PACT pharma	**20-614 Generation and characterization of T cells expressing PACT391C**	**Version 1.5**

Study #:	20-614
Study ID:	Generation and characterization of T cells expressing PACT391C
Responsible Scientist:	Andrew Conroy
Date:	30th November, 2020
Study Objective:	The goal of this study is to generate and characterize T cells expressing TCRs pulled down from patient PACT391C by the imPACT tetramer group. The edited T cells were assessed for TCR expression and IFNγ secretion in the comPACT coating assay.
Study Sheet Initiated by:	Oghene Efagene / 30th November, 2020
Study initially reviewed by:	Pankaj Tiwari / 2nd July, 2021
Study finally reviewed by:	SVC - / 15th March, 2021
Treatment:	Day 0: Activation Day 3:Nucleofection Day 4-14: Cytokine Treatment and Expansion
Readouts:	Gene editing Dextramer binding IFNγ secretion in the comPACT coating assay

1.0 MATERIALS AND METHODS

Reagent IDs:

Reagent ID	Reagent	Description	Vendor/Study/ Experiment	Container
PACT497	Cells	CD4/CD8 cells from Prodigy	EXP20002315	N/A
p001-020	HR DNA	pUCu-Kan TRAC(1k)_P2A.Neo12TRBC2opt.f-P2A.TRA(Va)opt	Nature Technology	ep15236
rnp001-063	RNPα	TRAC-1.Aldevron.sNLS-SpCas9-sNLS.Research Grade	20-614 /EXP20000119	ep15411
rnp002-060	RNPβ	TRBC-2.Aldevron.sNLS-SpCas9-sNLS.Research Grade	20-614 /EXP20000119	ep15412

Study Groups:

On day 0, cells were activated with TransACT in the presence of IL-7 and IL-15 (12.5 ng/mL each). On Day 2, cells were transfected.

Grp	n	Cells	Cuvette/Condition	Transfection Conditions	Expansion
1	1	7000000.00	Lonza X-Unit 100 µL	Neo12 + RNPα + RNPβ	24-well G-rex
2	1	7000000.00	Lonza X-Unit 100 µL	p959-PACT391C_TCR792 + RNPα + RNPβ	24-well G-rex
3	1	7000000.00	Lonza X-Unit 100 µL	p960-PACT391C_TCR793A01 + RNPα + RNPβ	24-well G-rex
4	1	7000000.00	Lonza X-Unit 100 µL	p961-PACT391C_TCR793A02 + RNPα + RNPβ	24-well G-rex

Experiment List:

Date	Experiment	Description	Initials
1st December, 2020	20002249	[PD-010] Receive isolated T cells and T cell activation	OE
4th December, 2020	20002250	[PD-010] Nucleofection	OE
7th December, 2020	20002293	PACT391C: neoTCR T cell comPACT Dextramer screening	TM
7th December, 2020	20002294	PACT391C: comPACT coating assay for cytokine secretion	TM

Key Identifiers:

Clinical Study ID	Clinical Patient ID	Patient Date of Birth	PACT ID
PACT-0101	0401	21st March, 1974	PACT391C

Notes:

This test conclusion.

2.0 RESULTS:
2.1 Dextramer Screening:

Table 1: Summary of the dextramer/2a data for all the neoTCRs tested

Plasmid-PACT-TCR Name	Matched comPACT ID tested	neoE Sequence	Gene	% Gene Edited (2a+) CD8 T cells	% Gene Edited (2a+) CD4 T cells	% cognate comPACT Dex+2a+CD8 T cells	% cognate comPACT Dex+2a+CD4 T cells	Pass CD8 binding	Pass CD4 binding	% mismatch comPACT Dex + 2a + CD8 T cells	% mismatch comPACT Dex + 2a + CD4 T cells
Neo12	PRO004	YLYHRVDVI	USP7	45.1	34.4	44.8	33.6	Yes	Yes	0.7	0.0
p959-PACT391C_TCR792	comPACT28036	SNNPLFQYTY	RNF145	37.2	34.2	35.9	20.3	Yes	Yes	0.8	0.0
p960-PACT391C_TCR793A01	comPACT27877	IQIETVLPAE	NUP210	14.8	11.9	0.7	0.1	No	No	1.0	0.0
p961-PACT391C_TCR793A02	comPACT27877	IQIETVLPAE	NUP210	13.8	9.7	0.7	0.0	No	No	0.8	0.1

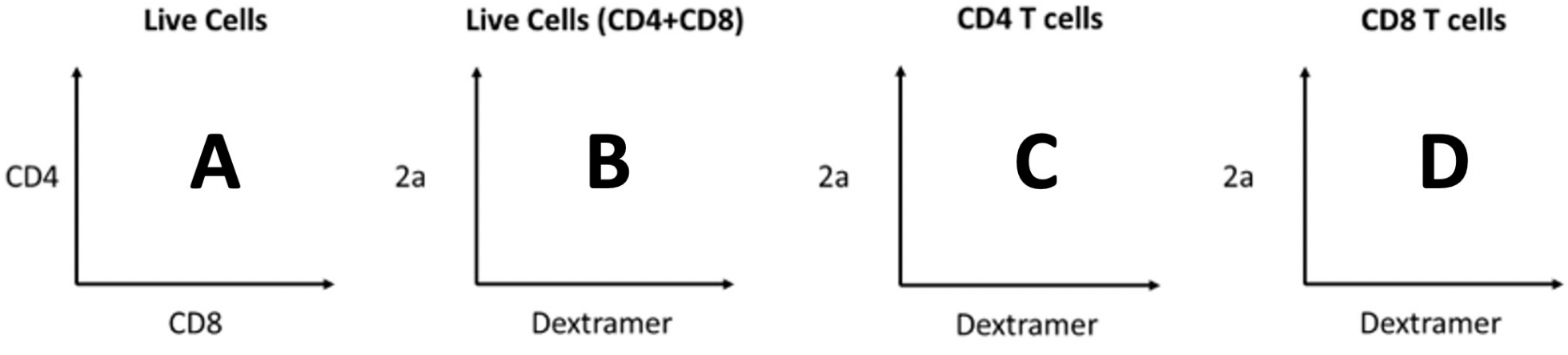
Results Summary:
3 of 3 PACT391C neoTCRs received were tested in the functional assays.

- 1 of the 3 PACT391C TCRs tested in this study exhibited dextramer binding in the CD4 T cell population (CD8-independent binding): TCR792.
- 2 of the 3 neoTCRs was negative for specific binding to CD8 and CD4 T cells: TCR793A01,TCR793A02

Definition of CD8-independent TCR: % Dextramer binding to CD4 T cells has to be at least 50% of Dextramer staining observed on CD8 T cells stained under same conditions for the TCR to pass this criterium.

2.2 Dextramer Screening Raw data:

Schema of the flow plots for Figures 1 to 4:



For the above gating schema, the ” Live Cells” gate (plots A and B) are derived from parental gates (not shown) ” Intact T cells → doublet discrimination → live cells” .

- Plot A illustrates the CD4 and CD8 composition of the live cell population.
- Plot B illustrates the dextramer and 2a peptide binding of the live cell population (combined CD4 and CD8 T cells).
- Plot C illustrates the dextramer and 2a peptide binding in the CD4 T cell population
- Plot D illustrates the dextramer and 2a peptide binding in the CD8 T cell population.

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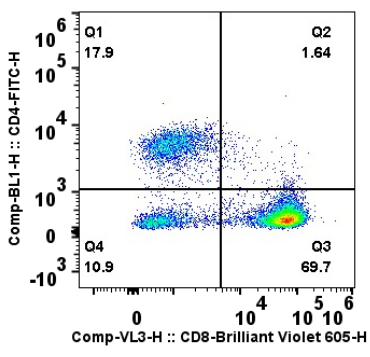
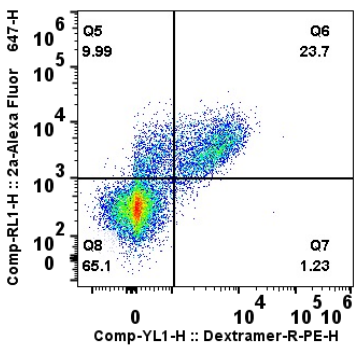
p959-PACT391C_TCR792

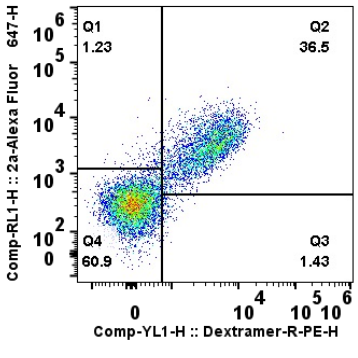
- Cognate comPACT: comPACT28039 (RNF145: SNNPLFQYTYL - HLA-A29:02) 11 mer



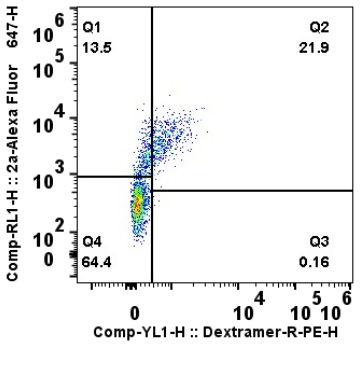
p959-PACT391C_TCR792+comPACT28039.fcs
Live Cells
13869



p959-PACT391C_TCR792+comPACT28039.fcs
Live Cells
13869

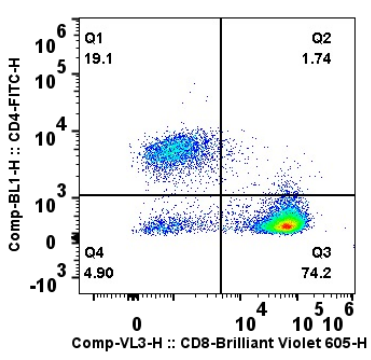


p959-PACT391C_TCR792+comPACT28039.fcs
Q3: CD8-Brilliant Violet 605-H+ , CD4-FITC-H-
9660

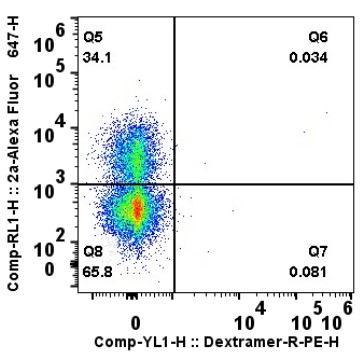


p959-PACT391C_TCR792+comPACT28039.fcs
Q1: CD8-Brilliant Violet 605-H- , CD4-FITC-H+
2477

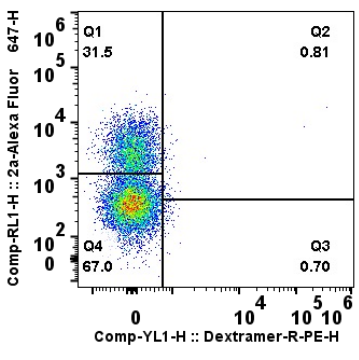
- Mismatch comPACT: comPACT6050 (ARHGAP44: TTDNMMLEFY- HLA-A29:02- HLA Allele Control)



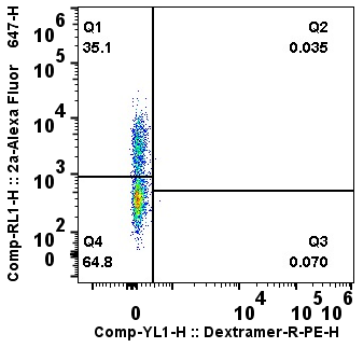
p959-PACT391C_TCR792+comPACT6050.fcs
Live Cells
14888



p959-PACT391C_TCR792+comPACT6050.fcs
Live Cells
14888



p959-PACT391C_TCR792+comPACT6050.fcs
Q3: CD8-Brilliant Violet 605-H+ , CD4-FITC-H-
11050



p959-PACT391C_TCR792+comPACT6050.fcs
Q1: CD8-Brilliant Violet 605-H- , CD4-FITC-H+
2849

Figure 1: Flow plots for **TCR792.**

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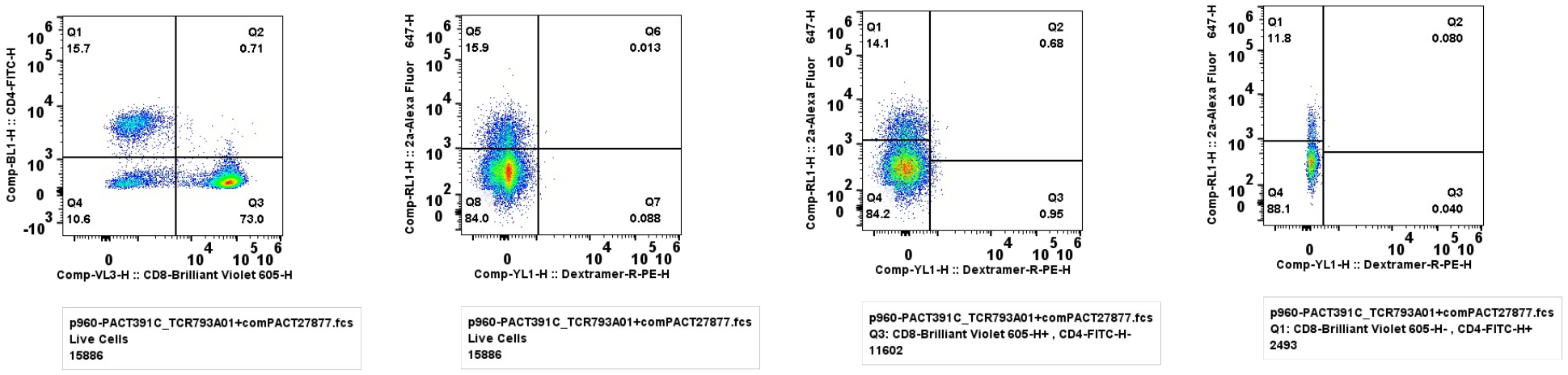
p960-PACT391C_TCR793A01

- Cognate comPACT: comPACT27877 (NUP210: IQIETVLPAE - HLA-B15:01) 10 mer



- Mismatch comPACT: comPACT1130 (ELF4: KQIFRTSEM- HLA-B15:01- HLA Allele Control)

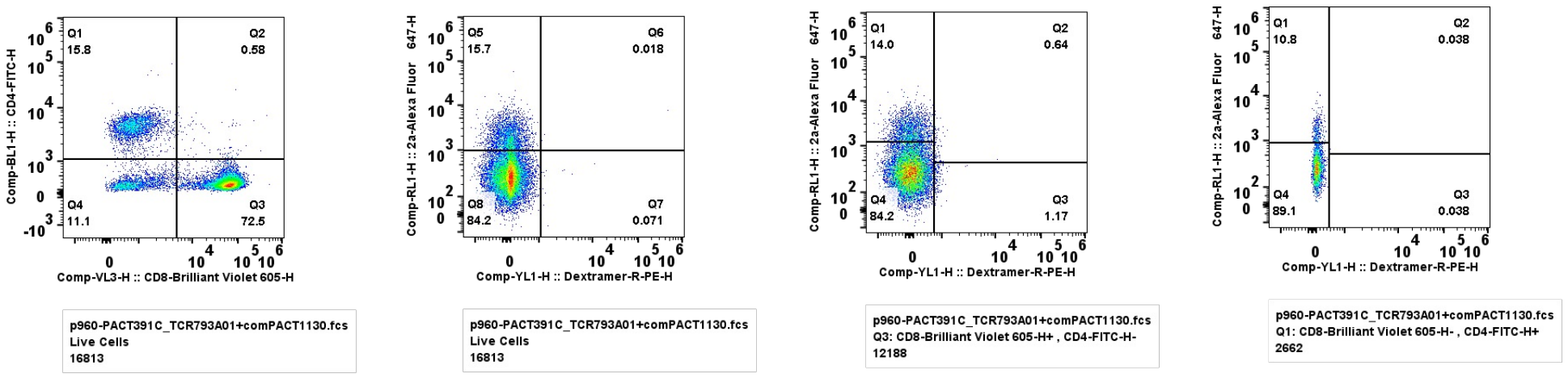


Figure 2: Flow plots for **TCR793A01.**

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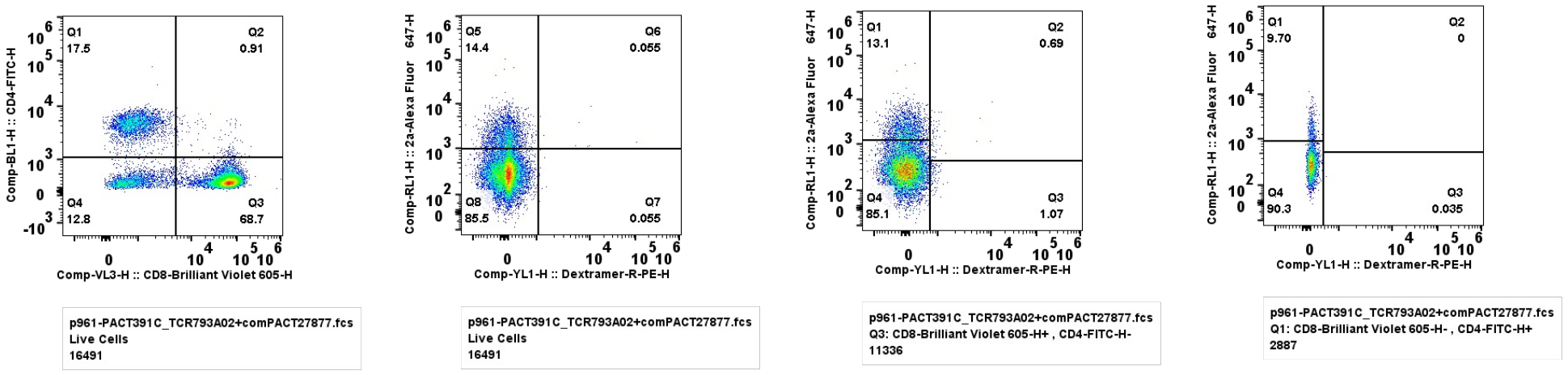
p961-PACT391C_TCR793A02

- Cognate comPACT: comPACT27877 (NUP210: IQIETVLPAE - HLA-B15:01) 10 mer



- Mismatch comPACT: comPACT1130 (ELF4: KQIFRTSEM- HLA-B15:01- HLA Allele Control)

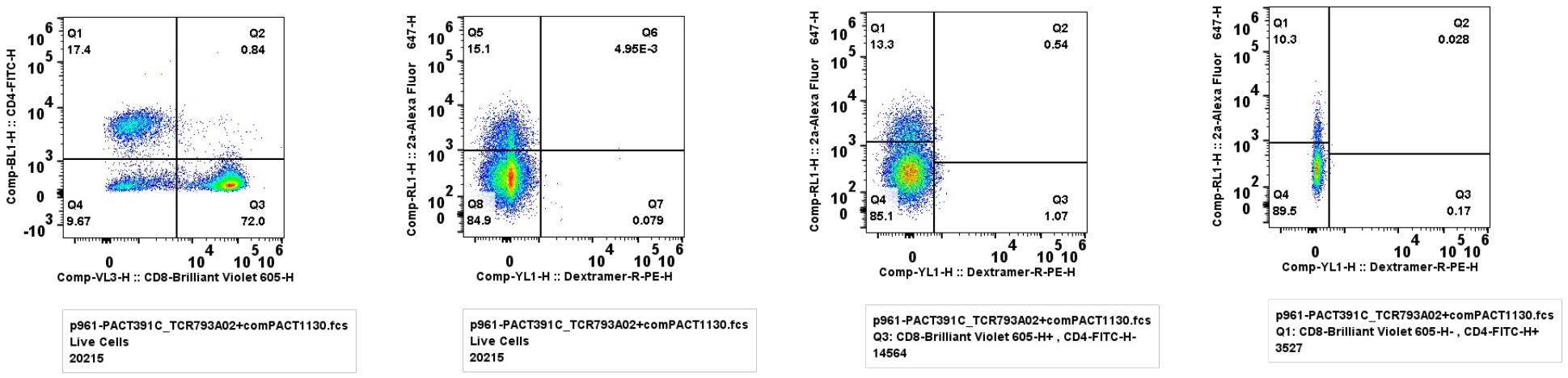


Figure 3: Flow plots for **TCR793A02.**

Neo12 Control TCR

- Cognate comPACT: PRO004 (USP7: YLYHRVDVI-HLA-A02:01) 9mer

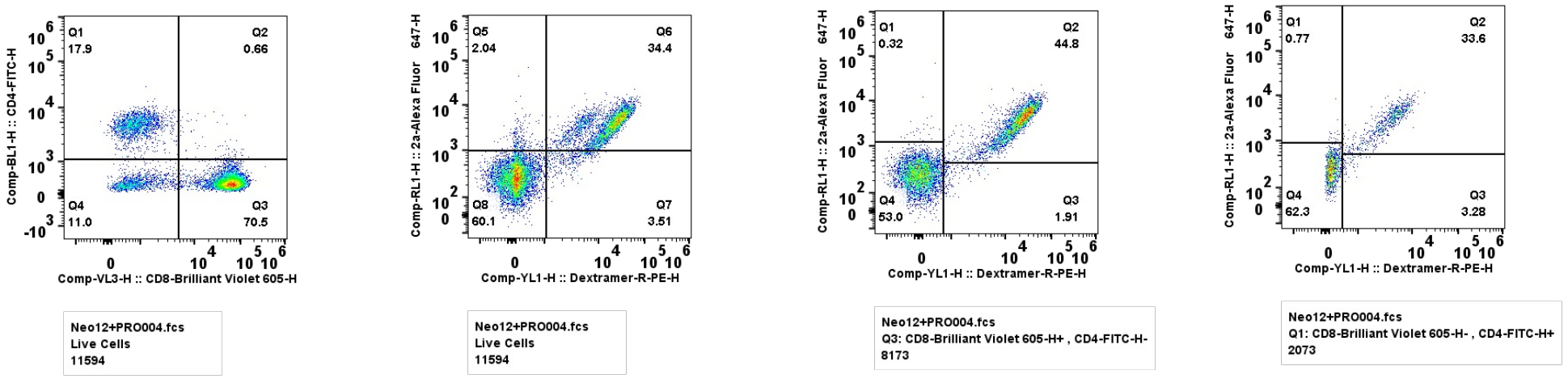


Figure 4: Flow plots for neo12 control TCR.

2.3 Results of functional characterization IFNγ secretion

Table 2: Summary of the IFNγ secretion

					IFNg(pg/10^3 edited cells)												
comPACT ID	**comPACT Name**	**neoE Sequence**	**Gene**	**TCR ID**	**1000**	**333**	**111**	**37.0**	**12.3**	**4.12**	**1.37**	**0.46**	**0.15**	**0.05**	**0**	**EC50 ng/ML**	**Mismatch pass(Y/N)**
PRO004		YLYHRVDVI	USP7	Neo12	25.9	24.7	25.4	25.3	24.6	21.5	15.4	5.1	0.6	0.0	0.0	1.1	Yes
comPACT28036	PACT391C_T_PP001370_O49_CP_HLA-A29:02	SNNPLFQYTY	RNF145	p959-PACT391C_TCR792	23.9	24.2	21.6	15.2	4.4	0.0	0.0	0.0	0.0	0.0	0.0	28.5	Yes
comPACT27877	PACT391C_T_PP001370_O150_CP_HLA-B15:01	IQIETVLPAE	NUP210	p960-PACT391C_TCR793A01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	ND	Yes
comPACT27877	PACT391C_T_PP001370_O150_CP_HLA-B15:01	IQIETVLPAE	NUP210	p961-PACT391C_TCR793A02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	ND	Yes

(EC_{50} with cognate comPACT and whether IFNγsecretion was observed in presence of mismatched comPACT) for all the neoTCR candidates tested.
ND = Not determined

Functional characterization (IFNγsecretion) raw data:

Figures 5 to 7 illustrate the potency data graphs illustrating the amount of IFNγ secreted by neoTCR expressing T cells stimulated with increasing concentration of the cognate comPACT or a single concentration of negative control mismatch comPACT.

IFNγ secretion upon stimulation with comPACT27877 (NUP210: IQIETVLPAE - HLA-B15:01)

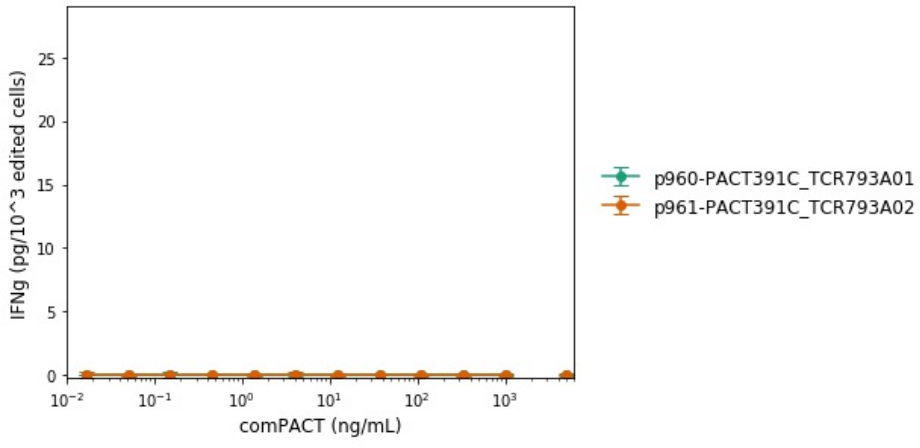


Figure 5: Potency data for TCR793A01, TCR793A02.

IFNγ secretion upon stimulation with comPACT28036 (RNF145: SNNPLFQYTY - HLA-A29:02)

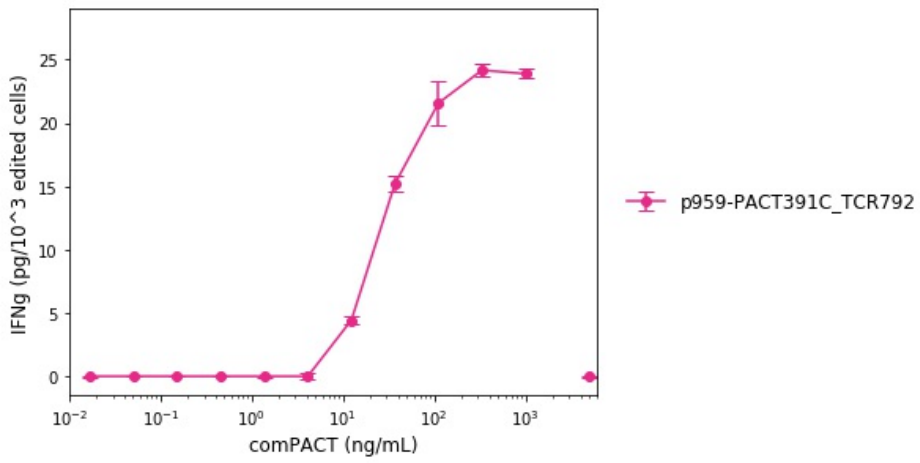


Figure 6: Potency data for Missing Data.

Positive control

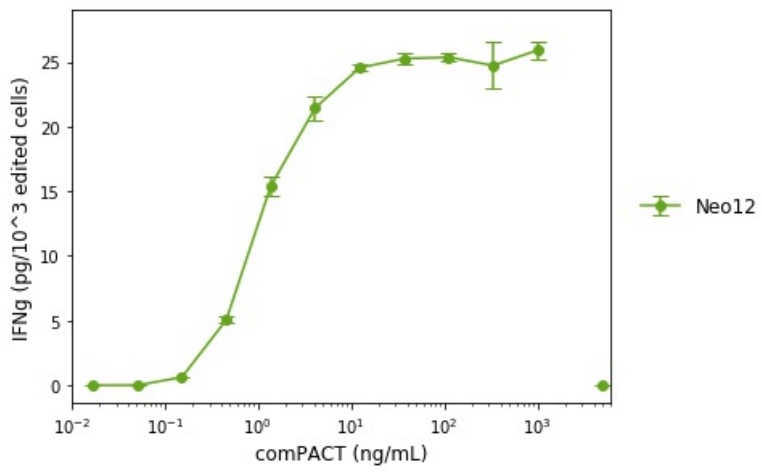


Figure 7: Potency data for the neoTCR positive control neo12.

3.0 CONCLUSIONS

Table 3: Summary of the dextramer screening data and the IFNγ secretion potency assay.

Plasmid-PACT-TCR Name	Matched comPACT ID tested	neoE Sequence	Gene	% Gene Edited (2a+) CD8 T cells	% Gene Edited (2a+) CD4 T cells	% cognate comPACT Dex+2a+CD8 T cells	% cognate comPACT Dex+2a+CD4 T cells	Pass CD8 binding	Pass CD4 binding	EC50 ng/ML	Pass mismatch
Neo12	PRO004	YLYHRVDVI	USP7	45.1	34.4	44.8	33.6	Yes	Yes	1.1	Yes
p959-PACT391C_TCR792	comPACT28036	SNNPLFQYTY	RNF145	37.2	34.2	35.9	20.3	Yes	Yes	28.5	Yes
p960-PACT391C_TCR793A01	comPACT27877	IQIETVLPAE	NUP210	14.8	11.9	0.7	0.1	No	No	ND	Yes
p961-PACT391C_TCR793A02	comPACT27877	IQIETVLPAE	NUP210	13.8	9.7	0.7	0.0	No	No	ND	Yes

The summary of the functional screening results for PACT-0101 0401 (PACT391C) are as follows:

3 out of the 3 PACT391C neoTCRs were screened in both the dextramer binding and potency assays.
Among the 3 neoTCRs screened:

- 1/3 passed all functional criteria for selection as TCR: CD8 independent dextramer binding and IFNγ secretion.
- 2/3 neoTCRs did not meet criteria for either cognate dextramer binding or IFNγ secretion.

The neo12 control neoTCR performed as expected in both the dextramer binding and IFNγ secretion assays.

Supporting Documents:

- 2541372_163633b3-89a9-4994-8b68-21f9b3cfcaf8.pdf