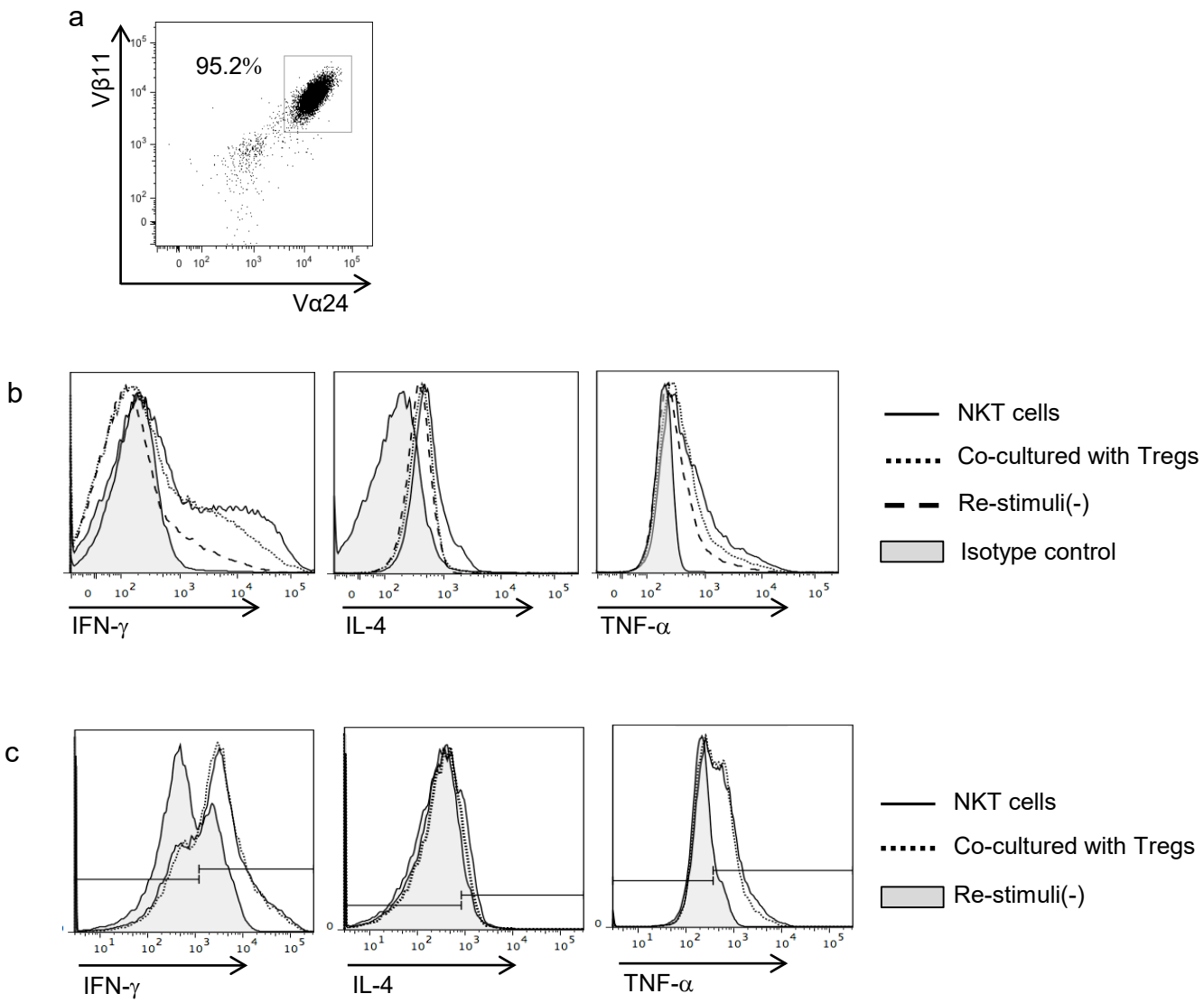

Fig. S1 Suppressive effects of Tregs on cytokine production of NKT cells
(**a**) Representative profile of isolated NKT cells. (**b**) Representative FACS profiles. The *solid line* histogram indicates NKT cells cultured without Tregs, the *dotted line* histogram indicates NKT cells co-cultured with Tregs, the *dashed line* histogram indicates NKT cells without re-stimulation [re-stimuli(-)], and the *gray-shaded* histogram indicates the isotype control. (**c**) The levels of IFN-γ, IL-4, and TNF-α secretion by NKT cells were evaluated with flow cytometry. The *solid line* histogram indicates NKT cells re-stimulated in the absence of Tregs, the *dotted line* histogram indicates NKT cells re-stimulated with Tregs, and the *gray-shaded* histogram indicates NKT cells without re-stimulation.

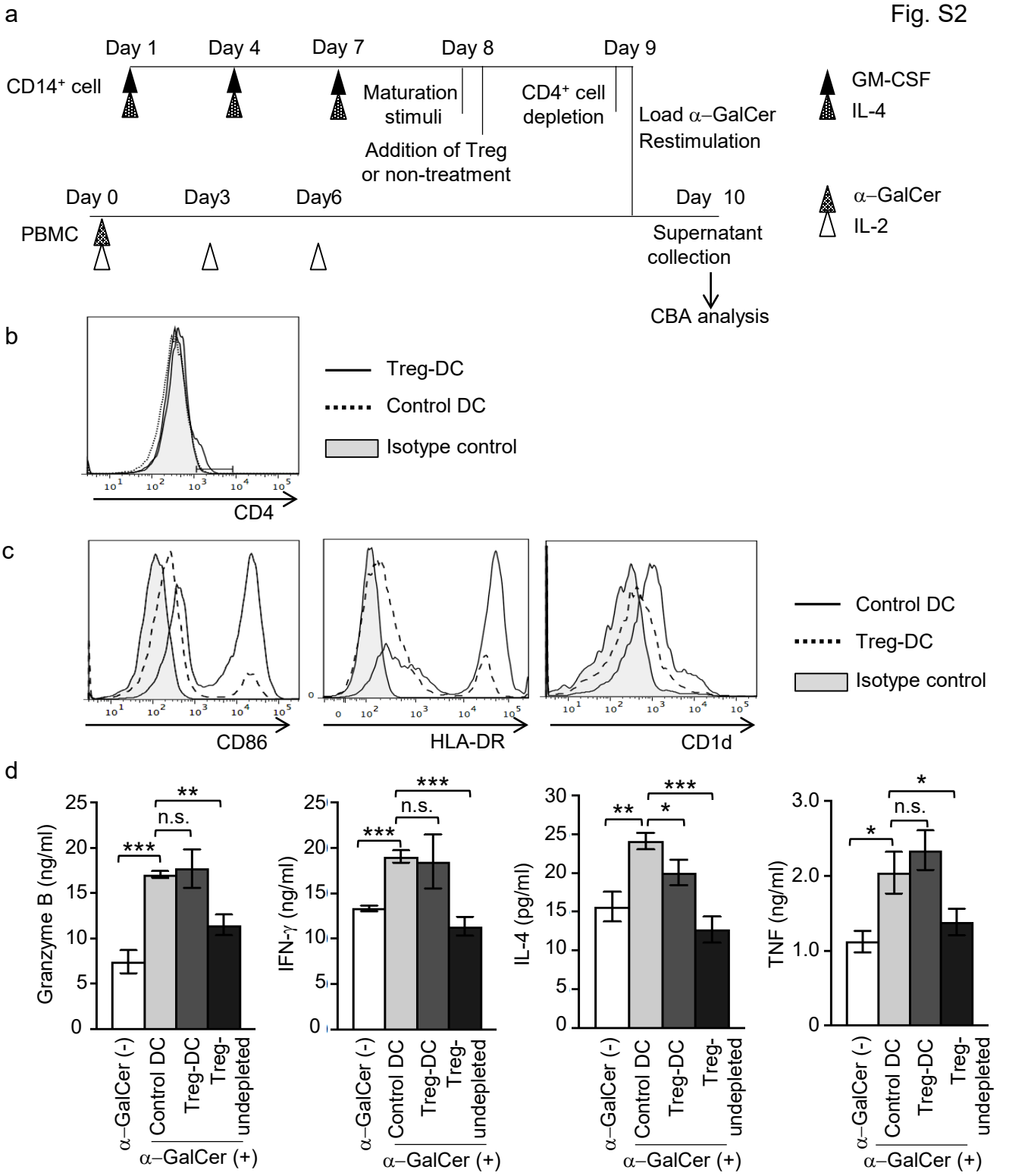

Fig. S2 Restimulation of NKT cells with DCs pre-incubated with Tregs

(a) The schedule of the assay. DCs derived from CD14⁺ cells were maturated upon OK-432 stimuli with (Treg-DC) or without Tregs (Control DC). After 24 hours, CD4⁺ cells were depleted from these cells using autoMACS. The cultured NKT cells were restimulated with α–GalCer-pulsed DCs (control-DC or Treg-DC). After 24-hours incubation, the supernatant was collected and CBA was performed to evaluate the cytokine production. (**b)** CD4 and **(c)** CD86, HLA-DR and CD1d expressions on DCs after CD4⁺ cell depletion using autoMACS; the *solid line* histogram indicates the control DC, the *dotted line* histogram indicates Treg-DC and the *gray-shaded* histogram indicates the isotype control. (**D**) The levels of granzyme B, IFN-γ, IL-4, and TNF in supernatants of NKT cell cultures are shown. Data are shown as means ± SD. Statistical comparisons were performed using unpaired *t*-tests. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. α–GalCer: α-galactosylceramide, CBA: Cytometric beads assay, n.s.: not significant.

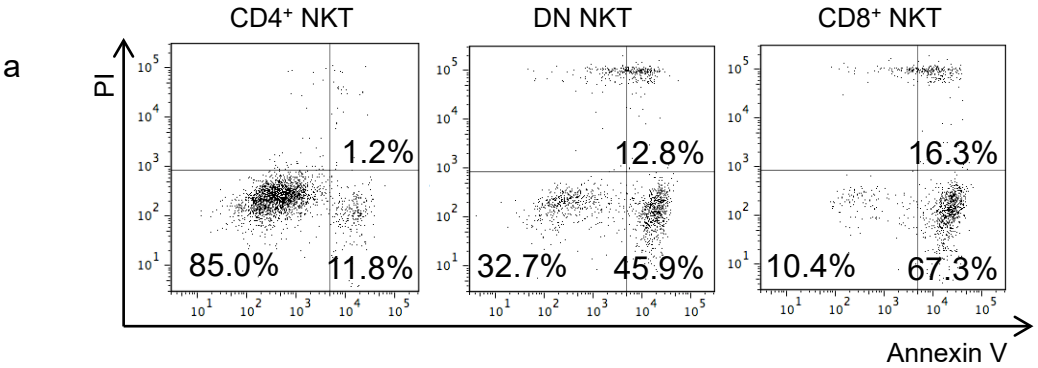

Fig. S3 Cytotoxic activity of each subset of NKT cells

(a) Representative Annexin V-PI staining pattern of each subset at an E:T ratio of 10:1 is shown. $CD4^+$ NKT:$CD4^+$ NKT cells, DN NKT:$CD4^-CD8^-$ NKT cells, and $CD8^+$ NKT:$CD8^+$ NKT cells

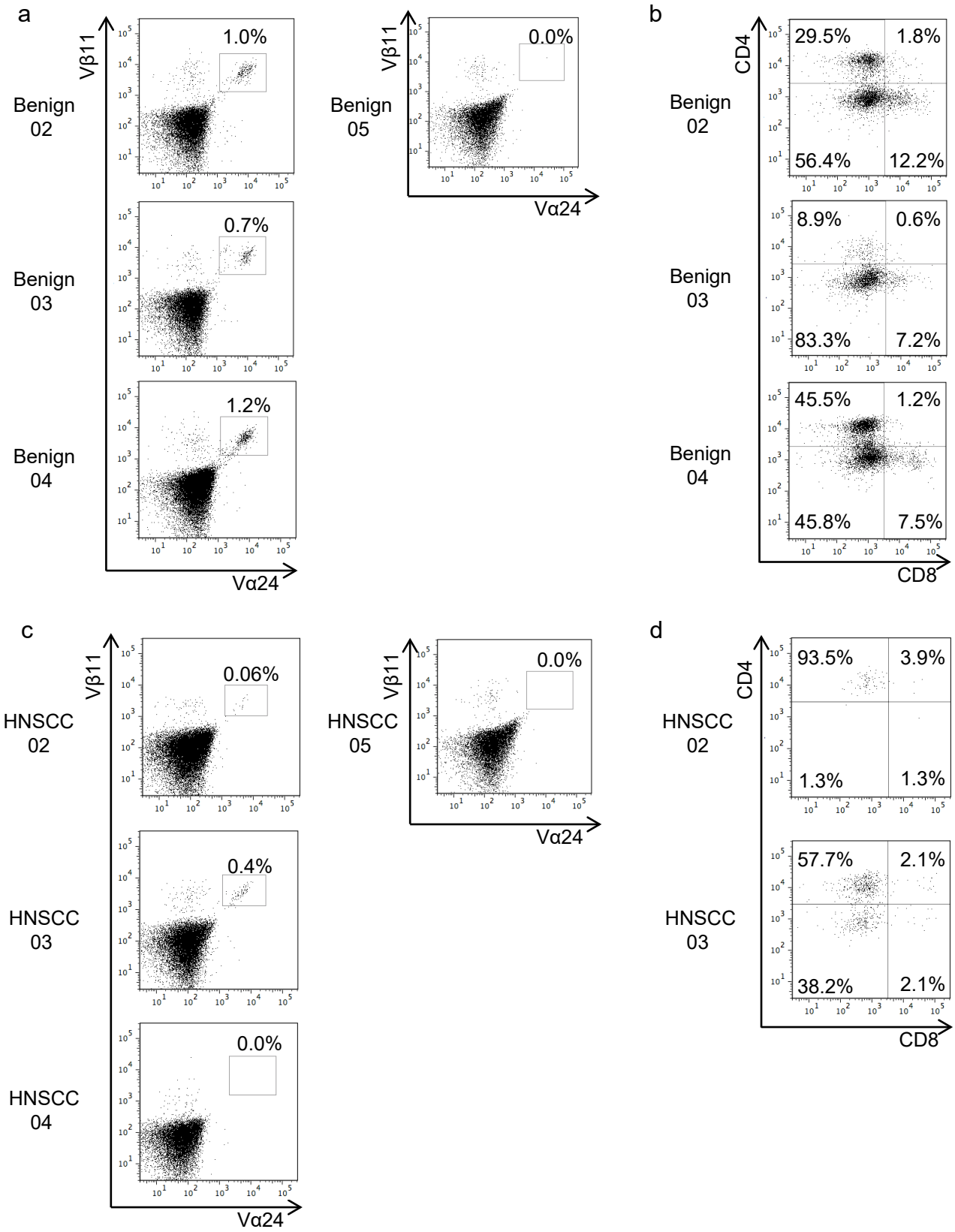

Fig. S4 Frequency and CD4/CD8 expression of NKT cells

The frequencies and the CD4/CD8 expressions of cultured NKT cells obtained from patients with benign tumors **(a and b)** and malignant tumors **(c and d)** are shown. The CD4/CD8 expressions of the three cases, Benign 05, HNSCC 04 and HNSCC 05, were not indicated because of their lack of NKT cell expansion. Benign: patients with benign tumors, HNSCC: patients with head and neck squamous cell carcinoma