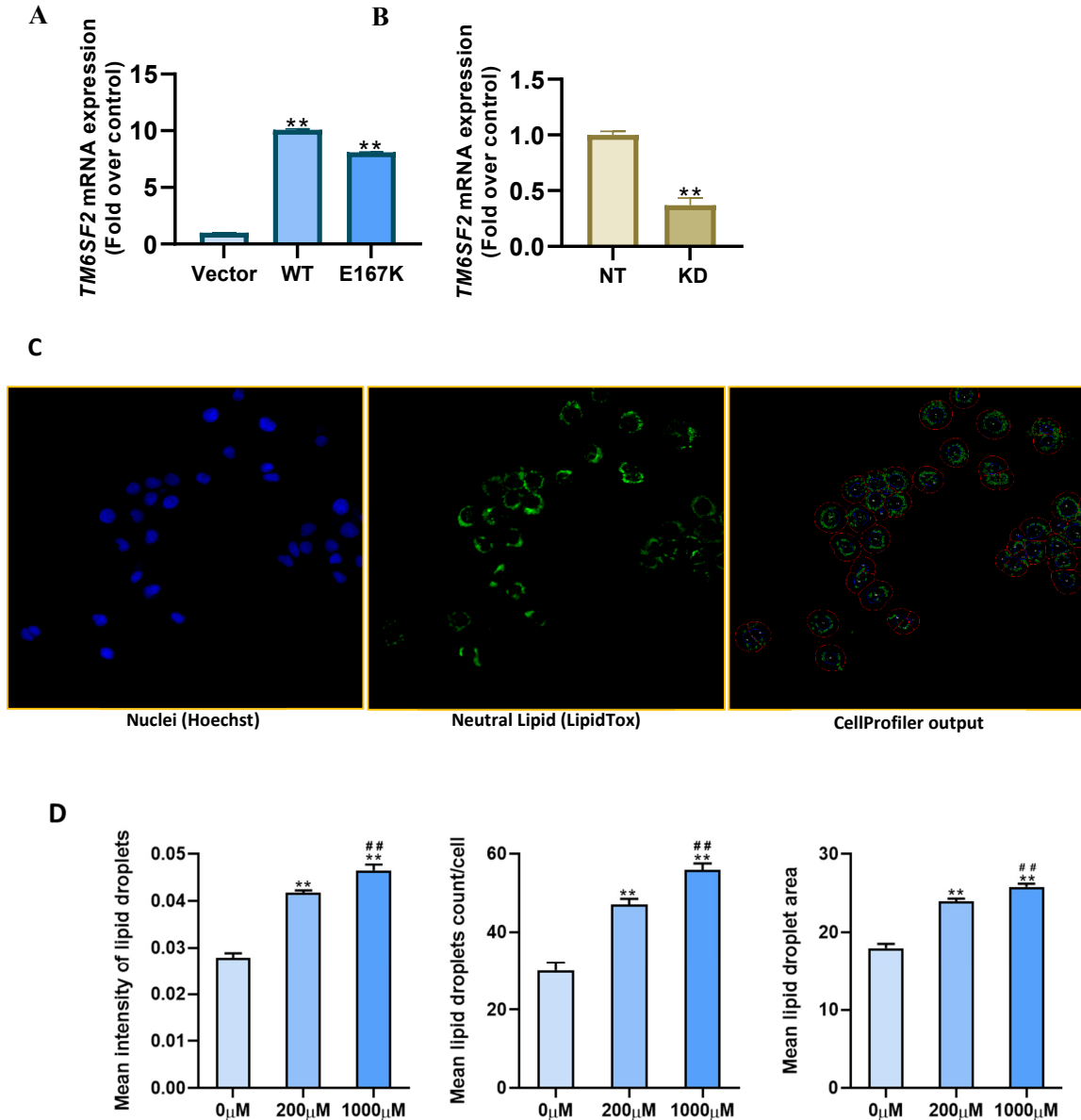


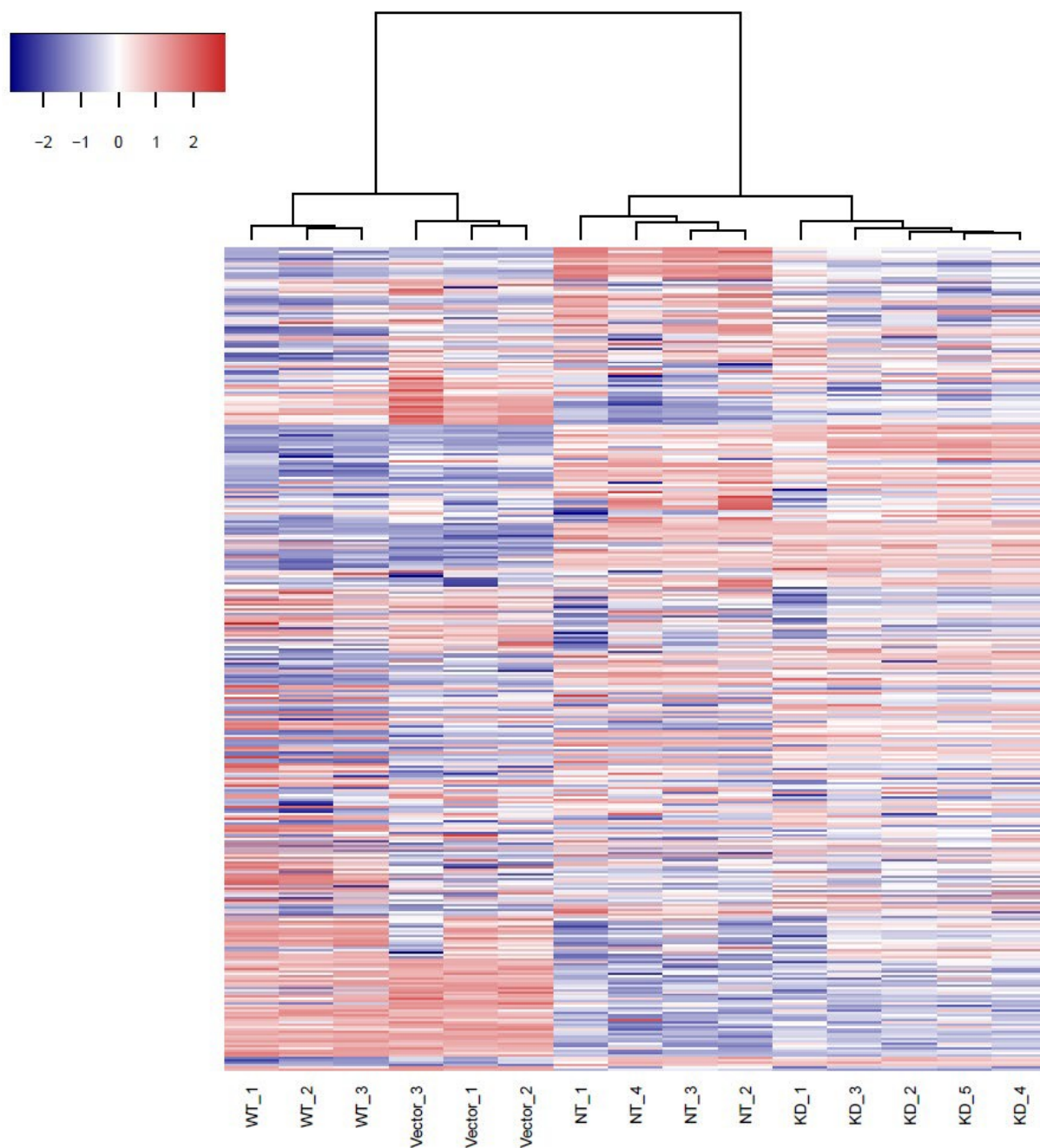
## Supplementary material

Supplementary Figure S1



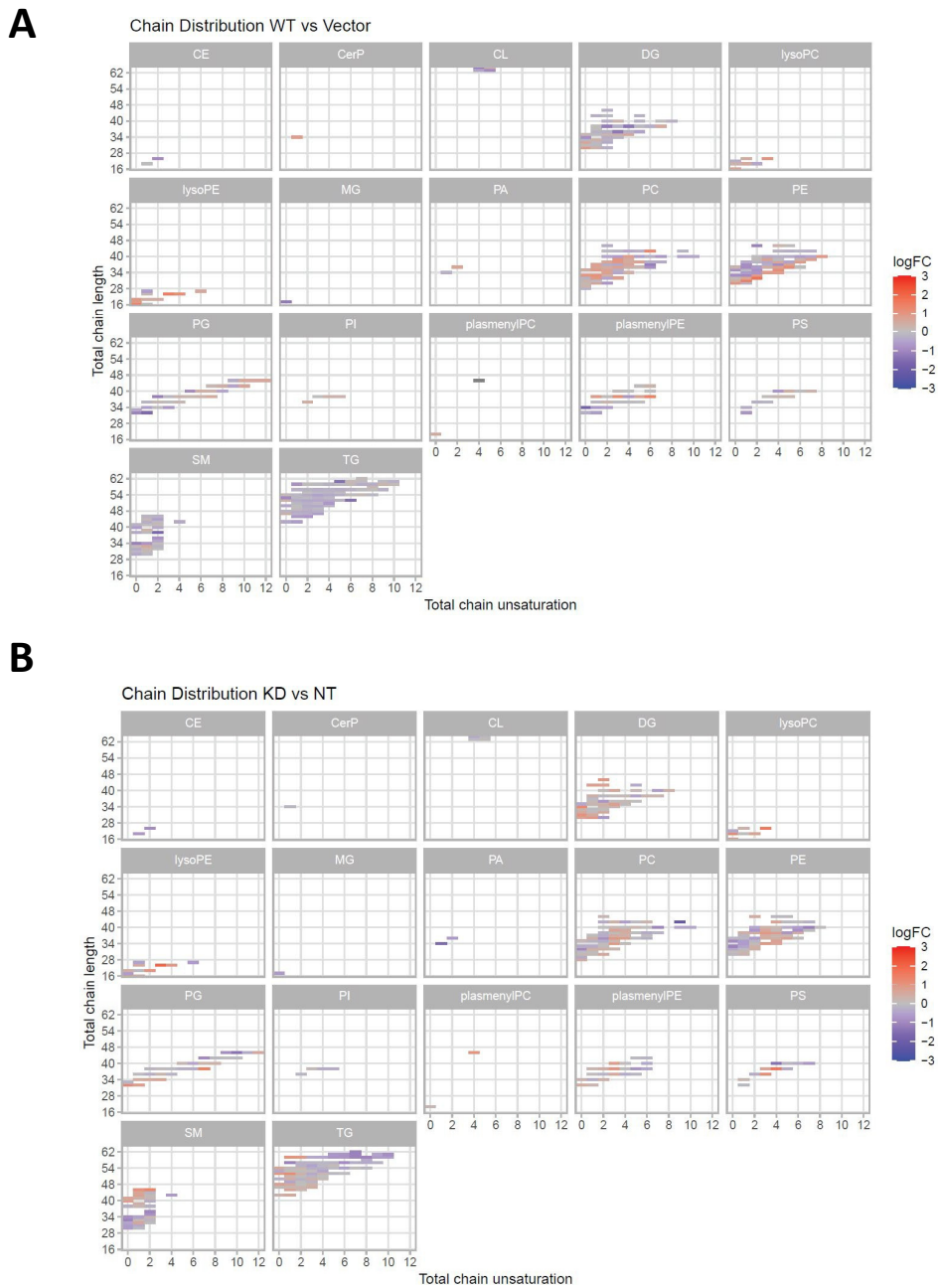
**Supplementary Figure S1: Validation of *TM6SF2* gene knockdown and *TM6SF2* overexpression in Huh-7 cells.** Quantitation of *TM6SF2* mRNA expression using RT-qPCR in (A) overexpression and (B) knockdown Huh-7 cell lines. (C) Microscopic image of cell nuclei stained with Hoechst (Blue; left panel) and neutral lipids by LipidTox Green (green; center panel). Right panel shows the areas for nuclei (blue circle), lipid droplets (green circles), and cell perimeter (red circle) identified by the CellProfiler pipeline. (D) Dose-dependent effects of oleic acid treatment on different lipid droplet variables in Vector control cells. For bar graphs, data is presented as mean+ S.E.M. from 3 independent experiments, \*\* significantly different than control,  $P < 0.01$ . ## significantly different than 200  $\mu\text{M}$  oleic acid  $P < 0.01$ . Wild-type *TM6SF2* (WT), E167K mutant variant *TM6SF2* (E167K), pCMV empty vector control (Vector), non-targeted shRNA control (NT), and *TM6SF2* knockdown (KD).

**Supplementary Figure S2:** Unsupervised Hierarchical clustering analysis for 350 lipid species.



**Supplementary Figure S2:** Red color represents the abundance of each lipid species (red: higher, blue: lower). WT (wild-type *TM6SF2* overexpression, Vector (empty vector control), NT (Non-targeted shRNA control), and KD (*TM6SF2* knockdown). Numbers at the ends refers to each replicate.

**Supplementary Figure S3:** Heatmap of side chain length and degree of unsaturation for lipid species identified in the lipidomic analysis.



**Supplementary Figure S3:** Each panel represents a lipid class and the title of the panel is the abbreviated name of the lipid class. Each box represents an individual lipid species where the colors indicate the relative ratio of the lipid (red: increased; white: no difference; blue: decreased) in (A) wild-type *TM6SF2* overexpression (n=3) compared to vector control (n=3) and (B) *TM6SF2* knockdown (n=5) versus non targeting control (n=4). CE (cholesterol esters), CerP (ceramide 1-phosphate), CL (Cardiolipin), DG (diacylglycerols), lysoPC (Lysophosphatidylcholines), lysoPE (lysophosphatidylethanolamine), MG (monoacylglycerols), PA (Phosphatidic acid), PC (phosphatidylcholine), PE (Phosphatidylethanolamine), PG (Phosphatidylglycerol), PI (phosphatidylinositol), plasmeylPC (Plasmeylphosphatidylcholines), plasmeylPE (Phosphatidylethanolamines), PS (Phosphatidylserines), SM (Sphingomyelines), TG (Triacylglycerols). WT (wild-type *TM6SF2* overexpression, Vector (empty vector control), NT (Non-targeted shRNA control), and KD (*TM6SF2* knockdown).

**Supplementary Table S1: List of differentially abundant lipid species identified through untargeted lipidomic analysis.**

| Comparison  | Molecule        | Class      | P.Val   | Fold Change | LogFC |
|-------------|-----------------|------------|---------|-------------|-------|
| KD - NT     | DG 31:0         | DG         | 0.00985 | 2.09        | 1.06  |
| KD - NT     | DG 34:0         | DG         | 0.01193 | 2.35        | 1.23  |
| KD - NT     | lysoPC 18:0     | lysoPC     | 0.00007 | 2.33        | 1.22  |
| KD - NT     | lysoPC 20:3     | lysoPC     | 0.00007 | 3.03        | 1.60  |
| KD - NT     | lysoPE 16:0     | lysoPE     | 0.00433 | 2.01        | 1.00  |
| KD - NT     | lysoPE 18:2     | lysoPE     | 0.00345 | 2.01        | 1.01  |
| KD - NT     | lysoPE 20:3     | lysoPE     | 0.00007 | 3.99        | 2.00  |
| KD - NT     | PA 34:1         | PA         | 0.01258 | -2.92       | -1.55 |
| KD - NT     | PC 42:9         | PC         | 0.00262 | -4.63       | -2.21 |
| KD - NT     | PC 40:7         | PC         | 0.00013 | -2.05       | -1.03 |
| KD - NT     | PE 40:7         | PE         | 0.00011 | -2.22       | -1.15 |
| KD - NT     | PE 33:0         | PE         | 0.00099 | -2.07       | -1.05 |
| KD - NT     | PG 44:10        | PG         | 0.00024 | -2.59       | -1.38 |
| KD - NT     | PG 32:0         | PG         | 0.01088 | 2.49        | 1.31  |
| KD - NT     | PG 38:7         | PG         | 0.00007 | 2.70        | 1.43  |
| KD - NT     | plasmeylPE 38:3 | plasmeylPE | 0.00007 | 2.04        | 1.03  |
| KD - NT     | PS 40:4         | PS         | 0.00066 | -2.69       | -1.43 |
| KD - NT     | PS 36:3         | PS         | 0.00619 | 2.28        | 1.19  |
| KD - NT     | PS 38:4         | PS         | 0.00046 | 3.12        | 1.64  |
| KD - NT     | SM 33:0         | SM         | 0.00007 | -2.10       | -1.07 |
| KD - NT     | SM 36:2         | SM         | 0.00013 | -2.04       | -1.03 |
| KD - NT     | SM 44:1         | SM         | 0.00324 | 2.11        | 1.08  |
| KD - NT     | SM 44:2         | SM         | 0.00020 | 2.60        | 1.38  |
| KD - NT     | TG 56:1         | TG         | 0.00319 | -2.23       | -1.16 |
| KD - NT     | TG 62:7         | TG         | 0.00007 | -2.18       | -1.12 |
| KD - NT     | TG 60:7         | TG         | 0.00007 | -2.14       | -1.10 |
| KD - NT     | TG 58:7         | TG         | 0.00008 | -2.13       | -1.09 |
| KD - NT     | TG 58:8         | TG         | 0.00007 | -2.08       | -1.06 |
| KD - NT     | TG 58:1         | TG         | 0.00016 | 2.13        | 1.09  |
| KD - NT     | TG 52:1         | TG         | 0.01003 | 2.30        | 1.20  |
| WT - Vector | CL 72:4         | CL         | 0.02068 | -2.31       | -1.21 |
| WT - Vector | DG 38:4         | DG         | 0.00272 | -2.46       | -1.30 |
| WT - Vector | DG 36:3         | DG         | 0.00272 | -2.44       | -1.28 |
| WT - Vector | DG 38:2         | DG         | 0.00390 | -2.35       | -1.23 |
| WT - Vector | lysoPE 20:4     | lysoPE     | 0.00666 | 2.21        | 1.14  |
| WT - Vector | lysoPE 16:0     | lysoPE     | 0.03878 | 2.74        | 1.46  |
| WT - Vector | lysoPE 20:3     | lysoPE     | 0.01081 | 2.87        | 1.52  |
| WT - Vector | MG 17:0         | MG         | 0.00390 | -2.40       | -1.26 |
| WT - Vector | PC 37:6         | PC         | 0.02634 | -2.25       | -1.17 |
| WT - Vector | PC 39:3         | PC         | 0.00721 | 2.04        | 1.03  |
| WT - Vector | PC 33:1         | PC         | 0.00272 | 2.14        | 1.10  |
| WT - Vector | PC 42:6         | PC         | 0.00882 | 2.45        | 1.29  |
| WT - Vector | PE 30:0         | PE         | 0.00305 | 2.00        | 1.00  |

| Comparison  | Molecule        | Class      | P.Val   | Fold Change | LogFC |
|-------------|-----------------|------------|---------|-------------|-------|
| WT - Vector | PE 35:4         | PE         | 0.00354 | 2.21        | 1.15  |
| WT - Vector | PE 31:1         | PE         | 0.00199 | 2.82        | 1.50  |
| WT - Vector | PE 32:2         | PE         | 0.00199 | 3.40        | 1.76  |
| WT - Vector | PG 32:1         | PG         | 0.00305 | -2.96       | -1.57 |
| WT - Vector | PG 38:2         | PG         | 0.00272 | -2.25       | -1.17 |
| WT - Vector | PG 32:0         | PG         | 0.04293 | -2.08       | -1.06 |
| WT - Vector | plasmeylPC 44:4 | plasmeylPC | 0.03364 | 10.85       | 3.44  |
| WT - Vector | plasmeylPE 34:0 | plasmeylPE | 0.00305 | -3.46       | -1.79 |
| WT - Vector | plasmeylPE 38:3 | plasmeylPE | 0.00438 | 2.24        | 1.16  |
| WT - Vector | plasmeylPE 38:6 | plasmeylPE | 0.01815 | 2.72        | 1.44  |
| WT - Vector | SM 38:2         | SM         | 0.03085 | -3.07       | -1.62 |
| WT - Vector | TG 52:6         | TG         | 0.00272 | -2.71       | -1.44 |
| WT - Vector | TG 60:5         | TG         | 0.00418 | -2.65       | -1.41 |

**Supplementary Table S 1:** Only the lipid species that were differentially abundant (adjusted  $p$  value  $< 0.05$  and log fold change  $> 0.5$  or  $< -0.5$ ) in cells with either wild-type *TM6SF2* overexpression or knockdown compared to their controls (vector or non-targeted controls respectively) are shown. WT (wild-type *TM6SF2* overexpression), Vector (empty vector control), NT (Non-targeted shRNA control), and KD (*TM6SF2* knockdown). Lipid species that were changed across two comparisons belonged to the following classes: DG (diacylglycerols), lysoPC (Lysophosphatidylcholines), lysoPE (lysophosphatidylethanolamine), MG (monoacylglycerols), PA (Phosphatidic acid), PC (phosphatidylcholine), PE (Phosphatidylethanolamine), PG (Phosphatidylglycerol), plasmeylPC (Plasmeylphosphatidylcholines), plasmeylPE (Phosphatidylethanolamines), PS (Phosphatidylserines), SM (Sphingomyelins), TG (Triacylglycerols).

**Supplementary Table S2: Alterations in *TM6SF2* expression results in differentially expressed lipid metabolizing genes.**

| Comparison  | Symbol   | Protein names  | P. Val   | LogFC | Fold Change |
|-------------|----------|--|----------|-------|-------------|
| WT - Vector | ALDH3B1  | Aldehyde dehydrogenase family 3 member B1              | 1.00E-06 | 1.37  | 2.58        |
| WT - Vector | APOD     | Apolipoprotein D                                       | 1.00E-06 | 2.02  | 4.05        |
| WT - Vector | RGL1     | Ral guanine nucleotide dissociation stimulator-like 1  | 6.39E-06 | 0.58  | 1.49        |
| WT - Vector | DAB2IP   | Disabled homolog 2-interacting protein                 | 6.54E-06 | 0.84  | 1.79        |
| WT - Vector | AGTR1    | Type-1 angiotensin II receptor                         | 2.45E-05 | -1.21 | 0.43        |
| WT - Vector | LIPH     | Lipase member H  | 7.58E-05 | 1.10  | 2.14        |
| WT - Vector | AKR1C1   | Aldo-keto reductase family 1 member C1                 | 9.92E-05 | -1.36 | 0.39        |
| WT - Vector | VAV3     | Guanine nucleotide exchange factor                     | 0.00071  | 0.70  | 1.63        |
| WT - Vector | AGMO     | Alkylglycerol monooxygenase                            | 0.00336  | -0.65 | 0.64        |
| WT - Vector | AKR1C4   | Aldo-keto reductase family 1 member C4                 | 0.00373  | -0.98 | 0.51        |
| WT - Vector | FDFT1    | Squalene synthase                                      | 0.00382  | -0.50 | 0.70        |
| WT - Vector | CAPN2    | Calpain-2 catalytic subunit                            | 0.00478  | 1.17  | 2.25        |
| WT - Vector | APOA2    | Apolipoprotein A-II                                    | 0.00517  | -0.58 | 0.67        |
| WT - Vector | HSD17B2  | 17-beta-hydroxysteroid dehydrogenase type 2            | 0.00778  | 0.53  | 1.45        |
| WT - Vector | AKR1C2   | Aldo-keto reductase family 1 member C2                 | 0.02397  | -1.13 | 0.46        |
| WT - Vector | TM6SF2   | Transmembrane 6 superfamily member 2                   | 1.00E-06 | 1.75  | 3.37        |
| KD - NT     | ACAA1    | 3-ketoacyl-CoA thiolase                                | 1.00E-06 | 0.81  | 1.75        |
| KD - NT     | AKR1B1   | Aldo-keto reductase family 1 member B1                 | 1.00E-06 | 2.73  | 6.64        |
| KD - NT     | ANXA1    | Annexin A1   | 1.00E-06 | 3.79  | 13.87       |
| KD - NT     | BMP2     | Bone morphogenetic protein 2                           | 1.00E-06 | 0.94  | 1.91        |
| KD - NT     | CYP2C19  | Cytochrome P450 2C19                                   | 1.00E-06 | -2.13 | 0.23        |
| KD - NT     | EHHADH   | Peroxisomal bifunctional enzyme                        | 1.00E-06 | -0.65 | 0.64        |
| KD - NT     | CCN1     | CCN family member 1                                    | 1.00E-06 | 1.73  | 3.32        |
| KD - NT     | MVD      | Diphosphomevalonate decarboxylase                      | 1.00E-06 | 0.66  | 1.58        |
| KD - NT     | PDGFRA   | Platelet-derived growth factor receptor alpha          | 1.00E-06 | -1.20 | 0.43        |
| KD - NT     | PPARG    | Peroxisome proliferator-activated receptor gamma       | 1.00E-06 | 0.69  | 1.62        |
| KD - NT     | PTGIS    | Prostacyclin synthase                                  | 1.00E-06 | -1.03 | 0.49        |
| KD - NT     | THRA     | Thyroid hormone receptor alpha                         | 1.00E-06 | -1.15 | 0.45        |
| KD - NT     | UBE2I    | SUMO-conjugating enzyme UBC9                           | 1.00E-06 | 0.83  | 1.78        |
| KD - NT     | VDR      | Vitamin D3 receptor                                    | 1.00E-06 | 1.66  | 3.16        |
| KD - NT     | PIK3R3   | Phosphatidylinositol 3-kinase regulatory subunit gamma | 1.00E-06 | 0.89  | 1.85        |
| KD - NT     | DGAT1    | Diacylglycerol O-acyltransferase 1                     | 1.00E-06 | -0.88 | 0.55        |
| KD - NT     | ABCC3    | ATP-binding cassette sub-family C member 3             | 1.00E-06 | 1.15  | 2.22        |
| KD - NT     | LPCAT3   | Lysophospholipid acyltransferase 5                     | 1.00E-06 | 0.54  | 1.45        |
| KD - NT     | VAV3     | Guanine nucleotide exchange factor                     | 1.00E-06 | 2.22  | 4.65        |
| KD - NT     | RGL1     | Ral guanine nucleotide dissociation stimulator-like 1  | 1.00E-06 | 0.97  | 1.96        |
| KD - NT     | ANKRD1   | Ankyrin repeat domain-containing protein 1             | 1.00E-06 | 3.56  | 11.80       |
| KD - NT     | TNFRSF21 | Tumor necrosis factor receptor superfamily member 21   | 1.00E-06 | 0.96  | 1.95        |
| KD - NT     | ANGPTL3  | Angiopoietin-related protein 3                         | 1.00E-06 | 1.05  | 2.08        |
| KD - NT     | ARSJ     | Arylsulfatase J  | 1.00E-06 | 1.20  | 2.30        |
| KD - NT     | ELOVL7   | Elongation of very long chain fatty acids protein 7    | 1.00E-06 | 1.26  | 2.39        |
| KD - NT     | DGAT2    | Diacylglycerol O-acyltransferase 2                     | 1.00E-06 | 0.67  | 1.59        |
| KD - NT     | SLC44A3  | Choline transporter-like protein 3                     | 1.00E-06 | 1.22  | 2.33        |
| KD - NT     | TTC39B   | Tetratricopeptide repeat protein 39B                   | 1.00E-06 | -1.32 | 0.40        |

| Comparison | Symbol   | Protein names   | P. Val   | LogFC | Fold Change |
|------------|----------|---|----------|-------|-------------|
| KD - NT    | AKR1C4   | Aldo-keto reductase family 1 member C4                              | 1.45E-06 | 1.48  | 2.79        |
| KD - NT    | SLCO1B3  | Solute carrier organic anion transporter family member 1B3          | 1.69E-06 | -1.35 | 0.39        |
| KD - NT    | EPHX2    | Bifunctional epoxide hydrolase 2                                    | 1.91E-06 | -0.60 | 0.66        |
| KD - NT    | SGMS1    | Phosphatidylcholine:ceramide cholinephosphotransferase 1            | 3.33E-06 | 0.82  | 1.77        |
| KD - NT    | STUB1    | E3 ubiquitin-protein ligase   | 6.40E-06 | 0.71  | 1.64        |
| KD - NT    | ABCC1    | Multidrug resistance-associated protein 1                           | 7.89E-06 | 0.87  | 1.83        |
| KD - NT    | LIPH     | Lipase member H   | 7.89E-06 | 1.33  | 2.51        |
| KD - NT    | ACBD4    | Acyl-CoA-binding domain-containing protein 4                        | 1.83E-05 | -0.70 | 0.61        |
| KD - NT    | B4GALNT1 | Beta-1,4 N-acetylgalactosaminyltransferase 1                        | 2.03E-05 | 0.50  | 1.42        |
| KD - NT    | HMGCS2   | Hydroxymethylglutaryl-CoA synthase                                  | 2.57E-05 | 0.98  | 1.98        |
| KD - NT    | LRP2     | Low-density lipoprotein receptor-related protein 2                  | 2.84E-05 | -0.75 | 0.60        |
| KD - NT    | GGT1     | Glutathione hydrolase 1 proenzyme                                   | 2.85E-05 | -0.95 | 0.52        |
| KD - NT    | PLA2G4A  | Cytosolic phospholipase A2  | 2.92E-05 | -1.05 | 0.48        |
| KD - NT    | UGT1A1   | UDP-glucuronosyltransferase 1A1                                     | 4.09E-05 | 1.64  | 3.13        |
| KD - NT    | HPGD     | 15-hydroxyprostaglandin dehydrogenase                               | 6.18E-05 | 1.18  | 2.26        |
| KD - NT    | STARD10  | START domain-containing protein 10                                  | 0.000117 | -0.54 | 0.69        |
| KD - NT    | SPTSSA   | Serine palmitoyltransferase small subunit A                         | 0.000153 | -0.71 | 0.61        |
| KD - NT    | PON3     | Serum paraoxonase/lactonase 3                                       | 0.000199 | -0.85 | 0.55        |
| KD - NT    | B3GALNT1 | UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1             | 0.000271 | 0.61  | 1.52        |
| KD - NT    | PPARGC1B | Peroxisome proliferator-activated receptor gamma coactivator 1-beta | 0.000291 | -0.61 | 0.66        |
| KD - NT    | LPCAT4   | Lysophospholipid acyltransferase LPCAT4                             | 0.000433 | -0.77 | 0.59        |
| KD - NT    | GDPD1    | Lysophospholipase D GDPD1   | 0.000553 | -0.55 | 0.68        |
| KD - NT    | SLC27A2  | Very long-chain acyl-CoA synthetase                                 | 0.000665 | -0.85 | 0.55        |
| KD - NT    | OSBPL1A  | Oxysterol-binding protein-related protein 1                         | 0.000748 | -0.74 | 0.60        |
| KD - NT    | CYP2R1   | Vitamin D 25-hydroxylase  | 0.000748 | -0.58 | 0.67        |
| KD - NT    | DAB2IP   | Disabled homolog 2-interacting protein                              | 0.000832 | -0.65 | 0.64        |
| KD - NT    | CAPN2    | Calpain-2 catalytic subunit   | 0.000931 | 1.31  | 2.48        |
| KD - NT    | ACP6     | Lysophosphatidic acid phosphatase type 6                            | 0.000962 | -0.53 | 0.69        |
| KD - NT    | SEC14L2  | SEC14-like protein 2  | 0.001312 | -0.84 | 0.56        |
| KD - NT    | APOA5    | Apolipoprotein A-V  | 0.001321 | -1.12 | 0.46        |
| KD - NT    | TGFB1    | Transforming growth factor beta-1 proprotein                        | 0.001356 | -0.90 | 0.54        |
| KD - NT    | LSR      | Lipolysis-stimulated lipoprotein receptor                           | 0.001498 | 0.51  | 1.43        |
| KD - NT    | PLIN5    | Perilipin-5   | 0.001606 | -0.90 | 0.54        |
| KD - NT    | SLCO1A2  | Solute carrier organic anion transporter family member 1A2          | 0.001779 | -0.63 | 0.65        |
| KD - NT    | G0S2     | G0/G1 switch protein 2  | 0.002284 | -0.91 | 0.53        |
| KD - NT    | SPHK1    | Sphingosine kinase 1  | 0.002309 | -0.53 | 0.69        |
| KD - NT    | SIRT3    | NAD-dependent protein deacetylase sirtuin-3                         | 0.002604 | -0.51 | 0.70        |
| KD - NT    | SULT2A1  | Sulfotransferase 2A1  | 0.002642 | -0.60 | 0.66        |
| KD - NT    | DECR2    | Peroxisomal 2,4-dienoyl-CoA reductase                               | 0.002888 | 0.56  | 1.48        |
| KD - NT    | PON1     | Serum paraoxonase/arylesterase 1                                    | 0.003019 | -0.77 | 0.59        |
| KD - NT    | PNPLA2   | Patatin-like phospholipase domain-containing protein 2              | 0.003203 | -0.69 | 0.62        |
| KD - NT    | PLA2G6   | Patatin-like phospholipase domain-containing protein 9              | 0.003506 | -0.85 | 0.55        |
| KD - NT    | ACOT7    | Cytosolic acyl coenzyme A thioester hydrolase                       | 0.003515 | 0.53  | 1.44        |
| KD - NT    | EGR1     | Early growth response protein 1                                     | 0.003783 | 0.84  | 1.78        |
| KD - NT    | PDK1     | 3-phosphoinositide-dependent protein kinase 1                       | 0.004379 | -0.63 | 0.64        |
| KD - NT    | PDK1     | Pyruvate dehydrogenase kinase isoform 1                             | 0.004379 | -0.63 | 0.64        |
| KD - NT    | SLC45A3  | Solute carrier family 45 member 3                                   | 0.004518 | 0.88  | 1.85        |

| Comparison | Symbol  | Protein names   | P. Val   | LogFC | Fold Change |
|------------|---------|---|----------|-------|-------------|
| KD - NT    | PDGFB   | Platelet-derived growth factor subunit B              | 0.005481 | 1.29  | 2.45        |
| KD - NT    | ID2     | DNA-binding protein inhibitor ID-2                    | 0.005720 | 0.77  | 1.71        |
| KD - NT    | MED4    | Mediator complex subunit 4                            | 0.006551 | -0.58 | 0.67        |
| KD - NT    | INPP5J  | Inositol polyphosphate 5-phosphatase J                | 0.008313 | 1.12  | 2.17        |
| KD - NT    | AKR1D1  | Aldo-keto reductase family 1 member D1                | 0.008674 | 1.33  | 2.52        |
| KD - NT    | PDGFA   | Platelet-derived growth factor subunit A              | 0.008825 | -0.55 | 0.68        |
| KD - NT    | APOC3   | Apolipoprotein C-III                                  | 0.009457 | -0.87 | 0.55        |
| KD - NT    | GNB3    | Guanine nucleotide-binding protein G                  | 0.010164 | -0.85 | 0.55        |
| KD - NT    | ALB     | Albumin   | 0.010572 | 0.55  | 1.46        |
| KD - NT    | ALB     | Fas-binding factor 1                                  | 0.010572 | 0.55  | 1.46        |
| KD - NT    | MED7    | Mediator of RNA polymerase II transcription subunit 7 | 0.011572 | -0.54 | 0.69        |
| KD - NT    | HSD17B8 | Estradiol 17-beta-dehydrogenase 8                     | 0.013272 | -0.71 | 0.61        |
| KD - NT    | LDLR    | Low-density lipoprotein receptor                      | 0.015460 | 0.55  | 1.47        |
| KD - NT    | ABHD4   | Alpha/beta hydrolase domain-containing protein 4      | 0.017082 | 0.56  | 1.47        |
| KD - NT    | FDX1    | Ferredoxin-1  | 0.020050 | 0.56  | 1.48        |
| KD - NT    | CIDEA   | Cell death activator CIDE-3                           | 0.028195 | 0.87  | 1.83        |
| KD - NT    | CYP2C9  | Cytochrome P450 2C9                                   | 0.029385 | -0.99 | 0.50        |
| KD - NT    | ACER3   | Alkaline ceramidase 3                                 | 0.031183 | 0.52  | 1.43        |
| KD - NT    | AKR1C2  | Aldo-keto reductase family 1 member C2                | 0.037809 | 1.00  | 2.00        |
| KD - NT    | TM6SF2  | Transmembrane 6 superfamily member 2                  | 0.428827 | -0.29 | 0.82        |

**Supplementary Table S2:** Genes that were differentially expressed (adjusted *p* value of less than 0.05 and an absolute log fold change of greater than 1 or 1.4-fold change) in cells with either wild-type *TM6SF2* overexpression or knockdown compared to their controls (vector or non-targeted controls respectively) are shown. WT (wild-type *TM6SF2* overexpression), Vector (empty vector control), NT (Non-targeted shRNA control), and KD (*TM6SF2* knockdown). Only the set of 1008 genes for the 749 proteins identified by Reactome as being part of the overall human Metabolism of lipids pathway (R-HSA-556833) and the 864 human proteins identified in GO as being involved in the regulation of lipid metabolic process (GO:0019216) were analyzed.



## Supplementary Method S1: CellProfiler Pipeline

<http://www.cellprofiler.org>

Version:5

DateRevision:413

GitHash:

ModuleCount:22

HasImagePlaneDetails:False

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Filter images?:Custom

Select the rule criteria:and (file does endwith ".C01")

Metadata:[module\_num:2|svn\_version:'Unknown'|variable\_revision\_number:6|show\_window:False|notes:['The Metadata module optionally allows you to extract information describing your images (i.e, metadata) which will be stored along with your measurements. This information can be contained in the file name and/or location, or in an external file.']]batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Extract metadata?:Yes

Metadata data type:Text

Metadata types: {}

Extraction method count:1

Metadata extraction method:Extract from file/folder names

Metadata source:File name

Regular expression to extract from file

name:^(?P<WELL>.\*)(?P<CELL>.\*)(?P<COND>.\*)(?P<srs>.\*)(?P<dye>.\*).C01

Regular expression to extract from folder name:(?P<Date>[0-9]{4}\_[0-9]{2}\_[0-9]{2})\$

Extract metadata from:All images

Select the filtering criteria:and (file does contain "")

Metadata file location:Elsewhere...|

Match file and image metadata:[]

Use case insensitive matching?:No

Metadata file name:

Does cached metadata exist?:No

NamesAndTypes:[module\_num:3|svn\_version:'Unknown'|variable\_revision\_number:8|show\_window:False|notes:['The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.']]batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Assign a name to:Images matching rules

Select the image type:Grayscale image

Name to assign these images:DNA

Match metadata:[{'Raw\_Lipid': 'CELL', 'Raw\_DNA': 'CELL'}, {'Raw\_Lipid': 'COND', 'Raw\_DNA': 'COND'}, {'Raw\_Lipid': 'WELL', 'Raw\_DNA': 'WELL'}, {'Raw\_Lipid': 'srs', 'Raw\_DNA': 'srs'}]

Image set matching method:Metadata

Set intensity range from:Image metadata

Assignments count:2

Single images count:0

Maximum intensity:255.0

Process as 3D?:No

Relative pixel spacing in X:1.0

Relative pixel spacing in Y:1.0

Relative pixel spacing in Z:1.0

Select the rule criteria:and (file does endwith "DNA.C01")

Name to assign these images:Raw\_DNA

Name to assign these objects:Cell  
Select the image type:Grayscale image  
Set intensity range from:Image metadata  
Maximum intensity:255.0  
Select the rule criteria:and (file does endwith "Lipid.C01")  
Name to assign these images:Raw\_Lipid  
Name to assign these objects:Nucleus  
Select the image type:Grayscale image  
Set intensity range from:Image metadata  
Maximum intensity:255.0

Groups:[module\_num:4|svn\_version:'Unknown'|variable\_revision\_number:2|show\_window:False|notes:['The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.']]batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Do you want to group your images?:No  
grouping metadata count:1  
Metadata category:None

RescaleIntensity:[module\_num:5|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]]  
batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Select the input image:Raw\_DNA  
Name the output image:DNA  
Rescaling method:Stretch each image to use the full intensity range  
Method to calculate the minimum intensity:Custom  
Method to calculate the maximum intensity:Custom  
Lower intensity limit for the input image:0.0  
Upper intensity limit for the input image:1.0  
Intensity range for the input image:0.0,1.0  
Intensity range for the output image:0.0,1.0  
Select image to match in maximum intensity:None  
Divisor value:1.0  
Divisor measurement:None

RescaleIntensity:[module\_num:6|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]]  
batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Select the input image:Raw\_Lipid  
Name the output image:Lipid  
Rescaling method:Match the image's maximum to another image's maximum  
Method to calculate the minimum intensity:Custom  
Method to calculate the maximum intensity:Custom  
Lower intensity limit for the input image:0.0  
Upper intensity limit for the input image:1.0  
Intensity range for the input image:0.0,1.0  
Intensity range for the output image:0.0,1.0  
Select image to match in maximum intensity:DNA  
Divisor value:1.0  
Divisor measurement:None

EnhanceOrSuppressFeatures:[module\_num:7|svn\_version:'Unknown'|variable\_revision\_number:7|show\_window:False|notes:[]]  
batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Select the input image:Lipid  
Name the output image:Enhanced\_Lipid  
Select the operation:Enhance  
Feature size:30  
Feature type:Speckles  
Range of hole sizes:1,10

Smoothing scale:2.0  
Shear angle:0.0  
Decay:0.95  
Enhancement method:Tubeness  
Speed and accuracy:Fast  
Rescale result image:Yes

IdentifyPrimaryObjects:[module\_num:8|svn\_version:'Unknown'|variable\_revision\_number:14|show\_window:False|  
notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:DNA  
Name the primary objects to be identified:Nuclei  
Typical diameter of objects, in pixel units (Min,Max):50,250  
Discard objects outside the diameter range?:Yes  
Discard objects touching the border of the image?:Yes  
Method to distinguish clumped objects:Intensity  
Method to draw dividing lines between clumped objects:Intensity  
Size of smoothing filter:10  
Suppress local maxima that are closer than this minimum allowed distance:7.0  
Speed up by using lower-resolution image to find local maxima?:Yes  
Fill holes in identified objects?:After both thresholding and declumping  
Automatically calculate size of smoothing filter for declumping?:Yes  
Automatically calculate minimum allowed distance between local maxima?:Yes  
Handling of objects if excessive number of objects identified:Continue  
Maximum number of objects:500  
Display accepted local maxima?:No  
Select maxima color:Blue  
Use advanced settings?:Yes  
Threshold setting version:12  
Threshold strategy:Global  
Thresholding method:Minimum Cross-Entropy  
Threshold smoothing scale:2  
Threshold correction factor:2  
Lower and upper bounds on threshold:0.17,1  
Manual threshold:0.0  
Select the measurement to threshold with:None  
Two-class or three-class thresholding?:Two classes  
Log transform before thresholding?:No  
Assign pixels in the middle intensity class to the foreground or the background?:Foreground  
Size of adaptive window:50  
Lower outlier fraction:0.05  
Upper outlier fraction:0.05  
Averaging method:Mean  
Variance method:Standard deviation  
# of deviations:2.0  
Thresholding method:Otsu

IdentifyPrimaryObjects:[module\_num:9|svn\_version:'Unknown'|variable\_revision\_number:14|show\_window:False|  
notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:Enhanced\_Lipid  
Name the primary objects to be identified:Lipid\_Droplets  
Typical diameter of objects, in pixel units (Min,Max):1,30  
Discard objects outside the diameter range?:Yes  
Discard objects touching the border of the image?:Yes  
Method to distinguish clumped objects:Intensity  
Method to draw dividing lines between clumped objects:Propagate  
Size of smoothing filter:10  
Suppress local maxima that are closer than this minimum allowed distance:7.0

Speed up by using lower-resolution image to find local maxima?:Yes  
Fill holes in identified objects?:After both thresholding and declumping  
Automatically calculate size of smoothing filter for declumping?:Yes  
Automatically calculate minimum allowed distance between local maxima?:Yes  
Handling of objects if excessive number of objects identified:Continue  
Maximum number of objects:500  
Display accepted local maxima?:No  
Select maxima color:Blue  
Use advanced settings?:Yes  
Threshold setting version:12  
Threshold strategy:Global  
Thresholding method:Minimum Cross-Entropy  
Threshold smoothing scale:1  
Threshold correction factor:2  
Lower and upper bounds on threshold:0.05,0.5  
Manual threshold:0.0  
Select the measurement to threshold with:None  
Two-class or three-class thresholding?:Two classes  
Log transform before thresholding?:No  
Assign pixels in the middle intensity class to the foreground or the background?:Foreground  
Size of adaptive window:50  
Lower outlier fraction:0.05  
Upper outlier fraction:0.05  
Averaging method:Mean  
Variance method:Standard deviation  
# of deviations:2.0  
Thresholding method:Otsu

IdentifySecondaryObjects:[module\_num:10|svn\_version:'Unknown'|variable\_revision\_number:10|show\_window:F  
alse|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input objects:Nuclei  
Name the objects to be identified:Lipid\_Cells  
Select the method to identify the secondary objects:Propagation  
Select the input image:Lipid  
Number of pixels by which to expand the primary objects:10  
Regularization factor:0.05  
Discard secondary objects touching the border of the image?:Yes  
Discard the associated primary objects?:Yes  
Name the new primary objects:LipidCellNoNuclei  
Fill holes in identified objects?:No  
Threshold setting version:12  
Threshold strategy:Global  
Thresholding method:Minimum Cross-Entropy  
Threshold smoothing scale:0.5  
Threshold correction factor:.9  
Lower and upper bounds on threshold:0.06,1.0  
Manual threshold:0.0  
Select the measurement to threshold with:None  
Two-class or three-class thresholding?:Two classes  
Log transform before thresholding?:No  
Assign pixels in the middle intensity class to the foreground or the background?:Foreground  
Size of adaptive window:50  
Lower outlier fraction:0.05  
Upper outlier fraction:0.05  
Averaging method:Mean  
Variance method:Standard deviation  
# of deviations:2.0

Thresholding method:Otsu

IdentifyTertiaryObjects:[module\_num:11|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the larger identified objects:Lipid\_Cells

Select the smaller identified objects:Nuclei

Name the tertiary objects to be identified:perimeter

Shrink smaller object prior to subtraction?:Yes

RelateObjects:[module\_num:12|svn\_version:'Unknown'|variable\_revision\_number:5|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Parent objects:Lipid\_Cells

Child objects:Lipid\_Droplets

Calculate child-parent distances?:None

Calculate per-parent means for all child measurements?:Yes

Calculate distances to other parents?:No

Do you want to save the children with parents as a new object set?:Yes

Name the output object:RelateObjects\_lipidCellLipidDroplets

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

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Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

Parent name:None

MeasureObjectIntensity:[module\_num:13|svn\_version:'Unknown'|variable\_revision\_number:4|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select images to measure:Raw\_Lipid

Select objects to measure:Lipid\_Cells, Lipid\_Droplets

MeasureObjectIntensity:[module\_num:14|svn\_version:'Unknown'|variable\_revision\_number:4|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select images to measure:Lipid

Select objects to measure:Lipid\_Cells, Lipid\_Droplets

MeasureImageIntensity:[module\_num:15|svn\_version:'Unknown'|variable\_revision\_number:4|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Select images to measure:Lipid  
Measure the intensity only from areas enclosed by objects?:Yes  
Select input object sets:Lipid\_Cells  
Calculate custom percentiles:No  
Specify percentiles to measure:10,90

CalculateMath:[module\_num:16|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Name the output measurement:Total\_Frame\_Lipid\_Intensity\_per\_Cell  
Operation:Divide  
Select the numerator measurement type:Image  
Select the numerator objects:None  
Select the numerator measurement:Intensity\_MeanIntensity\_Lipid\_Lipid\_Cells  
Multiply the above operand by:1.0  
Raise the power of above operand by:1.0  
Select the denominator measurement type:Object  
Select the denominator objects:Nuclei  
Select the denominator measurement:Number\_Object\_Number  
Multiply the above operand by:1.0  
Raise the power of above operand by:1.0  
Take log10 of result?:No  
Multiply the result by:1.0  
Raise the power of result by:1.0  
Add to the result:0.0  
How should the output value be rounded?:Not rounded  
Enter how many decimal places the value should be rounded to:0  
Constrain the result to a lower bound?:No  
Enter the lower bound:0.0  
Constrain the result to an upper bound?:No  
Enter the upper bound:1.0

GrayToColor:[module\_num:17|svn\_version:'Unknown'|variable\_revision\_number:4|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]  
Select a color scheme:RGB  
Rescale intensity:No  
Select the image to be colored red:Leave this black  
Select the image to be colored green:Lipid  
Select the image to be colored blue:DNA  
Name the output image:MergedColorImage  
Relative weight for the red image:1.0  
Relative weight for the green image:1.0  
Relative weight for the blue image:1.0  
Select the image to be colored cyan:Leave this black  
Select the image to be colored magenta:Leave this black  
Select the image to be colored yellow:Leave this black  
Select the image that determines brightness:Leave this black  
Relative weight for the cyan image:1.0  
Relative weight for the magenta image:1.0  
Relative weight for the yellow image:1.0  
Relative weight for the brightness image:1.0  
Hidden:1  
Image name:None  
Color:#ff0000  
Weight:1.0

OverlayOutlines:[module\_num:18|svn\_version:'Unknown'|variable\_revision\_number:4|show\_window:False|notes:[  
]]batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Display outlines on a blank image?:Yes  
Select image on which to display outlines:None  
Name the output image:ObjectOutlines  
Outline display mode:Color  
Select method to determine brightness of outlines:Max of image  
How to outline:Thick  
Select outline color:#0080FF  
Select objects to display:Nuclei  
Select outline color:yellow  
Select objects to display:Lipid\_Cells  
Select outline color:green  
Select objects to display:Lipid\_Droplets

Tile:[module\_num:19|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:True|notes:[]|batch\_state:  
array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select an input image:DNA  
Name the output image:TiledResults  
Tile assembly method:Within cycles  
Final number of rows:1  
Final number of columns:3  
Image corner to begin tiling:top left  
Direction to begin tiling:row  
Use meander mode?:No  
Automatically calculate number of rows?:No  
Automatically calculate number of columns?:No  
Select an additional image to tile:Enhanced\_Lipid  
Select an additional image to tile:ObjectOutlines

RescaleIntensity:[module\_num:20|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[  
]]batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:TiledResults  
Name the output image:RescaledTiledResults  
Rescaling method:Match the image's maximum to another image's maximum  
Method to calculate the minimum intensity:Custom  
Method to calculate the maximum intensity:Custom  
Lower intensity limit for the input image:0.0  
Upper intensity limit for the input image:1.0  
Intensity range for the input image:0.0,1.0  
Intensity range for the output image:0.0,1.0  
Select image to match in maximum intensity:DNA  
Divisor value:1.0  
Divisor measurement:None

SaveImages:[module\_num:21|svn\_version:'Unknown'|variable\_revision\_number:15|show\_window:False|notes:[]|ba  
tch\_state:array([], dtype=uint8)|enabled:False|wants\_pause:False]

Select the type of image to save:Image  
Select the image to save:RescaledTiledResults  
Select method for constructing file names:Single name  
Select image name for file prefix:None  
Enter single file name:\g<CELL>\_ \g<COND>\_ \g<WELL>\_ \g<srs>  
Number of digits:4  
Append a suffix to the image file name?:No  
Text to append to the image name:  
Saved file format:tiff

Output file

location:Elsewhere...|E:\Speliotes\Lipid\_Staining\_Pipeline\TM6SF2\2020\_02\_06\_TM6SF2\_KD\_Nof1\CellProfilerResultsRenamedCorrectlyRun1  
Image bit depth:8-bit integer  
Overwrite existing files without warning?:Yes  
When to save:Every cycle  
Record the file and path information to the saved image?:Yes  
Create subfolders in the output folder?:No  
Base image folder:Elsewhere...|  
How to save the series:T (Time)

ExportToSpreadsheet:[module\_num:22|svn\_version:'Unknown'|variable\_revision\_number:13|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the column delimiter:Comma (",")  
Add image metadata columns to your object data file?:Yes  
Add image file and folder names to your object data file?:No  
Select the measurements to export:No  
Calculate the per-image mean values for object measurements?:Yes  
Calculate the per-image median values for object measurements?:No  
Calculate the per-image standard deviation values for object measurements?:No  
Output file

location:Elsewhere...|E:\Speliotes\Lipid\_Staining\_Pipeline\TM6SF2\2020\_02\_06\_TM6SF2\_KD\_Nof1\CellProfilerResultsRenamedCorrectlyRun1\Excel  
Create a GenePattern GCT file?:No  
Select source of sample row name:Metadata  
Select the image to use as the identifier:None  
Select the metadata to use as the identifier:None  
Export all measurement types?:Yes  
Press button to select measurements:  
Representation of Nan/Inf:NaN  
Add a prefix to file names?:Yes  
Filename prefix:V3\_  
Overwrite existing files without warning?:Yes  
Data to export:Do not use  
Combine these object measurements with those of the previous object?:No  
File name:DATA.csv  
Use the object name for the file name?:Yes