

**S4. Optimization of anti-HMW CAR intracellular domain.** Three variations of the anti-HMW CAR extracellular domain were evaluated to determine the optimal format, based on transduction efficiency and cytokine release. Constructs differed in their T cell signaling motifs (28z = CD28 transmembrane / signaling domain and CD3 $\zeta$  signaling domain; 28BBz = CD28 transmembrane / signaling domain, 4-1BB signaling domain and CD3 $\zeta$  signaling domain; CD8.28BBz = CD8 transmembrane domain, CD28 signaling domain, 4-1BB signaling domain and CD3 $\zeta$  signaling domain); all contained the L2H (VL-218-VH) extracellular domain. Values in **bold-face type** are the greatest for that parameter with that donor or the mean of all donors.

	<b>28z</b>		<b>28BBz</b>		<b>CD8.28BBz</b>	
	<b>% Transduction<sup>1</sup></b>	<b>IFN-<math>\gamma</math> release<sup>2</sup></b>	<b>% Transduction<sup>1</sup></b>	<b>IFN-<math>\gamma</math> release<sup>2</sup></b>	<b>% Transduction<sup>1</sup></b>	<b>IFN-<math>\gamma</math> release<sup>2</sup></b>
<b>Donor 1</b>	<b>48</b>	<b>10345</b>	15	2405	12	9830
<b>Donor 2</b>	<b>65</b>	3385	18	2055	16	<b>4355</b>
<b>Donor 3</b>	<b>46</b>	27400	26	9735	15	<b>31400</b>
<b>MEAN (SEM)</b>	<b>53 (6)</b>	13710 (7134)	20 (3)	4732 (2504)	14 (1)	<b>15195 (8255)</b>

<sup>1</sup> Gene transfer efficiency in human PBL, as determined by flow cytometry on day 9 after transduction.

<sup>2</sup> IFN- $\gamma$  concentration in the supernatant of an overnight coculture between anti-HMW CAR-transduced PBL and the HMW-expressing melanoma line 1300. Values are in pg/mL.