Supplemental Data

Supplemental Figure Legends

Figure S1. Changes of Scribble expression and localization in the seminiferous epithelium of rat testes after adjudin treatment. Rats (n = 3 rats per time point) treated with a single dose of adjudin (50 mg/kg b.w.) by gavage at time 0h (control) and terminated at specified time points thereafter at 6h, 12h, 2D (day) and 4D. Testes were fixed in Bouin's fixative, embedded in paraffin, and cross-sections (5 μm thickness) were obtained with a microtome and process for immunohistochemistry. Scribble appears as reddish-brown immunoreactive substances in the seminiferous epithelium near the basement membrane, consistent with its localization at the BTB; and negative control using goat IgG (Gt IgG) was shown. A considerable time-dependent loss of Scribble at the BTB was detected. The boxed area in each micrograph was magnified and shown in an inset of the corresponding micrograph annotated from i to v. Scale bar = 40 μm, which applies to remaining micrograrphs; scale bar in inset in *i* = 20 μm, which also applies to other insets in *ii*-*v*.

Figure S2. Imaging analysis to assess changes in the expression of Scribble *versus* ZO-1 at the BTB during adjudin-induced germ cell loss from the seminiferous epithelium of adult rat testes. The histogram shown herein summarized findings shown in Fig. 2E (see *main text*) using dual-labeled immunofluorescence analysis, illustrating the adjudin-induced down-regulation of Scribble at the BTB in the seminiferous epithelium was associated with an up-regulation of ZO-1 based on imaging analysis as described in *Materials and Methods*. Each bar = mean±SD of n = 80 tubules randomly selected from 3 rat testes which had received a single dose of adjudin at 50 mg/kg b.w. by gavage at time 0, and rats were euthanized at specified time points. Normal rats at time 0 was arbitrary set at 1 and served as the control against which statistical analysis was performed *, $P < 0.05$; **, $P < 0.01$.

Figure S3. An *in vitro* study to examine the expression of Scribble complex components after treatment of Sertoli cells with adjudin. Sertoli cells were cultured alone for 4 days to form an intact epithelium with an established TJ-permeability barrier that mimicked the Sertoli cell BTB *in vivo*, possessing ultrastructures of TJ, basal ES, gap junction and desmosome under electron microscopy. Thereafter, these cells were treated with adjudin at 1 μg/ml and terminated at specified time points *vs.* vehicle control (ethanol). This concentration was selected based on recent studies illustrating that at this dose, it promoted the Sertoli cell TJ-permeability barrier function (Su et al., 2010). (*A*) Immunoblotting (top panel) using SC lysates obtained from cultures at specified time points after treatment with adjudin (1 μg/ml) with actin served as a protein loading control. The steady-state level of Scribble was found to

decline significantly *vs.* the vehicle control. The lower panel summarizes results of the immunoblotting in which the Scribble protein level at 0 h was arbitrarily set as 1. (*B*) Changes in the steady-state mRNA levels of Lgl2 and Dlg1 after adjudin treatment was analyzed by RT-PCR in Sertoli cells with S-16 served as an internal control. The expression of Lgl2 and Dlg1 was down-regulated after adjudin treatment *vs.* the vehicle control. Histograms in (*B*) summarize results of RT-PCR shown in the top panel. Each bar = mean±SD of $n = 3$ experiments using different Sertoli cell cultures. *, $P<0.05$; **, $P<0.01$ in which each treated group was compared with its corresponding control by ANOVA.

Figure S4. A study to assess any changes in BTB-associated proteins in Sertoli cell epithelium following specific knockdown of different components of the Scribble protein complex by RNAi. These histograms summarize results of immunoblotting shown in Fig. 3, illustrating no changes were detected in the steady-state levels of: (*A*) integral membrane proteins (*e.g.*, occludin, JAM-A) and adaptor protein ZO-1 at the TJ, (*B*) integral membrane protein N-cadherin and adaptor proteins (*e.g.*, α-catenin, β-catenin, γ-catenin) at the basal ES, and (*C*) polarity proteins (*e.g.*, aPKC, PAR3, PAR6 of the Par-based polarity protein complex) at the Sertoli cell BTB after single knockdown of Scribble, Lgl2 or Dlg1, and Scribble, Lgl2 and Dlg1 (SLD) triple-knockdown *versus* non-targeting control. Each bar = mean±SD of $n = 3$ independent experiments using different batches of Sertoli cell cultures for corresponding knockdown experiments. Target protein levels in controls were arbitrary set at 1 against which statistical analysis was compared between treatment and control groups. No statistical significance differences were found.

Figure S5. Changes in the distribution of occludin at the BTB in the seminiferous epithelium of stage V-VI tubules after knockdown of different Scribble complex components in adult rat testes *in vivo*. Expression of Scribble (green) in the seminiferous epithelium from rats of the Scribble single-knockdown (but not Lgl2 single-knockdown) and SLD triple-knockdown groups was found to be reduced *vs.* the non-targeting control group in stage V-VI tubules. However, expression of occludin (red) at the BTB near the basement membrane of the seminiferous epithelium remained relatively unaltered among all groups. Nuclei were stained with DAPI (blue). Scale bar = 80 μm, which applies to all remaining micrographs.

Reference

Su, L., Cheng, C. Y. and Mruk, D. D. (2010) Adjudin-mediated Sertoli-germ cell junction disassembly affects Sertoli cell barrier function *in vitro* and *in vivo*. *Int J Biochem Cell Biol* **42**: 1864-1875.

Figure S1 (Su et al.)

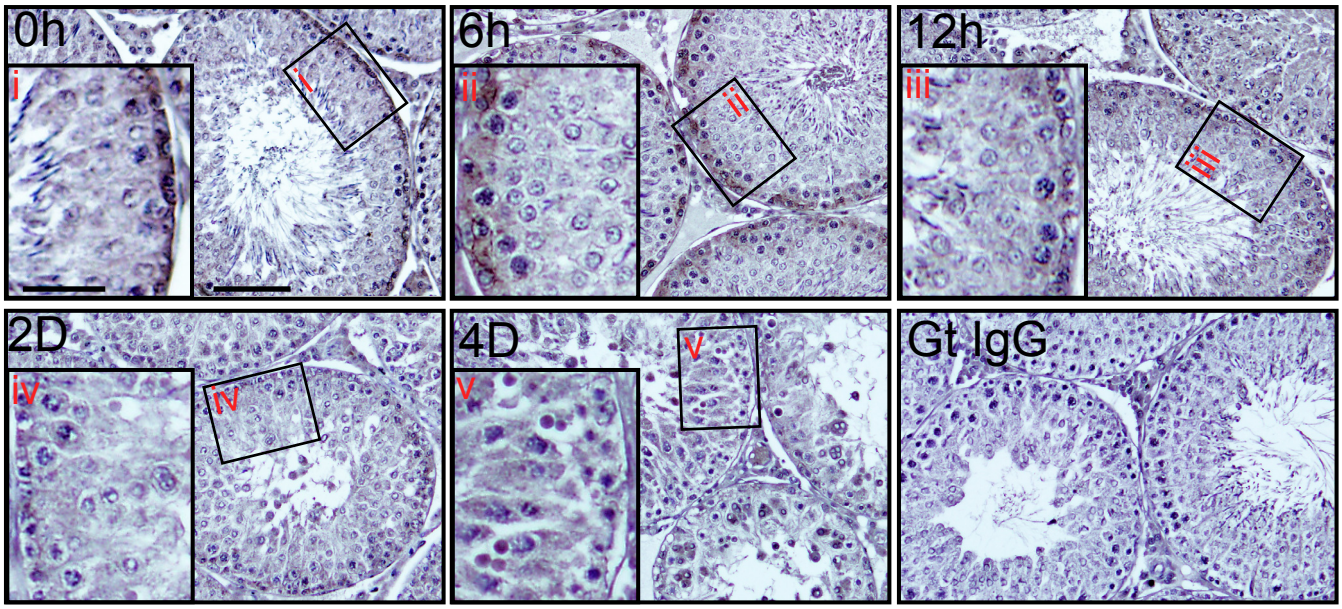


Figure S2 (Su et al.)

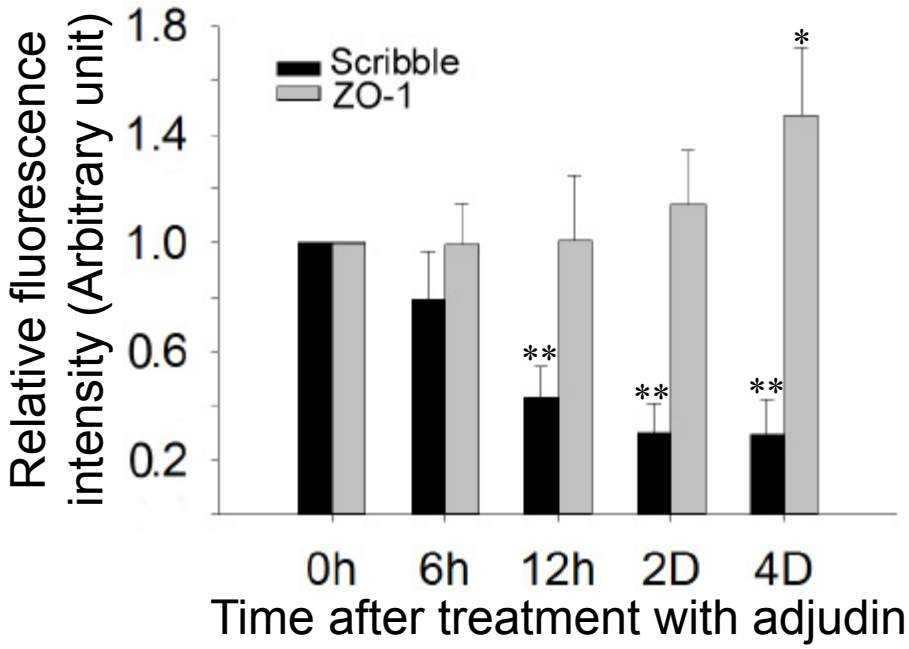
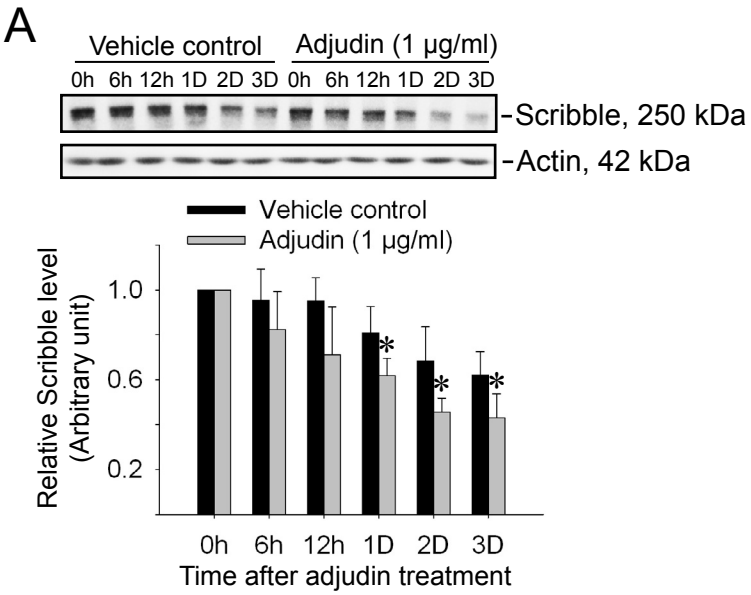


Figure S3 (Su et al.)



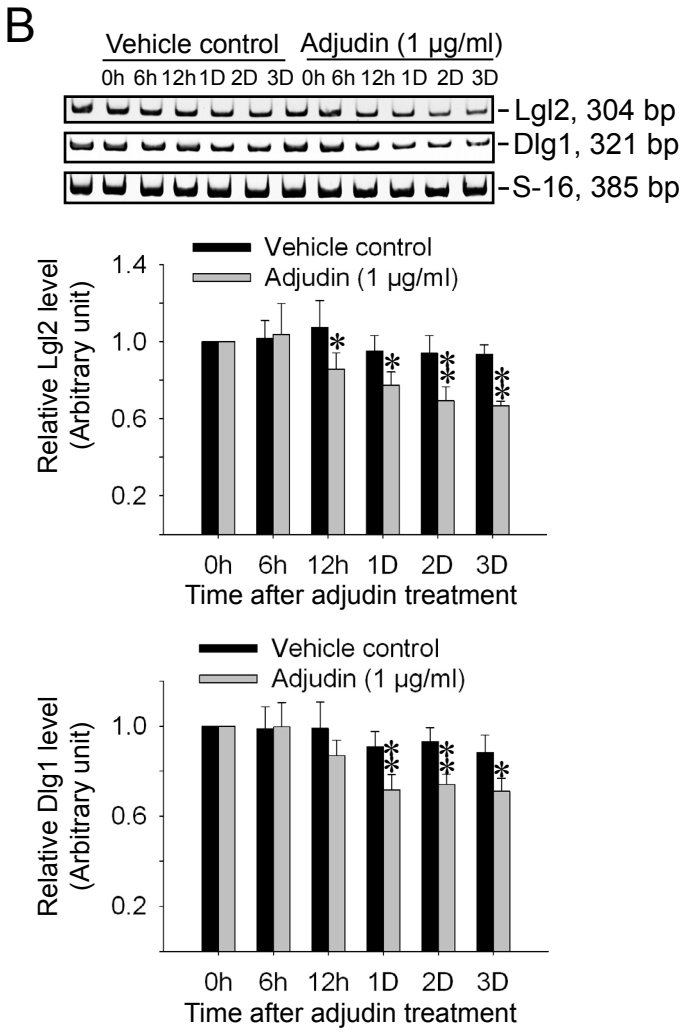


Figure S4 (Su et al.)

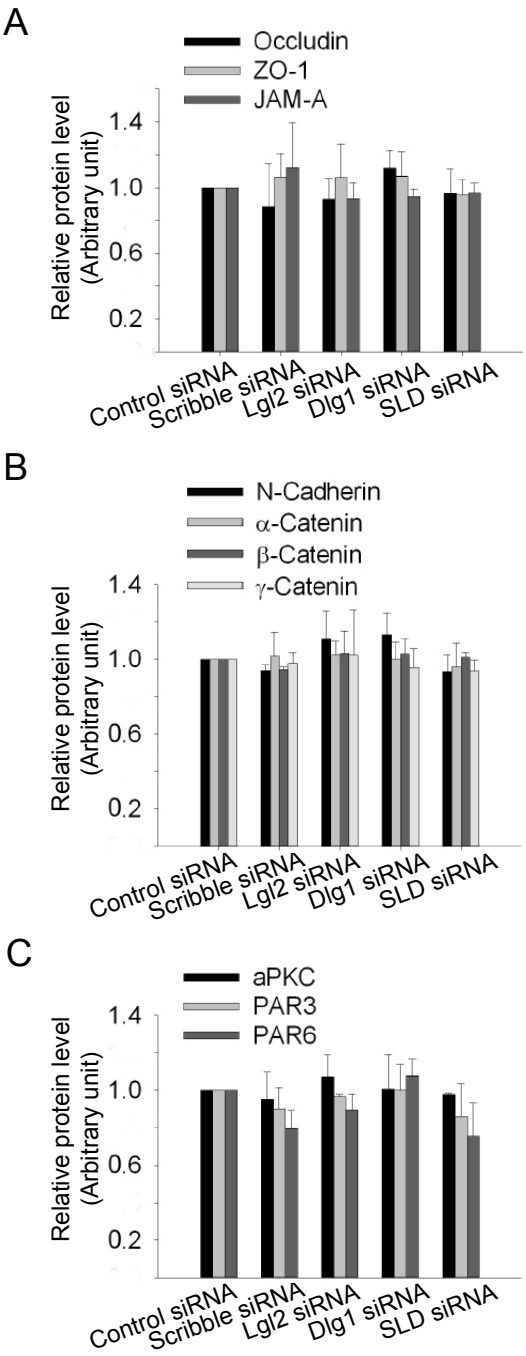


Figure S5 (Su et al.)

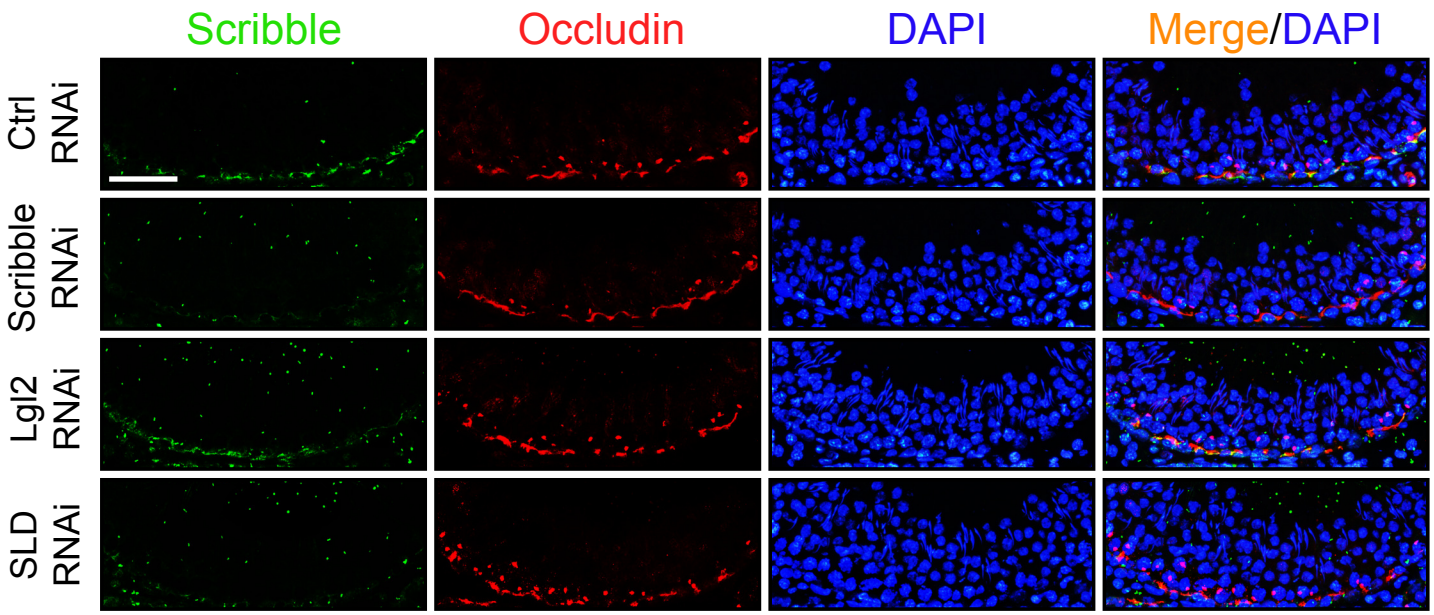


Table S1. Antibodies used for different experiments in this report*

Antibody	Host species	Vendor	Catalog Number	Working		Conjugation
				IB	IF	
Scribble	Goat	Santa Cruz Biotechnology	sc-11048	1:500	1:50	-
aPKC	Rabbit	Santa Cruz Biotechnology	sc-216	1:200		-
Par3	Rabbit	Upstate Biotechnology	07-330	1:500		-
Par6	Rabbit	Abcam	ab45394	1:1,000		-
Occludin	Rabbit	Zymed/Invitrogen	71-1500	1:250	1:50	-
ZO-1	Rabbit	Zymed/Invitrogen	61-7300	1:250	1:50	-
JAM-A	Rabbit	Zymed/Invitrogen	36-1700	1:300		-
N-Cadherin	Rabbit	Santa Cruz Biotechnology	sc-7939	1:300		-
α-Catenin	Rabbit	Santa Cruz Biotechnology	sc-7894	1:250		-
β-Catenin	Rabbit	Santa Cruz Biotechnology	sc-7199	1:250	1:50	-
γ-Catenin	Mouse	BD Transduction Laboratories	610254	1:1,000		-
Erk1/2	Rabbit	Cell Signaling	4695	1:1,000		-
pErk1/2	Rabbit	Cell Signaling	4370	1:1,000		-
Actin	Goat	Santa Cruz Biotechnology	sc-1616	1:250		-
Laminin γ3	Rabbit	Cheng Lab (1).			1:50	-
Rabbit IgG	Bovine	Santa Cruz Biotechnology	sc-2370	1:3000		HRP
Goat IgG	Bovine	Santa Cruz Biotechnology	sc-2350	1:3000		HRP
Mouse IgG	Bovine	Santa Cruz Biotechnology	sc-2371	1:3000		HRP
Goat IgG	Donkey	Invitrogen	A11055		1:100	Alexa Fluor 488
Rabbit IgG	Donkey	Invitrogen	A21206		1:100	Alexa Fluor 488
Rabbit IgG	Donkey	Invitrogen	A31572		1:100	Alexa Fluor 555

* IB, immunoblotting; IF, immunofluorescence microscopy. It is noted that all the antibodies cross-reacted with the corresponding proteins in rat as indicated by the manufacturer and tested in our laboratory in preliminary experiments. It is noted that for the anti-laminin γ3 antibody (IgG purified by sequential ammonium sulfate precipitation and DEAE anion-exchange chromatography), it was raised in a rabbit using purified recombinant rat laminin γ3 fragment (Yan and Cheng, 2006).

Reference

1. Yan, H. H. N. and Cheng, C. Y. (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.

Table S2. Primers used for RT-PCR and qPCR experiments in this report

Gene	GenBank accession number	Primer orientation	Primer sequence (5'-3')	Nucleotides position	Expected size (b.p.)
Scribble	XM_002726943.1	sense	5'- CTGGCACTGCTCACAGATCT -3'	724-743	257 bp
		antisense	5'- AGCACCTCAAGATGATTCCG -3'	961-980	
Lgl2	NM_001127549.1	sense	5'- TCCACCATCTCGAACACTCG -3'	442-461	304 bp
		antisense	5'- TGCTGGATGACAACAGCCTG -3'	726-745	
Dlg1	NM_012788.1	sense	5'- GTTGACCTCAGAGCTGCAAG -3'	2000-2019	321 bp
		antisense	5'- CCACCACTCGTCATCAGAAG -3'	2301-2320	
S-16	NM_001169146.1	sense	5'-TCCGCTGCAGTCCGTTCAAGTCTT-3'	87-110	385 bp
		antisense	5'-GCCAAACTTCTTGGATTCGCAGCG-3'	448-471	