Supporting Information

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SI Materials and Methods

Micro-PET/Computed Tomography Imaging. Mice were anesthetized with isoflurane at 2% and injected i.v. with 7.4 MBq (200 μCi) of [^{18}F]FHBG. One hour after, mice were scanned using a FOCUS 220 micro-PET scanner (Siemens) (energy window of 350–750 keV and timing window of 6 ns) as described previously (1). Image reconstruction was obtained using an iterative maximum a posteriori algorithm (1). The scan time was 15 min, with 10^6 events per acquisition. List-mode data were sorted into sinograms by Fourier rebinning and reconstruction performed with an algorithm based on ordered-subset expectation maximization iterative reconstruction without scatter or attenuation correction. Measurement of radioactive tracer amount in tumors or lymphoid organs was performed by drawing regions of interest (ROI) and obtaining the percentage injected dose per gram of tissue (%ID/g) from the PET image data with Amide (a medical imaging data examiner) software by use of a system calibration factor determined from an ^{18}F-filled mouse-size phantom. Specific signal quantification above the background was obtained by calculating the ratio of %ID/g in the ROI to %ID/g of a background region of the animal. Due to abdominal background caused by the predominantly hepatobiliary routes of [^{18}F]FHBG elimination, digital cropping to remove the very hot bladder, intestines, and gallbladder was done for image display. In the same session, mice were injected i.v. with Fenestra LC contrast agent (Advanced Research Technologies) and computed tomography (CT) images were acquired on a micro-CAT II instrument (Siemens). Images were reconstructed tridimensionally with the Feldkamp cone-beam filtered backprojection algorithm provided by the manufacturer. Total CT acquisition time was 10 min, and the spatial resolution was 250 μm.

1. Shu CJ, et al. (2009) Quantitative PET reporter gene imaging of CD8+ T cells specific for a melanoma-expressed self-antigen. *Int Immunol* 21:155–165.

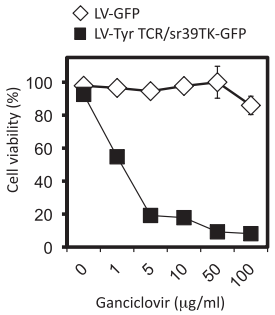


Fig. S1. Functionality of sr39tk in transduced cells. In vitro ganciclovir lysis assay was performed by adding titrated amounts of ganciclovir to 293T-CD3$^+$ cells transduced with negative control LV-GFP or LV-Tyr TCR/sr39TK-GFP vectors. Viable cells were analyzed by MTS assay.

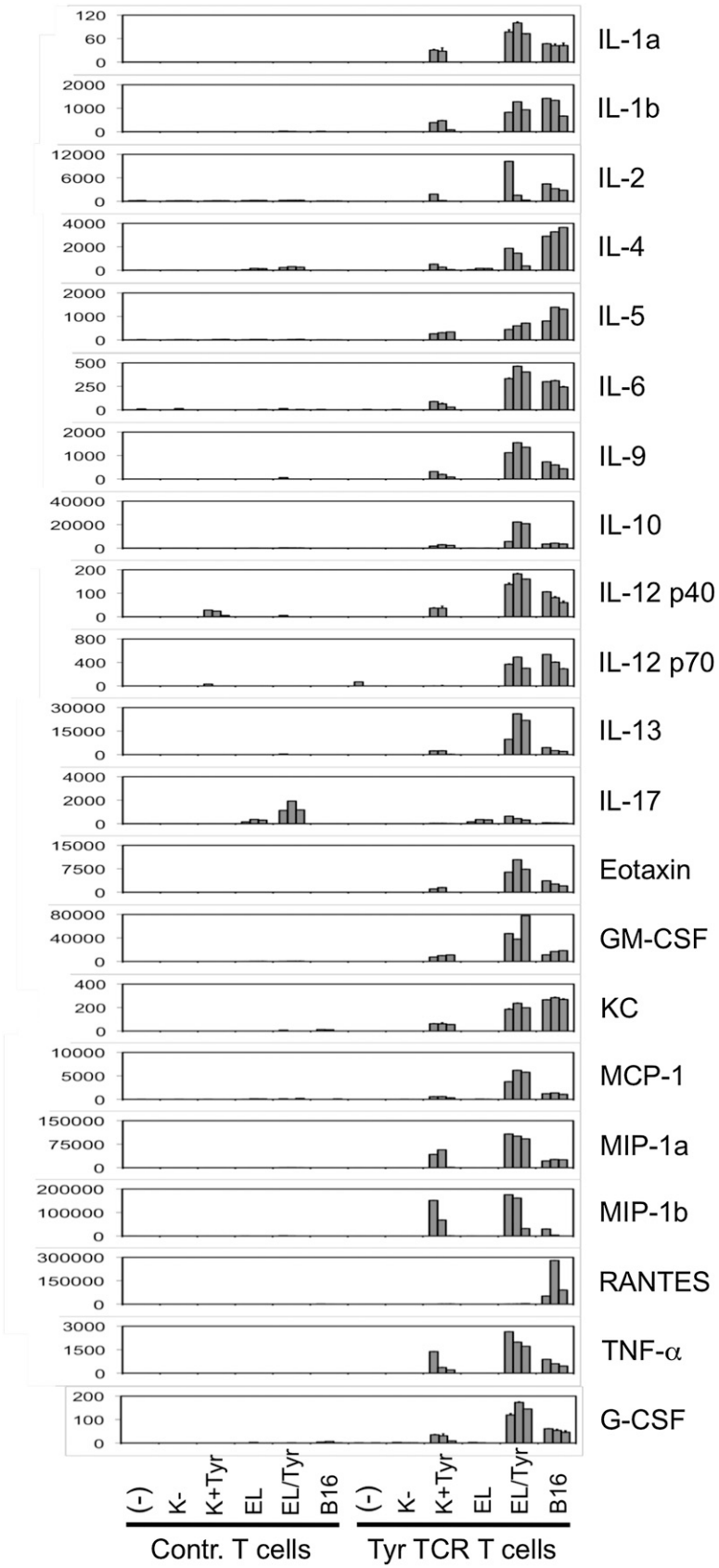


Fig. S2. Extended cytokine panel of 24, 48, and 72 h supernatant collections (first, second, and third bars for each group, respectively) in pg/mL of control T cells or tyrosinase TCR-transduced T cells coincubated with: (–), no cells; K^-, unpulsed K562-A2.1; K^+Tyr, tyrosinase$_{368–376}$ peptide-pulsed K562-A2.1; EL, EL4-A2/K^b; EL/Tyr, EL4-A2/K^b expressing tyrosinase; or B16, B16-A2/K^b cells.

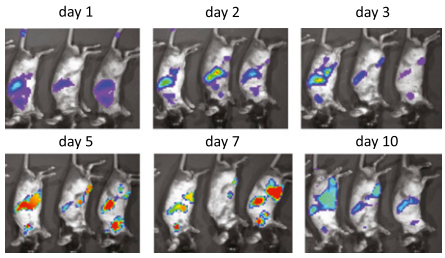


Fig. S3. Bioluminescence imaging of T cell trafficking in vivo. HLA-A2/K^b transgenic mice with inguinal s.c. EL4-A2/K^b-expressing tyrosinase (*Left*) and control EL4-A2/K^b (*Right*) tumors received the full protocol of adoptive cell transfer (ACT) with tyrosinase TCR/fLuciferase-transduced T cells. Mice were followed from day 1 to 10 post-ACT and bioluminescence signal of *Left* lateral views were recorded.

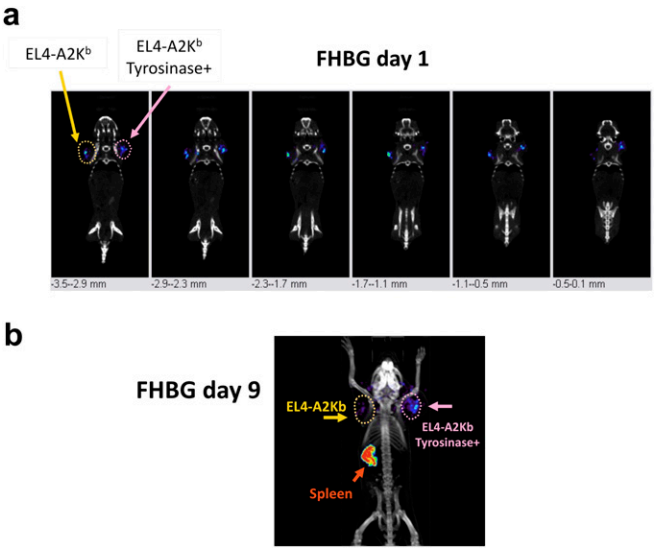
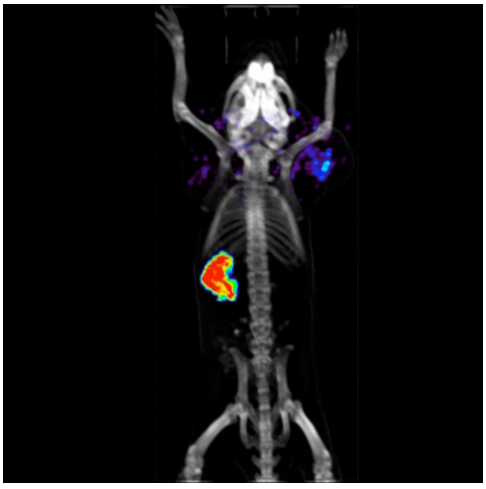


Fig. S4. PET CT imaging of T cell trafficking in vivo in mice with bilateral isogenic EL4-A2/K^b tumors with or without tyrosinase expression. (*A*) HLA-A2/K^b transgenic mice with thoracic dorsal s.c. EL4-A2/K^b-expressing tyrosinase (*Right*) and control EL4-A2/K^b (*Left*) tumors received the full protocol of adoptive cell transfer (ACT) with Tyr TCR/sr39TK/GFP-transduced T cells. On day 1 post-ACT, mice were injected with [^{18}F]FHBG and images captured through a series of microPET/CT scans. Specific signal quantification ratio above background: *Left* ROI = 1.47 ± 0.11%ID/g; *Right* ROI = 1.83 ± 0.15%ID/g. (*B*) Reconstructed tridimensional PET CT scan image at day 9 with the nonspecific signal from abdominal excretion of [^{18}F]FHBG subtracted from the final image. Specific signal quantification ratio above background: *Left* ROI = 1.69 ± 0.13%ID/g; *Right* ROI = 6.92 ± 0.5%ID/g.



Movie S1. Reconstructed tridimensional PET CT scan. HLA-A2/K^b transgenic mouse with thoracic dorsal s.c. EL4-A2/K^b-expressing tyrosinase (*Right*) and control EL4-A2/K^b (*Left*) tumors received the full protocol of adoptive cell transfer (ACT) with Tyr TCR/sr39TK/GFP-transduced T cells. PET CT scan at day 9 with [^{18}F] FHBG tracer injection.

Movie S1