# Primary cell-based phenotypic assays to pharmacologically and genetically study fibrotic diseases in vitro

S. Weigle, E. Martin, A. Voegtle, B. Wahl and M. Schuler

| Supplementary Information | Manuscript Section |
|---|---|
| Figure S1 | Results Section - TGFß-1-mediated fibroblast-to-myofibroblast transition assay |
| Figure S2 | Results Section - TGFß-1-mediated epithelial-to-mesenchymal transition assay |
| Table S1 | Methods Section - Fibroblast to myofibroblast transitions assay |
| Table S2 | Methods Section - Epithelial to mesenchymal transition assay |

**Figure S1**

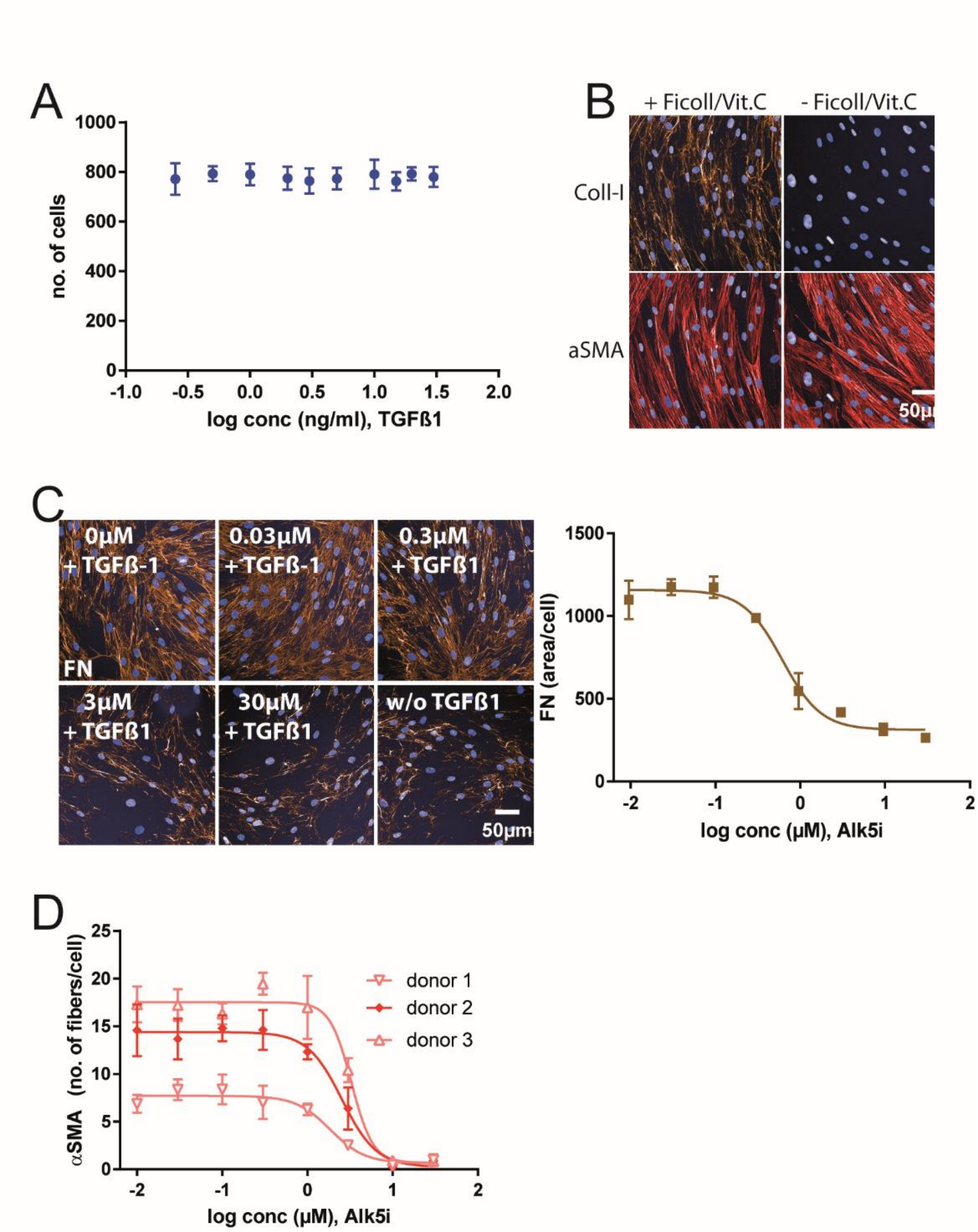

**Figure S1. A.** No effect of increasing TGFß-1 concentrations on cell counts in the FMT assay. **B.** Effect of the mixture of molecular crowding agent, vitamin-C, Ficoll-75 and -400 on αSMA and Coll-I expression. Only in the presence of the molecular crowding mixture Coll-I expression was detected. **C.** Images of NHLF cells (1000/well) treated with increasing doses of TGFß-1 and stained for nuclei, and fibronectin. Images were acquired with the IN Cell 2200 high content imager (right). Fibronectin area/cell was quantified with a custom protocol using Columbus software and plotted against increasing doses of TGFß-1. The Alki $IC_{50}$ was 0.6µM (left). **D.** Increasing Alk5i doses were added to different NHLF cell donors in the presence of 5ng/ml TGFß-1. αSMA expression was quantified 72h later. The potenty of the Alk5i was comparable for the 3 donors, but donor 1 showed a smaller maximal TGFß-1 effect.

Figure S2

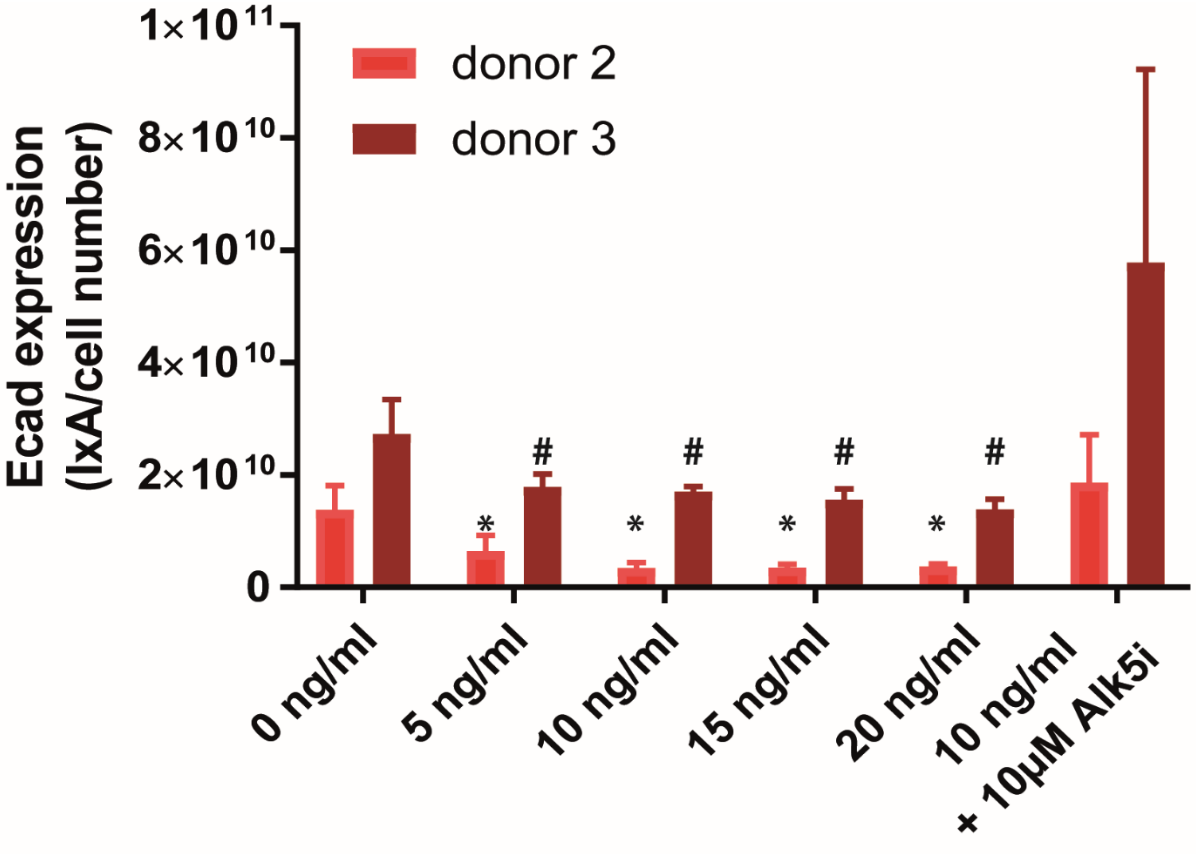

**Figure S2.** Increasing TGFß-1 doses and 10ng/ml TGFß-1 plus 10µM of the Alki SB-525334 were added to different hSAEC donors. Ecad expression was quantified 72h later. TGFß-1 at a concentration of 10ng/ml maximally inhibited Ecad expression, which was reversed by addition of an Alk5i. Compared to the other donor the assay window for the additional donors was smaller.

## Table S1

| | | | |
|---|---|---|---|
| **Find Nuclei** | **Channel : DAPI**<br>**ROI : None** | **Method : B**<br>Common Threshold : 0.4<br>Area : > 30 µm²<br>Split Factor : 7<br>Individual Threshold : 0.4<br>Contrast : > 0.1 | Output Population : Cells |
| **Find Cytoplasm** | **Channel : FITC2**<br>**Nuclei : Cells** | **Method : F**<br>Membrane Channel : Cy5<br>Individual Threshold : 0.1 (, numeric) | |
| **Find Surrounding Region** | **Channel : FITC2**<br>**Population : Cells**<br>**Region : Cell** | **Method : B**<br>Common Threshold : 0.2<br>Individual Threshold : 0.15<br>Include Input Region | Output Region : Full Cell |
| **Filter Image** | **Channel : Cy5** | **Method : Texture SER**<br>Filter : SER Ridge<br>Scale : 1 px<br>Normalization by : Region Intensity | Output Image : SER Ridge |
| **ABB: Find Simple Image Region** | **Channel : SER Ridge**<br>**Threshold : 0.1**<br>**Output Population : Brights** | | |
| **Select Region (2)** | **Population : Cells**<br>**Region : Full Cell** | **Method : Restrict by Mask**<br>Population : Brights<br>Mask Region : Region | Output Region : Candidate Fibers |
| **Modify Population** | **Population : Cells**<br>**Region : Candidate Fibers** | **Method : Cluster by Distance**<br>Distance : 0 px (fixed, menu)<br>Area : > 15 (, numeric) px² | Output Population : Candidates<br>Output Region : Region |
| **Calculate Intensity Properties** | **Channel : Cy5**<br>**Population : Candidates**<br>**Region : Region** | **Method : Standard**<br>Mean<br>Contrast | Output Properties : Alexa 647 |

| | | | |
|---|---|---|---|
| **Calculate Morphology Properties (3)** | **Population : Candidates**<br>**Region : Region** | **Method : Standard**<br>Area<br>Roundness<br>Length | Output Properties : length |
| **Select Population** | **Population : Candidates** | **Method : Filter by Property**<br>Alexa 647 Mean : > 300<br>Alexa 647 Contrast : > 0<br>length Length [µm] : > 10<br>length Roundness : <= 0.7<br>Boolean Operations : F1 and F2 and F3 and F4 | Output Population : Fibers |
| **Select Region (3)** | **Population : Cells**<br>**Region : Full Cell** | **Method : Restrict by Mask**<br>Population : Fibers<br>Mask Region : Region | Output Region : Fibers |
| **Calculate Morphology Properties** | **Population : Cells**<br>**Region : Fibers** | **Method : Standard**<br>Area | Output Properties : Fibers |
| **Select Region** | **Population : Cells**<br>**Region : Fibers** | **Method : Standard**<br>Skeleton | Output Regions : Fibers |
| **Calculate Morphology Properties (2)** | **Population : Cells**<br>**Region : Fibers Skeleton** | **Method : Standard**<br>Area | Output Properties : Fibers Skeleton |
| **Calculate Intensity Properties (2)** | **Channel : Cy5**<br>**Population : Cells**<br>**Region : Fibers** | **Method : Standard**<br>Mean | Output Properties : Fibers Alexa 647 |
| **Calculate Properties** | **Population : Cells** | **Method : By Related Population**<br>Related Population : Fibers<br>Number of Fibers | Output Properties : per Cell |
| **ABB: Find Simple Image Region (2)** | **Channel : TexasRed**<br>**Threshold : 2000 (, numeric)**<br>**Output Population : Fibronectin** | | |

| | | | |
|---|---|---|---|
| **Calculate Morphology Properties (4)** | **Population : Collagen/Fibronectin** | **Method : Standard** | Output Properties : Collagen/Fibronectin_Region |
| | **Region : Region** | Area | |
| | | Roundness | |

| | | | |
|---|---|---|---|
| **Select Population (2)** | **Population : Collagen/Fibronectin** | **Method : Filter by Property** | Output Population : Fibronectin Selected |
| | | Collagen/Fibronectin_Region Area [µm²] : > 25 | |

| | |
|---|---|
| **Define Results** | **Method** |
| | **Population : Cells** |
| | Number of Cells |
| | **Method** |
| | **Method : Formula Output** |
| | Formula : a/b |
| | Population Type : Objects |
| | Variable A : Fibers - Number of Objects |
| | Variable B : Cells - Number of Objects |
| | Output Name : Number of Fibers per Cell |
| | **Method** |
| | **Method : Formula Output** |
| | Formula : a/b |
| | Population Type : Objects |
| | Variable A :Collagen/Fibronectin Selected - Collagen/Fibronectin_Region Area [µm²] Sum (fixed, menu) |
| | Variable B : Cells - Number of Objects |
| | Output Name : Collagen/Fibronectin_SUM/Nuclei_SUM |

## Table S2

| | | | |
|---|---|---|---|
| **Find Nuclei** | **Channel : HOECHST 33342**<br>**ROI : None** | **Method : B**<br>Common Threshold : 0.4<br>Area : > 30 $\mu m^2$<br>Split Factor : 7<br>Individual Threshold : 0.4<br>Contrast : > 0.1 | Output Population : Cells |
| **Find Cytoplasm** | **Channel : Alexa 488**<br>**Nuclei : Cells** | **Method : F**<br>Membrane Channel : Alexa 488<br>Individual Threshold : 0.15 | |
| **Find Surrounding Region** | **Channel : Alexa 647**<br>**Population : Cells**<br>**Region : Cell** | **Method : B**<br>Common Threshold : 0.2<br>Individual Threshold : 0.15<br>Include Input Region | Output Region : Full Cell |
| **Calculate Morphology Properties (5)** | **Population : Cells**<br>**Region : Full Cell** | **Method : Standard**<br>Area<br>Roundness | Output Properties : Full Cell |
| **Calculate Intensity Properties (4)** | **Channel : Alexa 488**<br>**Population : Cells**<br>**Region : Full Cell** | **Method : Standard**<br>Mean | Output Properties : Alexa 647 |
| **Filter Image** | **Channel : Alexa 647** | **Method**<br>**Method : Texture SER**<br>Filter : SER Ridge<br>Scale : 2px<br>Normalization by : Region Intensity | Output Image : SER Ridge |
| **ABB: Find Simple Image Region** | **Channel : SER Ridge**<br>**Threshold : 0.02 (, numeric)**<br>**Output Population : Ecad** | | |
| **Select Region (2)** | **Population : Cells**<br>**Region : Full Cell** | **Method : Restrict by Mask**<br>Population : Ecad<br>Mask Region : Region | Output Region : Candidate Ecad |

| | | | |
|---|---|---|---|
| **Modify Population** | **Population : Cells**<br>**Region : Candidate Ecad** | **Method : Cluster by Distance**<br>Distance : 0 µm<br>Area : > 30 µm$^2$ | Output Population : Candidates Ecad<br>Output Region : Region |
| **Calculate Intensity Properties** | **Channel : Alexa 647**<br>**Population : Candidates Ecad**<br>**Region : Region** | **Method : Standard**<br>Mean<br>Sum | Output Properties : Alexa 647 |
| **Calculate Morphology Properties (3)** | **Population : Candidates Ecad**<br>**Region : Region** | **Method : Standard**<br>Area | Output Properties : A-Candidates_Ecad |
| **Calculate Properties (3)** | **Population : Candidates Ecad** | **Method : By Formula**<br>Formula : A*B<br>Variable A : Alexa 647 Sum<br>Variable B : A-Candidates_Ecad Area [µm$^2$] | Output Property : I*A_Candidates_Ecad |
| **Define Results** | **Population : Cells**<br>Number of Objects<br>**Method : Formula Output**<br>Formula : a*b/c<br>Population Type : Objects<br>Variable A : Candidates Ecad - Alexa 647 Sum Sum<br>Variable B : Candidates Ecad - A-Candidates_Ecad Area [µm$^2$] Sum<br>Variable C : Cells - Number of Objects<br>Output Name : I_Ecad_Sum*A_Ecad_sum/cell number<br>**Population : Ecad : None**<br>Population : Cells : None<br>Population : Candidates Ecad : None | | |