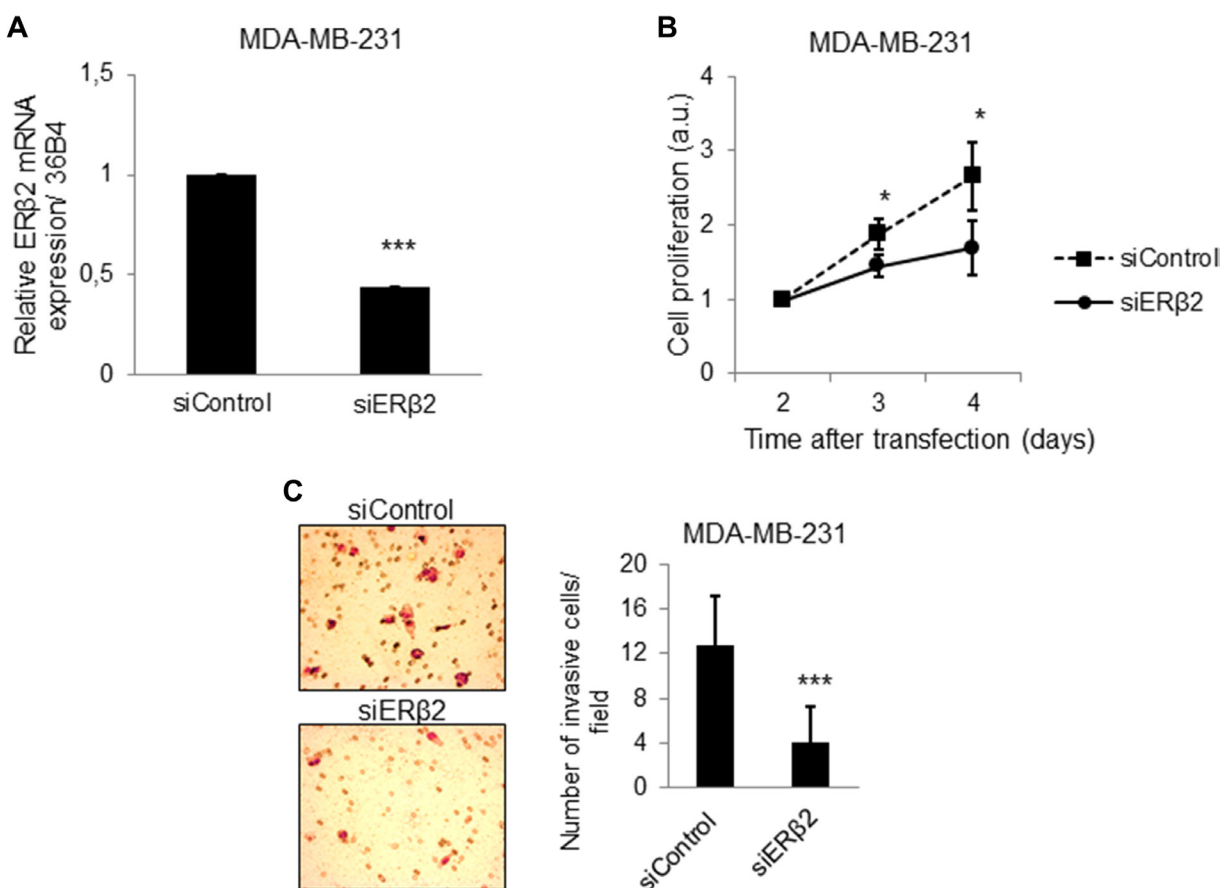
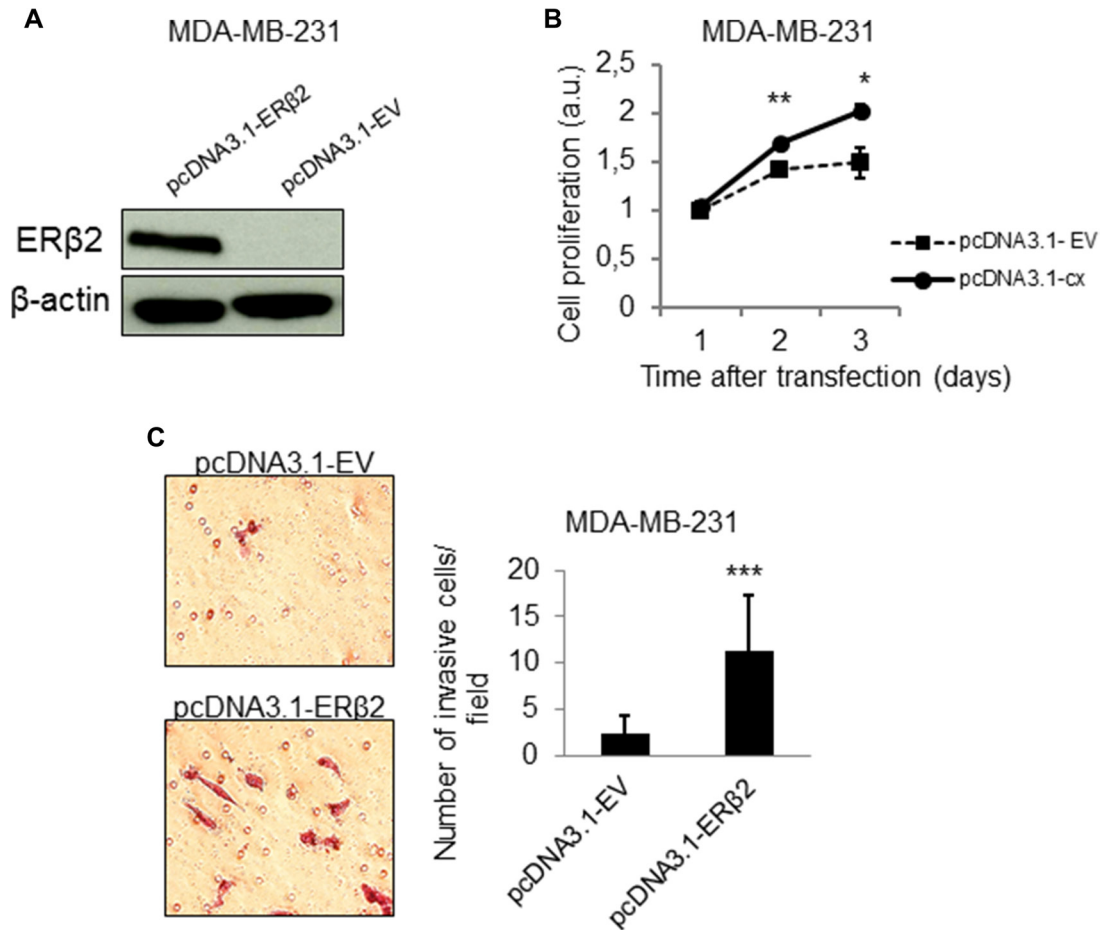


## Estrogen receptor $\beta 2$ induces proliferation and invasiveness of triple negative breast cancer cells: association with regulation of PHD3 and HIF-1 $\alpha$

### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1:** (A) ER $\beta 2$  siRNA down-regulates ER $\beta 2$  mRNA in MDA-MB-231 cells. ER $\beta 2$  mRNA level was determined by qPCR after transfection with control siRNA or ER $\beta 2$  siRNA. Data are normalized to 36B4 and shown as relative fold change compared to control siRNA  $\pm$  SD. \*\*\* $P < 0.001$ . (B) ER $\beta 2$  depletion reduces proliferation of the MDA-MB-231 cell line. MDA-MB-231 cells were transfected with control siRNA or ER $\beta 2$  siRNA. WST-1 assays as a measure of cellular proliferation were carried out at the indicated time points after siRNA transfection. Data are shown as means  $\pm$  SD. \* $P < 0.05$ . The experiment was repeated three times. One representative experiment is shown. (C) ER $\beta 2$  depletion reduces invasion of MDA-MB-231 cell line. MDA-MB-231 cells were transfected with control siRNA or ER $\beta 2$  siRNA, and cell invasion was evaluated by the BD Biocoat growth factor reduced Matrigel invasion chamber assay. Data represent means  $\pm$  SD. \*\*\* $P < 0.001$ . Experiment was repeated twice. One representative experiment is shown. A,B,C,  $p$  values were calculated by  $t$ -test.



**Supplementary Figure 2:** (A) Western blot analysis showing increased protein level of ERβ2 after transient overexpression of ERβ2 protein. ERβ2 was detected by the PPZ0506 antibody. β-actin was used as a loading control. (B) ERβ2 overexpression promotes cell proliferation in MDA-MB-231 cells. WST-1 assays of cell proliferation were carried out at the indicated time points after transfection of ERβ2 or empty vector (EV). Ratio of absorbance to day 1 is calculated. Data are shown as means of relative absorbance ± SD. \* $P < 0.05$ , \*\* $P < 0.01$ . Experiments were repeated three times. One representative experiment is shown. (C) ERβ2 overexpression promotes cell invasion in the MDA-MB-231 cell line. MDA-MB-231 cells were transfected with ERβ2 or EV, and cell invasion was evaluated by the BD Biocoat growth factor reduced Matrigel invasion chamber assay. Data represent means ± SD. \*\*\* $P < 0.001$ . Experiment was repeated twice. One representative experiment is shown. B,C,  $p$  values were calculated by  $t$ -test.