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SUPPLEMENTARY MATERIAL

corresponding to:

Zebrafish *grainyhead-like1* is a common marker of different non-keratinocyte epidermal cell lineages, which segregate from each other in a Foxi3-dependent manner

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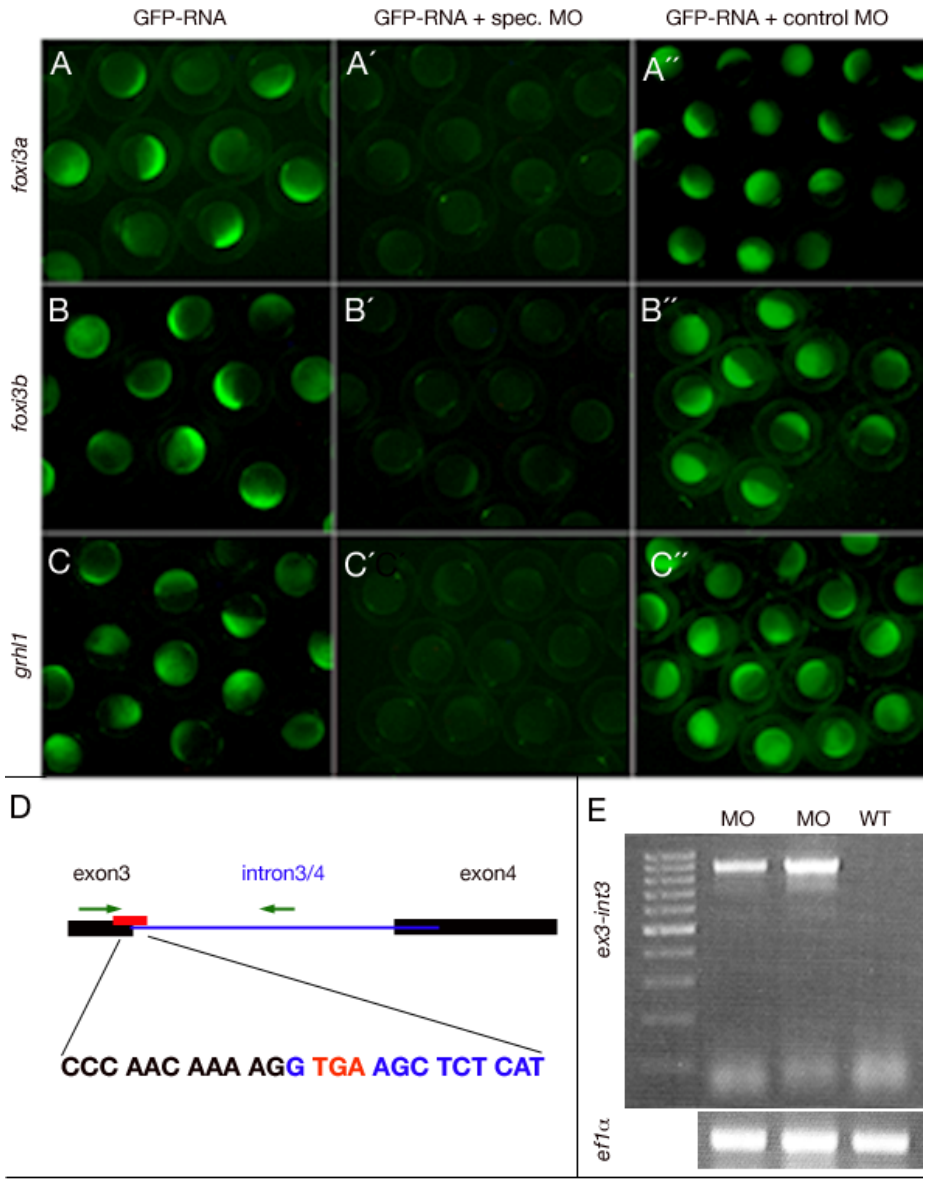
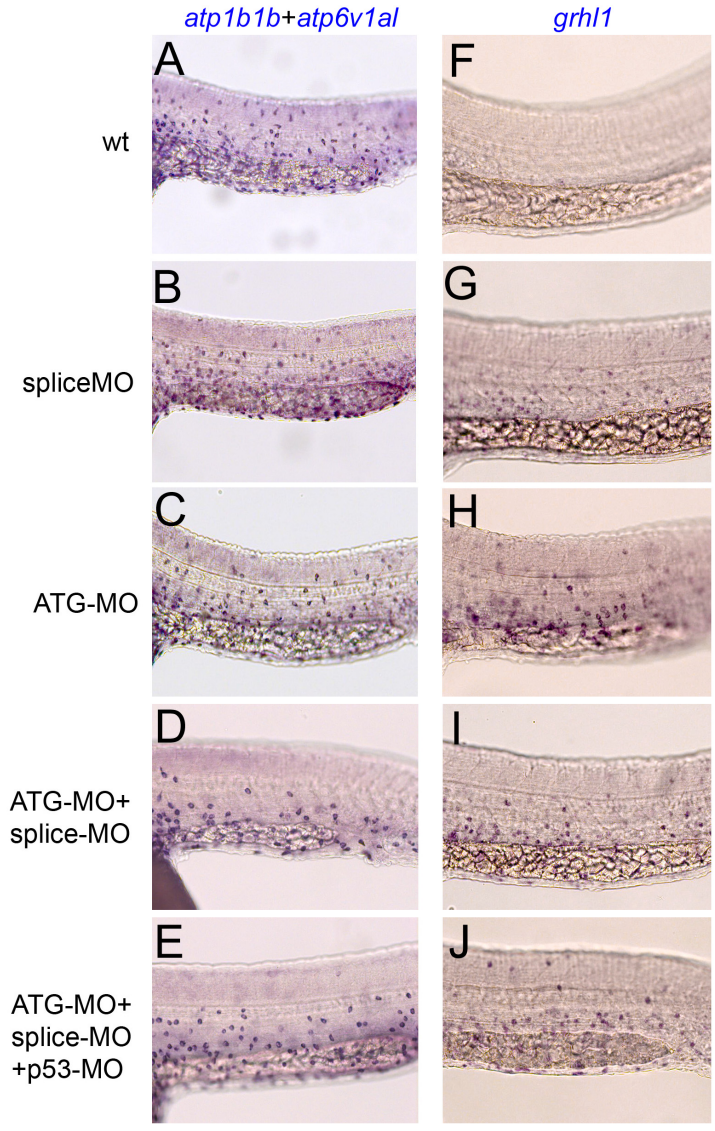
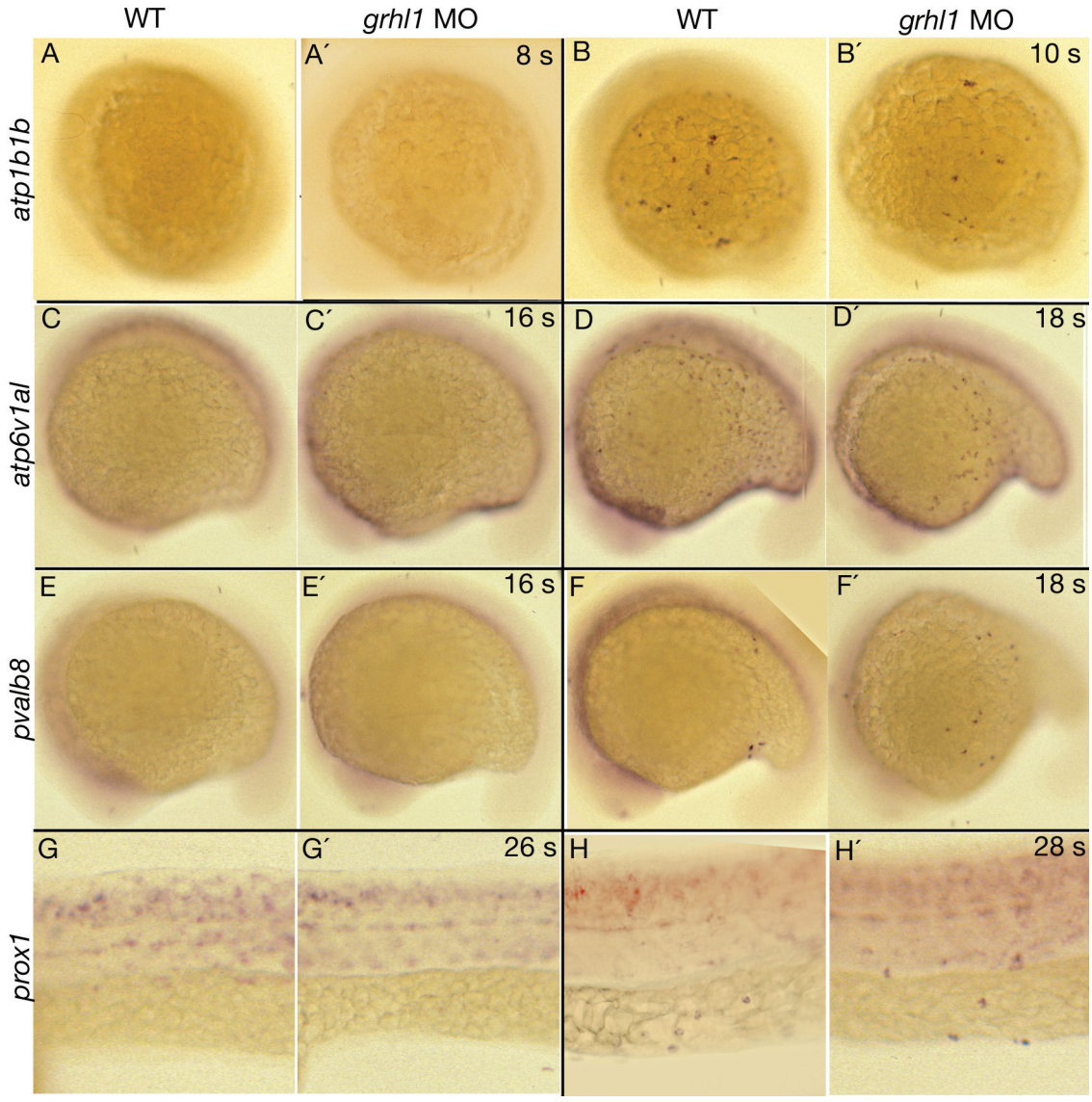


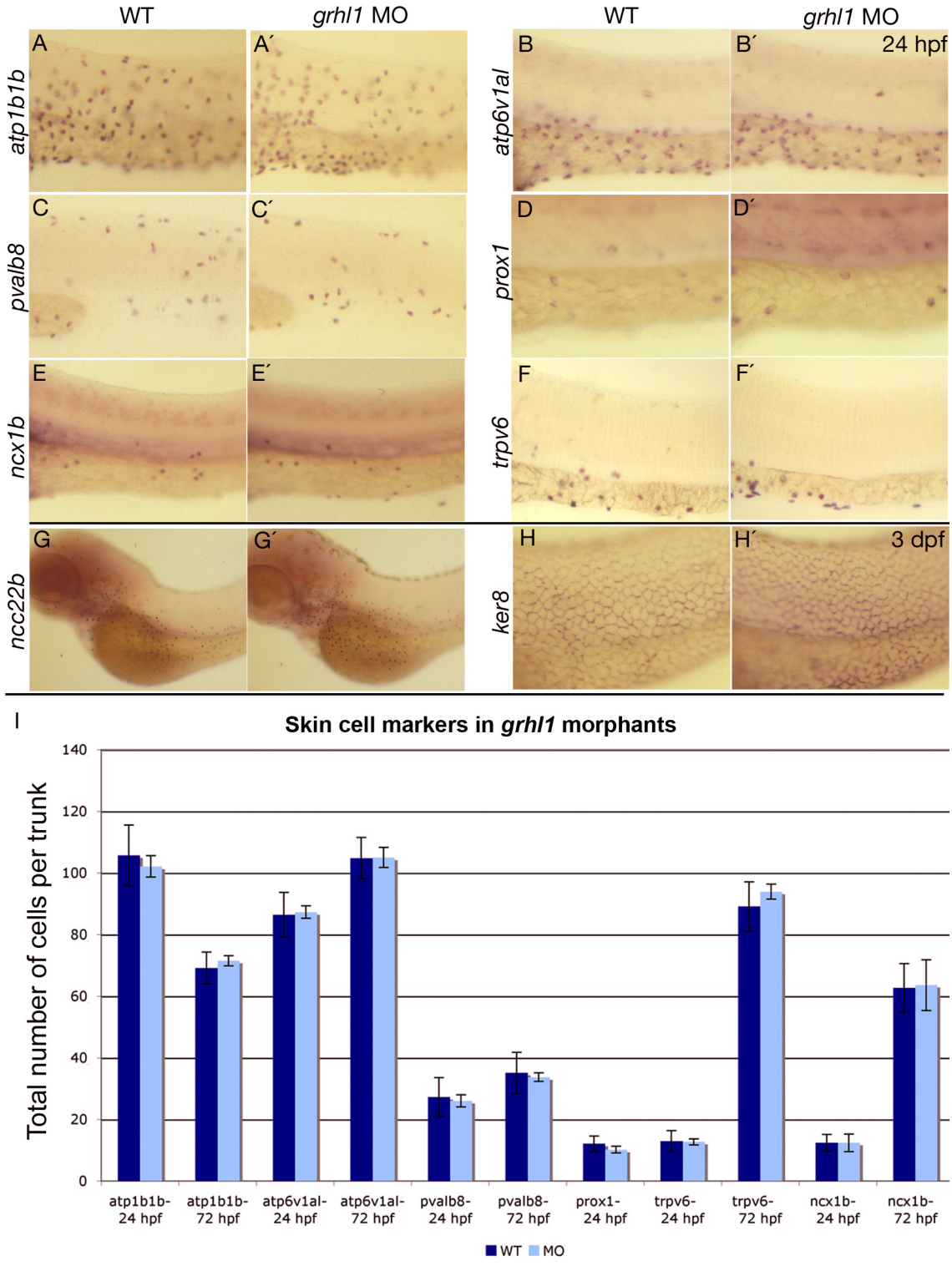
Supplementary Fig. S1. Efficacy controls of used ATG- and splice *grhl1* morpholinos. (A-C) *Live embryos at late blastula stages that were injected with constructs indicated at the top for genes indicated at the left side. These images show that the three ATG-MOs targeted specifically and efficiently the sequence they were designed against.* **(D)** *Graphical illustration of the* grhl1*-splice MO targeting site and the primer binding sites used to control the efficacy.* **(E)** *The* grhl1*-splice MO interfered with splicing of intron3-4, indicated by the RT-PCR amplification of an exon3-intron3/4 fragment from morphant (MO), but not from uninjected control WT embryos (WT). In reverse, a combination of an exon3 and an exon4 primer only gave an RT-PCR amplification product from WT, but not from morphant embryos (data not shown). Intron3/4 contains an in frame stop codon after the third exon (highlighted in red in D), which will yield a C-terminally truncated Grhl1 protein.*



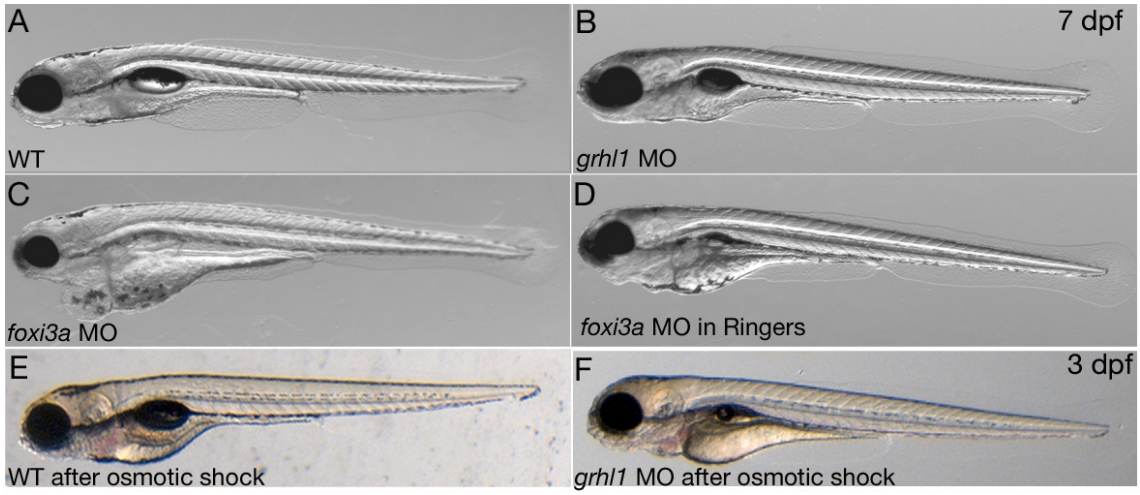
Supplementary Fig. S2. Embryos singly injected with *grhl1* splice or ATG-MO, and embryos co-injected with both, are similarly affected. *All panels show lateral views on the trunk of 24 hpf embryos at the level of the yolk tube, anterior to the left, after whole mount* in situ *hybridization with mixed* atp1b1b *and* atp6v1al *probes (left column) or with* grhl1 *probe (right column).* **(A,F)** *Uninjected control embryos;* **(B,G)** *embryos injected with* grhl1 *splice MO;* **(C,H)** *embryos injected with* grhl1 *ATG MO;* **(D,I)** *embryos co-injected with* grhl1 *splice and ATG MOs;* **(E,J)** *embryos co-injected with* grhl1 *splice MO,* grhl1 *ATG MO and* p53 *MO to suppress unspecific apoptotic effects.*



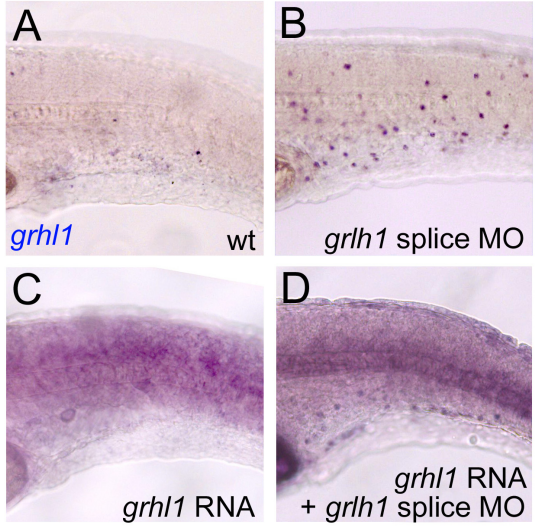
Supplementary Fig. S3. *grhl1* morphants display unaltered onset of expression of genes marking the different non-keratinocyte epidermal cell lineages. *All panels show lateral views (anterior to the left) of whole mount* in situ *hybridisations of wild-type siblings (WT, left columns) and* grhl1 *morphants (MO, right columns) with probes indicated at the left side and at stages indicated in the upper right corner (s = somites).* **(G-H´)** *The anterior part of the trunk. No difference in onset of expression could be detected between un-injected wild-type controls and morphant embryos.*



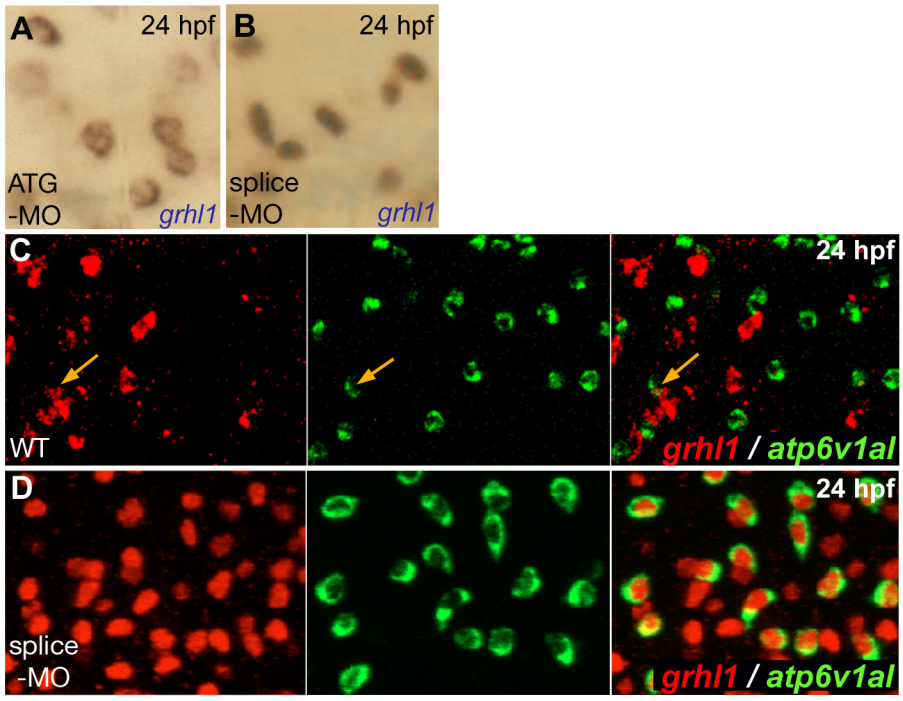
Supplementary Fig. S4. *grhl1* morphants display unaltered numbers of the different known non-keratinocyte epidermal cell types. (A-H´) *Lateral views of whole mount* in situ *hybridisations of WT (left columns) and* grhl1 *morphants (right columns) with probes indicated at the left side of the panels and at stages indicated in the upper right corner. All panels except (C,C') show the anterior part of the trunk.* **(C,C´)** *The posterior part of the trunk. No differences in expression were detected between WT and morphants.* **(I)** *Graphical illustration of the average total numbers of cells expressing markers indicated below the bars. Dark grey bars show WT, light grey bars show* grhl1 *morphants. Error bars indicate the standard error of the mean. According to the Student-*t *test, none of the subtle differences seen was below the 0.01 level.*



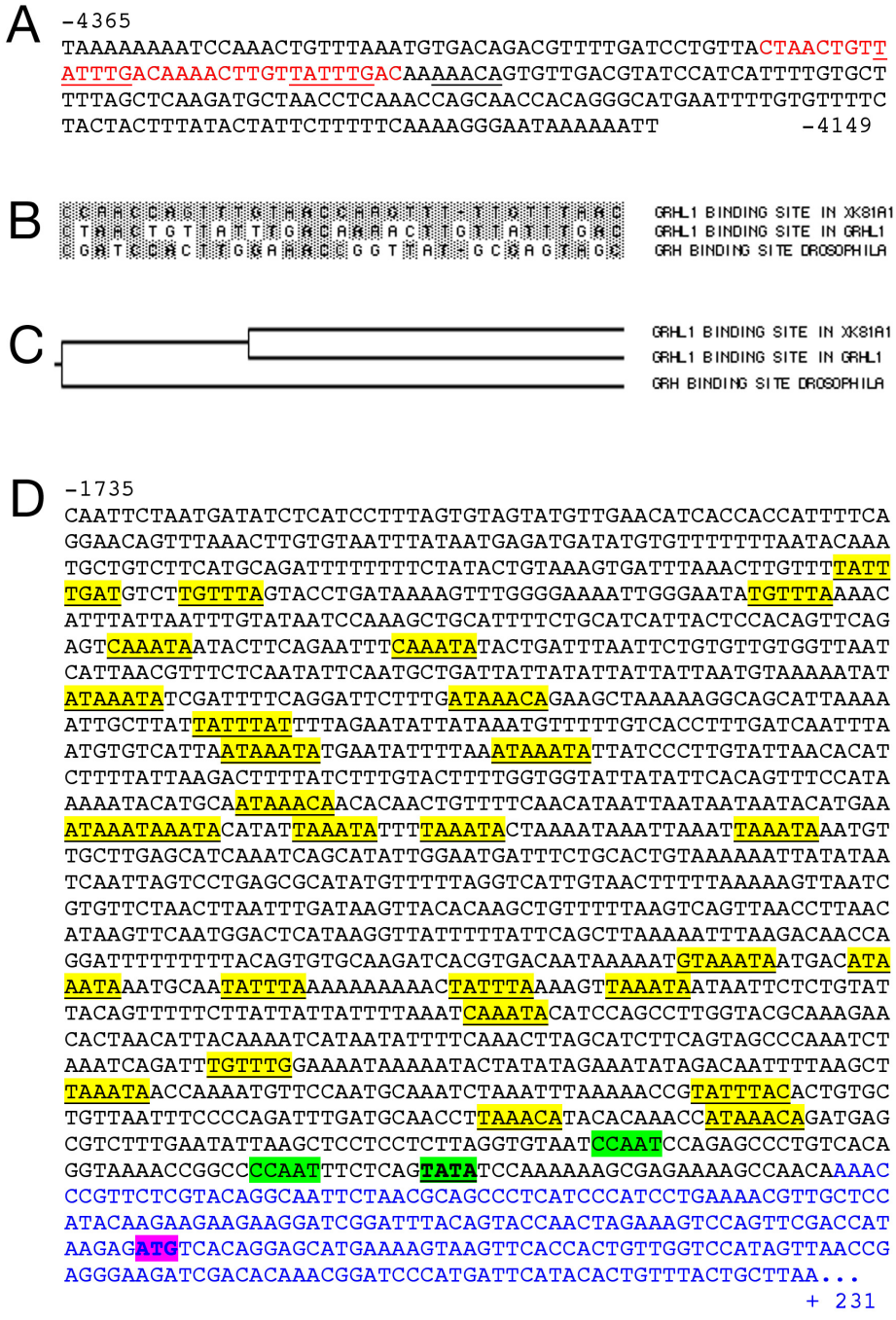
Supplementary Fig. S5. Loss of *grhl1* function does not affect skin morphology and osmoregulation. *All panels show live fish; stages are indicated in upper right corner, genotypes and/or treatments in the lower left corners.* **(A-D)** *Wild-type (WT) or morphant fish grown in regular E3 medium (hypotonic; A-C) or in Ringers medium (isotonic; D).* **(E-F)** *Larvae after osmotic shock (see Materials and Methods for details).*



Supplementary Fig. S6. Injection of *grhl1* mRNA leads to precocious loss of *grhl1* expression in wild-type embryos, and to a normalisation of *grhl1* expression in *grhl1* morphant embryos. *All panels show lateral views on trunk of embryos at 24 hpf, after* in situ *hybridisation with* grhl1 *probe.* **(A)** *Uninjected control;* **(B)** *embryo injected with grhl1 splice-MO;* **(C)** *embryo injected with* grhl1 *mRNA (20 ng/µl);* **(D)** *embryo coinjected with* grhl1 *MO and* grhl1 *mRNA. The weak ubiquitous staining in (C,D) is due to remnants of the injected synthetic mRNA.*



Supplementary Fig. S7. *grhl1* morphants display *grhl1* expression in all ionocytes, with nuclear localisation when *grhl1*-splice MO, and cytoplasmic localisation when *grhl1*-ATG MO is used. *All panels show* in situ *hybridisations at 24 hpf and with probes indicated in lower right corners.* **(A,B)** Grhl1 *transcripts were located in the nucleus upon injection of* grhl1*-splice-MO (B; also used in D), whereas they were cytoplasmic when* grhl1*-ATG-MO was used* **(A)**. **(C,D)** *Double fluorescent* in situ *hybridisations indicating that in wild-type embryos (WT), only few ATPase6v1al-positive ionocytes (in green) were* grhl1*-positive (in red) (C; orange arrows), whereas in the* grhl1 *morphant* **(D)**, *all ATPase6v1al-positive ionocytes contained nuclear* grhl1 *transcripts; anterior trunk region; left and middle pictures show single channels, right picture merged image.*



Supplementary Fig. S8. The 5′ upstream region of the zebrafish *grhl1* gene contains one putative Grhl1, and multiple putative Foxi1/3 binding sites. (A) *Blast searching of the zebrafish* grhl1 *gene and upstream sequences (ZFIN:ZDB-GENE-030131-3665; 152.592 bp; http://zfin.org) with the previously identified Grhl1 binding site of the* Xenopus XK81A1 *gene (Tao* et al., *2005) revealed a single related sequence of 33 bp (in red) 4283 to 4316 bp upstream of the putative transcription initiation site of the* grhl1 *gene. Putative Foxi1/3 binding sites (Kurth* et al., *2006) within and close to the motif are underlined.* **(B,C)** *Alignment (B) and phylogenetic tree (C) of the Grhl1 binding site of the* Xenopus XK81A1 *gene (Tao* et al., *2005) (row 1), the putative Grhl1 binding site of the zebrafish* grhl1 *gene (row 2) and the Drosophila Grh consensus binding site (Huang* et al., *1995) (row 3), as determined by Lasergene MegAlign software (DNA Star, Jotun Hein method).* **(D)** *Putative Foxi1/3 binding sites (Kurth* et al., *2006) in the promoter region of the zebrafish* grhl1 *gene, marked in yellow, some of which occur in palindromic organisation. The TATA box and two CAAT boxes of the promoter are marked in green, the 5′ portion of the* grhl1 *transcript (according to EST sequences) in blue, and the translational start codon in purple. Nucleotide numbers refer to the potential transcription initiation site, which is set to zero (also in A).*

SUPPLEMENTARY TABLE 1

PCR PRIMERS AND ENZYMES FOR RNA SYNTHESIS

Name	Forward primer (5' - 3')	Reverse primer (5' - 3')	Frag-ment size	Enzymes for RNA synthesis
z-grhl1-probe	CCCGTTCTCGTACAGGCAATTCTAACG	GCTTCAGTTGGTCATCTCTGGATTTC	1050 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl2a-probe (short)	AACGCTTTTCCGCTTGATCCTG	TTCACTTGTGAAGGCACGGC	287 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl2a-probe (long)	GCTTTTCCGCTTGATCCTGAATGTTTG	CAATGTTTCAACTGCTCATCCCGACAC	1070 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl2b-probe (short)	AGCAAAGTGGTTGAAATGTCAGACG	GCTTTTGCCGCAATGACTTG	505 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl2b-probe (long)	CCGCGGAGGAAGAGAAAATATGTCAC	GTATTTAAGTTGCTCATCTCGATTC	921 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl3-probe (short)	ATAGGCCCATCATTTCCAGG	CCTTACAAAAGCAGAGGCAG	520 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-grhl3-probe (long)	CCACAAGCACACCTCAGAGAGGAGACC	GCTCAGTGAATTGATGCCAATGTGCAC	1015 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
z-cp2-probe (short)	AATTGCAAACAAAATCACAG	ATAAACACGGGAAACATTAG	503 bp	*Sac*I, T7 (as) *Nco*I, Sp6 (s)
z-cp2-probe (long)	GTTGGTGAGGGAAAGACTACTGCGG	CCAGGAGATGGCGAGTTGTTTAC	1267 bp	*Sac*I, T7 (as) *Nco*I, Sp6 (s)
z-cp2-like1-probe (short)	AATGTGACACGAATGAATGT	TTTTCATTCTGATCAACAGA	385 bp	*Sac*I, T7 (as) *Nco*I, Sp6 (s)
z-cp2-like1-probe (long)	GTCTGCAGGTGGCGGACGGACACTCG	GGAGAGCAGTTTTCCTCTGGAAAGC	1047 bp	*Sac*I, T7 (as) *Nco*I, Sp6 (s)
z-lbp-1a-probe (short)	ATCACTGCCGCACCAAAAGTGT	CCATGCTGAAGTAAAAGTGG	300 bp	
grhl1-FL	CGAATTCGTACCAACTAGAAAGTC	GTCTAGATCTGTAGAAGGAGCTG	2069 bp	*EcoR*I, T3 (as) *Not*1, Sp6 (s)
ncc-chr18	CCTGTATGAGGAGTCCAGTGTGGACA	AATGAGTACTGCTCATGGAGTCTGCG		
ncc-chr22a	TCACCGCTTCTCCAAGCGTG	CTCTCGGGATGGCAATCTCC		
ncc-chr22b probe	CAGCAATGTCCCCCATCAAGGG	GACCGCTGCAGTGGATTGTGTA	1109 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
ncc-chr22c	GTGATATTCACAAGGATTAGCGCA	ACTTGCAGCGACGGTAATTGCA		
ncx1b-probe	GCCTTTACCATCCAAGGTGGATATCGA	CATGGCTGTCTTCAAAGTCCTCTCC	935 bp	*Nco*I, Sp6 (as) *Sac*I, T7 (s)
prox1-probe	AAGCCCCTTAGGTGCCCCAACA	GGCAAGGGGAAATGGGGTAAGG	938 bp	*Sac*I, T7 (as) *Nco*I, Sp6 (s)
grhl1-GFP	TAGGATCCAACCCGTTCTCGTACAGGCA	TATCTAGAGGTTTTCACTGAGTTGCTTGCT		*EcoR*I, T3 (as) *Not*1, Sp6 (s)

SUPPLEMENTARY TABLE 2

GENBANK ACCESSION NUMBERS OF *GRHL/CP2* GENES ANALYSED IN THIS WORK

Species	Gene	Accession-Number	First Reference
Danio rerio	*z-grhl1* (Chr 17)	XM_001923728 NC_007128	This study (EST Venkatesan *et al*., 2003)
Danio rerio	*z-grhl2a* (Chr 16)	NM_001030092 NC_007127	This study
Danio rerio	*z-grhl2b* (Chr 19)	NM_001083072 NC_007130	This study
Danio rerio	*z-grhl3* (Chr 17)	XM_001332902 NC_007128	This study
Danio rerio	*z-cp2* (Chr 23)	XM_001336446 NC_007134	This study (EST Venkatesan *et al*., 2003)
Danio rerio	*z-cp2-like* (Chr 9)	NM_001002214 NC_007120	This study
Danio rerio	*z-lbp-1a* (Chr 19)	NM_001114574 NC_007130	This study
Drosophila mel.	*d-grh*	NM_057496	Nüsslein-Volhard *et al*., 1984
Drosophila mel.	*d-cp2*	NM_136712	Wilanowski *et al*., 2002
C.elegans	*Ce-grh-1*	AC024797	Venkatesan *et al*., 2003
Homo sapiens	*h-GRHL1*	NM_014552	Huang and Miller, 2000
Mus musculus	*m-Grhl1*	NM_145890	Wilanowski *et al*., 2002
Gallus gallus	*g-GRHL1*	XM_426209	Venkatesan *et al*., 2003
Xenopus spec.	*X-Grhl1*	NM_001095602	Venkatesan *et al*., 2003
Homo sapiens	*h-GRHL2*	NM_024915	Wilanowski *et al*., 2002
Mus musculus	*m-Grhl2*	NM_026496	Wilanowski *et al*., 2002
Xenopus spec.	*X-Grhl2*	NM_001011338	Ting *et al*., 2003
Homo sapiens	*h-GRHL3*	NM_198173	Kudryavtseva *et al*., 2003
Mus musculus	*m-Grhl3*	NM_001013756	Kudryavtseva *et al*., 2003
Gallus gallus	*g-GRHL3*	XM_417842	This study
Xenopus spec.	*X-Grhl3*	NM_001005642	Ting *et al*., 2003
Homo sapiens	*h-CP2*	NM_005653	Kim *et al*., 1987
Mus musculus	*m-Cp2*	NM_033476	Lim *et al*., 1992
Gallus gallus	*g-CP2*	NM_204384	Murata *et al*., 1998
Xenopus spec.	*X-Cp2*	NM_001090493	Venkatesan *et al*., 2003
Homo sapiens	*h-CP2-like1*	NM_014553	Huang and Miller, 2000
Mus musculus	*m-Cp2-like1*	NM_023755	Rodda *et al*., 2001
Gallus gallus	*g-CP2-like1*	XM_422087	This study
Xenopus spec.	*X-Cp2-like1*	NM_001086908	Venkatesan *et al*., 2003
Homo sapiens	*h-LBP-1a*	NM_014517	Jones *et al*., 1988; Wu *et al*., 1988
Mus musculus	*m-Lbp-1a*	NM_001083319	Sueyoshi *et al*., 1995
Gallus gallus	*g-LBP-1a*	XM_426018	This study