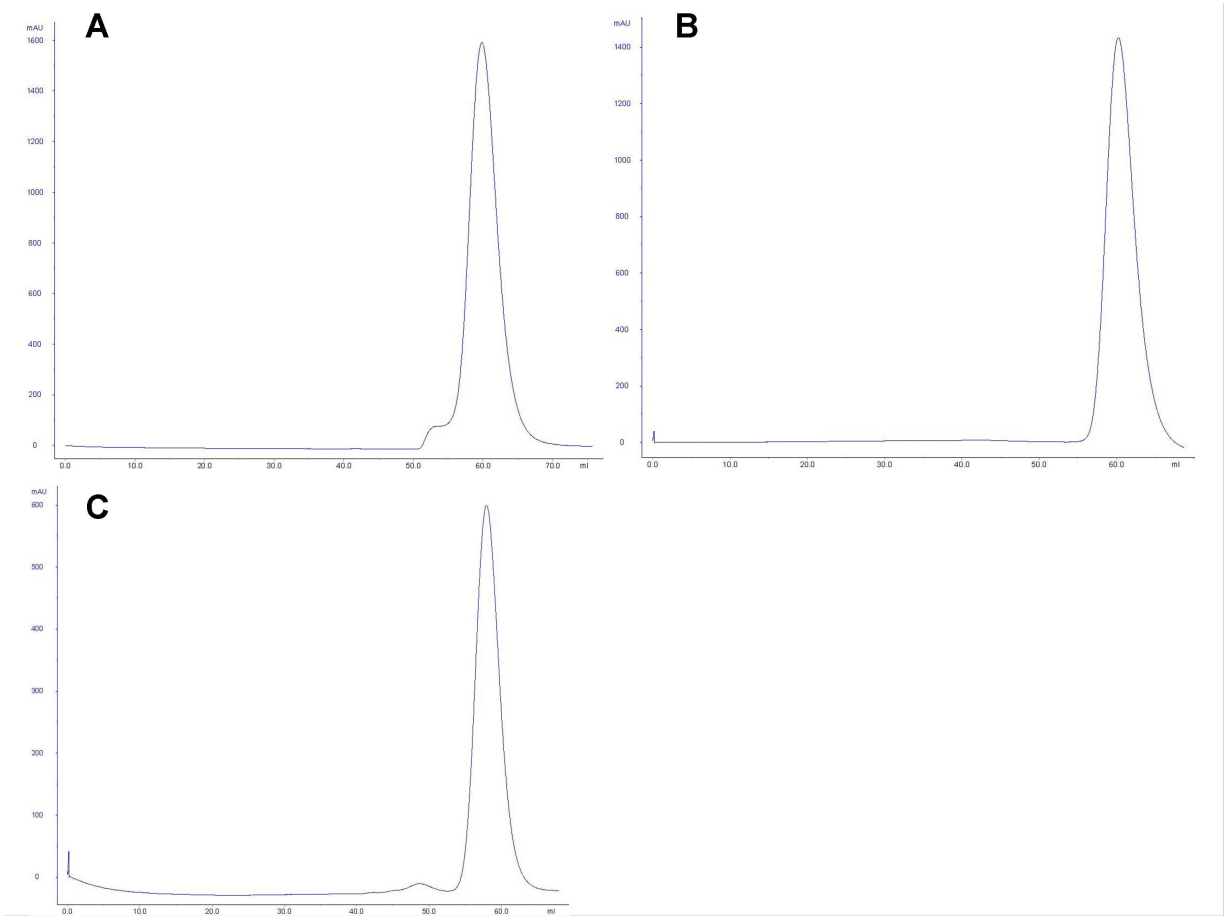


Figure S1: Gel filtration profile of rhTRAIL WT (**A**), G131R (**B**) and G131K (**C**). The rhTRAIL proteins were separated using a Hiload Superdex 75 16/60 column (GE Heathcare).

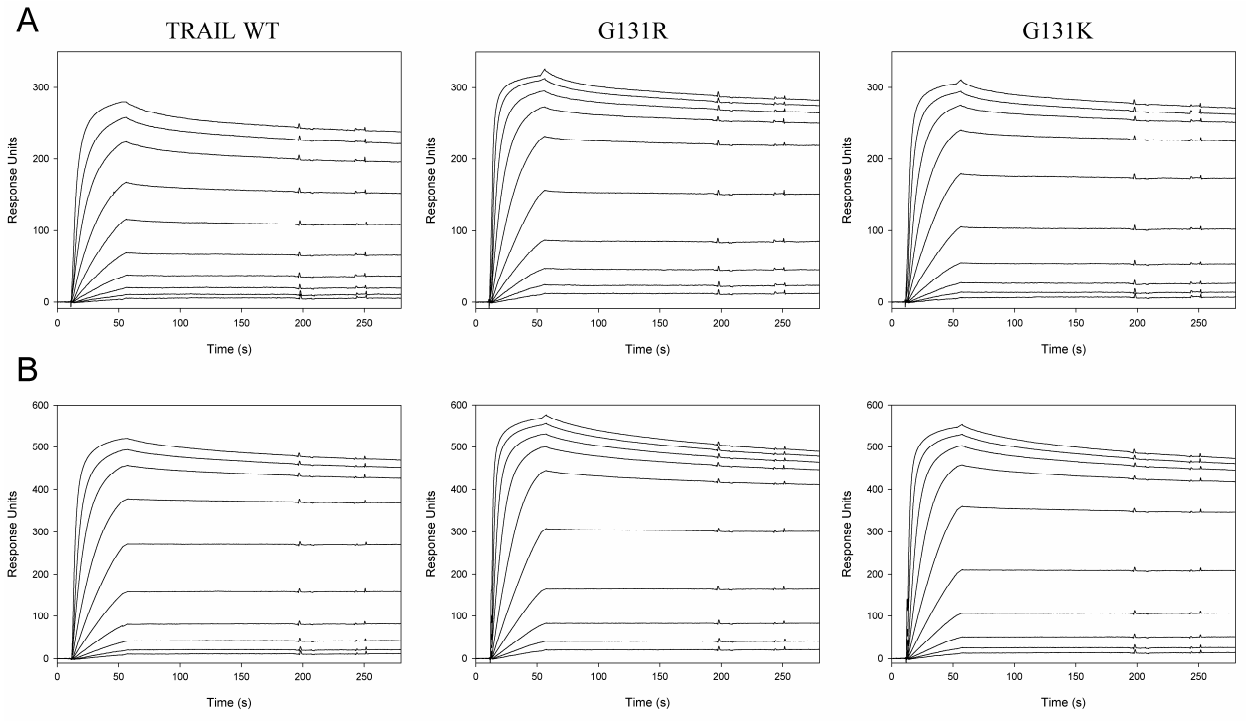


Figure S2: Time versus response SPR sensorgrams. Receptor binding of wild-type rhTRAIL and Gly-131 variants towards DR4-Ig (A), or towards DR5-Ig (B). DR4- and DR5-Ig receptor chimeras were immobilized at a level of ~ 800 RU. Purified wild-type rhTRAIL and Gly-131 variants were injected in three-fold at concentrations ranging from 250 nM to 0.5 nM at 70 µl/min flow rate using HBS-N supplemented with 0.005 % surfactant P20 (Biacore) as running and sample buffer. Binding of ligands to the receptors was monitored in real-time at 37^{o}C.

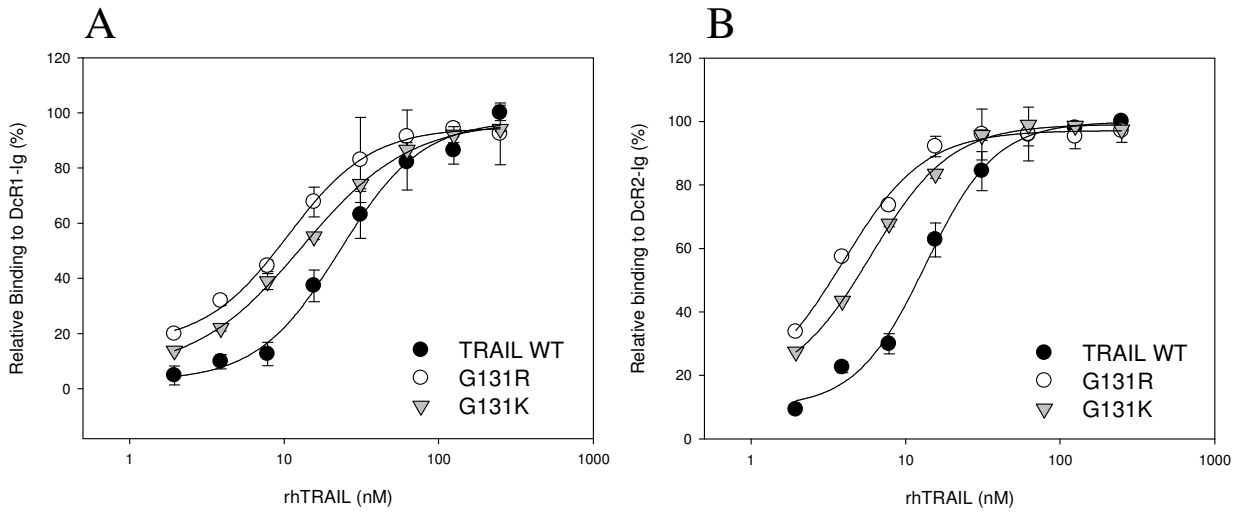
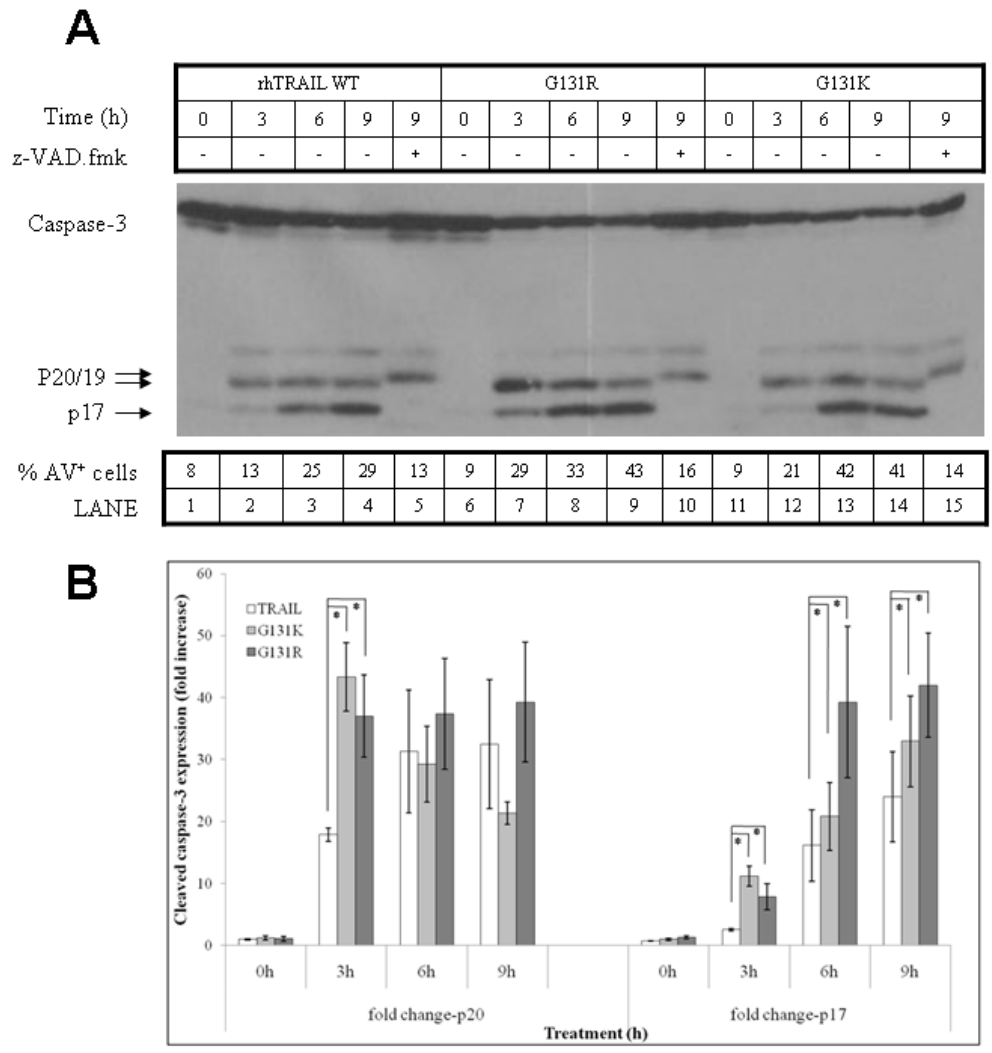


Figure S3: Receptor binding of wild-type rhTRAIL, G131R and G131K to DcR1-Ig as determined by SPR (A), or to DcR2-Ig (B). To obtain pre-steady state data that represent proper high-affinity complex formation, and assuming the initial fast off-rate to represent lower complexes, the response at each concentration was recorded 30 s after the end of the injections. Receptor binding was calculated relative to the response of wild-type rhTRAIL at 250 nM. The results are mean values +/- s.e.m.



Time (h)	0	3	6	9	9	0	3	6	9	9	0	3	6	9	9
z-VAD.fmk	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+

% AV+ cells	8	13	25	29	13	9	29	33	43	16	9	21	42	41	14
LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Figure S4: G131R and G131K induce processing of caspase-3 in ML-1 cells. (**A**) Cells were treated for the indicated times with either wild-type rhTRAIL (100 ng/ml), G131R (100 ng/ml) or G131K (100 ng/ml). Where indicated, cells were pre-treated with z-VAD.fmk (10 μM) for 30 min and then treated with 100 ng/ml of either wild-type rhTRAIL, G131R and G131K. Apoptosis was assessed by Annexin V staining (%AV+ cells). Cell lysates were also prepared for western blot analysis of caspase-3 processing. (**B**) Expression of the partially processed fragment p20 and the fully processed p17 fragment was quantified with densitometry and normalised to actin levels. The graph shows averaged fold increase in the expression of the p20 and p17 caspase-3 fragments ± S.E.M. from three independent experiments. The * indicates significant differences between samples ($p<0.05$).

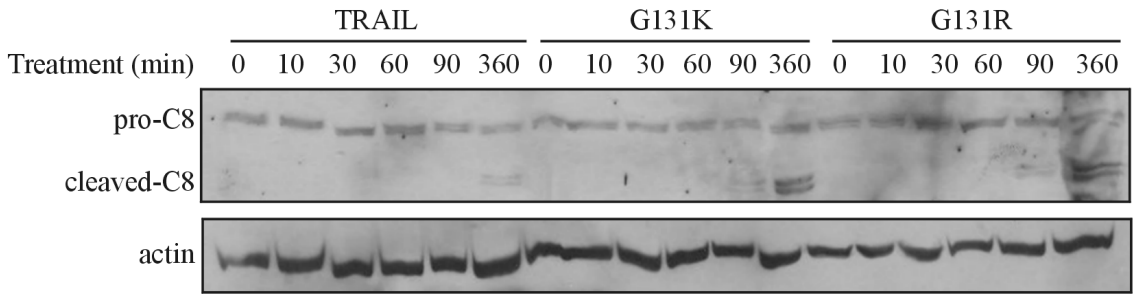


Figure S5: G131 variants are faster activators of TRAIL receptors than rhTRAIL WT. ML-1 cells were treated with 100 ng/ml of rhTRAIL WT, G131R and G131K for the times indicated after which cells were lysed and processing of pro-caspase-8 was detected with Western Blotting. Expression of actin was detected as a loading control.

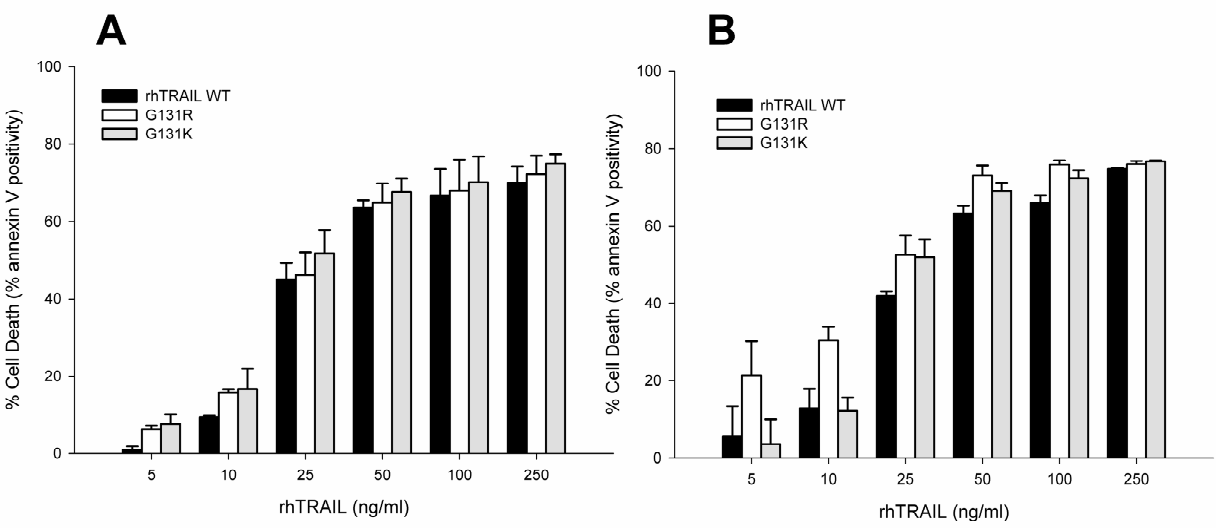


Figure S6: Apoptosis-inducing activity (Annexin V staining) of wild-type rhTRAIL or Gly-131 variants in HCT15 colon carcinoma and BxPC pancreatic cells. The results are mean values +/- s.e.m (n=3).