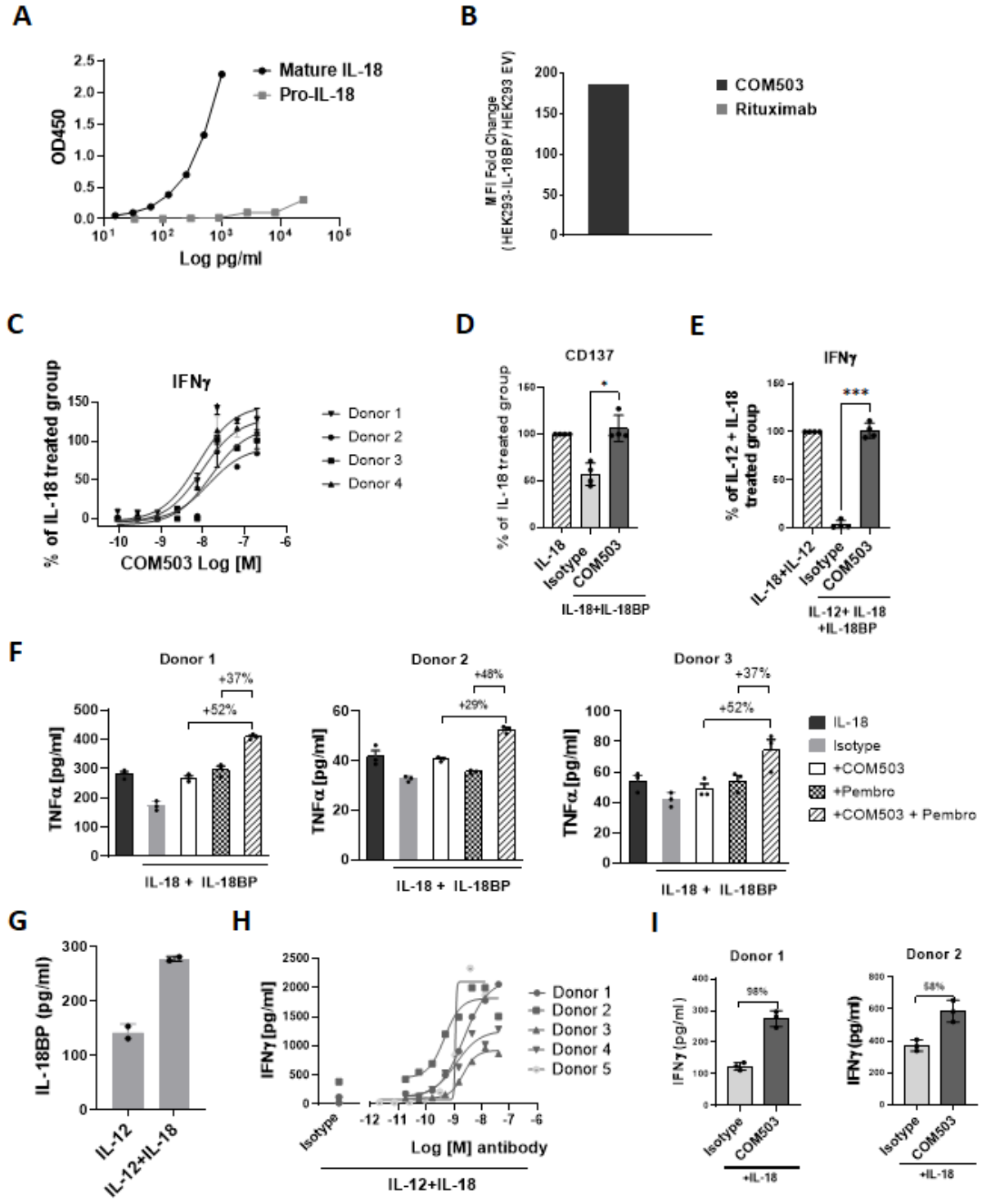
Supplemental Figure 2



Supplemental Figure S2. COM503 releases IL-18 activity in biochemical and functional assays. (A) Detection of mature and pro-IL-18 by human IL-18 ELISA assay. (**B)** Binding of COM503 (20ug/ml) or Rituximab (1ug/ml) to HEK293 cells overexpressing human IL-18BP measured by flow cytometry. **(C-D)** *Ex-vivo* stimulated human melanoma $CD8^+$ TILs (2 independent experiments, 4 donors) were co-cultured with melanoma antigen-expressing MEL624 cells and incubated with rIL-18BP (1ug/ml) and rIL-18 (30ng/ml) to allow the formation of an IL-18:IL-18BP complex before addition of 0.01-30ug/ml COM503 **(C)** or 10ug/ml COM503 **(D)** for 24 hours. IFNγ secretion was analyzed in cell supernatant **(C)** and CD137 expression was analyzed on cell membrane **(D).** Levels of IFNγ and CD137 in treatment groups were normalized to those detected in the IL-18 treated group. **(E)** Human NK cells (3 independent experiments, 4 donors) were stimulated with rIL-12 (10ng/ml) and incubated with rIL-18 (10ng/ml) and rIL-18BP (1ug/ml) to allow the formation of an IL-18:IL-18BP complex before addition of COM503 (10ug/ml) for 24 hours, after which supernatants were collected and analyzed for IFNγ secretion. Levels of IFNγ in treatment groups were normalized to those detected in the IL-18+IL-12 treated group. **(F)** $CD8^+$ CMV-specific T cells (4 independent experiments, 3 donors) were co-cultured with PD-L1-overexpressing MEL624 cells that were pre-pulsed with CMV pp65 peptide and incubated with rIL-18BP (1ug/ml) and rIL-18 (30ng/ml) to allow the formation of an IL-18:IL-18BP complex before addition of COM503 (10ug/ml), Pembrolizumab (aPD-1 Ab, 10ug/ml), or both agents for 24 hours. Supernatants were collected and analyzed for TNFα secretion. **(G)** Endogenous IL-18BP secreted from human PBMCs stimulated either with IL-12 (10ng/ml) or IL-12 (10ng/ml) plus IL-18 (300ng/ml) for 24 hours measured by ELISA assay. **(H)** Human PBMCs (6 independent experiments, 5 donors) were stimulated with rIL-18 (2ng/ml) and rIL-12 (10ng/ml) and incubated with COM503 (0.002-6ug/ml) for 24 hours, after which supernatants were collected and analyzed for IFNγ secretion. **(I)** *Ex-vivo* stimulated human melanoma $CD8^+$ TILs (2 independent experiments, 2 donors) were co-cultured with melanoma antigen-expressing MEL624 cells in the presence of rIL-18 (donor 1- 1.2ng/ml, donor 2- 3.7ng/ml) and incubated with COM503 (30ug/ml) for 24 hours, after which supernatants were collected and analyzed for IFNγ secretion. Bar graphs show the mean ± SEM; $P<0.05$*, $P<0.001$*** by One-way ANOVA followed by two-tailed t test. MFI- median florescence intensity.