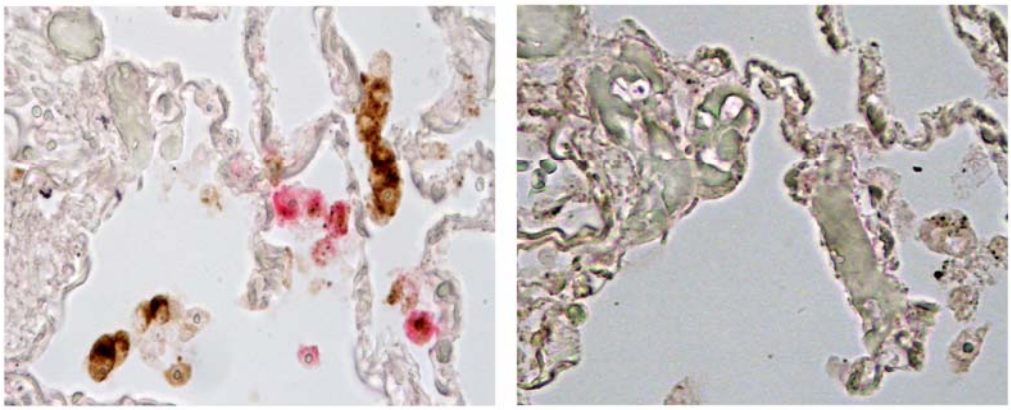

Supplementary Figure 7. CD68^{bright} cells did not express SARS-CoV mRNA in fatal SARS lung.

Paraffin-embedded lung tissue sections from fatal SARS patients were retrieved and de-waxed. After microwave heating and digestion with proteinase K (10μg/ml, Invitrogen, USA) for 15 minutes at 37°C, sections were incubated overnight at 46°C with a DIG-labeled SARS-CoV cRNA probe which encodes part of spike protein and corresponds to nucleotides 21428-23438 (Genbank Accession No. AY282752). Sections were subsequently incubated with peroxidase blocking solution containing 0.3% H_2O_2 and 0.03% sodium azide for 30 minutes at room temperature to inhibit endogenous peroxidase, followed by incubation with sheep anti-Digoxigenin-POD Fab fragment (1:50, Roche, PRC) for 2 hours at room temperature. Finally, the color was developed with DAB substrate (DakoCytomation, Denmark). After washing with PBS, immunostaining was performed on same sections by staining with a mouse monoclonal antibody against human CD68 (clone kp1, DakoCytomation, Denmark), a macrophage-specific marker and expressed by alveolar macrophages (Soilleux EJ et al, *J Leukoc Biol* 71, 445-57 (2002)) overnight at 4°C, followed by incubation with an anti-mouse universal immuno-AP polymer (ready-to-use, Nichirei corporation, Japan) for 2 hours at room temperature. The color was developed with Fast Red substrate (Sigma, USA). The DIG-labeled SARS-CoV spike protein cRNA probe was prepared from a DIG-RNA labeling kit (Roche, PRC) according to the manufacturer's instructions. Results clearly demonstrated that cells expressing CD68 (shown in red, left panel) was separable from cells containing SARS-CoV mRNA (shown in brown, left panel). Staining with isotype control antibody for anti-CD68 Ab and sense control probe for SARS-CoV gave negative results (right panel). Data were representative of samples from 3 autopsied SARS patients. This result is consistent with a report that SARS-CoV could not be found in CD68+ cells in the SARS lung (To KF et al, *J Pathol* 202, 157-63 (2003)).