Supplemental Data

Phosphorylation in the vicinity of the nuclear localization signal of human dUTPase abolishes nuclear import: structural and mechanistic insights

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Figure S1. Sequence alignment and shematic illustration of the two human dUTPase isoforms. **(A)** Sequence aligment of mitochondrial (mDUT) and nuclear (nDUT) human dUTPase isoforms. Mitochondrial import is paralleled with cleavage of the 69 amino acid long precursor sequence and results in the mature mitochondrial isoform. Nuclear import is mediated by the NLS sequence (boxed) and the Cdk1 phosphorylation site is marked with a black arrowhead (S11 residue in bold within the nuclear isoform). Note that the NLS is presumably non-functional in the mitochondrial isoform, as once imported into the mitochondrion, the protein is no longer available for importin. Gray arrowhead indicates the position (M24 in the nuclear isoform) from where protein atoms can be localized in the currently available human dUTPase 3D structures (PDB IDs: 1Q5H, 1Q5U, 2HQU, 3ARA, 3ARN, 3EHW). Due to their high flexibility, the N-terminal 23 residues are not seen in the electron density maps even if these are present in some of the above reported structures. **(B)** Schematic illustration of the two dUTPase isoforms where the NLS, mitochondrial processing site, Cdk1 phosphorylation site (S11), and the common (position 94-252 mDUT and 5-164 nDUT) and isoform-specific sequences (position 69-94 mDUT and 1-5 nDUT in lighter shades of gray), as well as the site from where the 3D structure has been determined are indicated.

A

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mDUT    MTPLCPRPALCYHFLTSLLRSAMQNARGTAEGRSRGTLRARPAPRPPAAQHGIPRPLSSA
nDUT    ------------------------------------------------------------

                    start of processed mDUT          NLS
mDUT    GRLSQGCRGASTVGAAGWKGELPKAGGSPAPGPETPAISPSKRARPAEVGGMQLRFARLS
nDUT    ---------------------------MPCSEETPAISPSKRARPAEVGGMQLRFARLS
                                     .  ****************************

mDUT    EHATAPTRGSARAAGYDLYSAYDYTIPPMEKAVVKTDIQIALPSGCYGRVAPRSGLAAKH
nDUT    EHATAPTRGSARAAGYDLYSAYDYTIPPMEKAVVKTDIQIALPSGCYGRVAPRSGLAAKH
        ************************************************************

mDUT    FIDVGAGVIDEDYRGNVGVVLFNFGKEKFEVKKGDRIAQLICERIFYPEIEEVQALDDTE
nDUT    FIDVGAGVIDEDYRGNVGVVLFNFGKEKFEVKKGDRIAQLICERIFYPEIEEVQALDDTE
        ************************************************************

mDUT    RGSGGFGSTGKN
nDUT    RGSGGFGSTGKN
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B

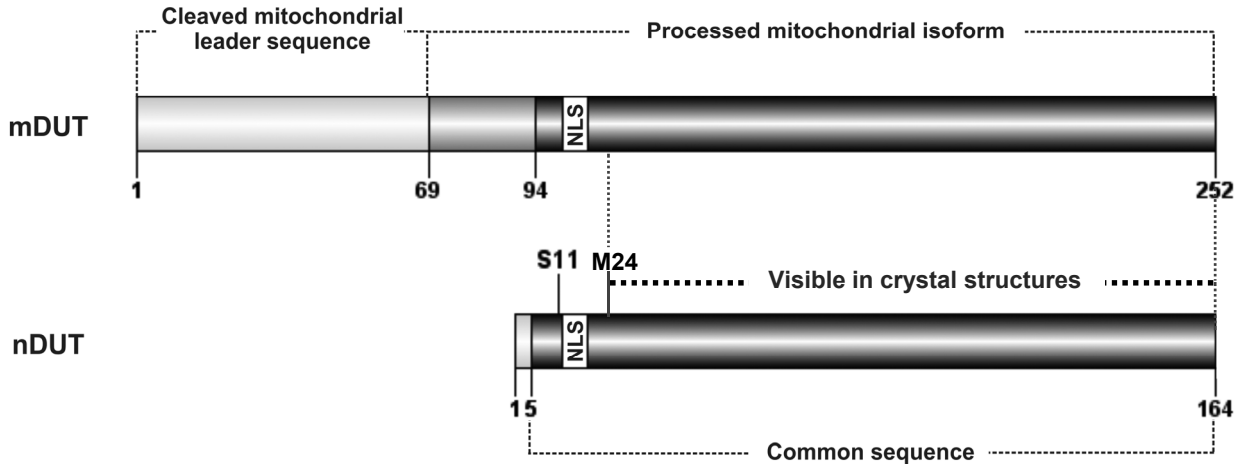

Figure S2. Phosphorylation-altered dUTPase localization pattern follows the same pattern in different cell lines

The DsRed-labeled WT human dUTPase (nuclear isoform) and hypophosphorylation mimicking S11Q mutant is mainly nuclear in asynchronous cell lines, whereas the P-mimicking S11E mutant is cytoplasmic. Localization pattern is the same in number of cell types with diverse genetic backgrounds. Scale bar represents 20 μm.

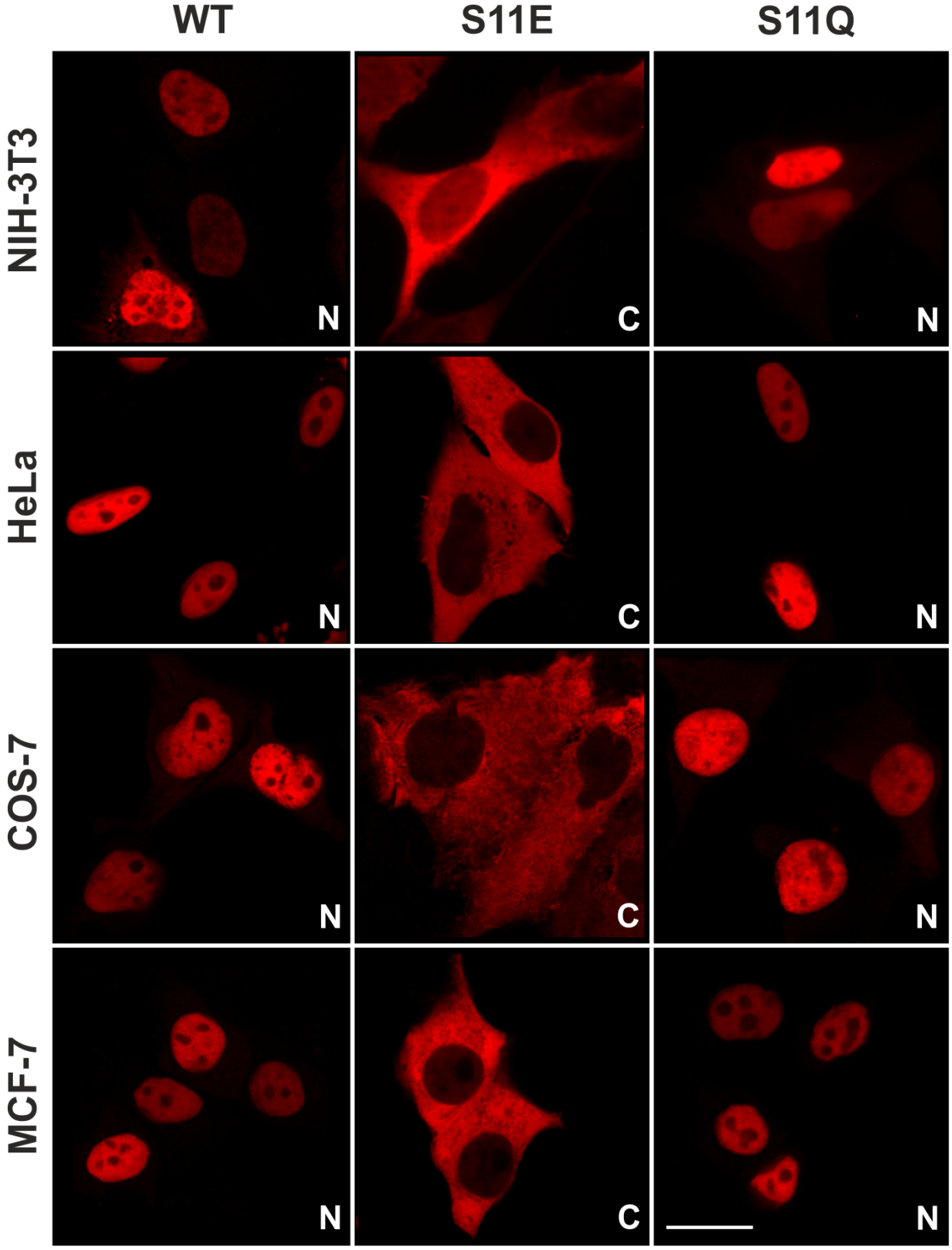

Figure S3. Phosphorylation-altered dUTPase localization pattern is nuclear export-independent

S11E mutant DsR-DUT expressing 293T cells were subjected to leptomycin B (a selective nuclear export inhibitor) treatment. No difference was observed in intracellular distribution of the protein compared to methanol mock-treated cells. Scale bar represents 20 μm.

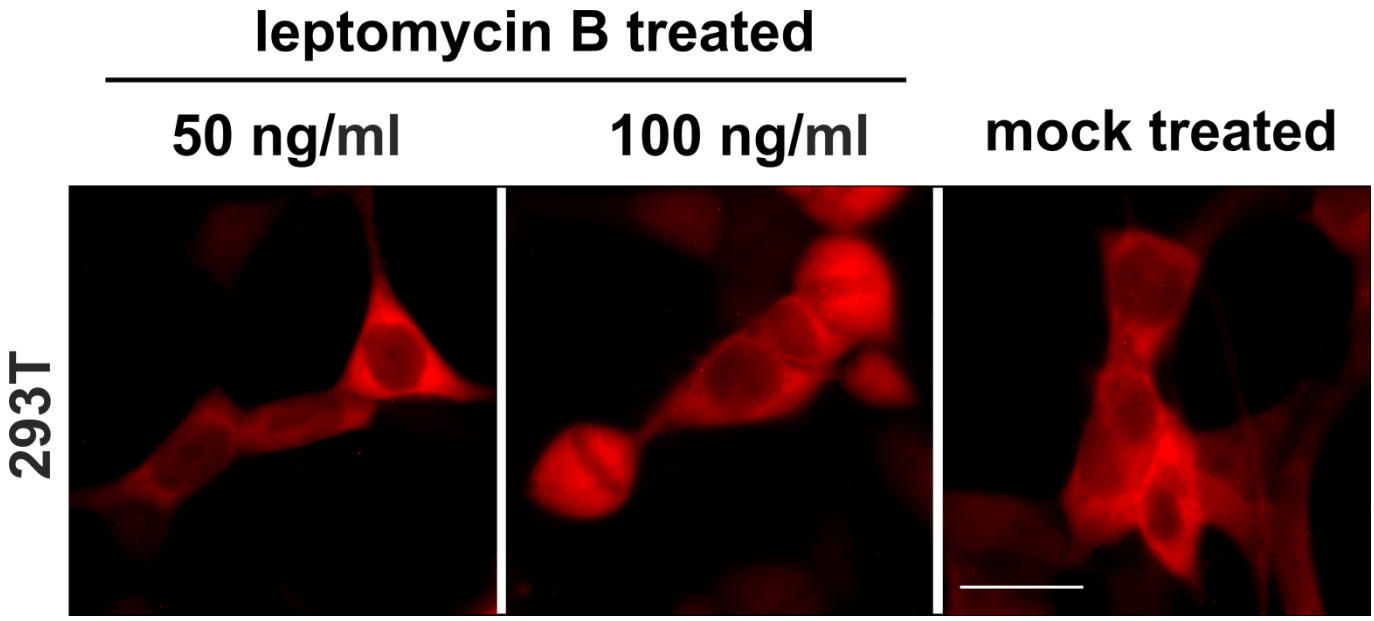

Figure S4. Stereo view of Figure 5. For legend, see main text.

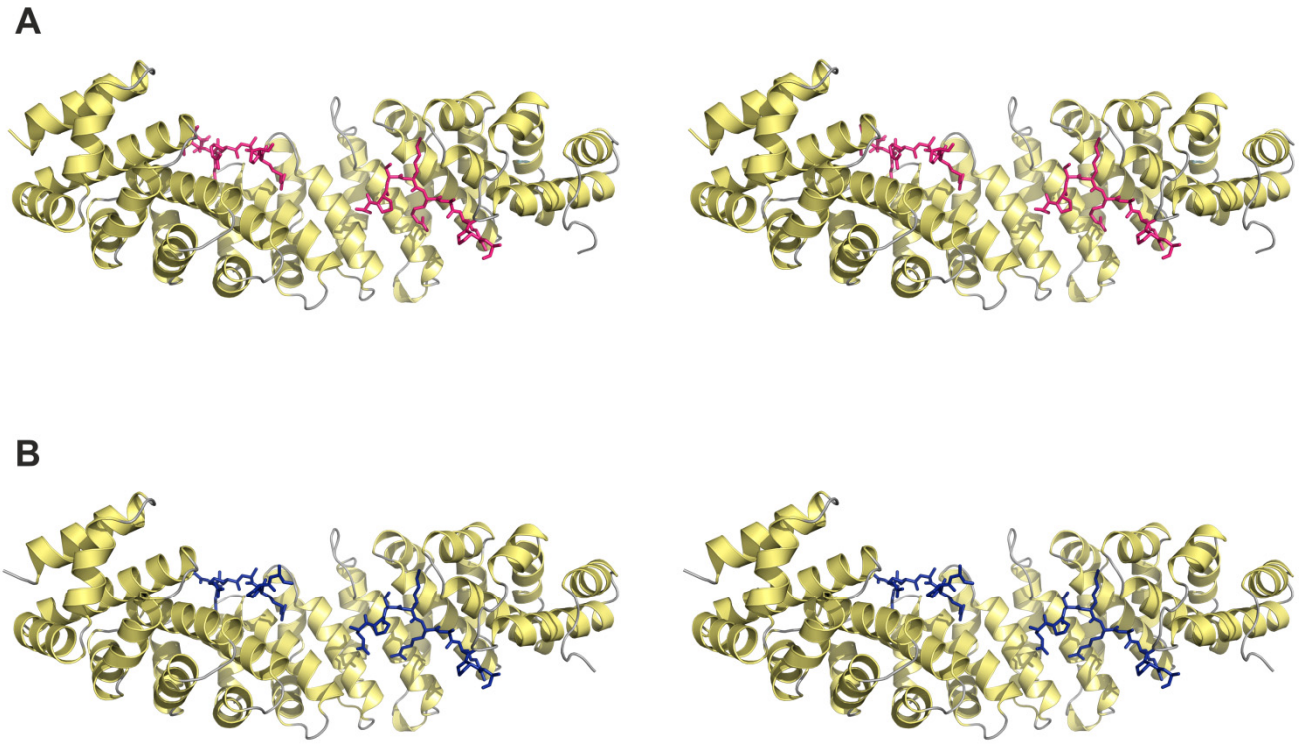

Figure S5. Stereo view of Figure 6. For legend, see main text.

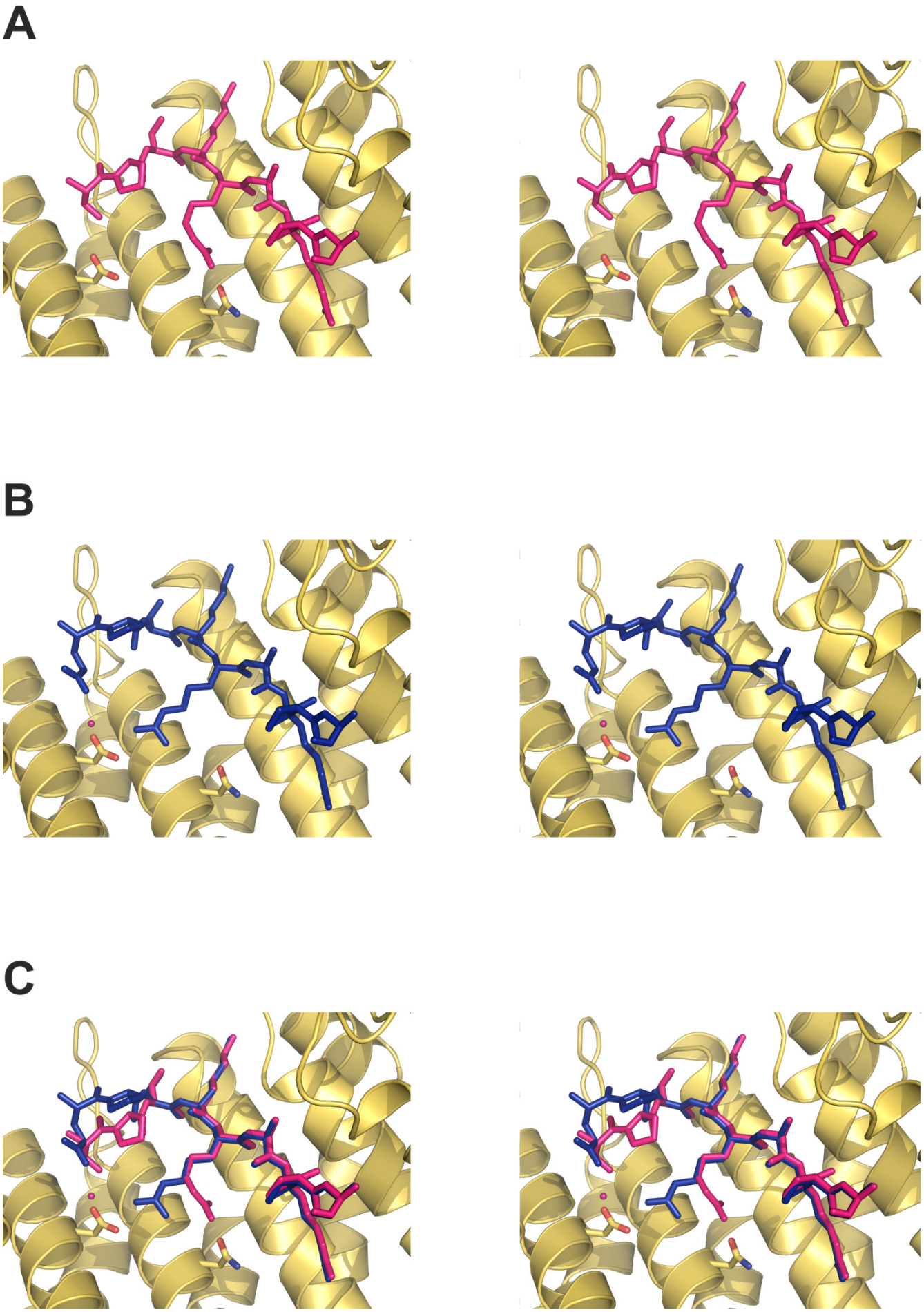

Table S1. Oligonucleotides and peptides used in the study.

	Oligonucleotide name	**Oligonucleotides 5'-3'**
Cloning into pDsRed-M N1	dutN1F	GATCTCGAGATGCCCTGCTCTGAAGAGAC
	dutN1R	ACGGTACCGCATTCTTTCCAGTGGAACCAAAAC
Mutagenesis	S11E_F	GAGACACCCGCCATTGAACCCAGTAAGCGGGC
	S11E_R	GCCCGCTTACTGGGTTCAATGGCGGGTGTCTC
	S11Q_F	GAGACACCCGCCATTCAACCCAGTAAGCGGGC
	S11Q_R	GCCCGCTTACTGGGTTGAATGGCGGGTGTCTC

	Peptide name	**Peptide sequence**
Crystallization	dutWT	AISPSKRARPAEV
	dutS11E	AIEPSKRARPAEV