Supplemental figures and tables for Loss of Fshr prevents testicular maturation in Atlantic salmon (Salmo salar L.)

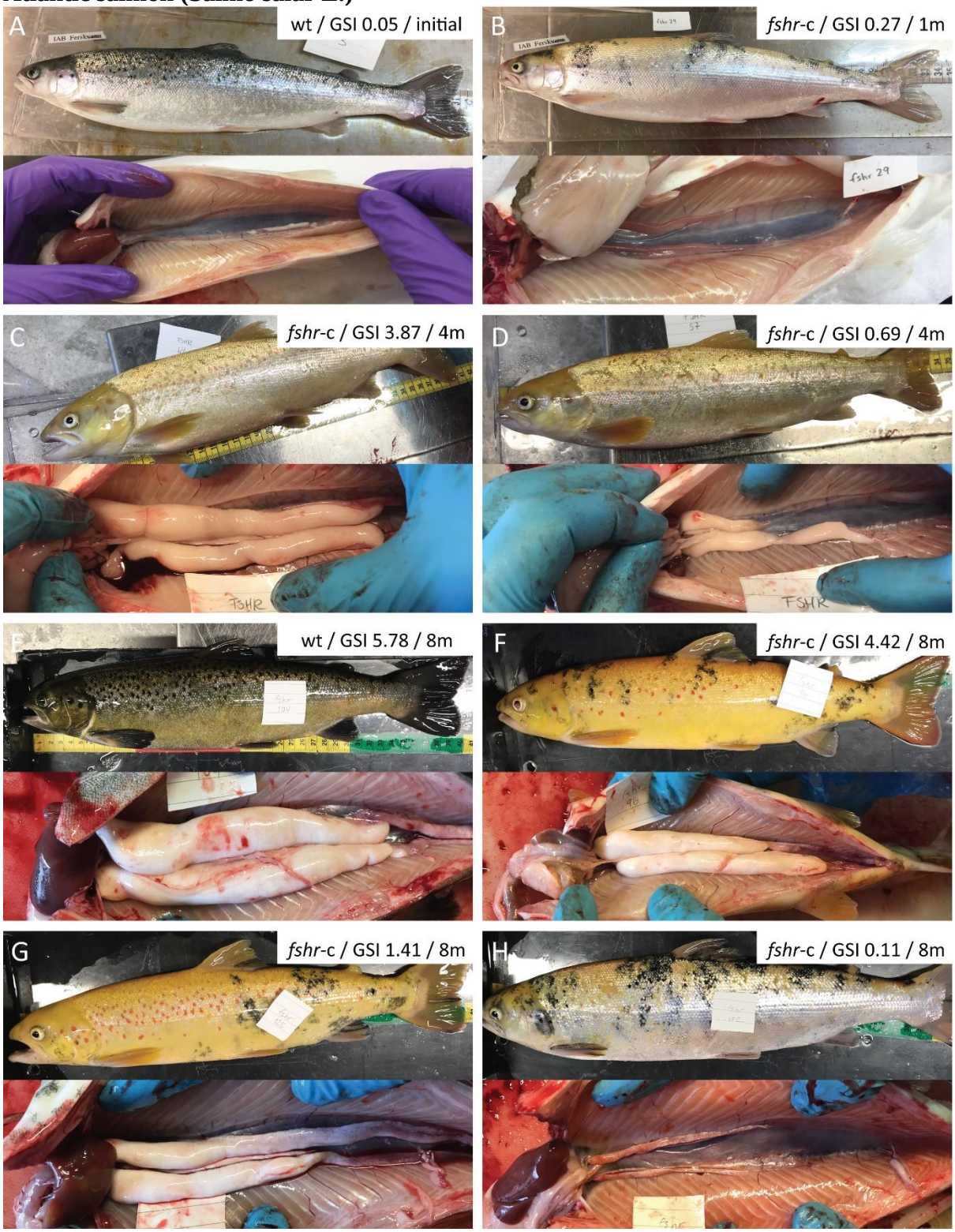

Figure S1. External appearance and macroscopic testes anatomy of wild-type (wt) control and *fshr* F0 crispant (*fshr*-c) fish sampled just before (A, initial) and 1 (B), 4 (C, D) and 8 (E-H) months after the start of the maturation-inducing treatment (6 weeks of constant light and 16°C water temperature, starting January 25, 2017). Detailed histological analysis of testis sections is given in Figure S2.

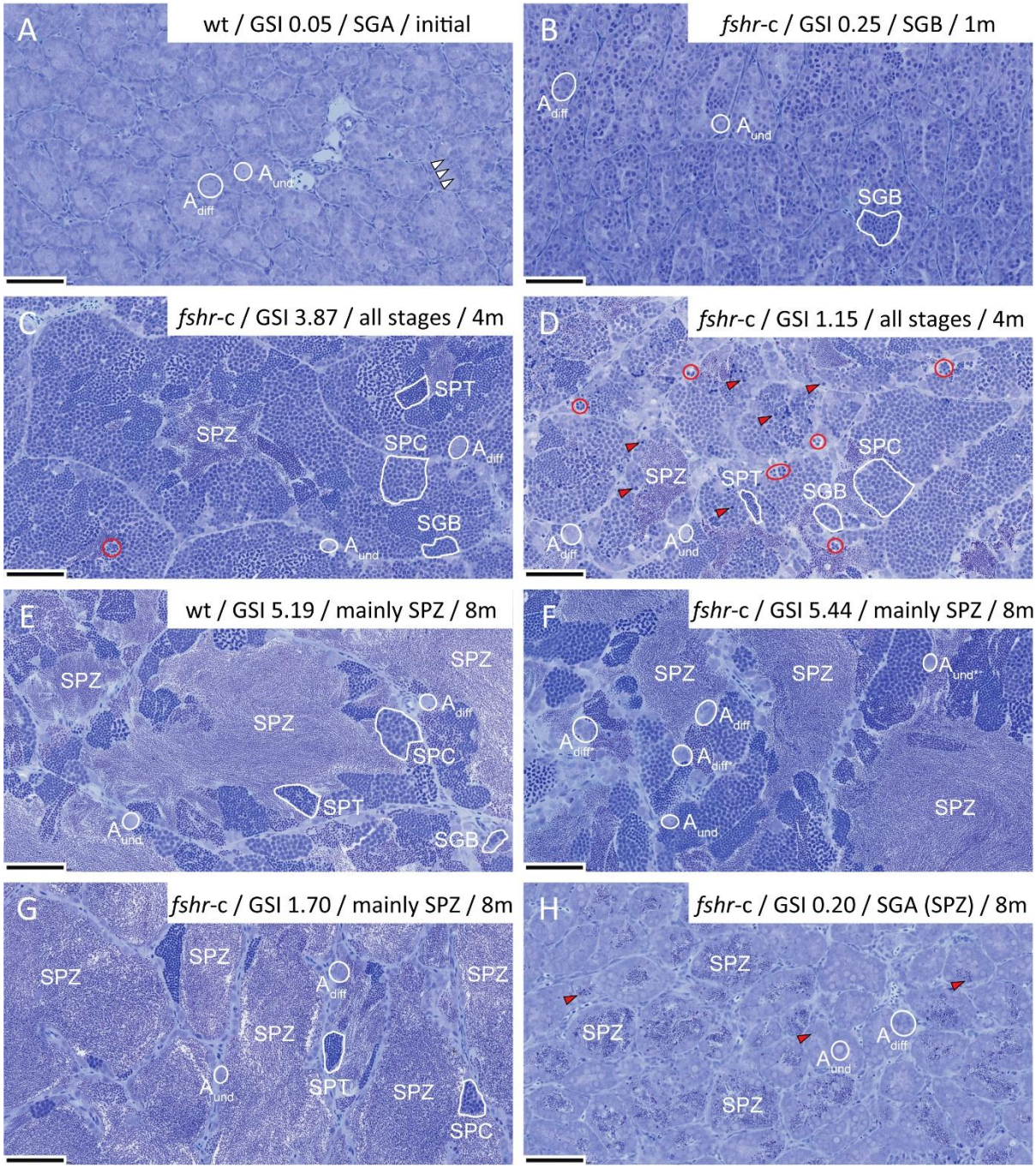

Figure S2. Wild-type control (wt) and F0 crispant (*fshr*-c) testes sampled just before (A, initial) and 1 (B), 4 (C, D), or 8 (E-H) months after the start of a maturation-inducing treatment (6 weeks of constant light and 16°C water temperature, starting January 25, 2017). The sections are labeled with the genotype, GSI value of the sampled male, the characteristic stage of germ cell development, and the time of sampling. Type A spermatogonia, being either single type A undifferentiated (A_{und}) or groups of type A differentiating (A_{diff}) spermatogonia; SGB – type B spermatogonia; SPC – spermatocytes; SPT – spermatids; SPZ – spermatozoa. Eight months after initiating the maturation regime, a subgroup among the crispants (**F**), characterized by GSI values as high as in wt controls, showed both types of type A spermatogonia also at unusual sites (indicated by A_{und}* and A_{diff}*, respectively). Red circles in C and D indicate groups of apoptotic germ cells, red arrowheads in D indicate single apoptotic germ cells. Red arrowheads in H indicate residual spermatozoa being resorbed by and hence found within Sertoli cells. Bars indicate 50 µm.

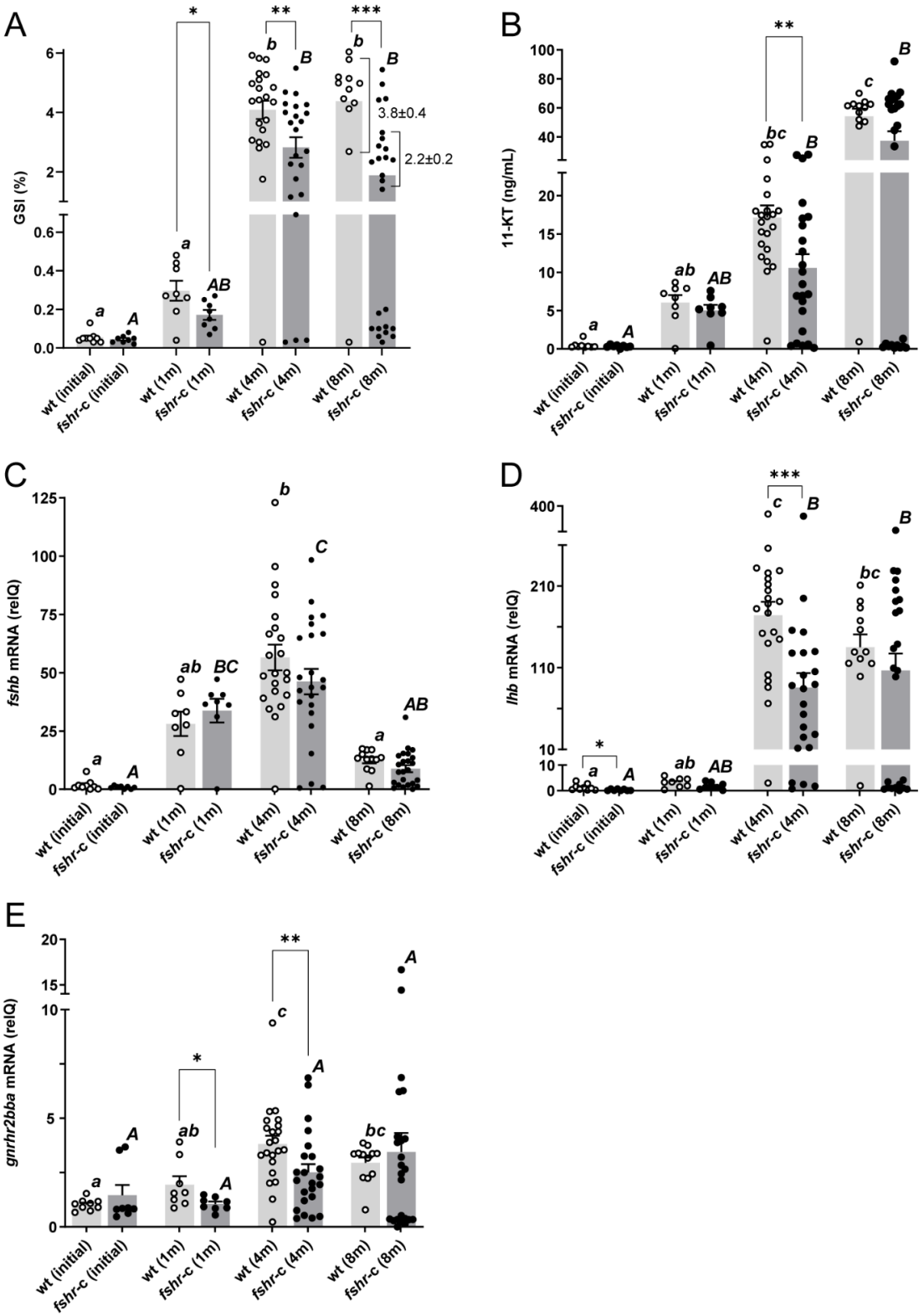

Figure S3. Comparison of (A) gonado-somatic index (GSI), (B) plasma 11-ketotestosterone (11-KT), (C) pituitary *fshb*, (D) *lhb*, (E) *gnrhr2bba* mRNA levels in F0 *fshr* wild-type (wt, o) and *fshr* crispants (*fshr*-c, •) at 0 (initial), 1, 4 and 8 months after the start of the maturation-inducing treatment (6 weeks of constant light and 16°C water temperature, starting January 25th 2017). Data are presented as mean ± SEM. Significant differences are indicated by asterisks (*P< 0.05, **P < 0.001, ***P < 0.0001) in the wt and *fshr*-c pairwise comparisons, and by different letters in the wt (lowercase) and *fshr*-c (uppercase) time-wise changes.

Table S1. Biometrics of F0 *fshr* crispant (*fshr*-c) and wild type (wt) males sampled just before (initial, January 24th), and 1 (February 21st), 4 (May 9th) and 8 months (September 26th) after the start of the maturation-inducing treatment (6 weeks of constant light and 16°C water temperature, starting January 25, 2017).

Sampling	ID	Group	Length (cm)	Weight (g)	Gonad weight (g)	GSI %
initial	3	wt	30.2	349	0.2	0.05
initial	4	*fshr*-c	24.5	206	0.1	0.04
initial	6	*fshr*-c	27.2	258	0.2	0.08
initial	8	wt	28.8	285	0.1	0.03
initial	9	wt	29.5	327	0.2	0.06
initial	10	*fshr*-c	31.0	391	0.2	0.05
initial	12	*fshr*-c	28.2	288	0.1	0.05
initial	14	wt	27.4	254	0.1	0.05
initial	15	wt	28.0	286	0.4	0.13
initial	16	*fshr*-c	29.5	303	0.1	0.02
initial	21	wt	27.7	278	0.2	0.05
initial	22	*fshr*-c	26.4	250	0.1	0.03
initial	24	*fshr*-c	26.2	229	0.2	0.06
initial	25	wt	25.5	218	0.1	0.04
initial	26	wt	30.0	349	0.2	0.05
initial	27	wt	28.6	282	0.1	0.03
1	28	*fshr*-c	26.9	228	0.2	0.07
1	29	*fshr*-c	21.7	456	1.2	0.27
1	30	wt	35.0	616	3.0	0.48
1	31	wt	32.0	436	1.2	0.27
1	32	*fshr*-c	31.0	436	0.7	0.15
1	33	*fshr*-c	30.5	416	1.0	0.25
1	34	wt	30.0	306	0.1	0.04
1	35	wt	29.6	360	1.0	0.26
1	36	*fshr*-c	28.5	306	0.4	0.12
1	37	*fshr*-c	33.5	550	1.0	0.19
1	38	wt	30.6	380	0.7	0.19
1	39	wt	34.6	558	2.5	0.44
1	40	*fshr*-c	28.5	314	0.7	0.22
1	41	*fshr*-c	30.5	352	0.4	0.10
1	42	wt	32.0	484	2.0	0.41
1	43	wt	35.5	596	1.7	0.28
4	44	wt	35.0	507	14.8	2.92
4	45	*fshr*-c	38.0	667	8.2	1.23
4	46	wt	40.0	790	13.8	1.75
4	47	wt	31.0	366	17.6	4.81
4	48	wt	35.0	536	31.2	5.82
4	49	*fshr*-c	35.0	476	20.4	4.29
4	50	*fshr*-c	34.5	473	16.2	3.42
4	51	wt	24.5	167	5.7	3.41
4	52	*fshr*-c	31.5	349	7.9	2.26

4	53	wt	32.5	396	4.0	1.01
4	54	*fshr*-c	32.0	394	16.8	4.26
4	55	wt	32.5	370	11.1	3.00
4	56	*fshr*-c	30.0	329	5.8	1.76
4	57	*fshr*-c	32.5	403	2.8	0.69
4	58	wt	35.0	550	15.4	2.80
4	59	*fshr*-c	34.0	505	27.7	5.49
4	60	wt	30.0	347	18.3	5.27
4	61	wt	32.5	387	22.9	5.92
4	62	wt	33.5	434	25.1	5.78
4	63	wt	33.0	462	20.4	4.42
4	64	*fshr*-c	37.0	604	23.4	3.87
4	65	wt	32.5	387	0.1	0.03
4	66	*fshr*-c	35.0	486	22.5	4.63
4	67	*fshr*-c	33.0	405	9.0	2.22
4	68	*fshr*-c	37.0	600	28.1	4.68
4	69	*fshr*-c	35.5	553	20.2	3.65
4	70	wt	35.0	514	27.3	5.31
4	71	*fshr*-c	36.0	595	25.0	4.20
4	72	wt	37.5	650	23.6	3.63
4	73	*fshr*-c	37.5	642	16.3	2.54
4	74	*fshr*-c	33.0	401	14.8	3.69
4	75	wt	36.5	614	31.3	5.10
4	76	wt	35.0	539	26.8	4.97
4	77	*fshr*-c	34.0	444	5.1	1.15
4	78	wt	32.0	416	18.8	4.52
4	79	wt	36.0	553	20.7	3.74
4	80	*fshr*-c	36.0	537	21.3	3.97
4	81	wt	32.0	386	11.8	3.06
4	82	wt	31.5	366	17.7	4.84
4	83	wt	31.0	361	15.7	4.35
4	84	wt	35.0	495	21.7	4.38
4	85	*fshr*-c	35.0	522	20.9	4.00
4	86	*fshr*-c	30.0	306	0.1	0.03
4	87	*fshr*-c	35.0	461	12.4	2.69
4	88	*fshr*-c	30.0	284	0.1	0.04
4	89	*fshr*-c	26.5	226	0.1	0.04
8	90	*fshr*-c	41.0	809	19.8	2.45
8	91	*fshr*-c	46.0	1104	34.5	3.12
8	92	*fshr*-c	41.0	777	14.6	1.88
8	93	*fshr*-c	39.0	731	0.5	0.06
8	94	*fshr*-c	45.0	1066	0.8	0.08
8	95	*fshr*-c	42.5	910	0.8	0.08
8	96	*fshr*-c	34.5	505	22.3	4.42
8	97	*fshr*-c	37.6	610	10.4	1.70
8	98	*fshr*-c	40.0	807	1.5	0.18

8	99	*fshr*-c	43.0	1003	2	0.20
8	100	wt	46.0	1225	0.4	0.03
8	101	*fshr*-c	48.0	658	32.6	4.95
8	102	wt	37.5	650	39.2	6.04
8	103	*fshr*-c	40.5	843	23.3	2.77
8	104	wt	38.5	678	39.2	5.78
8	105	wt	37.5	612	29.2	4.78
8	106	*fshr*-c	30.5	413	22.4	5.44
8	107	*fshr*-c	45.0	1130	1.1	0.10
8	108	wt	40.0	773	31.8	4.12
8	109	wt	46.5	1258	62.7	4.99
8	110	*fshr*-c	40.0	755	25.1	3.32
8	111	wt	42.3	854	38.1	4.46
8	112	*fshr*-c	42.0	938	1.1	0.11
8	113	wt	38.5	640	33.2	5.19
8	114	wt	35.0	419	11.2	2.68
8	115	*fshr*-c	37.5	584	0.2	0.03
8	116	wt	38.0	617	31.7	5.14
8	117	*fshr*-c	36.5	583	13.6	2.33
8	118	*fshr*-c	43.0	1054	47.0	4.46
8	119	wt	42.5	991	49.3	4.98
8	120	*fshr*-c	46.0	1187	0.7	0.06
8	121	*fshr*-c	30.4	861	0.8	0.10
8	122	wt	39.0	744	32.1	4.32
8	123	*fshr*-c	47.0	1173	1.2	0.10
8	124	*fshr*-c	40.5	787	22.6	2.87
8	125	*fshr*-c	39.0	668	9.5	1.41
8	126	*fshr*-c	40.0	752	18.6	2.48
8	127	*fshr*-c	40.5	788	18.9	2.40

Table S2. Evaluation of indel rate in a selection of F0 *fshr* crispant (*fshr*-c) males from the samplings 4 or 8 months after the start of the maturation-inducing treatment. Mutation analysis of fat fin tissue includes % wt sequence, % in-frame indels (del3), % frameshift indels and total reads.

Fish group	ID	WT (%)	In-frame (%)	Frameshift (%)	Total reads
fshr-c 4m	49	14.6	56.4	29.0	42184
fshr-c 4m	50	6.8	38.8	54.4	40404
fshr-c 4m	52	5.7	58.1	7.9	49969
fshr-c 4m	54	3.1	74.6	22.3	43060
fshr-c 4m	56	16.0	46.9	37.1	39242
fshr-c 4m	59	53.4	19.3	27.2	38578
fshr-c 4m	64	0.5	65.0	34.5	35726
fshr-c 4m	67	20.3	48.5	31.2	37427
fshr-c 4m	68	45.9	23.3	30.9	39771
fshr-c 4m	69	6.7	40.0	53.3	26691
fshr-c 4m	71	18.4	40.0	41.7	30691
fshr-c 4m	73	5.2	63.6	31.2	35399
fshr-c 4m	77	19.3	37.4	43.3	39894
fshr-c 4m	80	30.5	48.3	21.3	30088
fshr-c 4m	85	3.4	63.6	32.9	43440
fshr-c 4m	87	28.5	46.0	25.5	35900
fshr-c 8m	91	16.7	46.2	37.1	41384
fshr-c 8m	92	8.3	37.4	54.4	38548
fshr-c 8m	93	10.5	64.6	24.9	47453
fshr-c 8m	94	5.3	36.4	58.3	36781
fshr-c 8m	96	27.5	57.4	15.1	34670
fshr-c 8m	97	40.6	27.4	32.0	46637
fshr-c 8m	99	14.8	42.2	43.0	42958
fshr-c 8m	101	4.9	36.8	58.2	35206
fshr-c 8m	103	10.9	48.4	40.6	36255
fshr-c 8m	106	5.4	56.7	38.0	38590
fshr-c 8m	107	2.4	63.6	34.0	45788
fshr-c 8m	110	60.3	16.5	23.2	37179
fshr-c 8m	112	32.5	39.7	27.8	38884
fshr-c 8m	115	0.1	66.9	32.9	35636
fshr-c 8m	117	24.6	33.6	41.8	35386
fshr-c 8m	118	11.0	36.1	52.9	37894
fshr-c 8m	120	43.5	22.5	34.1	38127
fshr-c 8m	121	2.2	54.7	43.0	36297
fshr-c 8m	123	3.1	43.0	53.9	37392
fshr-c 8m	124	35.6	45.8	18.5	34644
fshr-c 8m	125	15.3	51.7	33.1	38307
fshr-c 8m	126	9.9	64.1	26.0	35309
fshr-c 8m	127	1.7	67.1	31.3	37846

Table S3. Evaluation of indel rate in *fshr* crispant broodstock chosen for crossings to produce an F1 generation. Mutation analysis of fat fin tissue includes % WT sequence, % in-frame indels, % frameshift indels and total reads.

Fish ID	WT (%)	In-frame (%)	Frameshift (%)	Total reads
Male 1	7.9	51.3	40.9	42377
Male 2	3.5	64.7	31.9	42489
Female 1	40.6	28.7	30.8	40271
Female 2	11.8	46.7	41.6	26762
Female 3	0.6	54.0	45.5	36575

Table S4. Biometrics of F1 *fshr* mutant and wt (+/+) males. Samplings took place after 8 months (October 15th) and 12 months (February 26th, 2021) after the start of the maturation-inducing treatment (6 weeks of constant light and 16°C water temperature, starting February 4th, 2020).

Sampling	ID	Length (cm)	Weight (g)	Gonad w. (g)	GSI (%)	Maturity	Genotype
8	269	44.5	1148	0.5	0.04	Immature	-/-
8	271	46.7	1480	0.9	0.06	Immature	-/-
8	280	46.5	1362	1.0	0.07	Immature	-/-
8	283	41.5	920	0.8	0.09	immature	-/-
8	287	44.5	1236	0.5	0.04	Immature	-/-
8	292	51.0	1676	3.1	0.18	Immature	-/-
8	294	48.2	1642	3.0	0.18	Immature	-/-
12	317	45.0	1256	0.6	0.05	immature	-/-
12	322	58.5	2953	1.3	0.04	Immature	-/-
12	324	51.0	1810	1.5	0.08	Immature	-/-
12	342	49.0	1675	0.6	0.04	Immature	-/-
12	344	46.0	1355	0.6	0.04	Immature	-/-
8	278	42.5	1094	0.8	0.07	Immature	if/if
8	279	42.3	972	42.0	4.32	Mature	if/if
8	284	42.0	920	72.0	7.83	Mature	if/if
8	288	46.5	1366	1.0	0.07	Immature	if/if
8	290	43.0	1014	0.4	0.04	Immature	if/if
8	291	41.5	934	0.9	0.09	Immature	if/if
8	293	42.5	1050	3.4	0.32	Immature	if/if
8	296	43.0	1090	4.4	0.40	Immature	if/if
8	297	41.0	880	3.7	0.42	Immature	if/if
8	299	41.1	914	40.0	4.38	Mature	if/if
12	310	48.5	1348	1.8	0.13	Immature	if/if
12	320	41.3	839	34.6	4.12	Mature	if/if
12	339	53.0	2322	2.7	0.11	Immature	if/if
12	340	48.0	1385	53.2	3.84	Mature	if/if
12	346	49.0	1547	0.8	0.05	Immature	if/if
12	349	40.0	874	44.9	5.13	Mature	if/if
12	350	44.0	1098	0.7	0.07	Immature	if/if
8	270	40.7	944	50.0	5.30	Mature	if/fs
8	281	36.0	572	22.0	3.85	Mature	if/fs
8	282	43.0	1198	1.7	0.14	Immature	if/fs
12	323	42.0	855	48.5	5.67	Mature	if/fs
12	348	47.5	1312	44.8	3.42	Mature	if/fs
8	273	41.0	870	71.0	8.16	Mature	if/wt
12	312	49.0	1241	46.1	3.71	Mature	if/wt
8	272	40.6	874	0.4	0.05	Immature	+/+
12	341	43.0	1041	37.7	3.62	Mature	+/+
8	275	33.4	574	46.0	8.01	Mature	+/+
8	276	33.3	480	29.0	6.04	Mature	+/+
8	286	37.5	768	40.0	5.21	Mature	+/+

8	303	44.8	1174	69.0	5.88	Mature	+/+
8	304	32.0	400	28.0	7.00	Mature	+/+
8	305	34.2	550	25.7	4.67	Mature	+/+
8	306	37.0	622	48.6	7.81	Mature	+/+
8	307	34.5	536	37.0	6.90	Mature	+/+
12	331	38.5	772	35.2	4.56	Mature	+/+
12	332	37.0	740	37.8	5.11	Mature	+/+
12	333	35.0	508	24.6	4.85	Mature	+/+
12	334	40.0	935	35.8	3.83	Mature	+/+
12	336	35.2	538	28.5	5.30	Mature	+/+
12	337	42.0	944	36.2	3.83	Mature	+/+
12	338	32.0	417	21.4	5.14	Mature	+/+