ROS-Mediated Apoptotic Cell Death of Human Colon Cancer LoVo Cells by Milk δ-Valerobetaine

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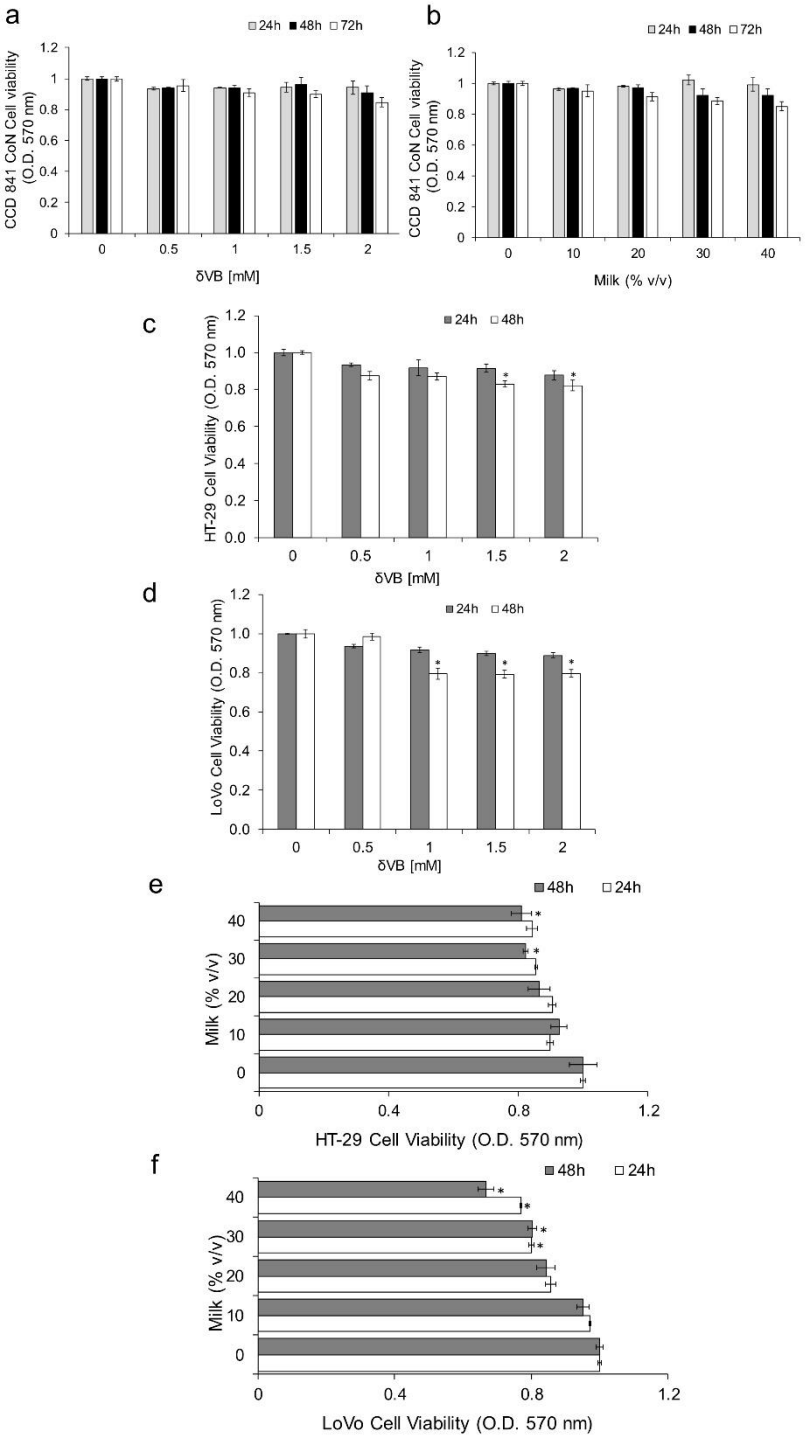

Figure. S1. Effects of milk-δVB on human non-malignant and colorectal adenocarcinoma cell viability. (**a, b**) CCD 841 CoN cells were treated with δVB (up to 2 mM) and milk (up to 40 % v/v) for 24, 48-72h. HT-29 and LoVo cells were treated with (**c, f**) increasing concentrations of δVB (up to 2 mM) and (**d, e**) milk (up to 40 % v/v) for 24-48 h. Control cells were grown in medium containing the same volume (% v/v) of HBSS-10 mM Hepes. Cell growth inhibition was assessed using MTT assay. Values represent the mean±SD of three independent experiments. **P*<0.05 *vs* Ctr.

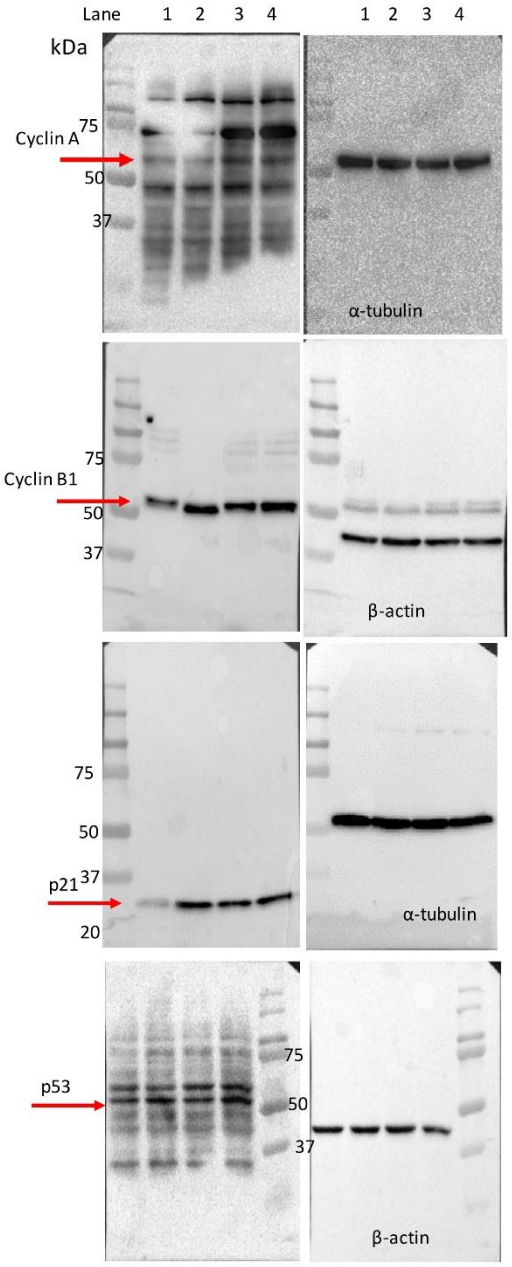

Figure. S2. LoVo cell cycle alteration induced by milk-δVB. The cropped blots are used in the main figure (Figure 2 c-f). Lane 1=Ctr; Lane 2=milk, Lane 3=δVB, Lane 4= milk+ δVB.

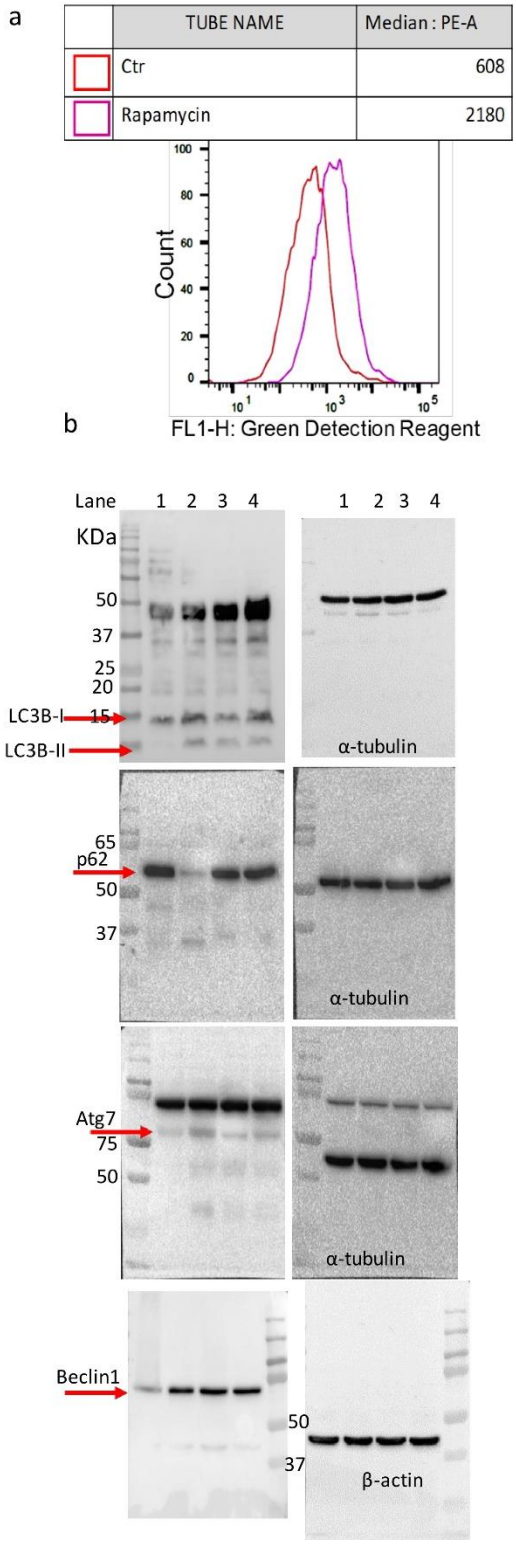

Figure. S3. Autophagy induced by milk-δVB. (a) Representative cytofluorimetric analysis of Green detection reagent performed in untreated LoVo cells (Ctr) and rapamycin-treated cell, as positive control of autophagic process. (b) The cropped blots are used in the main figure (Figure 3d-g). Lane 1=Ctr; Lane 2=milk, Lane 3=δVB, Lane 4= milk+ δVB.

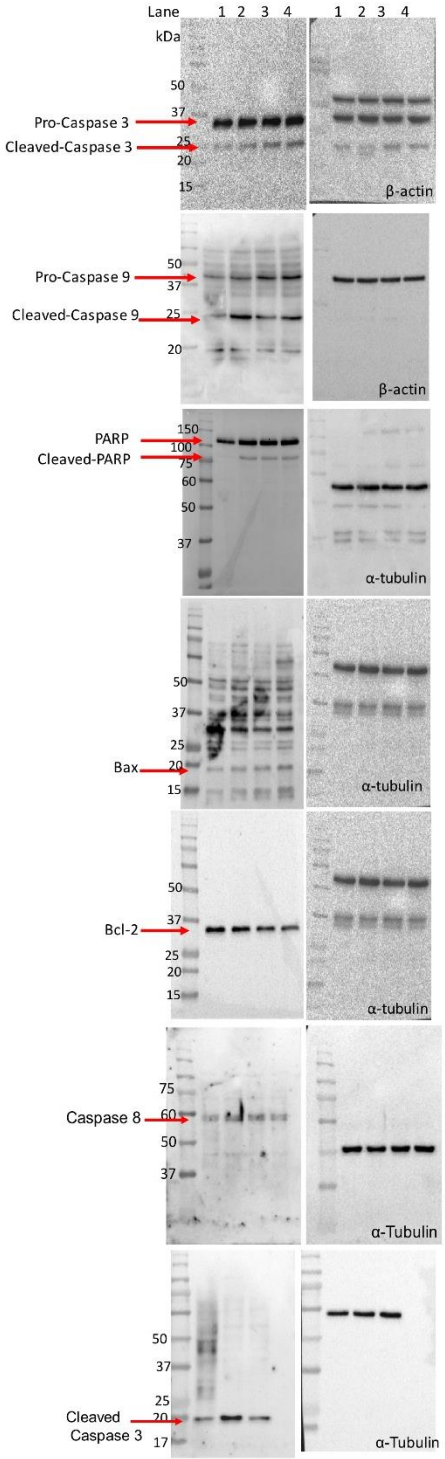

Figure. S4. Apoptotic mechanism. The cropped blots are used in the main figure (Figure 5c-h, n). Antibodies against Bax, Bcl-2 and SIRT6 (reported in the main Fig. 4) were blotted on the same filter and quantified by using the same loading control (α-tubulin). Lane 1=Ctr; Lane 2=milk, Lane 3=δVB, Lane 4= milk+ δVB. The cropped blots used in the main figure (Figure 5m). Lane 1=Ctr; Lane 2=milk+ δVB, Lane 2=Z-LEHD-FMK +milk+ δVB.

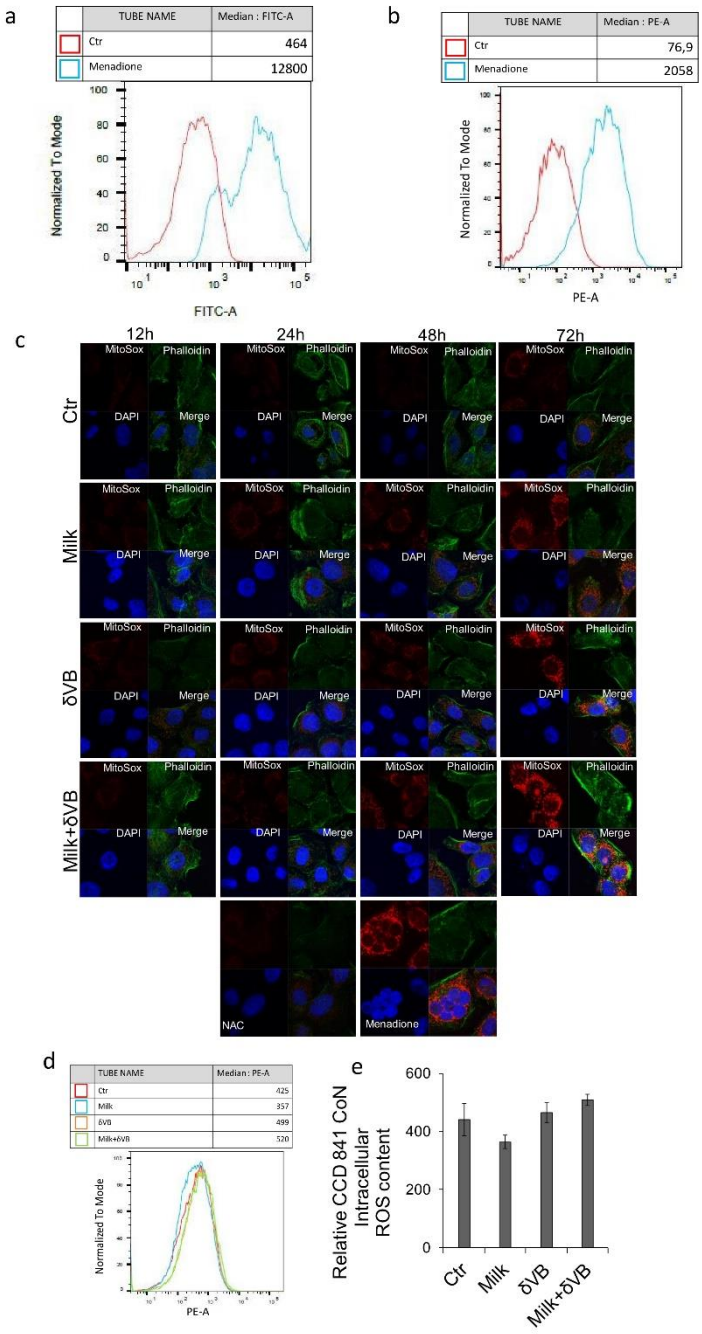

Figure. S5. Evaluation of redox homeostasis. Representative cytofluorimetric analysis of **(a)** DCFH-DA and (**b)** MitoSox assays performed in untreated LoVo cells (Ctr) and menadione-treated cells, as positive control of intracellular and mitochondrial ROS generation. **(c)** Representative confocal images peformed by 63X oil immersion objective of time-line (12, 24, 48, 72 h) mitochondrial ROS generation from milk (40% v/v), δVB (2 mM), milk+ δVB, or HBSS-10 mM Hepes (40% v/v) (Ctr) treated LoVo cells. Scale bars= 50 μm. **(d, e)** Representative FACS analysis and bar graph of DCFH-DA staining performed in CCD 841 CoN cells after 72 h of treatment with 40% v/v buffalo milk (milk), δVB (2 mM) and milk supplemented with δVB (milk+ δVB).

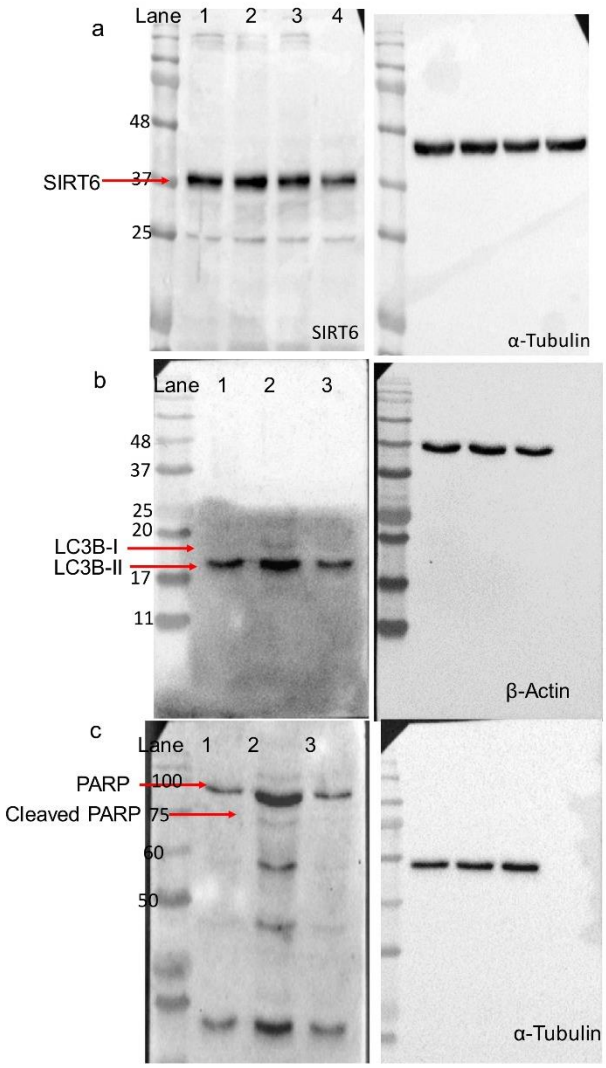

Figure. S6. The cropped blots are used in the main figure (Figure 7). (a) Lane 1=Ctr; Lane 2=milk, Lane 3=δVB, Lane 4= milk+ δVB. Lane 1= Ctr, Lane 2= Vehicle, Lane 3= Scramble, Lane 4= SIRT6-siRNA. (b, c) Lane 1= Ctr, Lane 2= milk+ δVB, Lane 3= SIRT6-siRNA+ milk+ δVB.