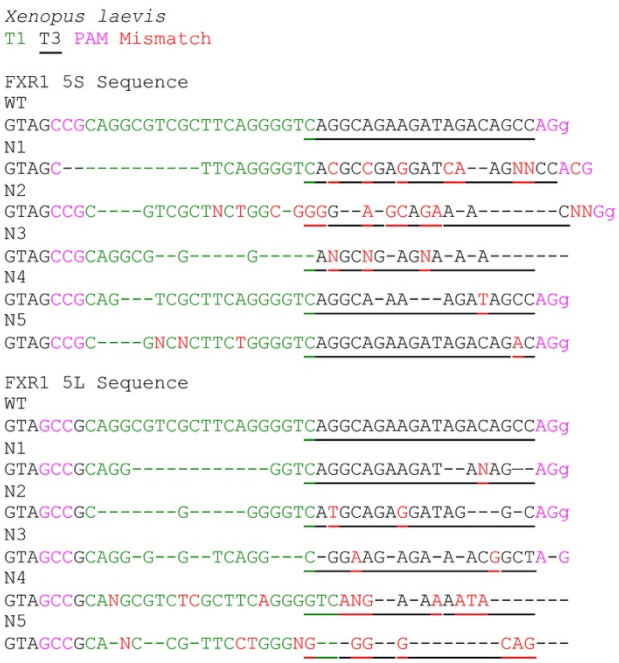
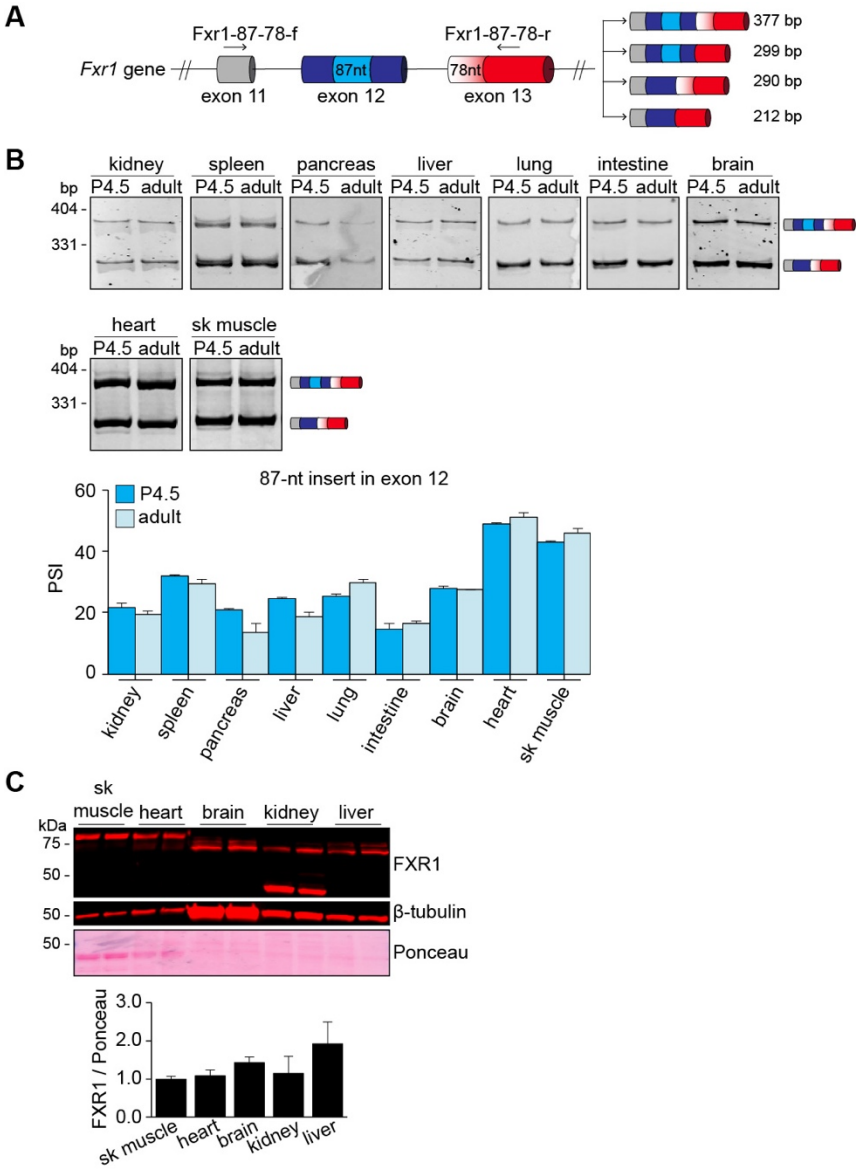
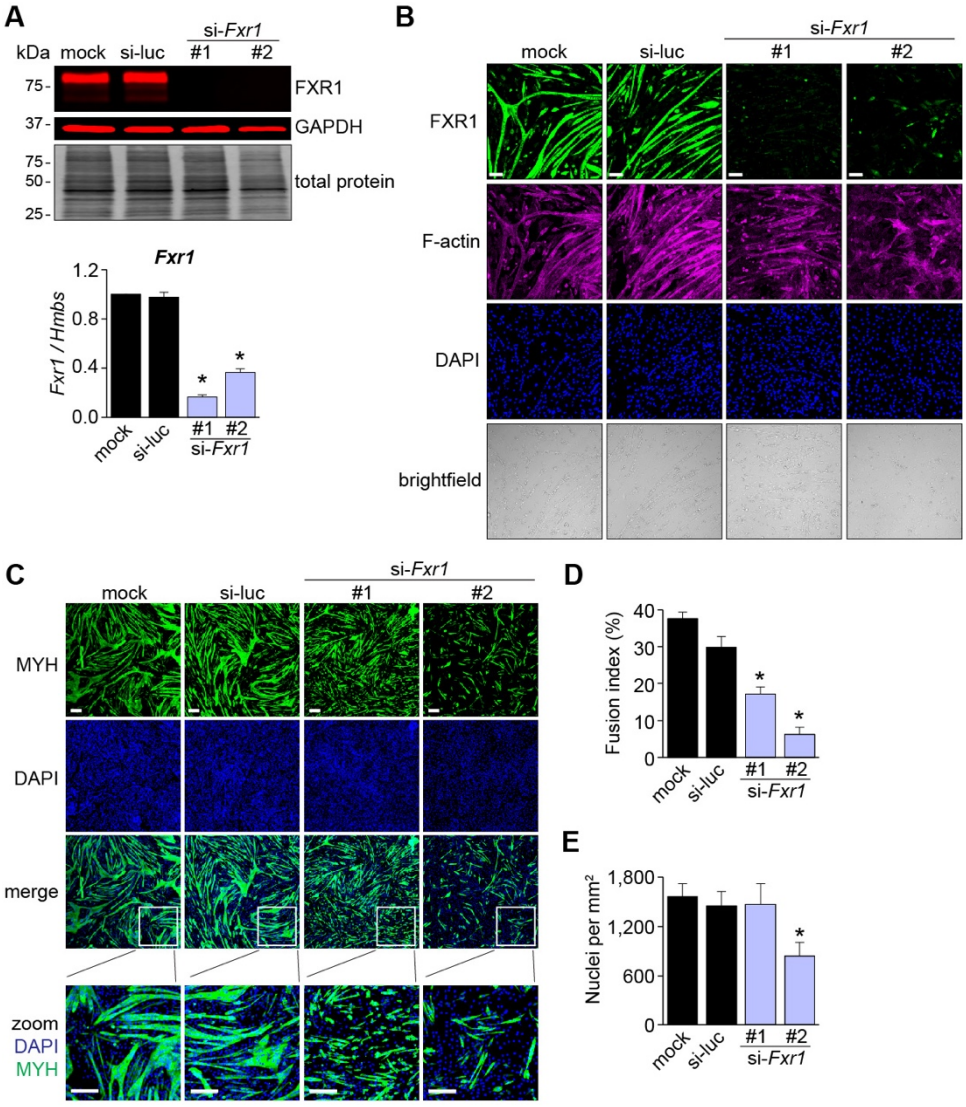
SUPPLEMENTAL FIGURES



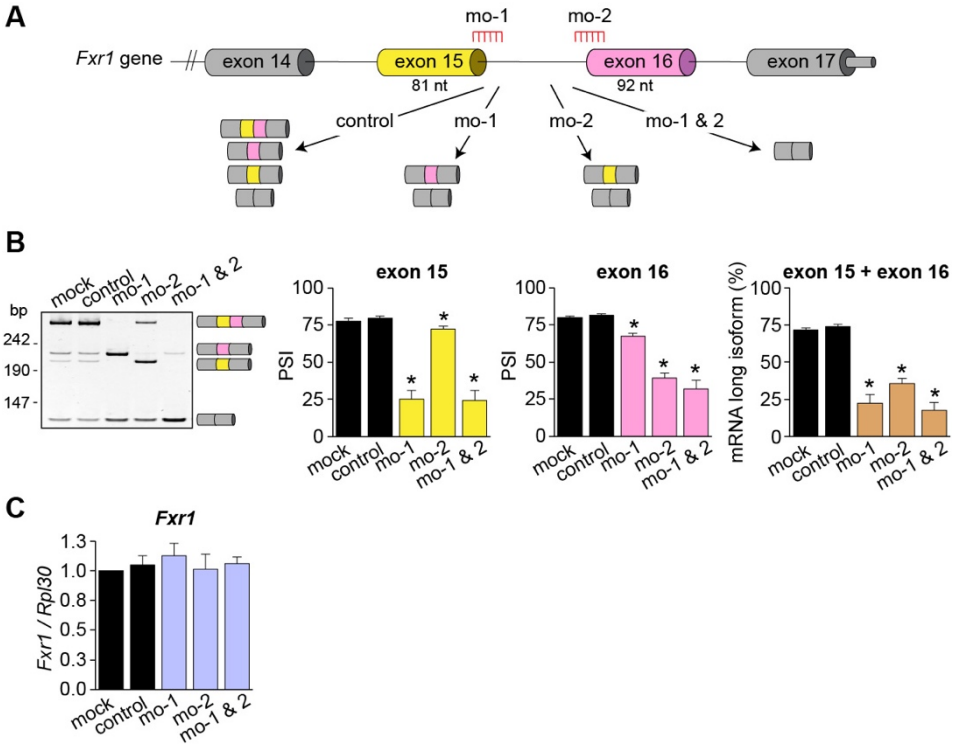
SUPPLEMENTAL FIGURE S1



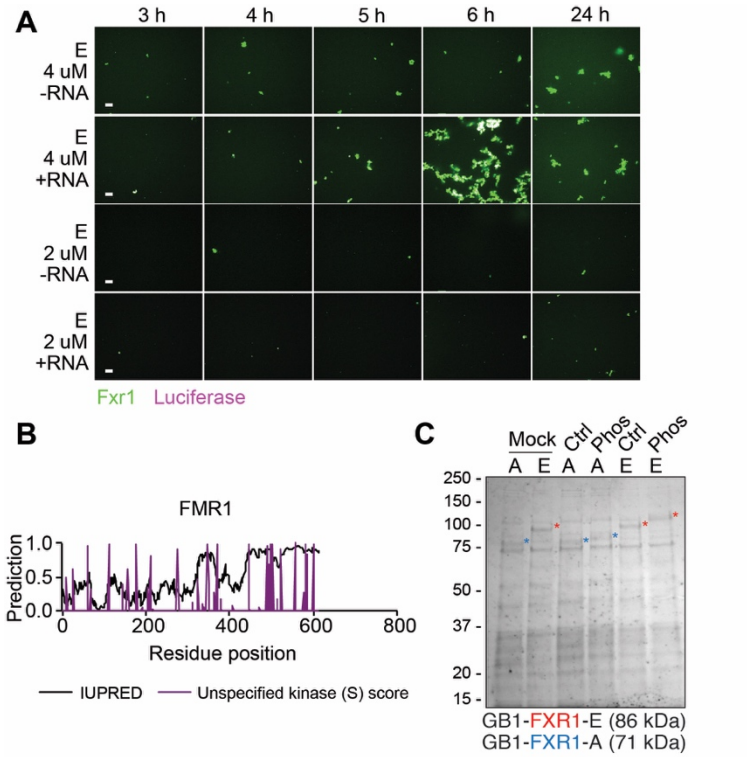
SUPPLEMENTAL FIGURE S2



SUPPLEMENTAL FIGURE S3



SUPPLEMENTAL FIGURE S4



SUPPLEMENTAL FIGURE S5

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Sequences of *fxr1* exon 15 from sgRNA treated embryos. Sequences of *fxr1.S fxr1.L* from five randomly selected *X. laevis* embryos injected with the sgRNA T1 (green) and T3 (underlined). sgRNA injected embryos had deletions (dashed) uncallable nucleotides (N) and mismatches (red) compared to reference sequences. Deletions roughly correspond to 3-4 nucleotides upstream of the PAM sequences (magenta). **Supplemental Figure S1** is linked to **Figure 1E-F**.

Supplemental Figure S2. Alternative splicing events in exons 12 and 13 of *Fxr1* pre-mRNA and expression of FXR1 protein isoforms during tissue development. A. Scheme of part of *Fxr1* gene showing the alternative splicing events in exons 12 (insert of 87 nt) and exon 13 (alternative splice site leading to the inclusion or skipping of a 78-nt region). To evaluate the splicing patterns by RT-PCR, the primers designed to target the constitutive flanking exons were Fxr1-87-78-f (forward) and Fxr1-87-78-r (reverse). **B.** Different tissues were collected from neonatal (postnatal day 4.5, P4.5) and adult (4 months) mice. Alternative splicing was evaluated by RT-PCR and quantified by densitometry. *N*=3-4 (P4.5 tissues) samples from 2-9 pooled neonates each depending on tissue type, *N*=4 (adult tissues) animals. **C.** Adult mouse tissues were evaluated by Western blots utilizing a monoclonal antibody against the N-terminus of FXR1 protein that thus recognizes all of the splice variants. *N*=2 animals. Results: mean ± s.e.m. *p≤0.05 (one-way ANOVA test with Bonferroni correction for multiple comparisons). bp: base pairs, sk. muscle: skeletal muscle. **Supplemental Figure S2** is linked to **Figure 2**.

Supplemental Figure S3. FXR1 is required for myogenesis. C2C12 myoblasts were transfected with two different si-RNAs targeting *Fxr1* (#1, #2) or a control siRNA (si-luc) and the next day differentiation was induced. **A.** Protein and RNA were collected at differentiation day 4 and FXR1 depletion was confirmed by Western blot and qPCR assays. **B.** Cells were evaluated by immunofluorescence to confirm knock down. Scale bars 100 μm. **C-D.** Myosin heavy chain (MYH) expression was evaluated by immunofluorescence (**C**), and the fusion index was estimated (**D**). **E.** The number of nuclei per field of view was estimated from the microscopy images. Results: mean ±

s.e.m. *p≤0.05 *versus* mock and si-luc (Student T-test), *N*=4-8 independent experiments. Scale bars 200 μm. **Supplemental Figure S3** is linked to **Figure 3**.

Supplemental Figure S4. Modulation of endogenous *Fxr1* splicing in C2C12 cells using morpholinos. Morpholinos (MOs) were delivered in undifferentiated myoblasts and the next day cells were differentiated for four days. **A.** Scheme of the MOs used to target exons 15 (mo-1) and 16 (mo-2). Control denotes cells treated with a MO control. **B.** Efficiency of MO action was evaluated by RT-PCR. **C.** Total *Fxr1* mRNA levels were evaluated by qPCR. Results: mean ± s.e.m. *p≤0.05 versus mock and control (Student T-test), *N*=3-8 independent experiments. **Supplemental Figure S4** is linked to **Figure 4**.

Supplemental Figure S5. RNA addition and protein phosphorylation influence FXR1 aggregation *in vitro*. A. Luciferase RNA accelerates isoform E aggregation. Representive images for either 4 μM or 2 μM isoform E with or without luciferase RNA after 3, 4, 5, 6, and 24 h. Scale bars 10 μm. **B.** FMR1 Predicted disorder (IUPRED) and unspecified kinase score for serine are marked for each amino acid. Predicted phosphorylated serines are uniformly distributed across the protein. **C.** Coomassie staining of the *in vitro* purified FXR1 isoforms A and E. Phosphorylation results in a band shift with respect to the control or mock treated samples. **D.** Representive images of 2 μM of FXR1 isoform E (shown in white) with or without RNA and with or without phosphorylation (phos) at 3, 4, 5, 6, and 24 h. Scale bars 10 μm. **Supplemental Figure S5** is linked to **Figure 6**.

SUPPLEMENTARY TABLES

Strain	Genotype	Parent	Reference
AGB828	P_{CMV}-*eGFP-FXR1* (HeLa isoform A) *KAN/NeoR* Amp^R	pEGFP-C1	Generous gift from Sumara group, IGBMC
AGB879	P_{CMV}-*Fxr1isoE-tGFP* Amp^R	pCMV6-AC-GFP	Origene
AGB1070	*mNeonGreen* Amp^R	pNCS	Allele Biotechnology
AGB1103	P_{CMV}-*mNeonGreen-Fxr1isoE* Amp^R	pCMV6-AC-GFP	This study
AGB1111	P_{CMV}-*mNeonGreen* Amp^R	pAGB1103	This study
AGB1139	$P_{CMV}2xTetO_2$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1162	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE* Amp^R Zeo^R	pcDNA4/TO	This study
AGB1189	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoA* Amp^R Zeo^R	pcDNA4/TO	This study
AGB1171	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{T236D\ K237D}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1172	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{K299D\ N300D}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1173	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{T236D\ K237D\ K299D\ N300D}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1174	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{\Delta 464-490}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1175	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE* $^{\Delta 464-490\ T236D\ K237D\ K299D\ N300D}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1176	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{1-374}$ Amp^R Zeo^R	pcDNA4/TO	This study
AGB1177	$P_{CMV}2xTetO_2$-*mNeonGreen-Fxr1isoE*$^{1-490}$ Amp^R Zeo^R	pcDNA4/TO	This study

Supplementary Table 1. Plasmids used in this study

Primer name	Primer sequence (5’ to 3’)
AGO2195	actggatccggtaccgaggaggagcaggtgcaggtgcaggtgCTCGAGATGGTGAGCAAG
AGO2196	caggaaacagctatgaccgcggagcaggtgcaggtgcaggtgGCGGCCGCTTACTTGTAC
AGO2202	gcatggacgagctgtacaaggcgcgtacgcggccgctcgagATGGCGGAGCTGACGGTG
AGO2203	caggaaacagctatgaccgctcaTGAAACACCATTCAGGACTGCTG
AGO2206	caggaaacagctatgaccgctcaCTGCTCATCAATCTGCAGACGTTC
AGO2207	caggaaacagctatgaccgctcaGCCACCACGTGGTCCACC
AGO1869	GGGCCTGGCGATAGGAGACGATGGCAGTAACATACAGCAAGC
AGO1870	GCTTGCTGTATGTTACTGCCATCGTCTCCTATCGCCAGGCCC
AGO1871	CCCAGGAATCTTGTTGGAAAAGTAATTGGAGACGATGGCAAAGTTATTCAAGAAATAGTGG
AGO1872	CCACTATTTCTTGAATAACTTTGCCATCGTCTCCAATTACTTTTCCAACAAGATTCCTGGG
AGO1873	GATGATCGAGAGACTCGACATAAATCCTCCATCAGTTCTGTGC
AGO1874	GCACAGAACTGATGGAGGATTTATGTCGAGTCTCTCGATCATC
AGO2350	cgtttaaacttaagcttggtaccgagctcgATGGTGAGCAAGGGCGAG
AGO2351	agcggccgccactgtgctggatatctgcagTCATGAAACACCATTCAGGACTG
AGO2374	agcggccgccactgtgctggatatctgcagTCACTGCTCATCAATCTGCAG
AGO2375	agcggccgccactgtgctggatatctgcagTCAGCCACCACGTGGTCC
AGO1863	CCTCAGTTAATGAGAATGGGCTAGGCAAGAGGTGCGACACGCGTACGCGGCCGCTCGAGATGGAGAGC
AGO1864	GCTCTCCATCTCGAGCGGCCGCGTACGCGTGTCGCACCTCTTGCCTAGCCCATTCTCATTAACTGAGG
AGO1867	GCAGCTGCGACAGATTGGTTCTAGGTCTTATAGTGGAAGAGG
AGO1868	CCTCTTCCACTATAAGACCTAGAACCAATCTGTCGCAGCTGC
AGO2486	gcggccgcgtacgcgcCTTGTACAGCTCGTCCATGC
AGO2487	gacgagctgtacaaggcgcgtacgcggccgctcgagATGGCGGAGCTGACGGTG
AGO2488	agcggccgccactgtgctggatatctgcagCTAGTCGCACCTCTTGCCTAG

Supplementary Table 2. Sequence of the primers (IDT) used for plasmid generation. Upper case denotes sequences that will directly base pair with the plasmid being amplified while lower case bases are overhangs for NEBuilder HiFi DNA assembly reaction (NEB).

Tissue	Sex	Age of individual	Company and catalog number	Lot number
heart	male	21 years	Amsbio (R1234139-50)	B707146
heart	male	51 years	Cell Applications Inc (1H30-50)	1369
heart	male	30-39 years	TakaRa (636532)	1610293A
skeletal muscle	female	48 years	Amsbio (R1234171-50)	B207200
skeletal muscle	male	58 years	Cell Applications Inc (1H60-50)	1376
skeletal muscle	male, female	20-68 years	TakaRa (636534)	1609179A
heart	female	22-week of gestation	Cell Applications Inc (1F30-50)	2739
heart	male	20-21 weeks of gestation	Agilent (540165)	0006262824
skeletal muscle	female	22-week of gestation	Cell Applications Inc (1F60-50)	2739
skeletal muscle	female	18 weeks of gestation	Agilent (540181)	0006260887

Supplementary Table 3. Commercially available human RNA samples.

Species	Primer name	Primer sequence (5' to 3')	Analyzed region	Expected bands (bp)
mouse	Fxr1-81-92-f	TGCTGTTCTGATGGATGGAC	exon 15 (81 nt) and exon 16 (92 nt)	300 219 208 217
mouse	Fxr1-81-92-r	GAAGCGCTAGTTGGACCATT		

mouse	Fxr1-87-78-f	AGAAAGCATTGGGAATGTGC	87-nt insert in exon 12 and alternative 3' splice site in exon 13	377 299 290 212
mouse	Fxr1-87-78-r	TGTCTCGCTGATGTCGAGTC		
human	FXR1-81-92-f	TGCTGTTCTGATGGATGGAA	exon 15 (81 nt) and exon 16 (92 nt)	298 217 206 125
human	FXR1-81-92-r	AGCACTAGTTGGGCCGTTTA		
X.tropicalis	fxr1-F1	TCTCACCACAACACAAACCG	exon 15 (81 nt)	266 185
X.tropicalis	fxr1-R1	GCGGAATCTACCTAAAGAACC		
X.tropicalis	eef1a1-F	CCCCTCTTGGTCGTTTTGCTGTCC	Forward primer spans exons 7-8 junction; reverse is end of coding sequence in exon 8.	135
X.tropicalis	eef1a1-R	TTGCCTTTCTGTGCTTTCTGAGCAG		

Supplementary Table 4. Sequence of the primers (Sigma, IDT) utilized to evaluate *FXR1* alternative splicing in mouse, human, and frog samples. eef1a1 was used as a loading control. bp: base pairs, nt: nucleotides.