### Supplementary Table 1. Quantitative PCR primer sequences

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Supplementary Figure 1

Table a shows the protein expression levels of mTOR, Raptor, and Rictor in DMSO and DSS treated young and adult mice. The signal intensity (a.u.) for mTOR, Raptor, and Rictor is indicated in the bar chart (b). The protein expression levels of mTOR, Raptor, Rictor, and α-tubulin are shown in (c). In (d), IgG and IP: anti-mTOR are used to detect the expression levels of mTOR, Raptor, GβL, mTOR, Raptor, GβL, and β-actin.
Supplementary Figure 1. mTORC1-independent Raptor is reduced in insulin-resistant liver

(a-c) Western blots from livers of (a) young (8-week-old) or adult (24-week-old) male mice, showing the whole blots corresponding to Figure 1c, and (b) normal chow diet (NCD) or HFD-fed male mice, crosslinked with DSS, and (c) quantitation of signal as a percentage of control (DMSO-treated liver lysate). (d,e) Western blots from livers of lean or ob/ob mice (8-week-old) following immunoprecipitation (IP) with anti-mTOR, (d) with or without prior crosslinking with DSP, (e) or in the presence of CHAPS to sustain mTOR-Raptor interaction. (f) Western blot from primary hepatocytes deprived of amino acids (-AA) or treated with rapamycin (Rapa) for 1 h prior to IP with anti-mTOR. (g) Western blot from Hepa1c1c7 cells, treated with varying concentrations of insulin for 24 hours, prior to DSS crosslinking. Statistical analysis were performed using two-way ANOVA. All data are shown as the means ± s.e.m. Blots are representative of three independent experiments, and samples within groups chosen randomly.
Supplementary Figure 2. Rescue of free Raptor reduces liver weight and TG content in older or obese mice

(a) Western blot from livers of young or adult Ad-GFP and Ad-Raptor mice sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). (b) Hepatic TG content in young (8-week-old), adult (24-week-old) or aged (10- to 12-month-old) male mice sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). (c,d) Liver weight in (c) aged (10- to 12-month-old, n=7/group) or (d) DIO (n=5 or 4/group) Ad-GFP and Ad-Raptor mice. *P < 0.05 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m. Blots are representative of three independent experiments, and samples within groups chosen randomly.
Supplementary Figure 3. Free Raptor reduces lipogenesis without affecting body weight, adiposity or fatty acid oxidation (a-e). Body weight (a), epidydimal fat pad (eWAT) weight (b), non-esterified fatty acid (NEFA) levels (c), liver mRNA expression (d) and plasma β-hydroxybutyrate levels (e) in young or adult Ad-GFP and Ad-Raptor mice, sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). (f) Srebp1c-dependent lipogenic gene expression in primary hepatocytes transduced with Ad-GFP or Ad-Raptor (n=4 biologic replicates). *P < 0.05 and **P < 0.01 as compared to the indicated control control by two-way ANOVA. All data are shown as the means ± s.e.m.
Supplementary Figure 4. Increase in free Raptor levels does not affect mTORC1 or mTORC2 activity
(a) mTORC1 kinase activity on 4E-BP1 substrate. (b-d) Western blots (b and d) and protein concentration (c) measured from livers of adult Ad-GFP and Ad-Raptor male mice, sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). (e-g) Western blots following IP with anti-mTOR antibody (e) from livers of young or adult Ad-GFP and Ad-Raptor male mice, sacrificed after a 16 h fast followed by 4 h refeeding (f), or after a 16 h fast with or without 4 h refeeding (g). Blots are representative of three independent experiments, and samples within groups chosen randomly.
**Supplementary Figure 5.** Free Raptor reduces hepatocyte Akt activity
(a, b) Western blots from liver of adult Ad-GFP or Ad-Raptor male mice, sacrificed after a 16 h fast with or without 4 h refeeding. (c) Liver mRNA expression in adult Ad-GFP and Ad-Raptor mice (n=6/group). (d) Western blots from eWAT of young or adult Ad-GFP and Ad-Raptor male mice sacrificed after a 16 h fast followed by 4 h refeeding. (e) Plasma insulin levels in young or adult Ad-GFP and Ad-Raptor male mice sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). **P < 0.01 as compared to the indicated control control by two-way ANOVA. All data are shown as the means Blots are representative of three independent experiments, and samples within groups chosen randomly.
Supplementary Figure 6. Raptor, but not mTORC1 activity, post-transcriptionally regulates PHLPP2

(a) Western blots of PHLPP isoforms from Hepa1c1c7 cells and liver. (b,c) Western blots from primary hepatocytes transduced with Ad-GFP or Ad-Raptor, then treated with vehicle, Rapamycin, or Torin1 (b), or Tsc+/+ and Tsc2-/− MEFs (c). (d,e) Western blot from adult (d) or HFD-fed (e) Raptorfl/fl liver transduced with AAV8-TBG-GFP or AAV8-TBG-Cre, normalized to β-actin. (f) Western blot from primary hepatocytes transduced with Ad-shControl, Ad-shRaptor, or Ad-shRictor. (g,h) Phlpp1 and Phlpp2 gene expression in Ad-GFP or Ad-Raptor-transduced liver (n=6/group) (g) or primary hepatocytes (n=4/group) (h). (i) Western blot of primary hepatocytes co-transduced with PHLPP1 or PHLPP2 and Ad-Raptor (or Ad-GFP control). (j) Western blot of Raptor (or GFP control)-transduced Hepa1c1c7 cells following immunoprecipitation with anti-PHLPP2, with or without MG-132. Blots are representative of two independent experiments. **P < 0.01 as compared to the indicated control control by two-way ANOVA. All data are shown as the means ± s.e.m. Blots are representative of three independent experiments, and samples within groups chosen randomly, unless otherwise stated.
Supplementary Figure 7. PHLPP2 knockdown increases Akt S473 phosphorylation and causes hepatic steatosis

(a) Western blot from primary hepatocytes transduced with Ad-shControl, Ad-shPhlpp1, or Ad-shPhlpp2. (b,c) Liver weight (b) and Oil-Red-O or H&E staining (c) in livers of adult Ad-shControl, Ad-shPhlpp1, or Ad-shPhlpp2 mice sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). **P < 0.01 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m. Blots are representative of three independent experiments, and samples within groups chosen randomly.
Supplementary Figure 8. Rescue of aging/obesity-reduced PHLPP2 levels prevents hepatic steatosis (a-c) body weight (a), eWAT weight (b), and liver weight (c) in adult, HFD-fed Ad-GFP or Ad-PHLPP2 male mice, sacrificed after a 16 h fast followed by 4 h refeeding (n=6 or 7/group). *P < 0.05 compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m.
Supplementary Figure 9. Metabolic effects of free Raptor are PHLPP2-dependent

(a-e) Body weight (a), eWAT weight (b), β-hydroxybutyrate (c), NEFA levels (d), and hepatic protein concentration (e) of adult Ad-GFP and Ad-Raptor mice co-transduced with control (Ad-shControl), Ad-shPHLPP1 or Ad-shPHLPP2 adenoviruses, sacrificed after a 16 h fast followed by 4 h refeeding (n=6/group). **P < 0.01 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m.
Supplementary Figure 10. Rescue of free Raptor or PHLPP2 does not affect glucose homeostasis.

(a,b) Blood glucose levels (a) and hepatic gluconeogenic gene expression (b) in young or adult Ad-GFP and Ad-Raptor male mice sacrificed after a 16 h fast with or without 4 h refeeding (n=6/group). (c) Intraperitoneal glucose tolerance test (GTT) in young or adult Ad-GFP and Ad-Raptor mice (n=6/group). (d,e) Blood glucose (d) and plasma insulin levels (e) in adult, HFD-fed Ad-GFP and Ad-PHLPP2 male mice, sacrificed after a 16 h fast followed by 4 h refeeding (n=6 or 7/group). All data are shown as the means ± s.e.m.
Supplementary Figure 11
Uncropped images of the original scans of representative immunoblots

Figure 1a
Figure 1c

Top

- mTOR
- Raptor
- Rictor
- α-tubulin

Bottom

- mTOR
- Raptor
- Rictor
- α-tubulin
Figure 3a

Stripped 45' (Restore), then re-probed for actin

Figure 3b

Stripped 45' (Restore), then re-probed for actin
Figure 3c

Akt kinase assay

Total lysates

Stripped 45' (Restore), then re-probed for total Akt

Figure 3d

PHLPP1

PHLPP2

β-actin

Raptor

β-actin
Figure 3e

Figure 3f

- β-actin
- p-S6 (S240/244)
- PHLPP2
- S6

- mTOR
- β-actin
- PHLPP2
Supplementary Figure 1b

Supplementary Figure 1d
Supplementary Figure 1e

Supplementary Figure 1f

Supplementary Figure 1g
Supplementary Figure 4b

- **p-S6K1 (T389)**
- **pS6 (S240/244)**
- **p4E-BP1-T37/46**

Supplementary Figure 4d

- **Raptor**
- **β-actin**
- **p-IRS1 (S636/639)**
Supplementary Figure 4e

IP

5% input

mTOR

Rictor

GβL

mTOR

Raptor

Rictor

GβL

β-actin

Supplementary Figure 4f

PKCα

pAkt (T450)

Stripped 45' (Restore), then re-probed for actin

β-actin
Supplementary Figure 4g

Supplementary Figure 5a

Stripped 45' (Restore), then re-probed for actin
Supplementary Figure 5d

Supplementary Figure 6a
Supplementary Figure 6f
Supplementary Figure 6i

Supplementary Figure 6j