S2. Optimization of anti-HMW CAR extracellular domain. Four variations of 225.28S-based scFv within the anti-HMW CAR extracellular domain were evaluated to determine the optimal format, based on transduction efficiency and cytokine release. Constructs differed in the orientation of the VL and VH of 225.28S and the presence of the 218 or G4S3 peptide linker (L2H = VL-218-VH, LGH = VL-G4S3-VH, H2L = VH-218-VL, HGL = VH-G4S3-VL); all contained the 28z intracellular domain. Values in **bold-face type** are the greatest for that parameter with that donor or the mean of all donors.

	L2H		**LGH**		**H2L**		**HGL**	
	% Transduction[1]	**IFN-γ release[2]**	**% Transduction[1]**	**IFN-γ release[2]**	**% Transduction[1]**	**IFN-γ release[2]**	**% Transduction[1]**	**IFN-γ release[2]**
Donor 1	**40**	4460	14	2470	28	**6370**	20	3905
Donor 2	**19**	3025	8	854	**19**	7385	14	**10995**
Donor 3	**61**	**18960**	36	8100	33	5975	17	6265
MEAN (SEM)	**40 (12)**	**8815 (5809)**	19 (9)	3808 (2196)	27 (4)	6577 (420)	17 (2)	7055 (2084)

[1] Gene transfer efficiency in human PBL, as determined by flow cytometry on day 6 after transduction.

[2] IFN-γ 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.