Inventory of Supplemental Materials

The following Supplemental information accompanies this paper:

Five Supplemental Figures (Figures S1 – S5) and figure legends

Supplemental references

Supplemental Tables as Dataset in a separate excel file
Legends of Supplementary Figures

Figure S1 (related to Figure 1)
Molecular Evolution of the *Zeus* locus
(A) protein sequence alignment of Zeus, Caf40 and their respective orthologs in *Drosophila*, representative vertebrates (human, mouse, chicken, frog) and yeast showing rapid evolution of Zeus protein and the high conservation of Caf40; (B) sequence evolution analysis showing elevated rate of replacement in the *Zeus* lineage; $Ka/Ks$ ratios were calculated for each branches and red branches indicated $Ka/Ks$ significantly greater than 1; (C) southern blot confirms the gene-duplication event of *Zeus*-Caf40; blue C, *Caf40* restriction fragment; orange Z, *Zeus* restriction fragment; (D) genomic region PCR of the three representative closely-related species confirms the insertion of the *Zeus* genomic region; primers of conserved flanking regions were used thus a 2kb band indicates the insertion of the *Zeus* genomic region; (E) Bayesian Empirical Bayesian inference of Zeus positively-selected aa sites.

Figure S2 (related to Figure 2)
Characterization of *Zeus* gene expression pattern and phenotype
(A) RT-PCR showing the transcript of *Zeus* in testis and accessory gland (AG); (B) Flyatlas gene expression of *Zeus* and *Caf40* in multiple tissues showing strong testis-specificity of *Zeus* and wide-spread expression of *Caf40*; (C) enhancer-trap line shows the testis and AG expression of Zeus-GFP; (D) expression of nanos-Gal4>>Zeus-GFP;
(E-F) schematic representation of point mutations (F) and P-element insertions (E) of Zeus presumptive null alleles; (G) sterility phenotype of Zeus presumptive null alleles; (H) defect of Zeus sperm storage by mated wildtype females

Figure S3 (related to Figure 3)
Characterization of Caf40 phenotype in cell differentiation and male fertility
(A) Cellular phenotype of Caf40 (using eye development as a model): top panel, morphology of the Control compound eye showing regular ommatidia and bristle cells; bottom panel, morphology of the Caf40-RNAi animals under photoreceptor-specific driver (GMR-Gal4) showing necrotic ommatidia and mis-differentiated bristle cells; (B) Germline-specific knockdown of Caf40 using nanosGal4 (nos-Caf40-RNAi) showed only a little effect in male fertility.

Figure S4 (related to Figure 4)
Evolution of sex-bias gene regulation by Zeus
(A) Sex-biased patterns of Zeus downstream genes: orange, percentage of male-biased genes; grey, percentage of unbiased genes; blue, percentage of female-biased genes; Zeus_RNAi_UP_genes, genes that are up-regulated under Zeus-RNAi (genes normally repressed by wildtype Zeus gene); Zeus_RNAi_DOWN_genes, genes that are down-regulated under Zeus-RNAi (genes normally activated by wildtype Zeus gene); random genes, randomly chosen genes in the genome; (B) Sex-biased patterns of Caf40 downstream genes; yellow, percentage of male-biased genes; darkred, percentage of unbiased genes; blue, percentage of female-biased genes; Caf40 RNAi Up genes, genes
that are up-regulated under \textit{Caf40}-RNAi; Caf40 RNAi Down genes, genes that are down-regulated under \textit{Zeus}-RNAi; random genes, randomly chosen genes in the genome.

Figure S5 Evolution of Zeus targets and networks (Related to Figure 5)

Integrated analysis of Zeus expression profiling and ChIP-chip showing putative direct targets of Zeus show primary signal of up-regulation in Zeus-RNAi; top right panel, Venn-diagram showing the number of genes and intersection between Zeus binding genes (from ChIP-chip) and differentially expressed genes under Zeus-RNAi; left panel, heat map showing the expression profiles of differentially expressed genes between Zeus-RNAi and control samples; bottom right panel, heat map showing the expression profiles of the putative Zeus direct target genes (bound and differentially expressed) between Zeus-RNAi and control samples; color keys were shown on top of each heatmap.

Supplemental References:

2. \url{http://www.dpgp.org/}.


Figure S2

A

AG  Testes

Zeus

B

Zeus  Caf40

C

Zeus::GFP

adult accessory gland  adult testis

D

nos>>Zeus-GFP  nos>>GFP

E

P11773

PG[2]ms229F07717

Chr. 2L

CG13102  Zeus (CG9573)  CG9562

F

G

H

Wt virgin spermathecae and seminal recepticle  Wt mated spermathecae

Zeus P-insertion mutant mated spermathecae

Zeus P-insertion mutant mated seminal recepticle

Wt mated seminal recepticle  Wt mated spermathecae
Figure S3

Germline knockdown fertility

A

Control

UAS-Caf40-IR/GMR-Gal4 (Caf40-RNAi)

B

Germline knockdown fertility

<table>
<thead>
<tr>
<th></th>
<th>Number of offspring</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>150</td>
</tr>
<tr>
<td>nos-Zeus-RNAi</td>
<td>60</td>
</tr>
<tr>
<td>nos-Caf40-RNAi</td>
<td>120</td>
</tr>
</tbody>
</table>
Figure S4

A  
Zeus downstream gene sex bias

- Female-biased Genes
- Unbiased Genes
- Male-biased Genes

B  
Caf40 downstream gene sex bias

- Male
- Unbias
- Female
Figure S5

Zeus differentially expressed gene set
Zeus ChIP-chip binding gene set

2325
63
259

Zeus-RNAi
Control

Zeus-RNAi
Control

Color Key

Color Key