



Supplementary Materials

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

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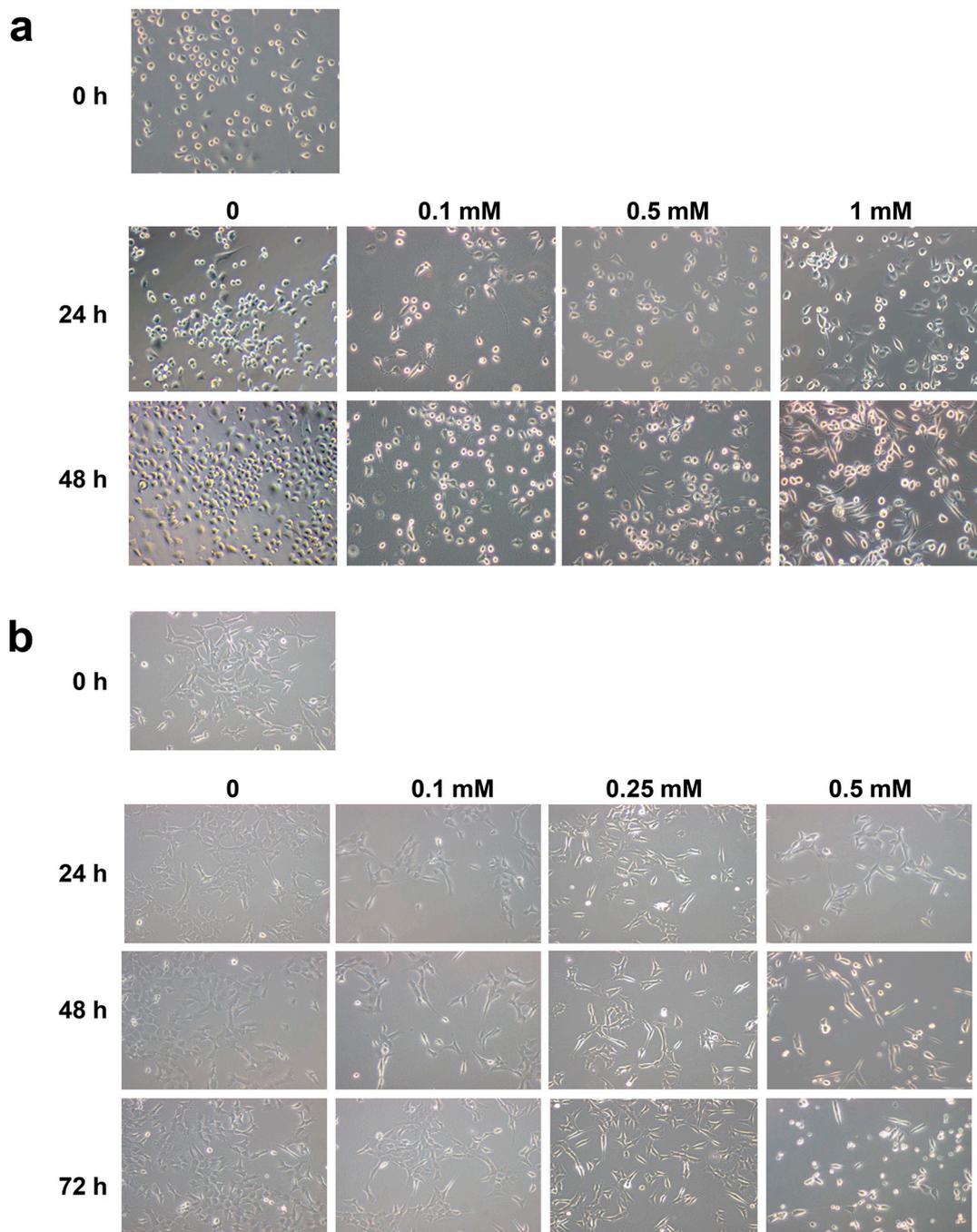
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**Table S1.** qPCR Oligonucleotides used for the quantitation of mRNA in this article. The sequences of the forward and reverse oligonucleotides are shown.

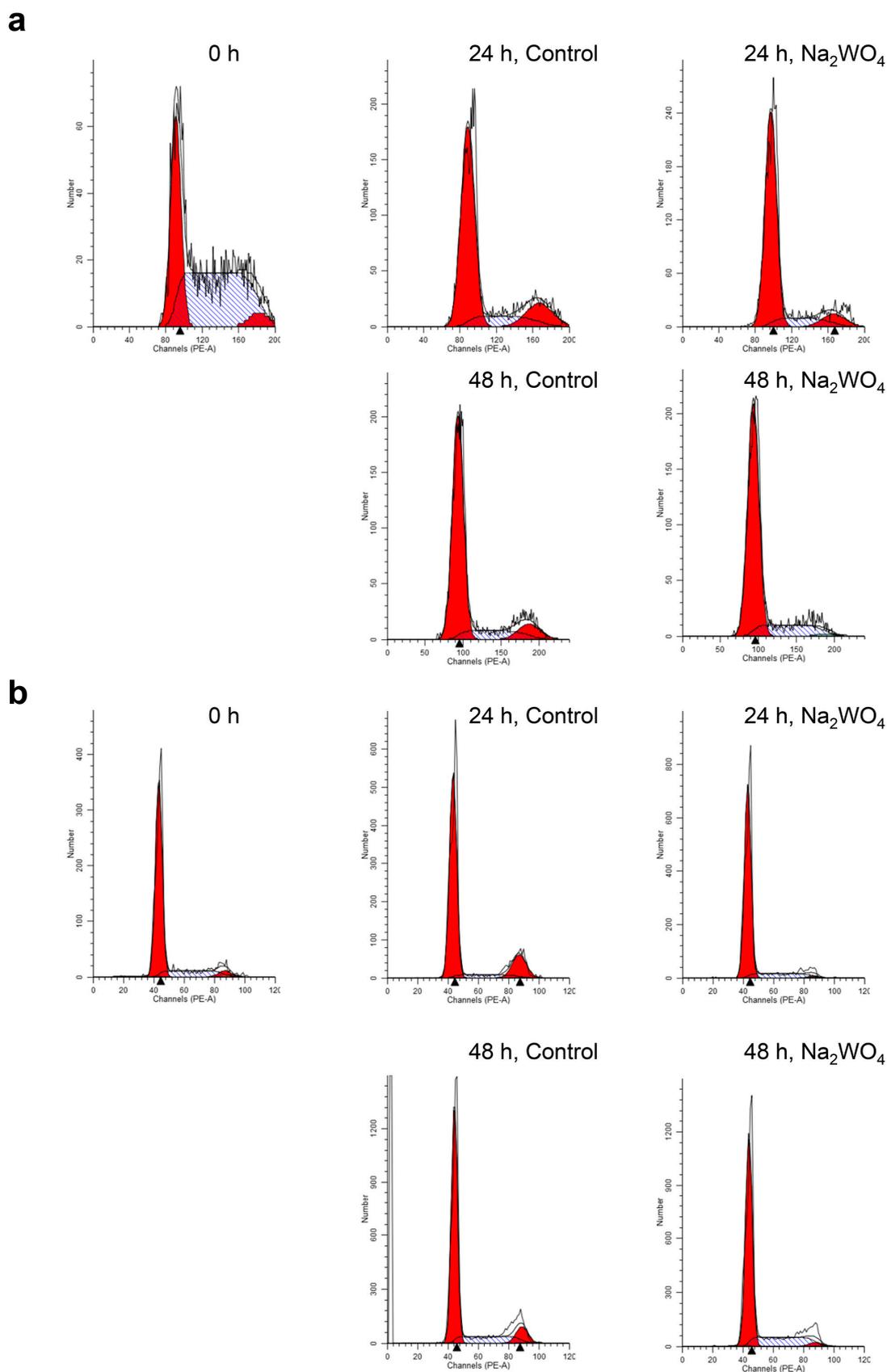
Gene	Specie	Name	Sequence
<i>Actin</i>	Mouse	FM1_Actb	5'-GATGTATGAAGGCTTTGGTC-3'
		RM1_Actb	5'-TGTGCACTTTTATTGGTCTC-3'
	Human	FH1_Actb	5'-GACGACATGGAGAAAATCTG-3'
		RH1_Actb	5'- <u>ATGATCTGGGTCATCTTCTC</u> 3'
<i>Choline O-acetyl transferase</i>	Mouse	FM1_Chat	5'-TCCTCTTAAAAGACTCCACC-3'
		RM1_Chat	5'-GACTTGTCATACCAACGATTC-3'
	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> CACTTTAATGTCCAAGTATCC-3'	<i>Sal</i> I
MEF2D K439Rf	5'CCCCACATAAGCATCAGATC <u>T</u> G <u>A</u> ACCAGTGTCCCCAAGTCG-3'	<i>Bgl</i> II
MEF2D K439Rr	5'-CGACTTGGGGACACTGGTTC <u>A</u> GATCIGATGCTTATGTGGGG-3'	<i>Bgl</i> II
Mef2D S444Af	5'-GCATCAAGTCAGAACCAAGTGG <u>C</u> CCCCGAGTCGTGAACGCAGCCCTGCACC-3'	<i>Ava</i> I
MEF2D S44Ar	5'-GGTGCAGGGCTGCGTTCACGACT <u>C</u> GGGG <u>C</u> CACTGGTTCTGACTTGATGC-3'	<i>Ava</i> I

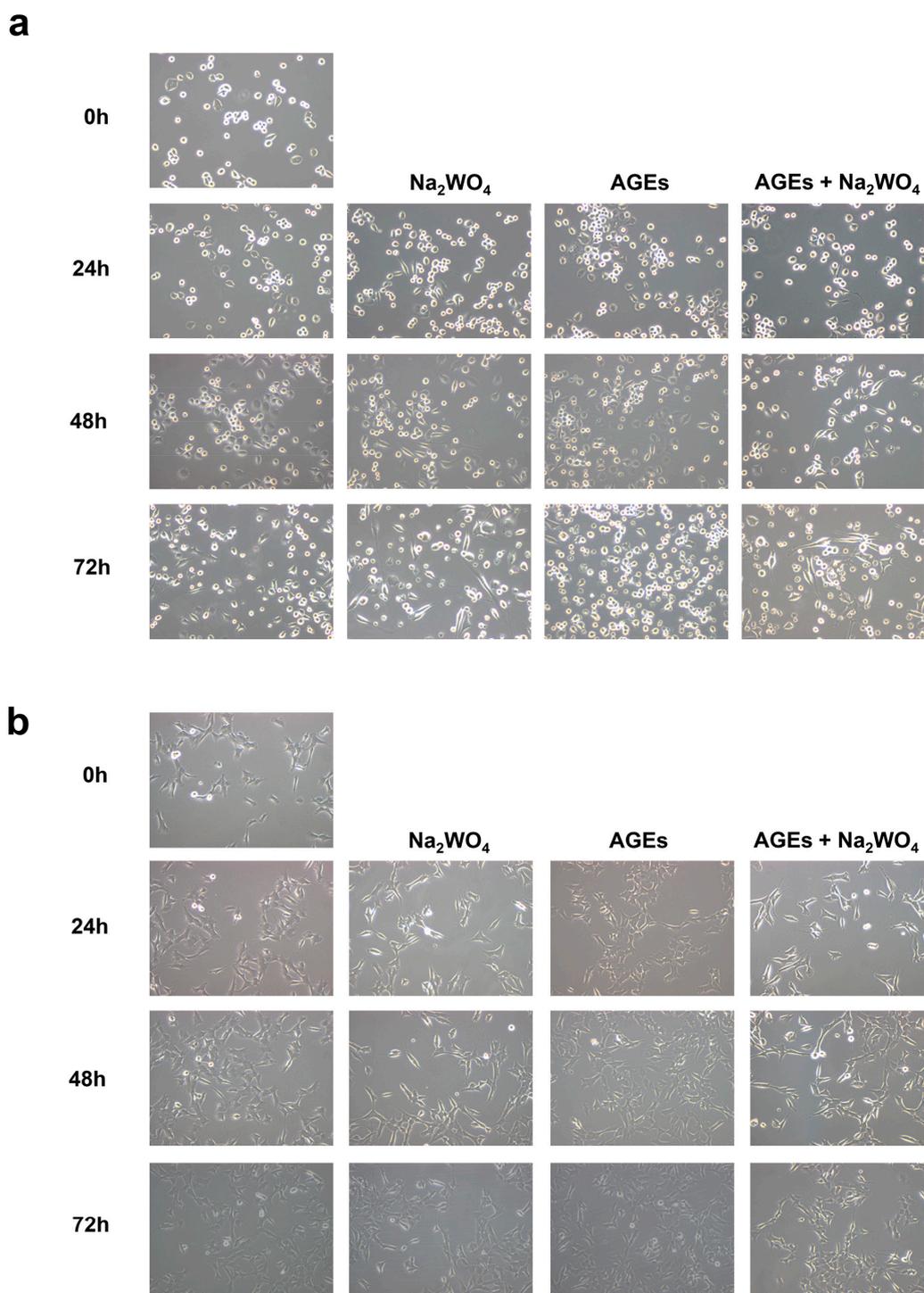


**Figure S1.** Representative images of  $\text{Na}_2\text{WO}_4$  effects on neurite outgrowth in Neuro2a (a) and SH-SY5Y (b) cells. Neuro2a (a) and SH-SY5Y (b) cells were incubated with different concentrations of  $\text{Na}_2\text{WO}_4$  and neurite outgrowth was determined by visible microscopy.

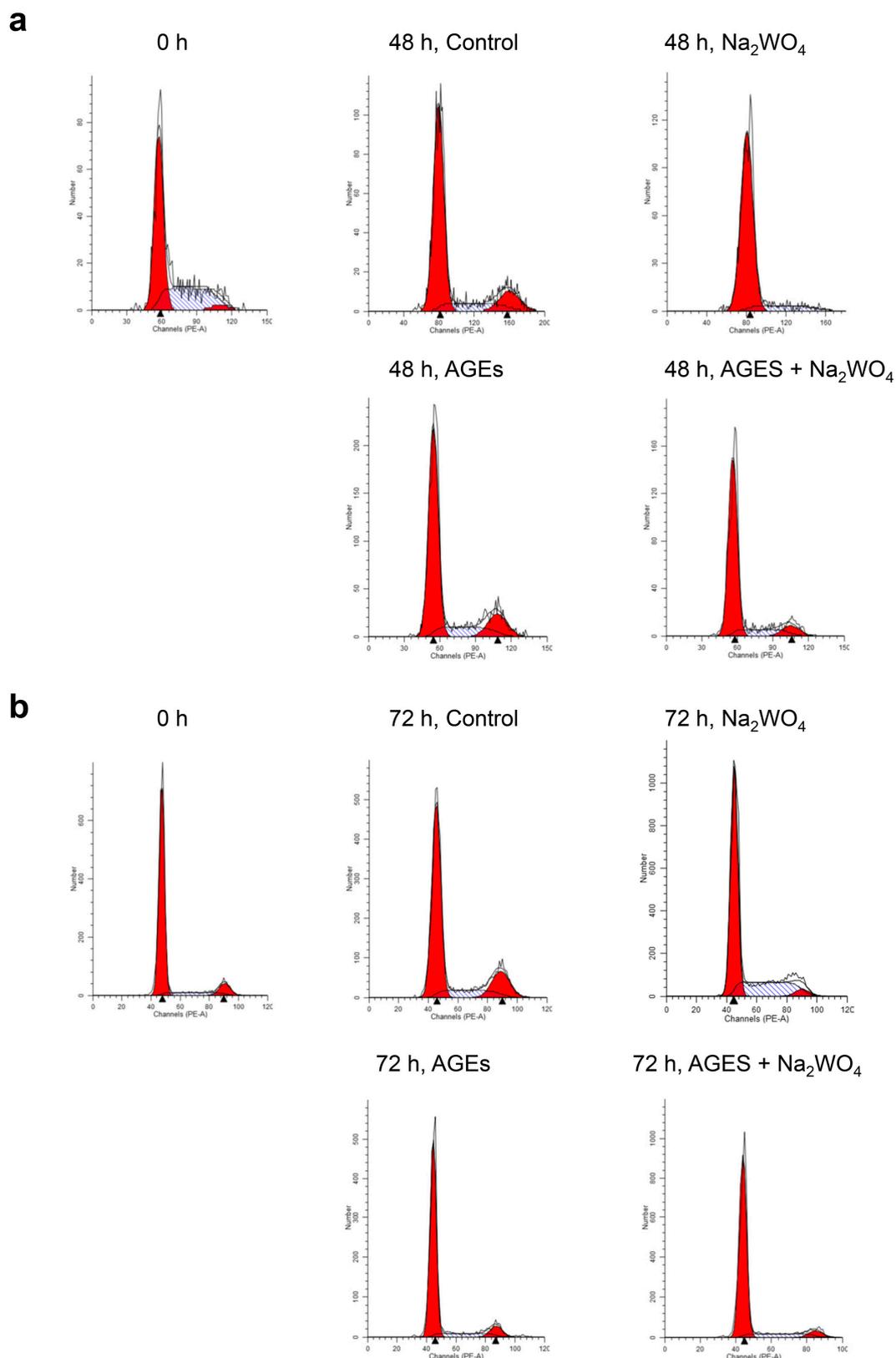


**Figure S2.** Flow cytometry analysis of the cell cycle. Fluorescence histograms of Neuro2a (a) and SH-SY5Y (b) cells incubated in the absence or presence of Na<sub>2</sub>WO<sub>4</sub> (1 mM for Neuro2a and 0.25 mM for SH-SY5Y) at 48 h.

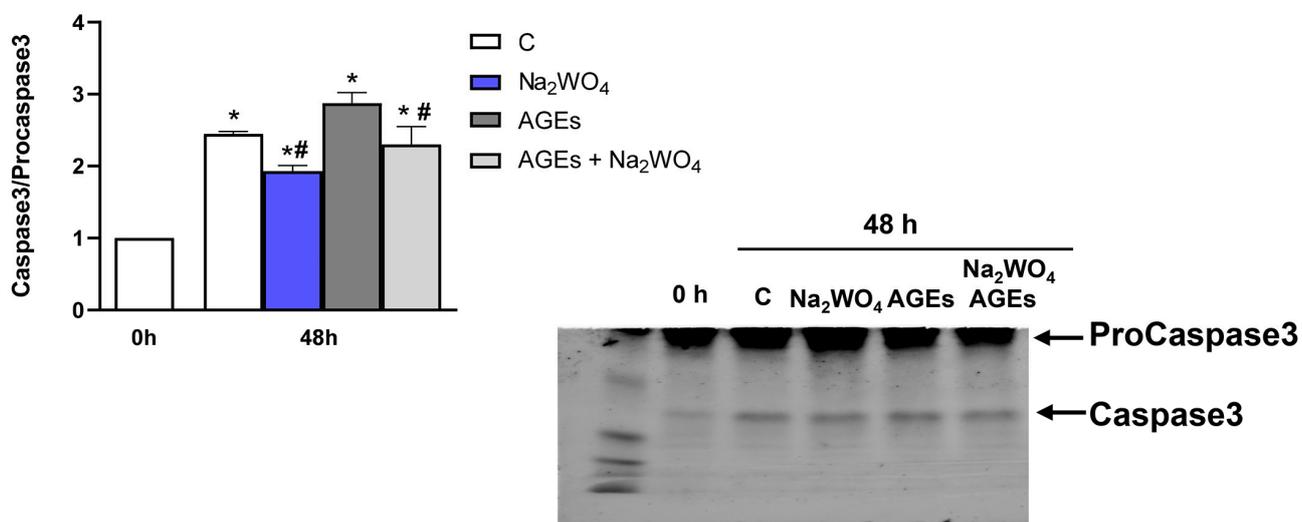




**Figure S5.**  $\text{Na}_2\text{WO}_4$  reverses the effects of advanced glycation end products (AGEs) on neurons. Neuro 2a and SH-SY5Y cells were incubated with 100  $\mu\text{g}/\text{mL}$  AGEs in the absence or presence of  $\text{Na}_2\text{WO}_4$ . **(a)** Cells with neurite outgrowth in Neuro 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.