Joelsson et al.:

Innovative *In Vitro* Method to Study Ventilator Induced Lung Injury

Supplementary Data

Tab. S1: Primers used in this study

Gene	RefSeq number
CAMP	NM_004345.4
HBD2	NM_004942.3
LCN2	NM_005564.4
CXCL8	NM_000584.3
TNFα	NM_000594.3
VEGFA	NM_001033756(18)
HIF1A	NM_001530(3)
SFPB	NM_000542(1)
RAGE	NM_001206966(10)
CHI3L	NM_001276(1)
IL-10	NM_000572.3
GAPDH	NM_002046(1)

Tab. S2: Biomarkers

Biomarkers	Literature – relevance to VILI/ARDS or innate immunity
VEGFA	Abadie et al., 2005; International HapMap et al., 2010; Desai and Cardoso, 2002; Maitre et al., 2001; Medford et al., 2009; Meyer et al., 2012; Shibuya, 2013; Thickett et al., 2001; Ware et al., 2005
HIF1A	N/A
RAGE	Jabaudon et al., 2015; Neeper et al., 1992; Shirasawa et al., 2004
SFPB	Agrawal et al., 2012; Cheng et al., 2003; Lin et al., 2000
IL-8	Baughman et al., 1996; Hildebrand et al., 2007; McClintock et al., 2008; Schutte et al., 1996
IL-10	Armstrong and Millar, 1997; Fiorentino et al., 1991; Hindorff et al., 2009; Meyer et al., 2012; Parsons et al., 2005; Schroeder et al., 2008
HBD2	Cederlund et al., 2011
TNFα	Cross and Matthay, 2011; Dada and Sznajder, 2007; Gong et al., 2005; Park et al., 2001; Piguet et al., 1990; Postlethwaite and Seyer, 1990; Roten et al., 1991
CAMP	Cederlund et al., 2011
YKL-40	Park et al., 2010

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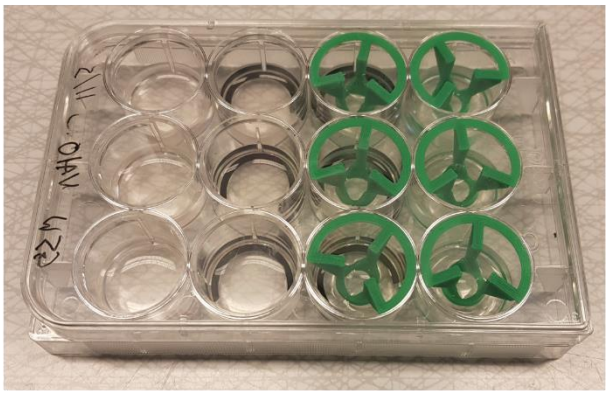

Ctrl

FKM Viton O-Ring

FKM Viton O-Ring + PLA

PLA

PLA

FKM Viton O-Ring

B

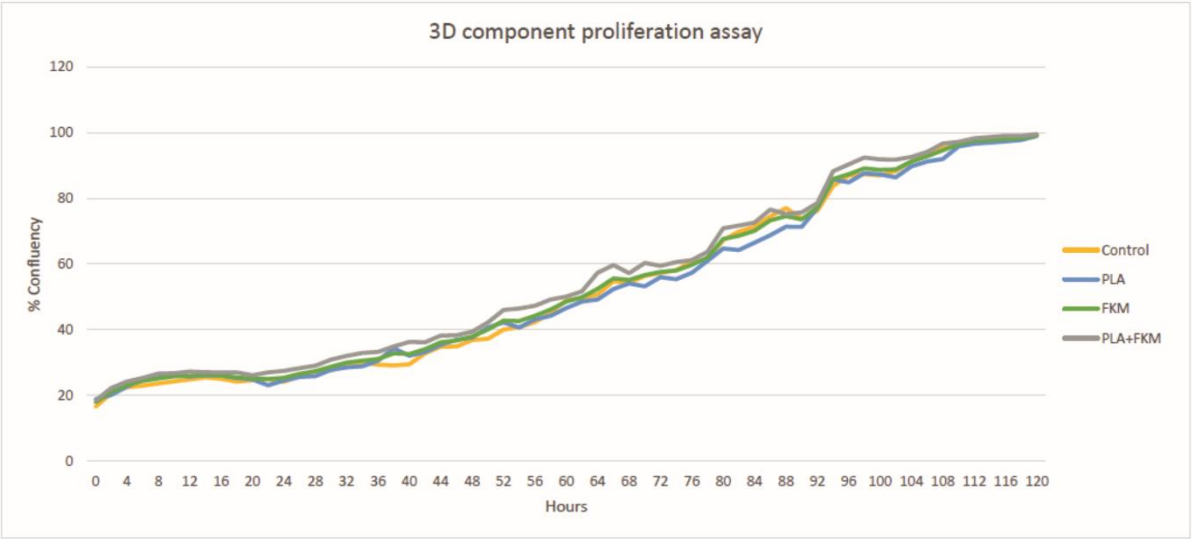

Fig. S1: 3D printed components and O-rings used in this research (Biocompatibility assay)
A, Polylactic acid (PLA), used for 3D printed components, along with FKM (Fleuroelastomer) Viton O-rings were tested for biocompatibility. Cells were grown in the presence of these components separately and together. B, Analyses of effects on proliferation in incucyte.

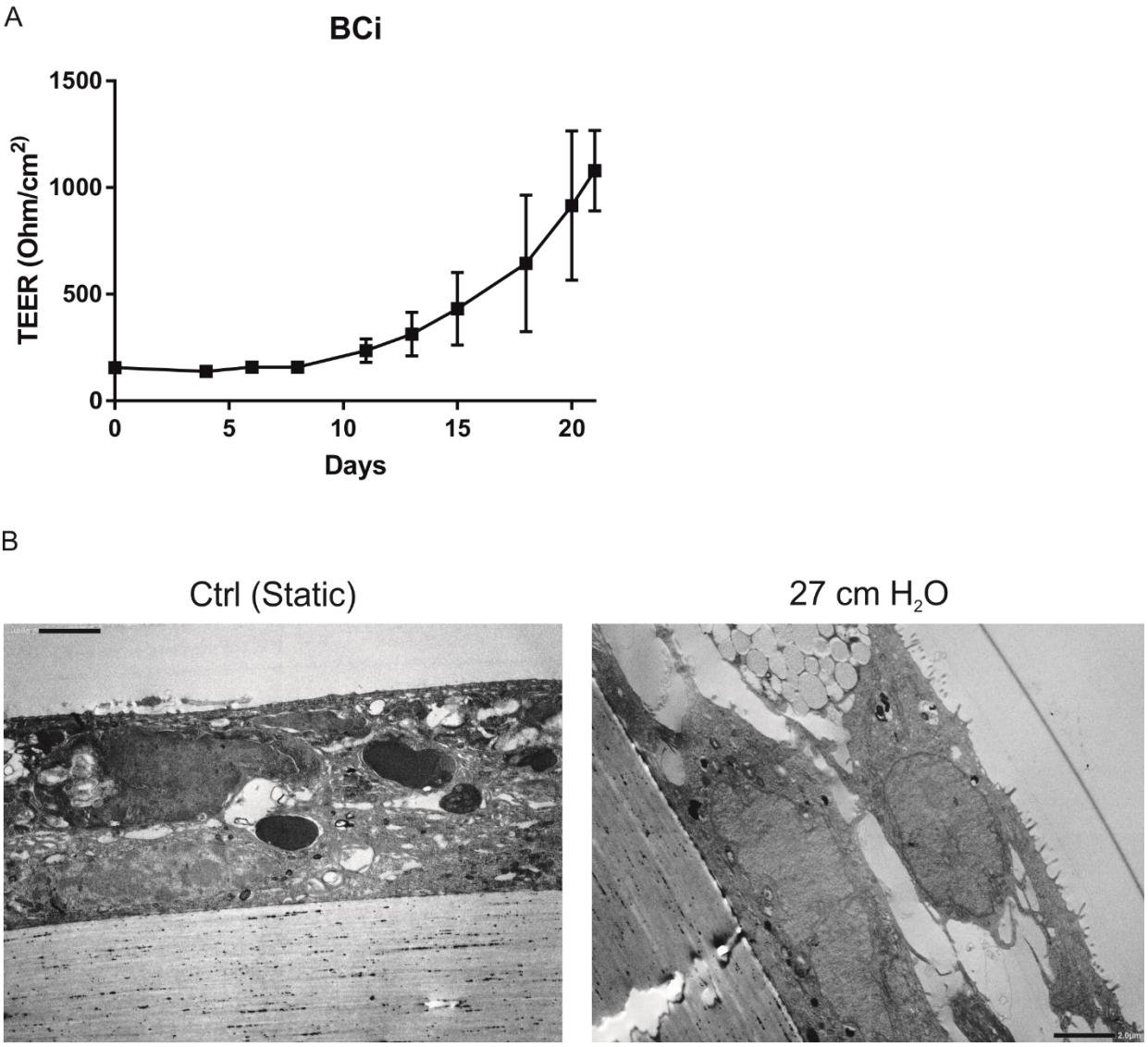

Fig. S2: Cyclical pressure induces similar phenotypical changes in BCi-NS1.1 as seen in VA10
A, TEER measurement of BCi ALI cultured cells. Graph represents results from four different 3-week ALI cultures (N = 4). B, Differentiated cell layer (left) and cells after cyclical stress (27 cm H_2O for 24 h) (right). Scale bars are 2 µm.

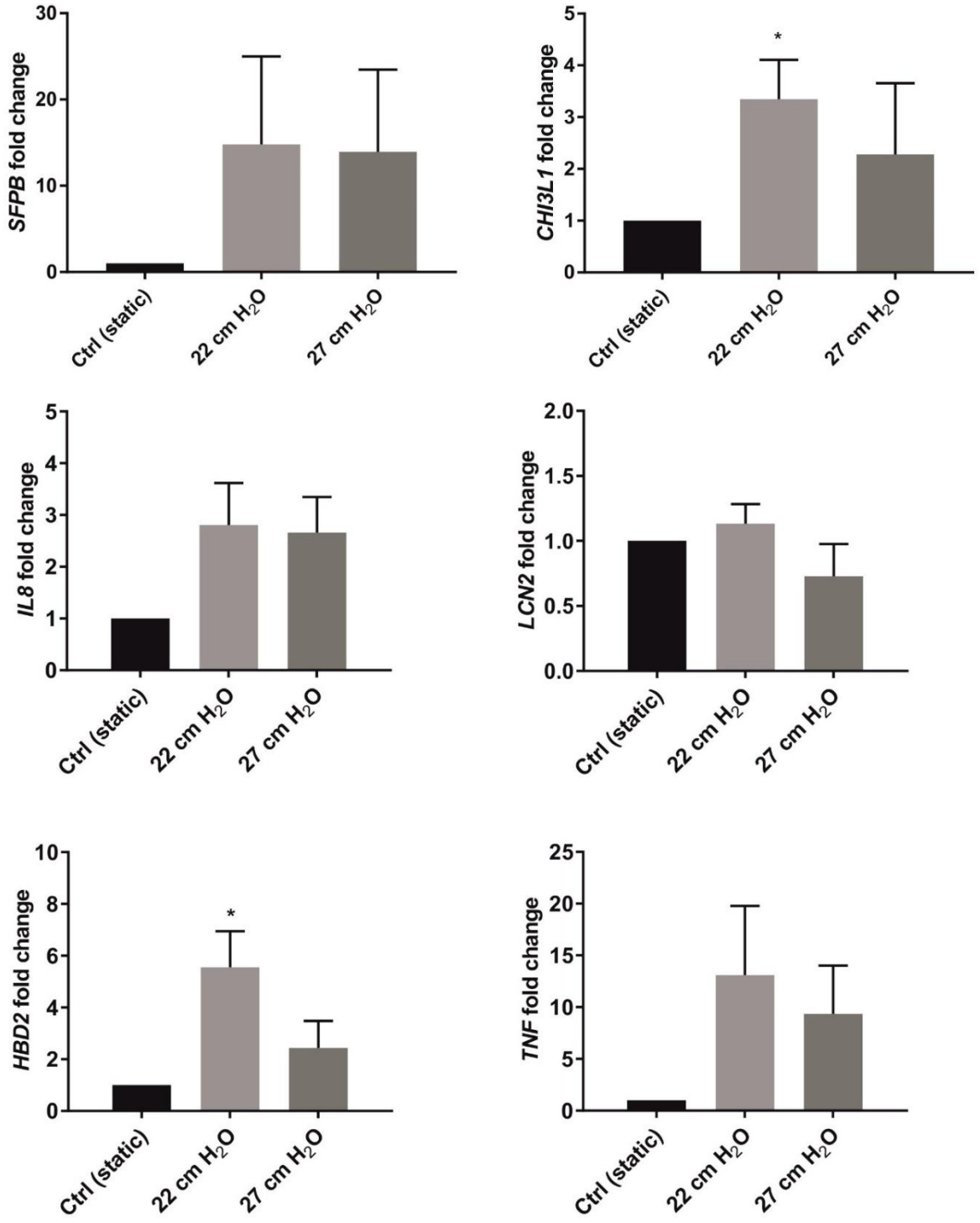

Fig. S3: Cyclical pressure applied to *in vitro* airway epithelium shows differential expression of VILI/ARDS and innate immunity associated markers
Expression of ARDS/VILI and innate immunity associated biomarkers *SFPB*, *CHI3L1*, *IL8*, *LCN2*, *HBD2*, *and TNFb* by qRT-PCR, N = 3, P ≤ 0.05 = *.

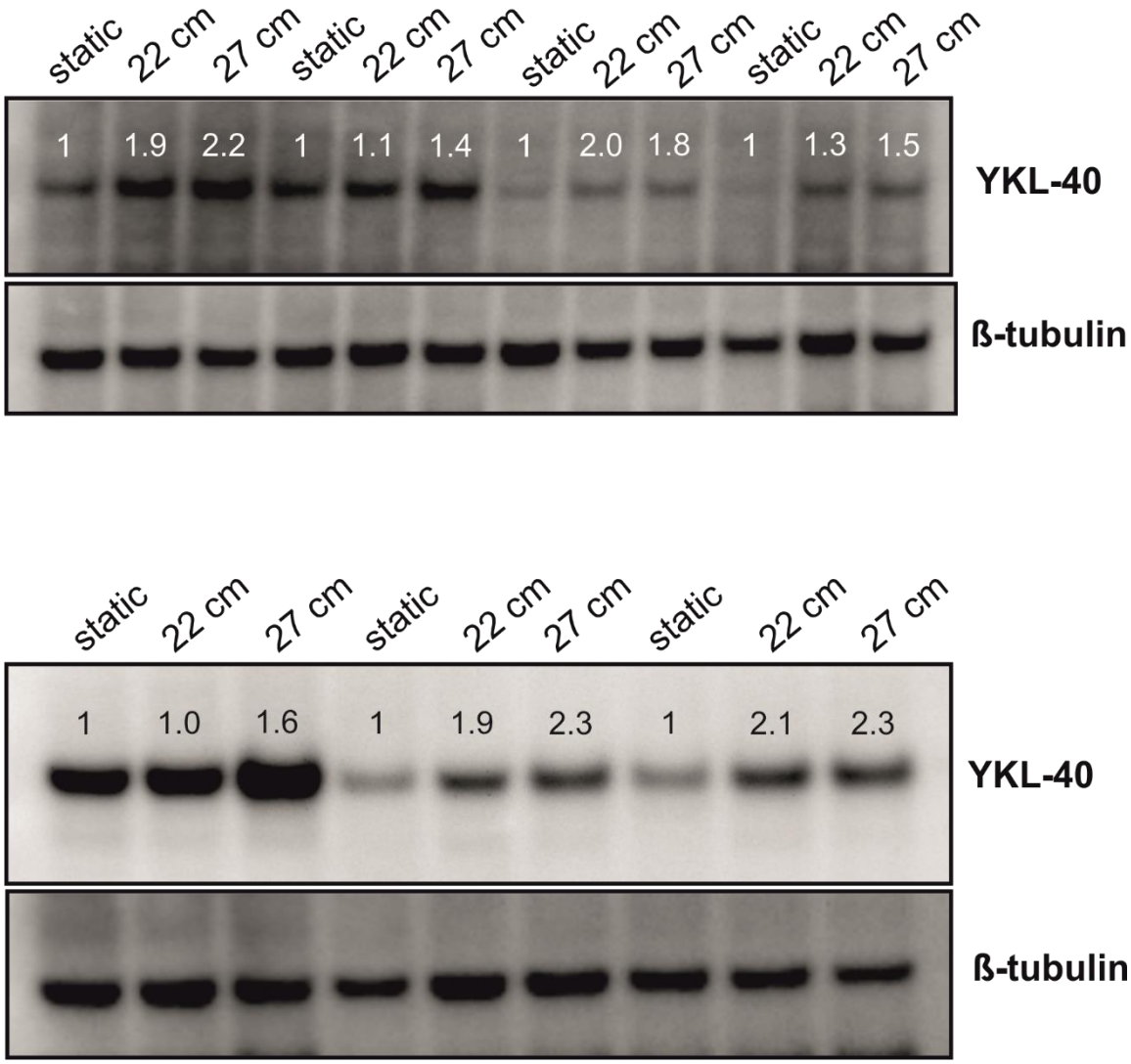

Fig. S4: Cyclically stressing ALI cultured VA10 cells results in increased protein expression of YKL-40
Western blots of YKL-40 from 7 experiments show an increase in protein expression when cells are cyclically stressed for 24 h in pressure chamber. Pressure values are 22 and 27 cm H_2O.

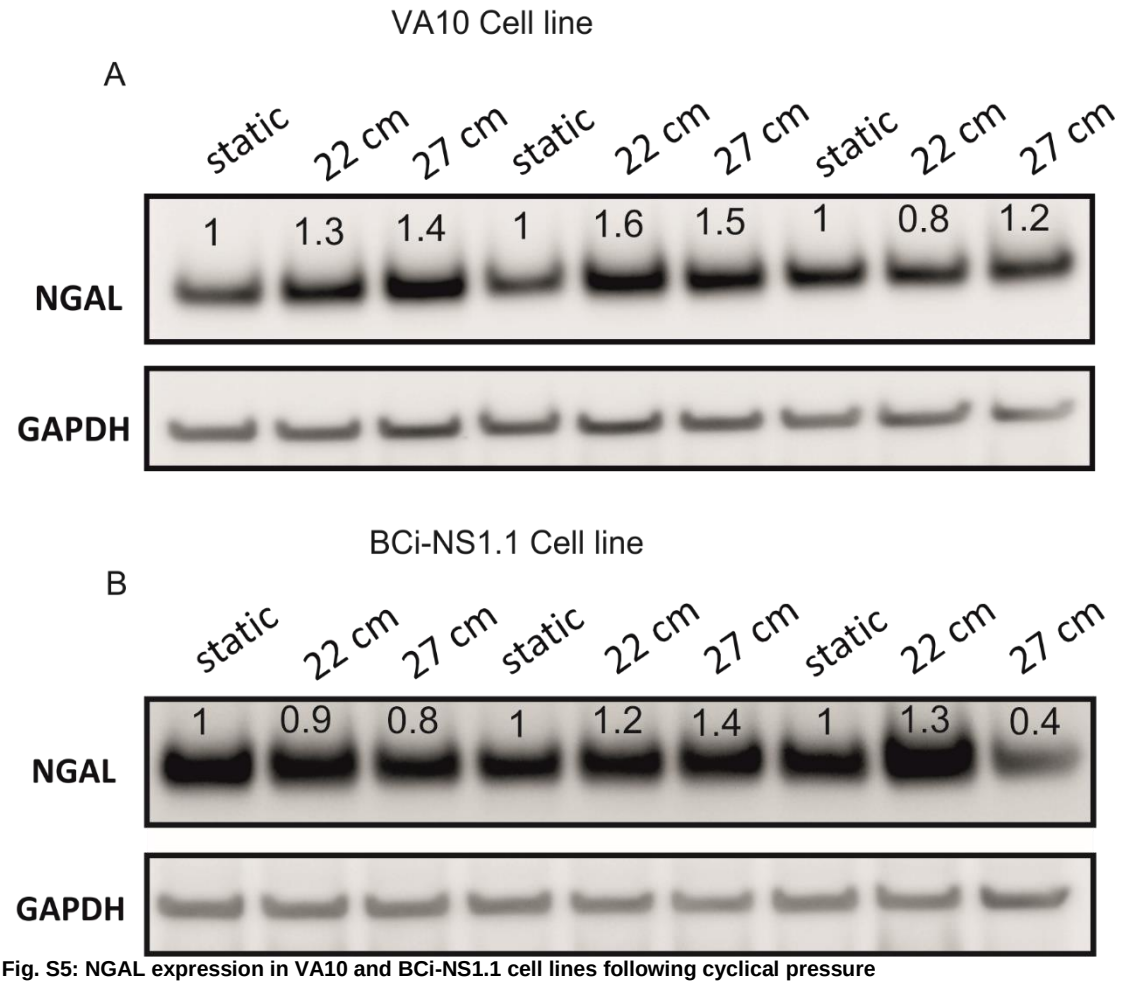

Fig. S5: NGAL expression in VA10 and BCi-NS1.1 cell lines following cyclical pressure
A, Western blots of NGAL expression in VA10 cell line at 22 and 27 cm H_2O. B, Western blots of NGAL expression in BCi cell line at 22 and 27 cm H_2O.

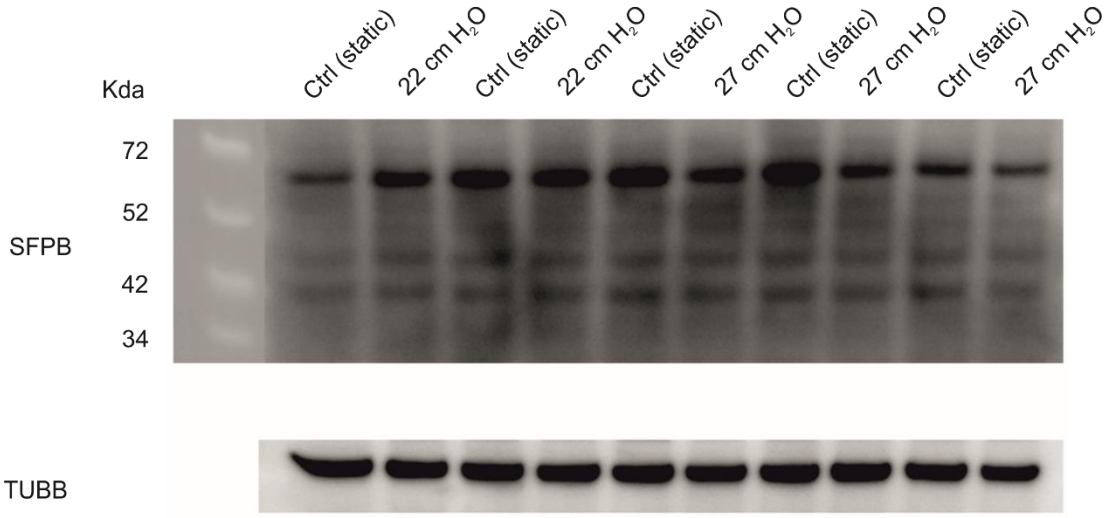

Fig. S6: SFPB expression in VA10 cell line following cyclical hyperbaric pressure
Western blots of SFPB expression in VA10 cell line at 22 and 27 cm H_2O.

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