# Supplementary Material

# Regulation of Epithelial-Mesenchymal Transition of A549 Cells by Prostaglandin $D_2$

Farzaneh Vafaeinik[a] Hye Jin Kum[a] Seo Yeon Jin[a] Do Sik Min[b]
Sang Heon Song[c] Hong Koo Ha[d] Chi Dae Kim[a] Sun Sik Bae[a]

[a]Gene and Cell Therapy Center for Vessel-Associated Disease, Medical Research Institute, and Department of Pharmacology, Pusan National University School of Medicine, Gyungnam, Republic of Korea, [b]Department of Pharmacy, Yeonsei University, Incheon, Republic of Korea, [c]Department of Urology, Pusan National University Hospital, Busan, Republic of Korea, [d]Department of Internal Medicine, Pusan National University Hospital, Busan, Republic of Korea

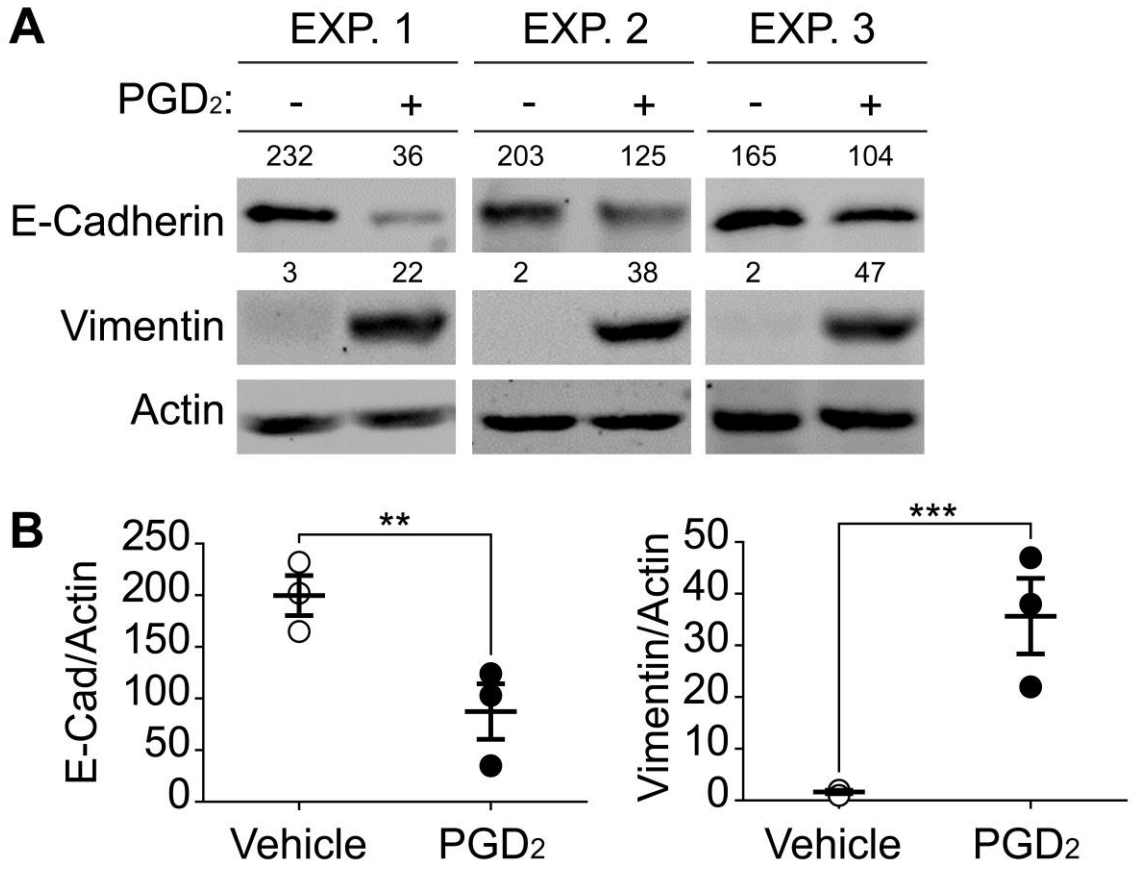
**Table 1 Sequences of primers for qPCR.**

| Target mRNA | Primer | Sequence |
|---|---|---|
| Slug | Forward | 5’-AGATGCATATTCGGACCCACA-3’ |
| | Reverse | 5’-CCTCATGTTTGTGCAGGAGAG-3’ |
| TGF-β1 | Forward | 5’-CAGCAACAATTCCTGGCGATA-3’ |
| | Reverse | 5’-GCTAAGGCGAAAGCCCTCAAT-3’ |
| TGFβR1 | Forward | 5’-TGGCTCAGGTTTACCATTGC-3’ |
| | Reverse | 5’-TTCTCCAAATCGACCTTTGC-3’ |
| GAPDH | Forward | 5’-GTCAGTGGTGGACCTGACCT-3’ |
| | Reverse | 5’-TGAGCTTGACAAAGTGGTCG-3’ |

**Table 2 Sequences of shRNA**

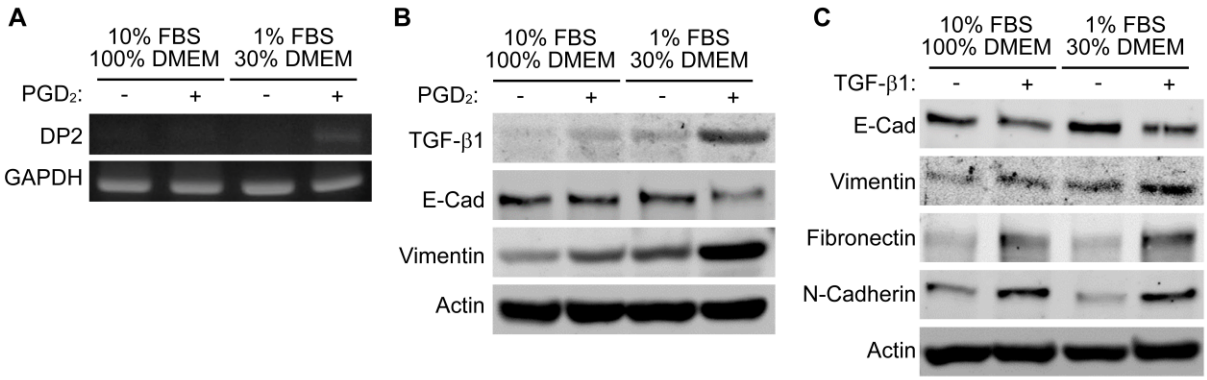
| Target Gene | Strand | Sequence |
|---|---|---|
| TGF-β1 | Forward | 5’-CCGGTACCAGAAATACAGCAACAATTCCTGCTCGAGCAGGAATTGTTGCTGTATTTCTGGTTTTTTG-3’ |
| | Reverse | 5’-AATTCAAAAAACCAGAAATACAGCAACAATTCCTGCTCGAGCAGGAATTGTTGCTGTATTTCTGGTA-3’ |
| TGFβR1 | Forward | 5’-CCGGTGGGTCTGTGACTACAACATCTCGAGATGTTGTAGTCACAGACCCTTTTTG-3’ |
| | Reverse | 5’-AATTCAAAAAGGGTCTGTGACTACAACATCTCGAGATGTTGTAGTCACAGACCCA-3’ |

## Supplemental Figures




**Online Figure S1**

**Figure S1. PGD2-dependent EMT of A549 cells. (A)** A549 cells were treated for 5 days in the presence or absence of PGD2 (15 μM) in DMEM (100%) with 0.5% FBS. Repeated experiments were performed at the same condition. Expression of epithelial and mesenchymal marker proteins were verified by western blot analysis. Actin-based band intensities were indicated at upper region of panel. **(B)** Band intensity was acquired by Li-COR odyssey instrument and actin-based band intensity was plotted. Data are means ± SEM of three independent experiments. Statistical significance compared to control group was determined by Student's t-test. **, $P < 0.01$; ***, $P < 0.001$.




# Online Figure S2

**Figure S2. Effect of serum and nutrtion on the EMT. (A)** A549 cells were treated for 5 days in the presence or absence of PGD2 (15 µM) in normal (10% FBS, 100% DMEM) and low serum/nutrition (1% FBS, 30% DMEM). Expression of DP2 was analyzed by RT-PCR. **(B)** A549 cells were treated for 5 days in the presence or absence of PGD2 (15 µM) in normal (10% FBS, 100% DMEM) and low serum/nutrition (1% FBS, 30% DMEM). Expression of TGF-β1 as well as other marker proteins were verified by western blot analysis. **(C)** A549 cells were treated for 5 days in the presence or absence of TGF-b1 (2 ng/ml) in normal (10% FBS, 100% DMEM) and low serum/nutrition (1% FBS, 30% DMEM). EMT of A549 cells was validated by western blot analysis.