



Supplementary Figure S6. Melanoma-secreted A β suppresses inflammatory signaling in astrocytes and protects melanoma from microglial phagocytosis. **A-F**, Representative images of TMEM119+, Iba1+ microglia surrounding sh-Scr (**A-C**) and sh-APP (**D-F**) melanoma cells. Fluorescent Markers: green= GFP (melanoma cells), orange=Iba1 (macrophage/microglia), blue = DAPI (nuclei), red = TMEM119 (microglia). Green outline = melanoma-associated microglia. **A,D**, Composite. **B,E**, Iba1 only. **C,F**, TMEM119 only. **G-P**, Example images illustrating three-dimensional brain slice immunofluorescence quantification. Fluorescent markers: green= GFP (melanoma cells), orange= Iba1 (microglia), blue = DAPI (nuclei), red = Arginase-1. Images **G-K** and **L-P** represent different two-dimensional levels (**G-K** level 21 of 61, **L-P** level 7 of 61) from a single confocal z-stack image of one cluster of 12-273 BM sh-Scr cells in the brain parenchyma. **G,L** – Image of melanoma cells in the brain parenchyma. **H,M** – Single channel image of Iba1 surrounding melanoma cells in the brain parenchyma. **I,N** – After binary transformation by thresholding of Iba1 expression, generation of three dimensional ROIs representing discrete melanoma-associated microglia. Each discrete microglia is marked with a distinct color. Matching colors between image **I** and **N** represent cross-sectional area of the same microglia at different two-dimensional levels. **J,O** – Identification of melanoma-associated (green outline) and control (white outline) microglia based on presence or lack of contact of three-dimensional ROIs with GFP-positive melanoma cells. **K,P** – Single channel image of Arginase-1 in melanoma-associated and control microglia in proximity to melanoma cells.