Supplementary Figure S1

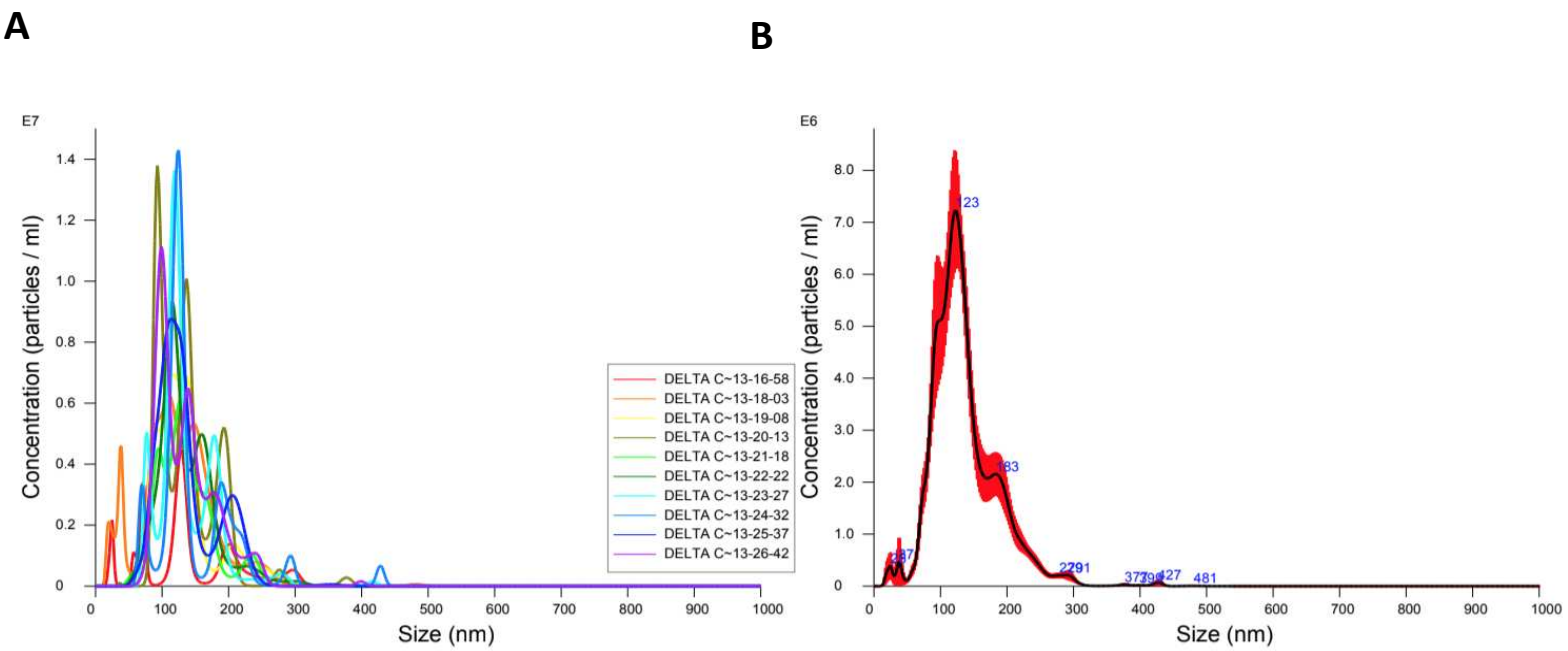

Figure S1

A 10 measurements of particle size and concentration of CFBE41o- EVs were taken (each line is showing each replicate). In **B** the average of **A** was obtained.

Supplementary Figure S2

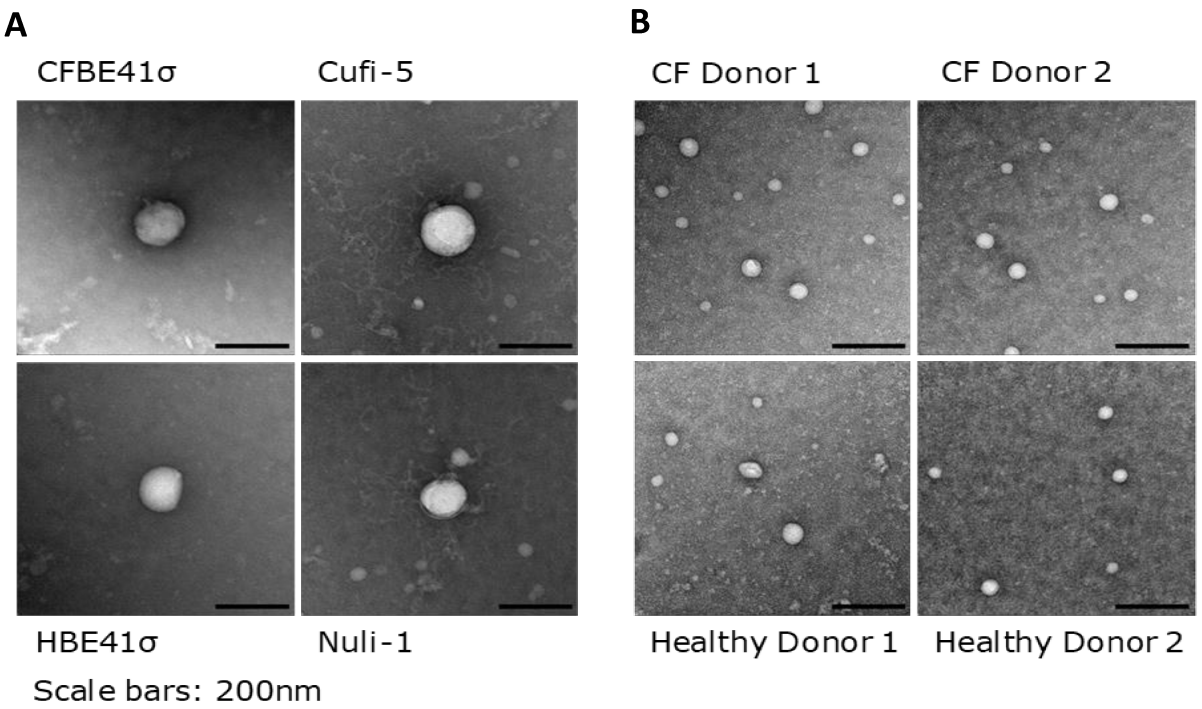

Figure S2

A. Representative TEM images of EVs obtained from CF bronchial cell lines (Cufi-5, CFBE41o-) and WT control (NuLi-1, HBE41o-) at 200 000X magnification

B. Representative TEM images of EVs obtained from CF BALF (2 donors-5-6yrs) and Healthy BALF (2 donors) at 200 000X magnification. Scale bar represents 200nm and is the same for all images.

Supplementary Figure S3

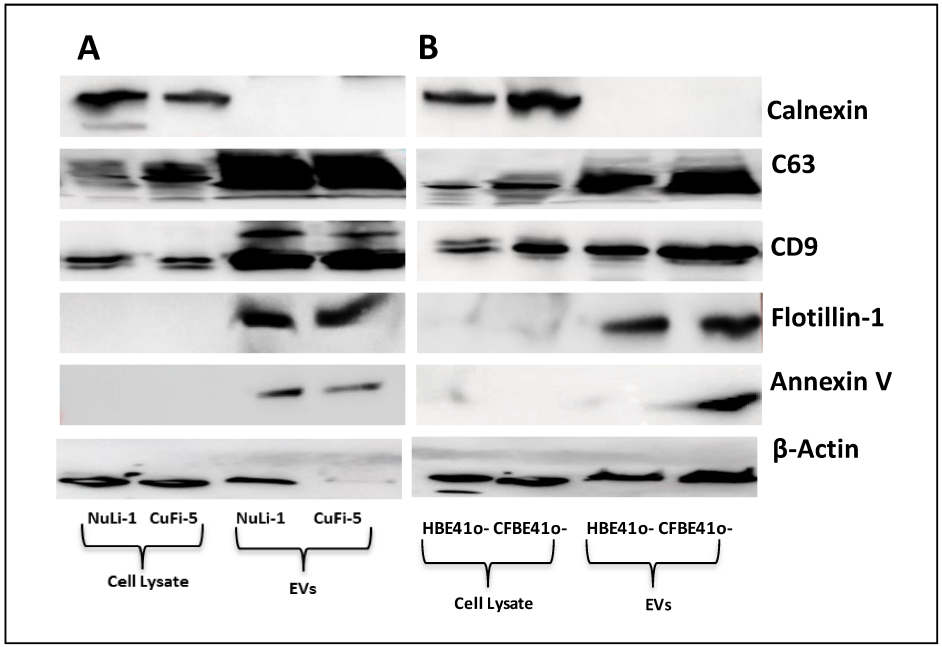

Figure S3
Protein expression of CD9, CD63. Flotillin-1, Annexin V and Calnexin in **A**. Cufi-5/Nuli-1 and **B.** CFBE41o-/HBE41o- cell lysates and isolated EVs.

Supplementary Figure S4

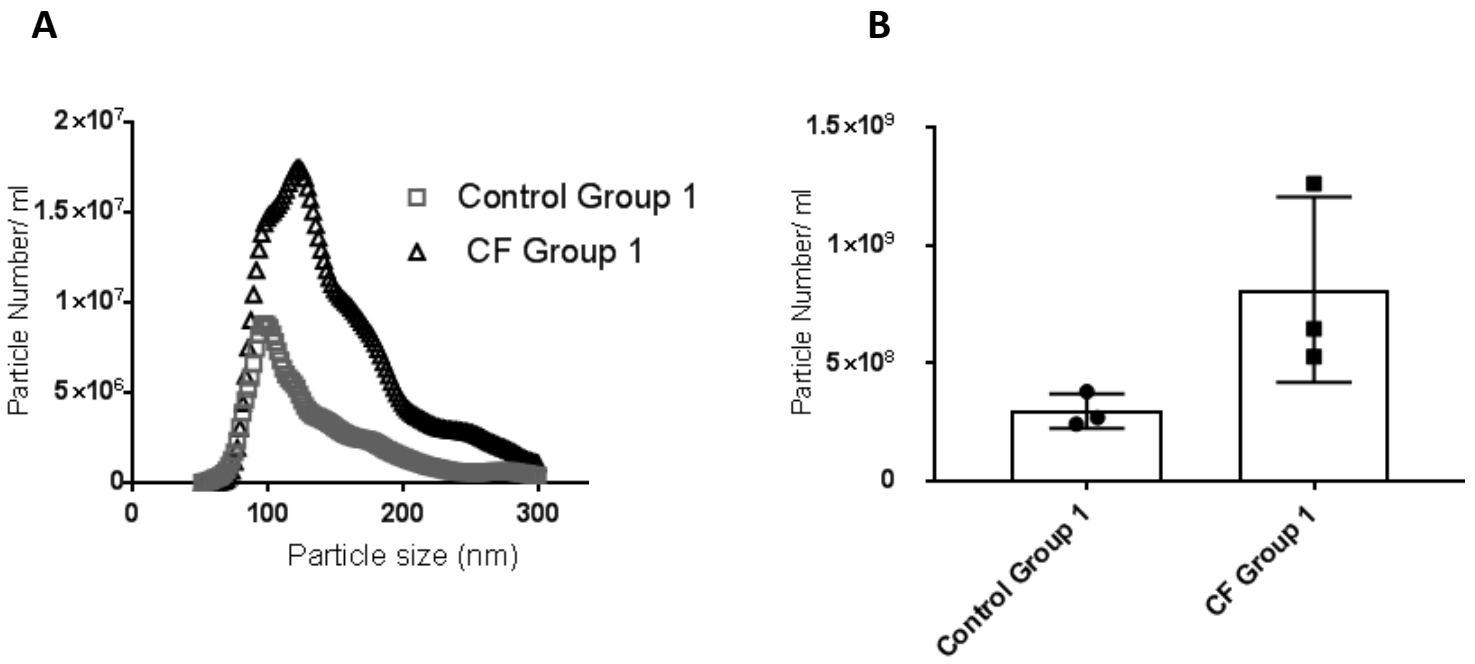

Figure S4

NTA analysis of EVs isolated from BALF of PWCF, age Group 1-2yrs and corresponding age matched controls (n=3). **A.** Distribution of particle size and **B.** Histograms representing the average of 3 experiments, error bars denote mean ± SD. Significance was determined by unpaired t-test; p=0.089 (not significant).

Supplementary Figure S5

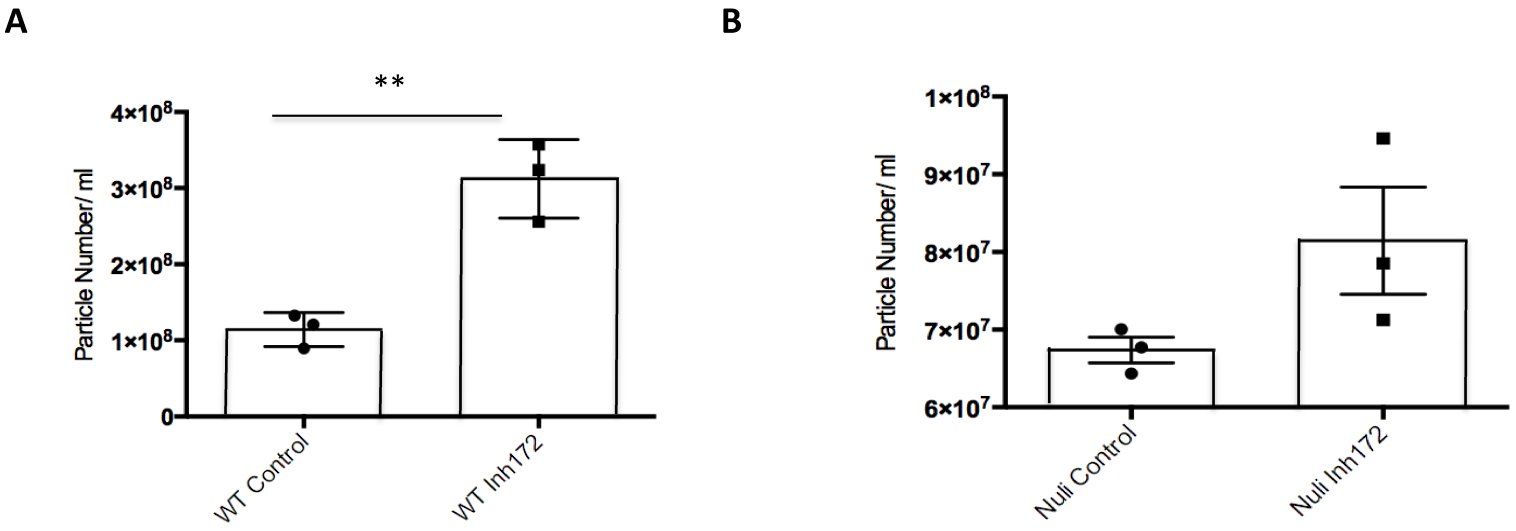

Figure S5

NTA analysis of EVs isolated from **A**. WT HBE41o- and **B**. NuLi-1 cells treated with 10uM of CFTR(inh)-172 for 4 hours Histograms represent the average of 3 experiments, error bars denote mean ± SD. Significance was determined by Student t-test; $p < 0.01$ (**).

Supplementary Figure S6

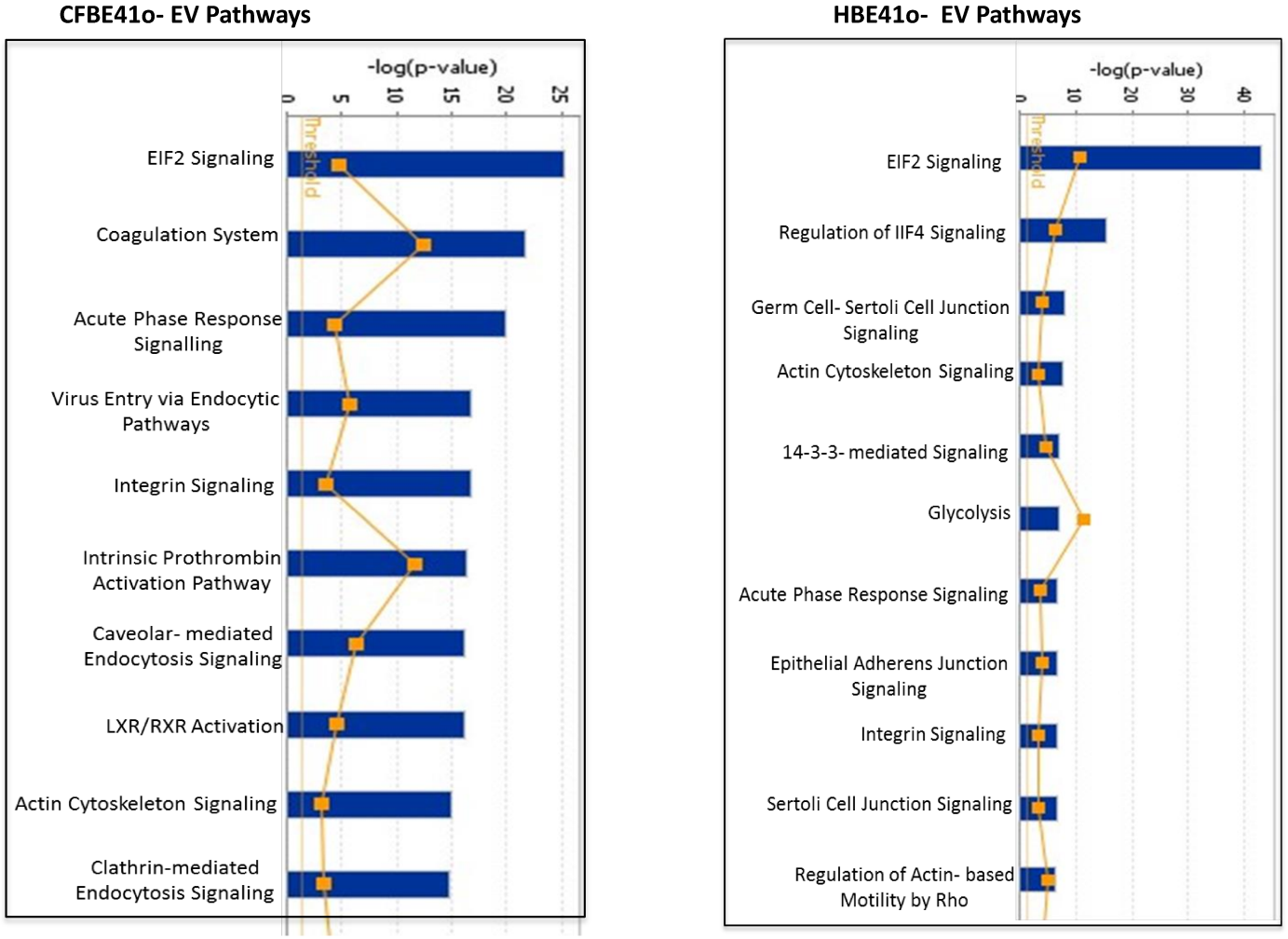

Figure S6

Proteins identified in the EVs were overlaid onto global molecular networks, using the Ingenuity knowledge database. Canonical pathways significantly associated with proteins ($p<0.05$) in WT HBE41o- EVs and F508del CFBE41o- EVs are displayed.

Supplementary Figure S7

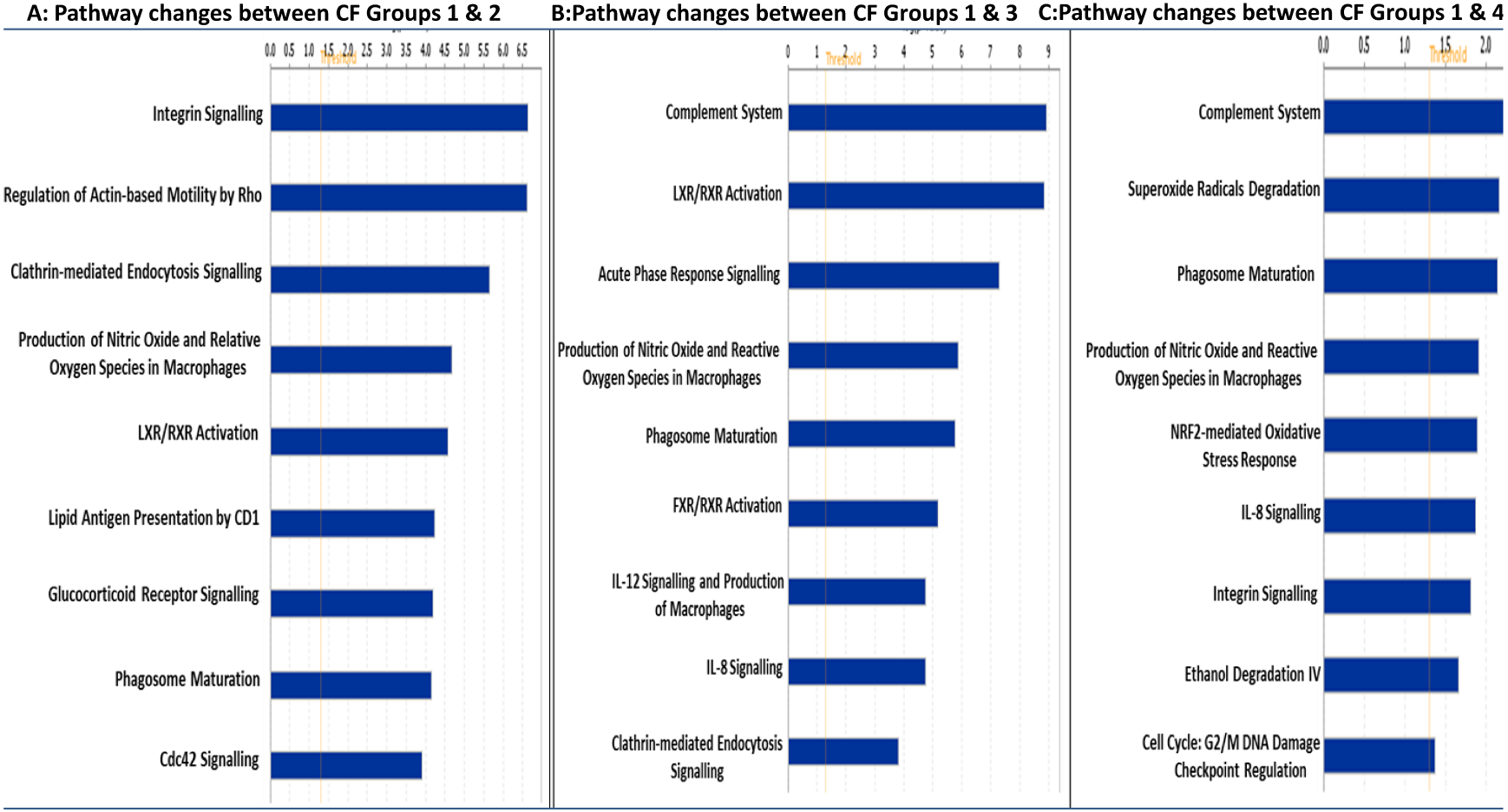

Figure S7

Proteins identified in the EVs that were significantly different between the different CF age groups were overlaid onto global molecular networks using the Ingenuity knowledge database. Canonical pathways significantly associated with proteins ($p<0.05$) are displayed. A- Group 1 vs Group 2 B- Group 1 vs Group 3 C- Group 1 vs Group 4

Supplementary Figure S8

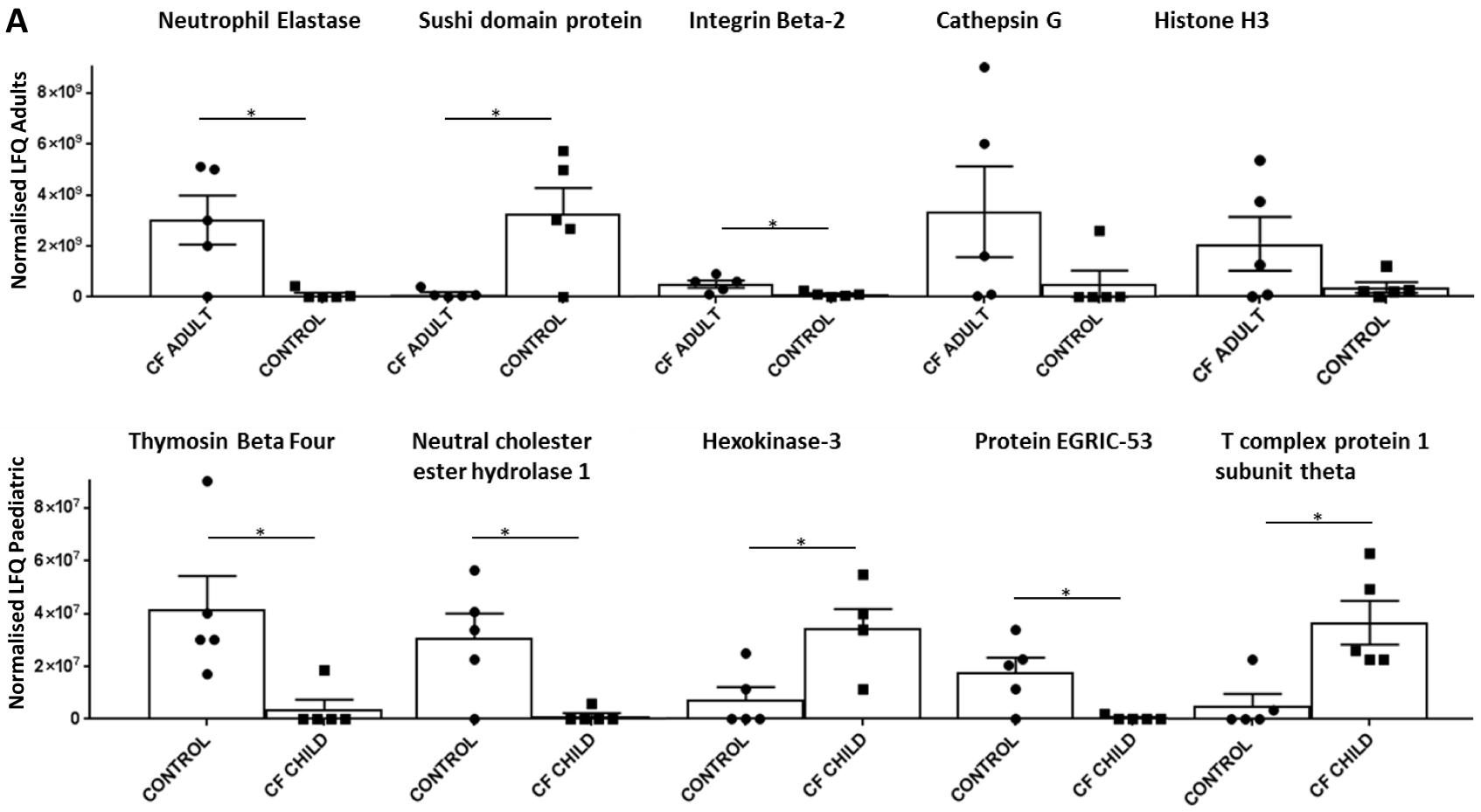

Figure S8

A LFQ intensities of 5 proteins identified in the Adult BALF EVs vs Adult Control EVs

B LFQ intensities of 5 proteins identified in the Paediatric BALF EVs vs Paediatric Control EVs

Histograms represent the average of 5 experiments, error bars denote mean ± SD.

Significance was determined by unpaired t-test; $p < 0.05$ (*).

Supplementary Figure S9

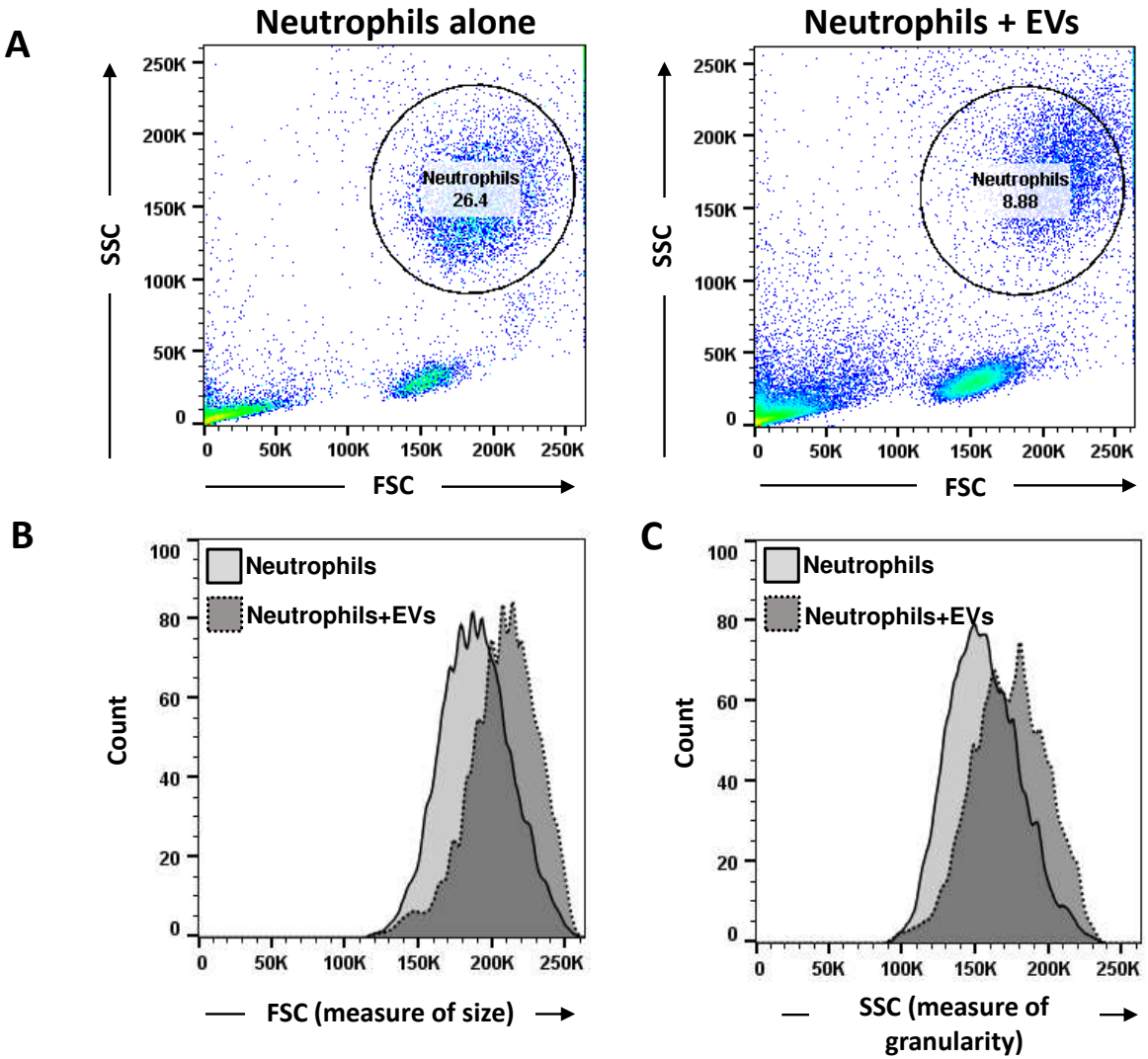

Figure S9

A Dotplots of Neutrophils +/- EVs incubation analysed by flow cytometry

B Forward scatter of Neutrophils +/- EVs, as a measure of cell size

C Side scatter of Neutrophils +/- EVs, as a measure of cell granularity

Supplementary Figure S10

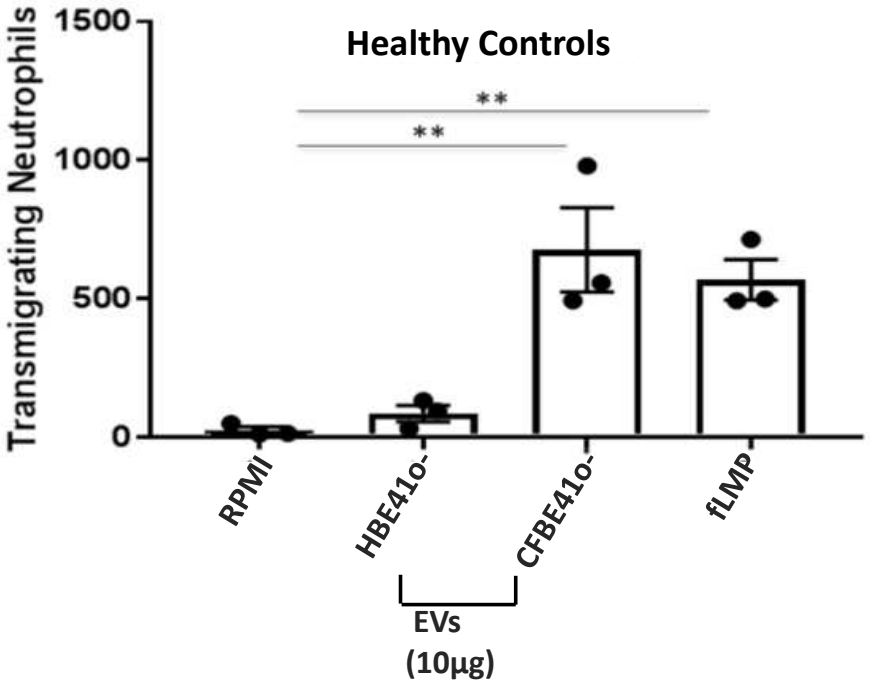

Figure S10 Neutrophil transmigration through through a trans-well chamber coated with CFBE41o- cells in response to treatment with CFBE41o-/HBE41o- EVs. . Histograms represent the average of 3 experiments, error bars denote mean ± SD . Statistical test used was one-way Anova, error bars denote mean± SD. $p<0.01$(**).

Supplementary Figure S11

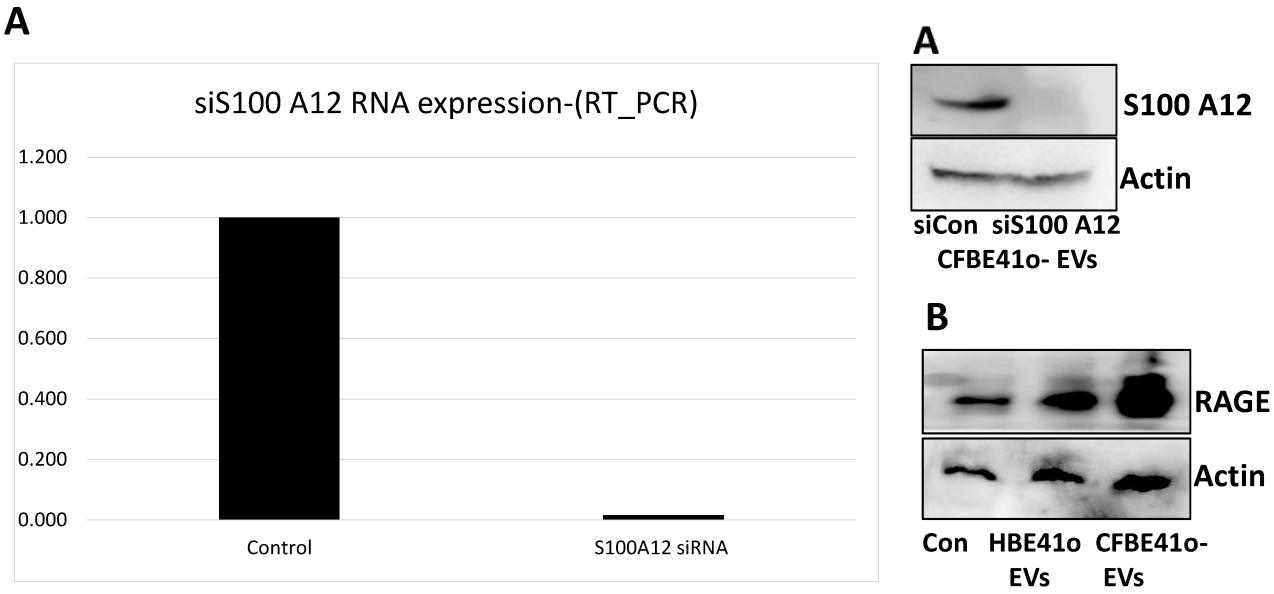

Figure S11A S100 A12 RNA and protein expression in EVs isolated from CFBE41o- cells treated with siRNA to S100 A12 and siCon for 96hrs. **S11B** RAGE expression in neutrophils from a person with CF after treatment with CFBE41o-/HBE41o- EVs.

Supplementary Figure 12

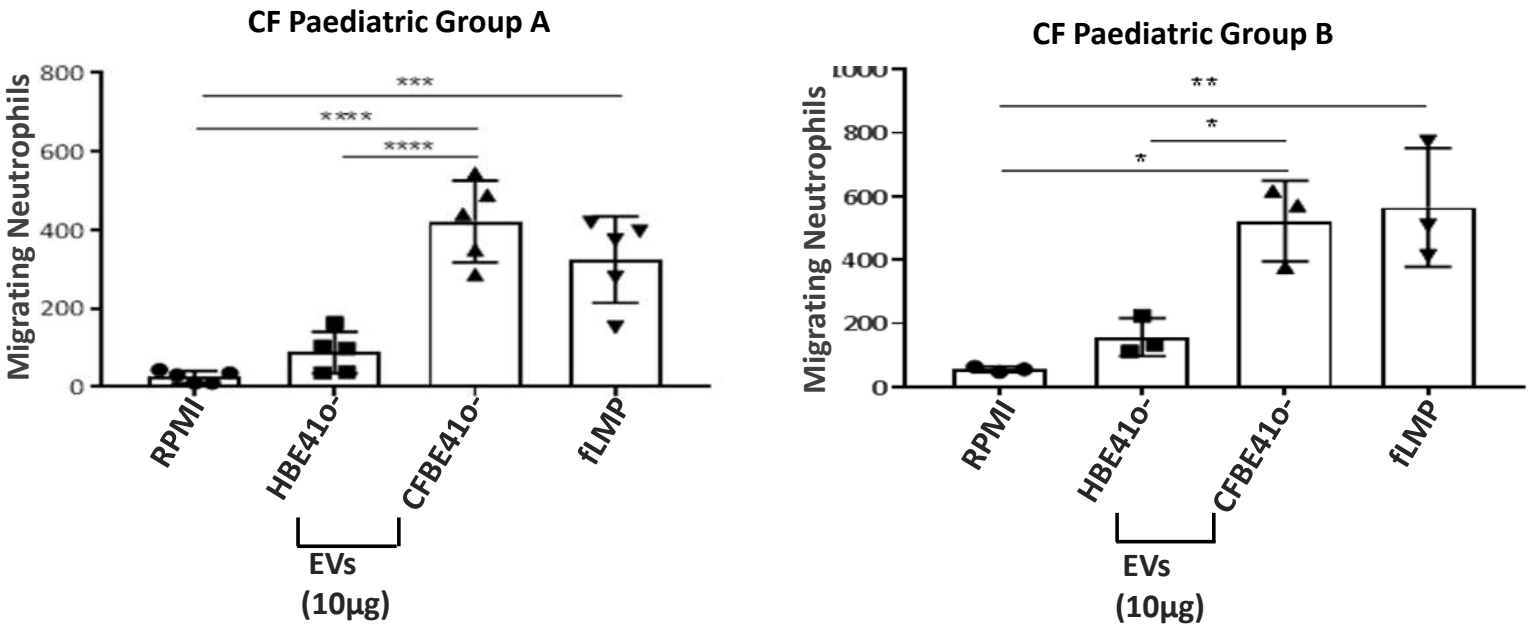

Figure S12 Chemotaxis of neutrophils from PWCF (Group A: 2-4yrs, Group B: 13-17yrs) in response to treatment with CFBE41o-/HBE41o -EVs. Histograms represent the average of 5 (Group A) 3 (GroupB) experiments, error bars denote mean ± SD. Statistical test used was one-way Anova, error bars denote mean± S.D. $p<0.05$ (*) $p<0.01$(**), $p<0.001$ (***).