

Supplementary Figure S5. Melanoma-secreted Aβ is Required for Growth and Survival in the Brain Parenchya. A-B, Images of brain slice immunofluorescence showing live (white arrow) and dead (yellow arrows) 12-273 BM cells. Fluorescent markers: yellow = anti-Cleaved-Caspase 3 (dead cells), green = GFP (melanoma cells), red = tomato lectin (blood vessels), blue = DAPI (nuclei). C, Two-dimensional quantification of melanoma-associated astrocyte perimeter. sh-Scr vs. sh-APP (* p<0.05). D-F, Example images demonstrating two-dimensional quantification method. Fluorescent markers: orange = GFAP (astrocytes), green = GFP (melanoma cells), blue = DAPI (nuclei). D, Image of melanoma cell in the brain parenchyma. E, conversion of image to binary image with thresholding of GFAP expression. F, Identification of discrete melanoma-associated (green outline) and control (white outline) astrocytes by two-dimensional ROI generation from the binary image.