SUPPLEMENTAL MATERIALI

la Sala et al., http://www.jem.org/cgi/content/full/jem.20080912/DC1

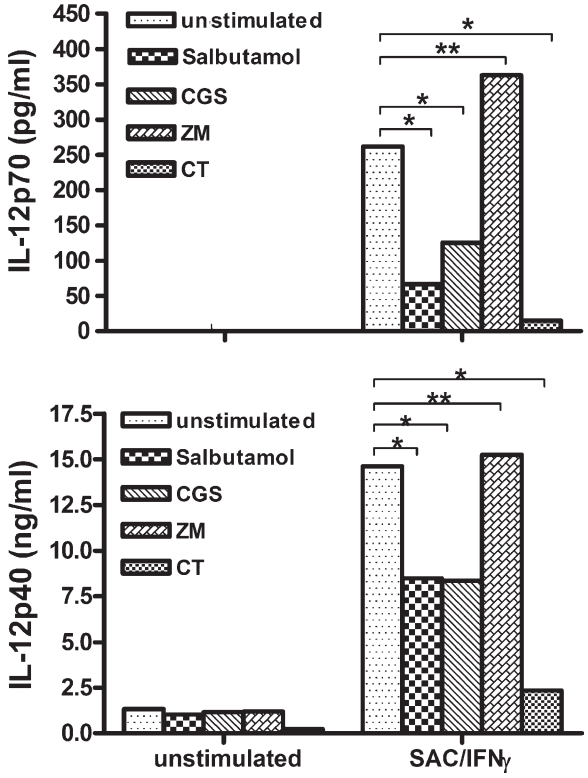

Figure S1. Regulation of IL-12 production from murine cDCs by Gs protein signaling pathways. Murine CD11c$^+$ splenic cDCs were stimulated with SAC (0.02%)/IFN-γ (10 ng/ml) alone or in the presence of the β_2 adrenergic agonist salbutamol (10 μM), the adenosine A2a receptor agonist CGS21680 (10 μM), the adenosine A2a receptor antagonist ZM241385 (1 μM), or CT (5 μg/ml). Culture supernatants were collected after 24 h, and IL-12 p40 and p70 concentrations were measured by ELISA. Results are shown from a representative experiment of two with similar results (*, $P \leq 0.02$; **, $P \geq 0.5$).

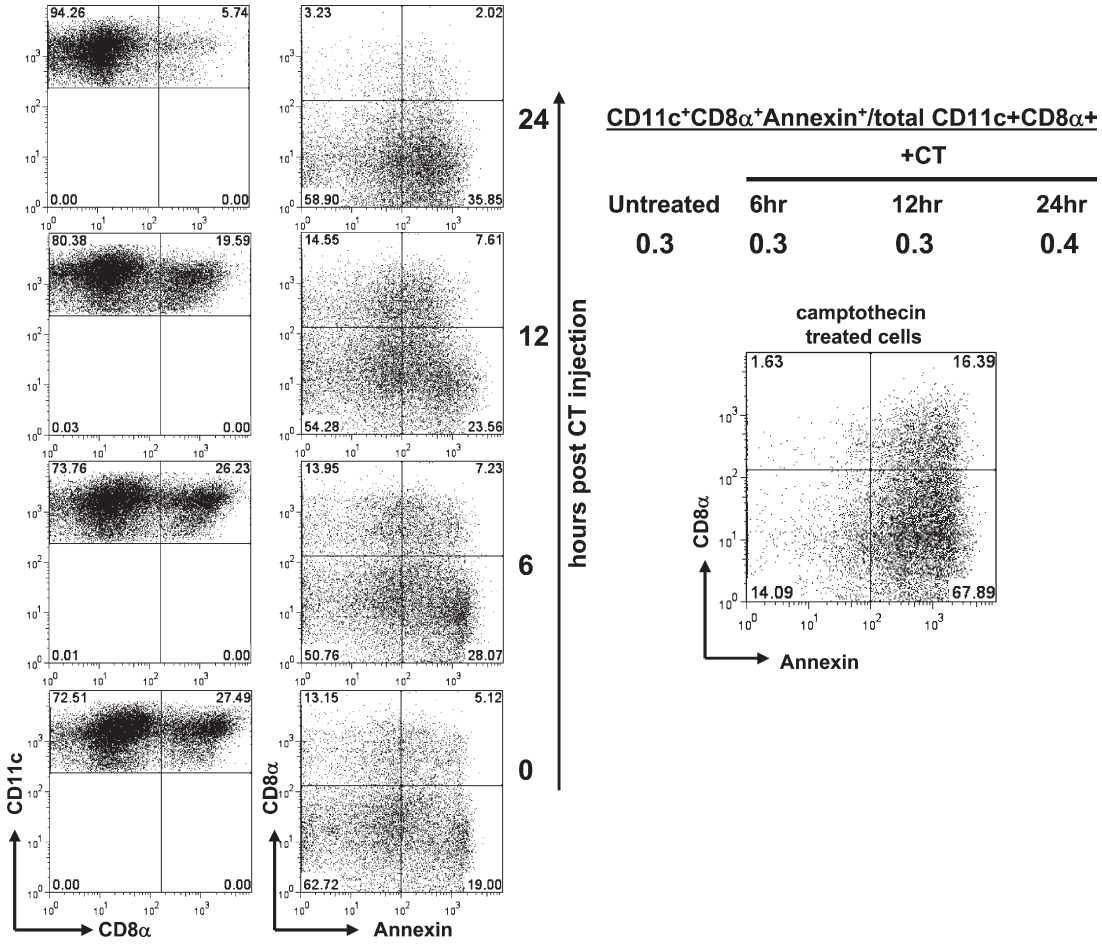

Figure S2. CT treatment does not enhance the apoptosis of CD11c$^+$CD8α^+ cells. After treatment of mice with CT (5 μg i.p.), CD11c$^+$ splenocytes were stained for CD8α, CD11c, and annexin V, and analyzed by flow cytometry. Ratio of annexin$^+$ cells within the CD11c$^+$ CD8$^+$ cell gate to total CD11c$^+$ CD8$^+$ cells is shown. Gates for annexin V staining were established using CD11c$^+$ splenocytes treated with camptothecin in vitro for 4 h at 37° C to induce apoptosis (bottom right). Data shown are representative of three experiments producing similar results.

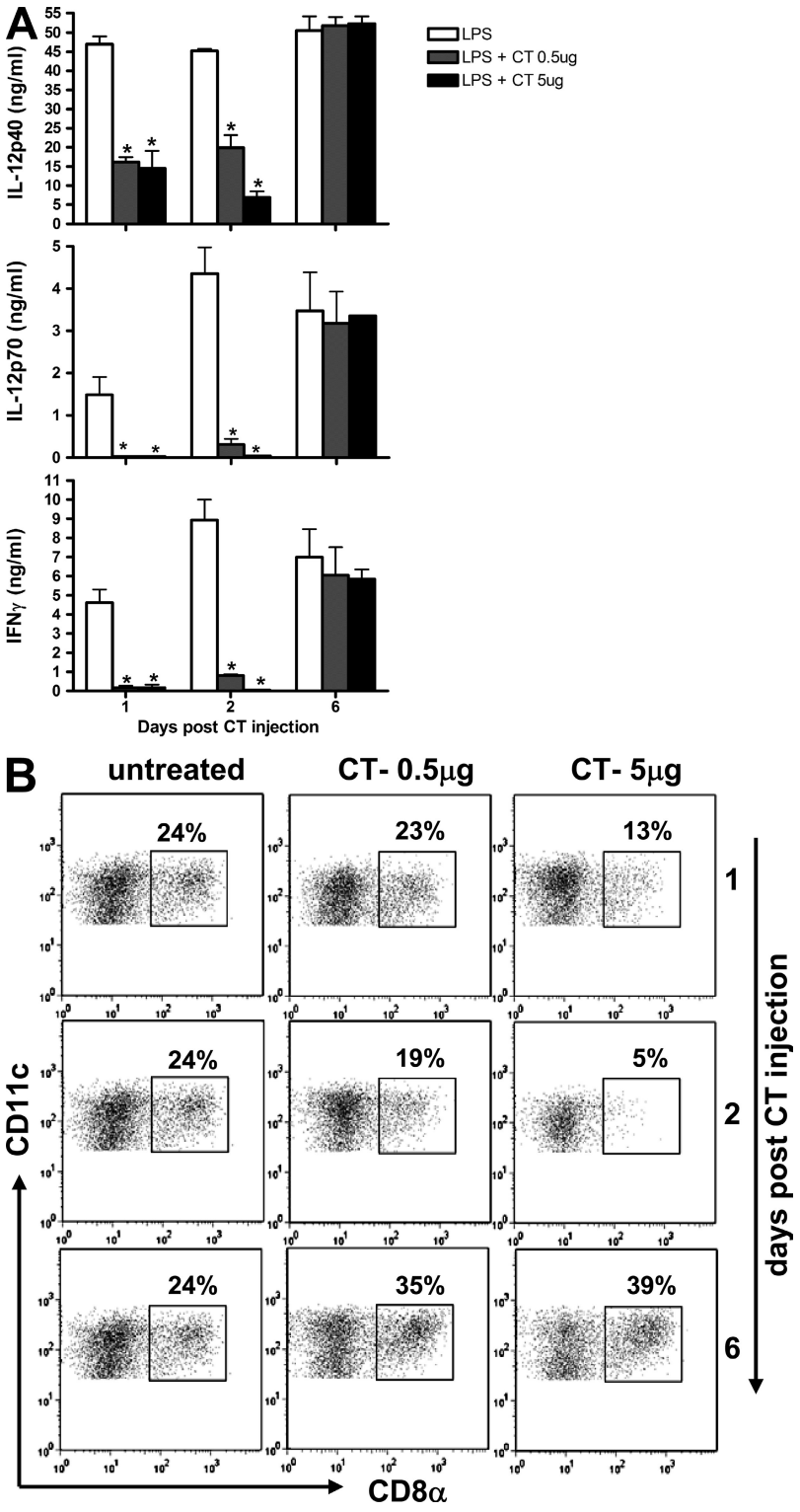

Figure S3. CT dose-dependent changes in serum IL-12 and IFN-γ concentration after systemic LPS administration and in the loss of CD8α⁺ cDCs. (A) BALB/c mice were injected i.p. with 5 or 0.5 μg CT or they were left untreated. At the indicated time points, mice were given LPS (250 μg) i.p. Sera were collected 6 h after LPS exposure and levels of serum IL-12p40, IL-12p70, and IFN-γ were measured by ELISA. Data are presented as mean ± SD of values from three individual mice for each conditions, and are representative of three independent experiments showing similar effects (*, $P \leq 0.02$). (B) Flow cytometry of CD11c⁺ cell–enriched spleen cells after CT administration as in A. Shown are data from cells pooled from three mice for each condition, and are representative of three experiments with similar results.

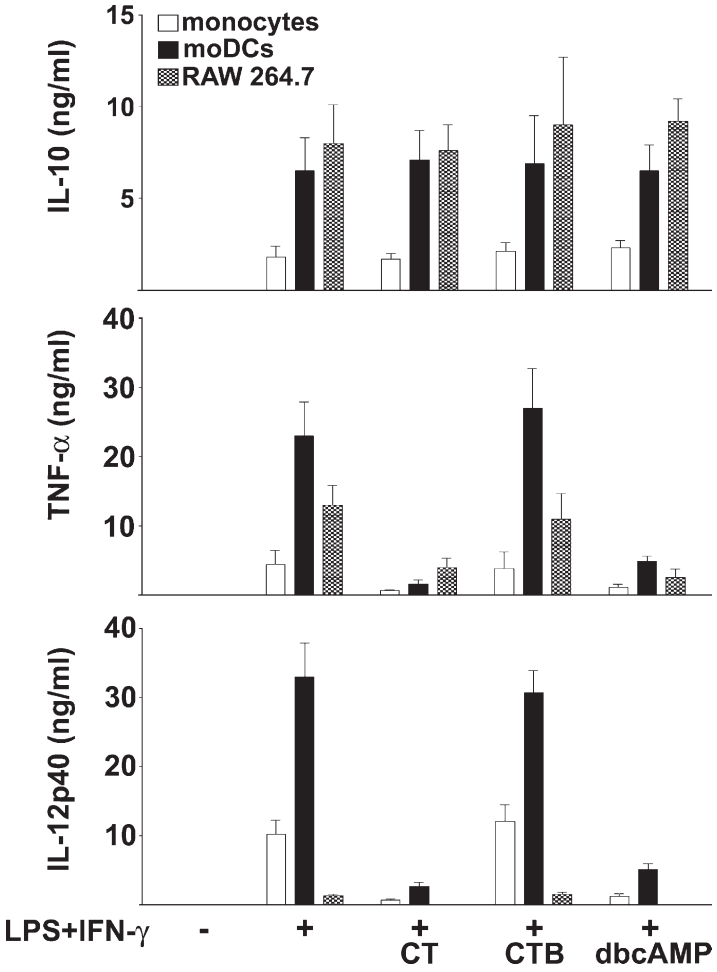

Figure S4. CT and dbcAMP inhibit the production of IL-12p40 and TNF-α but not IL-10 by different cell types. Human peripheral blood monocytes, monocyte-derived DCs (Mo-DC), or the RAW 264.7 cell line were stimulated with LPS and IFN-γ in the absence or presence of CT, CTB, or dbcAMP, and cytokines were measured by ELISA in cell culture supernatants after 24 h. Data are expressed as mean ± SD of triplicate cultures and are representative of two experiments showing similar results.