Combined Genetic Ablation of CD54 and CD58 in CAR Engineered Cytotoxic Lymphocytes Effectively Averts Allogeneic Immune Cell Rejection

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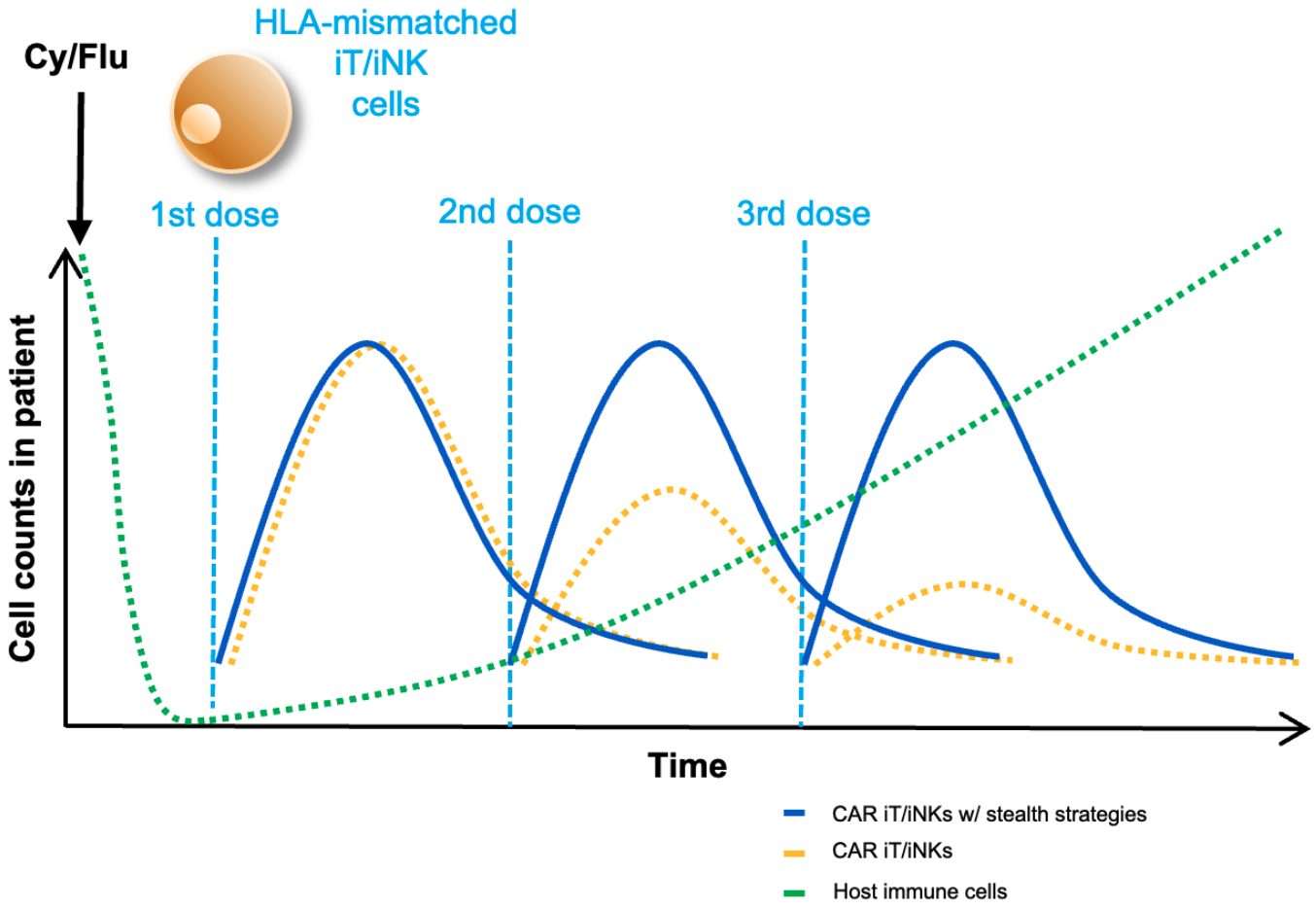
**equal contribution, #equal contribution*

Quirin Hammer

“Is an animal partially tolerant because all its reactive cells are almost completely debilitated, or because, while most of them are completely out of function, a minority retain full possession of their power”

Peter Medawar, Nobel Lecture 1960

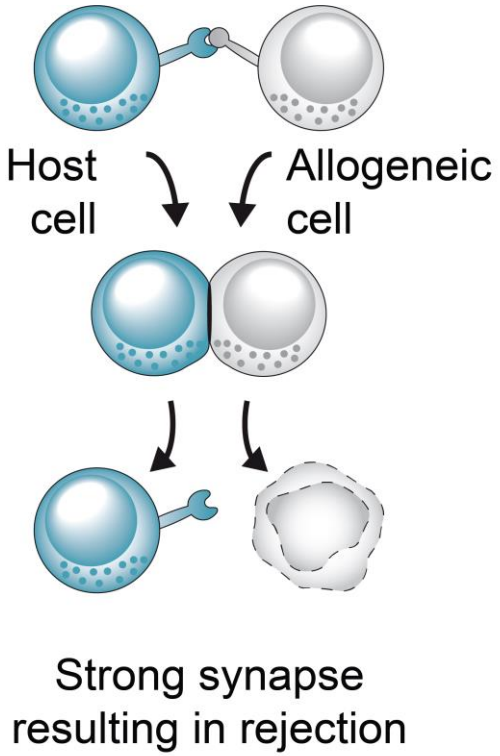
Rejection by the host is a critical consideration for multi-dosing of allogeneic cell therapy



Rejection by the host is a critical consideration for multi-dosing of allogeneic cell therapy

Reduced surface expression of human leukocyte antigen (HLA) triggers rejection by host natural killer (NK) cells by loss-of-inhibition

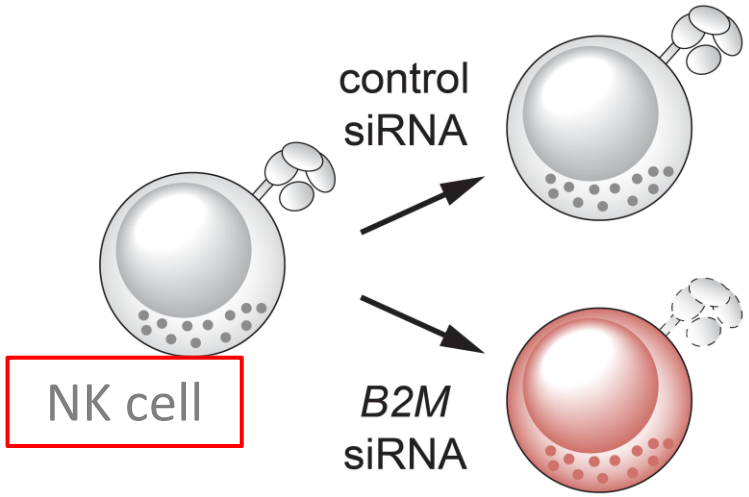
Naturally occurring escape strategies as blueprints for allogeneic cell products



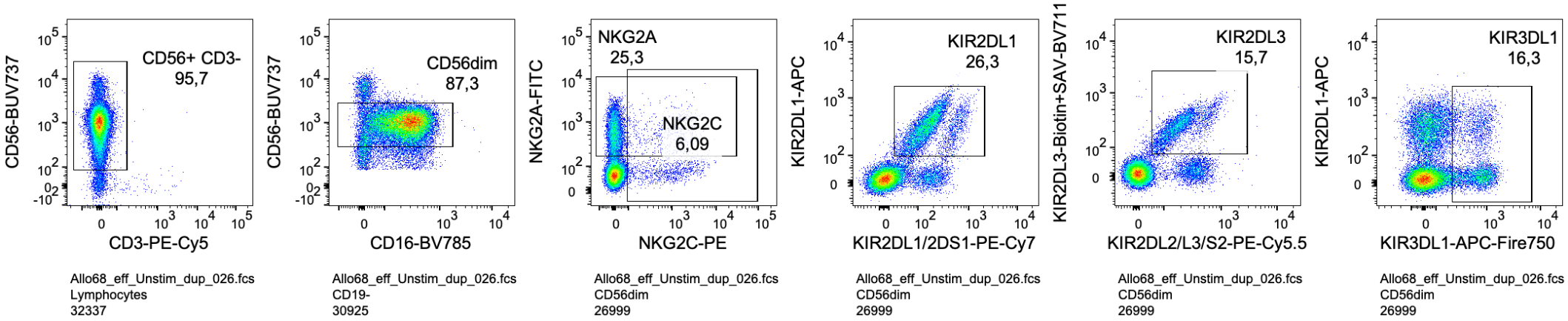
- CD11/18 and CD2 and their ligands **CD54** and **CD58** are crucial for synapse formation
- Burkitt's lymphoma down-regulates **CD54** and **CD58**
- Down-regulation of **CD58** renders *B2M*$^{-/-}$ B cell lymphoma resistant to NK cell killing
- Cytomegalovirus escapes NK cell surveillance by down-regulation of **CD58** on infected cells

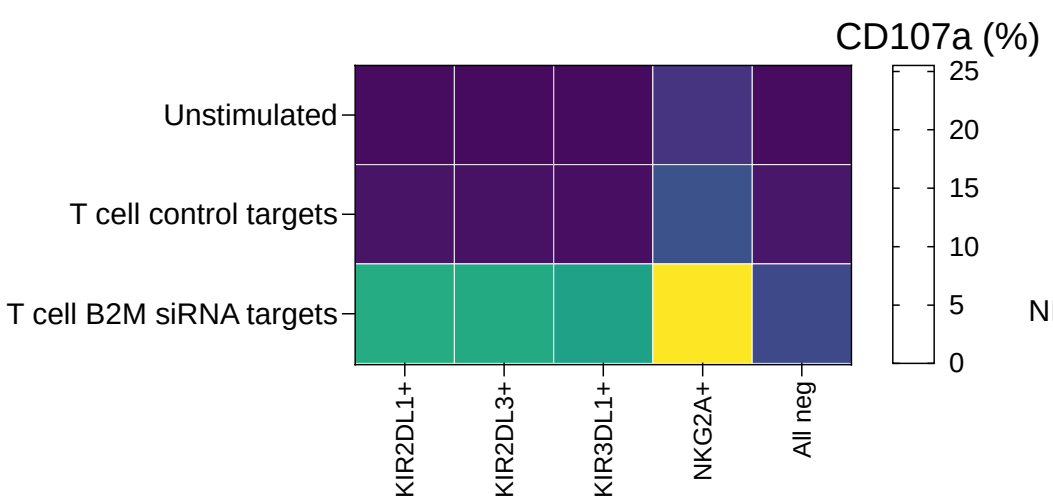
Orange *et al*., PNAS 2003; Billaud *et al*., Lancet 1987; Challa-Malladi *et al*., Cancer Cell 2011; Cao *et al*., Oncotarget 2016; Wang *et al*., PNAS 2018

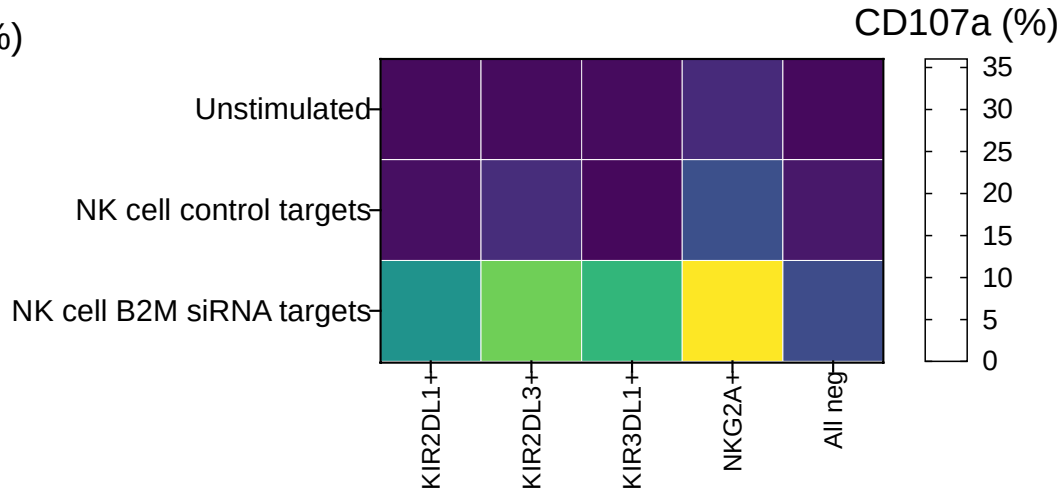
Decreased HLA class I expression triggers potent missing-self responses by allogeneic NK cells



Educated NK cells (NKG2A+ and KIR+) dominate the missing self response







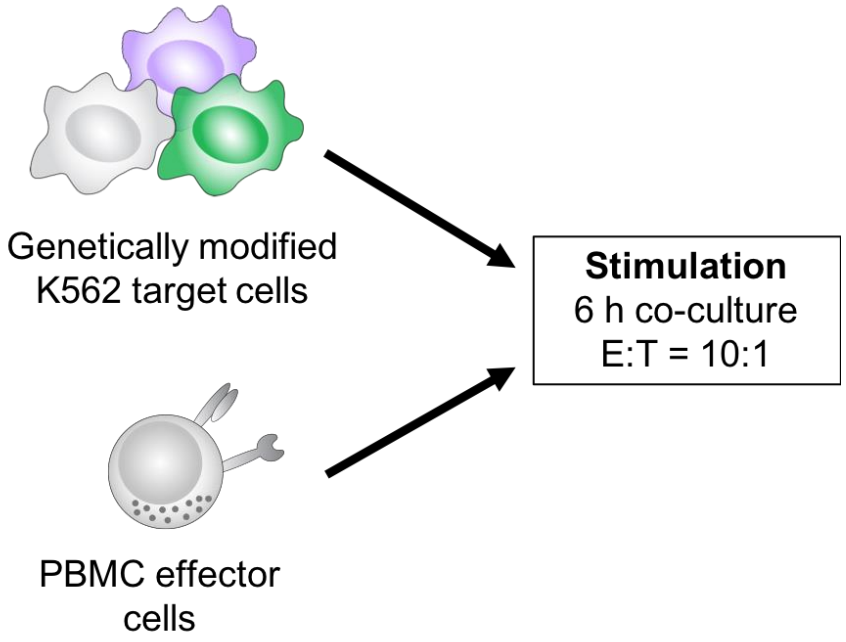
Head-to-head benchmarking of inhibitory strategies using K562

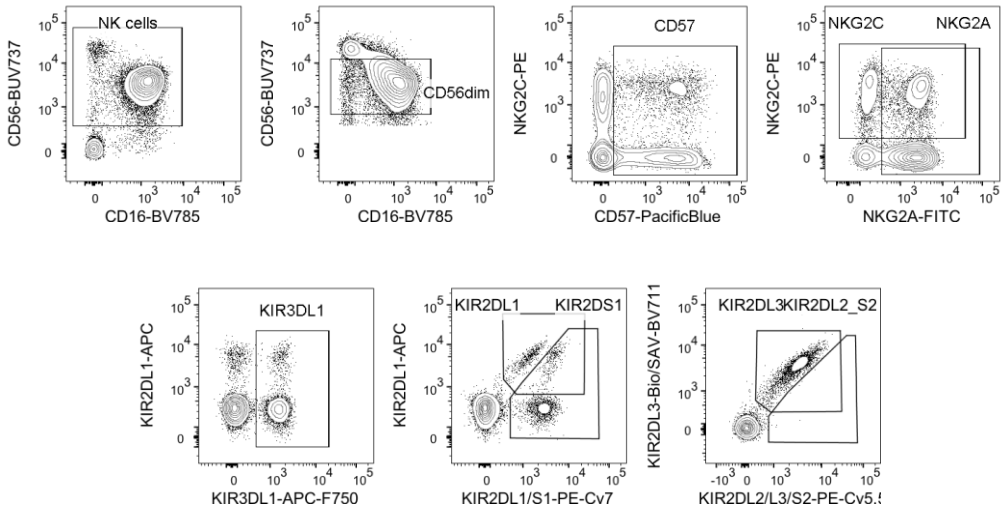
K562 targeted library

1. **K562-wt**
2. **K562-HLA-C*07:01:01** => ligand for inhibitory KIR2DL3
3. **K562-HLA-C*04:01:01** => ligand for inhibitory KIR2DL1
4. **K562-HLA-E** => ligand for inhibitory NKG2A
5. **K562-HLA-C*07/HLA-C*04** => ligands for KIR2DL3 and KIR2DL1
6. **K562-HLA-E/HLA-C*07/HLA-C*04** => ligands for NKG2A, KIR2DL3, and KIR2DL1
7. **K562-CD54KO** => absence of one adhesion ligand for CD11a/18
8. **K562-CD58KO** => absence of adhesion ligand for CD2
9. **K562-CD54/CD58KO** => absence of adhesion ligands for CD11a/18 and CD2

SCT: single chain trimer; SCD: single chain dimer
FTV1117/CD47: Deuse *et al*., Nat Biotechnol 2019; FTV1401/HLA-E single chain trimer: Gornalusse *et al*., Nat Biotechnol 2017

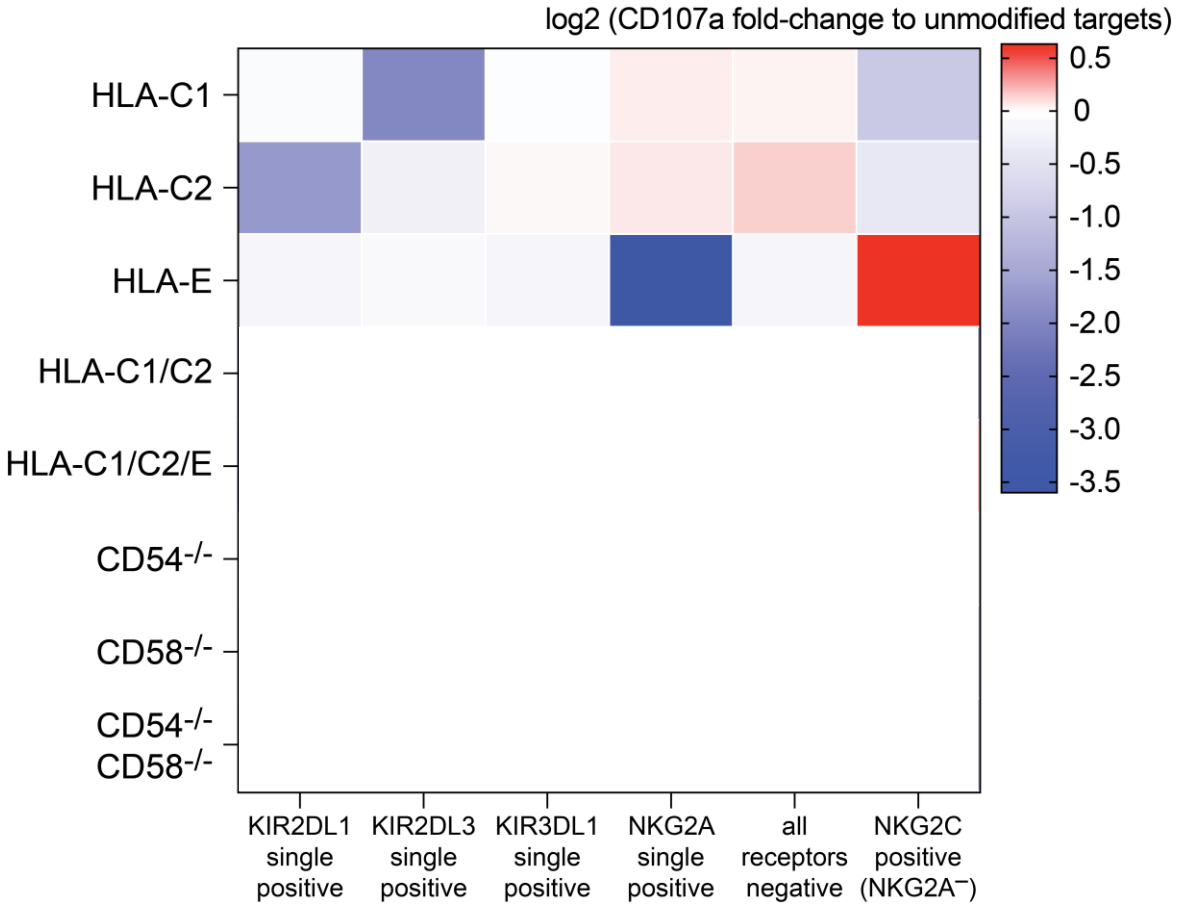
NK cell activation at the subset level





1. **CD56^{dim}**
2. CD56^{dim} NKG2C^{-} **NKG2A^{+}** KIR2DL1^{-} KIR2DS1^{-} KIR2DL3^{-} KIR2DL2/S2^{-} KIR3DL1^{-}
3. CD56^{dim} NKG2C^{-} NKG2A^{-} **KIR2DL1^{+}** KIR2DS1^{-} KIR2DL3^{-} KIR2DL2/S2^{-} KIR3DL1^{-}
4. CD56^{dim} NKG2C^{-} NKG2A^{-} KIR2DL1^{-} KIR2DS1^{-} **KIR2DL3^{+}** KIR2DL2/S2^{-} KIR3DL1^{-}
5. CD56^{dim} NKG2C^{-} NKG2A^{-} KIR2DL1^{-} KIR2DS1^{-} KIR2DL3^{-} KIR2DL2/S2^{-} **KIR3DL1^{+}**
6. CD56^{dim} NKG2C^{-} NKG2A^{-} KIR2DL1^{-} KIR2DS1^{-} KIR2DL3^{-} KIR2DL2/S2^{-} KIR3DL1^{-} **“all negative”**
7. CD56^{dim} **NKG2C^{+}** NKG2A^{-} KIR2DL1$^{+/-}$ KIR2DS1$^{+/-}$ KIR2DL3$^{+/-}$ KIR2DL2/S2$^{+/-}$ KIR3DL1$^{+/-}$

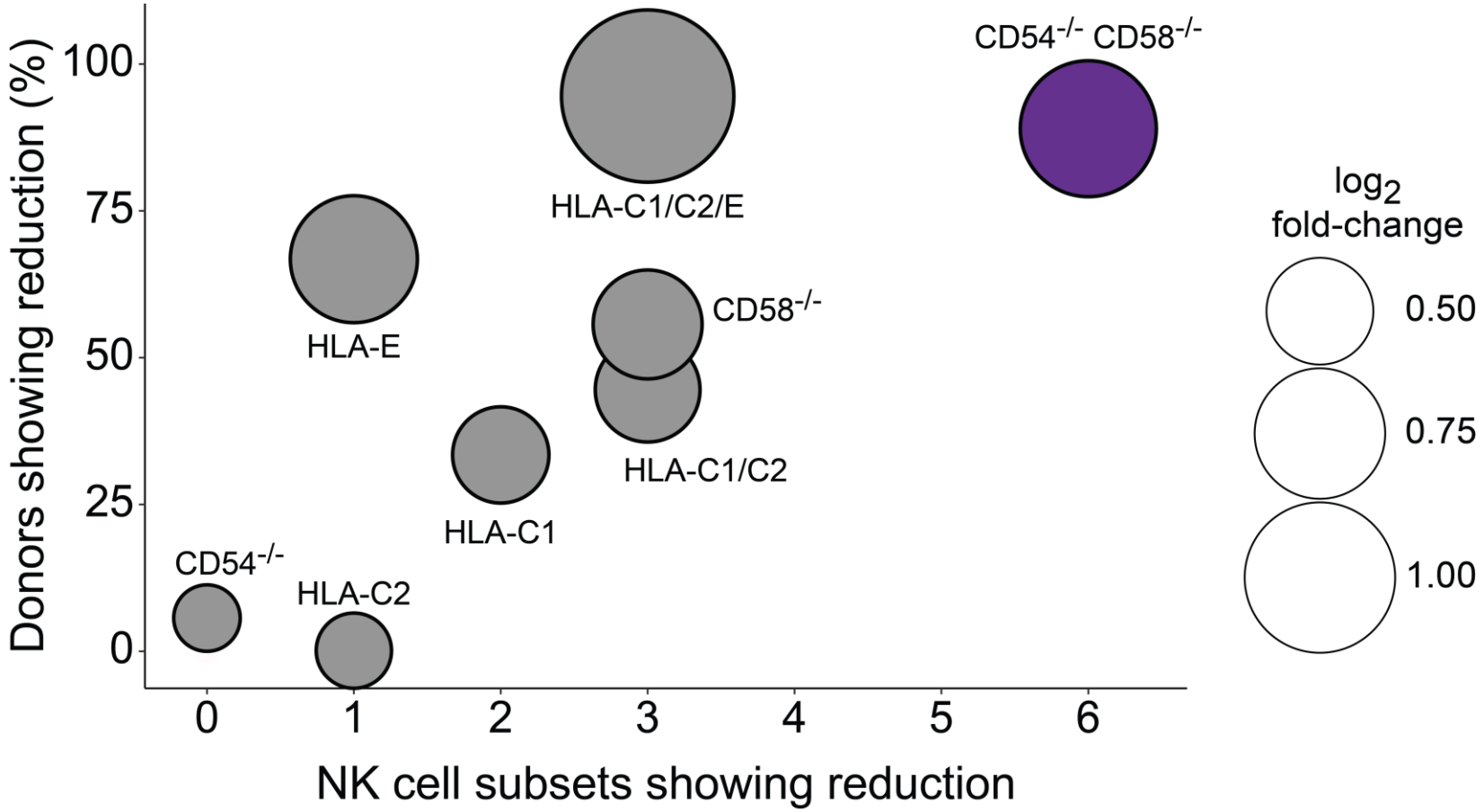
Combined deletion of *CD54* and *CD58* confers resistance across the spectrum of NK cell subsets



HLA-C1: ligand for KIR2DL3
HLA-C2: ligand for KIR2DL1
HLA-E: ligand for NKG2A (and NKG2C)

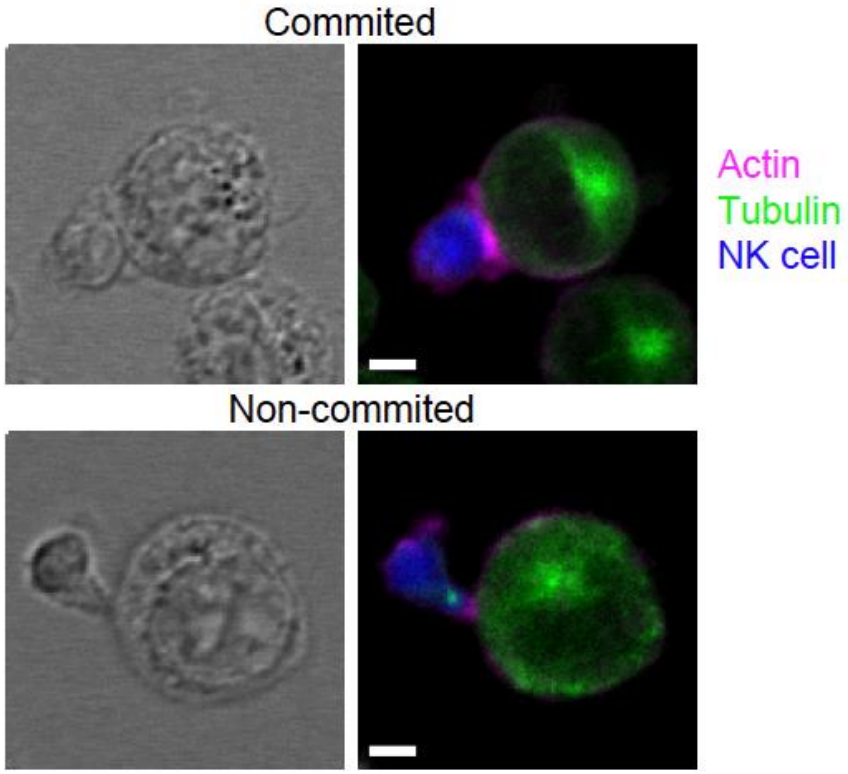
n=18 donors

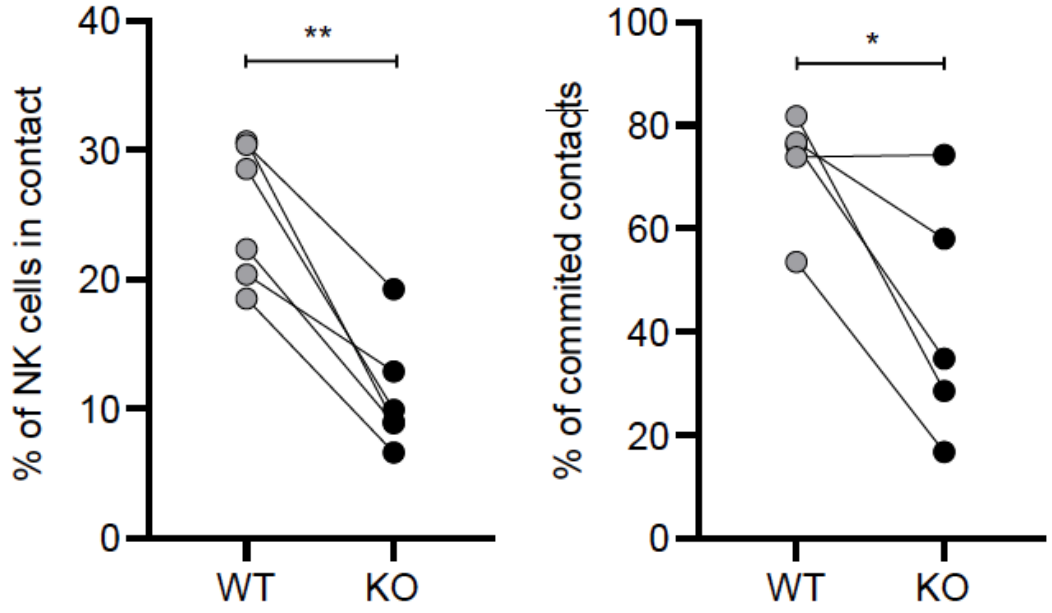
Combined deletion of CD54 and CD58 confers resistance across the spectrum of healthy donors



Quirin Hammer

$CD54^{-/-}$ $CD58^{-/-}$ target cells are resistant to NK cell killing due to reduced adhesion





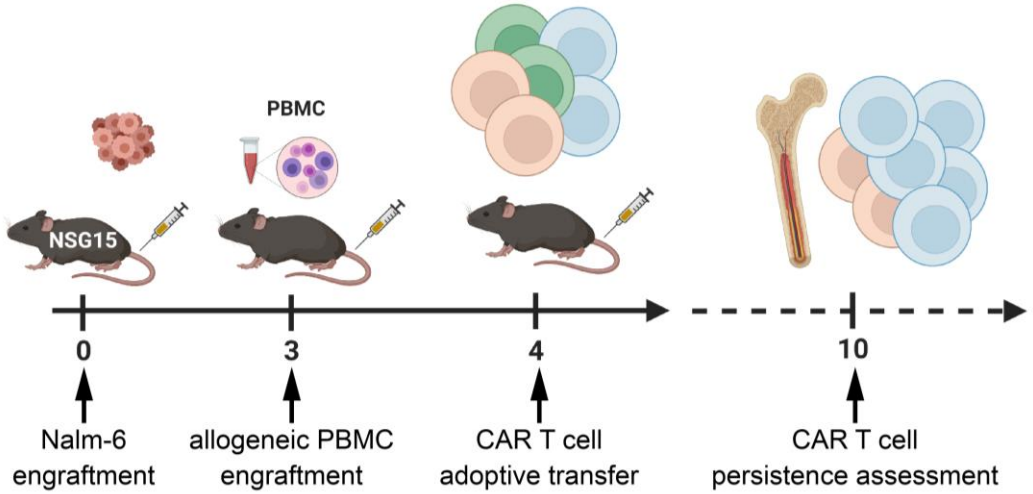
Hanna van Ooijen and Björn Önfelt

Reverse-engineering for therapeutic applications

CD54$^{-/-}$ *CD58*$^{-/-}$ *B2M*$^{-/-}$ CAR T cells maintain anti-tumor function and display persistence advantages *in vivo*

Memorial Sloan Kettering Cancer Center

Primary T cells expressing a 2nd generation CAR from the *TRAC* locus



Prof. Michel Sadelain and Karlo Perica, MD

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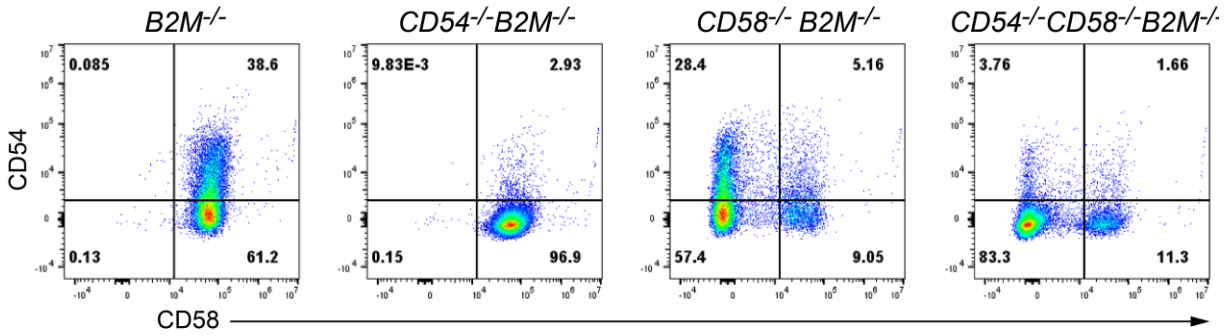
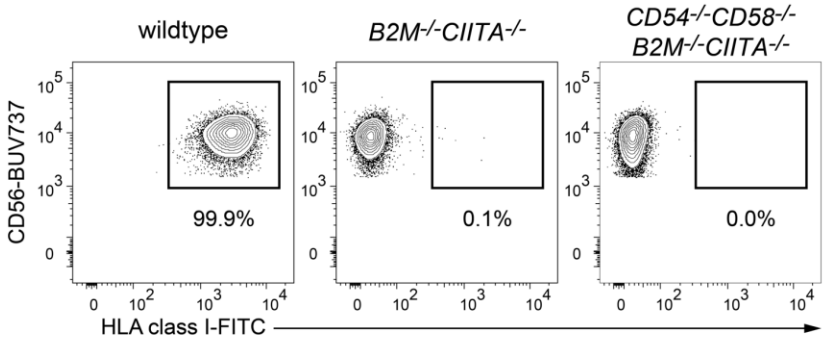


Prof. Michel Sadelain and Karlo Perica, MD

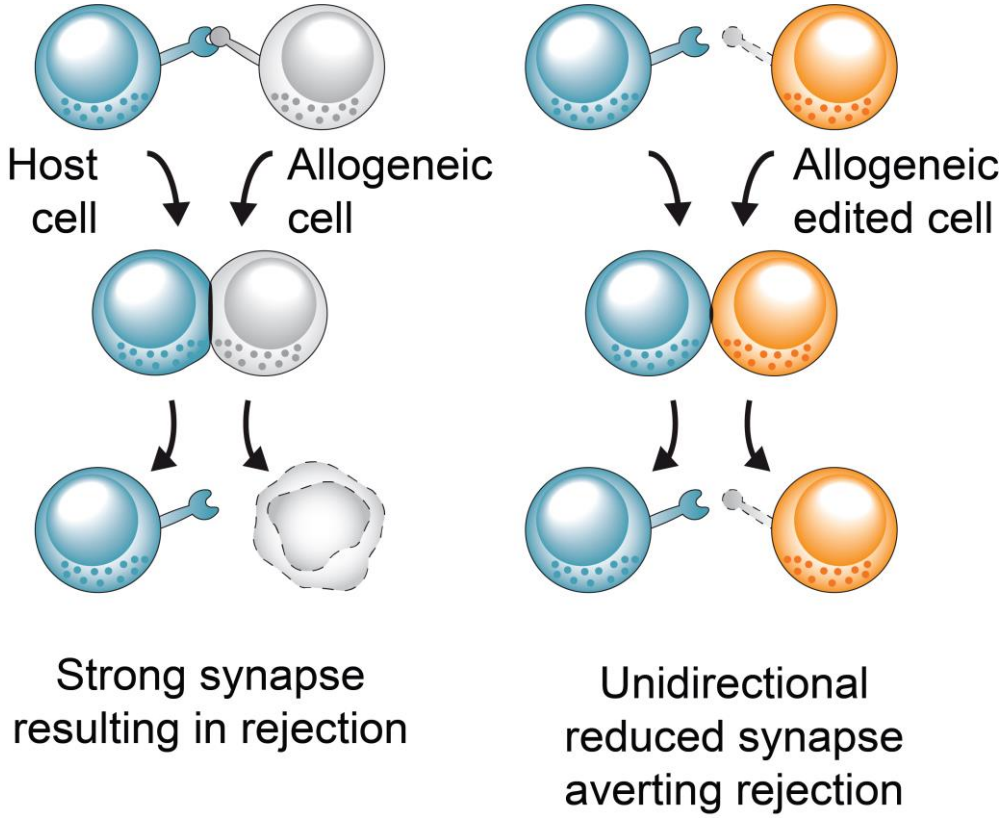
Deletion of *CD54* and *CD58* in a clonal line of multi-edited CAR iPSC-NK cells improves persistence *in vivo*

iPSC engineered with CD19-CAR, IL-15/IL-15Rα fusion, and high-affinity non-cleavable CD16
Differentiated into NK cells *in vitro*



Fate Therapeutics, Inc. (Rina Mbofung PhD, Anitha Somanchi PhD. Tom Lee PhD, Bahram Valamehr, PhD; Jode Goodridge, PhD)

Slippery NK



- Genetic deletion of the adhesion ligands CD54 and CD58 limits NK cell-mediated responses against HLA class I^{-} targets
- *CD54*$^{-/-}$ *CD58*$^{-/-}$ *B2M*$^{-/-}$ CAR T and CAR iPSC-NK cells display resistance to rejection by allogeneic immune cells

“Is an animal partially **tolerant because all its reactive cells are almost completely debilitated**, or because, while most of them are completely out of function, a minority retain full possession of their power”

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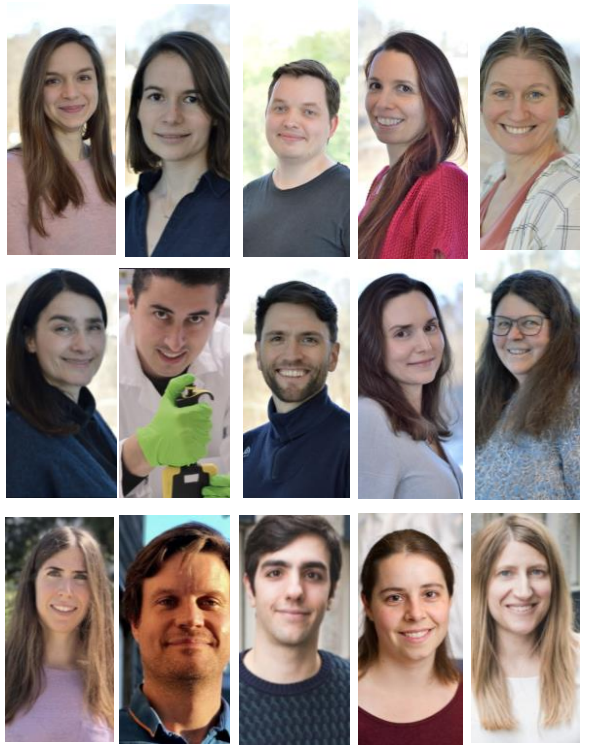

TEAM HAMMER

Forskningsrådet

MARIE CURIE ACTIONS

mature NK

MERCK

oncopeptides

Cancerfonden

Fate THERAPEUTICS