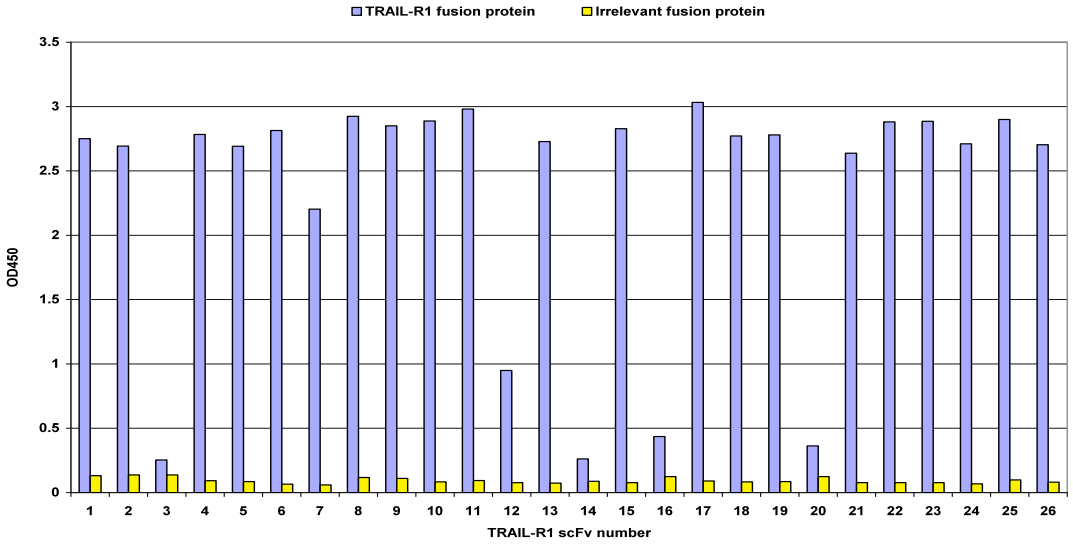
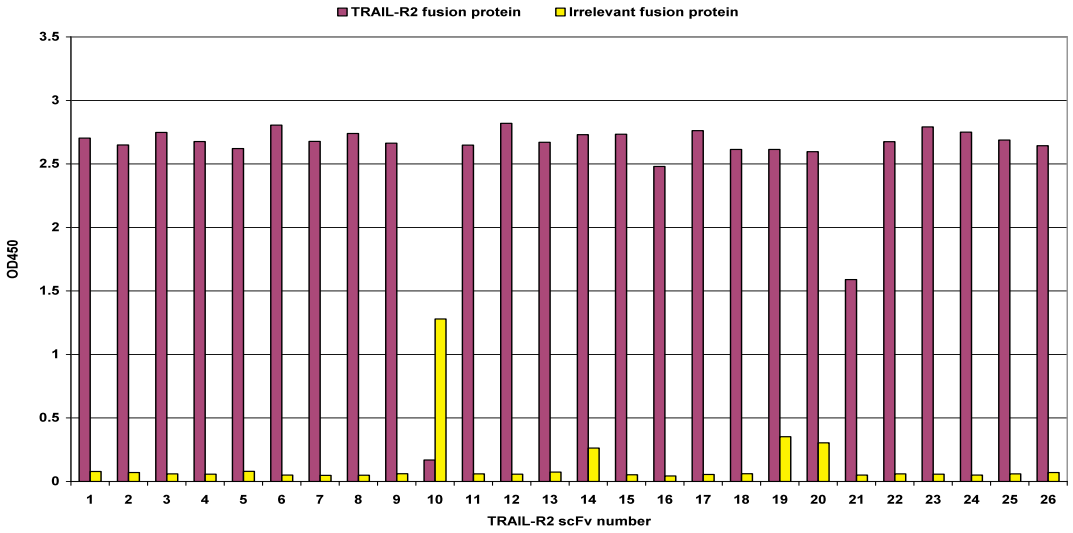
Fig.S1

(a)


TRAIL-R1 fusion protein
Irrelevant fusion protein
OD450
TRAIL-R1 scFv number

(b)


TRAIL-R2 fusion protein
Irrelevant fusion protein
OD450
TRAIL-R2 scFv number

(c)

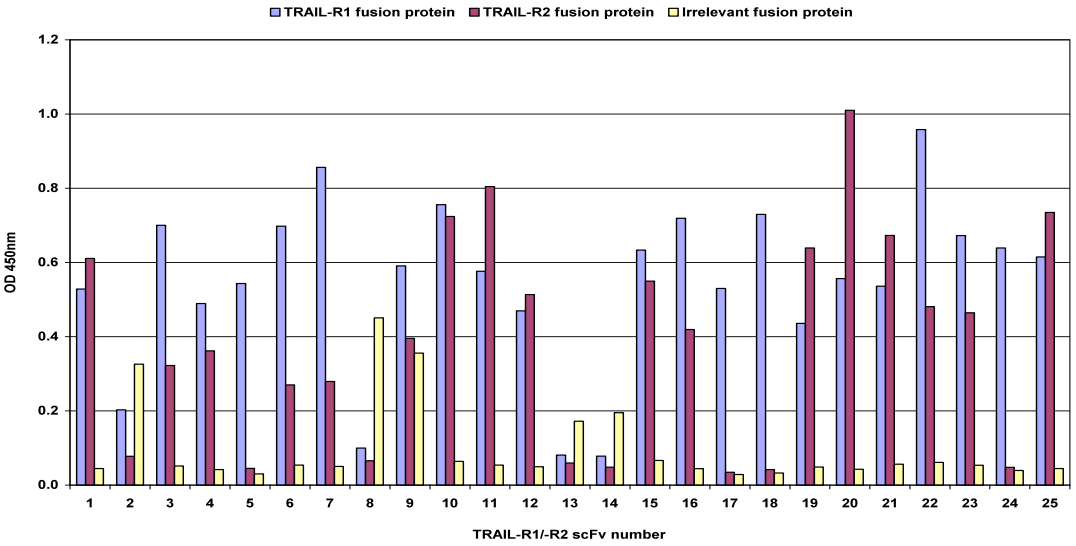

TRAIL-R1 fusion protein
TRAIL-R2 fusion protein
Irrelevant fusion protein
OD 450nm
TRAIL-R1/-R2 scFv number

Fig.S2

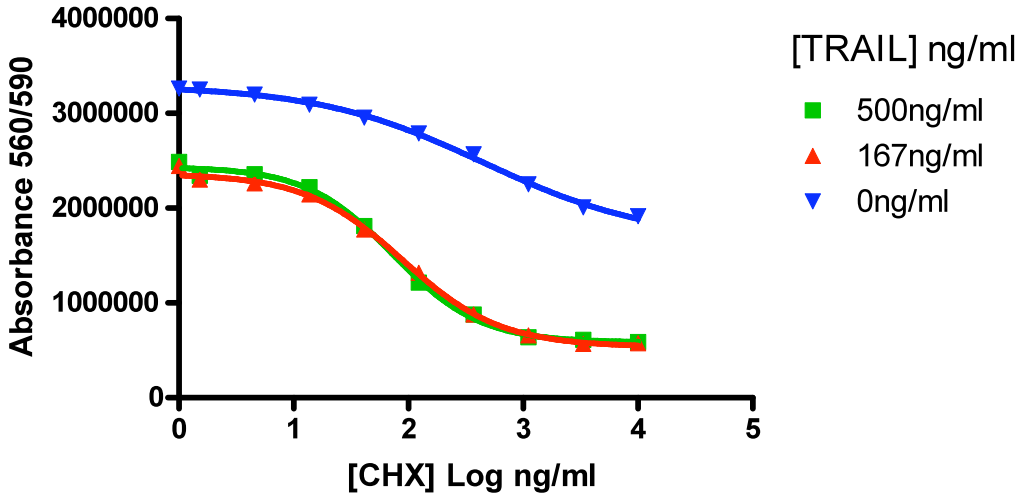
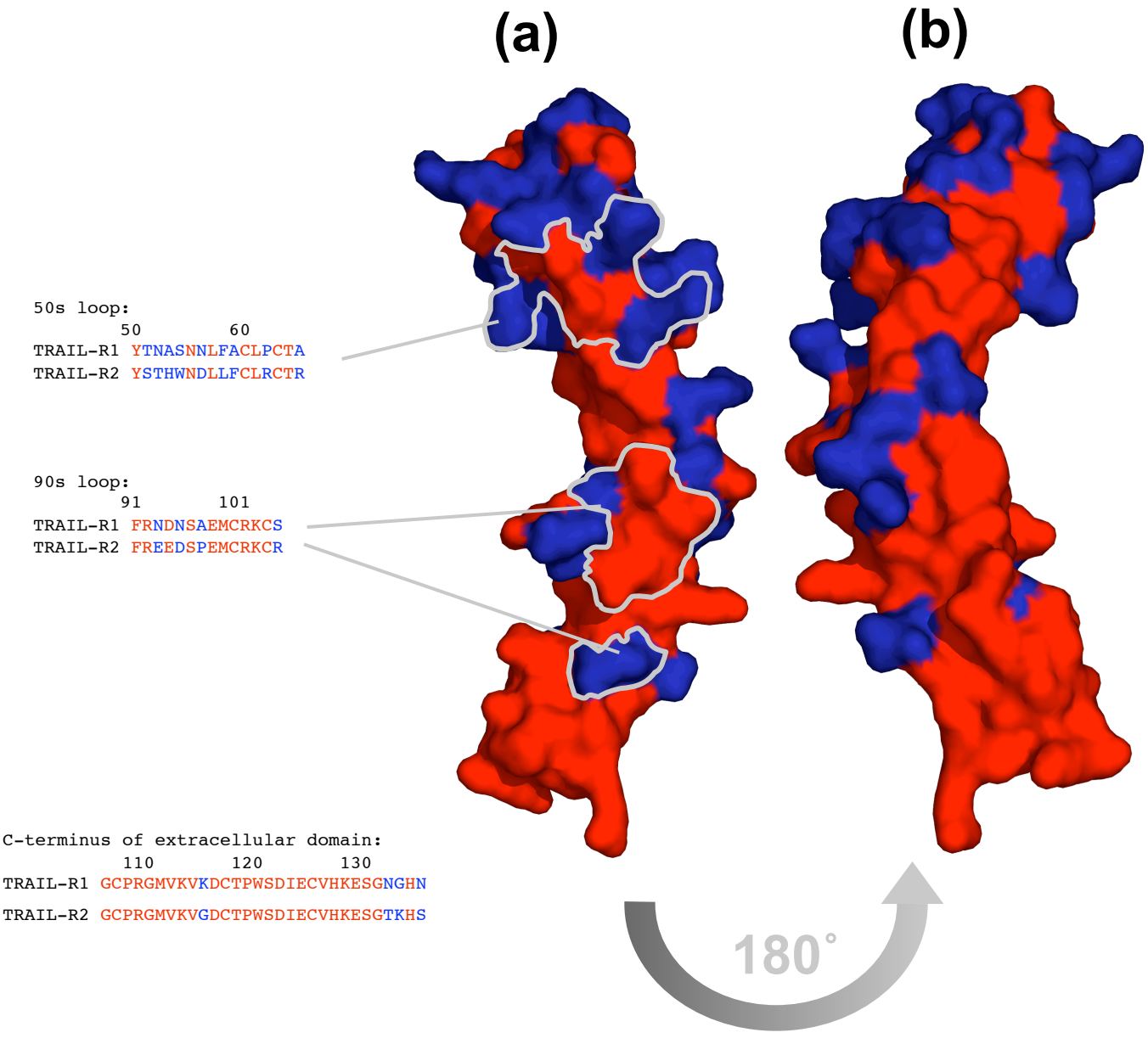

4000000
3000000
2000000
1000000
0
Absorbance 560/590
0
1
2
3
4
5
[CHX] Log ng/ml
[TRAIL] ng/ml
500ng/ml
167ng/ml
0ng/ml

Fig.S3


(a)
(b)
50s loop:
50 60
TRAIL-R1 YTNASNNLFACLPCTA
TRAIL-R2 YSTHWNDLLFCLRCTR
90s loop:
91 101
TRAIL-R1 FRNDNSAEMCRKCS
TRAIL-R2 FREEDSPEMCRKCR
C-terminus of extracellular domain:
110 120 130
TRAIL-R1 GCPRGMVKVKDCTPWSDIECVHKESGNGHN
TRAIL-R2 GCPRGMVKVGDCTPWSDIECVHKESGTKHS
180°

Supplementary Figure Legends

Figure S1. Example ELISA data demonstrating specificity of scFv produced from selections on (a) TRAIL-R1, (b) TRAIL-R2 and (c) both TRAIL-R1 and TRAIL-R2. Data are presented as histograms representing different intensities of ELISA signal against TRAIL-R1 (blue bars), TRAIL-R2 (red bars) and an irrelevant fusion protein (yellow bars). In these examples (a) 20/25 TRAIL-R1 specific scFv, (b) 24/25 TRAIL-R2 specific scFv, and (c) 16/25 TRAIL-R1 and TRAIL-R2 cross-reactive scFv were identified.

Figure S2. Optimization of TRAIL and cycloheximide concentrations to induce apoptosis of HeLa cells. HeLa cells were exposed to varying concentrations of cycloheximide (CHX) in the presence of 500ng/ml, 167ng/ml or 0ng/ml of TRAIL. Cell viability was measured by the addition of Alamar Blue™, which fluoresces in response to chemical reduction of the growth media resulting from cell proliferation. Minimal inhibition of cell proliferation was observed in response to TRAIL or cycloheximide alone, however TRAIL in combination with cycloheximide at 500ng/ml resulted in a significant inhibition of HeLa cell growth.

Figure S3. Regions of structural conservation between TRAIL-R1 and TRAIL-R2. A surface representation of the TRAIL-R2 molecule (PDB entry 1d0g) is shown with the N-terminus at the top and the membrane-bound C-terminus at the bottom. Residues are coloured red if conserved in a sequence alignment of TRAIL-R1 and TRAIL-R2 and blue if they are not conserved. (a) Residues of TRAIL-R2 known to be structural contacts with the ligand TRAIL [40] are highlighted on the protein surface with a grey perimeter. The sequence alignments for the corresponding regions, known as the 50s loop and the 90s loop, are shown to the left, with the TRAIL contact residues boxed in grey. Also shown on the left is the sequence alignment for the C-terminal regions of TRAIL-R1 and TRAIL-R2. On the

right hand side, TRAIL-R2 is rotated 180˚ to illustrate regions of conservation with TRAIL-R1 on the non-binding face of the receptor (b).