Supplemental Information

PRMT1 orchestrates with SAMTOR to govern mTORC1 methionine sensing via Arg-methylation of NPRL2

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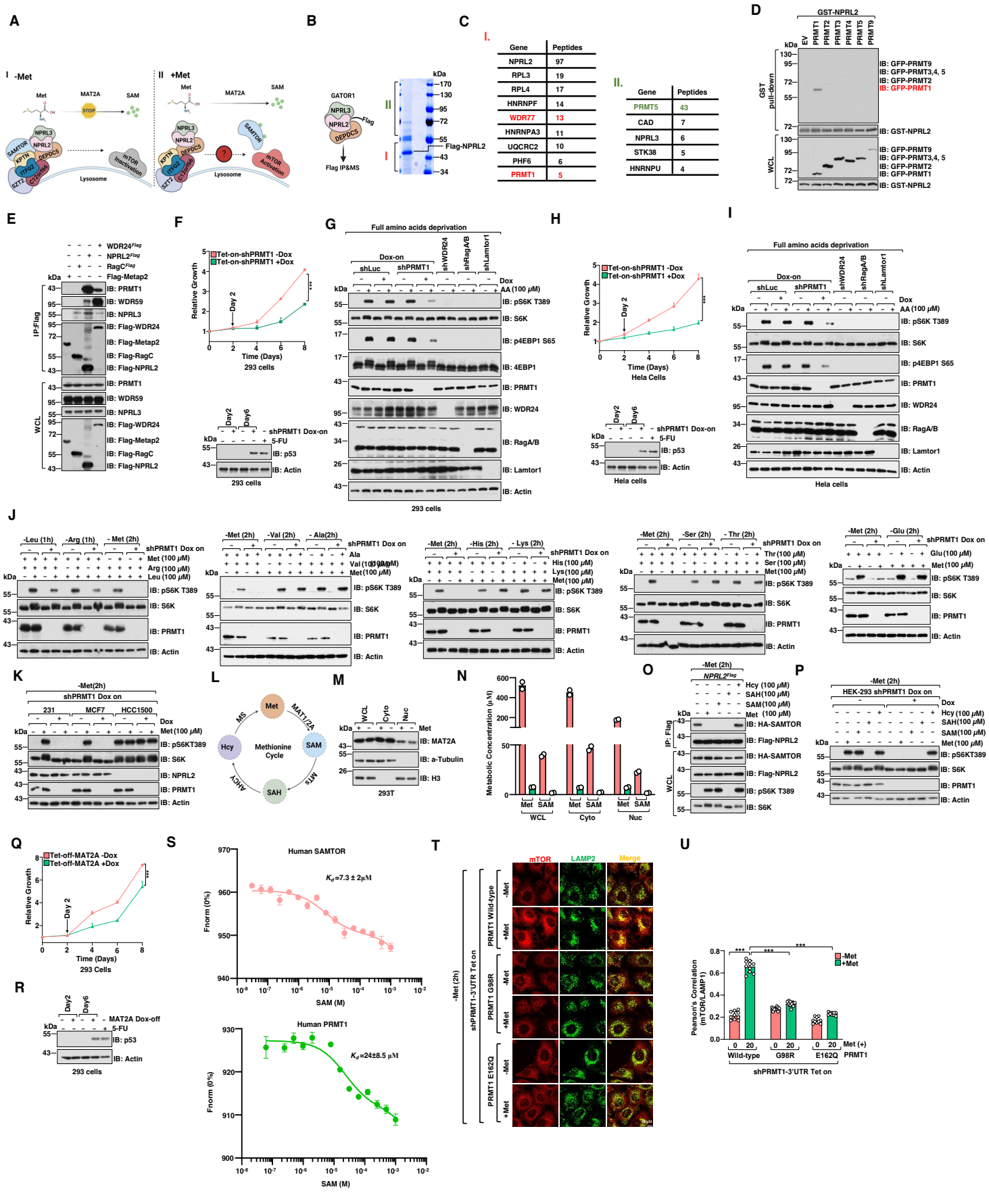

A
I -Met
II +Met
Met
MAT2A
SAM
STOP
NPRL3
NPRL2
SAMTOR
KPTN
DEPDC5
ITFG2
C12orf66
SZT2
mTOR Inactivation
mTOR Activation
Lysosome
B
GATOR1
Flag IP&MS
kDa
170
130
95
72
55
43
34
Flag-NPRL2
C
I.
Gene | Peptides
NPRL2 | 97
RPL3 | 19
RPL4 | 17
HNRNPF | 14
WDR77 | 13
HNRNPA3 | 11
UQCRC2 | 10
PHF6 | 6
PRMT1 | 5
II.
Gene | Peptides
PRMT5 | 43
CAD | 7
NPRL3 | 6
STK38 | 5
HNRNPU | 4
D
GST-NPRL2
EV
PRMT1
PRMT2
PRMT3
PRMT4
PRMT5
PRMT9
GST pull-down
WCL
IB: GFP-PRMT9
IB: GFP-PRMT3,4, 5
IB: GFP-PRMT2
IB: GFP-PRMT1
IB: GST-NPRL2
E
WDR24Flag
NPRL2Flag
RagCFlag
Flag-Metap2
IP:Flag
WCL
IB: PRMT1
IB: WDR59
IB: NPRL3
IB: Flag-WDR24
IB: Flag-Metap2
IB: Flag-RagC
IB: Flag-NPRL2
F
Tet-on-shPRMT1 -Dox
Tet-on-shPRMT1 +Dox
Relative Growth
Day 2
Time (Days)
293 cells
Day2
Day6
shPRMT1 Dox-on
5-FU
IB: p53
IB: Actin
G
Full amino acids deprivation
Dox-on
shLuc
shPRMT1
shWDR24
shRagA/B
shLamtor1
Dox
AA (100 μM)
IB: pS6K T389
IB: S6K
IB: p4EBP1 S65
IB: 4EBP1
IB: PRMT1
IB: WDR24
IB: RagA/B
IB: Lamtor1
IB: Actin
293 cells
H
Tet-on-shPRMT1 -Dox
Tet-on-shPRMT1 +Dox
Day 2
Time (Days)
Hela Cells
Day2
Day6
shPRMT1 Dox-on
5-FU
IB: p53
IB: Actin
Hela cells
I
Full amino acids deprivation
Dox-on
shLuc
shPRMT1
shWDR24
shRagA/B
shLamtor1
Dox
AA (100 μM)
IB: pS6K T389
IB: S6K
IB: p4EBP1 S65
IB: PRMT1
IB: WDR24
IB: RagA/B
IB: Lamtor1
IB: Actin
Hela cells
J
-Leu (1h)
-Arg (1h)
- Met (2h)
shPRMT1 Dox on
Met (100 μM)
Arg (100 μM)
Leu (100 μM)
-Met (2h)
-Val (2h)
- Ala(2h)
Ala
Val (100 μM)
Met(100 μM)
-Met (2h)
-His (2h)
- Lys (2h)
His (100 μM)
Lys(100 μM)
-Met (2h)
-Ser (2h)
- Thr (2h)
Thr (100 μM)
Ser (100 μM)
-Met (2h)
-Glu (2h)
Glu (100 μM)
IB: pS6K T389
IB: S6K
IB: PRMT1
IB: Actin
K
-Met(2h)
shPRMT1 Dox on
231
MCF7
HCC1500
Dox
Met(100 μM)
IB: pS6K T389
IB: S6K
IB: NPRL2
IB: PRMT1
IB: Actin
L
Met
MAT1/2A
SAM
MTS
SAH
AHCY
Hcy
MS
Methionine Cycle
M
WCL
Cyto
Nuc
Met
IB: MAT2A
IB: a-Tubulin
IB: H3
293T
N
Metabolic Concentration (u M)
600
400
200
50
0
Met
SAM
WCL
Cyto
Nuc
O
-Met (2h)
NPRL2Flag
Hcy (100 μM)
SAH(100 μM)
SAM(100 μM)
Met (100 μM)
IP: Flag
WCL
IB: HA-SAMTOR
IB: Flag-NPRL2
IB: pS6K T389
IB: S6K
P
-Met (2h)
HEK-293 shPRMT1 Dox on
Dox
Hcy (100 μM)
SAH(100 μM)
SAM(100 μM)
Met (100 μM)
IB: pS6KT389
IB: S6K
IB: PRMT1
IB: Actin
Q
Tet-off-MAT2A -Dox
Tet-off-MAT2A +Dox
Relative Growth
Day 2
Time (Days)
293 Cells
R
Day2
Day6
MAT2A Dox-off
5-FU
IB: p53
IB: Actin
293 cells
S
Human SAMTOR
Kd=7.3 ± 2μM
Fnorm (0%)
SAM (M)
Human PRMT1
Kd=24±8.5 μM
Fnorm (0%)
SAM (M)
T
mTOR
LAMP2
Merge
-Met (2h)
shPRMT1-3'UTR Tet on
PRMT1 Wild-type
PRMT1 G98R
PRMT1 E162Q
-Met
+Met
U
Pearson's Correlation (mTOR/LAMP1)
-Met
+Met
Wild-type
G98R
E162Q
Met (+)
PRMT1
shPRMT1-3'UTR Tet on

Figure S1. PRMT1 regulates methionine-dependent mTORC1 signaling. Related to Figure 1.

(**A**) A schematic model depicting the methionine sensing of mTORC1 signaling.

(**B**) A schematic illustration depicting the identification of GATOR1-interacting proteins by immunoprecipitation (IP) and mass spectrometry analysis. Coomassie blue staining of the immunoprecipitates (IPs) prepared from b. Brackets indicate protein bands analyzed by mass spectrometry.

(**C**) Proteins identified from fractions I and II as shown in **B**.

(**D**) PRMT1, but not other PRMTs, interacts with NPRL2. HEK 293T cells were transfected with the indicated plasmids. Cells were lysed and subjected to anti-Flag immunoprecipitation followed by immunoblotting.

(**E**) PRMT1 binds to endogenous GATOR1 complex. The indicated HEK293T knock-in cells were subjected to anti-Flag immunoprecipitation. The IPs were analyzed via immunoblotting**.**

(**F**) HEK293 cells were infected with either tet-on-sh*Luc* or tet-on-sh*PRMT1* lentiviruses and selected with puromycin for 3 days. Stable cell lines were pretreated with or without doxycycline (DOX) for the indicated times, and cell growth and p53 expression levels were analyzed. n=3 biological repeats, ***p<0.001, one-way ANOVA test.

(**G**) HEK293 cells were infected with the indicated shRNAs. For the inhibition of PRMT1, the stable cell lines were pretreated with or without DOX for an additional 2 days; for the inhibition of the other genes, cells were selected with puromycin for 72 hours. Cells were then deprived of complete amino acids for 1 hour and restimulated with amino acids for 10 min. Cell lysates were analyzed via immunoblotting with the indicated antibodies**.**

(**H**) HeLa cells were infected and analyzed in **F**, n=3 biological repeats, ***p<0.001, one-way ANOVA test.

(**I**) HeLa cells were treated and analyzed as in **G**.

(**J**) HEK293 cells were generated as in G, cells were deprived of the indicated amino acids for 2 hours and restimulated with the respective amino acids for 20 min. Cells were lysed and analyzed via immunoblotting with the indicated antibodies.

(**K**) Knockdown of *PRMT1* impairs methionine-mediated mTORC1 signaling in *NPRL2* competent cells (MDA-MB-231 and MCF7), but not in *NPRL2* depleted HCC1500 cells. The indicated cells were pretreated with or without DOX for an additional 2 days, deprived of methionine for 2 hours and restimulated with methionine (100 μM) for 20 min.

(**L**) A schematic illustration of the methionine cycle-related metabolites and enzymes.

(**M**) The expression of MAT2A in whole cell lysates (WCL), cytosol (Cyto), and nucleus (Nuc).

(**N**) HEK293 cells were incubated with or without methionine for 2 hours before sample preparation for ELISA analysis of methionine and SAM levels. n=2 biological repeats were presented.

(**O**) $NPRL2^{Flag}$ knock-in HEK293T cells were transfected with HA-SAMTOR, deprived of methionine for 2 hours and restimulated with methionine (100 μM), SAM (1 mM), SAH (1 mM), or Hcy (1 mM) for 20 min. Cells were lysed and subjected to immunoprecipitation (IP) and immunoblot analysis.

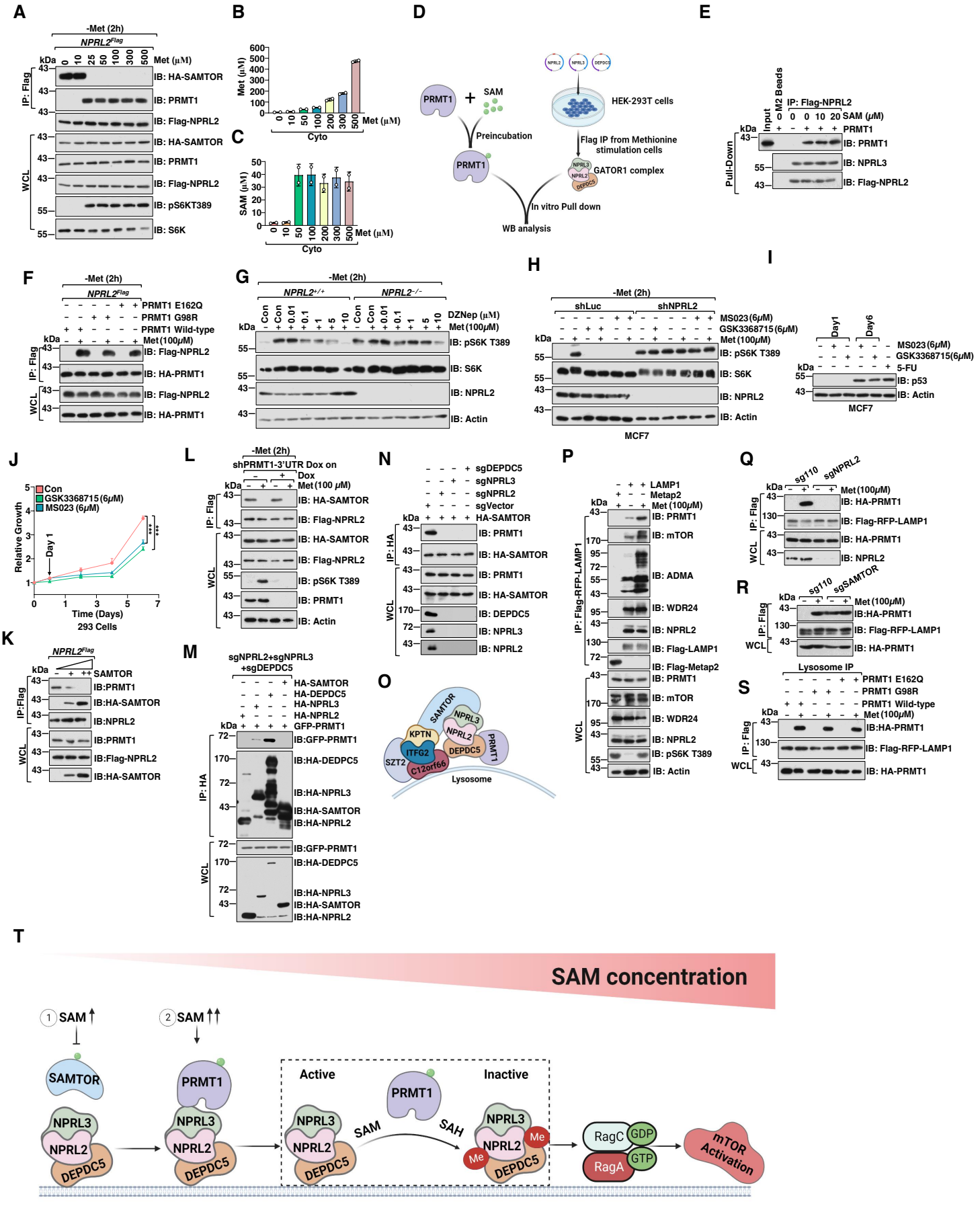
(**P**) Acute loss of *PRMT1* using a doxycycline-inducible (tet-on) system attenuated methionine- and SAM-meditated mTORC1 activation. HEK293-tet-on-shPRMT1 cells were treated with doxycycline (DOX, 100 ng/ml) for 48 hours prior to starvation and then stimulated with methionine (100μM), SAM (100μM), SAH (100μM), or Hcy (100μM) for 6 hours. Cells were lysed and analyzed via immunoblotting with the indicated antibodies.

(**Q**-**R**) *MAT2A*-Dox off cells are described in the Methods section. The stable cell lines were pretreated with or without DOX for the indicated times, followed by analysis of cell growth (**Q**) and p53 expression levels (**R**) via immunoblotting with the indicated antibodies. n=3 biological repeats, ***p<0.001, one-way ANOVA test.

(**S**) The K_d of human SAM-SAMTOR and SAM-PRMT1 was determined by the MST assay.

(**T**-**U**) Cells were deprived of methionine for 2 hours and restimulated with methionine (100 μM) for 20 min. The co-localization of mTORC1 and LAMP2 was analyzed via immunostaining (**T**). Scale bar, 10 μm.

Pearson's correlation analysis of mTOR and LAMP2 signals (**U**). 10 cells were analyzed for each condition, ***p<0.001, unpaired, two-tailed Student's t-test.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-Met (2h)
NPRL2Flag
Met (μM)
IP: Flag
WCL
IB: HA-SAMTOR
IB: PRMT1
IB: Flag-NPRL2
IB: pS6KT389
IB: S6K
B
Met (μM)
Cyto
C
SAM (μM)
D
PRMT1
SAM
Preincubation
HEK-293T cells
Flag IP from Methionine stimulation cells
GATOR1 complex
In vitro Pull down
WB analysis
E
IP: Flag-NPRL2
M2 Beads
SAM (μM)
Pull-Down
IB: NPRL3
F
PRMT1 E162Q
PRMT1 G98R
PRMT1 Wild-type
Met(100μM)
IB: HA-PRMT1
G
NPRL2+/+
NPRL2-/-
DZNep (μM)
IB: pS6K T389
IB: NPRL2
IB: Actin
H
shLuc
shNPRL2
MS023(6μM)
GSK3368715(6μM)
MCF7
I
Day1
Day6
5-FU
IB: p53
J
Con
GSK3368715(6μM)
MS023 (6μM)
Relative Growth
Day 1
Time (Days)
293 Cells
K
SAMTOR
IB:PRMT1
IB:HA-SAMTOR
IB:NPRL2
IB:Flag-NPRL2
L
shPRMT1-3'UTR Dox on
Dox
M
sgNPRL2+sgNPRL3
+sgDEPDC5
HA-SAMTOR
HA-DEPDC5
HA-NPRL3
HA-NPRL2
GFP-PRMT1
IB:GFP-PRMT1
IB:HA-DEPDC5
IB:HA-NPRL3
IB:HA-SAMTOR
IB:HA-NPRL2
N
sgDEPDC5
sgNPRL3
sgNPRL2
sgVector
IB: DEPDC5
O
SAMTOR
NPRL3
KPTN
NPRL2
ITFG2
DEPDC5
PRMT1
SZT2
C12orf66
Lysosome
P
LAMP1
Metap2
IB: mTOR
IB: ADMA
IB: WDR24
IB: Flag-LAMP1
IB: Flag-Metap2
Q
sg110
sgNPRL2
R
sgSAMTOR
S
Lysosome IP
T
SAM concentration
1 SAM
2 SAM
Active
Inactive
SAM
SAH
RagC
GDP
RagA
GTP
mTOR Activation

Figure S2. SAMTOR antagonizes the interaction of PRMT1 with the GATOR1 complex. Related to Figure 2.

(**A-C**) *NPRL2*^{Flag} knock-in HEK293T cells were deprived of methionine for 2 hours and restimulated with different concentrations for 30 min as indicated. Cells were lysed for analysis of NPRL2 and SAMTOR interaction (**A**) and quantification of methionine (**B)** and SAM (**C**). For the quantification of B and C, n=2 biological repeats were presented.

(**D**-**E**) SAM did not alter the binding status of PRMT1 with the GATOR1 complex *in vitro*. Recombinant PRMT1 was preincubated with various concentrations of SAM as indicated. The GATOR1 complex was immunoprecipitated from methionine-stimulated cells. The preincubated PRMT1 was then subjected to an *in vitro* pull-down assay with purified GATOR1 complex. Flag-IPs and cell lysates were analyzed via immunoblotting.

(**F**) HA-PRMT1 G98R and E162Q coimmunoprecipitated comparable amounts of GATOR1 as wild-type SAMTOR. *NPRL2* ^{Flag} knock-in HEK293T cells were transfected with the indicated plasmids. The cells were then subjected to anti-HA immunoprecipitation and the IPs were analyzed via immunoblotting.

(**G**) *NPRL2* wild-type and knockout cells were pretreated with various concentrations of DZNep for 24 hours. Cells were deprived of methionine for 2 hours, followed by restimulation with methionine for 20 min. Cells were lysed and analyzed via immunoblotting with the indicated antibodies.

(**H**) *NPRL2* knockdown MCF7 cells were pretreated with MS0223 (6 μM) and GSK3368715 (6 μM) for 48 hours. Cells were deprived of methionine for 2 hours and then restimulated with methionine for 20 min. Cells were lysed and analyzed via immunoblotting with the indicated antibodies.

(**I**-**J**) MCF7 cell lines were treated with MS0223 (6 μM) and GSK3368715 (6 μM) for the indicated times. The cell growth (**I**) was analyzed by CellTiter-Glo assay, and p53 expression levels were analyzed by immunoblotting (**J**). n=3 biological repeats were presented, ***$p<0.001$, one-way ANOVA test.

(**K**) SAMTOR blocked the association between GATOR1 and PRMT1.

(**L**) Knock down of *PRMT1* did not affect the interaction between GATOR1 and SAMTOR.

(**M**) HEK293T cells were infected with sgRNAs targeting *NPRL2*, *NPRL3*, or *DEPDC5,* respectively, to eliminate the effect of endogenous GATOR1. The resulting cells were co-transfected with GFP-PRMT1, HA-NPRL2, HA-NPRL3, HA-DEPDC5, or HA-SAMTOR. At 48 hours post-transfection, cells were lysed and analyzed via immunoprecipitation (IP) and immunoblotting.

(**N**) PRMT1 indirectly binds to SAMTOR via the GATOR1 complex. HEK293T cells infected with sgRNA targeting *NPRL2*, *NPRL3*, and *DEPDC5,* respectively, were then transfected with HA-SAMTOR. The interaction of PRMT1 and HA-SAMTOR was analyzed via immunoblotting.

(**O**) A schematic illustration depicting the interaction of GATOR1 (NPRL2/NPRL3/DEPDC5), KICSTOR (SZT2/KPTN/ITFG2/C12orf66), PRMT1, and SAMTOR.

(**P**) PRMT1 accumulation in lysosomes upon methionine stimulation. HeLa cells expressing Flag-Metap2 (Control) and Flag-RFP-LAMP1 were lysed and subjected to anti-Flag immunoprecipitation. Purified lysosomes were lysed and immunoblotted with the indicated antibodies.

(**Q**) The SAM-binding-deficient mutants G98R and E166Q localized to the lysosome at a level comparable to that of the wild-type PRMT1.

(**R**) PRMT1 localized to the lysosome in an NPRL2-dependent manner. Lysosome-associated PRMT1 in wild-type and *NPRL2* knockdown cells was analyzed via lysosome-IP and immunoblotting.

(**S**) SAMTOR prevented the lysosomal localization of PRMT1. The lysosome-associated PRMT1 in wild-type and *NPRL2* knockdown cells were analyzed via lysosome-IP and immunoblotting.

(**T**) The methionine sensing by mTORC1 is initially elicited by the dissociation of SAM-loaded SAMTOR (sensing the lower intracellular levels of SAM) from GATOR1, and SAM-loaded PRMT1(sensing the higher intracellular levels of SAM) then methylates NPRL2 and inactivates GATOR1.

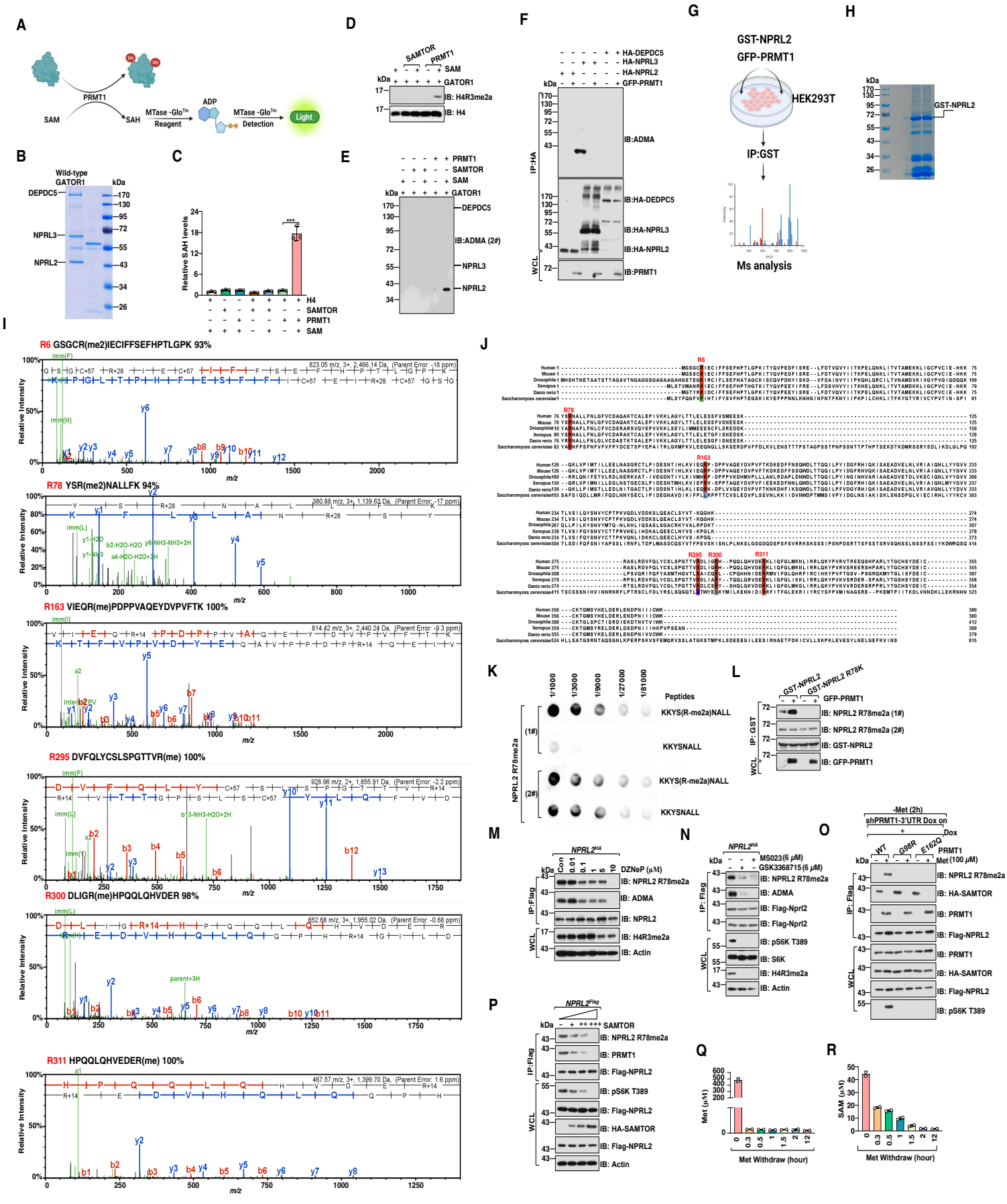
A
PRMT1
SAM
SAH
MTase -Glo™ Reagent
ADP
MTase -Glo™ Detection
Light
B
Wild-type GATOR1
kDa
DEPDC5
NPRL3
NPRL2
170
130
95
72
55
43
34
26
C
Relative SAH levels
***
H4
SAMTOR
PRMT1
SAM
D
SAMTOR
PRMT1
SAM
GATOR1
IB: H4R3me2a
IB: H4
E
PRMT1
SAMTOR
SAM
GATOR1
DEPDC5
IB:ADMA (2#)
NPRL3
NPRL2
F
HA-DEPDC5
HA-NPRL3
HA-NPRL2
GFP-PRMT1
IP:HA
IB:ADMA
IB:HA-DEPDC5
IB:HA-NPRL3
IB:HA-NPRL2
WCL
IB:PRMT1
G
GST-NPRL2
GFP-PRMT1
HEK293T
IP:GST
Ms analysis
H
GST-NPRL2
I
R6 GSGCR(me2)IECIFFSEFHPTLGPK 93%
R78 YSR(me2)NALLFK 94%
R163 VIEQR(me)PDPPVAQEYDVPVFTK 100%
R295 DVFQLYCSLSPGTTVR(me) 100%
R300 DLIGR(me)HPQQLQHVDER 98%
R311 HPQQLQHVEDER(me) 100%
Relative Intensity
m/z
J
R6
R78
R163
R295
R300
R311
K
1/1000
1/3000
1/9000
1/27000
1/81000
Peptides
KKYS(R-me2a)NALL
KKYSNALL
NPRL2 R78me2a
(1#)
(2#)
L
GST-NPRL2
GST-NPRL2 R78K
GFP-PRMT1
IP: GST
IB: NPRL2 R78me2a (1#)
IB: NPRL2 R78me2a (2#)
IB: GST-NPRL2
WCL
IB: GFP-PRMT1
M
NPRL2HA
DZNeP (μM)
Con
0.01
0.1
1
5
10
IP:Flag
IB: NPRL2 R78me2a
IB: ADMA
IB: NPRL2
IB: H4R3me2a
IB: Actin
N
NPRL2HA
MS023(6 μM)
GSK3368715 (6 μM)
IB: NPRL2 R78me2a
IB: ADMA
IB: Flag-Nprl2
IB: Flag-Nprl2
IB: pS6K T389
IB: S6K
IB: H4R3me2a
IB: Actin
O
-Met (2h)
shPRMT1-3'UTR Dox on
Dox
WT
G98R
E162Q
PRMT1
Met (100 μM)
IB: NPRL2 R78me2a
IB: HA-SAMTOR
IB: PRMT1
IB: Flag-NPRL2
IB: PRMT1
IB: HA-SAMTOR
IB: Flag-NPRL2
IB: pS6K T389
P
NPRL2Flag
SAMTOR
IB: NPRL2 R78me2a
IB: PRMT1
IB: Flag-NPRL2
IB: pS6K T389
IB: Flag-NPRL2
IB: HA-SAMTOR
IB: Flag-NPRL2
IB: Actin
Q
Met (μM)
Met Withdraw (hour)
R
SAM (μM)
Met Withdraw (hour)

Figure S3. PRMT1 methylates NPRL2 at R78 residue. Related to Figure 3.

(**A**) A schematic cartoon illustrating the analysis of the methylation event using the SAH generation as a readout.

(**B**) Coomassie blue staining of the purified GATOR1 complex.

(C-D) PRMT1, but not SAMTOR, promotes the methylation of Histone H4 *in vitro*. Histone H4 peptide (10 ng) was subjected to *in vitro* methylation assays for 1 hour in the presence of SAM (1 μM), PRMT1 (100 ng), and SAMTOR (100 ng), and the generation of SAH was analyzed via the MTase-Glo™ Methyltransferase Assay kit (**C**). Immunoblotting for the methylation levels of Histone H4 using H4R3me2a (**D**). n=3 biological repeats, ***p<0.001, unpaired, two-tailed Student's t-test.

(**E**) Immunoblotting for ADMA levels of GATOR1 complex component proteins from **A**; the antibody was from CST.

(**F**) PRMT1 promotes the asymmetric di-methylation of NPRL2, but not of other GATOR1 subunits (NPRL3 and DEPDC5). HA-NPRL2, HA-NPRL3, and HA-DEPDC5 were co-transfected with GFP-PRMT1 into HEK293T cells.

(**G**) A schematic cartoon illustration of the analysis of the NPRL2 methylation sites. HEK293T cells were transfected with GST-NPRL2 and GFP-PRMT1 and subjected to lysis and GST pull-down.

(**H**) Coomassie blue staining of the purified GST-NPRL2.

(**I-J**) The NPRL2 arginine methylation site identified by mass-spectrometry (**I**). Cross-species alignment of the NPRL2 protein sequences by Jalview. The identified arginine methylation site(s) were highlighted in red (**J**).

(**K**) Validation of NPRL2 R78me2a antibody using dot blot.

(**L**) GST-NPRL2 (wild-type or R78K mutant) was transfected with GFP-PRMT1 in HEK293T cells as indicated. The methylation of NPRL2 was analyzed via immunoblotting with the NPRL2 R78me2a antibody.

(**M**) *NPRL2*^{Flag} knock-in HEK293T cells were treated with DZNep at various concentrations for 24 hours and the methylation of NPRL2 was analyzed with NPRL2 R78me2a and ADMA antibodies.

(**N**) *NPRL2*^{Flag} knock-in HEK293T cells were treated with MS0223 (6 μM) and GSK3368715 (6 μM) for 48 hours, and the methylation of NPRL2 was analyzed as in **M**.

(**O**) Inactivation of PRMT1 inhibited the asymmetric di-methylation level of NPRL2. *NPRL2*^{Flag} HEK293T knock-in cell lines were infected with PRMT1 wild-type, G98R, and E162Q viruses, and cells were treated and analyzed as in **M**.

(**P**) SAMTOR inhibited NPRL2 methylation and the interaction between PRMT1 and GAOTR1 in a dose-dependent manner.

(**Q-R**) The cytosolic methionine (**Q**) and SAM (**R**) levels were analyzed via ELISA. n=2 biological repeats.

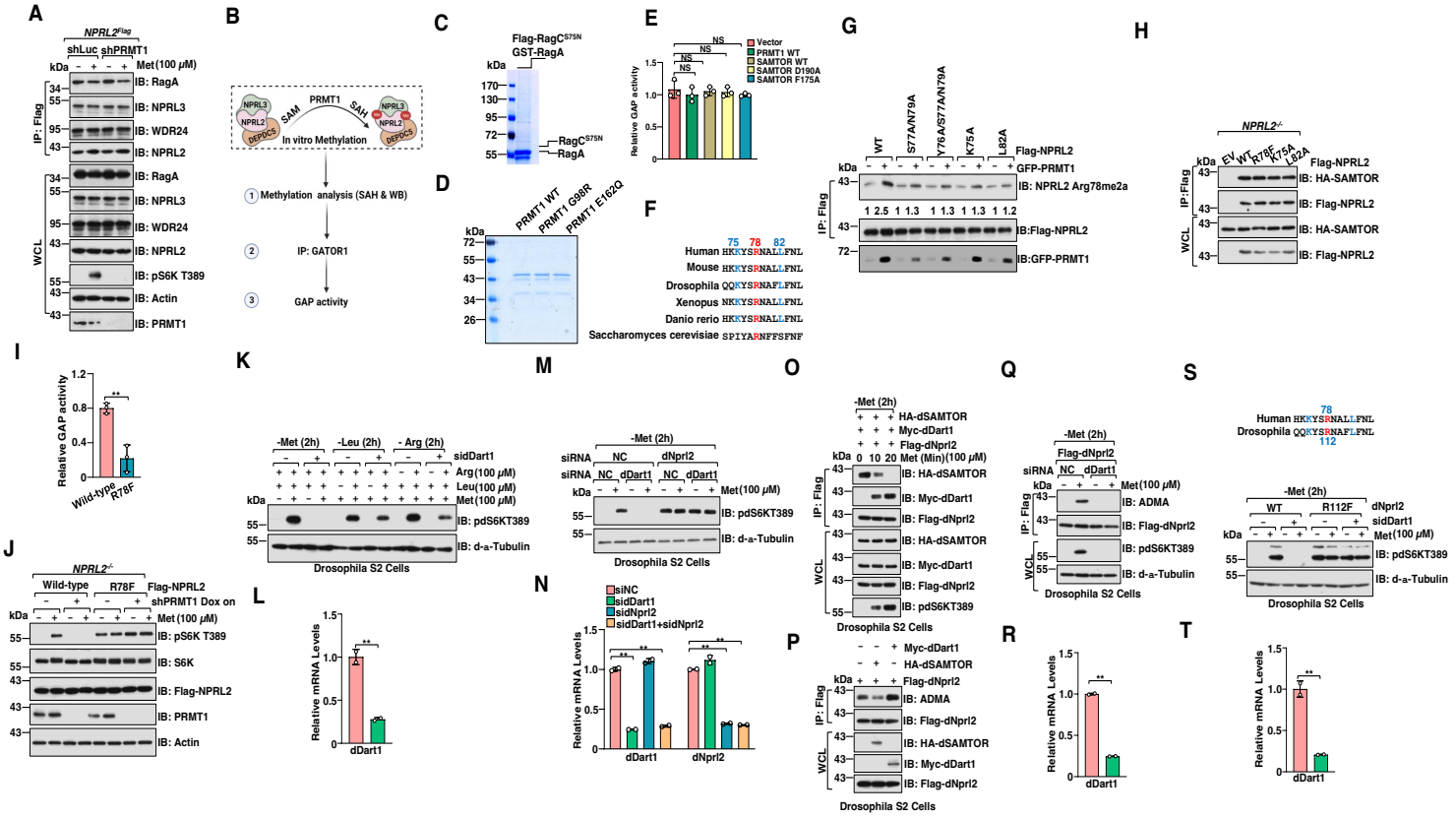

Figure S4. PRMT1 inhibits the GAP activity of GATOR1 and has a conserved role in Drosophila. Related to Figure 4.

(**A**) Knockdown of *PRMT1* did not alter the interaction of the GATOR1 complex with GATOR2 and RagA. *NPRL2*^{Flag} HEK293T cells were infected with shRNA targeting *PRMT1*. The interactions of GATOR1 with other components were analyzed via anti-Flag immunoprecipitation and immunoblotting with the indicated antibodies.

(**B**) A schematic model depicting how to analyze the methylation and GAP activity of GATOR1.

(**C**-**D**) Coomassie blue staining of the purified RagA/C complex (**C**) and PRMT1 wild-type, G98R, and E162Q derived from HEK293T cells (**D**).

(**E**) Direct incubation with PRMT1 or SAMTOR did not inhibit the GAP activity of GATOR1.GATOR1 was preincubated with PRMT1 or SAMTOR and subjected to GAP activity analysis. n=3 biological repeats, ns, no significant difference.

(**F**) Cross-species alignment of the NPRL2 protein sequences surrounding the R78 site by Jalview.

(**G**) Mutation of the sequence surrounding R78 inhibited PRMT1-mediated NPRL2 R78me2a modification.

(**H**) NPRL2 R78me2a modification did not affect the interaction between GATOR1 and SAMTOR.

(**I**) The NPRL2 R78F mutation exhibited no detectable GAP activity compared to wild type NPRL2. n=3 biological repeats, **p<0.01, unpaired, two-tailed Student's t-test.

(**J**) HEK293 *NPRL2* knockout cells were reconstituted with NPRL2 wild-type or R78 mutant. The cells were then infected with tet-on-sh*PRMT1* lentiviruses. The stable cell lines were pre-treated with or without doxycycline (DOX) for an additional 2 days before methionine starvation and restimulation. Whole cell lysates (WCL) were analyzed by immunoblotting with the indicated antibodies.

(**K**-**L**) *dDart1* deficiency inhibited methionine sensing in Drosophila S2 cells. S2 cells were transfected with the indicated siRNAs. At 48 hours post-transfection, cells were starved with methionine, leucine, and arginine

for 2 hours and restimulated with the same amino acids for 20 min. Cells were lysed for immunoblotting analysis (**K**) and dDart1 expression levels were analyzed via qPCR (**L**). n=2 biological repeats, **p<0.01, unpaired, two-tailed Student's t-test.

(**M**-**N**) S2 cells were transfected as indicated. Cells were starved of methionine for 2 hours and restimulated with methionine (100 μM) for 20 min, followed by immunoblotting (**M**) and qPCR analysis (**N**). n=2 biological repeats, **p<0.01, unpaired, two-tailed Student's t-test.

(**O**) S2 cells were transfected with HA-dSamtor, Flag-dNprl2, or Myc-dDart1. At 48 hours post-transfection, cells were starved of methionine for 2 hours and restimulated with methionine (100 μM) for the indicated time. The interaction of dSamtor, dNprl2, and dDart1 was determined via immunoblotting.

(**P**) dDart1, but not dSamtor, promoted the asymmetric di-methylation of dNprl2.

(**Q**-**R**) *dDart1* deficiency inhibited the asymmetric di-methylation of dNprl2 (**P**). The expression levels of dDart1 were analyzed via qPCR(Q). n=2 biological repeats, **p<0.01, unpaired, two-tailed Student's t-test.

(**S**-**T**) Alignment of human or Drosophila NPRL2 for the conserved catalytic arginine residues. S2 cells were transfected with dNprl2 wild-type or R112F mutant, followed by transfection with siRNA targeting *dDart1*. The indicated cells were deprived of methionine for 2 hours and restimulated with methionine for 20 min. Cell lysates were analyzed via immunoblotting with the indicated antibodies (**S**). The expression of *dDart1* was analyzed via qPCR (**T**). n=2 biological repeats, **p<0.01, unpaired, two-tailed Student's t-test.

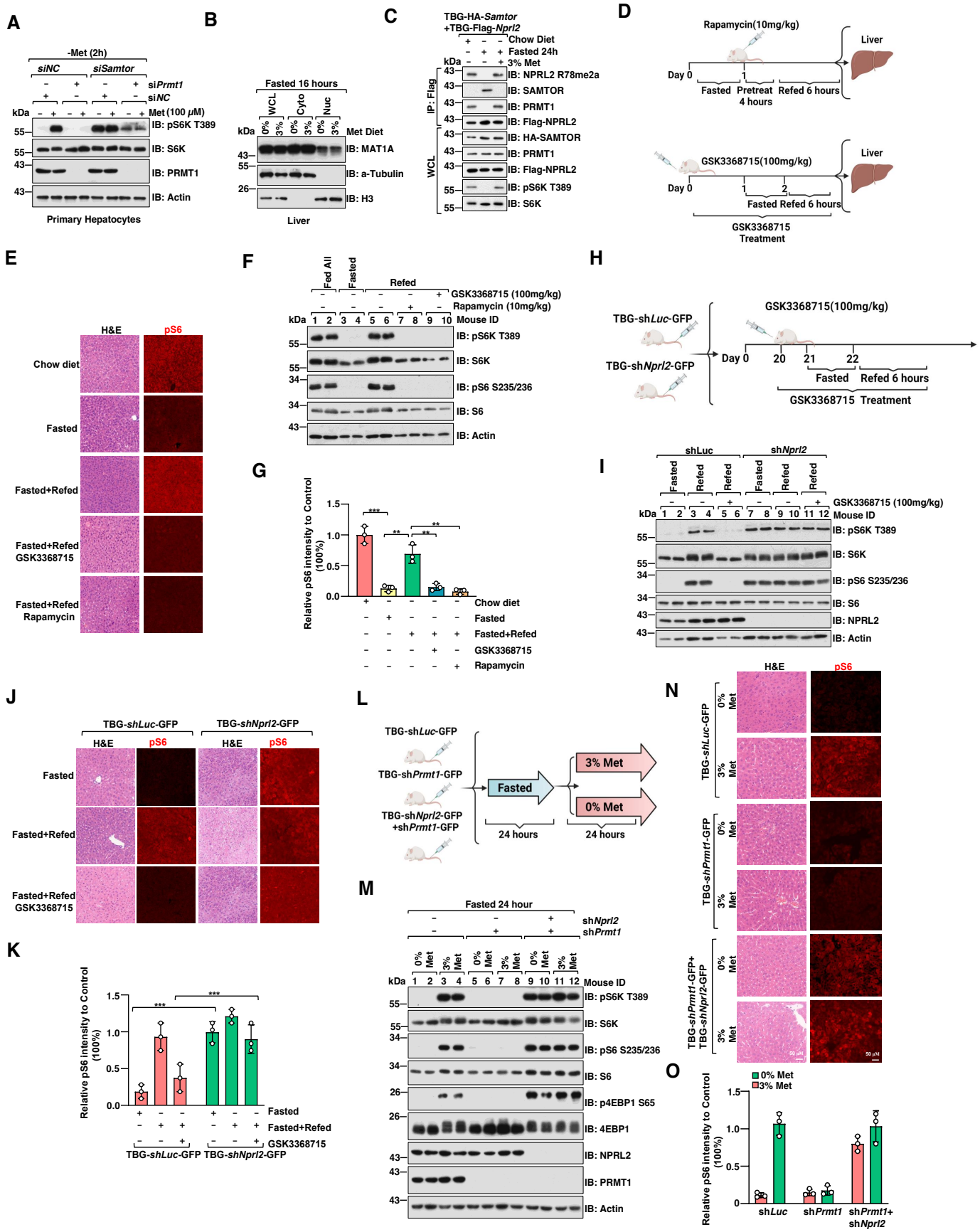
A
-Met (2h)
siNC
siSamtor
siPrmt1
siNC
kDa
Met (100 μM)
IB: pS6K T389
IB: S6K
IB: PRMT1
IB: Actin
Primary Hepatocytes
B
Fasted 16 hours
WCL
Cyto
Nuc
0%
3%
Met Diet
IB: MAT1A
IB: a-Tubulin
IB: H3
Liver
C
TBG-HA-Samtor
+TBG-Flag-Nprl2
Chow Diet
Fasted 24h
3% Met
IP: Flag
WCL
IB: NPRL2 R78me2a
IB: SAMTOR
IB: PRMT1
IB: Flag-NPRL2
IB: HA-SAMTOR
IB: PRMT1
IB: Flag-NPRL2
IB: pS6K T389
IB: S6K
D
Rapamycin(10mg/kg)
Liver
Day 0
Fasted
Pretreat
Refed 6 hours
4 hours
GSK3368715(100mg/kg)
Liver
Day 0
Fasted Refed 6 hours
GSK3368715
Treatment
E
H&E
pS6
Chow diet
Fasted
Fasted+Refed
Fasted+Refed
GSK3368715
Fasted+Refed
Rapamycin
F
Fed All
Fasted
Refed
GSK3368715 (100mg/kg)
Rapamycin (10mg/kg)
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: pS6 S235/236
IB: S6
IB: Actin
G
Relative pS6 intensity to Control (100%)
Chow diet
Fasted
Fasted+Refed
GSK3368715
Rapamycin
H
TBG-shLuc-GFP
TBG-shNprl2-GFP
GSK3368715(100mg/kg)
Day 0
Fasted
Refed 6 hours
GSK3368715 Treatment
I
shLuc
shNprl2
Fasted
Refed
GSK3368715 (100mg/kg)
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: pS6 S235/236
IB: S6
IB: NPRL2
IB: Actin
J
TBG-shLuc-GFP
TBG-shNprl2-GFP
H&E
pS6
Fasted
Fasted+Refed
Fasted+Refed
GSK3368715
K
Relative pS6 intensity to Control (100%)
Fasted
Fasted+Refed
GSK3368715
TBG-shLuc-GFP
TBG-shNprl2-GFP
L
TBG-shLuc-GFP
TBG-shPrmt1-GFP
TBG-shNprl2-GFP
+shPrmt1-GFP
Fasted
3% Met
0% Met
24 hours
24 hours
M
Fasted 24 hour
shNprl2
shPrmt1
0% Met
3% Met
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: pS6 S235/236
IB: S6
IB: p4EBP1 S65
IB: 4EBP1
IB: NPRL2
IB: PRMT1
IB: Actin
N
H&E
pS6
TBG-shLuc-GFP
TBG-shPrmt1-GFP
TBG-shPrmt1-GFP+
TBG-shNprl2-GFP
0% Met
3% Met
O
0% Met
3% Met
Relative pS6 intensity to Control (100%)
shLuc
shPrmt1
shPrmt1+
shNprl2

Figure S5. *Prmt1* deficiency impairs methionine sensing by mTORC1 in the mouse liver. Related to Figure 5.

(**A**) Primary hepatocytes were infected with shRNA as indicated. After 72 hours, cells were deprived of methionine for 2 hours and restimulated with methionine (100 μM) for 20 min. Cell lysates were analyzed by immunoblotting with the indicated antibodies.

(**B**) The expression of MAT1A in whole cell lysates (WCL), cytosol (Cyto), and nucleus (Nuc).

(**C**) Dietary methionine regulates Prmt1-Nprl2-Samtor interaction and Prmt1-mediated Nprl2 methylation in the liver. Wild-type male mice with hepatic expression of TBG-Flag-*Nprl2* and TBG-HA-*Samtor* for 21 days were fasted for 16 hours and refed with 3% or 0% methionine diet for 6 hours. Liver lysates were subjected to anti-Flag immunoprecipitation (IP), and whole cell lysates were analyzed by immunoblotting with the indicated antibodies.

(**D**-**G**) Eight-week-old male C57BL/6J mice were gavaged with the PRMT1 inhibitor GSK3368715 (100 mg/kg) and rapamycin (10 mg/kg) one day after a 24-hours fast followed by 6-hours refed. Schematic illustration of the treatment procedure in mice (**D**). Liver samples were analyzed by H&E staining and immunostaining for pS6 (**E**). Liver samples were analyzed via immunoblotting with the indicated antibodies (**F**). Quantification of the pS6 levels in **F**. Data are mean ± SD (n=3 per group), two-tailed t-test. **P < 0.01, ***P < 0.001. The intensity of pS6 was normalized to the chow diet group (Control) (**G**).

(**L**) A schematic illustration of the experimental setup used to study methionine sensing *in vivo*. Mice were injected with the respective adenoviruses for 21 days to deplete endogenous *Prmt1* and *Nprl2* in the liver as indicated. Mice were then fasted for 24 hours and refed with diets containing the indicated methionine contents for 24 hours, and the liver tissues were collected.

(**M**) Liver samples from **L** were analyzed by immunoblotting for the indicated proteins.

(**N**) Liver samples from **L** were analyzed by H&E staining and immunostaining for pS6.

(**O**) Quantification of pS6 levels in **N**. Data are mean ± SD (n=3 per group).

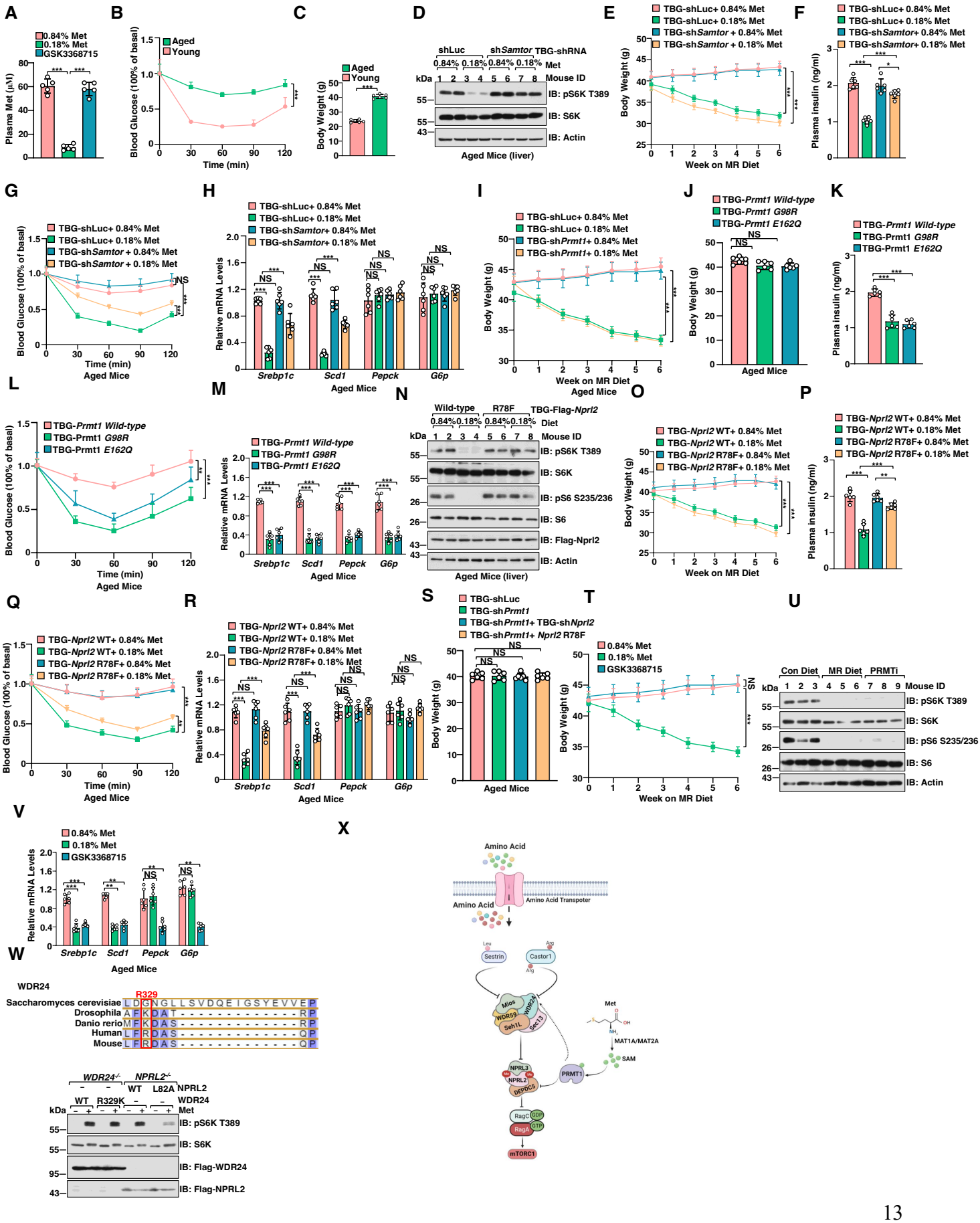
A
0.84% Met
0.18% Met
GSK3368715
Plasma Met (μM)
B
Aged
Young
Blood Glucose (100% of basal)
Time (min)
C
Aged
Young
Body Weight (g)
D
shLuc
shSamtor
TBG-shRNA
0.84% 0.18% 0.84% 0.18% Met
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: Actin
Aged Mice (liver)
E
TBG-shLuc+ 0.84% Met
TBG-shLuc+ 0.18% Met
TBG-shSamtor + 0.84% Met
TBG-shSamtor + 0.18% Met
Body Weight (g)
Week on MR Diet
F
TBG-shLuc+ 0.84% Met
TBG-shLuc+ 0.18% Met
TBG-shSamtor+ 0.84% Met
TBG-shSamtor+ 0.18% Met
Plasma insulin (ng/ml)
G
TBG-shLuc+ 0.84% Met
TBG-shLuc+ 0.18% Met
TBG-shSamtor + 0.84% Met
TBG-shSamtor + 0.18% Met
Blood Glucose (100% of basal)
Time (min)
Aged Mice
H
TBG-shLuc+ 0.84% Met
TBG-shLuc+ 0.18% Met
TBG-shSamtor+ 0.84% Met
TBG-shSamtor+ 0.18% Met
Relative mRNA Levels
Srebp1c
Scd1
Pepck
G6p
Aged Mice
I
TBG-shLuc+ 0.84% Met
TBG-shLuc+ 0.18% Met
TBG-shPrmt1+ 0.84% Met
TBG-shPrmt1+ 0.18% Met
Body Weight (g)
Week on MR Diet
Aged Mice
J
TBG-Prmt1 Wild-type
TBG-Prmt1 G98R
TBG-Prmt1 E162Q
Body Weight (g)
Aged Mice
K
TBG-Prmt1 Wild-type
TBG-Prmt1 G98R
TBG-Prmt1 E162Q
Plasma insulin (ng/ml)
L
TBG-Prmt1 Wild-type
TBG-Prmt1 G98R
TBG-Prmt1 E162Q
Blood Glucose (100% of basal)
Time (min)
Aged Mice
M
TBG-Prmt1 Wild-type
TBG-Prmt1 G98R
TBG-Prmt1 E162Q
Relative mRNA Levels
Srebp1c
Scd1
Pepck
G6p
Aged Mice
N
Wild-type
R78F
TBG-Flag-Nprl2
0.84% 0.18% 0.84% 0.18% Diet
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: pS6 S235/236
IB: S6
IB: Flag-Nprl2
IB: Actin
Aged Mice (liver)
O
TBG-Nprl2 WT+ 0.84% Met
TBG-Nprl2 WT+ 0.18% Met
TBG-Nprl2 R78F+ 0.84% Met
TBG-Nprl2 R78F+ 0.18% Met
Body Weight (g)
Week on MR Diet
P
TBG-Nprl2 WT+ 0.84% Met
TBG-Nprl2 WT+ 0.18% Met
TBG-Nprl2 R78F+ 0.84% Met
TBG-Nprl2 R78F+ 0.18% Met
Plasma insulin (ng/ml)
Q
TBG-Nprl2 WT+ 0.84% Met
TBG-Nprl2 WT+ 0.18% Met
TBG-Nprl2 R78F+ 0.84% Met
TBG-Nprl2 R78F+ 0.18% Met
Blood Glucose (100% of basal)
Time (min)
Aged Mice
R
TBG-Nprl2 WT+ 0.84% Met
TBG-Nprl2 WT+ 0.18% Met
TBG-Nprl2 R78F+ 0.84% Met
TBG-Nprl2 R78F+ 0.18% Met
Relative mRNA Levels
Srebp1c
Scd1
Pepck
G6p
Aged Mice
S
TBG-shLuc
TBG-shPrmt1
TBG-shPrmt1+ TBG-shNprl2
TBG-shPrmt1+ Nprl2 R78F
Body Weight (g)
Aged Mice
T
0.84% Met
0.18% Met
GSK3368715
Body Weight (g)
Week on MR Diet
U
Con Diet
MR Diet
PRMTi
kDa
Mouse ID
IB: pS6K T389
IB: S6K
IB: pS6 S235/236
IB: S6
IB: Actin
V
0.84% Met
0.18% Met
GSK3368715
Relative mRNA Levels
Srebp1c
Scd1
Pepck
G6p
Aged Mice
W
WDR24
R329
Saccharomyces cerevisiae L D G N G L L S V D Q E I G S Y E V V E P
Drosophila A F K D A T - - - - - - - - - - - - - R P
Danio rerio M F K D A S - - - - - - - - - - - - - R P
Human L F R D A S - - - - - - - - - - - - - Q P
Mouse L F R D A S - - - - - - - - - - - - - Q P
WDR24-/-
NPRL2-/-
WT
L82A NPRL2
WT
R329K
WDR24
kDa
Met
IB: pS6K T389
IB: S6K
IB: Flag-WDR24
IB: Flag-NPRL2
X
Amino Acid
Amino Acid
Amino Acid Transpoter
Leu
Sestrin
Arg
Castor1
Arg
Mios
WDR59
WDR24
Seh1L
Sec13
Met
MAT1A/MAT2A
SAM
NPRL3
NPRL2
DEPDC5
PRMT1
RagC
GDP
RagA
GTP
mTORC1

Figure S6. Hepatic Prmt1-Nprl2-mTORC1 dictates insulin sensitivity to dietary methionine restriction. Related to Figure 6.

(**A**) Plasma methionine levels in the indicated mice were determined by ELISA (n=6 per group, two-tailed t-test, ***P < 0.001).

(**B**) Intraperitoneal Insulin Tolerance Test (ITT) assay was performed with young mice (8 weeks, n=6 per group) or aged mice (13 months, n=6 per group, ***p<0.001, one-way ANOVA test).

(**C**) Body weight of young or aged mice used to perform ITT analysis in **B**. n=6 per group, ***p<0.001, one-way ANOVA test.

(**D**-**H**) Mice were injected with sh*Luc* or sh*Samtor* adenovirus to inhibit endogenous *Samtor*. The indicated mice were fed 0.84% or 0.18% methionine diet for 50 days (n=6 per group). Liver lysates were subjected to immunoblotting with the indicated antibodies (**D**). Body weight measurements during the first 6 weeks of the study (**E**). Plasma insulin levels were determined by ELISA (**F**), and the ITT was performed (**G**). The expression levels of the indicated genes were measured by qPCR (**H**). n=6 per group, ns, no significant difference, ***p<0.001, **p<0.01.

(**I**) Body weight measurements of mice presented in Figure 6D during the first 6 weeks of the study. n=6 per group, ***p<0.001, one-way ANOVA test.

(**J**-**M**) Mice were infected with the indicated adenovirus (Prmt1 wild-type, G98R, and E166Q) for 21 days (n=6 per group). Body weight measurements of mice (**J**). Plasma insulin levels were determined by ELISA (**K**) and ITT analysis was performed (**L**). The expression levels of the indicated genes were measured by qPCR (**M**). n=6 per group, ns, no significant difference, ***p<0.001, **p<0.01.

(**N**-**R**) Mice were infected with the indicated adenoviruses (Nprl2 wild-type, and R78F). The indicated mice were fed 0.84% or 0.18% methionine diet for 50 days (n=6 per group). Liver lysates were subjected to immunoblotting with the indicated antibodies (**N**). Body weight measurements during the first 6 weeks of the study (**O**). Plasma insulin levels were determined by ELISA (**P**), and the ITT was performed (**Q**). The expression levels of the indicated genes were measured by qPCR (**R**). n=6 per group, ns, no significant difference, ***p<0.001, **p<0.01.

(**S**) Mice were infected with the indicated adenovirus for 21 days (n=6 per group) and their body weights were measured.

(**T**) Mice with hepatic expression of TBG-Flag-*Nprl2*/TBG-HA-*Samtor* were fed with the indicated methionine diet (0.84% or 0.18%) or orally administered the PRMT1 inhibitor GSK3368715 for 50 days. Body weight measurements during the first 6 weeks of the study (n=6 per group, ns, no significant difference, ***p<0.001, **p<0.01).

(**U**) Liver lysates from **T** were subjected to immunoblotting with the indicated antibodies.

(**V**) Gene expression in liver prepared from **T** was measured by qPCR (n=6 per group, two-tailed t-test, ***P < 0.001, **P < 0.01, ns, no significant difference).

(**W**) Cross-species alignment of the WDR24 protein sequences. The identified arginine methylation site (R329) was highlighted in red. WDR24 methylation did not noticeably affect methionine sensing of mTORC1 in our experimental conditions. *NPRL2* or *WDR24* null HEK293T cells were infected with either wild-type NPRL2 or the indicated mutants, starved of methionine for 2 hours and restimulated with methionine (100 μM) for 20 min, and then analyzed via immunoblotting with the indicated antibodies.

(**X**) A schematic model depicting the physiological role of PRMT1 in the nutrient sensing of mTORC1 pathway.