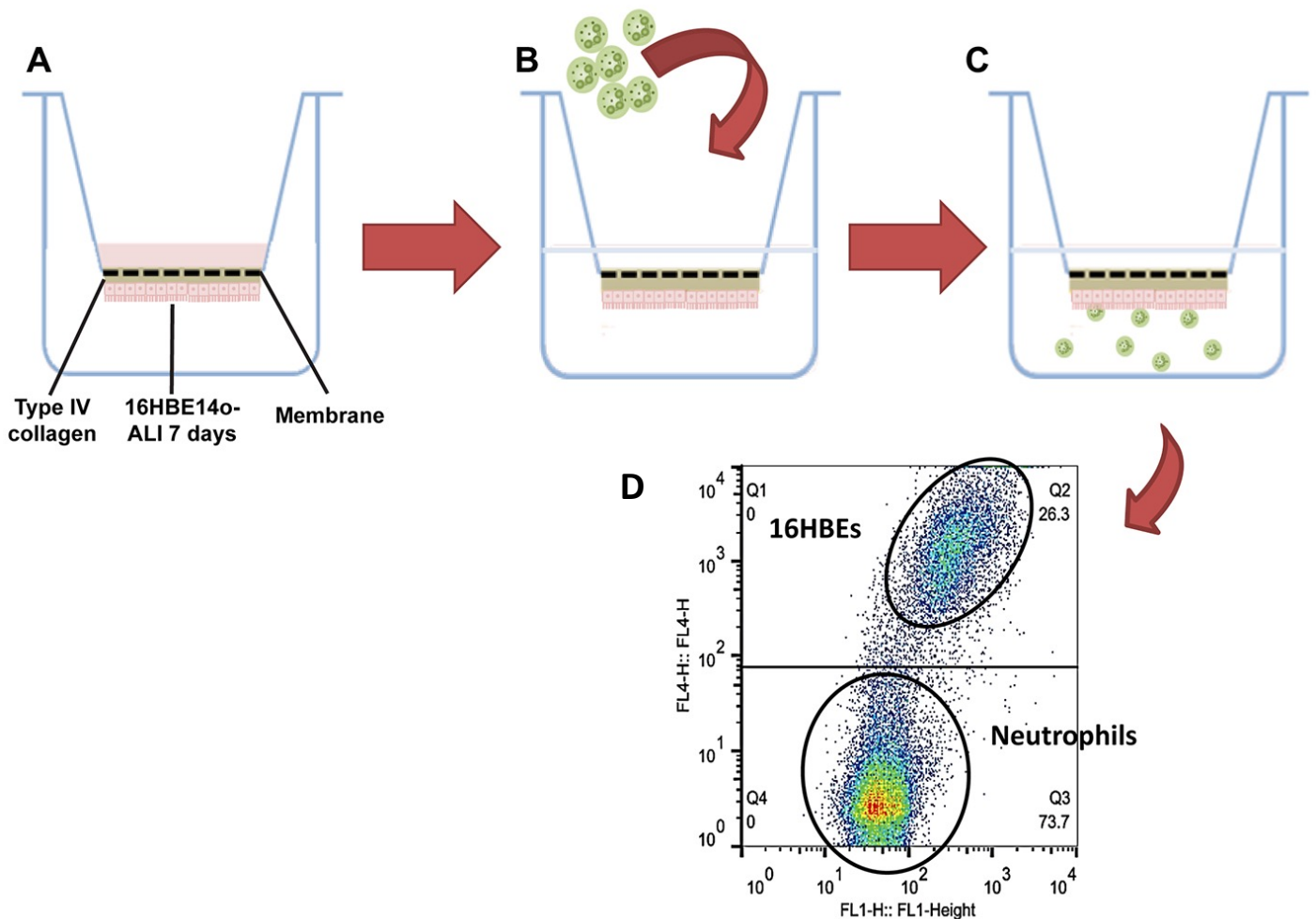


Supplemental Figure 1. FACS gating strategy for counting trans-epithelial migrated neutrophil *in vitro*.



A. Schematic of DiD-labelled 16HBE14o- monolayers grown at Air Liquid Interface (ALI) using a trans-well *in vitro* system.

B. CMFDA-labelled neutrophils isolated from human peripheral blood were added to the top of trans-well insert and the chemoattractant, fMLP added to the bottom of the well

C. Neutrophils were allowed to migrate towards fMLP in the bottom of the well for 3 h.

D. Cells were detached from the bottom of the trans-well upon the addition of 0.5 M EDTA and run through a FACS machine to distinguish between DiD-labelled 16HBE cells (FL4⁺) and CMFDA-labelled neutrophils (FL1⁺). The number of CMFDA-labelled neutrophils was counted and used to calculate percentage of TEpM relative to the fMLP positive control.