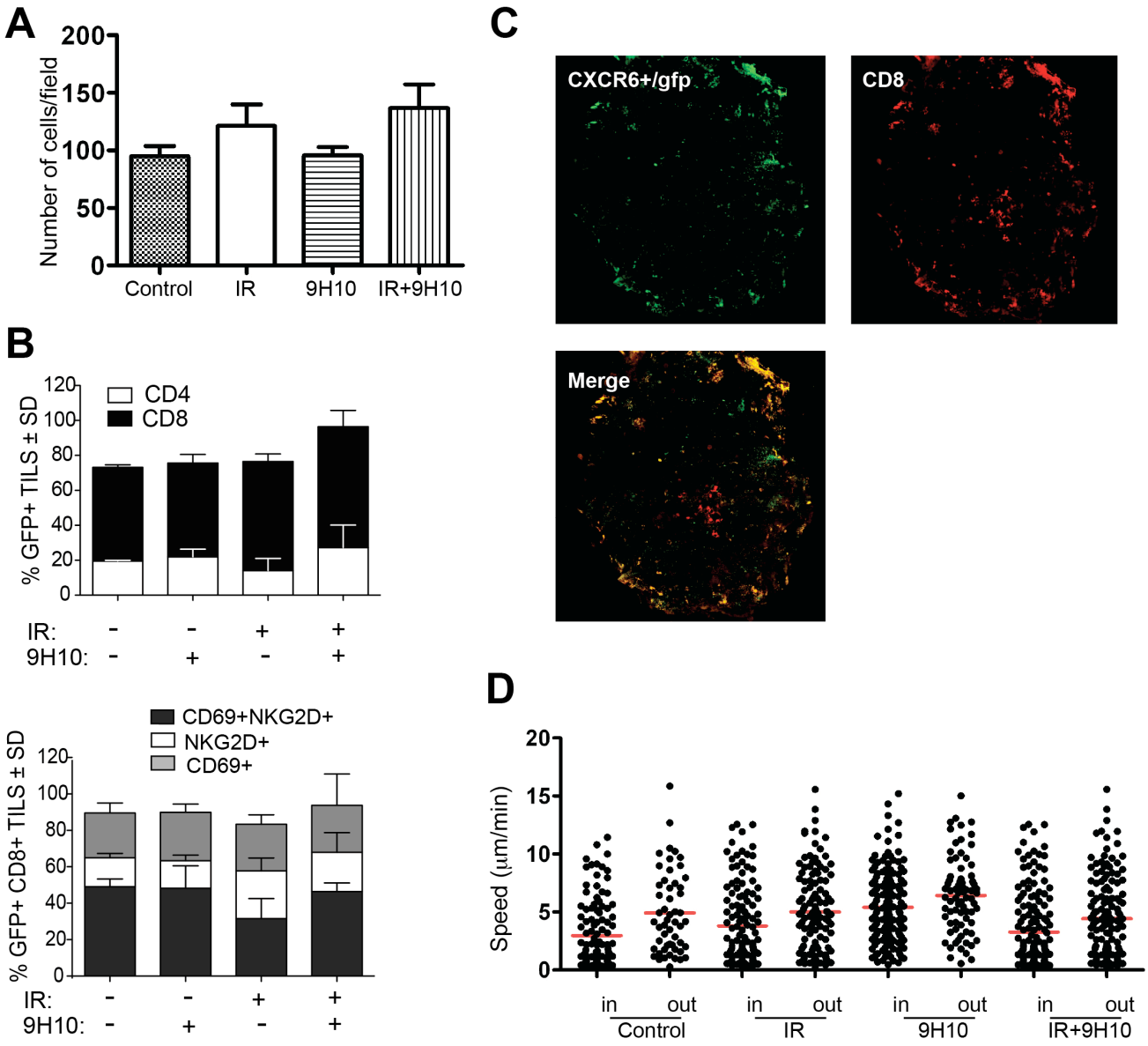
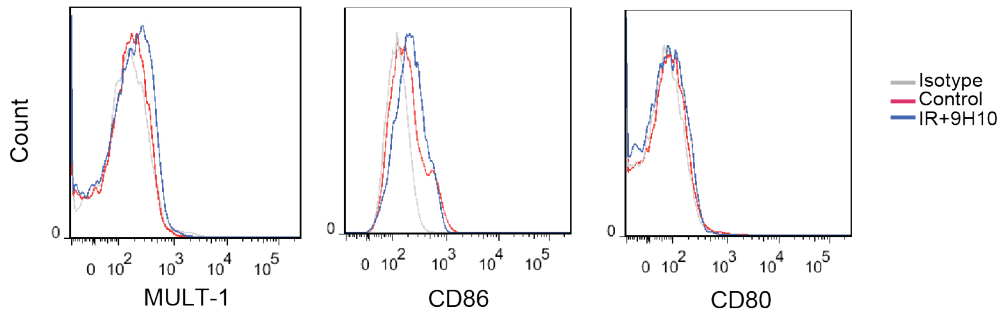
SUPPLEMENTAL MATERIAL.

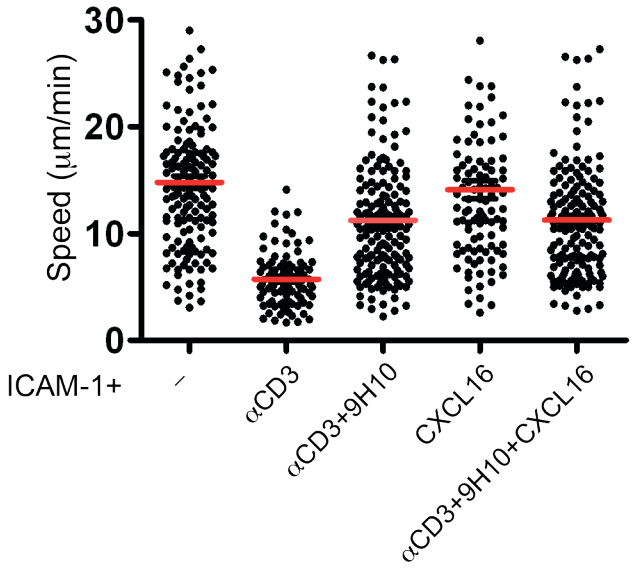


Supplementary Figure 1. Density, phenotype and behavior of GFP⁺ TILs in 4T1 tumors of mice treated with IR+9H10 analyzed at day 16. (A) Number of cells per field (Control=94.67±15.50, IR=121.3±31.9, 9H10=95.33±12.5, IR+9H10=136±35.56). The total number of GFP⁺ cells was determined in 3 different fields ($9x10^4$ μm^2) in areas rich in CFP⁺ tumor cells for each condition. (B) GFP⁺ TILs from dissociated tumors of mice treated with IR and/or 9H10, as indicated, were analyzed by flow cytometry after enrichment for CD45⁺ cells,

and gating on CD3. Percentage of $CD4^+$ (white bar) and $CD8^+$ (black bar) cells among GFP^+ TILs (top panel). Relative percentage of GFP^+CD8^+ cells expressing CD69 and/or NKG2D (bottom panel). Data are the mean ± SD of three independent samples/group. Differences between groups were non significant ($p>0.05$). (C) Representative tumor sections stained with PE-conjugated anti-mouse CD8α (Caltag) and digitally scanned using Leica SCN400F at 20x magnification. The majority of $CD8^+$ TILs present at the periphery of the tumor are GFP^+, while single-positive cells are seen mostly in deeper areas. (D) Comparison of T cell velocity in the CFP^+ cell rich areas (in) compared to areas with few or no CFP^+ 4T1 cells (out).



Supplementary Figure 2. 4T1 cells do not express MULT-1 and co-stimulatory molecules. The expression of NKG2D ligand MULT-1, and co-stimulatory molecules CD80 and CD86 was analyzed on 4T1 cells cultured in vitro and treated as indicated. MULT-1, CD80 and CD86 were not expressed on 4T1 cells, and radiation did not induce them.



Supplementary Figure 3. CXCL16 does not reverse the effect of 9H10 treatment on T cell motility in vitro. Preactivated CD8 T cells were stimulated in vitro with soluble anti-CD3 and/or 9H10 mAbs, as indicated, and analyzed for ability to migrate over an ICAM-1-coated glass surface. Recombinant CXCL16 was spotted on the glass surface together with ICAM-1. Scatter plots of T cell mean velocity for each condition. Each dot represents the average speed of a cell tracked over 15 min. Data are representative of 3 independent experiments.

LEGENDS FOR SUPPLEMENTARY MOVIES

Movies 1 and 2. In vivo TPLSM sequences of GFP$^+$ TILs in 4T1-CFP tumors at day 22. 3D time lapses were taken for the duration of 15 min and were acquired as a z-stack of 30 μm between 60 and 90 μm of depth below the capsule. Mice were mock treated (Control), treated with IR, 9H10, or IR+9H10, as indicated. T cells are green (GFP), 4T1 tumor cells are blue (CFP), blood vessels are red (Quantum dots). Each frame is a maximum pixel projection of 7

slices at 5 μm intervals. Time intervals were approximately 27 seconds. All movies played back at 10 frames per second. Scale bar, 58 μm.

Movies 3 and 4. In vivo TPLSM sequences of GFP$^+$ TILs in 4T1-CFP tumors at day 16. 3D time lapses were taken for the duration of 15 min and were acquired as a z-stack of 30 μm between 60 and 90 μm of depth below the capsule. Mice were mock treated (Control), treated with IR, 9H10, or IR+9H10, as indicated. T cells are green (GFP), 4T1 tumor cells are blue (CFP), blood vessels are red (Quantum dots). Each frame is a maximum pixel projection of 7 slices at 5 μm intervals. Time intervals were approximately 27 seconds. All movies played back at 10 frames per second. Scale bar, 58 μm.

Movies 5 and 6. In vivo analysis of T cell motility in the presence of NKG2D blocking mAb CX5 at day 16. 3D time lapses were taken for the duration of 15 min and were acquired as a z-stack of 30 μm between 60 and 90 μm of depth below the capsule. Mice were mock treated (Control) or treated with IR+9H10, as indicated. All mice received an i.p. injection of NKG2D blocking mAb CX5 minutes before imaging. T cells are green (GFP), 4T1 tumor cells are blue (CFP), blood vessels are red (Quantum dots). Each frame is a maximum pixel projection of 7 slices at 5 μm intervals. Time intervals were approximately 27 seconds. All movies played back at 10 frames per second. Scale bar, 58 μm.