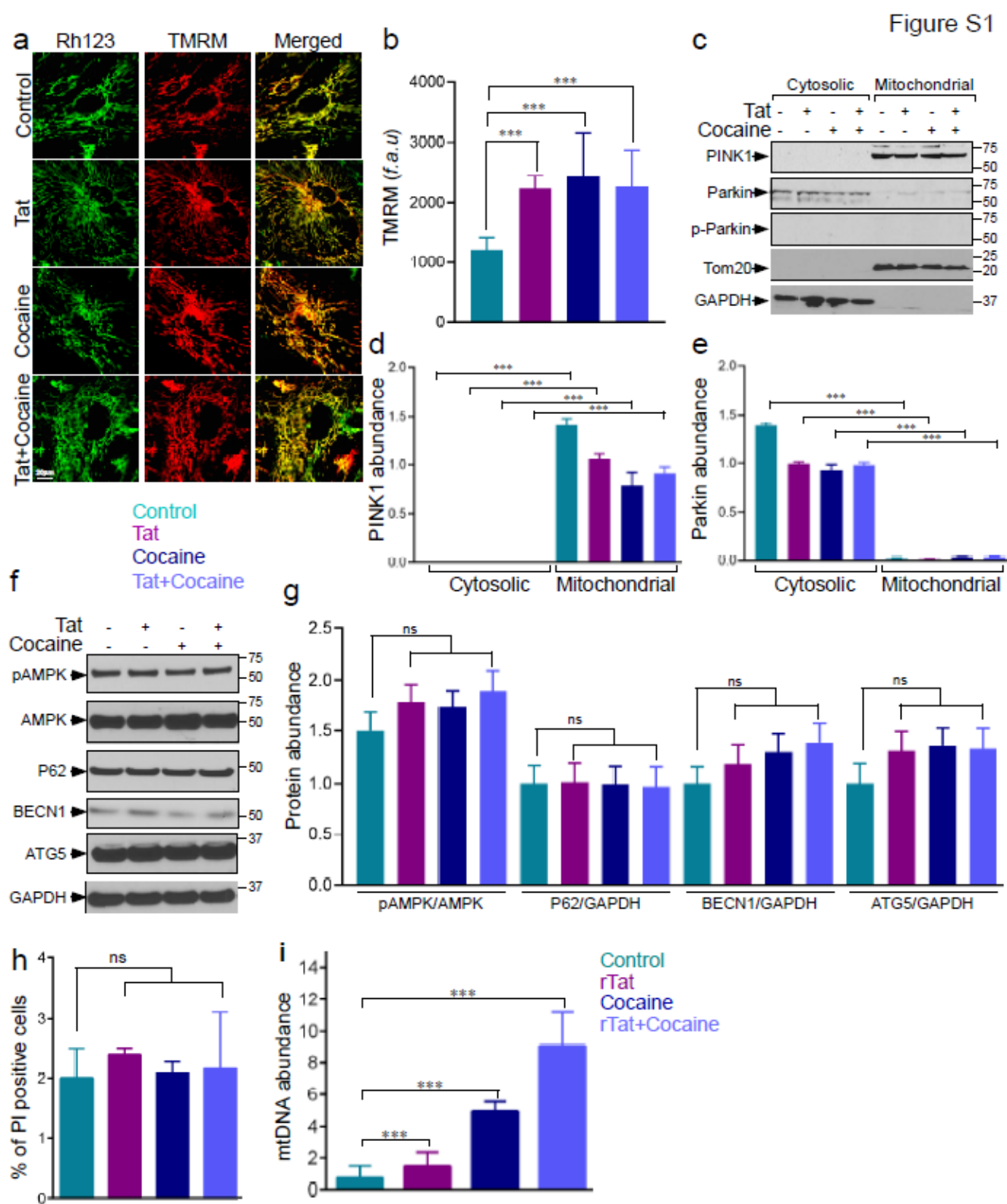


1 **ADDITIONAL INFORMATION**

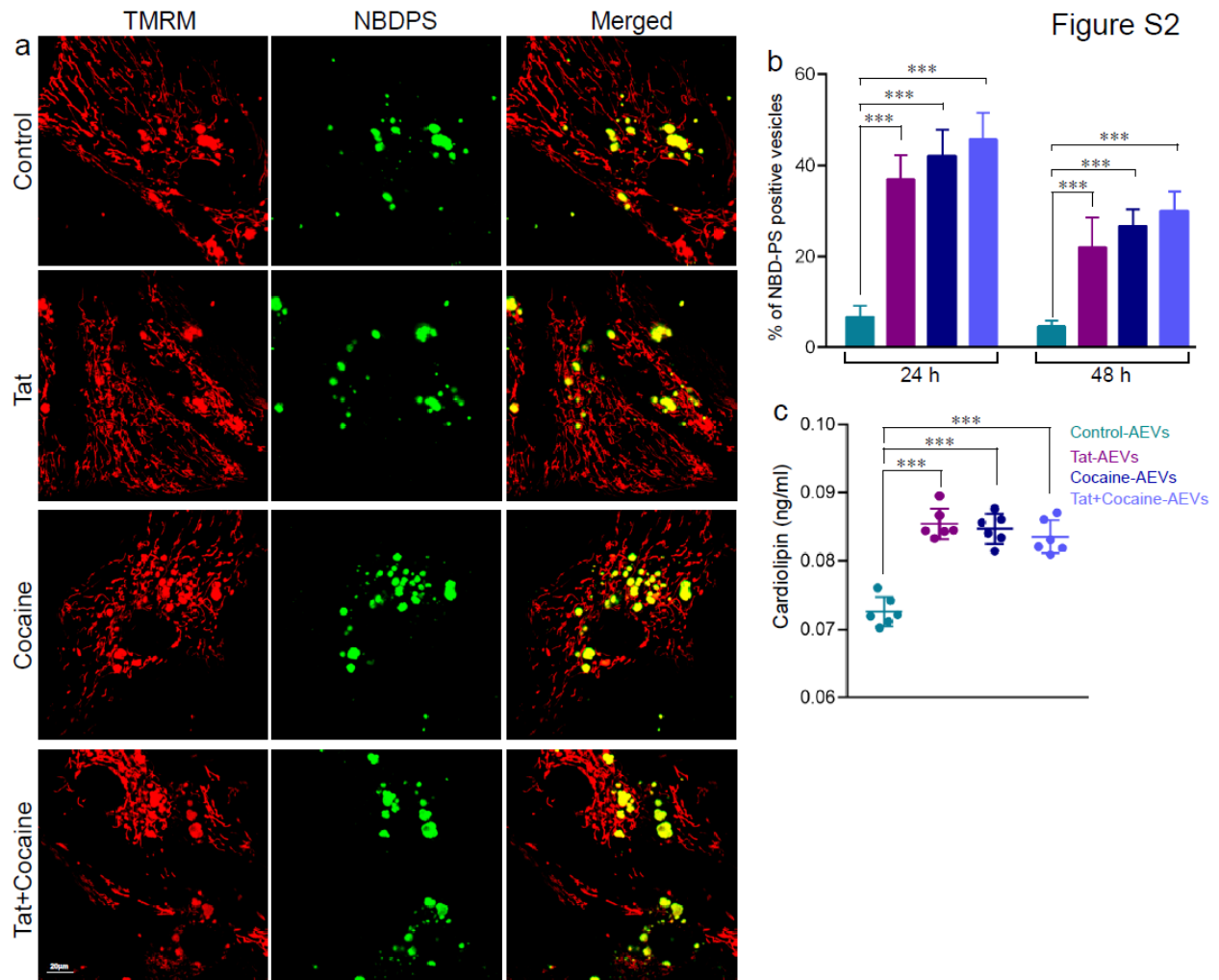


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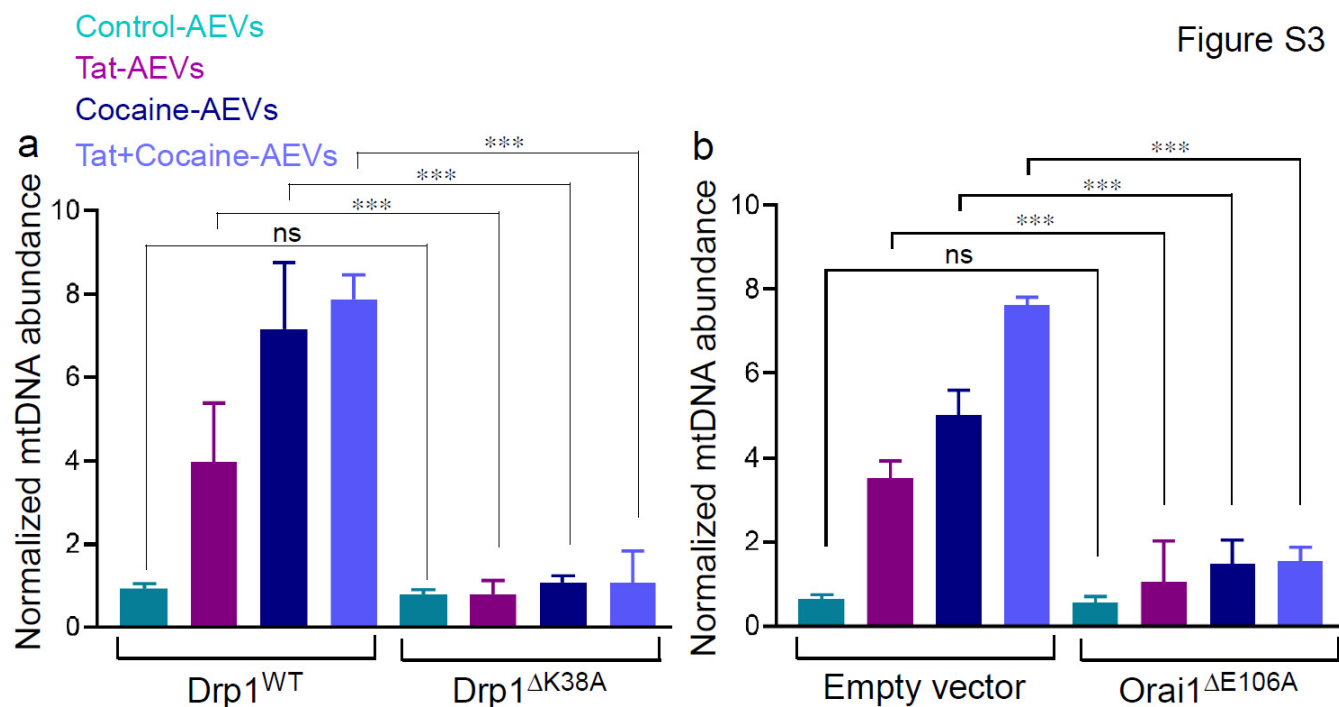
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Supplementary Figure S1. Astrocytes treated with Tat and/or cocaine have intact mitochondria.

(a, b) Astrocytes treated with or without Tat/cocaine for 48 h were loaded with TMRM (50 nM) and DHR123 (1 μ M) and imaged at 561 and 488 nm, respectively. Representative confocal images (a) and quantification of TMRM fluorescence (b) show increased mitochondrial membrane potential in astrocytes treated with Tat/cocaine. (c-e) Mitophagy was assessed in cytosolic and mitochondrial fractions obtained from astrocytes treated with or without Tat/cocaine via Western analyses for PINK1, Parkin, Tom20, and GAPDH. Representative Western blot (c) and the quantification of the normalized PINK1 (d) and Parkin levels (e) in cytosolic and mitochondrial fractions show no translocation of Parkin and thus no mitophagy. (f, g) Lysates prepared from astrocytes treated with or without Tat/cocaine were assessed by Western analyses for p-AMPK, AMPK, P62, BecN1, ATG5, and GAPDH to assess induction of autophagy in astrocytes. Representative Western blot (f) and the quantification of the normalized protein levels (g) show no processing of p62. (h) Astrocytes were treated with Tat/cocaine for 48 h and stained with propidium iodide (PI) to measure necrotic cell death. Hoechst33342 was used as a nuclear marker. Quantification of the percentage of PI positive cells show no cell death in astrocytes treated with Tat/cocaine. Astrocytes with no Tat or cocaine treatment served as control. Data represents Mean $\pm$ SEM; p-values with *p<0.05, **p<0.01, ***p < 0.001, n=3-4 independent experiments.



Supplementary Figure S2. Mitochondria are the membrane source for the AEVs. (a) To show the transfer of mitochondrial lipids to AEV membranes, astrocytes were loaded with 18:1-06:0 N-[7-Nitrobenz-2-oxa-1,3-diazol-4-yl] phosphatidylserine (NBD-PS), a fluorescent analog of PS, for 1h to allow accumulation of PS in the mitochondria. After 1h of incubation, astrocytes were treated with Tat/cocaine. (a) Representative confocal images show the accumulation of fluorescent PS in the mitochondria. (b) AEVs prepared from the ACM after 24 and 48h were subjected to FACS analysis. FACS was performed to sort NBD-PS labelled AEVs. Quantification of % of NBD-PS positive AEVs shows Tat/cocaine treatment to increase the NBD-PS labelled AEVs in the ACM. (c) Quantification of cardiolipin concentration in AEVs isolated from Tat/cocaine treated astrocytes using cardiolipin-specific ELISA. Data represents Mean $\pm$ SEM; One-way ANOVA with Tukey correction was performed to calculate p-values with ***p < 0.001, n=3-4 independent experiments.



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43 **Supplementary Fig. S3: Inhibition of mitochondrial fission in astrocytes prevents encapsulation**

44 **of mitochondria in AEVs.** (a, b) To assess whether inhibition of mitochondrial fission decreases the

45 mitochondrial particles released into the extracellular milieu, astrocytes expressing Drp1^{ΔK38A} or

46 Orai1^{ΔE106A} were treated with Tat/cocaine for 48 h. mtDNA abundance was measured in AEVs obtained

47 from Drp1^{ΔK38A} (a), and Orai1^{ΔE106A} expressing astrocytes (b). Quantifying mtDNA abundance shows

48 decreased mtDNA in AEVs obtained from Drp1^{ΔK38A} or Orai1^{ΔE106A} expressing astrocytes. Data

49 represents Mean ± SEM; One-way ANOVA with Tukey correction was performed to calculate p-values

50 with *p<0.05, **p<0.01, ***p < 0.001, n=3-4 independent experiments.

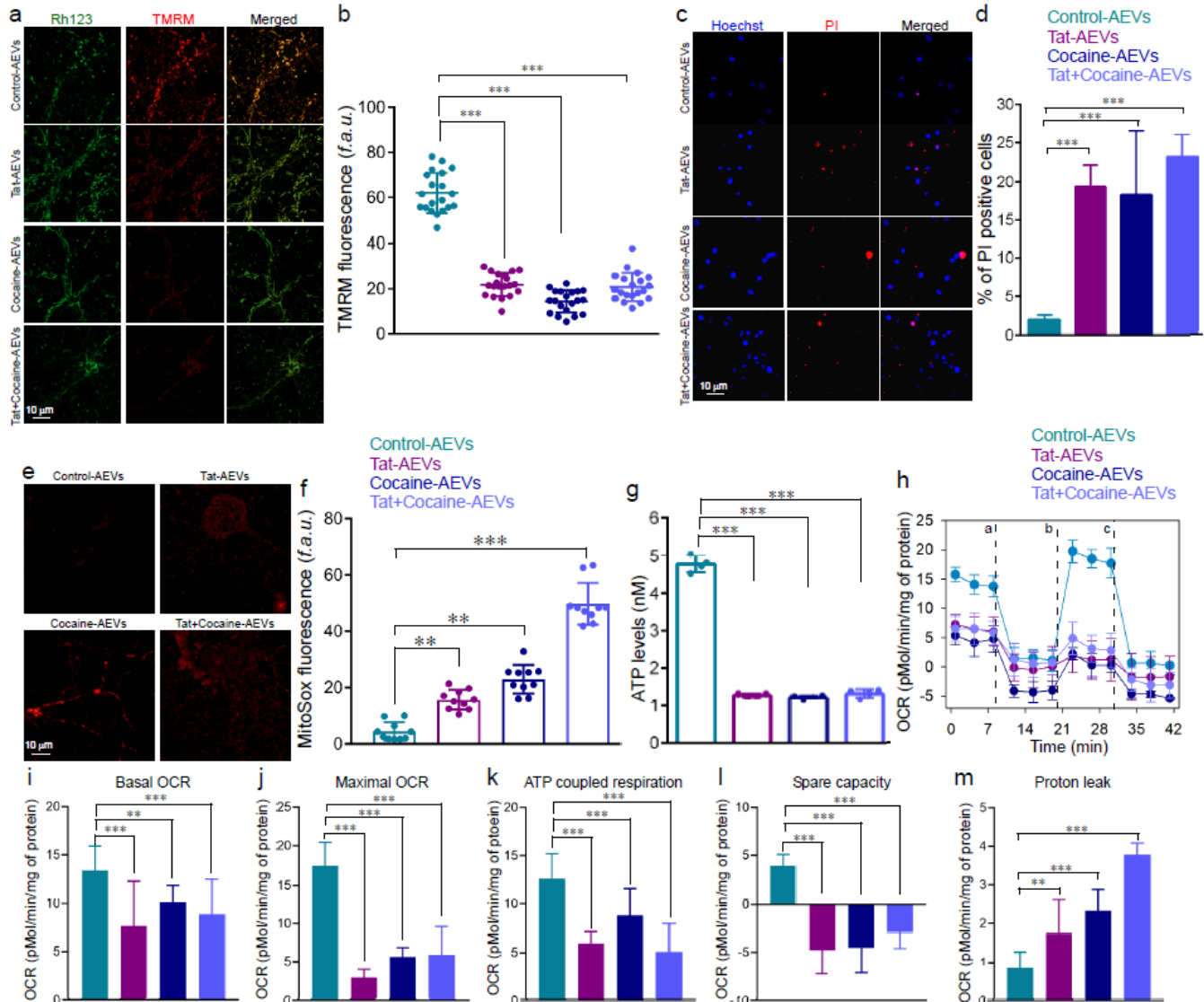
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Figure S4



Supplementary Fig. S4: Astrocyte-derived mitochondria are internalized by neurons, negatively affecting neuronal mitochondrial function. To assess whether the AEVs that carry mitochondria have neurotoxic effects, neurons were exposed to control-AEVs and Tat/cocaine-AEVs for 48 h. Forty-eight hours post-treatment, cells were stained with mitochondrial membrane potential indicator, TMRM (a, b), propidium iodide (PI) to measure necrotic cell death (c, d), MitoSox, to measure mROS (e, f), and neuronal ATP levels (g). Quantification of TMRM fluorescence, % of PI-positive cells, MitoSox fluorescence, and ATP levels shows AEVs to have a neurotoxic effect. (h-m) Measurement of oxygen consumption rate (OCR) in neurons exposed to control- AEVs or Tat/cocaine- AEVs for 48 h. After

64 basal OCR measurement, oligomycin (a), FCCP (b), and rotenone + Antimycin A (c) were added as
65 indicated. Representative OCR (**h**) traces in neurons exposed to control- AEVs and Tat/cocaine- AEVs
66 are shown. (**i-m**) Quantification of basal OCR (**i**), maximal OCR (**j**), ATP-coupled respiration (**k**), spare
67 capacity of the mitochondria (**l**), and proton leak (**m**) shows impaired mitochondrial respiration in
68 Tat/cocaine-AEVs-treated neurons. Data represents Mean $\pm$ SEM; One-way ANOVA with Tukey
69 correction was performed to calculate p-values with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 3-4$ independent
70 experiments.