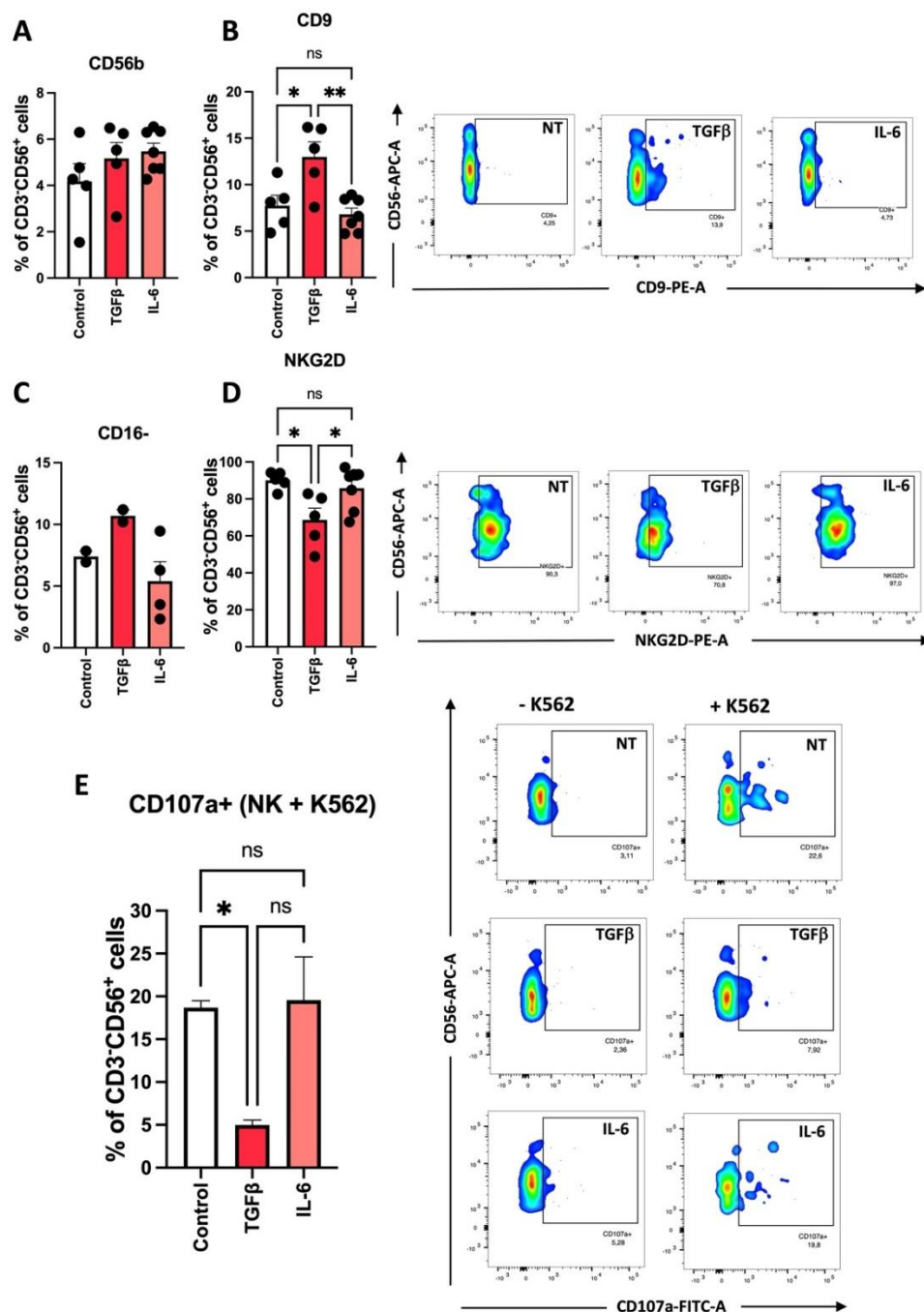


Supplementary Materials

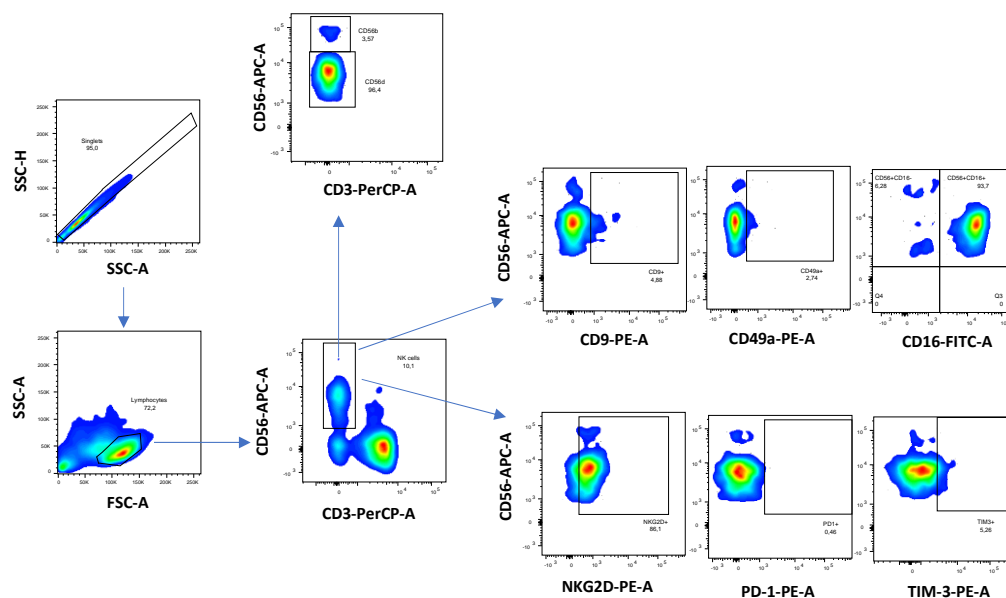
TIMP1 and TIMP2 Downregulate TGF β Induced Decidual-Like Phenotype in Natural Killer Cells

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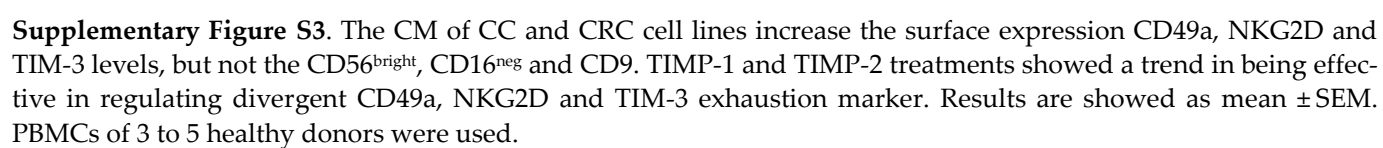


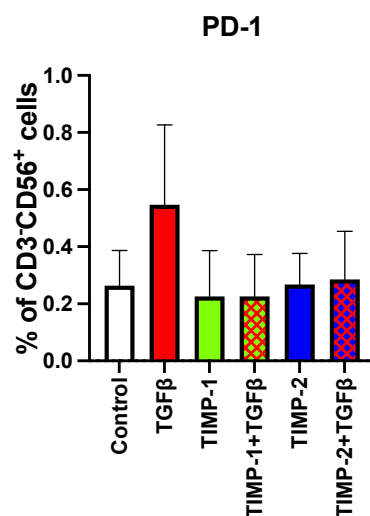
Supplementary Figure S1. Comparison of the effects of TGF β and IL-6 in generating of decidual-like/anergic/exhausted NK cells. We observed no differences in CD56^{bright} NK cell increase when compared TGF β with IL-6 (A), while NK cells exposed to TGF β has increased expression of CD9, compared with not treated and IL-6 exposed NK cells (B). Also, TGF β was more effective than IL-6 in increasing the frequency of CD16 NK cells (C), and in

downregulating NKG2D (D). TGF β significantly reduced NK cell degranulation capabilities against K562 cells, as compared to IL-6 (E). Results are showed mean \pm SEM, ANOVA, ns= not significant; *p < 0.05; **p < 0.01. Controls were cells in RPMI medium alone. PBMCs from 2–7 different healthy donors were used.

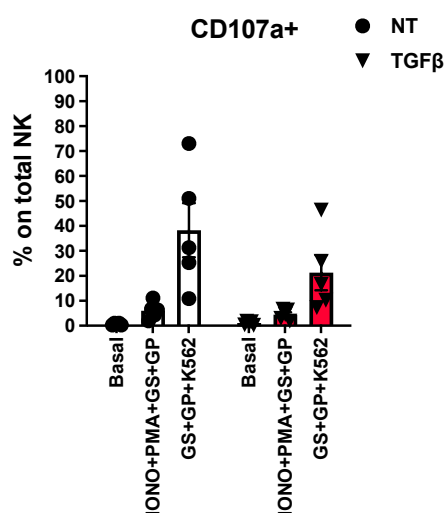


Supplementary Figure S2. Representative gating strategy for NK cell identification and selected decidual-like /CD56^{bright}, CD9, CD49a), activation (NKG2D) and exhaustion (PD-1, TIM-3) detection.





Supplementary Figure S4. TIMP-1 and TIMP-2 treatments showed a trend in contrasting the TGFβ-induced ability to increase PD-1 expression in cytolytic NK cells. PBMCs of 6 healthy donors were used. Results are showed as mean ± SEM.



Supplementary Figure S5. Internal controls for NK cell degranulation capability. The graph shows the degranulation capabilities of control and TGFβ treated NK cells at basal level (NK only in RPMI complete medium), NK cells with induced non-specific degranulation (NK only in RPMI complete medium, supplemented with Ionomycin, PMA, BrefeldinA and Monensin) and NKs with specific degranulation (NK cells and K562 cells at 1:1 effector/target ratio, in RPMI complete medium, supplemented with BrefeldinA and Monensin). Results are showed as mean ± SEM.