

Supplementary data figure legends.

Supplementary Figure 1. In-vitro expansion of EGFR-specific CTL in the presence of NK cells. Higher frequency of EGFR₈₅₃₋₈₆₁ peptide-specific CTL was observed on day 7 and day 14 in the condition when DC (from HLA-A2⁺ healthy donor) were cocultured with EGFR⁺, HLA-A2⁻ PCI-15B cells in presence of cetuximab (10µg/ml), with or without autologous NK cells (DC:NK: PCI-15B;1:1:1 ratio) in comparison to panitumumab. Representative figures of three independent experiments are shown.

Supplementary Figure 2. Enhancement of DC maturation by cetuximab-activated NK cells. Histogram analysis of the upregulation of maturation markers HLA-DR, CD80, CD83, CD86 on CD11c⁺ gated DC co-cultured with NK:PCI-15B in the presence of no treatment or panitumumab or cetuximab (each 10µg/ml, 48h). MFI values are indicated for each histogram. Representative figure of three independent experiments are shown.

Supplementary Figure 3: Importance of IFN-γ released by cetuximab activated NK cells in the enhancement of DC maturation. **(A-B)** Percentages of HLA-DR⁺ DC in DC preparations co-cultured with NK:PCI-15B (at 1:1:1 ratio) with no treatment or with IgG1 or cetuximab (each at 10µg/ml, 48h) were measured by flow cytometry. In parallel, anti-IFN-γ mAb or anti-IL-12p40/70 mAb (each at 10µg/ml) were added along with cetuximab to the co-culture of DC:NK:PCI-15B, and DC maturation markers (HLA-DR,CD80,CD83,CD86) were analyzed. Data are representative of three experiments from different donors.

Supplementary Figure 4: The absence of cetuximab in the NK:DC co-culture (without HNC cells) or DC:PCI-15B co-culture (without NK cells) abrogated the IFN-γ secretion. Levels of IFN-γ were measured after co-culture of NK:PCI-15B (1:1 ratio) or DC:NK:PCI-15B (1:1:1 ratio) or DC:PCI-15B (1:1 ratio) or DC: NK (1:1:1 ratio) with no treatment or with IgG1 or panitumumab or cetuximab (each at 10µg/ml) after 24h by ELISPOT assay. A two-tailed unpaired t-test was performed for statistical analysis.

Supplementary Figure 5: Enhancement of DC maturation by IL-2 activated NK cells. Histogram analysis of the upregulation of maturation markers CD80, CD86, and HLA-DR on DC co-cultured with rhIL-2 (100 units/ml,48h) or NK with and without IL-2 (100 units/ml, 48h). Thin line (untreated DC) and bold line (treated DC) are shown along with isotype (filled histogram). Representative figure of two independent experiments are shown.