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Supplemental Material to:

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Wip1 phosphatase positively modulates dendritic spine morphology and memory processes through the p38MAPK signaling pathway.

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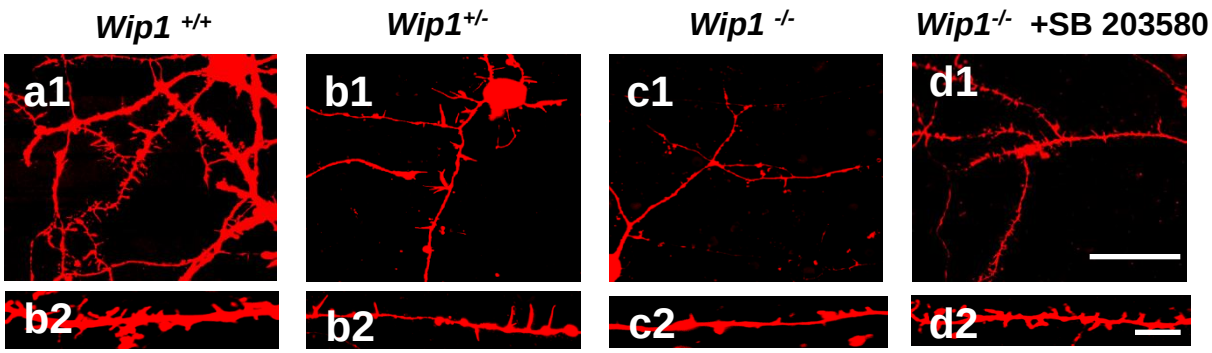
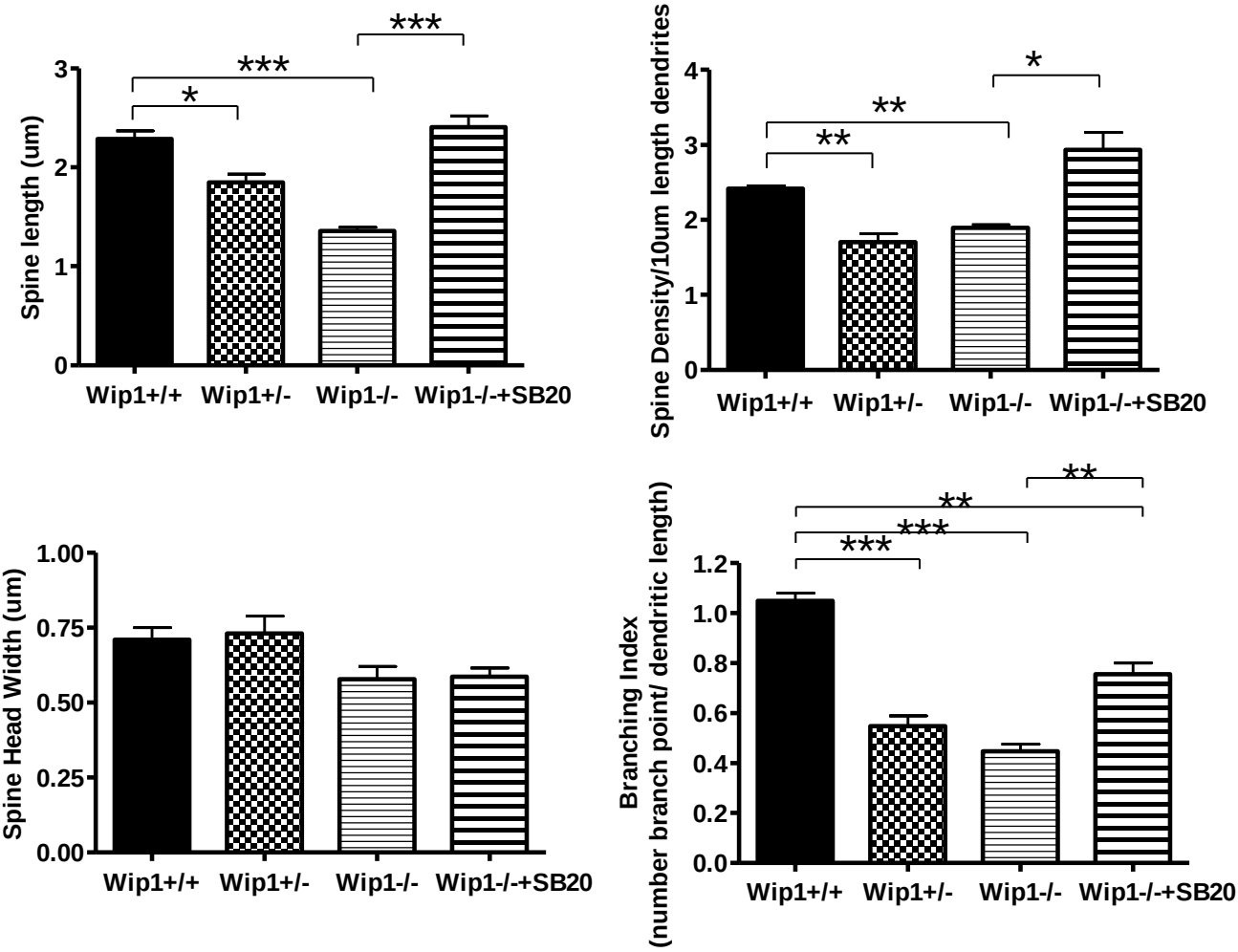
A

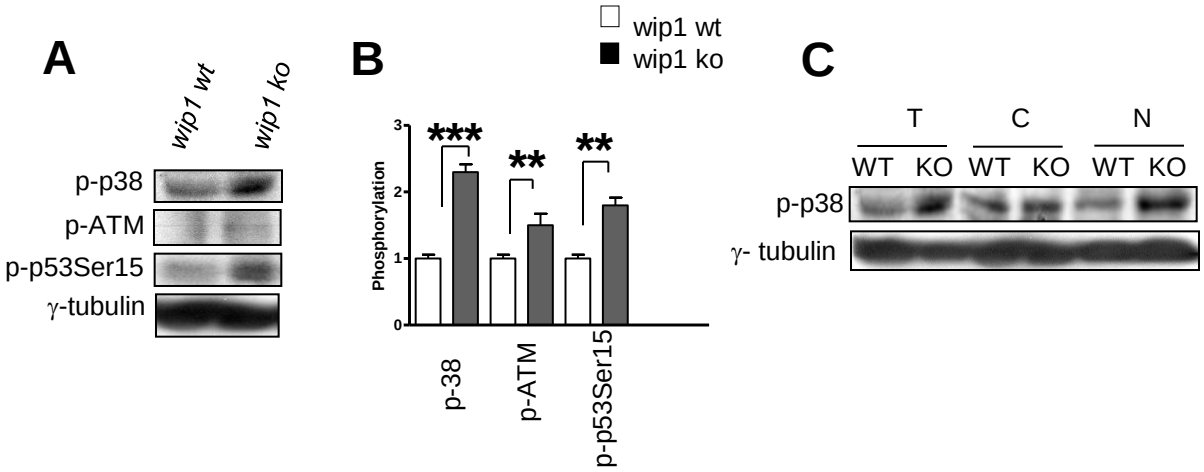


B



Supplementary Figure 1

Figure S1. Dendritic morphology analysis in cultured primary hippocampal neurons. (A) Rhodamine-Phalloidin staining of cultured primary hippocampal neurons treated with and without SB20. Images of dendrites from *wip1*$^{+/+}$ (a1), *wip1*$^{+/-}$ (a1), *wip1*$^{-/-}$ (a1), *wip1*$^{-/-}$ (treated with SB20) (a1) hippocampal neurons. Lower panels represent the magnification of fragments of dendrites (b_1). Lower panels represent the magnification of fragments of dendrites for each condition (a2, b2, c2, d2). Bars in series a: 20 μm and series b: 10 μm. (B) Confocal analysis of the cultured primary hippocampal neurons treated with and without p38MAPK inhibitor, SB203580 (SB20). Quantification of number of average dendrite length, spine head width, spine density/10 μm length dendrites and branching index in neurons from *wip1*$^{+/+}$, *wip1*$^{+/-}$, *wip1*$^{-/-}$ and *wip1*$^{-/-}$ (treated with SB20) mice. The measurement of the spine length, spine density and branching index in cells from *wip1*$^{+/+}$ mice (2.23 ± 0.15 μm, 2.4 ± 0.8 μm and 1.05 ± 0.05 μm, respectively) shows a significant decline compared with these indexes in cells from *wip1*$^{+/-}$ mice (1.84 ± 0.16 μm, 1.7 ± 0.2 μm and 0.54 ± 0.07 μm, respectively; p = 0.02, p = 0.0043, p = 0.0006 respectively) and *wip1*$^{-/-}$ mice (1.35 ± 0.06μm, 1.9 ± 0.07 μm and 0.44 ± 0.05 μm, respectively; p = 0.005, p = 0.009, p = 0.0001 respectively) hippocampal cells.



Supplementary Figure 2

Figure S2. Expression of p38MAPK, ATM, and p53 proteins in hippocampus. (A) Western blot analysis of protein extracts from the hippocampus of adult *wip1wt* and *wip1ko* mice. Antibodies used were directed against p-p38, wip1, phosphorylated-ATM Ser1981 (p-ATM Ser1981), phosphorylated-p53Ser15 (p-p53Ser15) and γ-tubulin (as loading control; A). The protein bands were quantified and compared from four independent experiments (B). **: $p < 0.01$. *: $p < 0.05$. Total (T), nuclear (N) and cytoplasmic (C) protein extracts of from the hippocampus of adult *wip wt* and *wip ko* mice were subjected to western blotting using antibodies against phosphorylated-p38 and γ-tubulin (as loading control; C).

Supplementary Methods

Immunofluorescence staining on cultured cells

The hippocampi of E18 d old mouse embryos were dissected and digested in papain containing HBSS buffer. Dissociated cells were plated on poly-l-lysine and lamine-coated 4-well dishes at the density of 5×10^4 per well in the neuronal basal culture medium containing B27 and L-glutamine supplement (Gibco, Invitrogen, Singapore). After 7 d in culture, cells were fixed with 2% paraformaldehyde in phosphate buffer for at least 2 h at room temperature. The staining protocol was the same as described above for the tissue sections with the exception that there was no cold acetone fixation. After staining, all sections and cultured neurons were imaged with a laser scanning confocal microscope (LSM510 META, Zeiss, Singapore). For low density culture of hippocampus neurons in 4 well dishes, cells were plated at a density of $(0.5–1) \times 10^3$ per well.