

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 SumoPlot™ 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.

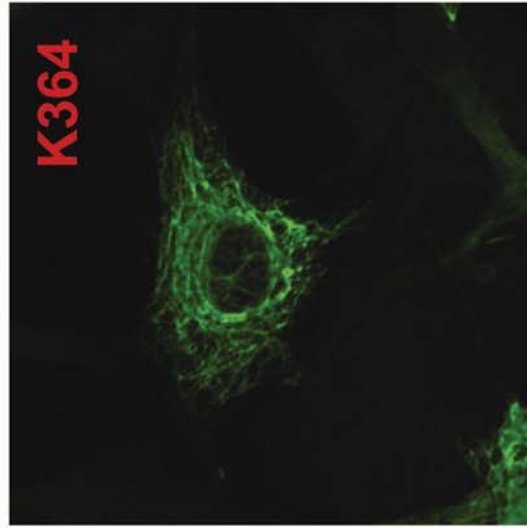
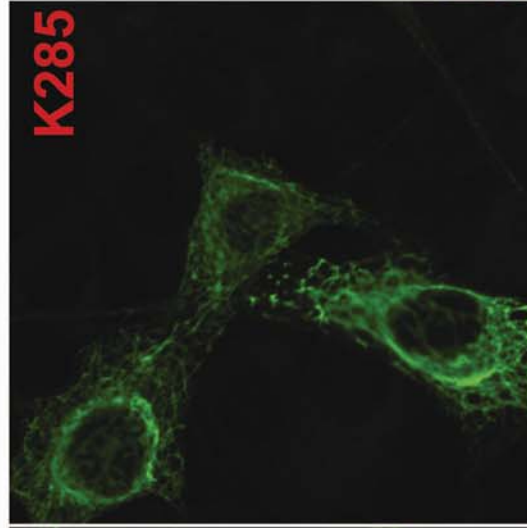
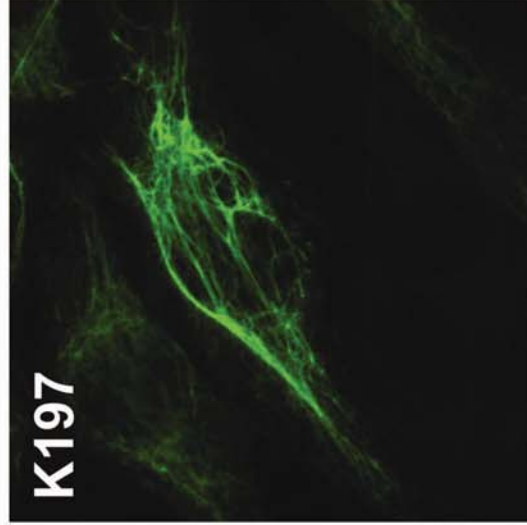
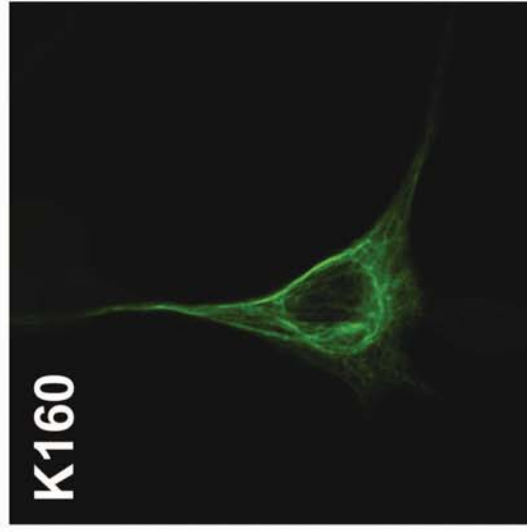
Supplemental Figure 4. Polyubiquitinated insoluble proteins are preferentially detected in diseased human livers. Human liver high salt extracts (HSEs) were prepared and analyzed as described in Experimental Procedures and Figure 8 legend.

K39	EAQVQS	LKEE	LLCL	LKNN	HKEE	INS	LQC	QLG	-ER	LDI	EVT	AAPS	AD--	LNQ	VLQ	EMRC	QYE	273				
K40	EAHVES	LKED	LLCL	LKKN	HEEE	VNLL	REQL	GLG	-DR	LSV	ELDT	APT	LD--	LNR	VLD	EMRC	QCE	266				
K14	EMQIES	LKEE	LAYL	LKKN	HEEE	EMNAL	RGQV	G-G	DVN	VEM	DAA	P	GVD--	LSR	ILN	EMRD	QYE	292				
K16	EMQIEG	LKEE	LAYL	LKKN	HEEE	EMLAL	RGQT	G-G	DVN	VEM	DAA	P	GVD--	LSR	ILN	EMRD	QYE	294				
K17	EMQIEN	LKEE	LAYL	LKKN	HEEE	EMNAL	RGQV	G-GE	IN	VEM	DAA	P	GVD--	LSR	ILN	EMRD	QYE	261				
K19	EMQIEG	LKEE	LAYL	LKKN	HEEE	I	STLR	GQV	G-G	QV	SVE	DS	AP	G	T	D--	LAK	ILSD	MRS	QYE	257	
K18	ETEIEA	LKEE	LLFM	LKKN	HEEE	E	VKGL	QAQ	IAS	SG	LTV	EV	DAP	KS	QD--	LAK	IMAD	IRA	QYD	257		
Lamina	ENRLQT	MKEE	LLDF	LQKN	IYSE	EL	RET	KRR	HET	RL	VEI	D	NGK	QRE	FES	R	LAD	ALQ	EL	RA	QHE	253

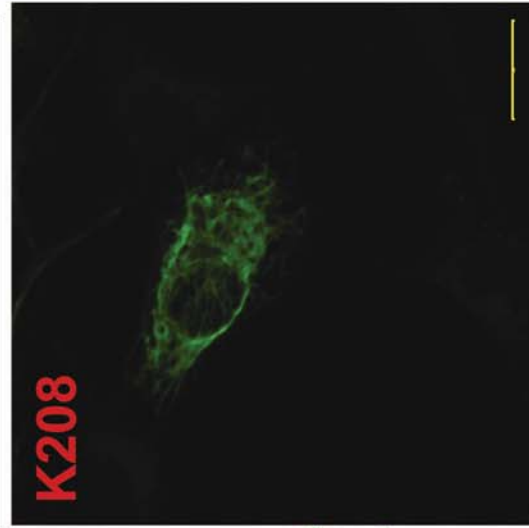
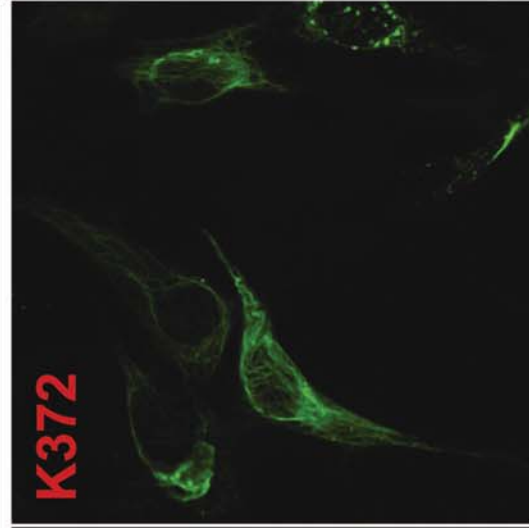
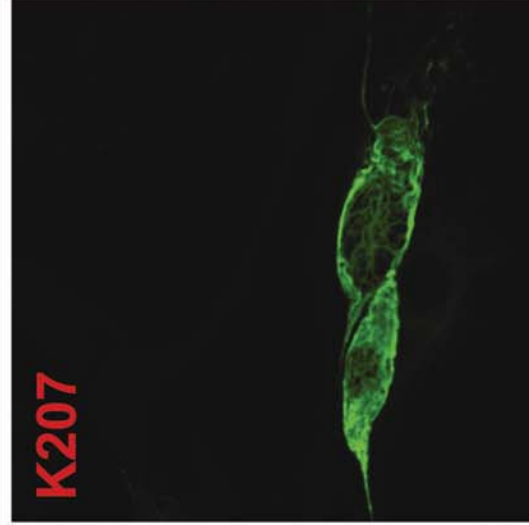
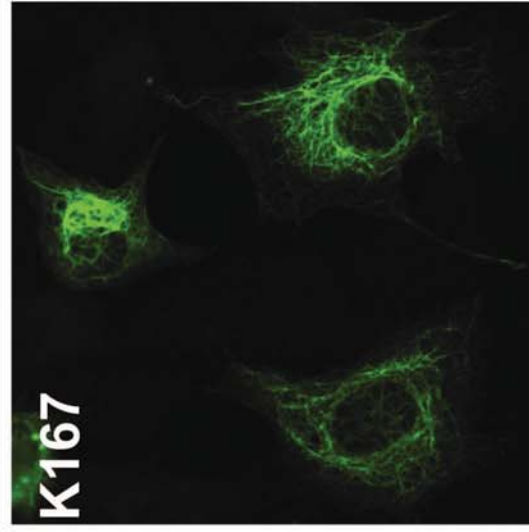


Supplemental Figure 1

K8

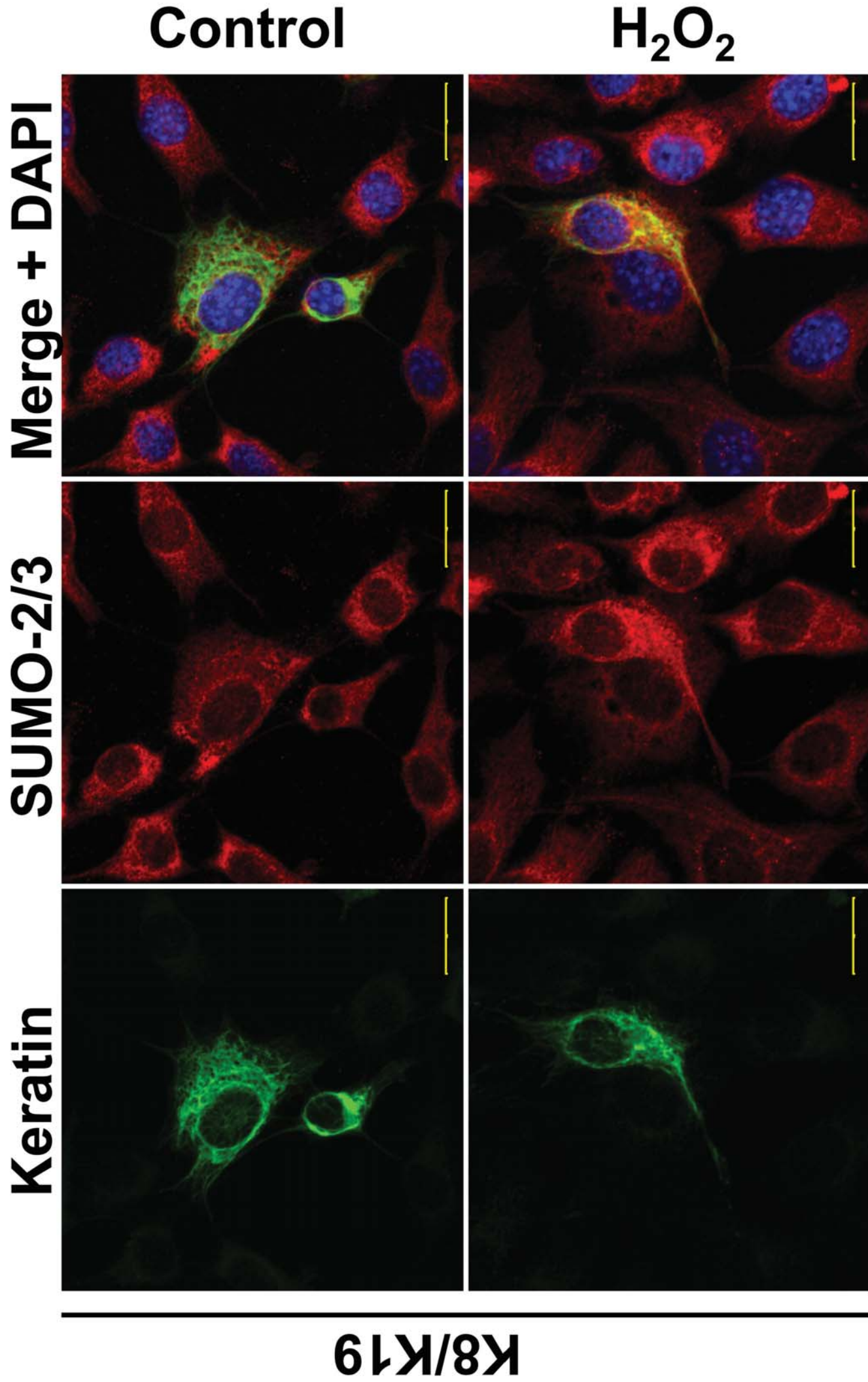


K18



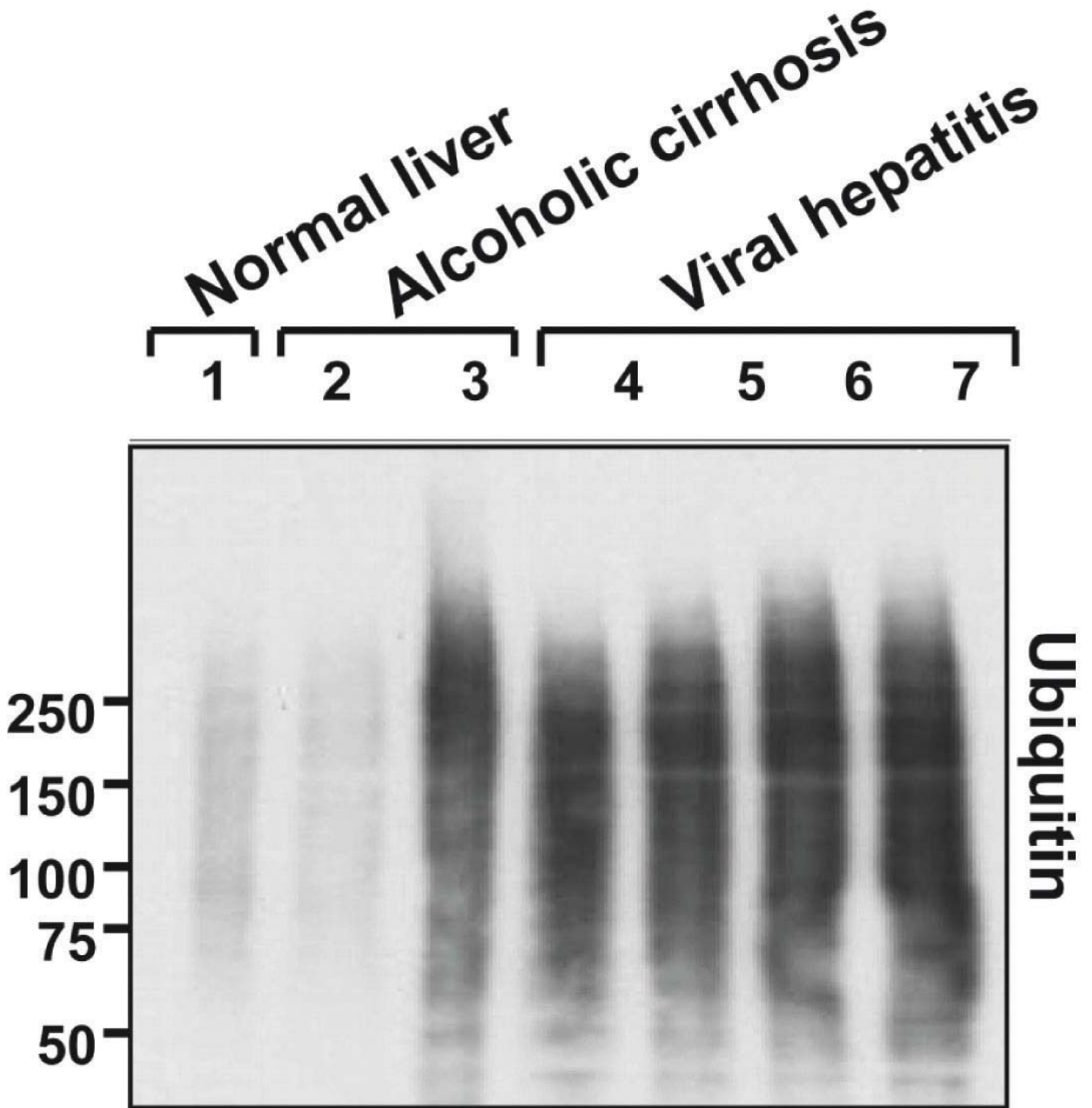
K19

Supplemental Figure 2



K8/K19

Supplemental Figure 3



Supplemental Figure 4