Supplementary Information for

An N-terminal di-proline motif is essential for fatty acid-dependent degradation of Δ9-desaturase in Drosophila

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Running title: Role of di-proline motif in Δ9-desaturase degradation

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SUPPLEMENTAL TABLE I. Primer pairs used for plasmid construction by site-directed mutagenesis.

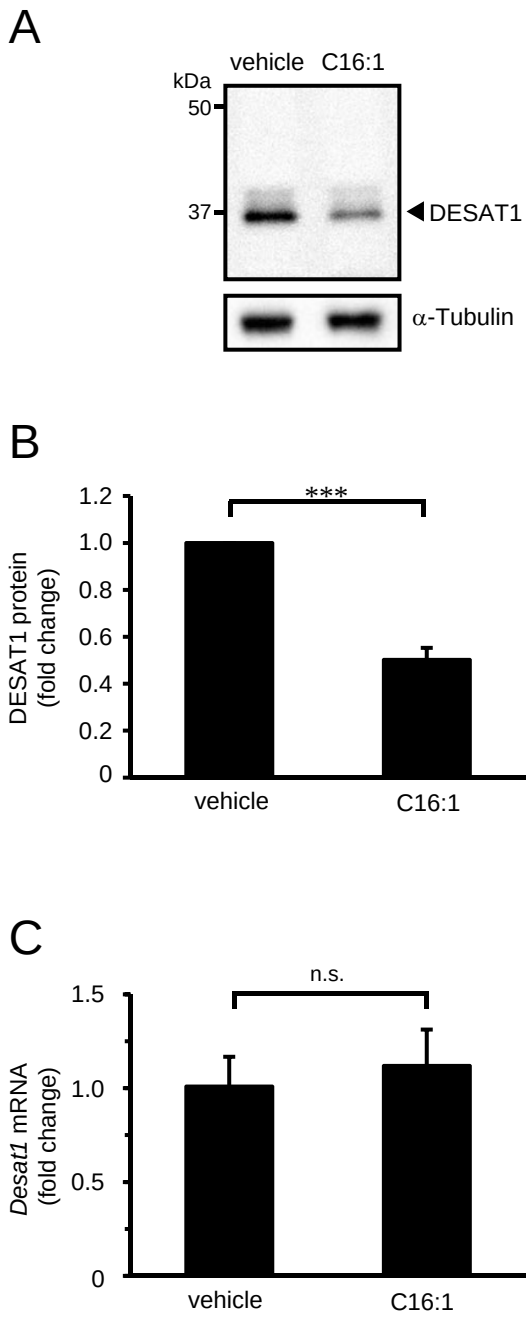
	Sequence (5' to 3')	Plasmid
Δ2-60-Fwd	TGAATGAATTCAACATGAAGAAGGCGAG	Δ2-60 DESAT1-FLAG^{C}
Δ2-41-Fwd	TAGAATTCAACATGTTCGAGTGCGATGTG	Δ2-41 DESAT1-FLAG^{C}
Δ2-21-Fwd	AGAATTCAACATGGCCGCCGCCGAC	Δ2-21 DESAT1-FLAG^{C}
Δ2-11-Fwd	CCGGAATTCAACATGATCTCAGACTCGCT	Δ2-11 DESAT1-FLAG^{C}
Δ2-6-Fwd	ATAGAATTCAACATGGCCGGTGCCCAGTC	Δ2-6 DESAT1-FLAG^{C}
C-term-Rev1	GGCGCTCTAGACTACTTGTCGTCGTCATC	Δ2-60 DESAT1-FLAG^{C}, Δ2-41 DESAT1-FLAG^{C}, Δ2-21 DESAT1-FLAG^{C}, Δ2-11 DESAT1-FLAG^{C}, Δ2-6 DESAT1-FLAG^{C}
N4A-Q6A-Fwd	CCCGCTGCTGCAGCCGGTGCCCAGTCCATC	DESAT1 (N4A/Q6A)-FLAG^{C}
N4A-Q6A-Rev	GGCTGCAGCAGCGGGCGGCATGTTGAATTCC	DESAT1 (N4A/Q6A)-FLAG^{C}
P2A-P3A-Fwd	TAGAATTCAACATGGCGGCCAACGCCCAAG	DESAT1 (P2A/P3A)-FLAG^{C}
P3A-Fwd	TAGAATTCAACATGCCGGCCAACGCCCAAG	DESAT1 (P3A)-FLAG^{C}
P2A-Fwd	TAGAATTCAACATGGCGCCCAACGCCCAAG	DESAT1 (P2A)-FLAG^{C}
C-term-Rev2	GCGCCCCTCTAGACTACTTGTCGTCGTCATCG TC	DESAT1 (P2A/P3A)-FLAG^{C}, DESAT1 (P3A)-FLAG^{C}, DESAT1 (P2A)-FLAG^{C}

SUPPLEMENTAL TABLE II. Primer pairs used for plasmid construction using an In-Fusion HD Cloning Kit.

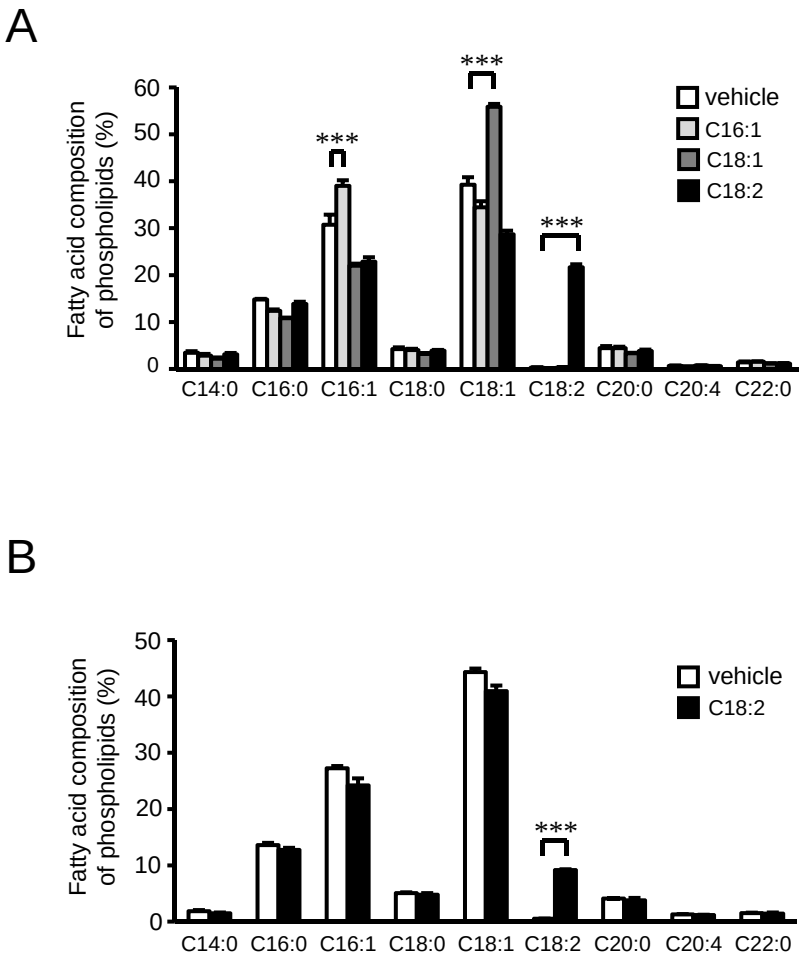
	Sequence (5' to 3')	Plasmid
Chimera-Fwd1	GCGTCATTTTGGCTCGAAATAGGCAATTAT	Chimera-FLAG^{C}, Chimera (AA)-FLAG^{C}
Chimera-Rev1	GATGTTCCTCCAGACGAGCTTCAGGCGGCG	Chimera-FLAG^{C}, Chimera (AA)-FLAG^{C}
Chimera-Fwd2	GTCTGGAGGAACATCATTCTCATGGTC	Chimera-FLAG^{C}, Chimera (AA)-FLAG^{C}
Chimera-Rev2	GAGCCAAAATGACGCATAAAAGGAAACGGC	Chimera-FLAG^{C}, Chimera (AA)-FLAG^{C}
Scd1-di-Pro-Fwd	ATGCCGCCCCACATGCTCCAAGAG	SCD1 (di-Pro)-FLAG^{C}
Scd1-di-Pro-Rev	CATGTGGGGCGGCATGGCGATCGC	SCD1 (di-Pro)-FLAG^{C}
Δ7-33-Fwd	GCCCAACAGGAGGACTCCACCGGAGTG	Δ7-33 DESAT1-FLAG^{C}
Δ7-33-Rev	GTCCTCCTGTTGGGCGTTGGGCGGCAT	Δ7-33 DESAT1-FLAG^{C}
Δ34-59-Fwd	ACCAAGCTGCAGAAGGCCGAGAAG	Δ34-59 DESAT1-FLAG^{C}
Δ34-59-Rev	CTTCTGCAGCTTGGTGGGGCTCTG	Δ34-59 DESAT1-FLAG^{C}
Δ7-59-Fwd	GCCCAAAAGAAGGCCGAGAAGCGCCGC	Δ7-59 DESAT1-FLAG^{C}
Δ7-59-Rev	GGCCTTCTTTTGGGCGTTGGGCGGCAT	Δ7-59 DESAT1-FLAG^{C}
Internal-FLAG-Fwd	AAAGACGATGACGACGACAAGGTCGATCTATCCGATCTG	DESAT1-FLAG^{Internal}
Internal-FLAG-Rev	GTCGTCATCGTCTTTGTAGTCCCCCTTGCCCTTGGCTTT	DESAT1-FLAG^{Internal}

SUPPLEMENTAL TABLE III. Primer pairs used for preparation of dsRNA.

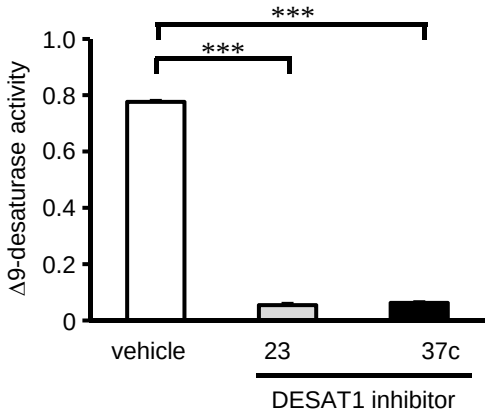
	Sequence (5’ to 3’)
Calpain-A-Fwd	TAATACGACTCACTATAGGGCAATTTGTCGCCGGACTC
Calpain-A-Rev	TAATACGACTCACTATAGGGATAAAGTGCGGCGATCTTC
Calpain-B-Fwd	TAATACGACTCACTATAGGGCTCGTTGCCCTATCCATCAT
Calpain-B-Rev	TAATACGACTCACTATAGGGAACATGTTCTGGTTCTCGGG
Calpain-C-Fwd	TAATACGACTCACTATAGGGCAGCTGGTGTGCCTGAA
Calpain-C-Rev	TAATACGACTCACTATAGGGTCTTGAAGAAGTCCTTCTGC
SOL-Fwd	TAATACGACTCACTATAGGGGCGAAGATCTGGTCAAGGAG
SOL-Rev	TAATACGACTCACTATAGGGACTGGCCTGTAGTGGAATGC
GFP-Fwd	ATTAATACGACTCACTATAGGGAGGAGGGCGAGGAGCTGTTCACC
GFP-Rev	ATTAATACGACTCACTATAGGGAGGCTCGTCCATGCCGAGAGTG



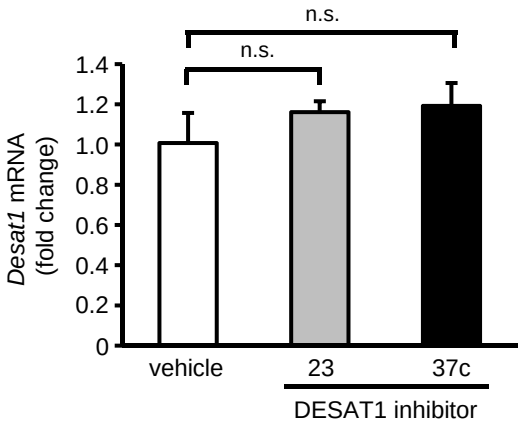
SUPPLEMENTAL FIGURE 1. Effect of unsaturated fatty acids on DESAT1 expression. S2 cells were treated with the indicated fatty acid (100 μM) for 6 h, and the amounts of DESAT1 and α-Tubulin protein were detected with anti-DESAT1 antibody and anti-α-Tubulin antibody, respectively (A, B). Band intensities were determined by Image J software, and levels of DESAT1 proteins are shown relative to the amount of DESAT1 protein in vehicle-treated cells (B). S2 cells were treated with the indicated fatty acid (100 μM) for 6 h, and the expression levels of *Desat1* mRNA were determined by real-time PCR (C). Mean ± SD (n = 3). ***$P < 0.001$; n.s., not significant.



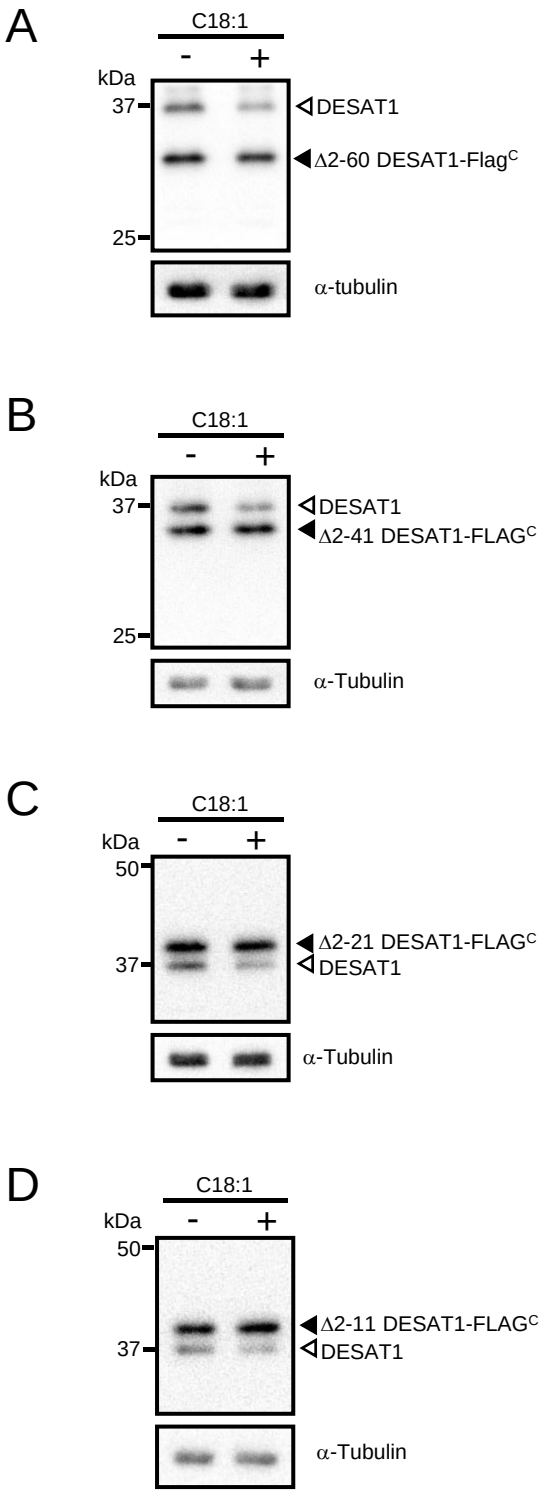
SUPPLEMENTAL FIGURE 2. Effect of unsaturated fatty acids on the fatty acid composition of cellular phospholipids. S2 cells were treated with the indicated fatty acid (100 μM) for 6 h (A) or 1 h (B), and fatty acid compositions of phospholipids were analyzed by gas chromatography. Mean ± SD (n = 3). ****P* < 0.001.



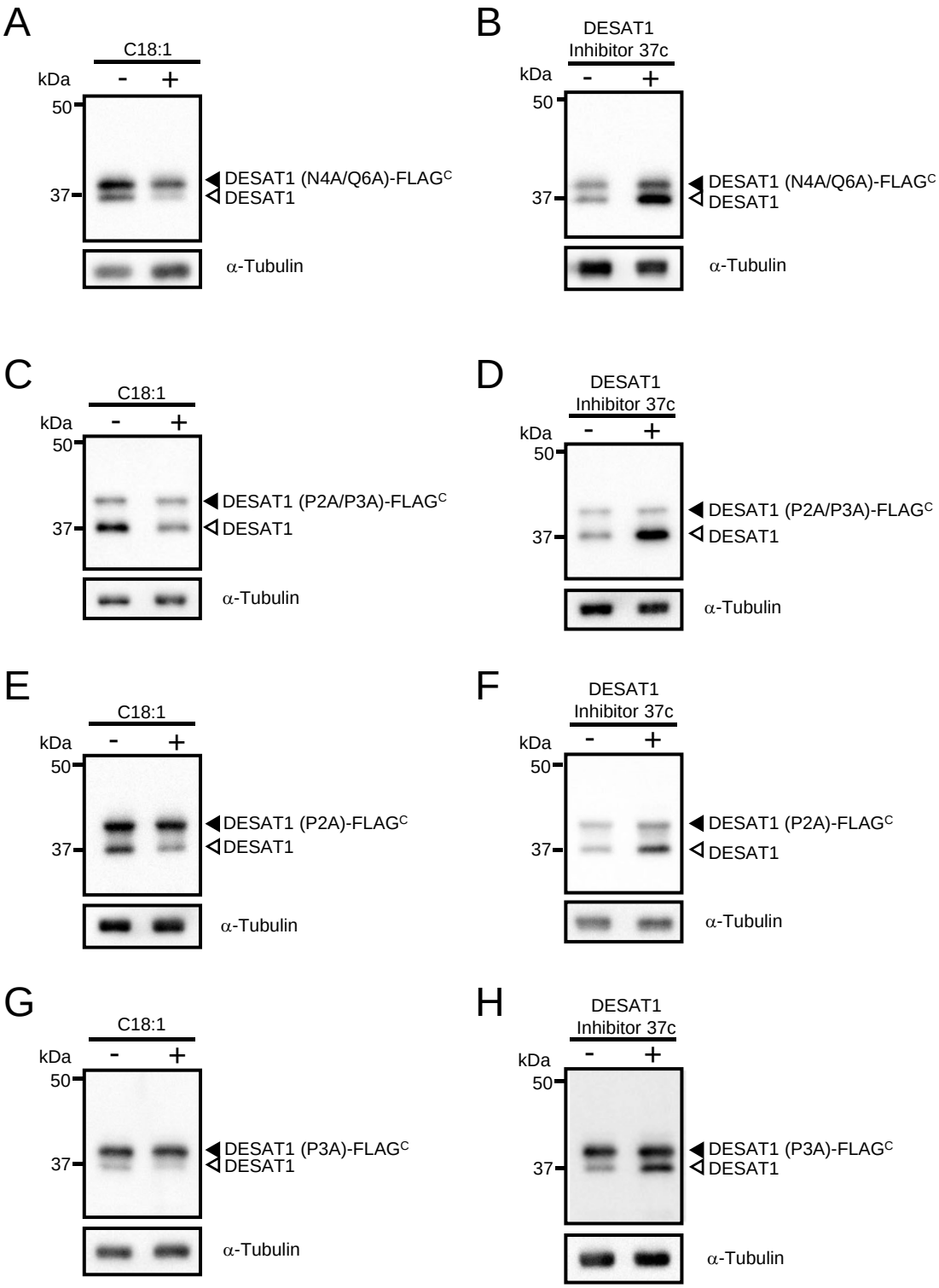
SUPPLEMENTAL FIGURE 3. Effect of SCD1 inhibitors on Δ9-desaturase activity of DESAT1. S2 cells were treated with SCD1 inhibitor 23 or 37c (1 μM) in the presence of C17:0 (20 μM) for 6 h. The conversion of C17:0 to C17:1 was determined by gas chromatography. Δ9-desaturase activity was defined as the ratio of the amount of C17:1 to the total amount of C17 fatty acid species. The proportions of incorporated 17-carbon length fatty acids (C17:0 and C17:1) in the acyl chains of phospholipids were 4.4 ± 0.2%, 4.7 ± 0.3%, and 3.8 ± 0.0% in vehicle-, SCD1 inhibitor 23-, and SCD1 inhibitor 37c-treated cells, respectively. Mean ± SD (n = 3). ****P* < 0.001.



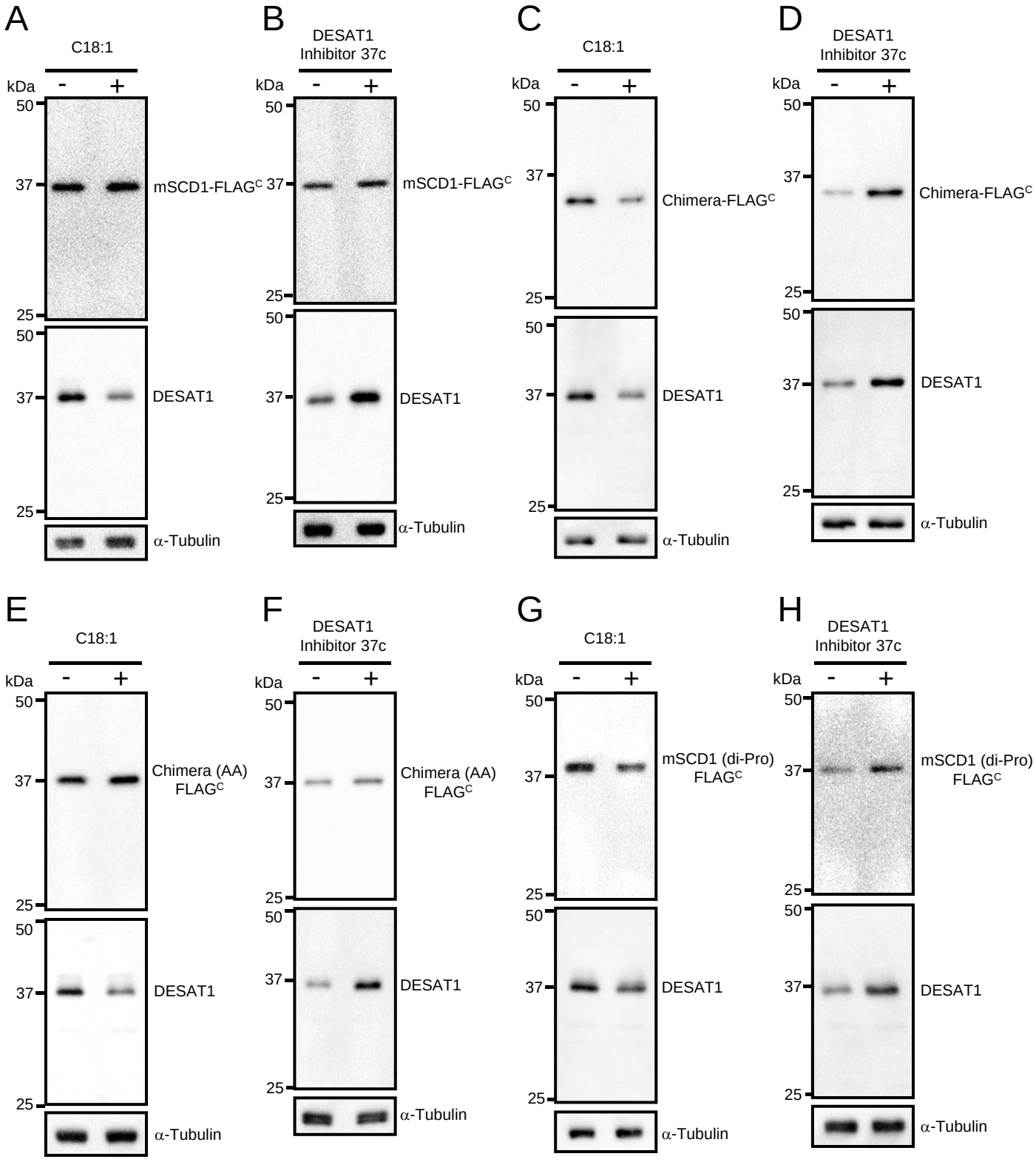
SUPPLEMENTAL FIGURE 4. Effect of DESAT1 inhibitors on DESAT1 expression. S2 cells were treated with DESAT1 inhibitor 23 or 37c (1 μM) for 16 h, and the expression levels of *Desat1* mRNA were determined by real-time PCR. Mean ± SD (n = 3). n.s., not significant.



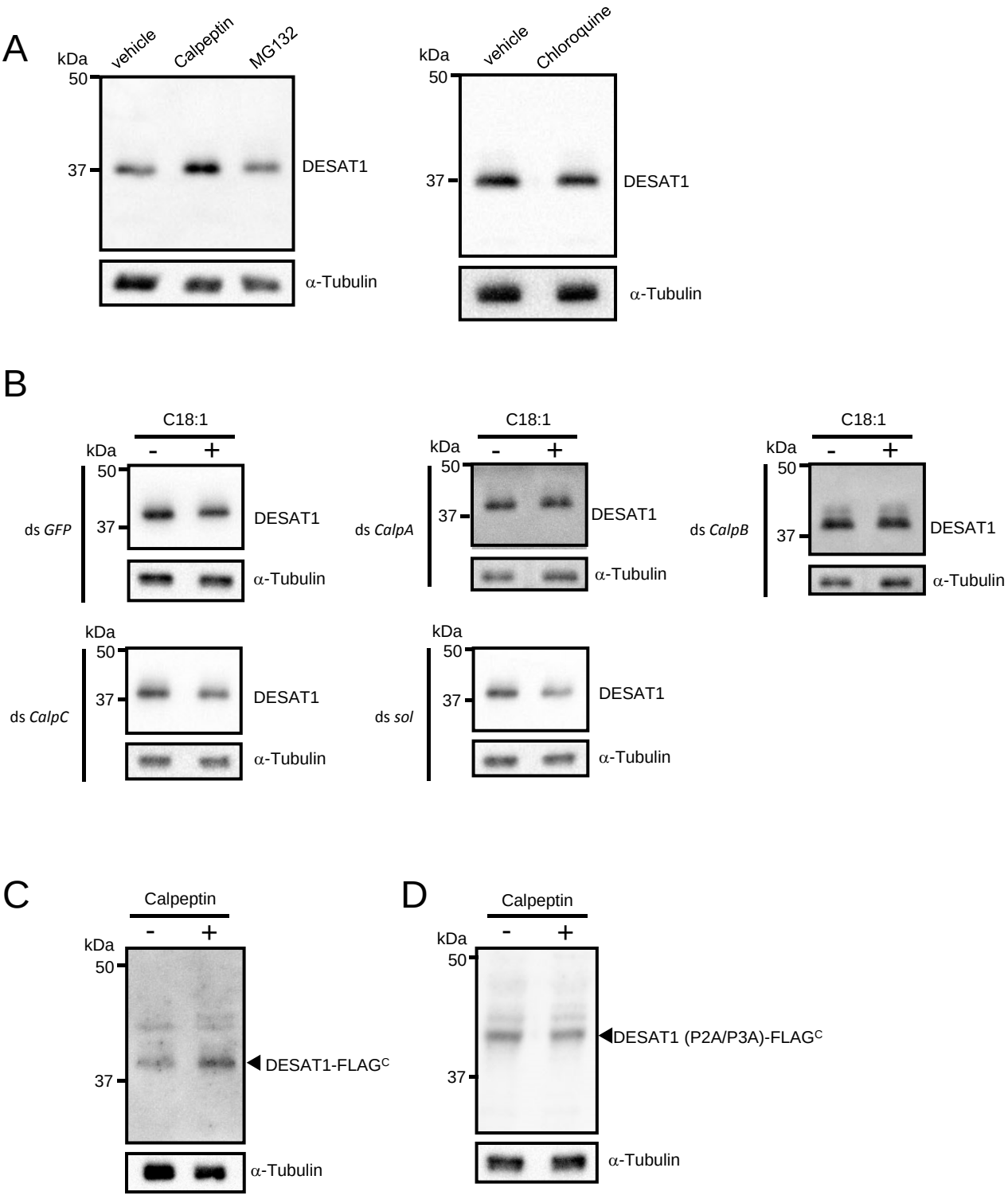
SUPPLEMENTAL FIGURE 5. Role of N-terminal domain of DESAT1 in the regulation of degradation of DESAT1. S2 cells expressing Δ2-60 DESAT1-FLAG^{C} (A), Δ2-41 DESAT1-FLAG^{C} (B), Δ2-21 DESAT1-FLAG^{C} (C), and Δ2-11 DESAT1-FLAG^{C} (D) were treated with C18:1 (100 μM) for 6 h, and the amounts of DESAT1 and α-Tubulin protein were detected with anti-DESAT1 antibody and anti-α-Tubulin antibody, respectively. Filled arrowhead, exogenously expressed DESAT1-FLAG^{C}; open arrowhead, endogenous DESAT1.



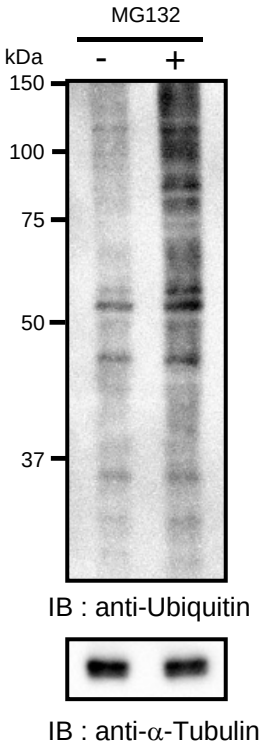
SUPPLEMENTAL FIGURE 6. Role of N-terminal residues of DESAT1 in the regulation of degradation of DESAT1. S2 cells expressing DESAT1 (N4A/Q6A)-FLAG^C (A, B), DESAT1 (P2A/P3A)-FLAG^C (C, D), DESAT1 (P2A)-FLAG^C (E, F), and DESAT1 (P3A)-FLAG^C (G, H) mutant were treated with C18:1 (100 μM) for 6 h (A, C, E, G) or DESAT1 inhibitor 37c (1 μM) for 16 h (B, D, F, H), and the amounts of DESAT1 and α-Tubulin protein were detected with anti-DESAT1 antibody and anti-α-Tubulin antibody, respectively. Filled arrowhead, exogenously expressed DESAT1-FLAG^C; open arrowhead, endogenous DESAT1.



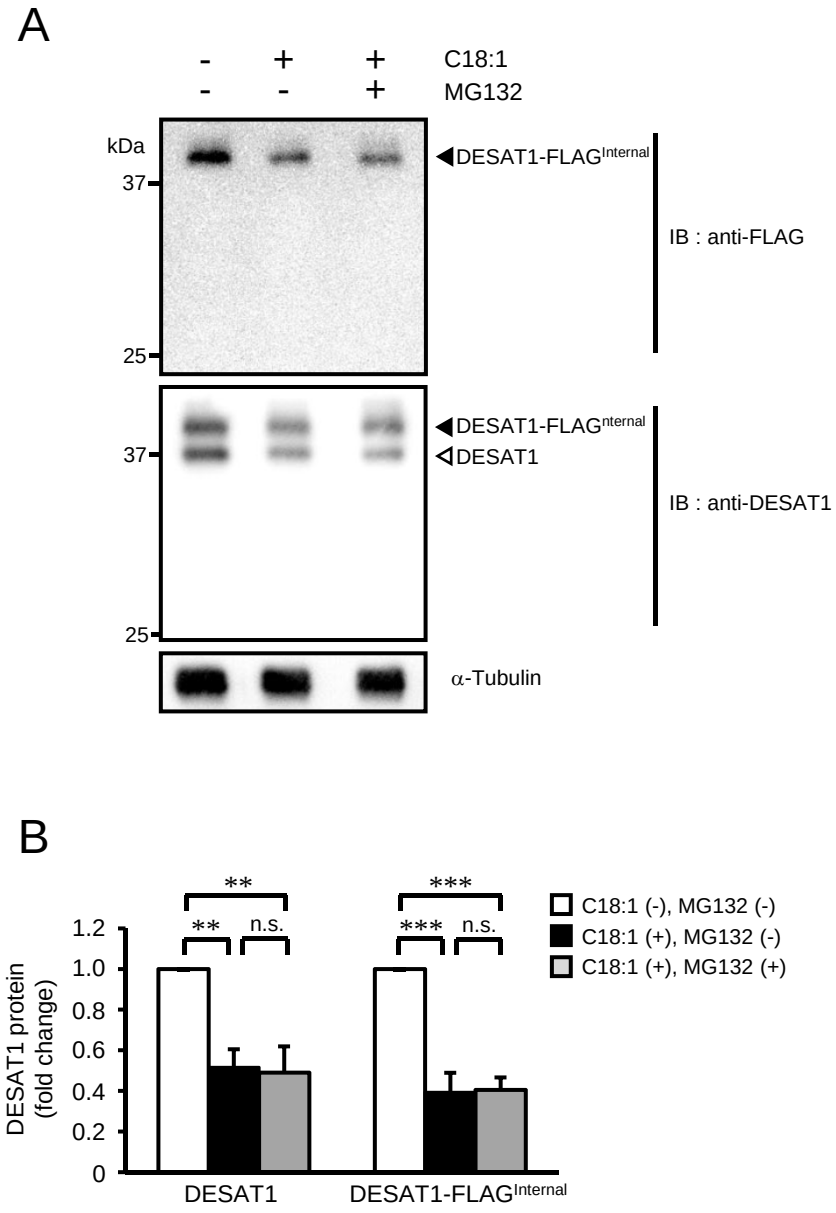
SUPPLEMENTAL FIGURE 7. Role of di-proline motif in the degradation of Δ9-desaturase. S2 cells expressing mouse SCD1-FLAG^C (A, B), Chimera-FLAG^C (C, D), Chimera (AA)-FLAG^C (E, F), and SCD1 (di-Pro)-FLAG^C (G, H) were treated with C18:1 (100 μM) for 6 h (A, C, E, G) or DESAT1 inhibitor 37c (1 μM) for 16 h (B, D, F, H), and the amounts of endogenous DESAT1, FLAG-tagged exogenously expressed SCD1, and α-Tubulin protein were detected with anti-DESAT1 antibody, anti-FLAG antibody, and anti-α-Tubulin antibody, respectively.



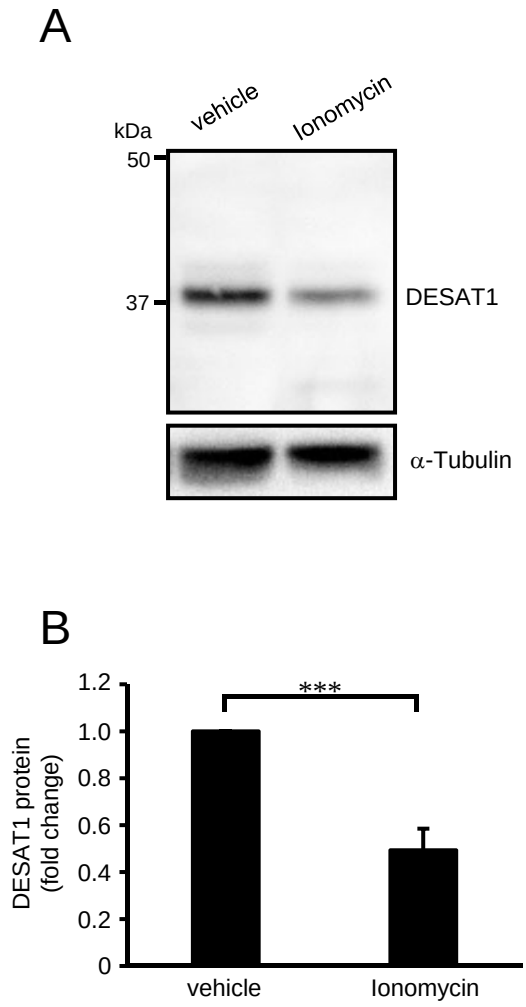
SUPPLEMENTAL FIGURE 8. Identification of protease involved in the degradation of DESAT1. S2 cells were treated with calpeptin (50 μM), MG132 (50 μg/ml), or chloroquine (50 μM) for 6 h (A). S2 cells were treated with dsRNA against *GFP*, *CalpA*, *CalpB*, *CalpC*, or *sol* for 72 h and then exposed to C18:1 (100 μM) for 6 h (B). S2 cells expressing DESAT1-FLAG^{C} (C), and DESAT1 (P2A/P3A)-FLAG^{C} (D) were treated with calpeptin (50 μM) for 6 h. The amounts of endogenous DESAT1, FLAG-tagged exogenously expressed DESAT1, and α-Tubulin protein were detected with anti-DESAT1 antibody, anti-FLAG antibody, and anti-α-Tubulin antibody, respectively (A-D).



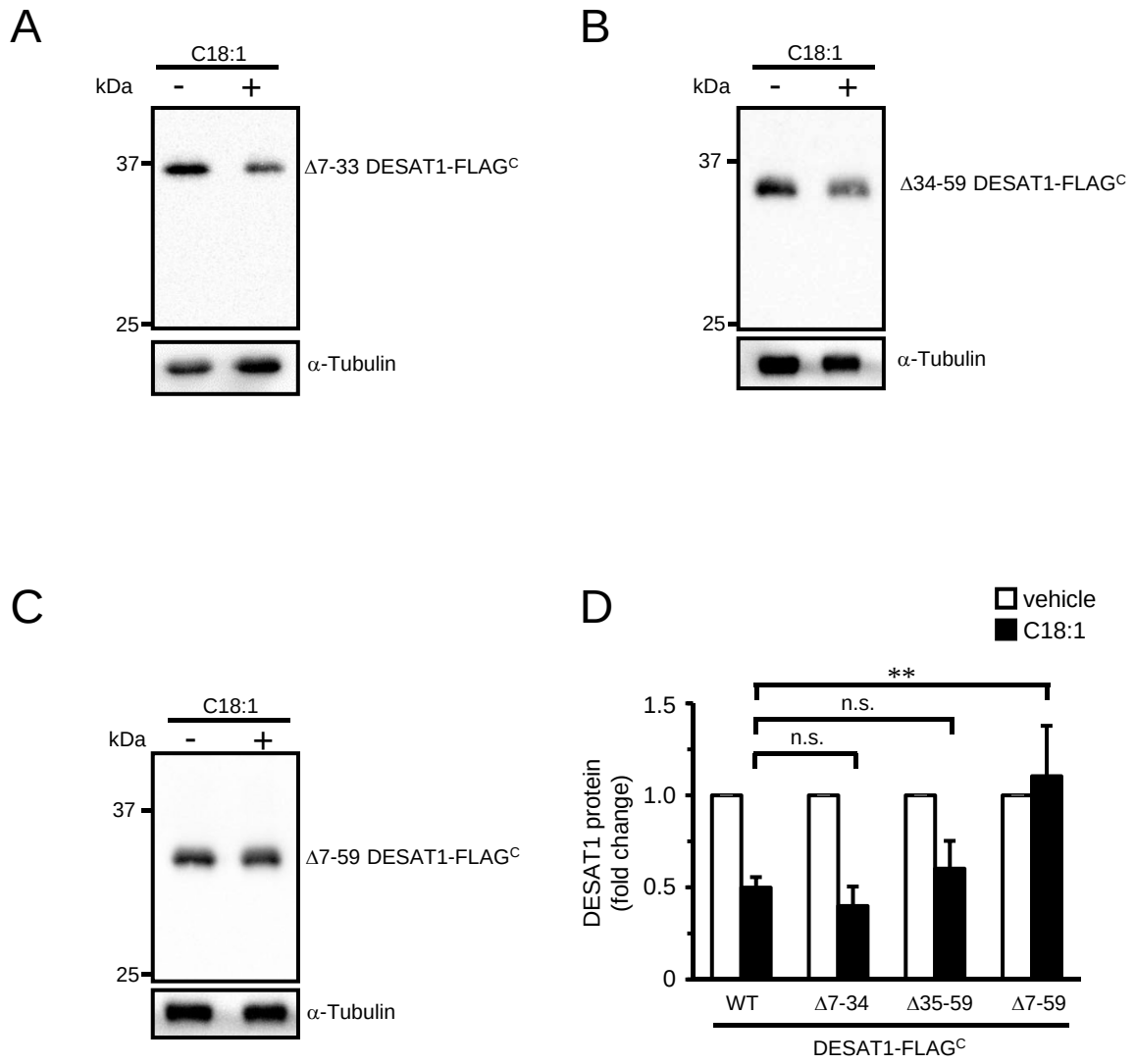
SUPPLEMENTAL FIGURE 9. Effect of MG132 on the removal of ubiquitinated proteins. S2 cells were treated with MG132 (50 μg/ml) for 6 h. The amounts of ubiquitinated proteins and α-Tubulin protein were detected with indicated antibodies.



SUPPLEMENTAL FIGURE 10. Effect of MG132 on the degradation of internally FLAG-tagged DESAT1. S2 cells expressing DESAT1-FLAG^{Internal} were treated with C18:1 (100 μM) and MG132 (50 μg/ml) for 6 h, and the amounts of endogenous DESAT1, FLAG-tagged exogenously expressed DESAT1, and α-Tubulin protein were detected with anti-DESAT1 antibody, anti-FLAG antibody, and anti-α-Tubulin antibody, respectively (A, B). Band intensities were determined by Image J software, and levels of DESAT1 proteins are shown relative to the amount of DESAT1 protein in vehicle-treated cells (B). Mean ± SD (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant.



SUPPLEMENTAL FIGURE 11. Effect of calcium ionophore on the degradation of DESAT1 protein. S2 cells were treated with ionomycin (5 μM) for 1 h, and the amounts of DESAT1 and α-Tubulin protein were detected with anti-DESAT1 antibody and anti-α-Tubulin antibody, respectively (A, B). Band intensities were determined by Image J software, and levels of DESAT1 proteins are shown relative to the amount of DESAT1 protein in vehicle-treated cells (B). Mean ± SD (n = 3). ****P* < 0.001.



SUPPLEMENTAL FIGURE 12. Role of residues 7-59 on the regulation of degradation of DESAT1. S2 cells expressing Δ7-33 DESAT1-FLAG^C (A, D), Δ34-59 DESAT1-FLAG^C (B, D), and Δ7-59 DESAT1-FLAG^C (C, D) were treated with C18:1 (100 μM) for 6 h, and the amounts of FLAG-tagged DESAT1 and α-Tubulin protein were detected with anti-FLAG antibody and anti-α-Tubulin antibody, respectively. Band intensities were determined by Image J software, and levels of DESAT1 proteins are shown relative to the amount of DESAT1 protein in vehicle-treated cells (D). The values for DESAT1-FLAG^C (Figure 3D) were shown for comparison (D). Mean ± SD (n = 3). $^{**}P < 0.01$; n.s., not significant.