Classification: Biological Sciences: Cell Biology

Supporting Information (SI):

Identification of a Degradation Signal at the Carboxy-Terminus of SREBP2: A New Role for this Domain in Cholesterol Homeostasis

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This section contains:

Materials

Reagents	Source	Identifier
Antibodies		
Rabbit monoclonal Anti- SREBP2	Ref. (1)	IgG-22D5 (used at 10 µg/mL)
Mouse monoclonal Anti-Scap	Ref. (2)	IgG-4H4 (used at 5 µg/mL)
Mouse monoclonal Anti-SREBP2	Refs. (3, 4)	IgG-1C6 (used at 1.6 µg/mL)
Mouse monoclonal Anti-Scap	This paper	IgG-2G10 (used at 5 µg/mL)
Mouse monoclonal anti-FLAG M2 clone	Sigma-Aldrich	Cat# F1804, RRID:AB_262044 (1:1000)
Rabbit monoclonal Anti-Actin	Sigma-Aldrich	Cat# A2066, RRID:AB_476693 (1:1000)
Rabbit polyclonal Anti-Calnexin	Novus Biologicals	Cat# NB100-1974, RRID:AB_10001873 (1:5000)
Mouse Anti-His clone HIS.H8	EMD Millipore	Cat# 05-949 RRID:AB_492660 (1:1000)
mouse monoclonal Anti-Myc clone 9B11	Cell Signaling Technology	Product # 2276S (1:1000)
Rabbit polyclonal Anti-GM130	Proteintech	Cat# 11308-1-AP (1:5000)
mouse monoclonal Anti-Sec61a	Santa Cruz	Cat# sc-393182 (1:500)
Cell Lines		
SV-589	Ref. (5)	N/A
SV-589 SREBP2-deficient	This study	TR-4411; See Methods
Sf9	Invitrogen	Cat# 11496015
CHO-K1	ATCC CCL-61	N/A
CHO-K1 Scap-null cells	Ref. (6)	SRD-13A
Chemicals, Peptides, and Recombinant proteins		
Benzonase	Sigma-Aldrich	Cat # E1014; CAS # 9025-65-4
3xFLAG peptide	APExBio	Cat# A6001
25-hydroxycholesterol	Steraloids	Cat# C6550-000
Sodium Compactin	Ref. (7)	N/A
Sodium Mevalonate	Ref. (7)	N/A
Sodium Oleate	Ref. (8)	N/A
Cycloheximide	Sigma-Aldrich	Cat# C1988, CAS# 66-81-9
Tris (2-Carboxyethyl) phosphine Hydrochloride (TCEP-HCl)	Goldbio	CAT# TCEP1 CAS# 51805-45-9

X-tremeGENE HP DNA transfection reagent	Millipore Sigma	Cat# XTGHP-RO
FuGENE 6 transfection reagent	Promega	E2692
Iodoacetamide	Sigma-Aldrich	I1149
Hydroxypropyl-β-cyclodextrin (HPCD)	Cyclodextrin Technologies Development	Cat# THPB-P
MG-132	Peptide Institute	Code 3175-v
N-Acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN)	Calbiochem	Cat # 208719 CAS# 110044-82-1
Digitonin	Calbiochem	Cat # 300410, CAS# 11024-24-1
NEBuilder HiFI DNA Assembly	NEB	Cat # E2621L
Protease Inhibitor Cocktail Set III, EDTA-Free	Calbiochem	Cat# 539134
Phenylmethylsulfonyl fluoride (PMSF)	Sigma-Aldrich	Cat # 93482 CAS# 329-98-6
Quickchange II XL mutagenesis kit	Agilent	Cat # 200521
Ni-NTA Agarose	Qiagen	Cat # 30250
Anti-FLAG M2 affinity resin	Sigma-Aldrich	Cat# A2220
Fetal Calf Serum	Sigma-Aldrich	N/A
Newborn calf lipoprotein-deficient serum (LPDS)	Ref. (8)	N/A
Software		
Prism 7	GraphPad	N/A
Image Studio v5.0	LiCor Biosciences	N/A
Recombinant DNA		
5'-CAATTTGTCAGTAATCAAGTGGG-3'	IDT	sgRNA upstream target for human SREBP2
5'-GCTGCATTCTGGTATATCAA-3'	IDT	sgRNA downstream target for human SREBP2
pTK-Scap (plasmid)	Ref. (9)	N/A
pTK-2xMyc-Scap CTD (plasmid)	This paper	NA
pTK-3xFLAG-SREBP2 14-1141 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-1068 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-1031 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-969 (plasmid)	This paper	N/A

pTK-3xFLAG-SREBP2 14-867 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-828 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-736 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-679 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 14-587 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP2 G1069A, E1070A, V1071A (plasmid)	This paper	Exon 19 mutant 1
pTK-3xFLAG-SREBP2 D1072A, A1073S, W1072A (plasmid)	This paper	Exon 19 mutant 2
pTK-3xFLAG-SREBP2 14-1141 P1075A, G1076A, Q1077A (plasmid)	This paper	Exon 19 mutant 3
pTK-3xFLAG-SREBP2 R1078A, E1079A, R1080A (plasmid)	This paper	Exon 19 mutant 4
pTK-3xFLAG-SREBP2 A1081S, T1082A, A1083S (plasmid)	This paper	Exon 19 mutant 5
pTK-3xFLAG-SREBP2 I1084A, L1085A, L1086A (plasmid)	This paper	Exon 19 mutant 6
pTK-3xFLAG-SREBP2 A1087S, C1088A, R1089A (plasmid)	This paper	Exon 19 mutant 7
pTK pTK-3xFLAG-SREBP2 H1090A, L1091A, P1092A (plasmid)	This paper	Exon 19 mutant 8
pTK- pTK 3xFLAG-SREBP2 L1093A, S1084A, F1095A(plasmid)	This paper	Exon 19 mutant 9
pTK 3xFLAG-SREBP2 L1096A, S1097A, S1098A (plasmid)	This paper	Exon 19 mutant 10
pTK 3xFLAG-SREBP2 P1099A, G1100A, Q1101A (plasmid)	This paper	Exon 19 mutant 11
pTK 3xFLAG-SREBP2 R1102A, A1103S, V1104A (plasmid)	This paper	Exon 19 mutant 12
pTK 3xFLAG-SREBP2 L1105A, L1106A, A1107S (plasmid)	This paper	Exon 19 mutant 13
pTK 3xFLAG-SREBP2 E1108A, A1109S, A1110S (plasmid)	This paper	Exon 19 mutant 14

pTK 3xFLAG-SREBP2 R1111A, T1112A, L1113A (plasmid)	This paper	Exon 19 mutant 15
pTK 3xFLAG-SREBP2 I1114A, K1115A, V1115A (plasmid)	This paper	Exon 19 mutant 16
pTK 3xFLAG-SREBP2 G1117A, D1118A, R1119A (plasmid)	This paper	Exon 19 mutant 17
pTK 3xFLAG-SREBP2 R1120A, S1121A, C1123A (plasmid)	This paper	Exon 19 mutant 18
pTK 3xFLAG-SREBP2 N1123A, D1124A, C1125A (plasmid)	This paper	Exon 19 mutant 19
pTK 3xFLAG-SREBP2 Q1126A, Q1127A, M1128A (plasmid)	This paper	Exon 19 mutant 20
pTK 3xFLAG-SREBP2 I1129A, V1130A, K1131A (plasmid)	This paper	Exon 19 mutant 21
pTK 3xFLAG-SREBP2 L1132A, G1133A, G1134A (plasmid)	This paper	Exon 19 mutant 22
pTK 3xFLAG-SREBP2 G1135A, T1136A, A1137S (plasmid)	This paper	Exon 19 mutant 23
pTK 3xFLAG-SREBP2 I1138A, A1139S, A1140S, S1141A (plasmid)	This paper	Exon 19 mutant 24
pTK 3xFLAG-SREBP2 I1129A, V1130A, K1131A, I1138A, A1139S, A1140S, S1141A (plasmid)	This paper	SREBP2(Deg-R)
pTK 3xFLAG-SREBP2 I1129A (plasmid)	This paper	N/A
pTK 3xFLAG-SREBP2 V130A (plasmid)	This paper	N/A
pTK 3xFLAG-SREBP2 K1131A (plasmid)	This paper	N/A
pTK 3xFLAG-SREBP2 I1138A (plasmid)	This paper	N/A
pTK 3xFLAG-SREBP2 A1139S (plasmid)	This paper	N/A
pTK 3xFLAG-SREBP2 A1140S (plasmid)	This paper	N/A
pTK3xFLAG-SREBP2 S1141A	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 V1032A, F1033A, L1034A (plasmid)	This paper	N/A

pTK-3xFLAG SREBP2 14-1068 H1035A, E1036A, A1037S (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 T1038A, V1039A, R1040A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 L1041A, M1042A, A1043S (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 G1044A, A1045S, S1046A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 P1047A, T1048A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 R1049A, T1050A, H1051A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 Q1052A, L1053A, L1054A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 E1055A, H1056A, (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 S1057A, L1058A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 R1059A, R1060A, R1061A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 T1062A, T1063A, Q1064A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 S1065A, T1066A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 K1067A, H1068A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 H1035A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 E1036A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 A1037S (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 T1038A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 V1039A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 R1040A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 L1041A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 M1042A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 A1043S (plasmid)	This paper	N/A

pTK-3xFLAG SREBP2 14-1068 G1044A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 A1045S (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 S1046A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 P1047A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 T1048A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 R1049A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 T1050A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 H1051A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 Q1052A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 L1053A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 14-1068 L1054A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 R1040A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 R1040S (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 R1040E (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 R1040E, I1129A, V1130A, K1131A, I1138A, A1139S, A1140S, S1141A (plasmid)	This paper	N/A
pTK-3xFLAG SREBP2 K555R K579R K630R K639R K640R K660R K681R K707R K725R K731R K768R K778R K782R K805R K817R K821R K822R K823R K842R K865R K901R K907R K918R K934R K989R K1031R K1067R K1115R K1131R plasmid)	This paper	All CTD lysine mutants
pTK-3xFLAG SREBP2 K555R K579R K630R K639R K640R K660R K681R K707R K725R K731R K768R K778R K782R K805R K817R K821R K822R K823R K842R K865R (plasmid)	This paper	lysine mutants Group 1

pTK-3xFLAG SREBP2 K901R K907R K918R K934R K989R K1031R K1067R K1115R K1131R (plasmid)	This paper	lysine mutants Group 2
pTK-3xFLAG SREBP2 - Myc (S1141+EQKLISEEDL-COOH)	This paper	N/A
pTK-3xFLAG SREBP2 - FLAG (S1141+DYKDDDDK-COOH)	This paper	N/A
pTK-3xFLAG SREBP2 - 6xHis (S1141+HHHHHH-COOH)	This paper	N/A
pTK-3xFLAG SREBP2 + A1142	This paper	N/A
pTK-3xFLAG SREBP2 + G1142	This paper	N/A
pTK-3xFLAG SREBP2 + S1142	This paper	N/A
pTK-3xFLAG SREBP2 + T1142	This paper	N/A
pTK-3xFLAG SREBP2 + C1142	This paper	N/A
pTK-3xFLAG SREBP2 + M1141	This paper	N/A
pTK-3xFLAG SREBP2 + P1142	This paper	N/A
pTK-3xFLAG SREBP2 + H1142	This paper	N/A
pTK-3xFLAG SREBP2 + K1142	This paper	N/A
pTK-3xFLAG SREBP2 + R1142	This paper	N/A
pTK-3xFLAG SREBP2 + N1142	This paper	N/A
pTK-3xFLAG SREBP2 + Q1142	This paper	N/A
pTK-3xFLAG SREBP2 + D1142	This paper	N/A
pTK-3xFLAG SREBP2 + E1142	This paper	N/A
pTK-3xFLAG SREBP2 + I1142	This paper	N/A
pTK-3xFLAG SREBP2 + L1142	This paper	N/A
pTK-3xFLAG SREBP2 + V1142	This paper	N/A
pTK-3xFLAG SREBP2 + F1142	This paper	N/A
pTK-3xFLAG SREBP2 + Y1142	This paper	N/A
pTK-3xFLAG SREBP2 + W1142	This paper	N/A
pTK-3xFLAG-SREBP-1a (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a 1-1071 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a 1-1034 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a 1-967 (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a R1043A (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a R1043E (plasmid)	This paper	N/A
pTK-3xFLAG-SREBP-1a R1043S (plasmid)	This paper	N/A
pEZT-2xMyc-TEV-SREBP2 (plasmid)	This paper	N/A

pEZT-2xMyc-TEV-SREBP2 R1040E (plasmid)	This paper	N/A
pEZT-Scap (plasmid)	This paper	N/A
pFastBac-10xHis-Scap CTD residues 751-1276	This paper	N/A
pFastBac-3xFLAG-SREBP2 CTD residues 793-1068 (plasmid)	This paper	N/A
pTK control plasmid	This paper	N/A
pcDNA 3.0 control plasmid	Invitrogen	N/A

Methods

Buffers and Media – Buffer A contains 10 mM Tris-HCl (pH 6.8), 100 mM NaCl, 1% (w/v) SDS, 1 mM EDTA, 1 mM EGTA, 200 µg/ml PMSF, 20 µg/ml ALLN, 25 mU/µl benzonase, and a 1:100 dilution of protease inhibitor cocktail set III. Buffer B contains 50 mM HEPES-NaOH (pH 7.5), 100 mM NaCl, 1% (w/v) digitonin, 1 mM TCEP, and 1 mM EDTA. Buffer C contains 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mg/mL iodoacetamide, 1 mM EDTA, 0.5 mM TCEP, and a 1:100 dilution of protease inhibitor cocktail set III. Buffer D contains 20 mM Tris-HCl (pH 7.5) and 150 mM NaCl. Buffer E contains 20 mM HEPES-NaOH (pH 7.5) and 150 mM NaCl. Buffer F contains 10 mM triethanolamine-acetic acid (pH 7.4), 250 mM sucrose, 1 mM sodium EDTA, and a 1:100 dilution of protease inhibitor cocktail set III. Buffer G contains 10 mM Tricine-NaOH (pH 7.4) and 150 mM NaCl. Medium A is Dulbecco's modified Eagle's medium (DMEM) (low glucose) supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin sulfate. Medium B is medium A supplemented with 10% (v/v) FCS (lipoprotein-rich serum). Medium C is medium B supplemented with 1 mM sodium mevalonate, 5 µg/ml cholesterol, 20 µM sodium oleate, and 0.4 µg/ml puromycin. Medium D is medium A supplemented with 5% (v/v) LPDS (lipoprotein-deficient serum), 50 µM compactin, and 50 µM mevalonate. Medium E is Medium D supplemented with 1% (w/v) HPCD. Medium F is a 1:1 mixture of Ham's F-12 and DMEM supplemented with 5% (v/v) FCS, 100 units/ml penicillin, and 100 µg/ml streptomycin sulfate. Medium G is medium F supplemented with 1 mM sodium mevalonate, 20 µM sodium oleate, and 5 µg/ml cholesterol. Medium H is a 1:1 mixture of Ham's F-12 and DMEM supplemented with 5% (v/v) LPDS, 100 units/ml penicillin, 100 µg/ml streptomycin sulfate, 50 µM compactin, 50 µM mevalonate, and 2% (w/v) HPCD.

Antibodies – To generate the monoclonal antibody IgG-2G10 against hamster Scap, BALB/c mice were immunized with purified recombinant hamster Scap (transmembrane domain, amino acids 1-767) (10) combined with Ribi Adjuvant System (Sigma-Aldrich) (25 μg primary injection, followed by two 50 μg boosts). Hybridoma cells were generated by fusing the splenic B lymphocytes with SP2-mIL6 mouse myeloma cells. After ELISA screening of hybridoma culture supernatants, one positive hybridoma, designated IgG-2G10 (subclass 1, k), was subcloned by serial dilution two times and purified by affinity chromatography using protein G-Sepharose4 Fast Flow columns. The source of all other antibodies is listed in the ***Materials*** section above.

Plasmids – An expression vector encoding human SREBP2 under the control of the weak thymidine kinase (TK) promoter (11) served as template and standard cloning methods were used to generate pTK-3xFLAG-SREBP2, an expression plasmid under control of the TK promoter that encodes, in sequential order from the NH_2-terminus, a 3xFLAG epitope tag (MDYKDDDDKGSDYKDDDDKGSDYKDDDDK), a 9-aa linker that includes a TEV protease cleavage site (ENLYFQGTG), and human SREBP2 (aa 14-1141). In all SREBP2 plasmids used in this study, two glycine residues in the original SREBP2 template, G961 and G1045, were each mutated to alanine to match the consensus human SREBP2 sequence (Uniprot Accession Q12772). pEZT-2xMyc-TEV-SREBP2 is an expression plasmid under control of the strong cytomegalovirus (CMV) promoter in the pEZT vector (12) that encodes, in sequential order from the NH_2-terminus, a 2xMyc epitope tag (MEQKLISEEDLEQKLISEEDL), a 9-aa linker that includes a TEV protease cleavage site (ENLYFQGTG), and human SREBP2 (aa 14-1141). pFastBac-3xFLAG-SREBP2-CTD is an expression plasmid in the pFastBac1 baculovirus expression vector that encodes, in sequential order from the NH_2-terminus, a 3xFLAG epitope tag and TEV protease cleavage site as described above, followed by a portion of the COOH-terminal domain (CTD) of human SREBP2 (aa 793-1068). An expression vector encoding HSV-tagged human SREBP1a under the control of the TK promoter (11) served as template and standard cloning methods were used to generate pTK-3xFLAG-SREBP1a, an expression plasmid under control of the TK promoter that encodes, in sequential order from the NH_2-terminus, a 3xFLAG epitope tag (MDYKDDDDKGSDYKDDDDKGSDYKDDDDK), a 9-aa linker that includes a TEV protease cleavage site (ENLYFQGTG), and human SREBP1a (aa 1-1147). pTK-Scap is a previously described expression plasmid encoding hamster Scap (aa 1-1276) under control of the TK promoter (9), and it served as template for generating the pEZT and pFastBac plasmids described

below. pEZT-3xFLAG-Scap is an expression plasmid under control of the CMV promoter in the pEZT vector that encodes, in sequential order from the NH_2-terminus, hamster Scap (aa 1-1276) and a 3xFLAG epitope tag (MDYKDDDDKGSDYKDDDDKGSDYKDDDDK). pTK-2xMyc-Scap-CTD is an expression plasmid under control of the TK promoter that encodes, in sequential order from the NH_2-terminus, a 2xMyc epitope tag (MEQKLISEEDLEQKLISEEDL), a 9-aa linker that includes a TEV protease cleavage site(ENLYFQGTG), and the COOH-terminal domain (CTD) of hamster Scap (aa 751-1276). pFastBac-His_{10}-Scap-CTD is an expression plasmid in the pFastBac1 baculovirus expression vector that encodes, in sequential order from the NH_2-terminus, a 13-aa linker that includes a His_{10}-epitope tag (MHHHHHHHHHHGGS) followed by the CTD of hamster Scap (aa 751-1276). pTK is an empty plasmid derived from pTK-HSV-SREBP2 (11) that served as a control plasmid in transfection experiments that used pTK-Scap and pTK-FLAG-SREBP2. pcDNA 3.0 served as a control plasmid in transfection experiments that used pEZT-2xMyc-TEV-SREBP2, pEZT-3xFLAG-Scap, and pTK-2xMyc-Scap-CTD. Truncations of the above plasmids, addition of epitope tags and single amino acids, and point mutations were generated by standard cloning methods. All plasmids were verified by sequencing the entire coding region. Primers used for construction of expression plasmids, site-directed mutagenesis, or subcloning are available upon request.

Sequence Analysis – Uniprot accession numbers for SREBP2 sequences that were compared in this study are: Q12772, *Homo sapiens* (human); XP_015005961.1, *Macaca mulatta* (macaque); Q60429, *Cricetulus griseus* (hamster); Q3U1N2, *Mus musculus* (mouse); NP_001192529.2, *Bos taurus* (cow); XP_025064703.1, *Alligator sinensis* (alligator); XP_015144523.1, *Gallus gallus* (chicken); NP_001116910.2, *Xenopus tropicalis* (frog). Uniprot accession numbers for SREBP1 sequences that were compared in this study are: P36956, *Homo sapiens* (human); F7E4A8, *Macaca mulatta* (macaque); Q60416, *Cricetulus griseus* (hamster); Q9WTN3, *Mus musculus* (mouse); A9CR99, *Bos taurus* (cow); A0A3Q0GKX1, *Alligator sinensis* (alligator); R4GLV5, *Gallus gallus* (chicken); A0A5G3IH17, *Xenopus tropicalis* (frog).

Cell Culture – SV-589 and SREBP2-deficient TR4411 cells were grown in a monolayer at 37°C and 5% CO_2 in medium B and medium C, respectively. CHO-K1 cells were grown in a monolayer at 37°C and 8% CO_2 in medium F. SRD-13A cells, a Scap-deficient cell line derived from CHO-7 cells (6), were grown in a monolayer at 37°C and 8% CO_2 in medium G. Sf9 insect cells were

grown in suspension in SF900 II SFM media (Gibco) at 27°C while shaking at 120 rpm. These environmental conditions were used for all experimental incubations, unless otherwise indicated. To guard against potential genomic instability, an aliquot of each cell line was passaged for only 4-6 weeks, after which a fresh batch of cells was thawed and propagated for subsequent experiments. All cell lines were confirmed to be free of mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza).

Generation of SREBP2-deficient cells – SREBP2-deficient TR4411 cells were derived from SV-589 human fibroblast cells using CRISPR-Cas9 technology (13). Briefly, two oligonucleotide pairs encoding the indicated 20-nucleotide guide sequences that targeted exon 2 of human SREBP2 (**Fig. S1A**) were each annealed and cloned into the pX459 v2.0 plasmid (Addgene). Both plasmids were co-transfected into SV-589 cells using FuGENE 6 as the transfection reagent according to the manufacturer's instructions. The transfected cells were subjected to selection with 1 μg/ml puromycin for two weeks, after which single surviving colonies were picked, expanded, and screened by PCR followed by sequencing. One colony with a 395-bp deletion in exon 2 of SREBP2 was chosen for further study. Single-cell clones from this colony were isolated by limiting dilution to establish the SREBP2-deficient TR4411 cell line.

Transient transfection of cells – SV-589 and TR4411 cells were transiently transfected with X-tremeGENE HP reagent according to the manufacturer's protocol using a 3:1 ratio of transfection reagent (μl):plasmid DNA (μg). CHO-K1 and SRD-13A cells were transiently transfected with FuGENE 6 reagent according to the manufacturer's protocol using a 3:1 ratio of transfection reagent (μl):plasmid DNA (μg). The only exception was the experiment described in **Fig. 7D**, where SRD-13A cells were transiently transfected using X-tremeGENE HP as described above.

Co-immunoprecipitation assays – On day 0, TR4411 cells were set up at a density of $1x10^6$ cells per 10-cm dish. On day 1, cells were transfected with plasmids as described in the Figure Legends. After incubation for 16 h, the medium containing transfection reagents was removed, cells were washed twice with PBS, and 10 ml of cholesterol-depleting medium E was added to each dish. After further incubation for 1 h, the medium was removed, cells were washed with PBS, and then harvested by scraping into 6 ml of ice-cold PBS. All subsequent steps were carried out on ice or at 4°C. Following centrifugation at 1000 x g for 5 min, cell pellets were resuspended in 1 ml of

solubilizing buffer B that had been freshly supplemented with protease inhibitors (160 µg/mL benzamidine, 200 µg/ml PMSF, 20 µg/ml ALLN, and a 1:100 dilution of protease inhibitor cocktail set III) and 25 mU/µl benzonase. After incubation for 1 h, the solubilized cells were subjected to centrifugation at 100,000 x g for 30 min, and the resulting supernatants were then incubated for 1 h with 50 µL of anti-FLAG M2 resin that had been pre-equilibrated in buffer B. The lysate/resin mixture was subjected to centrifugation at 1,000 x g for 5 min, after which the supernatant was removed, and the resin pellet was washed by resuspension in 1 ml of buffer B followed by incubation while rotating for 20 min. This wash step was repeated two additional times, after which bound proteins were eluted by incubation for 15 min with 200 µl of buffer B containing 400 µg/mL 3xFLAG peptide.

Organelle fractionation **–** On day 0, SV-589 cells were set up in medium B at a density of 1 x 10^6 cells per 10-cm dish. On day 1, cells were treated as described in the Figure Legends. After treatment, cells were washed twice with ice-cold PBS, harvested, and subjected to organelle fractionation by density gradient centrifugation as described previously (14). Briefly, cells from 3 10-cm dishes were harvested in 800 µL of buffer F and lysed by passage 13 times through a 25-gauge needle. The lysed cells were subjected to centrifugation at 3,000 x g for 10 min at 4°C to remove nuclei, after which 600 µl of the post-nuclear supernatant was collected. Discontinuous iodixanol gradients were generated by overlaying, in succession, 2.65 mL of buffers containing 24%, 19.33%, 14.66%, and 10% (v/v) iodixanol (buffers containing the desired percentages of iodixanol were made by diluting stock solutions of iodixanol (60% (v/v)) with buffer G). The gradients were allowed to stand at room temperature for 2 h and then subjected to centrifugation at 37,000 rpm in a TH641 swinging-bucket rotor (Thermo Scientific) for 4 h. The post-nuclear supernatant from above was then loaded on the top of the gradient and subjected to centrifugation at 37,000 rpm for 2 h in the TH641 rotor. After this step, a total of 12 fractions (800 µl each) were collected from the top and organelle enrichment was assessed by assaying equal volumes of all fractions for the presence of organelle markers by immunoblot analysis.

Immunoblot analysis – After treatments, cells were washed twice with PBS, and 200 µl of solubilizing buffer A was added to each well of a 6-well plate. Following incubation on a rotary shaker for 10 min at room temperature, solubilized cell lysates were collected and protein

concentrations were measured using the BCA assay kit. Equal amounts of cell lysate (by protein content) or equivalent volumes of lysates and eluates (after co-immunoprecipitation assays) were then mixed with 5x SDS-PAGE loading buffer, heated at 37°C for 10 – 20 min, and subjected to 10% SDS-PAGE. The electrophoresed proteins were transferred to nitrocellulose filters using the Bio-Rad Trans Blot Turbo system and subjected to immunoblot staining with the following primary antibodies: IgG 22D5 (1 μg/ml) to detect SREBP2, IgG 1C6 (2 μg/mL) to detect SREBP2, IgG 9B11 (1:1000 dilution in the experiment described in **Fig. 7D**) to detect Myc-tagged proteins, IgG 4H4 (5 μg/ml) to detect Scap, IgG 2G10 (5 μg/ml) to detect Scap, monoclonal anti-FLAG M2 (1:1,000 dilution) to detect FLAG-tagged proteins, polyclonal anti-GM130 (1:5000 dilution) to detect GM130, monoclonal anti-Sec61α (1:500 dilution) to detect Sec61α, monoclonal anti-Actin (1:1000 dilution in the experiments described in **Fig. 6C** and **Fig. 7D**, 1:5,000 dilution in all other experiments) to detect actin, and monoclonal anti-His clone His.H8 (1:1,000 dilution) to detect His_{10}-tagged proteins. Bound antibodies were visualized by chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, Thermo Scientific, Waltham, MA) using a 1:5000 dilution of anti-mouse IgG or anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). The filters were either exposed to Blue X-ray Film (Phoenix Research Products, Pleasanton, CA) for 1-30 s; or scanned using an Odyssey FC Imager (Dual-Mode Imaging System, Lincoln, NE; 2 min integration time) and analyzed using Image Studio ver 5.0 (LI-Cor, Lincoln, NE).

Protein overexpression in Sf9 cells – Using pFastBac-3xFLAG-SREBP2-CTD and pFastBac-His_{10}-Scap-CTD expression plasmids, baculoviruses were generated according to the manufacturer's guidelines (Invitrogen). On day 0, 20 ml cultures (for interaction studies) or 1 L cultures (for protein purification) of Sf9 cells were set up at a density of 1 x 10^6 cells/ml. After 4 h, cells were infected with 200 μl of each baculovirus (for interaction studies) or with 10 ml of each baculovirus (for protein purification). After 48 h (for interaction studies) or 72 h (for protein purification), cells were harvested by centrifugation at 4000 x g, washed with PBS supplemented with protease inhibitor cocktail III, and the pellets were either used immediately or frozen and stored at -80°C.

Purification of SREBP2-CTD/Scap-CTD complexes – All purification procedures were carried out on ice or at 4°C. Cell pellets from 12 L cultures were resuspended in Buffer C (40 ml/L of pellet), lysed with a Dounce homogenizer, and subjected to centrifugation at 20,000 x g for 30 min. The resulting supernatant was incubated with Ni-NTA beads (Qiagen) for 1 h, after which the Ni beads were collected by centrifugation at 1,000 x g for 5 min and transferred to an empty column. After settling in the column, the Ni beads were washed with 10 column volumes of buffer D containing 40 mM imidazole. Bound proteins were eluted with 2-3 column volumes of buffer D containing 300 mM imidazole directly onto anti-FLAG M2 resin, which had been pre-equilibrated in buffer D. The resin was then washed with 3 column volumes of buffer D, after which bound proteins were eluted with 2-3 column volumes of buffer D containing 100 μg/mL 3xFLAG peptide. The eluate was concentrated to 1 ml using a 30,000 MWCO Amicon concentrator, and subjected to gel filtration chromatography using a Superdex200 Increase column (GE Healthcare, Chicago, IL) that had been pre-equilibrated in buffer E. The typical yield for the SREBP2-CTD/Scap-CTD complex was 1-2 milligrams from 12 L of baculovirus-infected Sf9 cells.

Assay to measure interaction between SREBP2-CTD and Scap-CTD – Sf9 cell pellets from 20 ml cultures were lysed, subjected to centrifugation at 20,000 x *g* for 30 min, and the soluble supernatants were incubated with Ni-NTA beads. After 1 h, the supernatant/Ni bead mixture was transferred to an empty column and after settling, the beads were washed with 30 column volumes of buffer D containing 40 mM imidazole. Bound proteins were eluted with 3 column volumes of buffer D containing 300 mM imidazole.

Reproducibility of data – All results were confirmed in at least three independent experiments conducted on different days with different batches of cells. The only exceptions were the mutagenesis screen in **Fig. 2B** and **Fig. 5B**, which were conducted in duplicate.

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SI Appendix, Figure S1

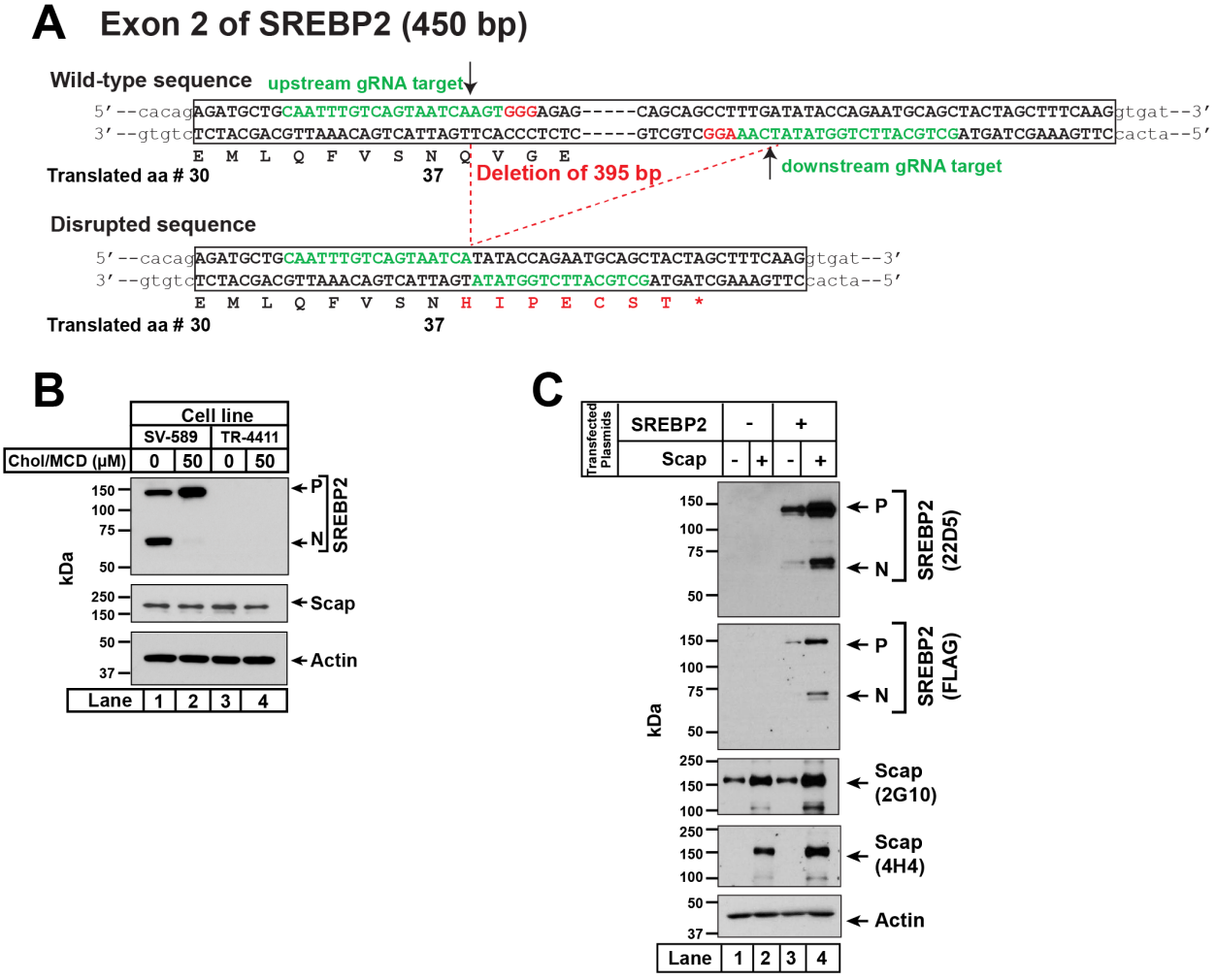

Figure S1. Characterization of an SREBP2-deficient cell line.

(**A**) Strategy for generating SREBP2-deficient SV-589 cells using CRISPR-Cas9 technology. Two guide RNAs were designed to target and disrupt exon 2 of human SREBP2 (20-nucleotide target sequences in *green* and NGG PAM sequences in *red*). The cleavage sites of Cas9 enzymes are indicated (*arrows*). A 395-bp deletion in exon 2 of SREBP2 resulted in a truncated transcript encoding amino acids 1 – 37 of SREBP2 followed by seven residues (*red*) and a stop codon (*). The cell line containing this disrupted version of SREBP2 is designated as TR-4411. (**B**) Characterization of SV-589 and TR-4411 cells in the absence or presence of sterols. On day 0,

cells were set up in medium C at a density of 700,000 cells/10-cm dish. On day 1, the medium was removed, and cells were switched to cholesterol-depleting medium E. After incubation for 1 h, cells were switched to medium D without or with 50 μM cholesterol/MCD complexes. After further incubation for 4 h, cells were harvested, and equal amounts of cell lysates were subjected to 10% SDS-PAGE (10 μg/lane) followed by immunoblot analysis for SREBP2 (IgG-22D5), Scap (IgG-2G10), and actin. (**C**) Transfection of TR-4411 cells. On day 0, TR-4411 cells were set up in medium C at a density of 400,000 cells/well of 6-well plates. After 6 h, cells were switched to fresh medium B and transfected as described in *Methods* with 0.5 μg of pTK-Scap and 1 μg of pTK-3xFLAG-SREBP2, as indicated. The total amount of DNA in each transfection was adjusted to 1.5 μg per well by addition of control pTK plasmid. After incubation for 40 h, the transfection medium was removed, and cells were switched to cholesterol-depleting medium E. After further incubation for 1 h, cells were harvested and equal fractions of whole cell lysates were subjected to immunoblot analysis of SREBP2 (IgG-22D5 or anti-FLAG), Scap (IgG-2G10 or IgG-4H4), and actin. *P*, precursor form of SREBP2; *N*, cleaved nuclear form of SREBP2.

SI Appendix, Figure S2

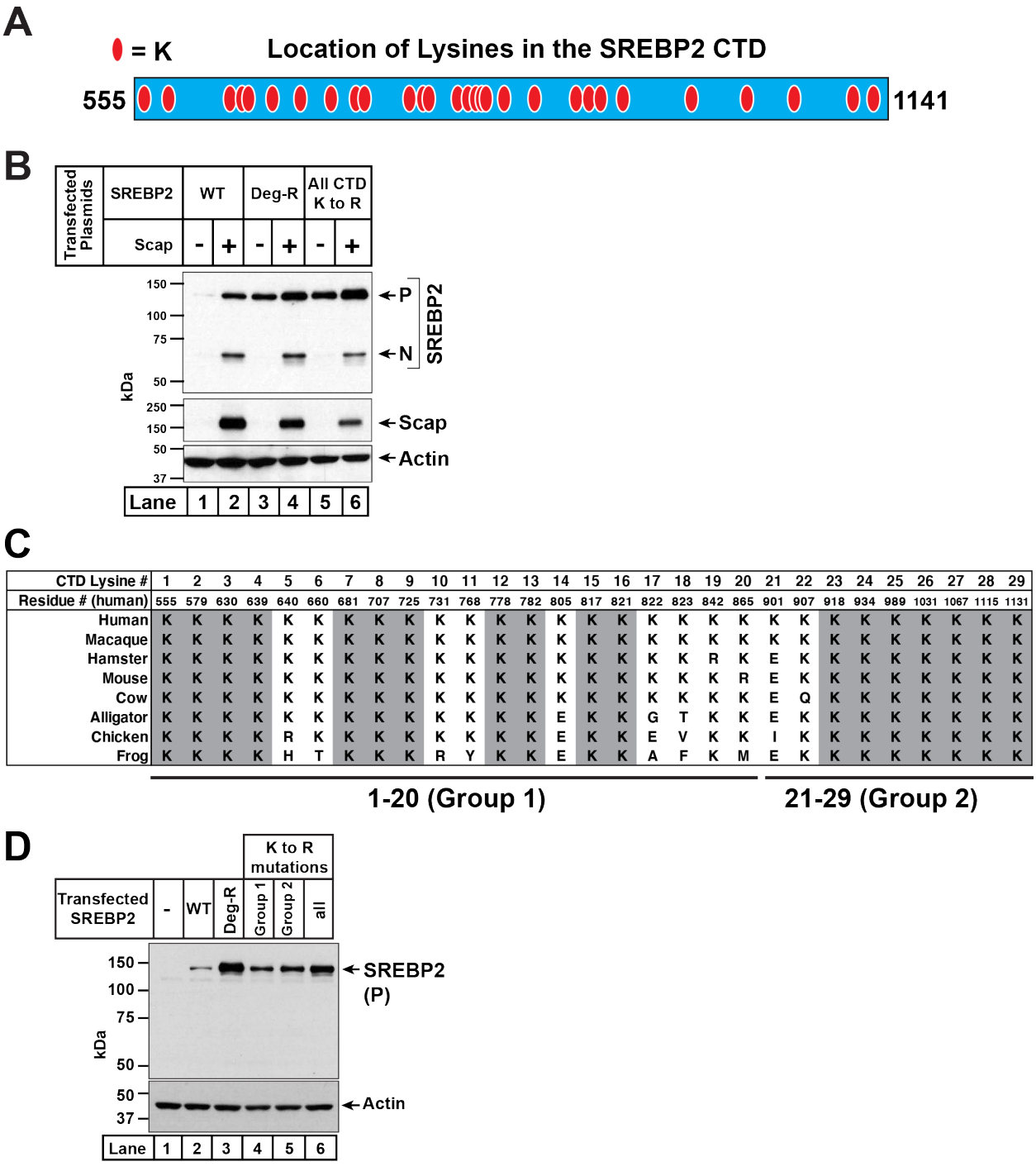

Figure S2. Analysis of lysines in the CTD of SREBP2.

(**A**) Schematic showing the relative locations of the 29 lysine residues in the COOH-terminal domain (CTD) of SREBP2 (aa 555 – 1141). (**B**) Stabilization of SREBP2 by mutation of all 29

lysines in its CTD to arginines. On day 0, SREBP2-deficient TR4411 cells were set up in medium C at a density of 400,000 cells/well of 6-well plates. After 6 h, cells were switched to fresh medium B and transfected as described in *Methods* with 0.5 μg of pTK-Scap and 1 μg of either WT or mutant versions of pTK-3xFLAG-SREBP2, as indicated. The total amount of DNA in each transfection was adjusted to 1.5 μg per well by addition of control pTK plasmid. After incubation for 40 h, the transfection medium was removed and cells were switched to cholesterol-depleting medium E for 1 h, after which cells were harvested and equal fractions of cell lysates were subjected to immunoblot analysis of SREBP2 (IgG-22D5), Scap (IgG-4H4), and actin. (**C**) Analysis of the conservation of the 29 lysines in the CTD of SREBP2. Residues that are identical in eight animal species are shaded (*gray*). Uniprot accession numbers for SREBP2 sequences are listed in *Methods*. Sequences were aligned using Clustal Omega. (**D**) Stabilization of SREBP2 by mutation of subsets of 29 lysines in its CTD to arginines. The 29 CTD lysines are divided into two groups as indicated in **C**. On day 0, SREBP2-deficient TR4411 cells were set up in medium C at a density of 400,000 cells/well of 6-well plates. After 6 h, cells were switched to fresh medium B and transfected as described in *Methods* with 1 μg of WT or the indicated mutant versions of pTK-3xFLAG-SREBP2. The total amount of DNA in each transfection was adjusted to 1 μg per well by addition of control pTK plasmid. After incubation for 40 h, the transfection medium was removed, cells were harvested and equal fractions of cell lysates were subjected to immunoblot analysis of SREBP2 (IgG-22D5) and actin. *P*, precursor form of SREBP2; *N*, cleaved nuclear form of SREBP2; *Deg-R*, degradation-resistant form of SREBP2.

SI Appendix, Figure S3

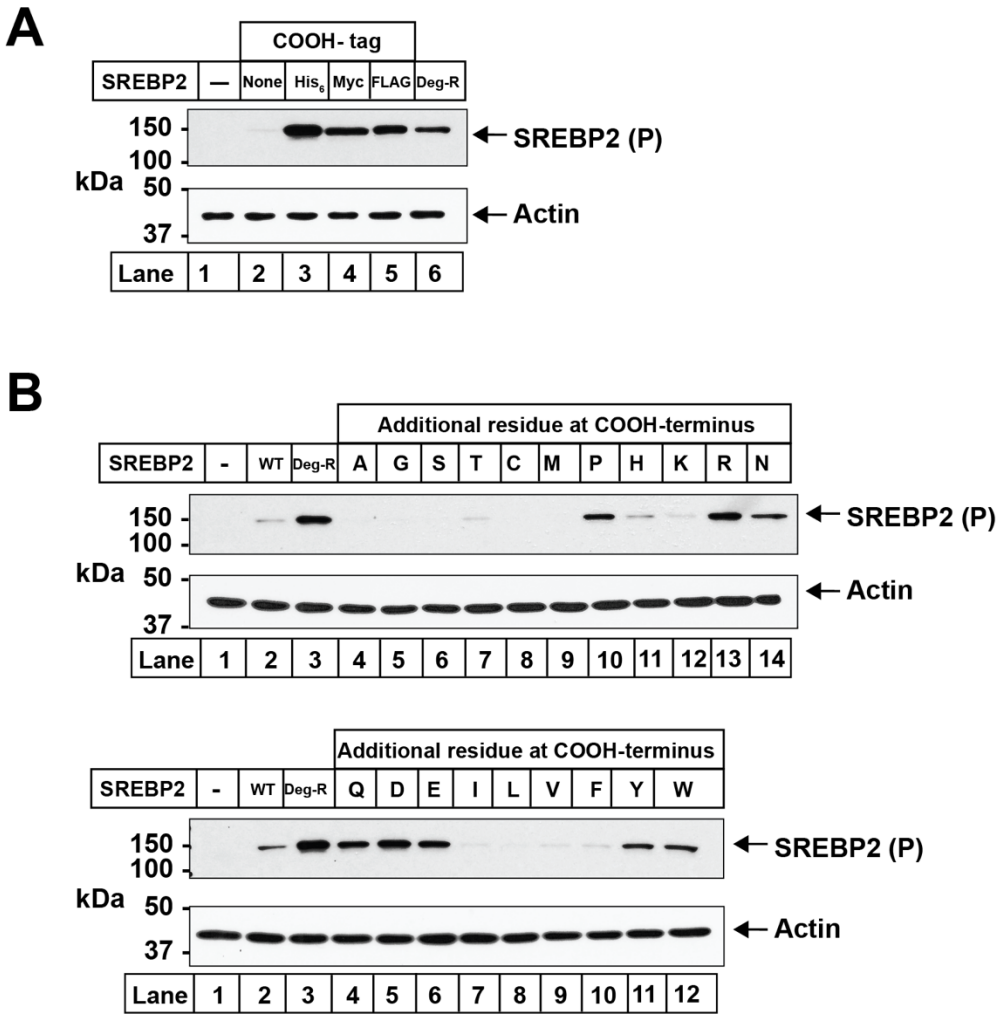

Figure S3. COOH-terminal additions inhibit degradation of SREBP2 in the absence of Scap. On day 0, Scap-deficient SRD-13A cells were set up in medium G at a density of 700,000 cells per 10-cm dish. On day 1, cells were switched to fresh medium F and transfected as described in *Methods* with 2 μg of either WT or indicated mutant versions of pTK-3xFLAG-SREBP2. The total amount of DNA in each transfection was adjusted to 2 μg per dish by addition of control pTK plasmid. On day 2, cells were harvested, and equal fractions of cell lysates were subjected to immunoblot analysis of SREBP2 (IgG-22D5) and actin. *P*, precursor form of SREBP2; *Deg-R*, degradation-resistant form of SREBP2.

SI Appendix, Figure S4

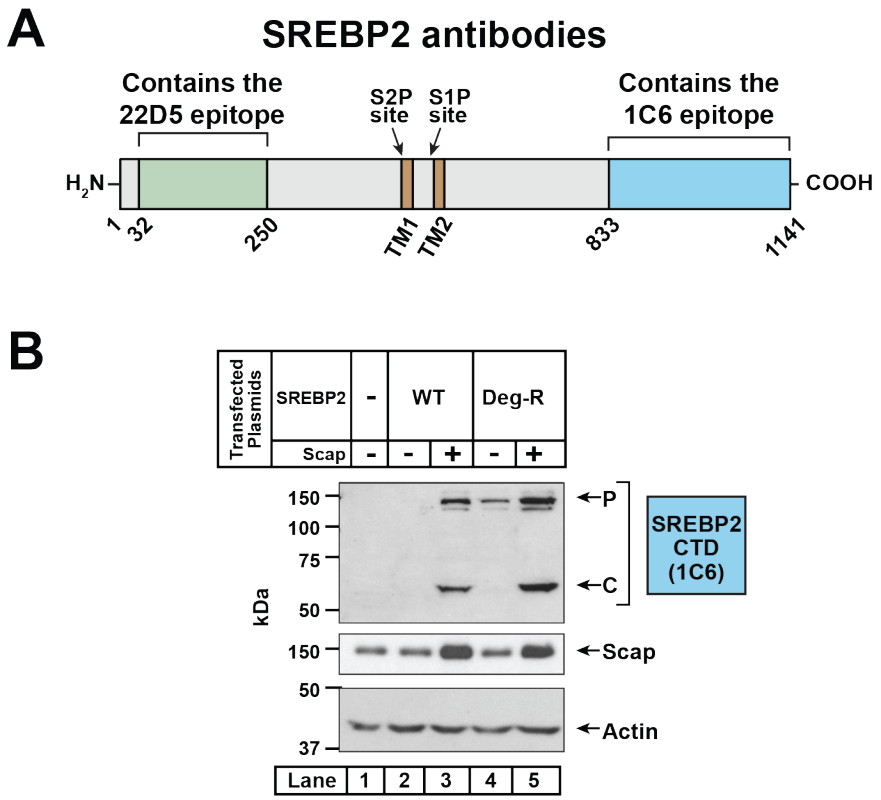

Figure S4. Antibody to detect cleaved COOH-terminal fragments of transfected SREBP2 in CHO-K1 cells.

(**A**) Diagram showing the locations of epitopes detected by IgG-22D5 (*green*) and IgG-1C6 (*blue*) in the NTD and CTD, respectively, of SREBP2. (**B**) Detection of the CTD of SREBP2. On day 0, CHO-K1 cells were set up in medium F at a density of 700,000 cells per 10-cm dish. On day 1, cells were switched to fresh medium F and transfected as described in *Methods* with 2 μg of pTK-Scap and 2 μg of the indicated version of pTK-3xFLAG-SREBP2. The total amount of DNA in each transfection was adjusted to 4 μg per dish by addition of control pTK plasmid. On day 2, the transfection medium was removed, and cells were switched to cholesterol-depleting medium H. After incubation for 1 h, cells were harvested and equal fractions of cell lysates were subjected to immunoblot analysis of SREBP2 (IgG-1C6), Scap (IgG-4H4), and actin. *P*, precursor form of SREBP2; *C*, cleaved CTD of SREBP2; *Deg-R*, degradation-resistant form of SREBP2; *CTD*, COOH-terminal domain.