

Supplementary Material

Highly sensitive detection of Zika virus non-structural protein 1 in serum samples by a two-site nanobody ELISA

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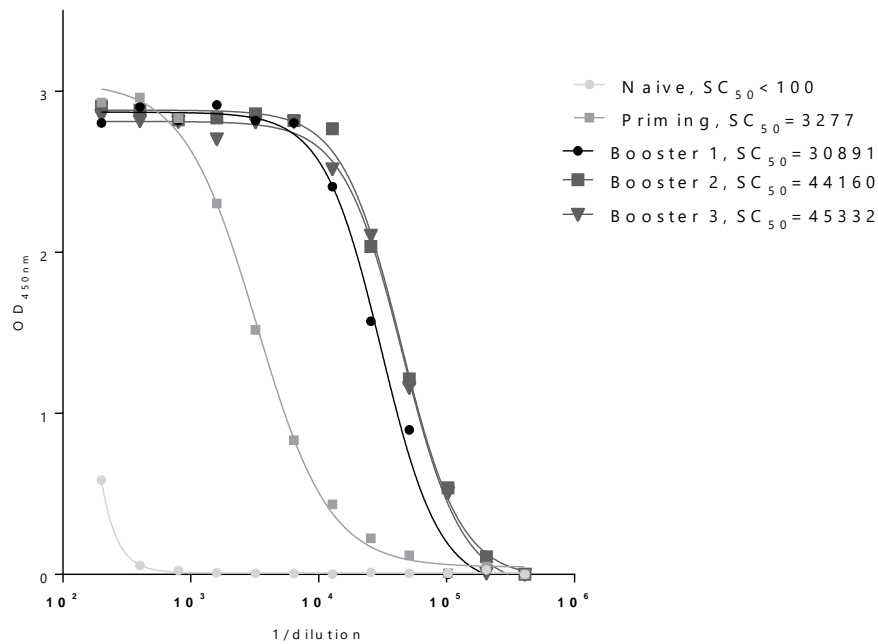


Figure S1. Serum titration against ZVNS1 along the immunization process. Serum samples were analyzed by ELISA on plates coated with ZVNS1. The titer, calculated as the concentration causing a 50% decrease in the maximum signal (SC_{50}) is shown in the insert and was used as an indicator of the progression of the llama immunity response.

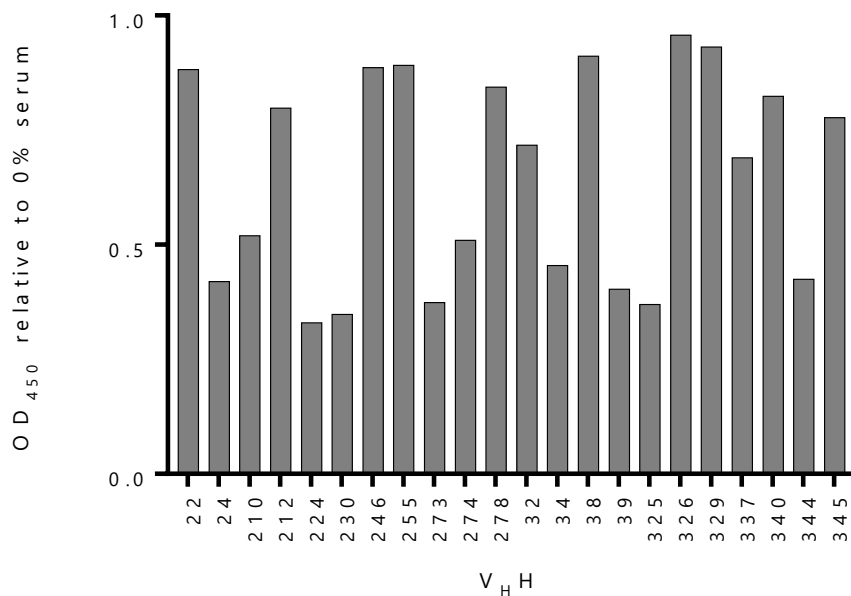


Figure S2- Performance of 22 Nb clones in the presence of human serum. The culture supernatant of each clone was assayed on wells coated with 100 ng/well of ZVNS1 in the presence or absence of 50% human serum. Bars represent the average of two measurements.

	-----FR1-----	CDR1	-----FR2-----	CDR2	-----FR3-----	CDR3	-----FR4-----
1 22	EVQLVDSGGGLVQTGGSLRLSCAAS	GTIFSTKA	MGWYRQAPGKRRREFVAL	IAPGGDI----	TYADSAEGRFTISRDSAKGTV--WLQMNLDKAEIDTAVYYC	NTVPRQD-----	WGQGTQVTVSS
51 278	QVQLVDSGGGLVQPGGSLRLSCAAS	GNIFSTKA	MGWYRQAPGKRRREFVAL	IDPAGST----	TYADSVGEGRFTISRDSAKGTV--WLQMNLDKAEIDTAVYYC	NTVPRQD-----	WGQGTQVTVSS
50 274	EVQLVDSGGGLVQTGGSLRLSCAAF	GSIFSRA	VGWYRQAPGKQREFVAL	IAPAGDT----	TYADSVGEGRFTISRDSATNTV--WLQMNLEPEDTAAYYC	NTIPRVKD-----	WGQGTQVTVSS
17 337	EVQLVDSGGGLVQTGGSLRLSCVTS	GVIFSRA	VGWYRQAPGKRRREFVAL	IDPAGTT----	TYADAVEGRFTISRDSAKNTV--WLQMNLDKAEIDTSVYYC	NTVPRKD-----	WGQGTQVTVSS (2)
68 326	QVQLVDSGGGLVQAGGSLTLCSDS	GSIFRHS	MGWYRQVPGKQREFVAL	ITKGGT-----	TYEDTVKGRFTISMSARNSTV--YLQMNLSLKPADTAVYYC	NAKWGYNNSDY-----	WGQGTQVTVSS
55 34	QVQLVDSGGGLVQAGGSLTLCSDS	GSILRFNS	MGWYRQAPGKQREFVAL	ITKGGSA-----	TYSDVMGRFTITMNSARNSTV--WMQMNLSLKPADTAIYYC	NAKWGYNNSDY-----	WGQGTQVTVSS
58 39	EVQLVDSGGGLVQAGGSLRLSCGRS	GSFSLNS	MGWYRQAPGKRRFIAS	ITIGGGTT----	RYADSVKGRFTISRDNKNTV--YLQMNLSLKPEDTAVYYC	NADAIVNNRRMQY----	WGQGTQVTVSS
3 210	QVQLVDSGGGLVQAGGSLRLSCAAS	GRFFSRYA	LGWYRQAPGKQREFVAL	ITNGDIT-----	DYANSVKGRFTISRDNKNTG--YLQMNLSLKPEDTAVYYC	NLGFGGTGGSSNF----	WGQGTQVTVSS (2)
43 255	QVQLVDSGGGLVQPGGSLRLSCAVS	GTFSSITA	MGWYRQAPGQREFVAL	FTSGGRT-----	NYVDSVKGRFTISRDNARNTVDLYLQMNLSLKPEDTAVYYC	NVEGLWNNRRERA----	WGQGTQVTVSS
72 340	QVQLVDSGGGLVQPGGSLRLSCAVS	GTFSSITS	MGWYRQAPGKQREFVAL	FTSGGRT-----	YVDSVKGRFTISRDNARNTVDLYLQMNLSLKPEDTAVYYC	NVEGLWNNRRERA----	WGQGTQVTVSS
67 325	EVQLVDSGGGLVQAGGSLRLSCAAS	GSIFSRGNAMASRQAPGKQREFVAL	ITSDDGA-----	YVDSVKGRFTISRDNKNTV--FLQMNLSLKPEDTAVYYC	NTLPRWA-----	WGQGTQVTVSS	
4 212	QVQLVDSGGGLVQAGGSLRLSCAAS	GNIFSSNAVGVRRAPGKQREFVAL	ITSGDST-----	HYADSVGEGRFTISGNAKNTV--YLQMDLSLKPEDTAVYYC	TTVPRGD-----	WGQGTQVTVSS	
64 32	EVQLVDSGGGLVQAGGSLRLSCAVS	GIDFSRYAITWNRQSPGNRRREFVAL	LPPADTT-----	VYADSVKGRFTISRDNKNTV--YLQMNLSLKPEDTAVYYC	ATSPRIHN-----	WGQGTQVTVSS	
26 230	QVQLVDSGGGLVQAGGSLRLSCAAT	RRVYDTKV	MGWYRQAPGKQREFVAL	FLIINGRVQRP	NYAAPVNGRFTISRDSAKDTV--DLQMNLSLKPEDTAVYYC	SMKLGYPVSEEY----	WGQGTQVTVSS
34 246	EVQLVDSGGGLVQAGGSLRLSCAGS	ARLSSSIKA	MGWYRQAPGKQREFVAL	VTGGST-----	IYADSVGEGRFTISRDNKNTV--YLQMNLSLKPEDTAVYYC	NEMPRIMP-----	WGQGTQVTVSS (3)
32 344	QVQLVDSGGGLVQPGGSLRLSCAAS	EFTFNITYF	MYWARQAPGKGLEWVST	ITPGGERT---	VYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	ARGLAAGIANSAAARY	RQGGTQVTVSS
49 273	QVQLVDSGGGLVQPGGSLRLSCAAS	GTFPSDAA	MTWARQAPGKGLEWVST	IRSDGS-T---	IYAESVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	RRPMPGPDGRDY----	RQGGTQVTVSS
6 224	QVQLVDSGGGLVQPGGSLRLSCAAS	GTFFAHYA	MTWARQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	SIGRPDWTR-----	RQGGTQVTVSS
2 24	QVQLVDSGGGLVQPGGSLRLSCAAS	GTFFAHYA	MTWARQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	GIGRPDWTR-----	RQGGTQVTVSS
57 38	QVQLVDSGGGLVQPGGSLRLSCAAS	GTFFAHYA	MSWYRQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	QIGRPVTR-----	RQGGTQVTVSS (4)
69 329	EVQLVDSGGGLVQPGGSLRLSCAAS	GTFPSLYA	MNWYRQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	ARGRPSSSQP-----	RGRGTQVTVSS (5)
33 345	EVQLVDSGGGLVQPGGSLRLSCAAS	GFAFSGYA	MTWARQAPGKGLEWVST	INSDGSDI---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	TIGRPTPNWTR-----	RQGGTQVTVSS (2)

Figure S3. Capture Nbs to ZVNS1 sequence alignment. The regions corresponding to the frameworks (FR) and the three CDRs are denoted on top. Numbers between brackets indicate the number of clones that have that sequence. Gaps are shown as dashes. The top and bottom sequences derived from VHH and VH genes, respectively. The hallmark residues of VHH are shown in bold and the GLEW motif and the Arg residue that substitus for W in FR4 of VH are also in bold. Sequence clone numbers (plain) and sequence names (bold) are shown on the left.

	-----FR1-----	CDR1	-----FR2-----	CDR2	-----FR3-----	CDR3	-----FR4-----
12 A12	EVQLVDSGGGLVQAGGSLRLSCGAS	GSFSSINS	MGWYRQAPGKRRREFVAL	TTLGGGGA---	KYADSVKDRFTISRDLAKMTV--FLQMSLSLKPEDTAVYYC	NADGI FNNRRMQY	WGQGTQVTVSS
31 C7	QVKLLESGGGLVQAGGSLRLSCGAS	GSFSSINS	MGWYRQAPGKRRREFVAL	TTLGGGTA---	NYAESVKDRFTISRDLVMTL--FLQMNLSLKPEDTAVYYC	NADAI FNNRRMQY	WGQGTQVTVSS (2)
20 B8	QVQLVDSGGGLVQPGGSLRLSCAAS	GRFFSRYA	LGWYRQAPGKQREFVAL	ITNGDIT---	DYANSVKGRFTISRDNKNTG--YLQMNLSLKPEDTAVYYC	NLGFGGTGGSSNF	WGQGTQVTVSS (2)
67 H3	EVQLVDSGGGLVQAGGSLRLSCAAS	GAIARVNT	MAWYRQAPGKQREFVAL	WTSTYSEGV	EYADSVKGRFTISRDSAKNT--YLQMNLSLKPEDTAVYYC	NLEQHGQLRGVY-	WGQGTQVTVSS
59 E10	QVQLVDSGGGLVQAGGSLRLSCGAS	ASVSSNKG	MQWSRQAPGKQREFVAL	VTGGST---	IYADSVGEGRFTISRDNKNTV--DLQMNLSLKPEDTAVYYC	NTMPTTMP-----	WGQGTQVTVSS
66 F6	EVQLVDSGGGLVQPGGSLRLSCAAS	GTFPSLYA	MNWRQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	ARGRPSSSQP---	RGRGTQVTVSS (3)
48 E1	QVQLVDSGGGLVQPGGSLRLSCAAS	GTFFAHYA	MSWYRQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	QIGRPVTR-----	RQGGTQVTVSS (2)
7 A7	EVQLVDSGGGLVQPGGSLRLSCAAS	GTFFAHYA	MTWARQAPGKGLEWVST	INSDGSDT---	AYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	SIGRPDWTR-----	RQGGTQVTVSS
42 D6	EVQLVDSGGGLVQPGGSLRLSCAAS	GFAFSGYA	MRWYRQAPGKGLEWVST	INSDGSDT---	WYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	AIGRTHDT-----	KGQGTQVTVSS
45 D9	EVQLVDSGGGLVQPGGSLRLSCAAS	GFAFSGYA	MRWYRQAPGKGLEWVST	INSDGSDT---	WYADSVKGRFTISRDNKNTL--YLQMNLSLKPEDTAVYYC	AQGRTHDT-----	KGQGTQVTVSS (2)

Figure S4. Detection Nbs to ZVNS1 sequence alignment. The regions corresponding to the frameworks (FR) and the three CDRs are denoted on top. Numbers between brackets indicate the number of clones that have that sequence. Gaps are shown as dashes. The top and bottom sequences derived from VHH and VH genes, respectively. The hallmark residues of VHH are shown in bold and the GLEW motif and the Arg residue that substitus for W in FR4 of VH are also in bold. Sequence clone numbers (plain) and sequence names (bold) are shown on the left.

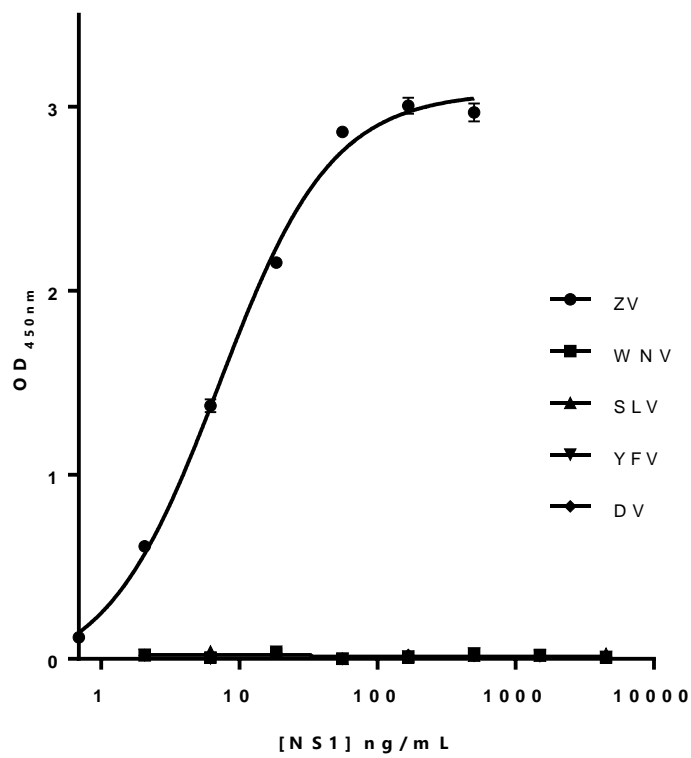


Figure S5. Analysis of cross-reactivity of the 32/D6 nanobody pair with non-Zika NS1. Each NS1 was individually tested in the 0.3-4500 ng/mL range. The results are the average values of triplicate measurements and the error bars represent the standard deviation.