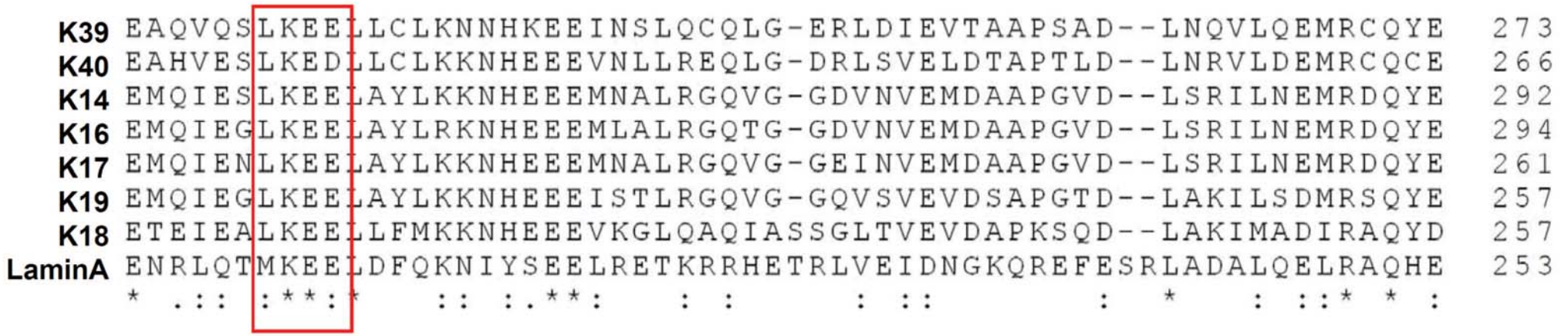
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Conservation of a SUMO consensus sequence in human Lamin A and type I keratin intermediate filaments. The sequence alignment was performed using the ExPASy Proteomics server (http://ca.expasy.org/sprot/). Highlighted in red are SUMO consensus sequences, as identified by the SumoPlotTM program. Lamin A K201 has previously been shown to be sumoylated.

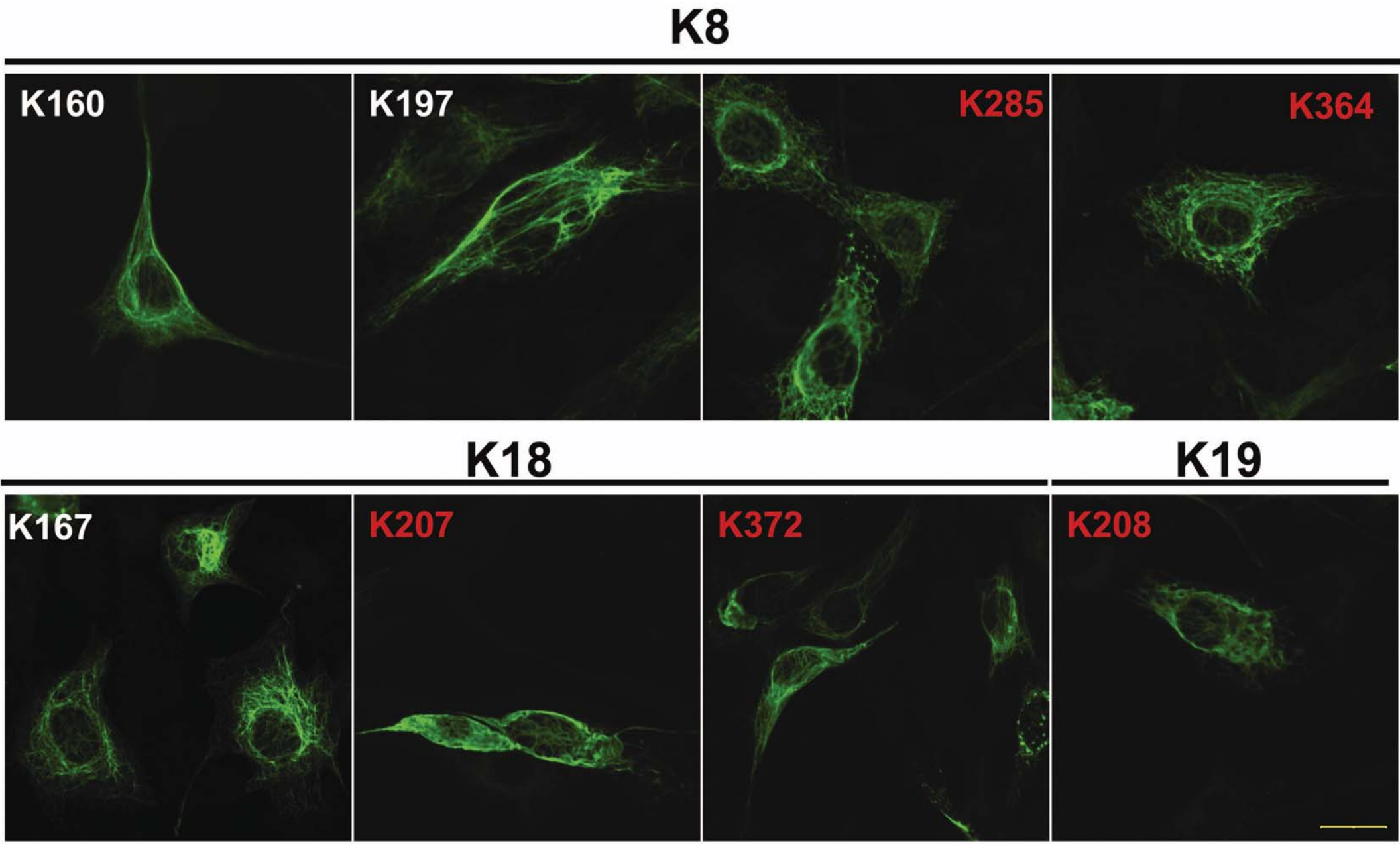
Supplemental Figure 2. Abnormal keratin filaments in SUMO target lysine to arginine mutants. NIH3T3 cells were transfected with a WT type I or type II keratin along with the designated mutant form of the corresponding type I or type II partner. Mutants labeled in red are those that decreased sumoylation, and mutants labeled in white are those that had no effect on sumoylation in the biochemical analysis shown in Figure 2B. Immunofluorescence staining was performed 24 hours after transfection. Primary mouse antibodies against K8, K18 and K19 were used to localize K8/K18 or K8/K19 heteropolymers, respectively, followed by secondary goat anti-mouse Alexa Fluor 488. Scale bar represents 20 μm.

Supplemental Figure 3. Keratins colocalize with SUMO-2/3 during oxidative stress. NIH-3T3 cells were transfected with human K8/K19 and were either left untreated (control), or were treated with 1 mM H2O2 for 45 min prior to immunofluorescence staining. Primary mouse antibody against K19 was used to localize K8/K19 heteropolymers along with rabbit anti-SUMO-2/3 antibody. Secondary goat anti-mouse Alexa Fluor 488 and goat anti-rabbit Alexa Fluor 594 antibodies were used to visualize the localization of keratins and SUMO by confocal microscopy. Shown are representative images from the single channels as well as a merged image with DAPI counterstain showing the cell nuclei. Scale bars = 20 μm.

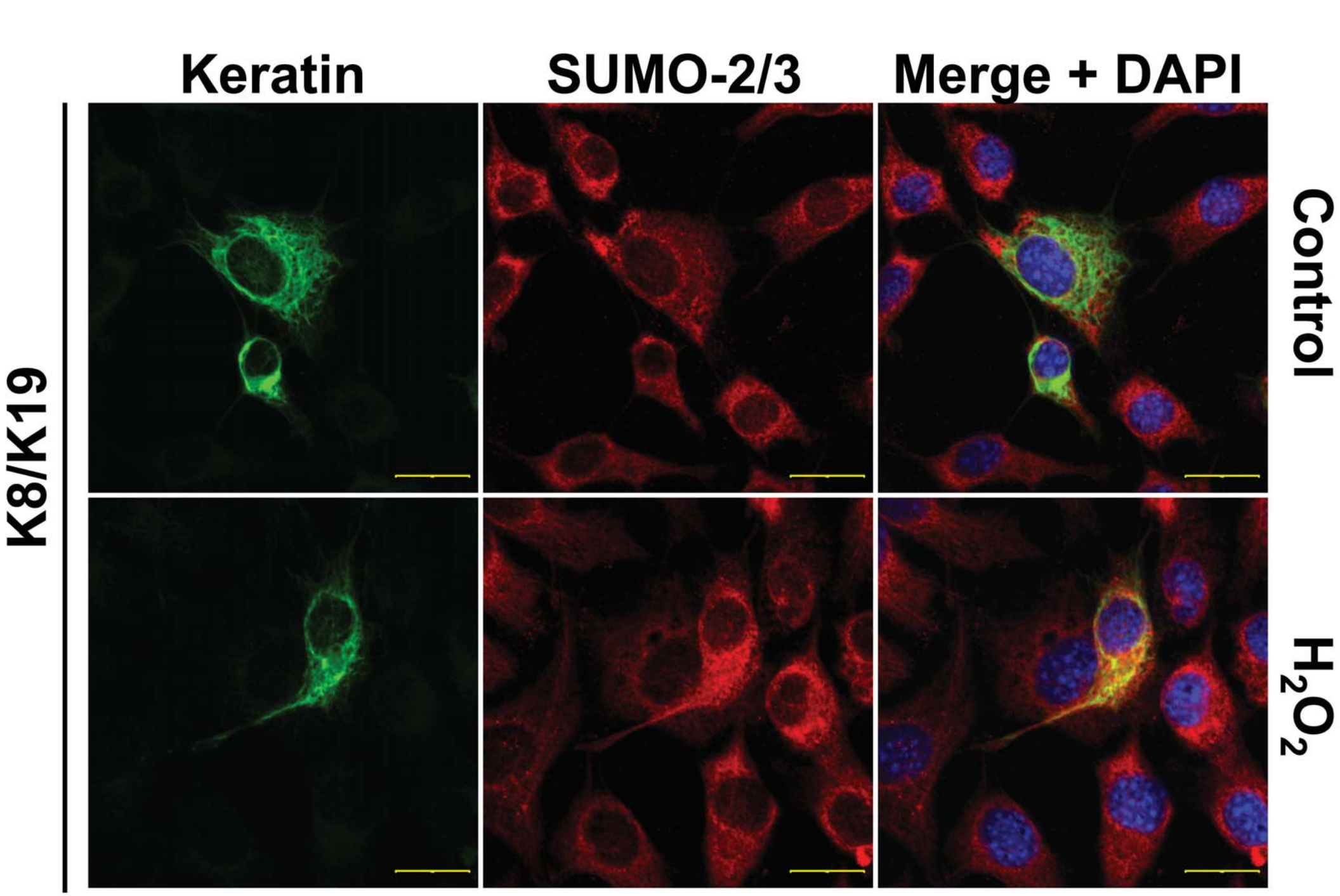
Supplemental Figure 4. Polyubiquitinated insoluble proteins are preferentially detected in diseased human livers. Human liver high salt extracts (HSEs) were prepared and analyzed as described in Experimental Procedures and Figure 8 legend.



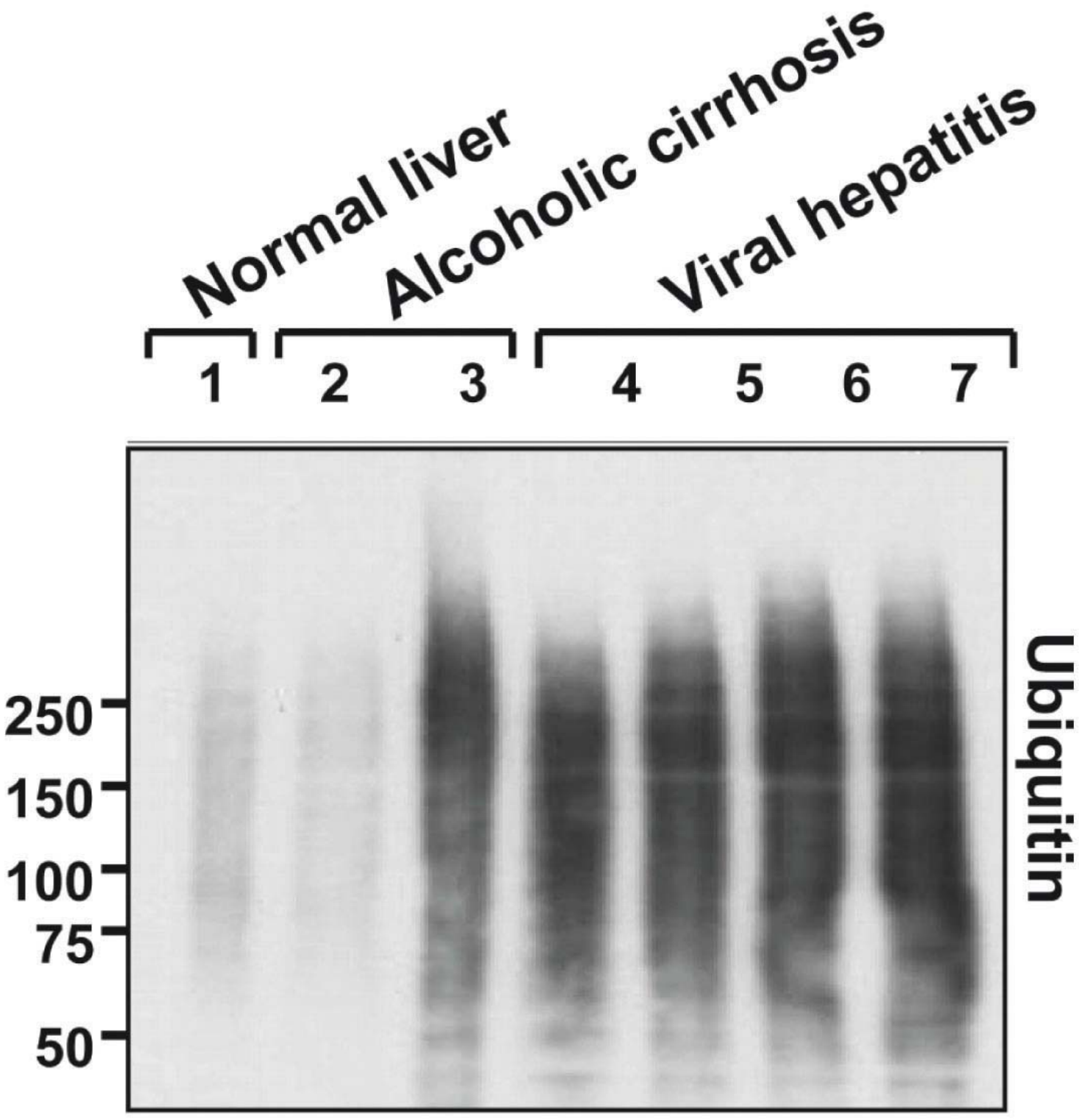
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4