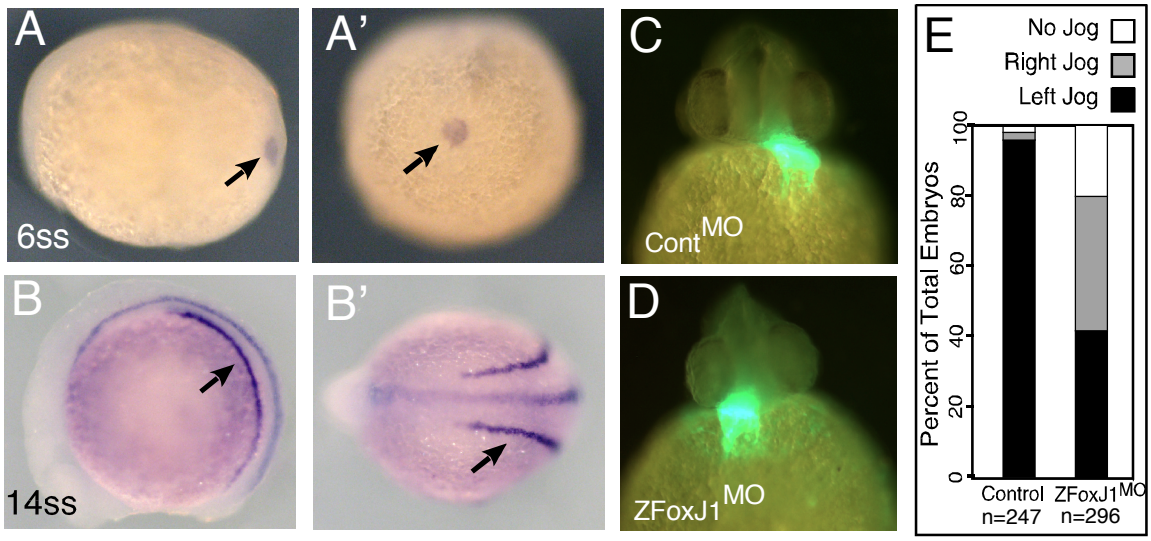
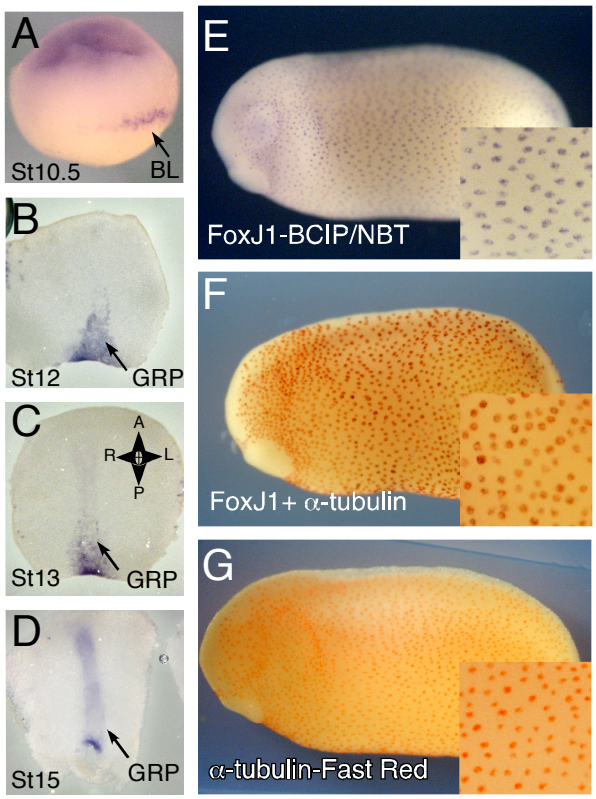


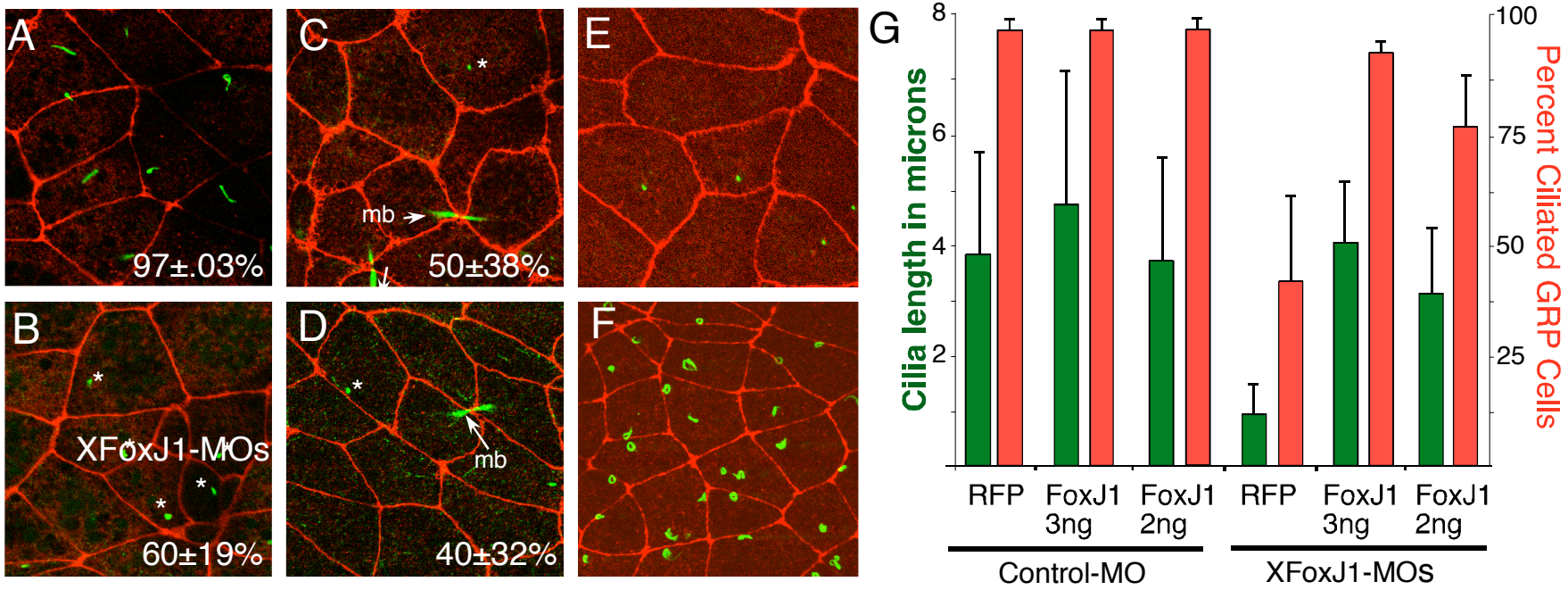
Supplementary Figure 1: *ZFoxJ1* RNA expression and function in ciliated cells of Zebrafish embryos.

(A-B) At the 6ss stage (A,A'), *ZFoxJ1* RNA is expressed prominently in KV (arrow). Shown are a side (A) and posterior (A') view. At the 14S stage (B,B'), *ZFoxJ1* is expressed prominently in the developing pronephros. Shown is a side (B) and dorsal (B') view. (C-E) Left-right organ orientation was measured in control morphants (C) and ZFoxJ1 morphants (D) using a transgenic line where GFP is expressed in the looping heart primordium under the control of myosin light chain 2A promoter. Control morphants have normal left-right asymmetry while that of ZFoxJ1 morphants is randomized (E).



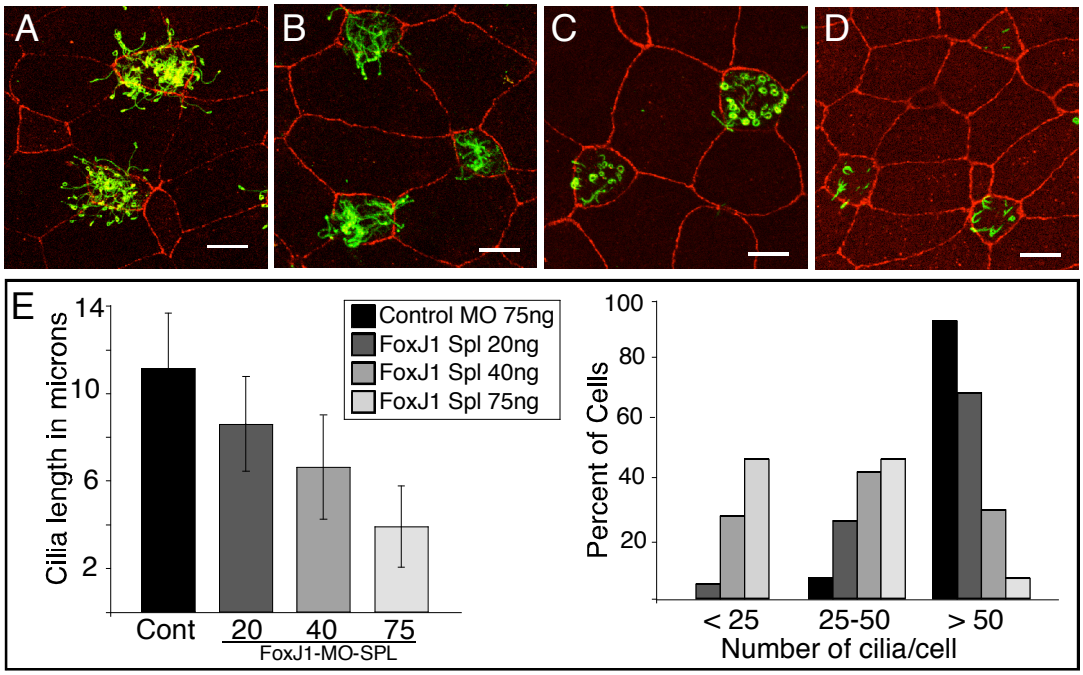
Supplementary Figure 2: Expression of *XFoxJ1* RNA in ciliated cells of Xenopus embryos.

(A) Shown is a stage 10.5 Xenopus embryo stained for the expression of *XFoxJ1* RNA using whole-mount in situ hybridization. Embryo is oriented with dorsal side to the right and animal pole up. Note expression of *XFoxJ1* RNA in cells located above the blastopore lip (BL) on the dorsal side, overlapping with the limit of involution. (B-D) Shown are dorsal explants at the indicated stage stained for the expression of *XFoxJ1* RNA. Note XFoxJ1 expression is detected in the GRP at stage 12 but disappears by stage 15, when expression is first detected at the midline most likely in the neural plate. Expression of *XFoxJ1* RNA extends from the GRP around the blastopore opening. All panels oriented as denoted in C. (E-G) Xenopus embryos at stage 26 were stained with a X*FoxJ1* probe using BCIP/NBT (E), with an α-tubulin probe using fast red (G), or with both probes together (F). Note that the cells expressing *XFoxJ1* are the ciliated cells stained for α-tubulin.



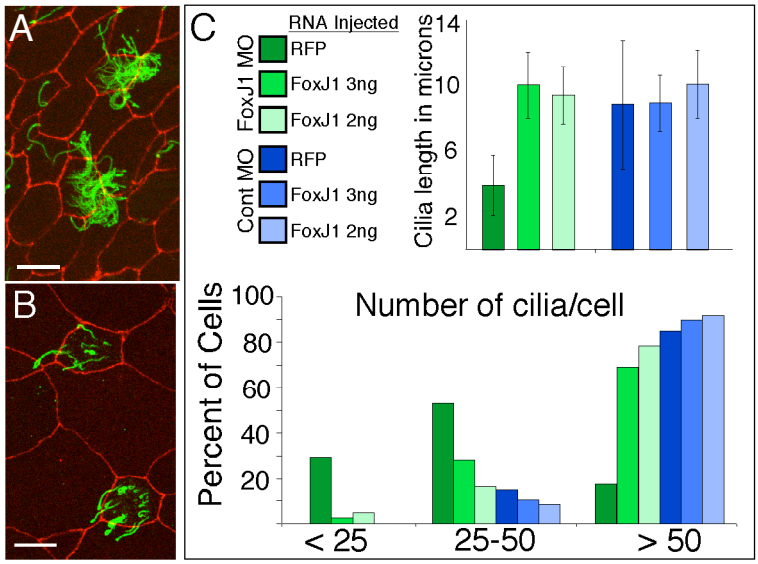
Supplementary Figure 3: Cilia formation and length in the GRP of FoxJ1 morphants.

(A-D) The GRP was imaged at stage 16 in embryos that were injected at the two-cell stage with a control-morpholino (Cont), with XFoxJ1-MO^{ATG} (atg), with XFoxJ1-MO^{SPL} (spl) or with a mixture of both morpholinos . In each case, cell junctions (red) are stained with a ZO-1 antibody while cilia (green) are stained with an antibody directed against acetylated-tubulin. Shortened cilia are marked with an asterisk, and midbodies (MB) that stain with the acetylated tubulin antibody are marked with arrows. The percentage of ciliated GRP cells (%ciliated±S.D.) was determined by scoring a total of 100-120 cells from the center of the GRP from 5- 6 embryos under each condition. (E-G) XFoxJ1 and control morphants were co-injected with 2 or 3ng of a flag-tagged form of *XFoxJ1* RNA or with RNA encoding red fluorescent protein (RFP) as a control. At stage 16, embryos were fixed, and processed for ZO-1 (red) and acetylated-tubulin staining (green) as above. Shown are cilia in the GRP cells of a XFoxJ1 morphant injected with *RFP* RNA (E), or with 3ng of flag-tagged *XFoxJ1* RNA (F). (G) The percentage of ciliated GRP cells (%ciliated ±S.D.), and cilia length (microns±S.D.) was determined by scoring a total of 100-120 cells from the center of the GRP of 5-6 embryos for each experimental condition.



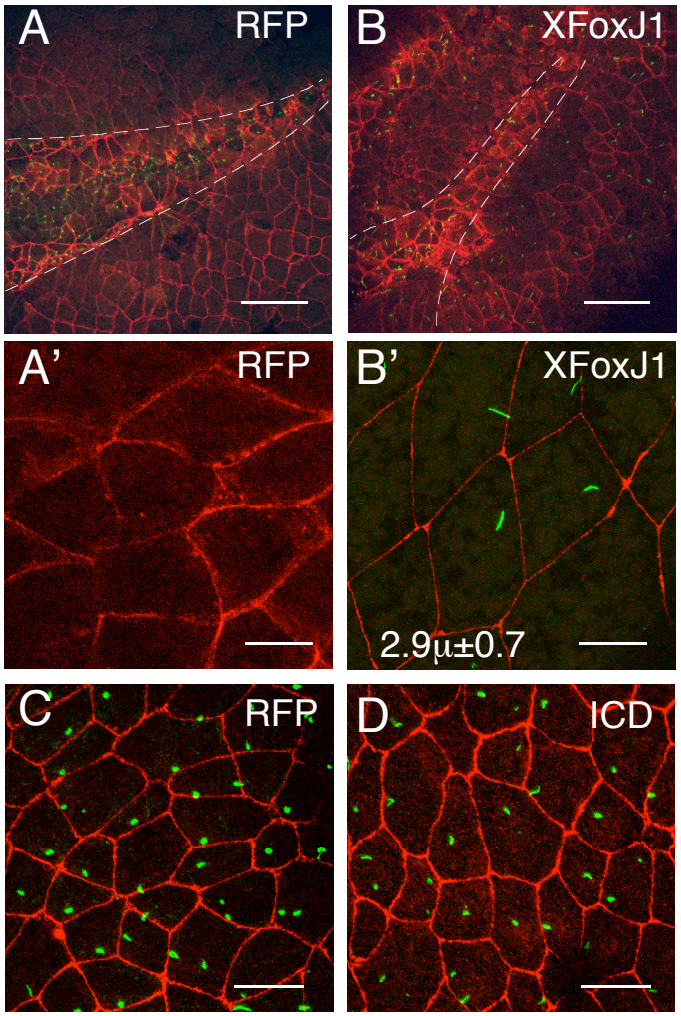
Supplementary Figure 4: Dose-dependent reduction of cilia number and length in the skin cells of XFoxJ1 morphants.

(A-D) Xenopus embryos were injected at the two-cell stage with 75ng of a control morpholino (A), or with 20ng (B), 40ng (C) or 75 ng (D) of XFoxJ1-MO^{SPL}. At stage 26, embryos were fixed and stained with ZO-1 (red) and acetylated-tubulin (green) to visualize cell junctions and cilia, respectively, of multi-ciliate cells. Scale bar=10μm. (E) Cilia length (left plot) and cilia number/cell (right plot) was measured in multi-ciliate cells by scoring 100 cilia and forty cells, respectively, under the indicated experimental conditions.



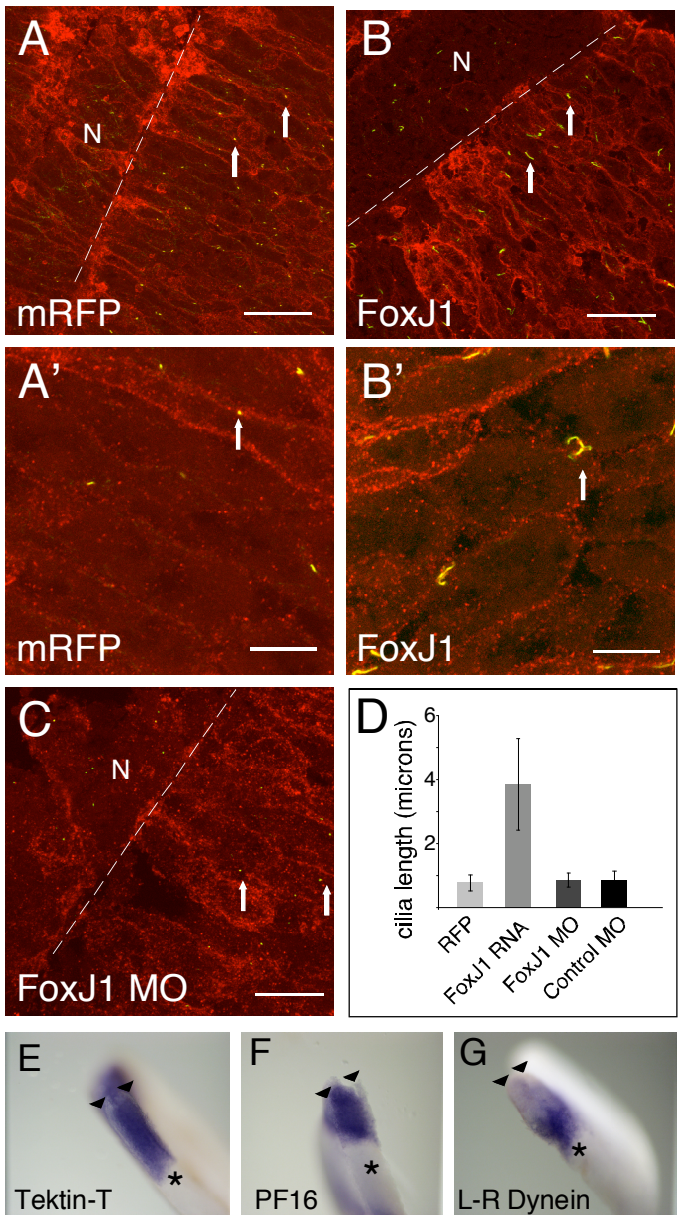
Supplementary Figure 5: *XFoxJ1* RNA rescues the cilia phenotypes in the skin cells of XFoxJ1 morphants.

(A-B) XFoxJ1 morphants were co-injected with 3ng of a flag-tagged form of *XFoxJ1* RNA (A) or with RNA encoding RFP (B) as a control. At stage 26, embryos were fixed and stained with ZO-1 (red) and acetylated-tubulin (green) to visualize cell junctions and cilia, respectively, in multi-ciliate cells. Scale bar=10μm. (C) XFoxJ1 morphants or control morphants were co-injected with 2 or 3ng of a flag-tagged *XFoxJ1* RNA or with 1ng of *RFP* RNA as a control. Under each condition, cilia length (top plot) and cilia number/cell (bottom plot) was measured in multi-ciliate cells, by measuring approximately 100 cilia and forty cells, respectively.



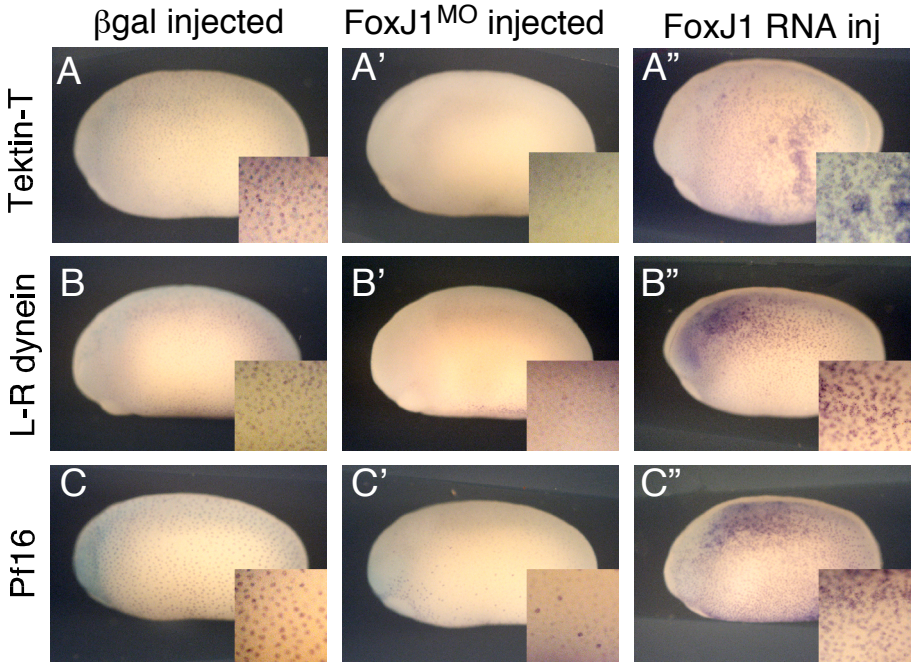
Supplementary Figure 6: Effects of XFoxJ1 and ICD on cilia formation on gastrocoel roof cells.

(A-D) Xenopus embryos were injected in the marginal zone at the two-cell stage with *RFP* RNA alone as a control or with *XFoxJ1* or *ICD* RNA along with *RFP* as a tracer, as indicated. At stage 17/18, dorsal explants were fixed and stained with ZO-1 and acetylated-tubulin antibodies to label cell junctions (red) and cilia (green), respectively. Panels A and B show low power images (scale bar= 100μm) with the GRP denoted within the dotted lines. Panels A' and B' show higher power images (scale bar=20μm) of cells in the lateral endoderm. The average length of ectopic cilia in the LEC is given in panel B' (μm ±S.D.). *ICD* RNA injected into the marginal zone has no apparent effect on ciliogenesis in the GRP (D) compared to control (C). Scale bar=20μm.



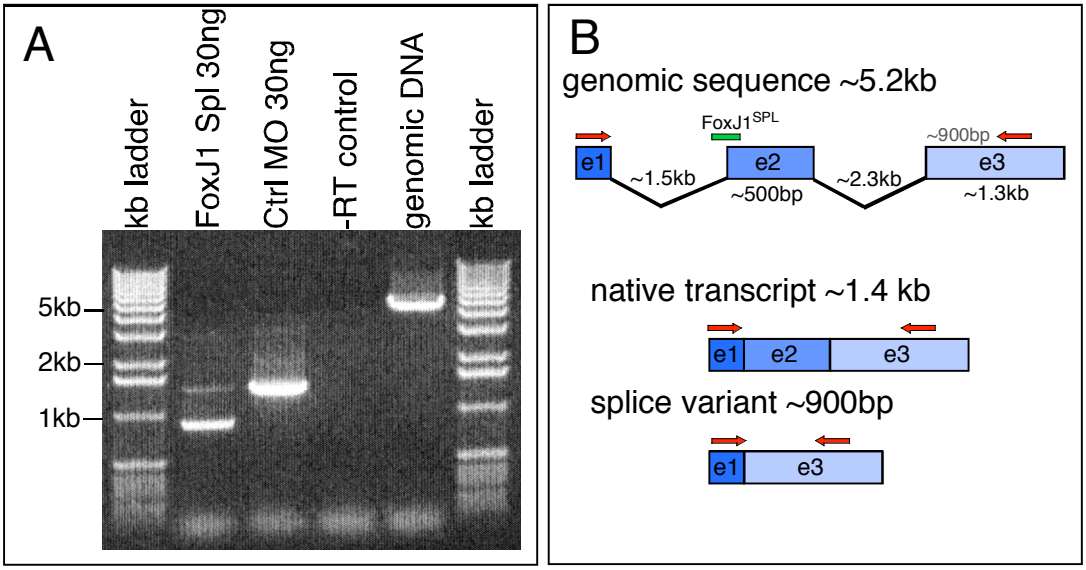
Supplementary Figure 7: Ectopic expression of *FoxJ1* RNA in mesodermal cells.

(A-D) Xenopus embryos were injected at the two-cell stage with both XFoxJ1-MOs, with a control morpholino or with 2ng of *XFoxJ1* RNA. *mRFP* RNA (red) was co-injected as a tracer to visualize cell membranes. At stage 12, Wilson explants were prepared from injected embryos to expose the dorsal mesoderm and cultured to stage 17/18 when they were fixed, and stained with the acetylated-tubulin antibody (green). In controls (A, A'), mesodermal cells form short, presumably primary cilia, that are unaffected in FoxJ1 morphants (C), but that increase in length in *FoxJ1* RNA injected embryos (B,B'). Average length of mesodermal cilia was determined under each experimental condition by measuring the length of 30-50 cilia (D). Boundary between the notochord (N) and somitic mesoderm is denoted with a dotted line (A-C) (E-G) *XFoxJ1* RNA was injected into one blastomere of Xenopu embryos at the two-cell stage. At stage 17, dorsal explants were fixed and stained for the expression of the indicated cilia genes using whole-mount in situ hybridization. After staining, th explants were cleared in glycerol, cut in cross-section and viewed on edge. Arrowheads denote the outer and inner epithelia and the asterisk marks the approximate position of the midline, whe injected side is oriented up and uninjected side oriented down. Note the dramatic upregulation c cilia gene expression in the mesoderm on the side injected with *FoxJ1* RNA. (A-C) Scale bar= 10μm (A',B') Scale bar=40μm



Supplementary Figure 8: Validation of gene expression regulated by FoxJ1.

(A-C) Expression of α-*tubulin*, *L-R dynein* and *PF16* (Spag6) was determined using whole-mount, in situ hybridization in β-gal injected control embryos or in embryos injected with *XFoxJ1* RNA or with XFoxJ1 morpholinos.



Supplementary Figure 9: XFoxJ1^{SPL} effectively blocks mRNA splicing
(A) Gel showing the reaction products generated during RT-PCR from total RNA from explants of embryos injected with 30ng of XFoxJ1-MO^{SPL}, or with a control morpholino. PCR reactions with –RT or with Xenopus genomic DNA are also shown as controls. The major band in Lane 3 corresponds to the mature XFoxJ1 transcript (~1.4kb) while that in lane 2 corresponds to a splicing variant (~900bp) as indicated in B. The major band in lane 5 shows the genomic fragment of ~5.2kb that corresponds to unspliced *XFoxJ1* mRNA. (B) Schematic of the *XFoxJ1* gene containing 3 exons and two introns. Red arrows denote primers used to amplify PCR fragments.
Location of XFoxJ-MOSPL morpholino is marked by the green box at the intron1/exon2 boundary. Sequencing of the ~900bp band in lane 2 indicates that XFoxJ1^{SPL} MO morpholino generates an alternate splice variant eliminating exon 2 and causing a frameshift in the ORF.

Supplementary Table 1

Microarray analysis of genes induced by *FoxJ1* in Xenopus ectoderm

Legend: Total RNA from explanted ectoderm was used to generate labeled complimentary RNA (cRNA) that was hybridized to *Xenopus laevis* Genome Array chips (Affymetrix #900491). Three data sets were generated using ectoderm injected with *ICD* RNA and two from ectoderm injected with both *XFoxJ1* and *ICD* RNA. Shown are the genes whose RNA levels changed greater than 10-Fold on average (Avg FC ratio) in a pairwise comparison of FoxJ1/ICD data sets to the ICD data sets. Structural Motifs and human homologs were identified based on assignment of Unigene identifiers to Homologene.

Genbank	Unigene	Avg FC Ratio	Ciliome	Structural Motifs	Human	Description
BC043831.1	Xl.66678	119.43	No	Coiled-Coiled	C14orf45	Coiled-coiled protein of unknown function with SMC2/SMC4 Homology
BJ045367	Xl.14154	84.45	No	None	LOC342346	Conserved protein of unknown function
BJ056774	Xl.14621	53.2	No	Coiled-Coiled	CCDC105	Coiled-coiled protein of unknown function
BJ087835	Xl.4890	48.5	No	Coiled-Coiled/SMC	CCDC78	Coiled-coiled protein of unknown function with SMC domain
BJ092720	Xl.72200	44.74	No	None	None	ESTs and homolog to Trop but no clear coding sequence
BC054332	Xl.12692	42.71	No	DCX	DCDC2B	Doublecortin related
BJ091326	Xl.5053	40.79	No	None	None	Unknown
BJ053550	Xl.64350	40.32	Yes	IQ calmodulin, AAA Atpase	IQCA	Tropicalis 155843, AAA Atpase, and IQ calmodulin
BM180891	Xl.14916	38.05	Yes	TPR	TTC12	Trop 175168, Conserved TPR domain protein of unknown function
BQ736249	Xl.15616	37.62	Yes	Tektin	TEKT3	Xenopus homolog of Tektin 3
BC043740.1	Xl.20447	37.62	Yes	Tektin	TEKT2	Xenopus homolog of Tektin 2
BC044268.1	Xl.66656	33.9	No	HLH-PAS	ARNT2	Xenopus homolog of ARNT2
BC049290.1	Xl.3026	33.13	Yes	Dpy-30 ADK	AK7	Xenopus homolog of adenylate kinase 7
BG346046	Xl.9077	30.55	No	WD40 Repeat	WDR16	WD-40 repeat conserved protein of unknown function
BQ398517	Xl.13942	30.2	Yes	Dynein	DNAH9	Dynein Arm Heavy Chain Axonemal
CD361045	Xl.26415	29.86	No	None	TEPP	testis/prostate/placenta-expressed protein, unknown function
BJ085862	Xl.2458	29.51	No	None	C15orf26	Conserved protein with no known function

Supplemental Table 1 Stubbs et al

BJ087968	XI.72243	29.18	No	Coiled-coiled	CCDC27	Coiled-coiled protein of unknown function
BJ076039	XI.52119	27.86	No	None	None	ESTs and homolog to Trop but no clear coding sequence
BG346004	XI.1504	27.86	Yes	Radial Spoke Protein	RSHL2	Xenopus homolog of Radial spokehead-like 2
BJ085557	XI.13305	27.22	No	None	C9orf116	Similar to C9orf116, little sequence
BJ054388	XI.15406	26.91	No	None	None	ESTs and homolog to Trop but no clear coding sequence
AW767950	XI.11087	26.91	Yes	DM10	EFHC1	Etiology JME
AF032383.1	XI.401	26.6	No	reprolyin ACR DISIN	ADAM22	metalloprotease-disintegrin
BJ082172	XI.15603	26.6	Yes	WD40 Repeat	WDR66	Trop 456177, WD-40 repeat conserved protein of unknown function
BG020671	XI.66678	25.11	No	Coiled-Coiled	C14orf45	Trop 03155
BG020958	XI.34888	24.53	No	MAP65_ASE1, coiled-coiled	CCDC87	Trop 172009: microtubule associated protein.
BG020534	XI.3108	24.53	No	L6 membrane protein	TM4SF18	transmembrane 4 superfamily member 18
BM192326	XI.25877	24.53	Yes	ADK	AK5	Xenopus homolog of adenylate kinase 1
BJ050954	XI.13352	23.97	Yes	Morn Domains	TSGA2	Testis specific A2 homolog
BI444329	XI.18717	23.7	No	L6 membrane protein	TM4SF1	tetraspanin-1
BJ051776	XI.16114	23.7	No	hormone beta chain	LHB	Trop 377323 Leutinizing hormone Beta
BG021827	XI.53283	23.7	Yes	None	C13orf26	Conserved protein with no known function
BJ048155	XI.12380	22.89	Yes	Coiled-Coiled	CCDC19	Similar to nasopharyngeal epithelium specific protein 1
BC041210.1	XI.7299	22.63	Yes	DUF1309	ODF3	Xenopus homolog of Shippo1, outer dense fiber
BG345897	XI.30012	21.36	Yes	Tektin	TEKT1	Xenopus homolog of Tektin 1
BJ090821	XI.46928	20.16	Yes	Armadillo repeats	Spag6	Xenopus homolog of Spag6
BF232441	XI.77966	19.93	No	Leucine-rich repeat	Leurine-rich 50	Related to regulator of chromosome condensation 1
BJ050529	XI.72323	19.93	No	7tm_3	GPR156	Trop 205043, G-coupled receptor of unknown function
BI313854	XI.24195	19.93	Yes	ADK	AK5	Xenopus homolog of adenylate kinase 1
BJ075928	XI.72612	19.7	No	SMC	C6orf97	Not trop, SMC containing protein of unknown function
BJ090136	XI.7994	19.7	Yes	Dynein Heavy Chain	DNAH8	Inner Dynein Arm Heavy Chain 1-alpha
BJ056722	XI.12819	19.25	No	GMP reductase	GMPR2	GMP reductase
BJ078477	XI.16776	19.03	No	Coiled-Coiled/SMC	CCDC63	Conserved Coiled-Coiled domain protein
BC042360.1	XI.57221	19.03	Yes	Armadillo repeats	Armc4	Conserved protein with no known function
AW643289	XI.16654	18.81	No	DUF667	C16orf80	Highly conserved protein of unknown function
BG816831	XI.17997	18.81	Yes	NDK Domain, Dyp-30	NME5	Xenopus homolog Nucleoside diphosphate kinase homolog 5
BJ078979	XI.16545	18.59	Yes	Tektin	TEKT4	Xenopus homolog of Tektin 4
BJ088741	XI.16441	17.75	No	WD40 Repeat	WDR16	WD-40 repeat conserved protein of unknown function
BJ080667	XI.13986	17.75	No	IQ-Calmodulin	IQCH	Trop268243, NYD-SP5
BJ085065	XI.16226	17.75	No	IQ-Calmodulin	None	trop 407388
BJ089527	XI.16412	17.55	No	WD40 Repeat	WDR49	WD-40 repeat conserved protein of unknown function

Supplemental Table 1 Stubbs et al

BJ043529	XI.16202	17.35	No	None	None	trop 165923, Protein not conserved, no motifs
BI446883	XI.18853	17.35	No	DPY-30	DYDC1	Conserved protein of unknown function
BJ078884	XI.16772	17.15	Yes	Dynein	DNALI1	dynein, axonemal, light intermediate chain
BC046664.1	XI.64437	17.15	Yes	DUF1309	ODF3	Xenopus homolog of Shippo1, outer dense fiber
AB085631.1	XI.21383	17.15	Yes	Ank Calcium Transporter	TRP channels	Xenopus calcium transporter 2
BJ086673	XI.15012	16.76	No	None	LOC463933	Tropicalis Coding, related to human hypothetical LOC463933
BF072033	XI.6973	16.56	No	Robl_LC7	DYNLRB2	Dynein-associated protein Roadblock
CD326460	XI.26316	16.56	No	DUF1042	C20orf28	Xenopus homolog of sperm flagellar one
BG159877	XI.11546	16.37	No	WD40 Repeat	None	Trop 156021
BI315451	XI.18412	16.19	Yes	IQ-Calmodulin, RIIa	SPA17	Trop 183347 PKCRegulatory subunit portion of type II PKA R-subunit
BJ055178	XI.16599	16	Yes	WD40 Repeat	DNAI1	Homolog of dynein, axonemal, intermediate chain 1
BJ089387	XI.12681	15.82	No	WD40 Repeat	WDR69	WD-40 repeat conserved protein of unknown function
BJ079557	XI.14126	15.82	No	None	None	Repetitive sequences
BI446799	XI.15888	15.82	No	Leucine-Rich Repeats	LRRC34	Trop 448354
BC049289.1	XI.14119	15.82	Yes	Spectrin repeats	SYNE1	Trop 349414
BI447010	XI.78572	15.28	No	Ankyrin Repeats	ANKRD45	Conserved ankyrin repeat protein
CD325641	XI.8555	14.93	No	Reticulon	RTN1	reticulon 1-C.2
BG017455	XI.9808	14.76	No	None	C14orf50	Conserved protein with no known function
BJ079815	XI.15440	14.76	No	C2H2 Zinc Finger gene model	C8orf70	Trop 171270
BJ086368	XI.13468	14.59	No	None	None	ESTs and homolog to Trop but no clear coding sequence
AW766365	XI.15197	14.42	No	WD40 Repeat	WDR38	WD-40 repeat conserved protein of unknown function
BC044984.1	XI.23512	14.42	No	Homeodomain	HOXA1	HoxA1
BJ098965	XI.15885	14.25	Yes	Leucine-Rich repeats	LRRC51	No known function
BJ091895	XI.72241	13.93	No	DUF1208	FAM92B	Little sequence no tropicalis, although conserved protein
BI348453	XI.78688	13.93	Yes	Ropporin-like	ROPN1L	Fibrous sheath of Sperm
BF071920	XI.10706	13.77	No	Tctex-1	TCTEX1D1	Dynein light chain
BJ046781	XI.51228	13.3	No	F-Box	FBXO31	Trop 169553
BJ054660	XI.16566	13.3	No	ADK, Adenylate Kinase	C9orf98	Unknown function
BJ055241	XI.15554	13.15	Yes	Armadillo, SRP1	RTDR1	Rhap Tumor deleted
AB022088.1	XI.1220	13	No	P450	CYP1A1	cytochrome P450
BJ043754	XI.73054	12.7	No	S-TKc	NEK5	Trop 467014, NIMA related kinase
BJ048763	XI.15384	12.7	No	None	C6orf206	Conserved protein with no known function
BJ083558	XI.6950	12.27	No	None	KIAA1370	Conserved protein of unknown function
BJ082817	XI.68845	12.27	No	Calponin, IG-Filamin	FLNC	Actin Binding, Filamin related
BQ399047	XI.80150	12.27	No	B41, SH2, TyrKc	JAK2	JAK2, Tyrosine-protein kinase

Supplemental Table 1 Stubbs et al

CB756314	XI.81323	12.27	Yes	PDEase_I,	PDE4D	3,5-cyclic phosphodiesterase type 9
BJ081503	XI.10830	12.13	No	None	C9orf68	Conserved protein with no known function
AW767983	XI.11177	11.99	No	WD40 Repeat	WDR63	Trop 304328 NYD-SP29 Conserved WD40 protein of unknown function
BJ075955	XI.13768	11.85	No	None	None	Little sequence no tropicalis
BF232515	XI.46978	11.71	No	C2H2 Zinc Finge	C8orf70	Trop 171270
BJ076059	XI.15389	11.58	No	None	None	Coding sequence, homolog to Trop sequence and to hypothetical genes
BG017110	XI.11474	11.58	No	Coiled-Coiled	CCDC96	Tr 468393
BJ083290	XI.17968	11.31	No	None	SPAG8	Similar to Spag8
BG019848	XI.9878	11.18	No	None	None	Coding sequence, homolog to Trop sequence and to hypothetical genes
BC046665.1	XI.9878	11.18	No	DCX	DCDC2	RU2S double cortin domains
BJ045936	XI.73464	11.06	No	None	None	Repetitive sequences
BJ043994	XI.13320	11.06	Yes	Radial Spoke Protein	RSHL3	Radial Spoke related
BJ049223	XI.73126	10.8	No	None	None	ESTs and homolog to Trop but no clear coding sequence
BJ048577	XI.22500	10.8	No	Leucine-rich repeat	LRRC27	LRR protein of unknown function
BF072242	XI.10381	10.56	No	Tyrosine Kinase Domain	RAGE	Related to Long flagellar protein 4
BC041731.1	XI.9439	10.56	No	Homeodomain	HOXA3	Xenopus homolog of homeo box A3
BJ084139	XI.15806	10.43	Yes	KIF9 Kinesin	KIF9	Trop 401497 Kinesin

Supplemental Table 2
Morpholino and Primer sequences used in this study

Morpholino

Name	Sequence
XFoxJ1-MO^{ATG}	5'-GCAGGTCAAACATTAATAAAGCCCT-3'
XFoxJ1-MO^{SPL}	5'-TGCTCCTACAATGCAA-GCAGAGAAT-3'
XControl	5'-GGATGGTGCAGAGTCTCCATCAGTA-3'
ZFoxJ1^{MO}	5'-CATGGAGAGCATGGTCCTGACAAAT-3'
ZControl	5'-CCTCTTACCTCAGTTACAATTTATA-3'

Primers

Primer Name	Sequence
ODC-F	5'-CTGCCGCCTCAGTGTGAA-3'
ODC-R	5'-TGCCCGCTCCAGAAGC-3'
DHC9-F	5'-CAGAATCCAGCCATCCGTG-3'
DHC9-R	5'-TTGTGAACCGAACACCAGTTG-3'
Pf16-F	5'-AAGAAAGCAGCAGCCTTTGTG-3'
Pf16-R	5'-CCCCAGAGTCCACTATTGACTGA-3'
Tektin-F	5'-CGCAATGAGACAAGCAACCA-3'
Tektin-R	5'-CACGTCATCTATGCGTTCATCA-3'
Splicing MO-F	5'-GCTGTTGCTGCTGCTTCCAGAACC-3'
Splicing MO-R	5'-TATATAGGAACCCAAGGACG-3'