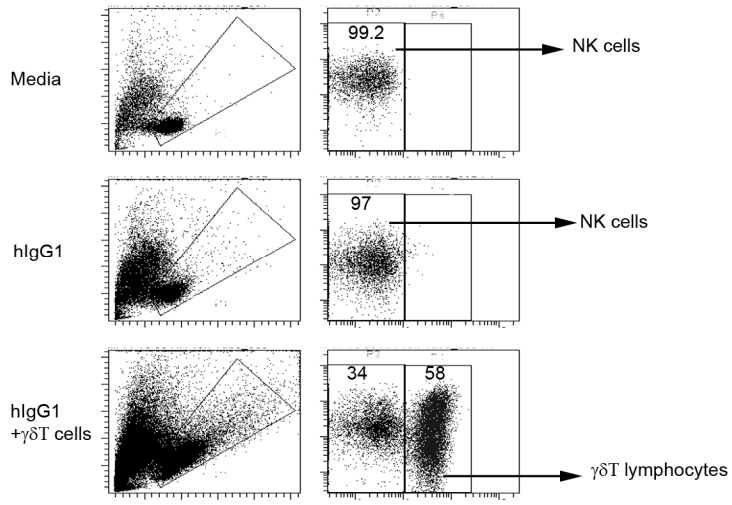

Figure S1. Gating strategy used in NK cells and γδ T lymphocytes coculture
An example of flow cytometry analysis shows the gating of NK cells and γδ T lymphocytes used in all NK activation and cytotoxicity experiments. Purified NK cells were cultured with media, in the presence of plate-immobilized hIgG1 (2.5 μg/ml) with or without the presence of IPP+IL-2 expanded γδ T lymphocytes at 4:1 ratio, after 48h cells were analyzed by flow cytometry. Dot plots represent forward and side scatter of gated T lymphocyte populations. NK cells and γδ T lymphocytes were further gated based on $CD3^{-}CD56^{+}$ NK cells and $CD3^{+}CD56^{+}$ and $CD3^{+}CD56^{-}$ γδ T lymphocytes. A representative data from twenty independent experiments is shown.

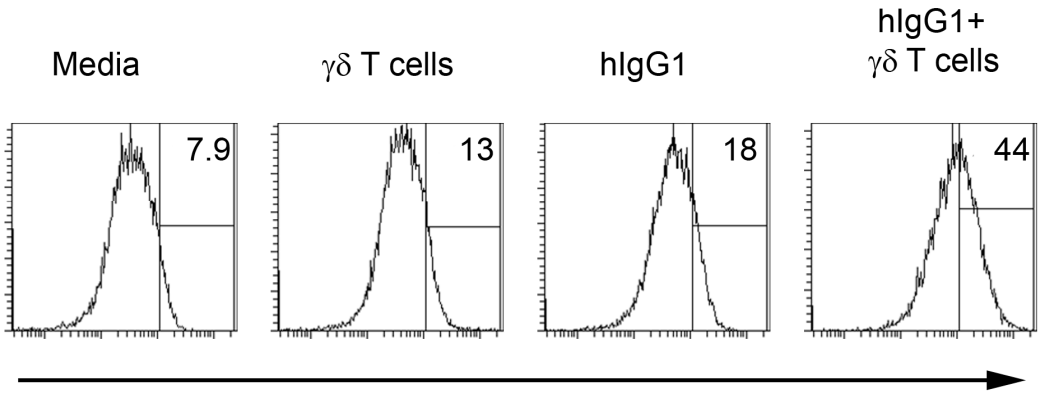

Figure S2. γδ T lymphocytes enhance hIgG1 induced activation of NK cells
Purified NK cells and IPP+IL-2 expanded γδ T cells were co-cultured at 4:1 ratio in the presence or absence of plate-immobilized hIgG1 (2.5 μg/ml) for 48 hours and the expression of CD54 was analyzed by flow cytometry. Histogram represents gated $CD3^{-}CD56^{+}$ NK cells. A representative data from twenty independent experiments is shown.

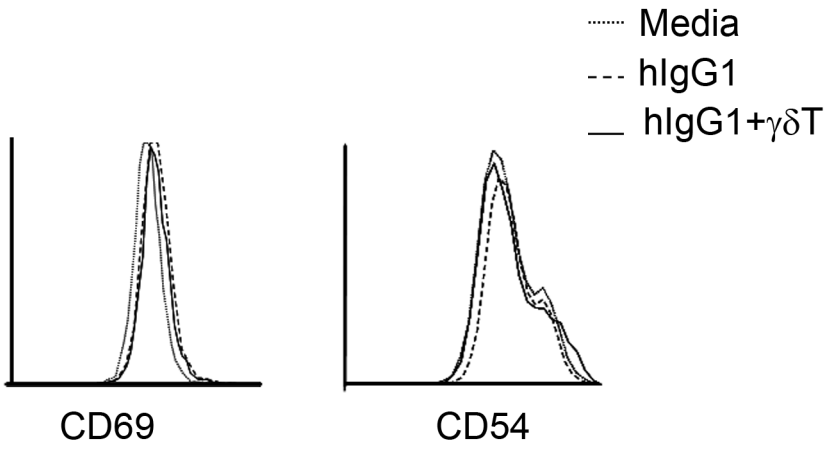

Figure S3. hIgG1 primed human NK cells fail to induce activation of gamma delta T lymphocytes
Purified NK cells (2×10^5 cells/well) were cultured with indicated numbers of IPP+IL-2 expanded γδ T lymphocytes in a presence of immobilized hIgG1 (2.5 μg/ml) for 48 hours. Expression of activation markers CD69 and CD54 were assessed by flow cytometry. Overlay of histograms representing gated $CD3^{+}\gamma\delta TCR^{+}$ T lymphocytes . Representative data from one of the ten independent experiments is shown.

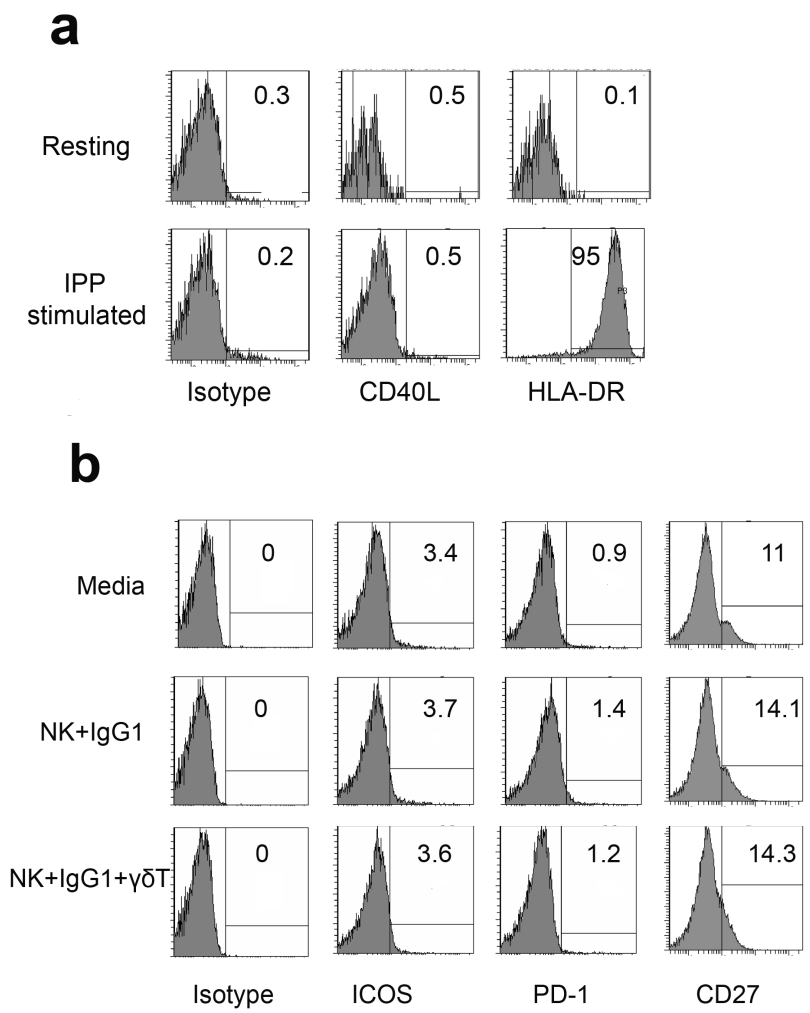

Figure S4. Expression of costimulatory ligands and receptors on γδ T lymphocytes and NK cells

(A) Fresh γδT lymphocytes from normal donors (top histograms) or γδT lymphocytes expanded in the presence of IPP+IL-2 (lower histograms) were stained with mAb specific for CD40L and HLA-DR. The expression of indicated costimulatory ligands on gated $CD3^{+}\gamma\delta TCR^{+}$ cells is shown. (B) NK cells cultured with media alone or immobilized hIgG1 with or without *in vitro* expanded γδ T lymphocytes for 48 h were stained with mAbs specific for ICOS, PD-1, and CD27. Overlay of histograms represents gated NK cells ($CD3^{-}CD56^{+}$) are shown. Data are representative of five independent experiments.

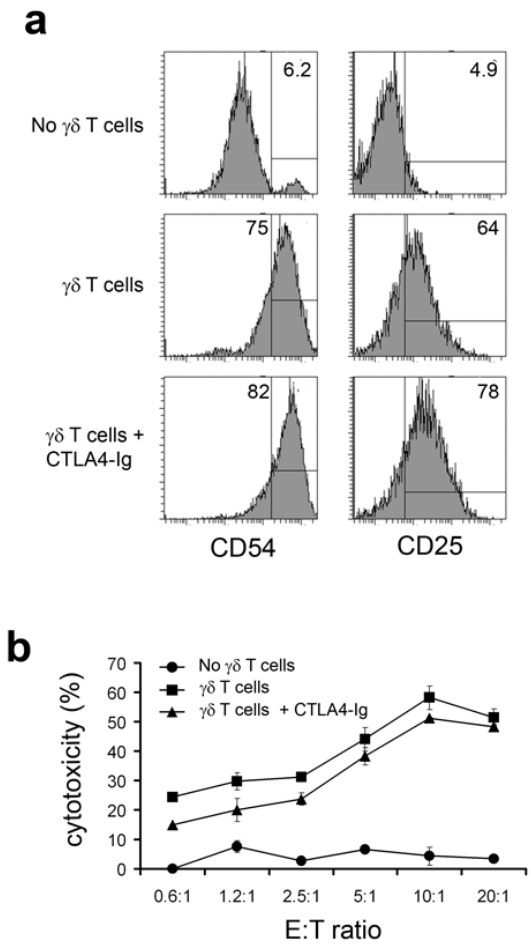

Figure S5. Blocking of B7-1/CTLA-4 does not inhibit γδ T lymphocytes induced activation of NK cells

(A) Purified NK cells (2×10^5 cells/well) were cultured with indicated numbers of IPP+IL2 expanded γδ T lymphocytes in a presence of immobilized hIgG1 (2.5 μg/ml) or hIgG1 alone for 48 hours. CTLA4-Ig (10 μg/ml) was added to wells containing NK cells and IPP+IL-2 expanded γδ T lymphocytes. After 48 h of culture, cells were stained for CD54 and CD25 expression. The histograms represent gated $CD3^{-}CD56^{+}$ NK cells. Data are representative of five independent experiments. (B) Soluble CTLA4-Ig fusion protein (10 μg/ml) was included during the culture of purified NK cells and γδ T lymphocytes (4:1 ratio) in hIgG1 pre-coated plates for 48 hours. Cytotoxicity of NK cells repurified using magnetic beads was analyzed in a standard 4 hour ^{51}Cr-release assay against TU167 SCCHN cell line. Data are presented as mean±SD of triplicate samples and representative of five independent experiments.

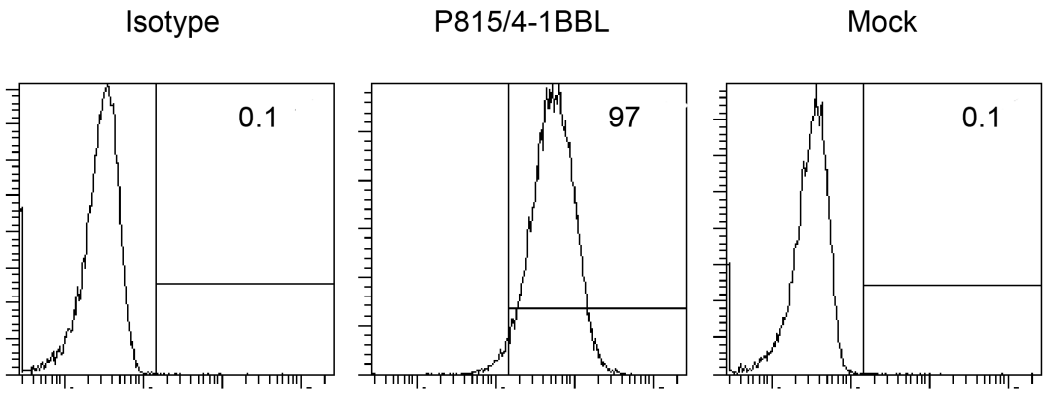

Figure S6. Characterization of P815/4-1BBL cells
P815/4-1BBL and mock cells (1×10^6 cells) were stained with anti–4-1BBL for 30 min and the expression of 4-1BBL was assessed by flow cytometry. Histogram represents gated p815 cells either expressing 4-1BBL or mock, isotype matched control was included for staining. A representative data from one of the five independent experiments is shown.

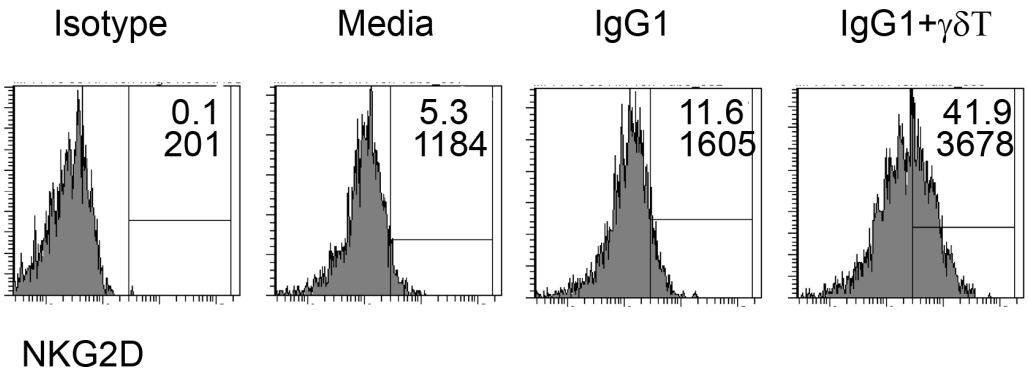

Figure S7. γδ T lymphocytes coculture with NK cells results in enhanced NKG2D expression
NK cells cultured with media alone or immobilized hIgG1 with or without *in vitro* expanded γδ T lymphocytes (4:1). After 48 hours of culture, the expression of NKG2D was assessed on NK cells. Histogram represent NKG2D expression on gated $CD3^{-}CD56^{+}$ NK cells. Percentage of NKG2D expression and MFI of NKG2D expression were presented, isotype matched antibody staining was included as negative control. A representative data from one of the ten independent experiments is shown.

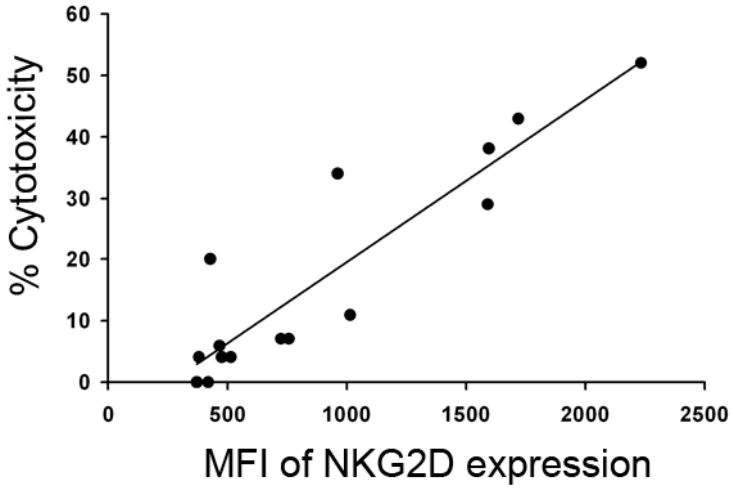

Figure S8. Correlation between NK cells NKG2D expression and in vitro cytolytic activity against HNSSC tumor

NK cells purified from PBMC of 15 individual donors were co-cultured in the presence of media alone, on plates pre-coated with hIgG1 with or with out expanded γδ T lymphocytes (4:1 ratio). After 48 h of culture, NK cells from media alone or IgG1 primed NK cells and NK cells repurified from γδ T lymphocyte coculture were tested for the cytolytic activity in a standard 4 h ^{51}Cr-release assay against indicated tumor targets. Graph is ploted as percentage of specific HNSSC tumor (Tu 167) killing against MFI of NKG2D expression. Linear coefficient comparing trend of all individuals, $R^2 = 0.82$.

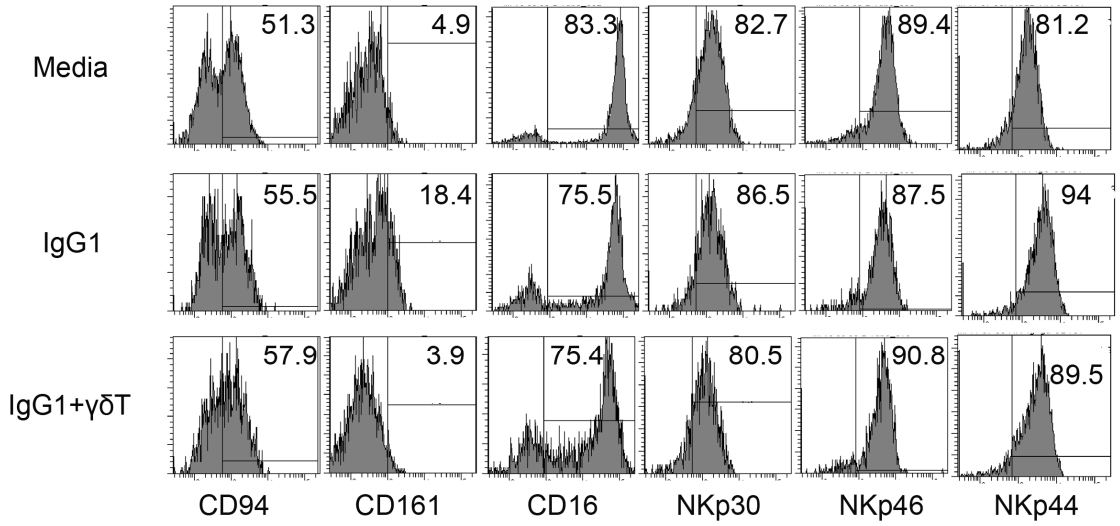

Figure S9. Regulation of NK receptors expression by γδ T lymphocytes
NK cells cultured with media alone or immobilized hIgG1 with or without *in vitro* expanded γδ T lymphocytes for 48 hours were stained for the classical NK activating and inhibitory receptors as CD16, NKp30, NKp46, NKp44, CD94, CD161 and analysed by Flow cytometry. Histogram represents $CD3^{-}CD56^{+}$ NK cells. A representative experiment from five is shown.

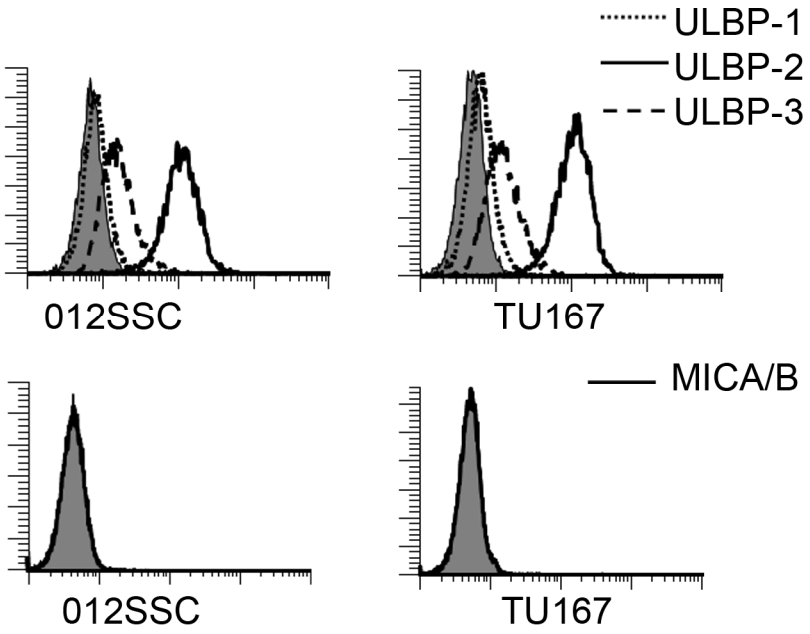

Figure S10. SSCHN cells express ligands for NKG2D
SSCHN cells (1×10^6 cells) were stained for NKG2D ligands i.e ULBPs and MICA/B for 30 min and the expression of ULBPs and MICA/B were assessed by flow cytometry. Histogram were gated on 012SSC or TU167 tumor cells expressing ULBPs or MICA/B, isotype matched control (Gray filled histogram) was included for staining. A representative data from one of the five independent experiments is shown.