to wells containing target cells (L6 myosatellite cells or HaCaT keratinocytes) and co-cultured for 24 hours.

RNA Extraction and Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from target cells (L6 or HaCaT) using TRIzol reagent and reverse-transcribed into cDNA using a Reverse Transcription Kit. qPCR was performed using SYBR Green Master Mix on a Bio-Rad CFX Connect system. Relative gene expression was calculated using the $2 - \Delta\Delta Ct$ method, with GAPDH serving as the internal control.

Results

Intrinsic regulatory programs underpin the spatial heterogeneity of CPTCs

To achieve a comprehensive cellular characterization of the abdominal midline, we performed single-cell RNA sequencing (scRNA-seq) using the 10X Chromium system on the skin, superficial fascia (SF), and deep fascia (DF) of normal rats' abdominal midline (Figure 1A1-A3). Unsupervised clustering identified diverse cell subsets, which were subsequently annotated according to specifically expressed or highly enriched marker genes. Among these populations, CPTCs (highlighted by the dotted box) were selected for further analysis to explore their heterogeneity across different anatomical layers. Dimensionality reduction revealed a striking layer-dependent segregation of CPTCs (Figure 1B-C). Notably, these distinct spatial identities persisted after Harmony batch correction, confirming they arise from intrinsic transcriptional differences rather than technical artifacts.

Initial pathway profiling via ClusterGVis provided a broad molecular overview (Figure 1D). To uncover the upstream drivers governing these molecular profiles, we focused on the underlying transcription factor (TF) regulatory networks. We identified high-confidence candidate TFs by intersecting pySCENIC-inferred regulon specificity scores (RSS) (Figure 1E) with layer-specific expression (Figure 1F). Subsequently, to validate the functional output of these drivers, we intersected their downstream targets (Table S1) with layer-enriched marker signatures. This strategy isolated a set of genuine, TF-regulated signature genes that exhibited distinct spatial segregation on UMAP (Figure 1G), while Venn analysis confirmed their strict exclusivity to each layer (Figure 1H).

GSEA confirmed that CPTCs execute layer-specific biological functions (Figure 1I). Skin CPTCs were predominantly enriched in pathways associated with epidermal homeostasis, such as keratinization and epithelium development. Superficial fascia CPTCs exhibited a dynamic transcriptional profile, highlighted by the regulation of locomotion and cellular response to chemical stimuli. In contrast, deep fascia CPTCs were primarily characterized by structural remodeling pathways, notably extracellular matrix organization and skeletal system development. Notably, despite these distinct spatial profiles, all CPTC populations universally maintained a conserved adhesion-related transcriptional program. Specifically, 'cell-cell adhesion' was prominently enriched in skin CPTCs, 'regulation of adhesion' in superficial fascia CPTCs, and 'cell adhesion' in deep fascia CPTCs. This conserved transcriptional axis suggests a

fundamental role for adhesion biology across all CPTC populations, implying that these cells may universally contribute to maintaining tissue structural integrity, facilitating intercellular communication, and transducing mechanical stimulus through adhesion-mediated signaling cascades.

CPTCs mediate layer-specific cell–cell communication as local signaling hubs

The conserved expression of adhesion molecules suggests that CPTCs are structurally primed for extensive intercellular contact. To determine whether this physical connectivity translates into functional signaling, we systematically mapped ligand–receptor interactions between CPTCs and their niche-specific neighbors.

In the cutaneous layer, CPTCs occupied a distinct niche surrounding hair follicles (Figure 2A-B) and were markedly enriched with the exosome marker Tsg101 (Figure 2C), implying a robust capacity for vesicle-mediated secretion. CellCall analysis substantiated this structural potential, revealing a dense communication network between CPTCs and epithelial lineages, particularly keratinocytes and hair follicle stem cells (HFSCs) (Figure 2D). Functional annotation of these interactions identified the enrichment of Hippo and Notch signaling modules-critical regulators of tissue homeostasis-alongside Estrogen signaling involved in cell cycle control (Figure 2E). Further mapping of the ligand-transcription factor axis (Figure 2F) demonstrated that CPTCs drive downstream programs via Rspo1, Fgf7, and Bmp4, thereby actively coordinating epithelial renewal and local immune surveillance.

In the superficial fascia, CPTCs functioned as integral components of the stromal reticular network. Immunofluorescence confirmed their physical coupling via connexin 43 (Cx43) gap junctions (Figure 3A) and cadherin 1 (Cdh1) adheren junctions (Figure 3B). Spatially, CPTCs exhibited intimate proximity to mast cells (Figure 3C) and nerve fibers (Figure 3D), suggesting a neuro-immune-stromal interactome. Ultrastructurally, CPTCs were observed enveloping microvasculature and releasing extracellular vesicles into the perivascular space (Figure 2E-F). Consistent with this spatial architecture, ligand–receptor analysis positioned CPTCs as key signaling senders to myofibroblasts, macrophages, and endothelial cells, predominantly through the Sema (e.g., Sema4g-Plxnb2) and Nectin axes (Figure 3G), underscoring their role in maintaining fascial integrity and vascular stability.

In the deep fascia, CPTCs displayed a distinct perifascicular distribution, maintaining tight associations with the perivascular stroma (Figure 4A). Communication network analysis revealed a preferential signaling axis directed toward myosatellite cells (Figure 4B, Table S2). GSEA enrichment highlighted MAPK signaling, focal adhesion as dominant interaction modules (Figure 4C). Mechanistically, this crosstalk was predicted to be mediated by specific growth factor ligands, including Fgf10, Fgf18, and Wnt5a, which converge on downstream transcription factors such as Foxo1 and Jun (Figure 4D). These data suggest that deep fascia CPTCs constitute a specialized niche component that modulates the activation and fate determination of myogenic progenitors.

Collectively, these findings establish CPTCs as versatile signaling hubs. CPTCs maintain layer-specific communication patterns consistent with their local microenvironments: supporting epithelial

homeostasis in the skin, coordinating neurovascular-immune niches in the superficial fascia, and modulating myogenesis in the deep fascia.

Superficial-fascia CPTCs function as an interfacial hub orchestrating communication across layered fascia-skin systems

Having established that CPTCs in each anatomical layer exhibit distinct communication patterns, we next sought to determine whether these populations are functionally connected across layers.

Histological staining (Masson and H&E) revealed the continuous architecture extending from the skin through the superficial to the deep fascia (Figure 5A1-A2), providing a structural basis for interlayer coordination. IF further demonstrated that CPTCs are distributed throughout these layers, forming a morphologically continuous network spanning from the skin layer through the superficial retinaculum to the deep fascia (Figure 5B–C). These findings suggested that SF-CPTCs may constitute an interconnected signaling continuum that bridges superficial and deep tissue layers, rather than acting as isolated local populations.

To resolve the hierarchical relationship within this continuum, we reconstructed the developmental trajectories of CPTCs. Monocle analysis positioned superficial-fascia (SF) CPTCs at the trajectory root (Figure 5D), a finding corroborated by RNA velocity, which showed transcriptional dynamics flowing from the SF population toward both skin and deep-fascia (DF) lineages (Figure 5E). Consistently, SF-CPTCs exhibited the highest developmental plasticity as indicated by CytoTRACE scoring (Figure 5F), suggesting they serve as a transitional reservoir or progenitor pool for the fascial network.

Consistent with this upstream developmental status, Cellcall analysis identified SF-CPTCs as a central signaling hub. While Skin and DF populations primarily engaged in intra-layer regulation, SF-CPTCs broadcasted directional signals to adjacent layers (Figure 5G). Specifically, SF-CPTCs communicated with the skin layer mainly through adherens junction pathways, while their interactions with the deep fascia were dominated by Wnt and PI3K-Akt signaling. Sankey visualization further elucidated the specific ligand-receptor-transcription factor axes (Figure 5G), highlighting the transcriptional programs through which SF-CPTCs orchestrate interlayer coordination.

To rigorously dissect the directionality of cross-layer signaling, we established a sequential co-culture model to determine whether SF-CPTCs can capture environmental cues and relay them to downstream targets. Specifically, SF-CPTCs were pre-conditioned with specific ligands (Wnt2 or Nectin) for 12 hours, followed by co-culture with deep-fascia (L6 myosatellite cells) or cutaneous (HaCaT keratinocytes) target cells for 24 hours (Figure 6A, D).

We first examined the Wnt signaling axis at the deep fascial interface. Strikingly, L6 myosatellite cells co-cultured with untreated SF-CPTCs showed no significant alteration in Wnt receptor (Lrp6) or effector (Myc) expression compared to monoculture controls (Figure 6B-C). However, co-culture with Wnt2-primed SF-CPTCs triggered a robust upregulation of both Lrp6 and Myc in L6 cells (Figure 6B-C).

Importantly, since Wnt2 was restricted to the pre-treatment phase, this response indicates that SF-CPTCs effectively integrated the Wnt signal and transduced it to the myogenic compartment.

A similar relay mechanism was observed at the cutaneous interface involving adhesive signaling. While untreated SF-CPTCs maintained basal levels of Nectin2 and Ctnnb1 in HaCaT keratinocytes, Nectin-primed SF-CPTCs elicited a potent upregulation of these targets (Figure 6E-F).

Collectively, these findings demonstrate that SF-CPTCs do not merely secrete constitutive factors; rather, they function as dynamic, bidirectional signal transduction hubs within the skin-fascia continuum. At the deep fascial interface, SF-CPTCs relay Wnt2 signals to activate the Wnt-Lrp6-Myc axis in underlying L6 myosatellite cells. At the cutaneous interface, Nectin-primed SF-CPTCs upregulate Nectin2 and Ctnnb1 in HaCaT keratinocytes, engaging an adhesion-mediated signaling cascade. Together, these results reveal that SF-CPTCs are capable of sensing transient microenvironmental stimuli and translating them into sustained, layer-specific regulatory outputs across the fascia-muscle and fascia-skin axes.

Discussion

CPTCs constitute a widely distributed population of interstitial stromal cells[22-24] that display remarkable plasticity across tissues[25]. Yet, how CPTCs diverge or align across distinct layers within the same anatomical region has remained largely unresolved[26]. By profiling CPTCs from the skin, superficial fascia, and deep fascia along the rat abdominal midline, we uncovered a clear pattern of cross-layer heterogeneity shaped by local microenvironmental cues. Through systematic analysis of layer-specific transcription factors and their downstream regulatory programs, we delineated a stratified regulatory architecture, revealing hierarchical TF–gene networks that correspond to the distinct physiological functions of each layer. Specifically, skin CPTCs exhibited immune- and barrier-oriented signatures, superficial fascia CPTCs showed mechanosensory features, and deep fascia CPTCs displayed matrix-remodeling and tensile-maintenance programs[27]. These findings indicate that contiguous tissues along the skin–fascia axis do not share a homogeneous CPTC pool, but instead host region-specific stromal states tailored to local demands[28].

Building on this transcriptional specialization, an important question is how these divergent identities translate into communication behaviors. Our analyses indicate that CPTCs form layer-specific communication hubs that regulate distinct cellular partners. In the skin, CPTCs interface with keratinocyte lineages and HFSC-associated pathways, supporting epithelial renewal and immune balance. Superficial fascia CPTCs, enriched in junctional proteins and embedded among vasculature, nerves, and immune cells, likely integrate mechanical cues with neurovascular and inflammatory signals. In contrast, deep fascia CPTCs exhibit signaling features linked to myogenic regulation and matrix homeostasis, consistent with their localization within collagen-dense muscle compartments. Collectively, these patterns demonstrate that each anatomical layer hosts a functionally distinct CPTC program—skin-resident CPTCs are tuned toward epithelial and immune equilibrium[29], superficial fascia CPTCs toward mechanosensory coordination[30], and deep fascia CPTCs toward structural and

regenerative adaptation[31,32]. Rather than acting as a monolithic stromal entity, CPTCs operate as layer-defined regulators with context-dependent functions.

Despite this functional divergence, a conserved adhesion-related program was consistently maintained across all CPTC subsets. Such conservation indicates that regional specialization does not diminish their capacity for intercellular cohesion[33]. Through their long telopodes, CPTCs may establish continuity across fascial planes, enabling the transmission of mechanical, inflammatory, or metabolic cues beyond their layer of origin[34]. This raises the possibility that local perturbations — such as mechanical stimulation induced by acupuncture— originating in the skin, superficial fascia, or deep fascia could propagate through a CPTC-based stromal network. In this view, fascia may rely on CPTCs as a primary stromal interface for information transfer, with cell–cell adhesion and telopodes continuity forming the structural basis for sustained cross-layer communication[35].

This interpretation aligns with prior descriptions of CD34+ membranous cells (CMC)[36]. Some investigators have proposed that the elongated 2D telopodes observed under transmission electron microscopy may represent sectional views of the thin, sheet-like 3D structures visualized as CMCs under scanning electron microscopy. This perspective, coupled with the overlapping morphological and functional features we observed, suggests that CPTCs and CMCs may occupy a similar regulatory niche —or potentially represent partially overlapping stromal identities within fascia-associated connective tissue.

Beyond layer-specific communication, our data indicate that CPTCs also possess the capacity to coordinate signals across fascial boundaries. In this context, superficial fascia CPTCs emerge as a particularly influential population, functioning predominantly as upstream signal initiators. Lineage inference consistently positioned them at the apex of CPTC trajectories—appearing as the predicted origin in pseudotime analyses, the primary inflow source in RNA velocity fields, and the subset with the highest CytoTRACE-defined developmental potential[37,38]. These results suggest that superficial fascia CPTCs may exist in a progenitor-like or state-flexible configuration, enabling them to transition between phenotypic states or retain a broad developmental potential that underlies the stromal continuum[39].

Importantly, our functional validation extends this concept of a "stromal continuum" from a developmental trajectory to a dynamic signaling mechanism. By employing a sequential co-culture model, we demonstrated that SF-CPTCs do not functionally isolate the fascial layers, nor do they act as primary generators of these morphogenic cues. Instead, they operate as directional signal relays, functioning analogous to an informational gateway that manages the transmission of microenvironmental inputs to distinct anatomical targets. The observation that SF-CPTCs require specific "priming" (e.g., by Wnt or adhesive ligands) to elicit responses in cutaneous or deep-muscular neighbors reveals a "context-dependent sensitization" strategy. This mechanism likely serves as a physiological gatekeeper, ensuring that powerful homeostatic pathways remain quiescent under basal conditions but are rapidly amplified across the tissue axis upon stimulation. Such a mode positions SF-

CPTCs not merely as passive structural components, but as an active distribution hub, capable of orchestrating the spatiotemporal propagation of signals across the skin-fascia continuum.

Finally, building on our previous work showing that superficial fascia CPTCs respond to acupuncture by strengthening adhesion interfaces and subsequently modulating macrophage behavior[40], the present findings provide a mechanistic rationale for this phenomenon. Their heightened state flexibility, combined with the conserved adhesion-related programs identified across layers, suggests that superficial fascia CPTCs are intrinsically equipped to translate mechanical cues into coordinated stromal responses[41]. This distinct combination of plasticity and stable adhesive architecture may underlie their ability to reorganize intercellular contacts and engage immune-regulatory circuits upon external stimulation, offering an integrated framework for understanding their role in acupuncture-induced macrophage modulation[40].

Taken together, both the layer-specific and cross-layer communication features of CPTCs suggest they operate along two coordinated axes: one supporting localized, microenvironment-tailored functions within each anatomical layer, and another maintaining long-range stromal continuity that facilitates communication across the skin–fascia continuum.

Declarations

CRediT authorship contribution statement

Yumeng Guo: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Zhenwei Zhang:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Yeping Shi:** Methodology, Investigation. **Jianming Yue:** Investigation. **Haixiang Huang:** Investigation. **Lu Mei:** Investigation. **Meng Dai:** Investigation. **Mingyu Bi:** Investigation. **Dong Hou:** Investigation. **Qiusheng Chen:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data Availability Statement

The single- cell RNA sequencing datasets generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO). Data for mock rat skin and superficial fascia are available under accession number GSE261489. The data for mock rat deep fascia are available under accession number GSE339178. All other data supporting the findings of this study are available within the article and its Supplementary Materials. Further details or materials necessary for data reanalysis can be obtained by contacting the corresponding author upon request

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have read and agreed to this declaration.

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References

1. Stecco, C., Pratt, R., Nemetz, L.D., Schleip, R., Stecco, A., and Theise, N.D. (2025). Towards a comprehensive definition of the human fascial system. J Anat *246*, 1084-1098. 10.1111/joa.14212.
2. Fede, C., Pirri, C., Fan, C., Petrelli, L., Guidolin, D., De Caro, R., and Stecco, C. (2021). A Closer Look at the Cellular and Molecular Components of the Deep/Muscular Fasciae. International Journal of Molecular Sciences *22*, 1411. 10.3390/ijms22031411.
3. Romano, E., Rosa, I., Fioretto, B.S., Lucattelli, E., Innocenti, M., Ibba-Manneschi, L., Matucci-Cerinic, M., and Manetti, M. (2020). A Two-Step Immunomagnetic Microbead-Based Method for the Isolation of Human Primary Skin Telocytes/CD34+ Stromal Cells. Int J Mol Sci *21*. 10.3390/ijms21165877.
4. Yonghong, S., Ruizhi, W., Yue, Z., Xuebing, B., Tarique, I., Chunhua, L., Ping, Y., and Qiusheng, C. (2020). Telocytes in Different Organs of Vertebrates: Potential Essence Cells of the Meridian in Chinese Traditional Medicine. Microscopy and Microanalysis *26*, 575-588. 10.1017/S1431927620001518.
5. Faussone-Pellegrini, M.S., and Gherghiceanu, M. (2016). Telocyte's contacts. Seminars in Cell and Developmental Biology *55*, 3-8. 10.1016/j.semcdb.2016.01.036.
6. Banciu, D.D., Banciu, A., and Radu, B.M. (2016). Electrophysiological Features of Telocytes. Advances in Experimental Medicine and Biology *913*, 287-302. 10.1007/978-981-10-1061-3_19.
7. Shim, W. (2016). Myocardial Telocytes: A New Player in Electric Circuitry of the Heart. Advances in Experimental Medicine and Biology *913*, 241-251. 10.1007/978-981-10-1061-3_16.
8. Radu, B.M., Banciu, A., Banciu, D.D., Radu, M., Cretoiu, D., and Cretoiu, S.M. (2017). Calcium Signaling in Interstitial Cells: Focus on Telocytes. International Journal of Molecular Sciences *18*, 397. 10.3390/ijms18020397.
9. Dawidowicz, J., Matysiak, N., Szotek, S., and Maksymowicz, K. (2016). Telocytes of Fascial Structures. Advances in Experimental Medicine and Biology *913*, 403-424. 10.1007/978-981-10-1061-3_26.
10. Bordoni, B., Mahabadi, N., and Varacallo, M.A. (2025). Anatomy, Fascia. In StatPearls.
11. Chambers, E.S., and Vukmanovic-Stejic, M. (2020). Skin barrier immunity and ageing. Immunology *160*, 116-125. 10.1111/imm.13152 PMID - 31709535.
12. Zügel, M., Maganaris, C.N., Wilke, J., Jurkat-Rott, K., Klingler, W., Wearing, S.C., Findley, T., Barbe, M.F., Steinacker, J.M., Vleeming, A., et al. (2018). Fascial tissue research in sports medicine: from

molecules to tissue adaptation, injury and diagnostics: consensus statement. Br J Sports Med *52*, 1497. 10.1136/bjsports-2018-099308 PMID - 30072398.

13. Kruglikov, I., Trujillo, O., Kristen, Q., Isac, K., Zorko, J., Fam, M., Okonkwo, K., Mian, A., Thanh, H., Koban, K., et al. (2016). The Facial Adipose Tissue: A Revision. Facial Plast Surg *32*, 671-682. 10.1055/s-0036-1596046 PMID - 28033645.
14. Sun, Z., Gepner, B.D., Cottler, P.S., Lee, S.H., and Kerrigan, J.R. (2021). In Vitro Mechanical Characterization and Modeling of Subcutaneous Adipose Tissue: A Comprehensive Review. J Biomech Eng *143*, 070803. 10.1115/1.4050286 PMID - 33625495.
15. Pirri, C., Caroccia, B., Angelini, A., Piazza, M., Petrelli, L., Caputo, I., Montemurro, C., Ruggieri, P., De Caro, R., and Stecco, C. (2023). A New Player in the Mechanobiology of Deep Fascia: Yes-Associated Protein (YAP). Int J Mol Sci *24*, 15389. 10.3390/ijms242015389 PMID - 37895068.
16. Findley, T.W. (2009). Second international fascia research congress. Int J Ther Massage Bodywork *2*, 1-6. 10.3822/ijtmb.v2i2.52 PMID - 21589727.
17. Rosa, I., Marini, M., and Manetti, M. (2021). Telocytes: An Emerging Component of Stem Cell Niche Microenvironment. J Histochem Cytochem *69*, 795-818. 10.1369/00221554211025489.
18. Popescu, L.M., Fertig, E.T., and Gherghiceanu, M. (2016). Reaching out: junctions between cardiac telocytes and cardiac stem cells in culture. J Cell Mol Med *20*, 370-380. 10.1111/jcmm.12719.
19. Díaz-Flores, L., Gutiérrez, R., Díaz-Flores, L., Jr., Goméz, M.G., Sáez, F.J., and Madrid, J.F. (2016). Behaviour of telocytes during physiopathological activation. Semin Cell Dev Biol *55*, 50-61. 10.1016/j.semcdb.2016.01.035.
20. Driskell, R.R., Lichtenberger, B.M., Hoste, E., Kretzschmar, K., Simons, B.D., Charalambous, M., Ferron, S.R., Herault, Y., Pavlovic, G., Ferguson-Smith, A.C., and Watt, F.M. (2013). Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature *504*, 277-281. 10.1038/nature12783.
21. Hatta, K., Huang, M.L., Weisel, R.D., and Li, R.K. (2012). Culture of rat endometrial telocytes. J Cell Mol Med *16*, 1392-1396. 10.1111/j.1582-4934.2012.01583.x.
22. Yonghong, S., Ruizhi, W., Yue, Z., Xuebing, B., Tarique, I., Chunhua, L., Ping, Y., and Qiusheng, C. (2020). Telocytes in Different Organs of Vertebrates: Potential Essence Cells of the Meridian in Chinese Traditional Medicine. Microsc Microanal *26*, 575-588. 10.1017/S1431927620001518.
23. Li, S., Shen, X., and He, S. (2019). Expression of toll-like receptors and their regulatory roles in murine cardiac telocytes. J Cell Mol Med *23*, 5360-5368. 10.1111/jcmm.14416.
24. Manole, C.G., Gherghiceanu, M., Ceafalan, L.C., and Hinescu, M.E. (2022). Dermal Telocytes: A Different Viewpoint of Skin Repairing and Regeneration. Cells *11*. 10.3390/cells11233903.
25. Horch, R.E., Weigand, A., Beier, J.P., Arkudas, A., and Boos, A.M. (2016). The Potential Role of Telocytes for Tissue Engineering and Regenerative Medicine. Adv Exp Med Biol *913*, 139-147. 10.1007/978-981-10-1061-3_9.
26. Iliopoulou, L., Tzaferis, C., Prados, A., Roumelioti, F., Koliaraki, V., and Kollias, G. (2025). Different fibroblast subtypes propel spatially defined ileal inflammation through TNFR1 signalling in murine

ileitis. Nat Commun *16*, 3023. 10.1038/s41467-025-57570-7.

27. Zhang, Y., Su, X., Dong, Y., Chen, T., Zhang, Y., Wu, B., Li, H., Sun, X., Xia, L., Zhang, D., et al. (2020). Cytological and functional characteristics of fascia adipocytes in rats: A unique population of adipocytes. Biochim Biophys Acta Mol Cell Biol Lipids *1865*, 158585. 10.1016/j.bbalip.2019.158585.
28. Su, X., Lyu, Y., Wang, W., Zhang, Y., Li, D., Wei, S., Du, C., Geng, B., Sztalryd, C., and Xu, G. (2016). Fascia Origin of Adipose Cells. Stem Cells *34*, 1407-1419. 10.1002/stem.2338.
29. Ceafalan, L., Gherghiceanu, M., Popescu, L.M., and Simionescu, O. (2012). Telocytes in human skin--are they involved in skin regeneration? J Cell Mol Med *16*, 1405-1420. 10.1111/j.1582-4934.2012.01580.x.
30. Huang, H., Shi, Y., Shen, B., Zhang, Z., Yue, J., Mei, L., and Chen, Q. (2025). Application of single-cell deep learning in elucidating the mapping relationship between visceral and body surface inflammatory patterns. Comput Struct Biotechnol J *27*, 4183-4200. 10.1016/j.csbj.2025.09.040.
31. Dawidowicz, J., Szotek, S., Matysiak, N., Mielanczyk, L., and Maksymowicz, K. (2015). Electron microscopy of human fascia lata: focus on telocytes. Journal of cellular and molecular medicine *19*, 2500-2506. 10.1111/jcmm.12665.
32. Ishiuchi, N., Nakashima, A., Maeda, S., Miura, Y., Miyasako, K., Sasaki, K., Uchiki, T., Sasaki, A., Nagamatsu, S., Nakao, N., et al. (2023). Comparison of therapeutic effects of mesenchymal stem cells derived from superficial and deep subcutaneous adipose tissues. Stem Cell Res Ther *14*, 121. 10.1186/s13287-023-03350-3.
33. Gherghiceanu, M., and Popescu, L.M. (2012). Cardiac telocytes - their junctions and functional implications. Cell and tissue research *348*, 265-279. 10.1007/s00441-012-1333-8.
34. Dawidowicz, J., Matysiak, N., Szotek, S., and Maksymowicz, K. (2016). Telocytes of Fascial Structures. Adv Exp Med Biol *913*, 403-424. 10.1007/978-981-10-1061-3_26.
35. Xuebing, B., Ruizhi, W., Yue, Z., Chunhua, L., Yonghong, S., Yingxin, Z., Baitao, D., Tarique, I., Ping, Y., and Qiusheng, C. (2020). Tissue Micro-channels Formed by Collagen Fibers and their Internal Components: Cellular Evidence of Proposed Meridian Conduits in Vertebrate Skin. Microsc Microanal *26*, 1069-1075. 10.1017/S1431927620024381.
36. Huang, H., Mei, L., Zhang, Z., Yue, J., Dai, M., Lu, L., Yang, M., Fan, L., Yang, T., and Chen, Q. (2025). Laminar CD34+ membranous cells with mechanosensitive properties in subcutaneous fascia of the abdominal midline. Sci Rep *15*, 39032. 10.1038/s41598-025-25651-8.
37. Sun, H., Wang, H., Zhang, W., Mao, H., and Li, B. (2023). Single-cell RNA sequencing reveals resident progenitor and vascularization-associated cell subpopulations in rat annulus fibrosus. J Orthop Translat *38*, 256-267. 10.1016/j.jot.2022.11.004.
38. Kang, M., Gulati, G.S., Brown, E.L., Qi, Z., Avagyan, S., Armenteros, J.J.A., Gleyzer, R., Zhang, W., Steen, C.B., D'Silva, J.P., et al. (2025). Improved reconstruction of single-cell developmental potential with CytoTRACE 2. Nat Methods *22*, 2258-2263. 10.1038/s41592-025-02857-2.
39. Wang, X., Lin, J., Liu, H., Zhao, C., Tu, Z., Xu, D., Zhang, E., Zhou, Z., Qi, X., Wang, X., and Lin, Z. (2024). Single-cell and spatial sequencing identifies senescent and germinal tumor cells in

adamantinomatous craniopharyngiomas. Cell Biosci *14*, 112. 10.1186/s13578-024-01299-1.

40. Zhang, Z., Huang, H., Yue, J., Zhu, X., Shi, Y., Shen, B., Zhu, Q., Mei, L., and Chen, Q. (2025). Ptgs2+ CPTC Function as a "Force-Immune Axis" by Responding to Acupuncture and Mediating M2 Macrophage Activation for Anti-Inflammatory Effects. FASEB Journal *39*, e71048. 10.1096/fj.202501175RR.
41. Sun, Z., Guo, S.S., and Fassler, R. (2016). Integrin-mediated mechanotransduction. J Cell Biol *215*, 445-456. 10.1083/jcb.201609037.

Figures

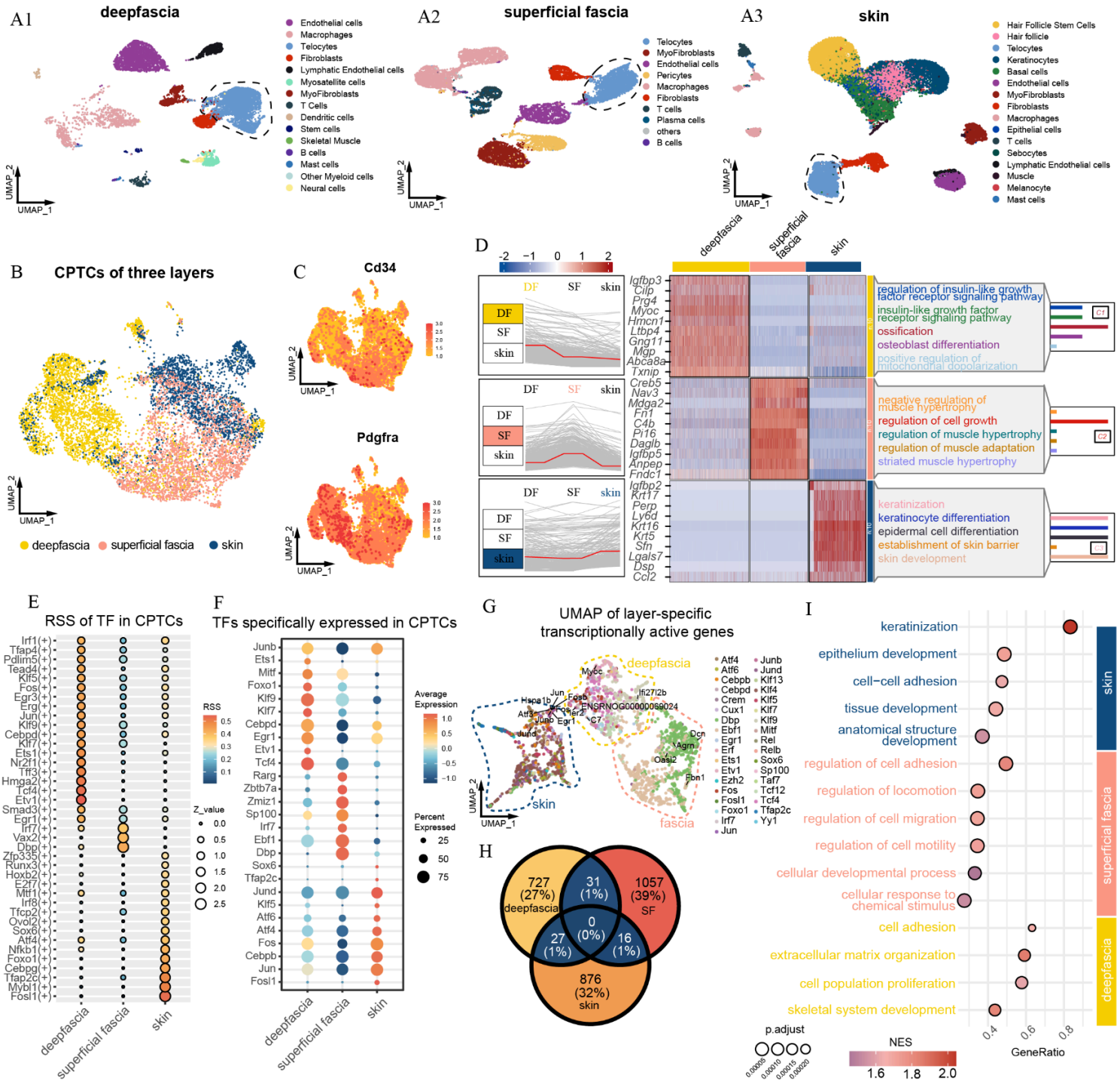


Figure 1

Single-cell transcriptomic atlas reveals layer-specific heterogeneity and conserved adhesion programs of CPTCs.

(A) UMAP visualization of scRNA-seq data from the deep fascia (A1), superficial fascia (A2), and skin (A3) of normal rats. CPTC populations are highlighted by dashed lines.

(B-C) UMAP projection of re-clustered CPTCs (B) showing distinct spatial segregation by layer, and feature plots (C) displaying the expression of canonical markers Cd34 and Pdgfra.

(D) Heatmap and trend analysis (ClusterGVis) displaying layer-enriched molecular signatures and corresponding GO biological processes.

(E-F) Analysis of transcription factor (TF) regulatory networks. Regulon Specificity Scores (RSS) of top candidate TFs (E) and dot plot of their specific expression patterns (F) in CPTCs. (G–H) UMAP visualization of TF-regulated signature genes

(G) and Venn diagram

(H) demonstrating the high exclusivity of these gene sets to their respective anatomical layers.

(I) GSEA enrichment analysis showing layer-specific functional modules alongside a universally conserved adhesion-related transcriptional program.

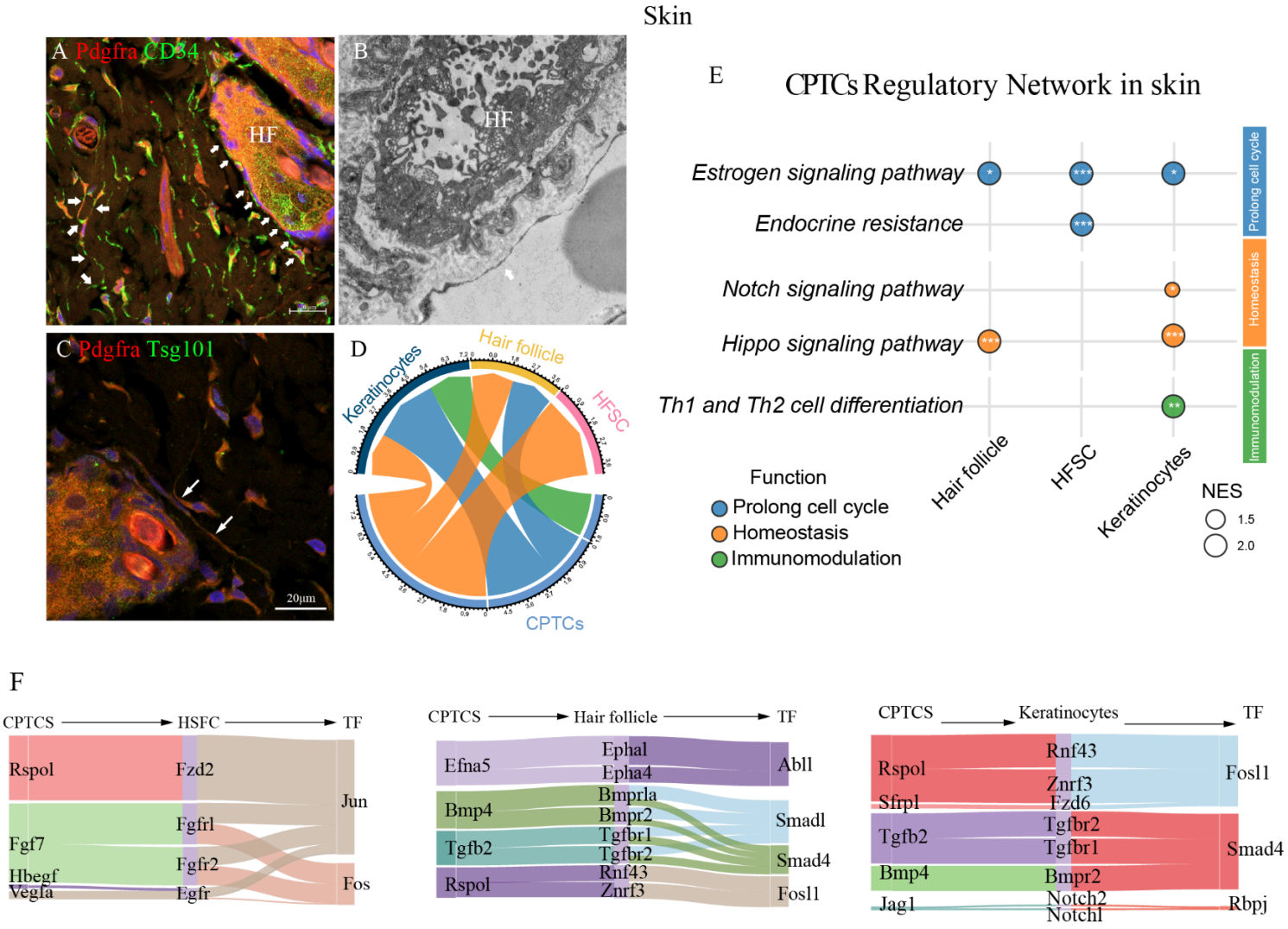


Figure 2

Skin-CPTCs occupy a follicular niche and orchestrate epithelial homeostasis.

(A-C) Structural identification and secretory characterization. Immunofluorescence (A) reveals CPTCs residing in the distinct niche surrounding hair follicles (HF), confirmed by transmission electron microscopy (B). Co-staining with Tsg101 (C) demonstrates significant enrichment of exosomal markers in CPTCs. Arrows:CPTCs. Scale bars: 20 μm.

(D) Chord diagram illustrating the dense intercellular communication network between CPTCs and epithelial lineages, including keratinocytes and hair follicle stem cells (HFSCs).

(E) Dot plot of the CPTC regulatory network. Functional annotation identifies key signaling modules governing homeostasis and cell cycle control in target cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

(F) Sankey diagrams mapping the ligand-receptor-transcription factor axis. CPTCs drive downstream transcriptional programs in epithelial targets via the secretion of ligands.

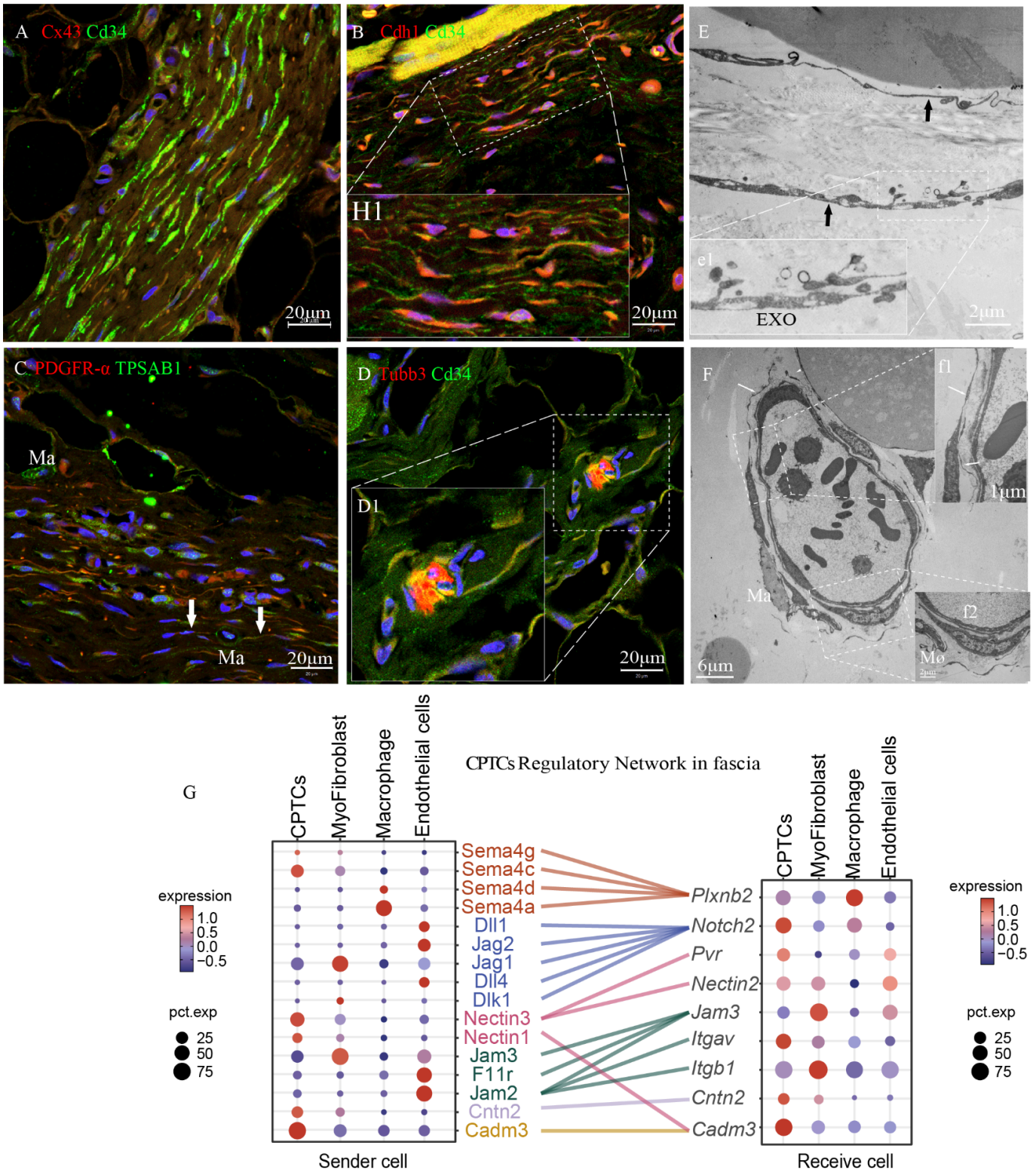


Figure 3

CPTCs orchestrate a neuro-immune-stromal interactome in the superficial fascia.

(A-B) IF visualization of CPTC connectivity. CPTCs maintain a reticular network through physical coupling via gap junctions(A) and adherens junctions (B). Scale bars: 20 μm.

(C-D) Spatial mapping of the stromal niche. CPTCs exhibit intimate proximity to mast cells (C) and nerve fibers (D).

(E-F) Ultrastructural characterization byTEM. Images reveal CPTC telopodes releasing extracellular vesicles (E) and enveloping adjacent cells (F), confirming their structural and communicative role.EXO: exosome; Ma: Mast cells

(G) Ligand-receptor interaction analysis. The dot plot identifies CPTCs as key signaling senders targeting myofibroblasts, macrophages, and endothelial cells.

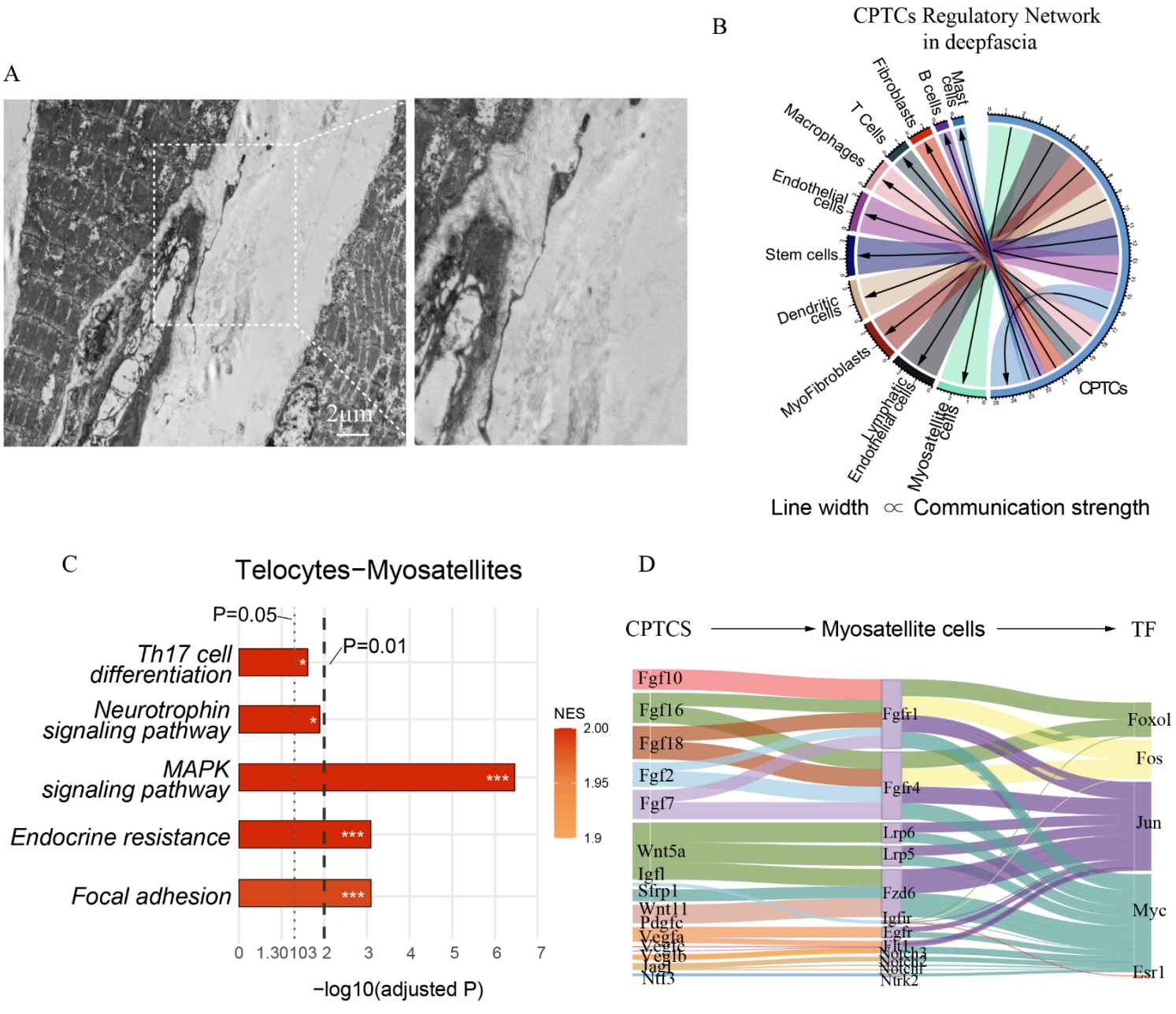


Figure 4

Deep fascia-resident CPTCs modulate myogenic progenitor fate via specific signaling axes.

(A) TEM visualizing the spatial niche of CPTCs in the deep fascia. Images show the unique intercalation of CPTC telopodes between muscle bundles and their close association with the perivascular stroma.

(B) Chord diagram illustrating the intercellular communication network. The width of the links is proportional to communication strength, highlighting a preferential signaling axis directed from CPTCs to myosatellite cells.

(C) Functional enrichment analysis of ligand-receptor pairs between CPTCs and myosatellite cells. Bar plot indicates dominant interaction modules, including MAPK signaling and Focal adhesion. *, $P < 0.05$; ***, $P < 0.001$.

(D) Sankey diagram mapping the specific ligand-receptor-transcription factor (L-R-TF) axis. CPTCs regulate myosatellite cell activation and fate determination through the secretion of ligands such as Fgf10, Fgf18, and Wnt5a, which converge on downstream transcription factors.

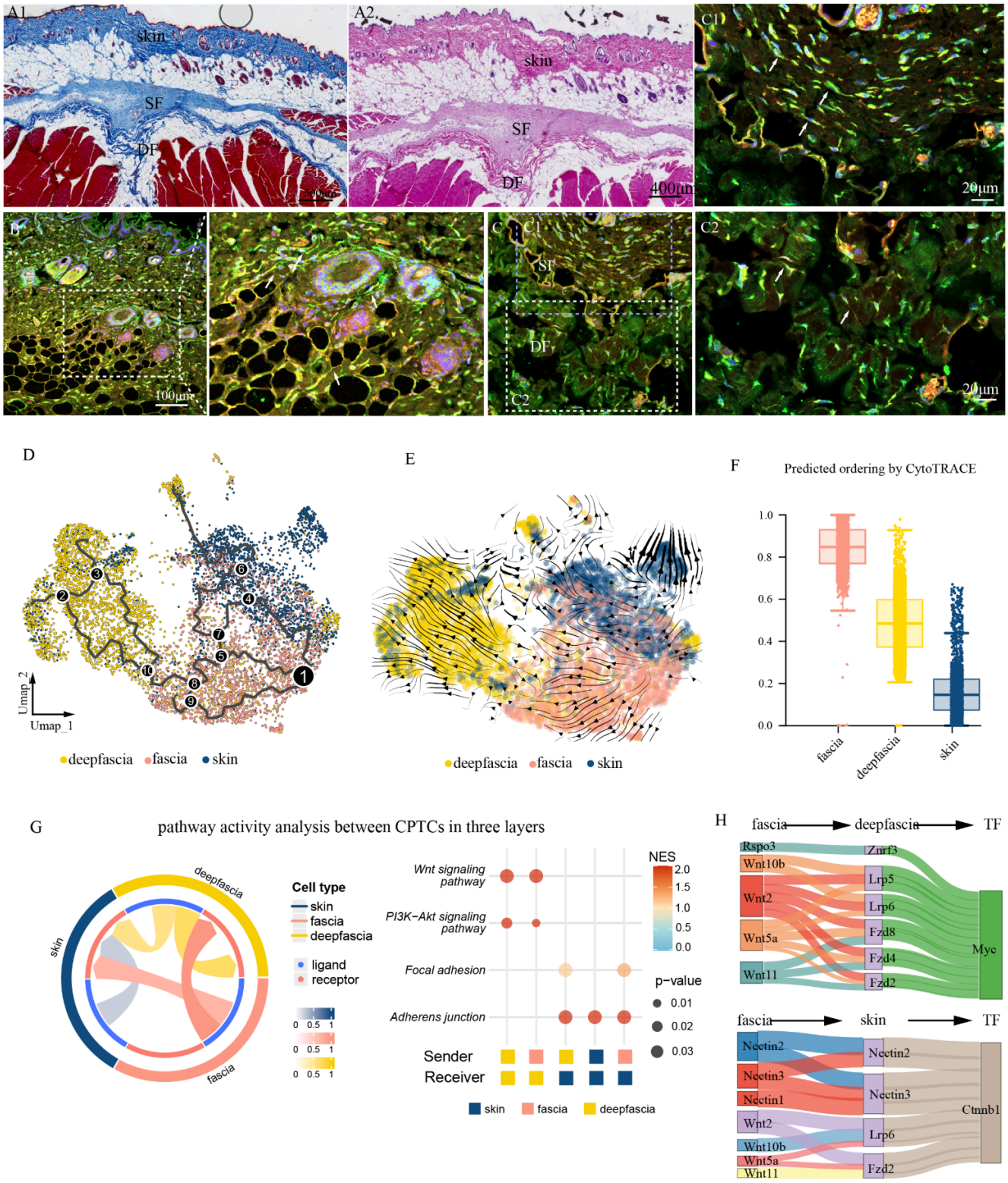


Figure 5

SF-CPTCs serve as a developmental reservoir and signaling hub bridging anatomical layers.

(A-C) Structural continuity of the fascial network. Masson trichrome (A1) and H&E (A2) staining illustrate the continuous architecture extending from skin to deep fascia (DF) via superficial fascia (SF). Immunofluorescence (B) and high-magnification views (C) reveal a morphologically continuous network

of CPTCs (arrows) spanning the superficial-skin interface (C1) and superficial-deep interface (C2). Scale bars: 400 μm (A), 100 μm (B), 20 μm (C).

(D-F) Reconstruction of developmental trajectories. Monocle pseudotime analysis (D) identifies SF-CPTCs at the trajectory root. RNA velocity stream plot (E) visualizes transcriptional dynamics flowing from the SF population toward both Skin- and DF-CPTC lineages. CytoTRACE scoring (F) confirms the superior developmental plasticity of SF-CPTCs compared to other subsets.

(G) Intercellular communication analysis via CellCall. The chord diagram (left) and dot plot (right) characterize the signaling flux, highlighting SF-CPTCs as a central hub broadcasting Wnt and PI3K-Akt signals to the deep fascia, and Adherens junction signals to the skin. (H) Sankey diagrams mapping the specific ligand-receptor-transcription factor axes. SF-CPTCs coordinate interlayer targets via distinct molecular programs, such as the Wnt–Myc axis for deep fascia and the Nectin/Wnt-Ctnnb1 axis for skin.

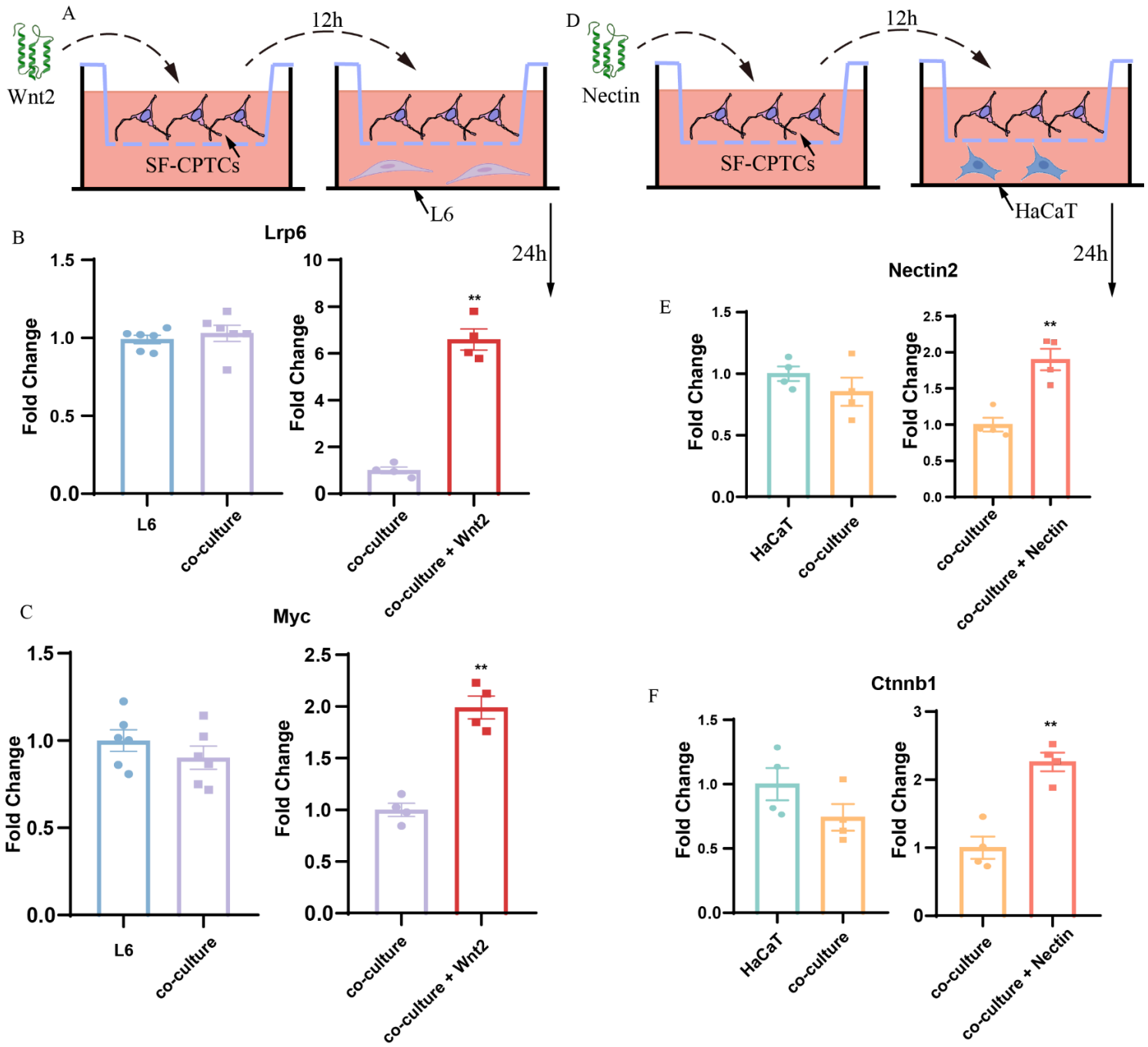


Figure 6

SF-CPTCs transduce signals to cross-layer targets.

(A) Schematic of the sequential co-culture assay. SF-CPTCs were pre-treated with Wnt2 (12 h), washed, and then transferred to co-culture with L6 cells for 24 h.

(B–C) qPCR analysis of Lrp6 (B) and Myc (C) in L6 cells. Comparisons show L6 cells co-cultured with either untreated SF-CPTCs or Wnt2-primed SF-CPTCs. Data represent fold change relative to L6 monoculture. **P < 0.01.

(D) Schematic of the sequential co-culture assay for the cutaneous interface. SF-CPTCs were pre-treated with Nectin protein (12 h) before co-culture with HaCaT cells.

(E–F) qPCR analysis of Nectin2 (E) and Ctnnb1 (F) in HaCaT cells, demonstrating the inductive effect of Nectin-primed SF-CPTCs compared to untreated controls. Data represent fold change. **P < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarytableS1.xlsx
- SupplementarytableS2.xlsx