Supplementary information 3

The fact that the amount of desmosterol in DRMs is not affected by changes in seladin-1 expression strongly suggests that the changes observed in DRM protein composition and DRM related functions (i.e. plasmin activity and APP-β-cleavage) in the seladin-1 heterozygous mice were not due to the increase found in desmosterol levels but to the decrease in the amount of membrane cholesterol. To confirm this, desmosterol content was 6-fold increased in human SH-SY5Y cells by the exogenous addition of desmosterol/methyl-β-cyclodextrin complexes (see methods and supplementary Figure 3 for details). In order to mimic as much as possible the situation found for both desmosterol and cholesterol in the brains of seladin-1 heterozygous mice we also set in SH-SY5Y cells an experimental condition in which 30% cholesterol was first extracted with mevilonin and methyl-β-cyclodextrin followed by the addition of exogenous desmosterol as mentioned above (suppl Figure 3A). The flotation profile of the DRM enriched protein flotillin 1, the activity of endogenous plasmin and the amount of APP-β-CTF were determined in these two experimental conditions and compared to untreated SH-SY5Y cells. The results revealed that while the addition of desmosterol has no effect in these variables, the extraction of cholesterol displaces flotillin 1 from DRMs, reduces plasmin activity and increases the amount of APP-β-CTF similarly to what we observed in the seladin-1 heterozygous mice brains (suppl. Figure 3B,C,D). Cell viability after these treatments was not affected as assayed by TUNEL staining (4.5±2.2%, 3.8±1.9% and 4.1±3% of apoptotic cells in untreated cells, cells treated with desmosterol and cells treated with mevilonin and desmosterol, respectively).

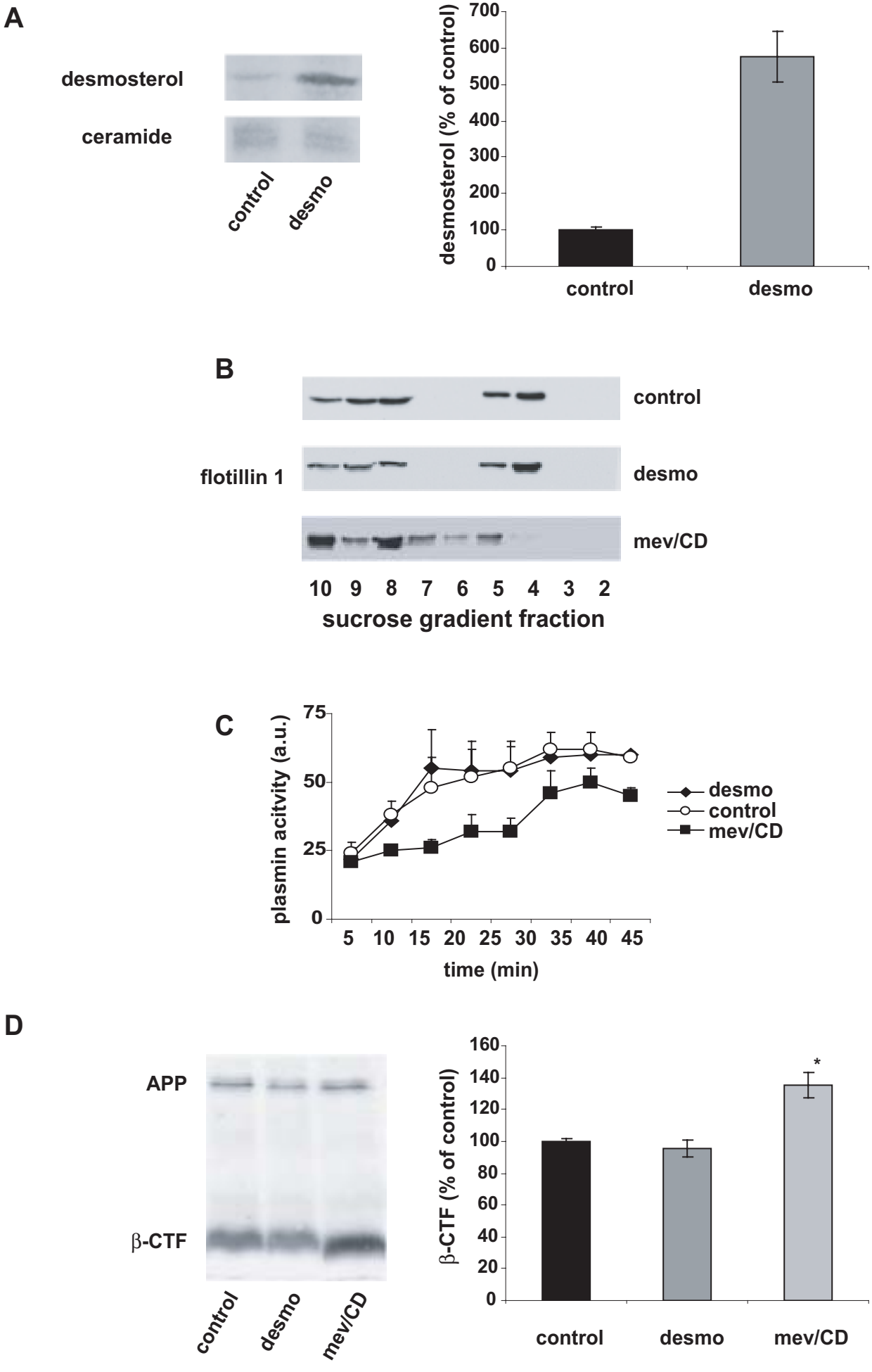
Supplementary Figure legend 3 The decrease in cholesterol but not the increase in desmosterol levels affects the flotation profile of the DRM component flotillin 1, plasmin activity and APP β-cleavage in SH-SY5Y cells.

TLC analysis of the same amount of treated cells with 0.3mM desmosterol/methyl-β-cyclodextrin inclusion complexes for 1 hour (desmo) or untreated (contol) SH-SY5Y cells. This treatment induced an average 6-fold increase in the amount of desmosterol. Ceramide levels, shown as a loading control, were not significantly changed. The graph on the right shows the mean values and standard error from the densitometric analysis of the TLCs from two independent experiments. Desmosterol content is given as the fold-increase in treated with respect to the corresponding level of the untreated cells (A).

Western blots using the antibody against the DRM enriched protein flotillin 1 of sucrose gradients of SH-SY5Ycells extracted in cold 1% TritonX-100 of untreated (control), treated with 0.3mM desmosterol/methyl-β-cyclodextrin complex for 1h to increase 6-fold cellular desmosterol levels (desmo) (see suppl. Figure 3A) and treated with 0.4μM mevilonin and 1mM cyclodextrin for 48 hours to reduce 30% of cellular cholesterol (Abad-Rodriguez et al., 2004) followed by incubation with 0.3mM desmosterol/methyl-β-cyclodextrin complexes for 1hour to increase desmosterol levels (mev/CD). These data showed that reduced cholesterol level, but not increased desmosterol levels resulted in displacement of the DRM marker protein from the DRM fractions (B).

Plasmin activity expressed in arbitrary units measured in 200 μg of freshly prepared membranes of SH-SY5Ycells previously treated as in B (control, white circles, desmo, black diamonds and mev/CD, black squares). Data correspond to mean values and standard errors from two independent experiments (C). These data show that cholesterol reduction, but not higher desmosterol levels in the DRMs, reduces the plasmin activity of cellular membranes.

Western blot using the 6E10 antibody that recognizes full length APP and its β-CTF specifically. The lanes correspond to total extracts with the same amount of protein from SH-SY5Ycells treated as indicated in B (control, desmo and mev/CD). The graph on the right shows the mean values and standard errors from the densitometric quantification of the Western blots from two independent experiments. Data were normalized to the amount of APP and are expressed as percentage of the values obtained in the untreated cells (D) and show that reduced DRM cholesterol content favors the β-cleavage of APP.


A
desmosterol
ceramide
control
desmo
desmosterol (% of control)
700
600
500
400
300
200
100
0
control
desmo
B
flotillin 1
control
desmo
mev/CD
10 9 8 7 6 5 4 3 2
sucrose gradient fraction
C
plasmin acitvity (a.u.)
75
50
25
0
5 10 15 20 25 30 35 40 45
time (min)
desmo
control
mev/CD
D
APP
β-CTF
control
desmo
mev/CD
β-CTF (% of control)
160
140
120
100
80
60
40
20
0
*
control
desmo
mev/CD