Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration

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In neurodegenerative diseases, debris of dead neurons are thought to trigger glia-mediated neuroinflammation, thus increasing neuronal death. Here we show that the expression of neurotoxic proteins associated with these diseases in microglia alone is sufficient to directly trigger death of naive neurons and to propagate neuronal death through activation of naive astrocytes to the A1 state. Injury propagation is mediated, in great part, by the release of fragmented and dysfunctional microglial mitochondria into the neuronal milieu. The amount of damaged mitochondria released from microglia relative to functional mitochondria and the consequent neuronal injury are determined by Fis1-mediated mitochondrial fragmentation within the glial cells.

The propagation of the inflammatory response and neuronal cell death by extracellular dysfunctional mitochondria suggests a potential new intervention for neurodegeneration—one that inhibits mitochondrial fragmentation in microglia, thus inhibiting the release of dysfunctional mitochondria into the extracellular milieu of the brain, without affecting the release of healthy neuroprotective mitochondria.

Glial activation in models of neurodegenerative diseases is inhibited by P110, a selective inhibitor of excessive mitochondrial fission and fragmentation. Using samples from the same mice described in our previously published studies12,18–20, we found evidence of activation of both microglia and astrocytes in murine models of AD, HD and ALS12,18–20. We therefore determined whether Drp1–Fis1-mediated excessive mitochondrial fission contributes to glial activation in mouse models of neurodegenerative diseases.

In neurodegenerative diseases, such as Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS) and Huntington’s disease (HD), the accumulation of neurotoxic proteins in neurons, which leads to neuronal dysfunction and eventually to neuronal death5. Although other cells, including microglia and astrocytes (glial cells), in the brain also express these proteins and therefore may be directly damaged, the contribution of this potential glial damage to neuronal injury is not fully understood. Much research has indicated that the role of glial cells in the brain is neuroprotective in nature; these cells release trophic factors, protect against oxidative stress and excitotoxicity, provide essential metabolites and remove damaging agents and cell debris in the milieu of the brain5–8. However, in neurodegenerative diseases, glia are activated to a state that triggers an increased secretion of proinflammatory factors, which leads to further neurotoxicity through a mechanism collectively termed neuroinflammation5–8. This glial activation and the consequent neuronal damage is attributed to unchecked glial stimulation via exposure to neuronal debris9. In animal models, the activated state of astrocytes, termed the A1 state, is rapidly induced by activated microglia following brain injury, and causes further neuronal injury and cell death10. However, the trigger for this unchecked inflammatory response and whether there are pharmacological agents to prevent A1 astrocyte formation and block neuronal injury have yet to be determined11. Here, we set out to address these questions.

There is a clear role for excessive mitochondrial fragmentation and dysfunction in neurons in neurodegenerative diseases, including in HD, AD, ALS, Parkinson’s disease, dementia, ataxia and hypertension-induced encephalopathy12–13. Indeed, mitochondrial fragmentation as a consequence of excessive dynamin-related protein 1 (Drp1)-induced mitochondrial fission is a prototypical feature of these experimental and clinical neurodegenerative diseases14–16. Interrupting excessive Drp1-mediated mitochondrial fission can prevent neuronal degeneration17. A heptapeptide (P110) that selectively inhibits the binding of activated Drp1 to one of its mitochondrial receptors, mitochondrial fission 1 (Fis1), without affecting physiological mitochondrial fission16 is neuroprotective in patient-derived cells and in genetic mouse models of AD, HD and ALS12,18–20. We therefore determined whether Drp1–Fis1-mediated excessive mitochondrial fission contributes to glial activation in mouse models of neurodegenerative diseases.

Results

Glial activation in models of neurodegenerative diseases is inhibited by P110, a selective inhibitor of excessive mitochondrial fission and fragmentation. Using samples from the same mice described in our previously published studies12,18–20, we found evidence of activation of both microglia and astrocytes in murine models of AD, HD and ALS (Fig. 1) when measured at the end of the study for each cohort (end point in Fig. 1a). We reported that sustained treatment with P110 (the timing and duration of which are indicated by the maroon bars in Fig. 1a) slowed down disease progression in 5XFAD mice18 (an AD model that expresses human APP (which encodes amyloid-β (Aβ) precursor protein) and PSEN1 (which encodes presenilin 1) transgenes with a total of five AD-linked mutations: the Swedish (K670N/M671L), Florida
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**Fig. 1** Inhibition of Drp1–Fis1-mediated mitochondrial fission in vivo reduces sustained microglia and astrocyte activation and subsequent proinflammatory responses in three models of neurodegenerative disease in mice. **a**, Age of initiation and length of sustained treatment (in months) with P110 or vehicle (Veh; dark red rectangles in the scheme) in model mice of AD (5XFAD), HD (R6/2) and ALS (SOD1-G93A). **b**, Representative brain sections immunostained for Gfap and Iba-1 (markers of astrocyte and microglia activation, respectively) in 5XFAD AD mice (hippocampus), R6/2 HD mice (striatum) and SOD1-G93A ALS mice (spinal cord) treated with vehicle or P110 (at 3 mg per kg per day) for the time indicated in the red bars in **a**. **c–j**, Expression profiles for select proinflammation-related and anti-inflammation-related genes and mitochondrial-biogenesis-related genes of each mouse model. Scale bars, 50 μm. Note that the tissue samples were from the mice used in our previous publications. Here, we show that this sustained P110 treatment also inhibited neuroinflammation in these models, as indicated by decreased levels of activated astrocytes and microglia in histological studies, whereby brain levels of glial fibrillary acidic protein (Gfap) and ionized calcium-binding adapter molecule 1 (Iba-1), respectively, were measured (Fig. 1b–d; Supplementary Fig. 1). The expression of proinflammatory genes (Fig. 1e) and the level of inflammatory cytokine accumulation in the **(1716V)** and London (V717I) mutations in APP, and the M146L and L286V mutations in PSEN1). This treatment also significantly decreased Aβ levels, improved mitochondrial health and short-term and long-term recall in an open-field test. Sustained P110 treatment also significantly increased the life span of SOD1-G93A mice (an ALS model; survival after paralysis: vehicle = 21 days, P110 = 41.5 days; P = 0.0039). Finally, sustained P110 treatment also improved the life span of R6/2 mice (a HD model; dead/survival at 13 weeks: vehicle = 6/12, P110 = 1/12; P = 0.0006) Here, we show that this sustained P110 treatment also inhibited neuroinflammation in these models, as indicated by decreased levels of activated astrocytes and microglia in histological studies, whereby brain levels of glial fibrillary acidic protein (Gfap) and ionized calcium-binding adapter molecule 1 (Iba-1), respectively, were measured (Fig. 1b–d; Supplementary Fig. 1). The expression of proinflammatory genes (Fig. 1e) and the level of inflammatory cytokine accumulation in the
Microglia are directly activated by neurotoxic proteins in a mechanism dependent on Drp1–Fis1-mediated excessive mitochondrial fission. P110 treatment in vivo may delay neuronal loss, which in turn reduces neuronal debris-induced gliosis. Alternatively, P110 treatment may directly inhibit glial activation. To examine these two possible mechanisms for the anti-inflammatory effect of P110, we performed in vitro studies in which microglia were exposed to neurotoxic proteins in the absence of neurons. Expression of a long track of neurotoxic polyglutamine (Q73) as a model for HD in the microglial cell line BV2 was sufficient to trigger mitochondrial fragmentation and dysfunction (Fig. 2a,b), and provoked P110-inhibitable inflammatory responses in microglia, as indicated by inflammasome formation, increased cellular reactive oxygen species (ROS) levels and reduced mitochondrial function (Fig. 2c–e) compared with cells expressing the non-pathological polyglutamine (Q23). The levels of the proinflammatory cytokines tumor necrosis factor-α (Tnf-α) and interleukin-1β (IL-1β) increased by approximately tenfold in microglia expressing Q73, and P110 treatment blunted the increases by more than 50% (Fig. 2f). We confirmed that P110 treatment of microglia expressing Q73 inhibited Drp1 activation, as indicated by the level of inhibition of Drp1 accumulation in the mitochondrial fraction and the decreased ratio of Drp1 phosphorylation on the activating site (S616) to that on the inactivating site (S637) (Fig. 2g,h). To show that these results were not simply due to the transient transfection of BV2, the responses induced by the expression of Q73 in BV2 microglia were similar to those observed in primary microglia isolated from R6/2 mice, a murine model of HD (Fig. 2i–k). As expected, mitochondrial dysfunction and cytokine release were lower in microglia from WT littermates relative to microglia from R6/2 mice (Fig. 2i–k). To confirm that these primary microglia are functional, we stimulated them with bacterial-derived lipopolysaccharide (LPS) and found a further decline in ATP production, greater levels of cellular and mitochondrial ROS production and a much greater cytokine release in R6/2-derived microglia relative to microglia derived from WT littermates (Fig. 2i–k). All markers of microglial activation were significantly reduced when excessive mitochondrial fission was inhibited by P110. Stimulation of primary rat microglia with 0.1 μg ml⁻¹ of LPS alone (Supplementary Fig. 2) or with 0.01 μg ml⁻¹ of LPS together with nigericin (an inducer of the NACH, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome) (Figs. 2l–o), in a two-signal model of inflammation in culture, produced similar blunting of microglial activation as that of inhibiting Drp1–Fis1 interactions.

Microglia propagate the proinflammatory response to activate astrocytes. Astrocytes, the other subset of glial cells in the brain, have a wide range of important physiological roles, including

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Fig. 2 | Drp1–Fis1-mediated mitochondrial fragmentation and dysfunction are required to induce microglial inflammatory responses in a model of HD (induced by cytotoxic Q73) or LPS activation in culture. a, Top: protocol of treatment for a–h. Bottom: representative photomicrographs of rat BV2 microglia transiently expressing Q23 (control) or Q73 (a model of HD) for 48 h, then treated with vehicle or P110 (1 μM added once, 24 h after transfection, in serum-free and antibiotic-free DMEM) and their mitochondria stained with anti-TOM20 (pseudocolored yellow for microglia) after 24 h. Right: mitochondrial aspect ratio was quantified using a macro in Fiji (ImageJ) (Fig. 2i–k). b, Intracellular ATP levels and mitochondrial ROS levels quantified after 16 h. c, Levels of inflammasome components (NLRP3 and apoptosis-associated speck-like protein containing a CARD (ASC)) present in mitochondrial fractions of Q23-expressing and Q73-expressing BV2 microglia after 24 h. d, Total cellular ROS levels were quantified after 24 h. e, Oxidative phosphorylation (OXPHOS) and glycolysis rate (using Seahorse) measured in BV2 mouse microglia cells transiently expressing Q23 or Q73 (HD model) for 48 h and treated without or with P110 (1 μM every 24 h). f, Tnf-α and IL-1β levels in cell supernatants from Q23-expressing and Q73-expressing BV2 microglia, treated as in a after 24 h. Con. control; g,h, Mitochondria-associated Drp1 levels in the above BV2 cells (g), as a measure of Drp1 activation, and Drp1 phosphorylation sites at S616-Drp1 (a phosphorylation site correlating with Drp1 activation) or S637-Drp1 (a site correlating with Drp1 inhibition) sites (h) in Q23-expressing and Q73-expressing BV2 microglia, treated as in a after 24 h. i, Levels of released Tnf-α and IL-1β in the culture media of primary mouse microglia treated as in i. j, Levels of released Tnf-α and IL-1β in culture media of primary mouse microglia treated with P110 (10 nmol ml⁻¹) for 24 h in serum-free and antibiotic-free DMEM. k, Mitochondrial ROS levels and total ROS levels quantified in R6/2 or WT primary mouse microglia treated as in i, k. l, Levels of released Tnf-α and IL-1β in culture media of primary mouse microglia treated as in i, l. m, Release of Nf-κB subunits (Nf-κBα, Nf-κBβ) in BV2 microglia treated with P110 (1 μM) and LPS (10 ng ml⁻¹) for 3 h followed by nigericin (1.2 μM) treatment (LPS/Nig) in the presence or absence of P110 (1 μM, added 30 min before LPS) treated for 21 h in serum-free and antibiotic-free DMEM. Right: quantification of the mitochondrial aspect ratio. n, Markers of microglial mitochondrial health, including intracellular ATP levels, mitochondrial ROS levels, OXPHOS, rate of glycolysis, spare respiratory capacity and mitochondrial membrane potential were determined in these control or LPS-primed nigericin-treated microglia. o, Levels of Tnf-α and IL-1α in culture media, and Cq RNA levels in these controls or LPS-primed nigericin-treated primary rat microglia in the absence or presence of P110. o, Nuclear translocation of nuclear factor-kB (Nf-kB) in primary rat microglia, treated as in l. Values underneath are fold change of Nf-κB to control microglia.
neuroprotective roles, when in the A2 state, and neuroinflammatory roles when activated to the A1 state. Because we noted activation of astrocytes in the murine models of AD, HD and ALS that correlated with the severity of the disease phenotypes (Fig. 1), supplementary Fig. 1),2,14,19, we assessed possible microglial-astrocyte cross-talk. We activated cultured microglia in the presence or absence of P110 (Figs. 2 and 3) and expressed Q73 or mutant SOD1-G93A (models of HD and ALS, respectively; see Fig. 4a,g for schemes), or used primary microglia from R6/2 mice and WT littermates (see Fig. 4n for the scheme). After culturing for 24 h, we transferred the microglial-conditioned media (MCM) to naive primary astrocytes (Fig. 4a–n) or to R6/2-derived astrocytes and their littermate WT astrocytes (Fig. 4o). Transfer of BV2 MCM of Q73 (HD model) or SOD1-A93G (ALS model) cultures to primary astrocytes induced pathological mitochondrial fragmentation in the astrocytes and evoked mitochondrial dysfunction, as evidenced by lower ATP levels, loss of normal inner mitochondrial membrane polarization, increased mitochondrial ROS production and increased cell death (Fig. 4a–e and 4g–k for HD and ALS, respectively). These treatments resulted in astrocyte activation to the A1 proinflammatory state (Fig. 4f and 4l for HD and ALS, respectively) and a greatly increased release of the proinflammatory cytokines Tnf-α and IL-1β (Fig. 4m). All these effects were blunted when the microglia alone were treated with P110 24 h before transferring their media to the naive astrocytes. As the half-life of P110 is ~15 min in culture, the data suggest that Drp1–Fis1 fragmentation in microglia generated a diffusible signal to the astrocytes, which resulted in the activation of astrocytes to the A1 state.

Importantly, to show that propagation of the inflammatory response is not simply due to the genetic manipulation of the BV2 microglial cell line, a twofold stronger propagation of the proinflammatory signal in astrocytes was induced by transferring conditioned media from R6/2 primary microglia to primary microglia from WT mice. These results show that there were increased mitochondrial and cell ROS levels in naive astrocytes and even higher cellular ROS levels in primary astrocytes isolated from R6/2 mice (Fig. 4n). The propagation of the R6/2 microglia signal in astrocytes was exacerbated by LPS treatment of the donor microglia and was blunted by treatment of the donor microglia with P110 (Fig. 4n). Increased cellular and mitochondrial ROS levels were also found when the proinflammatory microglial medium was transferred to R6/2-derived astrocytes (Fig. 4o), and the increase in cellular...
**Fig. 3** Drp1-Fis1-mediated mitochondrial fragmentation and dysfunction are required to induce microglial inflammatory response in a model of ALS (by expressing cytotoxic SOD1-G93A) or in a model of AD (induced by treatment with αAβ42) in culture. **a.** Top: protocol of treatment for **a**–**e.** Bottom: representative photomicrographs (pseudocolored yellow for microglia) and mitochondrial aspect ratios (right) of BV2 microglia cells transiently expressing human SOD1-WT or mutant SOD1-G93A (a model of ALS) for 48 h and then treated as indicated. **b–d.** Intracellular ATP (b), ROS levels (c) and mitochondrial OCR and ECAR, measures of mitochondrial OXPHOS capacity and glycolysis, respectively, as determined using Seahorse (d), in BV2 expressing WT or mutant SOD1-G93A after 24 h in serum-free and antibiotic-free DMEM. **e.** Levels of released Tnf-α and IL-1β the culture media of the BV2 cells treated as in **a**, Top: protocol of treatment for **f**–**k.** Bottom: representative photomicrographs (pseudocolored yellow for microglia) and mitochondrial aspect ratios (right) of BV2 microglia treated with or without αAβ42 (1 μM) or a model of AD) for 24 h in the presence or absence of P110 (1 μM added once together with αAβ42) in serum-free and antibiotic-free DMEM as a model of AD. **g–i.** Intracellular ATP (g), ROS levels (h) and metabolic health (i) in BV2 microglia treated with or without αAβ42 (1 μM) for 24 h in defined medium in the presence or absence of P110 (1 μM, added 15 min before αAβ42). Levels of released Tnf-α and IL-1β the culture media of cells treated as in **f–h.** Mitochondrial Drp1 levels and NLRP3 levels in BV2 microglia treated with or without oligomeric αAβ42 (1 μM) for 24 h in the presence or absence of P110 (1 μM added once together with αAβ42) in serum-free and antibiotic-free DMEM. Probability was calculated by one-way ANOVA (with Tukey’s post hoc test). All graphs represent the mean ± s.d., and P values are indicated. Scale bars, 5 μm. All experiments were performed in biologically independent replicates. **a.** n = 3, each point indicates a single mitochondrion; **b.** n = 3; **c.** n = 7 (MitoSOX), n = 4 (total ROS); **d.** n = 4; **e.** n = 5; **f.** n = 3, each point indicates a single mitochondrion; **g.** n = 3; **h.** n = 4; **i.** n = 4 (OXPHOS), n = 5 (glycolysis); j, n = 5; **k.** n = 3.

ROS production was significantly higher than that of WT littermate culture medium transferred to astrocytes (Fig. 4o versus 4n). We also confirmed that the activation of the mouse astrocytes was due to canonical activation of microglia, as shown by treatment of the microglia with bacterial LPS and transfer of the conditioned media to astrocytes (Fig. 4n, o; Supplementary Fig. 3). Primary
rat microglia similarly propagated the inflammatory response in primary rat astrocytes treated with LPS and nigericin (Fig. 5a–f) or by direct treatment of the astrocytes with the proinflammatory cytokines Tnf-α, IL-1α and C1q (Fig. 5g–l), which generated similar activation responses in astrocytes, thus serving as positive controls for the general immune activation of astrocytes. Therefore, propagation of the activation signal from all types of microglia to naive astrocytes or to R6/2 primary astrocytes was dependent on Drp1–Fis1–mediated mitochondrial fission in the microglia. Indeed, this effect was inhibited when the microglia were treated with P110 24 h before transfer of their conditioned media to the astrocytes (Figs. 4n,o and 5; Supplementary Fig. 3).

Direct treatment of astrocytes with P110 also inhibited their transition to the proinflammatory A1 state via direct stimulation with Tnf-α, IL-1α and C1q (Fig. 5g–l), thus indicating that Drp1–Fis1–mediated mitochondrial fragmentation also plays a critical role in the activation of astrocytes. Treatment of the astrocytes with a small-molecule inhibitor of Drp1 (Mdivi-1) or with a peptide that inhibits the physiological fission of mitochondria mediated by mitochondrial fission factor (Mff) and Drp1 (P259)25, exerted similar effects on mitochondrial fragmentation induced with Tnf-α, IL-1α and C1q (Fig. 5g, quantitated on the right). However, whereas Mdivi-1 improved mitochondrial functions and reduced cell death, P259, the inhibitor of physiological fission was injurious, causing a further reduction in mitochondrial membrane potential and higher mitochondrial ROS production in astrocytes activated by Tnf-α, IL-1α and C1q (Fig. 5i). Together, we found that a selective interruption of physiological mitochondrial fission is injurious to the astrocytes. Furthermore, our data indicate that induction of the pro-inflammatory phenotype in microglia or in astrocytes is dependent, at least in part, on Drp1–Fis1–mediated excessive mitochondrial fission and mitochondrial dysfunction, and that the propagation of the proinflammatory signal from activated microglia to naive or primed (R6/2 or WT) is similarly a Drp1–Fis1–dependent process.

Drp1–Fis1–dependent mitochondrial fission increases the ratio of dysfunctional/functional mitochondria released from mouse, rat and human microglia. Mitochondria can be released from multiple cell types27. We therefore posited that microglia may release mitochondria or their fragments into the extracellular milieu as physical vessels to propagate the neuroinflammatory signal. In support of this notion, functional mitochondria (with preserved inner membrane potential and ability to generate ATP) were present in the media of cultured naive primary microglia (Fig. 6b). By contrast, media of primary microglia activated with LPS and nigericin contained ~50% fewer functional mitochondria (as evidenced by reduced mitochondrial membrane potential and ATP levels in the extracellular mitochondria; Fig. 6b), but more than twofold greater extracellular mitochondrial protein content compared with media from naive primary microglia (as evidenced by western blot analyses for the mitochondrial proteins VDAC and TOM20; Fig. 6c, left). P110 treatment of LPS and nigericin–activated primary rat microglia did not significantly affect the amount of released mitochondria (Fig. 6c). However, P110 treatment of the microglia greatly reduced the loss of cytochrome c, a small (12 kDa) protein compartmentalized within the mitochondrial inter-membrane space and a marker for mitochondrial damage26 from the mitochondria released into the culture media (Fig. 6c). Consistent with the improved mitochondrial structure of the extracellular mitochondria, P110 treatment also inhibited the loss of the inner membrane potential and improved ATP production by these extracellular mitochondria, effects that are indicative of improved mitochondrial integrity (Fig. 6b). We can attribute the levels of JC-1 dye (used to assess mitochondrial membrane potential) and ATP measured in the MCM to the extracellular mitochondria, as filtering the media through a 0.2–µm filter11 (Fig. 6a) resulted in the loss of both the JC-1 signal and ATP (Fig. 6b; Mitoch).

We also found that microglia from R6/2 mice, BV2 microglia activated by Q73 expression (to model HD) and BV2 microglia expressing mutant SOD1 (to model ALS) released functional mitochondria into the culture media under basal conditions. However, the integrity of the released mitochondria, evaluated by measuring the mitochondrial membrane potential and ATP production, was lower than their corresponding controls (Fig. 6d,e). The amount of functional extracellular mitochondria was lower if the microglia were activated by expressing mutant huntingtin protein (in microglia derived from R6/2 mice), treated without or with LPS (Fig. 6d), or expressing Q73 or mutant SOD protein (Fig. 6e). As before, the extent of the dysfunction of these released mitochondria was greatly blunted if the cells were treated with P110 (Fig. 6d,e). Importantly, we found that a model of human microglia (differentiated from peripheral blood mononuclear cells29) also released functional mitochondria under basal conditions that produced ATP, and that activation of these human microglia with LPS and nigericin greatly reduced the integrity of the expelled mitochondria (Fig. 6f).

As before, treatment of the LPS-activated human microglia with P110...
improved the integrity and function of the released mitochondria (Fig. 6f). Together, our data show that inhibition of Drp1–Fis1-mediated mitochondrial fission by P110 reduces the dysfunction of the mitochondria released from activated microglia, but not the total amount of mitochondria or mitochondrial proteins released into the extracellular media.
Dysfunctional extracellular microglial mitochondria trigger the release of dysfunctional mitochondria from naive astrocytes. As previously reported, astrocytes also released functional mitochondria that can produce ATP (Fig. 6g,h, control versus MitoΔ). Moreover, astrocytes activated by conditioned media from LPS and nigericin-treated microglia released more damaged mitochondria, as evidenced by 50% lower mitochondrial membrane integrity and 50% lower ATP levels (Fig. 6g). The released astrocytic mitochondria exhibited a substantial loss of cytochrome c (Fig. 6h), which is suggestive of damage to the outer membrane of the extracellular mitochondria. As above, the release of these dysfunctional mitochondria from the astrocytes was greatly blunted if the microglia were activated in the presence of P110 (Fig. 6g,h). Again, because the half-life of P110 is very short, P110 did not exert its effect directly on the astrocytes. Structural abnormalities of the released astrocytic mitochondria, as assessed by electron microscopy analyses of the extracellular mitochondria, included reduced cristae structures and breaks in the mitochondrial outer membrane (arrows in Fig. 6i, middle panels versus left panels). By contrast, mitochondria isolated from astrocytes treated with conditioned media of LPS and nigericin-treated primary microglial cells co-treated with P110 showed less damage (Fig. 6i, right versus middle panels). These data reveal a role for excessive pathological mitochondrial fission in the genesis and delivery of dysfunctional mitochondria to the extracellular milieu.

To confirm that astrocytes directly release mitochondria, we examined the culture media of astrocytes activated by a mixture of the proinflammatory cytokines Tnf-α, IL-1β and C1q. This cytokine activation of the astrocytes reduced the function of the extracellular mitochondria by ~50% relative to those released from naive (control) astrocytes (Fig. 6j). Dysfunction of the extracellular astrocytic mitochondria was greatly blunted by treatment with P110, an inhibitor of Drp1–Fis1-mediated fission, or with Mdivi-1, an inhibitor of the catalytic activity of Drp1 (ref. 2). In contrast, similar to the results above, no benefit was observed after treatment with P259, a peptide inhibitor of the Drp1 and Mif interaction and inhibits physiological but not pathological fission (Fig. 6j). As the amount of mitochondria in the culture media was unaffected when the stressed cells were treated either with the cytoprotective peptide, P110, or the cell damaging peptide, P259 (see levels of lactate dehydrogenase (LDH) release in Figs. 5k and 6c,h), and Supplementary Fig. 4c), the mitochondrial content in the media is unlikely to be related to the extent of cell death. Examining the cytochrome c content of the extracellular mitochondria confirmed that the integrity of the mitochondria released from activated astrocytes treated with P110 or Mdivi-1 was better than those released from activated astrocytes treated with P259 or vehicle (Fig. 6k), thus indicating the selective role of Drp1–Fis1-mediated fission in this process.

Extracellular dysfunctional mitochondria propagate injury from microglia to astrocytes to neurons, whereas extracellular functional mitochondria are neuroprotective. As discussed above, much is known about the primary function of all glial cells in protecting and providing essential structural and metabolic support for neurons. Yet, we found that transfer of conditioned media of microglia-activated astrocytes (Fig. 7) or direct transfer of activated MCM to cultured primary neurons (Supplementary Fig. 4a) was sufficient to evoke neuronal damage. ATP production and mitochondrial inner membrane potential decreased in these neuronal cells, as did the production of neurotoxic ROS (Fig. 7a,b). Propagation of the injurious stimulus from the microglia to astrocytes, and from astrocytes to neurons, was consistent with whether microglia were activated nonspecifically with LPS (Fig. 7a,b) or by the recapitulation of genetic neurodegenerative diseases by expressing long polyglutamine (Q73, as a HD model) or SOD1-G93A (ALS model) (Fig. 7c). Extracellular fragmented mitochondria, but not free mitochondrial DNA, were the major mediators of the major neurotoxic signal released from activated astrocytes: filtering out mitochondrial particles from activated astrocyte-conditioned media (MitoΔ) attenuated the mitochondrial dysfunction in neurons, as assessed by measuring the level of neuronal mitochondrial respiration (Fig. 7d), and substantially decreased neuronal cell death (Fig. 7d). By contrast, enzymatic degradation of free mitochondrial DNA, a known contributor to cytotoxicity, using DNase treatment of the conditioned media had only a small effect (Fig. 7d). Finally, the addition of an enriched preparation of extracellular mitochondrial particulates to cultured neurons without soluble material from the conditioned media caused a fivefold increase in neuronal cell death, which was significantly reduced by P110 treatment of the primary microglia that propagated the injurious stimulus to the neurons via the astrocytic mitochondria (Fig. 7e). Therefore, extracellular mitochondrial alone propagate the injury from the activated microglia to the astrocytes and from the astrocytes to the neurons. We also confirmed results from a previous study, which showed that intact extracellular astrocytic mitochondria provide neuroprotection. That is, removal of the mitochondria from the naive or P110-protected astrocytic-conditioned media doubled the level of neuronal cell death (Supplementary Fig. 4b; compare the MitoΔ group to the “−” group).

The indirect propagation of the neurotoxic signal from the microglia to the astrocytes and subsequently to the neurons was not due to a transient expression of neurotoxic proteins in the microglia. Microglia isolated from the R6/2 HD mouse model also propagated the mitochondrial dysfunction and neuronal death through naive WT astrocytes (Fig. 8a) or through R6/2-derived and likely partially activated astrocytes (Fig. 8b). We observed more than fivefold higher ROS production in the neuronal mitochondria and 50% more neuronal cell death.
when the injurious stimulus from the microglia was propagated through R6/2-derived astrocytes compared with littermate WT astrocytes (Fig. 8b). Again, a greater propagation of neuronal injury was observed when the microglia were also cultured in the presence of LPS, and P110 treatment of the microglia (R6/2 or WT) was sufficient to greatly blunt the propagation of injury through the astrocytes (R6/2 or WT) to the primary neurons (Fig. 8a,b).
Loss of immune-privileged state of mitochondria in models of neurodegenerative diseases. Our results showed that loss of the immune-privileged state of extracellular damaged mitochondria correlates with an increased release of damaged mitochondria from the microglia, and that damaged extracellular mitochondria directly contribute to disease propagation by acting as effectors of the innate immune response by targeting adjacent astrocytes and neurons. We have previously reported that neurons harbor neurotoxic proteins\textsuperscript{22,33,34}; our data here showed that the Drp1–Fis1 inhibitory peptide P110 reduces mitochondrial fission and the consequent release of damaged mitochondria from the microglia, thus inhibiting the activation of astrocytes and protecting neurons from innate immune attack. Increased Drp1–Fis1-mediated mitochondrial fission in activated microglia triggers the formation of fragmented and damaged mitochondria that are released from these cells, thus inducing the innate immune response. Clinical and experimental studies have identified fragmented mitochondria in biofluids of patients afflicted by subarachnoid hemorrhage and of patients affected by stroke\textsuperscript{35–37}, which suggests that their presence in the extracellular space is a biomarker for neurodegeneration and disease severity\textsuperscript{38,39}. Our data showed a causal role of dysfunctional extracellular mitochondria in propagating neurodegenerative signals from microglia.

Activation of the innate immune response and neurotoxic protein-induced neuronal cell death in models of neurodegenerative diseases are both dependent on Drp1–Fis1-mediated excessive mitochondrial fragmentation. We have previously shown that expression of neurotoxic proteins in cultured neurons and the resulting cell death can be greatly blunted by inhibiting Drp1–Fis1-mediated mitochondrial fragmentation using P110 peptide\textsuperscript{16,20}. Here, we showed that the innate immune response in several models of neurodegenerative diseases is also dependent on pathological Drp1–Fis1-dependent mitochondrial fission in the microglia and the resulting release of dysfunctional mitochondria into the extracellular milieu. However, the mechanisms by which mitochondria are released to the extracellular space are still under investigation. For example, mitochondrial transfer may occur via release in extracellular vesicles\textsuperscript{40}, structures that are 50–1,000 nm in diameter\textsuperscript{41}, and astrocytes and microglia release extracellular vesicles\textsuperscript{42,43}. But other mechanisms, including direct exocytosis, may contribute to this release\textsuperscript{44,45}. Also not yet known is the minimal amount of damaged mitochondria required for the propagation of neuronal cell death and whether transfer of functional mitochondria between...
microglia and astrocytes and from glia to neurons has a role under physiological conditions. What we do know, however, is that extracellular mitochondria are critical in mediating this cell-to-cell propagation of pathological signaling.

The ratio between damaged mitochondria and functional mitochondria in the extracellular milieu governs the outcome of neurons. Although extracellular damaged mitochondria are injurious, transfer of functional mitochondria is protective (Supplementary Fig. 4), as has been previously shown, for example, in a mouse model of acute lung injury and in a model of stroke. Whether the extracellular damaged mitochondria enter the neurons, as has been suggested for functional mitochondria in a previous study, has not yet been determined. The novel finding of our study is that it is not the amount of the extracellular mitochondria but rather the ratio between damaged mitochondria and functional mitochondria in the extracellular milieu that governs the outcome of neurons, and is determined by the extent of pathological fission in the donor microglia. The structural and functional integrity of the released glial mitochondria were confirmed by measuring respiration, mitochondrial membrane potential, ATP production, content of cytochrome c (indicating mitochondrial outer membrane integrity) and by electron microscopy analyses. Although the amount of extracellular mitochondria was unaltered, all these parameters showed improved structure and function of the extracellular mitochondria released from microglia after treatment with P110.

Our data suggested that selective inhibition of pathological mitochondrial fission in the microglia (mediated by Drp1–Fis1) without affecting physiological mitochondrial fission (mediated by Drp1–Mff) reduced propagation of neuronal injury by two
mechanisms. First, P110 reduced activation of the innate immune response in microglia and in astrocytes and the cytokine-mediated neuronal cell death mechanisms in primary cortical neurons treated with activated rat microglia supported neuronal cell survival by P110 in the donor microglia.

Suppression of Drp1–Fis1-mediated mitochondrial fission is a readily translatable approach for interrupting this pathological microglia-to-astrocyte-to-neuron mitochondrial dysfunction, neuroinflammation and neuronal cell death mechanisms and supporting transfer of healthy mitochondria to neurons. However, we envision that any means of normalizing the balance between healthy and damaged mitochondria within the neuronal milieu, for example, by clearing damaged and fragmented mitochondrial with specific antibodies or by introducing healthy mitochondria, could also provide neuronal protection in neurodegenerative diseases.

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Fig. 8 | WT or R6/2 astrocytes induce Drp1–Fis1-dependent propagation of neuronal mitochondrial dysfunction and neuronal cell death from R6/2 microglia to naive mouse cortical neurons. a, Top: schematic of the experimental design using primary microglia (treated without or with LPS) that were isolated from R6/2 mice or their littermate WT mice. Bottom: mitochondrial ROS and LDH release in mouse primary cortical neurons treated with activated aACM from WT mouse astrocytes. b, Top: the treatment protocol is as in a, except that, in addition to the microglia, the primary astrocytes used were isolated from R6/2 mice or the WT littermates as indicated. Bottom: mitochondrial ROS and LDH release of these cells. All graphs represent the mean ± s.d., and P values are indicated. All experiments were performed in biologically independent replicates. a, n = 5 (MitoSOX), n = 4 (LDH); b, n = 5.

and associated accession codes are available at https://doi.org/10.1038/s41593-019-0486-0.

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References
1. Aguzzi, A. & O'Connor, T. Protein aggregation diseases: pathogenicity and therapeutic perspectives. Nat. Rev. Drug Discov. 9, 237–248 (2010).
2. Hayakawa, K. et al. Transfer of mitochondria from astrocytes to neurons after stroke. Nat. Neurosci. 19, 551–555 (2016).
3. Belanger, M. & Magistretti, P. J. The role of astroglia in neuroprotection. Dialogues Clin. Neurosci. 11, 281–295 (2009).
4. Chen, Z. & Trapp, B. D. Microglia and neuroprotection. J. Neurochem. 136, 10–17 (2016).
5. Crotti, A. & Glass, C. K. The choreography of neuroinflammation in Huntington’s disease. Trends Immunol. 36, 364–373 (2015).
6. Heneka, M. T. et al. Neuroinflammation in Alzheimer’s disease. Lancet Neurol. 14, 388–405 (2015).
7. Liu, J. & Wang, F. Role of neuroinflammation in amyotrophic lateral sclerosis: cellular mechanisms and therapeutic implications. Front. Immunol. 8, 1005 (2017).
8. Ransohoff, R. M. How neuroinflammation contributes to neurodegeneration. Science 353, 777–783 (2016).
9. Liddelow, S. A. et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487 (2017).
10. Itoh, K., Nakamura, K., Iijima, M. & Sesaki, H. Mitochondrial dynamics in neurodegeneration. Trends Cell Biol. 23, 64–71 (2013).
11. Reddy, P. H. Increased mitochondrial fission and neuronal dysfunction in Huntington’s disease: implications for molecular inhibitors of excessive mitochondrial fission. Drug Discov. Today 19, 951–955 (2014).
12. Joshi, A. U. et al. Inhibition of Drp1/Fis1 interaction slows progression of amyotrophic lateral sclerosis. EMBO Mol. Med. 10, e8166 (2018).
13. Khalil, B. & Liervans, J. C. Mitochondrial quality control in amyotrophic lateral sclerosis: towards a common pathway? Neural Regen. Res. 12, 1052–1061 (2017).
14. Reddy, P. H. et al. Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases. Brain Res. Rev. 67, 103–118 (2011).
37. Hayakawa, K. et al. Extracellular mitochondria for therapy and diagnosis in vivo. *Cell Death Differ.* 19, 1446–1458 (2012).

36. Hayakawa, K. et al. Protective effects of endothelial progenitor cell-derived extracellular mitochondria in brain endothelium. *Stem Cells* 36, 1404–1410 (2018).

35. Zussman, B., Weiner, G. & Ducruet, A. Mitochondrial transfer into the extracellular vesicle. *Nat. Rev. Mol. Cell Biol.* 19, 213–228 (2018).

34. Lai, R. C. et al. MSC secretes at least 3 EV types each with a unique permeation of membranes lipid, protein and RNA. *J. Extr. Vesicles* 5, 29828 (2016).

33. Taylor, A. R., Robinson, M. B., Gifondorwa, D. J., Tytell, M. & Milligan, C. E. Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. *Dev. Neurobiol.* 67, 1815–1829 (2007).

32. Qi, X., Qvit, N., Su, Y. C. & Mochly-Rosen, D. A novel Drp1 inhibitor provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

31. Grohm, J. et al. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *J. Neuroimmune Pharm.* 11, 622–628 (2016).

30. Cassidy-Stone, A. et al. Chemical inhibition of the mitochondrial division factor Drp1/Fis1 diminishes aberrant mitochondrial fission and neurotoxicity. *J. Cell Biol.* 213, 2655–2669 (2016).

29. Guo, X. et al. Inhibition of mitochondrial fragmentation diminishes Huntington’s disease-associated neurodegeneration. *J. Exp. Med.* 213, 5371–5388 (2013).

28. Gogvadze, V. et al. Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. *Dev. Neurobiol.* 67, 1815–1829 (2007).

27. Falchi, A. M. et al. Astrocytes shed large membrane vesicles that contain components act as damage-associated molecular pattern molecules in the cerebrospinal fluid in the setting of subarachnoid hemorrhage. *Nat. Med.* 23, 560–563 (2017).

26. Kornfeld, O. S. et al. Interaction of mitochondrial fission factor with dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Dev. Cell* 14, 193–204 (2008).

25. Sondag, C. M., Dhawan, G. & Combs, C. K. Beta amyloid oligomers and cell permeability. *Acta Histochem.* 121, 221–231 (2013).

24. Golovchenko, N. J. et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* 560, 198–203 (2018).

23. Sarkar, S. et al. Mitochondrial impairment in microglia amplifies NLRP3 inflammasome activation. *Cell Mol. Life Sci.* 75, 759–765 (2012).

22. Joshi, A. U., Saw, N. L., Shamloo, M. & Mochly-Rosen, D. Drp1/Fis1 interaction mediates mitochondrial dysfunction, bioenergetic failure and cognitive decline in Alzheimer’s disease. *Oncotarget* 9, 6128–6143 (2018).

21. Mishra, P. & Chan, D. C. Metabolic regulation of mitochondrial dynamics. *J. Cell Biol.* 212, 379–387 (2016).

20. Cassidy-Stone, A. et al. Chemical inhibition of the mitochondrial division factor Drp1/Fis1 diminishes aberrant mitochondrial fission and neurotoxicity. *J. Cell Biol.* 213, 5371–5388 (2013).

19. Disatnik, M. H. et al. Potential biomarkers to follow the progression and treatment response of Huntington’s disease. *Sci. Rep.* 8, 14034 (2018).

18. Falchi, A. M. et al. Astrocytes shed large membrane vesicles that contain components act as damage-associated molecular pattern molecules in the cerebrospinal fluid in the setting of subarachnoid hemorrhage. *Nat. Med.* 23, 560–563 (2017).

16. Qi, X., Qvit, N., Su, Y. C. & Mochly-Rosen, D. A novel Drp1 inhibitor provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

15. Knott, A. B., Perkins, G., Schwarzenbacher, R. & Bossy-Wetzel, E. Current knowledge on exosome biogenesis and function. *Nature* 520, 553–557 (2015).

14. Hooper, C. et al. Wnt3a induces exosome secretion from primary cultured rat microglia. *BMC Neurosci.* 13, 144 (2012).

13. Grohm, J. et al. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

12. Joshi, A. U., Saw, N. L., Shamloo, M. & Mochly-Rosen, D. Drp1/Fis1 interaction mediates mitochondrial dysfunction, bioenergetic failure and cognitive decline in Alzheimer’s disease. *Oncotarget* 9, 6128–6143 (2018).

11. Disatnik, M. H. et al. Potential biomarkers to follow the progression and treatment response of Huntington’s disease. *Sci. Rep.* 8, 14034 (2018).

10. Cassidy-Stone, A. et al. Chemical inhibition of the mitochondrial division factor Drp1/Fis1 diminishes aberrant mitochondrial fission and neurotoxicity. *J. Cell Biol.* 213, 5371–5388 (2013).

9. Falchi, A. M. et al. Astrocytes shed large membrane vesicles that contain components act as damage-associated molecular pattern molecules in the cerebrospinal fluid in the setting of subarachnoid hemorrhage. *Nat. Med.* 23, 560–563 (2017).

8. Gogvadze, V. et al. Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. *Dev. Neurobiol.* 67, 1815–1829 (2007).

7. Falchi, A. M. et al. Astrocytes shed large membrane vesicles that contain components act as damage-associated molecular pattern molecules in the cerebrospinal fluid in the setting of subarachnoid hemorrhage. *Nat. Med.* 23, 560–563 (2017).

6. Gogvadze, V. et al. Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. *Dev. Neurobiol.* 67, 1815–1829 (2007).

5. Grohm, J. et al. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

4. Hooper, C. et al. Wnt3a induces exosome secretion from primary cultured rat microglia. *BMC Neurosci.* 13, 144, 198–203 (2018).

3. Qi, X., Qvit, N., Su, Y. C. & Mochly-Rosen, D. A novel Drp1 inhibitor provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

2. Qi, X., Qvit, N., Su, Y. C. & Mochly-Rosen, D. A novel Drp1 inhibitor provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

1. Grohm, J. et al. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. *JAMA Neurol.* 72, 119–122 (2015).

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were differentiated for 10 days at 37 °C, 5% CO2 in RPMI medium 1640 GlutaMAX 4 mM glutamine, 1 mM sodium pyruvate and 10% FBS, and plated on uncoated stock no. 006494). In brief, dissociated cortical cells were suspended in DMEM/cerebral cortices of 2-day-old neonatal Sprague–Dawley rats or postnatal day R6/2 mice and their littermates. 

Primary astrocyte and microglia cultures from WT rats and mice and from mice. In brief, cortices were dissected and dissociated using a papain dissociation cerebral cortices of embryonic day 17 (E17) Sprague–Dawley rat embryos or E17 C57BL/6 Primary neuron cultures. 

were obtained 12–14 days after plating. Cultures were gently shaken, and floating cells (microglia) were collected, resulting in more than 95% pure culture of astrocytes. Astrocytes were dissociated by trypsinization and then reseeded at 3–5 x 10^4 cells per well in a XF 24-well cell culture microplate and cultured in Neurobasal medium (Invitrogen, cat. no. 21103-049) supplemented with B-27 and 0.5 mM glutamine. Cells were cultured at 37 °C in a humidified chamber of 95% air and 5% CO2. Cultures were used for experiments from 7 to 10 days after seeding. 

Primary astrocyte and microglia cultures from WT rats and mice and from R6/2 mice and their littermates. 

Primary astrocyte cultures were prepared from cerebral cortices of 2-day-old neonatal Sprague–Dawley rats or postnatal day 1–2 C57BL/6 mice or R6/2 or littermates (B6CBA-TgN (HD exon1)62; JAX stock no. 006494). In brief, dissociated cortical cells were suspended in DMEM/F12 50/50 (Life Technologies, cat. no. 11320-033) containing 25 mM glucose, 4 mM glutamine, 1 mM sodium pyruvate and 10% FBS. At 24 h after seeding, the medium was changed to Neurobasal medium supplemented with B-27 and 0.5 mM glutamine. Cells were cultured at 37 °C in a humidified chamber of 95% air and 5% CO2. Cultures were used for experiments from 7 to 10 days after seeding. 

Mitochondrial ROS production. To determine mitochondrial ROS production, cells were treated with 5 μM MitoSOX Red mitochondrial superoxide indicator (Invitrogen, cat. no. M5608) and 10 μg ml−1 of the nuclear staining dye Hoechst 33342 (Invitrogen, cat. no. B 2261) at the end of the experiment for 15 min at 37 °C according to the manufacturers’ protocols. Fluorescence was recorded for MitoSOX (excitation (Ex) 510 nm and emission (Em) 580 nm) and Hoechst-33342 (Ex 350 nm and Em 461 nm) in a SpectraMax M2e (Molecular Devices). The MitoSOX fluorescence signal was normalized to the Hoechst reading. Mitochondrial ROS levels in primary microglia were analyzed at 16 h for the HD and ALS models, 21 h for the LPS and nigericin model or 24 h for the AD model after individual stimuli. Mitochondrial ROS levels in primary astrocytes were analyzed either at 6 h for the HD and ALS models or 24 h for all other stimuli. Mitochondrial ROS levels in primary neurons were analyzed at 24 h after stimuli. 

ATP measurement. Relative intracellular or extracellular ATP levels were determined using an ATP-based CellTiter-Glo Luminescent Cell Viability kit (Promega, cat. no. G7570), which can perform cell lysis and generate a luminescence signal proportional to the amount of ATP present. In brief, for intracellular ATP levels, opaque-walled 96-well plates with cell lystate (50 μl) were proteins with single- versus multi-step reactions. The kit was added to each well and incubated for 30 min at room temperature. For measuring the ATP content in extracellular mitochondria, cell supernatant was cleared of cellular debris by centrifugation at 1,000 × g for 10 min and then centrifuged at 13,000 × g for 25 min followed by a wash with 1 ml of PBS. The pellet was resuspended in 1 ml of serum-free phenol-free DMEM or PBS before an equal volume of the single-step reaction provided in the kit with incubation for 30 min at room temperature. 

Mitochondria membrane potential measurement. To monitor mitochondrial health, JC-1 dye (Invitrogen, cat. no. T-3168) was used to assay the mitochondrial membrane potential. In brief, cell supernatant was cleared of cellular debris by centrifugation at 1,000 × g for 10 min at the end of the experiment (24 h). The cell supernatant was then loaded with 5 μl of JC-1 dye for 30 min at 37 °C. JC-1 dye exhibits potential-dependent accumulation in mitochondria, as indicated by a fluorescence emission shift from green (Ex 485 nm, Em 516 nm) to red (Ex 579 nm, Em 599 nm). Mitochondrial membrane potential was determined by measuring the fluorescence ratio using a SpectraMax M2e (Molecular Devices). Cells were incubated with tetra-methyl-rhodamine methyl ester (TMRM; Invitrogen, cat. no. T660) in HBSS (Hank’s balanced salt solution; Invitrogen, cat. no. at 6 h for the HD and ALS models or 24 h for all other stimuli. Mitochondrial ATP levels in primary neurons were analyzed at 24 h after stimuli. For extracellular mitochondria experiments, ATP levels in the pellet (after 13,000 × g centrifugation) were measured after the end of total stimulation (24 h). 

Mitochondria membrane potential measurement. To monitor mitochondrial health, JC-1 dye (Invitrogen, cat. no. T-3168) was used to assay the mitochondrial membrane potential. In brief, cell supernatant was cleared of cellular debris by centrifugation at 1,000 × g for 10 min at the end of the experiment (24 h). The cell supernatant was then loaded with 5 μl of JC-1 dye for 30 min at 37 °C. JC-1 dye exhibits potential-dependent accumulation in mitochondria, as indicated by a fluorescence emission shift from green (Ex 485 nm, Em 516 nm) to red (Ex 579 nm, Em 599 nm). Mitochondrial membrane potential was determined by measuring the fluorescence ratio using a SpectraMax M2e (Molecular Devices). Cells were incubated with tetra-methyl-rhodamine methyl ester (TMRM; Invitrogen, cat. no. T660) in HBSS (Hank’s balanced salt solution; Invitrogen, cat. no. at 6 h for the HD and ALS models or 24 h for all other stimuli. Mitochondrial ATP levels in primary neurons were analyzed at 24 h after stimuli.
Bioenergetic profiles. Cells were plated in a Seahorse XF 24-Cell Culture Microplate (Agilent). All Seahorse experiments using microalgae, astrocytes and neurons were performed at 24 h after individual stimuli. At the end of treatment, cells were washed twice with Agilent Seahorse XF Media (Agilent) supplemented with 1 mM pyruvate, 2 mM l-glutamine and 2 mM d-glucose. A final volume of 525 μL was placed in each well. Cells were then incubated in a 0% CO2 chamber at 37 °C for 1 h before being placed into a Seahorse XFe24 Analyzer (Agilent).

For oxygen consumption rate (OCR) and (extracellular acidification rate) ECAR experiments, cells were treated with 1 μM oligomycin, 2 μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and 0.5 mM rotenone/antimycin. A total of three OCR and pH measurements were taken after each compound was administered.

Immunocytochemistry and immunohistochemistry. Immunocytochemistry was performed as previously described. Mitochondrial structure analysis in microglia was performed after 24 h of the initial stimuli. For astrocytes, MCM was added to astrocytes for 24 h before analysis. Cells cultured on 8-well chamber slides were washed with cold PBS, fixed in 4% formaldehyde and permeabilized with 0.1% Triton X-100. After incubation with 2% normal goat serum (to block nonspecific binding), fixed cells were incubated overnight at 4 °C with anti-TOM20 primary antibody (1:500, Santa Cruz). Cells were washed with PBS and incubated for 60 min with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:500 dilution). The cells were then washed gently with PBS and counterstained with Hoechst 33342 (1:10,000 dilution, Molecular Probes) to visualize nuclei. The coverslips were mounted with Slowfade-antifade reagent (Invitrogen). Parameters of mitochondrial morphology were further quantified with Fiji (ImageJ) as previously described. A detailed workflow is presented in Supplementary Fig. 5. For the immunohistochemical studies, mice described in our previous publications were used. Adult B6SJL-Tg (SOD1-G93A) 1 Gpr1/ male mice with a high copy number of the mutant allele and their WT littermates at 120 days, 3XENAD transgenic male mice and their littermates at 6 months and hemizygous male R6/2 HD mice and their WT littermates at 12 weeks were analyzed. In brief, mice were killed, tissue dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Tissues were then paraffin‐ embedded, and sections were used for immunohistochemical staining of Gfap and Iba-1 (done by Histo‐tec Laboratory). The images were acquired using an All‐in‐One Fluorescence Microscope BX‐Z20 (Keyence). The level of Gfap or Iba-1 immunoreactivity was measured by using the mean optical density of diamobenzidine staining. The white balance of all images was standardized to eliminate color bias.

Isolation of mitochondria‐enriched fraction and lysis preparation for western blot analysis. Cells were washed with cold PBS at pH 7.4 and scraped off using a 27-gauge needle. The collected cells were passed through a 27-gauge needle to collect a mitochondria-enriched fraction, as previously described. The post-nuclear supernatant was further centrifuged at 10,000 g for 15 min to collect the mitochondria-enriched fraction, as previously described.

Electron microscopy. Pellets from ACM (precleared cell supernatant obtained after centrifugation at 10,000 g for 10 min) were washed twice with 10 mM Tris (pH 7.4) and were fixed in 2.0% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 for 1 h at room temperature on a rocker. They were rinsed in cacodylate buffer, gently scraped and pelleted and post-fixed in 1.0% osmium tetroxide in cacodylate buffer for 1 h on ice. They were then rinsed in buffer and stabilized with a small amount of 2% agaroze in PBS to hold them together. Samples were then dehydrated through a graded series of ethanol to 100%, followed by propylene oxide, 100% (Epon resin (Ted Pella) in a 1:1 solution of Epon: propylene oxide was added and then incubated overnight on a rocker at room temperature. The following day, samples were placed in fresh Epon for several hours and then embedded in Epon resin (Ted Pella) for 7 days. Ultrathin sections were cut using a diamond knife and viewed on an electron microscope at 80 kV and photographs were taken using a Gatan Multiscan 791 digital camera.

ELISA experiments. Mouse/rat IL-6 (cat. no. 5017218/50-183-46), mouse/rat IL-1α (cat. no. 501125107/5013531), mouse/rat TNF-α (cat. no. 50-112–8954/5017347) and IL-1β (cat. no. 50112974/50125471) ELISA kits (eBioscience) were used to quantify cytokine levels in mouse tissue and cell supernatant by using the equivalent cDNA amount of 1–2 ng total RNA used in cDNA synthesis. SYBRgreen master mix (Applied Biosystems, cat. no. 43-127-04) and a 2 μmol·L⁻¹ mix of forward and reverse primer sequences were used for 40 cycles of target gene amplification. The following primers were used in this study: 18S RNA: 5'-CTTGAAGGGGCAAAGGGGGC-3', 5'-AGCTGGCCAGGTGCTGCA-3', 5'-GCTTTGTCCGCTGCTGCTGCT-3', and 5'-AATGGACGTACGGATCCGGATCC-3'. GAPDH: 5'-GGCATGGACGGTCAACTTGG-3', 5'-CTGCAGTACCCCACTCTTGG-3', 5'-ATGGACGTACGGATCCGGATCC-3'. Microfluidic qPCR (pooled-cell samples). Total RNA was extracted from cells using a RNeasy Plus kit (QIAGEN, cat. no. 74136) and DNA synthesis was performed using a SuperScript VILO cDNA synthesis kit (Invitrogen) according to the suppliers’ protocols. Microfluidic qPCR was conducted using 1.25 μL of each cDNA sample pre-amplified using 2.5 μL of 2x Taqman pre-amplification master mix (Applied Biosystems) and 1.25 μL of the primer pool (0.2 pmol each primer per μL, negative primers to rat transcripts for reactive astrocyte subtype were used as previously described). Pre-amplification was performed (10 min of 95 °C denaturation step and 14 cycles of 15 s at 95 °C and 4 min at 60 °C) and reaction products were diluted 5× in TE buffer (TakaRa). Five microliters from a sample mix containing pre-amplified cDNA and amplification Master mix (20 μM MgCl₂, 10 mM dNTPs, FastStart Taq polymerase, DNA-binding dye loading reagent, 50 μL) were loaded into each sample well. The samples were loaded into a Dynamic Array chip (Fluidigm) and 5 μl from an assay mix containing DNA-assay loading reagent, as well as forward and reverse primers (10 pmol·L⁻¹) was loaded into each detector inlet. The chip was mixed and loaded in a NanoFlexTM 4-ICC Controller (Fluidigm) and processed in a BioMark Real-Time PCR System (Fluidigm) using a cycling program of 10 min at 95 °C followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72°C for 30 s. After completion of qPCR, a melting curve of amplified products was determined. Data were collected using BioMark Data Collection Software 2.1.1 build 20090519.0926 (Fluidigm) as the cycle of quantification (Cq), where the fluorescence signal of amplified DNA interacted with background noise. Fluidigm data were corrected for differences in input RNA using the geometric mean of three housekeeping genes (Aldh1L1, B2m, GAPDH). Data preprocessing and analysis were completed using Fluidigm Melting Curve Analysis Software 1.1.0 build 20100514.1234 (Fluidigm) and Real-time PCR Analysis Software 2.1.1 build 20090521.1135 (Fluidigm).

Western blotting. Protein concentrations were determined using the Bradford assay (Thermo Fisher Scientific). Proteins were resuspended in Laemmli buffer containing 2-mercaptoethanol, loaded onto SDS–PAGE gels and transferred onto nitrocellulose membranes, 0.45 μm (Bio‐Rad), as previously described. For extracellular mitochondria analysis, cell supernatant was cellularized by centrifugation at 1000 × g for 10 min and then centrifuged 13,000 × g for 20 min. The washed pellet was then resuspended in the equivalent cDNA amount of 1–2 ng total RNA used in cDNA synthesis. A glycerol gradient was used to separate extracellular mitochondria from the bulk of the supernatant. The supernatant was loaded onto 4% (w/v) agarose gel and run in 0.5× TBE buffer for 5 h. The gel was stained with 0.5% w/v ethidium bromide for 1 h and photographed using a GelDoc Imaging System (Bio‐Rad). The gel image was then scanned and the optical density was measured using a GelDoc Imaging System (Bio‐Rad). The relative intensity of the bands was assessed using the ImageJ software (National Institutes of Health).
buffer, pH 8.5. Scanned images of the exposed X-ray film or images acquired with Azure Biosystems C600 were analyzed using ImageJ to determine the relative band intensity (Supplementary Fig 6). Quantification was performed on samples from independent cultures for each condition. The following antibodies were used in this study: anti-ASC (B3) mouse monoclonal antibody (Santa Cruz Biotechnology, sc-514414; 1:200); anti-COX-2 rabbit polyclonal antibody (Abcam, Ab15191; 1:500); anti-cytochrome c (7H8.2C12) mouse monoclonal antibody (Abcam, ab13575; 1:1,000); anti-Drp1 (clone 8/DLP1 (RUO)) mouse monoclonal antibody (BD Transduction Laboratories, 611113; 1:500); anti-Enolase-1 rabbit polyclonal antibody (Cell Signaling Technology, 3810; 1:500); anti-GFAP rabbit polyclonal antibody (Abcam, ab9722; 1:200); anti-NLRP3 (D4D8T) rabbit monoclonal antibody (Cell Signaling Technology, 15101; 1:200); anti-p20 (D4) mouse monoclonal antibody (Santa Cruz Biotechnology, sc-398715; 1:1,000); anti-phospho SAPK/JNK (Thr183/Tyr185) rabbit polyclonal antibody (Cell Signaling Technology, 9251; 1:500); anti-phospho NFκB p65 (Ser536) (93H1) rabbit monoclonal antibody (Cell Signaling Technology, 2933; 1:500); anti-phospho-Drp1 (Ser616) rabbit polyclonal antibody (Cell Signaling Technology, 3455; 1:200); anti-phospho-Drp1 (Ser637) rabbit polyclonal antibody (Cell Signaling Technology, 4867; 1:200); anti-phospho-VDAC1 (20B12AF2) mouse monoclonal antibody (Abcam, 14734; 1:500); and anti-β-actin (8H10D10) mouse monoclonal antibody (Cell Signaling Technology, sc-47778; 1:500). Secundary antibodies, including anti-mouse IgG (NA931V) and anti-rabbit IgG (NA934V), were obtained from GE Healthcare. Detailed antibody validation profiles are available on the websites of the companies that the antibodies were sourced from.

Randomization and blinding. Animal and samples (mice) were randomly assigned to the various experimental groups, and mice were randomly selected for the analyses. In mouse imaging (immunohistochemistry and data acquisition from electron microscopy), the performer(s) was (were) blinded to the experimental design. Additionally, a few biological replicates for mitochondrial transfer experiments, mitochondrial health assays and Seahorse experiments were performed and analyzed by a person blinded to the experimental design.

Statistical analysis. Prism 8.0 (GraphPad Software) was used for the statistical analyses. Data shown are the mean ± s.d., with P < 0.05 considered statistically significant. Group differences were analyzed by one-way analysis of variance (ANOVA) followed by Sidak’s or Benjamini, Krieger and Yekutieli correction multiple comparisons tests or by two-way ANOVA followed by Tukey’s multiple comparisons test for multiple groups. No statistical methods were used to predetermine sample sizes, but our sample sizes were similar to those reported in previous publications. Data distribution was assumed to be normal, but this was not formally tested. No data or animals were excluded.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References
48. Stevens, J. C. et al. Modification of superoxide dismutase 1 (SOD1) properties by a GFP tag—implications for research into amyotrophic lateral sclerosis (ALS). PLoS One 5, e9541 (2010).
49. Minhas, P. S. et al. Macrophage de novo NAD+ synthesis specifies immune function in aging and inflammation. Nat. Immunol. 20, 50–63 (2019).
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- We used Excel 2015, Prism 6, 7, 8, Fiji ImageJ, BioMark Data Collection Software 2.1.1, Fluidigm Melting Curve Analysis Software 1.1.0, Real-time PCR Analysis Software 2.1

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- We used Prism 7/8 for Mac OS X Version 7.0d/8.2 for data analysis

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Category       | Disclosure                                                                 |
|----------------|----------------------------------------------------------------------------|
| Sample size    | No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications |
| Data exclusions| No samples were excluded from the study                                     |
| Replication    | All attempts were successful                                                |
| Randomization  | Animal/samples (mice) were assigned randomly to the various experimental groups. |
| Blinding       | In data collection and analysis (e.g., Seahorse, microfluidic qPCR, as well as imaging and data analysis of IHC and EM), the performer(s) was blinded with experimental design. |

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| n/a                       | Antibodies            |
|                           | Eukaryotic cell lines |
|                           | Palaeontology         |
|                           | Animals and other organisms |
|                           | Human research participants |
|                           | Clinical data         |

Methods

| Category    | Involved in the study |
|-------------|-----------------------|
| n/a         | ChIP-seq              |
|             | Flow cytometry        |
|             | MRI-based neuroimaging |

Antibodies

Antibodies used

- Anti-ASC Santa Cruz Biotechnology (Clone: B-3) sc-514414 1:200
- Anti-COX-2 Abcam ab15191 1:500
- Anti-cytochrome c Abcam [7Clone: H8.2C12] ab13575 1:1000
- Anti-Drp1 BD Transduction Laboratories 611113 (Clone: 8/DLP1 (RUO)) (Lot No: 7341636) 1:500
- Anti-Endoase-1 Cell Signaling Technology (Lot 2) 3810 1:500
- Anti-GFAP Abcam ab7260 1:500
- Anti-Iba-1 Abcam ab5076 1:1000
- Anti-IL1beta Abcam ab9722 1:200
- Anti-NLRP3 Cell Signaling Technology (Clone: D4D8T) 15101 1:200
- Anti-p20 Santa Cruz Biotechnology (Clone: D-4) sc-398715 1:100
- Anti-phospho JNK (Thr183/Tyr185) Cell Signaling Technology (Lot 23) 9251 1:500
- Anti-phospho NfkB Cell Signaling Technology (Clone: 93H1) 3033 1:500
- Anti-phospho p44/p42 MAPK (Erk1/2) (Thr202/Tyr204) Cell Signaling Technology 9101 (Clone: 197G2) 1:500
- Anti-phospho-Drp1 (Ser616) Cell Signaling Technology sc-11415 1:500
- Anti-VDAC1 Cell Signaling Technology 3700 (Clone: FL-145) 1:500
- Anti-ubiquitin Cell Signaling Technology 14734 1:500

Validation

Antibodies specific for the required antigens/epitopes were purchased from commercial sources.

- Anti-COX2 / Cyclooxygenase 2 antibody (ab15191) Suitable for: IHC-Fr, ICC/IF, Sandwich ELISA, IP, IHC-P, WB Reacts with: Mouse, Rat, Human, Syrian hamster

References provided on the vendor datasheet (127 total):

1. Soliman NA et al. The possible ameliorative effect of simvastatin versus sulfasalazine on acetic acid induced ulcerative colitis in adult rats. Chem Biol Interact 298:57-65 (2019).
2. Liu LM et al. Edaravone acts as a potential therapeutic drug against pentylentetrazole-induced epilepsy in male albino rats by downregulating cyclooxygenase-II. Brain Behav 9:e01156 (2019).
3. Kong Z et al. Artesunate alleviates liver fibrosis by regulating ferroptosis signaling pathway. Biomed Pharmacother 109:2043-2053 (2019).
References provided on the vendor datasheet (85 total):
1. Li Z et al. Protective roles of Amanita caesarea polysaccharides against Alzheimer’s disease via Nrf2 pathway. Int J Biol Macromol 121:29-37 (2019).
2. Zuo W et al. Hyperglycemia abolished Drp-1-mediated mitophagy at the early stage of cerebral ischemia. Eur J Pharmacol 843:34-44 (2019).
3. Lyu C, Zhang Y, Gu M et al. IRAK-M Deficiency Exacerbates Ischemic Neurovascular Injuries in Experimental Stroke Mice. Front Cell Neurosci Dec 21 2018 [PMID: 30622459] (WB, Mouse)
4. Doyle TM, Chen Z, Durante M, Salvemini D. Activation of sphingosine-1-phosphate receptor 1 in the spinal cord produces mecha-hyperosensitivity through the activation of inflammasome and IL-1β pathway J Pain Feb 22 2019 [PMID: 30802544] (WB, Rat)
5. Humphries, F., et al. 2018. The E3 ubiquitin ligase Pellino 2 mediates priming of the NLRP3 inflammasome. Nat. Commun. 9:1560.

Purified Mouse Anti-DLP1 Clone 8/DLP1 (RUO): 61113 - reactivity - Rat (QC Testing) Human, Mouse, Dog (Tested in developmental)
References provided on the vendor datasheet:
1. Bossya B, Bossy-Wetzel E, Pettrillia A, et al. S-Nitrosylation of DRP1 Does Not Affect Enzymatic Activity and is Not Specific to Alzheimer’s Disease. 2010; 20:5513-5526.
2. Estaquier J, Arnoult D. Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome c during apoptosis. Cell Death Differ. 2007; 14(6):1086-1094.
3. Jofuku A, Ishihara N, Mihara K. Analysis of functional domains of rat mitochondrial Fis1, the mitochondrial fission-stimulating protein. Biochem Biophys Res Commun. 2005; 333(2):650-659.
4. Karbowski M, Neutzen A, Youle RJ. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. J Cell Biol. 2007; 178(1):71-84.
5. Yoon Y, Pitts KR, Dahan S, McNiven MA. A novel dynamin-like protein associates with cytoplasmic vesicles and tubules of the endoplasmic reticulum in mammalian cells. J Cell Biol. 1998; 140(4):779-793.

Anti-GFAP antibody does not recognize unphosphorylated SAPK/JNK. This antibody may slightly cross-react with phospho-Erk1/2 or -p38

Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251; REACTIVITY; H M R Hm Mk Dm B Sc; Phospho-SAPK/JNK (Thr183/ Tyr185) reactions on Western Blots and Immunocytochemistry. Detects a protein band of 55kDa corresponding to GFAP and also a GFAP derived 48kDa band. Some customers have successfully used ab7260 on extracellular vesicles. Dis Model Mech 11:N/A (2018).

1. Chang LH et al. Blockade of soluble epoxide hydrolase attenuates post-ischemic neuronal hyperexcitation and confers resilience against stroke with TrkA activation. Sci Rep 8:118 (2018).
2. Yoshimura A et al. The Sox2promoter-driven CD63-GFP transgenic rat model allows tracking of neural stem cell-derived extracellular vesicles. Dis Model Mech 11:N/A (2018).

Anti-IL1 beta antibody (ab9722) Suitable for: WB, ELISA, Neutralising, IHC-P, ICC, IHC-Fr, IHC-FrFl, ICC/IF, WB, IHC-P, IHC - Wholemount, ICC.
References provided on the vendor datasheet:
1. Hu J et al. Activin A inhibition attenuates sympathetic neural remodeling following myocardial infarction in rats. Mol Med Rep 17:5074-5080 (2018).
2. Tu R et al. Soluble epoxide hydrolase inhibition decreases reperfusion injury after focal cerebral ischemia. Sci Rep 8:5279 (2018).

Caspase-1 p20 Antibody (D-4): sc-398715 caspase-1 p20 (D-4) is recommended for detection of caspase-1 precursor and p20 subunit of mouse and rat origin by Western Blotting.
References provided on the vendor datasheet:
1. Dolunay, A., et al. 2017. Inhibition of NLRP3 inflammasome prevents LPS-induced inflammatory hyperalgesia in mice: contribution of NfκB, caspase-1/11, ASC, NOX, and NOS isoforms. Inflammation 40: 366-386.
2. Zhang, X., et al. 2017. Resveratrol attenuates early brain injury after experi- mental subarachnoid hemorrhage via inhibition of NLRP3 inflammasome activation. Front. Neurosci. 11: 611.
3. Xiang, Y., et al. 2018. A high concentration of DMSO activates caspase-1 by increasing the cell membrane permeability of potassium. Cytotechnology 70: 313-320.
4. Hou, Y., et al. 2018. Nrf2 inhibits NLRP3 inflammasome activation through regulating Trx1/TXNIP complex in cerebral ischemia reperfusion injury. Behav. Brain Res. 336: 32-39.

Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251; REACTIVITY; H M R Hm Mk Dm B Sc; Phospho-SAPK/JNK (Thr183/ Tyr185) Antibody detects endogenous levels of p46 and p54 SAPK/JNK dually phosphorylated at threonine 183 and tyrosine 185. This antibody does not recognize unphosphorylated SAPK/JNK. This antibody may slightly cross-react with phospho-Erk1/2 or -p38 phosphorylated at the homologous residues. It will also react with SAPK/JNK singly phosphorylated at Thr183 and singly
phosphorylated at Tyr185.

References provided on the vendor datasheet: (1) Davis, R.J. (1999) Biochem Soc Symp 64, 1-12. (2) Ichijo, H. (1999) Oncogene 18, 6087-93. (3) Kyriakis, J.M. and Avruch, J. (2001) Physiol Rev 81, 807-69. (4) Kyriakis, J.M. (1999) J Biol Chem 274, 5259-62. (5) Leppä, S. and Bohmann, D. (1999) Oncogene 18, 6158-62. (6) Whitmarsh, A.J. and Davis, R.J. (1998) Trends Bio- chem Sci 23, 481-5. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody #9101; REACTIVITY; H M R Hm Mk Dm B Sc; Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated either individually or dually at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2). The antibody does not cross-react with the cor- responding phosphorylated residues of either JNK/SAPK or p38 MAP Kinase, and does not cross-react with non- phosphorylated Erk1/2.

References provided on the vendor datasheet: (1) Roux, P.P. and Blenis, J. (2004) Microbiol Mol Biol Rev 68, 320-44. (2) Baccarini, M. (2005) FEBS Lett 579, 3271-7. (3) Meloche, S. and Pouysségur, J. (2007) Oncogene 26, 2227-39. (4) Roberts, P.J. and Der, C.J. (2007) Oncogene 26, 3291-310. (5) Rubinfeld, H. and Seger, R. (2005) Mol Biotechnol 31, 151-74. (6) Murphy, L.O. and Blenis, J. (2006) Trends Biochem Sci 31, 268-75. (7) Dalby, K.N. et al. (1998) J Biol Chem 273, 1496-505. (8) Marais, R. et al. (1993) Cell 73, 381-93. (9) Cortenjann, M. et al. (1994) Mol Cell Biol 14, 4815-24. (10) Owens, D.M. and Keyse, S.M. (2007) Oncogene 26, 3203-13. Phospho-DRP1 (Ser616) Antibody #3455; Species Cross-Reactivity* R, (M, Mk) Phospho-DRP1 (Ser616) Antibody detects endogenous levels of DRP1 only when phosphorylated at Ser616.

References provided on the vendor datasheet: (1) Praefcke, G.J. and McMahon, H.T. (2004) Nat Rev Mol Cell Biol 5, 133-47. (2) Taguchi, N. et al. (2007) J Biol Chem 282, 11521-9. (3) Smirnova, E. et al. (2001) Mol Biol Cell 12, 2245-56. (4) Smirnova, E. et al. (1998) J Cell Biol 143, 351-8. (5) Koch, A. et al. (2003) J Biol Chem 278, 8597-605. (6) Knot, A.B. et al. (2008) Nat Rev Neurosci 9, 505-18. (7) Cereghetti, G.M. et al. (2008) Proc Natl Acad Sci U S A 105, 15803-8. (8) Zunino, R. et al. (2007) J Cell Sci 120, 1178-88. Phospho-DRP1 (Ser637) Antibody #3455; Species Cross-Reactivity* H, (M, R, Mk) Phospho-DRP1 (Ser637) Antibody detects endogenous levels of DRP1 only when phosphorylated at Ser637.

References provided on the vendor datasheet: (1) Praefcke, G.J. and McMahon, H.T. (2004) Nat Rev Mol Cell Biol 5, 133-47. (2) Baccarini, M. (2005) FEBS Lett 579, 3271-7. (3) Meloche, S. and Pouysségur, J. (2007) Oncogene 26, 2227-39. (4) Roberts, P.J. and Der, C.J. (2007) Oncogene 26, 3291-310. (5) Rubinfeld, H. and Seger, R. (2005) Mol Biotechnol 31, 151-74. (6) Murphy, L.O. and Blenis, J. (2006) Trends Biochem Sci 31, 268-75. (7) Dalby, K.N. et al. (1998) J Biol Chem 273, 1496-505. (8) Marais, R. et al. (1993) Cell 73, 381-93. (9) Cortenjann, M. et al. (1994) Mol Cell Biol 14, 4815-24. (10) Owens, D.M. and Keyse, S.M. (2007) Oncogene 26, 3203-13. Phospho-DRP1 (Ser637) Antibody #4867 Species Cross-Reactivity* R, (M, Mk) Phospho-DRP1 (Ser637) Antibody detects endogenous levels of DRP1 only when phosphorylated at Ser637.

References provided on the vendor datasheet: (1) Praefcke, G.J. and McMahon, H.T. (2004) Nat Rev Mol Cell Biol 5, 133-47. (2) Taguchi, N. et al. (2007) J Biol Chem 282, 11521-9. (3) Smirnova, E. et al. (2001) Mol Biol Cell 12, 2245-56. (4) Smirnova, E. et al. (1998) J Cell Biol 143, 351-8. (5) Koch, A. et al. (2003) J Biol Chem 278, 8597-605. (6) Knot, A.B. et al. (2008) Nat Rev Neurosci 9, 505-18. (7) Cereghetti, G.M. et al. (2008) Proc Natl Acad Sci U S A 105, 15803-8. (8) Zunino, R. et al. (2007) J Cell Sci 120, 1178-88. Tom20 Antibody (FL-145): sc-11415; Tom20 (FL-145) is recommended for detection of Tom20 of mouse, rat and human origin by Western Blotting.

References provided on the vendor datasheet: 1. Zong, W.X., et al. 2004. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev. 18: 1272-1282. 2. Usami, Y., et al. 2011. DJ-1 associates with synaptic membranes. Neurobiol. Dis. 43: 651-662. 3. De Marchi, U., et al. 2011. Uncoupling protein 3 (UCP3) modulates the activity of sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) by decreasing mitochondrial ATP production. J. Biol. Chem. 286: 12533-32541. 4. Singh, K., et al. 2011. Effect of denervation-induced muscle disuse on mitochondrial protein import. Am. J. Physiol., Cell Physiol. 300: C138-C145. 5. Pavlov, P.F., et al. 2011. Mitochondrial γ-secretase participates in the metabolism of mitochondria-associated amyloid precursor protein. FASEB J. 25: 78-88. 6. Bénard, G., et al. 2012. Mitochondrial CB1 receptors regulate neuronal energy metabolism. Nat. Neurosci. 15: 558-564. 7. Fernandes, R., et al. 2011. Uncoupling protein 3 (UCP3) modulates the activity of sarco-endoplasmic reticulum Ca2+ ATPase (SERCA) by decreasing mitochondrial ATP production. J. Biol. Chem. 286: 32533-32541. 8. Anti-VDAC1 / Porin antibody [20B12AF2] (ab14734); Reacts with: Mouse, Rat, Sheep, Goat, Cat, Dog, Human, Pig, Drosophila melanogaster, Fish, Quail, Common marmoset, Dogfish, Catshark.

References provided on the vendor datasheet: 1. Zhang D et al. Increased mitochondrial fission is critical for hypoxia-induced pancreatic beta cell death. PLoS One 13:e0197266 (2018). 2. Vantrouys E et al. Severe hepatopathy and neurological deterioration after start of valproate treatment in a 6-year-old child with mitochondrial tryptophanyl-tRNA synthetase deficiency. Orphanet J Rare Dis 13:80 (2018).
### Eukaryotic cell lines

**Cell line source(s)**

| BV2 microglia were a gift from Dr. Katrin Andreasson, Stanford University |

**Authentication**

None of the lines used were authenticated

**Mycoplasma contamination**

Cell lines were not tested for Mycoplasma contamination

### Animals and other organisms

**Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research**

**Laboratory animals**

| Adult B6SJL Tg [SOD1G93A] 1 Gur/J male mice with a high copy number of the mutant allele and their WT littermates at 4-6 weeks, 5XFAD transgenic male mice and their littermates at 3 months, and Hemizygous male R6/2 HD mice and their WT littermates at 4-6 weeks were used. |

**Wild animals**

| We did not use any wild animals |

**Field-collected samples**

| The study did not involve field collected samples |

**Ethics oversight**

| Animal husbandry and procedures were performed according to Stanford guidelines under IACUC approved protocols. |

*Note that full information on the approval of the study protocol must also be provided in the manuscript.*

### Human research participants

**Policy information about studies involving human research participants**

**Population characteristics**

| Peripheral blood mononuclear cells from de-identified healthy donors (35-65 years old) were obtained from the Stanford Blood center |

**Recruitment**

| N/A |

**Ethics oversight**

| N/A |

*Note that full information on the approval of the study protocol must also be provided in the manuscript.*