

Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1. Increase in cyclic guanosine monophosphate (cGMP) concentration of GC-G-expressing CHO-K1 or NG108 cells on exposure to coolness.

Two days after transfection of empty vector or GC-G-encoding plasmid in CHO-K1 (Chinese hamster ovary) or NG108 (mouse neuroblastoma) cells, cells were exposed to ambient temperatures (37 or 15°C) for 20 min and cellular cGMP concentration was determined. Data are mean \pm SD from 3 experiments. **, $P < 0.01$.

Supplementary Figure 2. Expression of full-length (FL) and diverse mutant GC-G proteins.

(a) Expression of FLAG-tagged GC-G FL and deletion mutants. Two days after transfection, cell lysates were examined by western blot analysis with anti-FLAG antibody to confirm the expression of the FL or indicated mutant protein.

(b) Expression of the chimeric GC-A/G protein. HEK-293T cells were transfected with an empty vector (Vector) or an expression plasmid encoding GC-A, GC-G, or chimeric GC-A/G. A FLAG epitope tag was added at the NH₂-terminus (FLAG.GC-A, FLAG.GC-G, or FLAG.GC-A/G). Two days after transfection, cell lysates were

immunoprecipitated, then examined by western blot analysis with anti-FLAG antibody to confirm the expression of the indicated FL or mutant proteins.

(c) Cell-surface localization of GC-A, GC-G, and the chimeric GC-A/G protein.

FACS analysis of a set of cells transfected as above, detached and stained with anti-FLAG antibody to determine the cell-surface localization.

(d) Recombinant GST.GC-G-CYC fusion protein was purified from the soluble fraction of bacterial lysates with glutathione sepharose beads and stained with Coomassie Brilliant Blue. Molecular mass is indicated on the left in kDa (a, b, and d).

Supplementary Figure 3. Grueneberg ganglion (GG) neurons from OMP-GFP/GC-G^{+/+} and OMP-GFP/GC-G-KO animals are viable after coronal section and respond to KCl with Ca²⁺ signals.

(a–b) Representative Ca²⁺ signals induced in GG neurons by perfusion with KCl (60 mM) were used as a control for viability and responsiveness of tissue slices from olfactory marker protein-green fluorescent protein (OMP-GFP)/GC-G^{+/+} or OMP-GFP/GC-G-KO animals. The numbers in the bottom right hand corners are number of cells with Ca²⁺ transient similar to that in the respective graph (left) and the total number of measured cells (right). ΔF represents changes in the ratio of the fluorescence intensity of Fura-2 at 340 nm/380 nm excitation. The experiments were

conducted at 37 °C.

Supplementary Figure 4. Expression of GC-G in thermosensory neurons is confined to the GG.

(a–f) *In situ* hybridization experiments with antisense probes for GC-G (a, c, e) and TRPM8 (b, d, f) on coronal sections through the GG (a-b), trigeminal ganglion (TG) (c-d) and dorsal root ganglion (DRG) (e-f) of mouse pups. While GC-G is expressed in the GG, it is absent from the TG and the DRG. By contrast, TRPM8 is present in a subset of neurons in the TG and DRG. The sections are representative of 3 experiments. Scale bars: 50 µm.

Supplementary Figure 5. GC-G is expressed in the GG but not in brain of mice.

(a) *In situ* hybridization on a sagittal section through a rather medial region of the head of a mouse pup incubated with an antisense probe for GC-G (position of the GG is indicated by an arrow and the outline of the brain is given by the broken line).

(b-c) Higher magnification of the anterior nasal region harboring the GG (arrows denote the GG). GC-G-expressing GG neurons are stained in dark purple. The section is representative of 5 pups. Scale bars: a = 2 mm; b = 1 mm; c = 0.2 mm.

Supplementary Figure 6. GC-G is absent from the mouse brain.

(a-c) *In situ* hybridization with an antisense probe for GC-G on sagittal sections through more lateral regions of the head of a mouse pup (outline of the brain is indicated by broken lines). The sections are representative of 5 pups. Scale bars: 2 mm.

Supplementary Figure 7. Temperature changes at the GG and the skin of mouse pups after exposure to a cool ambient temperature.

(a) Representative thermal images of pups at various times after exposure to a cool ambient temperature (15°C). In the top panel, the whole pup is depicted. The lower panel shows enlarged images of the head with the region harboring the GG indicated by arrowheads, and a representative spot of the skin at the body's periphery is labeled with squares. The color code of the thermal gradient scale is indicated to the right.

(b) Mean temperatures at the GG and the skin in P4 pups after exposure to 15°C. For these areas of the body, no clear differences regarding the temperature were observed comparing WT and GC-G-KO pups.

Supplementary Figure 8. Typical sonograms and peak frequency analyses of ultrasonic vocalizations (USVs).

(a) Representative sonograms from 3 categories of USV calls (chevron, downward, and complex) and (b) peak frequency of USV emitted by WT and GC-G-KO pups (P2). Data are mean \pm SD from WT (n=10 pups) or GC-G-KO (n=12 pups) animals.

Supplementary Figure 9. Generation of the targeted *loxP*-flanked (*Flox*) and cyclase deletion (Δ) alleles of the *Gucy2g* gene.

(a) The scheme for gene targeting and FLP/Cre-mediated recombination. We generated conditional *Gucy2g* mutant mice with one targeting vector that introduced two *loxP* sites flanking exons 16 to 18 coding for a critical part of the cyclase catalytic domain of *Gucy2g*. The external probe for Southern blot analysis is denoted by a thick line and the restriction site used (B, *Bam*HI) is indicated. We then removed the neomycin resistance gene cassette by transfection of the targeted ES cells with a FLP-recombinase encoding expression plasmid. The final targeted *Flox* allele contains a *loxP* site upstream of exon 16 and another *loxP* site downstream of exon 18 of the *Gucy2g* gene. ES cells were injected into C57BL/6 blastocysts to generate chimera. Germline transmission of the *Flox* allele was confirmed by PCR analysis. Heterozygous global *Gucy2g*^{A/+} mice were generated by crossing male Prm-Cre; *Gucy2g*^{Flox/+} to WT female mice. Small arrows indicate primers used in PCR analyses to confirm gene targeting (CU, FD, F1, R1) or Cre-mediated recombination (CU and

JD) for genotyping. Exons are numbered under open boxes. Filled triangles indicate *loxP* sites. Neo, neomycin resistance gene cassette; TK, thymidine kinase gene cassette for negative selection; WT, wild type.

(b) Genomic Southern blot to detect the targeted ES clones with *Bam*HI digestion using the external probe.

(c) Genotyping of the *Flox* allele by PCR analysis. PCR of genomic DNA from WT (+/+) or heterozygous (*Flox*/+) mice using primers CU+FD (5' *loxP* site) or F1+R1 (3' *loxP* site), respectively. Because an *Eco*RI restriction site was introduced along with the *loxP* site, digestion of *Eco*RI resulted in smaller PCR products indicating the presence of the *loxP* site.

(d) Genotyping of the Δ allele. Genomic DNA PCR analyses from +/+ or Δ /+ mice using primers CU and JD showing exons 15 to 18 deleted in the Δ allele.

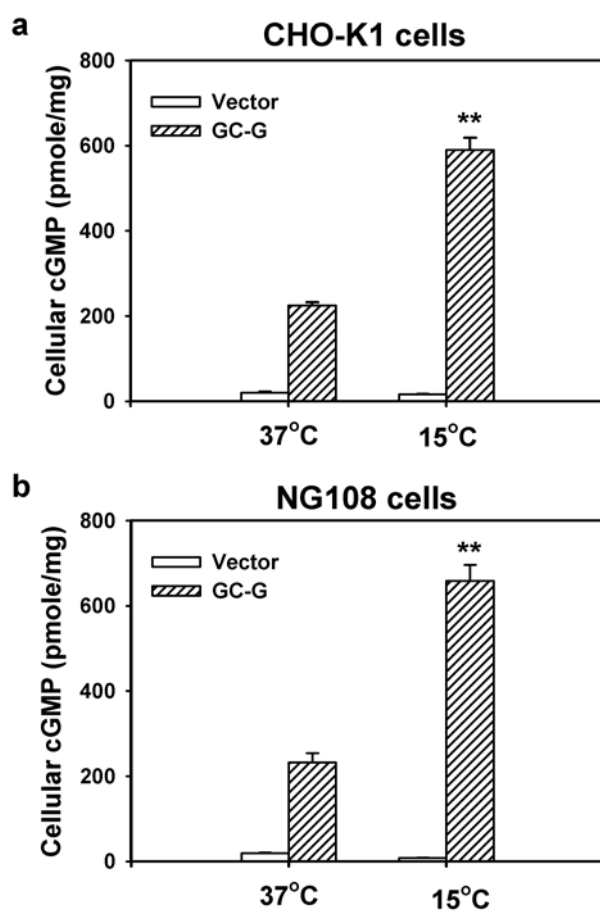
(e) Schematic graphs showing the domain composition encoded by WT or Δ transcript.

Locations of F2 and R2 primers flanking exons 16 to 18 used for RT-PCR analysis are marked. Peptide immunogen used to generate the anti-GC-G antibody for western blot analysis is indicated. SP, signal peptide; ECD, extracellular domain; TM, transmembrane region; KLD, kinase-like domain; CYC, cyclase domain.

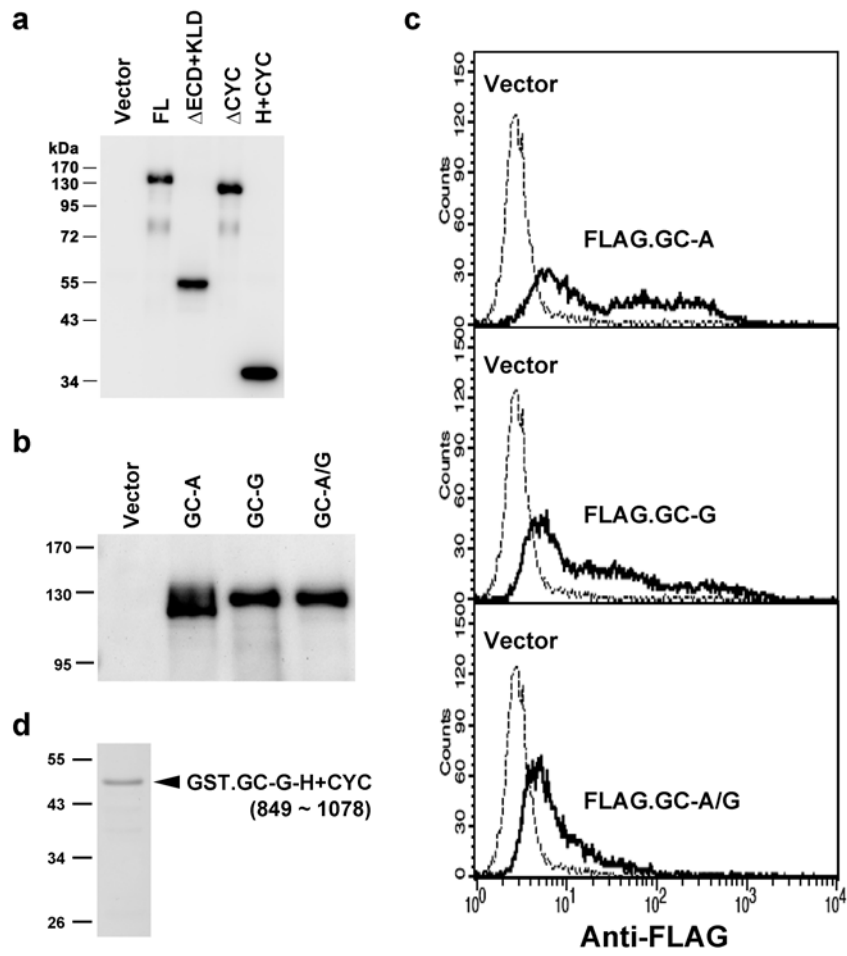
(f) Expression of GC-G +/+ and Δ / Δ mutant mRNA. RT-PCR analysis confirmed the deletion of exons 16 to 18 in the GC-G Δ / Δ mRNA.

(g) Expression of GC-G $+/+$ and Δ/Δ mutant protein. Western blot analysis of testis extracts from $+/+$ and Δ/Δ mice using the antibody specifically against the hinge region of GC-G, confirming the deletion of coding sequences from exons 16 to 18.

Suppl. FIG. 1

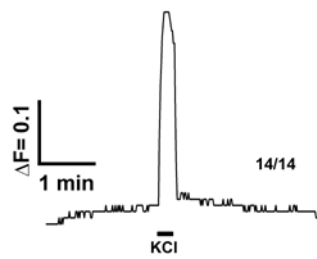


Suppl. FIG. 2

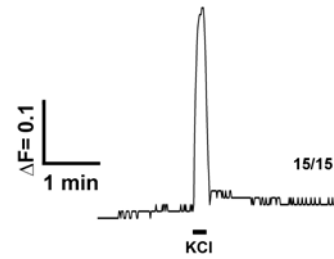


Suppl. FIG. 3

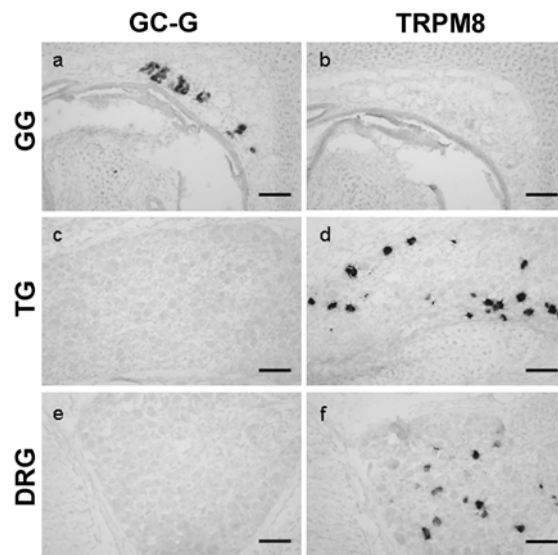
a OMP-GFP / GC-G^{+/+}
(GFP-positive cells)



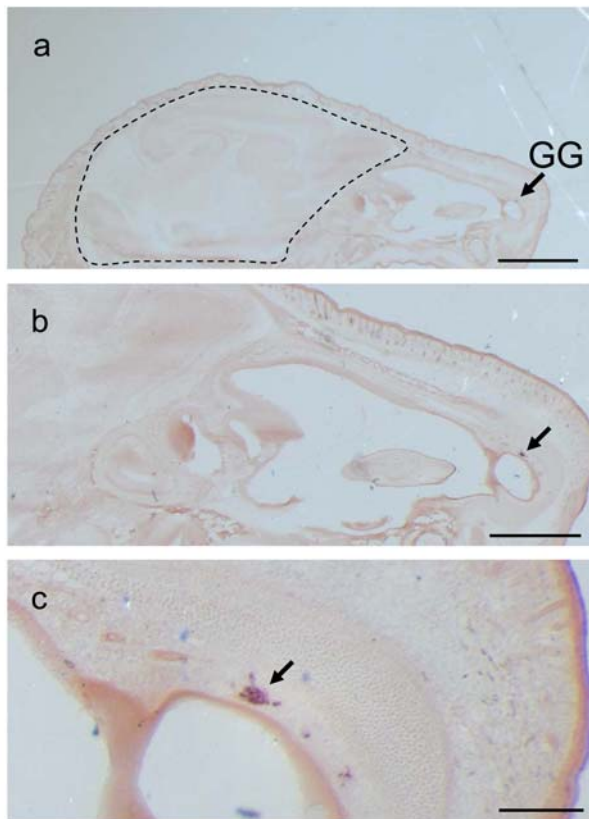
b OMP-GFP / GC-G^{-/-}
(GFP-positive cells)



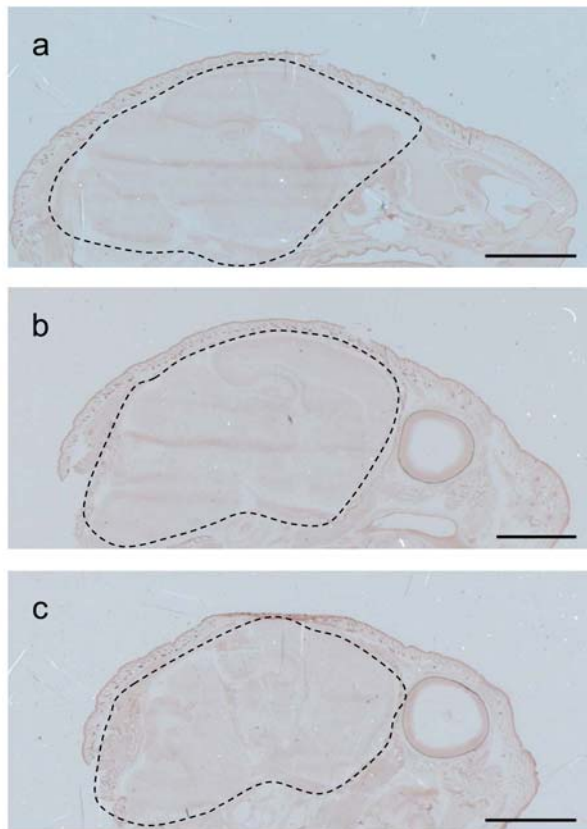
Suppl. FIG. 4



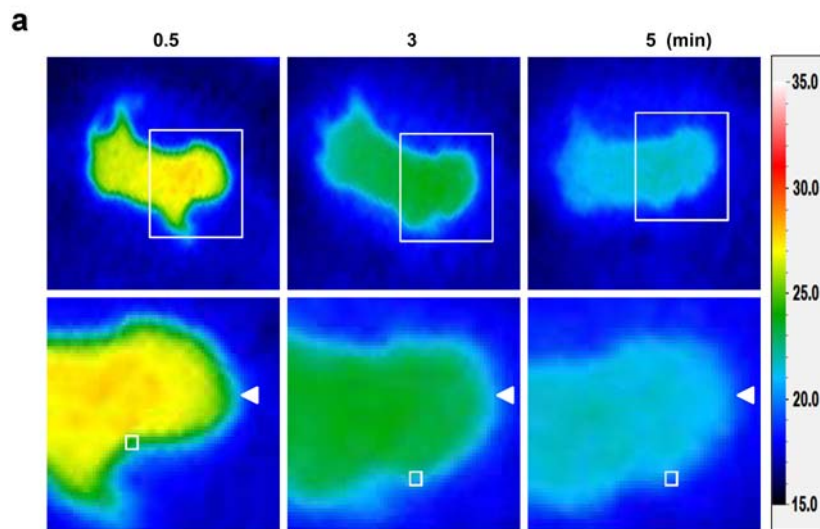
Suppl. FIG. 5



Suppl. FIG. 6



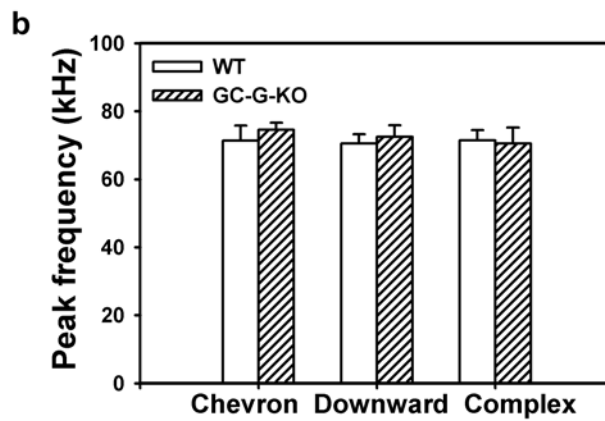
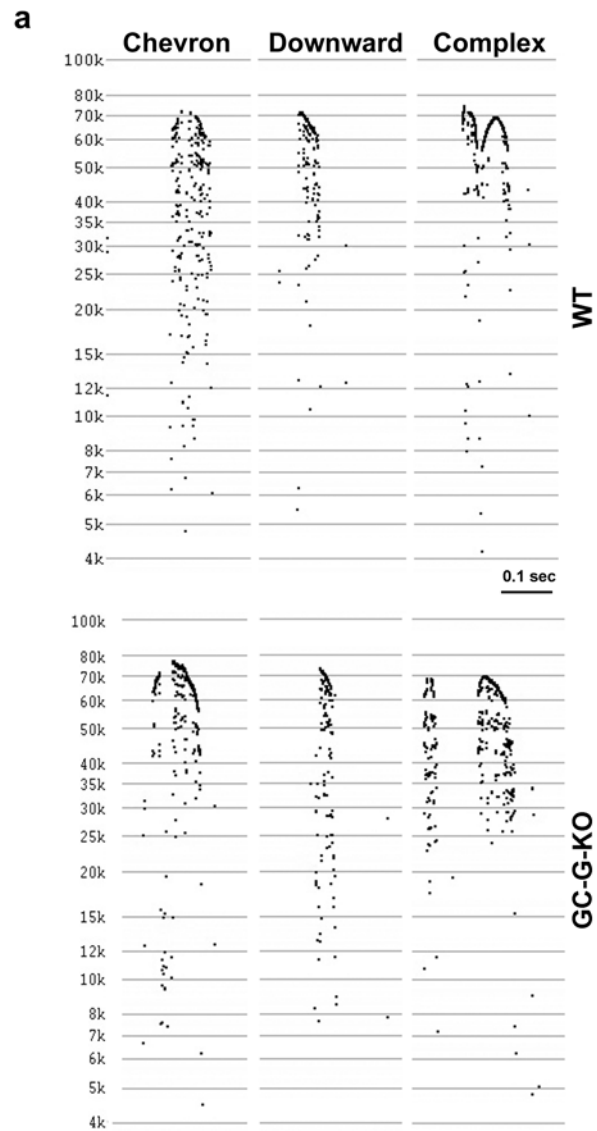
Suppl. FIG. 7



b

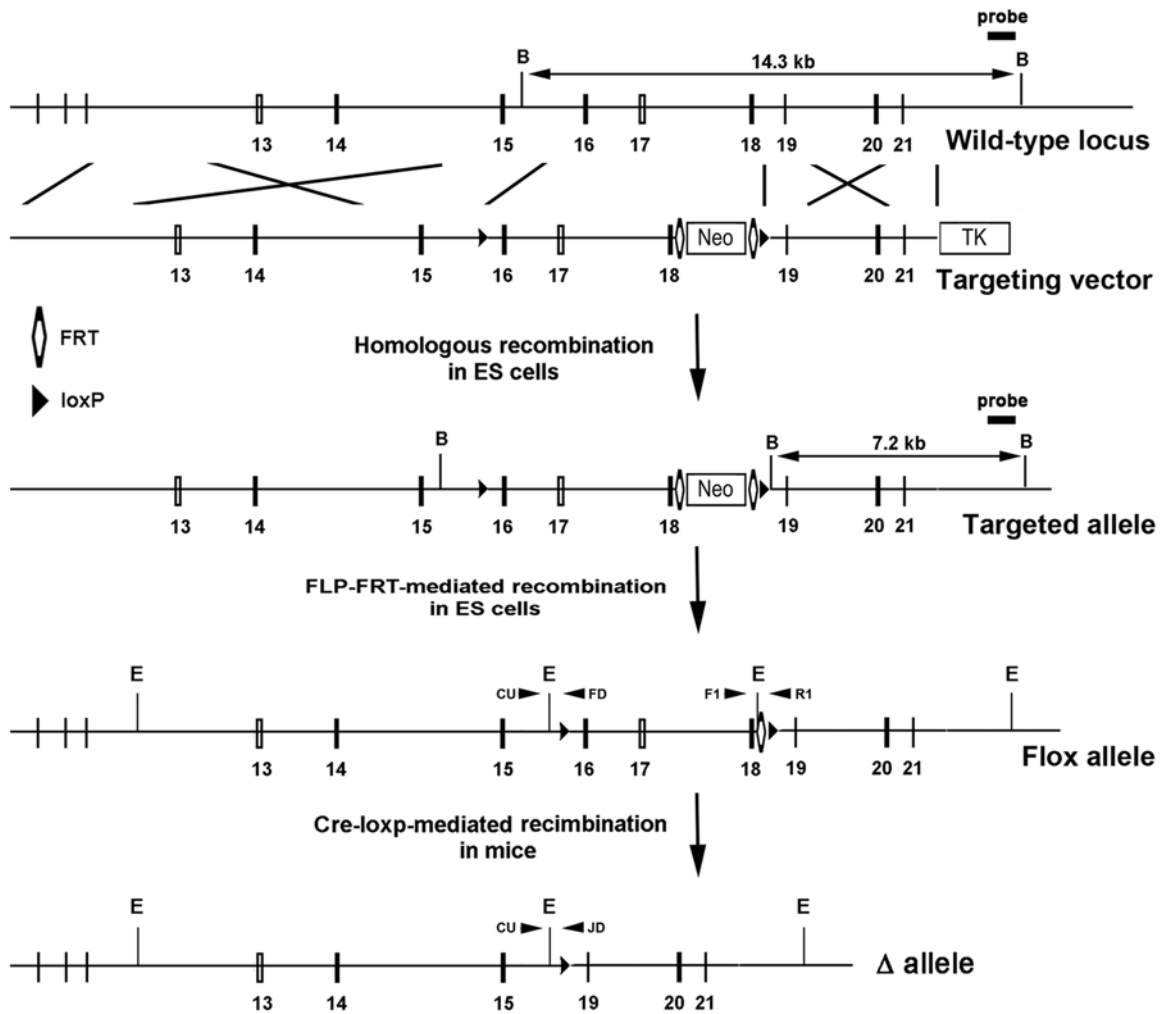
Genotype / min	GG			Skin		
	0.5	3	5	0.5	3	5
WT (n=3)	20.5 ± 0.1	19.5 ± 0.9	18.9 ± 0.3	23.3 ± 0.8	22.4 ± 0.2	20.8 ± 0.1
GC-G-KO (n=4)	20.5 ± 0.4	19.4 ± 0.9	18.6 ± 0.6	23.0 ± 1.3	21.6 ± 1.6	20.4 ± 0.9

Suppl FIG. 8

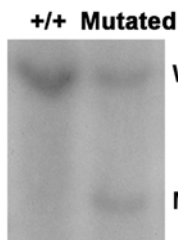


Suppl. FIG. 9

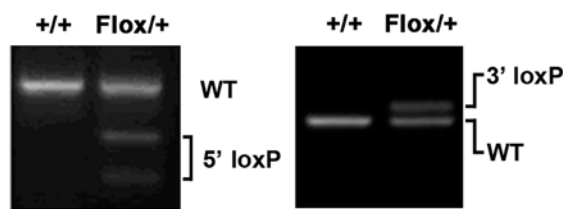
a



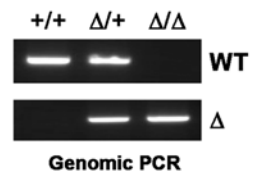
b



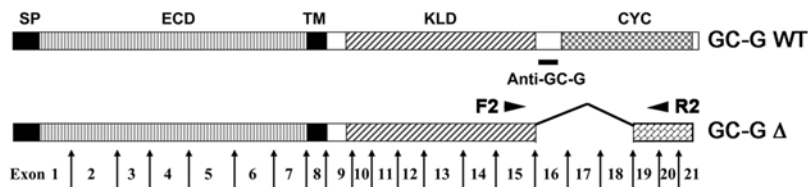
c



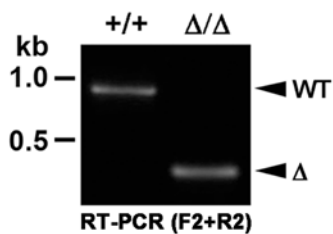
d



e



f



g

