Classical methods for detecting AER-α binding ERP-α

To illustrate the simplicity and label-free feature of the THz spectroscopy technology for detecting the binding reaction between AER-α and ERP-α, classical methods of immunohistochemistry (IHC) and Enzyme-linked immunosorbent assay (ELISA) were also employed to independently analyze the samples for comparison.

IHC is an important diagnostic pathology tool to demonstrate protein expression in the clinical and experimental researches. Here, mouse ovary embedded by paraffin was collected as sample slices and immunohistochemical staining was used to observe the histological sections.

A solution of paraformaldehyde was used to fix the mouse ovary tissue which contained antigen ER-α; the tissue sample was embedded in paraffin before sectioning. The antigen in the tissue was retrieved by boiling in sodium citrate buffer (pH 6.0) for 15 min. The mouse ovary slices were infiltrated into the 3% hydrogen peroxide for 20 min to block endogenous peroxidase. Normal goat serum blocking buffer was used to block specific proteins at 37°C for 30min.

Fig. S1(a) shows a large area of lime color, indicating that AER-α and ER-α in mouse ovary sample slide underwent substantial binding reaction. Moreover, premixing AER-α and ERP-α, which were then allowed to react with mouse ovary slide; the sporadic lime color indicates that premixed ERP-α brought competitive inhibition to ER-α in the mouse ovary sample (Fig S1(b)). These results serve to validate that AER-α and ERP-α have undergone sound binding reaction.

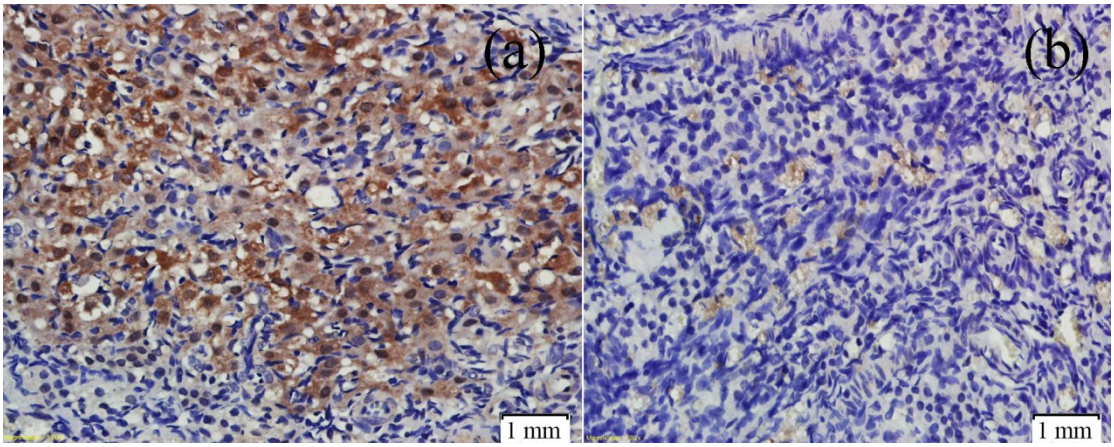

Fig. S1 Specificity and sensitivity measurements by IHC. (a) To remove normal goat serum from the slices, unconjugated AER-α was added and incubated overnight at 4 °C, followed by a conjugated secondary antibody (Streptavidin-Peroxidase(SP)) for 20 min and diaminobenzidine (DAB) staining; (b) After blocking AER-α and ERP-α

overnight at 4°C, the mixture was added onto the slices, and removed normal goat serum, incubated overnight at 4 °C, followed by a conjugated secondary antibody (SP) for 20 min and DAB staining.

In ELISA, Antigen: ERP-α, 0.2 ug/100 ul; Primary antibody: AER-α,100 ug/100 ul; dilution ratio 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, and 1:32000, respectively; Secondary antibody: Goat Anti-Rabbit IgG (bs-0295G-HRP) conjugated HRP at 1: 5000. The antibody was bound to the antigen on the solid phase carrier. After washing away the unbound antibody, the enzyme-labeled antibody (goat anti-rabbit IgG (bs-0295G) conjugated horseradish peroxidase (HRP)) was added. After washing the unbound enzyme-labeled antibody, the chromogenic substrate (o-phenylenediamine and hydrogen peroxide (OPD-H2O2)) was added. The optical density (OD) of the color is measured using a microplate reader, from which the amount of binding of antigen and antibody reaction can be quantitatively determined, with the larger OD value corresponding to greater amount of the binding of antigen and antibody.

The RBS (AER-α:ERP-α) was 0.023, 0.011, 0.0057, 0.0028, 0.0014, 0.035, 0.00071, and 0.00036, respectively, which did not cover the test range before. But the corresponding ELISA data could nonetheless help to judge the interaction of antigen-antibody even under a low concentration, such as the cases here. The approximate linear relation between optical density and dilution shown in the Fig. S2 illustrates that AER-α and ERP-α are obviously in binding reaction with each other.

Thus both of the independent ELISA and IHC tests had validated that AER-α and ERP-α were indeed undergoing binding reaction.

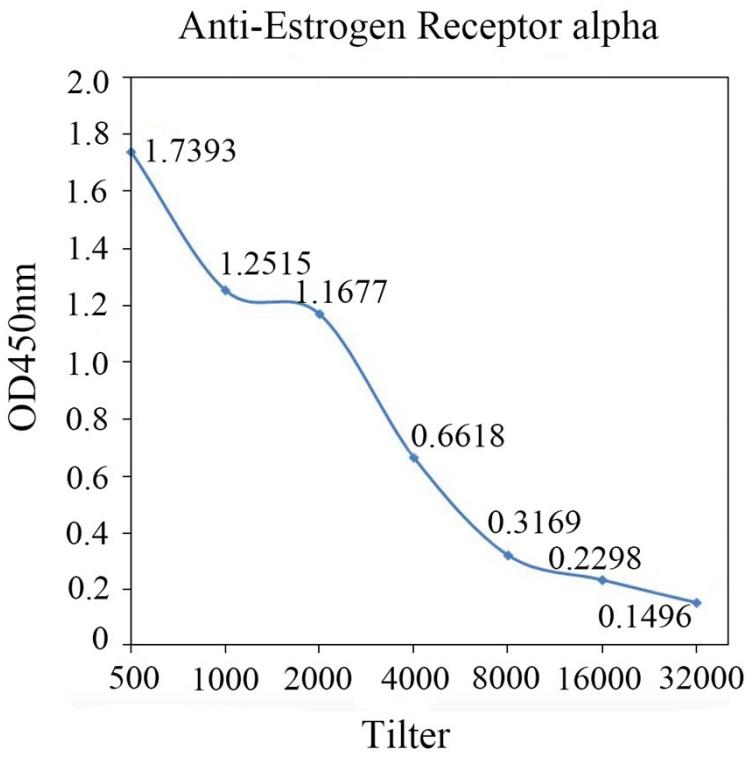

Fig. S2 Specificity and sensitivity measurements by ELISA. $OD450_{nm}$ is the optical density at 450 nm.