Supplemental Figure 1.



Supplemental Figure 1. Electron microscopic image showing evidence of autophagy in CNS1 tumor cells treated with Ad-TK/GCV. Arrows indicate autophagic vesicles, a hallmark of autophagy.

## Supplemental Figure 2.



## A DC yield from bone-marrow precursor cells





Supplemental Figure 2. Characterization of bone marrow-derived dendritic cells (DCs) induced by Flt3L+IL-6 or GM-CSF+IL-4. A, Yield of DCs from bone marrow precursor cells. Ten million bone marrow cells were cultured in RPMI conditioned media supplemented with 100 µg/ml Flt3L + 50 µg/ml IL-6 (FL-IL6), 100 µg/mL Flt3L (FL) or 10 µg/mL GM-CSF + 10 µg/mL IL-4 (GM-IL4) every 2-3 days. Loosely attached cells were harvested at day 7-8 and counted using trypan blue dye exclusion. Values indicate the average number of DCs yielded from bone marrow precursor cells (% DC yield). B, Micrographs show the morphology of FL-IL6-induced and GM-IL4-induced DCs after 7 days in culture. Insets show higher magnification images of cells that exhibit typical long cytoplasmic processes. Scale bar = 5µm. .

Supplemental Figure 3.



Supplemental Figure 3. Flt3L + IL-6 generated DC express high levels of CD161a rather than OX62 and consist mainly of conventional DC. Ten million bone marrow cells were cultured in RPMI conditioned media supplemented with 100  $\mu$ g/ml Flt3L + 50  $\mu$ g/ml IL-6 (FL-IL6). Loosely attached cells were harvested at day 7. Surface molecules OX-62, CD161a, CD3, CD4, CD45R, CD11c, CD45R and MHCII were stained and measured by flow cytometry. A, Representative histogram and corresponding percentage of OX62+ or CD161a+ cells are shown. B, Representative dot plot and the proportion of plasmacytoid DC (pDC; CD3- CD4+ CD45R+) and conventional DC (cDC; CD11c+ CD45<sup>high</sup> MHCII+) from FL-IL6 generated DCs are shown.

## **Supplemental Figure 4.**



**Supplemental Figure 4. Flt3L + IL-6 induced DC stimulates cytotoxic lymphocytes. A**, Schematic depicting T cell stimulatory activity of FL-IL6-induced DC. FL-IL6-induced BMDCs from naïve Lewis rats were loaded with Ad-TK/GCV treated dying tumor cells for 5h and then cocultured for 10 days with lymphocytes derived from the cervical lymph nodes from CNS1 tumor bearing rats treated with FL-IL6 DC vaccine. B, Photos of individual lymphocyte cultures after 10-day co-culture with tumor-loaded DCs, unloaded DCs or without DCs. Lymphocytes incubated with unloaded DCs, or without DCs, failed to proliferate and eventually died during the incubation . **C**, Cytotoxicity of lymphocytes pulsed with tumor-loaded DCs were tested by flow cytometry. CNS1 cells stained with VivoTag 680 were cultured with increasing numbers of lymphocytes pulsed with tumor-loaded DCs, or lymphocytes from a naïve rat as a control. After 5 h coincubation, cell death of CNS1 tumor cells was assessed by Annexin V-FITC and PI staining followed by flow cytometry. Cells positive for either Annexin-V or PI were considered to be dead. Data are represented as the percentage of dead tumor cells in the co-culture.