

Here we present the Results of the comparisons between ASD and CSR. Wake in these two conditions was enforced using different methods, thus the different changes observed in the ultrastructure could be due to a combination of different wake duration, use of caffeine, treadmill, social interaction and different exposure to light.

***Mitochondria***

Absolute size of mitochondrial population is statistically different between ASD and CSR (Wilcoxon rank sum test, W:  $p < 0.0001$ ) and the effect is mainly due to mitochondria of medium size ( $p = 0.049$ ; small mitochondria:  $p = 0.3038$ ; large mitochondria  $p = 0.32$ ).

The percentage of cytoplasm occupied by mitochondria and the percentage of hourglass mitochondria do not change significantly ( $p = 0.1253$  and  $p = 0.252$  respectively).

***Early endocytic pathway***

The density of early endosomes (EE) was lower in CSR than in ASD ( $p = 0.000087$ ), as well as the percentage of cytoplasm occupied by EE ( $p = 0.0005$ ).

***Lysosomes***

Primary lysosomes were larger ( $p < 0.0001$ ) and significantly less numerous ( $p = 0.0024$ ) in ASD than in CSR.

***Secondary lysosomes***

Secondary lysosomes were larger in ASD than in CSR ( $p < 0.0001$ ), but they were more numerous in CSR ( $p < 0.0001$ ). As a result, the percentage of cell cytoplasm occupied by active lysosomes was greater in CSR ( $p = 0.032$ ).

***Cell classification***

Multivariate logistic regression analysis was used to predict the probability of S, ASD, CSR, and RS cells of being classified in the correct experimental group using the 11 ultrastructural parameters listed below. For each parameter the following values are listed: 1) coefficient for the binary logistic equation (B); 2) standard error (s.e.); 3) relevance for the model (Wald test); 4) significance of the contribution to the model (Sig.); 5) fold change in the probability of being ASD for 1 unit increase in the parameter considered (Odds ratio).

ASD vs. CSR	B	s.e.	Wald	Sig.	Odds Ratio exp(B) of being ASD
cytoplasm occupied by mitochondria, %	-0.46	0.18	6.20	0.01	0.63
Quiescent lysosomes, size	0.33	0.17	4.03	0.04	1.4
Active lysosomes, size	0.88	0.29	8.72	0.003	2.42

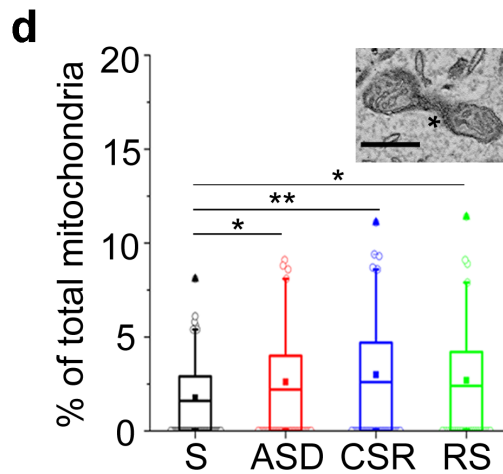
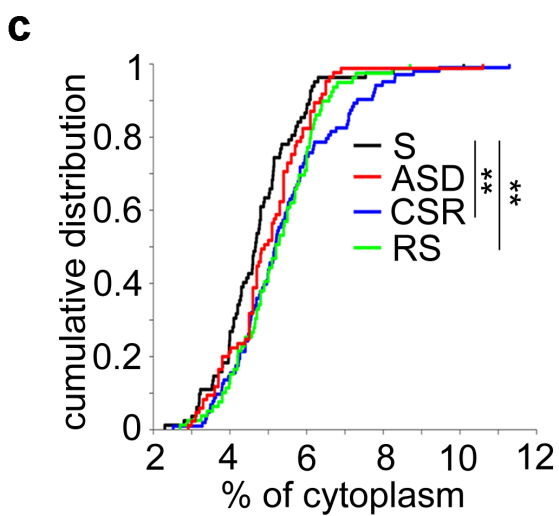
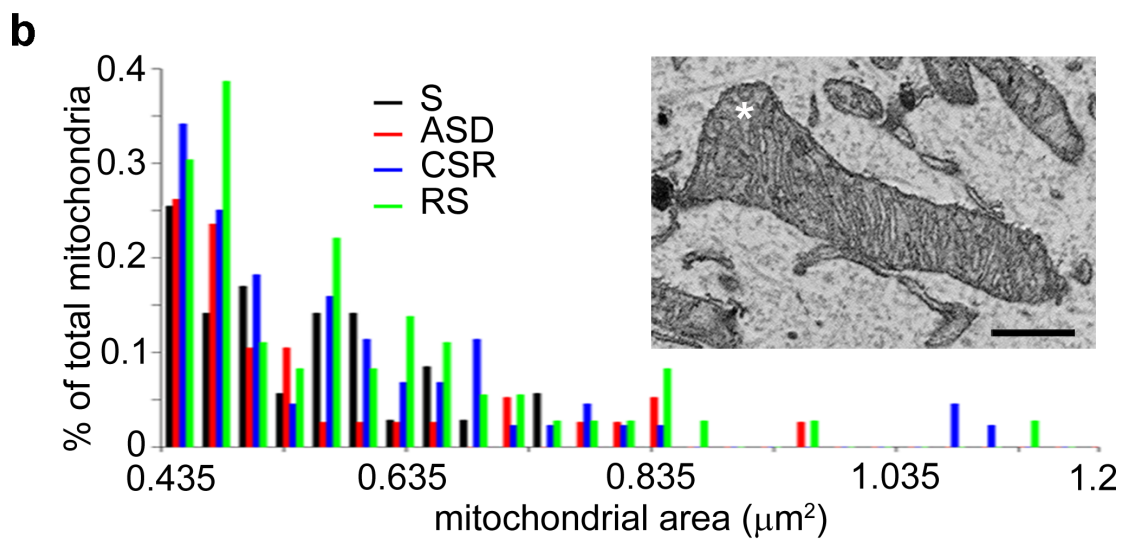
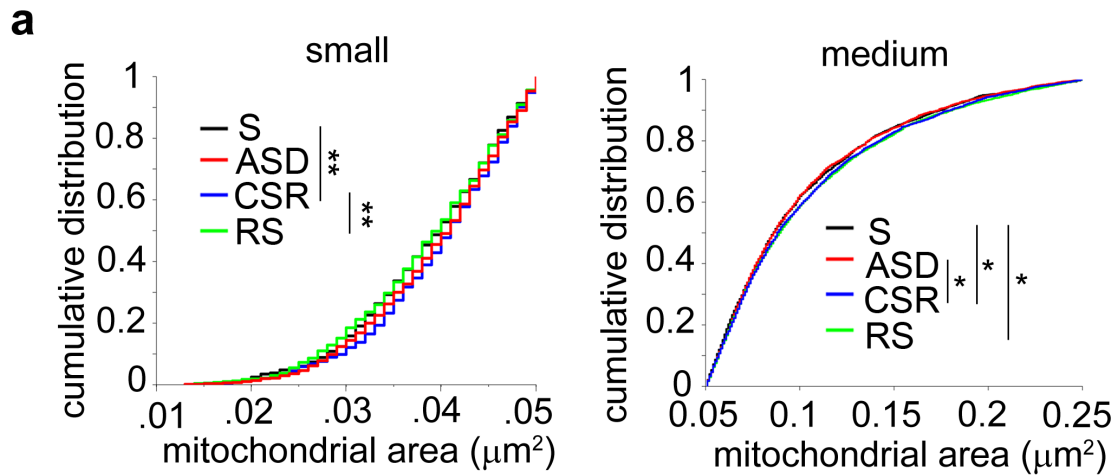
Quiescent lysosomes, density	-0.60	0.22	7.35	0.007	0.55
Active lysosomes, density	-1.31	0.30	18.65	0.00001	0.27
EE, density	0.65	0.25	6.79	0.009	1.91
MVBs, density	-0.42	0.23	3.17	0.07	0.66
hourglass mitochondria, %	0.03	0.19	0.04	0.84	1.04
Invaginating endosomes, density	0.08	0.23	0.15	0.70	1.09
Back-fusing endosomes, density	0.11	0.22	0.22	0.63	1.11
Lipofuscin granules, density	-0.21	0.2	1.09	0.3	0.81

<b>Cell classification: ASD vs CSR</b>			
	Predicted		
Observed	ASD	CSR	% correct
ASD = 86	62	24	72.1
CSR = 103	23	80	77.7

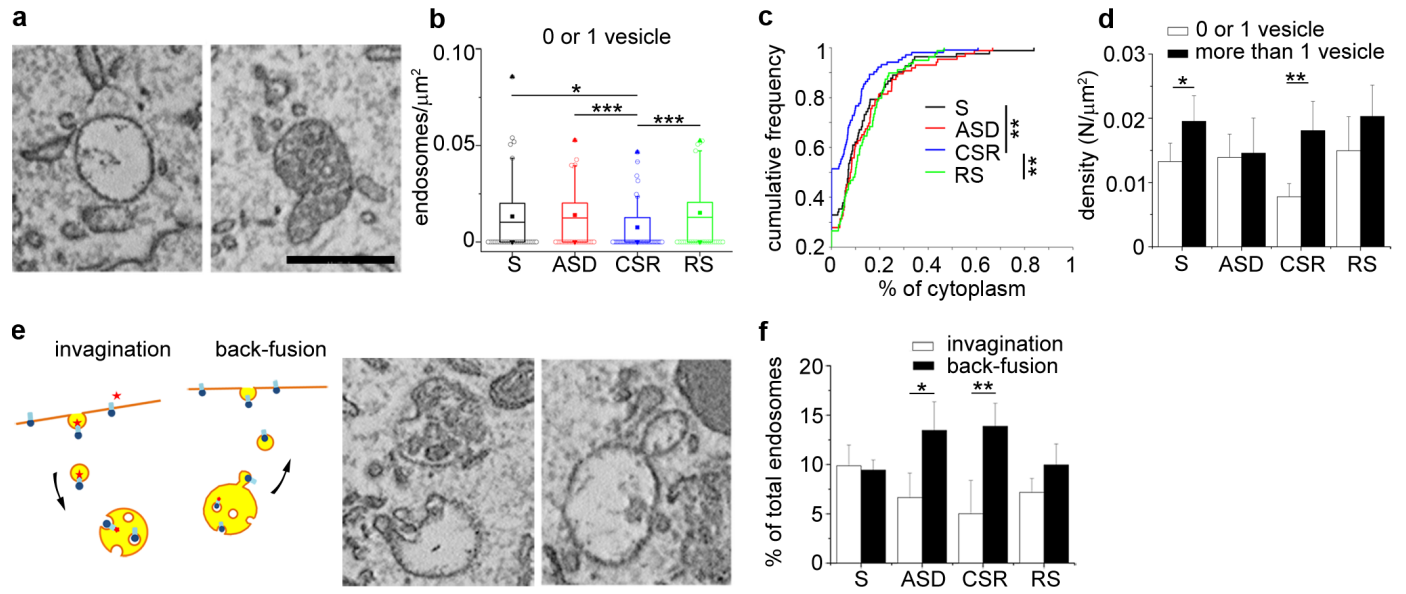
The probability distribution showed that from an ultrastructural standpoint CSR cells differed from ASD ( $p < 0.0001$ ). By computing the Euclidean distance (ED) between the probability distributions we found that the divergence between the distributions of ASD and CSR was higher than the divergence between S and ASD, or between CSR and RS ( $ED_{SvsASD}=29.4$ ,  $ED_{SvsCSR}=29.6$ ,  $ED_{SvsRS}=34.6$ ,  $ED_{ASDvsCSR}=43$ ,  $ED_{RSvsCSR}=39.2$ ).

**Supplementary figures 1-4; they correspond to figures 2-5 of the main text, the only difference being that in the supplementary version the direct comparison ASD vs. CSR is also shown.**

**Figure S1 (see Figure 2)**



**Figure S2 (see Figure 3)**



**Figure S3 (see Figure 4)**

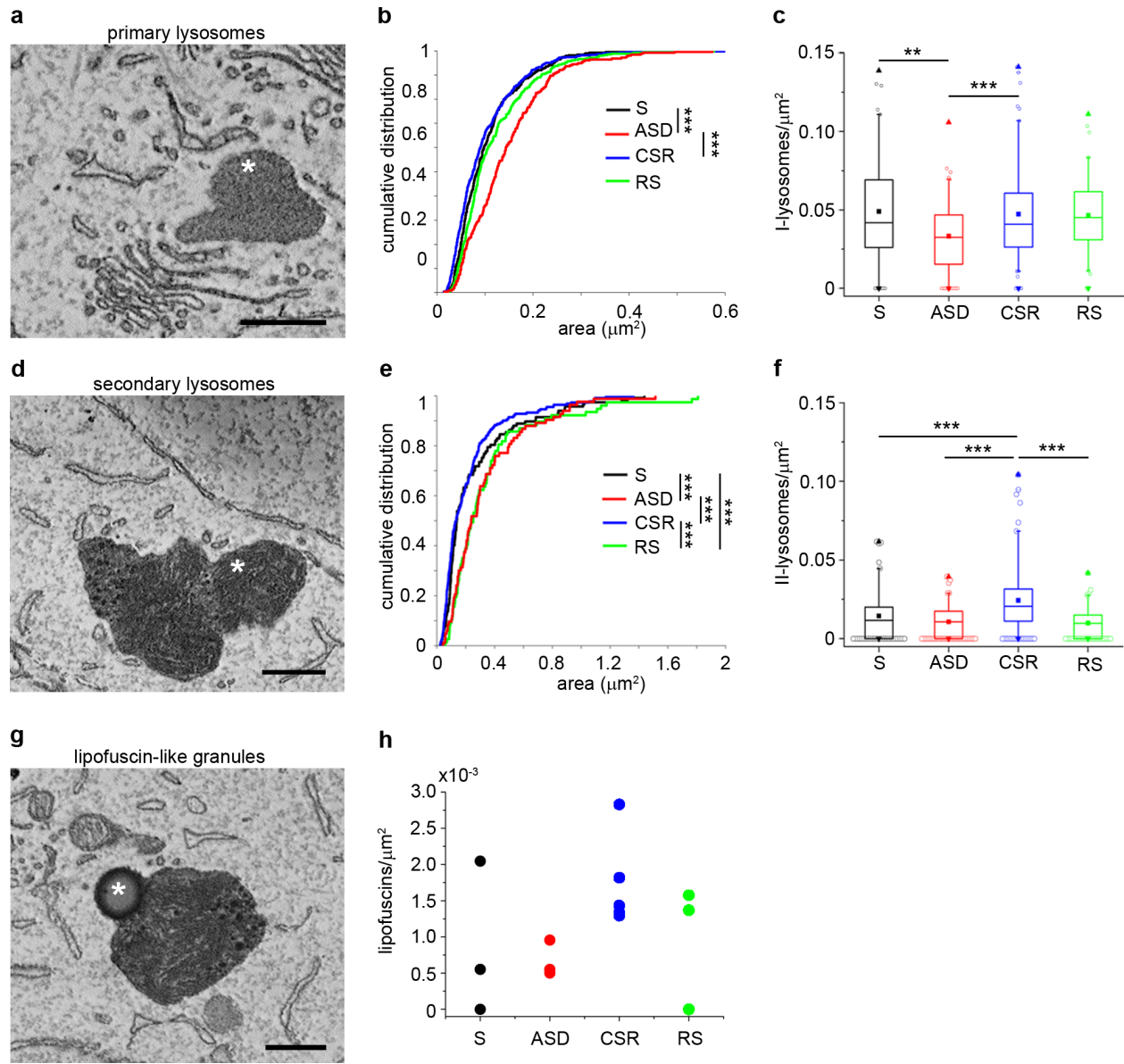


Figure S4 (see Figure 5)

