

## **Supplementary information**

### **Matrix Characterization**

#### **Cytochemistry**

Alcian blue staining for ECM-bound glycosaminoglycans (GAGs) before and after decellularization was done as follows. Cultures were fixed in 4% formaldehyde and permeabilized with 0.5% Triton-X100 for 30 min, washed two times with PBS and rinsed in 3% acetic acid (pH 2.5) for five minutes to equilibrate the pH. Samples were then incubated with 1% alcian blue for 2 h, washed in 3% acetic acid, followed by 3% acetic acid/50% ethanol, and two water wash. Presence of GAGs was verified by the accumulation of blue stain as observed under phase microscope with 10X magnification.

The retention of a collagenous matrix following the  $\text{NH}_4\text{OH}$  treatment was verified by positive staining for collagen by light green. Briefly, samples were washed three times in PBS and fixed in ice cold 75% ethanol for 10 min, incubated with light green stain at 25°C for 30 min on orbital shaker before being washed with ddH<sub>2</sub>O until no leaching was detected. This was followed by 75% ethanol wash to remove non-specific dye and then dried at 25°C and were examined for the presence of greenish-blue stain under phase microscope with 10X magnification.

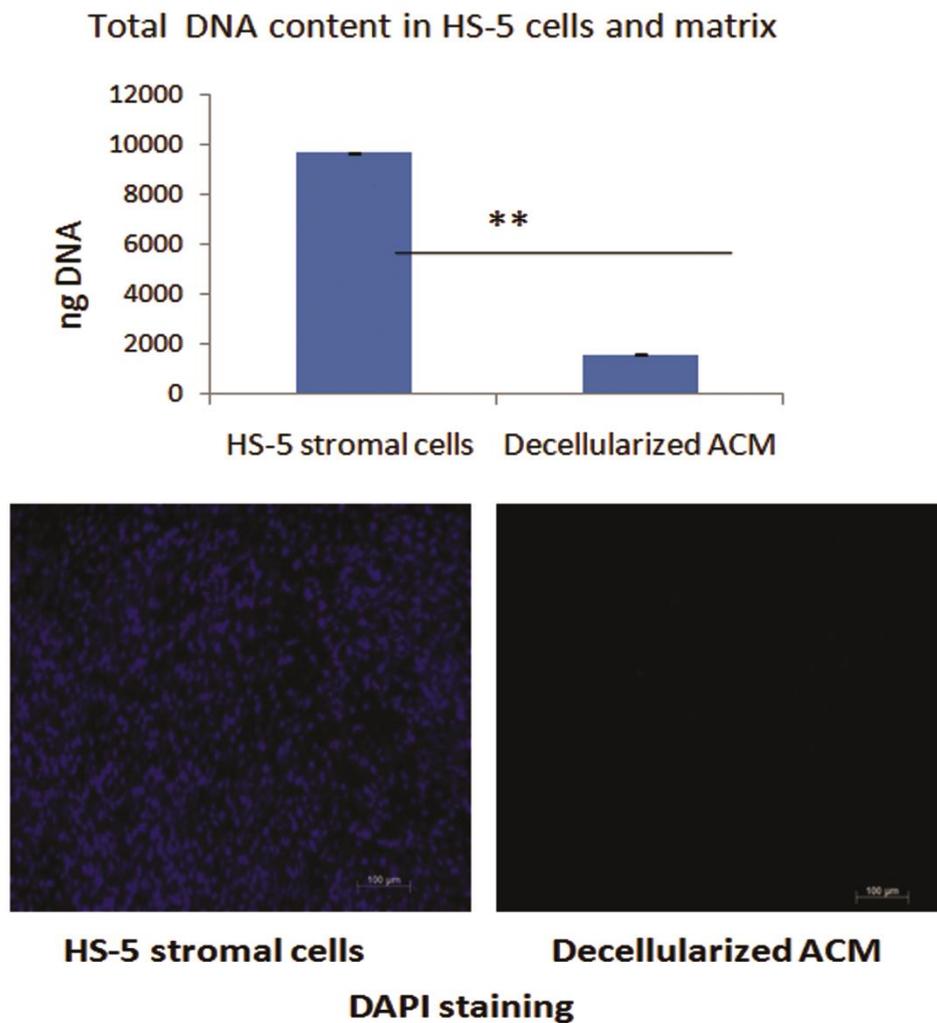
## **HSPC expansion**

### **Colony Forming Assays**

Colony forming assays were performed for all cell samples, as triplicate cultures in Methocult media (H4434) in 12 well plates, to assess the numbers of colony forming units (CFUs) for granulocyte, erythrocyte, macrophage, megakaryocyte common precursors (CFU-GEMM), granulocyte/macrophage common precursors (CFU-GM), and burst forming units (BFU-E) for pure erythroid precursors.  $2 \times 10^3$  viable cells were suspended in the media, plated per well as per the manufacturer's instructions and incubated for 14 days in 5% CO<sub>2</sub> and 20% O<sub>2</sub> at 37°C. CFUs were manually counted under light microscope with 5X magnification following 14 days of incubation. Statistical significance of the variation in CFU numbers was based upon data from three independent experiments. Fold expansion of CFUs in each culture was calculated as explained for marker-based cell expansion.

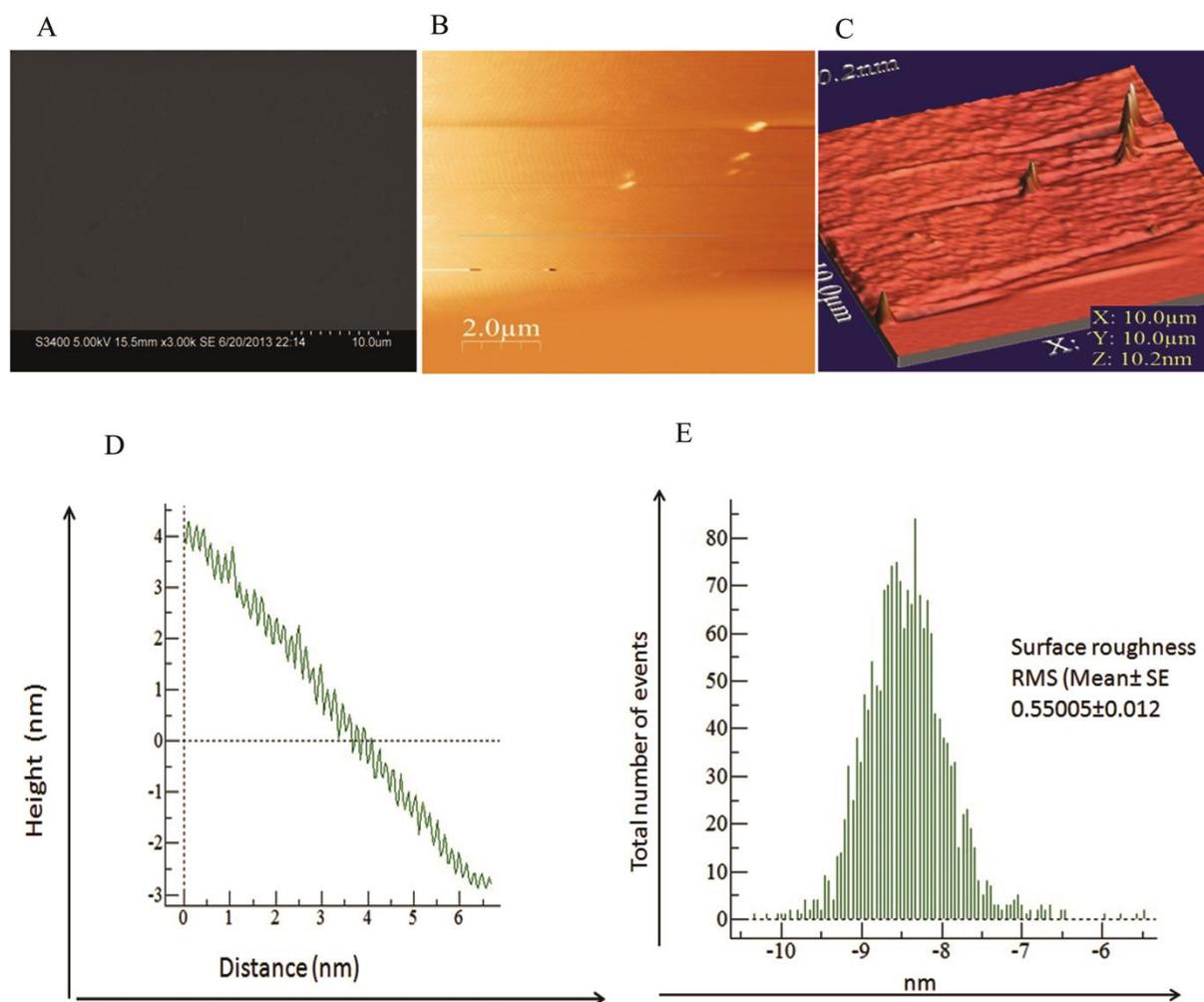
**Supplementary figures**

Figure S1-



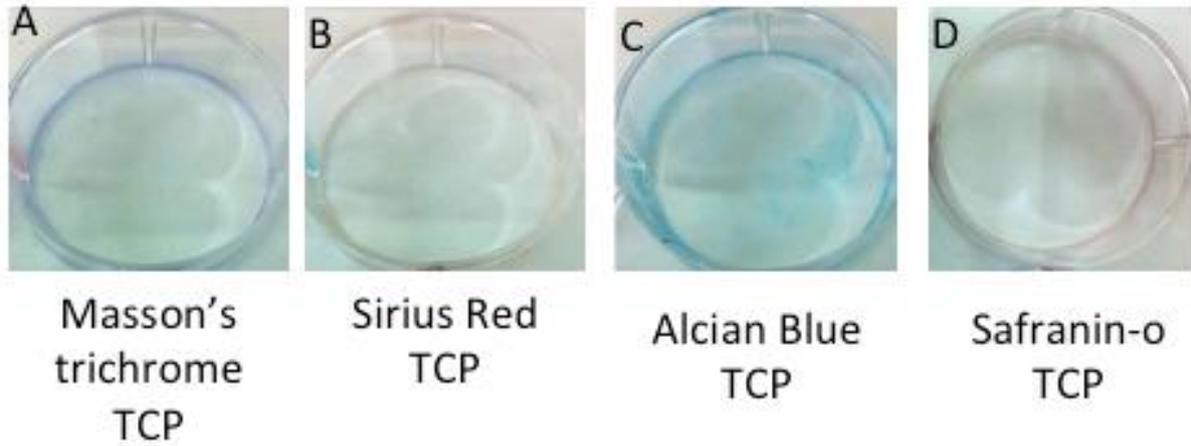
**Figure S1.** A) DNA quantification in HS-5 stromal cells and decellularized ACM. (\*\* $p < 0.01$ ). B) Nuclear staining with DAPI— left image depicts HS-5 stromal cells, right image depicts decellularized ACM.

Figure S2



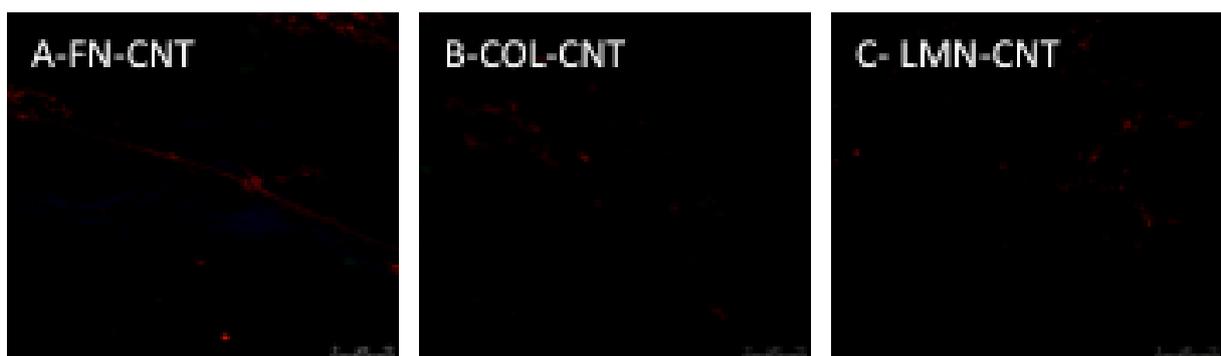
**Figure S2.** SEM and AFM analysis of blank coverslip. A) SEM image, B) 2D AFM image, C) 3D AFM image, D) Analysis of height variation in blank coverslip by AFM line scan, E) Surface roughness analysis of blank coverslip in AFM.

Figure S3



**Figure S3-** Cytochemical stainings on blank tissue culture plates as negative controls –A) Masson's trichrome , B) Sirius Red, C) Alcian Blue D) Safranin-o

Figure S4



**Figure S4-**Negative controls – Immunofluorescence stainings A) Fibronectin control B) Collagen control C) Laminin control stainings

## Supplementary Table

**Table S1.** Total protein estimation in decellularized ACM (each one well of 24 well tissue culture plate)

	1	2	3	Avg
µg/per well	44.6	58.8	67.2	56
STDEV	±9.2	±2.7	±15.8	±9

**Table S2.** Matrix surface thickness measured by AFM in native ACM and spin coated ACM

	Matrix Thickness Native ACM (nm)	Matrix Thickness SPIN COAT ACM (nm)
1	600	140
2	800	180
3	350	160
Avg (Mean ±SE)	583 ± 130	160 ±11

