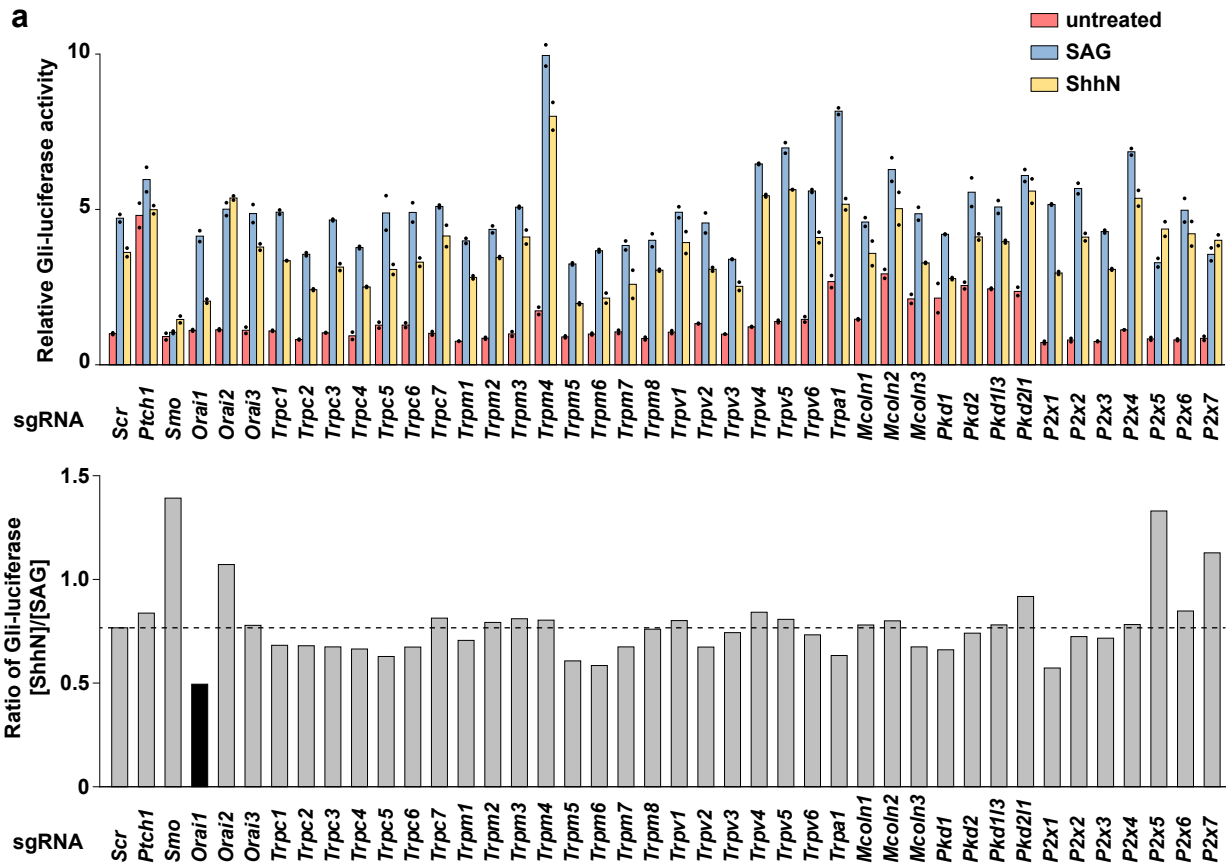
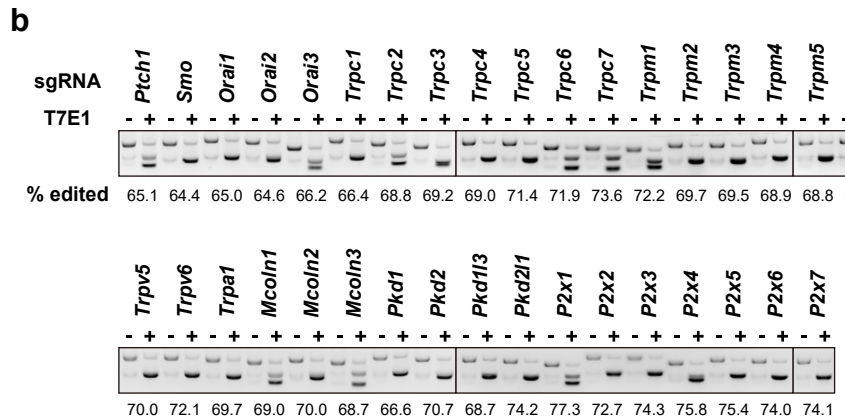
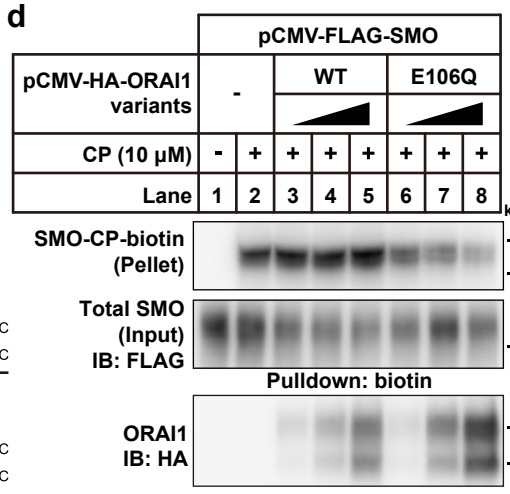
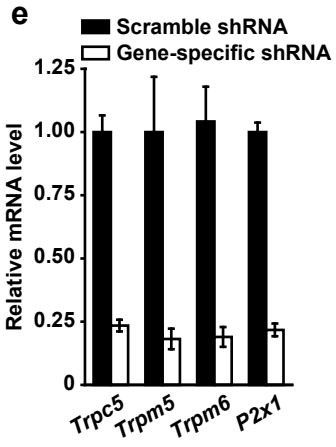
Fig. S4

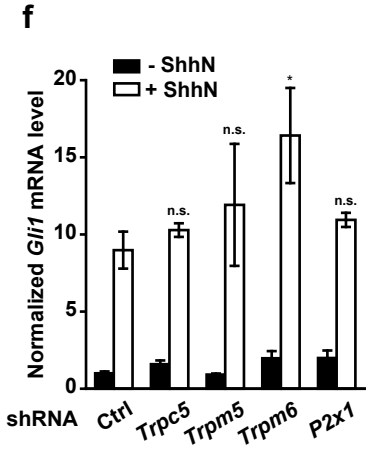

a
untreated
SAG
ShhN
Relative Gli-luciferase activity
sgRNA
Scr Ptch1 Smo Orai1 Orai2 Orai3 Trpc1 Trpc2 Trpc3 Trpc4 Trpc5 Trpc6 Trpc7 Trpm1 Trpm2 Trpm3 Trpm4 Trpm5 Trpm6 Trpm7 Trpm8 Trpv1 Trpv2 Trpv3 Trpv4 Trpv5 Trpv6 Trpa1 Mcoln1 Mcoln2 Mcoln3 Pkd1 Pkd2 Pkd1l3 Pkd2l1 P2x1 P2x2 P2x3 P2x4 P2x5 P2x6 P2x7
Ratio of Gli-luciferase [ShhN]/[SAG]


b
sgRNA
T7E1
% edited
65.1 64.4 65.0 64.6 66.2 66.4 68.8 69.2 69.0 71.4 71.9 73.6 72.2 69.7 69.5 68.9 68.8 68.8 68.6 73.5 70.7 69.6 71.3 70.4
70.0 72.1 69.7 69.0 70.0 68.7 66.6 70.7 68.7 74.2 77.3 72.7 74.3 75.8 75.4 74.0 74.1


c
Mouse Orai1 exon1, chr 5: 123153492-123153526
clone #1
clone #2
WT
allele 1: del 2 bp
allele 2: ins 1 bp
allele 1: del 64 bp


d
pCMV-FLAG-SMO
pCMV-HA-ORAI1 variants
WT
E106Q
CP (10 μM)
Lane
kDa
SMO-CP-biotin (Pellet)
Total SMO (Input) IB: FLAG
Pulldown: biotin
ORAI1 IB: HA


e
Scramble shRNA
Gene-specific shRNA
Relative mRNA level
Trpc5 Trpm5 Trpm6 P2x1


f
- ShhN
+ ShhN
Normalized Gli1 mRNA level
n.s.
shRNA Ctrl Trpc5 Trpm5 Trpm6 P2x1

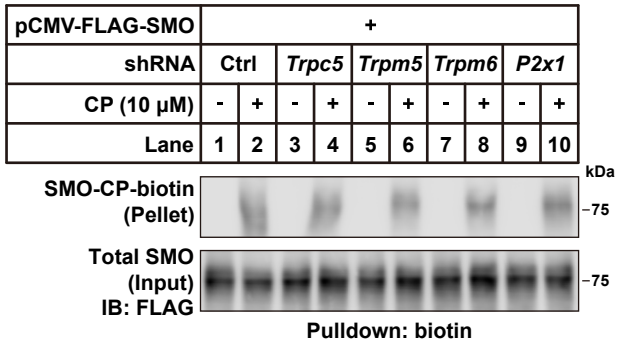

g
pCMV-FLAG-SMO
shRNA
Ctrl Trpc5 Trpm5 Trpm6 P2x1
CP (10 μM)
Lane
kDa
SMO-CP-biotin (Pellet)
Total SMO (Input) IB: FLAG
Pulldown: biotin

Fig. S4. Screening for Ca^{2+} channels responsible for ShhN-induced Ca^{2+} influx and validation of screening hit.

a. Upper panel: Relative luciferase activities of NIH3T3 cells stably expressing Gli-luciferase, Cas9-blasticidin and a constitutive EGFP as internal control (abbreviated as NIH3T3-GCE). Cells were transduces with pooled lentivirus containing indicated sgRNAs against a single gene, selected with 2 μg/mL puromycin, and treated with or without 100 nM SAG or 1:6 ShhN-conditioned media for 36 hours. Cells were then harvested for luciferase activity measurement according to the manufacturer's instructions. Two independent experiments were performed and plotted. Lower panel: calculated ShhN/SAG induction ratio. The value of cells receiving scramble sgRNA were normalized to 1.

b. Validation of editing efficiencies of sgRNAs by T7E1 endonuclease digestion.

c. Sanger sequencing of two monoclonal *Orai1* knockout cells. Primers flanking sgRNA targeting sites were used to amplify the target locus.

d. Analysis of SMO cholesterylation in HEK-293T cells co-expressing of WT or E106Q dominant-negative ORAI1. 1 μg SMO expression plasmid, along with 1, 2 or 4 μg ORAI1 expression plasmid were co-transfected into HEK-293T cells in a 10-cm dish, the total amount of transfected DNA was adjusted to 6 μg using pcDNA3.

e. Knockdown efficiencies of individual shRNAs against target genes.

f-**g.** The effects of *Trpm5*, *Trpm6*, *Trpc5* or *P2x1* knockdown on *Gli1* transcript level (**f**) or SMO cholesterylation (**g**). Sequences are provided in table S1.