Figure S1 Determination of the optimal route of administration for LV305. (a) Female BALB/c mice (n = 8 per group) were injected with 4 x 10¹⁰ vector genomes of LV305, SC, in the tail base, scruff of neck, or footpad; ID, in the lower back; IV; or IM. Splenic T cell responses were measured by ICS 14 days later. (b) Female BALB/c mice (n = 8 per group) were injected with LV305, SC, in the tail base, at the designated vector genome doses. Splenic T cell responses were measured by ICS 14 days later. Error bars represent mean ± SEM; bars represent mean. SC, subcutaneous; ID, intradermal; IV, intravenous; IM, intramuscular; ICS, intracellular cytokine staining; CTL, cytotoxic T lymphocyte; ** p < 0.005; *** p < 0.0005. Data are representative of 2 independent experiments.



Figure S2 Generation of CIN.23, a CT26 murine colon cancer cell line that stably expresses human NY-ESO-1 protein. CT26 cells were transduced with lentiviral vector encoding NY-ESO-1. Transduced cells were serially diluted to near single cell per well of a 96-well plate. Single-cell colonies were stained with anti-NY-ESO-1 antibody to analyze expression of NY-ESO-1 by flow cytometry. Data are representative of at least 3 independent experiments.



Figure S3 Adoptive transfer of CD8 T cells, CD4 T cells, or NK cells from LV305-immunized mice mediate anti-tumor immunity. On Day 0, female BALB/c mice (n = 5 per group) were injected with 1.5×10^5 CIN.23 cells, IV. On Day 3, tumor-bearing mice were injected, IV, with 1.0×10^6 cells (as designated in the figure) isolated from LV305- or mock-immunized donor mice. On Day 18, (a) tumor nodules in lungs were enumerated, and (b) splenic T cell responses were measured by ICS. Bars represent mean; error bars represent mean ± SEM. IV, intravenous; * p < 0.05. Data are representative of 2 independent experiments.

