THP-1 transduced with CD16: a tool to study Fc receptor functions and phagocytosis

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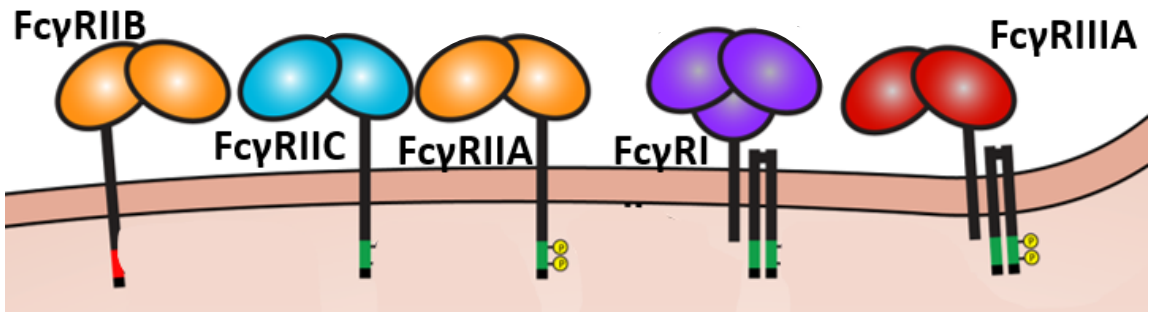
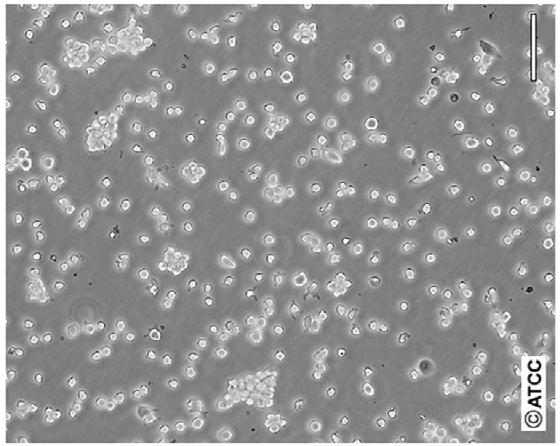
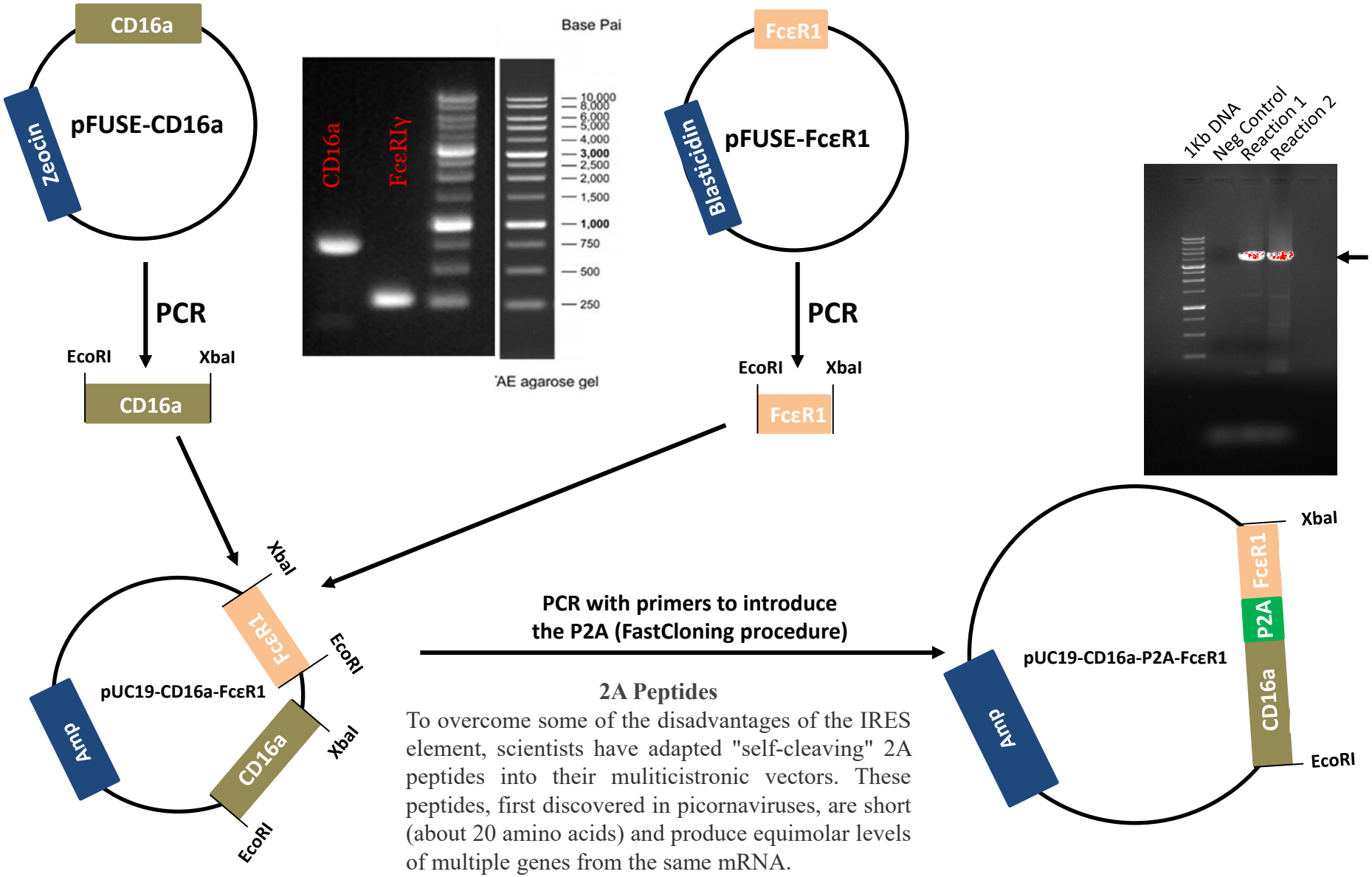
Introduction

- **Fc gamma receptors (FcγRs) are critical for mediating the effector functions of IgG and are associated with several antibody-mediated inflammatory disorders, such as ITP.**
- **Human FcγRIIIa may be an attractive therapeutic target due to its role in platelet clearance through macrophage phagocytosis.**
- **The absence of a human monocyte/macrophage cell line expressing this receptor has hampered the *in vitro* studies of the physiology of this FcγR and potential treatments.**
- **The human monocytic leukemia cell line (THP-1 cells) is an attractive target for the expression of this receptor.**





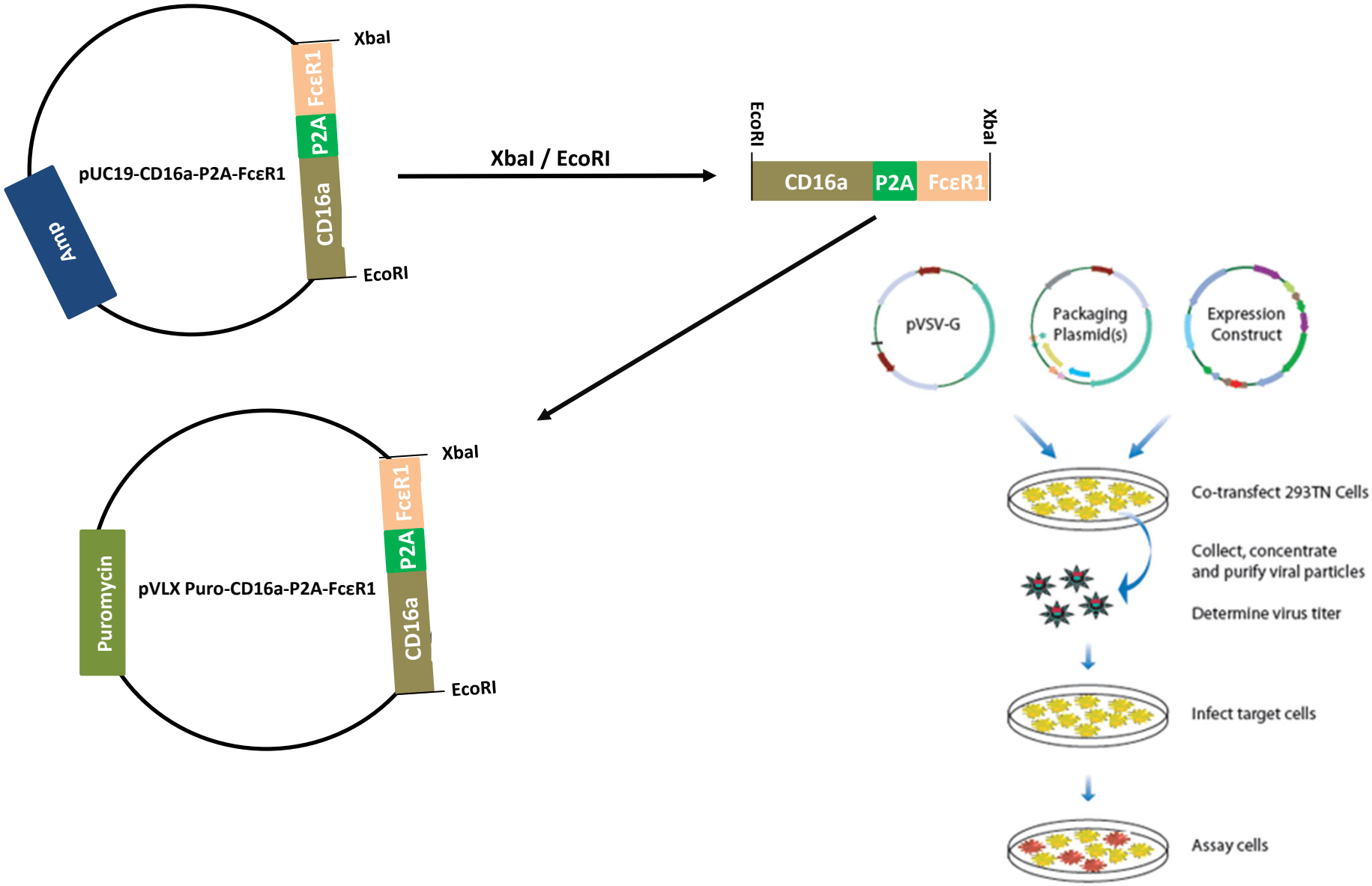
Lentiviruses as a tool to transduce THP-1 cells for the stable expression of the hFcγRIIIa



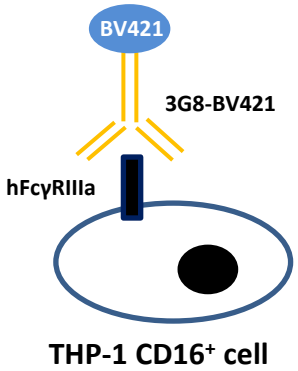
2A Peptides

To overcome some of the disadvantages of the IRES element, scientists have adapted "self-cleaving" 2A peptides into their muliticistronic vectors. These peptides, first discovered in picornaviruses, are short (about 20 amino acids) and produce equimolar levels of multiple genes from the same mRNA.

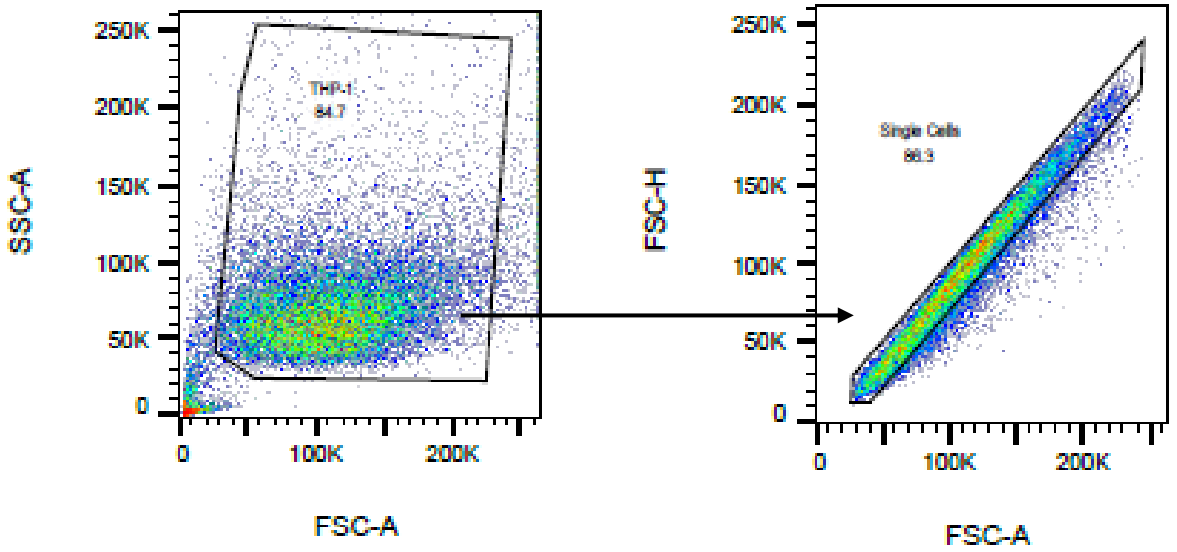
Lentiviruses as a tool to transduce THP-1 cells for the stable expression of the hFcγRIIIa



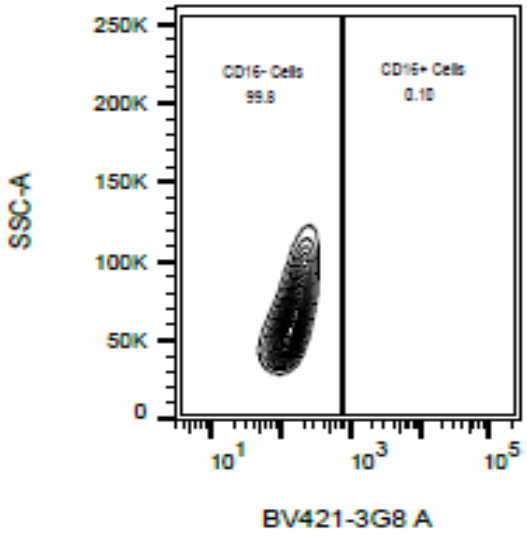
Expression of hFcγRIIIA on THP-1 cells transduced with lentivirus



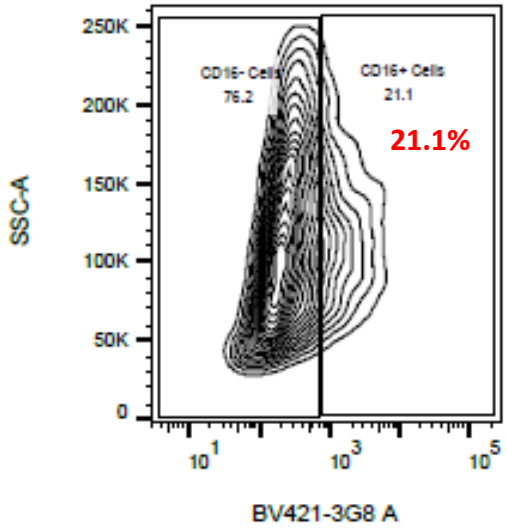
2 weeks after transduction



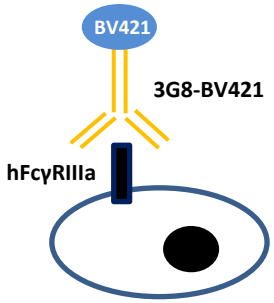
Control cells (non-transduced)



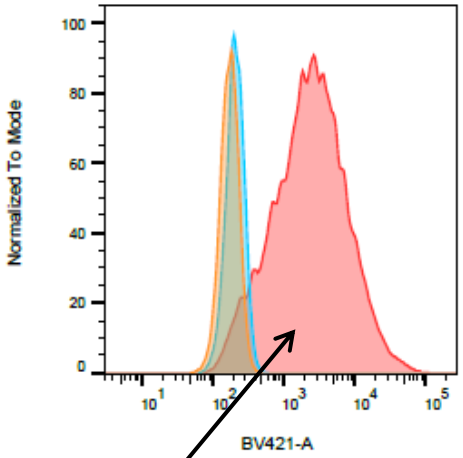
THP-1 cells transduced Puromycin-resistance-Lentivirus

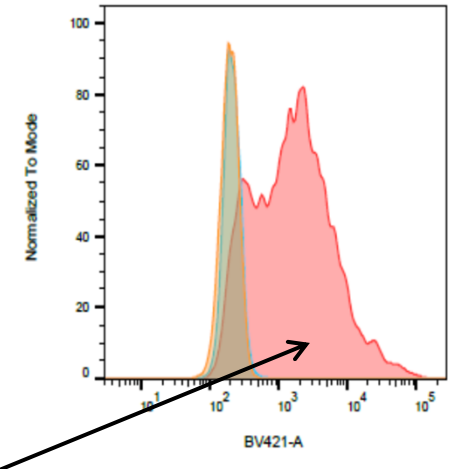


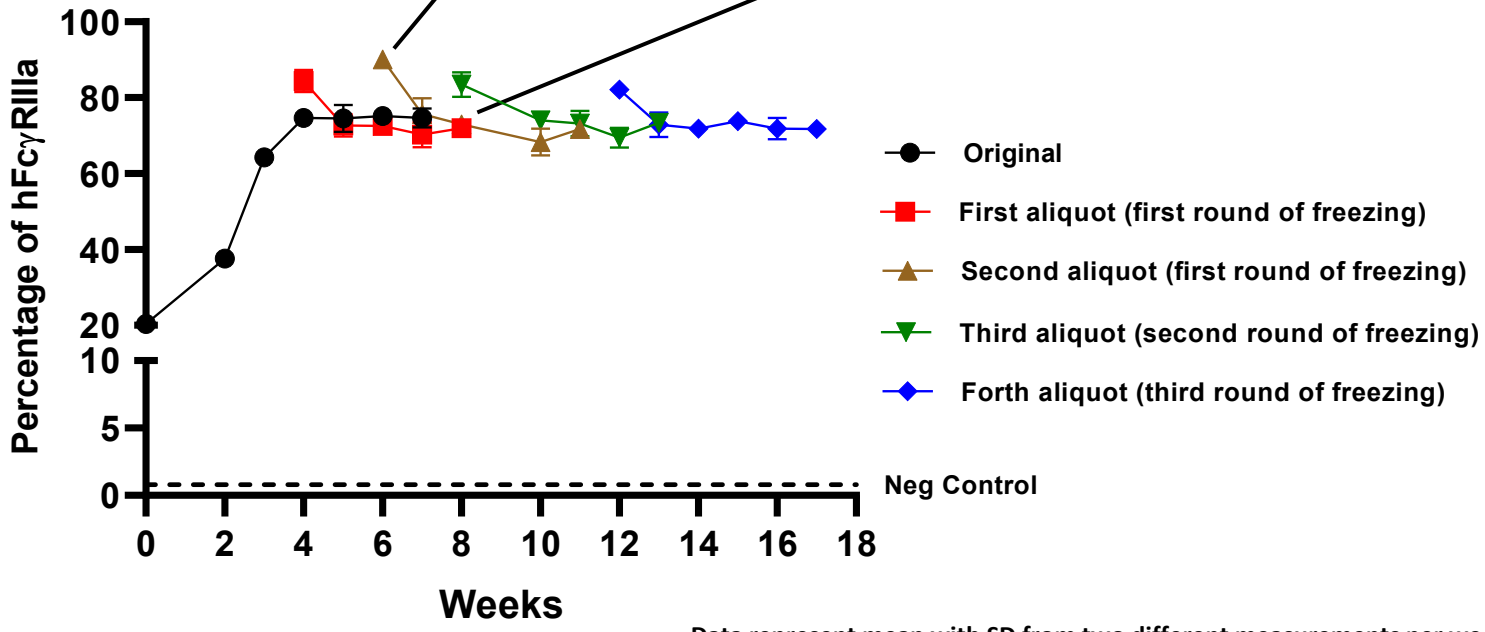
Stability of hFcγRIIIA expression on THP-1 CD16⁺ cells



THP-1 CD16⁺ cell



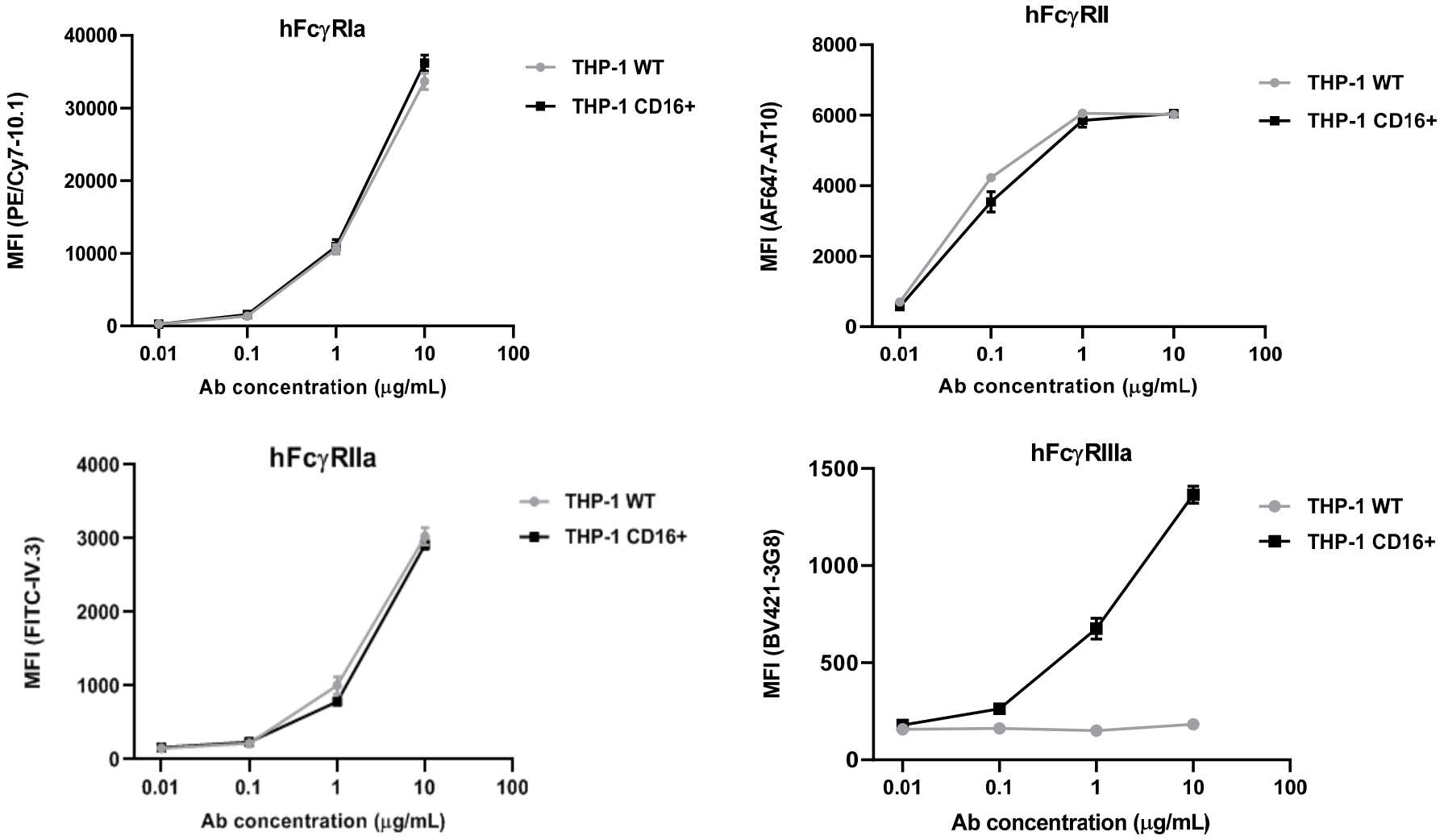




Data represent mean with SD from two different measurements per week

THP-1 CD16⁺ cells show a stable expression of the hFcγRIIIa

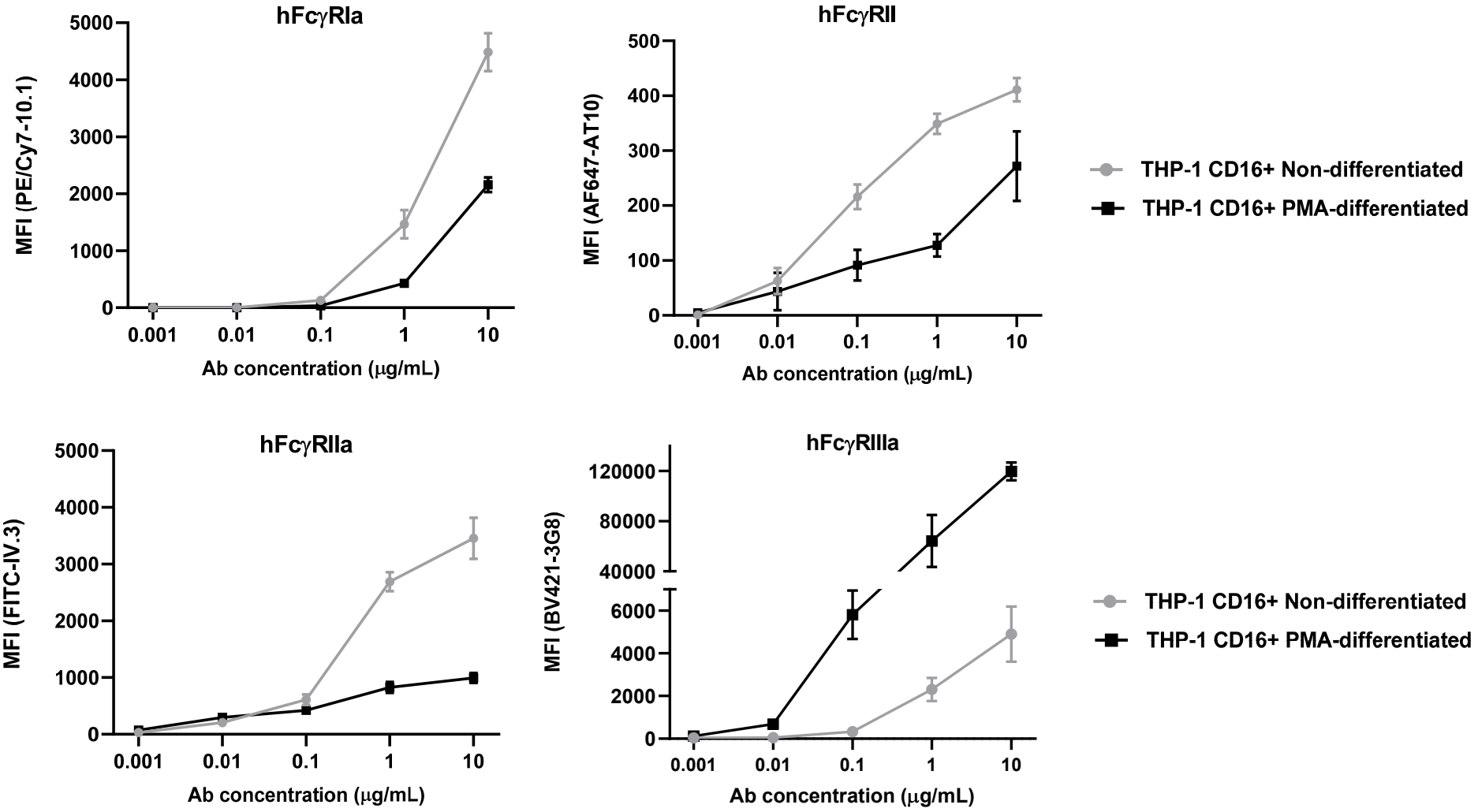
Expression of hFcγRs on THP-1 and THP-1 CD16^{+} cells



Data represent mean with SD from three different assays

THP-1 CD16^{+} cells show a similar pattern of hFcγRs expression to that observed in wild-type THP-1 cells

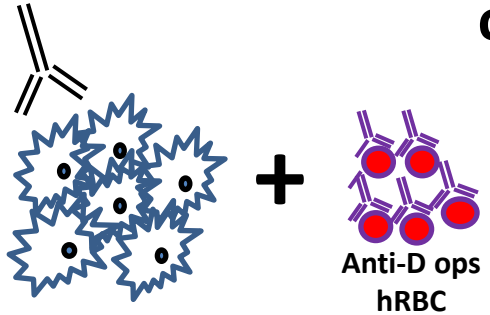
Expression of hFcγRs on non-differentiated or PMA-differentiated THP-1 CD16^{+} cells



Data represent mean with SD from three different assays

The PMA-differentiation of THP-1 CD16^{+} does affect the expression of human FcγRs

Phagocytosis assay and contribution of hFcγRs expressed on THP-1 or THP-1 CD16^{+} cells

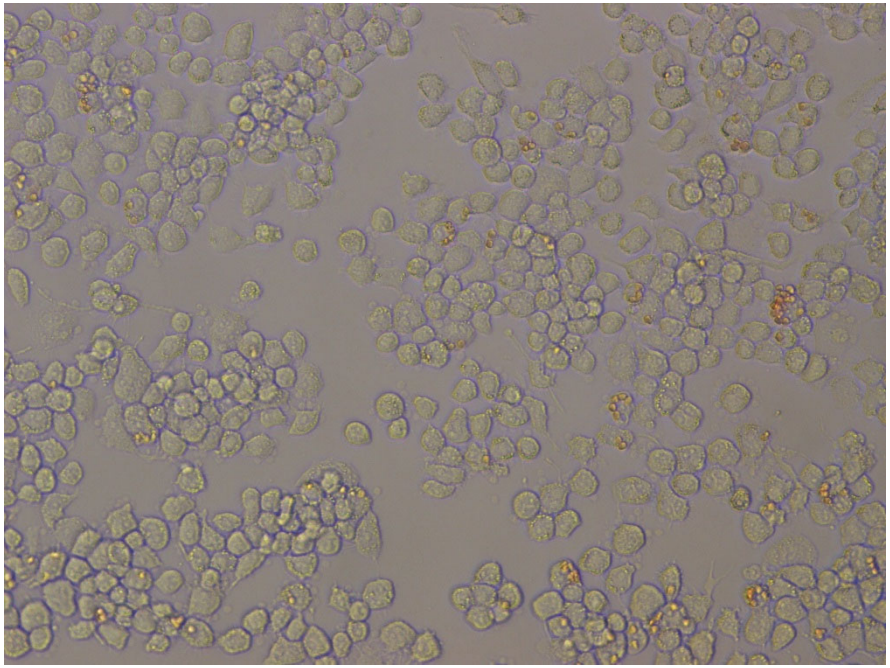


PMA-differentiated THP-1 CD16^{+} cells

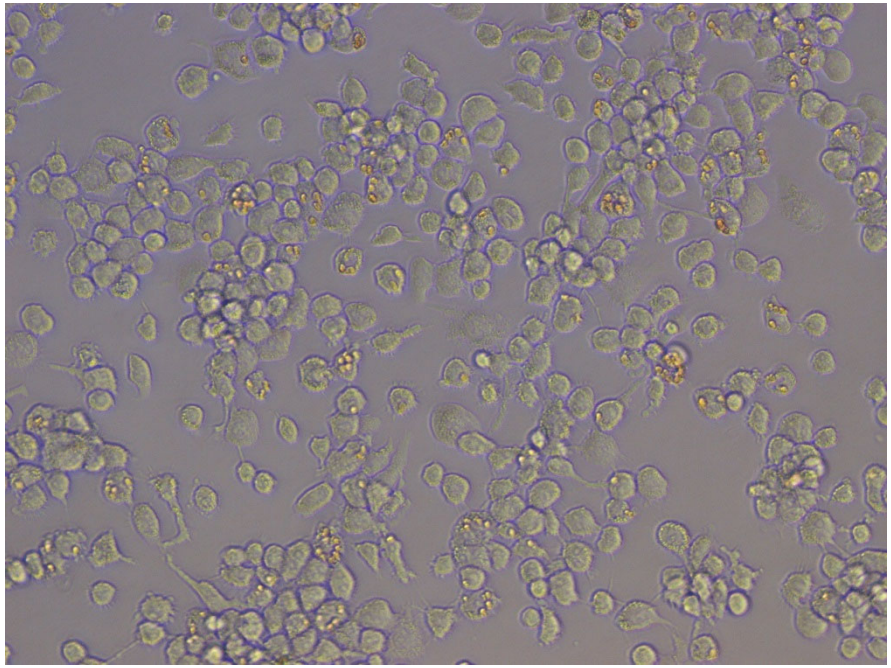
+

Anti-D ops hRBC

THP-1 cells

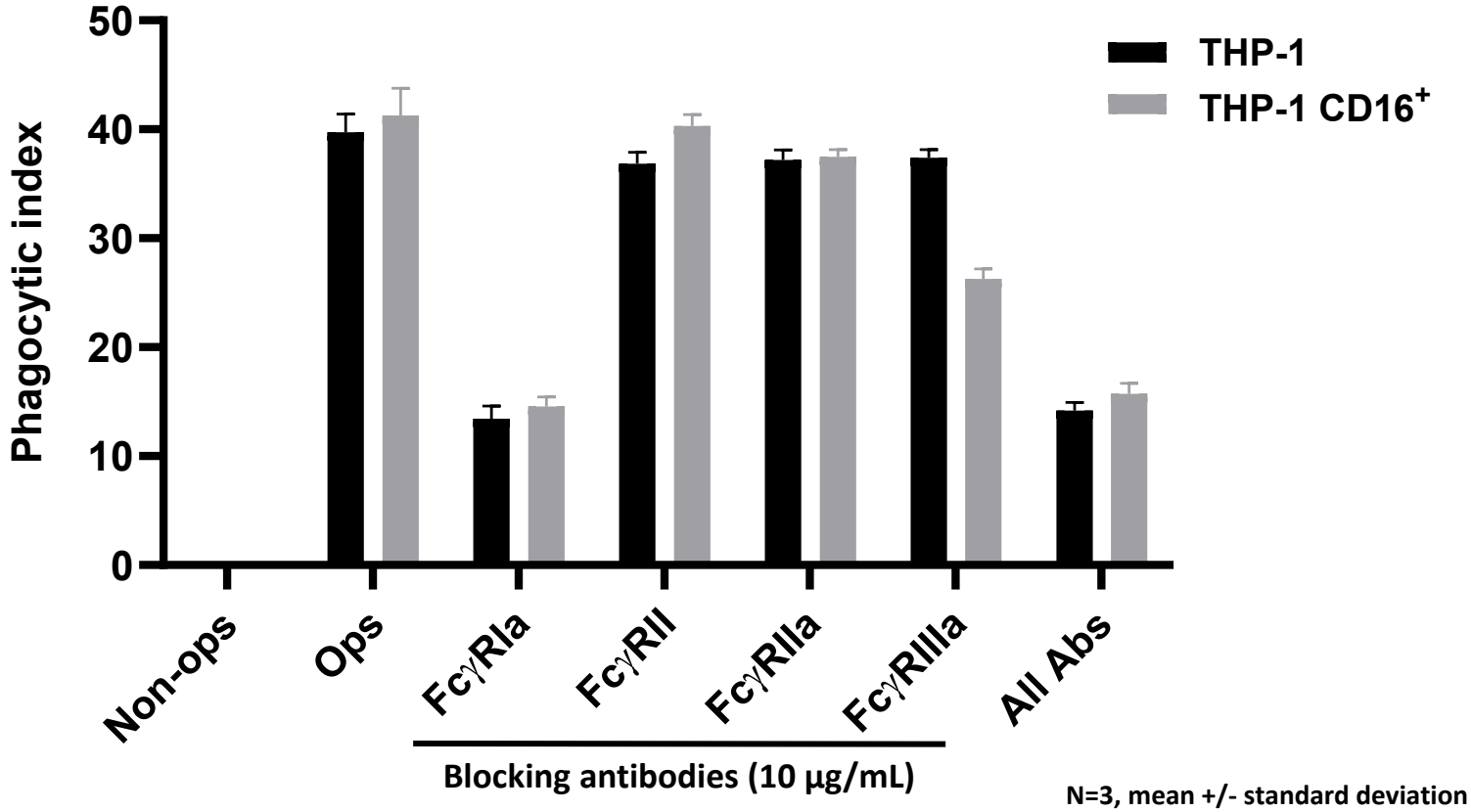


THP-1 CD16^{+} cells



THP-1 CD16^{+} can phagocytose anti-D-opsonized hRBCs similarly to wild-type THP-1 cells.

Phagocytosis assay and contribution of hFcγRs expressed on THP-1 or THP-1 CD16^{+} cells



N=3, mean +/- standard deviation

THP-1 CD16^{+} can phagocytose anti-D-opsonized hRBCs similarly to wild-type THP-1 cells. Transduced human FcγRIIIa contributes to phagocytosis.

Conclusions

- ✓ **THP-1 CD16^{+} cells stably express the hFcγRIIIa and the remaining hFcγRs.**
- ✓ **THP-1 CD16^{+} cells phagocyte hRBCs as well as wild-type THP-1 cells and FcγRIIIa contributes to phagocytic function.**
- ✓ **The transgenic cells constitute a valuable tool for further studies of FcγRIIIa-mediated effector functions and potential anti-CD16 treatments.**

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