Supplemental Materials

Analysis of substrate specificity of human DHHC protein acyltransferases using a yeast expression system

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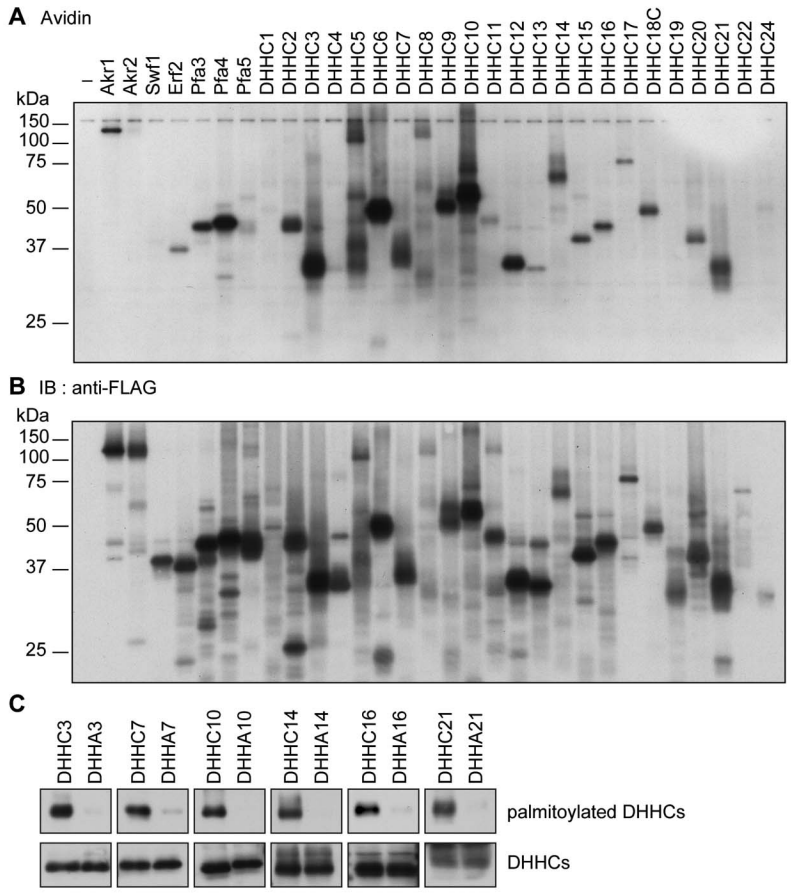

Fig. S1. Twenty human DHHC proteins form acyl intermediates in yeast. (*A* and *B*) SEY6210 (wild type) cells were transfected with the pAKNF314 (vector), pAKNF-AKR1 (*3xFLAG-AKR1*), pAKNF-AKR2 (*3xFLAG-AKR2*), pAKNF-SWF1 (*3xFLAG-SWF1*), pAKNF-ERF2 (*3xFLAG-ERF2*), pAKNF-PFA3 (*3xFLAG-PFA3*), pAKNF-PFA4 (*3xFLAG-PFA4*), pAKNF-PFA5 (*3xFLAG-PFA5*), pAKNF-DHHCx (*3xFLAG-DHHCx* with x representing the DHHC number; x=1-17, 19-22, and 24), or pLECF-DHHC18 (*DHHC18-3xFLAG*) plasmid. Total cell lysates were treated with *N*-ethylmaleimide (NEM) to block free Cys residues. After removal of palmitic acid from the palmitoylated Cys residues by incubation with hydroxylamine, the exposed free Cys residues were modified with biotin using Biotin-HPDP. The samples were then subjected to immunoprecipitation with anti-FLAG antibodies, followed by detection with streptavidin-HRP (*A*) or with immunoblotting using anti-FLAG antibody (*B*). (C) SEY6210 cells were transfected with pAKNF-DHHCx or pAKNF-DHHAx (x represents the DHHC number; x=3, 7, 10, 14, 16, and 21) plasmid. Total cell lysates were prepared and treated sequentially with NEM, hydroxylamine, and Biotin-HPDP. The biotinylated samples were precipitated with immobilized avidin beads and detected by immunoblotting with anti-FLAG antibody (upper panel). The DHHC proteins in the total lysates were also detected using immunoblotting with anti-FLAG antibody (lower panel).

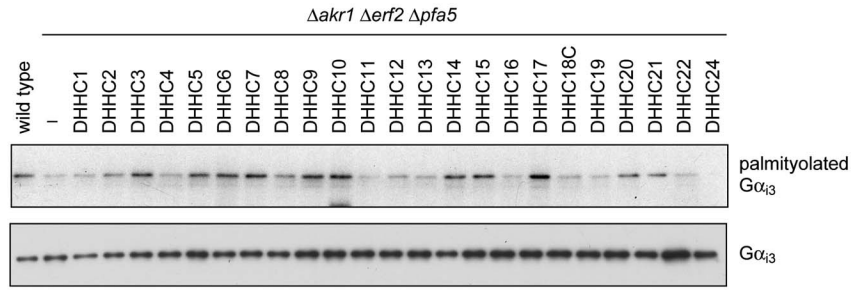

Fig. S2. Thirteen human DHHC proteins palmitoylate $G\alpha_{i3}$. The KAY251 (Δ*akr1* Δ*erf2* Δ*pfa5*) cells harboring the pYU-Gαi3 (*$G\alpha_{i3}$-9xMyc*) plasmid were transfected with the pAKNF314 (vector), pAKNF-DHHCx (*3xFLAG-DHHCx* with x representing the DHHC number; x=1-17, 19-22, and 24), or pLECF-DHHC18 (*DHHC18-3xFLAG*) plasmid. Total cell lysates were prepared from the transfected cells, as well as wild type (SEY6210) cells bearing the pYU-Gαi3 and pAKNF314 plasmids, and treated sequentially with NEM, hydroxylamine, and Biotin-HPDP. The DHHC proteins were immunoprecipitated with anti-Myc antibodies, and the precipitates were detected by Ultra Sensitive ABC Peroxidase Staining Kit (upper panel) or immunoblotting with anti-Myc antibody (lower panel).

Table S1. Human and mouse *DHHC* genes

DHHC[a]		ZDHHC[b]			Accession Number	
Human	Mouse	Human	Mouse	Other Name	Human	Mouse
DHHC1	*DHHC1*	*ZDHHC1*	*Zdhhc1*		NM_013304	BC026570
DHHC2	*DHHC2*	*ZDHHC2*	*Zdhhc2*	*REAM*	BC050272	NM_178395
DHHC3	*DHHC3*	*ZDHHC3*	*Zdhhc3*	*GODZ*	NM_016598	NM_026917
DHHC4	*DHHC4*	*ZDHHC4*	*Zdhhc4*		NM_018106	NM_028379
DHHC5	*DHHC5*	*ZDHHC5*	*Zdhhc5*		NM_015457	NM_144887
DHHC6	*DHHC6*	*ZDHHC6*	*Zdhhc6*		BC007213	NM_025883
DHHC7	*DHHC7*	*ZDHHC7*	*Zdhhc7*	*SERZ-β*	NM_017740	NM_133967
DHHC8	*DHHC8*	*ZDHHC8*	*Zdhhc8*		NM_013373	AY668947
DHHC9	*DHHC9*	*ZDHHC9*	*Zdhhc9*		BC012826	AK032233
DHHC10	*DHHC10*	*ZDHHC11*	*Zdhhc11*		NM_024786	AY668948
DHHC11	*DHHC11*	*ZDHHC23*	*Zdhhc23*	*NIDD*	NM_173570	AY668949
DHHC12	*DHHC12*	*ZDHHC12*	*Zdhhc12*		NM_032799	BC021432
DHHC13	*DHHC13*	*ZDHHC24*	*Zdhhc24*		NM_207340	BC071194
DHHC14	*DHHC14*	*ZDHHC14*	*Zdhhc14*		NM_024630	BC059814
DHHC15	*DHHC15*	*ZDHHC15*	*Zdhhc15*		BC103980	NM_175358
DHHC16	*DHHC16*	*ZDHHC16*	*Zdhhc16*	*APH2*	NM_032327	XM_129300
DHHC17	*DHHC17*	*ZDHHC17*	*Zdhhc17*	*HIP14*, *AKR1*	NM_015336	NM_172554
DHHC18	*DHHC18*	*ZDHHC18*	*Zdhhc18*		NM_032283	AY668950
DHHC19	*DHHC19*	*ZDHHC19*	*Zdhhc19*		NM_144637	BC049761
DHHC20	*DHHC20*	*ZDHHC20*	*Zdhhc20*		NM_153251	AY668951
DHHC21	*DHHC21*	*ZDHHC21*	*Zdhhc21*		NM_178566	NM_026647
DHHC22	*DHHC22*	*ZDHHC13*	*Zdhhc13*	*HIP14L*, *AKRL1*	AB024495	NM_028031
–[c]	*DHHC23*	–[c]	*Zdhhc25*		–	BC049767
DHHC24	*DHHC24*	*ZDHHC22*	*Zdhhc22*		NM_174976	NM_001080943

[a]The *DHHC* nomenclature followed the previous report (Fukata *et al.*, 2004).

[b]The *DHHC* genes are registered in GenBank as *ZDHHC* (zinc-finger, DHHC-type). Note that nomenclatures of *DHHC* are different from those of *ZDHHC* for some *DHHC* genes.

[c]The *DHHC23* gene does not exist in human genome.

Table S2. Yeast strains used in this study

Strain	Genotype	References
SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9*	(Robinson *et al.*, 1988)
SEY6211	*MAT**a** leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 ade2-101 suc2-Δ9*	(Robinson *et al.*, 1988)
KHY1028	SEY6210, Δ*yck2::YCK2-K-RASB URA3*	This study
KHY1031	SEY6210, Δ*yck2::YCK2-K-RASB*	This study
KHY1045	SEY6210, Δ*akr1::HIS3*	This study
KHY1046	SEY6210, Δ*yck2::YCK2-K-RASB Δakr1::HIS3*	This study
RHY59	*MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ90 ade2-101 suc2-Δ9 Δakr2::KanMX4 Δswf1::KanMX4 Δerf2::LEU2 Δpfa3::KanMX4 Δpfa4::LEU2 Δpfa5::KanMX4*	This study
IAY106	RHY59, Δ*agp1::pTEF-9xMYC-AGP1 URA*	This study
KAY39	KHY1046, Δ*yck1::pTEF-9xMYC-YCK1 URA3*	This study
KAY44	SEY6210, Δ*agp1::pTEF-9xMYC-AGP1 URA3*	This study
KAY47	SEY6210, *Δgpa2::pTEF-GPA2-9xMYC natNT2 URA3*	This study
KAY57	KHY1046, Δ*erf2::KanMX4 Δgpa2::pTEF-GPA2-9xMYC natNT2 URA3*	This study
KAY62	SEY6210, Δ*yck1::pTEF-9xMYC-YCK1 URA3*	This study
KAY110	SEY6210, Δ*sso1::pTEF-9xMYC-SSO1 URA3*	This study
KAY113	SEY6210, Δ*swf1::KanMX4 Δsso1::pTEF-9xMYC-SSO1 URA3*	This study
KAY251	KHY1046, Δ*erf2::LEU2 Δpfa5::KanMX4*	This study

Table S3. Primers used in this study

Primer	Nucleotide sequence
DHHC18C-F	5'-AGAATTCGCCACCATGAAGGACTGCGAG-3' (*Eco*RI)
DHHC18C-R	5'-AAGATCTTCCACCATGCTGGCATCAGG-3' (*Bgl*II)
DHHC24-F	5'-AAGGATCCATGCTGGCCCTGCGGCTGCTCAACG-3' (*Bam*HI)
DHHC24-R	5'-TGGATCCCTACTTATCCTGCTGCTTGGAGC-3' (*Bam*HI)
PSD95-F	5'-AGCCACCATGGACTGTCTCTGTATAGTGACAAC-3'
PSD95-R	5'-TGAATTCGAGAGTCTCTCTCGGGCTGGAACC-3'
SNAP25b-F	5'-TGGATCCATGGCCGAAGACGCAGACATGC-3' (*Bam*HI)
SNAP25b-R	5'-TTTAACCACTTCCCAGCATCTTTGTTGC-3'
Gαi3-F	5'-GCCACCATGGGCTGCACGTTGAGCGCCGAG-3'
Gαi3-R	5'-AACCCGGGCAAATAAAGCCCACATTCC-3' (*Sma*I)
AKR1-F	5'-AGGATCCATGGTGAACGAATTAGAGAATG -3' (*Bam*HI)
AKR1-R	5'-ACCTCATGAGCTCTCCTAATAGTCC-3'
AKR2-F	5'-AAGATCTATGACCAGTATGTCAATTATTG-3' (*Bgl*II)
AKR2-R	5'-AATCACGCAAATTTTTGTTGTGCAC-3'
ERF2-F	5'-GGATCCATGGCCTTGGTCTCTAGAAGGTCG-3' (*Bam*HI)
ERF2-R	5'-GCTGCACGTTGTCTGACATCTGAAGH-3'
SWF1-F	5'-GGATCCATGTCATGGAATCTACTATTTGTGC-3' (*Bam*HI)
SWF1-R	5'-CAGGACTAATTGCTGGTAGTATGGA-3'
PFA3-F	5'-GGATCCATGAATGACAGGCTTTCGTTGACAAG-3' (*Bam*HI)
PFA3-R	5'-TGGCTACAGTACCACAGTAGATTCT-3'
PFA4-F	5'-GGATCCATGCCAGTAAAGTTAAGGTGGCCTT-3' (*Bam*HI)
PFA4-R	5'-CGAGTGCTGGAAACCAGGGAATAGA-3'
PFA5-F	5'-GGATCCATGGCTCTATCATGGAATATTCGAA-3' (*Bam*HI)
PFA5-R	5'-GTGAAGATTGGAGACGTTGCCTCAC-3'
YCK2-F1	5'-GGAAACTACCAGAATACACAACGCC-3'
YCK2-R1	5'-CAATTGCCCGGGTTACATAATTACACACTTTGTCTTTGACTTCTTTTTCT TCTTTTTACCATCTCCTAGCTTACTGAAAAAACCTTTCG-3'
YCK2-F2	5'-AATAGAAAACGGAGGAGGTTTTTTG-3'
YCK2-R2	5'-CTATTTGTCTATCCAGCTAAACGTG-3'
YCK1-F	5'-CATACAAAAATTTCGCGCATTCGCTGGCCCTTTTCCTGCTCTCCTCTTCC CCATGGCCAAGCTTTTCAATTCATC-3'
YCK1-R	5'-TTATATTGGTGAGGTTGTTAACTGCTAGAGTGGTACTTGCTATGGGCAT GGACATAGATCTGCTAG-3'
GPA2-F1	5'-CGCCATCAAAGAAACAATTTTGGAAAATACATTGAAAGACTCTGGAGT GTTACAACGTACGCTGCAGGTCGACTC-3'
GPA2-R1	5'-ATAATAAAGGGGAGAAGAGGCATGCAGTTTTGTCTCTGTTTTAGCTGT GCATTCACATCGATGAATTCGAGCTCG-3'

GPA2-F2	5’-CAGCGAGCCTTATTGTTACAGCACAAATCACGCGTATTTTCAAGCAAA TATCATGGCCAAGCTTTTCAATTCATC-3’
GPA2-R2	5’-CGGTCTGCGTGTCAGGAGTGCTGCCGTTCTTTTCTGAAGATGCGCAGAG ACCCATTCCACTAGAAAACTTAG-3’
AGP1-F	5’-TGTAATCTTTATAGAAGAAGCACGCTAATATAGACAAAGATAGCTTCG CACAATGGCCAAGCTTTTCAATTCATC-3’
AGP1-R	5’-CTGTGGAGCTATTTTTCAAGTCTTTCAGTTCGTATAGAGACTTCGACGA CGACATAGATCTGCTAGTGGATCCG-3’
SSO1-F1	5’-AAGAAAACCCTTTTACAATTAAAAAAGGCAATTAAAAATAGAAACAA ATCAAATGGCCAAGCTTTTCAATTCATC-3’
SSO1-R1	5’-CCAACTCGTATGACTCTTCAAAAGGGGTTTCCAACTGGTACGGATTATT ATAACTAGATCTGCTAGTGGATCCG-3’

Supplemental References

Fukata M, Fukata Y, Adesnik H, Nicoll RA, Bredt DS (2004). Identification of PSD-95 palmitoylating enzymes. Neuron 44, 987-996.

Robinson JS, Klionsky DJ, Banta LM, Emr SD (1988). Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. Mol Cell Biol 8, 4936-4948.