Haploid genetic screens identify SPRING/C12ORF49 as a determinant of SREBP signaling and cholesterol metabolism

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The sterol-regulatory element binding proteins (SREBP) are central transcriptional regulators of lipid metabolism. Using haploid genetic screens we identify the SREBP Regulating Gene (SPRING/C12ORF49) as a determinant of the SREBP pathway. SPRING is a glycosylated Golgi-resident membrane protein and its ablation in Hap1 cells, Hepa1-6 hepatoma cells, and primary murine hepatocytes reduces SREBP signaling. In mice, Spring deletion is embryonic lethal yet silencing of hepatic Spring expression also attenuates the SREBP response. Mechanistically, attenuated SREBP signaling in SPRINGKO cells results from reduced SREBP cleavage-activating protein (SCAP) and its mislocalization to the Golgi irrespective of the cellular sterol status. Consistent with limited functional SCAP in SPRINGKO cells, reintroducing SCAP restores SREBP-dependent signaling and function. Moreover, in line with the role of SREBP in tumor growth, a wide range of tumor cell lines display dependency on SPRING expression. In conclusion, we identify SPRING as a previously unrecognized modulator of SREBP signaling.
Cellular sterol and fatty acid levels must be tightly controlled to ensure that these meet metabolic and growth demands. Accordingly, loss of lipid homeostasis is associated with a wide range of human conditions, including cancer, neurodegeneration, and cardiovascular disease. The sterol-regulatory element binding proteins (SREBPs) are a family of transcription factors that control all facets of lipid metabolism by regulating the expression of a panel of genes that contain a sterol regulatory element (SRE) in their respective promoter regions. There are three SREBP isoforms, SREBP1a, SREBP1c, and SREBP2, which are structurally similar, but activate a distinct set of genes and exhibit a divergent tissue distribution in vivo. SREBP1c primarily regulates genes implicated in fatty acid synthesis as well as cholesterol synthesis (FASN) and acetyl-CoA carboxylase (ACC). In contrast, SREBP2 is primarily implicated in the regulation of genes linked to cholesterol synthesis and uptake, including those encoding for the rate-limiting enzymes in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and squalene epoxidase (SQLE), and the low-density lipoprotein receptor (LDLR). The third isoform, SREBP1a, regulates genes involved in both sterol and fatty acid metabolism.

SREBPs are produced in their precursor form as membrane-associated endoplasmic reticulum (ER)-resident proteins that contain an N-terminal basic helix-loop-helix leucine zipper domain and a regulatory carboxyterminal region. Under conditions of sufficient cellular sterols, SREBPs are retained in the ER through formation of a tripartite complex with the sterol-sensing SREBP cleavage-activating protein (SCAP), and the ER anchor proteins insulin-induced genes 1 and 2 (INSIG1 and 2). A drop in cholesterol in the ER membrane leads to a conformational change in SCAP, which leads to dissociation from INSIG and facilitates COPII-mediated trafficking of the SREBP-SCAP complex to the Golgi. In the Golgi, SREBP is proteolytically activated through sequential cleavage by the proteases S1P and S2P, which release the transcriptionally active N-terminal domain of SREBP. Translocation of this domain to the nucleus leads to transcriptional activation of target genes containing an SRE. As SREBPs contain an SRE in their promoter region, they induce their own expression in a positive feedback mechanism.

The final step in the SCAP-SREBP cycle is the less-well understood COPII-mediated retrograde transport of SCAP from the Golgi to the ER, which allows SCAP to reiteratively associate with newly synthesized SREBPs and INSIGs in the ER to form a tripartite complex. To identify unknown determinants of the SREBP pathway we applied a set of mammalian haploid genetic screens and report here the identification of the SREBP Regulating Gene SPRING, as a previously unrecognized factor that governs SREBP activity in mammalian cells and in vivo by controlling the level of functional SCAP.

Results
Haploid genetic screens link SPRING to the SREBP pathway. The SREBP pathway is subject to exquisite regulation by a core set of molecular factors that include INSIGs, SCAP, MBTPS1, and MBTPS2. To identify unknown SREBP regulators we developed two SREBP-focused mammalian haploid genetic screens. Using this approach, we interrogated SREBP signaling in an unbiased manner reasoning that any unknown regulator should be found in independent screens.

In the first screen, we evaluated the cholesterol-dependent regulation of SQLE, a rate-limiting enzyme in cholesterol biosynthesis and a bona fide transcriptional target of SREBP2. To monitor the level of SQLE protein in live cells as a proxy for SREBP activity we engineered Hap1 cells in which we introduced mNeon into the last coding exon of the endogenous SQLE locus using CRISPR/Cas9-based microhomology-mediated end-joining integration (Fig. 1a and Supplementary Fig. 1A). Consistent with SREBP-dependent regulation of SQLE expression, the level of the SQLE-mNeon fusion protein was low in Hap1-SQLE-mNeon cells when cultured in sterol-containing medium yet was markedly increased upon sterol-depletion (Fig. 1b, c). Moreover, similar to untagged SQLE, the levels of chimeric SQLE-mNeon protein were subject to cholesterol-stimulated proteasomal degradation (Supplementary Fig. 1B). Using this cell line, we screened for positive genetic regulators that are required for SREBP signaling as well as for negative determinants essential for cholesterol-mediated degradation of SQLE, as illustrated in Fig. 1d. Briefly, following mutagenesis, Hap1-SQLE-mNeon cells were first sterol-depleted and subsequently treated with water-soluble cholesterol to induce SQLE-mNeon degradation. Mutants with the lowest and highest mNeon signal were isolated from FACS and the integration sites retrieved from genomic DNA and mapped, as previously reported. Validating our screening approach, we identified strong enrichment of gene-trap insertions in the SQLE locus in the mNeonLOW population, alongside a similar enrichment in the established positive regulators of the SREBP pathway SCAP, MBTPS1, MBTPS2, and SREBF2 itself (Fig. 1e). Conversely, the strongest negative regulator of SQLE-mNeon found in our screen was the E3 ubiquitin ligase MARC6, and its cognate E2 partner UBE2J2, which we have recently reported to be critical determinants of cholesterol-dependent degradation of SQLE. Additionally, as expected our screen also identified INSIG1, an established negative regulator of the SREBP pathway. As such, this screen faithfully reports on cholesterol-dependent regulation of SQLE by the SREBP pathway. Amongst the known core SREBP activating genes, identified as positive regulators of SQLE expression, we also found an uncharacterized gene, C12orf49, which is further referred to as SPRING.

In a parallel screen, we leveraged our recent finding that Hap1 cells tolerate loss of the key de novo fatty acid synthesis enzyme, FASN, which is a canonical SREBP1-regulated gene. We reasoned that in the absence of FASN and fatty acid synthesis Hap1 cells must rely on alternative survival pathways for acquiring fatty acids and growth. To test this idea, we generated independent Hap1-FASNKO cells (Supplementary Fig. 2A) and conducted a synthetic lethality screen, as previously reported. Briefly, Hap1-FASNKO cells were mutagenized and expanded in culture for 12 days to allow depletion of lethal mutations. Synthetic genetic interactions were thereafter analyzed by comparing the results obtained in Hap1-FASNKO cells and WT Hap1 cells treated in the same manner. A total of 72 genes showed a synthetic genetic interaction in Hap1-FASNKO cells (Fig. 1f). Amongst these, a prominent SREBP signature encompassing the gene encoding SREBF2 itself, SREBF2, the genes encoding the core SREBP-activation machinery (SCAP, MBTPS1, and MBTPS2) and the uncharacterized gene SPRING were apparent in Hap1-FASNKO cells. Notably, these genes appeared to be non-essential in WT Hap1 cells. Additionally, a set of SREBP target genes including LDLR, ACSL1, ACSL3, and FABP5, that are implicated in lipid uptake, trafficking, and metabolism also displayed a synthetic interaction in Hap1-FASNKO cells (Supplementary Fig. 3A). These observations suggest that in the absence of FASN, Hap1 cells depend on SREBP-dependent lipid uptake and further support the idea that SPRING may be implicated in SREBP signaling.

Previously, we have reported a haploid genetic screen that revealed that the entry of Andes viruses into target cells critically depends on SREBP signaling and on the presence of cholesterol in the host-cell membrane. When integrating the three SREBP-focused haploid screens we identify a core set of 5 shared genes...
necessary for SREBP signaling that next to SREBF2, SCAP, MBTPS1, MBTPS2 also includes SPRING (Fig. 1g). In aggregate, our three independent screens strongly point towards SPRING acting as a positive regulator of the SREBP pathway to govern lipid metabolism.

**SPRING is a determinant of the SREBP pathway in cells.** As SPRING was identified in three independent screens in conjunction with the core SREBP activation machinery we reasoned that it could play a role in SREBP-mediated transcriptional control of lipid metabolism. To test this, we engineered Hap1 cells lacking SPRING and tested their response to sterol-depletion (Fig. 2a, b). Cells lacking SPRING had reduced protein levels of the SREBP targets SQLE, SQS, and INSIG1 under basal culture conditions, and were unable to increase abundance of these under sterol-depletion condition. Similarly, in contrast to control Hap1 cells which, as expected, robustly increased the mRNA expression of the SREBP-transcriptional targets HMGCR, LDLR, SQLE, and FASN in response to sterol-depletion, in Hap1-SPRINGKO cells basal expression of these genes was reduced and the response to sterol-depletion was largely abrogated. Notably, mRNA and protein levels of SPRING were not sensitive to the cellular sterol status. As a functional consequence, Hap1-SPRINGKO cells exhibited markedly reduced levels of surface LDLR protein and failed to increase LDL uptake under sterol-deprived conditions (Fig. 2c, d and Supplemental Fig. 2E). Importantly, regulation of the SREBP
pathway was not restricted to Hap1 cells but was also observed in hepatocytes, which represent a physiological model for SREBP activity. Similar to our finding in Hap1 cells, loss of Spring in murine Hepa1-6 hepatoma cells and in primary mouse hepatocytes attenuated activity of the SREBP pathway (Fig. 2e and Supplementary Fig. 4).

Proteolytic processing of SREBPs is a prerequisite for their function as transcription factors, raising the possibility that SPRING may govern SREBPs at the level of their processing (Fig. 2f). Notably, we found that proteolytic processing of SREBP2 can occur in the absence of SPRING. However, we observed that absence of Spring profoundly reduced the precursor
and mature (i.e., processed) protein level of SREBP2, and consequently the level of its target genes in the absence of a change in the level or localization of Site 1 Protease (Supplementary Fig. 2B). We also considered the possibility that proteolytic cleavage by S1P or S2P could be affected by loss of SPRING. Similar to SREBPs, the ER-stress-related factor ATF6 undergoes proteolytic processing by these two proteases. We, therefore, tested whether basal- and Tunicamycin-induced ER stress signaling is altered in the absence of SPRING (Supplementary Fig. 2C). Basal ER-stress, as evaluated by expression of ATF6-driven genes and other ER stress-related genes, was not changed in cells lacking SPRING. Importantly, induction of ER stress by Tunicamycin, which promotes ATF6 translocation to the Golgi and subsequent proteolytic processing by S1P/S2P proteases was intact, albeit we did observe a small yet significant reduction. This suggests that the S1P/S2P axis is not globally abrogated in cells lacking SPRING and can still respond to physiological cues.

We also considered the possibility that loss of SPRING may result in an intrinsic lesion in SREBP activity (e.g., if it is directly required for transcriptional activation of SREBP). Yet this does not seem to be the case, as when we introduce an N-terminal constitutively active SREBP2 transcriptional domain into Hap1-SPRINGKO cells we were able to restore the basal and sterol-depletion-induced levels of SQLE (Fig. 2g). To evaluate the consequence of absence of SPRING we compared the transcriptional profile of Hap1-WT and Hap1-SPRINGKO cells (Fig. 3a and Supplementary Fig. 3B). This comparison confirmed the aberrant activation of the SREBP pathway in Hap1-SPRINGKO cells. We, therefore, proceeded to compare the global transcriptional response of these cells to sterol-depletion (Fig. 3b, c). While Hap1-WT increased expression of a panel of SREBPs-regulated target genes in response to sterol depletio, in the absence of SPRING the expression of these genes was refractory to this treatment. Importantly, the SREBP program could be restored by reintroducing SPRING in sterol-depleted Hap1-SPRINGKO cells (Fig. 3d). Collectively, this set of experiments establishes SPRING as a determinant of SREBP activation in mammalian cells.

**Spring attenuates hepatic SREBP signaling in vivo.** Spring is ubiquitously expressed in mouse tissues with slightly higher expression in the liver and kidney (Supplementary Fig. 5A). To study whether our observation in cells extends to the in vivo setting and to investigate the physiological role of Spring, we generated Spring−/− mice using CRISPR/Cas9 technology. Guide RNAs were designed to target the 5′ region of coding exon 2 and the 3′ region of coding exon 5. We obtained heterozygous mice with a deletion spanning exons 2 until 5 (Fig. 4a). However, heterozygous crosses failed to produce viable homozygous knockout offspring (Fig. 4b). The genetic distribution of offspring was, in fact, consistent with loss of Spr by being embryonic lethal and suggesting a critical role for **Spring** in mouse development. We confirmed this notion by analyzing embryos obtained in heterozygous crosses. We did observe abnormally developed embryos at day 7.5 dpc. These embryos were smaller and showed a poorly developed amniotic cavity and allantois in comparison with the adjacent to normally developing embryos in the mouse uterine horn. The abnormal embryos were confirmed to be homozygous null mutants by PCR genotyping (Supplementary Fig. 5B, C).

**Spring is a glycosylated Golgi-resident membrane protein.** **Spring** encodes for a 205 amino acid protein with no apparent homology to other proteins and is predicted to have a possible signal peptide, a single transmembrane-spanning domain, and a cysteine-rich motif (Fig. 5a). To gain insight into how SPRING influences SREBP activity we determined its cellular localization. We first considered the possibility that SPRING may be a secreted protein. However, our analysis indicates that this is not the case, but that SPRING is associated with cellular membranes (Supplementary Fig. 6A, B). In transduced HeLa cells SPRING was predominantly co-localized with the Golgi marker GM130 (Fig. 5b). Consistent with glycosylation of **Spring**, N-glycosidase (PNGase-F) removed glycans from SPRING protein (Supplementary Fig. 6C). Further, mutation of the single predicted glycosylation site (Asn-67) abolished SPRING glycosylation (Fig. 5c). To interrogate the nature of the glycan chain present on **Spring** we used Endoglycosidase-H (Endo-H), which removes glycans from ER- and cis medial Golgi-resident proteins, before they acquire complex modifications. We observed that unlike LDLR, SPRING glycosylation remained Endo-H-sensitive (Supplementary Fig. 6D). Together with the observed localization of **Spring** in the Golgi (Fig. 5b), sensitivity to Endo-H suggests that **Spring** is present in the cis medial Golgi, which is where the SCAP-SREBP-S1P machinery is located. To determine the topology of **Spring** in the Golgi membrane we performed protease protection assays. Unlike LDLR-HA, which has its C-terminus exposed to the cytoplasm and is hence sensitive to tryptic digestion, SPRING-V5 was refractory (Fig. 5d). Permeabilization of cellular membranes with Triton X-100 rendered SPRING-V5 sensitive to tryptic digestion. In aggregate with our observation on SPRING glycosylation, these results indicate that the C-terminus of SPRING faces the Golgi lumen.
Intrigued by the fact that cholesterol-sensing by SCAP occurs initially in the ER, we asked whether delivery of SPRING to the Golgi compartment was an absolute requirement for its ability to regulate SREBP activation. To address this, we generated a SPRING expression construct with a C-terminal (i.e., lumen-facing) ER-retention KDEL signal (SPRING<sub>KDEL</sub>). SPRING<sub>KDEL</sub> was largely retained in the ER, as evident by co-localization with the ER marker protein VAMP-associated protein A (VAPA, Fig. 5e). In contrast to WT SPRING, which could rescue sterol-dependent regulation of SQLE when introduced into Hap1-SPRING<sup>KO</sup> cells, SPRING<sub>KDEL</sub> failed to do so (Fig. 5f). Taken together, these results demonstrate that regulation of SREBP activity by SPRING requires its Golgi localization.

**SPRING governs SCAP localization in cells.** Trafficking to and subsequent proteolytic processing of SREBPs in the Golgi is critically dependent on a stoichiometric interaction with SCAP<sup>7,8</sup>. Although the levels of SREBP are drastically decreased, SREBP processing can still occur in SPRING<sup>KO</sup> cells (c.f. Fig. 2a, b, f). We, therefore, considered the possibility that SCAP function may be affected by SPRING. Regulation of SCAP retention in the ER requires interaction with INSIGs. This prompted us to investigate whether akin to INSIG, SPRING also interacts with SCAP. We used a co-immunoprecipitation approach and found that SCAP can interact with SPRING when the two are over-expressed in a model system (Fig. 6a). With this assay, we are unable to formally establish the cellular localization of this interaction. However, we point out that using the same approach we were unable to detect an interaction between SPRING and INSIG. The potential functional significance of the SCAP-SPRING interaction was then evaluated in CHO cells that stably produce SCAP-eGFP (CHO-SCAP-eGFP) in which Spring was ablated by CRISPR/Cas9-mediated genome editing. In control cells grown in sterol-containing media SCAP-eGFP was predominantly located in the ER and localization markedly shifted towards the Golgi upon sterol-depletion (Fig. 6b and Supplementary Fig. 7A). However, in CHO cells devoid of Spring, SCAP-eGFP appeared trapped in...
Fig. 4 SPRING is essential for mouse embryogenesis and for hepatic SREBP signaling. a Illustration of the genomic organization of murine SPRING (2410131K14Rik-201) and the allele obtained after CRISPR/Cas9 editing. The 5’ and 3’ gRNAs are indicated in red and green, respectively. Sanger sequencing of amplified genomic DNA was performed to confirm the deletion of exons 2–5. b Table showing an overview of the obtained genotypes from various crosses of heterozygous mice. *p < 0.005, lower than expected by Pearson’s Chi-square test with 2 df; **equal to expected when assuming embryonic lethality of homozygous null mice, Chi-square = 2.37, 0.9 > p > 0.1 with 1 df. c WT C57BL/6J mice were administered Ad-shCtrl or Ad-shSpring (N = 8/5 animals per group, respectively) via tail-vein injection. After 7 days, mice were fasted overnight and subsequently refed for 6 h. Total liver RNA was isolated and expression of the indicated genes was determined by qPCR. Each individual mouse is plotted within the box and whiskers plot that depicts the median line, the 25th and 75th percentile, and the min-max values. *p < 0.05, ***p < 0.001.
the Golgi irrespective of the cellular sterol status. Importantly, ER-localization of SCAP-eGFP could be restored in the SPRING\textsuperscript{KO} cells grown in the presence of sterols by introducing back SPRING expression. Mislocalization of SCAP to the Golgi in SPRING\textsuperscript{KO} cells likely results in depletion of functional SCAP in the ER, which is required at stoichiometric levels to support exit of SREBP towards the Golgi. However, these experiments were conducted with cells that stably over-produce SCAP-eGFP, and are thus highly suitable to track SCAP localization, but may mask effects on SCAP protein level. We, therefore, evaluated endogenous SCAP protein in Hap1 cells (Fig. 6c and Supplementary Fig. 2D). Remarkably, SCAP protein was reduced in Hap1
SPRINGKO cells. This is consistent with functional SCAP deficiency in these cells, and implies that increasing the level of SCAP should overcome the SREBP-signaling defect in SPRINGKO cells. In line with this idea we found that introducing SCAP into Hap1 SPRINGKO cells, similar to introducing back SPRING, fully restored the SREBP2-mediated sterol-dependent response (Fig. 6d). Functionally, introduction of SCAP was sufficient to restore LDL uptake and de novo cholesterol biosynthesis (Fig. 6e, f and Supplementary Fig. 7B).

Finally, it is well recognized that intact SREBP signaling is required for cell growth and proliferation. Moreover, there is increasing evidence that cancer cells activate SREBP signaling as a means to produce lipids to support their rapid growth.3,36 We, therefore, interrogated the Dependency Map (DepMap, www.depmap.org) repository which aims to identify genetic vulnerabilities in human cancer.37,38 Within the database of 342 cancer cell lines (27 distinct lineages) that were subjected to genome-wide CRISPR/Cas9 lethality, SPRING expression emerged as a selective gene in 337/342 of the evaluated cell lines (Supplementary Fig. 8A). This suggests that a wide-variety of tumor cells is dependent on SPRING expression for growth. Remarkably, in this panel of cell lines the top co-dependent genes with SPRING were those associated with the core SREBP machinery (Supplementary Fig. 8B), mirroring our haploid genetic screen results. This observation lends further support for a central role for SPRING in regulating the SREBP pathway and its potential role in proliferative diseases. Collectively, our in vitro and in vivo findings support the idea that SPRING is a Golgi-resident factor required for maintaining SCAP function, and that loss of SPRING results in functional depletion of SCAP in the ER and attenuation of SREBP signaling (Supplementary Fig. 9).

Discussion

The SREBP transcriptional network is a central determinant of homeostatic lipid metabolism. Dysregulation of this pathway underlies development of human conditions, exemplified by development of hypercholesterolemia and ensuing coronary artery disease due to mutations in the SREBP-regulated gene LDLR.25 Therefore, elucidating the mechanisms that govern the SREBP pathway is of outmost importance. Genetic approaches have been paramount in clarifying the molecular components that control cholesterol and fatty acid metabolism that are regulated by SREBPs.26 Mammalian haploid genetic screens have been applied to interrogate a variety of cellular processes and phenotypes, amongst others, pathogen entry,27,39–42 signal transduction,24 modes of toxin action,43 and gene essentiality.28 Yet this methodology is also well suited to address lipid-associated questions, and accordingly we recently applied this approach to investigate the control of sterol-stimulated degradation of HMGCR.44 In this study, by combining three independent SREBP-related screens we identify SPRING (C12orf49) as a previously uncharacterized regulator of the SREBP pathway.

SPRING is a Golgi-resident glycosylated membrane protein and together with its ortholog forms an uncharacterized protein family (Pfam UPF0454), which bears no substantial homology with other human proteins. We found that the primary phenotype of cells lacking SPRING is decreased basal levels of precursor and mature SREBPs and SREBP signaling, and an inability to enhance SREBP signaling so as to mount a homeostatic response to sterol-depletion. This is highly reminiscent of what is observed in cells lacking SCAP45 and in liver-specific Scap knockout mice.46 Accordingly, we have narrowed the primary lesion in SPRING signaling in SPRINGKO cells to SCAP functionality. Namely, in SPRINGKO cells SCAP levels are reduced and the protein is trapped in the Golgi irrespective of the cellular sterol status. Consequently, ectopic over-expression of SCAP rescues SREBP signaling in SPRINGKO cells, in line with functional depletion of SCAP in the ER. The COPII-mediated anterograde transport of SCAP from the ER to the Golgi is well-studied.45,47 However, despite being an essential part of SCAP’s life cycle, the molecular determinants and events that govern its Golgi activity and eventual retrograde COPI-mediated trafficking back to the ER have received only limited attention.

Upon delivery to the Golgi, the SCAP-SREBP complex is tethered to PAQR3.49 The interaction between SCAP and PAQR3 anchors the complex to the Golgi and is necessary to support proteolytic activation of SREBP and hepatic lipid synthesis. Cleavage by S1P is also a perquisite to release SCAP for COPI-mediated retrograde transport to the ER, as pharmacological or genetic inhibition of this process prevents SCAP retrograde trafficking, and instead directs SCAP towards lysosomal degradation.48 The phenotype of SPRINGKO cells is consistent with the potential involvement of SPRING in the process of retrograde transport of SCAP. Retrieval of proteins to the ER is classically dependent on the presence of a C-terminus -KXXX or -KDEL sequence44, both of which are absent in SCAP. Yet as these are also absent in SPRING we find it unlikely that SPRING directly acts as a retrieval adaptor protein. Alternatively, it is possible that SPRING is a licensing factor that is required for directing SCAP towards retrograde transport, possibly by releasing it from PAQR3 or other retention signals. However, PAQR3 was not identified in our screens, possibly reflecting functional redundancy between the 11 PAQR family members,50 or cell-type specific differences. It is also formally possible that SPRING governs SCAP by controlling the fraction that is directed towards the degradative pathway. Absence of SPRING, akin to preventing S1P-dependent cleavage of SCAP48, could result in functional depletion of SCAP. Our observation that the ATF6-mediated stress response - which like SREBP activation requires the sequential proteolytic processing by S1P and S2P yet does not require SCAP - is also reduced in the absence of SPRING provides support for this scenario and may explain reduced SCAP protein level in SPRINGKO cells. As such, SPRING could influence additional processes beyond modulating SCAP and the SREBP pathway. Finally, while speculative, it is intriguing to consider a potential role for SPRING’s cysteine-rich motif in its function. Notably, the cysteine-rich domain present in the ectodomain of the Hedgehog receptor Smoothened was recently reported to
**Fig. 6** SPRING modulates SCAP function. 

a HEK293T cells were transfected with the indicated expression constructs. Total cell lysates and immunoprecipitated fractions were analyzed by immunoblotting as indicated (N = 3). While we determined that SPRING was detected in the co-IP fraction with SCAP, its level was not enriched in this fraction relative to the level in total cells. 

b Representative fluorescence images of CHO-SCAP-eGFP-WT and CHO-SCAP-eGFP-SPRING-KO cells cultured in the presence or absence of sterols; scale bar, 10 μm. See also Supplementary Fig. 7A. Representative images of three independent experiments are shown. 

c An equal amount of crude membrane fractions from Hap1-WT and Hap1-SPRING-KO cells were immunoblotted as indicated (N = 4), with GM130 serving as loading control. 

d, e Hap1-WT and Hap-SPRING-KO cells that stably express SPRING or SCAP were treated as indicated and total cell lysates were immunoblotted as indicated (N = 3), and fluorescent LDL uptake was measured by FACS (N = 3 biologically independent samples). Note that the first 4 bars are from Fig. 2d and are shown for comparison. 

f Hap1-WT and Hap-SPRING-KO cells that stably express SPRING, SCAP, or GFP were cultured for 24 h in medium containing 10% LPDS, 3 mM β-methyl-cyclodextrin, and [13C]2-Sodium acetate (N = 3 biologically independent samples/group). Following lysis incorporation of [13C]2-acetate into cholesterol was determined by mass-spectrometry as described in the Methods section. All bars and errors represent mean ± SEM; *p < 0.05, **p < 0.001.
directly bind cholesterol, and this was sufficient to induce receptor activation.\textsuperscript{51,52} While the Smoothen and SPRING cysteine-rich domains differ in the number and organization of cysteines, it is possible that binding of cholesterol, or a related sterol, to this motif is required for regulation of SPRING function. Possibly, binding of a sterol to this domain may result in a regulatory conformational change akin to that occurring in SCAP\textsuperscript{1}. Evidently, addressing these possibilities and the detailed mechanism by which SPRING regulates the SREBP pathway will require the development of novel sensitive and quantitative assays to monitor, amongst others, SCAP trafficking.

To demonstrate that our findings in cells extend to a physiological in vivo setting we developed Spring knockout mice. Genetic ablation of Spring resulted in early embryonic lethality demonstrating that Spring is required for embryonic development in mice. The embryonic lethality associated with Spring is similar to that observed in mouse models of Srebf1 and Srebf2 deletion.\textsuperscript{53,54} albeit in these models embryonic lethality is not absolute. Whether the lethality observed in Spring\textsuperscript{−/−} embryos is related to its role in lipid metabolism remains to be investigated. Using adenoaviral-mediated silencing of hepatic Spring expression we found marked attenuation of SREBP2 signaling in the liver, with limited effect on the SREBP1 pathway. The effect on SREBP2 is similar to the phenotype observed in liver- or intestine-specific \textit{Scap} knockout mice,\textsuperscript{46,55} but the latter was somewhat unexpected given our results in primary hepatocytes and cell lines, and the functional requirement of an intact SREBP2 pathway to drive the SREBP1-controlled genetic program.\textsuperscript{53,56} This could possibly be due to the short-term duration of the experiment, to the presence of residual \textit{Spring} mRNA expression, or the lack of a dietary challenge. Nevertheless, these results demonstrate that \textit{Spring} is a regulator of the SREBP program in vivo in mice.

Finally, genetic variation in components of the SREBP genetic program, such as in the \textit{LDLR}, \textit{PCSK9}, and \textit{HMGCR}, is associated with lipid traits in humans.\textsuperscript{57} It remains to be seen whether genetic variation in \textit{Spring} contributes to lipid traits and associated diseases in humans. Our bioinformatic analysis suggests that a wide-variety of human tumor cells display dependency on expression of a core set of SREBP-centered genes, including \textit{Spring}, for their growth thereby expanding the potential involvement of \textit{Spring} to other lipid-associated conditions. In conclusion, we report here the identification of \textit{Spring} as a previously unrecognized regulator of the SREBP program. Our work in vitro and in vivo findings warrant further studies to evaluate the contribution of \textit{Spring} to lipid metabolism.

Methods

\textbf{Chemicals.} Simvastatin sodium salt was purchased from Calbiochem. All other reagents (including \textsuperscript{14}C-sodium acetate and methyl-\beta-cyclodextrin) were purchased from Sigma.

\textbf{Cell lines and culture.} HeLa, Hepa-1-6, and HEK293T cells were obtained from the ATCC and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 10,000 U/mL penicillin-supplemented with 10% LPDS, and 3 mM \textit{β}-methyl-cyclodextrin) were cultured in sterol-depletion medium (DMEM, IMDM, or DMEM/F12) supplemented with 10% lipoprotein-deficient serum (LPDS), 2.5 µg/mL simvastatin, and 100 µM mevalonate as indicated and previously described.\textsuperscript{39} For specifically evaluating cholesterol synthesis, cells were cultured in medium supplemented with 10% LPDS and 3 mM \textit{β}-methyl-cyclodextrin and cell viability was monitored using the MTT assay. To generate Hepa-1-6 murine hepatocytes that stably produce Cas9 (Hepa-1-6-Cas9), cells were transduced with a lentiviral construct encoding Cas9 and subsequently individual clones were selected and expression of Cas9 verified. Mouse hepatocytes expressing Cas9 were isolated from Cas9 knock-in mice.\textsuperscript{60,61} (#20857, The Jackson Laboratory) and cultured as described previously.\textsuperscript{51} Hepatocytes from wildtype (WT) livers were used as control WT cells.

\textbf{Generation of FASNKO and SPRINGKO cells.} To ablate expression of FASN and SPRING in human cell lines we used CRISPR/Cas9-mediated genome editing as previously described.\textsuperscript{62} Briefly, guide RNAs (sgRNAs) targeting an exon-coding region of the respective gene were designed and cloned into pX330 (Addgene #42330). The sequences of the sgRNAs are shown in Supplementary Data 1. Consequently, cells were transfected and independent clones obtained after selection. To ablate Spring in CHO-SCAP-eGFP cells sgRNAs targeting hamster Spring or the safe-harbor locus Ppp1r12c were cloned into lenticRIPSPRV2 (Addgene #352961). Lentiviral particles were produced in HEK293T cells and used to transduce and select individual clones CHO-SCAP-eGFP cells. Proper genome editing in all individual clones was confirmed by sequencing. To target Spring in mouse primary WT or Cas9 expressing hepatocytes, cells were isolated and cultured as described above. Cells were infected 4 h post infection with Ad-3xagRNA-Spring at an MOI of 20 for 24 h. Subsequently, cells were washed and sterol-depleted for 16 h after which cells were harvested for immunoblotting and gene expression analysis. To ablate Spring in Hepa-1-6-Cas9 cells were infected similarly at an MOI of 50 for 96 h.

\textbf{Generation of Hapi SQLE-mNeon cells.} To insert the mNeon-2A-Puro reporter cassette into the endogenous SQLE locus we used microhomology-based CRISPR/Cas9-CRIS-Pitch methodology.\textsuperscript{63} Briefly, this technique allows integration of a mNeon-2A-Puro cassette into the ultimate coding exon of SQLE and subsequent deletion of the murine resistant genes for mNeon2A-Puro. As we have recently reported for the \textit{HMGR}KO, the donor fragment containing the microhomology and mNeon-2A-Puro sequences as shown in Supplementary Data 1 and 2. Independent clones were expanded and genome editing was confirmed by sequencing, immunoblotting, and immunofluorescence.

\textbf{Human haploid genetic screens.} \textit{FASN} fitness screen. Hap1 FASNKO cells were mutagenized as previously described.\textsuperscript{51} In brief, retroviral gene-trap virus was produced in HEK293T cells. 40 million Hap1 FASNKO cells were transduced using virus combined from multiple harvests on 3 consecutive days. The obtained mutagenized library was cultured for 12–14 days while maintaining at least 4-fold library complexity. Afterward, cells were fixed with BD Fix buffer I (BD Biosciences) and stained for DNA content using 5 µg/mL propidium iodide (PI). To ensure that only gene-trap insertions were selected, cells were scored for FACS by G1 phase. Genomic DNA was isolated using the Qiagen DNA isolation kit. Insertions were mapped according to the protocol described in Blomen et al.\textsuperscript{64}

\textit{SQLE-mNeon} screen. In order to identify regulators of SQLE we prepared a library of mutagenized Hapi-SQLE-mNeon cells using a gene-trap retrovirus expressing blue fluorescent protein (BFP), as described previously.\textsuperscript{65,66} Briefly, 5 × 10\textsuperscript{6} Hapi-SQLE-mNeon cells were seeded and transduced with virus from two combined harvests on three consecutive days in the presence of 8 µg/mL protamine sulfate (Sigma). Mutagenized cells were expanded to thirty T75 flasks at a split ratio of 1:2 and cells were cultured under selection of 800 µg/mL puromycin for a total of 24 h and with 50 µg/mL \textit{β}-methyl-cyclodextrin-cholesterol (Sigma) during the last 6 h to stimulate cholesterol-dependent degradation of \textit{SQLE} in Hapi. At the end of this treatment, the cells were washed twice with PBS, dissociated with TrypLE (ThermoFisher), pelleted, and fixed with BD Fix Buffer I (BD biosciences) for 10 min at 37 °C. After washing twice with PBS containing 10% FCS, the cells were filtered through a 40 µm strainer (BD FalconTM) before sorting two populations of cells (i.e. SQLE-mNeon\textsuperscript{LOW} and SQLE-mNeon\textsuperscript{HIGH}) that represent ~3% of the lowest and highest SQLE-mNeon expressing cells from the total cell population, respectively. In addition, in order to reduce potential confounding effects of diploid cells, which are heterozygous for alleles carrying gene-trap integrations, the cells were sorted in parallel for haploid DNA content (G1 phase) by staining with propidium iodide. Cell sorting was carried out on a BD FACS C6Sorter C6 cell sorter isolated from 10 million cells of each population were collected. Sorted cells were pelleted and genomic DNA was isolated using a DNA mini kit (Qiagen). To assist de-crosslinking of genomic DNA the cell pellets were resuspended in PBS supplemented with Proteinase K (Qiagen) followed by overnight incubation at 56 °C with lysis buffer AL (Qiagen) with continuous agitation. Gene-trap insertion sites of each sorted cell population were amplified using a Linear Amplification polymerase chain reaction (LAM-PCR) on the total yield of isolated genomic DNA\textsuperscript{67}. Samples were subsequently submitted for deep sequencing and insertion sites were mapped and analyzed as previously described\textsuperscript{44,68}.

\textbf{Molecular cloning and generation of adenoviral particles.} The full human SPRING open reading frame was amplified from Hapi and HepG2 cDNA (ReSeq NM_002738) and sub-cloned into pDONR221 (Invitrogen) to create pDONR221-Spring and pDONR221-SPRING, respectively. The resulting entry constructs were used to generate plent6.3-SPRING and plent6.3-SPRING following LR gateway recombination with plent6.3-
DEST (Invitrogen). Site-directed mutagenesis was used to introduce an N67Q mutation in pDONR221-SPRING, pDONR221-SPRING and pDONR221-SPRINGG36E, respectively. SPRING cDNA was also cloned into the retroviral vector pBABE-Puro (Addgene #1764), mCherry (derived from pmCherry-C1 (Clontech)) was cloned at the C-terminus of SPRING (separated by an alanine linker) to create pBABE-mCherry-mCherry. Additionally, a C-terminal FLAG tag was added to pBABE-SPRING (separated by a LAV linker) to create pBABE-SPRING-FLAG. A KDEL retention signal (5’–AAGGACGGATG–3’) was added to the C-terminus of pBABE-SPRING-mCherry and pBABE-SPRING-FLAG to create pBABE-SPRING-mCherryG36E and pBABE-SPRING-FLAGG36E, respectively. The pcDNA-SCAP-GFP and pcDNA-SCAP-MyHCis plasmids were a kind gift from Andrew Brown (Sydney University, Australia). SCAP-MyHCis was amplified from pcDNA4-SCAP-MyHCis, cloned into pENTR (Invitrogen), and subsequently recombined into pLEnt6.3-DEST (Invitrogen) using Gateway cloning. All plasmids were verified by sequencing and transfected into cells using JetPrime reagent (Polyplus) according to the manufacturer’s protocol. To generate Ad-3xsgRNA-SPRING adenoviral particles a geneblock containing 3 guide RNAs targeting 3 different coding regions of SPRING were cloned into pTer-+(ENTR (Addgene #4301) that has been modified by addition of a CMV-GFP cassette (Supplementary Data 1). The resulting pTer-+(ENTR-GFP-shSPRING or pTer-+(ENTR-GFP-shScrambled were recombined into pAD-BLOCK-IT (Invitrogen) using Gateway recombination. Transfected 293T cells were screened using 5 μg/ml DyLight488-labeled LDL in IMDM supplemented with 1% FBS, 1% PAA, 10 mM HEPES, 0.075% NaN, 0.75% NaCl, 0.04% NaN, and 1% protease inhibitors (Roche). Lysates were cleared by centrifugation at 4 °C for 10 min at 10,000 × g. Subsequently, cleared lysates were separated on NuPAGE 4–12% Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes. Membranes were blocked with primary antibodies, which are listed in Supplementary Data 4. Secondary horseradish peroxidase (HRP)-conjugated antibodies (Invitrogen) were used and chemiluminescence was detected using a LAS4000 (GE Healthcare). In some experiments proteins were fractionated before immunoblotting as previously reported. For evaluating N-glycosylation, dithiothreitol (DTT) was added to total cell lysates –12°C for 6 h. Cells were then washed twice with PBS supplemented with 0.5% BSA, and resuspended in FACS buffer (2 mM EDTA and 5 mM NaN) for 6 h. Cells were then fixed with 4% paraformaldehyde and cellular LDL uptake was determined by flow cytometry on a CytoFLEX Flow cytometer (Beckman Coulter). Intact cells were gated by standard FSC vs SSC gating.

Surface LDLR assay. To determine the level of surface LDL-R, cells were treated as indicated in the figure legends, dissociated using Accutase (STEMCELL Technologies), and washed once with FACS buffer (2 mM EDTA, 0.5% BSA in PBS). Subsequently, cells were stained with an Allophycocyanin (APC)-conjugated mouse anti-human LDLR antibody (R&D; #472413, 10 μg/mL × 10⁶ cells) according to the manufacturer’s instructions. Subsequently, cells were washed three times with FACS buffer, fixed with 4% paraformaldehyde (FAA) and analyzed on a CytoFLEX Flow cytometer (Beckman Coulter). Relative surface LDLR levels were calculated from mean values after correction for background signal.

Cholesterol synthesis assay. Hap1 control and Hap1-SPRINGKO cells were cultured in 6-well plates in IMDM supplemented with 10% LPDS, 3 mM β-methyl-cyclohexan and 3 μM 12-Deoxy Acetate for 24 h. Cells were then washed twice with ice-cold PBS, once with 0.9% NaCl followed by addition of 1 mL of ice-cold methanol. For the extraction of sterols cells were scrapped and transferred to a 2-mL tube, sonicated with a tip sonicator at 8 W and 40 Joule and centrifuged at 14,000 × g for 10 minutes at 4°C. The supernatant was transferred to a new 1.5 mL tube and evaporated under nitrogen. The dried extract was dissolved in 100 μL methanol and analyzed by an Atmospheric Pressure Chemical Ionization (APCI) mass spectrometer equipped with a Turboionization source coupled to a Thermo Scientific iFunnel 6000 mass spectrometer (Thermo Scientific) with an Acuity UPLC HSS T3, 1.8 μm particle diameter (Waters, Milford Massachusetts, USA) coupled to a Thermo Q Exactive Plus Orbitrap mass spectrometer with an atmospheric-pressure chemical ionization (APCI) source. The column was kept at 30 °C and the flow rate was 0.2 μL/min. Cholesterol and other sterols were detected in a negative ion mode for a total runtime of 15 min. Data was acquired in full-scan positive ionization mode. Data interpretation was performed using the Xcalibur software (Thermo Fisher Scientific).
Yabe, D., Brown, M. S. & Goldstein, J. L. Insig-2, a second endoplasmic reticulum protein that binds cholesterol and stimulates transcription by binding to a sterol regulatory element. Nature 522, 1278–1285 (1997).

Duncan, E. A., Brown, M. S., Goldstein, J. L. & Sakai, J. Cleavage site for sterol-regulated protease localized to a leu-Ser bond in the luminal loop of sterol regulatory element-binding protein-2. J. Biol. Chem. 272, 12778–12785 (1997).

Duncan, E. A., Davé, U. P., Sakai, J., Goldstein, J. L. & Brown, M. S. Second-site cleavage in sterol regulatory element-binding protein occurs at transmembrane junction as determined by cysteine panning. J. Biol. Chem. 273, 17801–17809 (1998).

Horton, J. D. et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc. Natl Acad. Sci. USA 100, 12027–12032 (2003).

Takashima, K. et al. COPII-mediated retrieval of SCAPI is crucial for regulating lipopigmen formation in basal and sterol-deficient conditions. J. Cell. Sci. 128, 2805–2815 (2015).

Sharpe, L. J. & Brown, A. J. Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCAR). J. Biol. Chem. 288, 18707–18715 (2013).

Howe, V., Sharpe, L. J., Prabhu, A. V. & Brown, A. J. New insights into cellular cholesterol acquisition: promoter analysis of human HMGCAR and SQLE, two key control enzymes in cholesterol synthesis. Biochim Biophys. Acta Mol. Cell Biol. Lipids 1862, 647–657 (2017).

Flent, S., Stevenson, J., Kristiana, J. & Brown, A. J. Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMGCAR reductase. Cell Metab. 13, 260–273 (2011).

Zelcer, N. et al. The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. Mol. Cell. Biol. 34, 1262–1270 (2014).

Brockmann, M. et al. Genetic wiring maps of single cell protein states reveal an off-switch for GPCR signaling. Nature 546, 307–311 (2017).

Hobbs, H. H., Russell, D. W., Brown, M. S. & Goldstein, J. L. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu. Rev. Genet. 24, 133–170 (1990).

Goldstein, J. L., Rawson, R. R. & Brown, M. S. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. Arch. Biochem. Biophys. 397, 139–148 (2002).

Jae, L. T. et al. Deciphering the glycosylation of dystroglycanopathies using haploid screens for lassa virus entry. Science 340, 479–483 (2013).

Bloomen, V. A. et al. Gene essentiality and synthetic lethality in haploid human cells. Science 350, 1092–1096 (2015).

Kleinfelter, L. M. et al. Haploid genetic screen reveals a profound and direct dependence on cholesterol for hantavirus membrane fusion. MBio 6, e00801 (2015).

Hetz, C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat. Rev. Mol. Cell Biol. 13, 89–102 (2012).

Cao, L. et al. Global site-specific analysis of glycoprotein N-glycan processing. Nat. Protoc. 13, 1196–1212 (2018).

Nohturff, A., DeBose-Boyd, R. A., Scheek, S., Goldstein, J. L. & Brown, M. S. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. Proc. Natl Acad. Sci. USA 96, 11235–11240 (1999).

DeBose-Boyd, R. A. et al. Transport-depent proteolysis of SREBP: relocation of site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. Cell 99, 703–712 (1999).

Munro, S. & Pelham, H. R. A C-terminal signal prevents secretion of luminal ER proteins. Cell 48, 899–907 (1987).

Shao, W., Machamer, C. E. & Espenshade, P. J. Fatostatin blocks ER exit of SCAPI but inhibits cell growth in a SCAP-independent manner. J. Lipid Res. 57, 1564–1573 (2016).

Shimano, H. & Sato, R. SREBP-regulated lipid metabolism: convergent physiolog - divergent pathophysiology. Nat. Rev. Endocrinol. 13, 710–730 (2017).

Yu, C. et al. High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. Nat. Biotechnol. 34, 419–423 (2016).

Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. Nat. Genet. 49, 1779–1784 (2017).

Carette, J. E. et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick Cl. Nature 477, 340–341 (2013).

Jae, L. T. et al. Virus entry. Lassa virus entry requires a trigger-induced receptor switch. Science 344, 1500–1510 (2014).

Pillay, S. et al. An essential receptor for adenov-associated virus infection. Nature 530, 108–112 (2016).
Staring, J. et al. PLA2G16 represents a switch between entry and clearance of Picornaviridae. Nature 541, 412–416 (2017).

Carette, J. E. et al. Haploid genetic screens in human cells identify host factors used by pathogens. Science 326, 1231–1235 (2009).

Loregger, A. et al. Haploid Mammalian Genetic Screen Identifies UBXD8 as a Key Determinant of HMGCGR Degradation and Cholesterol Biosynthesis. Arteriosclerosis, Thrombosis, Vasc. Biol. 37, 2064–2074 (2017).

Slawson, R. B., Delbose-Boyd, R., Goldstein, J. L. & Brown, M. S. Failure to cleave sterol regulatory element-binding proteins (SREBPs) causes cholesterol auxotrophy in Chinese hamster ovary cells with genetic absence of SREBP cleavage-activating protein. J. Biol. Chem. 274, 28549–28556 (1999).

Matsuda, M. et al. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and hepatic elevation. Genes Dev. 15, 2105–2116 (2001).

Espenshade, P. J. & Hughes, A. L. Regulation of sterol synthesis in eukaryotes. Annu. Rev. Genet. 41, 401–427 (2007).

Shao, W. & Espenshade, P. J. Sterol regulatory element-binding protein (SREBP) cleavage regulates Golgi-to-endoplasmic reticulum recycling of SREBP cleavage-activating protein (SCAP). J. Biol. Chem. 289, 7554–7557 (2014).

Xu, D. et al. PAQR3 modulates cholesterol homeostasis by anchoring Scap/SREBP complex to the Golgi apparatus. Nat. Commun. 6, 8100 (2015).

Tang, Y. T. et al. PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. J. Mol. Evol. 61, 372–380 (2005).

Huang, P. et al. Cellular cholesterol directly activates smoothened in hedgehog signaling. Cell 166, 1176–1187.e14 (2016).

Byrne, E. F. X. et al. Structural basis of Smoothened regulation by its extracellular domains. Nature 535, 517–522 (2016).

Vergnes, L. et al. SREBP-2-deficient and hypomorphic mice reveal roles for SREBP-2 in embryonic development and SREBP-1c expression. J. Lipid Res. 57, 410–421 (2016).

Shimo, H. et al. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J. Clin. Invest. 100, 2115–2124 (1997).

McFarlane, M. R. et al. Scap is required for sterol synthesis and crypt growth in intestinal mucosa. J. Lipid Res. 56, 1560–1571 (2015).

Bong, S. et al. Expression of SREBP-1c Requires SREBP-2-mediated Generation of a Sterol Ligand for LXR in Livers of Mice. Elife 6, S15 (2017).

Global Lipids Genetics Consortium. et al. Discovery and refinement of loci associated with lipid levels. Nat. Genet. 45, 1274–1283 (2013).

Nothuhr, A., Yabe, D., Goldstein, J. L., Brown, M. S. & Espenshade, P. J. Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. Cell 102, 315–323 (2000).

Zelcer, N., Hong, C., Boyadjian, R. & Tontonoz, P. LXR regulates cholesterol uptake through ld1-dependent ubiquitination of the LDL receptor. Science 325, 100–104 (2009).

Platt, R. J. et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 159, 440–455 (2014).

Fedoseenko, A. et al. The COMMED family regulates plasma LDL levels and attenuates atherosclerosis through stabilizing the CCC complex in eosinophilic LDLR trafficking. Circ. Res. 122, 1648–1660 (2018).

Lackner, D. H. et al. A generic strategy for CRISPR-Cas9-mediated gene tagging. Nat. Commun. 6, 10237 (2015).

Nakade, S. et al. Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9. Nat. Commun. 5, 5560 (2014).

Brockmann, M. et al. Genetic wiring maps of single-cell protein states reveal an off-switch for GPCR signalling. Nature 546, 307–311 (2017).

Krycer, J. R., Phan, I. & Brown, A. J. A key regulator of cholesterol homeostasis, SREBP-2, can be targeted in prostate cancer cells with natural products. Biochem J. 466, 191–201 (2012).

Leichner, G. S., Avner, R., Harats, D. & Roittelman, J. Dislocation of HMGC-CoA reductase and Insig-1, two polytopic endoplasmic reticulum proteins, en route to proteasomal degradation. Mol. Biol. Cell 20, 3330–3341 (2009).

Zerenkurk, E. J., Sharpe, L. J. & Brown, A. J. DHCR24 associates strongly with the endoplasmic reticulum beyond predicted membrane domains: implications for the activities of this multi-functional enzyme. Biosci. Rep. 34, 666 (2014).

Loregger, A., Nelson, J. K. & Zelcer, N. in Cholesterol Homeostasis (eds Celissen, I. C. & Brown, A. J.) vol 1583, 53–63 (Springer, New York, 2017).

Fernández-Fernández, M., Rodríguez-González, P. & García Alonso, J. I. A simplified calculation procedure for mass isotopomer distribution analysis (MIDA) based on multiple linear regression. J. Mass Spectrom. 51, 980–987 (2016).

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Author contributions
The study was jointly conceived and designed by T.B. and N.Z. All authors contributed extensively to the work presented in this paper. A.L., M.R., J.N., L.v.d.H., J.S., I.H., S.H., M.v.d.B., and S.S. designed, performed, collected data and analyzed the cell-based experiments. M.R., J.N., and L.T.J. performed and analyzed the RNAseq experiments. M.v.d.B. measured and analyzed cholesterol synthesis. A.L., L.v.d.H., J.S., I.H., L.K., and R.O., and B.v.d.S. designed, performed, collected data and analyzed the mouse-based experiments. A.L., J.N., T.B., and N.Z. wrote the paper. All authors discussed the results and implications and commented on the manuscript.

Competing interests
The authors declare no competing interests.

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