Cell Reports, Volume *42*

Supplemental information

CDK5-PRMT1-WDR24 signaling cascade

promotes mTORC1 signaling and tumor growth

Shasha Yin, Liu Liu, Lauren E. Ball, Yalong Wang, Mark T. Bedford, Stephen A. Duncan, Haizhen Wang, and Wenjian Gan

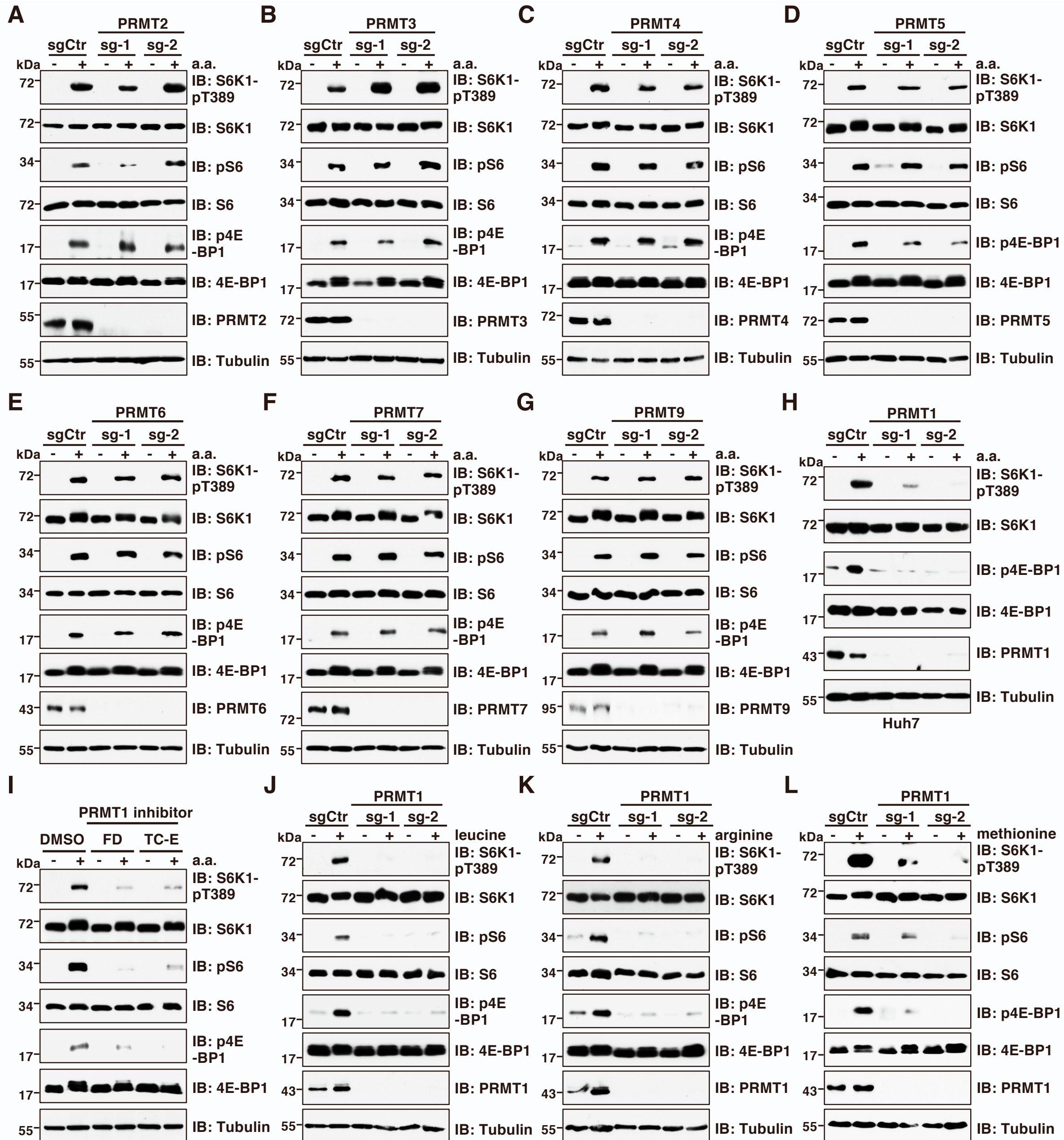

Figure S1. PRMT1, but not other PRMTs, promotes mTORC1 activation in response to amino acids (Related to Figure 1).

(A-G) Immunoblot (IB) analysis of whole cell lysates (WCLs) derived from HEK293 cells depleted of indicated PRMTs. Cells were infected with lentivirus expressing sgRNAs targeting individual PRMT and selected with 2 μg/ml puromycin for 4 days. Cells were starved of amino acids (a.a.) for 50 min or starved for 50 min and restimulated with a.a. for 10 min before harvesting.

(H) IB analysis of WCLs derived from Huh7 cells depleted of PRMT1. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting.

(I) IB analysis of WCLs derived from HEK293T cells treated with PRMT1 inhibitor furamidine dihydrochloride (FD) or TC-E 5003 (TC-E) for 24 hours. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.

(J and K) IB analysis of WCLs derived from HEK293T cells depleted of PRMT1. Cells were starved of leucine (J) or arginine (K) for 50 min or starved for 50 min and then restimulated with leucine (J) or arginine (K) for 10 min before harvesting.
(L) IB analysis of WCLs derived from HEK293T cells depleted of PRMT1. Cells were starved of methionine for 2 hours or starved for 2 hours and then restimulated with methionine for 20 min before harvesting.
All these experiments were repeated at least two times and similar results were obtained.

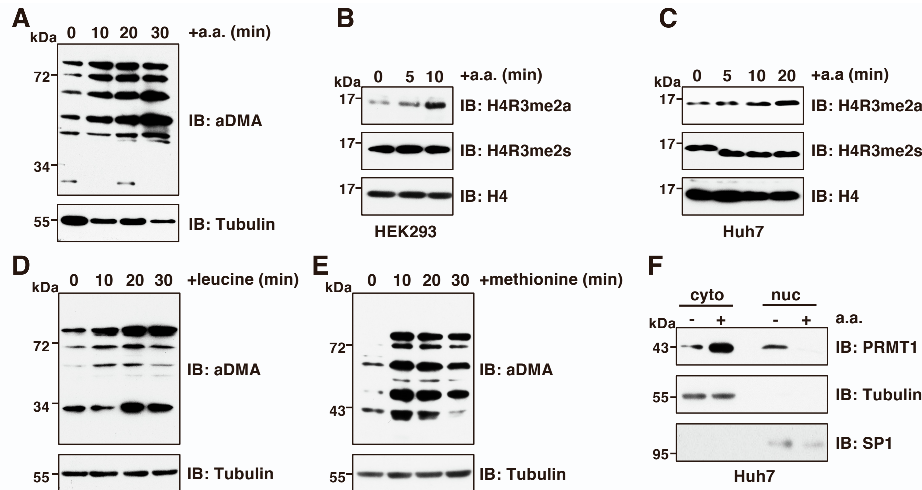

Figure S2. Amino acids promote PRMT1 activation and cytoplasm accumulation (Related to Figure 2).

(A) IB analysis of WCLs derived from HEK293T cells starved of a.a. for 50 min and then restimulated with a.a. for 0-30 min.

(B) IB analysis of histone extraction derived from HEK293 cells starved of a.a. for 50 min and then restimulated with a.a. for 0-10 min.

(C) IB analysis of histone extraction derived from Huh7 cells starved of a.a. for 2 hours and then restimulated with a.a. for 0-20 min.

(D) IB analysis of WCLs derived from HEK293T cells starved of leucine for 50 min and then restimulated with leucine for 0-30 min.

(E) IB analysis of WCLs derived from HEK293T cells starved of methionine for 2 hours and then restimulated with methionine for 0-30 min.

(F) IB analysis of cell fractions derived from Huh7 cells starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min.

All these experiments were repeated at least two times and similar results were obtained.

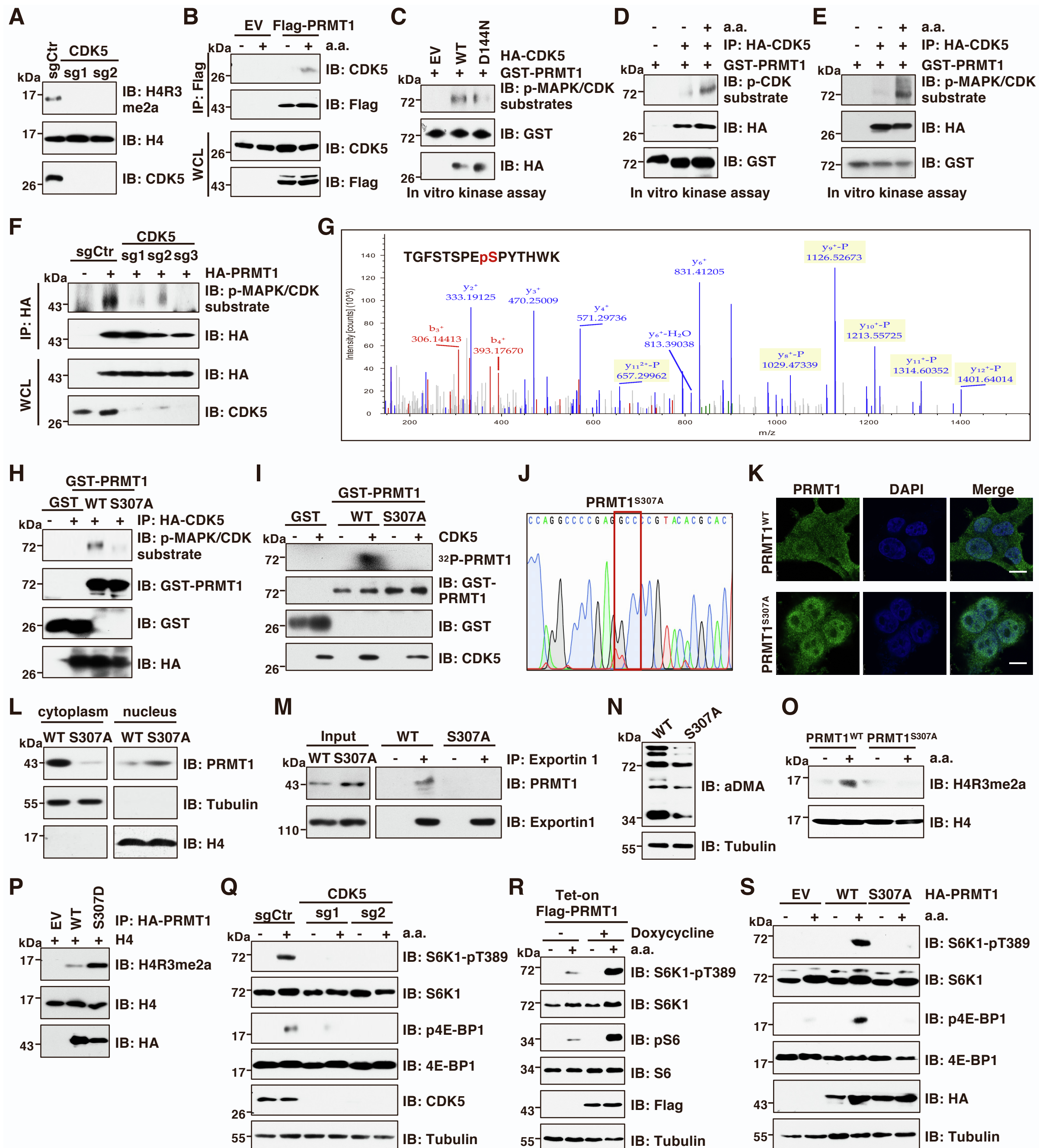

Figure S3. Phosphorylation of PRMT1 by CDK5 is required for its cytoplasmic localization and mTORC1 activation (Related to Figure 3).

(A) IB analysis of WCLs and histone extraction derived from HEK293T cells depleted of CDK5. Cells were infected with lentivirus expressing sgRNAs targeting CDK5 and selected with 2 μg/ml puromycin for 4 days.

(B) IB analysis of WCL and anti-Flag immunoprecipitates (IP) derived from HEK293T cells (EV) or PRMT1^{Flag} knock-in HEK293T cells. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.

(C) In vitro kinase assays using recombinant GST-PRMT1 protein as the substrate. HA-CDK5-

WT and HA-CDK5-D144N were immunopurified from HEK293T cells. The reaction products were subjected to IB analysis. PRMT1 phosphorylation was detected by p-MAPK/CDK substrate antibody.
(D and E) In vitro kinase assays using recombinant GST-PRMT1 protein as the substrate. HA-CDK5 derived from HEK293T cells was used as the kinase source. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting. The reaction products were subjected to IB analysis. PRMT1 phosphorylation was detected by p-CDK substrate antibody (D) and p-MAPK/CDK substrate antibody (E).
(F) IB analysis of WCL and anti-HA IP using p-MAPK/CDK substrate antibody. HEK293T cells were depleted of CDK5 (sgCtr as a control) and transfected with HA-PRMT1.
(G) Mass spectrometry analysis of PRMT1-S307 phosphorylation.
(H) In vitro kinase assays using recombinant GST-PRMT1-WT or GST-PRMT1-S307A proteins as substrate. HA-CDK5 protein purified from HEK293T cells was used as the kinase source. The reaction products were subjected to IB analysis. PRMT1 phosphorylation was detected by p-MAPK/CDK substrate antibody.
(I) In vitro kinase assays using recombinant GST-PRMT1-WT or GST-PRMT1-S307A proteins as substrate. Active CDK5 kinase was purchased from Promega. PRMT1 phosphorylation was detected by radioactive isotope, phosphorus-32 (^{32}P).
(J) Validation of $PRMT1^{S307A}$ knock-in HEK293T cells by Sanger DNA sequencing.
(K) Immunofluorescence (IF) analysis of PRMT1 localization in $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells. Scale bar, 10 μm.
(L) IB analysis of cell fractionations derived from $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells.
(M) IB analysis of Input and anti-Exportin 1 IP derived from $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells.
(N) IB analysis of WCLs derived from $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells.
(O) IB analysis of histone extraction derived from $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.
(P) In vitro arginine methylation assays using H4 protein as substrate. HA-PRMT1-WT or HA-PRMT1-S307D immunopurified from HEK293T cells were used as methyltransferase.
(Q) IB analysis of WCLs derived from Huh7 cells depleted of CDK5. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting.
(R) IB analysis of WCLs derived from $PRMT1^{S307A}$ knock-in HEK293T cells stably expressing Tet-on-Flag-PRMT1. Cells were treated with doxycycline for 48 hours to induce PRMT1 expression and then were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.
(S) IB analysis of WCLs derived from $PRMT1^{KO}$ Huh7 cells reconstituted with EV, PRMT1-WT or PRMT1-S307A. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting.
Experiments (A-F), (H and I), and (L-S) were repeated at least three times and similar results were obtained.

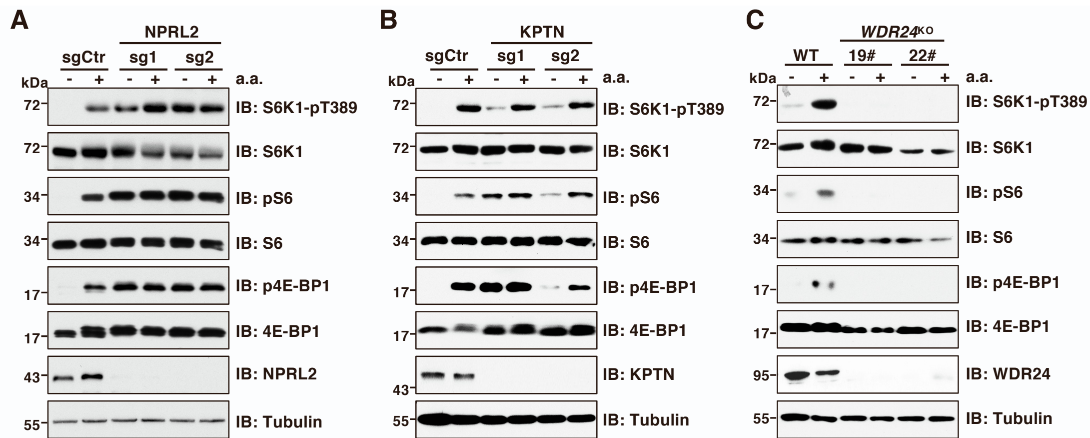

Figure S4. Loss of GATOR1 or KICSTOR activates mTORC1 whereas loss of GATOR2 inhibits mTORC1 (Related to Figure 4).

(A and B) IB analysis of WCLs derived from HEK293T cells depleted of NPRL2 (A) or KPTN (B). Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.

(C) IB analysis of WCL derived from $WDR24^{WT}$ or $WDR24^{KO}$ Huh7 cells. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting. These experiments were repeated at least three times and similar results were obtained.

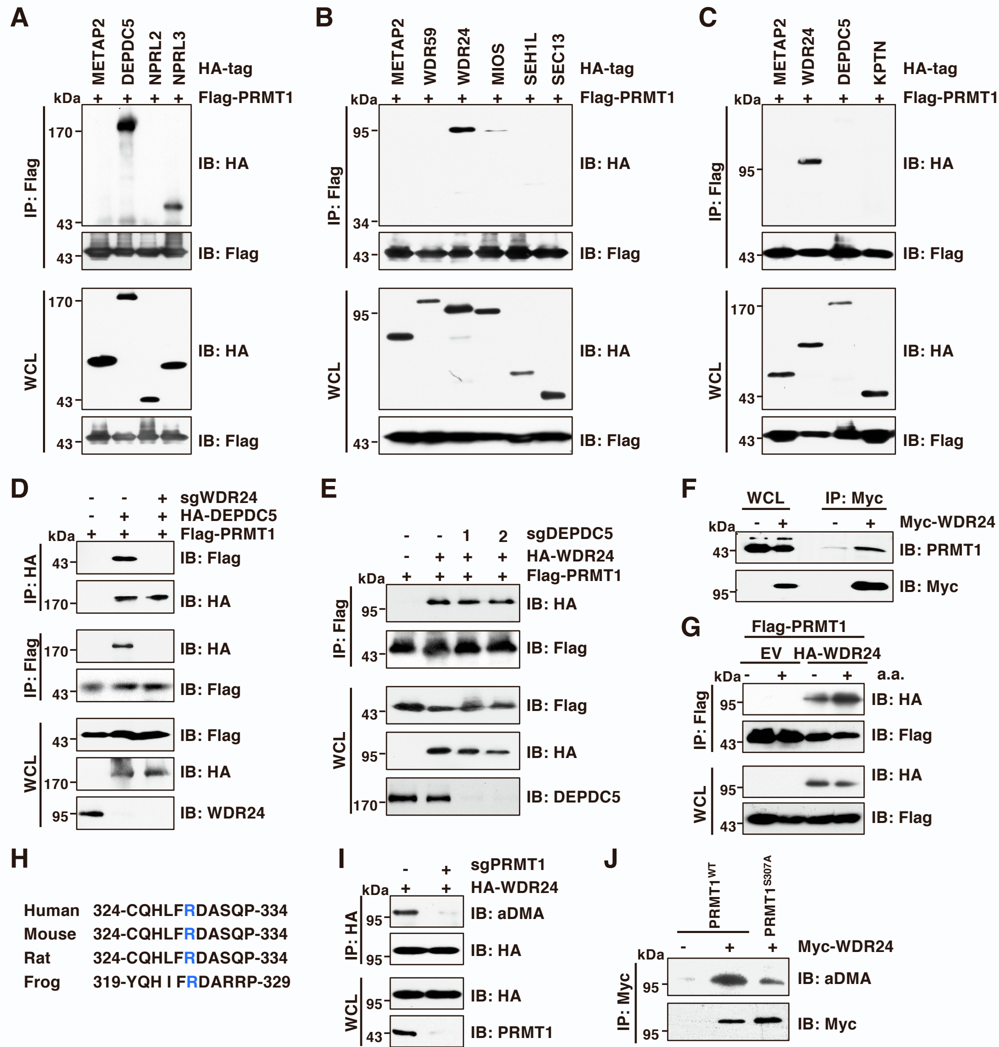

Figure S5. PRMT1 specifically interacts with and methylates WDR24 (Related to Figure 5).
(A-C) IB analysis of WCLs and anti-Flag IP derived from HEK293T cells transfected with indicated constructs.
(D) IB analysis of WCLs and anti-HA/anti-Flag IP derived from HEK293T cells depleted of WDR24 and transfected with indicated constructs.
(E) IB analysis of WCLs and anti-Flag IP derived from HEK293T cells depleted of DEPDC5 and transfected with indicated constructs.
(F and G) IB analysis of WCLs and anti-Myc (F) or anti-HA (G) IP derived from HEK293T cells transfected with indicated constructs. In (G), cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.
(H) A schematic presentation of the evolutionarily conserved R329 of WDR24.
(I) IB analysis of WCL and anti-HA IP derived from HEK293T depleted of PRMT1 and transfected with HA-WDR24.
(J) IB analysis of WCL and anti-Myc IP derived from PRMT1^{WT} and PRMT1^{S307A} knock-in HEK293T cells transfected with Myc-WDR24.
These experiments were repeated at least three times and similar results were obtained.

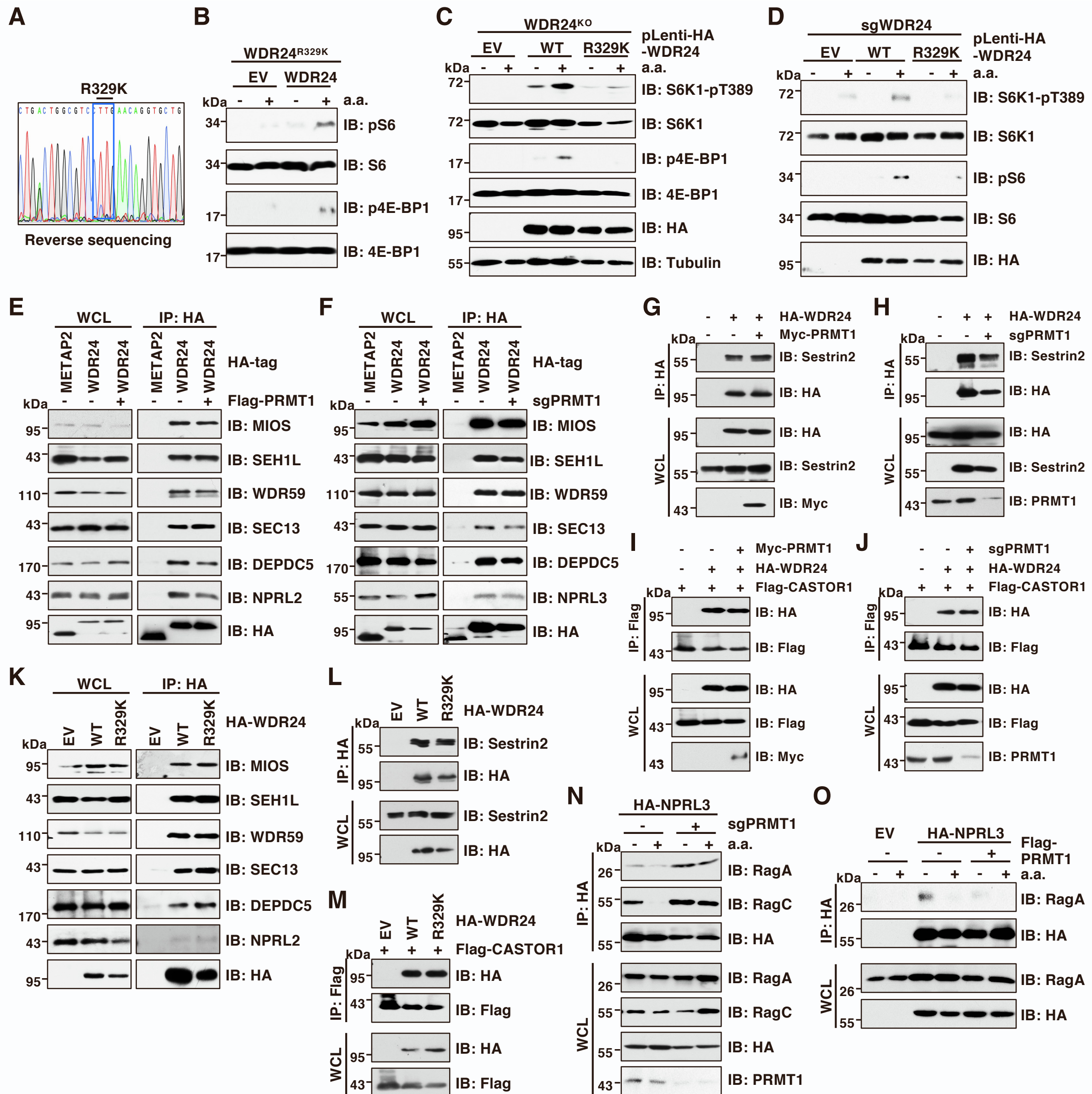

Figure S6. Deficiency in WDR24-R329 methylation inhibits mTORC1 signaling by enhancing interactions between GATOR1 and Rag GTPases (Related to Figure 6).

(A) Validation of WDR24^{R329K} knock-in Huh7 cells by Sanger DNA sequencing.

(B) IB analysis of WCL derived from WDR24^{R329K} knock-in Huh7 cells stably expressing empty vector (EV) or WDR24-WT. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting.

(C) IB analysis of WCL derived from WDR24^{KO} Huh7 cells reconstituted with EV, WDR24-WT or WDR24-R329K. Cells were starved of a.a. for 2 hours or starved for 2 hours and then restimulated with a.a. for 10 min before harvesting.

(D) IB analysis of WCL derived from WDR24-depleted HepG2 cells reconstituted with EV, WDR24-WT or WDR24-R329K. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.

(E) IB analysis of WCL and anti-HA IP derived from HEK293T cells transfected with HA-METAP2 (control) or HA-WDR24 with or without Flag-PRMT1.
(F) IB analysis of WCL and anti-HA IP derived from HEK293T cells with or without PRMT1 depletion and transfected with HA-METAP2 (control) or HA-WDR24.
(G) IB analysis of WCL and anti-HA IP derived from HEK293T cells transfected with HA-WDR24 with or without Myc-PRMT1.
(H) IB analysis of WCL and anti-HA IP derived from HEK293T cells with or without PRMT1 depletion and transfected with HA-WDR24.
(I) IB analysis of WCL and anti-Flag IP derived from HEK293T cells transfected with indicated constructs.
(J) IB analysis of WCL and anti-Flag IP derived from HEK293T cells with or without PRMT1 depletion and transfected with indicated constructs.
(K and L) IB analysis of WCL and anti-HA IP derived from HEK293T cells stably expressing EV, HA-WDR24-WT or HA-WDR24-R329K.
(M) IB analysis of WCL and anti-Flag IP derived from HEK293T cells transfected with Flag-CASTOR1 and EV, HA-WDR24-WT or HA-WDR24-R329K.
(N) IB analysis of WCL and anti-HA IP derived from HEK293T depleted of PRMT1 and transfected with HA-NPRL3. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.
(O) IB analysis of WCL and anti-HA IP derived from HEK293T transfected with HA-NPRL3 and Flag-PRMT1. Cells were starved of a.a. for 50 min or starved for 50 min and then restimulated with a.a. for 10 min before harvesting.
The experiments in (B-O) were repeated at least three times and similar results were obtained.

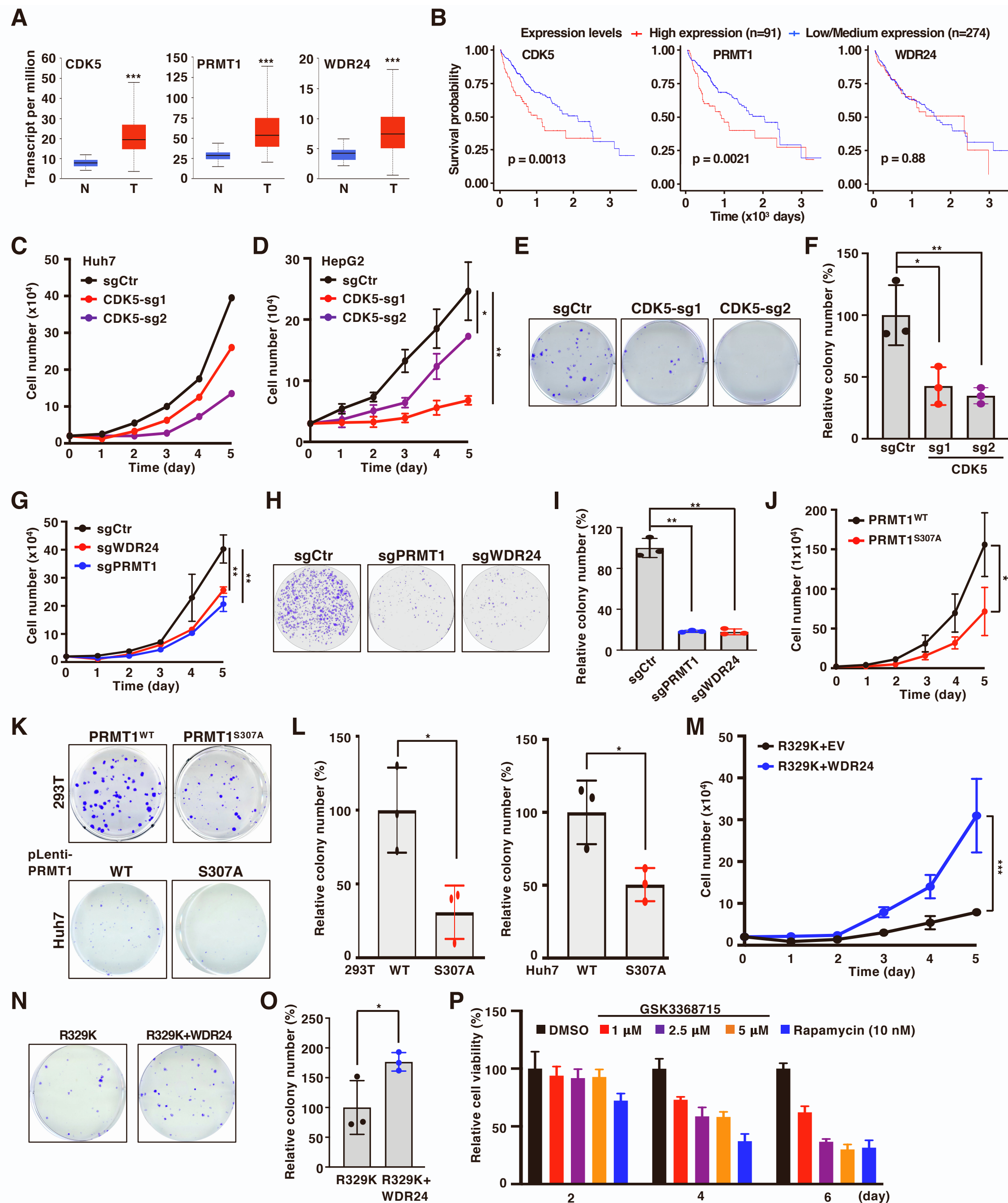

Figure S7. The CDK5-PRMT1-WDR24 axis is critical for HCC cell proliferation (Related to Figure 7).

(A) Analysis of mRNA levels of CDK5, PRMT1, and WDR24 in HCC samples (N = 371) and normal liver tissues (N = 50) in TCGA using the online tool UALCAN (http://ualcan.path.uab.edu/index.html). $^{***}P < 0.001$, t-test. N, normal. T, tumor.

(B) Analysis of correlation between CDK5, PRMT1, and WDR24 mRNA expression and HCC patient survival based on TCGA data using the online tool UALCAN.

(C and D) CDK5-depleted Huh7 cells (C) and HepG2 cells (D) were subjected to cell proliferation assays. Data in (D) are shown as the mean ± SD of n = 3 independent experiments. $^{*}P < 0.05$, $^{**}P$

< 0.01, two-way ANOVA.
(E and F) CDK5-depleted Huh7 cells were subjected to colony formation assays. Representative images were shown in (E) and quantification of colonies in (F). Data are shown as the mean ± SD of n = 3 independent experiments. *$P < 0.05$, **$P < 0.01$, two-tailed Student's *t* test.
(G) Huh7 cells depleted of PRMT1 or WDR24 were subjected to cell proliferation assays. Data are shown as the mean ± SD of n = 3 independent experiments. **$P < 0.01$, two-way ANOVA.
(H and I) Huh7 cells depleted of PRMT1 or WDR24 were subjected to colony formation assays. Representative images were shown in (E) and quantification of colonies in (F). Data are shown as the mean ± SD of n = 3 independent experiments. **$P < 0.01$, two-tailed Student's *t* test.
(J) $PRMT1^{WT}$ and $PRMT1^{S307A}$ knock-in HEK293T cells were subjected to cell proliferation assays. Data are shown as mean ± SD of n = 3 independent experiments. *$P < 0.05$, two-way ANOVA.
(K and L) $PRMT1^{WT}$ and $PRMT1^{S307A}$ HEK293T cells (Top) and PRMT1-depleted Huh7 cells reconstituted with PRMT1-WT or PRMT1-S307A (Bottom) were subjected to colony formation assays. Representative images were shown in (K) and quantification of colonies in (L). Data are shown as the mean ± SD of n = 3 independent experiments. **$P < 0.01$, two-tailed Student's *t* test.
(M) $WDR24^{R329K}$ knock-in Huh7 cells reconstituted with EV or WDR24-WT were subjected to cell proliferation assays. Data are shown as mean ± SD of n = 3 independent experiments. ***$P < 0.001$, two-way ANOVA.
(N and O) $WDR24^{R329K}$ knock-in Huh7 cells reconstituted with EV or WDR24-WT were subjected to colony formation assays. Representative images were shown in (N) and quantification of colonies in (O). Data are shown as the mean ± SD of n = 3 independent experiments. *$P < 0.05$, two-tailed Student's *t* test.
(P) Huh7 cells were treated with GSK3368715 or Rapamycin at indicated doses for 2, 4, or 6 days and then subjected to cell viability assays. Data are shown as the mean± SD of n=3 independent experiments.

Table S1. Ratio of S6K1-pT389/S6K1, pS6/S6, and p4E-BP1/4EBP1 in indicated Figures (Related to Figures 1, 3, 4, 6 and 7).

Fig. 1A		sgCtr -a.a.	sgCtr +a.a.	PRMT1/sg1 -a.a.	PRMT1/sg1 +a.a.	PRMT1/sg2 -a.a.	PRMT1/sg2 +a.a.
	S6K1-pT389/S6K1	0.00088	1.2	0.0017	0.0176	0.0019	0.00077
	pS6/S6	0.0016	0.79	0.0089	0.03	0.0015	0.004
	p4E-BP1/4E-BP1	0.0047	0.6	0.0043	0.27	0.004	0.008

Fig. 1B		sgCtr -a.a.	sgCtr +a.a.	PRMT1/sg1 -a.a.	PRMT1/sg1 +a.a.	PRMT1/sg2 -a.a.	PRMT1/sg2 +a.a.
	S6K1-pT389/S6K1	0.0060	0.5269	0.0076	0.0097	0.0101	0.0117
	pS6/S6	0.0130	0.6025	0.0119	0.0180	0.0150	0.0220
	p4E-BP1/4E-BP1	0.0024	0.1641	0.0016	0.0197	0.0016	0.0028

Fig. 1E		-Doxycycline -a.a.	-Doxycycline +a.a.	+Doxycycline -a.a.	+Doxycycline +a.a.
	S6K1-pT389/S6K1	0.004	0.56	0.02	1.15
	pS6/S6	0.02	0.33	0.017	1.35
	p4E-BP1/4E-BP1	0.004	0.3	0.0065	0.68

Fig. 3J		WT-a.a	WT+a.a	1#-a.a	1#+a.a	2#-a.a	2#+a.a	3#-a.a	3#+a.a
	S6K1-pT389/S6K1	0.0046	1.2	0.008	0.0053	0.0028	0.0042	0.0021	0.002
	p4E-BP1/4E-BP1	0.0725	0.72	0.043	0.38	0.029	0.15	0.01	0.05

Fig. 4B		Rap2a+ sgCtr-a.a	Rap2a+ sgCtr+a.a	Rap2a+ sgPRMT1-a.a	Rap2a+ sgPRMT1+a.a	RagB+ sgCtr-a.a	RagB+ sgCtr+a.a	RagB+ sgPRMT1-a.a	RagB+ sgPRMT1+a.a
	S6K1-pT389/S6K1	0.0023	0.78	0.013	0.39	1.26	1.9	1.42	1.39
	p4E-BP1/4E-BP1	0.005	0.84	0.009	0.2	1.1	1.04	0.86	0.84

Fig. 4C		sgCtr -a.a.	sgCtr +a.a.	sgPRMT1 -a.a.	sgPRMT1 +a.a.	sgPRMT1/ NPRL2-1-a.a.	sgPRMT1/ NPRL2-1+a.a.	sgPRMT1/ NPRL2-2-a.a.	sgPRMT1/ NPRL2-2+a.a.
	S6K1-pT389/S6K1	0.0022	0.48	0.0024	0.054	0.47	1.2	0.79	0.9
	p4E-BP1/4E-BP1	0.001	0.29	0.0009	0.063	0.42	0.68	0.53	0.77

Fig. 4D		sgCtr -a.a.	sgCtr +a.a.	sgPRMT1 -a.a.	sgPRMT1 +a.a.	sgKPTN -a.a.	sgKPTN +a.a.	sgPRMT1/ KPTN-a.a.	sgPRMT1/ KPTN+a.a.
	S6K1-pT389/S6K1	0.024	1.22	0.018	0.135	0.53	1.05	0.38	1.07
	p4E-BP1/4E-BP1	0.023	0.35	0.013	0.018	0.36	0.63	0.087	0.64

Fig. 4E		sgCtr -Dox-a.a	sgCtr -Dox+a.a	sgCtr +Dox-a.a	sgCtr +Dox+a.a	sgWDR24 +Dox-a.a	sgWDR24 +Dox+a.a
	S6K1-pT389/S6K1	0.0056	0.04	0.0054	0.46	0.0083	0.017
	pS6/S6	0.002	0.32	0.001	1.93	0.0022	0.089

Fig. 4F		sgCtr -Dox-a.a	sgCtr -Dox+a.a	sgCtr +Dox-a.a	sgCtr +Dox+a.a	sgWDR59 +Dox-a.a	sgWDR59 +Dox+a.a
	S6K1-pT389/S6K1	0.0007	0.37	0.001	0.59	0.0013	0.00065
	pS6/S6	0.009	0.035	0.0043	0.4	0.0055	0.0011

Fig. 6A		WT-a.a	WT+a.a	R329K-4# -a.a	R329K-4# +a.a	R329K-21# -a.a	R329K-21# +a.a
	S6K1-pT389/S6K1	0.008	0.38	0.001	0.0042	0.0017	0.023
	p4E-BP1/4E-BP1	0.038	0.81	0.039	0.023	0.031	0.16

Fig. 6E		WT-a.a	WT+a.a	R329K-a.a	R329K+a.a
	S6K1-pT389/S6K1	0.0087	0.75	0.05	0.42
	pS6/S6	0.13	0.9	0.32	0.09
	p4E-BP1/4E-BP1	0.049	0.57	0.028	0.13

Fig. 6F		WT-RagB -a.a	WT-RagB +a.a	WT+RagB -a.a	WT+RagB +a.a	R329K+RagB -a.a	R329K+RagB +a.a
	S6K1-pT389/S6K1	0.051	0.44	0.39	1.85	0.8	1.36
	pS6/S6	0.025	0.34	0.19	0.64	0.67	0.8
	p4E-BP1/4E-BP1	0.008	0.113	0.18	1.13	0.544	0.72

Fig. 6G		WT-sgNPRL2 -a.a	WT-sgNPRL2 +a.a	WT+sgNPRL2 -a.a	WT+sgNPRL2 +a.a	R329K+ sgNPRL2-a.a	R329K+ sgNPRL2+a.a
	S6K1-pT389/S6K1	0.06	0.51	0.96	3.1	1.92	2.2
	pS6/S6	0.027	0.49	0.34	0.62	0.67	1.2
	p4E-BP1/4E-BP1	0.001	0.58	0.42	0.4	0.12	0.88

Fig. 7G		WT-1	WT-2	WT-3	WT-4	R329K-1	R329K-2	R329K-3	R329K-4
	pS6/S6	0.66	0.65	0.34	1.1	0.033	0.014	0.023	0.15
	p4E-BP1/4E-BP1	0.27	0.95	0.5	0.026	0.005	0.008	0.006	0.003