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Supplemental Material to:

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Epigenetic regulation of planarian stem cells by the SET1/MLL family of histone methyltransferases

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SUPPLEMENTARY INFORMATION

Supplementary Methods

Quantitative real-time polymerase chain reaction (qPCR)

RNA was extracted with Trizol reagent (Invitrogen) from control worms and irradiated worms three days after treatment with 60 Gy of γ-irradiation. Samples were treated with TURBO DNA-free (Ambion) and then further purified using the RNeasy MinElute cleanup kit (Qiagen). cDNA was synthesized with GoScript Reverse Transcriptase (Promega) with an oligo dT_{15} primer. qPCR was performed on a Bio-Rad CFX Connect Real-Time System using SsoAdvanced SYBR Green Supermix (Bio-Rad) with a two-step cycling protocol and annealing/extension temperature of 58.5°C. Two technical replicate PCRs were performed on each of two separate control and irradiated sample sets. The relative amount of each *set1/mll* family target was normalized to that of *Smed-β-tubulin* (DN305397)[77] for each sample and averaged across the technical replicates before comparing between biological replicates.

Supplementary Figures

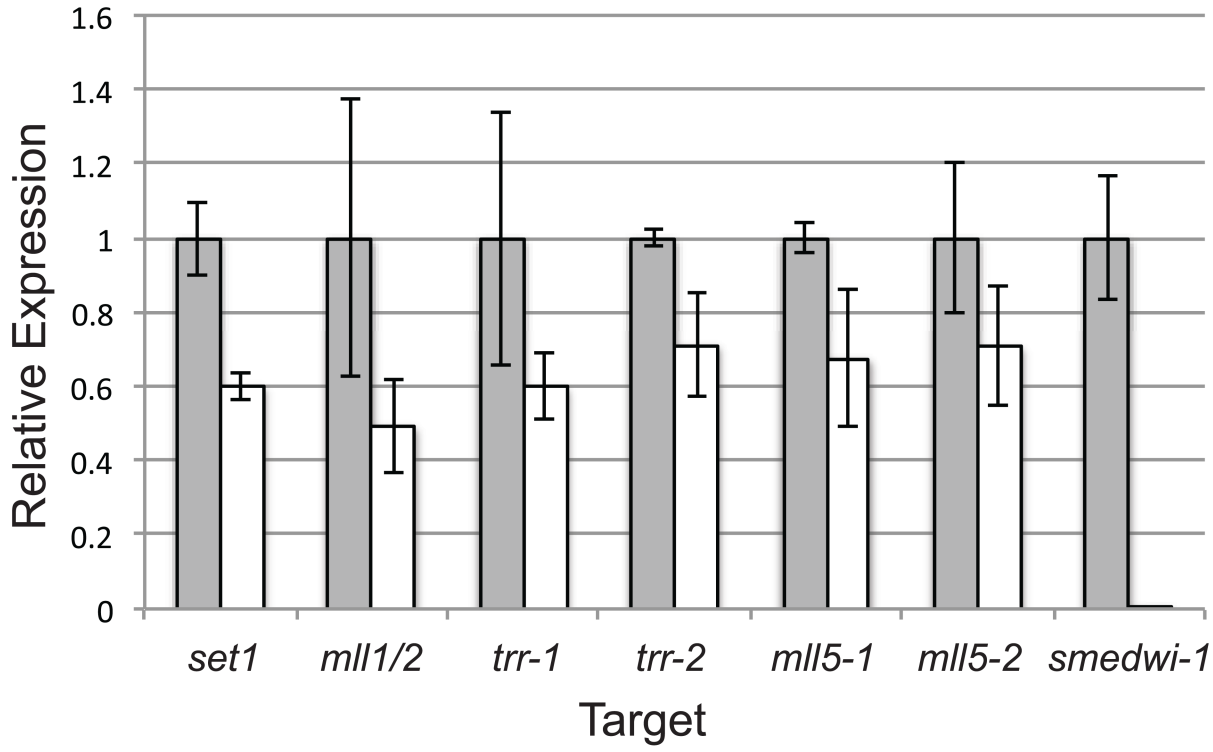

Figure S1: Reduction of *set1/mll* family mRNA expression following γ-irradiation. Relative expression levels of *set1/mll* gene family members measured in samples from control animals or animals treated with 60 Gy of γ-irradiation. Graphs show the mean of two biological replicates ± standard deviation, proportionately scaled to set the control means equal to one. *Smedwi-1*, which is expressed exclusively in the stem cells, was used a positive control for the γ-irradiation.

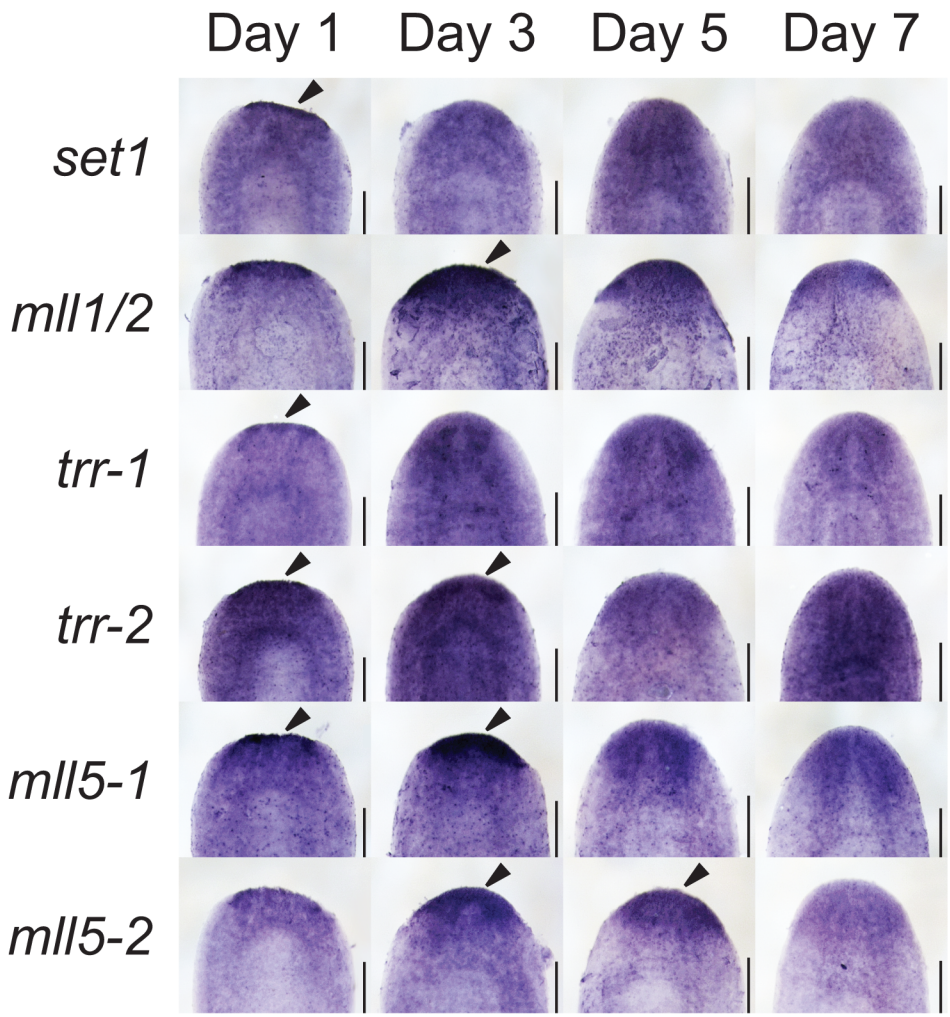

Figure S2: *set1/mll* mRNA expression patterns during regeneration. Whole-mount *in situ* hybridization to *set1/mll* family genes over a time course of regeneration. Images show the regenerating heads of trunk pieces that were amputated anterior to the pharynx fixed after the indicated number of days. Arrowheads point to blastema expression on the day(s) that show the strongest up-regulation in that tissue. Scale bars = 250 μm.

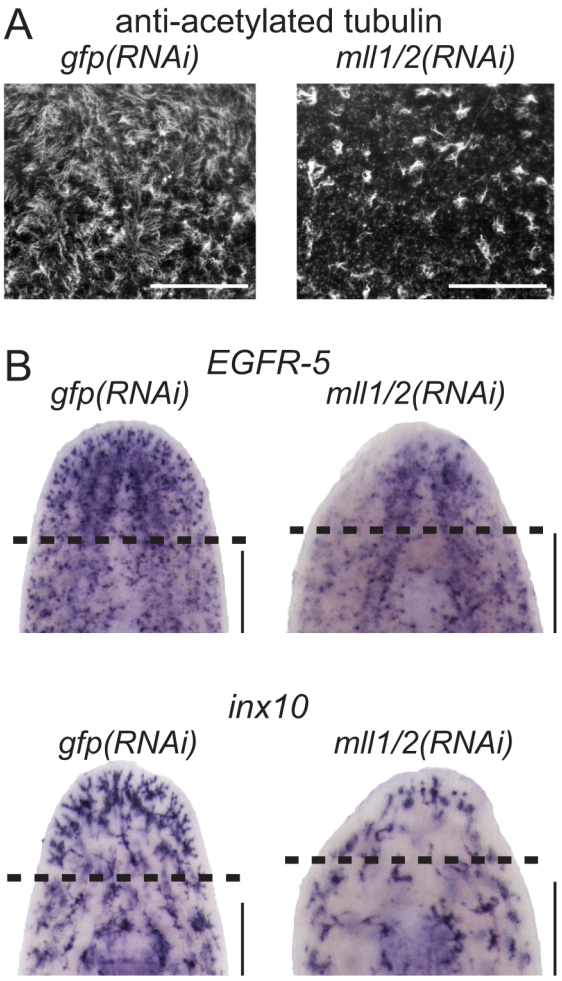

Figure S3: Effect of *Smed-mll1/2* RNAi on the ventral cilia and protonephridia. Planarians were fed six times over three weeks, amputated anterior to the pharynx, and allowed to regenerate for 10 days. (A) Anti-acetylated tubulin staining of the ventral cilia in *gfp(RNAi)* and *Smed-mll1/2(RNAi)* animals. The images were taken in an area to the right side of the pharynx. Scale bars = 100 μm. (B) Whole mount *in situ* hybridization to markers of ciliated portions of the protonephridia in *gfp(RNAi)* and *Smed-mll1/2(RNAi)* animals. Dashed lines indicate the plane of amputation. Scale bars = 250 μm.

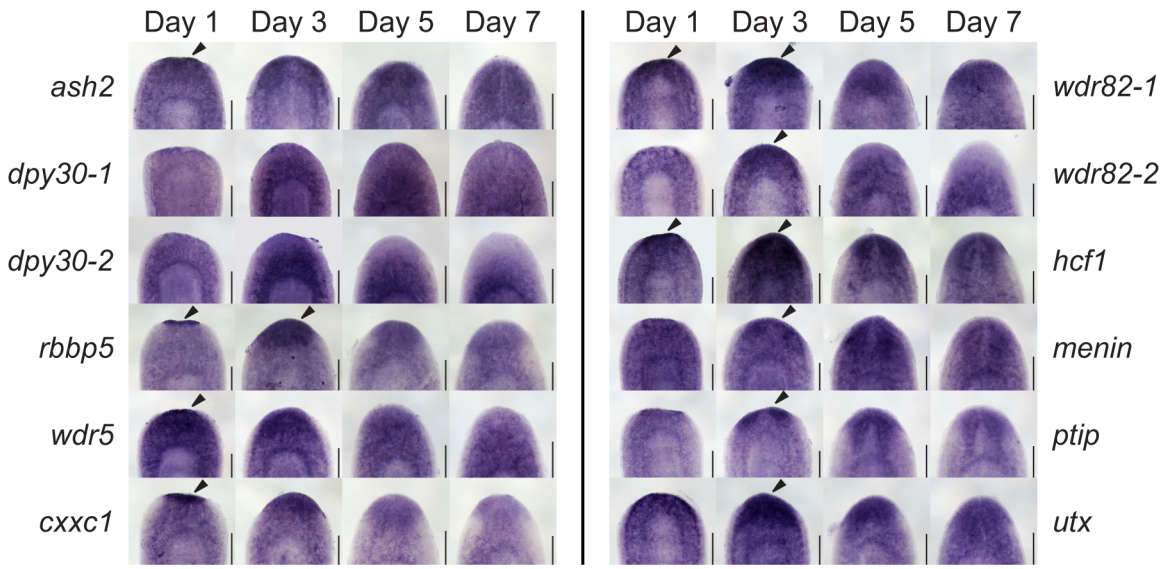

Figure S4: COMPASS expression during regeneration. Whole-mount *in situ* hybridization to COMPASS genes during a time course of regeneration. Worms were amputated anterior to the pharynx and fixed after the indicated number of days. The regenerating heads of trunk fragments are shown, and arrowheads point to blastemas with the highest up-regulation of expression. Scale bars = 250 μm.

Table S1: Summary of published transcriptome data for planarian *set1/mll* and COMPASS complex genes

Gene	Önal *et al.*	Resch *et al.*			Labbé *et al.*			
	Gene cluster	Stem cells	Progeny	Differentiated	Stem cells	Progeny	Differentiated	Class
Smed-set1	1, 2	35.58	19.15	20.30	17.74	21.63	7.43	--
Smed-mll1/2	1	73.96	49.33	30.91	67.28	77.20	11.41	SC+prog
Smed-trr-1	1	--	--	--	53.04	66.51	21.40	--
Smed-trr-2	1	--	--	--	21.59	37.42	11.22	--
Smed-mll5-1	1	45.68	33.83	32.80	25.45	41.15	13.58	--
Smed-mll5-2	1	83.22	59.79	64.57	--	--	--	--
Smed-ash2	1	29.09	17.63	14.63	73.61	49.22	12.39	SC-enriched
Smed-dpy30-1	4	--	--	--	1.30	0.55	6.14	--
Smed-dpy30-2	1	--	--	--	--	--	--	--
Smed-rbbp5	1	152.59	104.51	65.02	91.42	61.07	14.61	SC+prog, SC-enriched
Smed-wdr5	1	14.65	11.73	11.27	93.44	68.99	19.57	--
Smed-cxxc1	1	108.04	51.36	45.61	24.02	18.90	7.35	--
Smed-wdr82-1	1	160.08	107.05	83.74	117.80	103.55	17.80	SC+prog
Smed-wdr82-2	1	270.41	64.06	77.34	140.55	38.80	8.11	SC-enriched
Smed-hcf1	1	89.61	31.77	31.23	122.78	88.66	20.06	SC-enriched
Smed-menin	1	22.15	9.60	13.95	15.29	15.39	7.90	--
Smed-ptip	1, 3, 5	11.71	11.77	10.56	20.45	52.66	21.53	--
Smed-utx	1	113.34	66.15	61.61	42.72	54.77	13.02	--

Transcripts matching *set1/mll* or COMPASS genes were identified in each of three recently published transcriptomes by tblastn with the predicted full-length proteins. When more than one transcript was found for the same gene, the results were averaged. Önal *et al*. classified genes into one of six clusters based on their patterns of expression. Most of the *set1/mll* and COMPASS genes fall into Cluster 1, which contains the genes that are most highly expressed in stem cells. Average RPKM (reads per kilobase per million mapped reads) values are shown for transcripts from Resch *et al*. and Labbé *et al*. in stem cells, progeny, and differentiated tissues. Labbé *et al*. classified some of the genes into their stem cell enriched (SC-enriched) or stem cell and progeny (SC+prog) groups.

Table S2: Summary of RNAi phenotypes and penetrance

SET1/MLL family regeneration experiments

***Smed-set1(RNAi)* - 3 dsRNA feedings, 5 expts.**	
reduced blastema size	n = 18/36 trunks
no photoreceptors	n = 10/36 trunks
underdeveloped/delayed photoreceptors	n = 27/36 trunks
lesions	n = 3/47 heads or trunks
ventral curling	n = 8/47 heads or trunks
lysis	n = 25/72 heads or trunks
note: photoreceptors developed near amputation site or in pre-existing tissue	

***Smed-mll1/2(RNAi)* - 6 dsRNA feedings, 3 expts.**	
underdeveloped photoreceptors	n = 40/40 trunks
no movement/inching	n = 40/40 trunks
flat/ruffled appearance	n = 40/40 trunks

***Smed-trr-1(RNAi)* - 6 dsRNA feedings, 3 expts.**	
reduced blastema size	n = 50/50 trunks
underdeveloped photoreceptors	n = 23/50 trunks
delayed photoreceptors	n = 27/50 trunks
note: photoreceptors developed near amputation site or in pre-existing tissue	

***Smedi-trr-2(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal	n = 30/30 trunks

***Smedi-trr-2(RNAi)* - 9 dsRNA feedings, 1 expt.**	
normal	n = 10/10 trunks

***Smed-mll5-1(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal	n = 30/30 trunks

***Smed-mll5-1(RNAi)* - 9 dsRNA feedings, 1 expt.**	
normal	n = 10/10 trunks

***Smed-mll5-2(RNAi)* - 6 dsRNA feedings, 3 expts.**	
normal	n = 1/50 trunks
underdeveloped photoreceptors	n = 37/50 trunks
delayed photoreceptors	n = 12/50 trunks

Double knockdown regeneration experiments

***gfp(RNAi)/Smed-trr-1(RNAi)* - 6 dsRNA feedings, 1 expt.**	
reduced blastema size	n = 9/10 trunks
delayed photoreceptors	n = 10/10 trunks
note: photoreceptors developed near amputation site or in pre-existing tissue	

***gfp(RNAi)/Smed-trr-2(RNAi)* - 6 dsRNA feedings, 1 expt.**	
normal	n = 10/10 trunks

***Smed-trr-1(RNAi)/Smed-trr-2(RNAi)* - 6 dsRNA feedings, 1 expt.**	
reduced blastema size	n = 10/10 trunks
no photoreceptors	n = 8/10 trunks
underdeveloped photoreceptors	n = 2/10 trunks
ventral curling	n = 1/3 trunks
lysis	n = 17/20 heads or trunks
note: photoreceptors developed near amputation site or in pre-existing tissue	

***gfp(RNAi)/Smed-mll5-1(RNAi)* - 6 dsRNA feedings, 1 expt.**	
normal	n = 10/10 trunks

***gfp(RNAi)/Smed-mll5-2(RNAi)* - 6 dsRNA feedings, 1 expt.**	
normal	n = 4/10 trunks
underdeveloped photoreceptors	n = 3/10 trunks
delayed photoreceptors	n = 3/10 trunks

***Smed-mll5-1(RNAi)/Smed-mll5-2(RNAi)* - 6 dsRNA feedings, 1 expt.**	
normal	n = 6/10 trunks
delayed photoreceptors	n = 4/10 trunks

COMPASS and COMPASS-like regeneration experiments

***Smed-ash2(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
no photoreceptors	n = 26/30 trunks
underdeveloped photoreceptors	n = 4/30 trunks
ventral curling	n = 10/13 heads or trunks
head regression	n = 2/13 heads or trunks
lysis	n = 47/60 heads or trunks

***Smed-dpy30-1(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal regeneration	n = 20/20 trunks
inching movement	n = 20/20 trunks

***Smed-dpy30-2(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal	n = 14/40 trunks
reduced blastema size	n = 3/40 trunks
underdeveloped photoreceptors	n = 13/40 trunks
delayed photoreceptors	n = 13/40 trunks
ectopic photoreceptor	n = 2/40 trunks
note: ectopic photoreceptors formed between the normal ones and were confirmed by staining with VC-1 antibody, which detects photoreceptor neurons	

***Smed-rbbp5(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
no photoreceptors	n = 25/30 trunks
delayed/underdeveloped photoreceptors	n = 4/30 trunks
lesions	n = 2/36 heads or trunks
ventral curling	n = 22/36 heads or trunks
head regression	n = 10/36 heads or trunks
lysis	n = 24/60 heads or trunks

***Smed-wdr5(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
underdeveloped photoreceptors	n = 30/30 trunks
head regression	n = 7/30 trunks
lysis	n = 1/30 heads n = 0/30 trunks

***Smed-cxxc1(RNAi)* - 6 dsRNA feedings, 2 expts.**	
delayed photoreceptors	n = 30/30 trunks

***Smed-wdr82-1(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal	n = 10/10 trunks

***Smed-wdr82-2(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 28/30 trunks
no photoreceptors	n = 26/30 trunks
delayed/underdeveloped photoreceptors	n = 4/30 trunks
lesions	n = 7/50 heads or trunks
head regression	n = 6/50 heads or trunks
ventral curling	n = 18/50 heads or trunks
lysis	n = 10/60 heads or trunks

***Smed-hcf1(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
no photoreceptors	n = 30/30 trunks
curling	n = 5/7 heads or trunks
lysis	n = 53/60 heads or trunks

***Smed-menin(RNAi)* - 6 dsRNA feedings, 2 expts.**	
normal	n = 10/10 trunks

***Smed-ptip(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
underdeveloped photoreceptors	n = 30/30 trunks
lesions	n = 5/30 heads n = 1/30 trunks

***Smed-utx(RNAi)* - 6 dsRNA feedings, 2 expts.**	
reduced blastema size	n = 30/30 trunks
underdeveloped photoreceptors	n = 30/30 trunks
freckled blastema	n = 30/30 trunks
head regression	n = 8/30 trunks
curling	n = 1/30 trunks

Homeostasis experiments

***Smed-set1(RNAi)* - 4 feedings, 4 expts.**	
normal	n = 120/141 worms
head regression	n = 10/141 worms
lesions	n = 8/141 worms
curling	n = 2/141 worms

***Smed-set1(RNAi)* - 5 feedings, 1 expt.**	
normal	n = 2/50 worms
head regression	n = 46/50 worms
lesions	n = 6/50 worms
curling	n = 15/50 worms
lysis	n = 1/50 worms

***Smed-mll1/2(RNAi)* - 9 feedings, 1 expts.**	
no movement/inching	n = 29/29 worms
head regression	n = 2/29 worms
edema	n = 3/29 worms

***Smed-trr-2(RNAi)* - 9 feedings, 2 expts.**	
normal	n = 25/49 worms
reduced size	n = 4/49 worms
lysed	n = 20/49 worms

Phenotypes observed following knockdown of each gene and how often they occurred. Animals were fed dsRNA targeting each gene the indicated number of times over the stated number of experiments (expts.). Worms in homeostasis experiments were left intact; all others were amputated anterior to the pharynx and scored during 10-14 days of regeneration. "Underdeveloped photoreceptors" refers to cases where the photoreceptors never fully developed (i.e., were smaller or less pigmented than controls throughout the experiment). "Delayed photoreceptors" were initially abnormal but were indistinguishable from controls by day 10-14. Lesions, head regression, and ventral curling categories only include worms that were alive at the end of the experiment; some worms that lysed displayed these phenotypes first.

Table S3: Accession numbers and primers

Genbank accession numbers of cloned insert sequences

clone	accession number(s)
Smed-set1	DN301487, DN308184
Smed-mll1/2	KC262346
Smed-trr-1	KC262345
Smed-trr-2	DN309269, HO004937
Smed-mll5-1	DN314063, HO006509
Smed-mll5-2	KC262344
Smed-ash2	DN296978, DN305606, KC262336
Smed-dpy30-1 RNAi	KC262337
Smed-dpy30-1 riboprobe	KC262338
Smed-rbbp5	DN308991, HO007192
Smed-wdr5	DN315983, HO007009
Smed-cxxc1	DN310044, HO005298

clone	accession number(s)
Smed-wdr82-1	DN297595, DN305959
Smed-wdr82-2	KC262342
Smed-hcf1	KC262343
Smed-menin	KC262339
Smed-ptip	DN315791, HO008340, KC262340
Smed-utx	KC262341
EGFR-5	KC262347
inx10	KC262348
Smedwi-1	DN309285, HO004953
NB.32.1g	DN298711, DN306632
agat-1	DN290976, DN303276

Sequences below Genbank's 200bp cutoff

Smed-dpy30-2 RNAi	>*Schmidtea mediterranea Smed-dpy30-2* partial cDNA CAGCCAGGAATTCAATCGGATTATTCGGTCGTTGTTTTGCCAGAACTTTAAAACCACTAAGAACAACAGGTACAACAGTCTGATCTAAATATACTCTTGCCGATAA ATTCTGTAAATTTGCATCAGAACCAGAACCCTTGGATTTATC
Smed-dpy30-2 riboprobe	>*Schmidtea mediterranea Smed-dpy30-2* partial cDNA TCATGGCTGAAGAAAACGGTACAAATTACAGATATAATGATAAATCCAAGGGTTCTGGTTCTGATGCAAATTTACAGAATTTATCGGCAAGAGTATATTTAGATC AGACTGTTGTACCTGTTGTTCTTAGTGGTTTTAAAGTTCTGGCAAAACAACGACCGAATAATCCGATTGAATTCCTGGCTG

Primers used for cloning, with restriction sites underlined

clone	forward primer	reverse primer
Smed-mll1/2	T7 universal - amplified from cDNA cloned in pBS	ATAAGAATGCGGCCGCCCATACCGTTGCGTAGATTCCG
Smed-trr-1	T7 universal - amplified from cDNA cloned in pBS	ATAAGAATGCGGCCGCGGCTACCTCGTTCCGAATCAAC
Smed-mll5-2	CCGCTCGAGTACTGGCGCGTTGCTAGCTAAGAT	ATAAGAATGCGGCCGCTCGATTTGAACGTTGCACTGCTGG
Smed-dpy30-1 RNAi	GAAATTGAATTGCCAACTAGTG	ATAAGAATGCGGCCGCGCCATATATTCAATTGGATTGGGCGGTC
Smed-dpy30-1 riboprobe	GAAATTGAATTGCCAACTAGTG	ATAAGAATGCGGCCGCCATCACATAGATTCATAGGCA
Smed-dpy30-2 RNAi	CCGCTCGAGGATAAATCCAAGGGTTCTGG	CAGCCAGGAATTCAATCGGATTATTC
Smed-dpy30-2 riboprobe	TCATGGCTGAAGAAAACGGTAC	CAGCCAGGAATTCAATCGGATTATTC
Smed-wdr82-2	CCGCTCGAGCGTCTAATACTGCTATTCATGCTTCCAC	ATAAGAATGCGGCCGCGCAAATTTCGGGTTGAACGCAACG
Smed-hcf1	CCGCTCGAGTTCCGAGCCAACTCATTTGCAACC	ATAAGAATGCGGCCGCTATGGCACTTGGAGCTCCTGGAAA
Smed-menin	CCGCTCGAGAGCAATAACGCAGCAGTTGGG	ATAAGAATGCGGCCGCAACGGTAGCTTAATGGCAGGAACG
Smed-utx	CCGCTCGAGGGCTGCTTGGACAAATCTTGGTGT	ATAAGAATGCGGCCGCGCACAGTTGAGGCAACGAGTTTCA
EGFR-5	CCGCTCGAGTGCCTAAGATGCAAACACGCCAAG	ATAAGAATGCGGCCGCTCTGACCATGTGCTCCCAGTTTGA
inx10	CCGCTCGAGATTAGCAGCGCTCAAGACCCGAAT	ATAAGAATGCGGCCGCACAAACAGAATCAGCTGAAACGTGGA
LIC-BSins	GATGGTAGTAGGCCTCGAGGTCGACGGTATCGATA	GATGGTAGTAGGCAACAAAAGCTGGAGCTCCAC
LIC-pJC532vec	CCTACTACCATCGCACAGGTATTTATTCGGCGCAAAGC	CCTACTACCATCGCTGCAGGTCGATACAGTAGAAACTCG

qPCR primers

target	forward primer	reverse primer
Smed-set1	ATGCCATTGGTTCCCGCAACT	TTGTTCTGAGCGCGCTTCTCTT
Smed-mll1/2	AAATTCCACAAGACGCGTGTGACC	TCTGGCGATATGATCGGGTTTGGT
Smed-trr-1	TTGAATCGTTGCCGGGCTTAGAGA	CCGCTCATATTAATCGGGAGTGGCAT
Smed-trr-2	ATCACGACGCGTCAACTCATCGAA	TTCCGGCAATTGATTGATCCGCAC
Smed-mll5-1	ATGTGCTTGTGGTCACCCGAATTG	AGCGTCGTATTCATCCAACGGAGA
Smed-mll5-2	ATGCGGGAAATGTTTCCGGTCTGA	CGCCACATCGGTGAATGATTCCAT
Smedwi-1	GAAACGTGAGCCTAGAGAACG	TACACATCCCCCAGCTCTTC
Smed-β-tubulin	TGGCTGCTTGTGATCCAAGA	AAATTGCCGCAACAGTCAAATA