Supplementary Data for

Poloxamer-linked prodrug of a topoisomerase I inhibitor SN22 shows efficacy in models of high-risk neuroblastoma with primary and acquired chemoresistance

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This file includes Supplementary Figures S1 to S5.

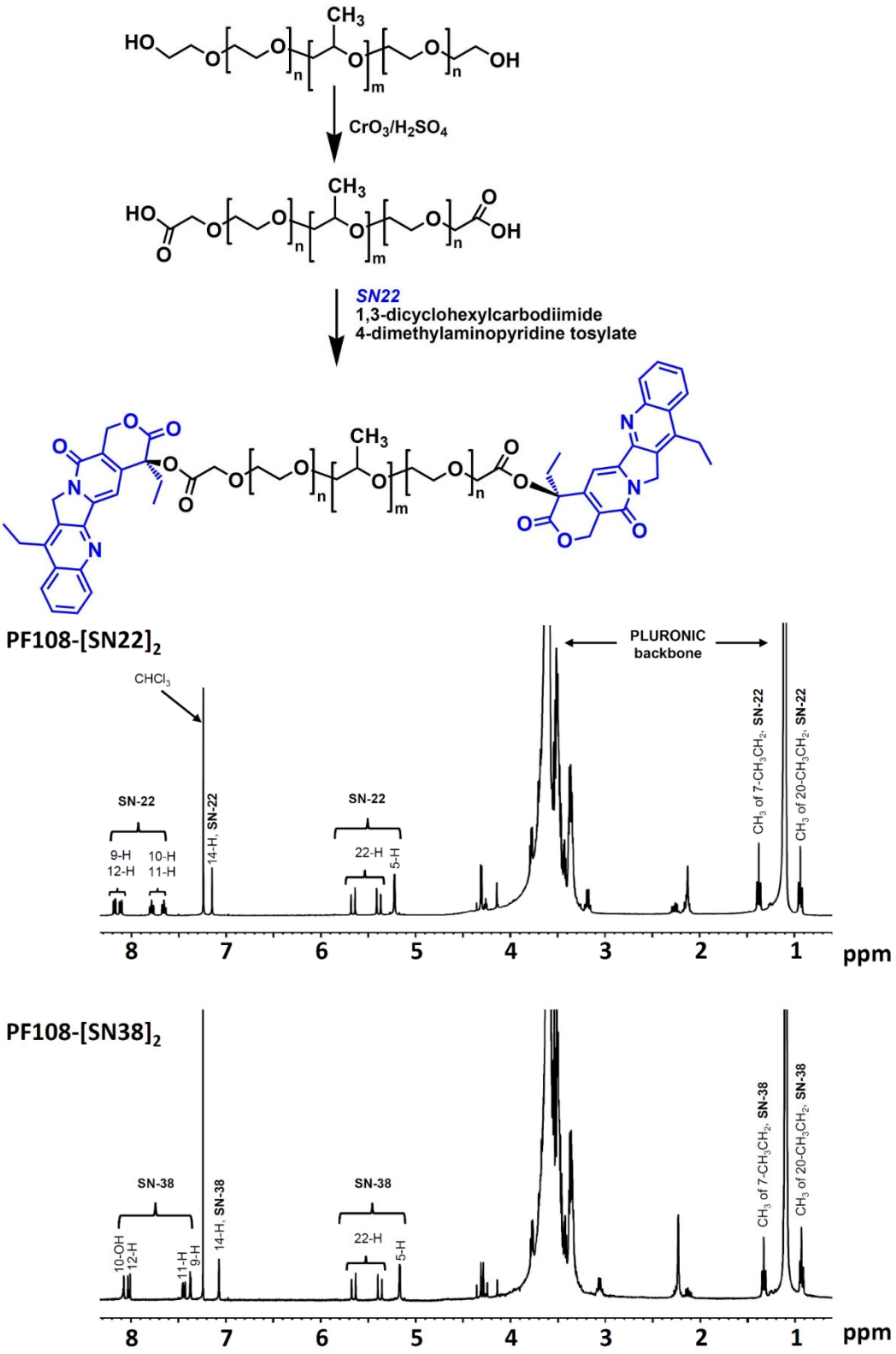

Fig. S1. The synthetic scheme and ^{1}H NMR spectra (400 MHz, $CDCl_3$) of Poloxamer-linked prodrugs of SN22 and SN38. The Poloxamer 338 (Pluronic F-108) scaffold has m~50 and n~135.

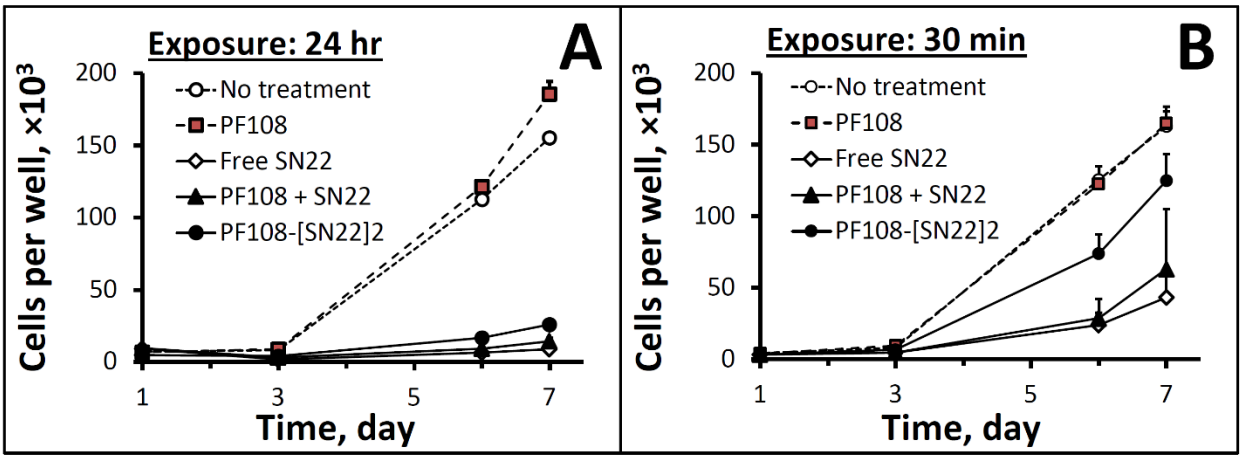

Fig. S2. Growth inhibition of *MYCN*-amplified NB cells exhibiting an acquired loss of p53 tumor suppressor function [BE(2)C] by PF108-$[SN22]_2$ prodrug vs. SN22 with or without Pluronic F-108 (doses equivalent to 100 nM of SN22). Cells untreated or treated with blank Pluronic F-108 were included as controls. Tested exposure durations included 24 hr and 30 min (**A** and **B**, respectively). Cell growth was monitored over time by bioluminescence. Results are shown as mean ± SD.

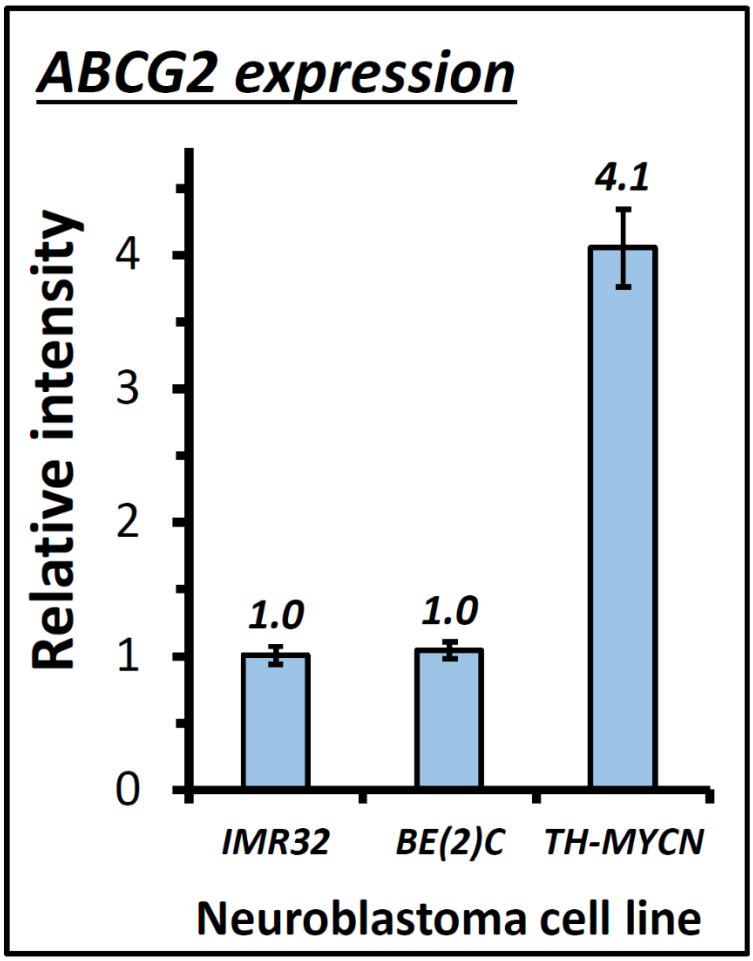

Fig. S3. ABCG2 transporter expression by NB cells representing different forms of *MYCN*-driven high-risk disease: *MYCN*-amplified IMR-32 and BE(2)C cells derived at diagnosis and at relapse after intensive chemoradiotherapy, respectively, and *MYCN*-overexpressing TH-*MYCN* NB cells derived from chemo-naïve primary tumors. ABCG2 expression was determined by Western blot densitometry using whole cell extract (50 μg) and a rat monoclonal antibody to ABCG2 (BXP-53, Santa Cruz Biotechnology) diluted 1:500 in 5% BSA/PBS-Tween 20 as a blocking agent. Densitometric analysis was performed with ImageJ software. The results normalized to β-actin are presented as mean ± SD.

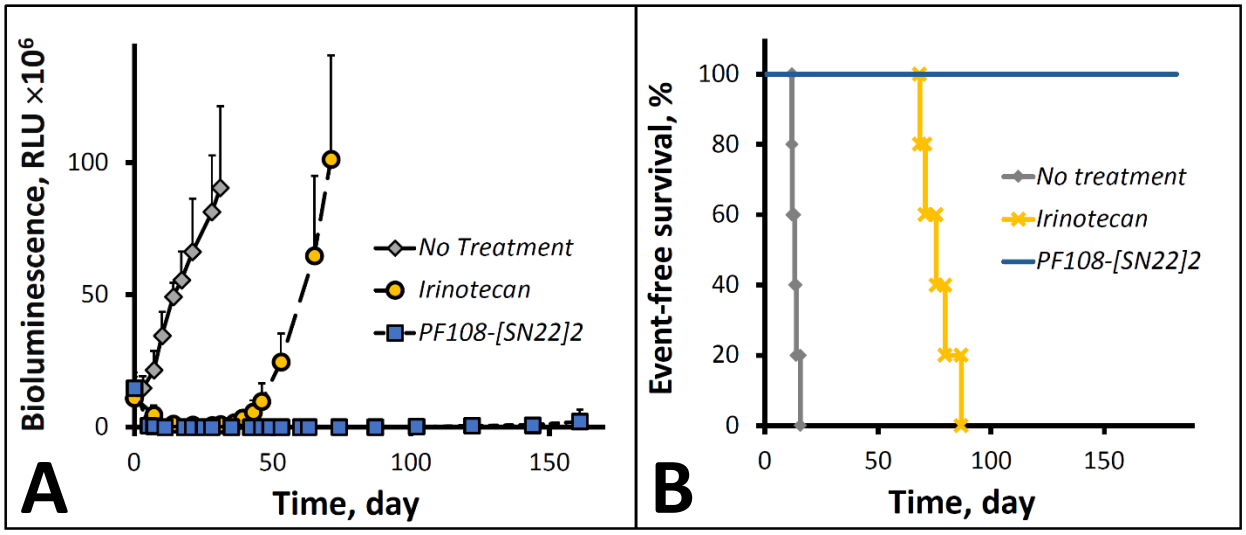

Fig. S4. Therapeutic efficacy of PF108-$[SN22]_2$ in an orthotopic model of chemo-naïve (newly diagnosed) *MYCN*-amplified NB. Mice were inoculated with 10^6 IMR-32 cells stably expressing luciferase. Treatment with PF108-$[SN22]_2$ was administered intravenously at a dose equivalent to 10 mg/kg of SN22 once a week for 4 weeks. Irinotecan administered twice a week at 15 mg/kg over 4 weeks was included as a control. Tumor-associated signal was monitored by quantitative bioluminescence (representative images taken at 0-9 weeks are shown in Fig. 3A of the main text). Quantitative data presented graphically in **A** are expressed as mean ± SD. The survival curves for respective animal groups are shown in **B**.

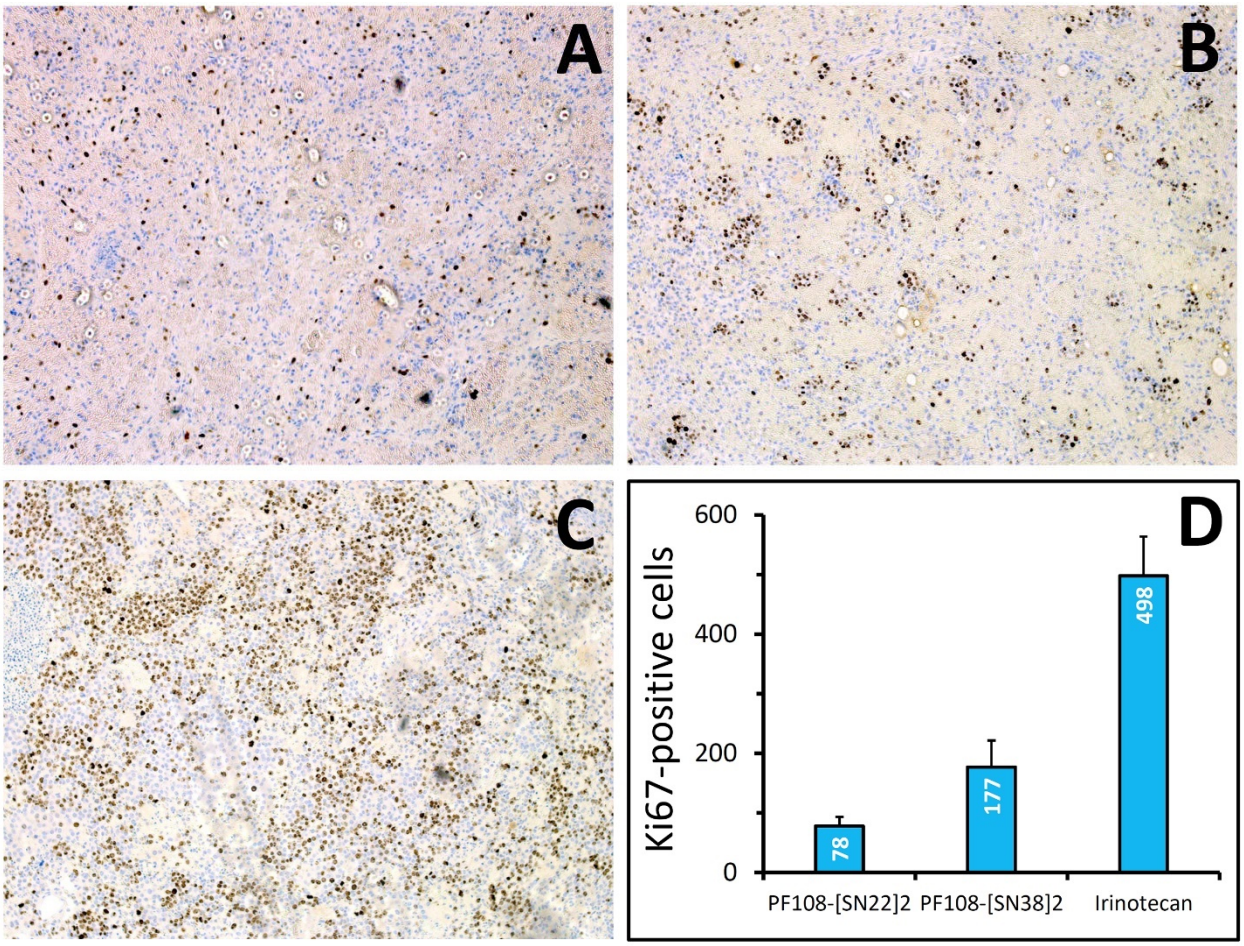

Fig. S5. The comparative effect of the prodrug treatment on tumor tissue histology and cell proliferation in a genetically engineered TH-*MYCN* mouse model of *de novo* resistant, *MYCN*-driven NB. PF108-$[SN22]_2$ (**A**), PF108-$[SN38]_2$ (**B**), or irinotecan (**C**) were administered intravenously to homozygous TH-*MYCN* mice with verified large NB tumors at doses corresponding to 10 mg/kg of SN22 or SN38. Tumors were harvested 24 hr post-treatment, formalin-fixed, embedded in paraffin and stained with an antibody to Ki67 (SP6, Abcam) using a Bond Max automated staining system (Leica Microsystems). Slides rinsed and dehydrated with ethanol and xylene were coverslipped and photographed at an original magnification ×200. The numbers of Ki67-positive cells in respective tumor samples (four fields per group) are expressed as mean ± SD (**D**).