

	cScap/4G10 ^{Fab}	cScap ^{D435V} /cInsig-1/4G10 ^{Fab}
Data collection and processing		
Magnification	46,296	46,296
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	60	60
Defocus range (μm)	1.6 – 2.6	1.6 – 2.6
Pixel size (Å)	1.08	1.08
Symmetry imposed	C1	C1
Initial particle images (no.)	9,052,025	11,542,708
Final particle images (no.)		
Global map	223,673	155,187
L1-L7/4G10 ^{Fab}	223,673	155,187
Global w/ordered TMs	20,931	80,678
Map resolution (Å)	2.9 – 4.1	3.5 – 4.6
FSC threshold	0.143	0.143
Refinement		
	L1-L7/4G10 ^{Fab}	L1-L7/4G10 ^{Fab}
Model composition		
Non-hydrogen atoms	4138	4178
Protein residues	511	516
Ligands	2	2
R.m.s. deviations		
Bond lengths (Å)	0.004	0.006
Bond angles (°)	0.653	0.940
Validation		
MolProbity score	1.60	1.84
Clashscore	5.26	8.59
Poor rotamers (%)	0.00	0.00
Ramachandran plot		
Favored (%)	95.39	94.47
Allowed (%)	4.61	5.53
Disallowed (%)	0.00	0.00

Table S1. CryoEM Data Collection and Refinement Statistics, Related to Figures 2 and 4

Location in Scap	Ortholog residue in chicken Scap	Mutation in hamster Scap	Effect on SREBP2 transport	Reference
TM2	Y298	Y298C	Constitutive	Nohturfft et al. 1998
TM3	L315	L315F	Constitutive	Yabe et al. 2002
TM4	D450	D443N	Constitutive	Hua et al. 1996
TM4	D435	D428A	No transport	Feramisco et al. 2005

Table S2. Mutations in Scap that Affect Function, Related to Figure 5

Key functional residues in chicken Scap and hamster Scap described in this paper. Introducing the Y298C, L315F, or D443N mutations into hamster Scap results in a Scap that does not bind Insigs and whose transport of SREBP2 is not inhibited by sterols. In contrast, mutating hamster Scap D428 to alanine or chicken Scap D435 to valine results in a Scap that does not transport SREBP2 and that binds to Insigs irrespective of sterol levels.

Location in Insig	Ortholog residue in chicken Insig-1	Mutation in human Insig-2	Effect of mutation on 25HC binding	Reference
TM1	G70	G39F	Reduced	Ren et al. 2015
TM2	C108	C77D	Reduced	Ren et al. 2015
TM2	A144	A113W	Not tested	Ren et al. 2015
	F146	F115A	Reduced	Radhakrishnan et al. 2007
	G148	G117F	Not tested	Ren et al. 2015
	H151	H120F	Not tested	Ren et al. 2015
TM4	Q163	Q132A	None	Radhakrishnan et al. 2007
	T167	T136A	Reduced	Radhakrishnan et al. 2007
	W176	W145A	None	Radhakrishnan et al. 2007
	D180	D149A	None	Radhakrishnan et al. 2007
TM6	G231	G200F	Reduced	Ren et al. 2015

Table S3. Mutations in Insig that Affect Function, Related to Figure 5

Key functional residues in chicken Insig-1 and human Insig-2 described in this paper. Introduction of each of the indicated mutations into human Insig-2 results in a protein that cannot bind to Scap. However, these mutations have variable results on binding of Insig-2 to 25-HC, as indicated.

Primers for PCR amplification of antibody regions		
mFG_1	AGGAACTGCAGGTGTCC	IgG Forward
mFG_2	CAGCTACAGGTGTCCACTCC	IgG Forward
mFG_3	TGGCAGCARCAGCTACAGG	IgG Forward
mFG_4	CTGCCTGGTGACATTCCCA	IgG Forward
mFG_5	CCAAGCTGTGTCCTGTC	IgG Forward
mFG_6	TTTTAAAAGGTGTCCAGKGT	IgG Forward
mFG_7	CCTGTCAGTAACTRCAGGTGTCC	IgG Forward
mFG_8	TTTTAAAAGGGGTCCAGTGT	IgG Forward
mFG_9	CGTTCCTGGTATCCTGTCT	IgG Forward
mFG_10	ATGAAGTTGTGGYTRAACTGG	IgG Forward
mFG_11	TGTTGGGGCTKAAGTGGG	IgG Forward
mRG*	AGAAGGTGTGCACACCGCTGGAC	IgG Reverse
mFK_1	RGTGCAGATTTTCAGCTTCTGCT	IgK Forward
mFK_2	TGGACATGAGGGCYCCTGCTCAGT	IgK Forward
mFK_3	CTSTGGTTGTCTGGTGTGAYGGA	IgK Forward
mFK_4	GTTGCTGCTGCTGTGGCTTACA	IgK Forward
mFK_5	GTATCTGGTACCTGTGG	IgK Forward
mFK_6	TGCTTTTCTGGATTTTCAGCCTCCAG	IgK Forward
mFK_7	TCAACTTCTGCTCTTCTGCTGTTT	IgK Forward
mFK_8	CTAGCTCYTCTCCTCAGYCTTCTTCTCCTC	IgK Forward
mFK_9	GTGMTGGTGCTBRTGGG	IgK Forward
mFK_10	STGYTGHTGYTCTGG	IgK Forward
mRK*	ACTGAGGCACCTCCAGATGTT	IgK Reverse

Table S4. Primers for Sequencing 4G10^{Fab}, related to STAR Methods