Distinct roles of bombesin peptides in itch transmission

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Supplementary Figure 1 *Nmbr* KO and their WT littermates showed comparable scratching responses to histamine ($P = 0.3479$), 48/80 ($P = 0.1858$), 5-HT ($P = 0.3346$), CQ ($P = 0.7368$), SLIGRL ($P = 0.6360$), and BAM8-22 ($P = 0.7080$). $n = 6$ per genotype.
Supplementary Figure 2 Normal projection of primary afferents in the dorsal horn of Nmb/Grp DKO mice.

(A) Density and innervation of CGRP+ (red) and IB4-binding fibers (green) in the superficial dorsal horn of the lumbar spinal cord is comparable between WT and Nmb/Grp DKO mice. (B and C) IHC of SP+ (B) and TRPV1+ (C) primary afferents in superficial dorsal horn of WT mice and Nmb/Grp DKO mice. (D) Normalized staining intensity of IB4-binding (P = 0.8477), CGRP (P = 0.4552), SP (P = 0.7632) and TRPV1 (P = 0.8790). Values are presented as mean ± SEM. n = 4 per genotype, unpaired t test. Scale bar, 100 µm.
Supplementary Figure 3  Normal pain behaviors of Grp KO mice.

(A and B) Mechanical pain threshold was comparable between Grp KO mice and their WT littermates as tested by non-noxious von Frey assay (P = 0.9351)(A) and noxious Randall Selitto assay (P = 0.6179)(B). n = 8 per genotype. (C-E) Grp KO mice showed normal responses to thermal stimuli in Hargreaves (P = 0.9844)(C), hotplate (P = 0.8183)(D) and tail immersion (P = 0.1223)(E) tests compared with WT littermates. n = 8 per genotype. (F-H) Licking/flinching responses induced by formalin (2%, 20 µl) were comparable between WT and Grp KO mice (P
= 0.9890, n = 6 per genotype) (F), capsaicin (2 µg, 20 µl, n = 7 per genotype) (P = 0.7805)(G) and MO (0.75%, 20 µl, n = 8 per genotype) (P = 0.2429)(H) were not different between Grp KO and WT littermates. (I and J) Grp KO and WT littermates developed similar extent of mechanical (P = 0.3214)(I) and thermal hypersensitivity (P = 0.4659)(J) after i.pl. injection of CFA (20 µl). n = 7 per genotype.

Values are presented as mean ± SEM. unpaired t test in (A-C, G and H), two-way repeated measure ANOVA in (D-F, I and J).
Supplementary Figure 4 Normal pain behaviors of *Nmb/*Grp DKO mice.

(A and B) Mechanical pain threshold tested by von Frey assay (*P* = 0.4615, *n* = 14-15 per genotype)(A) and Randall Selitto assay (*P* = 0.4614, *n* = 7-9 per genotype)(B) was comparable between *Nmb/*Grp DKO mice and their WT littermates. (C-E) *Nmb/*Grp DKO mice showed normal response to thermal stimuli in Hargreaves (*P* = 0.3589, *n* = 6-8 per genotype)(C), hotplate (*P* = 0.6178, *n* = 7-9 per genotype)(D) and tail immersion tests (*P* = 0.5485, *n* = 7-9 per genotype)(E). (F-H) Licking/flinching responses induced by formalin (2%, 20 µl) (*P* = 0.6178,
n = 6-7 per genotype) (F), capsaicin (2 µg, 20 µl) (P = 0.3074, n = 7-9 per genotype)(G) and MO (0.75%, 20 µl)(P = 0.9402, n = 7 per genotype)(H) were not different between Nmb/Grp DKO mice and WT littermates. (I and J) Nmb/Grp DKO mice and WT littermates developed comparable hypersensitivity to mechanical stimuli (P = 0.4348, n = 6 per genotype)(I) and thermal stimuli (P = 0.1406, n = 6 per genotype)(J) after i.pl. injection of CFA (20 µl).

Values are presented as mean ± SEM. unpaired t test in (A-C, G and H), two-way repeated measure ANOVA in (D-F, I and J).
Supplementary Figure 5 Normal pain behavior of Nmbr/Grpr DKO mice.

(A and B) Mechanical pain elicited by non-noxious von Frey assay ($P = 0.6511$, $n = 6$ per genotype) (A) and noxious Randall Selitto ($P = 0.2264$, $n = 6$ per genotype) (B) were comparable between Nmbr/Grpr DKO mice and their WT littermates. (C-E) Nmbr/Grpr DKO mice showed normal response to thermal stimuli in Hargreaves ($P = 0.3075$, $n = 12-14$ per genotype) (C), hotplate ($P = 0.4066$, $n = 6$ per genotype) (D) and tail immersion tests ($P = 0.5562$, $n = 6$ per
genotype)(E). (F-H) Licking/flinching responses induced by formalin ($P = 0.1229$, $n = 6$ per genotype)(F), capsaicin ($P = 0.27552$, $n = 6-7$ per genotype)(G) and MO ($P = 0.7216$, $n = 6$ per genotype)(H) were not different between Nmbr/Grpr DKO mice and WT littermates. (I and J) Nmbr/Grpr DKO mice and WT littermates developed comparable hypersensitivity to mechanical stimuli ($P = 0.7414$, $n = 6-7$ per genotype)(I) and thermal stimuli ($P = 0.1560$, $n = 6 - 7$ per genotype)(J) after i.pl. injection of CFA (20 μl).

Values are presented as mean ± SEM. Unpaired $t$ test in A, C, G and H, two-way repeated measure ANOVA in (D-F, I and J).