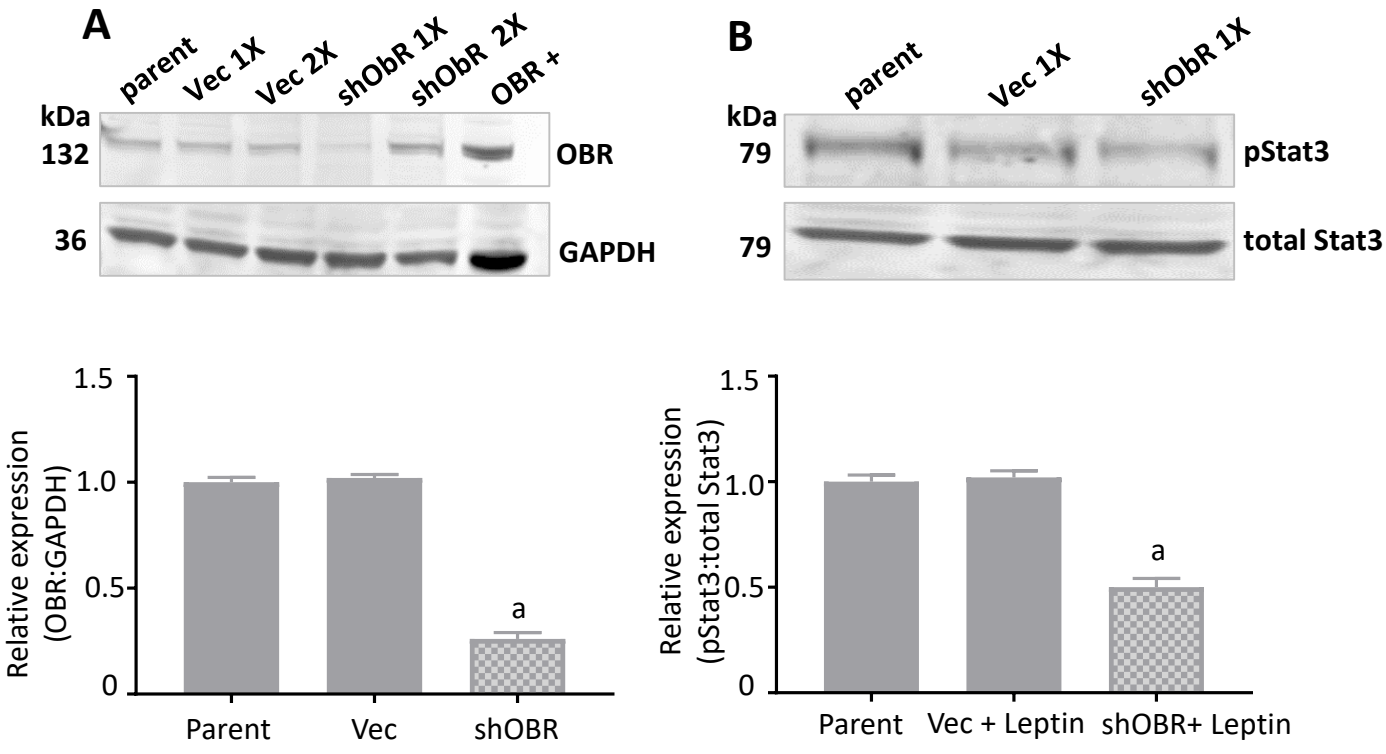


Figure S5. Knockdown of OBR by shRNA in triple negative breast cancer cells. (A) MDA-MB231 cells were transfected with shRNA vector control (Vec) and OBR shRNA [both at 10 µl (1X) and 20 µl (2X)]. The transfected cells were cultured (200,000 cells/well; 6-well plates) for 24 hrs. Western blot analysis (WB) of OBR expression in MDA-MB231 cells transfected with OBR shRNA or vector control (OBR+: positive control for OBR antibody). (B)WB analysis of leptin-induced pStat3 in knockdown and control MDA-MB231 cells. After 24hr serum starvation, transfected cells were incubated with leptin (2.5nM) for 5 min. WB was performed with 30 to 50µg of protein/lane on 4-15% polyacrylamide gels. Graphs represent quantitative analysis of OBR and pSTAT3. All data were derived from a minimum of three independent experiments using different cell preparations. “a”: $p \leq 0.05$ compared to Vec + leptin.