Supplemental Information

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

Yi Lu, Dongdong Lu, and Yu Hu
Figure S1

A

GLP2 (pg/ml)

rLV +               -
rLV-GLP2       - +

GLP2R

β-actin

BA

** 0 100 200 300 400 500 600 700 800 900

rLV rLV-GLP2

Figure S2

A

Tumor weight (gram)

rLV +               -
rLV-GLP2       - +

Tumor appearance time

** 0 2 4 6 8 10 12 14 16 18

rLV rLV-GLP2
Figure S3

| Treatment     | GLP2 | β-actin |
|---------------|------|---------|
| rLV          | +    | +       |
| rLV-GLP2     | -    | +       |

Figure S4

A

| GLP2 (pg/ml) |
|--------------|
| 0            |
| 200          |
| 400          |
| 600          |
| 800          |
| 1000         |
| 1200         |

B

| Treatment     | GLP2R | β-actin |
|---------------|-------|---------|
| rLV          | +     | +       |
| rLV-GLP2     | -     | +       |
| pcDNA3-NFκB  | -     | +       |

**Note:** The significance levels are indicated by asterisks: **p < 0.01.
Figure S7

A

B

rLV

rLV-GLP2

pGFP-V-RS-FOS

GLP2R

β-actin

Figure S8

α-MEM + L-ascorbic acid + β-GP + dexamethasone

MG63 cells

C-FOS mRNA

METTL3

C-FOS mRNA

Mtc

C-FOS

BMP

BALP

PICP

Promoter

Promoter

Osteoblast (OB)

GLP2

MG63

Promoter

NFκB

PKM2, C-myc, CyclinD1

MG63 growth

β-catenin

AP1

β-catenin

AP1

NFκB

DNApolβ
FIGURE LEGENDS

**Figure S1** 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. β-actin as internal control.

**Figure S2** A. Orthotopic osteosarcoma weight(gram) in rLV-GLP2 group or rLV group. Data were means of value from ten athymic Balb/c mice, mean±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±SEM, n=10, **,P<0.01.

**Figure S3** Western blotting with anti-GLP2 in MG63 infected with rLV-GLP2 or rLV. β-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-NFκB. Data were means of value from ten athymic Balb/c mice, mean±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-NFκB. β-actin as internal control.

**Figure S5** 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±SEM,
n=10, **,P<0.01. **. Western blotting with anti-GLP2R in OB cells induced from MG63 infected with rLV or rLV-GLP2. β-actin as internal control.

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

**Figure S7 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±SEM, n=10, **,P<0.01. **. Western blotting with anti-GLP2R in OB cells induced from MG63 infected with rLV, rLV-GLP2, rLV-GLP2 plus pGFP-V-RS-C-FOS. β-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 NFκB, 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.