Supplementary Materials

Lemberg K.M. et al. “Pro-905, a novel purine antimetabolite, combines with glutamine amidotransferase inhibition to suppress growth of malignant peripheral nerve sheath tumor.”

SUPPLEMENTAL METHODS

Detailed Pro-905 Synthesis and Characterization

Isopropyl ((((4*R*)-6-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate Cpd 902

A solution of 2´,3´-O-isopropylideneguanosine (500 mg; 1.55 mmol) in DMF (10 mL) was evaporated to ½ of the original volume. The cloudy solution was cooled to 0 °C and *tert*-butylmagnesium chloride (1M-solution in THF; 2.13 mL) was added under stirring. After 5 min, isopropyl [chloro(phenoxy)phosphoryl]-*L*-alaninate (758 mg; 2.48 mmol) was added as a solution in DMF (2 mL). The mixture was stirred at 0 °C for 5 min and then for 24 h at room temperature (monitored by TLC in system chloroform – methanol 9:1). The reaction mixture was evaporated, the residue coevaporated with toluene and chromatographed on a column of silica gel (150 mL) in system chloroform – methanol ((95:5), elution of by-products), followed by chloroform – methanol ((9:1), elution of product). Yield: 580 mg (63 %) of a white amorphous solid (2 diastereoisomers).

^{1}H NMR (DMSO-d_6, ppm) δ: 1.08-1.21 (m, 9H, $\underline{CH_3}$-CH-NH, CH($\underline{CH_3}$)$_2$), 1.29-1.36 (m, 3H, CH_3 isopropylidene), 1.49-1.54 (m, 3H, CH_3 isopropylidene), 3.67-3.83 (m, 1H, $\underline{CH}$-NH), 4.00-4.37 (m, 3H, H-4´, H-5´), 4.78-4.88 (m, 1H, O$\underline{CH}$(CH_3)$_2$), 5.10-5.16 (m, 1H, H-3´), 5.16-5.21 (m, 1H, H-2´), 5.96-6.05 (m, 2H, H-1´, P-$\underline{NH}$), 6.55 (bs, 2H, NH_2), 7.07-7.23 (m, 3H, H-2´´,H-4´´), 7.28-7.41 (m, 2H, H-3´´), 7.85 (s, 1H, H-8), 10.71 (bs, 1H, NH).

ESIMS, *m/z*: 615.2 $(M+Na)^+$ (100), 593.2 $(MH)^+$ (7).

HRMS (ESI): For $C_{25}H_{33}O_9N_6NaP$ $(M+Na)^+$ calculated: 615.19388; found: 615.19391. For $C_{25}H_{34}O_9N_6P$ $(MH)^+$ calculated: 593.21194; found: 593.21211.

Isopropyl ((((4*R*)-6-(2-amino-6-thioxo-1,6-dihydro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate Cpd 904

Trifluoroacetic anhydride (0.6 mL; 4.3 mmol) was added dropwise under argon to a 0 °C cold solution of guanosine derivative Cpd 902 (296 mg; 0.5 mmol) in dry pyridine (10 mL). The mixture was removed from ice bath and stirred at room temperature for 40 min. After that, a suspension of dry NaHS (0.87 g; 5 mmol) in DMF (15 mL) was added (dissolution occurred). The mixture was stirred at room temperature till disappearance of the starting compound (2 h;

TLC control in system 5% MeOH in $CHCl_3$). The mixture was evaporated and the residue was chromatographed on a column of silica gel (150 mL; short wide column) in a gradient of methanol in chloroform (0-5 %). The crude product was eluted by 5 % MeOH in $CHCl_3$, together with colored (purple) impurities. Final purification of the product was performed by additional chromatography on silica gel (50 mL) under the same conditions. Yield: 167 mg (55 %) of a white foam (2 diastereoisomers).

^{1}H NMR (DMSO-d_6, ppm) δ: 1.10-1.17 (m, 9H, $\underline{CH_3}$-CH-NH, CH($\underline{CH_3}$)$_2$), 1.31-1.52 (m, 6H, CH_3 isopropylidene), 3.69-3.78 (m, 1H, $\underline{CH}$-NH), 3.99-4.36 (m, 3H, H-4´, H-5´), 4.76-4.86 (m, 1H, O$\underline{CH}$(CH_3)$_2$), 5.11-5.15 (m, 1H, H-3´), 5.18-5.21 (m, 1H, H-2´), 5.96-6.05 (m, 2H, H-1´, P-$\underline{NH}$), 6.86 (bs, 2H, NH_2), 7.10-7.19 (m, 3H, H-2´´,H-4´´), 7.30-7.37 (m, 2H, H-3´´), 8.03 (s, 1H, H-8), 12.02 (bs, 1H, NH).

^{13}C NMR (DMSO-d_6, ppm) δ: 19.74-19.86 (m, NH-CH-$\underline{C}H_3$), 21.56-21.61 (m, CH_3 isopropyl), 25.46, 25.49, 27.18 and 27.20 (CH_3 isopropylidene), 49.94 and 50.12 ($\underline{C}$H-NH), 65.92 and 66.01 (2 x d, $J_{C,P}$ = 5.1, C-5´), 68.20 (O-$\underline{C}$H(CH_3)$_2$), 81.18 (C-3´), 83.83 and 83.92 (C-2´), 85.28 and 85.53 (2 x d, $J_{C,P}$ = 8.0 and 7.9, C-4´), 88.82 and 88.92 (C-1´), 113.47 and 113.49 (O-$\underline{C}$-O), 120.25-120.33 (m, C-2´´), 124.76 and 124.81 (C-4´´), 128.76 and 128.79 (C-5), 129.77 and 129.83 (C-3´´), 139.11 and 139.24 (C-8), 147.17 and 147.24 (C-4), 150.79 (d, $J_{C,P}$ = 6.3, C-1´´), 153.18 (C-2), 172.81 (d, $J_{C,P}$ = 4.5, COO), 172.92 (d, $J_{C,P}$ = 4.2, COO), 175.58 (C-6).

ESIMS, *m/z*: 631.1 $(M+Na)^+$ (100), 609.1 $(MH)^+$ (6).

HRMS (ESI): For $C_{25}H_{33}O_8N_6NaPS$ $(M+Na)^+$ calculated: 631.17104; found: 631.17129. For $C_{25}H_{34}O_8N_6PS$ $(MH)^+$ calculated: 609.18910; found: 609.18944.

Isopropyl ((((2*R*)-5-(2-amino-6-thioxo-1,6-dihydro-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate

Cpd 905

90% Trifluoroacetic acid (10 mL) was added to isopropylidene derivative Cpd 904 (140 mg; 0.23 mmol). The solution was stirred for 20 min at room temperature and evaporated. The residue was coevaporated with toluene (2 x 20 mL) and chromatographed on o column of silica gel (40 mL) in system methanol – chloroform (9:1). Final purification of the crude product was performed by additional chromatography in system ethyl acetate – acetone – ethanol – water (43:3:2:2). Appropriate fractions were evaporated and the residue crystallized from acetone. Yield: 80 mg (61 %) of white crystals (2 diastereoisomers).

^{1}H NMR (DMSO-d_6, ppm) δ: 1.12-1.21 (m, 9H, CH_3), 3.70-3.82 (m,1H, $\underline{CH}$-NH), 4.04-4.27 (m, 4H, H-3´, H-4´, H-5´), 4.41-4.44 (m, 1H, H-2´), 4.79-4.88 (m,1H, $\underline{CH}$(CH_3)$_2$), 5.32-5.36 (m, 1H,

3´-OH), 5.56-5.58 (m, 1H, 2´-OH), 5.72 and 5.73 (2 x d, 1H, $J_{1´,2´}$ = 6.0 and 5.9, H-1´), 5.99-6.06 (m, 1H, P-NH), 6.82 (bs, 2H, (NH_2), 7.14-7.22 (m, 3H, H-2´´, H-4´´), 7.32-7.38 (m, 2H, H-3´´), 8.03 and 8.04 (2 x s, 1H, H-8), 11.98 (bs, 1H, NH).

^{13}C NMR (DMSO-d_6, ppm) δ: 19.80 and 19.93 (2 x d, $J_{C,P}$ = 7.3 and 6.6, CH_3-CHNH), 21.56-21.62 (m, CH_3 isopropyl), 49.98 and 50.14 (CH-NH), 66.10 and 66.21 (2 x d, $J_{C,P}$ = 4.9 and 5.2, C-5´), 68.19 (CH isopropyl), 70.37 and 70.39 (C-3´), 73.28 and 73.36 (C-2´), 82.76-82.83 (m, C-4´), 86.58 and 86.69 (C-1´), 120.31 and 120.32 (2 x d, $J_{C,P}$ = 4.8, C-2´´), 124.72 and 124.75 (C-4´´), 128.52 and 128.54 (C-5), 129.75 and 129.80 (C-3´´), 138.39 and 138.47 (C-8), 148.14 and 148.17 (C-4), 150.83 and 150.86 (2 x d, $J_{C,P}$ = 6.2 and 6.3, C-1´´), 153.28 (C-2), 172.81 and 172.93 (2 x d, $J_{C,P}$ = 4.9 and 4.3, COO), 175.35 and 175.36 (C-6).

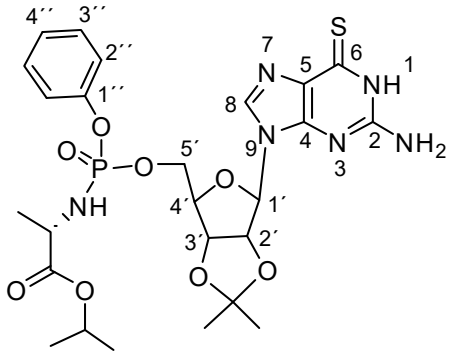
ESIMS, *m/z*: 591.1 $(M+Na)^+$ (100), 569.1 $(MH)^+$ (8).

HRMS (ESI): For $C_{22}H_{29}O_8N_6NaPS$ $(M+Na)^+$ calculated: 591.13974; found: 591.13983.

For $C_{22}H_{30}O_8N_6PS$ $(MH)^+$ calculated: 569.15780; found: 569.15796.

Elemental analysis (%): calcd for C, 46.48; H, 5.14; N, 14.78; P, 5.45; S, 5.64; found C, 45.91; H, 4.96; N, 14.64; P, 5.22; S, 5.47.

Figure. Structure numbering for NMR assignment.



Compound dose response and colony area formation in MPNST cells:

Dose response to JHU395 at 72h treatment was conducted in 96 well plates as previously described using Alamar blue [1]. 6-mercaptopurine activity in sNF96.2 or JH-2-002 cells was measured by trypan blue dye exclusion after one week of treatment.

Colony growth was conducted in 6 well plates. Cells were plated at 1000 cells/well and allowed to adhere to plates for ~24h. The following day media was replaced by media containing indicated concentrations of Pro-905, JHU395, the combination, or DMSO (control). Cells were incubated at 37°C, 5% CO_2 for approximately three weeks, with media containing compounds changed twice weekly. When control wells appeared 60-70% confluent by light microscope, cells were gently washed with warm PBS and fixed with 10% neutral buffered formalin. Cells were stained with crystal violet (0.05%) and washed with PBS. Plates were air dried overnight and then scanned. Quantification of percent area stained with cell colonies was calculated using the ColonyArea plugin for Fiji [2].

FGAR quantification:

Tumor cells (sNF96.2, JH-2-002) were cultured in 10 cm dishes. Cells were treated for indicated times with DMSO (control) or JHU395 (10 μM). For cell harvest, cells were washed with PBS, then trypsin. Cell suspension was pelleted at 1000 rpm, washed three times in cold PBS, and frozen at -80°C until bioanalysis.

Prior to extraction, frozen cell pellets were thawed on ice and weighed. FGAR quantification was completed as described in [3]. Briefly, sample preparation was performed using a single-step protein precipitation method. The cells were diluted 1:5 w/v with methanol containing 10 μM deuterated *N*-Acetyl Aspartic acid (NAA-d_3) as internal standard), followed by vortex mixing and centrifugation at 16,000 × *g* for 5 min at 4 °C. Supernatants were analyzed using UltiMate 3000 UHPLC coupled to Q Exactive Focus Orbitrap Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The mobile phase used for chromatographic separation consisted of 8 mM DMHA + 0.005% formic acid in water, pH 9 (A), and 8 mM DMHA in acetonitrile (B), delivered at a flow rate of 0.4 mL/min. A gradient LC method (time (min)/%B: 0-0.5/5, 2.5-3.5/95, 3.51-4.50/5) was used for the analyses. Separation of analytes was achieved using an Agilent Eclipse Plus C18 RRHD 2.1 × 100 mm, 1.8 μm particle size column (Agilent, Santa Clara, CA, USA). The mass spectrometer was operated with the capillary temperature setting at 350°C and a spray voltage of 4 kV. Nitrogen was used as the sheath and auxiliary gas set to 30 and 20 arbitrary units, respectively. Samples were subjected to ionization in negative mode and analyzed using the Full MS scan function. Parent ion of FGAR at m/z 313.0442 was used for quantitation and parent ion of NAA-d_3 at m/z 177.0596 was used as an internal standard.

Bioanalysis of purine metabolites from NPcis tumors:

For bioanalysis of purine nucleotides from tumors of NPcis mice, metabolites were extracted from tumor tissue (~100 mg) homogenized in ice cold 80% methanol:water (v/v). Samples were vortex mixed and stored at -80°C for 4 hours. Samples were thawed and centrifuged (13,000 × *g* for 10 min), and supernatant was isolated and dried under nitrogen gas, followed by resuspension in 50% acetonitrile solution. LC-MS based metabolomics profiling of selected purine synthesis intermediates was performed as previously described [1, 4] using an Agilent 1290 ultra high performance liquid chromatography system with detection on an Agilent 6520 Q-TOF mass spectrometer equipped with a dual electrospray (ESI) ion source, operated in negative ion mode.

In vivo Pro-905 tolerability:

Wild-type C57BL/6 male mice at approximately 10 weeks of age between 20-25 g were obtained from Envigo. Animals (n = 10/group) received Pro-905 (10 mg/kg i.p. or 1 mg/kg i.p. made in PBS + 1% Tween-80+ 10% ethanol), 6-MP (20 mg/kg i.p. was administered as a suspension in 1% carboxymethylcellulose with 5% DMSO), or vehicle. Drugs were dosed i.p. daily 5 days/week for two weeks. Weights were measured three times weekly. On day 12, one hour following dosing, all animals were euthanized by CO_2 inhalation. Blood samples were harvested by cardiac puncture. Complete blood counts and clinical chemistries were analyzed from a subset (n=5/group) of blood samples at the JHU Phenotyping Core Facility.

Nod scid gamma (NSG) (JAX #005557) female mice at 12 weeks of age between 20 and 30 g were obtained from Jackson Labs. Animals (n=5/group) received Pro-905 at 20 mg/kg (in PBS + 1% Tween-80 + 5% DMSO), 6-MP at 20 mg/kg (in 1% carboxymethylcellulose + 5% DMSO), or Vehicle (1% carboxymethylcellulose + 5% DMSO) i.p. daily 5 days/week. Weights and body condition scores were measured three times weekly. On day 16, two hours following dosing, all animals were euthanized via CO_2 inhalation with terminal blood collection. Complete blood counts and clinical chemistries were performed by the JHU Phenotyping Core Facility.

Western blotting:

Cells were cultured as described, treated with compounds at concentrations and times indicated, and harvested with 0.05% trypsin. Cells were washed with cold PBS, then lysed using ice cold CHAPS lysis buffer (40 mM HEPES, pH 7.4, 120 mM NaCl, 1 mM EDTA, 1% CHAPS, 10 mM glycerol 2-phosphate, 10 mM sodium pyrophosphate, 0.5 mM sodium orthovanadate and 50 mM NaF, and protease inhibitor cocktail was added just before lysis of cells). Equal quantities of total protein (10-20 µg/well) were separated by 4%–12% Bis-Tris SDS/PAGE (Thermo Fisher Scientific) and transferred by iBlot (Invitrogen) to either nitrocellulose or PVDF membrane. Rabbit cleaved PARP (#9541), rabbit γH2Ax (#9718), mouse anti-ß-tubulin (#86298), and mouse anti GAPDH (#97166) were purchased from Cell Signaling Technology. Detection was accomplished with Licor IRDye 800cw goat anti-rabbit (925-32211) and IRDye 680RD goat anti-mouse (925-68070) secondary antibodies using a BioRad ChemiDoc Imaging System.

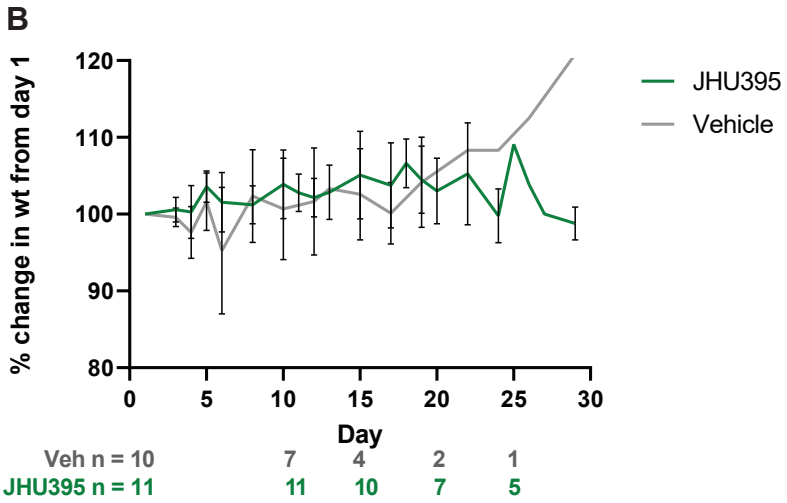
References (relevant to Supplementary Methods)

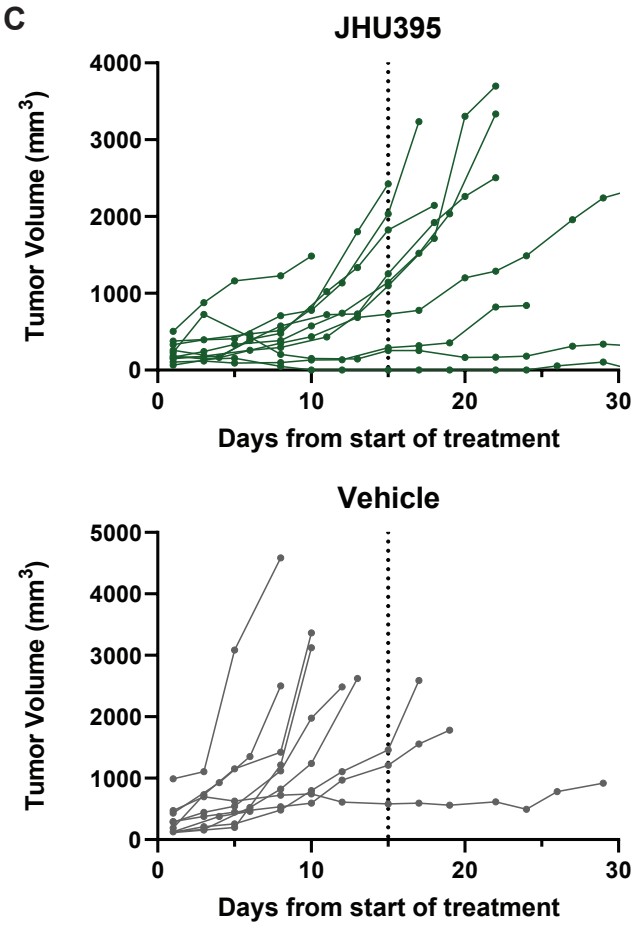
1. Lemberg, K.M., et al., *The novel glutamine antagonist prodrug JHU395 has antitumor activity in malignant peripheral nerve sheath tumor.* Mol Cancer Ther, 2019.
2. Guzman, C., et al., *ColonyArea: an ImageJ plugin to automatically quantify colony formation in clonogenic assays.* PLoS One, 2014. **9**(3): p. e92444.
3. Alt, J., et al., *Glutamine Antagonist GA-607 Causes a Dramatic Accumulation of FGAR which can be used to Monitor Target Engagement.* Curr Drug Metab, 2021. **22**(9): p. 735-745.
4. Rais, R., et al., *Discovery of DRP-104, a tumor-targeted metabolic inhibitor prodrug.* Sci Adv, 2022. **8**(46): p. eabq5925.

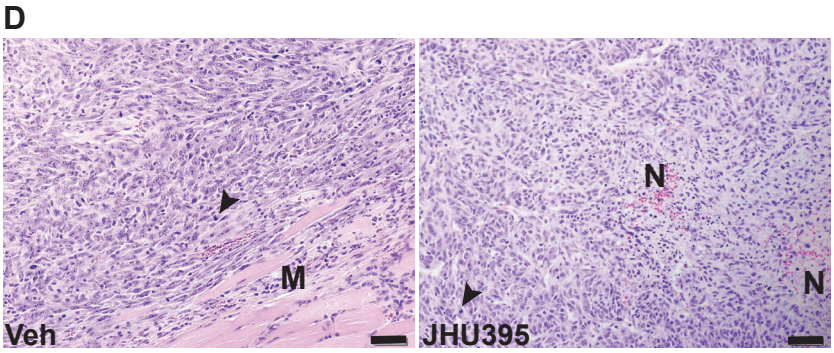
Supplemental Figure 1

A

Descriptor	Vehicle	JHU395
# male (%), # female (%)	4 males (40%), 6 females (60%)	3 males (27%), 8 females (73%)
median age at enrollment (IQR) (wks)	16.8 (14.2, 30.3)	18.1 (14.4, 23.8)
median weight at enrollment (IQR) (g)	27.5 (24.5, 28.8)	23 (22, 26.5)
median tumor vol at enrollment (IQR) (mm^3)	238.1 (128.3, 397)	185.2 (155, 294.9)

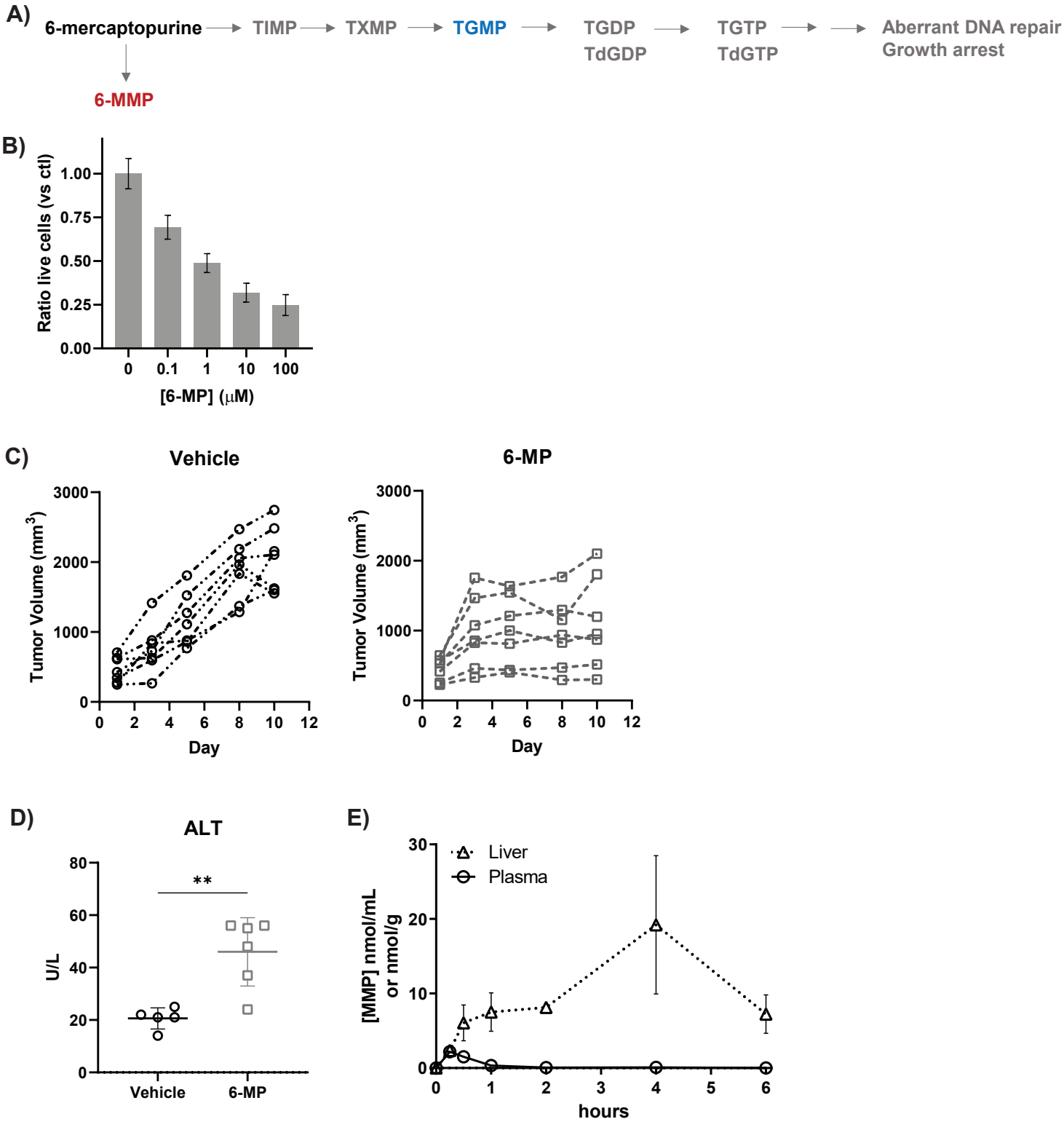






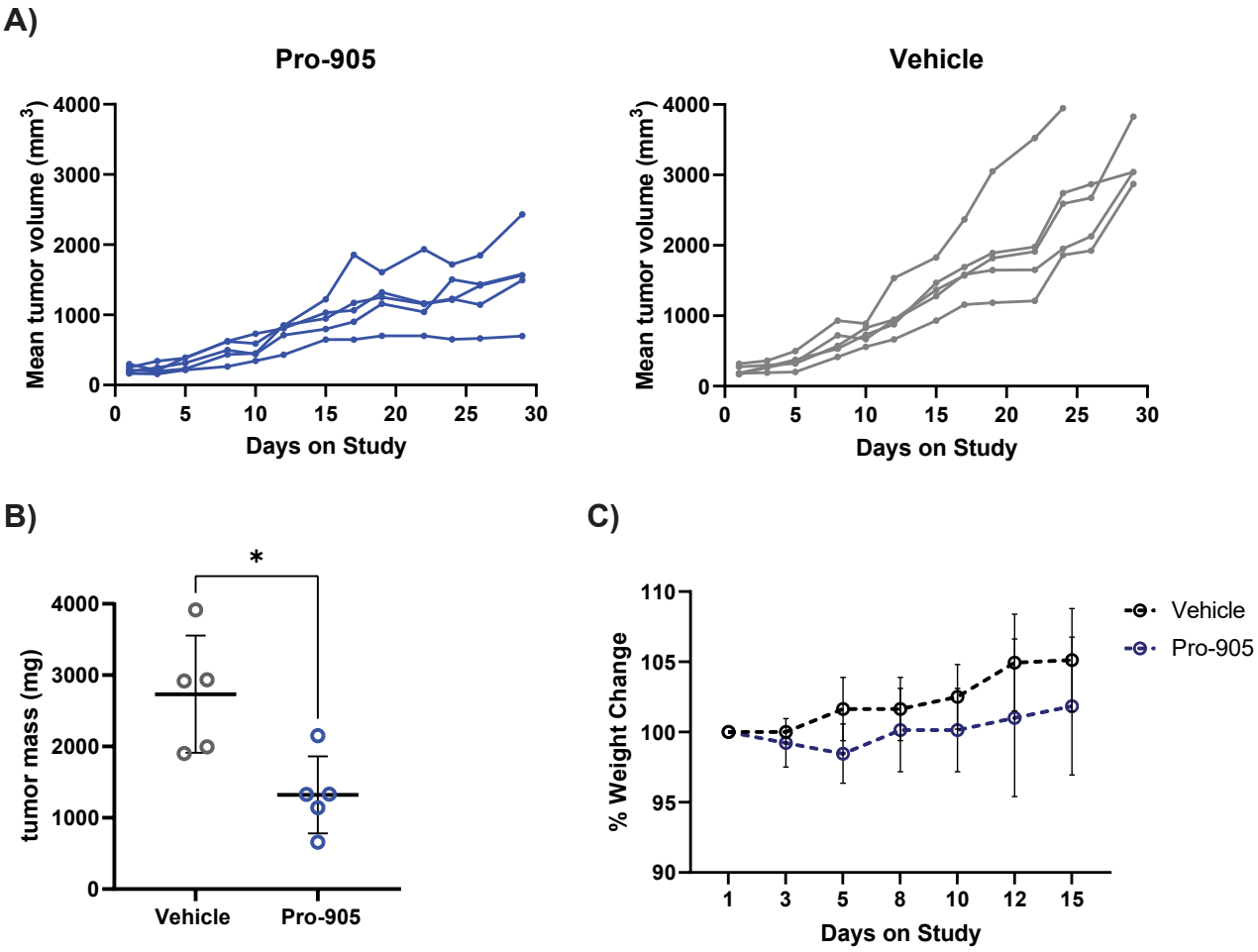
Supplemental Figure 1: Additional data on effect of JHU395 in NPcis mouse model A) Population description of NPcis mice enrolled on study. B) % change in weight from day 1 of treatment for mice on vehicle or JHU395 arms. Number of surviving mice at each increment shown beneath x-axis. Data are graphed as mean ± SD. C) Spider plots indicating tumor volume of each mouse measured over first thirty days of treatment. Dashed line shown at day 15, at which point 3/11 mice remained on vehicle arm and 9/11 mice remained on JHU395 arm. D) H&E stains of representative tumors at conclusion of treatment. Scale bar = 50 microns. M = muscle. N = necrosis. Arrowhead indicates dividing cells.

Supplemental Figure 2



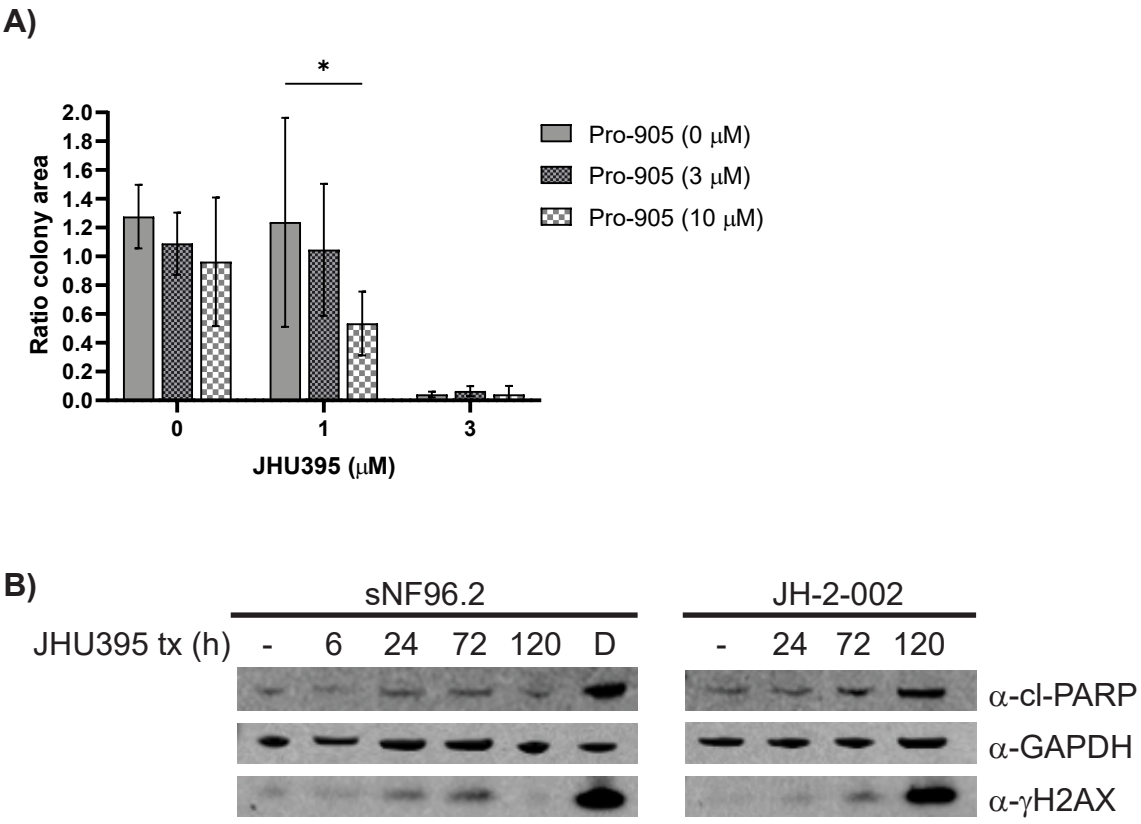
Supplemental Figure 2: Clinically used antimetabolite 6-mercaptopurine has partial antitumor activity in MPNST models with evidence for formation of toxic metabolites. A) Schematic of 6-mercaptopurine (6-MP) activation and metabolism in tumor cells. GI/hepatotoxic metabolite 6-MMP shown in red. Penultimate active metabolite TGMP shown in blue. B) Ratio live cells compared to control as assessed by Trypan blue dye exclusion from JH-2-002 cells treated with 6-MP at indicated concentrations for one week. C) Tumor volumes in murine flank MPNST treated with vehicle or 6-MP (20 mg/kg i.p. 5d/week) for 10 days. D) Alanine liver transaminase levels in plasma of vehicle and 6-MP treated mice after 10 days treatment. E) 6-methylmercaptopurine in liver and plasma from B6 mice treated with 6-MP (20 mg/kg i.p.) for indicated times.

Supplemental Figure 3



Supplemental Figure 3. Pro-905 has antitumor activity in a human MPNST patient-derived xenograft model. A) JH-2-031 patient derived xenograft tumor volumes and B) tumor weights at time of harvest in NSG mice during treatment with vehicle or Pro-905 (20 mg/kg i.p. 5 days per week for one month). C) Animal weight change from day 1 in NSG mice treated with vehicle or Pro-905 (20 mg/kg i.p. five days/week) for two weeks.

Supplemental Figure 4

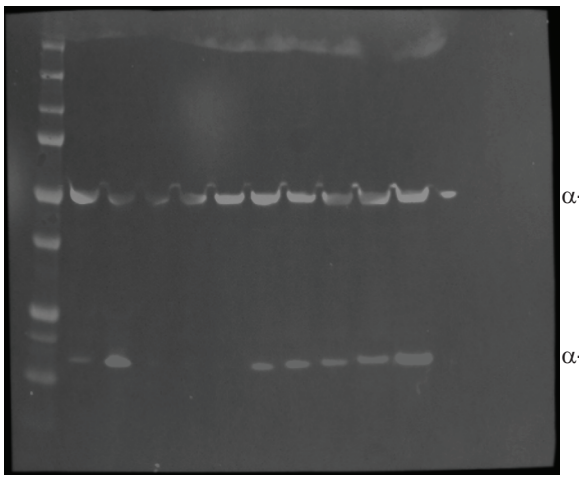


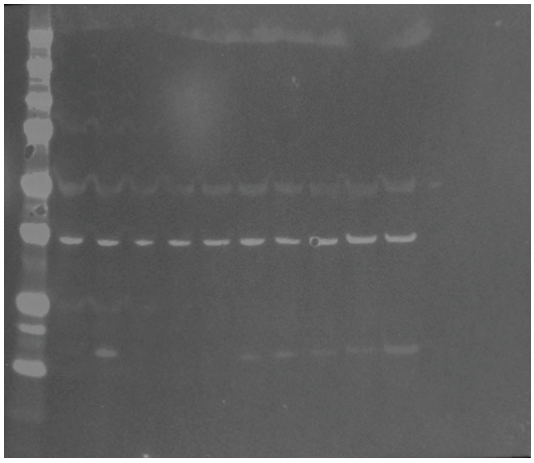
Supplemental Figure 4: Additional data on effect of JHU395 and Pro-905 in MPNST cells. A) Effect of combination JHU395 and Pro-905 on colony formation of sNF96.2 cells. Quantification performed using FIJI Colony Area plugin. B) Effect of single agent JHU395 (3 μM) on DNA damage (denoted by γH2AX) and induction of cleaved PARP at times indicated in sNF96.2 and JH-2-002 cells. GAPDH is a loading control. D=doxorubicin, positive control.

Supplemental Figure 5. Full scan images for western blots

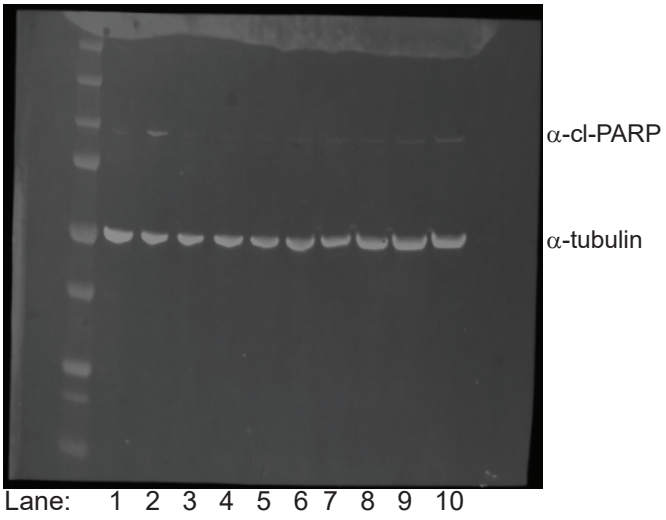
Full scan blot images for Figure 5D

Blot probed for tubulin, γ–H2AX initially. Then reprobed for GAPDH (2nd loading control)



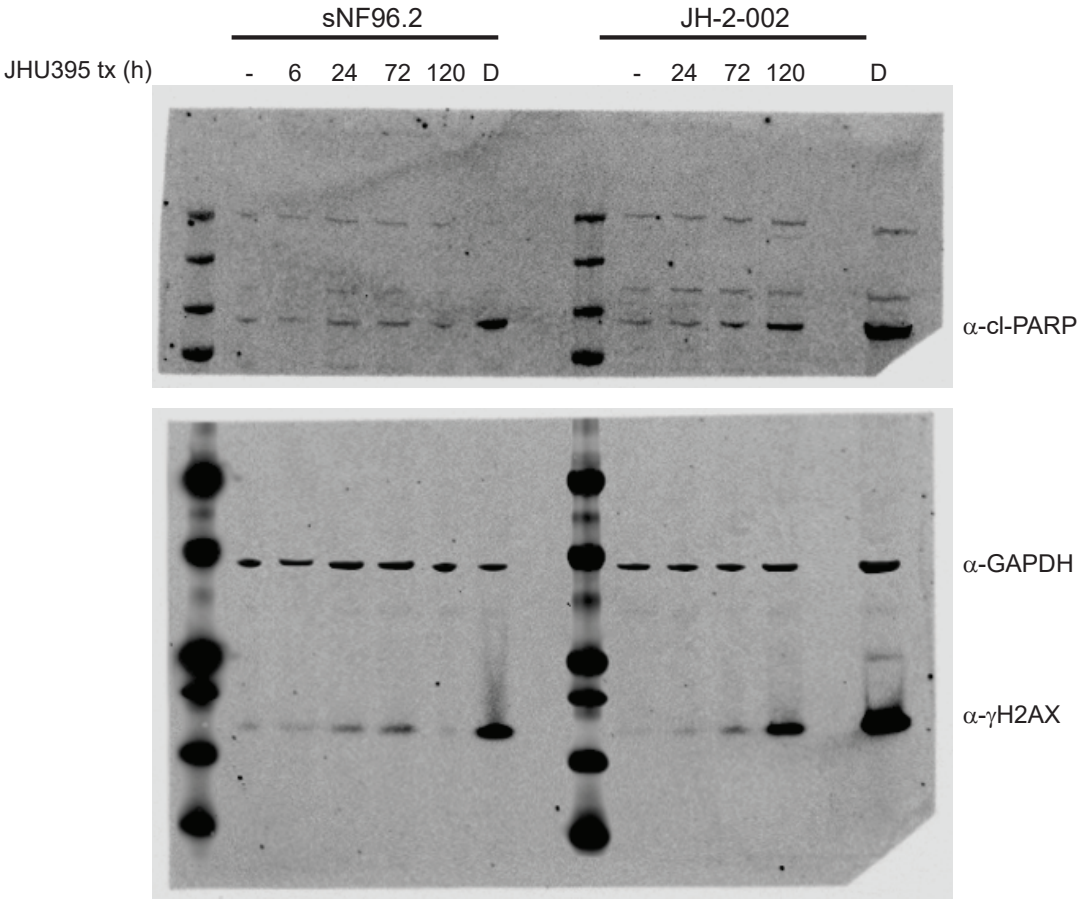


Same samples on a separate blot probed for tubulin, cleaved PARP



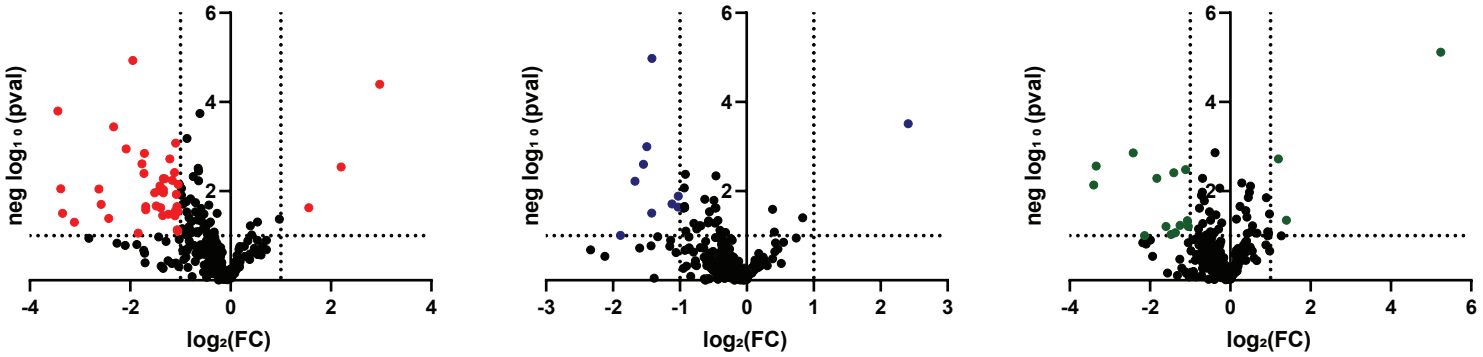
Supplemental Figure 5. Full scan images for western blots (continued)

Full scan blot images for Supplemental Figure 4B



Supplemental Figure 6

A



B

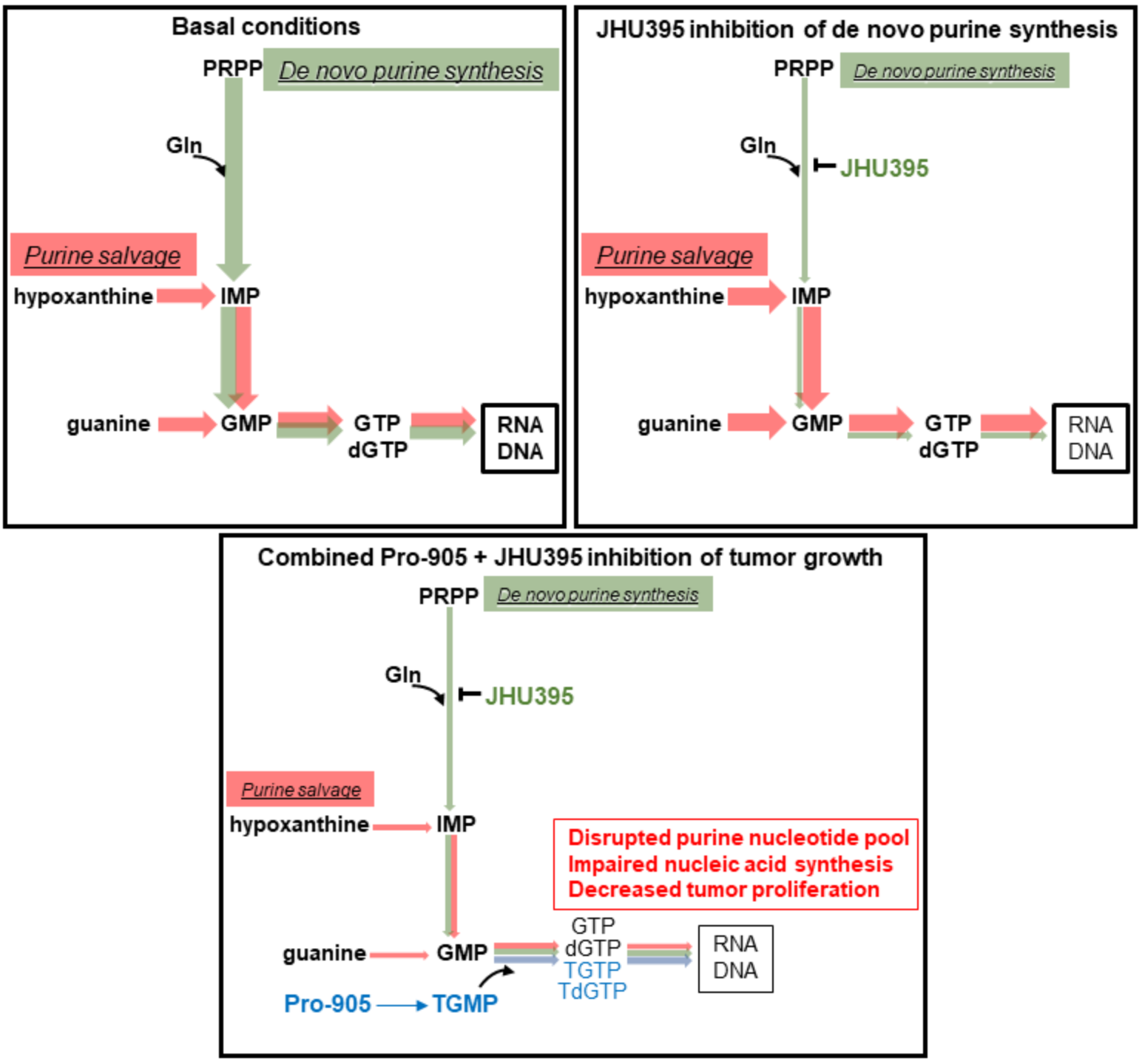
COMBO VS. VEH			
Metabolite	KEGG ID	log_2 FC	neg log_{10} pvalue
Geranyl-PP	C00341	2.97	4.40
putrescine	C00134	2.20	2.54
N-acetyl spermidine	C00612	1.56	1.63
dehydroascorbic acid	C05422	-1.02	2.15
allantoin	C01551	-1.05	2.15
glutathione-nega	C00051	-1.05	1.54
dUMP-nega	C00365	-1.06	1.10
acetoacetyl-CoA-posi	C00332	-1.07	1.13
dGMP	C00362	-1.07	1.65
trehalose-sucrose	C00089	-1.08	1.93
S-methyl-5-thioadenosine	C00170	-1.09	3.08
dTMP-nega	C00364	-1.10	1.56
D-gluconate	C00257	-1.11	1.45
UDP-D-glucuronate	C00167	-1.12	2.42
adenosine	C00212	-1.16	2.24
UDP-D-glucose	C00029	-1.21	2.72
NADPH	C00005	-1.24	1.48
glucono-?-lactone	C00198	-1.31	2.28
succinate	C00042	-1.34	1.96
4-aminobutyrate	C00334	-1.34	2.28
Methylmalonic acid	C02170	-1.34	2.03
dCDP-nega	C00705	-1.36	1.45
hydroxyproline	C01157	-1.39	1.63
cyclic-AMP	C00575	-1.41	2.12
Adenylosuccinate	C03794	-1.43	2.01
nega	C00021	-1.48	1.67
GDP-nega	C00035	-1.51	1.96
homoCysteine-posi	C00021	-1.69	1.65
cysteine	C00491	-1.69	1.58
aminoimidazole carboxamide ribonucleotide	C04677	-1.72	2.85
coenzyme A-posi	C00010	-1.73	2.40
glucosamine	C00329	-1.77	2.61
Cystine	C00491	-1.84	1.06
dihydroorotate	C00337	-1.95	4.93
sorbitol	C00794	-2.08	2.95
1-Methyladenosine	C02494	-2.19	
Deoxycholic acid	C04483	-2.33	3.44
NADH	C00004	-2.43	1.39
NADH-nega	C00004	-2.58	1.70
NADP+_posi	C00006	-2.63	2.05
2,3-Diphosphoglyceric acid	C01159	-3.11	1.30
dTDP-nega	C00363	-3.35	1.50
NADP+_nega	C00006	-3.38	2.05
acadesine	C04663	-3.44	3.80

PRO-905 VS VEH			
Metabolite	KEGG	log_2 FC	neg log_{10} pvalue
N-acetyl spermidine	C00612	2.41	3.51
N-carbamoyl-L-aspartate-nega	C00438	-1.03	1.89
ornithine	C00077	-1.03	1.64
D-glucono-?-lactone-6-phosphate	C01236	-1.12	1.71
dihydroorotate	C00337	-1.42	4.98
quinolinate	C03722	-1.42	1.51
acadesine	C04663	-1.49	3.00
glucosamine	C00329	-1.55	2.60
Flavone	C15608	-1.67	2.22
NADH-nega	C00004	-1.89	1.01

JHU395 VS VEH			
Metabolite	KEGG	log_2 FC	neg log_{10} pvalue
Geranyl-PP	C00341	5.24	5.12
5-phosphoribosyl-1-pyrophosphate	C00119	1.40	1.35
acetylphosphate	C00227	1.20	2.72
trehalose-sucrose	C00089	-1.05	1.21
5-methyl-THF	C00440	-1.07	1.34
xanthosine	C01762	-1.11	2.48
retinoic acid	C00777	-1.26	1.23
NADP+_posi	C00006	-1.37	1.07
aminoimidazole carboxamide ribonucleotide	C04677	-1.41	2.41
oxaloacetate	C00036	-1.47	1.03
NADP+_nega	C00006	-1.60	1.21
Deoxycholic acid	C04483	-1.83	2.29
NAD+_nega	C00003	-2.14	1.00
acadesine	C04663	-2.42	2.86
NADH-nega	C00004	-3.34	2.56
NADH	C00004	-3.40	2.14

Supplemental Figure 6: Additional data on effect of combination JHU395 and Pro-905 on metabolites in murine tumors. A) Volcano plots comparing observed changes in metabolites extracted from flank tumors in combination, Pro-905, and JHU395 treated mice compared to vehicle. Metabolites with absolute value (log_2fold change) > 1 and -log_{10} pvalue > 1 are highlighted in color. B) Tables of highlighted metabolites from each treatment group.

Supplemental Figure 7



Supplemental Figure 7: Model of purine nucleotide inhibition by JHU395 and Pro-905 in tumor cells. In a basal (untreated) state, both de novo purine synthesis (green) and purine salvage (red) contribute to the flux of substrates for purine nucleotide synthesis of DNA and RNA. JHU395 treatment primarily inhibits de novo purine synthesis, with substrate flux still emanating from purine salvage. Addition of Pro-905 delivers the active nucleoside antimetabolite TGMP (blue) to tumor cells, competing with delivery of substrates from purine salvage and contributing the antimetabolite to nucleic acid synthesis. The combination of Pro-905 and JHU395 disrupts the purine nucleotide pool, impairs nucleic acid synthesis, and decreases tumor cell proliferation in murine models.