## Supplemental Figures and Tables

Figure S1

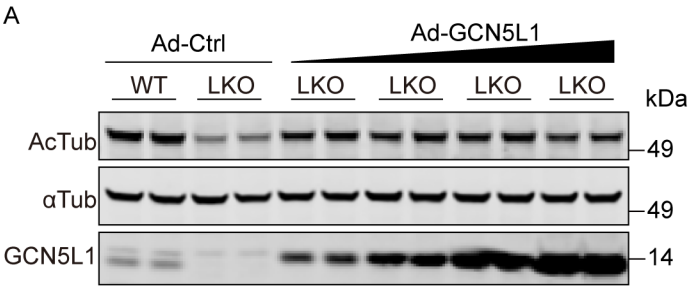


**Figure S1. Uniform α-tubulin acetylation in response to incremental doses of GCN5L1 rescue.** (A) WT and LKO hepatocytes were infected with control adenovirus (Ad-Ctrl) or with adenovirus coding for wildtype GCN5L1 (Ad-GCN5L1) and the Ac-Tub levels were analyzed by immunoblot.

Figure S2

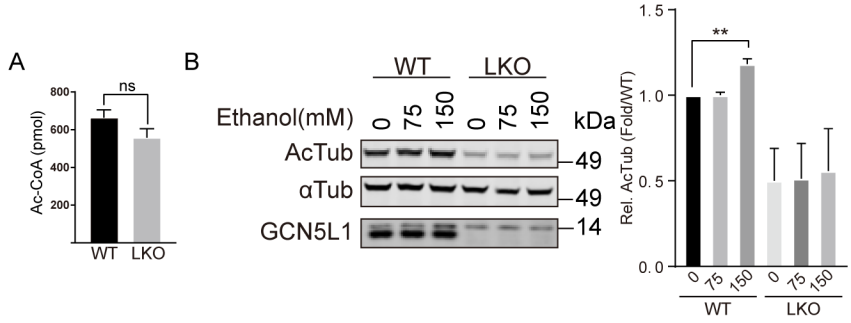


**Figure S2. Deletion of GCN5L1 did not impair cytosolic the acetyl-CoA.** (A) Ac-CoA content in WT or LKO hepatocytes were quantified by LC-MS as described in Material and Methods. (B) The supplementation of media with ethanol increased Ac-Tub levels in WT and LKO hepatocytes and levels of acetylation of α-tubulin (αtub) was assessed using immunoblot analysis and quantitation. The relative quantitation of representative immunoblot images are shown using three

independent experiments. (*p<0.05, *** p<0.001; unpaired students t-test was used to evaluate the statistical significance of the data).

Figure S3

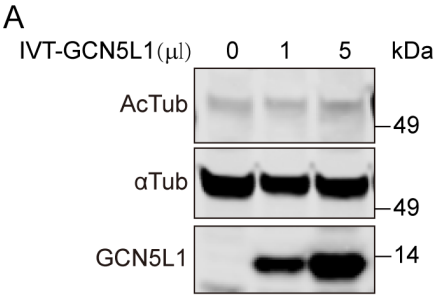


**Figure S3. GCN5L1 did not show α-Tubulin acetyltransferase activity *in vitro*.** (A) *In vitro* translated GCN5L1 (IVT-GCN5L1) were incubated with cytosolic α-tubulin in acetylation buffer for 1 hour at 30 ºC, then the reactants were analyzed by immunoblotting with antibodies against Ac-Tub, GCN5L1 and α-Tubulin.

Figure S4

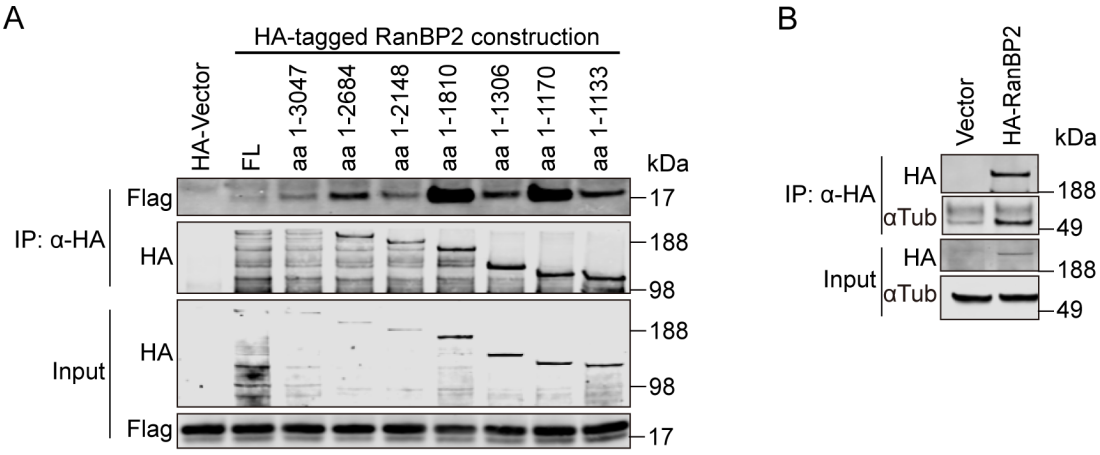


**Figure S4. Assay of GCN5L1-RanBP2 and RanBp2-a-tubulin protein interactions. (**A) The N-terminal region of RanBP2 was required for the association with GCN5L1. Different HA-tagged C-terminal deletion mutants of RanBP2 were transiently expressed in 293T cells with Flag-GCN5L1 and the cells lysates were subjected to immunoprecipitate using anti-HA antibody. The immunoprecipitates were probed with anti-Flag antibody to identify the region of RanBP2 that is required for interaction with GCN5L1. (B) RanBP2 co-immunoprecipitated with α-Tubulin. Control vector or HA-RanBP2 (1-1133 aa) transfected 293T cells were immunoprecipitated with anti-HA antibody. The immunoprecipitates were subjected to anti-HA and anti- α-Tubulin immunoblot analysis.

Figure S5

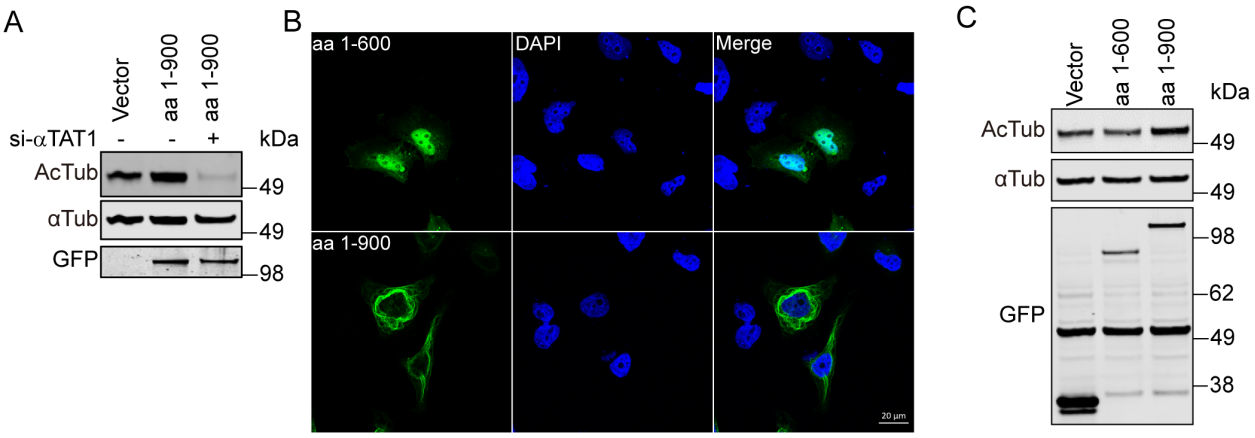


**Figure S5. N-terminal of RanBP2 was essential for its tubulin binding and acetylation regulation. (**A) The overexpression of the N-terminal of RanBP2 increased a-tubulin acetylation and this was abolished by the concurrent knockdown αTAT1. GFP-tagged N-terminal RanBp2 (aa 1-900) transfected HeLa cells were mock-transfected or transfected with αTAT1 siRNA (si-αTAT1), then the Ac-Tub levels were analyzed by immunostaing. The control vector (Vector) transfected cells were used as transfection control. α-Tubulin was used as loading control. (B) The aa 1-900 N-terminal region (aa 1-900) of RanBP2 is essential for its microtubule binding. Confocal microscopy of HeLa cells transfected with GFP-RanBP2 (aa 1-600) or GFP-RanBP2 (aa 1-900) showed that only the RanBP2 (aa 1-900) were recruited to microtubule. GFP fluorescence was visualized directly. DNA was visualized by DAPI staining (blue). Scale bar, 20 µm. (C) In parallel, immunoblot analysis shows that only the aa 1-900 and not the aa 1 – 600 region of RanBP2 promotes Ac-Tub. GFP-vector, GFP-RanBP2 (aa 1-600) or GFP-RanBP2 (aa 1-900) transfected HeLa cells were analyzed by immunoblot with antibodies against Ac-Tub, GFP and α-Tubulin. Representative immunoblots of three independent experiments.

Figure S6

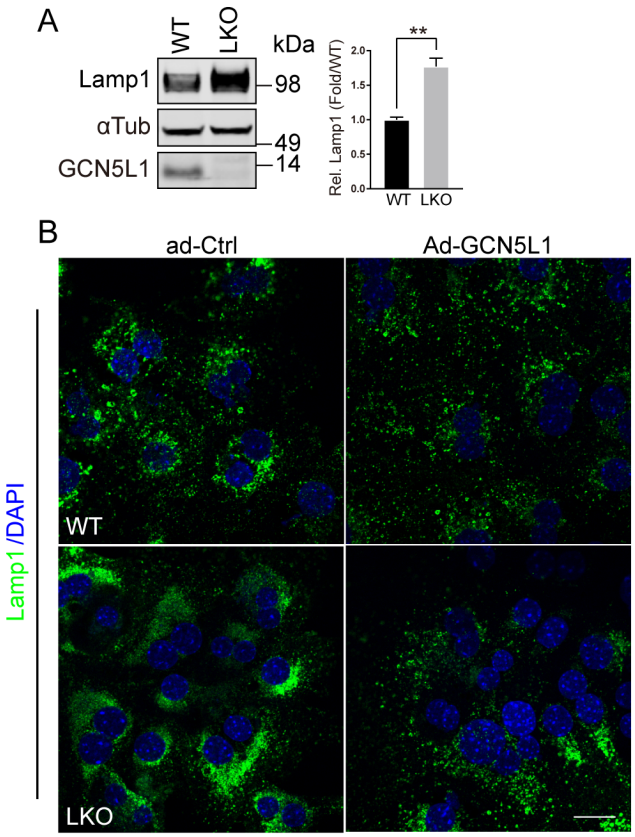


**Figure S6. GCN5L1 LKO associates with increased lysosome accumulation and GCN5L1 directs lysosomal positioning.** (A) Increase Lamp1 levels were detected by immunoblot analysis from LKO hepatocytes lysates compared to WT hepatocytes. α-Tubulin was used as loading control. The accompanying histogram represented the relative quantified ratio of Lamp1 to αTub normalized to the WT samples from three replicates. (B) Overexpression of GCN5L1 redistributed the lysosomes to the periphery in LKO hepatocytes. WT and LKO hepatocytes were infected with control adenovirus (Ad-Ctrl) or with adenovirus coding for wildtype GCN5L1 (Ad-GCN5L1) and the lysosomes distribution were visualized by immunostaining of Lamp1 (green). DNA was visualized by DAPI staining (blue). Scale bar, 10 µm. The confocal images are a representative image of three independent experiments. The relative quantitation of representative immunoblot images are shown using three independent experiments. (*p<0.05, *** p<0.001; unpaired students t-test was used to evaluate the statistical significance of the data).

# SUPPLEMENTAL TABLES:

**Supplementary Table 1.**
**Proteins detected by GCN5L1-BioID with high confidence (PSM>0.3).**

| Gene | PSM | p value |
|---|---|---|
| AHNAK | 4.0483 | 0.00074 |
| ERC1 | 3.61209 | 0.00047 |
| RAI14 | 3.55087 | 0.00219 |
| PTPN13 | 3.54868 | 0.00036 |
| TPR | 3.46795 | 7.5E-05 |
| TJP1 | 3.26973 | 0.00011 |
| AP2B1 | 2.88514 | 0.00058 |
| EPB41 | 2.88193 | 0.00097 |
| CSDE1 | 2.71322 | 6.6E-05 |
| EPB41L3 | 2.53716 | 0.00435 |
| AP2A1 | 2.47458 | 0.00098 |
| GTF3C1 | 2.33556 | 0.03464 |
| CAMSAP1 | 2.32755 | 0.05335 |
| XRN1 | 2.32387 | 0.03284 |
| PRPF8 | 2.29564 | 0.02711 |
| ZC3HAV1 | 2.22092 | 0.0496 |
| AP2A2 | 2.09248 | 0.00882 |
| UBAP2L | 2.01962 | 0.03632 |
| WDR6 | 1.9748 | 0.02672 |
| APC | 1.94771 | 0.01563 |
| MACF1 | 1.84952 | 0.03227 |
| HAUS5 | 1.81843 | 0.03019 |
| TP53BP2 | 1.80924 | 0.00086 |
| NAP1L1 | 1.77498 | 0.00394 |
| PPFIBP1 | 1.72264 | 3.4E-05 |
| MPRIP | 1.69073 | 0.01009 |
| CORO1B | 1.65314 | 0.00173 |
| HAUS3 | 1.63772 | 0.054 |
| EXOC1 | 1.63083 | 0.00742 |
| CCT8 | 1.59647 | 0.03466 |
| PCM1 | 1.58176 | 0.00029 |
| COPG2 | 1.55687 | 0.02304 |
| HAUS7 | 1.49368 | 0.0151 |
| SNRNP20( | 1.49087 | 0.03573 |
| EPS15L1 | 1.46274 | 0.0026 |
| PRPF19 | 1.46013 | 0.00199 |
| KIF11 | 1.45116 | 0.00114 |
| TNRC6B | 1.42092 | 0.03251 |
| EDC4 | 1.40928 | 0.01482 |
| DHX9 | 1.40485 | 0.05343 |
| MAP1B | 1.38818 | 0.00582 |
| EXOC2 | 1.3408 | 0.00583 |
| MAPRE2 | 1.33215 | 0.00416 |
| CNOT1 | 1.30654 | 0.01286 |
| PABPC1 | 1.29362 | 0.02568 |
| PRRC2C | 1.28858 | 0.05119 |
| AP2M1 | 1.2755 | 0.00606 |
| CD2AP | 1.26102 | 0.02161 |

**Supplementary Table 2.**
**The most enriched proteins detected by GCN5L1-BioID (PSM>1).**

| Gene | PSM |
|---|---|
| UTRN | 5.70679 |
| AHNAK | 4.0483 |
| MLLT4 | 3.79349 |
| ERC1 | 3.61209 |
| RAI14 | 3.55087 |
| PTPN13 | 3.54868 |
| TPR | 3.46795 |
| DST | 3.38584 |
| TJP1 | 3.26973 |
| CKAP5 | 3.26636 |
| AP2B1 | 2.88514 |
| EPB41 | 2.88193 |
| CSDE1 | 2.71322 |
| KIDINS22 | 2.71051 |
| EPB41L3 | 2.53716 |
| AP2A1 | 2.47458 |
| VPS50 | 2.45734 |
| CEP170 | 2.35419 |
| GTF3C1 | 2.33556 |
| CAMSAP1 | 2.32755 |
| XRN1 | 2.32387 |
| MYH9 | 2.32239 |
| PRPF8 | 2.29564 |
| ZC3HAV1 | 2.22092 |
| AP2A2 | 2.09248 |
| GOLGB1 | 2.05372 |
| UBAP2L | 2.01962 |
| WDR6 | 1.9748 |
| ANKHD1 | 1.94786 |
| APC | 1.94771 |
| EPB41L2 | 1.9084 |
| MACF1 | 1.84952 |
| ANKRD17 | 1.82509 |
| BIRC6 | 1.82139 |
| HAUS5 | 1.81843 |
| TP53BP2 | 1.80924 |
| KLC1 | 1.80402 |
| EXOC4 | 1.79825 |
| CCT3 | 1.79019 |
| NAP1L1 | 1.77498 |
| COPA | 1.72734 |
| FOCAD | 1.72605 |
| PPFIBP1 | 1.72264 |
| MTA2 | 1.71778 |
| MPRIP | 1.69073 |
| CORO1B | 1.65314 |
| GIGYF2 | 1.63984 |
| HAUS3 | 1.63772 |

Supplementary Table 3

| Recombinant DNA Constructs | | |
|---|---|---|
| **Vector** | **Insert** | **Notes** |
| C-Terminal-p3Xflag-CMV | GCN5L1 | This work. |
| pT7CFE1 | IVT-GCN5L1 | |
| pcDNA 3.1 | GCN5L1-BirA | GCN5L1-BirA was a gift from Dr. Iain Scott. |
| EF-HA plink | RanBP2 | Wälde, S., et al. (2012). |
| EF-HA plink | RanBP2(aa 1-1133) | |
| EF-HA plink | RanBP2(aa 806-1133) | |
| EF-HA plink | RanBP2(aa 806-1170) | |
| EF-HA plink | RanBP2(aa 806-1306) | |
| EF-HA plink | RanBP2(aa 1312-2557) | |
| EF-HA plink | RanBP2(aa 1350-2148) | |
| EF-HA plink | RanBP2(aa 2011-2445) | |
| EF-HA plink | RanBP2(aa 2307-2710) | |
| EF-HA plink | RanBP2(aa 1-2684) | |
| EF-HA plink | RanBP2(aa 1-2148) | |
| EF-HA plink | RanBP2(aa 1-1810) | |
| EF-HA plink | RanBP2(aa 1-1306) | |
| EF-HA plink | RanBP2(aa 1-1170) | |
| pEF5B-FRT-LAP-DEST | GFP-αTAT1 | pEF5B-FRT-GFP-αTAT1 was a gift from Maxence Nachury (Addgene plasmid # 27099) |
| pCMV6-Entry | Myc-Flag-αTAT1 | Origen (Cat:MR206707) |
| pEGFP-C2 | GFP-RanBP2(1-900) | Joseph, J. and M. Dasso (2008). |
| pEGFP-C2 | GFP-RanBP2(1-600) | |

| Antibodies | | | |
|---|---|---|---|
| **Antibody** | **Cat. number** | **Working dilution** | **Source** |
| Rabbit anti- Acetyl-α-Tubulin | #5335 | 1:1000(IB)<br>1:800(IF) | Cell signaling technology |
| Mouse anti- Acetyl-α-Tubulin | T7451 | 1:1000(IB)<br>1:800(IF) | Sigma |
| Mouse anti- α Tubulin | sc-8035 | 1:1000(IB) | Santa Cruz Biotechnology |
| Rabbit anti-GCN5L1 | Homemade | 1:1000(IB) | Scott, I., et al. (2012). |
| IRDye® 800CW Streptavidin | P/N 926-32230 | 1:10000(IB) | LI-COR |

| | | | |
|---|---|---|---|
| Mouse anti-flag | F3165 | 2 ug/sample (IP)<br>1:1000(IB)<br>1:400(IF) | Sigma |
| Mouse anti-HA | 11583816001 | 2 ug/sample (IP)<br>1:1000(IB) | Roche |
| Rabbit anti-HA | sc-7392 | 2 ug/sample (IP)<br>1:1000(IB) | Santa Cruz Biotechnology |
| Rabbit anti-GFP | sc-8334 | 1:1000(IB) | Santa Cruz Biotechnology |
| Mouse anti-Myc | M4439 | 2 ug/sample (IP)<br>1:1000(IB)<br>1:400(IF) | Sigma |
| Rabbit anti-LAMP1 | ab24170 | 1:1000(IB)<br>1:800(IF) | Abcam |
| GFP-Trap-MA | gtma-10 | 25 ul/sample (IP) | Chromotek |