Table S1. The number of round spermatids expressing a given gene

Gene	Chromosome	probe label	replicate	XY	2/3MSYq-	MSYq-
Ddx3x	X	biotin	replicate 1	16/102	17/100	32/105
		biotin	replicate 2	20/100	27/103	38/100
		biotin	replicate 3	20/101	26/101	36/101
Eif1ay	X	DIG	replicate 1	17/100	26/100	30/100
		DIG	replicate 2	22/100	24/101	31/100
		DIG	replicate 3	19/100	21/100	32/100
Fmr1	X	biotin	replicate 1	8/101	13/105	21/101
		biotin	replicate 2	11/101	11/100	21/101
		biotin	replicate 3	9/100	11/101	53/206
		DIG	replicate 4	11/100	N/A	19/100
Nxf2	X	DIG	replicate 1	20/101	30/100	36/103
		DIG	replicate 2	18/100	31/100	32/100
		DIG	replicate 3	17/100	33/100	30/100
Ott	X	biotin	replicate 1	21/101	23/100	30/106
		biotin	replicate 2	19/102	22/102	24/103
		biotin	replicate 3	20/101	25/100	35/101
Scml2	X	DIG	replicate 1	3/101	3/100	4/100
		DIG	replicate 2	3/100	7/100	9/100
		DIG	replicate 3	3/100	6/101	9/103
Slx	X	biotin	replicate 1	39/101	40/103	50/100
		biotin	replicate 2	66/156	48/106	57/112
		biotin	replicate 3	40/100	45/100	50/100
4930527E24RIK	X	biotin	replicate 1	37/101	45/106	69/135
		biotin	replicate 2	35/116	42/112	48/115
		biotin	replicate 3	37/100	40/100	51/100
Vsig1	X	biotin	replicate 1	13/102	17/101	35/104
		biotin	replicate 2	19/101	32/102	42/100
		biotin	replicate 3	18/101	31/101	39/100
		DIG	replicate 4	13/101	N/A	38/100
Xiap	X	biotin	replicate 1	0/101	0/100	0/100
		biotin	replicate 2	0/100	0/103	0/103
		biotin	replicate 3	0/102	0/103	0/102
Ube1y1/Zfy1	Y	biotin	replicate 1	33/105	39/100	47/101
		biotin	replicate 2	37/101	35/102	51/102
		biotin	replicate 3	37/100	40/100	50/100
Ube1y1 only	Y	DIG	replicate 1	29/100	N/A	47/100
Zfy1 only	Y	DIG	replicate 1	32/100	N/A	48/100

Gene	Chromosome	probe label	replicate	XY	2/3MSYq-	MSYq-
Uty	Y	biotin	replicate 1	9/101	13/101	27/107
		biotin	replicate 2	17/100	18/101	29/101
		biotin	replicate 3	20/103	23/100	27/100
Adam3	8	biotin	replicate 1	10/100	5/102	9/102
		biotin	replicate 2	0/101	0/100	0/100
		biotin	replicate 3	7/103	6/100	5/101
Atr	9	biotin	replicate 1	8/100	6/100	12/100
		biotin	replicate 2	12/103	13/100	21/100
		biotin	replicate 3	9/101	9/101	15/100
Brca1	11	biotin	replicate 1	72/103	71/100	73/100
		biotin	replicate 2	71/102	72/101	82/102
		biotin	replicate 3	71/100	71/103	70/100
Grhpr	4	biotin	replicate 1	61/101	N/A	52/100
		biotin	replicate 2	58/101	66/100	65/102
		biotin	replicate 3	54/100	52/100	54/102
Miwi	5	DIG	replicate	14/101	22/100	19/99
		DIG	replicate 2	16/102	16/100	16/100
		DIG	replicate 3	18/100	14/100	14/102
Pde6d	1	DIG	replicate	23/101	20/100	27/101
		DIG	replicate 2	27/100	26/100	25/101
		DIG	replicate 3	24/100	25/100	24/101
Prkdc	16	biotin	replicate 1	31/100	33/100	31/100
		biotin	replicate 2	27/100	27/103	16/75 *
		biotin	replicate 3	26/100	28/100	27/100
Zscan2	7	DIG	replicate	46/100	45/101	49/100
		DIG	replicate 2	45/100	46/103	47/105
		DIG	replicate 3	48/100	43/100	44/100

For each gene, RNA FISH was replicated three times per genotype using different individuals each time, and approximately 100 spermatids per slide were analysed for the presence of an RNA FISH signal. Within each replicate, the RNA FISH was performed under the same conditions using a probe labelled at the same time, and so the values are comparable. Spermatid expression counts were performed by two independent observers and combined to give the final values. Spermatid counts were similar whether digoxigenin or biotin probes were used.

Table S2. List of BAC, fosmid and long-range PCR products used as probes for gene-specific RNA FISH

Gene	BAC/Fosmid identifier	Source	Long range FP	Long range RP	Product size	Reference
Dbx3x	CITB-551M19	Research Genetics	N/A	N/A	N/A	Turner et al., 2006
Eif1ay	G135P63000G3	CHORI	N/A	N/A	N/A	
Fmr1	G135P65476A4	CHORI	CTGTCAGCAGGCAGCTTTTACATCCTGT	CTTGTGCGTGGACAGCATTTTGAGAGTA	12225	Mueller et al., 2008
	G135P65476A4	CHORI	ATGCCACCAAGTTCCCTACCTTCCAATA	GTGACAAATATCTCCTCCAACCCCAACA	12797	Mueller et al., 2008
Nxf2	G135P60619D5	CHORI	N/A	N/A	N/A	
Ott	RP24-278F7	CHORI	GGGTTCCTTTCTTCTTGATCTGTGTTTCC	GTCATTCACATGGATTGCTTTTGTGCAT	8893	Mueller et al., 2008
Scml2	RP24-204O18	CHORI	N/A	N/A	N/A	Mueller et al., 2008
Slx	RP23-470D15	CHORI	N/A	N/A	N/A	Reynard et al., 2007
4930527E24Rik	RP24-170G23	CHORI	TCATTCCCACCTCCTGAAAACTCCCTTA	TTTGTGATCATTCAGGCATAGTGCCAAC	8088	Mueller et al., 2008
	RP24-170G23	CHORI	TTCCTGAAGAAATCGTTGGAGATACACG	TCCGTACAAAAGGACTATTTGCCACTCA	9038	Mueller et al., 2008
Vsig1	RP24-291J23	CHORI	N/A	N/A	N/A	
Xiap	G135P601754F5	CHORI	N/A	N/A	N/A	Mueller et al., 2008
Ube1y1/Zfy1		Research Genetics	N/A	N/A	N/A	
Uty	CITB-246A22	Research Genetics	N/A	N/A	N/A	Turner et al., 2006
Ube1y1 only		Research Genetics	N/A	N/A	N/A	
Zfy1 only		Research Genetics	N/A	N/A	N/A	
Adam3	RP24-103I8	CHORI	N/A	N/A	N/A	
Atr	RP24-3994J4	CHORI	N/A	N/A	N/A	
Brca1	BMq-359C01	Gene Services	N/A	N/A	N/A	
Grhpr	RP24-460J15	CHORI	N/A	N/A	N/A	
Miwi	G135P68585C5	CHORI	N/A	N/A	N/A	
Pde6d	G135P601320F1	CHORI	N/A	N/A	N/A	
Prkdc	RP24-354G13	CHORI	N/A	N/A	N/A	
Zscan2	G135P60383D9	CHORI	N/A	N/A	N/A	Turner et al., 2006

Table S3. List of antibodies used to analyse the PMSC and chromocentre epigenetic marks

Antibody	Source	Catalogue number	Dilution
rabbit anti-SLX69-81	Eurogentec	N/A	1:100
mouse anti-_H2AX	Upstate	16-193	1:200
rabbit anti-_H2AX	Upstate	07-164	1:100
rabbit anti-H3K4me3	Abcam	ab8580	1:100
rabbit anti-H3K9Ac	Abcam	ab4441-50	1:150
rabbit anti-H3K9me3	Upstate	07-442	1:300
rabbit anti-H3K9me2	Upstate	07-212	1:100
rabbit anti-H3K36me3	Abcam	ab9050	1:200
rabbit anti-H4K8Ac	Upstate	06-760	1:100
rabbit anti-H4K12Ac	Upstate	06-761	1:150
rat anti-CBX1	Prim Singh	N/A	1:200
Alexa 488 chicken anti-mouse IgG	Molecular probes	A-21200	1:500
Alexa 488 chicken anti-rabbit IgG	Molecular probes	A-21441	1:500
Alexa 488 goat anti-rat IgG	Molecular probes	A-11006	1:500
Alexa 594 chicken anti-mouse IgG	Molecular probes	A-21201	1:500
Alexa 594 chicken anti-rabbit IgG	Molecular probes	A-21442	1:500