Supporting Information

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SI Text

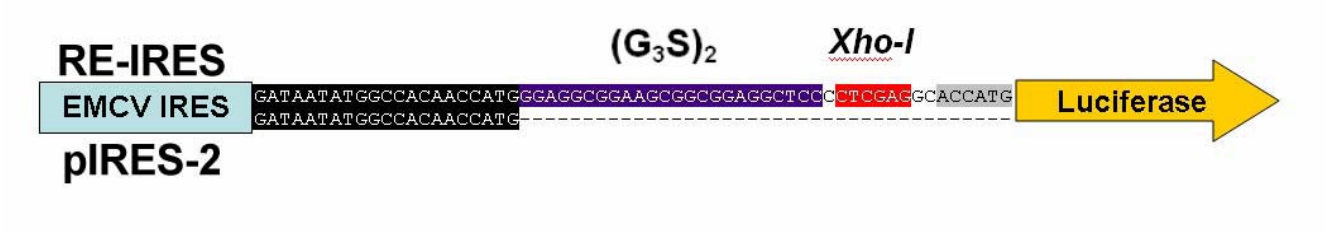

Fig. S1. Construction of the re-engineered (RE-IRES) EMCV IRES. pIRES-2 (Clontech) was modified to include a $(G_3S)_2$ linker (blue) and a *Xho-I* site (red) in frame with the native EMCV preferred transcriptional start site (immediately 5′ of the $(G_3S)_2$ linker).

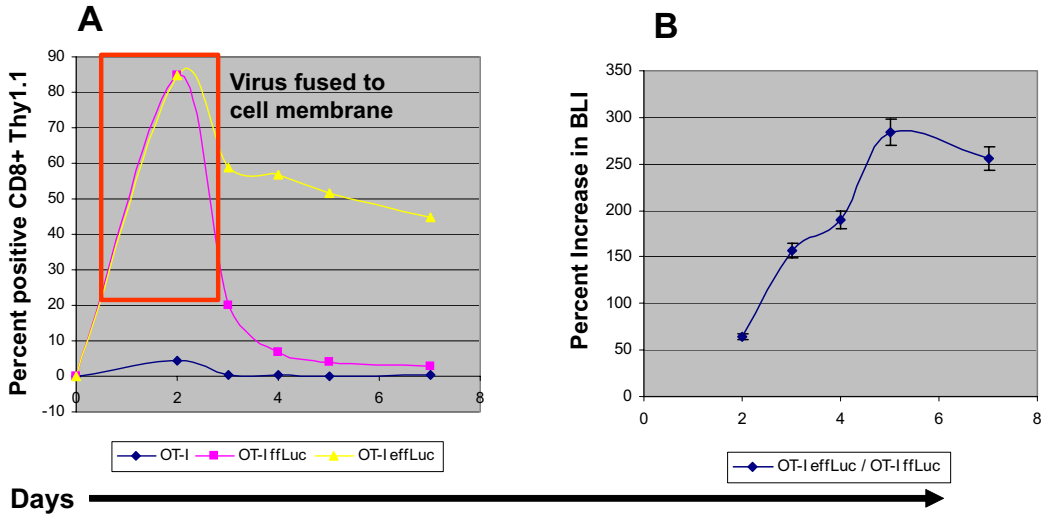

Fig. S2. Kinetics of Thy1.1 expression and firefly luciferase activity. OT-I T cells were transduced with v-ffLuc, v-effLuc or mock-transduced (as indicated) 24 h after activation. Thy1.1 expression (*A*) and bioluminescent activity (*B*) were measured for 7 days via flow cytometry and the *in vitro* bioluminescent assay (described in *Methods*), respectively. Early Thy1.1 expression (days 2–3) is caused by fusion of virus to target cell plasma membranes (pseudotransduction) and not true expression. Bioluminescent activity is expressed as the percentage difference of luminescence recorded from 1×10^5 effLuc-transduced OT-I T cells versus 1×10^5 ffLuc-transduced OT-I T cells.

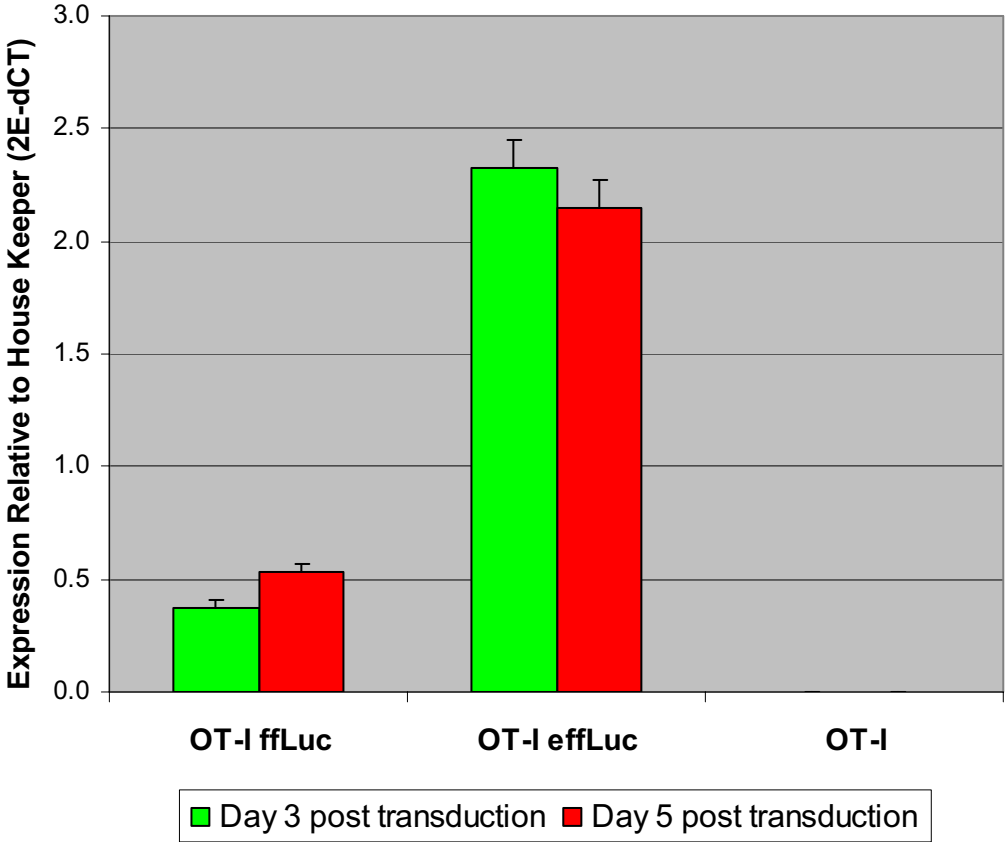

Fig. S3. Assessment of viral integration in OT-I T cells transduced with v-ffLuc or v-effLuc. OT-I T cells were transduced with v-ffLuc or v-effLuc (or mock-transduced; as indicated) and total DNA isolated via DNeasy (Qiagen) 3 (green bars) and 5 (red bars) days after transduction. Taqman quantitative PCR was performed to assess the degree of ffLuc and effLuc integration. For ffLuc the 5′ primer, 3′ primer and probe were TTTGAAGAAGAGCTGTTTCTGAGGAG, CACCAGCAGCGCACTTT and FAM-CCTTCAGGATTACAAGATTC, respectively. For effLuc the 5′ primer, 3′ primer and probe was TGCACAAGGCCATGAAGAGATA, AAATACTCGGCGTAGGTGATGTC and CACCATCGCCTTCACCG, respectively. The house keeping gene was mouse β-actin (VIC; Applied Biosystems).

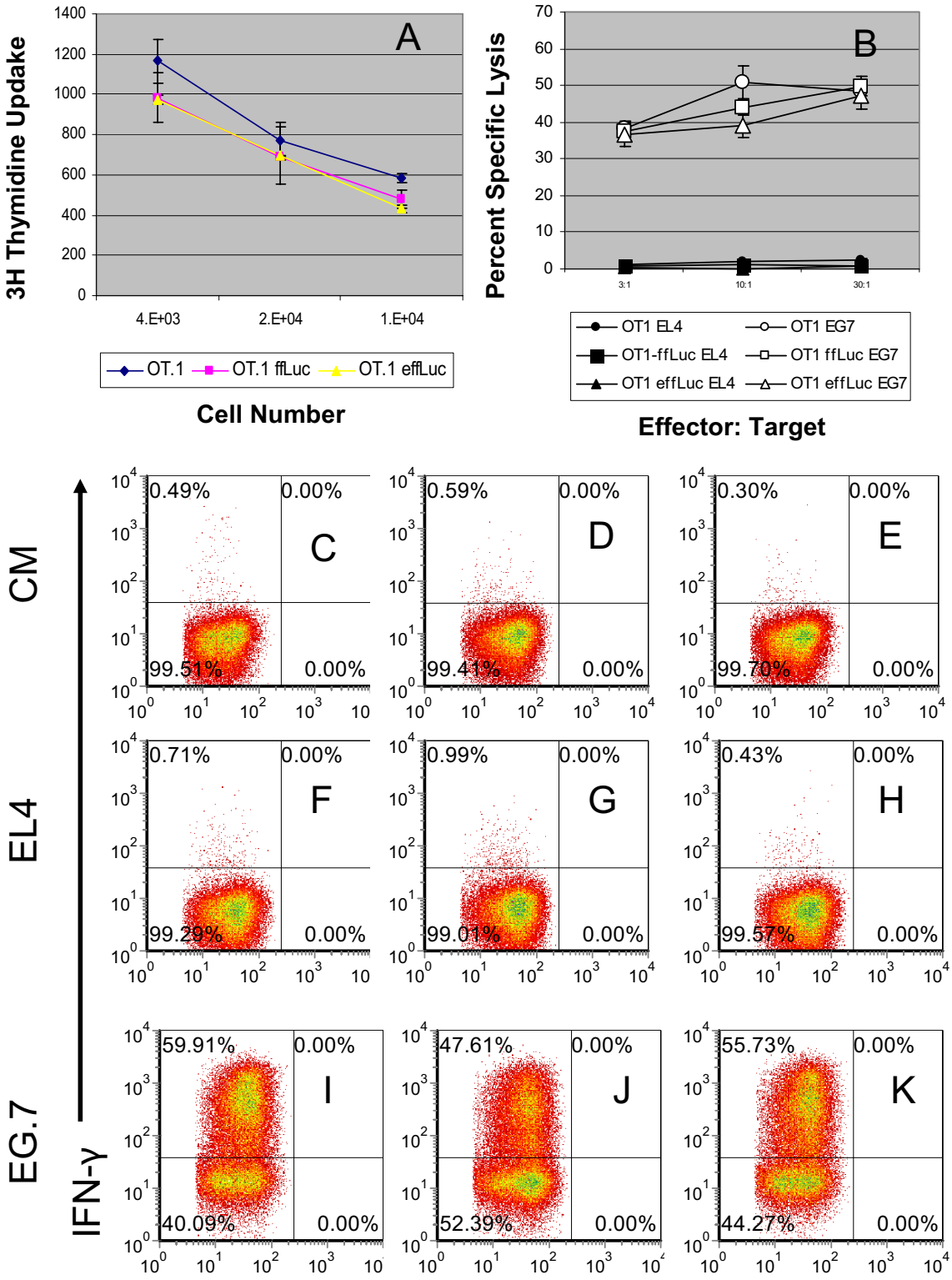

Fig. S4. *In vitro* immunological assessment of OT-I T cells transduced with v-ffLuc versus v-effLuc. OT-I T cells were transduced with v-ffLuc or v-effLuc (as indicated), sorted for Thy1.1^{+} cells 4 days later and subjected to functional assays 48 h later. These included proliferation (^{3}H Thymidine uptake) (*A*), specific cytotoxicity (chromium release assay using EL4 (negative control) and EG.7 (Ova-expressing) target cells and intracellular IFN-γ production after coculture (4 h) with EL4 or EG.7 (*B*). Note that EG.7 but not EL4 induced similar IFN-γ production in OT-I control T cells, ffLuc- and effLuc-expressing T cells. None of the functional assays demonstrated any significant differences between ffLuc-, effLuc-, and mock-transduced OT-I T cells.

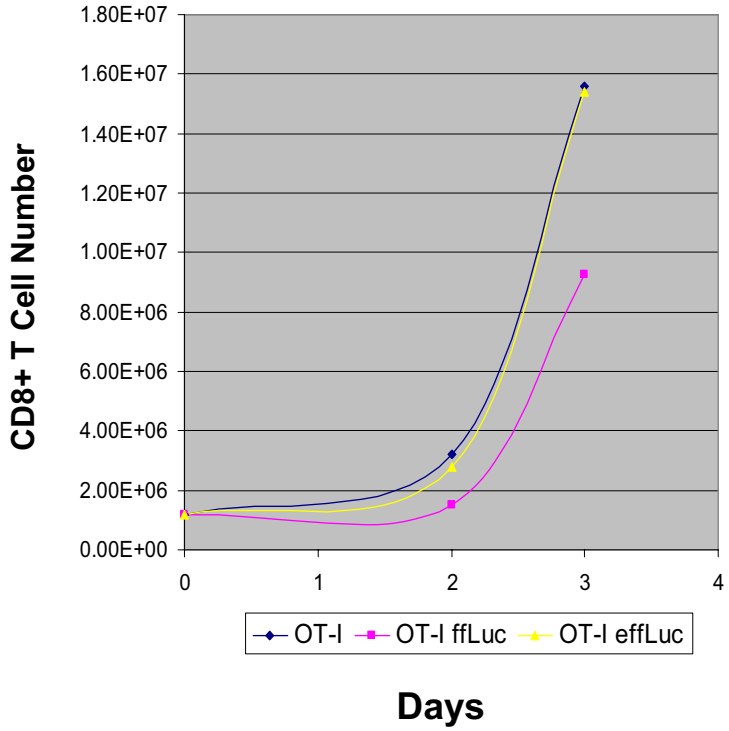

Fig. S5. Proliferation of v-ffLuc- and v-effLuc-transduced OT.1 T cells. OT-I T cells were transduced with v-ffLuc, v-effLuc or mock-transduced (as indicated) 24 h after activation and assessed for cell numbers. The first 3 days are shown.

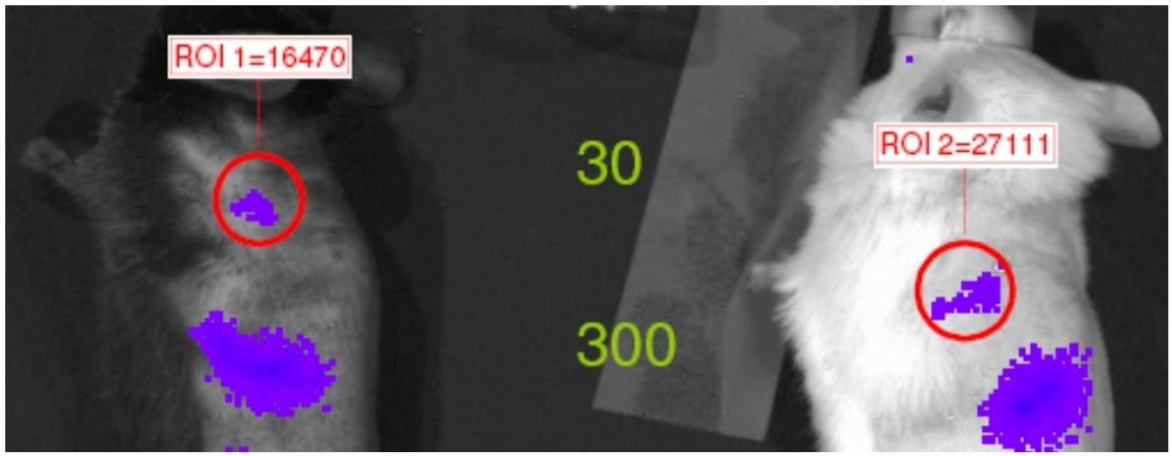

Fig. S6. Difference in photon flux transmission between black wild-type and albino C57BL/6 mice. Wild type C57BL/6 mice (*Left*) or albino C57BL/6 mice (*Right*) were injected s.c. with 30 or 300 effLuc-expressing T cells and photon flux measured.

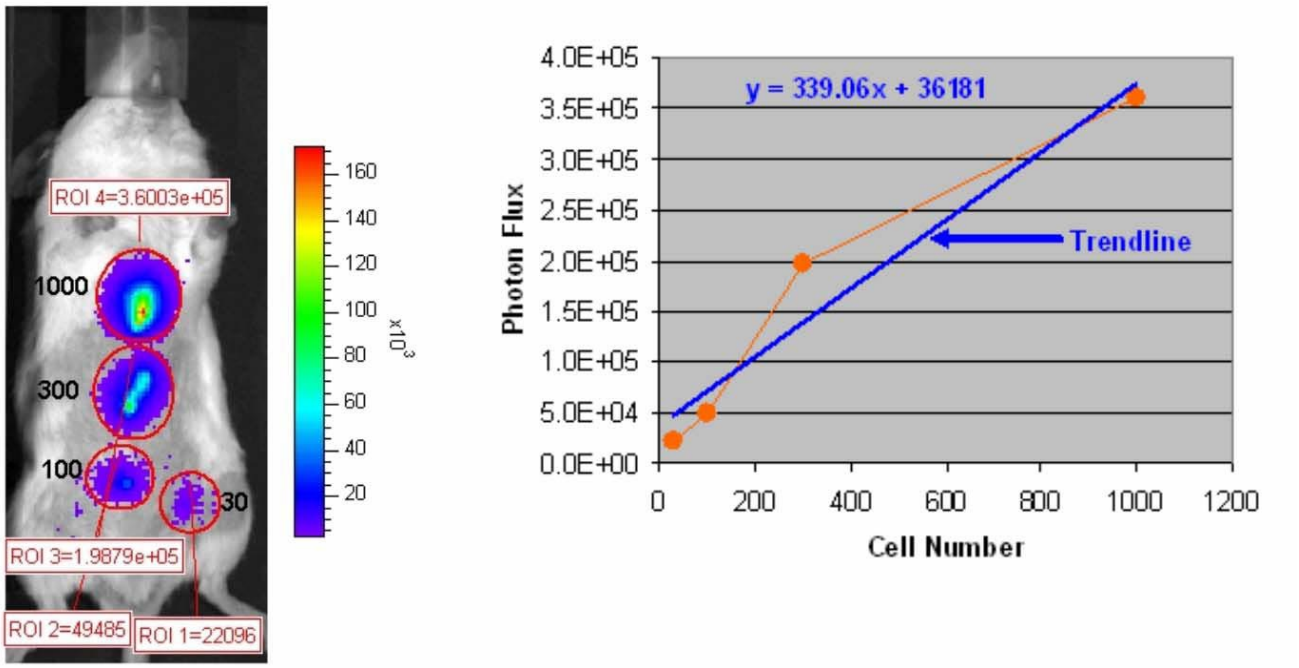

Fig. S7. Construction of standard curve to back calculate effLuc-expressing mouse T cells in s.c. tissue. OT.1 T cells were transduced with v-effLuc and sorted for Thy1.1+ cells, and a standard curve was generated for each experiment via s.c. injection of the ventral or dorsal skin (depending on experiment, ventral shown). The same litter of C57BL/6 albino mice were used per experiment. Here the litter was used for the Ova vaccination experiment shown in Fig. 3. T cells were injected at limiting dilution from 1,000 to 30 cells, as indicated and the photon flux measured. A standard curve of photon flux (*y* axis) versus number of cells injected (*x* axis) was generated, and a trend line and equation were calculated. The equation was used to back calculate the number of OT.1 T cells migrating to the vaccination site (*x*) based on the measured photon flux (*y*).

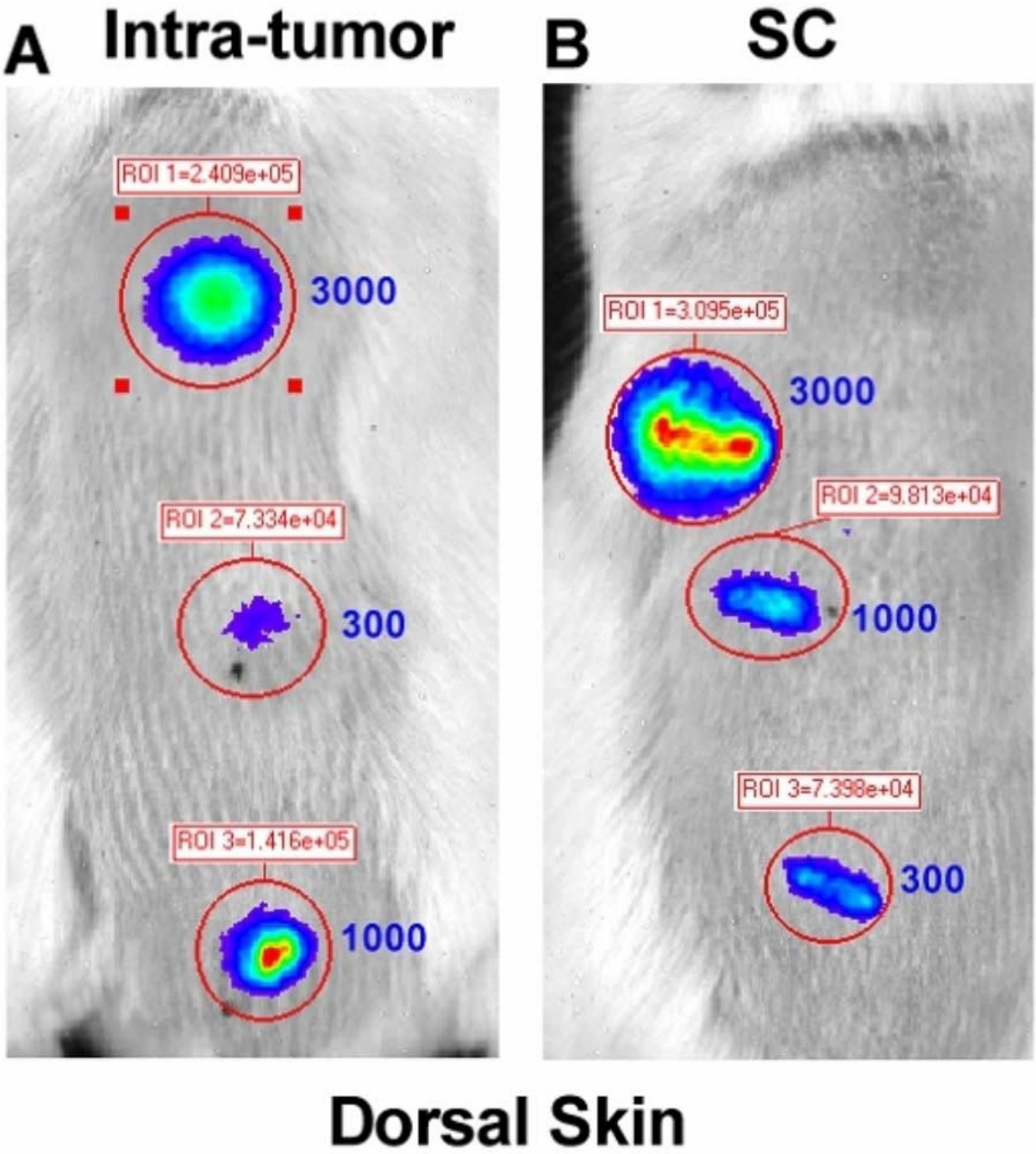

Fig. S8. Comparison of light emission of effLuc T cells injected via intra-tumor or s.c. injection. effLuc T cells were injected into the tumors of day 7 established EL4 tumors (*A*) or s.c. (*B*) at the indicated cell numbers. Bioluminescence is indicated as photons per second per ROI.

Table S1. Comparison of ffLuc and effLuc expression in cell lines representative of multiple mouse and human tissues

Tissue	Origin	effLuc (p/s/c)	ffLuc (p/s/c)	Fold difference	
				Intensity	Sensitivity
		Mouse			
Mouse T cells	OT-I	2,206.9 ± 229	38.9 ± 3.9	56.7 ± 0.6	100–110
EL4	Thymoma	456.8 ± 30	10.5 ± 1	43.5 ± 1.21	30–33
Wehi	Pre-B lymphoma	495.0 ± 41	15.9 ± 3.3	32.7 ± 4.1	48–52
P815	Mastocytoma	184.7 ± 14	19.7 ± 1.5	9.4 ± 0.68	14–16
SSCVII	Squamous cell carcinoma	1020.4 ± 153	128.3 ± 28	7.8 ± 0.4	17–20
3T3	Fibroblastoma	2,421 ± 91	32.6 ± 0.18	74.2 ± 2.41	100–110
MCA205	Fibrosarcoma	7,862.7 ± 856	252.5 ± 40	32.1 ± 2.5	39–43
CT26	colon carcinoma	2,361.1 ± 99.8	23.2 ± 1.65	103.0 ± 11.58	110–120
B16	Melanoma	1,399.6 ± 55	6.8 ± 0.8	212.1 ± 23	250–350
		Human			
Human T cells	PBMC	691.1 ± 54	61.3 ± 4.5	11.2 ± 0.3	10–12
Jurkat	T cell lymphoma	935.2 ± 63	3.8 ± 0.9	269.1 ± 24	>400
JM1	B cell lymphoma	175.4 ± 4	1.6 ± 0.18	108.3 ± 9.4	270–290
K562	Chronic myeloid leukemia	888.5 ± 45	42.5 ± 4.4	21.4 ± 1.9	19–22
293	Embryonic renal carcinoma	8,220.6 ± 298	300.5 ± 38	28.0 ± 2.59	37–40
Mel624	Melanoma	4,777.3 ± 159	140.6 ± 16	35.0 ± 3.6	38–42

Mouse and human cell lines (as indicated) were transduced at equivalent MOIs using VSV.G pseudotyped retrovirus. (Mouse T cells were transduced with ecotropic retrovirus). The table reports the observed signal intensity [photons per second per cell (p/s/c)], the fold difference in intensity and sensitivity for effLuc versus ffLuc. Note that the cell lines were not transduced to generate the highest possible signal intensities (i.e. higher MOIs or lentiviral substitution) and are thus not reflective of the highest obtainable with effLuc. Rather, v-ffLuc and v-effLuc were used at the same MOI (0.5) to facilitate intensity and sensitivity comparisons.