Figure S1. Isolation and purity validation of type A spermatogonia (SG-A), pachytene spermatocytes (pacSC), round spermatids (rST), and elongated spermatids (eST). (A) Phase contrast photos of isolated. Scale bar: 10 μm. (B, C) The purity of the four types of cells were validated by the unique expression patterns of proteins (B) and mRNAs (C) of two panels of germ cell marker genes detected by Western blots (B) and RT-PCRs (C). Names of the genes and the references (PMIDs) about their expression in the male germ cells are as the following: Plzf, promyelocytic leukemia zinc finger, 15156142, 15156143; Ret, ret proto-oncogene, 16237148; Soggy1, also named Dkkl1, dickkopf-like 1, 19596310; Sycp1, synaptonemal complex protein 1, 22761579, 15944401; Crem, cAMP responsive element modulator, 19910458, 20920259; Sohlh1, spermatogenesis and oogenesis specific basic helix-loop-helix 1, 22056784; Usp26, ubiquitin specific peptidase 26, 19305411; Miwi, piwi-like homolog 1, 12062093; Prm2, protamine 2, 11326282; Gapdhs, glyceraldehyde-3-phosphate dehydrogenase, spermatogenic, 15546993.

Figure S2. Scatter plots of protein data from two biologically independent iTRAQ experiments. Ratios of iTRAQ signals from one experiment were plotted against rations from the second experiment for pacSC (115/114), rST (116/114), and eST (117/114) with SG-A as reference (114). The 2008 quantitated proteins in the two iTRAQ experiments were plotted. The best-fit lines of the three cell types were shown. Note that the three scatter plots fit to the line y = x with R-square values ($R^2$) being 0.82, 0.89, and 0.89, respectively, indicating that our proteomic approach was highly reproducible.

Figure S3. Determination of cut-off values for protein level change based on the distribution of the 114/115 ratio of the same protein sample. (A) The experiment design shown by a flowchart. (B) Histogram of the distribution of protein change values, *i.e.*, the 115/114 ratios.

Figure. S4. Distributions of $ratio_{mp}$ at the pacSC/SG-A (A) and rST/pacSC (B) transitions as well as the null distribution. The null distribution is constructed by using mRNA and protein expression values of two biological replicates of pacSC and rST (1784 genes x 2 cell types to give 3568 entries). As the percentages of genes with ratio_mp <= 0.5 and ratio_mp >= 2 are 3% and 4%,

respectively, we regard that the false positive rates of these two groups of genes in the real distributions are all less than 5%.

Figure S5. Schematic representation of the spliceosome pathways of the mouse. Proteins involved in these pathways that were identified from our quantitative proteomic analysis were colored. Red for Cluster 1 proteins, and yellow for Cluster 2 proteins. Green labeled other proteins that were not identified by us but were recognized by the KEGG database.

Figure S6. Box plots of the protein and mRNA expression levels of five representative gene sets. Detailed information for these sets can be found in Fig. 2C.

Table S1. Sources and dilutions of the antibodies used for immunostainings.

| **Antibody** | **Company** | **Dilution** |
|---|---|---|
| α–LDHC | ProSci | 1:1000 |
| α–CLGN | PROTEINTECH | 1:1000 |
| α–SOD1 | PROTEINTECH | 1:1000 |
| α–FTH1 | Cell Signaling | 1:1000 |
| α–PLZF | Merck-Calbiochem | 1:1000 |
| α–RET | Santa Cruz | 1:1000 |
| α–SYCP1 | Novus Biologicals | 1:1000 |
| α–CREM | Santa Cruz | 1:1000 |
| α–SOGGY1 | Santa Cruz | 1:1000 |
| α–ACTB | Santa Cruz | 1:3000 |

Table S2. Oligonucleotide primers used for RT-PCR analysis.

| **Genes** | **For sense transcripts** | | **For antisense transcripts** | |
|---|---|---|---|---|
| | **Forward** | **Reverse** | **Forward** | **Reverse** |

| | | | | |
|---|---|---|---|---|
| Sohlh1 | TGACAACGCGCTCTGGCG | TGCCTCAGTTTGATGGCC | | |
| Gapdhs | TAGGGTGGTAGCAGTGAATGA | GATGAAATATGTGCCGAAGC | | |
| Miwi | ATGATCGTGGGCATC | AGGCCACTGCTGTCATA | | |
| Prm2 | ATGGTTCGCTACCGAATGA | TGATGGTGCCTCCTACATTTC | | |
| ActB | CAGCCTTCCTTCTTGGGTAT | TGGCATAGAGGTCTTTACGG | | |
| Usp26 | AATGTAACGAAGGGAGAAGTG | AGGCTTTGCCTTCTTATCGAG | | |
| Ldhc | TGCCCTTGTTGACGCTGATA | TCCACTGGGTTAGTGACGATA | GTCGAAGGTGGCGAGTTTAT | GCCAGGGAACTGATGGTATG |
| Adam5 | ACTTACTGGCATTTGAGGAG | CGTCATTAGGCAAACTTCTC | GCTGTGGGTTGGGTTGTTTT | GCCCTTACTTGTGATGATTT |
| Spa17 | GAAGGGCTGACACGGGAGATT | TGTTCCTTGAATGCGTGGTTG | CGGAAGAGGGACTGGATTTT | CAACCACGCATTCAAGGAAC |
| Tcp11 | TGTTCCTCAAGTGCTGCTTTG | CCTGCTGGTTGTGATGGGT | AGGAATCCTTGAGACAGCACC | AACACTCCGTCCAGTTTGAGC |
| Clgn | AGTCTAAAGCCAAGCACCACG | CACCTCCACAATCAATACCATCT | CCAAAAGAAAGATAGAAAGAAG | ACTGAAGTCATTGCGGAAGA |
| Dnajb3 | GAGCACCCGTGGAAGCAGAAG | CAATAACAAGAGGCACCGACAT | CTGCGATCCCAACAATAACAAG | AGGGTGGAAGTGGAAGAAGATG |
| Fh14 | GCAGAAGGATGGAGCCAACTA | CAGGCAACAAAGGTCTCAGTAG | TCAGGGAAGTAGGAGGTGGTA | AAAAGTGACAAACTAAATGGGTGG |
| Gk2 | TCCAATGAGCATCCTCCCAAAT | TAGCCAGCGAATAACAGCACC | CTCATGGGTTCCTGTAAGCAAG | GAGCAAGATGTGTCTCAACTGC |
| Pcdl2 | ATAGCTGCATTGAACACTACCA | CTTGAGATTTATCCCTCCACA | GGTTTTCTTCCAAGTCACTCTG | GTGAATAGCTGCATTGAACACT |
| Fabp9 | TCTTAGGCACCTGGAAACTGA | GGCTTTATTAAACATGCGACTT | CTAGCCACCTATTTCCCTCA | CCAAGCGTTAGTATTAGTTTCA |
| Fth1 | CGCCAGAACTACCACCAGGAC | AGAGCCACATCATCTCGGTCA | - | - |
| Sod1 | CGTCCGTCGGCTTCTCGTCTT | GTCACATTGCCCAGGTCTCCA | - | - |
| Fth1 piRNA1 | TCTCTATAAGACGGTACGGTCGAAGT | qiagen miScript Universal Primer | - | - |
| Fth1 piRNA2 | GGTGTAGTAGAGCCAGTTTTATTG | qiagen miScript Universal Primer | - | - |

| | | | | |
|---|---|---|---|---|
| Sod1 piRNA1 | GTGGCAGGAAAGGTCG TCAGT | qiagen miScript Universal Primer | - | - |
| Sod1 piRNA2 | CGGGTTCAGTAGAACA AAGAGT | qiagen miScript Universal Primer | - | - |

Table S3. Proteins and related peptides identified from the iTRAQ analysis.

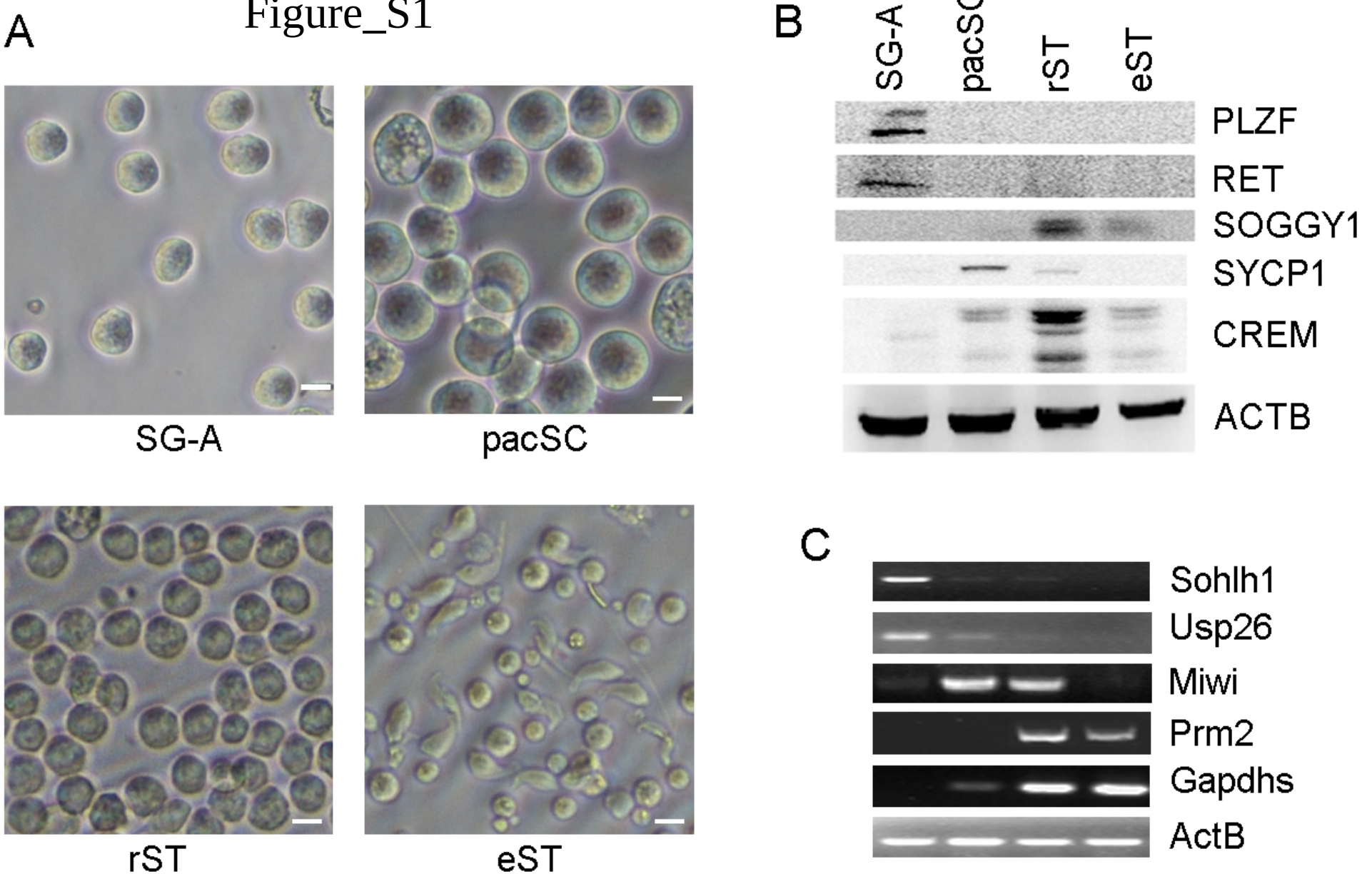
Table S4. A list of proteins identified in mouse male germ cells. These proteins were filtered out from the list of proteins in Table S3 by using criteria described in the text.

Table S5. Enriched functional annotation terms of the five clusters of proteins identified from four types of germ cells by using the iTRAQ analysis. The annotations were based on the KEGG and DAVID (Cellular Component, Biological Process) databases

Table S6. Top five enriched GO terms, percentages of PRMRs and antisense transcripts associated with genes of 15 (sub-) sets defined in Fig. 2C.

Table S7. The association of the five clusters of proteins identified from iTRAQ analysis with the (sub-) sets of genes using different regulatory mechanisms. Each cell defined by the intersection of a cluster and a set was split into two sub-cells, with the top one indicating the subsets of genes and the bottom one indicating the numbers of proteins/genes in each subset.

Figure_S1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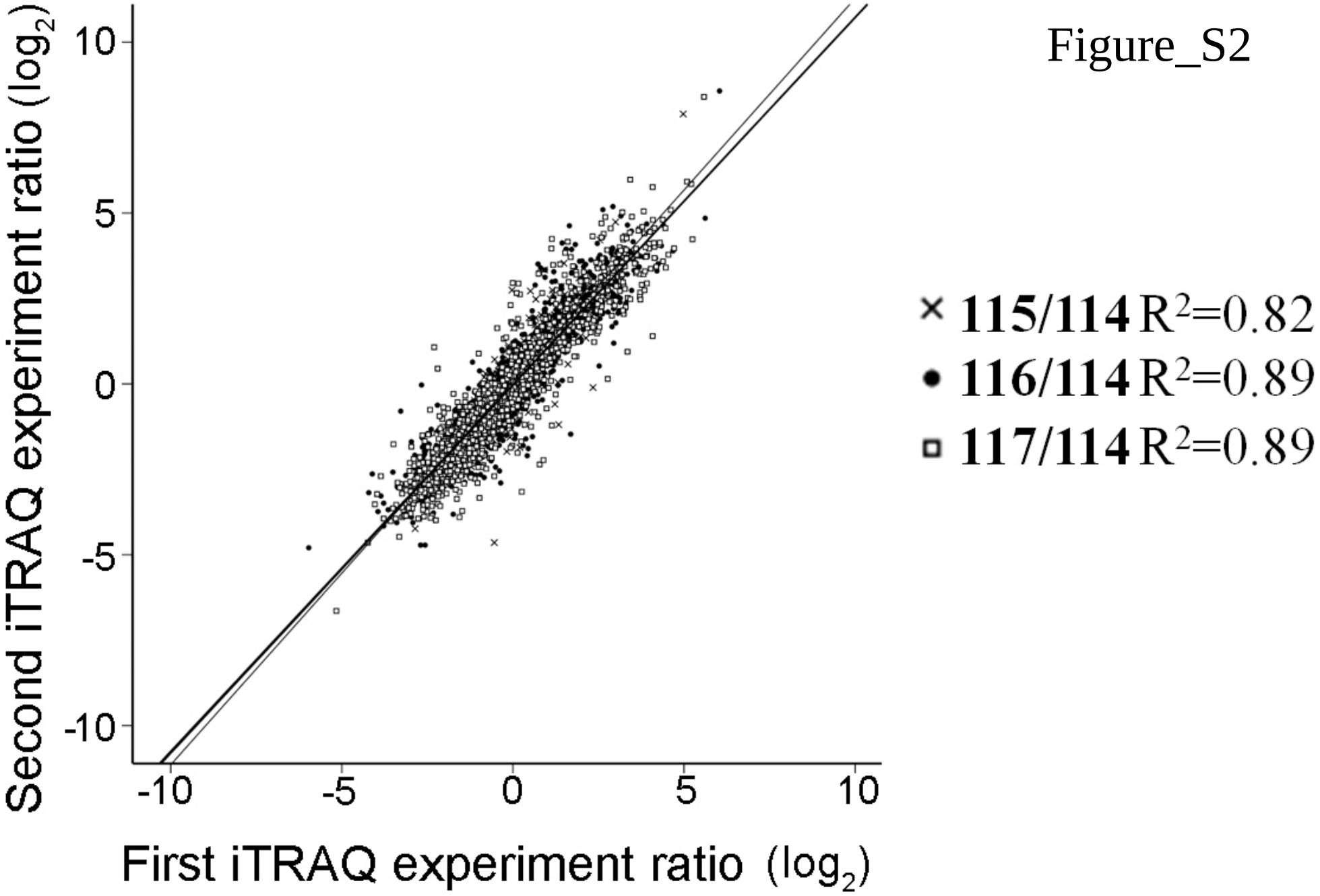
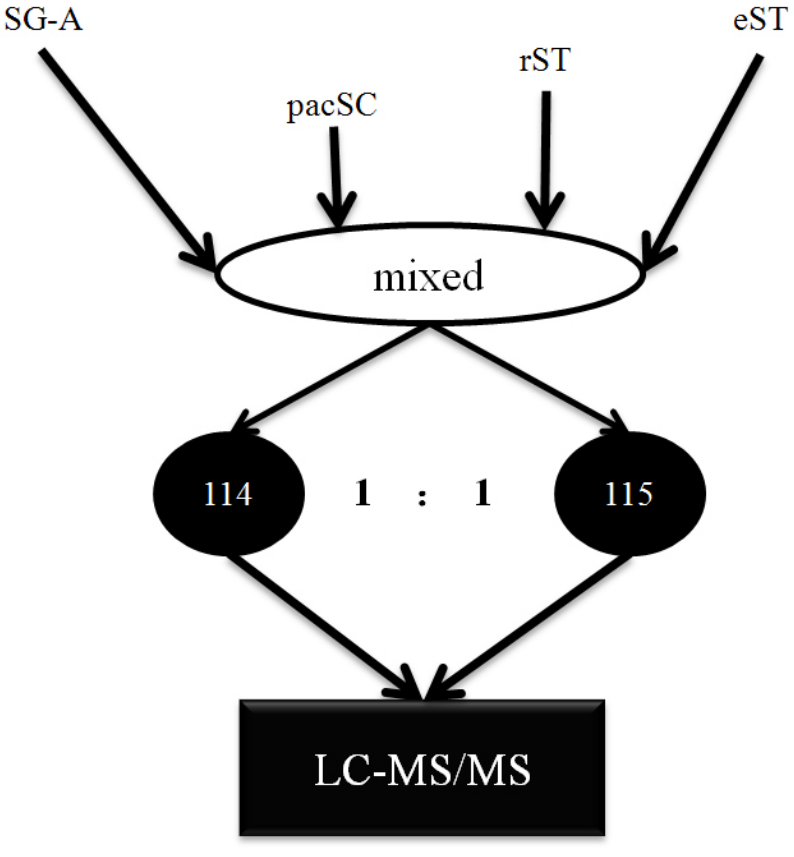
SG-A
pacSC
rST
eST
B
SG-A
pacSC
rST
eST
PLZF
RET
SOGGY1
SYCP1
CREM
ACTB
C
Sohlh1
Usp26
Miwi
Prm2
Gapdhs
ActB

Figure_S2


Second iTRAQ experiment ratio (log2)
First iTRAQ experiment ratio (log2)
10
5
0
-5
-10
-10
-5
0
5
10
× 115/114 R2=0.82
• 116/114 R2=0.89
▫ 117/114 R2=0.89

# Figure_S3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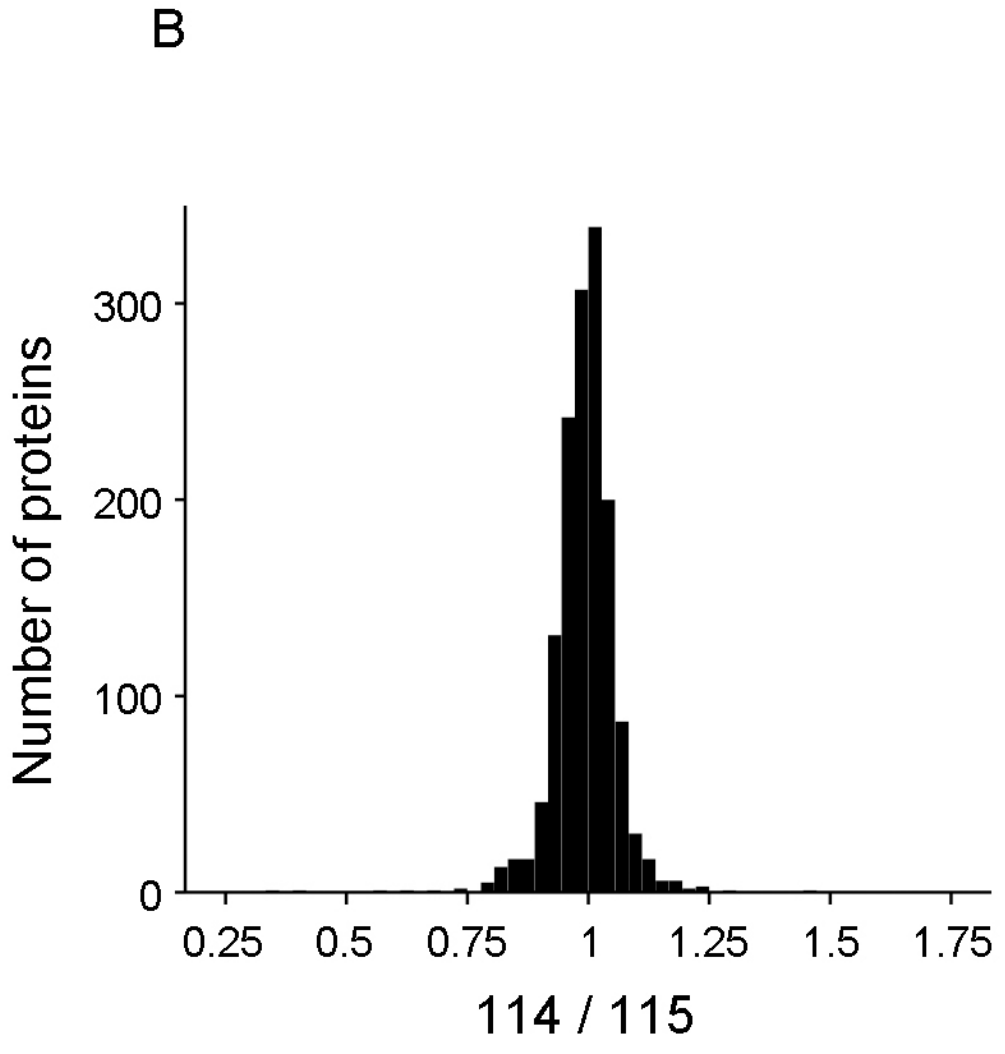
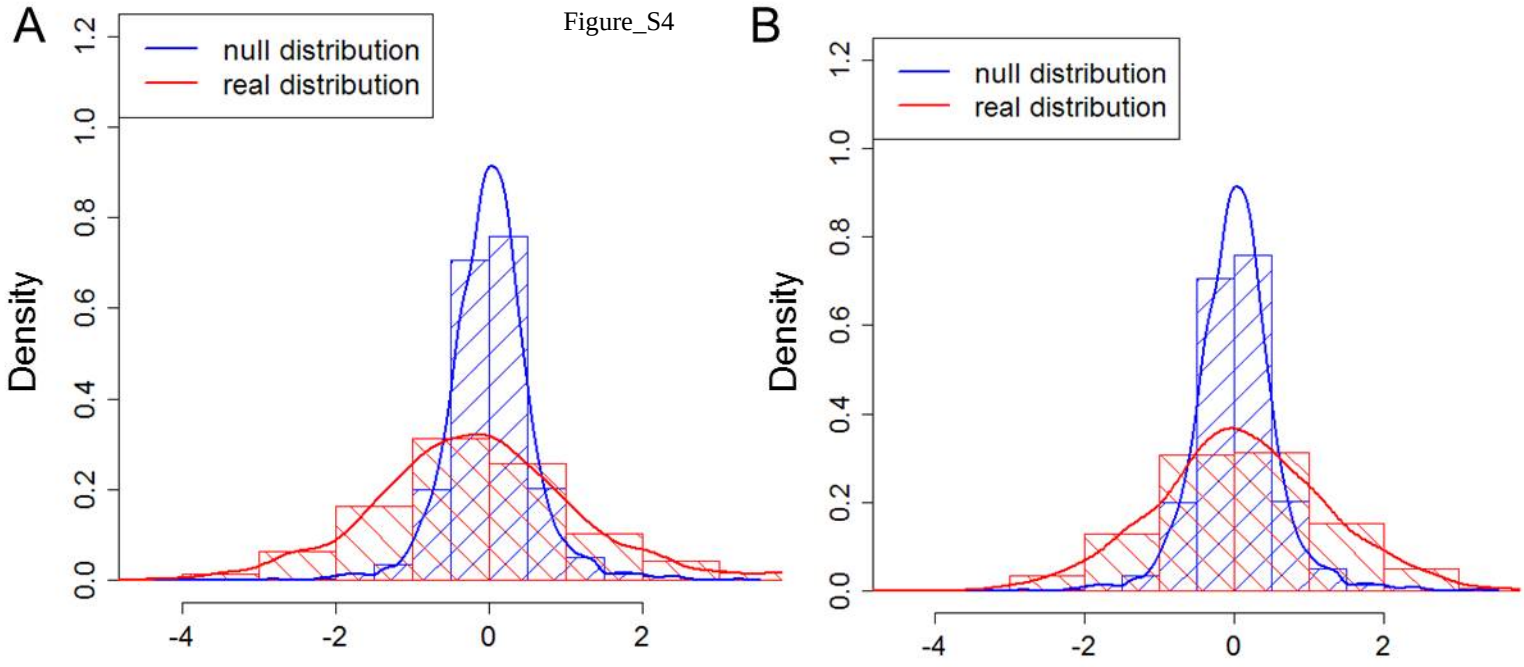
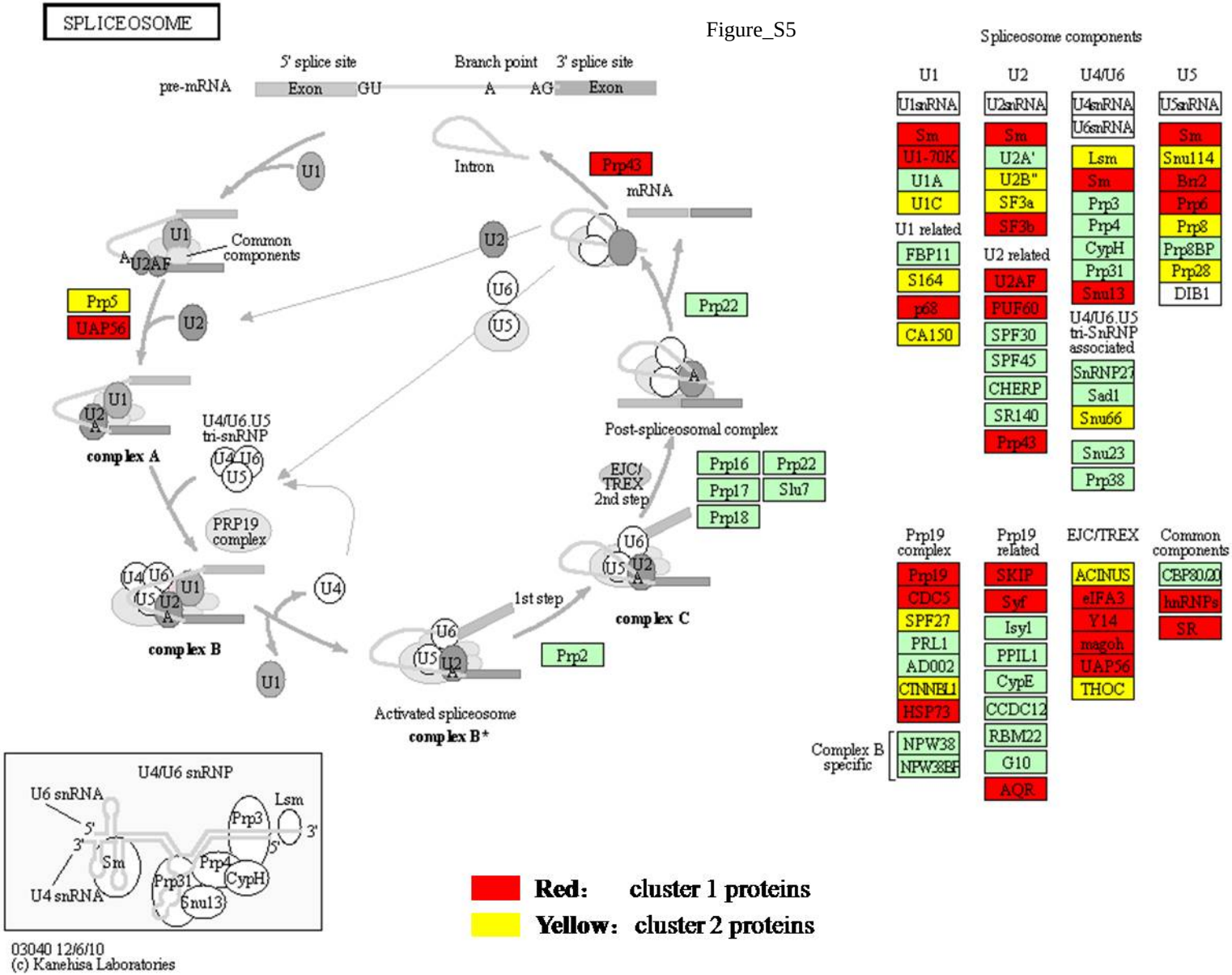
SG-A
pacSC
rST
eST
mixed
114
1 : 1
115
LC-MS/MS


B
Number of proteins
300
200
100
0
0.25
0.5
0.75
1
1.25
1.5
1.75
114 / 115

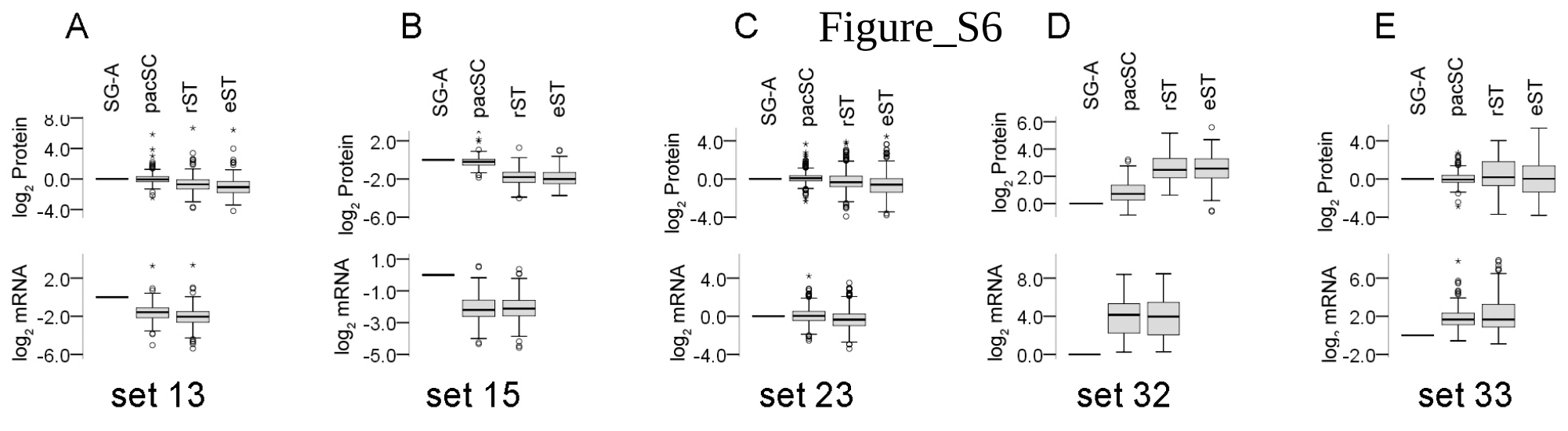
Figure_S4


A
null distribution
real distribution
Density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
-4
-2
0
2
B
null distribution
real distribution
Density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
-4
-2
0
2

Figure_S5



# Figure_S6


A
B
C
D
E
SG-A
pacSC
rST
eST
log2 Protein
log2 mRNA
set 13
set 15
set 23
set 32
set 33