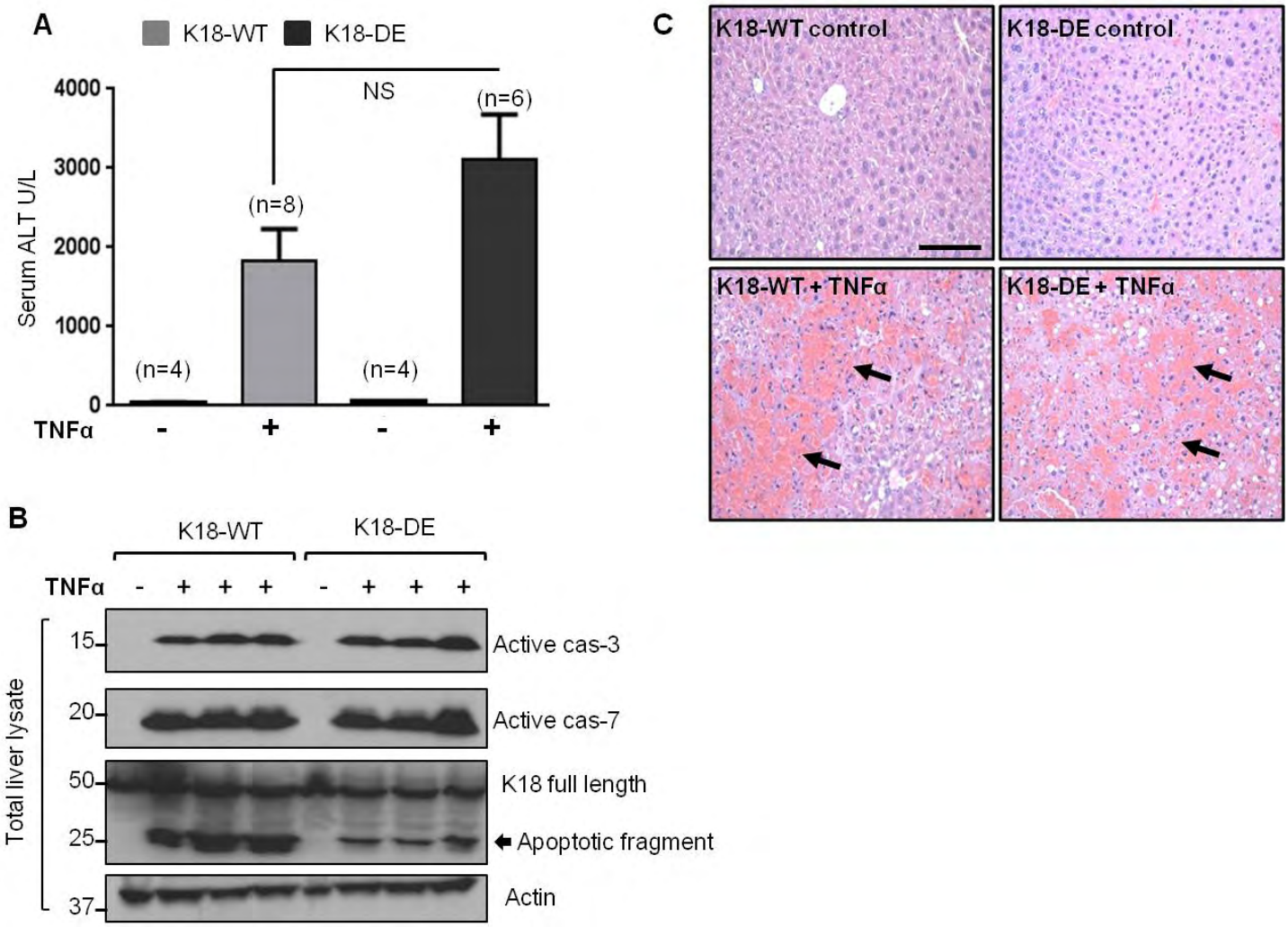

Fig. S1. K18-WT and K18-DE mice show similar liver damage in response to TNFα. Age and sex matched mice were fasted overnight and then injected intraperitonialy with saline or TNFα (15 ng/g of body weight) and actinomycin-D (1 ∀g/g of body weight) as described previously (Ku et al., 2003). (A) Serum ALT levels of K18-WT and K18-DE mice with or without TNF-α treatment (*n*=number of animals). Mice were euthanized 8 h after administration of TNFα followed by serum analysis. (B) TNFα induced caspase activation in liver. Total liver homogenates were prepared followed by immunoblot analysis of equal protein fractions using antibodies to K18 and active caspases 3 and 7. An immunoblot of actin is included to demonstrate equal protein loading. Each lane represents an individual mouse liver. (C) TNFα-induced liver damage was assessed by hematoxylin and eosin staining. Arrows highlight areas of hemorrhage. Scale bar=100 ∀m.

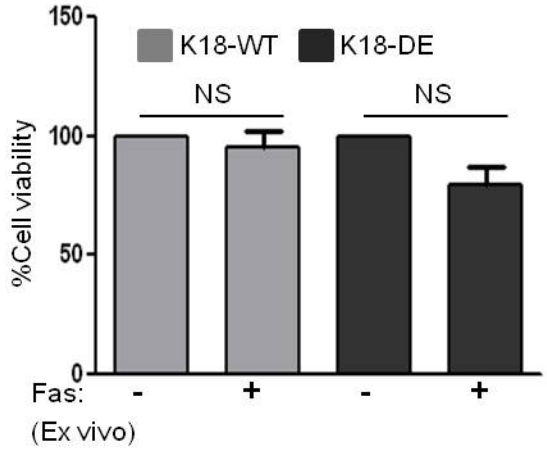

Figure S2: Primary cultured hepatocytes (ex vivo) are more resistant to in vivo Fas stimulation. Hepatocytes were isolated from K18-WT and K18-DE mice then challenged with Jo2 (0.5 µg/g) for 6h. Viable cells percentage was determined using trypan blue staining after trypsin treatment. Each bar represents the mean of 8 to 10 fields. Note that no significant cell death occurs with each genotype. NS=not significant.

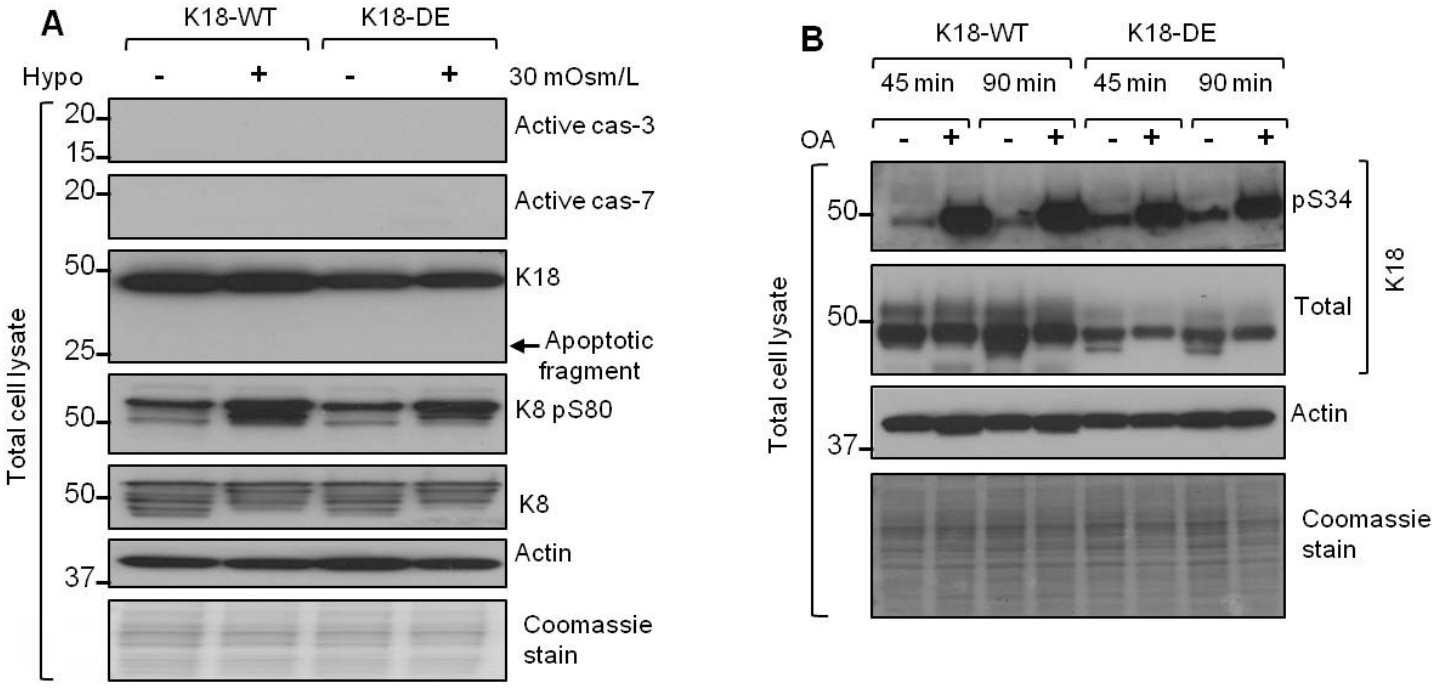

Fig. S3. Hypoosmotic stress does not result in caspase activation in cultured hepatocytes. (A) Hypoosmosis induced caspase activation. Isolated primary hepatocytes from K18-WT and K18-DE mice were cultured in the presence of isotonic (305 mOsm/L) or hypoosmotic (30 mOsml/Kg) conditions for 6 h. A total cell lysate was then analyzed by immunoblotting using antibodies to the indicated epitopes. An actin blot and Coomassie stain of the analyzed lysate are included as loading controls. Note that there is no detectable caspase activation under the strong hypoosmotic stress conditions we tested. (B) Effect of okadaic acid on K18 phosphorylation in cultured hepatocytes. Isolated primary hepatocytes from K18-WT and K18-DE mice were treated with okadaic acid (OA) for 45 or 90 min to induce keratin hyperphosphorylation. Total cell extracts were then prepared and analyzed by immunoblotting using antibodies to K18 pS34, K18 and actin. A Coomassie stain of the total lysate fraction is also included.

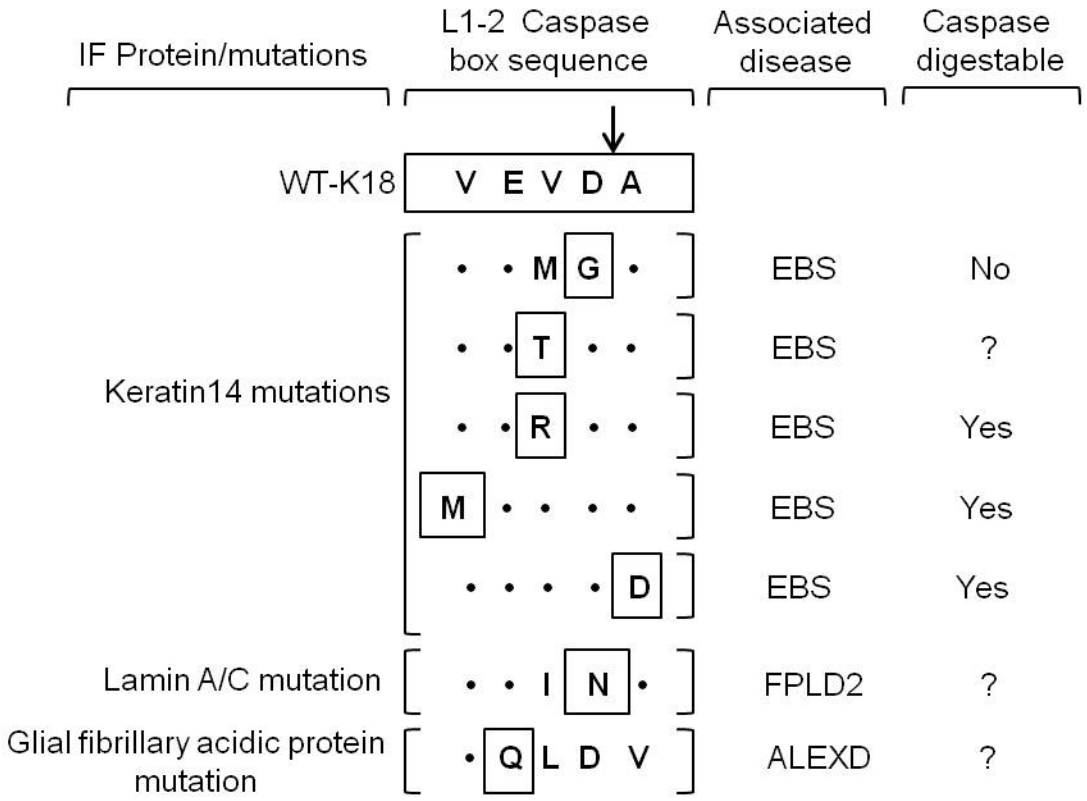

Fig. S4. Disease-causing L1-2 caspase box mutations of human IF proteins. Some of the disease-causing mutations reside in the linker 1–2 (L1–2) caspase cleavage motif of several IF proteins. The specific WT sequences for K18 (VEVDA), K14 (VEMDA), lamin A/C (VEIDA) and glial fibrillary acidic protein (VELDV) are shown. Dots indicate conserved amino acids that are identical to the K18-WT sequence. Boxes highlight the mutated amino acids in each IF protein in patients with the indicated diseases. The arrow points to the aspartate cut site. EBS, epidermolysis bullosa simplex (Ku and Omary, 2001; Coulombe et al., 2009); FPLD2, familial partial lipodystrophy type 2 (Lanktree et al., 2007); ALXAD, Alexander disease (Brockmann et al., 2003).