

Figure S1 (A) SEC of purified IgBD-TRAIL and TRAIL. (B) Identification of protein components in the novel protein peak (Lane 3) visualized by SEC in IgBD-TRAIL and hIgG mixture. Unmixed hIgG (Lane 1) and IgBD-TRAIL (Lane 2) were used as control. M: protein markers. (C) SEC of the IgBD-TRAIL preincubated with hIgG at different molar ratios. (D) Dynamic light scattering of hIgG and IgBD-TRAIL preincubated with different amount of hIgG.

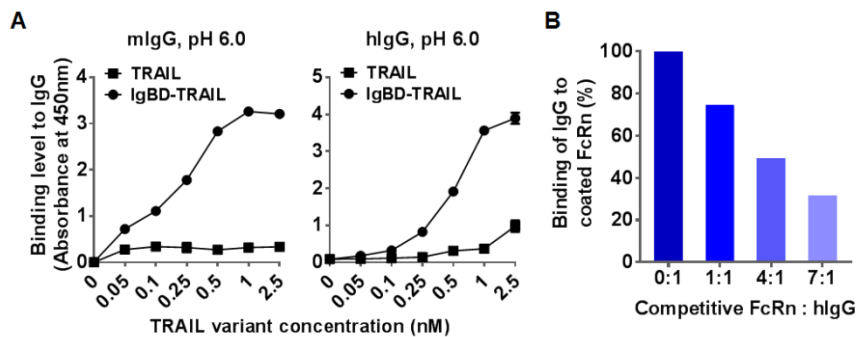


Figure S2 (A) ELISA for mIgG and hIgG binding of IgBD-TRAIL at pH 6.0. (B) Inhibition of FcRn binding of hIgG by addition of exogenous FcRn.

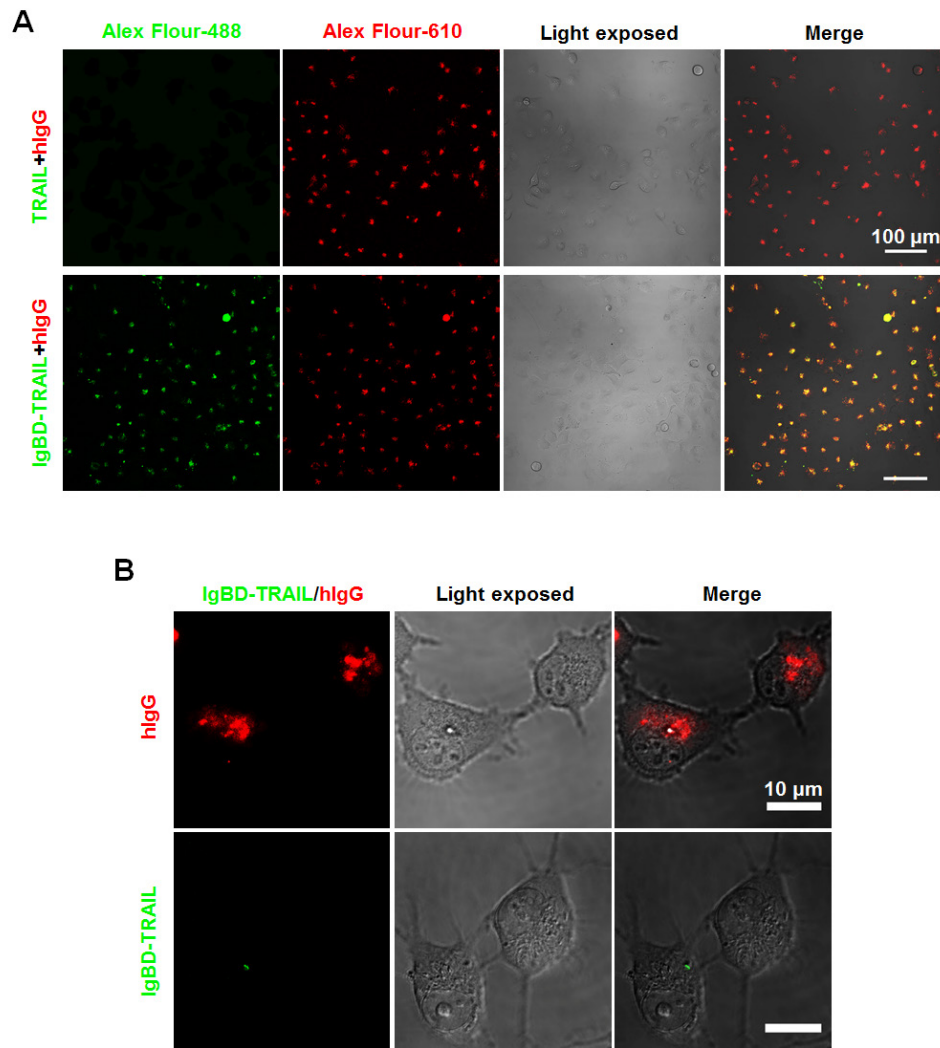


Figure S3 (A) Uptake and localization of TRAIL proteins preincubated with IgG in endothelial cells. **(B)** Cellular localization of IgBD-TRAIL and hIgG in endothelial cells.

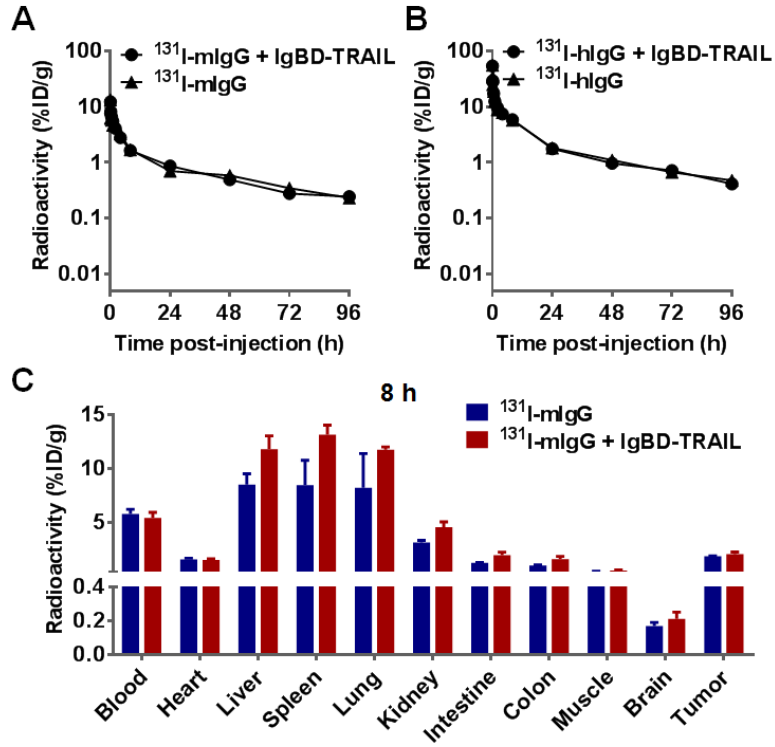


Figure S4 Pharmacokinetic and biodistribution of IgG. (A,B) Time-dependent clearance of ^{131}I -labeled mIgG (A) or hIgG (B) preincubated with or without IgBD-TRAIL at molar ratio of 1:1. (C) Biodistribution of ^{131}I -labeled mIgG in mice bearing COLO205 tumor grafts at 8 h post-injection.

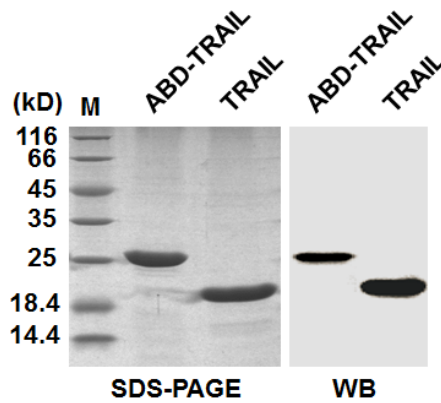


Figure S5 SDS-PAGE and western blotting of purified ABD-TRAIL.

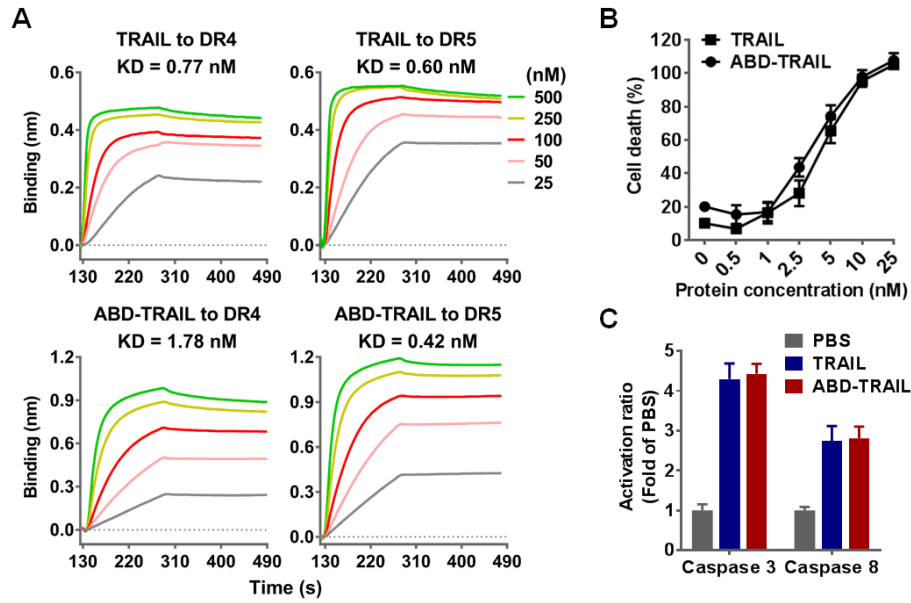


Figure S6 Death receptor binding and cytotoxicity of ABD-TRAIL. (A) Affinity of ABD-TRAIL and TRAIL for death receptor DR4 and DR5 measured by biolayer interferometry. (B) Cytotoxicity of ABD-TRAIL and TRAIL in COLO 205 tumor cells in absence of albumin. (C) Caspase activity of COLO 205 tumor cells treated with ABD-TRAIL and TRAIL in absence of albumin.

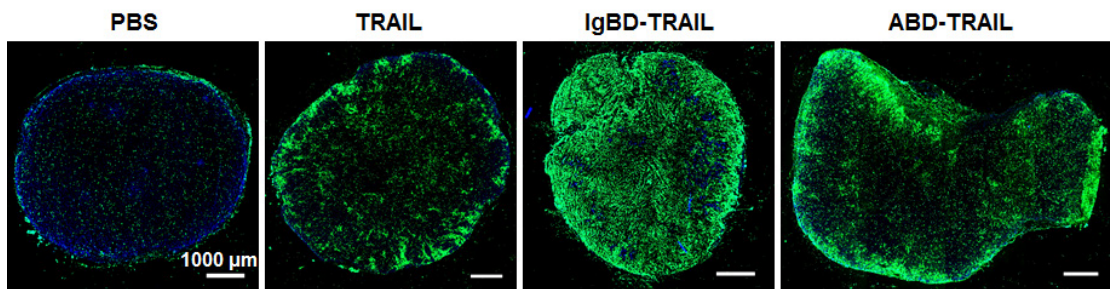


Figure S7 Comparison on apoptosis induction illustrated by TUNEL.

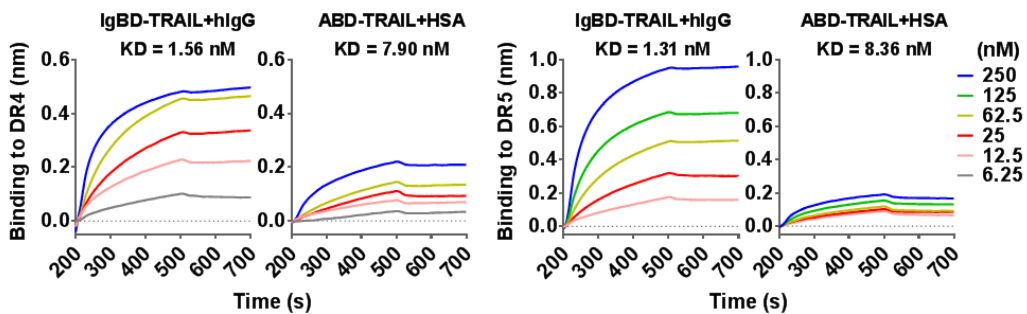


Figure S8 Affinity of IgBD-TRAIL preincubated with hIgG and ABD-TRAIL preincubated with HSA for death receptor DR4-Fc and DR5-Fc.

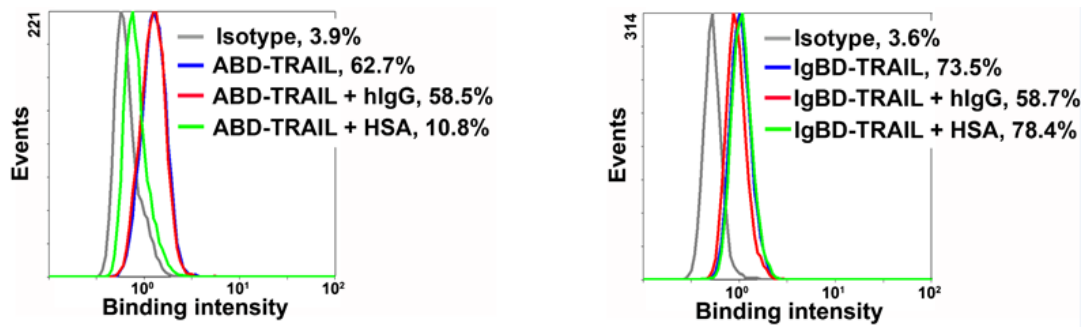


Figure S9 Cell binding of IgBD-TRAIL and ABD-TRAIL preincubated with or without hIgG or HSA. The positive rate was indicated.

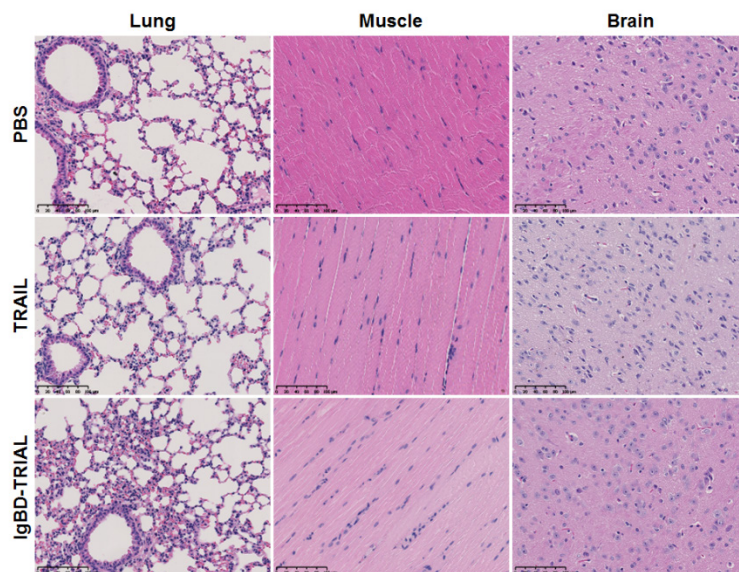


Figure S10 Histochemistry of tissues derived from mice for evaluation of short term acute toxicity of IgBD-TRAIL and TRAIL.

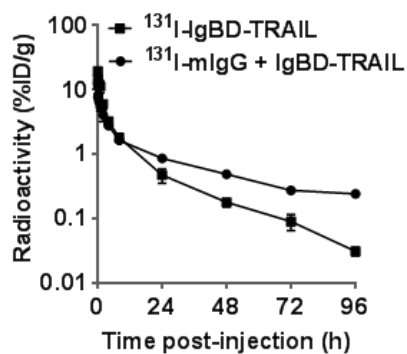


Figure S11 Blood clearance of ¹³¹I-IgBD-TRAIL and ¹³¹I-mIgG preincubated with IgBD-TRAIL.

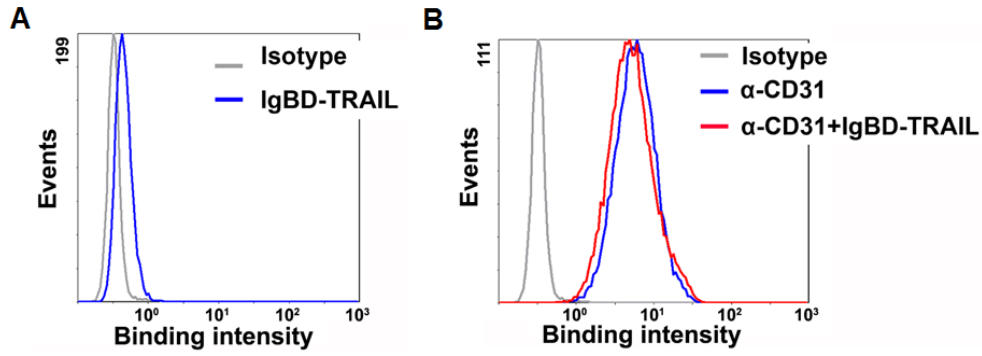


Figure S12 Impact of bound IgBD-TRAIL on the antigen binding of antibody against CD31. (A) Cell binding of IgBD-TRAIL preincubated without IgG. **(B)** Cell binding of antibody against CD31 preincubated with or without IgBD-TRAIL.

Table S1: Distribution of ¹³¹I-mIgG in mice, preincubated with or without IgBD-TRAIL

Organ/ Tissue	1 h		8 h		24 h		96 h	
	mIgG	mIgG+ IgBD-TRAIL	mIgG	mIgG+ IgBD-TRAIL	mIgG	mIgG+ IgBD-TRAIL	mIgG	mIgG+ IgBD-TRAIL
Blood	4.66±0.57	5.48±0.47	1.69±0.15	1.65±0.12	0.70±0.10	0.87±0.01	0.23±0.01	0.24±0.01
Heart	1.96±0.27	2.00±0.17	0.73±0.07	0.65±0.01	0.25±0.03	0.29±0.01	0.16±0.01	0.05±0.00
Liver	22.80±0.71	23.31±0.75	9.33±1.01	10.42±0.40	4.04±0.71	5.60±0.20	1.42±0.06	0.91±0.09
Spleen	7.36±0.58	9.06±0.86	5.69±1.27	6.74±1.02	2.80±0.15	3.48±0.11	0.92±0.05	0.70±0.21
Lung	34.17±3.78	31.36±0.64	3.30±0.42	3.64±0.41	0.74±0.04	1.20±0.16	0.26±0.00	0.14±0.02
Kidney	8.09±0.82	9.97±0.17	3.59±0.14	3.87±0.16	1.65±0.21	2.21±0.33	0.69±0.02	0.60±0.04
Colon	1.44±0.06	1.62±0.46	0.49±0.08	0.51±0.02	0.17±0.03	0.22±0.01	0.09±0.00	0.05±0.00
Intestine	1.00±0.04	1.21±0.07	0.37±0.03	0.39±0.04	0.15±0.01	0.26±0.02	0.05±0.01	0.06±0.00
Muscle	0.19±0.01	0.26±0.03	0.07±0.01	0.08±0.01	0.04±0.01	0.03±0.00	0.01±0.00	0.01±0.00
Brain	0.54±0.03	0.60±0.04	0.19±0.00	0.17±0.00	0.08±0.00	0.10±0.01	0.03±0.00	0.02±0.00

Data are expressed as mean ± SEM percent of injected dose per gram tissue (%ID/g, n=3)

Table S2: Distribution of ¹³¹I-hIgG in mice, preincubated with or without IgBD-TRAIL

Organ/ Tissue	1 h		8 h		24 h		96 h	
	hIgG	hIgG+ IgBD-TRAIL	hIgG	hIgG+ IgBD-TRAIL	hIgG	hIgG+ IgBD-TRAIL	hIgG	hIgG+ IgBD-TRAIL
Blood	12.48±0.40	12.51±0.078	5.74±0.33	5.86±0.28	1.83±0.01	1.77±0.14	0.48±0.04	0.41±0.04
Heart	3.10±0.01	2.80±0.11	1.69±0.10	1.65±0.08	0.49±0.02	0.49±0.02	0.18±0.01	0.17±0.01
Liver	24.57±1.07	24.11±0.48	7.74±0.22	7.62±0.36	2.27±0.13	2.29±0.14	0.78±0.09	0.81±0.02
Spleen	9.46±0.68	9.59±1.67	4.34±0.06	3.93±0.47	1.16±0.07	1.34±0.02	0.27±0.01	0.32±0.02
Lung	19.34±0.22	16.48±0.70	6.42±0.91	4.19±0.34	1.38±0.15	1.14±0.14	0.26±0.00	0.24±0.01
Kidney	5.82±0.12	5.80±0.62	3.32±0.03	3.52±0.21	0.79±0.03	0.84±0.06	0.27±0.01	0.28±0.02
Colon	2.60±0.12	3.02±0.54	1.68±0.06	1.84±0.05	0.27±0.02	0.26±0.01	0.12±0.01	0.10±0.00
Intestine	2.08±0.17	2.09±0.16	1.79±0.12	1.99±0.23	0.24±0.02	0.23±0.03	0.10±0.01	0.14±0.02
Muscle	1.30±0.06	1.13±0.08	0.93±0.04	1.03±0.17	0.22±0.02	0.24±0.02	0.12±0.00	0.12±0.03
Brain	0.51±0.02	0.48±0.04	0.29±0.01	0.36±0.05	0.06±0.01	0.07±0.01	0.03±0.00	0.03±0.00

Data are expressed as mean ± SEM percent of injected dose per gram tissue (%ID/g, n=3)