SUPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

Antibodies – The following antibodies are from BioLegend (San Diego, CA): Phycoerythrin (PE)-mouse anti-human ICAM-1/CD54, phycoerythrin-cyanine 7 (PE-Cy7)-mouse anti-human CCR2/CD192, peridinin chlorophyll protein–cyanine 5.5 (PerCP/Cy5.5)-mouse anti-human E-cadherin/CD324, fluorescein (FITC)-mouse anti-human HLA-ABC W6/32), FITC-mouse anti-human CD80, PerCP-Cy5.5-mouse anti-human CD11c, allophycocyanin (APC)-mouse anti-human MR/CD206, APC-mouse anti-human CD40, PerCP-Cy5.5-mouse anti-human MHCII/HLA-DR; isotype controls (PE-mouse IgG1 κ isotype control, PE-Cy7-mouse IgG2a κ isotype control, PerCP-Cy5.5-mouse IgG1 κ isotype control, FITC-mouse IgG2a κ isotype control, FITC-mouse IgG1 κ isotype control, PerCP-Cy5.5-mouse IgG2a κ isotype control, APC-mouse IgG1 κ isotype control). The following are antibodies used for exposed-*M.tb* AT intracellular trafficking studies: Rabbit anti-human LC-3 (MBL International, Woburn, MA), Goat anti-human LAMP-1 (Santa Cruz, Dallas, TX), Mouse anti-human ABCA1 (Abcam, Cambridge, MA), Rabbit anti-human ABCA3 (Abcam), Donkey anti-rabbit Alexa Fluor 647, Chicken anti-mouse Alexa Fluor 647, Donkey anti-goat Alexa Fluor 405, Donkey anti-mouse Alexa Fluor 405, and Donkey anti-goat Alexa Fluor 568 (all from ThermoFisher Scientific).

Human ALF isolation – Human alveolar lining fluid (ALF) was obtained from bronchoalveolar lavage fluid (BALF) as we previously described [1]. Briefly, BALF was obtained by BAL in sterile 0.9% NaCl, 0.2 μm-sterile filtered and subsequently concentrated 20-fold with a >10 kDa molecular mass cut off using a Centricon Plus (Millipore) filter at 4°C to achieve the volume of ALF present within the lungs devoid of surfactant lipids. ALF (defined as BALF >10 KDa fraction) [1-5] was frozen at -80°C until use.

Acidification of ALF exposed-M.tb containing intracellular compartments in ATs – *M.tb* compartment acidification was determined as we previously published [2,3]. Briefly, L- or H-ALF *M.tb*-infected ATs were washed with warmed phenol-red free medium and incubated at 37°C for 30 minutes with 75 nM LysoTracker (ThermoFisher Scientific). Following incubation, cells were fixed with cold 10% formalin for 10 minutes at room temperature. Quantification of co-localization of GFP-*M.tb* and LysoTracker was determined by counting ≥150 events per coverslip, performed in replicate, using the Olympus FV1000 Filter Confocal Microscope and Zeiss LSM 800 Confocal Microscope. All microscopy data were analyzed with the Olympus FluoView Viewer and Zeiss ZEN Software.

RNA isolation and gene expression - AT gene expression was determined as we previously described [2,4]. Briefly, L- or H-ALF *M.tb*-infected ATs were lysed in TRIzol reagent (ThermoFisher Scientific) for 15 minutes at room temperature with gentle shaking every 5 minutes. Total RNA was extracted, evaluated using spectrophotometric analysis for quality and quantity, and 1 μg RNA was reverse transcribed to cDNA by RT enzyme (SuperScript III) as we previously described. Gene expression was determined by qRT-PCR (BioRad CFX96 Real-Time System, Hercules, CA) using gene-specific primers and Taqman gene expression system. All gene expression values were normalized to housekeeping gene (β-actin) and fold change was calculated relative to uninfected ATs. All samples were run in duplicate by qRT-PCR.

SUPPLEMENTARY REFERENCES

1. Arcos, J., *et al.* Human lung hydrolases delineate *Mycobacterium tuberculosis*-macrophage interactions and the capacity to control infection. *J. Immunol* **187**, 372-381 (2011).

2. Arcos, J.*, et al.* Lung mucosa lining fluid modifies *Mycobacterium tuberculosis* to reprogram human neutrophil killing mechanisms. *J Infect Dis* **212**, 948-958 (2015).
3. Arcos, J.*, et al. Mycobacterium tuberculosis* cell wall released fragments by the action of the human lung mucosa modulate macrophages to control infection in an IL-10-dependent manner. *Mucosal Immunol* **10**, 1248-1258 (2017).
4. Scordo, J.M.*, et al. Mycobacterium tuberculosis* cell wall fragments released upon bacterial contact with the human lung mucosa alter the neutrophil response to infection. *Front Immunol* **8**, 307 (2017).
5. Moliva, J.I.*, et al.* Exposure to human alveolar lining fluid enhances *Mycobacterium bovis* BCG vaccine efficacy against *Mycobacterium tuberculosis* infection in a CD8(+) T-cell-dependent manner. *Mucosal Immunol* **11**, 968-978 (2018).

Supplementary Table 1*. Data for Multivariate Regression Analysis of Complement C3 Function

ALF#	Subset (L *vs* H)	Oxidation	Protein levels	*M.tb*-Binding
11	L	40.17008	3.050648	0.915998333
14	L	33.14912	5.81559	1.094339
12	L	117.7563	2.613571	0.824757
4	L	63.87487	2.950907	0.806304
5	H	93.38155	2.412589	0.692752
1	H	219.2849	4.558795	0.702768667
6	H	252.8068	3.489185	0.647114667
7	H	361.1781	9.277049	0.885315333

(*) Raw data used to perform multivariate regression analyses for each ALF sample shown (ALF#): ALF subset (Low *vs* High), oxidation (total protein tyrosine nitration) and innate protein levels (concentration) *versus* innate protein function (*M.tb*-binding, OD_{450}) for C3. All data shown has been normalized for 1 mg/mL ALF phospholipid content.

Supplementary Table 2*. Data for Multivariate Regression Analysis of Surfactant Protein A Function

ALF#	Subset (L *vs* H)	Oxidation	Protein levels	*M.tb*-Binding
11	L	40.17008	217.2859009	2.545135333
14	L	33.14912	Not Determined	2.188874333
12	L	117.7563	1.173810577	2.778242333
4	L	63.87487	61.03567849	2.393676667
5	H	93.38155	14.1991592	1.927447667
1	H	219.2849	37.97906461	1.815937
6	H	252.8068	Not Determined	1.963922333
7	H	361.1781	131.8583891	2.416066333

(*) Raw data used to perform multivariate regression analyses for each ALF sample shown (ALF#): ALF subset (Low *vs* High), oxidation (total protein tyrosine nitration) and innate protein levels (concentration) *versus* innate protein function (*M.tb*-binding, OD_{450}) for SP-A. All data shown has been normalized for 1 mg/mL ALF phospholipid content.

Supplementary Table 3*. Data for Multivariate Regression Analysis of Surfactant Protein D Function

ALF#	Subset (L *vs* H)	Oxidation	Protein levels	*M.tb*-Binding
11	L	40.17008	32.26231	0.93505
14	L	33.14912	13.9998	0.810345
12	L	117.7563	8.833574	1.062636
4	L	63.87487	3.260941	0.840977
5	H	93.38155	12.27038	0.468329333
1	H	219.2849	15.26958	0.603675333
6	H	252.8068	5.358528	0.68627
7	H	361.1781	50.11288	0.940030667

(*) Raw data used to perform multivariate regression analyses for each ALF sample shown (ALF#): ALF subset (Low *vs* High), oxidation (total protein tyrosine nitration) and innate protein levels (concentration) *versus* innate protein function (*M.tb*-binding, OD_{450}) for SP-D. All data shown has been normalized for 1 mg/mL ALF phospholipid content.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. L- and H-ALF-*M.tb* uptake by ATs. AT-*M.tb* uptake (2 h post-infection, 2h PI) was assessed by performing colony forming units (CFUs) for determination of intracellular *M.tb.* Data was normalized to AT cell count, which was calculated immediately prior to exposed-*M.tb* AT infection. Numbers 1-14 represent individual ALF samples, with each ALF from a different healthy human donor. Numbers correspond to the same ALF samples shown in Fig. 1. One-way ANOVA post-Tukey analysis, $p<0.05$ ALFs 2, 4-6 *vs.* ALFs 12-14; for ALF 1 & 3 *vs*. ALFs 13-14 #$p<0.05$.

Supplementary Figure S2. L-ALF and H-ALF does not alter *M.tb* trafficking within AT intracellular compartments. Quantification of GFP-*M.tb* co-localization with intracellular markers in infected ATs by confocal microscopy. (**A**) LAMP-1, (**B**) LC3, (**C**) ABCA1 and (**D**) ABCA3 co-localization at 3-day post-infection (DPI). Low ALFs (white bars) and High ALFs (black bars) are shown, with each ALF plotted individually.

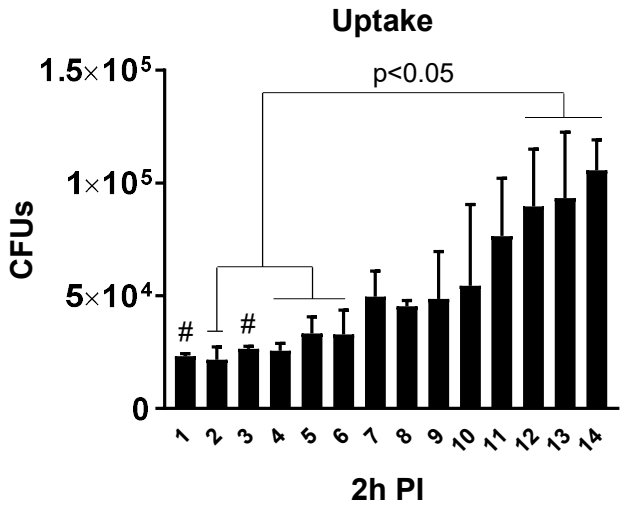
Supplementary Figure S3. L-ALF-*M.tb* induces more AT necrosis during late time points of infection. ATs were infected with L-ALF and H-ALF-*M.tb* for 5 days and AT supernatants were collected throughout infection. AT supernatants were tested for release of LDH, according to kit instructions. Shown is exposed-*M.tb* induced AT cell death by LDH release, as a measure of cell necrosis, during infection on (**A**) 2 DPI and (**B**) 4 DPI; n=2 in duplicate (mean $\pm$ SD). L=Low-ALF-*M.tb* , H=H-ALF-*M.tb* infected ATs. Student's *t* test, L-ALF- *vs.* H-ALF-*M.tb*; **$p<0.01$.

Supplementary Figure S4. Effect of L- and H-ALF-*M.tb* on AT immune mediator expression and production. ATs were infected with L-ALF or H-ALF-*M.tb* for 5 days for determination of AT immune mediator production (**A**), gene expression (**B**) or surface marker expression (**C&D**). (**A**) AT protein production in supernatants was determined by ELISA per kit instructions. N=3 low and

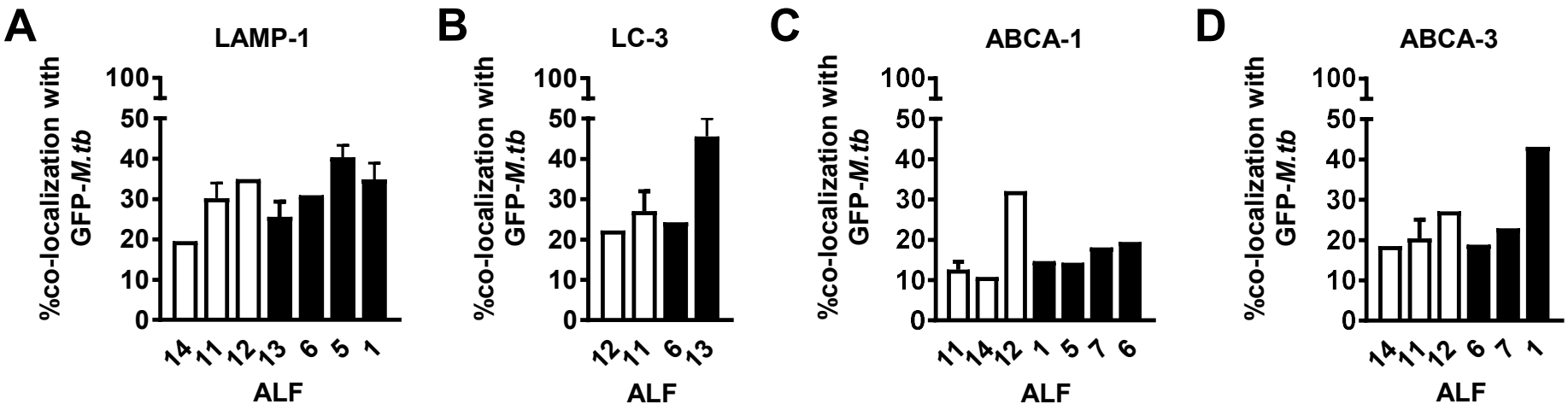
4 high ALFs in triplicate; mean ± SEM. (**B**) AT mRNA expression measured by qRT-PCR and shown as relative fold change vs uninfected ATs. N=3 low and 3 high ALFs, in duplicate; mean ± SEM. (**C**) AT surface expression and (**D**) Mean fluorescence intensity (MFI) measured by flow cytometry by counting >10,000 events per sample. Shown are n=1 low and 1 high ALF, in duplicate. L=Low-ALF-*M.tb* , H=H-ALF-*M.tb,* U=uninfected ATs. Student's *t* test, L-ALF- *vs.* H-ALF-*M.tb*; **p<0.01.

Supplementary Figure S5. Effect of supernatant from L- and H-ALF-*M.tb* infected ATs on macrophage surface expression. Resting macrophages were exposed to 0.2µm-sterile filtered supernatants from L- and H-ALF-*M.tb* infected ATs for 24 hours and macrophage surface expression of CD80, MR/CD206 and HLA-DR, was assessed by flow cytometry. MFI fold change (*versus* resting MDMs exposed to uninfected AT supernatants) is shown for n=3 L-ALFs and 3 H-ALFs with 2 different human MDM donors performed in duplicate (mean ± SD). L= macrophages incubated with L-ALF-*M.tb* infected AT supernatant & H= macrophages incubated with H-ALF-*M.tb* infected AT supernatant.

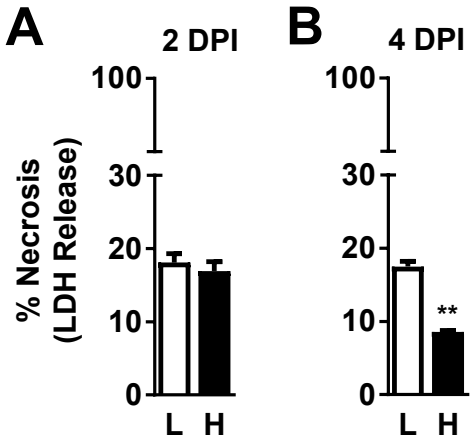
Supplementary Figure S1



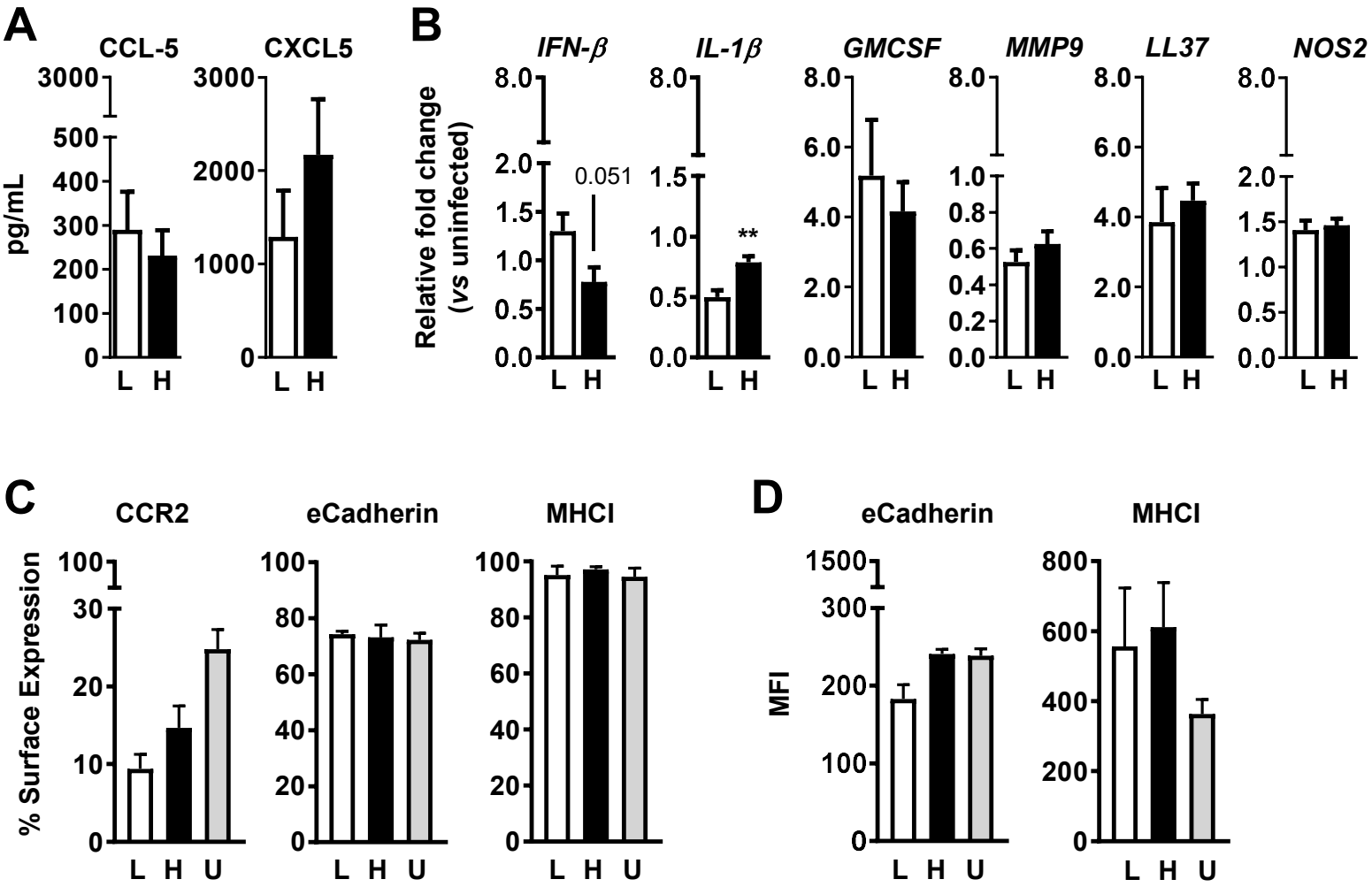
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5