Supplementary Materials and Methods

Germ cell separation

Preparation of enriched populations of pachytene spermatocytes and round spermatids was carried out according to our laboratory's established protocol (1). Briefly, a single cell suspension of spermatogenic cells was made from the testes of seven inbred pure 129 Sv/Ev mice. This was achieved by removing the tunica albuginea and placing the tubules in cold Dulbecco's phosphate buffered saline, pH 7.4, (DPBS) followed by incubation with 1 mg/ml collagenase (Sigma Cat# C0130) for 5 minutes at 37° and then washing the tubules twice in cold DPBS. The tubules were then incubated with 0.25 mg/ml trypsin (Sigma cat#T8003) and 1 µg/ml DNaseI (Sigma cat#DNEP) for 7 minutes at 37° and then passed through a 70 µm nylon filter (BD Falcon). The resulting single cell suspension was separated on a 2-4% BSA in DPBS gradient, and fractions containing ≥85% purity of pachytene spermatocytes or ≥92% purity of round spermatids were pooled.

Microarray analysis, quality control, and differential expression quantification

RNA concentration and purity were assessed using a NanoDrop 1000 Spectrophotometer (Thermo Scientific) and RNA quality was assessed using a Bioanalyzer (Agilent Technologies). Briefly, 2 µg of RNA from each sample was reverse transcribed into cDNA and purified. The cDNA was then transcribed into biotin-labeled cRNA, purified and fragmented. Fragmentation was confirmed using a Bioanalyzer (Agilent Technologies). The fragmented cRNA was hybridized to the microarray chip by Columbia University's Comprehensive Cancer Center Genomics Core facility.

The quality of the microarray data was assessed both by Normalized Unscaled Standard Error (NUSE) and the Relative Log Expression (RLE) (2). NUSE, which measures relative Chip quality, showed that all eight microarrays are of similar quality both as measured over the interquartile range (Supplementary Fig. S2A, boxes) and the median value (Supplementary Fig. 1A, dark lines). RLE gives an absolute measure

of variability and any median value that is not between 0.1 and -0.1 is considered an outlier (3). All eight microarrays have median RLE's within this range (Supplementary Fig. S2B, dark lines).

We identified the differentially expressed genes by computing the contrasts, which was achieved by fitting to the linear model, performing the hypothesis test, correcting for multiple testings, and controlling for the false discovery rate using the Benjamini-Hochberg correction with an accepted $\alpha \leq 0.05$ (4). The dataset was hierarchically clustered in an unsupervised manner, using average linkage Spearman correlation, limited to genes with an interquartile range (IQR) > 0.5, and a heatmap was generated to visually represent the levels of gene expression.

Quantitative real-time PCR

cDNA was synthesized from extracted RNA using random hexamers and the TaqMan Reverse Transcription Kit (Applied Biosystems) and qRT-PCR performed with the Power SYBR Green master mix (Applied Biosystems) on a ABI Prism 7500 (Applied Biosystems). Each reaction was performed in triplicate. The following primers were used:

Srsf2_coding forward: GACCGCTACACCAAGGAGTC; *Srsf2_coding reverse*: GAGACTTGGAGCGGCTGTAG; *Srsf2_UTR forward*: CCTCAGGTGGCAAACTTCAT; *Srsf2_UTR reverse*: GGCAATGTTGAGTCATTCTGC; *Ddx5_coding forward*: CGGGATCGAGGGTTTGGTG; *Ddx5_coding reverse*: GCAGCTCATCAAGATTCCACTTC; *Ddx5_UTR forward*: TCCAGGGCTGATAACATTGA; *Ddx5_UTR reverse*: GCCCCACAATCATTTCCATA; *Hnrnpk_coding forward*: CAGCTCCCGCTCGAATCTG; *Hnrnpk_coding reverse*: ACCCTATCAGGTTTTCCTCCAA; *Hnrnpk_UTR forward*: GGTTGGAAAGACTTGGCTTG; *Hnrnpk_UTR reverse*: CAGCATCAGCCACTTCCATA; *Tardbp_coding forward*: ATGAGAACGATGAACCCATTGAA; *Tardbp_coding reverse*: TGAGACACGGGATTCCGGTAG; *Tardbp_variant1 forward*: GAGCAGTTGGGGTATGATGG; *Hnrnpk_variant1 reverse*:

GGGCACCAGAATTAGAACCA; *actb forward*: CAGCTTCTTTGCAGCTCCTT; *actb reverse*: TTTGATGTCACGCAGGATTT.

Northern blot and *in situ* hybridization analysis

Fifteen micrograms of each sample were electrophoresed on 1.5% agarose gels containing 2.2 M formaldehyde at four volts/cm for 5-7 hours. Ethidium bromide staining of the 18S and 28S RNAs was used to determine equal loading for each sample. After electrophoresis the gel was washed and transferred onto Genescreen Plusr hybridization transfer membrane (Perkin Elmer) by blotting overnight in 10X Saline-Sodium Citrate (SSC) buffer. The blot was then rinsed, UV cross-linked and pre-hybridized for 4 hours in 7% Sodium Dodecyl Sulfate (SDS). The same forward primers and the *Srsf2_UTR reverse* primer that were used for quantitative real-time PCR primers were used to make PCR fragments for insertion into the pGEM ® -T Easy vector (Promega). The following reverse primers were used: N*Srsf2_coding reverse*: ACGGAGGAGGACTTGGACTT; N*Ddx5_coding reverse*: AATGGCAGGCAGCAAATAAG; N*Hnrnpk_coding reverse*: AAACCACCTCTTCCCCTCAT; N*Tardbp_coding reverse*: AAAGCCAAACCCTTTCGAGT. The gene fragments were then excised and ^{32}P-labeled RNA probes were transcribed using Prime-It® RmT random primer-labeling kit (Stratagene). After the probes were purified by passing through MicroSpin ® G-25 columns (GE Healthcare) they were hybridized overnight at 65° C in 7% SDS. After hybridization the blot was washed in 2X SSC, followed by 2X SSC and 0.1% SDS and finally in 0.1% SDS alone.

For *in situ* hybridization analysis slides were rehydrated and re-fixed in 4% PFA at room temperature for 10 minutes and then digested in Proteinase K (Roche cat#115879001) at 37° C for 7 minutes and re-fixed in 4% PFA at room temperature for 5 minutes. Pre-hybridization was carried out at 50° C for 2 hours in DIG Easy Hyb (Roche cat# 11796895001) hybridization solution in 50% formamide. Sense and anti-sense *Srsf2* digoxigenin (Dig) labeled probes were transcribed from a *Srsf2* coding fragment and *Srsf2* UTR fragment in a pGEM ® -T Easy vector (Promega) (see northern blot hybridization analysis) using

T7 and SP6 RNA polymerase and a DIG RNA labeling mix (Roche cat#11277073910). Slides were hybridized overnight at 50° C, rinsed in 5X SSC and washed in 0.2X SSC at 65° C for three hours. After pH adjustment the slides were exposed to the NBT-BCIP (Roche cat#11383213001 and 11383221001) color reaction for 3 days at 4° C.

Immunoblot analysis

Briefly, whole testis lysates were made with modified RIPA buffer [50 mM Tris-Hcl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and protease inhibitor cocktail (Roche)] and boiled for 5 minutes in 1X SDS loading buffer. They were separated on a 10% SDS-PAGE gel and transferred to a PVDF membrane. The blots were incubated with rabbit polyclonal anti-SRSF2 antibody (Abbiotec cat# 200181) at a concentration of 1:500, rabbit polyclonal anti-DDX5 antibody (GeneTex, cat# GTX100234) at 1:2000, mouse monoclonal anti-HNRNPK antibody (Abd Serotec, cat# MCA2622GA) at 1:3000, rabbit monoclonal anti-TARDBP(Epitomics cat# 5238-1) at 1:3000, and mouse monoclonal anti-β-actin antibody (Sigma, cat# A5316-.2ML) at 1:5000. Secondary antibodies used were goat-anti rabbit IgG-HRP (Santa Cruz, cat# sc-2004) and goat-anti mouse IgG-HRP (Santa Curz, cat# sc-2005) at 1:3000.

Immunofluorescence

Four percent PFA fixed control and mutant sections were used for immunofluorescence with SRSF2 antibody. The SRSF2 antibody (see above) was used at 1:100, and Alexa Fluor- 488 goat anti-mouse IgG secondary antibody (Molecular Probes, cat #A11029) was used at 1:300, followed by staining with DAPI.

Supplementary references

1. Wolgemuth, D.J., Gizang-Ginsberg, E., Engelmyer, E., Gavin, B.J. and Ponzetto, C. (1985) Separation of mouse testis cells on a Celsep (TM) apparatus and their usefulness as a source of high molecular weight DNA or RNA. *Gamete Res*, **12**, 1-10.
2. Brettschneider, J., Collin, F., Bolstad, B.M. and Speed, T.P. (2008) Quality Assessment for Short Oligonucleotide Microarray Data. *Technometrics* **50**, 241-264.
3. Kauffmann, A., Gentleman, R. and Huber, W. (2009) arrayQualityMetrics--a bioconductor package for quality assessment of microarray data. *Bioinformatics*, **25**, 415-416.
4. Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B*, **57**, 289–300.

Supplementary Table and Figure legends

Supplementary Figure S1. Microarrays on RNA of purified round spermatids are of high quality. (**A**-**B**) Quality metrics, Normalized Unscaled Standard Error (NUSE) and the Relative Log Expression (RLE) showing that all 8 microarrays are comparable and of sufficient quality to be examined for expression changes.

Supplementary Figure S2. *In situ* sense probes for *Srsf2* do not hybridize. *In situ* hybridization of control and mutant testicular sections with *Srsf2* sense probes. Neither probe shows the presence of sense transcripts.

Supplementary Table S1. A sortable list of all genes differentially expressed in *Brdt*$^{\Delta BD1/\Delta BD1}$ mutant round spermatids, regardless of adjusted Pvalue. A total of 1773 probe sets were differentially expressed in mutant round spermatids as compared to control round spermatids. Of these 1281 probes sets correspond to 1046 genes that are up-regulated and 492 probe sets correspond to 432 genes that are down-regulated. The Affymetrix probe set ID, the Log of the fold change, the adjusted P-Value, and the gene name are provided. The table can be sorted by any criteria.

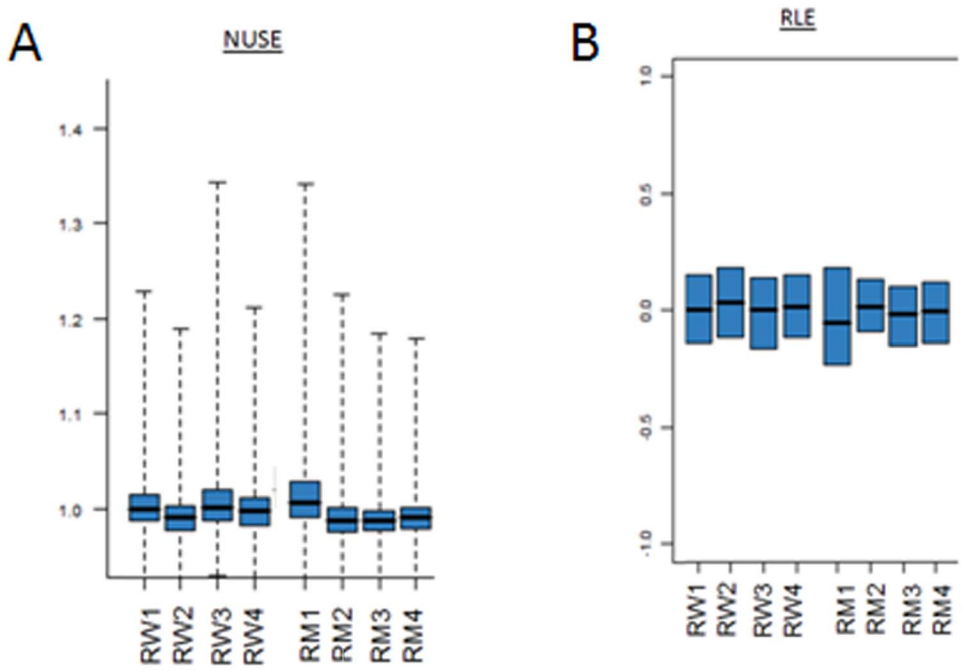
Supplementary Table S2. Pathway and function analysis of genes with an adjPVal ≤ 0.05, and up-regulated in *Brdt*$^{\Delta BD1/\Delta BD1}$ mutant round spermatids. A total of 234 probe sets, corresponding to 207 genes, were statistically significantly up-regulated in mutant round spermatids. These genes were analyzed for common pathways and functions using the DAVID genome software. Two pathways and seven functional categories (highlighted in red) were significantly enriched. The accession numbers of the enriched genes are listed for each pathway or category.

Supplementary Table S3. Pathway and function analysis of genes with an adjPVal ≤ 0.05, and down-regulated in *Brdt*$^{\Delta BD1/\Delta BD1}$ mutant round spermatids. A total of 222 probe sets, corresponding to 208 genes, were statistically significantly down-regulated in mutant round spermatids. These genes were

analyzed for common pathways and functions using the DAVID genome software. No pathways or functional categories were significantly enriched.

Supplementary Table S4. Pathway and function analysis of all genes up-regulated in *Brdt*$^{\Delta BD1/\Delta BD1}$ mutant round spermatids, regardless of adjusted Pvalue. A total of 1281 probes sets, corresponding to 1046 genes, were up-regulated in mutant round spermatids. These genes were analyzed for common pathways and functions using the DAVID genome software. Three pathways and forty functional categories (highlighted in red) were significantly enriched. The accession numbers of the enriched genes are listed for each pathway or category.

Supplementary Table S5. Pathway and function analysis of all genes down-regulated in *Brdt*$^{\Delta BD1/\Delta BD1}$ mutant round spermatids, regardless of adjusted Pvalue. A total of 492 probe sets corresponding to 432 genes that were down-regulated. No pathways or functional categories were significantly enriched.



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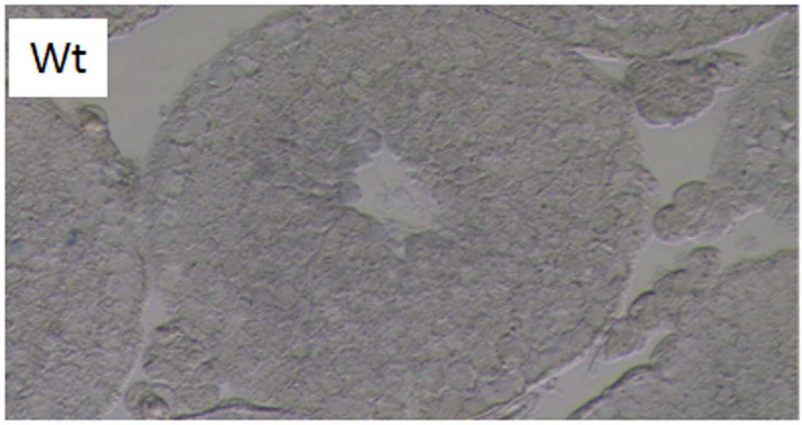
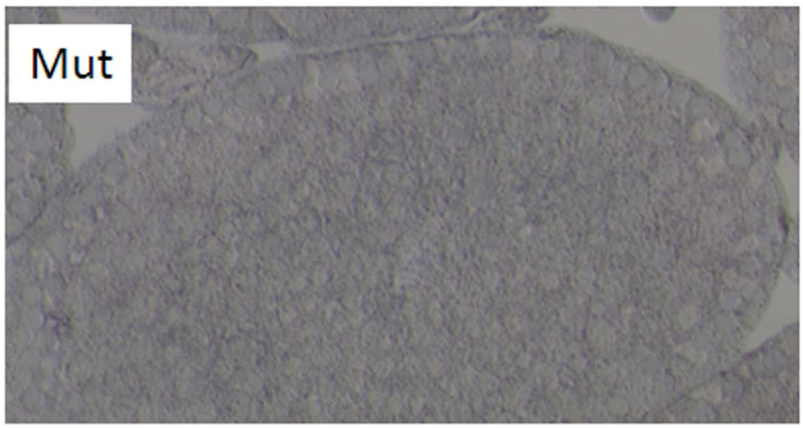
Srsf2 Coding Sense



Srsf2 Distal 3' UTR Sense



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