

Figure S2: (A) Representative live confocal images (20X air objective) of hPCLS incubated with Calcein AM (labels live cells) and Ethidium homodimer (Dead cells) and quantification live tissue (Calcein signal) and dead cell count (ethidium homodimer) Error bars represent SEM, and p-values were calculated using ordinary one-way ANOVA, $**p < 0.001$. Note each hPCLS was imaged only once during ex vivo culture. 3 hPCLS were analysed per day from 3 donors over all. Each dot represents a field of view imaged (5-6 fields of view were imaged/ hPCLS/ day). Scale bar 250 μ m (B) Significantly regulated genes on day 13 compared to day 1 were subjected to Cytoscape pathway analysis. The number of genes in each enriched GO terms present in our data set was plotted. Term Pvalue represents the pvalue for association of these genes to whole of GO term. % associated genes, is the percentage of our genes overlapping with all of the members of a given GO term. (C) Significantly regulated genes (day 13 vs. Day 1) were compared with unique cell type markers as established in LGEA²⁵ study. A, B, C and D mark comparisons of day4 vs day1, day7 vs day1, day10 vs day1 and day13 vs day1 respectively. Commonly used lung cell-type markers detected in our proteomics set-up (D) Volcano plots show Log₂FC of all the genes detected against their respective *fdr*. Red dots represent highly significant genes, while as blue are those regulated that are significant but moderate in the level of change. (E) Metabolites detected upon LC/MS were similarly plotted as in (D). (F) Represent individual metabolites and their change over days in ex vivo culture. (G) ATP level measured using ATP glo assay. Coloured dots represent hPCLS from the same donor.

Figure S2

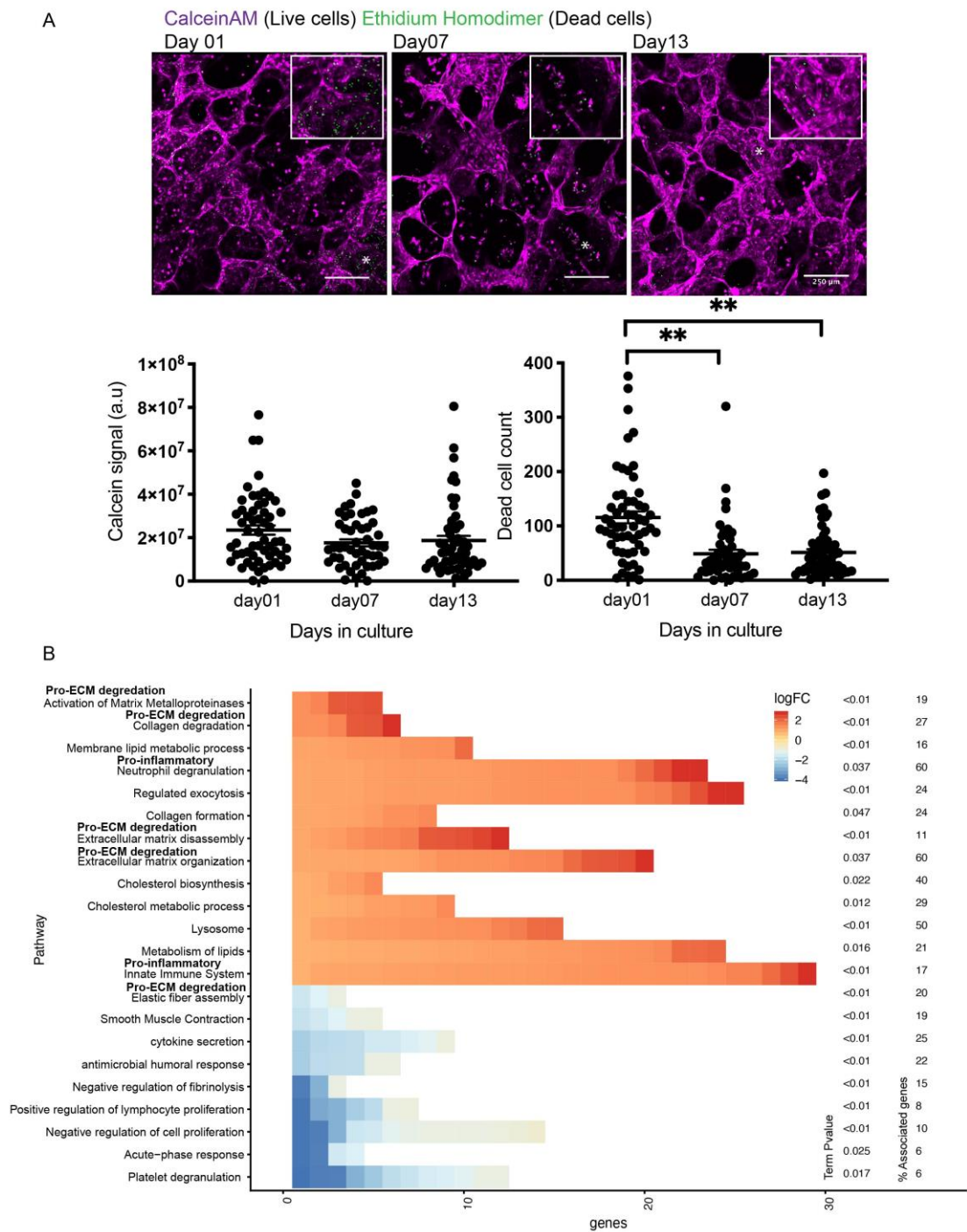


Figure S2 C

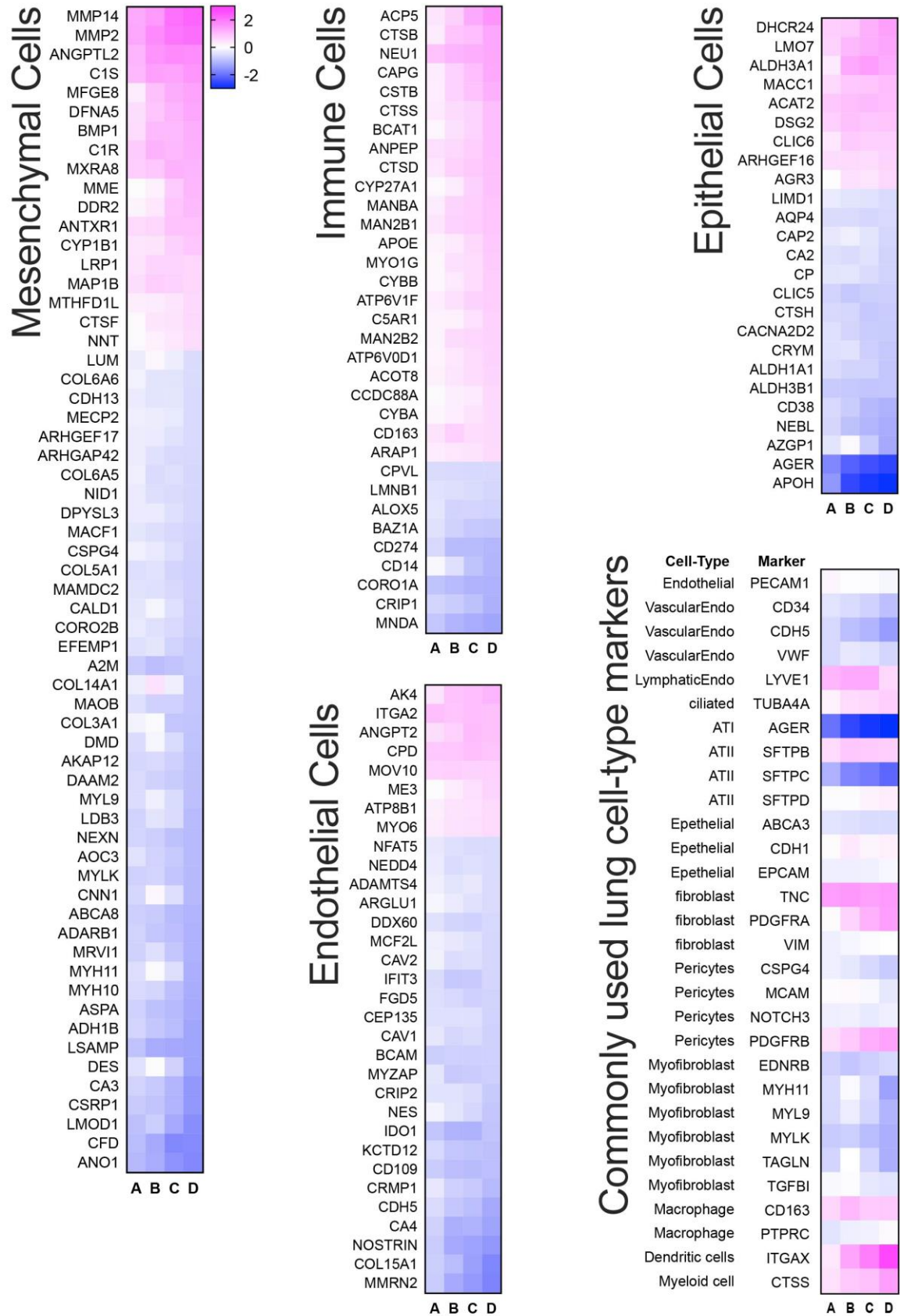


Figure S2

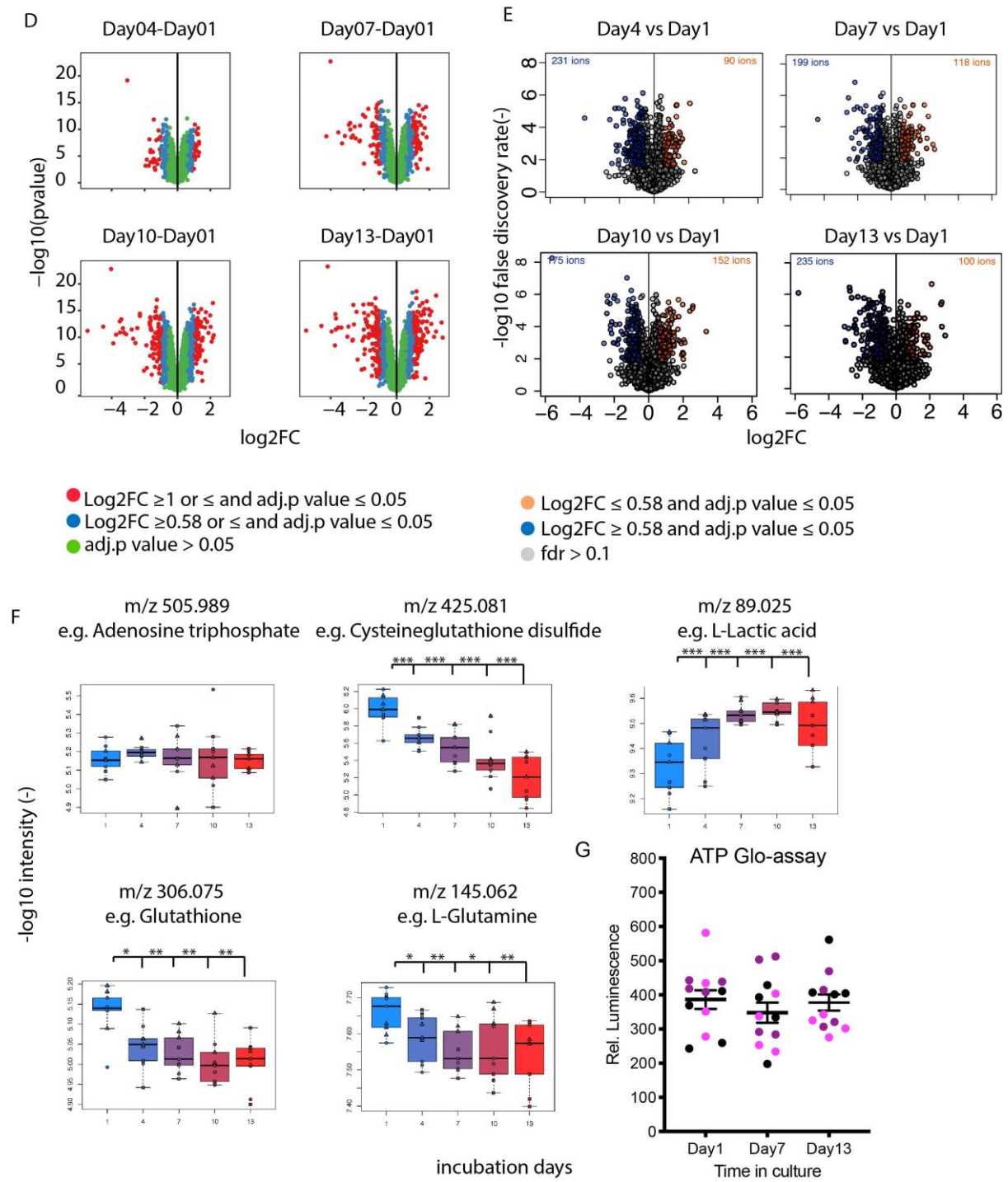
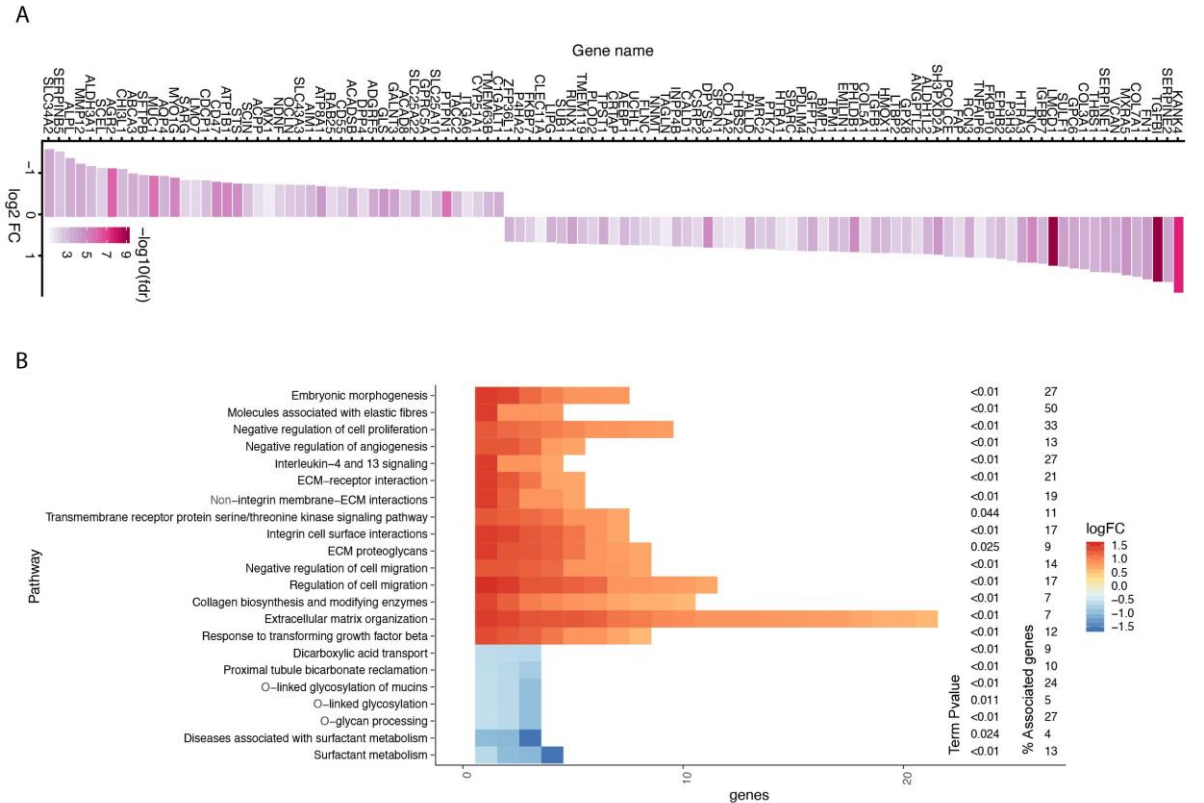


Figure S4: (A) List and Log2FC level of significantly regulated genes upon TGFβ1 treatment compared to unstimulated (day 13) (B) Pathway analysis of significantly regulated genes upon TGFβ1 treatment compared to unstimulated hPCLS (day 13). The analysis was carried out using Cytoscape. (C) Significantly regulated genes were compared to different cell-type markers as established by LGEA²⁵ study and log2FC were plotted as a heat map.

Figure S4



Significantly regulated proteome and related signalling pathways upon TGFbeta stimulation of ex vivo cultured hPCLS

Figure S4

C

Epithelial and Mesenchymal Cell class analysis of TGF β 1 (vs Day13 vehicle treated) treated hPCLS proteome

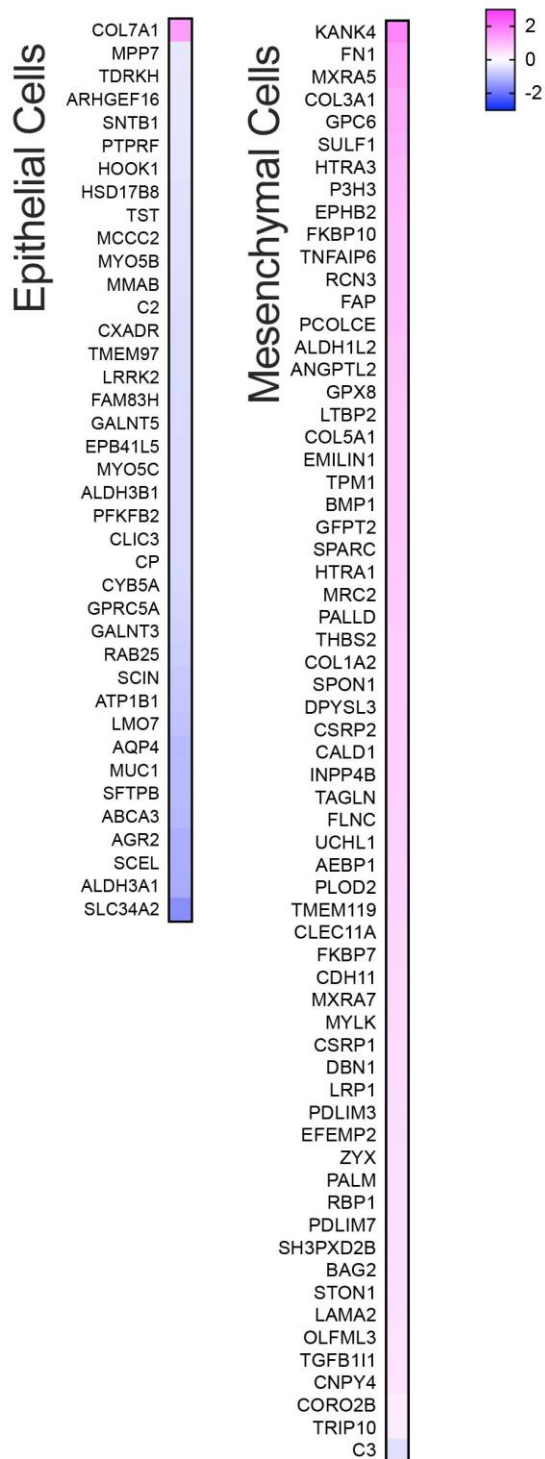


Figure S5: (A) Raw SHGintensity values of whole hPCLS from non-ILD patients and ILD patients. #, non-ILD SHGintensity values plotted on a different scale (B) Raw SHGintensity values of whole hPCLS from all 18 donors (unstimulated condition, 3-6 hPCLS/ donor). Color of the dots represent same donor. (Ci) Log2SHGi values of interstitial collagen (ROI analysis) to the amount of interstitial fibrillar collagen in vehicle-treated hPCLS on day 13. Upon global normalization (to mean of all donors), data shows below average fibrillar collagen amounts in male interstitium. (Cii) Log2SHGi values show that ex-smoker donors have significantly more interstitial collagen. (Ciii) Log2SHGi values show that donors grouped in age group equal to or above 65 years also show above average interstitial fibrillar collagen. Significance was calculated using one-way annova (*p value< 0.05). Donor id: All vehicle-treated donors from Figure 6 Bi. (D-E) Gender, smoking status and age specific breakdown of Log2SHGi values of fibrillar collagen of donors upon vehicle and TGFβ1 treatment.

Figure S5

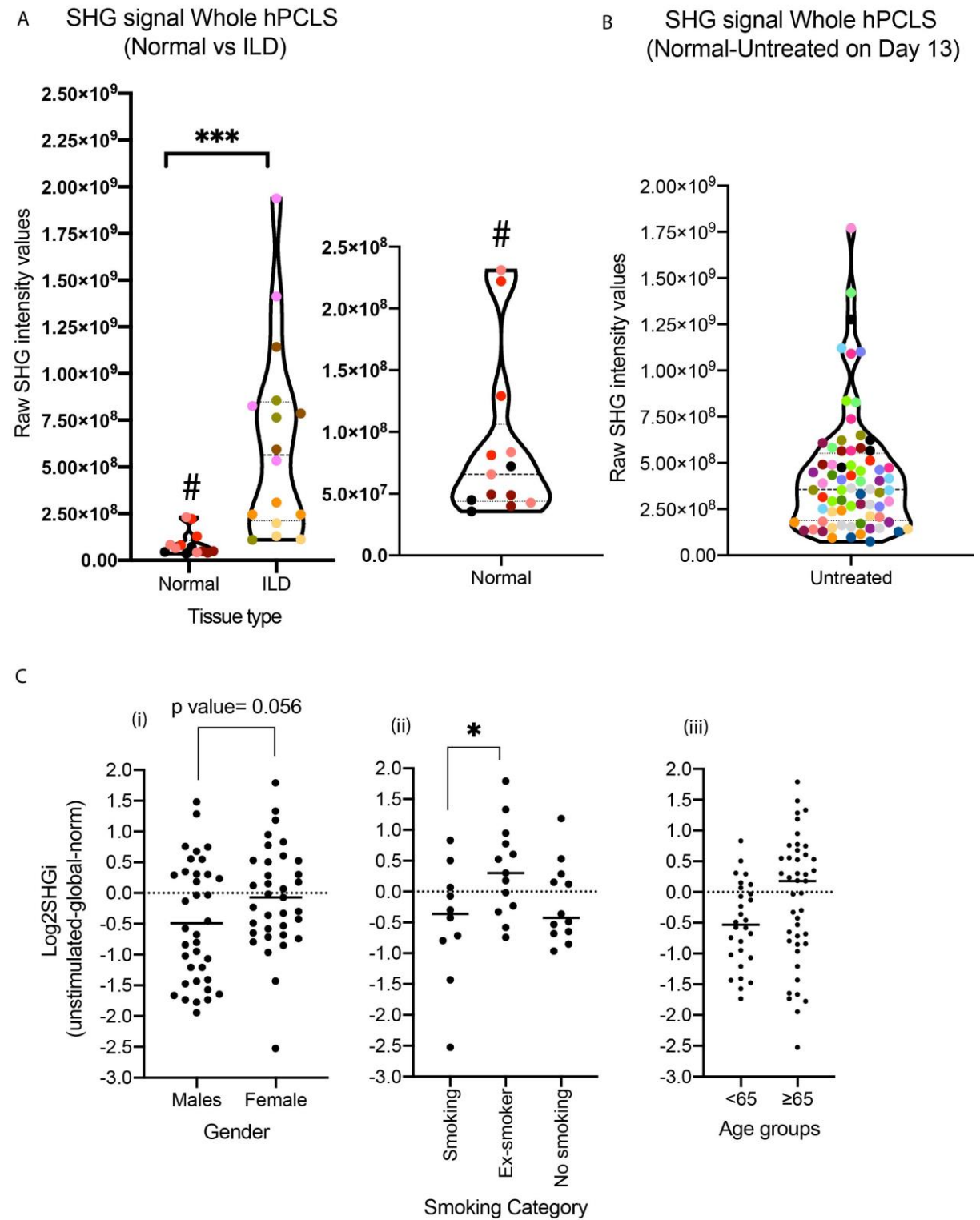
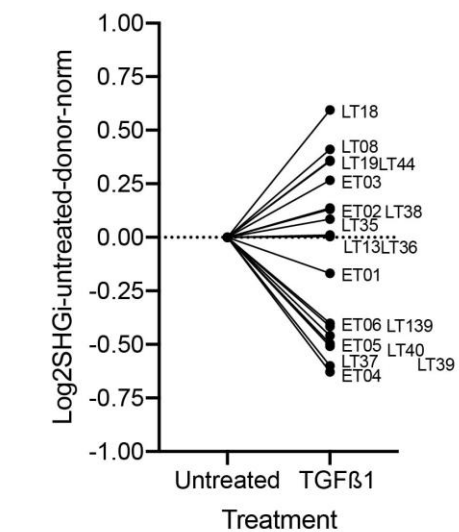


Figure S5D

Donor centric analysis of TGFβ1 response:



Gender centric analysis of TGFβ1 response:

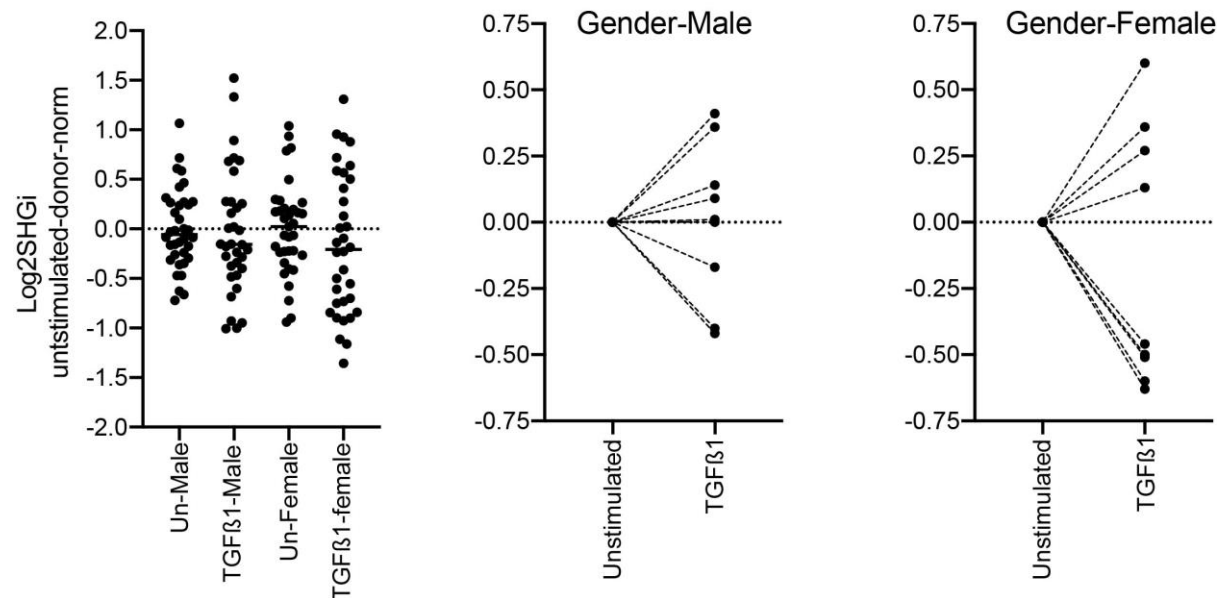
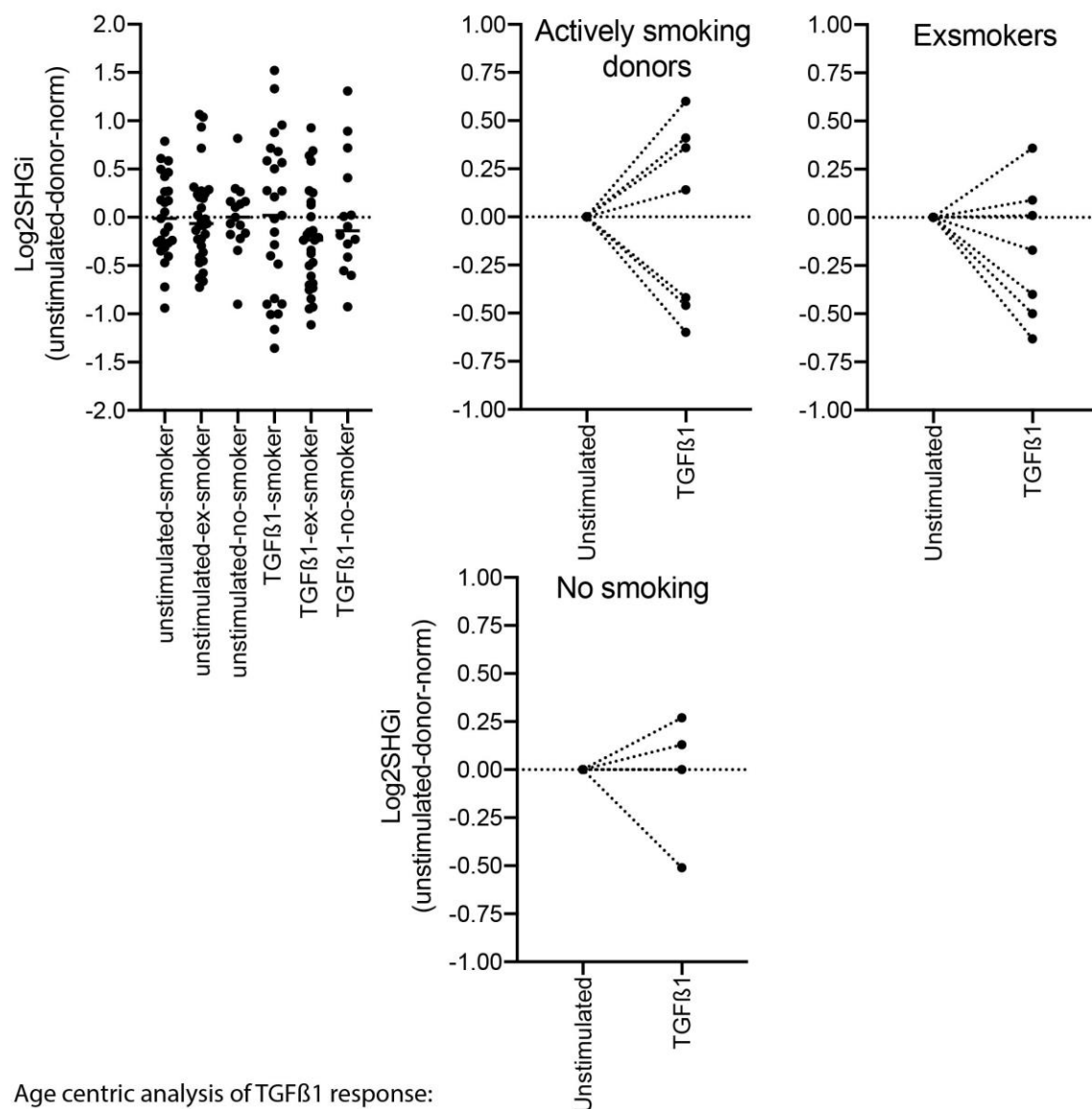


Figure S5E Smoking status centric analysis of TGF β 1 response:



Age centric analysis of TGF β 1 response:

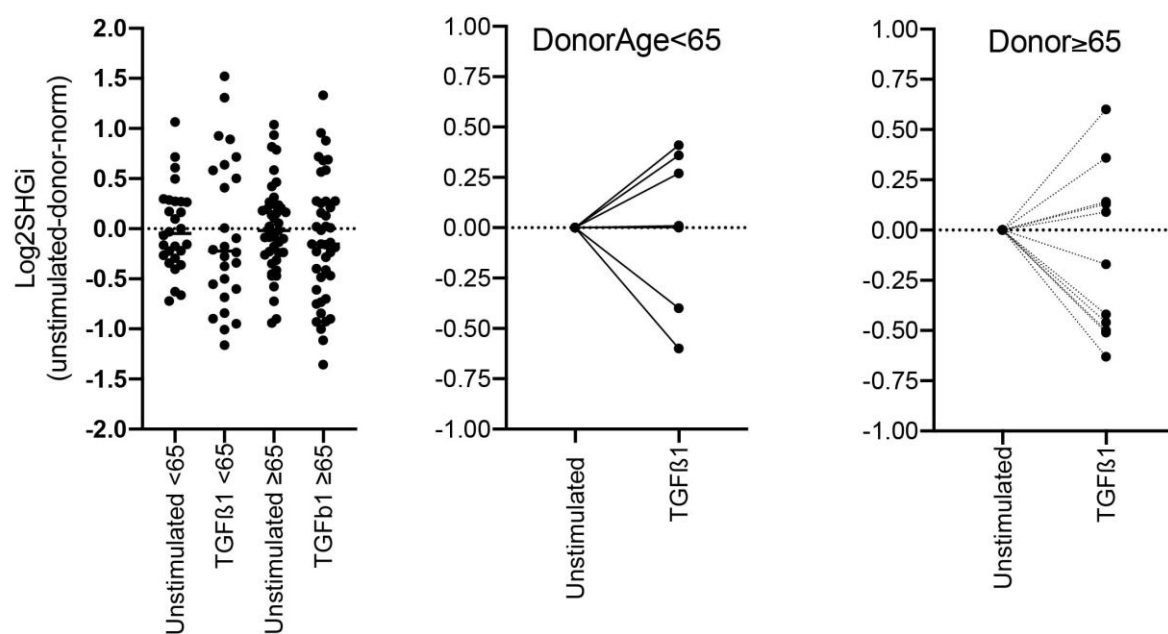


Figure S6: (A) Maximum projection images of 2-photon excited autofluorescence from the same hPCLS on different days shows that we can reliably find the same regions. Representative SHG images of hPCLS show that selective regions of hPCLS have increased fibrillar collagen deposition upon TGF β 1+MMPi treatment (white asterisks). B-C) Quantification of raw SHG values of hPCLS with only vehicle treatment (unstimulated) and, D-E) TGF β 1+MMPi treatment. B & D) Raw SHG intensity values on day 01 and day 13 in untreated and TGF β 1+MMPi treated hPCLS respectively. C & E) Day 01 normalised values on day 01 and day 13 in untreated and TGF β 1+MMPi treated hPCLS respectively. E) Day 1 normalised SHG values on day 13 show an increase in SHG signal upon TGF β 1+MMPi treatment for 2 weeks. F) Representative ROIs showing increased fibrillar collagen deposition (ROIs are zoomed asterisk marked region of Figure S6A SHG channel). Different colors represent different donors. Donor IDs: LT62, LT63. Scale bar 500 μ m. Note: For Untreated condition, only 1 hPCLS was analysed for LT62 donor.

Figure S6: Live time lapse imaging

