Supplementary tables

Table S1 related to figure 2: LiCl treatments of Et(-1.0otpa:mmGFP) embryos

Hpf	WT	Reduced or absent GFP expression in the dHb (48hpf)	n
22	16 (82%)	3 (18%)	19
24	0	80 (100%)	80
26	3 (20%)	12 (80%)	15
28	18 (77%)	6 (17%)	24
30	20 (90%)	2 (10%)	22

Table S2 related to figure 3: Effect of LiCl treatments on dHb neuron markers

Marker	WT	Reduced	Absent	n
HuC/D (48hpf)	1 (11%)	8 (89%)	0	9
Cxcr4b (48hpf)	28 (93%)	2 (7%)	0	30
kctd12.1(72hpf)	11 (20%)	24 (43%)	20 (37%)	55
Et(gata2a:EGFP) (72hpf)	8 (20%)	18 (45%)	14 (35%)	40
kctd8 (72hpf)	10 (20%)	32 (67%)	6 (13%)	48
Tg(hsp70-brn3a:GFP) (72hpf)	23 (85%)	4 (15%)	0	27
Kctd12.2 (72hpf)	39 (63%)	15 (24%)	8 (13%)	62

Table S3 related to figure 3, 4: Premature Wnt activation results in vIPN innervation

Treatment	WT	Only ventral	n
LiCl	4 (13%)	27 (87%)	31
Wif1 MO	5 (19%)	21 (81%)	26
BIO	1 (25%)	3 (75%)	4
tg(hsp70l:wnt8a-GFP)@22hpf	9 (100%)	0	9

Table S4 related to figure 4: Wif1 knock down phenocopies LiCl treatments

Marker	WT	Reduced	Absent	n
Et(-1.0otpa:mmGFP) (48hpf)	13 (29%)	5 (12%)	25 (59%)	43
HuC/D (48hpf)	0	5 (100%)	0	5
Cxcr4b (48hpf)	17 (94%)	1 (5%)	0	18
kctd12.1(72hpf)	26 (37%)	30 (42%)	13 (19%)	71
Et(gata2a:EGFP) (72hpf)	7 (23%)	7 (23%)	17 (54%)	31
kctd8 (72hpf)	21 (46%)	16 (38%)	9 (18%)	46
Tg(hsp70-brn3a:GFP) (72hpf)	6 (60%)	4 (40%)	0	10
Kctd12.2 (72hpf)	22 (58%)	14 (37%)	2 (5%)	38

Table S5 related to figure 4: *Wif1* expression is regulated by Wnt signaling

Treatment/ mutant	**WT**	**Reduced**	**Increased**	**n**
tg(hsp701:wnt8a-GFP) @22hpf	0	0	46 (100%)	46
LiCl@22hpf	10 (30%)	0	23 (70%)	33
BIO@22hpf	4 (44%)	0	5 (56%)	9
Axin1$^{-/-}$	8 (32%)	0	17 (68%)	25
IWR@22hpf	8 (24%)	36 (76%)	0	44
tg(hsDkk1:GFP) @22hpf	2 (6%)	31 (94%)	0	33
Tcf7l2 (whole incross clutches)	135 (100%)	0	0	135

Table S6 related to figure 4: Wif1 suppresses Wnt signaling during dHb development

Treatment/ *Kctd12.1*	**WT**	**Reduced**	**Absent**	**n**
hs - control	79 (95%)	4 (5%)	0	83
tg(hsp701:wnt8a-GFP) @22hpf	63 (71%)	25 (28%)	1 (1%)	89
wif1 MO	42 (88%)	6 (12%)	0	48
wif1 MO + tg(hsp701:wnt8a-GFP) @22hpf	14 (39%)	21 (58%)	1 (3%)	36
Treatment/ *Kctd8*	**WT**	**Reduced**	**absent**	**n**
hs - control	52 (70%)	22 (30%)	0	74
tg(hsp701:wnt8a-GFP) @22hpf	47 (57%)	36 (43%)	0	83
wif1 MO	40 (67%)	17 (28%)	3 (5%)	60
wif1 MO + tg(hsp701:wnt8a-GFP) @22hpf	20 (46%)	18 (42%)	5 (12%)	43

Supplementary Figures

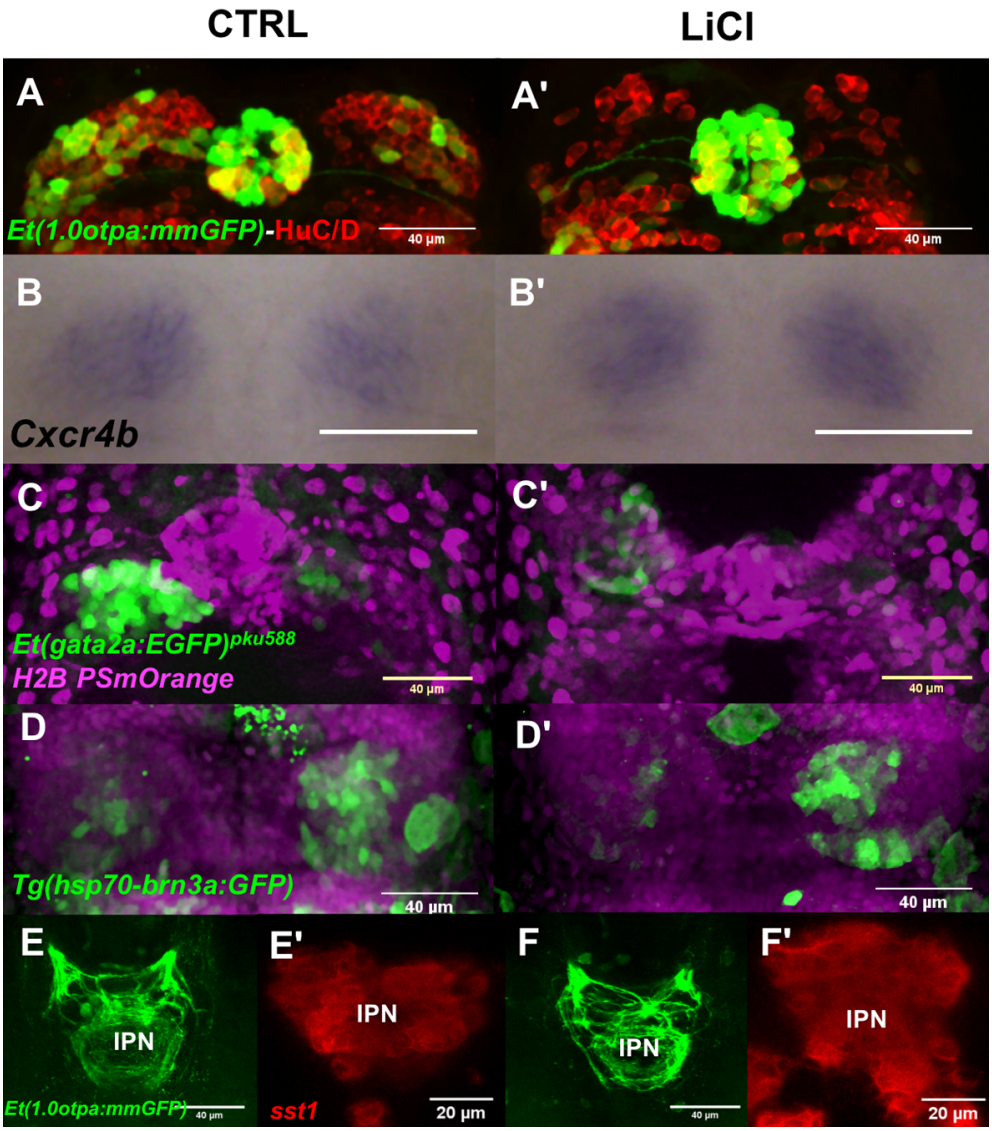

Figure S1, related to Figure 2 and 3: Premature activation of Wnt signaling affects dHb neuron differentiation

(A-F') Dorsal views, anterior to the top focused on the (A-D') developing habenulae and (E-F') IPN. (A, A', C-F') Projections of confocal z-stacks. (A-B') At 48 hpf, LiCl treated embryos exhibit the specific lack of GFP expressing habenular cells and a reduction in HuC/D positive differentiating neurons in the presence of unaffected *cxcr4b* expression in habenular precursor cells (Roussigne et al., 2009). (C, C') At 72 hpf, GFP expressing dHbl neurons and (D, D') less evident also dHbm neurons are reduced upon LiCl induced Wnt signaling activation. (E-F') Innervation of the normally formed IPN by habenular efferent axons as evidenced by normal expression of *somatostatin1* (sst1) in the IPN.

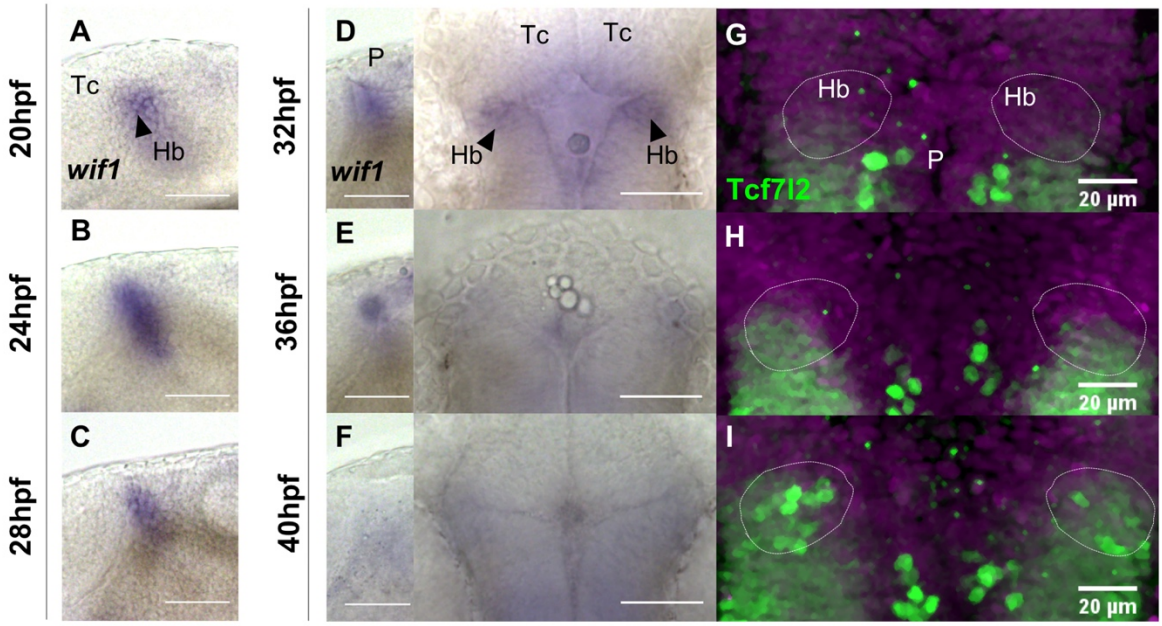

Figure S2: *Wif1* expression temporally complements Tcf7l2 expression

(A-C) and (D-F left) lateral views, anterior to the left and (D-F right) and (G-I) projections of confocal z-stacks (habenular region is encircled), dorsal views with anterior to the top focused on the diencephalon of wild type embryos at stages indicated on the left. *Wif1* is discretely expressed in the developing habenular region (arrowheads) until Tcf7l2 expression is initiated at 36 hpf. Hb, habenula; P, pineal; Tc, telencephalon.

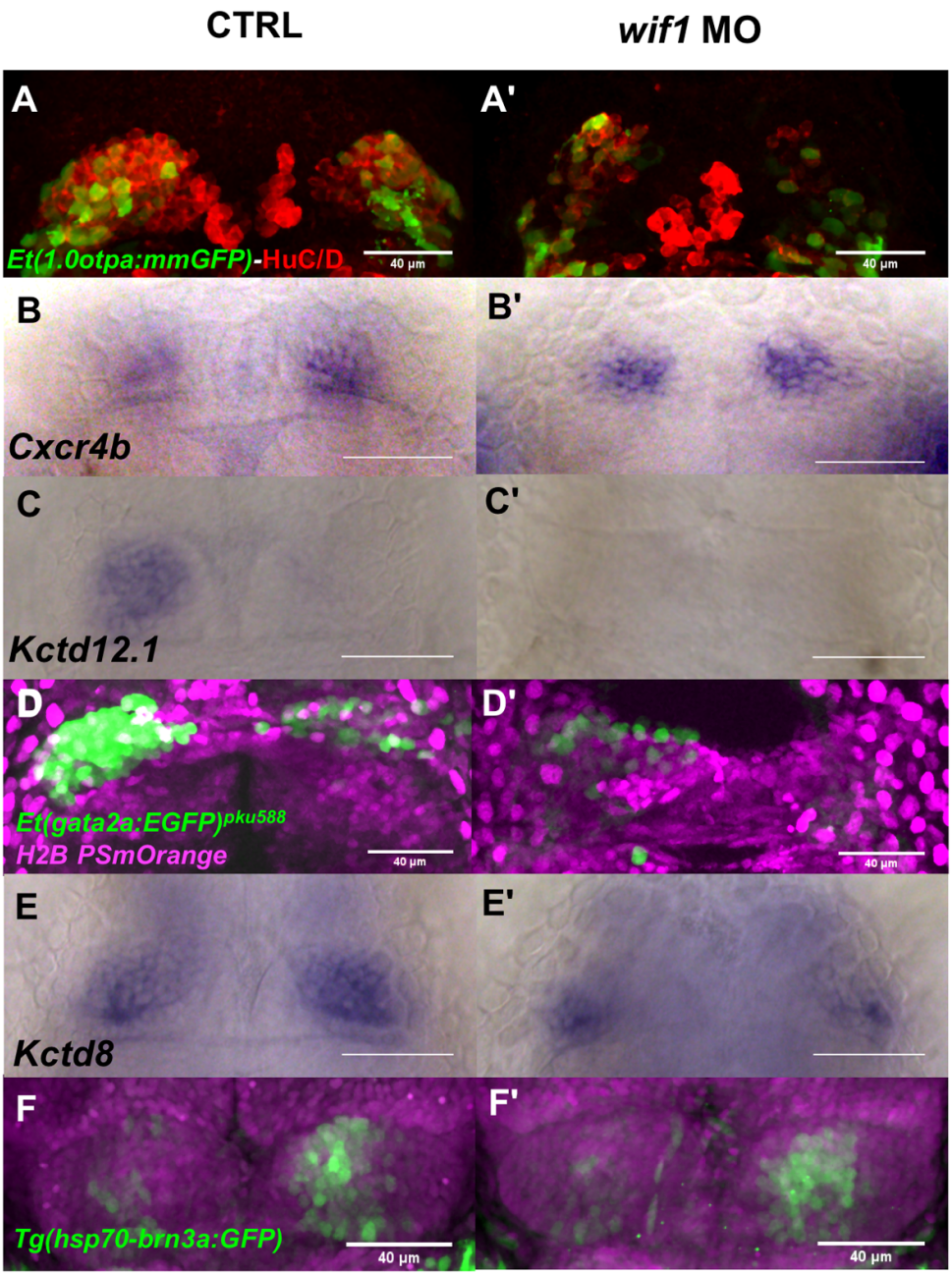

Figure S3, related to Figure 4: Wif1 interference alters habenular neuron differentiation

(A-F') Dorsal views, anterior to the top focused on the developing habenulae. (A, A', D, D' F, F') Projections of confocal z-stacks. (A-B') At 48 hpf, *Wif1* hypomorphic embryos exhibit the reduction of GFP expressing habenular cells and a reduction in HuC/D positive differentiating neurons in the presence of largely unaffected *cxcr4b* expression in habenular precursor cells (Roussigne et al., 2009). (C-F') At 72 hpf, the dHbl markers *kctd12.1* and *pku588* are reduced or absent upon *Wif1* knock down, while *kctd8* and *brn3a:GFP* in dHbm neurons are less strongly affected.

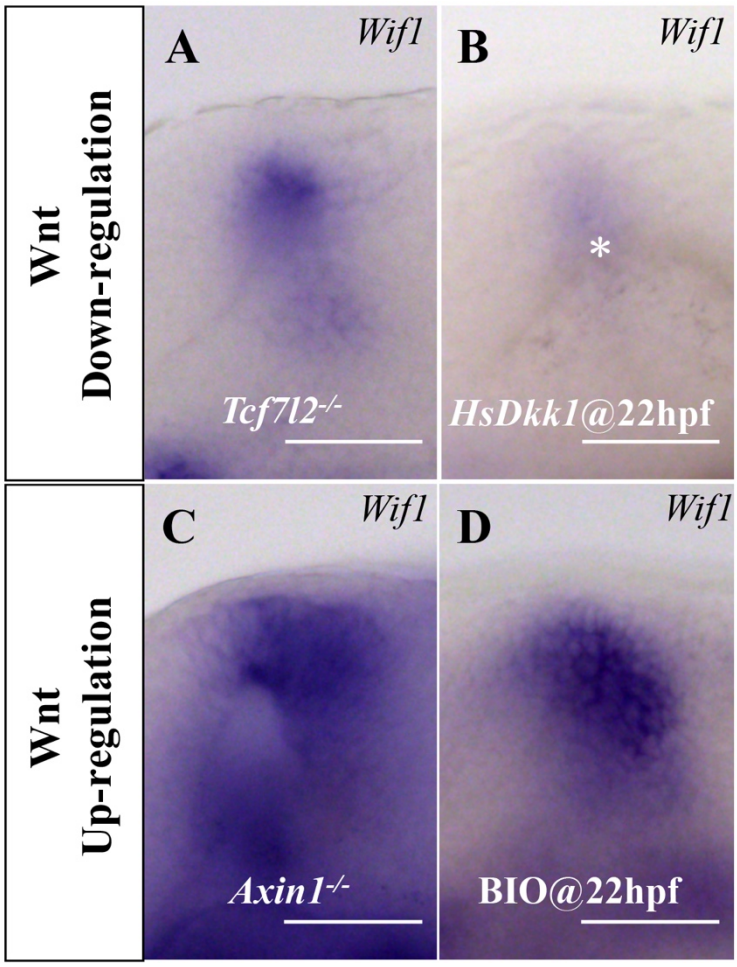

Figure S4, related to Figure 4: *Wif1* expression is regulated by Wnt signaling

(A-D) Lateral views with anterior to the left focused on the diencephalon at 26 hpf labeled for *Wif1* expression. (A) Wnt downregulation in *tcf7l2* mutants does not affect *Wif1* expression (compare also with the control embryo in Fig. 4G), which is consistent with the temporally complementing expression of Tcf7l2 and *Wif1*. (B) Transient transgenic Wnt suppression results in a decrease of *Wif1* expression in the habenulae (asterisk). (C, D) Wnt upregulation causes an increase of the *Wif1* expression domain. Labelling procedures were conducted in parallel for comparability.

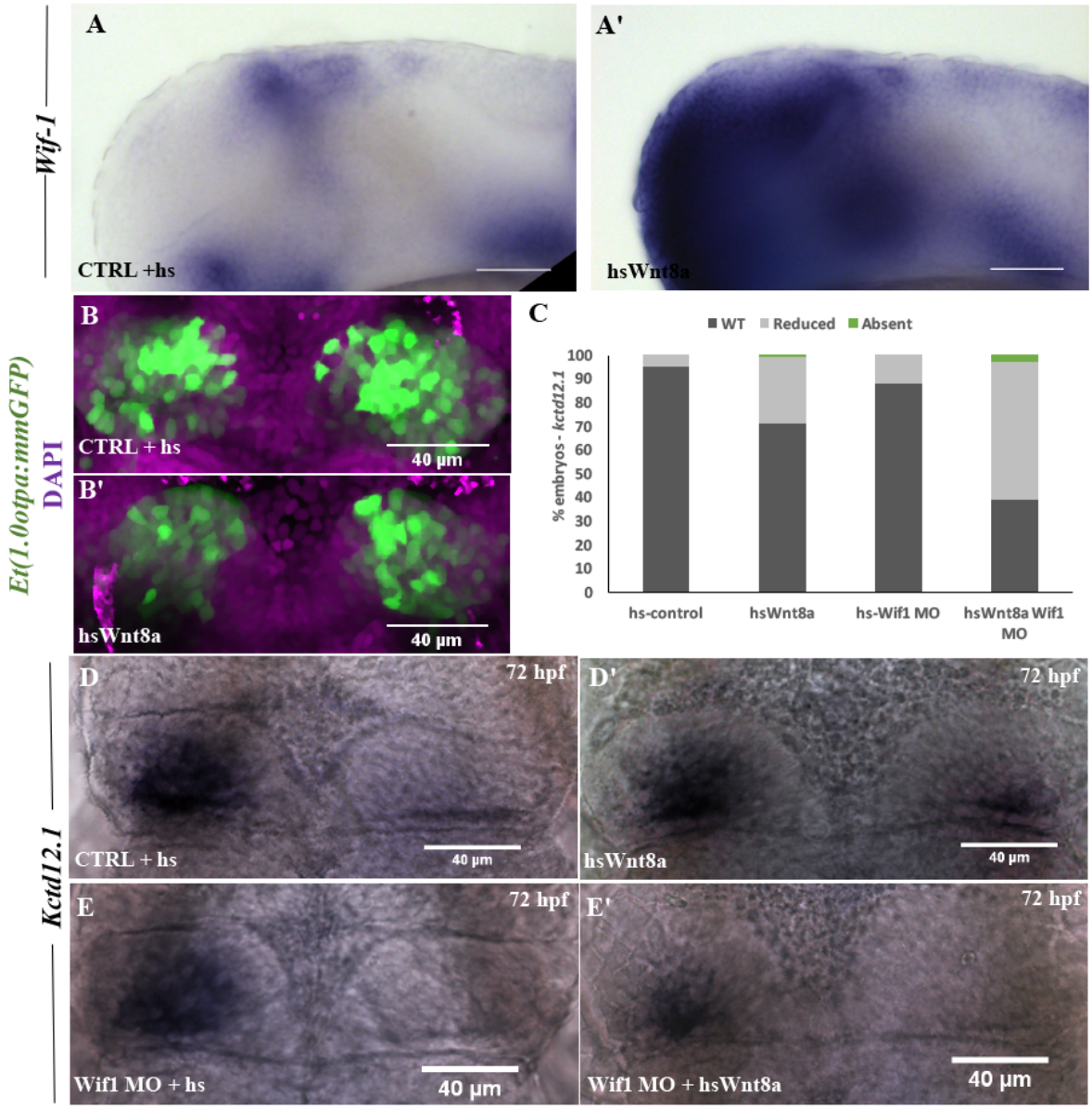

Figure 5, related to Figure 4: Transient *Wnt8a* activation affects habenular marker gene expression in Wif1 hypomorphic embryos

(A, A') Lateral view, anterior to the left focused on the head region. Heat shock activation of *Wnt8a* expression at 22 hpf strongly induces *Wif1* expression. (B, B', D-E') Dorsal views with anterior to the top focused on the diencephalon at (B, B') 48 hpf and (D-E') 72 hpf. (B, B') Projections of confocal z-stacks. (C-E') Activation of *Wnt8a* in mildly Wif1 hypomorphic embryos increases the number of embryos with reduced habenular marker gene expression.