

Supplementary Figure 1: IL-1 β , IL-2 and IL-4 were measured from the cell supernatant in response to NPM266-285cit and NPM266-285wt peptides in NPM266-285cit immunised HLA-DP4 mice.

Supplementary Figure 2: (A) To assess if NPM266-285cit vaccination provides anti-tumour activity against an aggressive model, HLA-DP4 mice were challenged with Lewis lung carcinoma cell 2 (LLC2) tumour cells expressing constitutive DP4 followed by immunisation with CpG/MPLA, NPM266-285cit or irrelevant peptides on day 4, 11 and 18. Tumour growth and survival was monitored, n= 10 mice/group. (A(ii)) Tumour volume on day 10.

Supplementary Figure 3: Tumour Growth curves of mice following B16F1iDP4 implant (A, B and C) and B16F1HHDII implant (D & E). Mice immunised with NPM266-285cit on day 1, 8 and 15 (C & E). Mice immunised with CpG/MPLA (B & D), and unimmunised (A). (A, B, D and E) n = 10. C, n = 20.

Supplementary Figure 4: Successful knock out of PAD2. ddPCR and FACS staining were used to assess if the knock out was successful. (A(i)) The absence of signal (blue) targeting exon 6-7 suggests PAD2 knock out in 2B10 clone but it was present in (A(ii)) the parental cells. Strong signal (orange) was observed targeting exon 13-14 indicating C terminal end of PAD2 still expressed in (B(i)) 2B10 clone and (B(ii)) parental cells. (C) The PAD2 KO clones (red) showed a PAD2 signal comparable to that of the parental line (blue) despite the lack of WT sequence. The signal was potentially due to the antibody binding site being in the C terminus while the KO indels lie in the first exon. (D) PAD4 expressed in both the parental cells (blue) and the PAD2 knock 2B10 clone (red). (E) PAD2 expression (band ~75 kd) was only observed in B16F1 parental cells and absent in PAD2KO cells in western blot.

Supplementary Figure 5: Tumour Growth curves of mice following B16F1PAD2KOcDP4 implant (A & B). Mice immunised with CpG/MPLA (A) or NPM266-285cit (B) on day 1, 8 and 15. n = 10.

Supplementary Figure 6: Successful depletion of CD25+ and CD45RO+ cells. Example plots showing (A) CD25 depletion, (B) CD45RO depletion.

Supplementary Figure 7: To assess if the NPM266-285cit responses in healthy donors are citrulline specific PBMCs from buffy coat were stimulated with NPM266-285cit for 11 days. Then restimulation responses to NPM226-285cit and NPM266-285wt were measured with IFN γ ELISpot assay. Quadruplicate wells were seeded, mean \pm SD, n =1.

Supplementary table 1: Antibodies used in the study.

Antibodies	Clone	Dilution	Source
anti-CD4 eFluo 450	RPA-T4	1:50	eBioscience #48-0049-42
anti-CD8-VioGreen	REA734	2:50	Miltenyi #130-110-684
anti CD25-PE	REA570	2:50	Miltenyi #130-109-075
Anti-CD45RO-VioGreen	REA611	5:50	Miltenyi #130-109-512
anti-CD134-PEcy7	REA621	5:50	Miltenyi #130-109-603
anti-IFN γ -APCe780	4S.B3	1:50	eBioscience #47-7319-42
anti-Granzyme B-PE	GB11	1:50	eBioscience #12-8899-41