

1 **SUPPLEMENTAL DATA**

2 **Supplemental note**

3 Clinical Report of Family A: In Family A, the index individual (III-1) and her maternal uncle
4 (II-3) showed several CFs of the VATER/VACTERL association: Individual III-1 presented
5 with anorectal malformation (A; vestibular fistula), tetralogy of Fallot (C), esophageal
6 atresia with tracheo-esophageal fistula (TE; Vogt type 3b), and unilateral renal agenesis
7 (R). Her uncle (II-3) showed anorectal malformation (A), esophageal atresia with tracheo-
8 esophageal fistula (TE; Vogt type 3b), unilateral renal agenesis (R), and aplasia of the
9 right radial bone and pre-axial polydactyly of the left hand (L). Both individuals showed
10 normal physical and neurocognitive development. At the time of assessment, the index
11 individual was a student of good standing in high school and her uncle had a degree from
12 university. Ultrasound examinations of I-1, I-2, II-2 and III-2 confirmed that these family
13 members were unaffected regarding their internal organ status.

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15 **Supplemental figures and tables**

16 **Figure S1. Results of molecular karyotyping in individuals II-2 and II-1 (Family C)**

17 **(A)** Chromosome Xp11.23p11.22 deletions of 1.07 Mb spanning chrX:g.49,375,617-
18 50,447,320 involving eight genes and six microRNAs is depicted in Genome Studio.

19 **(B)** The respective genomic segment is aligned to RefSeq genes (according to hg19)

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21 **Figure S2. Exon structure of zebrafish *shroom4* with Morpholino targets.**

22 **(A)** Schematic depiction of *shroom4* gene with transcripts *shroom4-201* and *shroom4-*
23 *202* and targets of MO. The *shroom4* MO is overlapping the exon (E) 2 splice donor site.

24 **(B)** RT-PCR amplified cDNA and Sanger sequencing showed excision of E2 for *shroom4-*
25 *201* and insertion of 41 base pairs (bp) from intron (I) 2 for *shroom4-202*.

26 **(C)** Pictures of ethidium-bromide-stained gel after performing RT-PCR with zfl harvested
27 at 2 dpf for MO and Ctrl MO. MO injected zfl show a weaker band at 397 bp and excision
28 of E2 for *shroom-201*. For *shroom4-202* a weaker band can be seen at 255 bp and the
29 insertion of 41 intronic bp is shown. *eef1a1* is used as a housekeeping gene control
30 indicating an equal expression of *eef1a1* in both samples.

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32 **Table S1. Summary of genetic and clinical features in individuals with *SHROOM4***
33 **variations**

34 This supplementary table depicts the genetic and clinical features of previously described
35 affected individuals. The table is divided in two subgroups: single nucleotide variants
36 (SNV), comprising missense and nonsense variants, and structural variations, including
37 deletions, duplications and complex rearrangements. Abbreviations are as follows: ID,
38 intellectual disability; MRI, Magnetic resonance imaging