

1 **Supplementary Material**

2 **Supplementary Figure legends**

3 **Supplementary Figure 1** Changes of junction proteins distribution in rat testis after Aroclor1254
4 administration. Testis cryosections from different groups of rats were used for
5 immunofluorescence analysis. The localization of BTB proteins were shown by Alexa Fluor
6 555-conjugated secondary antibody (*red*) with cell nuclei stained with DAPI (*blue*). An attenuated
7 signal of occludin (denoted by the *white arrows*) and “broader” signals of JAM-A and N-cadherin
8 (denoted by the *white brackets*) were observed in the Aroclor1254 group as compared with the
9 vehicle control, which were not shown in the Aroclor1254+SB203580 group. Scale bar=40 μ m,
10 which applied to all micrographs in Figure S1.

11

12 **Supplementary Figure 2** **Aroclor1254 did not alter Ser473 and Thr308 phosphorylation of PKB**
13 **in cultured SCs.** (a) Immunoblot analysis to assess the levels of PKB phosphorylation in SC lysates
14 prepared at different time points after Aroclor1254 treatment. (b) Histograms summarizing the
15 result shown in (a) after the level of phosphorylated PKB (p-PKB) was normalized to total PKB
16 expression. Protein level of the vehicle control group at time 0 was arbitrarily set at 1. Each bar
17 refers to mean \pm SD of n=3 experiments using different batches of SC lysates.

18

19 **Supplementary Figure 3** **Aroclor1254 did not alter the endocytosis and mRNA level of**
20 **occludin in cultured SCs.** (a) Immunoblot analysis of endocytosed occludin in SCs at different
21 time points after cell surface biotinylation in the presence of Aroclor1254 (or the mixture of
22 Aroclor1254 and SB203580). The endocytosed proteins were pulled-down by UltraLink

23 Immobilized NeutrAvidin Plus Resin. Total biotinylated proteins at 0 min without stripping were
24 detected as a positive control. Cell lysates without pull-down were also analyzed by immunoblot
25 to show the steady-state level of occludin with GAPDH served as the loading control. (b) Line and
26 scatter graphs summarizing the result shown in (a) by calculating the percentage of endocytosed
27 protein versus the total biotinylated protein, which was then normalized with the steady-state
28 level of occludin at each time point. (c) qPCR analysis of occludin mRNA level in SCs after
29 Aroclor1254 treatment for 48 h. Each bar refers to mean \pm SD of n=3 independent experiments
30 using SCs cultured from different rats.

31

32 **Supplementary Figure 4** The effects of Aroclor1254 on BTB protein levels in the presence of
33 cholesterol oxidase. (a) A schematic drawing illustrating the treatment regimen for both FigureS2
34 and Figure5. CO was added to the SC culture medium at 2 U/ml from day 4. 24 hours later,
35 randomly selected cultures were treated with 10 μ g/ml Aroclor1254 for another 48 h. (b)
36 Immunoblot analysis to assess the BTB protein levels in SC lysates on day 7 after treatment of
37 Aroclor1254 or CO+Aroclor1254, with GAPDH serving as a protein loading control. (c) Histograms
38 illustrating the result shown in (b) after each data point was normalized against GAPDH except
39 that the level of phosphorylated p38 MAPK (p-p38) was normalized to total p38 MAPK expression.
40 The protein level in the vehicle control group was arbitrarily set at 1. **, $P<0.01$, compared with
41 the vehicle control group. $\diamond\diamond$, $P<0.01$, compared with the CO-treated cells.

42

43 **Supplementary Figure 5** The effects of Aroclor1254 on BTB protein levels after Itch-silencing by
44 siRNA. (a) A schematic drawing illustrating the treatment regimen for both FigureS3 and Figure6.

45 The siRNA targeting at rat Itch or the non-targeting control siRNA was transfected into SCs on
46 culture day 3 for 24 hours. After another 24-hour interval, the SCs were treated with Aroclor1254
47 from day 5 for 48 h. **(b)** Immunoblot analysis to detect the BTB protein levels in SC lysates
48 harvested on day 7 after treatment, with GAPDH serving as a protein loading control. The
49 knock-down efficiency of Itch siRNA was found to be approximately 80%. **(c)** Histograms
50 illustrating the result shown in (b) after each data point was normalized against GAPDH except
51 that the level of phosphorylated p38 MAPK (p-p38) was normalized to total p38 MAPK expression.
52 The protein level in the control siRNA transfected SCs was arbitrarily set at 1. No significant
53 difference in occludin level was detected after Aroclor1254 treatment in Itch-silencing cells. **,
54 $P < 0.01$, compared with the control siRNA group. \diamond , $P < 0.01$, compared with the Itch siRNA group.
55 *N.S.*, no significant difference between two indicated groups.