Cold dispase digestion of murine lungs improves recovery and culture of airway epithelial cells

Piotr P Janas[1], Caroline Chauché[1], Patrick Shearer[2], Georgia Perona-Wright[2], Henry J McSorley[3] and Jürgen Schwarze[1*]

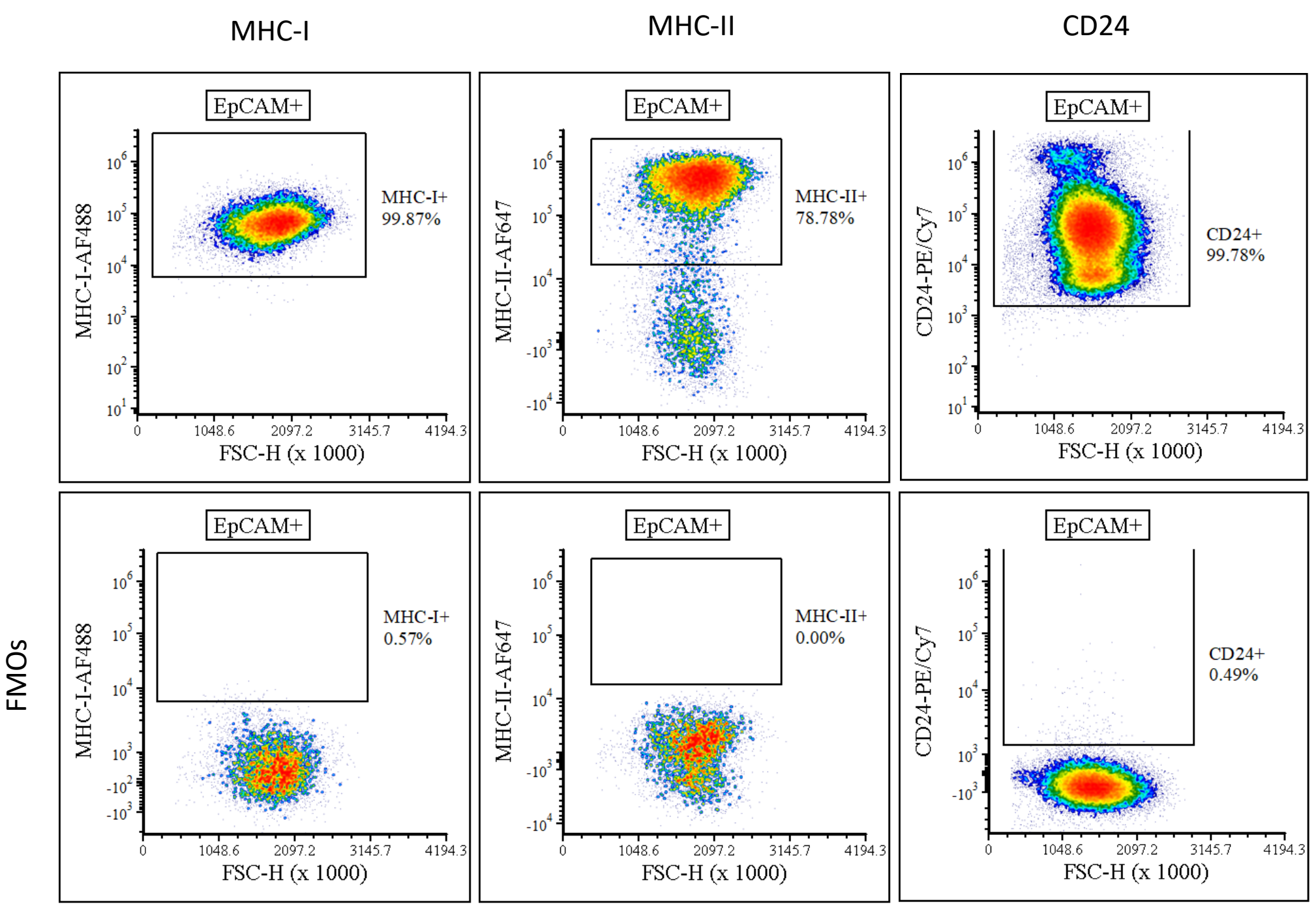
[1] Centre for Inflammation Research, The Queens Medical Research Institute, The University of Edinburgh, Edinburgh BioQuarter, Edinburgh, EH16 4TJ, United Kingdom

[2] Institute of Infection, Immunity & Inflammation, University of Glasgow, Glasgow, G12 8TA, United Kingdom

[3] Division of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, Dundee, DD1 5AA, United Kingdom.

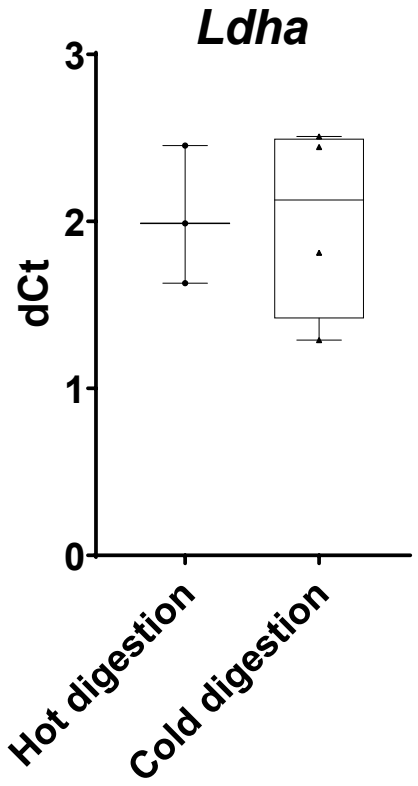
Corresponding author: Jürgen Schwarze, Jurgen.Schwarze@ed.ac.uk

Supplementary data

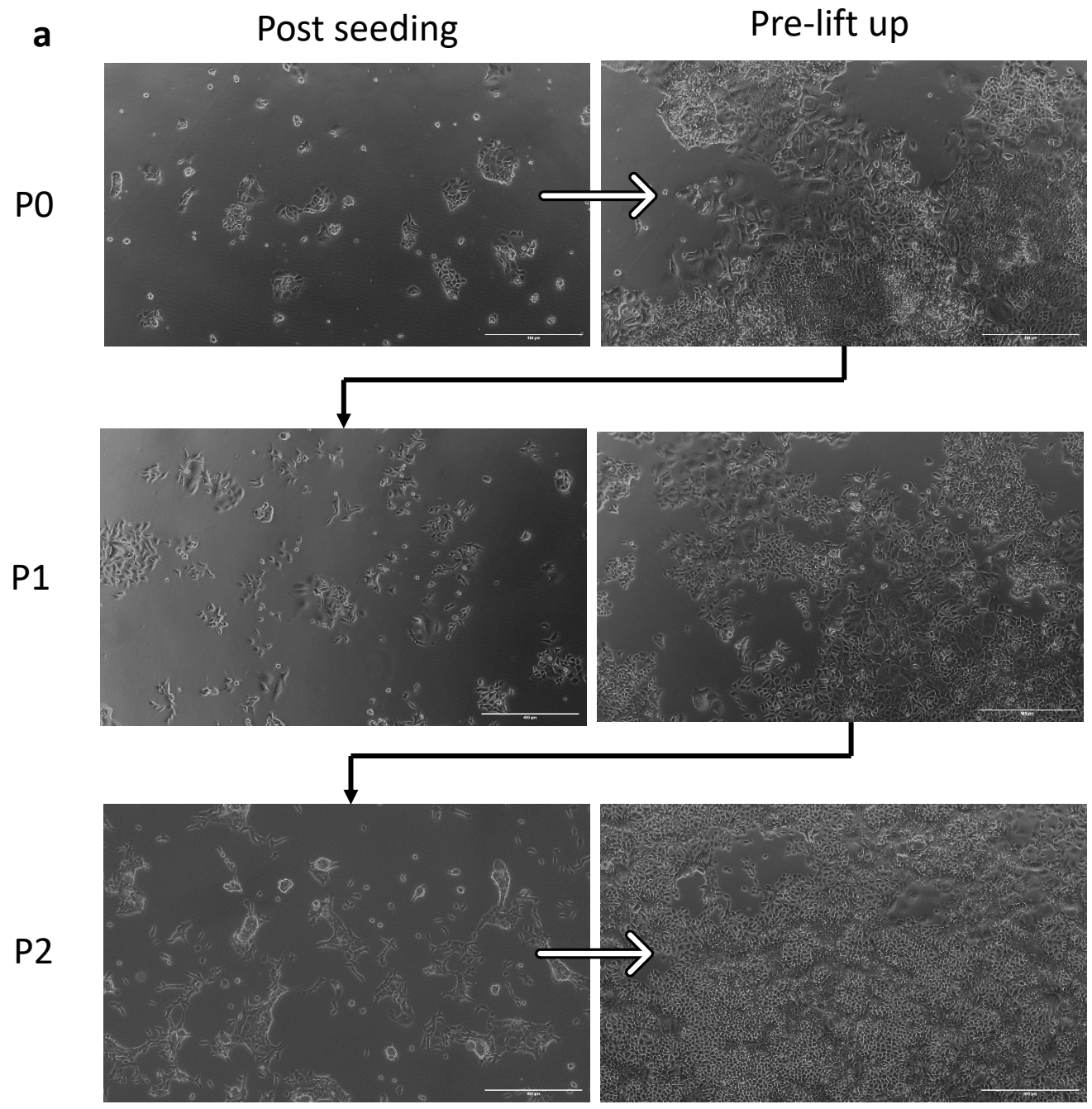


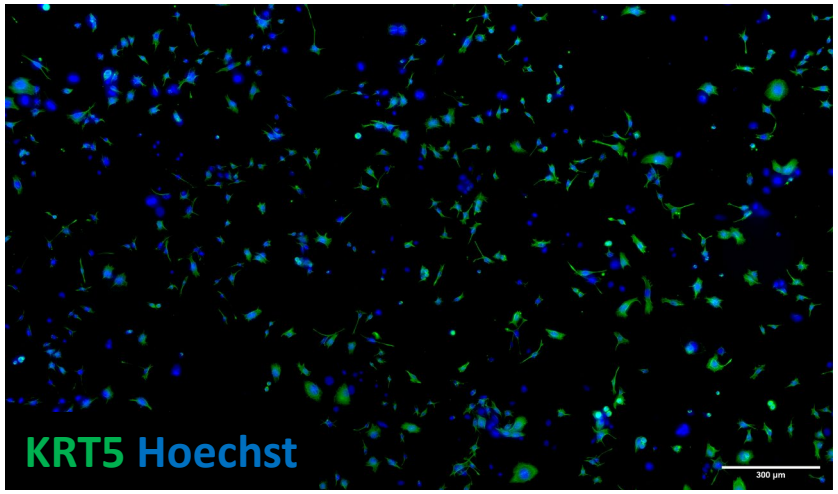
Supplementary figure 1: Cold digestion with dispase II does not remove MHC-I, MHC-II or CD24 from the surface of CD45-CD31-EpCAM+ cells.

Following debris exclusion (FSC/SSC), singlets gating (FSC-H/FSC-A), live cells gating (LIVE/DEAD fixable near-IR/FSC-H), CD45 and CD31 exclusion (CD45/CD31) and EpCAM+ gating (EpCAM/FSC-H) expression of MHC-I, MHC-II and CD24 was evaluated. Gating was based on fluorescence minus one (FMO) controls.



Supplementary figure 2: Digestion type does not affect cellular oxidative stress based on LDHa (lactate dehydrogenase) levels.
Following either hot or cold digestion CD45-CD31-EpCAM+ cells were MACS sorted and RNA isolated using Trizol and chloroform-based RNA isolation. SYBR green qPCR was performed, with Rpl37a as endogenous control. N=3-4





Supplementary figure 3: Murine AECs can be passaged (P0-2) following cold digestion and CD45-CD31-EpCAM+ MACS sorting.

(**a**) Following cold digestion, murine AECs ($CD34^{-}CD31^{-}EpCAM^{+}$) were MACS sorted and $2x10^5$ cells were seeded into a 24-well plate and cultured in a Stemcell PneumaCult Ex-Plus medium with 10µM Y27632, 3µM CHIR99021, 1µM A8301, 5µg/ml heparin and 50ng/ml FGF-10 for 14 days. Cells were passaged twice. Each time cells were detached using TrypLE and split 1:10 into a larger well plate starting from 24-well plate (P0), followed by 12-well plate (P1) and lastly a 6-well plate (P2). Once cells reached confluency at the end of passage 2, cells were lifted-up using TrypLE and $1.4x10^6$ live cells (trypan blue exclusion) were counted using haemocytometer. Six 10x objective phase-contrast single fields of view (3x2) were stitched together. Scale bar 400µm. N>2. (**b**) All cells express KRT5 (green) after three passages of CD45-CD31-EpCAM+ MACS sorted AECs. Scale bar, 300µm.