Supplementary Information

Ascorbate induces apoptosis in melanoma cells by suppressing Clusterin expression

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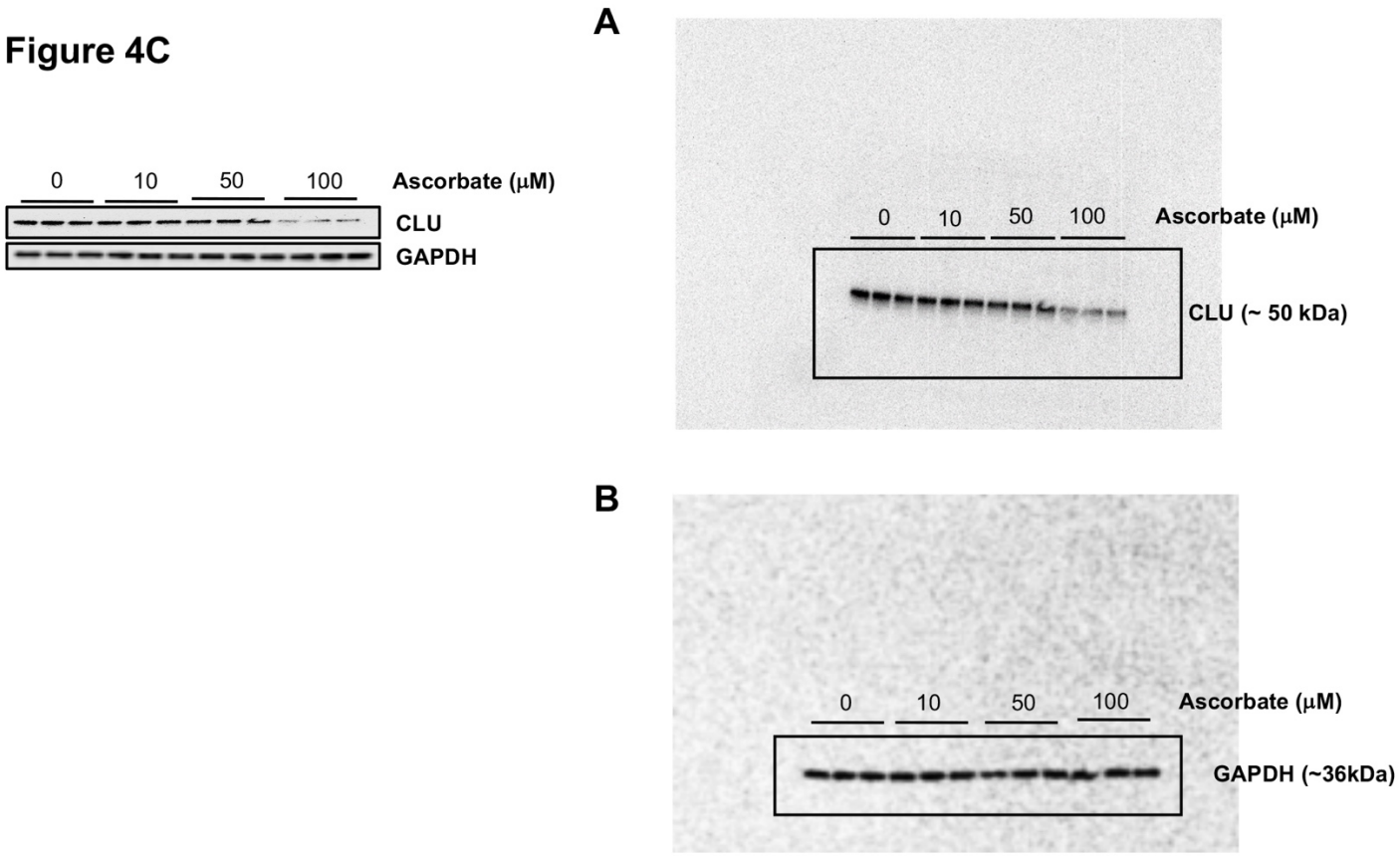
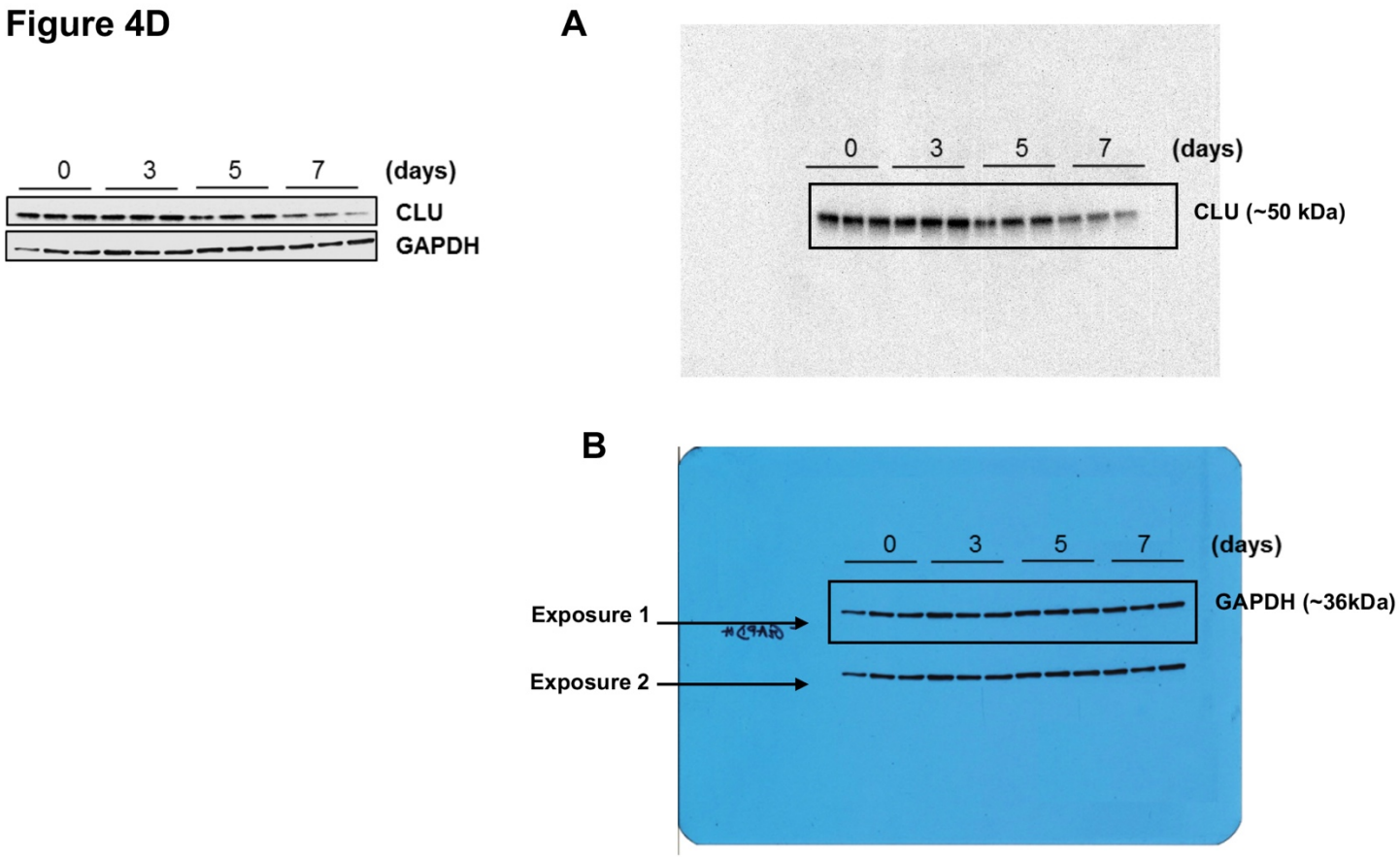
Supplementary Figure 1



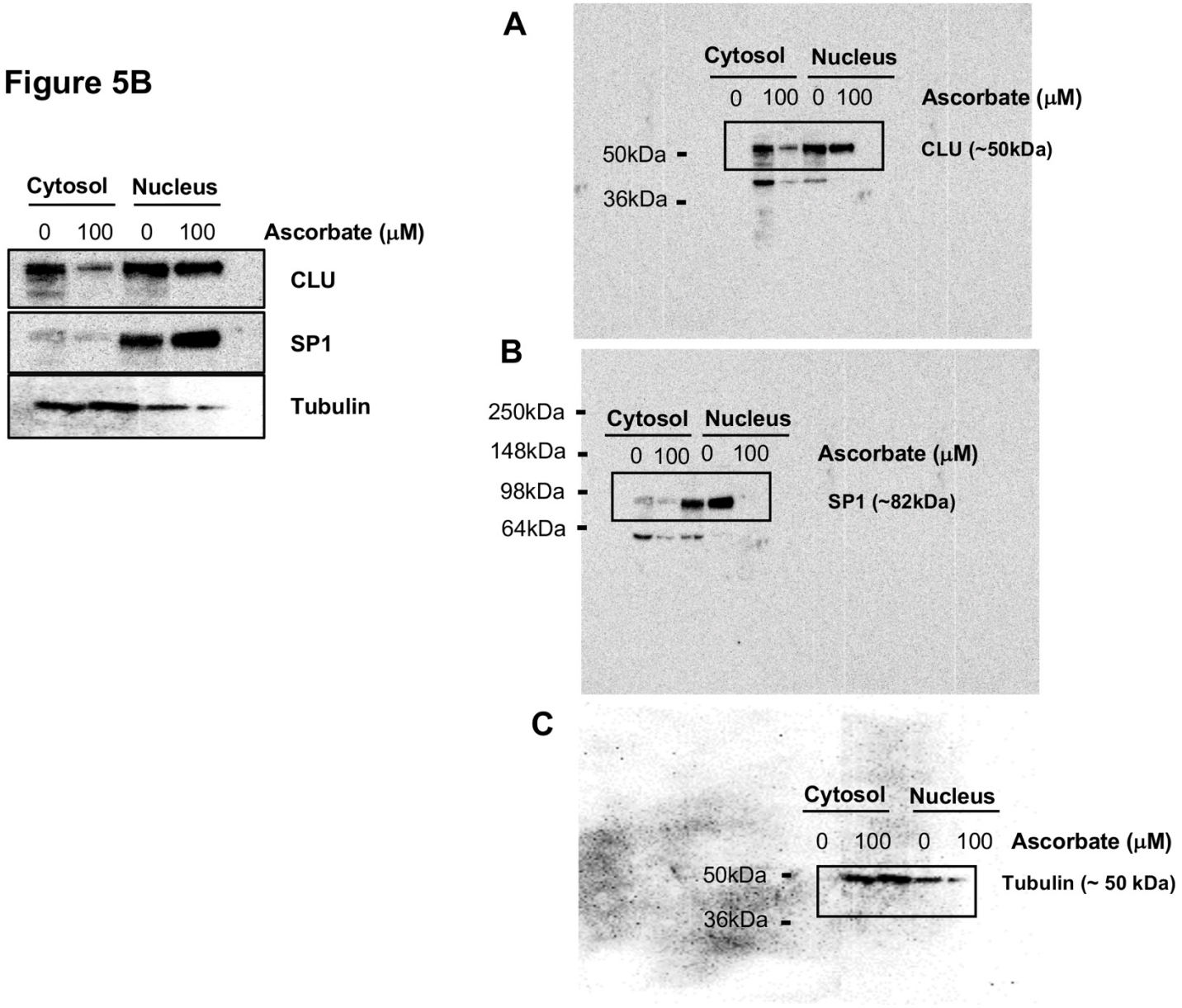
Supplementary Figure 1. Immunoblot membrane images of CLU expression in A2058 cells treated with different concentrations of ascorbate (0, 10, 50 and 100 μM). The cropped images are shown in **Figure 4C**. **(A)** Immunoblot membrane was probed with anti-CLU primary antibody and was imaged in FluorChemE system through Chemiluminescence channel. **(B)** Immunoblot membrane was then stripped and re-probed with anti-GAPDH primary antibody, subsequently imaged in FluorChemE system through Chemiluminescence channel.

Supplementary Figure 2



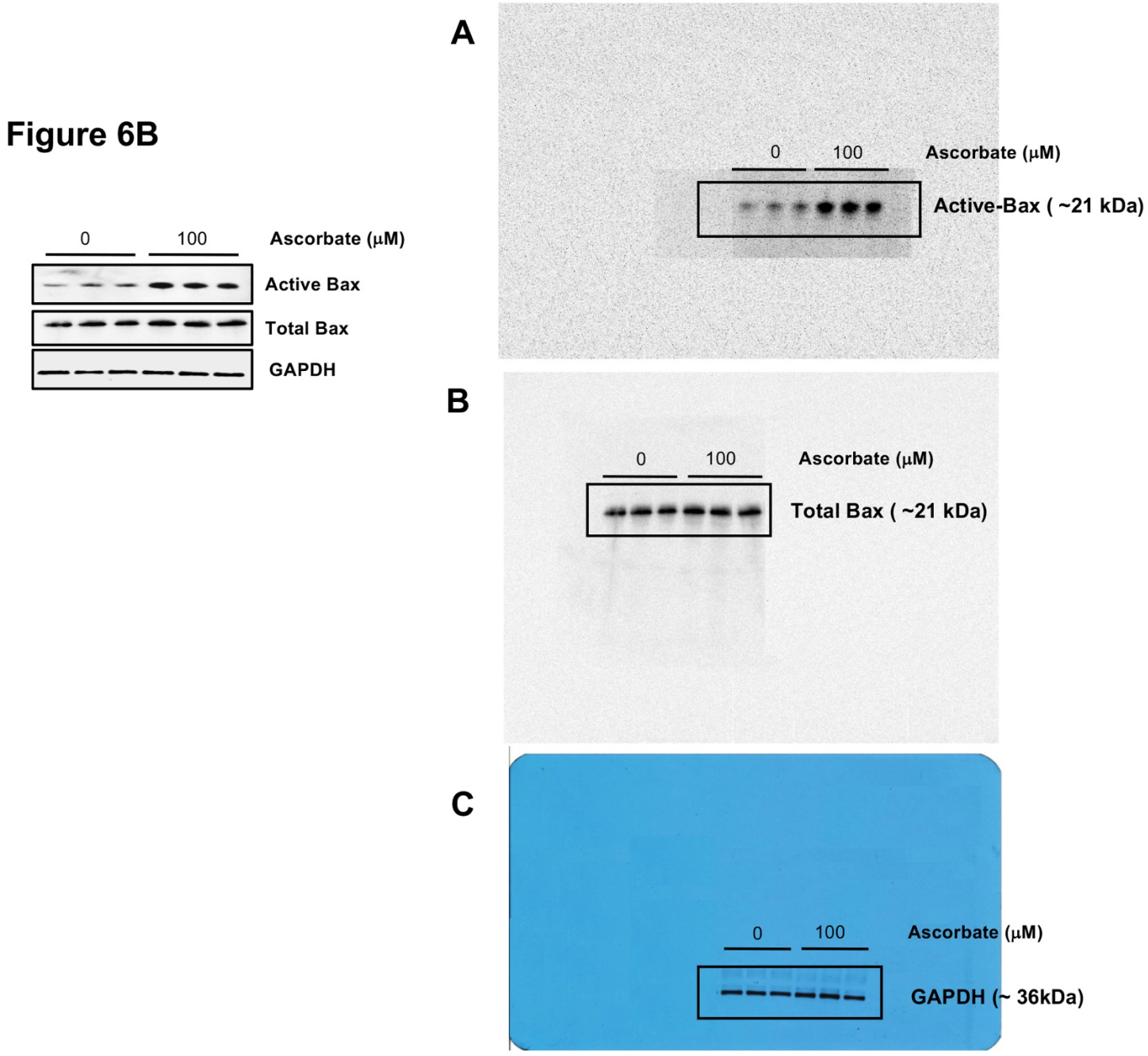
Supplementary Figure 2. Immunoblot membrane images of CLU expression in A2058 cells treated with ascorbate (100 μM) for a period of 0, 3, 5 and 7 days. The cropped images are shown in **Figure 4D**. **(A)** Immunoblot membrane was cut between ladder 50kDA and 36 kDA. The top half was probed with anti-CLU primary antibody and was imaged in FluorChemE system through Chemiluminescence channel. **(B)** The bottom half of the Immunoblot membrane probed with anti-GAPDH primary antibody was imaged on X-ray film for immunoblot imaging and developed with two different exposures.

Supplementary Figure 3



Supplementary Figure 3. Images of immunoblot showing CLU distribution in nuclear and cytoplasmic fractions with or without ascorbate treatment. The cropped images are shown in **Figure 5B**. **(A)** Immunoblot membrane was cut near 64kDa ladder size. The bottom part was probed with anti-CLU primary antibody and was imaged in FluorChemE system through Chemiluminescence channel. **(B)** Top part of the immunoblot membrane was probed with anti-SP1 primary antibody and imaged in FluorChemE system through Chemiluminescence channel. **(C)** Bottom part of the immunoblot membrane was further re-probed with anti-tubulin primary antibody and was imaged in FluorChemE system through Chemiluminescence channel.

Supplementary Figure 4



Supplementary Figure 4. Images of Immunoblot results showing active Bax and total Bax in A2058 cells with or without ascorbate treatment (100 μM). The cropped images are shown in **Figure 6B**. **(A)** Immunoblot membrane was cut into two parts along a line just above 36kDa ladder. The bottom half was probed with anti-active Bax (active monomer 6A7) primary antibody and was imaged in FluorChemE system through Chemiluminescence channel. The top half was probed for CLU proteins (data not shown). **(B)** Immunoblot membrane from another run of SDS-PAGE gel with same sample loading order was probed with anti-total Bax primary antibody and was imaged in FluorChemE system through Chemiluminescence channel. **(C)** Immunoblot membrane from active Bax probe was stripped and re-probed with anti-GAPDH primary antibody was imaged on X-ray film for Immunoblot imaging and developed in medical film developer.