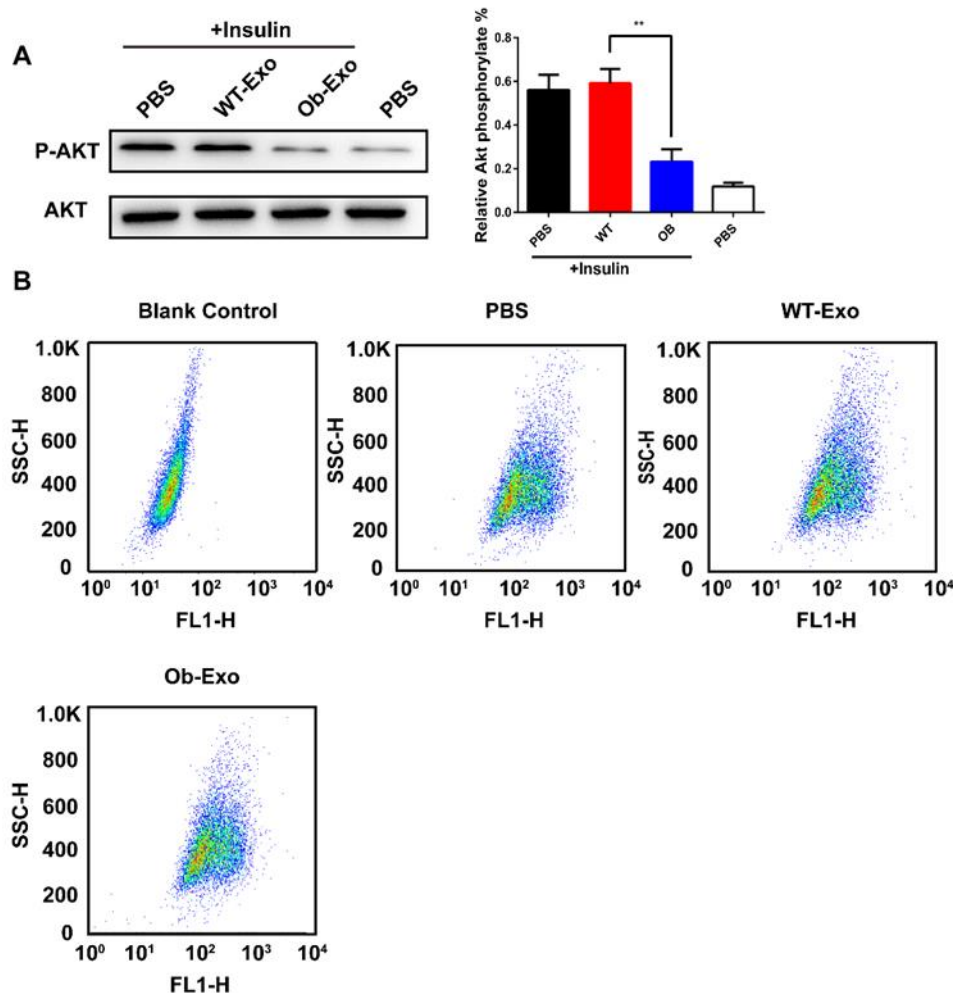


*Ob-exosomes affect the insulin sensitivity and glucose uptake of HepG2 cells*

To detect the effect of exosomes on insulin sensitivity and glucose uptake in HepG2 cells, HepG2 cells were treated with WT-exosomes, Ob-exosomes and PBS was used as a negative control. After insulin stimulation, phosphorylation of AKT (Ser473), a central player in the insulin signaling pathway, was detected by Western blot analyses (Supplement Figure 1A). The phosphorylation levels of AKT were lower in the HepG2 cells cultured with Ob-exosomes than in the HepG2 cells cultured with WT-exosomes, suggesting that the insulin responses was inhibited in the HepG2 cells absorbing Ob-exosomes. 2-NBDG, a fluorescent glucose analog was used to detect glucose uptake in HepG2 cells by flow cytometry. The results showed the spontaneous fluorescence intensity of HepG2 cells was  $31.10 \pm 3.21$ , the fluorescence intensity of PBS-, WT-exosomes-, Ob-exosomes-treated group was respectively  $222.26 \pm 1.03$ ,  $250.00 \pm 2.54$ ,  $210.31 \pm 3.70$ . Compared with WT-exosomes-treated group, the fluorescence intensity of Ob-exosomes- treated group was respectively decreased 15.9% (Supplement Figure 1B). These results showed that insulin-stimulated transport of glucose was inhibited in HepG2 cells treated with Ob- exosomes, indicating that insulin signal transduction in Ob-exosomes-treated HepG2 cells was impaired.



Supplementary Figure 1. Ob-exosomes impair activation of the insulin signaling pathway in vitro. (A) Effect of WT-, Ob-exosomes on AKT phosphorylation of insulin-induced HepG2 cells. TNF- $\alpha$  was used as a positive control and PBS was used as a negative control. \*\*p < 0.01, Ob-Exo vs WT-Exo. (B) Flow cytometry dot plots of 10000 HepG2 cells. The 2D plots indicating the relative FL1

fluorescence intensity (2-NBDG) of HepG2 cells. The Blank Control was HepG2 cells without 2-NBDG, showed the spontaneous fluorescence intensity of HepG2 cells. n=3 independent experiments. All values were expressed as means  $\pm$  SD.