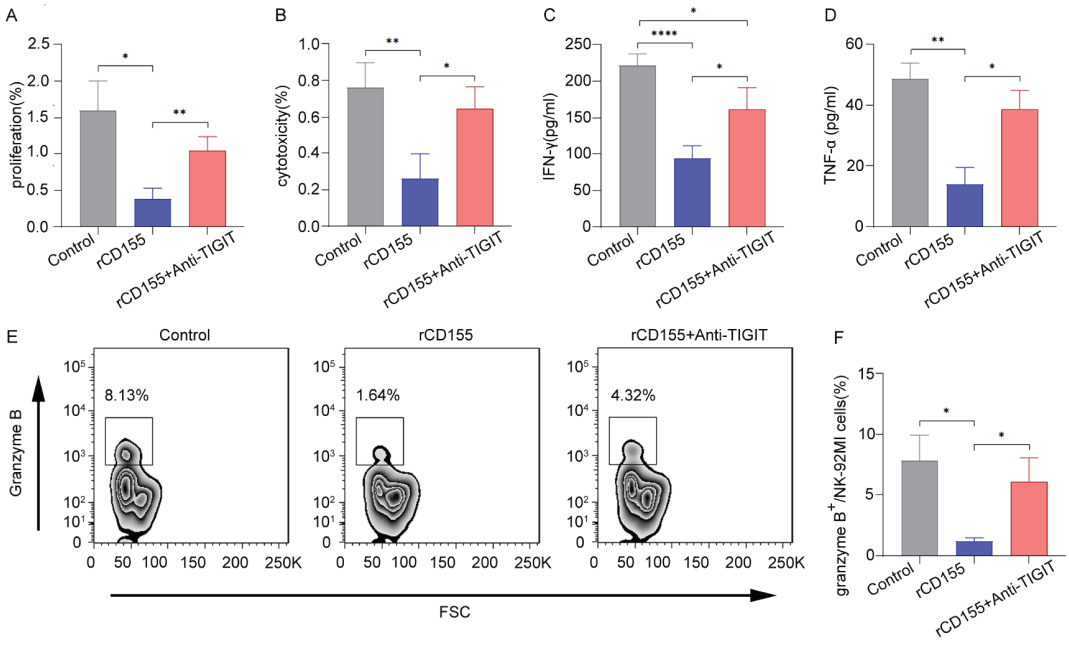
Recombinant CD155 Suppresses NK Cell Functions via the TIGIT Pathway

To investigate the role of CD155, NK-92MI cells were treated with recombinant CD155, with or without anti-TIGIT. These cells were activated with IL-15 (5 ng/mL) for 24 hours in the presence or absence of recombinant CD155 (5 μg/mL) and anti-TIGIT (20 μg/mL). Cell proliferation was assessed using the CCK-8 assay. For cytotoxicity assays, pre-activated NK-92MI cells under the same conditions were incubated with K562 cells at a ratio of 10:1 for 4 hours. Cytotoxicity was measured using the CytoTox 96® Non-Radioactive Cytotoxicity Assay kit. Additionally, after 24-hour activation, IFN-γ and TNF-α levels in the supernatants were quantified by ELISA, while intracellular granzyme B was detected via flow cytometry. The results showed recombinant CD155 significantly inhibited NK cell proliferation and cytotoxicity, which were restored by anti-TIGIT treatment (**Supplementary Fig. 1A-B**). The suppression of IFN-γ, TNF-α, and granzyme B production by recombinant CD155 was also inverted by anti-TIGIT (**Supplementary Fig. 1C-F**), demonstrating that CD155 suppresses NK cell functions via the TIGIT pathway.



Supplementary Fig. 1. Recombinant CD155/TIGIT pathway affects the immune functions of NK-92MI cells. NK-92MI cells were activated with IL-15 in the presence or absence of recombinant CD155 and anti-TIGIT, and then the immune functions of NK-92MI cells were assessed, including: (A) proliferation, (B) cytotoxicity, (C-D) levels of IFN-γ and TNF-α, and (E-F) granzyme B. rCD155, recombinant CD155. *P < 0.05; **P < 0.01; ****P < 0.0001. Kruskal-Wallis test followed by Dunn's test as a post-hoc test to ascertain the corrected significance of pairwise comparisons.