Fig. S1. Transgenic SLY expression in transfected cells. HEK293T cells were transfected with a Flag-*Sly1* and Flag-*Sly2* constructs and were subjected to immunostaining with an anti-FLAG antibody (green). SLY1 and SLY2 proteins were expressed, evidenced by green fluorescence. The cell nuclei are stained with DAPI (blue).

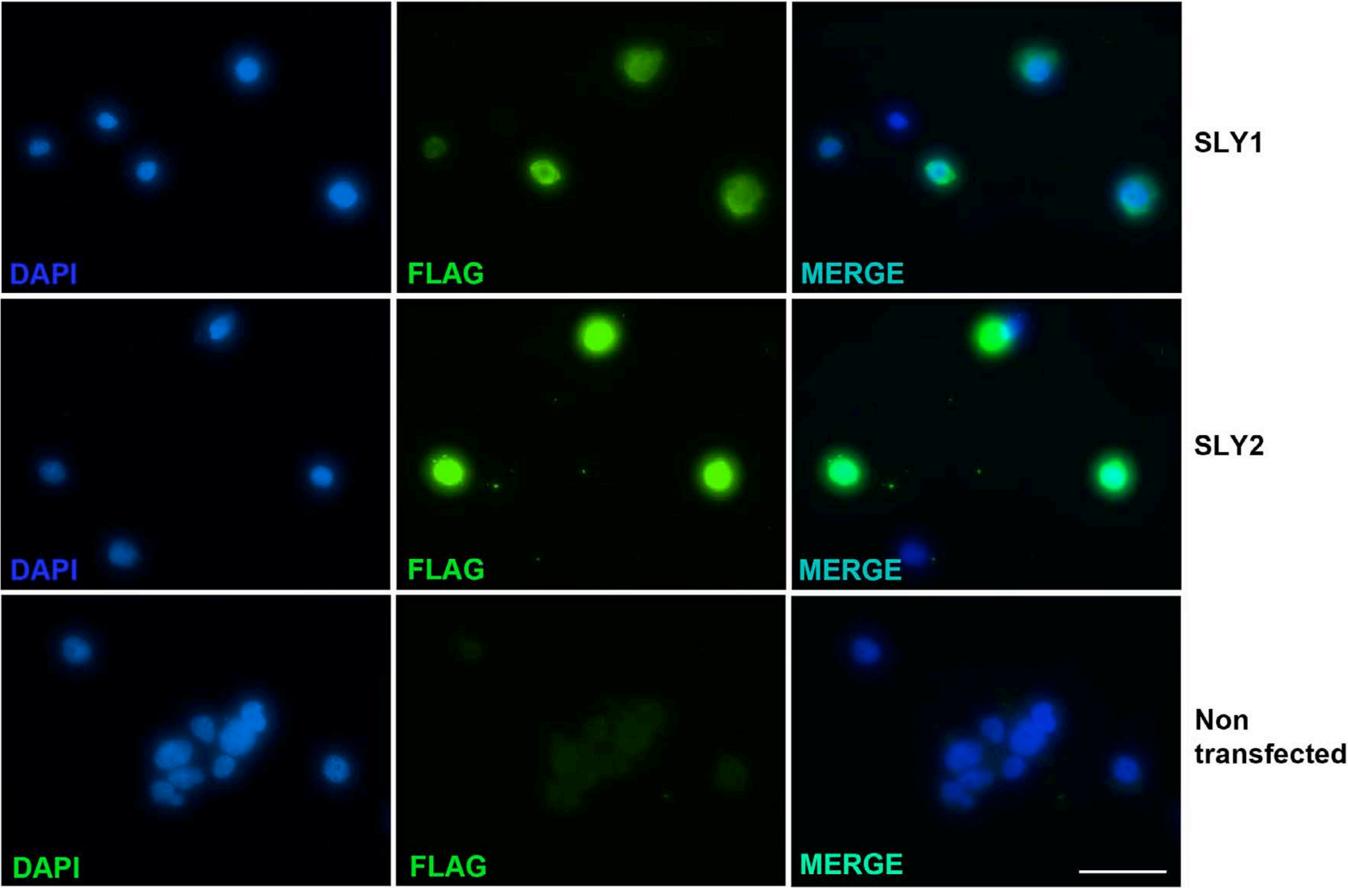


Fig. S2. *Sly* transcripts levels in whole testes from NPYq deficient and shSLY mice. *Sly* transcripts levels (A) from two *Sly*-deficient (shSLY) mouse lines, sh344 tsgic and sh367 tsgic and mice with moderate (2/3NPYq-) and severe (9/10NPYq- and NPYq-2) NPYq deficiency were compared by real-time RT-PCR with spermatid specific *Acrv* gene as a loading control. The controls were negative siblings of shSLY mice and XYRIII wild-type males; there were no differences between controls so the data from all controls were pooled. The number of males was n=3 for NPYq/*Sly*-deficient genotypes and n=10 for controls. In B, *Sly1* and *Sly2* transcript levels in whole testes from shSLY transgenic mice and 2/3NPYq- mice measured by semi-quantitative RT-PCR, quantified with ImageJ software, and normalized to spermatid specific *Acrv* gene are shown. The number of males was n=3 for sh344, sh367 and 2/3NPYq-, and n=6 for controls. All graphs are means ± SEM. Statistical significance (P<0.05): * vs. control, ** SLY1 vs. SLY2; columns marked with the same letter are not different from each other (t-test). Primer sequences are shown in Table S3.

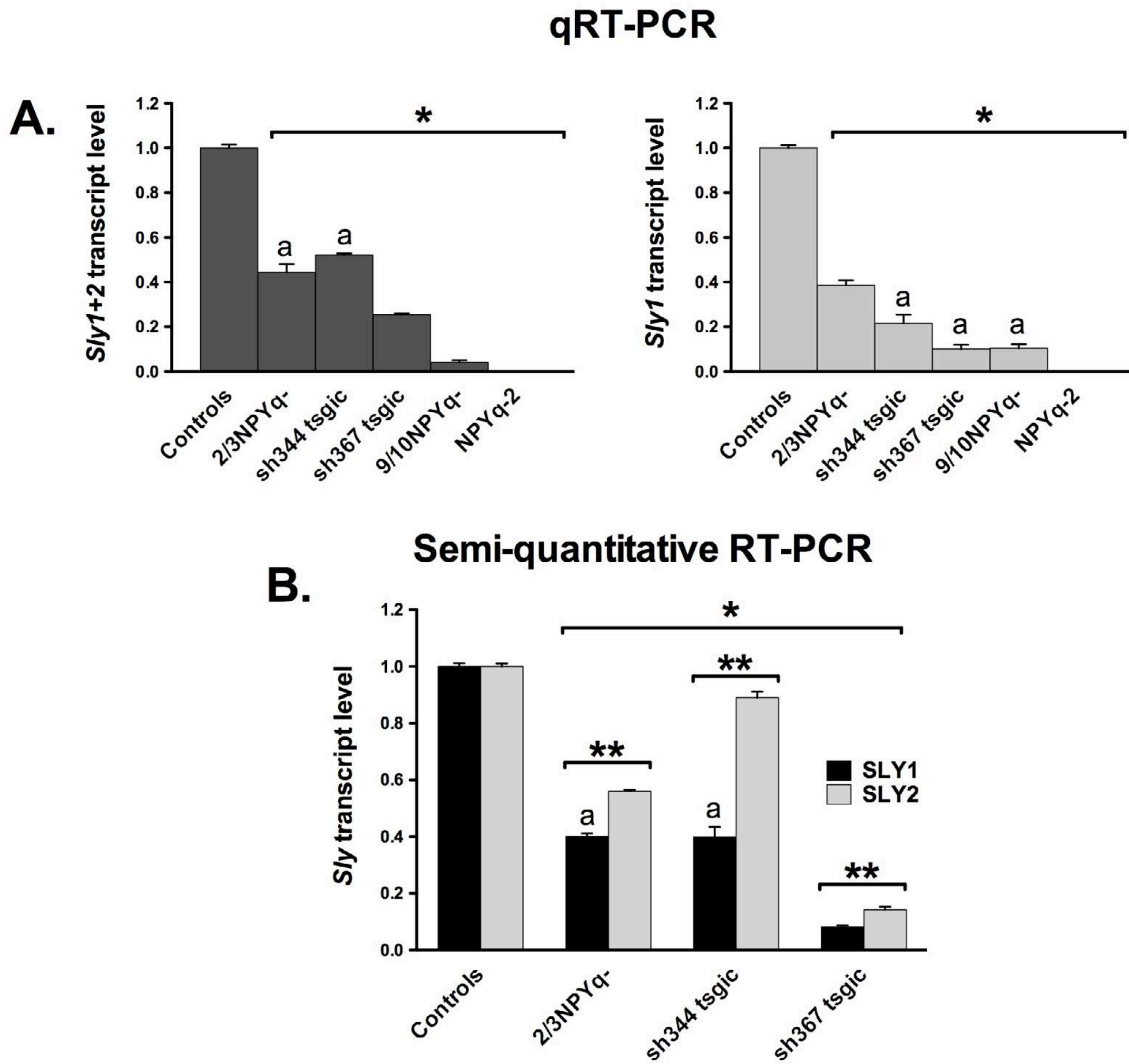


Fig. S3. Oocyte activation, arrest and chromosome normalcy after ICSI with sperm from *Sly*-deficient mice – comparison with NPYq deficient genotypes. The data show percentages of oocytes arrested (A), oocytes with normal paternal chromosomes (B), and aberration rate reflected by total number of aberrations divided by the number of oocytes examined (C). The percentages were calculated as explained in legend to Table S3. The number of examined males was: n= 12, n=3, n=5, for control, sh344, and sh367 and n=2 for 2/3NPYq-, 9/10NPYq- and NPYq-2.

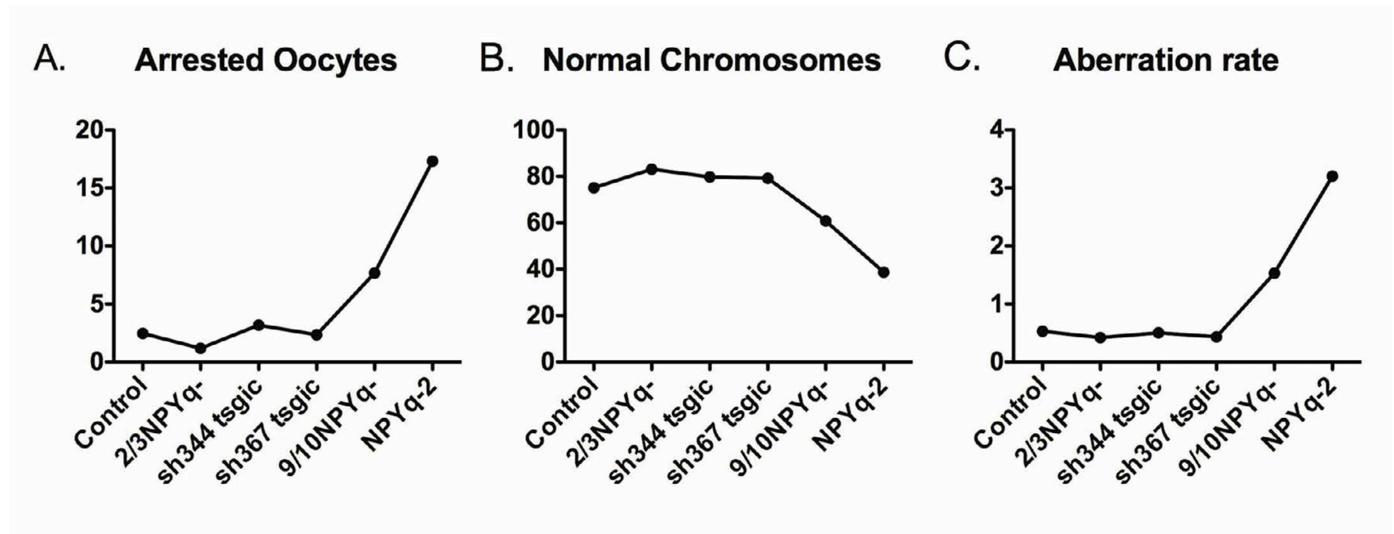


Figure S4. Oocyte activation, arrest and chromosome normalcy after ICSI with sperm from *Sly*-deficient mice, preselected according to the severity of headshape defects. Sperm originating from the same frozen-thawed cauda epididymal sperm samples were preselected during ICSI into two groups: sperm with slight (categories S1-2 shown in Fig. 3) and gross (G4-6 shown in Fig. 3) headshape defects. The data show percentages of oocytes activated, oocytes arrested, and oocytes with normal paternal chromosomes. The percentages were calculated as explained in legend to Table S1. Sperm samples were from 4 different *Sly*-deficient males, injections were performed 6 times, and at least 37 (range 37-69) oocytes were assessed for each measured parameter per group. No differences were noted between oocytes injected with sperm with slight and gross headshape defects (Fisher's Exact Test).

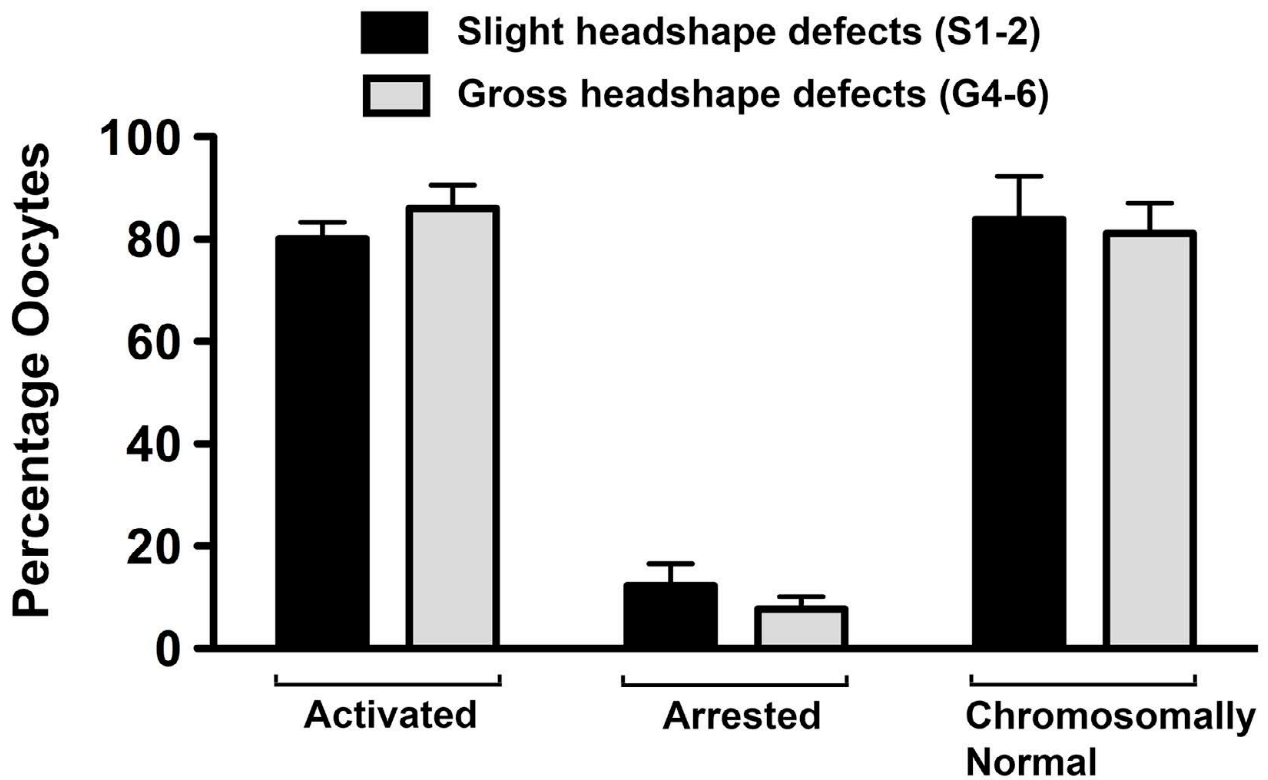
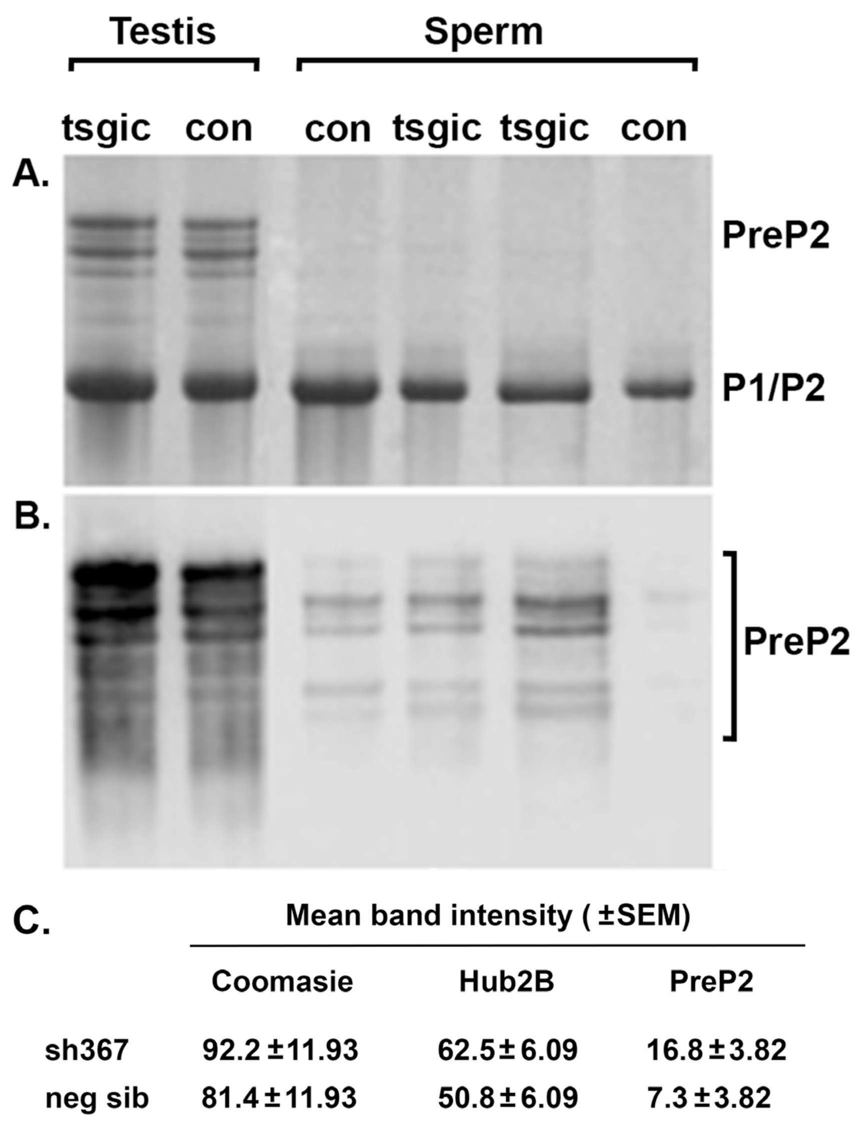


Figure S5. Sperm nuclear protein analysis. To test whether increased sperm DNA damage resulted from abnormal protamination of sperm chromatin we examined epididymal sperm from sh367 mice using the strategy described earlier for NPYq deficient mice. The figure shows a representative acid-urea gel separation of nuclear proteins extracted from cauda epididymal sperm corresponding to the same sperm number, and testes from *Sly*-deficient (tsgic) and non-transgenic negative siblings serving as controls (con). A: Coomasie blue stained gel; B: Immunoblot with preP2 antibody recognizing PreP2; C: Mean intensity of bands representing mature protamines (Coomasie and Hub2B) and immature protamines (preP2). The analysis was performed with two-way ANOVA, with gel and genotype as factors, from which the genotype means and errors were derived. No statistically significant differences between the genotypes were noted. P1 and P2 =protamine 1 and 2, respectively; PreP2 = premature forms of protamine 2 and the antibody detecting them, Hub2B antibody recognizing mature protamine 2; the Hub2B blot is not shown due to accidental photo loss. Three independent gels were run and 5 males were tested per genotype. No preP2 bands originating from sperm were detected on any of Coomassie blue stained gels. When the gels were blotted with anti-preP2 antibody, bands were detected in sperm from 4 out of 5 *Sly*-deficient males and in 2 out of 5 control males.



	Coomasie	Hub2B	PreP2
sh367	92.2 ± 11.93	62.5 ± 6.09	16.8 ± 3.82
neg sib	81.4 ± 11.93	50.8 ± 6.09	7.3 ± 3.82

Table S1. The results of ICSI with sperm from *Sly*- deficient mice.

Genotype	Sperm source	Sperm status	No of oocytes injected	No of oocytes survived (%)[1]	No of oocytes activated (%)[2]	No of oocytes arrested at PNs stage (%)[3]	No of metaphases examined	No of metaphases with normal chromosomes (%)[4]	Aberration rate
sh367 (n=5)	Epi	Fresh	146	110 (75)	102 (93)	1 (0.98)	62	51 (82)	0.48
	Testis		170	114 (67)	108 (95)	1 (0.93)	59	55 (93)[b]	0.18
	Epi	Frozen	155	126 (81)	107 (85)	6 (5.61)[a]	61	46 (75)	0.46
	Testis		172	119 (69)	109 (92)	2 (1.83)	43	26 (60)	1.35
sh344 (n=3)	Epi	Fresh	96	59 (61)	54 (92)	5 (9.26)	21	14 (67)	0.81
	Testis		95	74 (78)	68 (92)	1 (1.47)	30	29 (97)	0.03
	Epi	Frozen	97	63 (65)	60 (95)	2 (3.33)	37	30 (81)	0.49
	Testis		101	71 (70)	70 (99)	0 (0)	35	25 (71)	0.71
neg sib (n=8)	Epi	Fresh	307	189 (62)	179 (95)	6 (3.35)	77	58 (75)[c]	0.45
	Testis		277	163 (59)	150 (92)	6 (4.00)[b]	52	48 (92)[b]	0.15
	Epi	Frozen	278	177 (64)	158 (89)	2 (1.27)	83	62 (75)	0.40
	Testis		257	182 (71)	170 (93)	0 (0.00)	113	77 (68)	0.96

Percentage calculated from: [1] oocytes injected; [2] oocytes survived; [3] oocytes activated; [4] metaphases examined. Aberration rate = total number of aberrations divided by the number of oocytes examined. Statistical significance: [a] different from respective sperm type in neg sib (P<0.05, Fisher's Exact Test); [b] different from frozen testis within genotype; [c] different from fresh testis within genotype (P<0.05, Mantel-Haenszel test).

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Table S3. Primer sequences.

Gene name	Primer name	Primer sequence	Reference
Primers used to produce U6shSLY vector sequences			
U6 Forward primer: 5’-ATCCTCTTAAGTCGACGCCGCCATCTCTAG-3 sh344 Reverse primer: 5’-ATCCTCTTAAGAAAAAAGGACATGAAGTAGGCAGTATCCCATCTGTGGCTTTACAGATACTGCCTACTTCATGTCCAAACAAGGCTTTTCTCCAAGG-3’ [This study] sh367 Reverse primer: 5’-AAAAAAGGATAAATCTGGAGATGACACCCATCTGTGGCTTTACAGTGTCATCTCCAGATTTATCCAAACAAGGCTTTTCTCCAAGGG-3’ (Cocquet et al., 2009)			
Primers used to identify transgenic animals carrying U6sh344 and U6sh367 constructs			
sh344	*sh344-F*	5’-TAGCGCTACCGGACTCAGAT-3’	This study
	sh344-R	5’-GTCCTCCTTGAAGTCGATGC-3’	This study
sh367	*sh367-F*	5’-ACGTAAACGGCCACAAGTTC-3’	(Cocquet et al., 2009)
	sh367-R	5’-GTCCTCCTTGAAGTCGATGC-3’	
Real-time PCR primers			
Sly Global	*Sly Global-F*	CATTTATAAGACGCTTCACATAAAG	(Cocquet et al., 2009)
	Sly Global-R1	TCCTCCATGATGGCTCTTTC	
	Sly Global-R2	ATTCTCCATGATGGCTCTTTC	
Sly Long	*Sly Long-F*	GAAGACATGGGACATGAAGTAGG	(Cocquet et al., 2009)
	Sly Long-R1	Same as for *Sly* Global	
	Sly-Long-R2	Same as for *Sly* Global	
ß-actin	*ß-actin-F*	GGCACCACACCTTCTACAATG	(Garcia et al., 2006)
	ß-actin-F	GTGGTGGTGAAGCTGTAGCC	
Slx	*Slx-F*	TTCAGATGAAGAAGAAGAGCAGG	(Ellis et al., 2005)
	Slx-R	TCCATATCAAACTTCTGCTCACAC	
Slx-like	*Slxl1-F*	TTGGAGGACGCTCATTCTG	(Ellis et al., 2005)
	Slsl1-R	ACGACTTGTTGTTGATCATCTCC	
Actrt1	*Actrt1-F*	CTCAAAAATGGTCTGCAACAGC	(Ellis et al., 2005)
	Actry1-R	TCTTGATAGGGGTTCCCTCAAA	
Ssty1	*Ssty1-F*	AGAAGGATCCAGCTCTCTATGCT	(Ellis et al., 2005)
	Ssty1-R	CCAGTTACCAATCAACACATCAC	
Ssty2	*Ssty2-F*	CAGGTGCCATTCTTACAGGACTAT	(Ellis et al., 2005)
	Ssty2-R	ACCCAGGAACCTATTAAGAAGTCAT	
Asty	*Asty1-F*	GRGGAGTAGAACTCATCATC	(Ellis et al., 2005)
	Asty1-R	CAGGAGATGACTAACATAGCA	
Ubb	*Ubb-F*	GAGGGGTGGCTATTAATTATTCG	(Akerfelt et al., 2008)
	Ubb-R	CTAAACTTAAATTGGGGCAAGTG	
Mgclh	*Mgclh-F*	CCTTTACGTGTGACCTTTACCAG	(Ellis et al., 2005)
	Mgclh-R	CTGAATATGACATTTCGGATATGGT	
Semi-quantitative PCR primers			

Sly	*Sly-Orf-F*	ATGGCTCTTAAGAAATTGAAGGT	This study
Sly	*Sly-Orf-R*	TTCTTAGTTCTTGGTCCCCAAGTTC	This study
ß-actin	*ß-actin-F*	Same as for real-time PCR	(Garcia et al., 2006)
	ß-actin-F	Same as for real-time PCR	

References to Table S2

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