Supplemental Materials Molecular Biology of the Cell

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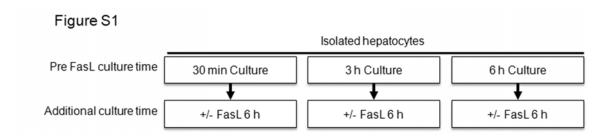


Fig S1. Experimental outline of hepatocyte pre-culture conditions prior to the addition of FasL. Primary hepatocytes were isolated from FVB/N, C57BL/6 and C3H/He mice by liver perfusion. Cells were cultured for 30 min, 3 h or 6 h following by the addition of Fas-L for 6h (0.5µg/ml).

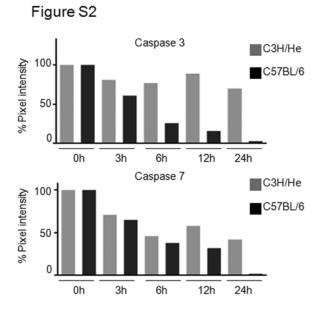


Fig S2. Densitometry analysis of the protein blots shown in Figure 2A. The data is representative of two independent experiments which showed similar findings.

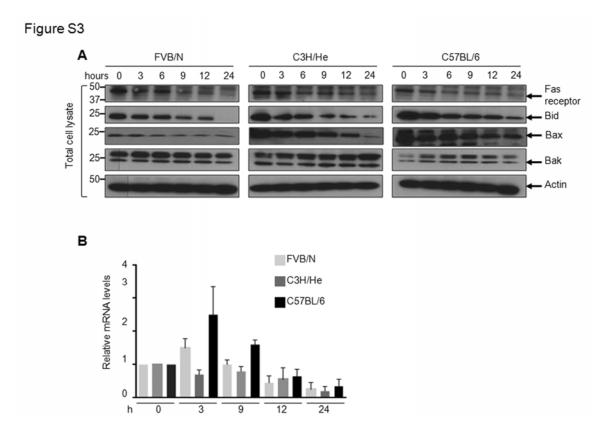


Fig S3. Assessment of FasR and Bcl-2 proteins and FasR mRNA in cultured hepatocytes. (A) Hepatocytes were isolated from FVB/N, C3H/He and C57BL/6 mice, then cultured for the indicated times. Total cell lysates were prepared and analyzed by immunoblotting using antibodies to the indicated antigens. (B) Relative expression of mRNA of Fas receptor from hepatocytes isolated from the indicated mouse strains (after 0, 3, 9, 12, and 24 hours of culture). The results shown are representative of two independent experiments.

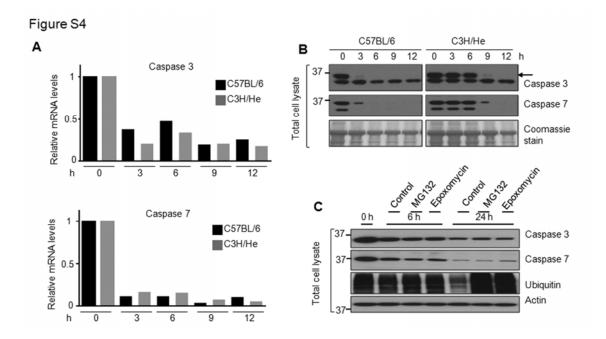


Fig S4. Relative mRNA and protein expression in hepatocytes from C57BL/6 and C3H/He mice, and effect of proteasome inhibition on caspase protein stability. (A) Changes in relative mRNA expression of caspase 3 (top) and caspase 7 (bottom) with culture time. Hepatocytes were obtained from the indicated mouse strains followed by isolation of total RNA and qPCR analysis as described in Materials and Methods. The results are from one experiment, with findings that are very similar to a repeat independent experiment. (B) Relative protein expression from the same hepatocytes used in panel A. (C) Hepatocytes were isolated from FVB/N mice and cultured in the absence or presence of the proteasome inhibitors MG132 (10μ M) and epoxomycin (10μ M). Hepatocytes were collected at the indicated time after treatment, and total cell lysates were prepared and analyzed by immunoblotting.