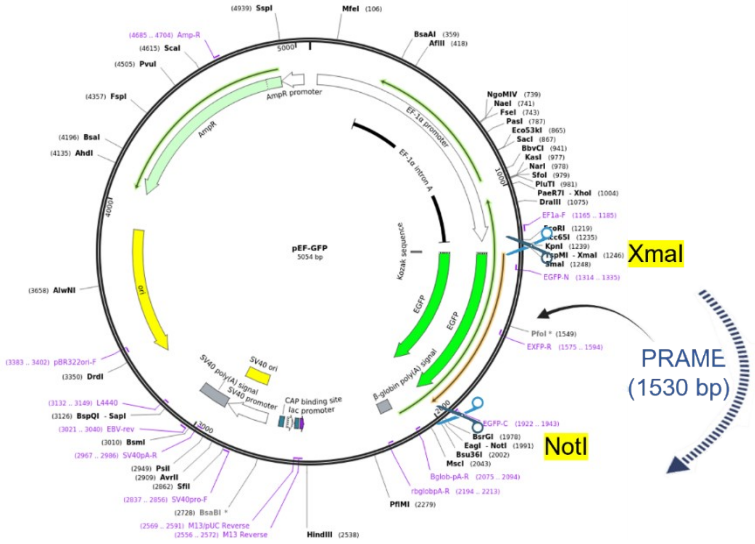
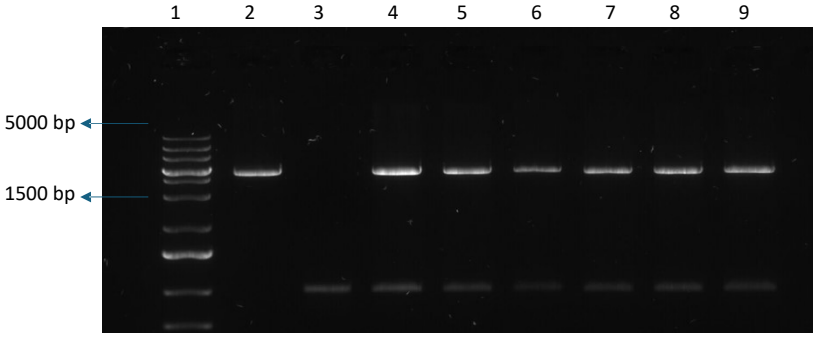
Supplementary figures and tables:



Supp. Figure 1: Vector map of the Plasmid encoding PRAME*. Cloning was designed to replace the GFP gene with PRAME at the XmaI and NotI restriction sites of the plasmid. The cloned plasmids were transformed and amplified in competent DHα cells as per the manufacturer's protocol. Colonies were then isolated and purified from the transformed DHα cells using PureLink Hi-Pure Plasmid Filter Maxiprep Kits. The purified Plasmid was then reconstituted in DNase/RNase-free water and*



stored for further use at -20°C.

Supp. Figure 2: Agarose gel electrophoresis assay for cloned PRAME Plasmids. *1.5% Agarose gel was prepared and used to run the products of ligation after cloning to verify the cloning process. All the ligation products showed successful cloning of the PRAME gene into the vector. Products from lanes 5 and 6 were sent for further sequencing. Lane 1: 1 kb plus DNA ladder; Lane 2: EF1α vector on its own*

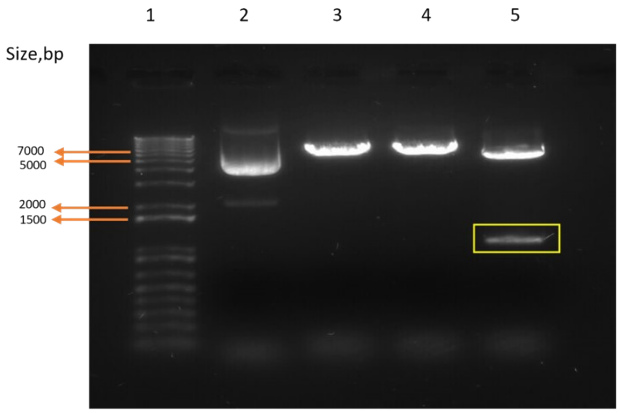
Lane 3: PRAME inserts on its own. Lane 4-9: Double digested mini prepped cloned products with restriction enzymes.

Percent Identity Matrix - created by Clustal2.1

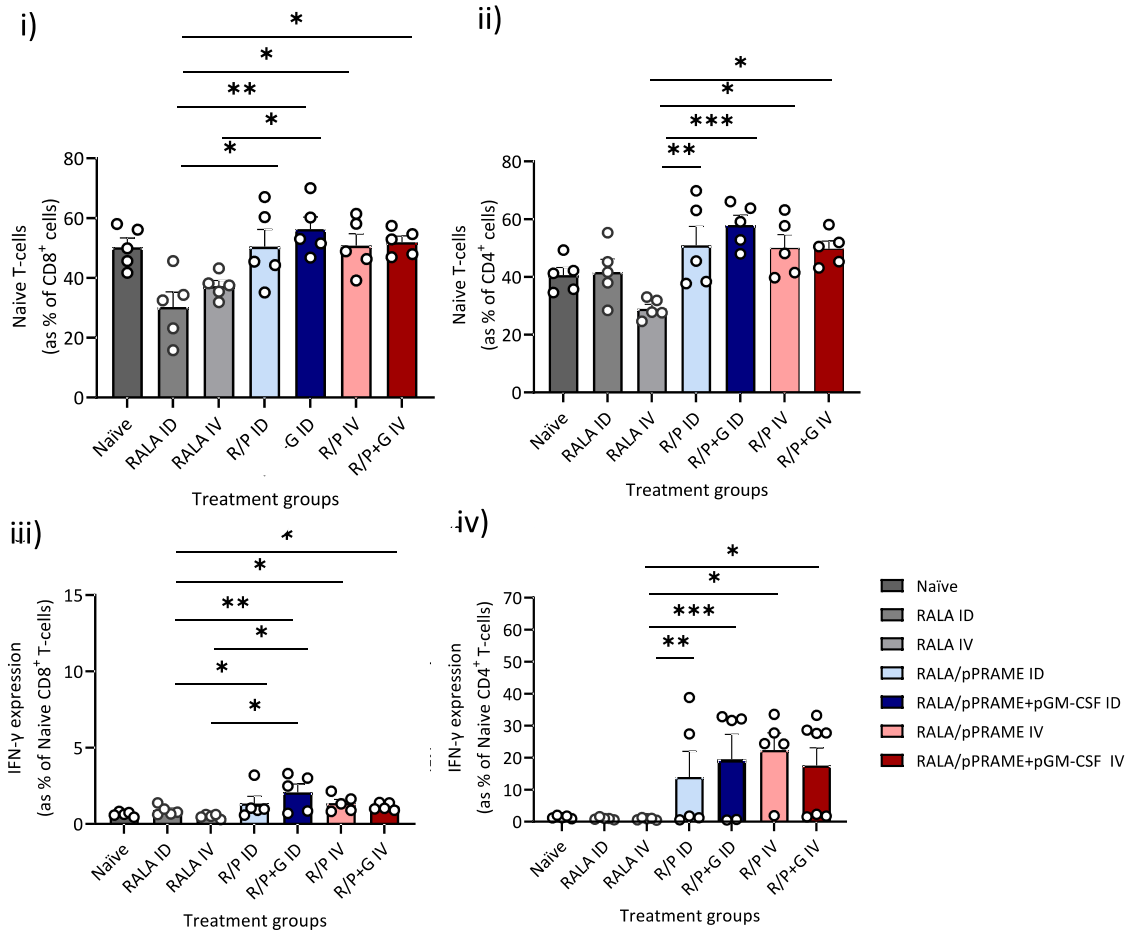
		Similarity with sequence 1 (%)	Similarity with sequence 2 (%)
PRAME	1: NM_001291715.2_187-1716	100.00	99.34
Product 2	2: FJE938_36189381_36189381	99.34	100.00

PRAME	1: NM_001291715.2_187-1716	100.00	50.82

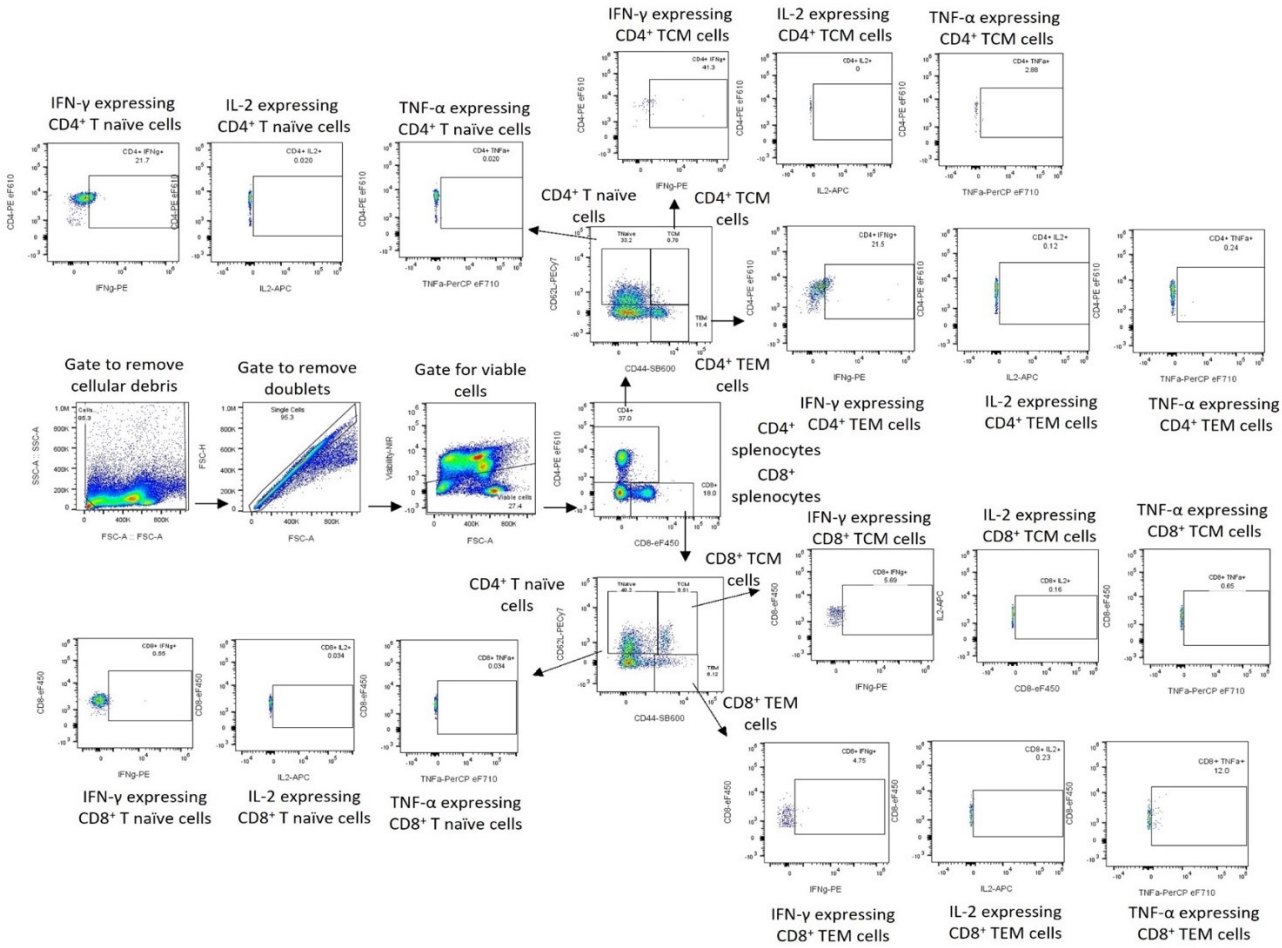
Supp. Figure 3: Percentage Identity Matrix from Clustal Omega Blast sequence. *The PRAME CDS sequence from NCBI and the sequence obtained from the Eurofins lab, UK were compared in the Clustal Omega multiple sequence alignment programme.*



Supp. Figure 4: Restriction digest of the PRAME Plasmid. *Lane 1= 1kb plus DNA Ladder, Lane 2= Control pDNA-PRAME, Lane 3= Single restriction by XmaI enzyme, Lane 4= Single restriction by NotI enzyme, Lane 5 Double restriction by using XmaI and NotI. The PRAME plasmid was subjected to restriction digest with XmaI and NotI and the size of the plasmid was determined via electrophoresis on a 1.5% w/v Agarose gel containing 15 µL of SYBR safe DNA gel stain. A current of 80 V for 60 min and the gel was then visualised under the UV light analyser (UVITEC) with the NineAlliance Mini HD9 Auto software. In line 5, the PRAME band, which is likely present as a linear plasmid, migrates more in the gel and is observed slightly below 1500 bp, consistent with the expected size for this form. The image shown is a representative of the three replicates.*



Supp Figure 5: Cellular immune response of T-naive splenocytes against PRAME vaccination: *i) Naïve $CD8^+$ T-cells ($CD62L^+$ $CD44^+$); ii) Naïve $CD4^+$ T-cells ($CD62L^+$ $CD44^-$); iii) & iv) IFN-γ expression by T-cells in PRAME vaccinated mice assessed in iii) Naïve $CD8^+$ T-cells and iv) Naïve $CD4^+$ T-cells. The PRAME antigen-specific immune response of splenic T-cells was analysed in C57BL/6 female immunocompetent mice. The mice were vaccinated either through intradermal or intravenous route with 3 doses of RALA/pRAME vaccine on Days 0,7, and 11. The NPs were prepared at N:P ratio 8 (20 µg), lyophilised with Trehalose cryoprotectant (10% in trehalose in reconstituted volume), and reconstituted to 1 mg/mL concentration prior to injecting. pGM-CSF was used along with pPRAME as an adjuvant for the vaccine. The spleens of the mice were extracted after 42 days of the 1st injection dose and the splenocytes were isolated, cultured and stained for intracellular markers of the immune cells. The stained splenocytes were analysed using flow cytometric analysis and the results are represented as the mean value in each group (N=5). Data represents the mean value (N=5) and are statistically evaluated by one-way ANOVA and the statistical difference is represented with '*.' *< 0.05, ** < 0.001, ***< 0.0001.*



Supp Figure 6: Gating strategy used for analysis of immune response in mice splenocytes. *Primary splenocytes extracted from C57BL/6 vaccinated mice were used for staining with antibodies for surface markers and intracellular immune cell markers. The gates were set accordingly using unstained control and FMO controls with respect to which the results were obtained for the stained samples.*

Table 1: Summary of PRAME protein in The Human Protein Atlas

The Human Protein Atlas PRAME summary	
Protein	PRAME nuclear receptor transcriptional regulator
Gene name	PRAME (CT130, MAPE)
Gene Location	22.q11.22 (22547701 – 22559361 bp)
Tissue specificity	Tissue enriched (Testis)
Tissue expression cluster	Testis (Mainly Spermatid Development)
Single-cell type specificity	Cell type enriched (Spermatogonia)
Single-cell type expression cluster	Spermatogonia and spermatocytes-Spermatogenesis (mainly)
Immune cell specificity	Not detected
Brain specificity	Not detected
Predicted location	Intracellular
Subcellular summary	Nucleoplasm, Plasma Membrane
Protein function	Functions as a transcriptional repressor, inhibiting the signalling of retinoic acid through the retinoic acid receptors RARA, RARB and RARG. Prevents retinoic acid-induced cell proliferation arrest, differentiation, and apoptosis.
Molecular function	Repressor
Biological process	Apoptosis, Differentiation, Growth regulation, Transcription, Transcription regulation
Gene summary	This gene encodes an antigen that is preferentially expressed in human melanomas and that is recognized by cytolytic T lymphocytes. It is not expressed in normal tissues, except testis. The encoded protein acts as a repressor of the retinoic acid receptor and likely confers a growth advantage to cancer cells via this function. Alternative splicing results in multiple transcript variants.