

# Azithromycin Has Lung Barrier Protective Effects in a Cell Model Mimicking Ventilator-Induced Lung Injury

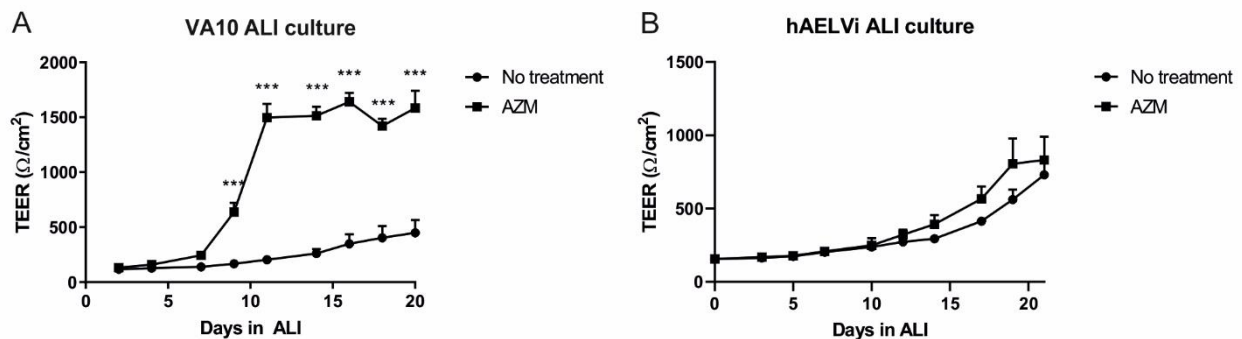
## Supplementary Data

Tab. S1: List of primers used in this study

Gene	RefSeq number
<i>CXCL8</i>	NM_000584.3
<i>TNF<math>\alpha</math></i>	NM_000594.3
<i>CHI3L</i>	NM_001276
<i>GAPDH</i>	NM_002046

Tab. S2: List of secondary antibodies used in this study

Primary antibody	Secondary antibody
NF $\kappa$ B p65 (Santa Cruz Biotechnology, sc-372)	Alexa Fluor 647 goat-anti rabbit IgG
E-Cadherin (BD Transduction Laboratories, 610182)	Alexa Fluor 647 goat-anti mouse IgG2a
Chitinase-3-like protein 1 (YKL-40) (Millipore, MABC196)	Alexa Fluor 647 goat-anti mouse IgG1
Surfactant protein B (Abcam, ab40876)	Alexa Fluor 647 goat-anti rabbit IgG

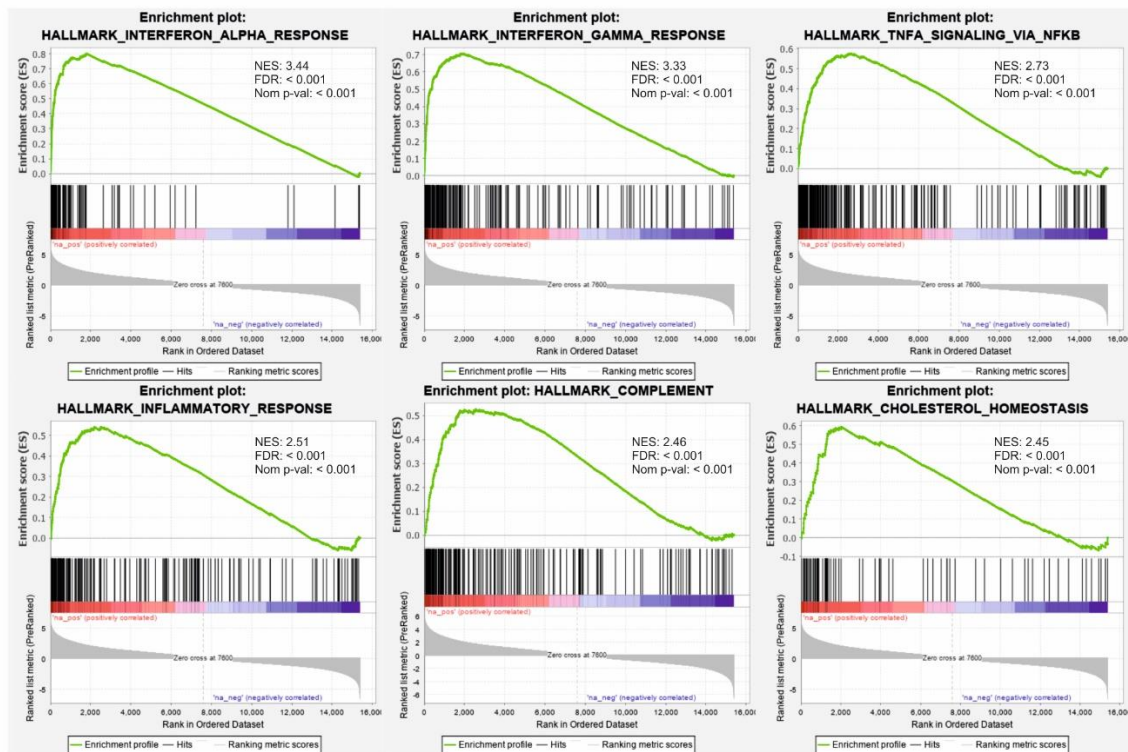


**Fig. S1: Trans-epithelial electrical resistance of ALI cultured cell lines with and without AZM**

TEER measurements of A) VA10 and B) hAELVi cell lines cultured in ALI conditions with and without AZM for 21 days (N = 3, P  $\leq$  0.001 = \*\*\*).

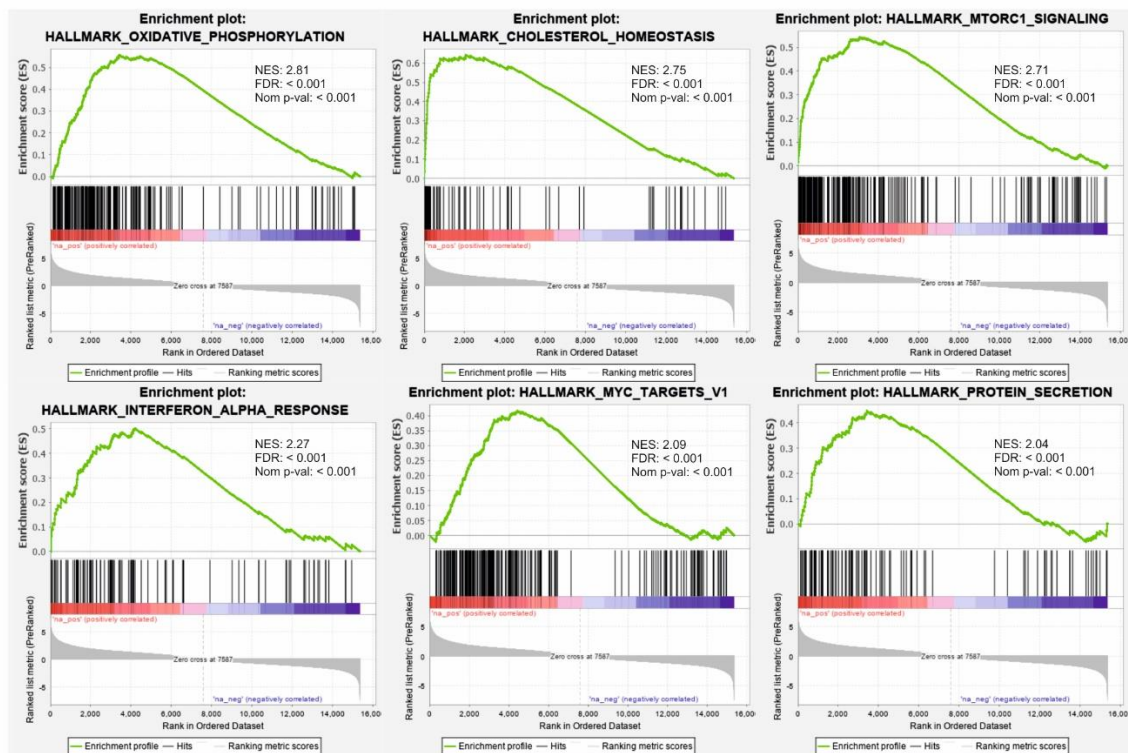
A

CON/ Static vs CON/ Stress



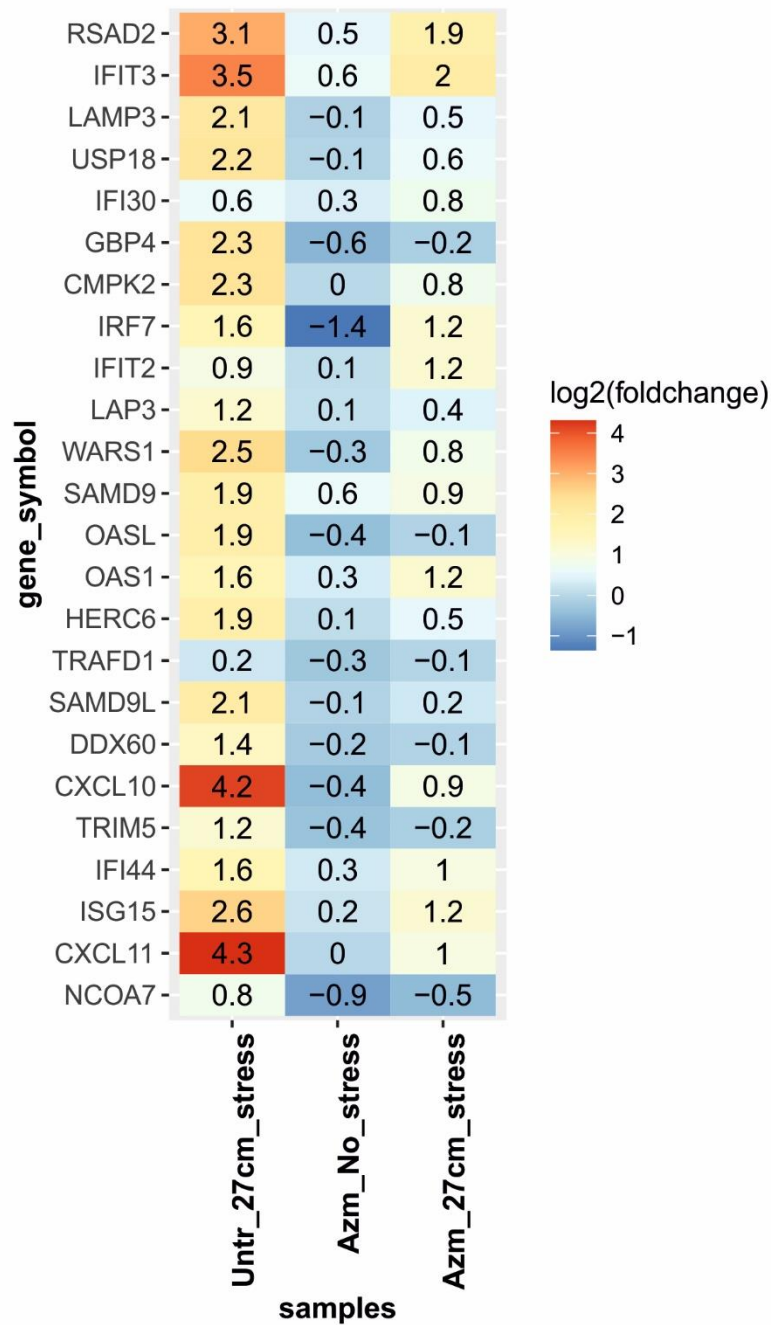
B

CON/ Static vs AZM/ Stress

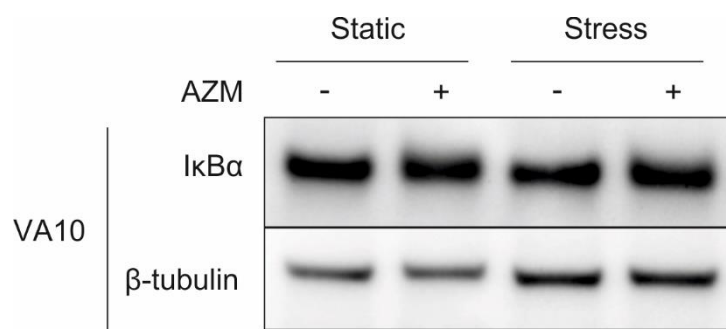


**Fig. S2: Gene set enrichment analysis showing the 6 highest positive enrichment plots of A) CON/Static vs CON/Stress and B) CON/Static vs AZM/Stress**

ALI-cultured VA10 cell layers with/without AZM treatment (CON/AZM) in static or stress conditions (Static/Stress) for 21 days. Normalized enrichment scores (NES), false discovery rate (FDR) and nominal p-value (Nom p-val) are shown.

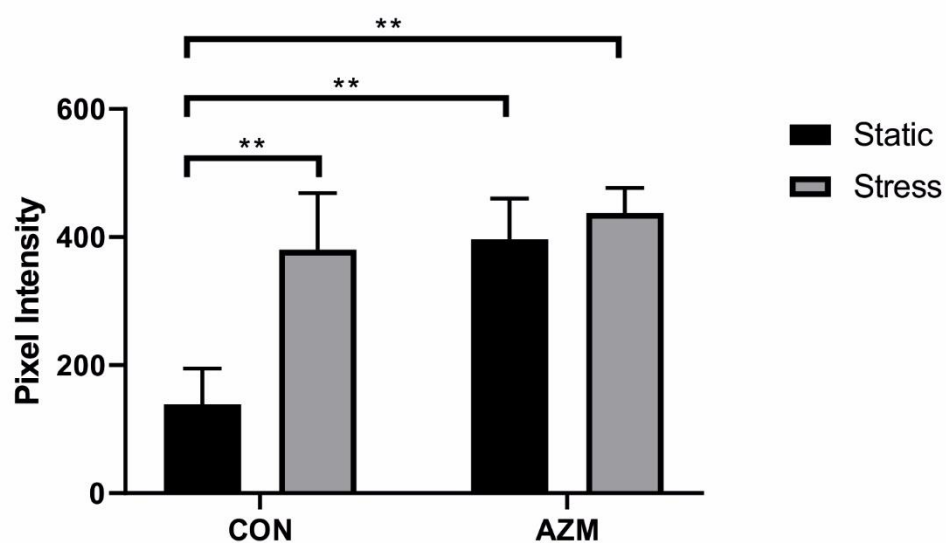


**Fig. S3: Comparison of the 24 most significantly differentially expressed genes in the IFN $\alpha$  pathway**  
ALI-cultured VA10 cell layers with/without AZM treatment in static or stress conditions for 21 days.



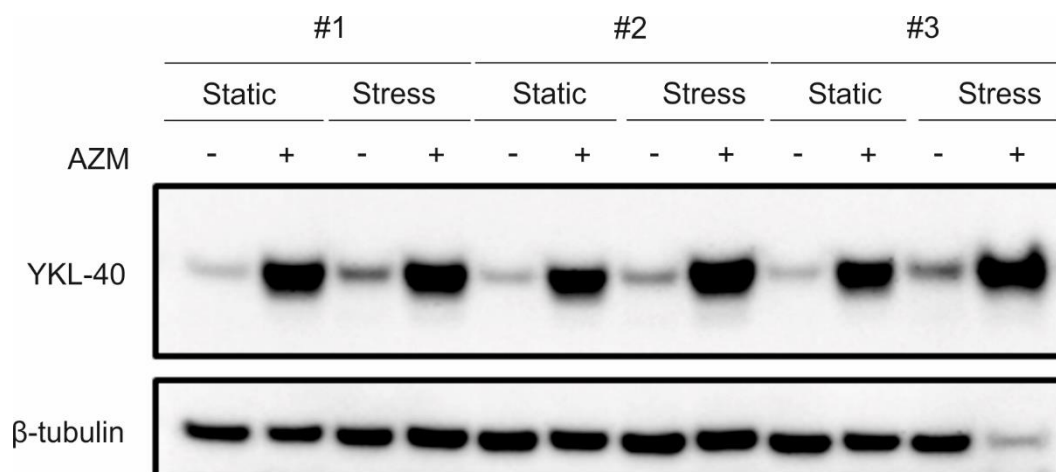
**Fig. S4: Western blot showing IκBα in VA10 cells**

ALI-cultured VA10 cell layers with/without AZM treatment in static or stress conditions for 21 days. β-tubulin shown as house-keeping gene. Representative images of 3 independent experiments are shown.



**Fig. S5: Pixel intensity quantification of Fig. 5A**

ImageJ was used to quantify pixel intensity of 3 replicates from Fig. 5A.



**Fig. S6: All YKL-40 Western blots of which one representative is shown in Fig. 5B**