**Figure S1**. **CD8+ T cells infiltrating 4T1 and MCA-OVA tumors exhibit high CD39 expression.** **A**, Frequency of CD39+ and CD73+ cells within CD3+CD8+ T cells from tumor-free and tumor-bearing mice (D17). **B**, Representative dot plots and graphs show frequency of CD3+CD8+ T cells expressing CD39 and CD73 in tumors, dLNs and spleens at days 28 p.i. for 4T1 (BALB/c) tumors and 17 p.i. for MCA-OVA (C57BL/6) tumors. **C**, Frequency and MFI of total CD39+ cells (CD39int + CD39high) and frequency of CD39high cells in CD3+CD8+ T cells from tumors, dLNs and spleens from 4T1 and MCA-OVA tumor-bearing mice. All results are representative of 3 to 5 independent experiments (*n*=4-6 mice per experiment). Data presented as mean ± SEM. ns, non significant; \**P*≤0.05; \*\**P*≤0.01; \*\*\**P*≤0.001; \*\*\*\**P*≤0.0001.

**Figure S2**. **CD39 is expressed on CD45+ cells infiltrating B16F10-OVA tumors. A**, Frequency of CD39int and CD39high cells within CD8+ T cells infiltrating B16F10 and B16F10-OVA tumors (D17). **B**, Immunohistofluorescence of tumor sections shows co-localization of CD45 (blue), CD39 (red) and CD8 (green). DAPI was used to stain cell nuclei. **C**, Pie chart shows mean frequency of immune cells infiltrating B16F10-OVA tumors (gate in CD45+ cells; inner circle): CD8+ T cells (CD3+CD8+), CD4+ Tconv cells (CD3+CD4+Foxp3-), Tregs (CD3+CD4+Foxp3+), NK cells (CD3-NK1.1+), granulocytic myeloid-derived suppressor cells (G-MDSCs; CD3-CD11b+Ly6G+), monocytic myeloid-derived suppressor cells (M-MDSCs; CD3-CD11b+Ly6G-CD11c-Ly6C+), dendritic cells (DCs; CD3-CD11b+Ly6G-CD11c+), B cells (CD3-CD19+) and NKT cells (CD3+NK1.1+). Outer circle depicts the frequency of CD39-, CD39int and CD39high cells within each cell population. **D**, Frequency of CD39+ immune cells that co-express CD73. **E**, Frequency of CD39high Tregs, G-MDSCs, M-MDSCs, NK cells and DCs at three different days p.i. (statistical difference between D17 and D24 *vs.* D10 in each group is indicated). All results are representative of 2 independent experiments (*n*=4-6 mice per experiment). Data presented as mean ± SEM. ns, non significant; \*, † or # *P*≤0.05; †† *P*≤0.01; \*\*\* *P*≤0.001; \*\*\*\* *P*≤0.0001.

**Figure S3**. **High expression of CD39 on CD8+ TILs is associated with low TNF and IL-2 production and expression of iRs in different experimental cancer models.**  **A**, Graphs show frequency of IFNγ+, TNF+, IL-2+, Granzyme B+ and CD107a+ cells within CD39highCD8+ TILs from B16F10 and B16F10-OVA tumor-bearing mice after PMA/Ionomycin stimulation and frequency of Ki-67+ cells after anti-CD3/anti-CD28 stimulation. **B**, Representative histograms show expression of the iRs PD-1, Tim-3, LAG-3 and TIGIT on CD3+CD8+ T cells (analyzed according to CD39 expression) from 4T1 tumors (D28) and MCA-OVA tumors (D17) (*n=*3-5). **C**, Representative histograms show expression of iRs on CD3+CD8+ T cells from dLNs and spleens from B16F10-OVA tumor-bearing mice. **D**, Graphs show expression of T-bet, Eomes and Blimp-1 in CD8+ TILs from B16F10-OVA tumors with different expression of CD39 (*n*=5). Data presented as mean ± SEM. ns, non significant; \**P*≤0.05; \*\**P*≤0.01; \*\*\**P*≤0.001.

**Figure S4**. **PD-1+Tim-3+ exhausted CD8+ T cells reach terminal exhaustion when re-stimulated *in vitro*.** **A** and **B**, Purified CD8+ T cells from B16F10-OVA tumors were sorted according to the expression of PD-1 and Tim-3 and re-stimulated *in vitro* with anti-CD3/anti-CD28 and rIL-2 for 72hs. **A**, Representative histograms show T-bet (numbers indicate MFI) and Ki-67 (frequency of Ki-67+ cells) expression in subsets of CD3+CD8+ T cells. **B,** Representative dot plots show the frequency of IFNγ+ cells in sorted CD3+CD8+ T cell subsets stimulated as above after boosting with PMA/Ionomycin (data representative of two independent experiments performed with pools of cells purified from tumors from 12 mice per pool).**C**, Frequency of IFNγ+ responder CD8+ T cells that were co-cultured with PD-1+Tim-3+CD8+ TILs in the presence of different concentrations of ARL67156 (*n=*6, three independent experiments). **D**, Frequency of IFNγ+ responder CD73KO CD8+ T cells that were stimulated alone or in co-cultures with sorted PD-1-Tim-3- or PD-1+Tim-3+CD8+ TILs (1:1 ratio) (n=3). **E**, Representative histogram shows CFSE-labeled responder CD8+ T cells unstimulated and stimulated alone or in co-culture with sorted PD-1-Tim-3- or PD-1+Tim-3+CD8+ TILs (1:1 ratio) (representative of 6 independent experiments, n=2-3 each). Data presented as mean ± SEM. ns, non significant; \**P*≤0.05; \*\**P*≤0.01.

**Figure S5**. **Human CD39+CD8+ T cells exhibit predominantly an effector/memory phenotype and co-expression of iRs**. **A**, Representative dot plot shows frequency of CD39+ and CD73+ cells within tumor-infiltrating CD4+ Tconv cells (Foxp3-) obtained from a breast cancer patient (n=9). **B**, Representative dot plots show frequency of CD39- and CD39+ CD8+ T cells expressing CCR7 and CD45RA in primary breast tumors or I-LNs as indicated. **C**, Pie charts show the mean proportion of cells expressing zero to three of the evaluated iRs (PD-1, TIGIT and BTLA) in non-naïve CD39- and CD39+ CD8+ T cells from breast tumors (left) and I-LNs (right). **D,** Representative histograms show the expression of PD-1 on CD39+CD73-, CD39-CD73- and CD39- CD73+ CD8+ T cells from I-LNs and NI-LNs.

**Figure S6**. **Human CD39+CD8+ T cells are increased in melanoma invaded/metastatic-LNs and exhibit features of exhaustion**. **A**, Representative dot plots and graphs show frequency of CD3+CD8+ T cells expressing CD39 and CD73 in NI-LNs and I-LNs from melanoma patients (*n=*3). **B,** Representative histograms and graphs show the expression of iRs on non-naïve (gating out CD45RA+CD27+ cells) CD39- (white histograms) and CD39+ (gray histograms) CD8+ T cells from I-LNs (*n=*2). **C**, Graphs show the frequency of cytokine-producing cells (TNF and IL-2) and CD107a+ cells in non-naïve CD39- and CD39+ CD8+ T cells from I-LNs after PMA/Ionomycin stimulation in each patient (*n=*2). Lines indicate that data are paired.

**Figure S7**. **CD39+CD8+ T cells induced *in vitro* maintain their capacity to produce cytokines.A**, Graphs show frequency of IFNγ+ and TNF+ cells among mouse CD39+CD8+ T cells induced in each condition, after boosting with PMA/Ionomycin. **B**, Graphs show frequency of IFNγ+ and TNF+ cells among human CD39+CD8+ T cells induced in each condition, after boosting with PMA/Ionomycin. **C**, Immunohistofluorescence of tumor sections shows localization of CD45+ cells (green), CD39+ cells (red), cells nuclei (DAPI), and hypoxic foci indicated by presence of Hypoxyprobe-1 adducts (H-1, gray). **D**, bar graph shows frequency of mouse CD39+CD8+ T cells induced after *in vitro* stimulation in normoxic or hypoxic (last 24 h in a 1.5% O2 atmosphere) conditions. **A** and **D**, Data presented as mean ± SEM. ns, non significant; \**P*≤0.05; \*\**P*≤0.01.

**Table S1.** Monoclonal antibodies, fluorochromes and clones used for mouse and human antigens.

**Table S2.** Frequency and MFI of CD39+CD8+ T cells from human tumor samples.

**Table S3.**Frequency of CD39+, PD-1+, TIGIT+ and Tim-3+ cells within CD8+ T cells that were purified from PBMCs from breast cancer patients and then stimulated *in vitro* with anti-CD3/anti-CD28 for 72 h in the presence or absence of recombinant IL-6 and/or IL-27.