	BCAP-TIR (I3C)
Data Collection	
Space group	P62 2 2
Cell dimensions	
$a = b, c (\text{\AA})$	135.04, 42.91
Resolution (Å)	44.2–2.5
Total reflections	99543 (9785)
Unique reflections	15066 (1489)
Ι/σΙ	24.8(7.2)
Completeness (%)	99(98)
Redundancy	6.6(6.6)
Wilson B-factor	53.84
R-merge	0.047 (0.24)
R-meas	0.051 (0.26)
CC1/2	0.99 (0.98)
CC*	1.0 (1.0)
Phaser-EP FOM	0.35
Refinement	
No. reflections	15066 (1489)
R_{work}/R_{free}	0.19/0.24
No. atoms	
Protein	1088
Ligands	32
Avg. B-factor	59.7
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (Degrees)	1.60
Validation	
Ramachandran favoured/allowed (%)	96/100
Rotamer Outliers (%)	7.8
Clashscore	7.7

Supplementary Table S1: Crystallographic Statistics for the N-terminal TIR domain of human BCAP

		βΑ	αΑ	βВ		αB
	10	。 → ⊉2.		40	50	60
BCAP MAL TLR1 TLR2 TLR6 TLR10	SRWSKD NIPLEELQRNLQ IC	CDILIVYSPD. YDVCVCHSEE. FHAFISYSGHI YDAFVSYSERI FHAFISYSEHI FHAFISY <mark>S</mark> EHI	AEEWCQYLQTLFLSS DLVAAQDLVSYLEGS SFWVKNELLPNLEKG AYWVENLMVQELENFI SAWVKSELVPVEKE SLWVKNELIPNLE.KI	RQV.RSQKILTHR TTPGGAIVSELCQ GMQICLHE NPPFKLCLHKI DIQICLHE EDSILICLYE	LGPEASFS RNFVPGKSIV RDFIPGKWII RNFVPGKSIV SYFDPGKSIS	AEDLSLF.L L.SS E.NIITCIE D.NIIDSIE E.NIINCIE E.NIVSFIE
	$\beta \xrightarrow{\beta C} 22$	αC' 2222••2 80	αC 222.2.22	ء ٩	βD	αD 22222 110
BCAP MAL TLR1 TLR2 TLR6 TLR10	STRCVVVLLSAE SHCRVLLITPG KSYKSIFVLSPN KSHKTVFVLSEN KSYKSIFVLSPN KSYKSIFVLSPN	LVQHFHKPS FLQDPWCK FVQSEW FVKSEW FVQSEW FVQNEW	SLLPL.L.Q.R.AF .YQMLQAL.T.EE .CH.Y.EL.Y.FA .SK.Y.ELDF.SH .CH.Y.ELYFA.HH .CH.Y.EF.YFAH.	HPPH GCT HHNLFHEG.SNSL .FRLFA NLFHEGSNNL	VRLICGVRD IPLLS.GL.S ILIL.EP.I ILIL.EP.I ILIL.EP.I ILIL.EP.I ILIL.EP.I	O.SEEFLD RAAYP.P PQYSI.PSS EKKAI.PQR PQNSI.PNK PFYCI.PTR
	120	βE	αE 00000000000 130 140	٤		
BCAP MAL TLR1 TLR2 TLR6 TLR10	FFPDWA.H.K E.LRFM.Y YHKLKSLMA.RRY FCKLRKIMN.TKY YHKLKALMT.QRY YHKLKALLE.KK	WQELTCDE YYVDGRGPDG. TYLEWPKE TYLEWPKE TYLQWPKE AYLEWPKE	DEPETYÜAAVKKA KSKRGLFWANLRAAI DEAQREGFWUNLRAAI KSKRGLFWANIRAAF DRRKCGLFW <mark>ANLRAAI</mark>	IS MRYLQTLS. NIKLTEQAK KS N		

Supplementary Fig. S1: Structural alignment of the BCAP-TIR and the TIR-domains of MAL (2y92), TLR1 (1fyv), TLR2 (1077), TLR6 (40m7) and TLR10 (2j67). The secondary structural elements shown are those of the BCAP-TIR domain (5for).



Supplementary Fig. S2: Size exclusion chromatography in combination with multi-angle light scattering (SEC-MALS) and differential refractometry data for the N-terminal TIR domain of BCAP (A), BCAP₃₃₀ (B) and BCAP_L (C). Plots show the normalised UV (280 nm) light scattering, refractive index and weight-averaged molecular mass (MW) variation across the peak in addition to the MW at the peak. The molecular weights obtained by SEC-MALS are in complete agreement with those obtained by sedimentation velocity.)



Supplementary Fig. S3: The highly conserved proline residue (P49) in the β B-loop of BCAP is not required for Mal/TIRAP interaction. HEK293T cells were transfected with FLAG-Mal, Myc-Mal and Myc-BCAP as described. Both β B loop proline mutations (P49A/P49D) allowed the BCAP-Mal/TIRAP interaction (lanes 4–5) to occur. Mutations in the α C' helix (L75A) and outside the TIR domain (L401A) both exhibit reduced interaction with Mal/TIRAP (lanes 2–3) while the D117A (lane 1) mutation which lies in the region connecting the α D helix and β D strand completely abolishes the BCAP and Mal/TIRAP interaction.



Supplementary Fig. S4: Interaction of BCAP-TDA and various TIR domain containing proteins determined by Yeast two-hybrid. Panels (A) and (C) were used to access transformation and mating efficiency on SD/-Trp/-Leu media. BCAP-TDA interacts with wild-type MAL/TIRAP and its mutants D96N and S180L (3, 5 and 7)(B) and with the TIR domains of TLR2 and TLR4 (D) (11 and 13) respectively on SD/-Trp/-Leu/-His/-Ade/+X- α -Gal+5mM 3-AT. The full matrix of the prey and bait plasmids used are shown below.

	pGADT7	pGADT7[TIRAP]	pGADT7[D96N]	pGADT7[S180L]
pGBKT7[BCAP-TDA]	1	3	5	7
pGBKT7	2	4	6	8
	pGADT7	pGADT7[TLR2]	pGADT7[TLR4]	pGADT7[TIRAP]
	1	I - I J	L	pointrilinen
pGBKT7[BCAP-TDA]	9	11	13	15

Supplementary Table S2: Plasmids and primers used for protein expression in bacteria, yeast and human cells. The restriction sites used, when relevant are shown.

Protein		Oligonucleotide Sequence $(5' \rightarrow 3')$	Vector
BCAP-TIR(F)		TACTTCCAATCCAATGCCCCCAGAGGATGCGACATCCTCATC	pMCSG7
BCAP-TIR(R)		TTATCCACTTCCAATGTTAGGAAATGGCTTTTTTCACAGCTGCCAC	
$BCAP_S(F)$		TACTTCCAATCCAATGCCATGGTGGTGCAGCCGGACCG	pMCSG7
$BCAP_{L/S}(R)$		TTATCCACTTCCAATGTTACTTCTCGAACTGGGGGGGGGACTC	pMCSG7
		CAGCGTCCTCTGGGTGG	
$BCAP_{L/330}(F)$		TACTTCCAATCCAATGCCATGGCAGCCTCAGGG	pMCSG7
BCAP ₃₃₀ (R)		TTATCCACTTCCAATGTTACTTTTCAAACTGCGGATGCGACCAG	
		GCACTGGCATTTGTCATCATATCTTCTTCTTCCAGCTGGTTG	
FLAG-BCAPL	(F)	ATCAAGCTTATGGCAGCCTCAGGGGTGCC (HindIII)	pCMV10
FLAG-BCAPL	(R)	ATCGGATCCTCAGCGTCCTCTGGGTGGAACAGG (BamHI)	
Myc-BCAP _L	(F)	Same as FLAG-BCAP _L (F) (HindIII)	pCMV-Myc
Myc-BCAP _L	(R)	ATCGGTACCGCGTCCTCTGGGTGGAACAGG (KpnI)	
Myc-BCAP-SV	' (F)	ATCAAGCTTATGGTGGTGCAGCCGGACCG (HindIII)	pCMV-Myc
Myc-BCAP-SV	' (R)	ATCGGTACCGCGTCCTCTGGGTGGAACAGG (KpnI)	
BCAP-TDA	(F)	ATCCATATGATGGCAGCCTCAGGGGTGCC (NdeI)	pGBKT7 AD
BCAP-TDA	(R)	ATCGGATCCTCACGAGCATTTCATAAGCAGGTCTGTGG (BamHI)	
MAL	(F)	ATCCATATGATGGCATCATCGACCTCCCTCCC (NdeI)	pGBKT7 AD
MAL	(R)	ATCGGATCCTCAAAGTAGATCAGATACTGTAGCTGAATCCCG (BamHI)	
TLR2-TIR	(F)	ATCCATATGAGCAGGAACATCTGCTATGATGCATTTGTTTC (NdeI)	pGBKT7 AD
TLR2-TIR	(R)	ATCGGATCCTCAGGACTTTATCGCAGCTCTCAGATTTACC (BamHI)	
TLR4-TIR	(F)	ATCCATATGGAAAACATCTATGATGCCTTTGTTATCTACTCAAG (NdeI)	pGBKT7 AD
TLR4-TIR	(R)	TCGGATCCTCATGATTTACCATCCAGCAGGGCTTTTCTG (BamHI)	