



GLI1 Promotes the Pro-Fibrotic Function of Monocyte-Derived Fibrocytes in Myelofibrosis

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Background

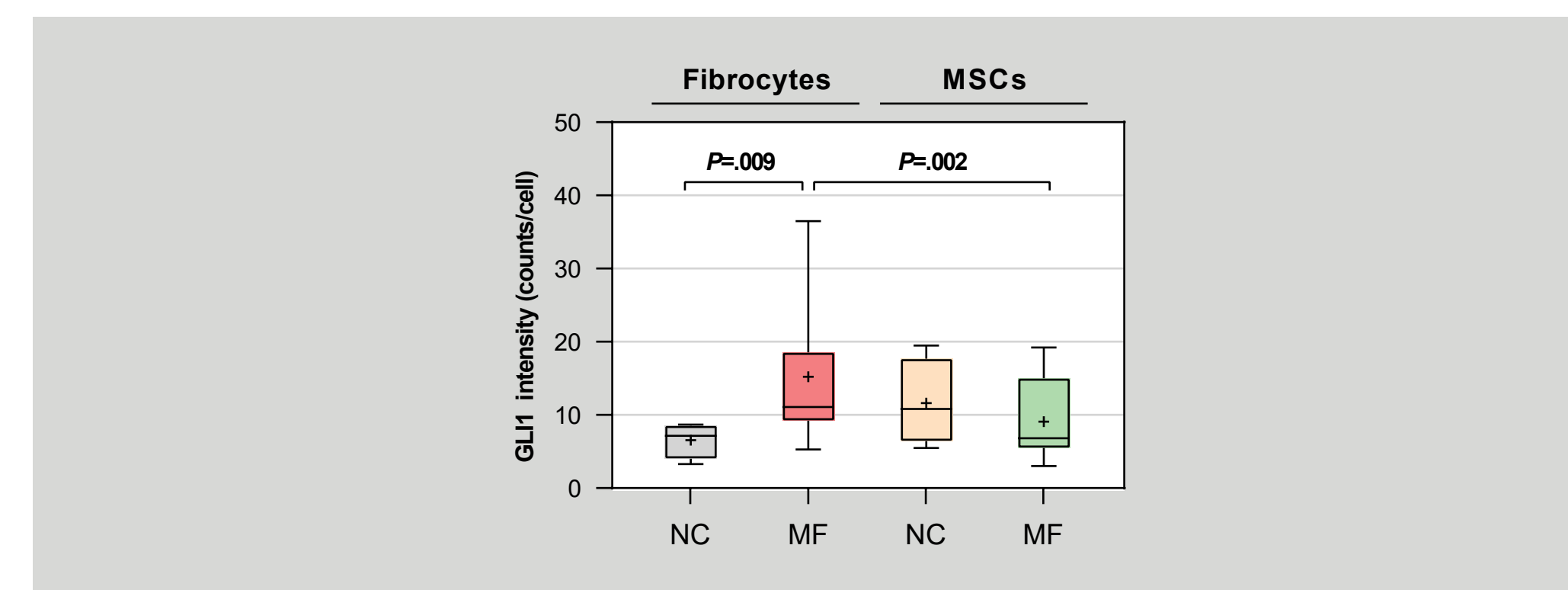
- Primary myelofibrosis (MF), post-polycythemia vera (PV) MF and post-essential thrombocythosis MF are myeloproliferative neoplasms (MPNs) characterized by progressive bone marrow (BM) fibrosis.
- A recent study suggested that glioma-associated oncogene-1 (GLI1), a downstream effector of the embryonic Hedgehog pathway, is implicated in the pathogenesis of BM fibrosis in MF.⁽¹⁾
- We and other investigators have found that monocyte-derived fibrocytes, rather than mesenchymal stromal cells (MSCs), induce BM fibrosis in MF.⁽²⁾
- In addition, we recently found that GLI1 expression in leukemic cells is induced by constitutively activated signal transducer and activator of transcription 3 (STAT3).⁽³⁾
- Therefore, we sought to determine the role of GLI1 in MF fibrocytes.

Methods

- BM biopsy sections of patients with MF were analyzed using multiplex fluorescence immunohistochemistry (mfiHC) and GLI1 levels were quantitated.
- Cultured BM fibrocytes or mesenchymal stromal cells (MSCs) were analyzed by quantitative PCR.
- Human MF BM cells were transplanted into NSG mice and analyzed using mfiHC.
- GLI was inhibited using non-specific GLI antagonist 61 (GANT61) and fibrocyte formation, viability, and gene expression were assessed.
- GLI1 was specifically silenced using small interfering RNA and fibrocyte apoptosis, viability, and gene expression were analyzed.
- Luciferase and STAT3 silencing assays were performed to assess the impact of STAT3 activity on GLI1 gene promoter in MF fibrocytes.

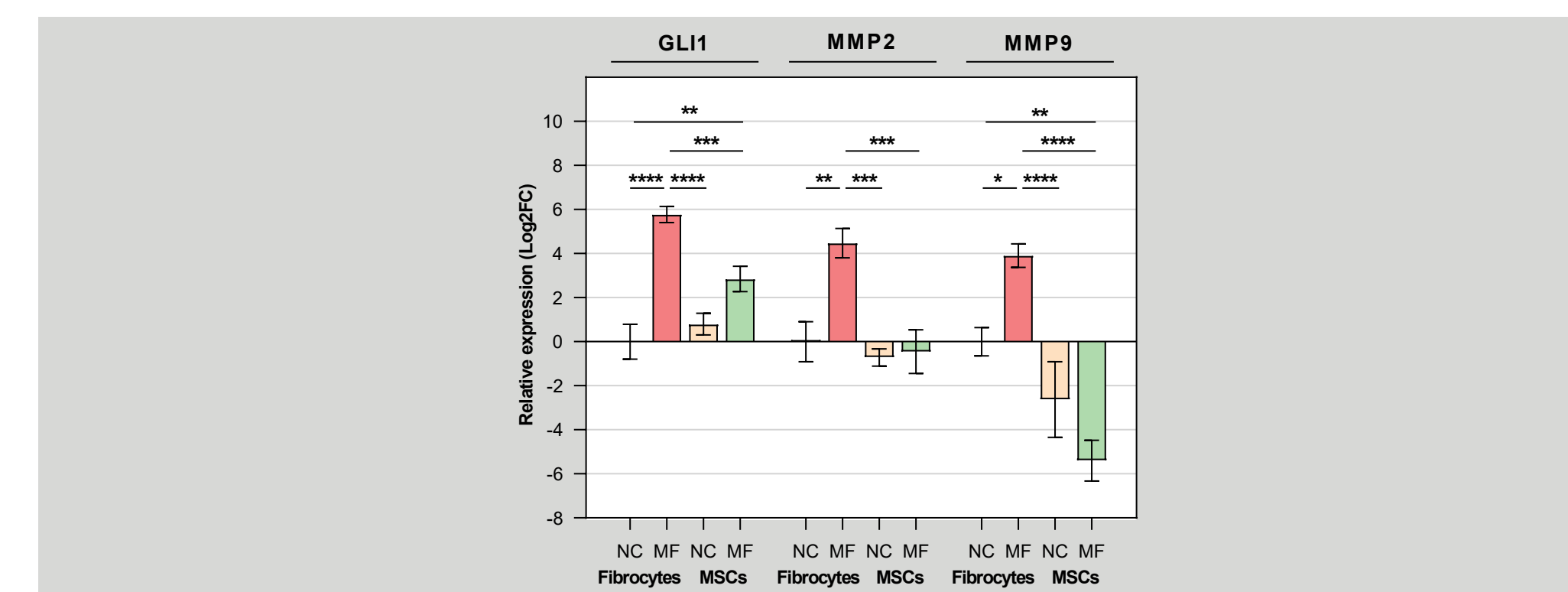
Results

1 GLI1 levels in MF fibrocytes and MSCs in situ



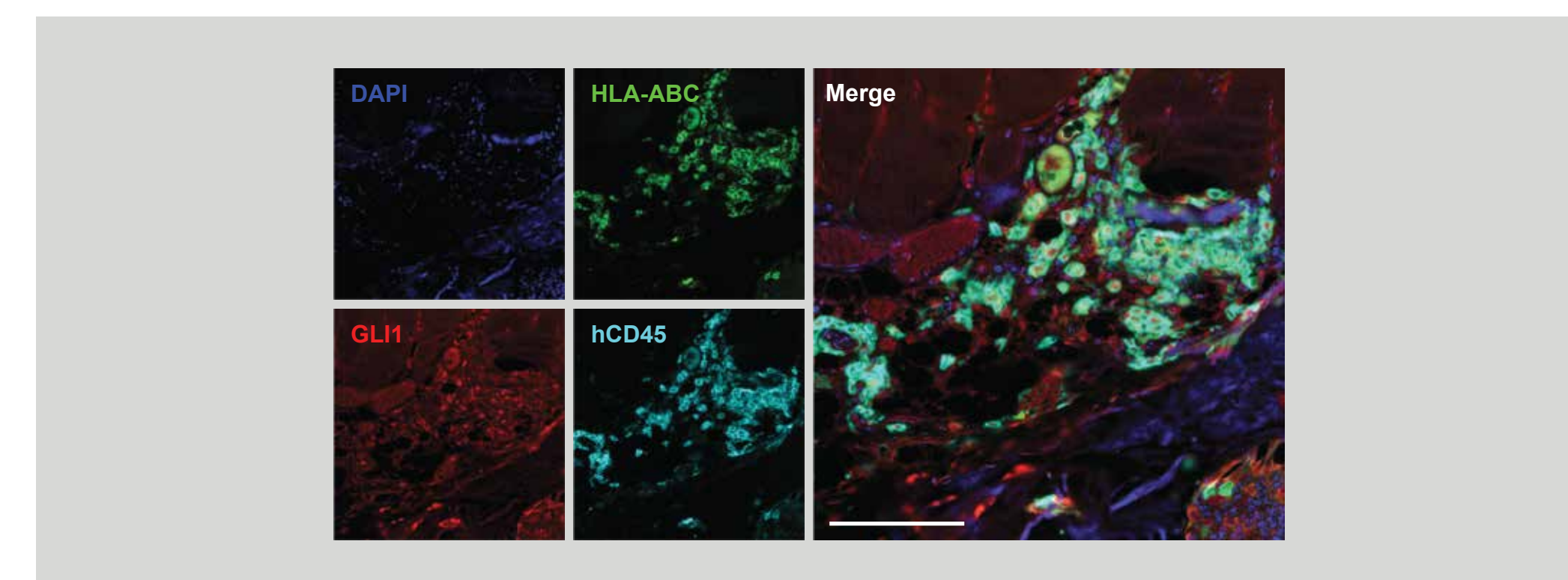
High levels of GLI1 were detected by mfiHC in CD45⁺/CD68⁺/procollagen I⁺ fibrocytes and low levels of GLI1 were detected in CD90⁺/CD105⁺ MSCs.

2 Gene expression in MF fibrocytes/MSCs in vitro



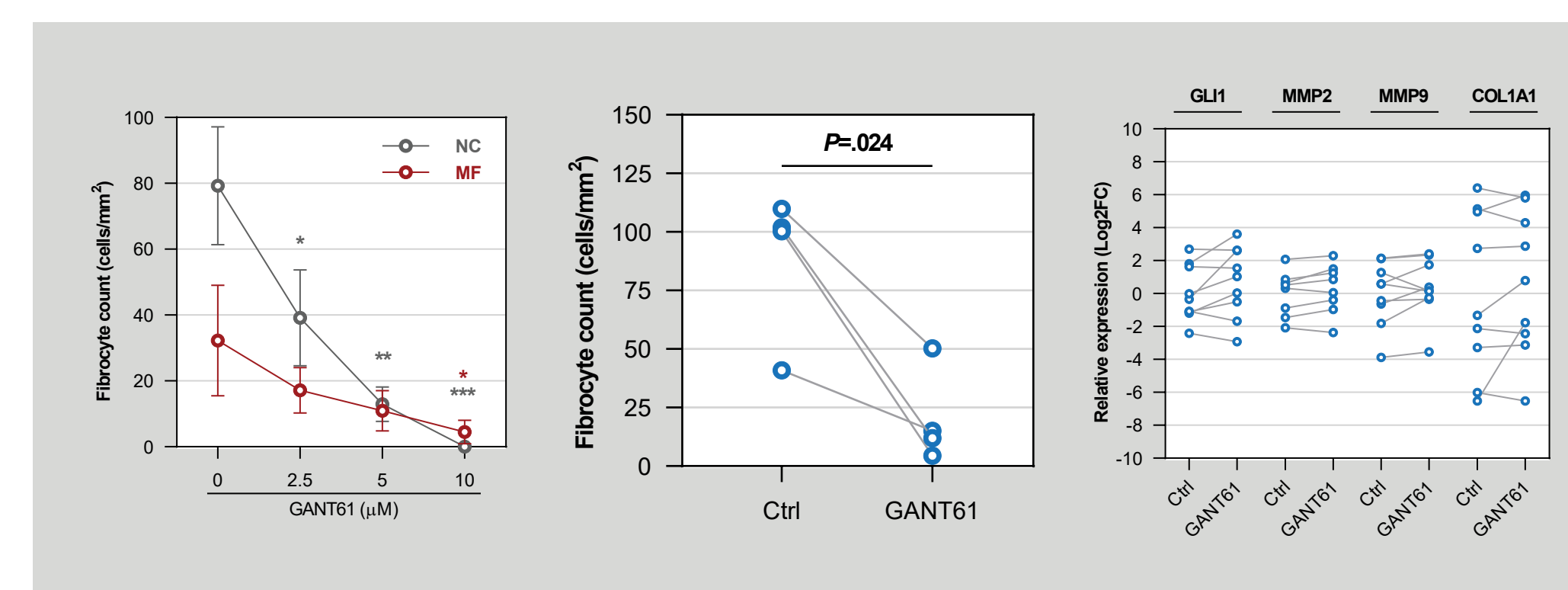
Significantly higher levels of GLI1 and GLI1-induced matrix metalloproteases (MMP) 2 and 9 were observed in MF fibrocytes than in MF MSCs or normal BM-derived fibrocytes.

3 Xenograft mouse model of GLI1⁺ MF fibrocytes



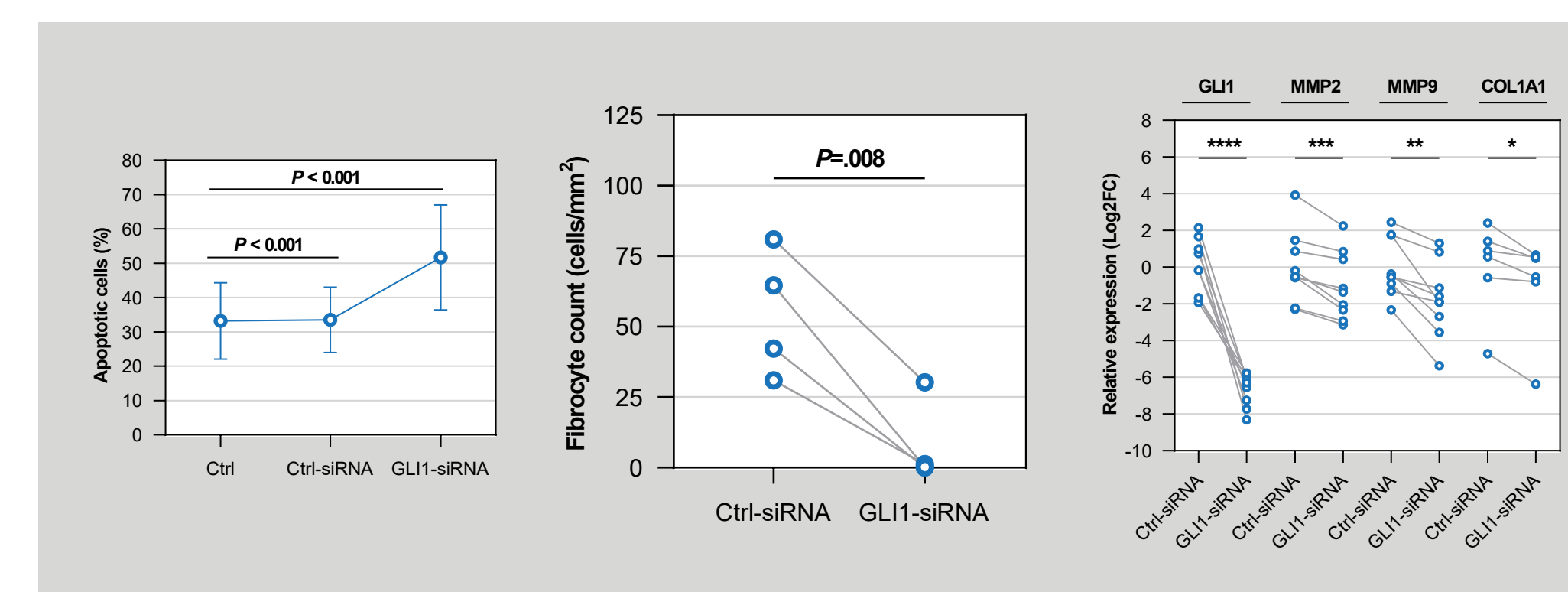
Engrafted human BM cells from MF patients differentiated into GLI1-expressing cells that induced BM fibrosis.

4 Effects of GLI inhibition on MF fibrocytes



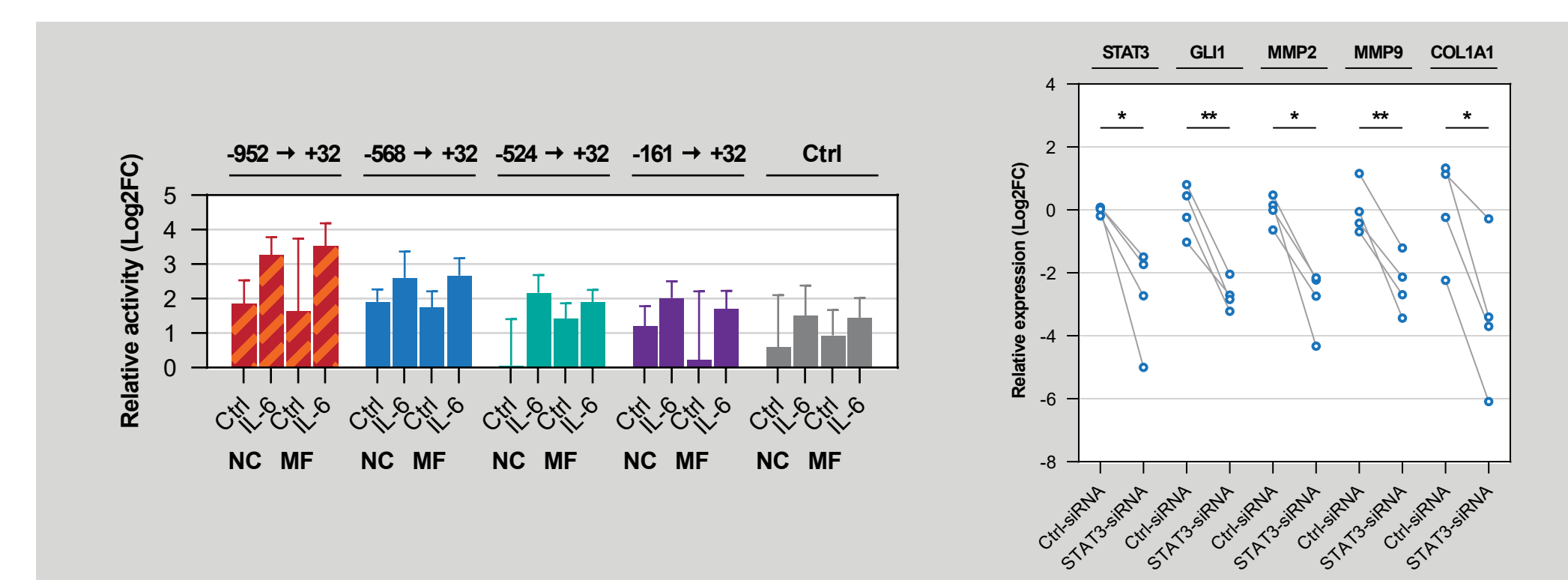
Non-specific GLI inhibition decreased the formation of both MF fibrocytes and normal fibrocytes.

5 Effects of GLI1 silencing on MF fibrocytes



Specific GLI1 silencing induced apoptosis of MF fibrocytes and fibrocyte-forming cells. In addition, GLI1 knockdown reduced expression of MMP2, MMP9, and procollagen I.

6 STAT3 binding to GLI1 promoter in MF fibrocytes



STAT3 bound to and activated multiple binding sites within the GLI1 gene promoter in MF fibrocytes.

Conclusion

GLI1, triggered by constitutively activated STAT3, activates pro-fibrotic signaling pathways in MF fibrocytes. Downregulation of GLI1 hampers fibrocyte survival and function, suggesting that GLI1 inhibition might alleviate BM fibrosis in patients with MF.

Key References

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- Schneider, R. K. et al. (2017). Cell Stem Cell. 20(6), 785-800.
- Rozovski, U. et al. (2021). Oncotarget. 12(5):401-411.