Supporting Information
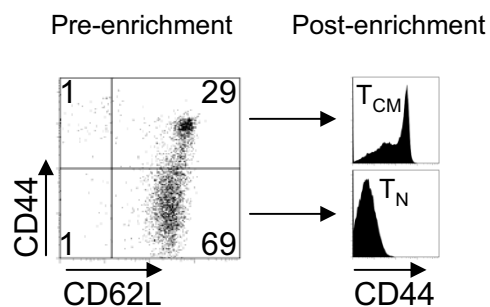
Hinrichs et al. 10.1073/pnas.0907448106

Fig. S1. CD8+ T cells enriched for naïve (CD62L+ CD44low) or central memory (CD62L+ CD44high) phenotype can be isolated from the spleens of pmel-1 mice. Flow cytometric analysis was performed on splenocytes from a 6-week-old pmel-1 mouse. The dot plot is gated on CD8+ lymphocytes. The histograms show expression of CD44 by the two subsets following enrichment. The experiment was independently repeated five times with similar results.
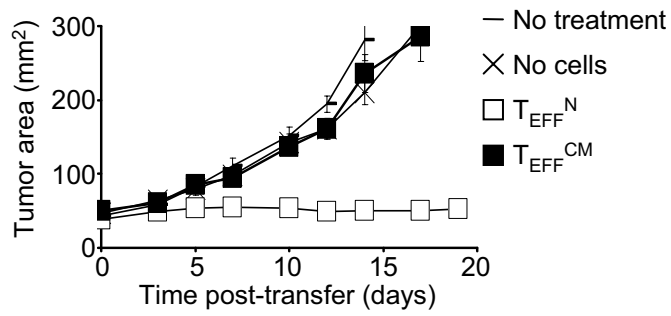
Fig. S2. Validation by real time RT-PCR of selected gene expression differences observed on microarray. Data shown are representative of at least two independent experiments.

Fig. S3. Adoptive transfer of effector cells generated directly from naïve cells improves tumor treatment. 2×10^7 effector cells generated by secondary stimulation were infused. Adjuvant vaccine and IL-2 were administered to all except the ''No treatment'' group. The standard error of the mean is indicated by the error bars. ($n = 5$ mice per treatment group). T_{EFF}^N versus T_{EFF}^{CM}, $P < 0.001$. The experiment was independently repeated with similar results.
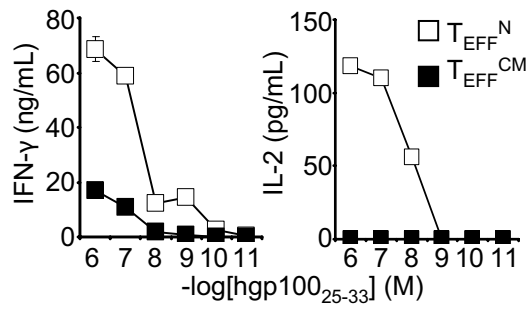
Fig. S4. Following infusion, T_{EFF}^{N} maintain greater production of IFN-γ and IL-2 than T_{EFF}^{CM}. Effector cells generated by secondary stimulation were adoptively transferred then reisolated from spleens 6 days following infusion. Cell numbers were equalized and production of IFN-γ and IL-2 in overnight coculture assays was determined. Error bars indicate the standard error of the mean. Experiment was independently repeated with similar results.

Fig. S5. Effector cells derived from naïve cells possess greater potential for clonal expansion. (*A* and *B*) The number of transferred cell present in the spleens and tumor draining lymph nodes, respectively, of mice treated with T_{EFF}^N or T_{EFF}^{CM} generated by secondary stimulation. Four mice per group and time point were analyzed. Error bars indicate the standard error of the mean. (*C*) The frequency of transferred cells in recipient spleens displaying the KLRG1^{hgih} phenotype 5 days following infusion. The transverse lines represent the means. $P < 0.0001$. Data shown are representative of at least two independent experiments.

Fig. S6. Effector cells derived from naïve or central memory cells persist in recipient mice. (*A*) The number of effector cells generated by primary stimulation present in the spleens of nontumor bearing recipient mice 30 days following infusion. Totals of 8×10^5 cells were initially transferred. $P = 0.1757$. (*B*) The number of effector cells generated by secondary stimulation present in the spleens of recipient mice 30 days following infusion. A total of 8.2×10^6 cells was administered. $P = 0.3911$. Sublethal irradiation, vaccine, and IL-2 were given for both experiments. The transverse lines represent the means for each group. Data shown are representative of at least two independent experiments.

Table S1. Differential expression of selected genes with described function in T cells

Fold Δ*	Gene product	Function
233	IL-2	Cytokine, activation and differentiation
27	Integrin-α_E	Adhesion molecule, mucosal homing
26	Slamf1	Costimulation
25	Ox40	Costimulation
16	Hivep3	Transcription factor, IL-2 regulation
8	Stat5a	Transcription factor, cytokine signaling
−5	CD62L	Adhesion molecule, lymphoid homing
−6	Eomes	Transcription factor, effector function
−14	Klrg1	Inhibitory receptor, senescence

*Increased expression T_{EFF}^N