**Title:** Paternal chronic folate supplementation induced the transgenerational inheritance of acquired developmental and metabolic changes in chickens

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**Supplementary Information**

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Figure S3 Altered antioxidant ability of breeder roosters and broiler offspring induced by

dietary folate supplementation in breeder roosters. (A) Altered T-AOC. (B) Altered malondialdehyde.

Figure S4 Dietary folate supplementation of breeder roosters induces differentially expressed genes and lncRNAs in the livers of breeder roosters and 1-day-old broiler offspring. (A-B) Hepatic differentially expressed mRNAs of breeder roosters and broiler offspring. (C-D) Validation of the accuracy of RIBO-Zero RNA-seq data by qRT-PCR. (E-F) Hepatic differentially expressed lncRNAs of breeder roosters and broiler offspring

Figure S5 Transgenerational regulatory roles of miRNAs in regulating lipid and glucose metabolism in response to paternal folate supplementation. (A-B): Differentially expressed miRNAs in the livers of breeder roosters and broiler offspring. (C-F) Differentially expressed miRNAs in the sperm of breeder roosters. (G) Validation of the accuracy of miRNA-seq data by qRT-PCR.

**Supplementary Table S1 Composition and nutrient level of the basal diet (air-dry basis) of breeder roosters.**

| Items | 0-6w | 7-15w | 16-40w | | 0-3w | 4-15w | 16-40w |
|---|---|---|---|---|---|---|---|
| Ingredient | | | | Nutrient Levels | | | |
| Corn | 60.50 | 63.55 | 63.78 | | | | |
| Soyabean meal | 33.00 | 30.00 | 16.00 | DM% | 87.00 | 87.00 | 82.20 |
| Soyabean oil | 2.50 | 2.50 | 0 | Crude protein% | 20.10 | 19.10 | 15.40 |
| Limestone | 1.50 | 1.50 | 1.40 | Calcium % | 0.96 | 0.94 | 0.88 |
| Dicalcium phosphate | 1.50 | 1.45 | 1.40 | Available P | 0.36 | 0.35 | 0.35 |
| Met | 0.27 | 0.25 | 0.04 | Total Lys | 1.173 | 1.124 | 0.699 |
| Lys | 0.19 | 0.22 | - | Total Met | 0.565 | 0.533 | 0.295 |
| Premix* | 0.54 | 0.53 | 0.50 | Total Met+Cys | 0.891 | 0.847 | 0.558 |

Note: * The premix provided the nutrients of diets including the VA, VD, VK3,VB1, VB2, VB6, VB12, Cu, Zn, Fe, and Mn, which were in accordance with the nutritional requirement of breeder rooster from each period； Specifically, vitamin premix in control groups were supplied without folic acid (Do Better Victory Bio LTD, shaanxi, china), and vitamin premix in four folate supplement groups were supplied with extra folic acid.

**Supplementary Table S2 Composition and nutrient level of the basal diet (air-dry basis) of broiler offspring.**

| Ingredients (%) | | Nutrient levels | |
|---|---|---|---|
| Corn | 60.50 | DM % | 87.00 |
| Soybean meal | 33.00 | Crude protein% | 20.10 |
| Soyabean oil | 2.50 | Calcium % | 0.96 |
| Limestone | 1.50 | Available P % | 0.36 |
| CaHPO4 | 1.50 | Total Lys, % | 1.17 |
| Salt | 0.30 | Total Met, % | 0.57 |
| choline | 0.02 | Total Met+Cys, % | 0.89 |
| Premix* | 0.13 | | |
| Methionine | 0.27 | | |
| Lysine | 0.19 | | |
| Threonine | 0.09 | | |
| Total | 100.00 | | |

Note: * The premix provided the nutrients of diets including the VA, VD, VK3,VB1, VB2, VB6, VB12, Cu, Zn, Fe, and Mn, which were in accordance with the nutritional requirement of broilers.

**Supplementary Table S3 Primer sequences for quantitative real-time PCR analysis and the PCR of the 3'UTR of PEPCK or ANGPTL4 mRNA.**

| Gene | Primer Sequence（5'-3'） | Tm/℃ | Product/ bp |
|---|---|---|---|
| PEPCK-3'UTR-F | ccgCTCGAGTCCACACATTCTGCAACAGGA | 59.86 | 219 |
| PEPCK-3'UTR-R | attGCGGCCGCTTCCCACAGCTGCTCAACAA | 60.11 | |
| ANGPTL4-3'UTR-F | ccgCTCGAGCTGGGAAAAACGCTCACAGC | 60.04 | 209 |
| ANGPTL4-3'UTR-R | attGCGGCCGCGTCAGAGCATACAGCTGGCA | 60.11 | |
| PEPCK-F | ACATCATGGTGGAGCAAGGC | 60.68 | 189 |
| PEPCK-R | CCTCTTCTGACATCCAGCGA | 59.18 | |
| MYCL -F | AAACTTTCCACGGGAACTCCG | 60.81 | 200 |
| MYCL –R | ACCGTGACCACATCAATCTCT | 59.10 | |
| ANGPTL4-F | AGCCATAATGTGAGCGTGGA | 59.46 | 199 |
| ANGPTL4-R | AGTCCACAGAGCCATCCGTA | 60.32 | |
| THRSP-F | CAGGTTCTGACCGACCTCAC | 60.04 | 137 |
| THRSP-R | ACGTCAGATCACCCTTGGAG | 59.10 | |
| β-actin-F | ATTGTCCACGCAAATGCTTC | 59.8 | 113 |
| β-actin-R | AAATAAAGCCATGCCAACTCGTC | 60.0 | |

**Supplementary Table S4 Effect of dietary folate supplementation on the reproductive performance of breeder roosters**

| Items | Folate concentration(mg/kg) | | | | | SEM | P value |
|---|---|---|---|---|---|---|---|
| | 0 | 0.25 | 1.25 | 2.50 | 5.00 | | |
| Volume of ejaculation | 0.64 | 0.62 | 0.73 | 0.68 | 0.73 | 0.040 | 0.853 |
| Sperm viability | 0.89 | 0.92 | 0.86 | 0.91 | 0.92 | 0.010 | 0.541 |
| Sperm motility | 0.82 | 0.79 | 0.81 | 0.85 | 0.79 | 0.010 | 0.343 |
| Sperm density; $\times 10^9$ | 3.19 | 3.89 | 3.11 | 3.40 | 2.82 | 0.140 | 0.161 |
| Sperm Plasma membrane integrity | 0.996 | 0.983 | 0.991 | 0.999 | 0.994 | 0.002 | 0.072 |
| Acrosomal integrity | 0.987 | 0.954 | 0.966 | 0.981 | 0.988 | 0.005 | 0.193 |
| Mitochondrial activity | 0.972 | 0.944 | 0.972 | 0.974 | 0.940 | 0.001 | 0.415 |
| Fertilization rate | 0.94 | 0.95 | 0.97 | 0.95 | 0.95 | 0.010 | 0.711 |
| Hatchability of fertile eggs | $0.85^{b}$ | $0.88^{ab}$ | $0.92^{a}$ | $0.92^{a}$ | $0.93^{a}$ | 0.010 | 0.016 |

Note: Data within a column without the same superscripts differ significantly ($P < 0.05$).

**Supplementary Table S5 Effect of dietary folate supplementation in breeder roosters on the lipid and glucose metabolism of breeder roosters and broiler offspring.**

| Items | | | Folate concentration(mg/kg) | | | | | P values |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 0.25 | 1.25 | 2.50 | 5.00 | |
| Plasma lipid metabolism | Total glyceride (mmol/L) | Breeder rooster | 0.33±0.05 | 0.45±0.05 | 0.44±0.05 | 0.31±0.05 | 0.36±0.01 | 0.132 |
| | | 1-d-old offspring | 0.68±0.01$^{a}$ | 0.81±0.10$^{a}$ | 0.86±0.08$^{a}$ | 1.15±0.09$^{b}$ | 0.69±0.07$^{a}$ | 0.001 |
| | | 21-d-old offspring | 0.30±0.02 | 0.24±0.04 | 0.29±0.01 | 0.28±0.04 | 0.36±0.03 | 0.181 |
| | Total cholesterol (mmol/L) | Breeder rooster | 2.37±0.19 | 2.51±0.39 | 1.99±0.13 | 2.34±0.14 | 2.13±0.14 | 0.487 |
| | | 1-d-old offspring | 8.49±0.59 | 9.52±0.46 | 9.93±0.48 | 8.35±0.39 | 8.60±0.58 | 0.145 |
| | | 21-d-old offspring | 5.73±0.65 | 5.09±0.78 | 4.13±0.67 | 4.17±0.39 | 4.12±0.30 | 0.224 |
| | HDL-C (mmol/L) | Breeder rooster | 2.38±0.12$^{a}$ | 1.99±0.07$^{b}$ | 1.82±0.19$^{b}$ | 1.90±0.10$^{b}$ | 1.87±0.18$^{b}$ | 0.043 |
| | | 1-d-old offspring | 3.08±0.17 | 3.07±0.37 | 2.33±0.18 | 2.25±0.24 | 2.49±0.35 | 0.113 |
| | | 21-d-old offspring | 1.94±0.23$^{a}$ | 1.48±0.06$^{bc}$ | 1.41±0.09$^{c}$ | 1.48±0.14$^{bc}$ | 1.87±0.10$^{ab}$ | 0.032 |
| | LDL-C (mmol/L) | Breeder rooster | 6.43±0.29$^{a}$ | 5.66±0.19$^{b}$ | 5.81±0.41$^{b}$ | 7.17±0.43$^{a}$ | 6.82±0.17$^{a}$ | 0.034 |
| | | 1-d-old offspring | 4.14±0.31$^{ab}$ | 4.97±0.22$^{a}$ | 4.51±0.44$^{ab}$ | 3.77±0.20$^{b}$ | 3.52±0.38$^{b}$ | 0.034 |
| | | 21-d-old offspring | 1.04±0.08$^{a}$ | 0.89±0.14$^{ab}$ | 0.63±0.10$^{b}$ | 0.63±0.05$^{b}$ | 0.68±0.03$^{b}$ | 0.014 |
| | VLDL-C (mmol/L) | Breeder rooster | 46.54±5.28$^{a}$ | 34.48±2.56$^{b}$ | 32.78±1.78$^{b}$ | 34.52±0.38$^{b}$ | 40.48±3.23$^{ab}$ | 0.037 |
| | | 1-d-old offspring | 72.03±7.36$^{a}$ | 44.25±4.46$^{bc}$ | 29.52±1.94$^{c}$ | 48.46±5.25$^{bc}$ | 53.30±10.87$^{ab}$ | 0.004 |
| | | 21-d-old offspring | 77.16±11.83 | 75.20±14.90 | 90.16±14.85 | 74.81±5.65 | 75.21±8.90 | 0.799 |
| | FFA (mmol/L) | Breeder rooster | 0.37±0.06 | 0.47±0.09 | 0.66±0.14 | 0.51±0.08 | 0.70±0.12 | 0.142 |
| | | 1-d-old offspring | 0.68±0.06$^{c}$ | 0.86±0.07$^{bc}$ | 0.89±0.08$^{bc}$ | 1.60±0.17$^{a}$ | 1.27±0.28$^{ab}$ | 0.003 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 21-d-old offspring | 0.78±0.06 | 0.90±0.08 | 0.93±0.09 | 0.92±0.09 | 0.97±0.07 | 0.571 |
| | Leptin | Breeder rooster | 13.87±3.93 | 9.04±1.77 | 10.19±0.98 | 9.17±1.35 | 12.48±2.03 | 0.484 |
| | (pg/L) | 1-d-old offspring | 25.99±6.22 | 92.55±56.08 | 38.03±22.96 | 28.17±10.93 | 18.54±4.77 | 0.360 |
| | | 21-d-old offspring | 161.18±42.60 | 69.13±9.04 | 117.31±23.75 | 305.30±201.94 | 75.87±42.01 | 0.398 |
| | Adiponectin | Breeder rooster | 342.21±41.89$^{a}$ | 230.09±20.26$^{b}$ | 210.78±8.90$^{b}$ | 202.15±18.48$^{b}$ | 198.04±24.64$^{b}$ | 0.003 |
| | (pg/L) | 1-d-old offspring | 228.99±25.77 | 293.51±67.45 | 338.68±40.66 | 300.91±68.59 | 258.42±33.67 | 0.605 |
| | | 21-d-old offspring | 286.54±41.82$^{a}$ | 198.13±14.28$^{b}$ | 178.12±13.48$^{b}$ | 173.69±6.59$^{b}$ | 178.35±28.00$^{b}$ | 0.018 |
| Hepatic lipid | TP | Breeder rooster | 6.08±0.40$^{b}$ | 5.97±0.20$^{b}$ | 6.48±0.12$^{ab}$ | 6.98±0.22$^{b}$ | 7.18±0.10$^{a}$ | 0.005 |
| metabolism | (mg/ml) | 1-d-old offspring | 10.83±0.61 | 10.82±0.29 | 11.62±0.36 | 10.06±0.36 | 11.25±0.35 | 0.129 |
| | | 21-d-old offspring | 12.20±1.09 | 11.62±0.26 | 11.97±0.73 | 11.37±0.14 | 11.80±0.32 | 0.899 |
| | TG | Breeder rooster | 0.106±0.008$^{b}$ | 0.140±0.003$^{a}$ | 0.138±0.005$^{a}$ | 0.144±0.014$^{a}$ | 0.161±0.015$^{a}$ | 0.015 |
| | (mmol/gprot) | 1-d-old offspring | 0.111±0.009 | 0.137±0.024 | 0.128±0.006 | 0.123±0.020 | 0.141±0.009 | 0.670 |
| | | 21-d-old offspring | 0.108±0.020 | 0.117±0.023 | 0.124±0.015 | 0.099±0.006 | 0.117±0.012 | 0.816 |
| | TC | Breeder rooster | 0.052±0.005$^{b}$ | 0.068±0.003$^{a}$ | 0.072±0.003$^{a}$ | 0.070±0.006$^{a}$ | 0.070±0.004$^{a}$ | 0.041 |
| | (mmol/gprot) | 1-d-old offspring | 0.226±0.009$^{b}$ | 0.276±0.014$^{a}$ | 0.272±0.009$^{a}$ | 0.280±0.013$^{a}$ | 0.292±0.017$^{a}$ | 0.020 |
| | | 21-d-old offspring | 0.035±0.004$^{b}$ | 0.048±0.003$^{a}$ | 0.048±0.002$^{a}$ | 0.052±0.005$^{a}$ | 0.045±0.002$^{b}$ | 0.030 |
| | HDL-C | Breeder rooster | 0.012±0.002$^{ab}$ | 0.013±0.002$^{a}$ | 0.010±0.002$^{abc}$ | 0.006±0.002$^{bc}$ | 0.004±0.002$^{c}$ | 0.033 |
| | (mmol/gprot) | 1-d-old offspring | 0.018±0.001 | 0.017±0.002 | 0.013±0.002 | 0.018±0.002 | 0.019±0.002 | 0.345 |
| | | 21-d-old offspring | 0.005±0.001 | 0.004±0.001 | 0.004±0.001 | 0.007±0.000 | 0.005±0.001 | 0.132 |
| | LDL-C | Breeder rooster | 0.018±0.001 | 0.024±0.001 | 0.022±0.001 | 0.022±0.002 | 0.023±0.002 | 0.339 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (mmol/gprot) | 1-d-old offspring | 0.084±0.011 | 0.099±0.002 | 0.091±0.012 | 0.088±0.017 | 0.084±0.005 | 0.475 |
| | | 21-d-old offspring | 0.014±0.001 | 0.015±0.001 | 0.013±0.000 | 0.013±0.000 | 0.013±0.001 | 0.462 |
| | VLDL | Breeder rooster | 49.55±3.74 | 49.32±4.40 | 46.61±3.12 | 41.64±2.73 | 39.54±2.07 | 0.153 |
| | (mmol/gprot) | 1-d-old offspring | 38.25±2.68 | 37.20±2.91 | 33.20±2.00 | 40.48±1.49 | 32.89±1.52 | 0.101 |
| | | 21-d-old offspring | 31.03±2.33 | 32.70±1.89 | 33.19±2.97 | 35.13±1.30 | 30.02±0.71 | 0.441 |
| | FFA | Breeder rooster | 0.050±0.005$^{b}$ | 0.046±0.005$^{b}$ | 0.070±0.009$^{ab}$ | 0.080±0.011$^{a}$ | 0.086±0.008$^{a}$ | 0.008 |
| | (mmol/gprot) | 1-d-old offspring | 0.071±0.008 | 0.079±0.014 | 0.073±0.002 | 0.080±0.013 | 0.089±0.018 | 0.866 |
| | | 21-d-old offspring | 0.033±0.005$^{c}$ | 0.044±0.011$^{bc}$ | 0.086±0.006$^{a}$ | 0.061±0.006$^{b}$ | 0.064±0.004$^{b}$ | 0.000 |
| | MDH | Breeder rooster | 33.09±1.39 | 38.39±2.33 | 39.35±4.16 | 35.25±1.82 | 41.89±3.46 | 0.240 |
| | (U/gprot) | 1-d-old offspring | 30.24±1.11$^{a}$ | 31.07±1.02$^{a}$ | 33.62±2.78$^{ab}$ | 38.84±3.75$^{bc}$ | 41.73±1.22$^{c}$ | 0.006 |
| | | 21-d-old offspring | 31.17±1.81 | 35.71±0.68 | 30.50±0.64 | 29.33±0.52 | 30.14±3.19 | 0.110 |
| | HL | Breeder rooster | 2,28±0.23 | 2.63±0.10 | 2.33±0.14 | 2.15±0.07 | 2.06±0.11 | 0.078 |
| | (U/gprot) | 1-d-old offspring | 2.13±0.11 | 2.18±0.04 | 1.99±0.07 | 2.29±0.10 | 2.08±0.11 | 0.221 |
| | | 21-d-old offspring | 1.84±0.15 | 1.97±0.05 | 1.84±0.10 | 1.86±0.05 | 1.84±0.04 | 0.795 |
| | Carnitine | Breeder rooster | 0.779±0.064$^{a}$ | 0.759±0.050$^{a}$ | 0.657±0.027$^{ab}$ | 0.565±0.042$^{b}$ | 0.601±0.025$^{b}$ | 0.009 |
| | (μg/gprot) | 1-d-old offspring | 0.558±0.044 | 0.474±0.028 | 0.489±0.019 | 0.542±0.012 | 0.503±0.032 | 0.238 |
| | | 21-d-old offspring | 0.486±0.039 | 0.452±0.037 | 0.479±0.048 | 0.532±0.027 | 0.480±0.016 | 0.619 |
| Hepatic glucose metabolism | PEPCK (U/mgprot) | Breeder rooster | 2.35±0.36$^{c}$ | 3.29±0.45b$^{c}$ | 3.67±0.55$^{bc}$ | 5.00±0.21$^{ab}$ | 5.61±1.02$^{a}$ | 0.006 |
| | | 1-d-old offspring | 2.31±0.32$^{b}$ | 4.18±0.52$^{a}$ | 4.41±0.78$^{a}$ | 4.80±0.38$^{a}$ | 4.17±0.38$^{a}$ | 0.021 |
| | | 21-d-old offspring | 0.75±0.07 | 0.61±0.22 | 0.90±0.15 | 0.91±0.18 | 0.78±0.09 | 0.621 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hexokinase (U/gprot) | Breeder rooster | 90.23±8.69 | 80.82±3.93 | 94.08±4.64 | 98.97±3.53 | 102.52±3.11 | 0.072 |
| | 1-d-old offspring | 51.50±2.91 | 51.47±1.36 | 55.26±1.72 | 47.84±1.69 | 53.47±1.64 | 0.129 |
| | 21-d-old offspring | 60.03±2.00 | 62.01±2.81 | 62.57±1.76 | 64.89±1.73 | 56.84±2.10 | 0.128 |
| Pyruvate kinase (mmol/gprot) | Breeder rooster | 430.86±105.75 | 595.24±110.54 | 312.56±32.83 | 368.35±77.20 | 514.13±52.45 | 0.147 |
| | 1-d-old offspring | 869.99±168.94$^{b}$ | 990.96±152.01$^{b}$ | 1121.25±215.69$^{b}$ | 1283.38±135.33$^{ab}$ | 1700.29±231.07$^{a}$ | 0.050 |
| | 21-d-old offspring | 780.37±64.07$^{b}$ | 807.96±41.32$^{b}$ | 855.20±110.72$^{b}$ | 974.50±13.99$^{ab}$ | 1081.65±66.34$^{a}$ | 0.026 |
| 6-phosphofructokinase (mmol/gprot) | Breeder rooster | 7.99±0.89 | 8.28±0.23 | 7.02±1.09 | 6.87±0.77 | 7.76±0.79 | 0.678 |
| | 1-d-old offspring | 6.93±1.66 | 8.32±2.23 | 5.74±0.75 | 7.72±1.90 | 6.26±0.39 | 0.765 |
| | 21-d-old offspring | 5.55±0.46 | 7.33±0.95 | 8.16±1.02 | 6.57±0.72 | 5.62±1.34 | 0.268 |
| Citroyl synthetase (mmol/gprot) | Breeder rooster | 22.55±1.78$^{b}$ | 16.67±5.22$^{c}$ | 28.27±1.91$^{a}$ | 27.78±1.10$^{a}$ | 29.00±0.73$^{a}$ | 0.000 |
| | 1-d-old offspring | 36.60±2.62 | 33.74±1.44 | 33.99±0.74 | 31.94±1.66 | 35.70±2.19 | 0.447 |
| | 21-d-old offspring | 30.39±1.92$^{c}$ | 39.38±1.09$^{ab}$ | 38.81±1.73$^{ab}$ | 41.42±1.87$^{a}$ | 34.80±2.60$^{bc}$ | 0.004 |
| Glucogen (mg/g liver) | Breeder rooster | 21.69±11.80 | 28.82±3.28 | 28.72±5.80 | 14.72±3.41 | 18.13±5.47 | 0.380 |
| | 1-d-old offspring | 5.95±0.60 | 5.79±0.16 | 6.27±0.39 | 7.02±0.60 | 6.14±1.04 | 0.681 |
| | 21-d-old offspring | 4.27±0.27 | 4.20±0.14 | 5.04±0.72 | 4.74±0.60 | 5.28±1.17 | 0.754 |

Note: Data within a column without the same superscripts differ significantly ($P < 0.05$).

**Supplementary Table S6 The key miRNAs and lncRNAs in sperm that regulated the glucose and lipid metabolism of broiler offspring.**

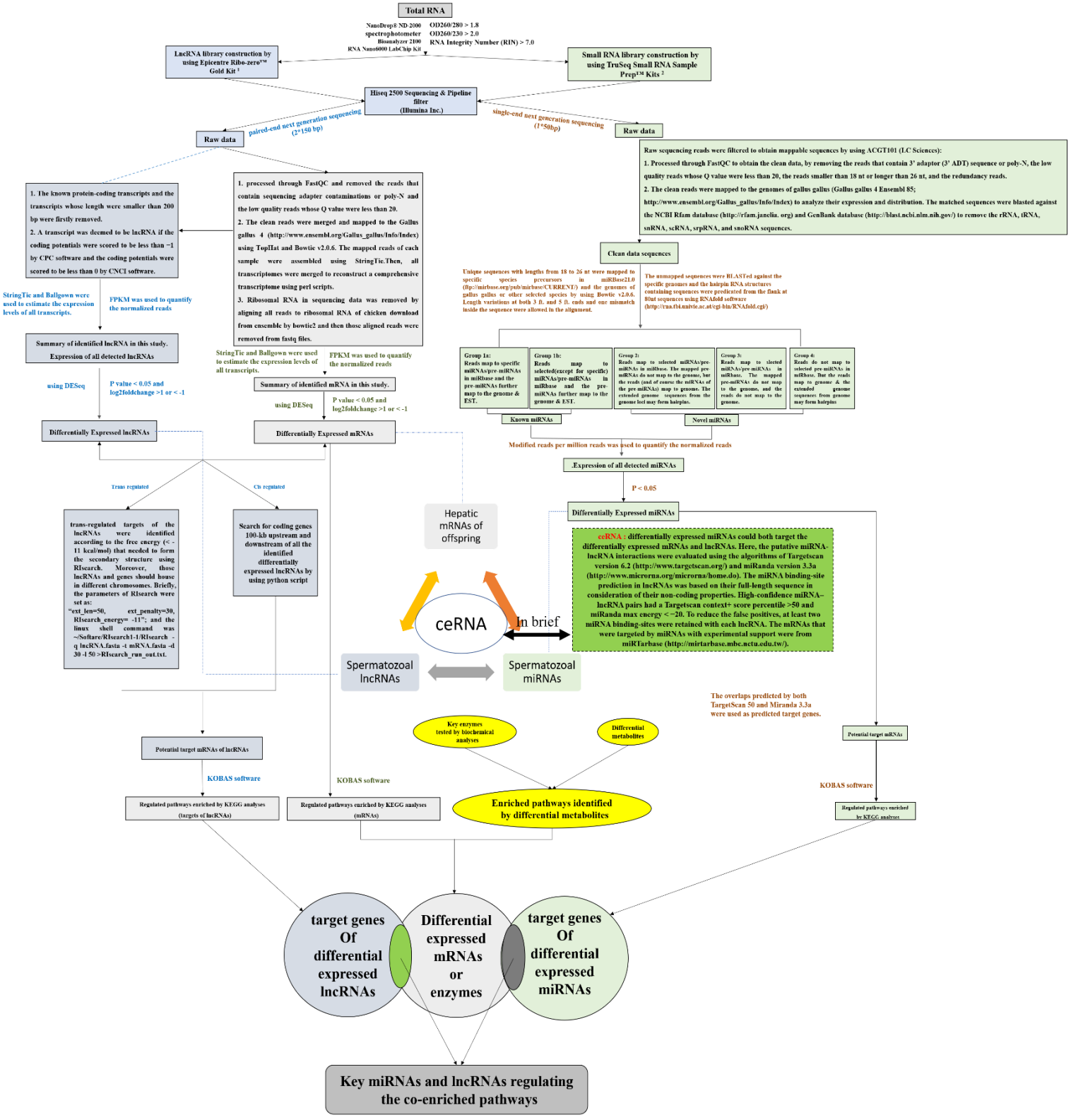
| key pathways | Hepatic differentially expressed mRNAs and significantly altered enzymes in offspring | Spermatozoal differentially expressed miRNA | Spermatozoal differentially expressed lncRNA |
|---|---|---|---|
| Glycolysis/ Gluconeogenesis | PEPCK | PC-5p-30232_97 | TCONS_00098732, TCONS_00098733, TCONS_00098734, TCONS_00099848 |
| | Hexokinase | PC-3p-165579_16, gga-miR-215-5p, bta-miR-192_R-3 | TCONS_00162819 |
| | 6-phosphofructokinase | PC-3p-199137_13, hsa-miR-143-3p_R+1, gga-miR-214_R+1 | TCONS_00082843, TCONS_00082844, TCONS_00082845, TCONS_00082846, TCONS_00082847, TCONS_00082848, TCONS_00082849, TCONS_00082850, TCONS_00082852, TCONS_00082855, TCONS_00082856, TCONS_00082857, TCONS_00082858, TCONS_00082859 |
| Pyruvate metabolism | Pyruvate kinase | PC-3p-353456_7, PC-5p-122036_22, PC-3p-15271_190 | TCONS_00045814, TCONS_00045815, TCONS_00045816, TCONS_00045817 |
| | MDH (ME3) | PC-3p-353456_7, PC-5p-46964_61, PC-3p-20654_141, gga-miR-135a-5p | TCONS_00021199, TCONS_00043468 |
| PPAR signaling pathway | PEPCK | PC-5p-30232_97 | TCONS_00098732, TCONS_00098733, TCONS_00098734, TCONS_00099848 |
| | ANGPTL4 | | TCONS_00107090, TCONS_00107092 |
| | FABP5 | age-miR-93_L+1R-2, PC-5p-309272_8, bta-miR-25, PC-5p-1083694_2, PC-3p-52806_54, bta-miR-378, gga-miR-214_R+1 | |
| | Adiponectin (ADIPOQ) | gga-miR-30a-3p_R-3, PC-3p-199137_13 | TCONS_00179256 |

**Supplementary Table S7 The spermatozoal miRNAs and lncRNAs that regulated ANGPTL4 (ENSGALT00000000877) expression through ceRNA mechanism.**

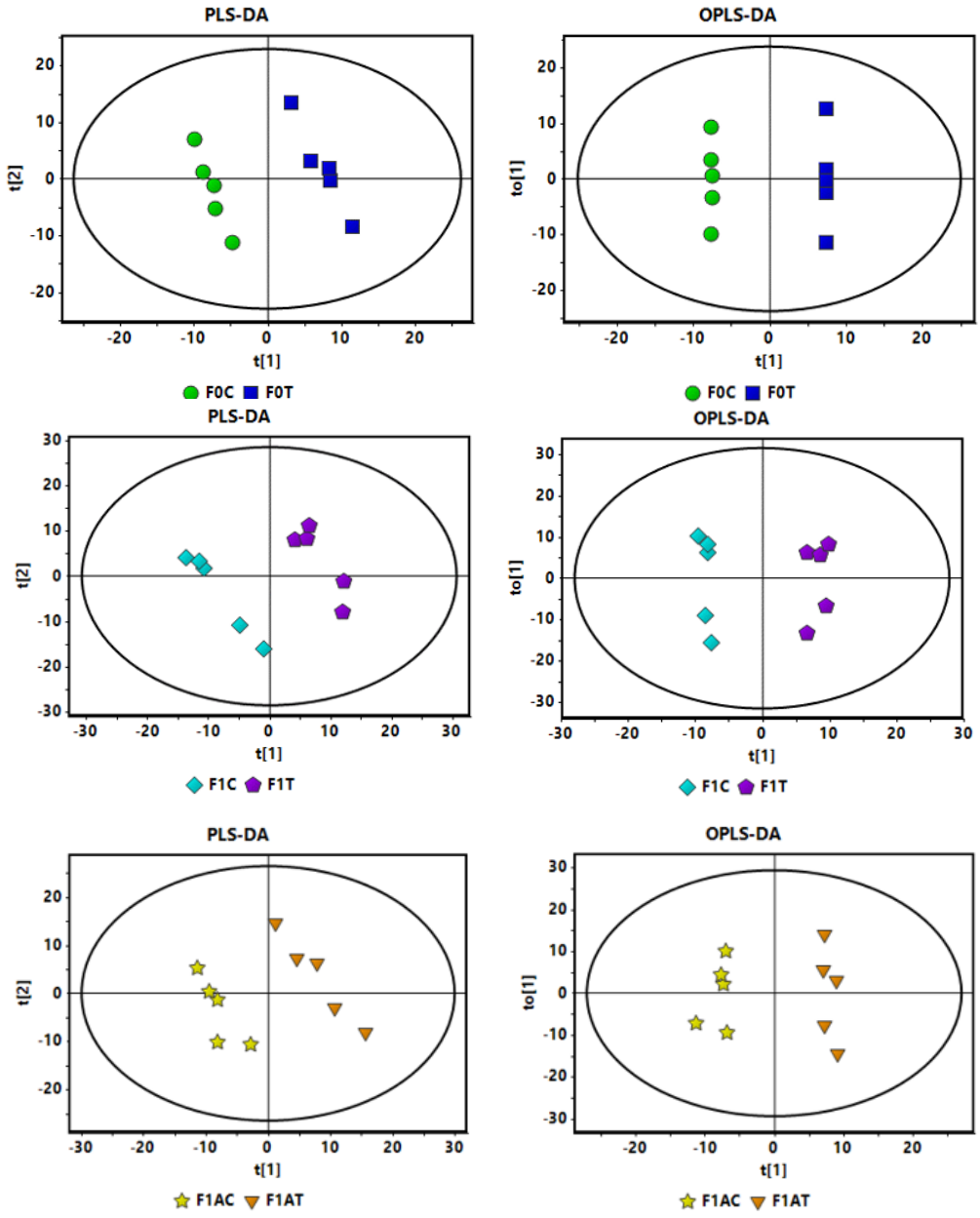
| mRNA | gene | KEGG | regulation | miRNA | regulation | lncRNA | regulation | p value |
|---|---|---|---|---|---|---|---|---|
| ENSGALT00000000877 | ANGPTL4 | PPAR signaling pathway | up | PC-3p-448507_5 | up | TCONS_00009366 | up | 0.03 |
| | | | | | | TCONS_00035238 | up | 0.02 |
| | | | | | | TCONS_00035244 | up | 0.03 |
| | | | | | | TCONS_00045762 | up | 0.00 |
| | | | | | | TCONS_00047758 | up | 0.05 |
| | | | | | | TCONS_00055846 | up | 0.03 |
| | | | | | | TCONS_00055870 | up | 0.03 |
| | | | | | | TCONS_00060475 | up | 0.02 |
| | | | | | | TCONS_00063365 | up | 0.05 |
| | | | | | | TCONS_00068695 | up | 0.01 |
| | | | | | | TCONS_00070335 | up | 0.01 |
| | | | | | | TCONS_00070570 | up | 0.04 |
| | | | | | | TCONS_00073624 | up | 0.01 |
| | | | | | | TCONS_00075331 | up | 0.05 |
| | | | | | | TCONS_00076442 | up | 0.01 |
| | | | | | | TCONS_00076790 | up | 0.02 |
| | | | | | | TCONS_00076814 | up | 0.03 |
| | | | | | | TCONS_00077751 | up | 0.00 |
| | | | | | | TCONS_00080115 | up | 0.04 |
| | | | | | | TCONS_00080408 | up | 0.02 |
| | | | | | | TCONS_00080666 | up | 0.02 |
| | | | | | | TCONS_00081862 | up | 0.01 |
| | | | | | | TCONS_00104587 | up | 0.03 |
| | | | | | | TCONS_00122312 | up | 0.04 |
| | | | | | | TCONS_00122792 | up | 0.04 |

| | | | |
|---|---|---|---|
| | **TCONS_00126809** | **up** | **0.02** |
| | **TCONS_00127001** | **up** | **0.03** |
| | **TCONS_00127158** | **up** | **0.03** |
| | **TCONS_00127515** | **up** | **0.03** |
| | **TCONS_00128356** | **up** | **0.04** |
| | **TCONS_00129449** | **up** | **0.03** |
| | **TCONS_00130849** | **up** | **0.02** |
| | **TCONS_00131456** | **up** | **0.02** |
| | **TCONS_00131821** | **up** | **0.01** |
| | **TCONS_00132033** | **up** | **0.05** |
| | **TCONS_00132465** | **up** | **0.01** |
| | **TCONS_00147310** | **up** | **0.01** |
| | **TCONS_00159338** | **up** | **0.04** |
| | **TCONS_00172934** | **up** | **0.02** |
| | **TCONS_00194782** | **up** | **0.03** |
| | **TCONS_00199637** | **up** | **0.02** |
| | **TCONS_00202693** | **up** | **0.04** |
| | **TCONS_00205049** | **up** | **0.02** |
| | **TCONS_00206316** | **up** | **0.04** |
| | **TCONS_00207843** | **up** | **0.01** |
| | **TCONS_00210911** | **up** | **0.02** |
| | **TCONS_00219282** | **up** | **0.04** |
| | **TCONS_00224432** | **up** | **0.00** |
| | **TCONS_00226237** | **up** | **0.02** |
| | **TCONS_00239341** | **up** | **0.01** |
| | **TCONS_00247523** | **up** | **0.01** |

Figure S1




**Supplementary Figure S1 Bioinformatic workflow used in the present study.**




**Supplementary Figure S2 Distribution of the liver samples of the CON and FS2 groups of breeder roosters, 1-day-old broiler offspring, and 21-day-old broiler offspring based on PLS-DA and OPLS-DA analyses.** Note: t(1), first principal component; t(2), second principal component; to(1), orthogonal component. F0, F1, and F1A represent the liver samples from breeder roosters, 1-d-old and 21-d-old broilers offspring, C and T represent the samples from control and 1.25 mg/kg folate supplementation groups.

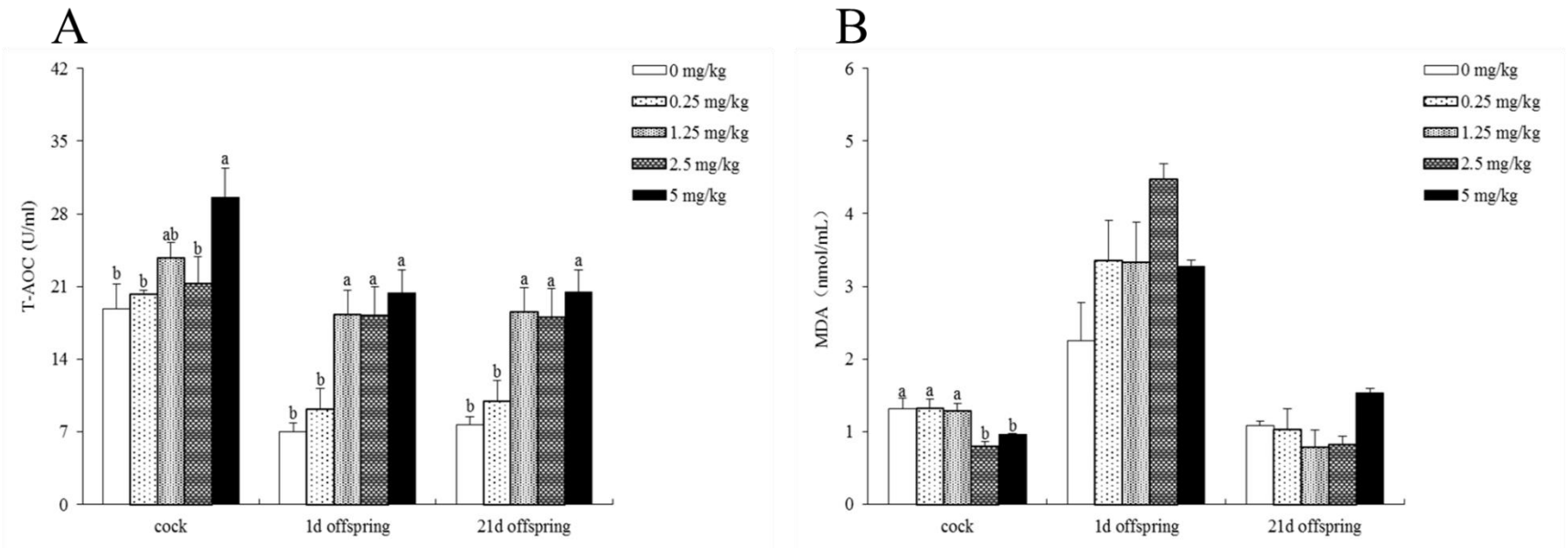



Figure S3 Altered antioxidant ability of breeder roosters and broiler offspring induced by dietary folate supplementation in breeder roosters. (A) Altered T-AOC. (B) Altered malondialdehyde.

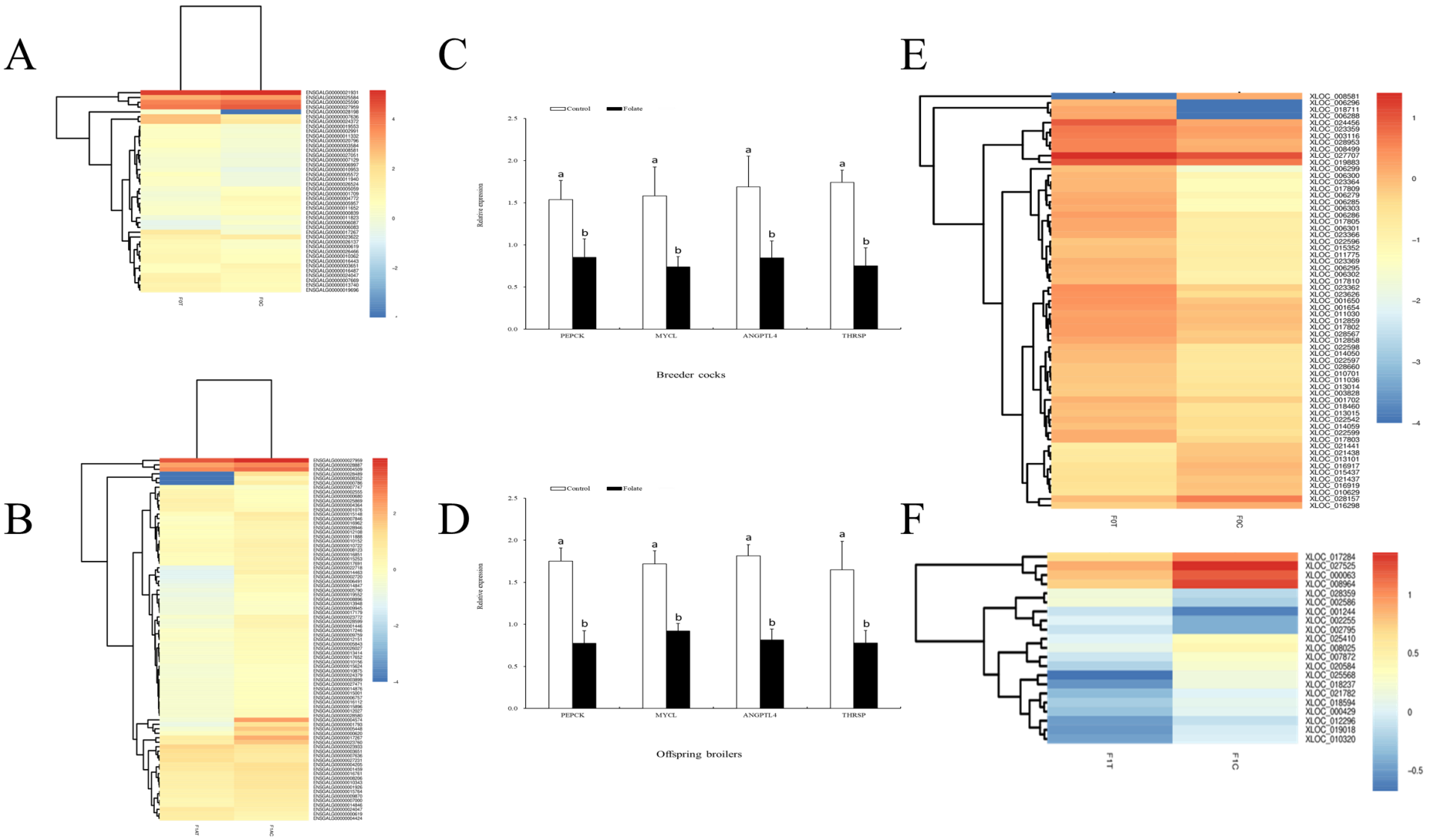



Figure S4 Dietary folate supplementation of breeder roosters induces differentially expressed genes and lncRNAs in the livers of breeder

roosters and 1-day-old broiler offspring. (A-B) Hepatic differentially expressed mRNAs of breeder roosters and broiler offspring. (C-D) Validation of the accuracy of RIBO-Zero RNA-seq data by qRT-PCR. (E-F) Hepatic differentially expressed lncRNAs of breeder roosters and broiler offspring

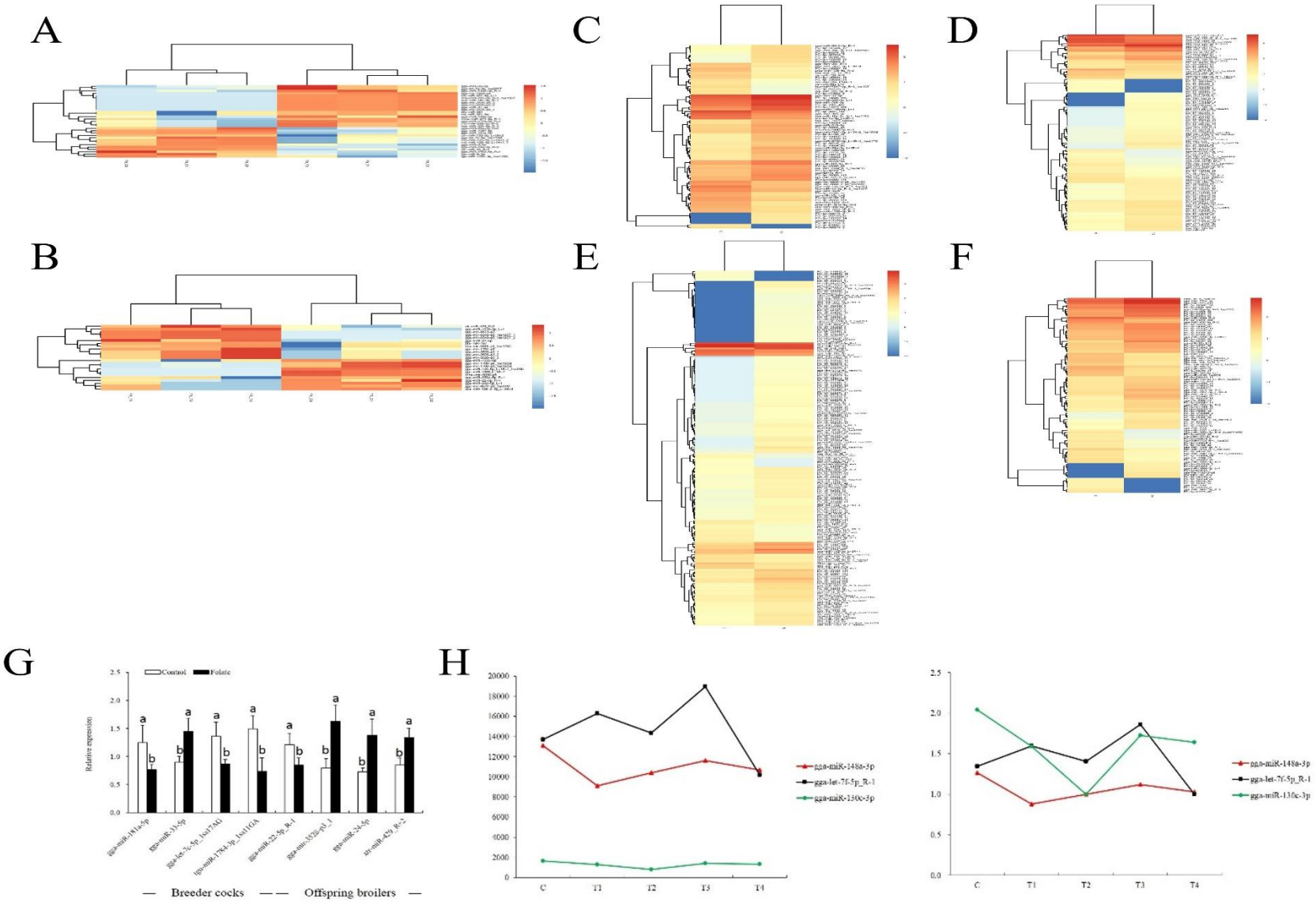

A
B
C
D
E
F
G
H
Control
Folate
Relative expression
Breeder cocks
Offspring broilers
gga-miR-148a-5p
gga-let-7f-5p_R-1
gga-miR-130c-3p
C
T1
T2
T3
T4

Figure S5 Transgenerational regulatory roles of miRNAs in regulating lipid and glucose metabolism in response to paternal folate supplementation. (A-B): Differentially expressed miRNAs in the livers of breeder roosters and broiler offspring. (C-F) Differentially expressed miRNAs in the sperm of breeder roosters. (G-H) Validation of the accuracy of miRNA-seq data by qRT-PCR; G for 8 hepatic differentially expressed miRNAs, and H for 3 spermatozoal differentially expressed miRNAs.