

## Supplementary Materials

# Immunization Effects of a Novel $\alpha$ -Synuclein-Based Peptide Epitope Vaccine in Parkinson's Disease-Associated Pathology

Jun Sung Park <sup>1,†</sup>, Riaz Ahmad <sup>1,†</sup>, Kyonghwan Choe <sup>1,2,†</sup>, Min Hwa Kang <sup>1</sup>, Tae Ju Park <sup>3</sup>  
and Myeong Ok Kim <sup>1,4,\*</sup>

<sup>1</sup> Division of Life Sciences and Applied Life Science (BK 21 Four), College of Natural Science, Gyeongsang National University, Jinju 52828, Republic of Korea; jsp@gnu.ac.kr (J.S.P.); riazk0499@gnu.ac.kr (R.A.); k.choe@gnu.ac.kr or k.choe@maastrichtuniversity.nl (K.C.); kmh1020@gnu.ac.kr (M.H.K.)

<sup>2</sup> Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs), Maastricht University, 6229 ER Maastricht, The Netherlands

<sup>3</sup> Haemato-Oncology/Systems Medicine Group, Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary & Life Sciences (MVLS), University of Glasgow, Glasgow G12 0ZD, UK; t.park.1@research.gla.ac.uk

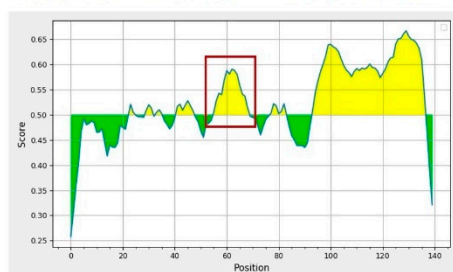
<sup>4</sup> Alz-Dementia Korea Co., Jinju 52828, Republic of Korea

\* Correspondence: mokim@gnu.ac.kr; Tel.: +82-55-772-1345

† These authors contributed equally to this work.

## Supplementary Figure

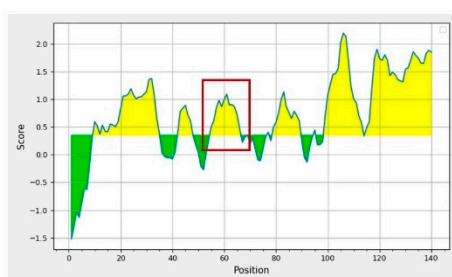
### (A) Bepipred Linear Epitope Prediction 2.0 Results



#### Predicted peptides :

No.	Start	End	Peptide	Legth
1	24	25	QG	2
2	30	32	AGK	3
3	34	36	KEG	3
4	42	48	SKTKEGV	7
5	56	69	AEKTKEQVTNVGGA	14
6	78	83	AQKTVE	6
7	94	137	FVKKQDLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYE	44

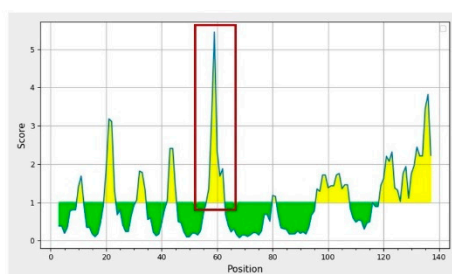
### (B) Bepipred Linear Epitope Prediction Results



#### Predicted peptides :

No.	Start	End	Peptide	Legth
1	10	35	KAKEGVVAAAEKTKQGVAAEAGKTKE	26
2	42	47	SKTKEG	6
3	55	66	VAEKTKEQVTNV	12
4	69	69	A	1
5	77	77	V	1
6	79	89	QKTVEGAGSIA	11
7	95	95	V	1
8	99	113	QLGKNEEGAPQEGIL	15

### (C) Emini Surface Accessibility Prediction Results



#### Predicted peptides :

No.	Start	End	Peptide	Legth
1	57	62	EKTKEQ	6
2	96	107	KKDQLGKNEEGA	12

**Supplementary Figure S1. Algorithms for predicting B cell epitope of  $\alpha$ -syn using the Immune Epitope Database (IDEB).** (A) Bepipred Linear Epitope Prediction 2.0 and the predicted peptides. (B) Bepipred Linear Epitope Prediction and the predicted peptides. (C) Emini Surface Accessibility Prediction and the predicted peptides.

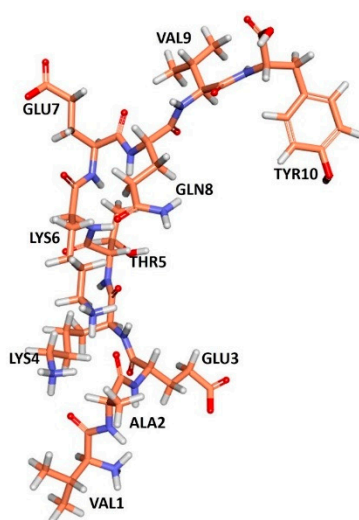
(A) Epitope Candidate's Docking Score

N o.	Epitope 1 (VAEKTKEQVT)	Epitope 2 (AEKTKEQVTN)	Epitope 3 (EKTKEQVTNV)
1	122.65	106.89	96.84
2	119.29	105.21	96.46
3	115.51	100	93.5
4	106.8	99.63	88.53
5	104.59	95.97	88.37
6	101.97	95.97	87.25
7	101.43	95.4	83.59
8	101.14	91.63	83.05
9	100.38	91.53	82.76
10	90.26	90.46	81.13

(B) Prediction of Toxic Peptides

Peptide Seq	QM score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
VAEKTKEQVT	-10.9	Non-Toxin	-0.32	-0.95	0.79	0	1132.42

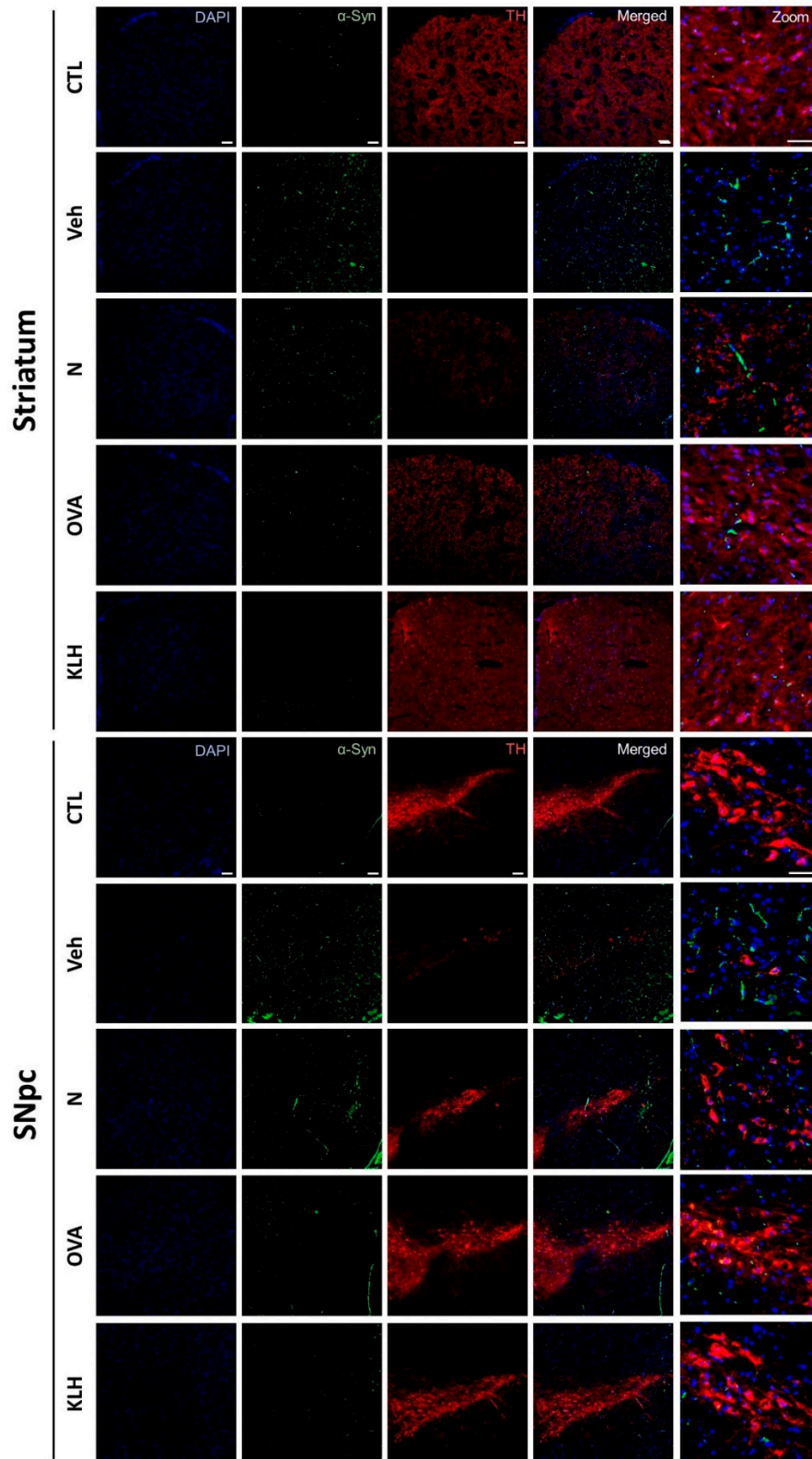
(C)



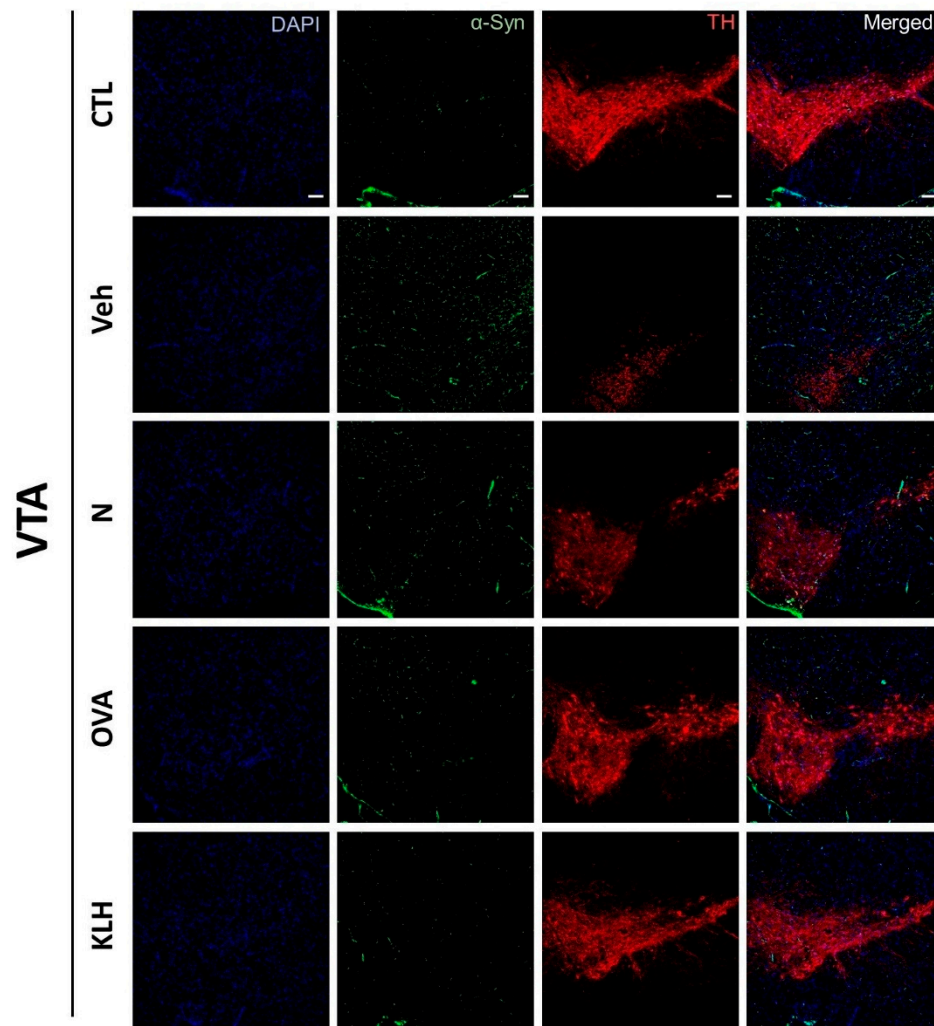
interaction	Chain	Interaction residues	interaction	Chain	Interaction residues
Conventional Hydrogen Bond	A	TRP33	Carbon Hydrogen bond	A	LYS31
		ASP56			ALA32
		HIS102			SER100
	B	TYR48		B	PRO54
		ARG53			SER55
		SER55		H	SER156
		ASN94	Pi-Donor Hydrogen Bond	B	PHE31
	H	ALA158	Pi-Sigma	B	TYR48
		THR160	Pi-Pi T-shaped	B	PHE31
			Alkyl	B	PRO90
			Pi-Alkyl	B	PHE31
					HIS33
				H	ALA158

Epitope (VAEKTKEQVT)

**Supplementary Figure S2. Examination of the three candidate peptides. (A)** Docking score via Genetic optimization for ligand docking (GOLD). **(B)** Selected epitope showing as non-toxic in the toxicity prediction model. **(C)** Structural conformation of the epitope and its interaction residues.

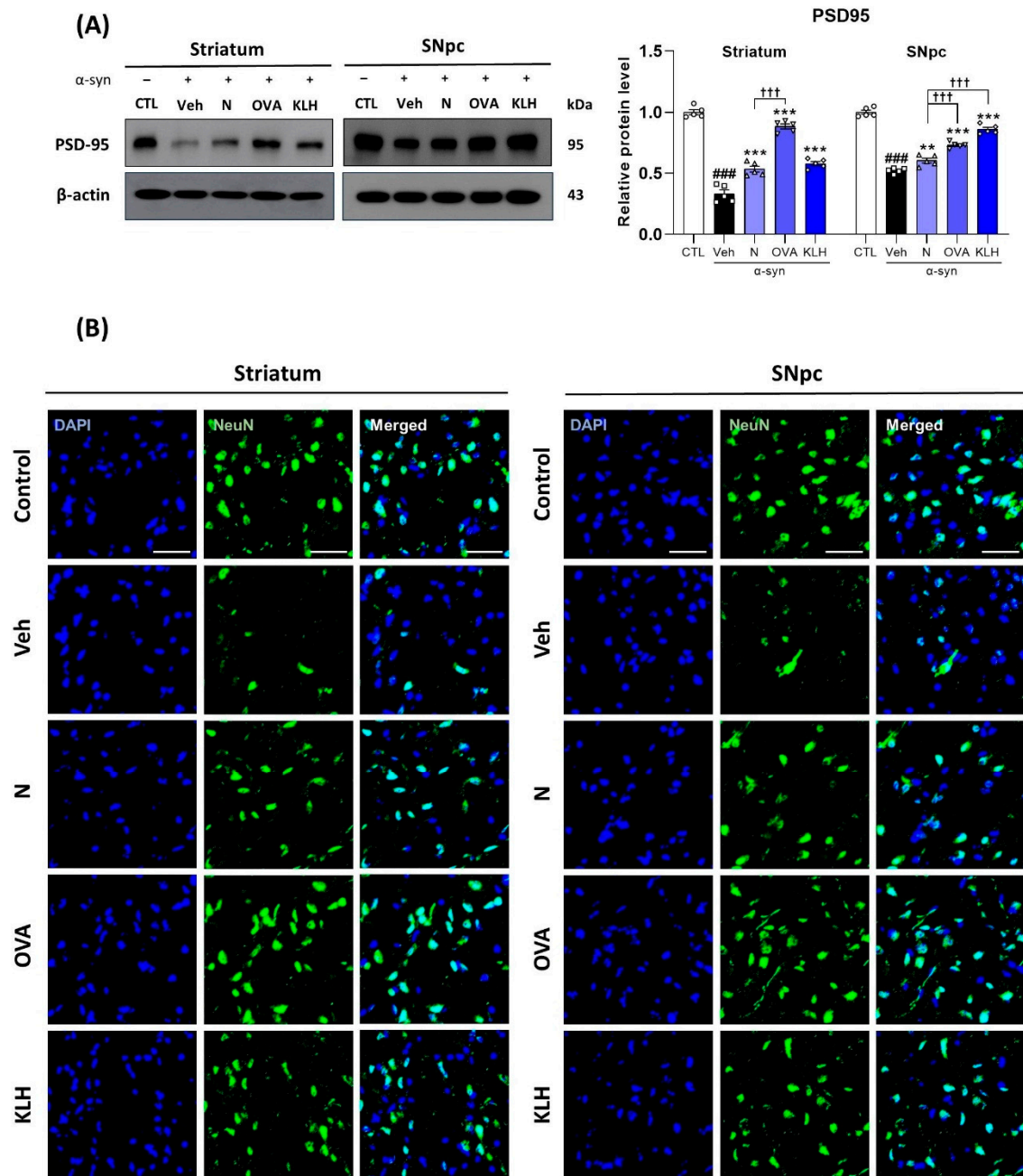


**Supplementary Figure S3. Immunofluorescence double staining of  $\alpha$ -synuclein ( $\alpha$ -syn) and tyrosine hydroxylase (TH) in the striatum and the substantia nigra pars compacta (SNpc). Blue represent DAPI, green represent  $\alpha$ -syn, and red represent TH. Scale bar represent 50  $\mu$ m and zoom is 20  $\mu$ m.**

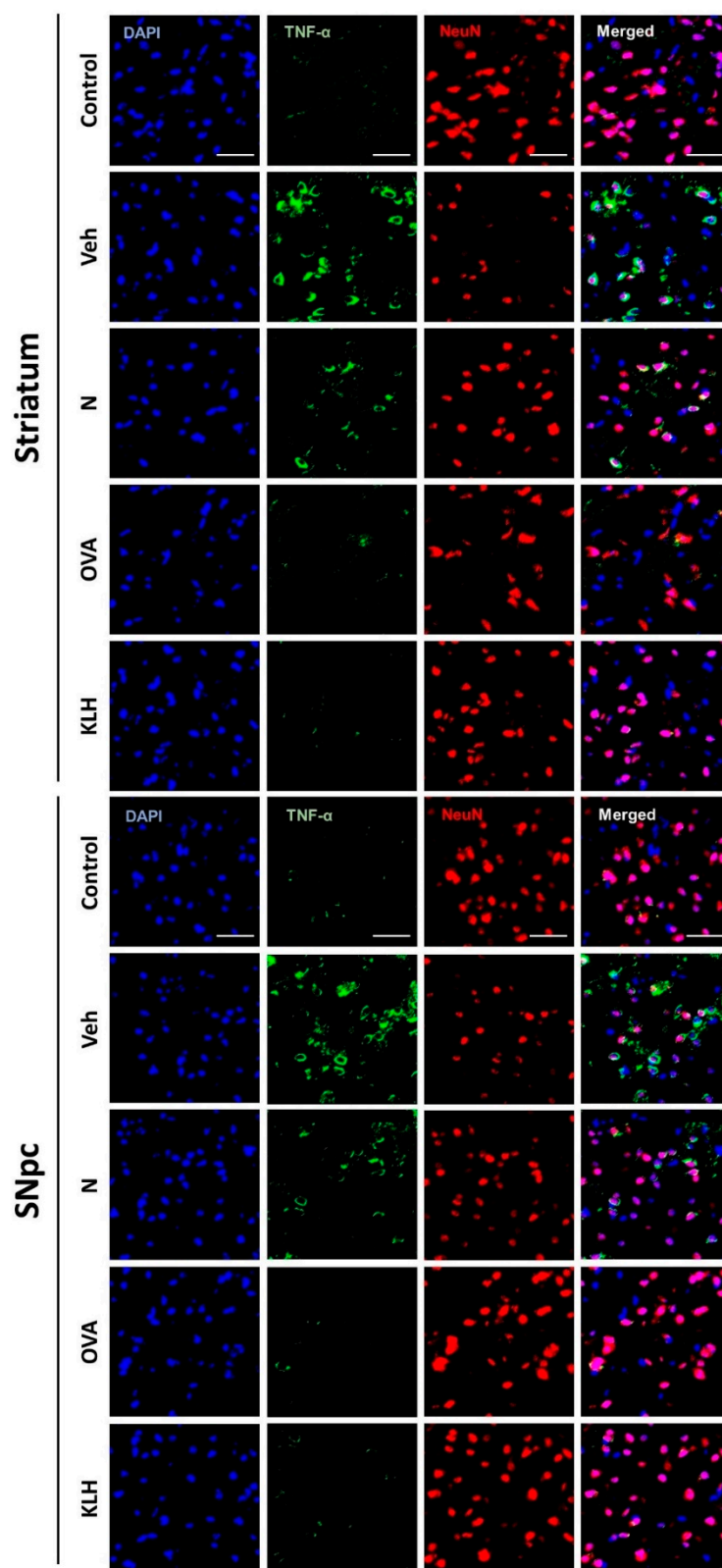


**Supplementary Figure S4. Immunofluorescence double staining of  $\alpha$ -synuclein ( $\alpha$ -syn) and tyrosine hydroxylase (TH) in the ventral tegmental area (VTA).** Blue represent DAPI, green represent  $\alpha$ -syn, and red represent TH. Scale bar represent 50 $\mu$ m.





**Supplementary Figure S5. Peptide-based epitope vaccines increased neurons in the striatum and substantia nigra pars compacta (SNpc).** (A) Western blot analysis showing increased expression of postsynaptic density protein 95 (PSD-95) in the striatum and SNpc (n = 5 per group). (B) Immunofluorescence staining of neuronal nuclei (NeuN) stained with DAPI in the striatum and SNpc. Scale bar represent 20 $\mu$ m. Comparisons: #control (CTL) with saline-treated (Veh)  $\alpha$ -syn-induced PD model; \*Veh group with epitope treated group [non-carrier protein (N) and carrier-protein (OVA and KLH)]; †Non-carrier protein (N) with carrier protein (OVA and KLH). Data are presented as mean  $\pm$  SEM. ###\*\*/†† p  $\leq$  0.01 and ####\*\*\*/††† p  $\leq$  0.001.



**Supplementary Figure S6. Immunofluorescence double staining of tumor necrosis factor alpha (TNFα) and neuronal nuclei (NeuN) in the striatum and substantia nigra pars compacta (SNpc).** Blue represent DAPI, green represent TNF-α and red represent NeuN. Scale bar represent 20μm.