Supporting Online Material for

A role for the ESCRT system in cell division in Archaea

Rachel Y. Samson ^{1,4}, Takayuki Obita ², Stefan M. Freund ³, Roger L. Williams ² and Stephen D. Bell ^{1,4}

¹MRC Cancer Cell Unit, Hills Road, Cambridge CB2 0XZ, UK

²MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, UK

³MRC Centre for Protein Engineering, Hills Road, Cambridge CB2 0QH, UK

⁴Present address Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE,

UK

* To whom correspondence should be addressed. E-mail: stephen.bell@path.ox.ac.uk
This PDF file includes
Materials and methods
Figs. S1 to S12
Tables S1 to S3
References

Materials and methods

Construct optimization for crystallization

Initially, we used His₆-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. ¹³C/¹⁵N-labeled protein was expressed in M9 minimal medium containing ¹³C-Glucose and ¹⁵NH₄Cl. 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. ¹³C/¹⁵N-labeled peptide was expressed in M9 minimal medium containing ¹³C-Glucose and ¹⁵NH₄Cl. 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 ¹³C/¹⁵N-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} ¹⁵N 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).

His₆FlAsH-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]. His₆FlAsHtagged 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 °C 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 °C. *S. solfataricus* PH1-16 used for genetic over-expression studies was grown at 75 °C in Brock's medium, pH 3.2 with 10 μ g/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 °C. Isolated colonies were grown in liquid SC-Leu-Trp medium to an $OD_{600} = 0.1.5 \mu l$ 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 His₆-tagged protein expression, cultures were grown in LB at 37 °C, 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 °C and

clarified by centrifugation at 35,000 g for 10 min. For purification of Saci1373-His₆, 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 °C. 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-His₆ in two goats and against purified Saci1372-His₆ 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 μ l bead volume containing 5 μ g of GST fusion protein were incubated with 2 μ g of either Vps4, MIT domain or Vps4 AAA+ domain as indicated. Incubations were performed for 30 minutes at 30 °C in 100 μ l of TBSTM (10 mM Tris pH 8.0, 150 mM NaCl, 0.1 % Tween-20, 5 mM MgCl₂). Beads were recovered by brief centrifugation, resuspended in 500 μ l 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 μ l 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 μ m 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 μ m 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 μ l RNase-free water. The tube was vortexed for 10 s to wash cells from the filter. 100 μ l of 6X lysis buffer (600mM sodium acetate, pH 5.2; 6 % SDS) and 600 μ l 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 °C 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 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 realplex² 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 μ l cells in 1 mm cuvettes using a time constant protocol with the following parameters: 1500 V, 25 μ F, and 400 Ω . Cells were allowed to regenerate in 1 ml Milli-Q water for 10 minutes at 75 °C before plating on solid Brock's medium lacking uracil. After incubation at 75 °C 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, $3 \mu g$ DNA were resolved on a 0.7% agarose gel. The gel was then agitated in dH₂O 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. ³²P-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 °C 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 OD₆₀₀ 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/µ1] (Invitrogen)). After 5 min on ice, paraformaldehyde was added to 2.5 % final concentration and the cells were incubated at 25 °C 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 °C 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 μ l PBS and 10 μ l 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 μ l VectraShield (Vector Co.) containing DAPI [1.5 ng/ μ l]. 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 μ m increments.



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

ST1214/1-266		
Mand 1671/1-270		
Thut 0797/1-286		
Smar 1277/1-260		
APE_0962/1-271		
Igni_0995/1-255		
5500881/1-221		
5500451/1-284	1 MFPSVFIFTLLYSSSFHLRSKYITTLSVDTKMSYSGSSSSLM	INVFGILLISDSNNLNSSHYVFDLIRV
571237/1-219	Notempore and a second s	
Saci 1416/1-210	1	SILITIFFSLIFKFILCIKFLIKFIK.
Saci 0451/1-214	1	MT.
Maod 1695/1-221		
Msod_2179/1-211		
mbut_1468/1-218		
Smar_0481/1-212		
APE_0143.1/1-223		
Ign1_1156/1-210		
Near 0816/1-216		
Near 0061/1-216		
CENSTa 1915/1-214		
CENSTA 1885/1-195		
\$\$00619/1-168		
ST1485/1-165		
Saci_1601/1-169	•••••••••••••••••••••••••••••••••••••••	
Mned_1969/1-165	•••••••••••••••••••••••••••••••••••••••	
Rout_1206/1-160		
Smar 0222/1-194		
CHMP6 HUMAN/1-201		
CIIM4A IIUMAN/1-222		
CHM4B_HUMAN/1-224		
CHM4C_HUMAN/1-233		
CHMP3_HUMAN/1-222	•••••••••••••••••••••••••••••••••••••••	
CIIM2A_HUMAN/1-222		
CIIM2B_HUMAN/1-213		
CHMPIA HUMAN/1-196	•••••••••••••••••••••••••••••••••••••••	
CHR15 H0808/1-199		
CHMPS HUMPN/I-219		
CIR95_IURAN/1-219	<u>h0</u>	h1
\$\$00910/1-259 \$71214/1-266	h0	
\$\$00910/1-259 \$71214/1-266 \$aci 1373/1-261		h1
SS00910/1-259 ST1214/1-266 Saci_1373/1-261 Maed_1671/1-270	hO MIDKLPTIFNNEKREKA MIDKISSFFNNDRKKKA MEDKLSIFNTORKKA	h1
SB00910/1-259 S71214/1-266 Saci_1373/1-261 Need_1671/1-270 Mut_0797/1-286	h0	h1
SS00910/1-259 ST1214/1-266 Saci 1373/1-261 Mead 1671/1-270 Ibut_0707/1-286 Sacr_1277/1-260	hO MIDKLPFIFNNIKRRMA MIDKISSFFNNDKRRMA MYDKLSIFNIFNKRMA MKLSIFNIFRKRMA MAIISRLGFLGFLFFFFFFFFF	h1
SS00910/1-259 S71214/1-266 Saci_1373/1-261 Need_161/1-270 Hbut_0797/1-286 Sana_1277/1-260 APE_0562/1-271 	hO MFDKLPTIFNNEKRKA MEDKISSFFNNDRKKA MEDKISSFFNNDRKKA MEDKISSFFNNDRKKA MEDKISSFFNNDRKKA MEDKISSFFNNBRKA MEDKISSFFNBRKA MEDKISS	h1
SB00910/1-259 S71214/1-266 Saci_1373/1-261 Mead_1671/1-270 Hbut_0707/1-286 Saar_1277/1-260 APR_0962/1-271 IgnI_0995/1-275 SenomIII(1.271	hO MPDKLPFIFNNEKEKKA MLDKISFFFNDEKEKA MFDKLSIFNITRKKI MKLSIFNITRKKI MKLSIFNITRKKI MKLSIFNITRKKI MKLSIFNITRKKI MKLSIFNITRKKI MKLSIFNITRKKI	h1
SS00910/1-259 S71214/1-266 Saci 1373/1-361 Meed_1671/1-270 Hbut_0797/1-286 Sam_1277/1-260 APE_0562/1-271 Igni_0995/1-255 SS00451/1-221 SS00451/1-286	hO MIDKLPFIFNNEXREMA MIDKISEFFNDEKEKA MEDKISIFFNDEKEKA MEDKISIFNEKEKE MERISIFNEKE MERISIFNEKE MERISIFNEKE MERISIFNEKE MERISIFNEKE MERISIFE MERISI	h1
SB00910/1-259 S71214/1-266 Saci_1737/1-261 Need_1671/1-270 Thut_0797/1-286 Saar_1277/1-260 APR 0962/1-271 IgnI_0995/1-255 S500081/1-221 S500081/1-219	hO MFDKLDFJFJNNEXREKA MEDKLDFJFJNNEXREKA MEDKLSFFJNNDKRKA MEDKLSFJNDKRKA MELSELPHUTEKKE MALISELEPLGVGEHKOJ MSTGIGS.RGIGWFGLFJGFJKEBKK MSTGIGS.RGIGWFGLFJGFJKEBKK MSTGIGS.CGFJLANFJKA MSTGIGS	h1
SS00910/1-259 S71214/1-266 Saci 1373/1-261 Maed_1671/1-270 Mbut_0707/1-286 Sanz_1277/1-286 Sanz_1277/1-255 SS00451/1-225 SS00451/1-284 S71237/1-284	hO MIDELPFIPNEXERAA MIDELSFPINEXERAA MIDELSFP	h1
SB00910/1-259 S71214/1-266 Saci_137/1-261 Macd_1671/1-270 Bbut_0797/1-286 APR 0962/1-271 TqmI 0995/1-255 S000021/1-221 S8000251/1-229 S70164/1-219 S70164/1-219	h0 MFDRLPFIFNNERREAL MEDRLSIFNNDREERA MEDRLSIFFNNDREERA MEDRLSIFFNDREERA MEDRLSIFFNDREERA MED	h1
SE00910/1-259 ST1214/1-266 Saci_1737/1-261 Mead_1671/1-270 Mbut_0707/1-286 Sanr 1277/1-286 SP00001/1-271 IgnI 0995/1-275 SP000051/1-271 SP000051/1-271 SP000051/1-271 SP000051/1-271 SP000051/1-271 Seci_0451/1-2719 Saci_0451/1-2719	hO MIDKLPFIFNNEKERKA MIDKLSFFFNNEKERKA MIDKLSFFFNEKKRKA MIDKLSFFFNEKKRKA MIDKLSFFFNEKKRKA MIDKLSFFFNEKKR MIDKLSFFFEKKRKA MIDKLSFFFEKKR MIDKLSFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSFFFEKKR MIDKLSF	h1
SS00910/1-259 ST1214/1-266 Saci 1373/1-261 Nsed_1671/1-270 Hbut_0797/1-286 Sar_1277/1-286 Sar_1277/1-286 SS00451/1-221 SS00451/1-221 SS00451/1-221 Saci_0451/1-221 Saci_0451/1-221	hO MIDNLISEPPINDERERA MIDNLISEPPINDERERA MEDNLISEPPINDERERA MEDNLISEPPINDERERA MEDNLISEPPINDERERA MEDNLISEPPINDERERA MEDNLISEPPINDERERA MEDNLISEPPINE MEDNLISEPPIN	h1
SE00910/1-259 S71214/1-266 Saci_1737/1-261 Need_1671/1-270 Hbut_0797/1-286 Saar_1277/1-260 APR 0962/1-271 Igni_0995/1-255 SE00081/1-221 SE00081/1-221 SE00081/1-219 Seci_1416/1-219 Saci_045/1-211 Need_1605/1-221 Need_1605/1-221	hO MFDKLPFIPNNEXREMA MEDKLPFIPNNEXREMA MEDKLSFPFNNDKRAM MEDKLSFPFNDDKRAM MEDKLSFPFNDKRAM MELSELPNTRKKS MALISELEPLGVGIEKOF MALISELEPLGVGIEKOF MALISELEPLGVGIEKOF MELSELPLGVGIEKOF MELSELPLGVGIEKOF MELSELPLGVGIEKOF MELSELPLGVGIEKOF MELSELPLGVGIEKOF MELSELFUNDE MELSE	h1
SE00910/1-259 S71214/1-266 Saci_1373/1-261 Maed_1671/1-270 Mbut_0707/1-286 Sanr 1277/1-286 Sanr 1277/1-285 SE00451/1-271 SE00451/1-214 Seci_1416/1-219 Saci_0451/1-214 Maed_1695/1-221 Maed_2179/1-211 Thut_1460/1-219 Sac_0451/1-214	hO MIDELPFIPNEXERAN MIDELPFIPNEXERAN MIDELSIPPIPNEXERAN MIDELSIPPIPNEXERAN MIDELSIPPIPNEXERAN MIDELSIPPIPREXER MIDELSIPPIRAL MIDELSIP	h1
SB00910/1-259 S71214/1-266 Saci_1373/1-261 Nacd_1671/1-270 Mbut_0797/1-286 Sanr 1277/1-286 Sanr 0995/1-251 S500081/1-221 S500081/1-221 S500081/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_045/1-211 Macd_1219/1-211 Macd_1219/1-211 Sanr 0401/1-212 Sanr 0401/1-212	h0 MFDKLDFIFNNEXERKA MEDKLDFIFNNEXERKA MEDKLSFFNNEXERKA MEDKLSFFNNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERKA MEDKLSFFNEXERK MED	h1
SE00910/1-259 ST1214/1-266 Saci_1373/1-261 Meed_1671/1-270 Hbut_0707/1-286 Sar_1277/1-286 Sar_1277/1-285 SE00001/1-221 SE000051/1-221 SE000051/1-221 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-211 Meed_2179/1-211 Ebut_1460/1-219 Sar_0401/1-212 AFR_0143.1/1-223 AFR_0143.1/1-223	hO MPDKLPPIPNKIKKKKKK MEDKLSPPINKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK	h1
SS00910/1-259 S71214/1-266 Saci 1373/1-261 Mand 1671/1-270 Mbut C707/1-286 San-1277/1-260 APE 0962/1-271 Ss00431/1-221 SS00431/1-224 S71237/1-294 S71237/1-294 S71237/1-294 Saci 0451/1-214 Mand 1605/1-221 Mand 1607/1-213 Saci 0451/1-214 Mand 1607/1-213 Saci 0461/1-213 Saci 0461/1-213	ho MFDRLPTIPNERERA MEDRLPTIPNERERA MEDRLSTP	h1
SE00910/1-259 ST1214/1-266 Saci_1737/1-261 Msed_1671/1-270 Hbut_0797/1-286 Sanr 1277/1-260 APR 0962/1-271 Igni 0995/1-255 SE00001/1-221 SE000051/1-221 SE000051/1-210 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-210 Msed_1605/1-221 Hbut_1660/1-211 Bbut_1660/1-211 Bbut_1660/1-212 APR 014.1/1-223 APR 014.1/1-223 APR 014.1/1-223 Msen_0029/1-215 Msen_0029/1-215	h0 	h1
SS00910/1-259 S71214/1-266 Saci_1737/1-261 Mead_1671/1-270 Hbut_0707/1-286 Sanr 1277/1-286 Ss00451/1-271 TgnI_0995/1-255 SS00451/1-214 Ssci_0451/1-214 Ssci_0451/1-214 Mead_21797/1-219 Saci_0451/1-214 Mead_21797/1-211 Hbut_1605/1-212 Mead_21797/1-213 Smar_0016/1-215 Smar_0016/1-216 Smar_0016/1-216	hO MIDELPFIPNEXERNA MIDELPFIPNEXERNA MIDELSFPINEXERNA MIDELSFPINEXERNA MIDELSFPINEXERNA MIDELSFPINEXERNA MIDELSFPINEXERNA MIDELSFPINEXERNA MIDELSFFINEXER MIDELSFFIN	h1
SB00910/1-259 S71214/1-266 Saci 1373/1-261 Need_1671/1-270 Thut_070/1-286 Sanr 1277/1-286 Sanr 1277/1-286 S500081/1-221 S5000851/1-221 S5000851/1-221 S5000851/1-219 S500180/1-219 S500180/1-219 S500180/1-219 S500180/1-219 S500180/1-218 S500180/1-218 S500180/1-218 S500180/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218 S500191/1-218	h0 MFDKLDFJFJPNNEXKRKA MEDKLDFJFJPNNEXKRKA MEDKLSFJPNNEXKRKA MEDKLSFJPNNEXKRKA MEDKLSFJPNEXKRKA MEDKLSFJPLOVENE MELSLIPNICK MEL	h1
SB00910/1-259 S71214/1-266 Saci_1737/1-261 Meed_1671/1-270 Hbut_0707/1-286 Sanr_1277/1-286 Sanr_0905/1-271 IgnI 0905/1-271 S00035/1-219 S70160/1-271 Saci_055/1-214 Meed_1095/1-219 Saci_055/1-214 Meed_109/1-211 Sbut_1609/1-211 Sbut_1609/1-213 Meed_2109/1-211 Shut_1609/1-213 Meed_2109/1-215 Meed_2109/1-215 Meed_000/1-216 CHWTm_1005/1-205 Saci_055/1-214 CHWTm_1005/1-216	hO MIDKLPFIPNEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNDEXKEK MIDKLSPFPNE	h1
SB00910/1-259 S71214/1-266 Saci_1373/1-261 Nacd_1671/1-270 Hbut_0797/1-286 Sanr 1277/1-286 Sanr 1277/1-286 Sarr 0995/1-271 S500081/1-221 S500081/1-221 S500081/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_0451/1-214 Macd_1095/1-221 Macd_1097/1-218 Sanr 0401/1-215 Sanr 0401/1-216 CHNUTA_195/1-216 CHNUTA_195/1-216 CHNUTA_195/1-266	ho MIDKLDFIJNNEXKENA MIDKLSJPNNEXKENA MIDKLSJPNINK MIDKLSJPNINK MIDKLSJPNIKK MIDKLSJPNIKK MIDKLSJPNIKK MIDKLSJPNIKK MIDKLSJPNIKK MIDKLSK	h1
SE00910/1-259 ST1214/1-266 Saci_1373/1-261 Maed_1671/1-270 Hbut_0797/1-286 Sanr 1277/1-260 APR 0962/1-271 IgnI 0995/1-255 SE00081/1-221 SE00081/1-221 SE00081/1-221 Saci_045/1-219 Saci_045/1-219 Saci_045/1-219 Saci_045/1-219 Saci_045/1-219 Saci_045/1-219 Maed_2179/1-211 Hbut_1660/1-210 Sanr 0401/1-212 APR 0143.1/1-223 Igni_1156/1-210 Sanr_0029/1-215 Sanr_0016/1-216 Sanr_005/1-216 Sanr_005/1-216 Sanr_005/1-216 Sanr_005/1-216	hO MFDRLPFIPNERREAD MEDRLPFIPNERREAD MEDRLSFPFNEDREREAD MEDRLSFFPNEDREREAD MEDRLSFFPNEDREREAD MEDRLSFFPERDEREAD MEDRLSFFPERDEREAD MEDRLSFFFERDEREAD MEDRLSFFERDEREAD MEDRLS	h1
SB00910/1-259 S71214/1-266 Saci_173/1-261 Maed_1671/1-270 Hbut_0797/1-286 Basr_1277/1-286 Saci_1277/1-286 Saci_242(-271 TqmI_0995/1-271 S5000000000000000000000000000000000000	ho MIDELIPITIONEXERN MEDELIPITIONEXERN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITPNEDEREN MEDELISITENEN MED	h1
SE00910/1-259 S71214/1-266 Saci_1737/1-261 Need_1671/1-270 Thut_079/1-286 Sar_1277/1-260 APR 0962/1-271 Igni 0995/1-255 S50008/1-211 S50008/1-211 S50008/1-219 Saci_045/1-219 Saci_045/1-219 Saci_045/1-219 Need_1605/1-210 Need_1605/1-210 Need_1607/1-213 Need_1607/1-213 Need_1607/1-213 Need_1607/1-213 Need_1607/1-213 Need_1607/1-213 Need_007/1-214 CHNUTA_1005/1-214 CHNUTA_1005/1-305 S7006197/1-65 S71405/1-165 Saci_1601/1-169 Need_1969/1-165	h0 MFDKLDFJFJPNNEXKRMJ MEDKLDFJFJPNNEXKRMJ MEDKLSFPFNNEXKRMJ MEDKLSFFJNNEXKRMJ MEDKLSFFJNEXKRMJ MEDKLSFFJRAKKK MEDKLSFFJRAKK MEDKLSFFJRAKKK MEDKLSF	h1
SB00910/1-259 S71214/1-266 Saci_1737/1-261 Mead_1671/1-270 Hbut_0797/1-286 Sar_1277/1-286 Sp000000000000000000000000000000000000	hO MIDELPFIFNERKERKE MIDELSFFFNERKERKERKERKERKERKERKERKERKERKERKERKERKE	h1
SB00910/1-259 S71214/1-266 Saci_173/1-261 Maed_1671/1-270 Thut_070/1-286 Sanr 1277/1-286 Sanr 1277/1-286 Sanr 0995/1-271 S500081/1-221 S500081/1-221 S500081/1-221 S500081/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-218 Sanr_0401/1-213 Sanr_02010/1-215 Sanr_0016/1-216 Sanr_0016/1-216 Sanr_0016/1-216 Sanr_0016/1-216 Saci_1601/1-160 S71405/1-165 S1405/1-165 Saci_1601/1-169 Nacd_1969/1-165 Sanr_0016/1-265	ho MFDKLDFIFNNEXKEKA MEDKLDFIFNNEXKEKA MEDKLSIFNEXKEKA	h1
SE00910/1-259 ST1214/1-266 Saci_1373/1-261 Meed_1671/1-270 Hbut_0797/1-286 Sanr_1277/1-260 APR 0962/1-271 TgmI 0995/1-271 SE00081/1-221 SE00081/1-219 Scril416/1-219 Saci_0451/1-214 Meed_1299/1-211 Hbut_1609/1-219 Sanr_0401/1-212 APR 0143.1/1-223 Igmi_1166/1-210 Mmar_0016/1-216 CHNUTA_1095/1-214 CHNUTA_1095/1-214 CHNUTA_1095/1-165 Saci_061/1-165 Saci_061/1-165 Saci_062/1-165 Saci_062/1-165 Saci_062/1-165 Saci_062/1-165 Saci_062/1-165 Saci_022/1-165 Saci_022/1-165 Saci_022/1-165 Saci_022/1-165	h0 MIDKLSPIPHNEXKKKS MIDKLSPIPHNEXKKKS MIDKLSIPHIPHNEXKKKS MIDKLSIPHIPHNEXKKKS MIDKLSIPHIPHNEXKKS MIDKLSIPHIPHKKKS MIDKLSIPHIPHKKKS MIDKLSIPHIPHKKKS MIDKLSIPHIPHKKKS MIDKLSIPHIPHKKKS MIDKLSIPH MAGUNAN MAD VNDIJANNAG, GR. GENTISHANNER MAGUNAN MAD VNDIJANNAG, GR. GENTISHANNER MAGUNAN MAD VNDIJANNAG, GR. GENTISHANNER MAGUNAN MAD VNDIJANNAG, GR. GENTISHANNER MAGUNAN MAD VNDIJANNAG, GR. GENTISHANNER MAGUNAN MAGUNA	h1
SB00910/1-259 S71214/1-266 Saci_1373/1-261 Nacd_1671/1-270 Hbut_0797/1-286 Sar_1277/1-286 Sar_1277/1-286 Sar_1277/1-286 Sar_1277/1-280 S5000001/1-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 S5000001-221 Macd_1059/1-221 Saci_1406/1-210 Saci_001/1-216 Smar_0001/1-216 ST1405/1-216 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-165 S5000007/1-160 S14007/1-165 S5000007/1-160 S14007/1-165 S5000007/1-160 S14007/1-160 S14007/1-160 S14007/1-160 S14007/1-222	ho MIDKLDFIJNNEXKERA MIDKLSJPNINK MIDKLSJPNINK MIDKLSJPNINK MIDKLSJPNINK MIDKLSJPNINK MIDKLSJPNIK MIDKLSKUP MIDK	h1
SE00910/1-259 ST1214/1-266 Ssci_1373/1-261 Msed_1671/1-270 Hbut_0797/1-286 Ssar_1277/1-260 APR 0962/1-271 IgmI 0995/1-255 SE000001/1-221 SE000001/1-221 SE000001/1-221 SE000001/1-221 Seci_045/1-214 Msed_2179/1-211 Hbut_1260/1-210 Smar_0401/1-212 APR 0643.1/1-222 Igmi_1156/1-210 Smar_0401/1-215 Smar_0401/1-215 Smar_0401/1-216 CINUTA_1005/1-205 Sci_1601/1-165 Sti455/1-165 Ssci_1601/1-169 Msed_1969/1-165 Sti455/1-165 Ssci_1601/1-169 Msed_1969/1-165 Igmi_1016/1/1-166 Sti455/1-165 Ssci_1601/1-169 Msed_1969/1-165 Igmi_1010/1/1-178 Ssci_0222/1-186 CINUTA_10104X1-222 CINUTA_10104X1-222	h0 MIDELPFIPHEREREA MEDELPFIPHEREREA MEDELPFIPHEREREA MEDELFFIPHEREREA MEDELFFIPHEREREA MEDELFFIPHEREREA MEDELFFIPHEREA MEDELFFIFTEREA	h1
SB00910/1-259 S71214/1-266 Saci_173/1-261 Maed_1671/1-270 Hbut_079/1-286 Sari 1277/1-286 Sari 1277/1-286 Sari 1277/1-286 Sari 0995/1-251 S500001/1-221 S500001/1-221 S500001/1-221 S5000000000000000000000000000000000000	MIDELIGNICAL PROPERTY AND A CONTRACT AND A CON	h1
SB00910/1-259 S71214/1-266 Saci_1737/1-261 Need_1671/1-270 Thut_070/1-286 Sari_1277/1-260 APR 0962/1-271 Igni 0995/1-255 S500081/1-221 S500081/1-221 S500081/1-221 S500081/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-210 Saci_0451/1-210 Saci_0451/1-210 Saci_051/1-210 Saci_051/1-216 Saci_051/1-165 S100519/1-221 CHNAT_HUMAX/1-221 CHNAT_HUMAX/1-221 CHNAT_HUMAX/1-223 CHNA_THUMAX/1-223 CHNA_THUMAX/1-223	h0 MEDELDFIFNNERKEN MEDELDFIFNNERKEN MEDELDFIFNNERKEN MEDELDFIFNNERKEN MEDELDFIFNERKEN	h1
SB00910/1-259 S12124/1-266 Saci_1373/1-261 Meed_1671/1-270 Hbut_0797/1-286 San_1277/1-260 APR 0962/1-271 Igni 0995/1-251 S00081/1-221 S00081/1-219 S00180/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-219 Saci_0451/1-214 Need_1299/1-211 Hbut_1609/1-219 San_0401/1-212 APR 0143.1/1-223 Igni_1166/1-210 Nmar_0016/1-216 CTHWTA_1035/1-216 ST1485/1-165 Saci_045/1-165 Saci_05/1-160 S1485/1-165 Saci_045/1-165 Saci_05/1-160 S1485/1-165 Saci_0601/1-178 Saci_022/1-186 S1485/1-165 Saci_01/1-178 Saci_022/1-186 S1485/1-165 Saci_01/1-178 Saci_022/1-186 S1485/1-165 Saci_022/1-186 S1485/1-221 CHMS4_IUMAX/1-222 CHMS4_IUMAX/1-222 CHMS4_IUMAX/1-222 CHMS4_IUMAX/1-223 CHMS4_IUMAX/1-222 CHM24_IUMAX/1-223	h0 MIDNLDFIFNNERKEN MIDNLDFIFNNERKEN MIDNLDFIFNNERKEN MIDNLDFIFNNERKEN MIDNLDFIFNNERKEN MIDNLDFIFNNERKEN MIDNLDFIFNERKEN MIDNLDFIFNERKEN MIDNLDFIFNERKEN MADUNDFURMERKEN MUNFFURMERKEN	h1
SB00910/1-259 S71214/1-266 Saci_173/1-261 Maed_1671/1-270 Thut_070/1-286 Sari_1277/1-260 APR 0962/1-271 IgnI 0995/1-255 S500081/1-221 S500081/1-221 S500081/1-221 S500081/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_1416/1-219 Saci_0451/1-211 Maed_1605/1-221 IgnI_156/1-210 Saci_021/1-216 Saci_021/1-216 Saci_001/1-216 Saci_1601/1-169 S100519/1-168 S100519/1-222 S100519/1-223 S10051	ho 	h1
SE00910/1-259 ST1214/1-266 Ssci_1373/1-261 Meed_1671/1-270 Hbut_0797/1-286 Ssci_1671/1-271 IgnI 0995/1-251 S500081/1-221 S500081/1-221 S500081/1-221 S500081/1-221 Ssci_065/1-221 Ssci_065/1-221 Meed_2179/1-211 Hbut_1260/1-219 Ssci_065/1-221 Meed_2179/1-211 Hbut_1260/1-210 Smar_0101/1-212 Smar_0101/1-215 Smar_0101/1-216 ST1455/1-165 Ssci_065/1-165 Sto00619/1-160 ST1455/1-165 Ssci065_11048x/1-222 CHN54_1005/1-126 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-160 Sto00619/1-222 CHN54_HUMAx/1-222 CHN54_HUMAx/1-223 CHN54_HUMAx/1-223 CHN54_HUMAx/1-190	h0 MIDELPFIPHEREMENT MEDELPFIPHEREMENT MEDELPFIPHEREMENT MEDELPFIPHEREMENT MEDELFFIPHEREMENT MEDELFFIPHEREMENT MEDELFFIPHEREMENT MEDELFFIFTER MEDELFFIFTER MEDEL	h1
SB00910/1-259 S71214/1-266 Saci_173/1-261 Maed_1671/1-270 Hbut_079/1-286 Sari 1277/1-286 Sari 1277/1-286 Sari 1277/1-286 Sari 1277/1-286 Sari 1277/1-280 S70168/1-221 S500081/1-221 S500081/1-221 S500081/1-219 S500081/1-219 S500081/1-219 S500081/1-219 S70168/1-219 Saci_1661/1-219 Saci_0451/1-219 Saci_0451/1-211 Maed_1059/1-221 Igni 1156/1-210 Marc 0029/1-215 Saci 0616/1-216 Saci_1601/1-165 Saci_1601/1-165 Saci_1601/1-165 Saci_0106/1-216 CHMUT_1055/1-221 CHMUT_1055 Saci_0101/1-178 Saci_021/1-185 Saci_0101/1-178 Saci_0101/1-178 Saci_0101/1-222 CHMUA_INUMX/1-222 CHMUA_INUMX/1-223 CHMUA_	MEDELPFIELD MEDILPFIELD MEDILPFIELD MEDILPFIELD MEDILFFIELD MEDILFFIE	h1

		h1	h2	h3
\$500910/1-259 \$71214/1-266 \$aci_1373/1-261 Nead_1671/1-270 Bbut_079/1-286 \$mar_1277/1-286 Apr_0562/1-271 Tgni_0995/1-255 \$50081/1-221	41 41 44 50 51 39 57	IRE KORDIELTIKVVRAQVEG IRE KORDIELTIKVIRAQIEG IRE KORDITIKVIRAQIEG IRE KERDEDITIKVIRAQIEG IRE KERDEDITIKVIRAQIEG IRE KERDEDITIKVRAQUEG IRE KERDEDITIKVRA IRE KERLE IRE KORDITIKE IRE KERLE IRE KORDIE IRE KERLE IRE KERL	D DAKAKIYAQ IAD CARI KVIYAPLA DAKAKIYAQ ISD CRMIKIYIYAPLA ISD XXAYYAQ ISD CRMIKIYYAPLA MTAATIYAQ ISD CRMIKIYYARLA MTAATIYAQ ISD CRMIKIYYARLA ROYAD TIRL KUKACLIYAPVI ROYAD TIRL KUKACLIYAPVI YOALIINA YUKAKIXALAY SEL PFEKAKLIQNI DO XMKIMKIKKIKI	TEXPELXED YOELGEVSL. EXVELXED YOELGEVSL. EXVELTION OF LOEVSL. EXVELTION OF LOEVSL. EXVELTION OF LOEVSL. EXVELTION OF LOEVSL. EXVELTION OF LOEVSL. EXTERNAL FROM OF LOVESL. EXTERNAL FROM OF LOVESL.
SS00451/1-284 S71237/1-219 S70169/1-251 Saci_0451/1-219 Saci_0451/1-219 Maed_1695/1-221 Maed_1695/1-221	124 55 92 55 55 56 54	ISRNOIZONALLFIRVVIAOMSX ISRLOIZONALLFIRVVIAOMSX IARNOIZONALFIRVVIAOMSX ISRLOIZONSLFIRVVISOISX ISRLOIZONSLFIRVVISOISX ISRNOIZONVLFIRVVISONSX ISRNOIZONVLFIRVVISONSX ISRNOIZONVLFIRVVISONSX	TT QRAANYANIVALIRKIIQULTOIA TARAANYANIVALIRKITYKULTOIA DRAANYANIVALIRKITYKULTTOIA DRAANYANIVALIRKITYULTOIA DRAANYANIALIRKITYULTOIA DRAANYANILALIRKITYULTOIA DRAARYANILALIRKITYULTOIA	EQVELALITYTELCDVFV. EQVELALITYTELCDVFT. EQVELALITYTELCDIFY. EQVELALITYTELCDIFT. EQVELALITYTELCDIFA. EQVELALITYTEVADVF8. EQVELALITYTEVADVF8.
Thut_1460/1-210 Smar_040/1-212 APR_0143.1/1-223 Ign1_1156/1-210 Nmar_0029/1-215 Nmar_0016/1-216 Smar_0061/1-216	56 56 55 52 50 52	LAKKIQADHALFIKYYQAQIIX LAKIQADHALFOXYDALIXX LAKIQAYDHALFIXYYALIXX LAKIQAYDHALFIXYYALIXX LAKIQAYDHALFIXYYALIXX LAKIQAYDHALFIXYYALIXX LAKIQAYDQLFIXIYAAIXAX MIXLAYXHDQLFIXIYAAIXAX LAKIQAYDAQLFIXIYAAIXAX LAKIQAYDAQLFIXIYAAIXAX	O TRAANYAA VALVALLEN XISLLTAIYA DIRXANYAAN LANVALUKIVA CUTVO YA DIRXANYAAN LANVAKIYA VIITVO YA DIRANYAAN LANVAKNA VIITVO YA DIRANYAAN LANVAKNA VIITA VA O TOTSIYA VICAN LANVA VIITA SARVA O TOTSIYA VICAN LANVA VIITA SARVA	IRVELALITANTNODVLV. EHASLKLITFLVFGDANK. ERVELALITANVFGDVA. ERVELALITANVFGDVA. EQVELALITASDLGDVV. EQVELALITSDLGDVV. EQVELALITSDLGDAVV.
CENSTa_1915/1-214 CENSTa_1095/1-195 SBO0619/1-160 ST1485/1-165 Saci_1601/1-165 Nmed_1969/1-165 Mut_1206/1-160	52 30 26 24 23 33	LAELKDRDAHNFGQIVTANQHH LVVENDRDHKLFARIVAATQOH INRMONKIGT.TWHIFNRNVAS IARHDNRINT.LELSIYHIGR GNRLSARAFQ.FKRLSANATX AARLRAAYQHTRKYLL	TTA AR BALSYLLADYR KYKKYL CHARMU DAIAG RYLAN LADYR KYKKYL CHARM PRILERNYAR SELLENYMOHL LTLEYT PYL SKYYX KI TIKKNI TYNL KYRYL PYLLSKYYX TILORKYI YTL VOLKIL YALLAD YAR SEQLENIL SYMDRLDYL ATLAD YAR SEQLENIL SYMDRLDYL	EQUELLTIDICETWV. ERIEMRISTCSDICDTVM. EILEIRIETIVICNIVT. EILEIRIETLMILNSLKD. EILEIRIETLMILNSLKD. EILEIRIETLMILNSLKD. ENVELKIETLVTIDVVSQ. EVLSVRLETLAQICTISSE
Igni_0101/1-178 Smar_0222/1-106 CHM95_HUMAS/1-201 CHM45_HUMAS/1-222 CHM45_HUMAS/1-224 CHM45_HUMAS/1-223 CHM67_HUMAS/1-222	25 32 41 44 47 47 41	LYEAKAKEHELMEKLYEAGENG MORIKSRHEMLI HAGIHAG LERERALANGLIRDGARIRAKI JOGELOAKKYGYK. MIRAALO INGELAAKKIGYK. MIRAALO INGELAAKKIGYO. MIRAALO	DELEARVYARIAKI, KEYYESIAALOVK TFLAKYARIAKI AKUKI IISELALUKU LIKIKAYOOOLURTINOI ISLIANVOOI LIKIKAYOOOLURTINOI ISLIANVOOI LIRIKAYESOLAOODOTLITIPORIA ALKIKAYEKOLTOI DOTLOTIIPORIA ALKIKAYEKOLTOI DOTLOTIIPORIA ALKIKAYEKOLTOI DOTLOTIIPORIA	ENTELKLOUVUNLODAGA. EXIVUSIEVANTIINF. EFTQIENKVMEG.LOFGNE ENANTNIVLKT.MELAAQ ENANTNIVLKT.METAAK ENSIITTVLKT.METAAK (XNQLAVLRVAGS.LOSSTE
CIMPA_INDEAN/1-213 CIMPPIA_HUMAN/1-213 CIMPIA_HUMAN/1-196 CIMP5_INDEAN/1-199 CIMP5_INDEAN/1-219 CIMP5_INDEAN(PDB 2GD5)	39 26 29 42	ING ILLIINKMANGONDAVAI ING ILLIINKMANIGNIALCKU SKAEQAKVKKALLOKNVICARV IKAENAKIKKAIOKGNNVARI LVKKDQIKKNRGCAKNVKQI	AAADU VERARIYARIYARIYAANIQAYSIRI LARQIYALINA KIRIFI AVSAYYI SANITQI YALINA IRKXII GYNNI RHASRVDA VASKI IALINA IRQXI QAYNI RHASRVDA VAARI KALRVI KOXRYI DORDI LAQOSI'NHI QA	VOTAVTMSOMENAGA.MSTTAR YOTAVTMSOVTXN.MAQVTR YOTAVTMSOVTXN.MAQVTR MYTIOSLEDTXTTVDAMXL
			02	
		h3	h4	h5
5500910/1-259 571214/1-266 Saci 1373/1-261 Med_1671/1-270 Hbut_0797/1-286 Sanz_1277/1-260	110 110 110 113 113	h3 VEYPVAKIEG, DIKDQIKGIAPI VEPVKKIEG, DIKDQIKGIAPI VEPVKRIEG, ELKEQVRCIAPI VEPVCRAEL, SIKEQVRCIAPI TEMVCRAEL, SVKDQITIIYP LEPEVCRE, SIKEPVKRIAPI	h4	h5
\$\$00910/1-259 \$T1214/1-266 \$aci_1373/1-261 Nscd_1671/1-270 Bbut_0797/1-286 \$mar_1277/1-260 APE 0962/1-271 Tgn1 0995/1-255 \$500081/1-221 \$5000451/1-219 \$701368/1-251 \$701368/1-251 \$70146/1-259	110 110 113 119 120 108 126 193 124 161	h3 VLYPVAKIEG, DIKDQIKGIAPI VLPVMKVLG, OLKOQIKGIAPI VLPVMKVLG, OLKOQIKGIAPI VLPVMKVLG, OLKOQIKGIAPI VLPVMKVLG, ULKOVRGVAPI TLMVFGAAL, ILKEOVRGVAPI TLMVFGAAL, IVKOQITIYPI LLPIVIGEL, SLKPUVKIAPI ALAPLIFAMS, ANDEYLAATSD' SUSKIISAPASNIHLLSIALBO SLIPVIGVIK, ILKAALBONGVAPI NI IPVIGVIK, ILKAALBONGVAPI SLIPVIGVIK, ILKAALBONGVAPI SLIPVIGVIK, ILKAALBONGVAPI SLIPVIGVIK, ILKAALBONGVAPI	h4	h5 . vp.avvdtGargildfag . vp.svdtGargildfag . vp
SS00910/1-259 ST1214/1-266 Saci 1373/-261 Mmad_1671/3-270 Tbut_0797/1-286 Smar_1277/1-260 APE_0962/1-271 Tgni_0995/1-255 SS00451/1-221 SS00451/1-221 ST1267/1-219 Saci_0451/3-221 Mmad_1695/3-221 Mmad_2179/3-221 Mmad_2179/3-221 Mmad_2179/3-221 Smar_0481/1-212 Smar_0481/1-212 Smar_0481/1-212	110 110 110 113 129 120 108 124 161 124 124 125 125 125 125 125	h3 VEYPVAXIEG DIKDQIKCIAPI VEPVMALG OLADQIKCIAPI VEPVMALG OLADQIKCIAPI VEPVMALG OLADQIKCIAPI VEPVMALG ILKEVAROVACIAPI VEPVGALE SLEVVALIATA NEPVICIC SLEVALIANA ALAPLEFAMI. ANDEYLAATSPI SEIPVICUE SLEVALIALALASPI SEIPVICUE SLEVALIALALASPI SEIPVICUE SLEVALIANAN NEIPVICUE SLEVALIANAN NEIPV	h4 valalosi i by NCIAVICA AINDROV valalosi py NCIAVICA AINDROV valalosi py NCIAVICA AINDROV valalosi py NCIAVICA VY ORTI LNLAPEL PY VANNIVER LSTMINUS REVY NIALTYN NO VCI IAARITSI VOSTRAVVERALTYNNIV IAARITSI VOSTRAVVERALTYNNIV ISLEVEN NEXT LOSSKAAL PY OTO DI ISLEARLES CLORVVERAC. DTGAN ISLEVEN NEXT LOSSKAAL PY OTO DI ISLEARLES CLORVVERAC. DTGAN ISLE ADLES CLORVVERAC. TEGANO ISSUE AND CLORVVERAC. TEGANO ISSUE ADLES CLORVVERAC. TEGANO ISSUE AND CLOR	h5 . vp. tvvde gargildeag . vp. tvvde gargildeag . vp. tvde gargildeag . vg. tasspearet teras . vg. tasspearet teras
SS00910/1-259 ST1214/1-266 Saci_137/1-261 Nsed_1671/1-270 Hbut_0797/1-286 Smar_1277/1-260 APE 0962/1-271 Ign1 0995/1-255 SS0080/1-221 SS0080/1-221 SS0080/1-221 SS00451/1-219 ST0160/1-219 ST0160/1-219 ST0160/1-219 Saci_0451/1-214 Nsed_1595/1-212 Msed_2179/1-211 Bbut_1669/1-212 APE 0143.1/1-223 Ign1_1156/1-210 Nser_0080/1-215 Nser_0080/1-215 Nser_0061/1-216 CENNTA_1915/1-214 CENNTA_1915/1-214	110 110 110 110 108 123 124 161 124 125 125 125 125 125 125 125 125 125 125	h3 VEYPVARIES DIRDINGTAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIAP VEPVRIS SIROURSIA SIPVIS SIROURSIA SISSING SISSING SIROURSIA SISSING SIROURSIA SISSING SISSING SIROURSIA SISSING SISSING SIROURSIA SISSING SISSING SISSING SIROURSIA SISSING SISSING SISSING SIROURSIA SISSING SISSING SISSING SIROURSIA SISSING SISSING SISSING SISSING SING AND STANTA SING SISSING SING SING SING SISSING SISSING SISSING SING SISSING SISSING SISSING SISSING SING SISSING SISSING SISSING SISSING SING SISSING SISSING SISSING SISSING SING SISSING SISSING SISSI	h4 VAIAADSI CREWNSIAVITS AINDROV VALABSI CREWNSIAVITS AINDROV VALABSI CREWNSIAVITS ABERT VALABSI CREWNSIAVITS ABERT VALABSI CREWNSIAVITS ABERT VALABSI CREWNSIAVITS ADETT VALABSI CREW	h5 . VP. AVVDEGARGILDEAG . VP. TVDEEAGKILNETK . VP. TVDEEAGKILNETK . VP. TVDEAGKILNETK . VP. TVDEARKILEEAK . VP. TVDEARKILEEAK . VP. TVDEARKILEEAK . VS
SS00910/1-259 ST1214/1-266 Seci_1373/1-261 Nsed_1671/1-270 Ibut_0797/1-286 Smar_1277/1-260 APR_0620/1-271 Tgn1_0995/1-255 SS00081/1-221 SS000851/1-214 SS000851/1-219 Seci_1416/1-219 Seci_1416/1-219 Seci_1416/1-219 Seci_1416/1-219 Seci_1416/1-219 Seci_0451/1-214 Msed_1095/1-221 Nsed_2109/1-215 Nser_0401/1-215 Nser_0401/1-216 SF1485/1-165 Seci_1601/1-169 Sf1485/1-165 Seci_1601/1-169 Nsed_109/1-165 Seci_1001/1-178 Secr_0322/1-186	110 110 110 120 126 126 124 124 124 125 125 125 125 125 125 125 125 125 125	h3 VEYPVAXIEG DIKDQIKCIAPI VEPVMRIEG DIKDQIKCIAPI VEPVMRIEG DIKDQIKCIAPI VEPVMRIEG DIKDQIKCIAPI VEPVMRIEGIEKCVROVAPI TEMVEGAALE EVKDQITIYPI LEPEVGED, SLEPVKIAPI VEPVGUEG, SLEPVKIAPI SEIPVLGVE, ELKANKCVMPI SEIPVLGVE, ELKANKCVMPI SEISVIJE, SEISSEN SEISVIJE, SEISSEN SEISVIJESSEN SEISVIJESSEN SEISVIJESSEN SEISSEN	h4 h4 h4 h4 h5 h5 h4 h5	h5 . VP. AVVDE CARGILDEAG . VP. TVADE CARGILDEAG . VP. TVADE CARGINE A . VY. ASS PHARE A . VY. ASS PHARE A . VY. ASS PHARE A . VY. ASS PHARE A . VY. TYAT A
SS00910/1-259 ST1214/1-266 Saci_1373/1-261 Nsed_1671/1-270 Hbut_0797/1-286 Smar_1277/1-260 APR 0962/1-271 Ign1 0995/1-271 SS0081/1-221 SS0081/1-221 SS0081/1-221 SS0081/1-219 Saci_0451/1-219 Saci_0451/1-219 Msed_1199/1-211 Hbut_1469/1-219 Smar_0401/1-212 APR 0143.1/1-223 Ign1_1156/1-210 Nser_0029/1-215 Nser_0016/1-216 SS00610/1-168 ST045/1-168 St00510/1-169 St00510/1-169 Msed_199/1-165 Ss00610/1-169 St00510/1-169 St00510/1-169 St00510/1-169 St00510/1-120 CHMSA_100488/1-221 CHMSA_100488/1-221 CHMSA_100488/1-223 CHMSA_10488/1-223	110 110 110 110 120 120 120 121 124 124 124 125 125 125 125 125 125 125 125	h3 VEYPVARIES DIRDIRGIAD VEYRVARIES DIRDIRGIAD VEXPVARIES DIRDIRG	h4 h	h5





Sequences were identified using the BLAST search program at <u>http://www-archbac.u-psud.fr/projects/sulfolobus</u>, aligned using ClustalW (<u>http://www.ebi.ac.uk/clustalw/</u>) 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 μ g 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 μ g 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 μ m. We have scored cells in which we observe two clearly segregated nucleoids. We detect structures at mid cell by immunocalization 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 μ m 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.



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)
$< I/\sigma > (last shell)$	16.2(3.7)
Unit cell P4 ₃	a=49.9 Å c=107.7 Å
Refinement statistics	
Resolution range	49.9 Ă –2.2Ă
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 ^t	
bonds	0.008 Å
angles	1.0°
dihedrals	4.8°

^aData 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.

^dr.m.s. deviations for bond angles and lengths in regard to Engh and Huber parameters (5)

Target	Forward Primer	Roverse Primer	Plasmid			
Target	r of war u i i mier	Keverse i fillet	Constructed			
Saci1373	GGATTCCCATATGTTTGATAAGT	GGGAATTCCTCGAGACCCTCAAGA	pET30 Sac1373			
Success	TATCGATAATTTTTAATAG	ACAATTAGACCCTTTTC	pGBK Sac1373			
			pGAD_Sac1373			
			1 —			
Saci1372	GTTAATGTCATATGTCTGCCCAA	GGGAATTCCTCGAGTTATAGAGCC	pET30 Sac1372			
	GTAATGCTTGAAGAGA	TTATACTTCTCGTGCCA	1 —			
Saci1373	GGATTCCATGGCAATGTTTGATA	GGGAATTCCTCGAGTTAACCCTCA	pET42_Sac1373			
	AGTTATCGATAATTTTTAATAG	AGAACAATTAGACCCTTTTC	-			
Saci1373	GAAACAGACCATGGAAGAAGCT	GGGAATTCCTCGAGTTAACCCTCA	pET42_Sac1373			
	CAGAAGATGGCTGAAG	AGAACAATTAGACCCTTTTC	tail			
Saci1373	CTGAAGCCATGGTTAGAGAATTG	GGGAATTCCTCGAGTTAACCCTCA	pET42_Sac1373			
	TTGCCAGAACTACC	AGAACAATTAGACCCTTTTC	RELL			
Saci1373	TACCCCCCATGGCTTCAGAGTTA	GGGAATTCCTCGAGTTAACCCTCA	pET42_Sac1373			
~	CCAAAGAGAG	AGAACAATTAGACCCTTTTC	SELP			
Saci1372	GTTAATGTCAATGTCTGCCCAAG	GGGAATTCCTCGAGTAGAGCCTTA	pET30_Sac1372minu			
6 :0.451			sMIT			
Saci0451			pGBK_Sac0451			
Saci1416			pGAD_Sac0451			
Saci1410	AATGATTTTCTGAG	TTCCCTTTCTTTTTCATCCACCCA	pGBK_Sac1410			
	AATOATTTCTOAO	AGTTGC	pOAD_3ac1410			
Saci1601	CGTATGTCCATATGAAGAAAAGG	GGATCTTCCTCGAGTTAAATCTCAA	pGBK_Sac1601			
Suchiool	ACGATTGCTGAATTACTCACTG	TTTTATAGTTTTCTTTAAGCTCCTTC	pGAD Sac1601			
		TTAGCC	F			
Sac pyrEF	(SacEF F)	(SacEF R)	pCRScript pyrEF			
1.2	CTTACAGCTCGAGTAACGCCCTT	AAGTCAAGATGCATCAAATCTGTT				
	AAATAAGGTTAGTC	GTGGGAACTTCACCGG				
Sso0909 and	(Sso0909 5')	(Sso0909 3'FLAG)	pSVA5_Sso0909 and			
pOP319	GAATTTCCCATGGGTGCACAAGT	ATGCGGGCCCTCACTTGTCGTCGTC	pSVA5_Sso0909E20			
	AATGTTAGAAG	GTCCTTGTAGTCTAATGCCTTAAAT	6Q			
01/45 0 0			D VG1 G 0000			
pSVA5_Sso0	(arau909_F)	$(ara0909_K)$	pRYS1_Ss00909			
909 and $pSVA5$ Scol		GTCGTCGTCCTTGTAGTC	pk 15_5s00909E206			
909F2060	CATATOTTIAGAGATO	UICUICUICUICUIUIAUIC	Q			
Target	Митаде	nesis Oligo	Plasmid			
Turget	l	Constructed				
pET30 Sac13	GGAATTAATTCCTGCTGATGGTGC	GTAGAATGGGAATGGTAAACACAG	pET30 Sac1372			
72		MIT –				
pET42_Sac13	GATGGCTGAAGTAAAAGTTTAAGA	pET42_Sac1373				
73			CORE1			
pET42_Sac13	GAACTACCCCATCCACCTTAAGAG	TTACCAAAGAGAGTA	pET42_Sac1373			
73 and			CORE2 and			
pET42_Sac13			pET42_MIM2			
73RELL						

Supplementary Table 2. Oligonucleotides used in this study

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				TA	FACATTGCTGCCCTTGACTG					
Saci1372	AAGCCGACAAAGAGGGAAAT				A	AGCTGTCGATCCGTCCCTAT					
Saci1373	CACAA	CACAAGAGATCTCGGACATACG				G	GCACCAGAGAAACTCCCTGT				
Saci1416	GGGCTGCAATGTACGCTAAT				CA	CAATAACCGGGACCAAGGAG					
Saci1460 (NusG)	TTGAAGCTACCGGACCTCAT				GCGGGTAATGCAACTGACTT						
Saci1601	CGCTAGAAAGATTGTCGCTGT				ΤT	TTTCCTCGTAAGCTCTGTTTCC					
Target Gene	0	5	30	60	9)	120	150	180	210	
Saci0451	1	0.88	0.51	0.51	0.5	55	0.51	1.25	1.66	1.68	1
Saci1372	1	0.72	0.68	0.72	0.7	78	1.06	2.07	2.16	1.73	1
Saci1373	1	1.08	0.79	0.88	1.0)7	1.58	2.46	2.97	2.18	1
Saci1416	1	1.07	0.81	0.80	0.9	91	0.87	1.80	2.86	2.16	1
Saci1460 (NusG)	1	1.05	1.27	1.02	0.9	96	0.92	1.19	1.19	1.16]
Saci1601	1	0.86	1.05	1.12	1.1	7	1.14	1.58	1.7	1.41	1

References for Supplementary Information

- 1) A.J. McCoy, Acta Crystallogr D Biol Crystallogr 63, 32 (2007).
- 2) A.G.W. Leslie, Joint CCP4 and ESF-EACMB Newsletter on Protein Crystallography 26, (1992).
- 3) CCP4, Acta Crystallogr. D **50**, 760 (1994).
- 4) A. Perrakis *et al.*, *Nat. Struct. Biol.* **6**, 458 (1999).
- 5) P. Emsley and K. Cowtan, *Acta Crystallogr. D* **60**, 2126 (2004).
- 6) G. Murshudov et al., Acta Crystallogr. D 53, 240 (1997).
- 7) I.G. Duggin, S. A. McCallum, S. D. Bell, *Proc. Natl. Acad. Sci. (USA)* **105**, 16737 (2008).
- 8) J. Sambrook and D.W. Russel, Molecular Cloning, CSH Press (New York).
- 9) I. Dionne, et al., Mol. Cell **11**, 275 (2003).
- 10) S. Berkner *et al.*, *Nucl. Acid. Res.* **5**, e88 (2007).
- 11) S.V. Albers *et al.*, *App. Env. Micro.* **72**, 102 (2006).
- 12) M. Jonuscheit *et al.*, *Mol. Micro.* **48**, 1241 (2003).
- 13) C. Schleper et al., Proc. Natl. Acad. Sci. (USA) 89, 7645 (1992).
- 14) N. Robinson *et al.*, *EMBO J.* **26**, 816 (2007).
- 15) T. Muziol *et al.*, *Dev. Cell* **10**, 821 (2006).
- 16) C. Kieffer *et al.*, *Dev. Cell* **15**, 62 (2008).
- 17) A. Dereeper *et al.*, *Nucl. Acid. Res.* **36**, W465 (2008).