Supplementary Data

Structure and regulatory role of the C-terminal winged helix domain of the archaeal minichromosome maintenance complex

Christoph Wiedemann¹, Anna Szambowska^{2,4}, Sabine Häfner¹, Oliver Ohlenschläger¹, Karl-Heinz Gührs³, and Matthias Görlach^{1,*}

¹Research Group Biomolecular NMR Spectroscopy, ²Research Group Biochemistry,

³Protein laboratory, Leibniz Institute for Age Research, Fritz Lipmann Institute, Beutenbergstr. 11, D-07745 Jena, Germany:

⁴Laboratory of Molecular Biology IBB PAS, affiliated with University of Gdansk, Wita Stwosza 59, Gdansk, Poland ^{*}Corresponding author: mago@fli-leibniz.de

Table S 1	Primers	used in	this st	udy
-----------	---------	---------	---------	-----

CW72 gtc cte gag cta gac tta ttt tta gta aca ttc rev Ssø MCM C-term. domain, Xhol site CW84 age cag gge tga gac tat ett aag gta tec rev Mth MCM C-term. domain, Xhol site CW84 age age gge tga gac tat ett aag gta tet rev Mth MCM C-term. domain, Xhol site CW82 geg egg cac cag goe get get gt gt ag gt gat gt rev thrombine to TEV site CW84 att aca te ta ca te ta gan ang rev 2 stop codons distal to Ssø MCM M604 CW84 cut ate and tee ta gan ang rev 2 stop codons distal to Ssø MCM M604 CW85 aca ata afg act ggt ant at ga cat gac ang cat at the dig an ang ace ggt at ta gat gt gt at tat gat cat at at rev 2 stop codons distal to Ssø MCM K618 CW80 gan ana cet ggt ang teg tat ag ga gt gt at at gac at get gat cat can ange tf rev Ssø MCM 1555 1557delinsDSD tta g rev Ssø MCM 1655 1657delinsDSD rev Ssø MCM 1604_E605ins(A)5, rev CW84 gga ana agt gg agt gt at at gg a at gg ge geg geg geg cat an fwd Ssø MCM 1604_E605ins(A)5, rev CW84 cW107 agt fag ga gtt gat at gg at an gat aca ata fwd Ssø MCM 1614_K61840 CW108 aca ata at gat ata gat aca cet aan age gt fwd Ssø MCM 1613_1614ins(A)5, rev CW110 agt gt ag ag gt gt at at gg at at ga ga gt gg a gag gg	Primer	Sequence 5'-3'	Construct ^a
CW81 age age gge get gg gg ee gg gge eg gas aac etg tat ttt cag fwd thrombine to TEV site GW82 gge gfa tta gaf gag rev thrombine to TEV site GW84 eg gg ga ca cag gee get gg tag gfg atg rev thrombine to TEV site GW84 eat ate aac tee ta ca ct et tag aan gt gg ana a fwd thrombine to TEV site GW84 eat ate aac tee tac act et tag ang tgg ana a fwd 2 stop codons distal to Sso MCM M604 CW85 aac ata atg act ggt aas taa gge gat act cea aga can fwd 2 stop codons distal to Sso MCM K618 CW85 gga aac cet gat age get gg gg ge gg ge gg ga agt fwd Sso MCM 1555_1557delinsDSD CW90 tgg tag ag gt gat atg gg at agt act agt act agt age agt gg at agt agt agt agt agt agt agt a	CW72	gtc ctc gag cta gac ttt ttt gta aca ttc	rev Sso MCM C-term. domain, XhoI site
ggc gta tita gat gag.CW82ggc ggc gc ac cag gc gc gt gt gt at g gt ag t gg at gg gg gt gat ag ag gt gat at gat at ga at gag ag t gat at ga at ag at ag as agCW83agt gta gga gtt gat at gt an tga ag tgg aaa aCW84cat at caac tee tas act et ta gg aa aagCW85aca at a at g act ggt aaa taa tga e ca aaa ag getCW80gaa aca eet gat age caa gat age gat act eca aga caatt agtt ace agt et et tag act tit et et atCW90tgg get at eag tgt tee tag act tit et ectgg a aaa ac eet gat age caa gag gg ge ge ge ge ge ge ge ga aatCW91gt ge cat ag gt gt at ag ga aa a gag tg a at ag ge aa at ag at ag ag aa ag ge	CW74	gtc ctc gag tca gac tat ctt aag gta tcc	rev Mth MCM C-term. domain, XhoI site
CW82gc gc gc gc ac cag gc gc t gt gt ag t ag	CW81	agc agc g gc ctg gtg ccg cgc gaa aac ctg tat t tt cag	fwd thrombine to TEV site
CW83 agt gta gga gtt gat att gta atg aag tga aaa fwd 2 stop codons distal to Sso MCM M604 CW84 cat at aca tee te ta act tet ta ga aag tga aag rev 2 stop codons distal to Sso MCM M604 CW85 aca ata atg act tegt aaa taa tga cet aaa age get fwd 2 stop codons distal to Sso MCM K618 CW80 tta ce agt cet at tigt ate tat at rev 2 stop codons distal to Sso MCM K618 CW90 tgg gat ace eca gat age gat act eca aga caa fwd 2 stop codons distal to Sso MCM K618 CW90 tgg cat ace agg tg tt tet ga get ttt ett cet rev Sso MCM I555_I557delinsDSD CW90 tgg ta gga gtt gat atg gat atg gat ace ata fwd Sso MCM M604_E605_ins(A)5, rev CW84 gga aa ga aaa ta at agt at ag at ace ata fwd Sso MCM I614_K618del CW107 agt gta gga gtt gat atg gat ata gat ace ace fwd Sso MCM I614_K618del CW108 aca ata atg at ata gat ace ace ge gee gee gat fwd Sso MCM I604_E605_I609del, rev CW84 CW109 gga aaa tag ta tag gat ata gat ace ace fwd Sso MCM I614_K618del CW110 gga tag agt tg at atg gat ata gat ace ace fwd Sso MCM E605_I609del, I614_K618del, rev CW84 CW110 gga tg ag agt tg at atg gat ata gat ace cet fwd Sso MCM E605_I609del, I614_K618del, rev CW84 CW110 gat ga gg at ga at gg agt aga agt aga agt gg a		ggc gta tta gat gag	
CW84 cat atc aac tcc tac act ctc tag gaa aag rev 2 stop codons distal to Sso MCM M604 CW85 aca ata atg act ggt aaa taa tga cct aaa agc gct fvd 2 stop codons distal to Sso MCM K618 CW80 gaa aca cct gat agc cag agt agc gat act cca aga caa fvd 2 stop codons distal to Sso MCM K618 CW80 gga aca cct gat agc cag at agc gat act cca aga caa fvd Sso MCM 1555_1557delinsDSD CW90 tgg gct atc agg tgt ttc tag act tt ctct rev Sso MCM 16155_1557delinsDSD CW91 gtc cca tgg aaa ttc tag at atg gcg gc gcg gcg gcg gcg ag agt fvd Sso MCM 604_E605ins(A)5, rev CW84 Ggra aa agt gta gga gtt gat atg ggt ag at ag at ag at aca ata fvd Sso MCM 1614_K618del CW109 gga aaa ata gat atg at aca cct aca agc gcc fvd Sso MCM 1614_K618del CW110 gat gt ag gat gat at ag at aca cct aca rev Sso MCM 1614_K618del CW110 ggt aga gtt gat atg gca gat gga aga gg aga gat gga aga gga gga	CW82	gcg cgg cac cag gcc gct gct gtg atg gtg atg	rev thrombine to TEV site
CW85 aca ata atg act ggt aaa taa tga cet aaa age get fwd 2 stop codons distal to Sso MCM K618 CW86 ttt acc agt cat tat tgt atc tat atc rev 2 stop codons distal to Sso MCM K618 CW80 tgg get acc agg tag ce ca gat age gat act cea aga caa fwd Sso MCM 1555_1557delinsDSD CW91 tgg get atc agg tgt tet tga get tt ett cet rev Sso MCM 1555_1557delinsDSD CW91 ggt cca tgg aaa tat gge gee gee gee geg gaa agt fwd Sso MCM 1604_E605ins(A)5, rev CW84 CW106 agt gta gga gtt gat atg gat ata gat aca ata fwd Sso MCM 1614_K618del CW107 agt gta gga gtt gat atg gat ata gat aca gee gee gee gee gee gee gee gee gee ge	CW83	agt g ta gga gtt gat atg taa tga aag tgg aaa a	fwd 2 stop codons distal to $Sso~{\rm MCM}~{\rm M604}$
CW86 ttt ace agt cat tat tgt atc tat atc rev 2 stop codons distal to Sso MCM K618 CW89 gaa aca cet gat age cag at age gat act cea aga caa tta g fwd Sso MCM I555_I557delinsDSD CW90 tgg et atc agg tgt tte tga get tte tte cet rev Sso MCM I555_I557delinsDSD CW90 get ace agg tgt tte tga get get geg gee geg gee geg gaa agt geg aaa fwd Sso MCM M604_E605ins(A)5, rev CW84 CW107 agt gta gga gtt gat atg gat ata gat aca ata age get fwd Sso MCM 618_P619ins(A)5, rev CW84 CW108 aca ata gat at gat aca cet aaa age get fwd Sso MCM 1614_K618del CW110 tgt at cat at tit tte cat tte cat at caa rev Sso MCM 1614_K618del CW111 gga aaa ta gat at gat at gat aca cet aaa age get fwd Sso MCM 6613_1614ins(A)5, rev CW10 atg at gt agg agt tgat at geg at aga gat aga at ag at aca cet aaa age get fwd Sso MCM 1604_K618del CW112 agt tag ga gt tgat at ge at age aca cet fwd Sso MCM 1603_1614_K618del CW112 agt gt agg agt tgat at gea tat gee age tgg age age agg tgg age age tgg tgg tgg age age age geg ce geg ce ceg geg fwd Sso MCM 1603_1614_K618del, rev CW84 CW112 agt gta gga tgg ceg tae ceg ag tgg age age age age age age age tgg tgg tgg tgg tgg tgg tgg tgg tgg t	CW84	cat atc aac tcc tac act ctc tag gaa aag	rev 2 stop codons distal to $Sso~{\rm MCM}~{\rm M604}$
CW89gaa aca cet gat age cea gat age gat act cea aga caa tta gfwd Sso MCM I555_I557delinsDSDCW90tgg get atc agg tgt tte tag get ttt ett cet gg at atc gg aat te cea tgg aag tte gat ag ge ge gee gee gee geg gaa agt gga aaarev Sso MCM I555_I557delinsDSDCW107agt gta gga gtt gat atg geg gee gee gee gee geg gaa agt gga aaafwd Sso MCM M604_E605ins(A)5, rev CW84 fwd Sso MCM K618_P619ins(A)5, rev CW84CW107agt gta gga gtt gat atg gat ata gat aca ata age getfwd Sso MCM I614_K618delCW109gga aaa ata gat ata gat aca geg gee gee gee gee gea ge at agt gat ggt gat atg gat ata gat aca ata age getfwd Sso MCM I614_K618delCW110tgt ate tat tet ttt te cat tte cat ate caac atg act ggtrev Sso MCM I614_K618delCW112agt gta gga gtt gat atg gat aga gac ag tgg acc gag tgt ge gag ttg at atg gat acc taa ag ce gag tgt ge gag ttg at atg ge age tgg age cag tgg acc gag tgt ge gag ttg at atg ge acc tag a gag tgt ge gag teg ce geg accfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW112agt gta gga gtt gat atg get age acc tag acc gag tgt ge gag tgg acc ceg gg accfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tat gge age tgg age ac gag tgg teg acc ceg gg tac cet ac ga tgg teg acc gg ga tec tag accfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW120tet aga gga teg ceg ga teg acc gag tgg teg acc ceg gg tac ceg agefwd Sso MCM E605_I609del, I614_K618del, rev CW84CW121get geg acc ceg gg tac ceg age ga tgg teg acc ceg gg tac ceg agefwd Sso MCM E605_I609del, I614_K618del, rev CW84CW121get cga tac ceg gg tac ceg age ceg acc c	CW85	aca ata atg act ggt aaa taa tga cct aaa agc gct	fwd 2 stop codons distal to $Sso~{\rm MCM}~{\rm K618}$
tta g tta g CW90 tgg get ate agg tgt tte tga get tte et get ace aga tgt agg agt tga agg agt tga tat gat ate aga tgt agg agt tga tat gat ate aga tga agg tge geg gee gee gee gee gee gee gee g	CW86	ttt acc agt cat tat tgt atc tat atc	rev 2 stop codons distal to $Sso~{\rm MCM}~{\rm K618}$
CW90tgg ct ate agg tgt tit tg ag tt tt et et etrev Sso MCM I555_I557delinsDSDCW91gt c ca tgg aa at te et agt a aa en ag tt g agfwd Sso MCM, Neol siteCW106agt gt agg agt gat at g ge ge ge ge ge ge ge ge ga aafwd Sso MCM M604_E605ins(A)5, rev CW84CW107agt gt agg agt gat at ag ga at ag at aa aa aafwd Sso MCM E605_I609del, rev CW84CW108aca ata at gat gat at ag at ace et aa aa ge ge gefwd Sso MCM E605_I609del, rev CW84CW109gga aaa at gat at ag at ace et aa age getfwd Sso MCM E614_K618delCW110gga aaa at ag at at ag at ace ge ge ge ge ge ge ge ge ge gefwd Sso MCM I614_K618delCW111gga aaa at ag at at gat at ag at ace ace ge	CW89	gaa aca cct gat agc cca gat agc gat act cca aga caa	fwd $Sso~{\rm MCM}$ I555_I557 delins DSD
CW91gtc cca tga aa ttc cta gta aac aga ttg acfwd Sso MCM, Ncol siteCW106agt gta gga gtt gat atg gc gc gc gc gc gc gc ga aatfwd Sso MCM M604_E605ins(A)5, rev CW84CW107agt gta gga gtt gat atg gat ata gat aca atafwd Sso MCM E605_I609del, rev CW84CW108aca ata atg act ggt aaa gc gc gc gc gc gc gc gc aaafwd Sso MCM E605_I609del, rev CW84CW109gga aaa ata gat ata gat aca ct aaa agc gctfwd Sso MCM I614_K618delCW110tgt atc tat cta ttt tc ca ct ttc cat atc aacrev Sso MCM I614_K618delCW111gga aaa ata gat ata gat aca gc gg gc gc gc gc gc gc gc gc gc ga tafwd Sso MCM E605_I609del, I614_K618delCW112agt gta gga gtt gat atg gat ata gat aca cctfwd Sso MCM E605_I609del, I614_K618del,CW112agt gta gga gt ga tag ga tt ga ta gg a aac ta aag cgfwd Sso MCM E605_I609del, I614_K618del,CW112agt gta gga gt ga tag ga ta ga aca ct aa aag cgfwd Sso MCM E605_I609del, I614_K618del,CW119gct cgg tac cg gg ga tg gc ga tag aca at ag cfwd double strand DNA for EMSA, rev CW120CW120tct aga gtg tag tag tag ga gt ga aca tag ag tc cc cg ggtfwd double strand DNA for EMSA, fwd CW121 ^b CW121tct ace tgg acg tag cc ga gt ga ga ga ga cgrev 3' overhang DNA for EMSA, fwd CW121 ^b CW122tcct tag ag tc ga cg tg gc cg tt ttt ttt ttt ttt ttt ttt		tta g	
CW106agt gta gga gtt gat atg gce gce gce gce gce gaa agt gga aaafwd Sso MCM M604_E605ins(A)5, rev CW84CW107agt gta gga gtt gat atg gat ata gat aca ata age gctfwd Sso MCM E605_1609del, rev CW84CW108gga aaa at gga ata gat ata gat aca cet aaa age gctfwd Sso MCM E618_P619ins(A)5, rev CW86CW109gga aaa ata gat ata gat aca cet aaa age gctfwd Sso MCM I614_K618delCW110gga aaa ata gat ata gat aca geg gce gce gce gce gce gce gce gaa afge cet ggtfwd Sso MCM I614_K618delCW112ggt ga gga gtt gat atg gat ata gat aca geg gce gce gce gce gce gce geg gg	CW90	tgg get ate agg tgt tte tga get ttt ett eet	rev Sso MCM I555_I557delinsDSD
gga aaaCW107agt gta gga gtt gat at gga at a gga ta ag gat at ag at a ag at ag at ag ag cg cg cg cg cg cg cg cd caafwd Sso MCM E605_1609del, rev CW84CW108aca at at ag at ag at ag gg cg cg cg cg cg cg cd caafwd Sso MCM K618_P619ins(A)5, rev CW86agc gctgga aaa at ag at at ag at aca cct aaa agc gcfwd Sso MCM I614_K618delCW110tgt at ct at at ct att tt cc act ttc cat at caacrev Sso MCM I614_K618delCW112agt gta gga gtt gat at ggt agt ag agt ag at ag at ag at ag at ag at ag ga tag agt ag agt gg ag cg cg cg gc cg cg cd cw84fwd Sso MCM T613_1614ins(A)5, rev CW110CW112agt gta gga gtt gat at ggc gag tgg ag cag tag aca agt gg cgfwd Sso MCM E605_1609del, I614_K618del,CW112agt gta gga gtt gat at ggc gag tgg ag cag tag aca cta aag cgfwd Sso MCM E605_1609del, I614_K618del,CW112agt gta gga gt tga tat ggc gag tgg ag cag tag aca cta aag cgfwd Sso MCM E605_1609del, I614_K618del,CW112agt gta gg agt tga tat ggc gag tgg ag cag tag aca ctafwd Sso MCM E605_1609del, I614_K618del,CW112ggt tgg ag tg cg ag tgg cgg taa acc taa aag cgfwd double strand DNA for EMSA, rev CW84CW120tct aga gga tcc cg gg acc cg gc cc cg gafwd double strand DNA for EMSA, fwd CW121bCW121ctt gca tgc cg ga cc gg gc tag cagrev 3' overhang DNA for EMSA, fwd CW121bCW122tct tct ag ag tc cc tag agt cgarev 3' overhang DNA for EMSA, fwd CW121bCW123gt gga gac tgc tag cd cg gg tag ct tt	CW91	gtc cca tgg aaa ttc cta gta aac aga ttg ac	fwd Sso MCM, NcoI site
CW107agt gta gag gtt gat at g gat at ag gat at ag at a gat at ag at a gat at ag ga g g g g	CW106	agt g ta gga gtt gat atg gcg gcc gcg gcc gcg gaa agt	fwd $Sso~{\rm MCM}~{\rm M604_E605ins(A)5},$ rev ${\rm CW84}$
$ \begin{array}{c} {\rm CW108} & {\rm aca} \ {\rm ata} \ {\rm atg} \ {\rm act} \ {\rm ggt} \ {\rm aca} \ {\rm atg} \ {\rm act} \ {\rm ggt} \ {\rm ggt} \ {\rm aca} \ {\rm atg} \ {\rm act} \ {\rm att} \ {\rm att} \ {\rm att} \ {\rm ttt} \ {\rm ttt$		gga aaa	
age getCW109gga aaa ata gat ata gat aca cat aaa age getfwd Sso MCM I614_K618delCW110tgt ate tat ate tat ttt tee cat tte cat ate aacrev Sso MCM I614_K618delCW111gga aaa ata gat ata gat aca geg gee geg gee geg ata atg act ggtfwd Sso MCM T613_I614ins(A)5, rev CW110CW112agt gta gga gtt gat atg gat ata ggat cat age gag tgg age age tggfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tat gge gag tgg age aag tggfwd E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg ate cte tag afwd double strand DNA for EMSA, rev CW120bCW120tet aga gga tec ccg ggt ace gag crev double strand DNA for EMSA, fwd CW119bCW121ctt gea tge cdg cag gea tge age tge agefwd 3' overhang DNA for EMSA, fwd CW121b fwd bubble DNA for EMSA, fwd CW121bCW122tee tag ag tee ccg gg ate cte tag agt cca carev 5' overhang DNA for EMSA, fwd CW121bCW123get cgg tac ccg gg ate cte tag agt cgarev 5' overhang DNA for EMSA, fwd CW121bCW124tet ace tgg ace gg ttt ttt ttt ttt ttt ttt ttt ttt ttt	CW107	agt gta gga gtt gat atg gat ata gat aca ata	fwd $Sso~{\rm MCM}$ E605_I609 del, rev ${\rm CW84}$
CW109gga aaa at ag at at ag at ac act aaa age gctfwd Sso MCM I614_K618delCW110tgt at c tat at c tat tt tc act tc at a ca acrev Sso MCM I614_K618delCW111gga aaa at ag at ag at ag at ac ag cg gc g gg gc gg at afwd Sso MCM T613_I614ins(A)5, rev CW110atg act ggtagt gt ag ag tt gat at gg at at ag at ac a cctfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tat gge gag tgg age aag tgg acefwd Sso MCM E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg at cct ct ag afwd double strand DNA for EMSA, rev CW120bCW120tct aga gga tcc ccg ggt acc cd ag gc ag tg gc ac ag tg ag ccfwd 3' overhang DNA for EMSA, fwd CW121bCW121ctt gca tge ctg cag gt cag ct ag ag t cga ag tg ag cag ag t cgafwd 3' overhang DNA for EMSA, fwd CW121bCW122tc ta cct gg ag ac cg gg at cct tag ag t cga acc carev 5' overhang DNA for EMSA, fwd CW121bCW123gct cgg tac ccg ggg at cct tag ag t cgarev 5' overhang DNA for EMSA, fwd CW121bCW124tct acc tg ag acc gg gt cct tt ttt ttt ttt ttt ttt ggarev bubble DNA for EMSA, fwd CW121bCW125tga tgg act gc gg tac ct tag agt cgarev for overhang DNA for EMSA, fwd CW124bCW126gct cgg tac ccg ggg at c ct tag agt ccc cgg gt accrev bubble DNA for EMSA, fwd CW124bCW126ttt ttt ttt ttt ttt ttt ttt ttt ttt t	CW108	aca ata at g act ggt aa a \gcd gcc \gcd gcc \gcd cc t aaa	fwd Sso MCM K618_P619ins(A)5, rev CW86
CW110tgt atc tat atc tat tit tcc act tic cat atc aac gg aaa ata gat ata gat ata gat aca geg gec geg ge geg gat atg act ggtrev Sso MCM I614_K618del fwd Sso MCM T613_I614ins(A)5, rev CW110 atg act ggtCW112agt gta gga gtt gat atg gat ata gat aca cctfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tat ggc gag tgg agc aag tgg agc gag tgg tgg cgg tag acc taa aag cgfwd Sso MCM E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg atc ctc tag a gag tgt cg ag tgg cgg taa acc taa aag cgfwd double strand DNA for EMSA, rev CW120bCW120tct aga gga tcc ccg ggt acc gag c acc gag cfwd double strand DNA for EMSA, fwd CW119bCW121ctt gca tgc ctg cag gtc gac tgc agg acc gag crev 3' overhang DNA for EMSA, rev CW122b acc gag cCW122tcc taga gtc gac ctg cag get gg act ct agg tcg acc gag cc gg tac ct agg tcg act tt ttt ttt ttt ttt ttt tttrev 5' overhang DNA for EMSA, fwd CW121b fwd bubble DNA for EMSA, fwd CW124b gtc gtc cag gta cag gc gtc cag gta gaCW125tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t		agc gct	
CW111gga aaa ata gat ata gat ata gat aca gcg gcc gcg gc gc gc gtfwd Sso MCM T613_I614ins(A)5, rev CW110atg act ggtagt gta gga gtt gat atg gat ata gat aca cctfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW112gag tgt agg agt tga tat ggc gag tgg agc aag tgg agcfwd E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg atc ctc tag afwd double strand DNA for EMSA, rev CW120 ^b CW120tct aga gga tcc ccg ggt acc gag cfwd double strand DNA for EMSA, fwd CW121 ^b CW121ctt gca tgc ctg cag gtc ga tgc aga tgc aga tgc agafwd 3' overhang DNA for EMSA, rev CW122 ^b acc gag crev 3' overhang DNA for EMSA, fwd CW121 ^b CW123gct cgg tac ccg ggg atc ctc tag agt cgarev 5' overhang DNA for EMSA, fwd CW121 ^b CW124tct acg ag cc gg ga tc gc ctt ttt ttt ttt ttt ttg ggc cag cag gtc cat carev 5' overhang DNA for EMSA, fwd CW121 ^b CW125tga tgg acc tgc tgg cc ttt ttt ttt ttt ttt ttt tt	CW109	gga aaa ata gat ata gat aca cct aaa agc gct	fwd $Sso~{\rm MCM}$ I 614_K618 del
atg act ggtCW112agt gta gga gtt gat atg gat atg gat atg ata gat acc cctfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tatg ggc gg tga gac aag tgg agc gag tgg tgc gag tgg cgg taa acc taa aag cgfwd E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg atc ctc tag afwd double strand DNA for EMSA, rev CW120 ^b CW120tct aga gga tcc cg ggt acc gag crev double strand DNA for EMSA, fwd CW119 ^b CW121ctt gca tgc ctg cag gtc gac tct aga gga tcc ccc ggt acc gag cfwd 3' overhang DNA for EMSA, fwd CW121 ^b CW122tcc tag agt cga ccg gga tc ct tag agt cga acc gag crev 3' overhang DNA for EMSA, fwd CW121 ^b CW123gct cgg tac ccg ggg atc ct tag agt cga acc ga gca ccg ggt cat carev 5' overhang DNA for EMSA, fwd CW121 ^b CW124tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ttg gge cag cag gtc cat carev bubble DNA for EMSA, fwd CW124 ^b CW125tga tgg acc tge tgg ccc ttt ag att ttt ttt ttt ttt tttfwd fork DNA for EMSA, fwd CW124 ^b CW126gct cgg tac ccg ggg atc ct cag agt agt ccc gg ta cccrev fork DNA for EMSA, fwd CW124 ^b CW127ttt ttt ttt ttt ttt ttt ttt ttt ttt t	CW110	tgt atc tat atc tat ttt tcc act ttc cat atc aac	rev $Sso~{\rm MCM}$ I 614_K618 del
CW112agt gta gga gtt gat atg gat atg gat ata gat aca cctfwd Sso MCM E605_I609del, I614_K618del, rev CW84CW118gag tgt agg agt tga tat ggc gag tgg agc aag tgg agcfwd E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg atc ctc tag afwd double strand DNA for EMSA, rev CW120bCW120tct aga gga tcc ccg ggt acc gag crev double strand DNA for EMSA, fwd double CW119bCW121ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggt acc gag crev 3' overhang DNA for EMSA, fwd CW121bCW122tcc tct aga gtc gac ctg cag gca ga tg cgarev 3' overhang DNA for EMSA, fwd CW121bCW123gct cgg tac ccg ggg atc ctc tag ag tcgarev 5' overhang DNA for EMSA, fwd CW121bCW124tct acc tgg acg acc ggg tt ttt ttt ttt ttt ttt ttt ttt tt	CW111	gga a aa ata gat ata gat aca gc g gcc gc g gcc gcg ata	fwd $Sso~{\rm MCM~T613_I614ins(A)5},$ rev ${\rm CW110}$
rev CW84 CW118 gag tgt agg agt tgg tag tag gag tgg agg a		atg act ggt	
CW118 gag tgt agg agt tga tat ggc agg tgg agc aag tgg gag tgg tgc gag tgg cgg taa acc taa aag cgfwd E605_T616delins(ASG)4, rev CW84CW119gct cgg tac ccg ggg atc ctc tag afwd double strand DNA for EMSA, rev CW120 ^b CW120tct aga gga tcc ccg ggt acc gag crev double strand DNA for EMSA, fwd 	CW112	agt g ta gga gtt gat atg gat ata gat aca cct	
gag tgg tgc gag tgg cgg taa acc taa aag cgCW119gct cgg tac ccg ggg atc ctc tag afwd double strand DNA for EMSA, rev CW120bCW120tct aga gga tcc ccg ggt acc gag crev double strand DNA for EMSA, fwd CW119bCW121ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggt acc gag cfwd 3' overhang DNA for EMSA, rev CW122b acc gag cCW122tcc tct aga gtc gac ctg cag gca tgc aag gc tcg tac ccg ggg atc ctc tag agt cgarev 3' overhang DNA for EMSA, fwd CW121bCW123gct cgg tac ccg ggg atc ctc tag agt cga c cg ag gtc cat carev 5' overhang DNA for EMSA, fwd CW121bCW124tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ttt ttt ttt t			rev CW84
 CW119 gct cgg tac ccg ggg atc ctc tag a CW120 tct aga gga tcc ccg ggt acc gag c CW121 tct aga gga tcc ccg ggt acc gag c CW122 tct ct aga gtc gac ctg cag gca tgc aag CW123 gct cgg tac ccg ggg atc ctc tag agt cga CW124 tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ttt ggc CW125 tga tgg acc tgc tgg cct ttt ttt ttt ttt ttt ttt t	CW118	gag t gt agg agt t ga tat ggc gag t gg agc aag t gg agc	fwd $E605_T616$ delins(ASG)4, rev CW84
 CW120 tct aga gga tcc ccg ggt acc gag c CW120 tct aga gga tcc ccg ggt acc gag c rev double strand DNA for EMSA, fwd CW121^b CW121 ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggt acc gg g c CW122 tcc tct aga gtc gac ctg cag gca tgc aag rev 3' overhang DNA for EMSA, fwd CW121^b CW123 gct cgg tac ccg ggg atc ctc tag agt cga rev 5' overhang DNA for EMSA, fwd CW121^b CW124 tct acc tgg acg acg ggt tt ttt ttt ttt ttt ttg ggc ac g gtc cat ca CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t		gag t gg t gc gag t gg c gg taa acc taa aag c g $% f_{\rm s}$	
CW121CW19bCW122ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggfwd 3' overhang DNA for EMSA, rev CW122bAcc gag crev 3' overhang DNA for EMSA, fwd CW121bCW123gct cgg tac ccg ggg atc ctc tag agt cgarev 5' overhang DNA for EMSA, fwd CW121bCW124tct acc tgg acg acc ggg ttt ttt ttt ttt ttt gg gcfwd bubble DNA for EMSA, rev CW125bCW125tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t	CW119	gct cgg tac ccg ggg atc ctc tag a	
acc gag cCW122tcc tct aga gtc gac ctg cag gca tgc aagrev 3' overhang DNA for EMSA, fwd CW121 ^b CW123gct cgg tac ccg ggg atc ctc tag agt cgarev 5' overhang DNA for EMSA, fwd CW121 ^b CW124tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ggc cag cag gtc cat cafwd bubble DNA for EMSA, rev CW125 ^b CW125tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t	CW120	tct aga gga tcc ccg ggt acc gag c	
 CW122 tcc tct aga gtc gac ctg cag gca tgc aag CW123 gct cgg tac ccg ggg atc ctc tag agt cga CW124 tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ggc CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t	CW121	ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggt	fwd 3' overhang DNA for EMSA, rev $\rm CW122^b$
 CW123 gct cgg tac ccg ggg atc ctc tag agt cga CW124 tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ggc CW124 tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ggc CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t		acc gag c	
 CW124 tct acc tgg acg acc ggg ttt ttt ttt ttt ttt ttg ggc fwd bubble DNA for EMSA, rev CW125^b cag cag gtc cat ca CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt ccg rev bubble DNA for EMSA, fwd CW124^b gtc gtc cag gta ga CW126 gct cgg tac ccg ggg atc ctc tag att ttt ttt ttt ttt ttt ttt fwd fork DNA for EMSA, rev CW127^b ttt CW127 ttt ttt ttt ttt ttt ttt ttt ttt ttt ccg gg gat ccc gg gta ccg gta ccg rev fork DNA for EMSA, fwd CW126^b agc CW134 agt gta gga gtt gat atg gaa acc gga aaa ata gat ata fwd substitute <i>Sso</i> MCM E605_K618 by <i>Mth</i> 	CW122	tee tet aga gte gae etg eag gea tge aag	rev 3' overhang DNA for EMSA, fwd CW121 ^b
 cag cag gtc cat ca CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt ccg rev bubble DNA for EMSA, fwd CW124^b gtc gtc cag gta ga CW126 gct cgg tac ccg ggg atc ctc tag att ttt ttt ttt ttt ttt ttt ttt ttt	CW123	get egg tae eeg ggg ate ete tag agt ega	rev 5' overhang DNA for EMSA, fwd CW121 ^b
 CW125 tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt ccg rev bubble DNA for EMSA, fwd CW124^b gtc gtc cag gta ga CW126 gct cgg tac ccg ggg atc ctc tag att ttt ttt ttt ttt ttt ttt ttt ttt	CW124	tet acc tgg acg acc ggg ttt ttt ttt ttt ttt ttt ttg ggc	fwd bubble DNA for EMSA, rev $\rm CW125^{b}$
gtc gtc cag gta ga CW126 gct cgg tac ccg ggg atc ctc tag att ttt ttt ttt ttt ttt ttt fwd fork DNA for EMSA, rev CW127 ^b ttt CW127 ttt ttt ttt ttt ttt ttt ttt ttt ttt t		cag cag gtc cat ca	
 CW126 gct cgg tac ccg ggg atc ctc tag att ttt ttt ttt ttt ttt fwd fork DNA for EMSA, rev CW127^b ttt CW127 ttt ttt ttt ttt ttt ttt ttt ttt cta gag gat ccc cgg gta ccg agc CW134 agt gta gga gtt gat atg gaa acc gga aaa ata gat ata fwd substitute <i>Sso</i> MCM E605_K618 by <i>Mth</i> 	CW125	tga tgg acc tgc tgg ccc ttt ttt ttt ttt ttt ttt t	rev bubble DNA for EMSA, fwd CW124 ^b
tttCW127ttt ttt ttt ttt ttt ttt ttt ttt ttt t		gtc gtc cag gta ga	
 CW127 ttt ttt ttt ttt ttt ttt ttt ttt cta gag gat ccc cgg gta ccg rev fork DNA for EMSA, fwd CW126^b agc CW134 agt gta gga gtt gat atg gaa acc gga aaa ata gat ata fwd substitute <i>Sso</i> MCM E605_K618 by <i>Mth</i> 	CW126	get egg tac eeg ggg ate ete tag att ttt ttt ttt ttt ttt	fwd fork DNA for EMSA, rev $\rm CW127^b$
agc CW134 agt gta gga gtt gat atg gaa acc gga aaa ata gat ata fwd substitute <i>Sso</i> MCM E605_K618 by <i>Mth</i>		ttt	
agc CW134 agt gta gga gtt gat atg gaa acc gga aaa ata gat ata fwd substitute Sso MCM E605_K618 by Mth	CW127	ttt tt t ttt ttt ttt ttt ttt cta gag gat ccc cgg g ta ccg	rev fork DNA for EMSA, fwd CW126 ^b
$\label{eq:cw134} CW134 \mbox{agt gta gga gtt gat atg gaa acc gga aaa ata gat ata \mbox{fwd substitute Sso MCM E605_K618 by Mth}$			
	CW134		fwd substitute Sso MCM E605_K618 by Mth
		gat aaa gtt gaa ggt cgt acc cct aaa agc gc	

Primer	Sequence 5'-3'	Construct ^a
CW143	agt gta gga gtt gat atg gag aca ggc aag ata gat ata	fwd substitute Sso E605_V686 by Mth MCM
	gac aag	E583_V666, rev CW74
CW144	gcc tgt ctc cat atc aac tcc tac act ctc tag gaa aag	rev substitute Sso E605_V686 by Mth MCM
		E583_V666, fwd CW91
CW145	agt g ta gga gtt gat atg gaa acc gga aaa ata gat ata	fwd Sso MCM S606T, rev $\mathrm{CW84}$
	gat	
CW146	ata gat ata gat aca ata ccg act ggt aaa	fwd Sso MCM M615P, rev $\operatorname{CW149}$
CW147	ata gat ata gat aca ata ggc act ggt aaa	fwd Sso MCM M615G, rev CW149
CW148	ata gat ata gat aca ata gcg act ggt aaa	fwd Sso MCM M615A, rev CW149
CW149	tat tgt atc tat atc tat ttt tcc act ttc	rev mutation of Sso MCM M615
CW150	agt g ga a aa ata gat ata gat cc g ata cc g act g gt cc g $\ $	fwd Sso MCM T613P, M615P and K618P
	cct aaa agc gc	
CW151	agt g ga aaa ata gat ata gat g gc ata g gc act g gt g gc $% f(x)$	fwd Sso MCM T613G, M615G and K618G
	cct aaa agc gc	
CW152	agt g ga aaa ata gat ata gat gcg ata gcg act ggt gcg $% f_{\rm s}$	fwd Sso MCM T613A, M615A and K618A
	cct aaa agc gc	
CW153	atc tat atc tat ttt tcc act ttc cat atc aac	rev mutation of Sso MCM T613 M615 and
		K618
CW156	ggt gat ccc ggt act gcc gcg tca caa atg	fwd Sso MCM K346 A Walker A- mutation
CW157	ggc agt acc ggg atc acc tat tat cag tat gtg	rev $Sso~{\rm MCM}$ K346A Walker A-mutation
CW158	cca ata tt g ata act cca gcg caa tta gag	fwd Sso MCM R560 A Sensor II- mutation
CW159	tgg agt tat caa tat tgg gct atc agg tgt ttc	rev Sso MCM R560A Sensor II-mutation
Y-shpF	$\rm (t)_{44}$ gct c gt gca gac gt c gag gt g agg acg agc tcc tcg	fwd Y-shapped DNA for helicase assay, rev Y-
	tga cca cg	$\rm shpR^{c}$
Y-shpR	cgt ggt cac gag gag ctc gtc ctc acc tcg acg tct gca	rev Y-shapped DNA for helicase assay, fwd Y-
	cga $gc(t)_{44}$	$\mathrm{shpF}^{\mathrm{c}}$

Table S 1: Primers used in this study

^a nomenclature for the description of sequence variations as recommended [1]

^b DNA oligo sequences for EMSA as published in [2]

^c DNA oligo sequences for helicase assay as published in [3]

Biophysical characterisation

Purified C-terminal domain of *Sso* MCM represents a folded and thermostable monomer

To obtain initial information about secondary structure elements of the purified C-terminal domain of Sso MCM far-UV CD spectra were recorded. The same initial biophysical characterization is provided for the *Mth* MCM C-terminal domain (Supplementary Figure S1, S2, S4-S5 and Table S2-S4). The spectra display two minima of ellipticity at 208 and 222 nm and one maximum at 192 nm (Supplementary Figure S1), respectively, that are typical for a high α -helical content (Supplementary Table S2). The secondary structure composition was calculated using CAPITO [4] and CDPro [5]. The heat induced protein unfolding as monitored by CD spectroscopy revealed that the isolated *Sso* MCM C-terminal domain is highly thermostable (Supplementary Figure S2 and Table S3).

Circular dichroism analysis

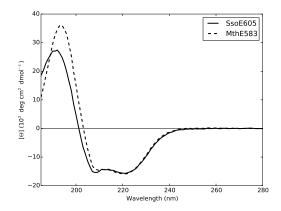


Fig. S 1: Far UV-CD spectra of S. solfataricus (solid line) and M. thermautotrophicus (dashed line) MCM C-terminal domains at 4°C. Circular dichroism (CD) spectra were collected on a JASCO J-710 CD spectropolarimeter at 4°C in a 1 mm quartz cuvette to evaluate the proper fold of the proteins and to estimate the secondary structure content. The instrument was calibrated with D-10-camphorsulphonic acid. Purified proteins were exchanged into pure water using NAP-5 columns (GE Healthcare). The protein concentration was in the 1–10 μ M range and verified spectrophotometrically at 280 nm with extinction coefficients calculated using ProtParam (http://web.expasy.org/protparam/). Each CD spectrum represents the average of 10 accumulated scans at 10 nm/min with a 1 nm slit width and a time constant of 4 s for a nominal resolution of 0.7 nm. Data were collected between 185 and 260 nm. No further zeroing was applied after background subtraction.

terminal domains derived nom OD spectra						
		Mth			Sso	
	helical	$\beta\text{-strand}$	irregular	helical	$\beta\text{-strand}$	irregular
CAPITO	46 - 57	6-20	30 - 34	34-44	12 - 26	39 - 54
$SELCON3^{a}$	52	9	38	47	9	45
$\operatorname{CONTIN}^{\mathrm{a}}$	47	13	40	46	8	46
$\rm CDSSTR^{a}$	53	11	37	51	9	40
$\mathrm{DSSP}^{\mathrm{b}}$	54	15	31	53	17	30

Table S 2: The secondary structure content of Mth and Sso MCM C-terminal domains derived from CD spectra

^a included in the software CDPro [5]

^b secondary structure determination based on the pdb entries [6] (*Mth* PDB id: 2MA3, *Sso* PDB id: 2M45)

 Table S 3: Heat induced unfolding

Construct	Denaturation temperature
Sso MCM	$\geq 85^{\circ}\mathrm{C}$
$Sso MCM\Delta WH$	$\geq 85^{\circ}\mathrm{C}$
Sso MCM WH	n. o. ^a
Mth MCM WH	$80^{\circ}\mathrm{C}$

 $^{\rm a}$ not observable in the temperature range up to 90°C

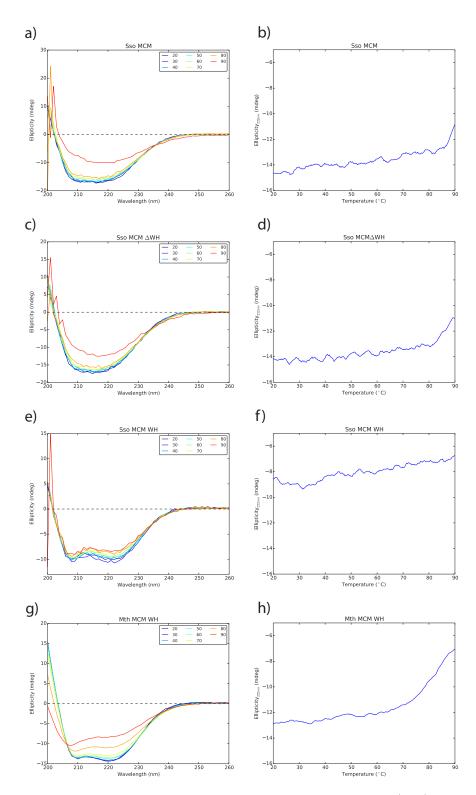


Fig. S 2: Heat denaturation as observed by CD spectroscopy of *Sso* MCM (a, b), *Sso* MCM∆WH (c, d), the *Sso* MCM C-terminal domain (WH) (e, f) and the *Mth* MCM C-terminal domain (WH) (g, h), respectively. At 20, 30, 40, 50, 60, 70, 80 and 90°C CD spectra were collected between 200 nm and 260 nm (left column). Each CD spectrum represents the average of 5 accumulated scans at 50 nm/min with a 1 nm slit width and a time constant of 1 s for a nominal resolution of 0.83 nm. Ellipticity was observed at 222 nm at temperatures ranging between 20–90°C (right column). Data points were collected in 0.2°C steps with a response time of 1 sec and a bandwidth of 1 nm. Temperature slope was 1°C/ min. Denaturation temperature was calculated with Spectra Manager[™] software (JASCO).

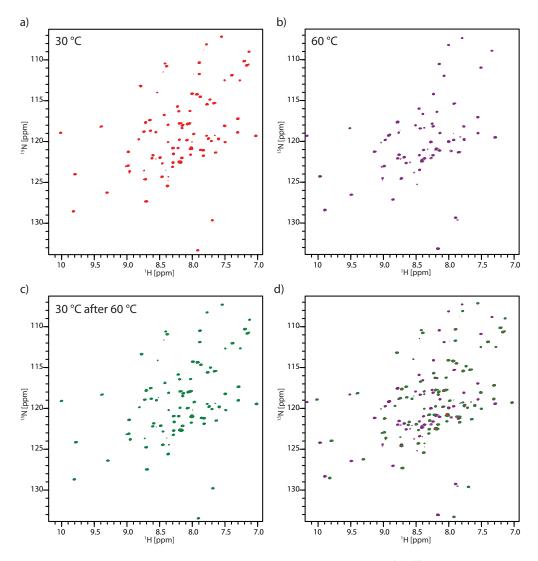


Fig. S 3: Temperature sensitivity of the Sso MCM C-terminal domain. [¹H, ¹⁵N]-HSQC spectra of an Sso MCM C-terminal domain sample (250 μ M) were recorded at 600 MHz with spectral widths of 6893 Hz sampled over 1024 complex points in the ω_2 (¹H) dimension, and 1944 Hz over 256 complex points in the ω_1 (¹⁵N) dimension with 4 scans per increment in the indirect dimension. The ¹H and ¹⁵N radio frequency carriers were set at 4.69 ppm and 116 ppm, respectively. Recycle time was 1 s. a) and b) [¹H, ¹⁵N]-HSQC spectra of Sso MCM C-terminal domain recorded at 30°C and 60°C. c) [¹H, ¹⁵N]-HSQC spectra of Sso MCM C-terminal domain recorded at 30°C of the spectra shown in a-c.

The observed [¹H,¹⁵N]-HSQC fingerprint spectra resulted in a highly similar signal pattern for both temperatures suggesting that the overall fold is not affected at higher temperatures.

Light scattering

To gain access to hydrodynamic parameters of Sso MCM C-terminal domain we performed dynamic and static light scattering analysis. The hydrodynamic radius derived from dynamic light scattering is 1.55 ± 0.12 nm (Supplementary Figure S4 and Table S4). This value is in line with the calculated one (R_h: 1.56-1.72 nm) as obtained for globular spherical proteins on the basis of their molecular weight by applying the Stokes' law [7]. The molecular weight estimated from R_h (9.3 kD) indicates that the purified, recombinant C-terminal domain of Sso MCM is monomeric in solution. Additionally, results obtained from static light scattering analysis showed that the *Sso* MCM C-terminal domain has a molecular mass of about 10.5 kD, which is consistent with the dynamic light scattering results and a calculated molecular weight of 9.5 kD (Supplementary Figure S5).

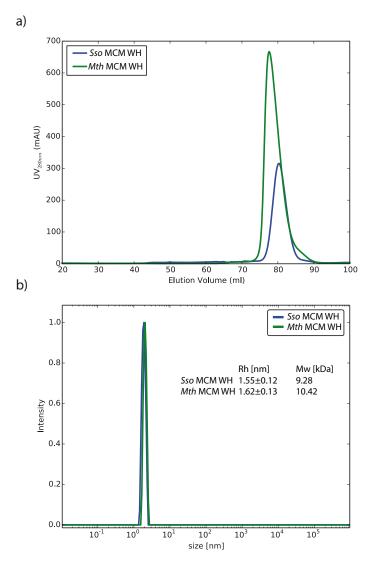


Fig. S 4: Size exclusion chromatography (a) and dynamic light scattering (b) of purified *Sso* (blue) and *Mth* MCM C-terminal domain (WH) (green), respectively.

a) Size exclusion chromatography was performed on a HighLoad 16/60 SD 75 column (GE Healthcare) equilibrated in 10 mM Na-Phosphate pH 6.2 and 150 mM NaCl. Flow rate was 1.5 ml/min.

b) Dynamic light scattering (DLS) on purified *Mth* and *Sso* MCM WH was performed on a Viscotek 802 DLS equipped with a 50 mW fiber coupled 830 nm diode laser. Fifty transients of 5 s each were recoreded at 20°C in a 50 μ l volume. Protein concentrations were in the range of 50 μ M in 10 mM Na-Phosphate, 150 mM NaCl, pH 6.2. Mass weighted distribution of hydrodynamic radii and a model of globular proteins were utilized for converting hydrodynamic radii to molecular weights. Mass weighted scattering intensity distribution from dynamic light scattering is plotted. OmniSIZE 3.0 software (Viscotek) was used for acquisition and analysis of DLS data.

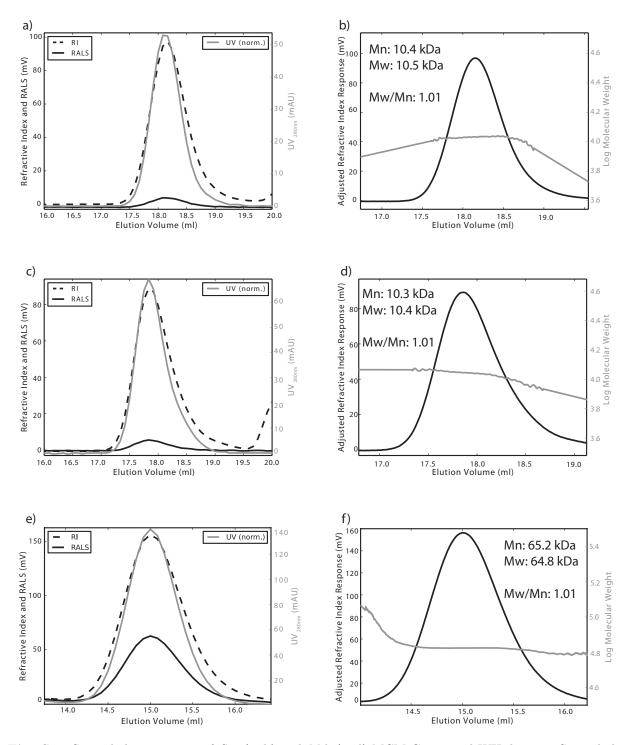


Fig. S 5: Static light scattering of Sso (a, b) and Mth (c, d) MCM C-terminal WH domain. Static light scattering (SLS) on purified Mth and Sso MCM WH were performed using a Superdex 200 10/300 GL column (GE Healthcare), equilibrated with the same buffer as for DLS, and 270 Dual detectors (Viscotek) coupled to an Äkta Explorer system (GE Healthcare). 100 μ l of purified Mth and Sso MCM WH were applied to the system with a flow rate of 0.5 ml/min. Protein concentration was in the range of 1 mg/ml. Bovine serum albumin standard (Thermo scientific) was used for calibration and the protein concentration was 2 mg/ml in a 0.9% aquaeous NaCl solution. The resulting data were evaluated with the OmniSEC software. Bovine serum albumin (e, f) was used for calibration. Refractive index (RI), right angle light scattering (RALS) and UV signal at 280 nm is shown on in a, c, and e. Adjusted refractive index and calculated molecular weight of the eluted protein are shown on in b, d, and f.

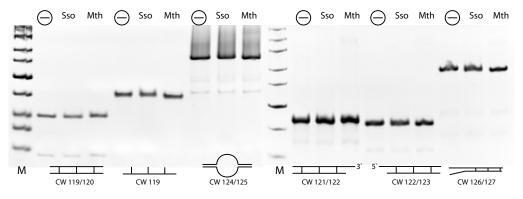


Fig. S 6: Electrophoretic mobility shift assay. DNA (10 μ M) of different topologies as indicated below the gels was incubated at room temperature for 30 min with a 100-fold molar excess of isolated archaeal MCM C-terminal domains of *Sso* or *Mth*, respectively. Samples were loaded onto a native 12.5% polyacrylamide gel. Gels were run at 20 mA for 2 h in 40 mM Tris-acetat, pH 7.2 at 4°C. Different incubation temperatures, buffer compositions and gel running temperatures were also tested with comparable outcome (not shown).

NMR relaxation parameters

 $\{^{1}\text{H}, ^{15}\text{N}\}$ relaxation parameters of selectively ¹⁵N-labeled *Sso* and *Mth* MCM C-terminal domains were acquired using two-dimensional, ¹H-detected heteronuclear NMR experiments by recording inversion recovery [¹H, ¹⁵N]-HSQC for T₁ measurements, CPMG [8] spin echo [¹H, ¹⁵N]-HSQC for T₂ measurements and heteronuclear steady-state NOE spectra, respectively. T₁ values were measured in a series of spectra with relaxation delays of 0.003, 0.05, 0.1, 0.15, 0.2, 0.35, 0.5, 0.7, 1.2, 2 and 4 s. T₂ measurements were taken with relaxation delays of 0.016, 0.033, 0.048, 0.065, 0.081, 0.114, 0.146 and 0.179 s. The relaxation delay for T₁ and T₂ measurements were 5 s and 3 s, respectively. To allow NOE evolution, ¹H-¹⁵N steady-state NOE values were measured with two different data sets, one collected without initial proton presaturation and a second with an initial proton presaturation period of 3 s. Protein concentrations were in the 1 mM range.

Estimates of the rotational correlation time, τ_c , of *Sso* and *Mth* MCM C-terminal domains were obtained from the ratios of ¹⁵N T₁ and T₂ values, using a modified equation as reported in [9]. The Debye-Stokes-Einstein equation was used to calculate the hydrodynamic radius from τ_c [10]. The hydrodynamic properties of *Sso* and *Mth* MCM C-terminal domains were also predicted from their NMR-derived structure ensembles using the software HYDROPRO [11].

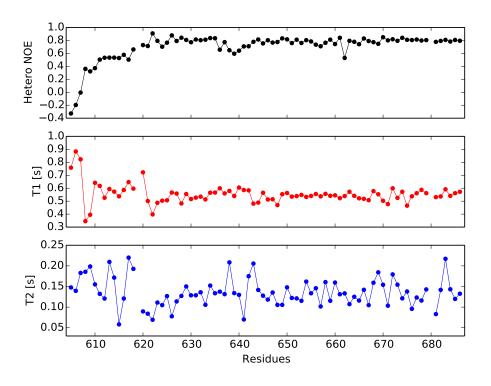


Fig. S 7: {¹H,¹⁵N} Hetero-NOE, relaxation times T_1 and T_2 for the *Sso* MCM C-terminal domain at 17.6 T plotted as a function of the residue number. T_1 and T_2 spectra were recorded with spectral widths of 8710 Hz sampled over 1024 complex points in the ω_2 (¹H) dimension, and 2280 Hz over 94 complex points in the ω_1 (¹⁵N) dimension with 8 scans for each increment in the indirect dimension. Heteronuclear steady-state NOE spectra were acquired with a spectral width of 8710 Hz over 1024 complex points in the ω_2 (¹H) dimension and 2280 Hz over 68 complex points in the ω_1 (¹⁵N) dimension with 32 scans for each increment in the indirect dimension. The ¹H and ¹⁵N radio frequency carriers were set at 4.69 ppm and 116 ppm, respectively. Temperature was at 30°C.

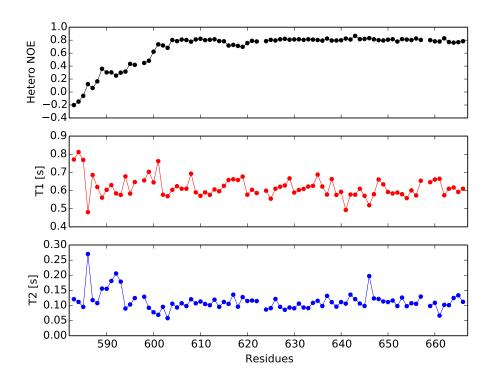


Fig. S 8: {¹H,¹⁵N} Hetero-NOE, relaxation times T₁ and T₂ for the *Mth* MCM C-terminal domain at 17.6 T plotted as a function of the residue number. T₁ and T₂ spectra were recorded with spectral widths of 7653 Hz sampled over 1024 complex points in the ω_2 (¹H) dimension, and 2204 Hz over 128 complex points in the ω_1 (¹⁵N) dimension with 4 scans for each increment in the indirect dimension. Heteronuclear steady-state NOE spectra were acquired with a spectral width of 7653 Hz over 1024 complex points in the ω_2 (¹H) dimension and 2204 Hz over 128 complex points in the ω_1 (¹⁵N) dimension with 8 scans for each increment in the indirect dimension. Heteronuclear steady-state NOE spectra were acquired with a spectral width of 7653 Hz over 1024 complex points in the ω_2 (¹H) dimension and 2204 Hz over 128 complex points in the ω_1 (¹⁵N) dimension with 8 scans for each increment in the indirect dimension. The ¹H and ¹⁵N radio frequency carriers were set at 4.69 ppm and 119 ppm, respectively. Temperature was at 30°C.

			Mth	Sso				
	DLS ^a	$\mathrm{SLS^b}$	HYDROPRO ^c	NMR ^d	DLS	SLS	HYDROPRO	NMR
$\tau_c^{\rm e}[{\rm ns}]$	$4.4{\pm}1.1$	_	7.7 ± 1.04	5.2 ± 1.4	$3.9{\pm}0.9$	_	5.2 ± 0.4	$4.4{\pm}1.2$
$R_h^{\rm f}[{\rm nm}]$	1.62 ± 0.13	_	$2.04{\pm}0.1$	$1.73 {\pm} 0.15$	1.55 ± 0.12	_	$1.79 {\pm} 0.04$	$1.64{\pm}0.15$
$M_W^{\rm g}[{\rm kD}]$	10.5	<u>10.4</u>	17.6 - 22.9	9.6 - 13.8	9.3	10.5	10.9 - 14.9	7.9 - 11.3
$M_W^{\rm h}[{ m kD}]$			10.1				9.5	

Table S 4: Comparison of hydrodynamic parameters of Mth and Sso MCM C-terminal domains

^a Dynamic light scattering

^b Static light scattering

^c calculation with HYDROPRO software [11] from NMR-derived structure ensembles, the average value is indicated

 $^{\rm d}$ τ_c was obtained from the $\rm T_1/T_2$ ratio [9] measured on a 750 MHz spectrometer at 30°C for Mth and Sso MCM-WH

 $^{\rm e}\,$ rotational correlation time

^f hydrodynamic radius

g for molecular weight calculation a hydration radius (0.16–0.32 nm) was subtracted from the protein hydrodynamic radius [7]

^h theoretical molecular weight based on amino acid composition

For DLS, SLS, HYDROPRO and NMR methods, τ_c , R_h and M_W , respectively, are mean values \pm standard deviations. The errors are obtained by applying the error propagation law. The underlined values indicate those determined from the experimental data. The nonunderlined values are determined using the Stokes-Einstein and the Debye-Stokes-Einstein equation [10].

MCM C-terminal domain	
Total distance restraints ^a	2494
NOE based distance restraints	
intra-residual (i-j =0)	291
sequential $(i-j = 1)$	652
medium-range $(2 \le i-j \le 4)$	812
long-range $(i-j \ge 5)$	701
Restrained H-bonds	38
Dihedral angle restraint	182
Constraint violations	
Distance constraints (Å)	$0.007 {\pm} 0.001$
Max. distance constraint violation (Å)	$0.20 {\pm} 0.08$
Dihedral angle constraints (°)	$0.21{\pm}0.14$
Max. dihedral angle violation ($^{\circ}$)	$3.22 {\pm} 2.25$
CYANA target function $(Å^2)$	$0.84{\pm}0.23$
AMBER energies (kcal mol^{-1})	-1437.1 ± 62.3
Deviations from idealized geometry	
Bond lengths (Å)	0.005
Bond angles (°)	1.450
mean global RMSD (Å)	
Total ^b (backbone)	$6.37 {\pm} 1.95$
Total ^b (heavy atoms)	$6.72{\pm}1.87$
Ordered ^c (backbone)	$0.92{\pm}0.28$
Ordered ^c (heavy atoms)	$1.82{\pm}0.31$
Ramachandran statistics $(\%)^d$	
Most favored	85
Additionally allowed	14
Generously allowed	1
Disallowed	0

Table S 5: NMR and refinement statistics for the MthMCM C-terminal domain

^a upper & lower distance restraints

^b Mth MCM WH: residues E583-V666

 $^{\rm c}~Mth$ MCM WH: residues K598-V666

^d PROCHECK classification [12]

Expression vector derived residues were not included in the structural statistics.

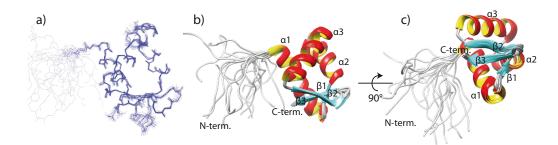


Fig. S 9: NMR solution structure of the *M. thermautotrophicus* MCM C-terminal domain. a) Superimposition of backbone traces for the 20 structures with the lowest energy after OPAL refinement. b and c) Ribbon representation of the structural ensemble with labeled secondary structure elements. Here, the less ordered residues 579–591 of the *M. thermautotrophicus* MCM C-terminal WH domain are omitted for clarity. The *Mth* MCM C-terminal domain was expressed and purified as reported [13]. The ¹H, ¹³C and ¹⁵N backbone and side chain chemical shift assignments for the *M. thermautotrophicus* MCM C- terminal WH domain are available from the BioMagResBank (accession numbers: 19187) [13]. The structural coordinates of the *M. thermautotrophicus* MCM C-terminal domain have been deposited to protein data bank (PDB: 2MA3). α 1-3: α helix 1-3; β 1-3: β strand 1-3

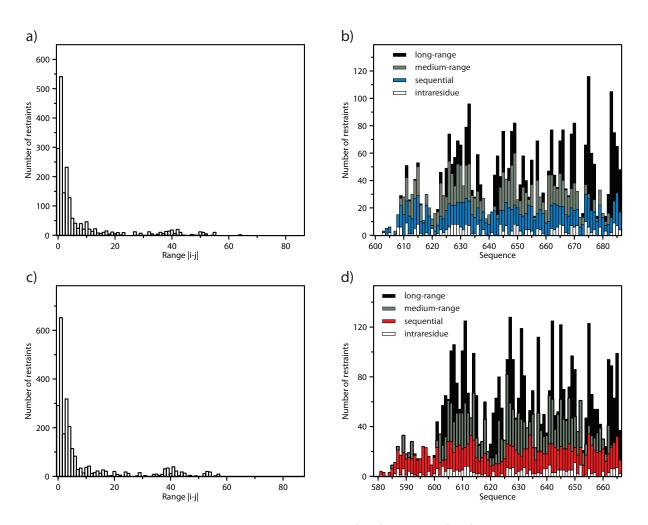


Fig. S 10: Distribution and number of NOEs of the *Sso* (a, b) and *Mth* (c, d) MCM C-terminal domain. a) and c) The panels show the number of NOEs as a function of residue |i-j| range. NOE constraints were classified into 4 range categories according to the sequential distance of involved residues (intraresidue k=i, sequential k=i±1, medium-range k=i±2-4, long-range k=i±n n>4). b) and d) The number of NOE constraints used in the structure calculation as a function of residue number.

No	$\rm PDB^{b}$	Z-score ^c	$\mathrm{rmsd}^{\mathrm{d}}$	$lali^{e}$	$\mathrm{nres}^{\mathrm{f}}$	$\% \mathrm{id}^\mathrm{g}$	Description
1	2m45-A	13.8	0.5	67	87	100	Minichromosome maintenance protein MCM
2	2ma3-A	10.9	1.1	64	88	20	Minichromosome maintenance protein MCM
3	406j-A	10.4	2.0	62	213	13	Iron-dependent transcription repressor related protein
4	2jt1-A	9.9	1.7	63	71	19	PefI protein
5	1dpu-A	9.9	2.3	64	69	14	Replication protein a (rpa32) c-terminal domain
6	4chu-B	9.9	1.7	63	127	17	HTH-type transcriptional regulator iscr
7	2heo-A	9.8	1.4	58	59	14	Z-DNA binding protein 1
8	4kmf-A	9.8	1.6	60	62	17	INF-inducible and ds-dep. eIF-2 kinase
9	3mwm-A	9.7	2.0	64	127	8	Putative metal uptake regulation protein
10	2xrn-B	9.7	1.5	61	241	7	HTH-type transcriptional regulator ttgv
11	4ija-A	9.6	1.6	59	363	17	XylR protein
12	2x4h-C	9.6	1.8	61	131	16	Hypothetical protein sso2273
13	1lva-A	9.5	1.8	62	258	13	Selenocysteine-specific elongation factor
14	10kr-B	9.5	1.5	61	122	23	Methicillin resistance regulatory protein MecI
15	4lb5-B	9.4	1.5	58	63	24	Protein kinase containing Z-DNA binding domains
16	1ldj-A	8.9	1.8	63	725	13	Cullin homolog 1
17	3dqv-D	8.8	1.8	63	378	11	NEDD8
18	2qww-A	8.8	2.4	60	146	17	Transcriptional regulator, MARR family
19	2mh2-A	8.7	1.9	60	64	18	Homologous-pairing protein 2 homolog
20	2g9w-A	8.6	1.7	62	119	11	Conserved hypothetical protein

Table S 6: 20 top ranked homologues of Sso MCM C-terminal domain^a returned by the DALI web server [14]

 $^{\rm a}\,$ the mean structure of the region K620-V686 was submitted $^{\rm b}\,$ Protein Data Bank entry code and chain identifier

^d root-mean-square deviation of C^{α} atoms in the least-squares superimposition of the structurally equivalent C^{α} atoms

^e number of structurally equivalent residues ^f total number of amino acids in the hit protein

^g percentage of identical amino acids over structurally equivalent residues

			-	~				
	No	$\rm PDB^b$	$\operatorname{Z-score}^{\operatorname{c}}$	$\mathrm{rmsd}^{\mathrm{d}}$	$lali^{e}$	$\mathrm{nres}^{\mathrm{f}}$	$\% \mathrm{id}^\mathrm{g}$	Description
-	1	2ma3-A	14.4	0.4	68	88	100	Minichromosome maintenance protein MCM
	2	2m45-A	10.5	2.7	67	87	19	Minichromosome maintenance protein MCM
	3	406j-A	9.8	2.1	62	213	18	Iron-dependent transcription repressor related protein
	4	2jt1-A	9.4	2.3	64	71	13	PefI protein
	5	1dpu-A	9.2	1.8	61	69	16	Replication protein A (rpa32) C-terminal domain
	6	4ija-A	9.2	1.6	57	363	16	XylR protein
	7	1hst-A	9.1	2.4	64	74	14	Histone H5
	8	3cta-A	9.0	1.7	60	187	23	Riboflavin kinase
	9	2mh2-A	8.9	1.8	61	64	11	Homologous-pairing protein 2 homolog
	10	2heo-A	8.8	1.7	58	59	16	Z-DNA binding protein 1
	11	1tbx-A	8.8	1.9	63	94	14	SSV1 F-93
	12	4chu-B	8.8	3.0	63	127	13	HTH-type transcriptional regulator ISCR
	13	1ldj-A	8.7	1.7	61	725	11	Cullin homolog 1
	14	1p6r-A	8.7	1.5	61	82	18	Penicillinase repressor
	15	3mwm-A	8.7	2.1	62	127	13	Putative metal uptake regulation protein
	16	2xrn-B	8.6	2.4	60	241	8	HTH-type transcriptional regulator ttgv
	17	4kmf-A	8.5	1.8	57	62	9	INF-inducible and ds-dep. eIF-2 kinase
	18	3bz6-A	8.4	2.1	66	168	14	UPF0502 protein PSPTO_2686
	19	1ust-A	8.4	2.3	64	92	14	Histone H1
	20	3eyy-A	8.3	1.8	61	133	15	Putative iron uptake regulatory protein

Table S 7: 20 top ranked homologues of Mth MCM C-terminal domain^a returned by the DALI web server [14]

^a the mean structure of the region K598-V666 was submitted

^b Protein Data Bank entry code and chain identifier

^c normalized score ^d root-mean-square deviation of C^{α} atoms in the least-squares superimposition of the structurally equivalent C^{α} atoms

 $^{\rm e}$ number of structurally equivalent residues $^{\rm f}$ total number of amino acids in the hit protein

^g percentage of identical amino acids over structurally equivalent residues

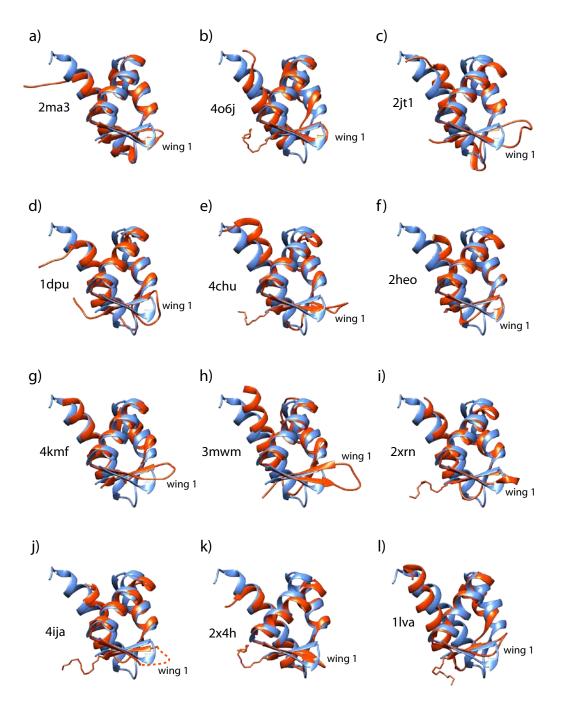


Fig. S 11: Superposition of the *Sso* MCM C-terminal truncated WH domain (blue) with homologous structures (red) found in a DALI search (see Tab. S6). Superpositions for the 12 structures exhibiting the highest Z score are shown.

Note that except for 2ma3 (*Mth* WH) and 2heo (mouse Z-DNA binding protein 1), the shown WH domains have a more extended wing 1 element and/or a C-terminal extension.

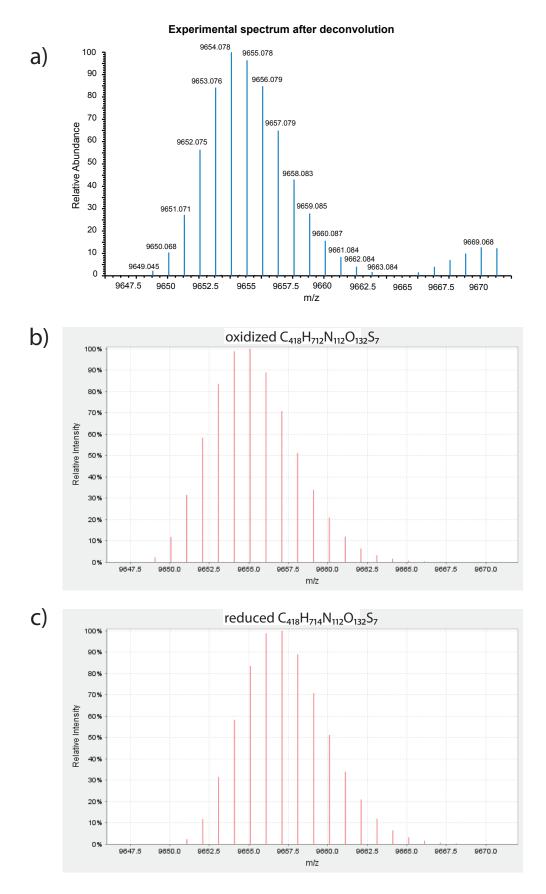


Fig. S 12: Mass spectrometry of the *Sso* MCM C-terminal WH domain. Isotope distribution of the *Sso* MCM C-terminal WH domain as experimentally determined by ESI mass spectrometry is shown in a). The experimentally determined isotope distribution was compared to b) predicted isotope distributions for the WH domain with disulfide bridge (oxidised) and c) with free cysteine residues (reduced), respectively. The predicted distributions were calculated using the freeware program "Isotope Pattern Calculator" (Pacific Northwest National Laboratory, http://omics.pnl.gov/software/ScalaBLAST.php).

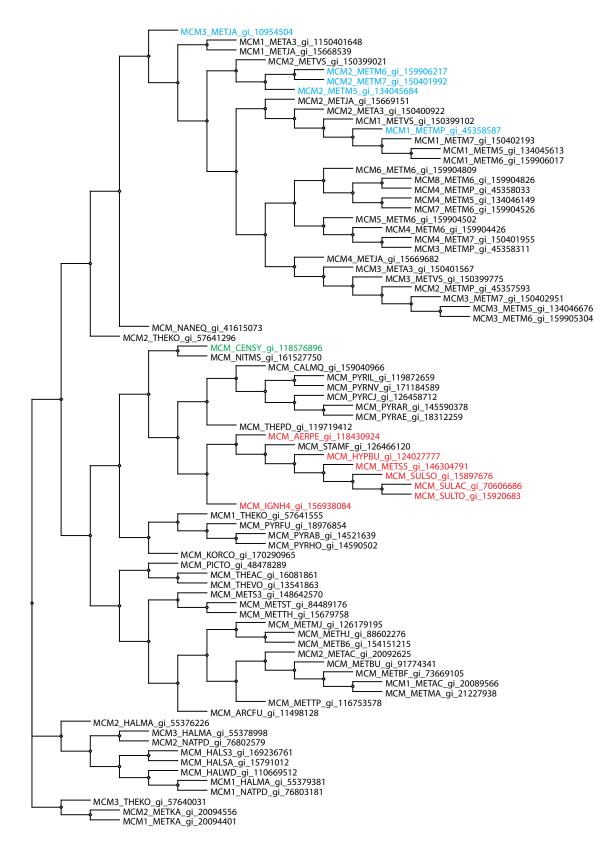


Fig. S 13: Phylogentic tree of of archaeal MCMs. The selection of species was reported [15]. The analysis was performed with MUSCLE [16, 17] under predefined settings on the EMBL-EBI web server [18]. Organism identification is based on the mnemonic UniProtKB entry code. For further information the NCBI protein sequence accession number (gi) is given. Protein sequences were cured by removing known intein sequences [19] where applicable. MCMs with two C-terminal cysteines are highlighted in red, those carrying a single cysteine \sim 20-30 residues proximal of the C-terminus in cyan, and the MCM with one cysteine at the very C-terminus in green, respectively.

	· · · · · · ·				
	· · · · ¥ ·	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			
· · · · · · · · · · · · · · · · · · ·					
e v		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		· < < < < < · · · · · · · · · · · · · ·	· · · · · · · · ZZ · · · · · · ·
<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>	- < < < < < < < < < < < < < < < < < < <	♡ − − − ∞ − × − > ∞ ∞ ∞ ∞ ∞ ∞ ∞ ∞ − − − − > − − − − −		> 2 < < < <	
<u></u>	z o z z o z	A A A A A A A A A A A A A A A A A A A	××××××××××××××××××××××××××××××××××××××		~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
	≺ – – – <	A Z G G G G G G G G G G G G G G G G G G			× > I > 4 I I I I I I + I O O O O O O O O O O O O
	<pre>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>></pre>		C X C X X X X X X X X U U U U U U U U U	××××××××××××××××××××××××××××××××××××××	× + + < × 0 0 0 0 0 0 0 × × × × × 0 0 0 0 0 0
_ ~ × < < v v v		**************			
>	- Z O O > L	Z < U > - > - = > - > > > > > > - > - > - > -			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
►					
00000000		0 < = = = 0 0 0 0 < < = = = = = 0 0 0 0 < < = = = =			> X X X 4 0 K K K K W I X K K 0 X
	X T L L V	<pre>A C C C C C C C C C C C C C C C C C C C</pre>			
		I > > > >			
	<u></u>	- O > Z = = 0 × X = = = = = = 0 = Z = Z	- 4		Z — — — — — Z — — — Z — — Z — — Z — — Z — — Z → Z →
>-><-u>	v <mark>u u u u</mark>	- > > < > > - < > > - < > > > > - < < < - > - < < < - > - < < < - > - < < < - > - < < < - > - < < < - > - < < < <	->->>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	< < > > > > > > 2 > > > .	
× m – N × N N m	200w0		<pre>< u O u u O u u u u u o u u u o u u u o u u u u</pre>		
	2 · · · × • · · · w	□ ★ ≮ Ⅲ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★	c ≪ ⊢ − m m m m m m m x x x	~~~~~	
د z س س س ص z	2 X X X X V	 4 σ ш ⊢ ν Ο × Ο Ο σ σ σ σ σ γ ν ⊢ ⊢ ≥ < 0 	- O O F ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~		~~~~~~~~~~~~~~~~~
		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	,0002000000000		0000000 0000 2200
- 2 4 4 M M M M M M M M M M M M M M M M M	0 ~ ~ 0 ~ ~	<pre></pre>	ХОА≻пппппк<××× ХОА≻ппппк<-<п		××××・TEEE・<×EEEEE
	N Z Z Q N	>>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		стологиййй состав	
	> < < < -	→ ≪ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → → → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ − → ∞ → ∞		< < < < < < < < > > > > > > > > > > > >	< 2 - 2 > < < < < - < < < < < <
******					
->		×			
	< < < < < < <	> 2 X X X Z M M C C C C C Q C A X Z C		- × « « « « > 0 0 0 × ≥ - « : 7 m 0 m 0 0 0 m 0 0 0 0 m 1 1 0 0 0 0 0 0 2 > > > 0 m 0 0 m 0	7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
« I « X X X X C	2 4 4 4 0 2 4 4 4 0	<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>	<pre>&lt; I f o &gt; 0 0 0 0 f f I x ; &gt; 2 m m m m m m m m m m &gt;</pre>	- ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	T 5 7 H 2 T 7 T 7 T 7 T 7 T 7 T 7 T 7 T 7 T 7 T
	л	F · · · · · · · · · · · · · · · · · · ·	× × × × × × × × × × × × × × × × × × ×		>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
0⊢00ш0шα 0		- 0 0 - 0 - 2 - 0 0 0 0 0 0 0 0 0 0			
0 0	4 4 Z		DZ III X Z H N N N D D K X Y	×2222	
M M M M M M M M M M M M M M M M M M M	S S		000×0000000000000000000000000000000000		· < = > · < Δ Η Δ · Ο = Ο = Ο = Ο = Ο = Ο = Ο = Ο = Ο = Ο
ш.х.п. т. п п.х.п. т. п.	⊔ · · · · ⊐ 9 · · · >	· « m · 米 m m « m · · · · · « · · · · · · · · · ·	0 × 0 × < < < < > < 0 × 0 × 0 × 0 × 0 × 0 × 0 × 0 × 0 × 0	× < × × × × × × × × × × × × × × × × × ×	× = 2 · · O = 2 · · · · · · · · · · · · · · · · · ·
	<pre>&lt;</pre>	· · · · · · · · · · · · · · · · · · ·		· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·
	2 · · · · · ·				
	~ ~ ~ ~ ~	4 4		······································	-<
			<pre>x x m x x x x x x m m m x x x x x m x x x x x x x x x x x x x x x x x x x x</pre>	<pre></pre>	
				>>	
>	- 0	※ ※ ペリン 目 > ペリ ※ × × × × × × × × × × × × × × × × × ×	~ > > >		- O Z & & O O Z & & Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
NTH>ZNK	> + + < 0				
		<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>		~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	Z & & & = O Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
~~~~~~~~~	ZQQI>				x 🗅 m m m m m m m m m m m m m m m m m m
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				* * Z Z Z Z X X X X X X X X X X X X X X	****Z \$\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
	r × ∝ ∝ ∑ σ < × ∝ ∝ ∝ ×	H G X G G O O O O O O O O O O O O O O O O	- ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	<pre></pre>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
< > × × × × × × ×		·>====================================	>>×-Опппп++ОО(	22++++3>>>>>>>>>>	
0000000 L>LLLL	0 > > > 0 0 c > > > > .	>>+Q==0000000000000000000000000000000000	-+>> · · · · · << ->>	> < = = = = = Z Z Z Z Z Z Z Z Z Z Z Z Z Z	20000000000000000000000000000000000000
≅≻≊≊≊≊≊ 	×××× v × ××	> → − − − − − − − − − − − − − − − − − −			- > > > < > > > >
	>>>> × ×		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	- * * * * * * * * * * * * * * * * * * *	****
>	- ~ ~ ~ ~ ~		>>>>		> < > < < < < - < < < < < < < < <
>		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		J~~~~~~~~~~~~~~~~	- ミメメミネネネネタミツミミミシー
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0 2 4 4 7 7 7 7 7 4 7 7 7	0 + > + C < < + + + < < < < < < < < < < < < <	,	, 000000000000000000000000000000000000	
→>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>		Q d m > Q > − + ∑ > − − > > ∾ d ∑ + d d d A m m X m X > Q m m m m m m Q − Q m m X r		· · · · · · · · · · · · · · · · · · ·	~~~~~~~~~~~~~~~~~~~
		<pre>4 0 × 0 × 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</pre>			
0000000	Q N N F K	< < = 0 = 0 = 0 = 0 = 0 = 0 = 0 = 0 = 0	< < < < < < < < < < < < < < < < < < <		< 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
- × v e z	$\vdash$	××00××	,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
	< + +	· · · · · · · · · · · · · · · · · · ·			
		· · · · · · · · · · · · · · · · · · ·		· · · · · · · · · 2ZZ · · · · · · ·	
		1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1     1 <td></td> <td></td> <td></td>			
		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			
24 884 5 5 777 3 91 3 92	5684 5684 6217 6217 1992 04 04 587	2226 2296 2001 2011 2012 2012 2012 2012 2012 201	75 555 555 9102 9102 5613 5613 5613 5613 5613 5613 5613 5613	022 033 033 053 053 053 055 1955 1955 1955 1955 1955 1955 1955	82 82 82 82 82 82 82 82 82 82
84305 6938( 6938( 8304) 89767 89767 89767 89767 89767 89767 89767 89768	027.00 13404 15040 15040 09545 15358	55376 7640. 11527. 7640. 70946 97199 97199 97199 97198 87265 84587 11845 87265 97182 87265 00928 18428 71842 87265 96186 00928 18428 71828 87265 96186 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17828 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 17888 178888 17888 17888 17888 17888 17888 17888 17888 17888 178888 178888 178888 178888 178888 178888 178888 178888 1788888 178888 178888 178888 178888 1788888 1788888 178888 178888 1788888 1788888 178888 1788888 1788888 178888 178888 17888888 1788888 178888 178888888 1788888 178888 178888888 1788888 178888 178888888 178888 178888 1	60179 6022. 7641. 75040 15040 15990 15990 15990 15990 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 556685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 56685 566855 566855 56685 56685 566855 56685 56685 56685 56685 5668	2002 15990 15990 15990 15990 15990 15990 15990 15990 15990 15990 15990 15990 15990 15990	56694 8642: 8642: 55378 6691( 7743 6691( 7743 79101 79101 79101 79101 79101 79101 79101 79101
91.11 91.15 91.16 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15 91.15	91 - 01 - 01 - 01 - 01 - 01 - 01 - 01 -	2010 2010 2010 2010 2010 2010 2010 2010	91 82 91 5 91 5 91 5 91 5 91 5 91 5 91 5 91		19-19-19-19-19-19-19-19-19-19-19-19-19-1
RPE C VH4 C TSS C TSS C LISO C USV C	ETMC ETMC ETMC ETMC ETMC	ALM HEKO RRCO RRCO RRCO RRCO RRCO RRCO RRCO RR	ETAS ETAS ETAS ETAS ETAS ETAS TEU-S TEU-S TEU-S TEU-S TEU-S	ETME ETME ETME ETME ETME ETME ETME ETME	1111-1111-1111-1111-1111-1111-1111-1111-1111
A SULAR	41 W 2 W 2 W 2 W 2 W 2 W 2 W 2 W 2 W 2 W	N N N N N N N N N N N N N N N N N N N		7 9 9 8 9 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9	877779787878787878787878787878787878787
		MOIQInterQ_g_17841280         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R         R			

Fig. S 14: Amino acid sequence alignment of archaeal MCM C-terminal domains for the species shown in Figure S12. The analysis was performed with MUSCLE [16, 17] using the default settings of the EMBL-EBI web server [18] and manually refined using Unipro UGENE [20]. Identification of organisms is based on the mnemonic UniProtKB entry code and the NCBI protein sequence accession number (gi) is given. Cysteine residues are highlighted in purple. Species with MCM C-terminal domains harbouring two cysteines are boxed in red, in blue for a single cysteine ~20-30 residues proximal of the C-terminus and green for one cysteine at the very MCM C-terminus, respectively.

## ATPase activity of Sso MCM derivatives

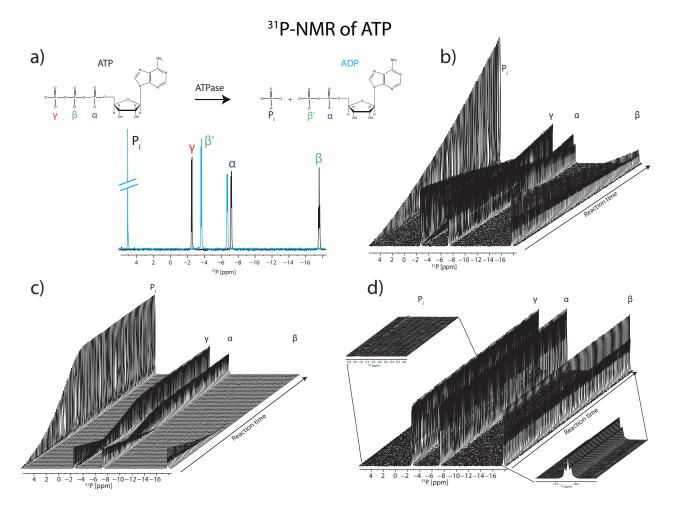


Fig. S 15: a) Hydrolysis of ATP into ADP and free inorganic phosphate. a, top) Schematic reaction scheme of ATP hydrolysis by an ATPase yields ADP and free inorganic phosphate. The  $\alpha$ -,  $\beta$ - and  $\gamma$ -phosphates of ATP and the released free inorganic phosphate (P_i) and the  $\alpha$ - and  $\beta'$ -phosphates of ADP are indicated. a, bottom) [³¹P]-NMR spectra of ATP (black) before and after (blue) complete hydrolysis into ADP and free inorganic phosphate. b-d) Time series of [³¹P]-NMR spectra indicating the progress of ATP hydrolysis catalysed by (b) *Sso* MCM, (c) *Sso* MCM $\Delta$ WH (E605_V686del, C-terminal domain) and (d) buffered ATP diluted into ATPase reaction buffer (see Material and Methods). Expanded regions in d) to show the time-course for free inorganic phosphate (d, top left) and  $\beta$ -phosphate of ATP (d, bottom right), respectively.

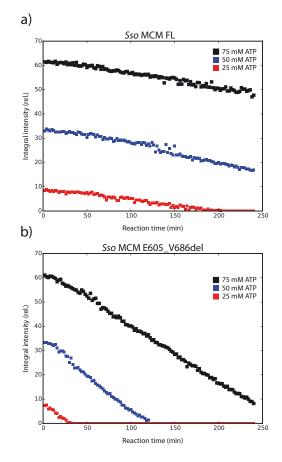


Fig. S 16: ATP hydrolysis by *Sso* MCM constructs under various ATP substrate concentrations. ATP hydrolysis was assayed by  $[^{31}P]$ -NMR spectroscopy. Time series of  $[^{31}P]$ -NMR spectra were recorded at 60°C. ³¹P chemical shifts at 60°C were referenced relative to 75 % H₃PO₄ at 0 ppm. Spectra were processed using Bruker software TOPSPIN V2.1. The integrated peak areas of the free phosphate (red dots, 25 mM ATP; blue, 50 mM ATP; black, 75 mM ATP) are plotted as a function of the reaction time.

Increasing the ATP concentration to 75 mM or decreasing it to 25 mM, respectively, did not significantly change the rate of ATP hydrolysis as judged from the slope of the decrease of the signal intensity integral over time: under all three ATP concentrations the rate for a) the full length wild type MCM was  $3.4 \text{ s}^{-1}$  and  $7.2 \text{ s}^{-1}$  for b) the MCM construct lacking the C-terminal WH domain, respectively. This indicates that the assays shown in Fig. S15 and S17 were conducted under saturating substrate conditions.

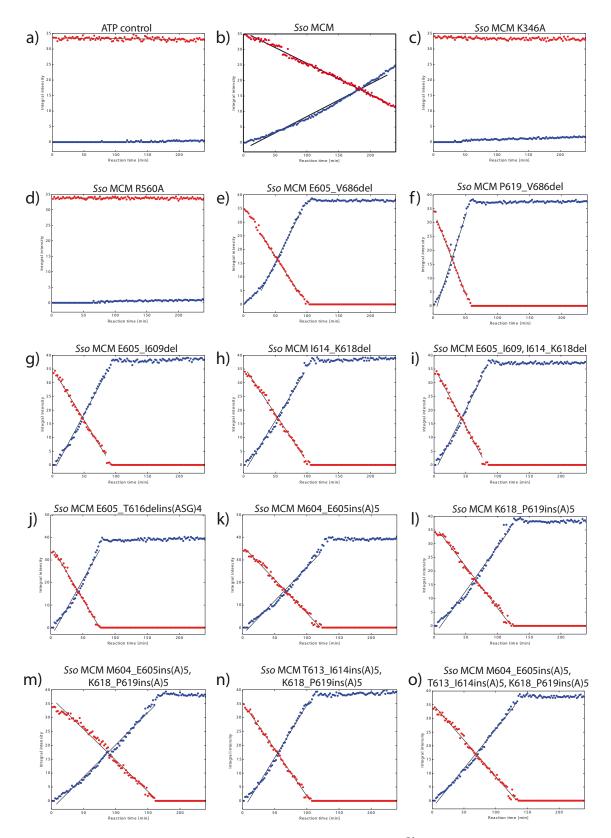


Fig. S 17: ATP hydrolysis by *Sso* MCM constructs. Time series of  $[^{31}P]$ -NMR spectra were recorded at 60°C. ³¹P chemical shifts at 60°C were referenced relative to 75 % H₃PO₄ at 0 ppm. Spectra were processed using Bruker software TOPSPIN V2.1. The integrated peak areas of the free phosphate (red dots) and the  $\beta$ -phosphate of ATP (blue dots) are plotted as a function of the reaction time. Results of the regression analysis for increase of free inorganic phosphate and decrease of  $\beta$ -phosphate of ATP given as black line.

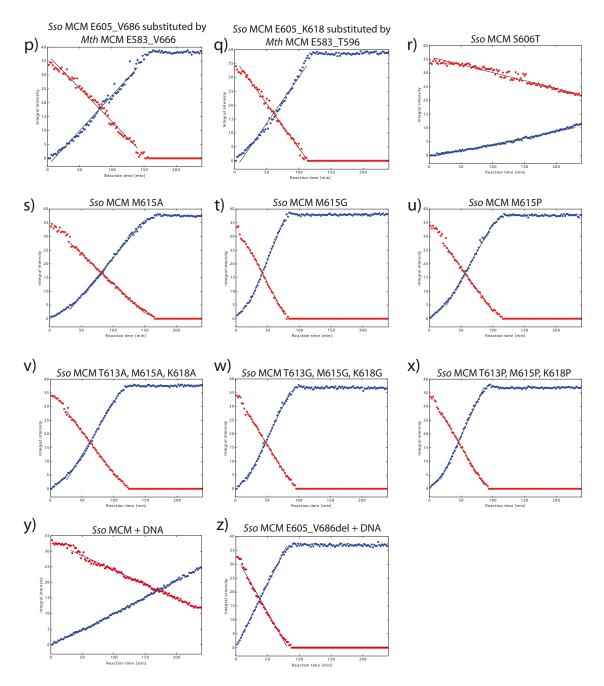


Fig. S 17: ATP hydrolysis by Sso MCM constructs continued.

$Sso MCM construct^{a}$	free $phosphate^{b}$	ATP $\beta$ -phosphate ^b	k _{cat}
	[mM/min]	[mM/min]	$[\min^{-1}]$
wild type (full length)	0.11	-0.10	$2 \pm 0.6^{c}$
K346A	0.02	-0.01	0.2
R560A	0.01	0.00	0.0
$E605_V686del$	0.46	-0.36	$7.2 {\pm} 0.5$
$P619_V686del$	0.71	-0.56	11.2
$ m E605_I609del$	0.42	-0.38	7.6
I614 K618 del	0.38	-0.34	6.8
$E605_{I609del}, I614_{K618del}$	0.46	-0.43	8.6
$E605_T616$ delins(ASG)4	0.50	-0.48	9.6
$M604_E605ins(A)5$	0.29	-0.29	5.8
$K618_{P619ins(A)5}$	0.32	-0.30	6.0
$M604_E605ins(\overline{A})5, K618_P619ins(A)5$	0.23	-0.22	4.4
T613_I614ins(A)5, K618_P619ins(A)5	0.37	-0.33	6.6
$\begin{array}{c} M604_E605ins(A)5,\ T613_I614ins(A)5,\\ K618_P619ins(A)5 \end{array}$	0.28	-0.27	5.4
substitute $E605_V686$ by <i>Mth</i> MCM $E583$ V666	0.25	-0.24	4.8
substitute $E605$ K618 by <i>Mth</i> MCM E583 T596	0.33	-0.31	6.1
$\overline{S606T}$	0.05	-0.06	1.2
M615A	0.28	-0.22	4.4
M615G	0.56	-0.44	8.8
M615P	0.41	-0.32	6.4
T613A, M615A, K618A	0.38	-0.30	6.0
T613G, M615G, K618G	0.48	-0.37	7.4
T613P, M615P, K618P	0.49	-0.39	7.8
wild type $+ \text{ DNA}^{d}$	0.11	-0.09	1.8
$ m E605_V686del + DNA^d$	0.45	-0.40	8.0

Table S 8: ATP hydrolysis of the Sso MCM constructs under saturating substrate concentrations

 $^{\rm a}$  nomenclature for the description of sequence variations as recommended [1]  $^{\rm b}$  slope of the regression line

^c standard deviation from three independent experiments
 ^d assay in the presence of 3-fold molar excess of sonicated pUC19-derived DNA (fragment size in the range of 80-160 bp)

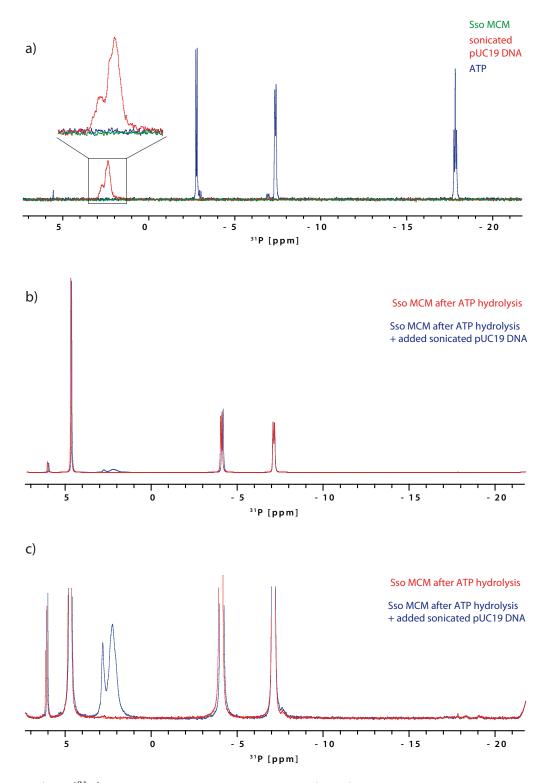


Fig. S 18: a) 1D [³¹P]-NMR spectra of purified *Sso* MCM (green), sonicated pUC19-derived DNA with a fragment size in the range of 80-160 bp (red) and ATP buffered to pH 7.2 (blue). Spectra were recorded at 600 MHz with a spectral width of 7042 Hz sampled over 1228 complex points in the  $\omega_1$  (³¹P) dimension. For both purified *Sso* MCM (green) and sonicated pUC19 derivate DNA (red) 60000 transients in the ³¹P-dimension were sampled. The ATP spectra was recorded with 40960 transients. b) [³¹P]-NMR spectra of *Sso* MCM after complete hydrolysis of ATP into ADP and free inorganic phosphate without DNA (red) and in the presence of DNA (blue). Spectral parameters same as in a) with 30000 transients in the ³¹P-dimension. c) Zoomed spectral region of b). The ³¹P radio frequency carrier was set at -10.5 ppm. Recycle time was 1 s and temperature was 60°C. ³¹P chemical shifts at 60°C were referenced relative to 75 % H₃PO₄ at 0 ppm. Concentrations of *Sso* MCM protein, ATP and sonicated pUC19 derivate DNA were 50  $\mu$ M, 50 mM and 150  $\mu$ M, respectively.

## DNA helicase activity of Sso MCM derivatives

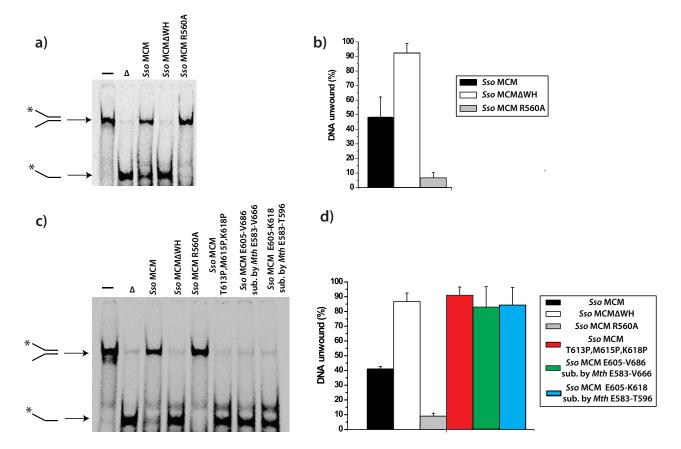


Fig. S 19: a, c) Helicase assays were performed with 2 nM 5'-labeled Y-shaped DNA substrate and 1  $\mu$ M of indicated *Sso* MCM constructs. After incubation for 30 min at 70°C, the reaction mixtures were cooled to 0°C and 10  $\mu$ l of helicase loading dye was added. The samples were electrophoresed through a 8% non-denaturing polyacrylamide gels in TBE buffer. Subsequently, gels were dried and exposed to a phosphoimaging screen. Gels were visualized using a phosphoimager (Typhoon Trio; GE Healthcare).  $\Delta$  refers to boiled DNA substrate (no protein). b, d) The bar graphs represents the percentage of unwound DNA for individual *Sso* MCM mutants as calculated using the Image Quant software. The mean standard error of three independent experiments is indicated.

### **Docking approach**

#### Paramagnetic relaxation enhancement

Proton relaxation rates  $(R_1)$  were obtained from a series of saturation-recovery  $[{}^{1}H, {}^{15}N]$ -HSQC experiments. Spectra were recorded at 30°C on a Bruker 750 MHz AvanceIII NMR system (5 mm triple resonance cryo-probe) with spectral widths of 8710 Hz sampled over 1024 complex points in the  $\omega_2$  ( ${}^{1}H$ ) dimension, and 2280 Hz over 256 complex points in the  $\omega_1$  ( ${}^{15}N$ ) dimension with 8 scans per increment in the indirect dimension. Data sets were acquired with recovery delays of 0.05, 0.1, 0.2, 0.5, 1, 1.5, 2, 4 and 6 s. The  ${}^{1}H$  and  ${}^{15}N$  radio frequency carriers were set at 4.69 ppm and 116 ppm, respectively. For determination of  ${}^{1}H$  solvent PREs, a stock solution of 0.5 M Gd(DTPA-bis(methylamide)) (Omniscan^{$\mathbb{M}$}, GE Healthcare) was added stepwise to a final concentrations of 0, 2, 4, 6, 8 and 10 mM. Spectra were processed using NMRpipe [21] and analyzed with CCPNmr Analysis [22]. Peak intensities were fitted using PREdator [23] to

$$I = I_0 (1 - e^{-R_1 t}).$$

 $I_0$  is the intensity after an infinite recovery delay, t is the recovery time and  $R_1$  is the longitudinal relaxation rate. PRE is defined as the increase in relaxation rate upon addition of a paramagnetic co-solvent. Here, PREs are represented by the slope of  $R_1$  as function of the concentration of Gd(DTPA-bis(methylamide). For a detailed description of PRE calculation, see [24–26].

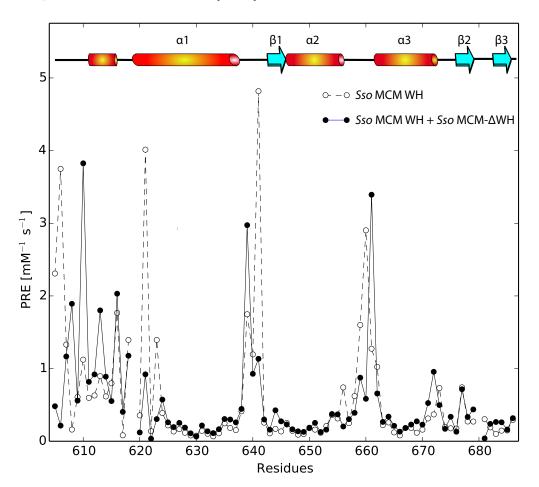


Fig. S 20: Paramagnetic relaxation enhancement (PRE) determined for the backbone amide protons of the *Sso* MCM C-terminal domain. PREs were measured for the *Sso* MCM C-terminal domain (100  $\mu$ M) in absence (dashed line) and presence (solid line) of a 3-fold molar excess of *Sso* MCM $\Delta$ WH. Secondary structure elements of the *Sso* MCM C-terminal domain NMR solution structure are indicated.

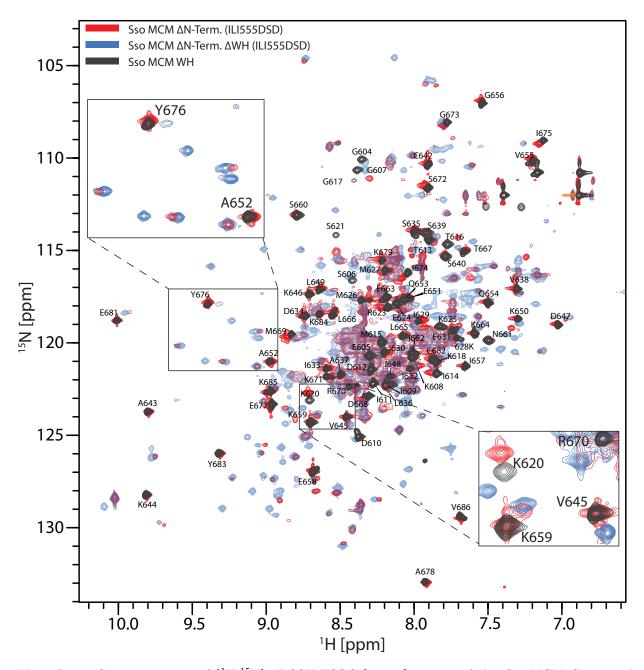


Fig. S 21: Superimposition of  $[{}^{1}\text{H}, {}^{15}\text{N}]$ -TROSY-HSQC [27, 28] spectra of the *Sso* MCM C-terminal domain (gray), *Sso* MCM $\Delta$ N-term (ILI555DSD) (red) and *Sso* MCM $\Delta$ N-term $\Delta$ WH (ILI555DSD) (blue). Spectra were recorded at 750 MHz with spectral widths of 9014 Hz sampled over 900 complex points in the  $\omega_2$  ( ${}^{1}\text{H}$ ) dimension, and 2432 Hz over 150 complex points in the  $\omega_1$  ( ${}^{15}\text{N}$ ) dimension with 32 scans for each increment in the indirect dimension. The  ${}^{1}\text{H}$  and  ${}^{15}\text{N}$  radio frequency carriers were at 4.74 ppm and 119.0 ppm, respectively. Recycle time was 2.5 s and temperature was 30°C. Protein concentrations were in the 300  $\mu$ M range.The assignment for the *Sso* MCM C-terminal domain is given.

Table S 9: Statistics of the HADDOCK docking run

HADDOCK score	$\mathrm{E}_{\mathrm{electrostatic}}$	$E_{\rm van-der-Waals}$	Buried surface area	Cluster size best (vs. total)
$0.1 \pm 5.4$	$-621.4 \pm 57.0$	$-26.8\pm9.8$	$1762.1 \pm 335.4$	134(191)

The program High Ambiguity Driven Protein-Protein Docking (HADDOCK) was used to generate a docked structure of *Sso* MCM including the C-terminal WH domain. Residues which are in close proximity to the potential domain interface were defined as active interacting residues (605, 606, 615, 616, 617, 620, 621, 623, 625, 626). Surrounding residues considered indirectly involved in or affected by the domain interaction were defined as passive interacting residues (673 and 674). Residue G601 of *Sso* MCM (PDB: 3F9V) was defined as active because it represents the most C-terminal residue of the AAA+ domain preceding the most N-terminal residue (V602) of the C-terminal WH domain. No further restrictions and structural assumptions were applied. HADDOCK clustered 191 structures into 9 clusters, which represent 95.5 % of the water-refined models generated by HADDOCK.

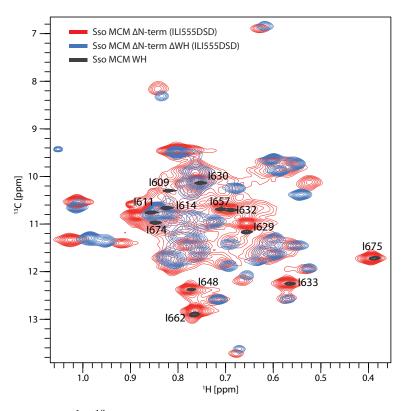


Fig. S 22: Superimposition of [¹H,¹³C]-HSQC spectra of the *Sso* MCM C-terminal WH domain (black), *Sso* MCM $\Delta$ N-term (ILI555DSD) (red) and *Sso* MCM $\Delta$ N-term $\Delta$ WH (ILI555DSD) (blue). In all samples the methyl groups of isoleucines are selectively labelled. For selective labeling of isoleucine methyl groups cells were grown in M9 media prepared in D₂O and supplemented with methyl-¹³C, 3,3-D₂  $\alpha$ -ketobutyric acid (10 mg/100 ml) and ¹²C-glucose as additional carbon source. The [¹H,¹³C]-HSQC spectra were recorded at 750 MHz with spectral widths of 6009 Hz sampled over 600 complex points in the  $\omega_2$  (¹H) dimension, and 1886 Hz over 100 complex points in the  $\omega_1$  (¹³C) dimension with 32 scans for each increment in the indirect dimension. The ¹H and ¹³C radio frequency carriers were at 3.76 ppm and 10 ppm, respectively. Recycle time was 2.5 s. Protein concentrations were in the 300  $\mu$ M range.

### **References Supplementary Data**

- J T den Dunnen and S E Antonarakis. Nomenclature for the description of human sequence variations. Human genetics, 109(1):121–124, July 2001.
- [2] Mariarita De Felice, Valentina Aria, Luca Esposito, Mariarosaria De Falco, Biagio Pucci, Mosè Rossi, and F Pisani. A novel DNA helicase with strand-annealing activity from the crenarchaeon Sulfolobus solfataricus. *Biochem. J*, 408:87–95, 2007.
- [3] Matthew J Moreau, Adam T McGeoch, Alan R Lowe, Laura S Itzhaki, and Stephen D Bell. ATPase Site Architecture and Helicase Mechanism of an Archaeal MCM. *Molecular Cell*, 28(2):304–314, October 2007.
- [4] Christoph Wiedemann, Peter Bellstedt, and Matthias Görlach. CAPITO—a web server-based analysis and plotting tool for circular dichroism data. *Bioinformatics (Oxford, England)*, 29(14):1750–1757, 2013.
- [5] Narasimha Sreerama and Robert W Woody. Estimation of Protein Secondary Structure from Circular Dichroism Spectra: Comparison of CONTIN, SELCON, and CDSSTR Methods with an Expanded Reference Set. Analytical biochemistry, 287(2):252–260, December 2000.
- [6] W Kabsch and C Sander. Dictionary of protein secondary structure: pattern recognition of hydrogenbonded and geometrical features. *Biopolymers*, 22(12):2577–2637, December 1983.
- [7] R M Venable and R W Pastor. Frictional models for stochastic simulations of proteins. *Biopolymers*, 27(6):1001–1014, June 1988.
- [8] S Meiboom and D Gill. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. Review of Scientific Instruments, 29(8):688, 1958.
- [9] L E Kay, D A Torchia, and A Bax. Backbone dynamics of proteins as studied by 15N inverse detected heteronuclear NMR spectroscopy: application to staphylococcal nuclease. *Biochemistry*, 28(23):8972–8979, November 1989.
- [10] Shenggen Yao, Jeffrey J Babon, and Raymond S Norton. Protein effective rotational correlation times from translational self-diffusion coefficients measured by PFG-NMR. *Biophysical Chemistry*, 136(2-3):145–151, August 2008.
- [11] A Ortega, D Amorós, and J García de la Torre. Prediction of hydrodynamic and other solution properties of rigid proteins from atomic- and residue-level models. *Biophysical journal*, 101(4):892–898, August 2011.
- [12] RomanA Laskowski, J AntoonC Rullmann, MalcolmW MacArthur, Robert Kaptein, and JanetM Thornton. AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. Journal of Biomolecular NMR, 8(4):477–486, December 1996.
- [13] Christoph Wiedemann, Oliver Ohlenschläger, Barbara Medagli, Silvia Onesti, and Matthias Görlach. (1)H, (15)N and (13)C chemical shift assignments for the winged helix domains of two archeal MCM C-termini. *Biomolecular NMR assignments*, 8(2):357–360, October 2014.
- [14] Liisa Holm and Päivi Rosenström. Dali server: conservation mapping in 3D. Nucleic Acids Research, 38(Web Server issue):W545–9, July 2010.
- [15] Nicholas Chia, Isaac Cann, and Gary J Olsen. Evolution of DNA replication protein complexes in eukaryotes and Archaea. PLoS ONE, 5(6):e10866, 2010.
- [16] Robert C Edgar. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC bioinformatics, 5:113, August 2004.

- [17] R C Edgar. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5):1792–1797, March 2004.
- [18] H McWilliam, W Li, M Uludag, S Squizzato, Y M Park, N Buso, A P Cowley, and R Lopez. Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Research*, 41(W1):W597–W600, June 2013.
- [19] Francine B Perler. InBase: the intein database. Nucleic Acids Research, 30(1):383–384, 2002.
- [20] Konstantin Okonechnikov, Olga Golosova, Mikhail Fursov, and UGENE team. Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics (Oxford, England)*, 28(8):1166–1167, April 2012.
- [21] F Delaglio, S Grzesiek, G W Vuister, G Zhu, J Pfeifer, and A Bax. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *Journal of Biomolecular NMR*, 6(3):277–293, November 1995.
- [22] Wim F Vranken, Wayne Boucher, Tim J Stevens, Rasmus H Fogh, Anne Pajon, Miguel Llinas, Eldon L Ulrich, John L Markley, John Ionides, and Ernest D Laue. The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins: Structure, Function, and Bioinformatics*, 59(4):687–696, June 2005.
- [23] Christoph Wiedemann, Peter Bellstedt, and Matthias Görlach. PREdator: a python based GUI for data analysis, evaluation and fitting. Source Code for Biology and Medicine, 9(21):1–4, 2014.
- [24] Michal Respondek, Tobias Madl, Christoph Göbl, Regina Golser, and Klaus Zangger. Mapping the Orientation of Helices in Micelle-Bound Peptides by Paramagnetic Relaxation Waves. *Journal of the American Chemical Society*, 129(16):5228–5234, April 2007.
- [25] Tobias Madl, Wolfgang Bermel, and Klaus Zangger. Use of Relaxation Enhancements in a Paramagnetic Environment for the Structure Determination of Proteins Using NMR Spectroscopy. Angewandte Chemie International Edition, 48(44):8259–8262, September 2009.
- [26] Tobias Madl, Thomas Güttler, Dirk Görlich, and Michael Sattler. Structural Analysis of Large Protein Complexes Using Solvent Paramagnetic Relaxation Enhancements. Angewandte Chemie International Edition, 50(17):3993–3997, March 2011.
- [27] K Pervushin, R Riek, G Wider, and K Wüthrich. Attenuated T2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. *Proceedings of the National Academy of Sciences*, 94(23):12366– 12371, November 1997.
- [28] R Riek, G Wider, K Pervushin, and K Wüthrich. Polarization transfer by cross-correlated relaxation in solution NMR with very large molecules. *Proceedings of the National Academy of Sciences*, 96(9):4918–4923, April 1999.