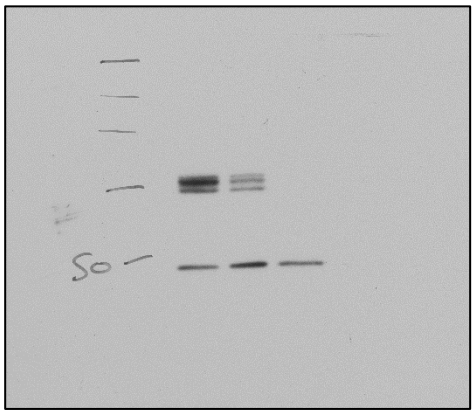
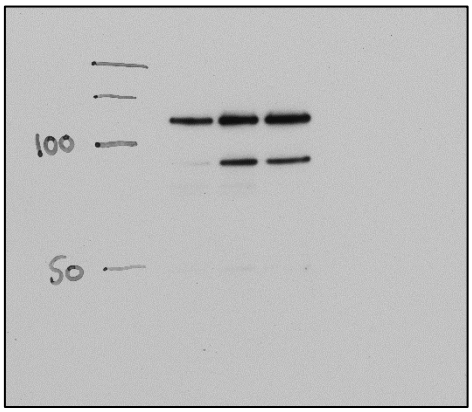
Figure S1. *Mthfr* knockout, heterozygous, and ~~wild type~~ liver probed with anti MTHFR (Abcam) and reprobed with anti-vinculin.

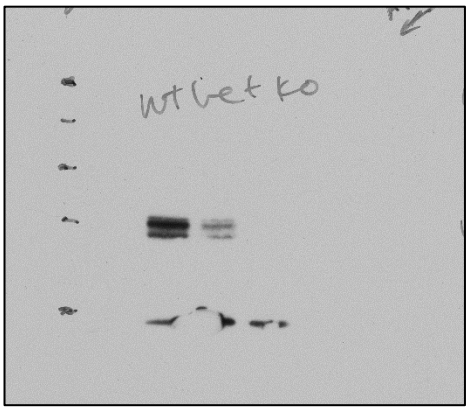
Rep 1. anti-MTHFR (Abcam ab203786) 1:1000 5 µg liver



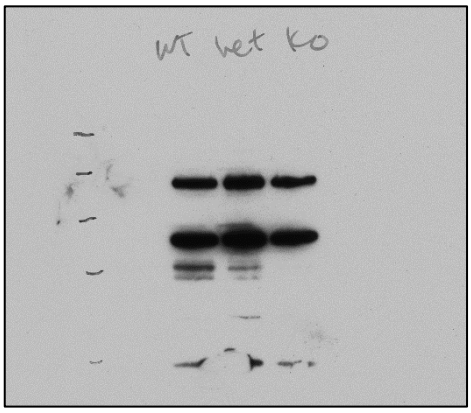
Rep 1. reprobed with anti-vinculin (Abcam ab129002) 1:500 5 µg liver



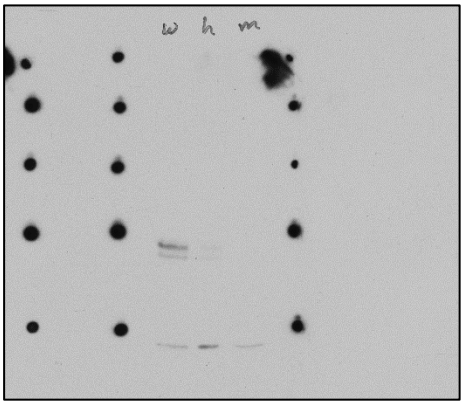
Rep 2. anti-MTHFR (Abcam ab203786) 1:1000 5 µg liver



Rep 2. reprobed with anti-vinculin (Abcam ab129002) 1:1000 5 µg liver



Rep 3. anti-MTHFR (Abcam ab203786) 1:1000 1 µg liver



ND

Figure S2. Varying number of GV oocytes probed with anti-MTHFR (Abcam)

anti-MTHFR (Abcam ab203786) 1:1000
Liver (5 µg), 10, 25, 45, 75, 100 GV oocytes

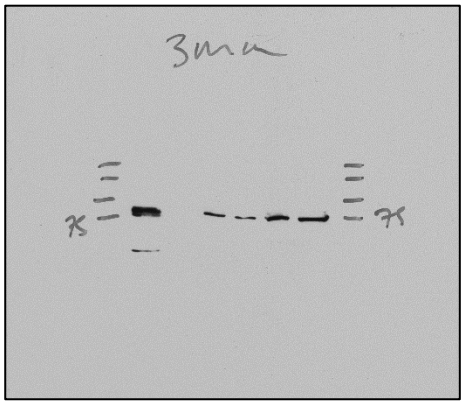
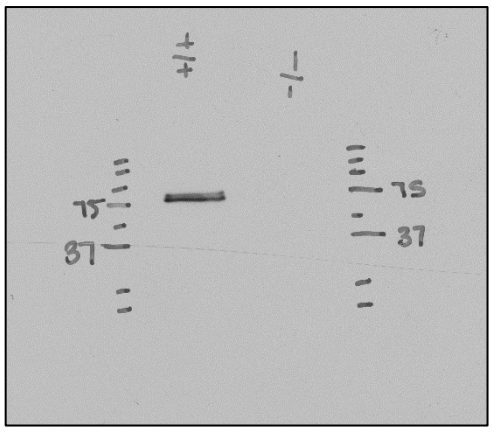


Figure S3. GV oocytes from *Mthfr* ~~wild type~~ and knockout females probed with anti-MTHFR (Abcam) and reprobed with anti-GAPDH.

Anti-MTHFR antibody (Abcam) 1:1000 in 5% milk (secondary, 1:5000) 25 GV oocytes; 10% gel



Reprobed with Anti-GAPDH (1:200)

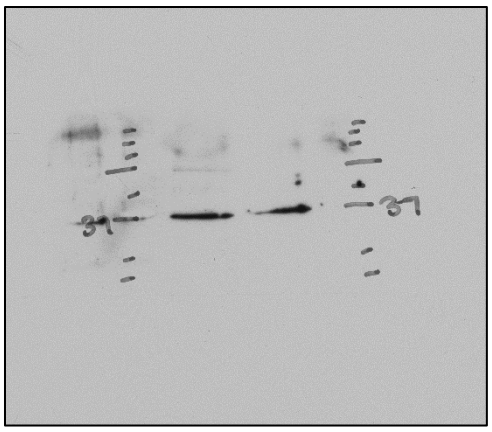
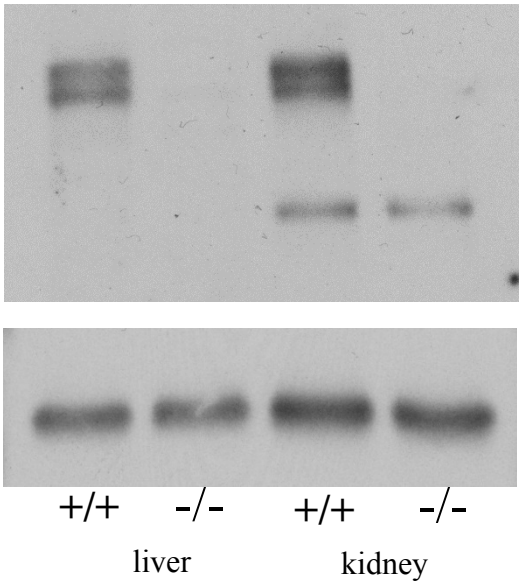
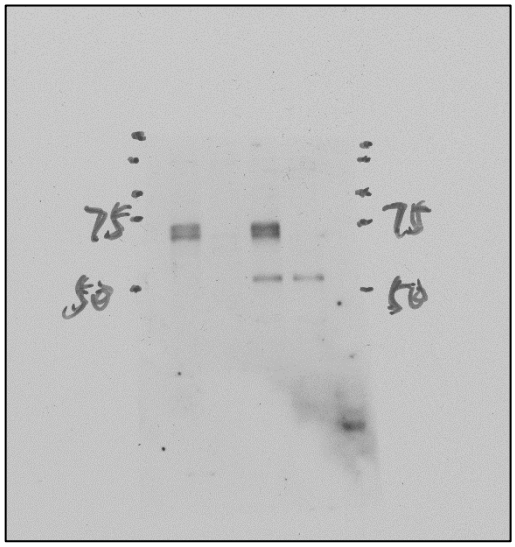


Figure S4. Liver and kidney lysates from *Mthfr* wild type and knockout mice probed with anti-MTHFR (Rima Rozen laboratory) and anti-GAPDH.

Liver and kidney lysates from knockout (-/-) and wild type (+/+) females probed with anti-MTHFR rabbit polyclonal from laboratory of Rima Rozen (McGill Univ.). Images were cropped from western blots shown at right that were adjusted to horizontal before cropping. Membrane was reprobed with anti-GAPDH (Santa Cruz).



Liver and kidney lysate from *Mthfr* wild type (+/+) and knockout (-/-) mice.
top: MTHFR from Rima Rozen (1:500)
bottom: reprobed with anti-GAPDH 1:200



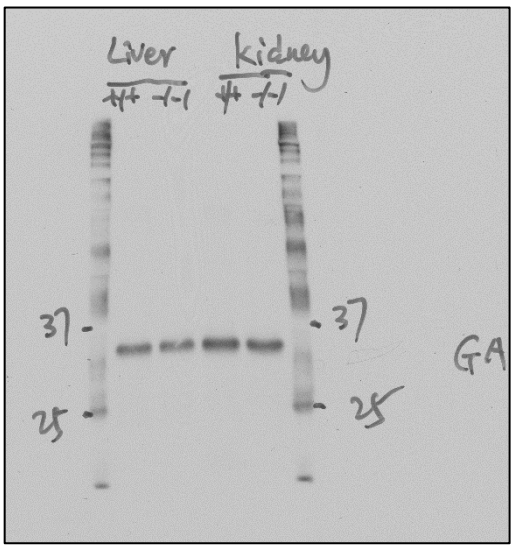
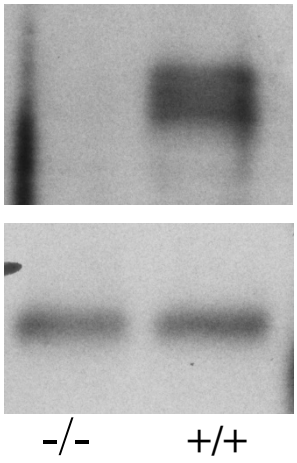


Figure S5. GV oocytes from *Mthfr* wild type and knockout females probed with anti-MTHFR (Rima Rozen laboratory) and anti-GAPDH.

GV oocytes from knockout (-/-) and wild type (+/+) females probed with anti-MTHFR rabbit polyclonal from laboratory of Rima Rozen (McGill Univ.). 100 GV oocytes per lane. Images were cropped from western blots shown at right that were adjusted to horizontal before cropping.



top: MTHFR from Rima Rozen (1:500)
bottom: anti-GAPDH 1:200
100 GV oocytes per lane
Knockout oocytes are in left lane (-/-), wild type oocytes at right (+/+).
Membrane was cut at ~50 kDa and top and bottom probed separately for MTHFR and GAPDH, respectively.

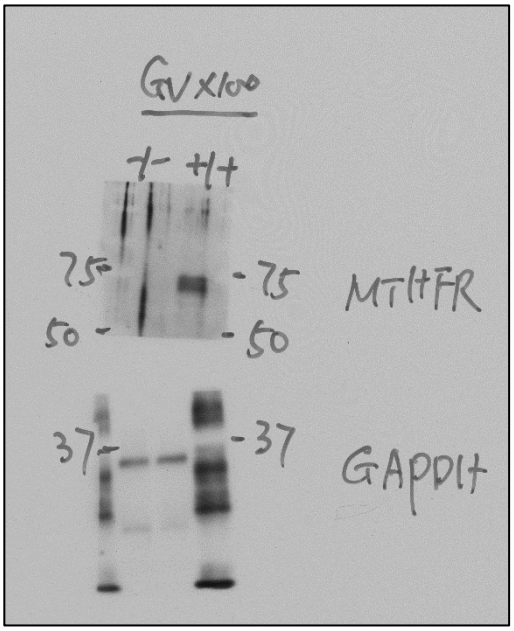
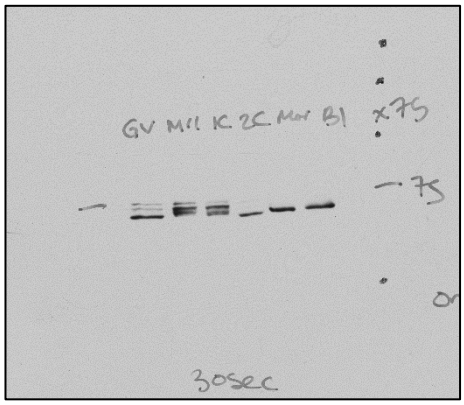
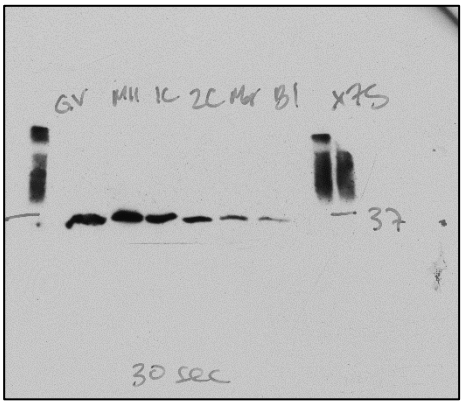


Figure S6. MTHFR in GV oocytes, MII eggs, and 1-cell, 2-cell, morula, and blastocyst stage emrbyos determined using Abcam anti-MTHFR.

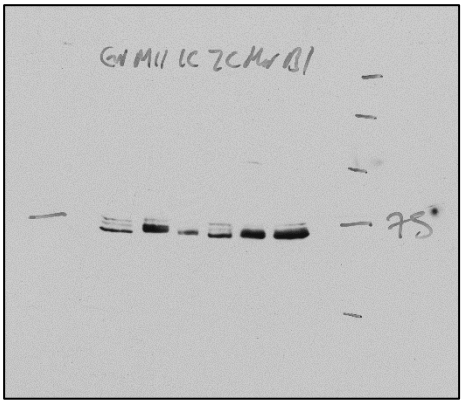
Rep 1. anti-MTHFR (Abcam) 1:1000
GV, MII,1c, 2c, M, Bl (75 each per lane)



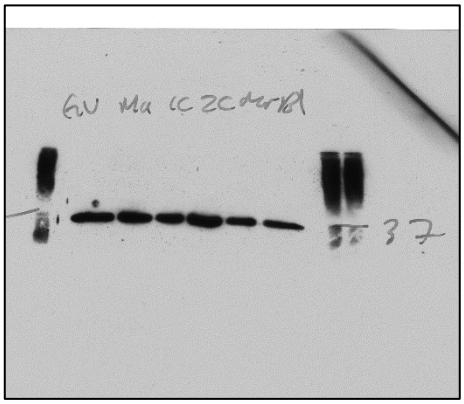
Rep 1. anti-GAPDH (Santa Cruz) 1:200
GV, MII,1c, 2c, M, Bl (75 each per lane)



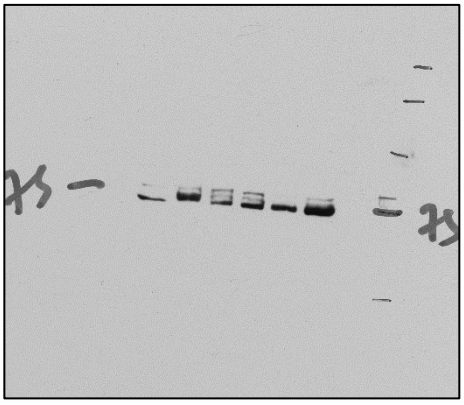
Rep 2. anti-MTHFR (Abcam) 1:1000
GV, MII,1c, 2c, M, Bl (75 each per lane)



Rep 2. anti-GAPDH (Santa Cruz) 1:200
GV, MII,1c, 2c, M, Bl (75 each per lane)



Rep 3. anti-MTHFR (Abcam) 1:1000
GV, MII,1c, 2c, M, Bl (75 each per lane)



Rep 3. anti-GAPDH (Santa Cruz) 1:200
GV, MII,1c, 2c, M, Bl (75 each per lane)

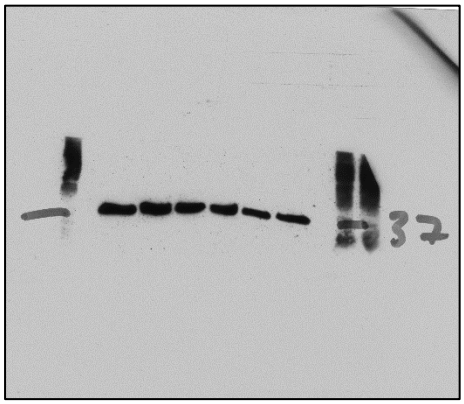
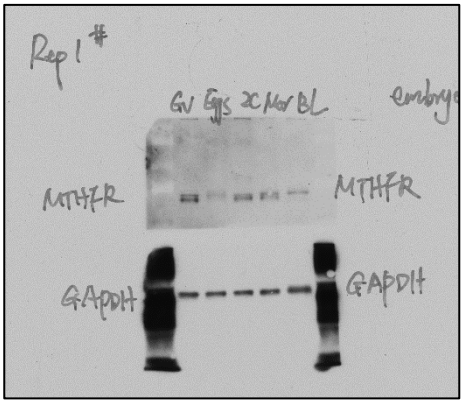
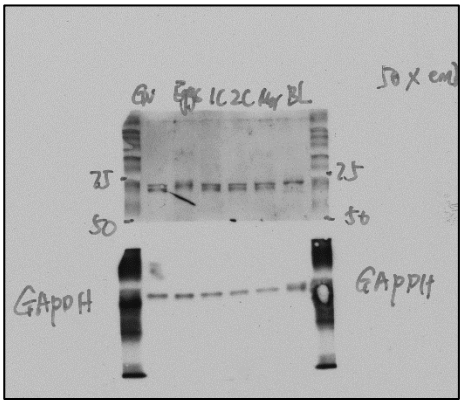


Figure S7. MTHFR in GV oocytes, MII eggs, and 1-cell, 2-cell, morula, and blastocyst stage emrbyos determined using anti-MTHFR from the laboratory of Rima Rozen.

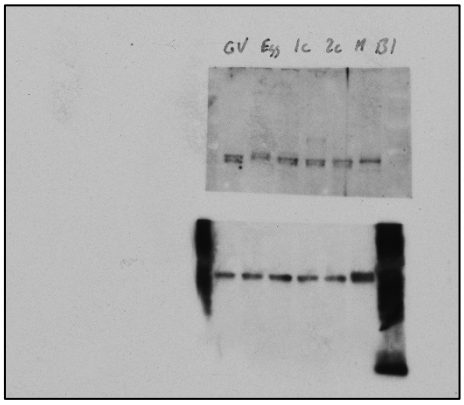
Rep 1. anti-MTHFR (Rozen) 1:500
GV, MII, 2c, M, Bl (50 each per lane). Membrane was cut at ~50 kDa and top probed for MTHFR (Rozen) and bottom for GAPDH (Santa Cruz)



Rep 2. anti-MTHFR (Rozen) 1:500
GV, MII, 1c, 2c, M, Bl (50 each per lane). Membrane was cut at ~50 kDa and top probed for MTHFR (Rozen) and bottom for GAPDH (Santa Cruz).



Rep 3. anti-MTHFR (Rozen) 1:500
GV, MII, 1c, 2c, M, Bl (50 each per lane). Membrane was cut at ~50 kDa and top probed for MTHFR (Rozen) and bottom for GAPDH (Santa Cruz).
Right and left are different exposures; left was used for MTHFR, right for GAPDH.



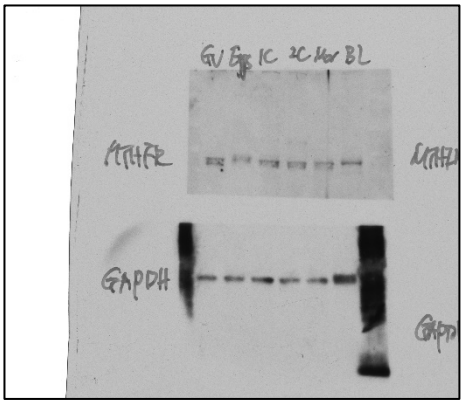
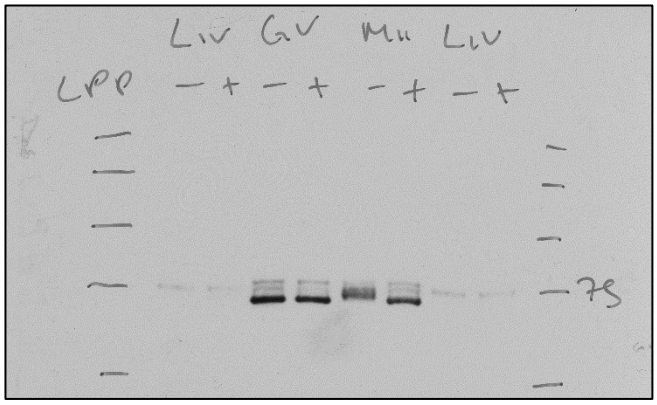
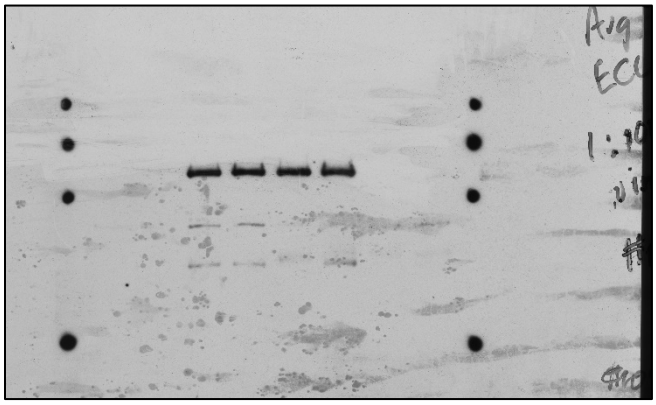


Figure S8. GV and MII oocytes treated with lambda protein phosphatase (LPP) and probed for MTHFR (Abcam). LPP treatment is described in Results.

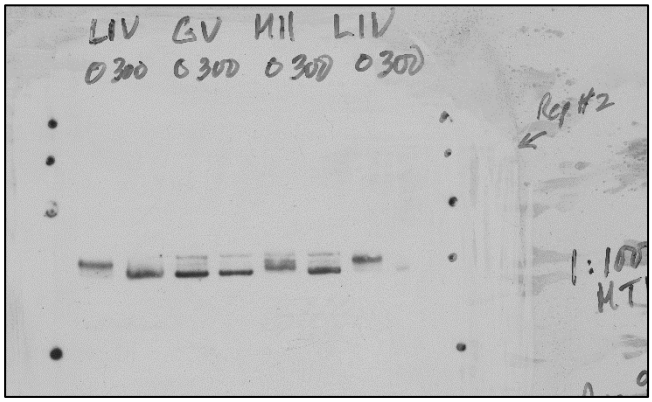
Rep 1. anti-MTHFR (Abcam) 1:1000
Liv, GV, MII, Liv +/- LPP (75 each per lane)



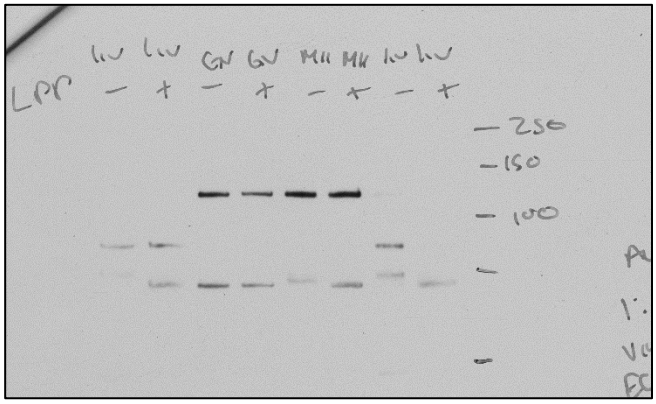
Rep 1. reprobed with anti-vinculin (Abcam) 1:1000



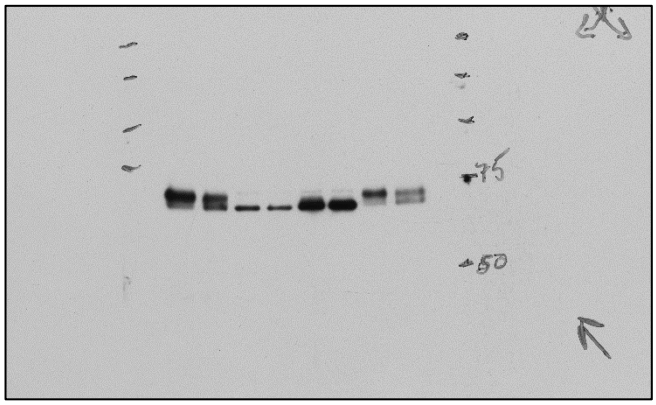
Rep 2. anti-MTHFR (Abcam) 1:1000
Liv, GV, MII, Liv +/- LPP (75 each per lane)



Rep 2. reprobed with anti-vinculin (Abcam) 1:1000



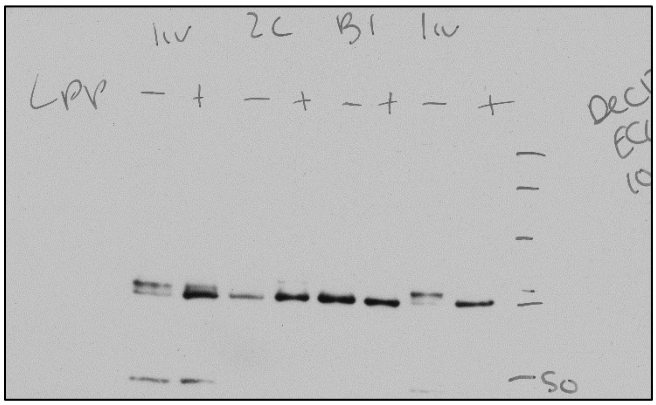
Rep 3. anti-MTHFR (Abcam) 1:1000
Liv, 2c, Bl, Liv +/- LPP (75 each per lane)



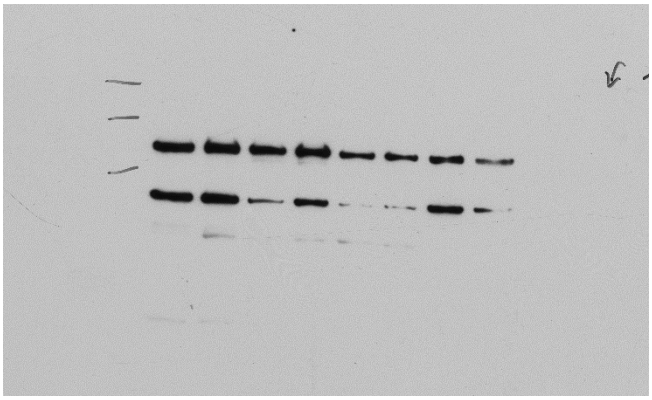
Rep 3. reprobed with anti-vinculin (Abcam) 1:1000

Figure S9. 2c embryos and blastocysts treated with lambda protein phosphatase (LPP).

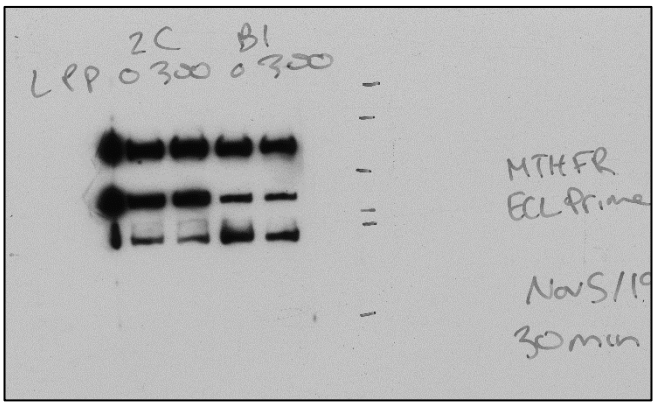
Rep 1. anti-MTHFR (Abcam) 1:1000
Liv, 2c, Bl, Liv +/- LPP (75 each per lane)



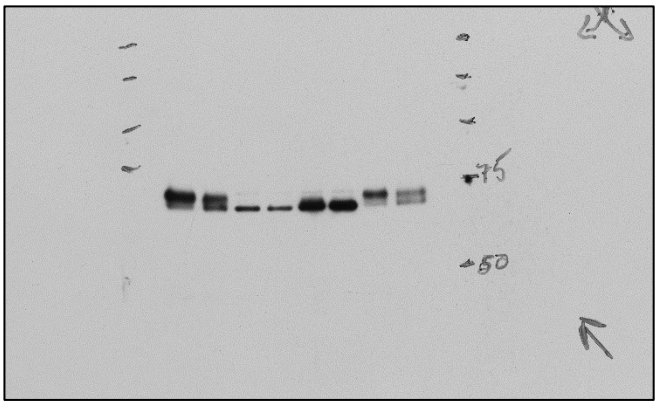
Rep 1. reprobed with anti-vinculin (Abcam) 1:1000



Rep 2. anti-MTHFR (Abcam) 1:1000
Liv, 2c, Bl, Liv +/- LPP (75 each per lane) and anti-vinculin (Abcam Ab129002) 1:1000
Membrane was probed simultaneously for MTHFR and vinculin.



Rep 3. anti-MTHFR (Abcam) 1:1000
Liv, 2c, Bl, Liv +/- LPP (75 each per lane)



Rep 3. reprobed with anti-vinculin (Abcam) 1:1000

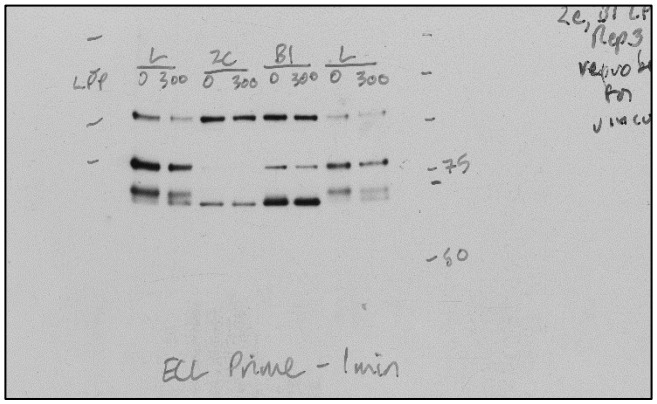
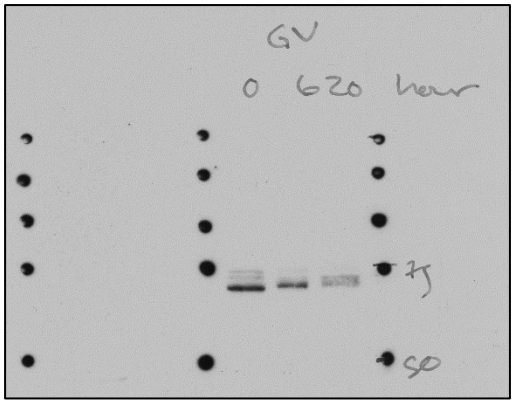
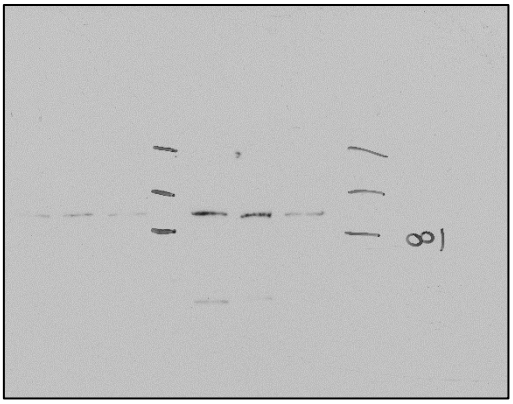


Figure S10. In vitro-matured GVoocytes at GV (0 hr, MI (6 hr) and MII (20 hr) probed for MTHFR.

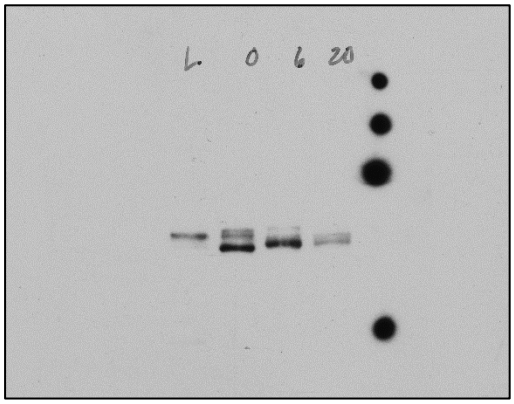
Rep 1. anti-MTHFR (Abcam) 1:1000
GV 0,6,20 hr (75 each per lane)



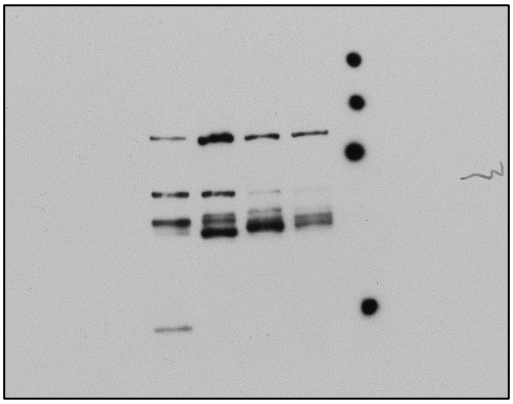
Rep 1. reprobed with anti-vinculin (Abcam) 1:1000



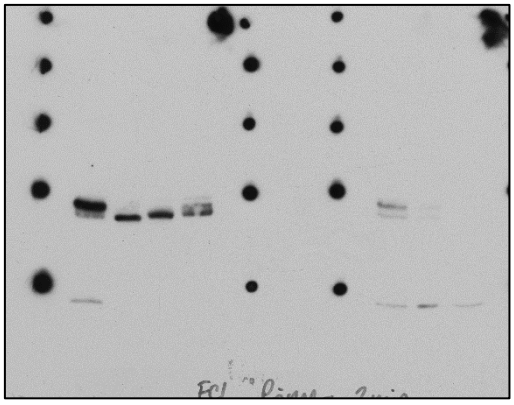
Rep 2. anti-MTHFR (Abcam) 1:1000
Liv, GV 0,6,20 hr (75 each per lane)



Rep 2. reprobed with anti-vinculin (Abcam) 1:1000



Rep 3. anti-MTHFR (Abcam) 1:1000
Liv, GV 0,6,20 hr (75 each per lane)



Rep 3. reprobed with anti-vinculin (Abcam Ab129002) 1:1000

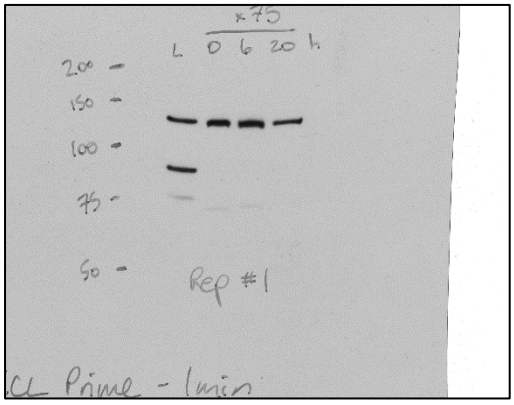
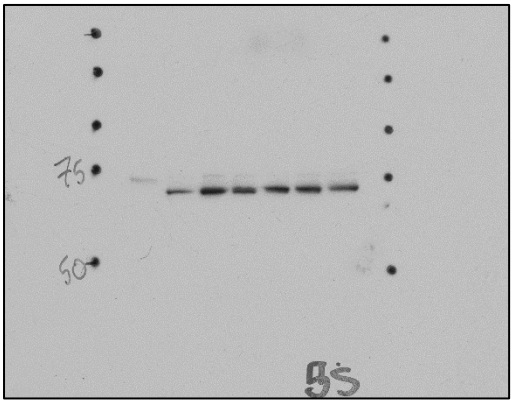
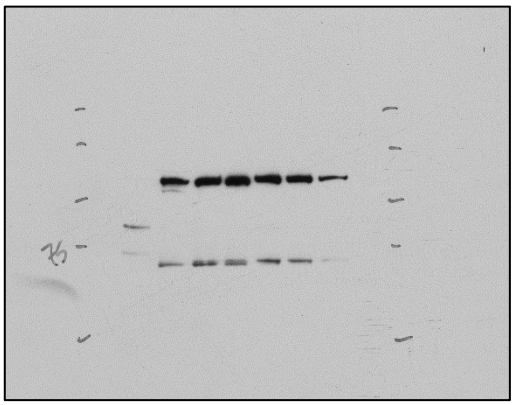


Figure S11. In vitro-matured GVoocytes during meiotic preogression into MI from 0-6 hr probed for MTHFR.

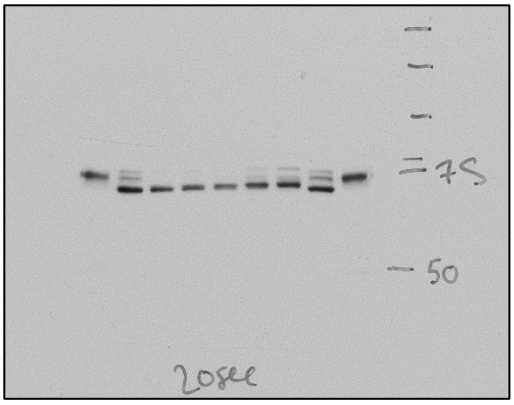
Rep 1. anti-MTHFR (Abcam) 1:1000
Liv, oocytes at 0,1GV, 1GVBD, 2,3,6 hr (75 each per lane)



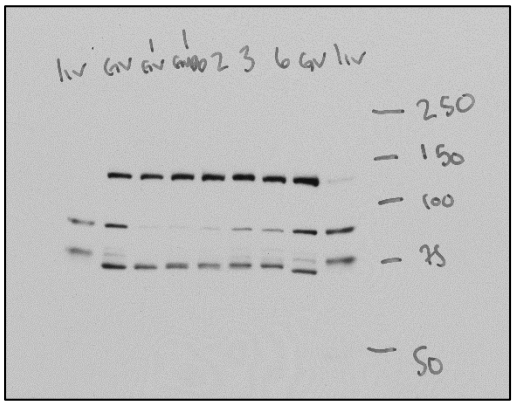
Rep 1. reprobed with anti-vinculin (Abcam) 1:1000. This was also used for the shorter MTHFR exposure.



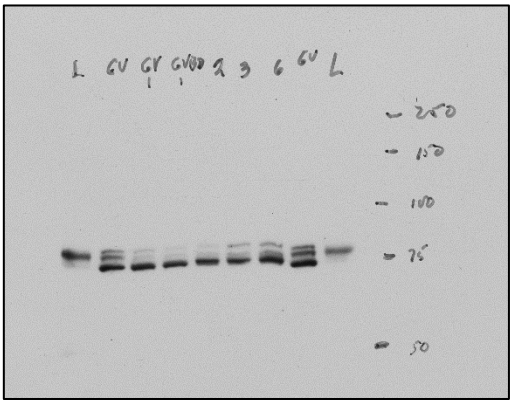
Rep 2. anti-MTHFR (Abcam) 1:1000
Liv, oocytes at 0,1GV, 1GVBD, 2,3,6 hr, GV at 0 hr (75 each per lane), and liver



Rep 2. reprobed with anti-vinculin (Abcam) 1:1000. This was also used for the shorter MTHFR exposure.



Rep 3. anti-MTHFR (Abcam) 1:1000
Liv, oocytes at 0,1GV, 1GVBD, 2,3,6 hr, GV at 0 hr (75 each per lane), and liver



Rep 3. reprobed with anti-vinculin (Abcam Ab129002) 1:1000. This was also used for the shorter MTHFR exposure.

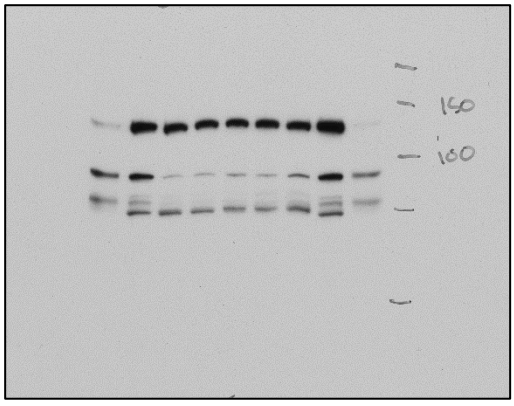
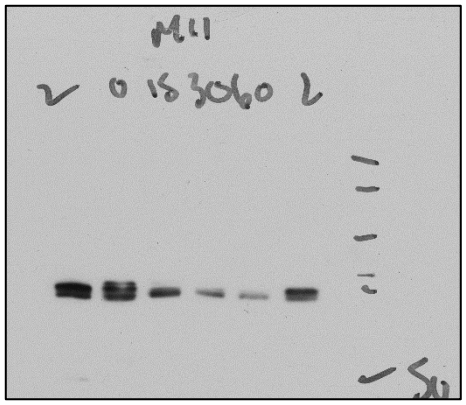
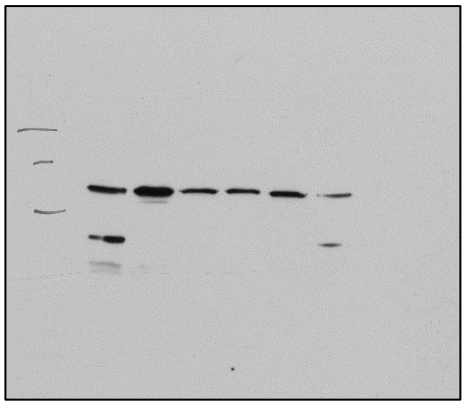


Figure S12. MTHFR probed 0-1 hr after parthenogenetic activation of MII eggs.

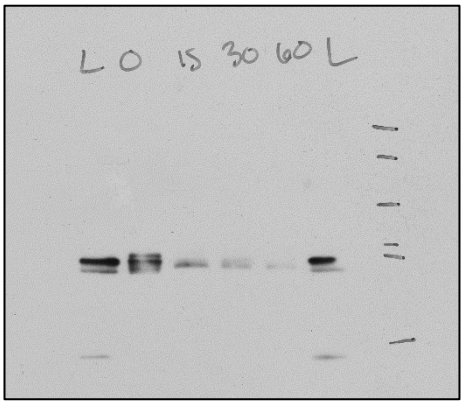
Rep 1. anti-MTHFR (Abcam) 1:1000
Liv, MII oocytes at 15, 30, 60 min (75 each per lane)



Rep 1. reprobed with anti-vinculin (Abcam Ab129002) 1:1000

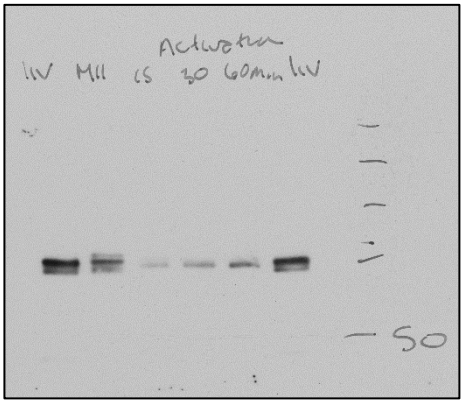


Rep 2. anti-MTHFR (Abcam) 1:1000
Liv, MII oocytes at 15, 30, 60 min (75 each per lane). Not reprobed for vinculin due to Covid-19-related laboratory shutdown.



ND

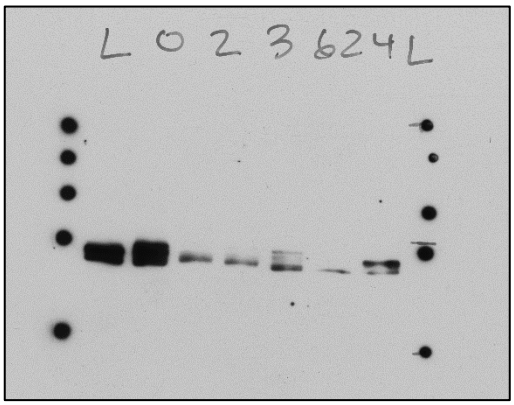
Rep 3. anti-MTHFR (Abcam) 1:1000
Liv, MII oocytes at 15, 30, 60 min (75 each per lane). Not reprobed for vinculin due to Covid-19-related laboratory shutdown.

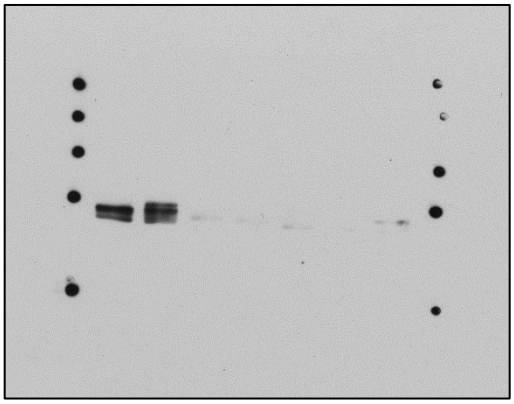


ND

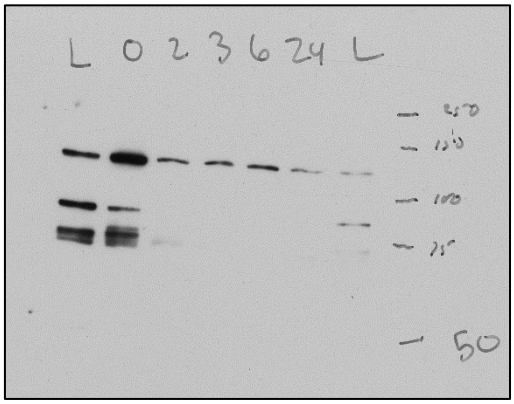
Figure S13. Activated eggs after parthenogenetic activation of MII eggs using $SrCl_2$ probed for MTHFR up to 24 h~~r~~ post-activation.

Rep 1. anti-MTHFR (Abcam) 1:1000
Liv, MII oocytes at 0, 2, 3, 6, 24 h~~r~~ (75 each per lane). Two exposures are shown, with the shorter exposure at the bottom.

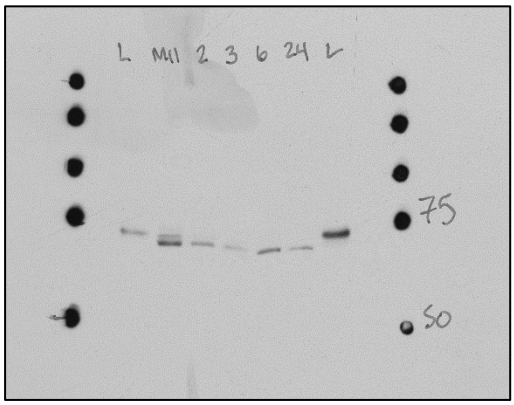




Rep 1. reprobed with anti-vinculin (Abcam Ab129002) 1:1000



Rep 2. anti-MTHFR (Abcam) 1:1000
Liv (5 µg), MII oocytes at 0, 2, 3, 6, 24 h~~r~~ (75 each per lane)



Rep 2. reprobed with anti-vinculin (Abcam Ab129002) 1:1000