

Cancer-associated fibroblast-specific expression of the matricellular protein CCN1 coordinates neovascularization and stroma deposition in melanoma metastasis

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1 Introduction

Melanoma is a highly aggressive form of skin cancer, characterized by rapid progression and a high rate of metastasis, making it one of the most lethal skin cancers. The role of CCN1, a matricellular protein produced by Cancer-Associated Fibroblasts (CAFs), in melanoma progression is relatively unexplored/

2 Aim

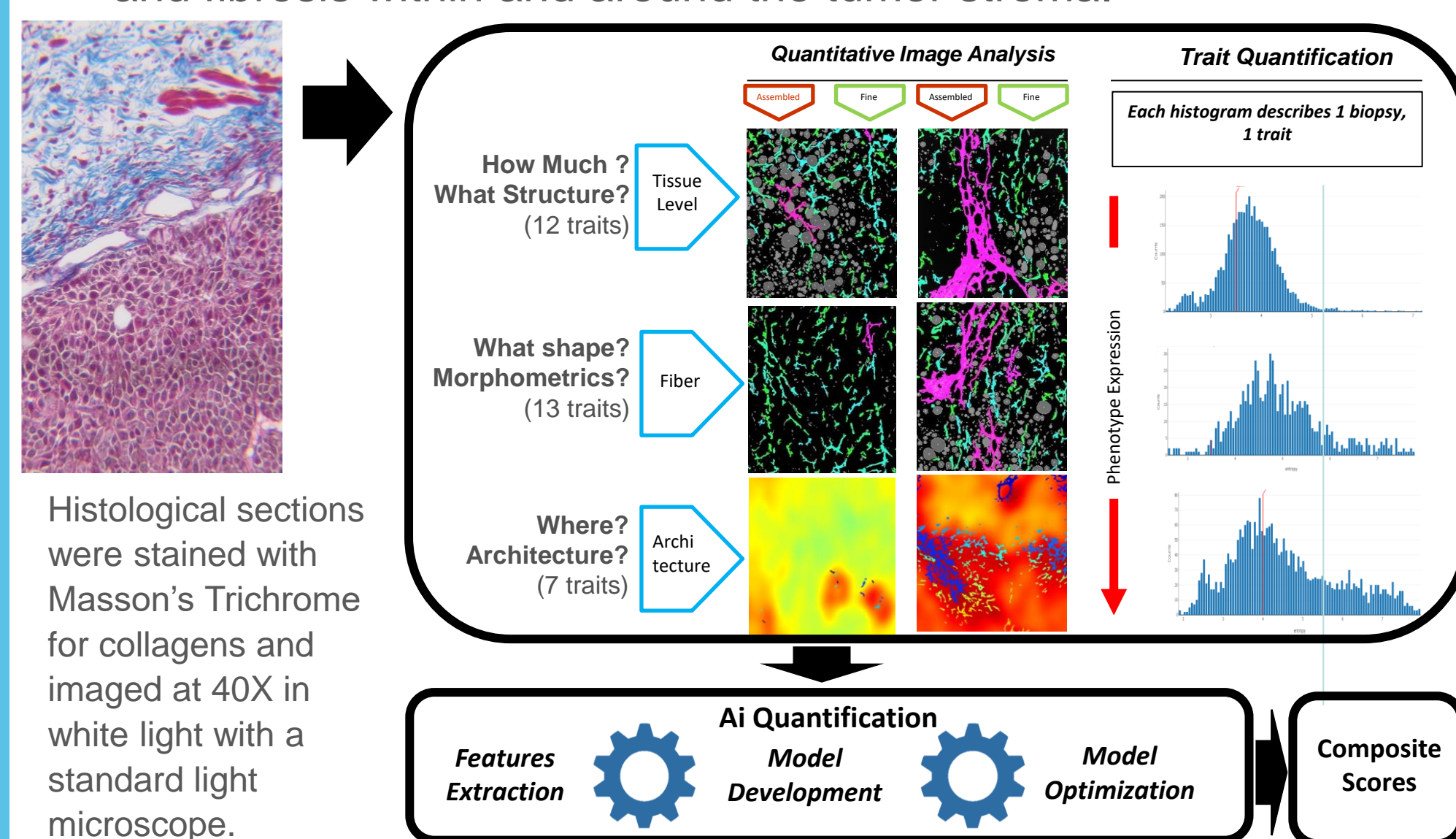
Here, we investigate the role of CCN1 expressed by CAFs in melanoma progression, to explore melanoma's aggressive nature and treatment resistance. This study is distinctive in its application of FibroNest, an innovative histological analysis tool, for detailed examination of the tumor microenvironment.

3 Method

- The study employed the B16-F10 murine melanoma cell line and C57BL6J mice possessing a targeted deletion of CCN1 in fibroblasts, providing a specific model to study CCN1's role in the tumor microenvironment.
- N=11 total
 - 6 Control-Wildtype CCN1 Mice
 - 5 CCN1 KO Mice

Histological analysis was conducted on harvested tumor tissues, including staining for CCN1, ECM components, immune cell markers, and standard histological staining (Masson's Trichrome).

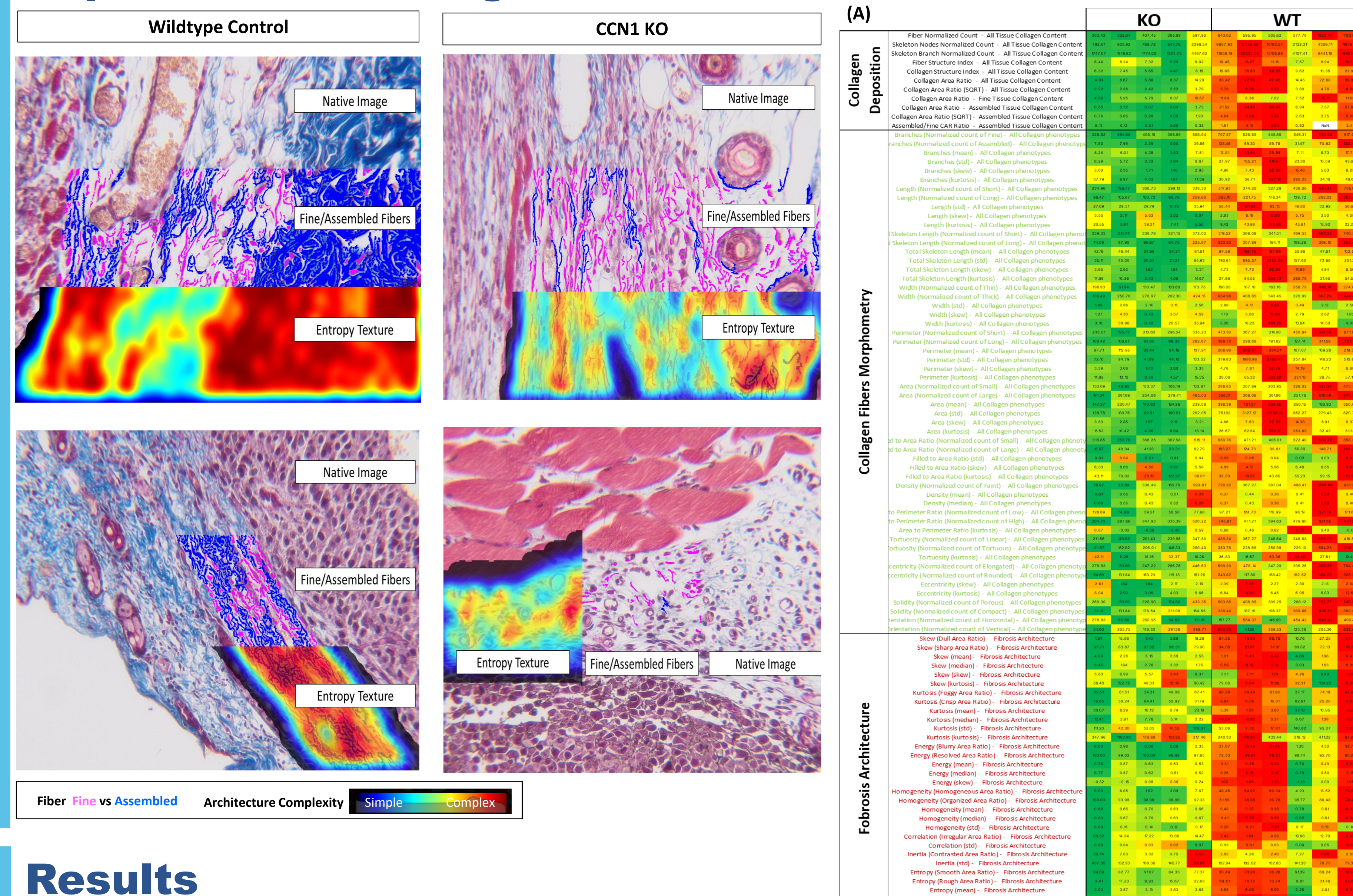
- This study used FibroNest, a digital pathology platform, for quantitative analysis of the collagen structure, ECM composition, and fibrosis within and around the tumor stroma.



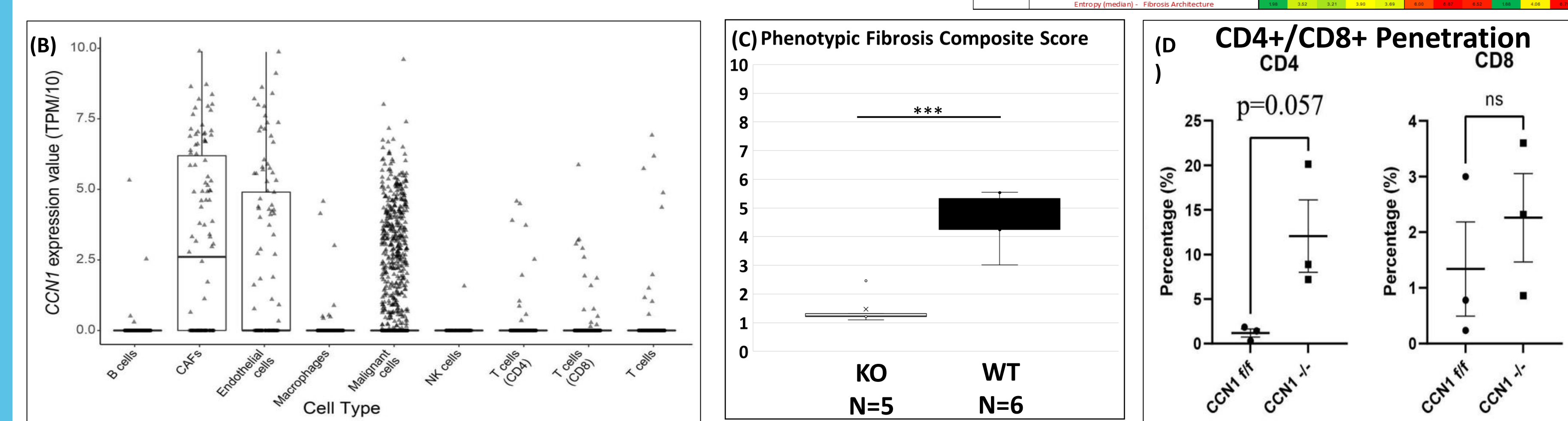
FibroNest™, a cloud-based image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for collagen content and structure, fiber morphometry, and architecture (measures the organization of the fibers). Principal quantitative fibrosis traits (up to 315 qFTs) are automatically detected and combined into a Phenotypic Composite Fibrosis Score (PT-Ph-FCS).

- PCR was used to examine gene expression changes, focusing on genes related to ECM organization, angiogenesis, and immune infiltration
- Flow cytometry quantified and characterized immune cell populations within the tumor, including CD4+ and CD8+ T cell infiltration.
- Microcomputed tomography (Micro-CT) scans assessed changes in tumor neovascularization and structure in vivo.

4 Representative Images



5 Results



- Analysis showed that CCN1 is predominantly expressed in CAFs within melanoma tumors (Fig A).
- FibroNest showed a significantly lower fibrotic footprint in the absence of CCN1 and revealed a substantial disruption in ECM elaboration and neovascularization, characterized by altered collagen organization and reduced vascular density in CCN1-deficient models (Fig B)
- Deletion of CCN1 from fibroblasts results in increased penetration of CD4+ and CD8+ T cells into tumors, as measured as a percent when tumors were subjected to flow cytometry with anti-CD4 anti-CD8 antibodies, suggesting its role in modulating immune responses (Fig C)

	KO		WT		Folds
	mean	Std	mean	Std	
Collagen Composite Score	2.0	0.6	6.1	1.7	3.0
Fibrosis Morphometric Composite Score - ALL	1.6	0.5	4.7	0.9	2.9
Fibrosis Morphometric Composite Score - FINE	1.3	0.8	3.8	1.3	3.0
Fibrosis Morphometric Composite Score - ASSBLD	2.6	0.6	5.9	1.0	2.3
Fibrosis Architecture composite Score	3.6	1.1	6.5	2.1	1.8
Phenotypic Fibrosis Composite Score	1.5	0.5	4.6	0.9	3.1

5 Conclusions

This study underscores the critical role of CCN1 in melanoma progression and its potential as a therapeutic target. The use of FibroNest for detailed analysis of tumor stroma offers new insights into the ECM and fibrosis changes, enhancing our understanding of cancer progression. Targeting CCN1, especially in CAFs, emerges as a promising approach in melanoma treatment, potentially improving the effectiveness of existing therapies and overcoming drug resistance.

