Supplementary Figure Legend

Supplementary Figure S1. Immunofluorescence staining (IF) of CD11b+ myeloid cells on spleen and tumor samples. Tumor-bearing mice were either untreated or treated with CTX+CD4 AT. 7 days after CTX treatment, mice spleens and tumor samples were collected and flash frozen in optimal cutting temperature solution. Frozen sections were stained with FITC-conjugated rat anti-mouse CD11b mAb (green). Representative images (x20 magnification) are shown.

Supplementary Figure S2. CTX+CD4 AT therapy induces an inflammatory immune milieu. Mice with established A20HA tumors were untreated or treated with CTX+CD4 AT. 7 days after CTX, total RNA was extracted from tumor masses and spleens and subject to quantitative real-time PCR (qRT-PCR) for the indicated genes. Data shown are representative of 3 independent experiments with similar results. ***, $P<0.001$.

Supplementary Figure S3. Chemoimmunotherapy leads to myeloid cell expansion in mice with lung metastasis of CT26HA colon cancer. $0.5x10^6$ CT26HA tumor cells were injected to mice via tail vein. 7 days after tumor inoculation, mice were either untreated (No Tx), or treated with CTX only, or treated with CTX followed by adoptive transfer of HA-specific CD4+ T cells (CTX+CD4 AT). 7 days after CTX treatment, spleens were processed for analyses. A, Representative dot plots showing the frequencies of different subsets of CD11b+ myeloid cells. The numbers represent the percentages of the gated CD11b+ population in total live cells. The results are summarized in bar graphs for cell frequency (B) and absolute cell number (C). Data are shown as mean ± SD with at least 3 mice per group. *, $P<0.05$. **, $P<0.01$. ***, $P<0.001$.

Supplementary Figure S4. Therapy-induced monocytes can suppress CD4+ T cell activation in response to either antigen-specific or nonspecific stimulation. Following the experimental procedures depicted in Fig. 1A, tumor-bearing mice were treated with CTX+CD4 AT. 7 days after CTX-treatment, monocytes ($CD11b^{+}Ly6C^{hi}$) and granulocytes ($CD11b^{+}Ly6C^{lo}$) were FACS-sorted from spleen cells. A, Responder cell proliferation measured by ^{3}H-thymidine incorporation upon antigen nonspecific stimulation. Spleen cells from HA-TCR Tg mice were used as responder cells. Responder cells were mixed with the indicated numbers of sorted myeloid cells, and stimulated with αCD3 and αCD28 mAbs. ^{3}H-thymidine was added to culture on day 3. 8 hrs later, cells were harvested to measure ^{3}H-thymidine uptake (CPM) shown as mean ± SD of triplicate cultures. B, Antigen-specific suppression mediated by monocytes. HA-specific CD4+ T cells purified from TCR-Tg mice were used as responder cells. CD11c+ DCs were isolated from a normal Balb/c spleen, pulsed with the cognate peptide and used as antigen-presenting cells. CD4+ responder cells were mixed with peptide-loaded DCs, in the presence or absence of variable numbers of sorted monocytes or granulocytes. Responder CD4+ T-cell proliferation was evaluated by ^{3}H-thymidine incorporation shown as mean ± SD of triplicate cultures. Results shown are representative of 2 independent experiments.

Supplementary Figure S5. Dose effect of CTX on myeloid cell induction. Naïve Balb/c mice were treated with a single injection of CTX at the indicated doses. 7 days later, spleen cells were harvested for FACS analysis. Representative dot plots are shown for co-staining of Ly6C and CD11b. Numbers in dot plots represent frequencies of the gated populations.

Supplementary Figure S6. Monocytic myeloid suppressor cells can be induced by doxorubicin (Dox) and melphalan (Mel). BALB/c mice were treated with a single dose of Dox (20 mg/kg via i.v.) or Mel (8 mg/kg via i.p.). 7 days later, spleen cells were harvested for analysis. A, Percent

of monocytic myeloid cells in treated mice. Cells were stained for CD11b and Ly6C. The bar graph summarizes the percent of monocytes (CD11b+Ly6C^{hi}) in each group. Data are shown as mean ± SD with 3-4 mice per group. ***, P<0.001. B, Monocytes induced by Dox or Mel suppress CD4+ T-cell proliferation. Monocytes were sorted from the spleens of mice treated with Dox or Mel. Spleen cells from HA-TCR Tg mice were labeled with CFSE and used as responder cells. Responder cells were mixed with equal numbers of sorted monocytes, and stimulated with αCD3 and αCD28 mAbs. After 3 days in culture, cells were stained for CD4 and analyzed by FACS. Proliferation of CD4+ responder cells was evaluated by CFSE dilution. Data shown are representative of 2 independent experiments.

Supplementary Figure S7. Low dose gemcitabine following CTX+CD4 AT therapy confers long-term survival benefit to mice with lung metastasis of CT26HA colon cancer. 0.5x10^6 CT26HA tumor cells were injected to mice via tail vein. 7 days after tumor inoculation, mice were untreated (No Tx), or treated as specified. Kaplan-Meier survival curve indicates the percentage of live mice as a function of time after tumor inoculation. The number of mice in each group is given.

Supplementary Figure S8. Low dose 5-fluorouracil (5-FU) reduces therapy-induced monocytes. Following the timeline depicted in Fig. 6 schema, mice with established A20HA tumors were treated with CTX on day 0, followed by CD4 AT on day 1. A cohort of mice received additional 5-FU treatment on day 4 and 5. On day 7, peripheral blood, spleen and tumor samples were collected for FACS analyses. Representative dot plots are shown, and the numbers represent the frequencies of the gated populations.