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# Effects of the MDM2 Inhibitor Nutlin-3a on Sensitivity of Pancreatic Cancer Cells to Berberine and Modified Berberines in the Presence and Absence of WT-TP53.

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Running title: Effects of BBR and NAX Compounds on PDAC Therapeutic Sensitivity.

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**Abstract**

Approaches to improve pancreatic cancer therapy are essential as this disease has a very bleak outcome. Approximately 80% of pancreatic cancers are pancreatic ductal adenocarcinomas (PDAC). A key regulatory gene frequently mutated (~75%) in PDAC is the *TP53* tumor suppressor gene which controls the transcription of multiple genes involved in cell cycle progression, apoptosis, cancer progression and other growth regulatory processes. The mouse double minute 2 homolog (MDM2) gene product is a nuclear-localized E3 ubiquitin ligase and negatively regulates the TP53 protein which results in its proteasomal degradation. Various MDM2 inhibitors have been isolated and examined in clinical trials, especially in patients with hematological malignancies. Nutlin-3a is one of the first MDM2 inhibitors isolated. Berberine (BBR) is a natural product found in many fruits and berries and used in traditional medicine for centuries. It has many biological effects, and some are anti-proliferative in nature. BBR may activate the expression of TP53 and inhibit cell cycle progression as well as other events important in cell growth. To understand more about the potential of compounds like BBR and chemical modified BBRs (NAX compounds) to sensitize PDAC cells to MDM2 inhibitors, we introduced either WT-TP53 or the pLXSN empty vector control into two PDAC cell lines, one lacking expression of TP53 (PANC-28) and one with gain-of-function mutant TP53 on both alleles (MIA-PaCa-2). Our results indicate that nutlin-3a was able to increase the sensitivity to BBR and certain NAX compounds. The effects of nutlin-3a were usually more substantial in those cells containing an introduced WT TP53 gene. These results highlight the importance of knowledge of the type of TP53 mutation that is present in cancer patients before the administration of drugs which function by stabilization of the TP53 protein.

## 1. Introduction

Pancreatic cancer accounts for many deaths world-wide (Muniraj et al., 2013; Siegel et al., 2013) The survival rate for pancreatic cancer is very poor. Pancreatic cancers are usually diagnosed late in the disease process. Most (~85%) of pancreatic cancers are pancreatic ductal adenocarcinomas (PDAC).

Treatment options for PDAC are limited. One common therapy for PDAC is surgical removal of the diseased portion of the pancreas (Kommalapati et al., 2018). However, the tumor frequently reappears and to complicate therapy, the tumor may have metastasized to other organs.

Treatment with chemotherapeutic drugs is an effective approach to treat certain types of cancer patients. However, a common problem is the development of drug resistance. Sometimes treatment of certain cancers with chemotherapeutic drugs leads to the selection of drug resistance cancer stem cells (CSCs) from the initial tumor (McCubrey et al., 2008; Davis et al., 2014; Zhang et al., 2016; McCubrey et al., 2017; Chappell et al., 2020). The CSCs may be less sensitive to the chemotherapeutic drug than the initial tumor cells. Chemotherapy is used to treat certain PDAC patients. However, treatment of PDAC patients with chemotherapy is usually a palliative approach as opposed to curative approach and only some patients respond (Ruarus et al., 2018; Müller et al., 2021).

Two of the most frequently mutated genes in PDAC are *KRAS* (~95%) and *TP53* (~75%) (Pu et al., 2019; Qian et al., 2020). Most *KRAS* oncogene mutations result in a constitutively active KRas protein which leads to Raf/MEK/ERK pathway that can promote uncontrolled cell growth (Waters & Der., 2018). Active KRas can also lead to the mobilization of other signaling pathways (Davis et al., 2014). Altered *TP53* tumor suppressor activity can also lead to loss of cell cycle regulation and other biochemical pathways which also effect cell growth (Grant et al., 2016). Some *TP53* point mutations result in novel activities for the TP53 protein. This class of mutations are referred to as gain of function (GOF) mutations. An additional class of *TP53*

mutation in PDAC results in deletion (either partial or full deletion) in one or both alleles and the full length TP53 protein is not expressed. Certain mutant TP53 proteins with GOF properties will activate oncogenic Ras signaling (Escobar-Hoyos et al., 2020). Thus, the mutations at *TP53* and *KRAS* can conspire to result in uncontrolled proliferation of PDAC as well as other cancer types.

Minute 2 homolog (MDM2) is a E3 ubiquitin ligase which negative regulates TP53 by ubiquitination. This results in targeting TP53 to the lysosome and subsequent proteasomal degradation. Nutlin-3 is one of the first MDM2 inhibitors developed. Multiple MDM2 inhibitors have been isolated and characterized. Some have been examined in clinical trials, especially in patients with hematopoietic cancers (Tisato et al., 2017; Konopleva et al., 2020).

BBR is a nutraceutical found in various fruits and berries. BBR has many properties and has been used in traditional medicine for centuries (McCubrey et al., 2017) BBR has anti-proliferative, anti-inflammatory, anti-diabetic, and more recently investigated, anti-cancer activities (Wang et al., 2012). In some models, BBR can induce oxidative DNA damage and impairs homologous recombination repair (Hou et al., 2017). In addition, BBR has been shown to have effects on TP53 activity (Liu et al., 2009).

Various attempts have been made to design modified berberines which may be more effective in various therapies. In previous studies, we determine that certain NAX compounds were more effective in inhibiting proliferation of PDAC cell than berberine (Abrams et al; 2019) Subsequently, we determined that the anti-type-2 diabetes drug metformin could be combined with BBR and certain NAX compounds to increase the inhibition of proliferation of PDAC cells (Akula et al., 2019). In the following studies, we determined the effects of treatment of pancreatic cancer cells containing WT TP53, GOF mutant TP53 and no detectable TP53 (TP53 null) with BBR, modified BBRs (NAX compounds) with a low dose of the MDM2 inhibitor nutlin-3a. Our results demonstrate that the presence of WT TP53 could increase the sensitivity PDAC cells to certain NAX compounds and BBR.

## 2. Materials and methods

### *2.1. Source of PDAC cell lines and cell culture conditions*

The MIA-PaCa-2 PDAC cell line (ATCC CRM-CRL-1420) was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were recovered from a 65-year-old Caucasian male PDAC patient (Deer et al., 2010). MIA-PaCa-2 cells have a mutation in the *KRAS* gene which results in a constitutively-active oncoprotein. Both *KRAS* alleles have codon 12 mutations (GGT → GAT) and GOF TP53 mutations (R248W) (Deer et al., 2010; Klemke et al., 2021). This is a very frequent TP53 mutation in human cancer.

The MDA-PANC-28 cell line was obtained from Dr. Shrikanth A. G. Reddy, MD Anderson Cancer Center MD Anderson Cancer Center (Houston, TX, USA). The MDA-PANC-28 cells were obtained from a tumor present in a female 69-year-old PDAC patient and established into a cell line at the MD Anderson Cancer Center (Frazier et al., 1996; Zhu et al., 2005). The MDA-PANC-28 cell line is frequently abbreviated PANC-28. PANC-28 have an activating mutation in the *KRAS* gene. PANC-28 cells are heterozygous for KRAS [protein: p. Gly12Asp, nucleotide (c.35G > A)]. No detectable TP53 protein was observed in PANC-28 cells (Fraiser et al., 1996; Zhu et al. 2005).

Cell culture medium for PDAC cell lines consisted of 5% (v/v) heat inactivated fetal bovine serum (FBS) (CellGrow-Mediatech, Herndon, VA, USA), 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA), 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA, USA and Thermo Fisher, Waltham, MA, USA), and 100 units/L penicillin G (Invitrogen) in Dulbecco's Modified Eagles Medium (DMEM) (Invitrogen).

### *2.2. Source of Nutlin-3A, BBR and NAX Compounds*

The MDM2 inhibitor nutlin-3a was obtained from Selleck Chemicals (Houston, Texas, USA). Berberine was purchased from Sigma/Aldrich (Saint Louis, Missouri, USA) The NAX compounds were developed by Dr. Lombardi at Naxospharma in Milan, Italy. The chemical structures of the NAX compounds have been presented previously (Abrams et al., 2019).

*2.3. Introduction of either WT-TP53 or a control plasmid into MIA-PaCa-2 and PANC-28 cells*

Plasmid DNA encoding WT-TP53 was generously provided by Dr. Moshe Oren (Eliyahu et al., 1989) (Rehovot, Israel). The WT-TP53 construct also encodes the resistance to geneticin (G418) an analog of neomycin. Plasmid pLXSN encodes resistance to G418 and was generously provided by A. Dusty Miller, Fred Hutchinson Cancer Center, Seattle, Washington, USA) (Miller and Rosman, 1989). MIA-PaCa-2 and PANC-28 cells containing either WT-TP53 or pLXSN have been previously described (Abrams et al., 2018; Abrams et al., 2021a; Abrams et al., 2021b).

*2.4. Cell proliferation assays in the presence of nutlin-3a, BBR and NAX compounds*

MIA-PaCa-2 + pLXSN, MIA-PaCa-2 + WT-TP53, PANC-28 + pLXSN and PANC-28 + WT-TP53 cells were seeded into 96-well cell culture plates (BD Biosciences, Bedford, MA, USA) at a density of 5,000 cells/well in 100 µl of phenol red free RPMI-1640 containing 1% FBS. Cell culture plates were incubated for one day to allow cells to adhere to the bottom of each well (Abrams et al., 2018). Treatment medium was prepared by performing ten two-fold serial dilutions to create a range of eleven concentrations of the different drugs, signal transduction inhibitors and nutraceuticals. After 72 hours of treatment (four days after seeding), the tetrazolium-based cell growth/viability assay was performed. The amount of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma-Aldrich) reduction in each well was quantified by dissolving the formazan crystals in 200 µl of dimethyl sulfoxide (DMSO) and reading the absorbance at 570 nM with a FL600 microplate fluorescence reader (Bio-Tek Instruments; Winooski, VT, USA). Control plates were read on day one and day four after seeding to provide a base line for cell growth. The mean and corresponding standard deviation of normalized adjusted absorbance was calculated from three replicate wells for each drug concentration. The inhibitory concentration of 50% ($IC_{50}$) is defined in this context as the concentration of the drug that causes MIA-PaCa-2 or PANC-28 cells to proliferate at a rate that is half as rapid as cells incubated in the absence of the drug.

## 3. Results

### *3.1. Abilities of a low dose of nutlin-3a to decrease the $IC_{50}$s of NAX compounds in MIA-PaCa-2 cells containing and lacking WT-TP53*

Previously, we determined that introduction of WT-TP53 increased the sensitivity of MIA-PaCa-2 cells to nutlin-3a and BBR (Abrams et al., 2018; Candido et al., 2019). We also determined that MIA-MaCa-2 cells are not as sensitive to NAX012, NAX014, and NAX075 (Abrams et al., 2019) as they to other modified BBRs namely, NAX035, NAX038, NAX042, NAX053, NAX060 and NAX111. In addition, we determined that MIA-PaCa-2 cells were not sensitive to NAX054 (Abrams et al., 2019). In the following experiments with MIA-PaCa-2 cells, we increased the concentration of NAX compounds to 5,000 nM, while starting concentrations of BBR and NAX compounds was 2,000 nM (Abrams et al., 2018; Abrams et al., 2019, Candido et al., 2019).

The $IC_{50}$ for NAX012 in MIA-PaCa-2 + pLXSN cells was 2,500 nM. When NAX012 was combined with a low dose of nutlin-3a, the $IC_{50}$ remained the same at 2,500 nM (Figure 1, Panel A). The $IC_{50}$ for NAX012 in MIA-PaCa-2 + WT-TP53 cells was 1,200 nM. When NAX012 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 120-fold lower at 12 nM (Figure 1, Panel B). The effects of combination of the different NAX compounds with low concentrations of nutlin-3a in the presence and absence or WT-TP53 are presented in Table 1.

The $IC_{50}$ for NAX014 in MIA-PaCa-2 + pLXSN cells was 600 nM. When NAX014 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 1.2-fold lower at 1,800 nM (Figure 1, Panel C). The $IC_{50}$ for NAX014 in MIA-PaCa-2 + WT-TP53 cells was 600 nM. When NAX014 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 15-fold lower at 12 nM (Figure 1, Panel D).

The $IC_{50}$ for NAX035 in MIA-PaCa-2 + pLXSN cells was 700 nM. When NAX035 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 2.3-fold lower at 300 nM (Figure 2, Panel A). The $IC_{50}$ for NAX035 in MIA-PaCa-2 + WT-TP53 cells was 450 nM. When NAX035 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 90-fold lower at 5 nM (Figure 2, Panel B).

The $IC_{50}$ for NAX038 in MIA-PaCa-2 + pLXSN cells was 450 nM. When NAX038 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 2.3-fold lower at 200 nM (Figure 2, Panel C). The $IC_{50}$ for NAX038 in MIA-PaCa-2 + WT-TP53 cells was 80 nM. When NAX038 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 4-fold lower at 20 nM (Figure 2, Panel D).

The $IC_{50}$ for NAX042 in MIA-PaCa-2 + pLXSN cells was 180 nM. When NAX042 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 9-fold lower at 20 nM (Figure 3, Panel A). The $IC_{50}$ for NAX042 in MIA-PaCa-2 + WT-TP53 cells was 80 nM. When NAX042 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 4-fold lower at 20 nM (Figure 3, Panel B).

The $IC_{50}$ for NAX053 in MIA-PaCa-2 + pLXSN cells was 100 nM. When NAX053 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 20-fold lower at 5 nM (Figure 3, Panel C). The $IC_{50}$ for NAX053 in MIA-PaCa-2 + WT-TP53 cells was 50 nM. When NAX053 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 4-fold lower at 20 nM (Figure 3, Panel D).

The $IC_{50}$ for NAX054 in MIA-PaCa-2 + pLXSN cells was >5,000 nM. When NAX054 was combined with a low dose of nutlin-3a, the $IC_{50}$ remained high >5,000 nM (Figure 4, Panel A). We have observed previously that NAX054 had very little growth inhibitory effects on MIA-PaCa-2 cells (Abrams et al., 2019). The $IC_{50}$ for NAX054 in MIA-PaCa-2 + WT-TP53 cells was 5,000 nM. When NAX054 was combined with a low dose of APR-246, the $IC_{50}$ was 5,000 nM (Figure 4, Panel B).

The $IC_{50}$ for NAX060 in MIA-PaCa-2 + pLXSN cells was 210 nM. When NAX060 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 4.6-fold lower at 45 nM (Figure 4, Panel C). The $IC_{50}$ for NAX060 in MIA-PaCa-2 + WT-TP53 cells was 80 nM. When NAX060 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 3.6-fold lower at 22 nM (Figure 4, Panel D).

The $IC_{50}$ for NAX075 in MIA-PaCa-2 + pLXSN cells was >5,000 nM. When NAX075 was combined with a low dose of nutlin-3a, the $IC_{50}$ remained high 5,000 nM (Figure 5, Panel A). The $IC_{50}$ for NAX075 in MIA-PaCa-2 + WT-TP53 cells was >5,000 nM. When NAX075 was

combined with a low dose of APR-246, the $IC_{50}$ was >1.5 fold lower at 3,500 nM (Figure 5, Panel B).

The $IC_{50}$ for NAX077 in MIA-PaCa-2 + pLXSN cells was >5,000 nM. When NAX077 was combined with a low dose of nutlin-3a, the $IC_{50}$ remained high >5,000 nM (Figure 5, Panel C). The $IC_{50}$ for NAX075 in MIA-PaCa-2 + WT-TP53 cells was >5,000 nM. When NAX075 was combined with a low dose of nutlin-3a, the $IC_{50}$ was >1. 3-fold lower at 4,000 nM (Figure 5, Panel D).

The $IC_{50}$ for NAX111 in MIA-PaCa-2 + pLXSN cells was 200 nM. When NAX111 was combined with a low dose of nutlin-3a, the $IC_{50}$ remained the same at 200 nM (Figure 6, Panel A). The $IC_{50}$ for NAX111 in MIA-PaCa-2 + WT-TP53 cells was 180 nM. When NAX111 was combined with a low dose of nutlin-3a, the $IC_{50}$ was 9-fold lower at 22 nM (Figure 6, Panel B). Thus, introduction of WT-TP53 increased the sensitivity to the combination of NAX111 and nutlin-3a.

*3.2. Effects of nutlin-3a on PANC-28 cells in the presence and absence of WT-TP53*

When PANC-28 + pLXSN and PANC-28 + WT-TP53 were treated with nutlin-3a, the $IC_{50}$s were >5,000 nM and approximately 2,000 nM respectively (Figure 7). Thus, introduction of a WT-TP53 gene increased the sensitivity to nutlin-3a >2.5-fold compared to PANC-28 cells which lacked WT-TP53. Thus, at these concentrations, nutlin-3a did not have significant effects on PDAC cells which lacked expression of WT-TP53. In addition, these experiments indicated that restoration of WT TP53 activity sensitized the PDAC cells to the nutlin-3 compound.

*3.3. Abilities of a low dose of Nutlin-3a to decrease the $IC_{50}$s of BBR in PANC-28 cells containing and lacking WT-TP53*

The abilities of a low dose of nutlin-3a to reduce the $IC_{50}$s of BBR were determined in PANC-28 + pLXSN and PANC-28 + WT-TP53 cells. The $IC_{50}$ for BBR in MIA-PaCa-2 + pLXSN cells was >2,000 nM (Figure 8, Panel A). When BBR was combined with a low dose of nutlin-3a, the $IC_{50}$ remained the same namely >2,000 nM. The $IC_{50}$ for BBR in PANC-28 + WT-TP53

cells was 1,000 nM (Figure 8, Panel B). When BBR was combined with a low dose of APR-246, the $IC_{50}$ was 500-fold lower at 2 nM. When WT-TP53 is present in PANC-28 cells, their sensitivity to the combined BBR and nutlin-3 treatment increases dramatically.

## 4. Discussion

PDAC is a devasting cancer for which there are few effective treatments. The expression of many genes is altered in PDAC. Various approaches have been attempted to restore TP53 activity. One approach is by targeting the MDM2 ubiquitin ligase which leads to stabilization of TP53. However, a problem in cells with mutant GOF *TP53* gene, the stabilized GOF TP53 protein may have some detrimental activities. Some MDM2 inhibitors have been examined in clinical trials (summarized in Tisato et al., 2017; Konopleva et al., 2020). Many of the clinical trials have been performed on patients with hematological cancers. The TP53 gene is mutated in some hematological cancers (Rivlin et al., 2011). The MDM2 protein is overexpressed in certain hematopoietic cancers that have WT-TP53 (Hayashi et al., 2019). Nutlin-3a can affect other TP53 related genes such as TP73 (Lau et al., 2008). Nutlin-3a was determined to increase TP73 activity which led to increased expression of pro-apoptotic Noxa and Puma expression as well as the cell cycle inhibitor $p21^{Cip}$. Nutlin-3a was observed to increase apoptosis in TP53-mutant neuroblastoma, colon carcinoma and osteosarcoma cells. Thus, a mechanism by which nutlin-3a could induce some anti-proliferative effects in cells with GOF-TP53 mutations is by the induction of TP73. Increased expression of TP73 expression has been shown to inhibit cell cycle arrest and induce apoptosis in MIA-PaCa-2 cell which have GOF mutant TP53. (Nakamura et al., 2016). Nutlin-3a can also induce the stabilization of TP73 (Lau et al., 2008; Shen & Maki., 2011)

BBR can induce many biochemical processes which inhibit cell proliferation (McCubrey 2017). We and others have demonstrated that BBR and nutlin-3a inhibitory effects in cells with mutant and wild type TP53 (Abrams et al., 2018; Candido et al., 2018; Abrams et al., 2019, Liu et al., 2020).

We have previously observed that certain NAX compounds are more toxic towards MIA-PaCa-2 cell than BBR namely NAX035, NAX038, NAX042, NAX053, NAX060, and NAX111. (Abrams et al., 2019; Akula et al., 2020). In contrast, MIA-PaCa-2 cells are less sensitive to NAX012, NAX014, NAX075 and NAX077 and not sensitive to NAX054. Addition of WT-TP53 increased the sensitivity of MIA-PaCa-2 cells to NAX012, NAX014, NAX035, NAX038 and NAX111 dramatically in comparison to MIA-PaCa-2 + pLXSN cells. In contrast, addition of WT-TP53 did not increase the sensitivity of cells to NAX042, NAX053 and NAX060 in comparison to MIA-PaCa-2 cells which had pLXSN. MIA-PaCa-2 cells were already highly sensitive to NAX042, NAX053 and NAX060.

The differences in chemical modifications of the BBR backbone structure of the various NAX is presented in (Abrams et al., 2019). The chemical modifications of the NAX compounds have (un)substituted (hetero) aromatic moieties bonded to position 13 of the parent alkaloid BBR skeleton through a hydrocarbon (C) linker of variable lengths.

NAX012 and NAX042 have additional phenyl moieties with two hydrocarbon (C) linkers of different lengths. NAX012 has a 3 C linker and has been prepared as iodide salt. NAX042 has a 4 C linker and has been prepared as chloride salt. NAX042 was more effective in suppressing proliferation of MIA-PaCa-2 cells much more effectively than NAX012. NAX012 and NAX014 suppressed proliferation of MIA-PaCa-2 cells moderately, however addition of WT-TP53 resulted in increased sensitivity to nutlin-3a substantively.

NAX035 and NAX053 have two phenyl moieties (benzhydryl) with hydrocarbon linkers of different lengths. Both compounds were very effective in suppressing the proliferation of MIA-PaCa-2 cells.

NAX075 and NAX077 have additional one heterocycle (pyridine) with hydrocarbon linkers of different lengths. These compounds are very similar in structure, and they had minimal effects on suppressing the proliferation on MIA-PaCa-2 cells.

NAX038 and NAX054 have multiple electron-releasing substituents on the introduced monophenyl moiety as well as hydrocarbon linkers of different lengths. NAX038 was effective in suppressing the proliferation of MIA-PaCa-2 cells. In contrast, NAX054 with In contrast, NAX054 with three methoxy (OCH3) groups on the added phenyl moiety did not suppress proliferation of MIA-PaCa-2 cells.

NAX014, NAX060 and NAX111 have multiple electron-withdrawing substituents on the introduced monophenyl moiety. NAX060 and NAX111 had similar effects on MIA-PaCa-2 cells. However, introduction of WT-TP53 increased the effects of addition of a low concentration of nutlin-3a when added with NAX111, but not NAX060.

These modifications of the BBR core structure had different effects on suppression of MIA-PaCa-2 growth and sensitivity to combination with nutlin-3a. It is possible, that the different modification of the BBR core structure affect the ability of the molecules to interact with the promoter regions of key genes involved in regulation of cell growth and induction of apoptosis.

Our studies point to the value of knowing what type of TP53 mutation(s) there/are is cancer patients that may be treated with nutlin-3a and next generation related compounds. These compounds may not have significant effects in cells which have deleted or silenced TP53 expression. While this observation does sound obvious, however, there are other TP53-related molecules such as TP63 and TP73 which could be activated by the nutlin-3a compound in PANC-28 + pLXSN cells. However, we observed that treatment of PANC-28 + pLXSN were not sensitive to the combination of BBR and nutlin-3a. In contrast, PANC-28 + WT-TP53 were highly sensitive to the combination of nutlin-3a and BBR.

Identification of novel reactivators of tumor suppressor genes is a critical basic and clinical research area (Zhang et al., 2016; Cluzeau et al 2021). Tumor suppressor genes are frequently inactivated in human malignancies. Nutlin-3a and structurally-related compounds which function by stabilizing tumor suppressor proteins such as TP53 represent import additions to effective cancer therapy.

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**Declaration of competing interest**

The authors declare that they have no conflict of interest with publication of this manuscript.

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## Figure Legends

**Figure 1. Effects of Combining a Low Concentration of 500 nM Nutlin-3a on the $IC_{50}$s of NAX012 and NAX014 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX012 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX012 and a low dose of 500 nM nutlin-3 (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentration of NAX012 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX012 and a low dose of nutlin-3a (blue triangles). Panel C) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX014 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX014 and a low dose of nutlin-3a (blue triangles). Panel D) MIA-PaCa-2 + WT-TP53 cells treated with different concentration of NAX014 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX014

and a low dose of nutlin-3a (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001, ** = *P* <0.005 NS = non significant.

**Figure 2. Effects of Combining a Low Concentration of 500 nM Nutlin-3a on the $IC_{50}$s of NAX035 and NAX038 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX035 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX035 and a low dose of nutlin-3a (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX035 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX035 and a low dose of nutlin-3a (blue triangles). Panel C) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX038 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX038 and a low dose of nutlin-3a (blue triangles). Panel D) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX038 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX038 and a low dose of nutin-3a (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001.

**Figure 3. Effects of Combining a Constant Concentration of 500 nM Nutlin-3 on the $IC_{50}$s of NAX042 and NAX053 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX042 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX042 and a low dose of nutlin-3a (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX042 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX042 and a low dose of nutlin-3a (blue triangles). Panel C) MIA-PaCa-2 + pLXSN cells treated with different concentration of NAX053 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX053 and a low dose of nutlin-3a (blue triangles). Panel D) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX053 (red squares) or MIA-

PaCa-2 + WT-TP53 cells treated with different concentrations of NAX053 and a low dose of nutlin-3 (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001.

**Figure 4. Effects of Combining a Constant Concentration of 500 nM Nutlin-3 on the $IC_{50}$s of NAX038 and NAX042 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX054 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX054 and a low dose of nutlin-3a (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX054 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX038 and a low dose of nutlin-3a (blue triangles). Panel C) MIA-PaCa-2 + pLXSN cells treated with different concentration of NAX054 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX054 and a low dose of nutlin-3a (blue triangles). Panel D) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX060 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX060 and a low dose of nutlin-3 (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001.

**Figure 5. Effects of Combining a Constant Concentration of 500 nM Nutlin-3 on the $IC_{50}$s of NAX075 and NAX077 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX075 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX075 and a low dose of nutlin-3a (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX075 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX053 and a low dose of nutlin-3a (blue triangles). Panel C) MIA-PaCa-2 + pLXSN cells treated with different concentration of NAX077 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX077 and a low dose of nutlin-3a (blue triangles). Panel D) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX077 (red squares) or MIA-

PaCa-2 + WT-TP53 cells treated with different concentrations of NAX077 and a low dose of nutlin-3 (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001.

**Figure 6. Effects of Combining a Constant Concentration of 500 nM Nutlin-3 on the $IC_{50}$s of NAX111 in MIA-PaCa-2 + pLXSN and MIA-PaCa-2 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentration of NAX111 (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of NAX111 and a low dose of nutlin-3a (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX111 (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of NAX111 and a low dose of nutlin-3 (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001. NS = non significant.

**Figure 7. Effects of Nutlin-3a on Growth of PANC-28 PDAC Cells Containing and Lacking WT-TP53.** PANC-28 + pLXSN cells (red squares) and PANC-28 + WT-TP53 cells (blue triangles). These experiments were repeated 3 times and similar results were obtained.

**Figure 8. Effects of Combining a Low Concentration of 500 nM Nutlin-3a on the $IC_{50}$s of BBR in PANC-28 + pLXSN and PANC-28 + WT-TP53 Cells.** Panel A) MIA-PaCa-2 + pLXSN cells treated with different concentrations of BBR (red squares) or MIA-PaCa-2 + pLXSN cells treated with different concentrations of BBR and a low dose of 500 nM nutlin-3 (blue triangles). Panel B) MIA-PaCa-2 + WT-TP53 cells treated with different concentration of BBR (red squares) or MIA-PaCa-2 + WT-TP53 cells treated with different concentrations of BBR and a low dose of nutlin-3a (blue triangles). They were repeated 3 times and similar results were observed. *** = *P* <0.0001.

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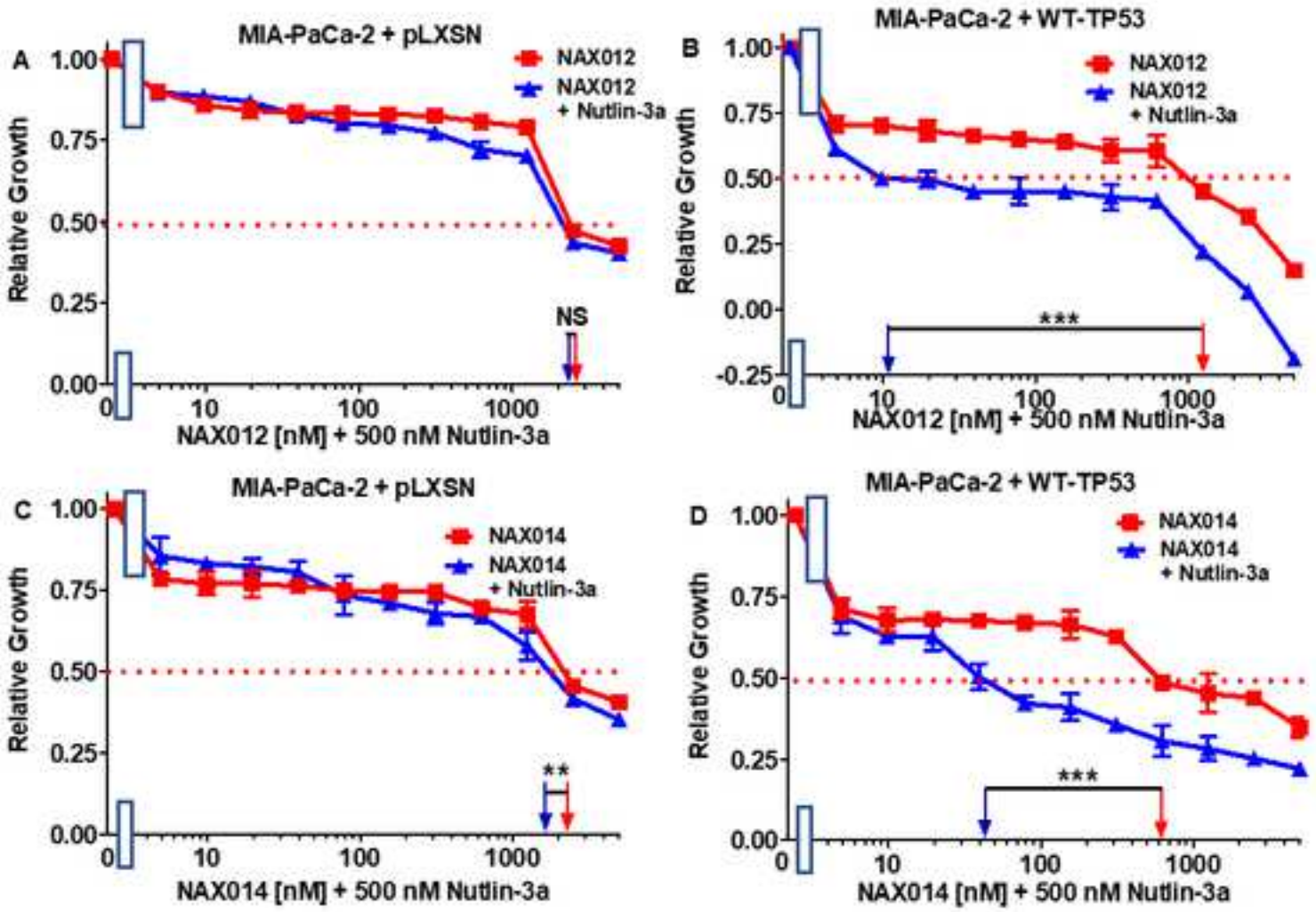

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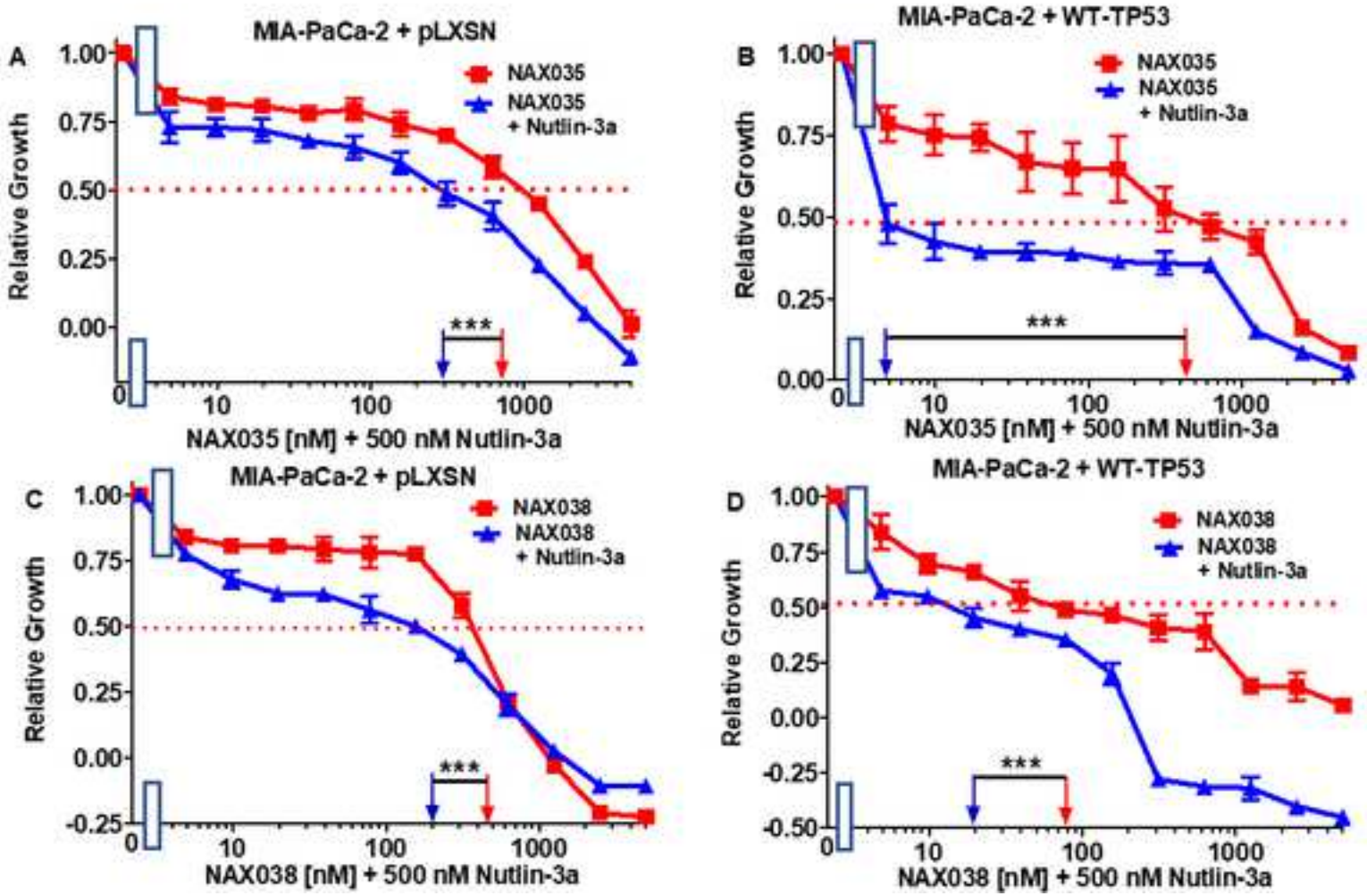

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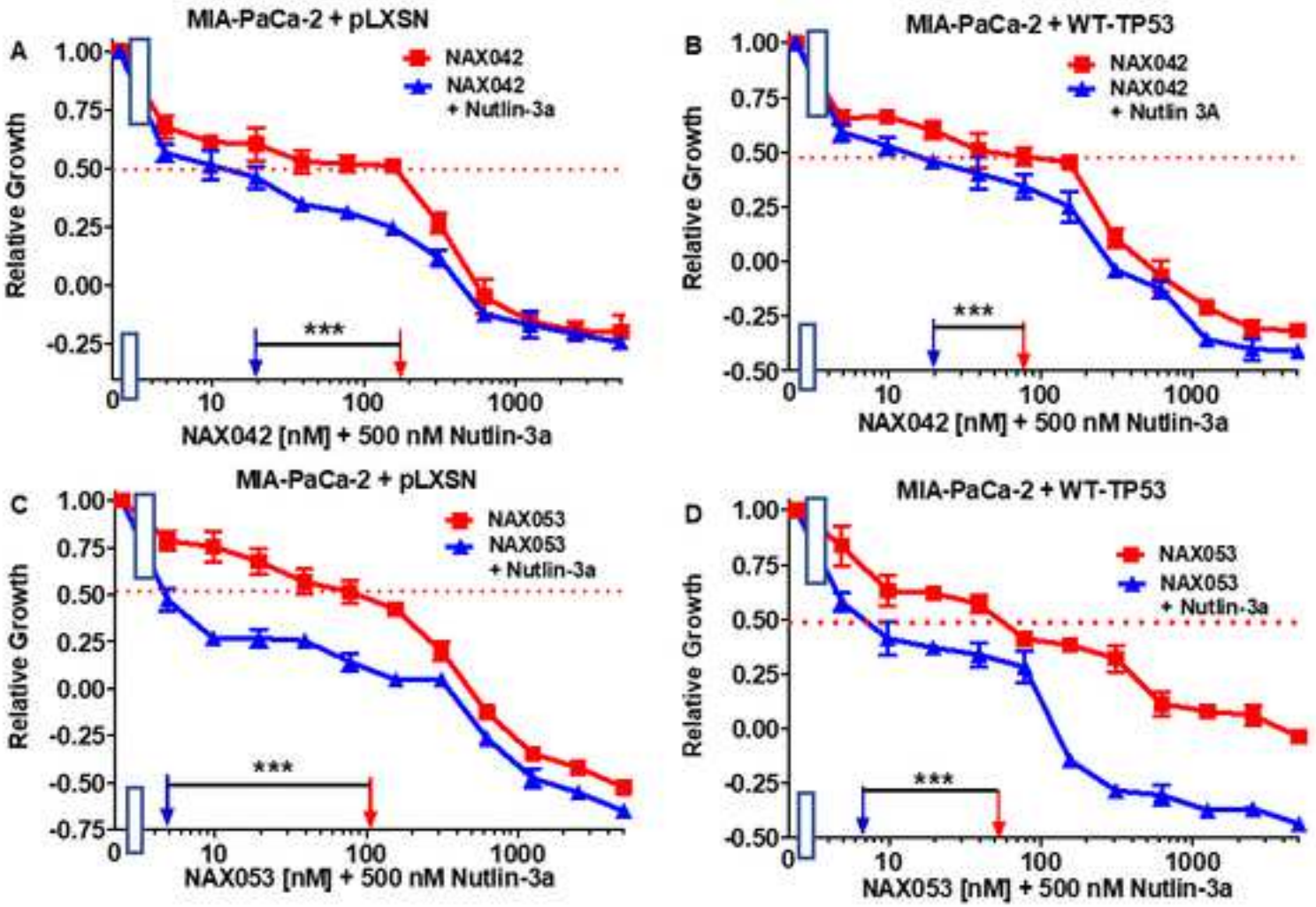

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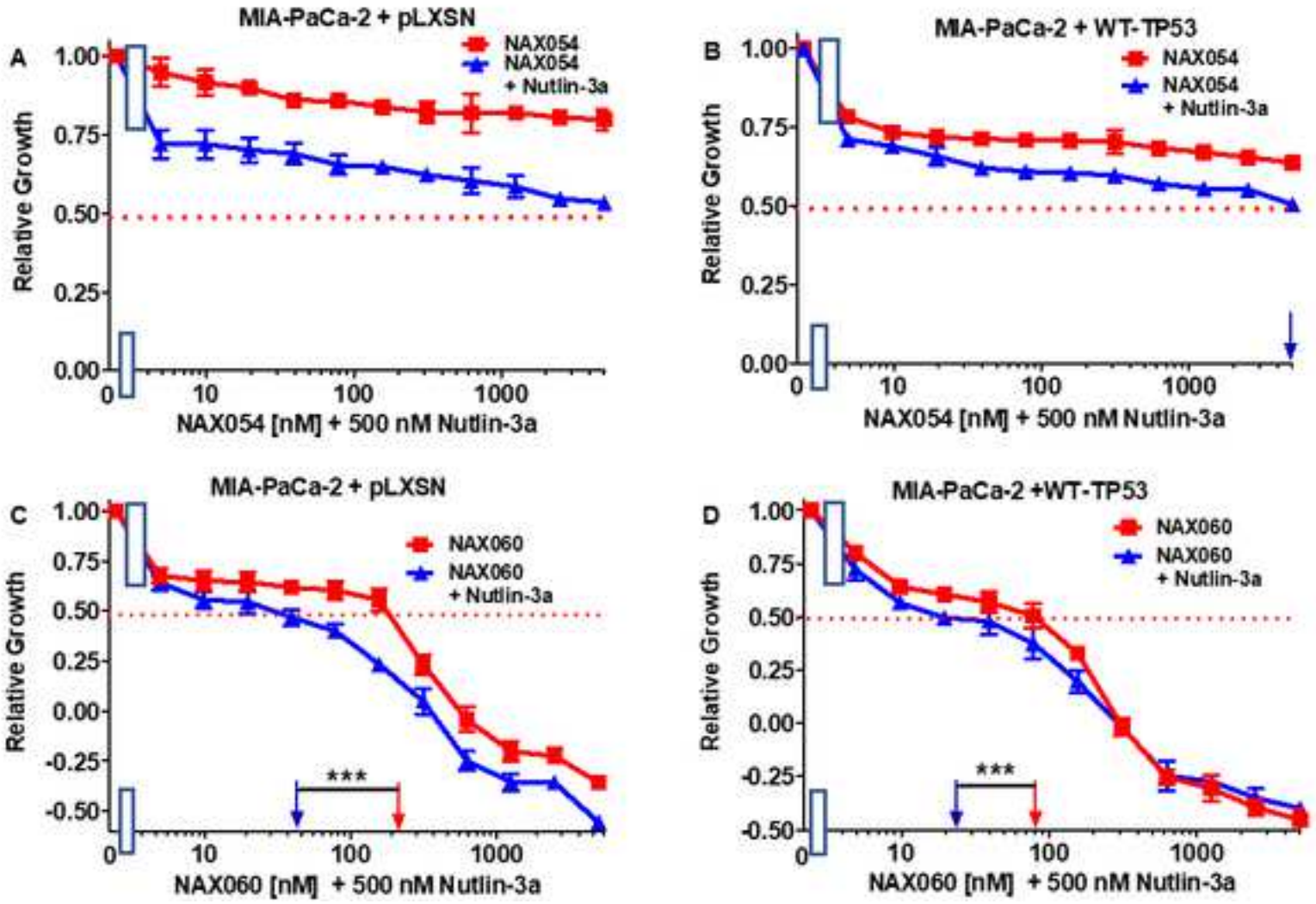

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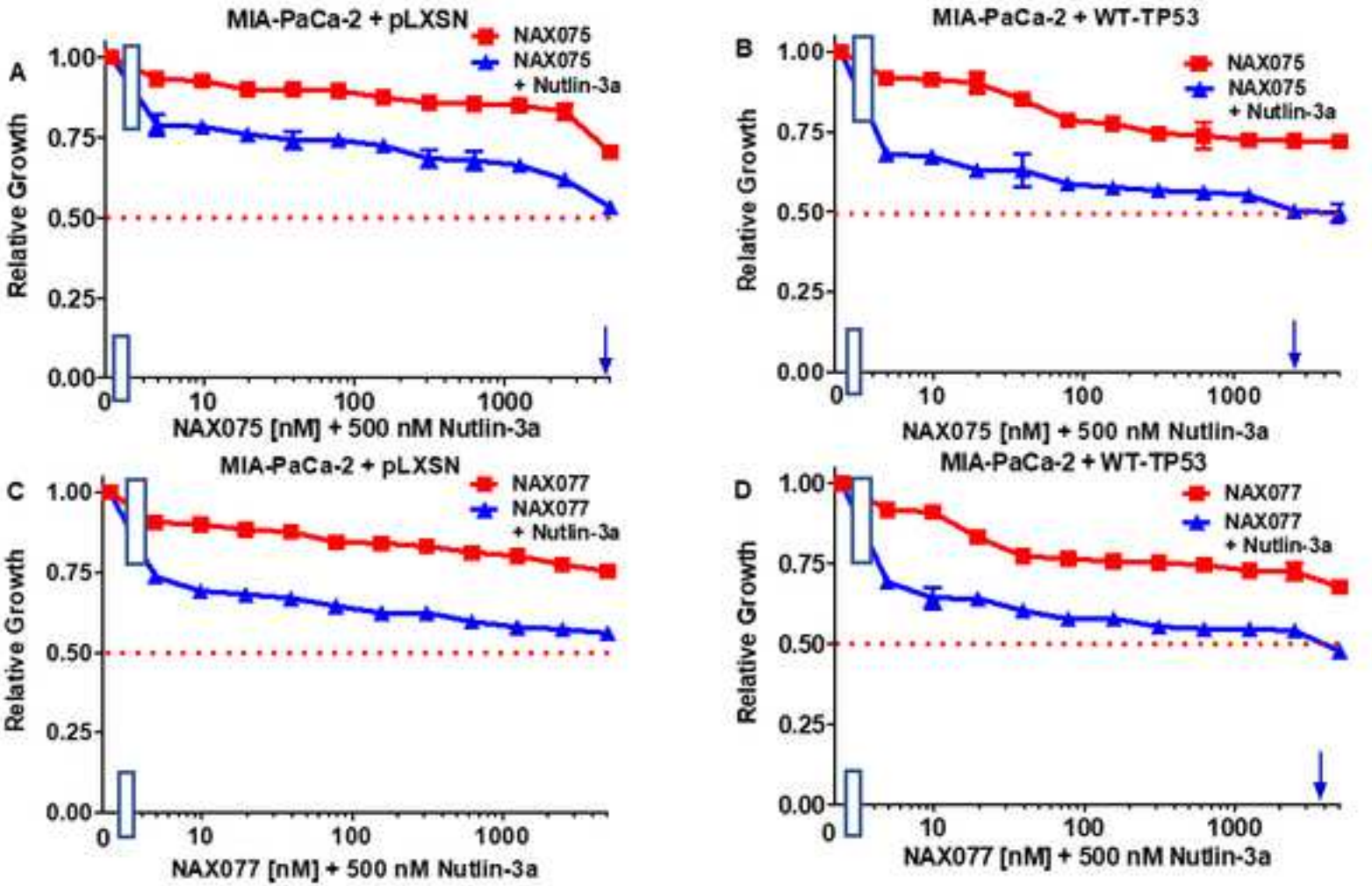

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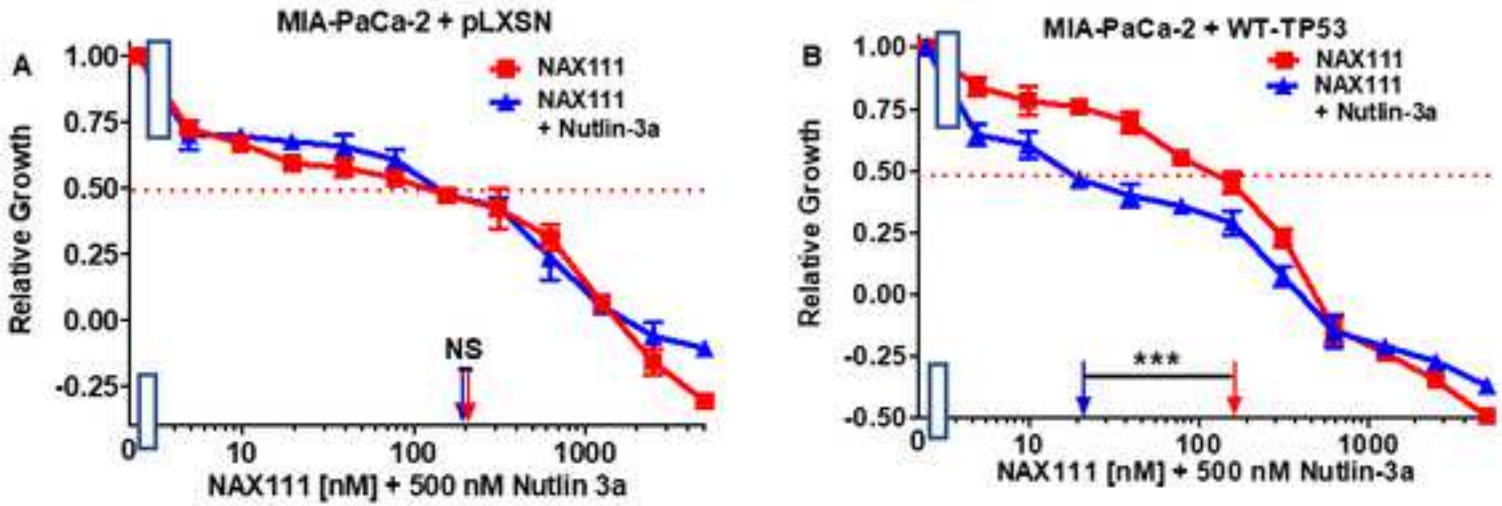

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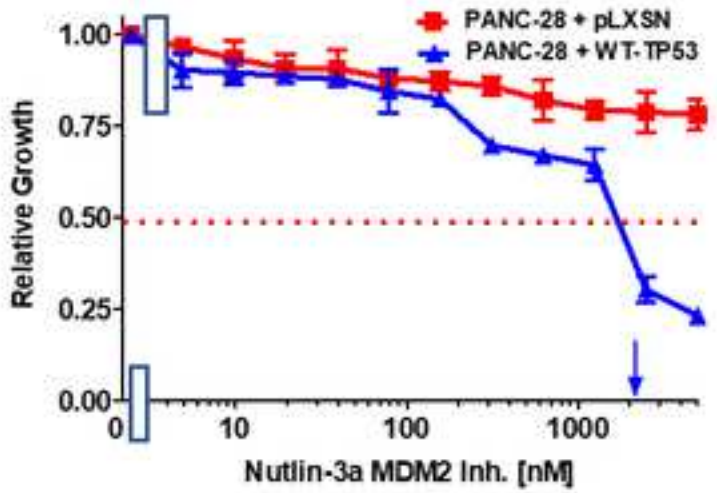

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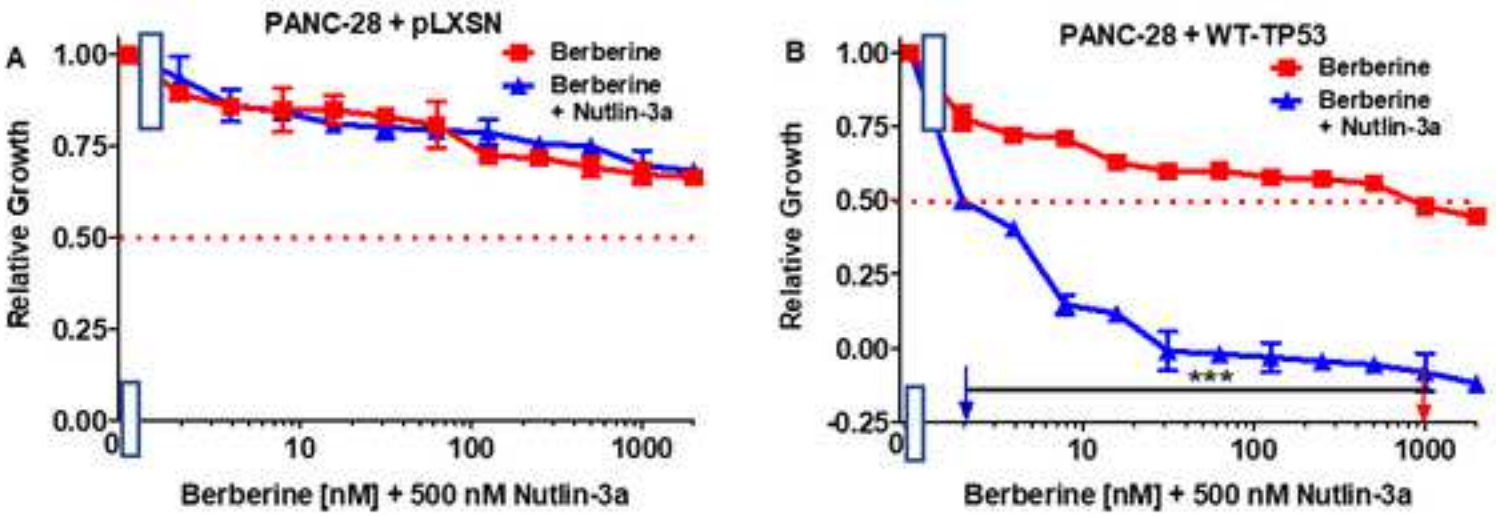

**Table 1. Effects of WT-TP53 on the Sensitivity of MIA-PaCa-2 and PANC-28 PDAC Cells to BBR, NAX Compounds and Nutlin-3a[1]**

| Berberine or NAX Compound | MIA-PaCa-2+ pLXSN (- Nutlin-3a) | MIA-PaCa-2 + pLXSN (+ 500 nM Nutlin-3a) | Fold Change + / - Nutlin-3a | MIA-PaCa-2+ WT-TP53 (- Nutlin-3a) | MIA-PaCa-2 + WT-TP53 (+ 500 nM Nutlin-3a) | Fold Change + / - Nutlin-3a |
|---|---|---|---|---|---|---|
| **NAX012** | 2,500 nM | 2,500 nM | 1 × | 1,200 nM | 10 nM | 120 × ↓ |
| **NAX014** | 2.200 nM | 1,800 nM | 1.2 × ↓ | 600 nM | 40 nM | 15 × ↓ |
| **NAX035** | 700 nM | 300 nM | 2.3 × ↓ | 450 nM | 5 nM | 90 × ↓ |
| **NAX038** | 450 nM | 200 nM | 2.3 × ↓ | 80 nM | 20 nM | 4 × ↓ |
| **NAX042** | 180 nM | 20 nM | 9 × ↓ | 80 nM | 20 nM | 4 × ↓ |
| **NAX053** | 100 nM | 5 nM | 20 × ↓ | 50 nM | 7 nM | 7.1 × ↓ |
| **NAX054** | >5,000 nM | >5,000 nM | 1 × | >5,000 nM | 5000 nM | 1 × |
| **NAX060** | 210 nM | 45 nM | 4.6 × ↓ | 80 nM | 22 nM | 3.6 × ↓ |
| **NAX075** | >5,000 nM | 5,000 nM | 1 × | >5,000 nM | 3,500 nM | >1.4 ×↓ |
| **NAX077** | >5,000 nM | >5,000 nM | 1 × | >5,000 nM | 4,000 nM | >1.3 ×↓ |
| **NAX111** | 200 nM | 200 nM | 1 × | 180 nM | 20 nM | 9 × ↓ |
| | | | | | | |
| **Berberine or NAX Compound** | **PANC-28+ pLXSN (- Nutlin-3a)** | **PANC-28 + pLXSN (+ 500 nM Nutlin-3a)** | **Fold Change + / - Nutlin-3a** | **PANC-28 + WT-TP53 (- Nutlin-3a)** | **PANC-28 + WT-TP53 (+ 500 nM Nutlin-3a)** | **Fold Change + / - Nutlin-3a** |
| **Berberine** | >2,000 nM | >2,000 nM | 1.8 × ↓ | 1,000 nM | 2 nM | 500 × ↓ |

[1]Determined as described in Abrams et al., 2021a