# *Supplementary Material*

**Supplementary Material S1** | Nucleotide sequences of the receptor binding domain (RBD, aminoacids 319-541) and the full-length stabilized version of the Spike protein (GeneBank: MN908947.3). Restriction enzymes used for the plasmid construction are marked in bold.

Target sequence: RBD

**CCATGG**GTAGAGTGCAACCTACAGAATCAATTGTGAGGTTTCCAAATATAACTAACTTG
TGCCCATTCGGAGAAGTGTTCAACGCTACAAGGTTCGCATCAGTGTACGCATGGAATAG
AAAAAGAATATCAAATTGCGTTGCTGATTACTCTGTGCTTTATAATTCAGCATCTTTCTC
AACTTTTAAATGTTATGGAGTTTCACCAACAAAATTGAATGATCTTTGTTTTACTAATGT
TTACGCAGACTCTTTCGTGATTAGGGGAGACGAGGTGAGGCAGATAGCTCCCGGACAGA
CTGGAAAGATTGCAGATTACAATTATAAGCTTCCAGATGACTTCACTGGATGCGTGATT
GCTTGGAATTCTAATAATTTGGACTCTAAAGTTGGTGGAAACTATAATTACCTTTATAGA
TTGTTTAGAAAGTCTAATTTGAAACCATTCGAAAGGGATATATCTACAGAAATTTACCA
AGCTGGATCTACACCTTGCAACGGAGTGGAGGGTTTCAACTGCTATTTCCCTCTTCAATC
TTATGGATTTCAACCTACTAATGGAGTTGGATACCAACCATATAGAGTGGTGGTGTTGTC
TTTCGAGTTGTTGCACGCTCCAGCTACAGTGTGTGGACCTAAAAAGTCTACTAACCTTGT
TAAGAATAAATGCGTTAACTTT**GTCGAC**

Target sequence: Spike

**CCATGG**GTCAGTGTGTGAATCTTACAACTAGGACACAGCTTCCACCAGCTTACACAAAC
TCATTCACTAGAGGTGTGTACTATCCAGATAAGGTGTTTAGATCTTCTGTTTTGCATTCA
ACTCAAGACTTGTTTCTTCCATTTTTCTCTAATGTTACTTGGTTTCATGCTATTCATGTGTC
TGGTACAAATGGTACTAAAAGATTCGACAATCCAGTGTTGCCTTTCAACGACGGTGTGT
ATTTCGCTTCTACAGAAAAATCAAACATTATAAGGGGTTGGATTTTTGGTACTACTCTTG
ATTCTAAAACACAGTCATTGTTGATTGTTAATAACGCTACAAACGTGGTTATTAAGGTTT
GTGAGTTTCAGTTTTGTAATGATCCTTTTTTGGGAGTTTACTACCATAAAAATAATAAGT
CTTGGATGGAATCTGAATTTAGAGTGTACTCTTCTGCAAACAATTGTACTTTTGAATATG
TTTCTCAGCCATTCCTTATGGACCTTGAGGGTAAGCAAGGAAACTTTAAGAACTTGAGG
GAATTTGTTTTTAAAAATATTGATGGATATTTCAAAATATACTCAAAGCATACACCAATA
AACCTTGTGAGGGACCTTCCACAAGGATTTTCTGCACTTGAGCCTCTTGTGGATTTGCCT
ATTGGAATTAATATTACAAGGTTCCAGACTTTGTTGGCTCTTCATAGATCTTACCTTACTC
CCGGAGACTCTTCATCTGGTTGGACAGCTGGAGCAGCTGCTTACTACGTTGGATACTTGC
AGCCAAGAACTTTCCTTTTGAAGTACAATGAGAATGGAACTATTACTGATGCTGTGGAC
TGCGCTCTTGACCCTCTTTCTGAGACTAAGTGCACTCTTAAATCTTTCACTGTTGAGAAA
GGTATATATCAGACATCTAACTTCAGAGTGCAACCTACAGAATCAATTGTGAGGTTTCC
AAATATAACTAACTTGTGCCCATTCGGAGAAGTGTTCAACGCTACAAGGTTCGCATCAG
TGTACGCATGGAATAGAAAAAGAATATCAAATTGCGTTGCTGATTACTCTGTGCTTTATA

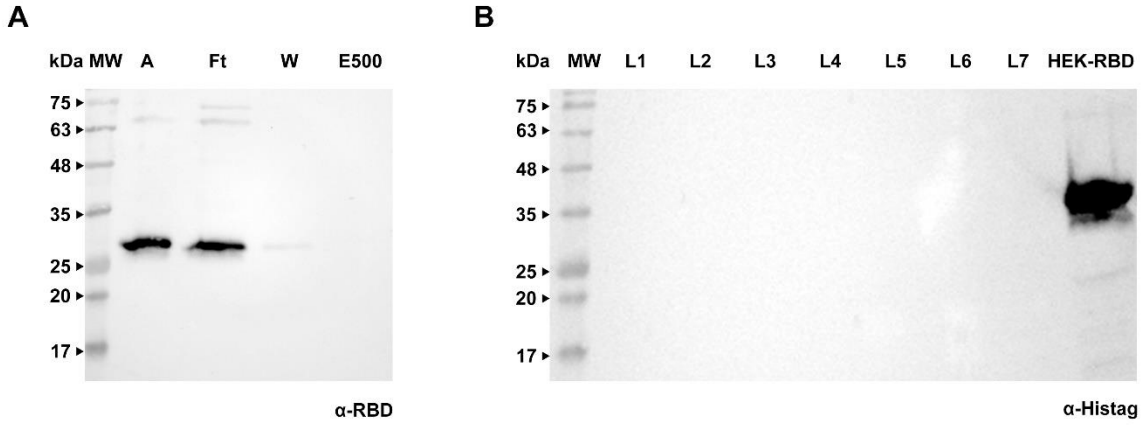
ATTCAGCATCTTTCTCAACTTTTAAATGTTATGGAGTTTCACCAACAAAATTGAATGATC
TTTGTTTTACTAATGTTTACGCAGACTCTTTCGTGATTAGGGGAGACGAGGTGAGGCAGA
TAGCTCCCGGACAGACTGGAAAGATTGCAGATTACAATTATAAGCTTCCAGATGACTTC
ACTGGATGCGTGATTGCTTGGAATTCTAATAATTTGGACTCTAAAGTTGGTGGAAACTAT
AATTACCTTTATAGATTGTTTAGAAAGTCTAATTTGAAACCATTCGAAAGGGATATATCT
ACAGAAATTTACCAAGCTGGATCTACACCTTGCAACGGAGTGGAGGGTTTCAACTGCTA
TTTCCCTCTTCAATCTTATGGATTTCAACCTACTAATGGAGTTGGATACCAACCATATAG
AGTGGTGGTGTTGTCTTTCGAGTTGTTGCACGCTCCAGCTACAGTGTGTGGACCTAAAAA
GTCTACTAACCTTGTTAAGAATAAATGCGTTAACTTTAATTTTAATGGTCTTACTGGAAC
TGGTGTTTTGACTGAATCAAACAAAAAGTTCCTTCCTTTTCAGCAGTTCGGAAGGGACAT
AGCAGACACTACTGACGCAGTGAGAGACCCACAAACTCTTGAGATTTTGGATATAACTC
CTTGTTCATTCGGTGGAGTTTCTGTTATTACACCCGGAACAAACACTTCTAACCAAGTTG
CTGTGCTTTACCAAGATGTGAACTGTACAGAGGTGCCAGTGGCTATTCACGCTGACCAG
TTGACACCAACTTGGAGAGTTTATTCTACTGGATCAAACGTGTTCCAGACTAGGGCTGG
ATGCCTTATTGGTGCAGAACATGTGAATAATTCTTATGAATGTGATATTCCAATTGGTGC
TGGAATATGCGCTTCATACCAGACACAGACAAACTCTCCAGCATCAGTGGCATCACAGT
CTATTATTGCTTACACAATGTCTTTGGGAGCAGAGAACTCTGTGGCTTACTCTAACAATT
CTATTGCTATACCAACTAACTTCACTATTTCTGTGACAACTGAAATATTGCCAGTGTCAA
TGACTAAGACTTCAGTTGATTGCACTATGTACATTTGTGGAGATTCAACAGAGTGTTCTA
ACTTGTTGCTTCAATACGGATCATTTTGTACTCAACTTAACAGAGCTCTTACTGGTATTG
CTGTGGAGCAAGATAAGAACACTCAAGAAGTTTTCGCACAAGTTAAGCAAATTTATAAG
ACACCTCCAATTAAGGATTTTGGTGGATTCAACTTCTCTCAGATACTTCCAGACCCTTCA
AAACCTTCTAAAAGATCATTCATTGAAGATTTGTTGTTCAATAAAGTGACACTTGCAGAC
GCTGGATTCATAAAGCAGTACGGTGACTGTTTGGGTGACATAGCTGCAAGGGACTTGAT
ATGCGCACAGAAATTCAACGGTTTGACAGTGTTGCCTCCACTTTTGACTGACGAGATGAT
TGCACAATATACTTCTGCACTTTTGGCTGGTACAATTACATCTGGATGGACATTCGGTGC
TGGAGCAGCTCTTCAAATTCCTTTCGCAATGCAAATGGCTTATAGGTTTAATGGTATTGG
TGTGACTCAAAATGTGCTTTATGAGAATCAAAAACTTATTGCAAACCAATTTAATTCTGC
AATTGGTAAAATACAAGATTCTTTGTCATCAACAGCATCTGCTCTTGGAAAGTTGCAAG
ATGTGGTGAACCAGAACGCACAAGCTTTGAACACTCTTGTGAAGCAACTTTCATCAAAC
TTTGGAGCAATATCATCTGTTCTTAACGATATTCTTTCTAGGCTTGACCCTCCAGAAGCA
GAAGTTCAGATTGATAGATTGATTACTGGAAGATTGCAATCACTTCAAACTTATGTTACT
CAGCAGCTTATTAGGGCTGCAGAGATAAGGGCATCTGCTAACCTTGCAGCTACTAAGAT
GTCTGAGTGCGTGCTTGGTCAATCTAAAAGGGTGGATTTCTGCGGTAAGGGATATCACC
TTATGTCTTTCCCTCAGTCAGCTCCTCACGGTGTGGTGTTCTTGCACGTGACTTACGTGCC
AGCTCAAGAAAAGAACTTCACTACTGCACCAGCTATTTGCCATGACGGTAAGGCACACT
TCCCAAGAGAGGGTGTGTTCGTGTCAAACGGAACACACTGGTTCGTGACTCAAAGAAAT
TTCTATGAGCCACAAATTATTACTACTGACAATACTTTTGTTTCTGGAAATTGCGACGTT
GTGATTGGTATTGTTAACAACACTGTTTACGACCCATTGCAGCCAGAATTGGATTCTTTT
AAGGAAGAATTGGATAAATACTTCAAGAATCACACTTCTCCAGATGTTGATTTGGGTGA
TATTTCTGGAATAAATGCATCAGTGGTTAACATTCAAAAGGAGATAGATAGGCTTAATG
AAGTGGCAAAGAATCTTAATGAATCTCTTATTGATCTTCAAGAGCTTGGTAAATACGAA
CAATATATAAAGTGGCCATCTGGTAGGTTGGTGCCTAGGGGATCTCCCGGATCTGGATA
CATTCCAGAAGCACCTAGGGACGGACAAGCTTACGTTAGGAAGGACGGAGAGTGGGTT
CTTCTTTCAACATTCTTGGGA**GTCGAC**

**Supplementary Material S2 |** List of N-glycan composition screened in Spike protein MS analysis. The database is composed of 69 types of glycans.

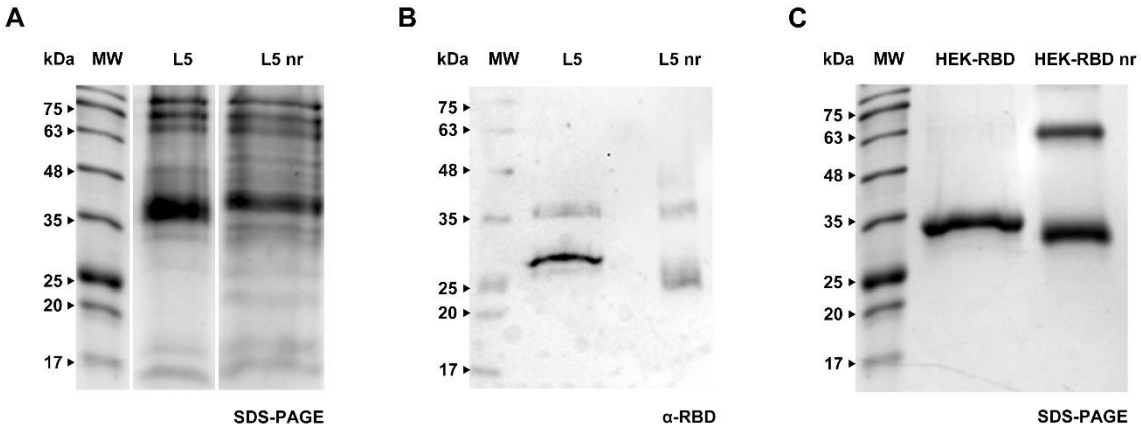
*Note: For glycosylation comparison between human and plant recombinant Spike, the numeric order of the 109 glycans identified by Castro et al. (2021) was maintained, and the 6 glycans identified in plants were added from position No. 110 to No. 115. For the screening, all glycans containing sialylation were removed.*

| Nº | COMPOSITION | Nº | COMPOSITION | Nº | COMPOSITION |
|---|---|---|---|---|---|
| **1** | HexNAc(2)Hex(9) | **31** | HexNAc(4)Hex(4)Fuc(2) | **80** | HexNAc(6)Hex(3)Fuc(2) |
| **2** | HexNAc(2)Hex(8) | **33** | HexNAc(4)Hex(5) | **81** | HexNAc(6)Hex(3)Fuc(3) |
| **3** | HexNAc(2)Hex(7) | **34** | HexNAc(4)Hex(5)Fuc(1) | **82** | HexNAc(6)Hex(4) |
| **4** | HexNAc(2)Hex(6) | **37** | HexNAc(4)Hex(5)Fuc(2) | **83** | HexNAc(6)Hex(4)Fuc(1) |
| **5** | HexNAc(2)Hex(5) | **39** | HexNAc(4)Hex(5)Fuc(3) | **84** | HexNAc(6)Hex(4)Fuc(2) |
| **6** | HexNAc(2)Hex(4) | **43** | HexNAc(4)Hex(6) | **86** | HexNAc(6)Hex(5) |
| **7** | HexNAc(2)Hex(4)Fuc(1) | **44** | HexNAc(4)Hex(6)Fuc(1) | **87** | HexNAc(6)Hex(5)Fuc(1) |
| **8** | HexNAc(3)Hex(5) | **46** | HexNAc(4)Hex(6)Fuc(2) | **91** | HexNAc(6)Hex(5)Fuc(2) |
| **9** | HexNAc(3)Hex(5)Fuc(1) | **47** | HexNAc(4)Hex(6)Fuc(3) | **92** | HexNAc(6)Hex(6) |
| **11** | HexNAc(3)Hex(6) | **49** | HexNAc(5)Hex(3) | **93** | HexNAc(6)Hex(6)Fuc(1) |
| **12** | HexNAc(3)Hex(6)Fuc(1) | **50** | HexNAc(5)Hex(3)Fuc(1) | **96** | HexNAc(6)Hex(6)Fuc(2) |
| **15** | HexNAc(2)Hex(3) | **52** | HexNAc(5)Hex(4) | **97** | HexNAc(6)Hex(6)Fuc(3) |
| **16** | HexNAc(2)Hex(3)Fuc(1) | **53** | HexNAc(5)Hex(4)Fuc(1) | **98** | HexNAc(6)Hex(7) |
| **17** | HexNAc(3)Hex(3) | **56** | HexNAc(5)Hex(4)Fuc(2) | **99** | HexNAc(6)Hex(7)Fuc(1) |
| **18** | HexNAc(3)Hex(3)Fuc(1) | **60** | HexNAc(5)Hex(5) | **107** | HexNAc(7)Hex(3)Fuc(1) |
| **19** | HexNAc(3)Hex(4) | **61** | HexNAc(5)Hex(5)Fuc(1) | **108** | HexNAc(7)Hex(4) |
| **20** | HexNAc(3)Hex(4)Fuc(1) | **64** | HexNAc(5)Hex(5)Fuc(2) | **109** | HexNAc(7)Hex(4)Fuc(1) |
| **23** | HexNAc(4)Hex(3) | **65** | HexNAc(5)Hex(5)Fuc(3) | **110** | HexNAc(2)Hex(3)Xyl(1) |
| **24** | HexNAc(4)Hex(3)Fuc(1) | **67** | HexNAc(5)Hex(6) | **111** | HexNAc(2)Hex(3)Fuc(1)Xyl(1) |
| **25** | HexNAc(4)Hex(3)Fuc(2) | **68** | HexNAc(5)Hex(6)Fuc(1) | **112** | HexNAc(3)Hex(3)Xyl(1) |
| **26** | HexNAc(4)Hex(3)Fuc(3) | **72** | HexNAc(5)Hex(6)Fuc(2) | **113** | HexNAc(3)Hex(3)Fuc(1)Xyl(1) |
| **28** | HexNAc(4)Hex(4) | **77** | HexNAc(6)Hex(3) | **114** | HexNAc(4)Hex(3)Fuc(1)Xyl(1) |
| **29** | HexNAc(4)Hex(4)Fuc(1) | **78** | HexNAc(6)Hex(3)Fuc(1) | **115** | HexNAc(4)Hex(3)Xyl(1) |

**Supplementary Material S3 |** Purification of RBD protein by immobilized metal affinity using a HisTrap™ High Performance column. **(A)** WB analysis of the eluted fractions of RBD protein with anti-RBD antibody, A - sample, Ft - flow through, W - wash, E500 - eluted fraction. **(B)** WB analysis of spent media from seven independent BY-2 lines (L1 to L7, samples corresponding to Figure 3E) with anti-His tag antibody.




**Supplementary Material S4 |** Comparison of RBD protein produced in BY-2 and HEK cells under non-reducing conditions. **(A)** Stained gel containing the spent medium of BY-2 cell line 5, L5- reducing conditions, L5 nr- non-reducing conditions. **(B)** Corresponding WB with anti-RBD antibody. **(C)** Stained gel containing the purified RBD from HEK cells, HEK-RBD- reducing conditions, HEK-RBD nr- non-reducing conditions.



**Supplementary Material S5 |** Detection of Spike native conformation in BY-2 spent medium. Samples for two independent lines (L3 and L4, same samples as in Figure 7B) were loaded into a 5 % polyacrylamide native gel and subjected to WB detection with anti-Spike antibody.