The Apoptotic Mechanism of Action of the Sphingosine Kinase 1 Selective Inhibitor, SKI-178, in Human AML Cell Lines.

Taryn E. Dick, Jeremy A. Hengst, Todd E. Fox, Ashley L. Colledge, Vijay P. Kale, Shen-Shu Sung, Arun Sharma, Shantu Amin, Thomas P. Loughran Jr., Mark Kester, Hong-Gang Wang, Jong K. Yun.

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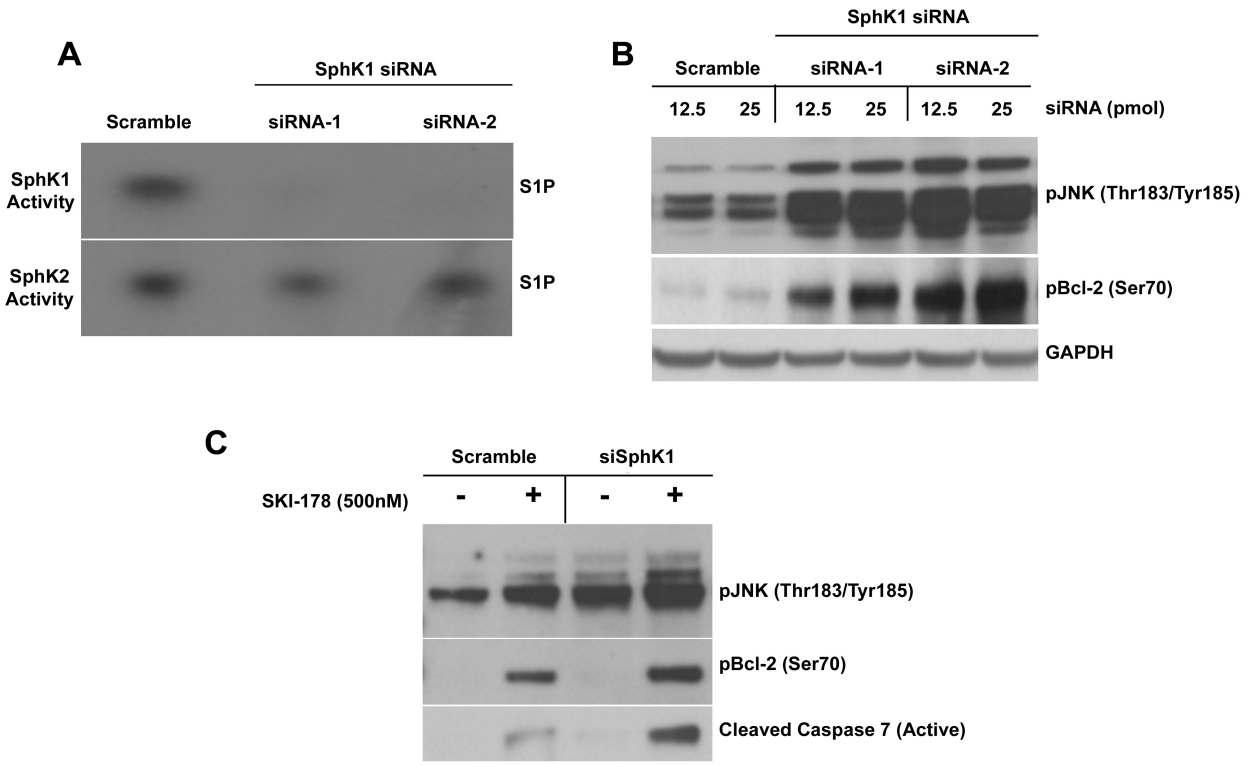
Supplementary Figure 1: siRNA knockdown of SphK1 recapitulates the effects of SKI-178: (A) MIA PaCa-2 cells were transfected with 25pmol siRNA for 72h. Scramble siRNA serves as a negative control. Whole cell lysate were prepared in buffers selective for SphK1 or SphK2 activity. SphK1 and SphK2 catalytic activity was subsequently determined by thin-layer chromatography. (B) MIA PaCa-2 cells were transfected with 12.5 or 25pmol siRNA for 72h. Scramble siRNA serves as a negative control. Western blot analysis was performed on whole cell lysate using indicated antibodies. (C) MIA PaCa-2 cells were transfected with 25pmol siRNA. After 48h, transfected cells were treated with 500nM SKI-178 and incubated for an additional 24h. Scramble siRNA serves as a negative control. Western blot analysis was performed on whole cell lysate using indicated antibodies. GAPDH serves as a loading control.



Supplementary Figure 1