

### **Supplementary Material:**

**Figure 1-Supplementary: UBXN2A is dominantly located in the nucleus under normal conditions in colon cancer cells despite the presence of the NES signal located in its C-terminus.** (A) UBXN2A protein has a potential conserved nuclear export signal (NES) located at the C-terminus of UBXN2A. (B-C) Total cytoplasmic and nuclear lysates from HCT-116 colon cancer cells were analyzed by WB. The total proteins in the nucleus fraction were 1/10 of the cytoplasmic fraction, as determined by a BCA protein assay. Results showed UBXN2A is dominantly localized in the nucleus in the absence of stresses in HCT-116 colon cancer cells.

**Figure 2-Supplementary: UBXN2A depletion by shRNA only interferes with the UVB-induced upregulation of p53 in the cytoplasm with no significant effect on nuclear p53.** (A) Two stable monoclonal HCT-116 cell lines using SilenciX technology (Tebu-bio) plus a control HCT-116 cell line expressing scrambled shRNA (Fig. 2, Panel F) were treated with 1 and 2 KJ/m<sup>2</sup> followed by WB analysis. (B) Quantification of signals followed by normalization performed separately for treatment groups revealed the absence of UBXN2A can interfere with UVB-induced upregulation of p53 levels in the cytoplasm affectedly at 1 KJ/m<sup>2</sup> and moderately at 2 KJ/m<sup>2</sup>. (C) The absence of UBXN2A had no effects on upregulation of nuclear p53 following UVB exposure. (D) HCT-116 cells were treated with Etoposide (50µM), LMB (25ng/mL), and combination of Etoposide and LMB for 24 hours. Cytoplasmic cell lysates were subjected to IP using anti-p53 polyclonal antibodies immobilized on IgA magnetic beads. Pulled down proteins were subjected to WB analysis using anti-mot-2 and anti-p53 antibodies. We normalized the mot-2 signal after IP to mot-2 inputs. While Etoposide can decrease mot-2-p53 binding (lane 3 versus lane 2), the presence of LMB increases mot-2 binding to p53 (lane 4 versus lane 3). In combination treatment, Etoposide partially neutralizes the LMB's effect, resulting in a decrease in mot-2 binding to p53 in comparison to LMB alone (lane 5 versus lane 4). However, the rescue effect induced by combination therapy failed to reach the reduction levels observed in Etoposide alone (lane 5 versus lane 3). Together, these results suggest inhibition of nuclear export by LMB may nullify UBXN2A-dependent disruption between mot-2 and p53 upon Etoposide stress.

**Supplementary Table 1:** Details of antibodies, manufacturer, and the dilution used for WB and flow-cytometry.

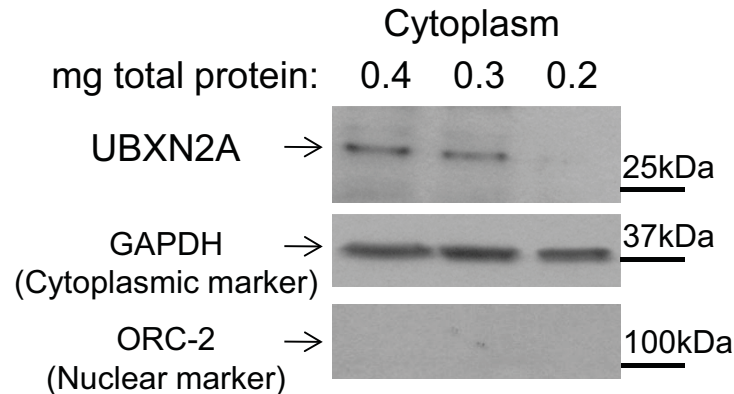
Name	Manufacturer	Dilution
Rabbit polyclonal anti-UBXN2A against #C-IQRLQKTASFRELS peptide located in the c-terminus of human UBXN2A (#NM_181713)	Pacific Immunology Corp	1:1000 (WB) 1:500 (IF)
Anti-p47 antibody	Santa Cruz biotechnology	1:1000
Anti-p97 antibody	Santa Cruz biotechnology	1:1000
Anti-p53 antibody (DO-1)	Santa Cruz biotechnology	1:1000
Anti-HSC70	Santa Cruz biotechnology	1:5000
Anti-cleaved PARP (Asp214)	Cell signaling	1:500
Mouse Anti-Human ORC-2 antibody	BD Biosciences	1:1000
Mouse anti-Glyceraldehyde-3-Phosphate Dehydrogenase antibodies	Millipore	1:20000
IRDye 800CW Goat anti-Rabbit IgG (H+L)	LI-COR Corporate	1:3000
IRDye 800CW Goat anti-Mouse IgG (H+L)	LI-COR Corporate	1:3000

**A**

1 mkdvdnlksikeewvcetgsdnqplggnqqsnceyfvdsllfeeaqkvssk  
 51 cvspae**qkkqv**dvniklwkn**gftvnddfrs**ysd**gasqqflnsikk**gelps  
 101 **elqgi**fdkeevdvkvedkkneic**l**stkp**vfqpfsgqghrlgsatpkivsk**  
 151 aknievenknnlsavplnnlepitnigiwlangkrivqkfnithrvshik  
 201 dfiekyqgsqrspffslatalpv**lrllde****tl**leeadlqnaviiqrlqkt  
 251 asfrelseh.

- 1) Bold sequence= SEP domain
- 2) Underlined sequence= UBX domain
- 3) "lrllde" sequence= NES

**B**



**C**

