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Supplemental information

**Transcriptional metabolic reprogramming
implements meiotic fate decision
in mouse testicular germ cells**

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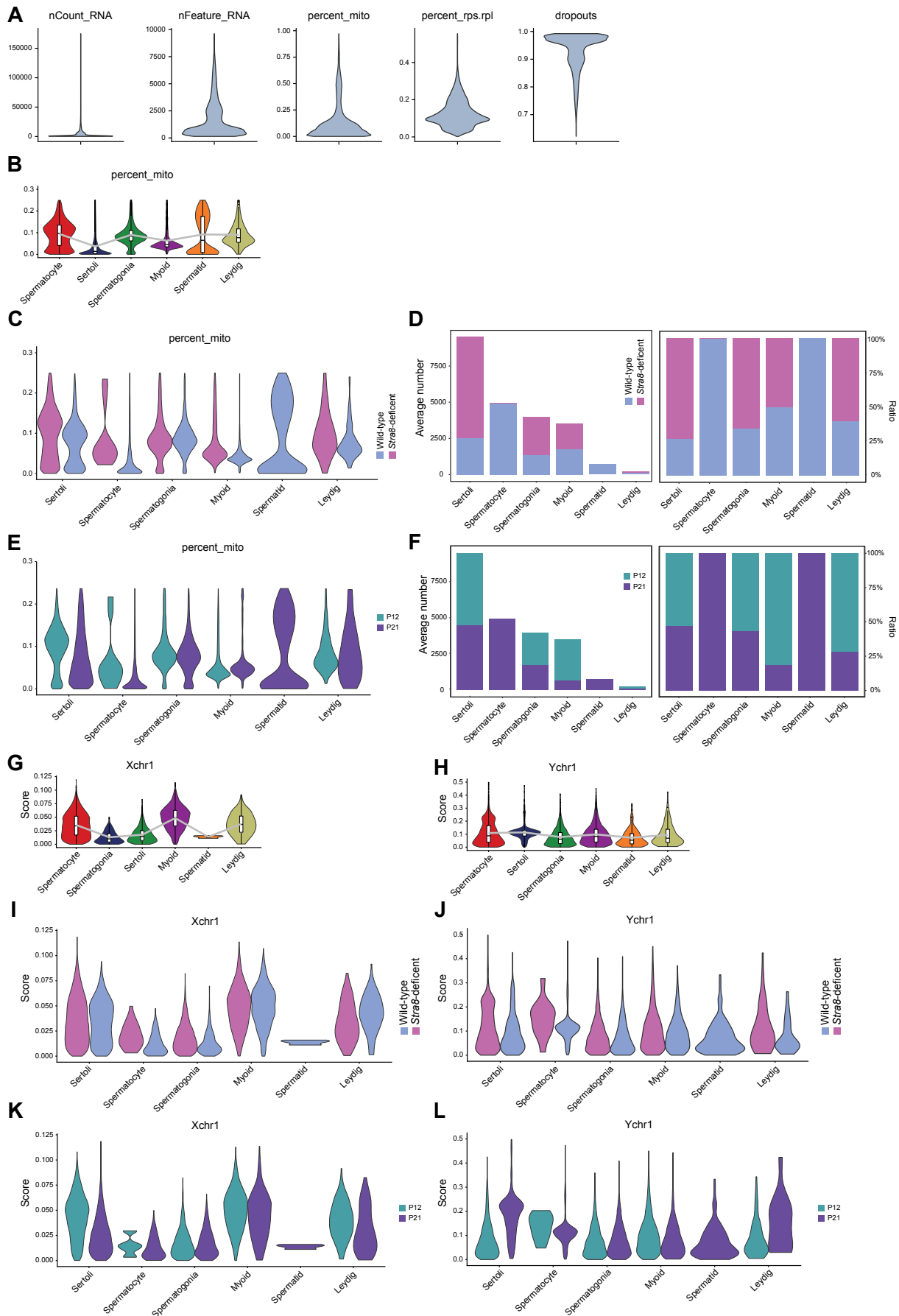


Figure S1

- 1 **Figure S1. 10X genomics quality control in scRNA-seq. Related to Figure 1.**
- 2 **A.** QC metrics showing number of counts, features, percentage of mitochondrial
- 3 genes, percentage of ribosome genes, and ratio of dropouts.
- 4 **B.** Distribution of mean expression level of mitochondrial genes for all cell types.
- 5 **C, D.** Distribution of mean expression level (**C**) and average number of mitochondrial
- 6 genes (**D**) for all major cell types between wild-type and *Stra8*-deficient
- 7 samples.
- 8 **E, F.** Distribution of mean expression level (**E**) and average number of mitochondrial
- 9 genes (**F**) for all major cell types between postnatal day 12 and day 21 samples.
- 10 **G.** Distribution of mean expression level of ChrX genes for all cell types.
- 11 **H.** Distribution of mean expression level of ChrY genes for all cell types.
- 12 **I, J.** Distribution of mean expression level of ChrX (**I**) and ChrY (**J**) genes for all major
- 13 cell types between wild-type and *Stra8*-deficient samples.
- 14 **K, L.** Distribution of mean expression level of ChrX (**K**) and ChrY (**J**) genes for all
- 15 major cell types between postnatal day 12 and day 21 samples.

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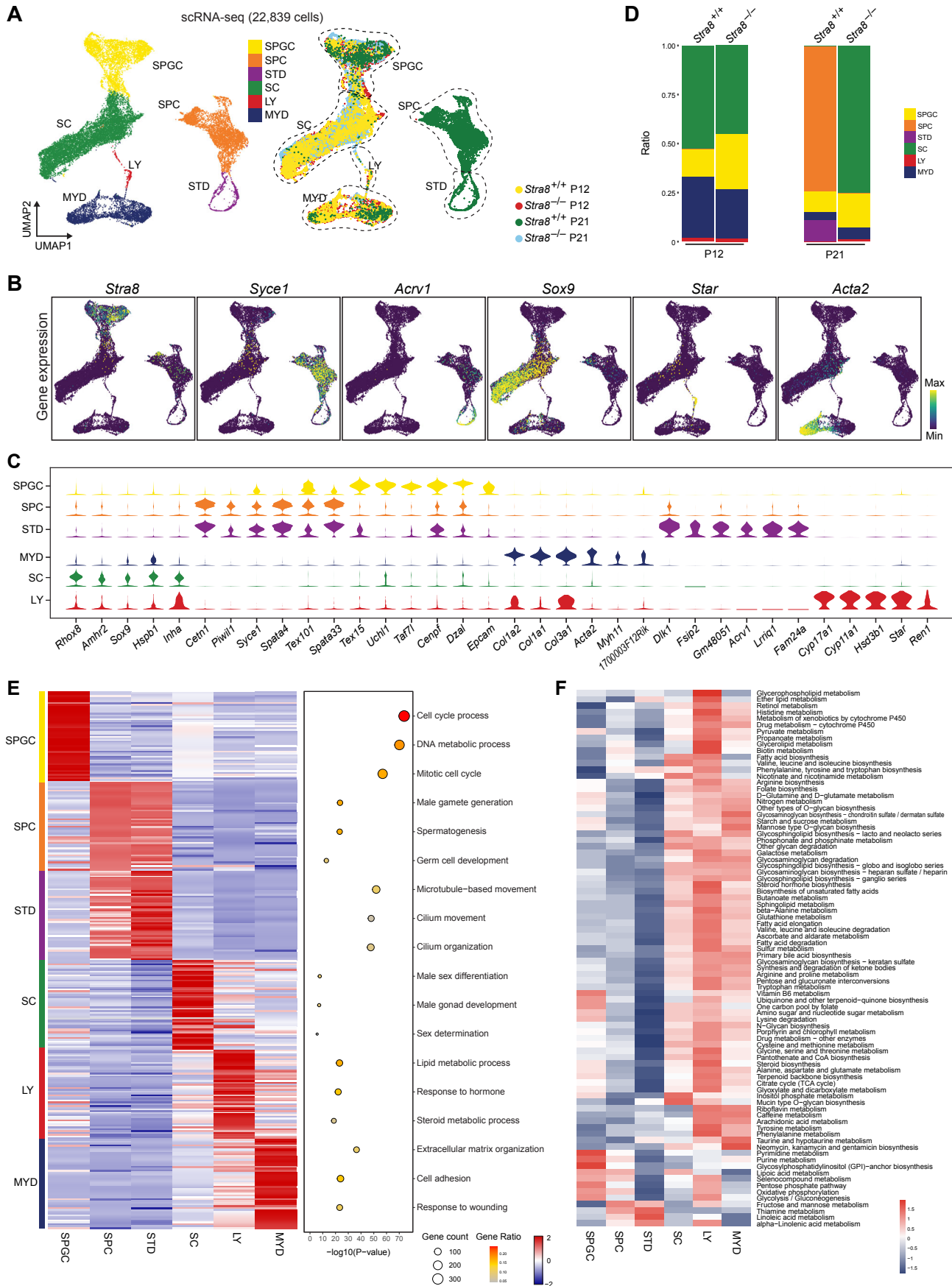


Figure S2

24 **Figure S2. scRNA-seq analysis of wild-type and *Stra8*-deficient mouse testes.**

25 **Related to Figure 1.**

- 26 **A.** Uniform manifold approximation and projection (UMAP) plot of cells captured
27 from wild-type and *Stra8*-deficient mouse testes colored by cluster and sample
28 identity. SPGC, spermatogonia/early spermatocyte. SPC, spermatocyte. STD,
29 spermatid. SC, Sertoli cell. LY, Leydig cell. MYD, myoid cell.
- 30 **B.** Histograms showing the percentage of each cell cluster (SPGC, SPC, STD, SC,
31 LY, MYD) in wild-type and *Stra8*-deficient samples at P12 and P21.
- 32 **C.** Distribution of known marker genes in each cluster cell shown as UMAP feature.
- 33 **D.** Violin plot showing the expression of representative gene expression for each
34 cell type.
- 35 **E.** Heatmap showing differential expression genes from each cell type. Top 3
36 enriched GO terms for each cell type are shown on the right.
- 37 **F.** Heatmap showing gene expression of metabolism signatures in indicated cell
38 types.

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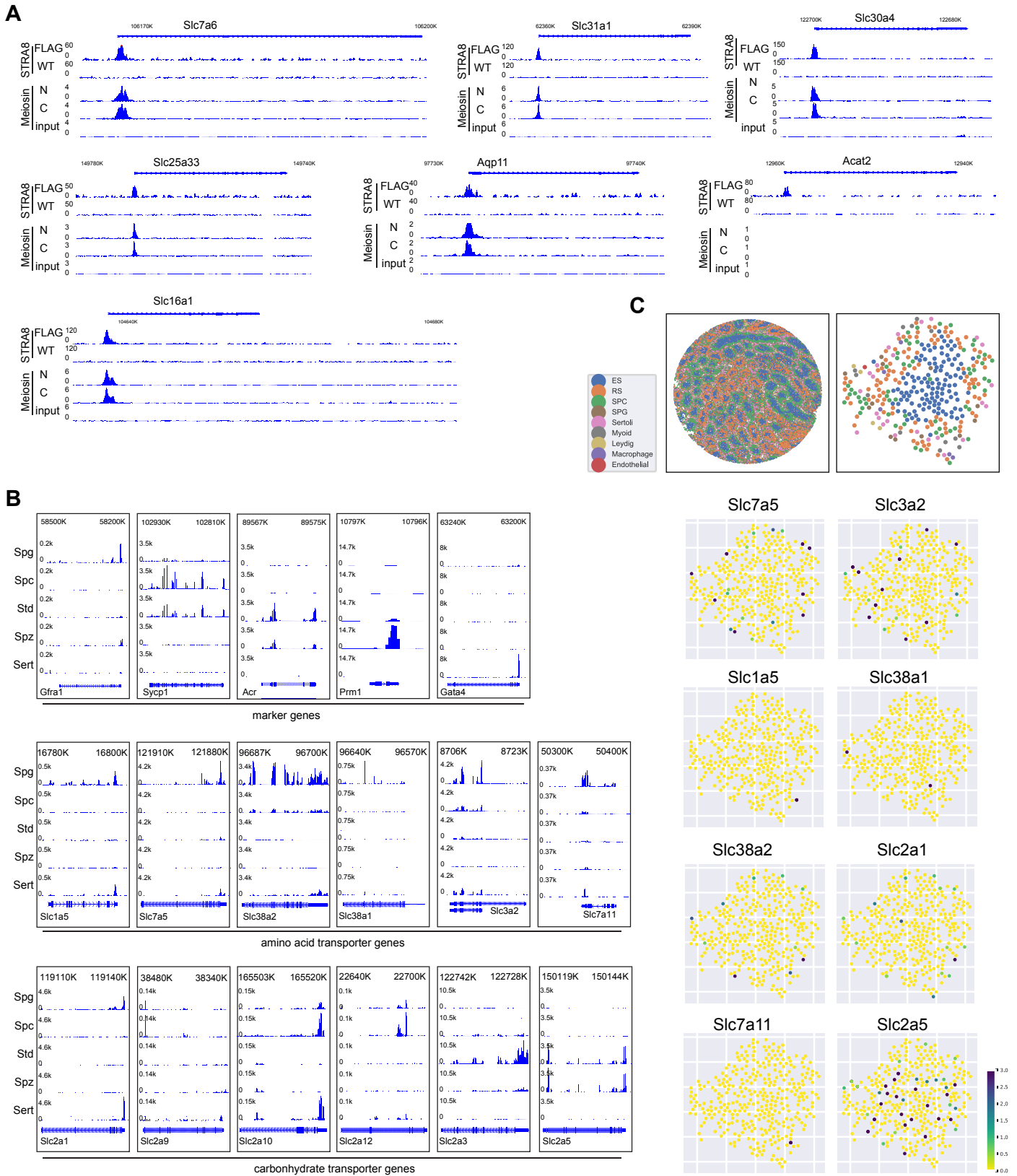


Figure S3

47 **Figure S3. Nutrient transporter gene expression in spermatogenesis. Related to**
48 **Figures 2 and 3.**

49 **A.** Genome tracks of *Slc7a6*, *Slc31a1*, *Slc30a4*, *Slc25a33*, *Aqp11*, *Acat2*, and *Slc16a1*
50 genes for Stra8 ChIP-seq (Kojima *et al*, 2019) and Meiosin ChIP-seq (Ishiguro *et al*,
51 2020). In Stra8 ChIP-seq, FLAG antibody was used to pull down Stra8 in FLAG-
52 tagged Stra8 knockin mouse. Wild-type mice, in which Stra8 is not tagged with
53 FLAG was used as the negative control. In Meiosin ChIP-seq, antibodies against N-
54 and C-terminal Meiosin were used to immunoprecipitate Meiosin. Input was used as
55 the negative control.

56 **B.** Gene expression from a published RNA-seq dataset (Soumillon, et al., 2013).
57 Upper panel, marker genes genome tracks of *Gfra1*, *Sycp3*, *Acr*, *Prm1*, and *Gata4*
58 for spermatogonia (Spg), spermatocyte (Spc), spermatid (Std), spermatozoa (Spz),
59 and Sertoli cell (Sert) from. Middle panel, genome tracks of amino acid transporter
60 genes, including *Slc1a5*, *Slc7a5*, *Slc38a2*, *Slc38a1*, *Slc3a2*, and *Slc7a11*, for
61 spermatogonia, spermatocyte, spermatid, spermatozoa and Sertoli cell. Lower
62 panel, genome tracks of carbohydrate transporter genes, including *Slc2a1*, *Slc2a9*,
63 *Slc2a10*, *Slc2a12*, *Slc2a3*, and *Slc2a5* genes for spermatogonia, spermatocyte,
64 spermatid, spermatozoa and Sertoli cell.

65 **C.** Gene expression from a published spatial transcriptome atlas (Chen, et al., 2021).
66 Upper panel, spatial mapping of testicular cell types in testicular section (left) and
67 one tubule (right). ES, elongating/elongated spermatid. RS, round spermatid. SPC,
68 spermatocyte. SPG, spermatogonia. Lower panel, spatial expression pattern of
69 *Slc1a5*, *Slc7a5*, *Slc38a2*, *Slc38a1*, *Slc3a2*, *Slc2a5*, *Slc2a1*, and *Slc7a11*.

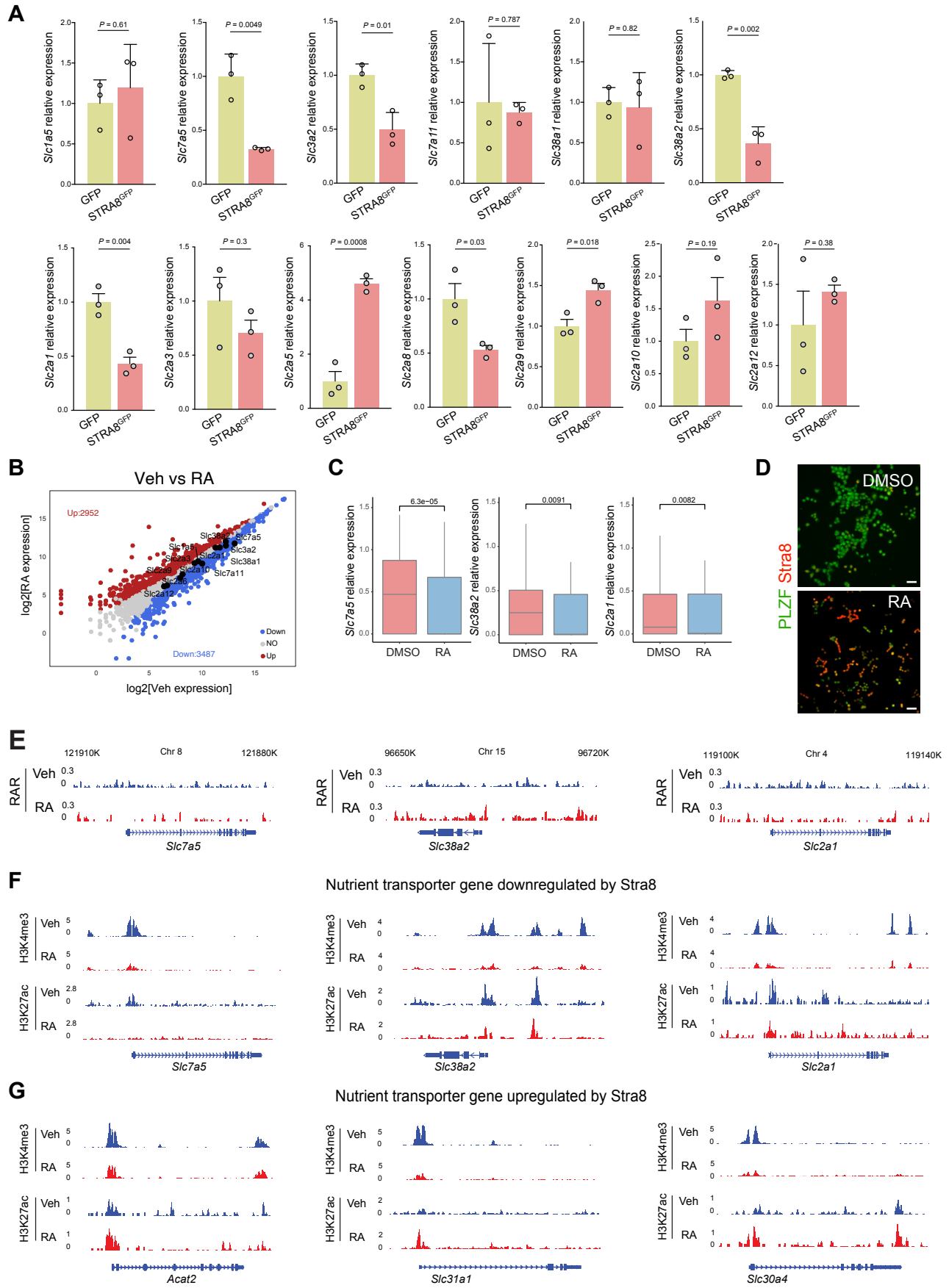


Figure S4

70 **Figure S4. Stra8 represses nutrient transporter expression. Related to Figures 2**
71 **and 3.**

72 **A.** qPCR of glutamine and glucose transporter genes in cultured F9 cells stably
73 transfected with GFP (control) or STRA8-GFP fusion protein. *P* value is the result
74 of Student *t*-test.

75 **B.** Volcano plot showing nutrient transporter genes of *Slc1a5*, *Slc7a5*, *Slc38a2*,
76 *Slc38a1*, *Slc3a2*, *Slc2a5*, *Slc2a1*, and *Slc7a11* expression in RNA-seq dataset of
77 cultured SSCs with indicated treatments. Veh, vehicle. RA, retinoic acid.

78 **C.** *Slc7a5*, *Slc38a2*, and *Slc2a1* genes expression in in vivo germ cell with RA
79 treatment from mouse scRNA-seq dataset. *P* value is the result of Student *t*-test.

80 **D.** Representative immunofluorescence images of Plzf and Stra8 are shown in
81 cultured spermatogonia stem cells (SSCs) with or without RA treatment (100 nM)
82 for 24 hours. Scale bar indicates 50 μ m.

83 **E, F.** Genome tracks of *Slc7a5*, *Slc38a2*, and *Slc2a1* genes in RAR ChIP-seq (**E**),
84 H3K4me3 ChIP-seq and H3K27ac ChIP-seq (**F**) with or without RA treatment in
85 the GEO ([GSE116798](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116798)).

86 **G.** Genome tracks of *Acat2*, *Slc31a1*, and *Slc30a4* genes in H3K4me3 ChIP-seq
87 and H3K27ac ChIP-seq with or without RA treatment from GEO ([GSE116798](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116798)).

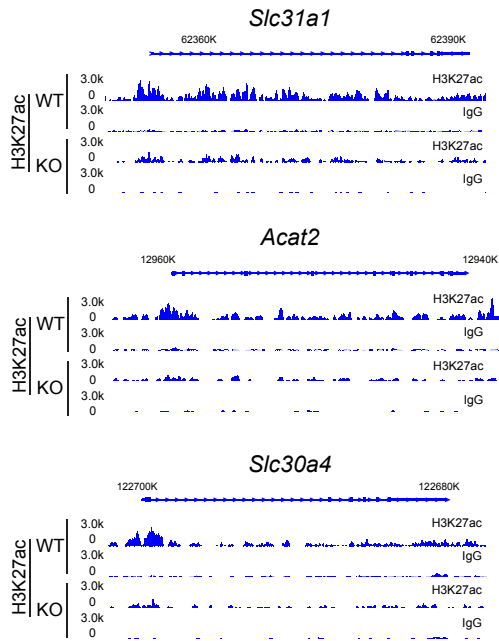
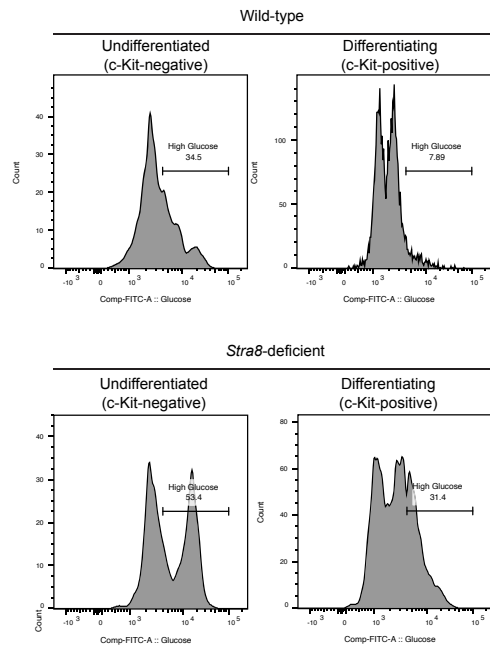
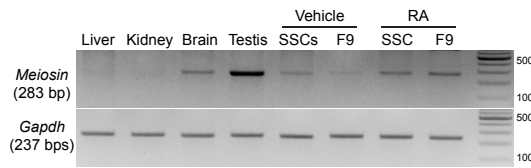
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A**C****B****Figure S5**

93 **Figure S5. Downregulation of glucose uptake during meiotic initiation requires**
94 ***Stra8*. Related to Figures 3 and 4.**

95 **A.** Genome tracks of *Slc31a1*, *Acat2*, and *Slc30a4* genes from CUT&RUN-seq for
96 H3K27ac using wild-type and *Stra8*-deficient testes at P12. Samples incubated
97 with preimmune IgG were used as the negative controls.

98 **B.** *Meiosin* (*Gm4969*) expression detected by RT-PCR analysis in indicated tissues
99 and in F9 cells and cultured SSCs with or without RA treatment.

100 **C.** FACS plots of FITC intensity indicative of glucose levels in indicated cell types
101 and genotypes. Freshly sorted cells were incubated with 2-NBDG for 15 minutes,
102 followed by FACS analysis.

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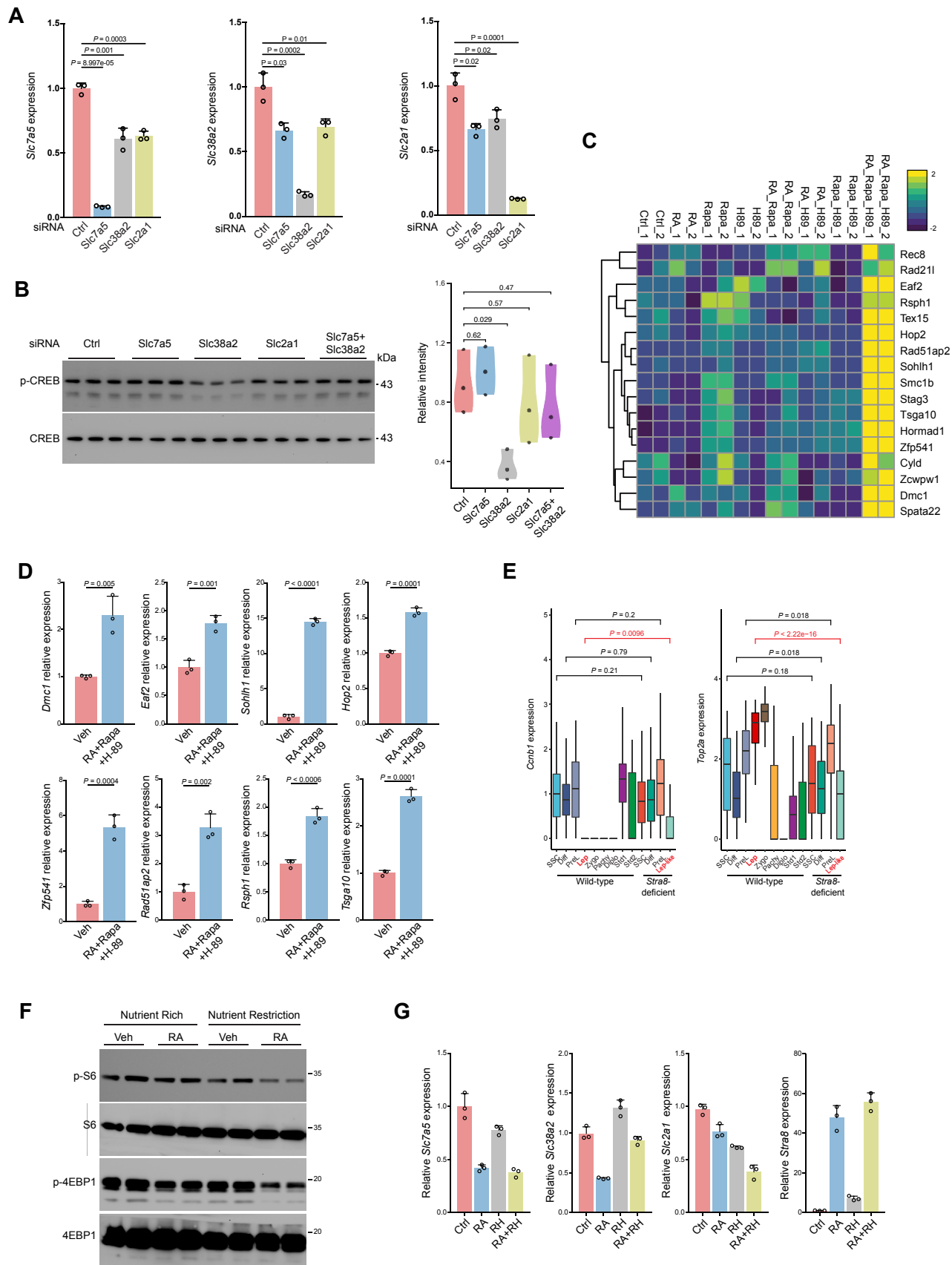


Figure S6

116 **Figure S6. Regulation of nutrient transporters. Related to Figures 5 and 6.**

- 117 **A.** qPCR of *Slc7a5*, *Slc38a2*, and *Slc2a1* after siRNA knockdown to show
118 knockdown efficiency in cultured SSCs. Statistical analyses between control
119 siRNA and siRNA against *Slc7a5*, *Slc38a2*, and *Slc2a1* were performed using
120 Student *t*-test with *P* values shown.
- 121 **B.** Representative immunoblotting analysis using total lysates from *Slc7a5*, *Slc38a2*,
122 *Slc2a1*, *Slc7a5+Slc38a2* knockdown SSCs. P-CREB are normalized to total
123 CREB. Statistical analyses between control siRNA and siRNA against *Slc7a5*,
124 *Slc38a2*, *Slc2a1* and *Slc7a5+Slc38a2* were performed using Student *t*-test with *P*
125 values shown.
- 126 **C.** Heatmap (qPCR) of selected meiosis-related gene expression from indicated
127 treatment normalized to *β-actin*.
- 128 **D.** qPCR analysis of indicated meiosis-related genes in mouse SSC cells after
129 indicated treatment normalized to *β-actin*. Data are representative of three
130 independent experiments. *P* value: Student *t*-test.
- 131 **E.** Box plot showing the expression level of *Ccnb1* and *Top2a* at different stages of
132 spermatogenesis in wild-type and *Stra8*-deficient germ cells. Statistical analyses
133 between wild-type and *Stra8*-deficient samples in each cell type were performed
134 using Student *t*-test with *P* values shown.
- 135 **F.** Representative immunoblotting analysis using total lysates from SSC cells
136 cultured in nutrient-rich (complete SSC media) and nutrient-restricted (50%
137 EBSS in complete SSC media) with or without RA treatment.

138 **G.** qPCR analysis of *Slc7a5*, *Slc38a2*, *Slc2a1*, and *Stra8* expression in cultured
139 SSCs with indicated treatment normalized to β -actin.

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Table S1. Primers used in this study

	Forward primer	Reverse primer
Slc1a5	CATCAACGACTCTGTTGTAGACC	CGCTGGATACAGGATTGCGG
Slc7a5	ATATCACGCTGCTCAACGGTG	CTCCAGCATGTAGGCGTAGTC
Slc38a1	CCTTCACAAGTACCAGAGCAC	GGCCAGCTCAAATAACGATGAT
Slc38a2	TAATCTGAGCAATGCGATTGTGG	AGATGGACGGAGTATAGCGAAAA
Slc3a2	TGATGAATGCACCCTTGTACTTG	GCTCCCCAGTGAAAGTGGA
Slc7a11	GGCACCGTCATCGGATCAG	CTCCACAGGCAGACCAGAAAA
Slc2a1	CAGTTCGGCTATAACACTGGTG	GCCCCGACAGAGAAGATG
Slc2a3	ATGGGGACAACGAAGGTGAC	GTCTCAGGTGCATTGATGACTC
Slc2a5	CCAATATGGGTACAACGTAGCTG	GCGTCAAGGTGAAGGACTCAATA
Slc2a8	CCCTTCGTGACTGGCTTTG	TGGGTAGGCGATTTCCGAGAT
Slc2a9	TTGCTTTAGCTTCCCTGATGTG	GAGAGGTTGTACCCGTAGAGG
Slc2a10	GCCTGACCTTCGGATATGAGC	TGCCATAGCAGTCAATGAGGA
Slc2a12	AGGTCCCAGCATGTTTACGTT	GGGCTAATAGCGTTCTGATCTG
Cyld	GGACAGTACATCCAAGACCGT	TCCTCACAGTTGGTAATTGCC
Hop2	CCCAGGACGTGTTTCGGAAAC	CACTGTGTCAAACCTGGTTCTGAT
Rsph1	AACGACCTTGGGGAGTACGAA	TGGCCGTGCTTTTTATTTTTGAC
tsga10	AACTGTGCTGTAGAGCTTTTGAA	GAGAGAACCTTGACGTTGCC
eaf2	GCAGAAGCTAGTCTTATGGACCA	GGGTGCTCCGAGCTGTATTC
Meiosin	GATGAACCGGAAGCAGTACATCCG	TCACTGGCAGTTAGGGGTGGGCAG