

Supplemental data

Supplemental Table 1: LC-MS was used to monitor TAG secreted by McA RH7777

cells. The TAG that elute from the column are converted to diacylglycerols during ionization. The diversity of diacylglycerols observed reflect the variety of secreted TAG. This table lists the molecular masses of the most prominent diacylglycerols observed (only 1 isomer of each listed) and their predicted fatty acid compositions (diacylglycerols with medium-chain fatty acids, fatty acids with an odd number of carbons and unsaturated fatty acids with *trans* configuration were omitted). The diacylglycerol ions observed using photospray ionization are $[M+H^+-H_2O]$. See supplemental figure 2 for the corresponding spectrum.

Mass	Mass (-H ₂ O)	Common Name	Systematic Name
568.5	551.5	DG(16:0/16:0/0:0)[<i>rac</i>]	1,2-dihexadecanoyl- <i>rac</i> -glycerol
592.4	575.4	DG(16:1(9Z)/18:1(9Z)/0:0) DG(16:0/18:2(9Z,12Z)/0:0)	1-(9Z-hexadecenoyl)-2-(9Z-octadecenoyl)- <i>sn</i> -glycerol 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)- <i>sn</i> -glycerol
594.5	577.5	DG(18:1(9Z)/16:0/0:0) DG(16:1(9Z)/18:0/0:0)	1-(9Z-octadecenoyl)-2-hexadecanoyl- <i>sn</i> -glycerol 1-(9Z-hexadecenoyl)-2-octadecanoyl- <i>sn</i> -glycerol
596.5	579.5	DG(18:0/16:0/0:0)	1-octadecanoyl-2-hexadecanoyl- <i>sn</i> -glycerol
618.5	601.5	DG(18:1(9Z)/18:2(9Z,12Z)/0:0) DG(18:0/18:3(9Z,12Z,15Z)/0:0) DG(16:1(9Z)/20:2(11Z,14Z)/0:0) DG(16:0/20:3(8Z,11Z,14Z)/0:0)	1-(9Z-octadecenoyl)-2-(9Z,12Z-octadecadienoyl)- <i>sn</i> -glycerol 1-octadecanoyl-2-(9Z,12Z,15Z-octadecatrienoyl)- <i>sn</i> -glycerol 1-(9Z-hexadecenoyl)-2-(11Z,14Z-eicosadienoyl)- <i>sn</i> -glycerol 1-hexadecanoyl-2-(9Z,12Z,15Z-eicosatrienoyl)- <i>sn</i> -glycerol
620.5	603.5	DG(18:1(9Z)/18:1(9Z)/0:0) DG(18:0/18:2(9Z,12Z)/0:0) DG(16:1(9Z)/20:1(11Z)/0:0) DG(16:0/20:2(11Z,14Z)/0:0)	1,2-di-(9Z-octadecenoyl)- <i>sn</i> -glycerol 1-octadecanoyl-2-(9Z,12Z-octadecadienoyl)- <i>sn</i> -glycerol 1-(9Z-hexadecenoyl)-2-(11Z-eicosenoyl)- <i>sn</i> -glycerol 1-hexadecanoyl-2-(11Z,14Z-eicosadienoyl)- <i>sn</i> -glycerol
622.6	605.6	DG(18:0/18:1(9Z)/0:0) DG(16:1(9Z)/20:0/0:0) DG(16:0/20:1(11Z)/0:0)	1-octadecanoyl-2-(9Z-octadecenoyl)- <i>sn</i> -glycerol 1-(9Z-hexadecenoyl)-2-eicosanoyl- <i>sn</i> -glycerol 1-hexadecanoyl-2-(11Z-eicosenoyl)- <i>sn</i> -glycerol
624.6	607.6	DG(18:0/18:0/0:0)[<i>rac</i>] DG(16:0/20:0/0:0)	1,2-dioctadecanoyl- <i>rac</i> -glycerol 1-hexadecanoyl-2-eicosanoyl- <i>sn</i> -glycerol
648.6	631.6	DG(18:2(9Z,12Z)/20:0/0:0) DG(18:1(9Z)/20:1(11Z)/0:0) DG(18:0/20:2(11Z,14Z)/0:0) DG(16:1(9Z)/22:1(13Z)/0:0) DG(16:0/22:2(13Z,16Z)/0:0)	1-(9Z,12Z-octadecadienoyl)-2-eicosanoyl- <i>sn</i> -glycerol 1-(9Z-octadecenoyl)-2-(11Z-eicosenoyl)- <i>sn</i> -glycerol 1-octadecanoyl-2-(11Z,14Z-eicosadienoyl)- <i>sn</i> -glycerol 1-(9Z-hexadecenoyl)-2-(13Z-docosenoyl)- <i>sn</i> -glycerol 1-hexadecanoyl-2-(13Z,16Z-docosadienoyl)- <i>sn</i> -glycerol

Supplemental Legends

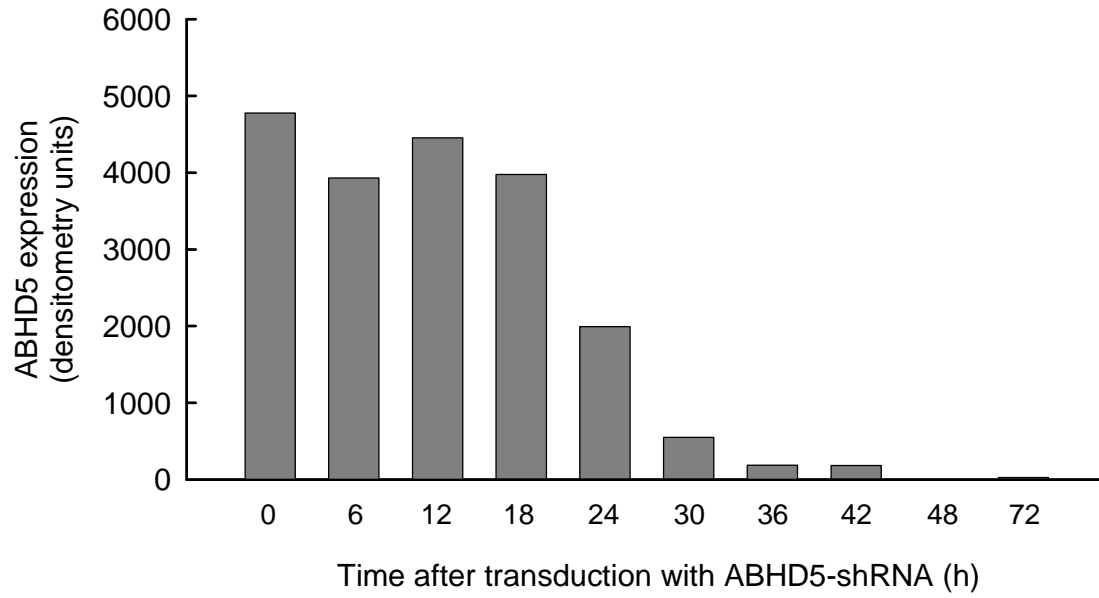
Supplemental Figure 1. Transduction of McA RH7777 cells with ABHD5-shRNA reduced the expression of ABHD5 to a minimum by 36 h. McA RH7777 cells were transduced with ABHD5-shRNA (25 plaque forming units per cell). At various times after transduction, cells were harvested and protein levels of ABHD5 were analyzed by immunoblotting. Data correspond to one experiment; the expression of ABHD5 at 48 h is representative of five independent experiments.

Supplemental Figure 2. LC-MS with photospray ionization facilitates evaluation of the fatty acid composition of TAG by analysis of diacylglycerol ions derived from triacylglycerol fragmentation. Lipids secreted by McA RH7777 cells deficient in ABHD5 (red) and controls (blue) were analyzed using LC-MS. The eluted TAG were ionized to diacylglycerols by the photospray source. (A) Spectrum of the mass of triacylglycerol-derived diacylglycerols with corresponding likely fatty acid composition. Individual ions are seen as a series of three peaks due to different charge species and the presence of naturally occurring heavy isotopes. (B) Expansion of spectrum of triacylglycerol-derived diacylglycerols with mass 603-606. See Supplemental Table 1 for a list of the observed diacylglycerol ions and their predicted fatty acid compositions.

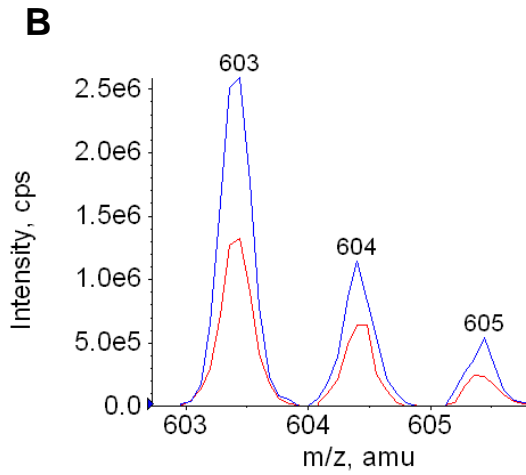
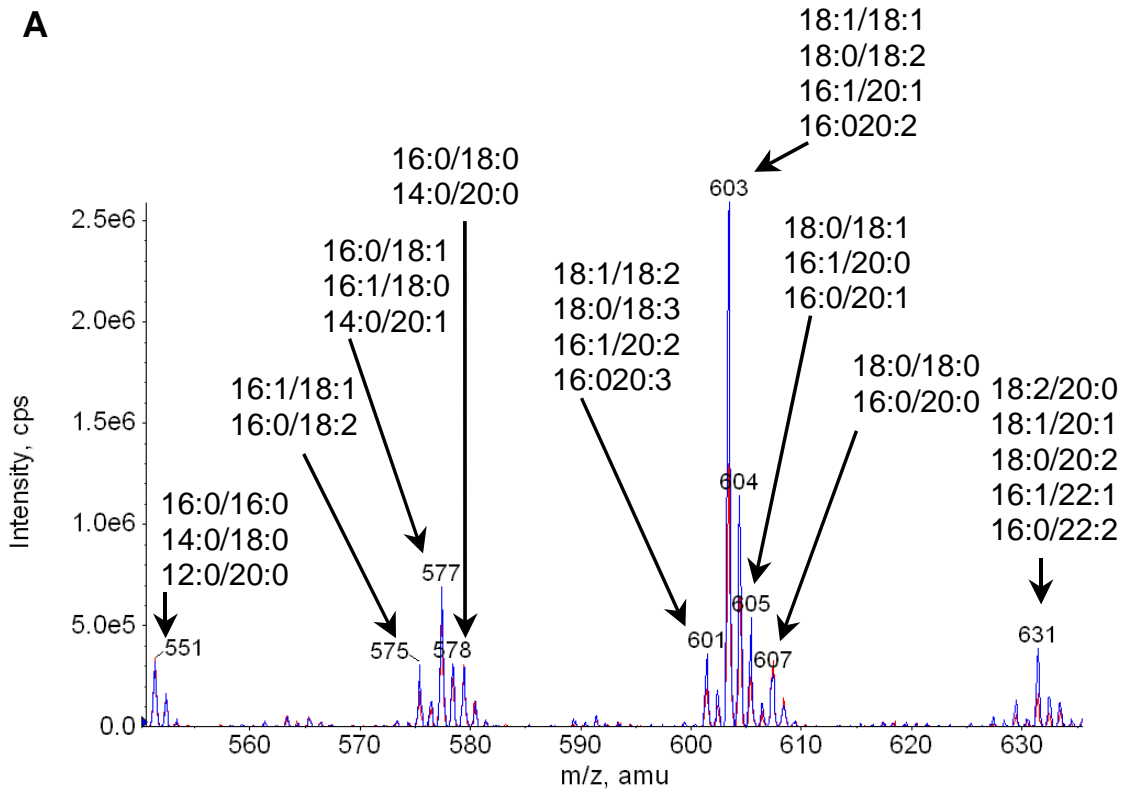
Supplemental Figure 3. The specific radioactivity of cellular TAG was comparable in McA RH7777 cells deficient in ABHD5 and controls. McA RH7777 cells transduced with ABHD5-shRNA or control-shRNA were treated as described in Figure 6. The radioactivity associated with TAG and the masses of TAG were measured and the

specific radioactivity of TAG calculated. The results represent the means and SEM from two to three experiments, each performed with triplicate samples.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

