Enhancing Large Molecule Design by Early Integration of MAPPs using Defined Allele Antigen Presenting Cells

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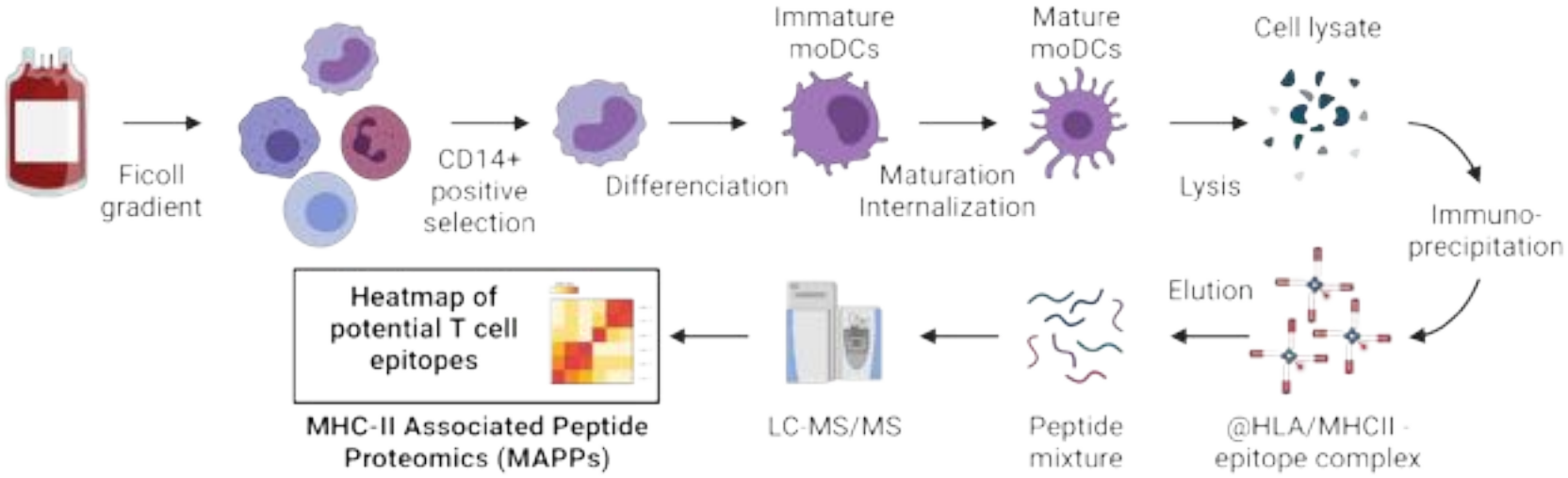
Executive Summary

- The current MAPPs methodology faces challenges
- One factor is the biological variability for MHC haplotypes of the donor material
- Another the labor-intensive process of dendritic cell generation
- Overcoming these hurdles would allow for the earlier and more routine use of MAPPs e.g. in the space of improving large molecule design
- Here we present the development and a first PoC for a mono allelic (artificial) antigen presenting cell line

Introduction

Why Artificial Antigen Presenting Cells?

- APCs for MAPPs are derived from PBMCs of healthy donors
- DC generation adds (labor intensive / time consuming) working steps to MAPPs protocol
- Input material is expensive and needs continuous replenishment (cyroPBMCs) or supply (Buffy Coat)
- HLA-II set is not fully controllable



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Introduction

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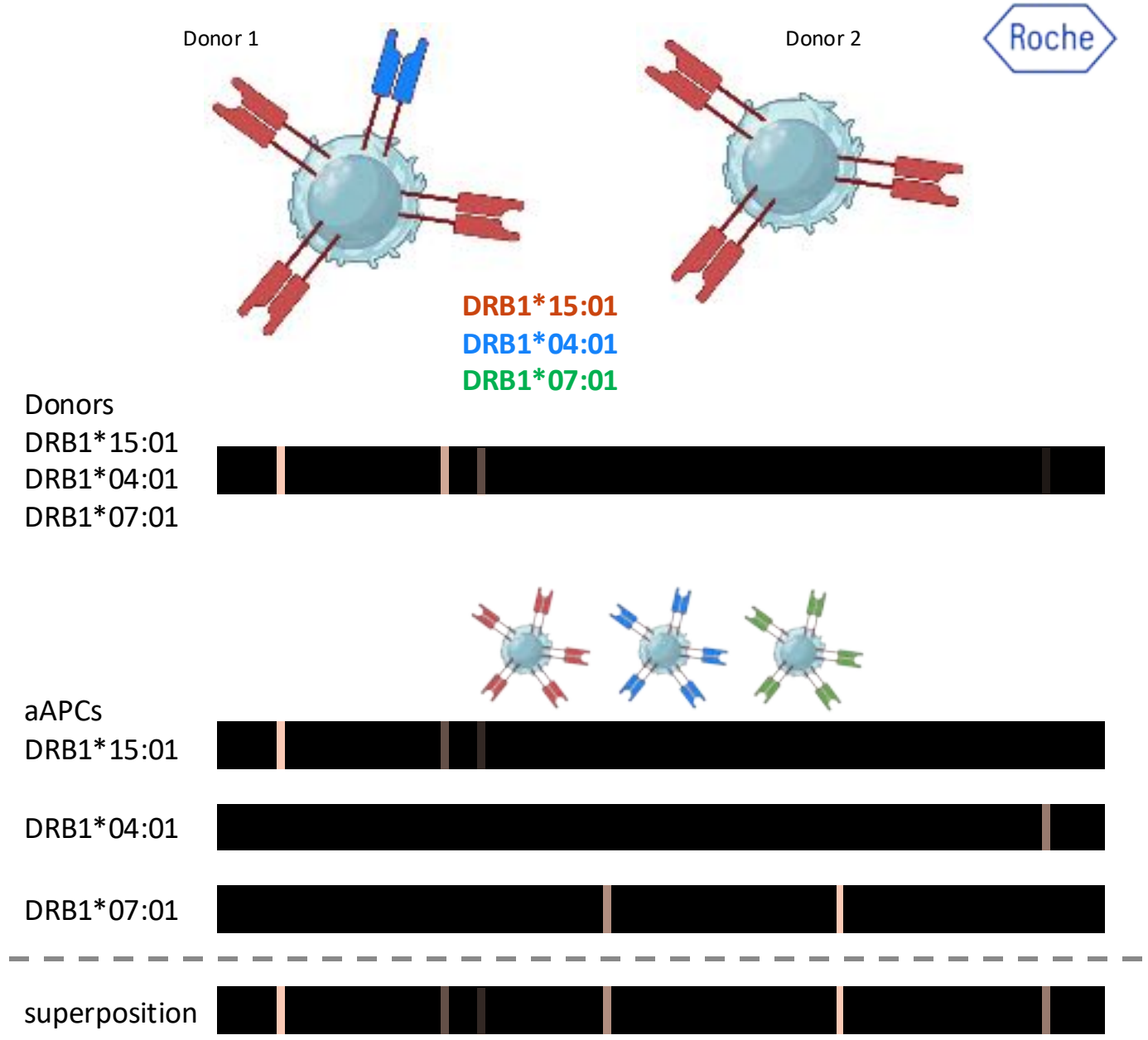


Introduction

Why Artificial Antigen Presenting Cells?

- aAPCs allow for
  - unlimited amount of cells with regular cell culture expansion without the need for differentiation
  - defined HLA-II alleles

Virtual Population

Table 4. HLA DRB1 allele frequencies in worldwide pop

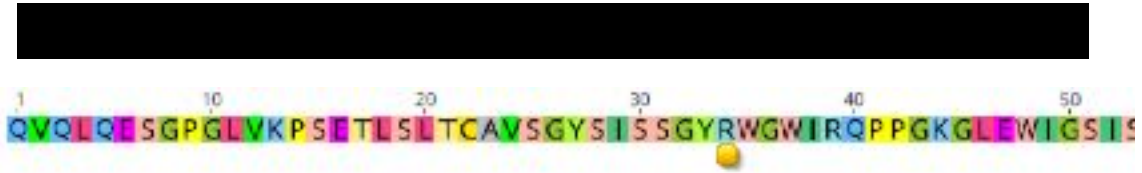
DRB1 alleles	Northern Europe		Western Europe		
	Denmark 2n=100	Ireland (North) 2n=2000	Portugal (North) 2n=92	Portugal (Centre) 2n=300	Portugal (South) 2n=98
*01	0.130	0.120	0.163	0.040	0.113
*03	0.100	0.160	0.130	0.160	0.141
*04	0.200	0.188	0.098	0.140	0.131
*07	0.168	0.160	0.174	0.140	0.153
*08	0.028	0.021	0.033	0.070	0.030
*09	–	0.007	0.011	0.020	0.010
*10	0.009	0.007	–	–	0.020
*11	0.018	0.041	0.098	0.110	0.132
*12	0.019	0.011	0.011	–	0.031
*13	0.139	0.081	0.130	0.160	0.171
*14	0.009	0.015	0.033	0.020	0.010
*15	0.185	0.185	0.109	0.110	0.020

DG Brum et al. 2007

Lead Optimization

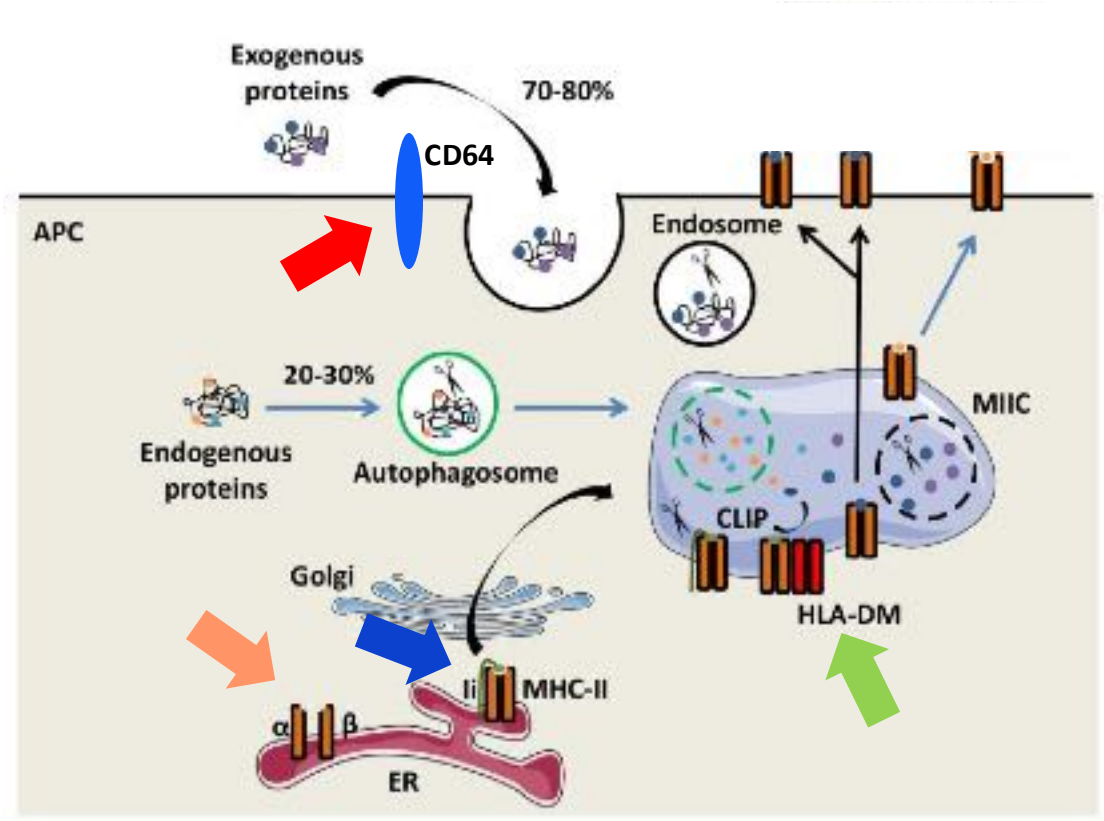
aAPCs
DRB1*15:01

aAPCs
DRB1*15-01



Methods

Components for aAPCs



Alexandre Couture et al. 2019

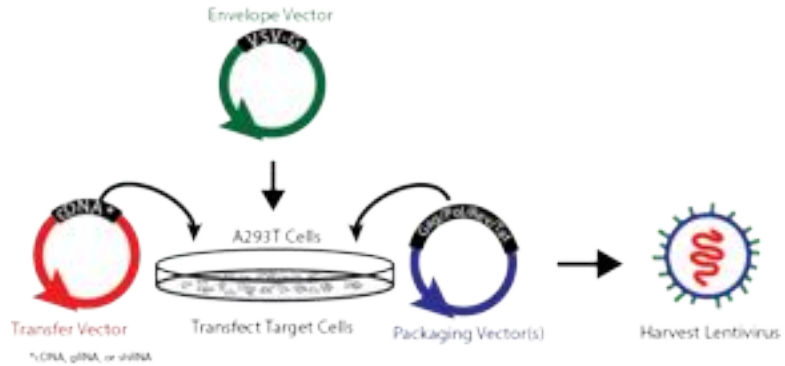
K562: HLA deficient B cell lymphoblast

CD64: high affinity FcgRI for improved uptake

CD74: Ii / CLIP for HLA stability

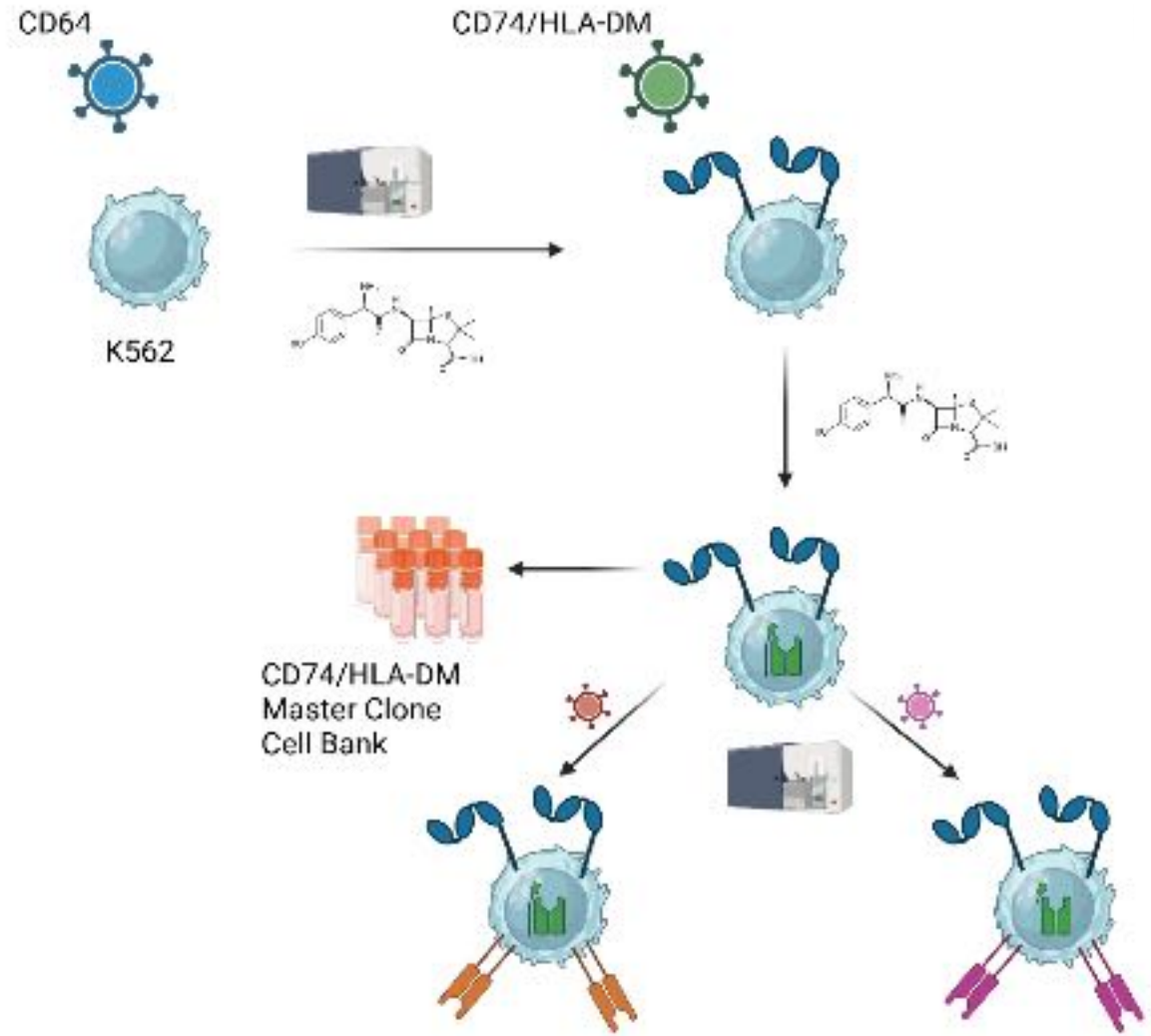
HLA-DM: HLA loading

HLA-D(R/P/Q): antigen presentation



Methods

Transduction Strategy



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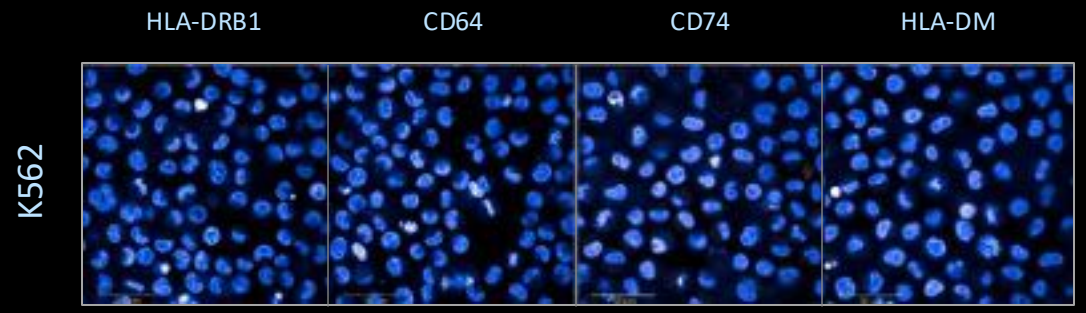
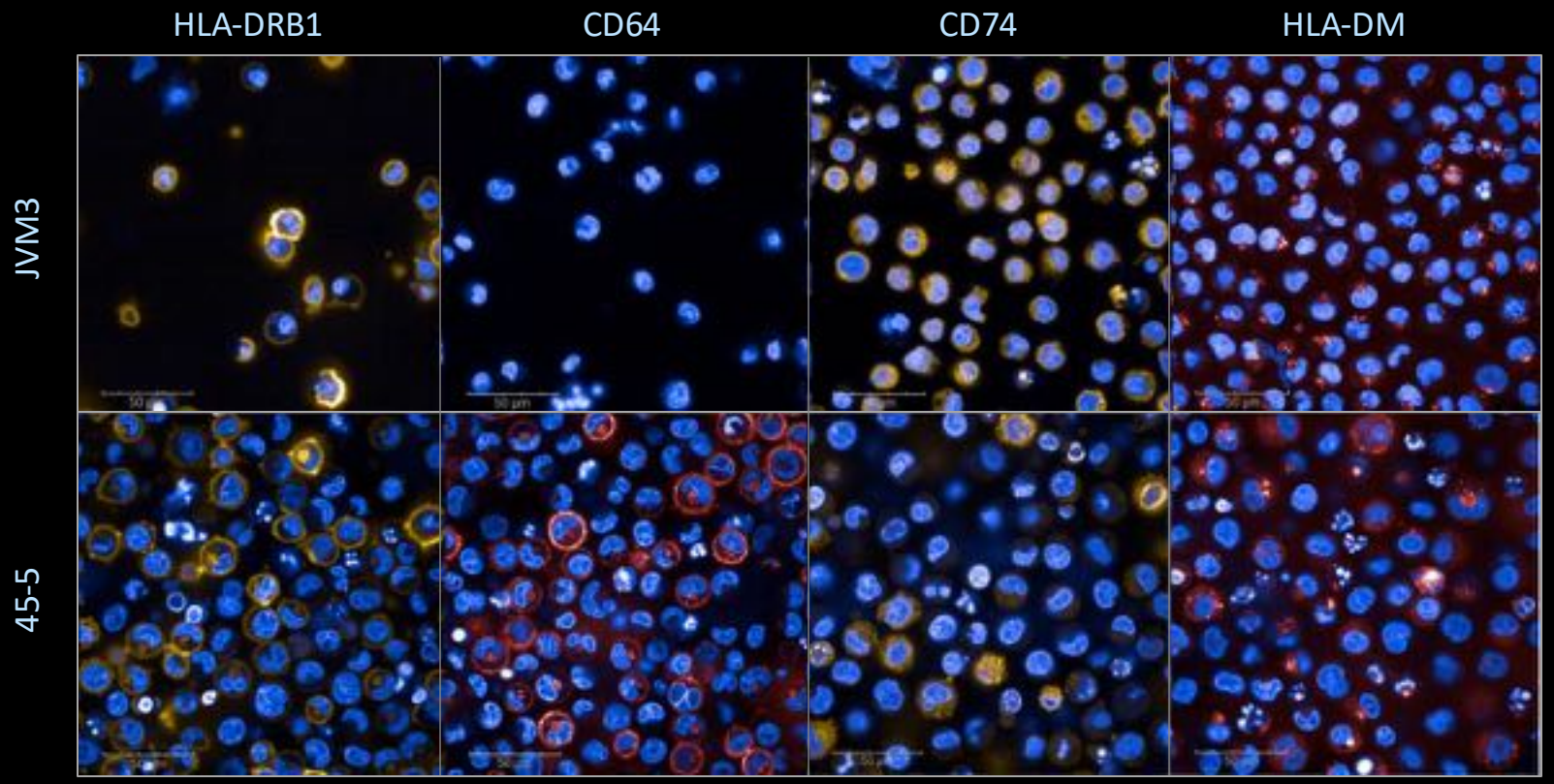
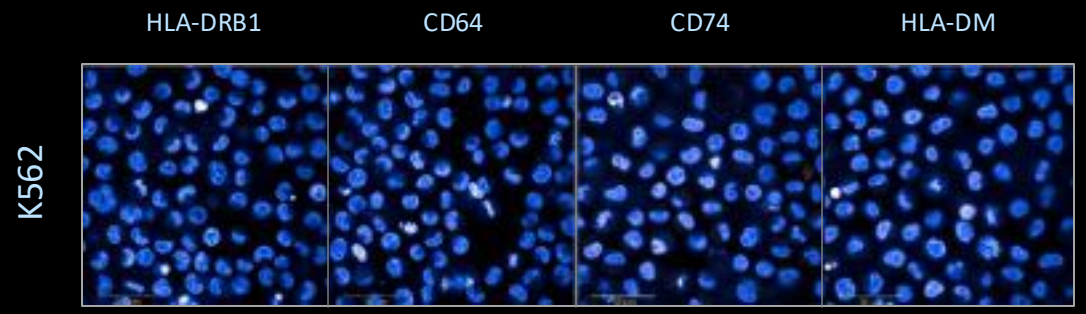
Results
Expression Data - Imaging

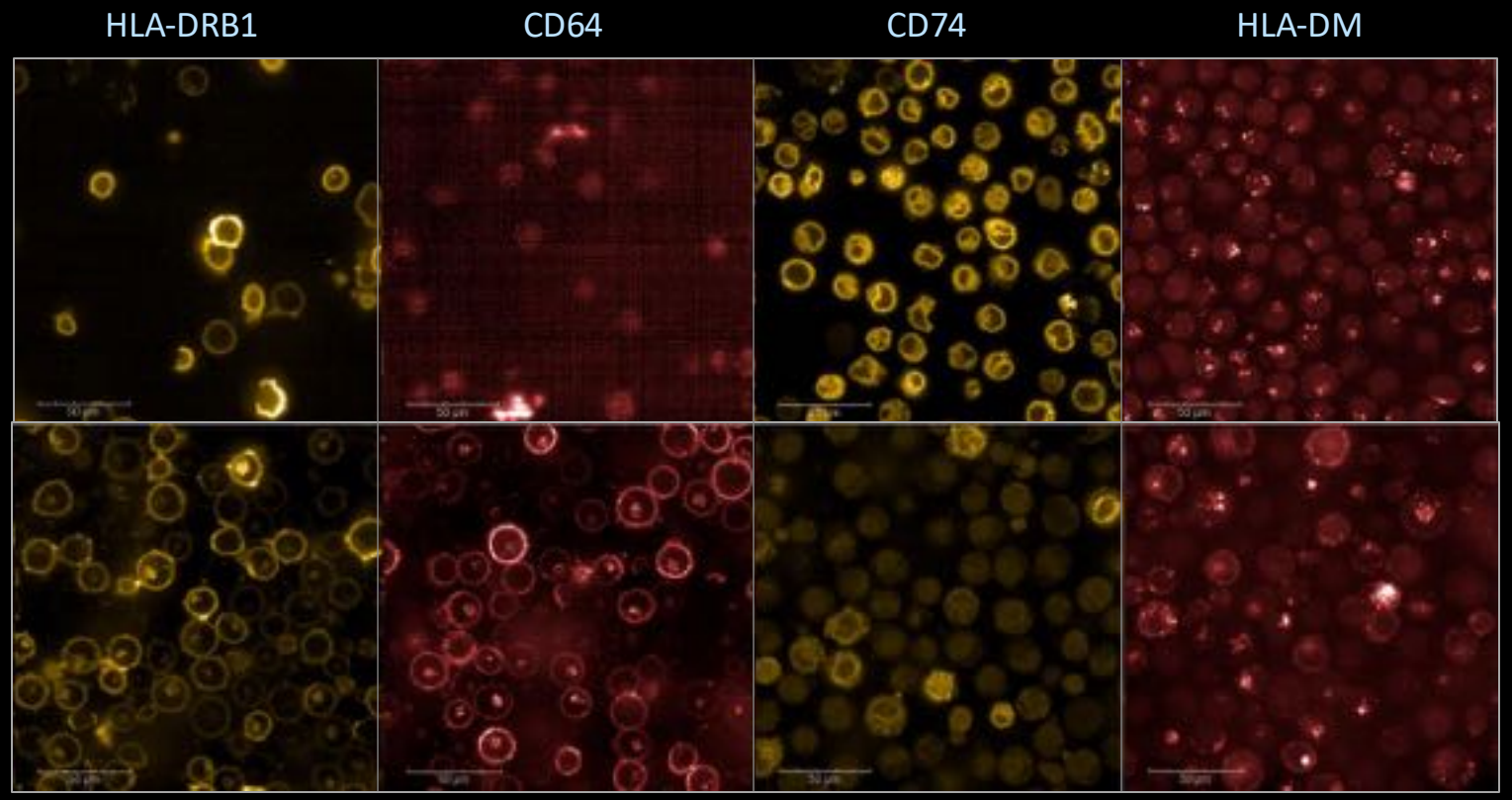




- All four constructs are expressed
- Expression pattern / compartmentation comparabel with JVM3
- Lower expression level for CD74 in aAPC

Results
Expression Data - Imaging





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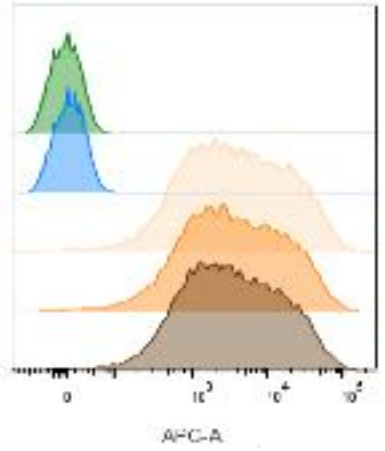
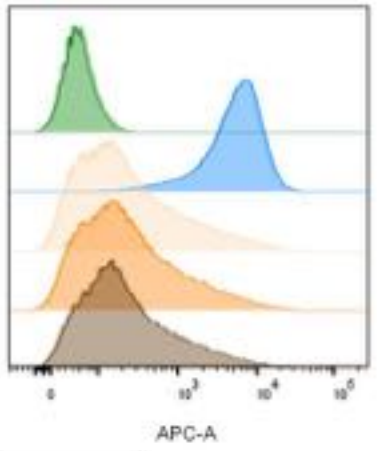
Results

Expression Data – Flow Cytometry

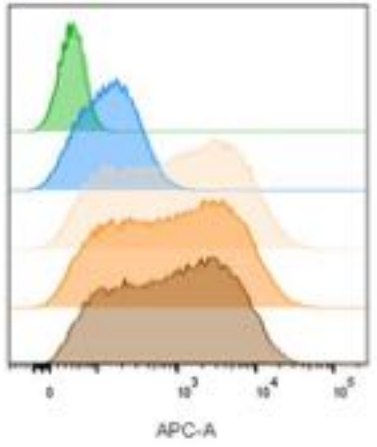
CD64



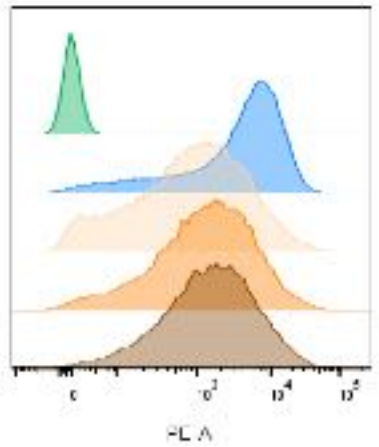
CD74

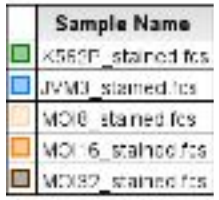


HLA-DM



DRB1*15:01

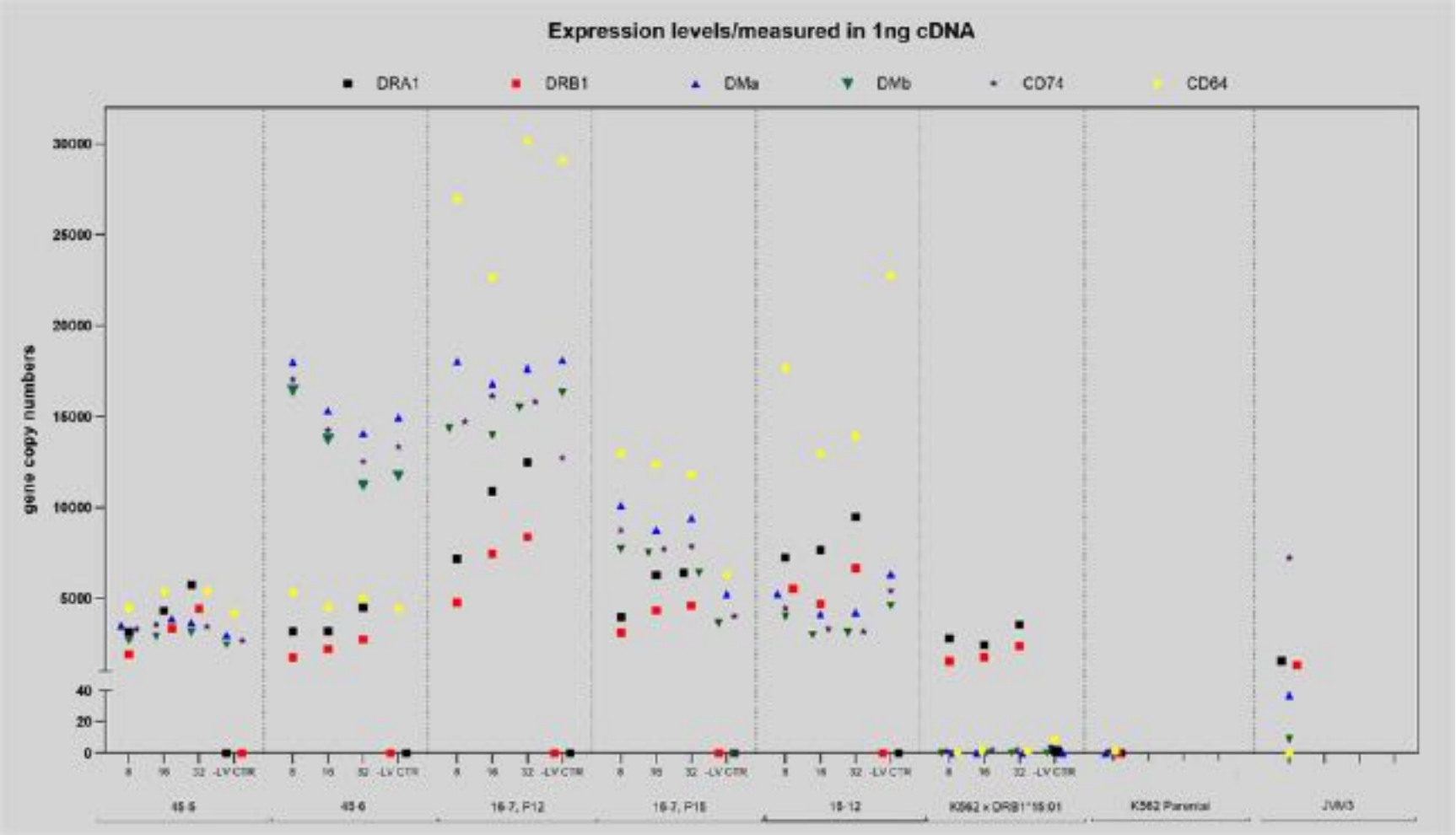




- Expression data consistant with microscopy
- Lower expression level for CD74
- Higher expressin of HLA-DM
- Comparable expression of DRB1

Results

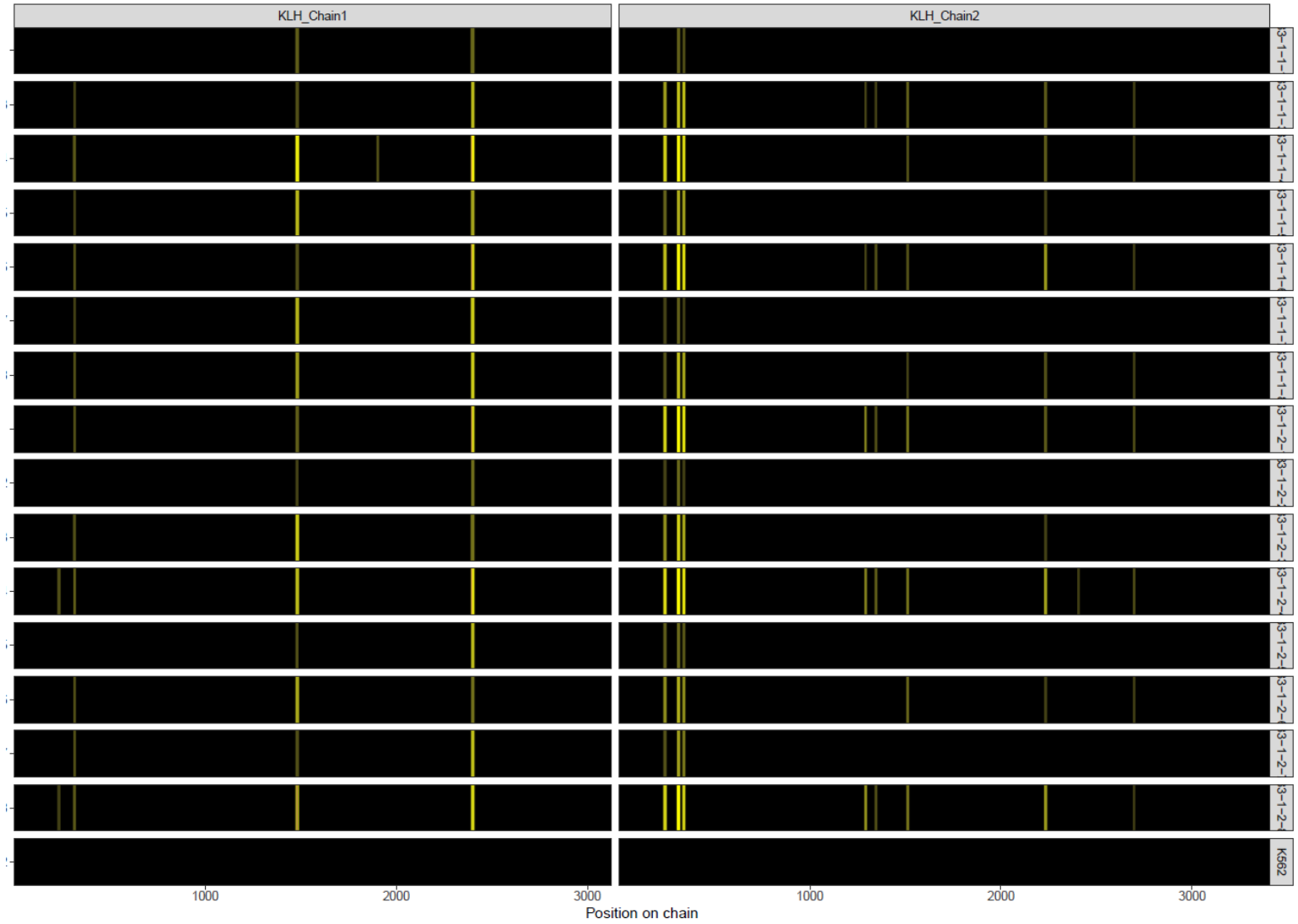
Expression Data – Genetic Copy Numbers



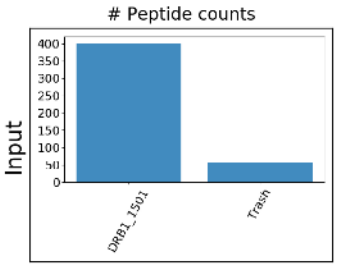
- Equal copy numbers for «paired» hetero dimeric components
- Copy numbers do not entierly reflect the observed expression levels

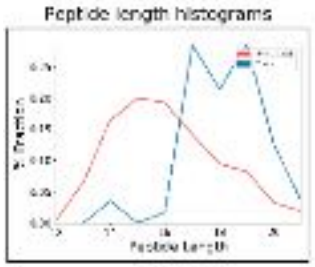
Results

MAPPs on PoC subClones 33 - KLH



- Consistant pattern over all subclones
- No clusters detected for parental cell line K562
- Majority of peptides maps well on DRB1*15:01

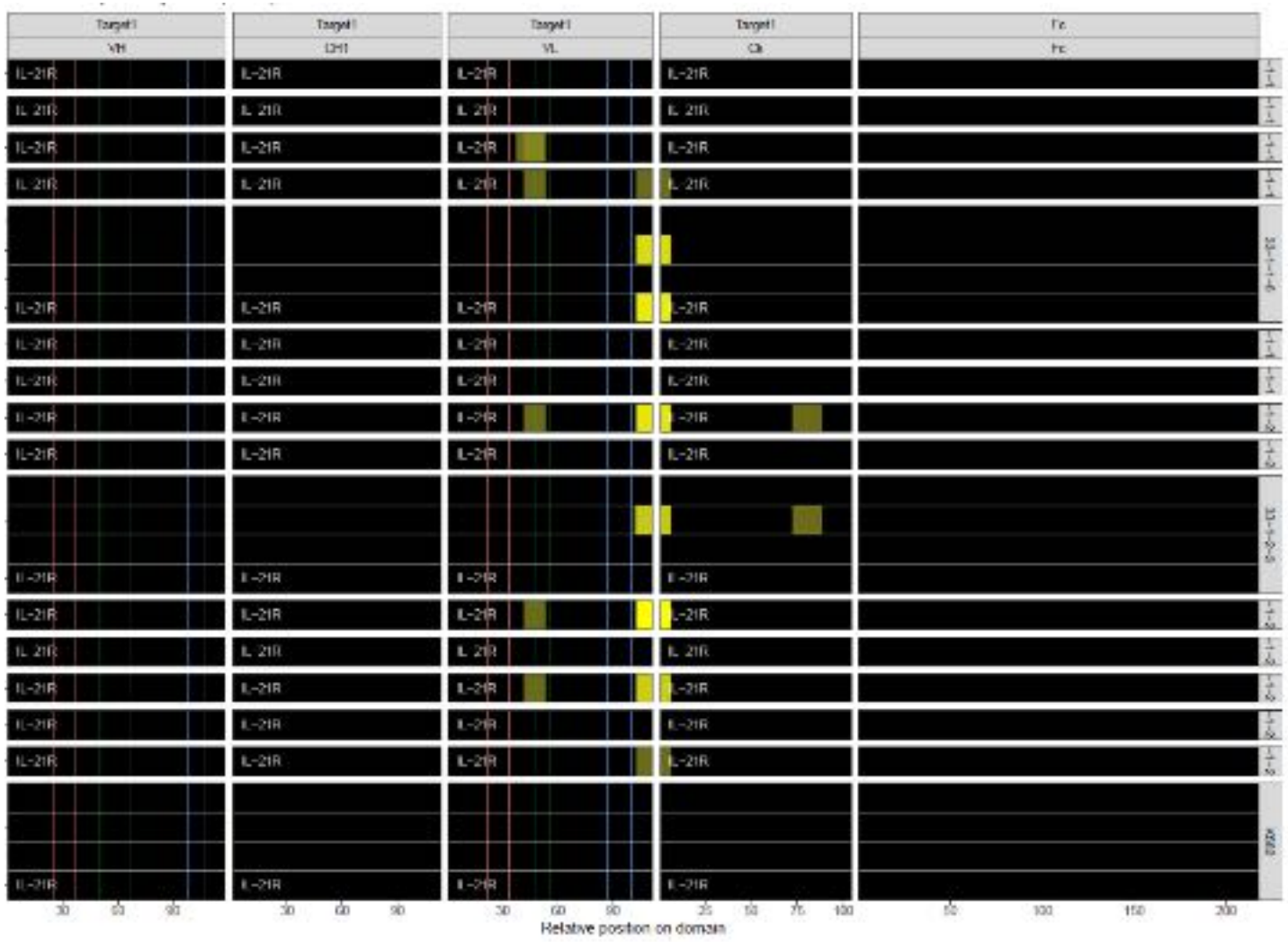




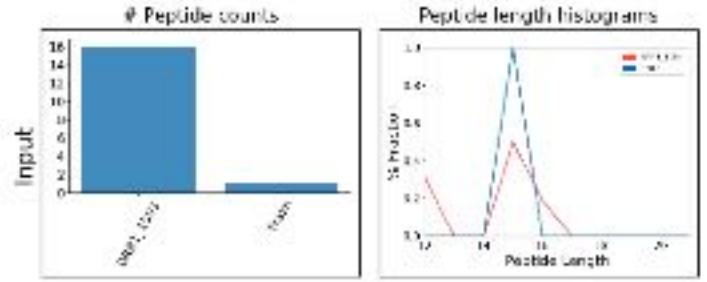
MHCMotifDecon - 1.2

Results

MAPPs on PoC subClones 33 – ATR-107



- Consistant pattern over all subclones (if clusters detected)
- No clusters detected for parental cell line K562
- Majority of peptides maps well on DRB1*15:01



MHCMotifDecon - 1.2

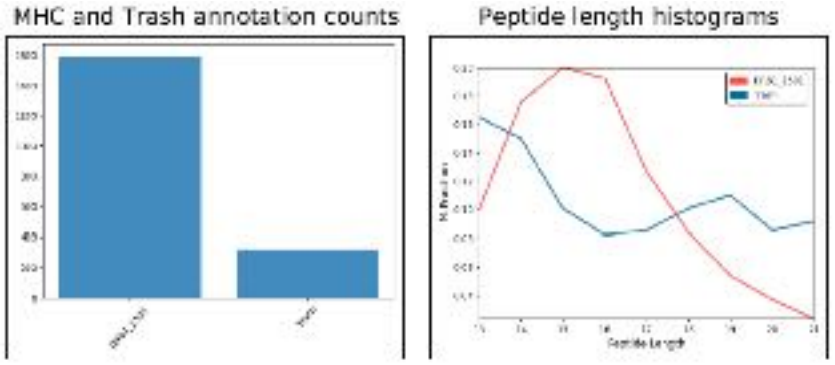
Results

MAPPs Fidelity on Endogenous Proteins

33-1-1-6

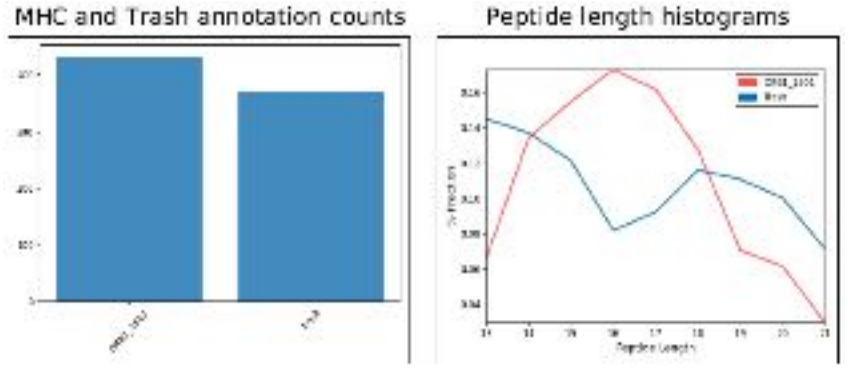
1600 →

400 →



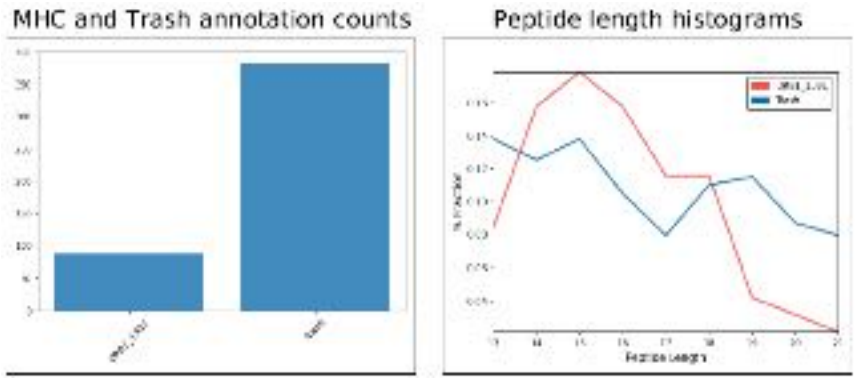
33-1-1-7

400 →



K562

400 →



- Wide fidelity spread for endogenous proteins for different (sub)-clones
- Low fidelity (sub)-clones tend to present overall less
- Lowest/Zero fidelity for K562 cell line

MHCMotifDecon - 1.2
S. Kaabinejadian et al. Front. Immunol. 2022

Final Thoughts

- We established a first PoC for a mono allelic (artificial) antigen presenting cell line for DRB1*15:01
- Feasibility for more HLAs including DP and DQ will be assessed
- Generation and testing of oligo allelic panels and cell mixtures will be tested
- Integration of the system for MAPPs supported lead optimization and other suitable applications to be assessed

Acknowledgments

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MS – 360 Labs
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Lilly von Muenchow

DTU
Morten Nielsen

Doing now what patients need next