Supporting Information

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SI Methods

Expression and Purification of Recombinant Proteins. Recombinant proteins of laminin α3, β3, and γ3 chains were expressed in *E. coli* BL21 (DE3) cells using the pET-46 Ek/LIC cloning kit (Novagen). In brief, cDNAs corresponding to different fragments of the laminin chains, including domain I of laminin β3 and γ3 and domain IV of laminin γ3 were amplified by PCR with Accuprime TaqDNA polymerase high fidelity (cat. no. 12346–086; Invitrogen) and primer pairs listed in Table S1. Complementary DNAs reverse transcribed from RNAs isolated from 90-day-old germ cells served as the template. Amplified cDNAs were then subcloned into pET-46 Ek/LIC vector. The cDNA insert was in frame with the expression vector and was confirmed by direct nucleotide sequencing at Genewiz. The expression vectors were transformed into *E. coli* BL21 (DE3) cells by heat shock method at 42°C. Recombinant proteins were induced in the presence of IPTG and produced with a $(His)_6$ tag at the N-terminus, and their authenticity was verified by a specific mouse anti-$(His)_6$ antibody as well as their corresponding antibodies by using an approach as described (1). Recombinant proteins were purified using $Ni^{2\dagger}$ column and the B-PER 6xHis fusion protein purification kit (Pierce) using the protocol provided by the manufacturer. For recombinant proteins that were expressed in the insoluble fraction of *E. coli*, proteins were extracted using the inclusion body solubilization reagent (Pierce) and were subsequently purified as described above. Protein purity was confirmed by SDS-PAGE with Coomassie blue staining. The identity of the corresponding laminin recombinant proteins was further confirmed by mass spectrometry at the Rockefeller University Proteomics Resource Center.

Treatment of Sertoli Cells with Purified Recombinant Laminin Fragments. Purified recombinant proteins were used for the following experiments to examine their effects on Sertoli cell–TJ barrier function. In short, Sertoli cells were plated on bicameral units or 12-well plates at a cell density of 1.2×10^6 or 0.5×10^6, respectively. Thereafter, 5 or 40 μg/ml recombinant laminin proteins was added to these cultures on day 3 after isolation with functional TJ barrier. Media were replaced every 24 hr, and cells were exposed to the same amount of recombinant protein daily (e.g., 40 μg/ml recombinant proteins) for 3 more days. Recombinant proteins were included in both the apical and basal compartments in the bicameral units. These culture conditions were determined from preliminary studies using different concentrations of recombinant proteins, and cells were treated for different time periods. The disruptive effect of domain I of laminin β3 on the Sertoli cell–TJ permeability barrier vs. controls was further assessed by measuring the TER across the Sertoli cell epithelium as described (2) in triplicate cultures. The TER experiment was repeated at least four times using different batches of Sertoli cells and recombinant proteins.

Endocytosis Assay. To estimate the loss of occludin from the Sertoli cell surface biochemically and to validate changes in the redistribution of occludin in Sertoli cells at the BTB as detected by fluorescent microscopy after β1-integrin knockdown by RNAi, the following endocytosis experiment was performed. In brief, Sertoli cells with a cell density at 0.45×10^6 cell/cm^2 were cultured on six-well plates, and cells were subjected to a hypotonic treatment on day 2 to remove residual germ cells. On day 4, when functional TJs and AJs that mimicked the BTB *in vivo* were formed, as confirmed by electron microscopy and TER measurement across the cell epithelium using procedures as earlier described (2, 3), cells were transfected with siRNA duplex specific to β1-integrin versus nontargeting control siRNA duplex as described in the main text. On day 7, when endogenous β1-integrin was shown to be silenced as verified by immunoblotting, Sertoli cell surface proteins were biotinylated with 0.5 mg/ml sulfo-NHS-SS-Biotin (Pierce) in PBS containing 0.9 mM $CaCl_2$ and 0.33 mM $MgCl_2$ (PBS/CM buffer) at 4°C for 30 min, and the free biotin was quenched with 50 mM NH_4Cl in PBS/CM buffer at 4°C for 15 min. Under these conditions at 4°C, it is known that biotinylated proteins failed to internalize, as such, and only cell surface biotinylated occludin was estimated (4, 5). Cell lysates were then prepared in immunoprecipitation buffer for extraction of occludin. Biotinylated proteins were pulled out with UltraLink Immobilized NeutrAvidin Plus beads (Pierce) from $\approx$400 μg of total protein of Sertoli cell lysates from each sample and subjected to immunoblot analysis using an anti-occludin antibody as described (6). All samples within an experiment group were processed simultaneously to eliminate interexperimental variations. Thus, only the cell surface occludin in Sertoli cells subjected to β1-integrin knock-down by RNAi was quantified and compared to cells transfected with control siRNA duplex.

Immunofluorescent Microscopy. Immunofluorescent microscopy was performed as described (1, 6) to examine the localization of β1-integrin in normal adult rat testes. Cross-sections (frozen sections at $\approx$6 μm thick obtained in a cryostat at $-$20°C) of testes were fixed with modified Bouin fixative, to be followed by an incubation of 1:75 dilution of a rabbit anti-β1-integrin (cat. no. AB1952, lot 22111029; Chemicon), together with either FITC-conjugated mouse anti-occludin (1:100; cat. no. 33-1511, lot 60706482), FITC-conjugated mouse ZO-1 (1:100; cat. no. 33-9111, lot 60706481; Invitrogen, Zymed), mouse anti-laminin α2 (1:300; cat. no. MAB1922Z, lot 0510012749; Chemicon), or mouse anti-vimentin (1:300; cat. no. sc-6260, lot D1805; Santa Cruz Biotechnology) at 35°C overnight. Thereafter, secondary antibodies conjugated with FITC or Cy3 (Zymed), diluted in PBS to 1:50, were incubated with sections on slides for $\approx$30 min. Rabbit anti-nidogen (1:500; cat. no. 481978, lot D22225; Calbiochem) was also used to colocalize with vimentin. Negative controls were performed using normal rabbit and mouse IgG instead of the primary antibodies. All sections from samples within a single experiment set were processed simultaneously (with approximatelytwo to three sections per glass slide) to avoid interexperimental variations.

Statistical Analyses. Statistical analyses were performed by ANOVA, followed by Tukey's honest significant difference test or Student's *t* test using GB-STAT Statistical Analysis software package (version 7.0, Dynamic Microsystems).

1. Yan HHN, Cheng CY (2006) Laminin α3 forms a complex with β3 and γ3 chains that serves as the ligand for α6β1-integrin at the apical ectoplasmic specialization in adult rat testes. *J Biol Chem* 281:17286–17303.

2. Lee NPY, Cheng CY (2003) Regulation of Sertoli cell tight junction dynamics in the rat testis via the nitric oxide synthase/soluble guanylate cyclase/3′,5′-cyclic guanosine monophosphate/protein kinase G signaling pathway: An *in vitro* study. *Endocrinology* 144:3114–3129.

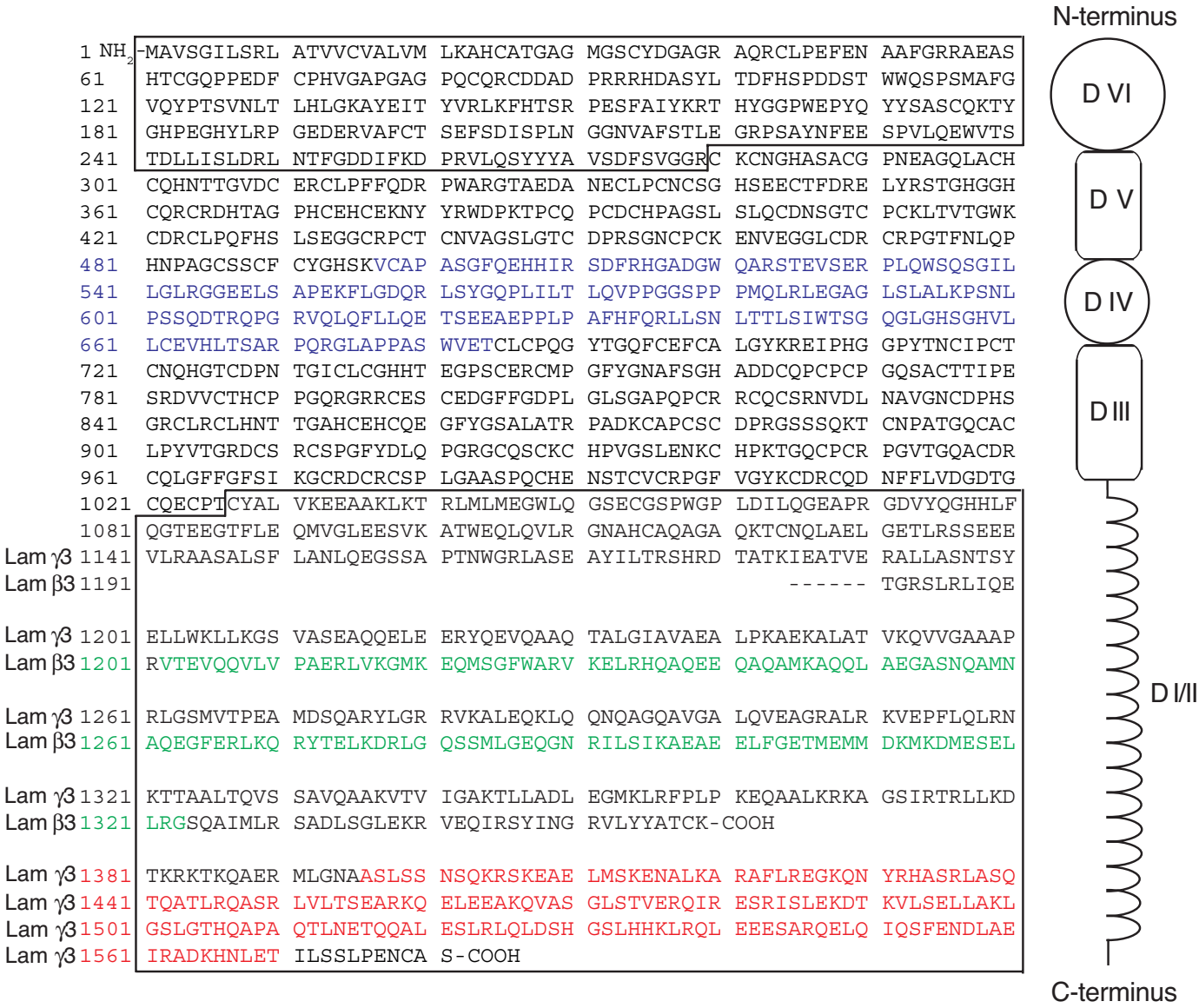

Fig. S1. Preparation of different cDNA constructs based on the primary amino acid sequences of rat laminin γ3 and β3 chains at selected domains for the production of recombinant proteins. Complementary DNA–deduced amino acid sequences of rat laminin γ3 (GenBank XM_231139) and part of rat laminin β3 sequence (GenBank XM_223087) are shown, which also correspond to the schematic drawing (*Right*) illustrating different functional domains of these two laminin chains. Domain IV of laminin γ3 (blue colored text), part of domain I of laminin γ3 (red colored text), and laminin β3 (green colored text) were selected to study the effect of laminin fragments on junction dynamics at the blood-testis barrier during spermatogenesis in rat testes. Boxed area near the N or the C terminus represents the corresponding domain VI and DI/II. Number on the left corresponds to the amino acid residue of the designated laminin chain from the N-terminus.

3. Siu MKY, Wong CH, Lee WM, Cheng CY (2005) Sertoli-germ cell anchoring junction dynamics in the testis are regulated by an interplay of lipid and protein kinases. *J Biol Chem* 280:25029–25047.
4. Yan HHN, Mruk DD, Lee WM, Cheng CY (2008) Blood-testis barrier dynamics are regulated by testosterone and cytokines via their differential effects on the kinetics of protein endocytosis and recycling in Sertoli cells. *FASEB J*, 10.1096/ 22:1945–1959.
5. Le TL, Yap AS, Stow JL (1999) Recycling of E-cadherin: A potential mechanism for regulating cadherin dynamics. *J Cell Biol* 146:219–232.
6. Yan HHN, Cheng CY (2005) Blood–testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors. *Proc Natl Acad Sci USA* 102:11722–11727.

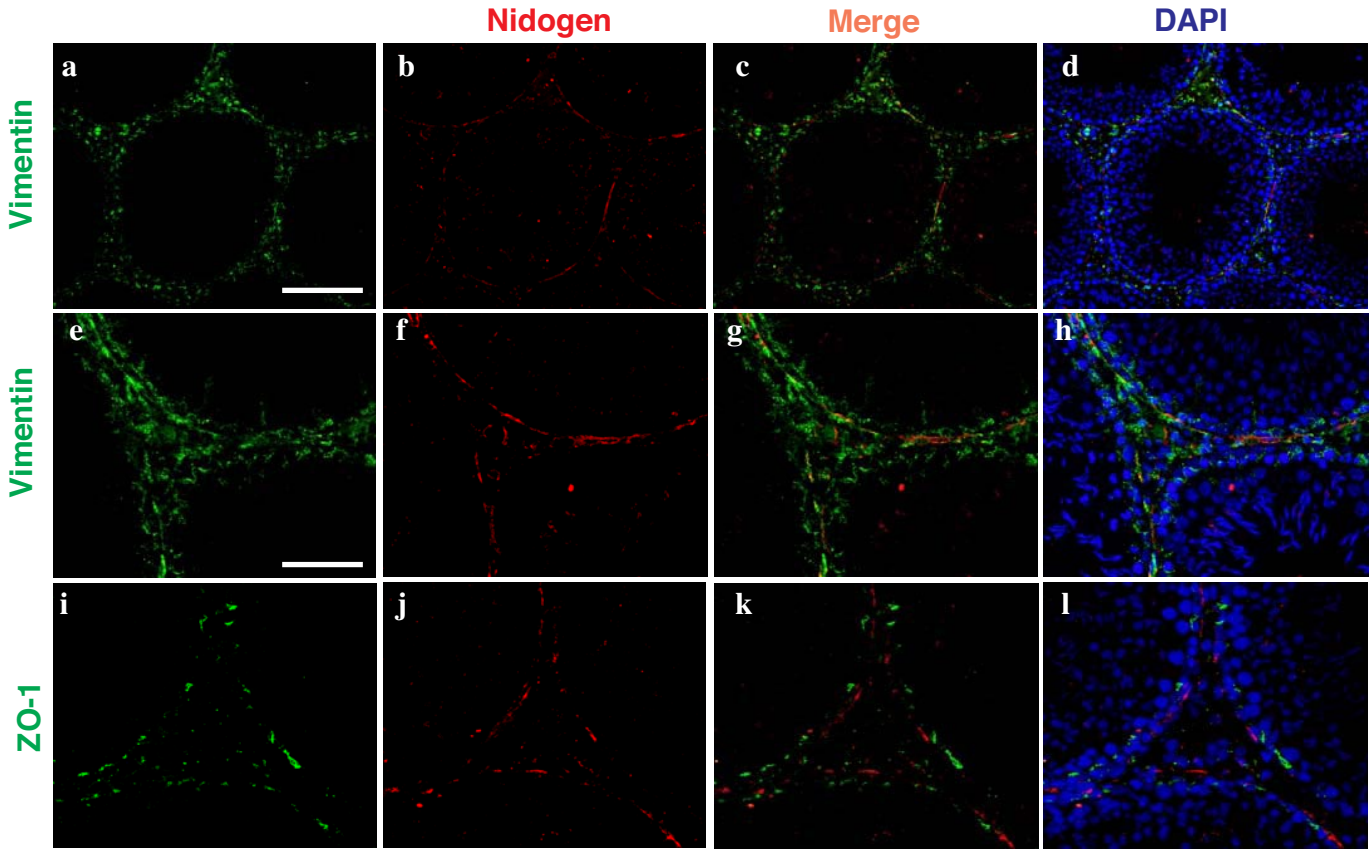

Fig. S2. A study to examine the localization and/or colocalization of vimentin with nidogen (entactin) and ZO-1 in the seminiferous epithelium of adult rat testes. Vimentin (green fluorescence, *a*, *e*), a structural component of the intermediate filament-based cytoskeleton that constitutes desmosomelike junction and hemidesmosome in testes, was shown to colocalize with nidogen (red fluorescence, *b*, *f*, *j*; also known as entactin, which is a 150-kDa glycoprotein found in all basement membranes, including rat testes that forms a stable 1:1 complex with laminins) as shown in *a–d* and *e–h*. However, ZO-1 (green fluorescence, i; a component of the BTB at the Sertoli–Sertoli cell interface) was found not to colocalize with nidogen as shown in *i–l* (see *k*), instead ZO-1 was localized "above" nidogen because BTB is spatially localized above the basement membrane. These results support the data shown in Fig. 3 that β1-integrin in the basal compartment is associated mostly with hemidesmosome instead of the BTB. *a*: 120 μm, which applies to *b–d*; in *e*:50 μm, which applies to *f–l*. DAPI, 4′, 6-diamidino-2-phenyl-indole, stained for cell nuclei.

Table S1. Primers used for expression of laminin recombinant proteins in *E. coli*, cloning of laminin fragments for their transient overexpression in Sertoli cells and for semiquantitative RT-PCR

Target gene name	Orientation	Primer sequence	Nucleotide position	Size, bp	Mr, kDa	Tm, °C	GenBank accession no.
r Lamβ3 Domain I	Sense; AS	5′-GACGACGACAAGATGGTTACTGAGGTTCAGCAG-3′; 5′-GAGGAGAAGCCCGGTCCCTCGAAGCAGCTCTGA-3′	3185–3202; 3532–3550	366	≈13.4	60	XM_223087
r Lamγ3 Domain IV	Sense; AS	5′-GACGACGACAAGATGGTGTGTGCCCCTGCTTCAGGG-3′; 5′-GAGGAGAAGCCCGGTGGTCTCCACCCAAGAGGCTGG-3′	1489–1509; 2032–2052	564	≈20.7	60	XM_231139
r Lamγ3 Domain I	Sense; AS	5′-GACGACGACAAGATGGCCTCGCTCTCCTCCAAC-3′; 5′-GAGGAGAAGCCCGGTGGTCTCCAAGTTGTGCTT-3′	4186–4203; 4692–4710	525	≈19.3	60	XM_231139
r Lamγ3 Domain Ia	Sense; AS	5′-GACGACGACAAGATGGCCTCGCTCTCCTCCAAC-3′; 5′-GAGGAGAAGCCCGGTCTACCGAGACTCTCGGATCTG-3′	4186–4203; 4432–4449	264	≈9.7	60	XM_231139
r Lamγ3 Domain Ib	Sense; AS	5′-GACGACGACAAGATGCGGATCTCCTTGGAGAAGGAC-3′; 5′-GAGGAGAAGCCCGGTCTAGGTCTCCAAGTTGTGCTTGTC-3′	4447–4467; 4690–4710	264	≈9.7	60	XM_231139
Lamγ3 Domain IV	Sense; AS	5′-AC*GCTAGC*ATGGTGTGTGCCCCTGCTTCAGGG-3′; 5′-AC*GAATTC*CTAGGTCTCCACCCAAGAGGCTGG-3′	1489–1509; 2032–2052	564	-	60	XM_231139
Lamγ3 Domain I	Sense; AS	5′-AC*GCTAGC*ATGGCCTCGCTCTCCTCCAAC-3′; 5′-AC*GAATTC*CTAGGTCTCCAAGTTGTGCTT-3′	4186–4203; 4692–4710	525	-	60	XM_231139
Lamβ3 Domain I	Sense; AS	5′-AC*GCTAGC*ATGGTTACTGAGGTTCAGCAG-3′; 5′-AC*GAATTC*CTACCCTCGAAGCAGCTCTGA-3′	3185–3202; 3532–3550	366	-	60	XM_223087
S16	Sense; AS	5′-TCCGCTGCAGTCCGTTCAAGTCTT-3′; 5′-GCCAAACTTCTTGGTTTCGCAGCG-3′	15–38; 376–399	385	-	*	XM_341815
GAPDH	Sense; AS	5′-ATCACCATCTTCCAGGAGCGA-3′; 5′-AGCCTTCTCCATGGTGGTGAA-3′	214–234; 298–318	105	-	*	BC087743

S16 and GAPDH were used for coamplification with other target genes at the specified annealing temperature. The ″red″ highlighted nucleotides are the corresponding start (ATG) and stop (CTA) codons. The ″blue″ and ″green″ italicized nucleotides are the corresponding restriction sites for NheI and EcoRI. The underlined nucleotide sequence represents the cloning site for the subsequent ligation of the PCR product to the pET-46 Ek/LIC vector with DNA ligase. Tm (°C), annealing temperature; Mr (kDa), molecular mass of recombinant protein.