Supplementary figure legends

Supplementary Figure 1. Flow cytometric analyses for the generation of NAC1-deficient tumor cells. (A) Cell sorting of NAC1/Cas9 plasmid-transfected mouse B16-OVA cells, gating on RFP+. **(B)** Cell sorting of NAC1/Cas9 plasmid-transfected human A2058 melanoma cells, gating on RFP+. **(C)** Viable cell numbers at day 0, day 1, day 2, and day 3 of mouse WT B16-OVA and NAC1 KO B16-OVA cells. **(D)** Viable cell numbers at day 0, day 1, day 2, and day 3 of human WT A2058 and NAC1 KO A2058 tumor cells. Data are the representatives of three identical experiments.

Supplementary Figure 2. Generation of tyrosinase-specific human CD8+ T cells. Human CD8+ T cells from human peripheral blood mononuclear cells (PBMCs) were retrovirally transduced with tyrosinase-specific TCR genes and analyzed for tyrosinase-specific TCR expression by the tetramer staining. Data is the representative of three identical experiments.

Supplementary Figure 3. Flow cytometric analysis of PD-1+TIM-3+ CD8+ T cells under treatment with WT B16-OVA CM or NAC1 KO B16-OVA CM. (A) After incubation with WT B16-OVA conditional medium (CM) or NAC1 KO B16-OVA CM for 24 hours, expression of TIM-3 on the live CM-treated OT-I CD8+ T cells was assessed by flow cytometry. One representative dot plot and bar graph of expression that were collected from three replicates experiment is shown. ***, $P \leq 0.001$ **(B)** Flow cytometric histogram analyses of TNF-α, IFN-γ, PD-1 and TIM-3 of CD8+ T cells after incubation in the CM obtained from mouse melanoma cells in combination with 2mM or 5mM of LA. **(C)** Flow cytometric histogram analyses of IFN-γ, TNF-α, Granzyme B, and PD-1 of CD8+ T cells after incubation in the indicated CM of mouse melanoma cells. **(D)** Flow cytometric gating strategy for analyzing the exhausted T cell (TIM-3+) with the WT or NAC1 KO B16-OVA CM for 24 hours. Representative flow cytometric dot plot collected from three replicate experiments is shown.

Supplementary Figure 4. Histological analyses of tumor CTL infiltration. (A) H&E staining of mouse WT or NAC1 KO B16-OVA tumor tissues following an ACT of OT-I CD8+ T cells. Scale represents 100 mm. **(B)** H&E staining of human WT or NAC1 KO A2058 tumor tissues following an ACT of human CD8+ T cell transfer. Scale represents 100 mm.

Supplementary Figure 5. Gating strategy for analyses of indicated cytokines and exhaustion markers of CD8+ T cells. (A) Flow cytometric gating strategy for analyzing the exhausted T cells (PD-1+, TIM-3+), and cytokine production (IFN-g, Granzyme B, and TNF-a) of CD8+ T cells with the treatment strategy indicated in Figure 5A & 5C. Representative flow cytometric dot plots collected from three replicate experiments is shown. **(B)** Flow cytometric gating strategy for analyzing the cytokine production (IL-2, Granzyme B) of CD8+ T cells in tumor tissues for flow cytometric analyses. Representative dot plot collected from three replicate experiments is shown.

Supplementary Figure 6. Immunoblotting analysis of LDHA overexpression. Overexpression of LDHA by retrovirus-mediated transduction in NAC1 KO tumor cells was examined by Western blots. LDHA and b-actin are shown. **(A)** Mouse NAC1 KO B16-OVA. **(B)** Human NAC1 KO A2058 cells.

Supplementary Figure 7. Gating strategy for analyses of infiltrating CD8+ T cells in WT or NAC1 KO B16-OVA tumor tissues. Flow cytometric gating strategy for analyzing the exhausted T cells (PD-1+, TIM-3+), and cytokine production (IL-2, Granzyme B) of Thy1.2+ CD8+ T cells in tumor tissues.

Supplementary Figure 8. Gating strategy for infiltrating CD8+ T cells in WT or NAC1 KO A20258 tumor tissues. (A) Flow cytometric gating strategy for analyzing IL-2, Granzyme B, and IFN-γ of CD8+ T cells in tumor tissues. Representative dot plot collected from three replicate experiments is shown. **(B)** Flow cytometric gating strategy for analyzing PD-1 and TIM-3 of CD8+ T cells in tumor tissues. Representative dot plot collected from three replicate experiments is shown.