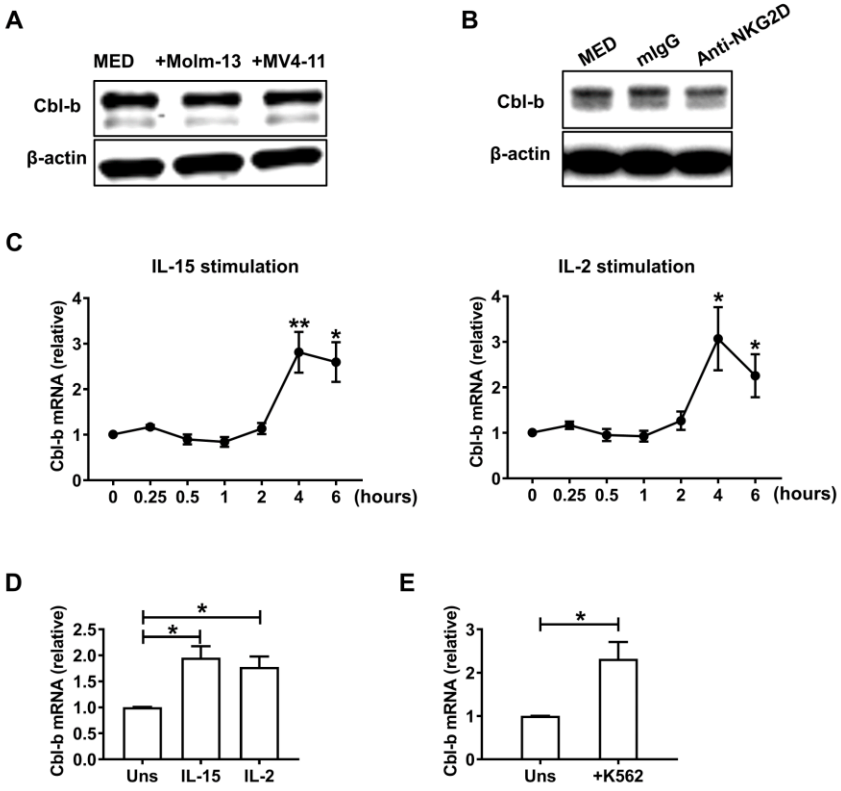
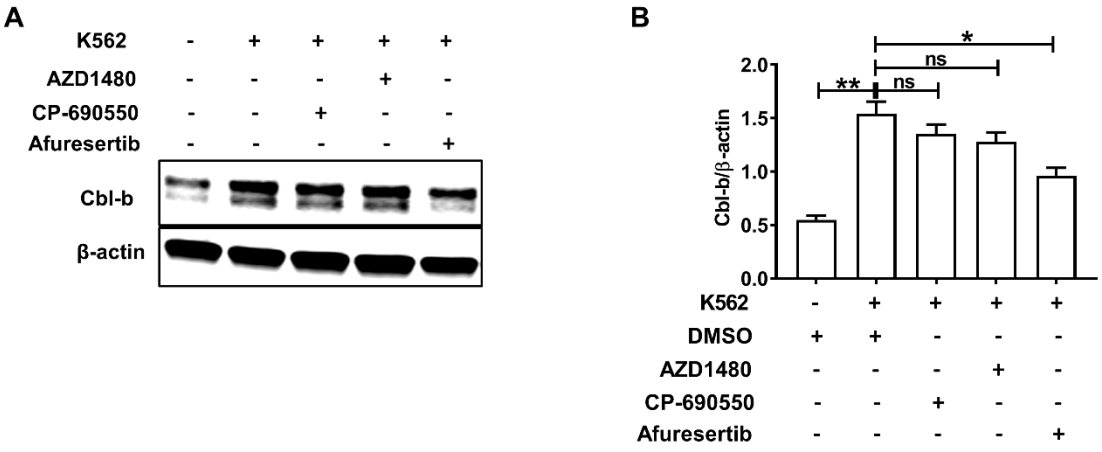
S Fig. 1



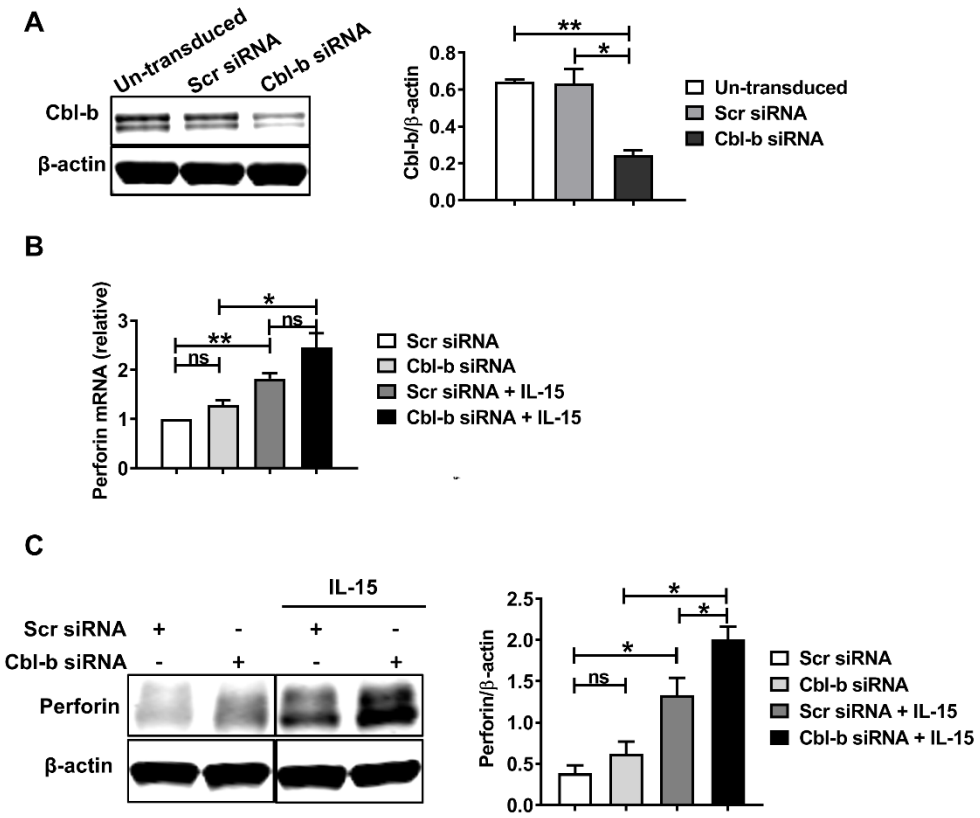
Supplemental Fig. 1. (A) Enriched primary human NK cells were cocultured with Molm-13 or MV4-11 leukemia cell lines (E/T ratio=10:1) for 24 h prior to assessment of Cbl-b by immunoblot analysis. Data are representative of three independent experiments. **(B)** Enriched primary human NK cells were cultured overnight with an anti-NKG2D activating antibody (clone 1D11) (10 μg/ml) or mouse IgG (10 μg/ml) as control prior to assessment of Cbl-b by immunoblot analysis. Data are representative of four donors with similar results. **(C)** The expression of Cbl-b was assessed by qRT-PCR after primary human NK cells were treated with IL-15 (10 ng/ml) or IL-2 (150 IU/ml) at different time point. Data are shown summarizes four independent experiments. **(D)** The expression of Cbl-b was assessed by qRT-PCR after primary human NK cells were treated with IL-15 (10 ng/ml) or IL-2 (150 IU/ml) for 24 h. Data are shown summarizes five independent experiments. **(E)** The expression of Cbl-b was assessed by qRT-PCR after primary human NK cells were incubated with K562 cells (E/T ratio=10:1) for 24 h. Data are shown summarizes five independent experiments. *P<0.05, **P<0.01, compared to the shortest time point. Data are presented as mean ± SEM.

S Fig. 2



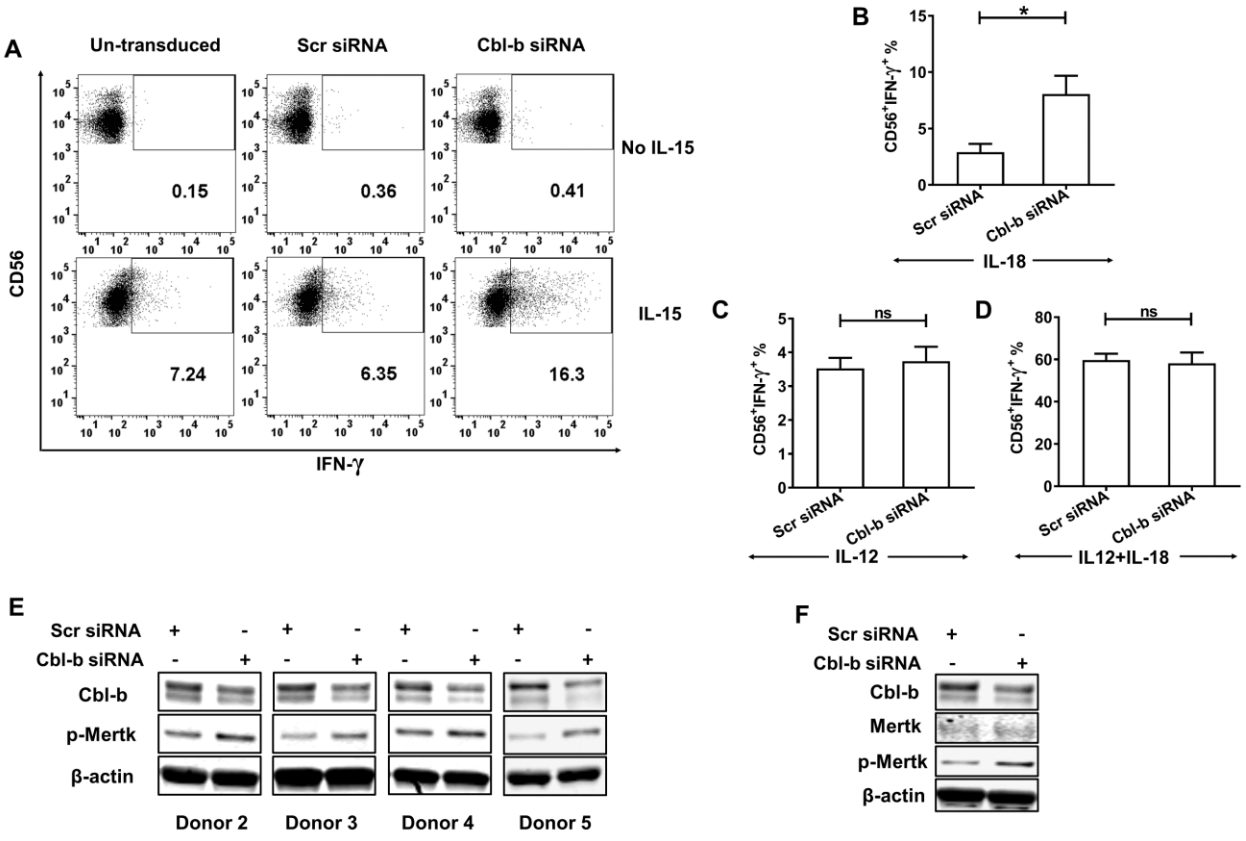
Supplemental Fig. 2. (A) Primary NK cells were pretreated with AZD1480 (10 uM), CP-690550 (10 uM) or Afuresertib (10 uM) for 90 min prior to a 24 h cocultured with K562 cells (E/T=10:1). The immunoblot data are representative of three donors in a similar fashion. Densitometric quantification assessing the ratio of Cbl-b protein levels to β-actin protein levels for three donors is summarized in **(B)**. *P<0.05, **P<0.01; ns, not significant. Data are presented as mean ± SEM.

S Fig. 3



Supplemental Fig. 3. (A) Primary human NK cells were transduced with Cbl-b siRNA or a scrambled siRNA (Scr siRNA) for 48 h, directly followed by immunoblot analysis to determine the efficiency of siRNA (n=3 donors). Densitometric quantification shows the ratio of Cbl-b protein levels to β-actin protein levels. **(B)** qRT-PCR was performed to quantify the level of perforin mRNA (n=7 donors) in Cbl-b siRNA- and Scr siRNA-transduced primary human NK cells incubated without or with IL-15 (10 ng/ml) for 16 h. **(C)** The perforin protein level in Cbl-b siRNA- and Scr siRNA-transduced primary human NK cells incubated without or with IL-15 (10 ng/ml) for 24 h was measured by immunoblot analysis. The data are representative of four experiments and are summarized in the right panel. Scr, scrambled; *P<0.05, **P<0.01; ns, not significant. Data are presented as mean ± SEM.

S Fig. 4



Supplemental Fig. 4. (A) Representative intracellular flow cytometric analysis of un-transduced $CD56^+$ primary human NK cells or those transduced with Scr siRNA or Cbl-b siRNA, each without or with incubation with IL-15 (10 ng/ml) for 24 h. **(B-D)** Intracellular flow cytometric analysis of $CD56^+$ primary human NK cells transduced with Scr siRNA or transduced with Cbl-b siRNA, incubated with IL-18 (10 ng/ml), IL-12 (10 ng/ml) or IL-18 (10 ng/ml) plus IL-12 (10 ng/ml) for 24 h. **(E)** The level of Mertk phosphorylation and Cbl-b in Cbl-b siRNA- or Scr siRNA-transduced primary human NK cells was measured by immunoblot analysis. Data of four different donors are presented. **(F)** The level of total Mertk expression in Cbl-b siRNA- or Scr siRNA-transduced primary human NK was measured by immunoblot analysis. Data presented are representative of three independent experiments. Scr, scrambled; *P<0.05, ns, not significant. Data are presented as mean ± SEM.