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Supplemental Information

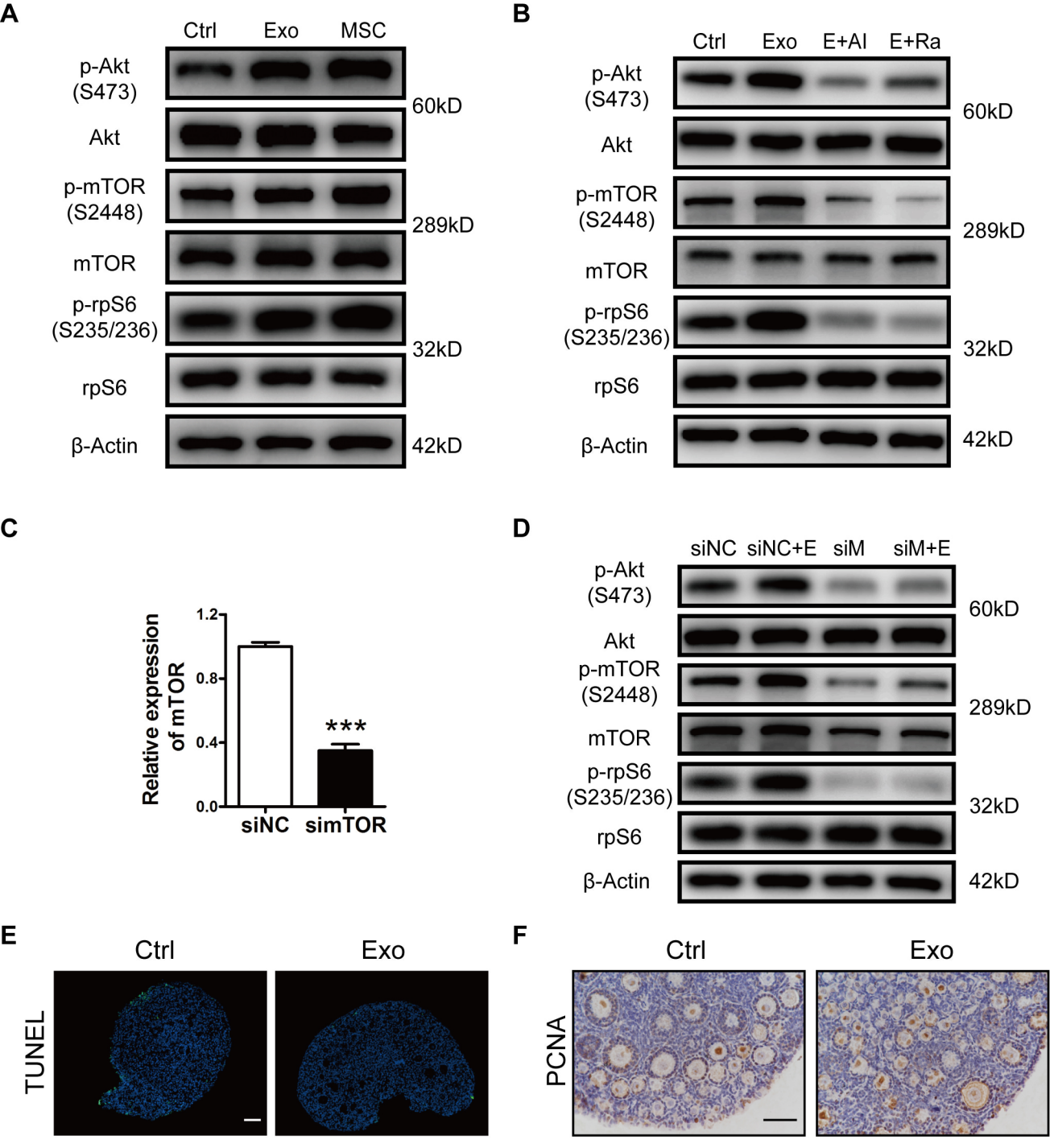
HucMSC-Derived Exosomes Mitigate the Age-Related Retardation of Fertility in Female Mice

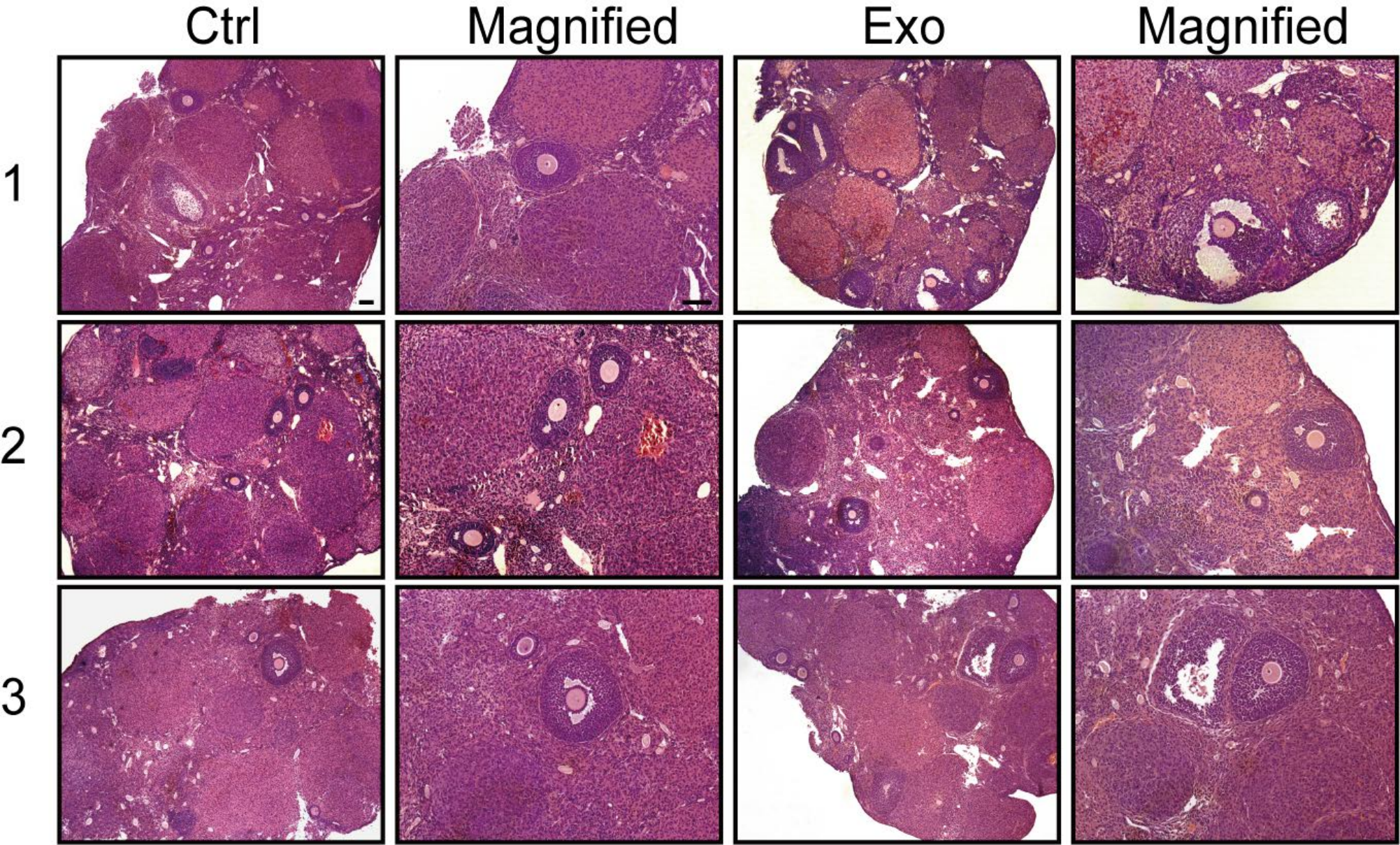
Weijie Yang, Jing Zhang, Boqun Xu, Yuanlin He, Wei Liu, Jiazhao Li, Songying Zhang, Xiaona Lin, Dongming Su, Tinghe Wu, and Jing Li

Supplementary Figure Legend

Figure S1. Specific effects of HucMSC-exos on follicular activation and development. (A) Western blot showing the expressions of p-Akt (Ser473), p-mTOR (Ser2448), p-rpS6 (Ser235/236) in ovaries treated with or without HucMSC-exos for 24 h. Ovaries co-cultured with HucMSC were used as positive control. The expressions of Akt, mTOR, rpS6, and β-actin were used as internal controls. Ctrl, PBS; Exo, HucMSC-exos 20 μg/mL; MSC, HucMSC. (B) Western blot of the expression of p-Akt (Ser473), p-mTOR (Ser2448), p-rpS6 (Ser235/236) in isolated primordial oocytes treated with HucMSC-exos with/without Akt inhibitor, Akt VIII (AI) and mTOR inhibitor, rapamycin (Ra) for 24 h. (C and D) Blocking effects of mTOR knockdown on HucMSC-exos induced activation of PI3K/mTOR signaling pathway. Newborn ovaries were pretreated with mTOR siRNAs for 60 h, following with 24 h of treatment with or without HucMSC-exos. RT-PCR showing the effective knockdown of mTOR mRNAs (C); Western blot showing the decreased expression of p-Akt (Ser473), mTOR and p-mTOR (Ser2448), p-rpS6 (Ser235/236) in treated newborn ovaries (D). The expressions of Akt, rpS6, and β-actin were used as internal controls. siNC, control siRNA; siNC+E, control siRNA+HucMSC-exos; siM, mTOR siRNAs; siM+E, mTOR siRNAs+HucMSC-exos. (E) TUNEL assay of apoptosis in ovaries treated with or without HucMSC-exos. Paired ovaries were separated and treated with or without HucMSC-exos for 24 h and transplanted under kidney capsules of the same recipient mice. Ovaries were collected 48 h after transplantation. Green, TUNEL positive cells; Blue, nuclear staining with Hoechst 33342. Bar=20 μm. (F) Ovarian PCNA staining in nuclei of oocytes and granulosa cells. Bar=50 μm.

Figure S2. Histology of follicular development between paired ovaries treated with or without HucMSC-exos. Mice at 10 month of age were intra-bursa injected with 20 μL HucMSC-exos in one lateral ovary and same volume of PBS in the other lateral ovary. Paired ovaries were collected 3 weeks later for morphological analysis. Representative three pairs of ovaries were shown. In each group, right panels are magnifications of left panels. Bar=50μm.


A
Ctrl
Exo
MSC
p-Akt (S473)
Akt
p-mTOR (S2448)
mTOR
p-rpS6 (S235/236)
rpS6
β-Actin
60kD
289kD
32kD
42kD
B
Ctrl
Exo
E+AI
E+Ra
p-Akt (S473)
Akt
p-mTOR (S2448)
mTOR
p-rpS6 (S235/236)
rpS6
β-Actin
60kD
289kD
32kD
42kD
C
Relative expression of mTOR
1.2
0.8
0.4
0.0
***
siNC
simTOR
D
siNC
siNC+E
siM
siM+E
p-Akt (S473)
Akt
p-mTOR (S2448)
mTOR
p-rpS6 (S235/236)
rpS6
β-Actin
60kD
289kD
32kD
42kD
E
Ctrl
Exo
TUNEL
F
Ctrl
Exo
PCNA


Ctrl
Magnified
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1
2
3

Supplementary Table S1

Antibody	Company	Catalog Number	Dilution
PE-conjugatedCD11b Antibody	BD	561689	1 test/10^6 cells
FITC-conjugatedCD19 Antibody	BD	560994	1 test/10^6 cells
FITC-conjugatedCD34 Antibody	BD	560942	1 test/10^6 cells
FITC-conjugatedCD44 Antibody	BD	560977	1 test/10^6 cells
FITC-conjugatedCD45 Antibody	BD	560976	1 test/10^6 cells
PE-conjugatedCD73 Antibody	BD	561014	1 test/10^6 cells
PE-conjugatedCD90 Antibody	BD	561970	1 test/10^6 cells
APC-conjugatedCD105 Antibody	BD	562408	1 test/10^6 cells
FITC-conjugatedHLA-DR/DQ Antibody	BD	555563	1 test/10^6 cells
β-Actin Antibody	CST	3700	WB 1:2000
GAPDH Antibody	CST	5174	WB 1:2000
Alix Antibody	Proteintech	12422-1-AP	WB 1:1000
Tsg101 Antibody	Abclonal	A2216	WB 1:1000
CD9 Antibody	Proteintech	20597-1-AP	WB 1:1000
Gm130 Antibody	BD	610822	WB 1:1000
rpS6 Antibody	CST	2217	WB 1:1000
p-rpS6（S235/236）Antibody	CST	2211	WB 1:1000
mTOR Antibody	CST	2983	WB 1:1000
p-mTOR Antibody	CST	5536	WB 1:1000
AKT Antibody	CST	2920	WB 1:1000
p-AKT Antibody	CST	4060	WB 1:1000
Foxo3a Antibody	CST	12829	IHC 1:200
PCNA Antibody	CST	13110	IHC 1:1000
Cx37 Antibody	Abcam	Ab181701	IF 1:200 WB 1:1000

Supplementary Table S2 Real-time PCR Primers

Gene	Forward primer	Reverse primer
Actin	5’-CCGTAAAGACCTCTATGCC-3′	5′-CTCAGTAACAGTCCGCCTA-3′
Gdf9	5′-TCTTAGTAGCCTTAGCTCTCAGG-3′	5′-TGTCAGTCCCATCTACAGGCA-3′
Zp3	5′-CATCTCAAAGTCGCGCCAG-3′	5′-GCCTGCGGTTTCGAGAAAC-3′
Bmp15	5′-TCCTTGCTGACGACCCTACAT-3′	5′-TACCTCAGGGGATAGCCTTGG-3′
Amhr	5′-GCAGCACAAGTATCCCCAAAC-3′	5′-GTCTCGGCATCCTTGCATCTC-3′
Kit	5′-CTCCCCCAACAGTGTATTCAC-3′	5′-TAGCCCGAAATCGCAAATCTT-3′
Kitl	5′-GAATCTCCGAAGAGGCCAGAA-3′	5′-GCTGCAACAGGGGGTAACAT-3′
Fshr	5′-CCTTGCTCCTGGTCTCCTTG-3′	5′-CTCGGTCACCTTGCTATCTTG-3′
Star	5′-ATGTTCCTCGCTACGTTCAAG-3′	5′-CCCAGTGCTCTCCAGTTGAG-3′