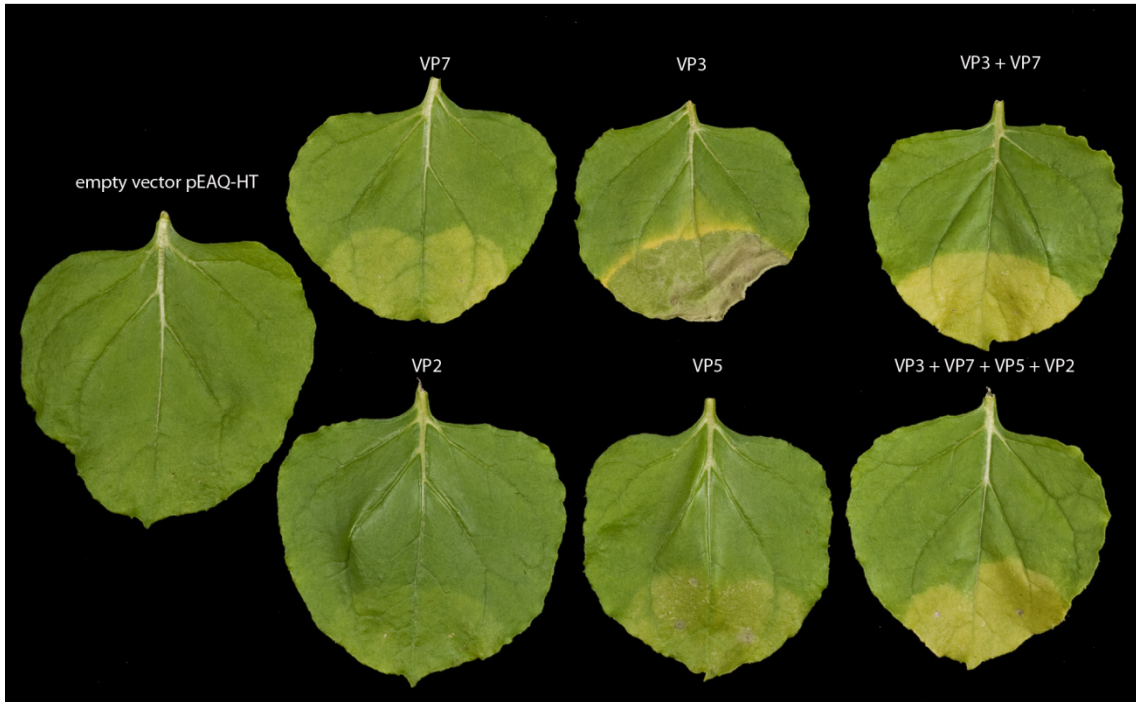


## SUPPORTING INFORMATION



**Figure S1:** Some BTV8 structural proteins cause symptoms when expressed in plants individually, but not when expressed in combination with their structural neighbouring proteins. Plants were infiltrated with suspensions of *Agrabacterium tumefaciens* harbouring pEAQ-*HT*-based plasmids for expression of BTV-8 structural proteins, either individually or in combinations as indicated. Leaf tissue was harvested 8 days post infiltration and a digital photograph taken.

Supporting Information: Figure S2

(a) VP2

Protein View: B4E549  
 SubName: Full=Outer capsid protein VP2  
 Database: SPtrEMBL  
 Score: 323  
 Taxonomy: Bluetongue virus 8

1	MEELAIPIYT	NVFP AELLDG	YDYIIDVSSR	<b>VEEEDPEVK</b>	<b>RHDVTEIPRN</b>
51	<b>SMFDIKDEHI</b>	<b>RDALIKPVN</b>	<b>NDGYVLPVVL</b>	<b>DITLKAFFDR</b>	<b>KRVVLNDGHS</b>
101	<b>EFHTKTNWVQ</b>	WMIDDAMDVQ	PLKVDIAHTR	<b>SRISHALFNC</b>	<b>TVRLHSHKAD</b>
151	<b>TASYHVEPVE</b>	<b>IESWGCNHTW</b>	<b>LSRIHHLVNV</b>	<b>ELFHCSQEA</b>	<b>YTLKPTYKII</b>
201	SNAER <b>ASTSD</b>	<b>SFNGTMIELG</b>	<b>RNHQIQMGDQ</b>	<b>GYQKLKEGLV</b>	<b>QVRIEGKTPL</b>
251	VIQEEITALN	<b>KIREQWIARN</b>	FDQREIKVLD	<b>LCRLLSTIGR</b>	KMCNTEEEPK
301	NEADLSVK <b>FQ</b>	<b>MELDEIFRPG</b>	<b>NNERTNIMGG</b>	GVHRKNEDRF	YVLIMIAASD
351	TNKGR <b>IWWSN</b>	<b>PYPCLRGALI</b>	<b>AAEVQLGDVY</b>	<b>NLLRNWFQWS</b>	<b>VRPTYVPYDR</b>
401	NR <b>ESDKYIYS</b>	<b>RINLFDSTLR</b>	PGDKIVHWEY	<b>KLLNEVREVS</b>	<b>INKGNECDLF</b>
451	<b>PEDEEFTTKF</b>	<b>HEARYTEMKN</b>	<b>QIIQSGWNQR</b>	<b>DFKMHKILED</b>	<b>GANVLTIDFE</b>
501	<b>KDAHIGTGSA</b>	<b>LSLPDYYNKW</b>	<b>IIAPMFNAKL</b>	<b>RITEVVIGTA</b>	<b>HTDDPAVGRS</b>
551	AK <b>AFTHDPFD</b>	<b>LQRYCLARYY</b>	<b>DVRPGMMGRA</b>	LSKQQNMSSM	TDKLSK <b>QEDY</b>
601	<b>AGIVSRILEY</b>	<b>KERENRCLTE</b>	<b>TAQYVFEKTC</b>	<b>LYVLELLSRH</b>	TMPSEDSEVT
651	FEHPTIDPSV	DIETWKIIDV	SQLIIFVFDY	LFENRKIVRD	TTEARWTLFK
701	IRSEVGRARI	<b>DAIEMTFPRF</b>	GRMLRNASQA	KINQDIACLN	FLPLLFIIIGD
751	NISYHRQWS	IPVLLYAADI	RIIPLEVGAY	<b>NNRFGLTSYL</b>	<b>EYMAFFPSYA</b>
801	<b>TRVAKIDESI</b>	<b>KCAIAMAEF</b>	<b>YMNTDIHSGS</b>	<b>VMSNVITTKR</b>	LLYETYLASL
851	CGGYSDDLW	<b>YLPITHPSKC</b>	<b>LVAFEVADDV</b>	<b>VPLSVRRERI</b>	LSRFPLSSRH
901	VK <b>GIALISVD</b>	<b>RNQKVSQTE</b>	<b>GIVTHRLCKK</b>	<b>NLLKYVCDVI</b>	<b>LFKFSGHVFG</b>
951	<b>NDEMLTKLLN</b>	V			

**(b) VP3**

*Protein View:* B4E550  
*SubName:* Full=Inner capsid protein VP3  
*Database:* SPtrEMBL  
*Score:* 476  
*Taxonomy:* Bluetongue virus 8

1	MAAQNEQRPE	RIK <b>TPYLEG</b>	<b>DVLSSDSGPL</b>	<b>LSVFALQEIM</b>	<b>QKVRQVQADY</b>
51	<b>MTATREVDFT</b>	<b>VPDVQK</b> ILDD	IK <b>ALAAEQVY</b>	<b>KIVKVPSISF</b>	<b>RHIVMQSRDR</b>
101	VLR <b>VDTYEE</b>	<b>MSQVGDVITE</b>	<b>DEPEK</b> FYSTI	IKK <b>VRFIRGK</b>	<b>GSFILHDIPT</b>
151	<b>RDHRGMEVAE</b>	<b>PEVLGVEFKN</b>	<b>VLPVLTAEHR</b>	<b>AMIQNALDGS</b>	<b>IIENGNVATR</b>
201	<b>DVDVFIGACS</b>	<b>EPIYRIYNRL</b>	<b>QGYIEAVQLQ</b>	<b>ELRNSIGWLE</b>	<b>RLGQRKRITY</b>
251	<b>SQEVLTDFRR</b>	<b>QDTIWVLALQ</b>	<b>LPVNPQVWVD</b>	<b>VPRSSIANLI</b>	<b>MNIATCLPTG</b>
301	<b>EYIAPNPRIS</b>	<b>SITLTQRITT</b>	<b>TGPFALITGS</b>	<b>TPTAQQLNDV</b>	<b>RKIYLALMFP</b>
351	<b>GQIILD</b> LKID	PGERMDPAVR	<b>MVAGVVGHL</b> L	<b>FTAGGRFTNL</b>	<b>TQNMARQLDI</b>
401	<b>ALNDYLLMY</b>	<b>NTRVQVNYGP</b>	<b>TGEPLDFQIG</b>	<b>RNQYDCNVFR</b>	<b>ADFATGTGYN</b>
451	GWATIDVEYR	<b>DPAPYVHAQR</b>	YIRYCGIDSR	<b>ELINP'TTYGI</b>	<b>GMTYHCYNEM</b>
501	<b>LRMLVAAGKD</b>	<b>SEAAEFRSML</b>	<b>PFHMVRFARI</b>	NQIINEDLHS	VFSLPDDMFN
551	ALLPDLIAGA	HQNADPVVLD	VSWISLWFAF	NR <b>SFEP</b> THR <b>N</b>	<b>EMLEIAPLIE</b>
601	<b>SVYASELSVM</b>	<b>KVDMRHLSLM</b>	<b>QRRFPDVLIQ</b>	ARPSHF <b>WKAV</b>	<b>LNDSPEAVKA</b>
651	<b>VMNL</b> SHSHNF	<b>INIRDMMRWV</b>	<b>LLPSLQPSLK</b>	<b>LALEEEAWAA</b>	<b>ANFEDLMLT</b>
701	<b>DQVY</b> MHRDML	<b>PEPRLDDIER</b>	<b>FRQEGFYITN</b>	<b>MLEAPPEIDR</b>	VVQYTYE <b>IAR</b>
751	LQANMQFRA	ALRRIMDDDD	WVREGGVLR <b>T</b>	VR <b>VKFFDARP</b>	<b>PDDILQGLPF</b>
801	<b>SYDTNEK</b> GGL	<b>SYATIKYATE</b>	TTIFYLIYNV	EF <b>SNT</b> PDSL <b>V</b>	LINPT <b>Y</b> TM <b>T</b> K
851	VFINKRIVER	VR <b>VGQILAVL</b>	<b>NR</b> RFVAYK <b>GK</b>	MR <b>IMDITQSL</b>	<b>KMGTKLAAPT</b>
901	V				

**(c) VP5**

Protein View: B4E553  
 SubName: Full=Outer capsid protein VP5  
 Database: SPtrEMBL  
 Score: 131  
 Taxonomy: Bluetongue virus 8

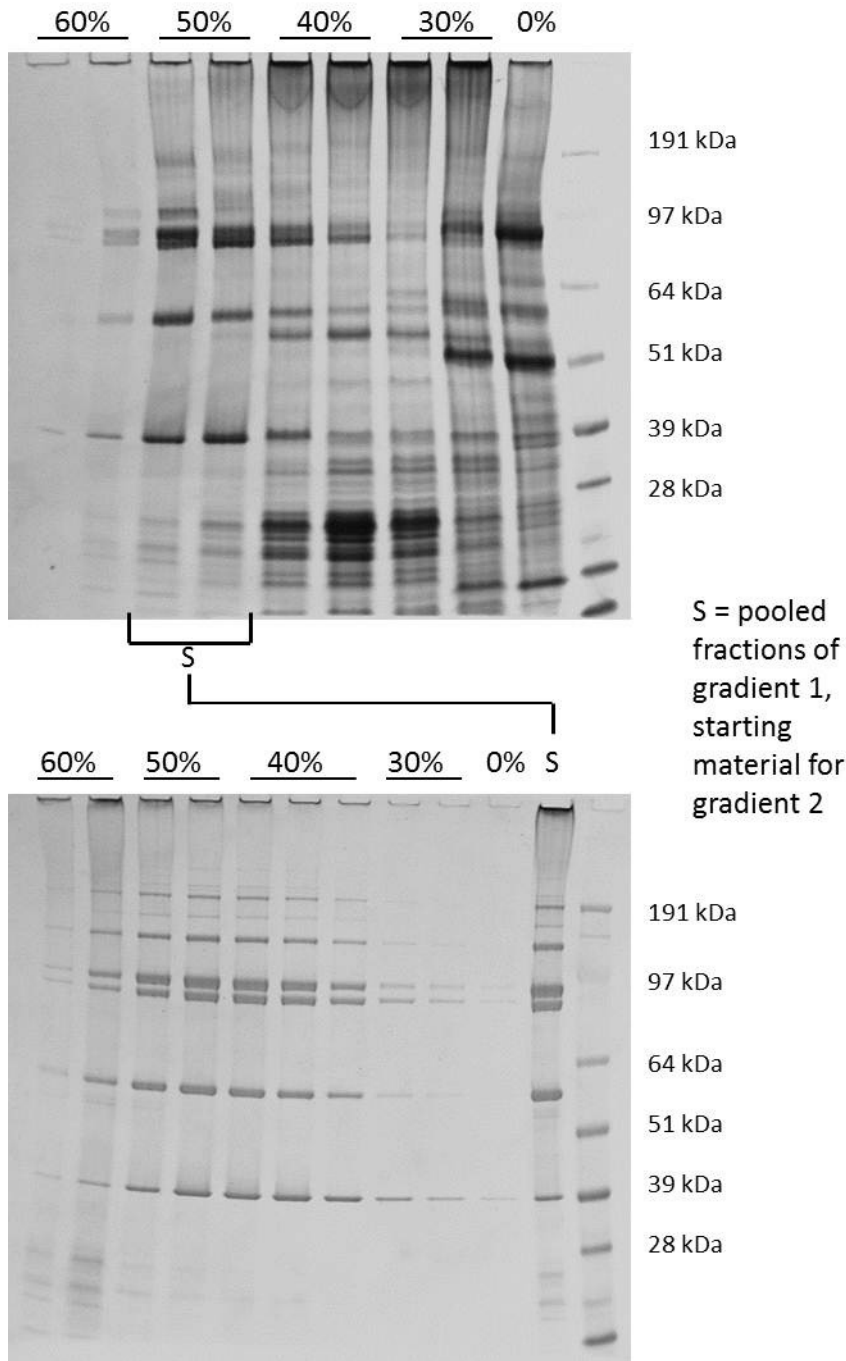
1	MGKIIKLSLR	FGKKVGNALT	SNTAKKIYST	IGKAAERFAE	<b>SEIGSAAIDG</b>
51	<b>LVQGSVHSLM</b>	<b>TGESYGESVK</b>	<b>QAVLLNVMGS</b>	<b>GEELPDPLSP</b>	<b>GERGMQTKIR</b>
101	<b>ELEDEQRNEL</b>	IRLKYNDKIK	<b>QKFGKELEEV</b>	<b>YEFMNGVAKQ</b>	EEDEEKHYDV
151	LKK <b>AVNSYDK</b>	<b>ILTEEEKQMR</b>	<b>ILATALQKEV</b>	KERTGTEAVM	VKEYRNKIDA
201	LK <b>EAIEVERD</b>	GMQEEAIQEI	AGMTADVLEA	ASEEVPLIGA	GMATAVATGR
251	AIEGAYKLKK	<b>VINALSGIDL</b>	<b>THLRTPKIEP</b>	<b>TIVSTVLDHK</b>	<b>FKDIPDEMLA</b>
301	<b>VSVLSKNRAI</b>	<b>EENHKEIIHL</b>	<b>KNEILPRFCK</b>	AMDEEKEICG	IEDKKIHPKV
351	MMKFKIPRTQ	QPQIHIYSAP	WSDDDVFFFH	CISHHHANES	FFIGFDLID
401	LVHYEDLTAH	WHALGAAQAA	VGRSLNEVYK	<b>EFLNLAINNT</b>	<b>YSSQMHHARM</b>
451	IRSK <b>TVHPIY</b>	<b>LGSLHYDISF</b>	<b>STLRSNAQRI</b>	<b>VYDEELQMI</b>	<b>LRGPLHFQRR</b>
501	AILGAIKHGV	<b>KILGTEVDIP</b>	<b>LFLRNA</b>		

**(d) VP7**

Protein View: B4E554  
 SubName: Full=VP7 core protein  
 Database: SPtrEMBL  
 Score: 177  
 Taxonomy: Bluetongue virus 8

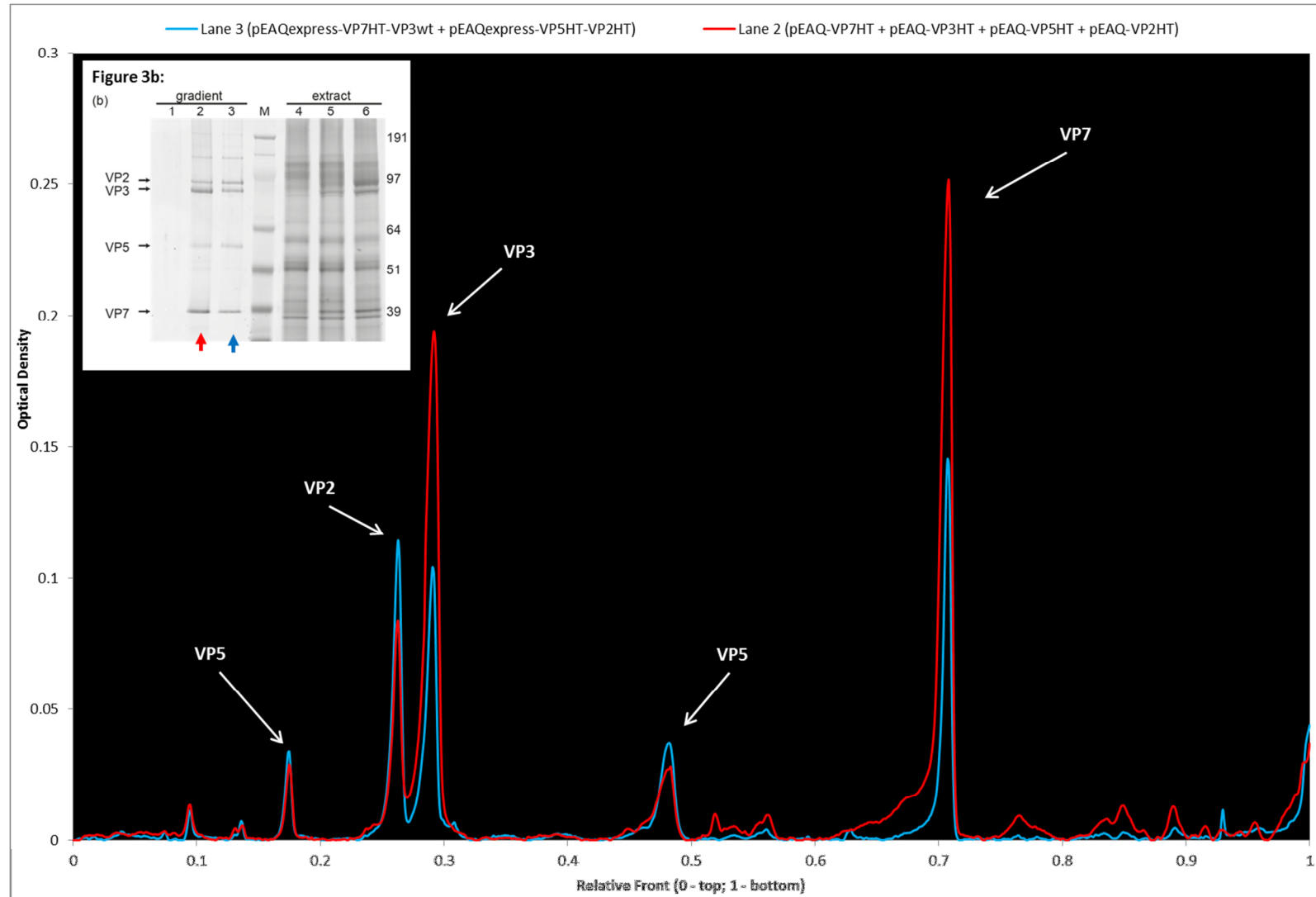
1	MDTIAARALT	VMR <b>ACATLQE</b>	<b>ARIVLEANVM</b>	<b>EILGIAINRY</b>	NGLTLRGV <b>TM</b>
51	<b>RPTS<b>LAQRNE</b></b>	MFFMCLD <b>MML</b>	SAAGIN <b>VGPI</b>	SPDY <b>TQHMAT</b>	IGVLAT <b>PEIP</b>
101	FTTEA <b>ANEIA</b>	R <b>VTGETSTWG</b>	<b>PARQPYGFFL</b>	<b>ETEETFQ<b>PGR</b></b>	WFM <b>RAAQAVT</b>
151	<b>AVVCGPDMIQ</b>	<b>VSLNAGARGD</b>	<b>VQQIFQGRND</b>	<b>PMMIYLVWRR</b>	<b>IENFAMAQGN</b>
201	<b>SQQTQAGVT<b>V</b></b>	<b>SVGGVDMRAG</b>	RIIA <b>WDGQAA</b>	LHVH <b>NPTQON</b>	AMVQ <b>IQVVFY</b>
251	ISMDK <b>TLNQY</b>	<b>PALTAEIFNV</b>	<b>YSFRDHTW<b>HG</b></b>	<b>LRTAILNR<b>TT</b></b>	<b>LPNMLPP<b>IFP</b></b>
301	<b>PND<b>R</b>DSILTL</b>	LLLSTL <b>ADVY</b>	TVLR <b>PEFAIH</b>	GVNP <b>MPGLT</b>	RAIAR <b>AAVY</b>

**Figure S2:** Mass spectrometry analysis of 4 main protein bands recovered from SDS-PAGE analysis of a density gradient fractions from leaf tissue co-infiltrated with constructs for co-expression of BTV-8 VP2, VP3, VP5 and VP7. The major protein hit of each band is presented (a-d). Matched peptides are shown in **red**. Scores >75 are considered significant ( $p < 0.05$ ).



**Figure S3:** Clarified extract from leaf tissue expression four BTV structural proteins, separated on two consecutive step sucrose gradients. Approximate sucrose concentration of steps indicated in per cent. Two fractions from sucrose gradient 1 (top) were pooled to provide starting material (S) for sucrose gradient 2 (bottom).

Supporting Information: Figure S4



**Figure S4:** Densitometric analysis of Coomassie-stained gel of Figure 3b (inset). Two lanes were analysed: purified VLP preparations prepared by co-infiltration of four pEAQ-*HT* constructs all under the control of a *HyperTrans* 5'UTR (red), or purified VLP preparation prepared by co-infiltration of two pEAQexpress constructs with VP3 under control of a wild-type 5'UTR (blue).