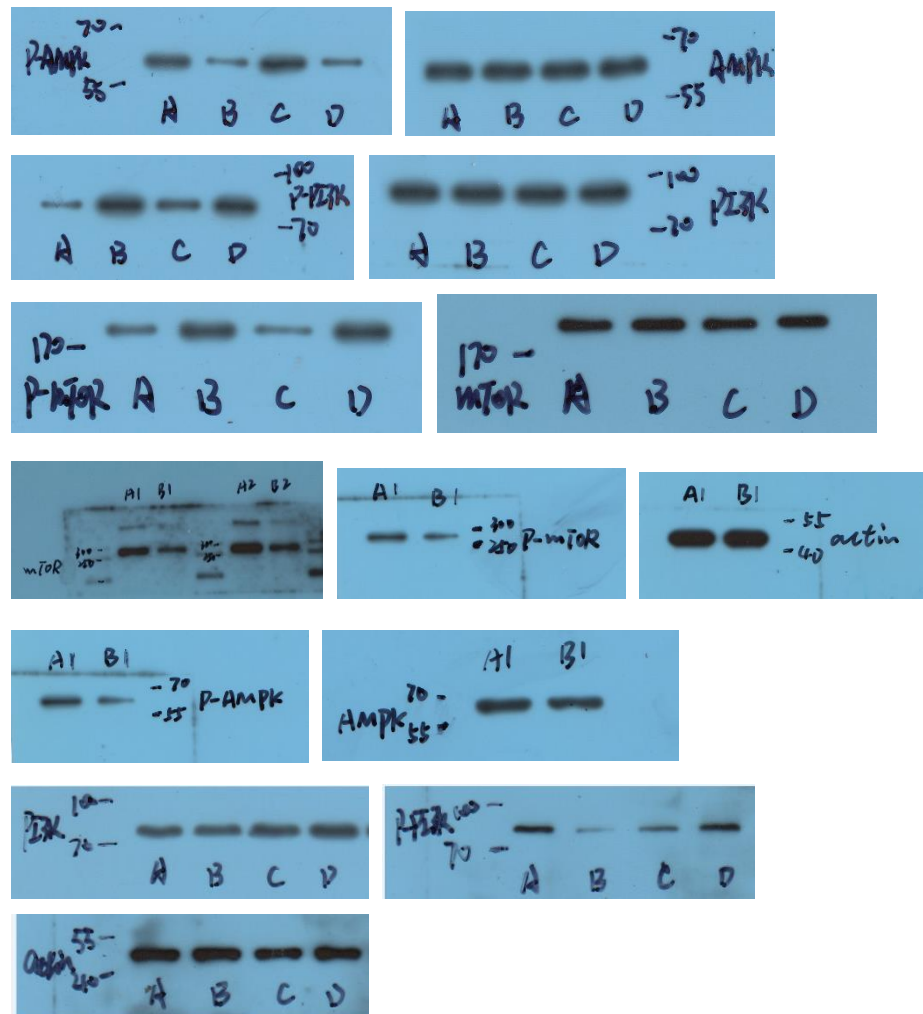


**Figure 3. Metformin inhibits mTOR phosphorylation in pathological B cells.**

A, phosphorylated (P-) AMPK (S487)/AMPK, P-PI3K p85 (Tyr458)/PI3K p85 and P-mTOR (S2481)/ mTOR in B cells between women with PCOS and control subjects.

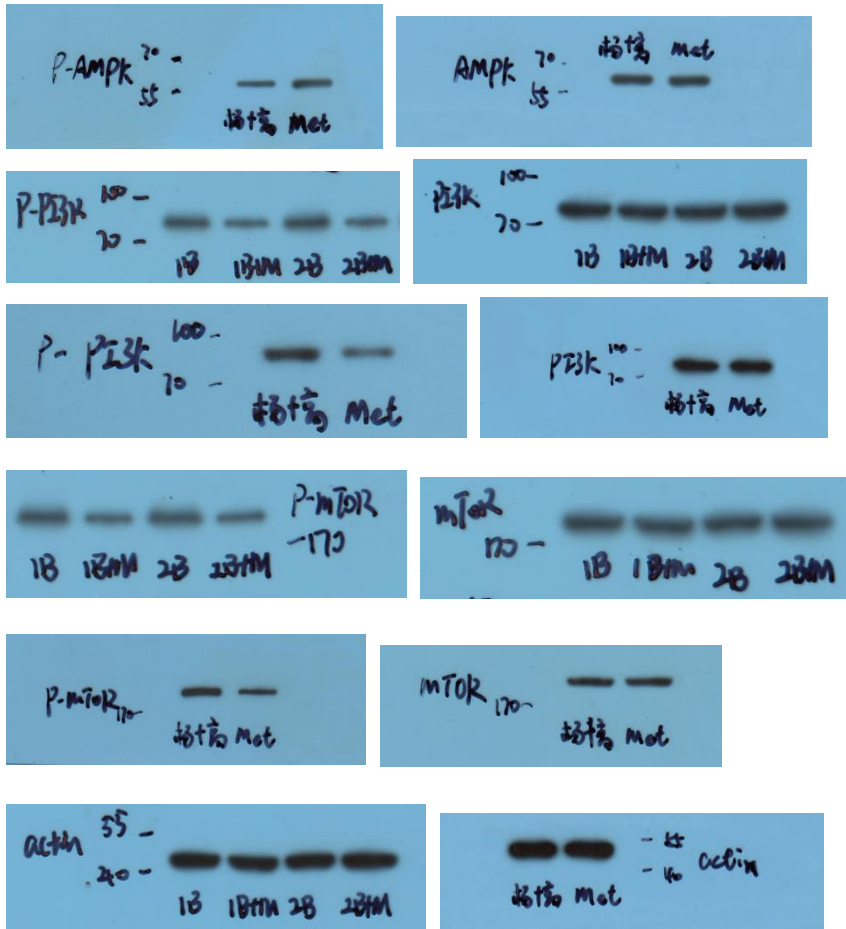


B-D, quantification analysis of the ratios of phosphorylated (P-) AMPK (S487)/AMPK, P-PI3K p85 (Tyr458)/PI3K p85 and P-mTOR (S2481)/ mTOR in B cells.

| P-AMPK (S487)/AMPK |      | P-PI3K p85 (Tyr458)/PI3K p85 |      | P-mTOR (S2481)/ mTOR |      |
|--------------------|------|------------------------------|------|----------------------|------|
| Control            | PCOS | Control                      | PCOS | Control              | PCOS |
| 0.4                | 0.08 | 0.26                         | 0.51 | 0.17                 | 0.69 |
| 0.81               | 0.11 | 0.04                         | 0.68 | 0.1                  | 0.81 |
| 0.43               | 0.2  | 0.08                         | 0.57 | 0.25                 | 0.4  |

E, P-AMPK (S487)/AMPK, P-PI3K p85 (Tyr458)/PI3K p85 and P-mTOR (S2481)/ mTOR in stimulated B cells from women with PCOS with or without metformin.

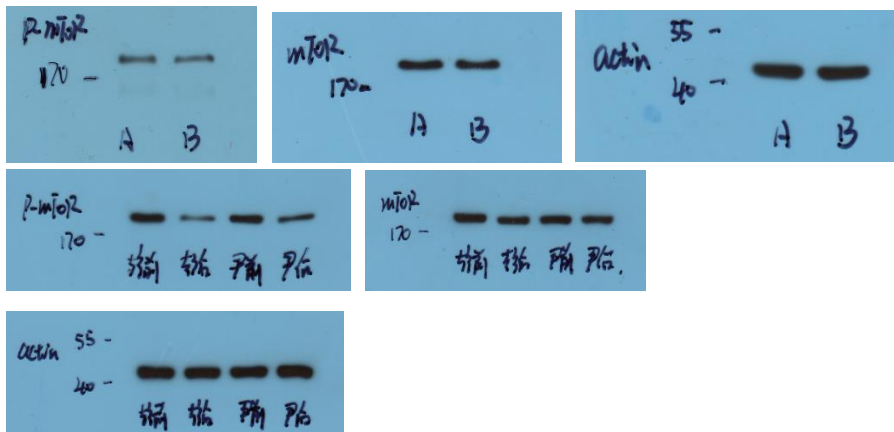




F-H, quantification analysis of the ratios of P-AMPK (S487)/AMPK, P-PI3K p85 (Tyr458)/PI3K p85 and P-mTOR (S2481)/ mTOR in stimulated B cells from women with PCOS with or without metformin.

| P-AMPK (S487)/AMPK |             | P-PI3K p85 (Tyr458)/PI3K p85 |             | P-mTOR (S2481)/ mTOR |             |
|--------------------|-------------|------------------------------|-------------|----------------------|-------------|
| B cells            | B cells+Met | B cells                      | B cells+Met | B cells              | B cells+Met |
| 0.33               | 0.46        | 0.41                         | 0.17        | 0.81                 | 0.38        |
| 0.31               | 0.5         | 0.65                         | 0.21        | 0.7                  | 0.36        |
| 0.27               | 0.48        | 0.53                         | 0.18        | 0.75                 | 0.45        |

I, P-mTOR (S2481)/ mTOR in B cells isolated from women with PCOS, before (pre) and after (post) treatment with metformin.



J, quantification analysis of the ratio of P-mTOR (S2481)/ mTOR in B cells isolated from PB of women with PCOS, before (pre) and after (post) treatment with metformin

| Pre Met | Post Met |
|---------|----------|
| 0.75    | 0.22     |
| 0.72    | 0.48     |
| 0.7     | 0.45     |