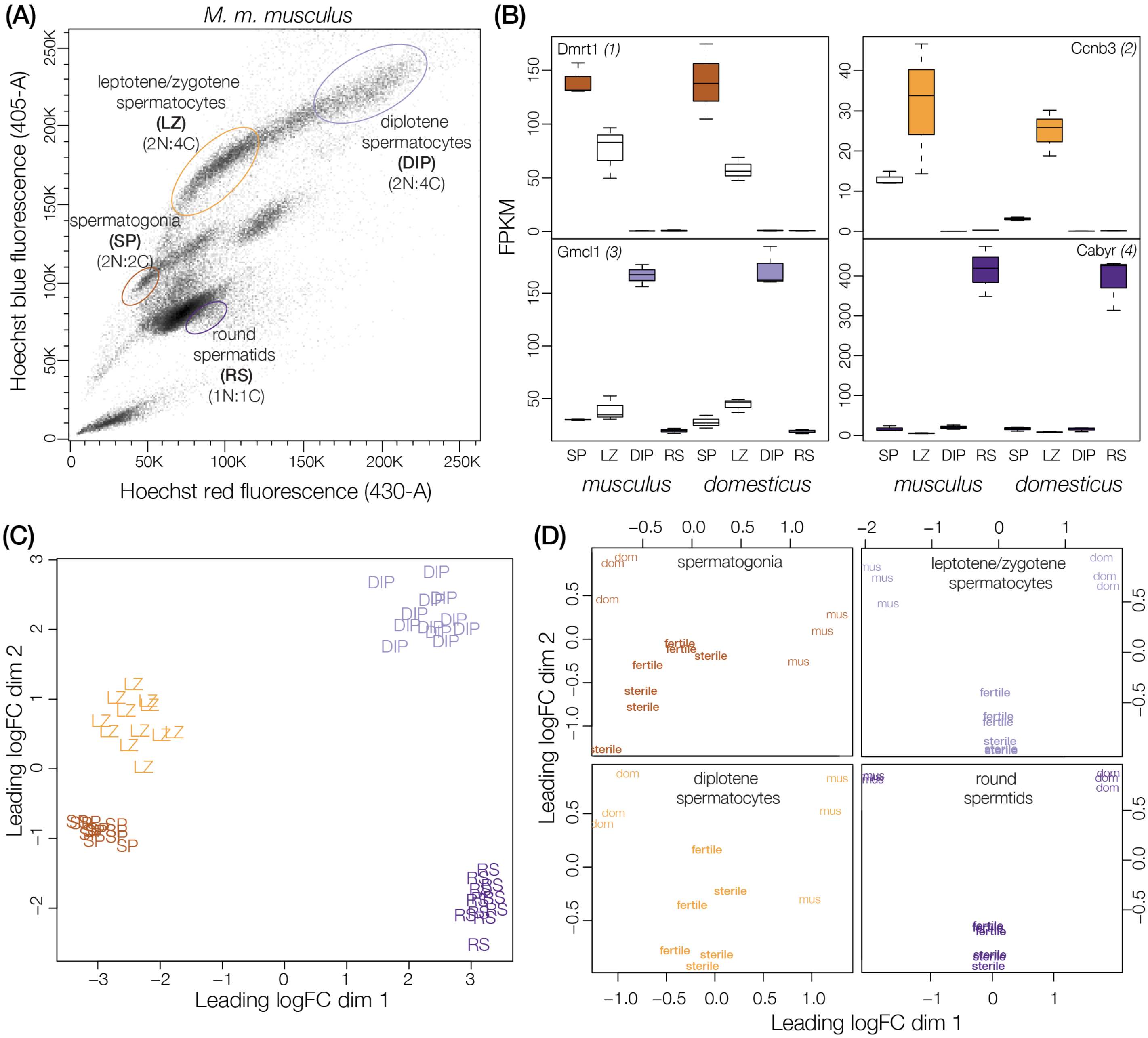

Figure S1. FACS enrichment of spermatogenesis cell populations. (A) Targeted cell populations, spanning spermatogenesis, highlighted on a profile of sorted testis cells from a fertile mouse. Dead cells were stained with propidium idodide, excited at 488 nm and detected with 585/42 bandpass filter. Live cells were stained with Hoechst, excited at 405 nm and detected with 450/40 (blue-shifted) and 610/20 (red-shifted) bandpass filters. FACS cell populations were gated to maximize the purity of each cell population. We optimized our protocol for cell purity using both gene expression metrics and microscopy. Due to the limited number of cells obtained per animal in each FACS experiment, we first assessed the purity of sorted cell populations using fertile mice, prior to collecting our final cell populations for experimental males. Initially populations were sorted into PBS with gating around boundary populations roughly corresponding to that of Getun et al. (2011). We then concentrated sorted cells using Cytospin 2, stained cells with hematoxylin and eosin, and visualized cell populations at 20X magnification. This process was iterated several times, with subsequent sorts resulting in better cell population separation, gating, and increased purity. We then further validated purity using a Thor Labs iCys Laser Scanning Cytometer to spot check sorts until fine-tuning was complete. **(B)** Gene expression (FPKM) in targeted cell populations for four autosomal genes with cell-specific expression patterns. (1) Raymond et al. 2000, *Genes Dev,* (2) Maekawa et al. 2004, *Arch Histol Cytol,* (3) Nguyen et al. 2002, *J Biol Chem,* (4) Li et al. 2007, *J Biol Chem*. **(C)** Multidimensional scaling plots of distances among cell populations and **(D)** among crosses. Distances are calculated as the root-mean-square deviation (Euclidean distance) of log2 fold changes among genes that distinguish each cell type/cross.

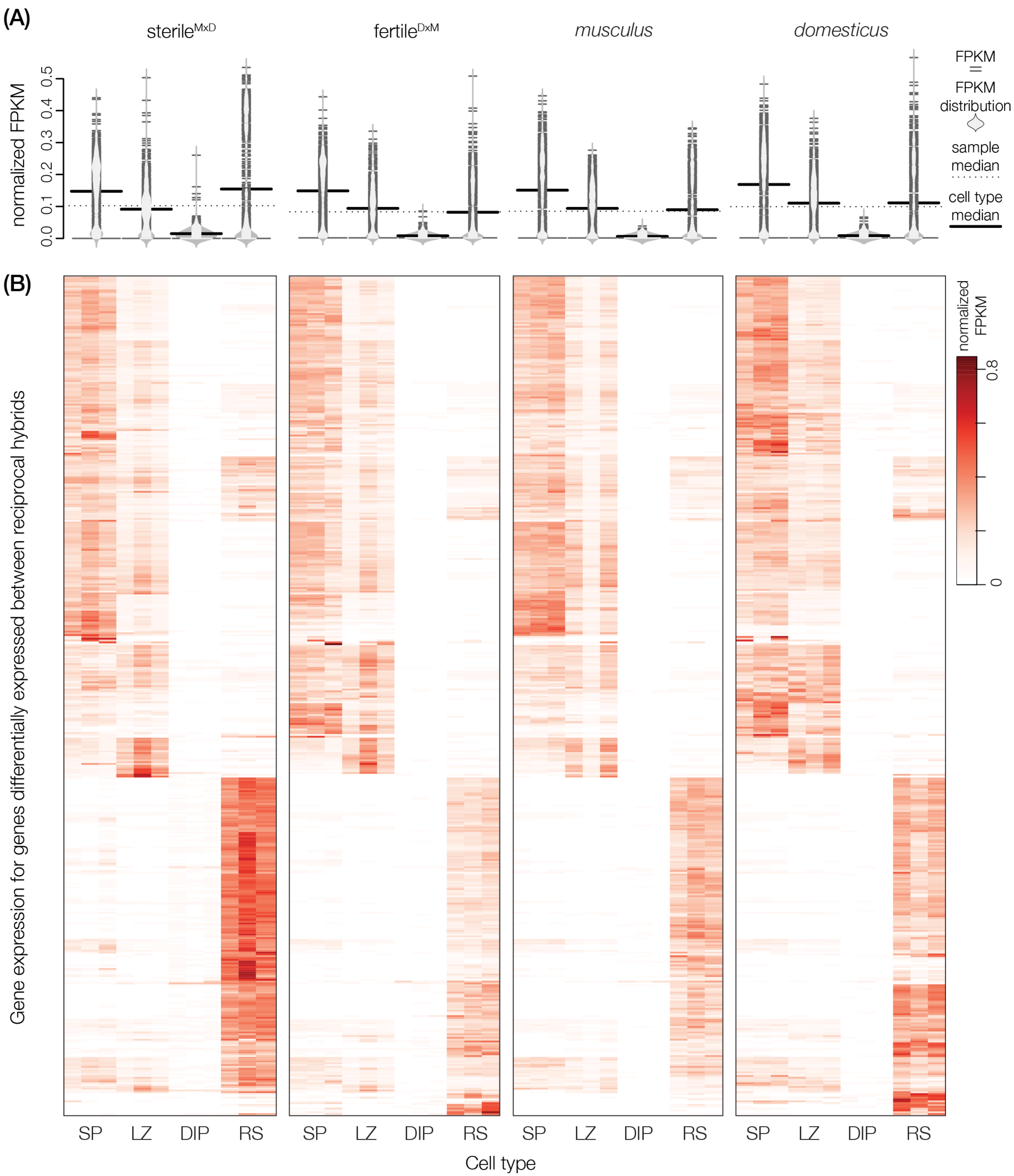

Figure S2. X-linked gene expression among different crosses. (A) Expression distributions for X-linked DE genes. Plotted with the R package beanplot (Kampstra 2008). **(B)** X-linked gene expression plotted as normalized FPKM values that are hierarchically clustered using Euclidean distance. FPKM values are normalized so that the sum of squares equals one, R package vegan (Oksanen et al. 2015).

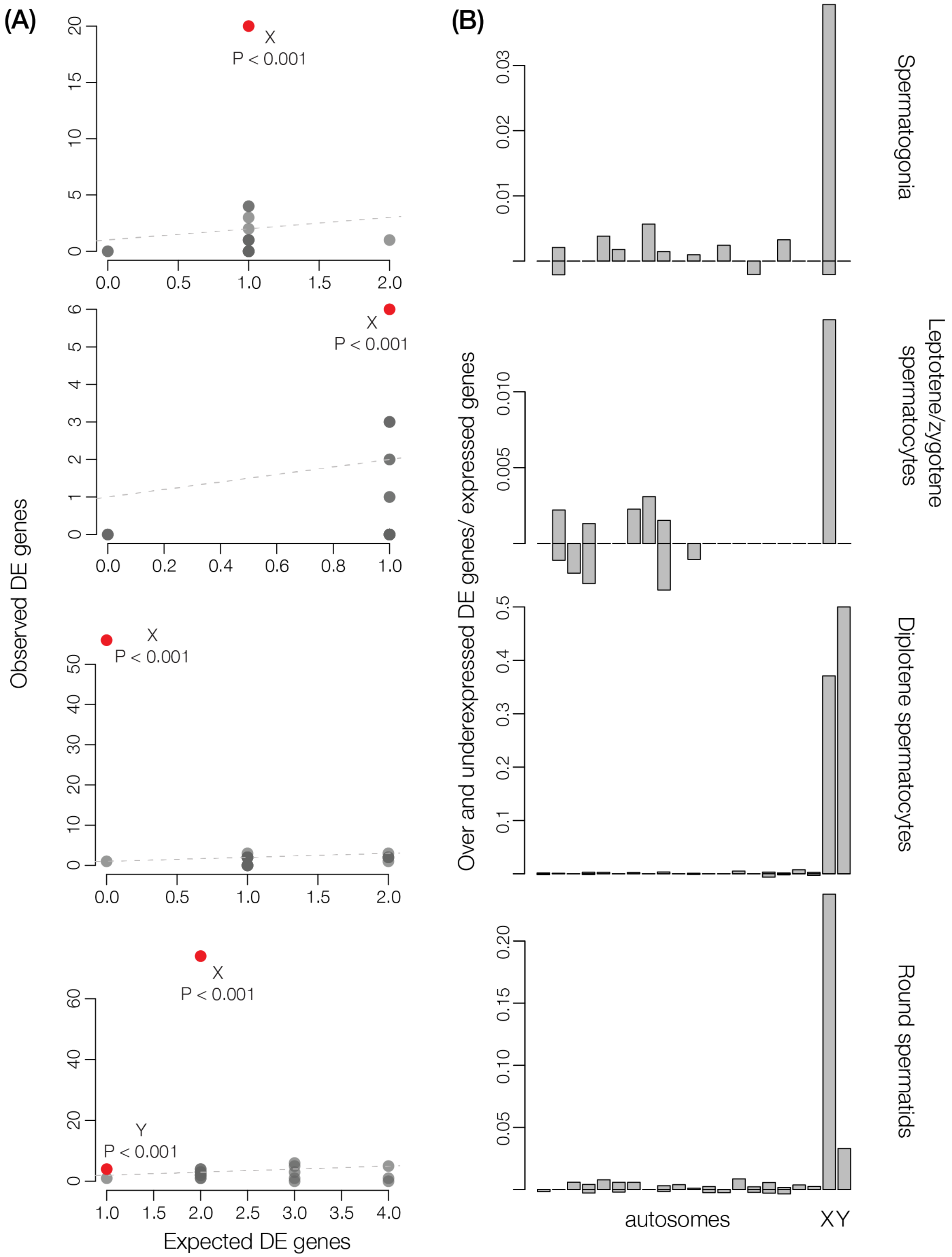

Figure S3. The X chromosome is enriched for genes with disrupted expression in sterile hybrids. (A) The ratio of observed and expected DE genes in comparisons between sterile^{MxD} hybrids and all fertile males. Expected values are based on the portion of expressed autosomal genes that are DE and are rounded to the nearest integer. Significance is based on chromosome-wise hypergeometric test for enrichment. **(B)** The proportion of over (above) or under (below) expressed genes that are DE per chromosome.

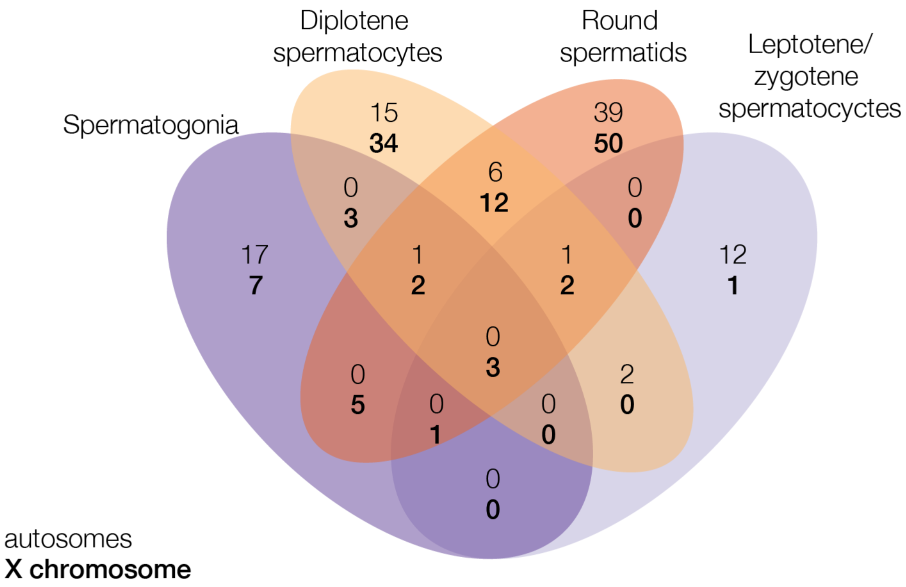

Figure S4. Disrupted gene expression varies with developmental stage. Venn diagram showing the overlap among autosomal and X-linked **(bold)** genes that were differentially expressed between sterile^{MxD} hybrids and all fertile males in different spermatogenic cell populations. There was very little overlap in DE genes among the developmental time points. Data were plotted with the R package VennDiagram (Chen and Boutros 2011).

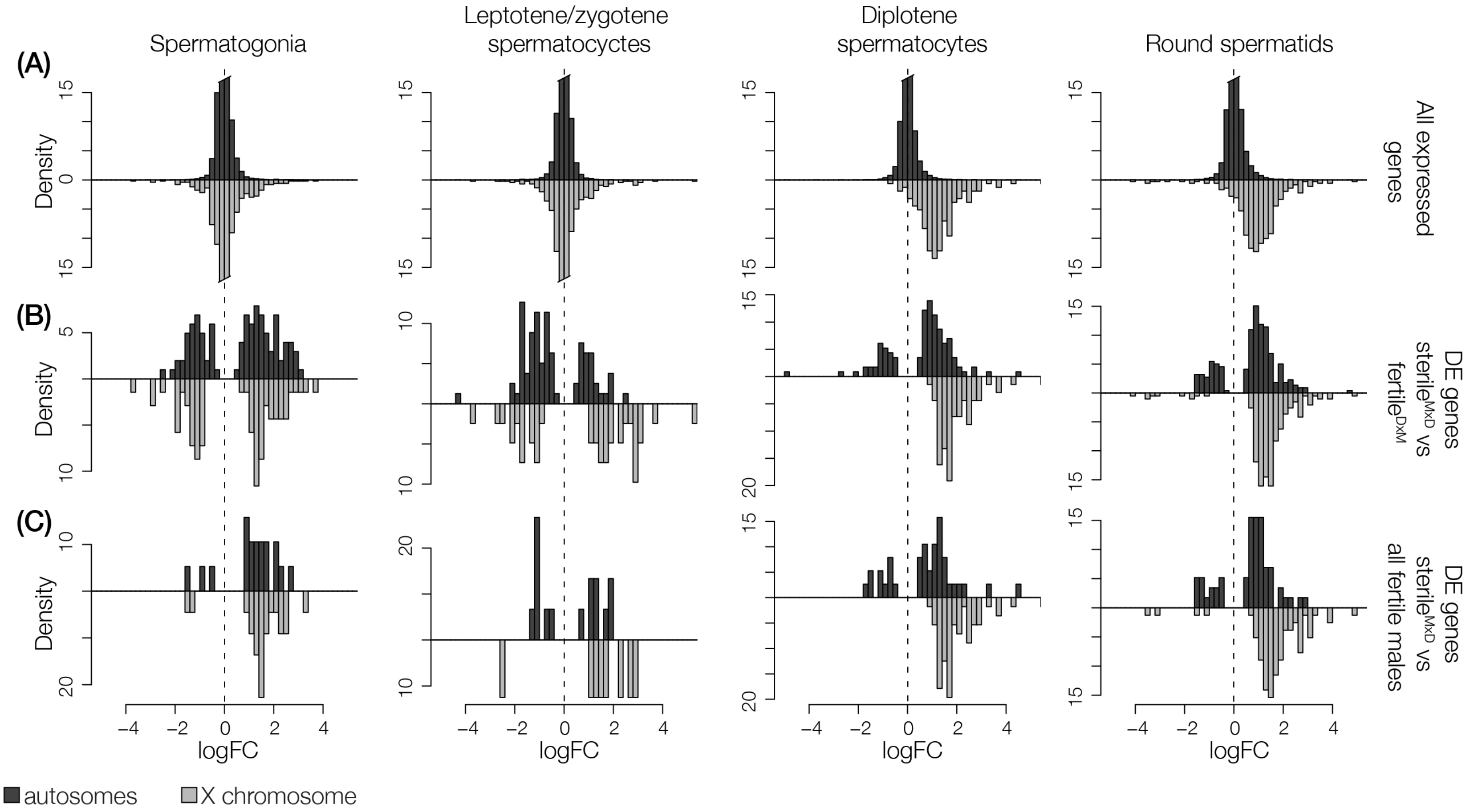

Figure S5. Genes with disrupted expression in sterile^{MxD} hybrids tend to be overexpressed. Density histograms of logFC between sterile^{MxD} and fertile^{DxM} hybrids for autosomal and X-linked genes expressed in each cell type. Each panels shows a different set of genes: **(A)** all expressed genes, **(B)** DE genes between sterile^{MxD} and fertile^{DxM} hybrids, **(C)** and DE genes between sterile^{MxD} and all fertile males.

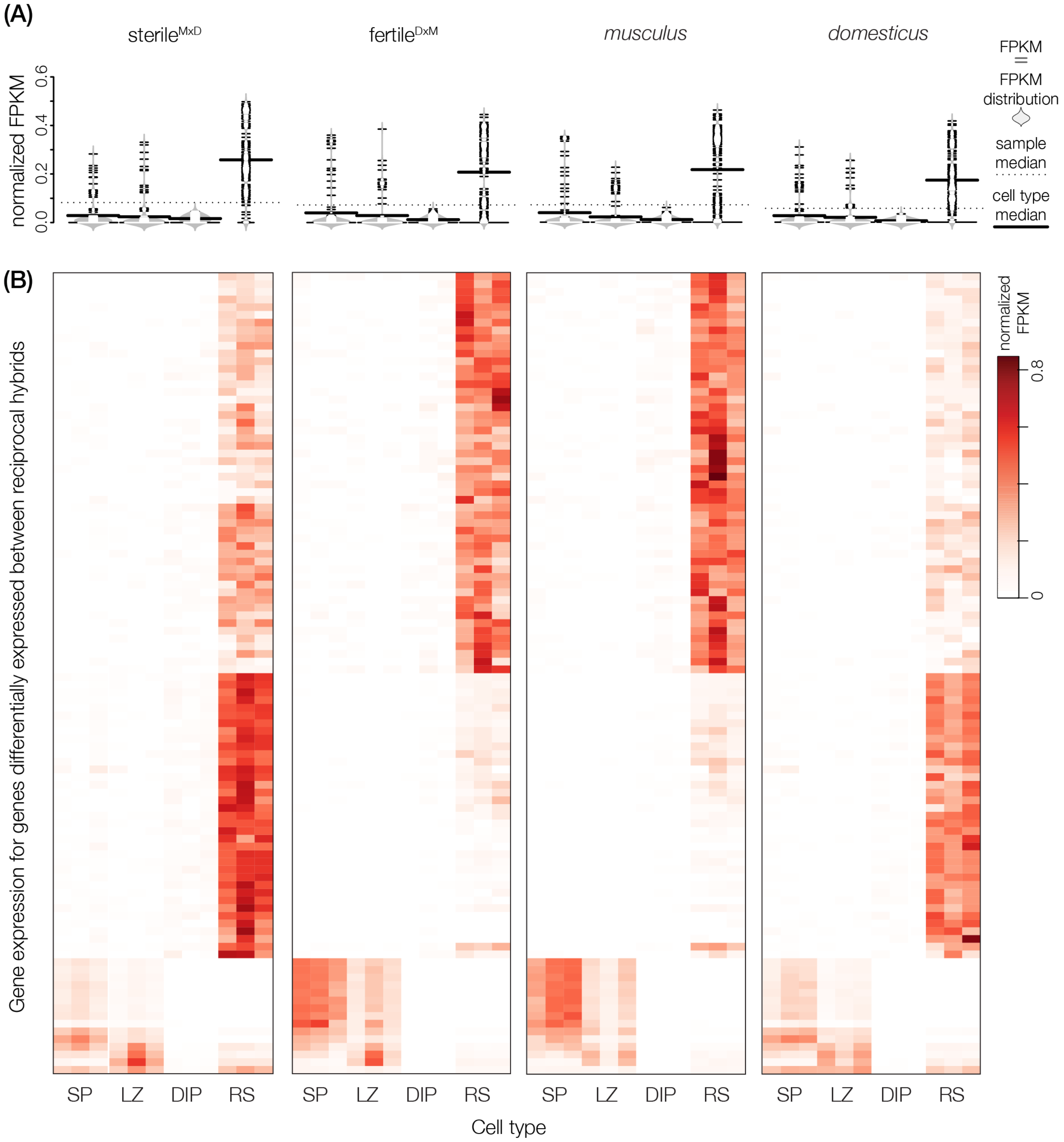

Figure S6. Y-linked gene expression among different crosses. (A) Expression distributions for Y-linked DE genes. Plotted with the R package beanplot (Kampstra 2008). **(B)** Y-linked gene expression plotted as normalized FPKM values that are hierarchically clustered using Euclidean distance. FPKM values are normalized so that the sum of squares equals one, R package vegan (Oksanen et al. 2015).

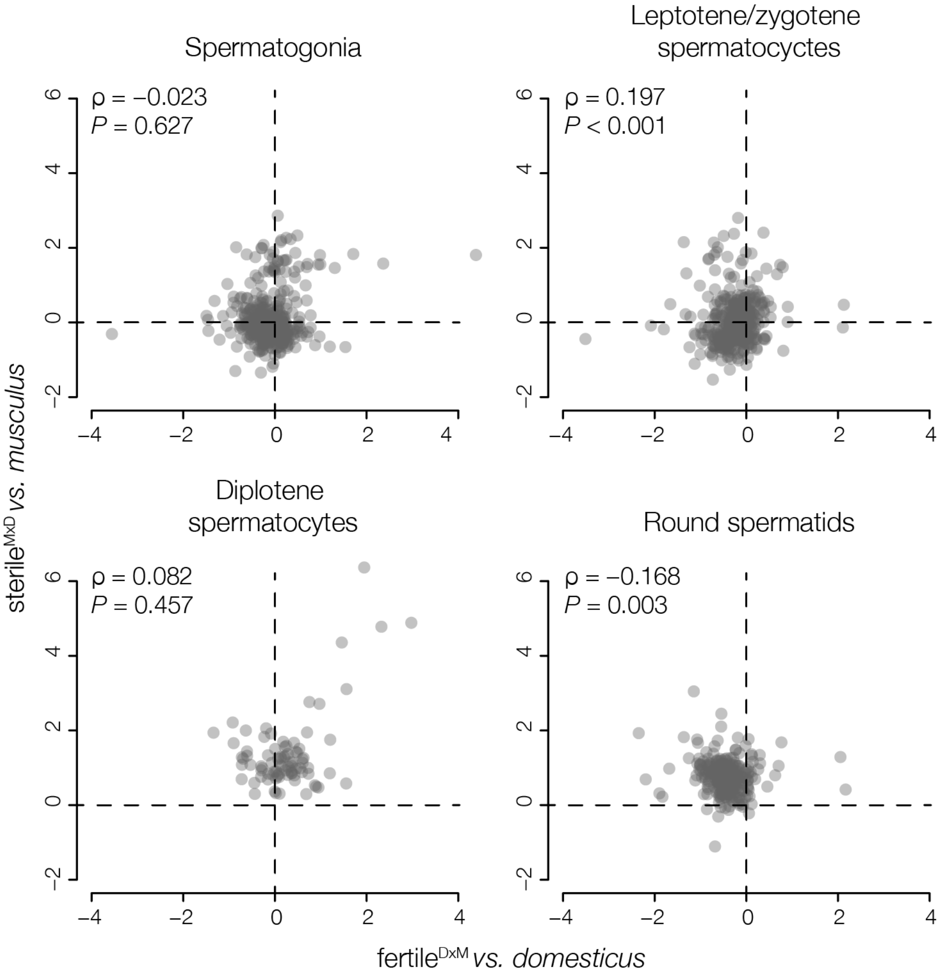

Figure S7. Correlation in X-linked gene expression between reciprocal hybrids relative to species-specific controls. Spearman's ρ of logFC between hybrids and conspecific chromosomes for genes expressed in both comparisons for each cell type.

Table S1. Sequencing of spermatogenic cell populations. *mus* = *M. m. musculus*, *dom* = *M. m. domesticus*, SP = spermatogonia, LZ = leptotene/zygotene spermatocytes, DIP = diplotene spermatocytes, RS = round spermatids.

Individual	Parents	Age (days)	Cell type	Raw paired reads	Trimmed paired reads	Pairs uniquely mapped		Assigned fragments	Final fragment counts[1]
						musculus	*domesticus*		
PPLL15.2	*mus* x *dom*	75	DIP	11823690	11198283	9986366	9870435	8814321	8022747
			RS	11564488	10904729	9659525	9559445	8604368	7841102
PPLL16.1		77	SP	11225816	10541448	9093009	8963739	7925116	7178330
			LZ	10640990	10007905	8831713	8702229	7626639	6929577
			DIP	10024328	9461558	8303698	8202947	7334175	6668364
			RS	9584197	8841160	7729943	7644411	7061275	6414681
PPLL17.1		67	SP	16843531	15883488	13865029	13664662	12347739	11142235
			LZ	15246316	14475968	12789457	12592268	11013956	9975352
			DIP	18634323	17514181	15372332	15173052	13780160	12515520
			RS	12638865	11855331	10353609	10224547	9536156	8676459
PPLL17.3		71	SP	11799101	11195470	9462191	9313113	8221694	7339907
			LZ	9871058	9317670	8171763	8053209	7139698	6452985
LLPP17.2	*dom* x *mus*	62	DIP	9994714	9491208	8446145	8500140	7442919	6786469
			RS	11262580	10624059	9370043	9442692	8554385	7827298
LLPP18.1		78	LZ	13908542	13141485	11651479	11645973	10008473	8997868
LLPP19.1		78	SP	16715111	15700417	13208648	13256853	11276479	10058279
			DIP	9029338	8566960	7510286	7569856	6723993	6101397
LLPP19.2		71	RS	9203792	8744380	7650046	7717771	6988852	6358231
LLPP19.3		90	DIP	9707845	9169603	8052666	8109772	7346172	6682953
			RS	9890829	9350563	8188883	8266029	7681904	7022427
LLPP22.7		69	SP	9169010	8561356	7492901	7507898	6697105	6078804
			LZ	10018389	9455050	8298644	8303072	7255947	6589418
LLPP22.8		67	SP	9648700	9065868	7732237	7771538	6816062	6073308
			LZ	11705944	11170568	9848274	9853137	8601365	7740602
WWLL3.1	*dom* x *dom*	64	SP	10552073	9978631	8232867	8795436	7234186	6484571
			DIP	10985159	10465262	9049623	9717157	8286803	7512520
			RS	11261501	10678254	9211837	9859085	8689767	7932244
WWLL4.1		61	LZ	10037623	9498288	8127464	8748569	7361891	6701277
WWLL6.1		68	RS	10194381	9567846	8054662	8727864	7784837	7105728
WWLL7.1		83	DIP	9987292	9407914	8034180	8665965	7474316	6795483
WWLL7.2		87	SP	12926098	12176326	10335353	11147407	9301722	8306411
			LZ	10043616	9604294	8301998	8958383	7436154	6671342
			DIP	10091404	9676530	8382821	9040406	7584753	6873746
			RS	10411193	9939188	8646042	9219674	8302435	7584827
WWLL7.3		89	SP	17756412	16772899	14150405	15191648	12742813	11274302
			LZ	20633018	19561703	16840391	18156243	15022061	13464643
CCPP21.1	*mus* x *mus*	65	SP	15875714	14848677	13127558	12285440	11053147	10013263
			LZ	13499123	12633183	11411863	10673548	9868505	9002647
			DIP	10738528	10200007	9363479	8767966	8078164	7378417
			RS	11097898	10502805	9576544	8997661	8491452	7802292
CCPP21.2		76	SP	14401271	13419249	11818296	11021435	10107916	9095887
			LZ	13274948	12559289	11368859	10578063	9774289	8834333
			DIP	18426482	17637976	16272253	15127992	13921878	12658466
			RS	11117634	10666428	9853080	9208465	8648569	7913516
CCPP21.3		62	SP	10875913	10336701	9178487	8557183	7936288	7196650
			LZ	10214496	9615769	8645697	7995249	7499243	6838283
			DIP	11234392	10680478	9749822	9052413	8534887	7793209
			RS	16056467	15109625	13805493	12748107	12398613	11381744

[1] Final fragment counts include multiply mapped reads for ampliconic/multicopy genes on the X and the Y chromosomes

Table S2. Number of DE genes between sterile^{MxD} hybrids and fertile^{DxM} hybrids or all fertile males controlling for different methodologies of counting multiply-mapped reads and different expression thresholds.

		Spermatogonia		Leptotene/ zygotene spermatocytes		Diplotene spermatocytes		Round spermatids		
		▼	▲	▼	▲	▼	▲	▼	▲	Method
fertile^{DxM}	Auto	32	54	45	24	30	74	51	144	uniquely mapping reads only
	X	22	34	11	23	1	58	15	154	
	Y	1	2	1	2	0	1	1	17	
all fertile	Auto	3	15	7	7	7	18	10	34	
	X	0	18	0	7	0	46	0	61	
	Y	0	0	0	0	0	1	0	0	
fertile^{DxM}	Auto	33	54	46	24	33	78	55	160	all reads, multiply-mapped included
	X	26	36	15	22	1	70	12	192	
	Y	9	1	9	1	0	1	8	39	
all fertile	Auto	3	15	8	7	8	19	11	40	
	X	1	17	0	6	0	61	0	78	
	Y	0	0	0	0	0	1	0	4	
fertile^{DxM}	Auto	32	53	45	23	30	77	53	146	multiply-mapped reads for multicopy X-linked genes, only one paralog/ gene family
	X	19	26	8	16	1	57	9	141	
	Y	9	1	9	1	0	1	8	39	
all fertile	Auto	3	15	7	7	7	18	11	34	
	X	1	18	0	5	0	46	0	59	
	Y	0	0	0	0	0	1	0	3	
fertile^{DxM}	Auto	8	13	15	9	12	29	17	59	expression threshold of FPKM > 5
	X	15	20	5	11	0	9	6	128	
	Y	9	1	8	1	0	1	0	23	
all fertile	Auto	0	4	2	2	3	9	11	16	
	X	0	7	0	2	0	8	0	53	
	Y	0	0	0	0	0	1	0	1	
fertile^{DxM}	Auto	3	7	10	5	3	8	4	30	expression threshold of FPKM > 10
	X	10	15	1	5	0	4	2	95	
	Y	8	1	7	1	0	0	0	11	
all fertile	Auto	0	3	1	1	1	3	0	10	
	X	0	5	0	1	0	4	0	44	
	Y	0	0	0	0	0	0	0	0	

Table S3. Summary of differential expression (DE) between sterile^{MxD} – fertile^{DxM} hybrid for genes involved in X chromosome inactivation (MSCI, MSUC, PSCR). The FDR corrected p-value is listed for DE genes and genes that are not DE are indicated as not significant (n.s.). Chr, chromosome; SP, spermatogonia; LZ, leptotene zygotene; DIP, diplotene; RS, round spermatid.

			Differentially expressed genes				
	Gene	Chr	SP	LZ	DIP	RS	Ref
X chromosome inactivation	*Atr*	9	n.s.	n.s.	n.s.	n.s.	1
	Brca1	11	n.s.	n.s.	n.s.	n.s.	1
	Cbx1	11	n.s.	n.s.	n.s.	n.s.	1
	Cbx3	6	n.s.	n.s.	n.s.	n.s.	1
	Cdk2	10	n.s.	n.s.	n.s.	n.s.	2
	H2afx	9	n.s.	n.s.	n.s.	n.s.	1
	H2afy	13	n.s.	n.s.	n.s.	n.s.	1
	H2afz	3	n.s.	n.s.	n.s.	n.s.	1
	Hormad1	3	n.s.	n.s.	n.s.	n.s.	2
	Hormad2	11	n.s.	n.s.	n.s.	n.s.	2
	Mdc1	17	n.s.	n.s.	n.s.	n.s.	1
	Smc1b	15	n.s.	n.s.	n.s.	n.s.	2
	Suv39h2	2	n.s.	n.s.	n.s.	n.s.	1
	Sycp3	10	n.s.	n.s.	n.s.	n.s.	2
	Ube2a	X	n.s.	n.s.	n.s.	n.s.	1
	Ube2b	11	n.s.	n.s.	n.s.	n.s.	1
Candidate hybrid sterility	4930447F04Rik	X	n.s.	n.s.	n.s.	0.050	3
	4933436I01Rik	X	0.020	0.002	n.s.	< 0.001	3
	Aff2	X	NA	NA	NA	NA	3
	Ctag2	X	n.s.	n.s.	0.033	0.012	3
	Fmr1nb	X	n.s.	n.s.	n.s.	< 0.001	3
	Mei4	9	n.s.	n.s.	n.s.	n.s.	3
	Msh3	13	n.s.	n.s.	n.s.	n.s.	3
	Prdm9	17	n.s.	n.s.	n.s.	n.s.	3
	Slitrk2	X	NA	NA	NA	NA	3
	Sycp1	3	n.s.	n.s.	n.s.	n.s.	3

[1] Turner J. M. A., 2007 Meiotic sex chromosome inactivation. Development 134: 1823–1831.

[2] Turner J. M. A., 2015 Meiotic Silencing in Mammals. Annu. Rev. Genet. 49: 395–412.

[3] Bhattacharyya T., Reifova R., Gregorova S., Simecek P., Gergelits V., Mistrik M., Martincova I., Piálek J., Forejt J., 2014 X chromosome control of meiotic chromosome synapsis in mouse inter-subspecific hybrids. PLoS Genet 10: e1004088.