

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure 1. OLFM4 has an anti-apoptotic property against the simvastatin-induced apoptosis in human gastric cancer cells. An OLFM4-knockdown cell line (OLFM4-Kd) was established from AGS human gastric cancer cell using pSUPER.retro vector and hairpin siRNA template and maintained in the medium containing puromycin (0.1 mg/mL), as previously described (Kim *et al.*, 2010). **(A)** OLFM4 mRNA was successfully knocked down with shRNA in AGS cells. Total RNA was analyzed by RT-PCR using OLFM4 primer sets. β -Actin mRNA was used as an internal control. **(B)** Immunoblot analysis showed a reduced OLFM4 protein level in OLFM4-Kd cells. α -Tubulin was used as a protein loading control. Non-targeting control shRNA was used in Mock. **(C and D)** AGS cell cycle was not affected by OLFM4 knockdown. Data represent percent distribution of cells at different phases of the cell cycle (n = 3). **(E)** MTT cell proliferation assay showed no significant difference between Mock and OLFM4-Kd cells. Measurements were converted to fold increase over the value obtained at day 1. **(F)** Percentages of the apoptotic cells were determined in Mock and OLFM4-Kd cells by flow cytometry following simvastatin treatment. **(G and H)** Mock or OLFM4-Kd cells were treated with combinations of 1 μ M simvastatin, 400 μ M mevalonate (product of HMG-CoA reductase), and 20 μ M Z-VAD-fmk (pan-caspase inhibitor). Percentages of apoptotic cells were then determined by FACS analysis. Caspase-3 activity was also measured using a fluorescent assay kit. Data in E-H are represented as mean \pm SD (n = 3).

Reference

Kim, K.K., Park, K.S., Song, S.B. and Kim, K.E. (2010) Up regulation of GW112 Gene by NF kappaB promotes an antiapoptotic property in gastric cancer cells. *Mol Carcinog* **49**, 259-270.

