# Supplemental Figures S1 – S5
# Supplemental Table S5

## Widespread formation of double-stranded RNA in testis

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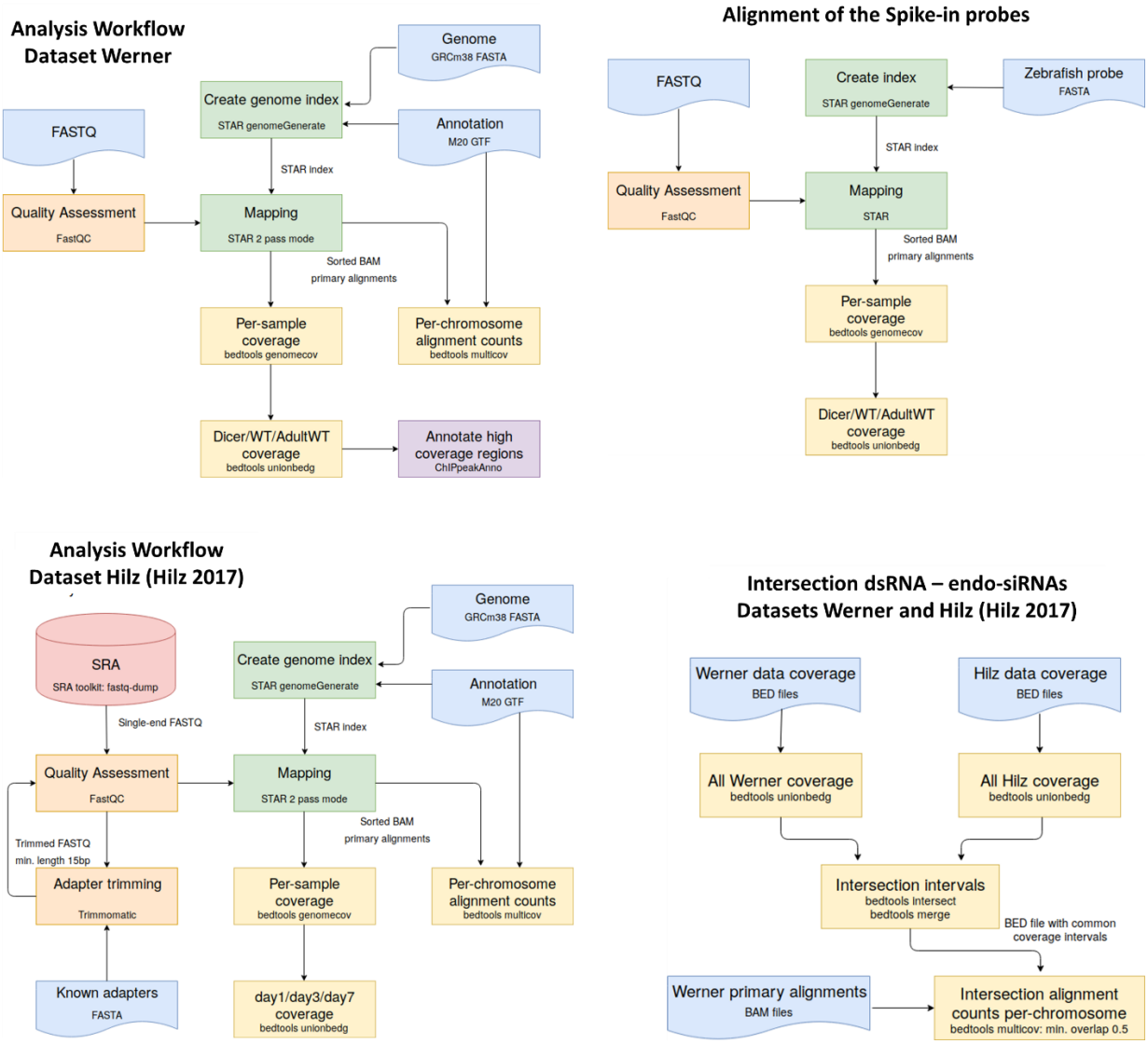
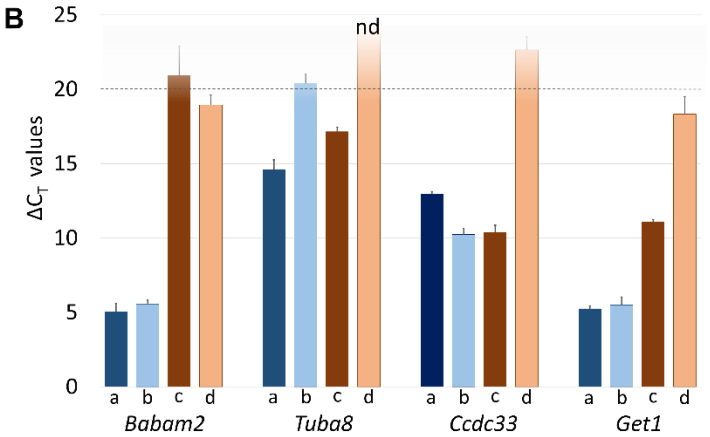
# Supplemental Figure S1




**Supplemental_Figure_S1**: Schematic representation of the bioinformatic workflow to analyse the different datasets. All code with annotation is available at (https://github.com/James-E-Clark/Masters-dsRNA-Project and https://github.com/jwcasement/dsRNA-Seq-project).

# Supplemental Figure S2

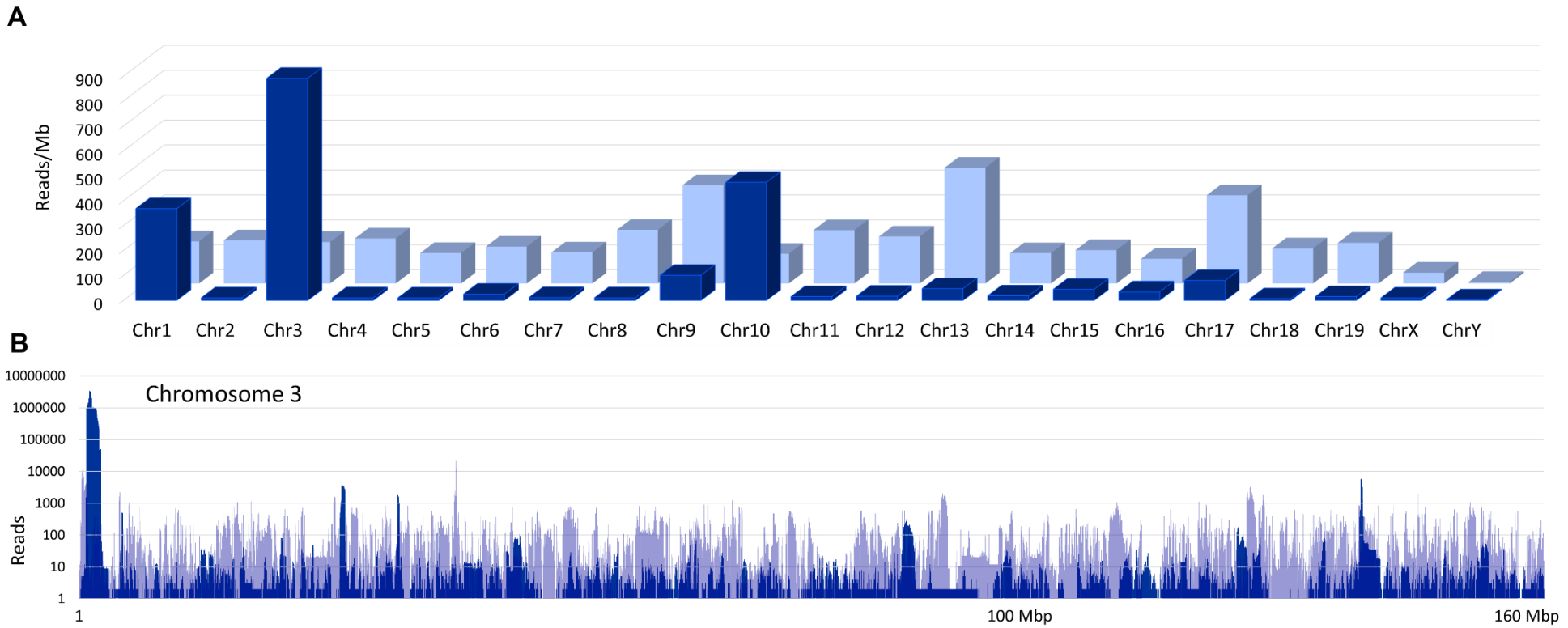

C

Source: FANTOM5 Project (Ensembl: Gene expression)

| **Sense** | ***Babam2*** | ***Tuba8*** | ***Ccdc33*** | ***Get1*** |
|---|---|---|---|---|
| TPM | 31 | 27 | 66 | 21 |
| **Antisense** | ***Gm17130*** | ***Gm15856*** | ***Gm16130*** | ***Gm15317*** |
| TPM | 0.6 | 0.5 | 1 | 0.6 |

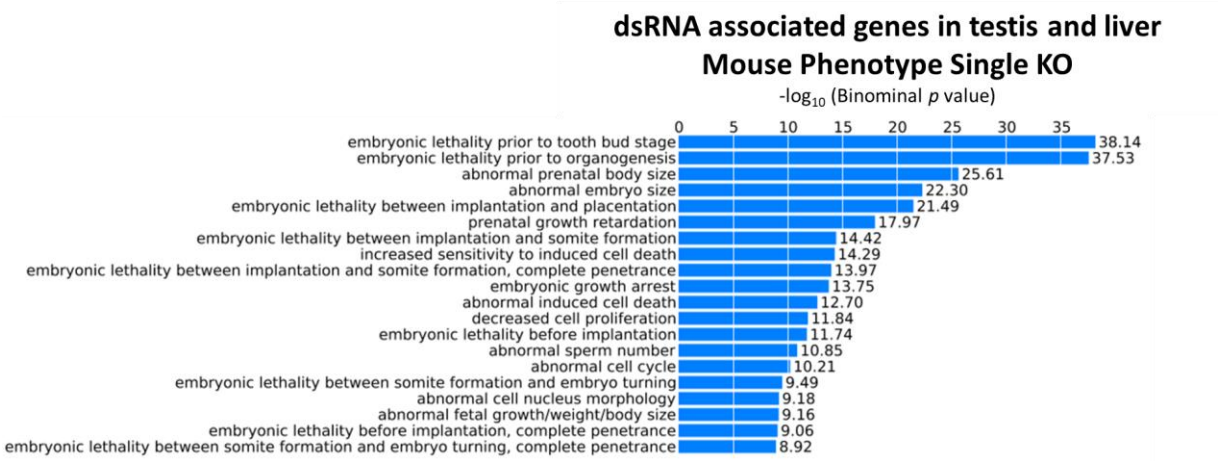
Supplemental_Figure_S2: RT-qPCR detection of 4 bi-directionally transcribed genes in juvenile mouse testis, Babam2, Tuba8, Ccdc33 and Get1. A) Four primer pairs were tested encompassing complementary and single stranded parts of the transcripts in both directions. All but two primer pairs spanned an intron to exclude amplification from genomic DNA. The amplicons from the protein coding sense transcript are in blue, the amplicons related to the complementary transcript are in brown. B) Bar graph indicating the ΔCT values of Babam2, Tuba8, Ccdc33 and Get1, and their complementary antisense pairs Gm17130, Gm15856, Gm16130, and Gm15317. ΔCT values relate to actin, hence high expression is reflected by small bars. Values above 20 are borderline detectable as indicated by the fading colors. The dark coloured bars (blue and brown) reflect amplification from the double stranded region, light colours from the single strand. N ≥ 6 from at least 2 independent experiments. C) Expression values for the assessed transcripts in TPM retrieved from Ensembl Gene expression (source: FANTOM 5 Project).

# Supplemental Figure S3



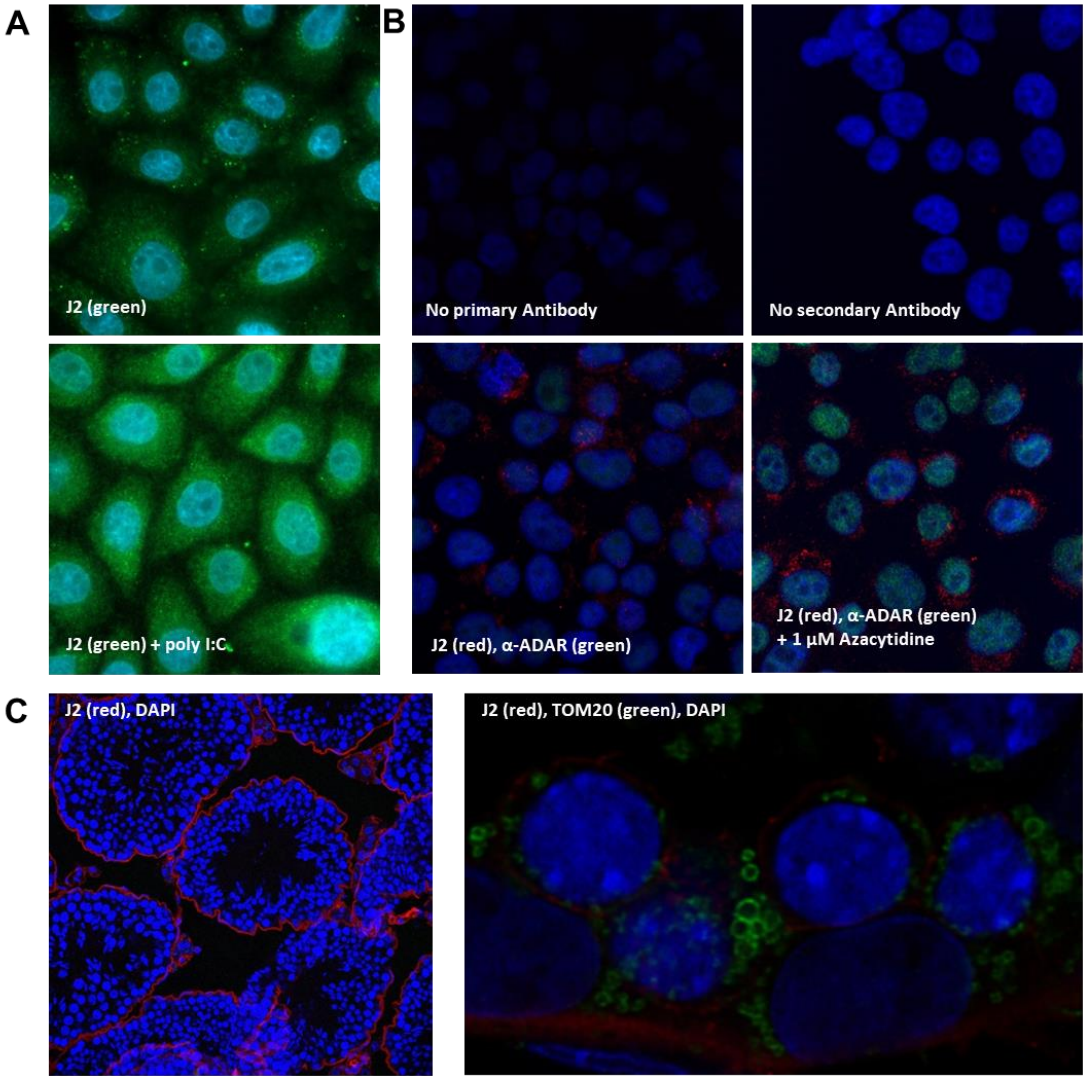

**Supplemental_Figure_S3:** Reads in both immune precipitated (RIP) and flow-through samples (FLOW) that intersect with endo-siRNAs (Hilz et al. RNA Biol 14: 219-235). A) Number of intersected reads per Mb on each chromosome compared between FLOW (dark blue) and RIP (light blue) samples. The Chromosomes 1, 3 and 10 are clear outliers in the FLOW samples. The sex chromosomes tend to show a lesser overlap between dsRNA and endo-siRNA formation even if the lower number of dsRNA peaks is taken into account. B) Visualization of reads on Chromosome 3 from FLOW (dark blue) and RIP samples (light blue) to demonstrate the disproportionate contribution of a single peak to the total read numbers. Read counts are presented at logarithmic scale.

# Supplemental Figure S4




Supplemental_Figure_S4: Consequences of dsRNA associated gene deletion on mouse phenotypes of dsRNA genes expressed in both testis and liver. The list of 1759 genes that form dsRNA was tested against all protein coding mouse genes (21,395). The mouse phenotype single KO database contains 9,170 entries that cover 9,466 or 44% of all genes.

# Supplemental Figure S5




Supplemental_Figure_S5: dsRNA detection using the J2 antibody. A) Keratinocytes (CCD cells) were stimulated with poly I:C, which is detected with the antibody (both control and poly I:C treated samples were processed and imaged using identical settings). In addition, dsRNA sensor proteins such as IFIH1, PKR and ADAR were upregulated in response to poly I:C (not shown). B) A375 melanoma cells stimulated with the demethylating drug Azacytidine which leads to dsRNA formation from transcription of repetitive elements. Both α-ADAR and J2 show enhanced staining after Azacytidine treatment. C) Mouse testis sections stained with J2 (red) and DAPI (blue, left) and J2, DAPI and the mitochondrial protein Tom20 (green, right; Abcam,ab78547). The red J2 staining is unspecific, possibly at membranes but absent in the cytosol of sperm cells and not found in structures that were expected to show dsRNA staining such as mitochondria (green circles in C) right) or possibly nucleus-associated structures (chromatoid body).

Supplemental_Table_S5: List of PCR primers used for RT-qPCR.

| Name | Sequence | Primer Length | Melting Temp. | Amplicon Length |
|---|---|---|---|---|
| Babam2.1F | TGCTGCTGATGTGGAAAGACT | 21 | 59.9 | |
| Babam2.1R | ATTTCCATCCCATCTGGGGC | 20 | 59.8 | 173 bp |
| Babam2.2F | GACCCTGGAGAAGATGTGGC | 20 | 60.1 | |
| Babam2.2R | GGGGAAAGCAGGGATGTGAA | 20 | 59.9 | 141 bp |
| Babam2.3F | CGTTGGAACCAAGTGAGCAG | 20 | 59.4 | |
| Babam2.3R | TTGTACGGTGCTCCAGCATT | 20 | 60 | 117 bp |
| Babam2.4F | TAAGCTCTCTGACCACCAGC | 20 | 59.1 | |
| Babam2.4R | GCATCTGCTCACTTGGTTCC | 20 | 59.2 | 124 bp |
| Tuba8.1F | ACCCAAGGACGTGAATGTCG | 20 | 60.3 | |
| Tuba8.1R | CGATTGCTGTGGTGTTGCTC | 20 | 60.1 | 182 bp |
| Tuba8.2F | TTCAGTGAGACTGGCAACGG | 20 | 60.25 | |
| Tuba8.2R | CGGGCGTAATTGTTAGCTGC | 20 | 60 | 158 bp |
| Tuba8.3F | AACTTATGGTCAAGGCGGGC | 20 | 60.7 | |
| Tuba8.3R | GGACTCGTGTGTCATCCTGT | 20 | 59.4 | 175 bp |
| Tuba8.4F | TCCGCCAAAGACAACGTCAG | 20 | 60.9 | |
| Tuba8.4R | TTAAGCTTGCTCTGAGGTCCC | 21 | 59.7 | 187 bp |
| Ccdc33.1F | GCTACTGCTGAACGAACTGG | 20 | 58.9 | |
| Ccdc33.1R | TGCTGAGCCTGGTACAGAAG | 20 | 59.4 | 120 bp |
| Ccdc33.2F | GACCCTGGCTGTTGACCTTT | 20 | 60.2 | |
| Ccdc33.2R | TCCAGGGACAAGATCCGACT | 20 | 60 | 198 bp |
| Ccdc33.3F | CCTGTCTCGCAGCCTCTTC | 19 | 60.2 | |
| Ccdc33.3R | AGAGGACATCCTGGCACTGA | 20 | 60.3 | 169 bp |
| Ccdc33.4F | GGCTTCCTGATAGAAGAGGGC | 21 | 59.9 | |
| Ccdc33.4R | GCAGCAGCCTAGAAGTTGTGA | 21 | 60.6 | 154 bp |
| Get1.1F | GTGTTGAGCTTCGTGTTCGG | 20 | 59.8 | |
| Get1.1R | TTTCCAGCCTGGCGTATCTG | 20 | 60.1 | 190 bp |
| Get1.2F | TAGACCGCCTGGTAGCCTT | 19 | 60 | |
| Get1.2R | TGAAAGGGTGGAGAACGATAGC | 22 | 60.1 | 105 bp |
| Get1.3F | GTTCCTTGTTCCCACGGACA | 20 | 60.2 | |
| Get1.3R | CCGAGCTTCTCACAGATCGC | 20 | 60.9 | 177 bp |
| Get1.4F | CCTTAGCTCGACTGTGAGGTG | 21 | 60.1 | |
| Get1.4R | AGGCCATACCGTTCTTGCAC | 20 | 60.7 | 187 bp |
| | | | | |
| ActB-F | GCTCTAGACTTCGAGCAGGAG | 21 | 59.7 | 226 bp |
| ActB-R | TAGAGGTCTTTACGGATGTCAAC | 23 | 57.2 | |