Supplementary section

UV-vis measurements set up

We analyzed the interaction between calmodulin (CaM, 4 $\mu$M) and melatonin (MEL, 1.3 $\mu$M) by UV–vis spectroscopy (figure 1s) into an i3 model spectrophotometer (Hanon Instruments Co., Ltd.) before (CaM) and after (CaM-MEL) promoting the coupling reaction.

CaM (120 $\mu$M) [1] and MEL (0.1 $\mu$M) interaction was assessed at different pH values (3.5, 4.5, 6.5 and 7.5), absorbance was measured in the wavelengths range of 250 – 320 nm (figure 2s) into an EnSpire (PerkinElmer) spectrophotometer in 96-well UV plate (Santa Cruz Biotechnology, Inc., sc-213228). The samples were measured by triplicate in independent experiments and measurements performed by spectrophotometers were done at room temperature.

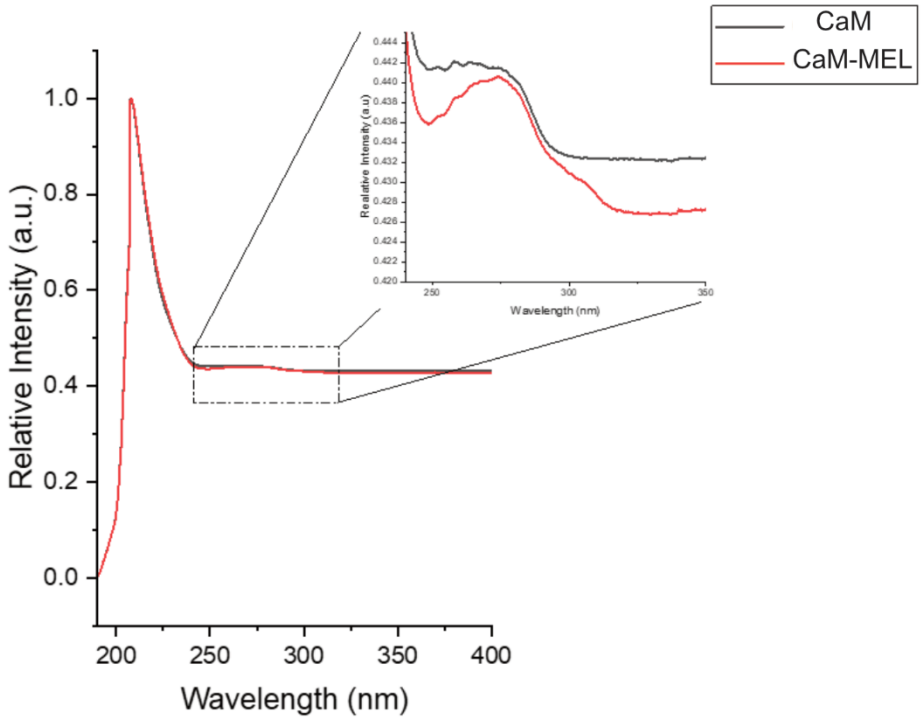


Figure S1**.** Comparison of the absorbance spectra recorded from calmodulin (CaM, 4 $\mu$M, black line) and CaM-melatonin (MEL, 1.3 $\mu$M, red line) by UV-vis spectroscopy in buffer Tris 20 mM, $MgCl_2$ 10 mM, $CaCl_2$ (0.1 mM) at pH 7.5. Note that the signals were normalized, and a representative experiment is shown.

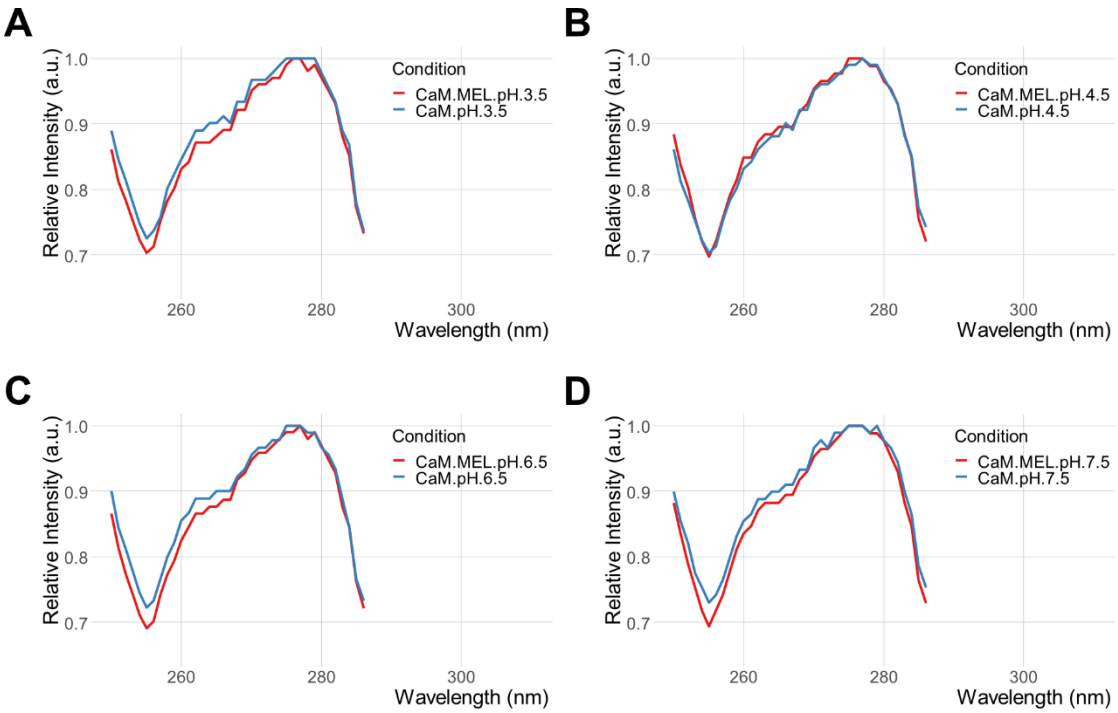


Figure S2**.** Comparison of the UV-vis absorbance spectra of $Ca^{2+}$-calmodulin and $Ca^{2+}$-calmodulin-melatonin at pH 3.5, 4.5, 6.5 and 7.5. Calmodulin (CaM, 120 $\mu$M) and CaM-melatonin (MEL, 0.1 $\mu$M) were incubated with $CaCl_2$ (0.1 mM) in Tris 20 mM and $MgCl_2$ 10 mM at pH, A) 3.5, B) 4.5, C) 6.5 and D) 7.5 at room temperature. Note that the signals were normalized, and a representative experiment is shown.

References

1. Stateva, S.R.; Salas, V.; Benaim, G.; Menéndez, M.; Solís, D.; Villalobo, A. Characterization of Phospho-(Tyrosine)-Mimetic Calmodulin Mutants. *PLOS ONE* **2015**, *10*, e0120798, doi:10.1371/JOURNAL.PONE.0120798.