# Supplementary Materials

# Willin/FRMD6 influences mechanical phenotype and neuronal differentiation in mammalian cells by regulating ERK1/2 activity

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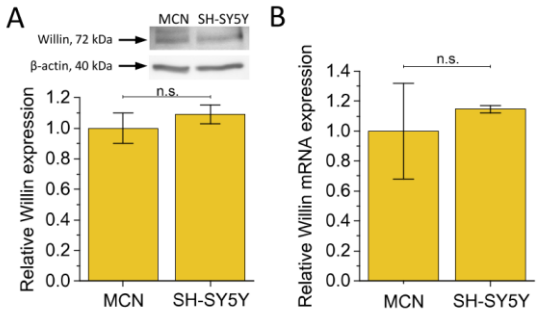
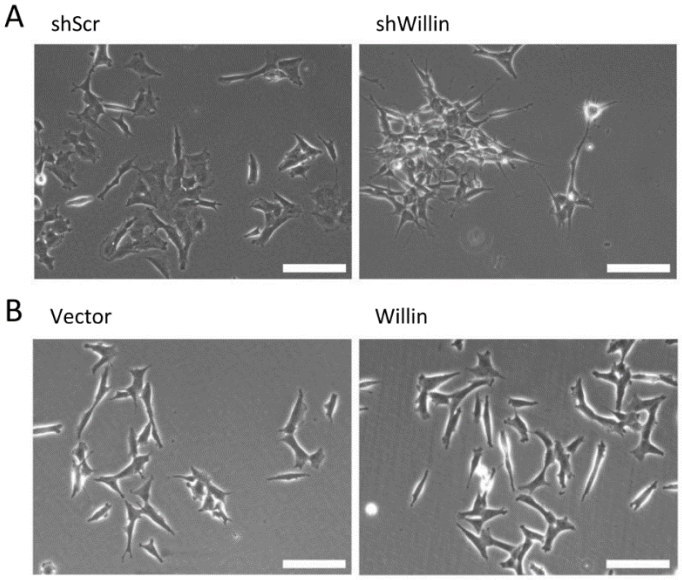
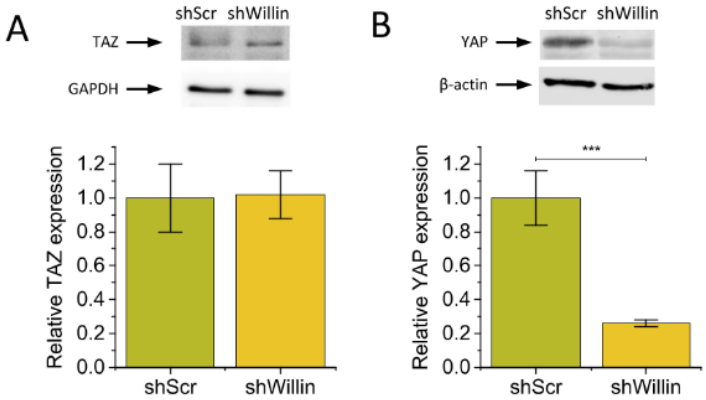
**Content:**



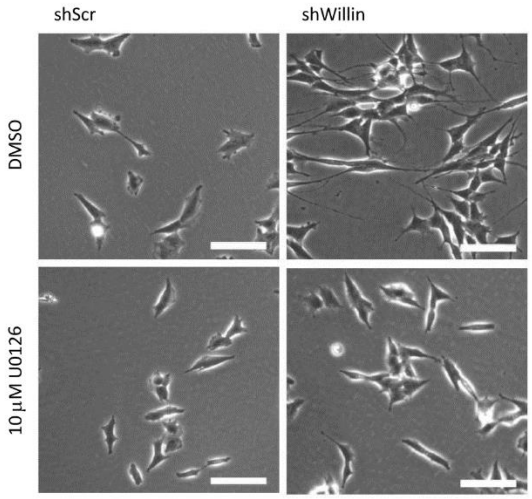

**Supplementary Figure 1 |** SH-SY5Y cells and primary mouse cortical neurons exhibit identical Willin/FRMD6 expression levels. **(A)** Quantitative Western blot analysis of Willin/FRMD6 expression in primary mouse cortical neurons (MCN) and SH-SY5Y cells. Means and SEM (error bars) were calculated from three independent experiments. **(B)** qPCR analysis of Willin/FRMD6 mRNA expression in primary mouse cortical neurons (MCN) and SH-SY5Y cells. Means and SEM (error bars) were calculated from two independent experiments, each of which was conducted in triplicates. Groups were compared using Student's *t*-test; n.s.: $p > 0.05$.



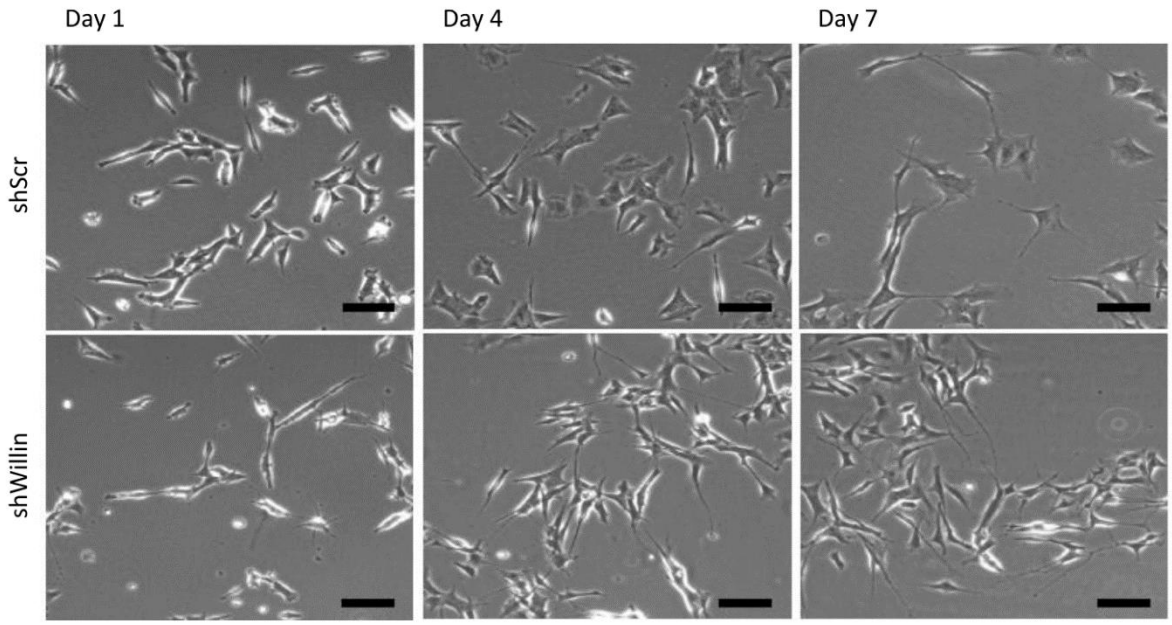

**Supplementary Figure 2 |** Willin regulates the morphology of SH-SY5Y cells. (A) Representative bright field images of *shScr* and *shWillin* cells grown in standard plastic cell culture dishes demonstrating the effect of Willin depletion on cellular morphology. Neurite-like extensions are observed for *shWillin* cells. (B) Representative bright field images of *Vector* and *Willin* cells grown in standard plastic cell culture dishes demonstrating that the overexpression of Willin has no effect on the morphology of SH-SY5Y cells. Scale bars: 20 µm.




**Supplementary Figure 3 |** Knockdown of Willin/FRMD6 does not affect total TAZ but reduces total YAP expression in SH-SY5Y cells. Quantitative Western blot analysis of **(A)** TAZ and **(B)** YAP expression in *shScr* and *shWillin* cells. Means were calculated from three technical repeats. Error bars represent ±SEM. Groups were compared using Student's *t*-test; ***: $p \leq 0.001$.




**Supplementary Figure 4 |** Blocking ERK1/2 activation via U0126-mediated MEK inhibition reduced the formation of neurite-like extensions in Willin/FRMD6 knock-down SH-SY5Y cells. Representative bright field images of *shScr* and *shWillin* cells grown in standard plastic cell culture dishes that were treated with DMSO or 10 µM U0126 for 24 hours. Scale bars: 20 µm.




**Supplementary Figure 5 |** RA-induced differentiation of SH-SY5Y cells. Representative bright field images of *shScr* and *shWillin* cells grown in standard plastic cell culture dishes before treatment with RA and after 4 and 7 days of treatment with 10 μM RA. RA treatment of *shWillin* cells increases the number of cells undergoing differentiation compared to *shScr* cells. Differentiated cells were defined as cells with neurites that were longer than 40 μm (see Methods and Materials). Scale bars: 20 μm.

**Supplementary Table 1 |** Primers used for qPCR analysis.

| Oligo | Sequence (5'-3') |
|---|---|
| hWillin FW | TGAAAACCTGCAGCTCAATG |
| hWillin RV | CTCTGGCCACGAAGCTTAAC |
| mWillin Fw | CGGCAATACGAAGTCACTTGGG |
| mWillin RV | TGCAATTCGGTCACTGATCAGC |
| HNeuroD1 FW p1 | GCGGCCCCAAAAAGAAGAAG |
| HNeuroD1 FW p2 | AGCCCTCTGACTGATTGCAC |
| HNeuroD1 RV p1 | TCCGACAGAGCCCAGATGTA |
| HNeuroD1 RV p2 | GTCTATGGGGATCTCGCAGC |