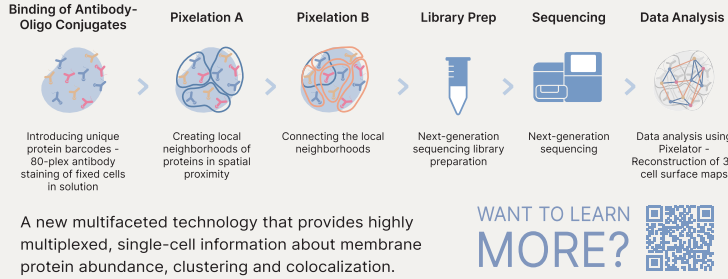


UNRAVELING THE MEMBRANE PROTEIN ARCHITECTURE OF CD19 CAR-T CELLS AT REST AND DURING TUMOR INTERACTION

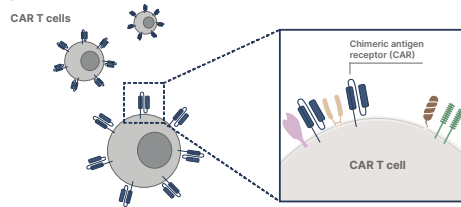
Divya Thiagrajan, Hanna van Ooijen, Filip Karlsson, Ludvig Larsson, Vincent van Hoef, Simon Fredriksson
Pixelgen Technologies AB, Stockholm, Sweden

MOLECULAR PIXELATION (MPX) WORKFLOW

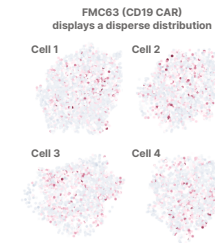


DECODING THE NANO-ORGANIZATION OF CD19 CAR-T CELL MEMBRANES USING MOLECULAR PIXELATION

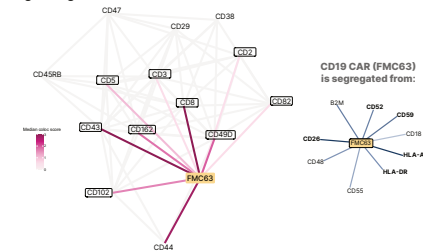
We utilized Molecular Pixelation (MPX) to comprehensively analyze the membrane protein organization of CD19 CAR-T cells in both resting conditions and during coculture with CD19-expressing tumor cells.



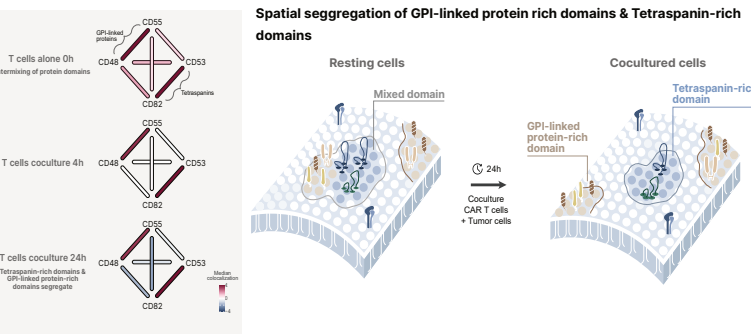
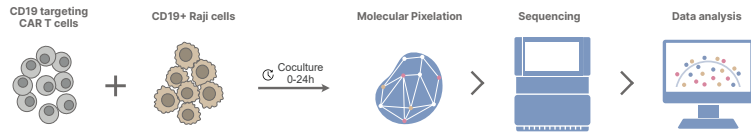
USING MPX TO CHARACTERIZE CAR-T CELLS



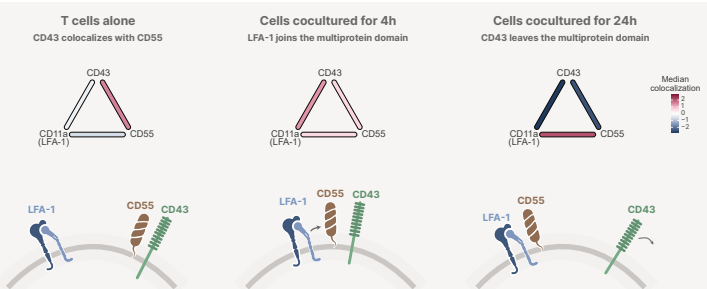
CAR construct colocalized with CD3 and its associated partners, yet its presence did not alter the organization of any proteins, demonstrating that the CD19 CAR construct integrates seamlessly into the same membrane nano-domains as CD3, preserving critical T cell signalling structures.



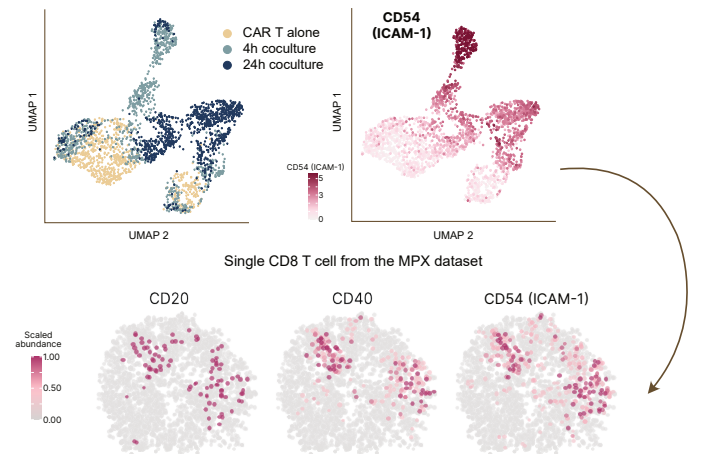
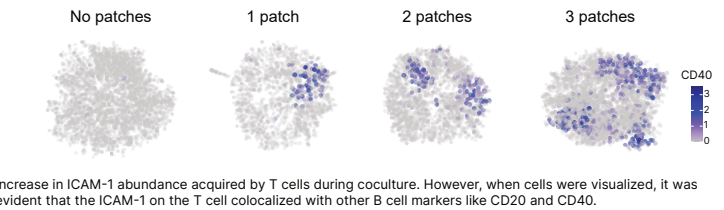
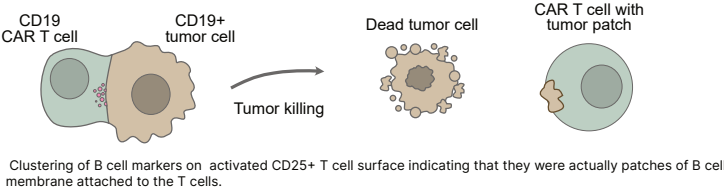
USING MPX FOR NANOSCALE MAPPING OF CAR RECEPTOR PROTEIN NEIGHBORHOODS



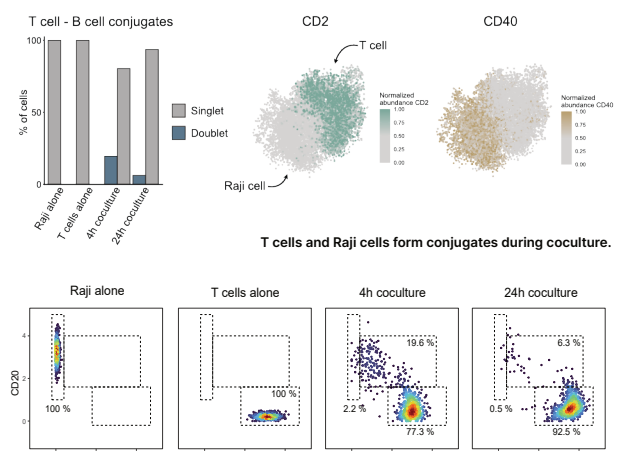
Reorganization of CD43 and LFA-1 could be seen during the coculture



USING MPX TO MEASURE CAR-T TROGOCYTOSIS BY PROTEIN CLUSTERING ANALYSIS



USING MPX TO QUANTIFY CAR-T CELL ACTIVITY - CONJUGATE DETECTION



SUMMARY

- Molecular Pixelation facilitates in differentiating **molecular mode of action of CAR-Ts**
- CAR-T trogocytosis** can be measured by protein clustering analysis using Molecular Pixelation
- Nanoscale mapping** of CAR receptor protein neighborhoods will help in understanding cell behavior and assessing therapeutic efficacy
- Molecular Pixelation can help uncover the **mechanistic foundations of CAR-T cell biology**, thus contributing to optimizing CAR-T cell therapies, in order to improve their efficacy and persistence in clinical settings