Editorial comments:

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.

We have retained the same format to match the journal style as requested.

2. Please address all specific comments marked in the manuscript.

We have addressed all editorial specific comments that marked in the manuscript.

3. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. Please remove the term Click-iT assay throughout the manuscript and refer it in the table of materials instead.

We have removed the term “Click-iT assay” from the whole manuscript and changed to “**Chemo-selective ligation reaction”** in both manuscript and table of materials.

4. Please remove the materials section from the protocol and add it to the table of materials.

We have transferred the materials section from the protocol and added it to the list of materials. Also, all buffer and medium preparations have been removed from the protocol section and added them in a separate Excel sheet as a **Table 1.**

5. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

Based on the JoVE Instructions, we have changed and adjusted the numbering throughout the protocol section.

6. Please ensure you answer the “how” question, i.e., how is the step performed? Also please use complete crisp sentences throughout the protocol section.

We have edited the protocol section in order to provide clear and complete sentences.

7. Please ensure that the highlight is no more than 2.75 pages including heading and spacing and forms the most cohesive story in the manuscript. Also aligns with the title.

We have highlighted in yellow the headings and steps that form the cohesive story in the manuscript.

8. Please provide a result to show that the cells obtained are hepatocytes and nothing else.

We have conducted western blotting analysis to demonstrate the ability of isolated primary mouse hepatocytes to express albumin protein as seen in liver lysate samples, but not in MEF and Hepa 1-6 cells (Figure 3).

9. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

We have made and provided an original schematic illustration (Figure 1), and have not used any figures from previous publications.

10. Please proofread the manuscript carefully before submitting.

We have proofread throughout the manuscript carefully several times before re-submitting.

1) Isolation of primary mouse hepatocytes

1.1 Pre-isolation preparations:

1.1.1 Prepare 450 ml of 40% density gradient buffer as described in **Table 1** and keep at 4 ^{o}C (15 ml/mouse).
1.1.2 Prepare 500 ml of Williams' Medium E as described in **Table 1** and keep at 4 ^{o}C
1.1.3 Prepare 500 ml of DMEM as described in **Table 1** and keep on ice (30 ml/mouse)
1.1.4 Prepare 500 ml of HBSS (-) Buffer as described in **Table 1** and keep at 42 ^{o}C in water bath (40 ml/mouse)
1.1.5 Prepare 500 ml of HBSS (+) Buffer with 0.3 mg/ml Collagenase Type X as described **in Table 1** with 30 min incubation at 42 ^{o}C in the water bath (40 ml/mouse)

2. Perfusion:

1.2.11 Spray 70% ethanol over the abdominal area of the mouse and then open the abdominal cavity by making a large transverse incision of the dermis and peritoneum by using sharp scissors and toothed forceps
1.2.12 Then, make a vertical cut of the abdominal layers until all abdominal organs are completely exposed by using sharp scissors and toothed forceps
1.2.13 Move small and large intestines toward the left side of the mouse with autoclaved cotton tip until liver, portal vein, and inferior vena cava (IVC) are clearly exposed
1.2.14 Insert a 24 G catheter into IVC just at the bifurcation with right renal vein carefully without shaking hands to avoid the injury of IVC and bleeding
1.2.15 Then remove the needle of the catheter and control the position of the cannula so that it should not get out of IVC
1.2.16 Connect 24 G cannula with perfusion tube tightly by using the connector
1.2.17 Start the perfusion of the liver with warm HBSS (-) at a flow rate of 4 ml/min
1.2.18 Then, cut off the splenic vein quickly to drain out the internal blood by using scissors
1.2.19 Continue perfusion with 35 ml of warm HBSS (-)

3. Digestion and Extraction

1.3.1 Perfuse the mouse liver with warm 35 ml of HBSS (+) including Collagenase Type X at a flow rate of 4 ml/min
1.3.3 Pour 5 ml of prepared warm HBSS (+) with Collagenase Type X into 100 mm Petri dish at the end of perfusion process
1.3.4 Cut off the perfused liver from the rest of the body before stopping the pump to avoid the backflow of blood into the liver
1.3.5 Remove the gallbladder by forceps, and then wipe the liver gently with paper towel to remove the blood
1.3.6 Transfer the liver to the Petri dish which contains 5 ml of prepared warm HBSS (+)
1.3.7 Remove the liver capsule gently using straight-tipped forceps
1.3.8 Disperse the parenchymal tissue carefully by using the forceps
1.3.9 Add 15 ml cold DMEM to the Petri dish
1.3.10 Shake the torn liver gently to release the residual parenchymal cells into the medium
1.3.11 Add another 15 ml cold DMEM to the Petri dish to get the rest of the cells

1.3.12 Transfer DMEM with the dissolved liver through 100 µm cell strainer into a 50 ml conical tube and keep in ice

4. Purification:

1.4.1 Centrifuge cell suspension at 60 *x g* for 2 min at 4 °C
1.4.2 Carefully remove the supernatant by vacuum aspiration
1.4.3 Resuspend the pellet in 10 ml DMEM
1.4.4 Add resuspended pellet as a thin layer on the top of 40% density gradient buffer
1.4.5 Centrifuge at 800 *x g* for 10 min without brake at 4 °C
1.4.6 Carefully remove supernatant including the upper layer (DMEM) and middle layer (dead or non-parenchymal cells), but not lower layer (Pellet: primary parenchymal hepatocytes)
1.4.7 Resuspend the primary cells with 2-3 ml William's medium E
1.4.8 Transfer the pellet with William's medium E to the new 50 ml tube to avoid contamination of dead cells on the wall of tube
1.4.9 Add 7-8 ml William's medium E, and mix gently by hand
1.4.10 Centrifuge at 800 *x g* for 1 min at 4 °C
1.4.11 Carefully remove supernatant by vacuum aspiration, and then resuspend pellet again with 10, 20 or 30 ml of William's medium E (according to the size of the pellet)
1.4.13 Seed 0.6 X 10^6 cells in a 6 well plate with William's medium E
1.4.14 Keep the plate in incubator at 37 °C for 2-3 h
1.4.15 Replace the medium with10% FBS DMEM medium with Anti-Anti to remove dead or unattached cells and for overnight incubation at 37 °C

2) Chemo-selective ligation reaction assay for detection of nascent protein synthesis

1. Metabolic labeling:

2.1.1 (Pre-treatment) Wash primary hepatocytes with 37 °C warm PBS twice, and add the methionine-free DMEM medium as described in **Table 1** for 30 min to deplete cytoplasmic methionine reserves
2.1.2 (Treatment) Wash primary hepatocytes with 37 °C warm PBS twice, and add the methionine-free DMEM medium with 25 µM AHA (L-Azidohomoalanine) for 4-6 h
2.1.3 Add any specific chemical (*i.e.* 10 µM rotenone) in the medium for 4-6 h in presence of AHA in order to investigate its effect on nascent protein expression levels
2.1.4 After incubation, remove the medium by vacuum aspiration, and wash primary hepatocytes with warm PBS three times
2.1.5 Add 200 µl lysis buffer to the plate and incubate for 15-30 min on ice.
2.1.6 Tilt the plate and take the whole cell lysate into 1.7 ml tube
2.1.7 Sonicate the cell lysate on ice for 5 sec three times by using a probe sonicator to solubilize the proteins and disperse the DNA
2.1.8 Vortex the cell lysate for 5 min
2.1.9 Centrifuge the cell lysate at 21130 *x g* for 10 min at 4 °C
2.1.10 Transfer the clear supernatant into a new tube and measure the protein concentration twice

2. Labeling the Azide-modified nascent protein:

2.2.3 Take 20-40 µg cell lysate and add 50 µl 2 X reaction buffer (component A+B), then adjust the volume to 80 µl by adding DDW and vortex for 5 sec

2.2.4 Add 5 µl $CuSO_4$ (component C), and vortex for 5 sec

2.2.5 Add 5 µl reaction buffer additive 1 (freshly prepared component D), then vortex for 5 sec and wait for 3 min

2.2.6 Add 10 µl reconstituted reaction buffer additive 2 (component E), then vortex for 5 sec

2.2.7 Rotate samples for 20 min at 4 ºC by using a multi-rotator machine

2.2.8 Add 300 µl methanol and vortex for 5 sec

2.2.9 Add 75 µl chloroform and vortex for 5 sec

2.2.10 Add 200 µl DDW and vortex for 5 sec

2.2.11 Centrifuge the samples at 21130 *x g* and 4 ºC for 5 min

2.2.12 Discard the aqueous supernatant and keep the pellet

2.2.13 Add 250 µl methanol to the tube, vortex and spin again for 5 min at 21130 *x g*

2.2.14 Discard the supernatant

2.2.15 Cover the samples with lint-free tissue and allow the pellets to air-dry for 15 min at room temperature

3. PAGE analysis and western blotting:

2.3.1 Solubilize the pellet in 20 µl of loading buffer **without** β-mercaptoethanol

2.3.2 Vortex for 10 min, and heat at 70 ºC by using heat block for 10 min

2.3.3 Load the samples into 10% SDS gel (1.5 mm) for Tetramethylrhodamine (TAMRA) detection

4. Detection:

2.4.1 Detect AHA signal using a variable mode laser scanner for precise quantitation of nascent proteins