

ADAM17 Inhibition Enhances Human NK Cell Proliferation by IL-15 in a Mouse Xenograft Model

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Abstract

Natural killer (NK) cells can kill tumor cells by direct and indirect means. Exploiting these cells for cancer therapies is a rapidly developing field, which includes autologous and allogeneic NK cell infusion strategies. L-selectin (CD62L) is a well described adhesion protein known to play a critical role in leukocyte adhesion, migration and signal transduction. The majority of CD56^{bright} and a subset of CD56^{dim} NK cells express CD62L, and these subsets appear to represent early and intermediate stages of NK cell maturation, respectively, and have a greater potential to proliferate than CD56^{dim} CD62L⁻ NK cells. IL-15 treatment following the adoptive transfer of NK cells enhances their expansion *in vivo*, and is an important strategy being investigated in the clinic to improve NK cell persistence. Using an *in vivo* xenogeneic model, the infusion of human IL-15 causes the expansion of adoptively transferred human NK cells, and we found that this was completely abrogated upon administering a CD62L blocking mAb. IL-15 is known to induce the downregulation of CD62L expression, and we show this can be blocked by ADAM17 inhibition. Interestingly, the administration of function blocking ADAM17 mAbs, including one specific to human ADAM17, dramatically increased NK cell expansion *in vivo* in the presence of IL-15. These findings demonstrate that CD62L is important for NK cell expansion by IL-15 in a xenograft model, and that this process is impaired upon CD62L shedding by ADAM17 in the cytokine-stimulated NK cells. Our findings could have clinical relevance for NK cell immunotherapies involving cytokine stimulation. By blocking ADAM17 function it may be possible to increase NK cell expansion and persistence as well as certain cytolytic activities to enhance their anti-tumor effector activities.

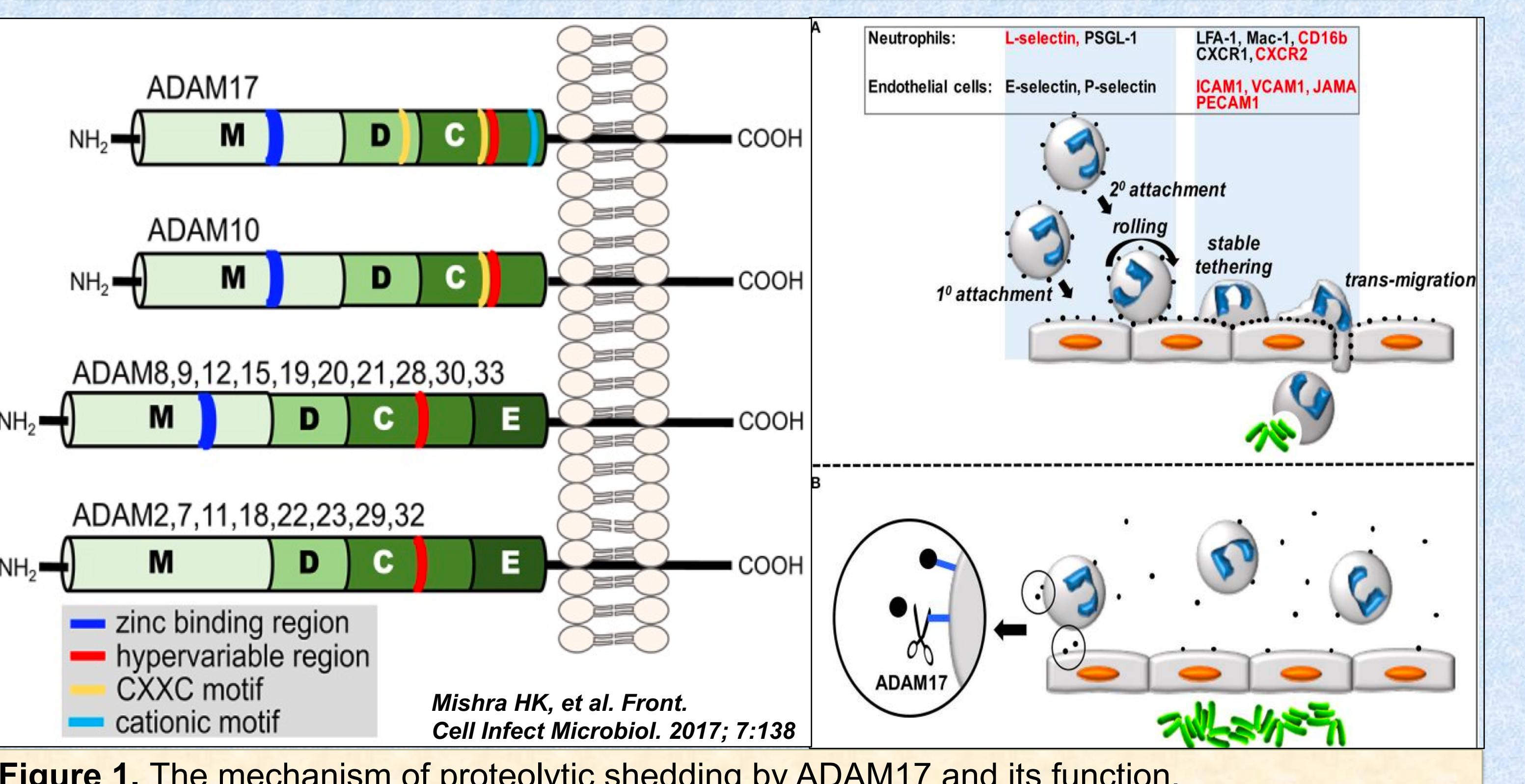


Figure 1. The mechanism of proteolytic shedding by ADAM17 and its function.

Results

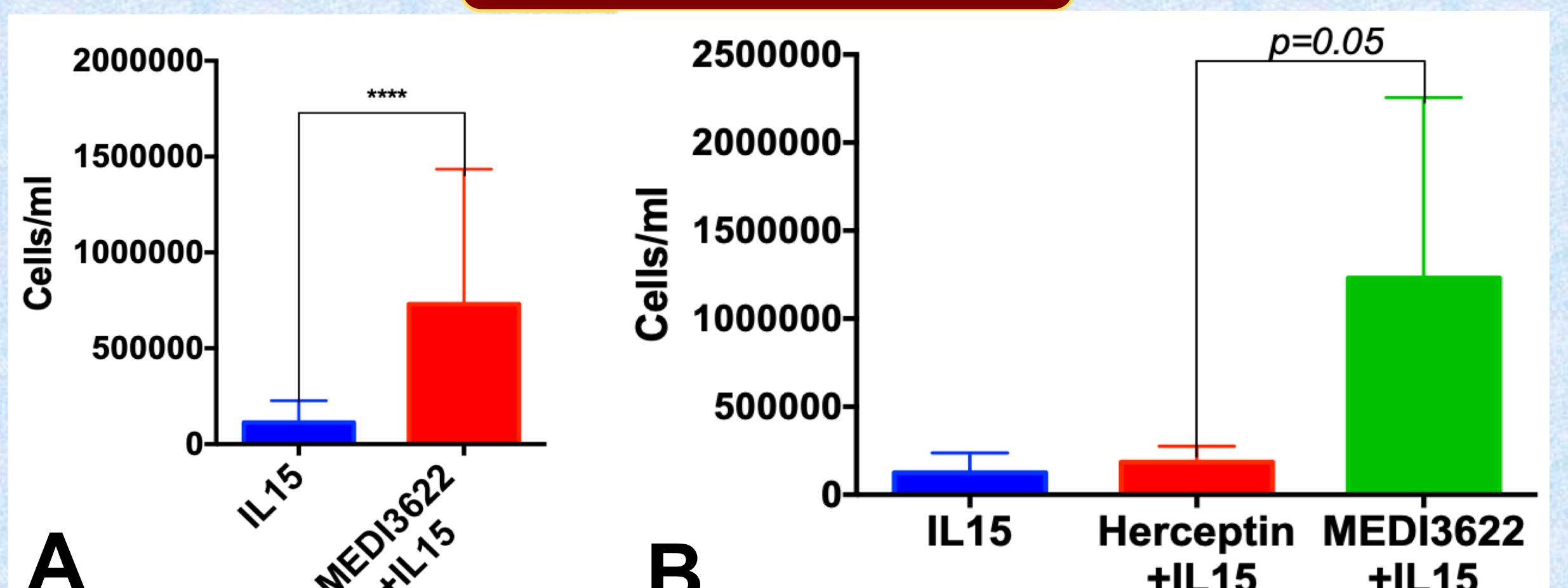


Figure 2. The inhibition of ADAM17 by mAb MEDI3622, which identify murine as well as human ADAM17, significantly enhances human NK cell proliferation.

Approx. 4×10^6 of untouched purified human NK cells/mouse (Stem Cell, Cat#19055) were injected intravenously in irradiated NSG mice. IL15 (source: NCI) was injected thrice weekly at the dosage of 5ug/mouse. MEDI3622 was administered at the dosage of 10mg/kg/5days. **A.** MEDI3622 treated mice showed significantly higher proliferation of NK cells compared to control group ($n=27$ each). **B.** In another set of experiments, MEDI3622 treated mice showed significantly higher proliferation than Herceptin treated mice at similar dose ($n=5$).

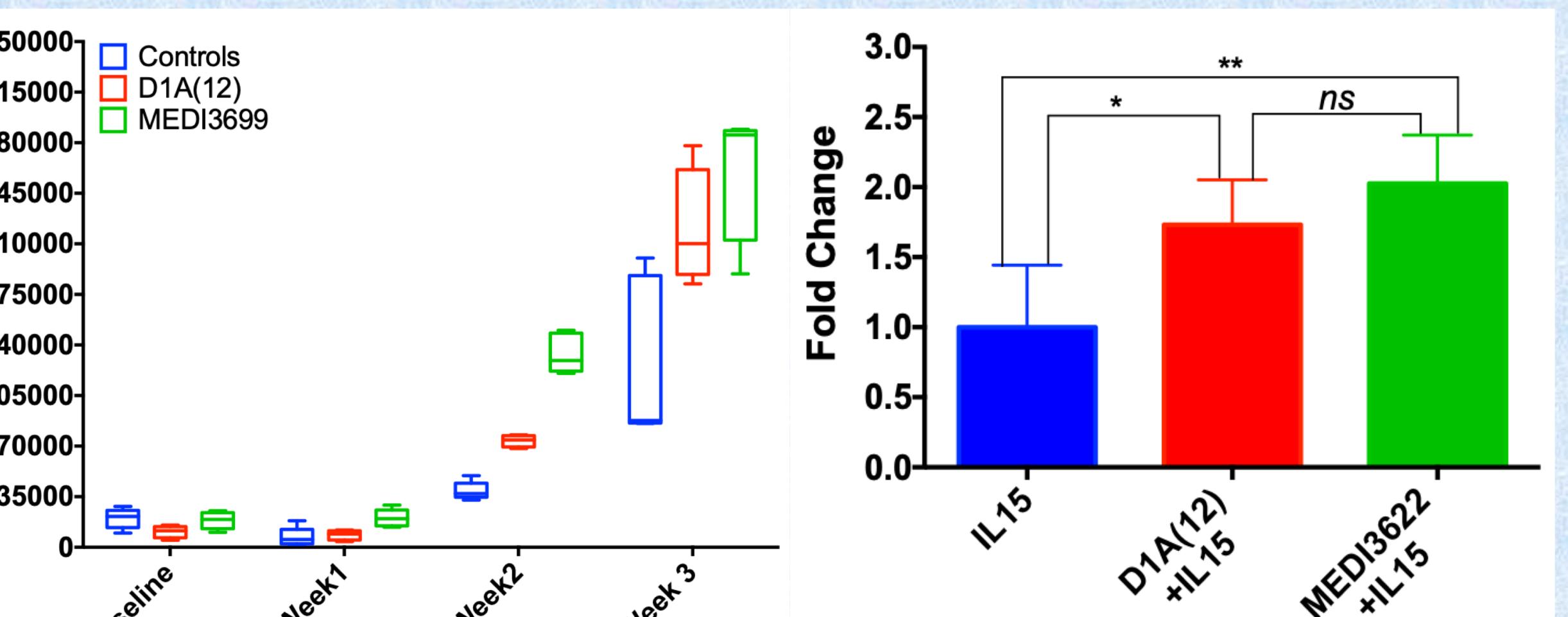


Figure 3. Inhibition of ADAM17 on human NK cells alone can trigger expansion. NK cell expansion was compared between a mAb, D1A(12), which only recognizes human ADAM17 and MEDI3622, which has cross-reactivity with both murine and human ADAM17. The expansion of NK cells were comparable between both antibodies treated group, whereas, both group exhibited significantly higher proliferation of NK cells in comparison to the control (IL15 alone) group.

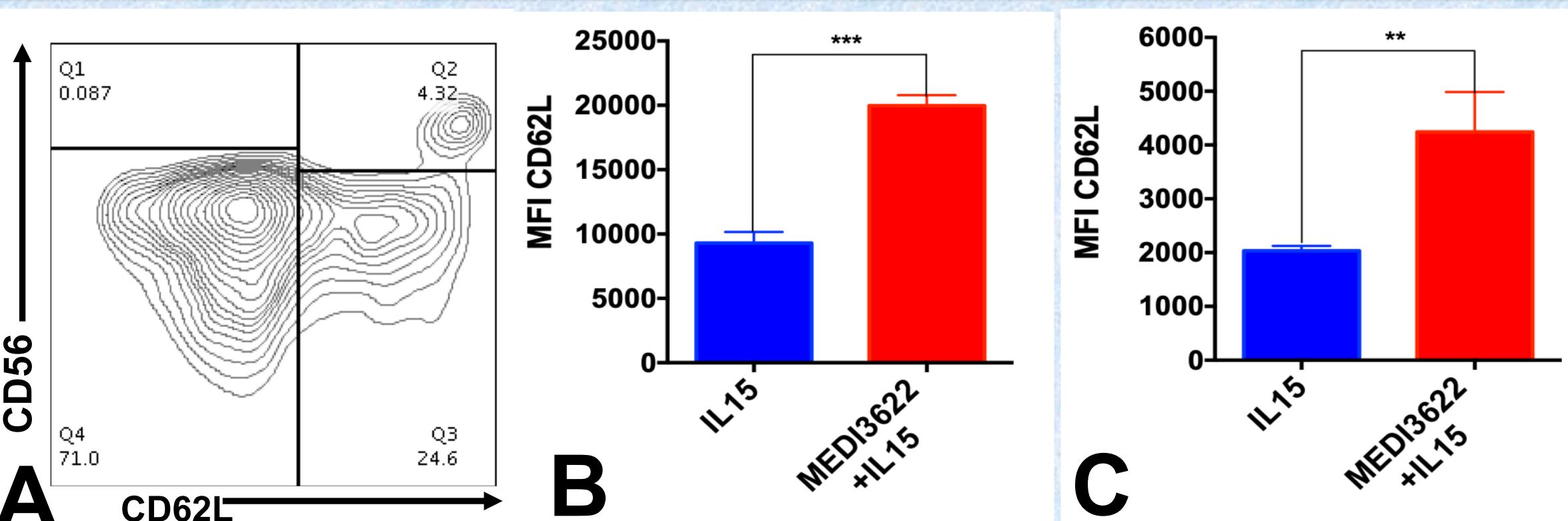


Figure 4. MEDI3622 prevents ADAM17 mediated ectodomain shedding of L-selectin (CD62L) from NK cells. (A). Representative contour plot showing the distribution of CD62L between different subsets of freshly isolated NK cells. The inhibition of ADAM17 by MEDI3622 (5ug/ml) significantly prevent the shedding of CD62L from the surface of CD56^{brights} (B) as well as CD56^{dim} (C) NK cells in an overnight human serum based media supplemented with 2.5ng/ml of IL15 (NCI).

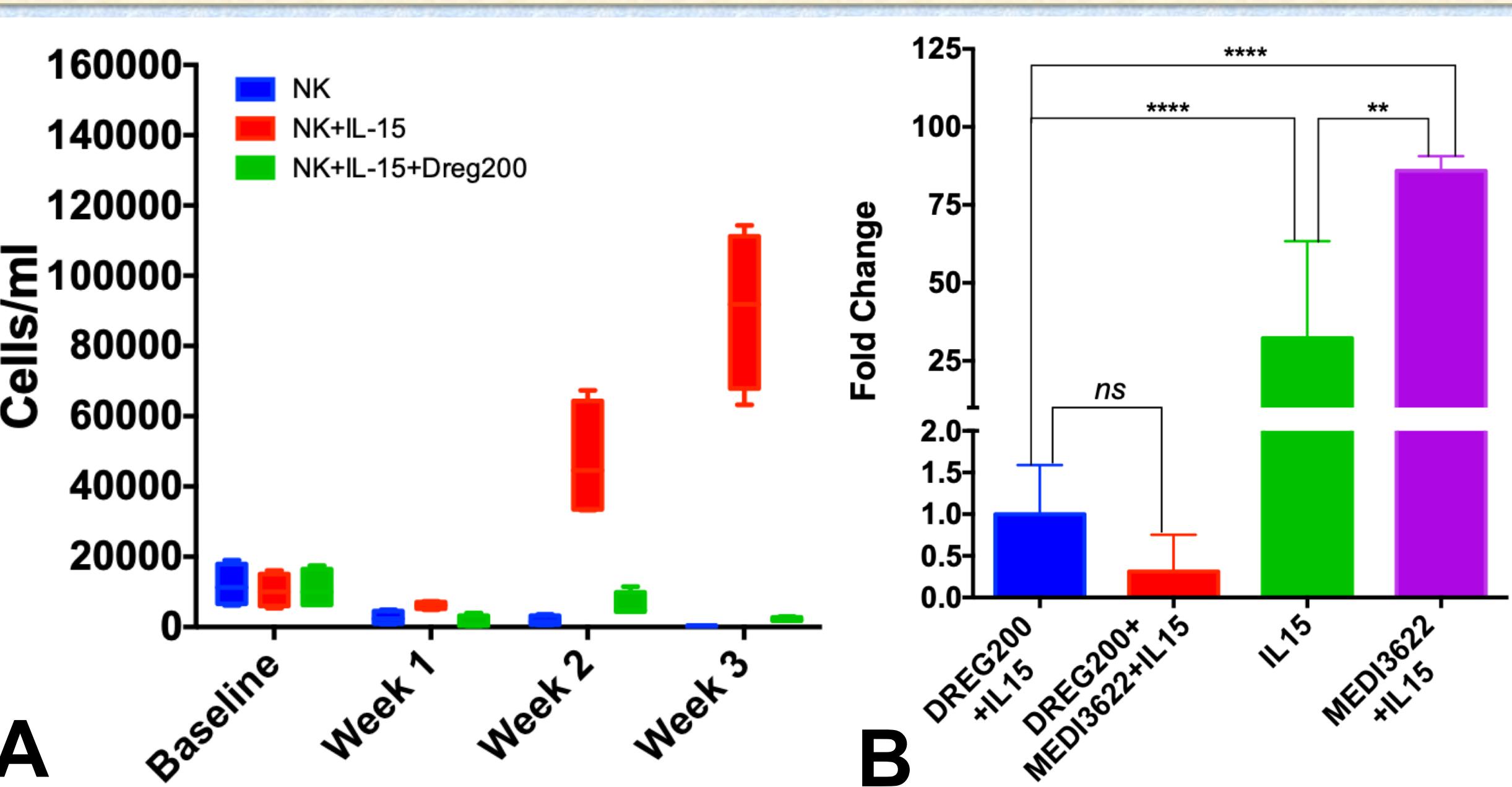


Figure 5. Blocking the function of CD62L by a mAb abrogate the expansion of NK cells. The mice were injected with a CD62L function block antibody called Dreg200 (at dose of 10 mg/kg/5-days) and their expansion was compared to MEDI3622 treated group at similar dose. The expansion of NK cells were abrogated in Dreg200 treated mice in comparison to MEDI3622 treated mice or even mice treated with IL15 alone.

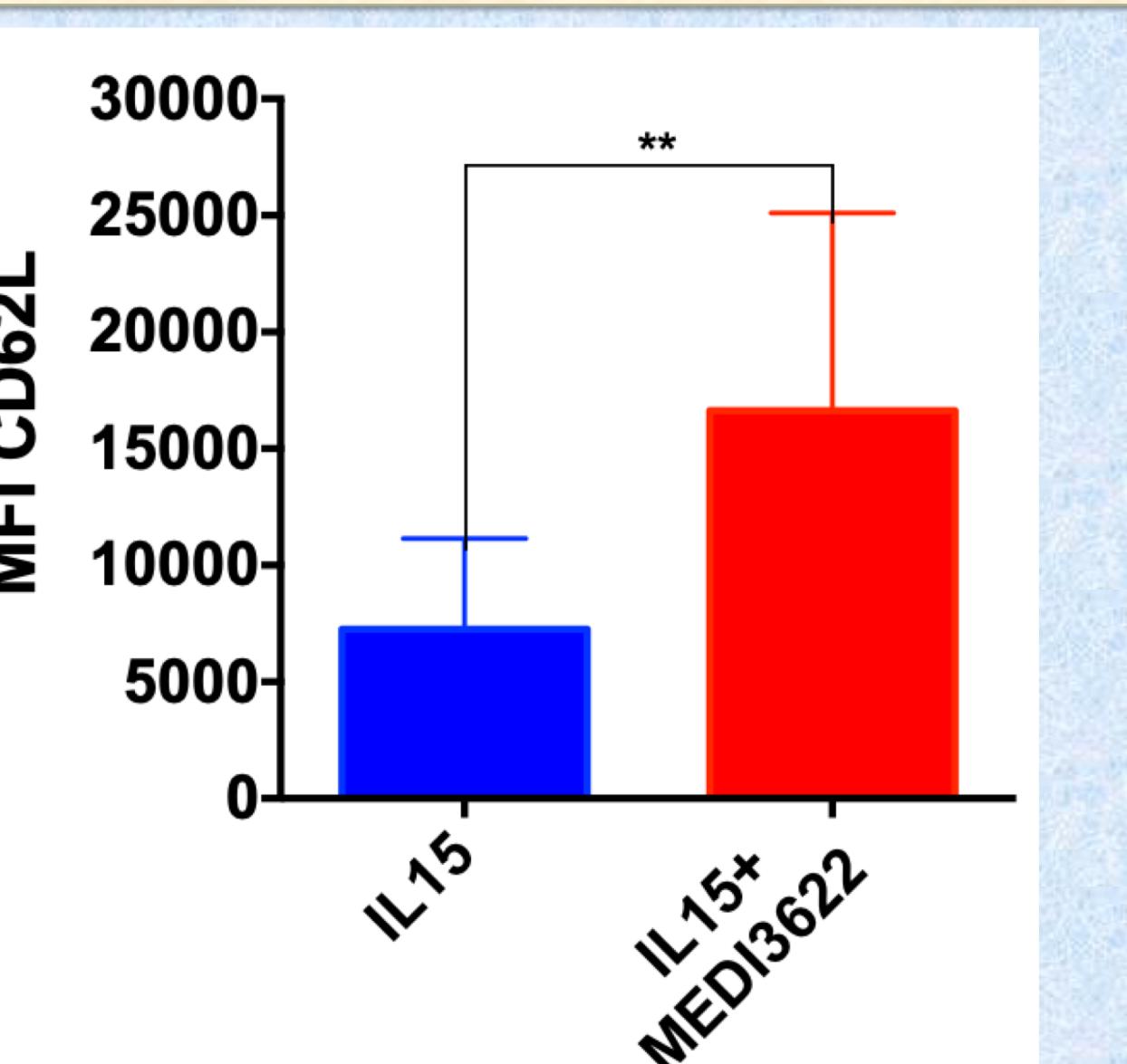


Figure 6. The mice treated with MEDI3622 showed significantly higher CD62L surface expression. MEDI3622 treated group showed significantly higher expression of CD62L on the surface of NK cells in comparison to the control group.

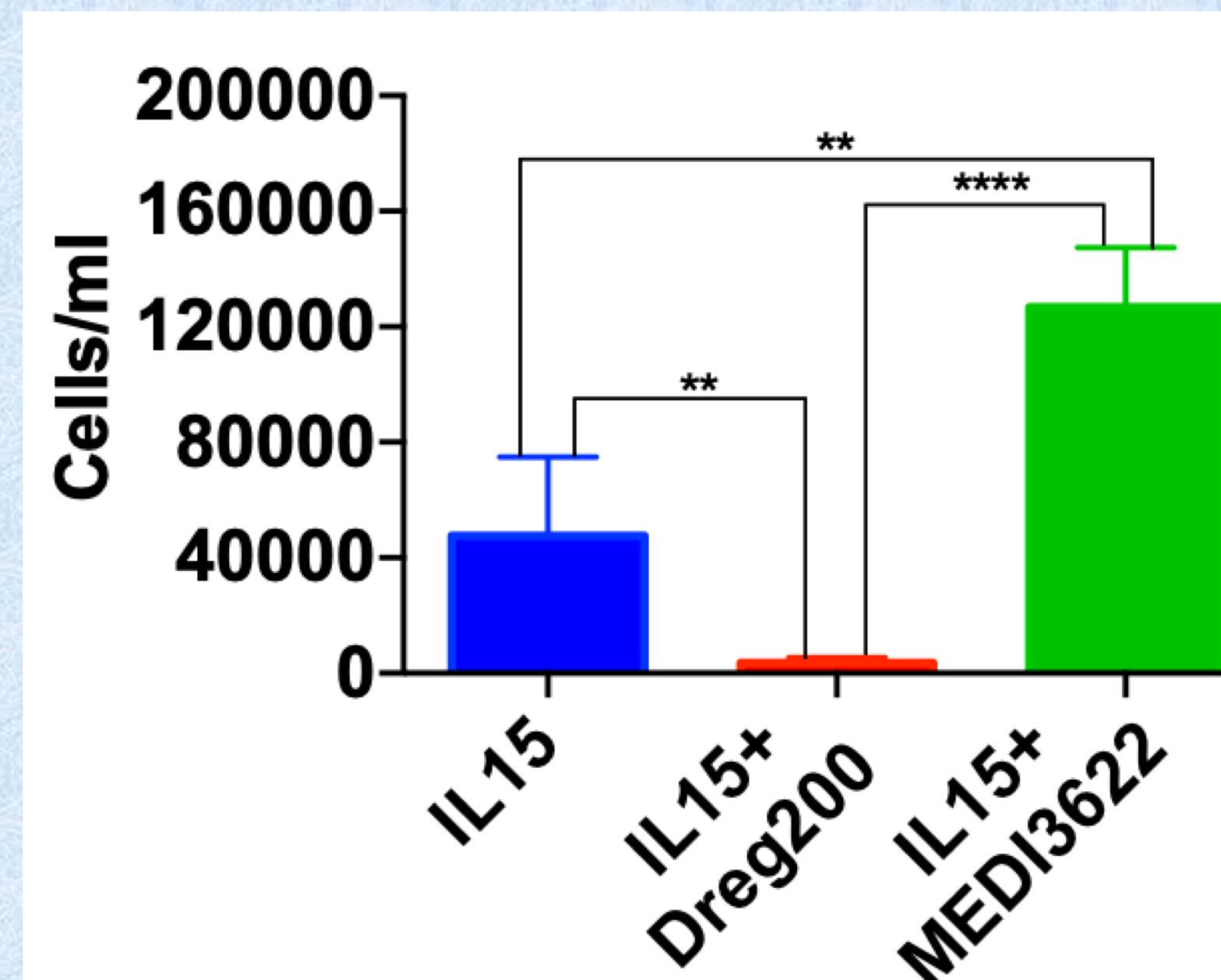


Figure 7. The pre-incubation of Dreg200 in NK cell is enough to abrogate the expansion of NK cells. NK cell were incubated with 5ug of either Dreg200 or MEDI3622 antibody and thereafter approx. 4×10^6 millions cell/mouse were injected intravenously in irradiated NSG mice. Once again, Dreg200 antibody was able to significantly abrogate the expansion of NK cells in comparison to MEDI3622 antibody and even IL15 alone treated mice.

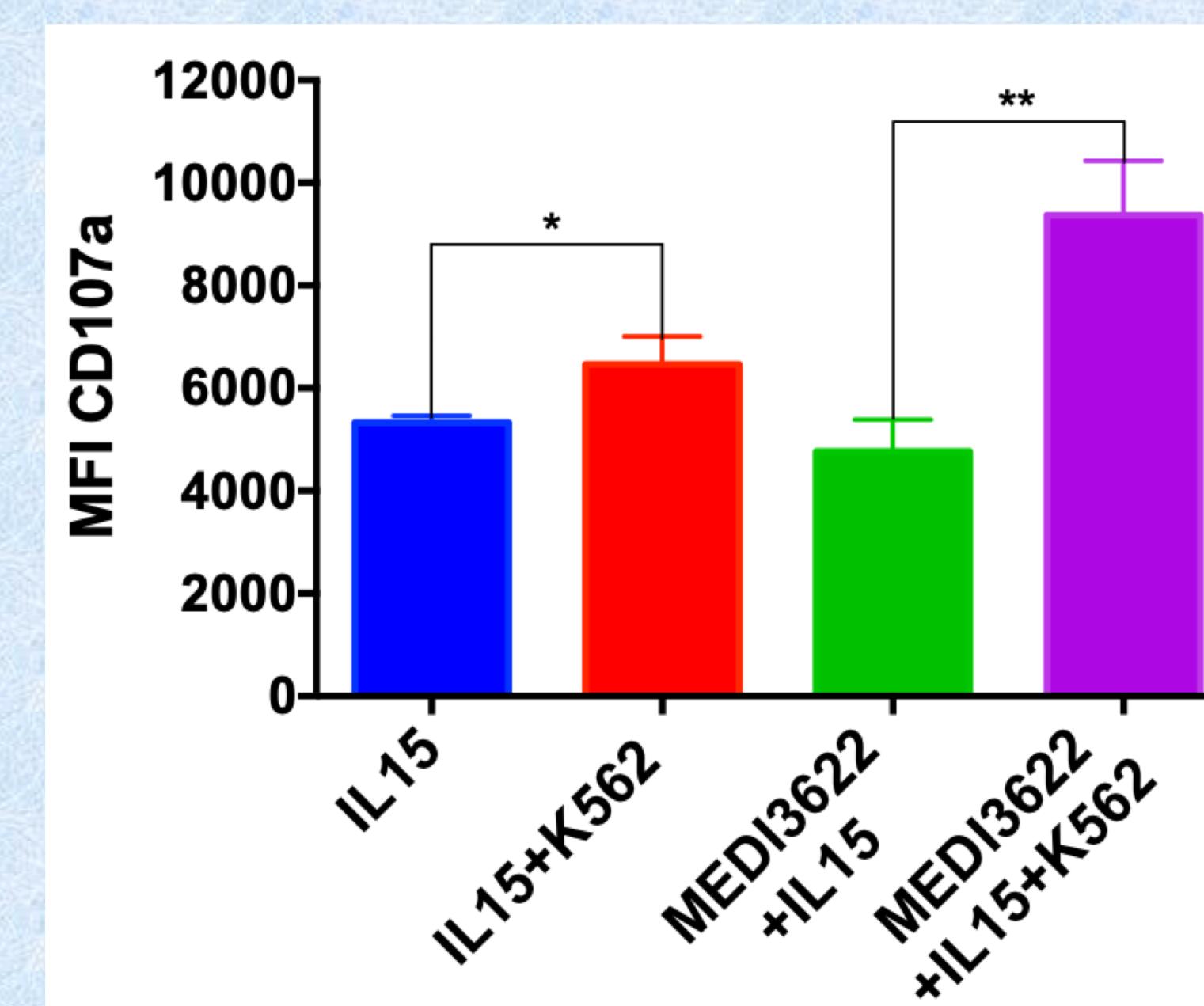


Figure 8. The expanded NK cells retain their natural cytotoxicity. The expanded NK cells were isolated from spleen of the NSG mice by using gentle MACS dissociator (as per manufacturer's protocol) and thereafter incubated with immortalized human myelogenous leukemia cell line called K562 to study their natural cytotoxicity. Approximately 80×10^3 NK cells were incubated with 8×10^3 K562 cells (10:1 Effector:Target ratio) in presence of Golgi stop and Golgi plug along with of CD107a antibody for 3 hours. Thereafter, CD56 cells were stained and CD107a MFI was measured and compared on the NK cell population. NK cells incubated with K562 showed significantly higher expression of CD107a indicating the retention of their natural cytotoxicity.

Conclusions

- The findings of our study suggests that ADAM17 inhibition by a mAb (MEDI3622) promotes the expansion of NK cells by preventing the downregulation of CD62L from the surface of NK cells in irradiated NSG mice.
- D1A(12), a mAb which only identify human ADAM17, promote the expansion of NK cells suggesting a primary role of ADAM17 during this process.
- The abrogation of NK cell expansion by a CD62L function block mAb (Dreg200) implicates the critical role of L-selectin in NK cell proliferation.
- The pre-incubation of freshly isolated NK cells with Dreg200, with no antibody injections in mice, can abrogate the process of expansion signifying its role in the early phases of homing and migration.
- Similarly, the pre-incubation of MEDI3622 also promoted NK cell expansion.
- The expanded NK cells retained their natural cytotoxicity.
- Our novel findings indicate the therapeutic potential of blocking ADAM17 on NK cells by MEDI3622 to promote their proliferation. This can be exploited in adoptive cell transfer based therapies where NK cell efficiency and persistence can be bolstered by their continuous proliferation and expansion.

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