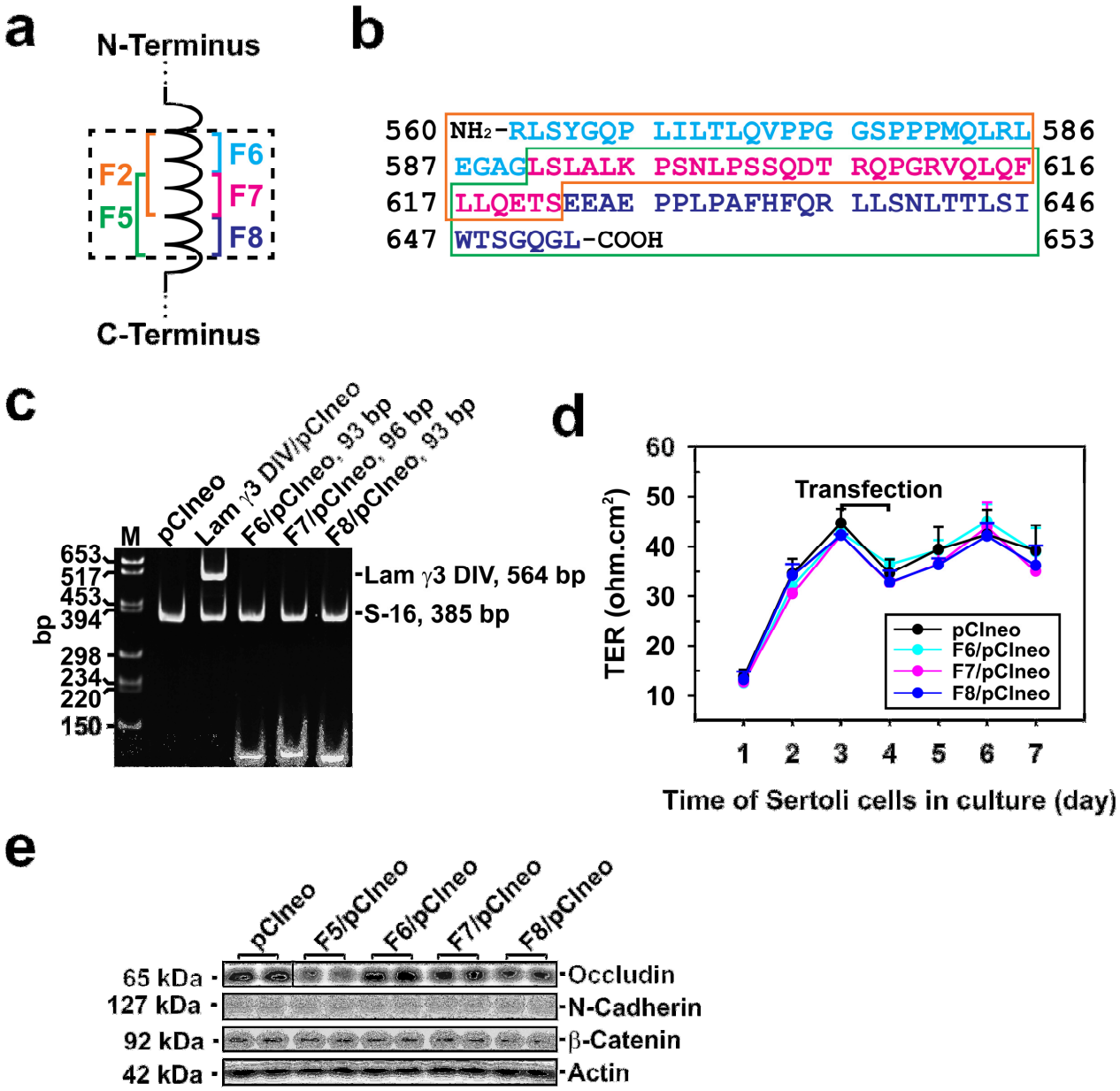
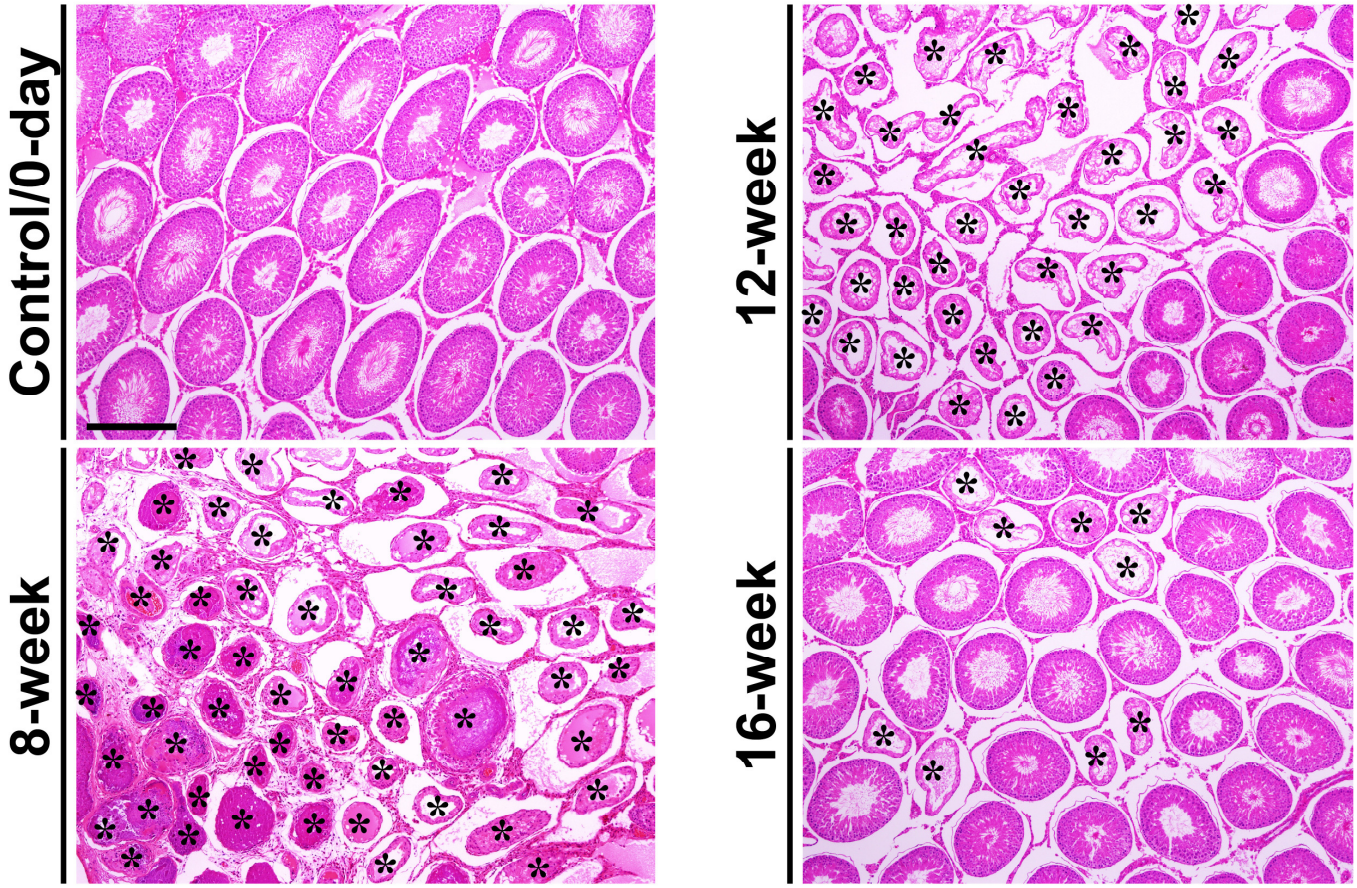
Supplementary Information

Supplementary Figure S1. Assessing biologically active domain(s) in the F5 fragment of laminin-γ3 domain IV.



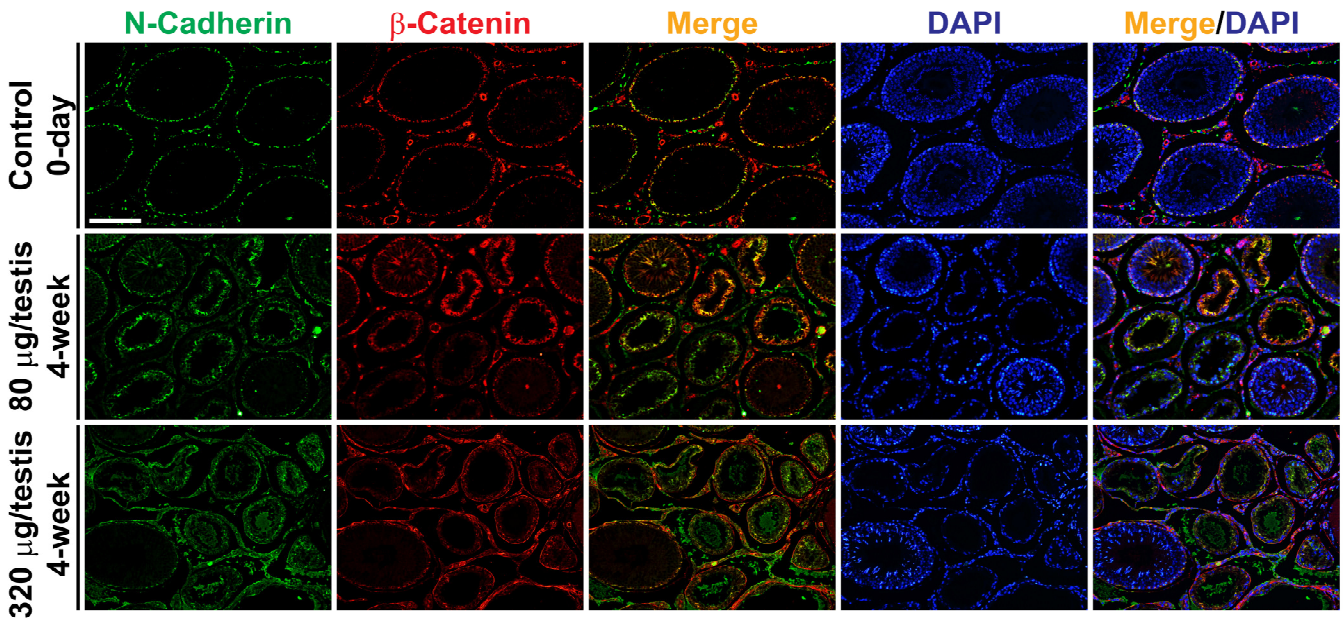
(**a**) Schematic drawing illustrating cDNA constructs that were prepared for F6, F7 and F8 derived from fragments 2 and 5 which were shown to have biological activities against the Sertoli cell BTB integrity. (**b**) Partial cDNA-deduced amino acid sequence of laminin-γ3 DIV (GenBank NM_001107830) is shown, from which F6 (blue), F7 (pink) and F8 (purple) cDNA constructs were prepared and cloned in pCIneo vector. Orange and green boxed areas are the corresponding sequences of F2 and F5 (see **a**). Numerals annotate amino acid residues of laminin-γ3 chain from the N-terminus. (**c**) RT-PCR showing the expression of F6, F7 and F8 in Sertoli cells after transfection for 3 days. Cells were transfected with pCIneo alone or Lam γ3 DIV/pCIneo to serve as negative or positive control, respectively. S-16 served as internal PCR control. The identity of the PCR product was confirmed by direct nucleotide sequence at GeneWiz. (**e**) Changes in Sertoli cell TJ-permeability barrier were assessed by quantifying TER across the cell epithelium cultured on Matrigel-coated bicameral units ($n = 3$) where cells were transfected with pCIneo alone, F6/pCIneo, F7/pCIneo or F8/pCIneo on day 3 for 24-hr, and lysates were obtained 3 days after transfection for immunoblottings shown in **e**. Each data point is a mean ±SD of $n = 3$ bicameral units of an experiment, which was repeated three times using different batches of Sertoli cells and yielded similar results. **e**) Immunoblots showing the steady-state levels of BTB-associated proteins in Sertoli cells following overexpression with different constructs by day 3 following transfection.

Supplementary Figure S2. Laminin F5-peptide induces reversible disruption of spermatogenesis in rats.



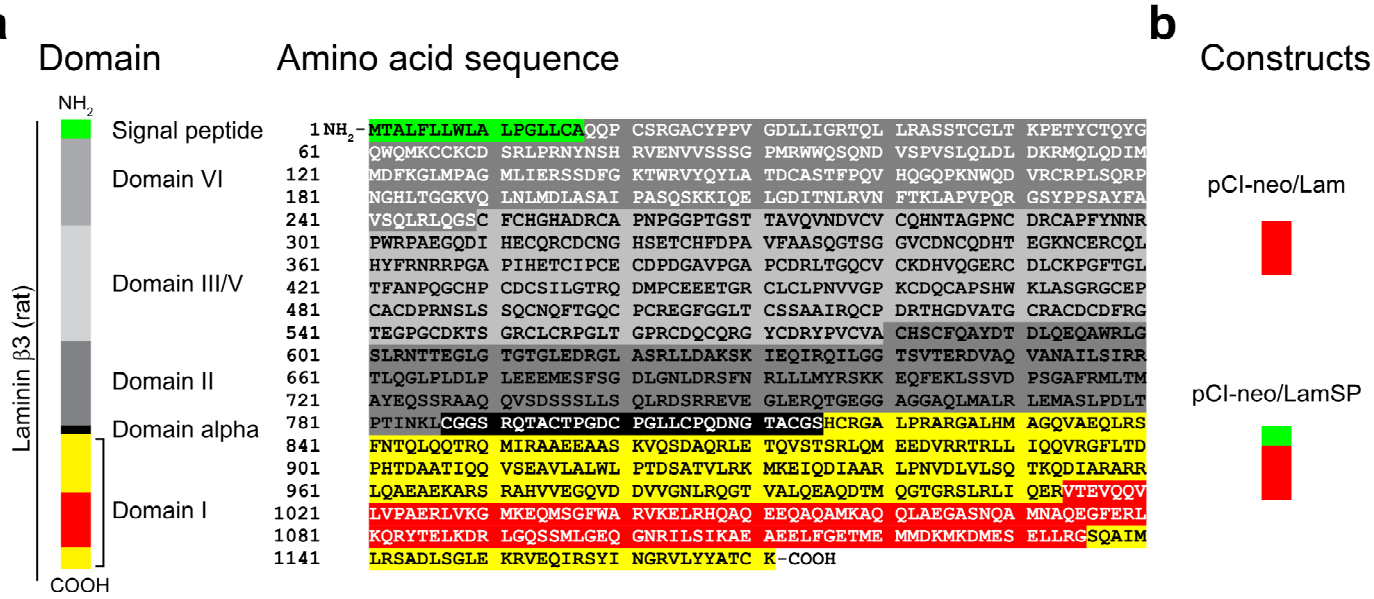
Laminin synthetic F5-peptide at low dose (80 µg per testis, ~10 µM) or vehicle control (0.89% NaCl) was injected into each testis of a group of adult rats (~300-350 gm b.w.) with n = 3 rats per time point in each treatment group *versus* controls at time 0 using a 28-gauge needle. Rats were terminated at specific time points at 8-, 12- and 16-week thereafter. Representative micrographs of paraffin sections of testes stained with hematoxylin and eosin. *, indicates damaged tubule showing signs of germ cell loss from the seminiferous epithelium. These findings also illustrate recovery of spermatogenic function in rats by 16-wk following synthetic F5-peptide treatment. Bar = 300 µm in control, which applies to all remaining micrographs.

Supplementary Figure S3. Laminin F5-peptide induces changes in protein distribution at the BTB.



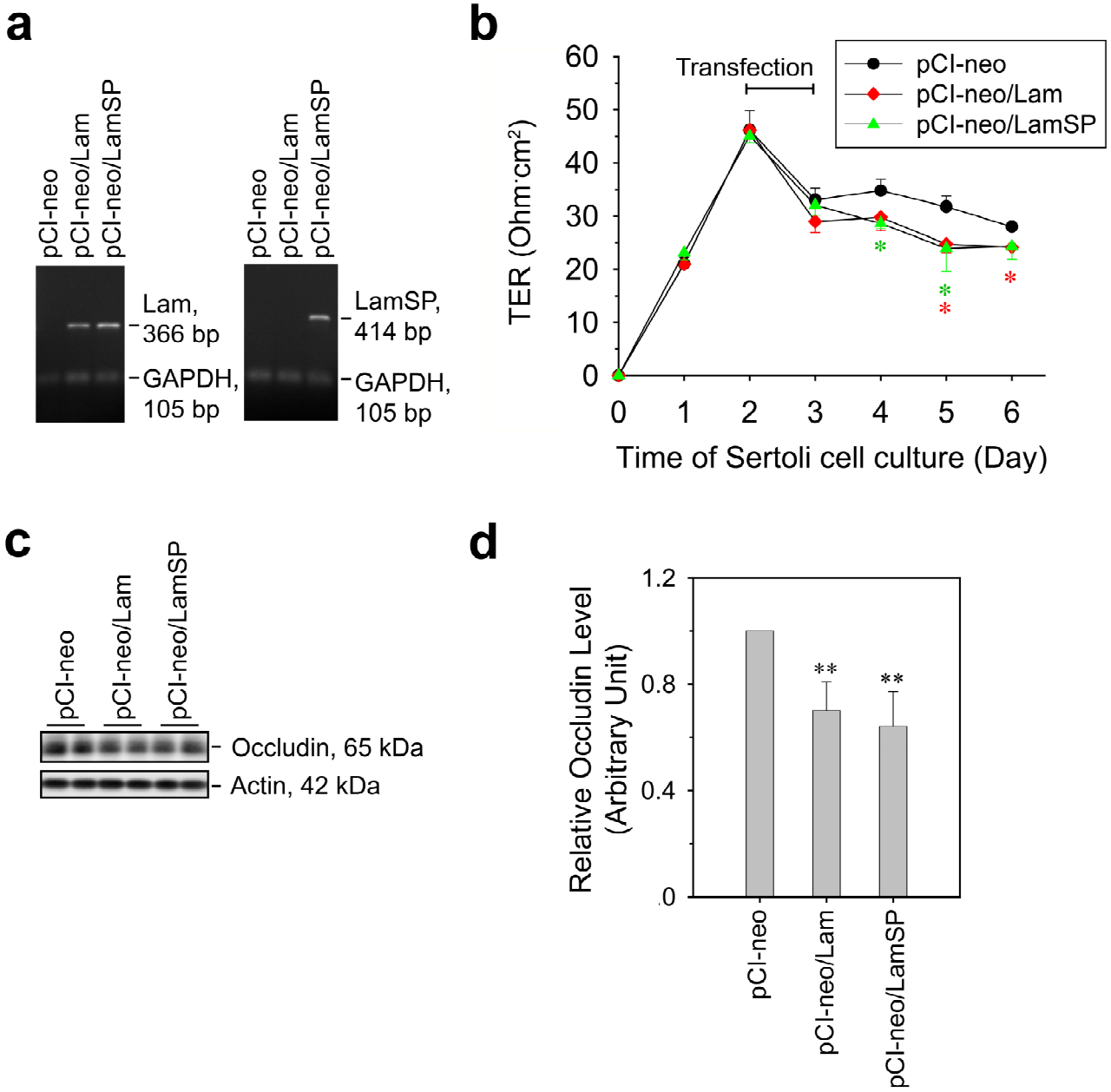
Normal rat testis *versus* testes treated with 80 μg F5-synthetic peptide per testis for 4-week, and testes treated with 320 μg peptide per testis for 4-week were shown. It is noted in damaged tubules, manifested by germ cell loss (see DAPI staining to visualize nuclei of Sertoli and germ cells in tubules) and a shrinkage of tubule diameter. Both N-cadherin and β-catenin no longer neatly confined to the BTB near the basement membrane, instead, they were mis-localized, analogous to neonatal rats at ~day 12-15 day postpartum (dpp), prior to the establishment of a functional BTB, when the seminiferous epithelium had few advanced germ cells as described.[51] Bar = 150 μm in control, which applies to all remaining micrographs.

Supplementary Figure S4. Laminin β3 domain I cDNAs that assess "inside-out" and "outside-in" signaling.



A biologically active proteolytic fragment of laminin-β3 domain I, which is cleaved from the surface of elongated spermatids during their release from the seminiferous epithelium at spermiation, was cloned and used for overexpression in primary cultured Sertoli cells. Secretion of this fragment was induced by the addition of an N-terminal signal peptide. (**a**) Schematic drawing illustrating the domains of full-length rat laminin-β3 precursor (Accession no. NM_001100841.1) and the corresponding amino acid sequence highlighted in matching colors. The fragment of interest (red) is located within Domain I, and the native signal peptide (green) is from the N-terminus. (**b**) Schematic drawing of constructs used for overexpression in Sertoli cells. The cDNA encoding the fragment of interest, either alone (Lam) or fused with the N-terminal signal peptide (LamSP), was cloned into the pCI-neo mammalian expression vector.

Supplementary Figure S5. Laminin β3 fragments on Sertoli cell TJ function and protein expression.



Sertoli cells were cultured for 2-day to allow the assembly of a tight junction (TJ) permeability barrier, and were subsequently transfected with Lam or LamSP constructs, with the empty vector pCI-neo as the control. (**a**) Total RNA was obtained from Sertoli cells (0.45 x 10^6 cells/cm^2) 3 days after transfection. The presence of Lam or LamSP mRNA was verified by RT-PCR. GAPDH was used as a loading control (**b**) Effects of Lam or LamSP expression on TJ integrity was monitored by daily measurements of transepithelial electrical resistance (TER) across the Sertoli cell epithelium (1.2 x 10^6 cells/cm^2). Each data point represents mean±SD (n = 3 replicates from a representative experiment). Similar results were obtained from 3 independent experiments. *$P < 0.05$ *vs.* pCI-neo; ANOVA followed by Dunnett's test. (**c**) Effects of Lam or LamSP expression on the steady-state level of blood-testis barrier (BTB) constituent proteins and signaling molecules. Immunoblots of Sertoli cell (0.45 x 10^6 cells/cm^2) lysates harvested 3 days after transfection. (**d**) Histogram summarizing immunoblotting results as in **a** from 3 independent experiments. Proteins that did not show a significant change were not represented. Each bar = mean±SD (n = 3). **$P < 0.01$ *vs.* pCI-neo; ANOVA followed by Dunnett's test.

Supplementary Table S1. Primers used for different experiments in this report.

Gene Fragment	Primer sequence	Orientation	Position	Length (b.p.)	Tm (ºC)	Cycle no.	GenBank accession no.
Lam γβ domain IV	5'- AC*CTCGAG*ATGGTGTGTGCCCCTGCTT-3' 5'- AC*GTCGAC*CTAGGTCTCCACCCAAGA-3'	Sense Antisense	1489-1504 2038-2052	564	56	27	NM_001107830
F1	5'- AC*CTCGAG*ATGGTGTGTGCCCCTGCTT-3' 5'- AC*GTCGAC*CTACTGGTCTCCCAGGAACTTCT-3'	Sense Antisense	1489-1504 1658-1677	189	56	27	NM_001107830
F2	5'- AC*CTCGAG*ATGAGACTCAGCTATGGACAGC-3' 5'- AC*GTCGAC*CTAAGAAGTCTCCTGCAGGAGGA-3'	Sense Antisense	1678-1696 1847-1866	189	56	27	NM_001107830
F3	5'- AC*CTCGAG*ATGGAAGAGGCAGAGCCTCCA-3' 5'- AC*GTCGAC*CTAGGTCTCCACCCAAGA-3'	Sense Antisense	1867-1884 2038-2052	186	56	27	NM_001107830
F4	5'- AC*CTCGAG*ATGGAGCGTCCTCTGCAATGG-3' 5'- AC*GTCGAC*CTAACCTGCCCCCTCCAGT-3'	Sense Antisense	1585-1602 1755-1770	186	56	27	NM_001107830
F5	5'- AC*CTCGAG*ATGTTATCTCTGGCTCTGAAACCC-3' 5'- AC*GTCGAC*CTACAGGCCTTGGCCACTG-3'	Sense Antisense	1771-1791 1944-1959	189	56	27	NM_001107830
F6	5'- AC*CTCGAG*ATGAGACTCAGCTATGGACAGC-3' 5'- AC*GTCGAC*CTAACCTGCCCCCTCCAGT-3'	Sense Antisense	1678-1696 1755-1770	93	56	27	NM_001107830
F7	5'- AC*CTCGAG*ATGTTATCTCTGGCTCTGAAACCC-3' 5'- AC*GTCGAC*CTAAGAAGTCTCCTGCAGGAGGA-3'	Sense Antisense	1771-1791 1847-1866	96	56	27	NM_001107830
F8	5'- AC*CTCGAG*ATGGAAGAGGCAGAGCCTCCA-3' 5'- AC*GTCGAC*CTACAGGCCTTGGCCACTG-3'	Sense Antisense	1867-1884 1944-1959	93	56	27	NM_001107830
S16	5'-TCCGCTGCAGTCCGTTCAAGTCTT-3' 5'-GCCAAACTTCTTGGTTTCGCAGCG-3'	Sense Antisense	15-38 376-399	385			XM_341815

* *CTCGAG*, restriction site for *Xho*I; *GTCGAC*, restriction site for *Sal*I; ATG, start codon; CTA, stop codon

b.p., base pairs; Tm, annealing temperature

Supplementary Table S2. Primary antibodies used for different experiments in this report*

Antibody	Catalog #	Lot #	Host	Vendor	Working dilution		
					IB	IF	IHC
Occludin	71-1500	944954A	Rabbit	Zymed/Invitrogen	1:250	1:50	1:50
ZO-1-FITC	33-9111	814986A	Mouse	Zymed/Invitrogen		1:50	
JAM-A	36-1700	463126A	Rabbit	Zymed/Invitrogen	1:250		
FAK	06-543	31701	Rabbit	Upstate/Millipore	1:1000		
p-FAK-Tyr^{407}	44650G	0400#7A	Rabbit	Zymed /Invitrogen	1:500	1:50	
N-Cadherin	sc-7939	G2109	Rabbit	Santa Cruz Biotechnology	1:200		
	33-3900	979580A	Mouse	Zymed/Invitrogen		1:50	
β-Catenin	71-2700	564500A	Rabbit	Zymed/Invitrogen	1:250	1:50	
Actin	sc-1616	F0809	Goat	Santa Cruz Biotechnology	1:200		

*Antibodies used herein were known to cross-react with the corresponding proteins in rats as indicated by the manufacturers.
Abbreviations used: IB, immunoblotting; IF, immunofluorescence microscopy; IHC, immunohistochemistry.

Supplementary Table S3. Primers used in studies to assess "inside-out" and "outside-in" signaling

a. Primers used for the cloning of Lam (laminin-β3 chain) and LamSP (laminin β3 chain with signal peptide)

Target sequence	GenBank Accession no.	Primer orientation and sequence (5' – 3')*		Nucleotide position**
Lam	NM_001100841.1	S	CTGAATTCATGGTTACTGAGGTTCAGCAG	3220-3237
		AS	CAGTCGACCTACCCTCGAAGCAGCTCTGA	3568-3585
LamSP	NM_001100841.1	S	CTGAATTCATGACGGCACTTTTCCTCTTGTGGCTTGCCT TGCCTGGCCTCCTGTGTGCCGTTACTGAGGTTCAGCAG	3220-3237
		AS	CAGTCGACCTACCCTCGAAGCAGCTCTGA	3568-3585

b. Primers used for checking mRNA expression in transfected Sertoli cells

Target sequence	Accession no.	Primer orientation and sequence (5' – 3')		Nucleotide position	Expected size (bp)
Lam	NM_001100841.1	S	GTTACTGAGGTTCAGCAG	3220-3237	366
		AS	CCCTCGAAGCAGCTCTGA	3568-3585	
LamSP	NM_001100841.1	S	ACGGCACTTTTCCTCTTG	184-201	414
		AS	CCCTCGAAGCAGCTCTGA	3568-3585	
GAPDH	BC087743	S	ATCACCATCTTCCAGGAGCGA	258-278	105
		AS	AGCCTTCTCCATGGTGGTGAA	342-362	

* Dotted underline, restriction enzyme recognition site; Single solid underline, start codon; Double solid underline, stop codon; Gray highlight, sequence for priming laminin β3 precursor; Wavy underline, sequence encoding the N-terminal signal peptide (17 amino acids including start codon).
** Nucleotide position corresponds to the sequence for priming laminin-β3 precursor marked by gray highlight.

Supplementary Materials and Methods

Primary Sertoli cell cultures. Sertoli cells were isolated from 20-day-old rat testes and cultured in serum-free F12/DMEM (Ham's F12 Nutrient's Mixture/Dulbecco's Modified Eagle's Medium) containing bacitracin, gentamicin, and other supplements [*e.g.,* human transferrin, bovine insulin, epidermal growth factor (EGF) to promote cell survival *in vitro*] as earlier described.[52-56] Sertoli cells cultured under these conditions from 20-day-old rat testes were differentiated and ceased to divide,[54,56] consistent with an earlier report that Sertoli cells ceased to divide by 20 days of age in rats;[57] and this system has been widely used in the field to study Sertoli cell function including the BTB function.[28,58-64] Recent studies have shown that the inclusion of serum (*e.g.,* fetal calf serum) in the spent media of Sertoli cell cultures induced de-differentiation in these cells from mice and humans, making them mitotically active again,[65,66] In our studies, Sertoli cells were plated on Matrigel [BD Biosciences, Bedford, MA; Matrigel diluted 1:7 in serum-free F12/DMEM (Sigma-Aldrich, St Louis, MO)]-coated: (i) 12-well dishes at 0.5 x 10^6 cells/cm^2 containing 3-ml F12/DMEM, (ii) coverslips (round) at 0.04 x 10^6 cells/cm^2 and placed in 6-well dishes containing 5-ml F12/DMEM, or (iii) bicameral units [Millicell-HA (Millipore, Bedford, MA) 12-mm diameter inserts, effective surface area 0.6 cm^2, 0.45 μm pore size] at 1.2 x 10^6 cells/cm^2, and placed in 24-well dishes containing 0.5-ml F12/DMEM each in the apical and basal compartment. Cells were cultured under serum-free F12/DMEM conditions at 35°C in a humidified atmosphere with 95% air/5% CO_2 (v/v) as described.[54] About 48 h after plating, cells were subjected to a hypotonic treatment (20 mM Tris, pH 7.4 at 22°C, 2.5 min) as described[67] to lyse residual germ cells. The purity of Sertoli cells in these cultures used for our studies had a purity of greater than 98% with negligible contamination of germ and/or Leydig cells when assessed by corresponding markers by RT-PCR and/or immunoblotting as described.[24] It is noted that these Sertoli cells were also indistinguishable from Sertoli cells isolated from adult rats morphologically and functionally.[68,69] Using this *in vitro* model, Sertoli cells were shown to form a functional TJ-permeability barrier; and having ultrastructures of TJ, basal ES, gap junction and desmosome within ~2-3 days when examined by electron microscopy,[25-27,70] mimicking the BTB *in vivo*, thus, this model has been widely used by investigators to study Sertoli cell BTB function.[28-30,63] Different cell densities were used for different experiments as listed in (i)-(iii) (see above) so that enough protein could be obtained in cell lysates for probing different target proteins by immunoblotting in (i), and a functional TJ-permeability barrier could be easily assessed by TER measurement in (ii). A lower cell density was used for fluorescence microscopy so that Sertoli cell nuclei were well separated spatially, and cell-cell interface was clearly discerned so that different adhesion proteins at or near the cell surface could be visualized and compared between treatment and control groups. It was noted that Sertoli cells cultured at cell densities at 0.5 to 1.2 x 10^6 cells/cm^2 as described (i) and (iii) were columnar in shape,[70,71] with ultrastructures of TJ, basal ES, gap junction and desmosomes, making them indistinguishable from those junctions found in the testis *in vivo*.[26,27]

Vectors used to assess "inside-out"/"outside-in" signaling. The coding sequence of a biologically active fragment from the domain I of rat laminin β3 precursor (Accession no. NM_001100841.1), herein referred as "Lam", was amplified from rat Sertoli cell cDNA by PCR (**Supplementary Table S3).** Alternatively, "LamSP" was obtained by including the coding sequence for the native 17-amino acid N-terminal signal peptide from laminin β3 precursor (nucleotides 181-231), namely NH_2-MTALFLLWLALPGLLCA-COOH) in the sense primer (**Supplementary Table S3**). Thus, expression of the LamSP construct would result in an N-terminal signal peptide fused directly to the Lam fragment without a linker. The amplified cDNA of Lam and LamSP were cloned into the EcoRI/SalI site of the pCI-neo Mammalian Expression Vector (Promega).

Assessing Sertoli TJ-barrier function after transfection. Primary Sertoli cells were isolated from 20-day-old male Sprague-Dawley rats, and were plated on Matrigel (BD Biosciences)-coated 12-well culture plates at 0.45 x 10^6 cells/cm^2 for harvesting cell lysates, or on Matrigel-coated Millicell-HA 12 mm culture plate inserts (Millipore) at 1.2 x 10^6 cells/cm^2 for transepithelial electrical resistance (TER) measurement. Sertoli cells were cultured for 2 days to allow the assembly of a TJ-permeability barrier mimicking the BTB *in vivo*. Thereafter, cells were transfected with plasmid DNA (1 μg for each well in a 12-well plate; 0.5 μg for each 12 mm insert) for 24 hours using Effectene Transfection Reagent (Qiagen; 15 μl per μg DNA) according to manufacturer's instruction. For RNA extraction and protein lysates, cells were harvested 3 days after transfection in Trizol Reagent (Invitrogen) and IP lysis buffer [50 mM Tris, pH 7.4 at 22˚C, containing 150 mM NaCl, 2 mM EGTA, 10% glycerol (v/v), 1% Nonidet P-40 (v/v), and freshly supplemented with Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts (1:100; Sigma-Aldrich) and Phosphatase Inhibitor Cocktails 2 and 3 (1:80; Sigma-Aldrich)], respectively. For TER, readings were taken daily throughout the entire period of culture. To assess the transfection efficiency using Effectene (Qiagen) and the Mammalian Expression Vector pCI-neo in Sertoli cells, luciferase reporter plasmid (pGL3-Control and pRL-TK, Promega) was co-transfected into Sertoli cells with plasmid DNAs at ~0.1-3 μg, and different cell densities 0.1-1.2 x 10^6 cells/cm^2 for 24-hr to monitor the transfection performance under each condition by assaying the luciferase reporter gene activity. We also assessed different ratios of plasmid DNA to Effectene, ranging between 1:10 and 1:25, for transfection efficiency using Effectene. Transfection efficiency was determined by counting cells (~ 400 cells in four randomly selected fields, ~100 cells/field) with positive signals using either fluorescence microscopy (for pTracer-CMV2 which contains a GFP reporter) or immunohistochemistry staining of laminin-β3 or -γ3 (for pCIneo) since Sertoli cells *per se* did not express laminin-β3 or -γ3.[10] It is noted that counting transfected cells with positive signals (*e.g.,* β-galactosidase or GFP) is a common approach to determine transfection efficiency.[72,73] Using this approach, the transfection efficacy was estimated to be ~15-20%.

BTB integrity assay. The integrity of the BTB *in vivo* was assessed in a functional assay by monitoring the ability of the BTB to restrict the diffusion of a small fluorescent probe (*e.g.,* FITC-inulin, Mr 4.6 kDa) that leaked out from the microvessels in the interstitium when administered at the jugular vein, traversing the immunological barrier and entered the adluminal compartment as described.[74] In short, rats (n = 3 rats per time point in both treatment and control groups)

(~250-275 g b.w.) received an intratesticular injection of either 80 or 320 μg peptide on the right testis using a 28-gauge needle in a final volume of 200 μl saline, and the left testis of the same rat received saline alone (200 μl, vehicle control) to serve as negative controls. For positive controls, rats received $CdCl_2$ (3 mg/kg b.w., i.p.) and terminated ~5-day thereafter, which is known to induce irreversible BTB damage.[32-34] Rats treated with saline alone or no treatment serving as negative controls. Rats at specified time points by 3-day, and 1-, 2- and 4-wk after synthetic peptide treatment including both the negative and positive controls were anesthetized by ketamine HCl (50 mg/kg b.w., administered i.m.) with xylazine as an analgesic (13 mg/kg b.w., i.m.) (Sigma-Aldrich) administered i.m. The surgical site was cleansed with 70% ethanol, and a ~1 cm incision was made to expose the jugular vein. Thereafter, inulin-FITC (Sigma-Aldrich) (5 mg/ml in PBS -10 mM sodium phosphate, 0.15M NaCl, pH 7.4 at 22 °C) in a final volume of 200 μl (*i.e.,* 1 mg/rat) was administered into the jugular vein using a 28-gauge needle. Thereafter, the surgical site was closed with a MikRon 9-mm Autoclip (Becton Dickinson, Sparks, MD), cleansed with 70% ethanol, and animals were allowed to recover. Approximately 90 min thereafter, rats were euthanized by CO_2 asphyxiation, testes were immediately removed and snap-frozen in liquid nitrogen. Testes were cut at a thickness of ~10-μm to obtain frozen cross-sections, collected onto poly-L-lysine-coated slides in a cryostat at -22^{o}C. ~100 tubules were randomly selected and examined from each rat testis with a total of 3 rats per time point. To yield semi-quantitative data to assess BTB integrity after F5-peptide treatment for statistical comparison, the distance of FITC-inulin that was diffused into the seminiferous epithelium beyond the BTB in tubules in treatment groups *versus* controls was measured and compared to the tubule radius (diffusion/radius) by fluorescence microscopy. For tubules that were obliquely sectioned, tubule radius was obtained using the mean of the shortest and the longest distance from the basement membrane. For this and pertinent experiments, all samples (*e.g.,* cross-sections of testes for fluorescence microscopy or immunohistochemistry, and lysates for immunoblot analysis) within an experiment group were processed simultaneously in a single experimental session to eliminate inter-experimental variations.

Histological analysis. Testes removed from rats were placed in Bouin's fixative for 24 h. Thereafter, testes were dehydrated using gradient ethanol, embedded in paraffin, and sectioned to 8-μm using a microtome. After de-

paraffinization in xylene at 60℃ for 1 h on a hot plate, and rehydration with xylene and gradient ethanol, cross-sections of testes were stained using hematoxylin and eosin for histological analysis as described.[27]

Dual-labeled immunofluorescence analysis. The distribution of integral membrane proteins in F5/pCIneo *versus* pCIneo alone transfected Sertoli cells was examined by dual-labeled immunofluorescence analysis. In brief, Sertoli cells were isolated and cultured on Metrigel-coated coverslips at 0.04 x 10^6 cells/cm^2, placed in 6-well dishes containing 5-ml F12/DMEM per well. On day 3, cells were transfected with 0.2 μg F5/pCIneo *versus* pCIneo plasmid DNA by using 3 μl Effectene and incubated for 24 h. Thereafter, the transfection mixture was removed and cells were cultured for an additional 48 h. Cells were then fixed in 4% paraformaldehyde in PBS (10 mM sodium phosphate, 0.15M NaCl, pH 7.4 at 22^{o}C), permeabilized with 0.1% Triton X-100 in PBS. After blocking with 10% normal goat serum (v/v) in PBS, cells were incubated with occludin (rabbit)/ZO-1(FITC, mouse) or β-catenin (rabbit)/N-cadherin (mouse) at 1:50 dilution in PBS (see **Table S2**). Following overnight incubation, cells were incubated with secondary antibodies conjugated with Cy3 or FITC (Invitrogen, 1:100 diluted in PBS) for 1 h. Subsequently, cells were mounted with ProLong anti-fade reagent containing DAPI (Molecular Probes, Eugene, OR) and fluorescent micrographs were obtained using an Olympus BX61 Fluorescence Microscope with an Olympus DB71 12.5 megapixel digital camera. To ascertain that cells were indeed transfected with DNA constructs when distribution of integral membrane proteins at the cell-cell interface were examined, Sertoli cells transfected with 0.2 μg F5/pCIneo or pCIneo plasmid DNA on day 3 were co-transfected with 0.07 μg *Lable*IT® Cy™3 Plasmid Delivery Control (Mirus). After 24 h incubation, transfection media were removed. Two days thereafter, cells were fixed and mounted as described above. Red fluorescence signals that were detected in transfected Sertoli cells by immunofluorescence microscopy illustrated successful transfection.

Immunohistochemistry. Immunohistochemical localization of occludin in rat testes was performed using a streptavidin-biotin based method. In short, frozen sections of testes were obtained in a cryostat with ~7-μm thickness at -22^{o}C and mounted onto poly-L-lysine-coated slides and air-dried. Sections were then fixed in Bouin's fixative for 5 min. Sections were treated with 1% hydrogen peroxide in methanol (v/v) for 15 min to block endogenous peroxidase activity, permeabilized with 0.1% Triton X-100 (v/v) in PBS for 10 min. Thereafter, sections were incubated with a rabbit anti-occludin antibody (**Supplementary Table S2**) overnight, to be followed by biotinylated goat anti-rabbit IgG (Invitrogen; 1:100 in PBS) and Streptavidin Horseradish Peroxidase (HRP) Conjugate (Invitrogen; 1:200 in PBS). Color development was performed using AEC mixture, counterstained with hematoxylin and mounted using Aqua Poly/Mount (Polysciences,

Cat#18606). Images were captured by using an Olympus DP71 12.5 MPx digital camera in an Olympus BX61 microscope (Olympus America, Inc., Melville, NY). Images were acquired using the Olympus MicroSuiteTM FIVE (Version 1224) software package (Olympus Soft Imaging Solutions Corp., Lakewood, CO), converted to TIFF format, and analyzed by Adobe PhotoShop in Adobe Creative Suite (Version 3.0, Adobe Systems, Mountain View, CA), such as merges of images to assess protein co-localization. Negative controls included the substitution of the primary antibody by normal rabbit IgG or the omission of the secondary antibody.

F-actin staining. Frozen sections (7 μm in thickness) of testes from F5-peptide treated *versus* control (normal) rats obtained in a cryostat at -22°C were fixed in 4% paraformaldehyde in PBS (w/v) for 10 min. Thereafter, sections were permeabilized in 0.1% Triton X-100 in PBS (v/v) and blocked with 1% BSA in PBS (w/v) for 30 min. This was followed by an incubation of FITC-conjugated phalloidin (Sigma-Aldrich, Cat. # P5282; 1:75 in 1% BSA) for 45-min at room temperature (22±1°C). Sections were mounted in ProLong Gold antifade reagent with DAPI (Invitrogen). To eliminate inter-experimental variations, all sections within an experimental group including treated and control testes were analyzed simultaneously by mounting ~2-3 sections onto a microscopic slide. Images were acquired with an Olympus BX61 fluorescence microscope as described above. All fluorescence images reported here were representative results of an experiment. Each experiment was repeated ~3-4 times using different sets of samples and yielded similar results.

Immunoblot analysis. Lysates were prepared from testes or Sertoli cells using the IP lysis buffer [10 mM Tris, pH 7.4 at 22°C, containing 0.15 M NaCl, 10% glycerol (v/v), 1% NP-40 (v/v)], supplemented with protease inhibitor cocktail (~25 μl/ml) and phosphatase inhibitor cocktails 1 and 2 (~15 μl/ml) (Sigma-Aldrich) as described.[49,75] Protein concentration in lysates was estimated using the *DC* protein assay kit (Bio-Rad Laboratories). Proteins were resolved by SDS-PAGE and the target proteins were probed with corresponding antibodies (**Supplementary Table S2**) by immunoblot analysis with actin served as protein loading control.

Statistical analysis. GB-STAT statistical analysis software (Version 7.0; Dynamic Microsystems) was used for statistical analysis. Each experiment was repeated at least three times using different batches of Sertoli cells; for *in vivo* study, at least 3 rats were used per time point including both treatment and control groups. Statistical significance was analyzed with Student's *t*-test, or one-way ANOVA coupled with two-tailed Dunnett's test. All values are expressed as mean ± SD.

DNA sequence accession codes. Sequence data were retrieved from the GenBank database under accession codes NM_001107838 (Laminin γ3), NM_001100841.1 (laminin β3), XM_341815 (S16) and BC087743 (GAPDH).

References

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Supplemental Figure Legends

Figure S1. Preparation of laminin-γ3 domain IV cDNA constructs to assess their biological activity. (**a**) Schematic drawing illustrating different functional domains of laminin-γ3 chain. Laminin-γ3 domain IV (DIV, in red) was selected based on earlier studies that this domain is biologically active in regulating BTB dynamics,[15] and it was further divided into 5 fragments of F1-F5 as shown in (**b**) to assess their effects on BTB function. (**c**) cDNA-deduced amino acid sequence of laminin-γ3 DIV (GenBank XM_231139) is shown, illustrating the sequences of F1 (blue boxed area), F2 (orange boxed area), F3 (purple boxed area), F4 (pink colored text) and F5 (green colored text). **(d)** RT-PCR illustrating the expression of laminin-γ3 DIV and the five different fragments (see **a**) in Sertoli cells after transfection with corresponding vectors *versus* pCIneo alone for 3 days. Cells transfected with pCIneo alone served as a negative control since Sertoli cells *per se* did not express laminin-γ3 chains, which are exclusively expressed by elongating/elongated spermatids.[10] S-16 served as an internal PCR control. Identity of the PCR product was confirmed by direct nucleotide sequencing at GeneWiz Inc (South Plainfield, NJ). M, DNA markers; bp, base-pair. (**e**) Changes in the Sertoli cell TJ-permeability barrier function following transient expression of different fragments of laminin-γ3 DIV *versus* DIV (positive control) and pCIneo alone (negative control) on day 3 for 24-hr by quantifying TER across the Sertoli cell epithelium in bicameral units (n = 3). This experiment was repeated three times during different batches of Sertoli cells and yielded similar results. *, P<0.05; **, P<0.01.

Figure S2. Laminin-γ3 fragments affect protein expression and localization at the Sertoli cell BTB. (**a**) On day 3, Sertoli cells with a functional BTB were transfected with different expression vectors for 24 hr and terminated 48 hr thereafter for immunoblotting, illustrating changes in the levels of integral membrane proteins (*e.g.,* occludin, JAM-A, N-cadherin), adaptor protein (*e.g.,* β-catenin) and regulatory proteins (*e.g.,* FAK, p-FAK-Tyr^{407}). (**b**) Bar graphs summarize results shown in **(a)**. Each bar is a mean±SD of n = 5 experiments, and data were normalized against actin. The protein levels in cells transfected with pCIneo alone were arbitrarily set at 1, against which one-way ANOVA and Dunnett's test were performed. *, P < 0.05; **, P < 0.01. (**c**) Overexpression of F5-peptide in Sertoli cells caused considerable re-distribution of basal ES-proteins at the BTB: β-catenin (red) and N-cadherin (green) at the Sertoli cell-cell interface, moving from cell surface into cell cytosol on day 3 after transfection. Overexpression of F5-peptide also changed localization and/or distribution of TJ proteins at the BTB: occludin (red) and ZO-1 (green) at the Sertoli cell-cell interface.

Co-localized proteins appeared as "orange" in merged images. Nuclei were visualized by DAPI (blue). Scale bar = 10 μm in **c** and **d**, which applies to all images in both panels.

Figure S3. Laminin F5-peptide perturbs Sertoli cell TJ function via p-FAK-Tyr^{407}. (**a**) To examine the mechanism by which the F5-peptide perturbs the Sertoli cell TJ-permeability barrier function as shown in **Figs. 1 and 2**, p-FAK-Tyr^{407}, a molecular "switch" in the apical ES-BTB axis to regulate BTB restructuring and apical ES adhesion,[23] was expressed either alone as a phosphomimetic mutant (FAK Y407E/pCIneo) or co-expressed with F5 [(F5+FAK Y407E)/pCIneo]. Co-expression of FAK phosphomimetic mutant FAK Y407E with F5-peptide in Sertoli cells blocked the F5-induced TJ-barrier disruption, which is likely mediated by the ability of p-FAK-Tyr^{407} to promote the distribution of occludin and ZO-1 at the Sertoli cell-cell interface shown in (**b**), thereby stabilizing the Sertoli TJ-barrier. Each data point is a mean±SD of n = 4 of a representative experiment, and this experiment was repeated 3 times which yielded similar results. *, $P < 0.05$; **, $P < 0.01$ Scale bar = 10 μm, which applies to all remaining micrographs.

Figure S4. Laminin F5-peptide reversibly perturbs BTB function *in vitro* and *in vivo*. (**a**) Amino acid residues (green) of the synthetic F5-peptide based on laminin-γ3 DIV. Numerals in "black" denote the sequence of laminin-γ3 chain (see Fig. **1a-c**). (**b**) Effects of the synthetic F5-peptide on the Sertoli cell TJ-permeability barrier. Peptide at 50 μg/ml (~10 μM) was added on day 3 ("green" arrow), which was either included in the daily replacement medium ("green") (n = 3) or removed by washing on day 4 ("pink" arrow) in medium without peptide ("pink") *versus* PBS control. When F5-peptide was removed, the disrupted TJ-barrier was "resealed", illustrating the disruptive effect was *reversible*. Each data point is a mean±SD of n = 3 of a representative experiment, and this experiment was repeated 3 times which yielded similar results. **, $P < 0.01$. (**c**) BTB integrity assay was performed to assess the ability of an intact BTB to block the movement of FITC-inulin across the BTB from the basal compartment near the basement membrane (annotated by "white" dotted line) to the adluminal compartment. Results of the BTB integrity assay are shown in normal testes (control) *versus* rats treated with $CdCl_2$ and different peptide treatment groups: 80 μg per testis (or ~10 μM) and 320 μg per testis (~40 μM). Scale bar = 150 μm in **c**, which also applies to all remaining micrographs. (**d**) Histograms summarizing data based on findings shown in **c** by comparing the distance of FITC-inulin diffused into the epithelium (D_{FITC}) (annotated by the "white bracket") *vs.* the radius of a seminiferous tubule (D_{STr}) (average of the longest and shortest axes for sections of oval-shaped tubules) (n = 200 tubules from testes of 3 rats in each group). **, $P < 0.01$; ns, not significantly different. (**e**) Immunoblot analysis of different target proteins at the BTB in adult rat testes at different time points after peptide administration. (**f**) Histograms summarizing data shown in **e** with n = 3 rats for each time point. *, $P<0.05$; **, $P<0.01$.

Figure S5. Laminin F5-peptide impairs spermatogenesis and induces germ cell loss from the testis. Peptide or vehicle control (0.89% NaCl, see **a**) was injected into each testis of a group of adult rats (~300-350 gm b.w.) with n = 3 rats per time point in each treatment group *versus* controls Rats were terminated at specific time points at 3-day and 1-, 2- or 4-wk thereafter. (**b-e**) Representative photographs of paraffin sections of testes stained with hematoxylin and eosin from rats treated with the synthetic F5-peptide at either 80 or 320 μg/testis. Vehicle control is shown in **a**. Morphometric

changes [e.g., shrinkiage of tubule, and % of damaged seminiferous tubules (ST) manifested by germ cell exfoliation – annotated by asterisks in tubules] of the tubules (n = 200 tubules from 3 rat testes) are summarized in the bar graphs shown in **f** and **g** . Bar = 150 μm in **a**, and the micrographs on the left panels in **b-e**, which applies to the micrographs on the right panels **b-e**. Bar = 50 μm in micrograph encircled in "blue" which applies to all micrographs encirculed in and "green" in **a-e**. The "blue" and "green" encircled micrographs are magnified images of the corresponding boxed areas of the lower magnification. ns, not significantly different by ANOVA; *, $P < 0.05$; and **, $P < 0.01$ when compared to normal control rats at time 0.

Figure S6. Laminin F5-peptide perturbs occludin disruption at the BTB. Normal rat testis (control, at time 0-day), displaying normal distribution of occludin at the BTB. *versus* rats received either 80 μg (~10 μM) or 320 μg (~40 μM) F5-peptide per testis by 1- and 4-week with noticeable germ cell loss in the tubules. Micrographs on the right panel are the corresponding magnified images of the boxed area shown on the left panel. Bar = 150 μm in the first micrograph on the left panel and scale bar = 50 μm in the first micrograph on the right panel, which apply to remaining micrographs in the same panel.

Figure S7. Laminin F5-peptide disrupts BTB and spermatid adhesion via p-FAK-Tyr^{407}. Testes received vehicle (control) at time 0 **(a)** *versus* synthetic F5-peptide at 80 μg/testis (~10 μM), and terminated at 3-day, and 1-, 2- and 4-week **(b)**, and positive control where rats were treated with $CdCl_2$ (5 mg/kg b.w., i.p., for 5 days) **(c)**. In control testes, p-FAK-Tyr^{407} was localized near the basement membrane consistent with its localization at the BTB [see "yellow" boxed area in the micrograph on the left, which was magnified in the right panel (top) in each row with the relative BTB location annotated by the "broken white-line"]; p-FAK-Tyr^{407} was also found in the adluminal compartment, restricted almost exclusively to the concave side of the spermatid head at the apical ES [see "blue" and "green" boxed areas in the micrograph on the third column, which were magnified in the right panel (bottom) in each row]. Following F5-peptide treatment, p-FAK-Tyr^{407} was down-regulated and mis-localized at the BTB, since it no longer restricted to the BTB, but diffused away (see "white" bracket on the top right panel of each column in treatment groups *vs.* control rats, which was widened over time in treatment groups). p-FAK-Tyr^{407} was also down-regulated and its localization at the apical ES also shifted from the concave to cover the convex side of the spermatid head by 3-day, and p-FAK-Tyr^{407} no longer associated with the apical ES in most elongated spermatids by 1- and 2-week, and by 4-week as elongated spermatids were not found in most tubules. In $CdCl_2$-treated rat testes **(c),** p-FAK-Tyr^{407} was no longer detectable at the BTB (see "open" white arrowheads), which was the magnified image of the "white" boxed area of the testis in the right column in **(c),** and p-FAK-Tyr^{407} was also considerably diminished. p-FAK-Tyr^{407} appeared as vesicle-like structures near the tubule lumen in rats after treatment (and also in $CdCl_2$-treated rats), representing cytoplasmic droplets from p-FAK-Tyr^{407}-positive materials engulfed by Sertoli cells.[23] Scale bar = 50 μm in **(a-c)**, which applies to all micrographs in the first three columns in **(a-c)**, ; scale bar in the "yellow" boxed micrograph = 20 μm, which applies to all "blue" and "green" boxed micrographs in **(a, b);** scale bar in the last micrograph in **(c)** = 80 μm.

Figure S8. Laminin F5-peptide induces spermatid loss via changes in F-actin distribution. Rat testes received synthetic F5-peptide at 80 μg/testis (~10 μM) at time 0, and terminated at 3-day, and 1-, 2- and 4-week *versus* control (normal rats). Frozen sections were used to visualize the distribution F-actin in the seminiferous epithelium. Relative location of the BTB was annotated by the "white" broken line in the panel stained for cell nuclei with DAPI. Actin filaments were most abundant at the BTB and the apical ES at the Sertoli-spermatid interface as shown in a stage VII and VIII tubule in control rats at time 0. By 3-day, 1-, 2- and 4-week after F5 peptide treatment, in tubules of stage ~VII-VIII, actin filaments were redistributed, progressively moved away from the BTB site (see the "white" brackets that illustrate the relatively "tightly" packed actin filaments, such as those found in controls, were "unbundled" and the "white" brackets were widened in treat rat testes). While F-actin was also restricted to the apical ES at these time-points, the actin filaments no longer tightly restricted to the Sertoli-spermatid interface at the apical ES as seen in control testes (see "magnified" images in the last column of the "boxed" areas in the third column) and the actin filaments no longer restricted to the elongated spermatids as shown in control testes, but were also found in early elongating spermatids (see "open" arrowheads). This mis-localization of F-actin network at the apical ES no longer supported spermatid adhesion, causing premature "spermiation". Scale bar = 50 μm in the micrograph in the first column, which applies to all micrographs in the first three columns, scale bar = 20 μm in the micrograph in the fourth column, which applies to all micrographs in this column.