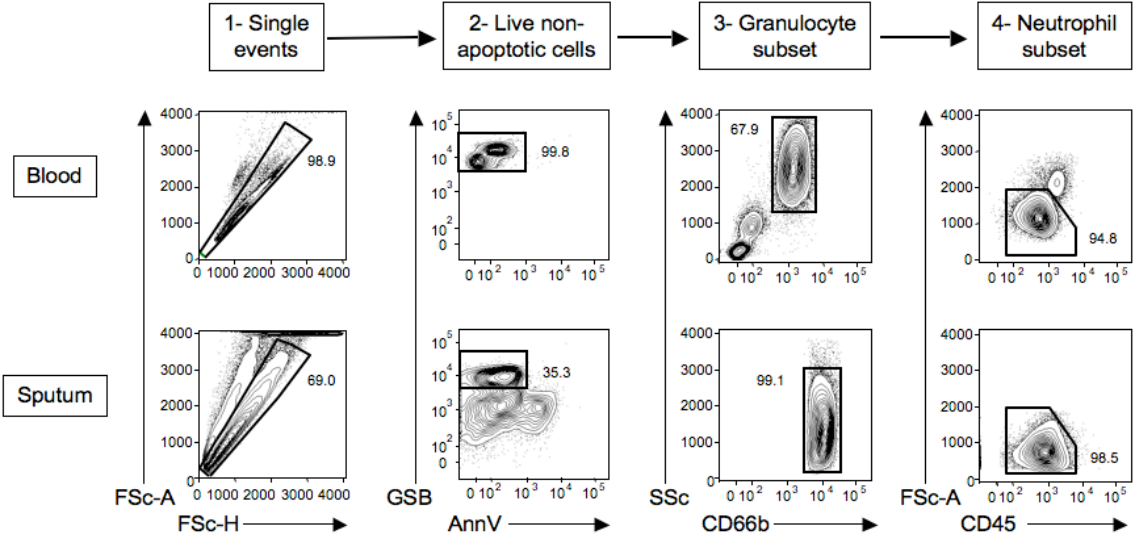
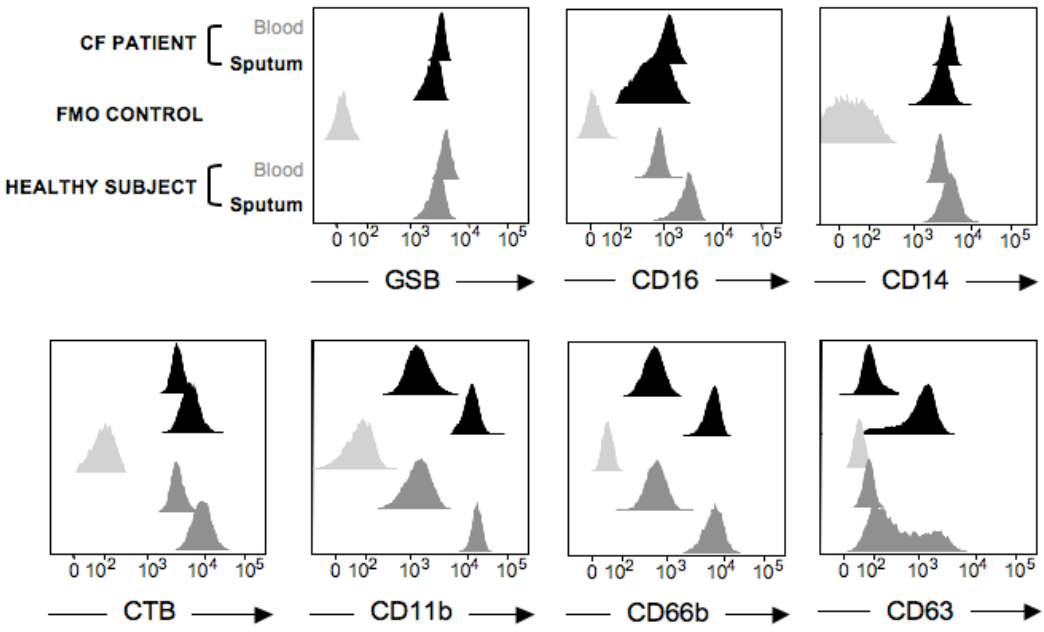

1- Single events
2- Live non-apoptotic cells
3- Granulocyte subset
4- Neutrophil subset
Blood
Sputum
98.9
99.8
67.9
94.8
69.0
35.3
99.1
98.5
FSc-A
FSc-H
GSB
AnnV
SSc
CD66b
FSc-A
CD45

SUPPLEMENTARY FIGURE 3. Compared activation patterns of viable neutrophils from CF and healthy airways. As shown here for comparison, neutrophils from CF and healthy subjects (1 representative subject for each group) show similar modulation when migrating from blood to sputum, e.g., decreased intracellular GSH levels (as measured by GSB adducts), increased lipid raft formation (as measured by CTB labeling), as well as surface mobilization of CD11b+ and CD66b+ vesicles and granules. Clear differences are seen in the degree of surface mobilization of CD63+ granules (massive in CF subjects and minor in healthy controls) and in surface CD14 and CD16 expression (both decreased in CF subjects and both increase in healthy controls). Fluorescence-minus one (FMO) controls are also shown for comparison (providing background staining levels, see Supplementary Methods, online).



SUPPLEMENTARY FIGURE 4. Surface mobilization of CD63+, NE-rich granules associates with loss of surface CD16 in viable CF airway neutrophils. Two upper and two middle panels represent X (CD63) by Y (CD16) representations of sputum neutrophils from 4 representative CF patients, showing massive surface mobilization of CD63 and Cd16 loss, as well as a clear negative correlation between the two surface markers. In healthy controls shown here for comparison (two lower panels), sputum neutrophils display a minor mobilization of CD63+ granules to the surface and do not decrease surface CD16 levels. Gates shown on all panels were defined so as to include all neutrophils in corresponding blood samples.