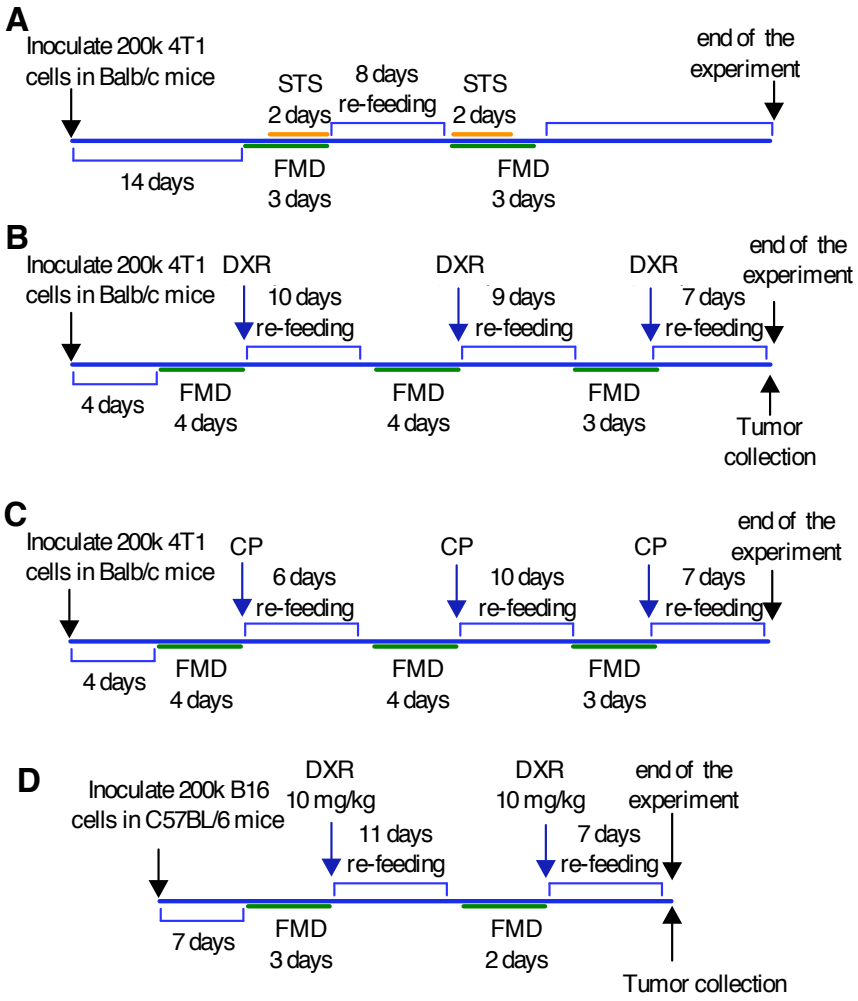

Figure S1. Related to Figure 1 . Schematic representation of tumor progression experiments.
Schematics of the experimental schedules in Figure 1 A, C-E are shown in A, B, C, and D, respectively**.** (**A**, **B**, and **C**) 12-week old female BALB/c and (**D**) C57BL/6 mice were inoculated with (**A**, **B**, and **C**) 200,000 4T1 murine breast cancer or (**D**) B16 murine melanoma cells in the right flank. FMD and STS groups underwent 3-4 days (FMD) or 2 days (STS) of dietary intervention followed (right at the end of the regimen) by DXR or CP injection. Between FMD/STS cycles, the animals were ad lib fed for a period of 8-11 days to allow for the recovery of the body weight lost. The third cycle of FMD was reduced to 3 days because of the faster rate of body weight loss. 7 days following the last administration of chemotherapy the animals were euthanized.

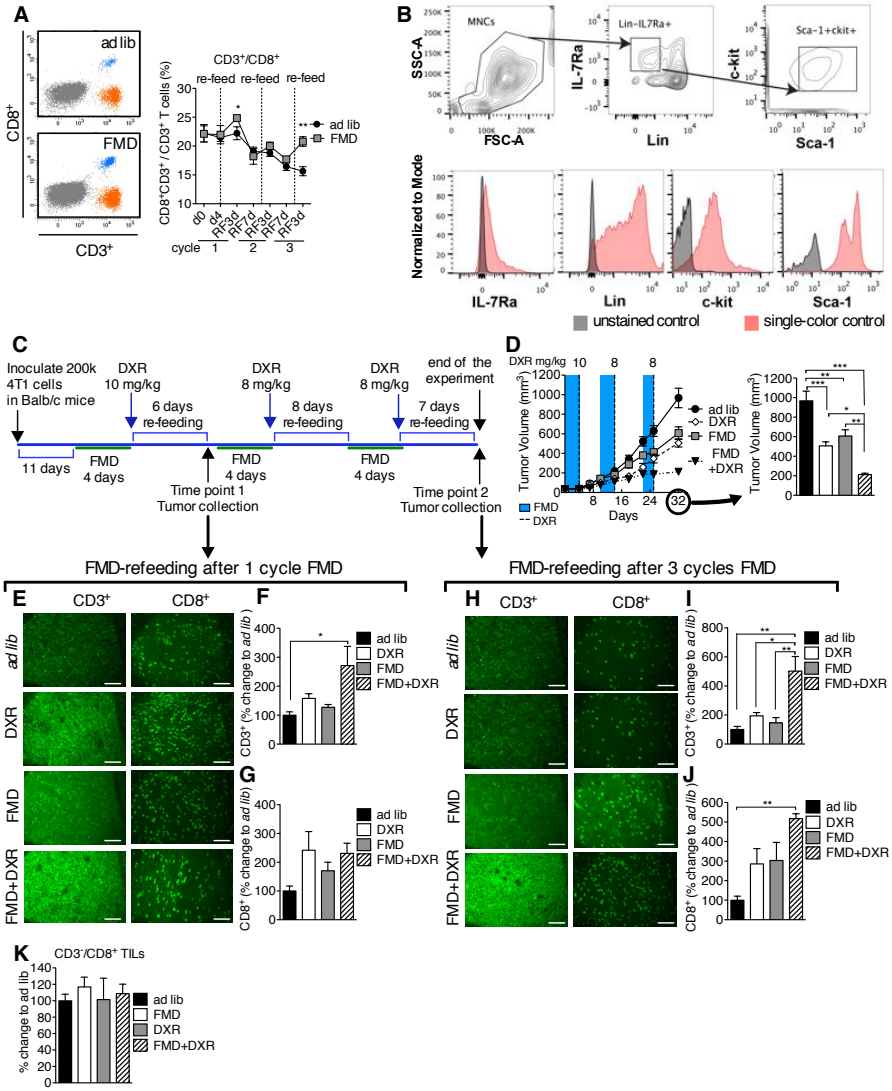

Figure S2. Related to Figure 2. The effect of FMD on $CD3^+/CD8^+$ TILs .

(A) Blood from BALB/c mice undergoing *ad lib* and FMD regimens was collected at different time points and analyzed with FACS (n=6) to assess levels of $CD3^+/CD8^+$ circulating cells. (**B**) Gating strategy for FACS analysis used to quantify Common Lymphoid Progenitors (CLP) from bone marrow collected from BALB/c mice undergoing FMD/DXR treatments (described in Figure S3A (n=6). **(C)** 7-month old female BALB/c mice were inoculated with 200,000 4T1 murine breast cancer cells in the right flank (n=10). One week following the inoculation, the animals were divided in the following groups: *ad lib* (*ad libitum* feeding), FMD, DXR and FMD+DXR. At this point the FMD groups underwent 4 days of dietary intervention followed by DXR injection at the end of FMD (day 4). Between FMD cycles, the animals were *ad lib* fed for a period of 8 to 11 days to allow for the recovery of the body weight lost. (**D**) Tumor progression and tumor volume at the end of the experiment. Tumor tissue was collected 7 days following the first DXR injection (time point 1) and 7 days following the third DXR injection (time point 2), processed and analyzed to assess the levels of tumor infiltrating lymphocytes (TILs). Infiltration of $CD3^+$ and $CD8^+$ TILs are shown at (**E-G**) time point 1 and (**H-J**) time point 2 (Scale bars, 75 µm). (**K**) FACS analysis of tumor-infiltrating $CD3^-/CD8^+$ lymphocites collected from animals in figure 6A is shown. Data represented as mean ± SEM. One-way ANOVA (Tukey post-analysis test) was performed. p-values <0.05, 0.01 and 0.001 are indicated as *, **, and ***, respectively.

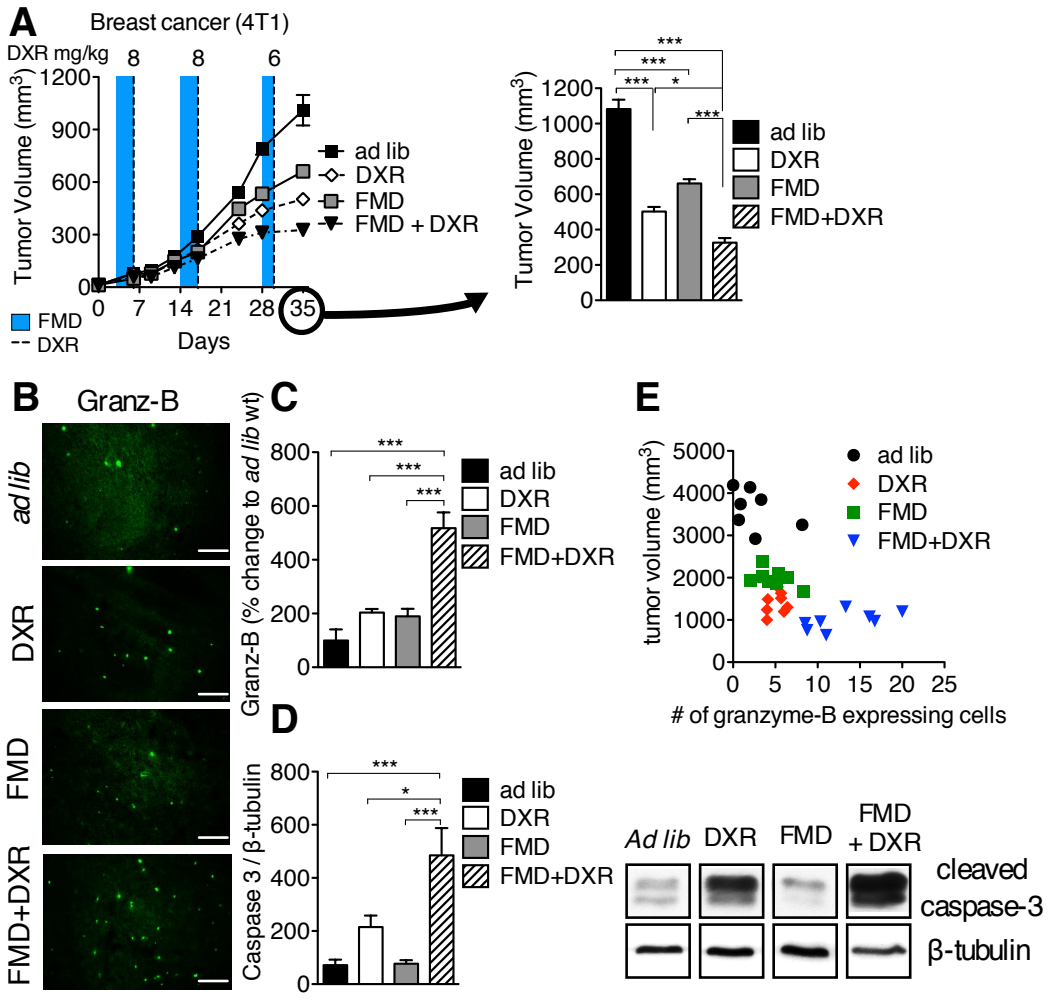

Figure S3. Related to Figure 3. Cytotoxicity markers are activated in TIL infiltrated 4T1 tumors.
12-week old female BALB/c mice were grafted with 200,00 4T1 murine breast cancer cells and underwent FMD/re-feeding regimen and were administrated DXR i.v. at the end of each cycle (n=15). (**A**) Tumor progression and tumor volumes measured at the end of the experiment. Tumors were collected at the end of the experiment and analyzed by (**B-C**) IHC to assess granzyme-B levels (Scale bars, 75 µm), and by (**D**) Western blotting to determine cleaved caspase-3 levels (n=8) (Blot was captured on film, and unedited, representative bands are shown). (**E**) Association between tumor volume at the end of the experiment and granzyme-B levels. Data represented as mean ± SEM. One-way ANOVA (Tukey post-analysis test) was performed. p-values <0.05, 0.01 and 0.001 are indicated as *, **, and ***, respectively.

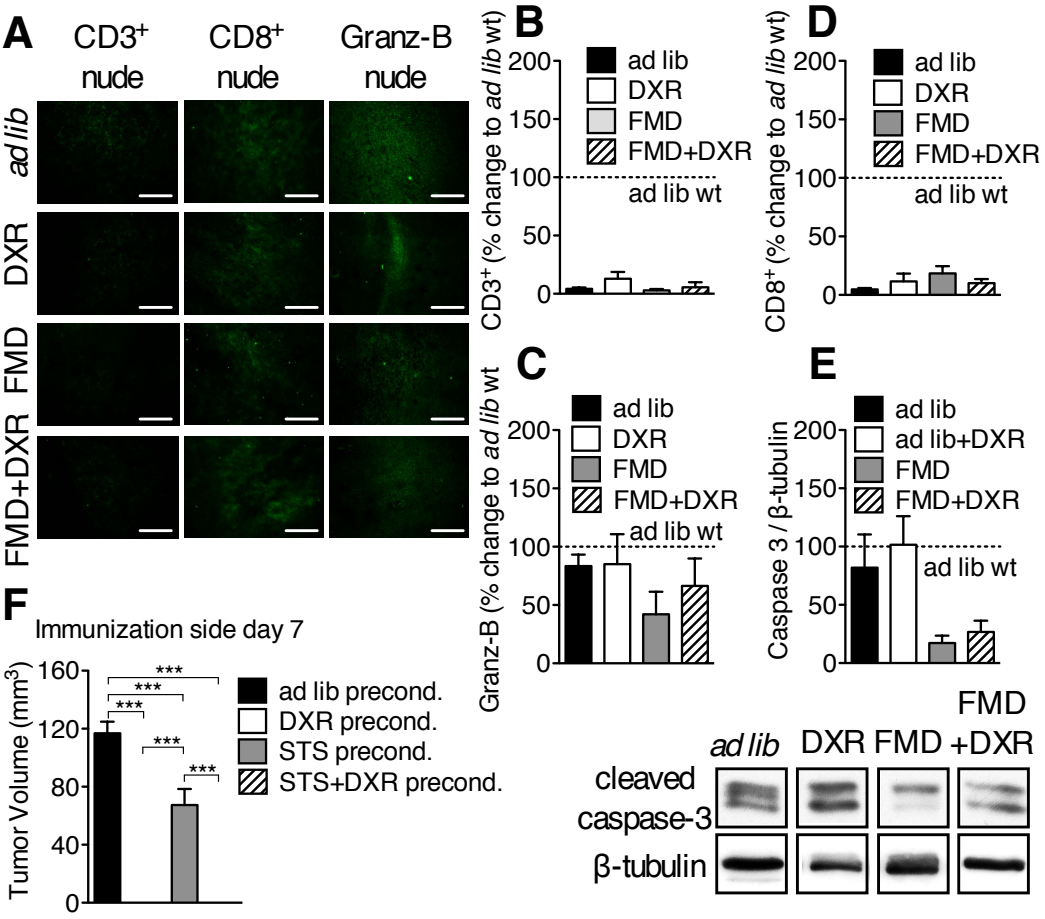

Figure S4. Consecutive cycles of FMD+DXR alter intratumoral lymphocyte composition.
Tumor tissues collected at the end of the experiment shown in Figure 2B were analyzed by (**A-D**) IHC to assess $CD3^+$ and $CD8^+$ TILs, and granzyme B (Scale bars, 75 μm), and by (**E**) Western blotting to assess caspase-3 levels (n=8) (Blot was captured on film, and unedited, representative bands are shown). (**F**) 4T1 cells pre-conditioned *in vitro* were inoculated in the left flank of BALB/c mice for the immunization experiment described in Figure 3I. Tumor volume at 7 days after inoculation is shown. Data represented as mean ± SEM. One-way ANOVA (Tukey post-analysis test) was performed. p-values <0.05, 0.01 and 0.001 are indicated as *, **, and ***, respectively.

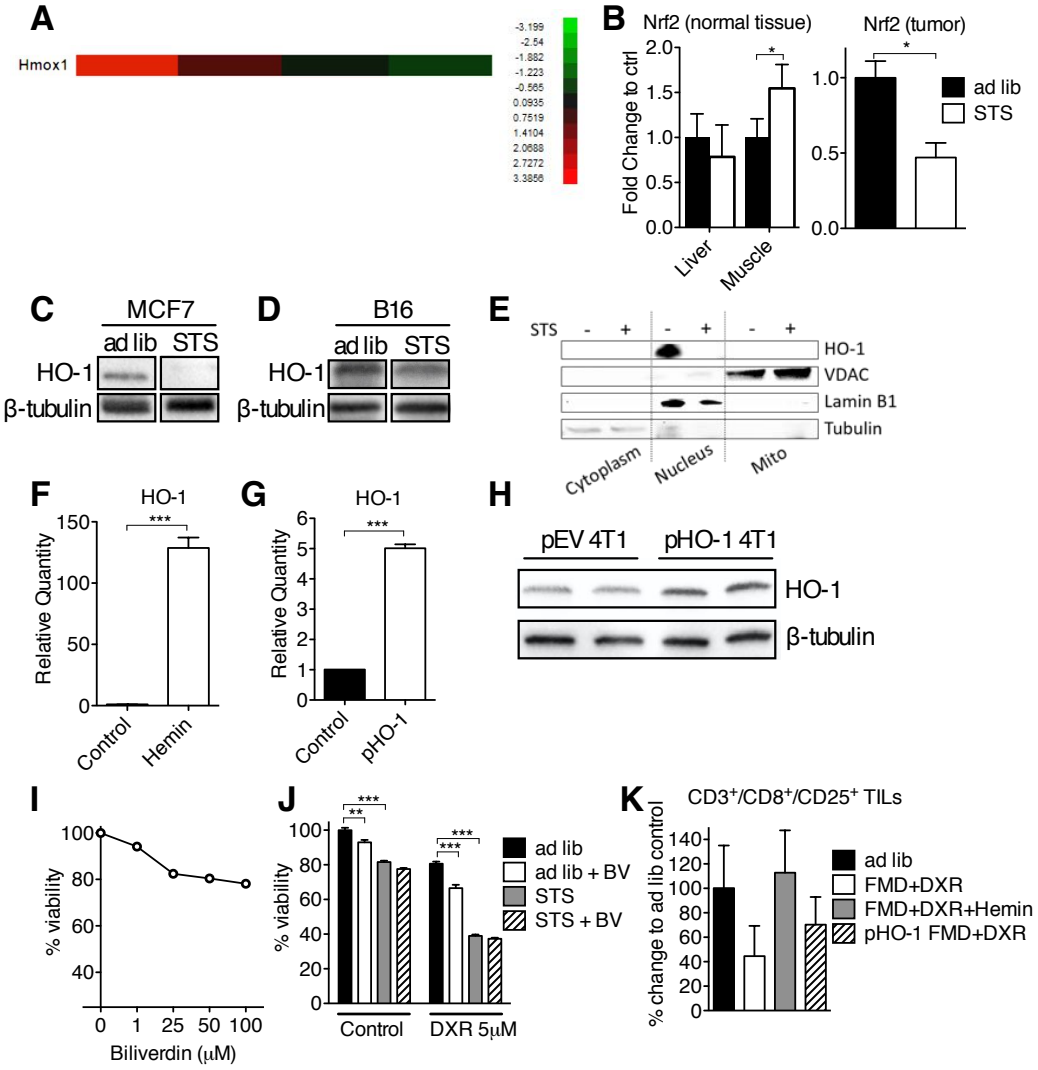

Figure S5. Related to Figure 4. Fasting differentially reduces tumor HO-1 and Nrf2 expression levels *in vivo* and *in vitro*.

Grafted breast tumor (4T1) and normal (liver and skeletal muscle) tissues were collected following a 48-hour STS and subject to an unbiased global gene expression microarray (n=6). (**A**) A representative image of Hmox1 (HO-1) levels from the microarray. (**B**) NRF2 expression levels were analyzed by qRT-PCR (n=3). (**C, D**) Down-regulation of HO-1 during *in vitro* STS is also shown by Western blotting in human breast adenocarcinoma (MCF7) and murine melanoma (B16) cells (n=3) (Blot was captured with Bio-Rad ChemiDoc, and unedited, representative bands are shown). (**E**) Subcellular localization of HO-1 protein in 4T1 cell before and after a 24-hour *in vitro* STS; voltage-dependent anion channel (VDAC; mitochondrial marker), lamin B1 (nuclear marker), and tubulin (cytoplasmic marker). (**F**) Hemin (20 uM) increased HO-1 expression levels in 4T1 cells as determined by qRT-PCR (n=3). (**G, H**) 4T1 cells that were stably transfected to over-express HO-1 had increased HO-1 expression as determined by (**G**) qRT-PCR and (**H**) Western blotting (n=3). (**I**) *In vitro* dose response to increasing concentrations of biliverdin ranging from 0-100 μM. (**J**) *In vitro* viability in response to biliverdin (BV) 1μM in 4T1 cells with and without STS and/or DXR, determined by MTT reduction (n=3). (**K**) FACS analysis of tumor-infiltrating $CD3^+/CD8^+/CD25^+$ Treg lymphocites collected from animals in figure 6A is shown. Data represented as mean ± SEM. Student's t-test was performed to compare two groups. p-values <0.05, 0.01 and 0.001 are indicated as *, **, and ***, respectively.

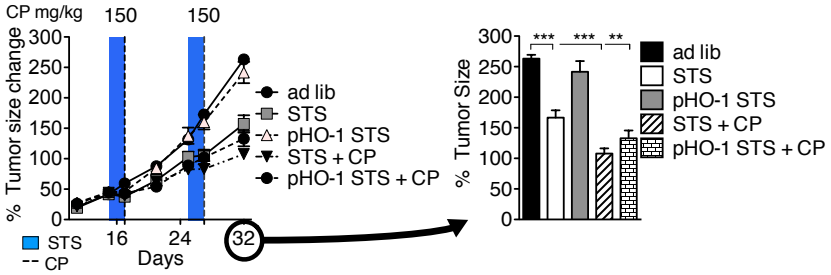

Figure S6. Related to Figure 5. STS sensitizes grafted 4T1 tumors to CP in a HO-1-dependent manner.
12-week old female BALB/c mice were grafted with 200,000 4T1 murine breast cancer cells that were stable transfected with a HO-1 expression (pHO-1) (n=10). Mice underwent two cycles of STS (48-hours) with or without CP (150 mg/kg, IP). Tumor progression was measured at multiple time points (left panel), including immediately prior to euthanasia (right panel). Data represented as mean ± SEM. Student's t-test was performed to compare two groups. p-values <0.05, 0.01 and 0.001 are indicated as *, **, and ***, respectively.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Doxorubicin administration: Doxorubicin HCL (DXR) (Pfizer Inc.) 2 mg/ml was injected intravenously (IV) at concentrations that varied based on the experiment setup and between animal strains (see results section for doses).

Cyclophosphamide administration: Upon reconstitution in sterile saline, cyclophosphamide (CP) (Baxter Healthcare Corp.) was injected intraperitoneally (IP) at concentration of 150 mg/kg per mouse.

Hemin administration: Hemin (Sigma-Aldrich Co.) was dissolved in 0.7N NH_4OH to final concentration of 50 mg/ml (stock solution). Working solution was prepared daily by diluting stock solution 1:10 in sterile saline. Hemin was administrated IP twice a day at 15 mg/kg (total of 30 mg/kg daily). Control mice were daily-injected IP with vehicle.

Zinc protoporphyrin administration: Zinc protoporphyrin (ZnPP) (Sigma-Aldrich Co.) was dissolved in DMSO to final concentration of 50 mg/ml (stock solution). Working solution was prepared daily by diluting stock solution in sterile saline. ZnPP was administrated IP once a day. Control mice were daily-injected IP with vehicle.

Biliverdin in vitro treatment: Biliverdin hydrochloride (Sigma-Aldrich Co.) was dissolved in DMSO to final concentration of 50 mg/ml (stock solution). For the *in vitro* use, the stock solution was dissolved in culturing media to a final concentration of 1-100μM.

Immunohistochemical Analysis: Tumor tissues were collected and snap frozen immediately after animal sacrifice and stored at -80°C until processed on a Leica CM1800 cryostat where 20 μm sections were serially collected on glass slides. The slides were fixed in 4% paraformaldehyde for 15 minutes, permeabilized in 1% Nonidet P-40 (Sigma-Aldrich) and finally blocked in 5% normal goat serum. The slides were then incubated overnight at 4°C with either one of the following antibodies: CD3, CD8 (Abcam plc), or granzyme-B (Cell Signaling Technology) primary antibodies. The incubation with secondary antibody was performed 1 hour at RT with either goat anti-mouse (Alexa Fluor® 488) or goat anti-rabbit (Alexa Fluor® 594). Glass slides from B16 tumors were prepared with mounting media containing DAPI (Abcam plc). Infiltration of positive cells in the tissue was detected with fluorescence microscopy (Nikon TE-DH) and then quantified with ImageJ (NIH software).

Protein Extraction and Western Blot Analysis: Tumor tissue was collected immediately after mouse sacrifice at the specified time points. The tissue was snap frozen in liquid nitrogen and stored at -80°C until utilized for the analysis. For the total protein extraction, the tumor tissue was homogenized in presence of

RIPA buffer (EMD Millipore), protease- and phosphatase inhibitors (Sigma Aldrich). Total protein extracts were quantified with BCA assay (Thermo Scientific Pierce), dissolved in NuPAGE buffer (life technologies) and denatured at 100°C. 40µg of total proteins were resolved in 18% polyacrylamide gel in presence of SDS and transferred to PVDF membrane before blocking with 5% milk in T-PBS. The membrane was incubated overnight at 4°C with either one of the following primary antibodies: Caspase-3, HO-1, Lamin B1, VDAC and β-tubulin (Cell Signaling Technologies). The incubation with secondary antibody anti-rat or anti-goat (Jackson ImmunoResearch Laboratories, Inc.) was performed for 1h at RT. The membrane was incubated with peroxidase substrate for enhanced chemiluminescence (ECL) for 2 minutes at room temperature and detection was performed by exposing the membrane to film (Figure S3D and S4E) or by detection with Bio-Rad ChemiDoc (Figure 4B, S5C, D, and H). Canon LiDE25 scanner was used for the acquisition of the films and all the raw images were inverted and quantified with ImageJ (NIH software). Raw images from Bio-Rad ChemiDoc were unedited and analyzed directly with ImageJ for quantification. For presentation purpose, representative images of bands belonging to each experimental group were collected and placed together in the figure. The high number of samples analyzed has required the use of multiple blots, which were processed in parallel following identical conditions. Because of the arrangement of the running lanes on the western blot, bands depicted to be next to each other in the final image might have been on different positions on the blot, or from different blots.

$CD8^+$ cells in vivo depletion: Complete depletion of $CD8^+$ CTL was achieved by intraperitoneal administration of neutralizing monoclonal antibody (αCD8; clone YTS 169.4) or rat IgG (BioXCell, USA) every 4 days. The depletion of circulating $CD8^+$ CTL over time was confirmed by FACS analysis. Lymphocytes from mice were collected using Histopaque 1077 following manufacturer's protocol (Sigma Aldrich, USA).

Flow cytometric analysis for CLP and T cells: Bone marrow cells collected from femurs and tibia were stained with antibodies against lineage markers (APC-Cy7), Sca-1(AF-700), C-kit(APC) and IL-7Ra(PE-Cy7). Peripheral blood cells collected from the tail vein were stained with antibodies against CD8a(AF488) and CD3(APC), according to manufacturer's instructions. All cell acquisition was performed with BD LSR II flow cytometer and analyzed with FACSDiva software (BD Biosciences, San Jose, CA). For the analysis of TILs, upon surgical removal the tumor was washed in ice-cold PBS, was mechanically minced to small fragments and incubated for 1h at 37°C under agitation with collagenase IV (Worthington; 300 units/mL in serum free DMEM) for enzymatic digestion. The digested tissue was then filtered and the isolated cells were washed and incubated with anti CD8, CD3, CD4 and CD25 antibodies (eBioscience, Inc.).

Microarray analysis: RNA from tissues was isolated according the procedures described by the manufacturer using the RNeasy kit (Qiagen). Then, RNA was hybridized to BD-202-0202 chips from Illumina Beadchips. Raw data were subjected to Z normalization as described (Cheadle et al., 2003). Briefly, for each pathway under each pair of conditions, a Z score was computed as [Z (pathway) = (sm mu)*pow(m,0.5)/delta], where mu = mean Z score of all gene symbols on the microarray, delta = standard deviation of Z scores of all gene symbols on the microarray, sm = mean Z score of gene symbols comprising one pathway present on the microarray, and m = number of gene symbols in a pathway present on the microarray. For each Z (pathway) a p value was also computed in JMP 6.0 to test for the significance of the Z score obtained. These tools are part of DIANE 1.0 and are available at http://www.grc.nia.nih.gov/branches/rrb/dna/diane_software.pdf. Parameterized significant analysis is finished according to the SAM protocol (Tusher et al., 2001) with ANOVA filtering (ANOVA $p<0.05$). Significant genes are selected for each pairwise comparison. Gene set enrichment was tested using the PAGE method as previously described (Kim and Volsky, 2005). Figures were selected based on the names and descriptions provided by Gene Ontology Database and Pathway Data Set (Subramanian et al., 2005). Further gene regularly relation and canonic pathway analysis is done by the Ingenuity Pathway Analysis System (Ingenuity Systems). All raw data are available in the GEO database.