# Supplementary Table S1

Host	Plasmids	Characteristics	Source
<i>strains</i> <i>L. lactis</i> NZ9000	_	<i>L. lactis</i> subsp. <i>cremoris</i> (derivative strain of MG1363, carrying <i>nisRK</i> genes on the	University Putra Malavsia
	170040	chromosome).	
L. lactis NZ9000	pNZ8048	L. lactis subsp. cremoris (derivative strain of MG1363, carrying <i>nisRK</i> genes on the chromosome).	Oniversity Putra Malaysia
L. lactis NZ9000	pNZ- Usp45- NucA	P <sub>nisA</sub> /Cm <sup>r</sup> pNZ8048 derivative containing Usp45-NucA	University Putra Malaysia
XL-10 GOLD <i>E. coli</i>	pIDT- SMART- 139A mimotopes	Amp <sup>R</sup> , pIDT-SMART derivative containing synthesized t <i>K-Ras</i> mimotopes	Present study
XL-10 GOLD <i>E. coli</i>	pIDT- SMART- TTD	Amp <sup>R</sup> , pIDT-SMART derivative containing synthesized toxoid	Present study
L. lactis NZ9000	pNZ- wt <i>KRAS</i>	P <sub>nisA</sub> /Cm <sup>r,</sup> pNZ8048 derivative containing wt <i>KRAS</i>	37
L. lactis NZ9000	pNZ-G12A	P <sub>nisA</sub> /Cm <sup>r,</sup> pNZ8048 derivative containing fusion of Usp45 and natural mutant G12A- <i>KRAS</i> in exon 2	Present study
L. lactis NZ9000	pNZ-Usp45	P <sub>nisA</sub> /Cm <sup>r</sup> pNZ8048 derivative containing Restriction site-modified Usp45	37
L. lactis NZ9000	pNZ- Usp45- G12A	P <sub>nisA</sub> /Cm <sup>r</sup> , pNZ8048 derivative containing fusion of Usp45 and natural mutant G12A- <i>KRAS</i> in exon 2	Present study
L. lactis NZ9000	pNZ- Usp45- 139A	P <sub>nisA</sub> /Cm <sup>r</sup> , pNZ8048 derivative containing fusion of Usp45 and 139A mimotopes	Present study
L. lactis NZ9000	pNZ- Usp45- 139A-TTD	P <sub>nisA</sub> /Cm <sup>r</sup> , pNZ8048 derivative containing fusion of Usp45 and 139A mimotopes- tetanus toxoid	Present study

# Bacteria host strains and plasmids used and constructed in this study.

# Supplementary Table S2

List of PCR oligonucleotide primers used in this study.

Name	Forward (5'-3')	Reverse( 5'-3')	$T_{m}$	Amplicon	Template			
NIZ0040			<u>(°C)</u>	Size (bp)				
pNZ8048	IAIIGICGAIAACGCGAGCAI	CGITICAAGCCIIGGIIIIC	59	Vary with	Recombinant			
				insert size	pNZ8048 plasmid			
SDM	TGGGTACTGCAGAAATGACTGAAT	CTATATACTAGTCTAATGATG	57	180	wt <i>KRAS</i> cDNA			
	ATAAACTTGTGGTAGTTGGAGCTG	ATGATGATGATGGGTTTCTCC						
	CTGCCGTAG	ATCAATTACTAC						
For construction pNZ-Usp45- therapeutic KRAS (139A)								
Usp45[a]	CACTCACCATGGGCATGAAAAAAA	TTATATTCGGCATGCAGCGTAA	56	110	pNZ8048-Usp45-			
1 1 1	AG	ACAC			Xgene			
Therapeutic	GCCTCTGGGTAGCATGCGAA	TAGAACTAGTCTAATGATGATG	59	193	pIDTSMART-139-			
KRAS	TATAAATTAG	ATGATGATGA			A			
mimotopes								
For construction pNZ-Usp45-mtKRAS (control)								
Usp45[b]	GCACTCACCATGGGCATGAAAAAA	GTTTATATTCAGTCATGCATGC	56	118	pNZ8048-Usp45-			
	AAGATTATCTC	AGCGTAAACACC			Xgene			
mtKRAS	TTACGCTGCATGCATGACTAATATA	AGCTTGAGCTCTCTAGAACTA	61	207	pNZ8048-G12A			
mimotopes	AACTTGTGG	GTCTAATGATGATG						
SOE-Usp45-	GCACTCACCATGGGCATGAAAAAA	AGCTTGAGCTCTCTAGAACTA	59	299	Two overlapping			
mtKRAS	AAGATTATCTC	GTCTAATGATGATG			PCR products			
For construction of pNZ-Usp45- therapeutic KRAS-TTD								
Mimotopes-	GCCTCTGGGTAGCATGCGAATATA	AAAATTATTACTAGTATGATG	67	98	pIDTSMART -139A			
TTD-B	AATTAG	ATGATGATGATGAGC			_			
TTD	CATCATCATCATCATCATACTAGT	AAGCTTCTAATGATGATGATG	62	144	pIDTSMART-TTD			
	AATAATTTTACAGTTTCATTTTGG	ATGATGTGTTTCTAAATGTGA			-			
		AGCTGA						
SOE-	GCCTCTGGGTAGCATGCGAATATA	AAGCTTCTAATGATGATGATG	66	179	Two overlapping			
mimotopes-TTD	AATTAG	ATGATGTGTTTCTAAATGTGA			PCR products			
±		AGCTGA			1			



Schedule of immunization and sample collection including blood, feces, GI tract and spleen. To evaluate immune response raised by 139A-tK-ras mimotopes and its counterparts, 5 groups (n=4) of BALB/c mice were tested. Each group were separately fed with: NaHCO<sub>3</sub>, recombinant *L. lactis* expressing control G12A-K-ras, 139A-tK-ras, 139A-tK-ras-TTD and empty vector pNZ8048. Each group was orally administered on day 1/2/3, and boosted on days 8/9/10, 15/16/17 and 22.



K-ras mimotopes produced after 6 hour of nisin induction. a) Concentration of K-ras mimotopes b) Image J analysis of Western blot bands representing intra- and extra-cellular K-ras mimotopes produced after 6 hour of nisin induction.

### **Supplementary Figure S3**



**Recombinant** *L. lactis* clones harbouring pNZ-Usp45-G12A, pNZ-Usp45-139A and pNZ-Usp45-139A-TTD. Western blot of expressed Usp45-G12A, Usp45-139A and Usp45-139A-TTD in both intracellular and extracellular protein fractions were detected via His-tag markers. M: Protein ladder; In: intracellular protein fraction; Ex: extracellular protein fraction; Usp45: lactococcal extracellular secretion signal peptide; TTD: tetanus toxoid.

### **Supplementary Figure S4**



Average weight of 4 mice during pre- and post-immunized. No significance weight different between control and experimental groups.