Supplemental Material

The Mature COC Promotes the Ampullary NPPC Required for Sperm Release from Porcine Oviduct Cells

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Supplementary Figures and Figure Legends

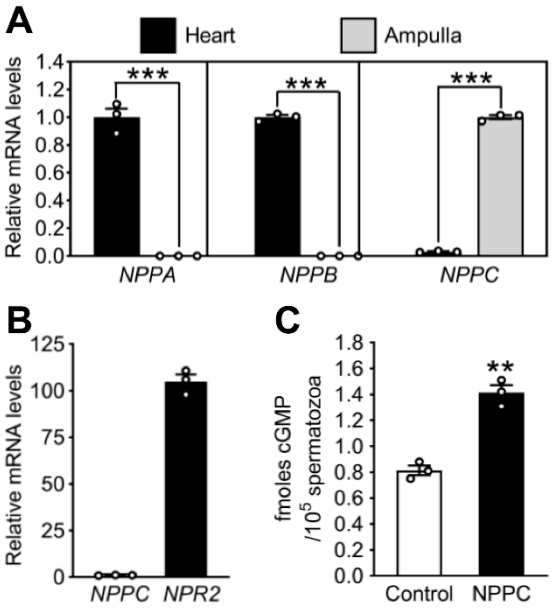


Figure S1. **NPPC expression and the cGMP levels in porcine spermatozoa**. (**A**) The mRNA levels of *NPPA*, *NPPB* and *NPPC* in the ampulla and heart (n = 3 independent experiments). Heart is the positive tissue for NPPA, NPPB and NPPC. (**B**) The mRNA levels of *NPPC* and *NPR2* in fresh porcine spermatozoa (n = 3 independent experiments). (**C**) Capacitated spermatozoa were incubated without or with 1 nM NPPC for 20 min, and the amounts of cGMP were measured using enzyme immunoassay kits. Bars indicate the mean ± SEM. n = 3 independent experiments. Statistical analysis was performed by two−tailed unpaired Student's *t*−test. ***$P < 0.001$ and **$P < 0.01$. Each data point represents a biologically independent experiment.

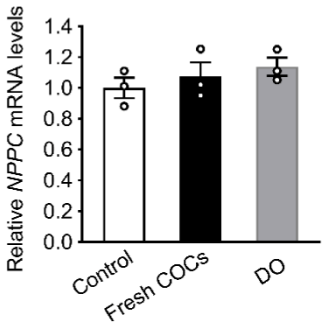


Figure S2. **The effect of fresh COCs or denuded oocytes on *NPPC* mRNA levels in the ampullary epithelial cells**. The ampullae isolated from oviducts at follicular phase were co−cultured with fresh COCs (immature COCs. 150 COCs/200 μL) or denuded oocytes (Dos, 150 oocytes/200 μL) for 3 h. At the end of the culture, ampullary epithelial cells were scraped using the edge of a microscope slide and used for *NPPC* mRNA measurement. Bars indicate the mean ± SEM. n = 3 independent experiments. Each data point represents a biologically independent experiment.

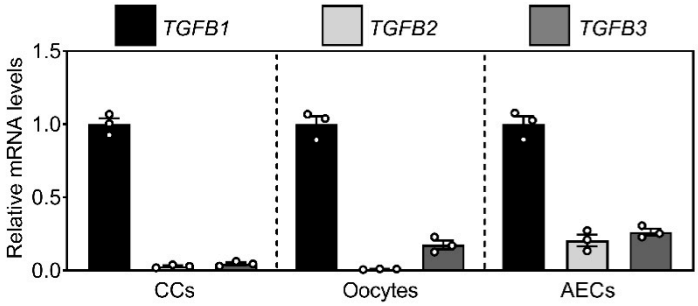


Figure S3. **Comparison of steady−state levels of *TGFB1*, *TGFB2*, and *TGFB3* mRNA in cumulus cells, oocytes, and ampullary epithelial cells**. Bars indicate the mean ± SEM. n = 3 independent experiments. Each data point represents a biologically independent experiment.

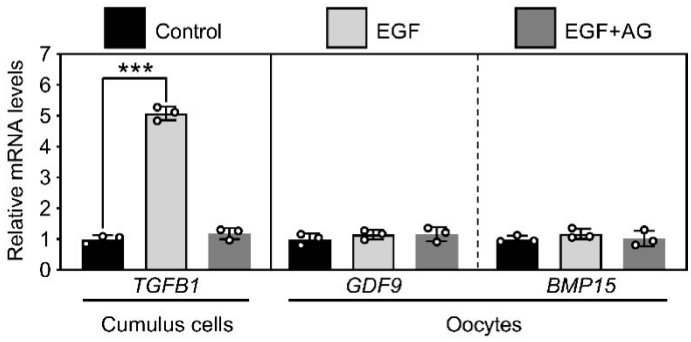


Figure S4. **The effect of EGF on the mRNA levels of *TGFB1*, *GDF9* and *BMP15* in COCs**. Immature COCs were cultured with EGF (10 ng/mL) and/or AG1478 (1 μM). n = 3 independent experiments. Statistical analysis was performed by two−tailed unpaired Student's *t*−test. ***$P < 0.001$. Each data point represents a biologically independent experiment. AG, AG1478, EGFR inhibitor.

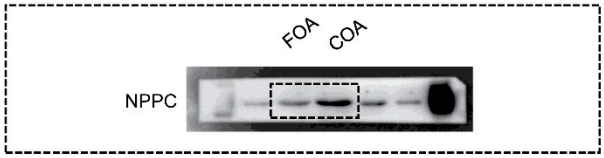


Fig. 1A

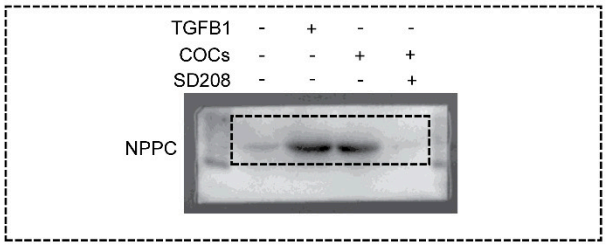


Fig. 5B

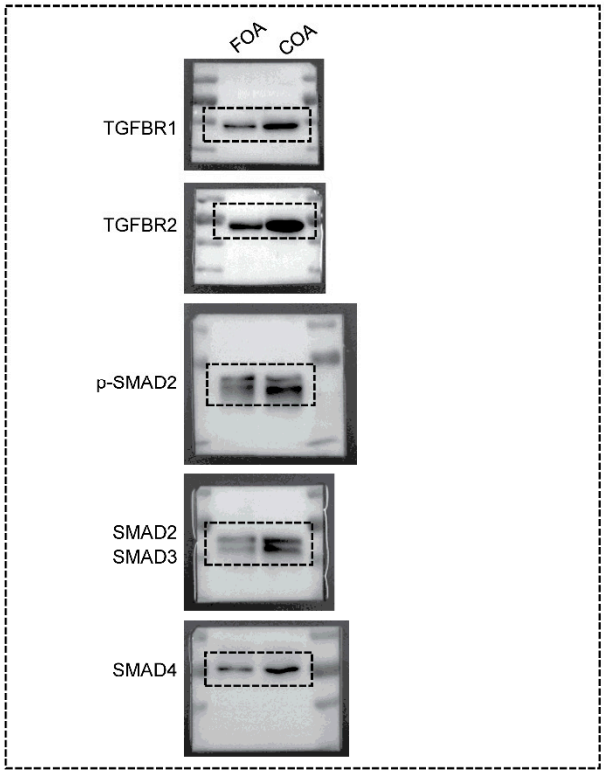


Fig. 5F

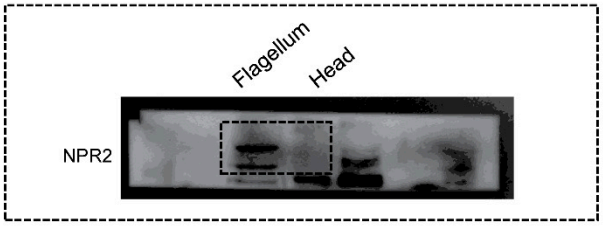


Fig. 2B

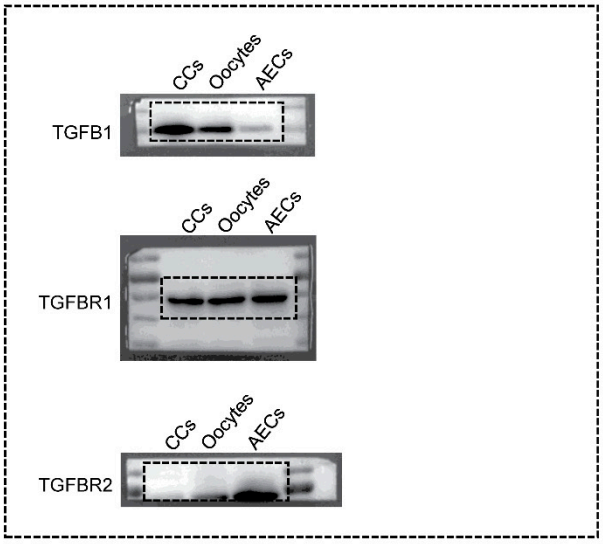


Fig. 5D

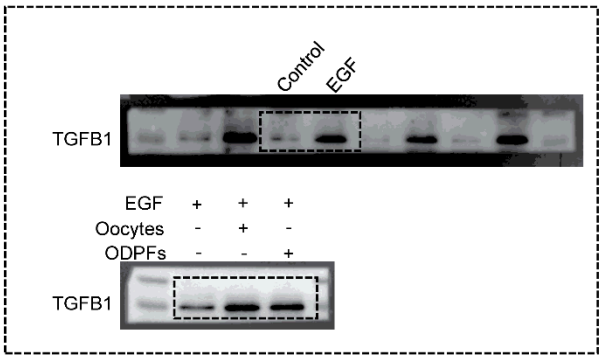


Fig. 6C

Figure S5. Uncropped scans of the Western blotting results.

Tables

Table S1. **The effect of NPPC on sperm motility**.

	Control	NPPC (0.01 nM)	NPPC (0.1 nM)	NPPC (1 nM)	NPPC (10 nM)
Motility%	60.1 ± 3.9	63.1 ± 3.7	66.11 ± 4.8	77 ± 3.1*	61.1 ± 4.3
VAP (μm/s)	72.66 ± 7.9	73.3 ± 7.6	75.9 ± 8.2	77.4 ± 12.0	73.6 ± 5.9
VSL (μm/s)	45.1 ± 6.5	45.9 ± 2.6	46.2 ± 3.7	47.2 ± 6.9	46.9 ± 3.9
VCL (μm/s)	152.4 ± 8.8	158.1 ± 6.3	157.6 ± 4.9	161.5 ± 10.2	155.3 ± 8.3
ALH	8.5 ± 0.8	9.0 ± 0.7	8.9 ± 0.6	9.0 ± 0.6	8.6 ± 0.9
BCF	36.9 ± 1.0	38.7 ± 2.1	37.2 ± 2.8	36.5 ± 1.5	37.4 ± 1.5
Straightness%	59.5 ± 4.7	60.5 ± 5.8	59.2 ± 4.8	58.1 ± 7.1	61.4 ± 6.7
Linearity%	28.5 ± 2.5	28.6 ± 3.5	28.4 ± 2.3	28.9 ± 3.1	28.8 ± 2.6
Elongation%	39.4 ± 1.9	38.4 ± 2.9	41.3 ± 5.9	39.0 ± 4.5	39.7 ± 4.1
Area μm/sq	17.3 ± 2.1	18.2 ± 3.2	17.5 ± 3.6	17.2 ± 2.8	18.0 ± 1.9

The motility parameters of spermatozoa were detected after incubation with different concentrations of NPPC. VAP, average path velocity; VSL, straight-line velocity; VCL, curvilinear velocity; ALH, amplitude of lateral head displacement; BCF, beat cross frequency. Bars indicate the mean ± SEM of three experiments. n = 500 for each sample. *$P < 0.05$.

Table S2. **Sperm motility parameters were determined after incubation with NPPC, 8−Br−cGMP, *l−cis*−D and NNC 55−0396**.

Treatments	Control	NPPC (1 nM)	8−Br−cGMP	*l−cis*−D	NNC 55−0396
Motility%	60.1 ± 2.6	76.9 ± 4.1*	75 ± 5.7*	61.0 ± 4.1	76.0 ± 3.6*
VAP (μm/s)	72.66 ± 7.9	77.1 ± 12.0	75.7 ± 11.7	69.0 ± 6.2	75.5 ± 12.3
VSL (μm/s)	45.1 ± 6.5	47.9 ± 6.9	46.8 ± 8.8	46.8 ± 7.6	46.7 ± 4.3
VCL (μm/s)	152.4 ± 8.8	161.7 ± 10.2	158.7 ± 11.5	154.8 ± 12.5	159.3 ± 18.1
ALH	8.5 ± 0.8	8.9 ± 0.7	8.7 ± 1.0	8.1 ± 0.8	8.8 ± 0.8
BCF	36.9 ± 1.0	36.6 ± 1.7	35.4 ± 1.9	35.1 ± 1.4	35.7 ± 1.3
Straightness%	58.5 ± 4.7	58.0 ± 7.2	56.4 ± 5.2	56.5 ± 3.2	57.0 ± 4.8
Linearity%	28.5 ± 2.5	28.0 ± 3.5	27.2 ± 5.5	27.1 ± 5.1	27.5 ± 4.5
Elongation%	39.4 ± 1.9	39.0 ± 4.7	37.8 ± 2.4	38.4 ± 2.8	38.2 ± 1.7
Area μm/sq	17.3 ± 2.1	17.0 ± 2.9	16.7 ± 4.9	16.4 ± 4.5	16.9 ± 3.9

Motility parameters of spermatozoa were detected after different treatment. The capacitated spermatozoa were incubated in dmTALP medium, supplement with different concentrations of NPPC (0.01-10 nM), NNC 55−0396 (NNC, 2 μM), 8−Br−cGMP (1 mM), and/or *l−cis*−Diltiazem (*l−cis*−D, 50 μM) for 30 min. VAP, average path velocity; VSL, straight-line velocity; VCL, curvilinear velocity; ALH, amplitude of lateral head displacement; BCF, beat cross frequency. Bars indicate the mean ± SEM of three experiments. n = 500 for each sample. *$P < 0.05$.

Table S3. **Information about the primary antibodies used in immune detection**.

Antibody	Catalog Code	Source	Host	Dilution	
				IHC/IF	WB
NPPC	Sc374043	Santa Cruz Biotechnology	Rabbit	1:50	1:1000
p−SMAD3	9520	Cell Signaling Technology	Rabbit		1:1000
p−SMAD2/3	8828	Cell Signaling Technology	Rabbit		1:1000
SMAD2/3	8685	Cell Signaling Technology	Rabbit	1:100	1:1000
SMAD4	46535	Cell Signaling Technology	Rabbit	1:100	1:1000
TGFB1	ab92486	Abcam	Rabbit	1:200	1:1000
TGFBR1	ab31013	Abcam	Rabbit	1:200	1:1000
TGFBR2	ab186838	Abcam	Rabbit	1:200	1:1000
GAPDH	5174	Cell Signaling Technology	Rabbit		1:1000

IHC, Immunohistochemistry; IF, Immunofluorescence; WB, Western blotting.

Table S4. **Primer sequences**.

Gene	Primer
NPPA	F: ACCTGATGGATTTCAAGAATTTGCT
	R: GGGGGCATAGCCTCATCTTC
NPPB	F: CAAGTCCTCCGGGGAATACG
	R: ACCTCCTGAGCACATTGCAG
NPPC	F: AGGCAACAAGAAGGGTTTGTC
	R: ACTAACATCCCAGGCCGCT
NPR2	F: CACCCACTTCATCAAGGCCAA
	R: GATTCGGGGGTTCTCGGTAG
TGFB1	F: AGCCAGAGGCGGACTACTAC
	R: AGGAATCATTGCTGTATTTCTGGT
TGFB2	F: TCCCCTCGGAAAATGCCATC
	R: TCGCCTTCTGCTCTCGTTTT
TGFB3	F: CTGTGCGTGAATGGCTCTTG
	R: CCTCACTGTCCACACCTTTGA
TGFBR1	F: GAGGCGACGGCATTCCAG
	R: AGGCCAGATGGTGGCTTTC
TGFBR2	F: AACAGTGGGCAGGTGGGAAC
	R: TTGGAACCAAAGGGTGGCTC
SMAD2	F: GTATGGACACAGGATCGCCA
	R: TTGGACGGATCTGTGAAGCC
SMAD3	F: ACTACCAGAGGGTGGAGACC
	R: AGAGGTTTGGAGAACCTGCG
SMAD4	F: TGTGGCTTCCACAAGTCAGC
	R: AGGCTGGAATGCAAGCTCAT
GAPDH	F: AGCAATGCCTCCTGCACCACCA
	R: TGAGTCCCTCCACGATGCCGAA
BMP15	F: AGGCCATTGGTTAATGGAGCA
	F: GTGACATCCATCTCCGTCCA
GDF9	F: CCTCTACAACACTGTCCGGC
	F: CACGATCCAGGTTAAACCGC

F, forward primer (5’-3’); R, reverse primer (5’-3’).