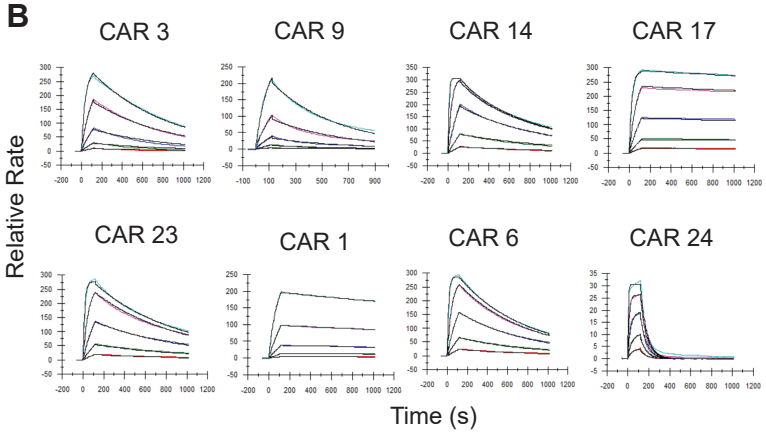
Fig. S2

A

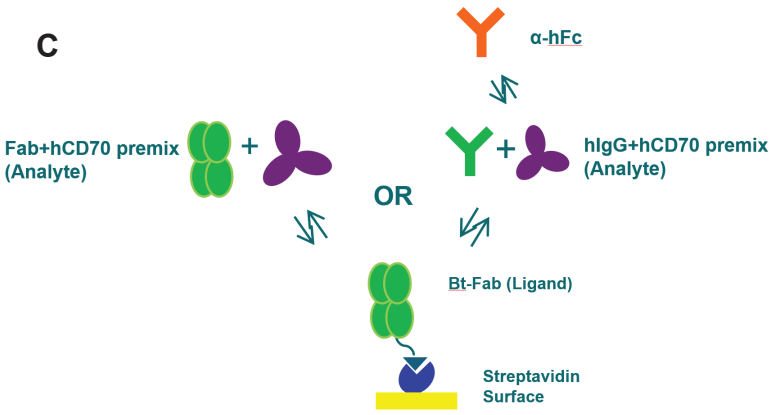
Clone	Fab				scFv			
	k_a ($x10^5$ $M^{-1}s^{-1}$)	k_d ($x10^{-3}$ s^{-1})	$t_{1/2}$ (min)	K_D (nM)	k_a ($x10^5$ $M^{-1}s^{-1}$)	k_d ($x10^{-3}$ s^{-1})	$t_{1/2}$ (min)	K_D (nM)
CAR 3	2.6 ± 0.4	1.34 ± 0.04	8.6 ± 0.3	5.2 ± 0.6	6.9 ± 0.4	0.84 ± 0.04	13.8 ± 0.6	1.2 ± 0.1
CAR 9	1.5 ± 0.2	2.8 ± 0.7	4 ± 1	19 ± 1				
CAR 14	11.9 ± 0.7	1.64 ± 0.02	7.07 ± 0.09	1.38 ± 0.06				
CAR 17	4.3 ± 0.3	0.06 ± 0.01	180 ± 20	0.15 ± 0.03	8.5 ± 0.5	0.091 ± 0.003	127 ± 4	0.108 ± 0.005
CAR 23	7 ± 1	1.3 ± 0.1	8.8 ± 0.9	1.9 ± 0.1	13.9 ± 0.1	1.24 ± 0.02	9.3 ± 0.1	0.89 ± 0.02
CAR 1	1.18 ± 0.05	0.17 ± 0.01	70 ± 3	1.4 ± 0.1				
CAR 6	6 ± 2	1.56 ± 0.02	7.4 ± 0.1	2.6 ± 0.7				
CAR 24	3.3 ± 0.2	15 ± 1	0.8 ± 0.1	47 ± 1	10 ± 1	19 ± 2	0.60 ± 0.05	19.1 ± 0.4

= not tested as scFv 10X His format did not express well

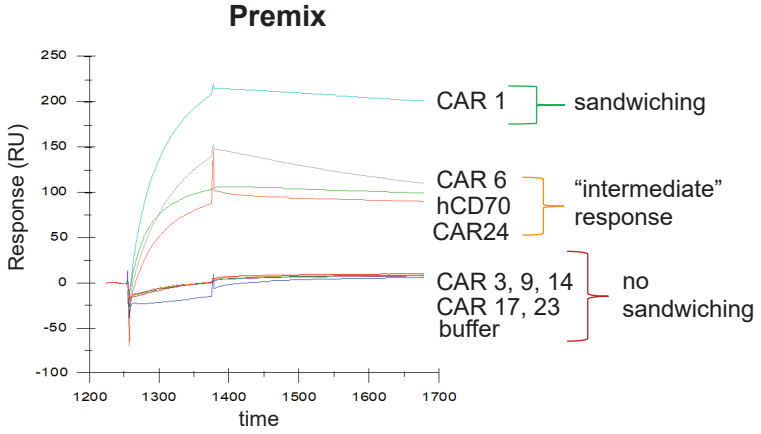
B



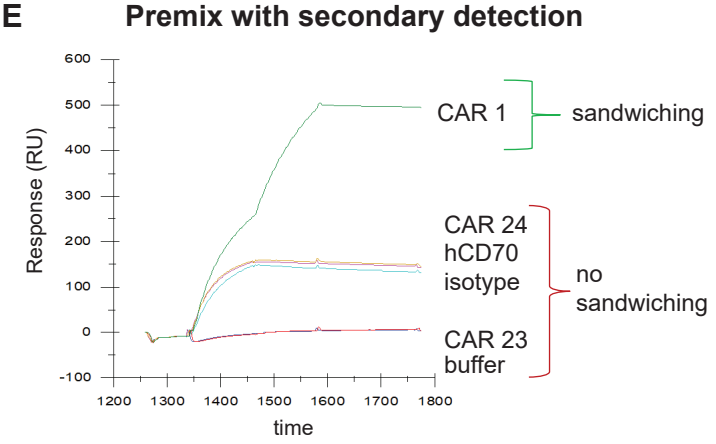
C



D



E



F

Ligand	Sandwiching with hCD70							
	Class 1					Class 2A		Class 2B
	CAR 3	CAR 9	CAR 14	CAR 17	CAR 23	CAR 1	CAR 6	CAR 24
CAR 3	SB	N	N	N	N	Y	Y	N
CAR 9	N	SB	N	N	N	Y	Y	N
CAR14	N	N	SB	N	N	Y	Y	N
CAR 17	N	N	N	SB	N	Y	Y	N
CAR 23	N	N	N	N	SB	Y	Y	N
CAR 1	Y	Y	Y	Y	Y	SB	N	N
CAR 6	Y	Y	Y	Y	Y	N	SB	N
CAR 24	N	N	N	N	N	N	N	SB

Y = sandwiching
N = no sandwiching
SB = self blocking

Figure S2. Kinetics and sandwiching data for anti-CD70 Fabs. **A,** Class 1 (red box) and class 2 (green box) anti-CD70 Fabs and/or scFv format(s) display binding affinity ranging from 0.15nM to 47nM. **B,** Double-referenced sensorgrams of anti-CD70 Fabs binding to hCD70. Triplicate measurements are consistent with one another; therefore, one replicate is shown **C,** Schematic view of premix binning assay formats with or without use of an anti-hFc secondary detection reagent (orange). **D,** Premix binning assay resulted in binding (sandwiching), no binding (no sandwiching) or "intermediate" binding which was then followed up with an anti-hFc secondary detection method. **E,** A representative plot is shown for CAR 23. **F,** Three sandwiching bins were identified.