

Fig. S1. **TWEAK activates cell death in HEC1A cells.** HEC1A cells were treated with the indicated concentrations of TWEAK for 48 h and assayed for viability.

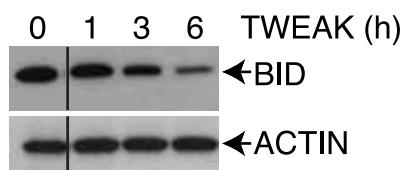


Fig. S2. **TWEAK induces BID cleavage, suggesting caspase-8 activation.** Cells were treated with TWEAK (10 $\mu\text{g}/\text{mL}$) for the indicated number of hours and subjected to immunoblot (IB) analysis with antibodies specific to BID. A vertical line denotes lane removal from the original image.

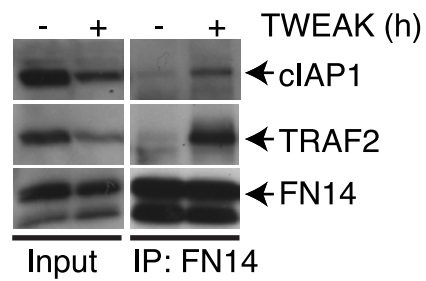


Fig. S3. TWEAK assembles a proximal signaling complex containing FN14, TRAF2 and cIAP1 in HEC1A cells. HEC1A cells were treated with TWEAK (3 $\mu\text{g}/\text{mL}$) for the indicated amount of time and subjected to immunoprecipitation (IP) of FN14 followed by IB with antibodies to FN14, TRAF2 and cIAP1.

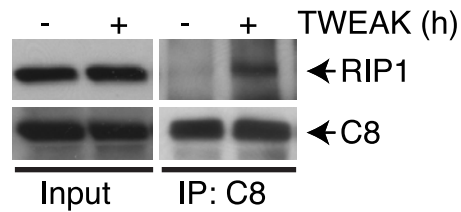


Fig. S4. **TWEAK induces association of a death-signaling complex containing RIP1, FADD and caspase-8 in HEC1A cells.** HEC1A cells were treated with TWEAK (3 μ g/mL) for the indicated time in the presence of zVAD-fmk (20 μ M) and subjected to IP of caspase-8 followed by IB of caspase-8 or RIP1.

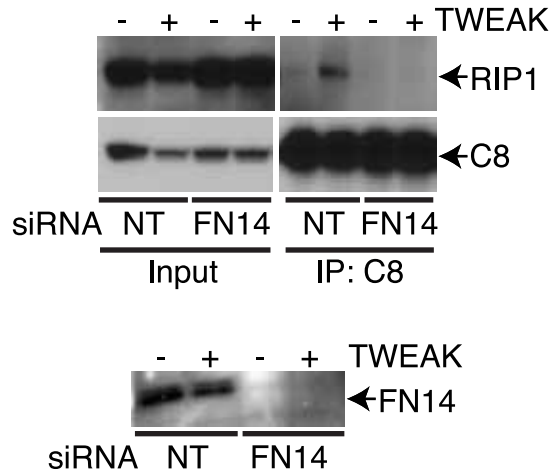


Fig. S5. FN14 is required for TWEAK-induced association of RIP1 and caspase-8. HSC3 cells were transfected with two siRNA oligonucleotides against FN14 for 48 h, treated with TWEAK (3 μ g/mL, 6 h) and subjected to IP of caspase-8 followed by IB of RIP1 or caspase-8.

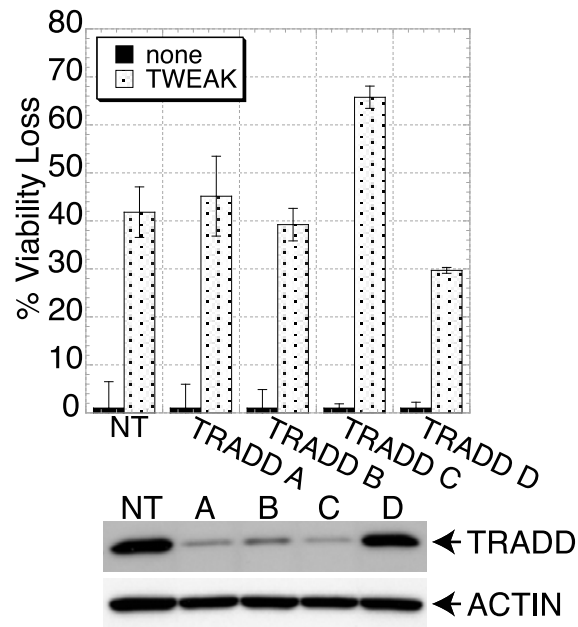
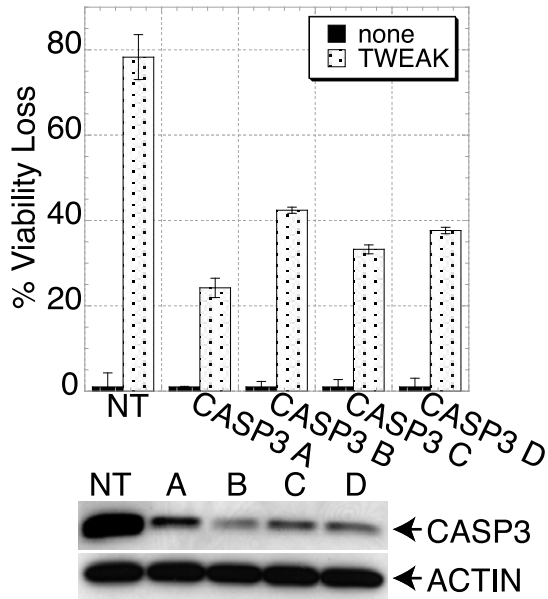


Fig. S6. **Knockdown of the adaptor protein TRADD does not inhibit TWEAK-induced apoptosis.** HSC3 cells were transfected with TRADD siRNA for 48 h, stimulated with TWEAK (3 μ g/mL, 48 h), and analyzed for viability. IB shows siRNA knockdown levels.

A



B

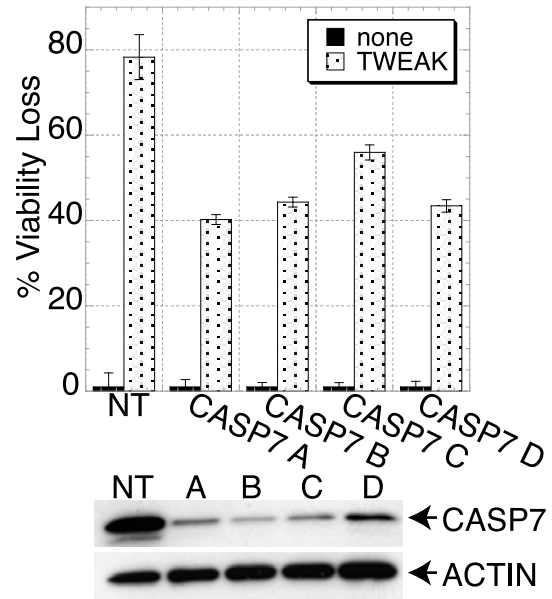


Fig. S7. **siRNA depletion of caspase-3 or -7 provides protection against TWEAK-induced cell death.** HSC3 cells were transfected with caspase-3 (A) or -7 (B) siRNA for 48 h, stimulated with TWEAK (3 μ g/mL, 48 h), and analyzed for viability. IB shows siRNA knockdown levels.

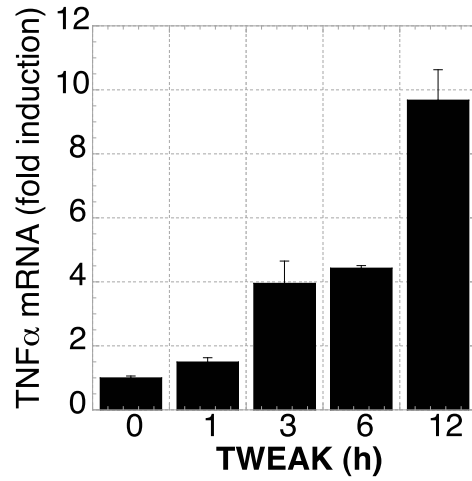


Fig. S8. **TNF α mRNA expression increases as early as 3 h after TWEAK stimulation.** HSC3 cells stimulated with TWEAK (3 μ g/mL) for the indicated times and analyzed by qRT-PCR for TNF α expression.

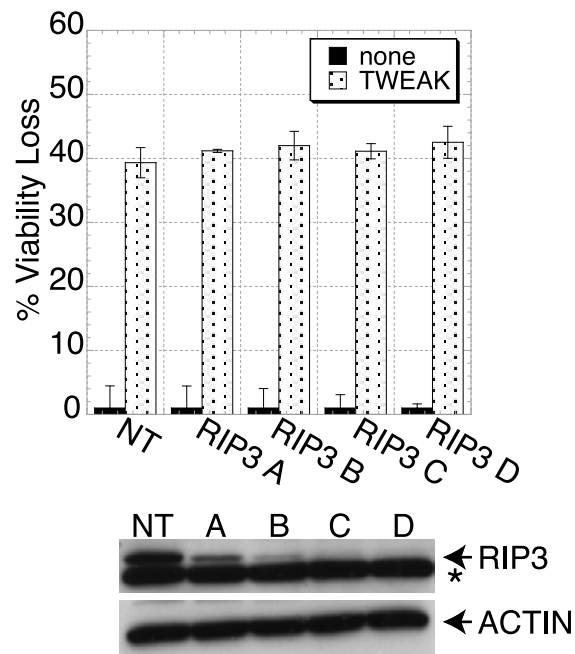


Fig. S9. **RIP3 is dispensable for TWEAK-induced cell death.** HSC3 cells were transfected with RIP3 siRNA for 48 h, stimulated with TWEAK (3 μ g/mL, 48 h), and analyzed for viability. IB shows siRNA knockdown levels. *Represents a non-specific band.

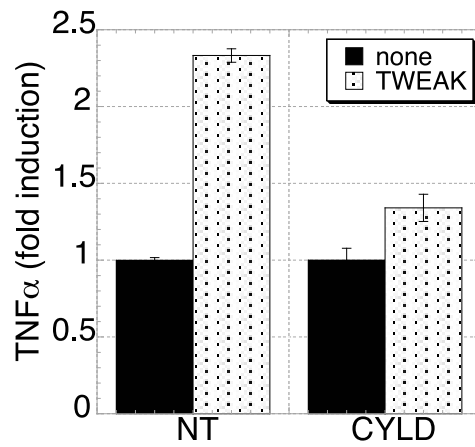


Fig. S10. **siRNA depletion of CYLD attenuates TWEAK-induced TNF α secretion.** HSC3 cells were transfected with CYLD siRNA for 48 h, stimulated with TWEAK (3 μ g/mL, 48 h), and cell supernatants analyzed for TNF α secretion by ELISA.