VIRAL-MEDIATED INHIBITION OF ANTIOXIDANT ENZYMES CONTRIBUTES TO THE PATHOGENESIS OF SEVERE RSV BRONCHIOLITIS

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ONLINE DATA SUPPLEMENT

Figure E1. Densitometric analysis of AOE expression in BAL. Densitometric analysis of Western blot band intensities of Fig.1 was performed using Alpha Ease software, version 2200 (2.2d) (Alpha Innotech Co., San Leandro, CA). Bands in RSV-infected samples were normalized to uninfected control sample background. Bar graphs are mean ± SEM. *$p < 0.05$; ** $p < 0.01$ and ***$p < 0.001$ relative to control mice.

Figure E2. **Close-up view of SYPRO Ruby-stained 2DE of BAL proteins, showing decreased AOE expression in RSV-infected mice.** BAL proteins were prepared from control and RSV-infected mice (day 3) and subjected to 2DE. The gels were stained with SYPRO Ruby. The regions of the gels containing the spots corresponding to SOD 1, Catalase, GPx 1 and GST-mu are shown enlarged and circled (one gel from control and two gels from RSV-infected mice for each AOE).

Figure E3. **Close-up view of SYPRO Ruby-stained 2DE of NPS proteins.** NPS proteins collected from three patients each with diagnosis of RSV-positive URTI (A), bronchiolitis (B), hypoxic bronchiolitis (C) and requiring ventilatory support (D) were subjected to 2DE. The regions of the SYPRO Ruby-stained gels containing the spots corresponding to SOD 1, Catalase, and Peroxiredoxin 1 are shown enlarged and circled, showing smaller protein spot volumes in more severe illness.

Figure E4. AOE are reduced in BAL of hMPV-infected mice. Groups of mice were infected with hMPV or sham inoculated with saline (Control) and BAL was collected at days 1, 3, 5, and 7. BAL proteins were resolved on 10% SDS-PAGE and Western blots were performed using

antibodies against SOD 1, 2, catalase and GST-mu. Membranes were stripped and reprobed for β-actin as an internal control for protein integrity and loading. Lanes 1-3 are BAL from three control and 4-6 from three hMPV-infected mice at each time point. The figure is representative of two independent experiments, each experiment with 3 mice/group/time point.

Fig. E1

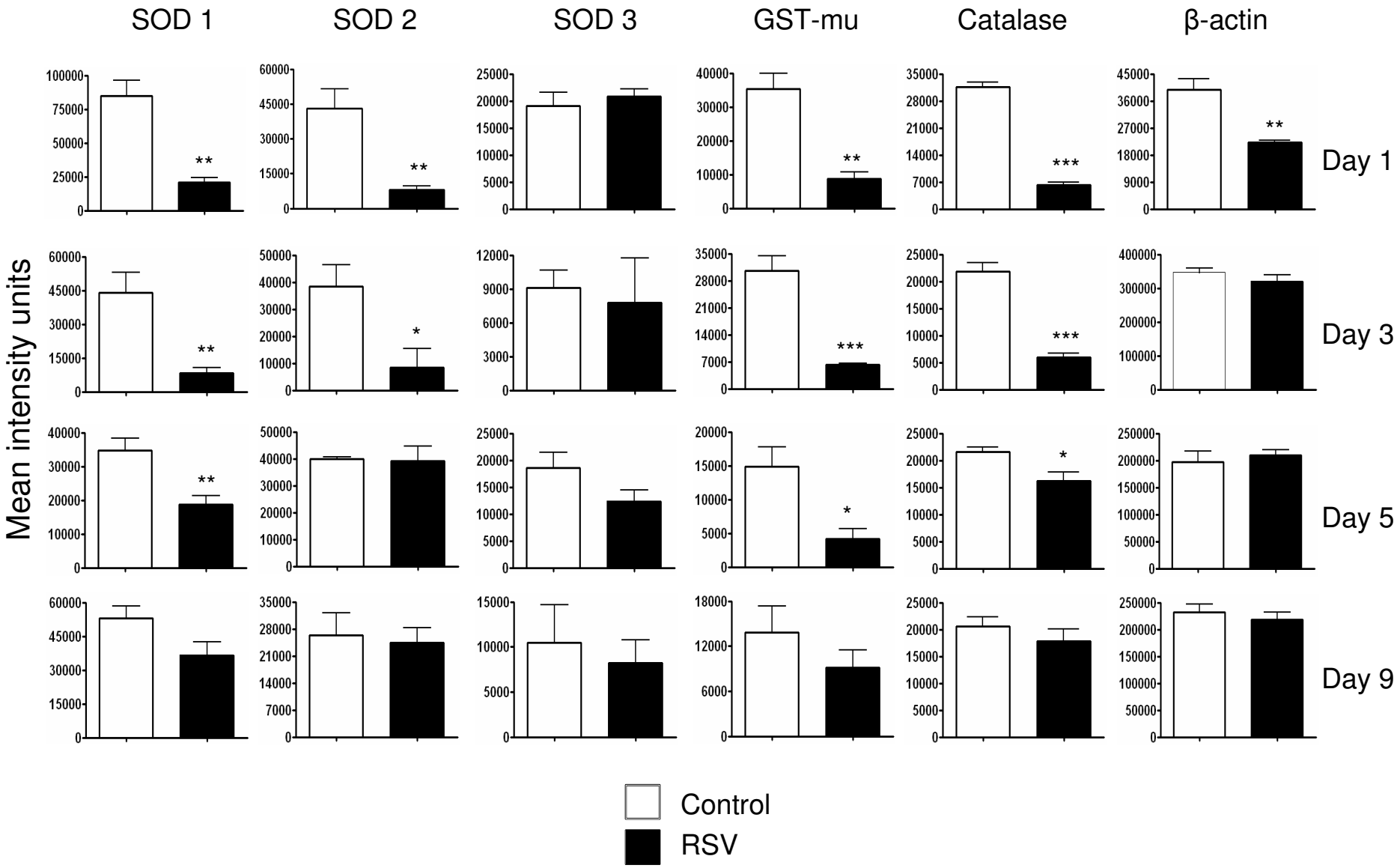

SOD 1
SOD 2
SOD 3
GST-mu
Catalase
β-actin
Day 1
Day 3
Day 5
Day 9
Mean intensity units
Control
RSV

Fig. E2

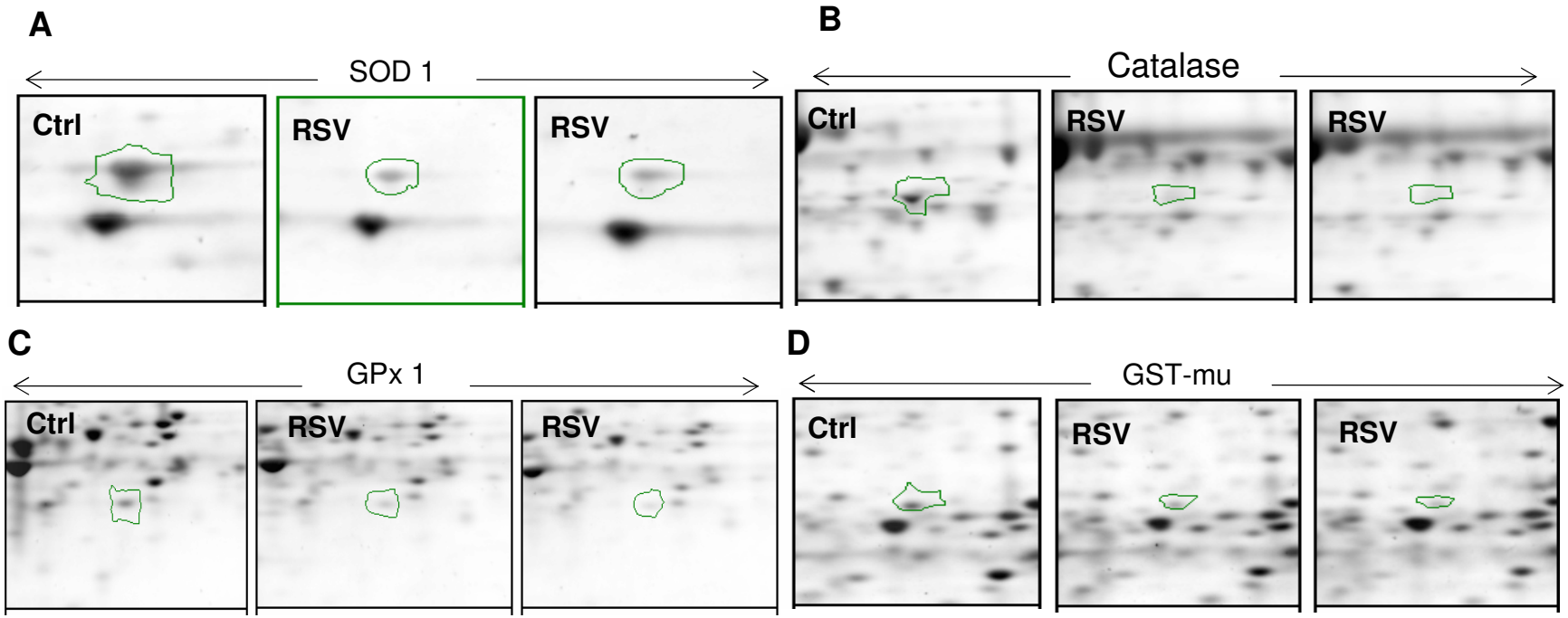

A
SOD 1
Ctrl
RSV
RSV
B
Catalase
Ctrl
RSV
RSV
C
GPx 1
Ctrl
RSV
RSV
D
GST-mu
Ctrl
RSV
RSV

Fig. E3

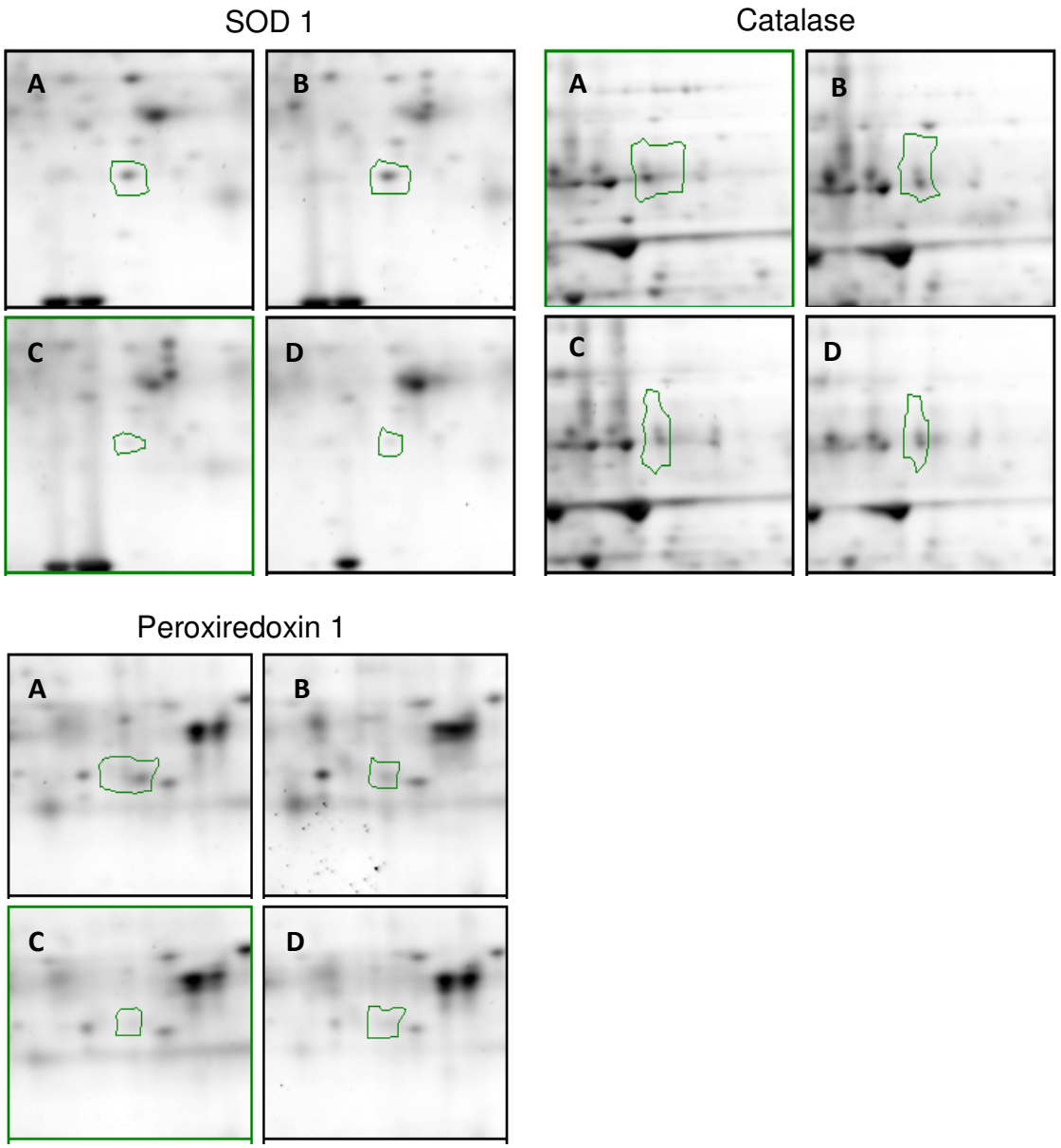
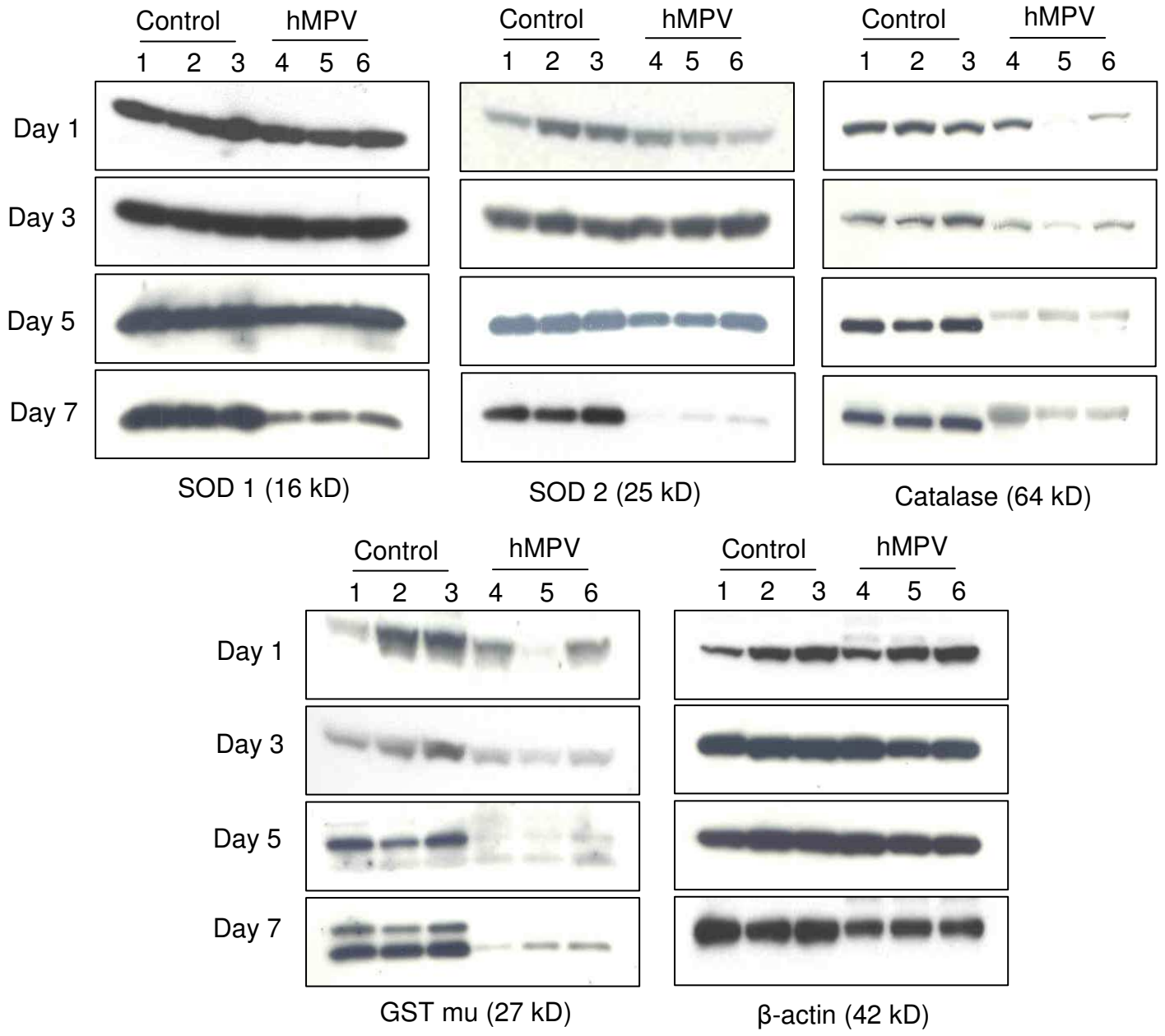


Fig. E4


Control
hMPV
1 2 3 4 5 6
Day 1
Day 3
Day 5
Day 7
SOD 1 (16 kD)
SOD 2 (25 kD)
Catalase (64 kD)
GST mu (27 kD)
β-actin (42 kD)