Supplemental materials

Loss of function and reduced levels of sphingolipid desaturase DEGS1 variants are both relevant in disease mechanism.

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Supplemental Table S1. Oligonucleotide primers used in the study.

Cloning primers

DEGS1 forward: 5′-CGCGAAGCTTGCGATCGCCATGGGGAGCCGCGTCTCGC

reverse: 5′-CGCGTCTAGAGTTTAAACTTACTCCAGCACCATCTCTCCTTTTTG

amplification program: 96°C 30s, [95°C 8s, 64°C 20s, 72° 45s]x30, 72°C 10 min

DEGS2 forward:5′-CGCGAAGCTTGCGATCGCCATGGGCAACAGCGCGAGCC

reverse:5′-CGCGTCTAGAGTTTAAACTCACAGACCATCTTTTGCCAGC

amplification program: 96°C 30s, [95°C 8s, 62°C 20s, 72° 45s]x30, 72°C 10 min

HygR forward: 5′ CGCGAAGCTTGCGATCGCCATGACACAAGAATCCCTGTTACTTCTC

HygR reverse: 5′ CGCGTCTAGAGTTTAAACTCAGGCGCCGGGGGCG

amplification program: 96°C 30s, [95°C 8s, 64°C 20s, 72° 45s]x30, 72°C 10 min

Forward primers: HindIII (underlined) and SgfI (double underlined) sites were added for cloning in pCDNA3 and pFN21A-HALO-Tag, respectively.

Reverse primers: XbaI (underlined) and PmeI (double underlined) sites were added for cloning in pCDNA3 and pFN21A-HALO-Tag, respectively.

Mutagenesis primers

c.320G>A forward: 5′-CAAAGCAATGTGGAATCGCT**A**GTTTGGAATGTTTGCTAATC

reverse: 5′-GATTAGCAAACATTCCAAAC**T**AGCGATTCCACATTGCTTTG

c.337A>G forward: 5′-CTGGTTTGGAATGTTTGCT**G**ATCTTCCTATTGGGATTCC

reverse: 5′-GGAATCCCAATAGGAAGAT**C**AGCAAACATTCCAAACCAG

c.395A>G forward: 5′-GTATCACATGGATCATC**G**TCGGTACCTTGGAGCTG

reverse: 5′-CAGCTCCAAGGTACCGA**C**GATGATCCATGTGATAC

c.517C>T forward: 5′-CCTCTCTTTTATGCCTTTT**T**GACCTCTGTTCATCAACC

reverse: 5′-GGTTGATGAACAGAGGTC**A**AAAGGCATAAAAGAGAGG

c.524T>A forward: 5′-CTTTTATGCCTTTCGACCTC**A**GTTCATCAACCCCAAACC

reverse: 5′-GGTTTGGGGTTGATGAAC**T**GAGGTCGAAAGGCATAAAAG

c.565A>G forward: 5′-CGTATCTGGAAGTTATC**G**ATACCGTGGCACAGGTC

reverse: 5′-GACCTGTGCCACGGTAT**C**GATAACTTCCAGATACG

c.764A>G forward: 5′-GCCTCTGAATTTACTTACCTTCA**G**TGTGGGTTATCATAATGAAC

reverse: 5′-GTTCATTATGATAACCCACA**C**TGAAGGTAAGTAAATTCAGAGGC

c.839C>T forward: 5′-CCACTGGTGAGGAAAATAG**T**AGCTGAATACTATGACAACC

reverse: 5′-GGTTGTCATAGTATTCAGCT**A**CTATTTTCCTCACCAGTGG

substituted nucleotides are in boldface.

qPCR primers

DEGS1 forward: 5′-GAGATCCTGGCAAAGTATCCA

reverse: 5′-GGCAATCTCATGAATAGCCAGA

DEGS2 forward: 5′-AGATACTGGCCAAGTACCCG
reverse: 5′-GATGTCGTGGATGGCCAGC

Supplemental Table S2. Mass spectrometry transition for evaluation of sphingolipid profile via MRM method. MS/MS 1 is considered the quantitative fragment ion, MS/MS 2 the qualitative fragment ion, DP the declustering potential applied to molecular ion and CE the collision energy applied to molecular ion to obtain the quantitative fragment ion MS/MS 1. When chemical standards of sphingolipids were not commercially available data were also confirmed by LC-HRMS.

[M+H]+	MS/MS 1	MS/MS 2	Name	DP (eV)	CE (eV)
482.4565	*264.2636*		*IS Cer 12*	*80*	*30*
647.5122	*184.0688*		*IS SM 12*	*80*	*40*
644.5096	*264.2636*		*IS GlucCer 12*	*80*	*50*
540.535	284.2879	266.277	DHCer 16	80	35
568.568	284.2879	266.277	DHCer 18	80	35
566.5524	284.2879	266.277	DHCer 18_1	80	35
596.6013	284.2879	266.277	DHCer 20	80	35
624.6343	284.2879	266.277	DHCer 22	80	35
652.6673	284.2879	266.277	DHCer 24	80	35
650.6517	284.2879	266.277	DHCer 24_1	80	35
538.5194	264.2636	520.51	Cer 16	80	35
566.5524	264.2636	548.54	Cer 18	80	35
564.5368	264.2636	546.54	Cer 18_1	80	35
594.5857	264.2636	576.57	Cer 20	80	35
622.6187	264.2636	604.60	Cer 22	80	35
650.6517	264.2636	632.63	Cer 24	80	35
648.6361	264.2636	630.62	Cer 24_1	80	35
703.5748	184.0688		SM 16	80	40
731.6078	184.0688		SM 18	80	40
729.5922	184.0688		SM 18_1	80	40
759.6411	184.0688		SM 20	80	40
787.6741	184.0688		SM 22	80	40
815.7071	184.0688		SM 24	80	40
813.6915	184.0688		SM 24_1	80	40
700.5722	264.2636	520.51	HexCer 16	80	50
728.6052	264.2636	548.54	HexCer 18	80	50
726.5896	264.2636	546.54	HexCer 18_1	80	50
756.6385	264.2636	576.57	HexCer 20	80	50
784.6715	264.2636	604.60	HexCer 22	80	50
812.7045	264.2636	632.63	HexCer 24	80	50
810.6889	264.2636	630.62	HexCer 24_1	80	50
862.625	264.2636	520.51	LacCer 16	80	60
890.658	264.2636	548.54	LacCer 18	80	60
888.6424	264.2636	546.54	LacCer 18_1	80	60
918.6913	264.2636	576.57	LacCer 20	80	60
946.7243	264.2636	604.60	LacCer 22	80	60
974.7573	264.2636	632.63	LacCer 24	80	60
972.7417	264.2636	630.62	LacCer 24_1	80	60
705.5948	184.068		DHSM 16	80	60
733.6278	184.068		DHSM 18	80	60
731.6122	184.068		DHSM 18_1	80	60
761.6611	184.068		DHSM 20	80	60
789.6941	184.068		DHSM 22	80	60

[M+H]+	MS/MS 1	MS/MS 2	Name	DP (eV)	CE (eV)
817.7271	184.068		DHSM 24	80	60
815.7115	184.068		DHSM 24_1	80	60
702.5922	266.2838	522.511	DHHexCer 16	80	60
730.6252	266.2838	550.541	DHHexCer 18	80	60
728.6096	266.2838	548.541	DHHexCer 18_1	80	60
758.6585	266.2838	578.571	DHHexCer 20	80	60
786.6915	266.2838	606.601	DHHexCer 22	80	60
814.7245	266.2838	634.631	DHHexCer 24	80	60
812.7089	266.2838	632.621	DHHexCer 24_1	80	60
864.645	266.2838	522.511	DHLacCer 16	80	60
892.678	266.2838	550.541	DHLacCer 18	80	60
890.6624	266.2838	548.541	DHLacCer 18_1	80	60
920.7113	266.2838	578.571	DHLacCer 20	80	60
948.7443	266.2838	606.601	DHLacCer 22	80	60
976.7773	266.2838	634.631	DHLacCer 24	80	60
974.7617	266.2838	632.621	DHLacCer 24_1	80	60
577.3639	264.2636	292.10	GM3 16	80	70
591.3796	264.2636	292.10	GM3 18	80	70
590.3718	264.2636	292.10	GM3 18_1	80	70
605.3953	264.2636	292.10	GM3 20	80	70
619.411	264.2636	292.10	GM3 22	80	70
633.4267	264.2636	292.10	GM3 24	80	70
632.4189	264.2636	292.10	GM3 24_1	80	70
1024.678	264.2636	520.51	Gb3 16	80	70
1052.711	264.2636	548.54	Gb3 18	80	70
1050.695	264.2636	546.54	Gb3 18_1	80	70
1080.744	264.2636	576.57	Gb3 20	80	70
1108.777	264.2636	604.60	Gb3 22	80	70
1136.81	264.2636	632.63	Gb3 24	80	70
1134.795	264.2636	630.62	Gb3 24_1	80	70

Supplemental Table S3. Mass spectrometry transition for evaluation of phytosphingolipid (Cer t18:0) profile via MRM method.

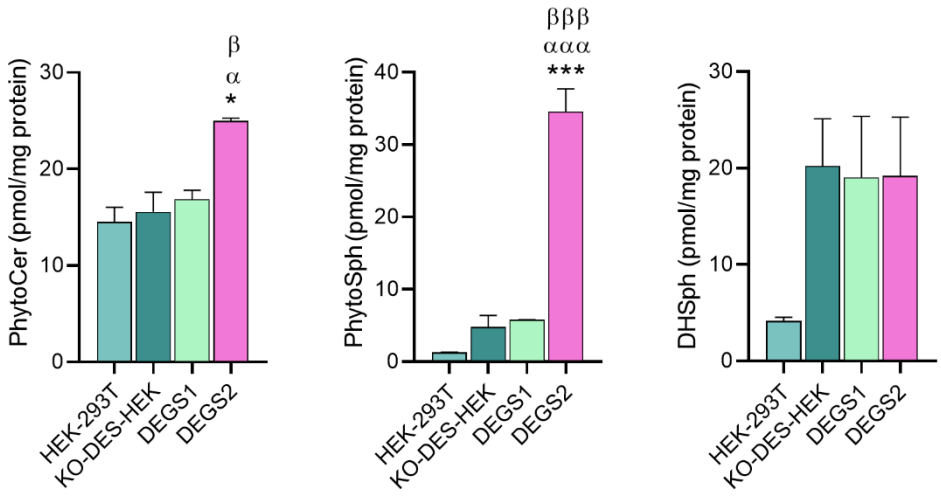
[M+H]+	MS/MS 1	MS/MS 2	Name	DP (eV)	CE (eV)
482.7	264.26		Cer IS	80	30
644.5	264.26		HexCer IS	80	50
556.53	300.2909	282.2791	Cer(t18:0/16:0)	80	35
584.5613	300.2909	282.2791	Cer(t18:0/18:0)	80	35
612.5926	300.2909	282.2791	Cer(t18:0/20:0)	80	35
640.6239	300.2909	282.2791	Cer(t18:0/22:0)	80	35
668.6552	300.2909	282.2791	Cer(t18:0/24:0)	80	35
666.5505	300.2909	282.2791	Cer(t18:0/24:1)	80	35

Supplemental Table S4. Mass spectrometry transition for evaluation of sphingoid bases profile via MRM method.

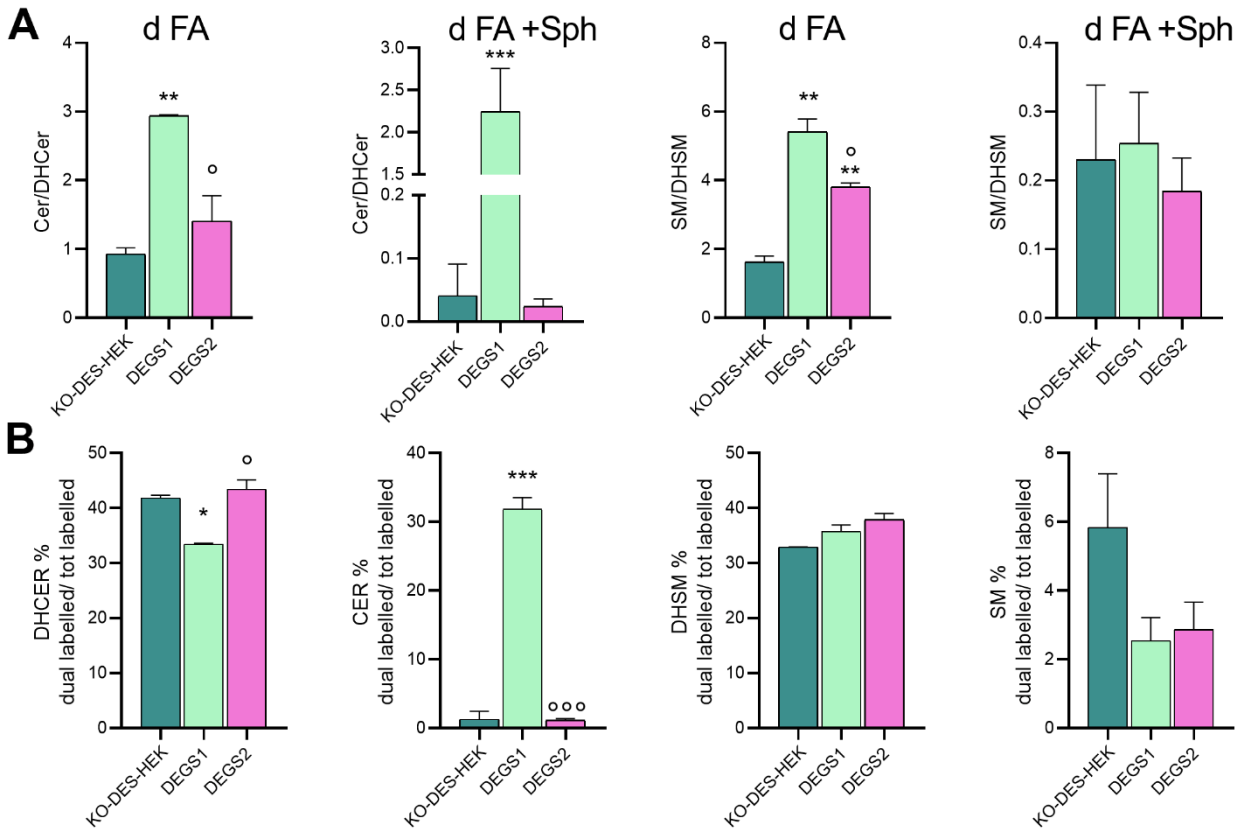
[M+H]+	MS/MS 1	MS/MS 2	Name	DP (eV)	CE (eV)
288.4	252.0		Sph IS	45	25
300.287	264.3	282.3	Sph	45	25
302.3053	266.3	284.3	DHSph	45	25
380.256	264.27	282.3	S1P	45	25
382.4	284.5	266.3	DHS1P	45	25
318.2993	282.3	264.3	PhytoSph	45	25

Supplemental Table S5. Mass spectrometry transition for ex novo synthesized sphingolipids by incorporation of d31 palmitic acid in both the sphingosine and fatty acid backbones (dual labelled) via MRM method.

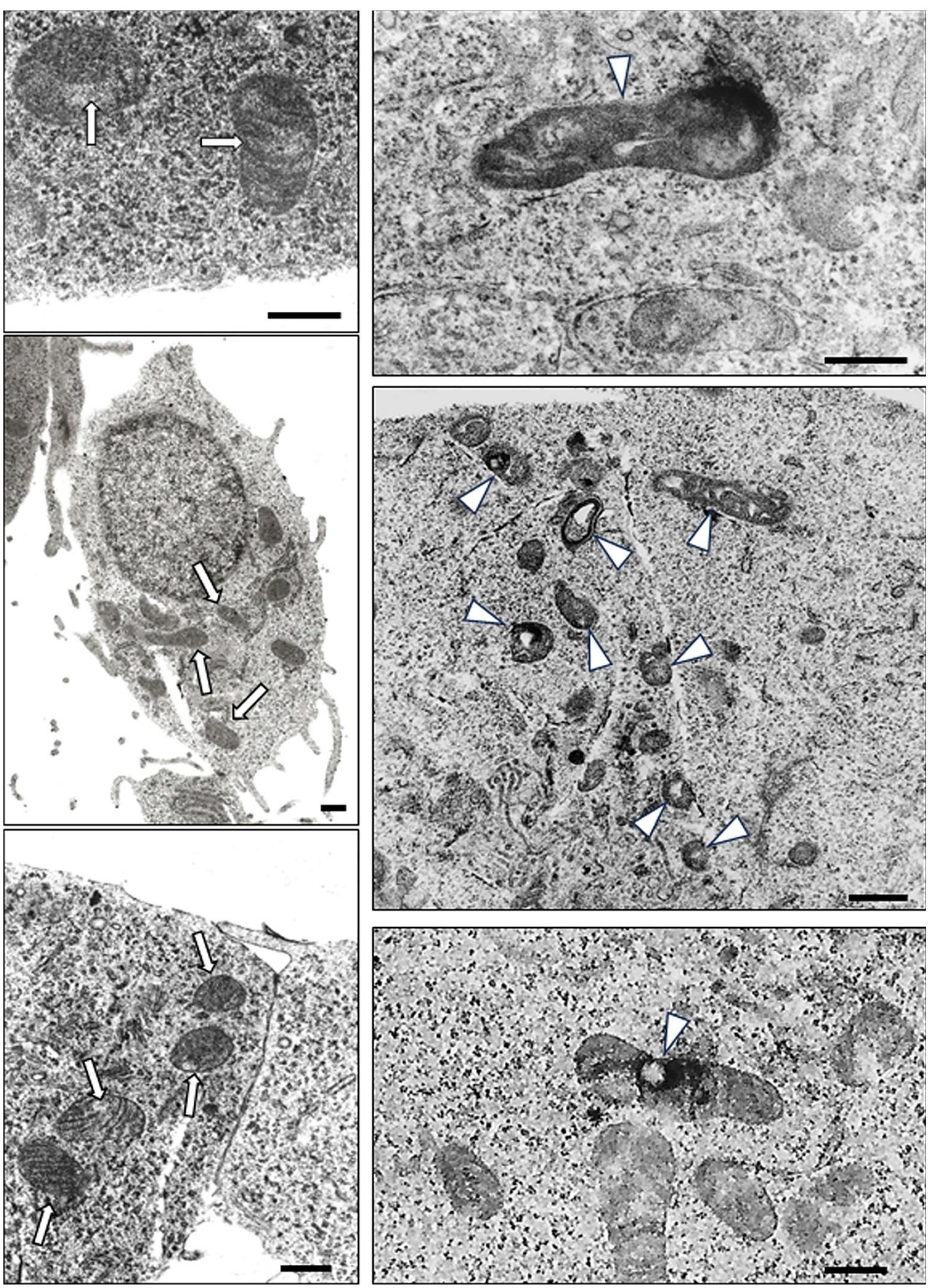
[M+H]+	MS/MS 1	Name	DP (eV)	CE (eV)
602.921	297.494	DHCer 16 dd31	80	35
598.9874	293.573	Cer 16 dd31	80	35
764.042	184.06	SM 16 dd31	80	40
767.98	184.06	DHSM 16 dd31	80	40
618.9189	331.49	phytoCer 16 dd31	80	40
761.040	293.573	HexCer 16 dd31	80	40
923.093	293.57	LacCer 16 dd31	80	40



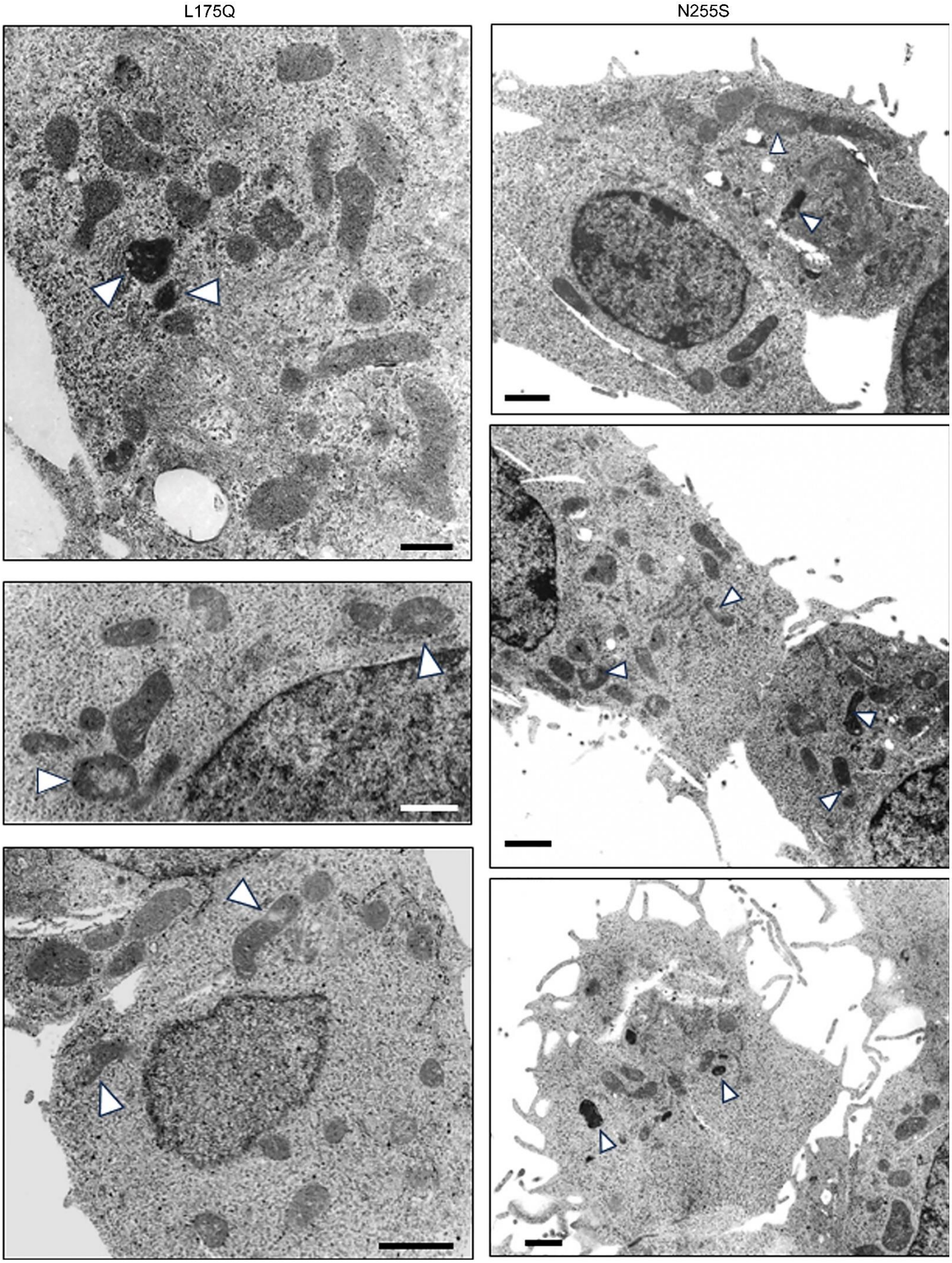
Supplemental Figure S1. Relevant phytosphingolipids detected in native HEK-293T cells, KO-DES-HEK, and KO-DES-HEK transfected with DEGS1 or DEGS2, as determined by LC-MS/MS. Significative results were investigated by t-tests and reported as * against HEK-293T, α against KO-DES-HEK, and β against DEGS1-transfected KO-DES-HEK.



Supplemental Figure S2. Comparison between the ex novo synthesis of sphingolipids that have incorporated d31 palmitic acid only in the fatty acid moiety or in both the sphingosine and fatty acid backbones (dual labelled). **(A)** Ratio of unsaturated on saturated sphingolipids detected in native KO-DES-HEK, and KO-DES-HEK transfected with DEGS1 or DEGS2 as determined by LC-MS/MS. **(B)** Percentage of dual labelled sphingolipids on the total amount of labelled sphingolipids. Significative results were investigated by one way ANOVA coupled with Bonferroni post hoc test and reported as * against KO-DES-HEK whereas ° against DEGS1.



Supplemental Figure S3: Larger view of Figure 6 of the main text for better evaluation of mitochondria in HEK-293T and KO-DES-HEK cells. Micrographs are presented at different magnification and identical scale bar (500 nm).



Supplemental Figure S4: Larger view of Figure 6 of the main text for better evaluation of mitochondria in L175Q and N255S clones. Micrographs are presented at different magnification and identical scale bar (500 nm).