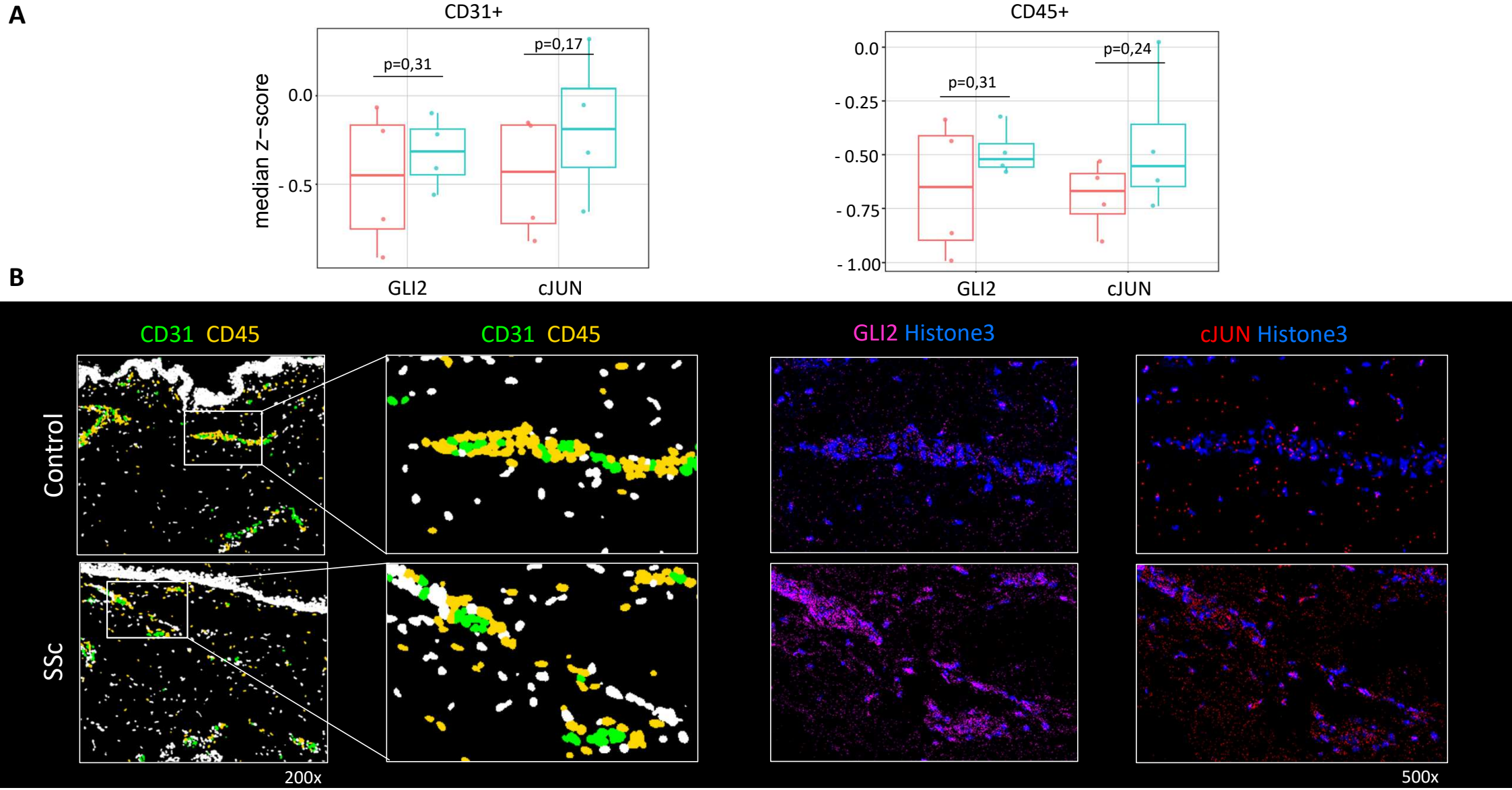
Mutual amplification of GLI2/Hedgehog and cJUN/AP1 signaling in fibroblast activation in Systemic Sclerosis (SSc) – potential implications for combined therapies

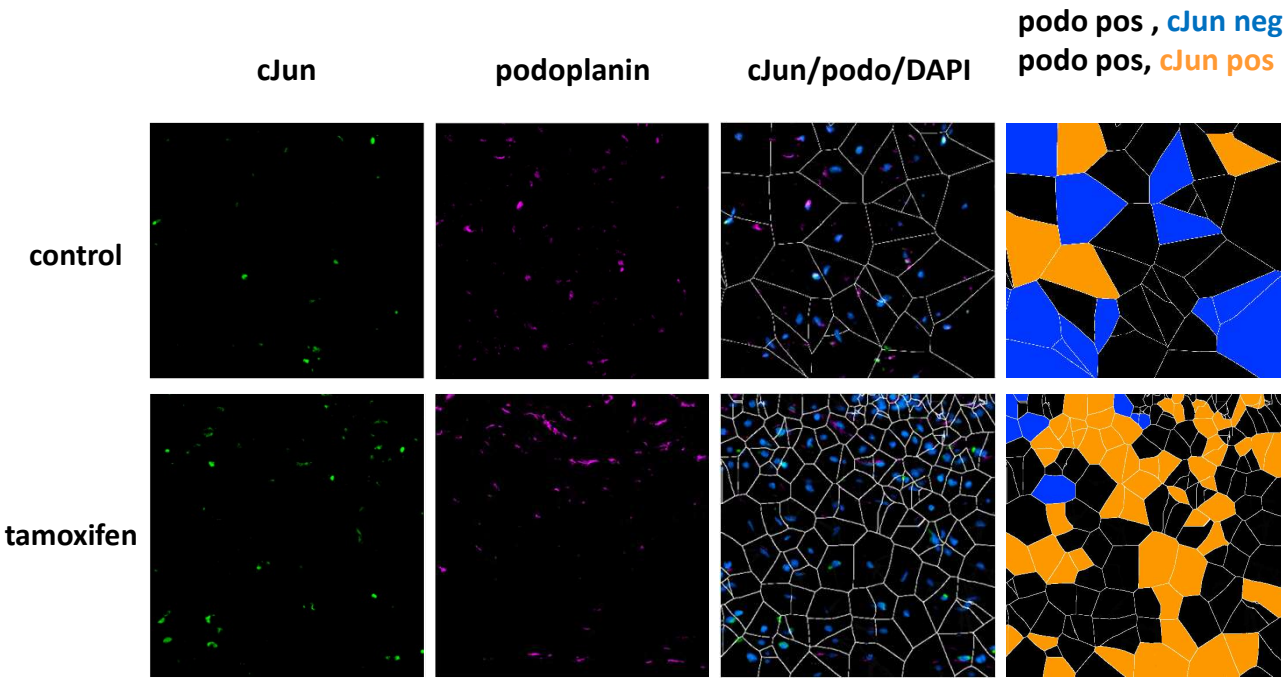
- Supplementary Figures -

Christina Bergmann[1,2] (MD)*, Sara Chenguiti Fakhouri[1,2] (MS), Thuong Trinh-Minh[3,4] (PhD), Tim Filla[3,4](MS), Aleix Rius Rigau[1,2] (PhD), Arif B. Ekici[5] (PhD), Benita Merlevede[1,2] (MD), Ludwig Hallenberger[1,2] (MD), Zhu Honglin[6] (MD), Clara Dees[1,2] (PHD), Alexandru-Emil Matei[3,4] (MD), Janina Auth[1,2] (MD), Andrea-Hermina Györfi[3,4] (MD), Xiang Zhou [3,4] (PhD), Simon Rauber[1,2] (PhD), Aline Bozec[1,2] (PhD), Nicholas Dickel[7] (MS), Liang Chunguang[7] (PhD), Meik Kunz[7,8,9] (PD), Georg Schett[1,2] (MD), Jörg H.W. Distler[3,4] (MD)

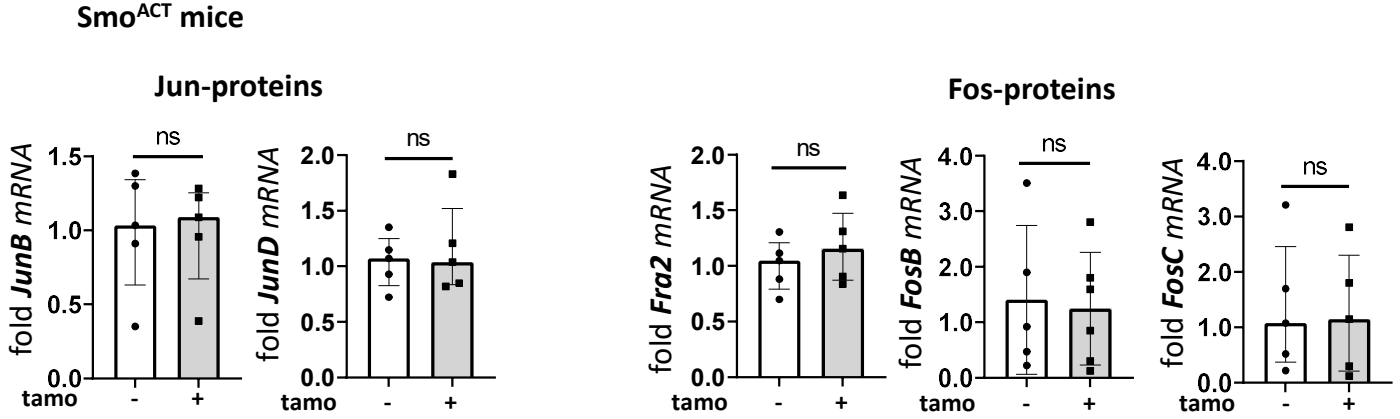
[1] Department of Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and Uniklinikum Erlangen, Erlangen, Germany; [2]Deutsches Zentrum für Immuntherapie (DZI), Friedrich-Alexander-Universität Erlangen-Nürnberg and Uniklinikum Erlangen; Erlangen, Germany; [3]Department of Rheumatology, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University, 40225, Düsseldorf, Germany; [4]Hiller Research Center, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University, 40225, Düsseldorf, Germany;[5]Institute of Human Genetics, Friedrich Alexander Universität Erlangen-Nürnberg and Uniklinikum Erlangen, Erlangen, Germany; [6]Department of Rheumatology and Immunology, Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China. 410008; [7]Chair of Medical Informatics, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany; [8]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany; [9]Fraunhofer Cluster of Excellence Immune-Mediated Diseases (CIMD), Hannover, Germany



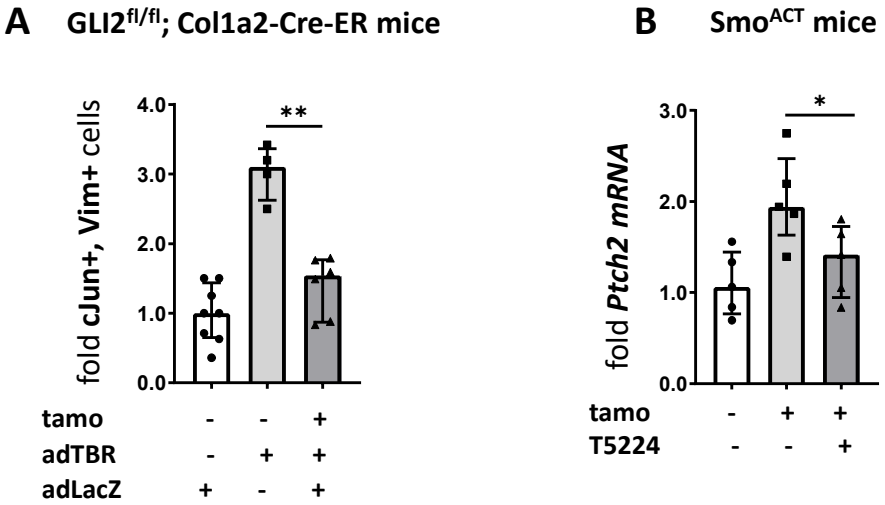
Supplementary figure 1: GLI2 and cJUN are upregulated in endothelial cells and immune cells in SSc skin compared to controls.
A. Median z-score of GLI2 and cJUN expression in CD31+ and CD45+ cell populations as detected by imaging mass cytometry (IMC) in SSc skin sections compared with healthy controls. **B.** Corresponding imaging mass cytometry (IMC) images with expression of CD31 (green), CD45 (yellow), GLI2 (magenta), cJUN (red) (n=4 individual samples)



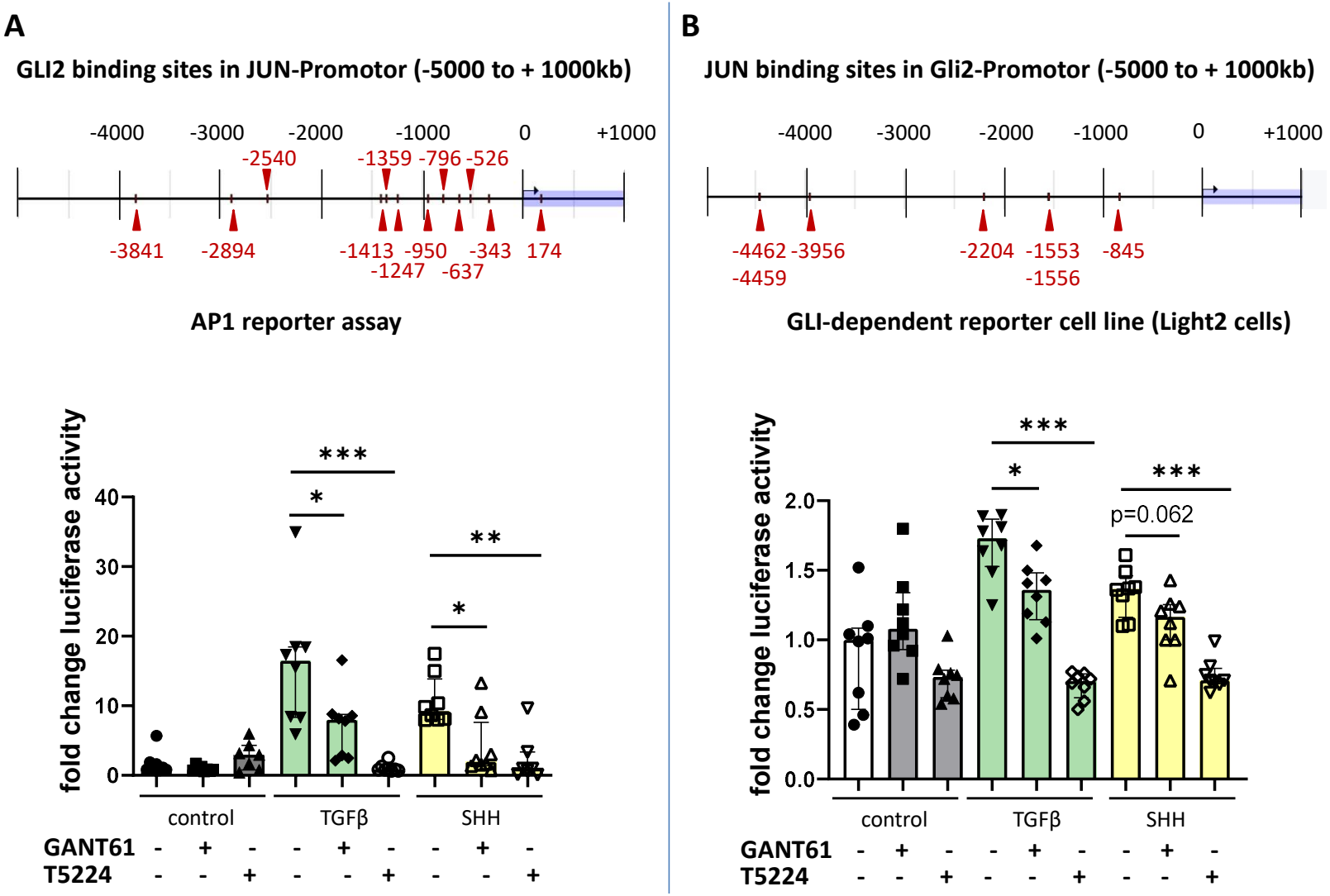
Supplementary figure 2: Immunofluorescence staining of cJun in Smo^{ACT} mice.
Upregulation of cJun+ fibroblasts in the skin of **Smo^{ACT} mice.** Representative immunofluorescence stainings for cJun (green), the fibroblast marker podoplanin (purple) and DAPI (blue) are shown at 1000-fold magnification (n=2 with 2 technical replicates). Voronoi tessellations are shown.



Supplementary figure 3: Analysis of the mRNA-expression of *JunB, JunD, Fra2, FosB, FosC* in Smo^{ACT} mice (n=5/group, 1-2 technical replicates). Data are presented as median with interquartile range. Tamo=tamoxifen. P-values < 0.05 were considered significant.

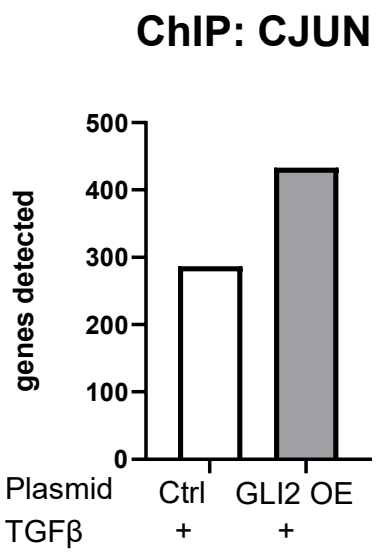


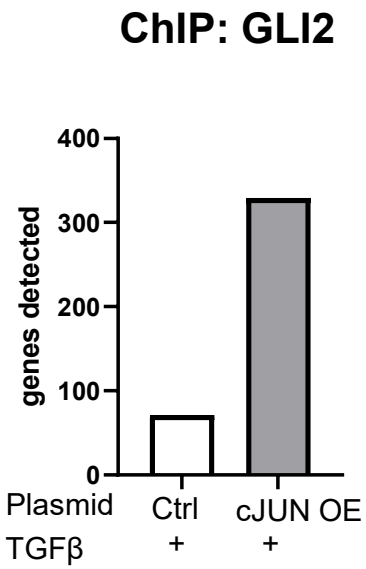
Supplementary figure 4: A. Fold cJun+, Vimentin+ cells in mice with conditional, fibroblast-specific knockout of Gli2 and additional treatment with adTBRvirus (n=4/group, 1-2 technical replicates) compared to mice treated with adLacZ. **B.** mRNA expression of Ptch2 in Smo^{ACT} mice (n=5/group). Data are presented as median with interquartile range. P-values < 0.05 were considered significant. Tamo=tamoxifen, adLacZ=attenuated adenovirus encoding for LacZ, adTBR= attenuated adenovirus encoding for constitutively active TGFβ receptor 1.



Supplementary figure 5: A. Top: Schematic representation of putative GLI2 binding sites in the JUN promoter region (-5000 to + 1000kb) as analyzed using the Eukaryotic Promoter database (EPD). **Bottom:** AP1 reporter assay: Inhibition of GLI2 reduces AP1-dependent transcription in fibroblasts stimulated with recombinant TGFβ or SHH (n=8 replicates per group). **B. Top:** Schematic representation of putative cJUN binding sites in the GLI2-promotor (-5000 to + 1000kb). **Bottom:** GLI-dependent reporter assay using Light2 cells: Inhibition of cJUN/AP1 reduces GLI-dependent transcription in hedgehog-responsive Light2-reporter cells stimulated with TGFβ or SHH (n=8 replicates per group). Results are presented as median with interquartile range.

A





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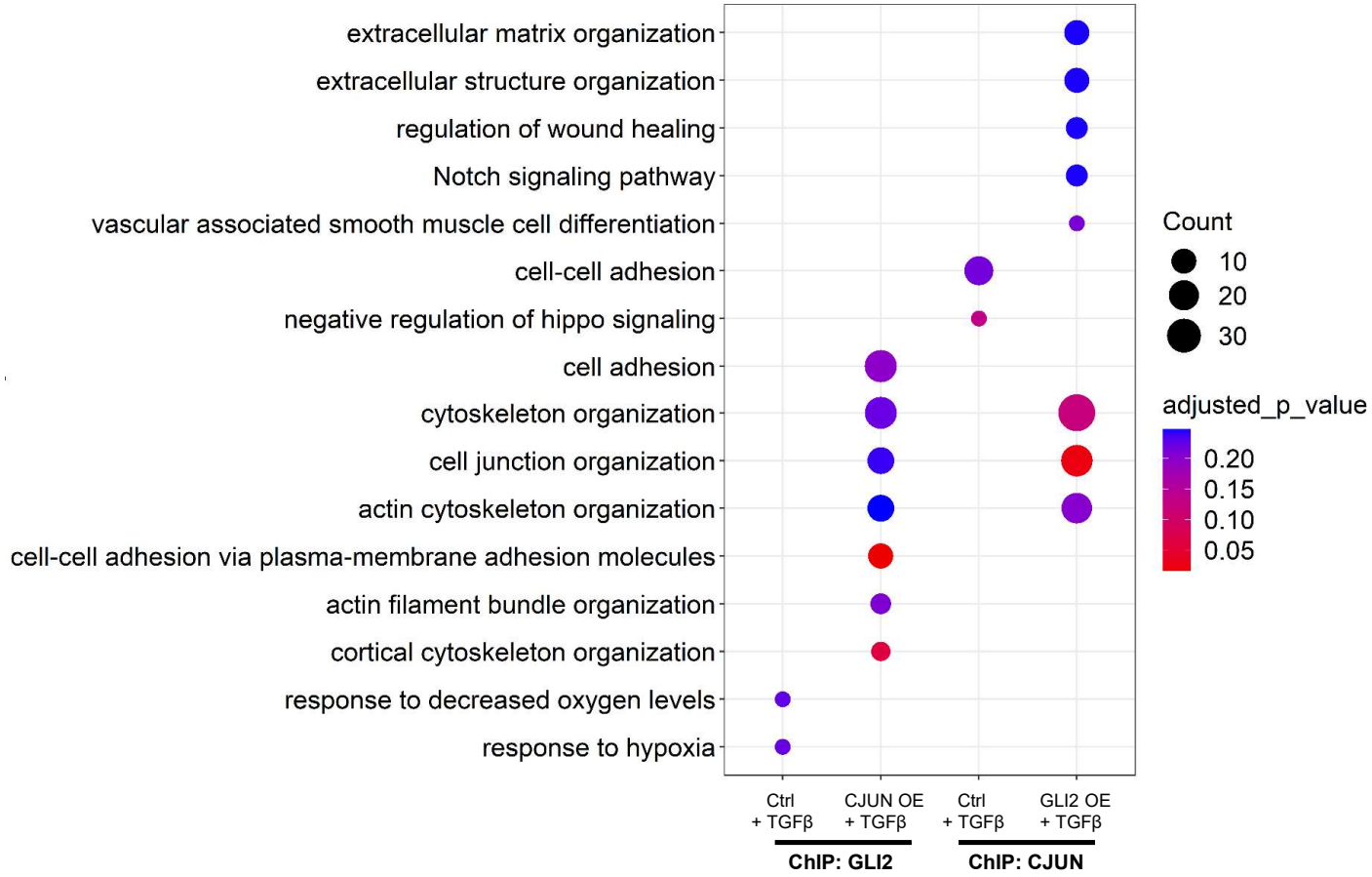

Supplementary figure 6: ChIP Sequencing of samples precipitated for cJUN upon overexpression of GLI2 and stimulation with TGFβ (gene counts of 2 cell lines included) compared to TGFβ alone (gene counts of 3 cell lines included) and ChIP sequencing of samples precipitated for GLI2 upon overexpression of cJUN and stimulation with TGFβ (gene counts of 2 cell lines included) compared to TGFβ stimulation alone (gene counts of 3 cell lines included). A. Bar graphs of genes detected in the respective comparisons across all cell lines. **B**. Results of GO-term analysis of the differentially regulated genes. Ctrl=control, OE=overexpression.