

## Ha *et al.* miR-10a/b-5p-NCOR2 Regulates Insulin-Resistant Diabetes in Female Mice

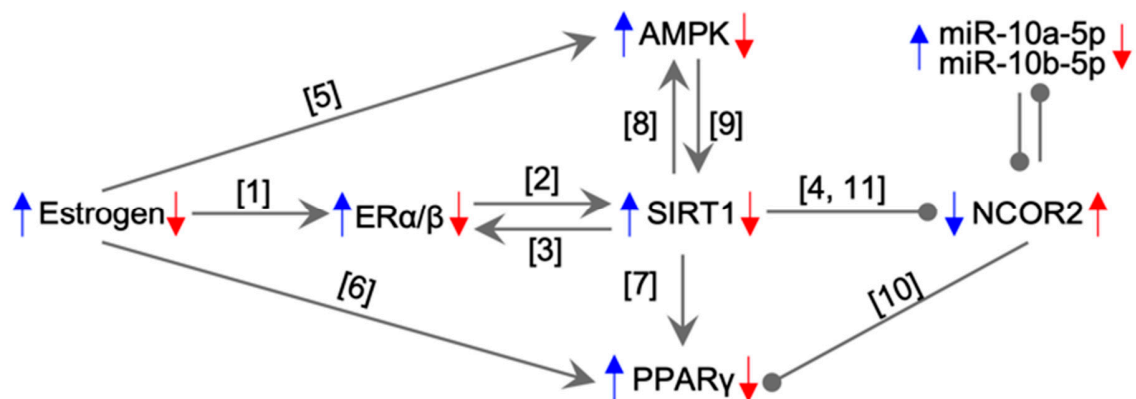
The supporting information contains the following items:

Supplementary Figure S1

Supplementary Tables S1–S3

Supplementary Reference





**Supplementary Figure S1.** Proposed molecular pathway illustrating how estrogen deficiency leads to increased NCOR2 expression, contributing to insulin resistance and type 2 diabetes (T2D) in females. This pathway is based on referenced studies supporting our findings regarding the estrogen-NCOR2-miR-10a/b-5p axis and its role in regulating insulin resistance in females. In the diagram, blue arrows represent upregulation or downregulation of hormones, proteins, and miRNAs in healthy states, while red arrows indicate in diabetic states.



**Supplementary Table S1.** Body weight and food intake in HFHSD mice after the injections of E2 or miR-10a/b mimic (mean  $\pm$  SD)**(A) Body weight**

| Group            | Diet |       | Body weight (g) |                |                |                |                |                |                | Change of body weight (g)<br>(Before vs. PI 6W) | Pvalue* |
|------------------|------|-------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|---|---------|
|                  |      |       | Before          | PI 1W          | PI 2W          | PI 3W          | PI 4W          | PI 5W          | PI 6W          |   |         |
| No injection     | OVX  | ND    | 30.1 $\pm$ 0.8  | 30.7 $\pm$ 0.9 | 31.4 $\pm$ 1.5 | 32.4 $\pm$ 2.2 | 31.9 $\pm$ 1.3 | 31.5 $\pm$ 0.4 | 32.6 $\pm$ 1.4 | 2.5 $\pm$ 0.8                                   | 0.0542  |
| Scrambled RNA    | OVX  | ND    | 31.9 $\pm$ 1.5  | 30.1 $\pm$ 2.2 | 31.5 $\pm$ 1.3 | 32.4 $\pm$ 1.4 | 32.2 $\pm$ 1.1 | 32.6 $\pm$ 0.5 | 32.9 $\pm$ 0.2 | 1.0 $\pm$ 1.1                                   | 0.1011  |
| E2               | OVX  | ND    | 31.5 $\pm$ 1.6  | 32.9 $\pm$ 2.8 | 33.1 $\pm$ 3.1 | 33.6 $\pm$ 4.4 | 33.9 $\pm$ 3.6 | 33.8 $\pm$ 4.1 | 34.2 $\pm$ 1.4 | 2.7 $\pm$ 0.6                                   | 0.0863  |
| miR-10a-5p mimic | OVX  | ND    | 31.7 $\pm$ 2.1  | 31.1 $\pm$ 2.1 | 32.6 $\pm$ 1.5 | 32.8 $\pm$ 2.3 | 33.2 $\pm$ 5.1 | 33.5 $\pm$ 2.6 | 33.9 $\pm$ 3.0 | 2.2 $\pm$ 1.3                                   | 0.2476  |
| miR-10b-5p mimic | OVX  | ND    | 30.7 $\pm$ 2.1  | 31.3 $\pm$ 2.7 | 32.3 $\pm$ 1.4 | 32.8 $\pm$ 1.9 | 33.6 $\pm$ 1.0 | 34.2 $\pm$ 0.9 | 34.3 $\pm$ 2.1 | 3.6 $\pm$ 1.4                                   | 0.1210  |
| No injection     | OVX  | HFHSD | 48.7 $\pm$ 3.8  | 49.4 $\pm$ 3.3 | 49.7 $\pm$ 5.1 | 50.2 $\pm$ 5.1 | 50.6 $\pm$ 5.4 | 51.1 $\pm$ 2.4 | 52.6 $\pm$ 3.6 | 3.9 $\pm$ 2.2                                   | 0.0812  |
| Scrambled RNA    | OVX  | HFHSD | 49.6 $\pm$ 2.6  | 49.8 $\pm$ 2.7 | 50.6 $\pm$ 2.5 | 51.2 $\pm$ 2.0 | 51.8 $\pm$ 1.9 | 52.3 $\pm$ 1.3 | 52.5 $\pm$ 1.4 | 2.9 $\pm$ 1.8                                   | 0.1090  |
| E2               | OVX  | HFHSD | 47.2 $\pm$ 3.6  | 46.9 $\pm$ 2.3 | 47.7 $\pm$ 2.7 | 48.1 $\pm$ 3.6 | 48.6 $\pm$ 2.1 | 49.0 $\pm$ 1.0 | 49.9 $\pm$ 1.3 | 2.7 $\pm$ 0.9                                   | 0.0762  |
| miR-10a-5p mimic | OVX  | HFHSD | 48.8 $\pm$ 4.9  | 49.0 $\pm$ 4.7 | 49.7 $\pm$ 3.4 | 50.1 $\pm$ 3.9 | 50.4 $\pm$ 3.7 | 50.7 $\pm$ 2.3 | 51.4 $\pm$ 2.2 | 2.6 $\pm$ 1.0                                   | 0.0563  |
| miR-10b-5p mimic | OVX  | HFHSD | 47.1 $\pm$ 2.4  | 48.1 $\pm$ 1.8 | 48.4 $\pm$ 3.9 | 49.8 $\pm$ 3.9 | 49.2 $\pm$ 2.5 | 50.0 $\pm$ 2.1 | 50.2 $\pm$ 1.5 | 3.1 $\pm$ 1.3                                   | 0.0618  |

n=5-7 per group

\* $p < 0.05$ , \*\* $p < 0.01$ , versus before injection or given no injection in OVX HFHSD fed-female mice.#PI, Post injection; W, week; OVX, ovariectomized; ND, a normal diet; HFHSD, a high-fat, high-sucrose diet; E2, 17 $\beta$ -estradiol**(B) Food intake**

| Group            | Diet |       | Food intake (g/day) |               |               | Pvalue* |
|------------------|------|-------|---------------------|---------------|---------------|---------|
|                  |      |       | Before              | PI 1W         | PI 3W         |         |
| No injection     | OVX  | ND    | 4.1 $\pm$ 1.3       | 3.6 $\pm$ 0.4 | 3.7 $\pm$ 0.7 | 0.5011  |
| Scrambled RNA    | OVX  | ND    | 3.5 $\pm$ 0.3       | 4.0 $\pm$ 0.5 | 3.8 $\pm$ 0.3 | 0.1002  |
| E2               | OVX  | ND    | 3.8 $\pm$ 0.4       | 3.9 $\pm$ 0.6 | 4.2 $\pm$ 0.2 | 0.0514  |
| miR-10a-5p mimic | OVX  | ND    | 3.5 $\pm$ 0.6       | 3.9 $\pm$ 0.4 | 3.3 $\pm$ 0.6 | 0.0632  |
| miR-10b-5p mimic | OVX  | ND    | 4.3 $\pm$ 0.5       | 4.0 $\pm$ 0.4 | 3.6 $\pm$ 0.4 | 0.0507  |
| No injection     | OVX  | HFHSD | 5.6 $\pm$ 8.7       | 6.0 $\pm$ 0.2 | 5.8 $\pm$ 0.3 | 0.2544  |
| Scrambled RNA    | OVX  | HFHSD | 6.1 $\pm$ 0.1       | 5.6 $\pm$ 0.3 | 5.8 $\pm$ 0.2 | 0.0653  |
| E2               | OVX  | HFHSD | 6.0 $\pm$ 0.8       | 5.8 $\pm$ 0.5 | 6.0 $\pm$ 1.3 | 0.5874  |
| miR-10a-5p mimic | OVX  | HFHSD | 4.7 $\pm$ 1.5       | 4.8 $\pm$ 0.6 | 5.1 $\pm$ 0.1 | 0.2052  |
| miR-10b-5p mimic | OVX  | HFHSD | 5.1 $\pm$ 0.7       | 4.9 $\pm$ 0.3 | 5.3 $\pm$ 0.4 | 0.3220  |

n=3 per group

\* $p < 0.05$ , \*\* $p < 0.01$ , versus before injection or given no injection in OVX HFHSD fed-female mice.



**Supplementary Table S2.** Clinical characteristics of female patients with type 2 diabetes and healthy donors

| Parameters               | Diabetes     | Healthy control |
|--------------------------|--------------|-----------------|
| Number of cases, n       | 37           | 32              |
| Age (years)              | 55 ± 6       | 43 ± 11         |
| A1C (%)                  | 8.0 ± 1.8    | 5.4 ± 0.5       |
| Glucose (mg/dL)          | 161.6 ± 56.5 | 89.4 ± 11.0     |
| Insulin (ng/mL)          | 2.35 ± 0.17  | 0.89 ± 0.4      |
| C-peptide (ng/mL)        | 2.1 ± 0.6    | 1.7 ± 0.7       |
| BMI (kg/m <sup>2</sup> ) | 23.7 ± 3.2   | 22.2 ± 3.5      |



**Supplementary Table S3.** Primary antibodies used in this study

| Name             | Vendor (Item #)          | Host   | Clonality  | MW  | Concentration        |
|------------------|--------------------------|--------|------------|-----|----------------------|
| p-AKT(Ser473)    | ThermoFisher (44-621G)   | Rabbit | Polyclonal | 55  | 1:200 WES            |
| Insulin          | Abcam (ab181547)         | Rabbit | Monoclonal | 12  | 1:100 IHC, 1:200 WES |
| Insulin receptor | ThermoFisher (PA5-27334) | Rabbit | Polyclonal | 156 | 1:150 WES            |
| NCOR2            | Invitrogen (PA1-843)     | Rabbit | Polyclonal | 250 | 1:50 WES             |
| GAPDH            | Cell signaling (S2118)   | Rabbit | Polyclonal | 36  | 1:500 WES            |



### Supplementary Reference

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