Nuclear cytosol isolation

NK cells (2×10^6/ml) were stimulated with the combination stimulus of IL-12 (10ng/ml) and IL-18 (10ng/ml) for 6h. The cells were washed with PBS and resuspended in cytosol lysis buffer (10 mM HEPES [pH 7.9], 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA supplemented with 1 mM DTT, 0.5 mM PMSF, and 10 ng/ml each aprotinin, leupeptin, and pepstatin) and allowed to swell on ice for 15 min; 200 µl of 10% Nonidet P-40 was added to the swollen cells, and the samples were vortexed vigorously for 10 s. Cells were centrifuged at 14,000 × g. The supernatant resulting from this centrifugation was the cytosolic fraction. The nuclear pellet resuspended in nuclear lysis buffer (20 mM HEPES [pH 7.9], 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA supplemented with protease inhibitors as in cytosol lysis buffer) and shaken vigorously at 4°C for 2 h. The samples were centrifuged at 14,000 × g to isolate the nuclear fraction.

Chromatin immunoprecipitation

NK cells (15×10^6) were stimulated with IL-12 (10ng/ml) and IL-18 (10ng/ml) for 6h. Cells were cross-linked with 1% formaldehyde at room temperature for 10 min, followed by incubation with 125mM glycine at room temperature for 5 min in order to quench formaldehyde and stop crosslinking. Cells were harvested, washed with cold 1× PBS and re-suspended in ChIP cytosol lysis buffer (20mM Tris-HCl [pH 8.0], 85mM KCl, 0.5% Nonidet P-40) in the presence of a protease inhibitor cocktail mixture (Sigma Aldrich, St. Louis, MO, USA), 1mM PMSF, 10mM methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone and 3mM sodium orthovanadate. The lysates were incubated on ice for 10 min, followed by centrifugation at 5000 × g for 5 min at 4°C. The nuclear pellet was re-suspended in ChIP nuclei lysis buffer (50mM Tris-HCl [pH 8.0], 10mM EDTA, 1% SDS) in the presence of protease and phosphatase inhibitors. The nuclear lysates were incubated on ice for 10 min and the chromatin was sheared to an average length of 600bp at 4°C in a Branson Sonifier 450. The sheared chromatin was cleared of debris by centrifugation at 14,000 × g for 20 min and then diluted 5-fold with ChIP dilution buffer (20mM Tris-HCl (pH 8.0), 167mM NaCl, 1.1mM EDTA, 1.1% Triton X-100, 0.01% SDS) in the presence of protease and phosphatase inhibitors and precleared with salmon sperm DNA/protein A agarose slurry (Millipore, Billerica, MA, USA). The diluted, pre-cleared chromatin was immunoprecipitated with IκBζ or control antisera at 4°C overnight. The protein-DNA complexes were collected with salmon sperm DNA/protein A agarose beads. The samples were washed twice with low salt buffer (20mM Tris-HCl [pH 8.0], 150mM NaCl, 2mM EDTA, 1% Triton X-100, 0.1% SDS), twice with high salt buffer (20mM Tris-HCl [pH 8.0], 500mM NaCl, 2mM EDTA, 1% Triton X-100, 0.1% SDS), once with LiCl buffer (20mM Tris-HCl [pH 8.0], 1mM EDTA, 1% deoxycholic acid, 1% Nonidet P-40, 250mM LiCl), twice with TE buffer (10mM Tris-HCl [pH 8.0], 1mM EDTA). The immune complexes were extracted in elution buffer (1% SDS, 100mM $NaHCO_3$) at room temperature and reverse cross-linked at 65°C for 6h in the presence of RNase A (0.5mg/ml) and 300mM NaCl. The DNA was precipitated with 100% ethanol overnight at −20°C and extracted with 500mM EDTA, 40mM Tris-HCl [pH 6.5] and proteinase K (200µg/ml) at 45°C for 2h. The DNA was purified using Qiaquick spin columns (Qiagen, Germany) and the purified DNA was used in as template in qPCR to assess the relative presence of the target sequences.

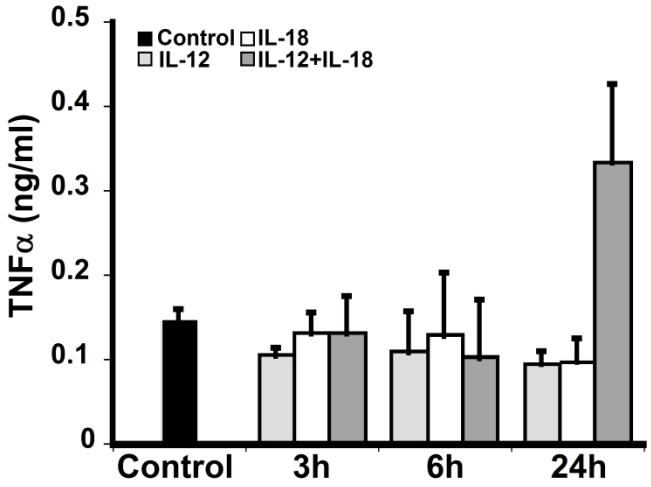

Figure S1. TNFα release by human NK cells
Human NK cells were isolated from PBMCs following positive selection for $CD56^+$ cells. Cells were stimulated (2×10^6 cells/ml) with recombinant IL-12 and IL-18 (10ng/ml each) for 3h, 6h and 24h. The cell supernatants were analyzed for TNFα release by ELISA. Values are expressed as mean ± SEM for three independent experiments.

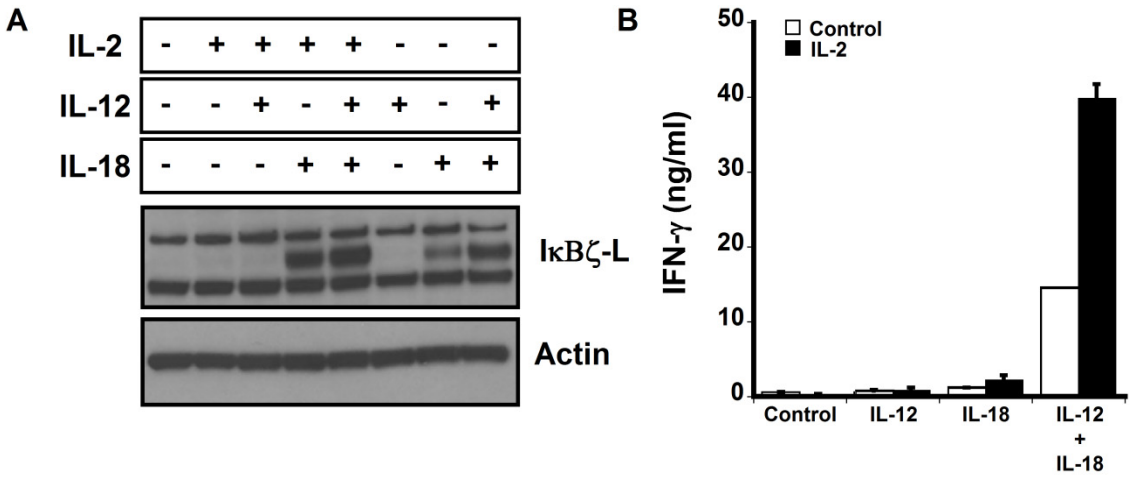

Figure S2. IκBζ expression and IFN-γ release in response to IL-2, IL-12, and IL-18 stimulation in human NK cells

$CD56^+$ human NK cells (2×10^6 cells/ml) were treated with recombinant IL-12 and IL-18 (10ng/ml each) in the presence or absence of IL2 (100U/ml) for 6h. Protein normalized cell lysates were immunoblotted for IkBz and actin. The cell supernatants were analyzed for IFN-γ release by ELISA. Immunoblot is representative of three independent experiments and bar graphs represent the mean ± SEM for two to three experiments.