

## SUPPLEMENTARY INFORMATION

### **The homodimerization domain of the StI repressor is crucial for efficient inhibition of mycobacterial dUTPase**

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## Supplemental note

Recently we have shown, that the experimentally correct setup of BLI studies require that Stl protein is immobilised on the sensor<sup>1</sup>. Table 2 in the main text shows the data for this experiment. As a comparison we also performed the BLI experiment in the setup where MtDUT is immobilised. Comparing data in Table 2 and Table S2 clearly indicates that dimerization of Stl<sup>WT</sup> interferes with the determination of the binding parameters.

## Supplemental tables

**Table S1. List of primers used for cloning and mutagenesis**

Name	Sequence (5' - 3')
Stl <sup>NT</sup> _FW	CGAAAAAAGCTAAATTA ACTATGAAACCCTGGCGAACACC
Stl <sup>NT</sup> _Rev	CATAGTTAATTTAGCTTTTTTCGGTATCGGTGCTCAGAATCTG
avi-MtDUT_FW	TATGCTCGAGTATGGGCAGCAGCCATCATC
avi-MtDUT_Rev	GCAGGTACCTCACAACTCGCATGTCCG
Stl-avi-mut-F-fin	GGCTCAGAAAATCGAATGGCACGAATAACTCGAGCGGCCGCAT
Stl-avi-mut-R-fin	TCGAAGATGTCGTTTCAGGCCGGACATGTTGGTATCTTTTTCCAGA ATAATTTTTTCTGATGTTC
GST-Stl-avi-pan4-F	TATTCTCGAGTATGTCCCCTATACTAGGTTATTGG
GST-Stl-avi-pan4-R	GTCCGGTACCTATTCGTGCCATTCGATTTTCTG
Stl <sup>NT</sup> -avi_F	ATGTCCGGCCTGAACGAC
Stl <sup>NT</sup> -avi_R	GCTTTTTTTCGGTATCGGTGC

**Table S2. BLI interaction analysis of sensor bound MtDUT titrated with Stl.**

Ligand	Analyte	$K_D^*$ (pM)	$k_{on}$ (M <sup>-1</sup> s <sup>-1</sup> )(10 <sup>5</sup> )	$k_{off}$ (s <sup>-1</sup> )(10 <sup>-5</sup> )
MtDUT <sup>WT</sup>	Stl <sup>WT</sup>	215±2	3.94±0.01	8.44±0.08
MtDUT <sup>Δloop</sup>	Stl <sup>WT</sup>	335±3	2.66±0.02	8.90±0.04
MtDUT <sup>WT</sup>	Stl <sup>NT</sup>	Heterogenous binding		
MtDUT <sup>Δloop</sup>	Stl <sup>NT</sup>	Heterogenous binding		

\*  $\chi^2$  value was below 0.4 in all cases.

**Table S3. Data collection and refinement statistics**

Structure	MtDUT <sup>Aloop</sup>	MtDUT-Stl <sup>NT</sup>
PDB ID	8CGA	8P8O
<b>Data collection</b>		
Space group	P6 <sub>3</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	55.249, 55.249, 83.751	104.669, 123.82, 170.229
$\alpha$ , $\beta$ , $\gamma$ (°)	90.00, 90.00, 120.00	90.00, 90.00, 90.00
Resolution (Å)	47.85-1.26 (1.33-1.26) *	46.38-3.40 (3.50-3.40)
<i>R</i> <sub>meas</sub>	0.04488 (0.4152)	0.156 (6.237)
<i>I</i> / $\sigma$ <i>I</i>	14.18 (2.69)	13.92 (0.48)
Completeness (%)	99.96 (99.97)	97.50 (93.20)
Redundancy	2.0 (2.0)	5.24 (5.21)
CC1/2	0.996 (0.763)	0.999 (0.314)
<b>Refinement</b>		
Resolution (Å)	47.85-1.30 (1.35-1.30)	46.38-3.40 (3.49-3.40)
No. unique reflections	35602 (3581)	30380 (2113)
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	0.1413 (0.1618) / 0.1707 (0.2127)	0.2439 (0.5497) / 0.2856 (0.5733)
No. atoms		
Protein	1074	11820
Ligand/ion	48	0
Water	117	0
<i>B</i> -factors		
Protein	14.33	145.65
Ligand/ion	13.18	145.65
Water	13.26	-
R.m.s deviations		
Bond lengths (Å)	25.29	-
Bond angles (°)	0.007	0.002
	1.03	0.48

\* Values for the highest resolution shell are shown in parentheses

**Table S4. MtDUT<sup>WT</sup>-Stl<sup>NT</sup> complex polar interactions**

MtDUT <sup>WT</sup>		Stl <sup>NT</sup>		Binding type
Residue	Atom type	Residue	Atom type	
His21	NE2	Asn102	OD1	Hydrogen bond
	ND1	Tyr106	OH	
Asp24	OD1			
	OD2			
Arg64	NH1	Ser115	O	
	NH2	Tyr112	O	
Ser65	OG			
Thr69	OG1	Ser114	O	
Arg70	NH1			
		Thr81	O	
Asp83	N			
Arg87	N	Tyr106	O	
Lys91	N	Tyr113	OH	
	NZ	Asp110	OD1	Salt bridge
Arg110	NH1	Asp117	OD1	Hydrogen bond
		Tyr116	O	
	NH2	Asp117	OD1	Salt bridge
				Hydrogen bond
Glu126	O	Asn56	ND2	Hydrogen bond
	N		OD1	

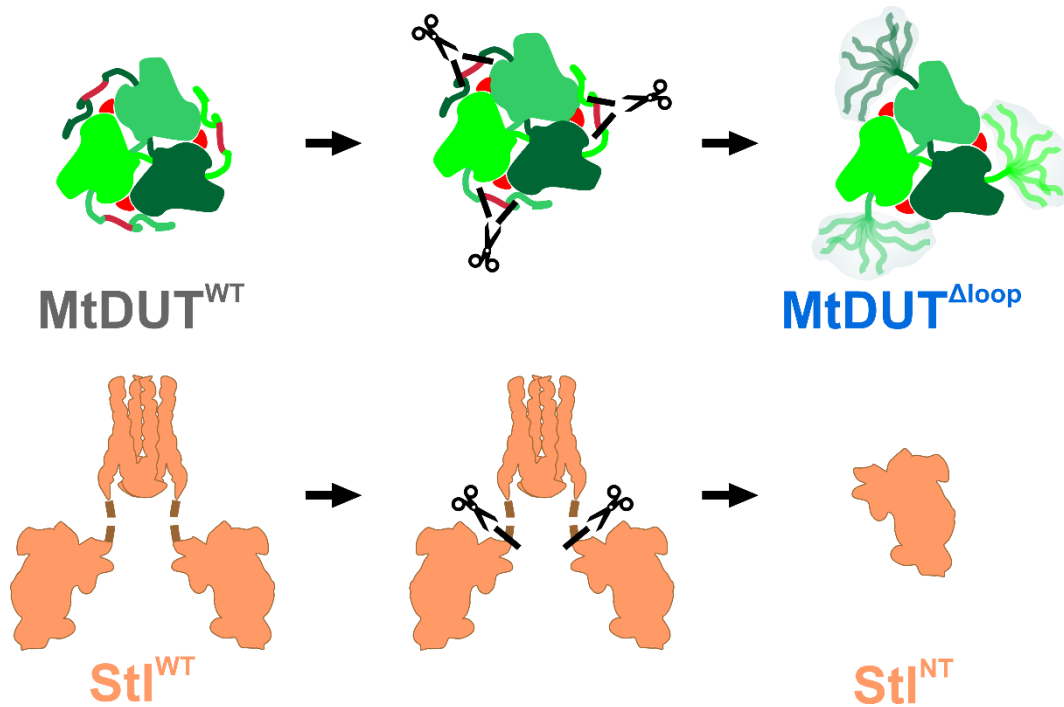
## Supplemental figures

	Motif I	Motif II	Motif III	
<i>C. burnetii</i>	-----MTHSVQLKILDKRLGSEFELPAYATRGSA <u>AGLDL</u> RACLDEPLKIEPDEETCLISTGLAIYLGHSNVAATIL <u>PRSG</u> LGHKHGIVLGNLVGLIDSDYQGELMV			99
<i>Y. pestis</i>	-----MKKIDIKILDPRVGNFELPYATRGSA <u>AGLDL</u> RACLDHAVELQPGQTLLPFGLAIHIGDSALAAVIL <u>PRSG</u> LGHKHGIVLGNLVGLIDSDYQGQLMV			98
<i>E. coli</i>	-----MMKKLDVKILDRPVGKEFELPYATRGSA <u>AGLDL</u> RACLNDAVELAPGDTLLVPTGLAIHIADPSLAAMML <u>PRSG</u> LGHKHGIVLGNLVGLIDSDYQGQLMI			99
<i>S. paratyphi</i>	-----MMKKLDVKILDRPVGQGFELPYATRGSA <u>AGLDL</u> RACLDVAELAPGATTLVPTGLAIHIADPSLAAMML <u>PRSG</u> LGHKHGIVLGNLVGLIDSDYQGQLMV			99
Human	MPCSEETPAISPSKRARPAEVGGMQLRFAR-----LSEHATAPTRGSA <u>AGLDL</u> YSA--YDVTIPMEKAVVKTDIQIALPSCG-YGRVA <u>PRSG</u> LAAKHFIDV--GAGVIDEDYRGNVGV			111
<i>H. pylori</i>	-----MKIKIQK-----IHPNALIPKYQTGSS <u>GF</u> DLHAV--EEMVIKPHSVGLVKIGICLSLEVGY-ELQV <u>TRSG</u> LALNHQVMVLSFGTVDNDYRGEIKV			90
<i>C. acnes</i>	-----MADVVFPT-----VAVPEAMPYRAMPDGA <u>ADL</u> TGR--HDVDLAPGERAMVETGVRVALPDGY-VGFVN <u>PRSG</u> LAARHGLSIVNAP <u>PTID</u> SGYRQINV			91
<i>N. guangzhouensis</i>	-----MPVALLR-----LDRDLVPVSPYAPGDA <u>ADL</u> MTT--VDVTLAPGERTLVPTGIAVALPEGY-VGLVH <u>PRSG</u> LAARHGLSIVNAP <u>PTID</u> AGYRGEIKV			90
<i>M. smegmatis</i>	-----MSTSLAVVR-----LDRELFMPTRAHGDG <u>AGVD</u> LYSA--ENVELAPGQALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LAARVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. leprae</i>	-----MSTSLAVVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSV--EDVKLAPGQALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LAARVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. ulcerans</i>	-----MSNSLAVVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSA--EDVVLPPGQALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LAARVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. marinum</i>	-----MSNSLAVVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSA--EDVVLPPGQALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LAARVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. tuberculosis</i>	-----MSTTLAIVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSA--EDVELAPGRRALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LATRVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. bovis</i>	-----MSTTLAIVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSA--EDVELAPGRRALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LATRVGLSIVNS <u>PTID</u> AGYRGEIKV			92
<i>M. avium</i>	-----MSTSLAIVR-----LDPGLELPSRAHGDG <u>AGVD</u> LYSA--EDVRLPEGRRALVSTGIAVAIPDHGM-VGLVH <u>PRSG</u> LAARVGLSIVNS <u>PTID</u> AGYRGEIKV			92
Phage $\phi$ 11	-----MTNTLQVRL-----LSENARMPERNHRTD <u>AGYD</u> IFSA--ETVVLPEQEKAVIKTDVAVSIPEGY-VGLLT <u>SRSG</u> VSSKTHLVI--ETGKIDAGYHGNLGI			90
Phage $\theta$ α	-----MTNTLQVKL-----LSKNARMPERNHRTD <u>AGYD</u> IFSA--ETVVLPEQEKAVIKTDVAVSIPEGY-VGLLT <u>SRSG</u> VSSKTHLVI--ETGKIDAGYHGNLGI			90

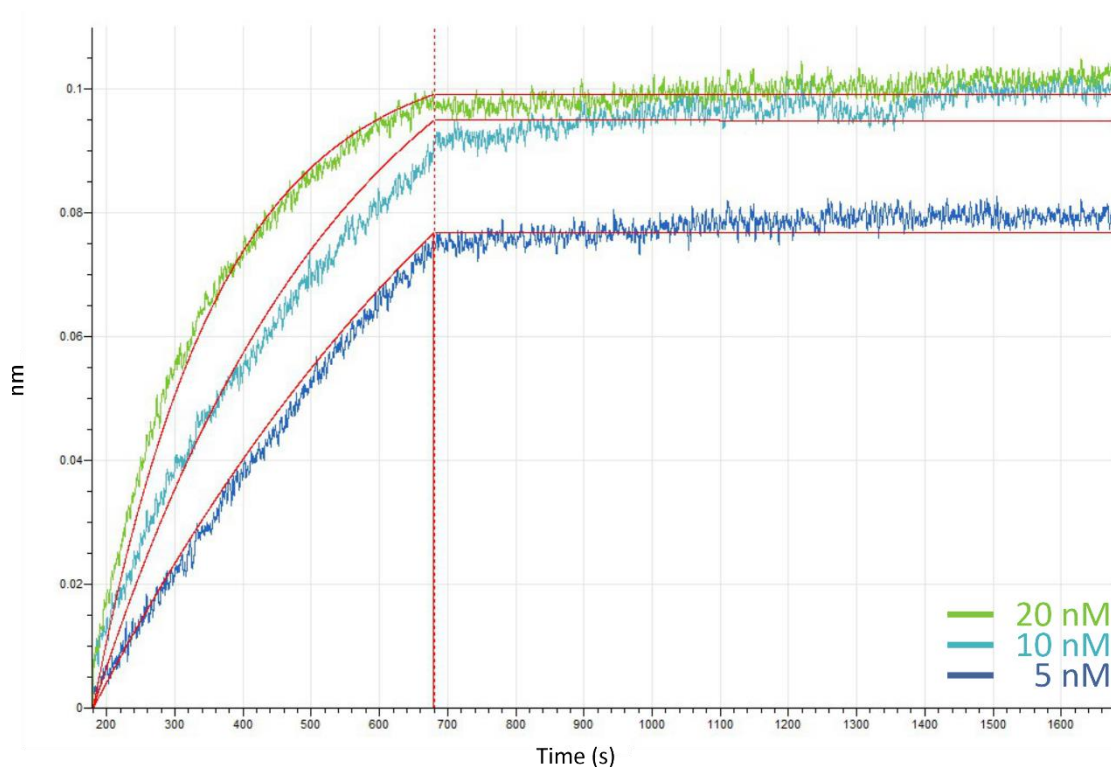
	Motif IV	Motif V	
<i>C. burnetii</i>	SCWNRGK-----EPYITNPG <u>RIAQ</u> LVVLPVILKAQFAVVEEFEL-----TERGAGGFGSSGQN-----		152
<i>Y. pestis</i>	SVWNRGQ-----QPFTIEPGE <u>RIAQ</u> MVFPVQAEFNILVEDFTD-----SERGTGGFGHSGRQ-----		151
<i>E. coli</i>	SVWNRGQ-----DSFTIQPGE <u>RIAQ</u> MFVFPVQAEFNILVEDFDA-----TDRGEGGFGHSGRQ-----		152
<i>S. paratyphi</i>	SLWNRGQ-----DSFTIEPGE <u>RIAQ</u> MVFPVQAEFNILVEAFDA-----TERCGEGFGHSGRK-----		152
Human	VLFNCK-----EKPELVK <u>RIAQ</u> LICERIFYPELIEVQALDD-----TERCSGCGFGSTGKN-----		164
<i>H. pylori</i>	ILANLSD-----KD- <u>FPYQVGR</u> <u>RIAQ</u> GVVQYTKAEPICEQLDE-----TSRSGGFGGSGVSKA-----		145
<i>C. acnes</i>	LLVNTDP-----REPVLHDAG <u>RIAQ</u> LVVFPVVEAIFEPVEDLDD-----TERGQGGYGGTGVSA <sup>MP</sup> FPVDG-----		152
<i>N. guangzhouensis</i>	CLVNLDP-----REPVLLHRGD <u>RIAQ</u> LVVQVVEQAQFLVSDSLDA-----SVRGAGCYGTCGFAGVETQRSAT		154
<i>M. smegmatis</i>	SLINLDP-----QTFVVISR <u>RIAQ</u> LVVQVVEPELVEVTSFDE <u>AGLAD</u> TRGDDGGHSGSGHASL-----		154
<i>M. leprae</i>	ALINLDP-----VEPLVVRGD <u>RIAQ</u> LVVQVVEVLELVVSSFDE <u>AGLAE</u> TSRGGGGHSGSGHASL-----		154
<i>M. ulcerans</i>	VLINLDP-----ATPIVVRGD <u>RIAQ</u> LVVQVVELELVVSSFDE <u>AGLAA</u> TSRGGGGHSGSGHASL-----		154
<i>M. marinum</i>	ALINLDP-----ATPIVVRGD <u>RIAQ</u> LVVQVVELELVVSSFDE <u>AGLAA</u> TSRGGGGHSGSGHASL-----		154
<i>M. tuberculosis</i>	ALINLDP-----AAPIVVRGD <u>RIAQ</u> LVVQVVEVLELVVSSFDE <u>AGLAA</u> TSRGGGGHSGSGHASL-----		154
<i>M. bovis</i>	ALINLDP-----AAPIVVRGD <u>RIAQ</u> LVVQVVEVLELVVSSFDE <u>AGLAA</u> TSRGGGGHSGSGHASL-----		154
<i>M. avium</i>	ALINLDP-----AEPIVVRGD <u>RIAQ</u> LVVQVVEVLELVVSSFDE <u>AGLAA</u> TSRGGGGHSGSGHASL-----		154
Phage $\phi$ 11	NIKNDALASNGY-IIPGVFDIKGEIDLSDAIRQYCTYQINEG <u>KLAQ</u> LVIVPITWTEFLKQVEEFES-----VSEERGEKGFSGSV-----		169
Phage $\theta$ α	NIKNDHEDDKM <sup>Q</sup> TEFLRNIDNEKIFEKERHLYKLSYRIKGE <u>RIAQ</u> LVIVPITWTEFLKQVEEFES-----VSEERGEKGFSGSV-----		170

**Figure S1. Sequence comparison of several trimeric dUTPase enzymes.** The conserved motifs of selected dUTPase sequences are highlighted in blue and indicated as a blue line. The mycobacteria-specific insert sequence elements are highlighted in yellow boxes. The multiple sequence alignment was performed using Clustal Omega.

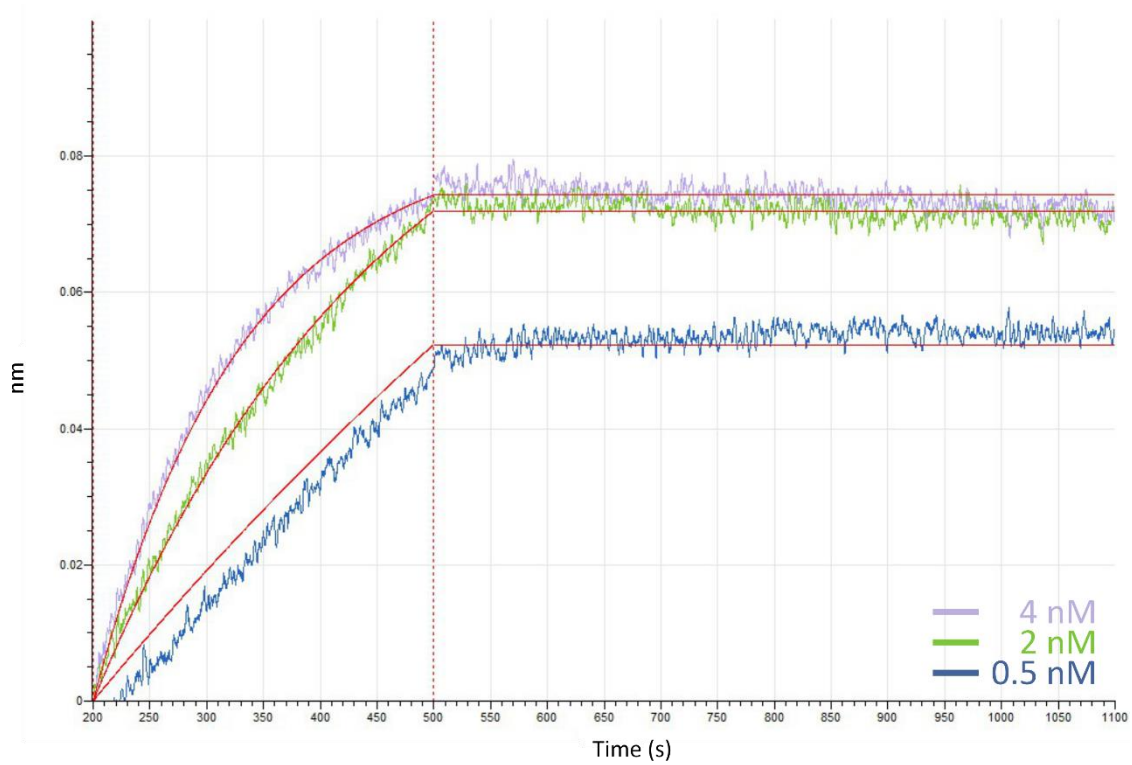


**Figure S2. Schematic figure explaining the truncation of MtdUT<sup>WT</sup> and Stl.** The deletion of the AGLAS surface loop of MtdUT<sup>WT</sup> resulting in MtdUT<sup>Δloop</sup> is shown on the top panel. MtdUT trimers are represented as green figures in three different shades (for the protomers)

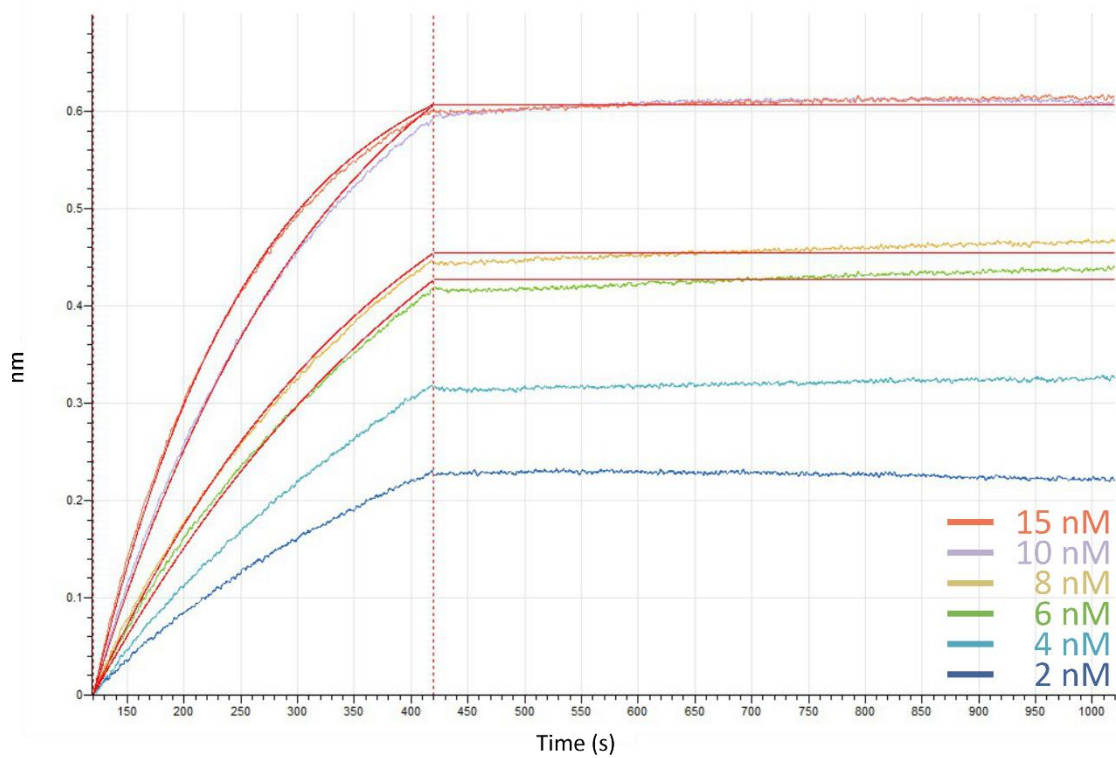
(based on crystal structures PDB ID 2PY4 and 8CGA). The C-terminal arm domains are represented as green lines, where the position of the AGLAS surface loop is highlighted in burgundy. For MtDUT<sup>Δloop</sup> the C-terminal arm domains are represented as partially transparent lines. The substrate is shown as red shape. The bottom of this panel shows the truncation of the C-terminal domain of Stl<sup>WT</sup> resulting in Stl<sup>NT</sup>. The Stl dimer is represented as a peach-colored figure, which consists of the C-terminal domain (based on crystal structure with PDB ID:6H48) (which is responsible for the dimer formation), the N-terminal domain (based on crystal structure with PDB ID:6H49) and a small hinge region<sup>2</sup> (represented as brown-coloured dashed line). Stl<sup>NT</sup> is shown as a peach-coloured figure.



**Figure S3.** BLI curves of Avi-tagged Stl<sup>WT</sup> and MtDUT<sup>WT</sup>. A 1:1 binding model has been fitted to the binding data ( $K_D < 1$  pM,  $\chi^2 = 0.194$ ).

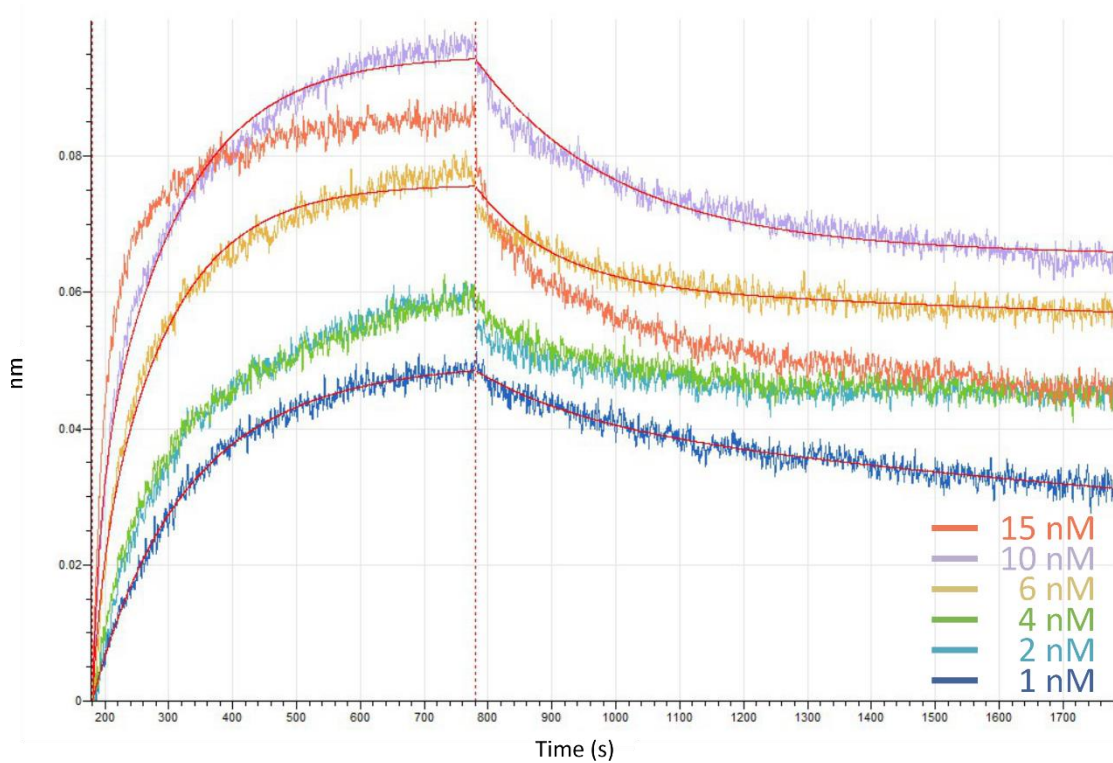


**Figure S4.** BLI curves of Avi-tagged Stl<sup>WT</sup> and MtDUT<sup>Δloop</sup>. A 1:1 binding model has been fitted to the binding data ( $K_D=33\pm 1$  pM,  $\chi^2=0.0374$ ).

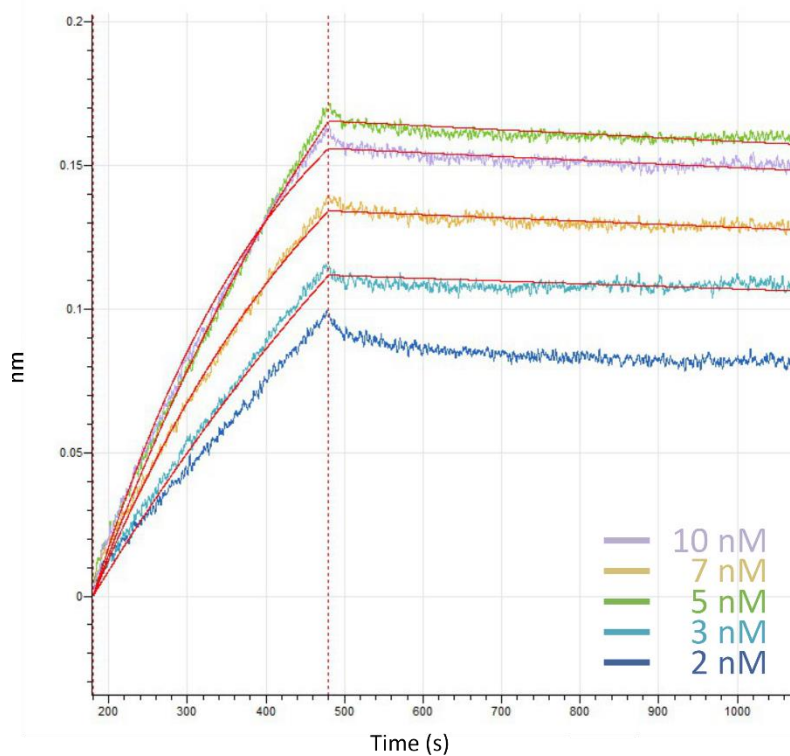


**Figure S5.** BLI curves of Avi-tagged Stl<sup>NT</sup> and MtDUT<sup>WT</sup>. A 1:1 binding model has been fitted to the binding data ( $K_D=190\pm 1$  pM,  $\chi^2=0.2024$ ).



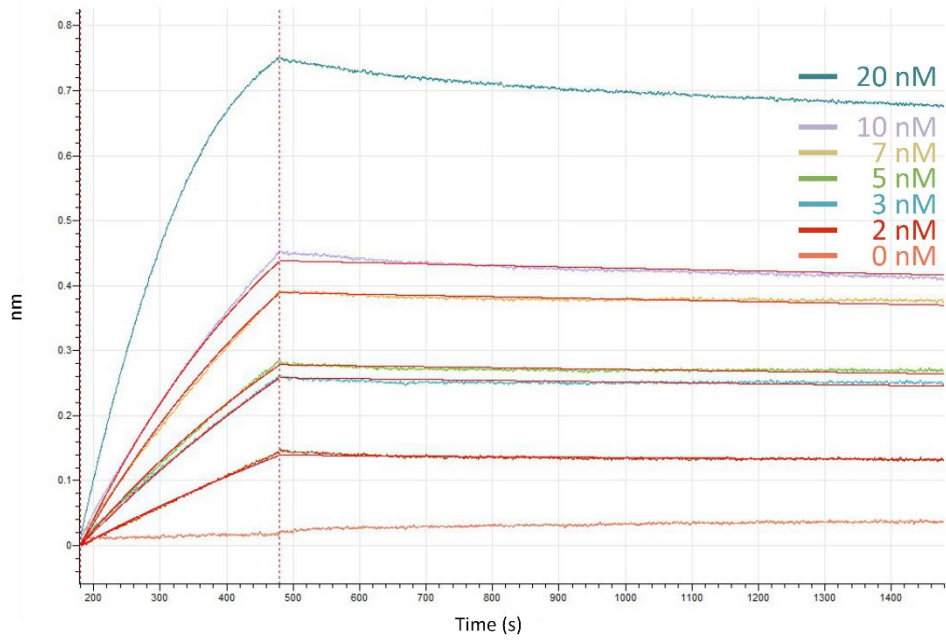


**Figure S6.** BLI curves of Avi-tagged  $Stl^{NT}$  and  $MtDUT^{Aloop}$ . The binding data suggest a case of heterogeneous binding.

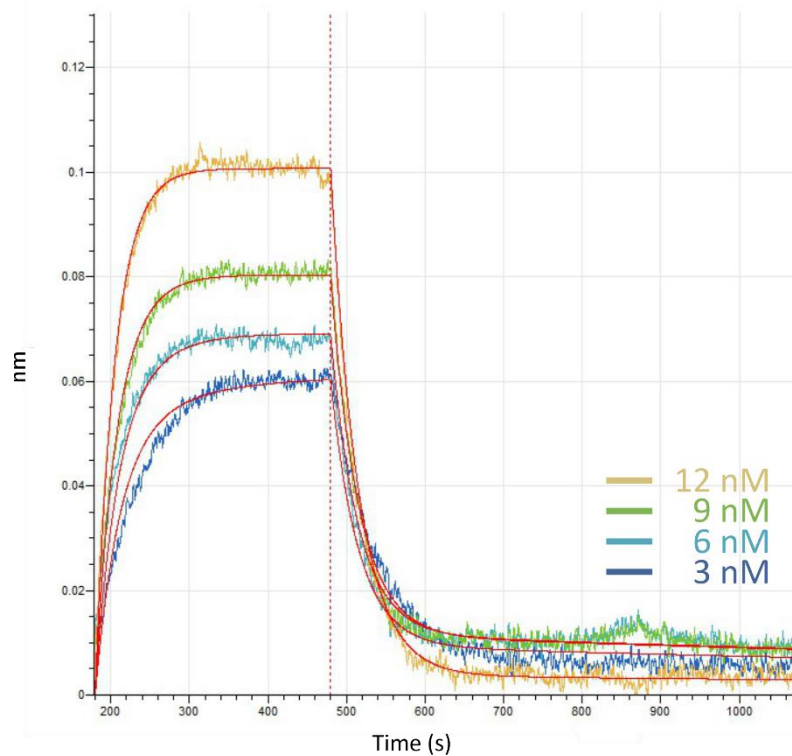


**Figure S7.** BLI curves of Avi-tagged  $MtDUT^{WT}$  and  $Stl^{WT}$ . A 1:1 binding model has been fitted to the binding data ( $K_D=215\pm 2$  pM,  $\chi^2=0.0964$ ).

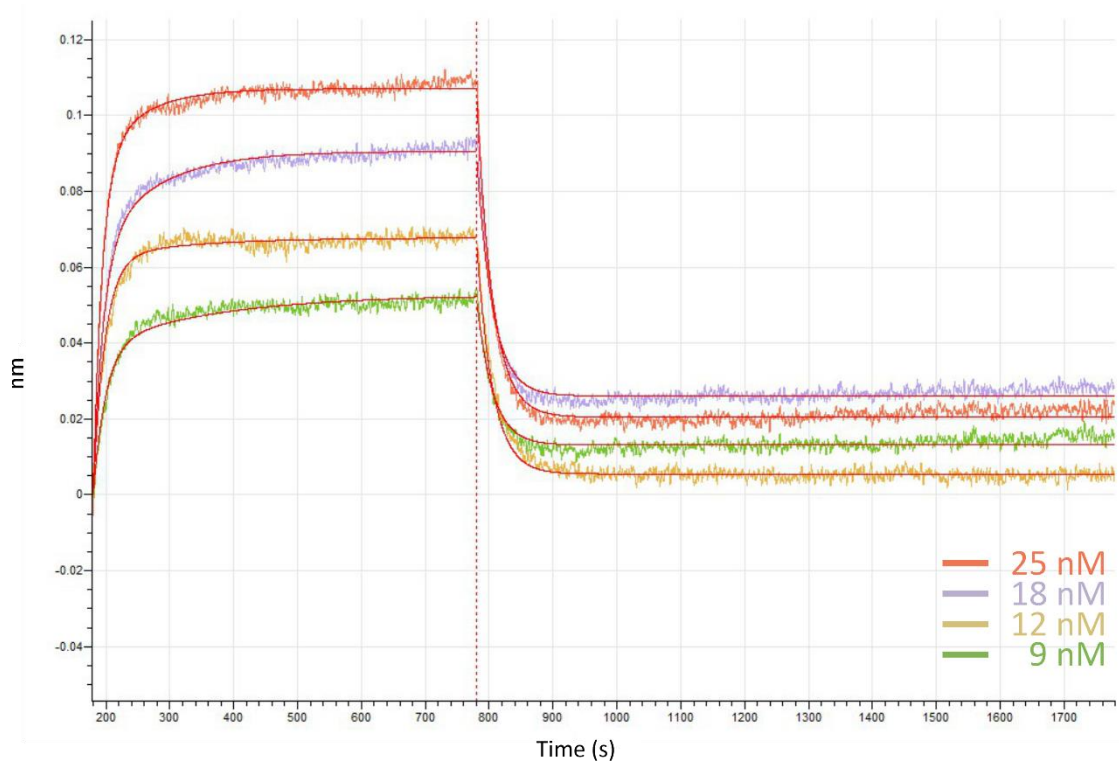




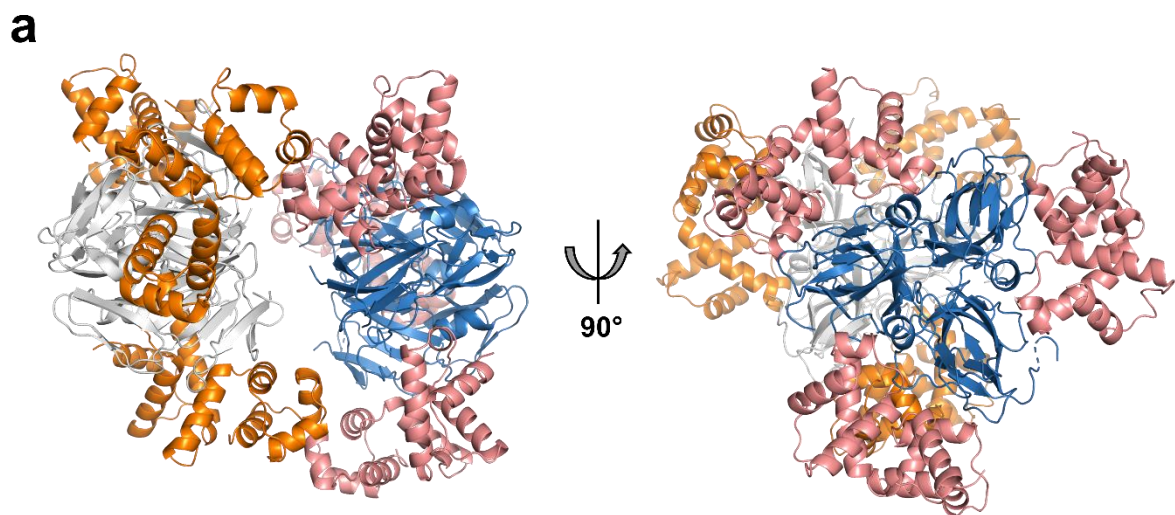
**Figure S8.** BLI curves of Avi-tagged MtDUT $\Delta$ loop and StI<sup>WT</sup>. A 1:1 binding model has been fitted to the binding data ( $K_D=335\pm 3$  pM,  $\chi^2=0.3497$ ).



**Figure S9.** BLI curves of Avi-tagged MtDUT<sup>WT</sup> and StI<sup>NT</sup>. The binding data suggest a case of heterogeneous binding.

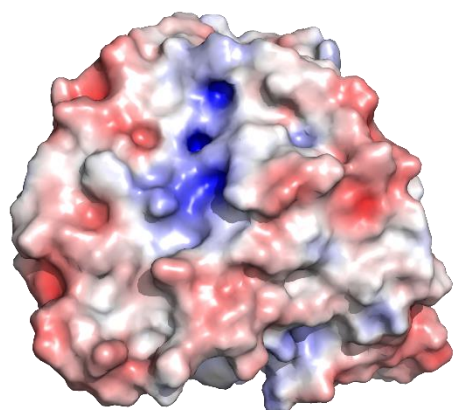


**Figure S10. BLI curves of Avi-tagged MtDUT<sup>Δloop</sup> and Stl<sup>NT</sup>.** The binding data suggest a case of heterogeneous binding.



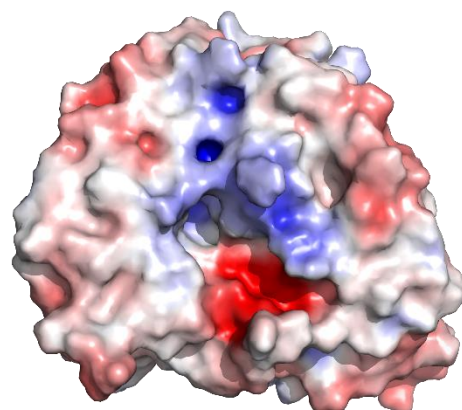
**b**

MtDUT<sup>WT</sup>  
PDB ID: 2PY4

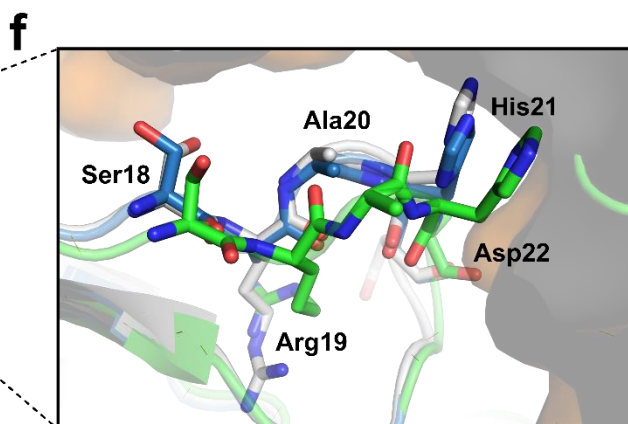
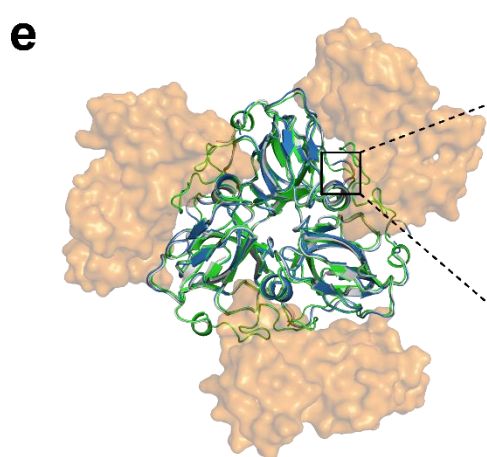


**c**

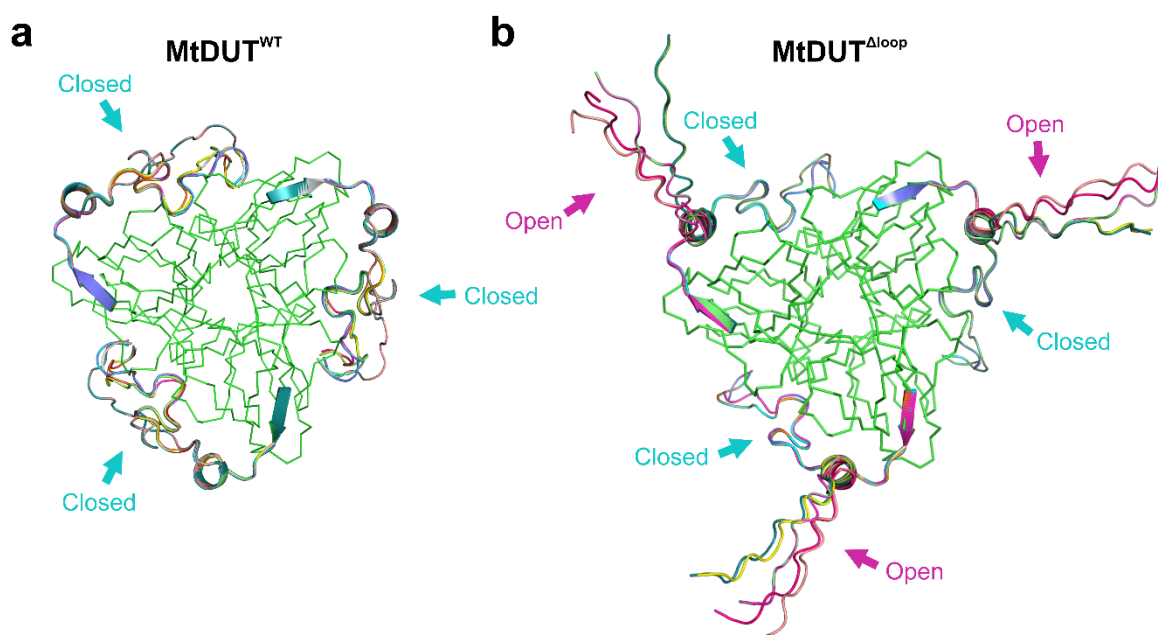
MtDUT<sup>WT</sup>  
PDB ID: 8P80



**d**

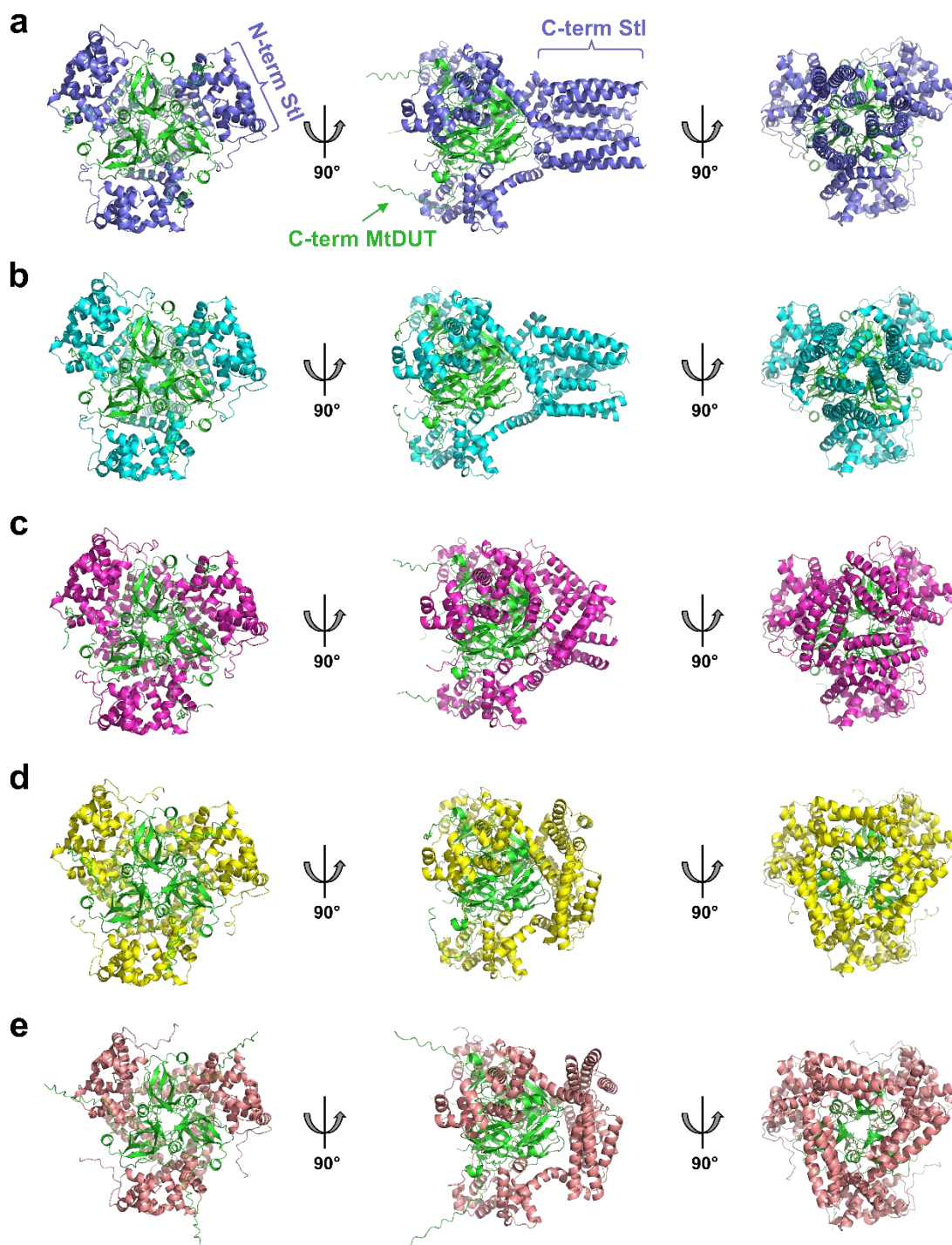


**Figure S11. Comparison of MtDUT<sup>WT</sup> structure in complex with substrate analogue and with Stl<sup>NT</sup>.** a) Composition of MtDUT<sup>WT</sup>-Stl<sup>NT</sup> structure asymmetric unit. The two MtDUT<sup>WT</sup> trimers are displayed as grey and blue cartoons, and Stl<sup>NT</sup> monomers are represented as orange and light pink cartoons. b-c) Electrostatic potential of the molecular surfaces of MtDUT<sup>WT</sup> in complex with dUPNPP substrate analogue (b) (the representation is the same as on Figure 4f) and MtDUT<sup>WT</sup> in complex with Stl<sup>NT</sup> (c). d) The colouring of the electrostatic surface potential scale. e) Overall fold comparison of MtDUT<sup>WT</sup> (PDB ID: 2PY4) and the two MtDUT<sup>WT</sup> trimers (PDB ID: 8P80) The two MtDUT<sup>WT</sup> trimers (PDB ID:8P80) share a high degree of overall similarity based on root mean square deviation (RMSD) of 0.31 Å for 362 C $\alpha$  atoms. The deviations from the substrate analogue bound structure based on RMSD values are 0.47 Å and 0.55 Å for 348 and 356 C $\alpha$  atoms, respectively. MtDUT<sup>WT</sup> (PDB ID: 2PY4) is represented as green cartoon, MtDUT<sup>WT</sup> trimers in complex with Stl<sup>NT</sup> (PDB ID: 8P80) are represented as grey and blue cartoon and Stl<sup>NT</sup> is represented as partially transparent orange surface. f) Conformational change of the N-terminal Ser18-Asp22 segment of MtDUT<sup>WT</sup> upon complex formation with Stl<sup>NT</sup>. The Ser18-Asp22 peptide segment is highlighted as sticks, the representation of molecules is the same as on panel (e). Individual panels were created using PyMOL 2.5.4 (Schrodinger, LLC; <https://www.pymol.org/>). The figure was assembled using CorelDRAW 2020 (Corel Corporation; <https://www.coreldraw.com>).



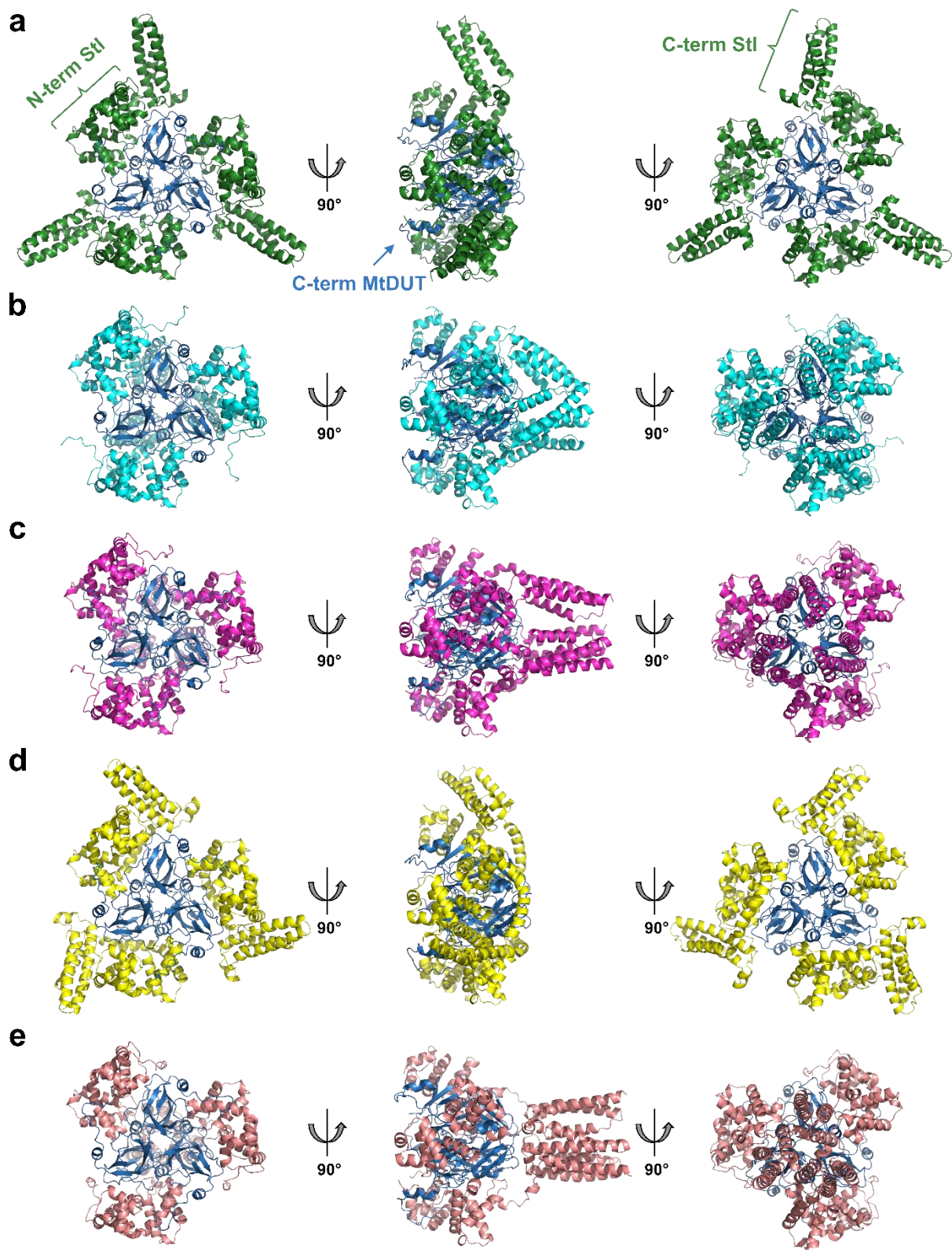
**Figure S12. Mobility analysis of C-terminal arm of MtDUT using AlphaFold.** a-b) Ten models representing possible conformations of MtDUT<sup>WT</sup> (a) and MtDUT<sup>Δloop</sup> (b) C-terminal arm. The protein core is shown as green ribbon, the C-terminal domains are shown from the last beta sheet secondary structure element and represented as multiple-coloured cartoons. The turquoise arrows indicate the closed or partially closed conformations of the C-terminal arm, while the magenta arrows indicate the open conformations of the C-terminal arm. The representation of protein structures was created using PyMOL 2.5.4 (Schrodinger, LLC; <https://www.pymol.org/>). The figure was assembled using CorelDRAW 2020 (Corel Corporation; <https://www.coreldraw.com>).





**Figure S13. AlphaFold modelling of MtDUT<sup>WT</sup>-Stl<sup>WT</sup> complex structure.**

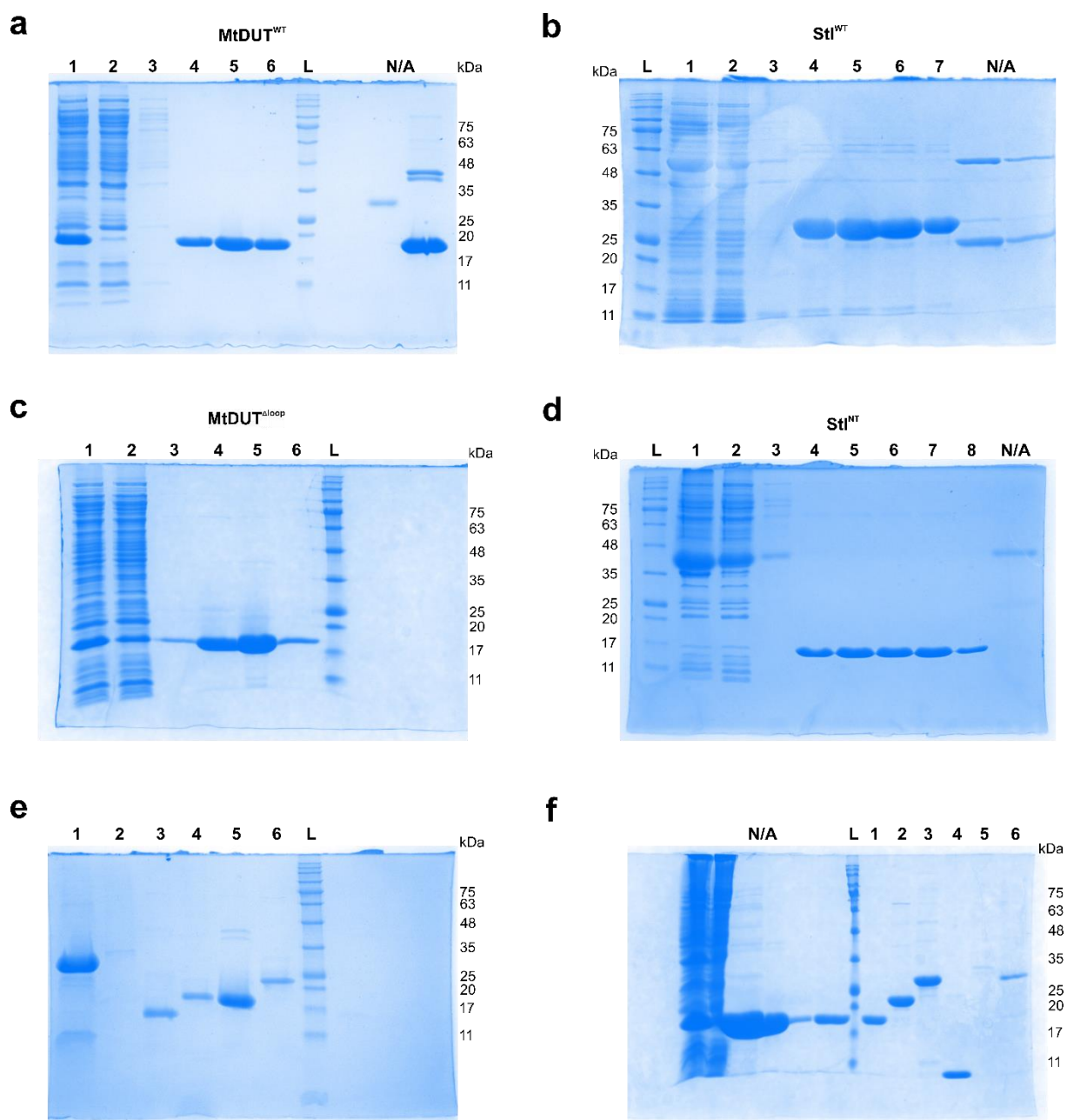
a-e) 5 MtDUT<sup>WT</sup>-Stl<sup>WT</sup> complex models showing possible complex assemblies of 3:3 binding stoichiometry. The models are shown in 3 different orientations, the MtDUT<sup>WT</sup> is displayed in green cartoon in all cases, while Stl is represented as different coloured cartoons. Predicted models are in descending order from (a) to (e) based on the prediction ranking scores. The representation of protein structures was created using PyMOL 2.5.4 (Schrodinger, LLC; <https://www.pymol.org/>). The figure was assembled using CorelDRAW 2020 (Corel Corporation; <https://www.coreldraw.com>).



**Figure S14. AlphaFold modelling of MtDUT<sup>Δloop</sup>-Stl<sup>WT</sup> complex structure.**

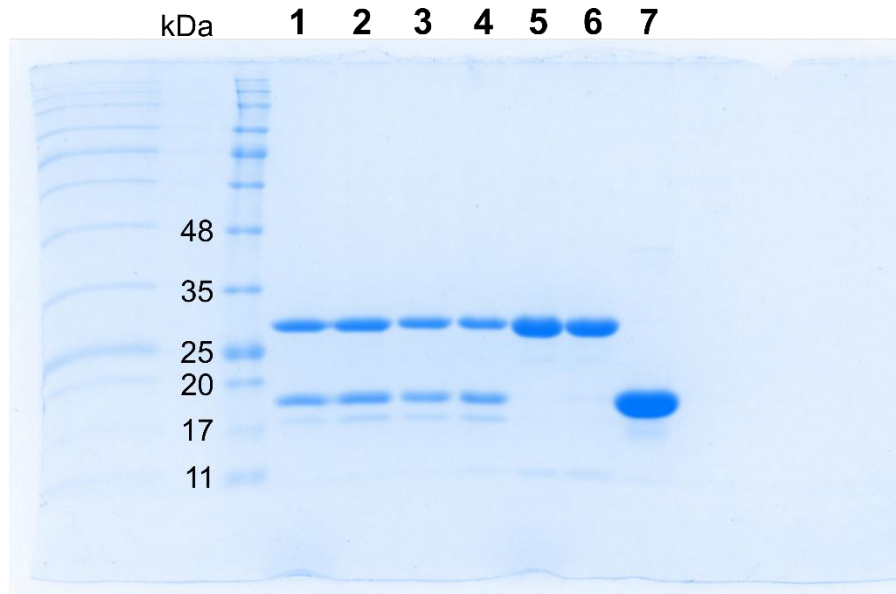
a-e) 5 MtDUT<sup>Δloop</sup>-Stl<sup>WT</sup> complex models showing possible complex assemblies of 3:3 binding stoichiometry. The models are shown in 3 different orientations, the MtDUT<sup>Δloop</sup> is displayed in dark blue cartoon in all cases, while Stl is represented as different coloured cartoons. Predicted models are in descending order from (a) to (e) based on the prediction ranking scores. The representation of protein structures was created using PyMOL 2.5.4 (Schrodinger, LLC;

<https://www.pymol.org/>). The figure was assembled using CorelDRAW 2020 (Corel Corporation; <https://www.coreldraw.com>).

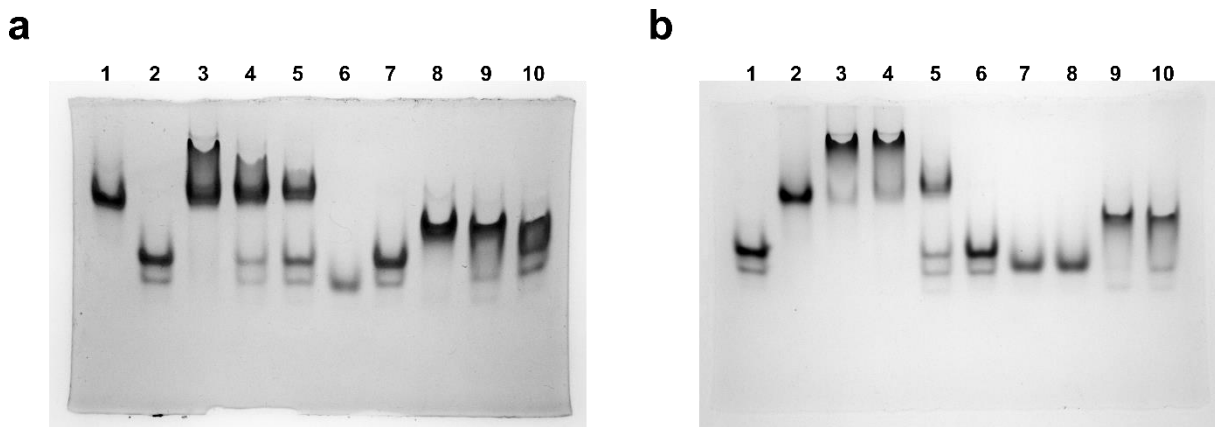


**Figure S15. SDS-PAGE images of protein preparations used in this study, purified by affinity chromatography.** a) MtDUT<sup>WT</sup> protein. 1. Supernatant fraction; 2. Flowthrough fraction; 3. Salt wash fraction; 4-6. Elution fractions; L. Protein ladder. b) Stl<sup>WT</sup> protein. L. Protein ladder; 1. Supernatant fraction; 2. Flowthrough fraction; 3. Salt wash fraction; 4-7. Elution fractions (N/A not applicable). c) MtDUT<sup>Δloop</sup> protein. 1. Supernatant fraction; 2. Flowthrough fraction; 3. Wash fraction; 4-6. Elution fractions; L. Protein ladder. d) Stl<sup>NT</sup> protein. L. Protein ladder; 1. Supernatant fraction; 2. Flowthrough fraction; 3. Wash fraction; 4-8. Elution fractions (N/A not applicable) e-f) Final protein preparations used in BLI experiments. e) 1. Stl<sup>WT</sup>; 2. Avi-tagged Stl<sup>WT</sup>; 3. Stl<sup>NT</sup>; 4. MtDUT<sup>WT</sup>; 5. MtDUT<sup>Δloop</sup>; 6. Avi-tagged MtDUT<sup>Δloop</sup>; L. Protein ladder. f) (N/A); L. Protein ladder; 1. MtDUT<sup>WT</sup>; 2. Avi-tagged MtDUT<sup>WT</sup>; 3. Stl<sup>WT</sup>; 4. N/A; 5. Avi-tagged Stl<sup>WT</sup>; 6. Avi-tagged Stl<sup>NT</sup>.





**Figure S16. SDS-PAGE image of MtDUT<sup>WT</sup>, Stl<sup>WT</sup> proteins and MtDUT<sup>WT</sup>:Stl<sup>WT</sup> protein complex analysed by size-exclusion chromatography.** 1-3. Protein samples of first peak (peak elution volume: 11.1 ml) of MtDUT<sup>WT</sup>:Stl<sup>WT</sup> complex. 4. Protein sample of second peak (peak elution volume: 13.0 ml) of MtDUT<sup>WT</sup>:Stl<sup>WT</sup> complex. 5-6. Protein samples of Stl<sup>WT</sup> peak (peak elution volume: 14.5 ml). 7. Protein sample of MtDUT<sup>WT</sup> peak (peak elution volume: 14.8 ml).



**Figure S17. Native gel electrophoresis images analysing MtDUT<sup>WT</sup>-Stl<sup>WT</sup> and MtDUT<sup>WT</sup>-Stl<sup>NT</sup> complex formation at different mixing concentrations.** a) 1. 9  $\mu$ M Stl<sup>WT</sup>; 2. 9  $\mu$ M MtDUT<sup>WT</sup>; 3. 9  $\mu$ M Stl<sup>WT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>; 4. 6  $\mu$ M Stl<sup>WT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>; 5. 3  $\mu$ M Stl<sup>WT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>; 6. 9  $\mu$ M Stl<sup>NT</sup>; 7. 9  $\mu$ M MtDUT<sup>WT</sup>; 8. 9  $\mu$ M Stl<sup>NT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>; 9. 6  $\mu$ M Stl<sup>NT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>; 10. 3  $\mu$ M Stl<sup>NT</sup> + 9  $\mu$ M MtDUT<sup>WT</sup>. b) 1. 9  $\mu$ M MtDUT<sup>WT</sup>; 2. 9  $\mu$ M Stl<sup>WT</sup>; 3. 4.5  $\mu$ M Stl<sup>WT</sup> + 4.5  $\mu$ M MtDUT<sup>WT</sup>; 4. 3.6  $\mu$ M Stl<sup>WT</sup> + 5.4  $\mu$ M MtDUT<sup>WT</sup>; 5. 2.25  $\mu$ M Stl<sup>WT</sup> + 6.75  $\mu$ M MtDUT<sup>WT</sup>; 6. 9  $\mu$ M MtDUT<sup>WT</sup>; 7. 9  $\mu$ M Stl<sup>NT</sup>; 8. 9  $\mu$ M Stl<sup>NT</sup>; 9. 4.5  $\mu$ M Stl<sup>NT</sup> + 4.5  $\mu$ M MtDUT<sup>WT</sup>; 10. 3.6  $\mu$ M Stl<sup>NT</sup> + 5.4  $\mu$ M MtDUT<sup>WT</sup>.

## References

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