Supplemental Figure S1

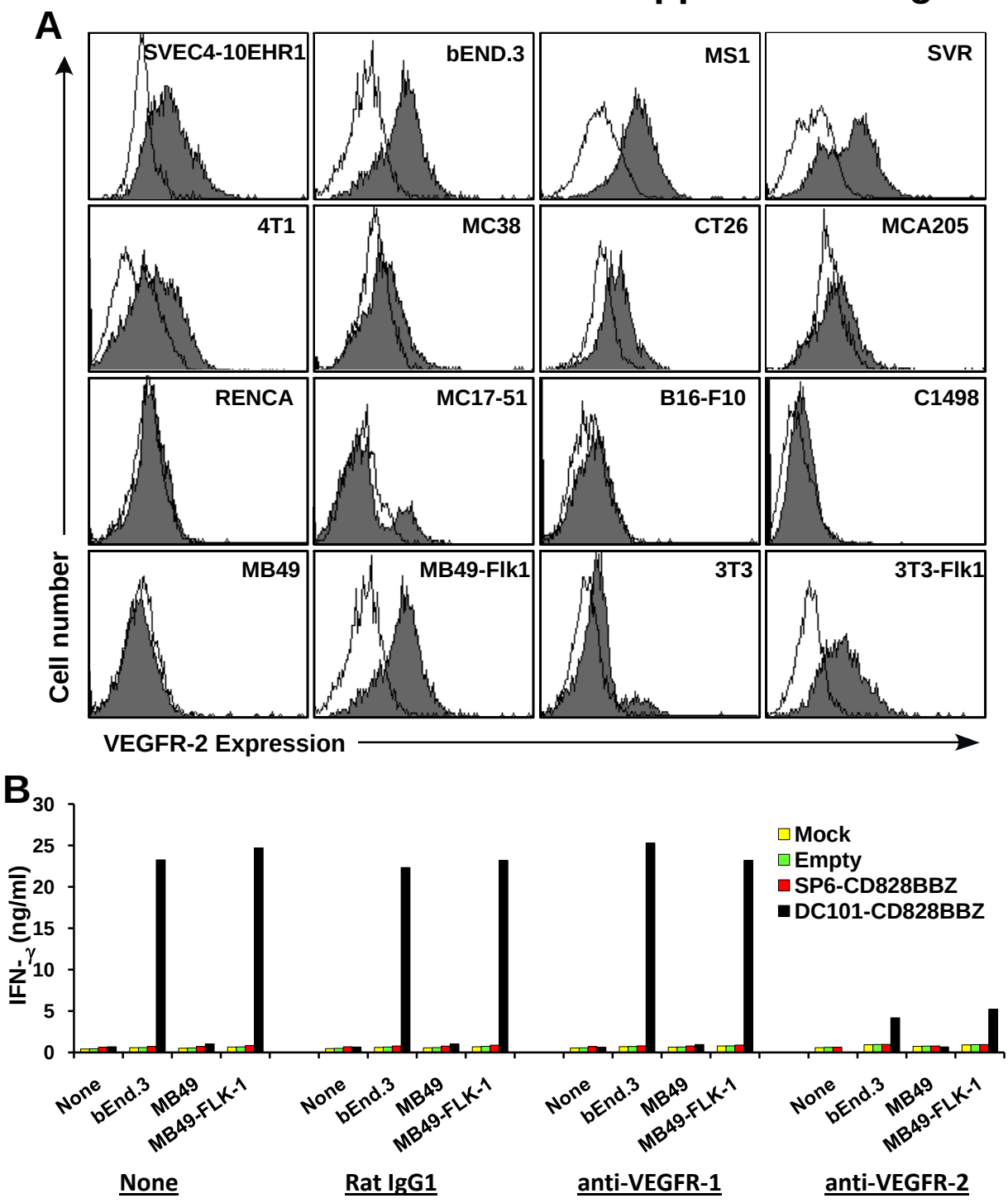

Figure S1

VEGFR-2 CAR transduced T cells specifically bind to VEGFR-2 on endothelial cells (**A**) Detection of cell surface expression of VEGFR-2 on mouse endothelial cells and tumor cells. Indicated mouse cell lines were incubated with PE-labeled rat anti-mouse antibody (BD biosciences) or isotype control antibody rat IgG2a, κ and analyzed by FACS. Filled histogram, VEGFR-2 specific staining; open histogram, staining with IgG2a, κ. Results shown are representative of two experiments. (**B**) Specificity of Indicated target cells were incubated with 10 μg/ml of either rat IgG1, anti-mouse VEGFR-1 antibody, or the anti-mouse VEGFR-2 antibody (DC101) at 37°C for 1 hour and then cocultured with empty vector, SP6-CD828BBZ CAR, or DC101-CD828BBZ encoding retroviral vector or mock transduced primary mouse T cells. Supernatants were harvested 24 hours later and assessed for IFN-γ by ELISA.

Supplemental Figure S2

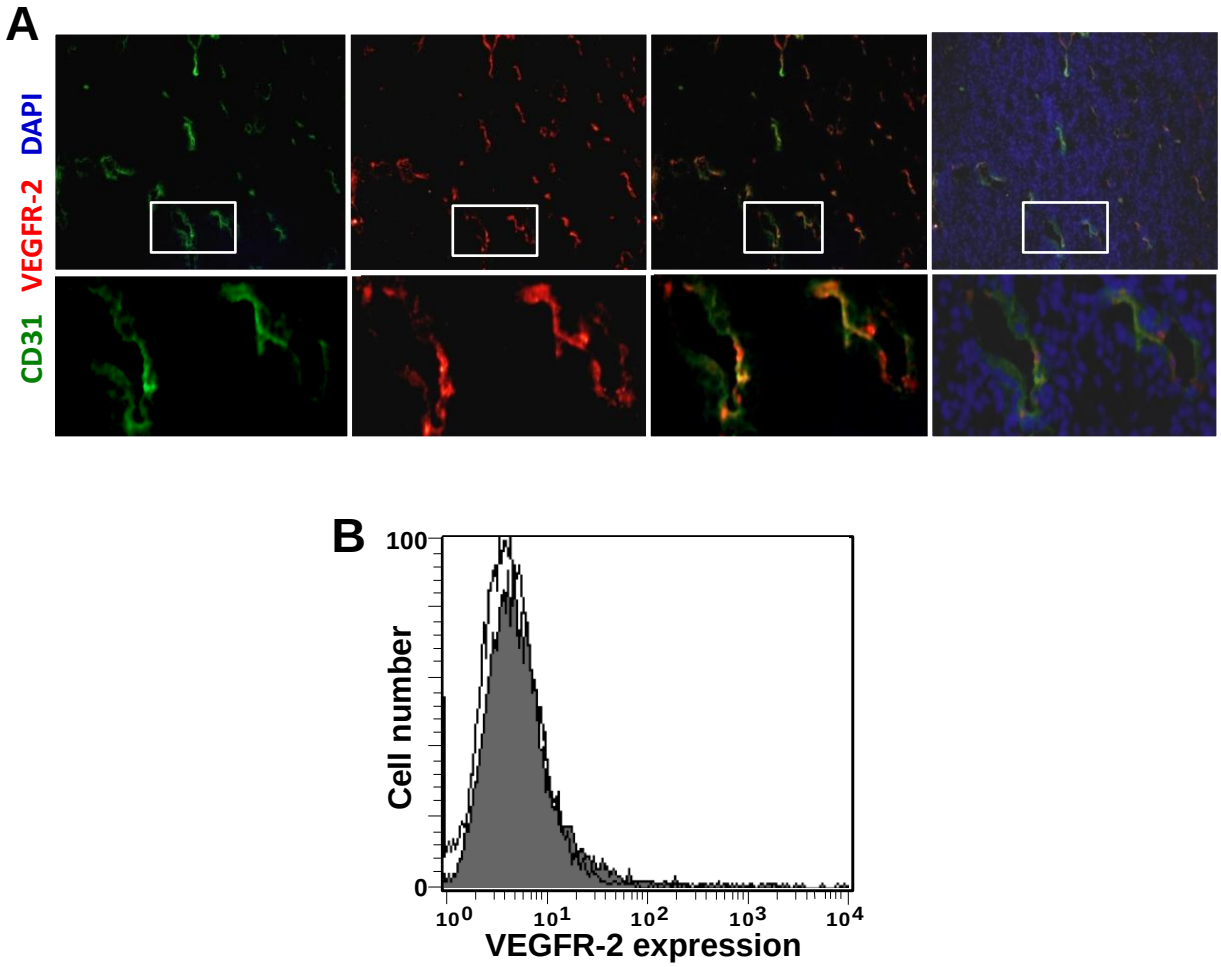

Figure S2

VEGFR-2 expression is restricted to endothelial cells in the tumor vessels of subcutaneous B16-F10 melanoma in C57BL/6 mice. (**A**) Immunofluoreseence analysis of VEGFR-2 expression in B16-F10 tumor. Tumor samples were obtained from C57BL/6 mice bearing 12 days-old subcutaneous B16-F10 tumors. Tumor sections were stained with FITC-conjugated antibody against the endothelial cell marker CD31 expressed on tumor vessels (green) or PE-labeled antibody against VEGFR-2 (red) together with DAPI (blue) to show the nucleus and analyzed using fluorescence microscopy. Yellow represents areas of colocalization of CD31 cell (green) and VEGFR-2 (red) on tumor vessels (10x). Boxed areas in the upper panel of the figure are presented in the bottom panel at higher magnification (40x). (**B**) FACS analysis of VEGFR-2 expression on B16-F10 tumor tissue. Single cell suspensions were prepared from tumor tissues obtained from C57BL/6 mice bearing 12 days-old subcutaneous B16-F10 tumor and analyzed for cell surface expression of VEGFR-2 by flow cytometry using a PE-labeled rat antibody against mouse VEGFR-2. Filled histogram, VEGFR-2 specific staining; open histogram, staining with the rat IgG2a, κ isotype control antibody. Results shown are representative of two experiments.

Supplemental Figure S3

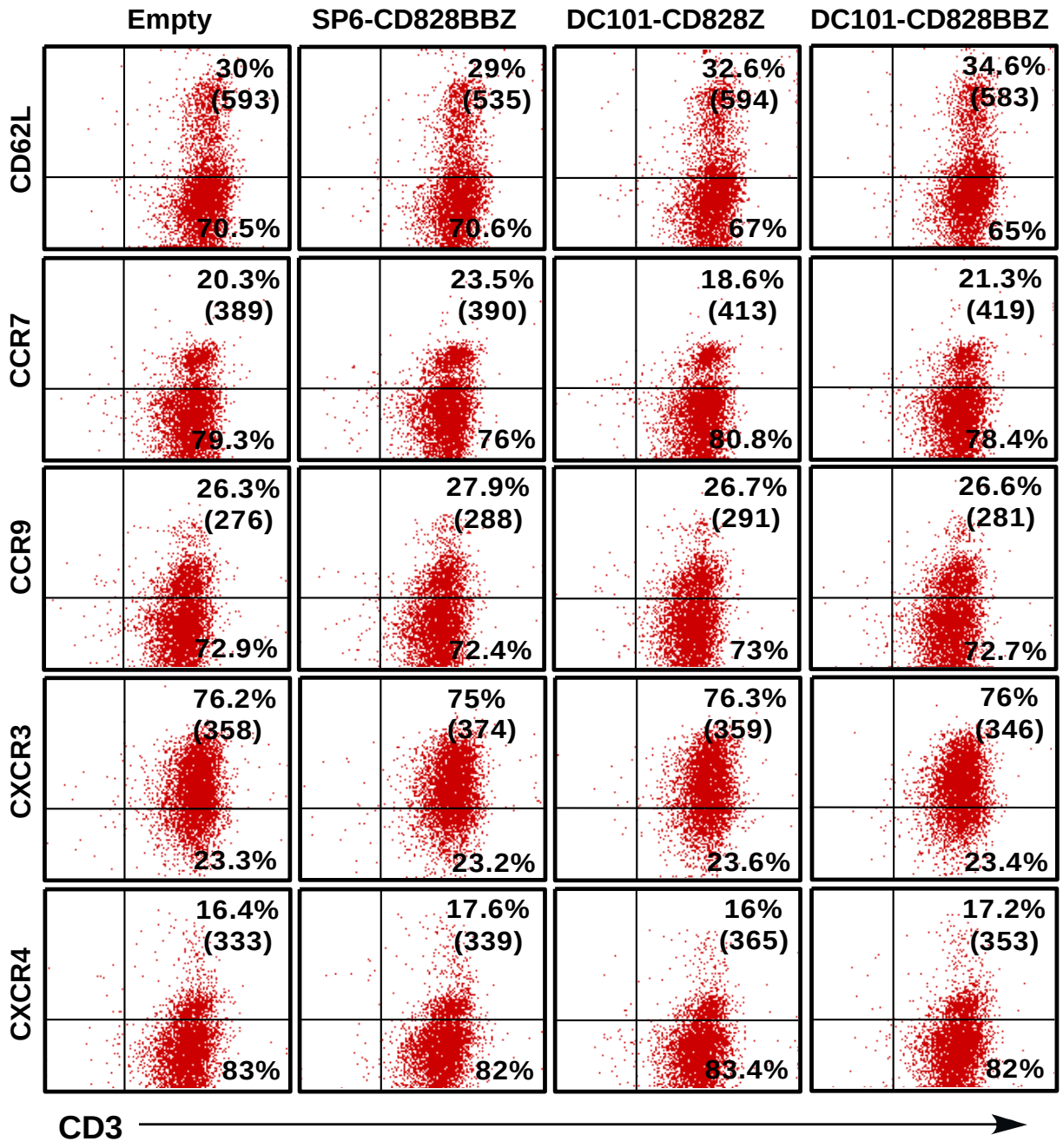

Figure S3

Phenotypic analysis of chemokine receptors expression on transduced mouse T cells. Enriched splenic $CD3^+$ T cells were transduced with the indicated retroviral vectors shown in the Figure. Five days after transduction cells were analysed by FACS for cell surface expression of CD62L molecule and indicated chemokine receptors that are known to be involved in homing and/or efficient trafficking of T cells in vivo. Representative FACS data showing the percentage of T cells in each quadrant with MFI of expression in parentheses are presented.

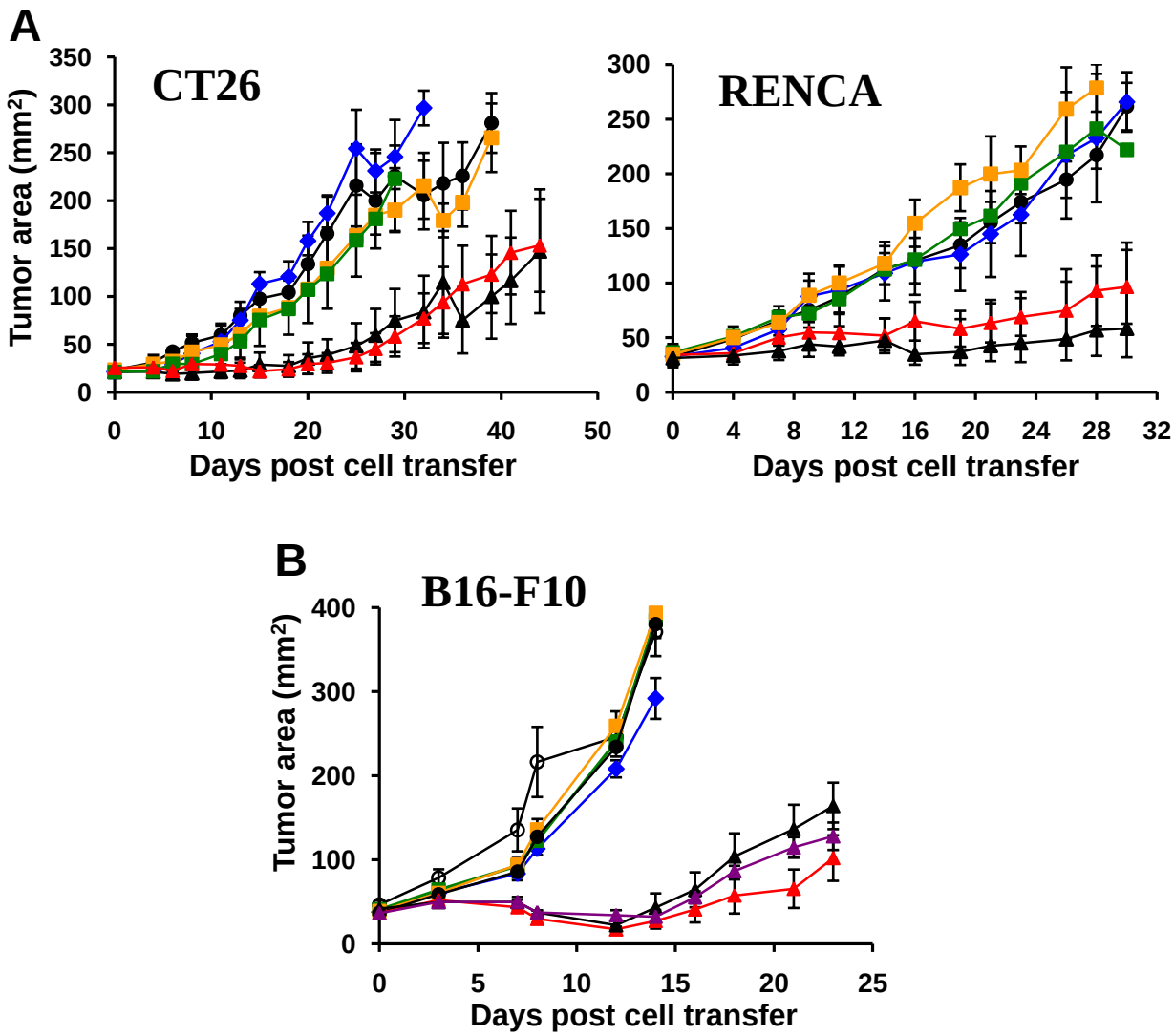

Figure S4

Purified CD8⁺ T cells transduced with DC101-CAR inhibited the tumor growth without any toxicity in BALB/c mice bearing established syngeneic tumors. BALB/c mice bearing established subcutaneous CT26 or RENCA tumors were sublethally irradiated at 5 Gy TBI and treated with a single dose of $5x10^6$ unseparated $CD3^+$ T cells transduced with the DC101-CD828BBZ (red triangles) or an empty vector (green squares) or $2x10^7$ purified $CD8^+$ T cells transduced with either DC101-CD828BBZ (black triangles) or an empty vector (orange squares). All T cell treatment groups received 2 daily doses of rhIL-2 for 3 days. Control groups received rhIL-2 alone (blue diamonds) or none (black circles). (**B**) C57BL/6 mice bearing 12 days-old subcutaneous B16-F10 melanoma were sublethally irradiated at 5 Gy TBI and treated with a single dose of $2x10^7$ unseparated $CD3^+$ T cells transduced with the DC101-CD828BBZ (red triangles) or an empty vector (green squares) or $2x10^7$ purified $CD8^+$ T cells transduced with either DC101-CD828BBZ (black triangles) or an empty vector (orange squares). Some groups received a mixture of 1e7 $CD4^+$ T cells and 1e7 $CD8^+$ T cells transduced with DC101-CD828BBZ (purple triangles) or an empty vector (purple squares). All T cell treatment groups received 2 daily doses of rhIL-2 for 3 days. Control groups received rhIL-2 alone (blue diamonds) or no treatment (black circles).

PRIMER	SEQUENCES
1121S1	GTGGGGGTCCTCGAGGCCA
1121S2	CCATGGACTTCCAGGTGCAGATCTTCTCTTTCCTGCTG
1121S3	ATTAGTGCCTCCGTCATCATGTCCAGAGGCGATATCCA
1121S4	GATGACACAGTCCCCCAGTTCAGTGTCCGCGTCCATCG
1121S5	GGGACCGTGTCACCATTACCTGTCGCGCCAGTCAAGGC
1121S6	ATCGACAACTGGCTGGGGTGGTACCAGCAAAAGCCAGG
1121S7	GAAGGCGCCAAAACTGCTGATCTATGATGCGAGCAACC
1121S8	TCGACACTGGAGTCCCCAGTAGGTTCTCCGGATCGGGG
1121S9	TCCGGCACGTACTTCACCTTGACCATCTCCAGCCTCCA
1121S10	GGCCGAGGATTTCGCTGTTTACTTCTGCCAGCAGGCAA
1121S11	AAGCGTTTCCGCCCACCTTCGGCGGGGGCACCAAGGTG
1121S12	GACATAAAAGGCAGTACCAGCGGATCCGGCAAGCCGGG
1121S13	CTCTGGCGAAGGTTCCGAAGTCCAGTTGGTGCAGTCCG
1121S14	GTGGTGGCCTGGTCAAGCCGGGTGGGAGTCTCAGGCTC
1121S15	TCCTGCGCCGCTTCAGGGTTCACATTCTCTTCGTATTC
1121S16	CATGAACTGGGTGCGCCAGGCGCCAGGCAAGGGGCTGG
1121S17	AGTGGGTCAGTTCGATTTCAAGTTCGTCCTCCTACATC
1121S18	TATTACGCCGACTCAGTCAAGGGCCGCTTCACAATCTC
1121S19	CAGAGACAACGCTAAGAACTCGCTGTACCTCCAAATGA
1121S20	ACTCGCTGCGCGCCGAAGACACCGCCGTGTACTATTGC
1121S21	GCGAGAGTGACTGATGCCTTCGACATATGGGGCCAGGG
1121S22	AACGATGGTGACCGTGAGTAGTGCGGCCGCAAGAGATC
1121AS1	GATCTCTTGCGGCCGCACT
1121AS2	ACTCACGGTCACCATCGTTCCCTGGCCCCATATGTCGA
1121AS3	AGGCATCAGTCACTCTCGCGCAATAGTACACGGCGGTG
1121AS4	TCTTCGGCGCGCAGCGAGTTCATTTGGAGGTACAGCGA
1121AS5	GTTCTTAGCGTTGTCTCTGGAGATTGTGAAGCGGCCCT
1121AS6	TGACTGAGTCGGCGTAATAGATGTAGGAGGACGAACTT
1121AS7	GAAATCGAACTGACCCACTCCAGCCCCTTGCCTGGCGC
1121AS8	CTGGCGCACCCAGTTCATGGAATACGAAGAGAATGTGA
1121AS9	ACCCTGAAGCGGCGCAGGAGAGCCTGAGACTCCCACCC
1121AS10	GGCTTGACCAGGCCACCACCGGACTGCACCAACTGGAC
1121AS11	TTCGGAACCTTCGCCAGAGCCCGGCTTGCCGGATCCGC
1121AS12	TGGTACTGCCTTTTATGTCCACCTTGGTGCCCCCGCCG
1121AS13	AAGGTGGGCGGAAACGCTTTTGCCTGCTGGCAGAAGTA
1121AS14	AACAGCGAAATCCTCGGCCTGGAGGCTGGAGATGGTCA
1121AS15	AGGTGAAGTACGTGCCGGACCCCGATCCGGAGAACCTA
1121AS16	CTGGGGACTCCAGTGTCGAGGTTGCTCGCATCATAGAT
1121AS17	CAGCAGTTTTGGCGCCTTCCCTGGCTTTTGCTGGTACC
1121AS18	ACCCCAGCCAGTTGTCGATGCCTTGACTGGCGCGACAG
1121AS19	GTAATGGTGACACGGTCCCCGATGGACGCGGACACTGA
1121AS20	ACTGGGGGACTGTGTCATCTGGATATCGCCTCTGGACA
1121AS21	TGATGACGGAGGCACTAATCAGCAGGAAAGAGAAGATC
1121AS22	TGCACCTGGAAGTCCATGGTGGCCTCGAGGACCCCCAC

Figure S5

Primers used to synthesis KDR1121 scFv. The KDR ScFv containing the mouse IgG kappa chain leader sequence followed by the codon optimized V_L and V_H sequences derived from KDR-1121 antibody linked by a 218 bp linker was designed using a web-based DNA codon optimization algorithm software and synthesized using the primer sequences shown in the figure.

Supplemental Table S1

Table S1. Retroviral mediated expression of chimeric antigen receptors (CAR) in primary mouse T lymphocytes

		DC101-CD8	DC101-CD828Z	DC101-CD828BBZ	SP6-CD828BBZ
% Tdxn	**$CD3^+CD4^+$**	83.2 ± 1.9	84 ± 1.7	79.0 ± 4.5	84.8 ± 2.7
	$CD3^+CD8^+$	80.8 ± 2.1	86 ± 1.6	78.2 ± 5.1	84.2 ± 3.9
MFI	**$CD3^+CD4^+$**	114.3 ± 7.8	95 ± 7.3	76.5 ± 4.9	86.8 ± 6.1
	$CD3^+CD8^+$	108.5 ± 9.4	92 ± 6.2	75.8 ± 5.6	83.5 ± 4.8

Table S1. Transduction efficiency of primary mouse T lymphocytes by CAR-expressing retroviral vectors. Enriched mouse splenic $CD3^+$ T cells were transduced with indicated retroviral vectors. Transduction efficiency and level of expression of CAR in transduced mouse T cells were determined on day 4 post-transduction by flow cytometry and presented as percent transduction (% Tdxn) and mean fluorescent intensity of expression (MFI) in the $CD4^+$ and $CD8^+$ T cell subsets. Data shown are mean SEM derived from 5 different experiments.