# **LEGENDS TO SUPPLEMENTARY FIGURES**

 **Figure S1. Comparison of** *HoxD* **regulatory landscapes in mammals and fishes. a.** *Hoxd* gene expression and regulation in mouse limb buds at E12.5. The *HoxD* cluster is flanked by two gene deserts, named according to their relative position (3' or 5') with respect to *Hoxd* gene orientation. The 3DOM regulatory landscape activates *Hoxd4* to *Hoxd11* in the proximal limb territory (green). The 5DOM activates *Hoxd10* to *Hoxd13* in the distal limb territory (blue). Schemes are based on ref.<sup>15</sup>. **b.**  Gene expression in fin buds at 40-60 hpf in the cognate zebrafish *hoxda* cluster. The fish cluster is also flanked by two gene deserts but their regulatory potentials are unknown (question marks). Fish *hoxd9a*  to *hoxd11a* are expressed in the preaxial fin territory (purple) whereas *hoxd11a* to *hoxd13a* are 422 expressed in a postaxial domain (orange). Schemes and WISH are inspired from <sup>14,53,54</sup>.

 **Figure S2. 3D chromatin conformation at the mouse and fish** *HoxD* **loci.** Contact frequency heatmaps at the mouse *HoxD* (E18.5 male UGS, one representative replicate out of two) and fish *hoxda* 426 (24 hpf and 48 hpf total embryos<sup>41,55</sup>) loci (top and bottom, respectively). The similarities in the constitutive structural organization of the mouse and the fish loci are underlined either by the position and relative extents of TADs (thick black lines), the presence of a sub-TAD boundary within 3DOM (asterisk), as well as by the positions and orientation of CTCF binding sites (red and blue arrowheads). *Hox* genes are in purple-scale rectangles and other genes are grey rectangles. Bin size is 10 kb. The 431 scales on the *x* axes were adjusted to comparable sizes for ease of comparison, yet the fish locus is more compact. Scale bars in both cases; 100 kb.

 **Figure S3. The** *HoxD* **locus is part of a large syntenic interval. a.** The mouse *HoxD* locus (mm39) is on top and the zebrafish *hoxda* locus (danRer11) is shown below. *Hox* genes are in purple-scale rectangles and annotated mouse enhancers are shown as either blue (5DOM) or green (3DOM) rectangles. Conserved sequences between the two gene deserts are shown as vertical black bars. Those conserved sequences overlapping with known murine enhancers were used to annotate the corresponding elements in zebrafish (blue rectangles). **b.** Synteny plot representing sequences conserved between the mouse and the zebrafish *HoxD* loci. On the *x* axis is the mouse locus (mm10, chr2: 73605690-75662521) and on the *y* axis is the zebrafish locus (danRer11, chr9: 1639965-2393397, inverted *y* axis). Despite a mouse locus that is in average 2.6 times larger than its zebrafish counterpart, the order of most conserved sequences is maintained, showing the absence of substantial genomic rearrangement at these gene deserts. **c.** Size comparisons between different regions of the zebrafish *hoxda* and the mouse *HoxD* loci. The left panel shows that the *Hox* clusters have maintained a similar size over time, while gene deserts have expanded in mouse and/or contracted in zebrafish. The right panel shows that the ratio between the sizes of 5DOM *versus* 3DOM is inverted in the two species.

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 **Figure S4. Chromatin profiles in zebrafish embryos. a.** Expression of *hoxd13a*, *hoxd10a* and *hoxd4a* in control zebrafish embryos by WISH. Stages are indicated on top of the panels. Scale bars; 200 µm. **b.** Dissection plan used for panel (**c**). PT, posterior trunk. **c.** ATAC-seq profile and both H3K27ac and H3K27me3 CUT&RUN profiles over the zebrafish *hoxda* locus in 16 hpf dissected heads (grey, one representative condition out of three) and 16 hpf posterior trunk cells (PT, blue, one representative replicate out of three). Both the *hoxda* cluster and 3DOM show specific open sites in posterior trunk, where *hoxda* genes are expressed, when compared to forebrain. The CUT&RUN profiles in posterior trunk cells show enrichment for H3K27ac (green coverage) on the central and anterior parts of the *hoxda* cluster, while H3K27me3 (red coverage) is enriched on the posterior part and on *evx2*. Scale bar; 100 kb.

 **Figure S5.** *Hoxd13a* **expression in control, heterozygous and homozygous mutant fin buds at 60 and 72 hpf. a.** Schematic of the deletion and spatial orientation of the fin buds**. b.** Various samples are shown to illustrate the variability observed. While a clear tendency is observed in the loss of the distal most expression in homozygous mutants, expression is still observed in some samples as well as in post-axial cells, unlike the situation in developing limb buds where expression is entirely absent in the comparable deletion. Scale bar: 50 µm.

### **Figure S6. WISH of** *hoxd13a***,** *hoxd10a* **or** *hoxd4a* **in zebrafish embryos lacking either 3DOM (a),**

 **or 5DOM (b).** The genotypes (in red, top) and genes analyzed (left) are shown as well as the stages (up left). **a.** Deletion of 3DOM. Black arrowheads (empty for no expression and full for expression) indicate differential gene expression in the cloacal region, whereas red arrowheads (empty for no expression and full for expression) point to the pectoral fin buds. Control and homozygote mutant embryos are shown side by side for each condition, except for *hoxd4a* where a heterozygous (Het) mutant is shown. Wild-type and homozygous embryos originate from the same clutch of eggs and were processed together. In Del(3DOM) mutant embryos (**a**), *Hoxd10a* and *hoxd4a* transcription is lost in fin buds, whereas *hoxd13a* transcripts in the cloaca are not affected. B, branchial arches; R, rhombomeres; Scale bars; 200 µm. **b**. In contrast, *hoxd13a* mRNAs are lost from the cloacal region in Del(5DOM) mutant animals at 36 hpf (red arrowheads), while still clearly detected in the fin buds, indicating that the 5DOM is necessary for *hoxd13a* transcription in the pseudo-cloacal region.

 **Figure S7.** *Hoxd* **gene expression in the mouse urogenital system. a.** Schematic representations of male and female urogenital systems. K: Kidney, B: Bladder, O: Ovary, T: Testis. The urogenital sinus (UGS) is indicated with a red circle. **b.** WISH of *Hoxd* genes in representative female and male urogenital systems. All *Hoxd* genes are expressed in anterior portions of the UGS including kidneys, the uterus, deferens ducts and the bladder, except *Hoxd13* transcripts, which are restricted to the UGS

 (see Fig. 3b). **c-d.** Schematic representation of two *HoxD* genomic configurations, The first one is a deletion of the entire *HoxD* cluster (**c**), whereas the second one is a random integration of a transgene carrying the same *HoxD* transgene plus some flanking sequences in 5' (**d**, thick red bar). *Hox* genes as in shades of purple and the deletion breakpoints are shown as vertical dashed red lines. Scale bar; 100 kb. **c.** WISH of *Hoxd13* in UGS from a transgenic *HoxD* cluster (TgBAC), while lacking both endogenous copies of the *HoxD* cluster. Expression is not detected from the transgenic cluster. **d.** Likewise, ß-gal staining of UGS transgenic for the *HoxD* cluster containing a *LacZ* reporter displays no activity in the UGS. By contrast, *LacZ* staining of mutant *Inv(nsi-Itga6)d11lac* embryos, which also includes a *lacZ* reporter confirms that 5DOM is necessary and sufficient to drive expression in the UGS. Scale bar: 1 mm.

 **Figure S8. Regulatory potential of sub-regions within 5DOM. a.** RNA-seq FPKM values for various mouse *Hoxd* genes in E18.5 UGS obtained from either wild-type or *Inv(nsi-Itga6)d11lac* mutant 498 embryos (see schematic in Fig. 3c). Data are shown separately for females ( $n=3$ , dots) and males ( $n=3$ , triangles). Drastic decreases are observed for *Hoxd10, Hoxd12* and *Hoxd13* when 5DOM is disconnected from the *HoxD* cluster. *Hoxd11* could not be assessed due to the presence of a transgenic copy of this gene in the *LacZ* reporter cassette. **b.** On top is a scheme of the 5DOM regulatory landscape on mm39 with *Hox* genes in purple. Blue rectangles indicate previously described 5DOM enhancers. The red arrowheads delimit the serial deletion breakpoints. The three consecutive deletions are depicted by red dashed lines. Below are RT-qPCR quantifications of expression levels relative to wild-type (n=4) in three mutant lines carrying serial deletions of 5DOM (n=3). The horizontal red line represents the value of 1 for reference. Severe reductions are observed for both the *Del(Rel5-SB1)* and *Del(Rel1-Rel5)* conditions, unlike in the *Del(SB1-Atf2)* deletion. Scale bar; 100 kb.

 **Figure S9. Sequence conservation in vertebrates of the GT2, islE and CsB UGS enhancers**. All three sequences are comprised in the box highlighted in Fig. 4a*.* The ATAC-seq and H3K27ac ChIP- seq profiles are shown with, below, their sequence conservation from fishes and mammals. The thick blue lines below the H3k27ac profiles indicate the extent of the transgenes assayed in Fig. 4. Scale bars; 1 kb.

 **Figure S10.** *hox13* **gene expression in the Daniocell atlas. a.** UMAP of endoderm cells using matrices 516 extracted from ref.<sup>56</sup>, colored by tissue. The black rectangle indicates the UMAP region which contains cellular clusters from the cloacal region. All other panels in the figure correspond to this rectangle. **b.** UMAP of endodermal cells and identities of their clusters. The colors indicate the identities of cells from both the cloacal region (red arrow) and the posterior intestine (dark green, arrow). **c.** UMAP of selected endoderm, clustered by developmental stages (color code below). **d.** UMAP as in panel b, with the expression in red of the various *hox13* paralogous genes. All cells with a normalized expression

 level above 2 are displayed in red. In panels **c** and **d**, arrowheads indicate *hox13* expressing cells in the cloacal region either at early (red) or late (black) timepoints. The black arrows point to *hox13* expression in intestinal cells.

 **Figure S11. Cloacal region phenotypes in** *hox13* **mutant zebrafish. a-f.** Confocal micrographs of mutant cloacal regions at 6 dpf shown in single channel (**a-c**) and pseudo color (**d-f**). **d.** Triple *hoxa13a*;*hoxa13b*;*hoxd13a* heterozygotes (n=6) exhibit wild-type patterning with separate openings for the hindgut (blue) and pronephric duct (yellow). **e.** Homozygous *hoxa13a* single mutants show wild- type patterning (n=4). **f.** Homozygous *hoxa13b* single mutants have wild-type patterning (n=4). **g-h.** Length and width of the hindgut and pronephric duct in wild-type and *hoxa13* mutant zebrafish embryos at 3 dpf. **g.** The length (red dotted lines) and width (white dotted lines) of the hindgut and pronephric complex at the median fin fold level were quantified in wild-type (n=4, left) and *hoxa13a*;*hoxa13b* double mutant embryos (n=5, right). **h.** The length difference of the hindgut and pronephric complex between wild-type and *hoxa13* double mutant embryos is statistically significant (\**p* = 0.0101, two- sided Welch's t-test). The error bars indicate the standard error of the mean. Scale bar length is 30 μm in **a-f** and 100 μm in **g**.

 **Table S1. Extent of the mouse and zebrafish domains.** Sizes are indicated in base pairs and were determined based on the transcription start sites of genes.

**Table S2. Accession numbers for re-analyzed data.** SRA accession numbers and reference of

publications for the re-analyzed data when previously published data was used.

**Table S3. RT-qPCR primers.** Lists of primers used in RT-qPCR experiments.

**File S1.** Sequences of the zebrafish probes used for WISH

**File S2.** Sequences of the mouse probes used for WISH

546 File S3. Sequences of the zebrafish *hoxda*<sup>Del(3DOM)</sup> and *hoxda*<sup>Del(5DOM)</sup> founder alleles













Hintermann<sup>\*</sup>, Bolt<sup>\*</sup> et al.<br>Figure S6











 $2.5$  $5.0$  $7.5$ 



## **Hintermann\*, Bolt\* et al.**

# **Sequences for zebrafish ISH probes**

### >hoxD4a\_probe\_danRer

GAGTGTGTGCGCGATCTCGATGCGACGCCGTCTTGTTAGATACCTGTTAAAATGAAACTCCTTCTCTAGTTCGAGAACCTGCTGTC TTGTGTAGGCTGTCCGAGAACGTTTGGGTTCAGGTCCTGTGTAATCCGGGTTCACCGTAGTAACGTGCACTTTCTTCATCCACGG GTAAACTACAGCAGGCTGCTTCGTTGGTATCCCGTTCTGGGTCTTTGTGTTTTGTTGTTGTCCACCAGTCCTCGATCCGGAAATCT GGACCGCGGGACACTGCTCTGTCTGTGCAGGGAAAGGGCTAGGGGTGCTGGCTTGATCCTGCACATGACCCCGCGGCTGCACC GACGAGCCCTGGACAGTGCTACAACTGTAAGGCTGTTCAGAGTAGTTTGACCGTGAATAGATTCCGGGATGCTGGAAATCAGTG TCCTGCGACGGACTGTAGTAGCCTGGGCTCTGTTCAGGTATATAGCTGTTCTGAGAATATTCCTCGCAAGGAGGAAATTTGGGATC CACATACTTGGAGTTCACCATGTACGAACTCATGGCCATTAATTTCTGAAGGTAGGAAATACTAATTTTTCTCGAGTTGTCTTTTTTT CCTCCCTCCATAAAGCCCTCC

### >hoxD10a\_probe\_danRer

CAGAACATGATTCGGGAATCAGGCTGGAGTACGCGGGTATCTCAGACGAACTGACCTTTGCTCTCTTGTCGGAGTACATACAACA GTTAGTCTCTTCTTTTATGCTTTGTGAAAATGTGCAGGTGGACATCTGGTTGAGCGGTTGCTCTAATCGGCAGGACCTGCTCGGAT CTGCCCAGGTATCAGTTTGAGGTATGTACGAGTGGACGGTCATGTCCATGTTTTGGTGGTTTACTTCCCCCCGTTTGCCAAGAGCC GGCAGGAGTCCACAGGTTTGCATTCCATAGGTTCCCATTTCTGCAGTGGCTGCTGGCATGTACATGTTACTGCTGGAATAAAAATC TGTTCTGCACGCCCCAATCAAGGAATCTACCAAAAAAGTGTTTGCTGCAGGAGAGCTGTTGGGAAAGGACATTTTGGATTTTTTT TTGTTTTCTTTTTTTGTTTTAAGAAGCAGAGAAGTGTCCTCTGTAAGCTGACATATCTAGCAAAACAATCCCTGTATAACCCAGCGA GTCTCTCTGCAACCACATGACACTCTCACCAATGAAATTTGAAAATGGCCTTGAGACGCAGG

### >hoxD13a\_probe\_danRer

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### **Sequences for mouse ISH probes**

#### >Hoxd4\_probe\_mouse

TAAGACTATTCACCATCTTAAGGTTTTTGGCACGCAGGTGTCGGTTATTTTCTGCAGTCCATTCCCCGA ACAAATAAATATTTAAGCAAATATAAACTAAACATAAAATAACCCGTTTCCTTCTTGGATTTATTTCTT TGGGCATGGGCCACTGGAAGCAAACAATTTCTTTCTTTCTTTTTTTTTTTTTTTTAAATCACAGAAGAAA TTCTATAGATAATCCAGAAAGTTCTGGGATTCTGGCTCAGCCTCTCCTGCTTGCACTGAAAGCACCTAG CACTGGCTGGCCTTCTGCCTCAACTCCCCCACAGTTACTAAGCCACACAGCTCCCTGAAGGGGGTGGT GTGGAGGAGACCATCCCGGTTCCCACTGGGGTAAAGCCTCCAACCTTCATTTGCAAATTCAATCCACC AGCAGAGATTTTCAGGAGTTTAATGACTCGCCAAGGGTTTATGTTCTCAGTAGCTCCTGCCATATGATA ATCTACTTAAAGAAGCCCGCCCACCCTCCCACCCTTCCCCACTTTAGGGAGGGGGCTGCCCTCGGGCA GGAAGGTAACCTAGTCCGAGGGCGGCCTGCAGGTCCCAGGTCCACCCCCACAGTACCTTAGAGCCCAG TGAGGTGACAGCAGGCCCCGCCTGCACCCGCAGCTTCGCCTCAACCTGGAGTGCAAGAAGGGATAGG CCCAGGGTCCCCACTTCTATAAGGTCGTCAGGTCCGTATGGTGGTCCTTGGCCATTGGCTGCAAATGCT GGCCCGGGGCAGCTGAAGAGGAGCAGGAAGATGAGGAAGAAGACCTGCCCTTGGTGTTGGGCAGTTT GTGGTCTTTTTTCCACTTCATCCTCCGGTTCTGGAACCAGATCCCCGGGCGAGCTCGAATTC

#### >Hoxd8\_probe\_mouse

TGCAGGCATAATTTTTAAAAACATTATTAATAAACTTCTTTTAATTTACTCACCATAAAGATTTAAAAT GTTCAAGTGCACTTGTTTGATTAATAAAGCATTTTATATTCAATCATCTAGCGCTGATCATTAGGAAGT TTATGTTGTTGAGGCAAACCACCAGCTCAGACGTTAAAAATTACCATTATAAAAATATAGCCACAAGG TCCGTATCAAATTACCCTTCTATAGGACAGCTCAAGGACTGCTTCTATGCGATACTACAAACTATTCTC TTTAAAAACACAGAAATCCCTTTCAAAGCGCAGAAACCTCAAGAGCAGTGACATTAAGGTAGCTTGAA ACACAGGTAAAGTCCGTTTCCAAAACACAAGAGGGAGGACCGGTGGTGTCCACCAGTATATACTTATA CTGATCATCAGTCTTAATAGTCTGTGGTAGAAGTGTAAAGGTAGAGTTTAATTTGTGGGGCAGCCTTCA GCTCCGTCTTCTTCCAGCCCCGAGACTTCCTTTTTGGGGTCTCCATCCTTTGCCTCCGGCCGGGACGCA GGAAACTTGTCTTTGTTGTTCTCCTTTTTCCATTTCATTCTCCTGTTCTGGAACCAGATTTTTACCTGTCT CTCCGTGAGGGCCAGAGTATGGGAGACCTCGATTCTCCTCTTCCTGGTCAGATAAGGGTTAAAAAGGA ATTC

#### >Hoxd9\_probe\_mouse

CTGAGTTGGATTTTAGTTGGGAGAGCAGGGTGGGGGTGTCCACACTCTGTCTTCCTTCAGATTCTCGGA ATTAGATCGTTGGCCCTGTTTGGGAGGCTGGATAGGGTGAGACTAAACTACAGCACTTTTCAGAAACA TGAGGGACATTTACATAGAATAGGGCTCCATCAAGCCTGCGTGCACTCACAAAATATAGCATTTCCTA AGGAATCAGGGGAGCAGGGAAGGGAGGGAGTGGGACAGGACAAGACTCCAAAGCCACTTGGTTTCTC CAATACATATGCGAGCAAATACAGTGTCCCCCAGATAACTCACAGAGACCAACCACACAGTCACTTCT ACCTAAAAAAAGAAGAAAGTCCGAGTCGCTGCAGAGTTTCTGAATCAAGCACCCACAAAGAAAACAA ACAACAACAAAAAACAAAAAACAAAACAAACAAACAAACAAACAAAAAAACCCGCTGCCTTTTGAG TGCTGCAGGCCAGACCCGCACTGGGTCAGTCTCCTTTAGGGCACTTCTCCTTGCTCATCTTTTTCATTTT CATCCTACGGTTCTGGAACCAGATTTTGACTTGTCTCTCTGTAAGGTTCAGAATCCTGGCCACCTCGTA GCGCCGGTCCCGGGTGAGGTACATGTTGAAGAGGAATTCCTTCTCCAGCTCTAGCGTCTGGTATTTGGT GTAGGGACAGCGCTTTTTCCGGGTGGAGCGAGCGTGGATC

#### >Hoxd10\_probe\_mouse

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CCTGTCGGTGAGGTTAACGCTCTTACTGATCTCTAGGCGGCGCTCGCGGGTGAGGTACATATTGAACA AGAACTCTTTTTCCAATTCCAGCGTTTGGTGCTTGGTGTAAGGGCACCTCTTCTTTCTGCCACTCTTTGC AGTGAGCCAATTGCTGGTTGGAGTATCAGACTTGATTTCCTCTTTGCTTTCCTTCTCCTGCACTTCGGGA CTGGACACGGAAACCTCAGCCAGGCAGCTCCTGTCTTCTGGAAGGCCGCCTTTGGCCTCGGGGCTCTC CACTTGGGAGACTTTAGTGGGCTCCTGGCCGCTCGCGCTTTCGTTCATCTTCTTTTCCATCTGCAGCTGG GCAGCCGAGAGCTGCGGCTTGGCCGCGCCACGAGGGTTGAGCTGGAGCATGACAGTGGAGCTGCCTT CGGGGCTGTTATTGCACTCTTGGGTTTCCCGGTGGCGTAGGTCTGACTCAGTCTAAA

#### >Hoxd11\_probe\_mouse

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#### >Hoxd12\_probe\_mouse

TGGACAGATTCTATAACAGGCGTAGAGCAACTTCATTCACAATTCAGAAAACCTAGGTTTCTCAAGGT AAACCTGGCAGGGCTTGGGGCCTTAAAAGCTTTACAAGAGCTGACCACCAGTCTCTGAGAAGAGACA CCCTAGCTAAGTTCTGATGGAGTTGGTGGGTGGTGGAGAGGATGGGGAAGAAGTGCTGGTTTTCTGTC CTTCAAGAGCAGCAACTACAGTCTGAGAATCTGAGAGGTTTCTTCTCCCACCCTTCAGACTCCTCAAGG AGGCATTTTTTTTGGGGGGGGGGTCTCTTCCTGTGGTGATACATTAAGGAAATTCAAGAAAGACAGAC ATATGGATAACTTCATTCATTCAAGAGGAGAGTCGTGGTTACAGAGTGCAGATCCTCCTCCGCCTCGCT TACTCTTCTTTCTTCCCTGTGCAGCCAAGCCTCAAAACAGGCCAGCAGCTCCCCTCACCCATAGCTCAG TTCTGGGGATCTAGTTACAGAGTGCTTGGCCTTGGCTCAGGGATAGGTGAGGCTGGAGCAGGGGAATT ATGACAGTCTAGAGACCATACTTTGTTTTGGAGTCCCAGTTTCTCTCCCTGTCTCTGAGAAGGAGATCT TCCAGCCTGTTTTACTCAGCAGGAGAACCTTTGAAGCGCTGAGGTTCCAGGGTATCCTTTCCTCCCCCC CCCCCCACAAGTCTGAAGGTCTAGGGTAGCTTTTGTCCTCCCAAATCATTCAGGGCAGATGGGGGTTG TGGAATCAGGCCCTTTCCTTCCTGCAGAGTGGAGAGCCCAGCTTTGTCACAAAGGGCTTCTGCTGCGA AGGGGCTAGAGTTATCCCCAGCCCCTTGCACTGCAGCGGGGTCTCAGGGCCTTTCTACCTGCACTTATA CCCGGAGCTCTAGCTAGGCTCCTGTTTCATGCAGAAAGAGCTGGATAGAGAAAGAAAAAGAAAAAGA ACACGTTGAAAACCGGAAAAAACAAACCCTCATACAGTGTTTCAATAGTGAGCCCCGGATGTAAACAT CATAGACAAGGACAGGTGACCCCCAGACACCATGCTGAATGTTTAAAGCCAGTGTTAGATTGCAATTC CCAAACACCTCTCAGGAGGGTCCCAAAGAGAGCTGAAAGCGAAGGGGGCTCCACTGGCCTCATC

### >Hoxd13\_probe\_mouse

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# Sanger sequences of the zebrafish founders for *hoxda<sup>Del(3DOM)</sup>* and *hoxda<sup>Del(5DOM)*</sup>

### >del3DOM founder1

NNNNNNNNNNNNGNNNANTNNNCTCNTGACGACTACAAAGTTATTTAATCTACAAACCAAGTTGATTTTATATACTCCATTG GGGTTTTGTAGCGTATTATTGACGTCTTGAATCCTTTTAGAGCGTGTGTGGTAATGTGTAGCCTCTTTACAGACTAATAAATGCT ACAGGCCTCTCACATGATATGATTAATAACTGTAAAAGAAAACGTTGCGCCACCAGAAGTGTCTAAAAATGATAATCTAGTCTC CCTATTGGAGGCCATAATGTAATGTGATGATGTATGGAGACCTGCTAACATTAACACAAGCCACACTACTCCTTTCACGTGCTA CACTAAAAAGAGAGAAATTGGTGGAAGGGCATTGCTCTTCAACTAAACATCACAAATGTGCCGTTTCAGCAGTGTTACAGCAA NTNC

### >del3DOM\_founder2

NNNNNNNNNNNNNNNNNTNNNCTCATGACGACTACNAAGTTATTTAATCTACAAACCAAGTTGATTTTATATACTCCATTGG GGTTTTGTAGCGTATTATTGACGTCTTGAATCCTTTTAGAGCGAGTGTGGTAATGTGTAGCCTCTTTACAGACTAATAAATGCTA CAGGCCTCTCACATGATATGATTAATAACTGTAAAAGAAAACGTTGCGCCACCAGAAGTGTCTAAAAATGATAATCTAGTCATT ATGGCCATGGAGGCCATAATGTAATGTGATGATGTATGGAGACCTGCTAACATTAACACAAGCCACACTACTCCTTTCACGTGC TACACTAAAAAGAGAGAAATTGGTGGAAGGGCATTGCTCTTCAACTAAACATCACAAATGTGCCGTTTCAGCAGTGTTACAGC AAANTNCCGGGAGA

### >del5DOM\_founder1

GGNNNNNNNNNNNNNNNNNNGGANTTCCNTGCATGCCNGAACATTTATGCTTCCTTTTCCCAAATGCCTGTAAAGCACAGA CATTTTTCCTCCTTAATTACTTCCCGAATTTACCCTACTTCTGTCCCAATTTTTTTAAACTTACTTCAAATATTTGAAGTGTGATTTT TGTGGCTGTCCTGAAGCGTTCAATCCATCTATAGCACAATCGCGTGTGCGTTTTATTTGAGTTTTGGGGATTTTCTGAGAGGAT CCCATTGCGAGGTCATGTGTCTTCCATTATCTGGGTGTTTTCTAATCAGCCTGCAGTGGCTGANATTAAACAGGGAAGAATTCT CCCATCAAACCGGACTGTCGTAAATCCAGCTCACCTTGGAGAGCAGAAGGGAAGTGCAGATCTGTAAACCCATCATCGCTCCG CTCCCCCACACACACTTATGCTCCACAANAGGACNNNNN

### >del5DOM\_founder2

NNNNNNNNNNNNNNNNNNNANNNNNNGCATGCCAGAACATTTATGCTTCCTTTTCCCAAATGCCTGTAAAGCACAGACAT TTTTCCTCCTTAATTACTTCCCGAATTTACCCTACTTCTGTCCCAATTTTTTAAAACTTACTTCAAATATTTGAAGTGTGATTTTTGT GGCTGTCCTGAAGCGTTCAGTCCATCTATAGCACAATCGCGTGTGCGTTTTATTTGAGTTTTGGGGATTTTCTGAGAGGATCCC ATTGCGAAGGTCATGTGTCTTCCATTATCTGGGTGTTTTCTAATCAGCCTGCAGTGGCTGANATTAAACAGGGAAGAATTCTCC CATCAAACCGGGCTGTCGTAAATCCAGCTCACCTTGGAGAGCAGAAGGGAAGTGCAGATCTGTAAACCCATCATCGCTCCAAC TCCCCCACACACACTTATGCTCCACAANAGGACCNNNN

# **Hintermann\*, Bolt\* et al. Table S1: Sizes of mouse and zebrafish domains**











# **Hintermann\*, Bolt\* et al. Table S2: Accession numbers for re-analysed data**



# **Hintermann\*, Bolt\* et al. Table S3: Sequences for primers and guides**

# **ZEBRAFISH**

**Genotyping primers**



### **crRNA**



# **MOUSE**

### **Genotyping primers**



# **RT-qPCR primers**



## **Primers to clone transgenes**

