

A Full-length alignments of *Aiptasia* and other *Npc2* proteins

Aiptasia *Npc2*D MKPAISLALVVIIAAVTTSIQAMKLFKDCGSQVGE--IVSLDVTPCTSDPCSLKRGGTNA
 Anemonia *Npc2*D MKFLVLLLCLQIIWSLS--EARKLSFKDCGSKVGK--LVSFDLSPCSQDPCIIKR-GSNA
 Human *Npc2* MRFLAATFLLLALSTAA--QAEPVQFKDCGSVDGV--IKEVNVSPCPTQPCQLSK-GQSY
 Drosophila *Npc2*A MLRYAVIACAALVVF----AGALEFSDCGSKTGKFRVAIEGCDTTKAECILKR-NTTV
 Aiptasia *Npc2*A -----
 Anemonia *Npc2*A MAKFFLIACMLYVLSLA--GAEVVDFDDCSGGKKGKGEIEKLEIIPCPTQPCQLKK-GSKV

Aiptasia *Npc2*D TVTINFKPHEQVTQSKIYVYAIIGIIPILPIPNPDAC TGHGLT C PLASGKDVELVVKQS
 Anemonia *Npc2*D TGTVTFIPSEEVTSKVMYAIIGFIPVPLPLPNTDGCCKGYGLT C PLKSGKPDELVFSHS
 Human *Npc2* SVNVTFTSNIQSKSSKAVVHGILMGVPVFPFPIPEPDGCKS-GINCP IQDKTYSYLNKLP
 Drosophila *Npc2*A SFSIDFALAE EATAVKT VVHGKVLGIEMPFPLANPDACVDSGLK C PLEKDESYRYTATLP
 Aiptasia *Npc2*A SVKRTFIPHENVTDAESSVHGKVMGFVVPFPLPNAHACKDSGVK C PLVAGSKYEYSSTLD
 Anemonia *Npc2*A QIKVTFVPHEDL TEATS VVHGEIGGFVVPFPLPNSNCCKDSGLT C PLKAGQKYVYTSALD

Aiptasia *Npc2*D IDSTFPAGKVTVKAE LKDQVQNNVLCGEVTLTLM---
 Anemonia *Npc2*D IDSTFPAGTVTLKGELKDQEENNIFCGKISLTLQ---
 Human *Npc2* VKSEYPSIKLVVEWQLQDDKNQSLFCWEIPVQIVSHL
 Drosophila *Npc2*A VLRSYPKVSVLVKWELQDQDGADIICVEIPAKIQ---
 Aiptasia *Npc2*A IKSAYPAISVVVKWQLQDGKGQDLYCFEVS AKIVS--
 Anemonia *Npc2*A VKSEYPAIKVVVKWEMQDKDNDVFCFKVATQIVS--

B Multiple-sequence alignment used to generate Npc2 phylogenetic tree

```

A digitifera_Npc2E      -KNCT--KNDDVTVESLDIN---PCSE-EP---CIFHK-GSTVSVTVAF-TPLEEVKSGE
A digitifera_Npc2F      -KNCA--SRKYALPLKVAIN---PCTK-QP---CTLHP-GKKASIAVVV-KPLVTIRRG
O_carmela_Npc2a         -SNCTSNPGPSTLGTKVNVTAVPDCD-AP---CVVHQ-GESLNVTVTF-VPNVAIENFT
A digitifera_Npc2D      -QTC---DKPSGRRLNSVDVT---PCNG-NP---CVFKR-GTNETITVTF-TPNEVVSCKG
Aiptasia_Npc2E          -KDC---GSKGATIVRLDIS---PCEE-EP---CNFKT-GTTVTGTLTF-VAKEYFTSGR
N_vectensis_Npc2B      -RDC---GSQGEIVGMDIS---PCDS-EP---CVLKR-GTSVDGSLTF-IPHEDLKRAK
Aiptasia_Npc2B          VVVL---VVVVGIVVVVDVD---QCTSDDP---CSLKR-GTNVTSTATM-IPLEEVQAT
Aiptasia_Npc2C          -TDC---GSSYLGEIHSLEVN---PCTS-DP---CVLKR-GDNMTSVISF-TPHEQVSAK
Aiptasia_Npc2D          -KDC---GSQVGEIVSLDVT---PCTS-DP---CSLKRGTNATVTINF-KPHQVTSQSK
N_vectensis_Npc2C      -QDC---GSKKGLIISVDLT---PCSS-DP---CVIKR-GANASGVITF-IPHEVVTSSK
A_viridis_Npc2D        -KDC---GSKVGLVSVFDSL---PCSQ-DP---CIIKR-GSNATGTVTF-IPSEEVTSK
M_faveolata_Npc2B      -ANCSVDTALEGLIISVDLT---PCPS-QP---CVPHK-GTNVTATIKF-SPEEMVTDGT
D_melanogaster_Npc2A   -SDC---GSKTKGFTRVAIE---GCDD-TK-AECILKR-NTTVSFSIDF-ALAEETAVK
M_faveolata_Npc2A      -ADC---GSL-AKINFDVVS---PCVM-EP---CELKK-GTNESIEIQF-IPNSNITEG
A digitifera_Npc2B      -RDC---GNKELSPAQVIIIT---PCPA-EP---CQLKK-GVNESVEVIF-KPTEVVTSAK
A digitifera_Npc2A      -SYI---GSKESISQVIVT---PCPA-EP---CQLKK-GVNESIEVIF-KPGEVVTSSK
Human_Npc2a             -KDC---GSSVDGVIKEVNVS---PCPT-QP---CQLSK-GQSYSVNVTF-TSNIQSKSSK
Mouse_Npc2A             -KDC---GSKVGIKEVNVS---PCPT-DP---CQLHK-GQSYSVNITF-TSGTQSQNST
Aiptasia_Npc2A          -----K-ELKSSVKRTF-IPHENVTDAE
N_vectensis_Npc2A      -KDCSG-GKGEIEVELDIS---PCPT-QP---CTLHK-GTTVSVNITF-VPHVTLDSGK
A_viridis_Npc2D        -DDCSG-GKGEIEIETLEII---PCPT-QP---CQLKK-GSKVQIKVTF-VPHEDLTEAT
H_magnipapillata_Npc2D -QNC---GHLDNNTI-VSIT---PCEK-EP---CTLVR-GSNATLEIQF-KAKHFSKQLK
H_magnipapillata_Npc2B -KPC---DMSSTVGDVAIS---PCDK-QP---CAFQR-GGSANIEISF-TAAKDADKLT
H_magnipapillata_Npc2A -KKCTS-PASSAVIGDVIIIT---PCDS-LP---CSFKR-GSGNLIKINF-QATKNSSELT
H_magnipapillata_Npc2C -KKCSS-PASSAVVGDVVIS---PCDN-QP---CQFIR-GGNANIQIHF-QAKKDNSNIT

LSVDAI-AFGHRLP-M--VRKE--NICEG--HGVT-----CPLEKGGKQTFPTINQKVERY
LELYGIHWLGIKFP-LS-VPNP--DICHG--YGTR-----CPMIANSRVVLSISQTLPSF
VVVHAS-VGIIHVP-YP-VTDP--NGCDTAVTGVT-----CPLKANVAVEWHHSFVSPI
ILLYAK-LVLGWIE-LS-LRNP--NICEG--YGLK-----CPLAKGVREELSVTERVPQV
VKAYAV-IEGVDLPLP-IPT--DACQG--YGLT-----CPINNGQTANFVIKQEIQAD
LSAHAI-IDKLPLP-LP-IPS--DACQG--YGLS-----CPVDSGVKSMFKIHQAIIESE
IYMHAT-VSGITIP-ID-IPNP--NACSG--HGLS-----CPLKSGETVELSMVLEVEAK
IDINAI-IAGSPH-VH-IPNP--NACDG--HGLK-----CPLKGGKVELVVSQVIRRS
IYVYAI-IGIIPIP-LP-IPNP--DACTG--HGLT-----CPLASGKDELVVKQSIDST
VLAYAI-FGLIPVP-LP-LPNS--DGCKG--YGLT-----CPLKSGKQVELVFEHYIDQT
VYMYAI-IGFIPVP-LP-LPNT--DGCKG--YGLT-----CPLKSGKDELVFSHSIDST
LQVYGF-IEGIKTP-PP-LEQP--DACKE--HGLE-----CPLKSGVTYSLEITLAIKPA
TVVHGK-VLGIEMP-PP-LANP--DACVD--SGLK-----CPLKEDESYRYTATLPVLR
TVVYGI-IEGVQVP-PP-VDNP--EVCKE--HGIT-----CPMPAECTQTFKATLPVKSE
VVIHGI-IEGVRFP-PP-FPHP--NGCKE--HGLE-----CPLKPNKEYTFKATLPVKRT
VVVHGI-IAGVVVP-PP-ISQP--NGCED--HGLD-----CPLQPNKEYTFKATLPVKS
AVVHGI-LMGVVP-PP-IPEP--DGCKS--GIN-----CPIQKDKTYSYLNKLPVKSE
ALVHGI-LEGIRVP-PP-IPEP--DGCKS--GIN-----CPIQKDKVYSYLNKLPVKNE
SSVHGK-VMGFVVP-PP-LPNA--HACKD--SGVK-----CPLVAGSKYEYSSTLDIKSA
AIVHGV-IAGIPVP-PP-LPNA--DVCKN--SGLK-----CPLPQKTYVYQSSLEVKTM
SVVHGE-IGGFVVP-PP-LPNS--NCCKD--SGLT-----CPLKAGQKYVYTSALDVKSE
TKVYVK--LLFWVPYYN-FGKE--DSCLD--NGIT-----CPVIEDEEYSYQSLSLHISK
TVVKGK-IGPIWVP-PP-LSQP--DACNN--EGLT-----CPIKSSQKTYQYSLPISSE
SVVKGK-IGPLWVP-PP-LSQP--DACQN--EGIT-----CPIKDGQSYLFSYDLPISTT
TIVKGG-IGPLWVP-PP-LSQP--DGCLN--DGII-----CPVKTDQQYVYSYDLPLSKS

YPLPI-DVEAYVENDNRK---ILC
VPMGSY-QLQAVMKDQLGR--M-VLC
APKGPVEIITWELQAPSKE--D-VAC
LPSSTR-EVKAKLVDQNGG--T-VVC
FPKVKL-QLKGEVMDPQGN--M-LFC
FPVGNL-TLKAAVTDSDTSS--QVVFC
FPRGKV-ILKTELKDQAKN--D-IFC
APPGRY-RIRTELKQYGI--D-VFC
FPAGKV-TVKAELKDQVQN--N-VLC
FPTGHL-TLKAELKDQSD--V-VIC
FPAGTV-TLKGELKQJEN--N-IFC
YPSIQL-VAQMDFKLPDDG--Y-LFC
YPKVSV-LVKWELQDQDGA--D-IIC
YPALQL-DVKWELHDQDAK--V-VYC
YQDVCM-I---RLL-----CSC
YDIKL-VVKWQLLDQAN--S-VFC
YPSIKL-VVEWQLQDDKNQ--S-LFC
YPSIKL-VVEWQLLEDDKKN--N-LFC
YPAISV-VVKWQLQDGGQ--D-LYC
YPSLKL-VVRWEIQDNKNK--D-VLC
YPAIKV-VVKWEMQDKDNN--D-VFC
NPKISI-FVKWLIQNEAEK--D-LVC
YPKINL-PVSWELKDEKGE--S-LVC
YPAISL-VVSWELQDENG--D-VVC
YPAISV-VVSWELQDENG--D-LVC

```

Figure S1 Alignments of Npc2 sequences from *Aiptasia* and other organisms. (A) Full-length alignment of selected Npc2-like proteins from *Aiptasia* sp. (this study), *A. viridis* (Ganot et al. 2011), human, and *D. melanogaster*. Red and green dots, amino acids that are identical (red) or similar (green: I,V,L; S,T; D,E; K,R; Q,N) between *Aiptasia* NpcD and human Npc2; pink shading,

amino acids whose mutation to alanine ablates the cholesterol-binding function of Npc2 in mammalian cells ([Ko et al. 2003](#)); blue shading, conserved cysteines used to identify conserved regions of the proteins for phylogenetic analysis; black underline, the conserved region used for phylogenetic analysis. (B, next page) The multiple-sequence alignment of the conserved regions used to produce the phylogenetic tree in Figure 2A.