Supplementary Information

Prostaglandin E_2 stimulates β1-integrin expression in hepatocellular carcinoma through the EP1 receptor/PKC/NF-κB pathway

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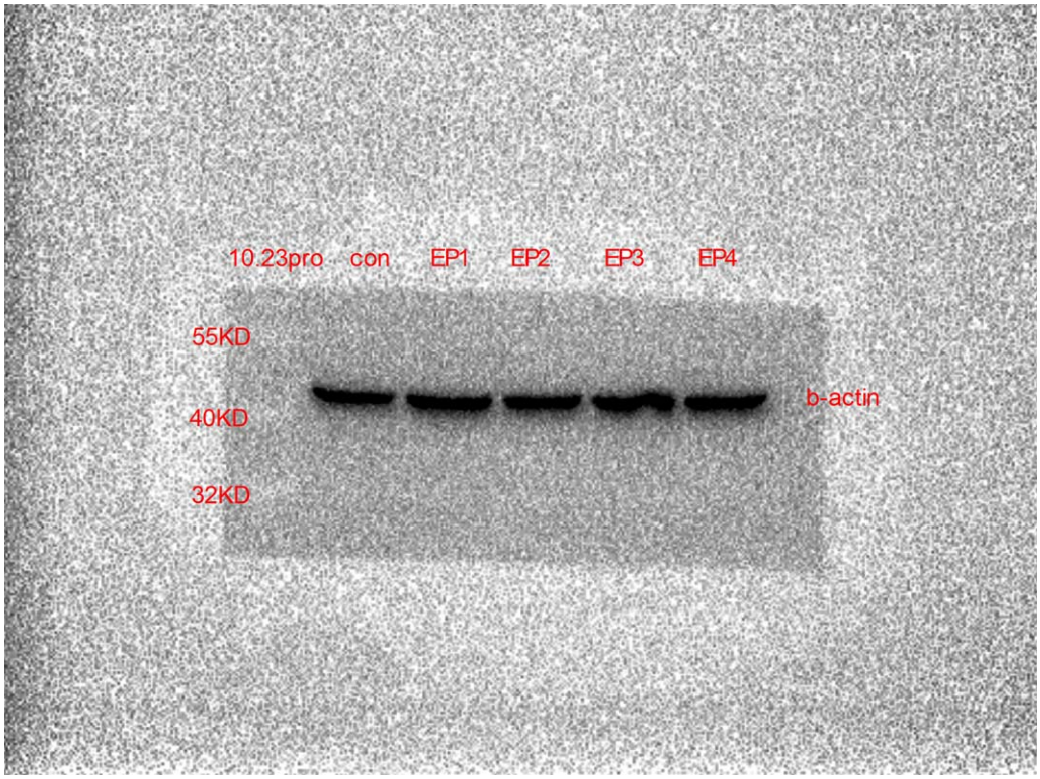

Figure S1. Effects of EP agonists on β1-integrin expression in Huh-7 cells.

Huh-7 cells were exposed to 5 μM EP1 agonist (17-PT-PGE_2), EP2 agonist (butaprost), EP3 agonist (sulprostone) and EP4 agonist (PGE1 alcohol) for 24 h, respectively. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β-actin antibody was added.

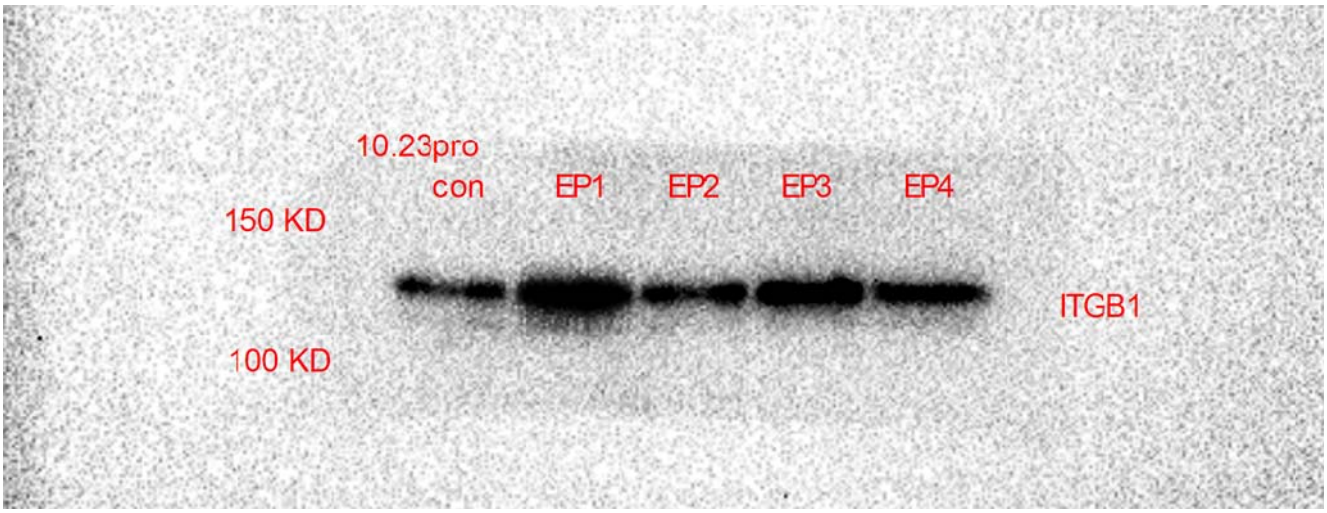

Figure S2. Effects of EP agonists on β1-integrin expression in Huh-7 cells.

Huh-7 cells were exposed to 5 μM EP1 agonist (17-PT-PGE_2), EP2 agonist (butaprost), EP3 agonist (sulprostone) and EP4 agonist (PGE1 alcohol) for 24 h, respectively. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β1 integrin antibody was added.

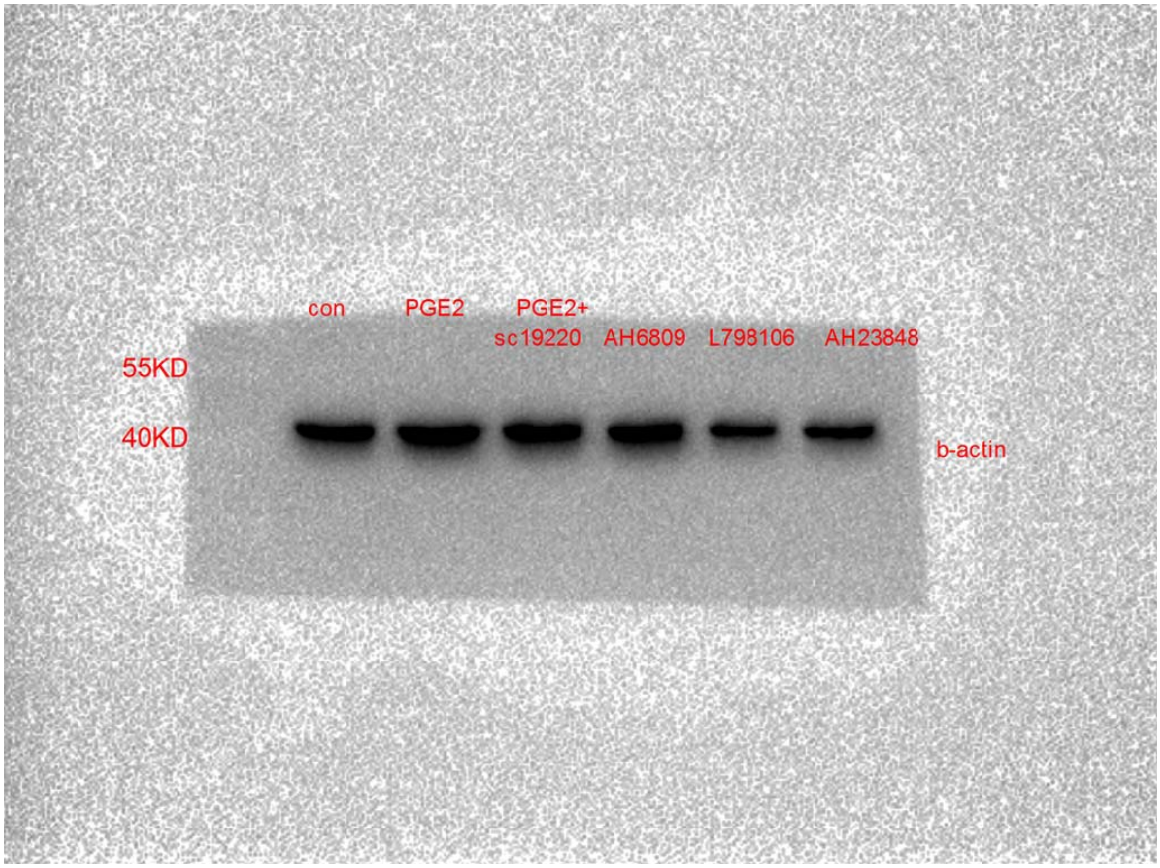

Figure S3. Effects of EP antagonists on PGE_2-mediated β1-integrin expression in Huh-7 cells.

Huh-7 cells were pretreated with various EP antagonists for 1 h, followed by PGE_2 for 24 h (EP1 antagonist sc19220, EP2 antagonist AH6809 and EP3 antagonist L-798106, EP4 antagonist AH23848). Total protein were collected. The gels have been run under the same experimental conditions. The anti-β-actin antibody was added.

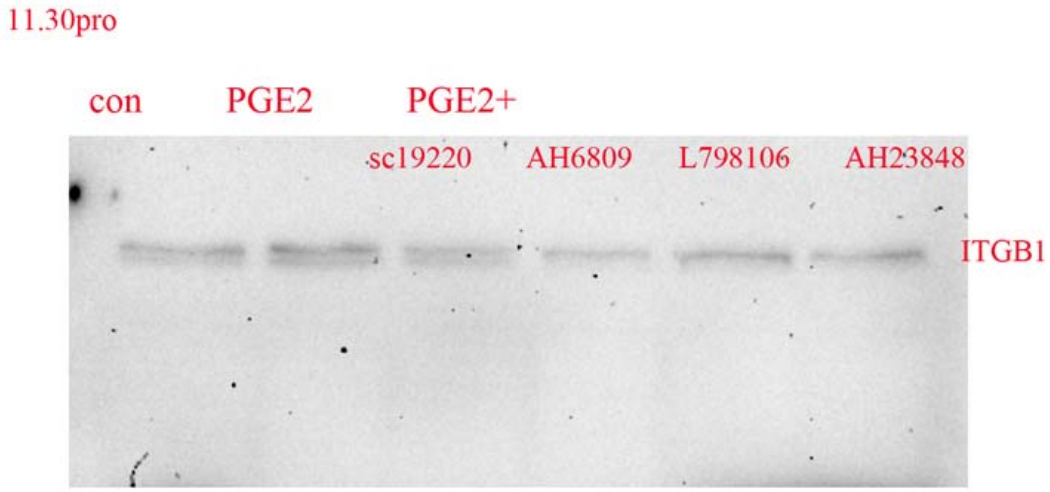

Figure S4. Effects of EP antagonists on PGE_2-mediated β1-integrin expression in Huh-7 cells.

Huh-7 cells were pretreated with various EP antagonists for 1 h, followed by PGE_2 for 24 h (EP1 antagonist sc19220, EP2 antagonist AH6809 and EP3 antagonist L-798106, EP4 antagonist AH23848). Total protein were collected. The gels have been run under the same experimental conditions. The anti-β1 integrin antibody was added.

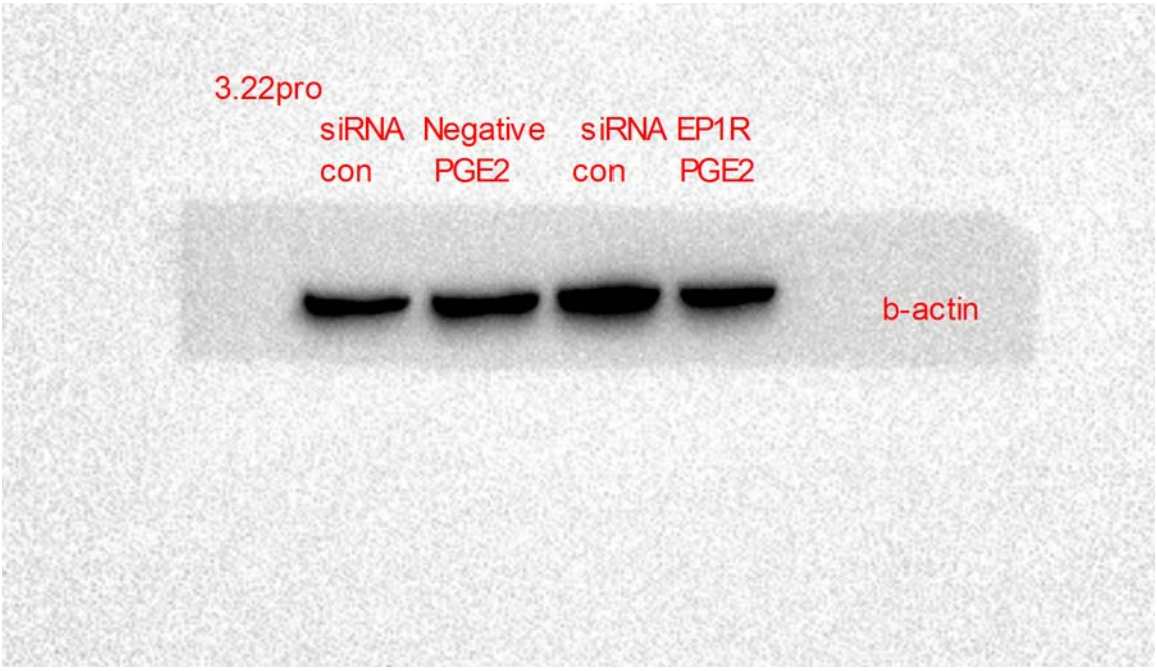

Figure S5. RNA interference targeting the EP1 receptor suppressed PGE_2-mediated β1-integrin upregulation in Huh-7 cells.

Huh-7 cells were transfected with an EP1R-siRNA. After 72h, the cells were exposed to PGE_2 for 24 h. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β-actin antibody was added.

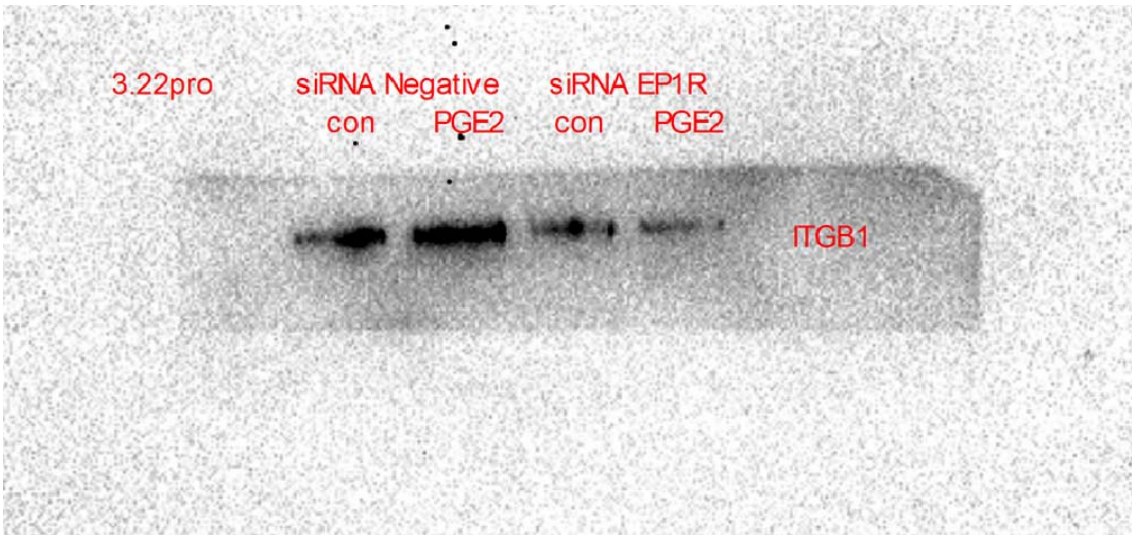

Figure S6. RNA interference targeting the EP1 receptor suppressed PGE_2-mediated β1-integrin upregulation in Huh-7 cells.

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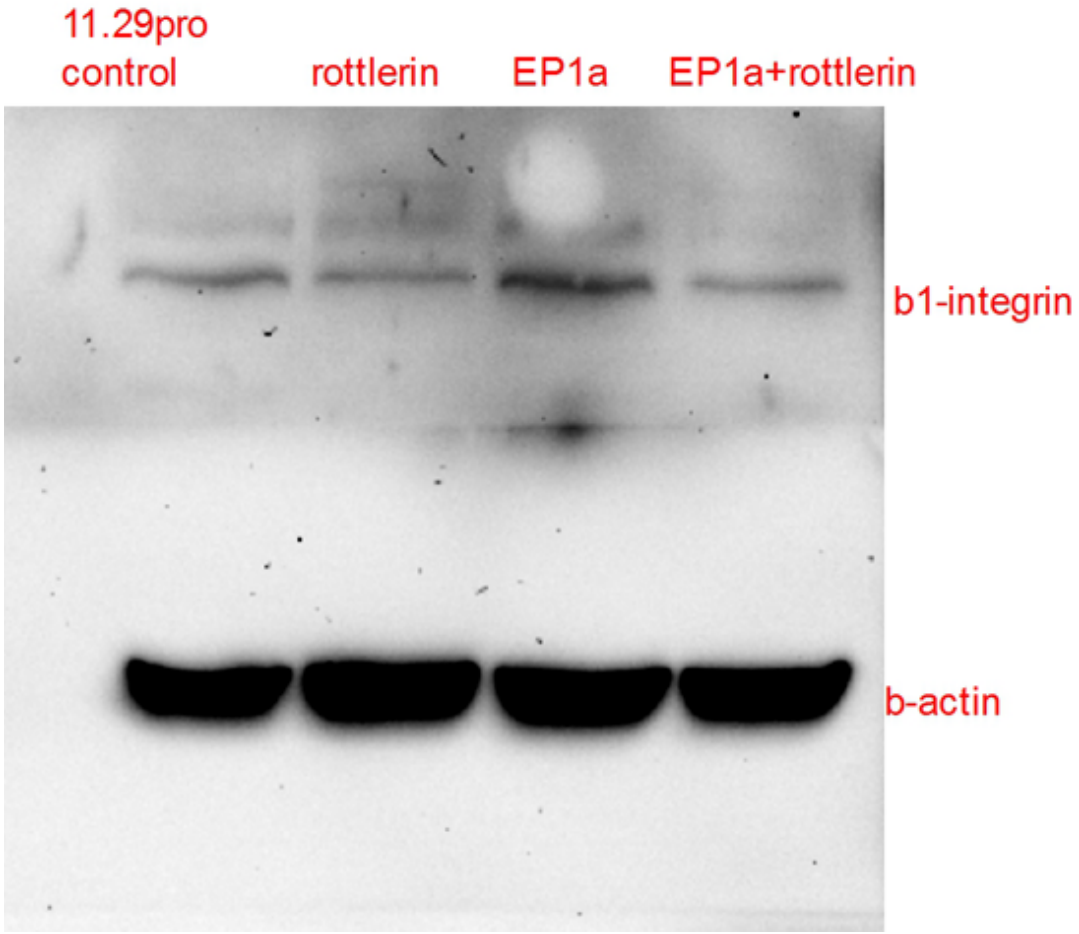

Figure S7. Effect of PKC inhibitor rottlerin on 17-PT-PGE_2-mediated β1-integrin expression in Huh-7 cells.

Huh-7 cells were treated with 17-PT-PGE_2 for 24 h, with or without pre-treatment of 5 μM rottlerin for 1 h. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β1 integrin antibody and anti-β-actin antibody were added at the same time.

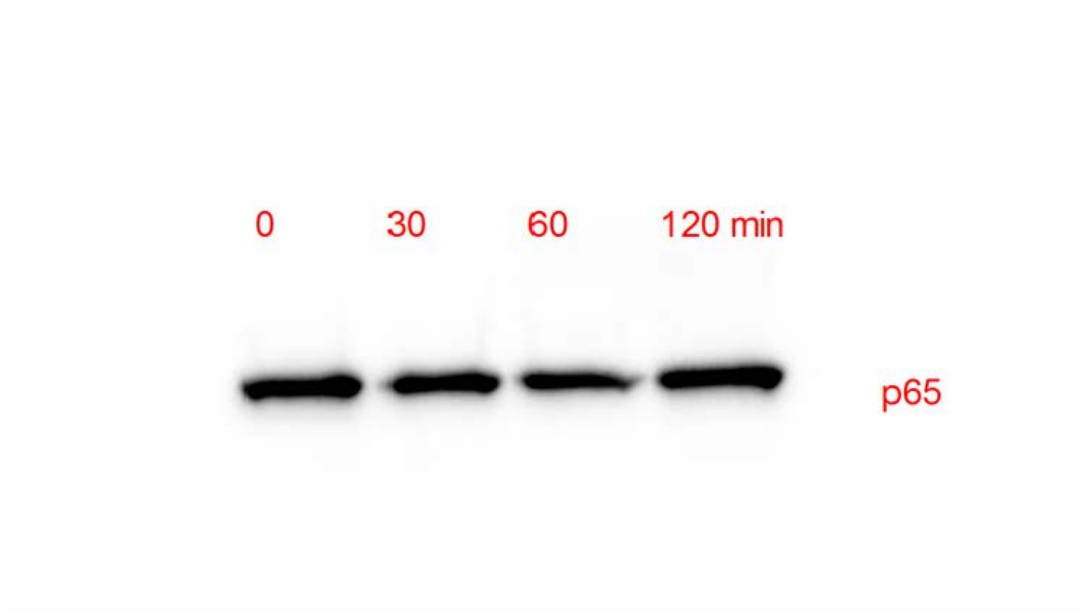

Figure S8. Effects of 17-PT-PGE_2 on NF-κB and IκB phosphorylation in Huh-7 cells. Huh-7 cells were treated with 5 μM 17-P-T-PGE_2 for 0, 30, 60, 120 min. Total protein were collected. The gels have been run under the same experimental conditions. The anti-p65 antibody was added.

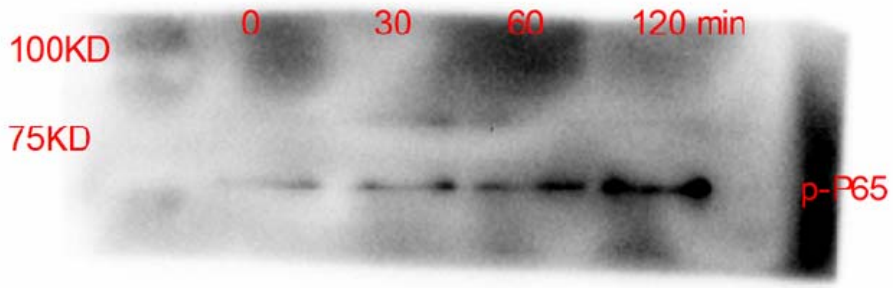

Figure S9. Effects of 17-PT-PGE_2 on NF-κB and IκB phosphorylation in Huh-7 cells. Huh-7 cells were treated with 5 μM 17-P-T-PGE_2 for 0, 30, 60, 120 min. Total protein were collected. The gels have been run under the same experimental conditions. The anti-phospho-p65 antibody was added.

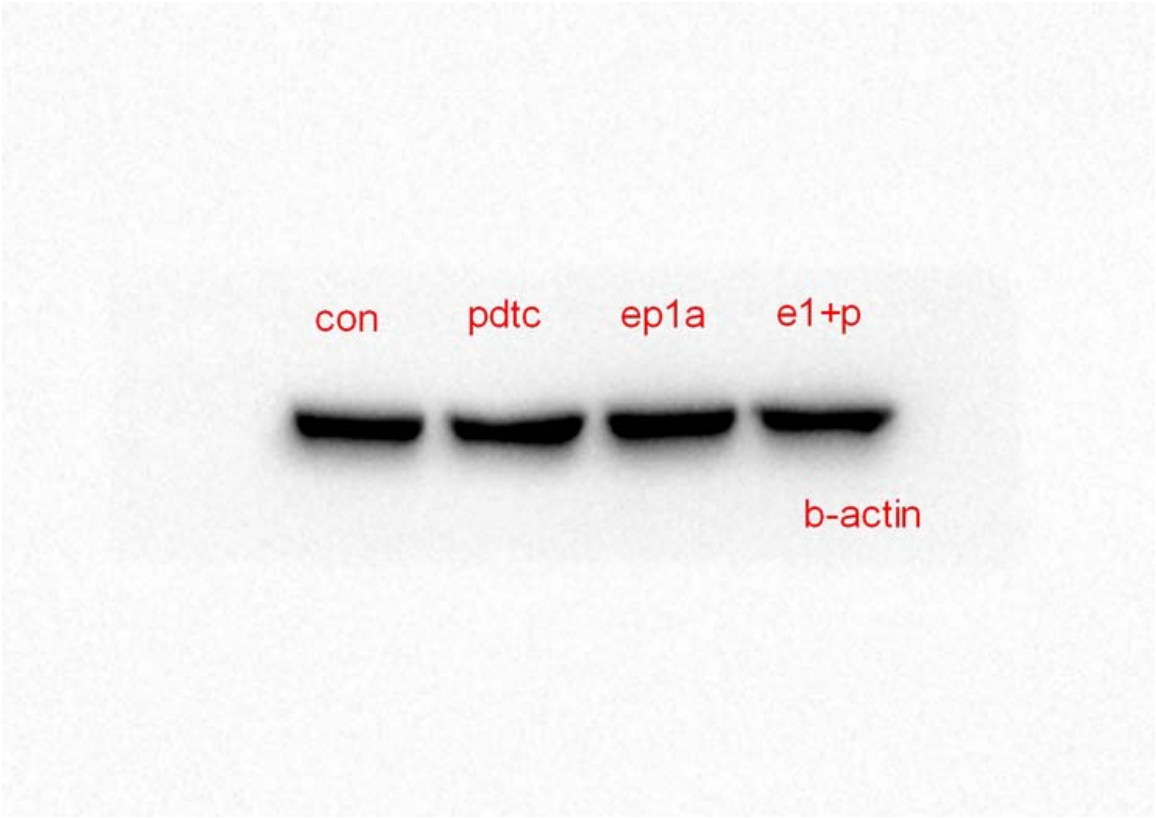

Figure S10. Effect of NF-κB inhibitor PDTC on 17-PT-PGE_2-mediated β1-integrin expression in Huh-7 cells.

Huh-7 cells were treated with 17-PT-PGE_2 for 24 h, with or without pre-treatment of PDTC for 24 h. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β-actin antibody was added.

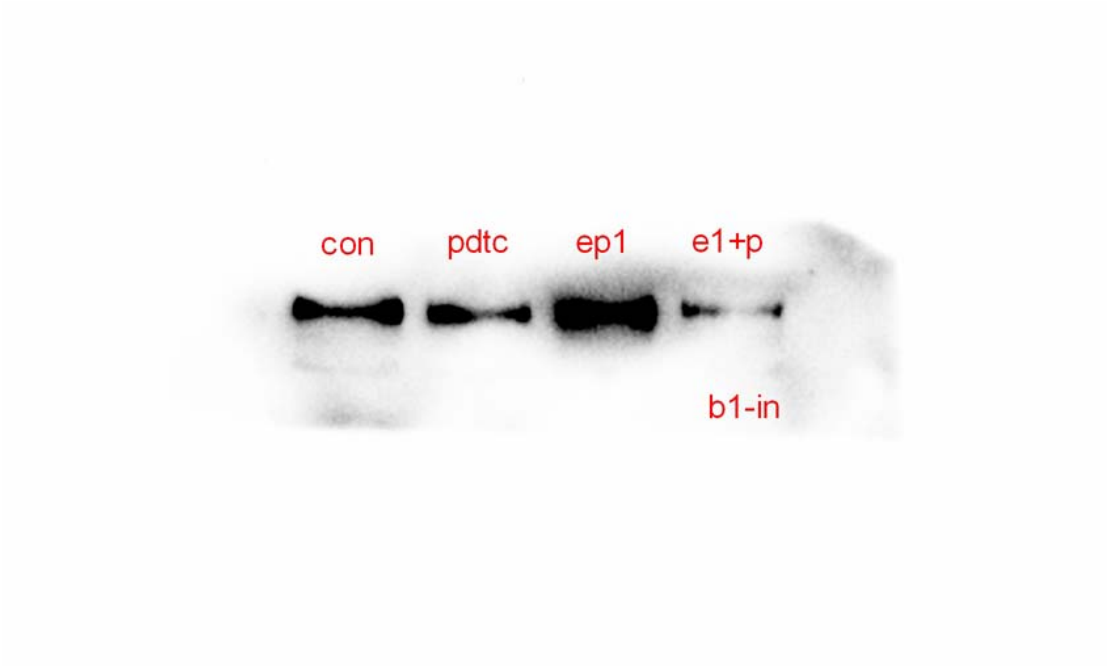

Figure S11. Effect of NF-κB inhibitor PDTC on 17-PT-PGE_2-mediated β1-integrin expression in Huh-7 cells.

Huh-7 cells were treated with 17-PT-PGE_2 for 24 h, with or without pre-treatment of PDTC for 24 h. Total protein were collected. The gels have been run under the same experimental conditions. The anti-β1 integrin antibody was added.

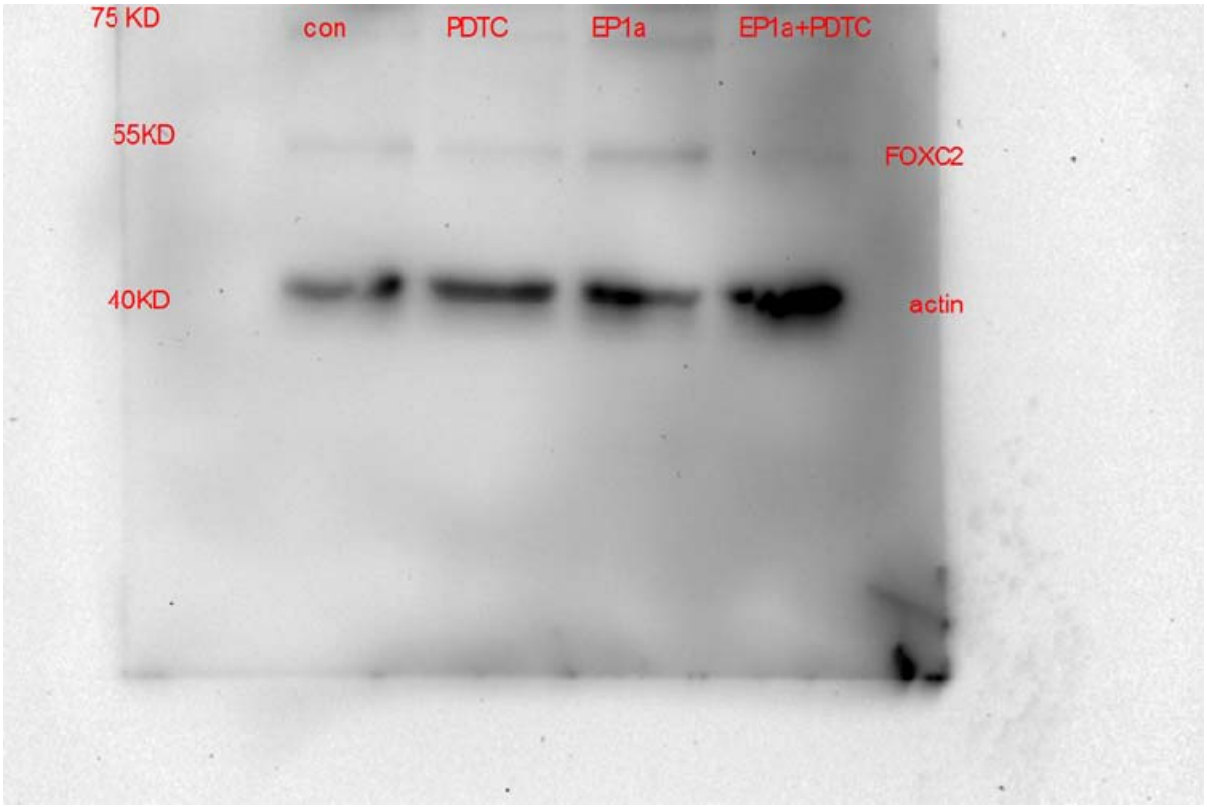

Figure S12. Effect of NF-κB inhibitor PDTC in EP1 receptor-mediated FoxC2 upregulation in Huh-7 cells.

Huh-7 cells were treated with 17-PT-PGE_2 for 24 h, with or without pre-treatment of PDTC for 24 h. Total protein were collected. The gels have been run under the same experimental conditions. The anti-FoxC2 antibody and anti-β-actin antibody were added at the same time.