

## Supplementary Figures

**Figure S1.** An ESI-MS/MS mass spectrum (positive ion precursor scan of  $m/z$  184.15) showing PC and SM detected in lipid extracts from Cav<sup>-/-</sup> MEFs without (top) and with (bottom) the addition of sodium hydroxide. The even  $m/z$  ions are PCs and the odd  $m/z$  ions are SM. PC, phosphatidylcholine; SM sphingomyelin; IS, internal standards

**Figure S2.** The levels of individual phosphatidylethanolamine (PE, A), phosphatidylglycerol (PG, B), phosphatidylserine (PS, C), phosphatidic acid (PA, D) and phosphatidylinositol (PI, E) in WT (filled bars) and Cav1<sup>-/-</sup> (hollow bars) MEFs. Data are presented as mean + standard error (n=4).

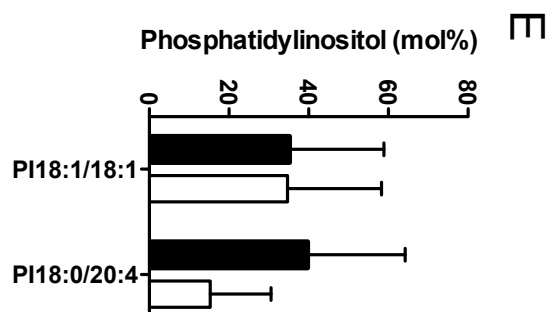
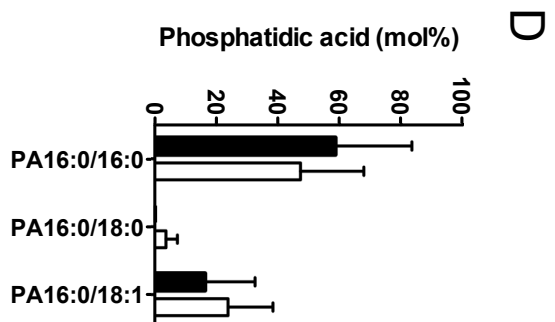
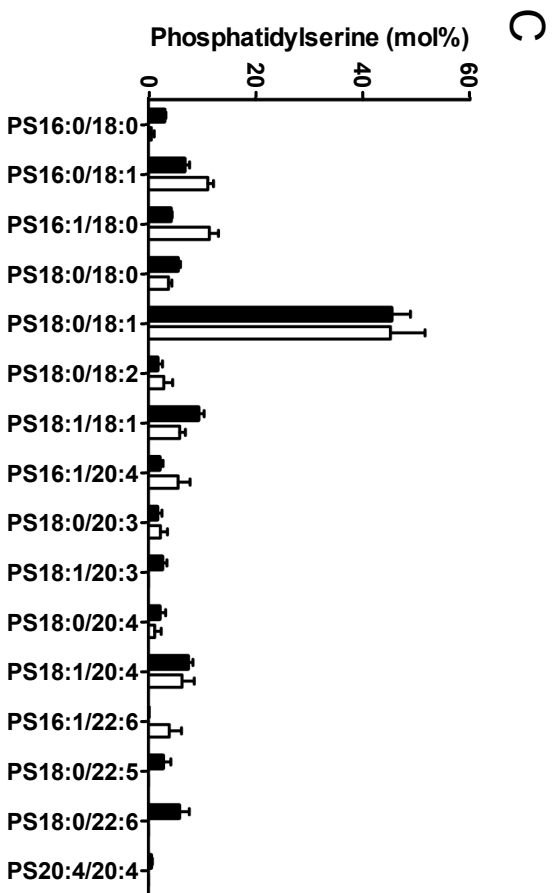
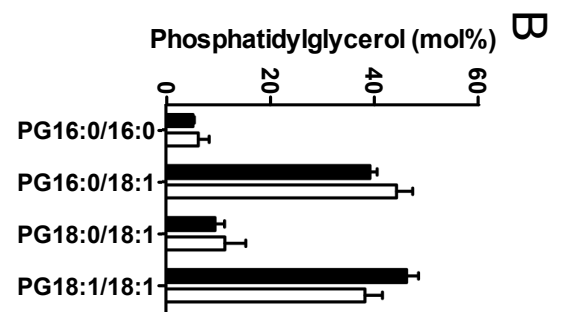
**Figure S3.** Protein distribution and lipid contents of DRMs from WT and Cav1<sup>-/-</sup> MEFs. MEF cell homogenates labeled with [<sup>14</sup>C]-acetate were extracted with 1% Triton, floated on Optiprep step gradients and eight fractions were collected from the top of the gradients.

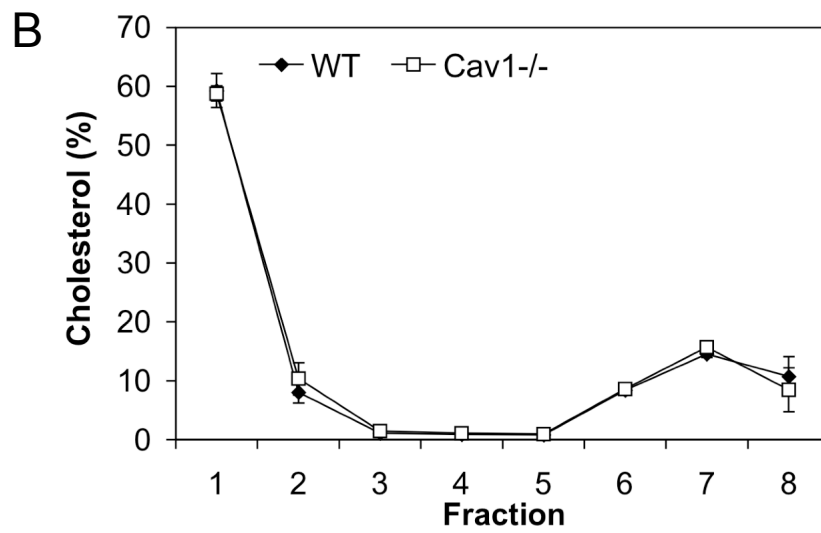
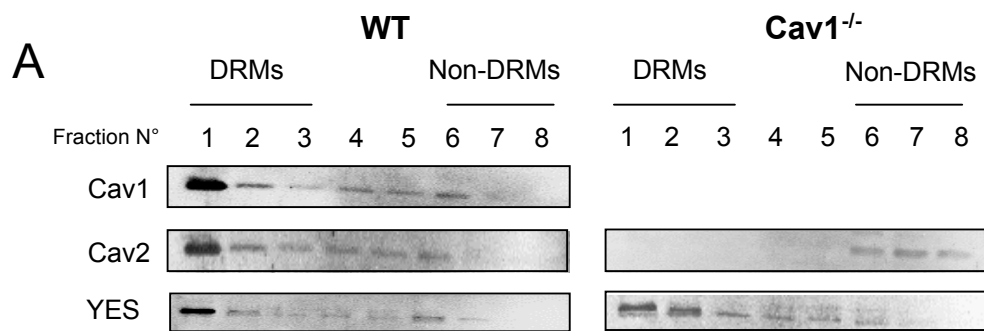
**A.** 20  $\mu$ l of each fraction was analyzed by immunoblotting for marker proteins YES, Cav1 and Cav2.

**B.** Lipids from DRM fractions were separated by TLC and <sup>14</sup>C-cholesterol counted for each fraction. Results are presented as the percentage of <sup>14</sup>C-cholesterol present in each fraction. The data show mean  $\pm$  standard deviation of three independent experiments.

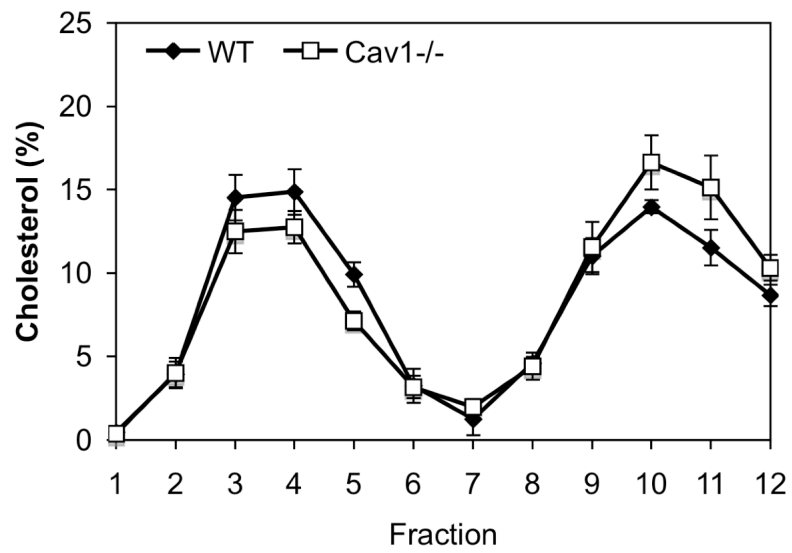
**Figure S4.** Cholesterol content in NDR and non-raft membranes in WT and Cav1<sup>-/-</sup> MEFs. Light and heavy membranes were prepared from whole cell homogenate by sonication and separation on a 5-45% sucrose gradient as described in *Methods*. Twelve fractions were collected from the top of the gradient. The distribution of cholesterol across the gradient was determined by fractionating WT (closed diamond) and Cav1<sup>-/-</sup> MEFs (open squares) with incorporated <sup>3</sup>H-cholesterol. The data show mean  $\pm$  standard deviation of three independent experiments.







Supplementary Figure S3.



Supplementary Figure S4.