Supplementary Information

Microtubule-dependent polarization and migration of microglia conserved by α-linolenic acid and extracellular Tau-mediated stimulation

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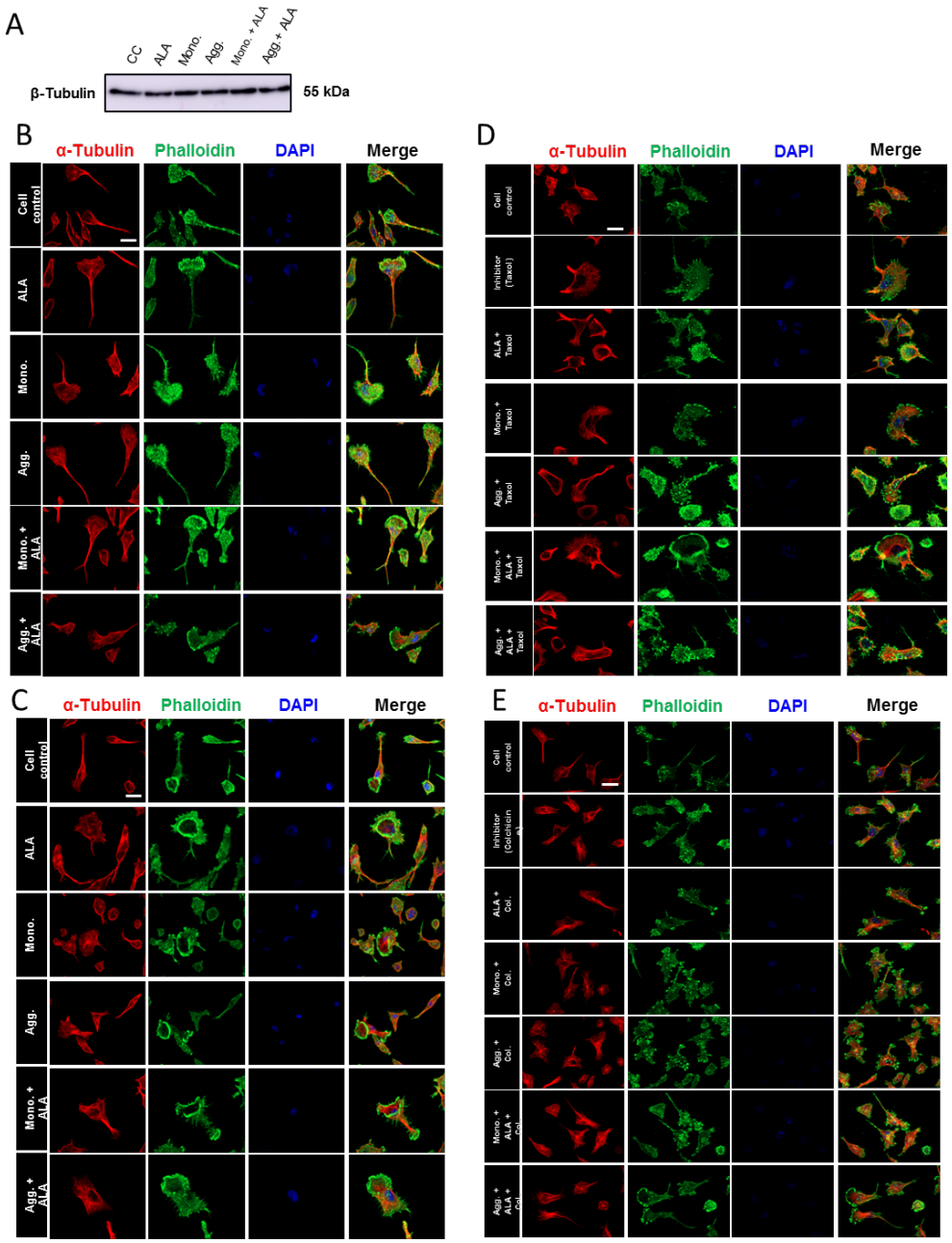
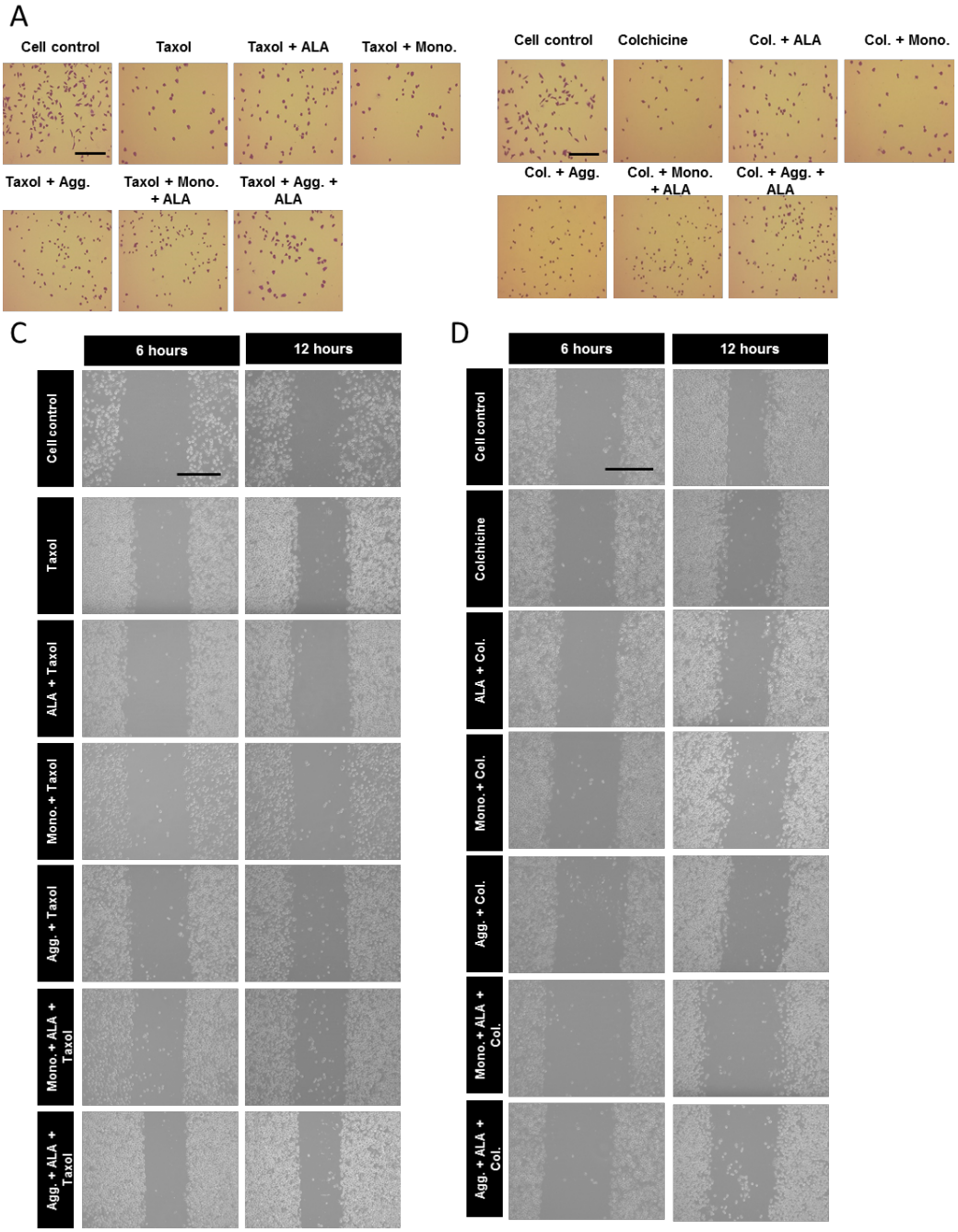
Supplementary Figure. 1



Supplementary figure 1. The polarization of microtubule network of Tau-mediated activation of microglia. A) Tau induced activated microglia show polarized morphology of the microtubule network where it network is more directed towards direction of migration at leading ends of the cell. **B)** The expression pattern of microtubule was analyzed upon Tau exposure through western blot analysis. **C)** Actin ring formation was observed in Tau-induced activated microglia cells the structures are supported by microtubules, which would help for migration processes. Scale bar is 10 µm. **D)** Microtubule-dependent polarization of microglia was assessed by pre-exposure of microglia with Taxol, which induces tubulin stabilization. Taxol-induced disruption of tubulin filaments that affected the polarization

of microglia. **E)** Colchicine-induced depolymerization of tubulin filaments increases the disruption of microtubule filaments and affected the polarized morphology. Morphological analysis was evaluated by phalloidin (green) and α-tubulin (red) staining. Scale bar is 10 µm.

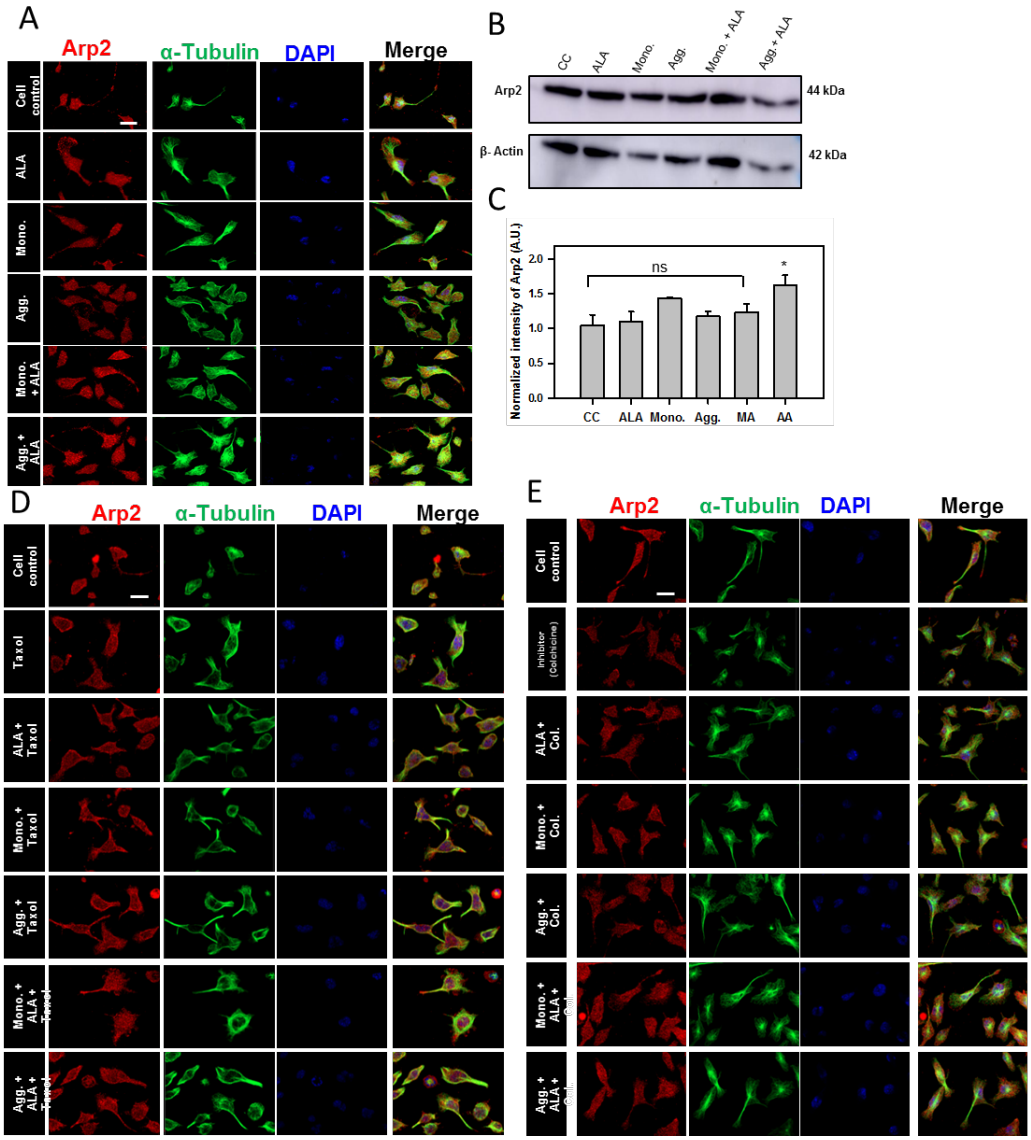
Supplementary Figure. 2



Supplementary figure 2. Microtubule-dependent adhesion and migration of microglia. A) Effect of microtubule stabilization on adhesion of microglia was studied upon pretreatment of Taxol; the adhered cells were fixed and stained with crystal violet. Number of adhered cells had been reduced due to Taxol treatment. **B)** Similarly, effect of microtubule depolymerization was analyzed upon pretreatment of

colchicine. The cells were further treated with Tau species and ALA and adhered cells were fixed and stained with crystal violet. Scale bar is 100 µm. C) Effect of microtubule stabilization and depolymerization was checked on time dependent 2D migration of microglia via **A)** Taxol **B)** Colchicine. The wound-scratch assay was carried out and cell migration was analyzed till 24 hours. The figure denotes analysis at 3 and 6 hours after wound scratch. Scale bar is 100 µm.

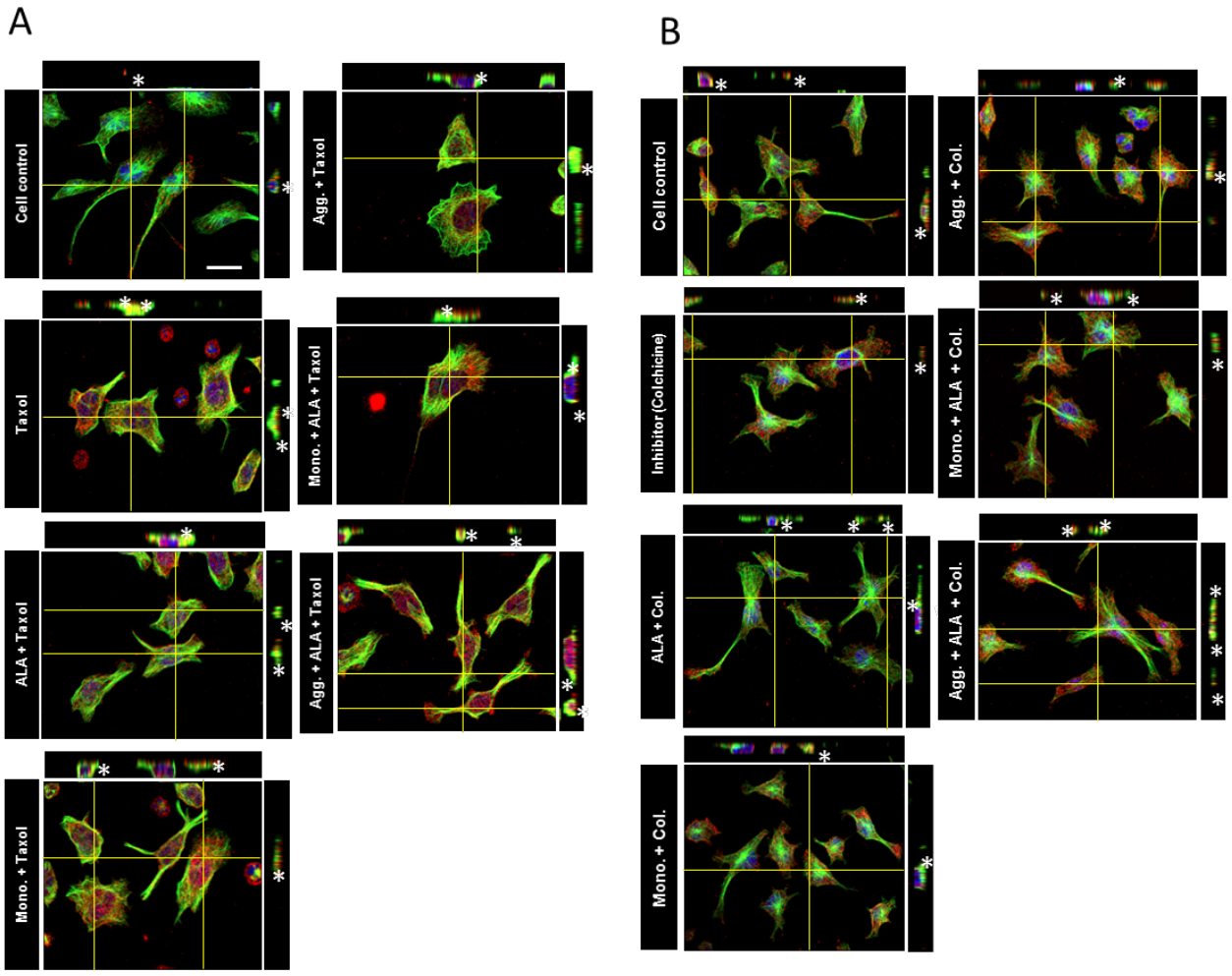
Supplementary Figure. 3



Supplementary figure 3. Crosstalk signaling between actin and microtubule cytoskeleton for microglial migration. A) Signaling between tubulin and actin was determined by association of tubulin with actin-binding proteins. Effect of Tau and ALA on crosstalk signaling was estimated via colocalization analysis. Arp2 (red) and Tubulin (green) colocalization was observed in the merge panel. B) Expression levels of Arp2 were evaluated by western blot analysis to check the increased expression during crosstalk signaling. C) The band intensity of Arp2 after Tau and ALA was evaluated and normalized with β-Actin. To understand the differences in the expression pattern during active signaling. Effect of microtubules disruption on crosstalk signaling of MT and actin was observed after pretreatment of tubulin stabilizer

and depolymerizing agents D) Taxol E) Colchicine on microglia. Tubulin and Arp2 association during activated signaling was evaluated by colocalization analysis. The colocalization between tubulin and Arp2 was calculated after taxol and colchicine treatment. Scale bar is 10 µm.

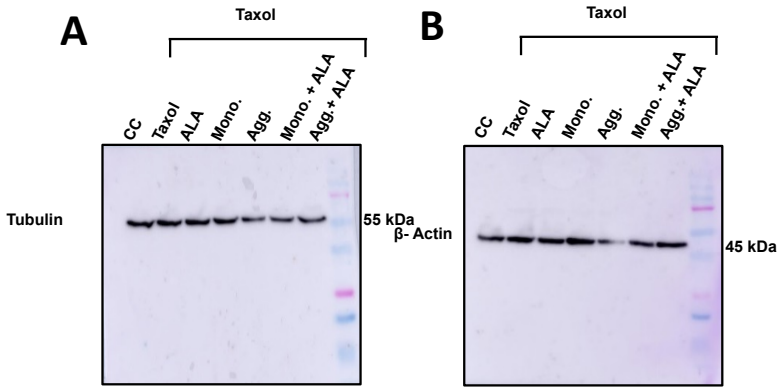
Supplementary Figure. 4



Supplementary figure 4. Involvement of Tubulin with Arp2 during crosstalk signaling in microglia. Microglia cells were treated with **A)** Taxol or **B)** Colchicine before treatment with Tau and ALA species. The colocalization between Arp2 and tubulin was evaluated by orthogonal sectioning from 3D experiment. The colocalization is shown on x and y-axis with the star mark. Scale bar is 10 µm.

Supplementary figure 6. Original uncut western blots

Corresponding to main figure 1 F



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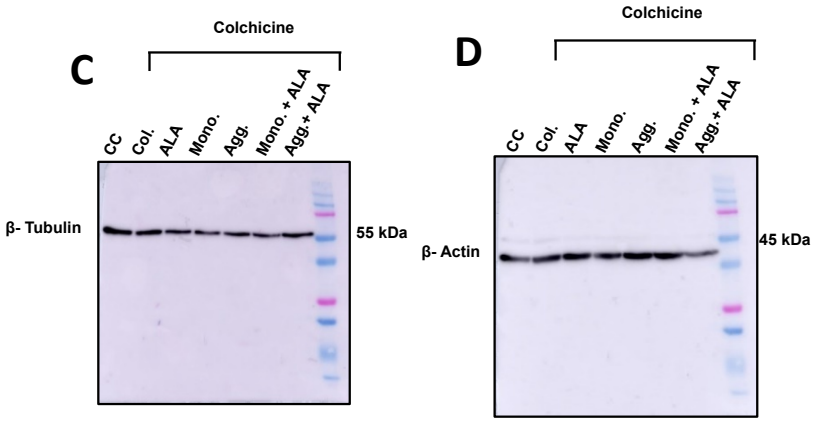
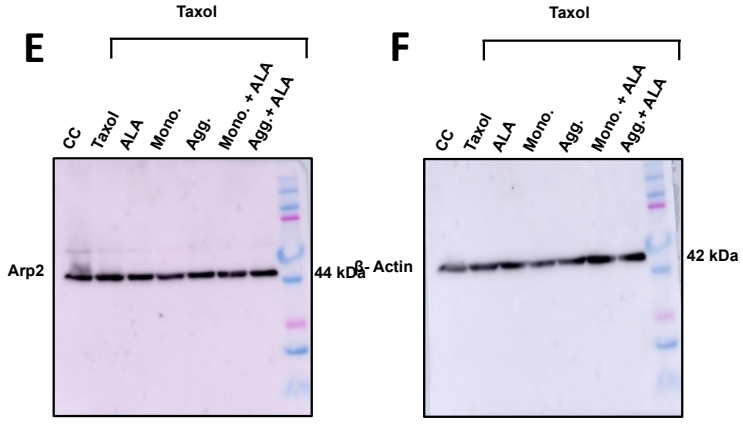
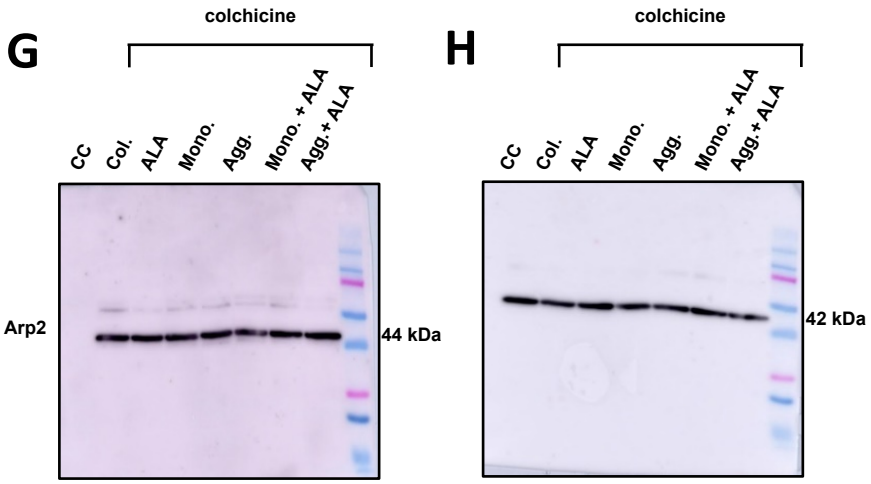


Corresponding to main figure 4 E



Supplementary figure 6.
Original uncut western blots contd…

Corresponding to main figure 4 I



Corresponding to supplementary figure 4B