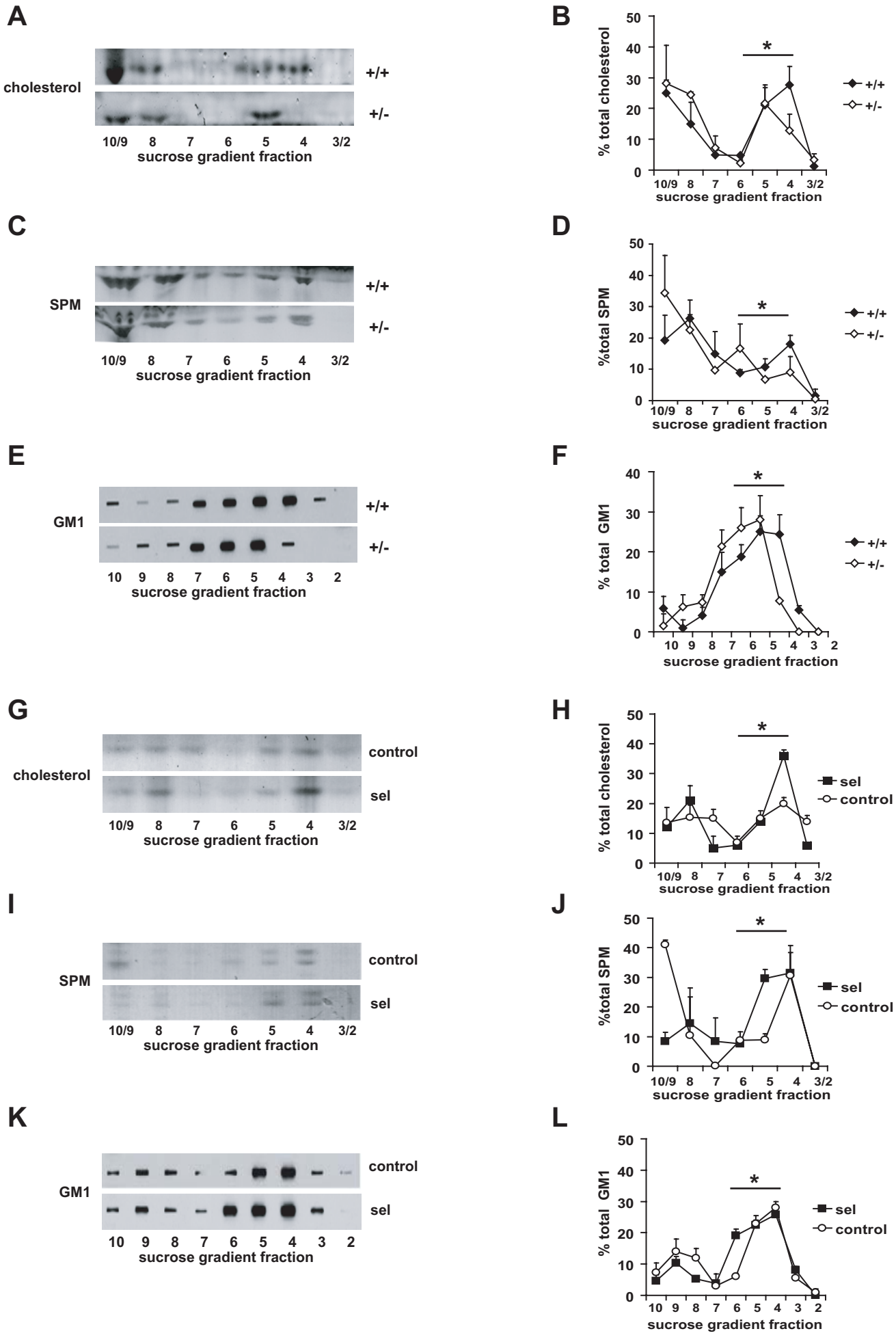
Supplementary information 1

To establish whether changes in protein content in DRMs were paralleled by changes in lipid composition, the levels of cholesterol, sphingomyelin (SPM) and the ganglioside GM1 were analyzed in the different gradient fractions. Thin Layer chromatography (TLC) (to detect cholesterol and SPM) and slot-blot using cholera toxin subunit B (that binds with high affinity to GM1) (Fishman et al., 1993) revealed that seladin-1 deficiency resulted in reduced amounts of DRM lipids in fractions 4-6 of seladin-1 heterozygous mouse brains (36.3±4.34% cholesterol, 17.4±4.28% SPM and 61.7±1.85% GM1) compared to wildtype mouse brains (53.5±5.38% cholesterol, 37.6±3.76% SPM, 71.8±3.23% GM1) (Suppl. Figure 1). Concurrently, seladin-1 overexpression resulted to the recruitment of more cholesterol (56±3.46%), SPM (68.7±6.35%) and GM1 (67.4±3.17%) in detergent-insoluble fractions 4-6 compared to control cells (42±3.53% cholesterol, 48.3±8.7% SPM and 57±2.83% GM1) (Suppl. Figure 1). All differences found were statistically significant ($P<0.05$). These results indicate that seladin-1 is required for the specific recruitment of DRM lipids into detergent insoluble membrane domains and that its deficiency results in DRM disorganization evident by re-localization of DRM-specific lipids and proteins.

Supplementary Figure legend 1 Seladin-1 expression levels affect the amount of lipids in DRMs. Total extracts of wildtype (+/+) and seladin-1 heterozygous (+/-) mouse brains as well as control and seladin-1 overexpressing SH-SY5Y cells were prepared as described in Figure 2. Where indicated fractions 2-3 and 9-10 were pooled and analyzed together. The distribution of cholesterol and SPM was examined by TLC while slot blot and cholera toxin B linked to peroxidase were used to detect GM1. Left panels show representative examples of the gradients. The graphs on the right show the amount of cholesterol, SPM and GM1 in each fraction as a percentage of total cholesterol, SPM and GM1, respectively, along the entire

gradient. Seladin-1 deficiency resulted in a decrease of the DRM components (cholesterol, SPM and GM1) in DRM-fraction 4-6 (A,B,C,D,E,F), whereas overexpression of seladin-1 in SH-SY5Y cells led to an increase of these lipids in DRMs (G,H,I,J,K,L). The graphs show the average and standard error from three different mouse brains for each condition and from three independent seladin-1 overexpressing and control SH-SY5Y cultures. Asterisks show statistical significance of the difference in the total amount of respective lipids in the DRM-fractions 4-6. *$P<0.05$.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