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Supplemental Information

Repurposing Pimavanserin, an Anti-Parkinson Drug for Pancreatic Cancer Therapy

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Supplementary methods section

LY294002 and GANT61 treatment

MIAPaCa2 or BxPC3 cells were seeded at a density of 0.2 x 10^6 cells per well in a 6-well plate and allowed to attach overnight. Following overnight incubation, cells were pre- treated with 50µM PI3K inhibitor LY294002 or 30µM Gli-1 inhibitor GANT61 for 4 hours following treatment with 7.5µM PVT for 48 hours and the cells were processed for western blot analysis.

Akt and Gli-1 silencing

AsPC1 and MIAPaCa2 cells were transfected with 100nm Akt siRNA (Cell Signaling Technologies) and 100nm Gli-1 siRNA (Santa Cruz) respectively using siPORT (Ambion Inc. Austin, TX) transfection reagent according to manufacturer's instructions. After 14 hours of transfection, cells were treated with 7.5µM PVT for 48 hours. Cells were then collected and whole cell lysates were subjected to western blotting.

Immunohistochemistry

FFPE tissues were subjected to IHC analysis. Expression of pAkt(S473), Gli-1, Oct-4, NANOG, cleaved caspase-3 and cleaved PARP was validated in subcutaneous and orthotopic pancreatic tumors.

TUNEL assay

Hydrated paraffin embedded tissues were sectioned as mentioned above. The sections were subjected to TUNEL analysis. TUNEL assay was carried out according to manufacturer's instructions (Calbiochem, San Diego, CA, USA).

Evaluation of Clinical Chemistry Parameters

After chronic administration of PVT for 24 days, mice were sacrificed and plasma was collected. Plasma samples were sent to Texas Veterinary Medical Diagnostic system, Amarillo, TX for analysis. Clinical chemistry parameters like ALT, AST, Total serum protein, Calcium, Albumin, Phosphorus, Glucose, BUN and Creatinine were analysed.

Mice behavioral analysis

In orthotopic experiment, the behavioral activity of mice was analysed after chronic administration of PVT for 49 days. The behavioral analysis was performed by Versamax (Accuscan Instruments Inc., Columbus, OH, USA).

Estimation of PVT in plasma, tumors and pancreas

The concentration of PVT was estimated in plasma, tumor lysates and pancreas. Plasma concentration of PVT was estimated in athymic nude mice treated with 10mg/kg PVT for 7 days. An aliquot of 50µL plasma or tumor lysate sample was crashed with 200µL of acetonitrile and rotenone solution. Samples were vortex mixed and centrifuged for 5 minutes at 12000rpm and 4°C. Clear supernatant, 180µL was transferred to an auto- sampler vial insert and 1µL sample was injected onto an analytical column (Kinetex C18, 50 x 2.1mm). 0.1% formic acid in water (pump A) and acetonitrile (pump B) was used as the mobile phase. Rotenone solution was taken as an internal standard (IS). The

standard curve was linear (r >0.995) over the concentration range of 1 to 200 ng/ml. The analytical data obtained was processed by Analyst software (version 1.6.2).

Plasma

Average plasma concentration is **0.0618µg/ml**

Sample	Plasma concentration (ng/ml)
Control sample	0.32
PVT sample 1	123.37
PVT sample 2	28.7
PVT sample 3	33.54
PVT sample 4	61.6

Table S1: Estimation of PVT concentration in plasma by LC/MS.

Tumor

Average concentration of PVT in tumor is **86.2ng/ml or 0.35µg/g tumor**

Sample	Concentration (ng/ml)
Control sample 1	0.17
Control sample 2	0.38
Control sample 3	0.57
PVT sample 1	78.26
PVT sample 2	105.48
PVT sample 3	116.42
PVT sample 4	44.62

Table S2: Estimation of PVT concentration in tumor by LC/MS**.**

Pancreas

Average concentration of PVT in pancreas is **120ng/ml or 0.48µg/g pancreas**

Sample	Concentration (ng/ml)
PVT sample 1	73.135
PVT sample 2	254.745
PVT sample 3	32.31

Table S3: Estimation of PVT concentration in pancreas by LC/MS**.**

Brain

Average concentration of PVT in brain is **78.46ng/ml or 0.31µg/g brain**

Sample	Concentration (ng/ml)
PVT sample 1	50.66
PVT sample 2	47.98
PVT sample 3	136.75

Table S4: Estimation of PVT concentration in brain by LC/MS.

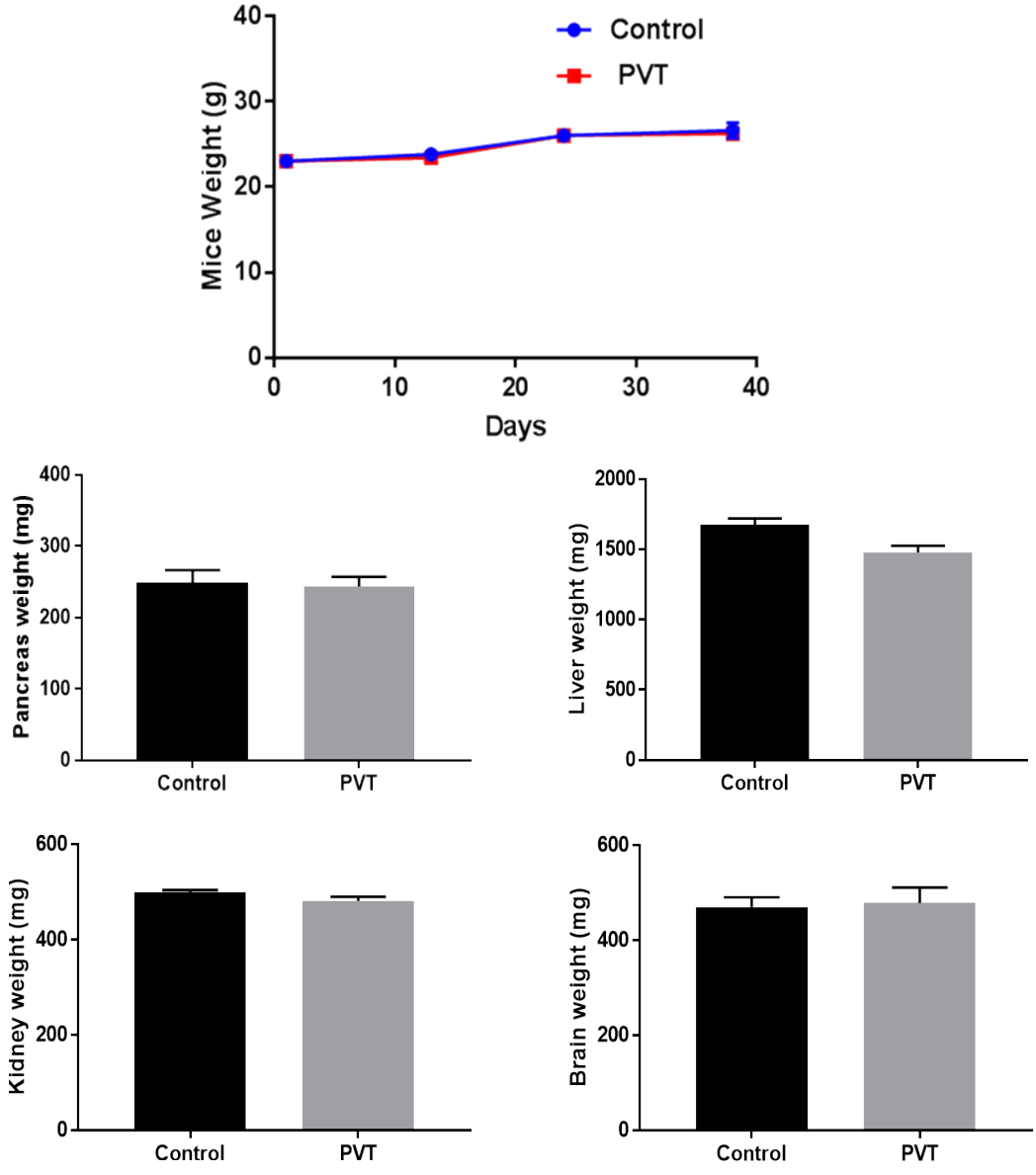


Figure S1: PVT did not alter the weight of mice and organs. Mice weight was recorded once in 10 days and plotted against days. Organs such as pancreas, liver, kidney and brain were aseptically removed at the day of sacrifice from control and PVT treated group mice.

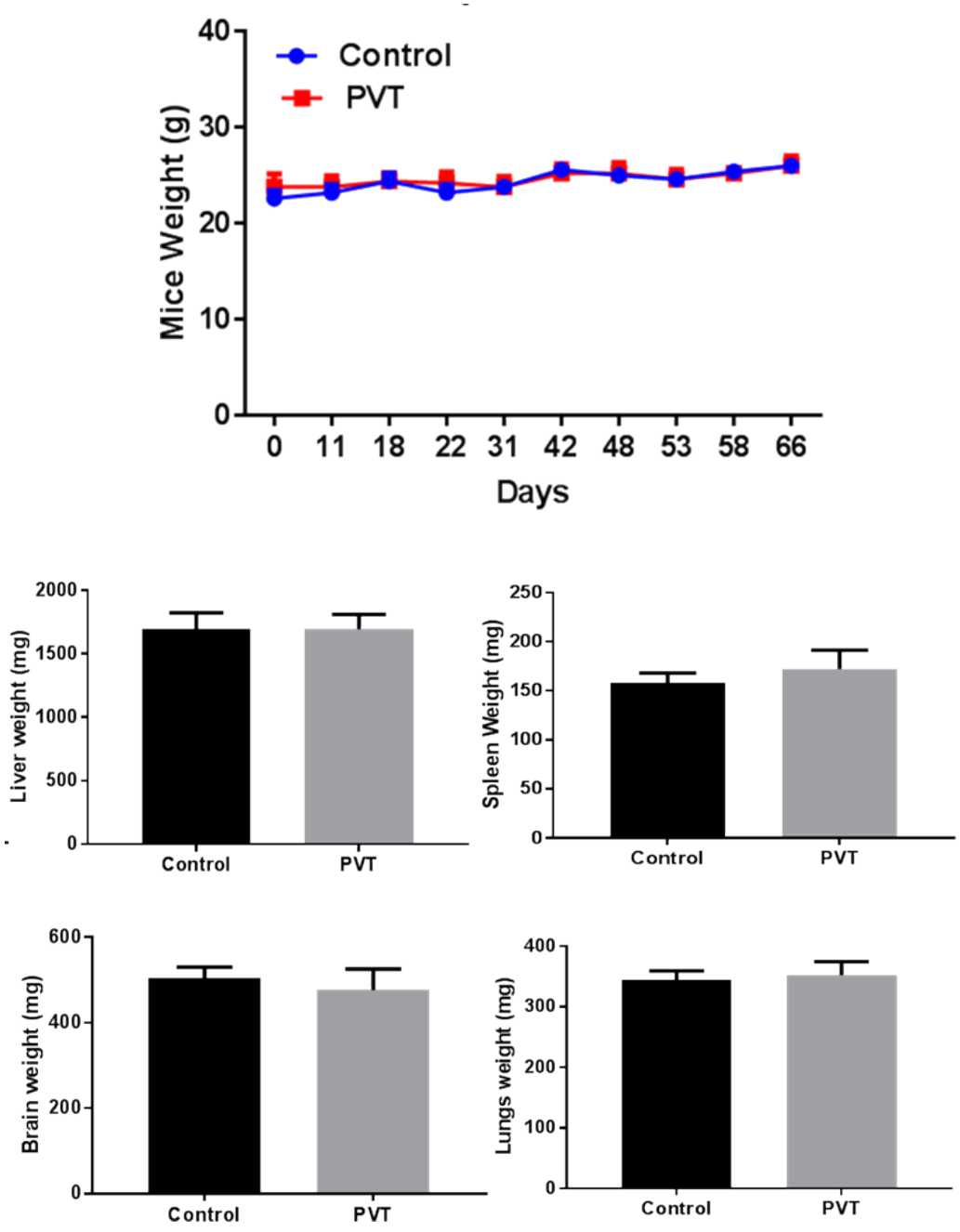
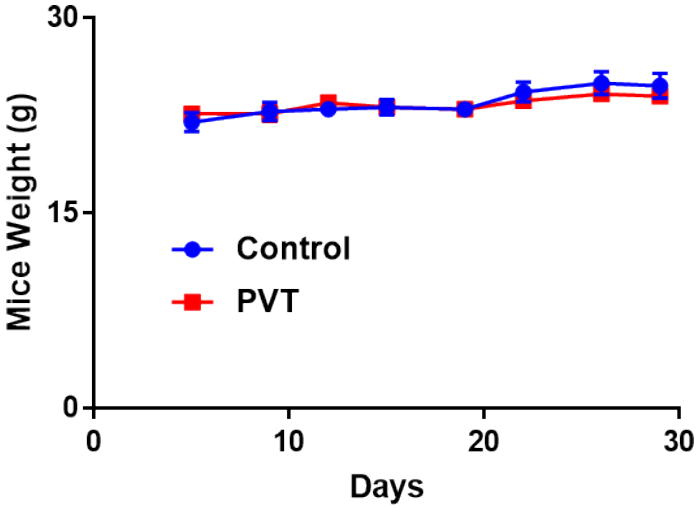
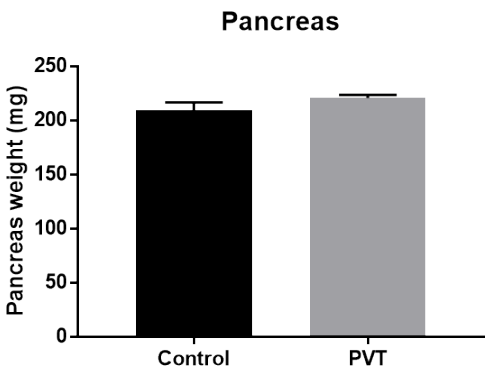


Figure S2: PVT did not alter the weight of mice and organs. Mice weight was recorded once in a week and plotted against days. Weight of aseptically removed organs like liver, spleen, brain and lungs were recorded in control and PVT treated group.





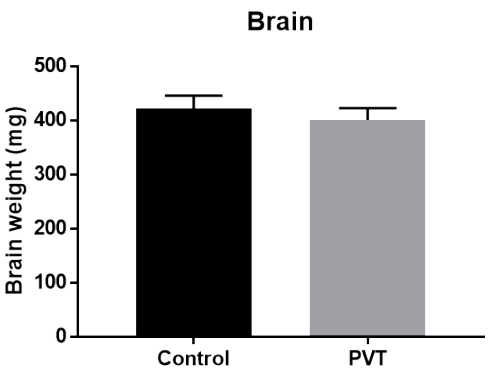


Figure S3: PVT did not alter the weight of mice and organs. Overall weight of the mice was recorded twice a week and plotted against days. Weight of aseptically excised pancreas and brain was recorded at the day of sacrifice.

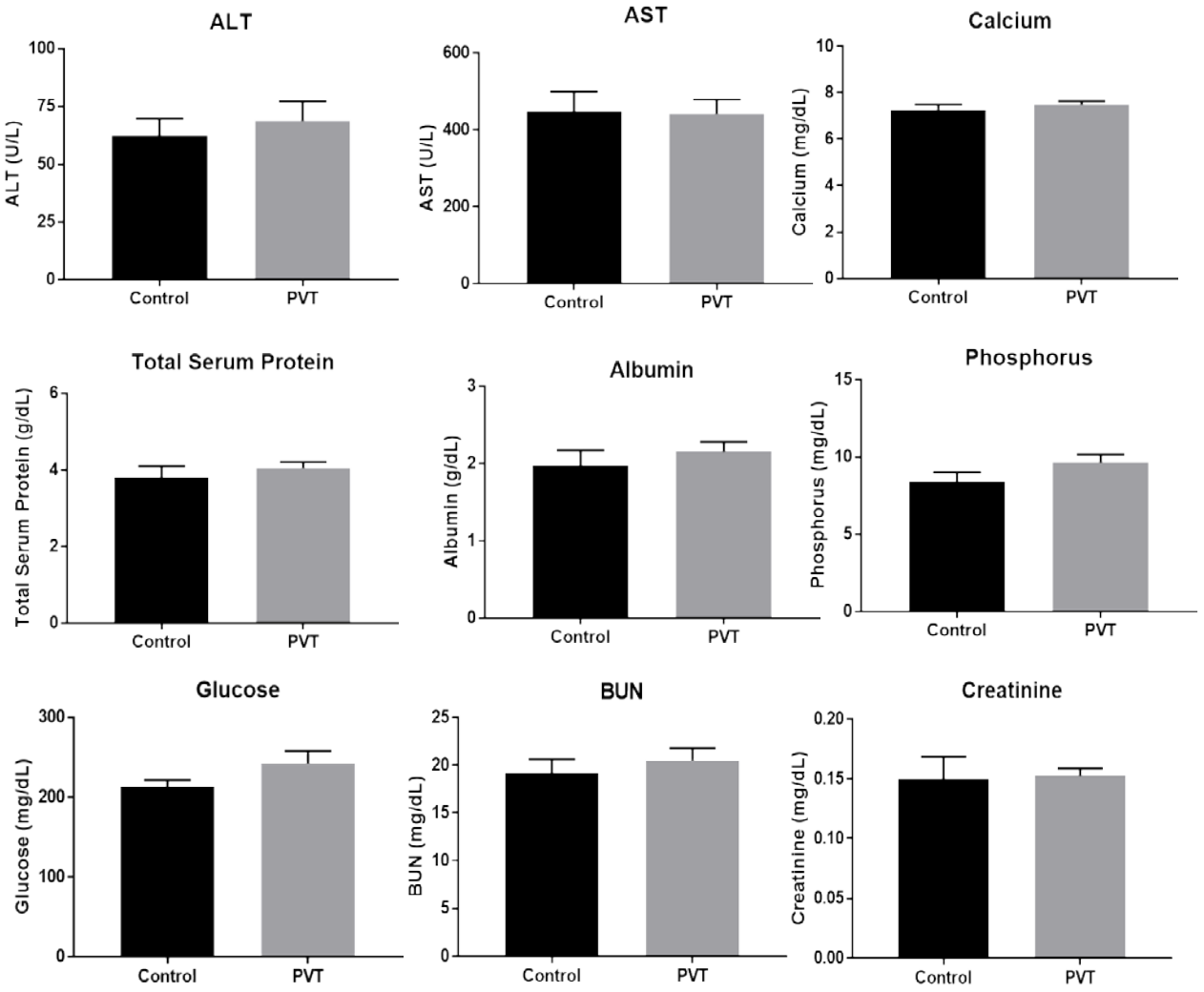


Figure S4: Clinical chemistry parameters remained unaltered with PVT treatment. ALT, AST, Total serum protein, Calcium, Albumin, Phosphorus, Glucose, BUN and Creatinine were analysed between control and PVT treated group.

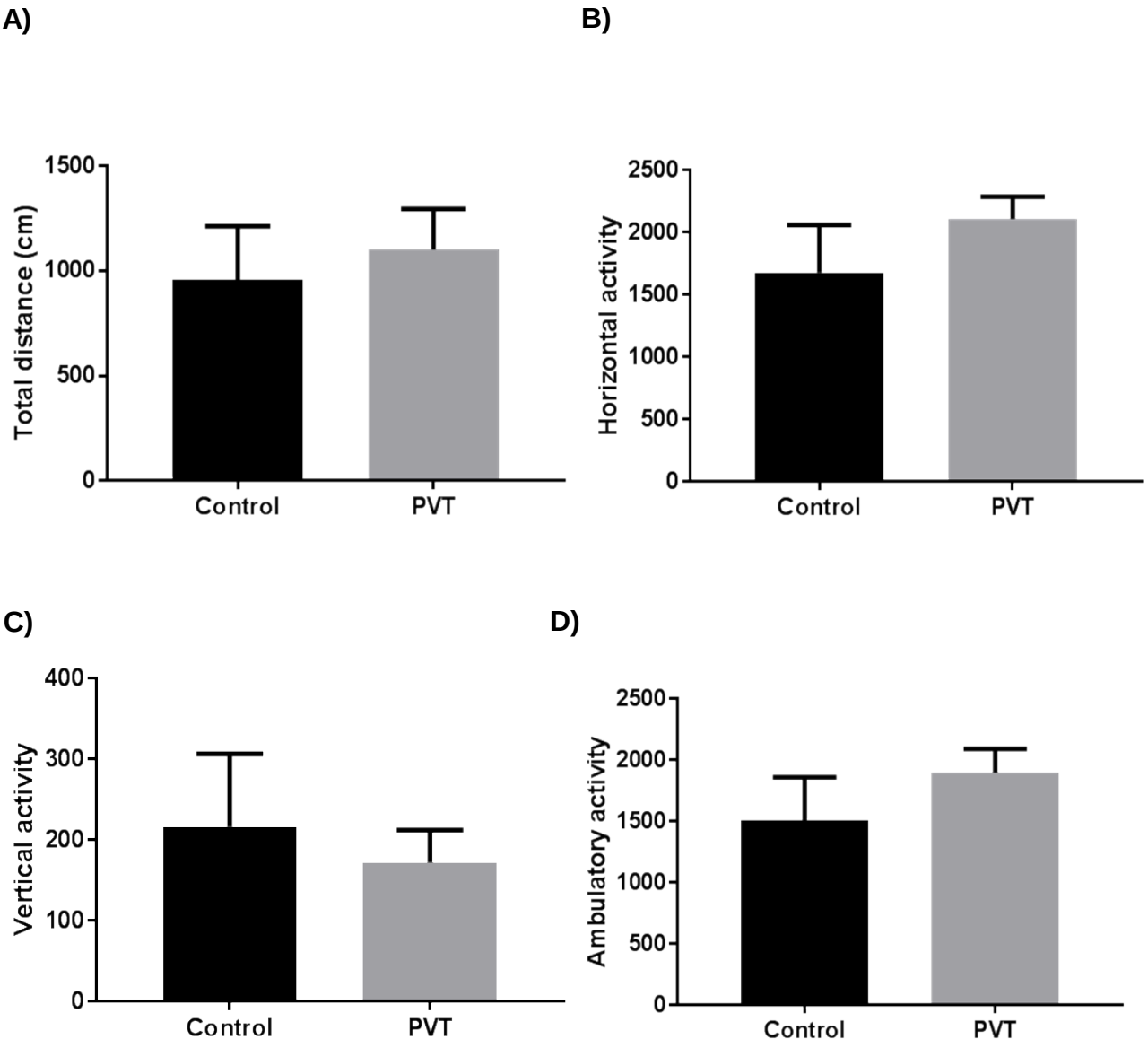


Figure S5: PVT did not affect the behavioral activity of mice. Behavioral activity parameters A) Total distance B) Horizontal activity C) Vertical activity D) Ambulatory activity were plotted between control and PVT treated group.

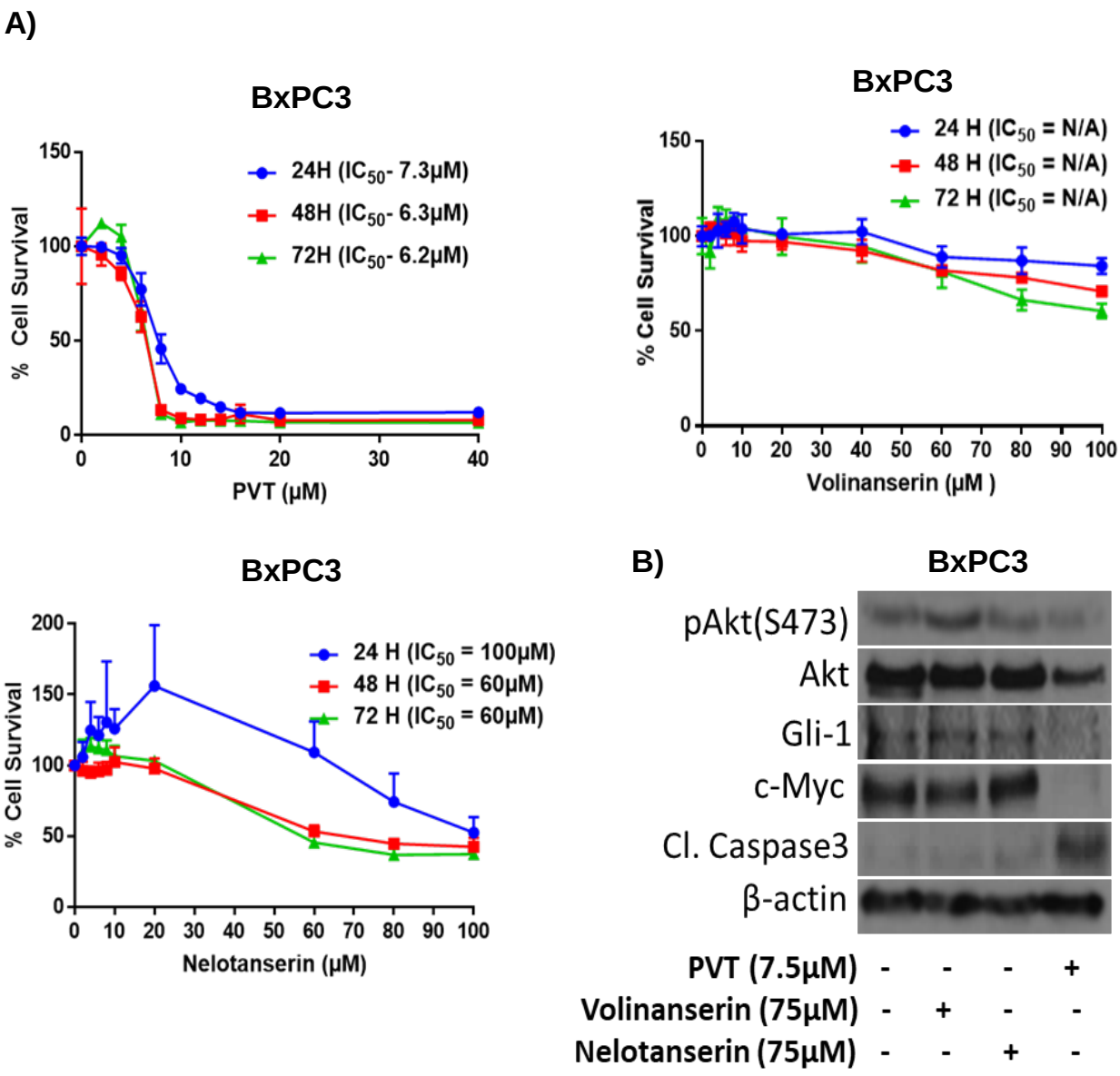


Figure S6: A) BxPC3 cells were treated with varied concentrations of PVT, Nelotanserin and Volinanserin for 24, 48 and 72 hours and cell survival was determined by SRB assay. B) Whole cell lysates of BxPC3 cells treated with 7.5µM PVT, 75µM nelotanserin and volinanserin for 48 hours was subjected to western blotting analysis.