

**Table S1. Mass-to-Charge Ratios (m/z) and LC Retention Times (RT) of Lipids that Change during Cytokinesis and at the Midbody, Related to Table 1**

For chemical structures of the lipids, see Fig. 1.

| m/z      | RT (min) | Lipid                                       | Fold increase during cytokinesis <sup>(a)</sup> | Fold increase at midbody <sup>(b)</sup> |
|----------|----------|---|---|---|
| 441.3344 | ~40      | sterol derivative                           | >27   | unchanged                               |
| 661.5191 | ~46      | phosphatidic acid ether/ester (O-18:0/16:0) | 40  | unchanged                               |
| 837.5525 | ~54      | phosphatidylinositol (16:0/18:0)            | 10  | unchanged                               |
| 538.5216 | ~70      | C16 diH-ceramide                            | >11   | unchanged                               |
| 566.5518 | ~72      | C18 diH-ceramide                            | accumulated <sup>(c)</sup>                      | unchanged                               |
| 594.5830 | ~73      | C20 diH-ceramide                            | accumulated <sup>(c)</sup>                      | unchanged                               |
| 622.6170 | ~75      | C22 diH-ceramide                            | accumulated <sup>(c)</sup>                      | accumulated <sup>(c)</sup>              |
| 650.6456 | ~77      | C24 diH-ceramide                            | accumulated <sup>(c)</sup>                      | accumulated <sup>(c)</sup>              |
| 620.5987 | ~77      | C22 ceramide                                | 4   | 4                                       |
| 648.6366 | ~79      | C24 ceramide                                | 4   | 14                                      |
| 698.5610 | ~68      | C16 hexosylceramide                         | >16   | 13                                      |
| 810.6783 | ~79      | C24 hexosylceramide                         | unchanged                                       | >36                                     |
| 647.4645 | ~48      | phosphatidic acid (16:0/16:0)               | unchanged                                       | 14                                      |
| 792.7113 | ~72      | triacylglycerol (16:1,12:0, 18:1)           | unchanged                                       | 54                                      |
| 810.5278 | ~49      | phosphatidylserine (18:0/20:4)              | unchanged                                       | 6                                       |

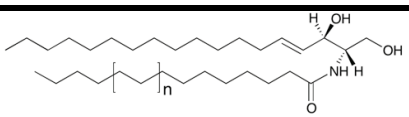
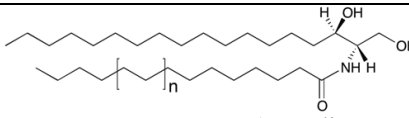
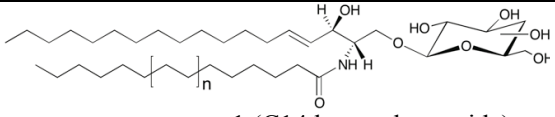
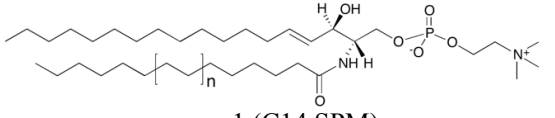
<sup>(a)</sup> Fold increase in S-phase vs cytokinesis cells is determined by  $[\text{Abundance}_{\text{cytokinesis}}] / [\text{Abundance}_{\text{S-phase}}]$  for each lipid. Abundance is the total ion count for a given ion. Each ion corresponds to a mass-to-charge ratio (m/z), which is used to assign the lipid species.

<sup>(b)</sup> Fold increase in midbody vs cytokinesis cells is determined by  $[\text{Abundance}_{\text{midbody}}] / [\text{Abundance}_{\text{purified lysate}}]$  for each lipid.

<sup>(c)</sup> A numeric value for fold increase could not be calculated due to the low abundance of these ions in S-phase or cytokinesis cells.

**Table S3. Targeted Analysis of the Sphingolipids in siSMPD4-, siGALC-, and siDGAT2-Treated Cells, Related to Figure 3**

Fold changes in ceramides, diH-ceramides, hexosylceramides and sphingomyelins were calculated in siRNA treated cells. Fold changes are an average of two independent experiments. For a graphical representation see Fig. S3A.

|                  |   | siSMPD4                                  | siGALC        | siDGAT2       |
|------------------|---|--|---------------|---------------|
|                  |   | Average fold changes relative to control |               |               |
| CERAMIDES        |    |  |               |               |
|                  | n=1 (C14 ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=2 (C16 ceramide)  | 4.5x increase                            | unchanged     | unchanged     |
|                  | n=3 (C18 ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=4 (C20 ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=5 (C22 ceramide)  | 8x increase                              | unchanged     | unchanged     |
|                  | n=6 (C24 ceramide)  | 3.5x increase                            | unchanged     | unchanged     |
| diH-CERAMIDES    |    |  |               |               |
|                  | n=1 (C14 diH-ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=2 (C16 diH-ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=3 (C18 diH-ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=4 (C20 diH-ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=5 (C22 diH-ceramide)  | not detected                             | not detected  | not detected  |
|                  | n=6 (C24 diH-ceramide)  | not detected                             | not detected  | not detected  |
| HEXOSYLCERAMIDES |  |  |               |               |
|                  | n=1 (C14 hexosylceramide)   | unchanged                                | unchanged     | unchanged     |
|                  | n=2 (C16 hexosylceramide)   | 3.1x increase                            | 3.2x increase | unchanged     |
|                  | n=3 (C18 hexosylceramide)   | unchanged                                | 2.1x increase | 3.5x increase |
|                  | n=4 (C20 hexosylceramide)   | unchanged                                | unchanged     | unchanged     |
|                  | n=5 (C22 hexosylceramide)   | unchanged                                | unchanged     | unchanged     |
|                  | n=6 (C24 hexosylceramide)   | 2.2x increase                            | 2.1x increase | unchanged     |
| SPHINGOMYELINS   |  |  |               |               |
|                  | n=1 (C14 SPM)   | unchanged                                | unchanged     | unchanged     |
|                  | n=2 (C16 SPM)   | unchanged                                | unchanged     | unchanged     |
|                  | n=3 (C18 SPM)   | unchanged                                | unchanged     | unchanged     |
|                  | n=4 (C20 SPM)   | not detected                             | not detected  | not detected  |
|                  | n=5 (C22 SPM)   | unchanged                                | unchanged     | unchanged     |
|                  | n=6 (C24 SPM)   | unchanged                                | unchanged     | unchanged     |