Lin et al, Characterization of a TUTase/nuclease complex required for Drosophila gametogenesis

Supplementary Figures and Tables

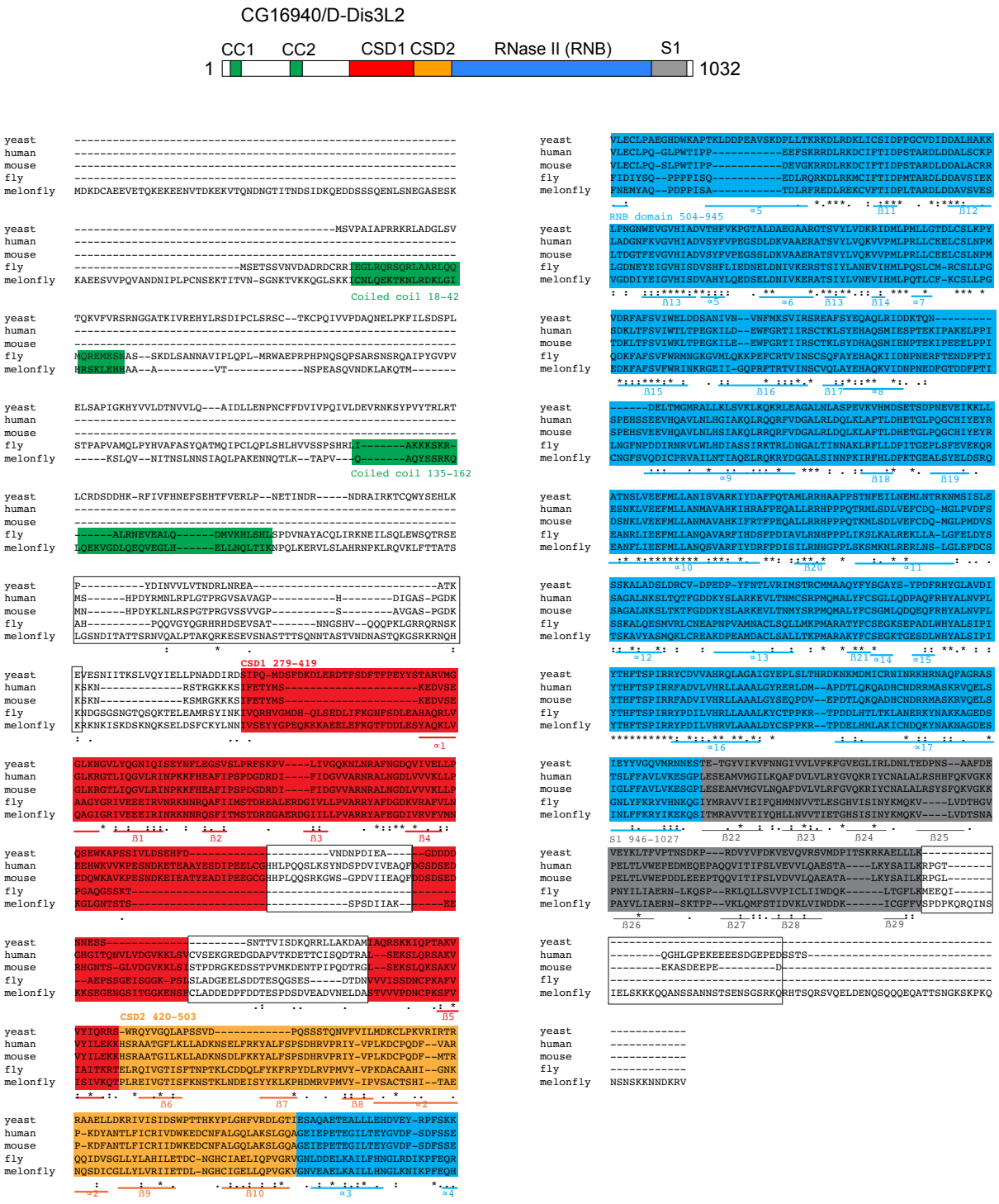

Figure S1. Domain comparison of Dis3L2 orthologs

The murine Dis3L2 structure (Faehnle, 2014) was used as a reference for structural modeling. The segments boxed in black were truncated in the crystallization study. All the domains shown are annotated according to sequence similarity as well as similar organization of alpha helices and beta sheets. Of note, the two cold shock domains (CSDs) found in other Dis3L2 orthologs were not predicted in fly CG16940 by several annotation servers, but the structural modeling provides support for their existence in CG16940. Dipteran Dis3L2 factors also contain N-terminal coiled-coil (CC) domains that are not shared by other species.

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Supplementary Figure 1

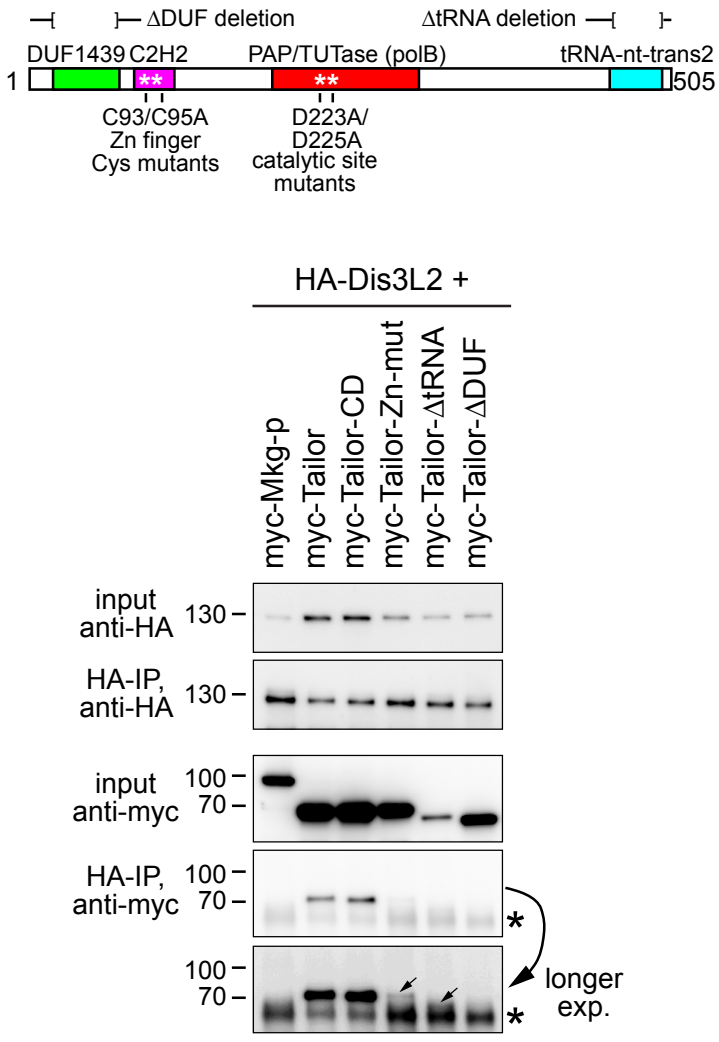

Figure S2. Tailor associates with Dis3L2 via Tailor N-terminal domains.

(A) Summary of Tailor domain structure and mutant variants analyzed in co-immunoprecipitation (co-IP) assays. (B) S2 cells were transfected with HA-Dis3L2 and the indicated Tailor or control (Mkg-p) constructs, and analyze by Western blotting. Input blots show the expression of the tagged proteins. Following HA-IP, Tailor and Tailor-CD are effectively co-IPed, whereas Zn finger mutant and ΔDUF variants are poorly co-IPed. TailorΔtRNA is relatively unstable, and considering its relative total accumulation, its association with Dis3L2 is more substantial than with Zn and ΔDUF mutants.

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Supplementary Figure 2

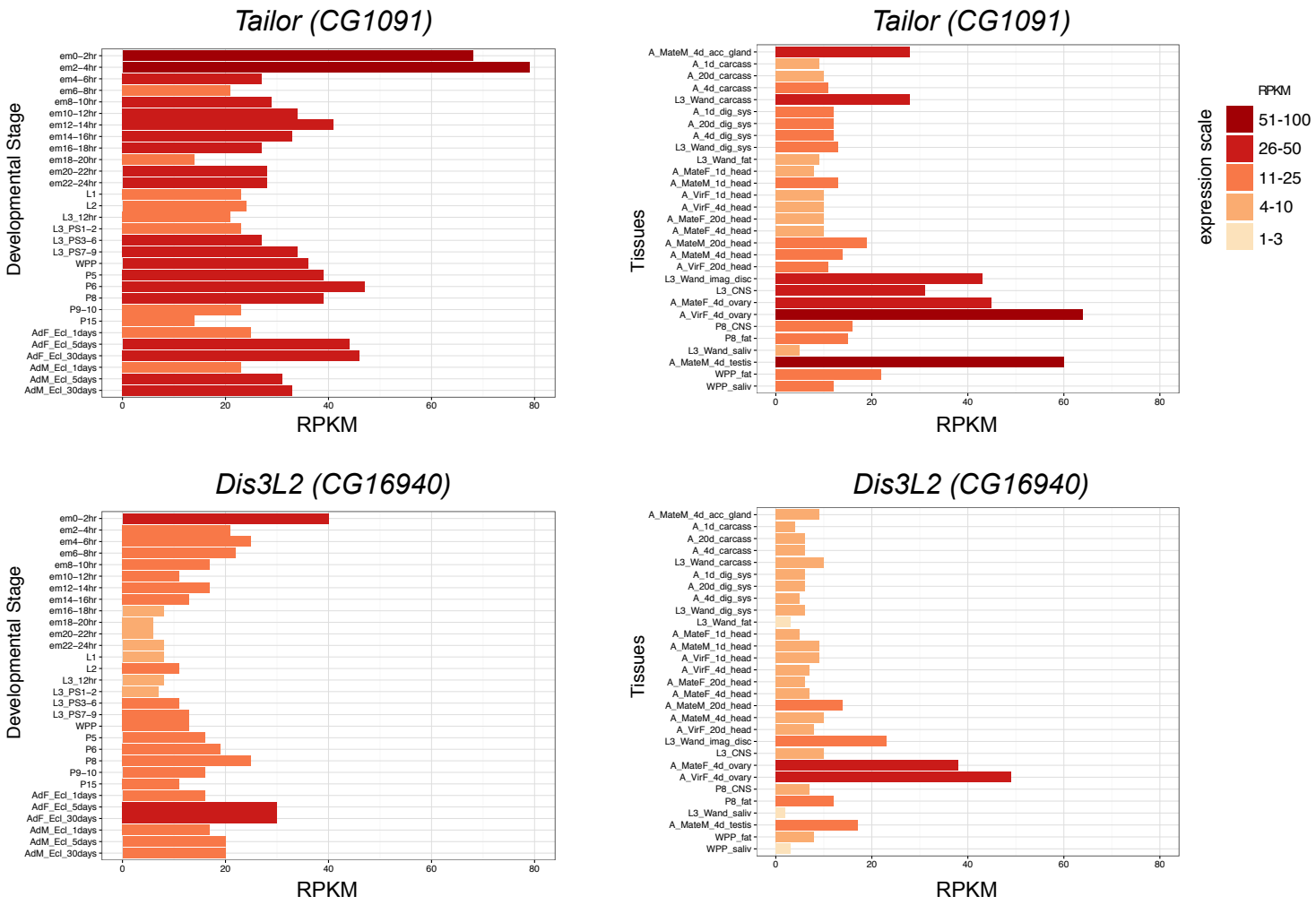

Figure S3. Developmental and tissue-specific expression of Tailor and Dis3L2.

Shown are summaries of modENCODE RNA-seq data for Tailor (CG1091) and Dis3L2 (CG16940) obtained from FlyBase (www.flybase.org). Both genes are broadly expressed, both temporally and spatially. Tailor is expressed at highest levels in adult ovary and testis, while Dis3L2 is highest expressed in ovaries. Consistent with this, both genes are also maternally deposited at a substantial level, compared to most other locations assessed.

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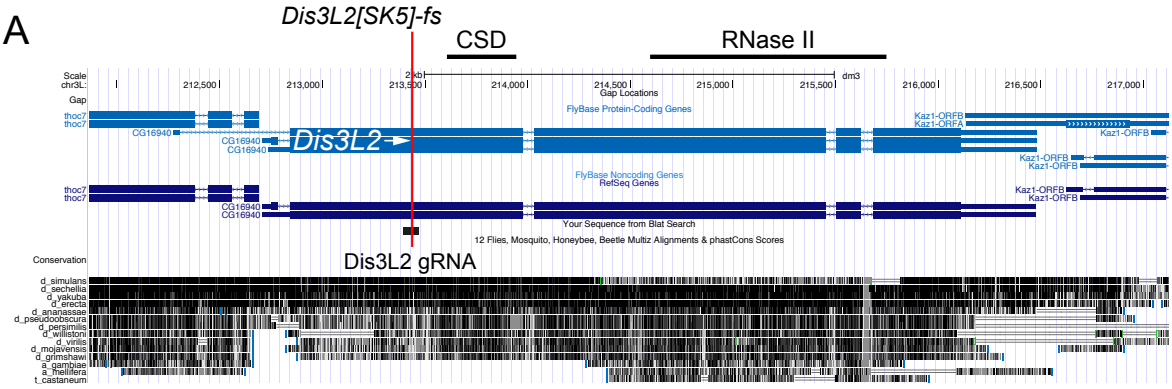
Supplementary Figure 3

A



B

```
Dis3L2 (CG16940) alleles
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#SK2    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCGCAACAGGTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC 0
#SK3    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCG------GTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC -6
#SK4    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCGCAca---TTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC -3
#SK5    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCGC----GGTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC -4
#SK6    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCGCAACAcaTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC 0
#SK7    TCGAGTGGAGTCAGACCAGGTCAGAAGCACATCCGCAACAGGTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTC 0
#SK8    Irregular sequence
```

C

```
          E  A  H  P  Q  Q  V  G  Y  Q  G  R  H  R  H  D  S  E  V  S  A  T  N  N  G  S  H  V  Q  Q  Q  P  K  L  G
WT        GAAGCACATCCGCAACAGGTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTCTCTGCTACAAACAATGGAAGTCATGTACAGCAGCAACCGAAACTAGGA
#SK5      GAAGCACATCCGC----GGTTGGTTACCAGGGGCGGCATCGCCACGATTCTGAGGTCTCTGCTACAAACAATGGAAGTCATGTACAGCAGCAACCGAAACTAGGA
          E  A  H  P     R  L  V  T  R  G  G  I  A  T  I  L  R  S  L  L  Q  T Met E  V Met Y  S  S  N  R  N ***
```

Figure S4. CRISPR mutagenesis of Drosophila Dis3L2.

(A) Genomic region of Dis3L2, and location of a transgenic gRNA directed against an N-terminal region that is upstream of the known Dis3L2 domains. (B) Summary of candidate lines subjected to genotyping. These tests revealed the transgenic gRNA induced high efficiency mutations, including both in-frame and out-of-frame indels. (C) Dis3L2[SK5] allele was used for detailed characterization, and showed similar homozygous and hemizygous phenotypes, suggesting that it is a null allele.

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Supplementary Figure 4

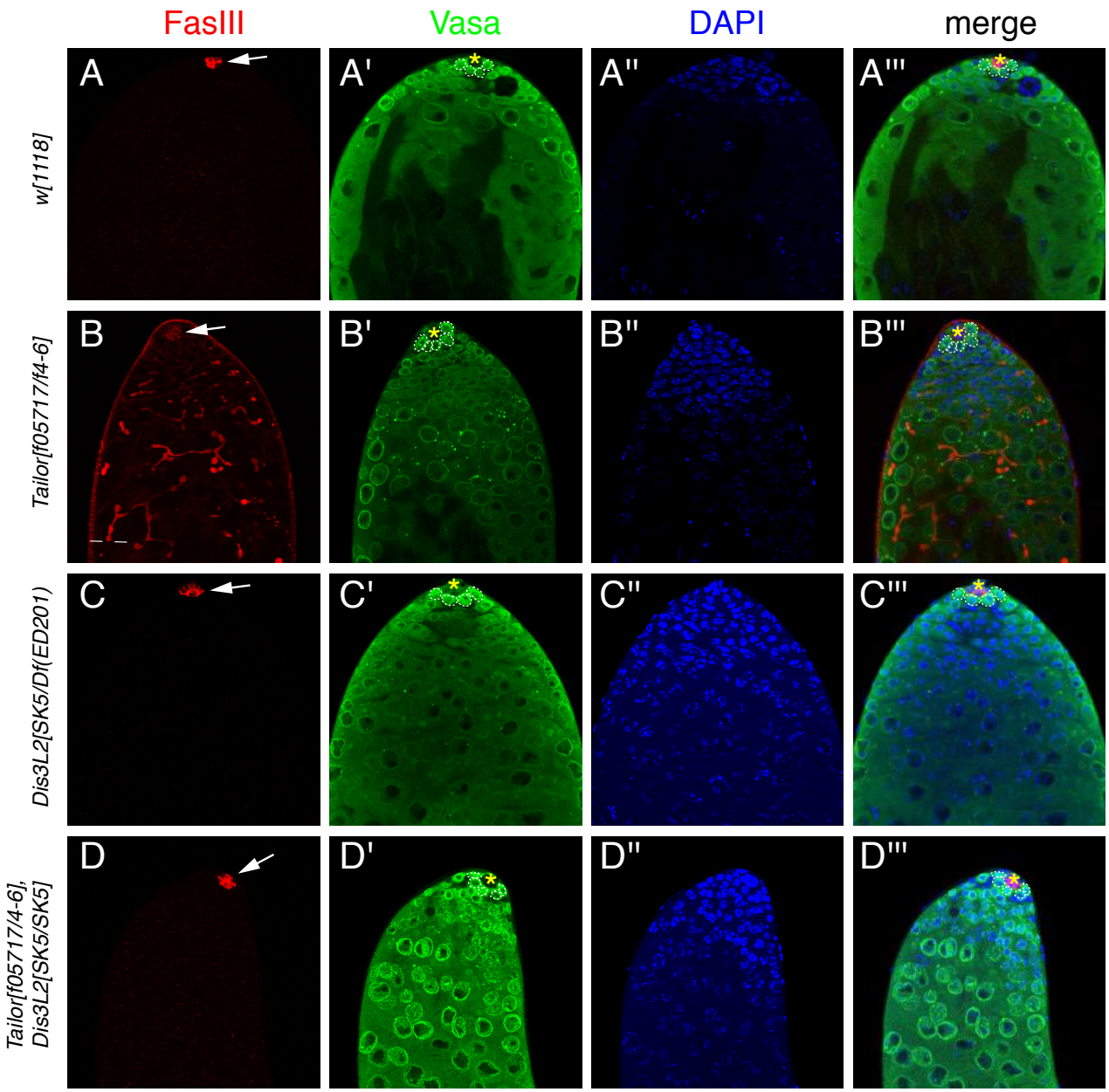

Figure S5. Analysis of germline stem cells in Tailor and Dis3L2 mutant testes.

Testes were dissected from five day old males and stained for Fas III (in red) to mark the somatic hub, the germline marker Vasa (in green), and the nuclear marker DAPI (in blue). Focusing on the germline stem cell (GSC) niche, the somatic hub is marked with arrows in A'-D', the GSCs that surround the hub are outlined with dotted circles in A"-D" and directly contact the hub (asterisk). As in control w[1118] testis (A), Tailor transheterozygote mutant (B), Dis3L2 hemizygous mutant (C) and Tailor, Dis3L2 double mutant all exhibit a clear hub surrounded by an appropriate number of GSCs.

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Supplementary Figure 5

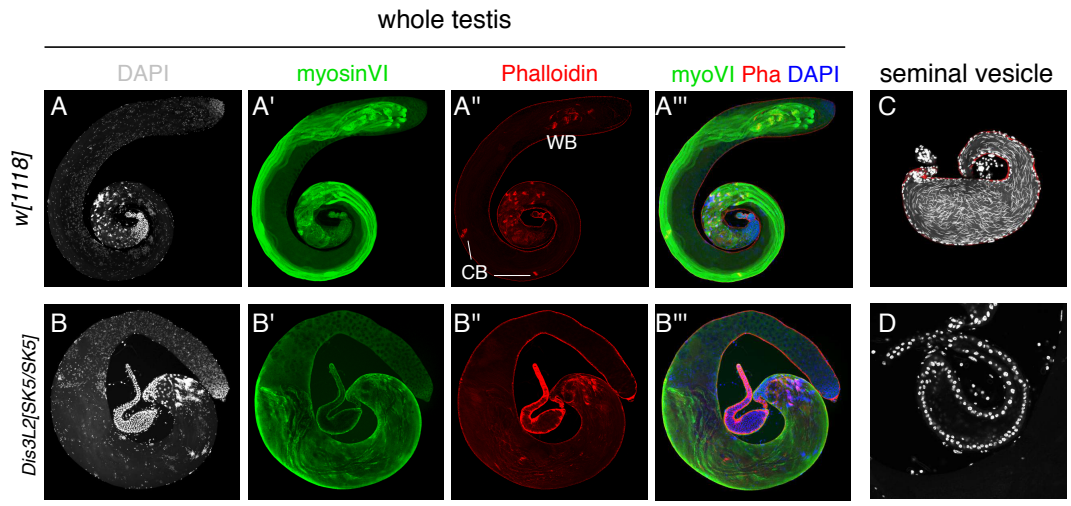

Figure S6. Testis defects of Dis3L2[SK5] homozygous mutants.

Shown are testis stainings for control w[1118] (top row) and Dis3L2[SK5/SK5] mutants. (A-B Staining of whole testes for DAPI (blue, shown in grayscale in single channel images), myosin VI (in green) and phalloidin (in red), and merged. (A) Control w[1118]; note that these images are the same as in main Figure 4. Panel A" highlights cystic bulges (CB) and wastebags (WB) of the maturing meiotic products. (B) Dis3L2 homozygous mutant completely lacks cystic bulges, and consequently produces no wastebags either. (C-D) Seminal vesicles, which are filled with mature sperm with needle-shaped DAPI labeled nuclei in control (C), whereas Dis3L2 homozygote has a small seminal vesicle that is devoid of mature sperm (D).

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Supplementary Figure 6

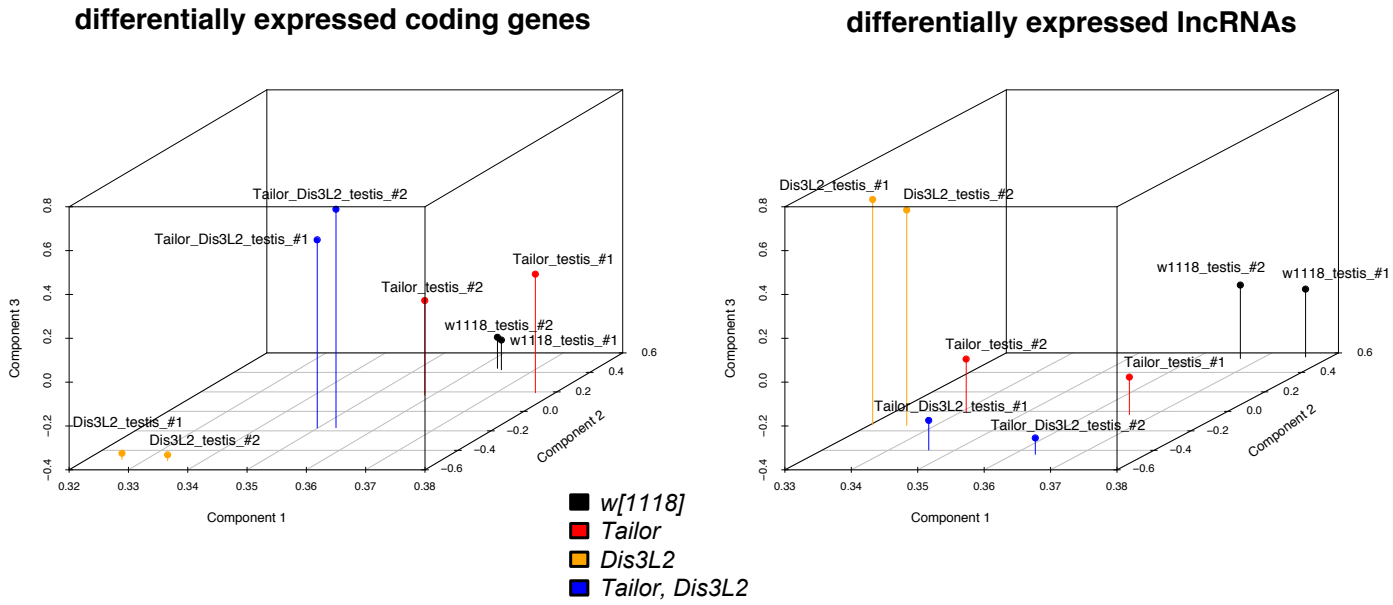

Figure S7. PCA analysis of Tailor, Dis3L2 and double mutant testes RNA-seq data

The left plot is based on protein-coding gene expression, while the right plot is based on lncRNA expression. In general, the replicate datasets are relatively closely related in the space, indicating the reproducibilty of data from the various mutants.

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Supplementary Figure 7

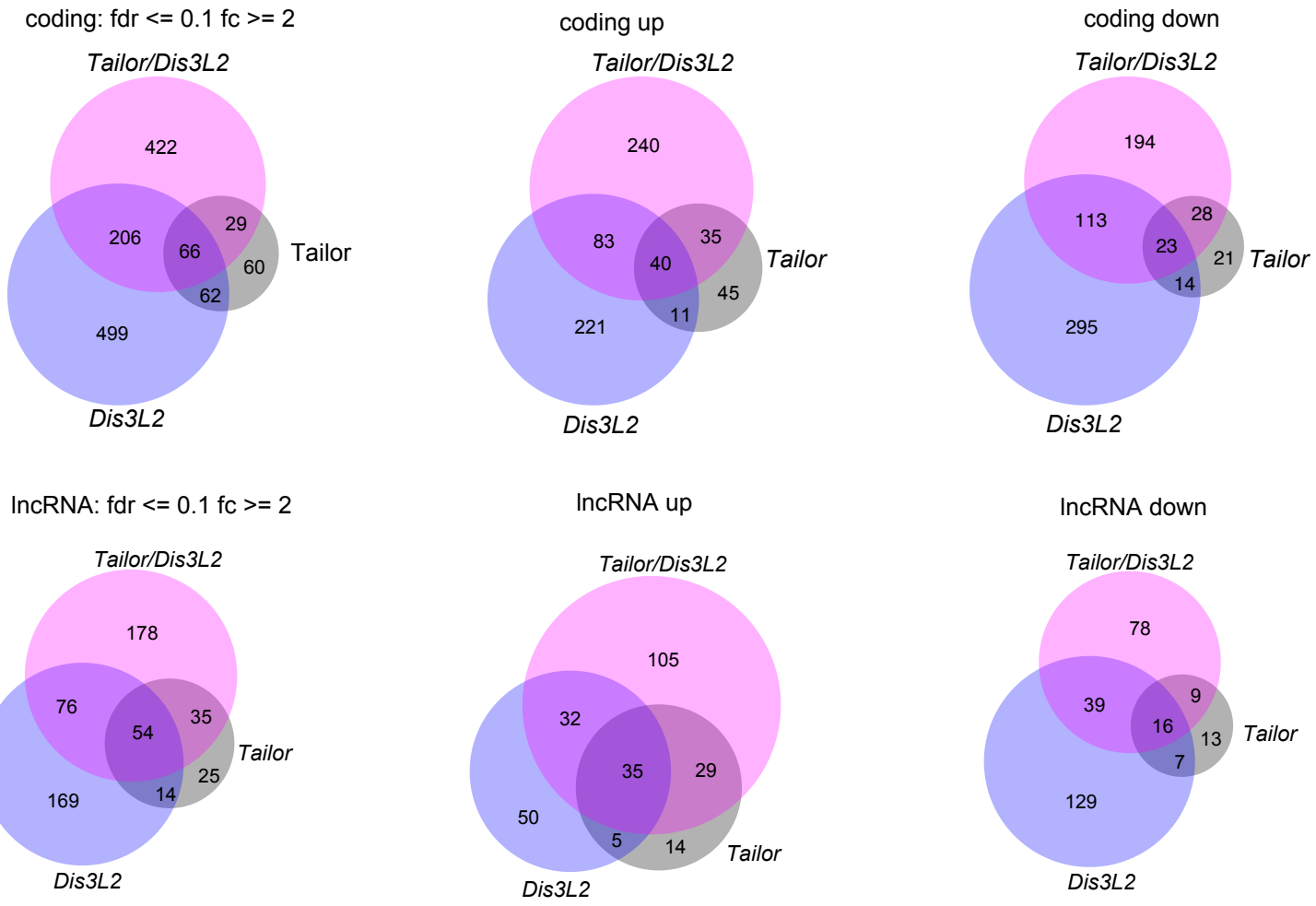

Figure S8. Venn diagrams summarizing shared changes amongst the Tailor and Dis3L2 mutant datasets.

Gene expression changes were called at ≥2 fold change and ≤0.1 false discovery rate. The top row of Venn diagrams represents the numbers of protein-coding genes with shared differential expression amongst the indicated mutants, shown as all changes (left), upregulated genes (middle) and downregulated genes (right). The same is diagrammed in the bottom row of Venn diagrams for lncRNAs.

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Supplementary Figure 8

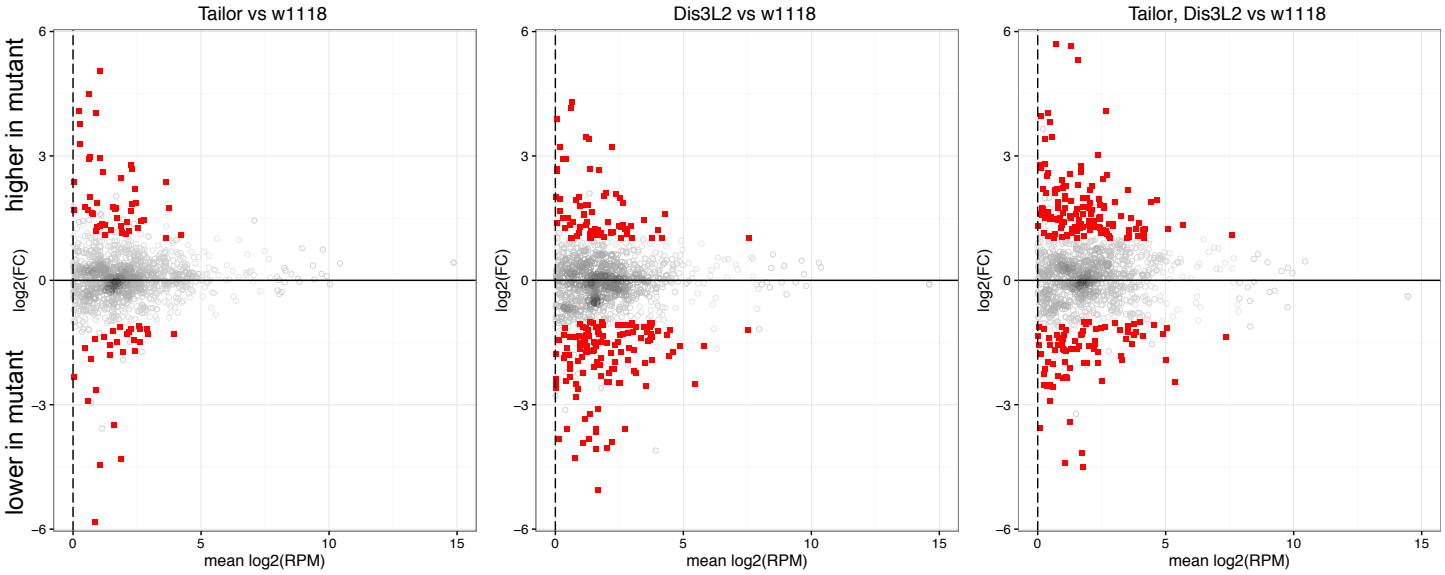

Figure S9. MA expression plots of lncRNA changes in Tailor, Dis3L2 and double mutant testis.

Biologically replicate RNA-seq datasets were made from each genotype. Red loci represent lncRNAs with FC≥2 at an FDR ≤0.1; all other loci are in grey. Note that more genes are differentially expressed in Dis3L2 and the Tailor, Dis3L2 double mutant, compared to the Tailor mtuant.

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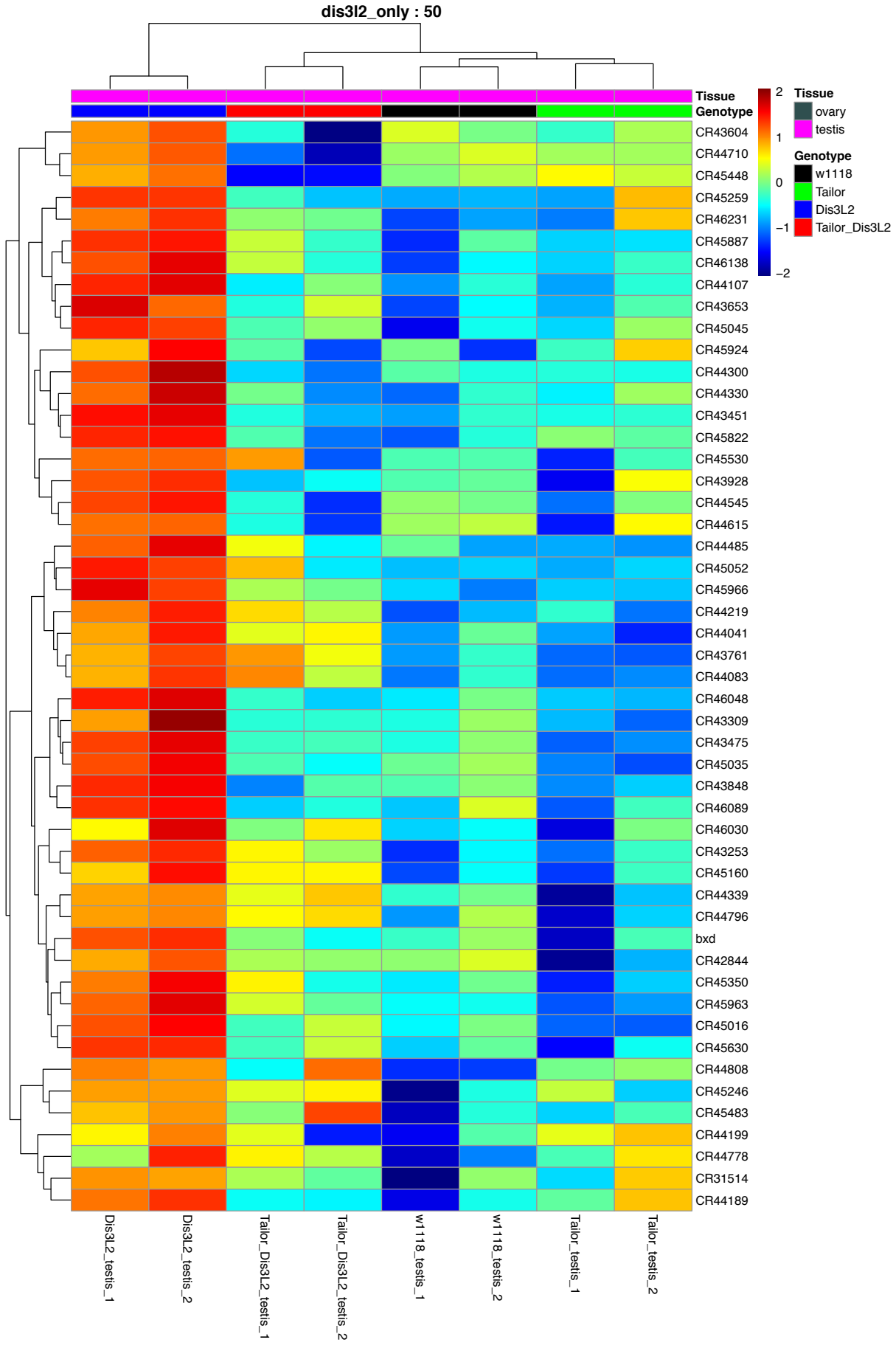
Supplementary Figure 9

Figure S10. Heatmaps of lncRNA clusters that are coordinately upregulated in *Tailor*, *Dis3L2*, or double mutants.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CR44485
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CR45160
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CR44796
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CR44808
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CR44778
CR31514
CR44189
Dis3L2_testis_1
Dis3L2_testis_2
Tailor_Dis3L2_testis_1
Tailor_Dis3L2_testis_2
w1118_testis_1
w1118_testis_2
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Tailor_testis_2
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Tissue
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Genotype
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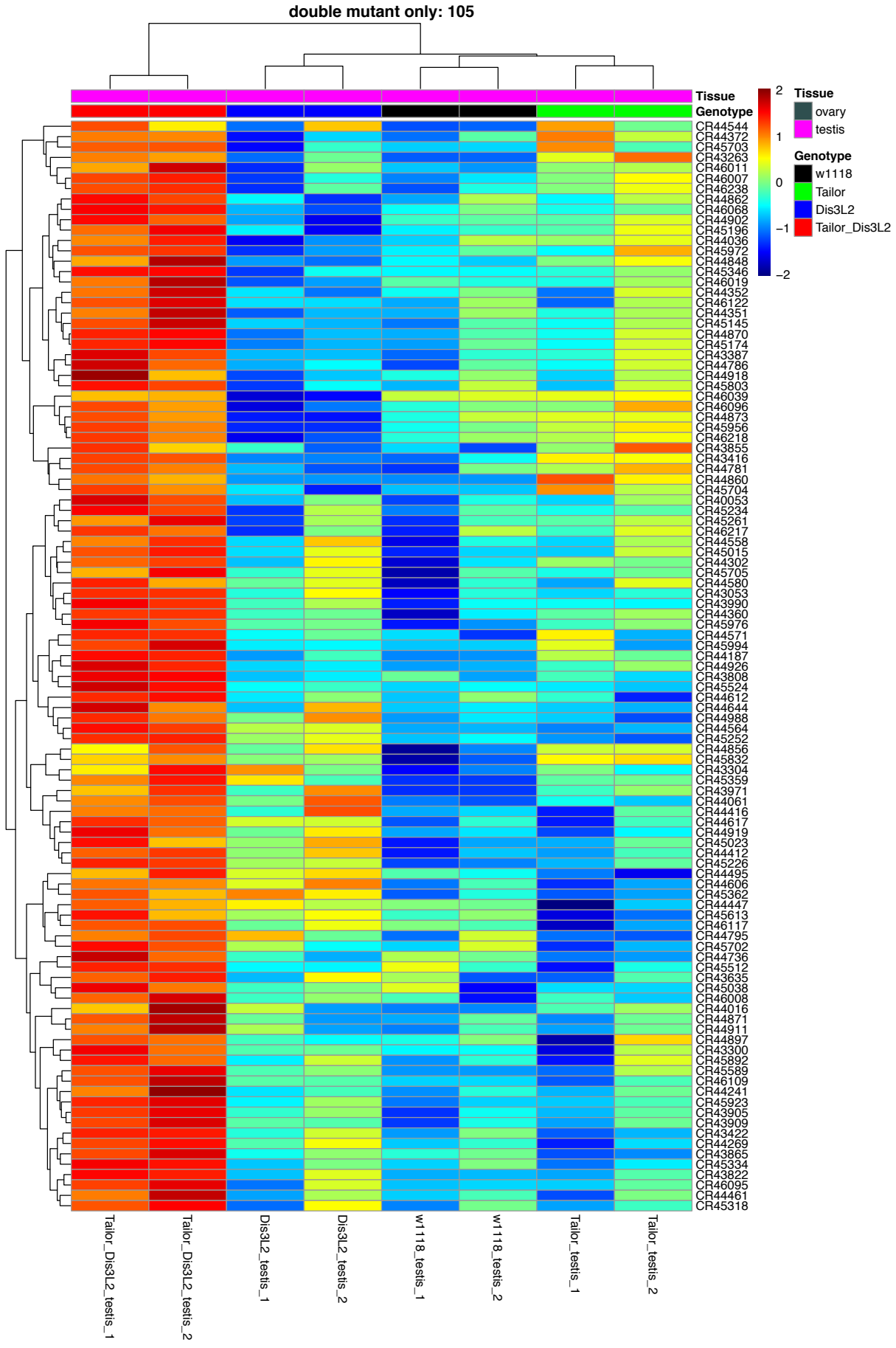
Tailor
Dis3L2
Tailor_Dis3L2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Tailor_Dis3L2
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CR46007
CR46238
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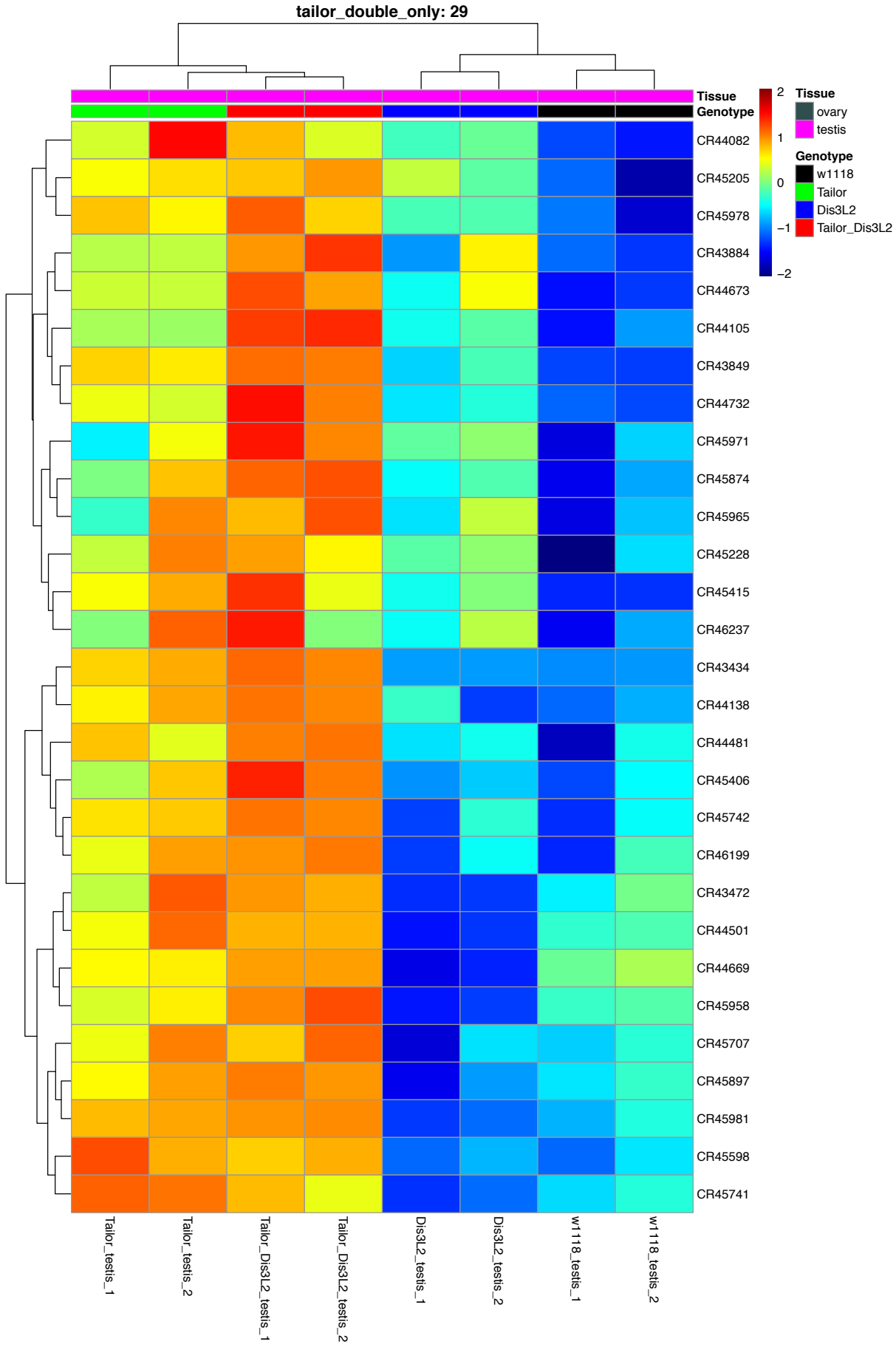
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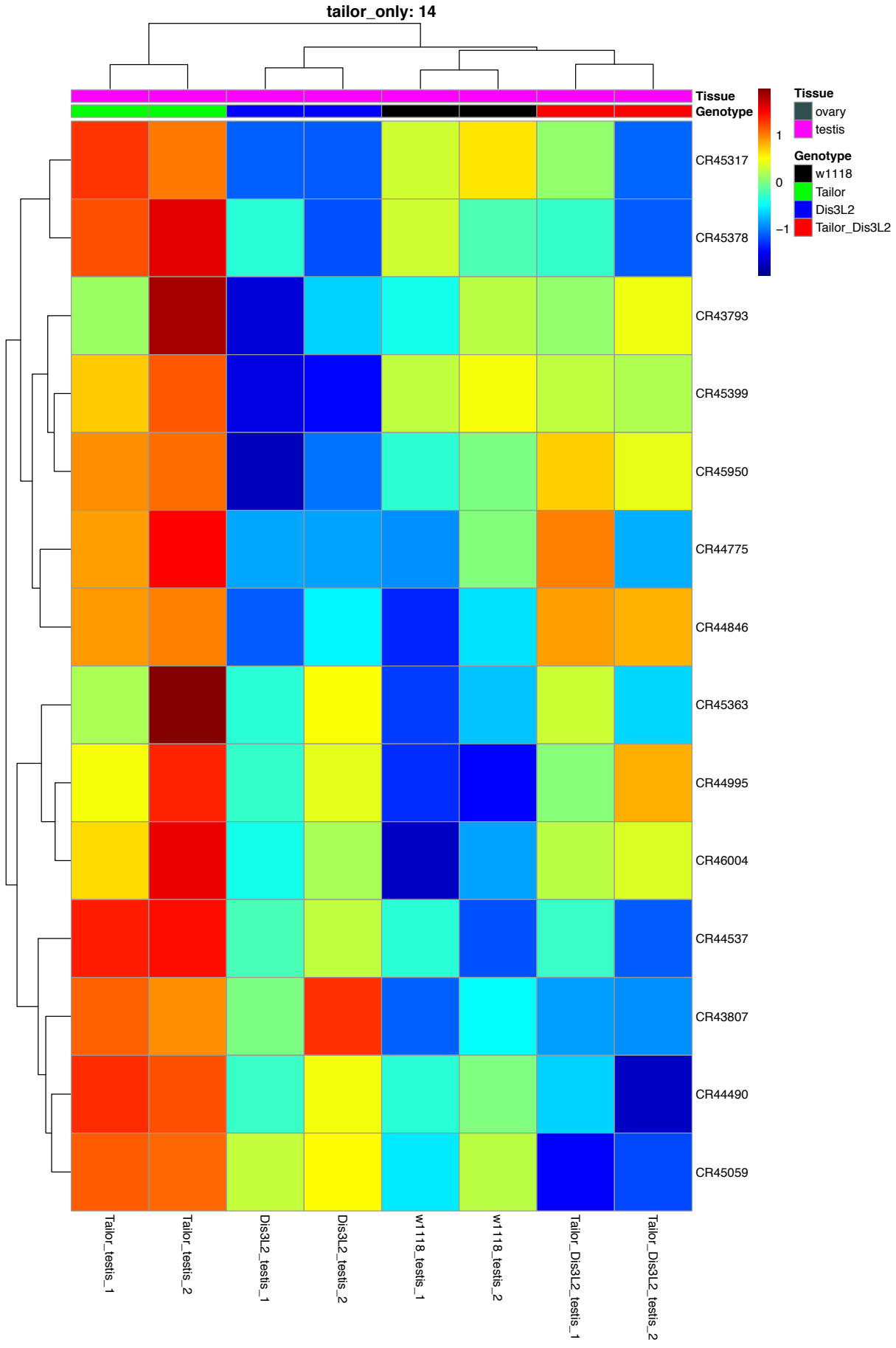
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Tailor_testis_2
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Dis3L2_testis_2
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Tailor_Dis3L2_testis_2
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0
−1
Tissue
ovary
testis
Genotype
w1118
Tailor
Dis3L2
Tailor_Dis3L2

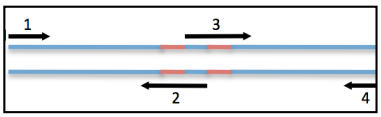
Supplementary Table 1. Statistics for total RNA-seq data produced in this study.

LabID	sample_genotype	genotype	gender	biorep	raw_reads	mapped_reads	mappability	mapped_reads (MAQ>2	mappability (MAQ>20)
G0028	w1118_male_1	w1118	male-testis	1	24944791	21006806	0.842	18217050	0.730
G0029	w1118_male_2	w1118	male-testis	2	26630888	22591202	0.848	20502054	0.770
G0032	Tailor.4-6_male_1	Tailor.4-6	male-testis	1	31545076	27454676	0.870	23462451	0.744
G0033	Tailor.4-6_male_2	Tailor.4-6	male-testis	2	31320624	27584818	0.881	24678401	0.788
G0034	Dis3L2.Df_male_1	Dis3L2.Df	male-testis	1	22954145	19410035	0.846	16981791	0.740
G0035	Dis3L2.Df_male_2	Dis3L2.Df	male-testis	2	25522306	20505373	0.803	18537043	0.726
G0038	Tailor_Dis3L2_male_1	Tailor_Dis3L2	male-testis	1	35456564	31435608	0.887	28806737	0.812
G0039	Tailor_Dis3L2_male_2	Tailor_Dis3L2	male-testis	2	11824793	10372208	0.877	9506974	0.804

MAQ>20: error of read alignment <= 1%

Table S2. Primer sequences for cloning and in vitro assays.

Tailor mutants



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-tRNANucl_rev	TTATTTCAGCTGGATCGAGCATTC
C93-95A_3	CATTCAGCgcTAGTTCCgcCAGCAACCGCATAGTGGGCAC
C93-95A_2	GCGGTTGCTGgcGGAACTAgcGCTGAATGTTCGCTTTATTG
G211A-S212A_3	CTACAAGTTCGcGgCCCGCATCACAGGCATTGG
G211A-S212A_2	GTGATGCGGGcCgCGAACTTGTAGACTCGCAGTG
D223A_3	CGATCCTCGGcCCTGGACCTCTTCGTCGACATC
D223A_2	GAGGTCCAGGgCCGAGGATCGATTTCCAATG
D223-225A_3	CGATCCTCGGcCCTGGcCCTCTTCGTCGACATCGG
D223-225A_2	GACGAAGAGGgCCAGGgCCGAGGATCGATTTCCAATG
H467A_3	ACAACTAAACgcCAATGTAACGAAAGCGGTGAC
H467A_2	CGTTACATTGgcGTTTAGTTGTATGGGATCCTG
Dis3L2 constructs	
3xHA tagged dis3l2	
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CG16940 3xHA EcoRI Fwd	GGACTATGCATATCCATATGACGTTCCA
	GATTACGCTATGCCTTACCCTTTATATCCCGCCCATA
CG16940 XbaI Rev	GACTCTAGACTAAATTTGTTCTTCCATCTTT
pUAS-3xHA-dis3l2-CD	
CG16940 D566A 569A Fwd	GATCCGATGACTGCTCGCGCTTTGGATGCCGCCGTTTCTATAGAGAAGC
CG16940 D566A 569A Rev	GCTTCTCTATAGAAACGGCGGCATCCAAAGCGCGAGCAGTCATCGGATC
Dis3L2 gRNA construct	
CG16940-F	CTTC GAAGCACATCCGCAACAGGT
CG16940-R	AAAC ACCTGTTGCGGATGTGCTTC