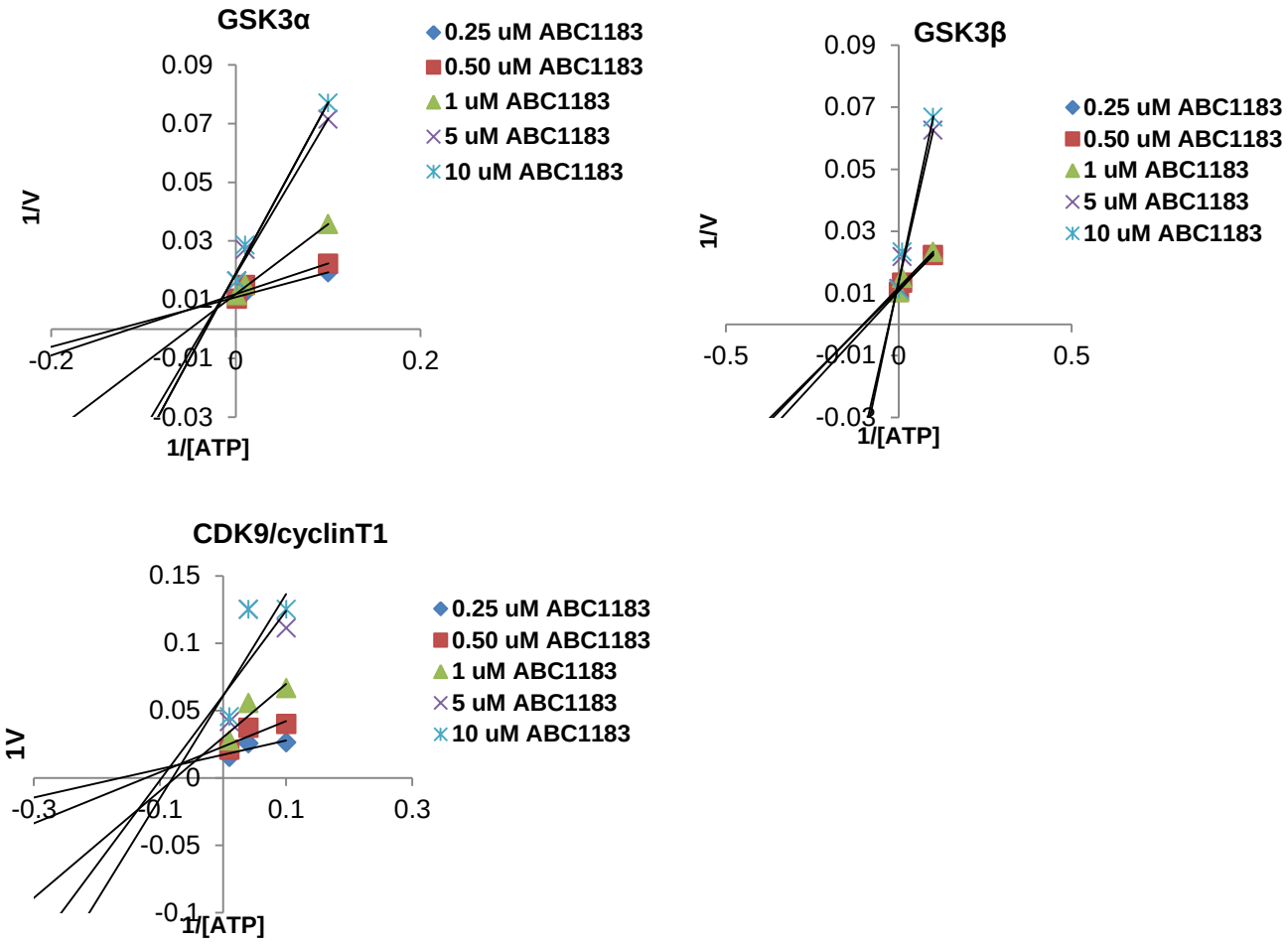
In vitro and in vivo anti-tumor and anti-inflammatory capabilities of the novel GSK3 and CDK9 inhibitor ABC1183. Randy S Schrecengost, Cecelia L Green, Yan Zhuang, Staci N Keller, Ryan A Smith, Lynn W Maines, Charles D Smith. The Journal of Pharmacology and Experimental Therapeutics.

Supplementary Fig S1



Supplementary Fig. S1. Inhibition mode of ABC1183 against GSK3 isoforms and CDK9. *in vitro* inhibition assays were carried out with various concentrations of ATP and fixed amounts of substrate in the presence of increasing concentrations of ABC1183. GSK3α and GSK3β are inhibited by ABC1183 in an ATP-competitive fashion while inhibition of CKD9 is ATP non-competitive.

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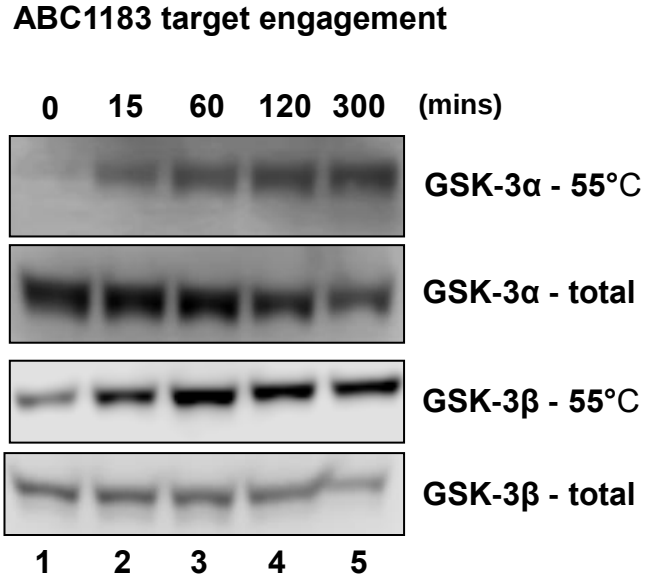
Supplementary Fig S2



Supplementary Fig. S2. ABC1183 physically engages with GSK3α and GSK3β. LNCaP cells were treated with 3 μM ABC1183 for indicated times, cells collected, resuspended in PBS with protease inhibitors, then heated for 3 minutes at 55°C. Cell suspensions were then immunoblotted with GSK3α and GSK3β antibodies. Unheated cell suspensions were also immunoblotted for loading controls. In the absence of inhibitor, heating degrades proteins. With physical association, proteins are more stable and are not degraded by heat.

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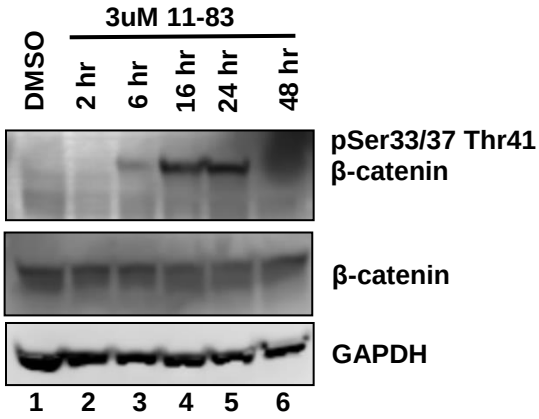
Supplementary Fig S3.



Supplementary Fig. S3. ABC1183 treatment induces proteasome targeting phosphorylation of β-catenin. FaDu cells were treated with 3 μM ABC1183 for 2 to 48 hours, as indicated. Cell lysates were immunoblotted with pSer33/37 Thr41 β-catenin, β-catenin and GAPDH.

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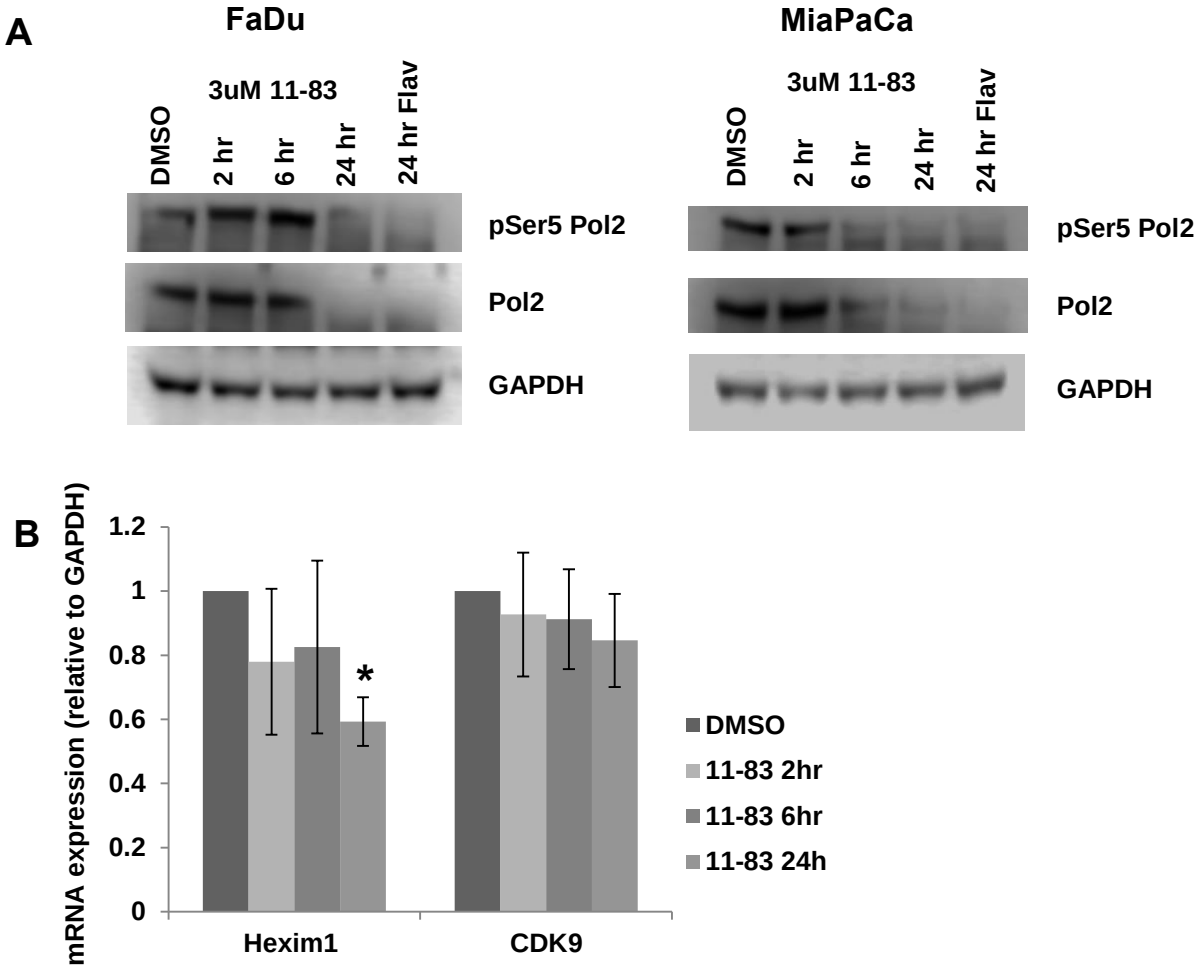
Supplementary Figure S4



Supplementary Fig. S4. Inhibition of CDK9 signaling by ABC1183. (A). FaDu and MiaPaCa cells were treated with 3 μM ABC1183 or Flavopiridol for 2 to 24 hours, as indicated. Cell lysates were immunoblotted with pSer5 Pol2, Pol2 and GAPDH. (B). FaDu cells were treated with 3uM ABC1183 for indicated times and mRNA transcript levels of Hexim1 and cdk9 were measured by qRT-PCR. * $p<0.05$.

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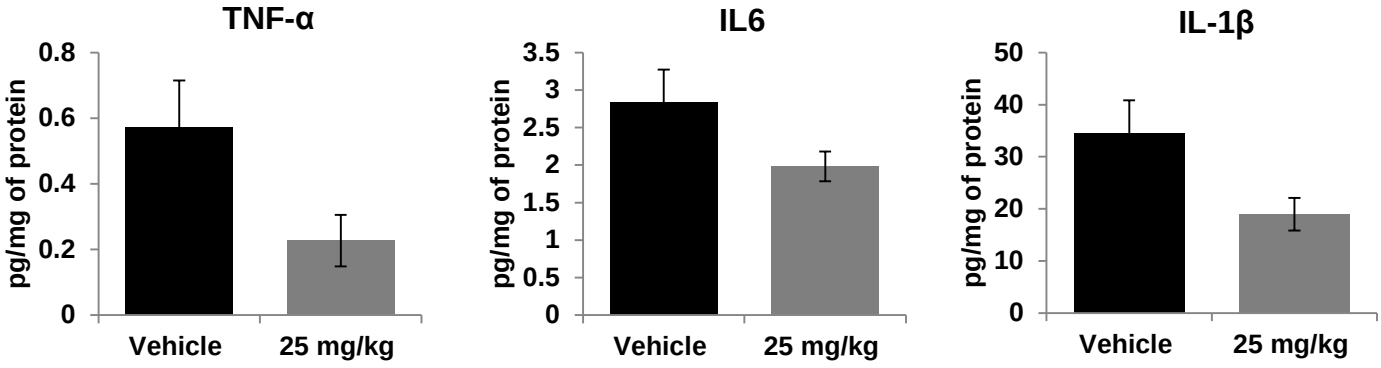
Supplementary Figure S5



Supplementary Fig. S5. Intratumoral cytokine expression is decreased following ABC1183 administration (A). Pan02 tumor-bearing C57BL/6 mice treated with 25 mg/kg ABC1183 or Vehicle for 12 hours, as indicated, followed by tumor extraction. Tumor lysates were prepared and cytokine levels of TNF-α, IL-6 and IL-1β were determined.