Supporting Information

Schmidt et al. 10.1073/pnas.1009069108

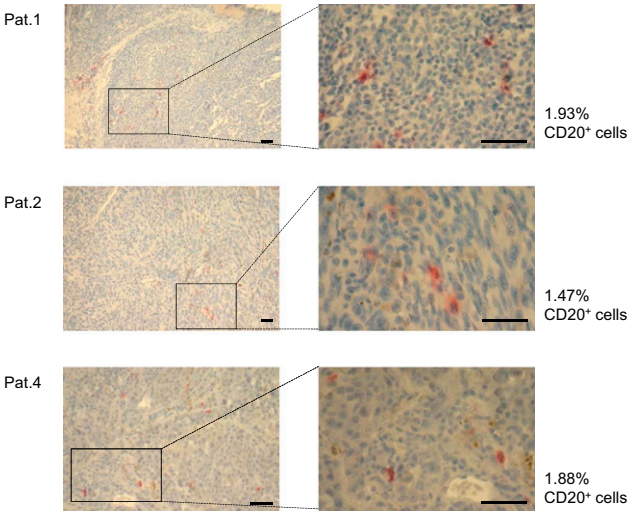

Fig. S1. Detection of CD20$^+$ cells in melanoma biopsies. Cryostat sections of melanoma biopsies were stained with the monoclonal anti-CD20 antibody L27 followed by the HRP-conjugated secondary antibody and visualized with AEC as substrate. Slides were counterstained with hematoxylin and the frequencies of CD20$^+$ cells were determined. A minimum of 1,000 melanoma cells were counted. (Scale bar, 10 μm.)

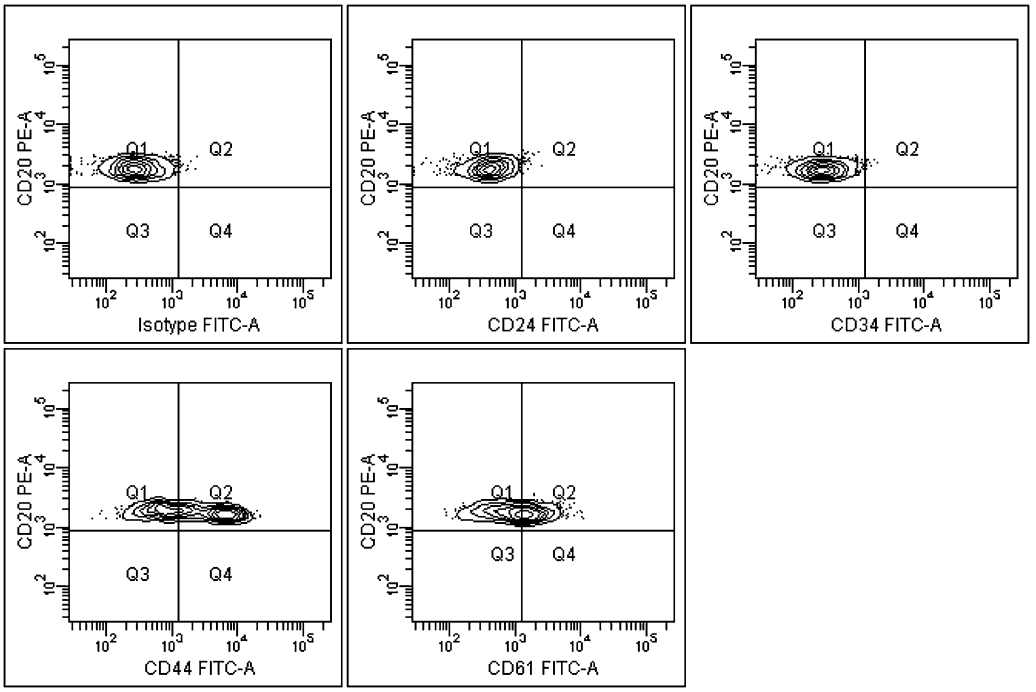

Fig. S2. Coexpression of CD20 and stem cell markers on melanosphere cells. Melanoma cells derived from patient 1 were cultivated in human embryonic stem cell medium to form melanospheres as described (1). Cells were stained with the PE-conjugated anti-CD20 mAb L27 and with FITC-conjugated anti-CD24, anti-CD34, anti-CD44, and anti-CD61 mAbs, respectively, and analyzed by flow cytometry. Staining with an isotype-matched antibody served as control. Expression of the respective stem cell marker was recorded for CD20$^+$ melanoma cells.

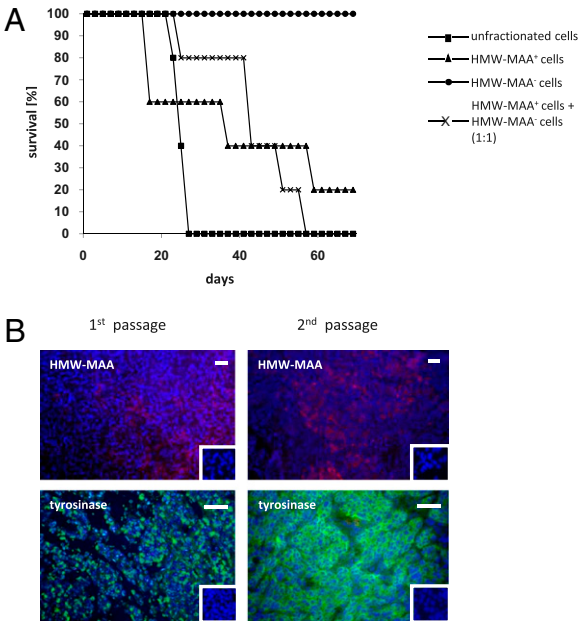

Fig. S3. Melanoma formation in immunodeficient mice. Melanoma cells from a patient-1 tumor lesion were flow-cytometry sorted into HMW-MAA$^+$ and HMW-MAA$^-$ cells using the PE-conjugated anti–HMW-MAA antibody EP-1. Cells of each subfraction as well as unfractionated cells (1×10^6 cells each) and a 1:1 reconstituted population of HMW-MAA$^+$ and HMW-MAA$^-$ cells (0.5×10^6 cells each), respectively, were s.c. injected into NIH-III mice ($n = 5$). (*A*) Overall survival of treated mice is presented in a Kaplan–Meier plot. (*B*) Sorted HMW-MAA$^+$ melanoma cells from first passage tumors established second passage tumors upon transplantation into NIH-III mice, whereas HMW-MAA$^-$ cells did not induce tumors. Melanomas from first and second passage were reanalyzed with respect to HMW-MAA and tyrosinase expression. Induced melanomas exhibited the same heterogeneous HMW-MAA expression as the parental tumor of the patient (Fig. 1*B*).

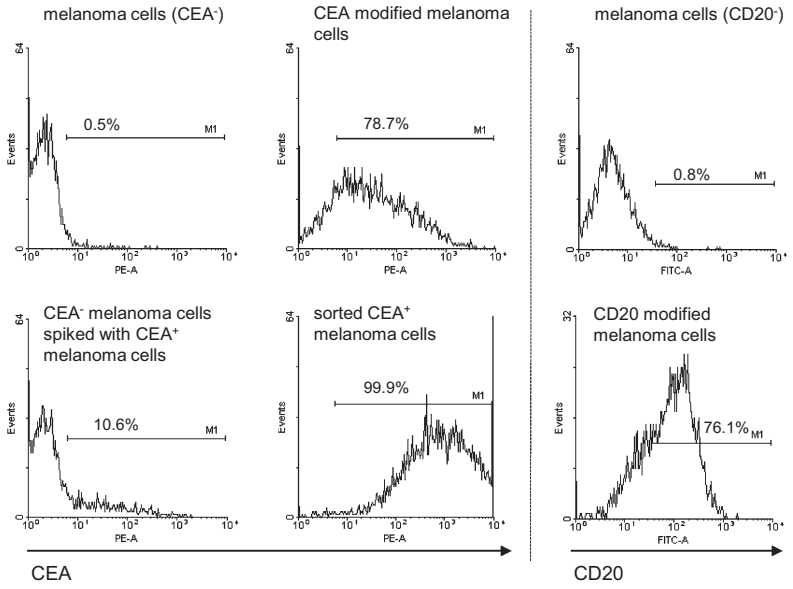

Fig. S4. Generation of the CEA$^+$ melanoma cell subpopulation. Melanoma cells from tumor lesions that lack carcinoembryonic antigen (CEA, CD66e) expression were retrovirally transduced to express CEA. CEA expression was confirmed by flow cytometry using the anti-CD66ε (CEA) antibody followed by PE-conjugated secondary antibody staining. CEA$^+$ melanoma cells were sorted to homogeneity using anti-PE MicroBeads as described in *Materials and Methods*. Unmodified melanoma cells from the same biopsy were spiked with modified, CEA$^+$ melanoma cells making up to 10% CEA$^+$ melanoma cells.

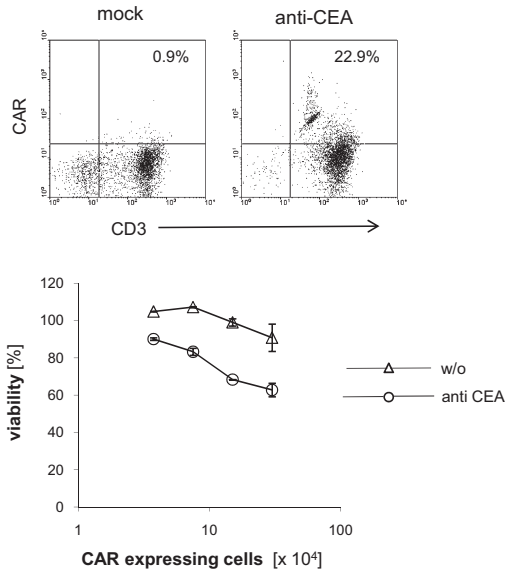

Fig. S5. Modified T cells with a CEA-specific CAR eliminate engineered CEA$^+$ melanoma cells. T cells from a healthy volunteer were engineered to express the CEA-specific CAR as monitored by flow cytometry using a PE-conjugated antihuman IgG1 mAb, which binds to the CAR extracellular IgG1 CH2CH3 spacer region. Mock-transduced T cells were used as controls. CAR-engineered T cells and unmodified T cells (w/o) were coincubated in serial dilutions with CEA-transfected melanoma cells (3×10^5 cells) for 24 h. Viability of tumor cells was recorded using the XTT-based viability assay.

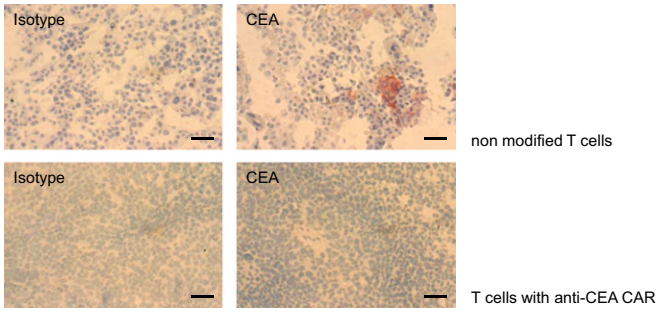

Fig. S6. Elimination of the spiked CEA$^+$ melanoma cell subpopulation from tumor lesions by anti-CEA CAR T cells. Engineered CEA$^+$ melanoma cells were spiked to nonmodified melanoma cells from the same biopsy (Fig. S4) and s.c. injected into immunodeficient mice for tumor formation. When tumors were grown to about ~20 mm^3, engineered T cells with CEA-specific CAR (1×10^6 CAR-expressing T cells) or nonmodified T cells were i.v. injected on 3 consecutive days. Tumors progressed during the following weeks. Mice were killed and tumors were recorded for CEA expression using the anti-CD66ε (CEA) antibody and staining with the HRP-conjugated secondary antibody and AEC as substrate. Staining with isotype antibodies served as controls. No CEA$^+$ melanoma cells were detected in spiked tumors treated with anti-CEA CAR T cells. Slides were counterstained with hematoxylin. (Scale bar, 10 μm.)

1. Fang D, et al. (2005) A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 65:9328–9337.