Deficiency in DGCR8-dependent canonical microRNAs causes infertility due to multiple abnormalities during uterine development in mice

Yeon Sun Kim[1], Hye-Ryun Kim[1], Hyongbum Kim[2], Seung Chel Yang[1], Mira Park[1], Jung Ah Yoon[3], Hyunjung J Lim[4], Seok-Ho Hong[5], Francesco J DeMayo[6], John P Lydon[6], Youngsok Choi[1], Dong Ryul Lee[1,3], Haengseok Song[1,3,*]

[1]Department of Biomedical Science, CHA University, Seongnam, Gyeonggi, Korea

[2]Department of Pharmacology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea

[3]Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Korea

[4]Department of Veterinary Medicine, College of Veterinary Medicine, Konkuk University, Seoul, 143-701 Korea

[5]Department of Internal Medicine, School of Medicine, Stem Cell Institute, Kangwon National University, Chuncheon, Kangwon 200-701, Korea

[6]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

*Correspondence should be addressed to: H Song, Ph.D. (hssong@cha.ac.kr) at Department of Biomedical Science, CHA University (Tel: 82-31-881-7150, Fax: 82-31-881-7249).

Figure S1. Gross histology and gonadotropin production in Dgcr8$^{d/d}$ mice. Gross histology (a) and RT-PCR results for FSHβ and LHβ subunits (b) expressed in the pituitary of Dgcr8$^{d/d}$ mice. The bottom panel in (a) shows higher-magnification images of the boxed area in the top panel. Scale bar: 50 μm.

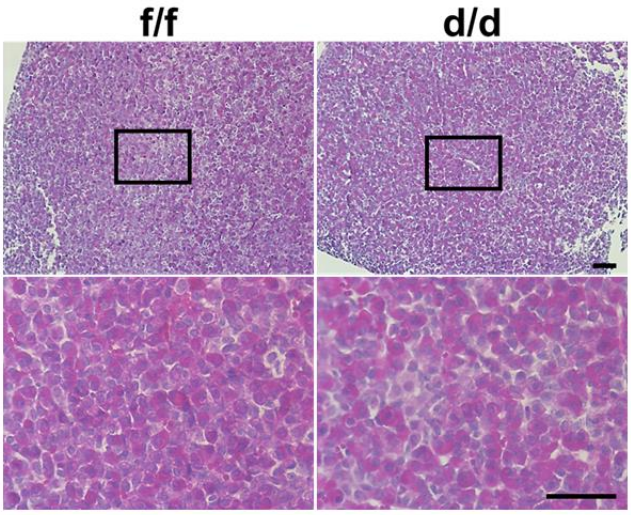
Figure S2. Histologic examination for acute immune responses in reproductive organs of Dgcr8$^{d/d}$ mice injected with oil. (a) Gross morphology of female reproductive organs in Dgcr8$^{f/f}$ and Dgcr8$^{d/d}$ mice 12 h after oil injection into uterine horns at post-hCG 9 h. Scale bar: 5 mm. (b) Histological analyses of female reproductive organs shown in (a). Note that there are no inflammatory cells such as neutrophils and eosinophils presented in Figure 3. Scale bar: 100 μm.

Figure S3. Expression profiles of miRNA biogenesis factors in mouse uterus. RT-PCR (a) and realtime RT-PCR (b) analyses for relative mRNA levels of miRNA biogenesis factors by E_2 (200 ng) and/or P_4 (2 mg) in the uteri of ovariectomized mice at different time points (n=5 for each group). Egr1 and Ltf were used as positive controls for early and late estrogen responses, respectively. Areg and Hoxa10, and Ptgs1 were for P_4 and E_2+P_4 responses, respectively.

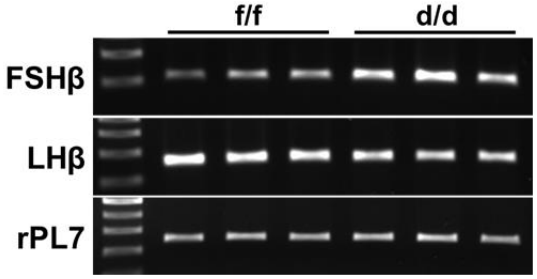
Figure S4. Analyses of E_2-dependent expression of miRNA biogenesis components via nuclear estrogen receptor(s). Expression patterns of Dicer, Argo2 and Xpo5 were analyzed by RT-PCR (a) and realtime RT-PCR (b-d) to examine whether nuclear estrogen receptor(s) are involved in E_2-dependent expression of these genes. Ovariectomized mice were treated with ICI 182, 780, a nuclear estrogen receptor antagonist, 30 min before E_2 (200 ng) treatments and sacrificed 3 h after E_2 administration (n=4 to 7 for each group). Unpaired Student *t*-test, *=$p<0.05$.

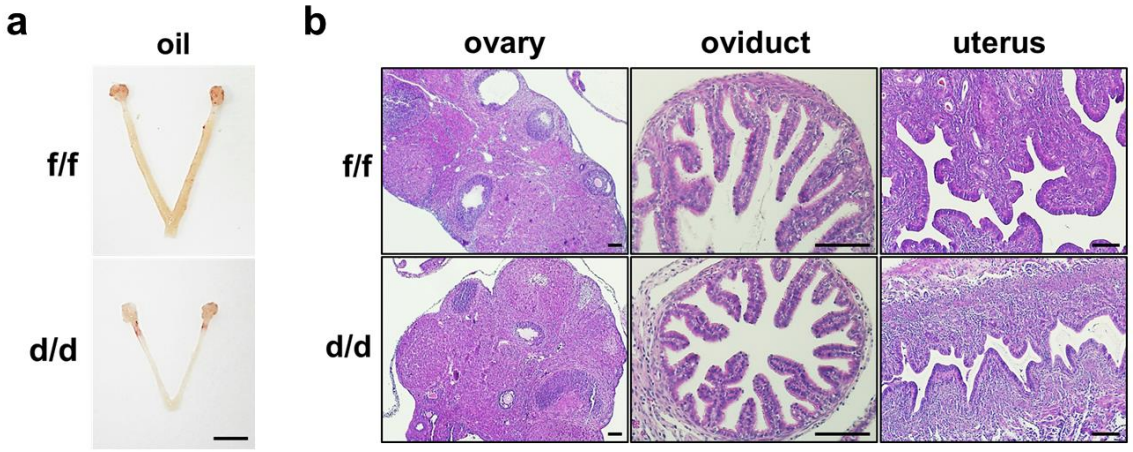
Figure S5. TUNEL assays for the uterine sections of 3- and 5-week-old Dgcr8$^{d/d}$ mice. (a) Microscopic images for apoptotic cells (green) counterstained with DAPI (blue) in uterine sections from Dgcr8$^{f/f}$ and Dgcr8$^{d/d}$ mice. Note that uteri of both Dgcr8$^{f/f}$ and Dgcr8$^{d/d}$ mice at 3 and 5 weeks of age were negative in the TUNEL assays. Scale bar: 50 μm. (b) Realtime RT-PCR for expression of apoptosis marker genes (Bcl2l11, Aldh1a3, and Fas) in the uteri of 3- and 5-week-old Dgcr8$^{f/f}$ and Dgcr8$^{d/d}$ mice.

a

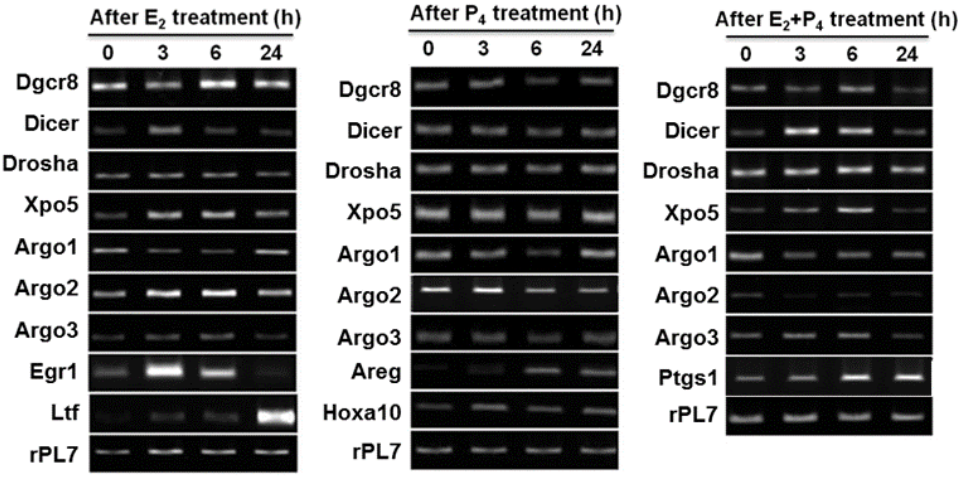


b

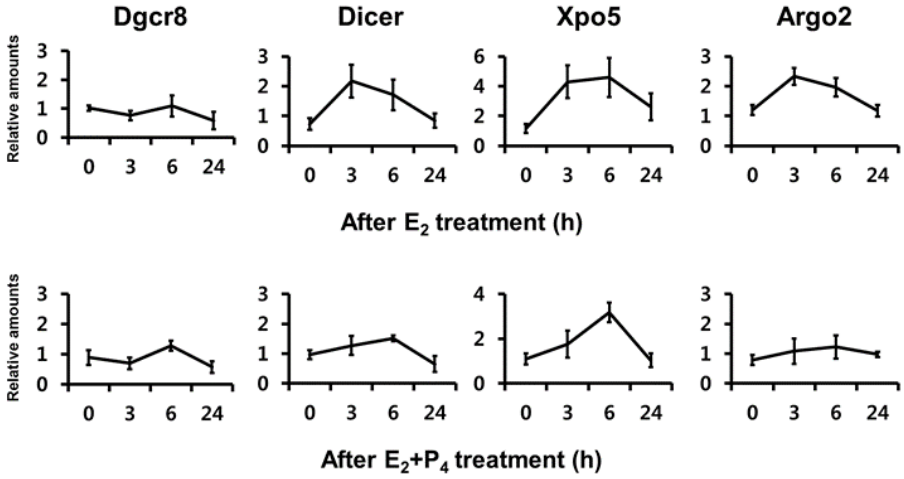


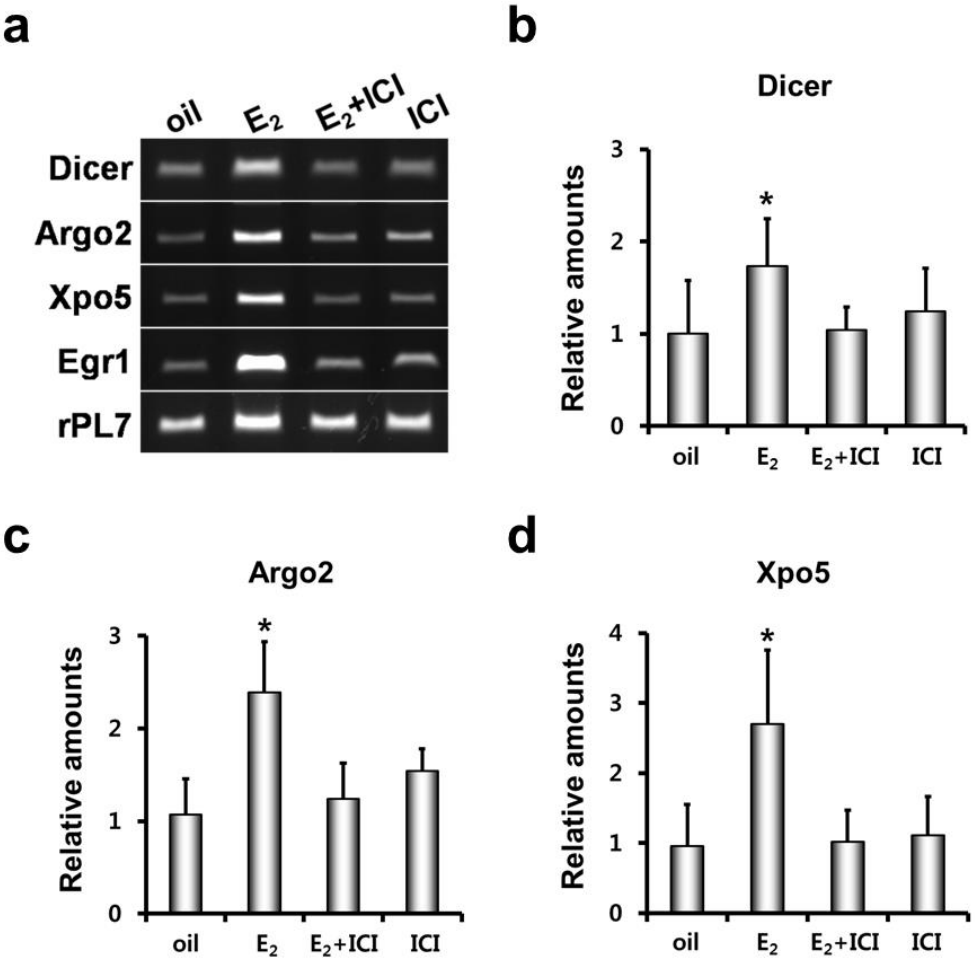

a
oil
f/f
d/d
b
ovary
oviduct
uterus
f/f
d/d

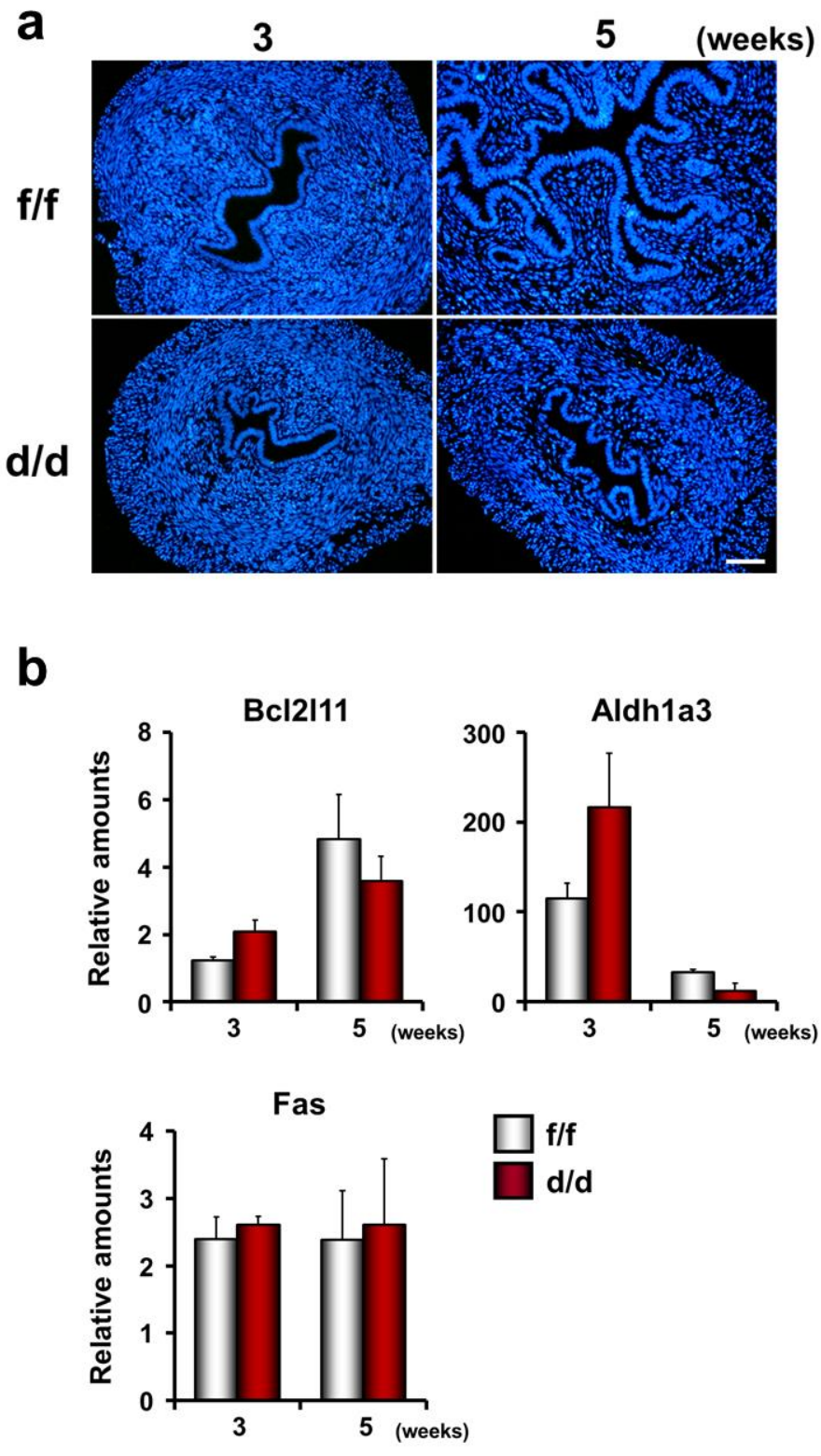
a


After E_2 treatment (h)
0 3 6 24
Dgcr8
Dicer
Drosha
Xpo5
Argo1
Argo2
Argo3
Egr1
Ltf
rPL7
After P_4 treatment (h)
0 3 6 24
Dgcr8
Dicer
Drosha
Xpo5
Argo1
Argo2
Argo3
Areg
Hoxa10
rPL7
After E_2+P_4 treatment (h)
0 3 6 24
Dgcr8
Dicer
Drosha
Xpo5
Argo1
Argo2
Argo3
Ptgs1
rPL7

b


Dgcr8
Dicer
Xpo5
Argo2
Relative amounts
After E_2 treatment (h)
Relative amounts
After E_2+P_4 treatment (h)


a
oil
E2
E2+ICI
ICI
Dicer
Argo2
Xpo5
Egr1
rPL7
b
Dicer
Relative amounts
3
2
1
0
*
oil
E2
E2+ICI
ICI
c
Argo2
Relative amounts
3
2
1
0
*
oil
E2
E2+ICI
ICI
d
Xpo5
Relative amounts
4
3
2
1
0
*
oil
E2
E2+ICI
ICI


a
3
5
(weeks)
f/f
d/d
b
Bcl2l11
Aldh1a3
Relative amounts
8
6
4
2
0
300
200
100
3
5
(weeks)
Fas
4
3
2
1
0
f/f
d/d