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Title: Pharmacological Inhibition of Pleckstrin Homology Domain Leucine-Rich Repeat Protein Phosphatase (PHLPP) is Neuroprotective: Differential Effects on Astrocytes.

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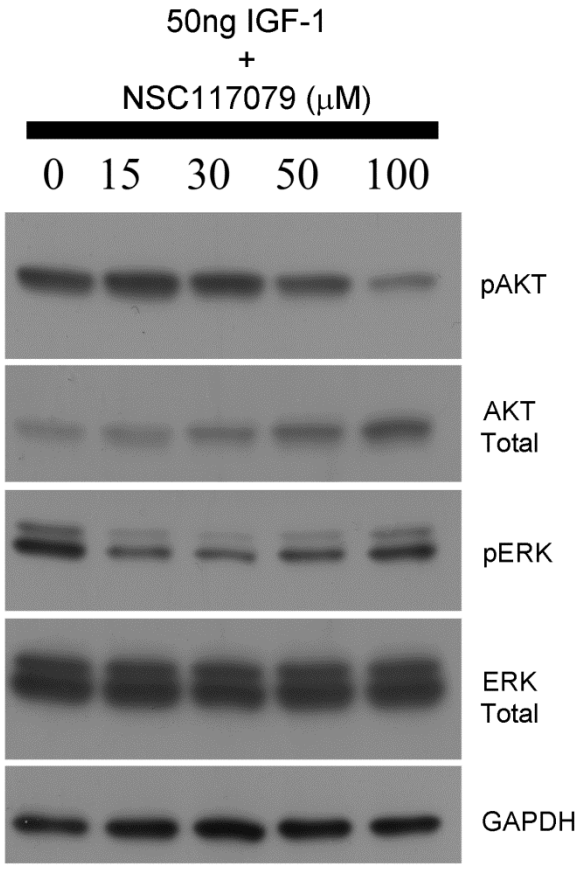
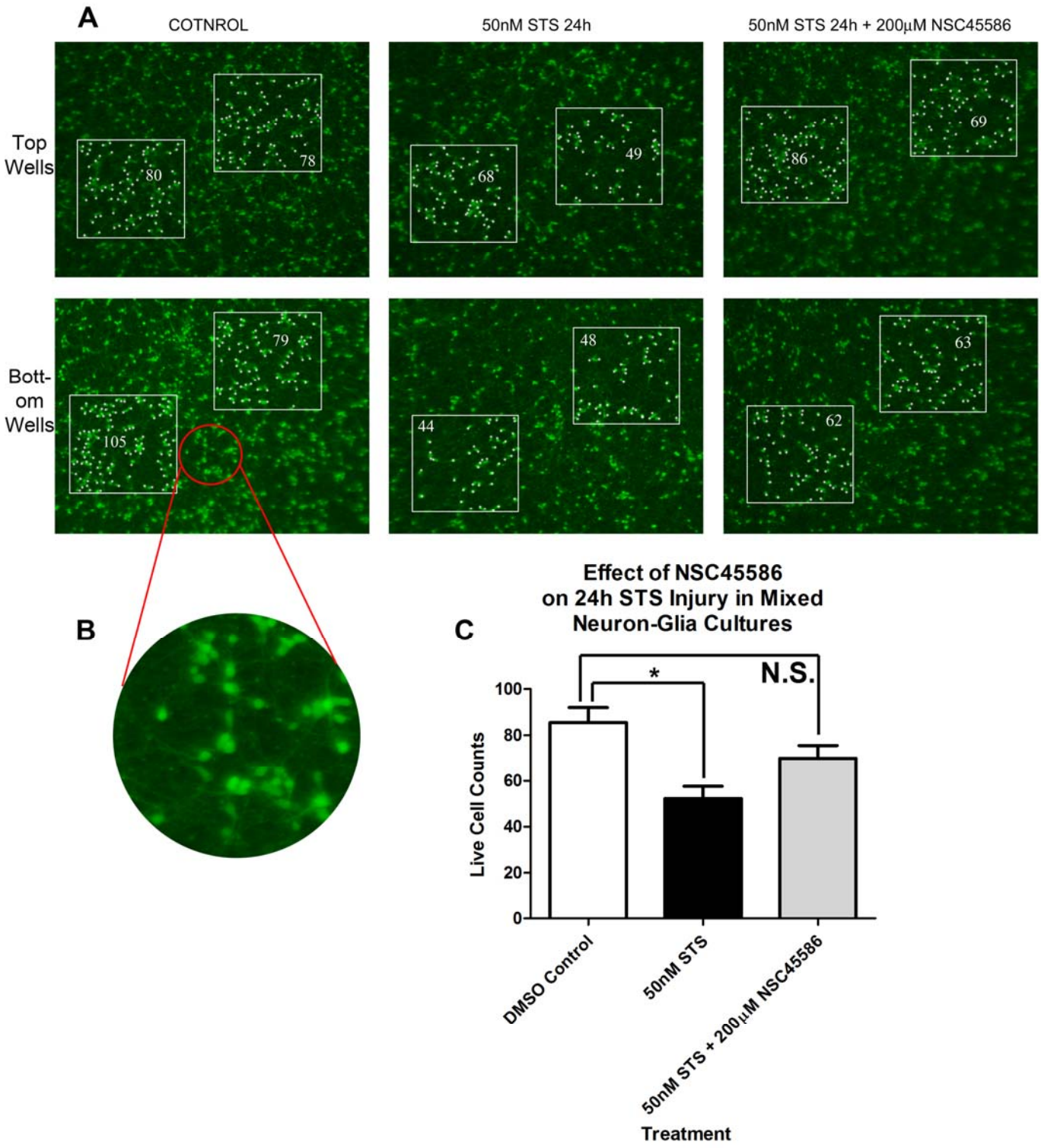
Supplementary Figure 1



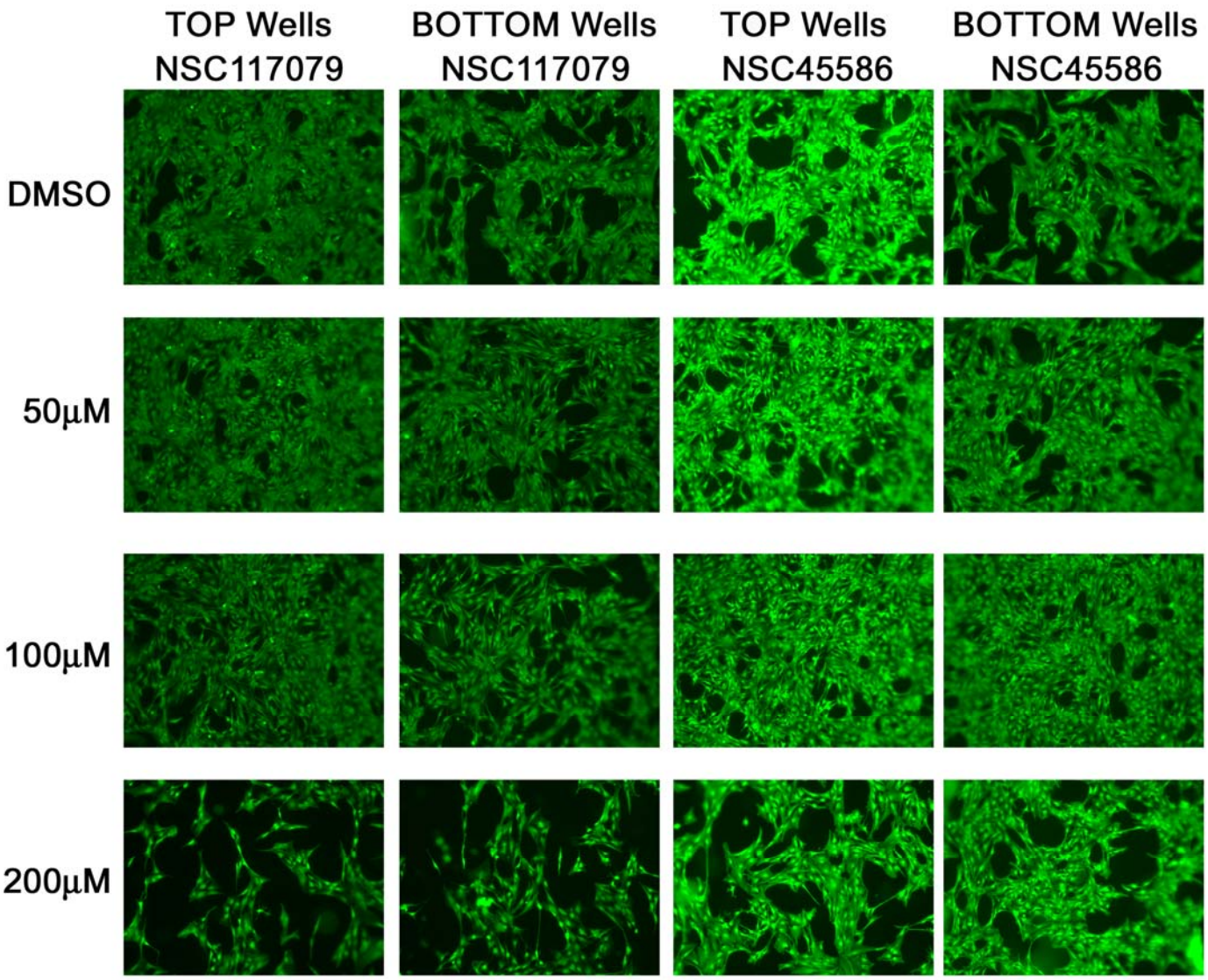
Supplementary Figure 1: PHLPP Inhibitor NSC117079 Inhibits IGF-1 Induced AKT Activation. Primary rat cortical neurons were grown onto 6-well plates, supplement starved 2h, and treated with 50ng/mL IGF-1 in the absence (control) or presence of increasing NSC117079 doses. Blots show that NSC117079 dose dependently decreases pAKT473 levels. pERK is altered by NSC117079 in a manner similar to non-stimulation conditions.

Supplementary Figure 2



Supplementary Figure 2: PHLPP Inhibitor NSC45586 Increases Neuronal Survival. Mixed primary rat cortical neurons and astrocytes were grown on an 8-well glass chamber slide. At DIV8 cultures were treated with DMSO (control), 50nM STS, or 50nM STS + 200μM NSC45586 for 24h; 2 wells per group (i.e. 1 top well and 1 bottom well of the chamber slide). After 24h injury, cells were washed twice with DPBS, and incubated with Calcein AM (2μM) dissolved in DPBS for ~10mins. **(A)** Live cell images were captured on a fluorescent microscope (10X objective). **(B)** An enlarged representative area from a control image shows green fluorescence in the neuronal soma and axons/dendrites. **(C)** Cell count analysis. 2 white boxes of equal size were randomly placed in each image field. Green (live) cells were counted in Photoshop. A white spot was placed next to each cell for counting accuracy. The total cell counts were averaged, and analyzed for each group in Prism software. The injury alone group (50nM STS) had significantly fewer live-green cells compared with uninjured DMSO control. Addition of NSC45586 reduced cell loss. Data were analyzed by ANOVA and Newman-Keuls Multiple Comparison post-hoc test. (*) indicates significant post-hoc difference ($p<0.05$) compared to non-injured control. Graphs show mean + SEM.

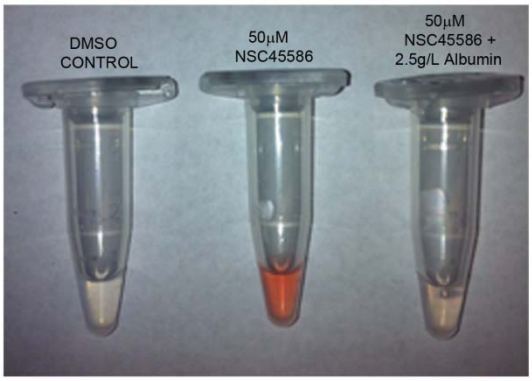
Supplementary Figure 3



Supplementary Figure 3: PHLPP Inhibitor NSC117079 Induces Astrocyte Cell Death. Primary rat astrocytes were seeded on (2X) 8-well chamber glass slides and grown for ~48h (to ~80-90% confluence). Cells were incubated for 24h with increasing

concentrations of NSC117079 or NSC45586. 24h later cells were washed twice with DPBS, and incubated with Calcein AM (2μM) dissolved in DPBS for ~10mins. Live cell images were captured using a fluorescent microscope (10X objective). 200μM NSC117079 altered astrocyte morphology, and induced cell death. NSC45586 also appeared to alter astrocyte morphology but these changes were not associated with obvious cell death.

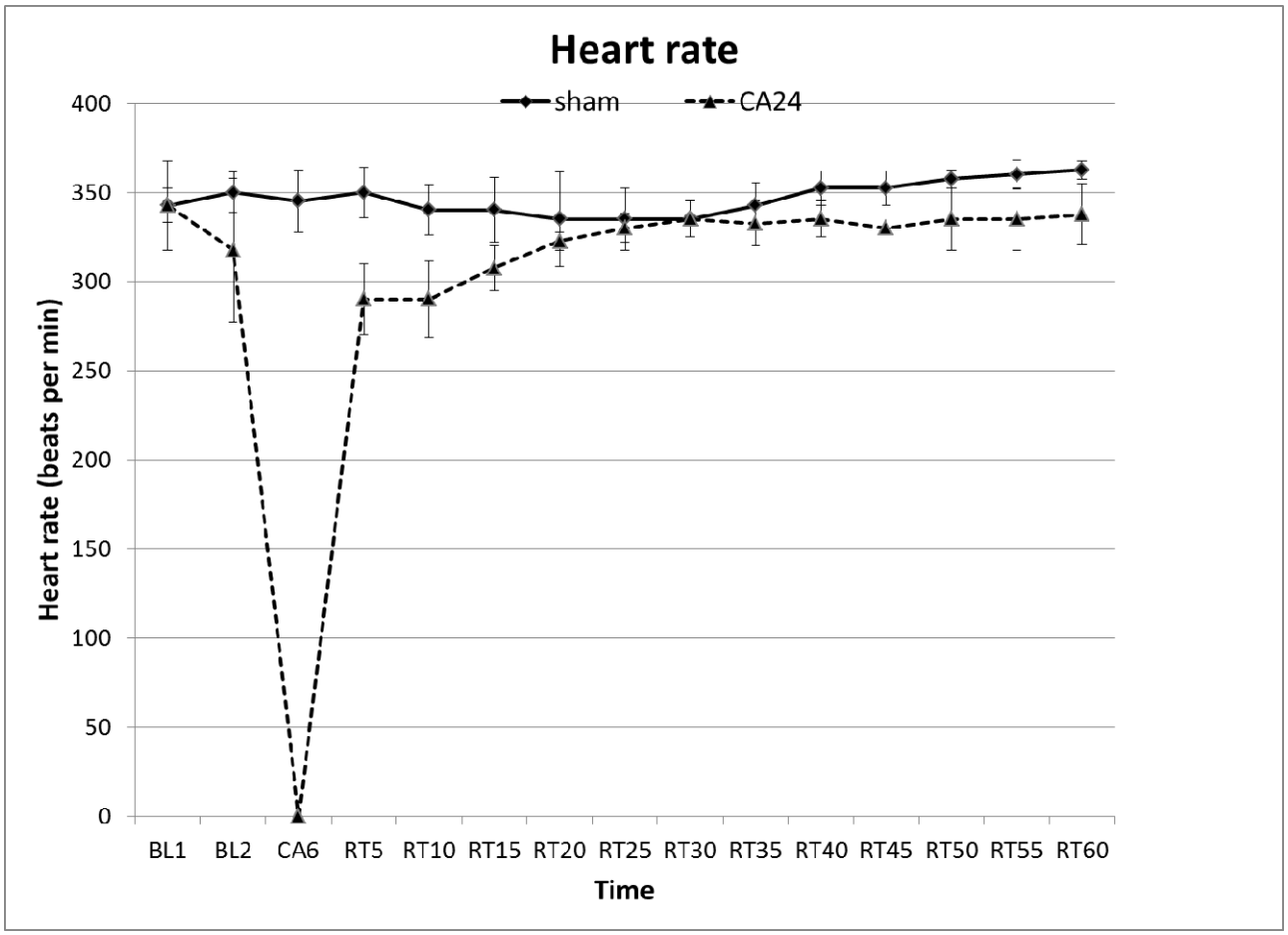
Supplementary Figure 4



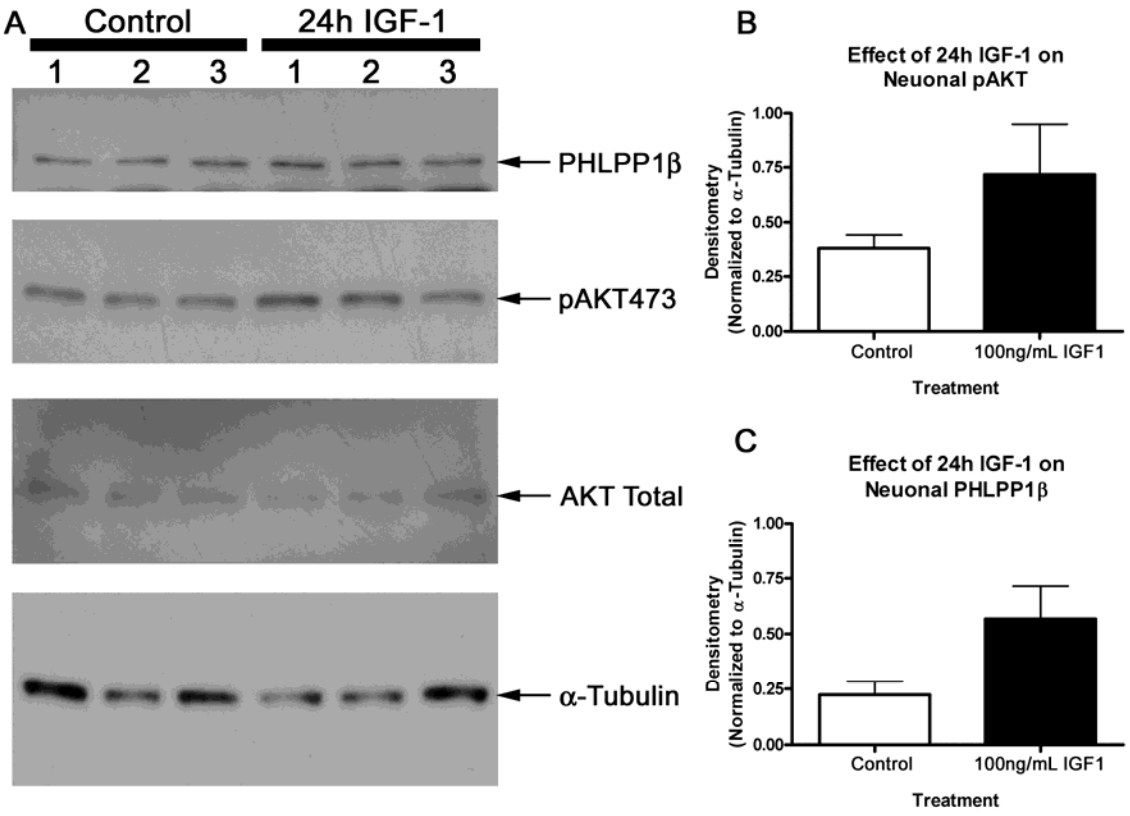
Supplementary Figure 4: Albumin Binds PHLPP Inhibitor NSC45586. Primary rat cortical neurons were grown on 6-well plates, supplement starved 2h, treated with 50μM NSC54486 for 35min in the absence or presence of bovine serum albumin, washed with PBS, and protein extracts harvested. Drug treatment stains the harvested protein extracts from clear (i.e. DMSO-control) to red/brown (i.e. 50μM NSC54486) by binding cellular membrane components and/or intracellular drug accumulation. However, in the presence of 2.5g/L albumin (i.e. the same concentration that is used in neuron growth B27 supplement) cellular disposition of NSC45586 is inhibited after 35min incubation – indicating preferential binding to albumin during the acute treatment period. Albumin interference may have decreased the efficacy of NSC45586 in STS injury experiments (i.e. because B27 supplement is a mandatory reagent needed for chronic 24h treatment

studies in neurons and it contains 2.5g/L albumin). In astrocyte viability studies, NSC45586 was also incubated over a 24h period but in the presence of 10% FBS. For comparison (to B27), compositional analysis of FBS stock (reported by the brand/company datasheet; Hyclone/Fisher Scientific) indicates albumin at a concentration of 1.9g/dL (i.e. ~1.9g/L when used at 10% final concentration).

Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 6: Chronic AKT activation Is Associated With PHLPP1 Upregulation in Primary Neurons. DIV 12 primary rat cortical neurons were treated 24h with 100ng/mL IGF-1. **(A)** Blots show changes in PHLPP1β, pAKT473, AKT total, and α-Tubulin after 24h IGF-1 stimulation. Densitometry (n=3/treatment) show that **(B)** PHLPP1 and **(C)** pAKT473 tend to increase after a 24h IGF-1 stimulation. Data were analyzed by T-test. Graphs show mean + SEM.

Supplementary Table 1

Physiology for VFCA Brain Injury Experiments

variable	group	BL1	BL2	RT5	RT15	RT30	RT45	RT60
pHa	**sham**	7.42±0.06	7.39±0.06	7.39±0.04	7.40±0.03	7.37±0.01	7.40±0.07	7.41±0.12
	CA24	7.39±0.03	7.39±0.03	7.16±0.07**	7.19±0.07	7.33±0.01	7.33±0.02	7.34±0.01
paO2	**sham**	202±43	97±20	294±111	354±49	332±45	229±108	245±88
mmHg	**CA24**	200±50	134±79	300±97	349±52	91±16	112±24	384±73*
paCO2	**sham**	38±7	38±7	37±3	35±6	39±3	35±6	34±13
mmHg	**CA24**	42±3	39±2	38±5	35±5	27±4**	37±4	36±9
BE	**sham**	-1.0±1.2	-2.0±0.5	-2.9±0.7	-2.0±1.5	-2.8±1.7	-2.8±1.3	-3.5±2.3
mEq/L	**CA24**	0.5±1.0	-1.5±3.1	-14.2±2.5**	-13.8±2.9**	-10.3±1.7**	-5.6±1.3**	-5.8±3.7
Lactate	**sham**	1.0±0.3	0.9±0.3	0.8±0.2	0.7±0.2	0.8±0.3	0.6±0.2	1±0.8
mmol/L	**CA24**	1.2±0.4	1.1±0.4	11.9±1.6**	8.1±0.6**	5.5±0.6**	2.7±1.0**	1.8±0.5
Hct	**sham**	43±2	40±3	39±2	40±3	39±2	40±3	38±3
	CA24	42±2	39±2	44±2*	41±4	40±1	43±4	39±6
Glucose	**sham**	140±17	119±13	111±10	109±3	113±16	110±12	111±15
mg/dL	**CA24**	172±31	158±43	156±109	143±86	130±56	135±35	135±39
Na	**sham**	140±1	142±1	143±1	143±2	144±1	145±3	145±4
mmol/L	**CA24**	140±2	142±2	146±2	146±2	145±1	146±3	147±5
K	**sham**	4.5±0.4	3.9±0.1	3.7±0.1	3.8±0.3	3.8±0.2	3.5±0.1	4.2±1.9
mmol/L	**CA24**	4.3±0.4	3.8±0.4	5.6±0.5*	3.4±0.4	4.0±0.0	4.2±0.4*	3.7±0.8
Cl	**sham**	110±3	115±2	116±1	117±3	117±2	117±3	119±4
mmol/L	**CA24**	107±3	112±5	113±3	115±5	117±2	117±4	119±10
Osmol	**sham**	288±3	291±1	293±3	293±1	293±1	296±4	296±6
mosm/L	**CA24**	289±5	294±5	301±9	300±5	298±5	300±8	303±10

BL = baseline. RT = resuscitation time (mins). BE = base excess.
(*) $p<0.05$ vs. sham; (**), $p<0.01$ vs. sham

Supplementary Table 2

Mean Arterial Pressures (MAP) for VFCA Brain Injury Experiments

group		BL1	BL2	CA6	RT5	RT10	RT15
CA24	Mean	81.50	86.25	5.50	67.50	63.75	53.75
	N	4	4	4	4	4	4
	Std. Deviation	11.358	9.394	2.082	9.292	18.945	8.995
	Std. Error of Mean	5.679	4.697	1.041	4.646	9.473	4.498
sham	Mean	74.25	87.00	89.75	93.25	90.50	87.75
	N	4	4	4	4	4	4
	Std. Deviation	2.630	12.649	7.676	9.179	10.083	10.340
	Std. Error of Mean	1.315	6.325	3.838	4.589	5.041	5.170

Continued Below

group		RT20	RT25	RT30	RT35	RT40	RT45	RT50	RT55	RT60
CA24	Mean	52.25	53.00	56.75	62.50	59.25	59.00	61.25	63.00	62.75
	N	4	4	4	4	4	4	4	4	4
	Std. Deviation	5.620	9.309	7.182	7.326	7.365	10.296	12.093	9.055	9.845
	Std. Error of Mean	2.810	4.655	3.591	3.663	3.683	5.148	6.047	4.528	4.922
sham	Mean	80.50	75.50	76.75	100.00	93.75	91.00	90.50	89.00	86.25
	N	4	4	4	4	4	4	4	4	4
	Std. Deviation	8.963	8.505	8.057	13.441	9.251	9.416	7.594	6.683	8.846
	Std. Error of Mean	4.481	4.252	4.029	6.721	4.626	4.708	3.797	3.342	4.423

a.VAR = MAP