Supplementary Materials to:

A sensitivity scale for targeting T cells with Chimeric Antigen Receptors (CARs) and Bispecific T cell Engagers (BiTEs)

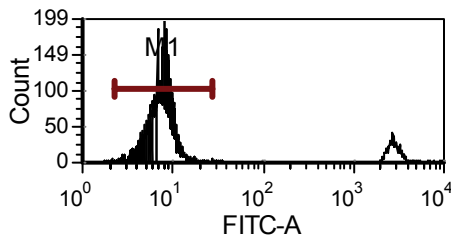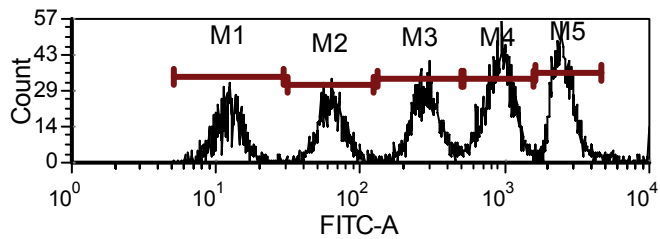Jennifer D. Stone, David H. Aggen, Andrea Schietinger, Hans Schreiber, and David M. Kranz

Supplementary Figure 1
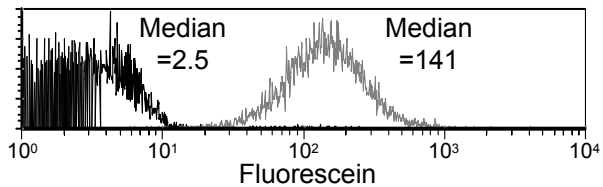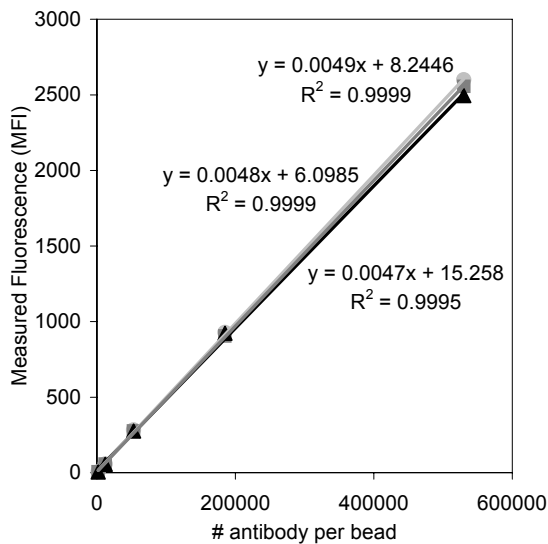
Dako Qifi Kit
Mouse IgG2a beads (K0078)
Stained with included FITC anti-mouse

Bead population	# Ab bound per bead
A	0 (set-up beads)
B	1,800
C	12,000
D	53,000
E	185,000
F	530,000

Marker	Median	Geometric Mean	Arithmetic Mean
M1	7.64	7.45	7.86

Marker	Median	Geometric Mean	Arithmetic Mean
M1	12.41	12.34	12.75
M2	63.21	63.52	65.22
M3	283.87	282.63	290.51
M4	930.57	912.76	935.62
M5	2502.87	2563.08	2607.71

$y = 0.0049x + 8.2446$
$R^2 = 0.9999$

$y = 0.0048x + 6.0985$
$R^2 = 0.9999$

$y = 0.0047x + 15.258$
$R^2 = 0.9995$

Median =2.5   Median =141

Average measurements in this experiment: 12,000 ± 1000

Average over three independent experiments: 13,600 +/- 2300

Supplementary Figure 1. Quantification of Antibodies Bound to Cells By Comparison to Calibrated Beads. Ag104A and beads with the capacity to bind different levels of murine antibodies (Dako) were stained with saturating levels of 237 antibody, and detection was carried out with a fluorescein-labeled anti-mouse antibody.

Supplementary Figure 2
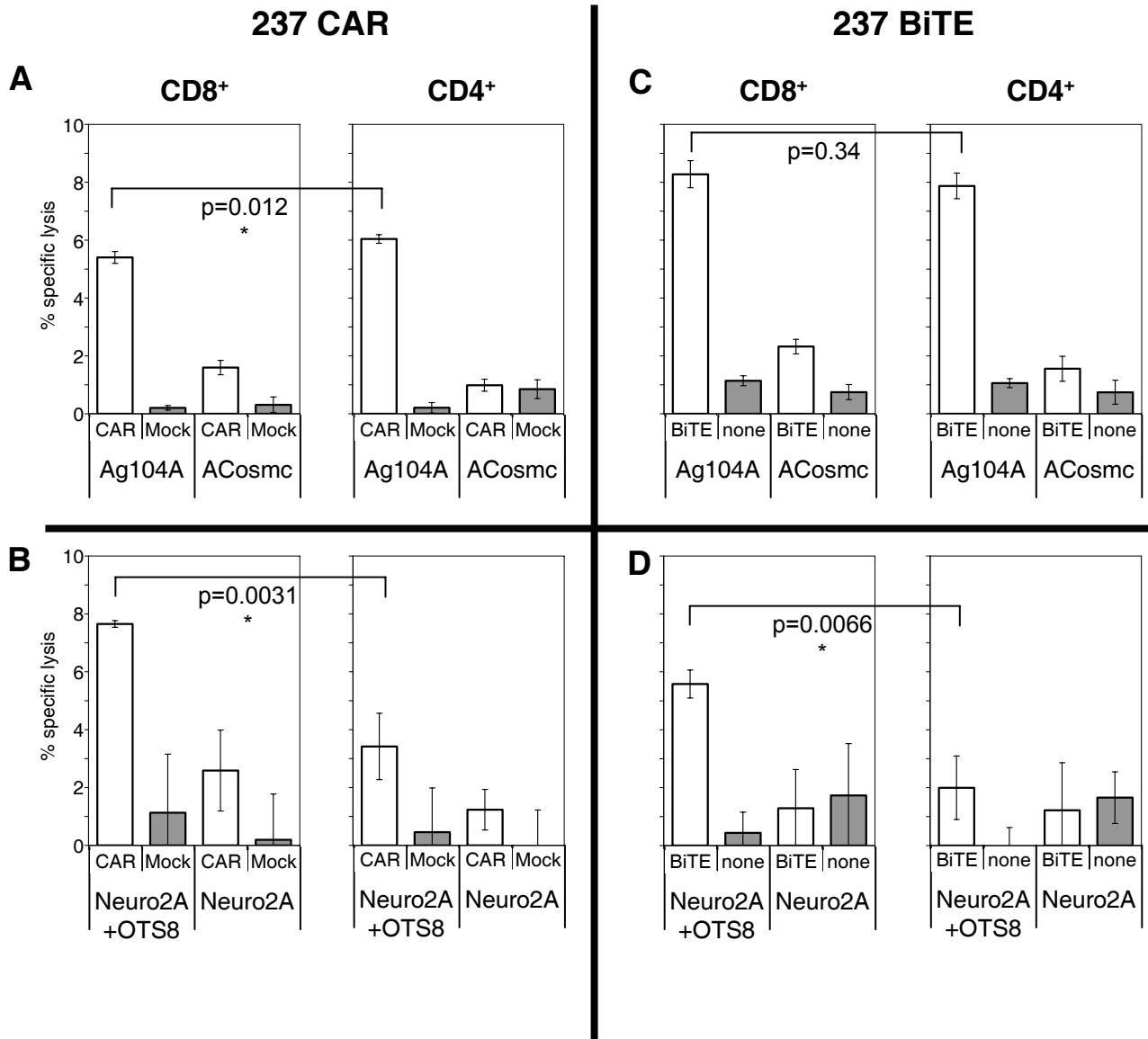
Supplementary Figure 2. Target Cell Lysis Stimulated By 237 Targeting Strategies. Lysis of murine target cells by *(A,B)* 237 CAR- or Mock-transduced T cells, or *(C,D)* T cells with or without 100nM added 2C11:237 BiTE; where the specific target cells are *(A,C)* murine fibrosarcoma cells Ag104A (237^{high}, open bars) or ACosmc (237^{low}, shaded bars); *(B,D)* murine neuroblastoma cells Neuro2A+OTS8 (237^{high}, open bars) or Neuro2A (237^{low}, shaded bars). In each panel, the responses of CD8+ T cells are shown in the left graph, and the responses of CD4+ T cells are shown in the right graph. Lysis was measured by ^{51}Cr release into the culture medium. The Students' unpaired, two-tailed t test was used to calculate p values indicating the difference between CD8$^+$ and CD4$^+$ responses.