



Supplementary Materials

# Sodium Tungstate Promotes Neurite Outgrowth and Confers Neuroprotection in Neuro2a and SH-SY5Y Cells

Nora Montero-Martin, María D. Girón \*, José D. Vilchez and Rafael Salto

Department of Biochemistry and Molecular Biology II, School of Pharmacy, University of Granada, E18071 Granada, Spain; nora84@ugr.es (N.M.-M.); damasovr@gmail.com (J.D.V.); rsalto@ugr.es (R.S.)

\* Correspondence: mgiron@ugr.es

## Electronic Supplementary Materials

- 1.- **Table S1.** *Oligonucleotides for qPCR used in this article.* Page 2
- 2.- **Table S2.** *Oligonucleotides used for the cloning and mutagenesis of rat MEF2D.* Page 2
- 3.- **Figure S1.** *Representative images of Na<sub>2</sub>WO<sub>4</sub> effects on neurite outgrowth in Neuro2a (a) and SH-SY5Y (b) cells.* Page 3
- 4.- **Figure S2** *Flow cytometry analysis of the cell cycle.* Page 4
5. **Figure S3** *Effects of Na<sub>2</sub>WO<sub>4</sub> effects on cell viability measured as LDH release.* Page 5
6. **Figure S4.** *Na<sub>2</sub>WO<sub>4</sub>-induced neurite outgrowth is mediated by PI3K/Akt and ERK1/2 signaling pathways in Neuro2a cells.* Page 5
7. **Figure S5.** *Na<sub>2</sub>WO<sub>4</sub> reverses the effects of advanced glycation end products (AGEs) on neurons.* Page 6
8. **Figure S6.** *Na<sub>2</sub>WO<sub>4</sub> normalizes the cell cycle on cells incubated with advanced glycation end products (AGEs) on neurons.* Page 7
9. **Figure S7.** *Na<sub>2</sub>WO<sub>4</sub> normalizes the procaspase 3 processing on cells incubated with advanced glycation end products (AGEs) on neurons.* Page 8

**Citation:** Montero-Martin, N.; Girón, M.D.; Vilchez, J.D.; Salto, R. Sodium Tungstate Promotes Neurite Outgrowth and Confers Neuroprotection in Neuro2a and SH-SY5Y Cells. *Int. J. Mol. Sci.* **2024**, *25*, 9150. <https://doi.org/10.3390/ijms25179150>

Academic Editors: Anna-Leena Sirén and Aleksey Zaitsev

Received: 11 July 2024

Revised: 13 August 2024

Accepted: 21 August 2024

Published: 26 August 2024



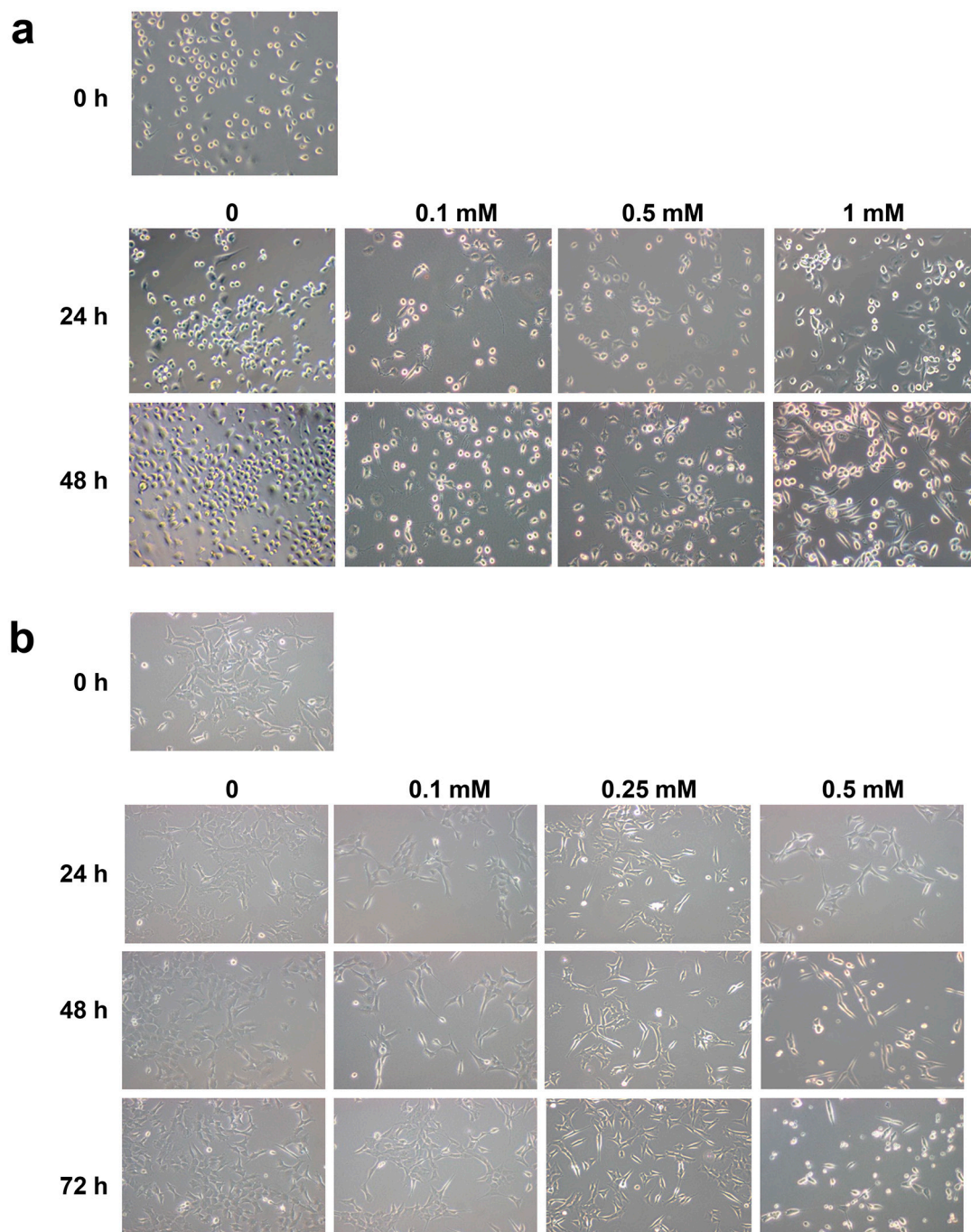
**Copyright:** © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Table S1.** *qPCR Oligonucleotides used for the quantitation of mRNA in this article.* The sequences of the forward and reverse oligonucleotides are shown.

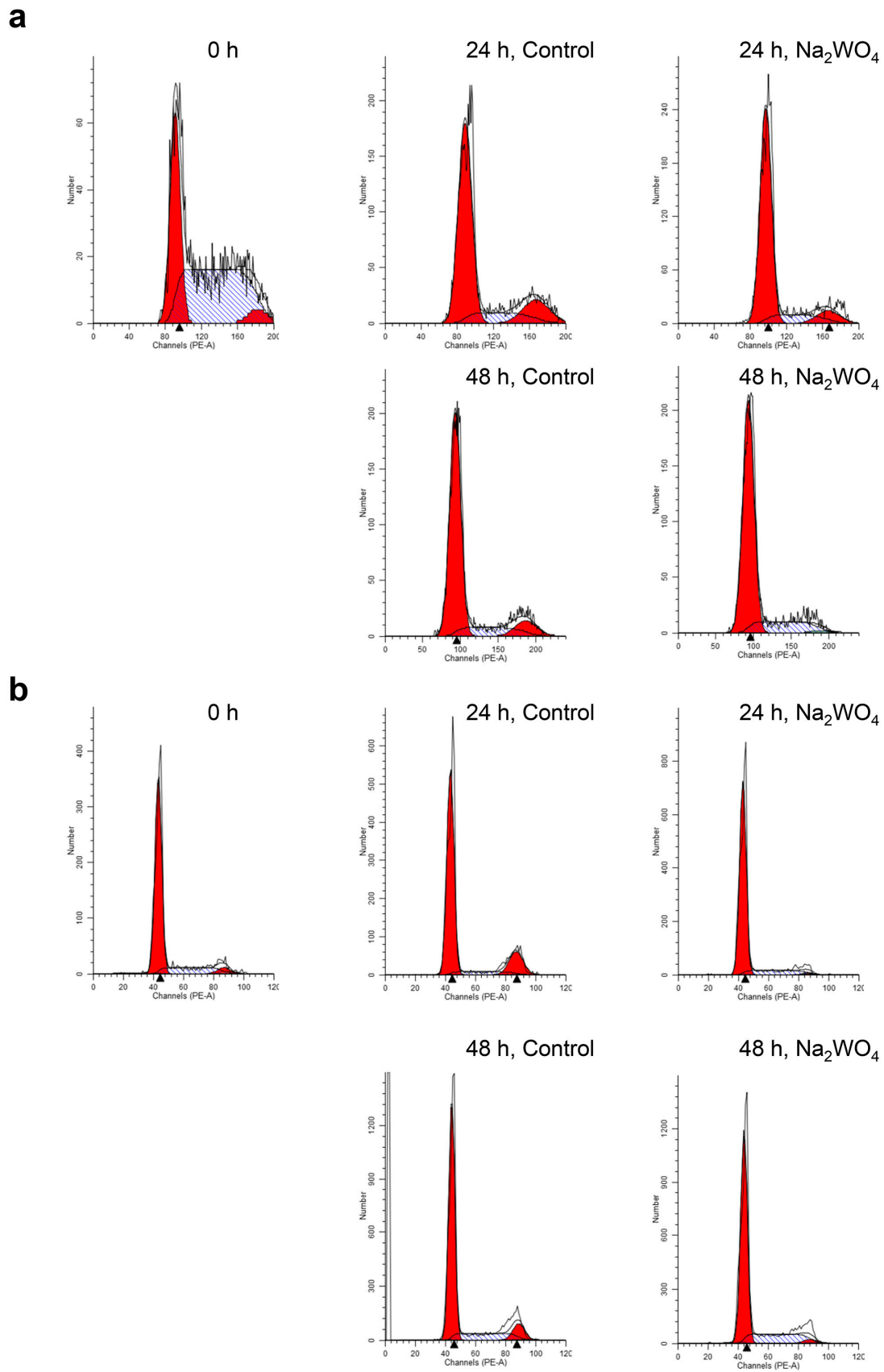
Gene	Specie	Name	Sequence
<i>Actin</i>	Mouse	FM1_Actb	5'-GATGTATGAAGGCTTTGGTC-3'
		RM1_Actb	5'-TGTGCACTTTTATTGGTCTC-3'
	Human	FH1_Actb	5'-GACGACATGGAGAAAATCTG-3'
		RH1_Actb	5'- <u>ATGATCTGGGTCATCTTCTC</u> 3'
<i>Choline O-acetyl transferase</i>	Mouse	FM1_Chat	5'-TCCTCTTAAAAGACTCCACC-3'
		RM1_Chat	5'-GACTTGTCATACCAACGATTC-3'
	Human	FH1_Chat	5'-TCAGTTCTTTGTCTTGGATG-3'
		BH1_Chat	5'-TGGAAGCCATTTTGACTATC-3'
<i>Nurr1</i>	Mouse	FM1_Nurr1	5'-CTATGGTCACAGAGAGACAC-3'
		RM1_Nurr1	5'-GCTTTGAGAACATGGACTAC-3'
	Human	FH1_Nr4a2	5'-GACTATCAAATGAGTGGAGATG-3'
		BH1_Nr4a2	5'-GACCTGTATGCTAATCGAAG-3'
<i>Tyrosine Hydroxylase</i>	Mouse	FM1_Th	5'-ATGGAAATGCTGTTCTCAAC-3'
		RM1_Th	5'-GTCTCTAAGTGGTGGATTTTG-3'
	Human	FH1_Th	5'-CAAAATCCACCATCTAGAGAC-3'
		BH1_Th	5'-CTGACACTTTTCTTGGGAAC-3'
<i>MEF2D</i>	Mouse	FM1_Mef2d	5'-ATGAACTGATCACTAGTCCC-3'
		RM1_Mef2d	5'-CCTTCTTCATCAGTCCAAAC-3'

**Table S2.** *Oligonucleotides used for the cloning and mutagenesis of rat MEF2D.* The sequences of the forward and reverse oligonucleotides are shown. Underlined nucleotides correspond to introduced mutations or new restriction sites.

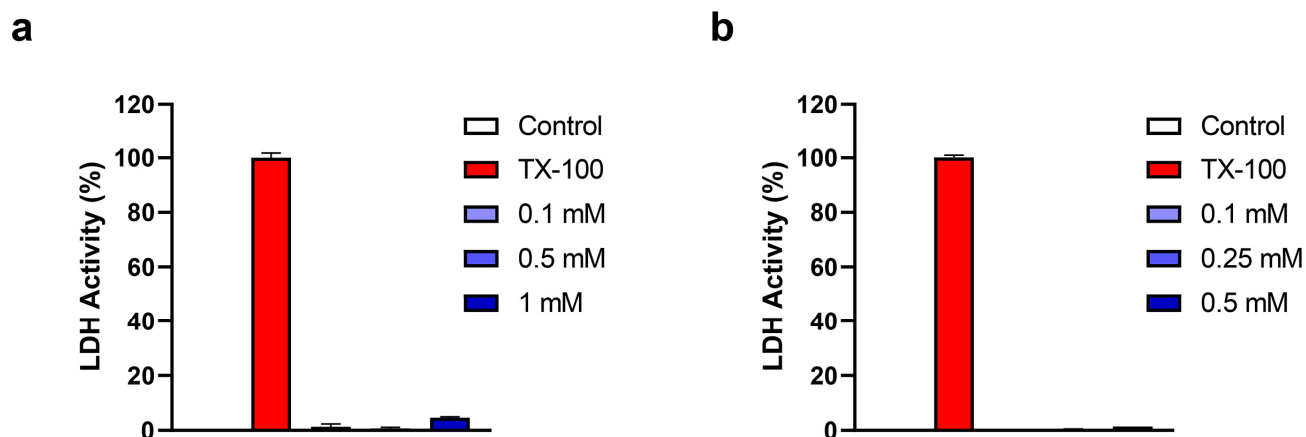
Name	Sequence	Site
MEF2Df	5'- <u>AGATCTAT</u> TGGGGAGGAAAAAGATTTCAG-3'	<i>Bgl</i> II
MEF2Dr	5'- <u>GTCGACT</u> CACCTTTAATGTCCAAGTATCC-3'	<i>Sal</i> I
MEF2D K439Rf	5'CCCCACATAAGCATCAGATCTGAACCAGTGTCCCCAAGTCG-3'	<i>Bgl</i> II
MEF2D K439Rr	5'-CGACTTGGGGACACTGGTTCA <u>GATCT</u> GATGCTTATGTGGGGG-3'	<i>Bgl</i> II
Mef2D S444Af	5'-GCATCAAGTCAGAACCAAGTGGCCCCGAGTCGTGAACGCAGCCCTGCACC-3'	<i>Ava</i> I
MEF2D S44Ar	5'-GGTGCAGGGCTGCGTTCACGACTCGGGG <u>C</u> CACTGGTTCTGACTTGATGC-3'	<i>Ava</i> I



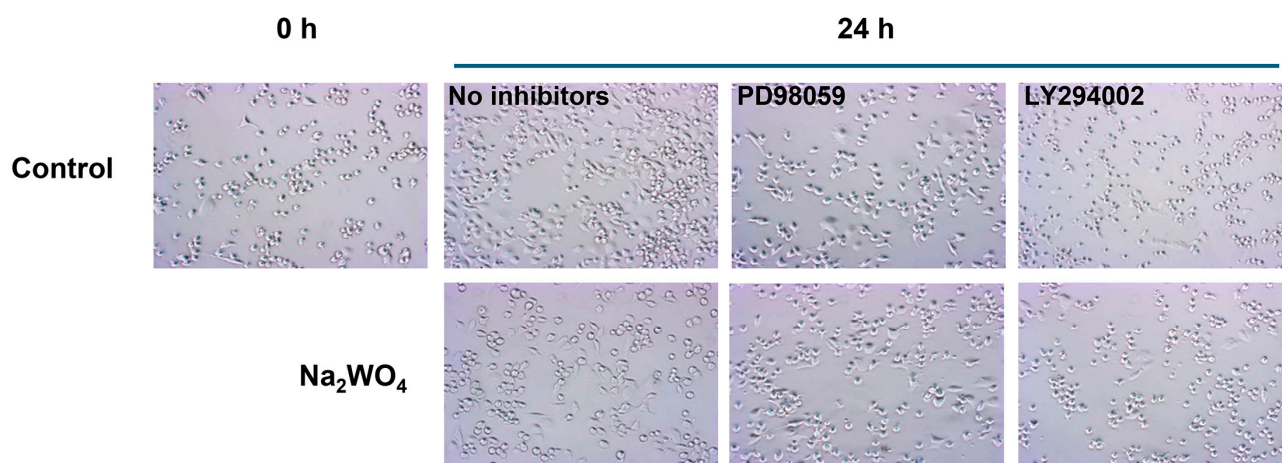
**Figure S1.** Representative images of  $\text{Na}_2\text{WO}_4$  effects on neurite outgrowth in Neuro2a (a) and SH-SY5Y (b) cells. Neuro2a (a) and SH-SY5Y (b) cells were incubated with different concentrations of  $\text{Na}_2\text{WO}_4$  and neurite outgrowth was determined by visible microscopy.



**Figure S2.** Flow cytometry analysis of the cell cycle. Fluorescence histograms of Neuro2a (**a**) and SH-SY5Y (**b**) cells incubated in the absence or presence of Na<sub>2</sub>WO<sub>4</sub> (1 mM for Neuro2a and 0.25 mM for SH-SY5Y) at 48 h.

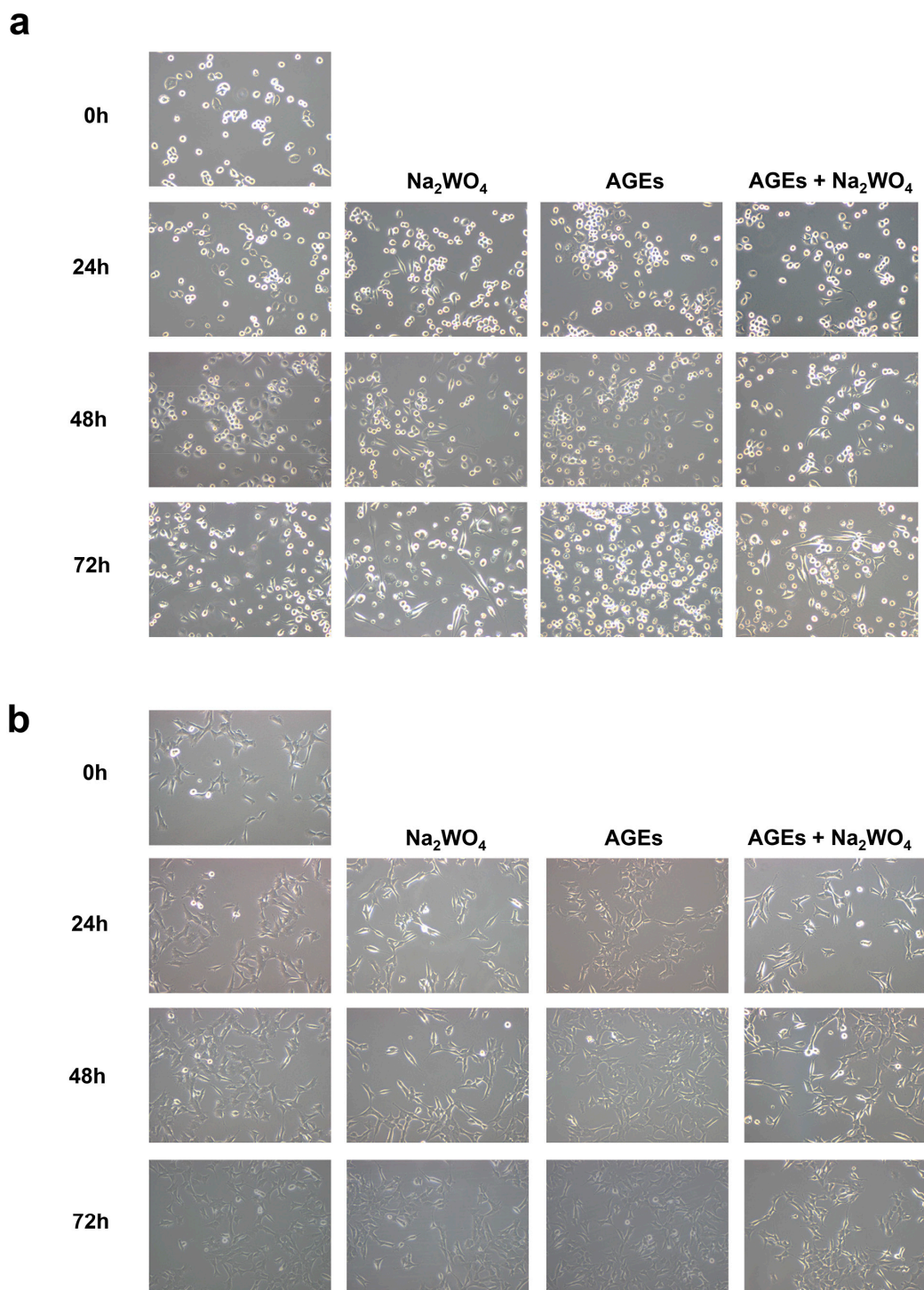


**Figure S3.** Effects of  $\text{Na}_2\text{WO}_4$  effects on cell viability measured as the LDH release. Neuro2a (a) and SH-SY5Y (b) cells were incubated in the absence or presence of  $\text{Na}_2\text{WO}_4$  (48 h for Neuro2a and 0.25 mM for SH-SY5Y) at 48 h.

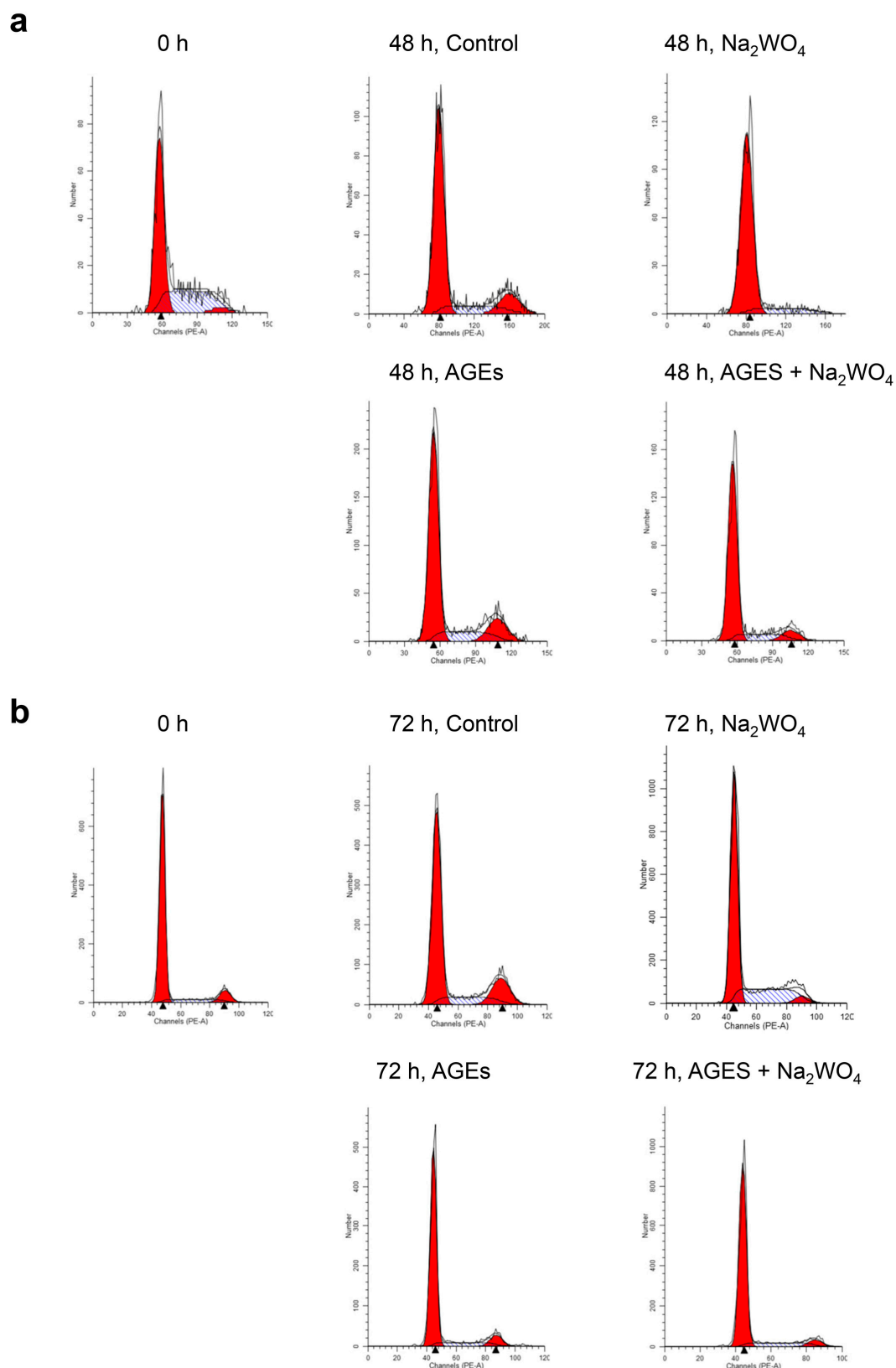


**Figure S4.**  $\text{Na}_2\text{WO}_4$ -induced neurite outgrowth is mediated by PI3K/Akt and ERK1/2 signaling pathways in Neuro2a cells. Neuro2a cells were pre-treated with PD98059 10  $\mu\text{M}$  or LY294002 20  $\mu\text{M}$  and then treated with 1 mM  $\text{Na}_2\text{WO}_4$  for 24 h (a) or 30 min (b). Inhibitors were maintained during the experiment. The percentage of differentiated cells was determined by analysis of the cell morphology.

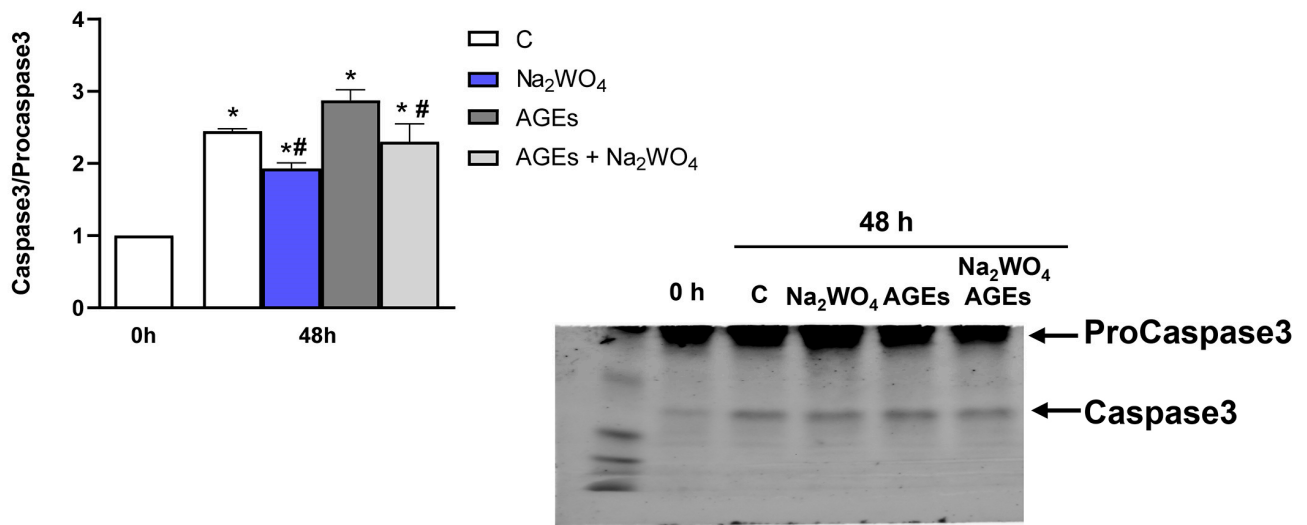




**Figure S5.**  $\text{Na}_2\text{WO}_4$  reverses the effects of advanced glycation end products (AGEs) on neurons. Neuro 2a and SH-SY5Y cells were incubated with 100  $\mu\text{g}/\text{mL}$  AGEs in the absence or presence of  $\text{Na}_2\text{WO}_4$ . **(a)** Cells with neurite outgrowth in Neuro 2a **(a)** or SH-SY5Y cells **(b)** incubated with the effectors for 72 hours. The percentage was determined by analysis of the cell morphology. **(b)** Fluorescence histograms of Neuro2a and SH-SY5Y cells incubated with AGEs in the absence or presence of  $\text{Na}_2\text{WO}_4$  (1 mM for Neuro2a and 0.25 mM for SH-SY5Y).



**Figure S6.** Na<sub>2</sub>WO<sub>4</sub> normalizes the cell cycle on cells incubated with advanced glycation end products (AGEs) on neurons. Neuro2a and SH-SY5Y cells were incubated with 100 µg/mL AGEs in the absence or presence of Na<sub>2</sub>WO<sub>4</sub>. Fluorescence histograms of Neuro2a (**a**) and SH-SY5Y cells (**b**) incubated with AGEs in the absence or presence of Na<sub>2</sub>WO<sub>4</sub> (1 mM for Neuro2a and 0.25 mM for SH-SY5Y).



**Figure S7.** Na<sub>2</sub>WO<sub>4</sub> normalizes the procaspase 3 processing on cells incubated with advanced glycation end products (AGEs) on neurons. Neuro2a cells were incubated with 100 µg/mL AGEs in the absence or presence of Na<sub>2</sub>WO<sub>4</sub> (1 mM). Procaspase and caspase 3 were analyzed by Western blot using antibodies that recognize both forms (Cell Signalling). Results represent means ± SEM (n = 4). \*p<0.05 vs untreated cells; #p<0.05 vs AGEs treated cells.