Supplementary Material

TCF12 haploinsufficiency causes autosomal dominant Kallmann syndrome and reveals network-level interactions between causal loci

Davis, Balasubramanian, et al.

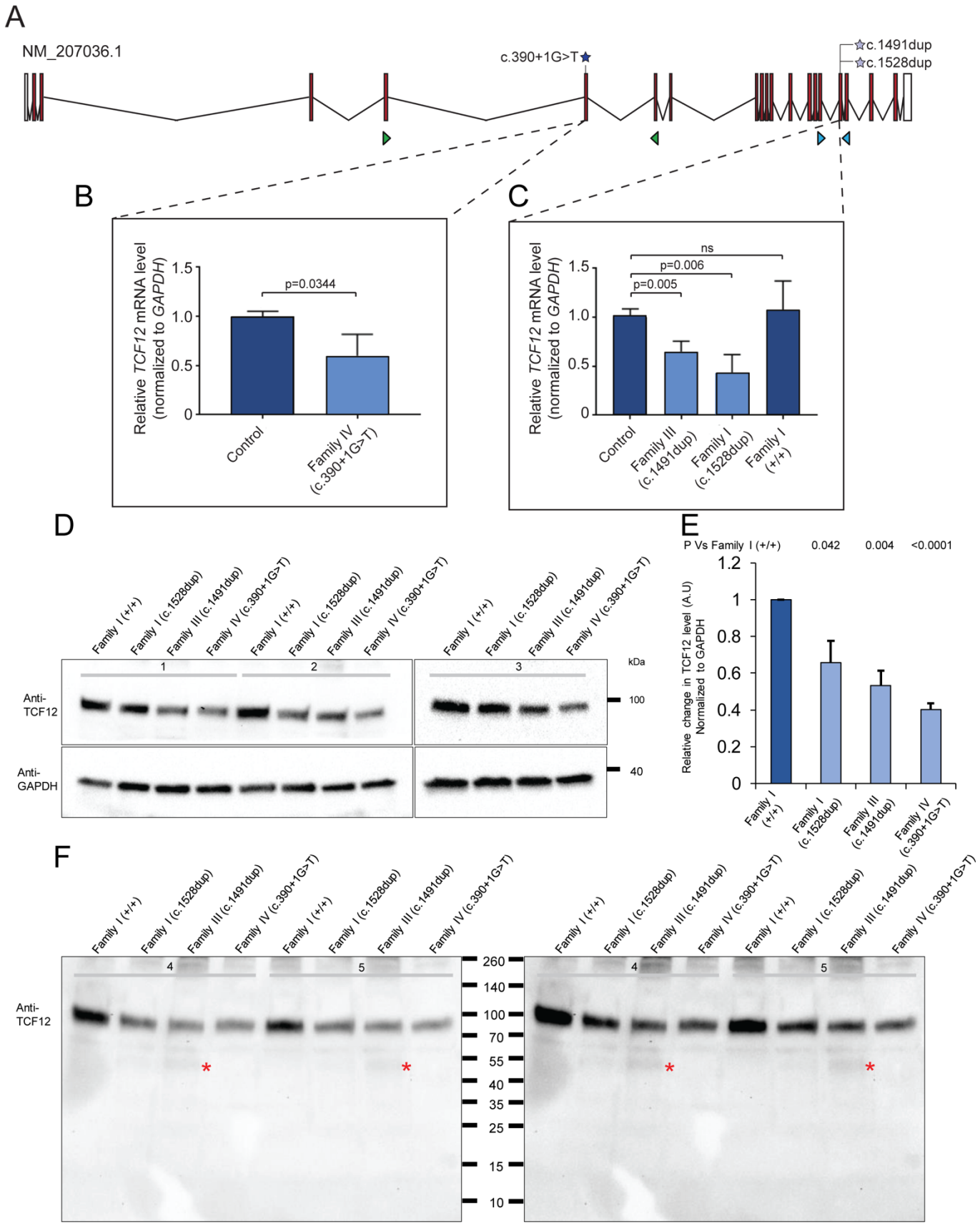

A
NM_207036.1
c.390+1G>T
c.1491dup
c.1528dup
B
Relative TCF12 mRNA level (normalized to GAPDH)
p=0.0344
Control
Family IV (c.390+1G>T)
C
Relative TCF12 mRNA level (normalized to GAPDH)
ns
p=0.006
p=0.005
Control
Family III (c.1491dup)
Family I (c.1528dup)
Family I (+/+)
D
Family I (+/+)
Family I (c.1528dup)
Family III (c.1491dup)
Family IV (c.390+1G>T)
kDa
Anti-TCF12
Anti-GAPDH
100
40
E
P Vs Family I (+/+)
0.042
0.004
<0.0001
Relative change in TCF12 level (A.U) Normalized to GAPDH
F
Anti-TCF12
260
140
100
70
55
40
35
25
15
10

Figure S1

Fig. S1. Individuals with *TCF12* variants have significantly reduced *TCF12* mRNA abundance. (A) Schematic of the human *TCF12* locus (GRCh37/hg19, chr15:57210833-57580714; GenBank ID: NM_207036.1). White boxes, untranslated regions; red boxes, coding exons; black lines, introns; blue stars, variants identified in cases; green or blue triangles, primers used to for qPCR of regions encompassing exons 6 and 17, respectively. (B and C) qPCR data from lymphoblastoid cell lines (LCL) derived from KS cases: Patient 1 (Family I), Patient 4 (Family III) and Patient 5 (Family IV), an unaffected family member (Family I; cousin of Patient 1 and sister of Patient 2), or an unrelated matched control. *TCF12* transcript levels are significantly reduced in individuals with LOF mutations (35-65% reduction vs control), indicating degradation of mutant mRNA. ns, not significant. Error bars, standard deviation of the mean. (D) Western blot was performed on whole-cell protein extracted from LCLs derived from KS cases in three independent biological replicates. For each sample 50 μg lysate was immunoblotted and anti-TCF12 and anti-GAPDH antibodies were used for detection, respectively. Expected molecular weight of TCF12 proteins are: WT, 73 kDa; Mutant in Family I, 55 kDa; Mutant in Family III, 54 kDa; and Mutant in Family IV, 14 kDa (assuming exon 6 exclusion, but likely would not be detectable with this TCF12 antibody). (E) Quantification of Western signal and normalization to GAPDH indicates a significant reduction in TCF12 protein abundance in samples from affected individuals compared to an unaffected individual (Family I +/+). Statistical differences were calculated using student's t-test; Error bars indicate the standard error of mean. (F) Full gel images for TCF12 immunoblot show trace amounts of putative truncated protein for Family I and Family III (red stars). Left, 250 seconds of exposure; right, 250 seconds of exposure with increased image contrast.

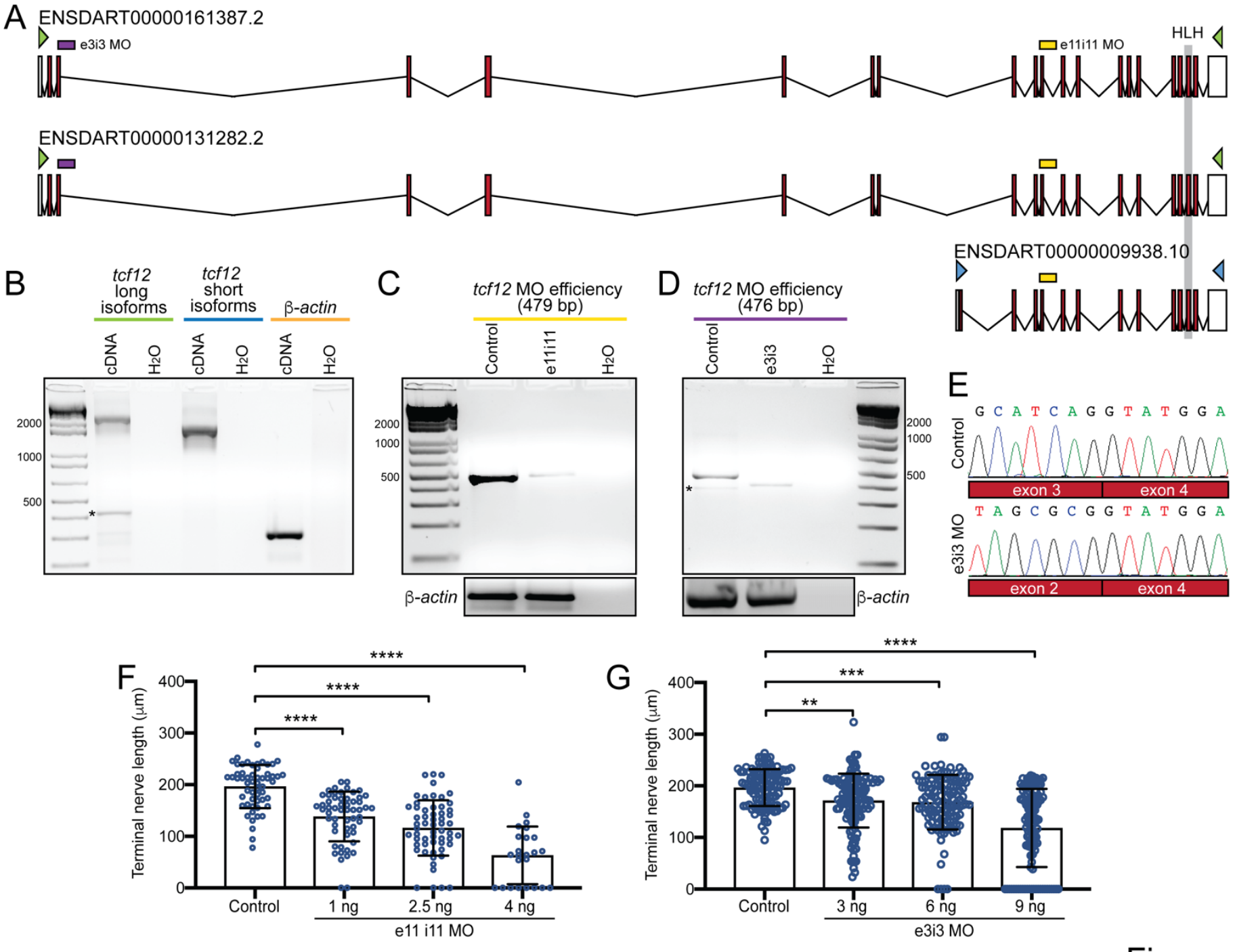

A
ENSDART00000161387.2
e3i3 MO
e11i11 MO
HLH
ENSDART00000131282.2
ENSDART00000009938.10
B
tcf12 long isoforms
tcf12 short isoforms
β-actin
cDNA
H2O
2000
1000
500
C
tcf12 MO efficiency (479 bp)
Control
e11i11
β-actin
D
tcf12 MO efficiency (476 bp)
e3i3
E
G C A T C A G G T A T G G A
Control
exon 3
exon 4
T A G C G C G G T A T G G A
e3i3 MO
exon 2
exon 4
F
Terminal nerve length (μm)
400
300
200
100
0
****
Control
1 ng
2.5 ng
4 ng
e11 i11 MO
G
***
**
3 ng
6 ng
9 ng
e3i3 MO

Figure S2

Fig. S2. Characterization of *D. rerio tcf12* transcript isoforms and transient suppression reagents. (A) Schematic of the zebrafish *tcf12* locus at chr7: 52,241,636-52,434,865 (GRCz10) depicting three different splice isoforms detected by RT-PCR in zebrafish embryos at 1 day post-fertilization (dpf). Red boxes, exons; white boxes, untranslated regions; black lines, introns. Triangles indicate primers to generate PCR products shown in panel B (green, long isoforms; blue, short isoforms, respectively). Morpholinos (MO) targeting splice donor sites are indicated with purple (e3i3) and yellow (e11i11) rectangles. Gray shaded exon indicates the sequence encoding the helix-loop-helix (HLH) domain. (B) RT-PCR products amplified from the cDNA of 1 dpf whole embryo cDNA. Colored labels correspond to primers used in panel A; amplicons were purified and sequenced and shown to correspond to Ensembl accession ID numbers shown in panel A; (*) is a non-specific PCR product. (C and D) MO efficiency was determined by injecting 6 ng of either e11i11 (C) or e3i3 MO (D) into embryo batches, followed by RT-PCR of flanking regions on cDNA generated from 1 dpf morphant or uninjected control embryos. (*) is a non-specific PCR product in panel D. (E) Chromatograms show that the e3i3 morphant RT-PCR product results in exclusion of exon 3 (73 bp) resulting in a frameshift and premature termination of transcripts ENSDART00000161387.2 and ENSDART00000131282.2. (*F* and *G*) Dose-dependent response of e11i11 or e3i3 MOs as indicated by measurement of terminal nerve axon length in 2 dpf embryos immunostained with anti-GnRH antibody. (****), $p<0.0001$; (***), $p<0.001$; (**), $p<0.01$. Statistical differences were calculated using a nonparametric Kruskal-Wallis test; error bars represent standard deviation (s.d.).

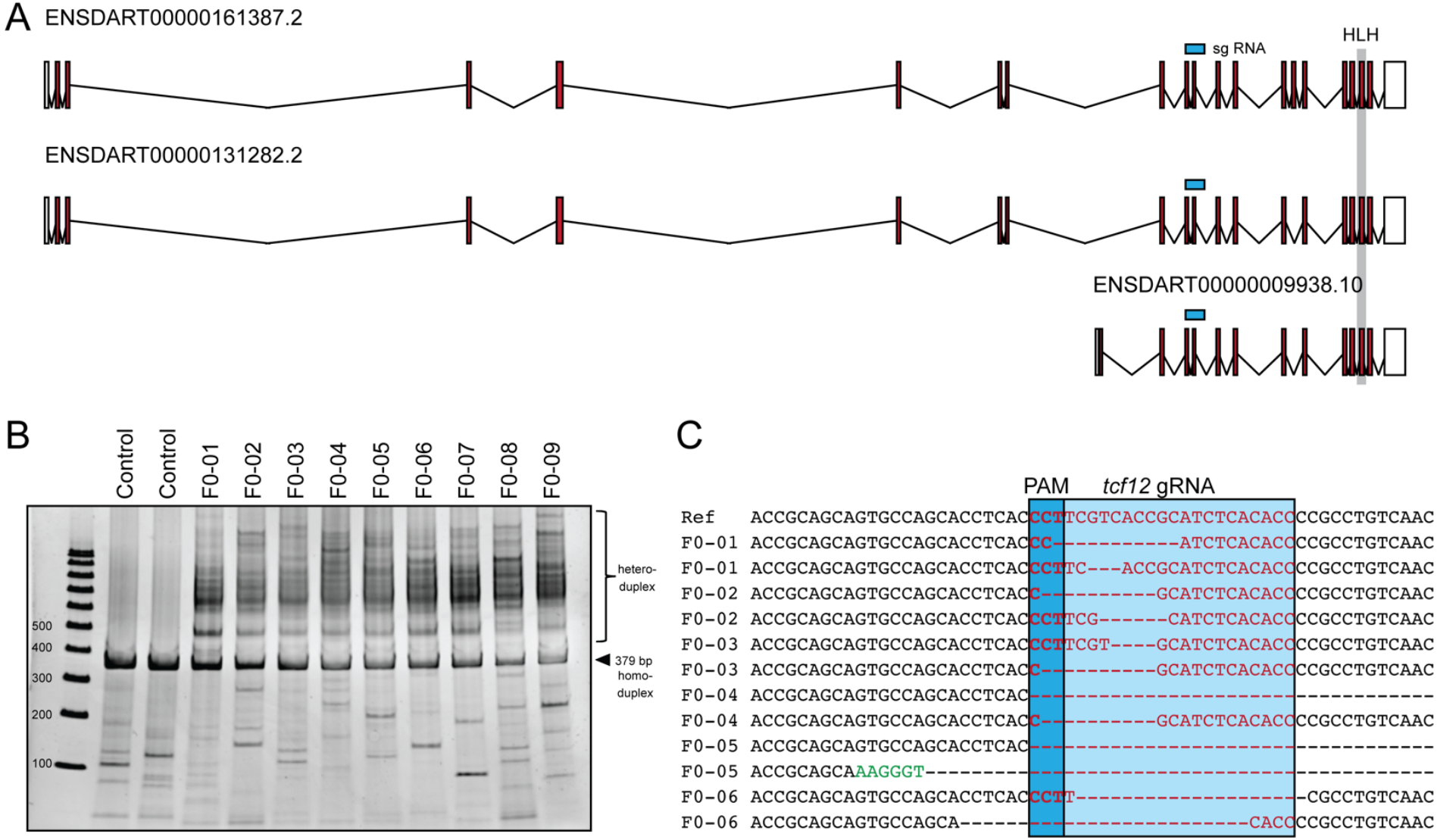
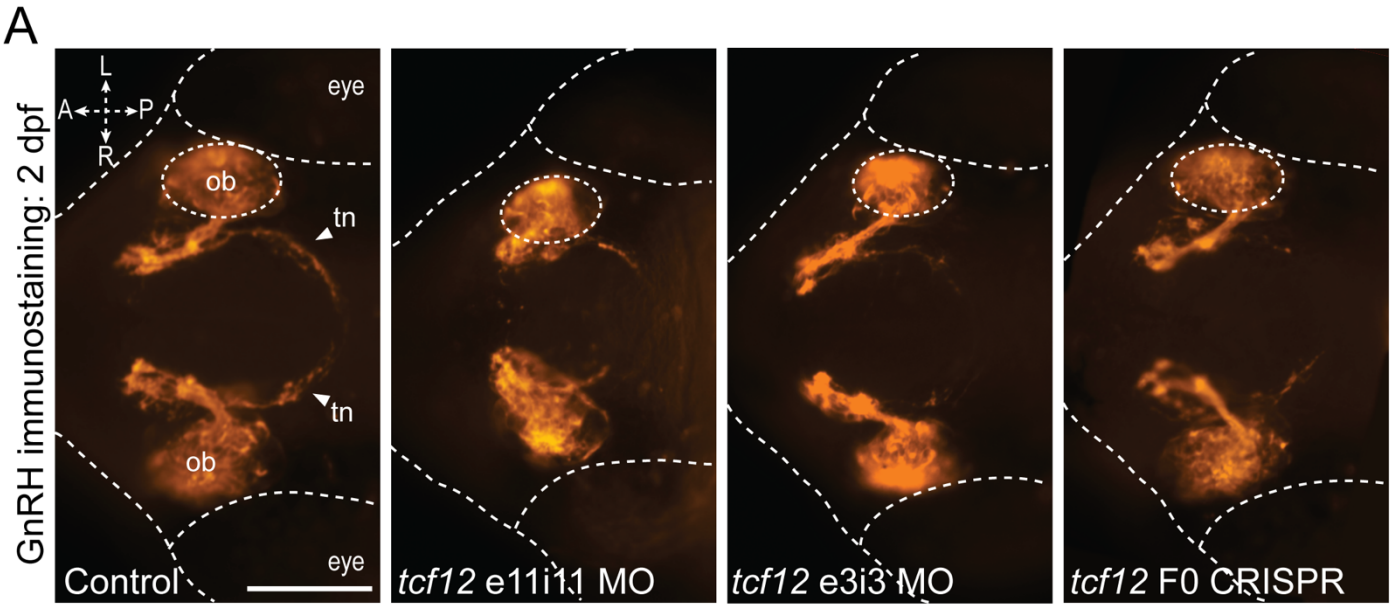

A
ENSDART00000161387.2
sg RNA
HLH
ENSDART00000131282.2
ENSDART00000009938.10
B
Control
Control
F0-01
F0-02
F0-03
F0-04
F0-05
F0-06
F0-07
F0-08
F0-09
500
400
300
200
100
hetero-duplex
379 bp homo-duplex
C
PAM
tcf12 gRNA
Ref ACCGCAGCAGTGCCAGCACCTCACCCTTCGTCACCGCATCTCACACCCCGCCTGTCAAC
F0-01 ACCGCAGCAGTGCCAGCACCTCACCC----------ATCTCACACCCCGCCTGTCAAC
F0-01 ACCGCAGCAGTGCCAGCACCTCACCCTTC---ACCGCATCTCACACCCCGCCTGTCAAC
F0-02 ACCGCAGCAGTGCCAGCACCTCACC----------GCATCTCACACCCCGCCTGTCAAC
F0-02 ACCGCAGCAGTGCCAGCACCTCACCCTTCG------CATCTCACACCCCGCCTGTCAAC
F0-03 ACCGCAGCAGTGCCAGCACCTCACCCTTCGT----GCATCTCACACCCCGCCTGTCAAC
F0-03 ACCGCAGCAGTGCCAGCACCTCACC----------GCATCTCACACCCCGCCTGTCAAC
F0-04 ACCGCAGCAGTGCCAGCACCTCAC----------
F0-04 ACCGCAGCAGTGCCAGCACCTCACC----------GCATCTCACACCCCGCCTGTCAAC
F0-05 ACCGCAGCAGTGCCAGCACCTCAC----------
F0-05 ACCGCAGCAAAGGGT----------
F0-06 ACCGCAGCAGTGCCAGCACCTCACCCTT----------CGCCTGTCAAC
F0-06 ACCGCAGCAGTGCCAGCA----------CACCCCGCCTGTCAAC

Figure S3

Fig. S3. CRISPR/Cas9 genome-editing of the zebrafish *tcf12* locus. (A) Schematic of the zebrafish *tcf12* locus at chr7: 52,241,636-52,434,865 (GRCz10) depicting three different splice isoforms detected by RT-PCR in zebrafish embryos at 1 day post-fertilization (dpf). See Fig. S2 for details. Red boxes, exons; white boxes, untranslated regions; black lines, introns. Gray shaded exon indicates the sequence encoding the helix-loop-helix (HLH) domain. Blue rectangle indicates single guide RNA (gRNA) target site on exon 11 (according to isoform ENSDART00000161387.2). (B) Heteroduplex analysis of PCR product encompassing the *tcf12* gRNA target site indicate that all embryos injected are targeted by CRISPR/Cas9. Polyacrylamide gel is shown; uninjected controls do not display heteroduplexes, whereas heteroduplexes are present in nine different F0 embryos harvested at 2 days post-fertilization (dpf). (C) Representative sequences from cloned PCR fragments flanking the *tcf12* gRNA target that were obtained from 6 different F0 embryos. gRNA target is indicated with red text and blue rectangle; PAM, protospacer adjacent motif; green text, insertion. 12 clones were sequenced per embryo to result in an estimated 46% mosaicism.


A
GnRH immunostaining: 2 dpf
L
A
P
R
eye
ob
tn
tn
ob
eye
Control
tcf12 e11i11 MO
tcf12 e3i3 MO
tcf12 F0 CRISPR

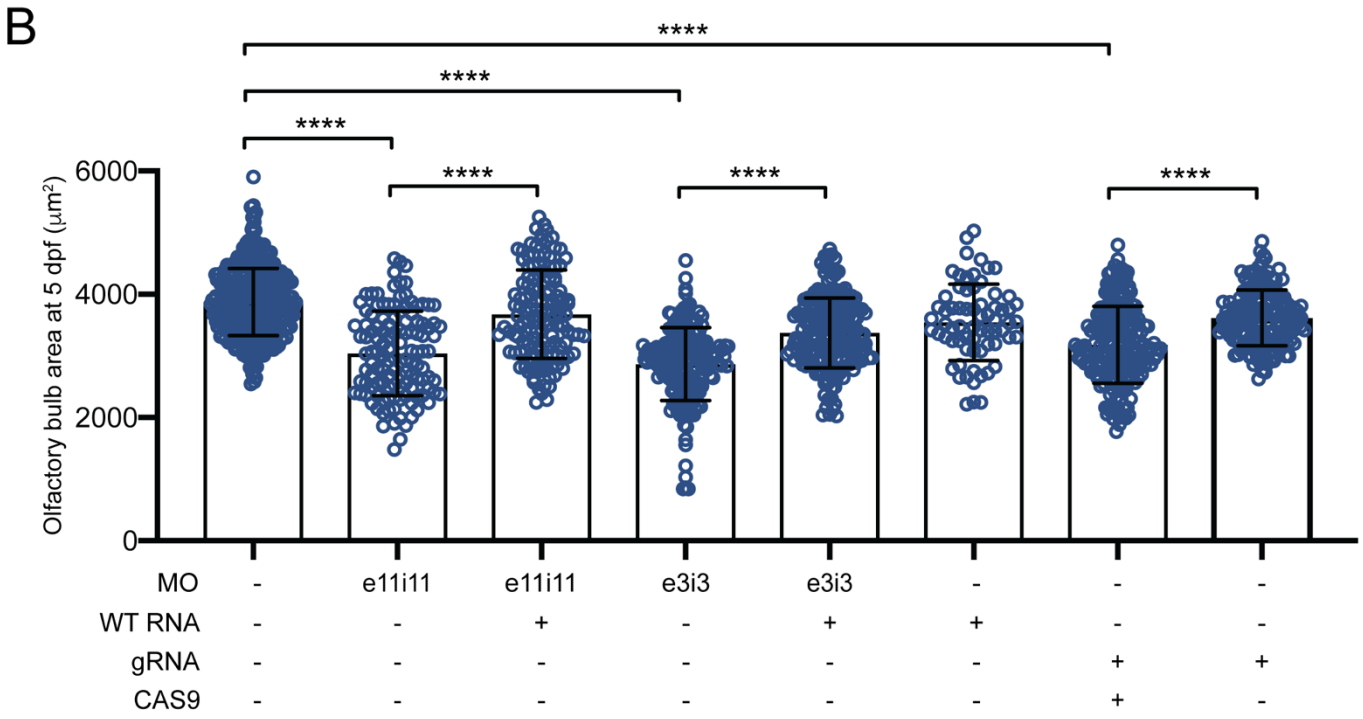
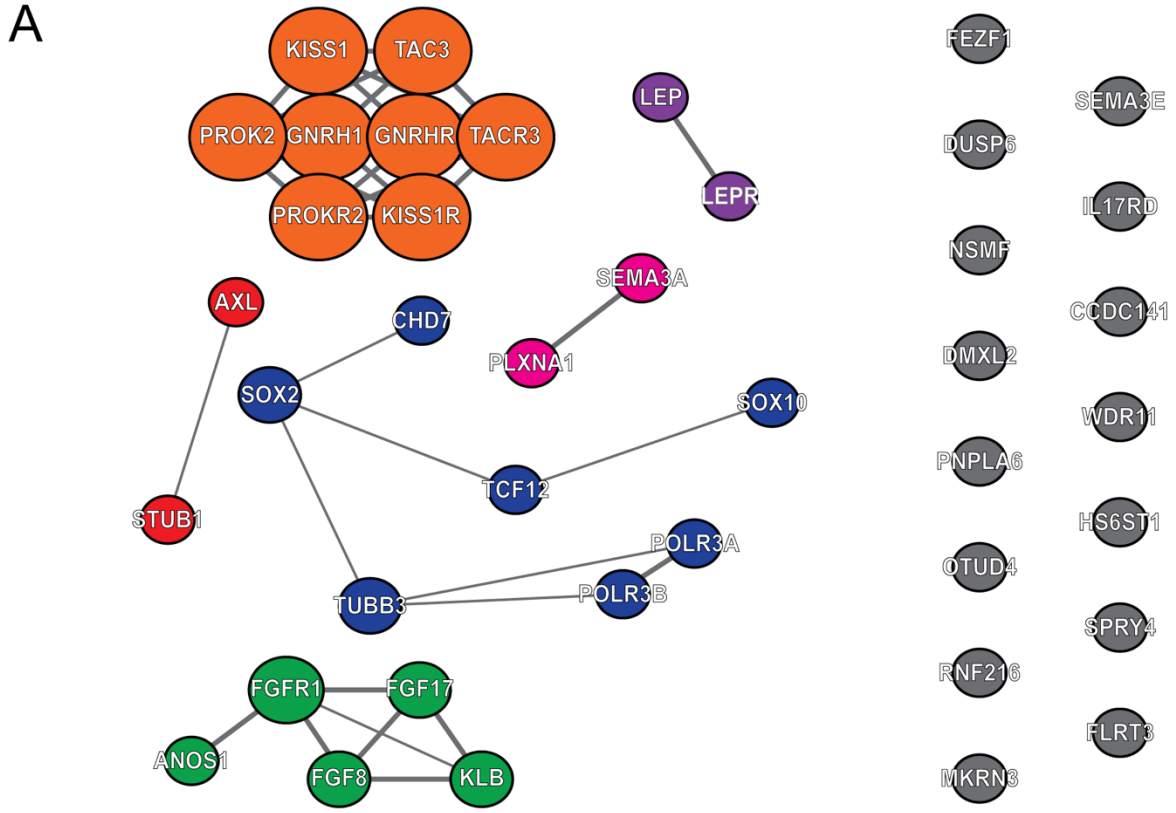

B
****
****
****
****
****
****
Olfactory bulb area at 5 dpf (μm²)
6000
4000
2000
0
MO - e11i11 e11i11 e3i3 e3i3 - - -
WT RNA - - + - + + - -
gRNA - - - - - - + +
CAS9 - - - - - - + -

Figure S4

Fig. S4. *Tcf12* morphants and F0 mutants display a reduction in olfactory bulb size. (A) Representative ventral views of fluorescence immunostained (anti-GnRH antibody) *tcf12* morphants (MO) or F0 CRISPR mutant embryos at 2 days post-fertilization (dpf). Dashed ovals indicate olfactory bulb area that was measured. Abbreviations: ob, olfactory bulb; tn, terminal nerve; A, anterior; P, posterior; L, left; R, right; scale bar 100 μm, equivalent scale across panels. (B) Measurement of olfactory bulb area in GnRH antibody stained embryos. Statistically significant differences were calculated using a nonparametric Kruskal-Wallis test; (****), $p<0.0001$; error bars represent standard deviation (s.d.). n=34-196 embryos per injection batch.


A
KISS1
TAC3
PROK2
GNRH1
GNRHR
TACR3
PROKR2
KISS1R
LEP
LEPR
SEMA3A
PLXNA1
AXL
CHD7
SOX2
SOX10
TCF12
STUB1
POLR3A
POLR3B
TUBB3
FGFR1
FGF17
ANOS1
FGF8
KLB
FEZF1
DUSP6
NSMF
DMXL2
PNPLA6
OTUD4
RNF216
MKRN3
SEMA3E
IL17RD
CCDC141
WDR11
HS6ST1
SPRY4
FLRT3

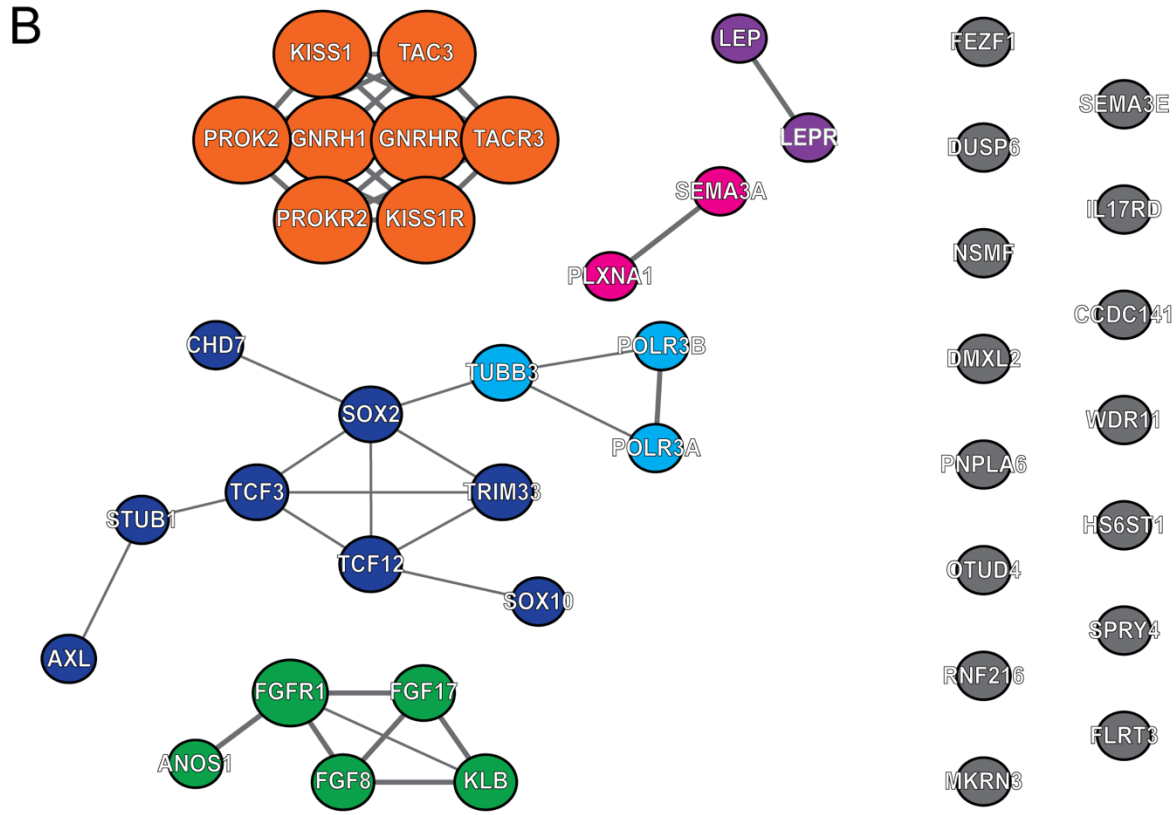

B
KISS1
TAC3
PROK2
GNRH1
GNRHR
TACR3
PROKR2
KISS1R
LEP
LEPR
SEMA3A
PLXNA1
CHD7
TUBB3
POLR3B
SOX2
POLR3A
TCF3
TRIM33
STUB1
TCF12
SOX10
AXL
FGFR1
FGF17
ANOS1
FGF8
KLB
FEZF1
DUSP6
NSMF
DMXL2
PNPLA6
OTUD4
RNF216
MKRN3
SEMA3E
IL17RD
CCDC141
WDR11
HS6ST1
SPRY4
FLRT3

Figure S5

Fig. S5. Schematic of the TCF12 protein-protein interaction network according to GeNets. (A) Network generated with 40 IGD genes (Table S5). (B) Network generated with 40 IGD genes plus two TCF12 interactors identified with an integrative multi-species algorithm (TCF3 and TRIM33; see Fig. S6). Similar colored nodes indicate protein communities more likely to interact with one another than with genes in other modules. Gray circles indicate proteins that are not associated with any community.

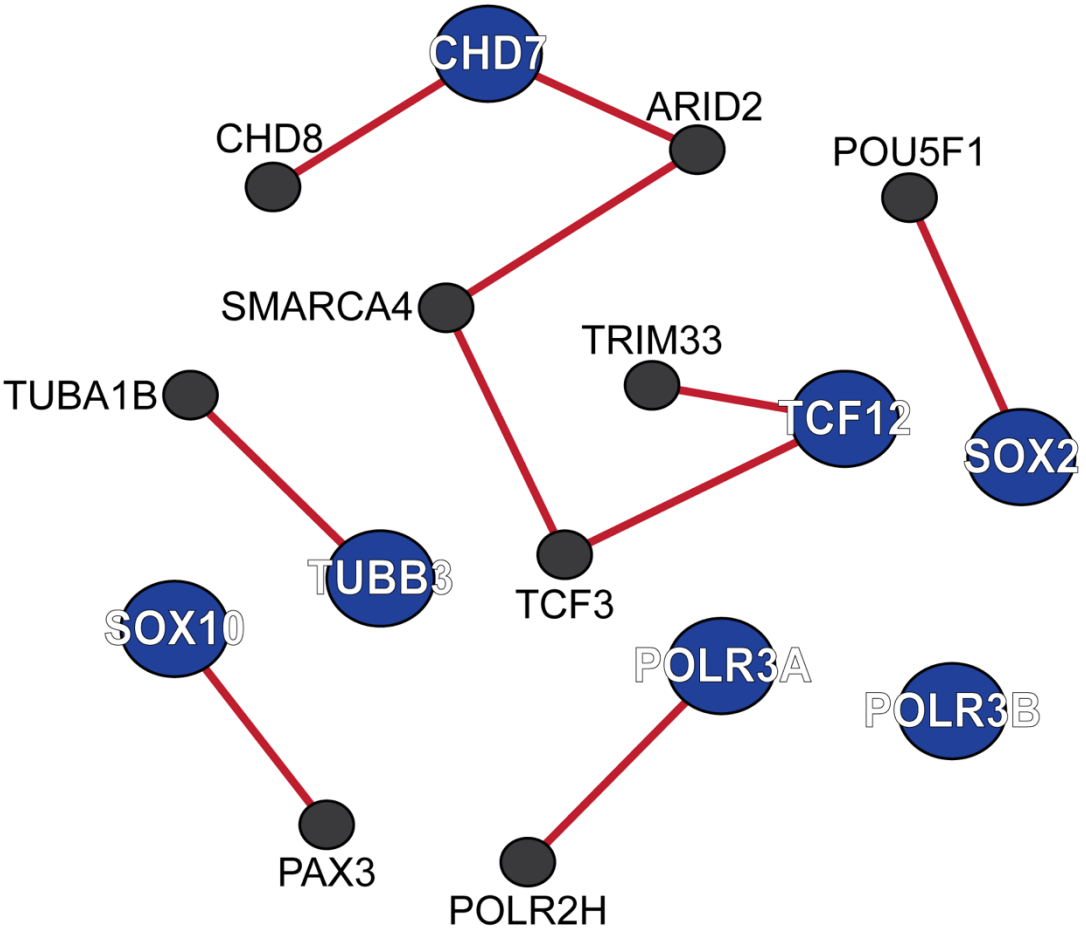

CHD7
ARID2
CHD8
POU5F1
SMARCA4
TRIM33
TUBA1B
TCF12
SOX2
TUBB3
TCF3
SOX10
POLR3A
POLR3B
PAX3
POLR2H

Figure S6

Fig. S6. Expanded TCF12 affinity network predicted by an integrative multi-species prediction algorithm (IMP). We applied predicted IGD genes in the TCF12 protein community identified by GeNets (blue circles; Fig. S5A) to IMP and identified two additional direct interactors, TCF3 and TRIM33.

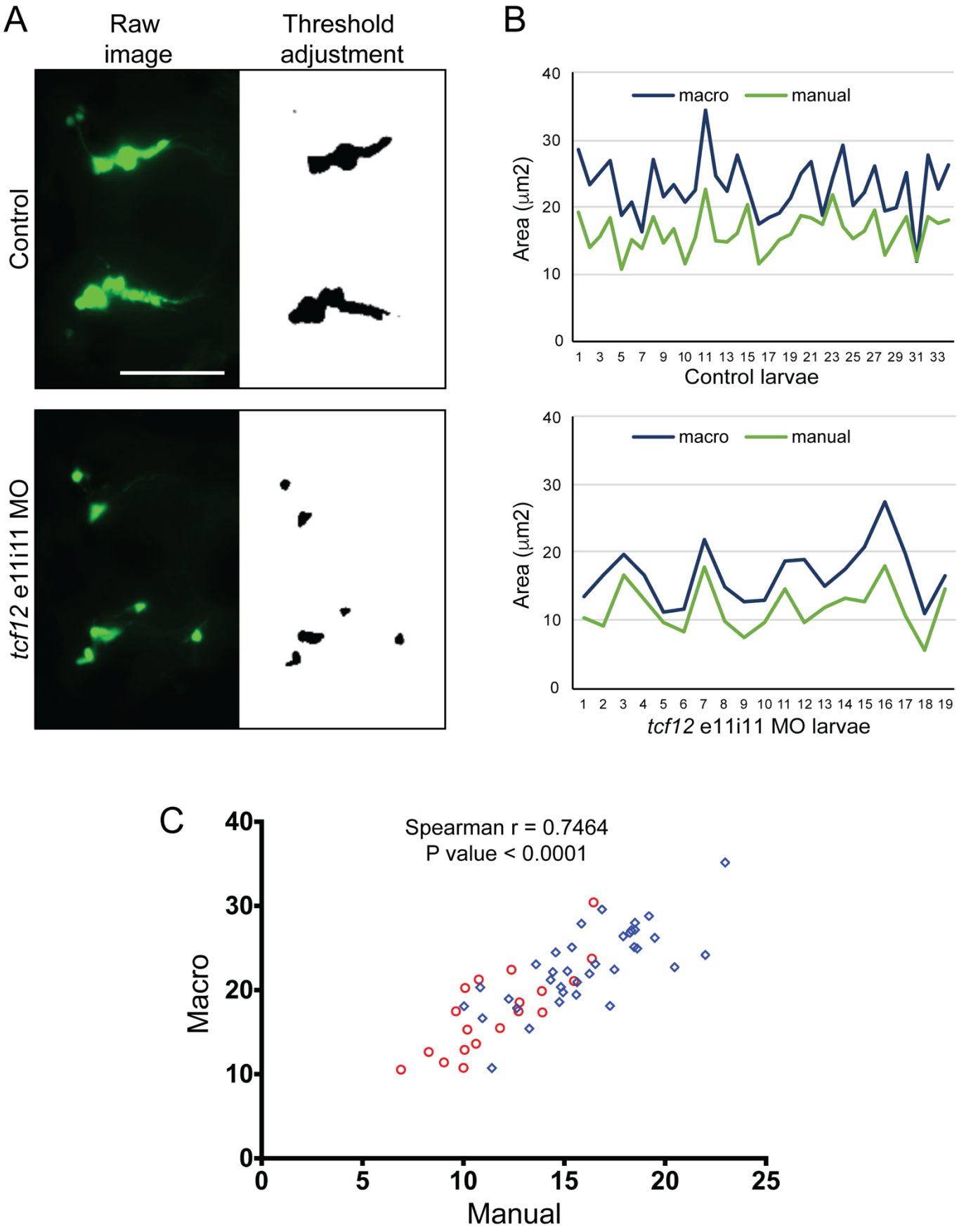

A
Raw image
Threshold adjustment
Control
tcf12 e11i11 MO
B
macro
manual
Area (µm2)
Control larvae
tcf12 e11i11 MO larvae
C
Spearman r = 0.7464
P value < 0.0001
Macro
Manual

Figure S7

Fig. S7. Development of an automated image measurement paradigm for gnrh3:egfp neuronal patterning. (A) Representative dorsal images acquired from *tg(gnrh3:egfp)* larvae at 5 dpf using the VAST Bioimager. Left, raw fluorescence image; right, post-processing image with ImageJ (NIH) threshold adjustment; scale bar 100 μm, equivalent scale across panels. (B) Comparison of manual measurement versus automated measurement using the ImageJ macro plotted for individual larvae across two different experimental conditions; n=34 control larvae and 20 control larvae. (C) Correlation between two different measurement paradigms using Spearman's rank correlation. Blue diamonds, controls; red circles, *tcf12* morphants.

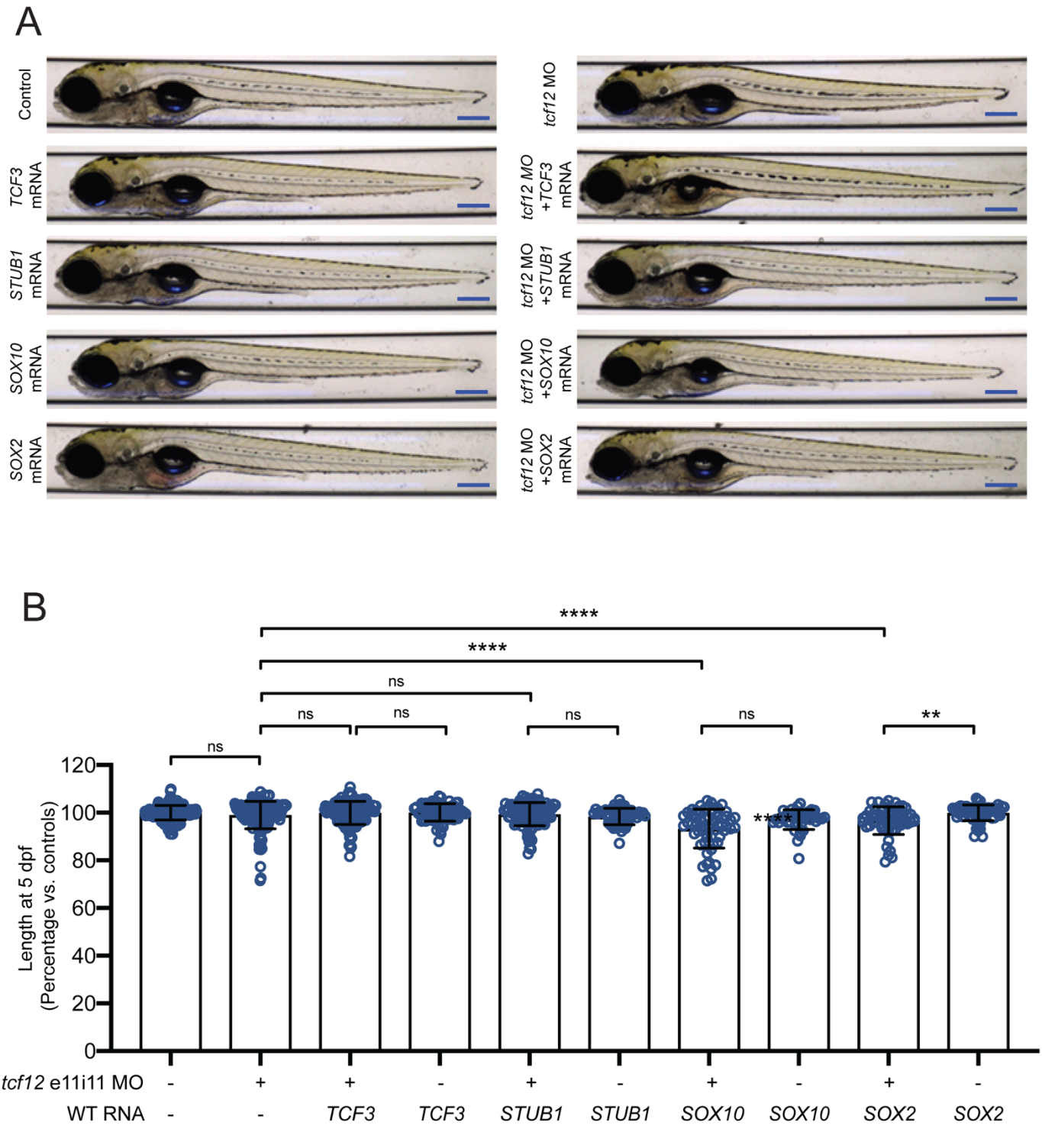

A
Control
TCF3 mRNA
STUB1 mRNA
SOX10 mRNA
SOX2 mRNA
tcf12 MO
tcf12 MO +TCF3 mRNA
tcf12 MO +STUB1 mRNA
tcf12 MO +SOX10 mRNA
tcf12 MO +SOX2 mRNA
B
****
****
ns
ns
ns
ns
ns
ns
**
****
Length at 5 dpf (Percentage vs. controls)
120
100
80
60
40
20
0
tcf12 e11i11 MO - + + - + - + - + -
WT RNA - - TCF3 TCF3 STUB1 STUB1 SOX10 SOX10 SOX2 SOX2

Figure S8

Fig. S8. Zebrafish larvae assessed in TCF12 network studies display minimal overt morphological defects. (A) Representative lateral bright field images acquired from *tg(gnrh3:egfp)* larvae at 5 dpf using the VAST Bioimager on-board camera. Scale bar 300 μm, equivalent scale across panels. (B) Quantification of larval body length represented as a percentage versus the mean of controls for *in vivo* complementation studies of *tcf12* e11i11 MO with WT human mRNA; see Fig. 4c for gnrh3-GFP area measurements. We used FishInspector software to measure larval length; ns, not significant; ****p<0.0001; **p<0.01 (nonparametric Kruskal-Wallis test); error bars represent standard deviation (s.d.). n=44-189 larvae/batch, repeated.

Table S1. Rare sequence variants (RSV)s shared between the affected male cousins in Family I.

Gene	Cytogenetic location	Protein name	UniprotKB	OMIM ID	Phenotype in OMIM	hg19 coordinate	Transcript (GenBank / ENSEMBL)	Nucleotide change	Protein change	Frequency in controls (ExAC)	pLI score	Missense z-score
SLC12A4	16q22.1	Solute carrier family 12 (Potassium/ chloride transporter), member 4	Q9UP95	604119	NA	chr16: 67979422	NM_005072.4	c.2882G>A	p.Arg961Gln	0.000182 (22/120872 alleles)	0	1.81
SRCIN1	17q12	SRC kinase signaling inhibitor 1	Q9C0H9	610786	NA	chr17: 36704828	NM_025248.2	c.3235G>A	p.Glu1079Lys	0.0005985 (72/120310 alleles)	1.00	4.43
FOSB	19q13.32	V-fos FBJ murine osteosarcoma viral oncogene homolog B	P53539	164772	NA	chr19: 45974273	ENST00000590335.1	c.513G>A	p.Met171Ile	0	0.5	2.81
MUC16	19p13.2	Mucin 16	Q8WXI7	606154	NA	chr19: 9064390	NM_024690.2	c.23056C>T	p.Pro7686Ser	0.00003351 (4/119366 alleles)	NA	NA
TCF12	15q21.3	Transcription factor 12	Q99081	600480	NA	chr15: 57554351	NM_207036.1	c.1528dup	p.Thr510Asnfs*12	0	0.97	0.35
WIF1	12q14.3	WNT inhibitory factor 1	Q9Y5W5	605186	NA	chr12: 65461473	NM_007191.4	c.634+2T>A	NA	0	0	-0.16

Abbreviations: NA, Not available.

Table S2. Rare sequence variants (RSV)s identified in our KS cohort with stringent exome filtering criteria (pLI >0.09 or missense Z-score >3; absent from ExAC).

Gene	Cytogenetic location	Protein name	Uniprot KB	OMIM ID	Phenotype in OMIM	hg19 coordinate	Transcript (GenBank / ENSEMBL)	Nucleotide change	Protein change	Frequency in controls (ExAC)	pLI score	Missense z-score
Family I, Individual 1												
NPR2	19p.13.3	Atrial natriuretic peptide receptor 2	P20594	108961	Acromesomelic dysplasia, Maroteaux type; Epiphyseal chondrodysplasia, Miura type; Short stature with nonspecific skeletal abnormalities	chr9: 35802582	ENST00000342694	c.2048 T>C	p.Tyr598Cys	0	0.99	4.22
ZBTB20	3q13.31	Zinc finger and BTB domain-containing protein 20	Q9HC78	606025	Primrose syndrome	chr3: 114099124	ENST00000474710 *	c.139del	p.Ala47Pro fs*9	0	0.98	5.72
MKRN1	7q34	E3 ubiquitin-protein ligase makorin-1	Q9UHC 7	607754	NA	chr7: 140159508	ENST00000494939	c.351dup	p.Thr118Asp fs*7	0	0.98	1.23
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57554351	NM_207036.1	c.1528dup	p.Thr510Asnf s*12	0	0.97	0.35
Family I, Individual 2												
TLN1	9p13.3	Talin-1	Q9Y490	186745	NA	chr9: 35707068	ENST00000314888	c.4955+1 G>T	Splice Variant	0	1	5.13
TMEM57	1p36.11	Macoilin	Q8N5G 2	610301	NA	chr1: 25812190	ENST00000399763	c.326G>A	p.Arg109Gln	0	0.99	3.72
PLEKHA6	1q32.1	Pleckstrin homology domain-containing family A member 6	Q9Y2H 5	607771	NA	chr1: 204230579	ENST00000414478 *	c.439C>T	p.Gln147*	0	0.98	-0.74
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57554351	ENST00000559703	c.1528dup	p.Thr510Asnf s*12	0	0.97	0.35
Family II, Individual 3												
SEMA6B	19p13.3	Semaphorin-6B	Q9H3T3	608873	NA	chr19	ENST00000586965	c.538C>G	p.His180Ala	0	0.74	3.41

TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15	ENST00000559703	c.1490_1491del	p.Ser497Cysfs*12	0	0.97	0.35
Family III, Individual 4												
HERC1	15q22.31	Probable E3 ubiquitin-protein ligase HERC1	Q15751	605109	Macrocephaly, dysmorphic facies, and psychomotor retardation	chr15: 63954109	ENST00000443617	c.9013A>G	p.Ser3005Gly	0	1	3.82
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57554314	ENST00000559703	c.1491dup	p.Val498Cysfs*12	0	0.97	0.34
Family IV, Individual 5												
PITPNM2	12q24.31	Membrane-associated phosphatidylinositol transfer protein 2	Q9BZ72	608920	NA	chr12: 123475208	ENST00000542749	c.2453C>T	p.Pro818Leu	0	0.99	4.67
ANAPC2	9q34.3	Anaphase-promoting complex subunit 2	Q9UJX6	606946	NA	chr9: 140076270	ENST00000323927	c.1331G>C	p.Gly444Ala	0	0.98	3.51
MAP2K7	19p13.2	Dual specificity mitogen-activated protein kinase kinase 7	O14733	603014	NA	chr19: 7968949	ENST00000545011*	c.120G>T	p.Arg40Ser	0	0.98	3.55
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57458665	ENST00000557947	c.390+1G>T	Splice Variant	0	0.97	0.34
Family V, Individual 6												
B3GAT1	11q25	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 1	Q9P2W7	151290	NA	chr11: 134253872	ENST00000524765	c.323C>T	p.Thr108Met	0	0.79	3.27
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57543622	ENST00000559703	c.1188+1G>A	Splice Variant	0	0.97	0.35
Family VI, Individual 7												
KIAA2018	3q13.2	Basic helix-loop-helix domain-containing protein USF3	A0A1W1GCQ4	617568	NA	chr3: 113378610	ENST00000478658	c.1918_1919dup	p.Pro640Hisfs*8	0	1	-1.2
DDX3X	Xp11.4	ATP-dependent RNA helicase DDX3X	O00571	300160	Mental retardation, X-linked 102	chrX: 41193929	ENST00000542215*	c.84G>T	p.Trp28Cys	0	1	5.13

CHMP7	8p21.3	Charged multivesicular body protein 7	Q8WUX9	611130	NA	chr8: 23112839	ENST00000397677	c.551del	p.Ser184Thrfs*38	0	0.98	2.22
REPS2	Xp22.2	RalBP1-associated Eps domain-containing protein 2	Q8NFH8	300317	NA	chrX: 16965152	ENST00000357277	c.168_169dup	p.Glu59Argfs*19	0	0.97	1.23
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57545652	ENST00000559703	c.1453C>T	p.Arg485*	0	0.97	0.35
Family VII, Individual 8												
DSCAM	21q22.2	DS Cell Adhesion Molecule	O60469	602523	NA	chr21: 41416097	ENST00000400454	c.5291T>C	p.Leu1764Pro	0	1	4.35
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57545467	ENST00000559703	c.1270dup	p.Met424Asnfs*10	0	0.97	0.35
Family VIII, Individual 9												
ACAN	15q26.1	Aggrecan core protein	P16112	155760	?Spondyloepimetaphyseal dysplasia, aggrecan type; ?Spondyloepiphyseal dysplasia, Kimberley type; Short stature and advanced bone age, with or without early-onset osteoarthritis and/or osteochondritis dissecans	chr15: 89388814	ENST00000559004	c.1130G>A	p.Trp377*	0	1	1.03
CNTN1	12q12	Contactin-1	Q12860	600016	?Myopathy, congenital, Compton-North	chr12: 41422964	ENST00000551295	c.2923G>T	p.Glu975*	0	1	1.25
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57543567	ENST00000559703	c.1136dup	p.Gly380Trpfs*11	0	0.97	0.35
Family IX, Individual 10												
INTS3	1q21.3	Integrator complex subunit 3	Q68E01	611347	NA	chr1: 153744392	ENST00000435409	c.2673_2674dup	p.Ser893Valfs*20	0	1	5.19
JARID2	6p22.3	Protein Jumonji	Q92833	601594	NA	chr6: 15487747	ENST00000397311	c.364C>T	p.Arg122Trp	0	1	3.7

KCNQ5	6q13	Potassium voltage-gated channel subfamily KQT member 5	Q9NR82	607357	Mental retardation, autosomal dominant 46	chr6: 73332001	ENST00000370392	c.84_85dup	p.Arg31Alafs*69	0	1	4.62
PLXNB1	3p21.31	Plexin-B1	O43157	601053	NA	chr3: 48453998	ENST00000358536	c.4885_4886dup	p.Phe1629Serfs*22	0	0.85	3.07
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57535737	ENST00000537840	c.1103C>G	p.Ser368*	0	0.97	0.35
Family X, Individual 11												
SLC30A10	1q41	Zinc transporter 10	Q6XR72	611146	Hypermanganes-emia with dystonia 1	chr1:220101381	ENST00000366926	c.401del	p.Phe134Serfs*58	0	0.97	-0.56
PRR23D1	8p23.1	Proline-rich protein 23D1	E9PI22	NA	NA	chr8:73992599	ENST00000533250	c.697A>G	p.Ser233Gly	0	0.42	3.22
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15:57524615	ENST00000537840	c.812C>G	p.Ser271*	0	0.97	0.35
Family XI, Individual 12												
KALRN	3q21.1-q21.2	Kalirin RhoGEF kinase	O60229	604605	NA	chr3: 124180798	ENST00000240874	c.3910T>G	p.Leu1304Val	0	1	4.67
TENM2	5q34	Teneurin transmembrane protein 2	Q9NT68	610119	NA	chr5: 167419969	ENST00000403607	c.968C>A	p.Ser323Tyr	0	1	3.4
TCF12	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57525003	ENST00000537840	c.920dup	p.Tyr301*	0	0.97	0.35
ZNF236	18q23	Zinc finger protein 236	Q9UL36	604760	NA	chr18: 74625740	ENST00000253159	c.2941C>T	p.His981Tyr	0	1	4.89
KCNG1	20q13.13	Potassium voltage-gated channel modifier subfamily G member 1	Q9UIX4	603788	NA	chr20: 49626733	ENST00000371571	c.143C>T	p.Pro48Leu	0	0.27	5.81
PC	11q13.2	Pyruvate carboxylase	P11498	608786	Pyruvate Carboxylase Deficiency	chr11: 66639539	ENST00000393960	c.92G>T	p.Arg31Leu	0	0.72	4.05
Family XIII, Individual 14												
CUL9	6p21.1	Cullin-9	Q8IWT3	607489	NA	chr6: 43191839	NM_015089.4	c.7291C>G	p.Arg2431Gly	0	0.97	2.9

DNM1	9q34.11	Dynamin-1	Q05193	602377	Epileptic encephalopathy, early infantile, 31, Autosomal dominant	chr9: 130982483	ENST00000372923.3	c.712A>G	p.Ser238Gly	0	1	5.97
CACNA1C	12p13.33	Voltage-dependent L-type calcium channel subunit alpha-1C	Q13936	114205	Timothy syndrome, Autosomal dominant; Long QT syndrome 8; Brugada syndrome 3	chr12: 2800073	NM_199460.3	c.6374T>C	p.Ile2125Thr	0	1	6.41
ZFHX2	14q11.2	Zinc finger homeobox protein 2	Q9C0A1	617828	Marsili syndrome	chr14: 24000703	NM_033400.3	c.2787G>T	p.Gln929His	0	1	2.95
*TCF12***	15q21.3	Transcription factor 12	Q99081	600480	Craniosynostosis 3	chr15: 57484409	NM_207036.2	c.445del	p.Ser149Glnfs*96	0	0.97	0.35

Abbreviations: NA, Not available. *Variant impacts a non-canonical transcript. **In homozygosity

Table S3. Loss of function *TCF12* variants identified in the KS cohort.

Family	Individual	hg19 coordinates	Gene	Ref allele	Alt allele	Nucleotide change	Protein change	Exon	Zygosity
I	1 and 2	chr15:57554351-57554351	*TCF12*	T	TA	c.1528dup	p.Thr510Asnfs*12	17	het
II	3	chr15:57554313-57554314	*TCF12*	ACT	A	c.1490_1491del	p.Ser497Cysfs*12	17	het
III	4	chr15:57554314-57554314	*TCF12*	C	CT	c.1491dup	p.Val498Cysfs*12	17	het
IV	5	chr15:57458665-57458665	*TCF12*	G	T	c.390+1G>T	NA	6	het
V	6	chr15:57543622-57543622	*TCF12*	G	A	c.1188+1G>A	NA	14	het
VI	7	chr15:57545652-57545652	*TCF12*	C	T	c.1453C>T	p.Arg485*	16	het
VII	8	chr15:57545469-57545469	*TCF12*	A	AA	c.1270dup	p.Met424Asnfs*10	16	het
VIII	9	chr15:57543569-57543569	*TCF12*	C	CC	c.1136dup	p.Gly380Trpfs*11	14	het
IX	10	chr15:57535737-57535737	*TCF12*	C	G	c.1103C>G	p.Ser368*	13	het
X	11	chr15:57524615-57524615	*TCF12*	C	G	c.812C>G	p.Ser271*	10	het
XI	12	chr15:57525003-57525003	*TCF12*	A	AA	c.920dup	p.Tyr307*	11	het
XII	13	chr15:57523366-57523366	*TCF12*	C	CC	c.596dup	p.Asn200Lysfs*4	9	het
XIII	14	chr15:57484409-57484409	*TCF12*	TT	T	c.445del	p.Ser149Glnfs*96	7	hom

Abbreviations: Ref, reference; Alt, alternate; het, heterozygous; hom, homozygous; NA, not available. Variants are named according to GenBank ID: NM_207036.2.

Table S4. Representative zebrafish morphometric data

Injection	Measurement			p-value		
	n	Mean	S.E.M.	vs Control	vs. MO	vs. gRNA alone
GnRH terminal nerve axon length at 2 dpf (μm; 2 measurements/embryo): Fig. 3*C*						
Controls	215	196.00	2.07	-	-	-
2 ng *tcf12* e11i11 MO	79	132.96	5.45	<0.0001	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *TCF12* RNA	79	174.41	3.81	0.0020	<0.0001	-
6 ng *tcf12* e3i3 MO	93	159.43	5.14	<0.0001	-	-
6 ng *tcf12* e3i3 MO + 12.5 pg WT *TCF12* RNA	94	182.13	3.69	0.1246	0.0157	-
12.5pg WT *TCF12* RNA	58	191.68	4.32	>0.999	-	-
100 pg *tcf12* gRNA + 200 pg Cas9	98	162.59	4.23	<0.0001	-	<0.0001
100 pg *tcf12* gRNA	82	195.12	3.37	>0.999	-	-
Area of GFP+ neurons in *gnrh3:gfp* larvae at 5 dpf (μm²): Fig. 3*D*						
Controls	125	23.09	0.88	-	-	-
2 ng *tcf12* e11i11 MO	56	17.93	1.20	<0.0001	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *TCF12* RNA	56	23.09	1.06	>0.999	<0.0001	-
6 ng *tcf12* e3i3 MO	84	17.34	0.71	<0.0001	-	-
6 ng *tcf12* e3i3 MO + 12.5 pg WT *TCF12* RNA	81	19.97	0.70	>0.999	0.0380	-
12.5pg WT *TCF12* RNA	40	23.09	1.07	>0.999	-	-
100 pg *tcf12* gRNA + 200 pg Cas9	66	18.76	0.55	0.049	-	0.017
100 pg *tcf12* gRNA	67	22.65	0.70	>0.999	-	-
Area of GFP+ neurons in *gnrh3:gfp* larvae at 5 dpf (μm²): Fig. 4*C*						
Controls	224	23.09	0.42	-	-	-
2 ng *tcf12* e11i11 MO	170	13.98	0.72	<0.0001	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *TCF3* RNA	71	15.31	0.78	<0.0001	>0.9999	-
12.5pg WT *TCF3* RNA	43	19.26	1.04	0.0107	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *STUB1* RNA	78	19.68	0.9	0.0050	<0.0001	-
12.5pg WT *STUB1* RNA	72	18.62	0.66	<0.0001	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *SOX10* RNA	57	16.58	1.09	<0.0001	0.3376	-
12.5pg WT *SOX10* RNA	45	19.69	0.9	0.0387	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *SOX2* RNA	58	15.25	1.11	<0.0001	>0.9999	-
12.5pg WT *SOX2* RNA	44	19.89	1.12	0.0347	-	-
GnRH terminal nerve axon length at 2 dpf (μm; 2 measurements/embryo): Fig. S2*F*						
Controls	68	196.44	4.41	-	-	-
1 ng *tcf12* e11i11 MO	28	138.22	6.44	<0.0001	-	-
2.5 ng *tcf12* e11i11 MO	29	116.23	7.03	<0.0001	-	-
4 ng *tcf12* e11i11 MO	24	63.00	11.40	<0.0001	-	-
3 ng *tcf12* e3i3 MO	60	171.40	4.76	0.0006	-	-
6 ng *tcf12* e3i3 MO	59	168.35	4.89	<0.0001	-	-
9 ng *tcf12* e3i3 MO	57	127.12	11.22	<0.0001	-	-
Olfactory bulb area at 2 dpf (μm²; 2 measurements/embryo): Fig. S4*B*						
Controls	196	3875.1	25.57	-	-	-
2 ng *tcf12* e11i11 MO	62	3039.6	61.79	<0.0001	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *TCF12* RNA	69	3676.8	61.44	0.0038	<0.0001	-
6 ng *tcf12* e3i3 MO	93	2867.9	43.35	<0.0001	-	-
6 ng *tcf12* e3i3 MO + 12.5 pg WT *TCF12* RNA	94	3372.4	41.43	<0.0001	<0.0001	-

12.5pg WT *TCF12* RNA	34	3544.1	75.14	0.0014	-	-
100 pg *tcf12* gRNA + 200 pg Cas9	98	3179.0	44.50	<0.0001	-	<0.0001
100 pg *tcf12* gRNA	82	3615.2	35.29	0.0007	-	-
Larval length at 5 dpf (percentage vs control): Fig. S7*B*						
Controls	189	100	1.55	-	-	-
2 ng *tcf12* e11i11 MO	164	99.01	3.15	>0.9999	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *TCF3* RNA	137	99.91	2.93	>0.9999	>0.9999	-
12.5pg WT *TCF3* RNA	69	100.07	3.09	>0.9999	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *STUB1* RNA	110	99.39	3.28	>0.9999	>0.9999	-
12.5pg WT *STUB1* RNA	54	98.37	3.31	0.1323	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *SOX10* RNA	59	93.28	7.47	<0.0001	<0.0001	-
12.5pg WT *SOX10* RNA	61	97.12	3.77	0.0017	-	-
2 ng *tcf12* e11i11 MO + 12.5 pg WT *SOX2* RNA	55	96.64	5.51	0.0012	0.0043189)	-
12.5pg WT *SOX2* RNA	40	99.96	3.65	>0.9999	-	-

Table S5. Established IGD genes used in the GeNets analysis

Gene	Cytogenetic location	Protein name	UniprotKB	OMIM ID	Phenotype in OMIM
AXL	19q13.2	AXL RECEPTOR TYROSINE KINASE	P30530	109135	N/A
CCDC141	2q31.2	COILED-COIL DOMAIN CONTAINING 141	Q6ZP82	616031	N/A
CHD7	8q12.1-q12.2	CHROMODOMAIN HELICASE DNA-BINDING PROTEIN 7	Q9P2D1	608892	Charge Syndrome, Hypogonadotropic hypogonadism 5 with or without anosmia
DMXL2	15q21.2	DMX-LIKE 2	Q8TDJ6	612186	Polyendocrine-polyneuropathy syndrome
DUSP6	12q21.33	DUAL-SPECIFICITY PHOSPHATASE 6	Q16828	602748	Hypogonadotropic hypogonadism 19 with or without anosmia
FEZF1	7q31.32	FEZ FAMILY ZINC FINGER 1	A0PJY2	613301	Hypogonadotropic hypogonadism 22, with or without anosmia
FGF17	8p21.3	FIBROBLAST GROWTH FACTOR 17	O60258	603725	Hypogonadotropic hypogonadism 20 with or without anosmia
FGF8	10q24.32	FIBROBLAST GROWTH FACTOR 8	P55075	600483	Hypogonadotropic hypogonadism 6 with or without anosmia
FGFR1	8p11.23	FIBROBLAST GROWTH FACTOR RECEPTOR 1	P11362	136350	Encephalocraniocutaneous lipomatosis, Hartsfield syndrome, Hypogonadotropic hypogonadism 2 with or without anosmia, Jackson-Weiss syndrome, Osteoglophonic dysplasia, Pfeiffer syndrome, Trigonocephaly 1
FLRT3	20p12.1	FIBRONECTIN-LIKE DOMAIN-CONTAINING LEUCINE-RICH TRANSMEMBRANE PROTEIN 3	Q9NZU0	604808	Hypogonadotropic hypogonadism 21 with anosmia
GNRH1	8p21.2	GONADOTROPIN-RELEASING HORMONE 1	O94923	152760	Hypogonadotropic hypogonadism 12 with or without anosmia
GNRHR	4q13.2	GONADOTROPIN-RELEASING HORMONE RECEPTOR	P30968	138850	Hypogonadotropic hypogonadism 7 without anosmia
HS6ST1	2q14.3	HEPARAN SULFATE 6-O-SULFOTRANSFERASE 1	O60243	604846	{Hypogonadotropic hypogonadism 15 with or without anosmia}
IL17RD	3p14.3	INTERLEUKIN 17 RECEPTOR D	Q8NFM7	606807	Hypogonadotropic hypogonadism 18 with or without anosmia
ANOS1	Xp22.31	KAL1 GENE	P23352	300836	Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1)
KISS1	1q32.1	KISS1 METASTASIS SUPPRESSOR	Q15726	603286	Hypogonadotropic hypogonadism 13 with or without anosmia
KISS1R	19p13.3	KISS1 RECEPTOR	Q969F8	604161	Precocious puberty, central, 1; Hypogonadotropic hypogonadism 8 with or without anosmia
KLB	4p14	KLOTHO, BETA	Q86Z14	611135	N/A
LEP	7q32.1	LEPTIN	P41159	164160	OBESE, MOUSE, HOMOLOG OF
LEPR	1p31.3	LEPTIN RECEPTOR	P48357	601007	Obesity, morbid, due to leptin receptor deficiency
MKRN3	15q11.2	MAKORIN 3	Q13064	603856	Hypogonadotropic hypogonadism 18 with or without anosmia
NSMF	9q34.3	NMDA RECEPTOR SYNAPTONUCLEAR SIGNALING AND NEURONAL MIGRATION FACTOR	Q6X4W1	608137	Hypogonadotropic hypogonadism 9 with or without anosmia
OTUD4	4q13.2	OTU DOMAIN-CONTAINING PROTEIN 4	Q01804	611744	Hypogonadotropic hypogonadism 7 without anosmia
PLXNA1	3q21.3	PLEXIN A1	Q9UIW2	601055	N/A

PNPLA6	19p13.2	PATATIN-LIKE PHOSPHOLIPASE DOMAIN-CONTAINING PROTEIN 6	Q8IY17	603197	Laurence-Moon syndrome, Boucher-Neuhauser Syndrome, Oliver McFarlane Syndrome, Spastic paraplegia 39, autosomal recessive
POLR3A	10q22.3	POLYMERASE III, RNA, SUBUNIT A	O14802	614258	Leukodystrophy, hypomyelinating, 7, with or without oligodontia and/or hypogonadotropic hypogonadism
POLR3B	12q23.3	POLYMERASE III, RNA, SUBUNIT B	Q9NW08	614366	Leukodystrophy, hypomyelinating, 8, with or without oligodontia and/or hypogonadotropic hypogonadism
PROK2	3p13	PROKINETICIN 2	Q9HC23	607002	Hypogonadotropic hypogonadism 4 with or without anosmia
PROKR2	20p12.3	PROKINETICIN RECEPTOR 2	Q8NFJ6	607123	Hypogonadotropic hypogonadism 3 with or without anosmia
RNF216	7p22.1	RING FINGER PROTEIN 216;	Q9NWF9	609948	Cerebellar ataxia and hypogonadotropic hypogonadism
SEMA3A	7q21.11	SEMAPHORIN 3A	Q14563	603961	Hypogonadotropic hypogonadism 16 with or without anosmia
SEMA3E	7q21.11	SEMAPHORIN 3E	O15041	608166	CHARGE syndrome
SOX2	3q26.33	SRY-RELATED HMG-BOX GENE 2	P48431	184429	Microphthalmia, syndromic 3, Optic nerve hypoplasia and abnormalities of the central nervous system
SOX10	22q13.1	SRY-BOX 10	P56693	602229	PCWH syndrome; Waardenburg syndrome, type 2E, with or without neurologic involvement; Waardenburg syndrome, type 4C
SPRY4	5q31.3	SPROUTY RTK SIGNALING ANTAGONIST 4	Q9C004	607984	Hypogonadotropic hypogonadism 17 with or without anosmia
STUB1	16p13.3	STIP1 HOMOLOGOUS AND U BOX-CONTAINING PROTEIN 1	Q9UNE7	607207	Spinocerebellar ataxia, autosomal recessive 16
TAC3	12q13.3	TACHYKININ 3	Q9UHF0	162330	Hypogonadotropic hypogonadism 10 with or without anosmia
TACR3	4q24	TACHYKININ RECEPTOR 3	P29371	162332	Hypogonadotropic hypogonadism 11 with or without anosmia
TUBB3	16q24.3	TUBULIN BETA 3 CLASS III	Q13509	602661	Cortical dysplasia, complex, with other brain malformations 1, Fibrosis of extraocular muscles, congenital, 3A
WDR11	10q26.12	WD REPEAT-CONTAINING PROTEIN 11	Q9BZH6	606417	Hypogonadotropic hypogonadism 14 with or without anosmia