

Unraveling Chain Specific Ubiquitination in Cells Using Tandem Ubiquitin Binding Entities

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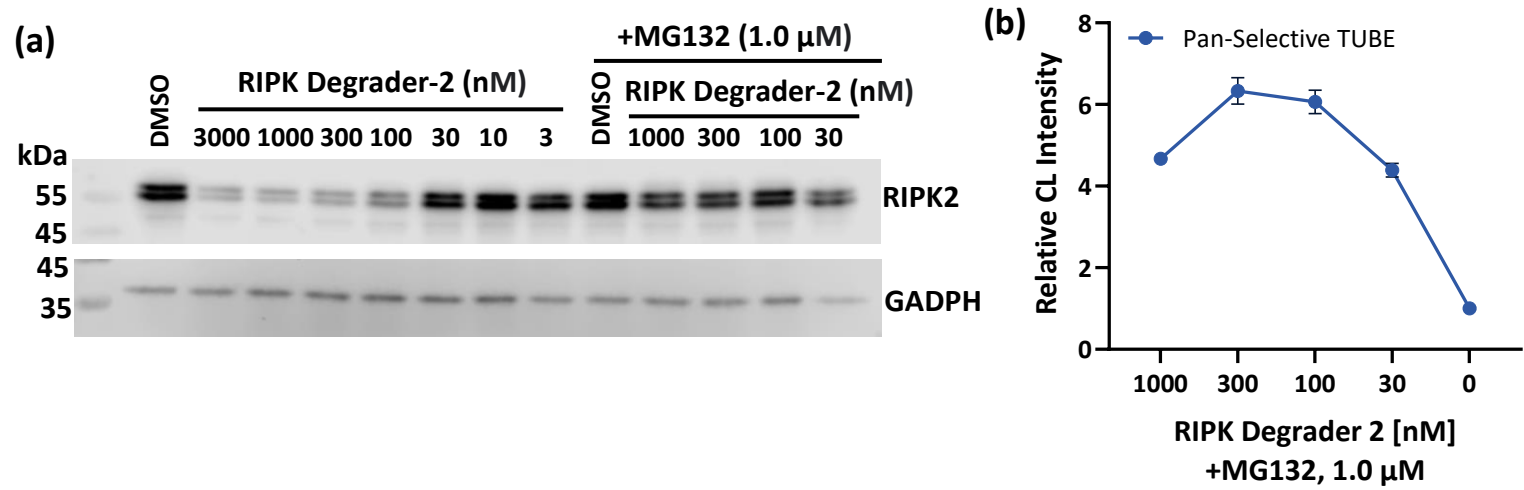


Fig. S1. RIPK-Degrader 2 Mediates Proteasomal Degradation of RIPK2. K562 cells (1.5×10^6 cells/ml) were treated with RIPK-Degrader-2, PROTAC at indicated doses in absence or presence of $1.0 \mu\text{M}$ MG132 for 45 minutes. Cell lysates ($30 \mu\text{g}$) were separated on SDS-PAGE and probed with anti-RIPK2 antibody to detect RIPK2 degradation. Immunoblotting with anti-GAPDH antibody was used for loading control **(a)**. Original western blot images are presented in Supplementary Figure S1. **(b)**. Lysates ($15 \mu\text{g}/\text{well}$) from RIPK Degrader-2 co-treated with MG132 were captured on Pan-selective TUBE followed by detection with anti-RIPK2 antibody. Signal were developed using enhanced chemiluminescence (CL) substrate and luminescence was read using BMG LabTech Clariostar plate reader. Relative CL intensities were calculated by dividing raw CL signals from PROTAC treatment to the DMSO control and plotted using GraphPad Prism. Error bars represent standard deviation ($n = 3$).

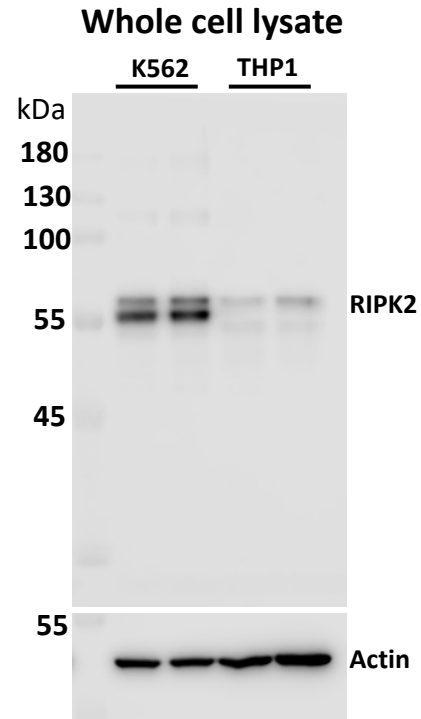


Fig. S2. Expression of RIPK2 in K562 and THP1 cells. Whole cell lysates (30 μ g) from untreated K562 cells and THP1 were separated on SDS-PAGE and probed with anti-RIPK2 antibody to detect RIPK2 levels. Immunoblotting with anti-Actin antibody was used for loading control (n = 1).

Fig. 2 Uncropped Western Blot Images

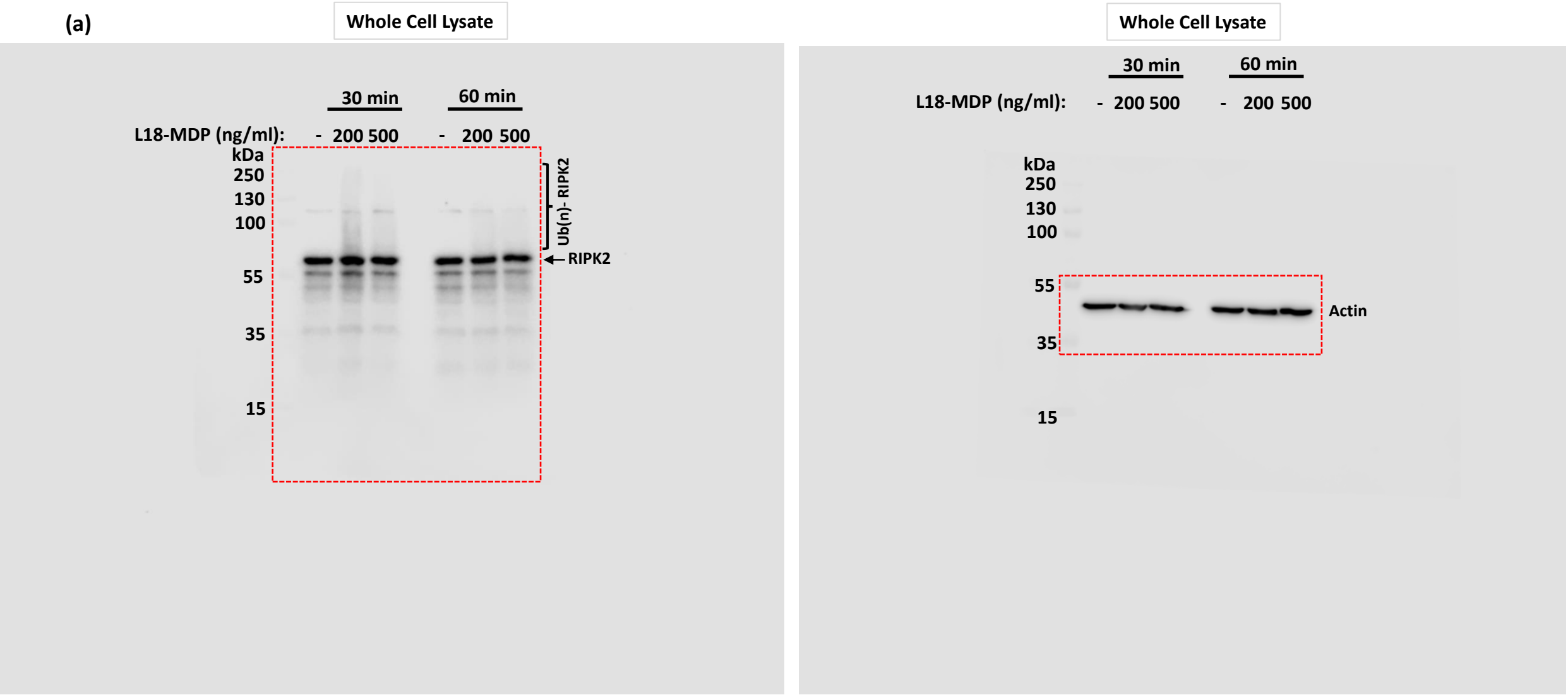


Fig. 2 Uncropped Western Blot Images

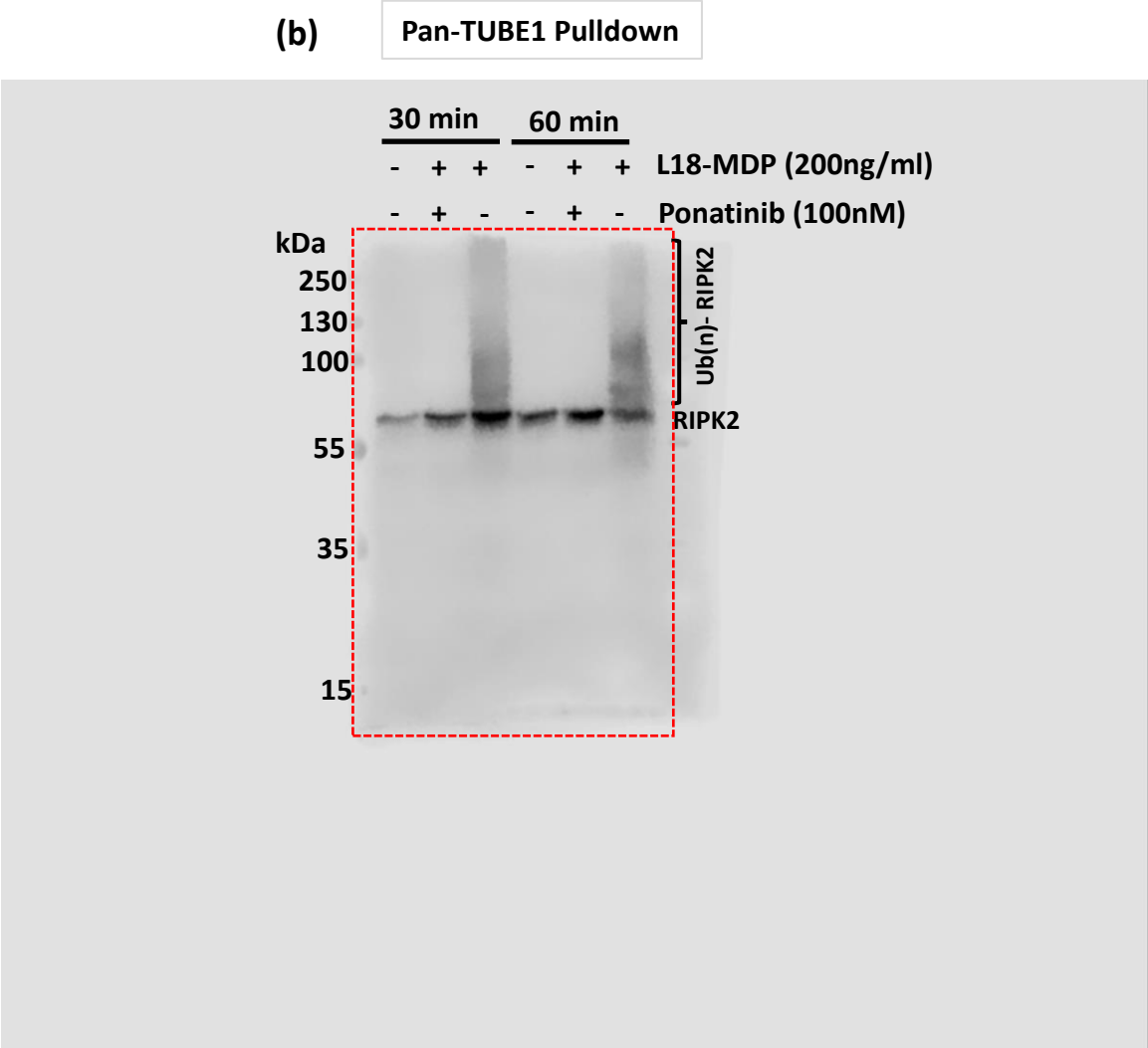


Fig. 4 Uncropped Western Blot Images

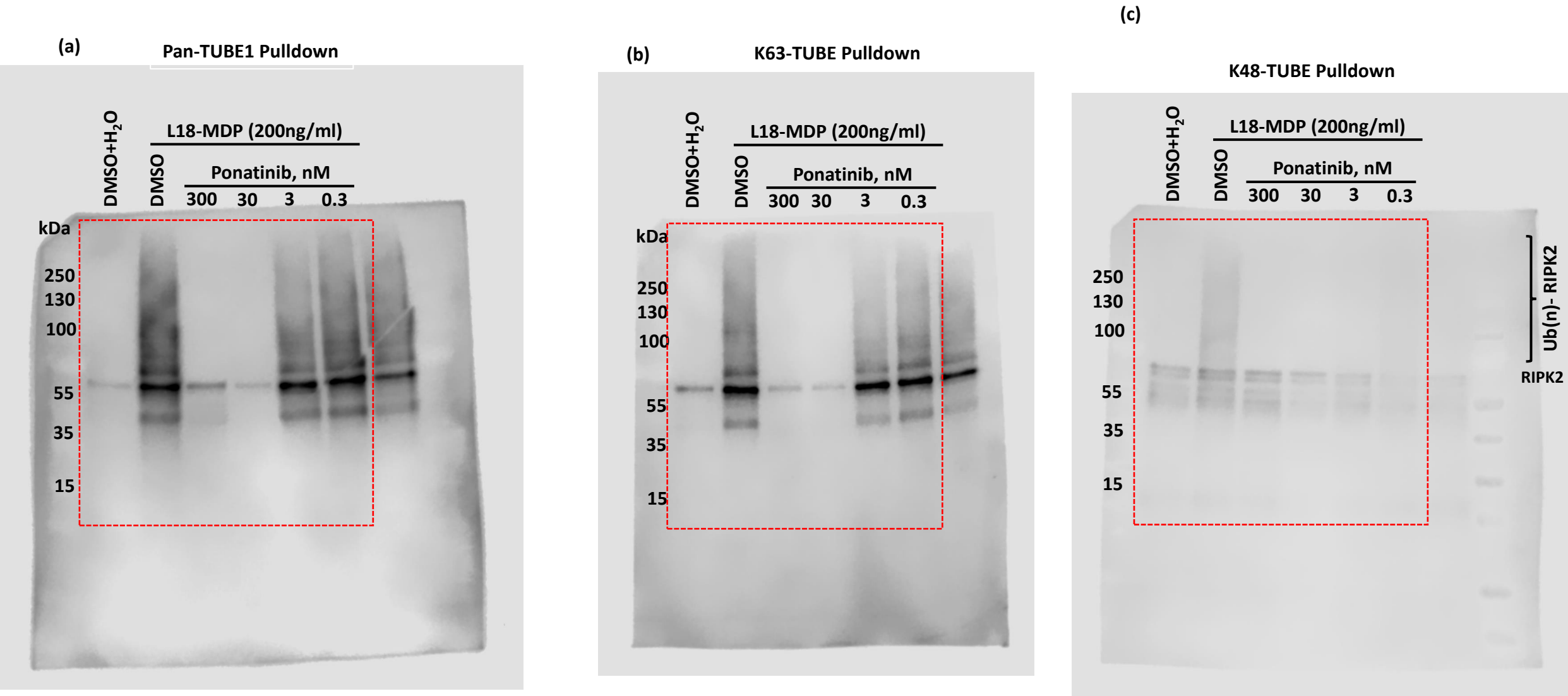


Fig. 4 Uncropped Western Blot Images

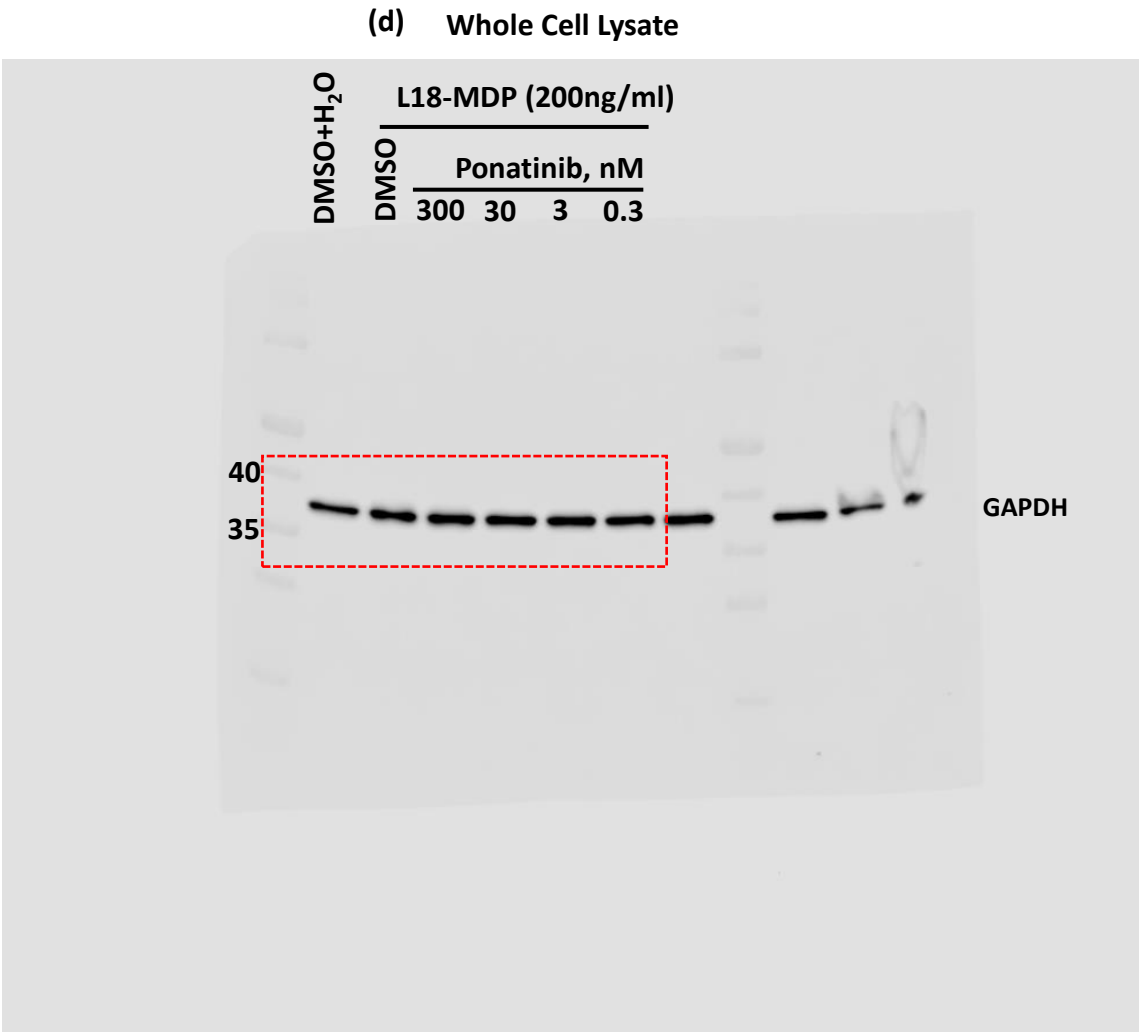
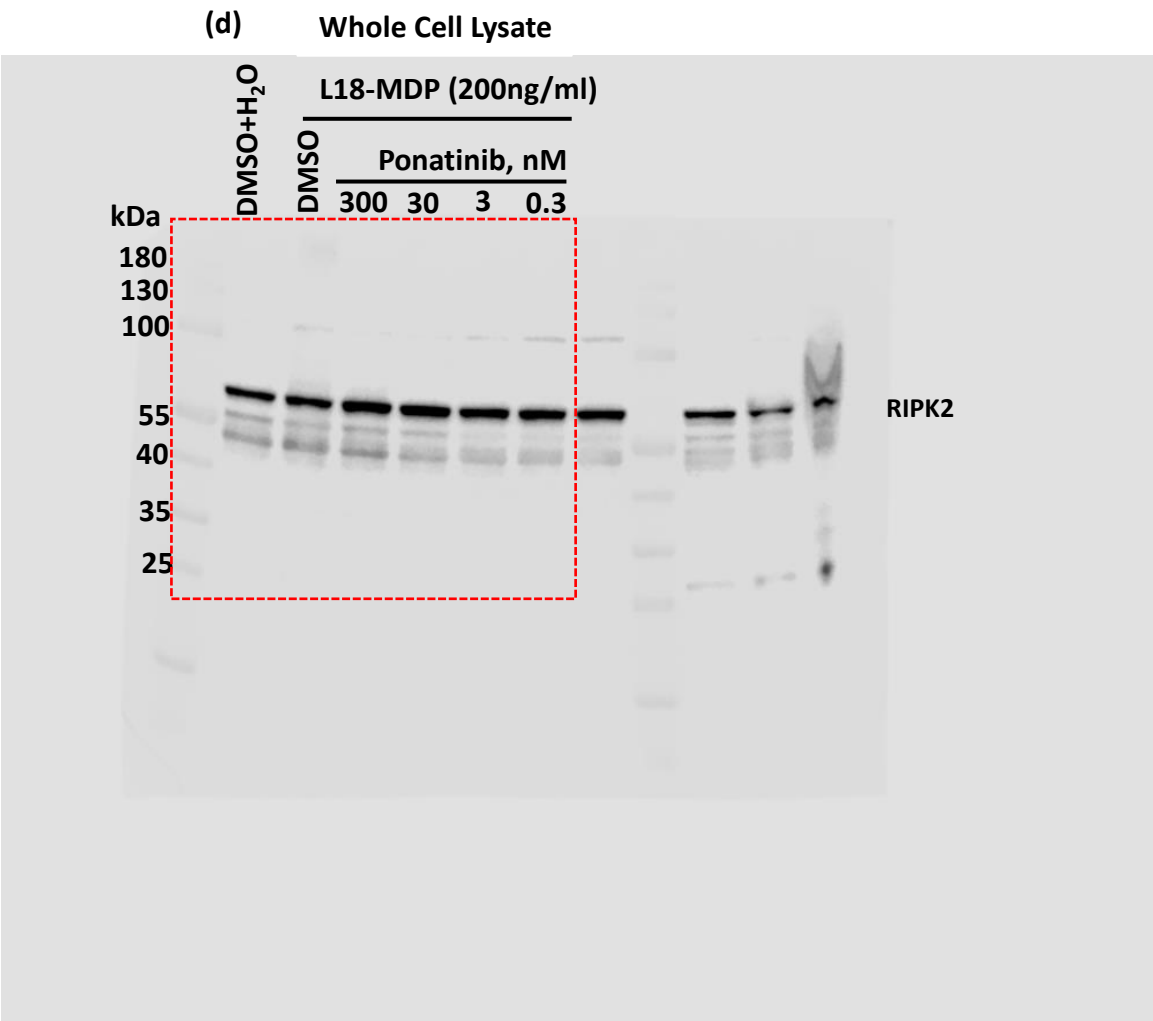


Fig. 5 Uncropped Western Blot Images

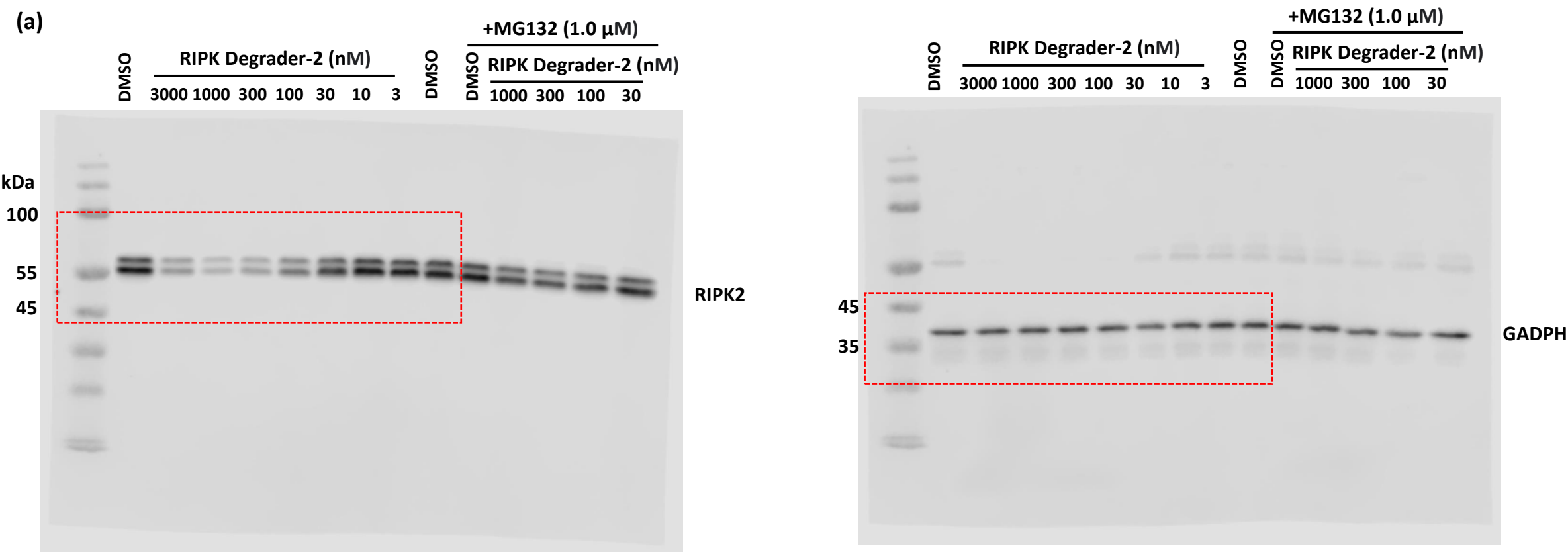
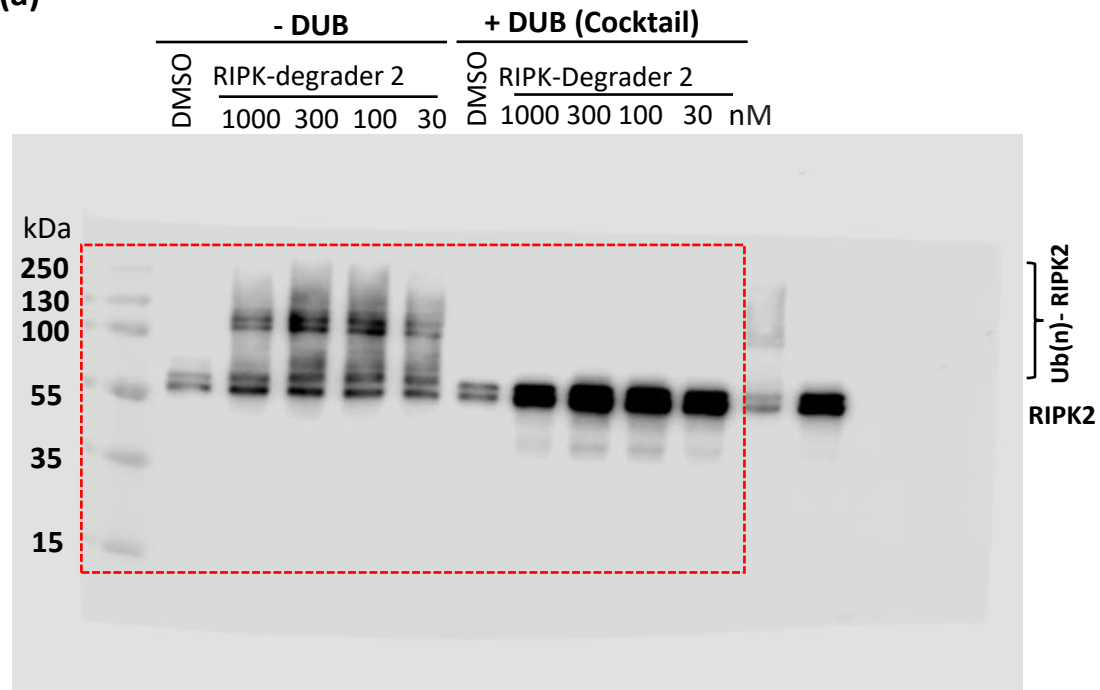


Fig. 6 Uncropped Western Blot Images

(a)



(b)

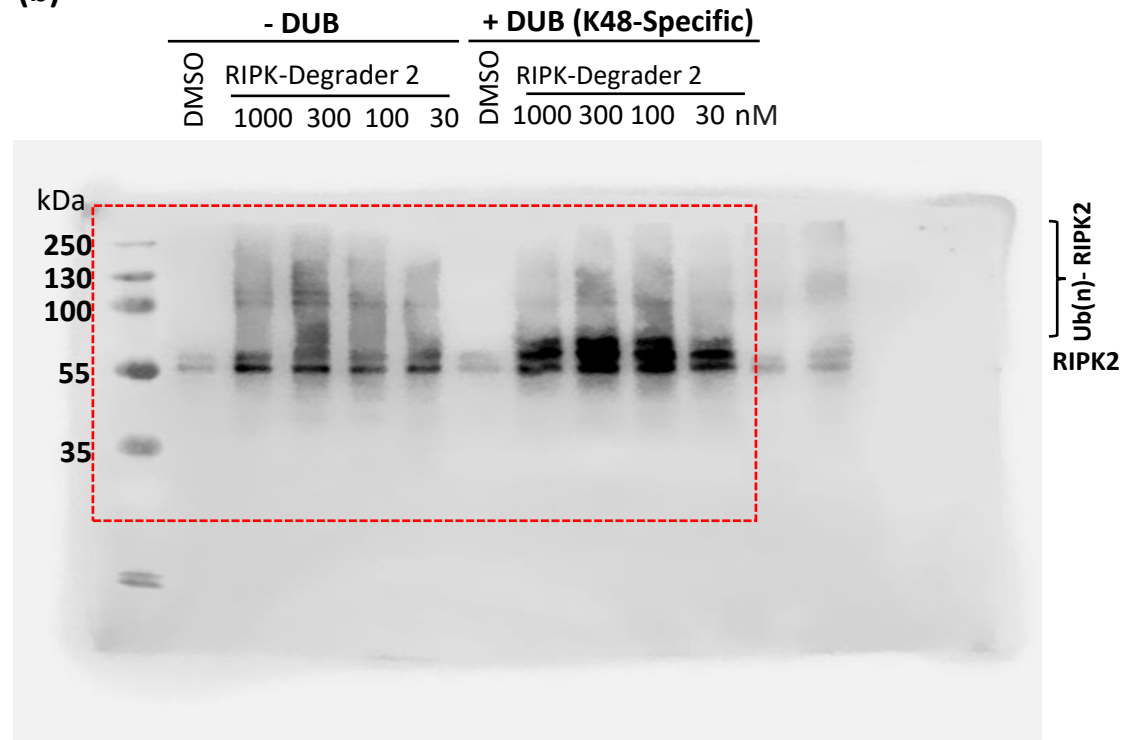


Fig. 6 Uncropped Western Blot Images

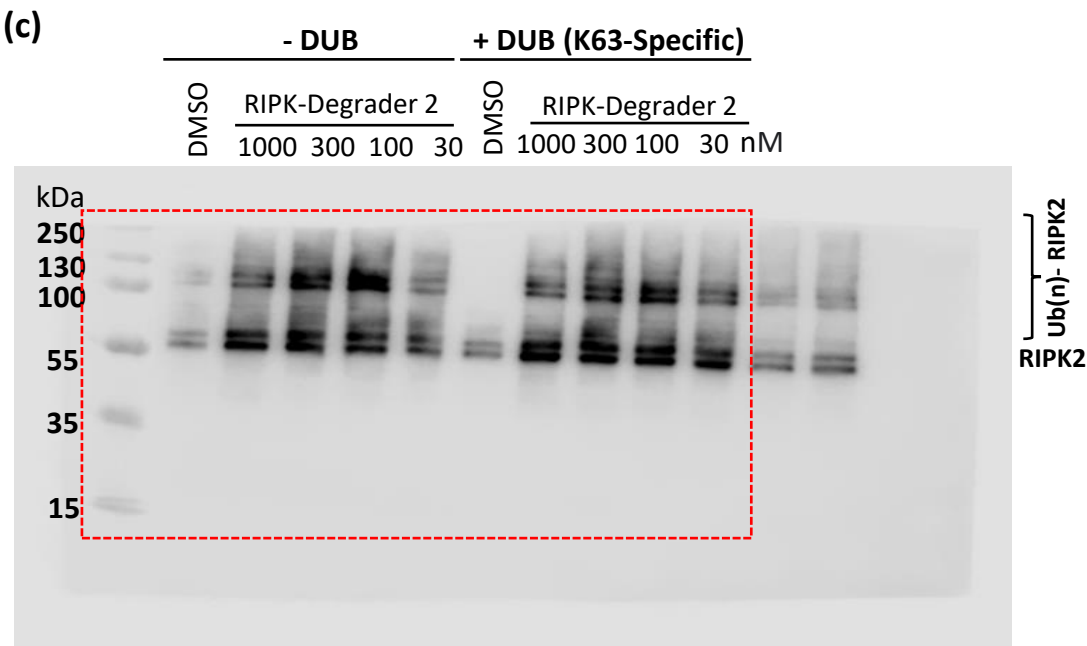


Fig.S1 Uncropped Western Blot Images

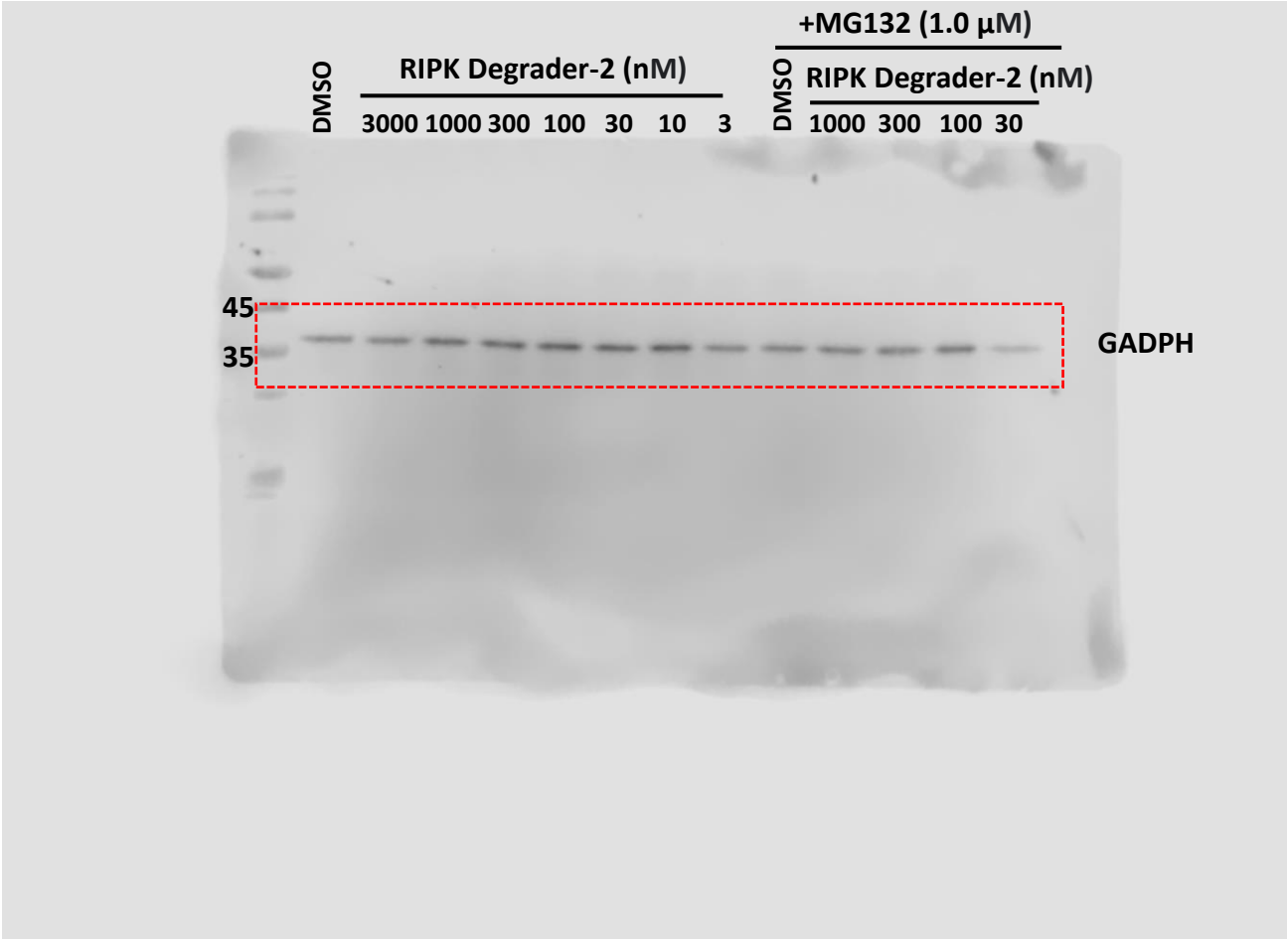
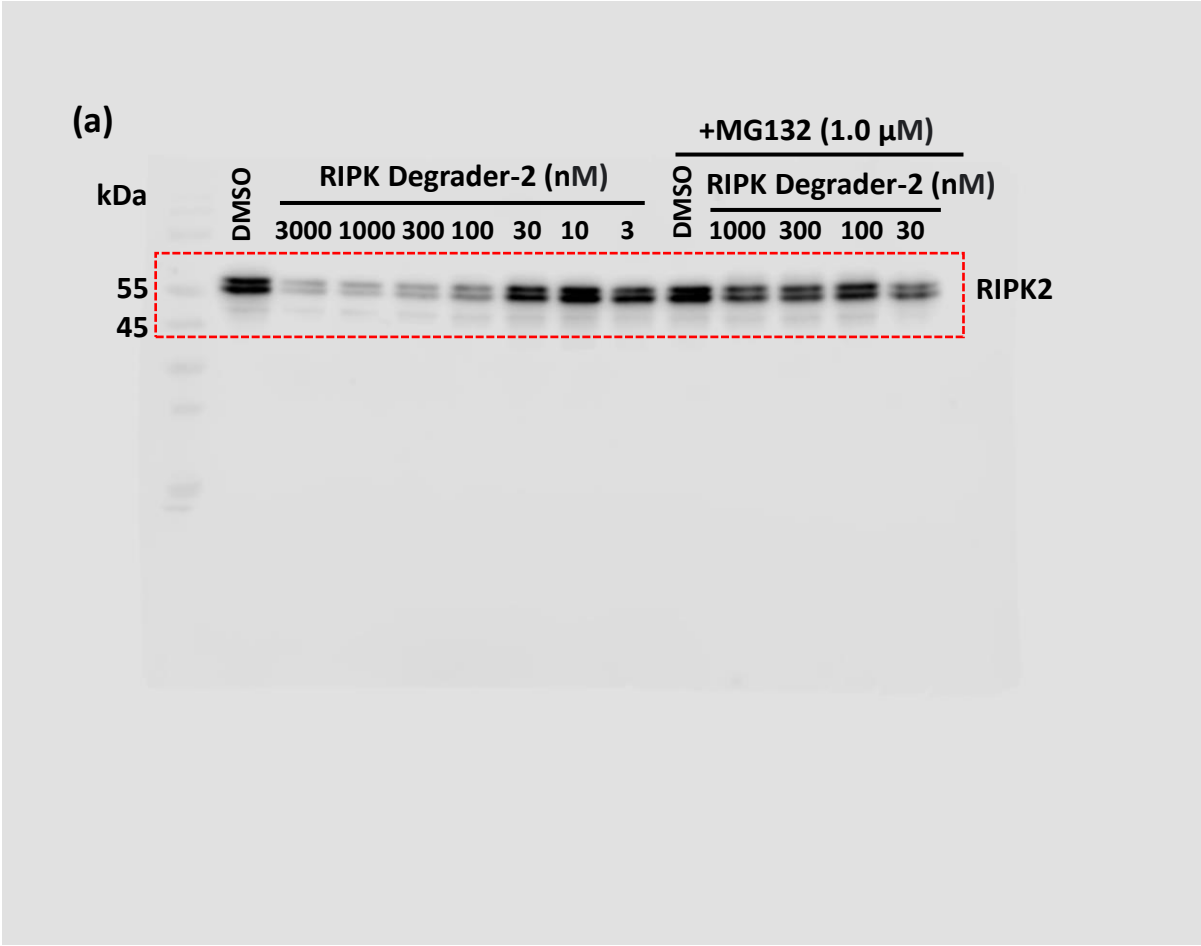


Fig.S2 Uncropped Western Blot Images

