SUPPLEMENTARY FIGURES.

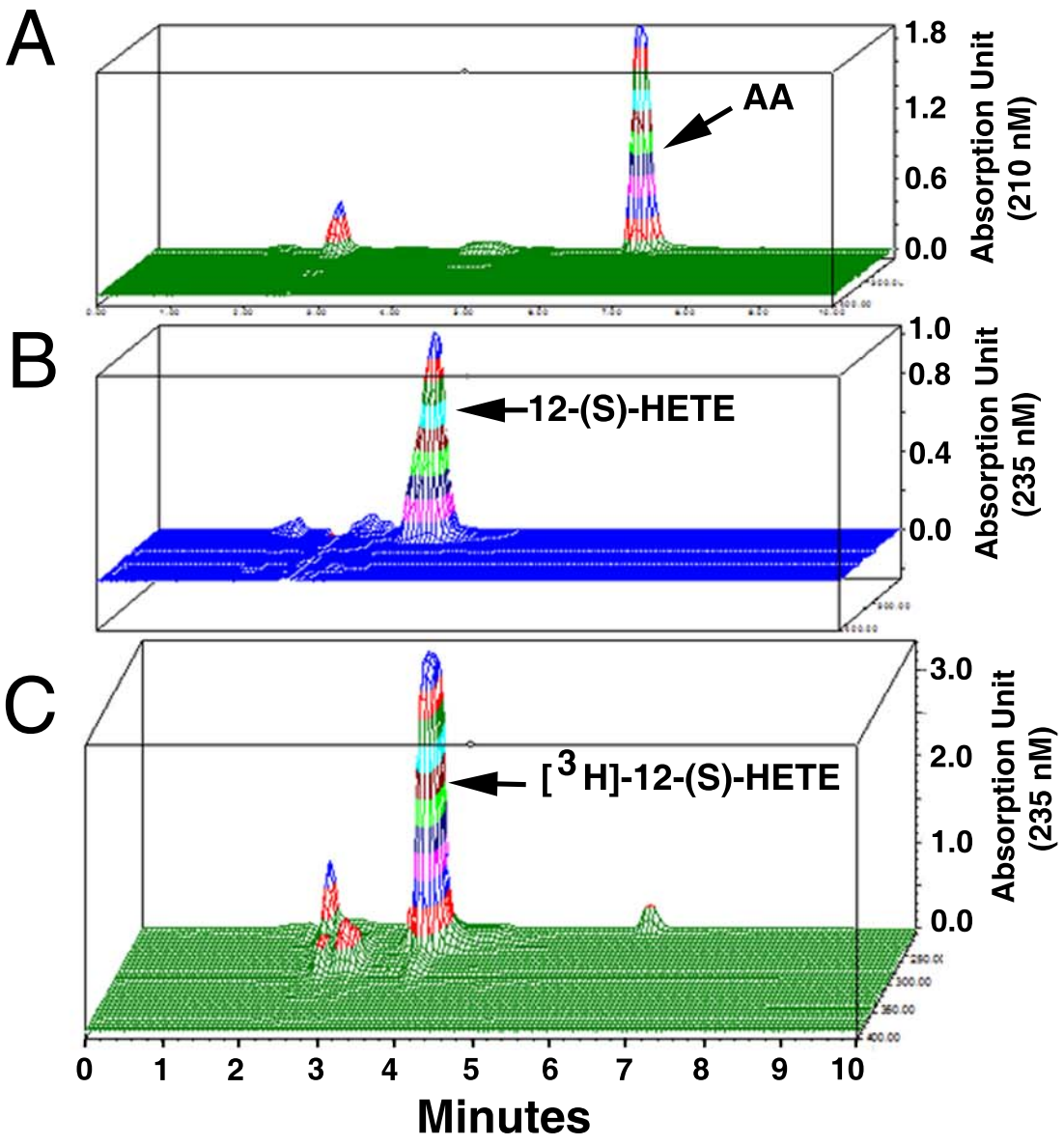
Supplemental Figure 1. Synthesis of [^{3}H]-12-(S)-HETE. The HPLC elution profile of arachidonic acid (*A*), 12-(S)-HETE standards (*B*), or synthesized [^{3}H]-12-(S)-HETE (*C*). The synthesized [^{3}H]-12-(S)-HETE was collected in fractions eluted between 3.75-4.75 min (*C*).

Supplemental Figure 2. *A.* The mRNA level of *GPR31* expression is 2.92 ±1.9 fold higher in PC3 cells than that in the DU145 cells (*, $p < 0.05$). The specific binding of [^{3}H]-12-(S)-HETE is 6.8 ± 2.9 fold higher in PC3 cells than that in the DU145 cells (*, $p < 0.05$). *B.* Specific binding of [^{3}H]-12-(S)-HETE in *GPR31*, *BLT2* and *pcDNA* vectors transfect CHO cells. Note that the specific binding of [^{3}H]-12-(S)-HETE in *GPR31* expressing cells is markedly higher than that in BLT2 expressing cells.

Supplemental Figure 3. 12-(S)-HETE/GPR31 signaling is unable to induce intracellular Ca^{+2} mobilization. (*A*) Ca^{+2} mobilization was measured as we previously described (1). Note that various concentrations of 12-(S)-HETE was unable to stimulate intracellular Ca^{+2} mobilization in PC-3 cells. (*B*) Expression of GPR31 receptors in a panel of endothelial cells. (*C*) 12-(S)-HETE was unable to induce Ca^{+2} mobilization in HUVEC cells. In a control, sphingosine-1-phosphate (S1P) is able to induce Ca^{+2} mobilization in HUVEC. HUVEC, human umbilical vein endothelial cells; HBMEC, human brain microvascular endothelial cell, HPAC, human pulmonary aortic endothelial cells.

Reference

1. Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI, Hla T (1999). Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. Cell 99: 301-312.



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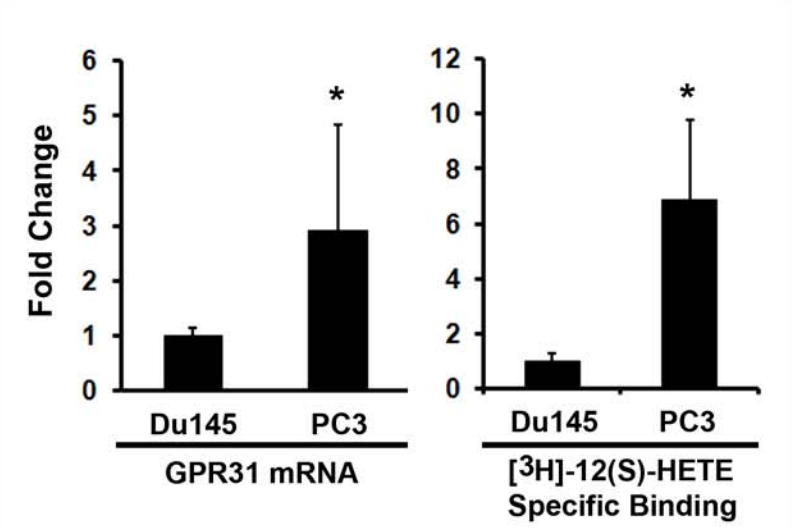
A



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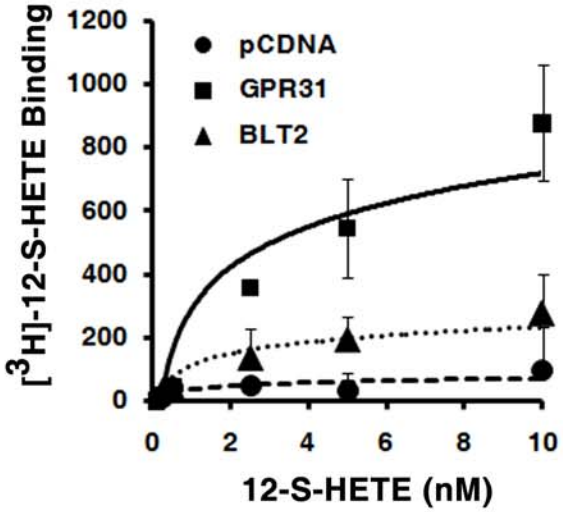
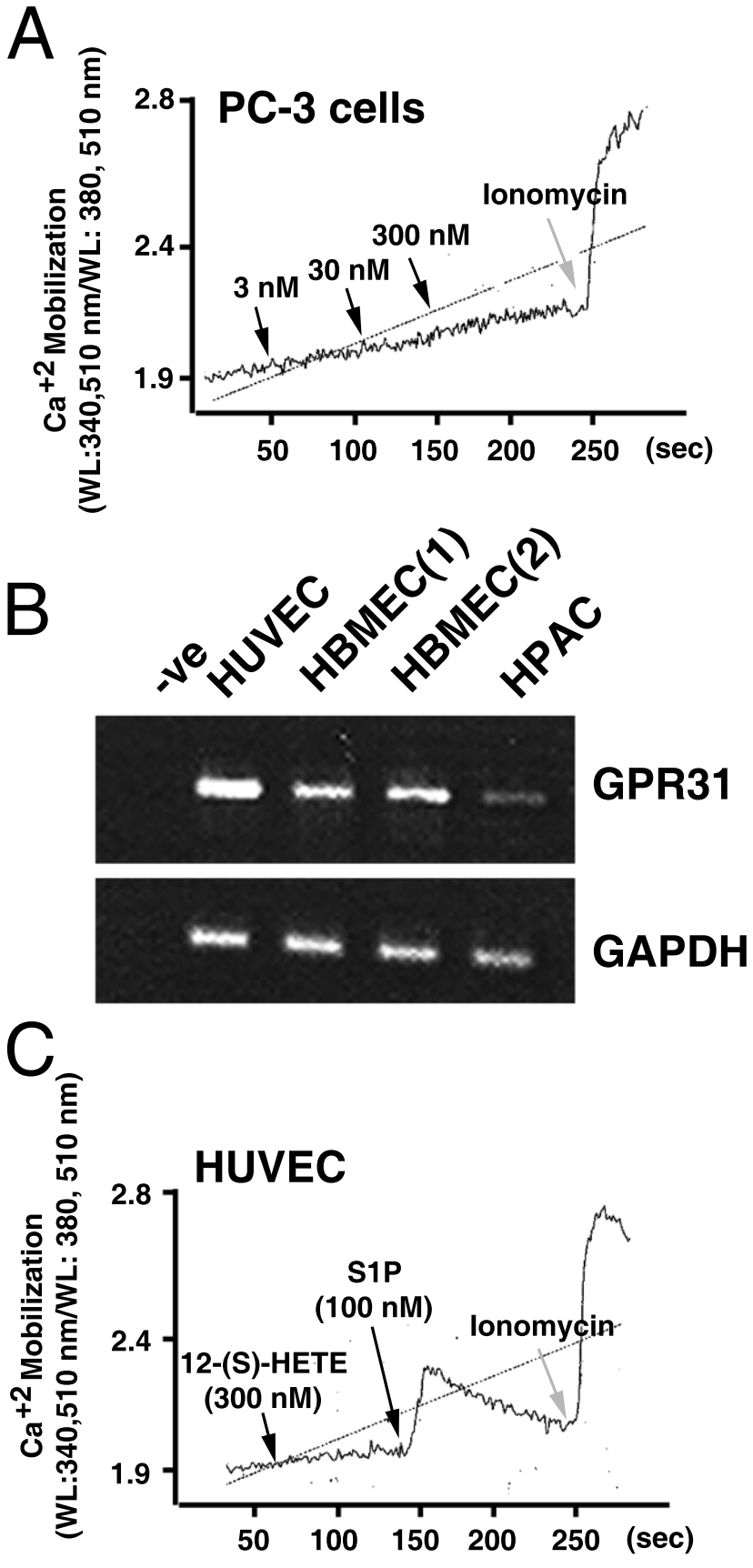


Guo et al., Suppl. Fig 2



Guo et al., Suppl. Fig. 3