Supplemental Figure 1

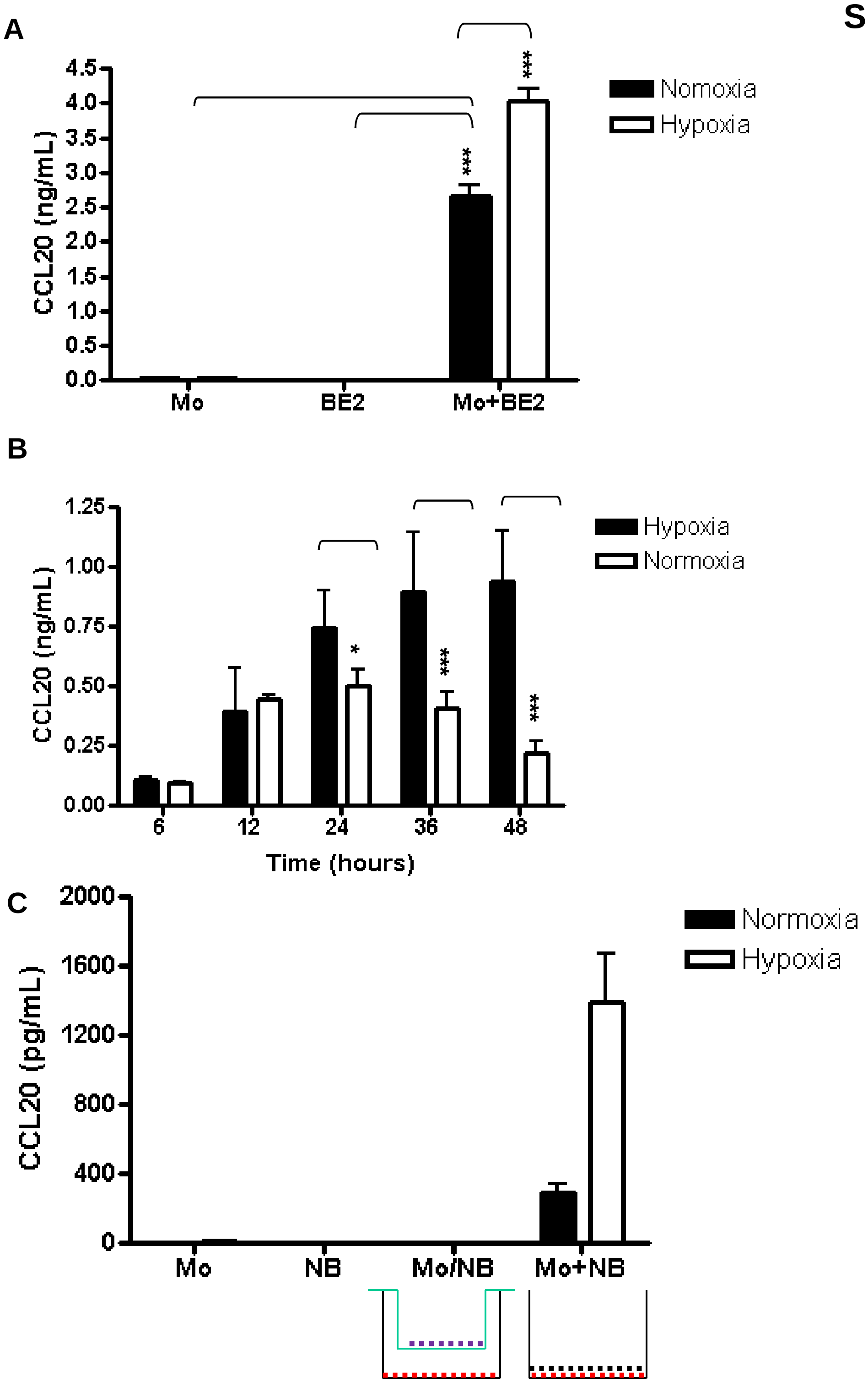

Figure S-1: Contact with NB cells and hypoxia synergistically induce CCL20 in human monocytes. (A) Monocytes and SK-N-BE2 NB cells were cultured alone or combined in hypoxic or normoxic conditions for 48 h. CCL20 concentration was quantified in the supernatants from indicated conditions using ELISA. Data are from a representative of three experiments. **(B)** Monocytes were co-cultured with CHLA-255 NB cells under normoxic or hypoxic conditions. Supernatants were collected at indicated time points and CCL20 concentration was measured by ELISA. Data are from a representative of three experiments. **(C)** Monocytes and NB cells were cultured alone or co-cultured in the same wells or in Transwell chambers, separated by 0.4 µm membrane for 36 hrs. CCL20 concentration was measured by ELISA. Data are from a representative of three experiments.

Supplemental Figure 2

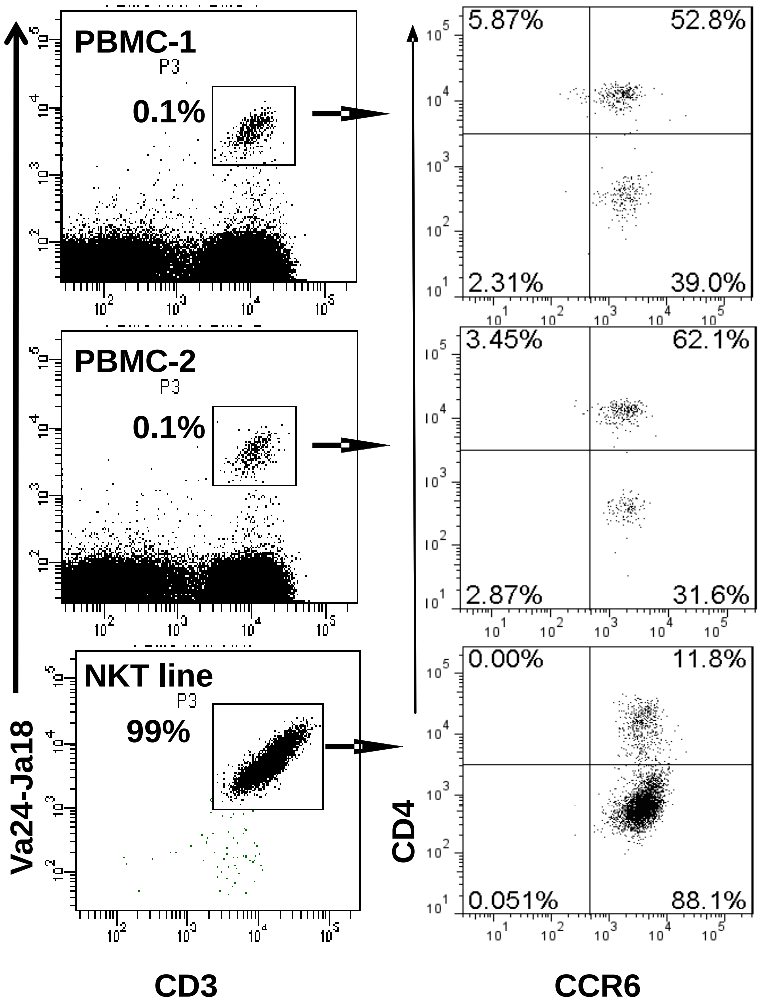

Figure S-2: CCR6 expression on NKT cells. Primary NKT cells from freshly isolated PBMC of two individuals (PBMC-1 and -2) and ex vivo expanded NKT cells (NKT line) were identified by FACS as CD3+Vα24-Jα18+ events (left panel) and analyzed for CD4 and CCR6 expression (right panel).

Supplemental Figure 3

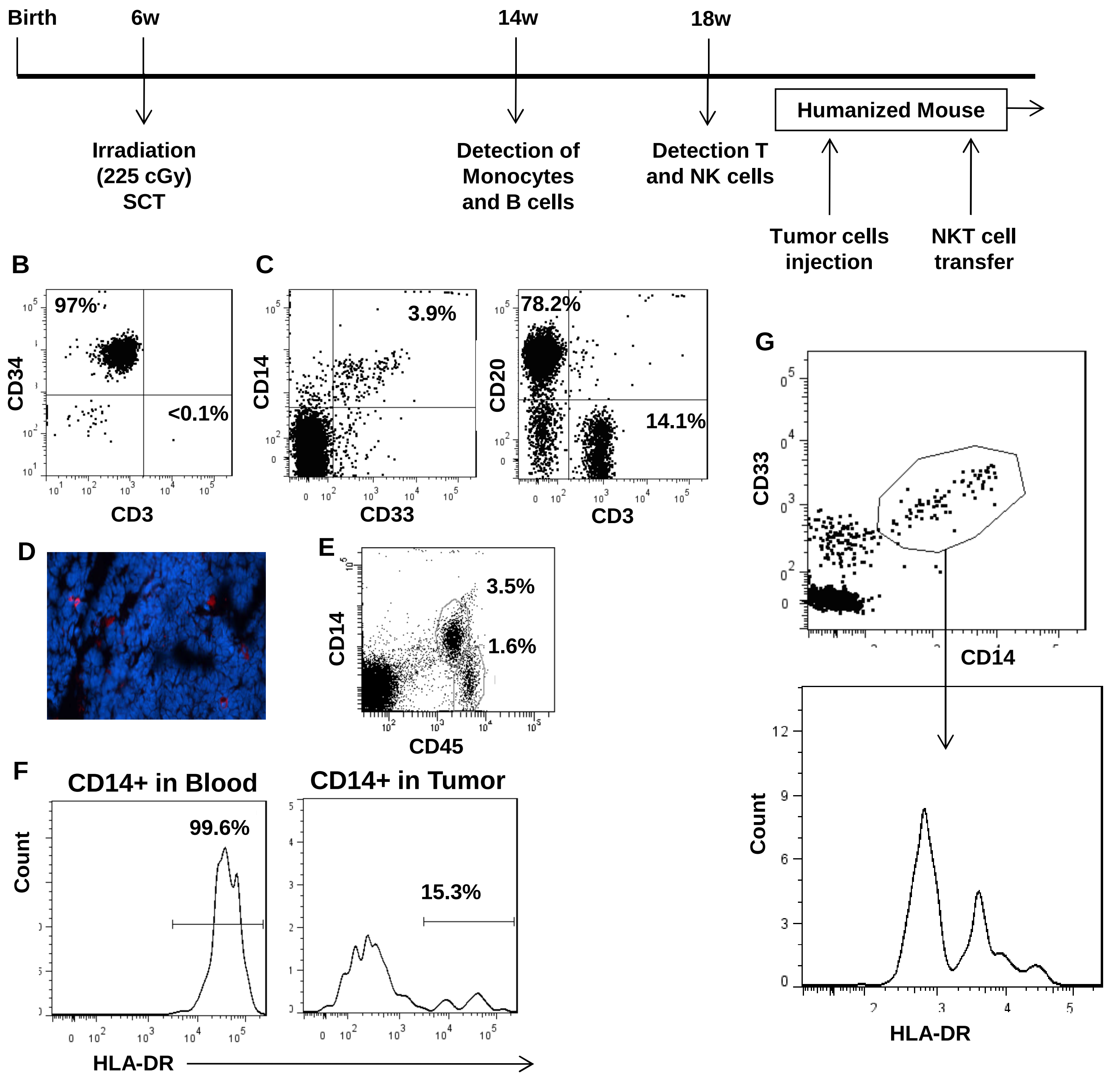

Figure S-3: Neuroblastoma model in hu-NSG mice. (A) Six-week old NSG mice were sublethally irradiated and transplanted with human cord blood CD34+ hematopoietic stem cells (SCT). Human monocytes and B cells apperaed in peripheral blood at 2 months and T cells at 3 months after SCT. NB cells were injected either under the renal capsule or intravenously (metastatic model) at 3.5 months after SCT. The adoptive transfer of NKT cells was performed at different intervals after tumor cell injection depending on the experimental setup. **(B)** After two rounds of positive selection, cord blood stem cell preparations had >95% CD34+ stem cells and <0.1% CD3+ T cells. **(C)** Representative plots demonstrate a typical reconstitution of monocytes, T and B lymphocytes in peripheral blood of hu-NSG mice at the time of NB-cell injection. **(D)** IF staining of NB graft with anti-human CD45 mAb (red), X20 magnification. **(E)** FACS analysis of viable cells in tumor suspension for human CD45 and CD14. **(F)** CD14+ monocytic cells were analyzed for the level of HLA-DR expression in peripheral blood (left) and in NB tumor grafts of the same animals. Shown is a representative of five mice. **(G)** HLA-DR expression (bottom) was analyzed in TAMs from primary human NB tumor after gating on CD45+CD33+CD14+ cell (top). Shown is a representative of three primary tumors.

Supplemental Figure 4

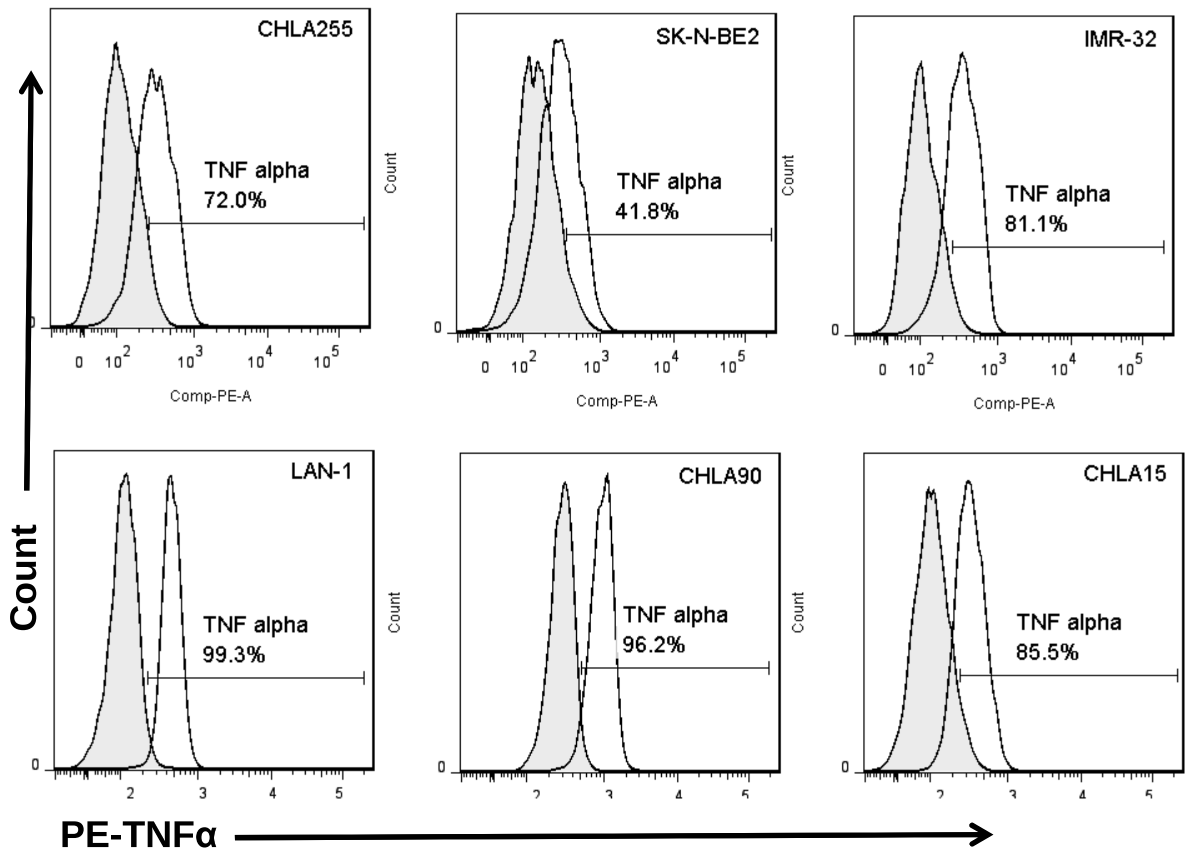

Figure S-4: Intracellular TNFα expression in NB cell lines. Cultured cells from the indicated human NB cell lines were fixed and permeabilized followed by intracellular staining with PE-conjugated anti-TNFα (transparent) or isotype control (grey) mAbs. Shown are representative FACS plots from one of three independent experiments.

Supplemental Figure 5

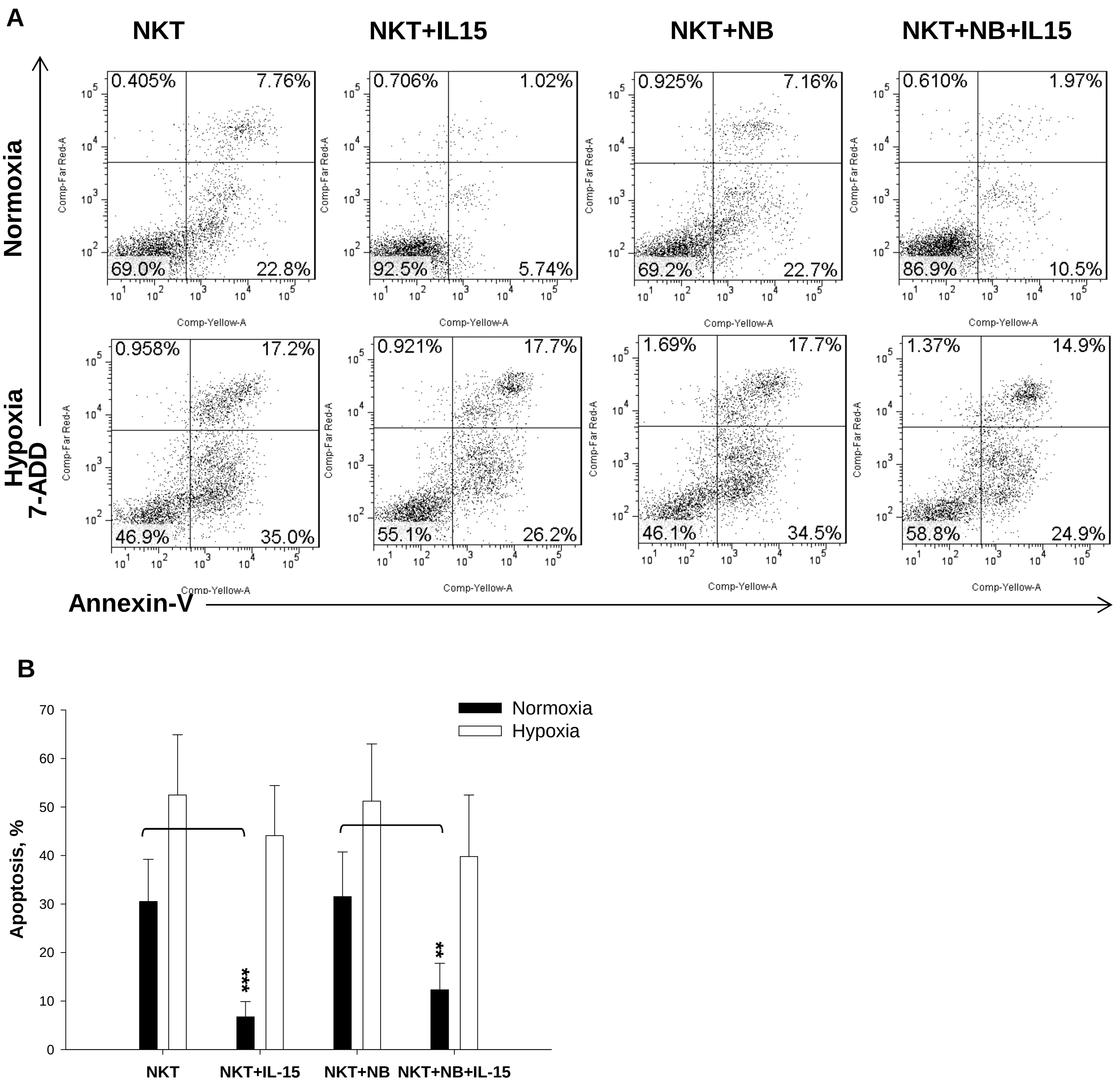

Figure S-5: Differential effect of IL-15 on NKT-cell apoptosis in normoxia and hypoxia. (A) NKTs were expanded from PBMC using stimulation with αGalCer and IL-2 for 7 days, then washed and cultured in the absence or presence of NB cells (1:1 ratio) in normoxia or hypoxia, with or without IL-15 at 10 ng/ml for 24 h followed by staining for Annexin-V and 7-AAD. Shown are representative FACS plots from one of four independent experiments. **(B)** Mean ± SD from 4 experiments, $^{**}P<0.01$ $^{***}P<0.001$.

Supplemental Table 1

Real-time PCR primers

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Length
LAPTM5	GGTCACACCTCTGAGTATG	GTGGAGGAGAAGAGAAACTC	128 bp
CCL3	TGGCTGCTCGTCTCAAAGTA	TGCAACCAGTTCTCTGCATC	116 bp
CCL20	CGTGTGAAGCCCACAATAAA	GCTGCTTTGATGTCAGTGCT	122 bp
CXCL12A	CAGAGCTGGGCTCCTACTGT	GCATTGACCCGAAGCTAAAG	117 bp
CXCL10	GCAGGTACAGCGTACGGTTC	CAGCAGAGGAACCTCCAGTC	124 bp
CCL17	TGGAGCAGTCCTCAGATGTC	CTTCTCTGCAGCACATCCAC	129 bp
CCL19	CATCATTGGTGCCACTCAGA	ACACAGATCCTGCACACACC	148 bp
CXCL11	ATGCAAAGACAGCGTCCTCT	CAAACATGAGTGTGAAGGGC	103 bp
CXCL16	CAATCCCCGAGTAAGCATGT	CTACACGAGGTTCCAGCTCC	119 bp
CCL5	TGTACTCCCGAACCCATTTC	TACACCAGTGGCAAGTGCTC	100 bp
CCL4	GGATTCACTGGGATCAGCAC	CTTCCTCGCAACTTTGTGGT	114 bp
CCL2	AGGTGACTGGGGCATTGAT	GCCTCCAGCATGAAAGTCTC	109 bp