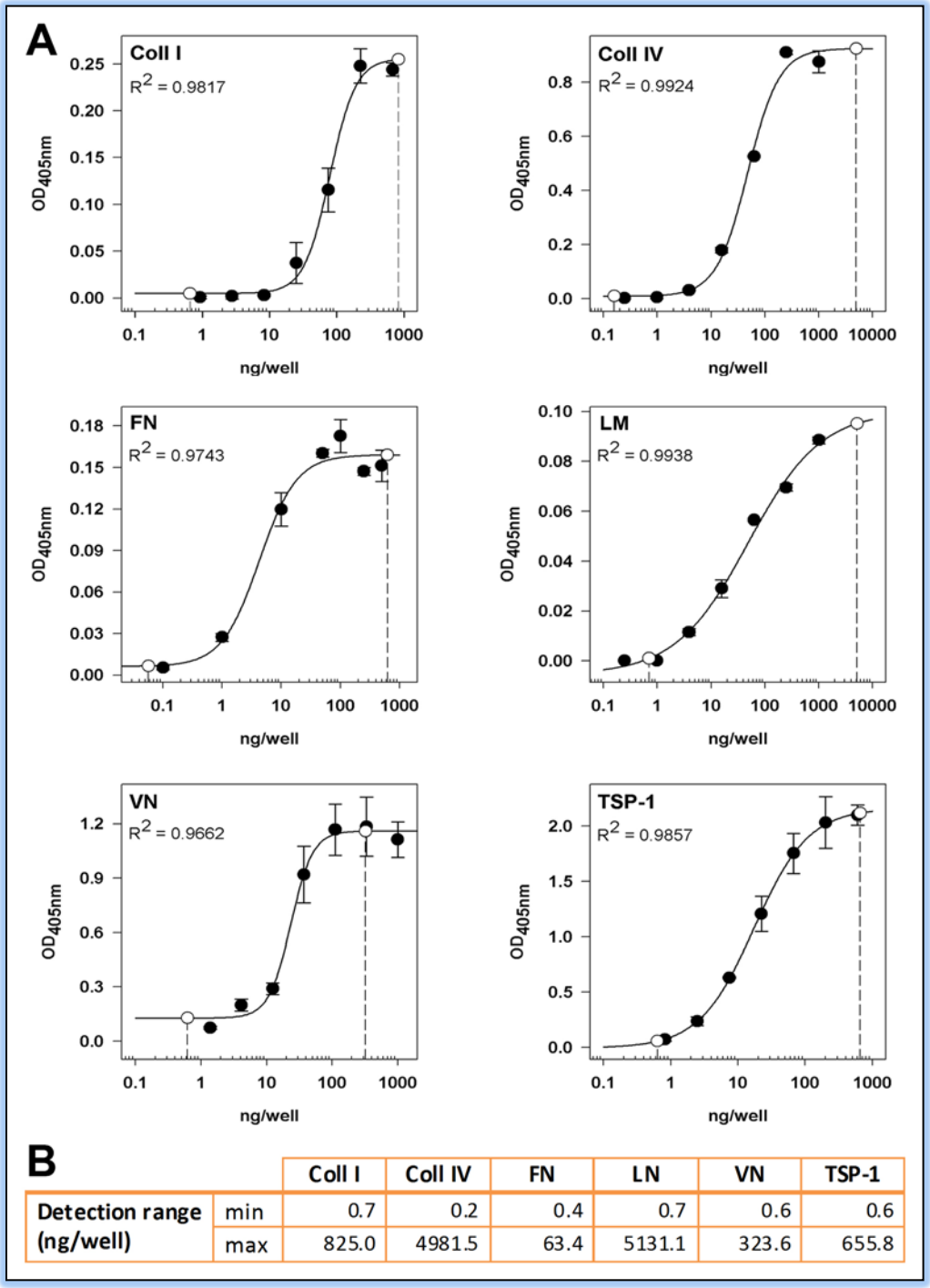
Supplemental Figures

S-Fig 1

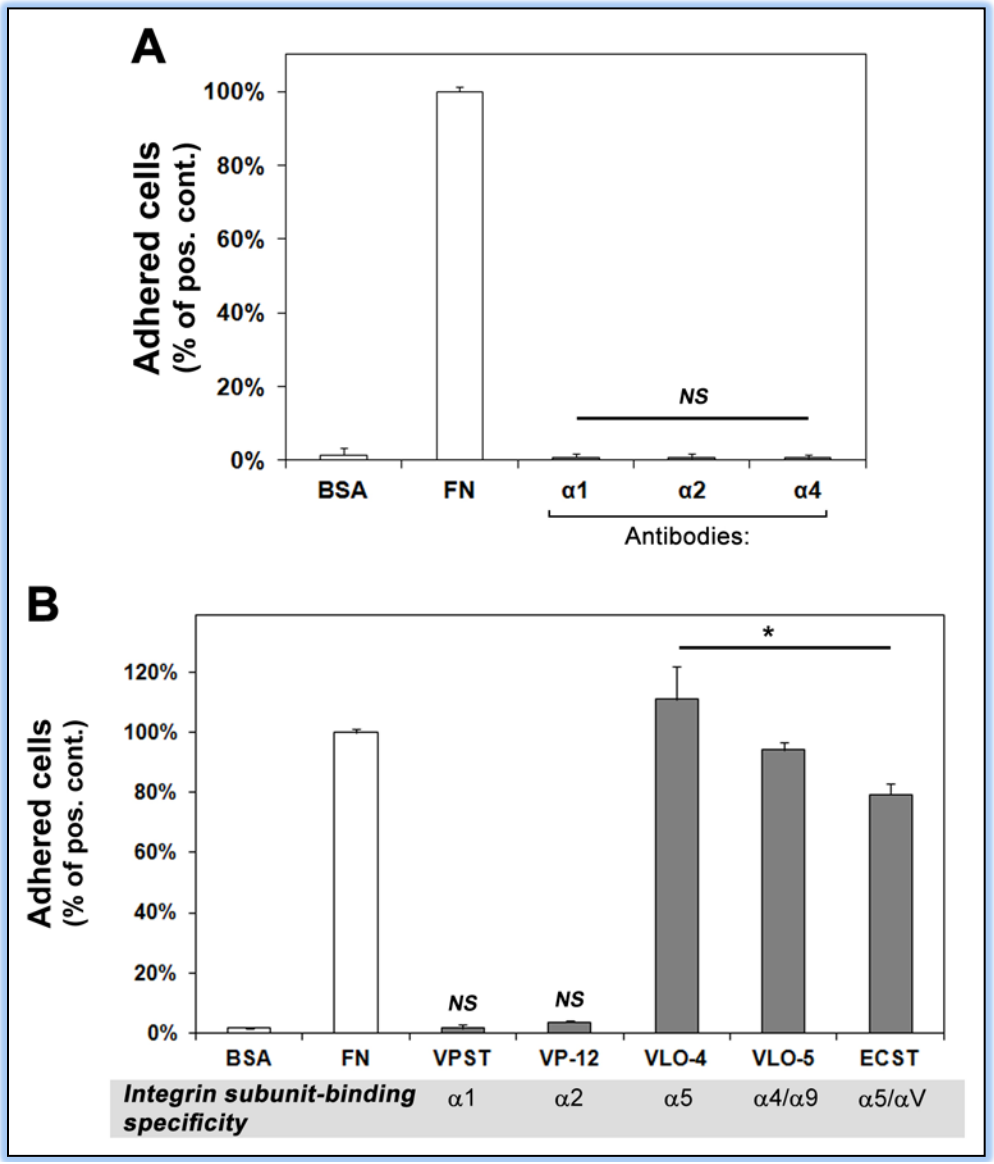


B

		Coll I	Coll IV	FN	LN	VN	TSP-1
Detection range (ng/well)	min	0.7	0.2	0.4	0.7	0.6	0.6
	max	825.0	4981.5	63.4	5131.1	323.6	655.8

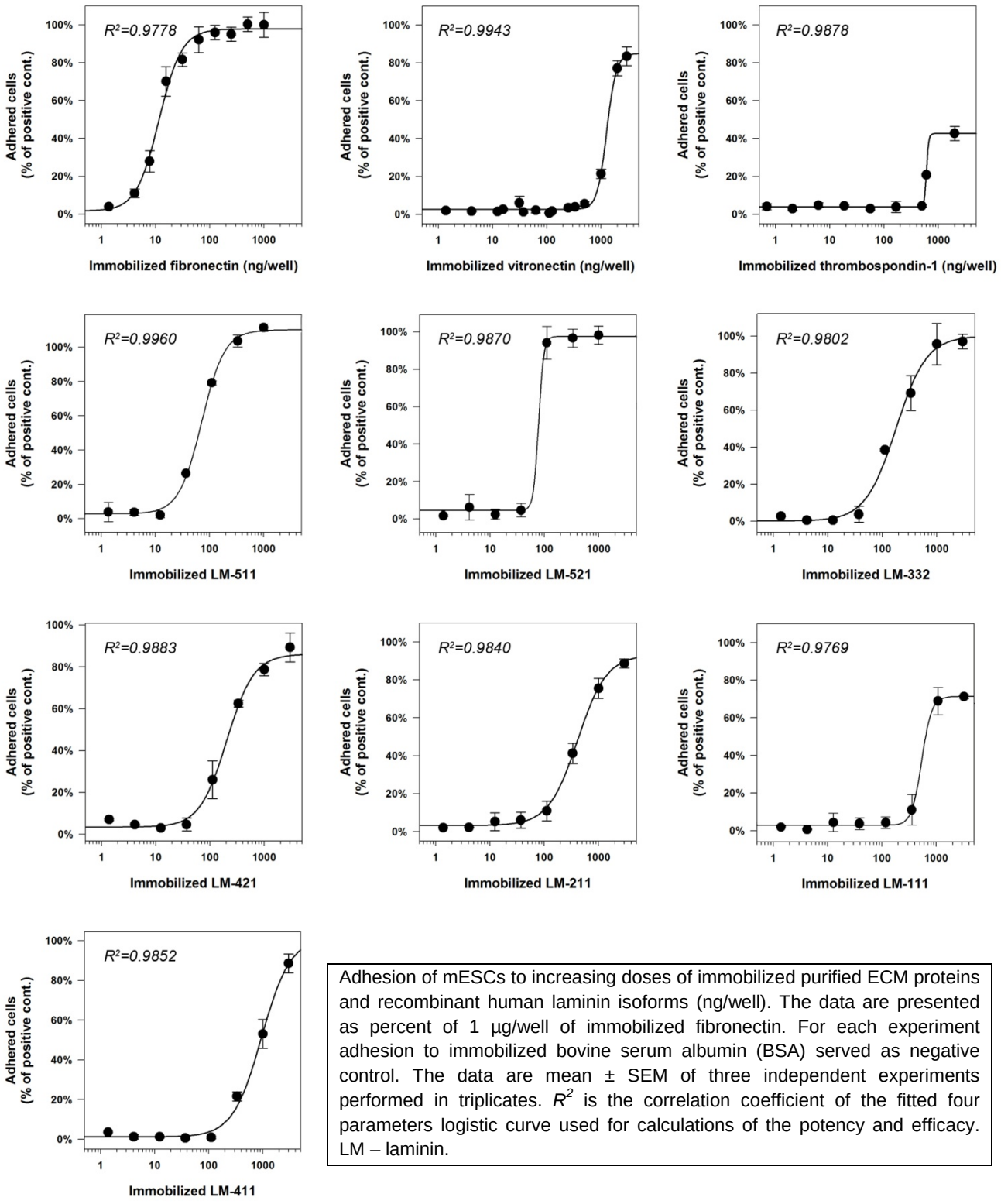
(**A**) Calibration curves of ELISA assays for immobilized collagen type I (Coll I), collagen type IV (Coll IV), fibronectin (FN), laminin (LM), vitronectin (VN) and thrombospondin (TSP-1). The calibration curves were prepared independently for each experiment and the data are mean ± SD of a representative experiment (n=3).. Black circles – concentrations used for generating the calibration curve. White circles – detection limits. R^2 is the correlation coefficient of the four parameters logistic curve fitted to calculate detection range and the unknown concentration of ECM proteins in CMs. (**B**) Detection range (ng/well) of the ECM proteins in ELISA assays. Values that fell below the minimal value were considered undetectable, while in case of values above the maximal detection limit the samples were diluted and independently reprobed.

S-Fig 2



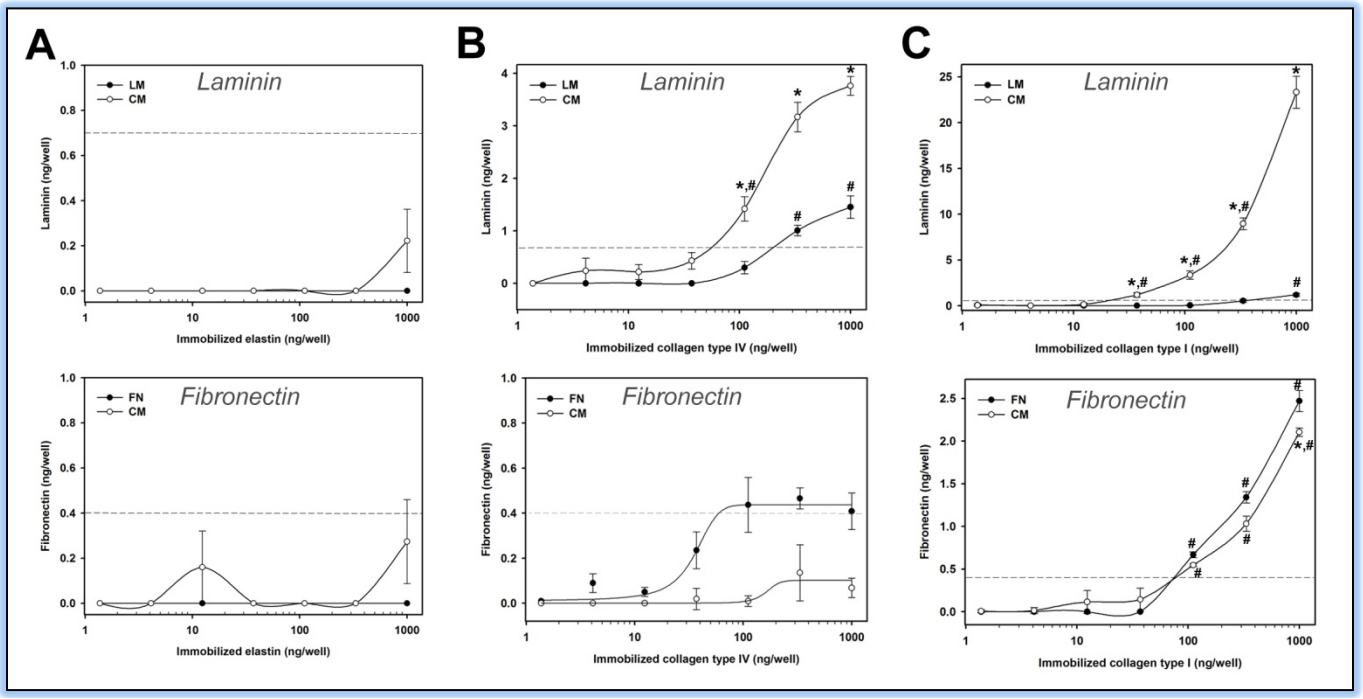
Adhesion assay of mESCs on immobilized 1 ug/well of bovine serum albumin (BSA) and fibronectin (FN), white bars; and (**A**) immobilized antibodies specific for α1, α2 and α4 integrin receptor subunits (grey bars); and (**B**) immobilized 2 ug/well of the disintegrins viperistatin (VPST); VP-12; VLO-4; VLO-5 and echistatin (ECST). The data presented as percent of 1 ug/well of immobilized FN, which served as positive control while BSA was used as negative control. The data are mean ± SEM of least three independent experiments in triplicates. *$p<0.01$ vs. BSA. *NS* – not significant vs. BSA.

S-Fig. 3



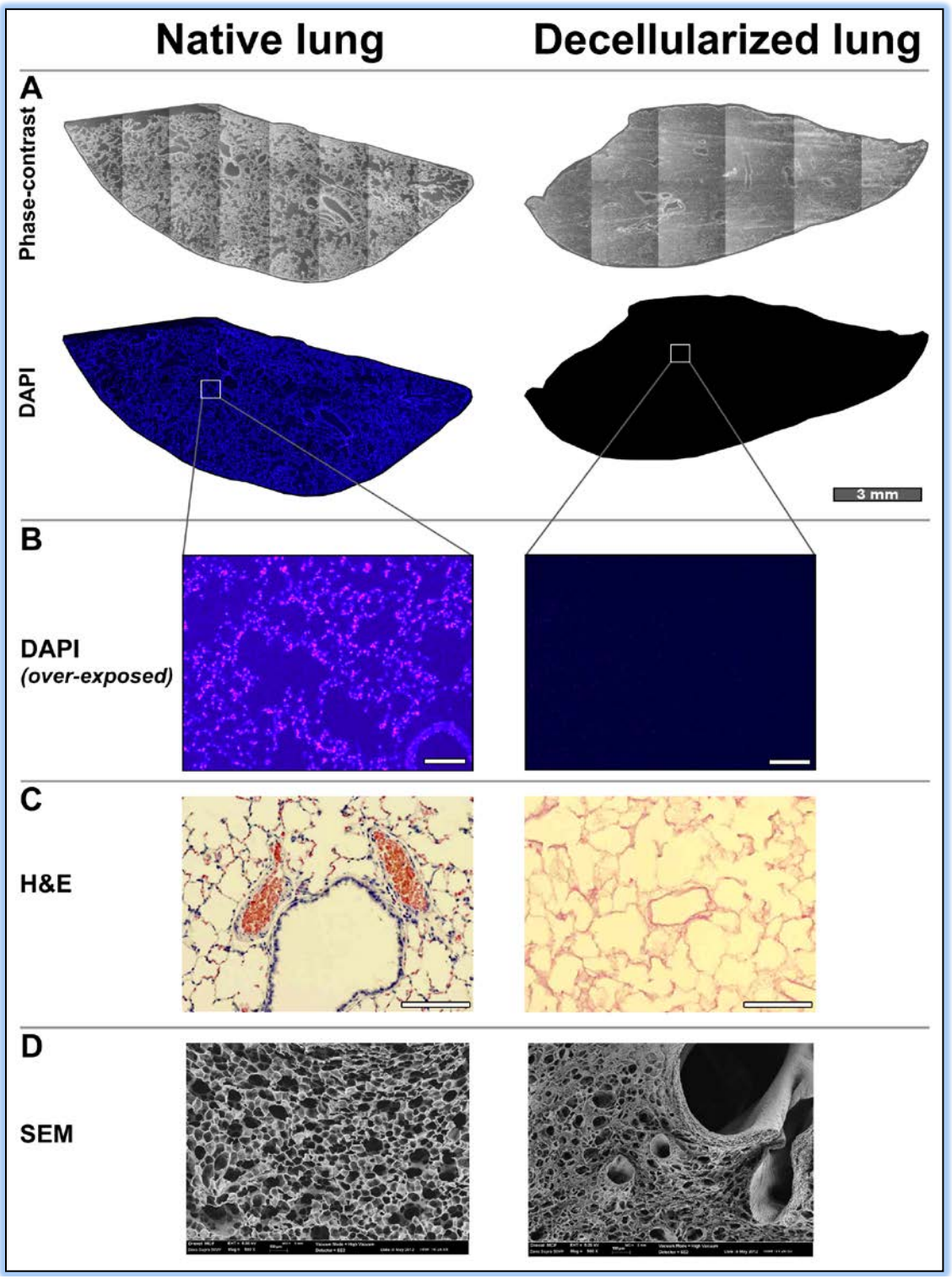
Adhesion of mESCs to increasing doses of immobilized purified ECM proteins and recombinant human laminin isoforms (ng/well). The data are presented as percent of 1 µg/well of immobilized fibronectin. For each experiment adhesion to immobilized bovine serum albumin (BSA) served as negative control. The data are mean ± SEM of three independent experiments performed in triplicates. R^2 is the correlation coefficient of the fitted four parameters logistic curve used for calculations of the potency and efficacy. LM – laminin.

S-Fig. 4



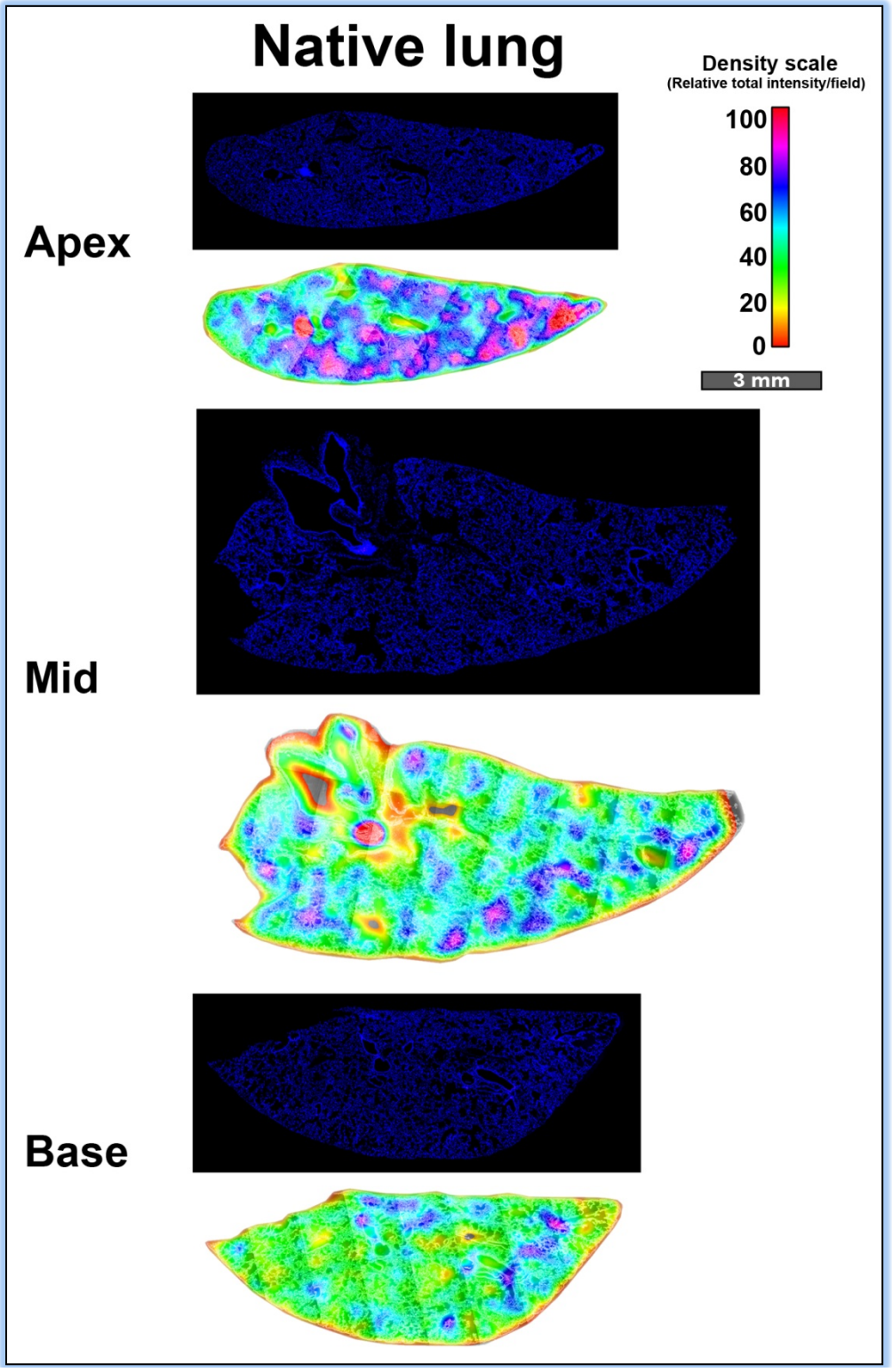
Quantitative analysis using ELISA of the interactions of CM-derived laminin (upper panel) and fibronectin (lower panel) with increasing doses of immobilized elastin (**A**), collagen type IV (**B**) and collagen type I (**C**). Upon immobilization of the indicated ECM proteins, 100 ul of 100% CM or 1 ug/well of laminin (LM) or fibronectin (FN) were allowed to interact for 1 h and followed by specific quantitation. The dashed line is the lowest detection limit for each antibody. The data are mean ± SEM of at least three independent experiments in duplicates.. **p<0.05* vs. pure protein; *#p<0.05* vs. lower detection limit.

S-Fig. 5



Histological slices of native (left panel) and decellularized (right panel) left lungs at mid position were prepared and used for subsequent analyses. (**A**) Slices mounted in DAPI-containing mounting solution. Fluorescent (blue – DAPI staining) and phase-contrast photomicrographs were stitched at 42× magnification to contain the whole slice. Scale bar – 3 mm. (**B**) Fluorescent photomicrographs acquired at 100× magnification under over-exposure conditions to visualize absence of nuclear staining in decellularized lung. Scale bar – 150 µm. (**C**) Hematoxylin and eosin staining of native and decellularized lungs slices acquired at 200× magnification. Scale bar – 100 µm. (**D**) Scanning electron microscopy (SEM) images of native and decellularized lung tissue acquired at 500× magnification. Scale bar – 100 µm .

S-Fig. 6



Histological slice of native left lung at apex, mid and base positions were prepared and mounted in DAPI-containing mounting solution. Fluorescent (blue – DAPI staining) and phase-contrast photomicrographs were stitched at 4.2× magnification to contain the whole slice. The phase-contrast photomicrographs are overplayed with representative density maps of relative fluorescent signal intensity per 114,255 μm^2 of the corresponding fluorescent image. The color scale is in arbitrary units to maximize the relative mean signal difference in each slice (Density scale) from 0 (transparent) until 100 (red), (size bar – 3 mm).

Supplemental Tables

S-Table 1 – List of antibodies used for adhesion assays, Western blotting and ELISA.

Ab:	Cat. No.	Company	Dilution / Application	Conjugate
α1	555001	BD Pharmingen	10ug/ml / Adhesion	
α1	AB1934	Millipore	1:1000 / Western	
α2	SC-9089	Santa-Cruz	10ug/ml / Adhesion 1:200 / Western	
α3	AB1920	Millipore	1:1000 / Western	
α4	SC-14008	Santa-Cruz	10ug/ml / Adhesion	
α4	AB1924	Millipore	1:1000 / Western[1]	
α5	SC-10729	Santa-Cruz	1:200 / Western	
α6	SC-10730	Santa-Cruz	1:500 / Western	
α9	N/A[5]	Chemicon	1:1000 / Western	
αV	SC-10719	Santa-Cruz	1:500 / Western	
β1	MAB 1997	Millipore	10ug/ml / Adhesion	
β1	AB1952	Millipore	1:1000 / Western	
Actin	SC-7210	Santa-Cruz	1:500 / Western	
Coll I	AB745	Millipore	1:1000 / Western	
Coll IV	MAB1910	Millipore	1:500 / Western	
Fibronectin	AB1945	Millipore	1:1000 / Western	
Laminin	AB19012	Millipore	1:1000 / Western	
Thrombospondin-1	clone 2D11	Kindly provided by Dr. D. Roberts (NIH, Bethesda, MD).	1:1000 / Western[2]	
Vitronectin	AB19014	Millipore	1:1000 / Western	
Goat anti-rabbit	7074S	Cell Signaling Technology	1:10,000 / Western	HRP[3]
Goat anti-mouse	7076S	Cell Signaling Technology	1:10,000 / Western	HRP
Goat anti-rabbit	A3687	Sigma	1:500 / ELISA	ALP[4]
Goat anti-mouse	A3562	Sigma	1:1000 / ELISA	ALP

[1] Western blotting was performed using non-reduced proteins.

[2] All non-fat dry milk solutions were replaced with BSA at similar concentrations.

[3] HRP – horseradish peroxidase

[4] ALP – alkaline phosphotase

[5] N/A – not available. Rabbit polyclonal antibody against cytoplasmic fragment commercially developed as a service.

S-Table 2 – Quantitative interaction characteristics of CM-derived LM or FN with elastin, collagen type IV and collagen type I.

		Laminin		Fibronectin	
		Purified LM	**CM**	**Purified FN**	**CM**
Elastin	**pg/ng of Elastin**	UD[1]		UD	
	Efficiency (% on 1ug)				
Collagen type IV	**pg/ng of Coll IV**	2.4 ± 0.8	9.4 ± 4.0*	UD	
	Efficiency (% on 1ug)	0.15 ± 0.02%	0.94 ± 0.11%*		
Collagen type I	**pg/ng of Coll I**	1.4 ± 0.3	28.0 ± 3.8*	4.2 ± 1.8	3.4 ± 1.4
	Efficiency (% on 1ug)	0.12 ± 0.02%	5.86 ± 0.44%*	0.25 ± 0.01%	65.57 ± 1.55%*

The data are mean ± SEM of at least three independent experiments in duplicates (n = 6-8). **p<0.05* CM-derived vs. purified LM or FN. CM – conditioned media; LM – laminin; FN – fibronectin. [1]UD – undetected, below lower detection limit.