Supplementary Figures



Supplementary Figure 1. 5'- and 3'-RACE analyses for porcine lincRNA-NORFA. Gel images showing the nested PCR amplified products obtained from 5'-RACE (**a**) and 3'-RACE (**b**) analyses. Black arrows indicate the products at 584 bp and 692 bp in length, respectively. DNA molecular weight markers are also represented.





Supplementary Figure 2. Identification of the conservation of pig NORFA structure. (a) Diagram depicting the investigation strategy for pig NORFA conservation. (b) The secondary structure of pig NORFA was analyzed by RNAfold and RNAstructure software. H: helice, J: junction. (c) The conservation of NORFA secondary structure, diagram showing the conservation of H21 of pig NORFA by UCSC database among different species. (d) The tertiary structure and domains of pig NORFA were predicted and the conservation of these domains were detected using UCSC. (e) The potential ORFs within pig NORFA (sense and anti-sense) were predicted and their conservation were detected by UCSC. (f) The conservation of pig NORFA primary structure was detected. (g) The conservation of desert region between *EDF1* and *TRAF2* (red box) among different mammal species were detected.



Supplementary Figure 3. NORFA physically interacts with miR-126 in granulosa cells. (a) The negative control for RNA FISH assays. (b) The signals of *NORFA* in porcine granulosa cells after transfection with *NORFA*-siRNA (si*NORFA*) for different time (24 h, 36 h and 48 h) were detected by FISH assays. (c) The expression levels of NORFA and miR-126 in NORFA overexpressed porcine granulosa cells were detected by qRT-PCR. (d) The enrichment of miR-126 on biotin-labeled NORFA in NORFA overexpressed porcine granulosa cells was detected by RNA pull-down. (e) The expression levels of miR-126 and NORFA in miR-126 overexpressed porcine granulosa cells were detected by qRT-PCR. (f) The enrichment of miR-126 on biotin-labeled NORFA in miR-126 on biotin-labeled NORFA in miR-126 on biotin-labeled NORFA in miR-126 and NORFA in miR-126 on biotin-labeled NORFA in miR-126 and NORFA in miR-126 on biotin-labeled NORFA in miR-126 and NORFA in miR-126 overexpressed porcine granulosa cells were detected by qRT-PCR. (f) The enrichment of miR-126 on biotin-labeled NORFA in miR-126 overexpressed porcine granulosa cells was detected by RNA pull-down. Data in c-f are shown as mean \pm S.E.M. with three independent experiments. *P*-values were calculated by a two-tailed Student's *t*-test. **,##*P* < 0.01.



Supplementary Figure 4. Multiple-sequence alignment of pre-miR-126 from different species. The multiple-sequence alignment of pre-miR-126 from 10 different species were shown and asterisks in red indicate the sequence of mature sequence of miR-126.



Supplementary Figure 5. Forced expression and silencing of miR-126 in porcine granulosa cells. (a) The expression levels of miR-126 were measured by qRT-PCR after porcine granulosa cells transfection with mimics NC (n=3) or miR-126 mimics (n=3). (b) qRT-PCR was performed and miR-126 expression levels were detected in porcine granulosa cells transfected with inhibitor NC (n=3) or miR-126 inhibitor (n=3). Data are shown as mean \pm S.E.M. by a two-tailed Student's *t*-test.



Supplementary Figure 6. NORFA affects the sensitive of porcine granuolasa cells to TGF- β 1. (a) The apoptosis rate of porcine granulosa cells treated with TGF- β 1 and NORFA-siRNA (siNORFA) or/and pcDNA3.1-NORFA (NORFA^{OE}) were measured by FACS. (b) The protein levels of TGFBR2, p-SMAD3, SMAD3 and PCNA in porcine granulosa cells treated with TGF- β 1 and siNORFA or/and NORFA^{OE} were determined by western blotting and normalized to GAPDH. GAPDH was used as a loading control. (c) The proliferation levels of porcine granulosa cells treated with TGF- β 1 and siNORFA or/and NORFA^{OE} were measured by CCK-8. Data are shown as mean ± S.E.M. with three independent experiments. *P*-values were calculated by a two-tailed Student's *t*-test. ***P* < 0.01.



Supplementary Figure 7. NORFA/miR-126/TGFBR2 axis regulates TGF- β signaling pathway in granulosa cells. (a) Construction of pcDNA3.1-NORFA-mut vector with miR-126 response element mutation. (b) The expression levels of NORFA, miR-126 and TGFBR2 in porcine granulosa cells transfected with pcDNA3.1-NORFA-mut vectors were measured by qRT-PCR. (c) The protein levels of TGFBR2 and p-SMAD3 in porcine granulosa cells treated with NORFA^{OE} or NORFA-mut^{OE} were detected by western blot. Data were shown as mean ± SEM with three independent experiments. *P* values were calculated by using a two-tailed Student's t-test. **P*<0.05, ***P*<0.01 and ns indicates no significance.



Supplementary Figure 8. Identification of *NORFA* **variants in Erhualian, Yorkshire and Landrace sow groups.** A 19-bp duplication (g.-1193 indel) and 5 point variants (g.-12C>T, g.249A>G, g.269A>G, g.290G>A and g.380G>A) were identified within *NORFA*. Sanger sequencing traces for these variants were shown and their locations were indicated by red arrows.



Supplementary Figure 9. Characterization of the 19-bp duplication variant within NORFA. (a) Schematic showing the luciferase reporters containing different fragments of NORFA 5'-flanking sequences. The core promoter of NORFA was identified using dual-luciferase activity assay. The transcription start site (TSS) is indicated with black arrow and the 19-bp duplication is represented with red diamond. (b) The linkage disequilibrium (LD) analyses. The LD values of these six variants within NORFA from Erhuanlian, Yorkshire and Landrace sow groups were calculated by SHEsis software.



Supplementary Figure 10. Characterization of the core promoter of porcine *NORFA*. (a) The core promoter sequence of *NORFA* containing 19-bp duplication was shown. The binding motifs of the potential transcription factors (TFs) were labeled with underlines. (b) The binding motifs of TFs within 19-bp duplication alleles were analyzed by JASPAR. 5 novel binding sites for NFIX, E2F6, E2F4, PAX2 and GABPA were identified, and their locations (red) with prediction scores were represented.



Supplementary Figure 11. NFIX is differentially expressed during follicular atresia. Heatmap showing the mRNA levels of NFIX in porcine granulosa cells from healthy follicles (HF) and atretic follicles (AF), analyzed by qRT-PCR (n=10). The expression levels of NFIX in AF were normalized to that in HF.



Supplementary Figure 12. The orignal images of gels and blots. (a) The orignal gel image for Figure1e. (b-q) The orignal blot images in the main figures of this manuscript. Figure6h (b), Figure6i (c), Figure7b (d), Figure7d (e), Figure7e (f), Figure8d-g (g), Figure8h (h), Figure8i (i), Figure8j (j), Figure8k (k), Figure8l (l), Figure8m (m), Figure9e (n, o), Figure10c (p) and Figure10d (q). The dotted rectangles in red, green, blue and brown indicated the representative images in the main text and the dotted rectangles in black showed the orignal images involved the corresponding experiments in this research.

Supplementary Tables

Name	Sequence (5' to 3')	Source	
Mimics NC	UUGUACUACACAAAAGUACUG	This study	
MiR-126	UCGUACCGUGAGUAAUAAUGCG	This study	
Inhibitor NC	CAGUACUUUUGUGUAGUACAA	This study	
MiR-126 inhibitor	CGCAUUAUUACUCACGGUACGA	This study	
NC-siRNA	Sense: UUCUCCGAACGUGUCACGUTT	This study	
	Anti-Sense: ACGUGACACGUUCGGAGAATT		
TGFBR2-siRNA	Sense: GCCAACAACAUCAACCACATT	This study	
	Anti-Sense: UGUGGUUGAUGUUGUUGGCTT		
NORFA-siRNA	Sense: CAGACAGAUGUGGAUGAAUTT	This study	
	Anti-Sense: AUUCAUCCACAUCUGUCUGTT		
NFIX-siRNA	Sense: CCAACCGGUUUGUCAGCAUTT	This study	
	Anti-Sense: AUGCUGACAAACCGGUUGGTT		

Supplementary Table 1 Oligonucleotide sequences used in this study

Supplementar	v Table 2	. Primers	designed	for gRT-PC	R
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	Supplementary Table 2. Primers designed for QRT	-PCR
Genes	Primer sequence (5'-3')	Usage
miR-126	CCTGTTGTCTCCAGCCACAAAAGAGCACAATATTTCAGGAGAC	Reverse-transcription ¹
10.400	AACAGGCGCALIA	
<i>mIR-126</i>		
ECELZ		
EGFL/	P. TCCGCTGTTGTGGGGGTTT	qRI-PCR
AGPAT2	E: CGCTCGTGCGTCATCATCT	aRT-PCR
//0////2	R: TCCAGCACCTCCACCTTGAT	qui i en
LCN12	F: CGTTTCTTTCCCGCTTCTGT	aRT-PCR
	R: CCTGAGTGTATCCTCCCTGTGC	·
C8G	F: CGGACTACCGGAGCTTTGC	qRT-PCR
	R: AGCGTCGCAGAAGCCGTAC	
FBXW5	F: CCAGAATCTCAACGCCAGCAC	qRT-PCR
	R: TGCCGCCCAGGTCAAAGA	
TRAF2		qR1-PCR
EDFI		qRI-PCR
MAMDC4	F: ACCGTTCGGATGAGGACACA	aRT-PCR
	R: GACCCATGCAGATAGTGATAGAAGA	4
PHPT1	F: CCACGGGATTGGTCACAGA	gRT-PCR
	R: ACGGCGTGTACTCGAATCAG	
LCN8	F: CGAGCAATACGCCATCCTG	qRT-PCR
	R: CCGTGTCTGCCGTCAACTC	
LCN5	F: CTGGTACGAGATCGCCTTGG	qRT-PCR
10116	R: ACGATGGCGTAGGTCCTGTA	
LCN6		qRT-PCR
LCN10	F: TGTCAGGCGGCTCTGTGCTT	qRT-FCR
	R: CTGAAGAGCAGCAGCGTCTTG	
FALACOD	Ε΄ ΤΤΟΓΩΟΔΑΔΩΩΟΔΤΟΔΤΟΤΟΔ	qRT-PCR
FAM69B		
	R: TGCCCTGCCAGAGCCCACT	
TMEM141	F: ATCAGGCTGTCTCACGCTCTT	qRT-PCR
	R: CCGTCGCCTGTGATTGGTAG	
CCDC183	F: CCAAGAACAAGGCGACGAT	qRT-PCR
	R: ACGCGGTCGAAGACGTACTT	
BAX	F: CCGAAATGTTTGCTGACG	qRT-PCR
	R: AGCCGATCTCGAAGGAAGT	
BCL2	F: TTCTTTGAGTTCGGTGGGG	qRT-PCR
	R: CCAGGAGAAAICAAAIAGAGGC	
TGFBR2	F: GTGCCCAAGCAGGTCATTCA	qRT-PCR
	R: CTCCTCAGGGCTTCGGTCA	
GAPDH	F: GATGGTGAAGGTCGGAGTG	qR1-PCR
	R: CGAAGTTGTCATGGATGACC	
U6	F: TTATGGGTCCTAGCCTGAC	qRT-PCR
	R: CACTATTGCGGGTCTGC	
Linc-NORFA	F: AGGAACCTGCGTGGGAATC	qRT-PCR
	R: CCGATCTCCCAGCATGAAA	
Rplp0	F: TCCAGGCTTAGGCATCACC	qRT-PCR
	R: GGCTCCCACTTTGTCTCCAG	
NFIX	F: CGAGGAGCGAGCGGTGAA	qRT-PCR
	R: TGGCGAAGGCAGTCAATCC	
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¹. Stem-loop reverse-transcriptional primers designed for detect the expression level of mature miRNAs. F: Forward primers; R: Reverse primers.

Supplementary Table 3.	Primers used for	plasmids	construction

Plasmids	Primer sequence (5'-3')	Vector	Usage
NORFA-MRE-wt	F: CCTGGGAAGCAAGACTTTGTC	pmirGLO	vector
	R: GGAGGCTGAGTTTGCCACA		construction
NORFA-MRE1-mut	F: AAAATGTGTTTGGTATGAGCGCGCTGAGATTGGCAGTCC	pmirGLO	Mutation
	R: GGGGGCGGAGCGCTGGGGACAGGGAGCTTGGCAAGG		
NORFA-MRE2-mut	F: AGGAACCCTTTGGAGGTGGTGTATTGTTGCCGGCCTC	pmirGLO	Mutation
	R: GCTGCCGAGGGGAGCTTCACCTGCAGCACTGCACCCCT		
TGFBR2-MRE-wt	F: TGACCTATAGGACTTGCTCGGA	pmirGLO	vector
	R: CACCTTCACTGTGTGCTAAAATT		construction
TGFBR2-MRE-mut	F: TCTCTGATGTCTTCTCCGCGCGCGATTGGGACCGTGCATT	pmirGLO	Mutation
	R: AACCCAACATGAGAGACCATCTATGACATAACTGGTCTGAA		
pNORFA-1752	F: CGGGGTACCGGTCGTCATGGAATATGCTCG	pGL3-basic	
	R: CCGCTCGAGCCTCCCAATACGATCTACTTCCTG		
pNORFA-1233	F: CGGGGTACCGGGTTTGTGAGTCCAAAGCAA	pGL3-basic	
	R: CCGCTCGAGCCTCCCAATACGATCTACTTCCTG		
pNORFA-793	F: CGGGGTACCACCTGTAATCCTGCTGGTGAGA	pGL3-basic	vector
	R: CCGCTCGAGCCTCCCAATACGATCTACTTCCTG		construction
pNORFA-280	F: CGGGGTACCCCAGCATCGCGTCGTCTTT	pGL3-basic	
	R: CCGCTCGAGCCTCCCAATACGATCTACTTCCTG		
pcDNA3.1-NFIX	F: GCCCCCAACTCACAACTCTG	pcDNA3.1	
	R: GGGGATTTTTCCACGTCTCAA		

F: Forward primers; R: Reverse primers.

Primers	Sequence (5'-3')	Temp	Product size
NORFA	F: GCAAAGTCCCCACGGAGACCA	62 °C	139 bp for A1/A1
	R: AGCCTGGCGGGTCCTCAGGC		120 bp for A2/A2
SBEX	F:CAGCAAAGGGTGGCAAGGT	60 °C	145 bp
	R:ATGCCTGTGCCACTTTCAACT		

Supplementary Table 4. Primers used for ChIP assay

Primers	Sequence (5'-3')	Annealing temp	Product size
NORFA-P1	F: TGGTCCAGGAGGGCAAGC	62 °C	637 bp
	R: CCCCGTTTGCGTGCTTGT		
NORFA-P2	F: GGGTTTGTGAGTCCAAAGCAAG	60 °C	796 bp
	R: GGTTTCCACGTCCCTCGGTAT		
NORFA-P3	F: TTCCAGAGCCTCCAAAGTGA	62 °C	730 bp
	R: CCTTTGGGAGACCAGCACA		
NORFA-P4	F: GCAGGAAGTAGATCGTATTGGG	62 °C	630 bp
	R: GGGATTCCCACGCAGGTT		
NORFA-P5	F: CGTAGGGAGCAGCTAATGGG	62 °C	840 bp
	R: CCAGCTCTAACGGACCTAAACG		
NORFA-P6	F: CCTGGGAAGCAAGACTTTGTC	61 °C	539 bp
	R: GCAGGCTTTGAAGTTCTGTGATT		

Supplementary Table 5. Primers used for NORFA polymorphism detection

Supplementary Methods

FACS analysis

The apoptosis rate of porcine granulosa cells were assessed by FACS, as described in the methods with Annexin V-FITC and PI dyes. For gating strategy, cells without dye label and two single dye label were respectively usd to detect the FSC/SSC gates, which is shown in Reference 24. Q1 mainly includes the dead cells, Q2 includes the late apoptotic cells, Q3 mainly includes early apoptotic cells, and Q4 mainly includes healthy cells. Besides, the right part of FSC gating (Q2+Q3) was considered as postive apoptotic cells.