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## **Melanoma-secreted Amyloid Beta Suppresses Neuroinflammation and Promotes Brain Metastasis**

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## 25 **Summary**

26

27 Brain metastasis is a significant cause of morbidity and mortality in multiple cancer  
28 types and represents an unmet clinical need. The mechanisms that mediate metastatic  
29 cancer growth in the brain parenchyma are largely unknown. Melanoma, which has the  
30 highest rate of brain metastasis among common cancer types, is an ideal model to  
31 study how cancer cells adapt to the brain parenchyma. We performed unbiased  
32 proteomics analysis of melanoma short-term cultures, a novel model for the study of  
33 brain metastasis. Intriguingly, we found that proteins implicated in neurodegenerative  
34 pathologies are differentially expressed in melanoma cells explanted from brain  
35 metastases compared to those derived from extracranial metastases. This raised the  
36 exciting hypothesis that molecular pathways implicated in neurodegenerative disorders  
37 are critical for metastatic adaptation to the brain.

38

39 Here, we show that melanoma cells require amyloid beta ( $A\beta$ ), a polypeptide heavily  
40 implicated in Alzheimer's disease, for growth and survival in the brain parenchyma.  
41 Melanoma cells produce and secrete  $A\beta$ , which activates surrounding astrocytes to a  
42 pro-metastatic, anti-inflammatory phenotype. Furthermore, we show that  
43 pharmacological inhibition of  $A\beta$  decreases brain metastatic burden.

44

45 Our results reveal a mechanistic connection between brain metastasis and Alzheimer's  
46 disease – two previously unrelated pathologies, establish  $A\beta$  as a promising therapeutic  
47 target for brain metastasis, and demonstrate suppression of neuroinflammation as a  
48 critical feature of metastatic adaptation to the brain parenchyma.

49

## 50 **Main**

51

52 Brain metastasis is the most common form of adult intracranial malignancy<sup>1</sup> and results  
53 in severe morbidity and mortality. 40-75% of Stage IV melanoma patients develop brain  
54 metastasis<sup>2,3</sup>, reflecting melanoma's striking ability to colonize the brain. Brain  
55 metastases are less responsive than extracranial metastases to current cancer  
56 therapies<sup>4-6</sup>, and the majority of patients succumb to disease in less than one year<sup>7</sup>.  
57 Furthermore, patients with brain metastasis are often excluded from clinical trials and  
58 urgently need new clinical options. In recent years, research has started to elucidate the  
59 molecular mechanisms contributing to the multi-step process of brain metastasis. Most  
60 findings have focused on cancer extravasation across the blood-brain barrier (BBB),  
61 which cannot be leveraged therapeutically given that the vast majority of brain  
62 metastasis patients will present with extravasated cancer cells at the time of cancer  
63 diagnosis. The main bottleneck in the brain metastatic process has been shown to be  
64 the successful expansion of a single cell in the brain parenchyma to form a macro-  
65 metastasis<sup>8</sup>. Recent studies have begun to demonstrate the role of the brain  
66 microenvironment in this process. In particular, reactive astrocytes have been shown to  
67 interact with cancer cells in the brain<sup>9,10</sup> and exhibit both pro- and anti-brain metastatic  
68 activity<sup>11,12</sup>. Astrocytes have several roles in normal brain physiology, including  
69 neurotransmitter uptake<sup>13</sup>, metabolic support<sup>14</sup>, and response to injury<sup>15</sup>. Furthermore,  
70 astrocytes have been heavily implicated as both neurotoxic and neuroprotective in a  
71 variety of neurodegenerative pathologies, including Alzheimer's disease<sup>16-18</sup>. These  
72 findings suggest the intriguing possibility of a functional connection between  
73 neurodegenerative pathologies and brain metastasis, which has not yet been explored.

74

75 Here, we demonstrate that melanoma cells require amyloid beta ( $A\beta$ ), a polypeptide  
76 heavily implicated in Alzheimer's disease, for survival and late growth in the brain  
77 parenchyma. Melanoma cells cleave Amyloid Precursor Protein (APP) to produce and  
78 secrete  $A\beta$ , and  $A\beta$  secreted from cancer cells triggers local astrocytes to adopt a pro-  
79 metastatic, anti-inflammatory phenotype. Targeting  $A\beta$  production by pharmacologic

80 inhibition of  $\beta$ -secretase activity suppresses metastatic growth of human cancer cells in  
81 the brain parenchyma of mice.

82

### 83 **Proteomics analysis links melanoma brain metastasis and neurodegeneration**

84 To study mechanisms of brain metastasis, we leveraged pairs of brain metastasis-  
85 derived (BM) and non-brain metastasis-derived (NBM) melanoma short term cultures  
86 (STCs) obtained from the same patient (Figure 1a) as a novel model of brain  
87 metastasis. Comparing patient-matched BM and NBM STC pairs reduces the  
88 confounding inter-patient heterogeneity characteristic of melanoma. Upon intracardiac  
89 injection in immunocompromised mice, BM STCs exhibited an increased ability to  
90 metastasize to the brain than their paired NBM STCs (Figure 1b-d, Extended Data  
91 Figure 1a,b). Therefore, any molecular differences between paired BM and NBM STCs  
92 can be associated with differential brain metastatic ability.

93

94 Using a cohort of 15 BM and 11 NBM derived STCs, which included 3 isogenic pairs,  
95 we performed an unbiased mass spectrometry analysis of whole cell protein lysates to  
96 identify novel candidate pathways and proteins that may mediate melanoma brain  
97 metastasis. KEGG pathway analysis of differentially expressed proteins revealed an  
98 enrichment in proteins related to neurodegenerative pathologies and oxidative  
99 phosphorylation in the BM vs NBM STCs (Figure 1e-g). Proteomics results were  
100 validated by Western blot analyses (Extended data Fig. 1c,d). Metabolic profiling  
101 demonstrated that BM STCs have increased mitochondrial fusion and electron density  
102 (Figure 1h,i), elevated mitochondrial oxygen consumption (Fig. 1j and Extended data  
103 Figure 1e,g,i), and decreased glycolysis (Extended data Figure 1f,h,j) than their  
104 respective paired NBM. These results provide further evidence of a recently reported  
105 connection between melanoma brain metastasis and oxidative phosphorylation<sup>19,20</sup>,  
106 supporting the capability of our proteomic results to reflect phenotypic differences  
107 related to brain metastatic ability.

108

### 109 **APP is specifically required for melanoma brain metastasis**

110 We performed an *in vivo* brain metastasis mini-screen to identify novel mediators of  
111 melanoma brain metastasis. We first selected three proteins (PRKAR2B, SCARB1,  
112 XPNPEP3) found consistently increased in both paired and unpaired BM STCs relative  
113 to NBM STCs (Figure 1g), and functionally related to neurodegeneration and/or  
114 mitochondrial metabolism. Additionally, a review of the literature of top differentially  
115 expressed proteins from the proteomics results revealed several connections with  
116 Alzheimer's disease, APP cleavage, and A $\beta$ . Although APP was not identified as  
117 significantly increased in BM vs NBM STCs, proteomics analysis of whole cell lysates  
118 cannot detect secreted proteins such as A $\beta$  cleaved from APP. Given that APP and its  
119 cleavage products can have profound effects on the brain microenvironment in the  
120 development of Alzheimer's disease <sup>21</sup>, we hypothesized that melanoma cells may  
121 require APP and/or its cleavage products for survival or growth in the brain  
122 parenchyma.

123

124 Using lentiviral shRNA, we silenced the four selected candidates in a STC, 12-273 BM,  
125 and measured effects on *in vitro* proliferation (Figure 2b, Extended data fig. 2a-d) and  
126 metastatic capability upon intracardiac injection in mice. Silencing of APP and  
127 PRKAR2B resulted in a significantly decreased brain to body luminescence signal  
128 (Figure 2a), indicating that these proteins have differential effects on metastatic  
129 potential to the brain as compared to other organs. However, since mice injected with  
130 PRKAR2B-depleted cells had increased metastasis to extracranial organs (Extended  
131 Data Figure 1e-g), we decided to narrow our focus to APP. Silencing of APP did not  
132 affect the proliferative capacity of melanoma cells *in-vitro* (Figure 2b), but resulted in  
133 reduced colonization of the brain and a decreased brain/body luminescence signal  
134 (Figure 2c,d). *Ex-vivo* MRI of mouse brains revealed that loss of APP decreases overall  
135 brain tumor burden (Figure 2e,f; Supplementary Video 1), number of brain metastases  
136 (Figure 2g), and average brain metastasis size (Fig. 2h). Metastatic burden to specific  
137 organs was further quantified by NuMA immunohistochemistry, which specifically labels  
138 the nuclei of human cells within mouse organs. Loss of APP resulted in a dramatic  
139 reduction of brain metastatic burden (Figure 2i,j), but had no significant effect on  
140 metastasis to either the liver (Figure 2k) or the kidneys (Figure 2l). Silencing of APP in a

141 second STC, WM-4265-2 BM<sup>22</sup>, also inhibited brain metastasis without significant  
142 effects on metastasis to other organs (Extended Data Figure 2h-j) Thus, APP is  
143 required for melanoma to colonize the brain but not to metastasize to other organs.

144

#### 145 **Amyloid Beta is the form of APP required for melanoma brain metastasis**

146 APP can be processed to produce amyloid beta (A $\beta$ ), a polypeptide heavily implicated  
147 in Alzheimer's disease, through sequential cleavage by beta and gamma secretases.  
148 Although APP is expressed in a wide variety of normal tissues and tumor types,  
149 potential A $\beta$  production by cancer cells has never been explored. Therefore, we sought  
150 to investigate whether melanoma cells can produce A $\beta$  and if so, whether A $\beta$  production  
151 is altered in BM vs NBM STCs. Using probe specific gamma-secretase assays<sup>23</sup>, we  
152 established that melanoma cells can cleave APP with gamma secretase and observed  
153 consistently increased cleavage of APP in BM vs paired NBM STCs (Figure 3a).  
154 Notably, increased cleavage of NOTCH, a canonical gamma secretase substrate, was  
155 not consistently observed in paired BM vs NBM STCs (Figure 3b), which suggests a  
156 specific association between APP cleavage and brain metastasis. Analysis of  
157 melanoma conditioned media demonstrated that melanoma cells are able to produce  
158 and secrete A $\beta$ , and that A $\beta$  secretion is increased in BM relative to paired NBM STCs  
159 (Figure 3c). Therefore, we posited that A $\beta$  is the specific form of APP required for  
160 melanoma brain metastasis.

161

162 To test this hypothesis, we asked whether A $\beta$  is sufficient to rescue the inhibition of  
163 metastasis observed upon APP silencing. We cloned SPA4CT-T43P<sup>24</sup>, a heavily  
164 truncated (>75% of APP amino acid sequence removed), mutant form of APP that  
165 retains the ability to produce A $\beta$  but cannot produce other major cleavage products,  
166 such as sAPP- $\alpha$  and sAPP- $\beta$  (Figure 3d). The substitution of proline for threonine at  
167 position 43 partially inhibits gamma secretase cleavage<sup>25</sup> and prevents excessive A $\beta$   
168 generation (Extended Data Figure 3a,b). We knocked out APP in 12-273 BM STC using  
169 CRISPR/Cas9 and reintroduced either APP-770, the major full-length APP isoform  
170 expressed in melanoma cells (Extended Data Figure 3c), or SPA4CT-T43P (Figure 3e).  
171 We confirmed that knockout of APP reduces A $\beta$  secretion and that both wild type APP

172 (APP-770) and SPA4CT-T43P are able to rescue A $\beta$  secretion to near physiologic  
173 levels (Figure 3f). *In-vivo*, the loss of brain metastasis observed upon APP knockout  
174 was rescued by introduction of either APP-770 or SPA4CT-T43P (Figure 3g,h),  
175 demonstrating that A $\beta$  is the form of APP required for melanoma brain metastasis.

176

### 177 **Melanoma-secreted Amyloid Beta is required for late growth and survival in the** 178 **brain parenchyma**

179 To investigate how A $\beta$  functions in melanoma brain metastasis, we began by identifying  
180 which step of the brain metastatic process A $\beta$  is required for. First, we established the  
181 timeline of brain metastasis in 12-273 BM STC by *ex-vivo* immunofluorescence analysis  
182 of brain slices (Figure 4a upper panels, Supplementary Videos 2-6. At day 1 post  
183 intracardiac injection, melanoma cells are arrested in the brain microvasculature. By day  
184 3, melanoma cells have extravasated into the parenchyma and remain adhered to the  
185 surface of blood vessels with a rounded morphology. From days 3 to 7, cells begin to  
186 divide and spread along the vasculature in an elongated morphology, a process known  
187 as vascular co-option<sup>8</sup>. By day 7, approximately two thirds of the melanoma cells that  
188 had initially reached the brain at day 1 have died. This occurs when melanoma cells  
189 either fail to extravasate (Figure 4c) or undergo apoptosis upon entering the brain  
190 parenchyma (Figure 4d). From days 7-14, surviving melanoma cells begin to proliferate  
191 in a rounded morphology independent of the vasculature to form micro-metastases.  
192 After day 14, cells rapidly divide, forming small macro-metastases visible to the naked  
193 eye by day 21.

194

195 When comparing melanoma cells with and without A $\beta$ , we did not observe differences in  
196 the number of live cells from day 1 through day 7 post intracardiac injection (Figure 4b),  
197 indicating that A $\beta$  is not required for vascular arrest, extravasation, or early survival of  
198 melanoma cells in the brain parenchyma. Melanoma cells lacking A $\beta$ , however, were  
199 unable to successfully proliferate to form micro- and macro-metastases by days 14 and  
200 21 respectively (Figure 4b), and instead underwent apoptosis (Extended Data Figure  
201 4a). Therefore, A $\beta$  is required for melanoma cells to progress from vascular co-option to  
202 successful metastatic colonization of the brain parenchyma.

203

#### 204 **Melanoma-secreted A $\beta$ stimulates local reactive astrocytosis**

205 Given that reactive astrocytes are important regulators of brain metastasis<sup>10</sup> and that  
206 amyloid beta can influence astrocyte physiology<sup>26,27</sup>, we hypothesize that A $\beta$  secreted  
207 by melanoma cells triggers a reactive phenotype in surrounding astrocytes that supports  
208 melanoma growth in the brain. GFAP staining revealed an increase in the presence of  
209 reactive astrocytes surrounding melanoma cells over time (Figure 4a, lower panels),  
210 with significant physical contacts between melanoma cells and astrocytes developing  
211 from days 7 to 14. By day 21, reactive astrocytes form a glial scar-like structure that  
212 envelops the growing brain metastases (Figure 4a, bottom right; Supplementary Video  
213 7). Notably, the time period during which melanoma cells lacking A $\beta$  fail to survive  
214 overlaps with the time in which astrocytes begin to extensively interact with melanoma  
215 cells. We analyzed astrocytes surrounding live melanoma cell clusters with and without  
216 A $\beta$  in the brain at day 10 post-injection by GFAP staining. Clusters of cells lacking A $\beta$   
217 display significantly decreased local reactive astrocytosis than control cells (Figure 4e,f;  
218 Extended data figure 4b), indicating that melanoma-secreted A $\beta$  stimulates local  
219 reactive astrocytosis.

220

221 We further interrogated how melanoma-derived A $\beta$  affects the phenotype of astrocytes  
222 by exposing primary rat astrocytes to melanoma-conditioned media. Primary rat  
223 astrocytes were isolated and maintained at rest *in-vitro* in serum-free media, as  
224 previously reported<sup>28</sup>. We exposed astrocytes to melanoma conditioned media (CM)  
225 lacking A $\beta$ , either by genetic silencing of APP in melanoma cells (sh-APP IgG) or by  
226 immunodepletion of A $\beta$  from control melanoma CM (sh-Scr Anti-A $\beta$ ), and compared  
227 them to astrocytes exposed to control melanoma CM (sh-Scr IgG) (Figure 5a).  
228 Astrocytes exposed to media with A $\beta$  displayed more elongated branches, a phenotype  
229 related to astrocyte reactivity<sup>29</sup>, as compared to those exposed to media without A $\beta$   
230 (Figure 5b,c).

231

#### 232 **Melanoma-secreted A $\beta$ induces an anti-inflammatory response in astrocytes**



233 When comparing global transcriptomic changes in astrocytes exposed to melanoma CM  
234 with and without A $\beta$ , we found a high degree of correlation ( $r=0.64$ ) between changes  
235 induced by APP silencing in melanoma cells and those induced by A $\beta$  immunodepletion  
236 from CM of control melanoma cells (Figure 5d,e). This demonstrates that melanoma-  
237 secreted A $\beta$  accounts for the majority of APP-mediated transcriptomic changes that  
238 melanoma cells induce in astrocytes, and establishes secreted A $\beta$  as a direct mediator  
239 of crosstalk between melanoma cells and astrocytes.

240

241 To further characterize how melanoma-derived A $\beta$  influences astrocytes, we performed  
242 gene set enrichment analysis of differentially expressed transcripts in astrocytes  
243 exposed to melanoma CM with and without A $\beta$ . Intriguingly, results showed a  
244 significantly decreased enrichment score in multiple pathways related to inflammatory  
245 signaling in astrocytes exposed to media with A $\beta$  as compared to those exposed to  
246 media without A $\beta$  (Figure 5f).

247 Therefore, we hypothesized that a key function of melanoma derived-A $\beta$  could be to  
248 stimulate astrocytes to an anti-inflammatory phenotype that helps prevent immune-  
249 mediated clearance of melanoma cells in the brain parenchyma. Using a cytokine array,  
250 the levels of astrocyte-derived secreted factors were quantified in supernatants from  
251 astrocytes exposed to melanoma CM in the presence or absence of A $\beta$ . This analysis  
252 revealed that A $\beta$  both stimulates astrocyte secretion of anti-inflammatory cytokines and  
253 suppresses astrocyte secretion of cytokines with known pro-inflammatory activity in the  
254 central nervous system (Figure 5g)<sup>30-39</sup>, thus demonstrating that melanoma-secreted  
255 A $\beta$  stimulates astrocytes to an anti-inflammatory phenotype.

256

### 257 **A $\beta$ suppresses microglia activation and phagocytic clearance of melanoma cells**

258 Several of the identified astrocyte-secreted cytokines have documented roles in  
259 microglial chemotaxis<sup>40</sup>, activation<sup>31</sup>, and M1 polarization<sup>41</sup>. We therefore sought to  
260 examine the effect of melanoma-secreted A $\beta$  on the recruitment of microglia to the  
261 metastatic site. Resident microglia surrounding melanoma cells with and without A $\beta$   
262 were visualized by Iba1 immunofluorescent staining in brain slices. Microglia

263 surrounding melanoma cells lacking A $\beta$  exhibited a more ameboid morphology (Figure  
264 5h), which signifies increased microglial activation <sup>42</sup>. Furthermore, we observed a  
265 significantly increase in microglial phagocytosis of melanoma cells lacking A $\beta$  (Figure 5i,  
266 Extended data Figure 4c, and Supplementary Video 8), demonstrating that A $\beta$  secretion  
267 protects melanoma cells from phagocytic clearance by microglia.

268

### 269 **A $\beta$ is a Promising Therapeutic Target for Treatment of Brain Metastasis**

270 To assess if targeting A $\beta$  could be a promising therapeutic strategy for treatment of  
271 melanoma brain metastasis, we investigated whether A $\beta$  is required for growth and  
272 survival of established brain metastases. Using a doxycycline inducible shRNA system,  
273 we depleted APP in growing melanoma brain macro-metastases (Extended data Figure  
274 5a; Figure 6a). Loss of ability to produce A $\beta$  in pre-existing brain metastases resulted in  
275 decreased brain metastatic burden (Figure 6b-d). Many therapeutic approaches  
276 efficiently targeting amyloid beta have been developed and tested for the treatment of  
277 Alzheimer's disease, including anti-A $\beta$  antibodies <sup>43</sup> and  $\beta$ -secretase (BACE) inhibitors  
278 <sup>44</sup>. We tested LY2886721, a BACE inhibitor that blocks A $\beta$  production <sup>45</sup> by inhibiting the  
279 rate limiting step in its generation (Figure 6e). Treatment of mice with LY2886721 at a  
280 dose of 75 mg/kg/day in food resulted in a 75% reduction in plasma A $\beta$  levels (Extended  
281 data Figure 5b). Pharmacological inhibition of A $\beta$  production decreased brain metastatic  
282 burden in both a patient-derived short-term culture (12-273BM; Figure 6f-h) and an  
283 established melanoma cell line (131/4-5B1; Figure 6i-k). Our data support A $\beta$ -targeting  
284 as a promising therapeutic approach against melanoma brain metastasis.

285

286

### 287 **Discussion**

288

289 Identifying and characterizing mechanisms that mediate survival of cancer cells in the  
290 brain parenchyma has been historically challenging. The majority of studies aiming to  
291 identify mechanisms of brain metastasis have utilized cell lines originally derived from  
292 extracranial metastases and examined transcriptomic changes after serial

293 transplantation in mice to increase brain tropism<sup>12,46-48</sup>. While these models have been  
294 an invaluable tool, the process by which they are generated varies greatly from the  
295 brain metastatic process occurring in patients<sup>49</sup>. In contrast, STC pairs exhibiting  
296 differential brain tropism (Figure 1b-d and Extended data fig. 1a,b) are derived directly  
297 from naturally arising metastases in patients<sup>50</sup>. Recently, some studies have directly  
298 profiled gene expression of surgically resected patient tumors<sup>19,51</sup>. While this method  
299 has several advantages, such as strong clinical relevance and ability to capture effects  
300 mediated by the brain microenvironment, the limited amount of material available per  
301 sample can restrict the type of analyses that are technically feasible. Furthermore, direct  
302 use of patient biopsies makes it difficult to establish whether genes identified as  
303 upregulated in CNS metastases represent contamination from brain tissue or neural  
304 mimicry by cancer cells. Proteomic screening of short term cultures – which no longer  
305 contain normal brain tissue – circumvents this issue and allowed us to identify an  
306 association between brain metastasis and proteins implicated in Alzheimer's disease.

307

308 Several studies have demonstrated that reactive astrocytes support the formation of  
309 brain metastasis<sup>10,11</sup>, provided early antagonistic interactions can be overcome<sup>12</sup>.  
310 Here, we show that A $\beta$  secreted by melanoma cells stimulates astrocytes to exhibit a  
311 pro-metastatic, anti-inflammatory phenotype. It was demonstrated that cancer cells in  
312 the brain induce a subpopulation of pro-metastatic Stat3 positive reactive astrocytes in  
313 their microenvironment by an unknown mechanism<sup>52</sup>. In addition, Stat3<sup>+</sup> reactive  
314 astrocytes contribute to the pathology of acute brain injury and several  
315 neurodegenerative diseases, including Alzheimer's disease. Further investigation is  
316 warranted to address whether melanoma-secreted A $\beta$  specifically induces Stat3  
317 activation in astrocytes.

318

319 Astrocytes have been shown to regulate microglial activity in response to inflammatory  
320 insults<sup>53</sup>. A similar role for astrocytes has been theorized in the brain metastatic  
321 microenvironment but remains largely unexplored<sup>54</sup>. Here, we establish that melanoma-  
322 secreted A $\beta$  directly suppresses astrocyte secretion of several cytokines that recruit and  
323 activate microglia, such as CCL2, a potent chemoattractant that polarizes microglia to a

324 pro-inflammatory, anti-tumorigenic M1 phenotype<sup>41</sup>. Furthermore, we demonstrate that  
325 melanoma-secreted A $\beta$  inhibits microglial phagocytosis of melanoma cells. Taken  
326 together, these results suggest that astrocytes regulate microglial activity in the brain  
327 metastatic microenvironment. It is also possible that melanoma-secreted A $\beta$  impacts  
328 microglial response to melanoma cells directly. A recent study showed that acute  
329 exposure to A $\beta$  initially stimulated microglial phagocytosis of microparticles, but re-  
330 exposure of the same microglia to A $\beta$  days later instead inhibited phagocytosis<sup>55</sup>. Given  
331 the high A $\beta$  concentrations used and lack of a continuous exposure of microglia to A $\beta$ , it  
332 is difficult to interpret that study's findings in the context of the brain metastatic  
333 microenvironment. Additional studies are needed to clarify whether melanoma-secreted  
334 A $\beta$  directly impacts microglial function.

335

336 The role of A $\beta$ , both in Alzheimer's disease and in normal physiology, remains  
337 controversial. Most studies investigating the function of A $\beta$  have been performed in the  
338 context of Alzheimer's pathology using transgenic mice that mimic the human disease  
339<sup>56</sup>. It is well established that insoluble aggregates of A $\beta$  contribute to pathological  
340 astrocyte- and microglia-mediated neuroinflammation in Alzheimer's disease<sup>57</sup>.  
341 Furthermore, A $\beta$  oligomers have been shown to act as anti-microbial peptides and  
342 protect against CNS infection<sup>58,59</sup>. Intriguingly, we demonstrate an anti-inflammatory  
343 function of soluble A $\beta$  in the context of brain metastasis. The majority of A $\beta$  produced by  
344 melanoma cells is A $\beta$ -40 (Figure 3 c,f, data not shown), the less aggregative form, and  
345 none of our models of brain metastasis gives rise to A $\beta$  plaques (data not shown).  
346 Instead, we hypothesize that melanoma-derived A $\beta$  acts in the form of soluble  
347 monomers or oligomers to suppress astrocyte-driven inflammation. Indeed, both  
348 monomers and oligomers of A $\beta$  have been shown to affect phenotypic changes in  
349 astrocytes<sup>26,27</sup>. Whether soluble A $\beta$  also acts as an anti-inflammatory mediator in the  
350 brain during normal physiology and other pathophysiologic contexts is an important  
351 question that requires further investigation. If present, a similar function of soluble A $\beta$   
352 could profoundly impact our understanding of Alzheimer's development and shed light

353 on the reported lack of efficacy of anti-A $\beta$  agents against advanced Alzheimer's  
354 disease.

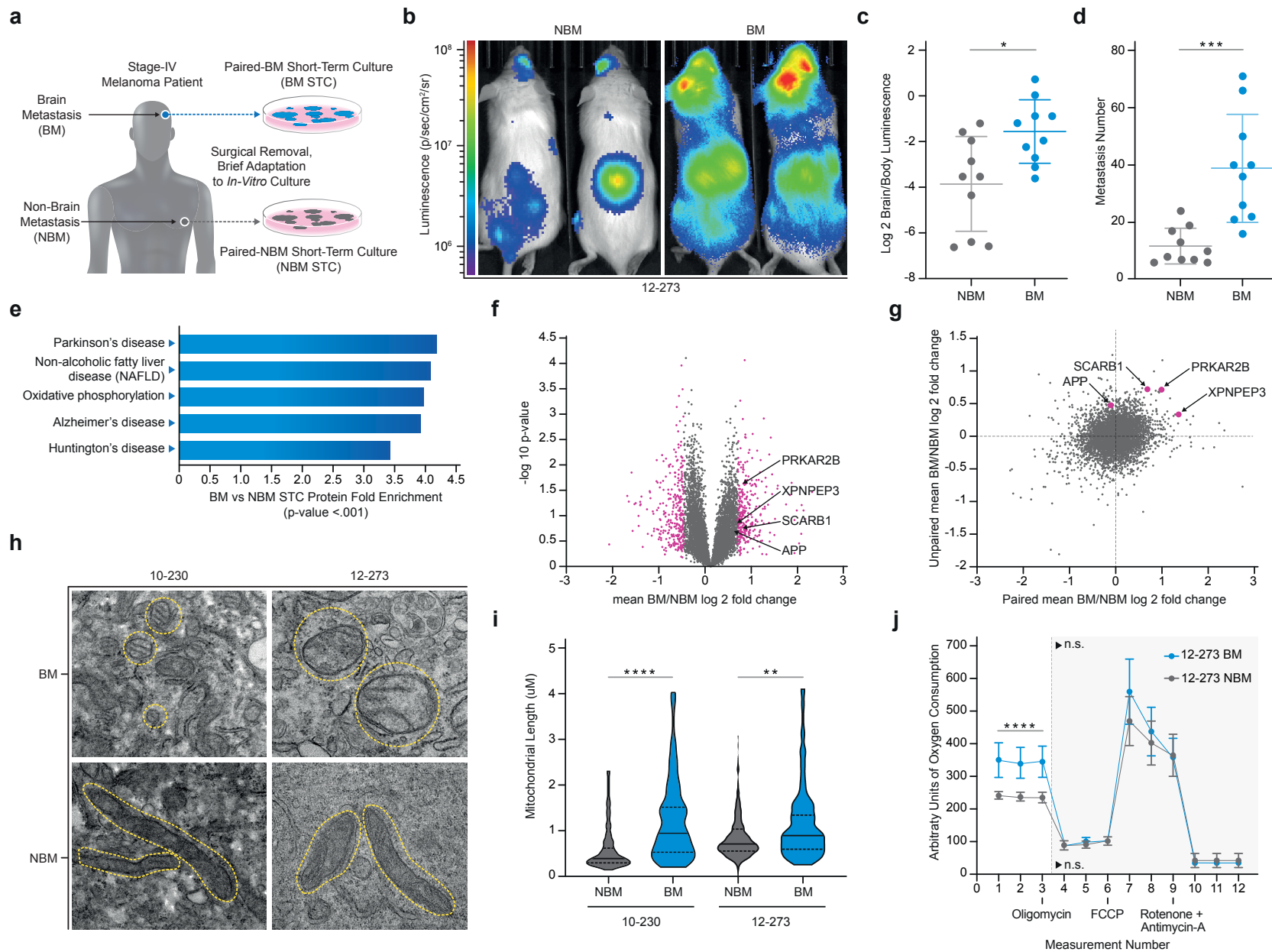
355

356 Our studies show A $\beta$  is a highly promising therapeutic target for melanoma brain  
357 metastasis. The brain represents an immune privileged environment and is often a site  
358 of treatment resistance or relapse in patients <sup>4</sup>. Although checkpoint blockade  
359 immunotherapy, the standard-of-care for metastatic melanoma, can be efficacious in  
360 brain metastasis, the rates of on-treatment progression are higher for intracranial than  
361 for extracranial metastases<sup>5</sup>. Targeting A $\beta$ , which suppresses neuroinflammation, in  
362 combination with immune checkpoint inhibitors could result in a more robust anti-tumor  
363 immune response and improve patient outcomes. Several therapeutic agents targeting  
364 A $\beta$  have been developed and extensively tested in clinical trials for treatment of  
365 Alzheimer's disease and could be repurposed for treatment of melanoma brain  
366 metastasis. One possibility includes BACE small molecule inhibitors, exemplified by  
367 LY2886721, which provided proof-of-principle efficacy in our preclinical models. Another  
368 attractive approach is the use of anti-A $\beta$  antibodies, which have been extensively tested  
369 in clinical trials for Alzheimer's disease. Anti- A $\beta$  antibodies successfully sequestered A $\beta$   
370 in Alzheimer's patients <sup>60</sup> but lacked clinical efficacy <sup>61</sup>. In Phase III clinical trials, anti-  
371 A $\beta$  antibodies were tolerated at high doses for extended periods of time without an  
372 increase in rates of adverse effects over placebo <sup>61</sup>. Given that dose limiting toxicities  
373 and adverse effects are major drivers of failure in cancer clinical trials, targeting A $\beta$  in  
374 melanoma brain metastasis with anti- A $\beta$  antibodies represents a particularly promising  
375 treatment strategy.

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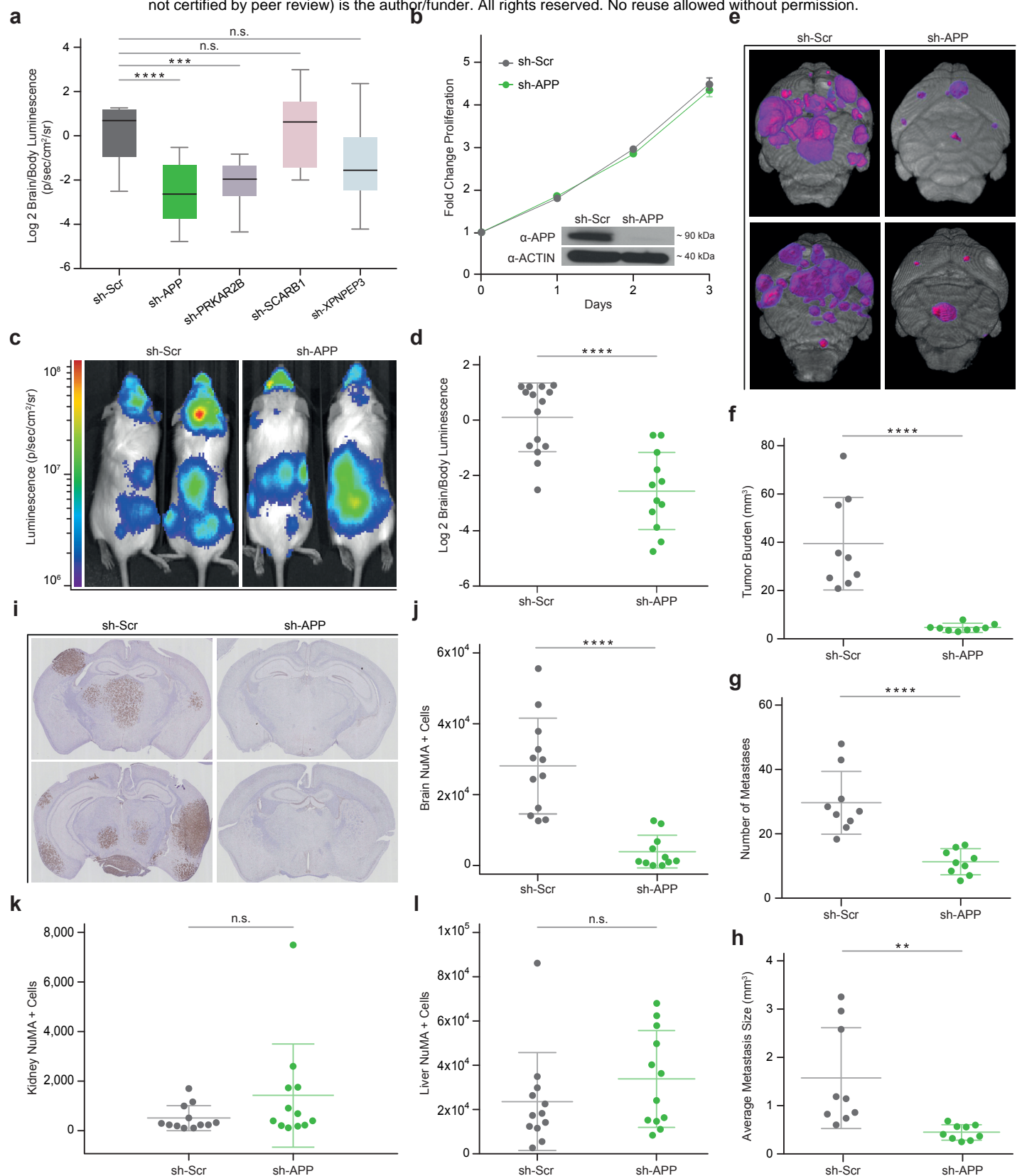
377 In summary, our studies reveal an unexpected role for tumor-secreted A $\beta$ , a polypeptide  
378 heavily implicated in Alzheimer's disease, in the adaptation of melanoma cells to the  
379 brain microenvironment and provide proof-of-principle of A $\beta$  targeting as a novel  
380 therapeutic avenue against this devastating condition.

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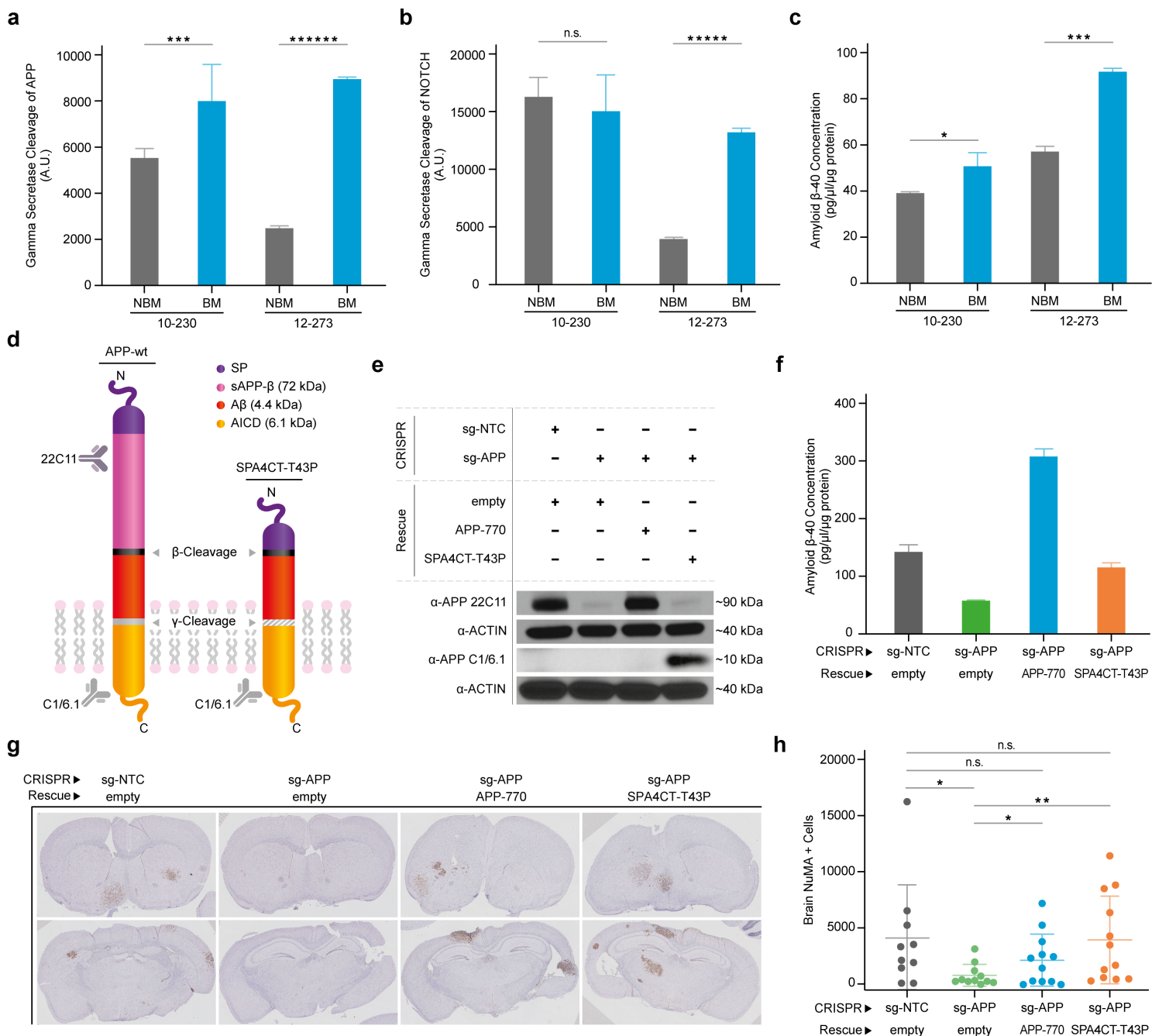
**Figure 1: Proteomics Links Melanoma Brain Metastasis and Neurodegeneration**

**a**, Diagram of the generation of patient-matched STC pairs. **b**, Representative IVIS images of 12-273 STC pair at 29 days post-intracardiac injection in mice. **c**, Quantified brain/body luminescence ratio on day 29 on 12-273 NBM vs BM (\*  $p < 0.05$ ). **d**, Number of brain metastases quantified by microscopy of serial FFPE sections with H&E staining. (12-273 NBM vs BM,  $p < 0.0005$ ;  $n = 2$  independent experiments with 10 mice per group, representative experiment shown). **e-g**, Mass spectrometry analysis of whole cell lysates from a cohort of 14 BM and 11 NBM STCs, including 3 patient-matched pairs. **e**, Top enriched pathways ( $p < 0.001$ ) in BM vs NBM STCs identified from KEGG Pathway analysis of global protein levels. **f**, Volcano plot of mean  $\text{Log}_2$  BM/NBM fold change of global protein levels and  $-\text{Log}_{10}$  p-values. Pink – proteins with mean  $\text{Log}_2$  BM/NBM fold change  $> 0.6$  (1.5 BM/NBM fold change) or  $< -0.6$  (2/3 BM/NBM fold change). **g**, Comparison of mean paired STC BM/NBM  $\text{Log}_2$  fold change to unpaired STC BM/NBM mean  $\text{Log}_2$  fold change. Pink – candidates selected for in-vivo mini-screen. **h**, Representative electron microscopy images of paired STCs. Yellow circles outline mitochondria. **i**, Quantification of average mitochondrial length in paired STCs. 10-230 NBM vs BM (\*\*\*\*  $p < .00005$ ), 12-273 NBM vs BM (\*\*  $p < .005$ ). **j**, Seahorse MitoStress analysis of oxygen consumption rate in 12-273 NBM and BM. Basal oxygen consumption rate of 12-273 NBM vs BM (\*\*\*\*  $p < .00005$ ;  $n = 3$  independent experiments, 4-6 biological replicates per group per experiment. representative experiment shown)



**Figure 2: APP is Specifically Required for Melanoma Brain Metastasis**

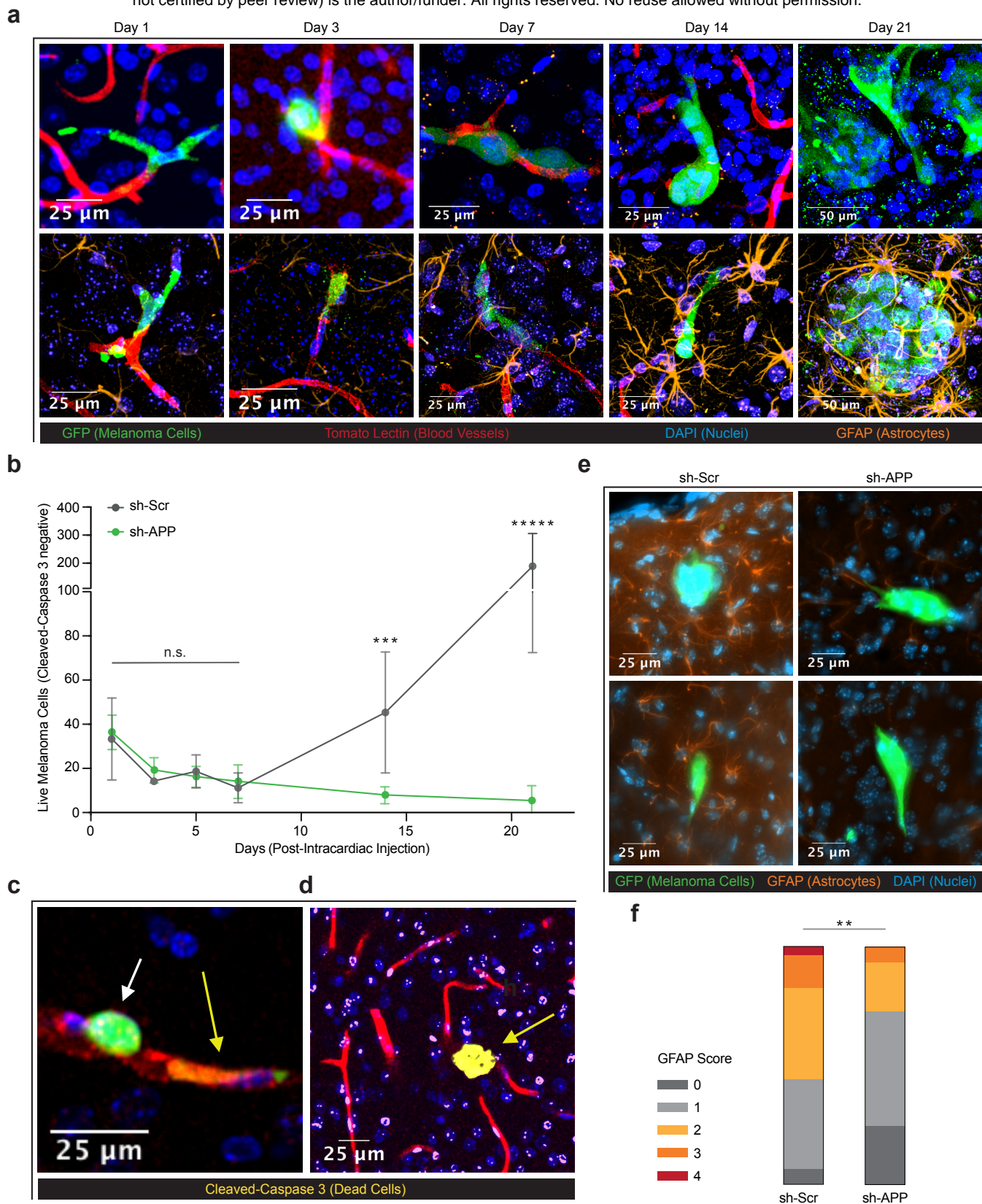
**a**, Quantified Log<sub>2</sub> Brain/Body luminescence 35 days post-intracardiac injection in mice of 12-273 BM with shRNA-mediated silencing of selected candidates or scrambled hairpin control (sh-Scr). sh-Scr vs sh-APP (\*\*\*\*  $p < 0.00005$ ), sh-Scr vs sh-PRKAR2B (\*\*  $p < 0.005$ ). (n = 1 experiment, 10-12 mice per group. Box = Interquartile range. Error bars = min to max.) **b**, Fold change in-vitro proliferation and western blot analysis of 12-273BM cells transduced with sh-APP vs sh-Scr. **c**, Representative IVIS images at day 35. **d**, Quantified Log<sub>2</sub> Brain/Body luminescence at day 35 of mice injected with 12-273BM cells transduced with sh-Scr vs sh-APP lentivirus (\*\*\*\*  $p < 0.00005$ ). (e-g), Ex-vivo brain MRI of mice injected with 12-273BM sh-Scr vs sh-APP: (n=1 experiment, 9 mice per group). **e**, Representative images. Pink-purple – brain metastasis. Quantification of **f**, brain metastatic burden (\*\*\*\*  $p < 0.00005$ ); **g**, number of brain metastases (\*\*\*\*  $p < 0.00005$ ) and **h**, average metastasis size by MRI. (\*\*  $p < 0.005$ ) (i-l), Labeling of metastatic cells by anti-NuMA immunohistochemistry on FFPE brain slides of mice injected with sh-Scr vs sh-APP: **i**, Representative brain images; (**j-l**), Quantification of NuMA+ metastatic cells in (**j**) mouse brain (\*\*\*\*  $p < 0.00005$ ), (**k**) kidneys, and (**l**) livers.



**Figure 3: Amyloid Beta ( $A\beta$ ) is the form of APP required for Melanoma Brain Metastasis**

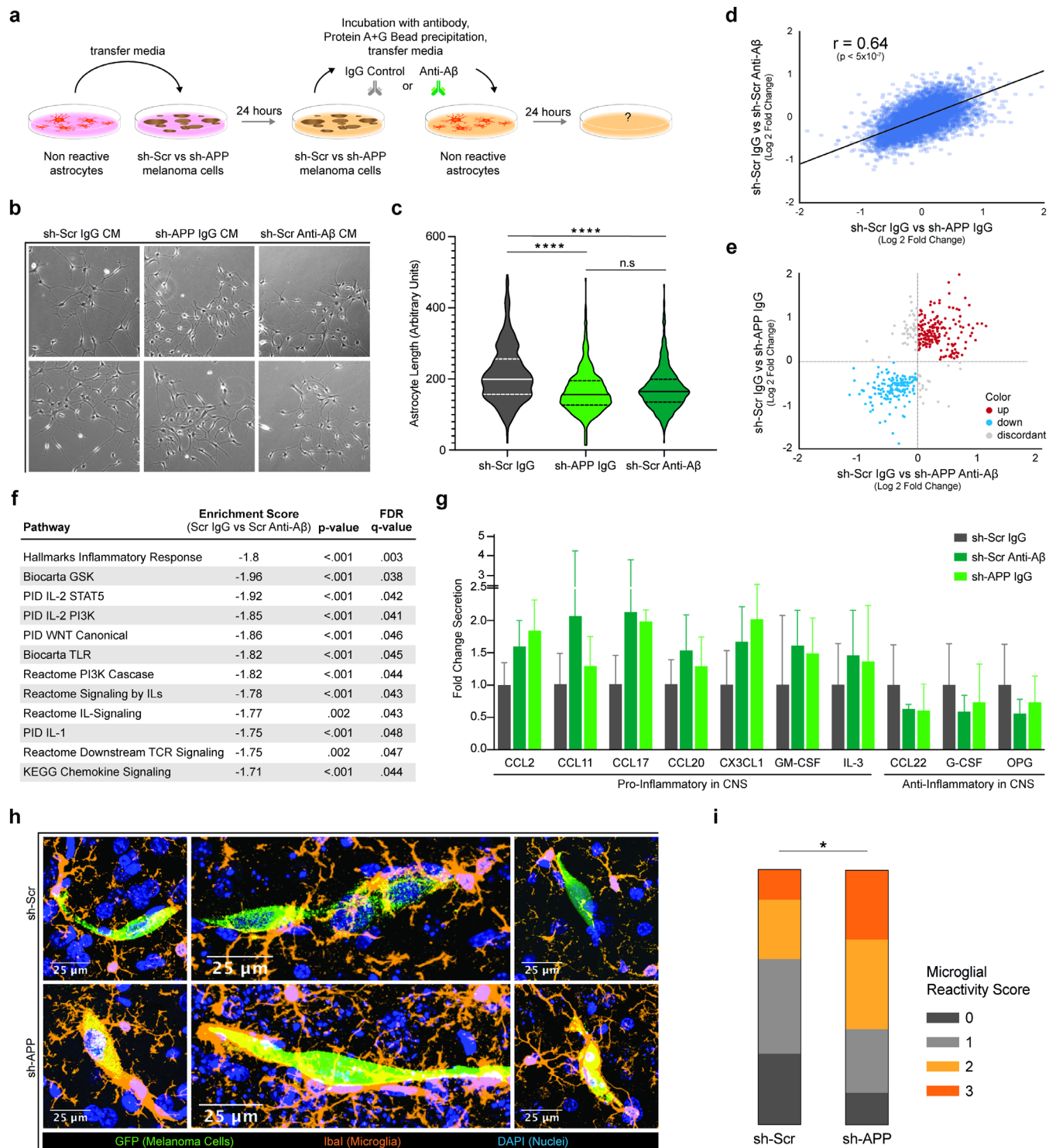
**a**, Quantification of gamma-secretase cleavage of APP. 10-230 NBM vs BM (\*  $p < 0.05$ ), 12-273 NBM vs BM (\*\*\*\*\*  $p < 0.000005$ ). **b**, Quantification of gamma-secretase cleavage of NOTCH. 12-273 NBM vs BM (\*\*\*\*\*  $p < 0.000005$ ) (two independent experiments,  $n = 4$  biological replicates for group, representative experiment shown). **c**, Quantification of  $A\beta$  secretion by ELISA. 10-230 NBM vs BM (\*  $p < 0.05$ ), WM-4071 NBM vs BM (\*\*\*  $p < 0.0005$ ) (two independent experiments,  $n = 2-4$  biological replicates for group, representative experiment shown). **d**, Diagram of wildtype APP and SPA4CT-T43P. **e**, Western blot analysis using anti-APP 22C11 (using actin as loading control) in 12-273 BM infected cells. **f**, Quantification of  $A\beta$  secretion by ELISA in 12-273 BM infected cells. **g**, Representative images of FFPE brain slides with labeling of metastatic cells by anti-NuMA immunohistochemistry. **h**, Quantification of NuMA+ metastatic cells in mouse brains. sg-NTC vs sg-APP (\*  $p < 0.05$ ), sg-APP vs APP-770 (one sided t-test \*  $p < 0.05$ ), sg-APP vs SPA4CT-T43P (one sided t-test \*\*  $p < 0.005$ ). ( $n = 10-12$  mice per group).





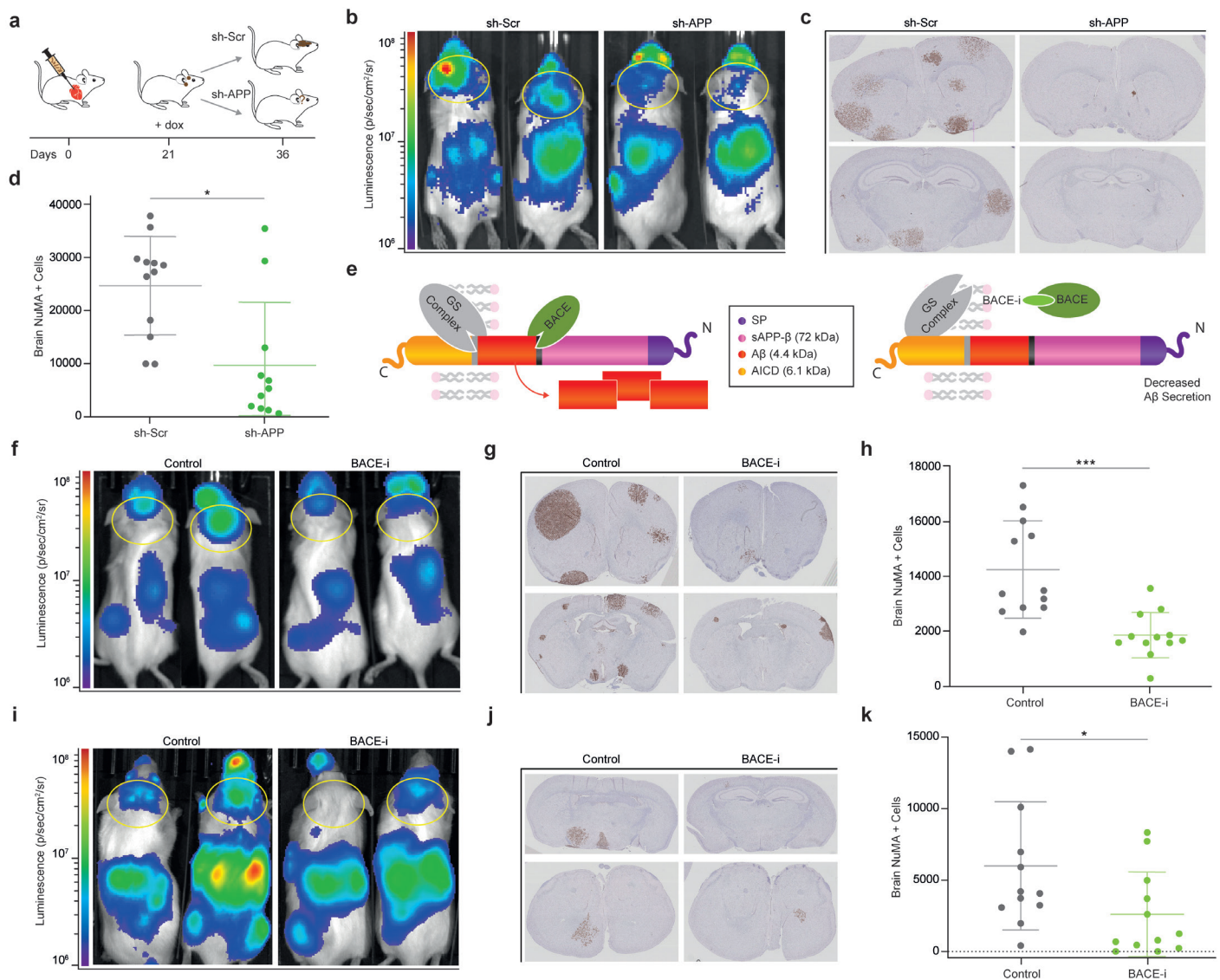
**Figure 4: Melanoma-secreted A $\beta$  is Required for Late Growth and Survival in the Brain Parenchyma**

**a**, Representative images of brain slice immunofluorescence of 12-273 BM cells at days 1, 3, 7, 14 and 21. Fluorescent markers: green = anti-GFP (melanoma cells), red = tomato lectin (blood vessels), blue = DAPI (nuclei), orange = anti-GFAP (astrocytes). (Images are from 3 mouse brains per group per time point per experiment, 2 independent experiments). **b**, Quantification of live 12-273 BM cells in the brain parenchyma over time after intracardiac injection. Day 14 sh-Scr vs. sh-APP (\*\* $p < .0005$ ), day 21 sh-Scr vs. sh-APP (\*\*\*\* $p < .000005$ ). ( $n = 2$  independent experiments, 6 mice per group in total). **c, d**, Images of brain slice immunofluorescence showing live (white arrow) and dead (yellow arrows) cells. Fluorescent marker: yellow = anti-Cleaved-Caspase 3 (dead cells). **e**, Representative images of brain slice immunofluorescence at day 10 post intracardiac injection. **f**, Quantification of qualitative scoring of astrocyte reactivity to live melanoma cells at day 10 post-intracardiac injection. sh-Scr vs sh-APP (chi-square 0-1 = negative, 2-4 = positive \*\* $p < 0.005$ ) ( $n = 2$  independent experiments, 4 mouse brains per group, representative experiment shown)



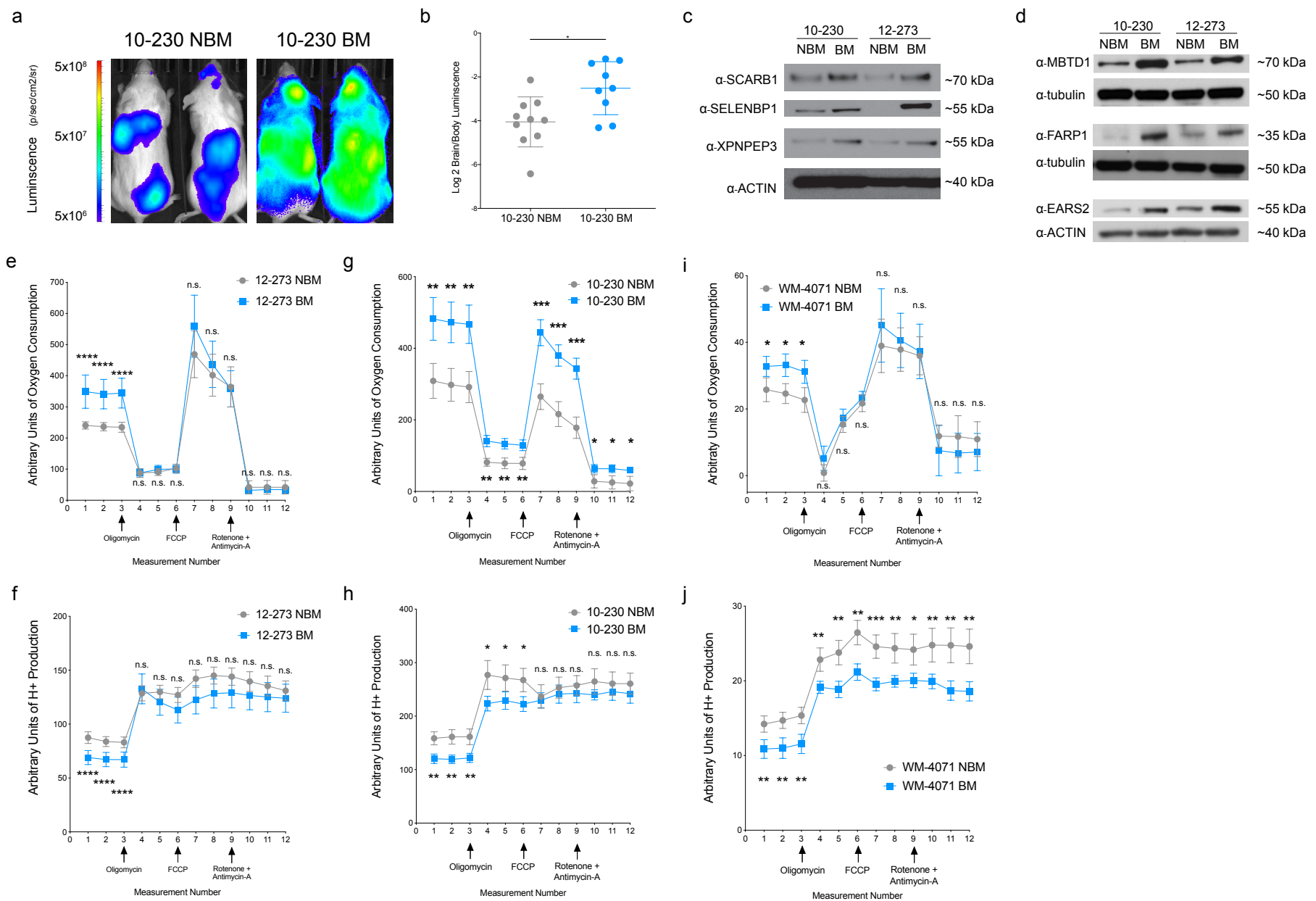
**Figure 5: Melanoma-secreted A $\beta$  Induces an Anti-Inflammatory Response in Astrocytes and Inhibits Microglial Activation**

a, Diagram of experimental method used to expose astrocytes to melanoma-conditioned media with and without A $\beta$ . b, Representative images of astrocytes after 24-hour exposure to melanoma-conditioned media (CM). c, Quantification of astrocyte length. Sh-Scr IgG CM vs sh-APP IgG CM (\*\*\*\*  $p < 0.00005$ ), sh-Scr IgG CM vs sh-Scr Anti-A $\beta$  (\*\*\*\*  $p < 0.00005$ ). (n = two independent experiments, 4 biological replicates per group, representative experiment shown). d, e Gene expression changes induced in astrocytes upon removal of A $\beta$  from melanoma conditioned media by genetic silencing of APP in melanoma cells (x axis – sh-Scr IgG CM vs sh-APP IgG CM) compared to changes induced by direct immunoprecipitation of A $\beta$  from conditioned media (y axis – sh-Scr IgG CM vs sh-Scr Anti-A $\beta$  CM). f, Enriched pathways identified from GSEA analysis of global gene expression changes in astrocytes exposed to sh-Scr IgG CM vs sh-Scr Anti-A $\beta$  CM. (n = 2 biological replicates per group) g, Quantification of cytokine secretion by cytokine array in astrocytes exposed to melanoma conditioned media (two independent experiments, 4 biological replicates per group, representative experiment shown). h, Representative images of microglia surrounding 12-273 melanoma cells at day 10 post intracardiac injection. Fluorescent markers: green = anti-GFP (melanoma cells), orange = anti-Ibal (microglia), blue = DAPI (nuclei). i, Quantification of qualitative scoring of microglial reactivity to melanoma cells at day 10 post-intracardiac injection. sh-Scr vs sh-APP (chi-square 0-1 = negative, 2-3 = positive \*  $p < 0.05$ ). (n = 4 mouse brains per group)



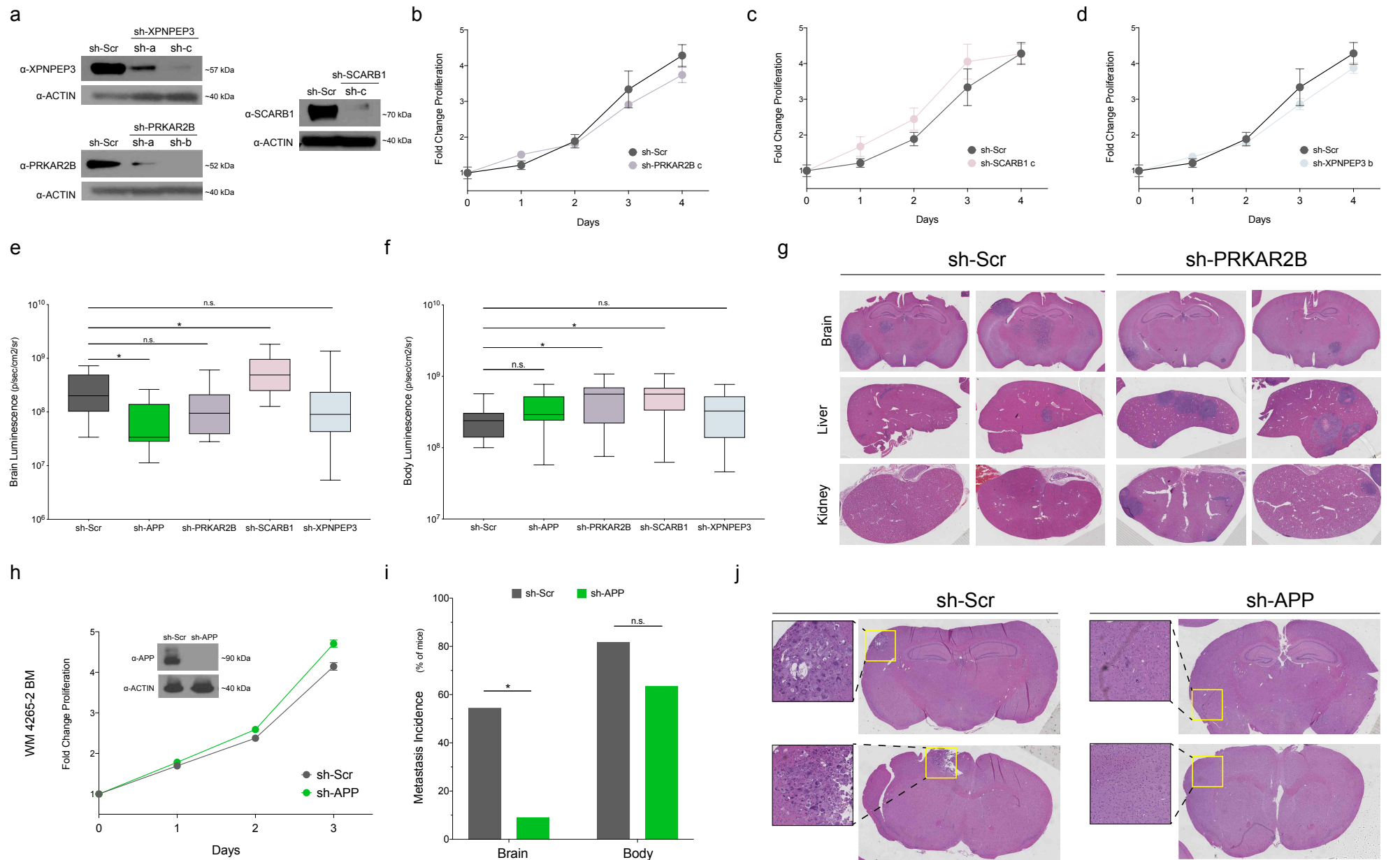
## Figure 6: A $\beta$ is a Promising Therapeutic Target for Treatment of Brain Metastasis

**a**, Diagram of therapeutic simulation experiment inducing silencing of APP in established brain metastases. **b**, Representative IVIS images at day 37 post-intracardiac injection. **c**, Representative images of FFPE brain slides with labeling of metastatic cells by anti-NuMA immunohistochemistry. **d**, Quantification of NuMA+ metastatic cells in mouse brains. sh-Scr vs sh-APP (\*  $p < 0.05$ ). (n = 10-12 mice per group) **e**, Diagram of APP cleavage and beta-secretase inhibition of A $\beta$  production. **f,i** Representative IVIS images at Day 28 post intracardiac injection with 12-273 BM STC (**f**) and 5B1 melanoma cell line (**i**). **g,j**, Representative images of FFPE brain slides with labeling of metastatic cells by anti-NuMA immunohistochemistry (**g** – 12-273 BM, **j** – 5B1). **h,k** Quantification of NuMA+ metastatic cells in mouse brains. 12-273 BM Control vs BACE-i (**h** \*\*\*  $p < 0.0005$ ), 5B1 Control vs BACE-i (**k** \*  $p < 0.05$ ) (n = 10-12 mice per group)



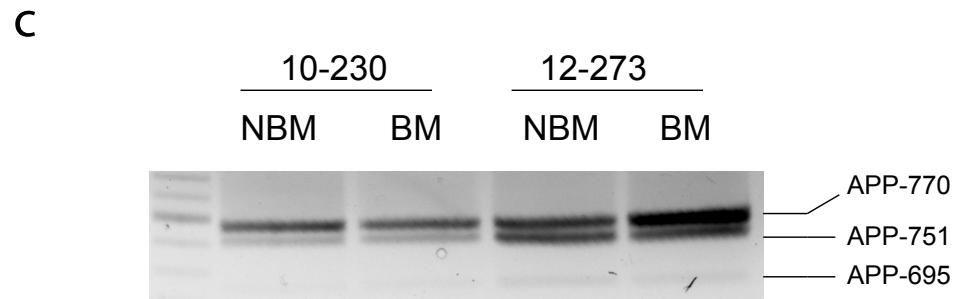
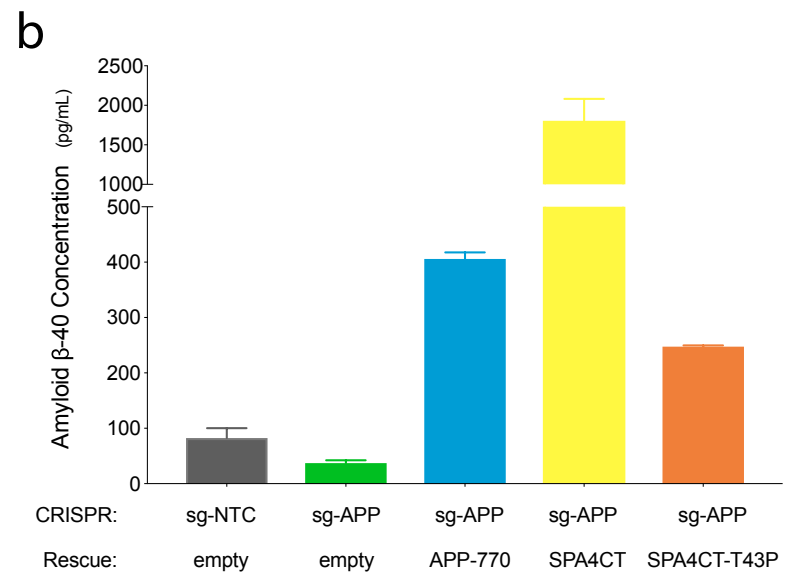
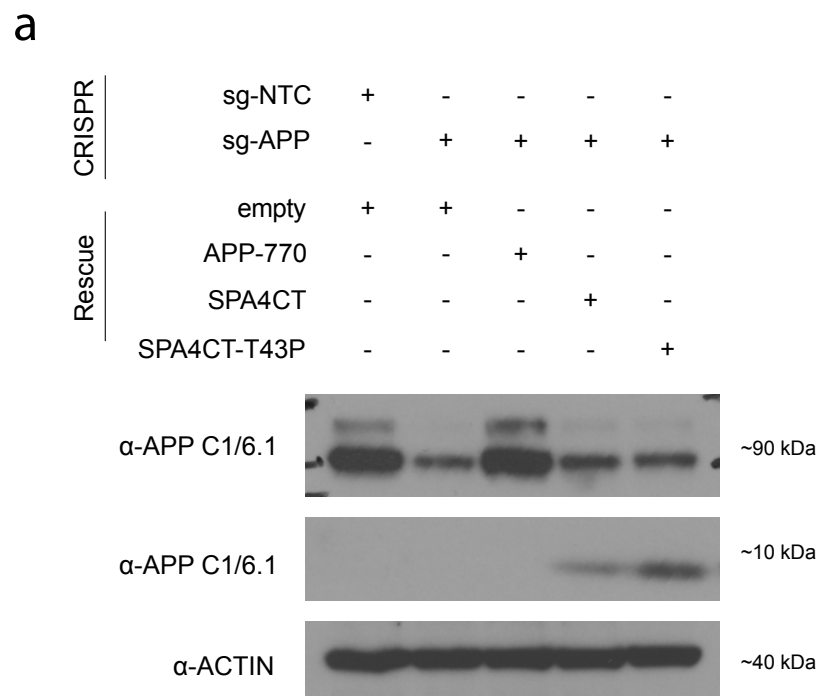
### Extended Data Figure 1:

**a**, Representative IVIS images of 10-230 STC pair at 35 days post-intracardiac injection in mice. **b**, Quantified brain/body luminescence ratio on day 35. 10-230 NBM vs BM (\*  $p < 0.05$ ). (n = 2 independent experiments, 10 mice per group, representative data shown). **c,d** Western blot analysis of STC pairs for differentially expressed proteins identified in proteomics. **e-j**, Seahorse metabolic analysis of STC pairs. 12-273 pair oxygen consumption (**e**, \*\*\*\*  $p < 0.00005$ ) and H<sup>+</sup> production (**f**, \*\*\*\*  $p < 0.00005$ ), 10-230 pair oxygen consumption (**g**, \*\*  $p < 0.005$ ) and H<sup>+</sup> production (**h**, \*\*  $p < 0.005$ ), WM-4071 pair oxygen consumption (**i**, \*  $p < 0.05$ ) and H<sup>+</sup> production (**j**, \*\*  $p < 0.005$ ). (10-230 pair, 12-273 pair, n = 3 independent experiments, 4-6 biological replicates per group, representative experiment shown. WM 4071 pair n = 1 experiment, 4-6 biological replicates per group)



### Extended Data Figure 2:

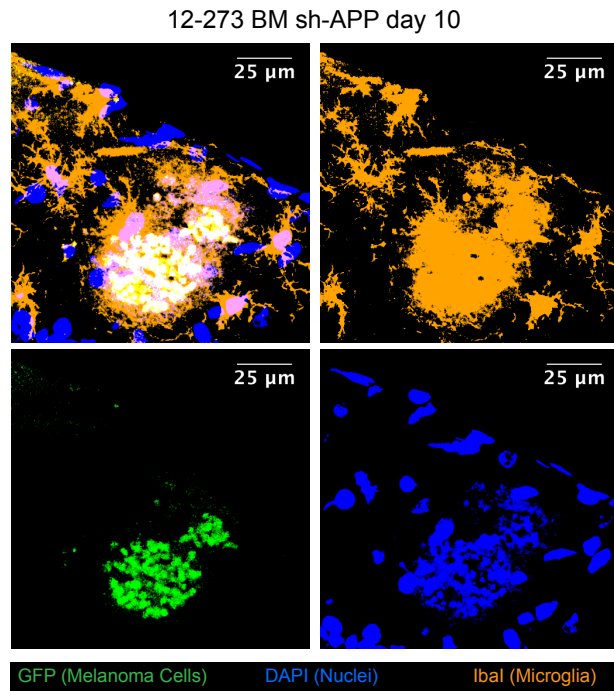
**a**, Western blot of analysis of 12-273 BM cells transduced with the indicated sh-RNA carrying lentivirus. The following sh-RNAs were used in the miniscreen: sh-XPNPEP3-c, sh-PRKARB-c, sh-SCARB1-c. **b-d**, Fold change in-vitro proliferation and western blot analysis of 12-273BM cells with **(b)** sh-Scr vs sh-PRKAR2B, **(c)** sh-Scr vs sh-SCARB1, **(d)** sh-Scr vs sh-XPNPEP3. **e**, Quantified brain luminescence in mice 35 days post-intracardiac injection of 12-273 BM with shRNA-mediated silencing of selected candidates or sh-Scr. sh-Scr vs sh-APP (\*  $p < 0.05$ ), sh-Scr vs sh-SCARB1 (\*  $p < 0.05$ ). (Box = Interquartile range. Error bars = min to max.) **f**, Quantified body luminescence in mice 35 days post-intracardiac injection of 12-273 BM with shRNA-mediated silencing of selected candidates or sh-Scr. sh-Scr vs sh-PRKAR2B (\*  $p < 0.05$ ), sh-Scr vs sh-SCARB1 (\*  $p < 0.05$ ). (Box = Interquartile range. Error bars = min to max.) **g**, Representative H&E-stained FFPE sections of brains, livers, and kidneys from mice injected with 12-273 BM sh-Scr and sh-PRK-AR2B. Each column contains images from the same mouse. ( $n = 1$  experiment, 10-12 mice per group). **h**, Fold change in-vitro proliferation and western blot analysis of WM 4265-2 BM cells with sh-Scr vs sh-APP. **i**, Incidence of brain and body metastasis in mice injected with WM 4265-2 BM at 88 days post-intracardiac injection. sh-Scr vs sh-APP brain (chi square, \*  $p < 0.05$ ). **j**, H&E stained FFPE sections of brains from mice injected with WM-4265-2 BM sh-Scr and sh-APP. Upper sh-APP image is from the single sh-APP mouse with brain metastasis. ( $n = 10-12$  mice per group). **k**, Video of 3D projections from MRI images of representative of mouse brains. Pink-purple – brain metastasis (12-273 BM). Top row: sh-Scr. Bottom row: sh-APP.



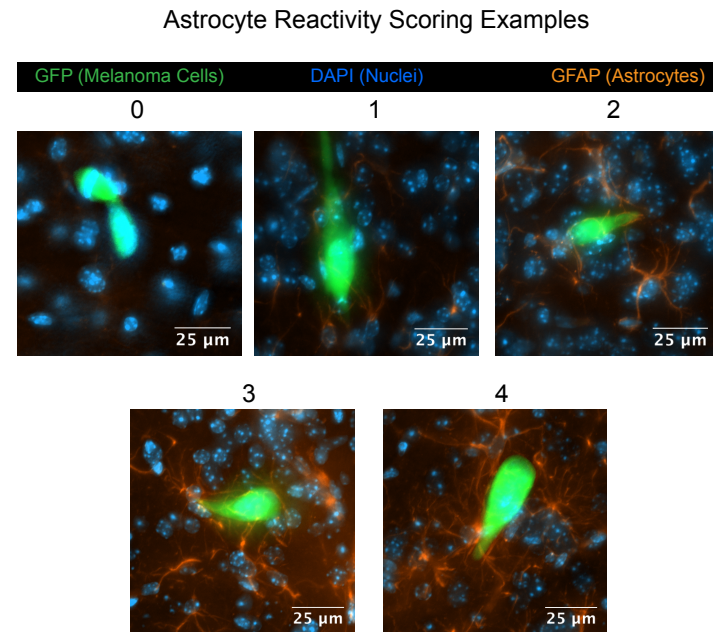
### Extended Data Figure 3:

**a**, Western blot analysis of 12-273 BM infected cells for APP and SPA4CT. **b**, Quantification of A $\beta$  secretion by ELISA in 12-273 BM infected cells. **c**, Semi-quantitative RT-PCR of APP isoforms in 10-230 and 12-273 STC pairs.

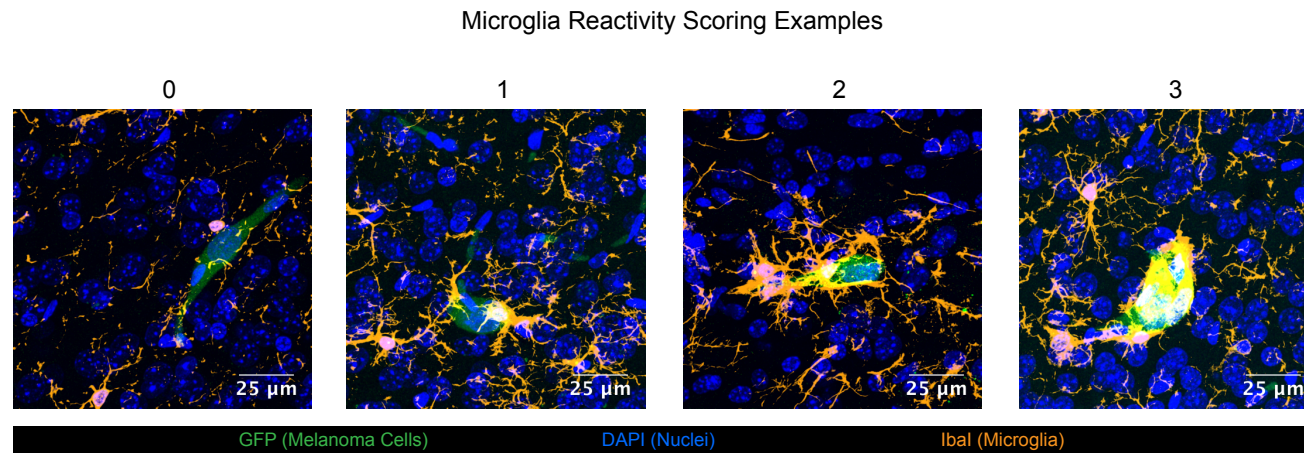
a



b



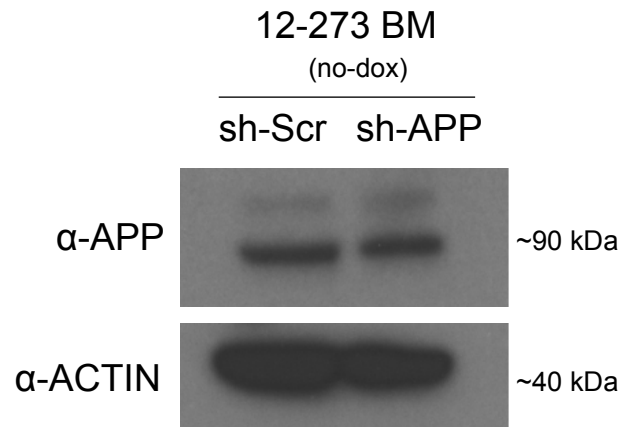
c



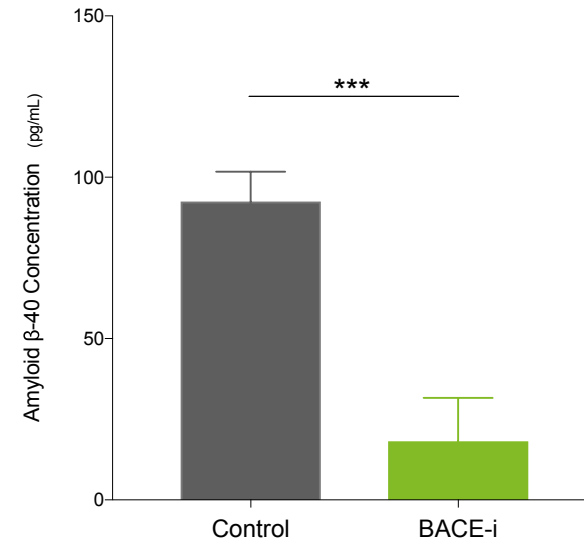
#### Extended Data Figure 4:

**a**, Image of microglial phagocytosis of apoptotic bodies from brain slices of mice instilled with 12-273 BM sh-APP cells 10 days post-intracardiac injection. Fluorescent markers: green = anti-GFP (melanoma cells), blue = DAPI (nuclei), orange = anti-Iba1 (microglia). **b**, Representative images of astrocyte reactivity scores. Fluorescent markers: green = anti-GFP (melanoma cells), blue = DAPI (nuclei), orange = anti-GFAP (astrocytes). **c**, Representative images of microglia reactivity scores. Fluorescent markers: green = anti-GFP (melanoma cells), blue = DAPI (nuclei), orange = anti-Iba1 (microglia). **d-i**, 3D projection of confocal images of melanoma cells in brain parenchyma at Day 1 (**d**), Day 3 (**e**), Day 7 (**f**), Day 14 (**g**), and Day 21 (**h-i**). Fluorescent markers: green = anti-GFP (melanoma cells), blue = DAPI (nuclei), orange = anti-GFAP (astrocytes).

**a**



**b**



### Extended Data Figure 5

**a**, Western blot analysis of 12-273 BM sh-Scr and sh-APP cells cultured without doxycycline on day of intracardiac injection. **b**, Quantification of plasma A $\beta$  levels in Control vs BACE-i treated mice at 28 days post-intracardiac injection with 12-273 BM cells.



382

## 383 **Supplementary Videos**

384

385 **Supplementary video 1** – Representative 3D renderings from MRI images of brains  
386 from mice at 35 days after intracardiac injection with 12-273 BM. Pink/purple =  
387 metastasis. Top row: sh-Scr. Bottom row: sh-APP.

388

389 **Supplementary Videos 2-6** – 3D renderings from confocal images of 12-273 BM sh-  
390 Scr cells in mouse brain parenchyma at the following times after intracardiac injection:  
391 day 1 (Video 2), day 3 (Video 3), day 7 (Video 4), day 14 (Video 5), and day 21 (Video  
392 6). Fluorescent markers: green = anti-GFP (melanoma cells), red = tomato lectin (blood  
393 vessels), blue = DAPI (nuclei).

394

395 **Supplementary Video 7** – 3D rendering from confocal image of 12-273 BM sh-Scr cells  
396 in mouse brain parenchyma at 21 days after intracardiac injection. Fluorescent markers:  
397 green = anti-GFP (melanoma cells), red = tomato lectin (blood vessels), blue = DAPI  
398 (nuclei), orange = anti-GFAP (astrocytes).

399

400 **Supplementary Video 8** – 3D rendering from confocal image of 12-273 BM sh-APP  
401 cells at 10 days after intracardiac injection. Video depicts melanoma cells that have  
402 been completely phagocytized by microglia. Fluorescent markers: green = anti-GFP  
403 (melanoma cells), orange= anti-Ibal (microglia), blue = DAPI (nuclei)

404

405

406

## 407 **Methods**

408

### 409 **Cell culture**

410 *Melanoma short-term cultures and cell lines.* Low passage melanoma short-term  
411 cultures (STCs), derived in the Osman laboratory as described <sup>50</sup>. were grown in DMEM

412 with 10% fetal bovine serum (FBS), 1 mM Sodium Pyruvate, 4 mM L-Glutamine, 25 mM  
413 D-Glucose, 1% Nonessential Amino Acids (NEAA), 100 units/mL penicillin, and 100  
414 ug/mL streptomycin. Additional STCs, a kind gift of the Herlyn lab at the Wistar Institute,  
415 were grown in Tu2% media (80% MCDB153, 20% Leibovitz's L-15, 2% FBS, 5ug/ml  
416 Insulin (Bovine), 1.68 mM calcium chloride, 100 units/mL penicillin, 100 ug/mL  
417 streptomycin). STCs were kept below lifetime passage number of 40 for all experiments.  
418 For details of STCs utilized, see Extended Data Table 1. HEK293T cells (for lentivirus  
419 production) and 131/4-5B1 (hereafter 5B1, <sup>46</sup>) melanoma cells were grown in DMEM  
420 with 10% fetal bovine serum (FBS), 1 mM Sodium Pyruvate, 4 mM L-Glutamine, 25 mM  
421 D-Glucose, 100 units/mL penicillin, and 100 ug/mL streptomycin. Cell lines were  
422 maintained in a 5% CO<sub>2</sub> incubator at 37 °C and were routinely tested for Mycoplasma  
423 contamination.

424

425 *Astrocytes isolation and culture.* Astrocytes were purified by immunopanning and  
426 cultured in serum-free conditions as previously described <sup>62</sup>. Briefly, cortices from 5-6  
427 postnatal day 4-6 Sprague Dawley rat pups (Charles River) were dissected out and  
428 meninges and choroid plexus removed. The cortices were minced with a scalpel and  
429 digested in Papain for 40 min at 34°C under constant CO<sub>2</sub>/O<sub>2</sub> gas equilibration. The  
430 digested brain pieces were washed with CO<sub>2</sub>/O<sub>2</sub>-equilibrated Ovomuroid inhibitor  
431 solution, triturated, and spun down through a cushion gradient containing low and high  
432 Ovomuroid inhibitor layers. The resulting cell pellet was passed through a 20 µm nylon  
433 mesh to create a single cell suspension. The cells were then incubated in a 34°C water  
434 bath for 30-45 mins to allow cell-specific antigens to return to the cell surface. Negative  
435 selection was performed using Goat anti-mouse IgG + IgM (H + L), *Griffonia*  
436 (*Bandeiraea simplicifolia* lectin 1 (BSL-1), Rat anti-mouse CD45, and O4 hybridoma  
437 supernatant mouse IgM <sup>63,64</sup>, followed by positive selection for astrocytes using mouse  
438 anti-human integrin β5 (ITGB5). Purified astrocytes were detached from the panning  
439 plate with trypsin at 37°C for 3-4 min, neutralized by 30% fetal calf serum, counted,  
440 pelleted, and resuspended in 0.02% BSA in DPBS. All isolation and immunopanning  
441 steps occurred at room temperature, except for the heated digestion, incubation, and  
442 trypsinization steps. Cells were plated at 50,000 or 70,000 cells per well in 6 well plates

443 containing 2 mL/well of serum-free Astrocyte Growth Medium (50% Neurobasal  
444 Medium, 50% Dulbecco's Modified Eagle Medium (DMEM), 100 U/mL Penicillin, 100  
445  $\mu\text{g/mL}$  Streptomycin, 1 mM Sodium pyruvate, 292  $\mu\text{g/mL}$  L-Glutamine, 5  $\mu\text{g/mL}$  *N*-  
446 Acetyl-L-cysteine (NAC), 100  $\mu\text{g/mL}$  BSA, 100  $\mu\text{g/mL}$  Transferrin, 16  $\mu\text{g/mL}$  Putrescine  
447 dihydrochloride, 60 ng/mL (0.2  $\mu\text{M}$ ) Progesterone, and 40 ng/mL Sodium selenite.  
448 Immediately before plating, the astrocyte trophic factor Heparin-binding EGF-like growth  
449 factor (HBEGF) was added (5 ng/mL) and media equilibrated to 37°C in a 10% CO<sub>2</sub>  
450 incubator). Cells were incubated at 37°C in 10% CO<sub>2</sub>. Media was changed (50% well  
451 volume) once per week.

452

453 *Exposure of Astrocytes to Melanoma Conditioned Media.* Astrocytes were plated at  
454  $5 \times 10^4$  astrocytes/well in 6-well plates. 12-273 BM melanoma cells were plated at  
455  $2.5 \times 10^5$  cells/well in 6-well plates. Astrocyte-conditioned media (ACM) was obtained by  
456 culture of astrocytes in astrocyte media for 1 week. 1.5mL of ACM were then transferred  
457 and incubated with 12-273 BM cells in 6-well plates for 24 hours. Media was then  
458 removed and incubated with either 4.5 ug of mouse IgG or 2.25 ug of anti-amyloid beta  
459 antibody N25 and 2.25ug of anti-APP 6E10 antibody for 1 hr at 4 degrees and 1 hr at  
460 room temperature with rotation mixing. 75ul of protein A/G beads (Pierce 88803) were  
461 washed twice with PBS, added to 1.5mL of conditioned media, and incubated for 1 hour  
462 at room temperature with rotation mixing. Beads were precipitated using a magnetic  
463 tube holder and 1.2 mL of conditioned media was transferred per well to astrocytes in a  
464 6-well plate. Astrocytes were incubated for 24 hours.

465

466 *Astrocyte Length Quantification.* After 24-hour incubation in melanoma conditioned  
467 media, pictures of astrocytes were randomly captured on light microscopy at 10x.  
468 Average astrocyte length was quantified by measurement of longest dimension of each  
469 astrocyte using ImageJ.

470

471 *Astrocyte RNA-Seq and Analysis.* After 24-hour incubation in melanoma conditioned  
472 media, RNA was harvested from astrocytes using the RNeasy Mini Kit (Qiagen 74104).  
473 RNA-Seq library preps were made using the Illumina TruSeq® Stranded mRNA LT kit

474 (Cat #RS- RS-122-2101 or RS-122-2102), on a Beckman Biomek FX instrument, using  
475 100 ng of total RNA as input, amplified by 12 cycles of PCR, and run on an Illumina  
476 4000 as single read 50. FastQC v0.11.7  
477 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to check fastq  
478 files for poor sequencing quality; all samples had high quality. Illumina adapter  
479 sequences and poor-quality bases were then trimmed using trimmomatic v0.36<sup>65</sup>.  
480 Trimmed sequences were mapped to mm10 using STAR v2.6.0a<sup>66</sup>, indexed using  
481 samtools v1.9<sup>67</sup>, then quantified for UCSC genes using HTSeq-count v0.11.1<sup>68</sup>.  
482 Comparative analysis between conditions was performed using DESeq2 v1.24.0<sup>69</sup> with  
483 default parameters.

484

485 *Measurement of Astrocyte-Secreted Cytokines.* After 24-hour incubation in melanoma  
486 conditioned media, media was removed from astrocytes and levels of cytokines were  
487 measured using the Rat XL Cytokine Array Kit (R&D Systems ARY030) using 1 mL of  
488 conditioned media per membrane. Using ImageJ, mean integrated density from spots  
489 representing different cytokines was quantified. For each detected cytokine, mean  
490 densities of sh-Scr anti-AB and sh-APP IgG were normalized to the mean sh-Scr IgG  
491 density and plotted as a mean fold change.

492

### 493 **Proteomics Analysis of Short-Term Cultures (STCs)**

494

495 *Protein isolation.* STCs at ~80% confluence at ~24 hours post-media change were  
496 scraped from 10 or 15cm plates on ice, washed once with cold PBS, and lysed in cold  
497 RIPA buffer supplemented with protease inhibitor, for 15 minutes with vortexing every 5  
498 minutes. Protein concentration was determined using Micro BCA Protein Assay Kit  
499 (Thermo Scientific 23235).

500

501 150 µg of each protein lysate were proteolytically digested and subjected to quantitative  
502 mass spectrometry on an Orbitrap Fusion Lumos mass spectrometer using isobaric  
503 tandem mass tags (TMT) similar to previous studies<sup>70,71</sup>.

504

505 *Sample preparation for mass spectrometry analysis.* 150 µg of each protein lysate were  
506 prepared using the filter-aided sample preparation (FASP) method <sup>72</sup>. Briefly, each  
507 sample was reduced with DTT (final concentration of 20 mM) at 57°C for 1 hour and  
508 loaded onto a MicroCon 30-kDa centrifugal filter unit (Millipore) pre-equilibrated with 200  
509 µl of FASP buffer [8 M urea and 0.1 M tris-HCl (pH 7.8)]. Following three washes with  
510 FASP buffer, lysates were alkylated on a filter with 50 mM iodoacetamide for 45 min in  
511 the dark. Filter-bound lysates were washed three times each with FASP buffer followed  
512 by 100 mM ammonium bicarbonate (pH 7.8). The samples were digested overnight at  
513 room temperature with trypsin (Promega) at a 1:100 ratio of enzyme to protein. Peptides  
514 were eluted twice with 100 µl of 0.5 M NaCl. The tryptic peptides were subsequently  
515 desalted using an UltraMicro Spin Column, C18 (Harvard Apparatus) and concentrated  
516 in a SpeedVac concentrator.

517

518 *TMT labeling.* The dried peptide mixture was re-suspended in 100 µl of 100 mM  
519 Triethylammonium bicarbonate (TEAB) (pH 8.5). Each sample was labeled with TMT  
520 reagent according to the manufacturer's protocol. In brief, each TMT reagent vial  
521 (0.8 mg) was dissolved in 41 µL of anhydrous ethanol and was added to each sample.  
522 The reaction was allowed to proceed for 60 min at room temperature and then  
523 quenched using 8 µL of 5% (w/v) hydroxylamine. The samples were combined at a 1:1  
524 ratio and the pooled sample was subsequently desalted using strong-cation exchange  
525 and strong-anion exchange solid-phase extraction columns (Strata, Phenomenex) as  
526 described<sup>2</sup>.

527

528 *Global Proteome Analysis.* A 500 µg aliquot of the pooled sample was fractionated  
529 using basic pH reverse-phase HPLC (as described) <sup>71</sup>. Briefly, the sample was loaded  
530 onto a 4.6 mm × 250 mm Xbridge C18 column (Waters, 3.5 µm bead size) using an  
531 Agilent 1260 Infinity Bio-inert HPLC and separated over a 90 min linear gradient from 10  
532 to 50% solvent B at a flow rate of 0.5 ml/min (Buffer A = 10 mM ammonium formate, pH  
533 10.0; Buffer B = 90% ACN, 10 mM ammonium formate, pH 10.0). A total of 120  
534 fractions were collected and non-concatenated fractions combined into 40 final

535 fractions. The final fractions were concentrated in the SpeedVac and stored at -80 °C  
536 until further analysis.

537

538 *LC-MS/MS analysis.* An aliquot of each final fraction was loaded onto a trap column  
539 (Acclaim® PepMap 100 pre-column, 75 µm × 2 cm, C18, 3 µm, 100 Å, Thermo  
540 Scientific) connected to an analytical column (EASY-Spray column, 50 m × 75 µm ID,  
541 PepMap RSLC C18, 2 µm, 100 Å, Thermo Scientific) using the autosampler of an Easy  
542 nLC 1000 (Thermo Scientific) with solvent A consisting of 2% acetonitrile in 0.5% acetic  
543 acid and solvent B consisting of 80% acetonitrile in 0.5% acetic acid. The peptide  
544 mixture was gradient eluted into the Orbitrap Lumos Fusion mass spectrometer  
545 (Thermo Scientific) using the following gradient: 5%-23% solvent B in 100 min, 23% -  
546 34% solvent B in 20 min, 34% -56% solvent B in 10 min, followed by 56%- 100%  
547 solvent B in 20 min. Full scans were acquired with a resolution of 60,000 (@  $m/z$  200), a  
548 target value of 4e5 and a maximum ion time of 50 ms. After each full scan the most  
549 intense ions above 5E4 were selected for fragmentation with HCD using the “Top  
550 Speed” algorithm. The MS/MS were acquired in the Orbitrap with a resolution of 60,000  
551 (@  $m/z$  200), isolation window of 1.5  $m/z$ , target value of 1e5, maximum ion time of  
552 60 ms, normalized collision energy (NCE) of 35, and dynamic exclusion of 30 s.

553

554 *Data analysis.* The MS/MS spectra were searched against the UniProt human reference  
555 proteome with the Andromeda<sup>73</sup> search engine integrated into the MaxQuant<sup>74</sup>  
556 environment (version 1.5.2.8) using the following settings: oxidized methionine (M),  
557 TMT-labeled N-term and lysine, acetylation (protein N-term) and deamidation  
558 (asparagine and glutamine) were selected as variable modifications, and  
559 carbamidomethyl (C) as fixed modifications; precursor mass tolerance was set to  
560 10 ppm; fragment mass tolerance was set to 0.01 Th. The identifications were filtered  
561 using a false-discovery rate (FDR) of 0.01 using a target-decoy approach at the protein  
562 and peptide level. Only unique peptides were used for quantification and only proteins  
563 with at least two unique peptides were reported. Data analysis was performed using  
564 Perseus. Protein levels were median centered and log2 normalized. To identify proteins  
565 differentially expressed proteins between the BM and NBM cohorts, a Welch’s t-test

566 was performed between unpaired 14 BM and 11 NBM unpaired samples and a paired t-  
567 test was performed on the 3 sample pairs. Top differentially expressed genes (defined  
568 here as p-value < 0.05) were assessed at the pathway level using DAVID GO-term  
569 enrichment with an FDR < 0.001 (ref).

570

## 571 **Animal Studies**

572 All experiments were conducted following protocols approved by the NYU Institutional  
573 Animal Care Use Committee (IACUC) (protocol number s16-00051). NOD/SCID/IL2yR<sup>-/-</sup>  
574 male mice (Jackson Labs, Cat# 05557) at 6-8 weeks were used for in-vivo studies.

575

576 *Long-term brain metastasis assays.* 1 x 10<sup>5</sup> 12-273 NBM, 1 x 10<sup>5</sup> 12-273 BM, 1 x 10<sup>5</sup>  
577 10-230 NBM, 1 x 10<sup>5</sup> 10-230 BM, 2 x 10<sup>5</sup> 5B1 cells, or 1.5 x 10<sup>5</sup> WM-4265 BM cells  
578 suspended in 100 ul of PBS were injected with ultrasound guidance (Visualsonics Vevo  
579 770 Ultrasound Imaging System) into the left ventricle of mice anesthetized with  
580 isoflurane. Mice were monitored weekly for metastatic progression by in-vivo Bio-  
581 luminescent imaging (BLI). Upon substantial weight loss and/or signs of distress  
582 (neurological signs, abnormal locomotion) in any experimental mice, experimental  
583 endpoint was established. At experimental endpoint, all mice in all experimental groups  
584 were euthanized by perfusion.

585 For all long-term brain metastasis assays, 12 mice per group underwent intracardiac  
586 injection with cancer cells. Experimental group sizes vary from 10 to 12 mice due to  
587 infrequent instances of unsuccessful intracardiac injection.

588

589 *In-vivo BLI.* 10 minutes prior to imaging, luciferin substrate (150mg/kg) was  
590 administered to mice by intraperitoneal injection. Mice were anesthetized with isoflurane  
591 and imaged by IVIS Illumina instrument (PerkinElmer) for an automatically-determined  
592 duration (1-120 sec). Signal was quantified by measurement of total luminescent flux  
593 (p/sec/cm<sup>2</sup>/sr) in drawn brain and body regions of interest.

594

595 *Perfusion.* Mice were anesthetized with a ketamine (100mg/kg) and xylazine (10 mg/kg)  
596 cocktail by intraperitoneal injection. The heart was exposed by gross dissection and an

597 incision was made in the right atrium. Subsequently, 10 mL of PBS followed by 10 mL of  
598 4% PFA was injected into the left ventricle.

599

600 *Short-term brain metastasis assays and brain slice immunofluorescence.*  $1 \times 10^5$  (for live  
601 melanoma cell quantification) or  $5 \times 10^5$  (for astrocyte and microglial scoring) 12-273 BM  
602 cells were introduced by intracardiac injection. Mice were euthanized by perfusion at  
603 specified time points post-intracardiac injection. Where relevant, 100 ug of Dylight 647  
604 labeled Lycopersicon Esculentum (Tomato Lectin) were injected into the left ventricle 3  
605 min prior to perfusion. For these assays, 3-6 mice with successful intracardiac injection  
606 were used per group per time point.

607

608 *Drug treatment experiments.* Protocol for long-term brain metastasis assay (as  
609 described above) was performed. LY288671 (75mg/kg/day) was administered to mice in  
610 food pellets starting one week prior to intracardiac injection of cancer cells and  
611 continuing through the experimental endpoint.

612

613 *Continuous shRNA-mediated gene silencing.* Protocol for long-term brain metastasis  
614 assay (as described above) was performed. Doxycycline hyclate (200mg/kg/day) was  
615 administered to mice in food pellets starting two days prior to intracardiac injection of  
616 cancer cells and continuing for the experimental duration.

617

618 *Induction of gene silencing in growing metastases.* Protocol for long-term brain  
619 metastasis assay (as described above) was performed. Mice were administered a  
620 normal diet at the beginning of the experiment. 21 days after intracardiac injection of  
621 cancer cells, gene silencing was induced by administration of Doxycycline hyclate  
622 (200mg/kg/day) in food pellets.

623

#### 624 **Mouse tissue processing, histology and NuMA Immunohistochemistry**

625 Organs harvested from PFA-perfused mice were fixed in 10% formalin for 48 hours.  
626 Prior to embedding, brains were sectioned grossly into thirds coronally and livers were  
627 sectioned by lobe. Organs were embedded in paraffin and cut into 5uM thick sections.



628

629 *Histological Analysis Comparing Short-Term Culture Pairs.* The embedded brain thirds  
630 were sectioned coronally through the entire length of tissue at an interval of 50uM. All  
631 sections were stained with H&E. Number of unique metastases present per brain was  
632 assessed by a pathologist by identifying and tracking metastases through serial  
633 sections, ensuring each metastasis was counted only once. Slides were blinded to  
634 pathologist (R. R.) prior to analysis.

635

636 *Sectioning and NuMA Immunohistochemistry.* For kidney and liver, sections were  
637 obtained from one and two representative levels respectively. One section per level was  
638 stained with H&E. For brain, sections were obtained from 2 (12-273 BM) or 4 (5B1)  
639 evenly space levels of the embedded coronal thirds. In total, this resulted in 6(12-273  
640 BM) or 24 (5B1) serial coronal brain section levels for metastatic quantification. One  
641 section per level was stained with H&E. Chromogenic Immunohistochemistry was  
642 performed on a Ventana Medical Systems Discovery XT instrument with online  
643 deparaffinization and using Ventana's reagents and detection kits unless otherwise  
644 noted. Unconjugated, polyclonal rabbit anti-human Nuclear Mitotic Apparatus Protein  
645 (NuMA; Abcam Cat# 97585 Lot# B115626 RRID: AB\_1855299) was used for labeling.  
646 Sections were deparaffinized in xylene and rehydrated in graded ethanol followed by  
647 rinsing in deionized water. Epitope retrieval was performed in a 1200-Watt microwave  
648 oven at 100% power in 10 mM sodium citrate buffer, pH 6.0 for 10 minutes. NuMA  
649 antibody was diluted 1:7000 in Tris-BSA (25 mM Tris, 15 mM NaCL, 1% BSA, pH 7.2)  
650 and incubated for 12 hours. Primary antibody was detected with goat anti-rabbit HRP  
651 conjugated multimer, and the complexes were visualized with 3,3 diaminobenzidine  
652 and enhanced with copper sulfate. Slides were washed in distilled water, counterstained  
653 with hematoxylin, dehydrated and mounted with permanent media. Appropriate positive  
654 and negative controls were run in parallel to study sections.

655

656

657 *NuMA+ Cell Quantification.* Scanned images of slides were analyzed using Viziopharm  
658 software. Briefly, for brain, regions on interest were drawn to include all brain

659 parenchymal tissue and exclude leptomeningeal areas. For kidney and liver, regions of  
660 interest were drawn to include all organ parenchyma. A cell identification and binary  
661 categorization algorithm was used to quantify the total number of NuMA-positive cells  
662 per slide. Settings for successful discrimination between NuMA-positive and NuMA-  
663 negative cells were established for each organ through testing on positive and negative  
664 control areas in multiple slides.

665

### 666 **Brain Slice Immunofluorescence**

667 Brains from perfused mice were fixed overnight in 4% PFA. Brains were sectioned using  
668 a vibratome (Leica) into 50 $\mu$ M-thick slices. Slices were taken from 3-4 levels evenly  
669 spaced through the cortex. Slices were incubated in blocking buffer (10% Normal Goat  
670 Serum, 2% BSA, 0.25% Triton) in PBS for 2 hours at room temperature. Primary  
671 antibodies were incubated overnight at 4 degrees in blocking buffer and were washed 4  
672 times for 5 min in 0.25% Triton in PBS. Slices were incubated in secondary antibody in  
673 blocking solution for 2 hours and were washed 4 times for 5 min in 0.25% Triton in PBS.  
674 Nuclei were stained with DAPI at 1:1000 for 4 min in PBS. Brain slices were mounted to  
675 glass slides and coverslipped in Dako Fluorescence Mounting Medium (Agilent S3023).  
676 Confocal images were captured in z-stack using a Zeiss-770 microscope at 60x in oil.  
677 Non-confocal images were captured using a Zeiss-880 microscope at 20x or 40x. The  
678 same voltages were used for image capture across all images and groups within each  
679 experiment. Images were processed using ImageJ. Within each experiment, brightness  
680 and contrast values were kept the same across all images and groups.

681

682 The following primary antibodies were used: Anti-GFP AlexaFluor 488 Conjugated Ab  
683 (Santa Cruz sc-9996 AF488) 1:200, Anti-Cleaved Caspase-3 AlexaFluor 555 Conjugate  
684 (Cell Signaling 9604S) 1:100, Anti-GFAP (Aves Labs) 1:2000, Anti-Iba1 (Wako  
685 Chemicals 019-19741) 1:500.

686

687 The following secondary antibodies were used: Goat anti-Chicken IgY Alexa Fluor 568  
688 (Thermo Fisher A-11041) 1:500, Goat-anti-Rabbit IgG Alexa Fluor 568 (Thermo Fisher  
689 A-11011) 1:500

690

### 691 *Astrocyte + Microglia Scoring*

692 Confocal (microglia) or non-confocal (astrocyte) images were captured randomly of live  
693 cancer cells and their surrounding brain parenchyma. Blinded images were scored  
694 according to a qualitative scoring system. For astrocytes, the criteria used to assign a  
695 score of 0-4 were brighter GFAP staining than nearby brain parenchyma, extent of  
696 astrocyte branching, and extent of GFAP interaction with cancer cells (see Extended  
697 Data Figure 4b for example score images). For microglia, the criteria used to assign a  
698 score of 0-3 were reactive morphology (less ramified, more ameboid)<sup>42</sup> compared to  
699 nearby brain parenchyma, number of microglia in physical contact with cancer cells, and  
700 degree of phagocytosis of cancer cells by microglia (see Extended Data Figure 4c for  
701 example score images).

702

### 703 **Ex-Vivo Magnetic Resonance Imaging**

704 After perfusion, the skulls of mice were removed and fixed in formalin for 72 hours. MRI  
705 experiments were performed on a Biospec 7030 micro-MRI system (Bruker) composed  
706 of an Avance-3 HD console and a zero-boil off 7-Tesla (7 T) (300 MHz) 300-mm  
707 horizontal bore magnet equipped with an actively shielded gradient coil insert (Bruker  
708 BGA-12S-HP; ID 114-mm, 660-mT/m gradient strength, 130- $\mu$ s rise time). All scans  
709 were performed with a Bruker transmit-receive whole mouse body radiofrequency coil  
710 (Bruker 1P T20071V3: OD=59mm, ID=38mm, L=40mm) tuned to 300.16 MHz, the <sup>1</sup>H  
711 proton Larmor frequency at 7-T commercial. This rf probe enabled the acquisition of 3D  
712 datasets with sub-millimetric isotropic resolution (<150 $\mu$ m) during overnight scans  
713 spanning from 8 to 12-hours. As previously described<sup>22</sup>, tumor burden was detected  
714 using multiple sequences. Hyper-intense signal detected by a T<sub>2</sub>-weighted. Rapid  
715 Imaging with Refocused Echoes (RARE) sequence recognizes edema surrounding  
716 tumors. The 3D RARE sequence was performed with the following acquisition  
717 parameters: (120  $\mu$ m)<sup>3</sup> isotropic resolution, acquisition time 5h 27 min., repetition time  
718 TR = 500 ms, echo spacing ES=12.7 min., Turbo factor TFx=12, effective echo time  
719 TE<sub>eff</sub>=76.2 ms, bandwidth BW= 75 KHz, Matrix size = 284<sup>3</sup>, field of view FOV= (4.0  
720 mm)<sup>3</sup>, number of averages Nav=6. Pigmented metastases were detected with signal

721 brightening when using a T<sub>1</sub>-weighted 3D Gradient echo sequence with the parameters  
722 as follow: (120 μm)<sup>3</sup> isotropic resolution, acquisition time 2hrs 41 min., repetition time  
723 TR = 20 ms, echo time TE=4.0 ms, flip angle FA= 18°, bandwidth BW= 75 KHz, Matrix  
724 size = 284<sup>3</sup>, field of view FOV= (34.0 mm)<sup>3</sup>, number of averages Nav=6. Unpigmented  
725 and/or hemorrhagic metastases were detected with a hypo-intense signal when  
726 acquired under a T<sub>2</sub>\*-weighted multi-gradient echo (MGE) sequence (3D MGE, [120  
727 μm]<sup>3</sup> isotropic resolution, acquisition time 3h 35 min., repetition time TR = 40 ms, echo  
728 time TE=3.6 ms, echo spacing ES=3.2 ms, 4 echoes, flip angle FA= 20°, bandwidth  
729 BW= 100 KHz, Matrix size = 284<sup>3</sup>, field of view FOV= (34.0 mm)<sup>3</sup>, number of averages  
730 Nav=4. All 3 sequences were used to quantify tumor burden. Identified tumor areas  
731 from analysis were cross referenced with histological sections to ensure accuracy.

732

### 733 **Transmission Electron Microscopy**

734 Cultured cells were fixed in 0.1M sodium cacodylate buffer (pH 7.2) containing 2.5%  
735 glutaraldehyde and 2% paraformaldehyde for 2 hours and post-fixed with 1% osmium  
736 tetroxide for one hour, then block stained in 1% aqueous uranyl acetate, dehydrated  
737 using a gradient of ethanol and embedded in EMbed 812 (Electron Microscopy  
738 Sciences, Hatfield, PA). Ultrathin sections (60 nm) were cut, mounted on copper grids  
739 and stained with uranyl acetate and lead citrate. Stained grids were examined under  
740 Philips CM-12 electron microscope and photographed with a Gatan (4k x2.7k) digital  
741 camera. Images were analyzed in ImageJ by measuring the largest visible  
742 mitochondrial dimension (length or width) for each mitochondrion present in randomly  
743 selected images.

744

### 745 **Seahorse Metabolic Analysis**

746 4.5 x 10<sup>4</sup> 12-273 NBM, 3.5 x 10<sup>4</sup> 12-273 BM, 8 x 10<sup>4</sup> 10-230 NBM, 1x10<sup>5</sup> 10-230 BM, 3 x  
747 10<sup>4</sup> WM-4071 NBM, or 3 x 10<sup>4</sup> WM-4071 BM cells were plated on a XF24 Cell Culture  
748 Microplate coated with Cell-Tak (Corning). Simultaneously, cells were also plated from  
749 same master mix in 12-well plates for later use for normalization. Seahorse MitoStress  
750 test protocol was followed using a Seahorse XF24 instrument (Agilent). Concentrations  
751 of inhibitors injected are as follows: 12-273 pair – 1uM oligomycin, 1.5 uM FCCP, .5uM

752 antimycin A and rotenone; 10-230 pair .5 uM oligomycin, 1.5uM FCCP, .5uM antimycin  
753 A and rotenone; WM-4071 pair - .75 uM oligomycin, 2 uM FCCP, .5 uM antimycin A and  
754 rotenone. After MitoStress test, protein was harvested from the normalization 12-well  
755 plates using RIPA buffer lysis and quantified by BCA assay. Oxygen consumption rate  
756 (OCR) and extracellular acidification rate (ECAR) values for every STC were normalized  
757 to average protein concentration of the corresponding normalization wells.

758

## 759 **Plasmid Generation**

760

761 CMV-Luciferase-EF1 $\alpha$ -copGFP (GFP-luc) Lentivector Plasmid was purchased from BD  
762 Biosciences (BLIV511PA-1)

763

764 *sh-RNA plasmids*. Tet-pLKO-puro was purchased from Addgene (21915). sh-RNAs  
765 were cloned as previously described ([https://mcmanuslab.ucsf.edu/protocol/cloning-  
766 small-hairpins-lentiviral-vectors](https://mcmanuslab.ucsf.edu/protocol/cloning-small-hairpins-lentiviral-vectors)) into Tet-pLKO-puro using AgeI and EcoRI restriction  
767 sites. pLKO tet-on scrambled (sh-Scr) was purchased from Addgene (47541). See  
768 Extended Data table 2 for sh-RNA sequences.

769

770 *CRISPR plasmids*. pLenti-Cas9 was purchased from Addgene (52962).

771 pLentiGuide-Puro was purchased from Addgene (52963). sg-RNA sequences were  
772 designed using the GPP sgRNA Designer (Broad Institute) and cloned into pLentiGuide-  
773 Puro using the BsmBI restriction site. See Extended Data table 2 for sg-RNA  
774 sequences.

775

776 *APP Expression plasmids*. pLVX-IRES-tdTomato (pLVX) was purchased from Clontech  
777 (631238). APP-770 was purchased from Genecopoeia (EX-Z2553-M02) and subcloned  
778 into pLVX using SpeI and NotI restriction sites.

779

780 *SPA4CT plasmids*. A cloning strategy was designed and implemented to generate the  
781 SPA4CT sequence<sup>24</sup>. pLVX APP-770 was digested with EcoRI and NotI to generate a  
782 366 base pair terminal APP insert. Complementary oligos (purchased from Integrated

783 DNA Technologies) were annealed to form an insert with XhoI and EcoRI overhangs.  
784 pLVX was digested with XhoI and NotI and religated together with the two inserts. For  
785 specific sequences, see Extended Data Table 2. SPA4CT-T43P was generated using  
786 the Q5 Site-Directed Mutagenesis Kit (NEB E0554S) using pLVX SPA4CT as a  
787 template. For specific sequences of primers and inserts, please see Extended Data  
788 Table 2.

789

### 790 **Lentiviral Production and Infection**

791 HEK293T cells at 80% confluency were co-transfected with 12 µg of lentiviral  
792 expression constructs, 8 µg of psPAX2 and 4 µg pMD2.G vectors using Lipofectamine  
793 2000 (Invitrogen) following manufacturer's recommendations. At 48 hr post transfection,  
794 supernatants were collected, filtered (0.45 µm) and stored at -80°C. Melanoma cells  
795 were infected with lentiviral supernatant supplemented with polybrene at a final  
796 concentration of 4 µg/mL. 24 hours after infection, the following selection methods were  
797 used for infected cells:

798 Cell sorting by GFP fluorescence for GFP-luc plasmid.

799 Culture in puromycin (2ug/mL) for sh-RNA and sg-RNA plasmids.

800 Culture in blasticidin (10ug/mL) for Cas9 plasmid.

801 Successful pLVX infection was verified by RFP expression on microscopy; cells were  
802 not sorted based on RFP expression.

803

### 804 **In-vitro Proliferation Assay**

805 2 days prior to assay, genetic silencing was induced by addition of doxycycline (1  
806 µg/mL) to media.  $1 \times 10^4$  12-273 BM or  $1 \times 10^4$  WM-4265 BM cells were plated in four  
807 24-well plates. After allowing cells to adhere overnight, a baseline plate was obtained by  
808 removal of media and fixation in .1% glutaraldehyde for 15 minutes. Remaining plates  
809 were fixed every 24 hours thereafter. Wells were stained with .5% crystal violet in PBS  
810 for one hour and washed extensively with water. Crystal violet retained by cells was  
811 then eluted by incubation in 250uL 15% acetic acid for 1 hour with shaking. 100uL from  
812 each well was transferred to a 96 well plate and absorbance at 590 nm was measured.

813 Absorbances were normalized to the absorbances from the baseline plate to obtain a  
814 fold-change value.

815

### 816 **Western Blot Analysis**

817 Cells were harvested in cold RIPA lysis buffer supplemented with protease inhibitor and  
818 protein was quantified by BCA assay. Cell lysates (15-20 ug of protein) were resolved in  
819 4%-12% Bis-Tris gels (Invitrogen) and transferred to PVDF membranes using wet  
820 transfer. Membranes were blocked in either 5% non-fat milk or 5% BSA in Tris-buffered  
821 saline 1% Tween 20 (TBST) for 1 hour. Membranes were incubated overnight with  
822 primary antibodies diluted in 5% milk or BSA TBST. Membranes were washed 3 times  
823 for 10 mins in TBST and incubated in secondary antibody for 1 hour in 5% Milk or BSA  
824 TBST. Membranes were washed 3 times for 10 minutes in TBST and incubated with  
825 ECL substrate for 3 min and exposed to film for imaging.

826

827 The following primary antibodies were used:

828 Anti-APP 22C11 (Thermo Fisher Scientific 14-9749-82) 1:1000 in milk, Anti-APP C6/1.1  
829 (Gift from Mathews Lab) 1:5000 in BSA, anti-PRKAR2B (Thermo Fisher Scientific  
830 PA5-28266) 1:1000 in milk, anti-XPNPEP3 (Atlas Antibodies HPA000527) 1:500 in milk,  
831 anti-SCARB1 (Abcam ab52629) 1:1000 in milk, anti-SELENBP1 (Abcam ab90135)  
832 1:1000 milk, anti-EARS2 (Santa Cruz sc-271728) 1:500 in milk, anti-FARP1 (Santa  
833 Cruz sc-293249) 1:1000 in milk, anti-MBTD1 (Thermo Fisher 730065) 1:1000 in milk,  
834 Anti-Beta-Actin-Peroxidase (Sigma Aldrich A3854) 1:100000 in milk, Anti-tubulin (Sigma  
835 T9026) 1:10000 in milk.

836

837 The following secondary antibodies were used:

838 Goat anti-Rabbit IgG-Peroxidase (Sigma-Aldrich A0545) 1:5000, Goat anti-mouse IgG  
839 kappa-light chain (Santa Cruz sc-516102) 1:1000.

840

### 841 **Membrane purification and $\gamma$ -secretase activity assay**

842 Cell membrane preparation and  $\gamma$ -secretase assays were described previously <sup>75-78</sup>.  
843 Briefly, cells were harvested from T75 flask 90% confluency and collected by centrifuge

844 (800 g, 10 min). Cell pellets were resuspended with hypotonic buffer (5mM Tris, pH 7.4),  
845 incubated in ice for 30 min and homogenized with Glass Teflon Homogenizer. Samples  
846 were centrifuged at 1000 g, 30 min and the supernatants that contain the total membrane  
847 fraction were collected. Pellets were resuspended with hypotonic buffer, homogenized  
848 and centrifuged again. Combined supernatants were centrifuged at 100,000g for 60  
849 min. Resulted pellets referred to as membrane fractions were resuspended and washed  
850 with MES buffer (50 mM MES, pH 6.0, 150 mM KCl, 5 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, and  
851 protease inhibitors) and spun down (100,000g for 60 min). Membrane fractions were  
852 dissolved in MES buffer and protein concentration was determined by the DC assay kit  
853 (Biorad). For  $\gamma$ -secretase assays, Sb4 substrate (1  $\mu$ M) or NTM2 substrate (0.4  $\mu$ M) and  
854 membrane fractions (50  $\mu$ g/ml) were incubated in PIPES Buffer (50 mM PIPES, pH 7.0,  
855 150 mM KCl, 5 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>) and 0.25% CHAPSO detergent at 37°C for 3  
856 hours.  $\gamma$ -Secretase products were detected by AlphaLISA methods using G2-10 or  
857 SM320 antibodies for A $\beta$ 40 or NICD, respectively.

858

### 859 **Amyloid Beta ELISA**

860 Media was conditioned with melanoma cells for 24-72 hours and concentrated 10x  
861 using Amicon Ultra 3-kDa Concentrators (Millipore Z740169). Amyloid Beta-40 ELISA  
862 was performed using the Human AB40 ELISA Kit (Invitrogen). Secretion values were  
863 normalized to protein content of wells as measured by RIPA harvest and BCA protein  
864 quantification.

865

### 866 **APP Isoform Semi Quantitative qPCR**

867 RNA was isolated from 10-230 NBM, 10-230 BM, 12-273 NBM, and 12-273 BM cells at  
868 80% confluence in 6 well plates using the RNeasy Mini Kit (Qiagen 74104). 600 ng of  
869 RNA was subjected to DNase I treatment and reverse transcription. PCR was  
870 performed with primers as previously described<sup>79</sup> to generate bands of different sizes  
871 corresponding to the following isoforms: APP-770 – 461bp, APP-751 – 417bp, APP-695  
872 235bp

873

### 874 **Statistical Analysis**



875 Statistical analyses were performed with Prism 8 (GraphPad Software). Unless  
876 otherwise stated, the Student's t-test was used for experiments. P-values <0.05 were  
877 considered to be statistically significant. Unless otherwise stated, values are averages  
878 and error bars are +/- standard deviation.

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## 1100 **Acknowledgements**

1101 E.H. is supported by NIH R01CA2022027, P01CA206980, a Leveraged Finance Fights  
1102 Melanoma-MRA Team Science Award and a NIH Melanoma SPORE (NCI P50  
1103 CA016087; PI: I.O.). K.K. is supported by F30CA221068, and previously by the NIH/NCI  
1104 5 T32 CA009161-37 (Training Program in Molecular Oncology and Immunology, PI:  
1105 D.E. Levy), NIGMS 5 T32 GM007308-41 (Medical Scientist Training Program, PI: M.  
1106 Philips), and a Vilcek Foundation Scholarship. A.F. is supported by a Fundacion Ramon  
1107 Areces fellowship. YM.L. is supported by RF1AG057593. S.L. is funded by Cure  
1108 Alzheimer's Fund, Anonymous Donors and the NYU School of Medicine.  
1109 We thank Drs. Clemens Krepler and Meenhard Herlyn for patient-derived short-term  
1110 cultures. We thank Dr. Rana Moubarak and members of the Hernando lab for critical  
1111 reading of the manuscript. We thank the NYU Center for Biospecimen Research and  
1112 Development (CBRD), the Experimental Pathology Core Facility (Director, Dr. Cindy  
1113 Loomis), the Immunohistochemistry Core Laboratory (Dr. Luis Chiriboga), the Microscopy  
1114 core (Dr. Alice Xiang), the Genomics Technology Center (Dr. Adriana Heguy), and the  
1115 Flow Cytometry Core (Dr. Peter Lopez), supported in part by the Laura and Isaac  
1116 Perlmutter Cancer Center Support Grant NIH/NCI P30CA016087, and National Institutes  
1117 of Health S10 Grants NIH/ORIP S10OD01058 and S10OD018338.

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## 1119 **Author Contributions**

1120 K.K., R.J.S. and E.H. conceived and designed the experiments. K.K., assisted by G.L.,  
1121 performed the experiments and analyzed the data. G.L. performed *in-vitro* and *in-vivo*

1122 experiments with WM-4265 BM. E.W. conducted gamma-secretase *in-vitro* assays,  
1123 supervised by Y.L. F.G-E. assisted with generation of mutant APP constructs and A $\beta$   
1124 ELISA analysis. R.V-I. and D.A. assisted with in-vivo experiments. I.R. isolated  
1125 astrocytes, supervised by S.L. L.B. performed bioinformatics analysis of RNA-seq data,  
1126 supervised by K.R. A.F. performed pathway analysis of proteomics data and western blot  
1127 analysis of STC pairs. J.R. and J.C. analyzed ex-vivo MRI data, supervised by Y.Z-W.  
1128 A.D. performed mass spectrometry analysis, supervised by B.U. E. deM. and I.O.  
1129 generated and provided patient-derived STCs. R.R performed histological analysis of in-  
1130 vivo experiments using paired STCs. P.M. assisted with design of A $\beta$  immunoprecipitation  
1131 from melanoma conditioned media. K.R. performed statistical and pathway analysis of  
1132 proteomics data. S.L. assisted with design and analysis of *in-vitro* astrocyte experiments.  
1133 R.D. provided BACE-i and assisted with BACE-i experimental design. K.K and E.H. wrote  
1134 the manuscript.

#### 1135 **Competing Interests**

1136 R.D. is a full-time employee at Eli Lilly. All other authors have no financial interests.  
1137 E.H., R.J.S. and K.K. are inventors on a pending International Patent Application No.  
1138 PCT/US2019/033377 filed on May 21, 2019. SAL is a Founder of AstronauTx Ltd, a  
1139 company making therapies to target astrocytes in neurodegenerative disease.

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