SUPPLEMENTAL INFORMATION

Jaspine B induces non apoptotic cell death in gastric cancer cells independently of its inhibition of ceramide synthase

Francesca Cingolani[1,&,#], Fabio Simbari[1], Jose Luis Abad[1], Mireia Casasampere[1], Gemma Fabrias[1], Anthony H. Futerman[2], Josefina Casas[1#]

[1] Research Unit on BioActive Molecules (RUBAM), Department of Biomedicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), 08034 Barcelona, Spain. [2]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel.

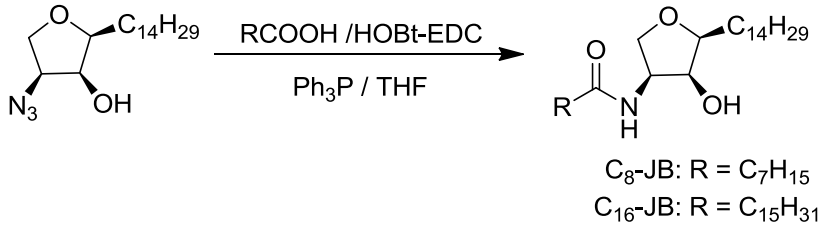
Running title: Biological effect of Jaspine B in cancer cells

& Current address: Division of Digestive Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA.

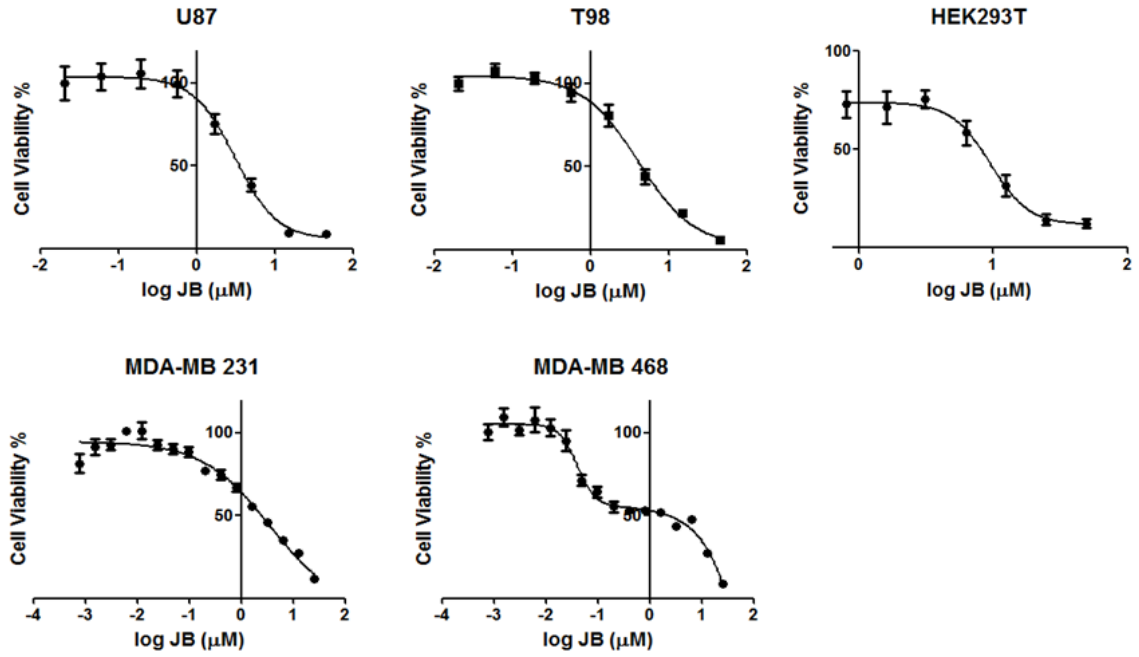
Corresponding authors: Josefina Casas (fina.casas@iqac.csic.es) and Francesca Cingolani (fcingol@emory.edu)

Address correspondence to Josefina Casas, Department of Biomedicinal Chemistry. IQAC-CSIC. Jordi Girona 18, 08034-Barcelona, Spain. Tel. (+34)-934 006 115; FAX (+34)932 045 904. E-mail: fina.casas@iqac.csic.es.

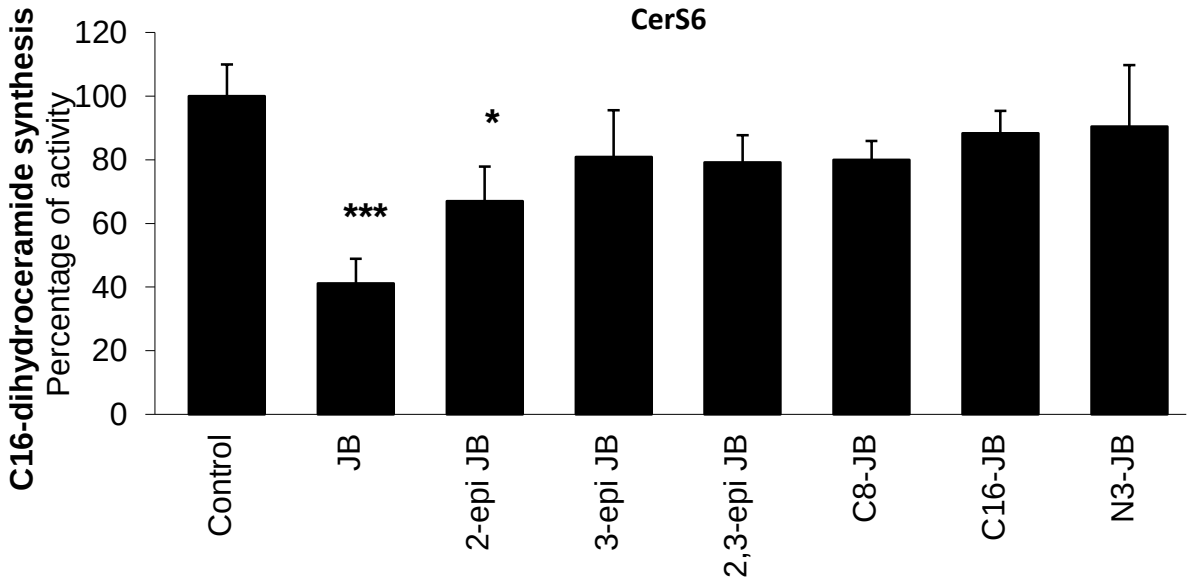
LIST OF SUPPLEMENTAL DATA



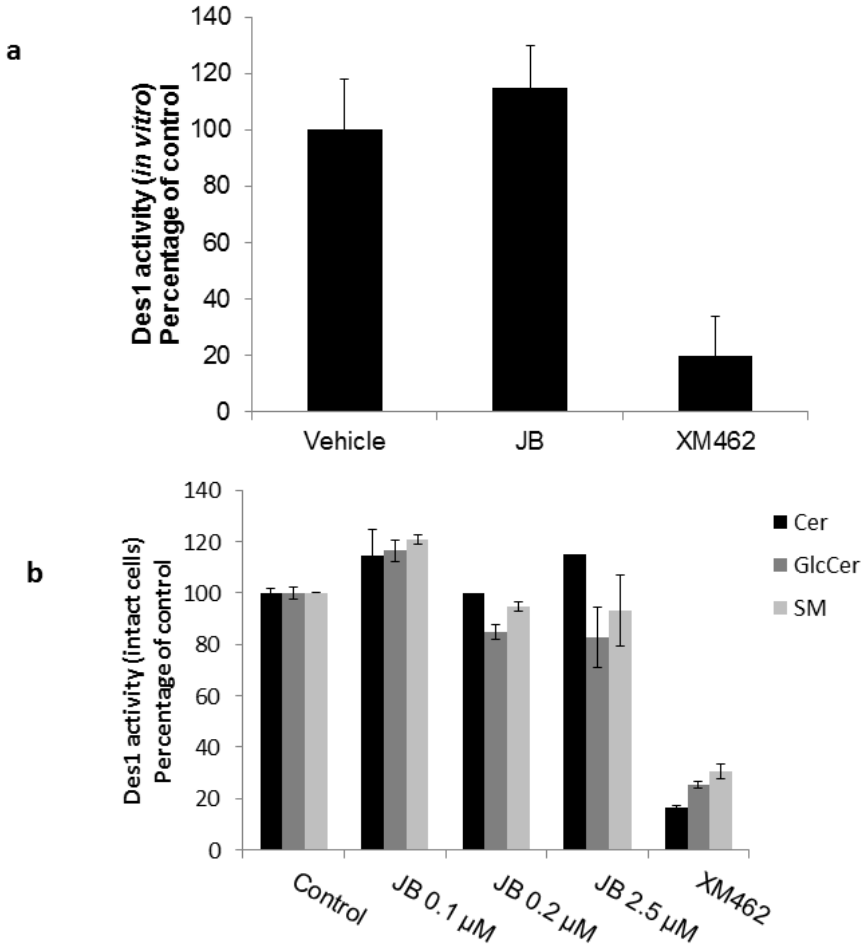
Supplemental Figure S1. *N*-octanoyl-JB (C_8-JB) and *N*-hexadecanoyl-JB (C_{16}-JB) synthesis (Van den Berg RJBHN, Boltje TJ, Verhagen CP, Litjens REJN, van der Marel GA, Overkleeft HS. 2006. An efficient synthesis of the natural tetrahydrofuran pachastrissamine starting from D-ribo-phytosphingosine. *J Org Chem*. **71**:836–839.)



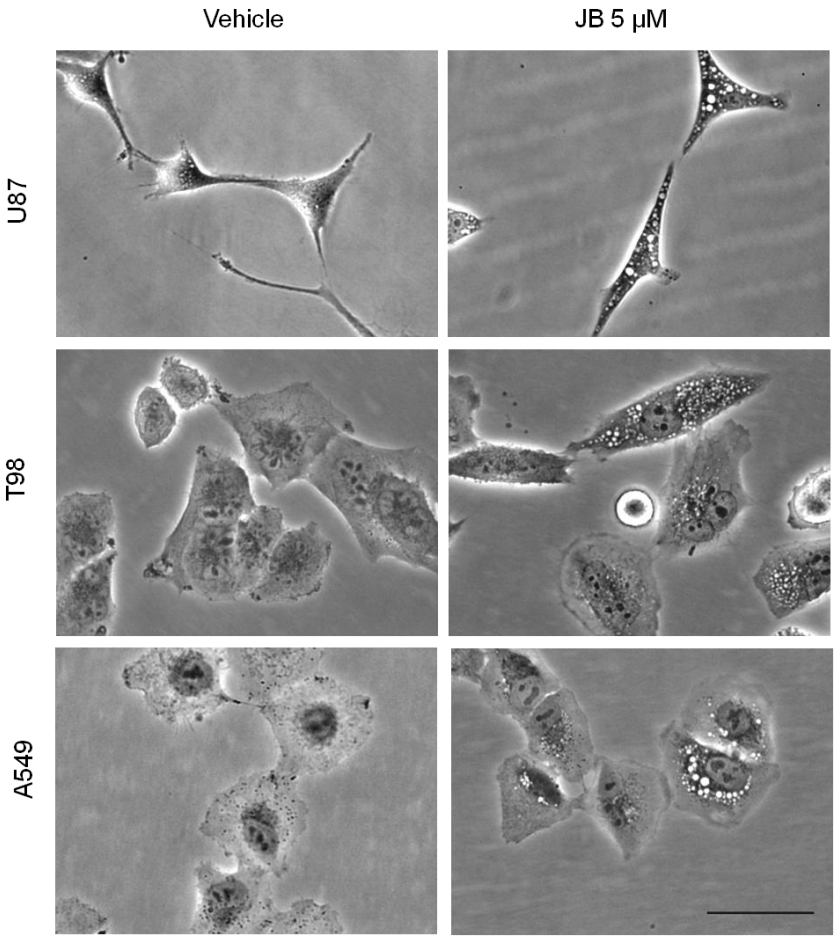
Supplemental Figure **S2.** Effect of JB on cell viability. U87, T98, MDA-MB-231, MDA-MB-468 and HEK293T cells were incubated with different concentrations of JB or ethanol (≤0.25%) for 24 h. Viability was determined by MTT assay, and expressed as a percentage of the control value. Data are means ±SD of 2-3 independent experiments performed in triplicate.



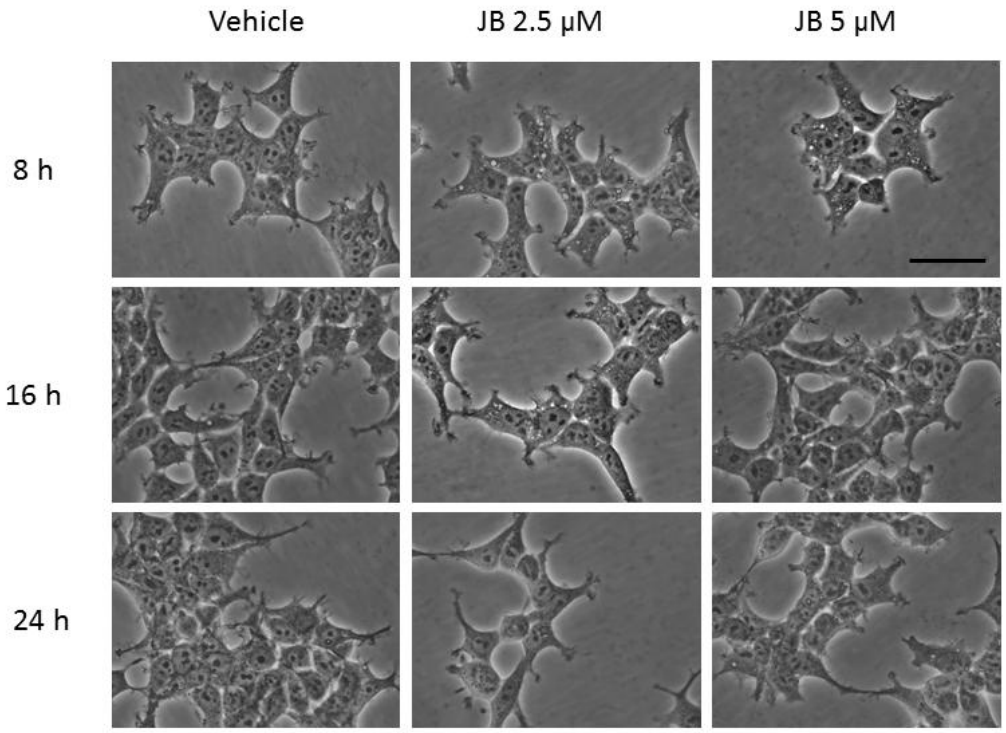
Supplemental Figure **S3.** CerS6 inhibition by JB, JB stereisomers, acyl-JB and N3-JB. Cell lysates were incubated with the indicated compounds (5μM) or ethanol for 5 minutes and then incubated for 10 minutes with NBD-Sa. Lipids were extracted and NBD-dhCer was separated by TLC. Results are means ± SD of two experiments in duplicates (*, $p<0.05$; ***$p<0.001$, t test)



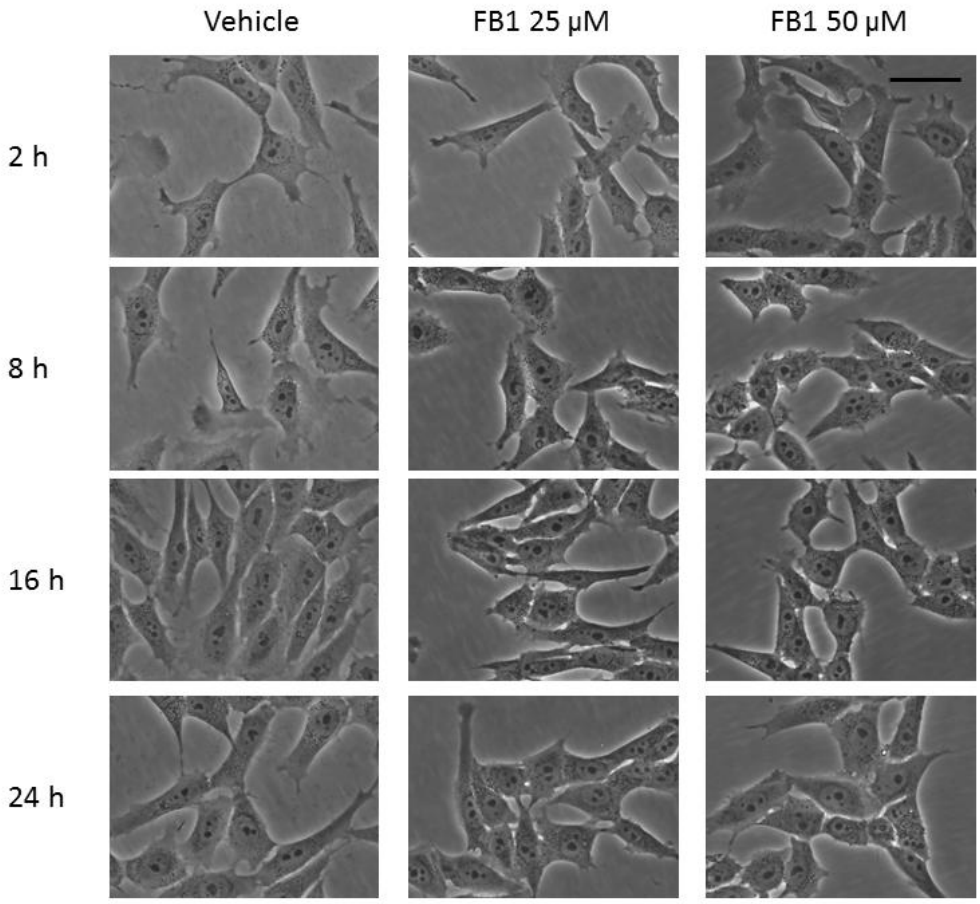
Supplemental Figure **S4.** Jaspine B does not inhibit Des1. (**a**) HGC-27 cell lysates were incubated with equimolar amount of JB and dhCer-C6-NBD (10 μM). Samples were processed and analyzed as detailed in the Supplementary Materials and Methods section. Data are means ± SD of three independent experiments performed in triplicate. (**b**) HGC-27 cells were incubated with DHCer-C6-NBD (10 μM) and Jaspine B for 4 hours and *Des1* activity was calculated as detailed in the Supplementary Materials and Methods section. Data are means ± SD of one representative experiment with triplicates. In both intact and cell lysates experiments XM462 (8 μM) was included as a positive control. Cer: Ceramide-C6-NBD, GlcCer: Glucosylceramide-C6-NBD, SM: Sphingomyelin-C6-NBD.



Supplemental Figure **S5.** JB induces cell vacuolation in various cancer cells. Phase contrast pictures of cell lines incubated with JB 5 µM or ethanol after 4 hours (U87), 6 hours (T98) and 8 hours (A549). Images are representative of two different experiments. Scale bar: 50 µm.



Supplemental Figure **S6.** JB does not induce cell vacuolation in HEK293T cells. Phase contrast pictures of HEK293T cells incubated with JB 2.5 µM, 5 µM or ethanol as a vehicle after 8 hours, 16 hours and 24 hours. Images are representative of the cell phenotype observed in two different experiments. Scale bar: 50 µm.



Supplemental Figure **S7.** FB1 does not induce cell vacuolation in HGC-27 cells. Phase contrast pictures of HGC-27 cells incubated with FB1 25 µM, 50 µM or ethanol as a vehicle after 2 hours, 8 hours, 16 hours and 24 hours. Images are representative of the cell phenotype observed in two different experiments. Scale bar: 50 µm.

Supplemental Experimental Procedure

Determination of *Des1* activity *in vitro* was performed as previously reported (1). For Des1 activity determination in intact cells, cells were seeded at a density of $2x10^5$cells/well in 6 well-plates. Twenty four hours later, the medium was removed and fresh medium containing*N*-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-D-*erythro* dihydrosphingosine (dhCerC6NBD, 10 μM) and JB or XM462 or ethanol was added. After 4 h incubation, cells were collected and trypsinized. After adding 1 ml of methanol, 100 μl of the cell lysate were injected into the HPLC-FD equipment. Equipment and analysis conditions were as detailed previously (1).

Reference

1. Munoz-Olaya, J. M., X. Matabosch, C. Bedia, M. Egido-Gabás, J. Casas, A. Llebaria, A. Delgado, and G. Fabrias. 2008. Synthesis and biological activity of a novel inhibitor of dihydroceramide desaturase. *ChemMedChem* **3**:946–953.