

Appendix 1: Prediction of protein A) secondary and B) 3D structures. 1) Nef-Tat^(exons 1+2), 2) Nef-Tat^(exon 1).

A)

1)

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1 ***** 60
MGGKWSKSSVIGWPAVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNAACAWLEA
CCCCCCCCCCCCCHHHHHCCCCCCCCCCCCCHHHHHCCCCCCCCCCCCCHHHH
988766677777667888888577788876588999998587778988776478999999

61 ***** 120
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHTQGY
HCCCCCCCCCEEEECCHHHHHHHHHHHCCCCCEECCHHHHHHHHHHHHCC
74556777777777877656899999999987599887577557767777654566657

121 ***** 180
FPDWQNYTPGPGVRYPLTFGWCKLVPVEPKVEEANKGENTSLHHPVSLHGMDPEREV
CCCCCEECCECECECECECHHHCCCCCCCCCCCCCCCCCCCCCEEC
6677766777777666655888777688567654567888876566667888887579

181 ***** 240
LEWRFDRLAFHHVARELHPEYFKNCLGSMPEVDPRLPEWKHPGSPKTACTIONCYCKKC
EEEEECCHHCHHHHHCCCCCCCCCCCCCCCCCCCCCECCCCCCCCCCCCCCCC
999977789999999984555577777655666777777766664455554444

241 ***** 296
CFHCQVCFMTKALGISYGRKKRRQRRRAHQNSQTHQASLSKQPTSPRGDPTGPKE
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCECCCCCCCC
5545555656777665444555566677766645556666787777766677778

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2)

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1 ***** 60
MGGKWSKSSVIGWPAVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNAACAWLEA
CCCCCCCCCCCCCHHHHHCCCCCCCCCCCCCHHHHHCCCCCCCCCCCCCCCC
988766677788678999998577778766566788887678778988777666655555

61 ***** 120
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHTQGY
CCCCCCCCCCCCCEEEECCHHHHHHHHHHHCCCCCEECCHHHHHHHHHHHHCC
555667777777778776568999999999975998786445655667866467566

121 ***** 180
FPDWQNYTPGPGVRYPLTFGWCKLVPVEPKVEEANKGENTSLHHPVSLHGMDPEREV
CCCCCEECCECECECECHHHHHCCCCCCCCCCCCCCCCCCCCCEEC
6777667777777666665898788588578887656888886666557888887669

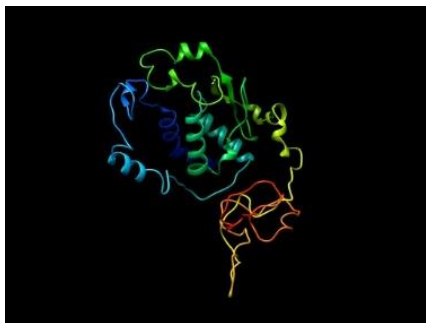
181 ***** 240
LEWRFDRLAFHHVARELHPEYFKNCLGSMPEVDPRLPEWKHPGSPKTACTIONCYCKKC
EEEEECCHHCHHHHHCCCCCCCCCCCCCCCCCCCCCECCCCCCCCCCCCCCCC
99997668999999998454557777666444555666677777777777645677

241 ***** 281
CFHCQVCFMTKALGISYGRKKRRQRRRAHQNSQTHQASLSK
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
7666655444455666556667556655477666667889

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B)

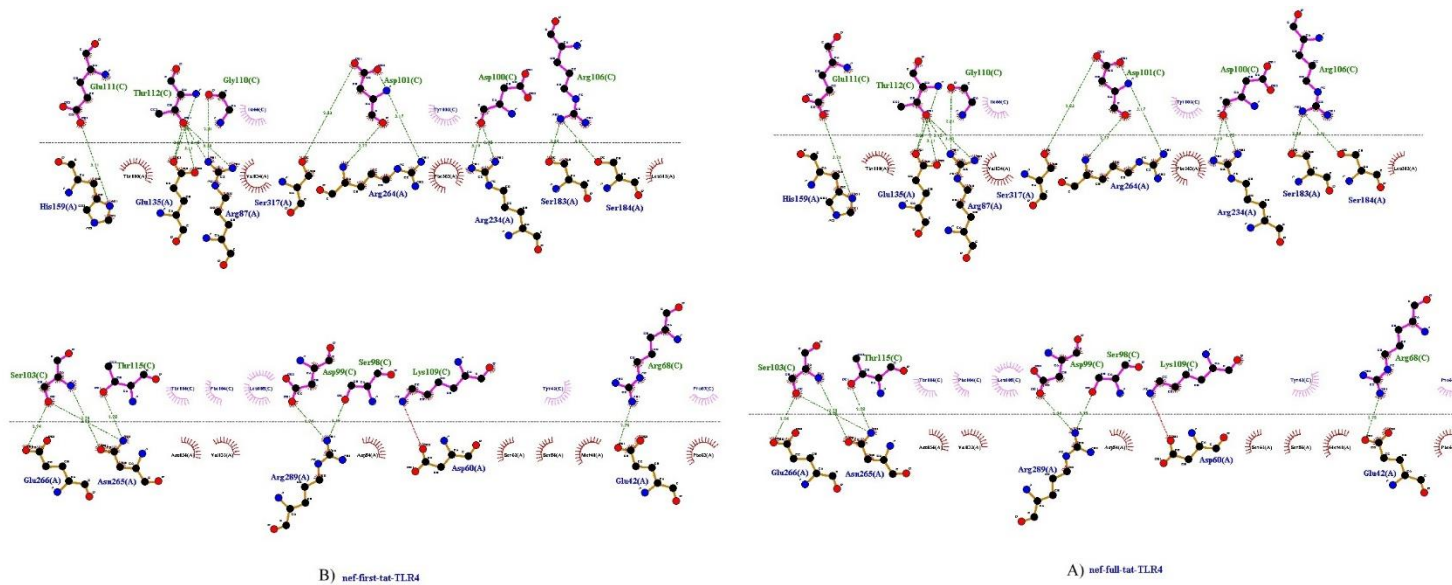
1)



2)

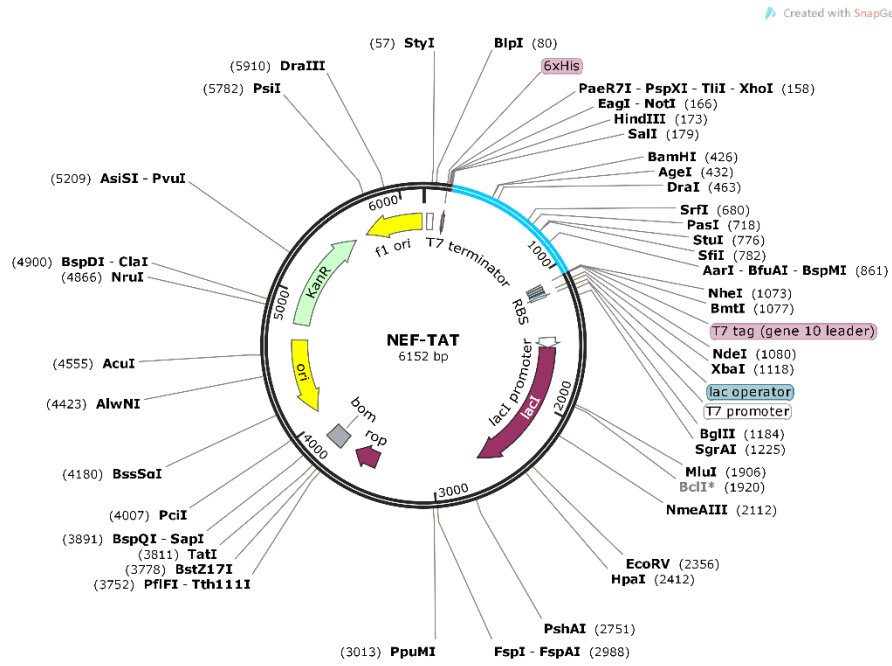


Appendix 2: Interactions (hydrogen and other non-covalent bonds) between TLR-4 and A) Nef-Tat (exons 1+2), B) Nef-Tat (exon 1) using ligplot software.

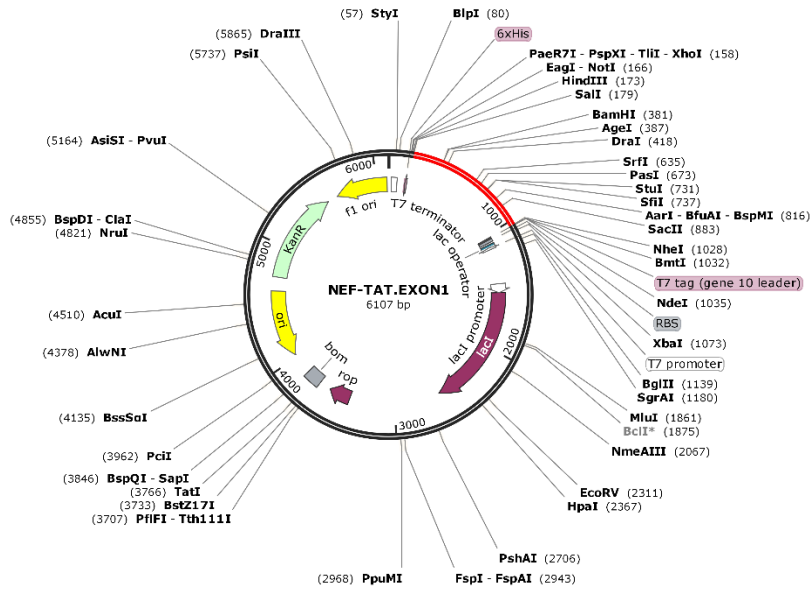


Appendix 3: *In silico* cloning of vaccine constructs into pET24a (+) prokaryotic expression vector. A) Nef-Tat^(exons 1+2) and B) Nef-Tat^(exon 1) cloned into pET24a (+) vector by *SalI*/*NheI* restriction enzymes and marked in blue and red, respectively.

A)



B)



Appendix 4: The Fusion protein constructs sequences

construct	Protein sequence
Nef-Tat ^(exons 1+2)	MGGKWSKSSV IGWPAVRERM RRAEPAADGV GAVSRDLEKH GAITSSNTAA NNAACAWLEA QEEEEVGFPV TPQVPLRPMT YKAAVDLSHF LKEKGGLEGLIHSQRRQDIL DLWIYHTQGY FPDWQNYTPG PGVRYPLTFG WCYKLVPVEPDKVEEANKGE NTSLLHPVSL HGMDDPEREV LEWRFDSRLA FHHVARELHPEYFKNCLEGS MEPVDPRLEP WKHPGSQPKT ACTNCYCKKC CFHCQVCFMTKALGISYGRK KRRQRRRAHQ NSQTHQASLS KQPTSQPRGDPTGPKE
Nef- Tat ^(exon 1)	MGGKWSKSSV IGWPAVRERM RRAEPAADGV GAVSRDLEKH GAITSSNTAANNAACAWLEA QEEEEVGFPV TPQVPLRPMT YKAAVDLSHF LKEKGGLEGLIHSQRRQDIL DLWIYHTQGY FPDWQNYTPG PGVRYPLTFG WCYKLVPVEPDKVEEANKGE NTSLLHPVSL HGMDDPEREV LEWRFDSRLA FHHVARELHPEYFKNCLEGS MEPVDPRLEP WKHPGSQPKT ACTNCYCKKC CFHCQVCFMTKALGISYGRK KRRQRRRAHQ NSQTHQASLS K

Appendix 5: The structural refinement of the of Nef-Tat^(exons 1+2) and Nef-Tat^(exon 1) using Ramachandran and ERRAT2 Plot

Constructs	Ramachandran Plot						ERRAT2
	Residues in most favored regions	Residues in disallowed regions	Number of glycine residues	Number of proline residues	Total number of residues	Quality model	Overall quality factor
Nef-Tat ^(exons 1+2)	82.7%	2.4%	22	24	296	Good	82.051
Nef-Tat ^(exon 1)	87.4%	0.8%	20	20	281	Good	92.692

Appendix 6: The physicochemical and immunological characteristics of constructs

Construct	Molecular weight (kDa)	pI	Negative charge residue	Positive charge residue	Solubility	Allergenicity	Antigenicity
Nef-Tat ^(exons 1+2)	33.6 kDa	8.62	35	40	Soluble	Non-allergen	0.61
Nef- Tat ^(exon 1)	32 kDa	8.62	33	38	Soluble	Non-allergen	0.62