Supplemental Materials

Molecular Biology of the Cell

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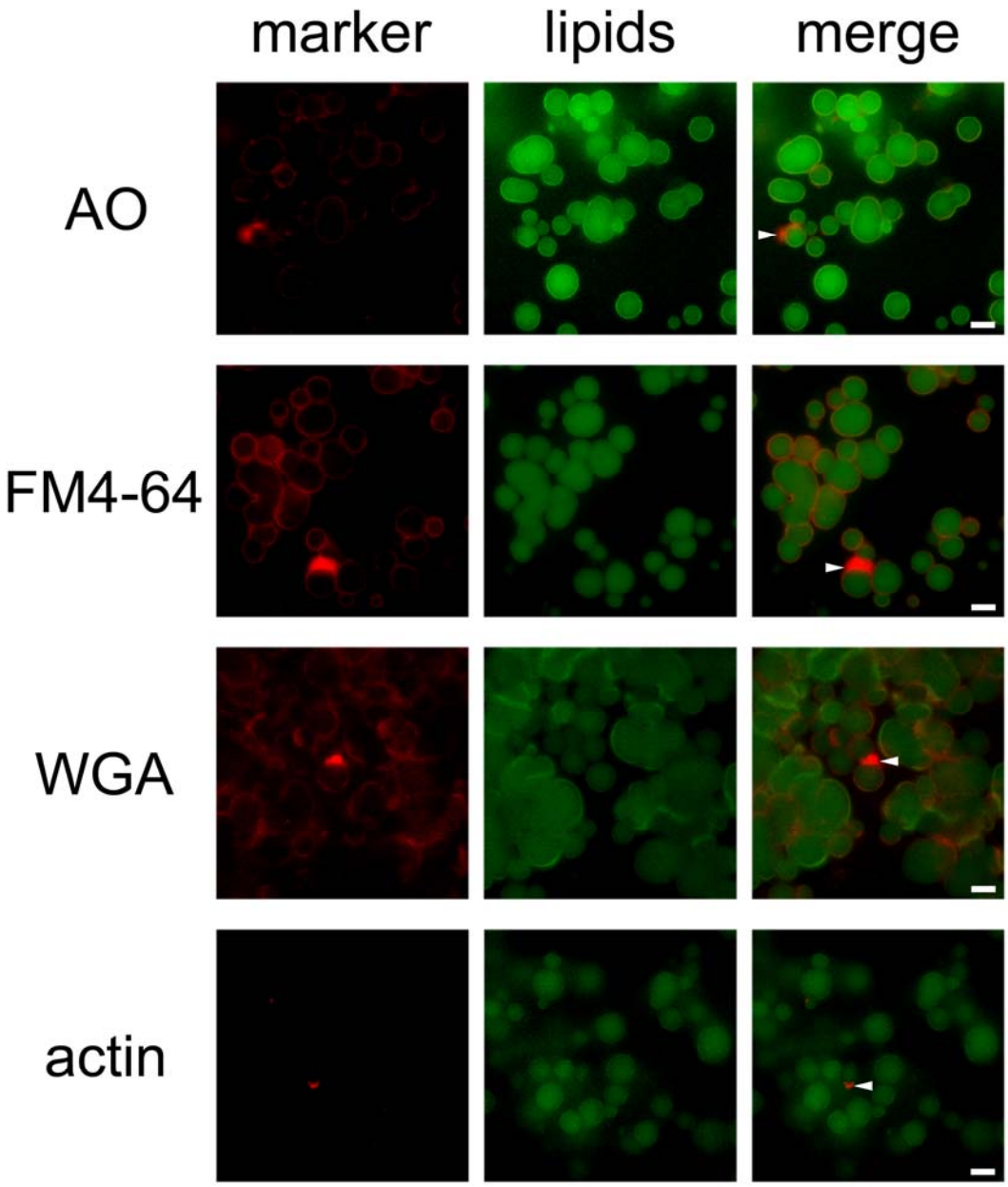
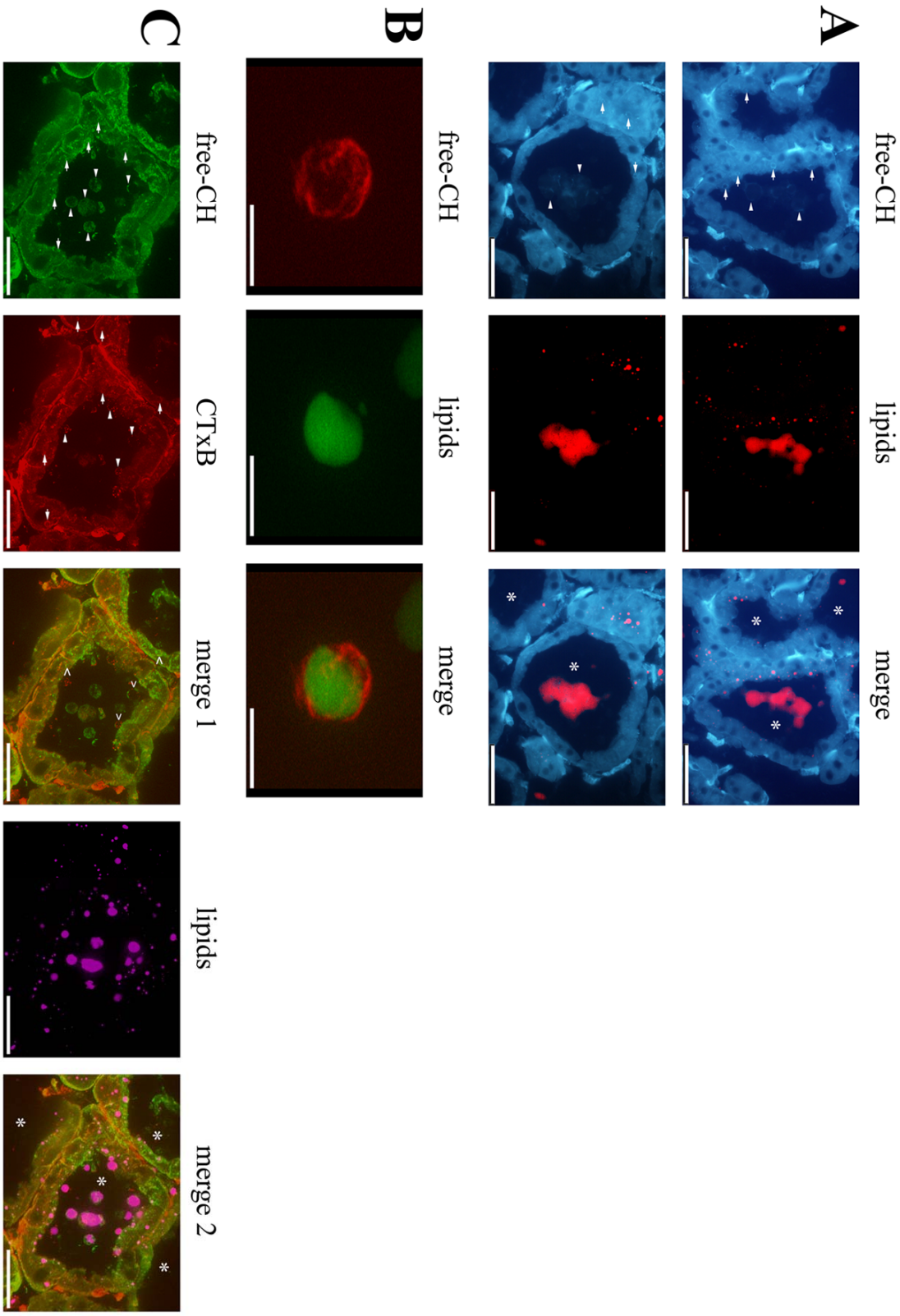


Supplemental Figure 1. Incidence of crescent in mouse MFGs.
Freshly collected whole mouse milk (day 10 of lactation) was diluted 1:4 in sterile water, centrifuged 15 min at 10000xg at 4°C and the floating cream was washed three times with 0.9% NaCl. MFGs were then labeled with acridine orange (AO, 0.1%), FM4-64 (1 mg/ml), Alexa594-conjugated wheat germ agglutinin (WGA, 1 mg/ml), or rhodamine-conjugated phalloidin (1 mg/ml) diluted in PBS for 30 min at 4°C. Neutral lipids were concomitantly counterstained with BODIPY 493/503 (3 µg/ml). 50 µl of each stained sample was mixed with 50 µl of 1% low melt agarose solution, placed on a glass slide and topped with a coverslip. About twenty pictures were randomly acquired for each label and MFGs containing or not cytoplasmic crescents were counted using ImageJ. Cytoplasmic crescents (arrowheads) were estimated to be present in 2.95%, 2,91%, 3,15% and 2.63% (2,91% in average) of the MFGs labeled with AO (n=5496), FM4-64 (n=6662), WGA (n=5528) or phalloidin (n=5474), respectively.



Supplemental Figure 2. Localization of free cholesterol in mouse mammary gland and in mouse MFGs.

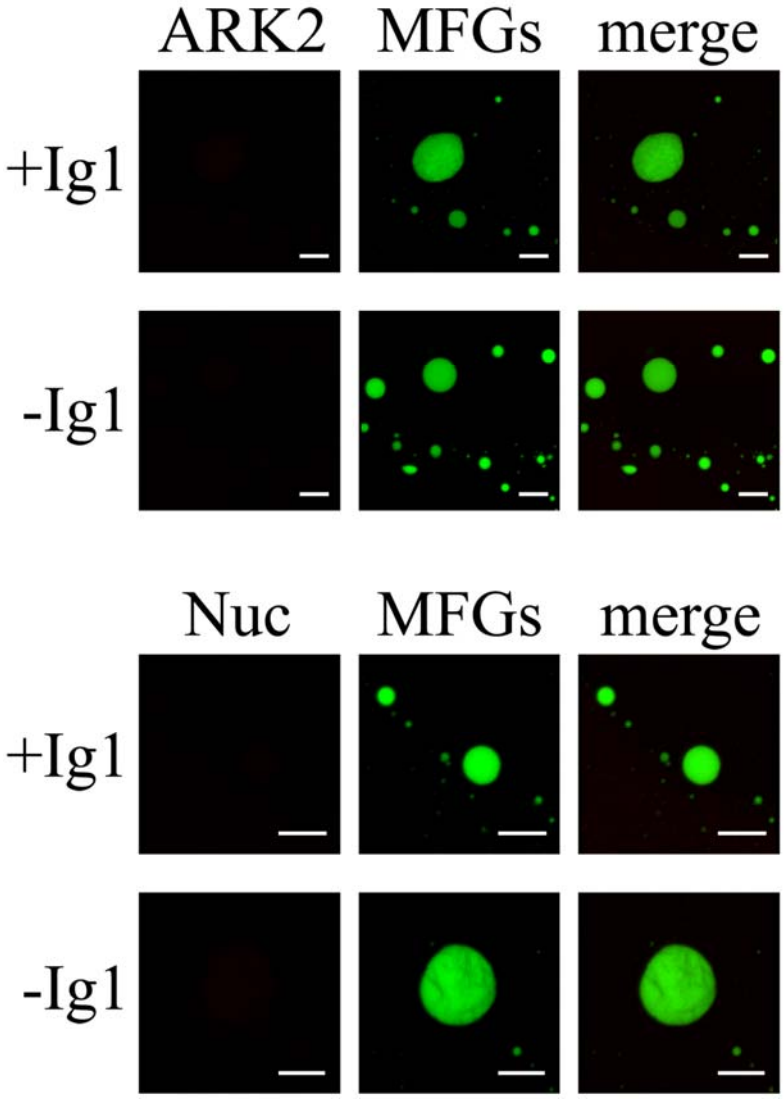
The localization of free CH was studied by fluorescence microscopy on tissue sections from the mammary gland of mice at day 10 of lactation (L10) in the absence of pups and on

isolated MFGs using filipin. Neutral lipids were stained with BODIPY 493/503 and GM1 ganglioside was detected using Alexa594-conjugated Cholera Toxin B subunit (CTxB). BODIPY 493/503 is visualized in red false color (A) or magenta false color (C). Asterisks indicate lumens.

A: Free CH (blue) was observed throughout MESCs with clear accumulations surrounding the CLDs (arrows), as well as on the surface of MFGs (arrowheads). Note that some cells surrounding the alveolae were also strongly labeled. Merge shows the picture resulting from the superposition of the two previous ones. Epifluorescence microscopy pictures. Scale bar = 60µm.

B: Free CH (red false color) was visualized on the surface of purified MFGs (green). Merge shows the picture resulting from the superposition of the two previous ones. Pictures are Z projections (Y-axis) of Apotome stacks. Scale bar = 2µm.

C: The codetection of free CH (green false color) and GM1 ganglioside (red) on mouse mammary gland sections at L10 showed they were observed around some CLDs (arrows) as well as on the surface of MFGs (arrowheads). Note that free CH and GM1 ganglioside were well colocalized at the basolateral side of MESCs (merge 1, yellow) but were only sparsely colocalized around CLDs or MFGs (merge1, open arrowheads). Merge 2 shows the superposition of free CH, CTxB and lipid labelings. Pictures are Z projections (Y-axis) of Apotome stacks. Scale bar = 60µm.



Supplemental Figure 3. Negative controls for IIF analysis of purified mouse MFGs.
Two irrelevant primary antibodies were used: a mouse monoclonal IgG directed against the nucleolar protein nucleolin/C23 and a rabbit polyclonal IgG directed against the cytosolic kinase ARK2/Aurora. None of these primary antibodies or the negative controls without primary antibody showed any labeling. All pictures are Z projections (Y-axis) of confocal stacks. Scale bar = 5 µm.

Supplemental Table 1. List of proteins identified by LC-MS/MS in mouse CLDs and MFGMs.
The proteins identified by LC-MS/MS with at least two peptides in CLDs (260 proteins, CLD proteins identification and peptides identification) or MFGMs (395 proteins, MFGM proteins identification and peptides identification) were classified by either their biological function (CLD function and MFGM function) or their cellular localization (CLD localization and MFGM localization), according to the GO terms of the Uniprot KB database. The proteins common to CLDs and MFGMs were also classified by either their biological function (common function) or their cellular localization (common localization). Proteins identified by LC-MS/MS in mouse CLDs and MFGMs and associated with lipid rafts, as they were already found in these membrane microdomains in other studies (1, (Foster *et al.*, 2003); 2, (Zhai *et*

al., 2009); 3, (Poston *et al.*, 2011); 4, (Yu *et al.*, 2008)), were classified for their cellular localization, according to the GO terms of the Uniprot KB database (CLD, MFGM and common raft-associated proteins).