## The *Sporomusa* type Nfn is a novel type of electronbifurcating transhydrogenase that links the redox pools in acetogenic bacteria

## SUPPLEMENTARY INFORMATION

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**Supplementary Figure 1. Gene clusters encoding the central metabolism of** *S. ovata.* Gene cluster I contains the genes for methenyl-THF cyclohydrolase (*fchA*), the methylene-THF dehydrogenase (*folD*), the formyl-THF synthetase (*fhs*) and the methylene-THF reductase (*metVF*). Further the CODH/ACS (*acsA* and *acsB*), the corrinoid/iron sulfur protein (*acsCD*), a corrinoid activation and regeneration protein (*acsV*), the CoFeSP methyltransferase (*acsE*), the CODH maturation factor (*Cooc*) and the CODH nickel-insertion protein (*acsF*) are encoded in gene cluster I, as well as the genes *hdrCBA* and *mvhD* which share similarities to subunits of the heterodisulfide reductase and methyl viologen reducing hydrogenase from methanogenic archaea. A hypothetical protein is encoded by *orf1*. Gene cluster II contains the electron-bifurcating hydrogenase (*hydCEDBA*). Gene cluster III contains the genes *atpIBEFHGDC* which code for a H<sup>+</sup>-dependent F<sub>0</sub>F<sub>1</sub>-ATP synthase. In V the genes coding for a formate dehydrogenase (*fdh*), the acetae kinase (*ack*) and the phosphotransacetylase (*pta*) are depicted.



Supplementary Figure 2. Measurement of the Rnf activity in the membrane fraction of *S. ovata.* The Rnf activity of the membrane fraction was measured in 50 mM MOPS, pH 7, containing 10 mM NaCl and 20 mM MgSO<sub>4</sub>. (a) 30  $\mu$ M Fd were added to the buffer (1) and reduced with CO by purified CODH from *A. woodii* (2). Reduction of Fd was followed by measuring the absorbance at 430 nm. After addition of 1 mM NAD<sup>+</sup>. The reaction was started by adding the membrane fraction to the assay (3). The reduction of NAD<sup>+</sup> was measured by following the absorbance at 340 nm. (b) The enzyme assay was performed as in (a) but NADP<sup>+</sup> rather than NAD<sup>+</sup> was used as electron acceptor. (c) The assay was performed as in (a) but ferredoxin was used to start the assay.



Supplementary Figure 3. Measurement of the methylene-THF dehydrogenase activity in the cytoplasmic fraction of *S. ovata*. The MTHFDH activity of the cytoplasmic fraction was measured in 100 mM Tris/HCl, pH 7.5. (a) 0.5 mM THF and 1.5 mM formaldehyde were added to the buffer to form methylene-THF non-enzymatically (1, 2). After the addition of 1 mM NADP<sup>+</sup> the measurement was started by adding cytoplasmic fraction to the assay (3). The reduction of NADP<sup>+</sup> was measured by following the absorbance at 340 nm. (b) The assay was performed as in (a) but NAD<sup>+</sup> was used as an electron acceptor. As a control NADP<sup>+</sup> was added after no increase of  $A_{340}$  (NAD<sup>+</sup> reduction) was observed.



Supplementary Figure 4. Native- and subsequent SDS-PAGE of the purified Stn complex. The preparation of the Stn complex was separated by native PAGE (a) and the protein complexes with molecular masses of 232, 142 and 91 kDa were cut out of the gel and further separated by denaturating SDS-PAGE (b) together with  $10 \mu g$  of the purified Stn complex (Stn).



Supplementary Figure 5. pH and temperature optima of the Stn complex. Simultaneous reduction of NAD<sup>+</sup> and Fd<sub>ox</sub> with NADPH was measured as described in methods. To determine the pH optimum of the Stn complex a combined buffer containing 25 mM MES/MOPS/Tris/CHES, 10 mM NaCl and 20 mM MgSO<sub>4</sub> was used with different pH values as indicated and the measurements were performed at 30°C (a). To determine the temperature optimum of the Stn complex the measurement was performed in 50 mM Gly-Gly buffer, pH 8 (b). 100% corresponds to 1.6 (a) and 3.5 (b) µmol ferredoxin reduced x min<sup>-1</sup> x mg<sup>-1</sup>. All data points are mean  $\pm$  SD (n = 3).



Supplementary Figure 6. K<sub>m</sub>-value determination of the simultaneous reduction of NAD<sup>+</sup> and Fd with NADPH and for the Fd<sub>red</sub>- and NADH-dependent reduction of NADP<sup>+</sup>. The assays were performed as described in methods using varying amounts of either NADPH (a), NAD<sup>+</sup> (b), NADP<sup>+</sup> (c) or NADH (d). All data points are mean  $\pm$  SD (n = 3).



**Supplementary Figure 7. Sequence alignment of StnA and homologs.** Black triangles indicate amino acids responsible for coordinating the FeS-cluster. White characters with black background show strictly conserved amino acids. Bold black characters indicate amino acids with similar characteristics. Sequence alignments were performed with ClustalOmega multiple sequence alignment and visualized with ESPript 3.0<sup>1</sup>.



**Supplementary Figure 8. Sequence alignment of StnB and homologs.** Black circles indicate the conserved glycines of the NADH-binding motif. The FMN binding site is indicated. Black triangles indicate amino acids responsible for coordinating the FeS-clusters. White characters with black background show strictly conserved amino acids. Bold black characters indicate amino acids with similar characteristics. Sequence alignments were performed with ClustalOmega multiple sequence alignment and visualized with ESPript 3.0<sup>1</sup>.



**Supplementary Figure 9. Sequence alignment of the N-terminal sequence of StnC and homologs.** The FAD- and NADP(H)-binding sites are indicated. Grey triangles indicate residues of cysteine-rich motifs known from NsoC. Black triangles indicate amino acids responsible for coordinating the FeS-clusters. White characters with black background show strictly conserved amino acids. Bold black characters indicate amino acids with similar characteristics. Sequence alignments were performed with ClustalOmega multiple sequence alignment and visualized with ESPript 3.0<sup>1</sup>.

StnC NsoC NuoG SfrA	449 445 1 1	SG <b>NI</b> IVNO RGKAVVDI MATIHVO MVSLTIDO	GGAFTT EVTLQTS GKEYEVN GKDITVZ	VRDKVFA 51 EGVFA VGA 4 KE	AGGDAV1 AGGDLII	IGPKIA LGPSTV	IDAIAQ IESIAT	GKNAAQ GRRAAL	VIDSY MIDLY . DNL TTI	LNGC <b>L</b> V LKGK <b>L</b> E LEAC <b>L</b> S LDAA <b>A</b> L	P H A D S K A K E V 	LIEPSK	HIDEI	VRDEDL	L
StnC NsoC NuoG SfrA	508 525 27 27	FDLK <b>PY</b> N GLDI <b>PY</b> F GITI <b>P</b> TL	TQKDITA HWKDVTE CWHPALC CWLKKVS	AADLADE EDYEHV GSVGACE SPTGACE	AKA. QCAVK( VCAVE)	QYQNAE IE	PR DTRGRI .GVDRP	VSLTVE VKSRLK VMSCMT MTACNT	DAEVRI PVEER PASDG PVKDG	NKS IKG IFISID IKVTTQ	FM FV DEEAK SEKLS	QVAKTF EVEESL QFRESV RIRQKI	TEEEAI TEEEVI VEWLM1 MELMLV	RESKR KEAER INHPHD NHPLD	CLEC CMSC CPVC CPVC
StnC NsoC NuoG SfrA	566 587 107 102	GCRDYFE GCMEVFR EEGGN DAGGE	CQLIKYJ CKLREYA CHLQDMJ CDLQNAC	IQDYDVS Atlye <b>a</b> k IVMTGHS CYGLG <b>A</b> A	TEKDS QDRFK FRRYRE KQEYGA	QVECH <b>K</b> G.ETN <b>R</b> F.TKRT A.VLE <b>R</b>	TTEFDN FEIDET HRNQDI RKIRYD	IHPFIER HPSVVL GPFISH WPLIES	NPDKCY DNNKCY EMNRC DPNRC	VLCGLC VLCGRC IACYRC ILCEKC	VRVCD VNLTH VRYYK VKVDH	EVVGAT EIVGEG DYADGT EIVGCN	AIGLV VVDYL DLGVY AIRVV	S <b>RG</b> FD <b>S</b> F <b>RG</b> FK <b>T</b> SAHDNV N <b>RG</b> EA <b>T</b>	VIMP RISP YFGR IIDT
	C-	rich moti	f								[4F	<sup>-</sup> e-4S]-o	diclust	er dor	nain
StnC NsoC NuoG SfrA	646 666 184 179	EFKLPLSI PLGNTLGI PEDGTL. VDGNPL.	ETACISC DMDGVFI ESEFS NCEFC	GOCVDV GDMIDV GDMLVEI GNCVAA	CPTGA CPTGA CPTGVE CPTGTI	CM <b>EK</b> QV IS <b>EK</b> LP FT <b>DK</b> TH LIS <b>K</b> PF	S.Y <mark>K</mark> QI F.I <b>K</b> PG SERYNR K.F <b>R</b> GR	PANMDS PWKTTP KWDMQF PWAFTT	MASVC VKTVC APSIC TPSVC	SYCGVG NGCSFA QQCSIG PFCATG	CNVNI CEMNI CNISP CQIEY	EY <b>K</b> GDV EVYNDM GE <b>R</b> YGE HS <b>R</b> NGR	VERVTE LVRASS LRRIEN VERVTS	DRV SVAG Nryngi Sd.dsi	'NDDG WNNG VNHY YNSG
										[4Fe-4	S] Mo	lybdop	terin (	<b>DR</b> fan	nily
StnC NsoC NuoG SfrA	723 743 261 254	WLCQRGK HLCDICR FLCDRGR NLCINGR	GLGHAN KRPWAE GYGYVN GYSYIN	NDKA <b>RL</b> I SDLI NLKDRPF NSPD <b>RL</b> A	APVIK FPLLN QPVQR EPMVK	RNGQFV GE RGDDFI GQKADW	K <b>V</b> DWNE K <b>V</b> EWDK T <b>L</b> NAEÇ NT	ANLEVV VREF AMQGAA AMGTAA	KRLQAV DI TALKQ:	VVA <b>A</b> YG <b>i</b> es <b>l</b> rq IVASHG	KDSIG HEDIA SKKVI ADAVA	VVVSPR LILTPE GIGSPR GEGSPR	LTNEE LTNEE ASVESI VTNED	L <b>FL</b> AG <b>K</b> VEYFK <b>R</b> N <b>FA</b> LRE N <b>YL</b> FQ <b>K</b>	LADA VAEE LVGE LMRS
StnC NsoC NuoG SfrA	803 806 335 330	VNTTIKT: K ENFYTGI AIGTGNII	SYSVDG AHGEQEF DSEARL	SGLG. FKLG. LQLALK FA <b>A</b> TQK	SV AF VLREG VLREM	GYDAS EDGIS GIY GIAGA	TNSFAE TATLED TPALRE STTIDA	LDNSDF Ikkakk IESYDA IDRATA	VLTLG VLLKAI VLVLG VLVVG	KVKENH D. <b>I</b> EKY ED <b>V</b> TQT CD <b>L</b> NAE	GARVA ATGME	LAVRQA YRVIKA	VKGKAH ATK	REMAAA	QKVA
StnC NsoC NuoG SfrA	852 842 412 398	DWQIAAI	LNIGQRA	PVL PYL AKHPLFV NNAKLVI	FKI VLI TNVDD AAMRD	IRLSGV LKGKEV IRLDDI IKLKKF	CSVAWP VEEGYE AAWI ANSHLK	OSLANT VAIVEA YR <b>A</b> PVE YR <b>P</b> GNE	ADMK <b>V</b> PAEP <b>L</b> DQAR <b>L</b> T. L <b>L</b>	FLK NVPT GFAIAH INALTK	<b>A</b> L A L.DNS <b>A</b>	. LLNLG . ILHEG APAVDG . VLEEG	· VDENKV VNEVGI IEPEL LENKEP	VAE.KI LLKLGV Q FCSANI	regFa 7kg <b>l</b> p 2sk <b>i</b> d sn <b>l</b> s
StnC NsoC NuoG SfrA	904 896 485 458	ELKASLA EA VIVQALA DLTAALA	DVKVSEE GAKKPLJ GVSIAD <i>I</i>	EI Isgina A <b>a</b> a	QALA AGSLEVI ATGV7	AQKYAK EAYVV IQAAAN IEADLR	AAKPLI IGKPRK VAKALK AAARLV	VIDEDT GLKGDV GRGADV VGGKK	VSA GITMIA GVAV	A ARSVNS	MGLGI FGAEL	MGGGSL MRGGNT	EAVKLM EEA. DAVKAI	MAYAAV	/ITGK
StnC NsoC NuoG SfrA	958 917 553 525	IGAAYR <mark>G</mark> LPS <b>G</b> TAGDTG <b>G</b>	IILVRTH LW LFPVYEH	KNNTQ <b>G</b> A . AEKE <b>G</b> I 	VDMGFV INAFN LDMGV	/M NM APDHFP	GHQTDG	TTFEKA	WGKKLI	PAAAGK	P <b>V</b> SAV E <b>L</b> RVN  D <b>L</b> WQI	AQGIES  LTELET IEGIEQ	GKIKAI GRADAV GSVKAI	LVVIGE KAR VVIEN LYLLGC	DPAA EP.E IDL.H CDPVA
StnC NsoC NuoG SfrA	1011 947 573 605	YPQI YSV RHASAIR SFPEGER	ESA <b>LL</b> QF K VNA <b>AL</b> AF IRK <b>AL</b> EF	KISFLVV EI KAPLVMV KIELLIV	YDMFM1 FSL VDHQR1 QDPFPC	ГКТ <b>А</b> ТА  ГАІ <b>М</b> ЕМ ЗЕА <b>А</b> КМ	ADMV  AHLV AHVV FP	LVSS <b>AE</b> AASF <b>AE</b> SSVA <b>AE</b>	VNGTY SDGTY KNGTF	IRSDR <b>R</b>  INNEG <b>R</b> ITIDG <b>R</b>	IQAVR  AQRFF V	AAIQPK QVYDPA	TGKATI	LQ IVMLES	WRWL
StnC NsoC NuoG SfrA	1079 653	.ILIETLI HSLHSTLI	KSLGIK) LSREVDØ	ZDTIADV VTQLDHV	VRAAIAS	SEVSNY AKIPEL	AGMDAA AGIKDA	ADFGTT.	RIRGQI	KLAREP	 HRYSG	RTAMRA	NISVHE	₩PN 	DIDT
StnC NsoC NuoG SfrA	1129 733 660	LYTDGFA MFTFSI	TEGQKAI MEGNNQE	IL <b>A</b> AVGI T <b>A</b> HRSÇ PL <b>A</b> K	VPVF.	VE APGWNS APSGDA	KKK <b>Y</b> ds •••• PQA <b>W</b> NK RED <b>W</b> DI	VEMN FQD LTELYN	FVNGR  EVGGKI IRLTGES	QSL LRF SRI					

**Supplementary Figure 10. Sequence alignment of the C-terminal sequence of StnC and homologs.** Grey triangles indicate residues of cysteine-rich motifs known from NsoC. Black triangles indicate amino acids responsible for coordinating the FeS-clusters. White characters with black background show strictly conserved amino acids. Bold black characters indicate amino acids with similar characteristics. Sequence alignments were performed with ClustalOmega multiple sequence alignment and visualized with ESPript 3.0<sup>1</sup>.



Supplementary Figure 11. Thin layer chromatography of flavins extracted from the Stn complex. The flavin-content of ~1 nmol purified Stn complex was loaded onto the TLC plate, 1 nmol FAD and FMN were used as standards. Separation of the flavins was achieved by using 60% [v/v] n-butanol, 15% [v/v] glacial acetic acid and  $25\% [v/v] H_2O$  as the mobile phase.

Supplementary Tabl	1. Purification o	of the Stn complex of S. ov	ata.
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	Protein [mg]	NADPH: MV <sub>ox</sub> OR [U*/mg]	Total activity [U]	Yield [%]	Purification [x-fold]
Cytoplasm	1127	3.0	3381	100	1.0
Q-Sepharose	361	11.3	4079	120	3.7
Phenyl-Sepharose	65	35.7	2321	69	11.9
Superdex 200	8	217.5	1740	51	72.5
Blue-Sepharose	6	278.2	1669	49	92.7

\*1 Unit is defined as 1 µmol NADPH oxidised per minute.

## **Supplementary References**

1 Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* **42**, W320-324 (2014).