Supplementary Information

Ascorbate induces apoptosis in melanoma cells by suppressing Clusterin expression

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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. 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. 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. 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.