Supplementary Information to

β-catenin is a molecular switch that regulates transition of cell-cell adhesion to fusion

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- Supplementary Figures S1 – S7

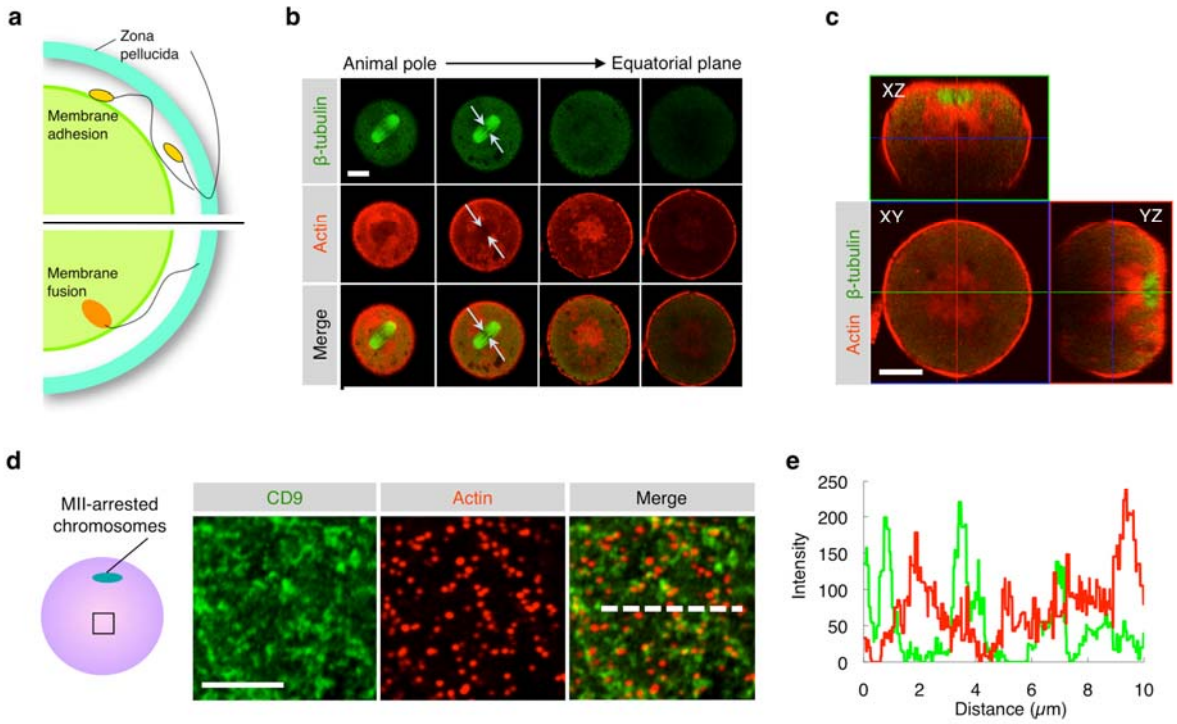

Figure S1. Possible involvement of actin microfilaments in fertilization.

a, Schematic model of membrane adhesion and fusion between sperm and oocytes at fertilization. **b**, Localization of β- and γ-actin isoforms and β-tubulin in superovulated C57BL/6N oocytes. Images of serial sections were captured from a region containing metaphase II-arrested (MII) chromosomes (indicated by arrows) to the inner portion of the oocyte, and among them, selected images are shown. Scale bar: 20 μm. **c**, Two-dimensional images (shown as XY, XZ and YZ axes) after reconstruction of the three-dimensional (3D) images in **b.** Scale bar: 20 μm. **d**, Diverse localization of β- and γ-actin isoforms and CD9 on the oocyte cell membrane. The membrane region captured is indicated in a box on the left. Scale bar: 5 μm. **e**, The fluorescent intensity of β- and γ-actin isoforms and CD9 on the oocyte cell membrane after scanning along the dotted line shown as 'Merge' in **d.** Red and green lines indicate fluorescence intensities for actin and CD9, respectively.

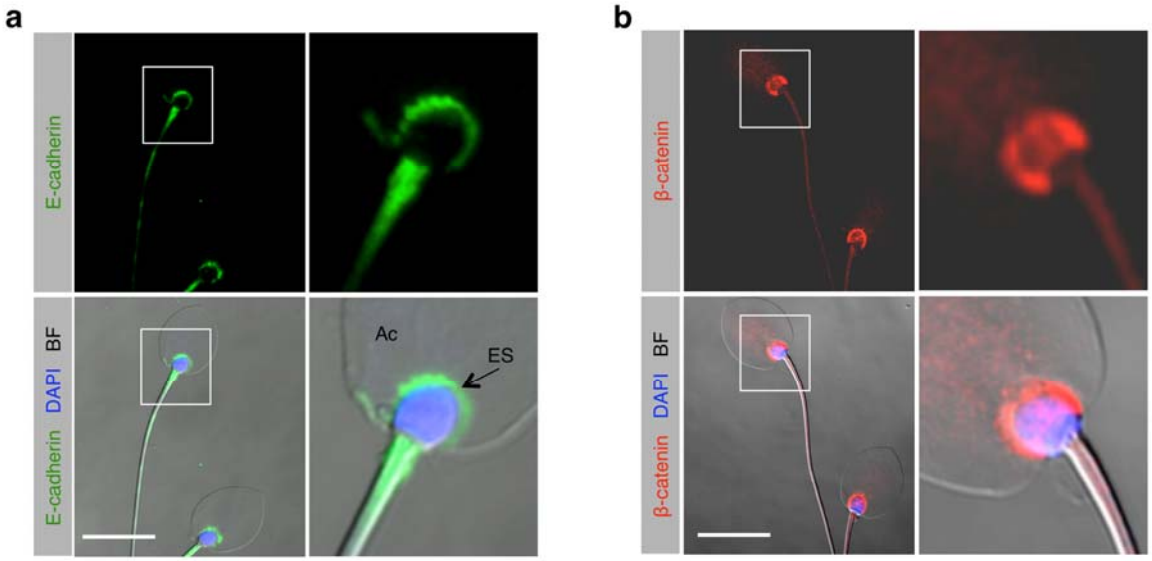

Figure S2. Localization of E-cadherin and β-catenin in epididymal sperm of an insectivore, *Suncus murinus*.

a, Localization of E-cadherin in *Suncus* sperm (permeabilized). Ac, acrosome; ES, equatorial segment. **b**, Localization of β-catenin in *Suncus* sperm. In each panel, boxes in left sets of panels were enlarged and shown on the right. Scale bar: 5 μm.

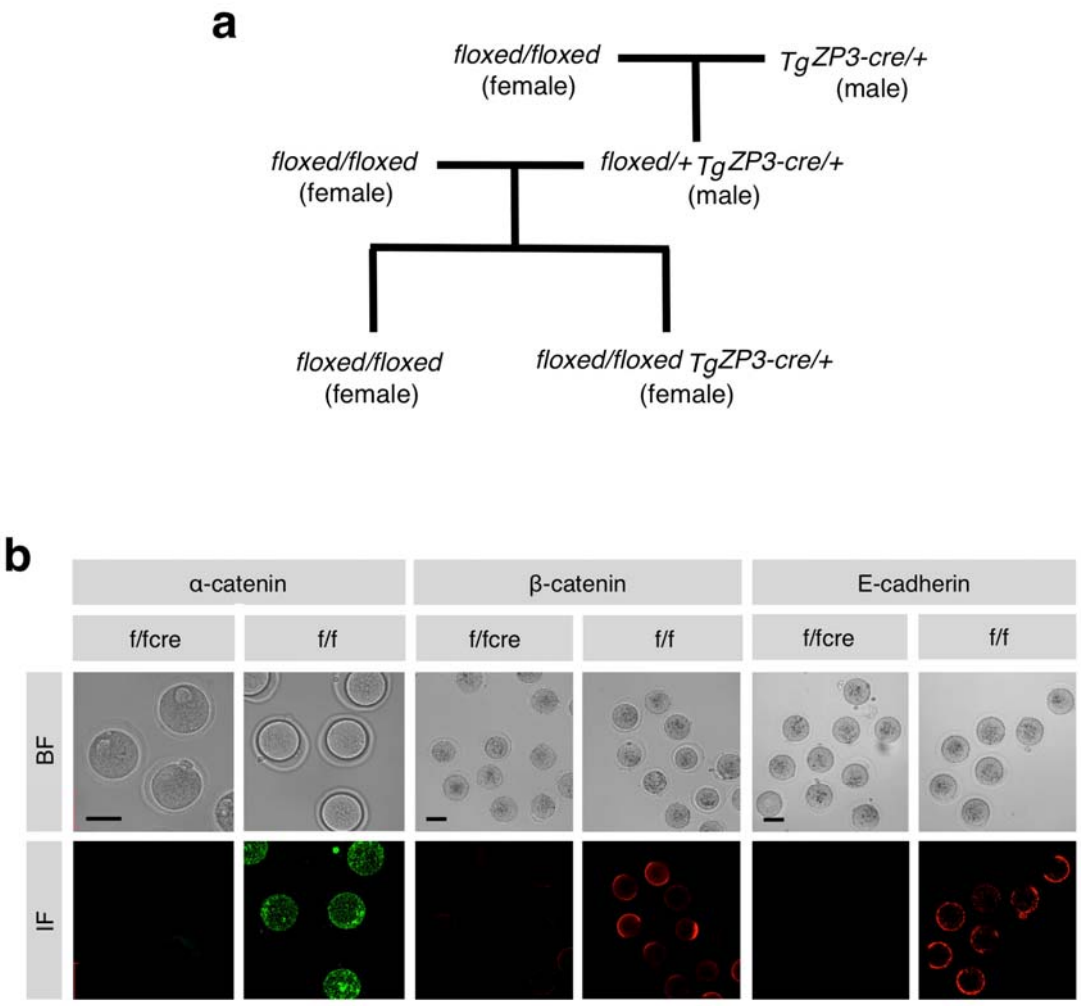

Figure S3. Generation of oocytes lacking expression of β-catenin, α-catenin or E-cadherin.

a, Procedure for generation of female mice with oocytes lacking expression of each type of gene. Three strains of floxed mice (*E-cadherin*$^{floxed/floxed}$, *β-catenin*$^{floxed/floxed}$, and α-*catenin*$^{floxed/floxed}$) were intercrossed with mice carrying a transgene expressing cre-recombinase (*Tg*$^{ZP3\text{-}cre/+}$) in oocytes (see Experimental Procedures). **b**, Expression of β-catenin, α-catenin or E-cadherin in oocytes from offspring of floxed mice mated to cre-recombinase-expressing Tg mice. Ovulated oocytes were immunostained with each mAb. Note that oocytes (f/fcre) lacking each of these genes exhibit no target protein expression. BF, bright field; IF, Immunofluorescence. Scale bars: 50 μm.

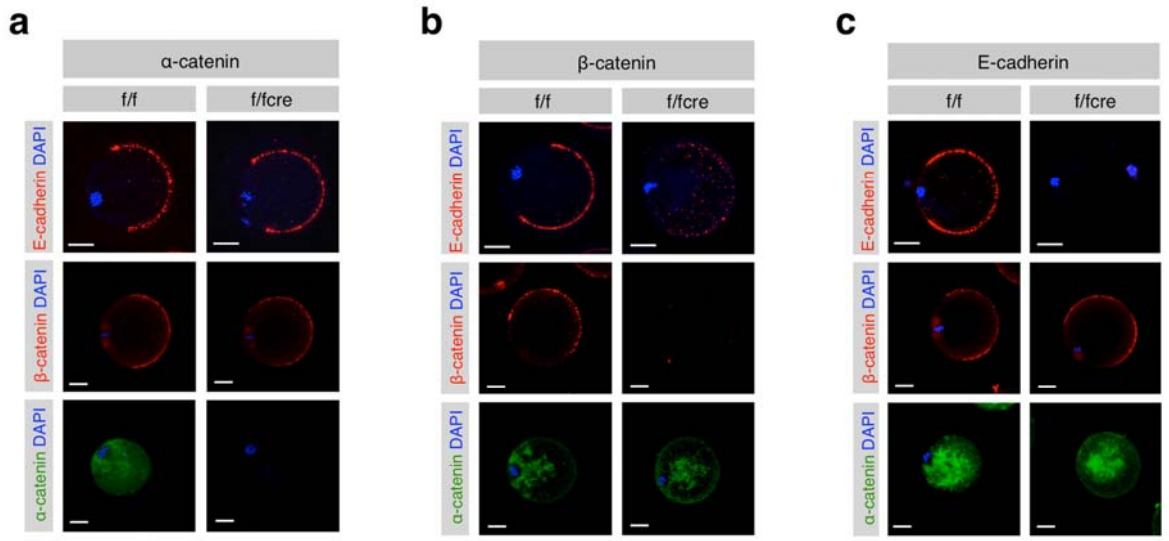

Figure S4. Close interaction between β-catenin and E-cadherin on the oocyte cell membrane.

Ovulated oocytes lacking each of *α-catenin* (a), *β-catenin* (b) and *E-cadherin* (c) genes were immunostained with mAbs against these three proteins. Mutual expression patterns in oocytes (f/fcre) lacking each of *α-catenin*, *β-catenin* and *E-cadherin* genes were compared with those in oocytes (f/f) of control floxed mice. Scale bars: 20 μm.

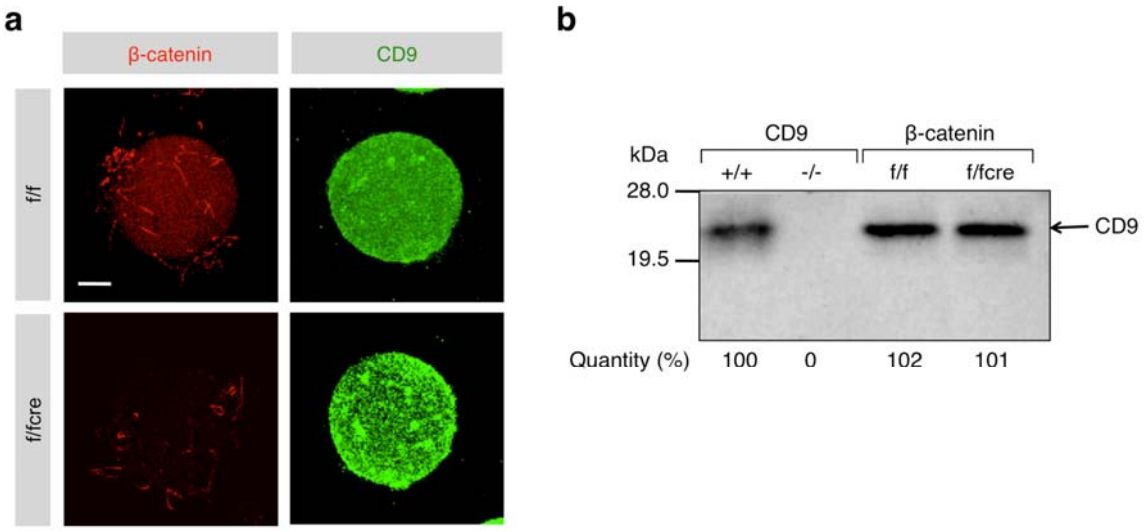

Figure S5. Expression of CD9 in β-catenin-deficient oocytes.

a, Immunocytochemical localization of CD9 and β-catenin in the *β-catenin*$^{floxed/floxed}$*Tg*$^{ZP3\text{-}cre/+}$ (f/fcre) and *β-catenin*$^{floxed/floxed}$ (f/f) oocytes with adhered sperm. Note that β-catenin expression is lacking in f/fcre oocytes, but present in the adhered normal sperm. Furthermore, CD9 expression is still seen in f/fcre oocytes. Scale bar: 20 μm. **b**, Quantitative analyses of CD9 in wild-type (+/+), CD9 KO (-/-), *β-catenin*$^{floxed/floxed}$ (f/f) and *β-catenin*$^{floxed/floxed}$*Tg*$^{ZP3\text{-}cre/+}$ (f/fcre) ovulated oocytes. Cell extracts of 10 oocytes were applied in each lane. The amounts of CD9 were densitometrically measured, normalized by the levels of CD9 expressed in +/+ oocytes, and shown as Quantity (%) below the figure.

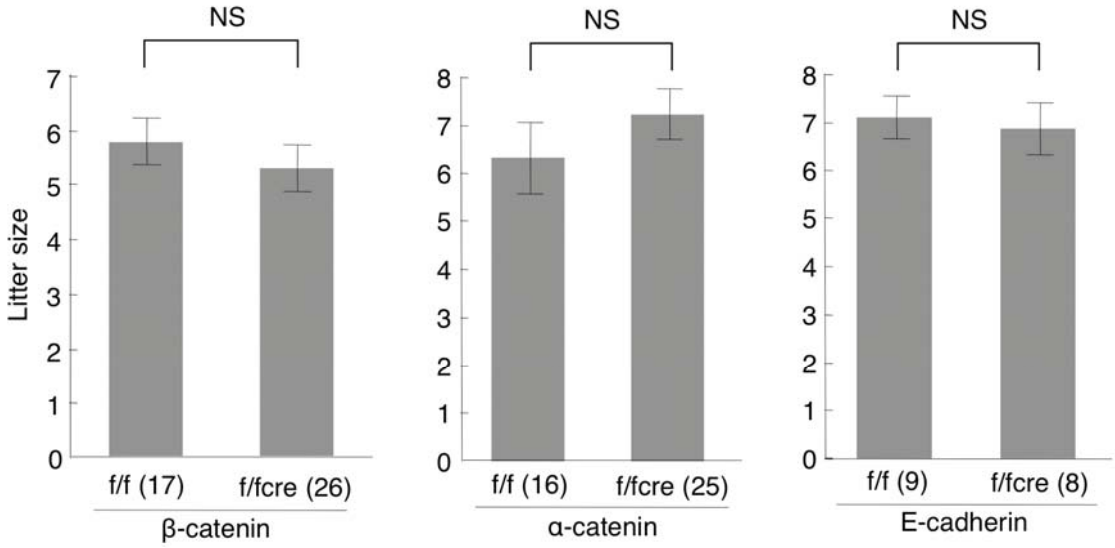

Figure S6. Litter sizes of female mice lacking each of *β-catenin*, *α-catenin*, and *E-cadherin* genes in oocytes.

Each of these female mice was mated with C57BL/6N males, and their mean litter sizes were determined (see Methods). Parentheses indicate the total number of female mice examined in each group. NS, not significant. Values are the mean ± SE.

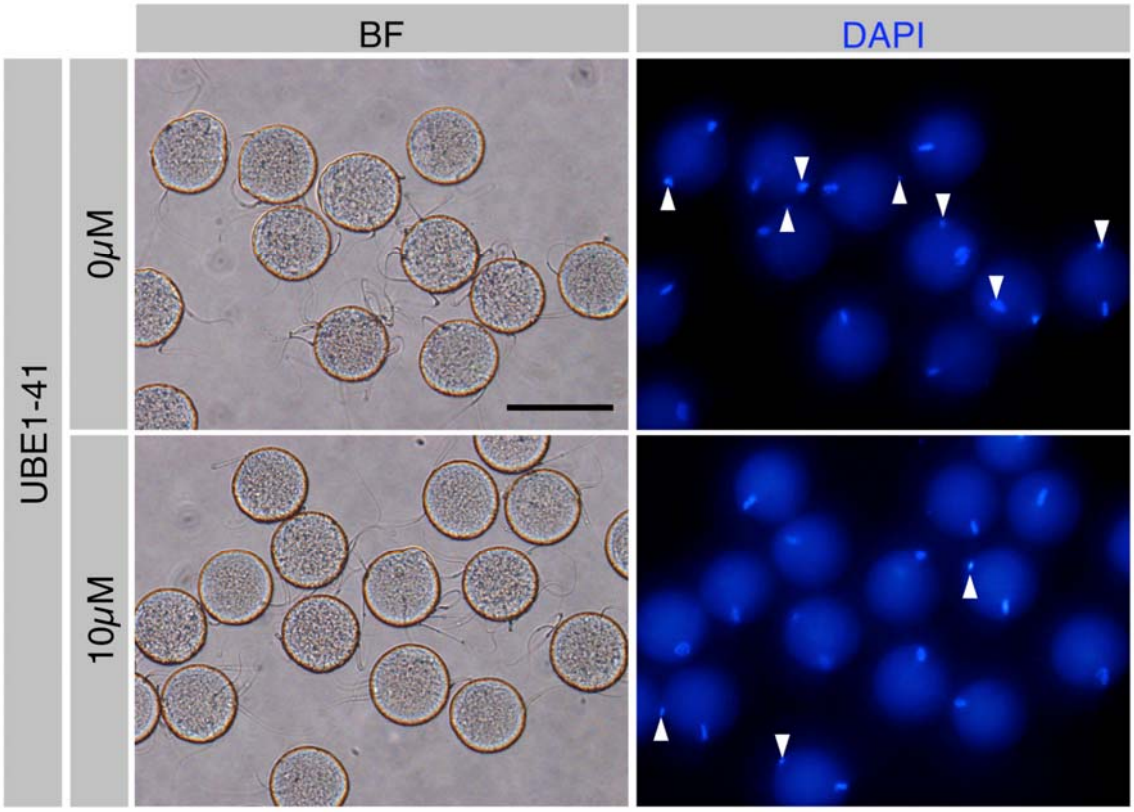

Figure S7. Reduced fusing ability of "zona-free" oocytes treated with UBE1-41.

After treatment of oocytes in the presence or absence of UBE1-41, oocytes were subjected to ZP removal, stained with DAPI, and then incubated with C57BL/6N sperm for 30 min. Note that in UBE1-41-treated oocytes, the number of DAPI-positive sperm (shown by arrowheads) is decreased. BF, bright field. Scale bar: 100 μm.