DNA templates, modified nucleotides and immune responses to candidate mRNA vaccines against *Mycobacterium tuberculosis* and SARS-CoV-2

Patrick Arbuthnot

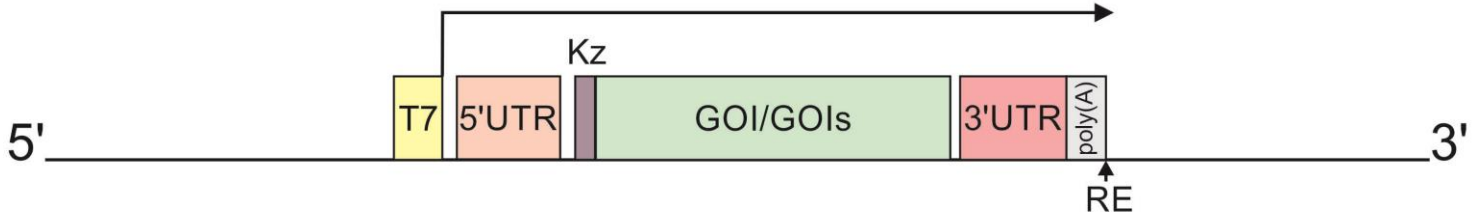
Wits/SAMRC Antiviral Gene Therapy Research Unit
Infectious Diseases and Oncology Research Institute (IDORI)
Faculty of Health Sciences, University of the Witwatersrand
SOUTH AFRICA
www.wits.ac.za/agtru/

Background

- Gene therapy for HBV infection
  - Developed use of mRNA encoding anti-HBV proteins (TALENs)
- Emphasis on freedom to operate and new IP for sustainability
  - Plasmids
  - Modified nucleotides (pseudouridine and N1-methylpseudouridine)
  - Evaluation for SARS-CoV-2 and Mtb responses

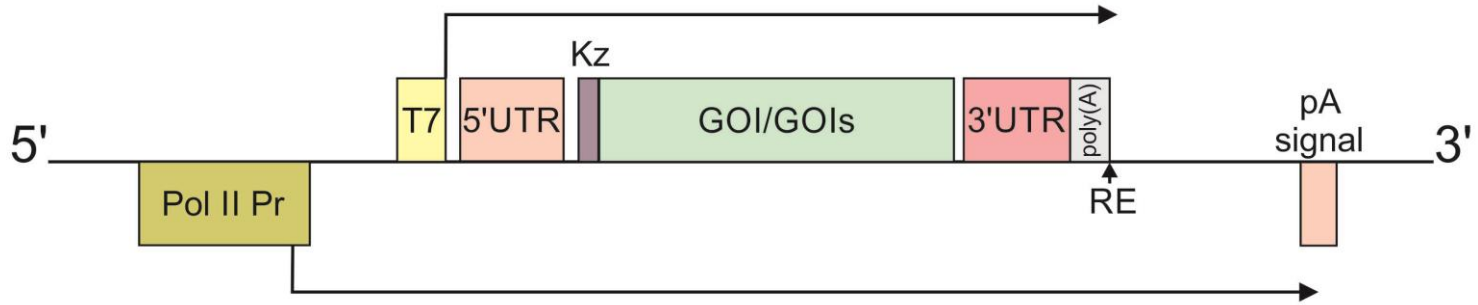
Plasmid properties

- In-house design of destination plasmid
- Components
  - T7 promoter
  - 5’ UTR
  - Kozak sequence
  - MCS
  - 3’ UTR
  - polyA sequence
  - Kana R or Amp R



Plasmid design

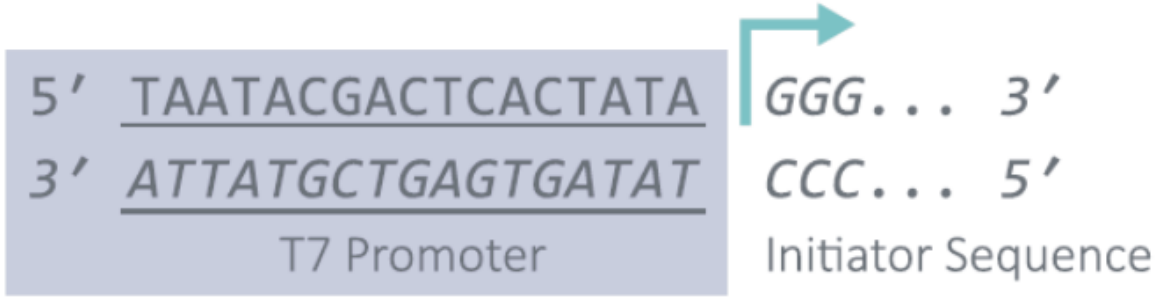
- UTRs: borrow from nature
- Scarless cloning of insert
- Multiprotein production
- Insert: uridine-depleted, codon optimised and secondary structure optimised
- Eukaryotic promoter an and polyA (bGH) signal too



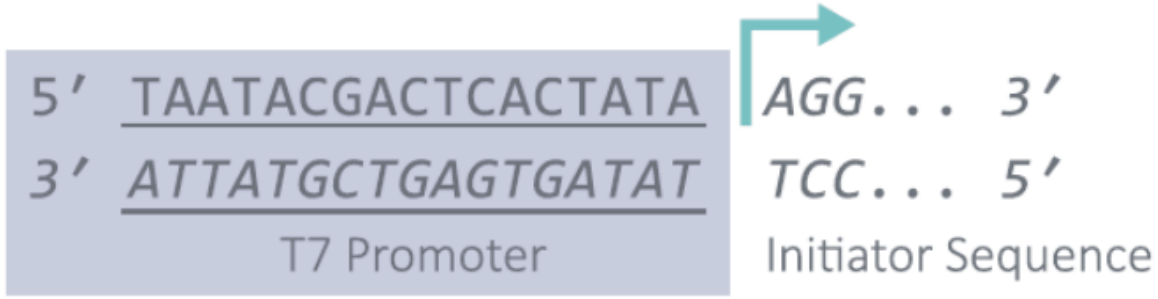
T7 promoters

- Facilitates post-transcriptional/enzymatic and co-transcriptional capping
  - pT7(GG) vectors → post-transcriptional capping and co-transcription capping (ARCA)
  - pT7(AG) vectors → co-transcriptional capping (CleanCap® technology)

Post-transcriptional capping and co-transcriptional capping

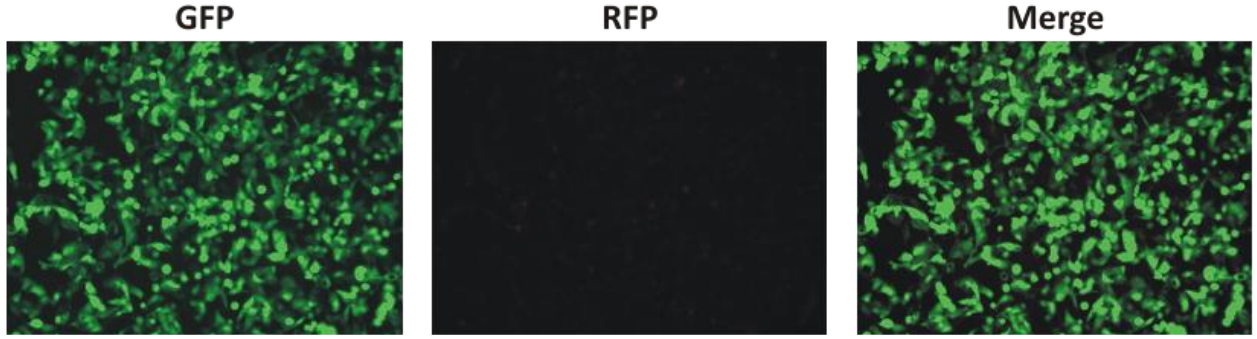


Co-transcriptional capping (CleanCap® AG)

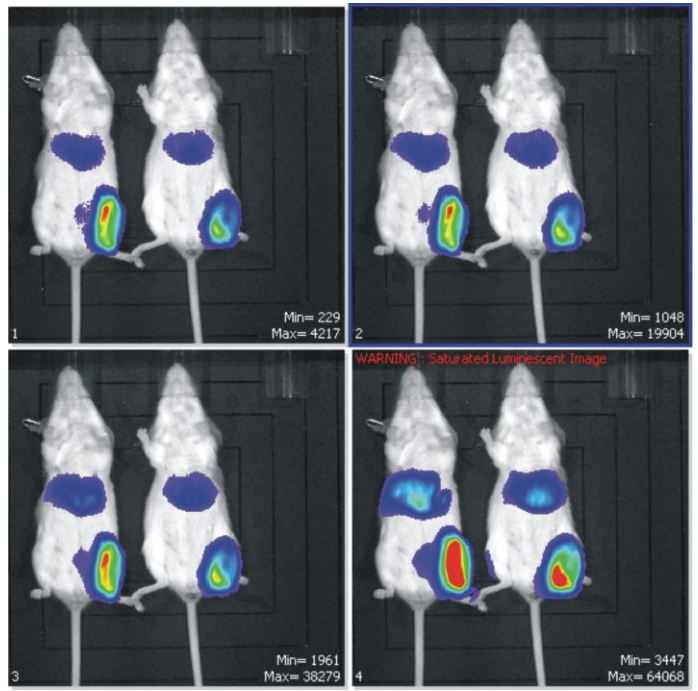


Reporter systems

Dual protein expression



Firefly Luciferase Reporter



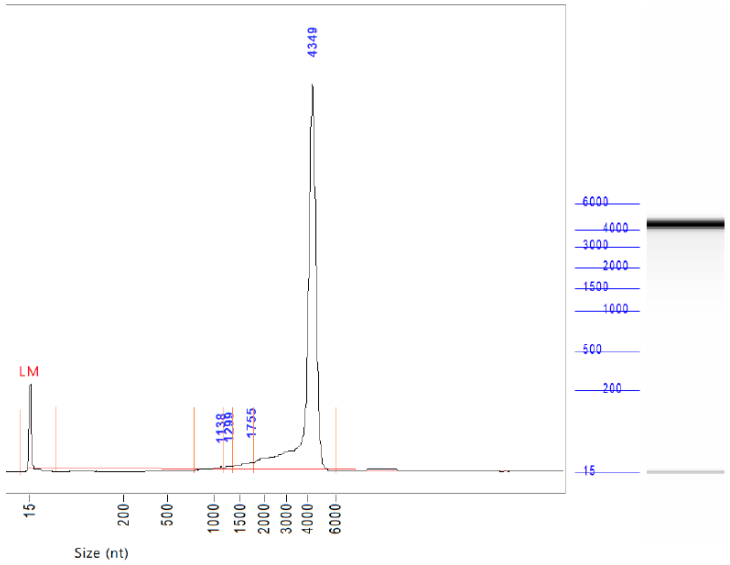
Modified nucleotides in COVID vaccines

- Evaluation of U vs ψ vs N1-methyl-ψ
- Inserted into mRNA from proprietary AGTRU backbone plasmid
- Combinations of variants
    - Original D614G strain (re-design)
    - Omicron 2P BA1
    - WIV-1 (Bat SARS-like coronavirus WIV1)

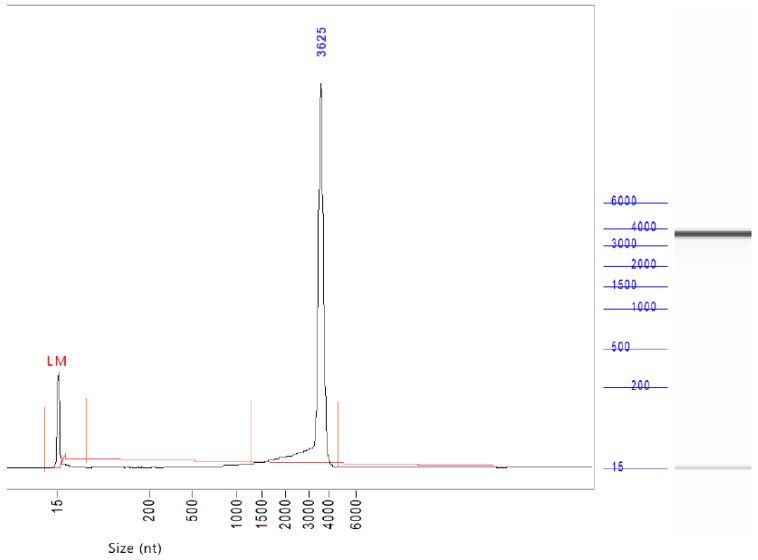
CGE of spike mRNAs containing modified nucleotides

COS-2P

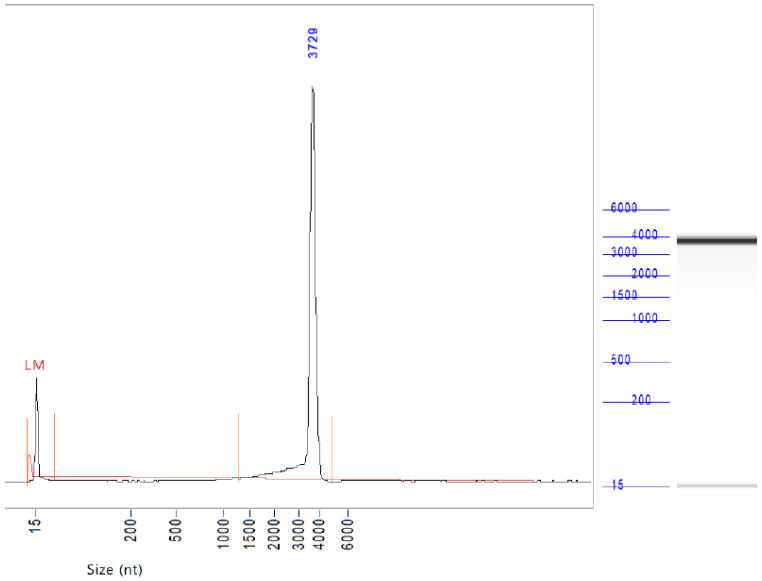
Uridine



Pseudouridine

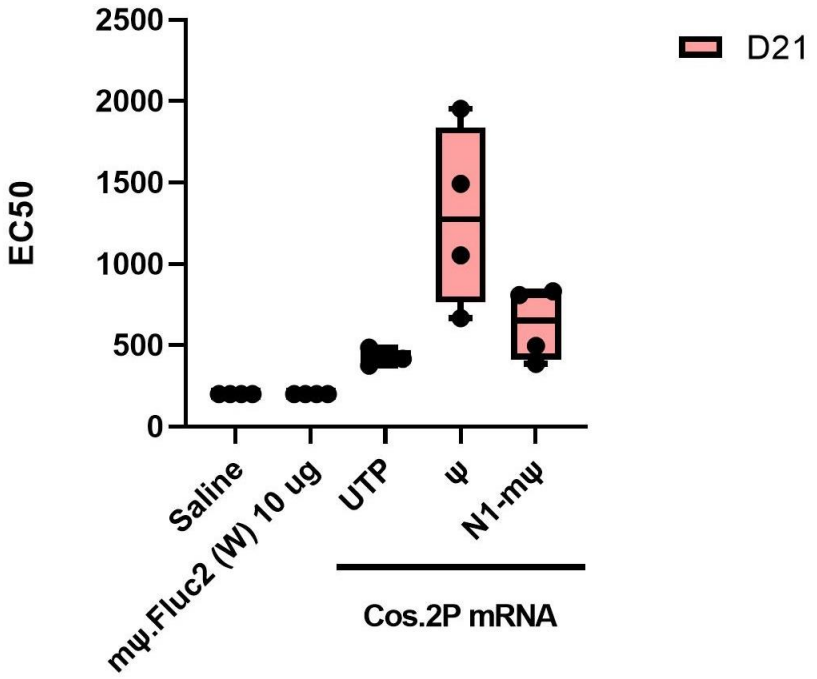


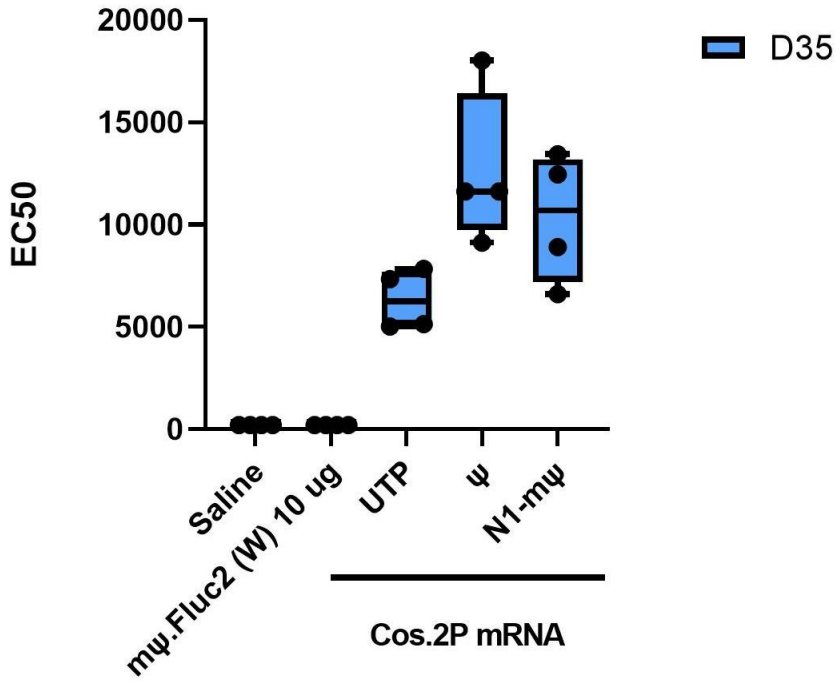
N1-Methylpseudouridine



Formulated (SM102) and evaluation of immunogenicity

Antibody titers: Comparison of UTP, ψ, N1-me-ψ for COS-2P



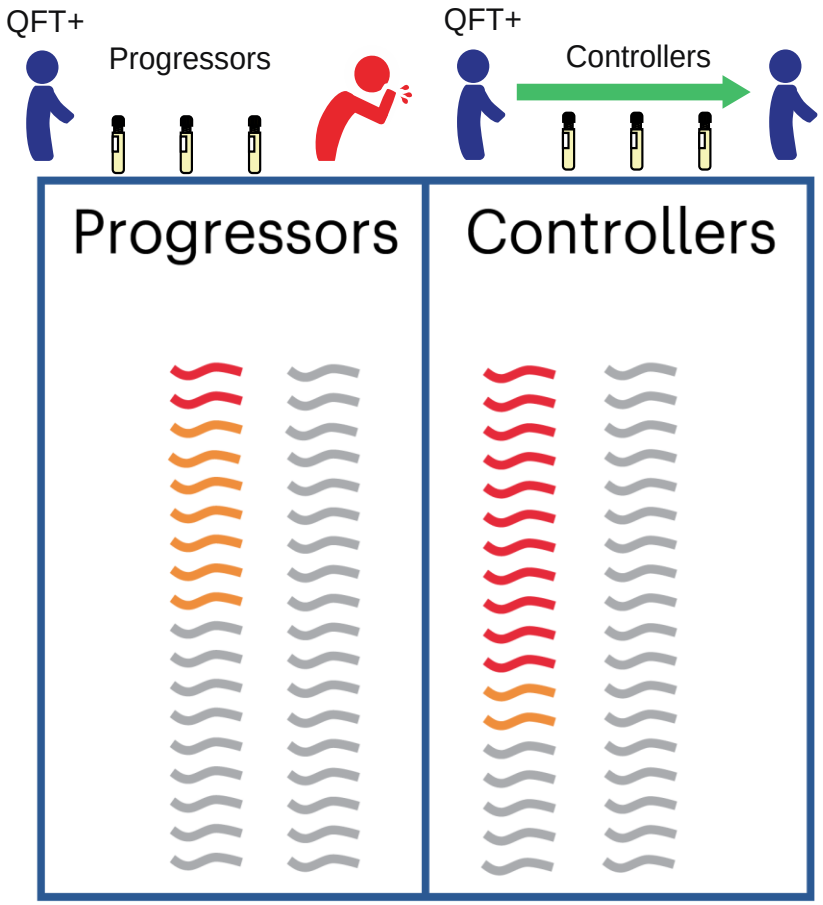


Neutralisation

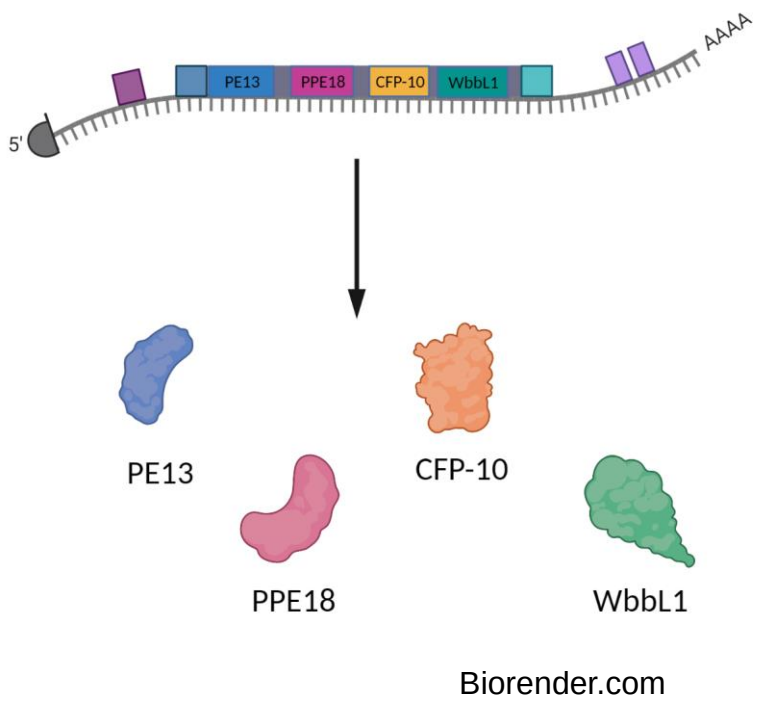
		ID50							
		Day 21				Day 35			
		D614G	BETA	OMICRON BA1	WIV-1	D614G	BETA	OMICRON BA1	WIV-1
UTP- Cos.2P	954	50	50	280	50	169	244	38235	2686
	956	50	50	1417	50	190	648	25136	765
	958	50	50	117	50	371	856	30274	147
	959	50	50	951	50	130	468	23398	2962
ψ-Cos.2P	1080	50	54	Depleted	50	262	146	28312	699
	1076	50	50	3598	50	322	433	32552	2097
	1075	50	50	1894	50	384	725	17812	151
	1073	50	50	3637	50	116	580	15271	1024
N1.Cos.2P	957	50	50	1122	77	516	1145	252199	2327
	955	50	50	1011	50	446	2183	37146	1601
	952	50	50	801	50	466	193	20057	1425
	1079	512	390	809	82	162	335	11987	568

ID50
µg/ml
<50
50 - 100
101 - 300
301 - 1000
>1000

Data-driven antigen selection informed by controllers of M.tb infection



Musvosvi, M., et al. Nat Med 29, 258–269 (2023)



TITAN-01 mRNA TB vaccine

Mtb mRNA vaccine development

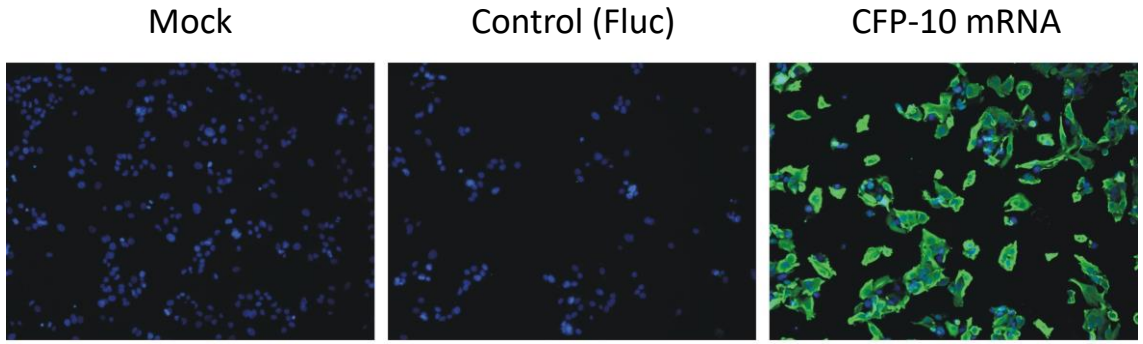
Objective 1: To determine the immunogenicity and optimize PE13, CFP-10, WbbL1, and PPE18 mRNA vaccine construct design. All generated using pseudouridine with proprietary plasmid backbone

Individual mRNA vaccines

Polyprotein mRNA vaccines

Objective 2: To determine protection against M.tb in mice vaccinated with PE13, CFP-10, WbbL1, PPE18 containing mRNA vaccines or a construct containing all four antigens

Objective 3: To compare frequencies, functions and phenotypes of antigen-specific T cell response in controllers or progressor-associated T cells in healthy uninfected adults, healthy M.tb infected adults, and persons with active tuberculosis



Immunogenicity studies: Individual antigens

BALB/c 

C57BL/6 

C3HeB/FeJ (Kramnik) 

PE-13 mRNA ($3x10^{13}$ copies)

PPE-18 mRNA ($1,2x10^{13}$ copies)

CFP-10 mRNA ($3x10^{13}$ copies)

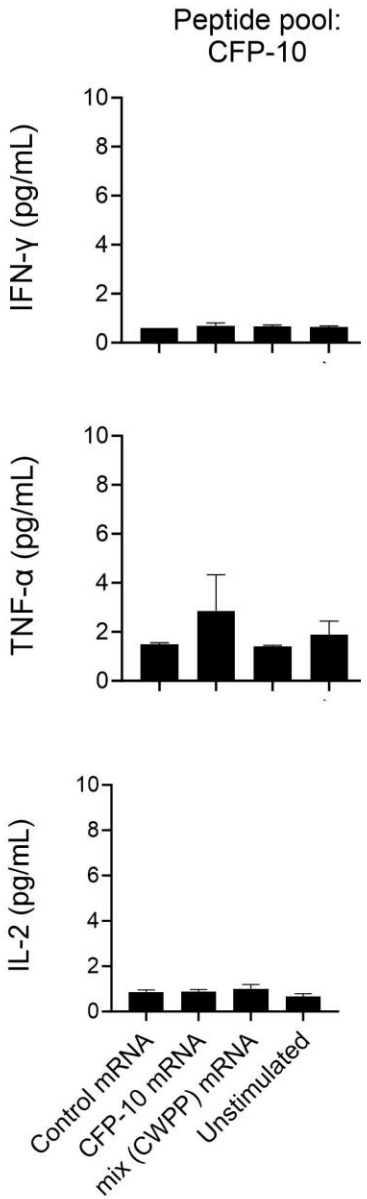
WbbL1 mRNA ($1,5x10^{13}$ copies)

Mixed (CWPP) mRNA (DP mix)

Individual antigens (10 µg) and mixed (2.5 µg of each)

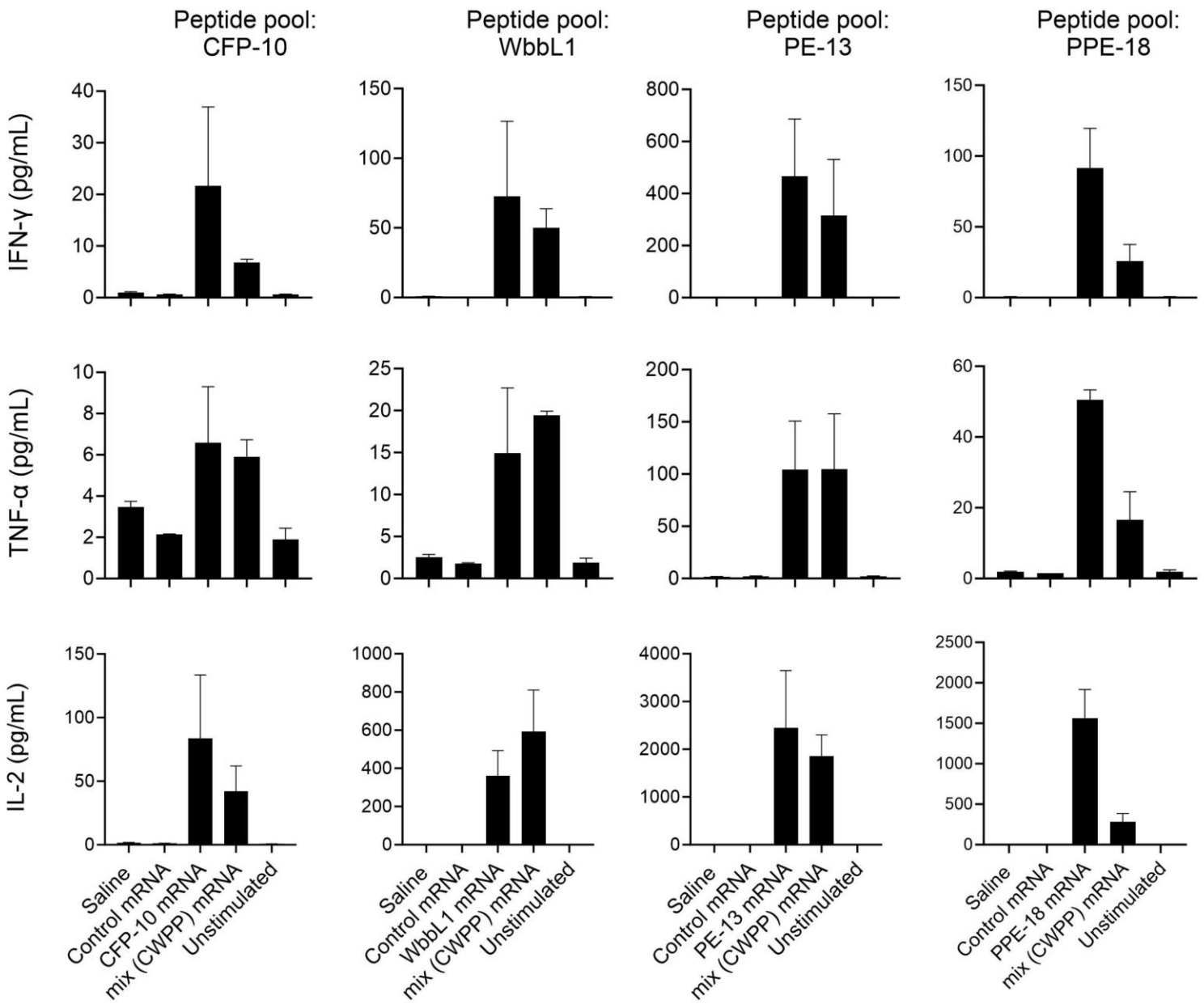
- Splenocytes from vaccinated mice restimulated with peptides
- Secreted cytokine profiles

BALB/c mice



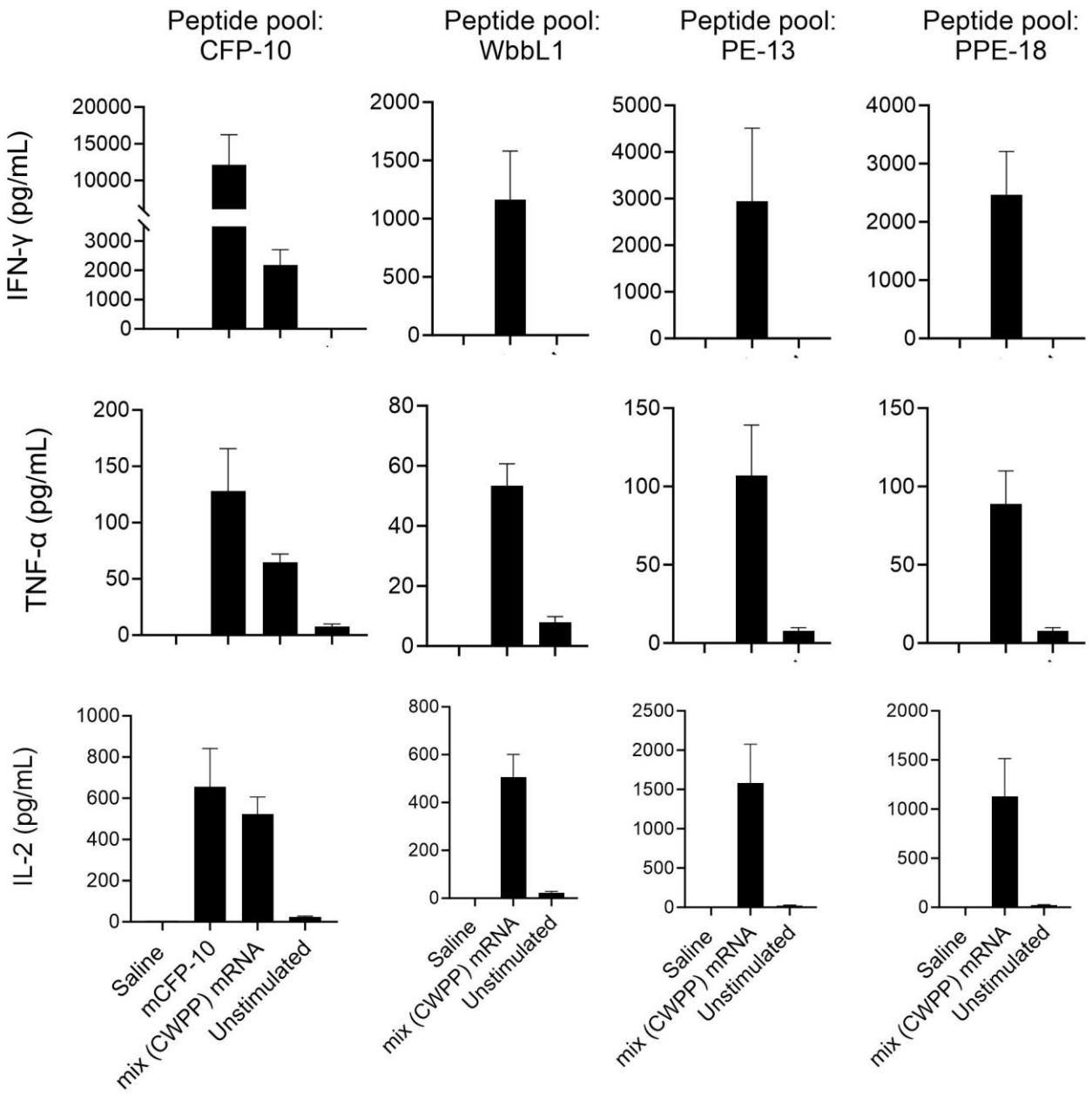
mRNA	Individual				Mix (CWPP)				
Peptide pool	CFP-10	WbbL1	PE-13	PPE-18	CFP-10	WbbL1	PE-13	PPE-18	mix (CWPP)
BALB/c									
C57BL/6									
Kramnik									

C57BL/6 mice



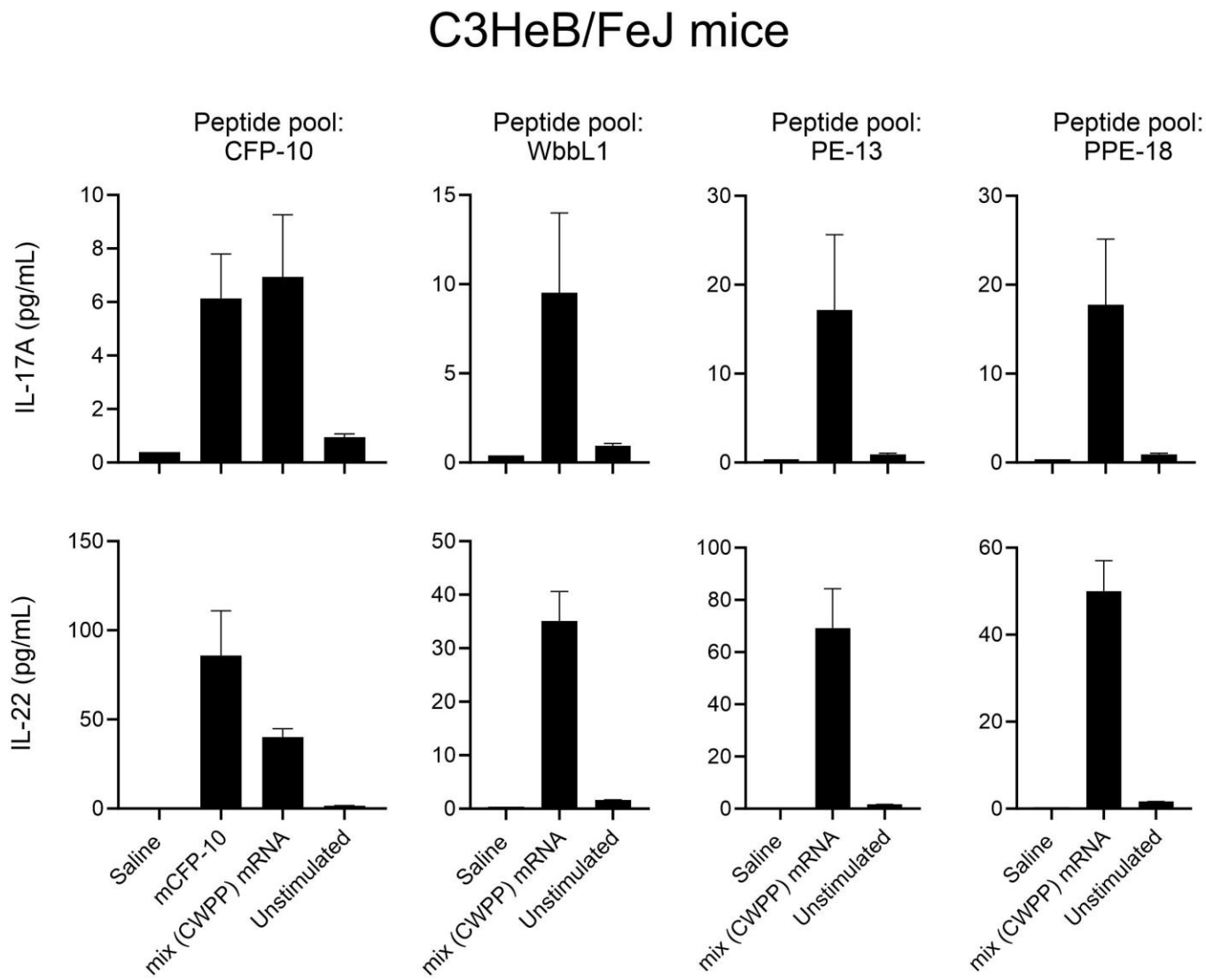
mRNA	Individual				Mix (CWPP)				
Peptide pool	CFP-10	WbbL1	PE-13	PPE-18	CFP-10	WbbL1	PE-13	PPE-18	mix (CWPP)
BALB/c									
C57BL/6									
Kramnik									

C3HeB/FeJ mice



mRNA	Individual				Mix (CWPP)				
Peptide pool	CFP-10	WbbL1	PE-13	PPE-18	CFP-10	WbbL1	PE-13	PPE-18	mix (CWPP)
BALB/c									
C57BL/6									
Kramnik									

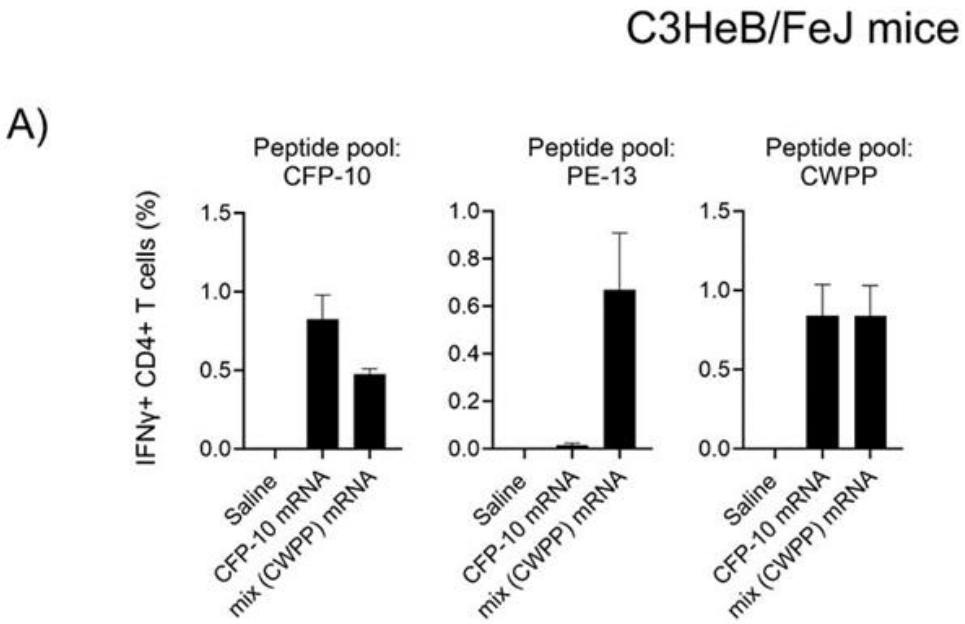
CD4+ Th17 cell markers



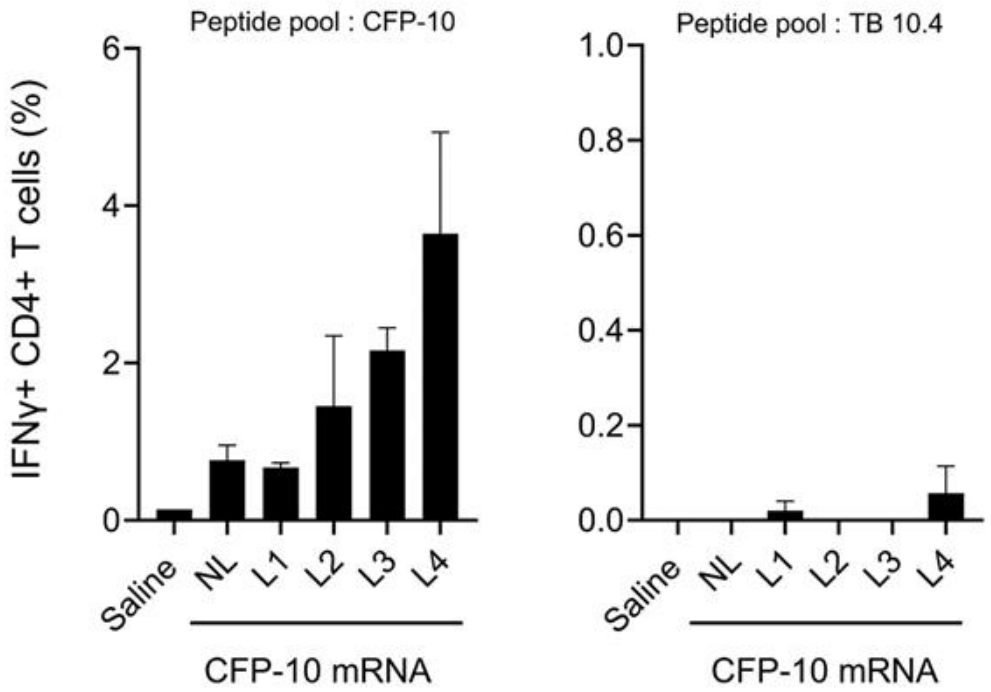
mRNA	Individual				Mix (CWPP)				
Peptide pool	CFP-10	WbbL1	PE-13	PPE-18	CFP-10	WbbL1	PE-13	PPE-18	mix (CWPP)
BALB/c									
C57BL/6									
Kramnik									

Immunogenicity studies: Intracellular IFNγ

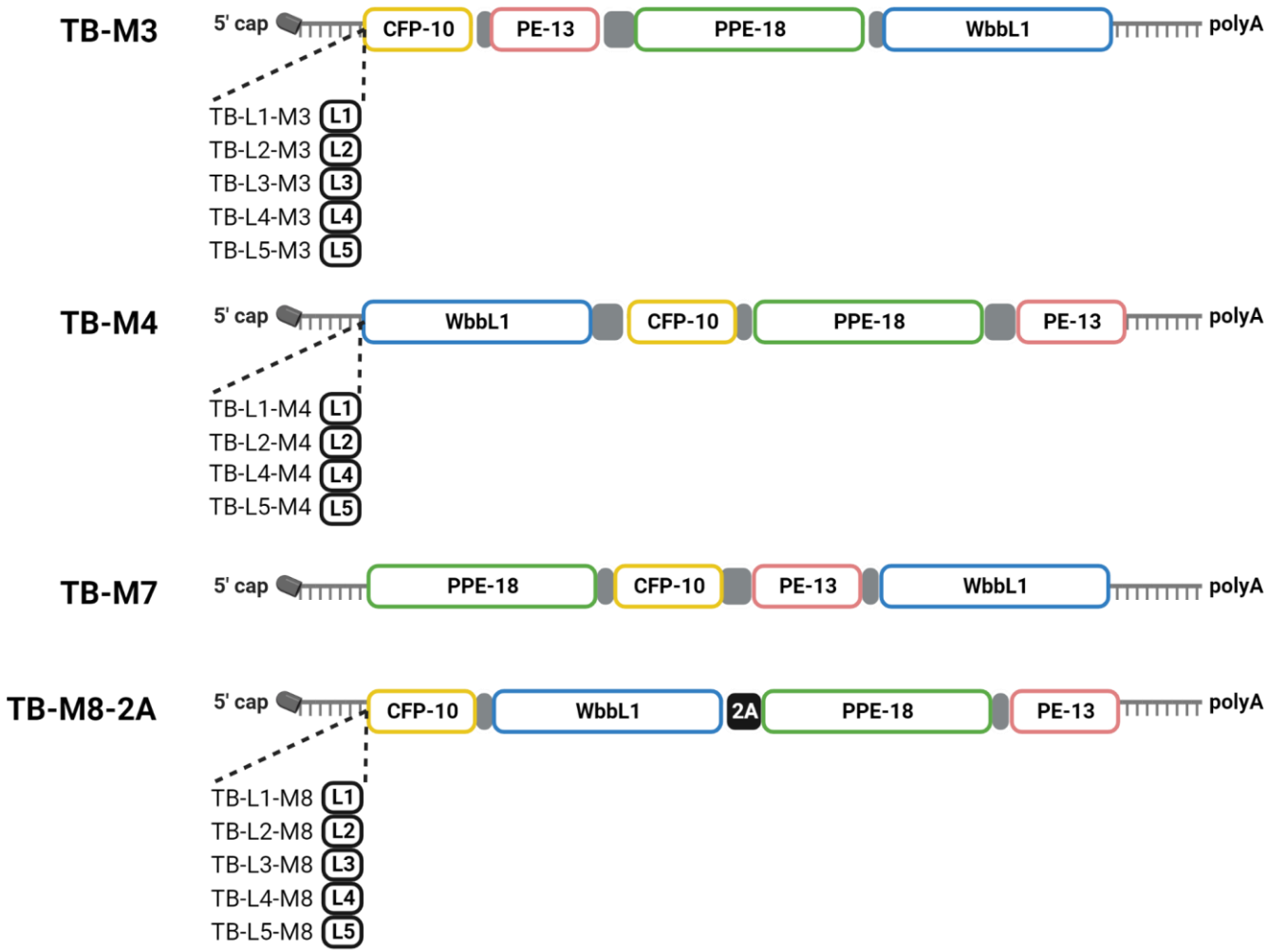
- Splenocytes from vaccinated mice restimulated with peptides
- Measure IFNγ-positive cells amongst CD4 or CD8 T populations

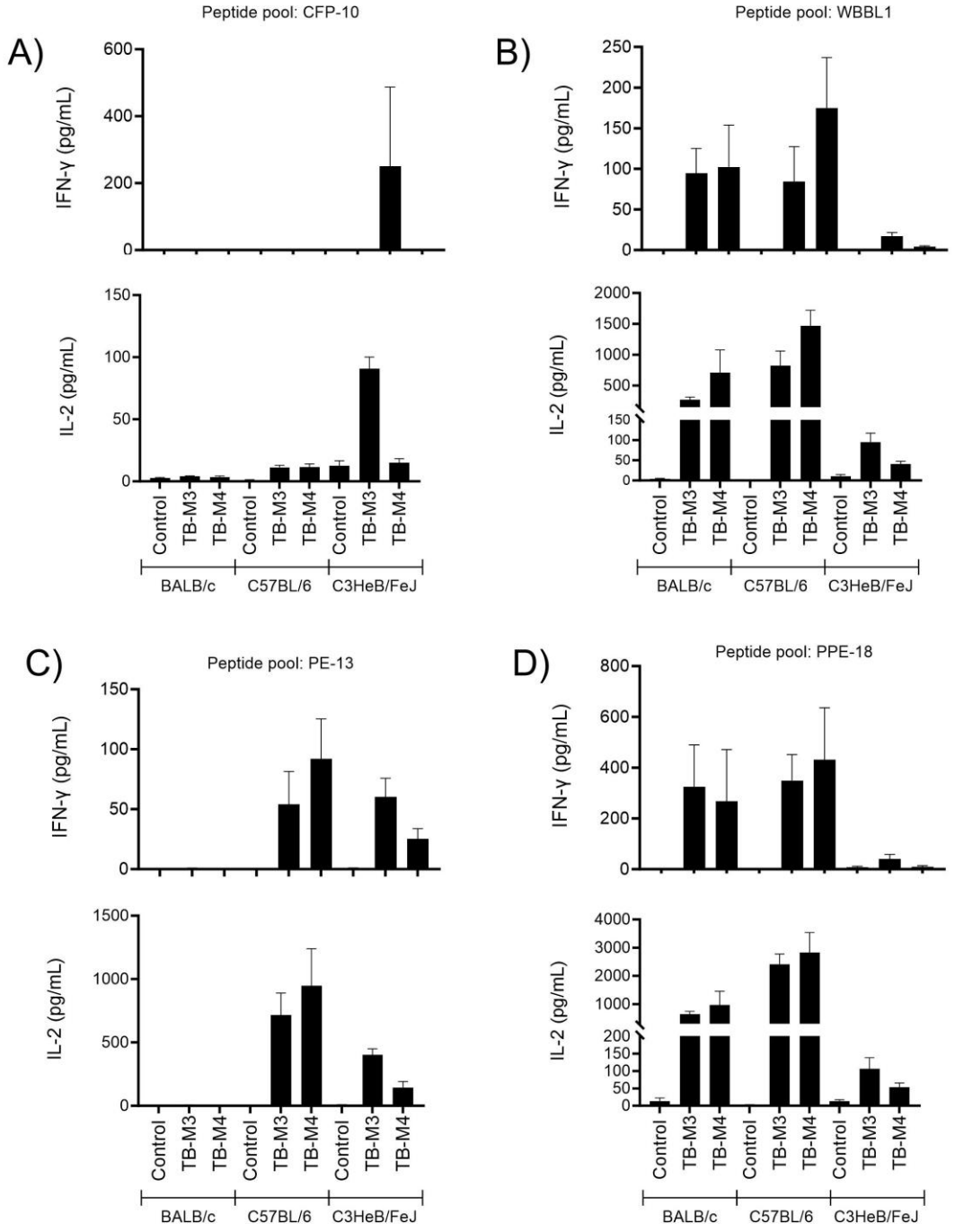


Immunogenicity studies: *M.tb* Leaders



TB mRNA polyprotein constructs





mRNA	TB-M7				
Peptide pool	CFP-10	Wbbl1	PE-13	PPE-18	mix (CWPP)
BALB/c					
C57BL/6					
Kramnik					
mRNA	TB-M3				
BALB/c					
C57BL/6					
Kramnik					
mRNA	TB-M4				
BALB/c					
C57BL/6					
Kramnik					

Data in progress

Data in progress

Summary and next steps

- mRNA candidate vaccines based on antigens produced by controllers
- No evidence of toxicity
- Proprietary plasmid efficient (also with reporters and SARS-CoV-2)
- Presence of ψ in mRNA
- Immunogenic in mice dependent on strain
  - Single proteins
  - Polyproteins

- Further immunogenicity and Challenge studies

- Fast-track development of TB antigens on mRNA platform (partnership with Afrigen)

Acknowledgements



Munya Musvosvi &Tom Scriba (Mtb)

University of Cape Town, South Africa

RWC 2023

Partners and Funders

World Health Organization

Johnson & Johnson INNOVATION

NRF

saMRC advancinglife

medicines patent pool

Afrigen Biologics & Vaccines

Roche

BIOVAC science of protecting life