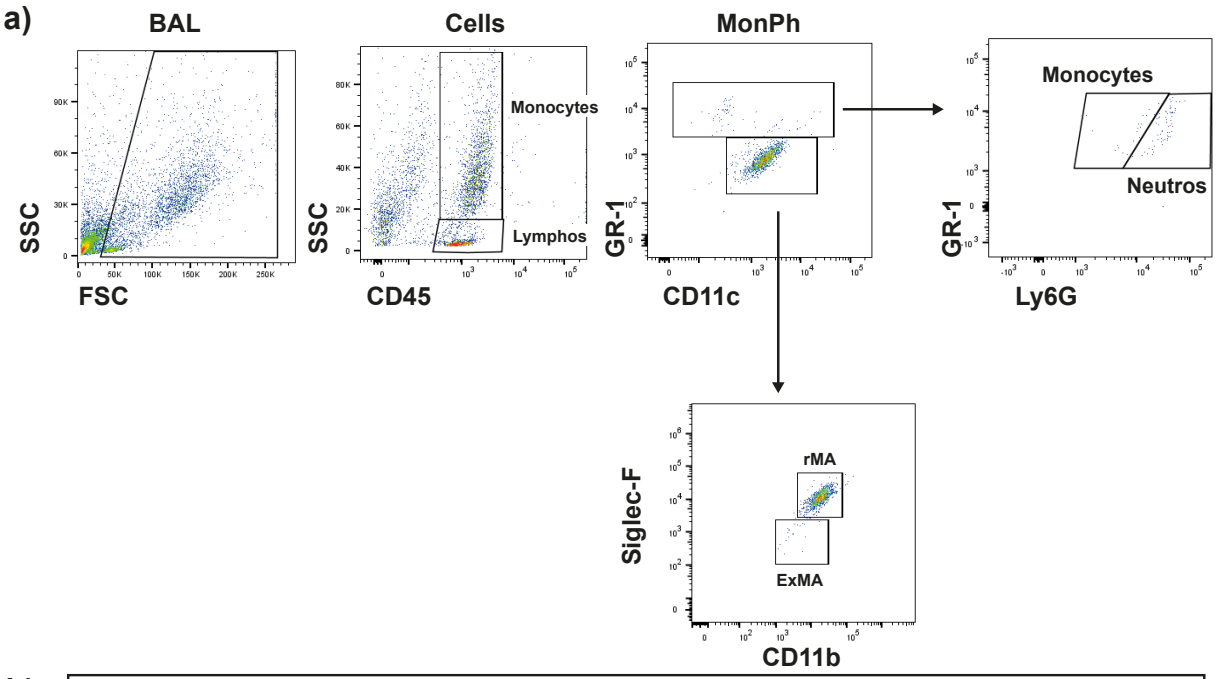
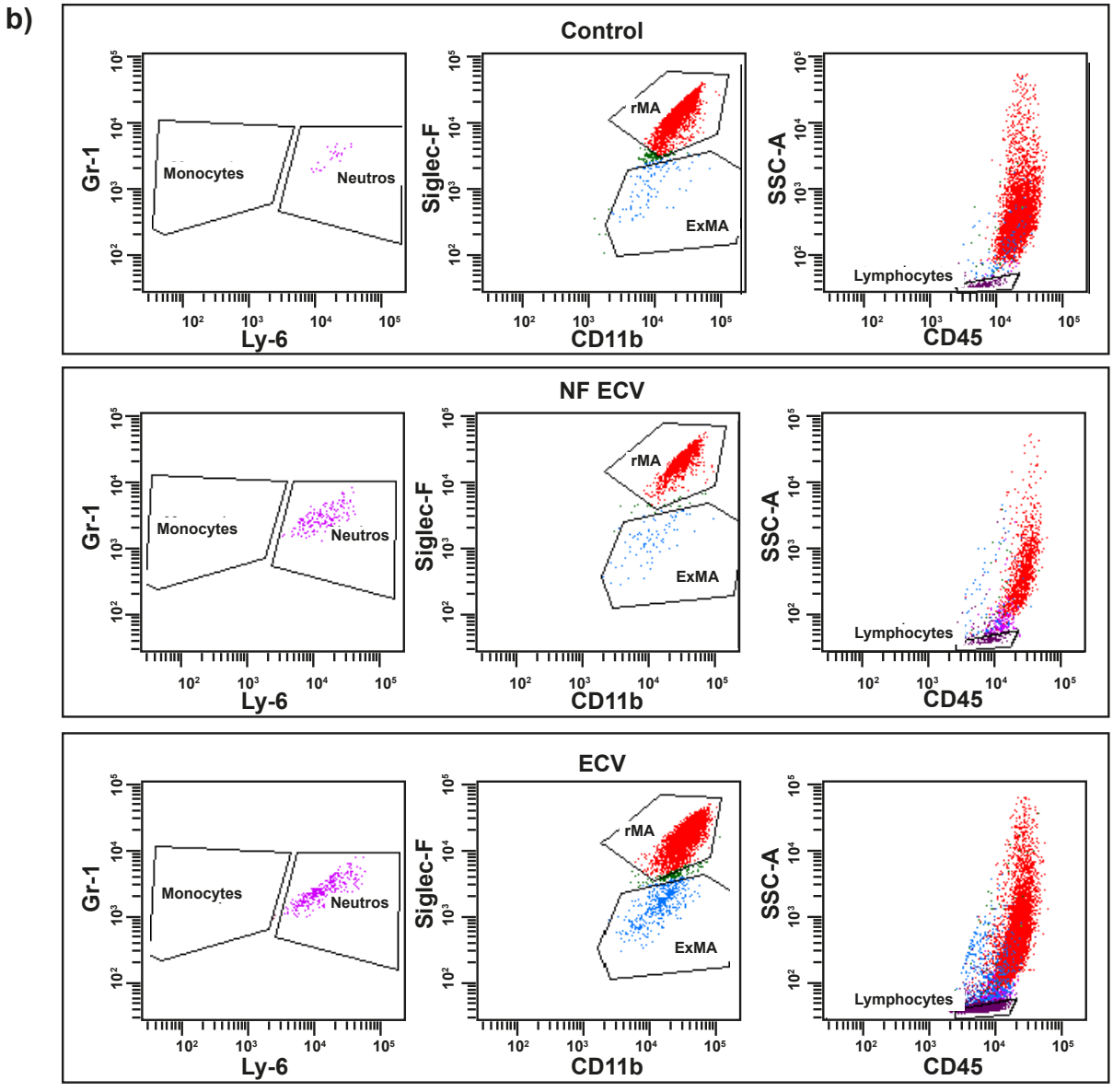

a)
BAL
Cells
MonPh
SSC
FSC
SSC
CD45
Monocytes
Lymphos
GR-1
CD11c
Monocytes
Neutros
GR-1
Ly6G
Siglec-F
CD11b
rMA
ExMA


b)
Control
NF ECV
ECV
Gr-1
Ly-6
Monocytes
Neutros
Siglec-F
CD11b
rMA
ExMA
SSC-A
CD45
Lymphocytes

Supplementary figure S6. Effect of long-term *in vivo* exposure to ECV or NF ECV on immune cell composi-tion of bronchoalveolar lavage (BAL).

a) Flow cytometry gating strategy. First, the total BAL cells were gated with forward scatter (FSC) and side scatter (SSC). From the total BAL cells, the lymphocytes were gated based on low FSC and low SSC within the CD45+ cluster. The mononuclear phagocytes (MonPh) were gated based on the higher FSC and/or SSC within the CD45+ cluster. From the MonPh cluster, the $CD11c^{+}Gr\text{-}1^{-}$ population was sub-gated to differentiate resident macrophages (rAMs: $CD11b^{hi}Siglec\text{-}F^{hi}$) and exudate macrophages (ExMAs: $CD11b^{hi}Siglec\text{-}F^{low}$). From the MonPh cluster, the $CD11c^{-}Gr\text{-}1^{+}$ population was sub-gated to identify neutrophils ($GR\text{-}1^{+}Ly6G^{hi}$) and monocytes ($GR\text{-}1^{+}Ly6G^{-}$).

b) Representative flow cytometry plots of the bronchoalveolar lavage (BAL) from mice exposed to either nicotine-free e-cigarette vapour (NF ECV) or nicotine-containing e-cigarette vapour (ECV) for 8 months. Control animals received room air only.