

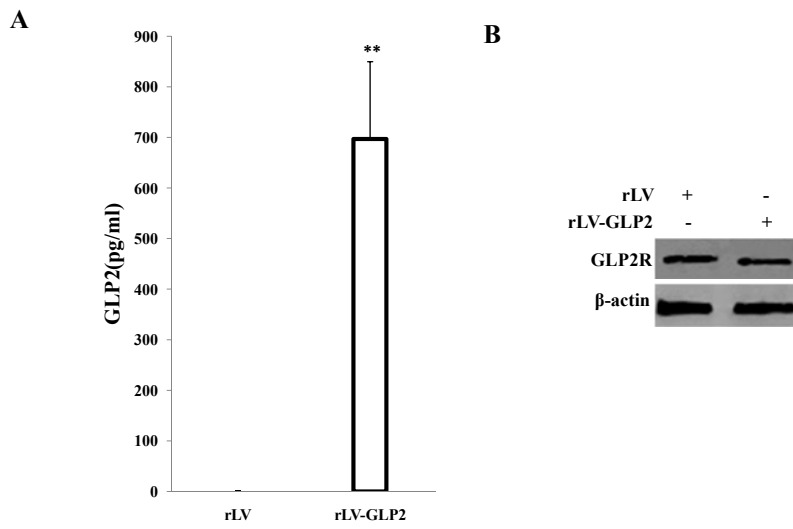
OMTN, Volume 10

## **Supplemental Information**

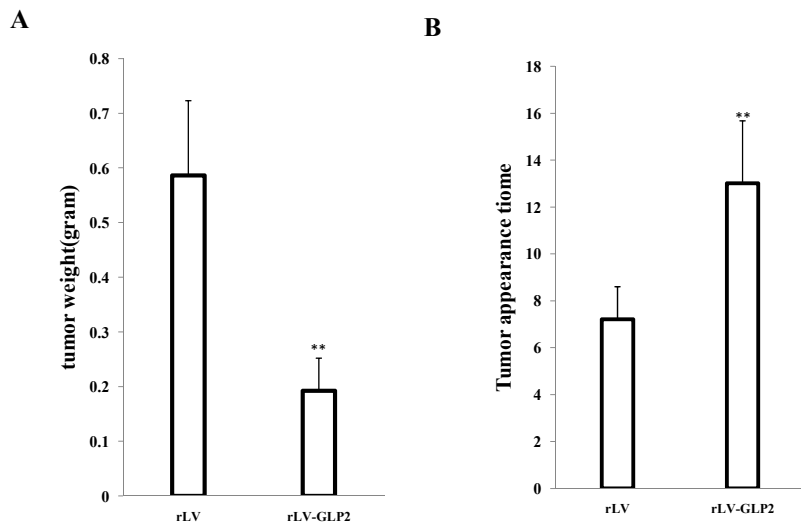
### **GLP2 Promotes Directed Differentiation from Osteosarcoma Cells to Osteoblasts and Inhibits Growth of Osteosarcoma Cells**

**Yi Lu, Dongdong Lu, and Yu Hu**

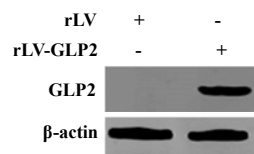
**FigureS1**



**FigureS2**

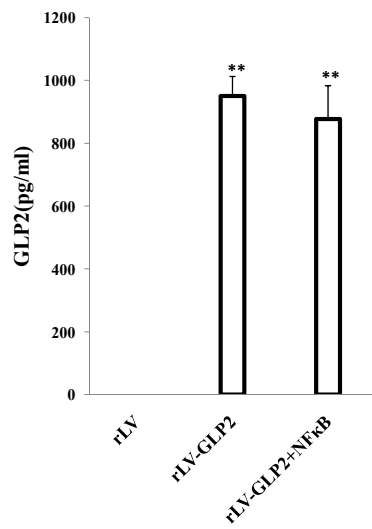


FigureS3

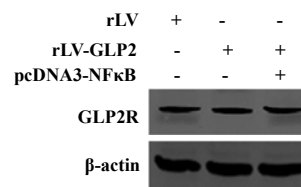


FigureS4

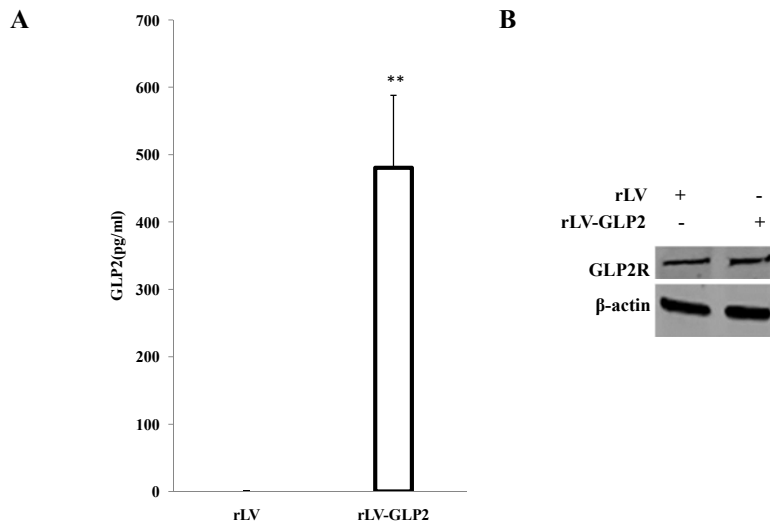
A



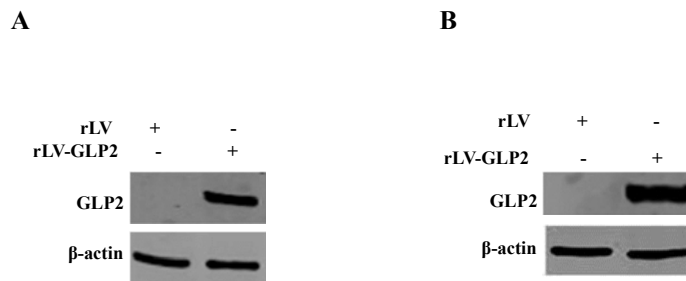
B



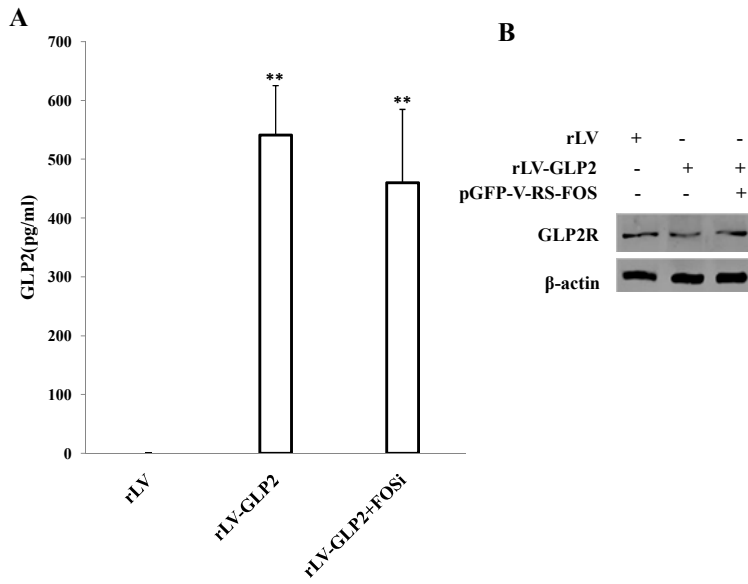
**FigureS5**



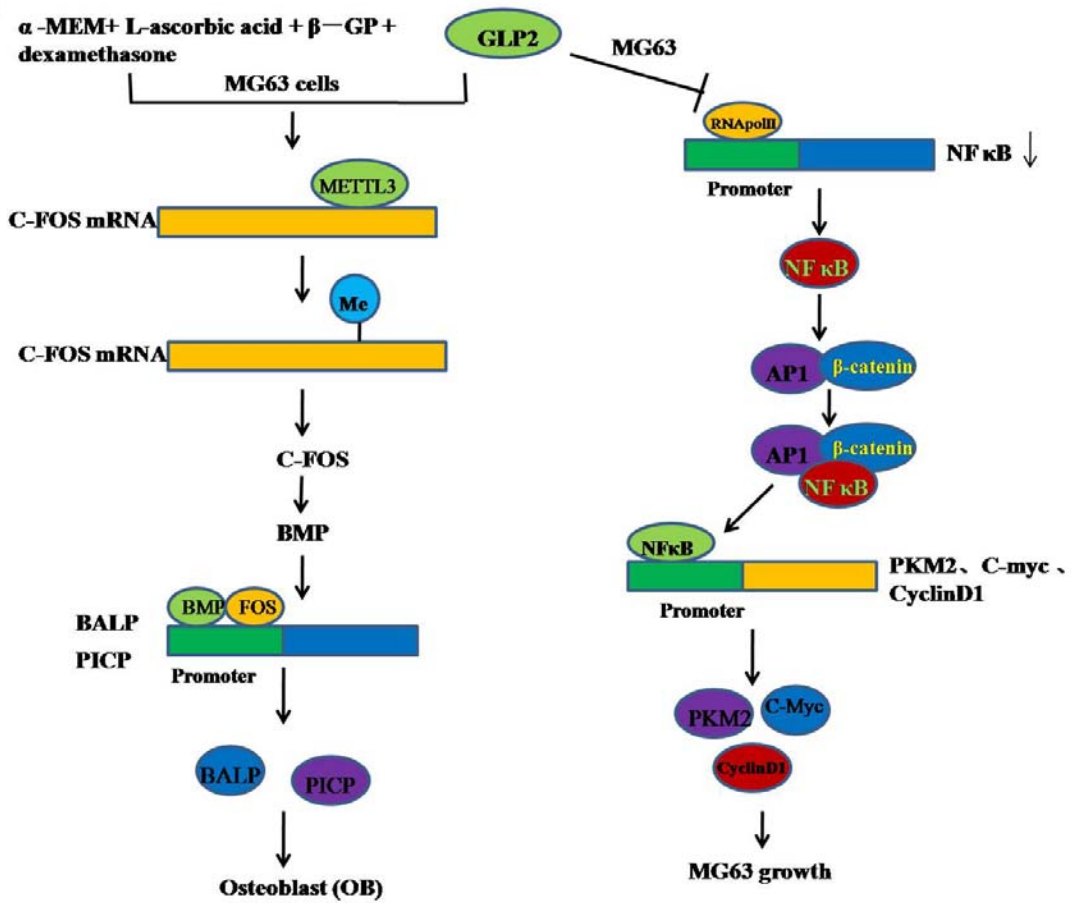
**FigureS6**



FigureS7



FigureS8



## FIGURE LEGENDS

**FigureS1** **A.** ELISA assay of released level of GLP2 in osteosarcoma MG63 infected with rLV-Green-GLP2 or rLV. **B.** Western blotting with anti-GLP2R in MG63 infected with rLV-GLP2 or rLV.  $\beta$ -actin as internal control.

**FigureS2** **A.** Orthotopic osteosarcoma weight(gram) in rLV-GLP2 group or rLV group . Data were means of value from ten athymic Balb/c mice, mean $\pm$ SEM, n=10, \*\*,P<0.01. **B.** The Orthotopic osteosarcoma appearance time in rLV-GLP2 group or rLV group. Data were means of value from ten athymic Balb/c mice, mean $\pm$ SEM, n=10, \*\*,P<0.01.

**FigureS3** Western blotting with anti-GLP2 in MG63 infected with rLV-GLP2 or rLV.  $\beta$ -actin as internal control.

**Figure S4** **A.** ELISA assay of released level of GLP2 in osteosarcoma MG63 infected with rLV, rLV-Green-GLP2,rLV-GLP2 and rLV-GLP2 plus pcDNA3-NF $\kappa$ B. Data were means of value from ten athymic Balb/c mice, mean $\pm$ SEM, n=10, \*\*,P<0.01. **B.** Western blotting with anti-GLP2R in MG63 infected with rLV, rLV-Green-GLP2 and rLV-GLP2 plus pcDNA3-NF $\kappa$ B.  $\beta$ -actin as internal control.

**FigureS5** **A.** ELISA assay of released level of GLP2 in OB cells induced from MG63 infected with rLV or rLV-GLP2. Data were means of value from ten athymic Balb/c mice, mean $\pm$ SEM,

n=10, \*\*,P<0.01. **B.** Western blotting with anti-GLP2R in OB cells induced from MG63 infected with rLV or rLV-GLP2.  $\beta$ -actin as internal control.

**Figure 6 A.** Western blotting with anti-GLP2 in OB cells induced from MG63 infected with rLV or rLV-GLP2.  $\beta$ -actin as internal control. **B.** Western blotting with anti-GLP2 in OB cells induced from MG63 infected with rLV or rLV-GLP2.  $\beta$ -actin as internal control.

**FigureS7 A.** ELISA assay of released level of GLP2 in OB cells induced from MG63 infected with rLV, rLV-GLP2, rLV-GLP2 plus pGFP-V-RS-C-FOS. Data were means of value from ten athymic Balb/c mice, mean $\pm$ SEM, n=10, \*\*,P<0.01. **B.** Western blotting with anti-GLP2R in OB cells induced from MG63 infected with rLV, rLV-GLP2, rLV-GLP2 plus pGFP-V-RS-C-FOS.  $\beta$ -actin as internal control.

**Figure S8** The schematic illustrates a model of GLP2 inhibits osteosarcoma carcinogenesis and promotes the differentiation of MG63 to osteoblast (OB). GLP2 inhibits the expression and activity of NF $\kappa$ B, triggering the decrease of C-Myc, PKM2, CyclinD1 in osteosarcoma cells. On the other hand, excessive GLP2 significantly increased the expression of osteogenesis-associated genes (e.g. Ocn and PICP) dependent on C-Fos-BMP signaling which promotes directed-differentiation from osteosarcoma cells to osteoblast.