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Supplemental Information

Galanin Signaling in the Brain Regulates Color Pattern Formation in Zebrafish

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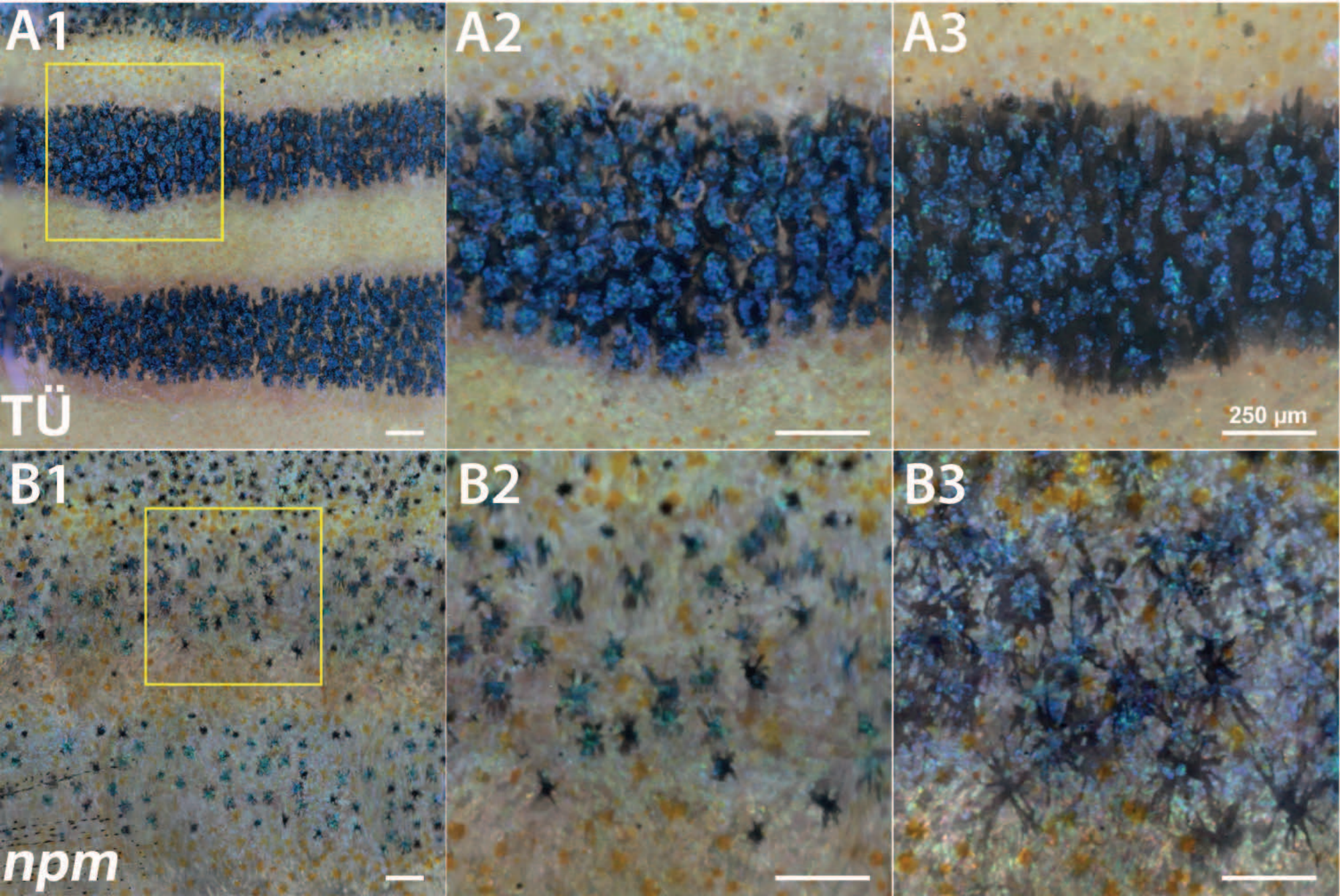
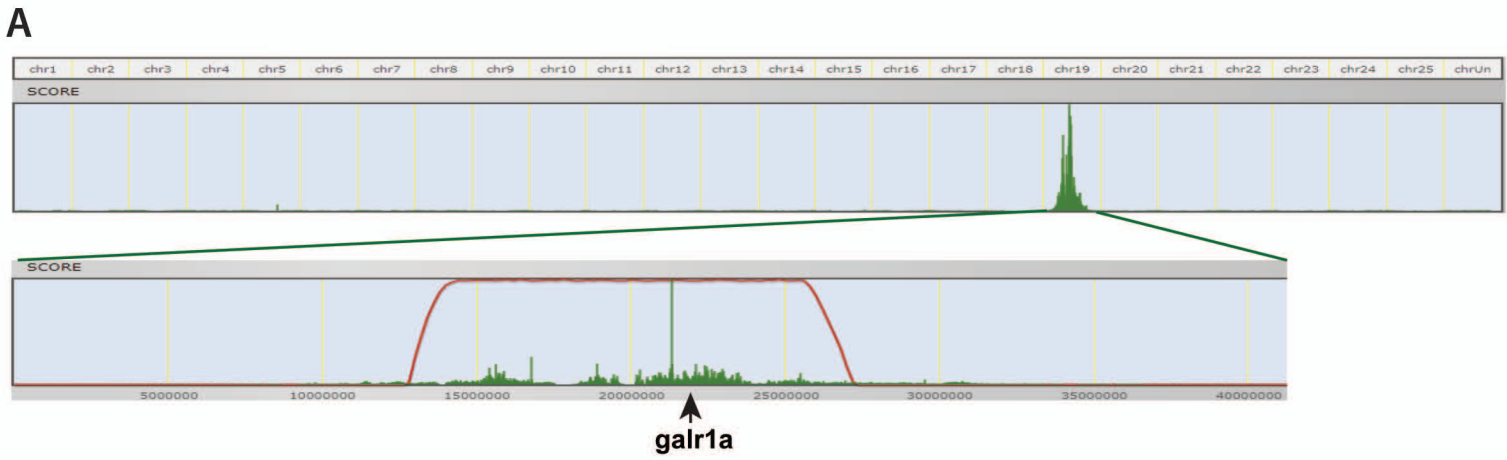
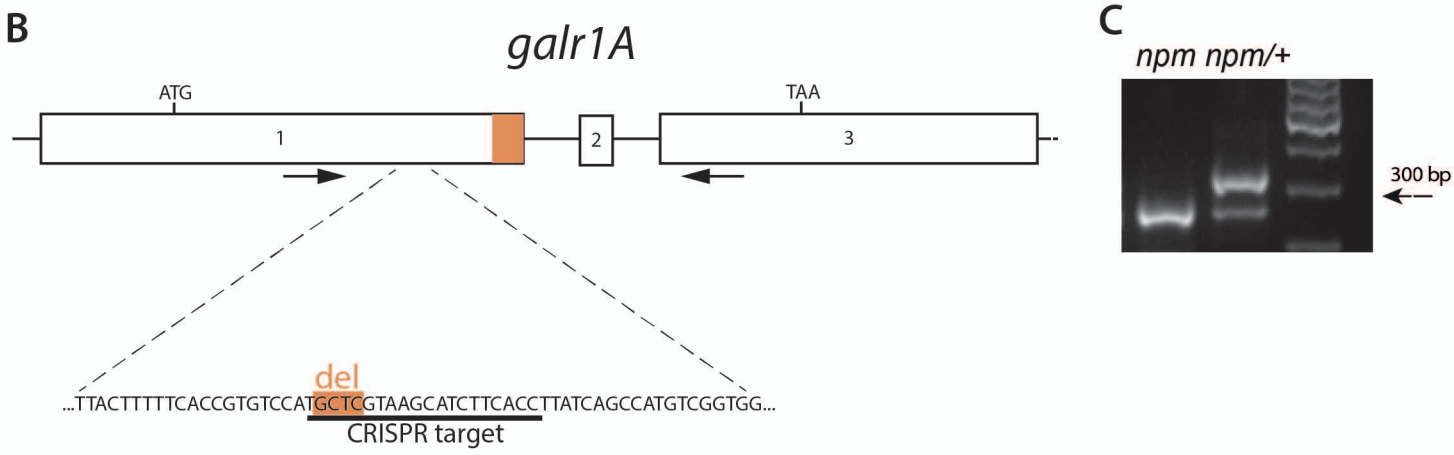

A1
A2
A3
TÜ
250 µm
B1
B2
B3
npm

Figure S1. Melanophore shape in *npm* mutants, Related to Figure 1. In wild type (A) melanophores display a compact shape and fill the dark stripe area; melanosomes are strongly dispersed in the cells after short anaesthesia (2 min, A2), and fully dispersed after extended periods of anaesthesia (30 min, A3). In *npm* mutants (B) melanosomes only disperse after prolonged anaesthesia (B3) revealing a much more stellate shape of melanophores, and showing that they contact one another.





D

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GALR1_H.sap       1 MELAVGNLSEGNASWPEPPAPEPGPLFGIGVENFVTLVVFGLIFALGVLGNSLVITVLARSKPGKPRSTTNLFILNLSIADLAYLLFCIPFQATVYALPT 100
Galr1a_D.rer      1 MGTQNNSDLDRPRSNIDLEAPEKN-LFGIGTDNLVTLLIFGLIFTLGVLGNSLVITVLAQRKPGQQRSTTNIFILNLSVADLSYLLFCIPFQSTVYMLPT  99
Galr1A in npm     1 MGTQNNSDLDRPRSNIDLEAPEKN-LFGIGTDNLVTLLIFGLIFTLGVLGNSLVITVLAQRKPGQQRSTTNIFILNLSVADLSYLLFCIPFQSTVYMLPT  99
Galr1A_mut        1 MGTQNNSDLDRPRSNIDLEAPEKN-LFGIGTDNLVTLLIFGLIFTLGVLGNSLVITVLAQRKPGQQRSTTNIFILNLSVADLSYLLFCIPFQSTVYMLPT  99

                    *.************************.********.****.*.***.**.*.**.**  ** ** ***.**** **.      * ***** ***  ..* *
GALR1_H.sap     101 WVLGAFICKFIHYFFTVSMLVSIFTLAAMSVDRYVAIVHSRRSSSLRVSRNALLGVGCIWALSIAMASPVAYHQGLFHPRASNQTFCWEQWPDPRHKKAY 200
Galr1a_D.rer    100 WILGAFICKFIHYFFTVSMLVSIFTLSAMSVDRYIAIVHCRKSSSIRVARHALIGVLVIWVLSFAMATPVAYYQGIVES-EDNSTFCWEVWPDHDRRKIY 198
Galr1A in npm   100 WILGAFICKFIHYFFTVSMLVSIFTLSAMSVDRYIAIVHCRKSSSIRVARHALIGVLVIWVLSFAMATPVAYYQGIVES-EDNSTFCWEVWPDHDRRKIY 198
Galr1A_mut      100 WILGAFICKFIHYFFTVSM*                                                                               118

                    *********.***.**.**************.*.********************** .*******..*** *** ***. ***. *..**************
GALR1_H.sap     201 VVCTFVFGYLLPLLLICFCYAKVLNHLHKKLKNMSKKSEASKKKTAQTVLVVVVVFGISWLPHHIIHLWAEFGVFPLTPASFLFRITAHCLAYSNSSVNP 300
Galr1a_D.rer    199 VVCTFVFGYVLPLILISFCYAKVLNHLHKKLRNVSKKSEASKKKTAQTVLVVVVVFCLSWLPHHVVHLWVEFGSFPLNQASFVLRVAAHCLAYSNSSVNP 298
Galr1A in npm   199 VV----------------------LNHLHKKLRNVSKKSEASKKKTAQTVLVVVVVFCLSWLPHHVVHLWVEFGSFPLNQASFVLRVAAHCLAYSNSSVNP 277

                    .**********.******.*..  ..   ...* **. .. ****** *
GALR1_H.sap     301 IIYAFLSENFRKAYKQVFKCHIRKDSHLSDTKESKSRIDTPPSTNCTHV 349
Galr1a_D.rer    299 VIYAFLSENFRQAYKQVFRCQVASECPTNEAREMKSKTEAAPSTNCTTV 347
Galr1A in npm   278 VIYAFLSENFRQAYKQVFRCQVASECPTNEAREMKSKTEAAPSTNCTTV 326
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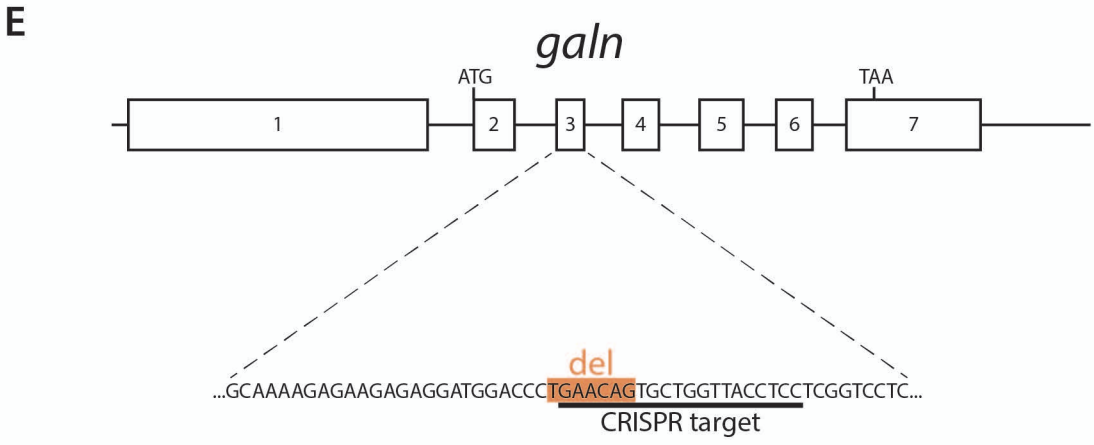

F

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                      * *    .  **...* *. . *.   *****************.  *** ***.**. ***.  ..
prepro-GALN H.sap     1 MARGSALLLASLLLAAALSASAGLWSPAKEKRGWTLNSAGYLLGPHAVGNHRSFSDKNGLTSKRELRPED  70
prepro-Galn D.rer     1 MHRCVGGVCVSLIVCAFLTETLGMVIAAKEKRGWTLNSAGYLLGPHAIDSHRSLSDKHGLAGKREMPLDE  70
Galn_mut              1 MHRCVGGVCVSLIVCAFLTETLGMVIAAKEKRGWTLLVTSSVLVVLITLYRLRILPVQGGEKICLVNMPL  70

                      * * *.    * .  ...***.***.*.*** ****    **.. .**.* .
prepro-GALN H.sap    71 DMKPGSFDRSIPENNIMRTIIEFLSFLHLKEAGALDRLLDLPAAASSEDIERS 123
prepro-Galn D.rer     71 DFKTGALR--IADEDVVHTIIDFLSYLKLKEIGALD---SLPSSLTSEEISQP 118
Galn_mut              71 LVTGASATNTDWQEREKCL  89
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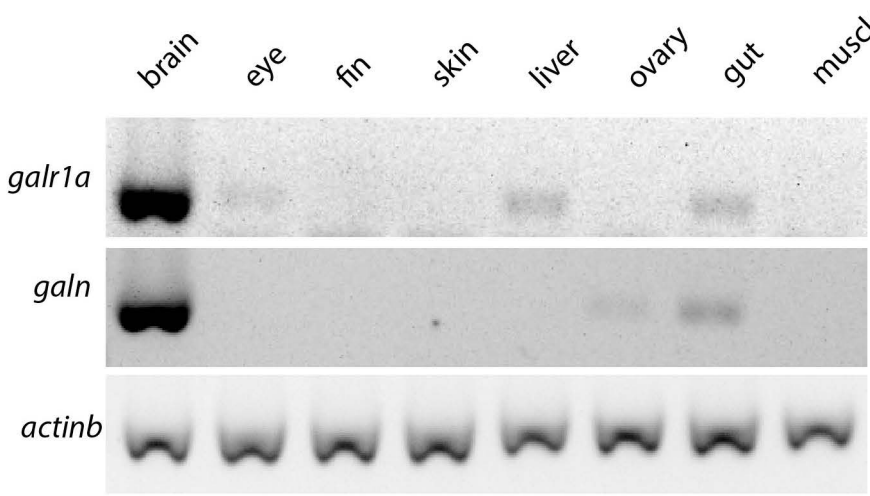
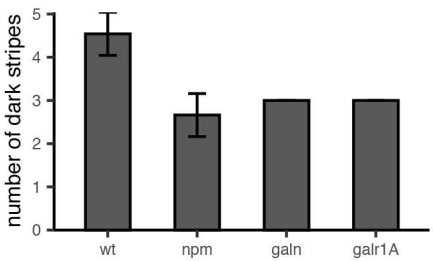
Figure S2. Mapping of *npm* and details of *galr1A* and *galn* mutations, Related to Figure 4. *npm* maps to chromosome 19 (A) as shown by the output of the SNPTrack software. In (B) a schematic representation of the *galr1A* gene is shown, exons are boxes, introns are not drawn to scale. The region of exon1 that is lost from the transcript by aberrant splicing in *npm* mutants is shown in orange. The CRISPR target site and the induced 4 bp deletion are indicated. (C) RT-PCR results showing a shorter transcript in *npm* mutants, primers used are indicated as arrows in (B). (D) protein sequence alignment of Galr1A from human and zebrafish wild-type, *npm* and $galr1A^{k.o.}$ mutants. Identical amino acid positions are indicated by asterisks, similarities by dots, the seven transmembrane regions are shaded in yellow. In *npm* 21 amino acids are missing. The k.o. allele has a premature stop. In (E) a schematic representation of the galn gene is shown, exons are boxes, introns are not drawn to scale. The CRISPR target site in exon 3 and the induced 7 bp deletion are displayed. (F) shows the protein sequence alignment of the Galn precursors (prepro-Galn) from human and zebrafish and the generated k.o. allele. Identical amino acid positions are indicated by asterisks, similarities by dots, the mature peptide is highlighted in yellow. The grey box covers the altered amino acids present in the k.o. allele.

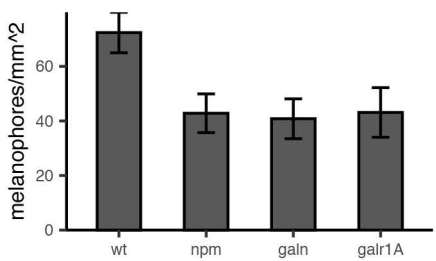
A



B



C



D

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prepro-Gal D.re     1 MHRCVGGVCVSLIVC-AFLTETLGMVIAAKEKRGWTLNSAGYLLGPHAIDSHRSLSDKHGLAGKREMP-L  68
si-rp71-1c10.8      1 MQSSCALLCISLCVFTAHLSSIHGMTLMNPEKKGWTLNSAGYLLGPYA---HRSLNVRHRASGKRDTGNE  67


                      * * .     .  . .*.  *.**.*** *  .    * ..*  . *
prepro-Gal D.re    69 DEDFKTGALRIADEDVVHTIIDFLSYLKLKEIGALDSLP-SSLTSEEISQP 118
si-rp71-1c10.8     68 NSSFPTSSY---NDSYLLSILGHLAYLRLKEKGMTEDFSGSFINSGNVKQ- 114
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Figure S3. Expression of *galr1A* and *galn*, Related to Figure 4. RT-PCR results showing the expression of *galr1A* and *galn* in different tissues of adult zebrafish (A). Both genes are highly expressed in the brain. The numbers of dark stripes (B) and the numbers of melanophores (C) in *npm*, *galn* and *galr1A* mutants are shown. (D) shows the alignment of prepro-Galn with the potential Galn-like peptide from zebrafish, si-rp71-1c10.8. Identical amino acid positions are indicated by asterisks, similarities by dots, the mature Galn-peptide is highlighted in yellow.

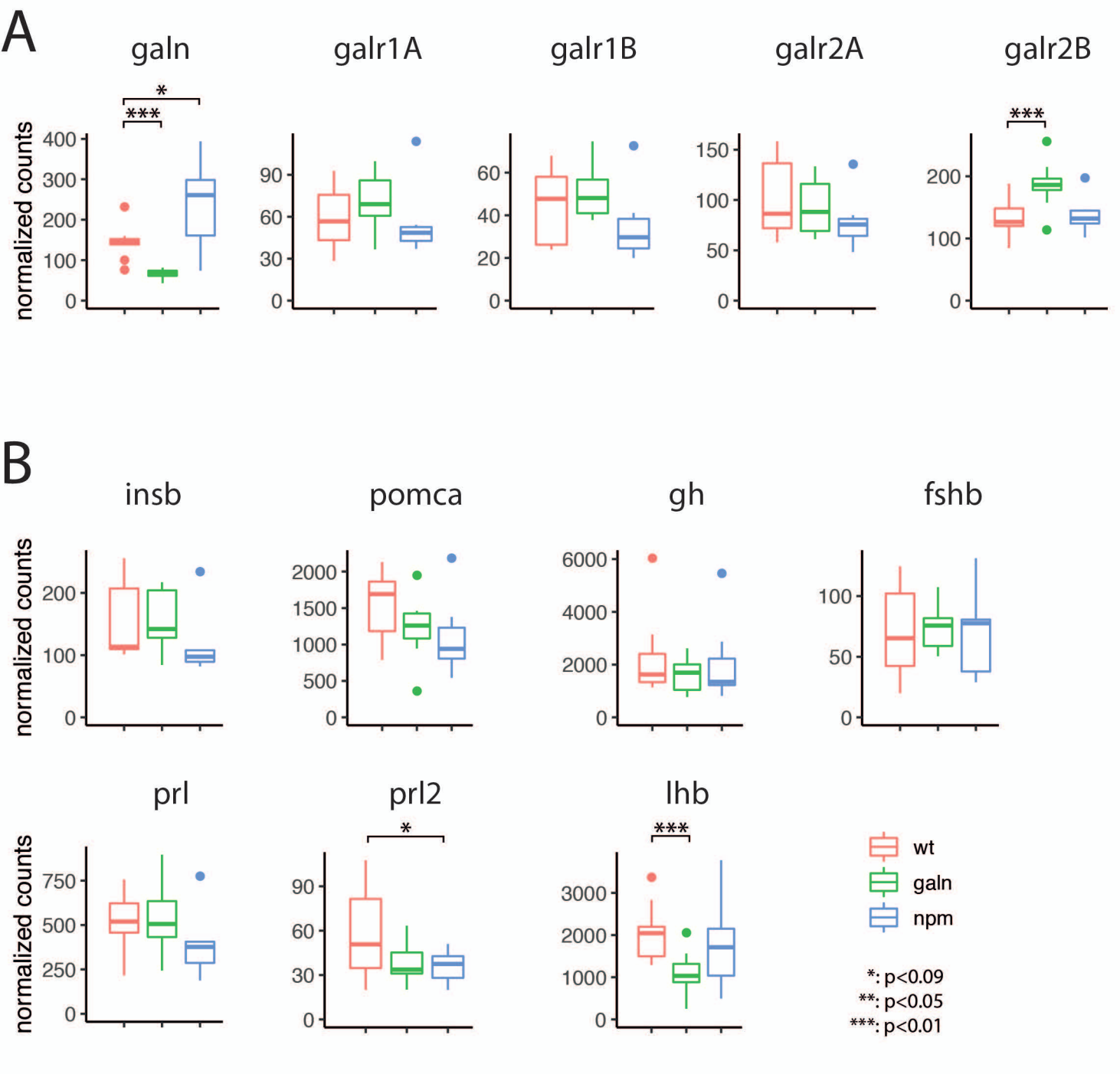
A
galn
galr1A
galr1B
galr2A
galr2B
normalized counts
*
***
***
B
insb
pomca
gh
fshb
prl
prl2
lhb
*
***
wt
galn
npm
*: p<0.09
**: p<0.05
***: p<0.01

Figure S4. Expression levels in *galn*$^{k.o.}$ and *npm* mutants, Related to Figure 4. (A) *galn* and galanin receptor transcript abundance measured by Nanostring. *galn* levels are low in *galn*$^{k.o.}$ mutants, presumably due to nonsense-mediated decay; they are possibly upregulated in *npm* mutants. The expression of the different receptor genes is not changed in the mutants with the exception of galr2B in *galn*$^{k.o.}$. The expression levels of hormones known to be influenced by Galanin signalling in mammals are mostly unchanged in *galn*$^{k.o.}$ and *npm* mutants (B). Only luteinizing hormone b and (possibly) prolactin2 are affected. The plots depict the mean values (horizontal line), the first and third quartiles (box) and the lowest and highest values no further than 1.5 x IQR (inter-quartile range) from the hinge (whiskers); outliers are plotted as individual points (*: $p<0.09$; **: $p<0.05$; ***: $p<0.01$). n=9 for wild-type, n=8 for *galn*$^{k.o.}$, n=7 for *npm*.

Primer_name	5'-sequence-3'	CRISPR Target site
galr1A_CRISPR1	TAGGTGAAGATGCTTACGAGCA	GGTGAAGATGCTTACGAGCA
galr1A_CRISPR2	AAACTGCTCGTAAGCATCTTCA	
galn_CRISPR1	TAGGATGGACCCTGAACAGTGC	GGATGGACCCTGAACAGTGC
galn_CRISPR2	AAACGCACTGTTCAGGGTCCAT	
galr1A_seq_f	TCTGTCCTACCTGCTCTTC	
galr1A_seq_r	AAAGGCAATCCACCAACC	
galn_seq_f	GTGAATACATTTTGTGTAAAACAGG	
galn_seq_r	ACAAGGTAATAACAAGGATGAAATC	
galr1A_cdna_f	GTCGCCTACTATCAGGGCAT	
galr1A_cdna_r	TTCAGGGGAAAGGAGCCAAAC	
galn_cdna_f	CAGAAACACTCGGGATGGTGA	
galn_cdna_r	CTTCTCGCCCCCTTGCAC	

Table S1. List of primers used in this study. Related to STAR Methods.

Gene Name	Accession	Position	Target Sequence
actb2	NM_181601.3	1648-1747	CCTGGGCATATTGTAAAAGCTGTGTGGAACGTGGCGGTGCCAGACATTTGGTGGGGCCAACCTGTACACTGACTAATTCAATTCCAATAAAAGTGCACAT
galn	NM_001346239.1	322-421	GGCAGGAAAGAGAGAAATGCCTTTAGATGAGGATTTCAAGACAGGAGCTCTGAGGATAGCAGATGAGGATGTCGTCCATACCATCATTGACTTTCTTTCG
galr1a	XM_691123.5	528-627	AAAACAACAGTGATTTGGACAGACCTAGAAGCAACATAGACTTAGAAGCACCTGAAAAAAACCTATTTGGCATCGGCACAGACAACTTAGTCACGCTTCT
gapdh	NM_001115114.1	490-589	CTCACAGTTGTAAGCAATGCCTCCTGCACCACCAACTGCCTGGCTCCTTTGGCAAAGGTCATCAATGATAACTTTGTCATCGTTGAAGGTCTTATGAGCA
tg	NM_001329865.1	3086-3185	TCTGTTGGTGTGTTGATGAAGAGGGTCAATACATCGCTGACTCTCTGACGTCTCGTTCCTCACTACCTCAGATGTGCCAAACTTTATGCCAGAGGCTCCA
trh	NM_001012365.2	691-790	GGTGAACGGACGCTTCAGTAAGTGTTCGGGAAACACACCTGTCTCTCCATGTGCCAGTTTTCGAAGCGACAGTTCAAAGCCTCATTCACCTGTGACGCGT
insb	NM_001039064.1	127-226	TTCTCCATCCCAGCATCTGTGTGGTTCAAGCCTGGTGGATGCGCTTTACCTAGTGTGTGGGCCTAGAGGTTTCTTCTACACCAACAGAGGCAGGAGAGAC
tshba	NM_181494.2	243-342	TTTATTGTTCAGAGGGGATGCACTTATCAGGAAGTTGAGTACCGGACAGCCGTCTTGCCGGGATGCCCTTCACATGCAGATCCTCACTTCACCTACCCAG
galr1b	NM_001327843.1	672-771	TAACCGGAGGAATGCGCTTATTGGCGTCTGTGTCATTTGGATGCTTTCCTTTATCTTTGCCGTCCCGGTTGCTCAGCACCAAATTTTGACGGATCACCCC
galr2a	XM_021480396.1	629-728	CTCCAGGCCACTATCTACACCATGGACGAGTGGGTTTTCGGCGCGTTCGTGTGCAAAGCCGTGCACTTTATTATTTACCTGACCATGTACGCGAGCATCT
galr2b	XM_021474712.1	1344-1443	TGAAAATAAAGTGCACGGCTAATGATGCCTTACAGAAGCCTTATTAAAAGAAAACACTCAAAGTGAGCCGGAGGATTTCTGCAGCTTTGATCGACATCAC
pomca	NM_181438.3	916-1015	AAGGGGGAGAGGTTGTTATAGGGGGATGTTTTGAATATACTTTCTTCCCAGCAAACTTCTGGATGAGAGGTTCCTATCATGCATAGAAACGAAGGTGGGC
gh1	NM_001020492.2	490-589	ATGGACAGCCAAATATGGATGACAACGACTCCCTGCCGTTGCCTTTTGAGGATTTCTACCTGACCGTAGGGGAGACCAGTCTCAGAGAGAGCTTTCGCCT
lhb	NM_205622.2	435-534	CCCGACTTCTGCATGTCCCAGAGAGAGGATTTCCCCGCATACTAGACCTCGGACAACTCACATCAACCTACACACACAGTCGAGCTCAGCATTATTAGAC
fshb	NM_205624.1	261-360	TGTACAAGAGCTACGAGTTTAAAGGCTGTCCTGCAGGGGTTGATTCAGTCTTCGTGTACCCCGTGGCTCTGAGCTGTGAGTGCAACCAGGTTAACTCAGA
prl	NM_181437.3	490-589	CGTACACAAGATGGGCTCGTCTTCTGACAACCTGTCCACTCTCCCGTTTAATGGCAACAACCTTGGTCAGGATAAAACGTCTCGACTTGTCAATTTCCAC
prl2	NM_001162854.1	576-675	CCATGCTTCCGATTTCTGACAGCGGAGAAGCCATGAGTGACTACGATCTCCTCTACTGCTTTCGCCGCGACTCCAACAAAGTCCAGAACTATCTAAAAAT

Table S2. List of transcripts and target sequences used for Nanostring expression profiling. Related to STAR Methods.