

Supplementary data, Mignard et al.

Supplementary Figure S1:

Volume of buffer used to resuspend the different fractions during U251 cells or liver fractionation.

Supplementary Figure S2:

Western blot analysis of mice brains fractionation (see material and methods). The blots shown are representative of three independent fractionations.

Supplementary Figure S3:

Statistical data from the spectrometric measurement of liver or U251 cells extracted lipids are shown (Tukey's multiple comparison test, GraphPad Prism).

Supplementary Figure S4:

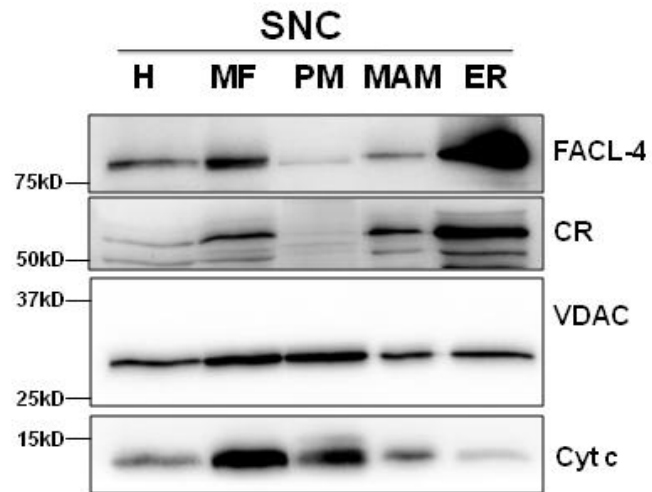
(A) Cell fractionation was performed as described in material and methods, either on U251 control cells (sham control) or on U251 cells treated by STS for 4h. ASM or NSM activity was measured as described in material and methods in each fraction and expressed as pmol of hydrolysed SM/ μ g protein/h. The results shown in the graphs are the mean (\pm SEM) of three independent experiments. (B) Transmission electron microscopy was done on isolated mitochondrial fraction (MF, see material and methods) obtained from U251 cells (the bar represents 0.2 μ m). M: mitochondria; ER: endoplasmic reticulum; L: lysosome. (C) Western blot analysis of the fractions obtained in STS-treated U251 cells for the co-purification of Golgi (GM130) and lysosomes (LAMP2). The blots are representative of three independent fractionation experiments.

Supplementary Figure S5:

U251 cells were spotted on sterile glass coverslips the day before treatment. Control (NT) and treated cells (STS, 0.5 μ g/ml 4h) were fixed by paraformaldehyde (4% in PBS, 15min at room temperature). Cells were permeabilized, saturated (5% BSA in PBS) and incubated with antibodies raised against TOM20 (Pharmingen 612278) and ASM (Thermo Fisher Scientific PA5-77047) in 1% BSA-0.05% saponin-PBS overnight at 4°C. The secondary antibodies used were coupled to a fluorochrome (Alexa 488 and 647 respectively, Molecular Probes). Images were collected on a Nikon confocal A1 RSi microscope in the Cellular and Tissular Imaging Core Facility of Nantes University (MicroPICell). Each line is a series of several optic sections in the z axis (green: TOM20, red: ASM).

Supplementary Figure S1: Mignard et al.

Fractions		Volume (Fractionation from cells)	Volume (Fractionation from liver)
H	Homogenate	3.5 mL Store 50µL	28 mL Store 100 µL
MF	Mitochondrial Fraction	800 µL Store 50 µL	2 mL Store 200 µL
PM	Pure Mitochondrial Fraction	60 µL	300 µL
MAM	Mitochondri-associated membranes	200 µL	200 µL
ER	Endoplasmic Reticulum	200µL	500 µL
Cyto	Cytosol	3 mL Store 1 mL	10 mL Store 1mL



Liver lipids measurement statistics

Tukey's Multiple Comparison Test	ceramide	sphingomyelin	sphingosine	sphinganine	glucosyl ceramide	lactosyl ceramide
H vs MF	**	ns	*	ns	*	*
H vs PM	ns	ns	**	ns	ns	*
H vs MAM	***	**	ns	ns	**	ns
H vs ER	***	*	ns	ns	ns	ns
H vs Cyto	**	ns	ns	ns	ns	ns
MF vs PM	*	ns	ns	ns	ns	ns
MF vs MAM	****	*	ns	ns	ns	ns
MF vs ER	***	ns	ns	ns	ns	**
MF vs Cyto	***	**	*	ns	**	**
PM vs MAM	***	***	ns	ns	**	ns
PM vs ER	***	**	*	ns	ns	*
PM vs Cyto	***	ns	**	ns	*	**
MAM vs ER	*	ns	ns	ns	*	ns
MAM vs Cyto	***	***	ns	ns	***	*
ER vs Cyto	***	***	ns	ns	**	ns

ns: not significant *: p<0.05 **: p<0.01 ***: p<0.001

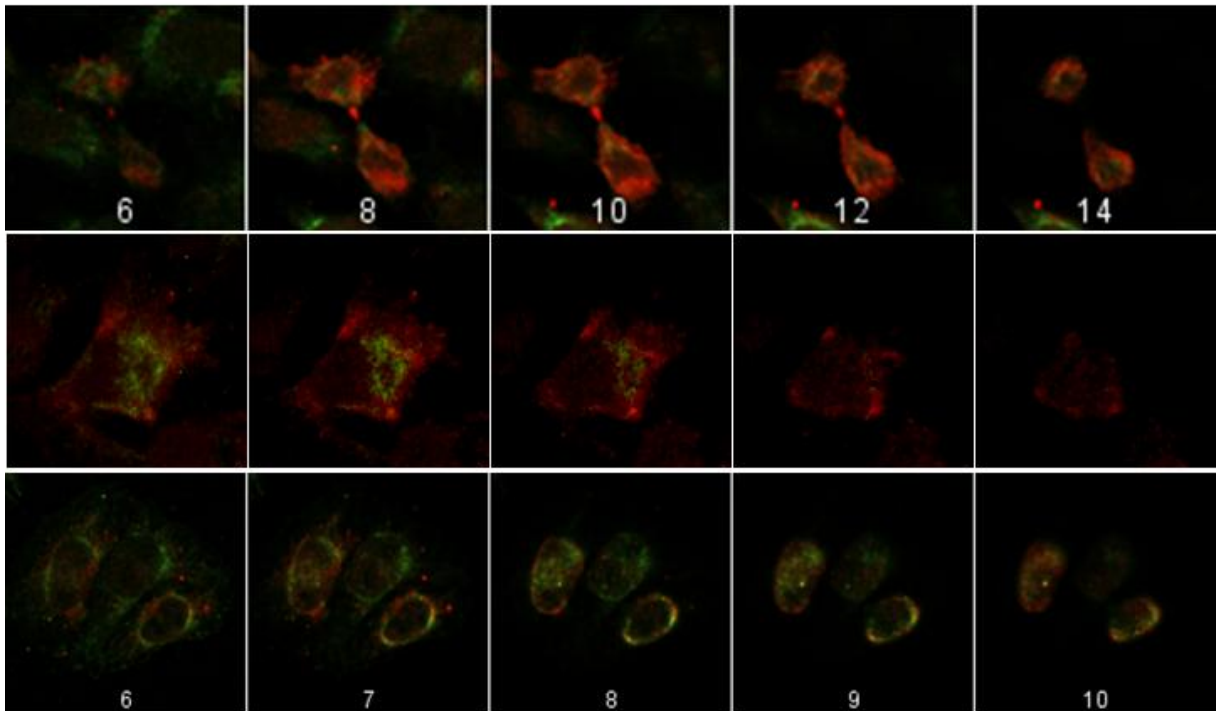
U251 lipids measurement statistics

Tukey's Multiple Comparison Test	ceramide	sphingomyelin	sphingosine	sphinganine	glucosyl ceramide	lactosyl ceramide
H vs MF	ns	***	ns	**	***	ns
H vs PM	*	ns	*	***	***	ns
H vs MAM	ns	***	ns	ns	***	**
H vs ER	ns	*	ns	ns	**	ns
H vs Cyto	ns	ns	ns	ns	ns	ns
MF vs PM	ns	***	ns	***	ns	ns
MF vs MAM	ns	ns	ns	*	**	ns
MF vs ER	ns	*	ns	**	***	ns
MF vs Cyto	ns	***	ns	**	***	*
PM vs MAM	ns	***	ns	***	ns	*
PM vs ER	ns	ns	*	***	***	ns
PM vs Cyto	*	*	ns	***	***	ns
MAM vs ER	ns	**	ns	ns	**	ns
MAM vs Cyto	ns	***	ns	ns	***	**
ER vs Cyto	ns	***	ns	ns	***	ns

ns: not significant *: p<0.05 **: p<0.01 ***: p<0.001

Supplementary Figure S5: Mignard et al.

NT



STS

