Supplemental Figure

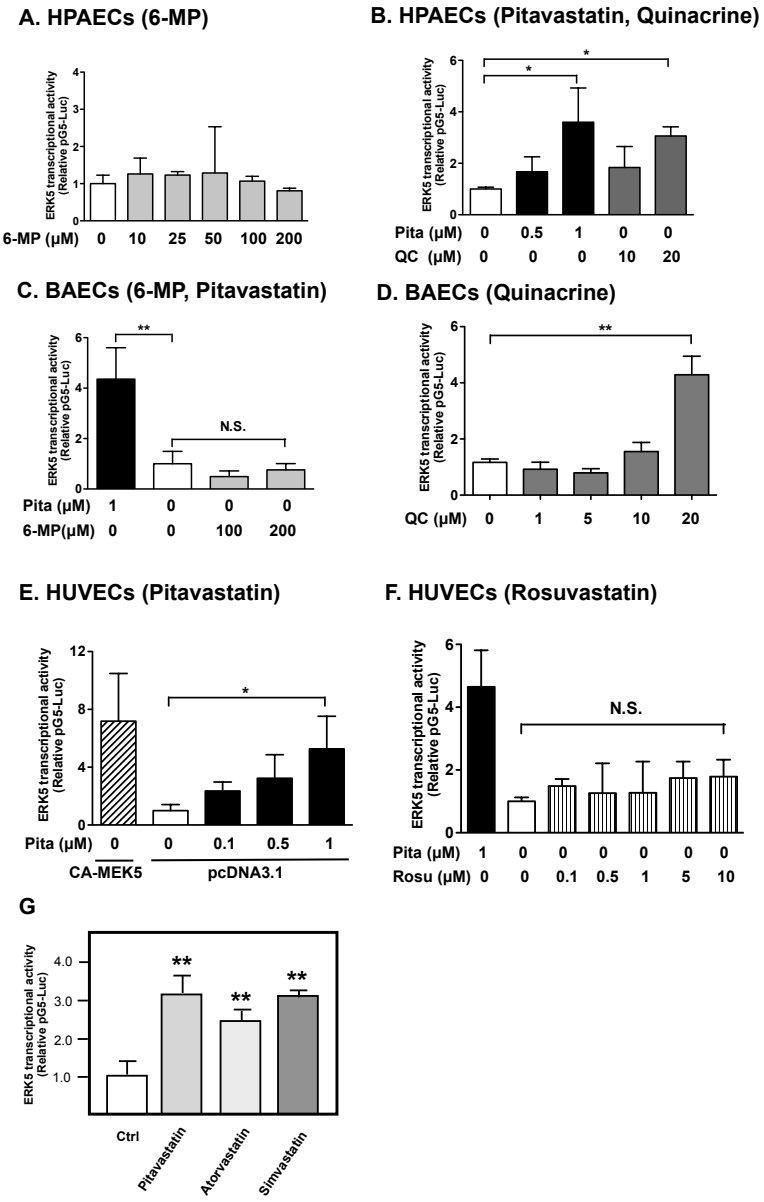

Figure S1: Activation of ERK5 transcriptional activity induced by 6-mercaptopurine (6-MP), quinacrine (QC), pitavastatin (Pita), and rosuvastatin (Rosu) in various types of ECs.

HPAECs (**A** and **B**), BAECs (**C** and **D**) and HUVECs (**E** and **F**) were transfected with pG5-Luc and pBIND-ERK5 constructs as described in Fig.1. Twenty-four hours later, transfected cells were treated with 6-MP, pitavastatin, QC and rosuvastatin as indicated for 24 hrs. Luciferase activity was assayed as described in Fig.1. Values represent firefly luciferase:*Renilla* luciferase ratios. Values are mean ± SD (n=3) * $p<0.05$, ** $p< 0.01$ versus non-treated control. (**G**) ERK5 transcriptional activation induced by various statins in HUVECs. HUVECs were transfected with pG5-Luc and pBIND-ERK5 constructs as described in Fig.1. Twenty-four hours later, transfected cells were treated with pitavastatin, atorvastatin, or simvastatin for 24 hrs. Luciferase activity was assayed as described in Fig.1. Values represent firefly luciferase:*Renilla* luciferase ratios. Values are mean ± SD (n=3), ** $p< 0.01$ versus untreated control.

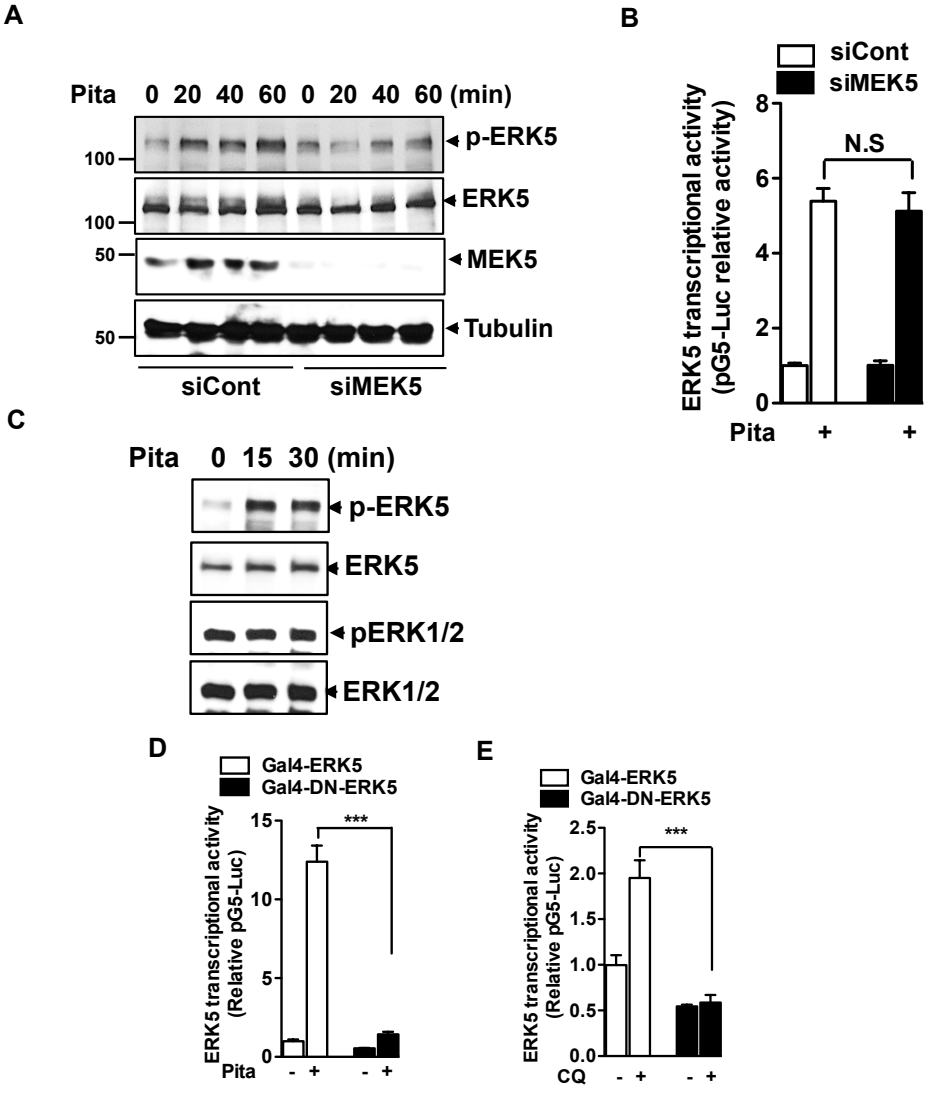

Figure S2: ERK5 TEY motif phosphorylation, but not activation of ERK5 transcriptional activity, was inhibited by MEK5 siRNA transfection. (**A**) HUVECs were transfected with MEK5 siRNA (siMEK5, 100 nM) or control siRNA (siCont) for 48 hrs prior to pitavastatin stimulation as indicated. p-ERK5, ERK5, MEK5, and tubulin expression were assayed by Western blotting with each specific antibodies. Representative blots from three independent experiments are shown. (**B**) HUVECs were transfected with siMEK5 (100 nM) or siCont for 48 hrs. Cells were then transfected with pG5-Luc and Gal4-ERK5 as described in the methods. Cells were then stimulated with pitavastatin as indicated, and luciferase activity was assayed. Values are mean ± S.D, n=3 (non-treated groups), n=6 (treated groups). (**C**) **Pitavastatin increased ERK5 TEY motif phosphorylation, but not ERK1/2 phosphorylation.** HUVECs were treated with pitavastatin as indicated. p-ERK5, ERK5, p-ERK1/2, and ERK1/2 expression were assayed by Western blotting with each specific antibodies. Representative blots from three independent experiments are shown. (**D-E**) **ERK5 TEY motif phosphorylation mutant (AEF) inhibited pitavastatin (pita) and chloroquine (CQ)-induced ERK5 transcriptional activity.** HUVECs were transfected with pG5-Luc and Gal4-ERK5 as described in the methods. Cells were then stimulated with pitavastatin (D) or CQ (E) as indicated, for 24 hrs, and luciferase activity was assayed. Values are mean ± S.D (n=6).