cancers

MDPI

*Article*

# Mambalgin-2 induces cell cycle arrest and apoptosis in glioma cells via interaction with ASIC1a

**Supplementary material:**

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**Table S1.** Primers, used for qPCR.

| Gene | Primer | | Amplicon Size, bp |
|---|---|---|---|
| | Forward | Reverse | |
| *β-actin* | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT | 88 |
| *GPDH* | ACAACTTTGGTATCGTGGAAGG | GCCATCACGCCACAGTTTC | 73 |
| *RPL13a* | TCAAAGCCTTCGCTAGTCTCC | GGCTCTTTTTGCCCGTATGC | 104 |
| *ASIC1a* | CGAAGCAGGCATCAAAGTGC | TTTGGATGATAGGGAGCCACG | 642 |
| *ASIC2* | CACCAAGACTTCACCACAGTGTTT | TGTAGCGGGTCTCACAGTCA | 409 |
| *ASIC3* | TACAAGAACTGTGCCCACCC | GGTCTTCGGAACAGAGCAGA | 502 |
| *ASIC4* | GAGGAGAGAGACAAGCGGCA | GTCCAGCATGATCTCCAGGC | 930 |
| *α-ENaC* | CCAGGCCGCTGCACCT | GCCGATCTTCCAGTCCTTCC | 750 |
| *γ-ENaC* | GAGTGACGTGCCAATCAGGA | TCTCCGAAACCACAGATGGC | 305 |

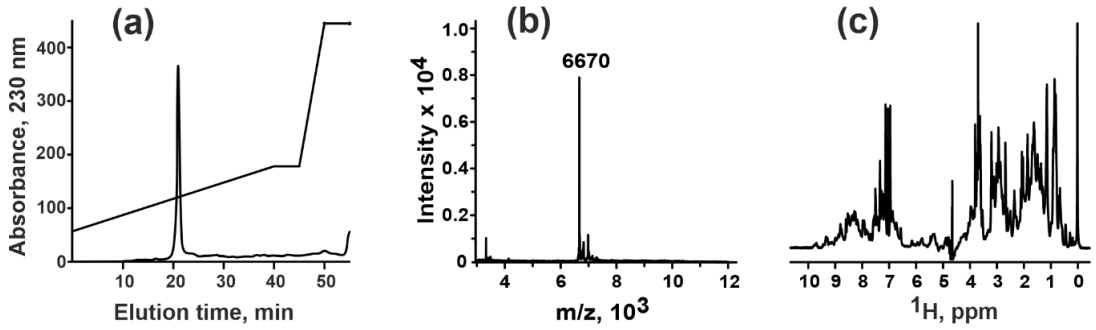


**Figure S1.** Characterization of the refolded mambalgin-2: (a) HPLC analysis of mambalgin-2 homogeneity and purity; (b) Mass-spectrometry analysis of mambalgin-2; (c) $^{1}$H-NMR spectra of mambalgin-2.

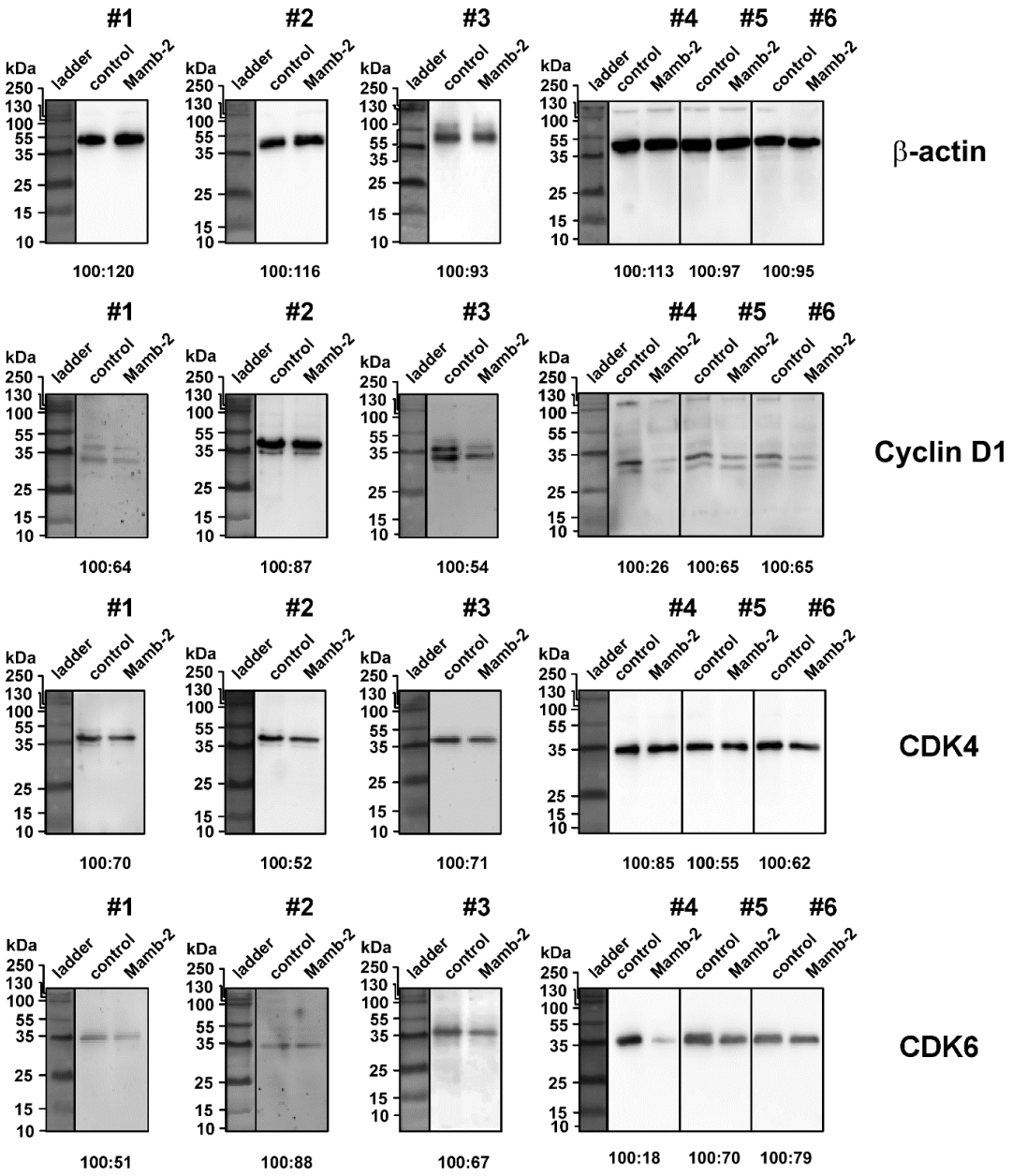


**Figure S2.** Western blots from 6 independent portions of U251MG cells, showing the mambalgin-2 influence on phosphorylation of Cyclin D1 (pSer90), CDK4 (pThr172), and CDK6 (pTyr24) expression. Cells were incubated with 1 μM mambalgin-2 or 0,1% DMSO for 72 h (see methods) and protein phosphorylation/expression was analyzed by western blot. Cell portions of cells are shown as #1-#6. Portions #1-#3 were analyzed on separate nitrocellulose membranes, portions #4-6 were analyzed on the same membrane. Protein ladder (Thermo Fisher, 26619) from optical channel is shown on the left of each membrane. Optical density ratio of the protein bands corresponded to the untreated cells (control) and mambalgin-2 treated cells (Mamb-2) is presented below the lanes.

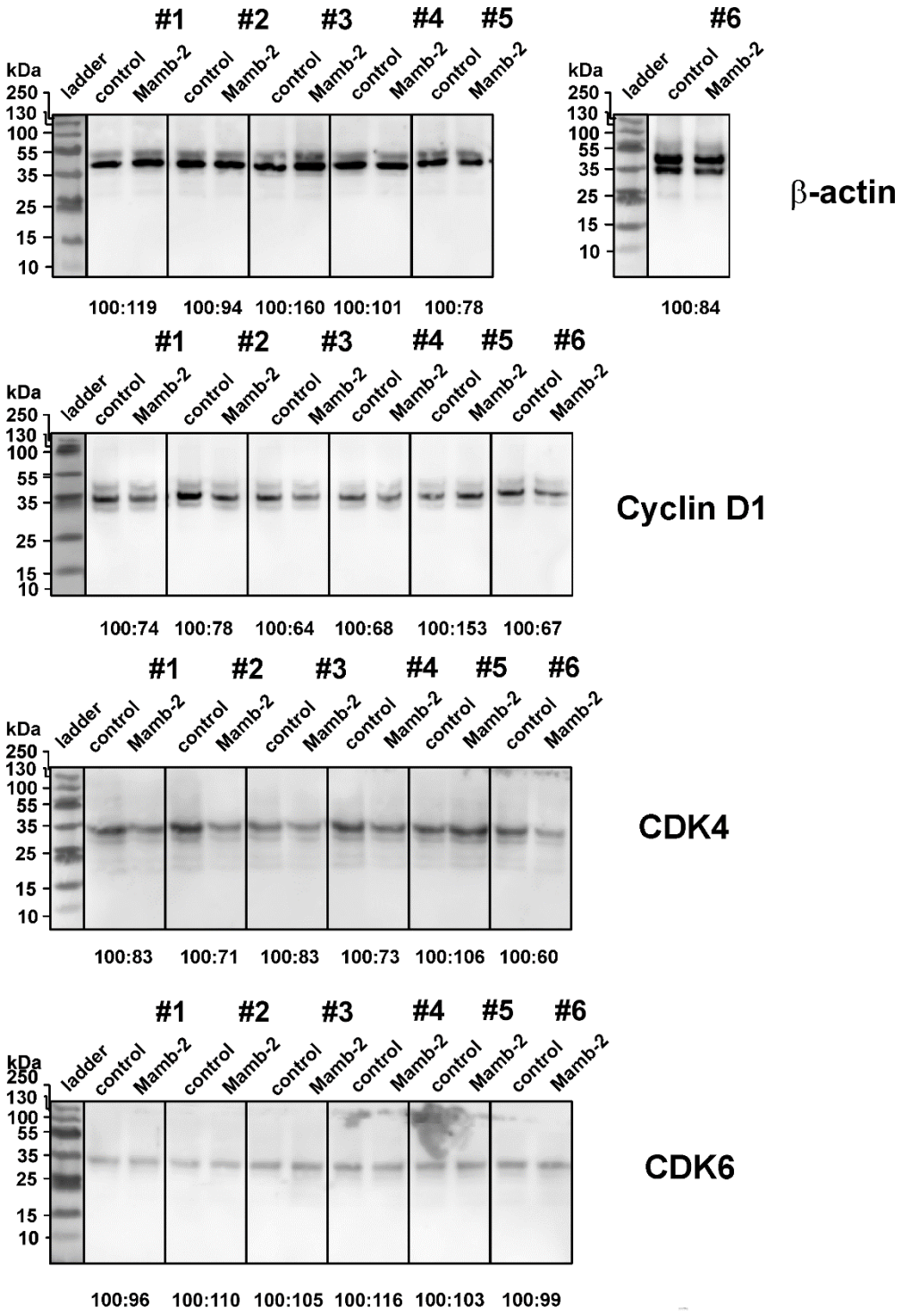


**Figure S3.** Western blots from 6 independent portions of A172 cells, showing the mambalgin-2 influence on phosphorylation of Cyclin D1 (pSer90), CDK4 (pThr172), and CDK6 (pTyr24) expression. Cells were incubated with 1 μM mambalgin-2 or 0,1% DMSO for 72 h (see methods) and protein phosphorylation/expression was analyzed by western blot. Cell portions of cells are shown as #1-#6 and were analyzed on the same membrane. Protein ladder (Thermo Fisher, 26619) from optical channel is shown on the left of each membrane. Optical density ratio of the protein bands corresponded to the untreated cells (control) and mambalgin-2 treated cells (Mamb-2) is presented below the lanes.