Elys and Lamin B/C depletions in spermatogonia and spermatocytes

1. RNA-seq in larval testes. Differential expresion and TPM comparison.

1.1. Comparison of all genes

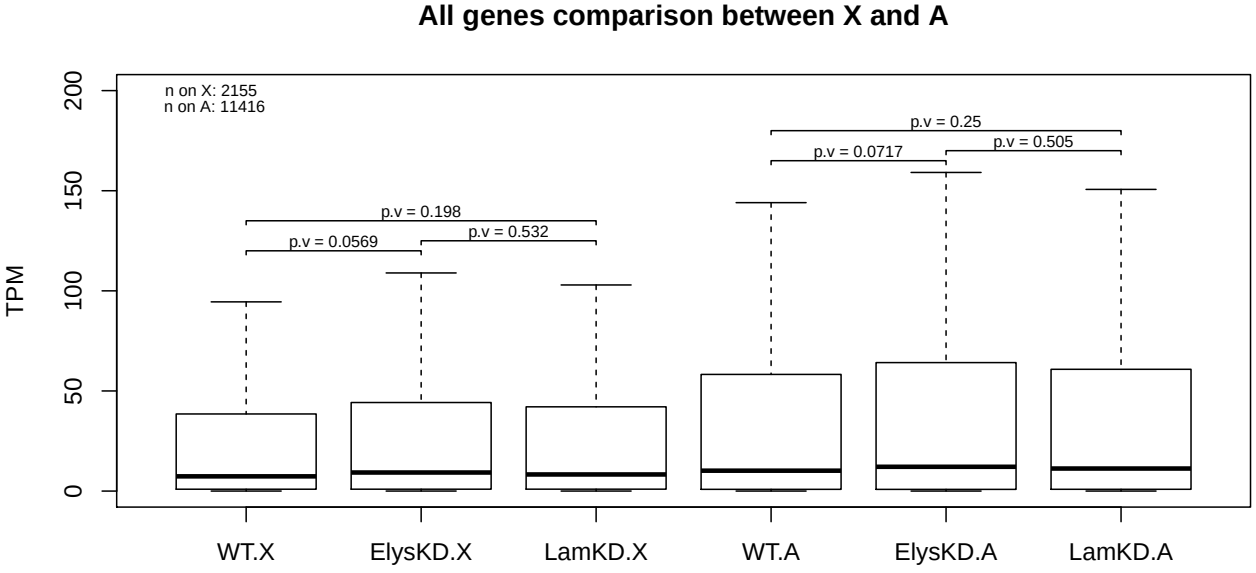
We used a BDGP5.78 annotation for *Drosophila* genes:

chr	start	end	strand	id	gene_name	tss
chr2L	7529	9484	+	FBgn0031208	CG11023	7529
chr2L	9839	21376	-	FBgn0002121	l(2)gl	21376
chr2L	21823	25155	-	FBgn0031209	Ir21a	25155
chr2L	21952	24237	+	FBgn0263584	CR43609	21952
chr2L	25402	65404	-	FBgn0051973	Cda5	65404
chr2L	65999	66242	+	FBgn0266878	CR45339	65999

Using salmon we obtained read counts per gene and normalized TPM values:

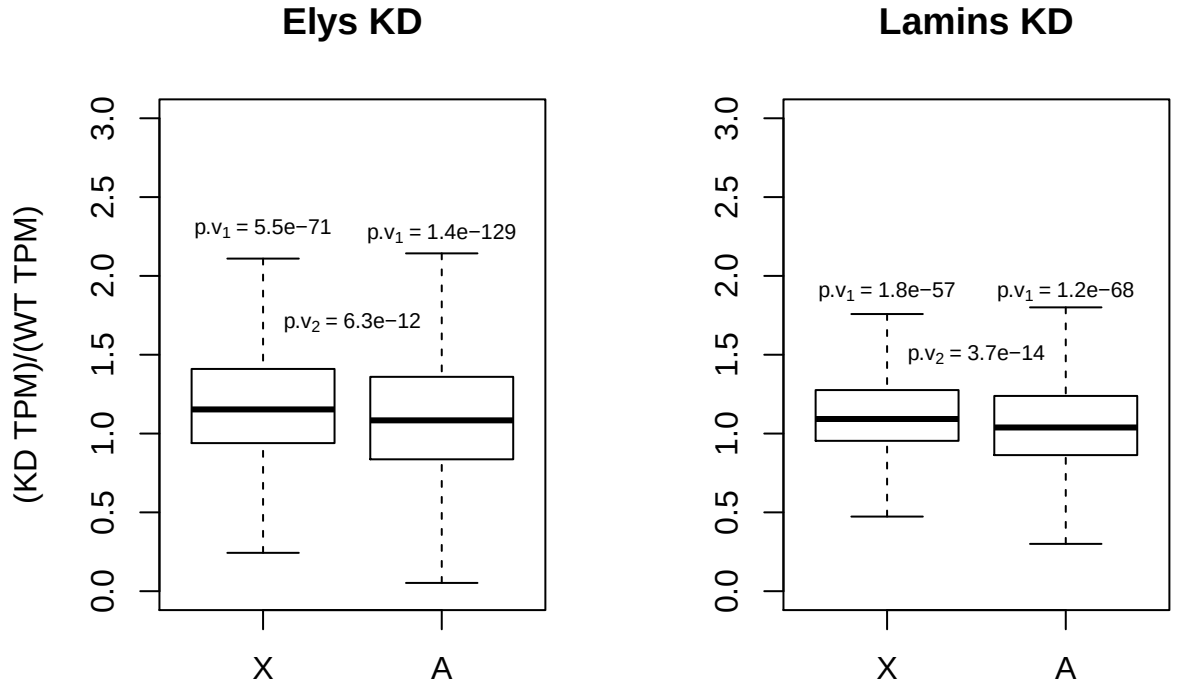
chr	start	end	strand	gene_name	WT	ElysKD	LamKD
chr2R	18024473	18060339	+	a	6.7153633	5.6267533	5.8948233
chr3R	12632936	12655771	-	abd-A	7.6534000	9.6282000	9.5642133
chr3R	12752932	12797958	-	Abd-B	0.4616433	0.6147677	0.6040280
chr3L	16608966	16640982	-	Abl	9.4840567	12.5862000	10.4057300
chr2L	10973443	10975293	-	abo	27.6684000	41.1312667	36.6294333
chrX	264064	264980	+	ac	0.2590723	0.0034933	0.0034933

The main question that we investigate is whether the expression of genes on X-chromosome is higher than of those on autosomes in spermatocytes? And do Elys and/or Lamins knockdowns alter this discordance?

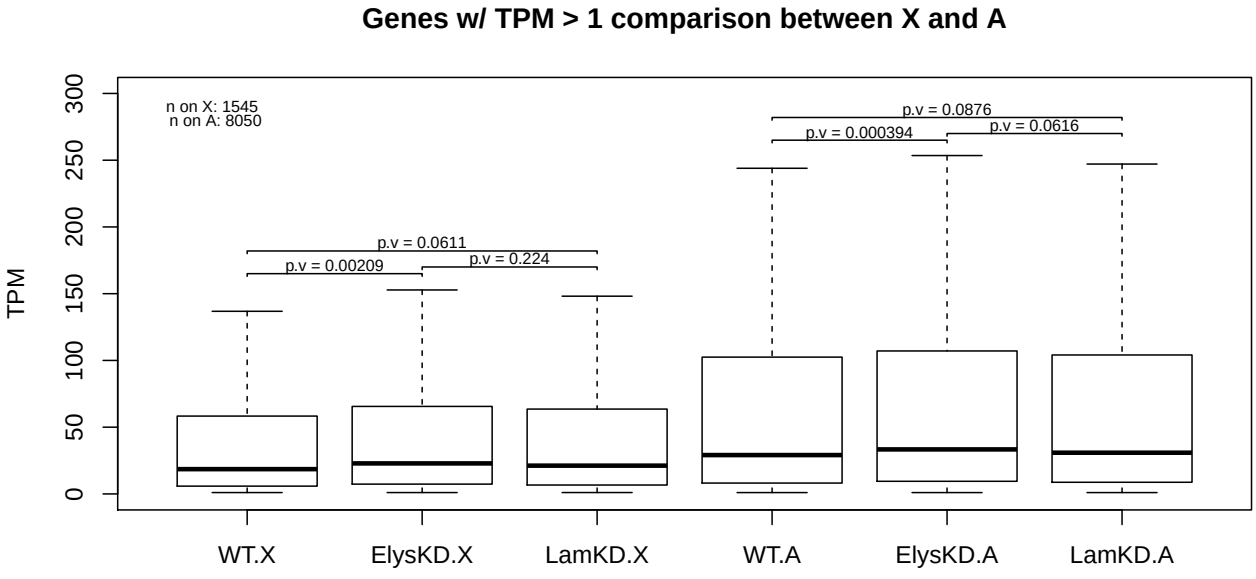


Median ratio of WT X to WT A is equal to 0.72

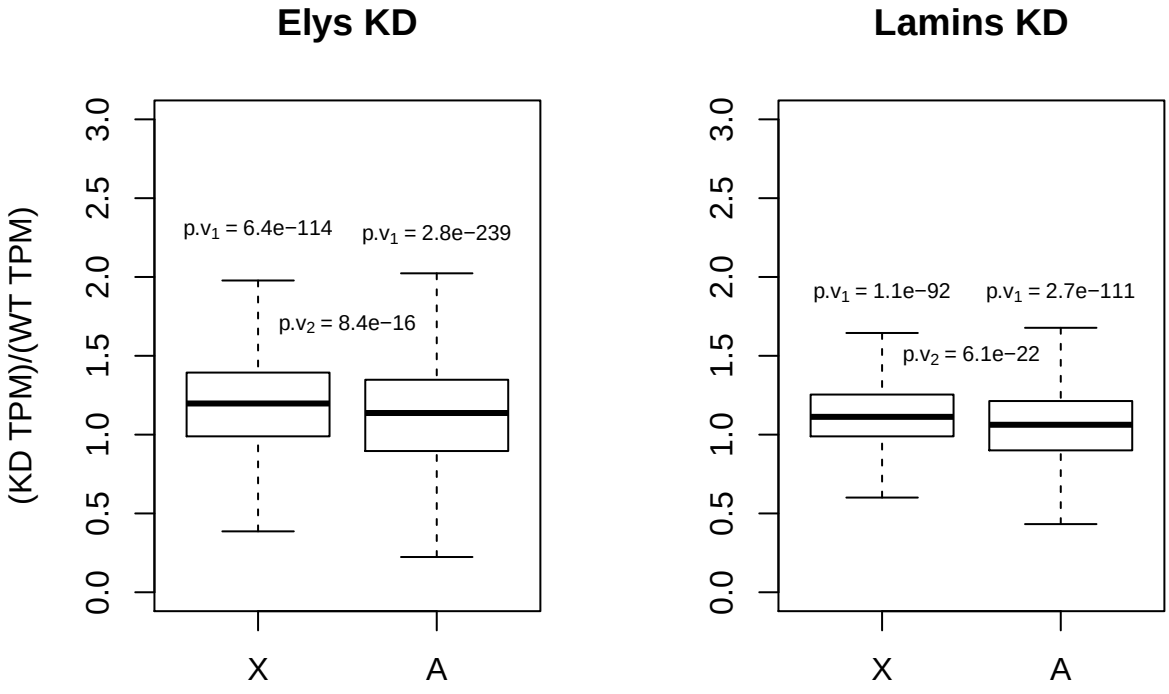
Let's see the ratios of TPMs in case of Elys and Lams knockdowns. $p.v_1$ represents one-tailed comparison of each sample's medians with 1 via wilcoxon test, $p.v_2$ represents two-tailed two-sample comparison using the same test



1.2. Comparisons of genes that have at least one TPM in all treatments



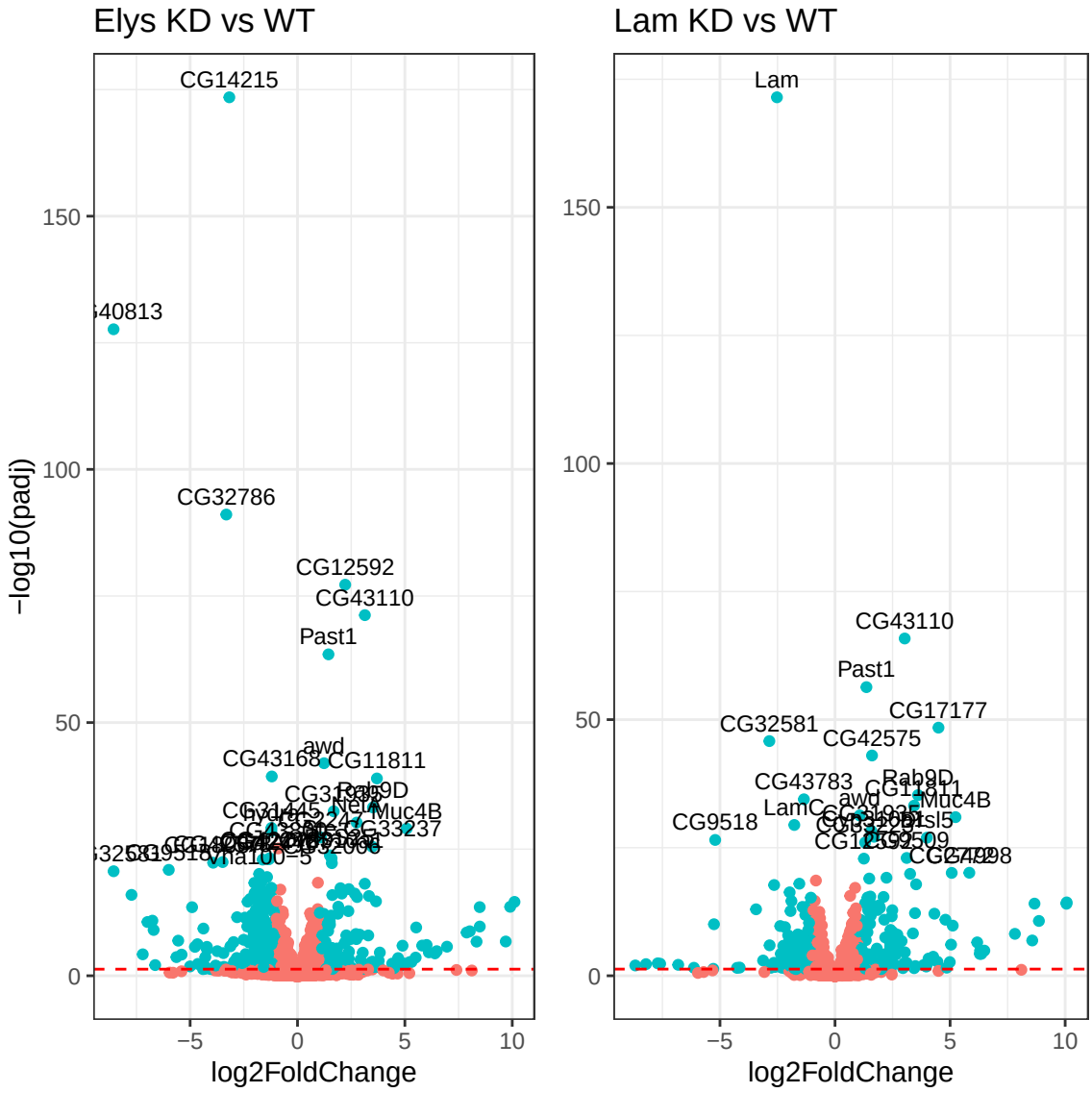
Median ratio of WT X to WT A for these genes is equal to 0.64


Elys KD
(KD TPM)/(WT TPM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
p.v1 = 6.4e−114
p.v1 = 2.8e−239
p.v2 = 8.4e−16
X
A
Lamins KD
p.v1 = 1.1e−92
p.v1 = 2.7e−111
p.v2 = 6.1e−22

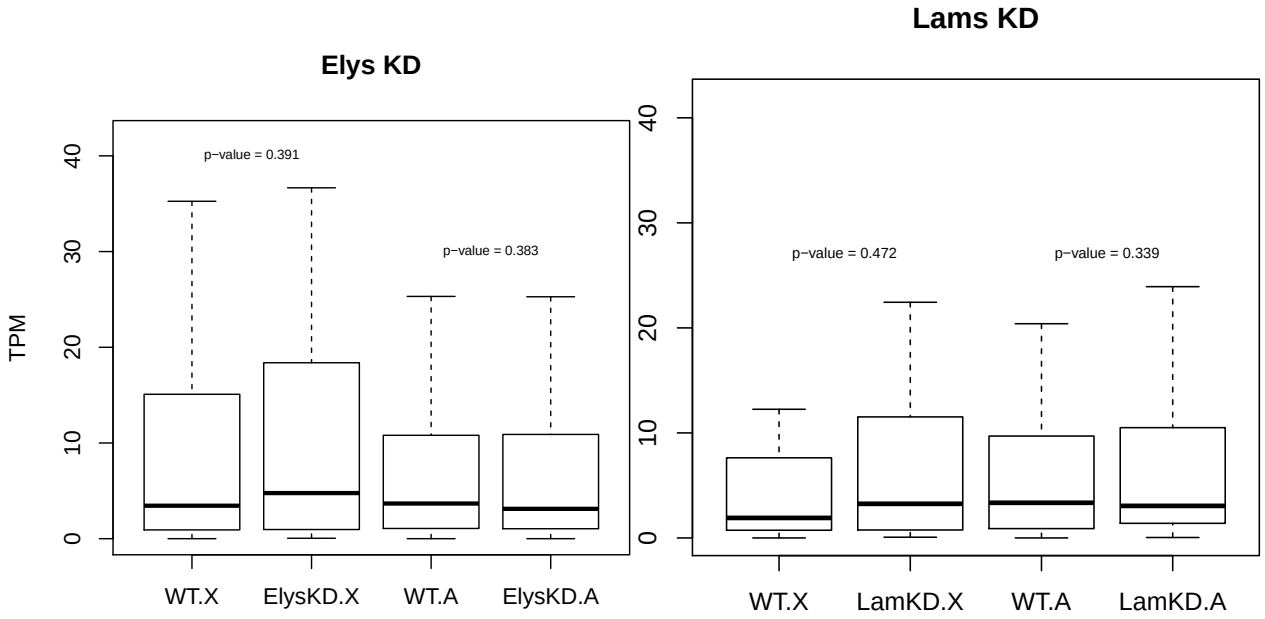
1.3. Differential expression analysis

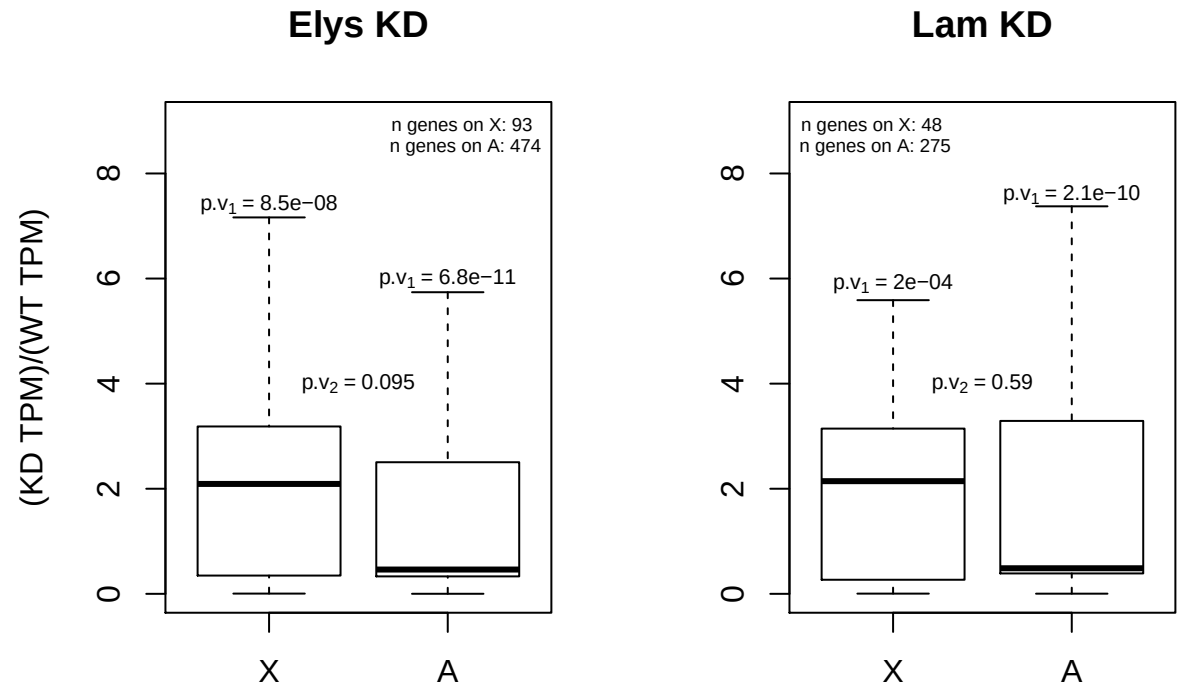
Differential expression analysis was performed using DESeq2.

Volcano plot for Elys KD:

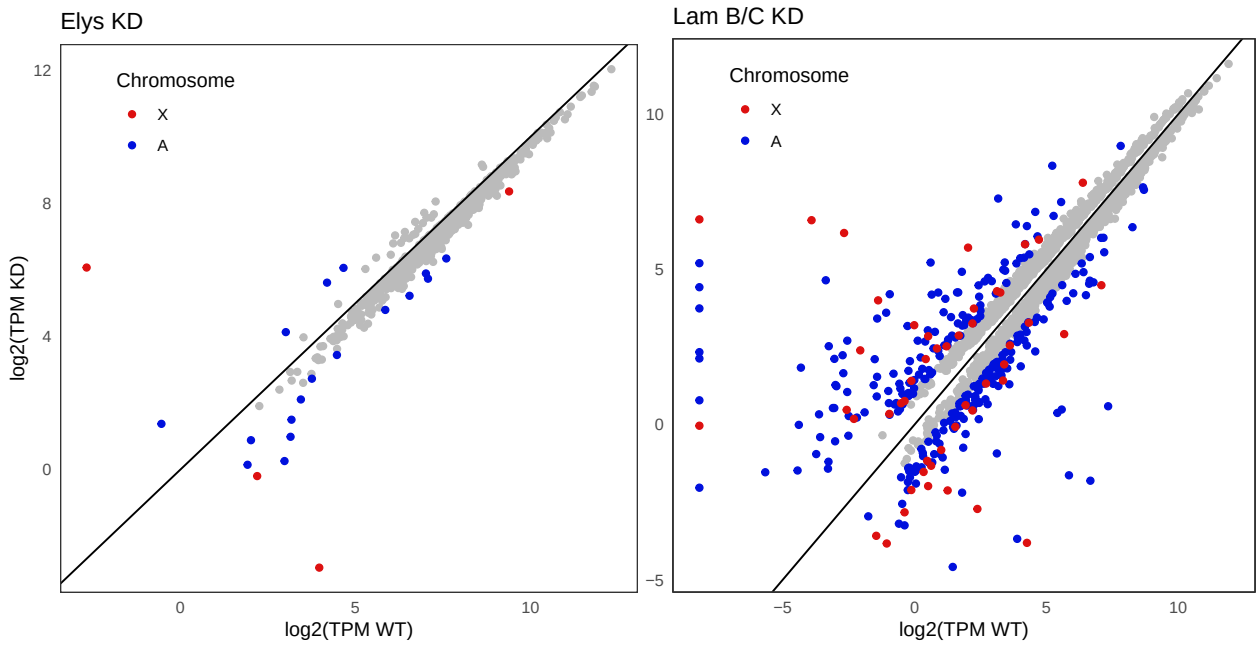


Here are similar boxplots for differentially expressed genes (via DESeq2 and TPM ratio > 2:

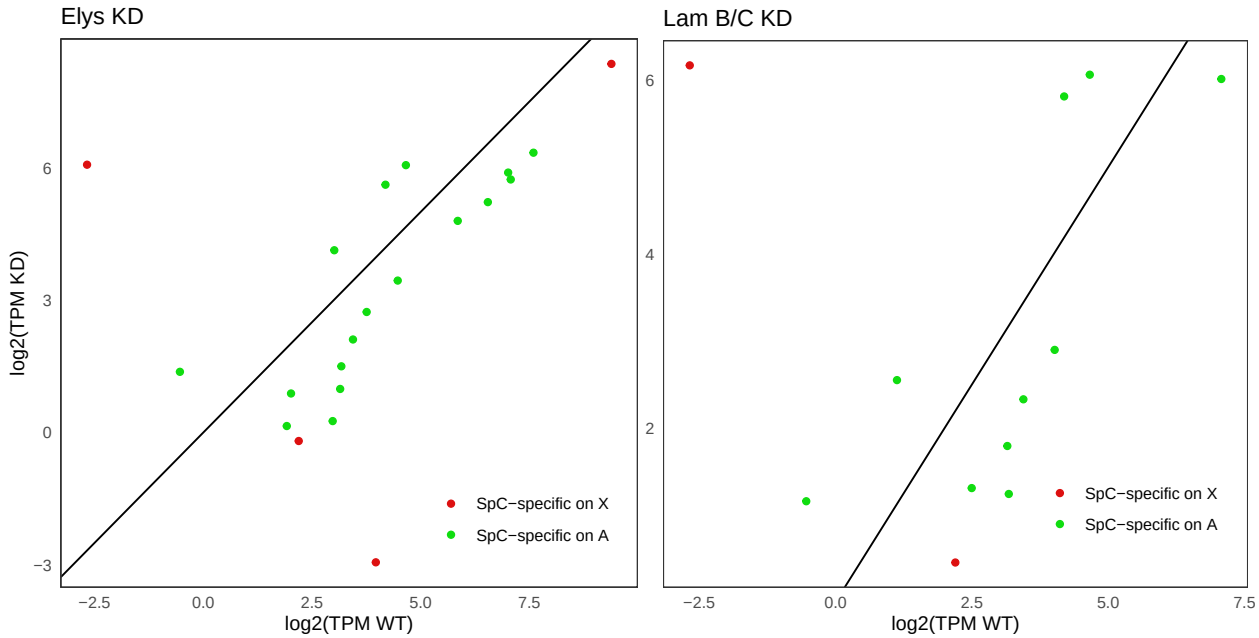




Dot plots for TPMs of differentially expressed genes (TPM ratio > 2):

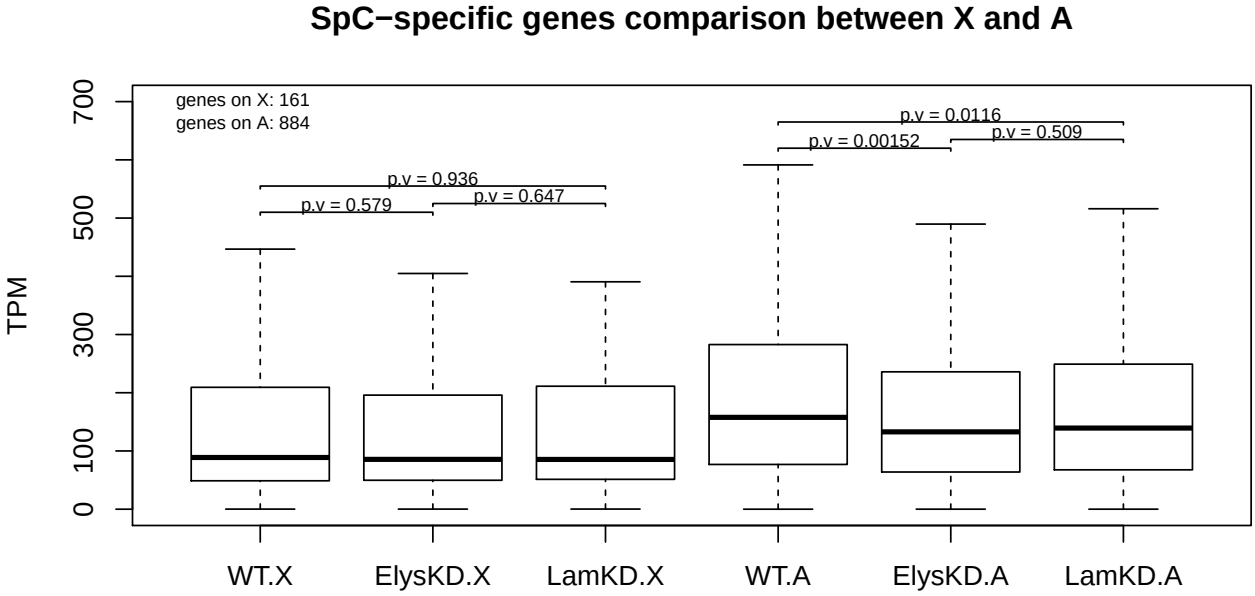


SpC-specific genes:



1.4 Spermatocyte-specific genes

Boxplot with TPMs for SpC-specific genes (p-values are from two-sample two-sided wilcoxon test):



Median ratios of WT X to WT A for SpC-specific genes is equal to 0.56

Ratios ($p.v_1$ represents testing if medians are lower than 1):