Supporting Online Material for

A role for the ESCRT system in cell division in Archaea

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Materials and methods

Construct optimization for crystallization

Initially, we used His_6-tagged MIT domain (residues 1-82) of Saci1372 in a complex with various length of peptides (e.g., residues 164-208) of Saci1373 for crystallization, however, none of them formed any crystals. We therefore determined the precise domain boundary of both constructs by NMR spectroscopy. We identified the flexible regions of both constructs, and we designed core constructs for Saci1372 (residues 1-75) and Saci1373 (residues 183-195), which are both necessary and sufficient for binding. Using these minimal constructs, we obtained crystals of the complex that enabled structure determination.

Protein cloning, expression, purification for NMR spectroscopy

Saci1372 Vps4 MIT domain (residues 1-82) was cloned with an N-terminal His6 tag in the pOPTH vector. $^{13}C/^{15}N$-labeled protein was expressed in M9 minimal medium containing ^{13}C-Glucose and $^{15}NH_4Cl$. Cells were lysed in buffer C [20 mM Tris pH 8.0 (4 °C), 20 mM imidazole, 100 mM NaCl and 1 mM β-mercaptoethanol]. The protein was purified on a 5 ml His-Trap-FF column equilibrated with the same buffer. The column was washed in the same buffer with 0.1% Triton X-100 and the protein was eluted with an imidazole gradient. Subsequent purification was conducted on a HiTrap-Q column (Amersham) in buffer D [20 mM Tris pH 8.5 (20 °C), 1 mM DTT] with an NaCl gradient. The eluted protein was purified by gel filtration on a Superdex 75 16/60 in buffer B [20 mM Tris pH 7.4 (20 °C), 100 mM NaCl and 1mM DTT]. Saci1373 (residues 177-195) was cloned with an N-terminal GST tag in the pOPTG vector. $^{13}C/^{15}N$-labeled peptide was expressed in M9 minimal medium containing ^{13}C-Glucose and $^{15}NH_4Cl$. Cells were lysed in buffer A [20mM Tris pH 7.5 (4 °C), 100mM NaCl and 1mM DTT] and incubated with glutathione Sepharose 4B (GE Healthcare) for one hour. After washing, the GST-fusion was digested with TEV protease [100:1(*w*:*w*)] at 4 °C for 12 h on the resin. The TEV-eluted protein was further purified by gel filtration on a Superdex 75 16/60 in buffer B.

NMR spectroscopy

NMR spectra were recorded at 25 °C on Bruker DRX 600 spectrometers. Samples contained 0.5 mM $^{13}C/^{15}N$-labeled protein/peptide in a complex with 1.0 mM unlabelled ligands in 20 mM Na Citrate (pH 3.0) and 50 mM NaCl. Resonance assignments were obtained using standard methods. For backbone dynamics, $\{^1H\}$ ^{15}N steady-state heteronuclear NOE values were acquired at 25 °C.

Protein cloning, expression and purification for crystallography

Saci1372 Vps4 MIT domain (residues 1-75) was cloned with an N-terminal GST tag in the pOPTG vector and expressed in C41(DE3)RIPL cells. Cells were lysed in buffer A [20mM Tris pH 7.5 (4 °C), 100mM NaCl and 1mM DTT] and incubated with glutathione Sepharose 4B (GE

Healthcare) for one hour. After washing, the GST-fusion was digested with TEV protease [100:1(*w*:*w*)] at 4 °C for 12 h on the resin. The TEV-eluted protein was further purified by gel filtration on a Superdex 75 16/60 in buffer B [20 mM Tris pH 7.4 (20 °C), 100 mM NaCl and 1mM DTT]. All constructs were verified by sequencing. Saci1373 MIM2 (residues 183-195) was chemically synthesized (Sigma-Aldrich).

His_6FlAsH-tagged Saci1372 Vps4 MIT was cloned in the pOPTHF vector and expressed in C41(DE3)RIPL cells. Cells were lysed in buffer C [20 mM Tris pH 8.0 (4 °C), 20 mM imidazole, 100 mM NaCl and 1 mM β-mercaptoethanol]. The protein was purified on a 5 ml His-Trap-FF column equilibrated with the same buffer. The column was washed in the same buffer with 0.1% Triton X-100 and the protein was eluted with an imidazole gradient. Subsequent purification was conducted on a HiTrap-Q (Amersham) in buffer D [20 mM Tris pH 8.5 (20 °C), 1 mM DTT] and a NaCl gradient. The eluted protein was purified by gel filtration on a Superdex 75 16/60 in buffer E [20 mM Tris pH 7.4 (20 °C), 100 mM NaCl and 1mM TCEP]. His_6FlAsH-tagged Saci1372 Vps4 MIT (10 nmoles) was labelled with 10 nmoles of Lumio Green detection reagent (Invitrogen, LC6090) in buffer F (20 mM Tris pH 7.4 (20 °C), 100 mM NaCl and 5 mM β-mercaptoethanol) in a final reaction volume of 1 ml for 2 hours at 4 °C. The sample was dialysed overnight against buffer F at 4 °C using a 3.5 kDa Slide-A-Lyzer membrane (Pierce).

Crystallization

The LMB nanolitre crystallisation robotic facility was used for a broad initial screen of 1440 crystallisation conditions. Optimal crystals for the complex of the Saci1372 Vps4 MIT (residues 1-75) with Saci1373 MIM2 (residues 183-195) were obtained at 17°C by vapour diffusion from a protein solution at 18 mg/ml containing a 1:2 molar ratio of MIT:MIM2 and a reservoir solution containing 15% PEG4000 and 100 mM Tris pH 7.5 (20 °C). Crystals were cryoprotected by adding glycerol to a final concentration of 25% and frozen by dunking in liquid nitrogen.

Crystallographic structure determination

Diffraction data were collected at ESRF beamline ID29. The complex crystallized in space group $P4_3$ with two complexes *per* asymmetric unit. Phases were derived from molecular replacement using the program PHASER (*1*) and the structure of the Sso909 MIT domain as an initial model (PDB ID 2V6Y). Images were integrated using MOSFLM (*2*) and scaled with SCALA (*3*). An initial model was automatically built using ArpWarp (*4*). The model was adjusted manually using COOT (*5*) and refined with REFMAC 1 (*6*). The final crystallographic statistics are given in Supplementary Table 1.

Fluorescence titration binding assay

Analyte protein was titrated into a cuvette containing N-terminally labelled with Lumio Green (FlAsH-MIT) in 1.1 ml binding buffer (20 mM Tris pH 7.4 (20 °C), 100 mM NaCl, and 5 mM β-

mercaptoethanol). Fluorescence was measured using a Perkin-Elmer LS-55 spectrophotometer with an excitation wavelength of 490 nm and an emission wavelength of 530 nm. Excitation and emission slits were 10 and 10 nm, respectively. Anisotropy was measured with an integration time of 5 s. A 1 mM protein analyte was titrated into a cuvette with a Hamilton-MicroLab titrator, allowing 50 s stirring after each titration step and a pause of 10 s before the anisotropy was recorded. Titration of binding buffer alone resulted in no overall change in intensity or anisotropy. The K_d values were calculated from direct fitting of the titration data to a single-site model. At least two independent titrations were conducted to determine K_d values.

Strains and Growth Conditions

S. acidocaldarius DSM639 was grown in Brock's medium, pH 3.2 at 75 ºC and synchronized as described in (*7*). Specifically, 100 ml of culture at approximately OD_{600} = 0.15 were applied to a poly-D-lysine-coated membrane within the baby machine apparatus. After pumping Brock's medium through the apparatus for 3 h at 0.75 ml/min, newly divided cells were collected on ice over a period of one hour. Synchronized grow-out was initiated by transferring the vessel containing the cells from ice to a water bath heated to 75 ºC. *S. solfataricus* PH1-16 used for genetic over-expression studies was grown at 75 ºC in Brock's medium, pH 3.2 with 10 μg/ml uracil before transformations and without uracil afterwards. All *S. solfataricus* plating was done on Brock's medium, pH 3.2 + 0.2 % tryptone + 0.2 % D-(+)-galactose solidified with 0.7 % Gelrite (Serva). *Saccharomyces cerevisiae* AH109 was grown in YPED medium (*8*) and plated on solid selective media, as indicated.

Yeast Two-Hybrid Assays

S. cerevisiae AH109 cells were co-transformed with plasmids encoding the indicated proteins fused to the GAL4 DNA-binding domain, pGBKT7, or the GAL4 activation domain, pGADT7 (Clonetech). Co-transformants were selected following growth on SC-Leu-Trp agar for 2 days at 30 ºC. Isolated colonies were grown in liquid SC-Leu-Trp medium to an OD_{600} = 0.1. 5 μl of each culture were spotted on both SC-Leu-Trp (as a control for growth) and on SC-Leu-Trp-His agar; growth after 3 days on the medium lacking histidine was considered a positive result for protein-protein interactions.

Protein Purification, Antibody Production and Western Blotting

The plasmids used for protein purification were generated by PCR-mediated cloning using the primers listed in Table 2 and were transformed into *E. coli* Rosetta cells (Novagen) for protein expression. Oligonucleotide-mediated site-directed mutagenesis was performed according to the QuickChange protocol (Stratagene). For His_6-tagged protein expression, cultures were grown in LB at 37 ºC, induced with 1 mM IPTG at OD_{600} = 0.6, and harvested by centrifugation 3 hours post-induction. Cells were lysed into 20 mM Tris (pH 8.0), 300 mM NaCl using a French press set to 20,000 psi (ThermoFisher). Cell extract was then heat treated for 20 min at 75 ºC and

clarified by centrifugation at 35,000 g for 10 min. For purification of Saci1373-His_6, the insoluble pellet was resuspended in 20 mM Tris (pH8.0), 300 mM NaCl, 8 M urea. Proteins were then purified over Ni-NTA agarose (Qiagen) as described in the QIAexpressionist handbook (Qiagen). GST-fusion proteins were purified according to protocols described in the Recombinant Protein handbook (GE Healthcare). Briefly, Rosetta cells (Novagen) containing the appropriate expression vector were induced with 1 mM IPTG at OD_{600} = 0.4 for 3-4 hours at 37 ºC. Cells were harvested by centrifugation and washed in TBS (10 mM Tris, pH 8.0; 150 mM NaCl). Cells were then lysed by French press in TBS-T (TBS, 0.1 % Tween-20) and purified in batch mode using glutathione sepharose according to the manufacturer's instructions.

Polyclonal antisera were raised against purified Saci1373-His_6 in two goats and against purified Saci1372-His_6 in two rabbits (Covalab). Standard western blotting procedure was followed using polyclonal primary antibodies and either Immunopure mouse anti-goat IgG-HRP (Pierce) or donkey anti-rabbit IgG-HRP (Pierce) as secondary antibodies. Results were visualized using ECL reagents (GE Healthcare). Rabbit anti-FLAG M2 monoclonal primary antibody (Sigma) was used in westerns to detect FLAG-tagged protein overexpression in *S. solfataricus*.

GST Pulldowns

GST pulldowns were performed essentially as described (*9*). Briefly, a ~10 μl bead volume containing 5 μg of GST fusion protein were incubated with 2 μg of either Vps4, MIT domain or Vps4 AAA+ domain as indicated. Incubations were performed for 30 minutes at 30 °C in 100 μl of TBSTM (10 mM Tris pH 8.0, 150 mM NaCl, 0.1 % Tween-20, 5 mM $MgCl_2$). Beads were recovered by brief centrifugation, resuspended in 500 μl TBSTM and transferred to clean tubes. The wash step was repeated 3 times before the pellet was resuspended in 1 ml 1X SDS-PAGE loading buffer and boiled. 10 μl samples were electrophoresed on 11.25% SDS-PAGE gels and Vps4 detected following western blotting.

Affinity Purification of Antibodies

1.5 mg purified Saci1373 or Saci1372 were coupled to 1-ml HiTrap NHS-activated HP columns in standard coupling buffer (200 mM sodium carbonate; 500 mM NaCl, pH 8.3) according to the manufacturer's protocol (GE Healthcare). 1 % SDS was included in the coupling buffer to solubilise Saci1373.

Goat anti-Saci1373 and rabbit anti-Saci1372 polyclonal sera were diluted 1:10 in 1X TBS, passed through a 0.45 μm filter, and recirculated through the appropriate affinity column at <1 ml/min for 45 min at room temperature. The columns were washed with 1X TBS, TBS-T, and then eluted with 100 mM glycine, pH 2.5 into Tris, pH 8.5, 100 mM final molarity. Fractions were spotted onto Immobilon-P membrane (Millipore) and those containing antibody were detected with the appropriate secondary antibody.

RNA Purification

At the time points indicated, 7 ml of *S. acidocaldarius* culture were removed from the growth vessel by syringe. The culture was passed through a 0.45 μm nitrocellulose filter held in a reusable syringe filter unit (Sartorius). The filter was removed from the unit and placed in a microfuge tube containing 500 μl RNase-free water. The tube was vortexed for 10 s to wash cells from the filter. 100 μl of 6X lysis buffer (600mM sodium acetate, pH 5.2; 6 % SDS) and 600 μl saturated phenol, pH 4.3 were added to the resuspended cells and the tube was vortexed for 2 min using a Vortex-Genie 2 TurboMix (Scientific Industries, Inc.). The extract was then centrifuged at 15,000 g for 2 min. The aqueous phase was transferred to a new tube and extracted a second time with phenol, pH 4.3. The aqueous phase was then transferred to a new tube and precipitated overnight at -20 ºC in an equal volume of isopropanol and 300 mM sodium acetate, pH 5.2. The nucleic acids were then pelleted, washed with 75 % ethanol, and allowed to air dry. After resuspending in RNase-free water, the pellets were treated with DNase I (Invitrogen) according to the manufacturer's instructions. The extracted RNA was further purified with an RNeasy kit (Qiagen) according to the RNA cleanup protocol in the manufacturer's manual.

Quantitative RT-PCR

All primers (Table S3) were designed using Primer3 (v.0.4.0) software (http://fokker.wi.mit.edu/primer3/input). Potential amplimers produced by primer pairs were determined using Primersearch software (http://bioinfo.hku.hk/EMBOSS). Primer secondary structure was predicted using OligoAnalyzer 3.1 mfold (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer). First strand cDNA was synthesized from 250 ng of purified total RNA using 250 ng random hexamers (Invitrogen) and SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. No RT controls were prepared using water in place of the RNA. SYBR detection assays were performed in triplicate using 5.5 % of cDNA reaction as template, forward and reverse primers at 125 nM final concentration, and 2.5X RealMasterMix/SYBR Solution (5 PRIME) according to the manufacturer's instructions. Standard curves were prepared in duplicate using purified PCR products as template. qPCR reactions and melting curves were performed and analyzed using a Mastercycler ep realplex^2 machine and included analysis software (Eppendorf).

Construction of pRYS1

pyrEF genes were amplified from *S. acidocaldarious* genomic DNA with primers SacEF_F and SacEF_R (Table S2). Purified PCR products were digested with XhoI and NsiI and ligated into pCRScript that was digested with SalI and PstI. The *S. acidocaldarius* vector pJ, generated by Lipps and colleagues (*10*), was digested with HindIII and NotI and the resulting pRN1-derived fragment was gel-purified (Qiagen) and the DNA ends filled in with T4 DNA polymerase. pCRScript_*pyrEF* was digested with SrfI and ligated to the pRN1 fragment. *Sso0909* and *Sso0909* E206Q were amplified from *S. solfataricus* genomic DNA and pOP319 respectively

with the primers Sso0909 5' and Sso0909 3'FLAG. The PCR products and vector pSVA5 (*11*) were digested with NcoI and ApaI, gel-purified, and ligated to generate pSVA5_Sso0909 and pSVA5_Sso0909E206Q. The genes were then re-amplified from the pSVA5 vectors using the ara0909_F and ara0909_R primers. The gel-purified PCR products and the pRYS1 vector were digested with SacII and NotI and ligated to generate pRYS_Sso0909 and pRYS_Sso0909E206Q.

Transformation and Southern Blotting

S. solfataricus PH1-16 cells were prepared for transformation as described (*12,13*). 200-600 ng of plasmid DNA were electroporated into 50 μl cells in 1 mm cuvettes using a time constant protocol with the following parameters: 1500 V, 25 μF, and 400 Ω. Cells were allowed to regenerate in 1 ml Milli-Q water for 10 minutes at 75 ºC before plating on solid Brock's medium lacking uracil. After incubation at 75 ºC for approximately 5 days, colonies were picked and restreaked on Brock's plates for isolation. Colonies able to grow on plates lacking uracil were grown in liquid medium so genomic DNA could be prepared (*8*). After digestion with NcoI, 3 μg DNA were resolved on a 0.7% agarose gel. The gel was then agitated in dH_2O for 10 min, denaturation buffer (1.5 M NaCl, 0.5 M NaOH) for 30 min, and neutralization buffer (0.5 M Tris, pH 7.5; 1.5 M NaCl) for 30 min. DNA was then transferred to Hybond-XL membrane (GE Healthcare) by capillary blotting according to the manufacturer's instructions. ^{32}P-labelled probe was prepared according to the manufacturer's instructions using the NEBlot kit (New England Biolabs) and either linearized pCRScript or the Sso0909 (Vps4) MIT domain PCR product as template. See Fig. S10 for an example of the probing with Sso0909. Hybridization was performed in Denhardt's buffer (5X SSC, 5X Denhardt's solution, 0.5% SDS) at 65 ºC and washes were performed as described in the Hybond-XL manual. Membranes were exposed to phosphoimager screens which were subsequently scanned on an FLA-5000 phosphoimager (Fujifilm). Data was analyzed using AIDA Image Analyzer software (Raytest).

Protein Overexpression in *Sulfolobus*

S. solfataricus PH1-16 transformants were grown in Brock's medium containing 0.2 % tryptone and 0.4 % D-(+)-galactose to early logarithmic phase. The cells were pelleted and washed twice with Brock's medium containing 0.1 % tryptone. The cells were then resuspended in the same medium to an OD_{600} of 0.15. Expression of protein from the pRYS1 plasmid was either induced with 0.4 % D-(-)-arabinose or repressed with 0.4 % D-(+)-galactose. Samples were collected at 0 and 44 h for spectrophotometry, flow cytometry, western blotting, and microscopy.

Flow Cytometry

Sampling and staining of cells as well as flow cytometry were performed as described in (*14*).

Immunofluorescence Microscopy

For samples enriched in dividing cells, 2 ml were collected from synchronized *S. acidocaldarious* cultures at 180 min of grow-out. For *S. solfataricus* cells overexpressing versions of Vps4, 500 μl were collected from cultures 44 hours post-induction. Cells were gently pelleted at 6,000 g for 3 min and then resuspended in 420 μl FM4-64X buffer (36 mM sodium phosphate, pH 7.6; FM4-64X [12 ng/μl] (Invitrogen)). After 5 min on ice, paraformaldehyde was added to 2.5 % final concentration and the cells were incubated at 25 ºC for 45 min. Cell pellets were then washed with 1X PBS, pH 7.4. To permeablize the cells, pellets were suspended in 50 mM glucose; 20 mM Tris, pH 7.5; 10 mM EDTA; 0.2 % Tween-20 and incubated at 25 ºC for 15 min. Cells were then washed three times with 1X PBS to thoroughly remove all traces of detergent. Pellets were resuspended in 20-100 μl PBS and 10 μl were spread onto poly-D-lysine-coated coverslips. After air drying, the coverslips were washed with PBS and covered with blocking buffer (2 % BSA in 1X PBS) for 30 min at room temperature. The coverslips were washed three times with PBS and then covered with primary antibody, either 1:20 dilution of affinity-purified anti-Saci1373 or 1:50 dilution of affinity-purified anti-Saci1372 (diluted in 2 % BSA in 1X PBS, 0.05 % Tween-20) for 1 hr at room temperature. Coverslips were washed with PBS and then incubated at room temperature for 1 hr with Alexa 488-conjugated anti-rabbit or anti-goat secondary antibody diluted 1:1000 in 2 % BSA in 1X PBS, 0.05 % Tween-20. After washing with PBS, the coverslips were inverted onto microscope slides spotted with 25 μl VectraShield (Vector Co.) containing DAPI [1.5 ng/ μl]. The edges were sealed with clear nail polish. All centrifugation steps were performed for 3 min at 6,000 g and all incubations were done in the dark.

Slides were observed using a Zeiss Axioplan 2e epifluorescence microscope fitted with a 100X Plan-Neofluar oil immersion lens, numerical aperture 1.3. Images were captured with a CoolSNAP HQ CCD 16-bit camera (Roper Scientific) and analyzed using Metamorph (v.6.2.4) software (MDS Inc.). Images were processed for presentation using Photoshop software (Adobe). Movies displaying three-dimensional images were created using AutoQuant X software, version X2.0.0 (Media Cybernetics, Inc., Bethesda, MD, USA). Three-dimensional representations were constructed using the blind 3D deconvolution function of the software from 10 Z-stacked images taken at 0.1 μm increments.

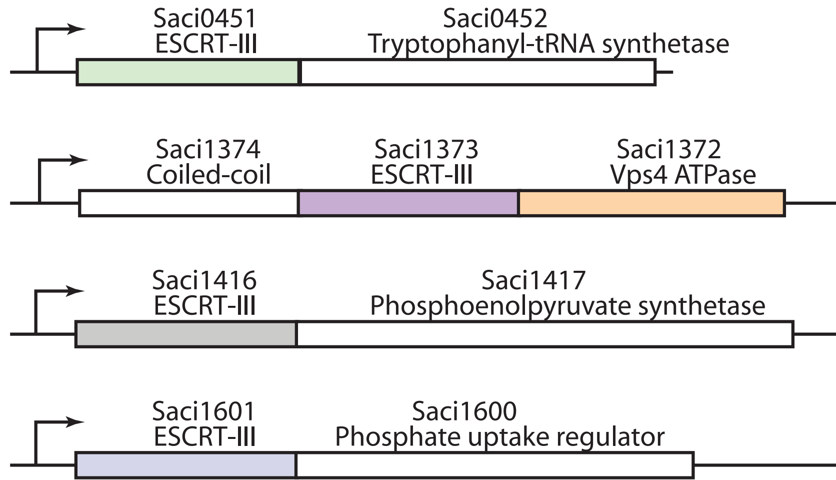

Figure S1. Cartoon of the genetic loci encoding ESCRT-III and Vps4 homologs in *Sulfolobus acidocaldarius*.

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SSO0910/1-259           ......................................................................
ST1214/1-266            ......................................................................
Saci_1373/1-261         ......................................................................
Mmed_1671/1-270         ......................................................................
Mbut_0797/1-286         ......................................................................
Smar_1277/1-260         ......................................................................
APE_0962/1-271          ......................................................................
Igni_0995/1-255         ......................................................................
SSO0881/1-221           ......................................................................
SSO0451/1-284        1  MFPSVFIPTLLYSSSFHLRSKYITTLSVDTKNSYSGSSSSLMNVFGILLISDSNNLNSSHYVFDLIRVDE
ST1237/1-219            ......................................................................
ST0168/1-251         1  .............................MSISTSGSSVMVGILYTYFFSLTFKPILCIKPLYKFIM...
Saci_1416/1-219         ......................................................................
Saci_0451/1-214      1  .................................................................MT...
Mmed_1695/1-221         ......................................................................
Mmed_2179/1-211         ......................................................................
Mbut_1468/1-218         ......................................................................
Smar_0481/1-212         ......................................................................
APE_0143.1/1-223        ......................................................................
Igni_1156/1-210         ......................................................................
Nmar_0029/1-215         ......................................................................
Nmar_0816/1-216         ......................................................................
Nmar_0061/1-216         ......................................................................
CENSYa_1915/1-214       ......................................................................
CENSYa_1885/1-195       ......................................................................
SSO0619/1-168           ......................................................................
ST1485/1-165            ......................................................................
Saci_1601/1-169         ......................................................................
Mmed_1969/1-165         ......................................................................
Mbut_1206/1-160         ......................................................................
Igni_0101/1-178         ......................................................................
Smar_0222/1-186         ......................................................................
CHMP6_HUMAN/1-201       ......................................................................
CHM4A_HUMAN/1-222       ......................................................................
CHM4B_HUMAN/1-224       ......................................................................
CHM4C_HUMAN/1-233       ......................................................................
CHMP3_HUMAN/1-222       ......................................................................
CHM2A_HUMAN/1-222       ......................................................................
CHM2B_HUMAN/1-213       ......................................................................
CHMP1A_HUMAN/1-196      ......................................................................
CHM1B_HUMAN/1-199       ......................................................................
CHMP5_HUMAN/1-219       ......................................................................
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                                                   h0                 h1
SSO0910/1-259        1  .........................MFDKLPFIFNNEKRRKAQ..LGKI...LTEISLKLKDQQTRLEEA
ST1214/1-266         1  .........................MLDKISSFFNNDRKRKAQ..LGKI...ITEISLKLKDQQDRLDEA
Saci_1373/1-261      1  .........................MFDKLSIIFNSDRKRKVH..LSKA...ITEISLKLKEQQDRLDEA
Mmed_1671/1-270      1  ...........................MKLSSLFNITRKKDEQGLLASK...ITEISIKLKDQQDKLDET
Mbut_0797/1-286      1  ......................MSAIISRLLGFLGVGEHKQPS..LKER...IDHAIVKLELLEDRVAEL
Smar_1277/1-260      1  ...............MSTLGIGS.RGIGWFGRLFGFFKRSKDP..VTRS...IYEAIAVLDKMINSIEST
APE_0962/1-271       1  ..................MVIDIVRKLGIISWSPGAKAGHEPG..YGEEGVDYDEILARLQLTKTEVDSM
Igni_0995/1-255      1  ...........................MQRTMVFFFRKKKERR..LKDK...YFERLYEAFEWGERLAKY
SSO0881/1-221        1  ......MASSKVEDFVKNWG.GK.QEPSIGERIKNAFKP.QQP..LRYR...LVMANYRLRTMVSRLDVY
SSO0451/1-284       71  VRYILANLG..KD.FQKMWG.YE.ENKFKIKG......S.KEP..LKYK...LVQAHYKISSMISRLDAY
ST1237/1-219         1  ......MAG..VNDFIKNWN.GK.QEPTIAEKVKNLFKP.QQP..LRYR...LVMAHYRLKTTISRLDVY
ST0168/1-251        39  PRYKLPNLG..KEDFQKIWA..G.NEKVKIPK......S.KEP..LKYR...LIQAQYKIRSMISRLDSY
Saci_1416/1-219      1  ......MAD..VNDFLRNWG.GR.QEPTISEKIKNLFKS.QQP..LRYR...LVMANYRLRTTISRLDVY
Saci_0451/1-214      3  AIYILTNIS..KE.FQKIWE..G.NQKVKIPK......S.KDP..LKYR...LVQAQYRVRSMVSRLDVY
Mmed_1695/1-221      1  ......MTS.KVEDFVRNWN.GR.QEVGIGERVKNAFKP.KQP..LKYK...LVTANYKLRTTINRLEVY
Mmed_2179/1-211      1  ......MLG..KD.FQKYWA.GS.DDKSAWEGFKGAFKS.KEP..LKYR...IVQARYKLGSMINRLDVH
Mbut_1468/1-218      1  ......MAR..VEDFAKVWS.PQPKEPGIIEKIKNTINP.PPP..LRHK...LAIALYKLRVQNNKLEYI
Smar_0481/1-212      1  ................MSNIWF.GN.NQQTIGDKIKSLFKNDREP..IEKK...AIIAHYRVKTALGRINNY
APE_0143.1/1-223     1  ......MG...LESWAKKWNPPESTTETVGQKLRNFISP.PPP..IRNQ...IIQAVYRIQGQISKLDYS
Igni_1156/1-210      1  ......MDA..IVRFSRIWE.EG.SKGGRKFLIFGTKES.EEP..LKKK...VFKARYLIEKQIDRIGYT
Nmar_0029/1-215      1  ...........MANLSNKWT.KP.PTTSITEKIGDTVKP.KGA..LKPR...VQEGVKRLRLQIQKLDKM
Nmar_0816/1-216      1  .............MLSNSWN..NTGGESISQKVMGKVKP.DEP..LKNK...IDFAQKKLQFQITKLEGI
Nmar_0061/1-216      1  ...........MPNFDKTWA.RQ.ETQSVTGKLREAVKP.QGA..LKPR...IQTAVNKLQVQISKMDSM
CENSYa_1915/1-214    1  ...........MPSFDKTWA..QQETPGITEKIRGAVKP.EGP..LKPR...IQNAVNKLQVQISKLDLM
CENSYa_1885/1-195    1  ...................................MFRP.QGP..LKPR...VQTAITRLRKQITKLDGM
SSO0619/1-168        1  .............................................MKKKGKSLAELLIDVRIARNKVQNI
ST1485/1-165         1  .............................................MKKKG..LPEIITDIKLTIHRIDAF
Saci_1601/1-169      1  .............................................MKKRT..IAELLTDIRMAKYKIDMW
Mmed_1969/1-165      1  .............................................MKRD...LQRLLVDVKIARGKIRLW
Mbut_1206/1-160      1  ................................MIFKRRRQVSPR.VK.....LLELAVATRYAVSRLGLQ
Igni_0101/1-178      1  .........................................MG..LRVK...VVEALRKTHRTRRRLEAY
Smar_0222/1-186      1  ................................MVFRRRK.VNP..YKET...LYAIYNVKQGN.SRIEAL
CHMP6_HUMAN/1-201    1  ..............................MGNLFGRKKQSRVTEQDKAILQLKQQRDKLRQYQKRIAQQ
CHM4A_HUMAN/1-222    1  ........................MSGLGRLFGKG...KKEKGPTPEEAIQKLKETEKILIKKQEFLEQK
CHM4B_HUMAN/1-224    1  ........................MSVFGKLFGAGGGKAGKGGPTPQEAIQRLRDTEEMLSKKQEFLEKK
CHM4C_HUMAN/1-233    1  ........................MSKLGKFFKGGGSSKSRAAPSPQEALVRLRETEEMLGKKQEYLENR
CHMP3_HUMAN/1-222    1  ..............................MGLFGKTQEKPPKELVNEWSLKIRKEMRVVDRQIRDIQRE
CHM2A_HUMAN/1-222    1  ...............................MDLLFGRRKTPEELLRQNQRALNRAMRELDRERQKLETQ
CHM2B_HUMAN/1-213    1  ...............................MASLF.KKKTVDDVIKEQNRELRGTQRAIIRDRAALEKQ
CHMP1A_HUMAN/1-196   1  .............................................MDDTLFQLKFTAKQLEKLAKKAEKD
CHM1B_HUMAN/1-199    1  ..........................................MSNMEKHLFNLKFAAKELSRSAKKCDKE
CHMP5_HUMAN/1-219    1  .............................MNRLFGKAKPKAPPPSLTDCIGTVDSRAESIDKKISRLDAE
CHMP3_HUMAN (PDB 2GD5)                                                         h1
```

h1 h2 h3

Sequence	Start
SSO0910/1-259	41
ST1214/1-266	41
Saci_1373/1-261	41
Msed_1671/1-270	41
Mbut_0797/1-286	44
Smar_1277/1-260	50
APE_0962/1-271	51
Igni_0995/1-255	39
SSO0881/1-221	57
SSO0451/1-284	124
ST1237/1-219	55
ST0168/1-251	92
Saci_1416/1-219	55
Saci_0451/1-214	55
Msed_1695/1-221	56
Msed_2179/1-211	54
Mbut_1468/1-218	56
Smar_0481/1-212	50
APE_0143.1/1-223	56
Igni_1156/1-210	55
Hmar_0029/1-215	52
Hmar_0816/1-216	50
Hmar_0061/1-216	52
CENSYa_1915/1-214	52
CENSYa_1885/1-195	30
SSO0619/1-168	26
ST1485/1-165	24
Saci_1601/1-169	24
Msed_1969/1-165	23
Mbut_1206/1-160	33
Igni_0101/1-178	25
Smar_0222/1-186	32
CHMP6_HUMAN/1-201	41
CHM4A_HUMAN/1-222	44
CHM4B_HUMAN/1-224	47
CHM4C_HUMAN/1-233	47
CHMP3_HUMAN/1-222	41
CHM2A_HUMAN/1-222	40
CHM2B_HUMAN/1-213	39
CHMP1A_HUMAN/1-196	26
CHM1B_HUMAN/1-199	29
CHMP5_HUMAN/1-219	42

CHMP3_HUMAN (PDB 2GD5) h1 h2

h3 h4 h5

Sequence	Start
SSO0910/1-259	110
ST1214/1-266	110
Saci_1373/1-261	110
Msed_1671/1-270	110
Mbut_0797/1-286	113
Smar_1277/1-260	119
APE_0962/1-271	120
Igni_0995/1-255	108
SSO0881/1-221	126
SSO0451/1-284	193
ST1237/1-219	124
ST0168/1-251	161
Saci_1416/1-219	124
Saci_0451/1-214	124
Msed_1695/1-221	125
Msed_2179/1-211	123
Mbut_1468/1-218	125
Smar_0481/1-212	119
APE_0143.1/1-223	125
Igni_1156/1-210	124
Hmar_0029/1-215	121
Hmar_0816/1-216	119
Hmar_0061/1-216	121
CENSYa_1915/1-214	121
CENSYa_1885/1-195	99
SSO0619/1-168	94
ST1485/1-165	92
Saci_1601/1-169	92
Msed_1969/1-165	91
Mbut_1206/1-160	82
Igni_0101/1-178	94
Smar_0222/1-186	99
CHMP6_HUMAN/1-201	110
CHM4A_HUMAN/1-222	112
CHM4B_HUMAN/1-224	115
CHM4C_HUMAN/1-233	115
CHMP3_HUMAN/1-222	110
CHM2A_HUMAN/1-222	109
CHM2B_HUMAN/1-213	108
CHMP1A_HUMAN/1-196	95
CHM1B_HUMAN/1-199	98
CHMP5_HUMAN/1-219	112

CHMP3_HUMAN (PDB 2GD5) h3 h4 h5

Figure S2. Sequence alignment of crenarchaeal ESCRT-III homologs. Human ESCRT-III subunits are also shown for reference.

Sequences were identified using the BLAST search program at http://www-archbac.u-psud.fr/projects/sulfolobus, aligned using ClustalW (http://www.ebi.ac.uk/clustalw/) and adjusted manually. The archaeal ESCRT-III subunits are divided into three different groups (coloured green, red and blue), based primarily on their C-terminal sequences that are clearly distinct among the three groups. The secondary structure prediction for SSO0910 is shown above the alignment and the secondary structure of human CHMP3 (from the crystal structure, PDB ID

2GD5, ref. 15) is shown below the alignment. The MIM2 motif of Saci1373 and the related MIM2 of human CHMP6 (PDB ID 2K3W, ref. 16) are highlighted.

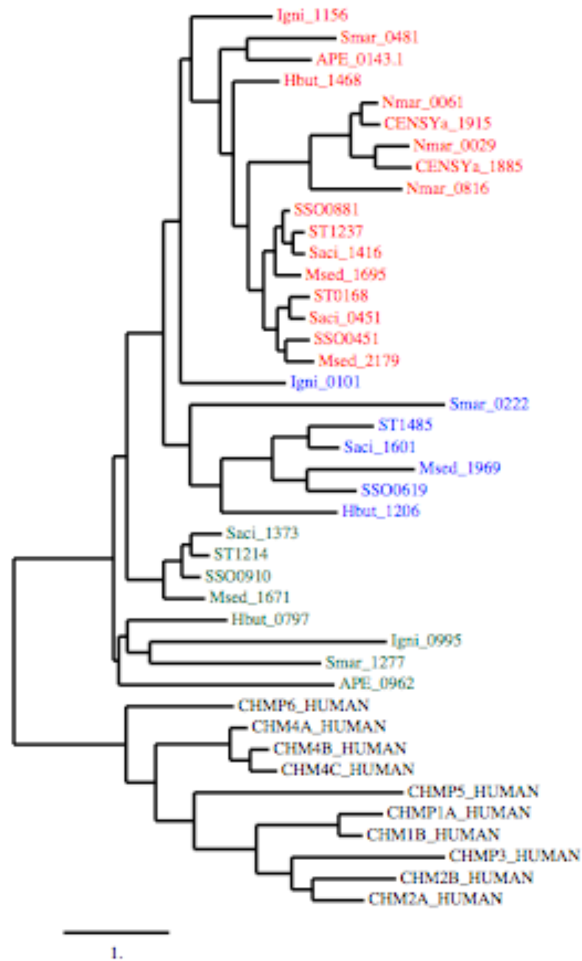


Figure S3. Phylogenetic tree of crenarchaeal ESCRT-III homologs and human ESCRT-III subunits was calculated based on the alignment shown in Figure S2 using the http://www.phylogeny.fr website (*17*). The groups are colored as in Figure S2. Species abbreviations are Igni – *Ignicoccus hospitalis*; Saci – *Sulfolobus acidocaldarius*; Msed - *Metallosphaera sedula*; Sso – *Sulfolobus solfataricus*; ST – *Sulfolobus tokodaii*; Hbut – *Hyperthermus butylicus*; Smar - *Staphylothermus marinus*; APE – *Aeropyrum pernix*; CENSY – *Cenarchaeum symbiosum*, Nmar – *Nitrosopumilus maritimus*.

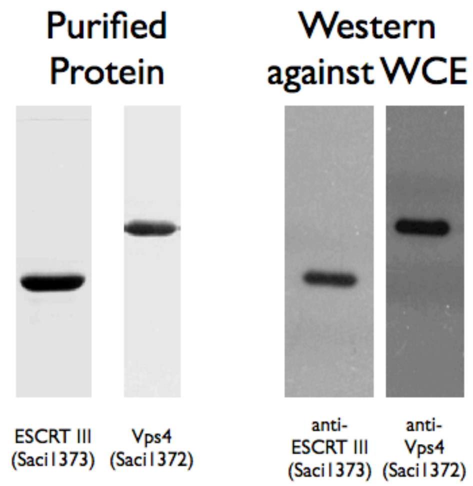
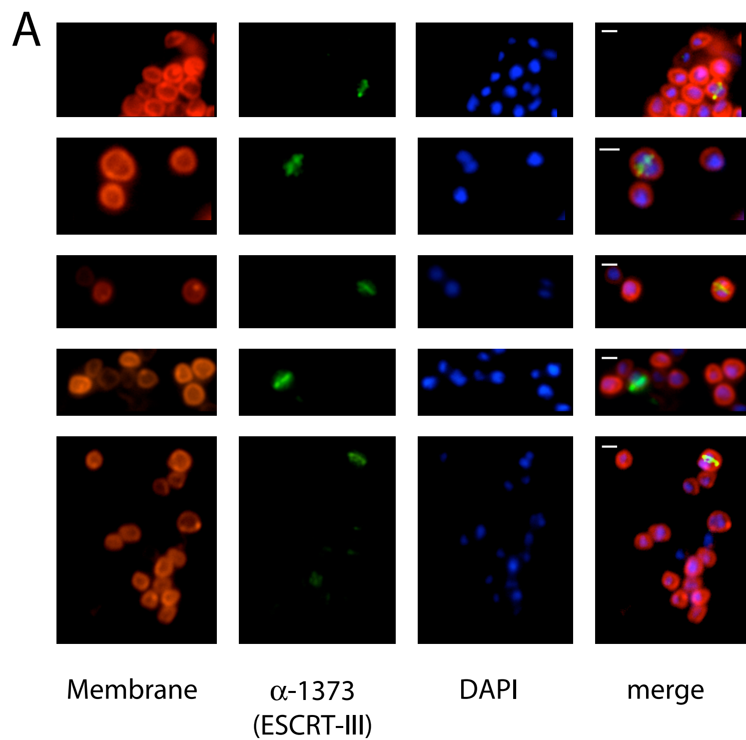


Figure S4. 5 μg of purified recombinant proteins run on 11.25% SDS-PAGE and detected by Coomassie staining. Detection of endogenous *S. acidocaldarius* ESCRT-III (Saci1373) and Vps4 (Saci1372) in 50 μg of *S. acidocaldarius* whole cell extract by immunoaffinity purified antibodies raised against the purified recombinant proteins (see Supplementary methods for description of preparation).



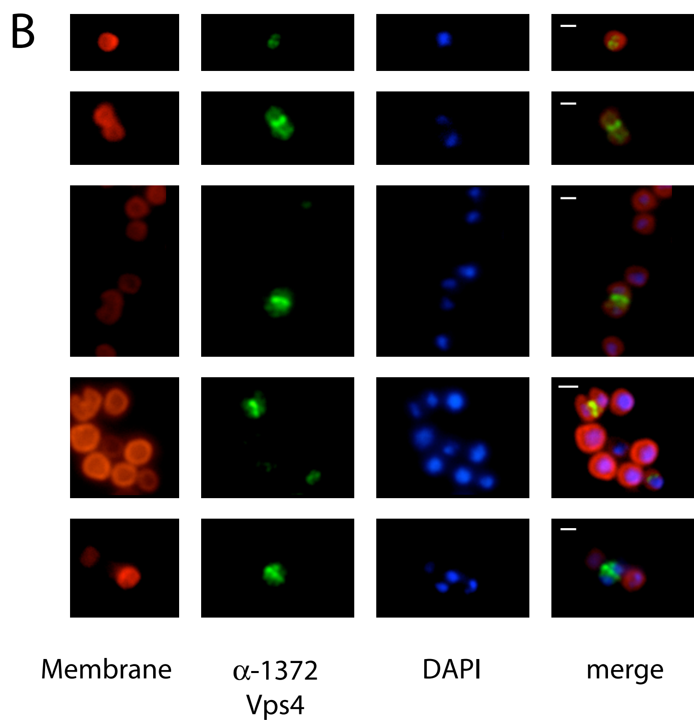


Figure S5. Immunolocalization of (A) Saci1373 (ESCRT-III) and (B) Saci1372 (Vps4). White bars indicate 1 μm. We have scored cells in which we observe two clearly segregated nucleoids. We detect structures at mid cell by immunlocalization in 56% of cells (n=215) with Saci1373 (ESCRT-III) and 58% of cells (n=110) with Saci1372 (Vps4).

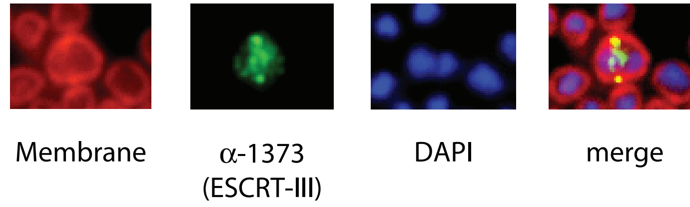


Figure S6. Immunolocalization of Saci1373. A series of 10 fluorescent images showing ESCRT-III localization generated in incremental 0.1 μm steps through the Z-plane were processed by 3D deconvolution. A 3D representation was generated - see Movie S1.

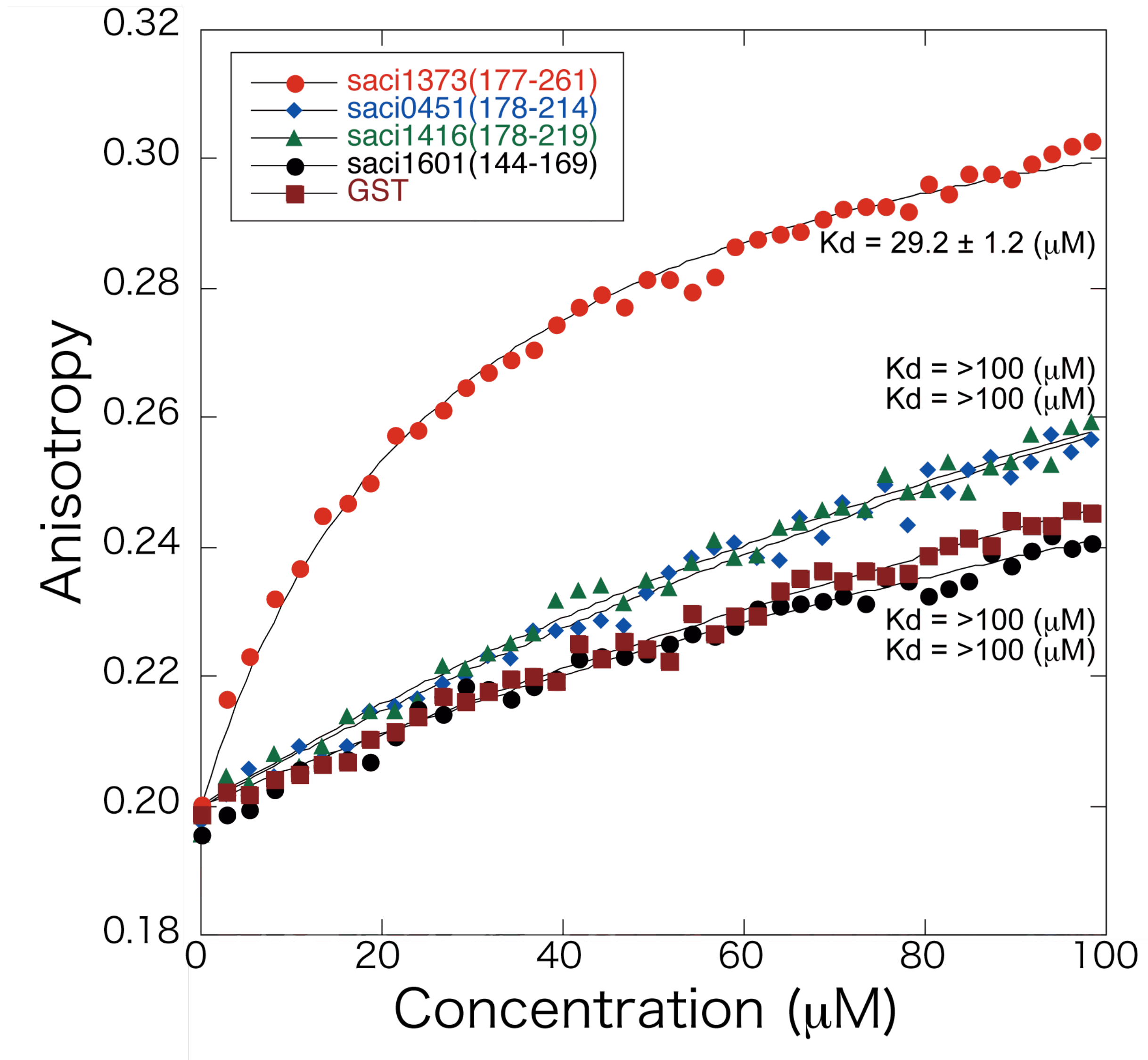
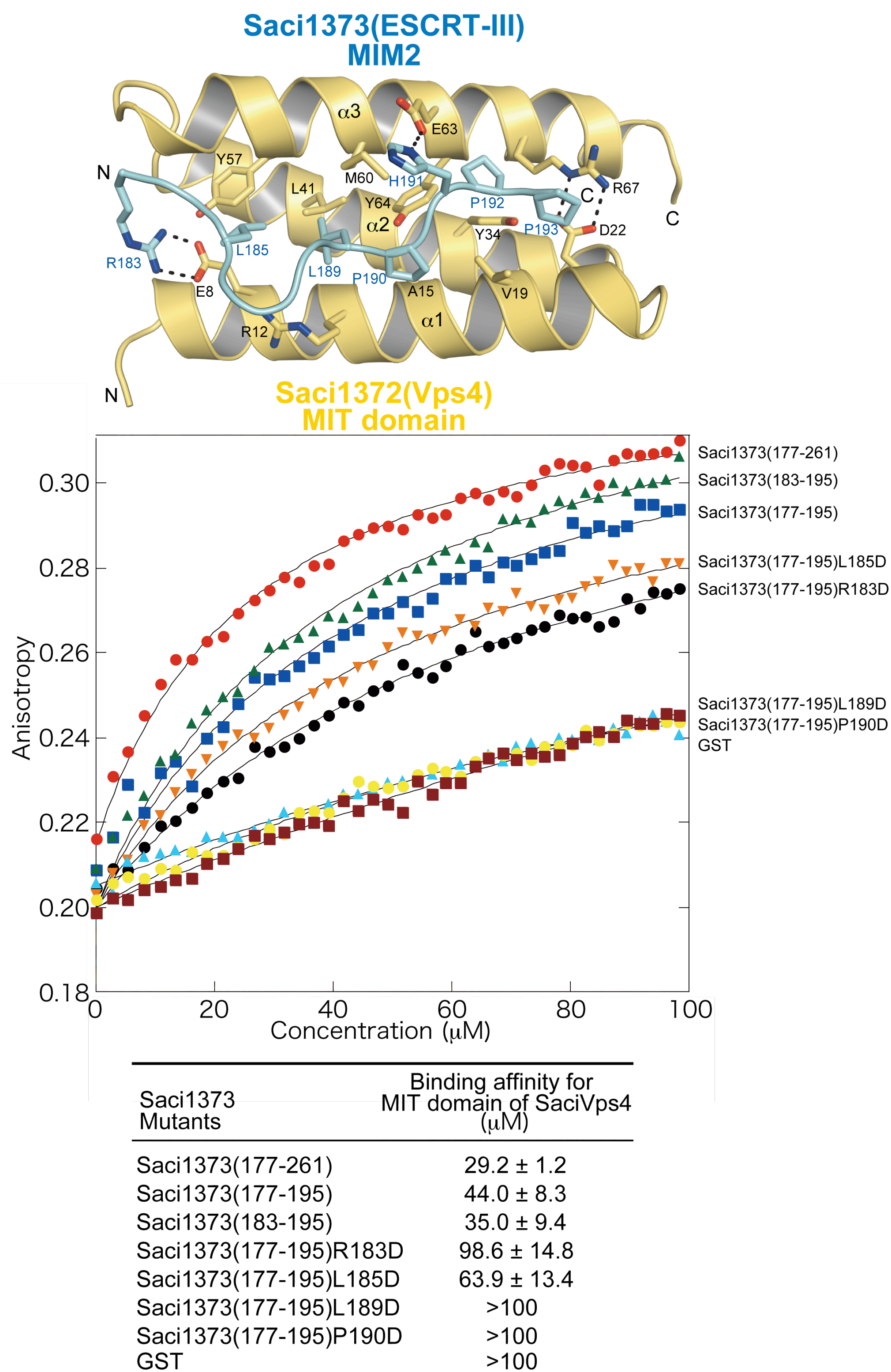


Figure S7. The affinities of peptides from the four ESCRT-III-related subunits of *S. acidocaldarius* for the Saci1372 MIT domain. Solutions containing peptides were titrated into a cuvette containing FlAsH-tagged MIT domain and the fluorescence anisotropy was followed (Supplementary methods). The Saci1373 peptide shows the greatest affinity for the Saci1372 Vps4 MIT domain.



Saci1373 Mutants	Binding affinity for MIT domain of SaciVps4 (μM)
Saci1373(177-261)	29.2 ± 1.2
Saci1373(177-195)	44.0 ± 8.3
Saci1373(183-195)	35.0 ± 9.4
Saci1373(177-195)R183D	98.6 ± 14.8
Saci1373(177-195)L185D	63.9 ± 13.4
Saci1373(177-195)L189D	>100
Saci1373(177-195)P190D	>100
GST	>100

Figure S8. The affinities of Saci1372 MIT domain for peptides derived from the Saci1373 ESCRT-III subunit. Solutions containing peptides were titrated into a cuvette containing FlAsH-tagged MIT domain and the fluorescence anisotropy was followed. Mutations of the core residues (189-LP-190) of Saci1373 observed in contact with the MIT domain in the structure of the

complex result in loss of binding. The detailed interactions between Saci1373(177-195) and the Saci1372 (Vps4) MIT domain are illustrated in the upper panel.

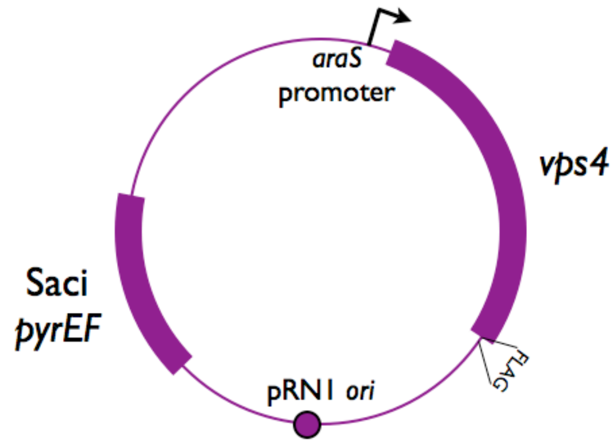


Figure S9. Design of expression construct. The vector was based on the *S. acidocaldarius* expression vector designed by Lipps and colleagues (*10*). The *S. acidocaldarius* pyrEF genes were included in this vector for selection in *S. solfataricus* PH1-16 (*12*). The arabinose promoter was added for controllable expression in *S. solfataricus* (*11*) and downstream of this either wild-type or Walker B (E206Q) mutant *S. solfataricus vps4* was inserted. The *vps4* open reading frame was fused to a C-terminal FLAG (DYKDDDDK) tag. [Note that *S. solfataricus* was used in these experiments as no inducible promoters have been developed for use in *S. acidocaldarius*]

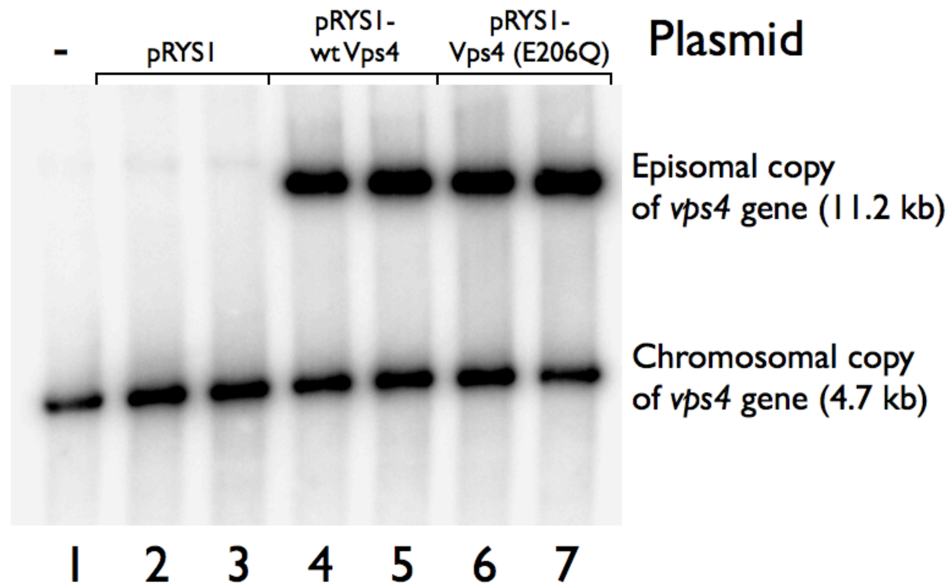


Figure S10. Southern blotting confirming the presence of an episomal copy of the Sso0909 *vps4* gene. Genomic DNA was prepared from PH1-16 (lane 1) or two separate transformants of PH1-16 pRYS1 (lanes 2 and 3), PH1-16 pRYS-Vps4 (lanes 4 and 5) or PH1-16 pRYS1-Vps4(E206Q) – lanes 6 and 7. The presence of the *vps4* gene in PH1-16 pRYS-Vps4 or PH1-16 pRYS1-Vps4(E206Q) is revealed by the upper band. Quantitation of the relative intensity of the episomal and chromosomal copy of the *vps4* genes reveals that the episome is present in a 3-fold to 4-fold higher copy number than the chromosomal copy.

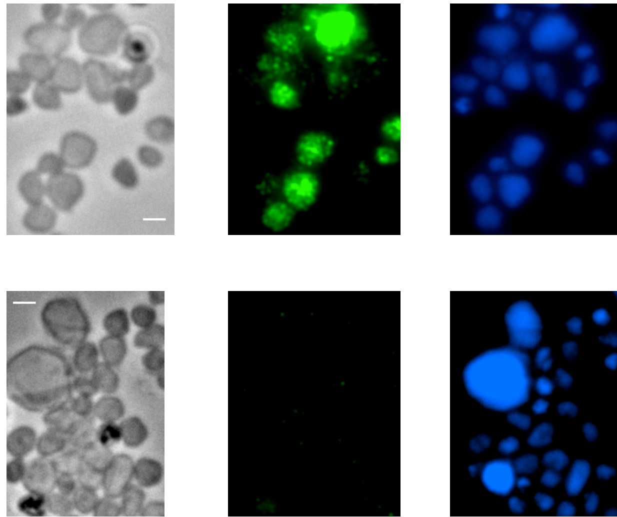


Figure S11. Immunolocalization of Vps4 (top panels) and Saci1373 (ESCRT-III) in cells over-expressing the Walker B mutant Vps4. No discernable accumulation of Saci1373 (ESCRT-III) is apparent, suggesting that dismantling of ESCRT-III lattices is not a prerequisite for their cell cycle modulated alterations in abundance.

Supplementary Table I. Data collection, structure determination and refinement statistics for the Saci1372 MIT/Saci1373 (ESCRT-III) peptide complex

	Native data[a]
Data collection statistics	
Resolution	2.2 Å
Completeness (last shell)	99.5 (99.5)
R_{merge}[b] (last shell)	0.141 (0.52)
Multiplicity (last shell)	7.2 (7.4)
< I/σ > (last shell)	16.2(3.7)
Unit cell $P4_3$	a=49.9 Å c=107.7 Å
Refinement statistics	
Resolution range	49.9 Å –2.2Å
Number of reflections	12626
Cutoff (F/σ)	None
Completeness	99.5%
Protein atoms	1370
Average B factor	16.8 $Å^2$
(Wilson B factor)	(22 $Å^2$)
Waters	118
R_{cryst}[c]	0.23
R_{free}[c] (% data used)	0.27 (5.1)
r.m.s.d. from ideality[d]	
bonds	0.008 Å
angles	1.0°
dihedrals	4.8°

[a]Data sets were collected at ESRF beamline ID29 at λ=1.00 Å using an ADSC Q315 detector.

[b]$R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

[c]R_{cryst} and $R_{free} = \sum ||F_{obs}| - |F_{calc}|| / \sum|F_{obs}|$; R_{free} calculated with the percentage of the data shown in parentheses.

[d]r.m.s. deviations for bond angles and lengths in regard to Engh and Huber parameters (*5*)

Supplementary Table 2. Oligonucleotides used in this study

Target	Forward Primer	Reverse Primer	Plasmid Constructed
Saci1373	GGATTCCCATATGTTTGATAAGTTATCGATAATTTTTAATAG	GGGAATTCCTCGAGACCCTCAAGAACAATTAGACCCTTTTC	pET30_Sac1373 pGBK_Sac1373 pGAD_Sac1373
Saci1372	GTTAATGTCATATGTCTGCCCAAGTAATGCTTGAAGAGA	GGGAATTCCTCGAGTTATAGAGCCTTATACTTCTCGTGCCA	pET30_Sac1372
Saci1373	GGATTCCATGGCAATGTTTGATAAGTTATCGATAATTTTTAATAG	GGGAATTCCTCGAGTTAACCCTCAAGAACAATTAGACCCTTTTC	pET42_Sac1373
Saci1373	GAAACAGACCATGGAAGAAGCTCAGAAGATGGCTGAAG	GGGAATTCCTCGAGTTAACCCTCAAGAACAATTAGACCCTTTTC	pET42_Sac1373 tail
Saci1373	CTGAAGCCATGGTTAGAGAATTGTTGCCAGAACTACC	GGGAATTCCTCGAGTTAACCCTCAAGAACAATTAGACCCTTTTC	pET42_Sac1373 RELL
Saci1373	TACCCCCCATGGCTTCAGAGTTACCAAAGAGAG	GGGAATTCCTCGAGTTAACCCTCAAGAACAATTAGACCCTTTTC	pET42_Sac1373 SELP
Saci1372	GTTAATGTCAATGTCTGCCCAAGTAATGCTTGAAGAGA	GGGAATTCCTCGAGTAGAGCCTTATACTTCTCGTGCCA	pET30_Sac1372minusMIT
Saci0451	CGATTCTGCATATGACCGCAATATATATATTGACCATGATAAGC	CCATGTCACTCGAGTTAGCTCTTTTGTTGCTGTGTAATTCC	pGBK_Sac0451 pGAD_Sac0451
Saci1416	GTTACGATCATATGGCAGATGTGAATGATTTTCTGAG	CTGCAGTGCTCGAGTTATTTTTGATTTGCGTTTGTTTTTGATCCACCGAAGTTGC	pGBK_Sac1416 pGAD_Sac1416
Saci1601	CGTATGTCCATATGAAGAAAAGGACGATTGCTGAATTACTCACTG	GGATCTTCCTCGAGTTAAATCTCAATTTTATAGTTTTCTTTAAGCTCCTTCTTAGCC	pGBK_Sac1601 pGAD_Sac1601
Sac pyrEF	(SacEF_F) CTTACAGCTCGAGTAACGCCCTTAAATAAGGTTAGTC	(SacEF_R) AAGTCAAGATGCATCAAATCTGTTGTGGGAACTTCACCGG	pCRScript_*pyrEF*
Sso0909 and pOP319	(Sso0909 5') GAATTTCCCATGGGTGCACAAGTAATGTTAGAAG	(Sso0909 3'FLAG) ATGCGGGCCCTCACTTGTCGTCGTCGTCCTTGTAGTCTAATGCCTTAAATTTCTCTGTCC	pSVA5_Sso0909 and pSVA5_Sso0909E206Q
pSVA5_Sso0909 and pSVA5_Sso0909E206Q	(ara0909_F) GAATTTCCCGCGGCCTAGGGCACCATATGTTTAGAGATG	(ara0909_R) ACTTATGCGCGGCCGCTCACTTGTCGTCGTCGTCCTTGTAGTC	pRYS1_Sso0909 pRYS_Sso0909E206Q
Target	**Mutagenesis Oligo**		**Plasmid Constructed**
pET30_Sac1372	GGAATTAATTCCTGCTGATGGTGCGTAGAATGGGAATGGTAAACACAG		pET30_Sac1372 MIT
pET42_Sac1373	GATGGCTGAAGTAAAAGTTTAAGAATTGTTGCCAGAACTACC		pET42_Sac1373 CORE1
pET42_Sac1373 and pET42_Sac1373RELL	GAACTACCCCATCCACCTTAAGAGTTACCAAAGAGAGTA		pET42_Sac1373 CORE2 and pET42_MIM2

Supplementary Table 3. Oligonucleotides used in quantitative PCR (upper table).
The number of molecules of a particular gene at a given time point was extrapolated from its corresponding standard curve. Triplicate experimental values were averaged and all time points were normalized to 0 minutes.

Target Gene	Forward Primer	Reverse Primer
Saci0451	TACAGGCTTGTTCAGGCTCA	TACATTGCTGCCCTTGACTG
Saci1372	AAGCCGACAAAGAGGGAAAT	AGCTGTCGATCCGTCCCTAT
Saci1373	CACAAGAGATCTCGGACATACG	GCACCAGAGAAACTCCCTGT
Saci1416	GGGCTGCAATGTACGCTAAT	CAATAACCGGGACCAAGGAG
Saci1460 (NusG)	TTGAAGCTACCGGACCTCAT	GCGGGTAATGCAACTGACTT
Saci1601	CGCTAGAAAGATTGTCGCTGT	TTTCCTCGTAAGCTCTGTTTCC

Target Gene	0	5	30	60	90	120	150	180	210
Saci0451	1	0.88	0.51	0.51	0.55	0.51	1.25	1.66	1.68
Saci1372	1	0.72	0.68	0.72	0.78	1.06	2.07	2.16	1.73
Saci1373	1	1.08	0.79	0.88	1.07	1.58	2.46	2.97	2.18
Saci1416	1	1.07	0.81	0.80	0.91	0.87	1.80	2.86	2.16
Saci1460 (NusG)	1	1.05	1.27	1.02	0.96	0.92	1.19	1.19	1.16
Saci1601	1	0.86	1.05	1.12	1.17	1.14	1.58	1.7	1.41

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