SUPPORTING INFORMATION

Method S1. Membrane yeast two-hybrid assay.

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Table S3. Primers used in the current study. A restriction site or linker is shown in **bold and underlined** and a Kozak translation initiation sequence for yeast expression is shown in *italic*.

Figure S1. **Alignment of DGAT1 from different species.** Alignment was virtualized by Geneious v5.3 (Drummond *et al.*, 2010).

Figure S2. **Prediction of intrinsic disorder profile (blue) of the N-terminal region of DGAT1 from representative algae, plant and animals and its likelihood to participate in protein-protein interaction (red).** The analyses were performed by DISOPRED (Ward *et al.*, 2004). *At*, *Arabidopsis thaliana*; *Kn*, *Klebsormidium nitens*; *Cz*, *Chromochloris zofingiensis*; *Pt*, *Phaeodactylum tricornutum*; *Tp*, *Thalassiosira pseudonana*.

Figure S3. DGAT activity of CzDGAT1 variant enzymes at high oleoyl-CoA concentrations. A-D, DGAT activities of the full-length CzDGAT1 ($DGAT1_{1-550}$), N-terminal

truncated CzDGAT1 ($DGAT1_{81-550}$) and their corresponding acyl-CoA binding protein (ACBP) fused proteins (ACBP-$DGAT1_{1-550}$ and ACBP-$DGAT1_{81-550}$) at oleoyl-CoA concentration from 0.1-25 μM. Data were fitted to the allosteric sigmoidal equation or a previously proposed kinetic model that accounts for sigmoidicity and substrate inhibition (Xu *et al.*, 2017) using GraphPad Prism. The combined kinetic model is the preferred model for $CzDGAT1_{1-550}$, ACBP-fused $CzDGAT1_{1-550}$, and ACBP-fused $CzDGAT1_{81-550}$, but not $CzDGAT1_{81-550}$. Data represent means ± S.D. (n = 3).

Figure S4. Probing possible self-interaction of CzDGAT1 variants using membrane yeast two-hybrid assay. DNA sequences encoding $CzDGAT1_{1-550}$, $CzDGAT1_{81-550}$ and $CzDGAT1_{107-550}$ were ligated to the Lex A- C-terminal fragment of ubiquitin (C_{ub}) and the N-terminal fragment of ubiquitin containing an Ile/Gly point mutation ($N_{ub}G$), yielding C_{ub}-bait and $N_{ub}G$-prey, respectively. Serial dilutions of yeast cells producing each bait/prey combination were spotted on synthetic drop-out (SD) agar plates lacking Ade, His, Leu and Trp (SD-A-H-L-T).

Figure S5. Enzyme activity of CzDGAT1 variants in the presence of Coenzyme A (CoA). The DGAT activities of $CzDGAT1_{1-550}$, and acyl-CoA binding protein (ACBP) fused enzyme (ACBP-$DGAT1_{1-550}$) were assayed at 5 μM oleoyl-CoA in the absence or presence of 50 μM CoA. Data represent means ± S.D. (n = 3).

Figure S6. Illustration of the N-terminal fusion of acyl-CoA binding protein (ACBP) to CzDGAT1. The three-dimensional structure of *Arabidopsis thaliana* ACBP6 was generated with the SWISS-MODEL software. *A. thaliana* ACBP6 consists of four α helixes for acyl-CoA binding and is proposed to facilitate the feeding of acyl-CoA to the catalytic pocket of CzDGAT1 via capturing cytosolic acyl-CoAs or acyl-CoAs partitioned into the membrane lipid bilayer and subsequently channeling them to DGAT by proximity.

Method S1. Membrane yeast two-hybrid assay.

Possible self-interaction of CzDGAT1$_{1\text{-}550}$ and its N-terminal truncated mutants (CzDGAT1$_{81\text{-}550}$ and CzDGAT1$_{107\text{-}550}$) were tested using the membrane yeast two-hybrid system (kindly provided by Dr. Igor Stagljar, University of Toronto) with the method described by Snider et al. (Snider *et al.*, 2010). Briefly, cDNAs encoding CzDGAT1$_{1\text{-}550}$, CzDGAT1$_{81\text{-}550}$ and CzDGAT1$_{107\text{-}550}$ were amplified by PCR and cloned into the pBT3N bait vector or pPR3N prey vector, respectively. The pBT3N:bait was then co-transformed with the pPR3N:prey, Ost-N_{ub}I 'positive' control prey or Ost-N_{ub}G 'negative' control prey into the yeast strain NMY51 [*MATa, his3*Δ*200, trp1-901, leu2-3,112, ade2, LYS2::(lexAop)4-HIS3,ura3::(lexAop)8-lacZ,ade2::(lexAop)8-ADE2, GAL4*]. The possible interaction was then assayed on synthetic drop-out (SD) agar plates lacking Ade, His, Leu and Trp (SD-A-H-L-T) by 1: 10 serial dilution of cell cultures starting from an OD600 value of 0.4.

Table S1. DGAT1 proteins used for multiple sequence alignment. The topology organization of DGAT1 was predicted using TMHMM (Krogh *et al.*, 2001). TMD, transmembrane domain.

Name	Organism	Phytozome/Genbank accession number/JGI protein ID	Length of N-terminus (amino acid residues)	# of TMD
ApDGAT1	*Auxenochlorella protothecoides*	XP_011402032	326	7
AtDGAT1	*Arabidopsis thaliana*	NM_127503	132	9
BnDGAT1	*Brassica napus*	JN224473	113	8
BtDGAT1	*Bos taurus*	AAL49962	83	9
CaeDGAT1	*Caenorhabditis elegans*	NM_001269372	70	9
CheDGAT1	*Chlorella ellipsoidea*	KT779429	260	9
CreDGAT1	*Chlamydomonas reinhardtii*	Cre01.g045903	158	7
CsDGAT1	*Camelina sativa*	XM_010417066	132	9
CsuDGAT1	*Coccomyxa subellipsoidea C-169*	54084	1	9
CvDGAT1	*Chlorella vulgaris*	ALP13863.1	20	9
CzDGAT1A	*Chromochloris zofingiensis*	MH523419	289	9
CzDGAT1B	*Chromochloris zofingiensis*	Cz09g08290	107	9
DmDGAT1	*Drosophila melanogaster*	AF468649	132	8
DrDGAT1	*Danio rerio*	NM_199730	86	9
EaDGAT1	*Euonymus alatus*	AY751297	111	9
GmDGAT1	*Glycine max*	AY496439	102	9
HaDGAT1	*Helianthus annuus*	HM015632	110	9
HsDGAT1	*Homo sapiens*	NM_012079	86	9
JcDGAT1	*Jatropha curcas*	DQ278448	123	9
KnDGAT1	*Klebsormidium nitens*	GAQ91878	260	9
LuDGAT1	*Linum usitatissimum*	KC485337	111	9
MdDGAT1	*Monodelphis domestica*	XM_007488766	227	8
MmDGAT1	*Mus musculus*	AF078752	95	9
MtDGAT1	*Medicago truncatula*	XM_003595183	142	9
NoDGAT1	*Nannochloropsis oceanica*	KY073295	29	9
NtDGAT1	*Nicotiana tabacum*	AF129003	140	9
NvDGAT1	*Nematostella vectensis*	XM_001639301	54	9
OeDGAT1	*Olea europae*	AY445635	136	9
OsDGAT1	*Oryza sativa*	NM_001061404	142	8
PbDGAT1	*Paracoccidioides brasiliensis*	EEH17170.1	79	10
PfDGAT1	*Perilla frutescens*	AF298815	138	9
PotDGAT1	*Populus trichocarpa*	XM_006371934	96	9
PpDGAT1	*Physcomitrella patens*	XM_001770877	51	9
PtDGAT1	*Phaeodactylum tricornutum*	HQ589265	149	8

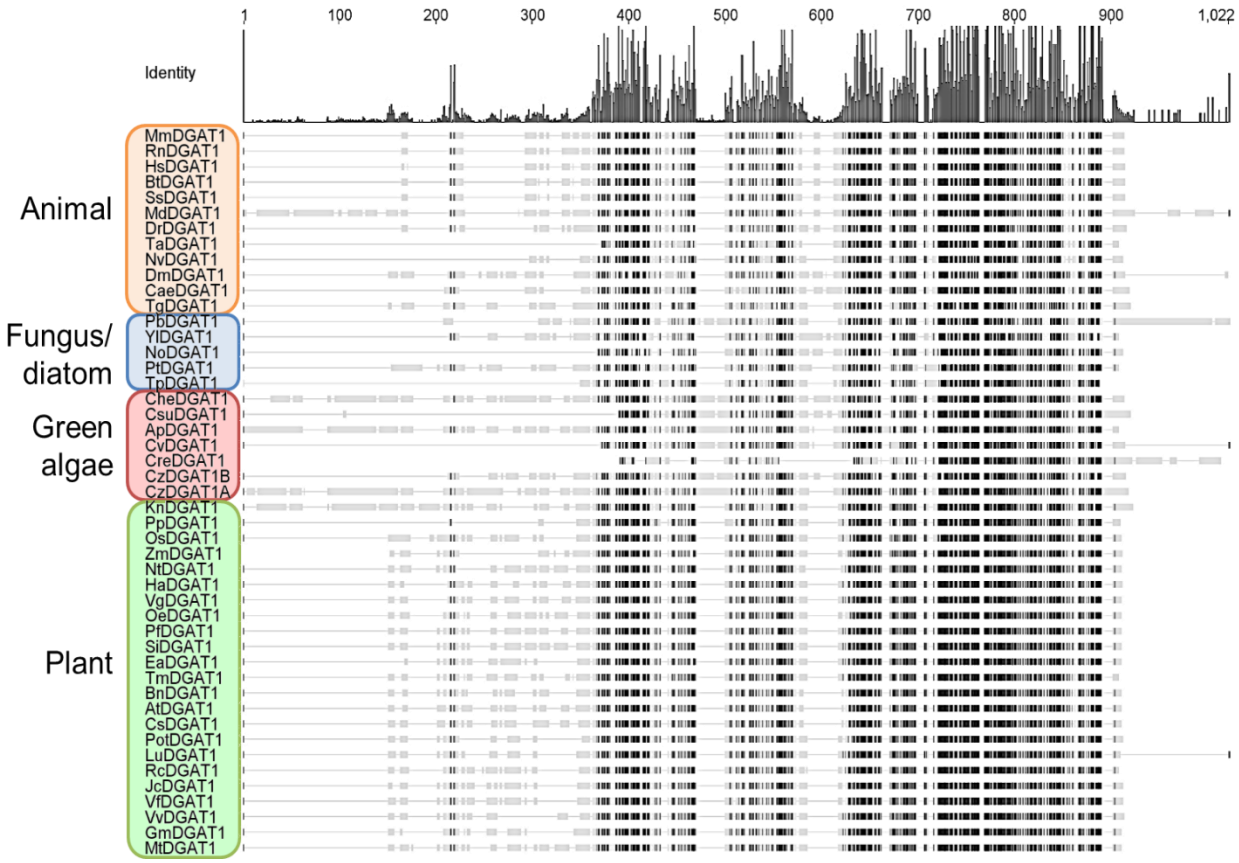
RcDGAT1	*Ricinus communis*	NM_001323734	128	9
RnDGAT1	*Rattus norvegicus*	AB062759	97	9
SiDGAT1	*Sesamum indicum*	JF499689	147	9
SsDGAT1	*Sus scrofa*	NM_214051	83	9
TaDGAT1	*Trichoplax adhaerens*	XM_002111989	11	9
TgDGAT1	*Toxoplasma gondii*	AY327327	117	9
TmDGAT1	*Tropaeolum majus*	AY084052	124	9
TpDGAT1	*Thalassiosira pseudonana*	XM_002287179	38	9
VfDGAT1	*Vernicia fordii*	DQ356680	129	9
VgDGAT1	*Vernonia galamensis*	EF653276	127	9
VvDGAT1	*Vitis vinifera*	CAN80418	103	9
YlDGAT1	*Yarrowia lipolytica*	XM_502557	98	8
ZmDGAT1	*Zea mays*	EU039830	97	8

Table S2. Apparent kinetic parameters of CzDGAT1 variants using a combined model accounting for sigmoidicity and substrate inhibition (Xu *et al.*, 2017)**.** DGAT activity was examined at increasing oleoyl-CoA concentration from 0.1 to 25 μM. Data were fitted to a nonlinear regression using a combined model accounting for sigmoidicity and substrate inhibition with the GraphPad Prism software. Data shown are means ± S.D. (n=3).

Enzyme	$DGAT1_{1-550}$	$DGAT1_{81-550}$	ACBP-$DGAT1_{1-550}$	ACBP-$DGAT1_{81-550}$
Preferred model	Combined model	Allosteric sigmoidal	Combined model	Combined model
Apparent V_{max} (pmol TAG/min/mg protein)	314.20±16.11	16.82±0.17	502.50±23.75	79.38±27.04
Hill coefficient	1.31±0.08	1.76±0.06	1.72±0.12	1.12±0.14
Apparent $S_{0.5}$ (μM)	1.76±0.15	1.78±0.04	1.10±0.08	4.76±2.52
Apparent K_i (μM)	58.74±11.85	-	38.44±7.03	9.03±4.41
Goodness of Fit/R2	0.992	0.995	0.980	0.961

Table S3. Primers used in the current study. A restriction site or linker is shown in **bold and underlined** and a Kozak translation initiation sequence for yeast expression is shown in *italic*.

Primer name	Oligonucleotide sequence
Primers used for cloning of cDNAs for yeast expression	
CzDGAT1$_{1\text{-}550}$-F (to pYES2.1)	gcaga**GCGGCCGC***GAA*ATGGAGGGTGCACGAATC
CzDGAT1-R (to pYES2.1)	tat**GTCGAC**GTGTGACATAAGAGCAGCATTCC
CzDGAT1$_{81\text{-}550}$-F (to pYES2.1)	gcaga**GCGGCCGC***GAA*ATGATTGGCAGCAGTCCTCT
CzDGAT1$_{107\text{-}550}$-F (to pYES2.1)	gcaga**GCGGCCGC***GAA*ATGGATGGACTGGTCAACTTGGC
AtACBP6-F (to pYES2.1)	gcaga**GCGGCCGC***GAA*ATGGGTTTGAAGGAGGAATTTG
AtACBP6-R (to pYES2.1)	tat**GTCGAC**GGTTGAAGCCTTGGAAGCA
AtACBP6-Linker-R (to pYES2.1)	**GAATTCCGT**GGTTGAAGCCTTGGAAGCA
Linker-CzDGAT1$_{1\text{-}550}$-F (to pYES2.1)	**ACGGAATTC**ATGGAGGGTGCACGAATC
Linker-CzDGAT1$_{81\text{-}550}$-F (to pYES2.1)	**ACGGAATTC**ATGATTGGCAGCAGTCCTCT
Linker-CzDGAT1$_{107\text{-}550}$-F (to pYES2.1)	**ACGGAATTC**ATGGATGGACTGGTCAACTTGGC
AtACBP6-F (to pESC-leu2d empty)	tata**GGATCC***GAA*ATGGGTTTGAAGGAGGAATTTG
AtACBP6-R (to pESC-leu2d empty)	tata**CTCGAG**TCAGGTTGAAGCCTTGGAAGCA
Primers used for cloning of cDNAs for membrane yeast two-hybrid assay (MYTH)	
CzDGAT1$_{1\text{-}550}$-F (to MYTH vector)	ac**GGCCATTACGGCC**ATGGAGGGTGCACGAATC
CzDGAT1$_{81\text{-}550}$-F (to MYTH vector)	ac**GGCCATTACGGCC**ATGATTGGCAGCAGTCCTCT
CzDGAT1$_{107\text{-}550}$-F (to MYTH vector)	ac**GGCCATTACGGCC**ATGGATGGACTGGTCAACTTGGC
CzDGAT1-R (to MYTH vector)	tat**GGCCGAGGCGGCC**TCAGTGTGACATAAGAGCAGCATTCC
Primers used for cloning of cDNAs for plant expression	
AtWRI-F (to pGreen)	CTAG**ACTAGT**ATGAAGAAGCGCTTAACCAC
AtWRI-R (to pGreen)	CGC**TCTAGA**TCAGACCAAATAGTTACAAGAAAC
AtACBP-F (to pGreen)	TATA**ACCGGT**ATGGGTTTGAAGGAGGAATTTG
AtACBP-R (to pGreen)	CGC**TCTAGA**TCAGGTTGAAGCCTTGGAAGCA
CzDGAT1 $_{1\text{-}550}$-F (to pGreen)	TATA**ACCGGTACTAGT**ATGGAGGGTGCACGAATC
CzDGAT1 $_{81\text{-}550}$-F (to pGreen)	TATA**ACCGGT**ATGATTGGCAGCAGTCCTCT
CzDGAT1 $_{107\text{-}550}$-F (to pGreen)	TATA**ACCGGT**ATGGATGGACTGGTCAACTTGGC
CzDGAT1-R (to pGreen)	CGC**TCTAGA**TCAGTGTGACATAAGAGCAGCATTCC
Venus-F (to pGPTVII)	cgc**TCTAGA**ATGGTGAGCAAGGGCGA
Venus-R (to pGPTVII)	TATA**ACTAGTACCGGT**CTTGTACAGCTCGTCCAT
AtACBP-R (to pGPTVII)	tata**CTCGAG**TCAGGTTGAAGCCTTGGAAGCA
CzDGAT1-R (to pGPTVII)	tata**CTCGAG**TCAGTGTGACATAAGAGCAGCATTCC
Linker-SCFP3A-F (to pGPTVII)	**ACGGAATTC**ATGGTGAGCAAGGGCGAGG
SCFP3A-R (to pGPTVII)	TATA**CTCGAG**TTACTTGTACAGCTCGTCCATGCC
AtGPAT9-F (to pGPTVII)	CGC**TCTAGAGTCGAC**ATGAGCAGTACGGCAGGGAG
AtGPAT9-linker-R (to pGPTVII)	**GAATTCCGT**CTTCTCTTCCAATCTAGCCAGGA



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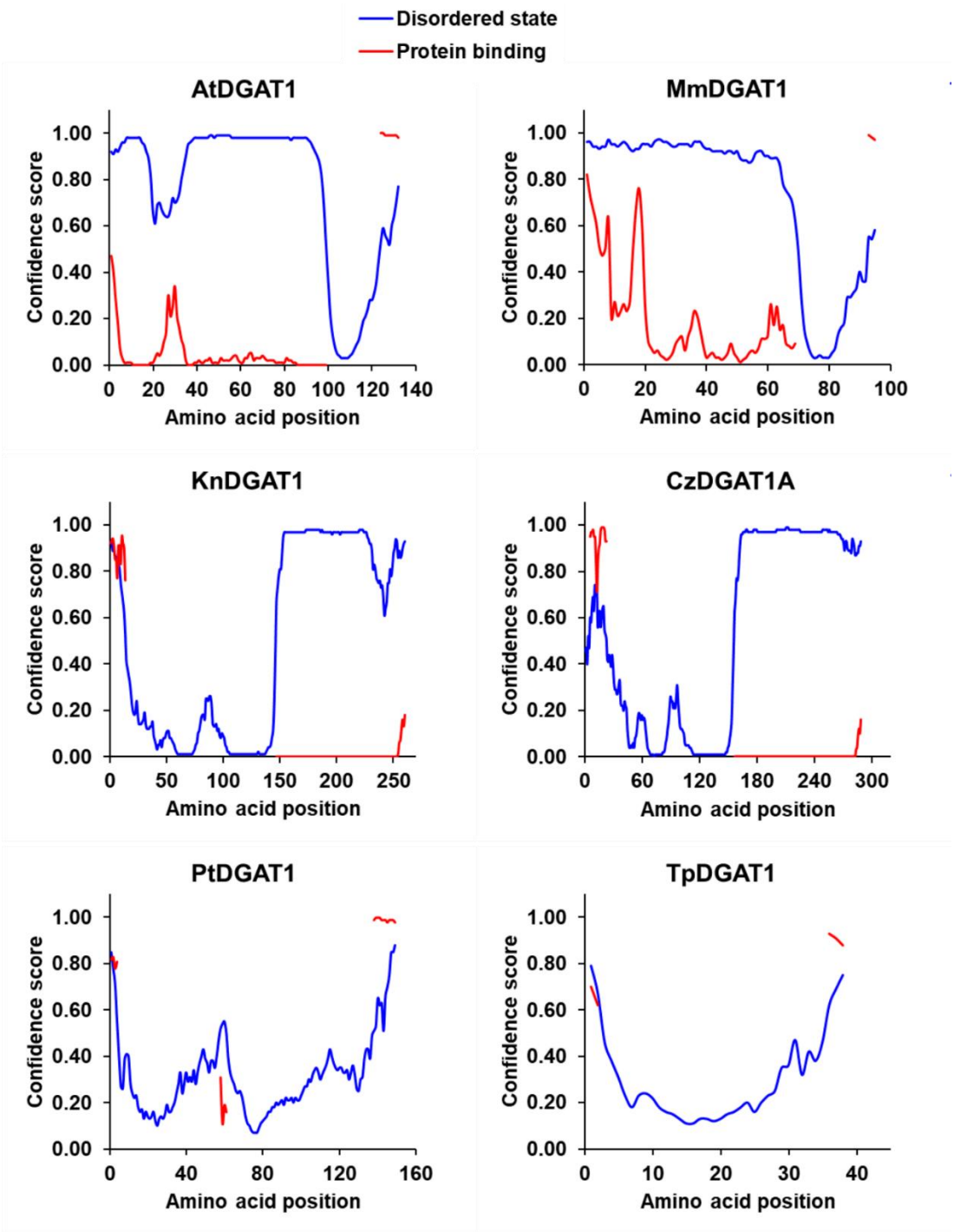

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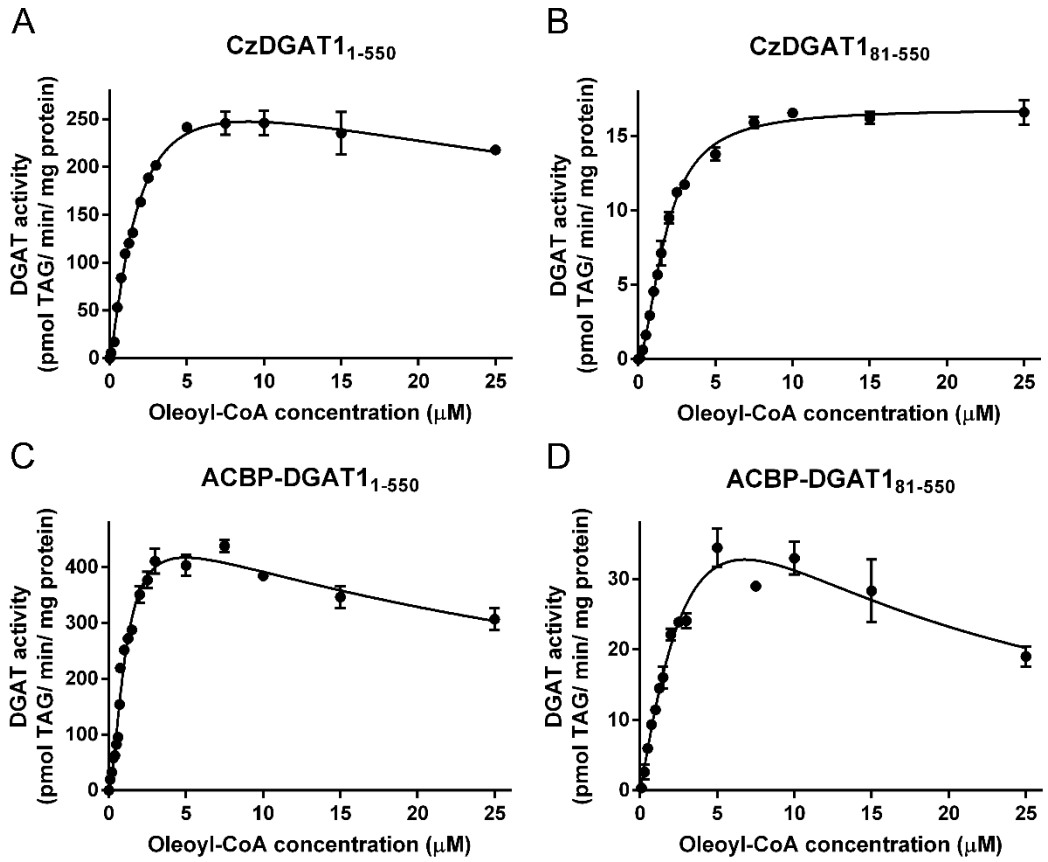

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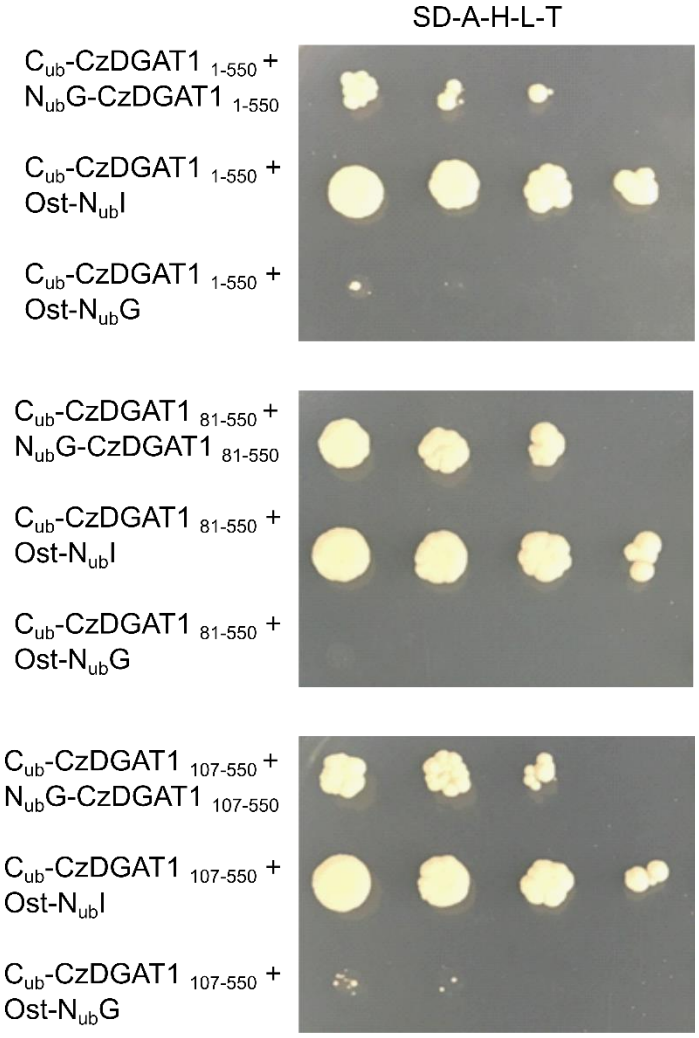

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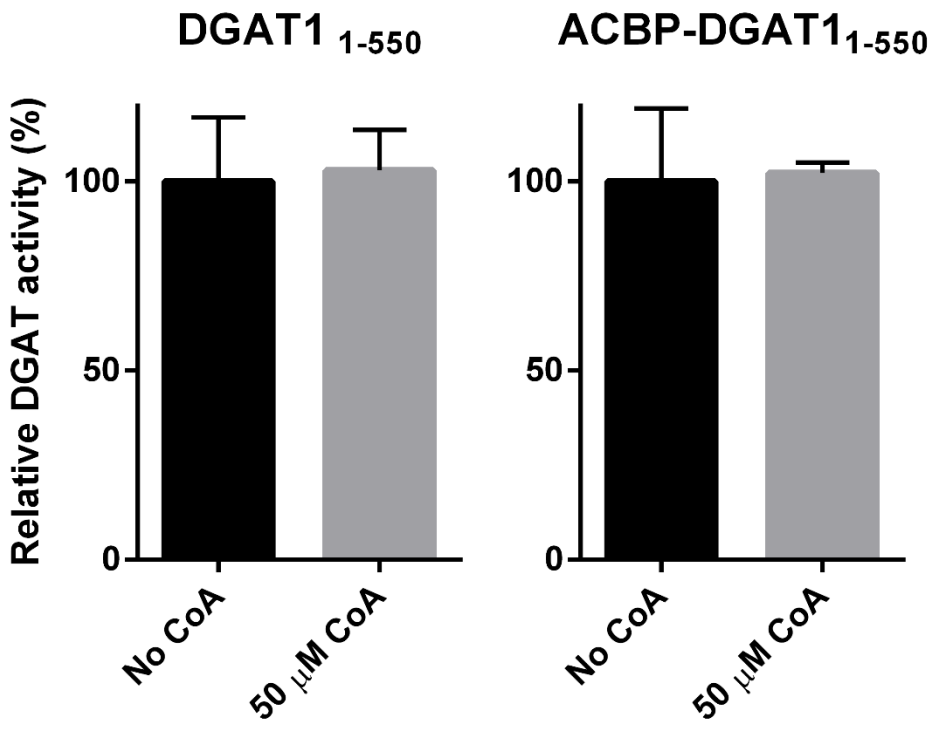

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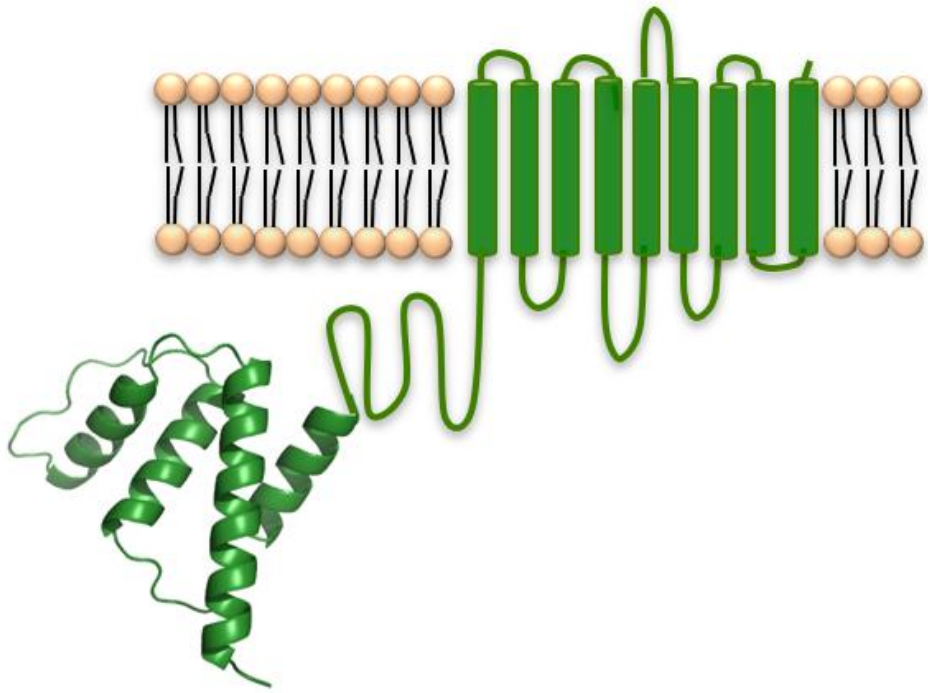

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