Snider et al., http://www.jcb.org/cgi/content/full/jcb.201209028/DC1

A

Identified by global analysis in cell lines	Confirmed by mass spec on normal liver K8	Residue conserved in K8 across species	Residue conserved in type II human keratins
Lys-117 [rod]	Yes	100%	60%
Lys-207 [rod]	**Yes**	**100%**	**90%**
Lys-295 [rod]	No	100%	15%
Lys-325 [rod]	**Yes**	**100%**	**100%**
Lys-347 [rod]	No	83%	90%
Lys-472 [tail]	No	83%	10%
Lys-483 [tail]	No	67%	10%

B

Type I	
K9	**Lys**
K10	**Lys**
K12	Arg
K13	**Lys**
K14	Arg
K15	Arg
K16	Arg
K17	Arg
K18	Arg
K19	Arg
K20	**Lys**
K23	Thr
K24	Arg
K25	Arg
K26	Thr
K27	Arg
K28	Arg
K31	**Lys**
K32	**Lys**
K33a	Arg
K33b	Arg
K34	**Lys**
K35	**Lys**
K36	**Lys**
K37	**Lys**
K38	**Lys**
K39	**Lys**
K40	**Lys**

Type II	
K1	**Lys**
K2	**Lys**
K3	**Lys**
K4	**Lys**
K5	**Lys**
K6	**Lys**
K7	**Lys**
K8	**Lys**
K71	**Lys**
K72	**Lys**
K73	**Lys**
K74	**Lys**
K75	**Lys**
K76	**Lys**
K77	**Lys**
K78	**Lys**
K79	Arg
K80	Arg
K81	**Lys**
K82	**Lys**
K83	**Lys**
K84	**Lys**
K85	**Lys**
K86	**Lys**

Type III	
DESMIN	Arg
GFAP	Arg
PERIPHERIN	Arg
SYNCOILIN	Arg
VIMENTIN	Arg
Type IV	
α-INTERNEXIN	Arg
NFM	**Lys**
NFL	Arg
NFH	Arg
NESTIN	Arg
SYNEMIN	Arg
Type V	
LAMIN A/C	Arg
LAMIN B1	**Lys**
LAMIN B2	Arg
Type VI	
PHAKININ	**Lys**
FILENSIN	His

C

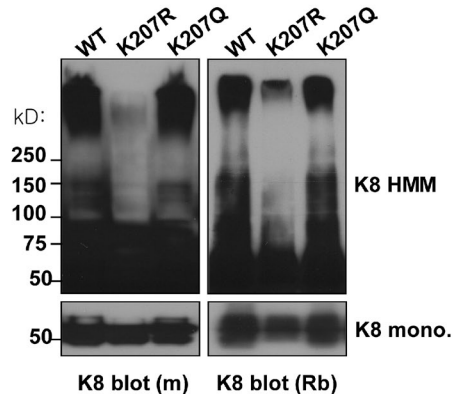

Figure S1. **K8 Lys-207 is highly conserved and affects the formation of HMM K8 complexes.** (A) Comparison of putative human K8 acetylation sites, previously identified by proteomic studies in cell lines, for their presence on K8 from normal liver of human K8 overexpressing mice. The latter were identified by mass spectrometry analysis as described in Materials and methods. Highlighted in bold are residues that are highly conserved and acetylated in liver K8. Sequence alignment was performed to compare zebrafish, marbled lungfish, potoroo, mouse, rat, bovine, and human K8, or human type II keratins (K1–K8 and K71–K86). (B) Conservation of K8 Lys-207 in Type I–VI IFs. Protein sequence analyses and alignments were performed using UniProt Knowledgebase. (C) Immunoblot for K8 HMM species of WT, K207R, and K207Q K8 using a mouse (m) or a rabbit (Rb) monoclonal antibody to K8.

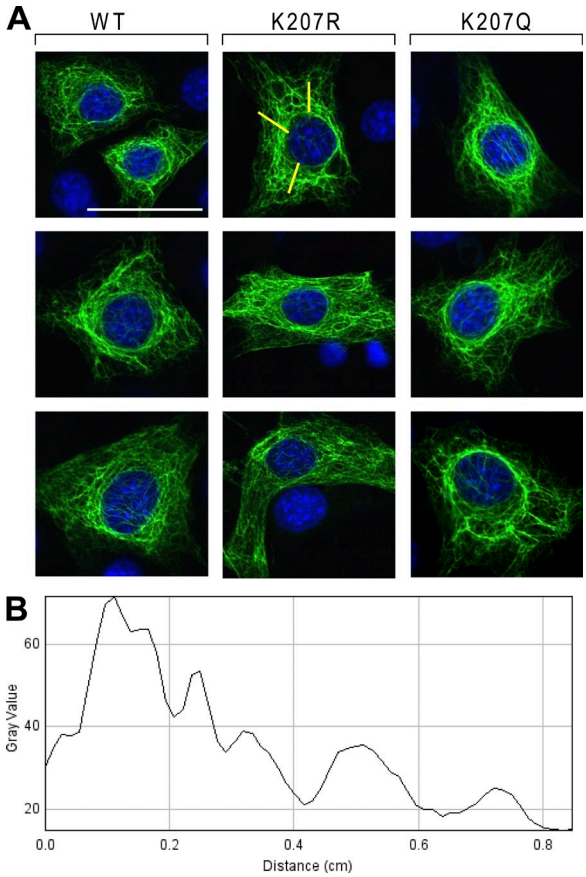

Figure S2. **K8 Lys-207 is important for perinuclear filament organization.** (A) Representative images of the cells analyzed in Fig. 2 B. Each cell was analyzed using ImageJ software by drawing three lines (originating from the nucleus; example shown in top middle panel) in random directions and quantifying the signal intensity along these lines. Bar, 20 µm. (B) Example of a single intensity plot from A obtained using ImageJ software. Note that the lower limit of the software-generated y-axis is automatically set to the lowest signal and does not go to zero. The raw numbers from each plot were averaged to obtain a measure for the signal intensity for each cell (n = 3 intensity plots per cell).

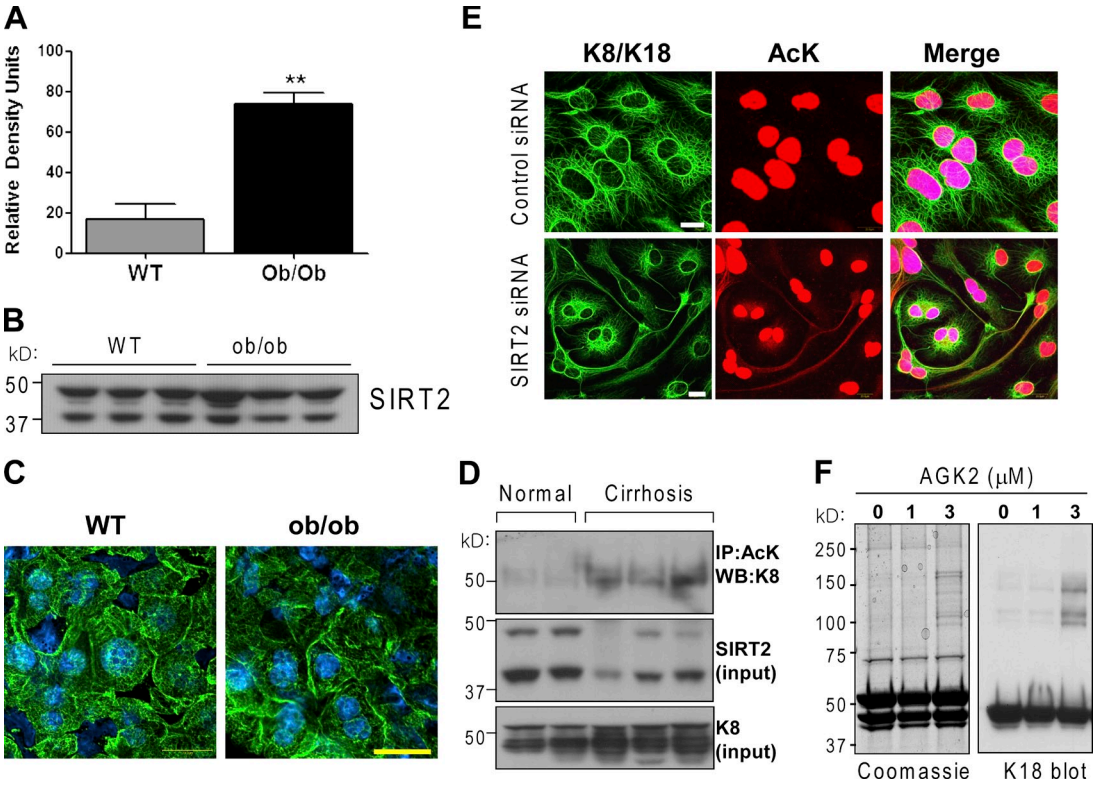

Figure S3. **K8 acetylation is regulated by glucose levels and SIRT2.** (A) Quantification of the band intensities for acetylated K8 from Fig. 3 B. **, $P < 0.01$, unpaired *t* test. The results are presented as the mean and the standard deviation. (B) Immunoblot for SIRT2 of the same samples shown in Fig. 3 B. (C) K8/K18 filament organization in WT and ob/ob mouse liver. Bars, 20 μm. (D) Increased acetylation (as assessed by rabbit anti-AcK immunoprecipitation and K8 immunoblot) of K8 in cirrhotic human liver explants is associated with decreased SIRT2 expression. (E) K8/K18 filament organization and rabbit anti-AcK staining in HepG2 cells transfected with control or SIRT2 siRNA for 48 h. Bars, 20 μm. (F) Coomassie stain and immunoblot of K18 in HSEs of HT29 cells after treatment with vehicle (0) or AGK2 for 18 h.