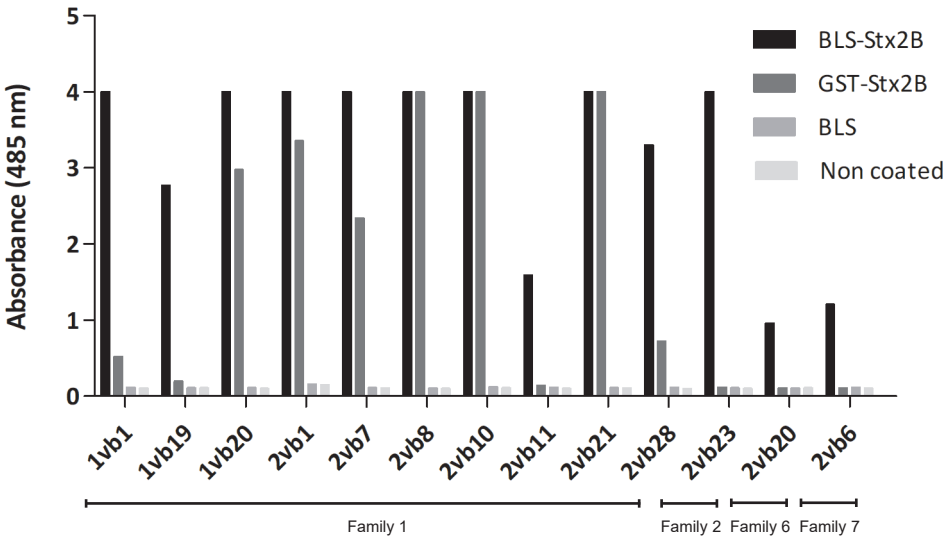

Figure S1. Analysis of the thirteen anti-Stx2B VHHs selected for the development of the capture ELISA. VHHs were evaluated by direct ELISA against the recombinant proteins BLS-Stx2B and GST-Stx2B. Wells coated with BLS, GST or non-coated were used as controls. Reactivity values obtained coating the microplate wells with GST were similar to those obtained with BLS (data not shown). The family to which each VHH belongs is indicated.

A

B

		FR1	CDR1	FR2	CDR2
Family 1	2vb27	QVQLQESGGGLVQPGGSLKLACAAS	GITFRNKAIG	WYRQAPGKGRELVA	RIDSFDTTDYADSVKG
	1vb20	..............A....R.S....	.....R....	........Q.....	....D.I.........
	1vb1	..........I.......R.S.V..	..LLDR..MA	........A.DR..	T..AS...N.P.....
	2vb8	..............A....R.S....	.....R....	........Q.....	....D.I.........
	2vb19	..................R.S....	..L..S..V.	.......TQ..W..	Y.TGD.L.S.E.F...
	2vb1	..................R.S.V..	.FS..R....	........Q.....	....D.I.N.......
	2vb7	..............A....R.S....	..I.......	........Q.....	....A.I.N.P.....
	2vb10	..............A....R.S....	..I..G....	........Q.....	...AG.I.N.......
	2vb28	..............A....R.S....	.....R..V.	........Q.....	....D.I.N.......
	2vb21	..............A....R.S....	...L...SV.	.......RQ....G	Y.SGD.I.N.V....A
	2vb11	..................R.S....	..I.KS..V.	.......TQ..W..	Y.TGD.S.S.Q.F...
Family 2	2vb23	QVQLQESGGGLVQAGGSLRLSCTAS	GSIFNTATMA	WSRQAPGKQRELVA	SITQGRITYPVDSVKG
Family 6	2vb20	QVQLQESGGGLVQPGGSLRLSCAAS	GFTIDYYAIA	WFRQAPGEEREWVS	CIRSGDGSTWYVDSVKG
Family 7	2vb6	QVQLQESGGGLVQPGGSLRLSCAAS	GIIFRSKSVG	WYRQAPGTQREWVA	YISGDDSTNYEDFVKG

		FR3	CDR3	FR4
Family 1	2vb27	RFSISRDNAKNTVYLQMNSIKSEDTAVYYC	NLRGSNY	WGQGTQVTVSS
	1vb20	....	....	....
	1vb1	..T.....Q..L.........P....D.R.	..L.T.I	....
	2vb8	...........M..................	....	....
	2vb19	..T.........M.........P........	....	....
	2vb1	.............F................	....	....
	2vb7	....	....T..	....
	2vb10	.......H.....................	....T..	....
	2vb28	.......H.....................	....T..	....
	2vb21	....	....A..	....
	2vb11	..T..............N..P........	....	....
Family 2	2vb23	RFTLSRDNSKNTVYLQMNSLEPEDTAVYYC	GVDTIPTSRPRY	WGQGTQVTVSS
Family 6	2vb20	RFSISSDNAKNAVYLQISSLKPEDTAVYYC	AASRGSPYCPAVIDYDY	WGQGTQVTVSS
Family 7	2vb6	RFTISRDNAKNTVYLQMNSVKPEDTAIYYC	AADYRDYDELLLPVPPPYDY	WGQGTQVTVSS

Figure S2. Genetic organization and sequence analysis of VHHs. (A) Genetic organization of VHHs. The four conserved framework regions (FR) are indicated in grey. Hypervariable complementary determining regions (CDR) are indicated in different colors. FRs maintain the tertiary structure of the paratope while the CDRs form the hypervariable loops that directly interact with the antigen epitope. (B) Amino acid sequence alignment of selected VHHs recognizing Stx2. Amino acid sequence analysis revealed that selected VHHs belong to four different families. Members of Family 1 were aligned considering the sequence of 2vb27 VHH as the reference sequence. The points indicate identical amino acid residues.

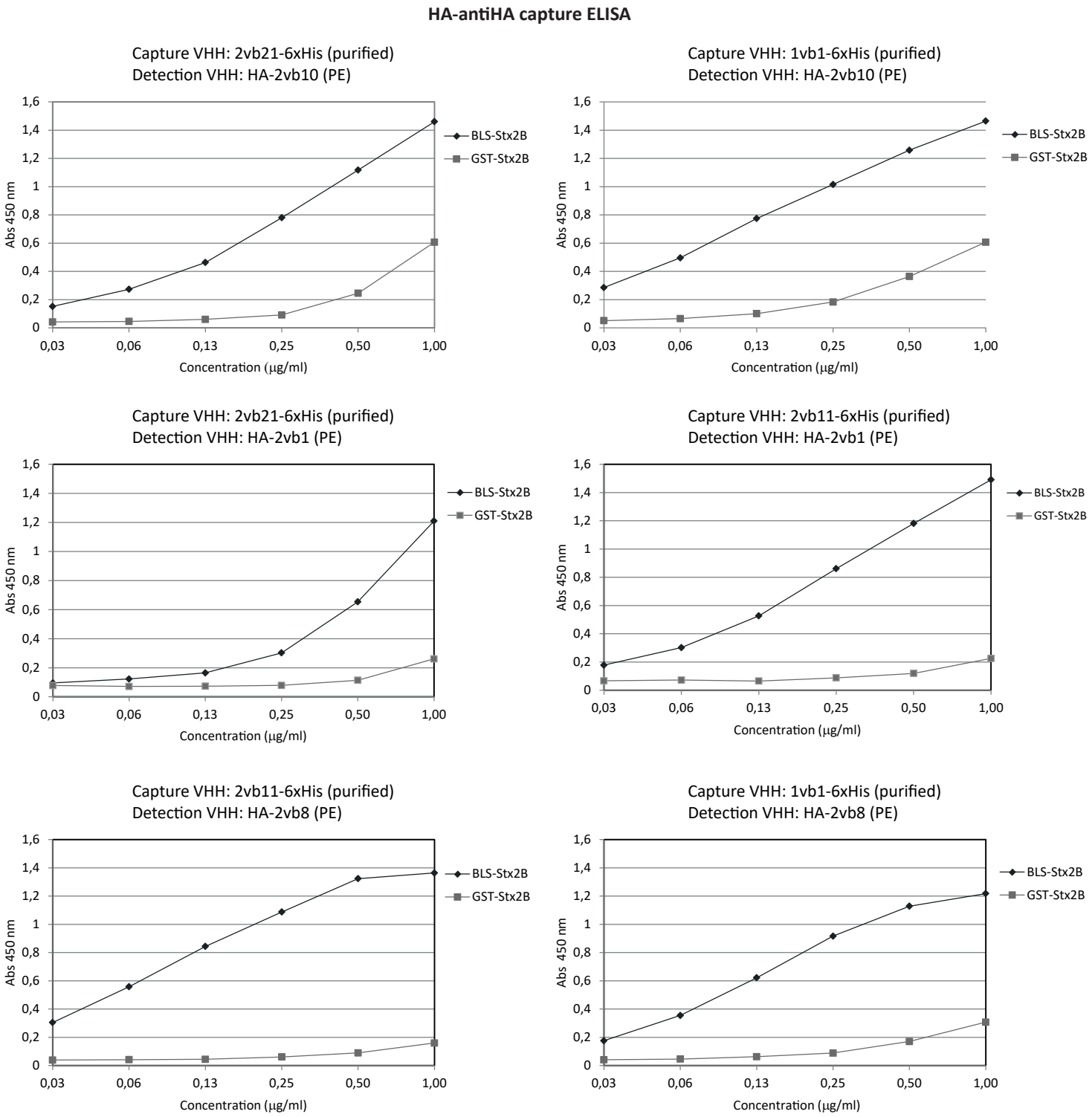

Figure S3. Determination of the detection limit of the six selected VHH pairs for the recombinant proteins BLS-Stx2B and GST-Stx2B by the HA-antiHA capture ELISA. Capture VHHs (VHH-6xHis) were purified and detection VHHs (HA-VHHs) were obtained from the periplasmic extracts (PE) enriched in the corresponding VHH.

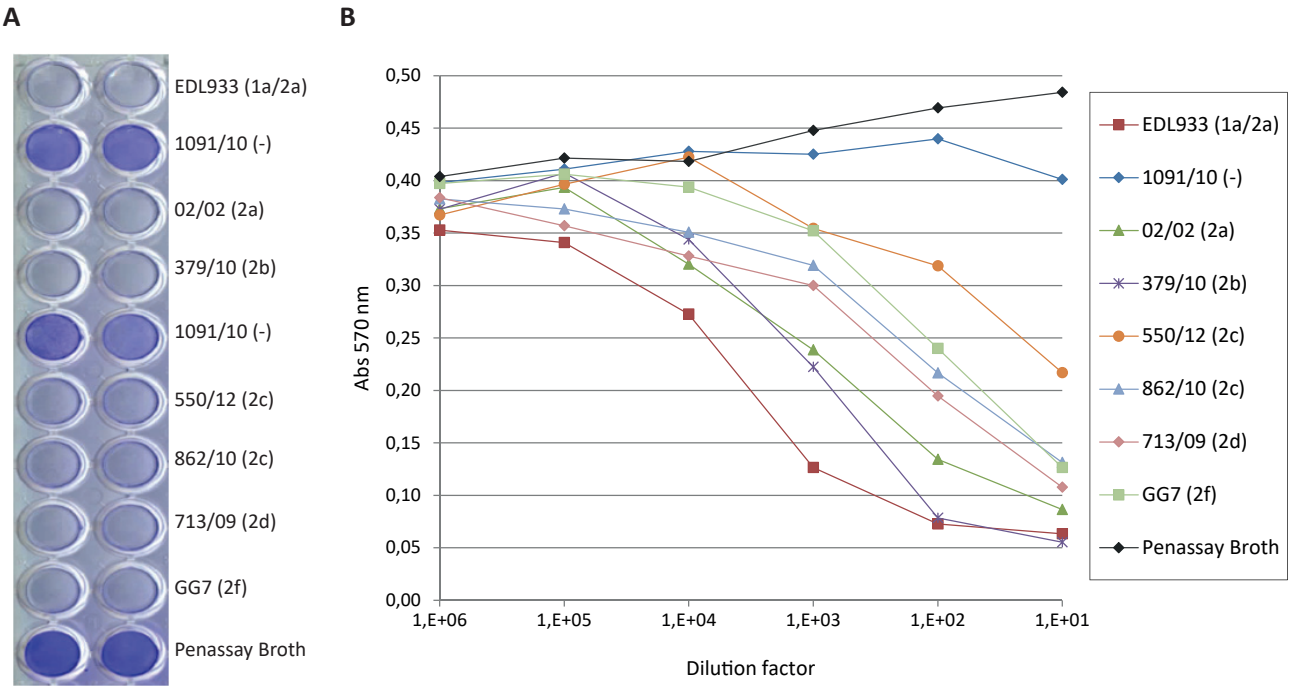

Figure S4. Analysis of the cytotoxic activity of culture supernatants obtained from different STEC strains. (A) Vero cell cytotoxicity assay. The picture of the wells was taken after incubating the cells with the culture supernatant of the indicated strain and staining with Crystal Violet. (B) MTT viability assay. Vero cells monolayers were incubated with different dilutions of the culture supernatants of the indicated strains. After incubating with MTT (4,5-dimetiltiazol-2,5-difeniltetrazolium), which is reduced to formazan in living cells, dimethyl sulfoxide was added to solubilize the formazan product and the absorbance was determined at 570 nm and 655 nm.

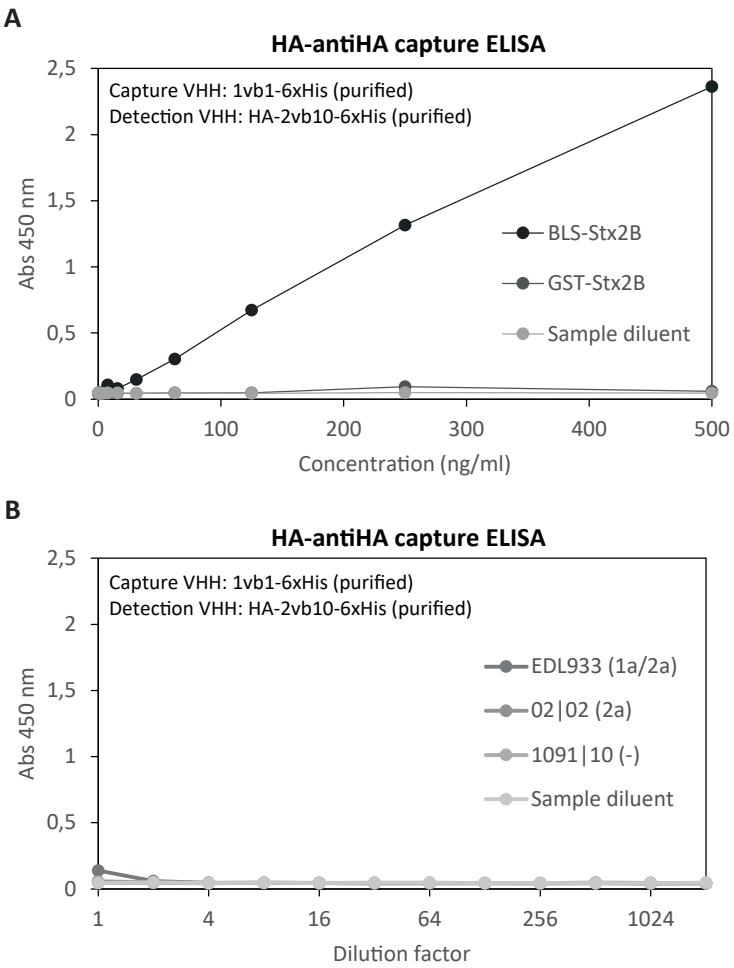

Figure S5. Detection of recombinant and native Stx2 by the HA-antiHA capture ELISA using the purified HA-2vb10-6xHis VHH as detection antibody. (A) Detection of BLS-Stx2B and GST-Stx2B recombinant proteins. (B) Detection of native Stx2a. Serial dilutions of the culture supernatants of the indicated strains were evaluated.