Supplementary Figure 1: Sequence alignment of CTNNBL1 from diverse animal species. Numbering and secondary structures for human CTNNBL1 are shown on top of the alignment. NAM, NTD, ARM, CTD stand for the N-terminal anchoring motif, N-terminal domain, ARM domain, and C-terminal domain respectively. Residues are color-coded based on properties. The highly conserved residues ( $80 \%$ or more) are highlighted by background coloring.








|  |  | ARM5 H3 | ARM6 H1 | ARM6 H2 |  |  | ARM6 H3 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 360 | 370 | 380 | 390 |  | 400 | 410 |  |
| Human | 352 | SALKVLDHAMIGPEG |  |  |  | K K V G T T | K EH |  | 41 |
| Gorilla | 352 | P E | DNCHKFVDI | RTIFP | K | K K V | EKEHEEHV |  | 411 |
| Chimpanzee | 35 | GPE | DNCHKFVD | RTIFP | K | K K V G T | ekeheehv |  | 411 |
| Orangutan | 352 | 1 GPE | D N | K | K | K K V G T | Keheehvo | C S 1 | 411 |
| Macaque | 352 | SALKVLDHAMIGPE | DNCHKFVDI | RTIFPL | PR ${ }^{\text {P }}$ IK K | K | EKEHEEHVC | CS IL | 411 |
| Marm | 352 | ALKVLDHAMIGPEG | DNCHKFVD | RTIFPLFM | K | K K V | EKEHEEHVC | S 1 | 411 |
| Cow | 352 | GPE | DNCHKFVDI | RTIFP | K | K K V G T | ekeheehv | C S 1 | 411 |
| GuineaPig | 352 | KVLDHAMIGPEG | TDNCHKFVDI | R | RKIK | KKVGTTE | EKEHEEHVC | C S 1 | 411 |
| Dog | 354 | SALKVLDHAMIGPEG | TDNCHKFVDILGL | RTIFPL | K | K KVG T | EKEHEEHVC | C S 1 | 413 |
| Horse | 353 | SALKVLDHAMIGPEG | TDNCHKFVDI | RTIFPL | K | K | EKEHEE | S | 412 |
| Elephant | 354 | SALKVLDHAMIGPEG | TDNCHKFVDI | RTIFPL | K | K | ekeheehv | CS 1 | 413 |
| Rat | 352 | SALKVLDHAMIGPEG | ADNCHKFVDILG | RTIFPL | RKIK | K K V G T | EKEHEEHVC | c | 11 |
| ous | 352 | SALKVLDHAMIGPEG | TDNCHKFVDI | RTIFPL | PRK I K | K KVGT | EKEHEEHVC | C S 1 | 11 |
| Opossum | 357 | SALKVLDHAMIGPEG | TDNCHKFVDI | RTIFPL | PKK I K | KKVGTTE | EKEHEEHV | CS IL | 416 |
| Chick | 353 | KVLDHAMIGPEG | TDNCHKFVDI | RTIFPL |  | K KVGT | EKEHEEHVC | CS 1 | 412 |
| Coelacanth | 350 | SALKVLDHAMIGPEG | TDNCHKFVDI | RTIFPL | K | KKAGV | EKEHEEHVC | CS | 09 |
| Bat | 352 | SALKVLDHAMIGPEG | THCCRKFVDILGL | RTIFPL | K | KKVGTTE | EKEQEEHVC | A | 411 |
| Zebrafis | 35 | SALKVLDYGMIGPEG | SDNCHKFVDI | RTIFPL |  | KKVGISD | DKQHEEHV |  | 411 |
| Toad | 344 | SALRVLDHAMIGPE | DNCHKFVDI | R TIFPL |  | KKTGVS | EKEHEEHVC |  | 03 |
| Salmon | 350 | SALRVLDHGMIGPEG | SDNCHKFVDI | P L |  | RKAGAAD | dKEHEEHVC | C | 409 |
| Tick | 354 | GALKVLDFATSNMEG | TDNCNKFVDI | RTVFPLFMQ | K | KKKVASP | PEEHEEHVC | C | 413 |
| Mosquito | 363 | GSLKVLDHAVSGPDG | RDNCNKFVDI | RTIFPLFMK |  | KKRLLSt | TDEHEEHVV |  | 422 |
| FruitFly | 376 | KVLDHAMAGQDG | RDNCNKFVEILG | PL | NK | KQRLISA | ADEHEEHV |  | 435 |
| Beetle | 356 | GSLKVLDYAMSGPHG | KDNCNKFVDILG | P | KKNR | RKKVLS | TEEHEEHVT | TS | 15 |
| Placozoa | 357 | GAIKTLTYVLSNYNG | pdCAVKFIELFg | RSLFPL | KMF | KKLTNE | ESEHIEHIC | CSIM | 415 |
| Bee | 380 | GSLKVLDHAMNGPDG | KDNCSKFVDILG | RTIFPL | KNR | RKRMLTA | AEEHEEHVI |  | 39 |
| Flea | 356 | GALKVLNHALSGEAG | KDNCTKFVDILG | RTIFPLFM | L K | KRKGM | AEEYEEHIV |  | 415 |
| Ant | 381 | GSLKVLDYAMNGPDG | KDNCSKFVDILG | RTIFPLFMK | TNNR | RKKMLTA | AEEHEEHVV | vs I | 440 |
| SilkWorm | 354 | GSLKVLDHALVGPDG | RDNCNKFVDILGL | RTVFPL |  | RKRLLTV | VDQHEEHVV | vs I | 11 |
|  | 355 | GSLKVLDHALAGPDG | KDNCNKFVDILG | RTVFPLFM |  | RKRILTL | LDQHEEHVV |  | 2 |
| Lice | 322 | GSLKVLDHAMSGPEG | KDNCNKFVDILGL | RTIFPLFM | KNR | RKKVITV | veehemhvi | LS IV | 381 |
| Worm | 335 | KVLNHATSGDEG | IENCNKLVEMLGL | RTIFPLFMR |  | KRKDT ${ }^{\text {c }}$ | deheehvc | ctil | 394 |





Figure S2: a) Preparation of the full-length CTNNBL1 and CTNNBL1 ${ }^{\text {SF }}$ protein samples. Lane 1: overexpressed full-length CTNNBL1 in the supernatant; lane 2: full-length CTNNBL1 after purification by NTA resin; lane 3: full-length CTNNBL1 after the fusion tag was cleaved by HRV 3C protease; lane 4: fulllength CTNNBL1 after the fusion tag was separated from the target protein; lane 5: pure full-length CTNNBL1 after purification by SP-Seharose chromatography; lane 6: pure CTNNBL1 ${ }^{\mathrm{SF}}$ after purification by SP-Seharose chromatography. b) Verifying the presence of full-length protein in crystals. Lane 1: full-length CTNNBL1 before crystallization; lane 2: protein sample from washed and re-dissolved crystals.
a) b)


Figure S3: Superimposition of the structures of full-length CTNNBL1 (red) and CTNNBL1 ${ }^{\text {SF }}$ (blue). NAM, N-terminal anchoring motif; NTD, N-terminal domain; ARM, armadillo domain; CTD, C-terminal domain. The numbers 1-6 indicate the six ARM repeats within the ARM domain.


NAM

Figure S4: Comparison of the structures of CTNNBL1 and p115 (pdb code 2W3C).


