Melatonin reduces GSK3β-mediated Tau phosphorylation, enhances Nrf2 nuclear translocation and anti-inflammation

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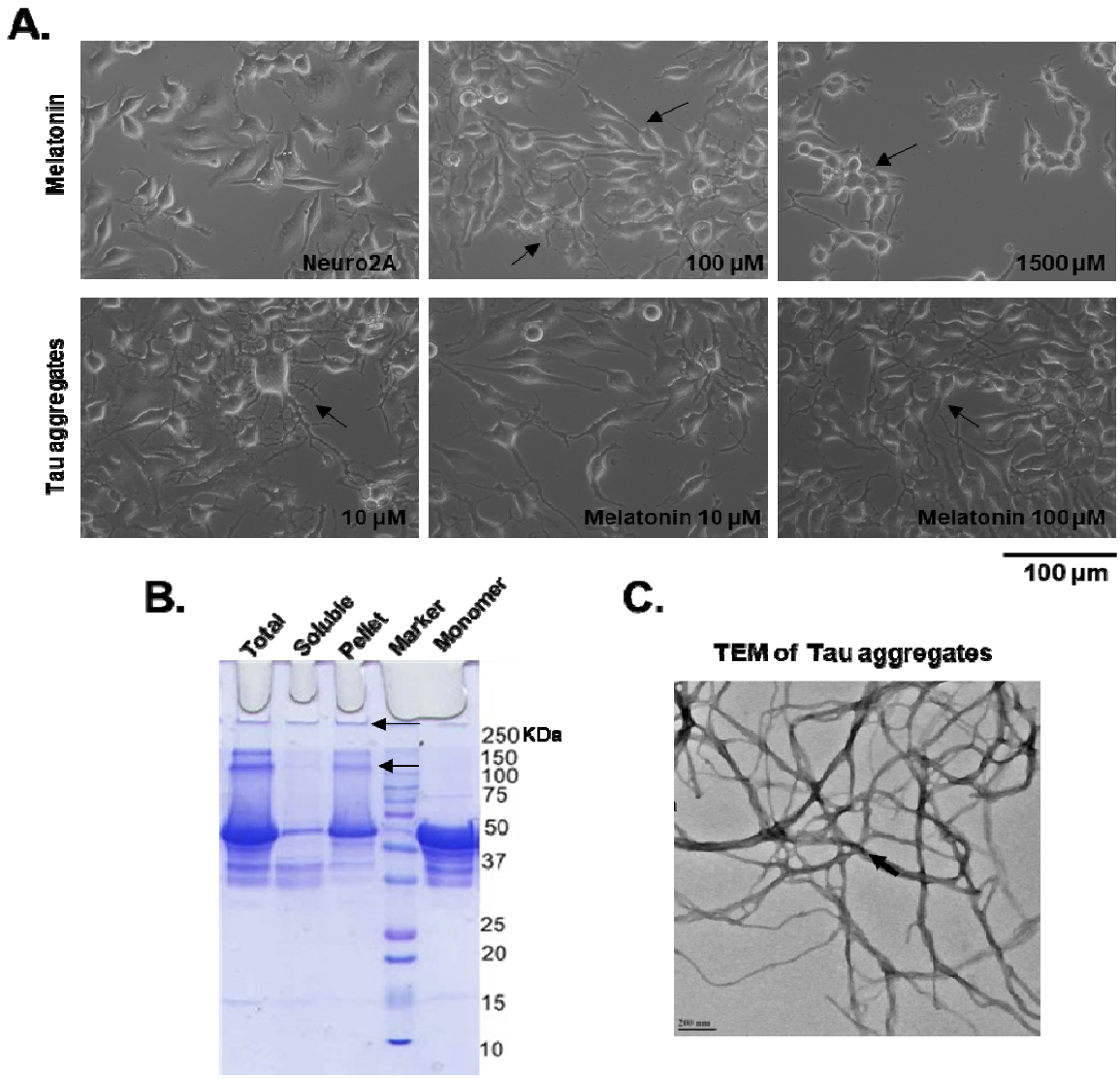
Supplementary Figure 1

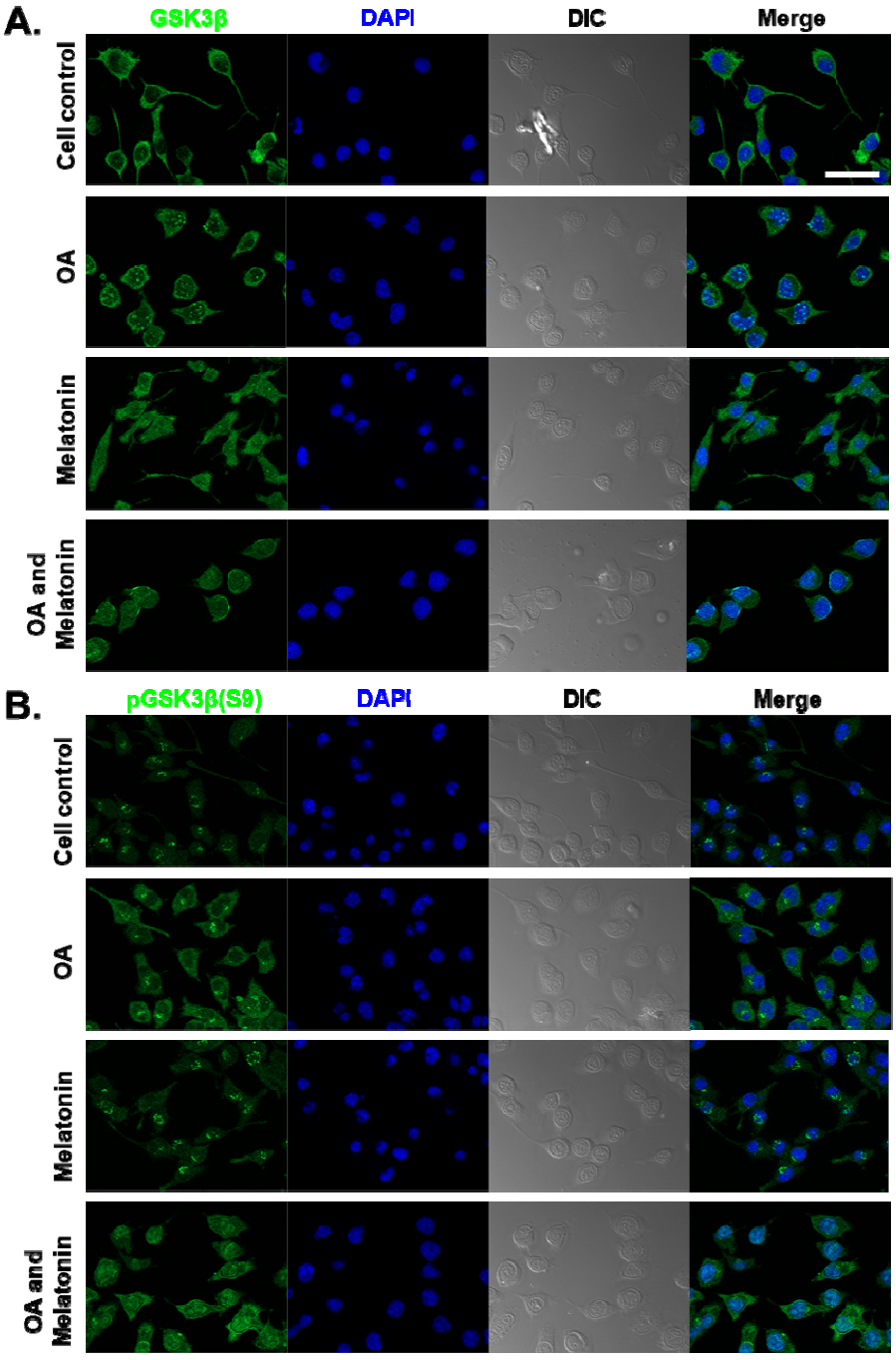
Phase contrast study and aggregation of Tau



Supplementary Fig. 1. Phase contrast study of Neuro2A cell morphology and SDS-TEM analysis of Tau aggregates. (A) Cells were found to maintain neuronal connection at 100 µM concentration of Melatonin whereas at higher concentration of 1500 µM, cell toxicity was observed as indicated by arrow. The cell viability assay of Melatonin in presence of Tau aggregates was validated by phase contrast study. (B, C) Pre-formed Tau aggregates were analyzed by SDS-PAGE. Higher order aggregates are pointed by 'arrow' on SDS-PAGE and TEM images. On SDS-PAGE, the fractions of pelleting assay were loaded and indicated as Total without pelleting assay, Supernatant, Pellet.

Supplementary Figure 2

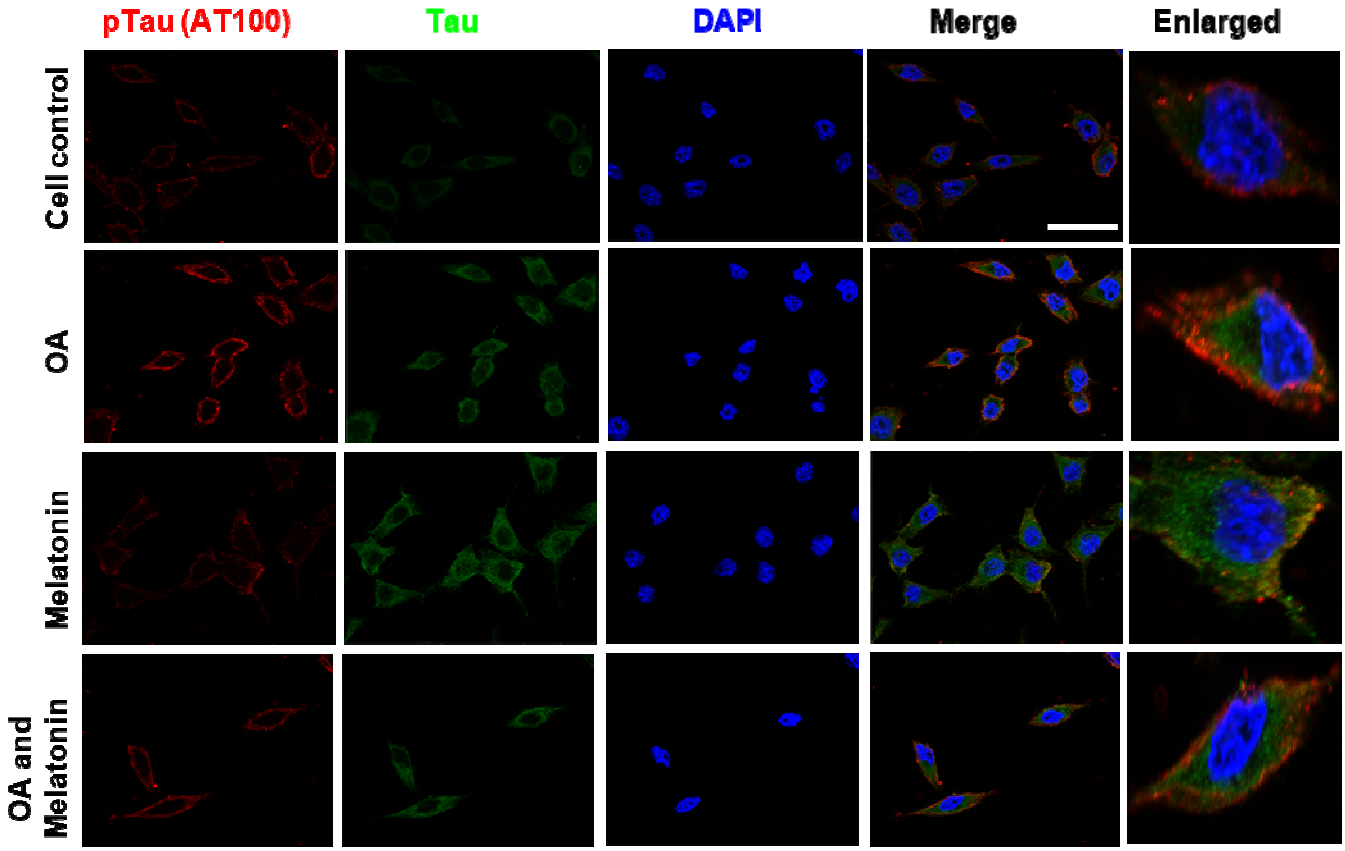
Level of pGSK3β (pS9) and total GSK3β in Neuro2A cells



Supplementary Fig. 2. Immunofluorescence study of Neuro2A for GSK3β and pGSK3β (Ser^9) expression . (A) The cellular level of GSK3β and pGSK3β were observed in Melatonin and OA treated cells, where it was observed that total GSK3β level was reduced by Melatonin treatment but pGSK3β (Ser^9) level remain insignificant compared to OA-stress. The scale bar is 50 µm.

Supplementary Figure 3

Melatonin reduces OA-induced Tau phosphorylation



Supplementary Fig. 3. Immunofluorescence study of phospho-Tau level (AT100) on Neuro2A cells . The level of phospho-Tau (AT100) was observed in Melatonin and OA treated cells, where pTau was found to be localized at the periphery of the cells as neuronal puncta. The OA treated group showed more accumulation of AT100-Tau which was reduced by Melatonin treatment. The scale bar is 50 μm.