SUPPLEMENTARY INFORMATION

A Kinesin dependent Mechanism for Controlling Triglyceride Secretion from the Liver
Priyanka Rai et. al.

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SUPPLEMENTARY METHODS

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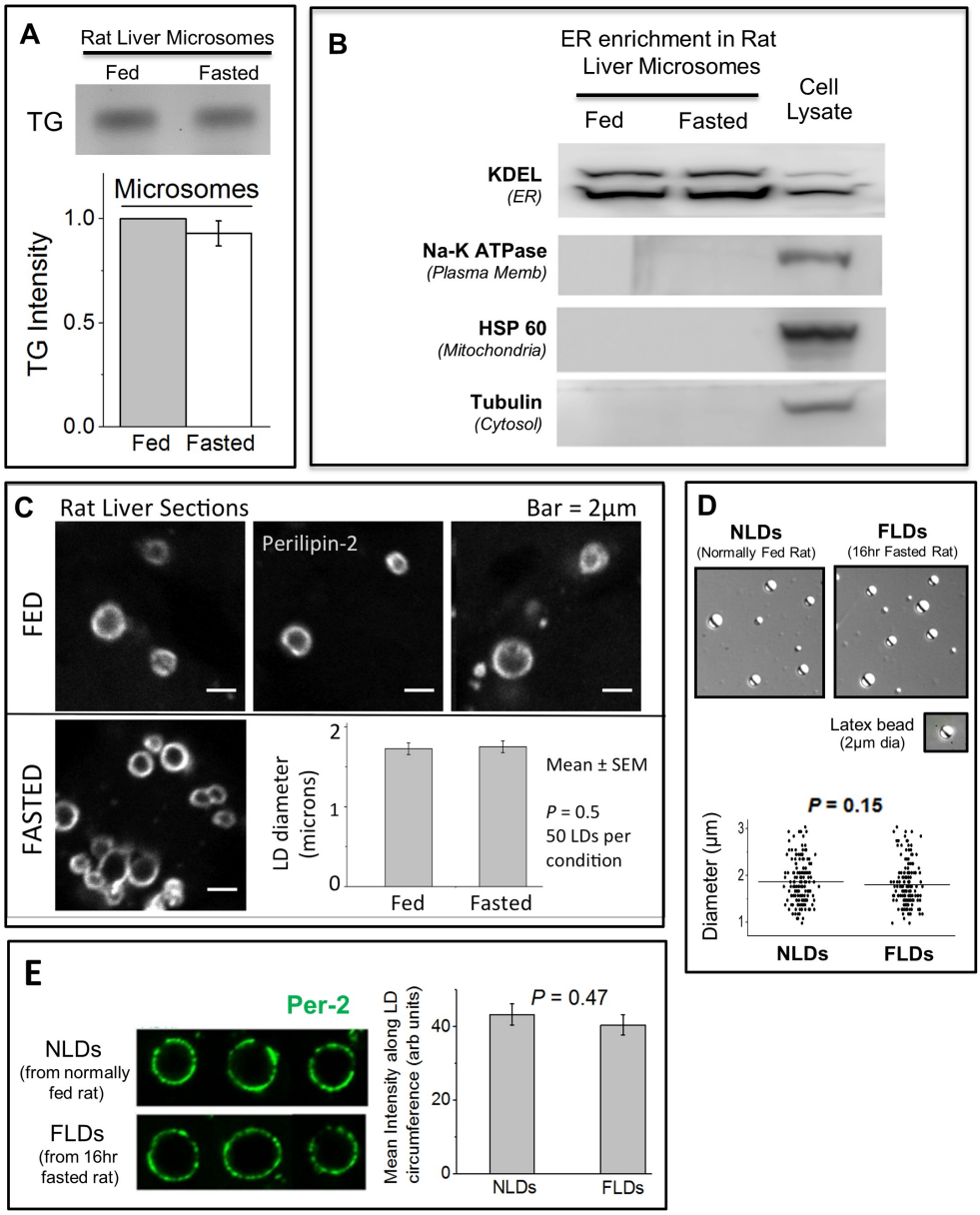


SUPPLEMENTARY FIG S1

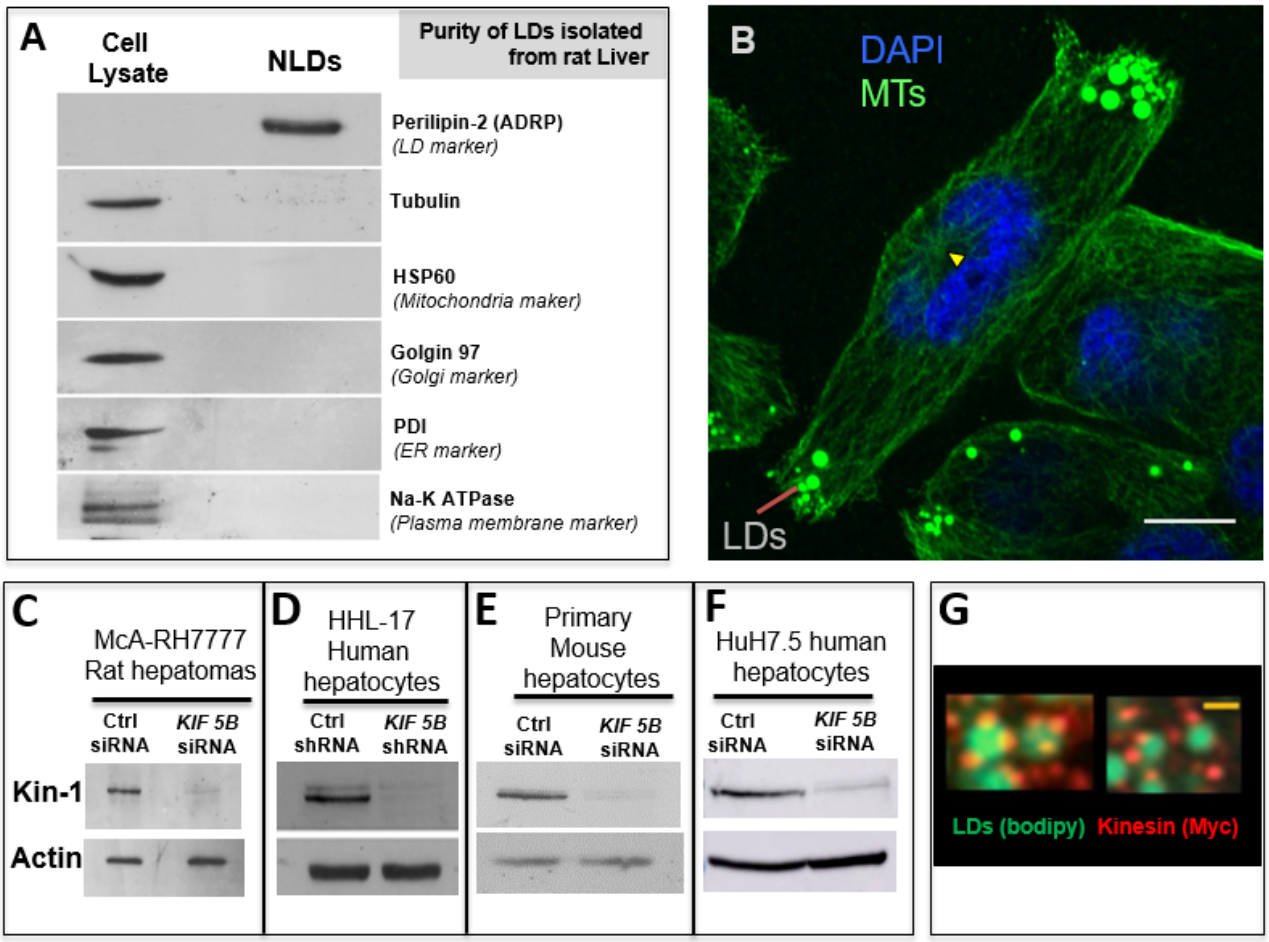
A) TLC experiment shows no difference in TG content between microsomes purified from liver of fed and fasted rats (*P* = 0.72). Samples having equal (total) protein were used. This experiment was repeated thrice (Error bar = SEM).

B) Microsomes prepared from rat liver (fed and fasted) are subjected to western blotting against known markers. Enrichment of an ER marker (KDEL) is seen in the microsome fraction. Proteins present in plasma membrane, mitochondria and cytosol could not be detected in the microsome fraction. Cell lysate was loaded as a positive control.

C) Rat liver sections are immunostained with an antibody against perilipin-2 to label LDs. Representative LDs are shown for fed rats (upper three panels) and fasted rat (lower panel). Bar = 2 microns. The size of LDs in both states is statistically same.

D) Differential interference contrast images show no difference in size between LDs purified from normally fed and fasted rat liver. Image of a 2 micron (dia) latex bead is also shown. Lower panel shows a scatterplot of LD diameters measured from these images. Horizontal lines are the mean values.

E) Left panel :- Immunofluorescent staining of LDs purified from normal-fed (NLDs) and fasted rat liver (FLDs) against perilipin-2 under identical imaging conditions. Three LDs from both the conditions are shown. Note the uniform staining of perilipin-2 on LD, suggesting that the membrane is intact. Right Panel :- Fluorescence intensity measured along the circumference of NLDs (20 used) and FLDs (18 used) suggests no significant difference in the perilipin-2 amount. Error bars are SEMs.



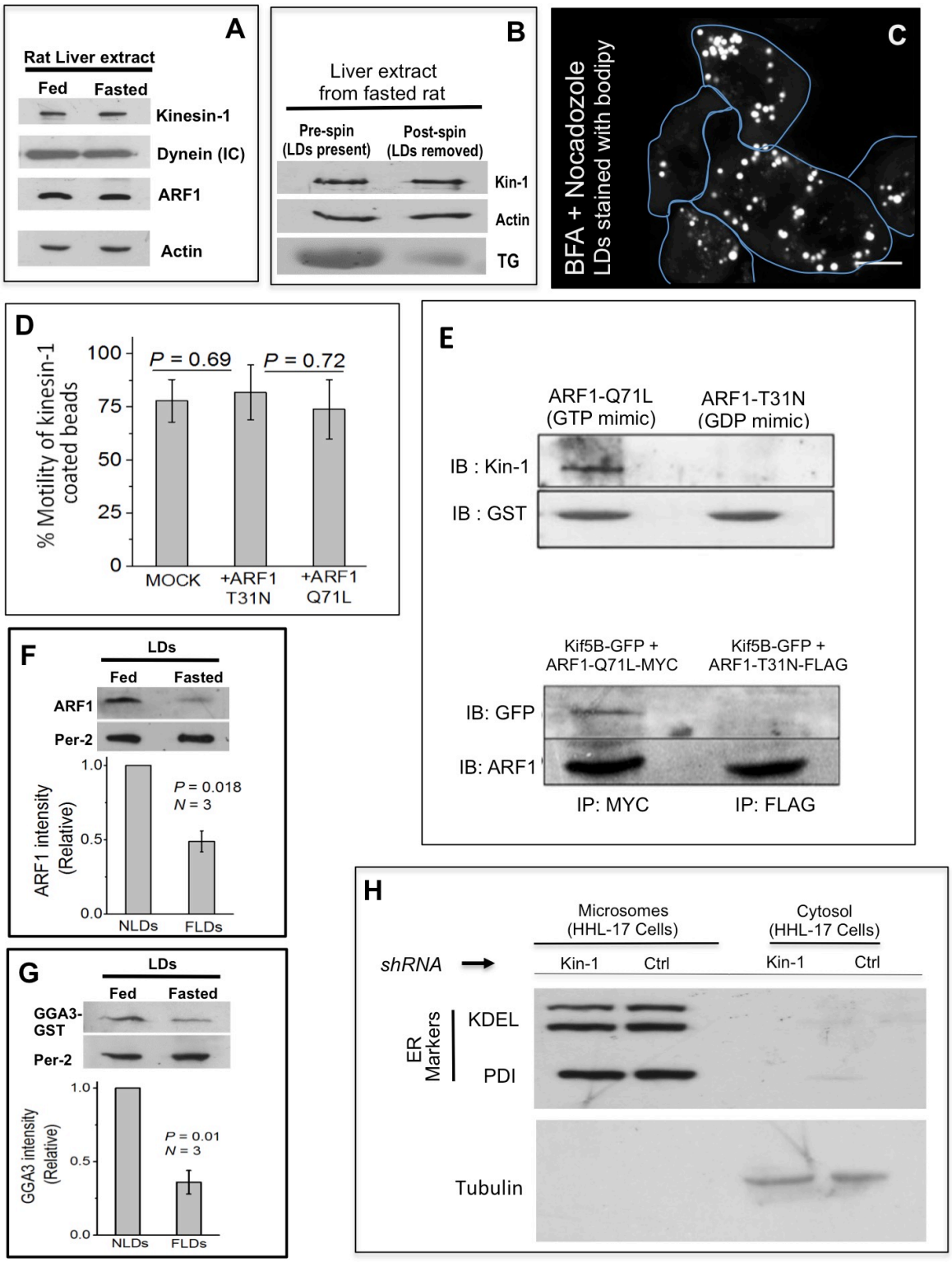
SUPPLEMENTARY FIG S2

A) Purity of LD samples from liver of normally fed rat. Western blots show the presence of perilipin-2 (Lipid droplet marker; also called ADRP) on LDs. Perilipin-2 is not detectable in the cell lysate fraction. Equal amount of LD protein and cell lysate was loaded. Tubulin (cytoplasmic marker), HSP 60 (mitochondrial marker), Golgin 97(Golgi marker), PDI (ER marker) and Na-K-ATPase (plasma membrane marker) could not be detected on the purified LD samples, suggesting that these samples are largely free of contamination.

B) McA-RH7777 hepatoma cells showing immunofluorescence staining of microtubules (MTs) using an anti-tubulin antibody. LDs are stained using bodipy and nucleus using DAPI. The yellow arrowhead indicates a focal point from where the MTs emanate (presumably the MT organizing centre).

C,D,E,F) Western blotting after kinesin-1 knock down in different cells types from different species (mentioned in the figure). Kinesin-1 knockdown was done using siRNA (in rat and mouse species) or adenoviral-shRNA (in human cell lines; has a different sequence from siRNA). The result indicates more than 80% knock down in all cases. Actin is used as loading control.

G) HeLa Cells were transfected with KIF5b-myc, and 36 hrs after transfection cells were loaded overnight with 200μM of oleic acid (OA). After fixation cells were stained for KIF5B-Myc (red) and bodipy (green). Scale Bar: 1μm



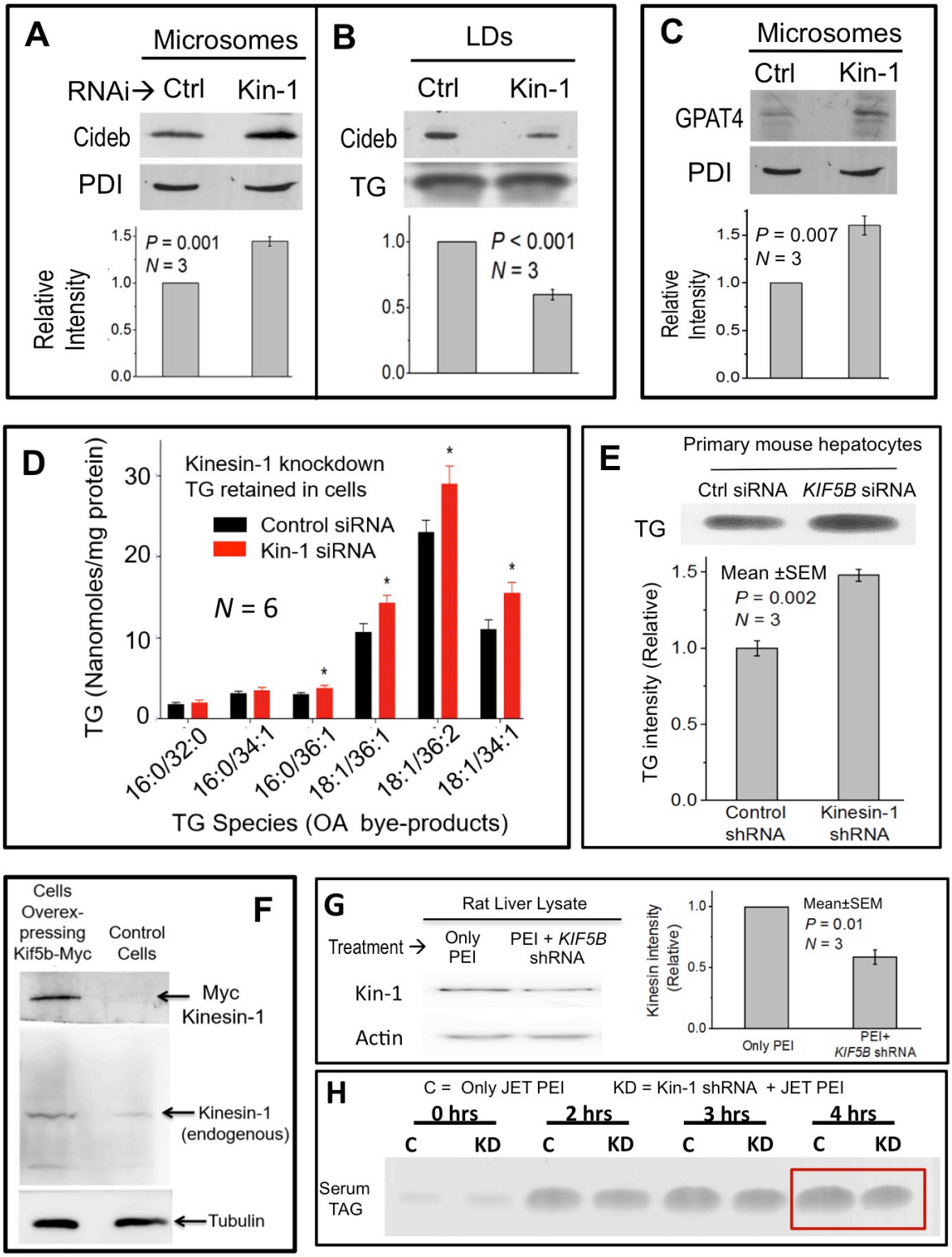
SUPPLEMENTARY FIG S3

A) Western blots show equal amount of kinesin-1, dynein (dynein IC74 antibody used) and ARF1 in liver extract prepared from rats in fed and fasting condition. Actin used as loading control.

B) Western blotting against kinesin-1 of rat liver extract prepared from a fasted rat. Pre-spin sample contains LDs. LDs were removed from the post-spin sample by flotation (see reduction in TAG). Equal intensity of kinesin-1 in both samples shows that the kinesin-1 associated with LDs is a negligible fraction of the total available in liver cytosol.

C) McA-RH7777 cells treated with Brefeldin-A and Nocadozole were stained with bodipy to image LDs.

D) There is no effect of recombinant ARF1 (mimicking both active GTP and inactive GDP bound states) on the motion of kinesin-1 coated beads in an *in vitro* bead motility assay.
E) UPPER:- Pull down of kinesin-1 from cell lysate using GST-ARF1-Q71L (GTP mimic) and GST-ARF1-T31N (GDP mimic). Experiments done in three biological replicates. Error bars are SEM. LOWER:- KIF5B-GFP and ARF1 mutants (MYC or FLAG tagged) were overexpressed in HeLa cells, followed by OA loading. Co-immunoprecipitation was performed using HeLa cell lysate. Western blotting showed that only ARF1-GTP (Q71L) binds to KIF5B-GFP (detected using anti-GFP antibody). This experiment was repeated twice with similar results.
F) Western blot against ARF1 on NLDs and FLDs. Perilipin-2 bands show equal loading. Experiments done in four biological replicates. Error bars are SEM.
G) ARF1 activity on NLDs and FLDs from GGA3-GST binding. Western blotting against perilipin-2 used as loading control. Experiments done in three biological replicates. Error bars are SEM.
H) Enrichment of ER components in microsome fractions prepared from HHL-17 hepatocytes that were subjected to control and kinesin-1 specific shRNA. Microsomes were enriched in KDEL and PDI (ER markers), but tubulin could not be detected.



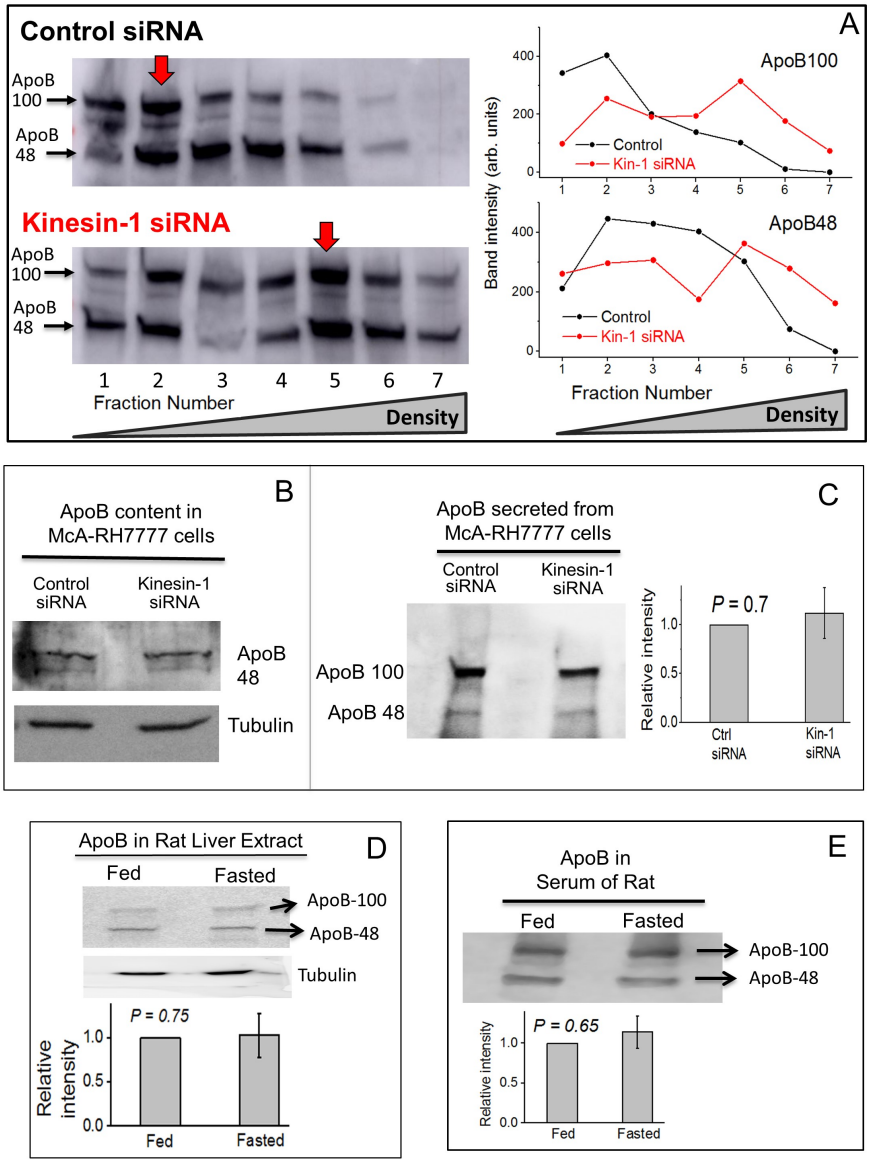
SUPPLEMENTARY FIG S4

A) HHL-17 hepatocytes (control or *Kif-5b* shRNA treated) were used to prepare microsomes. Equal loading confirmed by western blotting against PDI (ER marker). Cideb was retained on microsomes after kinesin-1 knockdown. Experiments done in three biological replicates. Error bars are SEM.

B) LDs were purified from HHL-17 hepatocytes (control or *KIF-5B* shRNA treated). Cideb was reduced on LDs after kinesin-1 knockdown. TLC for TG done to ensure equal loading of LDs. Experiments done in three biological replicates. Error bars are SEM.

C) HHL-17 human hepatocyte cell line (control shRNA or kinesin-1 shRNA treated) was used to prepare ER-enriched microsomes. Western blotting was done using antibody against GPAT4. GPAT4 in microsomes increases after kinesin-1 depletion. Experiment was repeated thrice. Equal loading confirmed by western blotting against PDI (ER marker).

D) Cellular levels of TG species following kinesin-1 knockdown in McA-RH7777 cells. Data represent mean±sem for 6 biological replicates. *$P < 0.05$.
E) Kinesin-1 knockdown by siRNA leads to accumulation of TAG in mouse primary culture hepatocyte cells. Quantification of band intensities shows ~1.5 fold more TAG in kinesin-1 knockdown cells.
F) Western blots for McA-RH7777 cells after transfection with Myc-Kif5B plasmid. 48 hrs post transfection cells and media was used for VLDL-TAG analysis. Equal amount of protein from control and Myc-Kif5B overexpressing cell lysate was used to check for kinesin overexpression using antibodies against Myc and Kinesin-1. Tubulin was used as loading control.
G) Western blotting against kinesin-1 in liver lysate of rats injected with only Jet PEI transfection reagent (control) or kinesin-1 shRNA+PEI. A partial (~40%) knockdown of kinesin-1 is observed in the liver of rat after PEI-shRNA treatment. Actin is used as loading control.
H) TLC of serum prepared at different time points from rats injected with only PEI (control) or (Kinesin-1shRNA+PEI). Tyloxapol was injected 72 hrs after shRNA-PEI injection in order to block VLDL catabolism. Zero hour implies the timepoint of tyloxapol injection. As time passes, there is significantly less serum TG in the knockdown animal when compared to control (e.g., compare intensities between “C” and “KD” within the red box). This suggests that VLDL-TAG secretion is inhibited after kinesin-1 knockdown in the liver.



SUPPLEMENTARY FIG S5

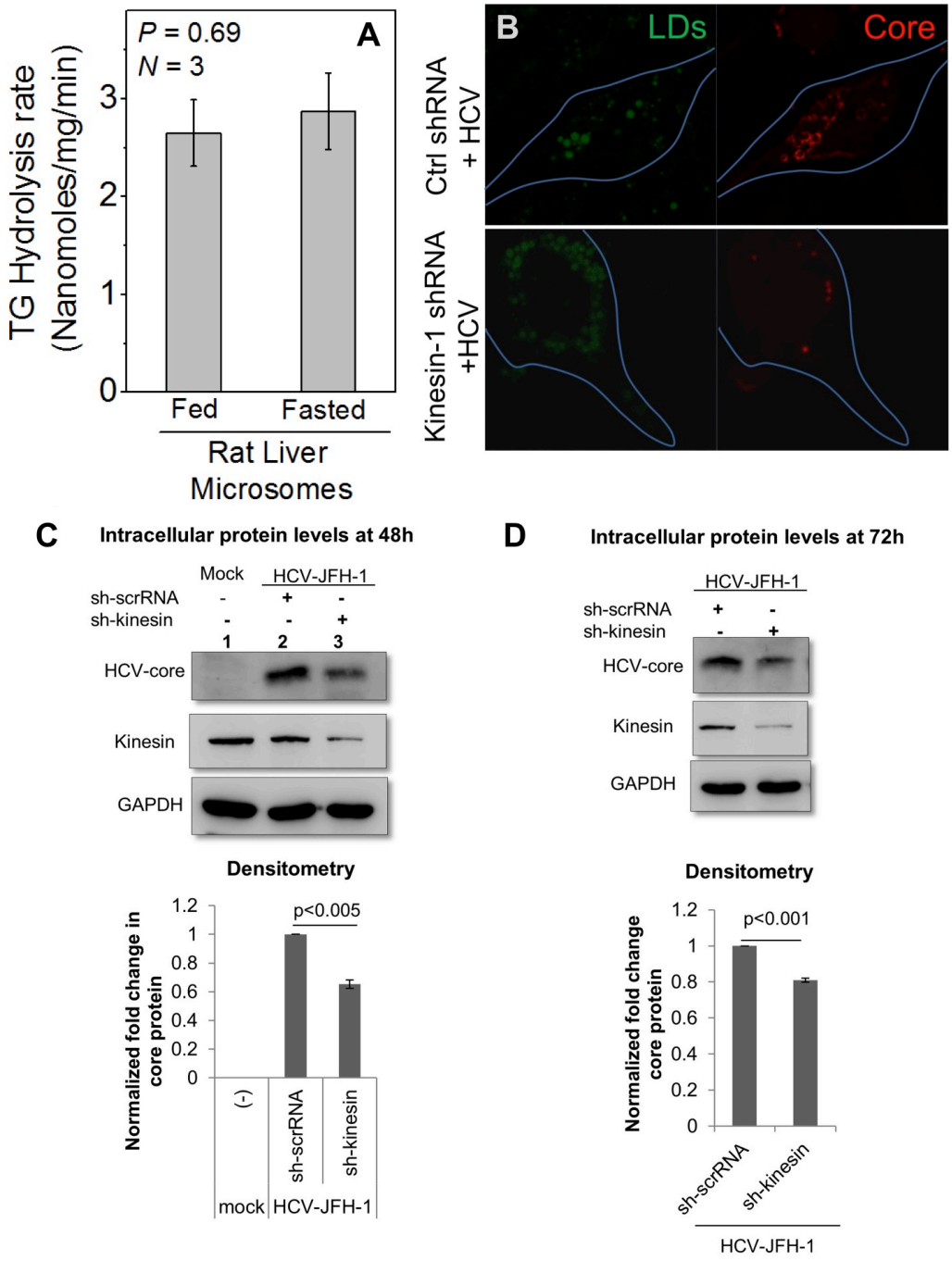
A) Serum from control and kinesin-1 shRNA injected rats were subjected to sucrose density gradient ultracentrifugation to separate apoB-containing lipoproteins. ApoB was immunoprecipitated from each fraction, separated by SDS-PAGE and assessed by Western blotting against antibodies to ApoB. Lanes 1-7 correspond to fractions from top to bottom of sucrose gradient (fraction 1 = lowest, fraction 7 = highest density). Red arrows indicate fraction with highest ApoB. The ApoB48 and ApoB100 band intensity change across fractions is also plotted (right panels). Peak shifts towards right (higher density) after kinesin-1 knockdown. These results are representative of three independent experiments.

B) Western blotting against ApoB shows no significant effect of kinesin-1 knockdown on the levels of ApoB-48 in McA-RH7777 cells. Tubulin is a loading control.

C) Western blotting against ApoB shows no effect of kinesin-1 knockdown on ApoB secretion from McA-RH7777 cells. ApoB was immunoprecipitated from culture medium after a chase (see main text). Total protein loaded was same in both samples.

D) Western blotting of rat liver extract against ApoB shows no change in ApoB levels in the liver between fed and fasted states. Tubulin was used as a loading control.

E) Western blotting of serum from rat against ApoB shows that ApoB-100 and ApoB-48 secretion from the liver is unchanged between fed and fasted states.



SUPPLEMENTARY FIG S6

A) LC-MS based triglyceride hydrolysis activity of membrane lysates of microsomes harvested from fed and fasted rat livers. These experiments were done in three biological replicates with reproducible results. Error bars are SEM.

B) Confocal imaging of HuH7.5 cells treated with control shRNA or shRNA against kinesin-1. The borders of cells are indicated. LDs are stained with the MDH dye and HCV-core is stained using an antibody against core.

C, D) Western Blot showing the effect of partial knockdown of kinesin on the levels of HCV-core protein in the infected cells at 48h and at 72h timepoints post-shRNA addition to cells. GAPDH is used as loading control. Combined data from three independent western blots has also been plotted as the fold change in densitometry (lower panels). Error bars are SEM.

Supplementary TABLE S1

List of multiple reaction monitoring (MRM) transitions used in the targeted TAG profiling using LC-MS/MS. TAG = Triglyceride. Lipid species targeted by MRM are shown.

TAG Species	Adduct	Precursor ion	Product ion
17:0/34:1 (IS)	$[M+NH_4]^+$	864.8	577.5
16:0/32:0	$[M+NH_4]^+$	824.8	551.5
16:0/34:1	$[M+NH_4]^+$	850.8	577.5
16:0/34:0	$[M+NH_4]^+$	852.8	579.5
16:0/36:2	$[M+NH_4]^+$	876.8	603.5
16:0/36:1	$[M+NH_4]^+$	878.8	605.6
16:0/36:0	$[M+NH_4]^+$	880.8	607.5
16:0/36:4	$[M+NH_4]^+$	872.8	599.5
16:0/38:4	$[M+NH_4]^+$	900.8	627.5
18:0/32:0	$[M+NH_4]^+$	852.8	551.5
18:0/34:1	$[M+NH_4]^+$	878.8	577.5
18:0/34:0	$[M+NH_4]^+$	880.8	579.5
18:0/36:2	$[M+NH_4]^+$	904.8	603.5
18:0/36:1	$[M+NH_4]^+$	906.8	605.6
18:0/36:0	$[M+NH_4]^+$	908.8	607.6
18:0/36:4	$[M+NH_4]^+$	900.8	599.5
18:0/38:4	$[M+NH_4]^+$	928.8	627.5
18:1/32:0	$[M+NH_4]^+$	850.8	551.5
18:1/34:1	$[M+NH_4]^+$	876.8	577.5
18:1/34:0	$[M+NH_4]^+$	878.8	579.5
18:1/36:2	$[M+NH_4]^+$	902.8	603.5
18:1/36:1	$[M+NH_4]^+$	904.8	605.6
18:1/36:0	$[M+NH_4]^+$	906.8	607.6
18:1/36:4	$[M+NH_4]^+$	898.8	599.5
18:1/38:4	$[M+NH_4]^+$	926.8	627.5
18:2/34:2	$[M+NH_4]^+$	872.8	575.5
20:4/34:1	$[M+NH_4]^+$	898.8	577.5
20:4/36:2	$[M+NH_4]^+$	924.8	603.5
20:4/36:1	$[M+NH_4]^+$	926.8	605.6
20:4/36:0	$[M+NH_4]^+$	928.8	607.6
22:6/34:0	$[M+NH_4]^+$	924.8	579.5
22:6/36:0	$[M+NH_4]^+$	952.8	607.6
22:6/36:1	$[M+NH_4]^+$	950.8	605.6
22:6/36:2	$[M+NH_4]^+$	948.8	603.6

Supplementary TABLE S2

TAG species identified and quantified in targeted LC-MS/MS TAG profiling of McA-RH7777 cells following kinesin-1 knockdown.

	Control siRNA			Kinesin-1 siRNA			p-value	Significant	
Cells (nmol/mg protein)	Mean	SEM	N	Mean	SEM	N			
16:0/32:0	1.9	0.12	6	2.1	0.22	6	0.44336	NS	
16:0/34:1	3.2	0.23	6	3.6	0.32	6	0.33403	NS	
16:0/36:1	3.1	0.15	6	3.9	0.22	6	0.01324	*	
18:1/36:1	10.8	0.92	6	14.4	0.85	6	0.01655	*	
18:1/36:2	23.1	1.4	6	29.1	2.1	6	0.03879	*	
18:1/34:1	11.1	1.1	6	15.6	1.2	6	0.01998	*	
Media (pmol/ mg protein)	Mean	SEM	N	Mean	SEM	N			
16:0/32:0	108.9	14.5	6	112.3	13.4	6	0.86671	NS	
16:0/34:1	165.2	13.4	6	109.2	9.8	6	0.00708	**	* p < 0.05
16:0/36:1	243.4	22.3	6	156.7	11.3	6	0.00604	**	** p < 0.01
18:1/36:1	321.2	29.4	6	201.3	23.2	6	0.00947	**	*** p < 0.001
18:1/36:2	549.1	23.1	6	366.2	32.2	6	0.00096	***	
18:1/34:1	175.6	13.2	6	109.8	12.3	6	0.00448	**	

SUPPLEMENTARY METHODS

1) Reagents

All reagents were purchased from Sigma-Aldrich, India unless otherwise mentioned. HBSS was from Invitrogen (1X, Invitrogen, 14175). Collagenase type IV was used for isolation of primary mouse hepatocytes (Worthington, 4188). FBS and Lipofectamine 2000 were from Invitrogen. Tyloxapol (Triton WR-1339) was from MP Biomedicals (Cat No. 157162). Oleic Acid was purchased from Sigma (Cat # O1383). PLD inhibitor (VU0155056) and lipids were purchased from Avanti Polar (Alabaster, AL). Triglyceride was purchased from Sigma Aldrich. In vivo-jetPEI (Polyplus-transfection, SA) reagent was used to knock down kinesin-1 in rats. The inhibitory kinesin tail domain peptide (KTD) has been described earlier (1, 2).

Antibodies

ARF1 (Cat. # ab108347), KDEL (Cat. # ab12223), GPAT4 (Cat. #ab 76707) and Na-K ATPase (Cat. # ab7671) from Abcam (Cambridge, MA). Actin (Cat. # AAN01-A) antibody was from Cytoskeleton, Inc. (Denver, CO). PDI (Cat. # 610946) antibody was from BD Biosciences (San Jose, CA). MYC antibody (Cat. # 06-549) from Upstate (Billerica, MA). Cideb (Cat. # PRS2319) and FLAG (Cat. # F1804) antibody was from Sigma-Aldrich, India. Golgin 97 (Cat. # A21270) was from Invitrogen. Perilipin-2 (Cat # 651102) was from Progen Biotechnik (Heidelberg, Germany). Kif3A (kinesin-2) antibody was from BioLegend (San Diego, CA) (Cat # MMS 198P). Phospho-PLD1 (Thr147) Antibody and PLD1 Antibody #3832 were obtained from Cell Signalling. HCV-core antibody was from Abcam (ab2740). Anti-Apolipoprotein B antibody AB742 was from Merck.

An affinity purified rabbit polyclonal antibody was raised against the peptide CDKNRVPYVKGCTER (rat KIF5c amino acids 159–172) by GenScript, Piscataway, NJ. This antibody has been reported and tested before (3).

siRNA and shRNA against Kinesin-1 (in Human, Rat and Mouse species)
Human:- *KIF*-5B shRNA adenovirus (Cat. # shADV-213107; Ad-GFP-U6-h-KIF5B-shRNA) and control-shRNA adenovirus (Cat. # 1122N ; Ad-GFP-U6-shRNA) were purchased from Vector Biolabs (Malvern, PA).

Rat:- On-TARGETplus *KIF-5B* (117550) siRNA-SMART Pool (Cat. # L-090783-01-0010) and ON-TARGETplus Non-targeting Pool (Cat. # D-001810-10-20) were purchased from Dharmacon (Lafayette, CO). Rat *KIF-5B* shRNA was generated by Vector Biolabs (Malvern, PA) against the following targeting sequence: CGUCCAAGCCUUAUGCAUU. This shRNA plasmid was used with the in vivo-jetPEI (Polyplus-transfection, SA) reagent to knock down kinesin-1 in the animal (rat).

Mouse:- ON-TARGET plus SMARTpool against mouse *KIF-5B* was purchased from Dharmacon (Cat No. L-040710-01-0010, Dharmacon, Lafayette, CO).

2) Animals and Animal procedures.

Sprague Dawley (SD) rats were bred and maintained by the animal house facility at Tata Institute of Fundamental Research, Mumbai. All animal protocols were approved by the Institutional Animal Ethics Committee (IAEC) formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

3-4 month old male SD rats were used for all the experiments. Rats from the same litter were used for a fed-fasted pair. Rats were maintained on a regular light(12h)/dark(12h) cycle and fed a standard laboratory chow diet. Animals in Fed group had *ad libitum* access to food and water. Animals in fasted group were fasted for 16h, with *ad libitum* access to water.

3) Cell Culture

HHL-17, BRL3A (ECACC -Cat No. 85111503), HeLa (Cat No. ATCC CCL-2^{TM}) and McA-RH7777 cells (Cat No. ATCC® CRL-160™) were used in this study. HHL-17 hepatocytes were a generous gift from Dr Arvind Patel, University of Glasgow Centre for Virus Research, UK. BRL3A cells were maintained in Coon's Media supplemented with 5% FBS. HHL-17 and HeLa cells were maintained in DMEM containing 10% FBS. McA-RH7777 cells were maintained in DMEM containing 20% FBS. All cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37^0C. Huh cells were maintained in DMEM (Sigma) with 10% Fetal Bovine Serum (GIBCO, Invitrogen)

4) Instrumentation and data acquisition

The instrument and detection system has been described earlier (1, 4). Lipid droplet motility experiment was performed at 37°C using a custom-developed differential interference contrast microscope (Nikon TE2000-U) using a 100X oil objective with numerical aperture (NA = 1.4). The microscope is placed in an acoustically protected room on an optical table (Newport). Image frames were acquired at video rate of 30 frames/sec (no binning) with a Cohu 4910 camera. Each pixel measured 98nm × 98nm. Image frames were digitized and saved as AVI files using an image acquisition card (National Instruments). No image-processing hardware or software was used for image enhancement. The positions of lipid droplets and beads were tracked frame by frame using custom-written software in LabVIEW (National Instruments), as described (5). An Olympus confocal microscope (FV1000) equipped with 405, 488, 561 and 633nm lasers for excitation and spectral/band-pass emission filters was used for acquisition of confocal images.

5) Purification of Lipid droplets from rat liver for *in vitro* motility

Purification of lipid droplets was done as previously reported (1, 6). Briefly, a 3–4 month old male SD rat was anesthetized using sodium thiopentone. The liver was perfused with ice-cold PBS through the hepatic portal vein and dissected out. Liver was homogenized using a Dounce

homogenizer in 1 M MEPS buffer (35mM PIPES, 5mM EGTA, 5mM $MgSO_4$ and 1M sucrose, pH 7.1) with 2X protease inhibitor cocktail, 4mM PMSF, 2µg/ml of pepstatin A and 4mM DTT. Homogenization was done in 1 M MEPS buffer at a ratio of 1:1.5 (w/v). The homogenate was centrifuged (1800*g,* 4°C, 10 min) to obtain a post- nuclear supernatant (PNS). PNS was loaded on a Sephacryl S-200 column (40-ml bed volume) after adding all the protease inhibitors. This step was done to separate organelles from the cytosolic protein fraction. The first 4 ml of eluate was collected, supplemented with protease inhibitors and mixed with 5.6ml of 2.5 M MEPS (2.5 M indicates the molarity of sucrose). This eluate was loaded at the bottom of a step gradient consisting of 2 ml each of 1.4M, 1.2M and 0.5M MEPS. The gradient was centrifuged in a SW41 Beckman rotor tube at 120,000*g* at 4 °C for 1hr. The topmost 0.5M MEPS fraction containing lipid droplets was collected and flash frozen in liquid nitrogen. The aliquots were subsequently used in motility experiments.

6) *In vitro* Lipid droplet and bead motility assays

Tubulin (5–10 mg/ml) was purified from goat brain as described (7). Taxol-stabilized microtubules were polymerized in BRB80 buffer containing 1mM GTP and 20µM taxol at 37 °C. Motility was observed in flow chambers of ~10µl volume prepared by sticking poly-l-lysine–coated coverslips to a microscope slide using two strips of double-stick tape. Lipid droplet motility was performed in a motility mix buffer that contains 38 µl of the LD fraction and 2µl of a 20× ATP regenerating system (20mM ATP, 20mM $MgCl_2$, 40 mM creatine phosphate and 40 U/ml creatine kinase). The final ATP concentration was 1mM. The motility mixture was introduced in a flow chamber containing microtubules. LD motion was analyzed by placing them on microtubules using an optical trap. Each slide was observed for 20 min. Further details can be found in (1, 6)

To study the effect of ARF1 mutants on *in vitro* motion of lipid droplets, purified LDs were incubated with GST-tagged ARF1 mutant proteins at 17µM final concentration. Motility assay was performed as described in previous section. Each slide was observed for maximum of 20 minutes at 37°C. To rule out of the possibility of buffer affecting the motile fraction of LDs, motility experiments were also performed only in presence of buffer (buffer control).

To assess effects of Kinesin tail domain peptide (KTD), Kinesin-1 (KHC) and Kinesin-2 (Kin-2) antibody on LD motility, a 40µl aliquot of LDs was thawed on ice. 250µM (final conc.) of KTD peptide or KHC/Kin-2 antibody (80µg/ml) was added and incubated on ice for 15 min. 20X ATP regenerating system (20 mM ATP, 20mM $MgCl_2$, 40mM creatine phosphate and 40 U/ml creatine kinase) was added to the LD-KTD mixture. Motility was assayed as described above. Each slide was observed for maximum of 20 minutes at 37°C. To rule out of the possibility of buffer affecting the motile fraction of LDs, motility experiments were also performed only in presence of buffer (buffer control).

In vitro motility of kinesin-1 coated beads was also done using a similar assay. Kinesin-1 was purified from goat brain.

7) Imaging of cells and analysis of LD motility

An Olympus confocal microscope (FV1000) equipped with 405, 488, 561 and 633nm lasers for excitation and spectral/band-pass emission filters was used for acquisition of confocal images. An Olympus 63X NA 1.4 oil immersion objective was used. For LD-Cideb colocalization studies, cells were transfected with Cideb-mcherry plasmid. 24 hrs post transfection, cells were stained with bodipy and imaged.

For analysing LD motility, HHL-17 cells were infected either with ctrl-shRNA or *Kif5b* shRNA AAV. After 72h, imaging was done using a custom-developed differential interference contrast microscope (Nikon TE2000-U). For LD distribution analysis, McA-RH7777 cells were either transfected with siRNA (scrambled or against *Kif5b*), or treated with BFA (5µg/ml for 5 hours). After 72h of transfection or 5hrs of BFA treatment, cells were stained with bodipy and used for imaging.

All the images were analyzed with help of ImageJ (NIH) and Fiji software. The movement of LDs were tracked using the TrackMate plugin. The threshold for size (~1µm) and intensity of the spots was set to select all the LDs visible in the frame. Appropriate linking and gap closing

distances (~1-2 μm) were entered to track the movements of the LDs. The track statistics were analysed in OriginPro 9.1.0 (OriginLab Corporation).

8) Isolation of Lipid droplets from rat liver for biochemical assays

For biochemical assays LD purification was similar to *in vitro* motility, with some modifications. Liver was lysed, and the PNS was prepared in 1M MEPS buffer. 5ml PNS was mixed with 7ml of 2.5M MEPS. This was loaded at the bottom of a sucrose step gradient and overlaid by 5ml each of 1.4 M, 1.2 M, and 0.5 M sucrose in MEP. The topmost layer is 5ml MEP (no sucrose). The gradient was centrifuged in a SW32 Beckman rotor tube at 120,000*g* at 4°C for 1h. The topmost fraction was collected and concentrated to 100μl and used for biochemical assays. This fraction was enriched in lipid droplets, as confirmed by the detection of Perilipin-2 by western blotting.

9) Normalization of Lipid droplet samples by Optical density

We invested significant effort into developing a method that ensures two LD samples (e.g. NLD and FLD) have approximately equal number of LDs. LDs isolated from normal-fed (NLDs) and fasted (FLDs) rats were prepared and triglyceride in these samples was estimated using a triglyceride estimation kit (Sigma). These samples were then adjusted by dilution to obtain an NLD sample and an FLD sample with equal triglyceride/unit volume. We next measured OD_{400} (optical density at 400 nm) to see if OD_{400} is a good parameter to normalize two LD samples. We have used this method extensively to normalize bead phagosome samples (8). We therefore expected that the method could also be applied to LD samples that have similar size and refractive index as latex beads (see Supplementary Fig S1-C). Indeed, the OD_{400} was almost equal for NLD and FLD samples (that had been adjusted to obtain equal triglyceride/unit volume; see above).

To further verify this method, we subjected these samples to western blotting against perilipin-2 (marker for LDs) using an anti perilipin-2 antibody. Imaging of purified NLDs or FLDs was done using a microchamber immunofluorescence assay (See section 20 of Supplemental experimental procedures). We observed equally intense staining of perilipin-2 on individual NLDs and FLDs (Supplementary Fig S1-D). We therefore believe that perilipin-2 amounts are similar on individual NLDs and FLDs. Western blotting of NLD and FLD samples with equal OD_{400} consistently showed equal amount of perilipin-2 (Fig 2A).

The triglyceride content of LDs (as a mol% of total lipids) does not change between fed and fasted states (9). Therefore, the NLD and FLD samples (having equal OD_{400} and equal perilipin) were doubly confirmed to have equal triglyceride by a TLC experiment (Fig 2A). Taken together, we believe that LD samples normalized for OD_{400} also have equal (total) surface area and equal volume (because they have equal surface-associated perilipin-2 and triglyceride inside the LD).

To determine the linearity and sensitivity of OD_{400} method for normalization of LD samples, we measured the OD_{400} of serially diluted NLD samples. A linear relationship was observed between OD_{400} and concentration. Therefore, LD amounts were normalized using OD_{400} and validated either by perilipin-2 western blotting or triglyceride estimation. Such normalized NLD and FLD samples were used to infer difference in protein levels between two samples (e.g., see Fig 2A). We ensured that OD_{400} of NLD/FLD samples was within the linear range. We estimate that >10% difference in LD amount between two samples would be detected using OD_{400}.

10) Protein extraction and western blotting

Equal amounts of LDs isolated from rat liver (see above) were taken and proteins were extracted by sonicating in 2X SDS sample buffer for 20 min. The samples were further boiled for 10 min and resolved by SDS-PAGE. For some experiments, LD proteins were precipitated by mixing LDs with a chloroform:acetone solution (1:1 v/v ratio).

For preparing liver tissue extract, liver was minced and homogenized using a Dounce homogenizer in 1M MEPS buffer with protease inhibitors. The lysate was centrifuged (200*g*, 4°C, 5 min) before use for Western blotting. To prepare a fraction without LDs, the extract was subjected to ultracentrifugation at 500000*g* at 4°C for 60 min. At the end of centrifugation, LD fraction (topmost layer) was carefully removed with the help of syringe and rest of the supernatant was mixed with the pellet.

The proteins were transferred to PVDF membrane for western blot analysis as previously described (1). Primary antibodies for Kinesin-1 (1:2500), ARF1 (1:2500), GFP (1:1000), CideB (1:2000), Tubulin (1:2000), ADRP (1:1000), HCV-core (1:250) and actin (1:2500) were used. The secondary antibody used was conjugated to horseradish peroxidase (HRP) (1:20000). The blots were developed using the ECL kit (Millipore, Billerica, MA). Exposed films were scanned and bands of interest were quantified using Image J software.

11) Thin Layer Chromatography

Purification of lipid droplets was done as previously described. Lipids were extracted according to method of (10). Briefly, 0.8ml aqueous sample containing LDs were mixed with 2ml of methanol and 1ml chloroform followed by vortexing and overnight lipid extraction. 1ml each of chloroform and water were added the next day, which resulted in phase separation; the lower being the organic phase. The organic phase was transferred to a new glass tube, dried under a stream of N_2 and resuspended in 20µl of chloroform. The silica TLC plates (Merck) were pre-cleaned using Chloroform followed by air drying and heating to $100^{O}C$ for 15mins. The sample was then spotted onto these plates using a glass capillary. The solvent system used was that of Wilfling et al (11) with minor modifications. The first solvent was a mixture of n-hexane/diethyl ether/acetic acid (70:30:1). The first solvent was run halfway upto the top of the plate, after which the plate was air-dried. The plate was then run in solvent mixture of n-hexene/ diethyl ether (59:1). The plate was dried and visualized by spraying with 10%$CuSO_4$ in 8%H_3PO_4 followed by baking in the oven above $150^{0}C$ for 15-20 minutes. The plates were scanned and quantified using Image J-software.

12) GST-tagged protein purification

The GST fusion proteins were expressed in bacteria (*E. coli,* BL21) and purified by glutathione-Sepharose (Cat No. 27-4574-01, Qiagen) as per manufacture instructions. The purified proteins were dialyzed against PBS. The samples were checked for quality by running on SDS-PAGE followed by Coomassie Brilliant Blue staining.

13) GGA3-GST based measurement of ARF1 activity on lipid droplets

ARF1-GTP binds with higher affinity to lipid membranes using its myristoyl group and interacts with its effector protein, GGA3 to control downstream signalling cascades (12). Thus the amount of GGA3 that binds to LDs reflects activity of ARF1 on LDs. Equal amount of NLDs and FLDs (by OD_{400}) were incubated with purified GGA3-GST protein for 2hr at $25^{O}C$ with constant mixing. At the end of incubation the LDs were subjected to sucrose density gradient as described earlier. GST western blotting was performed using these LDs samples to check for amount of GGA3-GST bound to LDs.

14) Pull down and Co-immunoprecipitation experiments

BRL3A cells were lysed in buffer containing 25mM Tris-HCl, pH 7.4, 150mM NaCl, 1.0% Triton X-100, 1mM PMSF, 0.1% BSA, 5mM EDTA and protease inhibitor cocktail. The lysate was incubated with recombinant ARF1-GST for 4hr followed by incubation with gluthathione beads for 2hr. The beads were washed with wash buffer (20mM HEPES, pH 7.4, 150mM NaCl, 0.1% Triton X-100, 10% Glycerol, 1mM PMSF and protease inhibitor cocktail) and lysed in 20µl of 2X SDS sample buffer. The samples were resolved on 8% SDS-PAGE and subjected to Western blot analysis as described above. For co-immunoprecipitation experiment, Hela cells were transfected with the GFP-KIF5b and Myc-ARF1-Q71L or FLAG-ARF1-T31N plasmids. The cells were then loaded with 200 µM BSA conjugated oleic acid. Cells were lysed at 4°C for 20 minutes in a buffer containing 25mM Tris-HCl, pH 7.4, 150mM NaCl, 1.0% Triton X-100, 1mM PMSF, 0.1% BSA, 5mM EDTA and protease inhibitor cocktail (Roche). Lysates were centrifuged at 10,000*g* for 10 minutes at 4°C and the supernatant was used for immunoprecipitation using 2µg of myc or FLAG conjugated agarose beads. Complexes were washed with wash buffer and lysed in 20µl of 2X SDS sample buffer. The samples were resolved on 8% SDS-PAGE and subjected to Western blot analysis.

15) Artificial Lipid Droplets (ALD) experiments for Recruitment of Arf-1 and Kinesin-1

ALDs were prepared by using a freeze-thaw cycle (13). Glyceryl trioleate and PC were taken in acid washed glass tubes. The lipids were dried in nitrogen gas. HKM buffer [50 mM HEPES (pH 7.4 with KOH), 120 mM Potassium acetate and 1mM $MgCl_2$] was added to the tubes and vortexed for 10 minutes. The whitish emulsions were poured into ultra-low temperature resistant tubes and frozen in liquid nitrogen. The frozen contents were immediately transferred to a water bath at 55°C. This freeze-thaw was repeated for 4-5 cycles with intermediate vortex mixing. Formation of ALDs could be seen by the contents turning increasingly white after every freeze-thaw cycle.

The resultant solution was incubated with freshly prepared rat liver lysates (<3 days old; stored at -80°C) to allow recruitment of proteins to the ALDs and other membranes. A step gradient was set up next to purify ALDs from unilamellar vesicles. The solution was supplemented with 2.5 molar MEPS buffer (1:1 v/v ratio) containing Roche protease Inhibitor, and added to the bottom of an SW32 tube. It was then overlaid with 5ml each of 1.2M, 1M, 0.5M and 0M MEPS buffer and centrifuged at 120000*g* for 1hour at 4°C. Buoyant triglyceride-containing ALDs (along with recruited proteins) formed a dense layer at the top of the centrifuge tube. ALDs were collected with an 18G needle and centrifuged (20,000*g*, 10mins, 4°C). MEPS buffer from the bottom was collected with 20G needle and discarded to obtain a concentrated ALD sample.

For PLD1 inhibitor assays, ALDs were incubated with rat liver lysate pre-treated either with 30 mM butanol-1 or 10μM PLD1/2 inhibitor (VU0155056) for 90 min at 37^0C. At the end of incubation, the mix was subjected to sucrose gradient centrifugation to separate the ALDs. ALD samples were collected and normalized using OD_{400} as described for NLDs and FLDs earlier. Equal volume of concentrated ALDs was used for protein precipitation with chloroform acetone (1:1 v/v ratio). Western blotting of the precipitated proteins was performed. Western blot quantification (densitometry) was performed using ImageJ software (NIH).

16) Cellular Fractionation

HHL-17 cells were infected with *KIF*-5b shRNA adenovirus or control shRNA adenovirus. 72 hrs post infection cells were used for subcellular fractionation as described earlier (11). Briefly, cells were harvested and washed with ice-cold PBS, resuspended in 3ml hypotonic buffer (10 mM Hepes [pH 7.4], 1 mM EDTA supplemented with protease inhibitor cocktail). Cells were incubated for 20 min at 4^OC. At the end of incubation, the cells were centrifuged at 1000 g for 10 min. The cell pellet was resuspended in isotonic buffer (250 mM sucrose, 10 mM Hepes [pH 7.4], 1 mM EDTA, protease inhibitor cocktail), followed by lysis in a cell cracker (Isobiotec; 18 micron clearance). The lysate was centrifuged at 1000*g* for 10 min to pellet cell debris. The resulting supernatant was centrifuged again at 12,000*g for* 15 min to pellet mitochondrion. The resulting supernatant was fractionated into LDs, soluble, and membrane fractions (enriched in microsomes) by using a two-layer gradient with following details:- **Bottom layer,** 1.5 ml: 250 mM sucrose, 10mM Hepes [pH 7.4], 1 mM EDTA, Roche complete protease inhibitor tablet. **Upper layer,** 3 ml: 50 mM sucrose, 10 mM Hepes [pH 7.4], 1 mM EDTA, protease inhibitor cocktail). Centrifugation was done at 100,000*g* for 6hr. Protein concentration was determined using BCA method (Sigma).

17) Kinesin-1 knockdown in Rat Liver and measurement of VLDL-triglyceride in Rat Serum

We used the in vivo JET PEI reagent for expressing an shRNA plasmid directed against the rat *KIF-5B* gene in rat liver. This technique for knockdown of proteins in the liver has been described (14). Intravenous injections and blood sample collection were carried out with the animals under anesthesia. Rat Kif5b shRNA plasmid (300μg) was diluted in 5% glucose and complexed with linear polyethylenimine (In vivo JET PEI) at an N/P ratio of six, following the manufacturer's protocol. The shRNA/PEI complex was injected into rats via the tail vein 72hrs before blood collection. A second dose of the complex was administered 24hrs after the first dose. At the end of 72 hrs, 0.75 ml Tyloxapol (Triton WR-1339; 10% v/v solution in 0.85% sodium chloride) was injected through the tail vein. This is the zero-hour time point in Fig 4D. Blood samples were collected at the time of tyloxapol injection (0 hr), followed by collection after every 1hr. After the final collection at 4hrs, the animal was sacrificed and liver samples were

collected for Western blot analysis. Serum was prepared from blood samples and used for triglyceride estimation either by TLC or by GPO method (Chemistry Analyzer C73, BeneSphera Brand).

18) Liver sectioning

Normally fed or 16 hr fasted SD rats (3-4 month old male) were anesthetized using sodium thiopentone. The liver was perfused with ice-cold PBS through the hepatic portal vein and dissected out. A small part of liver was taken out and kept in 4% PFA in 1X PBS for 24 hrs at 4^0C for fixation. The tissue was further kept in 30% sucrose and 4% PFA in 1X PBS overnight, or till the tissue sinks. The tissue was used to prepare 30μM section using a freezing microtome. The sections were either stained with bodipy (1:1000 in 1X PBS) or immunostained with perilipin-2 antibody and imaged at 63X NA 1.4 oil immersion objective using an Olympus confocal microscope (FV1000).

19) Microchamber immunofluorescence assay

We have used a microchamber immunofluorescence assay for immunostaining of isolated LDs. The method has been adapted from (15) with some modifications. First, microchambers were prepared using coverslip attached to glass slides using double stick tape. The LD samples (30ul) were infused into these microchambers. LDs were allowed to float and bind to the surface of coverslips by incubating the microchambers in humidified box for 20 minutes with the coverslip facing upwards. Blocking was done by passing 30ul of blocking solution of 6% BSA in 1X PBS through the microchamber for 30min. This was followed by 3 washes by passing 30μl of 1x PBS solution. 30ul of Perilipin 2 (undiluted) antibody was flown in the microchamber and incubated for 30 min followed by wash with 3x30μl 1XPBS solution. Next 30μl of secondary antibody (Alexa 488; 1:250 dilution) was flown and incubation is done for 30 minutes. Another wash with 3x30μl of 1XPBS is given to remove any nonspecific antibody binding. Imaging was done immediately to avoid bleaching of the signal. Imaging was done at 100X NA 1.4 oil immersion objective using an LSM 510 microscope (Carl Zeiss Microimaging, Jena).

20) Isolation/Culture of Primary mouse hepatocytes and Kinesin-1 knockdown

Isolation and culture of primary mouse hepatocyte was done as described (16, 17). Six to eight week-old male C57BL/6 mice were used. The hepatocytes were plated on collagen-coated (density 5 μg/cm^2) 35 or 60 mm plates at an initial density to attain a 75-80% confluent monolayer after overnight culture. Cells were allowed to attach for 4-5 h at 37°C in a humidified CO_2 incubator, washed once with 1X PBS before changing the culture media to DMEM supplemented with Pen/Strep and 10% FBS.

Mouse primary hepatocytes at 70-80% confluency on collagen coated 60mm dishes were used. The cells were transfected with 200nM siRNA (scrambled or *Kif5b* siRNA) using Lipofectamine 2000 transfection reagent according to manufacturer's instructions. The hepatocytes were then cultured for 72hrs in DMEM supplemented with 10% FBS. The experimental design for TAG storage/secretion was similar to a protocol described earlier (18). The cells were loaded with 0.4mM oleic acid (OA) conjugated with FA-free BSA (at a molar ratio of 6:1) in incomplete media for 6 hrs. At the end of incubation cells were washed with incomplete media with 0.5% fatty acid free BSA. During the chase period, the cells were incubated in incomplete media with 0.5% fatty acid free BSA and chased for 4 hrs. The cells were collected and used for TLC as previously described.

21) Sample preparation and targeted LC-MS triglyceride profiling

For measuring the cellular and secreted triglyceride, McA-RH7777 cells were transfected either with 200nM siRNA (scrambled or *Kif5B*) or Myc-Kif5b plasmid (for kinesin-1 overexpression) 72hrs post transfection (for kinesin-1 siRNA) or 48hrs post transfection (for kinesin-1 overexpression), cells were loaded with 0.4mM oleic acid (OA) conjugated with FA-free BSA (at a molar ratio of 6:1) in incomplete media for 6 hrs. At the end of incubation cells were washed with incomplete media with 0.5% fatty acid free BSA. During the chase period, the cells were incubated in incomplete media with 0.5% fatty acid free BSA and chased for 4hrs. The cells and media were then collected and used for LC-MS analysis.

C17:0/17:1/17:0 TAG (triglyceride internal standard, Avanti Polar Lipids Inc., Cat: LM-6000) was used as an internal MS quantitation standard. For measurement of cellular triglyceride, the cell pellets were washed with PBS (x 3 times), and transferred into a glass vial using 1mL PBS. 3mL of 2:1 (vol/vol) $CHCl_3$: MeOH with the triglyceride internal standard (100 pmol per sample) was added, and the mixture was vigorously vortexed. The two phases were separated by centrifugation at 2800 x *g* for 5 minutes. The organic phase (bottom) was collected, and dried under a stream of N_2 gas. The lipidome was re-solubilized in 500 µL of 2:1 (vol/vol) $CHCl_3$: MeOH, and 10 µL was used for the targeted LC-MS analysis. For measurement of the secreted triglyceride, 2mL of phenol red free media supplemented with BSA was collected from the cells, transferred to a glass vial, to which 6 mL of 2:1 (vol/vol) $CHCl_3$: MeOH with the triglyceride internal standard (100 pmol per sample) was added, and the mixture was vigorously vortexed. The two phases were separated by centrifugation at 2800 x *g* for 10 minutes, the organic phase (bottom) was collected and dried using a stream of N_2 gas. Thereafter the secreted lipidome was re-solubilized in 30 µL of 2:1 (vol/vol) $CHCl_3$: MeOH, and 20 µL were used for the targeted LC-MS analysis.

All the triglyceride species analyzed in this study were quantified using the multiple reaction monitoring (MRM) method on an AbSciex QTrap 4500 LC-MS with a Shimadzu Exion-LC series quaternary pump. All data was collected using the Acquisition mode of the Analyst software, and analyzed using the Quantitate mode of the same software. The LC separation was achieved using a Gemini 5U C-18 column (Phenomenex, 5 µm, 50 x 4.6 mm) coupled to a Gemini guard column (Phenomenex, 4 x 3 mm, Phenomenex security cartridge). The LC solvents were: buffer A: 95:5 (vol/vol) H_2O: MeOH + 0.1% formic acid + 10 mM ammonium formate, and buffer B: 60:35:5 (vol/vol) iPrOH: MeOH: H_2O + 0.1% formic acid + 10 mM ammonium formate. All the MS based TAG estimations was performed using an electrospray ion source in the positive ion mode using the following MS parameters: ion source = turbo spray, collision gas = medium, curtain gas = 20 L/min, ion spray voltage = 4500 V, temperature = 400^{o}C. The MRM transition method for the triglyceride standard yielded the following voltages: declustering potential = 150 V, entrance potential = 10 V, collision energy = 35 V, collision cell exit potential = 10 V. A typical run consisted of 55 minutes, with the following solvent run sequence post injection: 0.3 ml/min 0% buffer B for 5 minutes, 0.5 ml/min 0% buffer B for 5 minutes, 0.5 ml/min linear gradient of buffer B from 0 – 100% over 25 minutes, 0.5 ml/min of 100% buffer B for 10 minutes, and re-equilibration with 0.5 ml/min of 0% buffer B for 10 minutes. A detailed list of all the species targeted in this MRM study, describing the precursor parent ion mass and adduct, the product ion targeted can be found in **Supplementary Table S1**. All the endogenous triglyceride species were quantified by measuring the area under the curve in comparison to the triglyceride internal standard, and then normalizing to the total protein content. All the data is represented as mean ± s. e. m. of 6 biological replicates (**Supplementary Table S2**).

22) Measurement of ApoB levels in Cell culture and in Animals

ApoB in Cell culture For measuring the cellular and secreted ApoB levels, McA-RH7777 cells were transfected with 200nM siRNA (scrambled or Kif5B). 72h post transfection, cells were loaded with 400µM oleic acid (OA) conjugated with FA-free BSA (at a molar ratio of 6:1) in incomplete media for 6 hrs. At the end of incubation cells were washed with incomplete media with 0.5% fatty acid free BSA. During the chase period, the cells were incubated in incomplete media with 0.5% fatty acid free BSA and chased for 4 h. The cells and media were collected and used for ApoB westerns. For detecting ApoB levels in media, ApoB immunoprecipitaion was done. Briefly, the media was centrifuged to remove any cell debris and then 10X IP buffer (250mM Tris-HCl, pH 7.4, 1.5M NaCl, 10% Triton X-100, 10mM PMSF, 50mM EDTA and protease inhibitor cocktail) was added to make final concentration 1X. The media was then incubated with 15 µl of ApoB antibody overnight. At the end of incubation, 50µl of protein A/G plus agarose beads (Thermo) were added for another 4hrs at 4°C. After three washes in wash buffer (20 mM Hepes, pH 7.4, 0.1% Triton X-100, 150 mM NaCl, 10% (v/v) glycerol, 1 mM PMSF and protease inhibitors), the beads containing immune complexes were boiled in sample buffer. The samples were resolved on 4-15% SDS-PAGE and subjected to western blot analysis.

ApoB in Animals Normally fed and 16hrs fasted rats were injected with Tyloxapol as described earlier. Liver and blood samples were collected after 4 hrs. Serum was prepared from blood samples and used for ApoB100 western and triglyceride estimation using TLC. Liver sample was also used for ApoB100 western.

23) Sucrose gradient separation of apoB-containing lipoproteins from serum Separation of serum apoB-containing lipoproteins was performed as described (19). Rats were injected with kinesin shRNA as described before (section 17). 72 hrs post infection, rats were injected with Tyloxapol. 4 hrs after Tyloxapol injection, blood was collected and serum was prepared. Serum was adjusted to a sucrose concentration of 12.5% (w/v). The adjusted serum was placed on the top of a step gradient consisting of 1.9 ml of 49% sucrose and 1.9 ml of 20% sucrose. Next, 2.8 ml of phosphate-buffered saline (PBS) was layered on the top of the supernatants. All solutions contained protease inhibitors. After centrifugation at 35,000 rpm for 65hours at 10°C in a Beckman SW41 rotor, 10 fractions (1 ml each) with different densities were collected from the top of the tube. ApoB was immunoprecipitated from each fraction. The apoB distribution patterns in control and kinesin knock down condition were determined by immunoblotting, and the intensities of the bands were determined semiquantitatively using Image J software.

24) Preparation of ER-enriched Microsomes from Rat liver
Microsomes were isolated from liver following a published protocol (20). Liver tissue was chopped into fine pieces and homogenised in homogenization buffer (20mM Tris-HCL, pH- 7.4, 250 mM sucrose and 1 mM EDTA) in a Dounce homogenizer by applying 20 strokes. The liver homogenate was then centrifuged at 500*xg* for 15 minutes to remove cell debris. The supernatant was collected and centrifuged again at 15000*xg* for 10 minutes to pellet down mitochondria. This step was repeated three times. The supernatant was then centrifuged at 106,000*xg* for 1 hour at 4^{o}C. The resulting supernatant was removed and microsomes were collected as a pellet. The microsome pellet was resuspended in 1 ml of buffer containing 10mM Tris-HCl, pH 7.4 and 1 mM EDTA, aliquoted and stored at -80^{o}C.

25) Substrate hydrolysis assays for measurement of Microsomal Lipase activity in Liver
Microsomal preps were performed as described earlier and an LC-MS based triglyceride substrate hydrolysis assay (14) was used to measure lipase activity. The microsomal preparations were lysed by bead beating using a Bullet Blender (Next Advance, USA) as per manufacturer's instructions. Centrifugation at 100,000 *g* for 1 hour separated the soluble and membrane associated proteomes. The soluble fraction was discarded, and the membrane pellet was washed 3 times using 1 mL PBS, following which the membrane pellet was re-suspended in 0.5 mL PBS by sonication to generate the membrane proteome. 10 µg of membrane proteome obtained from microsomal preparations was incubated with 100 µM triglyceride (glyceryl trioleate, Sigma-Aldrich Cat: T7140) in a reaction volume of 100 µL in PBS at 37^{o}C with constant shaking. After 45 minutes the reaction was quenched with 350 µL of 2:1 (vol/vol) $CHCl_3$: MeOH, doped with 250 pmol internal standard, cis-10-heptadecenoic acid (C17:1 FFA). The mixture was vortexed, and centrifuged at 2800 x *g* for 5 minutes to separate the aqueous (top) and organic (bottom) phase. The organic phase was collected and dried under a stream of N_2 gas, re-solubilized in 100 µL of 2:1 (vol/vol) $CHCl_3$: MeOH, and subjected to LC-MS analysis. A fraction of the organic extract (~ 20 µL) was injected onto an AbSciex QTrap 4500 LC-MS with a Shimadzu Exion-LC series quaternary pump. LC separation was achieved using a Gemini 5U C-18 column (Phenomenex, 5 µm, 50 x 4.6 mm) coupled to a Gemini guard column (Phenomenex, 4 x 3 mm, Phenomenex security cartridge). The LC solvents were: buffer A: 95:5 (vol/vol) H_2O: MeOH + 0.1% ammonium hydroxide, and buffer B: 60:35:5 (vol/vol) iPrOH: MeOH: H_2O + 0.1% ammonium hydroxide. A typical LC run consisted of 15 minutes post-injection: 0.1 mL/min 100% buffer A from for 1.5 minutes, 0.5 mL/min linear gradient to 100% buffer B over 5 minutes, 0.5 mL/min 100% buffer B for 5.5 minutes, and equilibration with 0.5 mL/min 100% buffer A for 3 minutes. All MS analysis was performed using an electrospray ionization source in a MS1 scan negative ion mode for product formation (free fatty acid from glyceryl trioleate). All MS parameters were same as described in the MS-based TAG profiling method, except that ion spray voltage was -4500V. Measuring the area under the peak, and normalizing it to the internal standard quantified the product release for the lipid substrate hydrolysis assays. The substrate hydrolysis rate was corrected by subtracting the non-enzymatic rate of hydrolysis, which was obtained by using heat-denatured proteome (15

minutes at 95^{o}C, followed by cooling at 4^{o}C for 10 mins x 3 times) as a control. All data is represented as mean ± s. e. m. of 3 biological replicates.

26) Effect of Kinesin-1 knockdown on hepatitis-C virus (HCV) replication and secretion

Cell lines used

Huh7.5 cells (generous gift from C.M. Rice, Rockefeller Institute) were used. Cells were maintained in DMEM+10%FBS.

Constructs used

Full length HCV-JFH-1 containing plasmid construct was used to synthesize full length HCV RNA. (generous gift from Takaji Wakita, Tokyo Metropolitan Institute for Neuroscience, Japan).

In vitro transcription

Plasmid containing full length HCV-JFH-1 cDNA was digested with XbaI. Linearized DNA was used for in vitro transcription of full length HCV RNA in a run off transcription reaction with T7 polymerase (Fermentas). The reaction was carried out in standard conditions (Fermentas protocol) with 3µg of linearized DNA per reaction. The reaction was incubated for 4h at 37°C followed by DNase I treatment for 1h. Samples were treated with phenol:chloroform and then precipitated using absolute ethanol. The RNA pellet was dissolved in 20µl of nuclease free water.

Transfection

Lipofectamine 2000 was used for RNA tranfections (Invitrogen).

qRT-PCR

Total RNA was isolated using Tri-Reagent from Sigma. RNA isolated from both the cells and cell culture supernatant were used to prepare cDNA using M-MuLV-Reverse transcriptase enzyme (Thermofisher) using standard protocols. cDNAs were further used for quantitative PCR. For this, DyNAmo Flash SYBR Green qPCR kit was used (Thermofisher). Both intracellular and extracellular HCV RNA copies were determined using the specific primers mentioned below. The relative amount was plotted after normalization with the levels of internal control, GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) RNA. This procedure has been described earlier (21).

Primers

HCV forward: 5’ TGCGGAACCGGTGAGTACA 3’
HCV reverse: 5’ CTTAAGGTTTAGGATTCGTGCTCAT 3’
GAPDH forward: 5’ CATGAGAAGTATGACAACAGCCT 3’
GAPDH reverse: 5’ AGTCCTTCCACGATACCAAAGT 3’

27) Statistical Methods

The relevant statistical details have been mentioned within Figures and Figure captions. Student’s t-test (two-tailed or one-tailed, depending on the comparison) was used to calculate statistical significance. *P*-values are reported at 95% confidence. A Kolmogorov-Smirnov test was done to ascertain statistical significance in Fig 1E because the underlying distribution of run-lengths was not normal. One-way ANOVA was used to ascertain differences in the multiple comparison of Fig 1F. Details are reported in the Figure caption.

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