Supplementary material for:

Who's in, who's out? Re-evaluation of lipid raft residents

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**Supplementary Table 1.** Statistical analysis of immunoreactivity quantification data in selected regions of mouse cerebellum following Triton X-100 or Brij O20 detergent treatment compared to control non-detergent protocol.

| Tukey's multiple comparisons test | Summary | Adjusted P Value |
|-----------------------------------|---------|-----------------|
| **Gigantocellular reticular nucleus** |         |                 |
| GM1                               |         |                 |
| NO DETERGENT vs. Brij O20         | N.S.    | 0.0851          |
| NO DETERGENT vs. Triton X-100     | *       | 0.0104          |
| Brij O20 vs. Triton X-100         | **      | 0.0030          |
| GD1a                              |         |                 |
| NO DETERGENT vs. Brij O20         | N.S.    | 0.2257          |
| NO DETERGENT vs. Triton X-100     | *       | 0.0449          |
| Brij O20 vs. Triton X-100         | *       | 0.0358          |
| GD1b                              |         |                 |
| NO DETERGENT vs. Brij O20         | N.S.    | 0.8895          |
| NO DETERGENT vs. Triton X-100     | N.S.    | 0.0844          |
| Brij O20 vs. Triton X-100         | N.S.    | 0.1446          |
| GT1b                              |         |                 |
| NO DETERGENT vs. Brij O20         | N.S.    | 0.6399          |
| NO DETERGENT vs. Triton X-100     | *       | 0.0307          |
| Brij O20 vs. Triton X-100         | *       | 0.0177          |
| Tukey's multiple comparisons test       | Summary | Adjusted P Value |
|----------------------------------------|---------|-----------------|
| **Inferior cerebellar ponsuncle**      |         |                 |
| GM1                                    |         |                 |
| NO DETERGENT vs. Brij O20              | *       | 0.0234          |
| NO DETERGENT vs. Triton X-100          | *       | 0.0329          |
| Brij O20 vs. Triton X-100              | **      | 0.0095          |
| GD1a                                   |         |                 |
| NO DETERGENT vs. Brij O20              | N.S.    | 0.0861          |
| NO DETERGENT vs. Triton X-100          | N.S.    | 0.0849          |
| Brij O20 vs. Triton X-100              | *       | 0.0378          |
| GD1b                                   |         |                 |
| NO DETERGENT vs. Brij O20              | N.S.    | 0.3842          |
| NO DETERGENT vs. Triton X-100          | N.S.    | 0.9330          |
| Brij O20 vs. Triton X-100              | *       | 0.0330          |
| GT1b                                   |         |                 |
| NO DETERGENT vs. Brij O20              | N.S.    | 0.4688          |
| NO DETERGENT vs. Triton X-100          | N.S.    | 0.0552          |
| Brij O20 vs. Triton X-100              | *       | 0.0405          |
| **Arbor vitae - Lobules IV-V**         |         |                 |
| GM1                                    |         |                 |
| NO DETERGENT vs. Brij O20              | N.S.    | 0.9898          |
| NO DETERGENT vs. Triton X-100          | N.S.    | 0.0531          |
| Brij O20 vs. Triton X-100              | *       | 0.0263          |
| GD1a                                   |         |                 |
| NO DETERGENT vs. Brij O20              | N.S.    | 0.7454          |
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|                      |       |       |
|----------------------|-------|-------|
| NO DETERGENT vs. Triton X-100 | *     | 0.0462|
| Brij O20 vs. Triton X-100    | *     | 0.0380|
| **                    |       |       |
| NO DETERGENT vs. Triton X-100 |   **  | 0.0095|
| Brij O20 vs. Triton X-100    | N.S.  | 0.1311|
| ***                    |       |       |
| NO DETERGENT vs. Triton X-100 | **** | <0.0001|
| Brij O20 vs. Triton X-100    | ***   | 0.0008|

N.S. = not significant; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001
Supplementary Figure 1. Negative control sections of immunohistochemical staining. Cerebellar tissue sections treated in parallel with sections shown in Figure 2, except primary antibodies were omitted.
Supplementary Figure 2A. Imaging mass spectrometry of 699.4927, 747.4938, 790.5328, and 885.5433 m/z following Brij O20 and Triton X-100 treatment of mouse cerebellar tissue. n(animals) = 4; n (technical replicates) = 12.

Supplementary Figure 2B. Imaging mass spectrometry of 806.5374, 862.5997, 878.5972, and 888.6160 m/z following Brij O20 and Triton X-100 treatment of mouse cerebellar tissue. n(animals) = 4; n (technical replicates) = 12.
Supplementary Table 2. Identified molecular species that correspond to dominant individual m/z mass spectrometry signals of the most abundant simple phospholipids and glycolipids. PA: phosphatidic acid; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PC: phosphatidylcholine. Identified according to The Human Metabolome Database (HMDB) (Wishart et al. 2007; Wishart et al. 2009; Wishart et al. 2013; Wishart et al. 2018)

Adduct Type:
M-H
M-H$_2$O-H

| Molecular Weight | Adduct Type | Identification                                                                 |
|------------------|-------------|-------------------------------------------------------------------------------|
| 699.4927         | PA          | Phosphatidic acid                                                             |
| 747.4938         | PA          | Phosphatidic acid                                                             |
| 790.5328         | PE          | Phosphatidylethanolamine                                                      |
| 885.5433         | PI          | Phosphatidylinositol                                                          |
| 806.5374         | 3-O-Sulfogalactosylceramide (d18:1/18:0), PE |                                                                                  |
| 862.5997         | 3-O-Sulfogalactosylceramide (d18:1/22:0) |                                                                                  |
| 878.5972         | PC,PE       | Sulfogalactosylceramide                                                       |
| 888.6160         | 3-O-Sulfogalactosylceramide (d18:1/24:1(15Z)), PC |                                                                                  |

M+Cl
M+HAc-H

Molecular Weight Tolerance ±: 0.01 Da

Supplementary references

Wishart, D. S., Feunang, Y. D., Marcu, A. et al. (2018) HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res* **46**, D608-D617.

Wishart, D. S., Jewison, T., Guo, A. C. et al. (2013) HMDB 3.0--The Human Metabolome Database in 2013. *Nucleic Acids Res* **41**, D801-807.

Wishart, D. S., Knox, C., Guo, A. C. et al. (2009) HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* **37**, D603-610.

Wishart, D. S., Tzur, D., Knox, C. et al. (2007) HMDB: the Human Metabolome Database. *Nucleic Acids Res* **35**, D521-526.