#### **Supporting Information.**

**Fig. S1. Domain architectures of T4CPs.** T4CPs are composed of an N-terminal transmembrane domain (NTD), a nucleotide-binding domain (NBD), an all- $\alpha$ -domain (AAD), and in some cases a C-terminal domain (CTD). The schematics present domain structures of the TrwB<sub>R388</sub> prototype and of T4CPs characterized in this study (listed at top). Upper: NTDs bearing two predicted transmembrane domains; residue numbers mark the boundaries of the membrane-spanning  $\alpha$ -helices (gray cylinders). IM, inner membrane. Middle: Soluble domains composed of NBDs and AADs. At left, the solved structure of the TrwB<sub>R388</sub> protomer lacking its NTD, with flanking residue numbers below. Predicted structures of corresponding regions of TraJ and VirD4 homologs determined by Phyre2 modeling; NBDs are multicolored, AADs are in green. Lower: CTD information. TrwB<sub>R388</sub> and TraJ<sub>pKM101</sub> lack CTDs. CTDs of the VirD4 homologs from *A. tumefaciens* (At), *A. phagocytophilum* (Ap), and *W. pipientis* (Wp) vary in length and have a high proportion of acidic residues. CTD sequences are presented at the bottom; acidic Glu and Asp residues are in bold letters.

**Fig. S2. Sequence alignments of T4CPs.** Alignments of **A**)  $\operatorname{TraJ}_{pKM101}$  with VirD4 subunits from *A. tumefaciens, A. phagocytophilum*, and *W. pipientis*; **B**)  $\operatorname{TraJ}_{pKM101}$  with  $\operatorname{TrwB}_{R388}$ ; **C**) VirD4 subunits from *A. tumefaciens, A. phagocytophilum*, and *W. pipientis*; **D**) alphaproteobacterial VirD4 homologs with *L. pneumophila* DotL. Boxed regions denote N-terminal transmembrane domains (NTD), nucleotide binding domains and all- $\alpha$ -domains (NBD/AAD), and C-terminal domains (CTD). Red lettering, sequence identities; blue lettering, sequence similarities. **E**) Sequence alignments of the CTDs carried by VirD4 homologs from the *Wolbachia* species shown. Panel A: Schematic depiction showing variability in sequence lengths of the CTDs. Panel B: Sequence alignments of the CTDs showing variability in sequence compositions. Color scheme: Red, polar/acidic residues; Blue, polar/basic residues; Green, polar/neutral residues; Black, nonpolar residues.

**Fig. S3.** C-terminal sequences of known or candidate T4SS effectors. A. The *A. tumefaciens* effectors carry clusters of positively-charged Arg residues at their C termini. A systematic mutational analysis of VirF established the importance of Arg residues in an Arg(7x)Arg(x)Arg(x)Arg motif (marked in red letters) for translocation through the *A. tumefaciens* VirB/VirD4 T4SS. A few Arg residues critical for VirF translocation are conserved near the C termini of *A. tumefaciens* VirE2 and VirE3, and one of these is also conserved among the *A. phagocytophilum* Ats-1 and the *W. pipientis* WD0636, WD0811 and WalE1 effectors (red letters). The C termini of the effectors shown carry other positively-charged Arg or Lys residues (blue letters) and, notably, a high proportion of hydrophobic residues (underlined). Previous work identified clusters of hydrophobic residues at the C termini of effectors translocated through the *Legionella pneumophila* Dot/Icm T4SS, and a systematic mutational analysis established the importance of such residues for RalF translocation.

Fig. S4. Interactions of the VirE2CT100 fragment with VirD4<sub>At</sub> domains as assessed by isothermal calorimetry (ITC). His<sub>6</sub>-tagged VirE2CT100, MBP-tagged VirD4<sub>At</sub> deleted of its N-terminal transmembrane domain and the AAD (designated NBD/CTD), and the MBP-tagged AAD<sub>VirD4At</sub> were purified by sequential rounds of affinity chromatography and gel filtration chromatography using a Superose 6, 10/300 GL size exclusion column from GE Life Sciences. Binding of VirE2CT100 (185  $\mu$ M) to: Panel A, buffer alone; Panel B, the NBD/CTD variant; and Panel C, the AAD. Five microliter injections were used for each experiment, with 2 min between each injection to allow for peaks to reach baseline levels. The upper graphs show the raw data and the lower graphs show the normalized integration of the data. While apparent binding was observed in both B and C, low concentrations of NBD/CTD and AAD (higher concentrations could not be achieved due to aggregation) resulted in a low Wiseman "c" parameter, and could not be used to derive accurate thermodynamic information.



Fig. S1 Whitaker/Christie

						Ν	TD					1	NBD/A	AD
	1	10	20	30	40	50	60	70	80	90	100	110	120	130
pKM101TraJ AgroYirD4 YirD4Ap YirD4Hp Consensus	MNSS MHSS MSHSG <sup>S</sup> .	KTSPQRMTI NHIRNILYF NHLRNILI(	_SIVCSLAAG FCGLFCLLE 3GVVAFSILE	HDDRERGLAFL FCAASCYVTFF FCFYISGYLF\ FCFYLSGILF\ fcgF.	LFAITLPPYM RRGFNGEAIM /LLVNGYDYLI /LFVDGPDGLI gp\$	VHFLYAKF TFDYF DFNAINPSYSDF DFKAIRPGLTPF .ff	TYGIDPS AFHYEI PHRLHPTJ PQALHPTJ 	TAKYLIPYLYKN TPLYLGYAS CFQYISKHHGDPI CFDHIQNCHHHPI yl	TFSLHPLHS TVFHRGLSV KLFGIANSL ELYSLELKI t1.	ALIAGHFIGY YIFTSLAYLS KLHGSFFAPI KLIISSALPY .li.s.fv	GGLIAFIIY SQLIISLRN GLYCLYAHN YVLMIILHN g.lin	KSRYFKGE KHHGTA-RWF RHYLIDWRPF RERIIEWRPF k.rr.	RFKKIYRGTE EIGEHQHAGY KKKESLHGD RKKESLHGD , r . ke hg	LYRARTL LQRYSRI SRNASERD SKNASEKE
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
pKM101TraJ AgroYirD4 YirD4Ap YirD4Wp Consensus	ADKTR KGPIF IRKIG IRKAG	ERGYNQLTY GKTCGPLHF LRSRRGILI LRSKKGLLI	/ANIPIPT GSYLT _GKDQRGYLY _GKDKRGYFI .gy.t	YAENLHFSIAO Ngeophslyyf Adgyohallff Adgyohallff eh.1a	GTTGTGK PTRAGKGVG PTGSGKGVG PTGSGKGVG GPTg.GKgvg	TTIFNELLFKS- LVIPTLLTFKGS FVIPNLLFHEDS FVIPNLLFHTDS .VIPNLLFHTDS	-IIRGGKN VIALDVKC VIVHDVKL VIVHDIKL VIVHDIKL	ITALDPNGGFLKI GELFELTSRARKI LENYDLTSGHRKI LENYEITSGHREI .e.,#.tsg.rk	NFY ASGDAVFKF KIGQEYYYH RQGQKYYYH gY	RPGDYILN SPLDSERKTH NPAQPDGISH NPAQPDGISH .P#.i.h	AYDKRTEGHV CYNPVLDIAT CYNPLDHISK CYNPLEHISE CY#pi.	/FFNEIRRSYL LPPERQFTE1 (KPGQhyde KPGQhyde 	)YERLYNSIY IRRLAANLITF )YQKIANLINF )YQKIANLINF JYQKIANLINF	QES 1KGKGAEG 2EQDF 2EQDF 2EQDF
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
pKM101TraJ AgroYirD4 YirD4Ap YirD4Mp Consensus	PDMAT FIDGA HYNEA HQNEA	EEHFGYGRL RDLFYAGII RSLFYGYYL RSLFYGYYL r.1Fv.g.L	IFSEVSKKL TCIERGT YLLAVPEKV YLLAAPEKV	HSLYSTVTH- Ptigavydlff Ksfgevvrthf Ksfgevvrthf Ksfgevvrthf	EEVIHHA AQPGEKYKLFA RSDDVVYNLA RSDDVVYNLA RSDDVVYNLA	CNYDQKKLKEFL AQLAEESLNKEF YYLDTIGKKJ YYLDTMGKIJ	MGTPAEA) IQRI-FDN HPVAYMN HPVAYMN	LFSGSE <mark>KAYGSA</mark> IAGNDT <mark>KILTSY</mark> LAAFLQKADKER LAAFLQKADKER LaKas.	RFVLS TSVLGDG SGVISTMNS SGVVSTMNS Vls	KNLAPHL GLNLWADPLI SLELWANPLI SLELWANPLI ,1,1wa,Pli	KMPEGNFSLR KAATSRSDFS DTATASSDFN DTATASSDFN k.atsdf.	RDHLDDG <mark>K</mark> PG1 SYYDLRRKKTC IIQDFKRKRYS IILDFKRKKY1 drKt	LFITHQEEM JYLCVSPNDI Svyvgvtpdni Vyvgltpdni .*******	KRSLNPLI EVLAPLM TRLRPLM NRLKPLM
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
pKM101TraJ Agro¥irD4 ¥irD4Ap ¥irD4Hp Consensus	SCHLD RLLFQ QVFYQ QVFYQ #	SIFSIY QLYSILQRS QATEFLCRI QATEFLCRI qsil.r.	-LGMGEKESR Slpgkdeche Flpsddepyg Klpsddepyg .lp#e	INVFIDELESL VLFLLDEFKHL VLFLMDEFPTL VLFLMDEFPTL !lfl.DEfL	_QFLPNLNDA _GKLEAIETA _GKMEQFQTG _GKMEQFQTG _gk\$e#La	TKGRKSGLCYY TTIAGYKGRFN LAYFRGYRYRLF LAYFRGYRYRLF LAYFRGYRYRLF itrgyr.	AGYQTYSO FIIQSLSF LIIQDTEO LIYQDTEO +i+Q++so	LYKYYGRDMAQ LTGTYDDAGKQ LKGIYEEAGMM LKGIYEEAGMM LKGIYEEAGMM	TILANMRSN NFLSNTGVQ SFLSNSTYR SFLSNSTYR .flsn	IYLGGSRLGD YFMATADD ITFAANNI ITFAANNI !ad	ETLDQMSRSL ETPTYISKAI ETANLISQLI ETANLISQLI ETANLISQLI	GEIEGEVER Geytfkarst GNKTVSQESL GNKTVQQESL GNKTVQQESL G#.tes	(ESDPQK [SYSQAS] _NRPKFLDLN _NKPKFLDLN	PHIYRKRR IFDHNIQI PASRSLHI PASRSLHI OMBRSLHI
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
pKM101TraJ AgroYirD4 YirD4Ap YirD4Mp Consensus	DVKVV SDQGA SDTQR SETQR SETQR	RAVTPTEIS ALLRPEQVE ALLLPQEVI ALLLPQEII ALLLPQEII all.P.#!	SMLPNLTGYL RLLDDQSEIY CMLPKDEQIL CMLPRDEQII S <sup>\$</sup> Lpil	ALPGDMPYAKA LIKGRPPL LIESTYPI LIESTYPI LIESTYPI	KAKHYKYHRI -Klrkvqyysi -Ksrkikyfei -Kskkilyysi .K.kk!ky	KNPYPGIELREJ DRTL-KRLFERG DKTFTRRLLKST DNTFTRKLLKQT dnt11	GSLPEPF IPTQEP1 VPTQEP1	-PLML /DPEKIRGGYEGI /DPNKYFSGA	SDYS EAGIVSEAS NKSK	NDQYQSHLAE Ensedlkalm Ydnkendate	IANFNEDAAP GSSSAENPPE GPNAADYLYE	PRNRTVAEDHO Raeygyeddo RaQtki	SYKYGADIPE DLYDREEFEI DAYYDESEIDI	ERV-MGIN DLYGIGEE DELKEEEI
	651	660	670	680	690	700	710	720	730	740	750 7	/55		
pKM101TraJ AgroYirD4 YirD4Ap YirD4Wp Consensus	GDEDQ PEDDL DDEDV d.	ADAREIPPE EDLDESDDY DGFDDEEDY	ESYYPPELTL /FHDEDDYL- /GKLKGDGKP	ALAAQQQLLDQ NDEDYDDPYEI KEEKYDDEFDI	QIIALQQRSR: DDMELEGDGDI NEFEDEDDGDI	SAPAQPAK Ideneafedell Kpksngk	.DDEYYDDE	EFDSLEPEEDED	FEEDDSLEE	EDEFGEDRPT	DDNDSSNGRL	.KS		

CTD

Fig. S2 Whitaker/Christie

Α

# Sequence Alignments of VirD4 Homologs

- N I	-		
- 1 1		. 1	
1.			
		 _	

# NBD/AAD

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
pKM101TraJ	HDDRE	RGLAFL	FAITLPPVH	IVHFLYAKFT	/GIDPSTAKYI	IPYLYKNTF	SLHPLHSALI	GHFIGYGGLI	AFJ CYDKSR	YFKGERFKKI	YRGTELYRAR	TLADKTRERG	Y <mark>NQL TYANIPI</mark>	PTYAENLHF
R388TrwB	HHPDDO	RKYSAG	IVIVLPL	IFHITAYQK1	[EYLGSPKLL[	ALHELMKLTP	QKPILLLSALO	GLAYGYLFYH	LLN SYGQGE	-FGGAPFKRF	LRGTRIYSGG	KLKRIITREKA	-KQVTYAGYPI	PRDAEPRHL
Consensus	•hdDr4	Rglaag	iaItLP\$	!ffitAkqkt	ce!dgSpalla	aipelmKnTf	qlpiLllaai	Gla!GYgglia	alr sydqgr	.FgGarFKri	1RGTriYrar	KLarkTREra	.nQl TYAn !Pi	PrdAEnrH1
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
pKM101TraJ	SIAGTI	FGTGKTT	IFNELLFKS	IIRGGKNIAL	DPNGGFLKN	YRPGDVILN	NYDKRTEGHVI	FNEIRRSYDY	ERLYNSIYQ	ESPDNATEEN	FGYGRLIFSE	VSKKLHSLYS	TVTMEEVIHHF	CNYDQKKLK
R388TruB	LYNGA	FGTGK <mark>S</mark> y	LLRELAYTG	LLRGDRHVI	DPNGDMLSKI	GRDKDIILN	Pydqrtkghsi	FNEIRNDYDH	QRYALSYYP	RGKTDEAEEN	ASYGRLLLRE	FAKKL-ALIG	TPSMRELFHHT	TIATFDDLR
Consensus	1!aGal	FGTGK <mark>st</mark>	ilrELa%kg	iiRGdrn!al	LDPNGdmLkn	gRdgD!ILN	aydqrteghsi	FNEIRrdyDu	#R1anS!Yq	rgkddaaEEN	agYGRLilrE	taKKL.aLig	TpsMrEliHHa	cnadqddLr
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
pKM101TraJ	EFLMG1	TPAEAIF	SGSEKA	VGSARFYLS	KNLAPHLKMPI	EGNFSLRDHL	DDGKPGTLFI	FHQEEMKRSLN	PLISCHLDS	IFSIYLGMGE	K-ESRINYFI	DELESLQFLP	NLNDALTKGRK	(SGLCYYAGY
R388TrwB	GFLEG1	TLAESLF	AGSNEASKA	LTSARFYLS	KLPEHYTMPI	)GDFSTRSHL	EDPNGGNLFI	FHREDMGPALR	PLISANVDV	YCTSILSLPE	EPKRRLHLFI	DELASLEKLA	SLADALTKGRK	(AGLRYYAGL
Consensus	eFLeG1	TLAEaiF	aGS#KA	LgSARFYLS	InLaeH1kMP	#G#FS1RdHL	#DgngGnLFI	FHrE#MgraLrI	PLISAN1Ds	!csi!Lg\$gE	e.erRin1FI	DELaSL#kLa	nLaDALTKGRK	(aGLrYyAG1
	391	400	410	420	430	440	450	460	470	480	490	500	510	514
pKM101TraJ	QTYSQI	.VKYYGR	DHAQTILAN	MRSNIVLGG	SRLGDETLDQI	1SRSLGETEG	EVERKESDPQI	(PHIVRKRRDV	KYVRAVTPT	EISHLPNLTG	YLALPGDMPVI	IKFKAKHYKY	HRKNPYPGIEL	REI
R388TruB	Q <mark>st</mark> sqi	.DDYYGY	KEAQTLRAS	FRSLVVLGG	SRTDPKTNEDI	1SLSLGEHEV	ERDRYSKNTGI	(HHSTGRALER	VRERVVMPA	EIANLPDLTA	YVGFAGNRPII	IKYPLEIKQF	ANRQPAFYEGI	I
Consensus	Q <mark>st</mark> sqi	.ddYYGr	deAQTirAn	MRSnVLGG	SR1ddeTn##1	1SrSLGEhEg	Er #Rkek #pql	(hhitrar#rl	kreRaVnPa	EIanLP#LTa	YlalaG#rP!I	IKfkaehkq%	arr#Pafgee]	r

Fig. S2 Whitaker/Christie

# Sequence Alignments of VirD4 Homologs

							NID						NBD/A	AD
	1	10	20	30	40	50	60	70	80	90	100	110	120	130
Agro¥irD4 Anaplasma¥irD4 WolbachiavirD4 Consensus	MNSSK MHSSN MSHSGN • mhSsr	(TSPQRMTL HIRNILYF HLRNILIG h.r#i\$	SIVCSLAAC FCGLFCLLF GVVAFSILE	GFCAASCYVTF FCFYISGVLF FCFYLSGILF FCFYLSGILF FCfy.sg!lF	RRGFNGEAI VLLVHGVDY YLFVDGPDG .1.v.G.d.	HTFDYF LDFNAINPS' LDFKAIRPGI \$dF.ai.p.	SDFPMRLAF SDFPMRLAF TPFPQALAF fp.alAF	TIFQYISKHH TIFDHIQNCH Lif.yi	YASTYFARGL GDPKLFGIAN HHPELYSLEL	SVVIFT <mark>SLAV</mark> SLKLHGSFFA KIKLIISSAL s.klS.a.	LSSQLIISLRN PIGLVCLVANN PVVVLMIILNN P···l·i.lwN	KHHGTA-R RHYLIDHR RERIIEHR rhi.HR	HAEIGENQHAG PFKKKESLHGD PFRKKESLHGD pf.kkEslHgd	YLQRYSRI SRHASERD SKHASEKE s.waser.
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
AgroYirD4 AnaplasmaYirD4 HolbachiavirD4 Consensus	KGPIFO IRKIGL IRKAGL irkig]	iKTCGPLHF RSRRGILL RSKKGLLL Irs.,g111	GSYL GKDQRGYL GKDKRGYF Gkd.rgY1.	FNGEQPHSLYY VADGYQHALLF EADGYQHALLF adgyqHaLlf	APTRAGKGY APTGSGKGY APTGSGKGY APTgsGKGY	GIVIPTLLTI GFVIPNLLFI GFVIPNLLFI GFVIPNLLFI	FKGSVIALDV HEDSVIVHDV HTDSVIVHDI HTDSVIVHDI HTDSVIVHDI	KGELFELTSR KLENYDLTSG KLENYEITSG KIEn%#1TSg	ARKASGDAVF HRKKIGQEVY HRERQGQKVY HRERQGQKVY	KFSPLDSERK VHNPAQPDGI VHNPAQPDGI VHNPAQPDGI vHnPa#p#gi	THCYNPYLDIF Shcynpldhig Shcynplehig Shcynplehig Shcynpl.wig	ITLPPERQFT KKPGQHV EKPGQHV kPgQnv	ETRRLAANLIT DDVQKIANLIM DDVQKIANLIM #dvqkiANLIM	AKGKGAEG PEQDF PEQDF peq#f
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
Agro¥irD4 Anaplasma¥irD4 WolbachiavirD4 Consensus	FIDGAF Hyneaf Honeaf W, #eaf	ROLFYAGIL RSLFYGYYL RSLFYGYYL RsLFYgy!L	TCIERG YLLAYPEK YLLAAPEK ylla,pekv	IPTIGAVYDLF /KSFGEVVRTM /KSFGEVVRTM /ksfGeVvrtm	AQPGEKYKL RSDDYYYNL RSDDYYYNL rsddyyYnL	FAQLAEESLI AYYLDTI AYYLDTH AYYLDTH avvL#t.	NKEAQRI-FE GKKIHPYAYN GKIIHPYAYN gK.ihp!a%r	NMAGNDTKIL NIAAFLQKAD NIAAFLQKAD NIAAFLQKAD NiAaflqKad	TSYTSYLG KERSGYISTM KERSGYVSTM kersgY.stm	DGGLNLHADP NSSLELHANP NSSLELHANP #SSLELHAPP	LIKAATSRSDF LIDTATASSDF LIDTATASSDF LIDTATASSDF	SYYDLRRKK NIQDFKRKR NILDFKRKK n!.DfkRKk	TCIYLCYSPND VSVYVGYTPDN VTVYVGLTPDN v.!YvgvtP##	LEVLAPLH LTRLRPLH LNRLKPLH L.rl.PLM
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
AgroYirD4 AnaplasmaYirD4 HolbachiavirD4 Consensus	RLLFQC QYFYQC QYFYQC qyf%QC	LVSILQR9 ATEFLCR1 ATEFLCRk AtefLcR,	LPGKDECHE LPSDDEPY( LPSDDEPY( LPsdDEpy;	EVLFLLDEFKH GVLFLMDEFPT GVLFLMDEFPT gVLFL\$DEFpt	LGKLEATET Lgkmeqfqt Lgkmeqfqt Lgk\$eqf#t	AITTIAGYK GIAYFRGYR GIAYFRGYR gIayfrGYr	GRFHFIIQSL /RLFLIIQD1 /RLFLIYQD1 /RLFLIYQD1	SALTGTYDDA EQLKGIYEEA EQLKGIYEEA eqLkGiY##A	GKQNFLSNTG GMNSFLSNST GMNSFLSNST Gn#sFLSNst	VQVFHATADD YRITFAANNI YRITFAANNI yr!tfAan#i	ETPTYISKAIG ETANLISQLIG ETANLISQLIG ETANLISQLIG ETANLISQLIG	iEYTFKARST inktysqesl inktyqqesl inktyqqesl i#ktv.qesl	SYSQASHF NRPKFLDLNPA NKPKFLDLNPA n.pk%1#1npa	DHNIQISD SRSLHISD SRSLHISE srslhIS#
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
AgroYirD4 AnaplasmaYirD4 HolbachiavirD4 Consensus	QGAALL TQRALL TQRALL LgrALL	RPEQYRLL LPQEYIHL LPQEIIHL 1P##!i\$L	DDQSEIYL] PKDEQILL] PRDEQIIL] p.#e#I.L]	IKGRPPLKLRK IESTYPIKSRK IESTYPIKSKK IestyPiKsrk	VQYYSDRTL Ikyfedktf Ilyysdntf !.y%sd.tf	-KRLFERQI TRRLLKSTI TRKLLKQT trrLlk.t.	GSLPEPA-PL EPTQEPYDPE PTQEPYDPN .ptqEPydP.	ML KIRGGYEGEA KYFSGA kg	SDYSNDQ GIYSEASENS NKSKYDN ss.#.	VQSHLAEIAN EDLKALMGSS KENDATEGPN .#a.eg.n	FNEDAAPRNR1 SAENPPERAE1 AADYLVEA 1.a#er	TVAEDHGSVK /GYEDDGDLV QTKDAVY #d.g.v.	VGADIPERV-M DREEFEDLYGI DESEIDDELKE d#i.#	GINGDEDQ GEEPEDDL EEIDDEDY ge##D.
	054	000	670	680	690	700	710	720	730	740	750			
	651	660	0/0		-	_		-						
AgroYirD4 AnaplasmaYirD4 HolbachiavirD4 Consensus	651 ADAREJ EDLDES DGFDDE .d.d#,	EBU EPPESVVPF DDYFHDEE EDYGKLKO dy	ELTLALAAQ DVL-NDED DGKPKEEK #e.	QQLLDQTIAL YDDPYEDDMEL YDDEFDNEFED y####el	QQRSRSAPA EGDGDADEN EDDGDKPKS #.dgd	QPAK EAFEDELLDI NGK #	DEVVDDEFDS	LEPEEDEDFE	EDDSLEEEDE	+- Fgedrptddn	IDSSNGRLKS			

#### NITO

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Fig. S2 Whitaker/Christie

С

# Sequence Alignments of VirD4 Homologs

	-	_	-	
NI	_ 1			
	_ 1			
	_		_	

# NBD/AAD

	1	10	20	I	30	40		50	6	50	70		80	90	0	100	1	110	120		130
DotL AgroYirD4 YirD4Anapl YirD4Wolb Consensus	MHRGI HNSSI MHSSI HSHSGI +n+sg.	EDSRHELD KTSPQRMT NHIRNILY NHLRNILI	PTLLLRD LSIVCSL FFCGLFC GGVVAFS	TRTFTQR AAGFCAAS LLEFCFY ILEFCFY	LADFFA SCYVTF ISGVLF LSGILF	DPTNISI RRGFNGEF VLLVHGVE VLFVDGPE	/LISLAA\ AINTFDVF DYLDFNAJ DGLDFKAJ	/SYYF Enpsy Erpgl	SEAATFL SDFPMRL TPFPQAL S	LIMGG HYETP HPTIF HPTIF	ilfflysy Lylg Qyiskhh Dhiqnch	TRKQKL YASTYF GDPKLF HHPELY	PFRLP HRGLS GIANS SLELK	QISRAKD VYIFTSLI SLKLHGSFI (IKLIISSI	YNDLKP AVLSSQ FAPIGL ALPVVV	GINKPN LIISLR VCLVAN LMIILN	I RGITI KHHG RHYLI N RERII	FFGNDR FA-RHA EDHRPF EEHRPF	KTGEELH EIGEMQH KKKESLH RKKESLH k.gE.1h	Fanddhi Agylqry Gdsrhas Gdskhas	SRI ERD
DotL AgroYirD4 YirD4Anapl YirD4Wolb Consensus	131   LIFGST KGPIF( IRKIGI IRKAGI	140 IGSGKTET GKTCGPLH RSRRGIL RSKKGLL s.k	150 +++ LVSLSYN FGS LGKDQRG LGKDKRG 1gs	ALVQGSGI YLTNGEQI YLVADGY( YFIADGY( ylv.g	160 FIYVDG PHSLVV QHALLF QHALLF .h.1	170 KGDNSLYF APTRAGKO APTGSGKO APTGSGKO apt,sgkg	IKVFSHVF GVGIVIPI GVGFVIPI GVGFVIPI GVGFVIPI	L80 RSMGR TLLTF NLLFH NLLFH NLLFH	19 EDDLLLI KGSVIAL EDSVIVH TDSVIVH edsvi	00 INFHTG DVKGE DVKLE DVKLE DIKLE , <b>#.k.</b> e	200 ARDIVGP LFELTSR NYDLTSG NYEITSG	QEKRLS ARKASG HRKKIG HRERQG •rkr•g	210 + NTLNP DAVFK QEVYV QKVYV #.V	220 FCQGSSSI FSPLDSEI WNPAQPDO WNPAQPDO f.ps.	0 HLTQLV RKTHCY GISHCY GISHCY thcy	230 VSLMGS NPVLDII NPLDHI NPLEHI npli	SGQSSDI ATLPPEI SKKPG SEKPG SEKPG	240 GDMHKG RQFTET -QMYDD -QMYDD -QMYDD .#n	250 RAIAF RRLAANL YQKIANL YQKIANL raanl	YEALMRI ITAKGK( IMPE IMPE !_a	260 1 .LYY iAEG •QDF •QDF
DotL AgroYirD4 YirD4Anapl YirD4Wolb Consensus	261   HRDEGI FIDGAI HYNEAI HQNEAI ++ <sup>#ear</sup>	270 AILLDANT RDLFVAGI RSLFVGVV RSLFVGVV r.Lfva	280 IRNYFDL LTCIE LYLLAVP LYLLAAP 1	QRLESIY RGTPTI- EKYKSF- EKYKSF- si.	290 + -GAVYD -GEVVR -GEVVR -GEVVR .g.V	PRDDQESVI PRDDQESVI DLFAQPGEI RTMRSDDV\ RTMRSDDV\	IETIPKI (YKLFAQI /YNLAVVI /YNLAVVI .y.l	310 VTDP AEES DT DT	LRNYLNT LNKEAQR IGKKIHF MGKIIHF 1.k	20 TLPGYN RI-FDN YVAYMN YVAYMN	330 IKEKKGKQ IMAGN IIAAF IIAAF	VSQYLE DTKILT LQKADK LQKADK k.1.	340 QHGFI SYTSY ERSGY ERSGY	JSU THQLYRSI LGDGGI ISTMNSSI VSTMNSSI	U FSSLAD LNLHAD LELHAN LELHAN LELHAN	360 TYGHII PLI PLI PLI PLI PLI	RTNLAE KAATSR DTATAS DTATAS Lata.	370 VDFKDV SDFSVY SDFNIQ SDFNIL SDFNIL	JBU VLNRRIL DLRRKKT DFKRKRY DFKRKKY dl.Rk	VVLLPAL CIYLCVS SVYVGV1 TVYVGL1 •!yl	390 1 .EKS PND IPDN IPDN IPDN
DotL AgroYirD4 YirD4Anapl YirD4Wolb Consensus	391   PDELSI LEVLAI LTRLRI LNRLKI 1L.I	400 NLGKIIVS PLHRLLFQ PLHQVFYQ PLHQVFYQ PLHQVFYQ	410 SLKAMMA QLVSILQ QATEFLC QATEFLC ql\$.	AGLGEEVI RSLPGKDI RTLPSDDI RKLPSDDI r.Lpd	420 GDYRD 	430 ••••••••••••••••••••••••••••••••••••	IAPTPYMC CHEVLF PYGVLF PYGVLF	440 CILDE FLLDE FLMDE FLMDE F1\$DE	45 YGYYA-V FKHLGKL FPTLGKH FPTLGKH %lgk.	50 /QGFAY EAIET 1EQFQT 1EQFQT 1EQFQT	460 VPAQARS AITTIAG GIAYFRG GIAYFRG .iarg	LGFSAI YKGRFM YRVRLFI YRVRLFI 9++F++	470 + FAGQD FIIQS LIIQD LIVQD fi.Qd	480 LPAFQKAS LSALTGT TEQLKGI TEQLKGI I1.al.g.	0 \$KEEAA YDDAGK YEEAGM YEEAGM YEEAGM Y.#ag.	490 -SIGAN QNFLSN NSFLSN NSFLSN SFLSN	TNIKIC TGVQVF STYRITI STYRITI	500 1KLEDP 1ATAD- FAANN- FAANN- na#.	510 TETHDFF DETPTYI IETANLI IETANLI .ETi	TKTAGE( Skaige Sqlign Sqlign Sqlign Sk.ig#,	520   IYVT .YTF .KTV .KTV .yt.
DotL AgroYirD4 YirD4Anapl YirD4Wolb Consensus	521   KYDSF( KARST! SQESLI QQESLI kS.	530 TKETSIA 5YSQA NRPKFLDL NKPKFLDL	540 NSYMDTK SMFDHNI NPASRSL NPASRSL	SSSFEKRI QISDQGAI HISDTQRI HISETQRI .iSrl	550 ARVDLL ALLRPE ALLLPQ ALLLPQ ALLLPQ ALL_PQ	560 DLKEQTE( QVRLLDD( EVIMLPKI EIIMLPR( #1	EAHIFF SEIVLI EQILLIE EQIILIE ,e,iili	570 SKI- KGRPF STYF STYF STYF STYF	VRARH LKLRKVQ IKSRKIK IKSKKIL	30 <b>IFYANP</b> QYYSDR (YFEDK _YYSDN .%%, #.	590 PKPVKQLK TL-KRLF TFTRRLL ITFTRKLL Lk.L.	INQ LK ERQ GS KST IP KQT VP 9	600 VEPPP LPEPA TQEPY TQEPY eP.	610 DDYLMKLO PLML DPEKIRGO DPNKVFSO dp	0 + QKQLAS GVEGEA GA	620 FQSILE SDY GIVSEA NKSI	5GDLSI 5NDQYQ 5ENSEDI KYDNKEI 8.#1	530 NKAYEN SHLAEI LKALMG NDATEG nka.e.	640 EEITLIS ANFNEDA SSSAENP PNAADYL	KALKEST Aprnrty Peraey( Vea	650 1 'IVE 'AED iYED QT
DotL AgroYirD4 YirD4Anapl YirD4Holb Consensus	651   PIERG HGSVK DGDLYI KDAVYI	660 VAALIAFH VGADI DREEF DESEI V.a.i	670 	VEDIVEE HGINGDE IGEEPED EEEIDDE .e.i.##	580 EVEGAL DQADAR DLEDLO DVDGFO #vega.	690 TIFSKLRJ REIPPESV\ DESDDYFHI DEEDYGKL	(DPNAPP) /PPELTLf )EDDYL-H _KGDGKPH .+P <sup>#</sup> ++P	700 CLVAD ALAAQ ADEDY KEEKY L.a.	71 KEVFSEP QQLLDQI DDPYEDD DDEFDNE .#.f.#.	LU -+ PLLPIN [I )M F	720 IETRNQMI Alqqrsr Elegdgd Ededdgd	TIERLA Sapaqp Hdenea Kpksng ea	730 GAKDK AK FEDEL K	74 (Yagtvani LDDEVYDI	U ELIKDF DEFDSL	750 QIATSYI EPEEDEI	PPEERD' DFEEDD ee.d	760 710YQE 6LEEED	LTGIIRD EFGEDRP	LSAKISA TDDND <mark>S</mark> S	780 1 IERE NGR
DotL AgroYirD4 YirD4Anap1 YirD4Wolb Consensus	781   KANKKI LKS	791 +  RAEELT							CTD	)							Fiç	g. S2	Whita	.ker/C	hristi

D

E (A)

CTD



(B)



Fig. S2 Christie/Whitaker

		Charge	pl
VirE2	RLPADAAGVLGEAADK <u>YS</u> RD <u>FVRP</u> EPASRPISDSRRIYES <mark>RP</mark> RSQSVNSF	+2	9.98
VirE3	DYH <u>LSAS</u> EQEN <u>LL</u> NQ <u>LLSVPLPIPSPKPKSARSMIF</u> E <u>GS<mark>RP</mark>RERS</u> T <u>S</u> R <u>GF</u>	+2.1	10.25
VirF	N <u>VAEPIMF</u> NE <u>ISALEVMA</u> EVRPI <u>A</u> RSIKT <u>A</u> HDD <u>ARA</u> E <u>LMSA</u> D <mark>RPRSTRGL</mark>	-0.9	5.54
Ats-1	<u>AAMQQAVLSAARGLS</u> DVSHDD <u>SAQTQGNP</u> TVT <u>PLVSA</u> QN <mark>RGP</mark> ETH <u>G</u> KGTR	+0.2	8.01
WD0636	<u>IVEELVKAGAEIEQADKFGMTAMDYAKNSKEVTEVLKKETDRIEKLFEKL</u>	-3.0	4.54
WD0811	T <u>ASKRTEQPKNDAPKKAGEP</u> ENNNH <u>S</u> TLFEQIRGGTTLKRVGSNKILQMN	+4.1	10.65
WalE1	<u>SSTLTRKQVLPLKEEFDRELEEKLAKRLASL</u> DQPSAEPVN <u>SRA</u> TATPGTV	0	6.99

Fig. S3 Whitaker/Christie



Fig S4. Whitaker/Christie