SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Domain structure of Stbd1. The architecture of Stbd1 based on primary sequence is indicated, with the N-terminal hydrophobic segment, a putative leucine zipper and the N-terminal CBM20 domain. Shown also is W293, a residue conserved throughout the Stbd1 family and also present in the laforin CBM20 domain. The truncated forms of Stbd1 shown were identified in a yeast two-hybrid screen using a portion of Stbd1 itself as bait; the relative β -galactosidase activities are indicated alongside the catch.

Supplemental Figure 2. Sequence alignment of mammalian Stbd1s. Sequences were aligned with the Clustal algorithm, with darker shading denoting greater degrees of conservation among species. The orange box indicates the highly conserved hydrophobic N-terminal twenty four residues. The purple box encloses the putative leucine zipper motif. The red box encloses the conserved CBM20 domain.

Supplemental Figure 3. Subcellular localization of endogenous Stbd1 with respect to organelle markers in FL83B cells and Rat1Neo5 fibroblasts. FL83B cells (A) or Rat1Neo5 (B) cells were immunostained with antibodies directed towards mStbd1 (middle panels) and antibodies towards LAMP1, a lysosomal marker (lower left panel), HDEL, as an endoplasmic reticulum marker (middle panel), or against β -tubulin as a microtubule marker (right panel). The upper panels show merges of the images, with Stbd1 (red) and the corresponding organelle marker (green), with nuclei stained with Hoechst (blue). The scale bars are 20 µm.

Supplemental Figure 4. Subcellular localization of Stbd1 over-expressed in COS M9 cells with respect to organelle markers. Cells overexpressing full-length hStbd1 were immunostained with antibodies directed towards mStbd1 (middle

panels) and antibodies towards LAMP1, a lysosomal marker (A, lower panel), towards HDEL, as an endoplasmic reticulum marker (B, lower panel), towards syntaxin 6 as a Golgi marker (C, lower panel) or against β -tubulin as a microtubule marker (D, lower panel). The upper panels show merges of the images, with Stbd1 (red) and the corresponding organelle marker (green), with nuclei stained with Hoechst (blue). The scale bars are 20 µm.

Supplemental Figure 1



Supplemental Figure 2.

Homo sapiens Pan troglodytes Macaca mulatta Cavia porcellus Dipodomys ordii Mus musculus Rattus norvegicus Monodelphis domestica Ochotona princeps Felis catus Dasypus novemcinctus Loxodonta africana Erinaceus europaeus Equus caballus Pteropus vampyrus Bos taurus

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| _ | 10 | 20 3 | 0 40 | . 50 | . 60 | 70 |
|-----------------------|--|---|---|--|--|--|
| | MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG | ALFVWLLR3GPGD ALFVWLLR3GPGD ALFVWLLR3DPGD ALLVWLLR3GPGD ALFVWLLR3DPG ALFVWLLR3DSGA | G K D G D A E Q E K D A G K D G D A E Q E K D A G K D G D A K Q E K D A G K D G D A K Q E K D A O K D A E P R K G A P P O K D G V A E P P Q K G | PL-GGAAIPG-G PL-GGAAIPG-G PL-GAAAVPG-G RGSGTSPARG-G GEASAPG-G APPGEAAAPGDG | H 0 5 6 5 - 5 6 L 5 P H 0 5 6 5 - 5 6 L 5 P H 0 5 6 5 - 6 6 L 5 P P D 4 6 6 V - 6 6 KWR H 0 0 6 6 6 - 6 6 L 5 P P 6 6 6 6 5 - 6 6 L 5 P | GPSGQELVTKPEH GPSGQELVTKPEH GPSGQELVTKPEH GPCGLQIVIKLEH GLCKQELITKPEH EPSDRELVSKAEH |
| | MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGSLAG MGAVWSALLVGGSLAG MGAVWSALLVGGGLAG MGAFWSALLVGG <mark>S</mark> LAG | ALILWLLR DSGA ALFIWLLR DSGA ALFAWLLR DSPGD ALFVWLLR SGAGD ALFVWLLR DGAGD MLFVWLLR DGPGD | ≥GKDGGAEP・LKD GQDAGPRELLPA AGISRGAEPDKEA AGNESDAER・・NA AGNEGGTEQKKDA FGNEGAAEPK・DA | APPGEAAAPGGG ER - APAARG - G PT - REAATPGGR .PQ - GEAAAPG - S .PL - GETAVPG - G .PP - GETAGPG - G | PGGGGS-GGLSP DGGGNSGDPLDP PGGGDS-SGLSP DQGGG-GGPSP DQGGG-GGLSP DQGGG-GCS | EPSDRELVSKAEH KPKQRRISRNYH GPGKRELDSKAEQ VPSRRELVTKAEH GPSRLELVTKPEH VPFGLEPVTKPDH |
| 80 | MGAIWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG MGAVWSALLVGGGLAG 9 90 | ALFVWLLR····G ALFIWLLR····D ALFVWLLR····D ALFVWLLR····D 100 | KGQEGDAEPEKDS IGKEGDAEQEKDA IGKEGDAEKEKDV SGKEGNAEQRKDA 110 120 | LR - GEAEPLS - G SP - GEAATAG - G (SP - GEAAAAG - G PP - REAVAPG - G 130 | DDGGG GLSS DDGGG GGLSP DEGAR GGLSP DDGGGR - GGLSP 140 | GPSRPEPIRKAEH GPSKPELVTKPEH GPSTQELVTKPEY GPSAKPVIIGYEH 150 |
| | QESNGHLISKTKDLG QESNGHSISKTKDLG QESNGHLISETK QESNGHLISASKGLD RESNGHLISESKDLG | NLQAASWRLQN NLQAASWRLQN NLQAASWRLQN NLQEAMCAQKN NLPEAQR··LQ | PS REVCDNSRE PS REVCDNSRE LC GADGNKTR NV GADWVNARE | HVLSGQFPDTEA HVSSGQFPDTEA HCPAAQILDTH1 FVPVGKIPDTHS | PATSETSNSRSH SATSETSNSRSY LAASETGNSAGY RAD | SEVSRNESLESPM SEVLRNESLKSPM NEASENESLESHV SEALRNKSLESHG SEAARNQSPGSHG |
| L H Y L L L | RESNGHLISESKDLG- QESNGCLVSESKSS- QESNGSLISETRDLG- QESNGCLVSETKGHG- QESNGCLISETKGLG- QESNGCLISETKGLG- | | N V • • G • • • • NARE SSTQNLNCLRPG AT • • GG VHTG SQ PSG KDG DCD SSR PSG KNG NCDNSR PSG ED SD CDNSR | VVPVGKVPDTHS QVYSSQMLKMEF PVPSAN HVPSGQFPDTES HVPSGQLPDTES HVPSGQSPDTKS | 8 R AN P | ISETSRNOSPESRV ISEVLNSES GSHSGSQSLESPR /SAVSRNERLQSPL /SEVSGNES 'SDVSSNVSLDPK. |
| | QGSNGCLVSETKESG QESNGCLISETKGPG QESNGCLVSEPSGPG QESNGCLVSETKGP 30 170 | - NWQEAVWRQON - SLQAAACRRO - NAHEAAWRLOS - DLPGAAWRQOS 180 | PTGENGNCDOSRE KDGDCVSPR PSGEGGDCGSSRE PSGEAGNPDSSR 190 200 | YTPSRWLPDIGS HVPSGQFPETES HVPSGWFPDKES /HVPSGPFPDTES 210 | TAASEASNSRDY LTTSETGHSKGY LATSATGNSKSY LTTSETGNS 220 | 'PNVSGNESHESS 'SRNEREE 'SEVSRNESCES DISRHESLGSPM 230 |
| G G G E G | EWGFOKGOEISAKAA EWGFOKGOEISAKAA EWGFOKGOEISAKAA EKKFOKGOEIPAKAA ERSFOKGOMTPASAA EWRLPKGOETAVKVAG | TCFAEKLPSSNLLK TCFAEKLPSSNLLK TCFAEKLPSSNLFM TCFADKLPFNSPPV TCFGKKLSSSDLPV SVAAKLPSSSLLV | NRAKEEMSLSDLI NRAKEEMSLSHLI DRAKEEASLSHLI DRAKKGN-QTQLI DRVG-VS-HAQLI DRAKAVS-QDQ- | NSQDRVDHEEWEN NSQDRVDHEEWEN NSQDRVDHKEWEN DTQDPAGQEDWEN DSQAPADQEDWEN | AVPRHSSWGDVGV AVSRHSSWGDVGV AVSRHSSWGDVGV AVSRHSSWGDVGL /VSRHSSWGDVGL | /GGSLKAPVLNL /GGSLKAPVLNL /GGSLKAPVLSL .GGNLEVSGVNL .GGRIESSGLNV |
| G G A | EWRLPKGHETAVKVAG LEPSLPTAN EWRFQHEREVLAKAG EWGFQKGQETLAKAAF FQKGQETSAKAAT | SVAEKLPSSSPLM VHSSGKFPSDNSFM QQLPSSKTPV PCFAEKLYSSNLVV TCFAGKLPSSNLLM | DRAEAAS - LAQS DKPEEQVRALQVI DSIEVGV - TQLI DRGKEVS - LAELS DKAKEVG - LAQLI | SQDEDDEDWEN | /VSRHSSWG <mark>S</mark> VGL /VPRHSLWRDAD /VSRHSSWGDIGL /VSRHSSWGDVGL /VSRHSSWGDVGL | GGSLEASRLSL GKTSEPSHSGVSI GGNLEQG GGGLEAPGLSP SGSLEAPVLGP - |
| - - - 22 | EWGLOKGHKTPVKAAT RGFOKGOETPAKAAT HIRFHKRODTPAKAAF ERGLOKGOETPSKAVF 10 250 | CFAKKLPSSNMLL CFAGKLPSSNLVT YFVGKLPSSNLLI CLAEKFPSSNLLL 260 | DRGEEEALI DRAKEDLI DRVKEEVSRAQLI GRAKEVS-PAEPI 270 280 | RHSADSDDWE DHQTPADHEDWEN DSQDMADQEDWEN DSQTRANHEDWEN 290 | VSRHSSWODIGL VSRHSSWGDVGL VSRHSSWGDIGL VSRHSSWGDVGL 300 | GDSPEAFMLNP GGSLKAPVLNP SGSPESPTLSP GDGLGSPVLSS 310 |
| | - NQGMDNGRSTLVEAF - NQGMDNGRSTLVEAF - SPEMHDNRSSLVEAF - SQGMDCDN-TCVELF - NQRMDCDN-TCVELF - NQRMDDSTNSLVGG - NQGMDESRNSLVGG - NQGMDESRNSLVGG | GQQVHGKMERVAV GQQVHGKMERVAV DQQVHGKTERVAV DWEVDGKRKSAQ GWEADQKVR | MPAG SQ DVSVRFQ MPAG SQ DVSVRFQ VSSESK DVSVRFQ - SSD SQ DVSVRFQ - SSL KPQ DVSIQFQ - SVKPRDVSIQFQ VAAVSQ DVNVQFQ | EVHYVTSTDVQFI IVHYVTSTDVQFI IVHYTSTDVQFI IVHYTSTDVQFI IVHYTSTGAQFL IVHYTNTDVQFI IVHYSTSTDVQFI IVHYVTMSDASLV | AVTGDHECLGRW AVTGDHECLGRW AVTGDHEYLGRW AVTGDHESLGGW AVTGDHESLGGW AVTGDHESLGGW AVTGDHESLGGW | NTYIPLHYNKDGF NTYIPLHCNKDGF NTYIPLHYNKDGF DTYIPLHHKDGI KTYIPLHYKDGI NTYIPLHYCKDGL NTYIPLHYCKDGL HSYIPLQCGKDWF |
| | OGMD CGRSTLMEP N GGMD CGRSTLMEP N GGMD GGRSSAAEAF S GGMD YGRSVLVES H QGMN SGRSTLEEAF N QGMD YGRSTLVAAF N QGLD YGRSTLVAAF K QGKDWD RSMLVAAF 10 320 | 0 READ VKPGRVVA 6 QEMD VKRKKGVA 6 QEVNIKTEKVIS 10 REVD VKTKKVVE 10 REVD VKTKKVVE 10 QEMD VKQKRV 10 QEKE | ASA E A D UVSIRFO WISSESO UVSIRFO ISSESO UVNVKFO VFPEPO UVSITFO MISS-A D UVNVTFO SRUVSVRFO MISSESO UVSVRFO C R UVSVRFO C R UVSVRFO C R UVSVRFO | IVHYVTSTDVEFI IVHYTSTGGDFI IVHYTTSTGEDFI IVHYTSTGEDFI IVHYTSTGEVOFI IVHYTSTGVDFI IVHYTSTGVDFI IVHYTSTGVDFI IVHYTSAGVDFI | AVTGDHEHLGRW AVTGDHESLGRW AVTGDHESLGRW ATGDHESLGRW ATGDHESLGRW AVTGDHESLGRW AVTGDHESLGRW AVTGDHESLGRW | NTYIPLHCNKDGL NTYIPLQYSKDGF NTYIPLQYSKDGF NTYIPLQYSKDGF NTYIPLQGSKDGF NTYIPLQGSKDGF KSYIPLQGSRDGF |
| | P L H Y N K D G F WS H S I F P L H C N K D G F WS H S I F P L H H K D G L WS H S V L P L H H K D G L WS H S V L P L H Y K K D G L WS H S V F P L H Y C K D G L WS H S V F P L Y C K D G L WS H S V F P L Q C G K D W F WS R S V P P L H C N K D G L WS H S V L P L Q Y S K D G F WS H S V S P L Q Y S K D G F WS H S V S | PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVLV PADTVVEWKFVVV PADTVVEWKFVVV PADTVVEWKFVVV PADTVVEWKFVVV | ENGGVTRWEECSI ENGGVTRWEECSI ENGGVARWEECSI ENGKVTRWEECSI ENGKVTRWEECSI ENKEVTRWEECSI ENGSIIRWEECSI ENGGVTRWEECSI DNGKITRWEECSI ENGEVIRWEECSI ENGEVIRWEECSI | NRFLETGHEDKVV NRFLETGHEDKVV NRFLUTGHEDKVV NRFLUTGHEDKVV NRFLUTGHEDKVV NRFLUTGHEDKVV NRFLETGHEDKVV NRFLETGREDKVV NRFLETGLEDKVV NRFLETGLEDKVV | /HANUWGIH /HANUWGIH /HANUWGIH /HANUWGVH /HANUWGVH /HGNUWGIH /HGNUWGIH /YKNUWGCH /RKNUWGIP /QENUWGIH /QENUWGIH | |
| 1 | PLQYGKDGLWSHSVSI PLQGSKDGFWSHSVSI PLQYSKDGFWSRSVYI PLQCSRDGFWSRSVSI | _ PAGAVVQWKFVVV _ PADTVVEWKFVVV _ PADTMVEWKFVVV _ PADTVVEWKFVVV | ENGGITRWEECSI ENGEVTRWEECSI ENGKVTRWEECSI ENGEVARWEECSI | NRFLDTGHEDKEN NRFLETGHEDKVV NRLLETGYEDKVV NRFLETGHEDKVV | /HKMWGIH /HKMWGIP /HKSWGIH /HRMWGIH | |

Suplemental Figure 3.



В



Supplemental Figure 4.

