Supplementary methods:

Reagents:

Rabbit polyclonal anti-IL-15, anti-IL-15R were purchased from R&D Systems. Human IL-21 was obtained from Peprotech. The IL-15/IL-15Rα complex was a gift from Drs. Cristina Bergamaschi, George Pavlakis, and Barbara Felber at NCI-Frederick. Ribonuclease protection assays were purchased from Pharmingen/BD Biosciences.

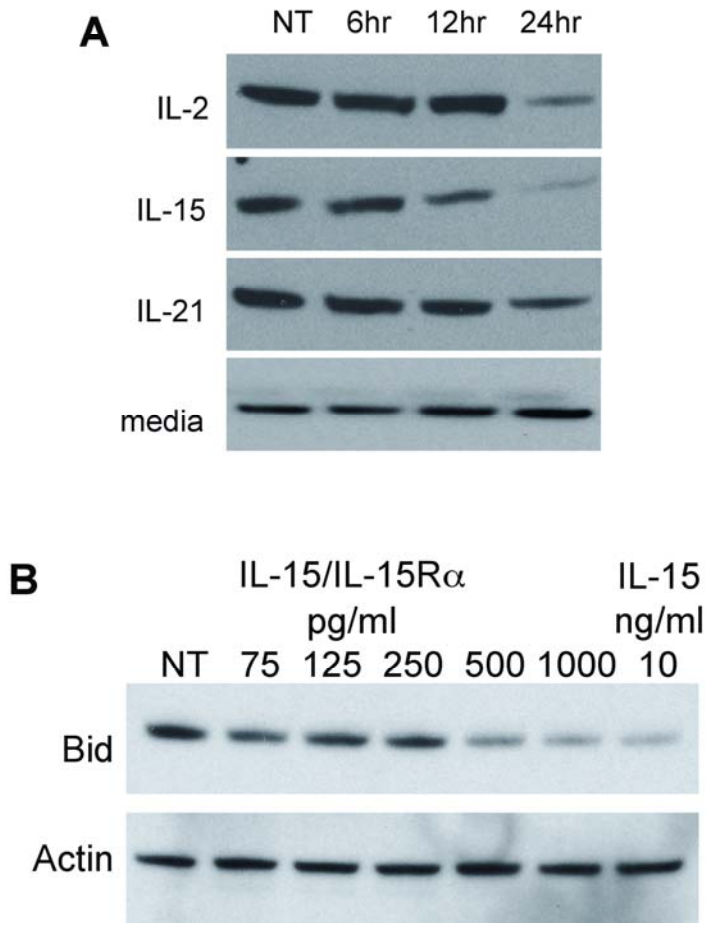
mRNA isolation and expression analysis:

Total RNA was isolated from primary NK cells by Trizol extraction (Invitrogen) according to manufacturer's protocol. Ribonuclease protection assays were performed using a ^{33}P labeled mAPO-2b multiprobe set as previously described (1).

Supplementary figure legends:

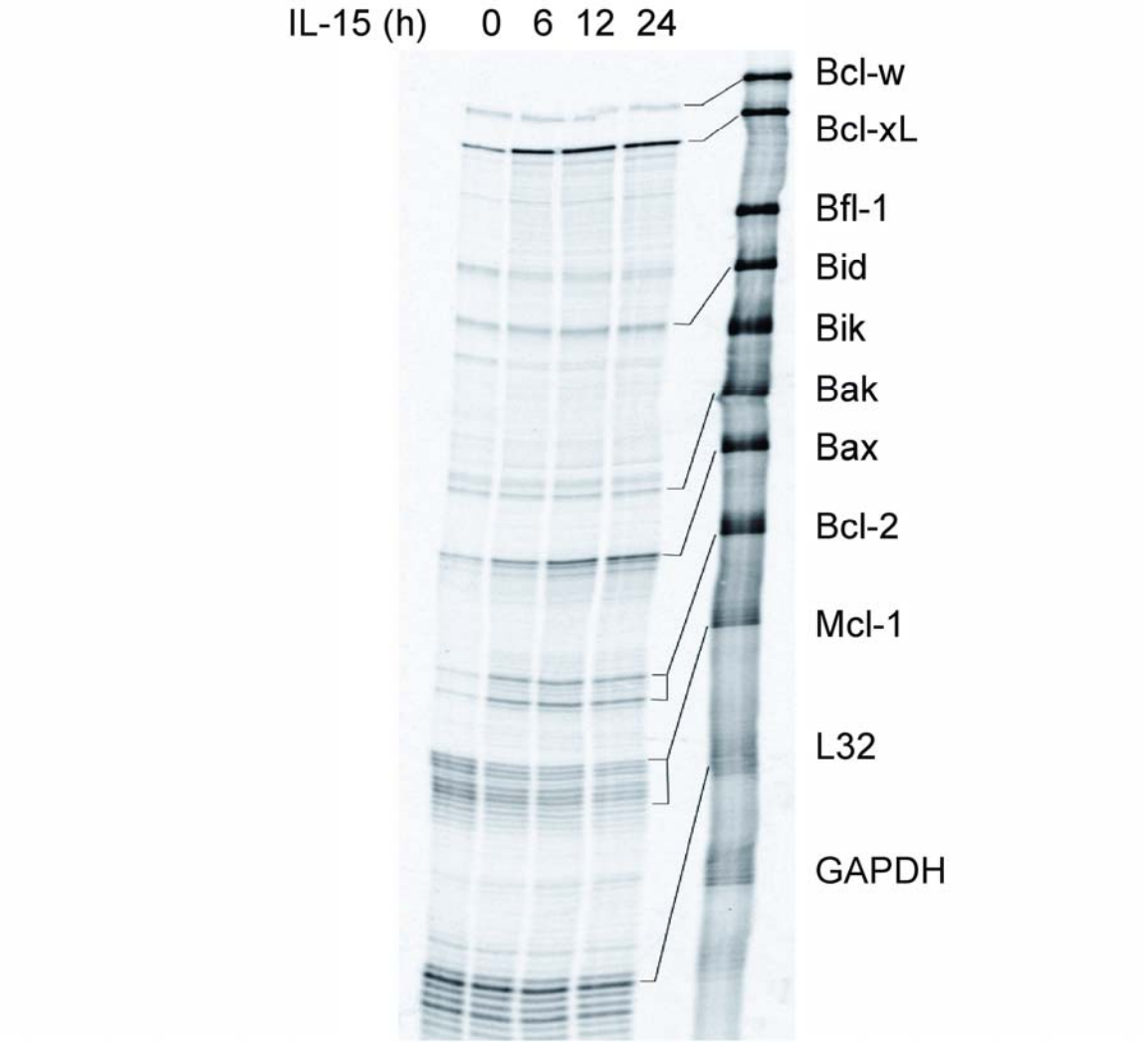
Supplementary Figure 1. Bid expression is altered by other IL-2 family members and by IL-15/IL-15Rα complex. (A) Primary human NK cells were treated with 100U IL-2, 20 ng/ml IL-15, and 100 ng/ml IL-21 for the indicated times. Cells were lysed in TTX-100 buffer and Western analysis performed with a polyclonal anti-Bid antibody. As a control, Bid was examined in the absence of cytokines with results demonstrating no change in Bid when cells were maintained in media alone. (B) Primary human NK cells were treated with IL-15/IL-15Rα complex and IL-15 at the indicated concentrations for 12 hours. Cell lysates were analyzed by Western analysis performed with a polyclonal anti-Bid antibody. The blot was reprobed with anti-actin as a control. Results are representative of analyses from two individual donors.

Supplementary Figure 1 Hodge et al



Supplementary Figure 2. Bid mRNA expression is unchanged following IL-15 treatment. Donor NK cells were treated with IL-15 for the indicated times. Total cellular RNA was prepared and analyzed by ribonuclease protection assay using a ^{33}P labeled hAPO-2b multiprobe. Bcl-2 and Bcl-xL mRNAs are positive controls for IL-15 induction of gene activity. L32 mRNA is an internal control for sample variation. The results are representative of RNA from 2 individual donors.

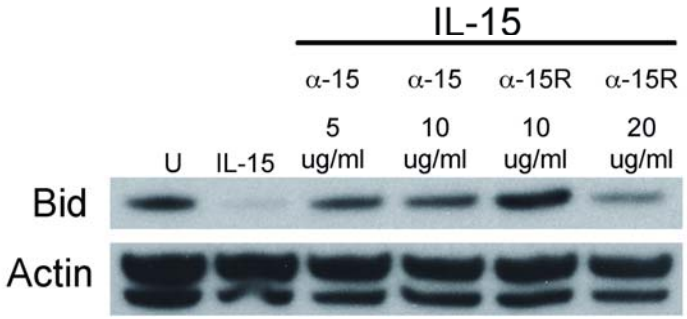
Supplementary Figure 2 Hodge et al



Supplementary Figure 3. Alterations in Bid expression in primary LGL leukemia cells are blocked with IL-15Rα blocking and IL-15 neutralizing antibodies. T-LGL donor PBMCs were pretreated for one hour with IL-15 and IL-15Rα blocking antibodies before 12 hours of IL-15 treatment. Western analysis was performed using a rabbit polyclonal anti-Bid antibody. Blots were reprobed with goat anti-actin as a control. Similar Bid

increases were observed in other LGL donor cells treated with the blocking antibodies (data not shown).

Supplementary Figure 3 Hodge et al



Reference

1 Hodge DL, Martinez A, Julias JG, et al. Regulation of nuclear gamma interferon gene expression by interleukin 12 (IL-12) and IL-2 represents a novel form of posttranscriptional control. Mol Cell Biol 2002;22:1742-53.