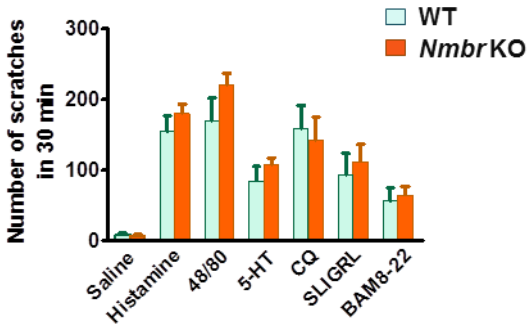
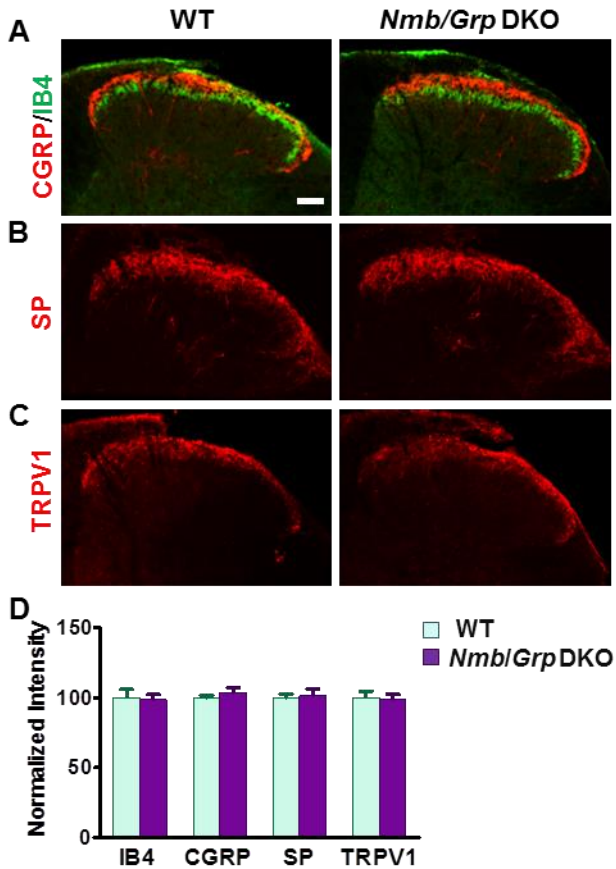


## **Distinct roles of bombesin peptides in itch transmission**

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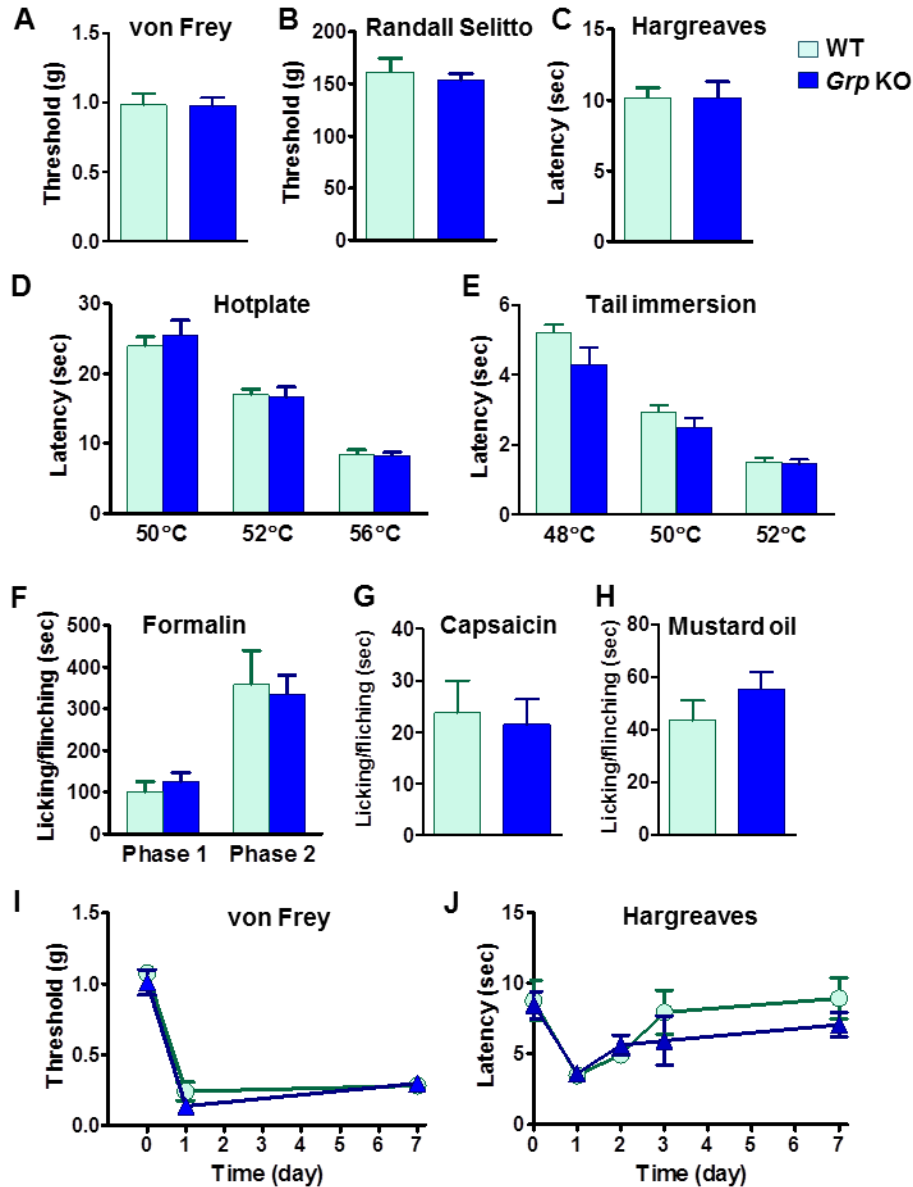


**Supplementary Figure 1** *Nmbr* KO and their WT littermates showed comparable scratching responses to histamine ( $P = 0.3479$ ), 48/80 ( $P = 0.1858$ ), 5-HT ( $P = 0.3346$ ), CQ ( $P = 0.7368$ ), SLIGRL ( $P = 0.6360$ ), and BAM8-22 ( $P = 0.7080$ ).  $n = 6$  per genotype.



**Supplementary Figure 2 Normal projection of primary afferents in the dorsal horn of *Nmb/Grp* DKO mice.**

(**A**) Density and innervation of CGRP<sup>+</sup> (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<sup>+</sup> (**B**) and TRPV1<sup>+</sup> (**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  $\pm$  SEM.  $n = 4$  per genotype, unpaired  $t$  test. Scale bar, 100  $\mu$ m.

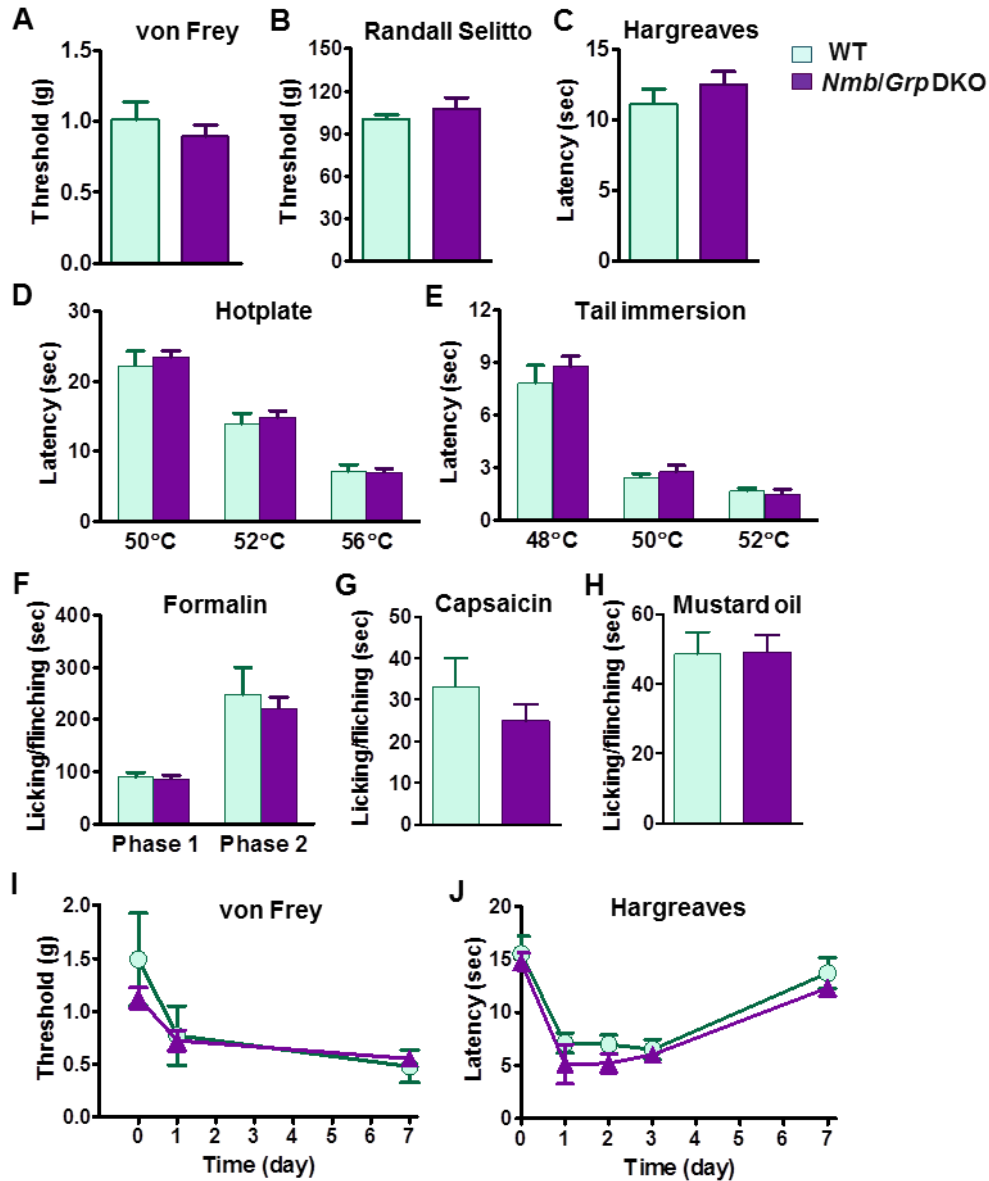


### Supplementary Figure 3 Normal pain behaviors of *Grp* KO mice.

(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  $\mu$ l) were comparable between WT and *Grp* KO mice ( $P$

= 0.9890,  $n = 6$  per genotype) (**F**), capsaicin (2  $\mu\text{g}$ , 20  $\mu\text{l}$ ,  $n = 7$  per genotype) ( $P = 0.7805$ )(**G**) and MO (0.75%, 20  $\mu\text{l}$ ,  $n = 8$  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  $\mu\text{l}$ ).  $n = 7$  per genotype.

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

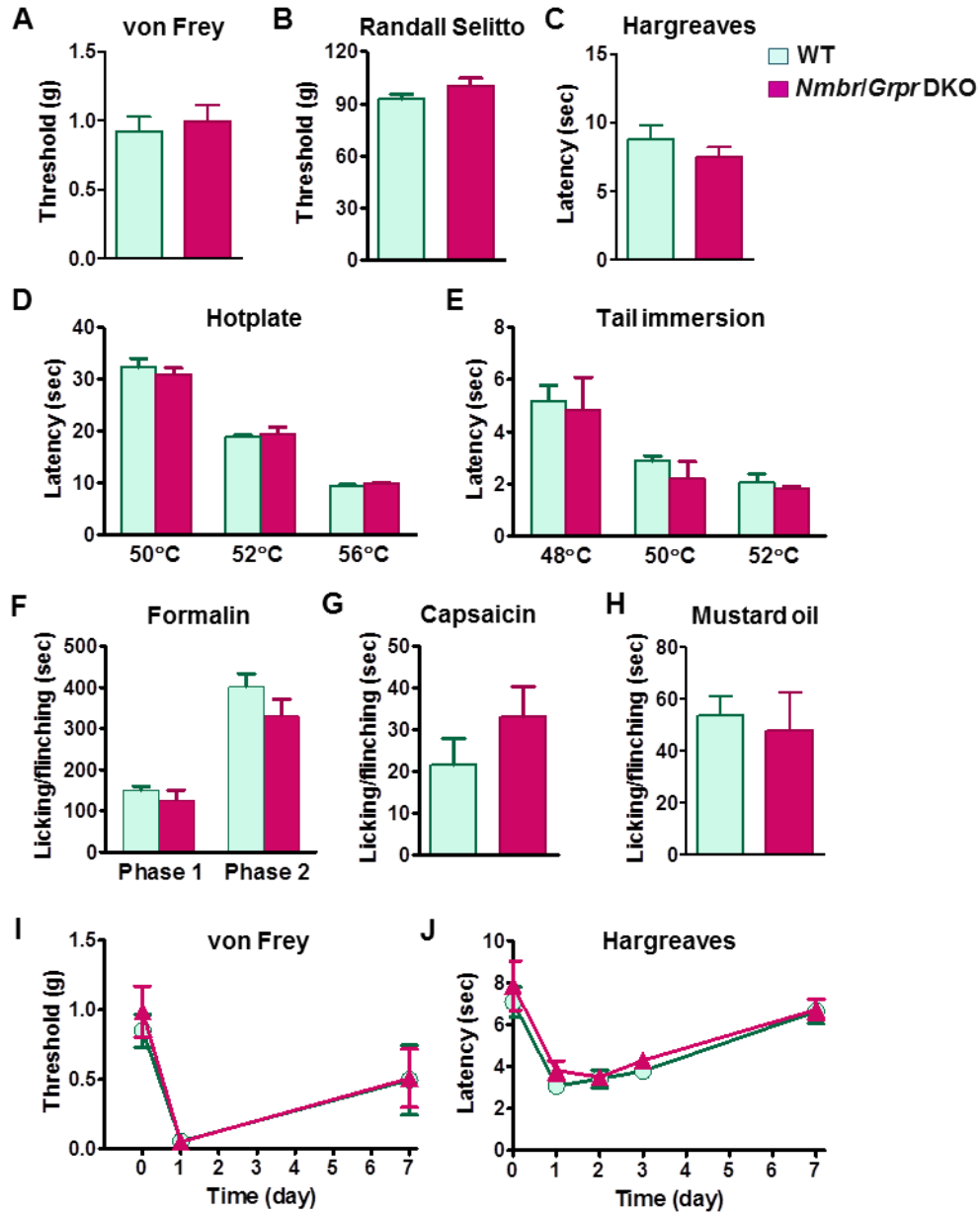


**Supplementary Figure 4 Normal pain behaviors of *Nmb/Grp* DKO mice.**

(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  $\mu$ l) ( $P = 0.6178$ ,

$n = 6-7$  per genotype) (**F**), capsaicin (2  $\mu\text{g}$ , 20  $\mu\text{l}$ ) ( $P = 0.3074$ ,  $n = 7-9$  per genotype)(**G**) and MO (0.75%, 20  $\mu\text{l}$ )( $P = 0.9402$ ,  $n = 7$  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  $\mu\text{l}$ ).

Values are presented as mean  $\pm$  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  $\mu$ l).

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