Supplemental Materials for

Discovery of a human testis-specific protein complex TEX101-DPEP3 and selection of disrupting antibodies

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Supplemental Materials include:

Figure S1. Identification of antibody clones disrupting TEX101-DPEP3 complexes by hybrid immunoassay.

Figure S2. Optimization of testicular tissue lysis

Figure S3. Identification of TEX101 interactome by T*2* (34ED229) mAb co-immunoprecipitation coupled with mass spectrometry.

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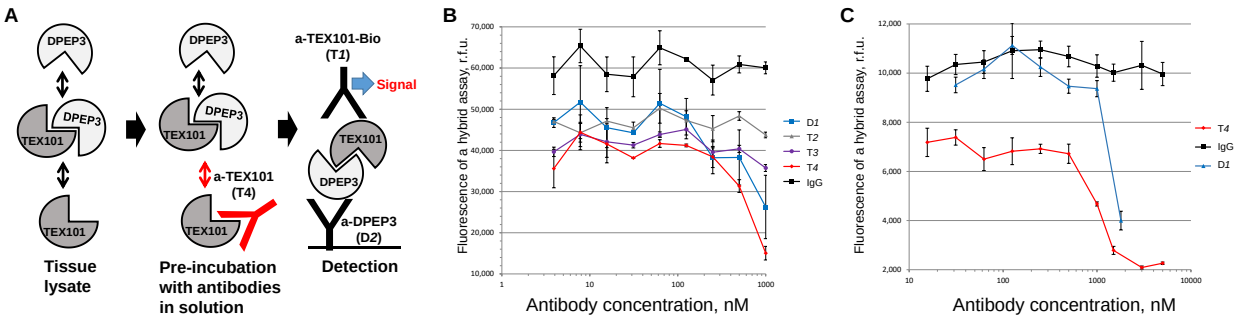
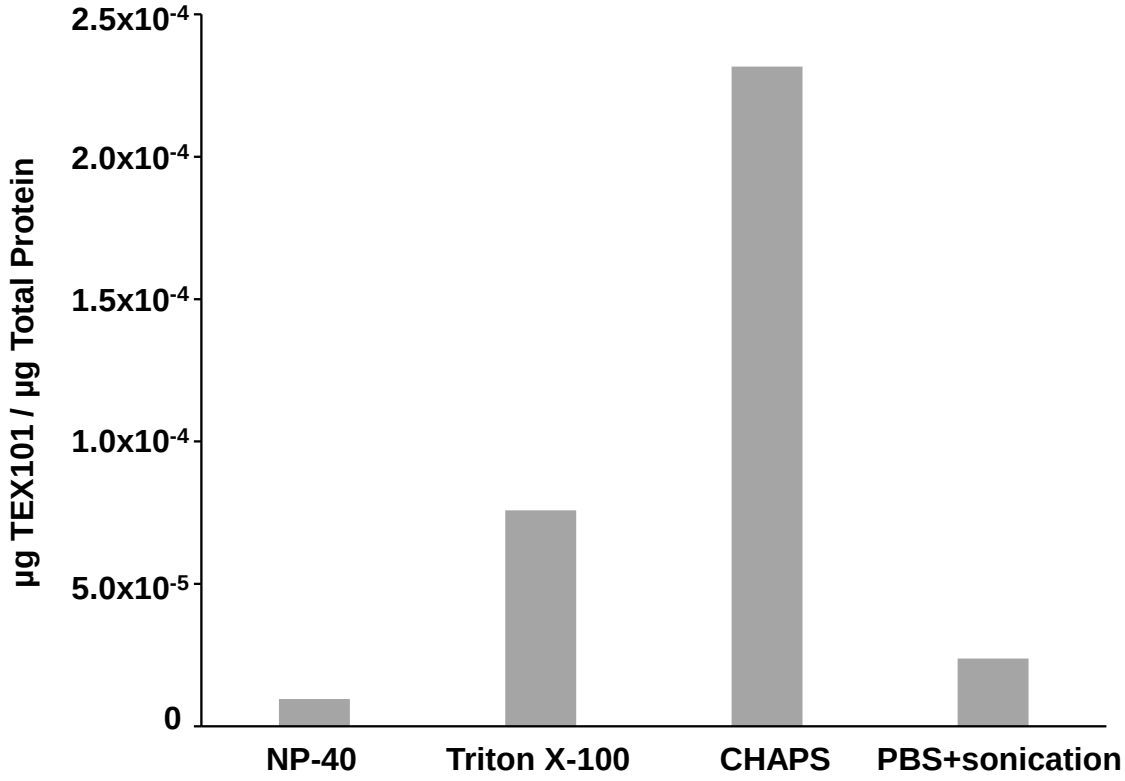
Supplemental Figures

Supplemental Figure S1



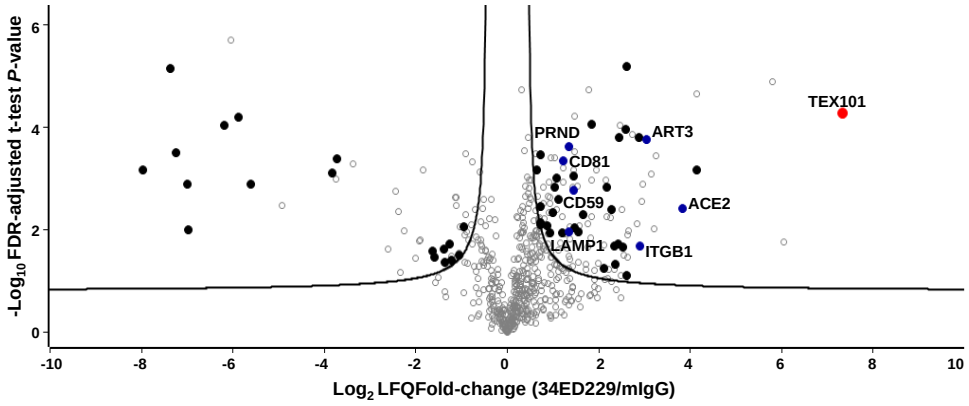
Supplemental Fig. S1. Identification of antibody clones disrupting TEX101-DPEP3 complexes by a hybrid immunoassay. (**A**) Schematic representation of screening for anti-TEX101 and anti-DPEP3 inhibitory antibodies. Testicular tissue lysates (65 µg total protein) were pre-incubated overnight with increasing concentrations of selected clones (*T2, T4, T3, D1*), and non-specific mouse IgG. Relative abundance of TEX101-DPEP3 complexes in equilibrium were determined using the hybrid immunoassay. (**B**) Antibody clones *T4* and *D1* revealed dose dependent decrease of fluorescent signal. No effect was detected when testicular tissue lysates were pre-incubated with T2, T3 and non-specific mouse IgG antibodies. (**C**) EC50 values were determined as 1080 nM [95%CI 454-2550] for *T4* antibody and ~2000 nM for *D1* antibody. High concentrations of antibodies were required to observe the decrease of fluorescence signal, due to the presence of high amounts of free unbound TEX101 and DPEP3 in the testicular tissue lysates. Error bars represented the standard deviation of the duplicates (B) and triplicates (C).

Supplemental Figure S2



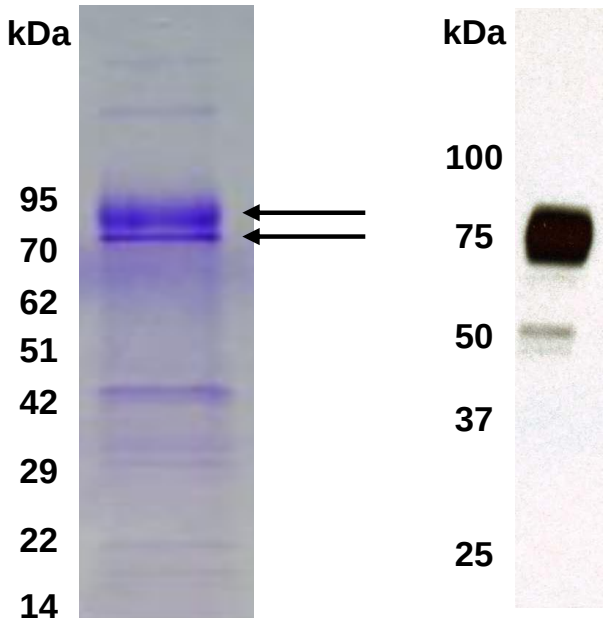
Supplemental Fig. S2. Optimization of testicular tissue lysis. After physical disruption, pulverized tissue was incubated in lysis buffers containing various mild non-denaturing non-ionic (NP-40, Triton X-100) and zwitterionic (CHAPS) detergents. Tissue lysis in PBS with sonication (no detergent) was used as a control. Efficiency of tissue lysis and protein solubilisation was assessed by BCA total protein assay and in house TEX101 immunoassay. Highest recovery of TEX101 protein from testicular tissue was achieved by lysis with CHAPS (1% w/v).

Supplemental Figure S3



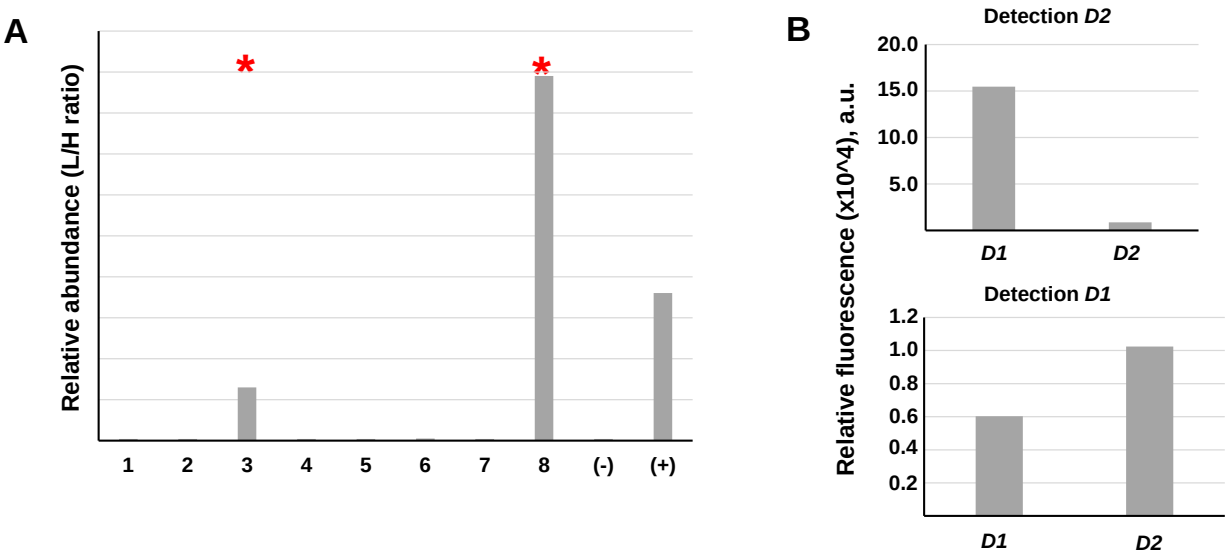
Supplemental Fig. S3. Identification of TEX101 interactome by *T2* (34ED229) mAb co-immunoprecipitation coupled with mass spectrometry. Volcano plot (-log10(*p*-value) versus log2(fold change)) revealed proteins co-enriched with target protein TEX101 by anti-TEX101 monoclonal antibody *T2* (34ED229) compared to the mouse IgG negative control, from normal testicular tissue with active spermatogenesis. Statistical analysis was performed from 3 biological replicates in Perseus. Fold change and *p*-value cut-offs are indicated by the hyperbolic selection curve in black (fold change was assessed for the dataset by the variance correction (s0), FDR-adjusted t test p values <0.01). Anti-TEX101 antibody isolated 28% of membrane/secreted proteins among all significantly enriched proteins from testicular tissue, while isotype control mouse IgG antibody isolated 38% of membrane/secreted proteins. Target protein TEX101 is plotted in red, membrane/secreted proteins significantly enriched are plotted in black, and significantly enriched membrane/secreted proteins expressed by testicular germ cells (candidate interactors of TEX101) are plotted in blue. See **Supplemental Table S4** for the full list of significantly enriched proteins in testicular tissue, seminal plasma and spermatozoa, respectively.

Supplemental Figure S4



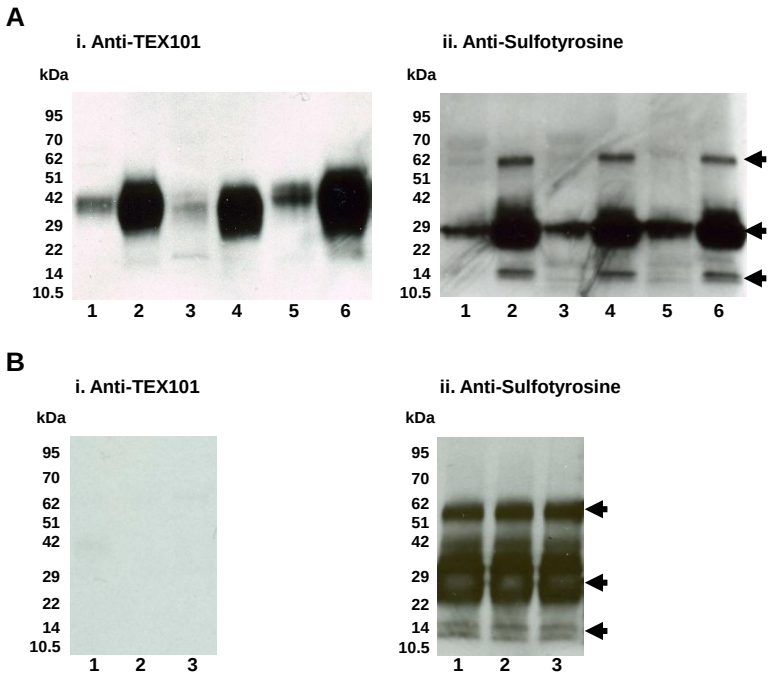
Supplemental Fig. S4. SDS-PAGE and Western blotting analysis of purified recombinant DPEP3 protein. Arrows indicate the bands that were excised for MS analysis. Majority of DPEP3 protein migrated around 75-85 kDa.

Supplemental Figure S5



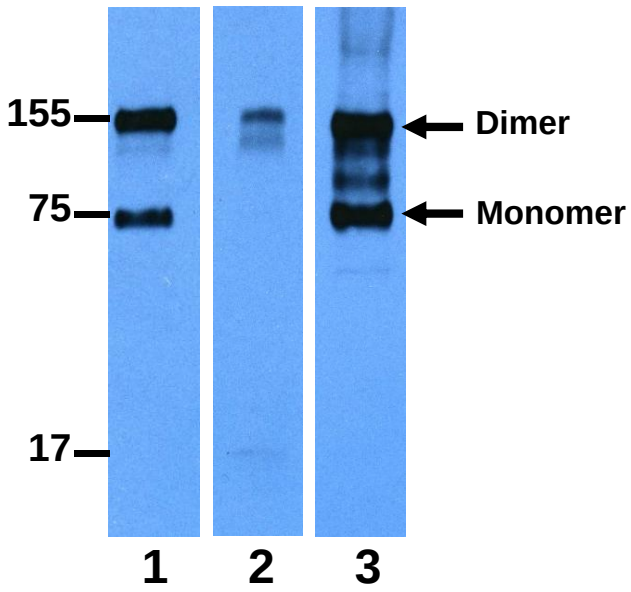
Supplemental Fig. S5. Production of mouse monoclonal antibodies against different epitopes of native DPEP3 protein. **(A)** Mice were immunized with purified recombinant DPEP3. Immunocapture-SRM was used for the screening of hybridoma colonies and the selection of mouse monoclonal antibodies against native DPEP3 protein, present in seminal plasma pool. Lane (+) indicates the positive control (serum of immunized mouse) and lane (-) indicates the negative control (sheep anti-mouse IgG- Fcγ fragment-specific antibody only). Asterisks mark the clones that had positive reaction with native DPEP3. **(B)** The two mouse monoclonal anti-DPEP3 antibodies, *D1* and *D2*, were paired in a sandwich immunoassay, targeting two different epitopes on native DPEP3 protein.

Supplemental Figure S6



Supplemental Fig. S6. Investigation of TEX101 sulfation in testicular tissue, seminal plasma and spermatozoa. (**A**) Immunoprecipitation of TEX101 protein from testicular tissue lysate, seminal plasma and spermatozoa lysate by *T1* mAb, followed by immunoblotting with commercial rabbit polyclonal anti-TEX101 antibody (i) or mouse monoclonal anti-sulfotyrosine antibody (ii). Samples were as follows: (1) input testicular tissue lysate; (2) testicular tissue after IP; (3) input seminal plasma; (4) seminal plasma after IP; (5) input spermatozoa lysate; (6) spermatozoa lysate after IP. (**B**) Control immunoprecipitation was performed in parallel by using an isotype control mouse IgG antibody. Samples were as follows: (1) testicular tissue lysate after IP; (2) seminal plasma after IP; (3) spermatozoa lysate after IP. Black arrows indicate proteins enriched by *T1* TEX101-specific antibody and isotype control mouse IgG, and detected by anti-sulfotyrosine antibody. Protein marker is indicated to the left of each blot.

Supplemental Figure S7



Supplemental Fig. S7. Western blotting analysis of endogenous DPEP3 protein using the commercial rabbit polyclonal anti-DPEP3 antibody (HPA058607) for DPEP3 detection in: (1) testicular tissue lysate; (2) seminal plasma; (3) spermatozoa lysate (~40 µg total protein/well). Protein marker is indicated to the left of the blot. DPEP3 migrates to 75 kDa (monomer) and 155 kDa (dimer) in testicular tissue and spermatozoa lysate. In seminal plasma, an additional band migrates to 17 kDa which may indicate degradation of DPEP3 after it is shed from sperm surface.