Supplementary Figures

CARM1 Suppresses *de novo* Serine Synthesis by Promoting PKM2 Activity

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5 supplementary figures

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Figure S1

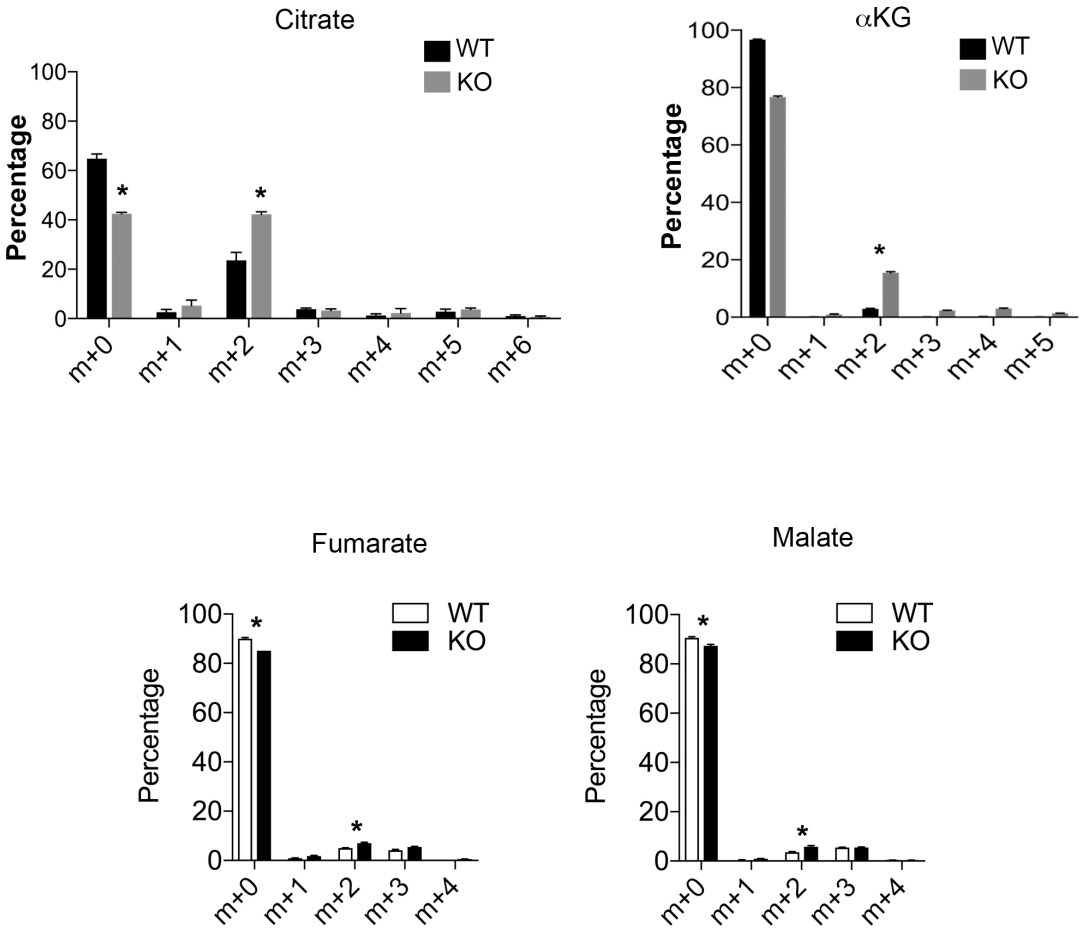

Figure S1. Glucose flux in wild type (WT) and Carm1-/- (KO) MEFs as determined by GC-MS. The levels of glucose-derived Citrate, alpha-Ketoglutarate (aKG), Fumarate, and Malate in WT and Carm1-/- (KO) MEFs were measured using GC-MS. Data represent mean ± SEM, n = 3).

Figure S2

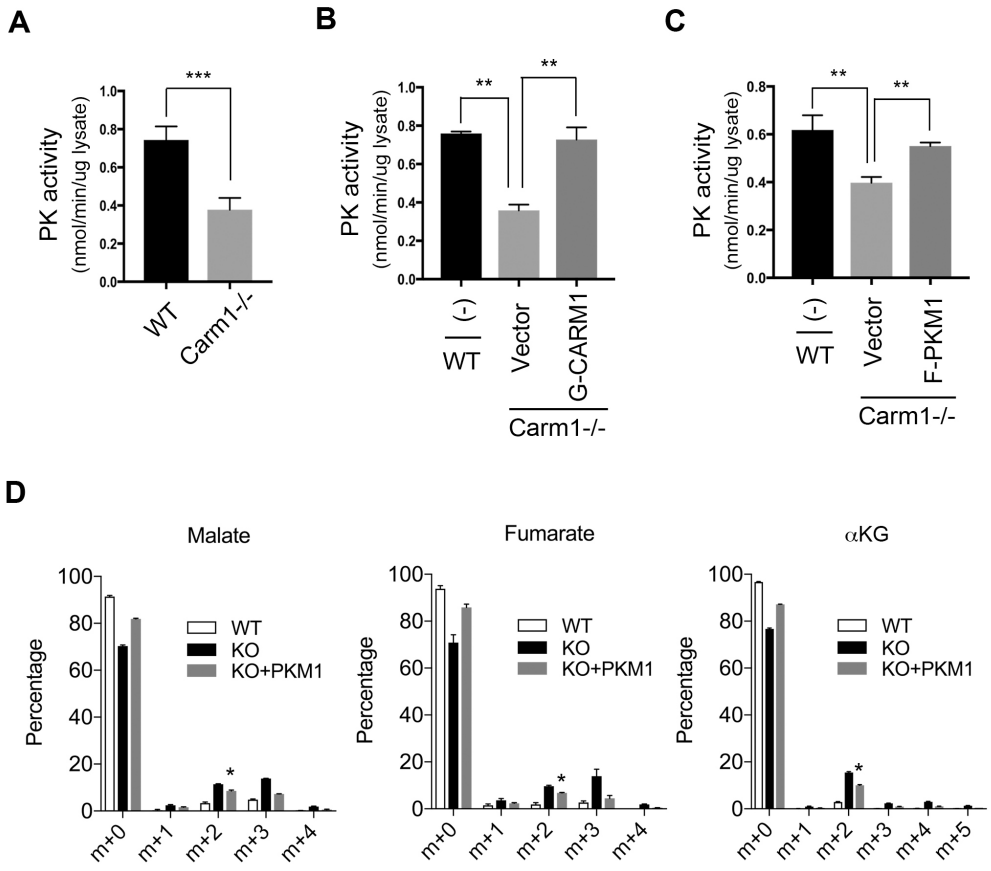

Figure S2. CARM1 promotes pyruvate kinase activity and restoration of PK activity by overexpressing PKM1 in CARM1-/- cells suppresses the elevation of glucose-derived TCA metabolites. (A) Carm1 knockout (-/-) MEFs exhibit lower pyruvate kinase (PK) activity than wild type (WT) MEFs. The PK activity was presented as the turnover of NADH calculated as the unit of nmol/min/ug cell lysate. **(B)** Re-expression of GFP-CARM1 in Carm1-/- MEFs restores PK activity. **(C)** Re-expression of Flag-PKM1 (F-PKM1) in Carm1-/- MEFs restores PK activity. **(D)** The levels of glucose-derived Malate, Fumarate, and alpha-Ketoglutarate (αKG) in wild type (WT), CARM1-/- (KO), and Flag-PKM1 transfected CARM1-/- (KO+PKM1) MEFs were measured using GC-MS. Data represent mean ± SEM, n = 3.

Figure S3

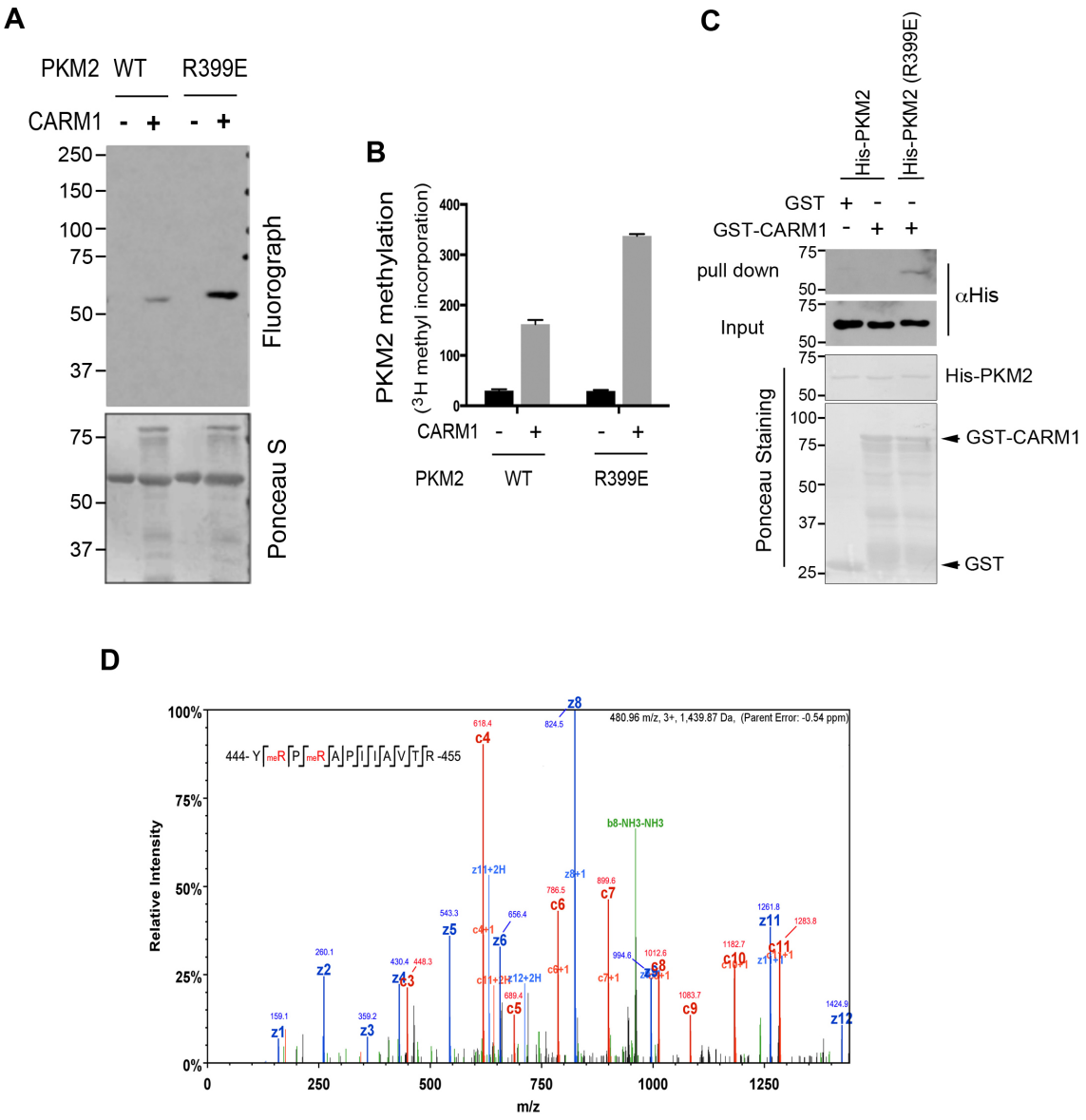

Figure S3. Characterization of PKM2 arginine methylation by CARM1. (**A**) Dimeric PKM2 is strongly methylated by CARM1. *In vitro* methylation assay was performed by incubating CARM1 with wild type (WT) or dimeric mutant PKM2 (R399E). (**B**) The methylation of WT and R399E mutant PKM2 was quantified by scintillation counting of the methylation products from (**A**). (**C**) CARM1 prefers to interact with dimeric (R399E) than the WT PKM2. An *in vitro* pull down experiment was performed by incubating GST-CARM1 with His-tagged WT or dimeric mutant (R399E) PKM2. (**D**) LC-MS/MS analysis of CARM1-methylated PKM2 protein identified mono-methylation of R445 and R447.

Figure S4

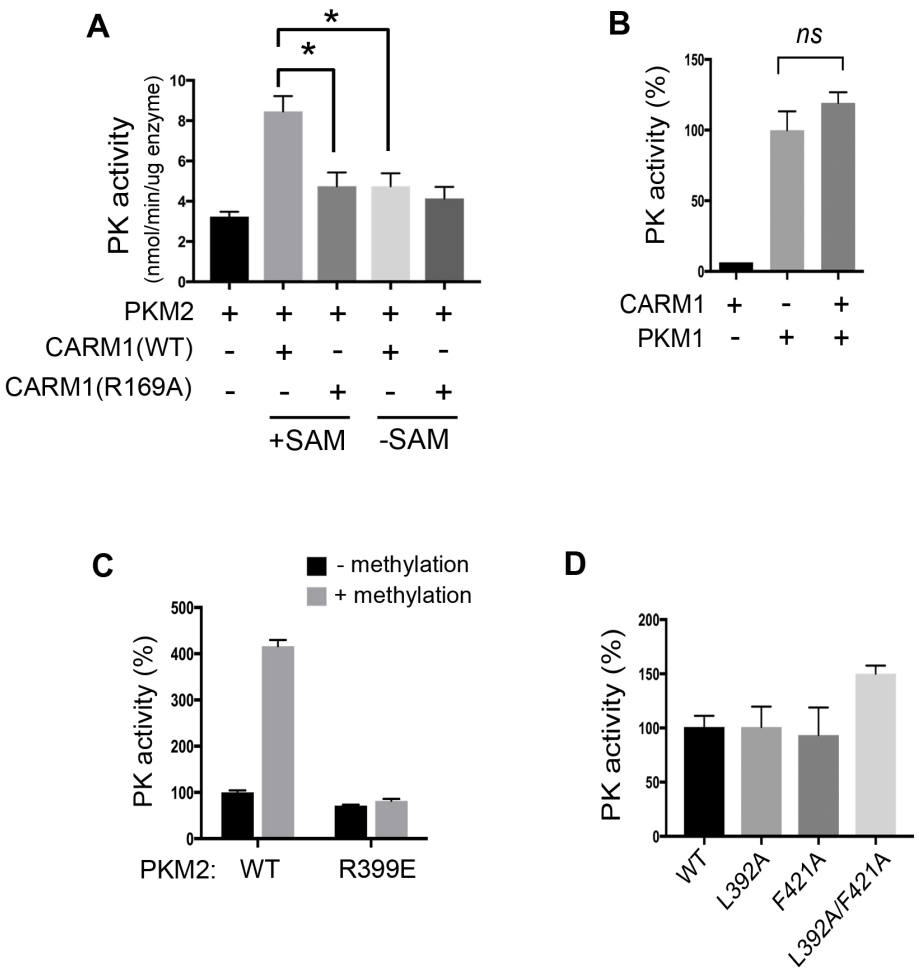

Figure S4. Characterization of PKM2 arginine methylation on its PK activity. (A) Arginine methylated PKM2 exhibits significantly greater PK activity than unmethylated PKM2. The PK activity was presented as the turnover of NADH calculated as the unit of nmol/min/ug recombinant enzyme. (**B**) Subjecting PKM1 to CARM1 methylation has no impact on its PK activity. An *in vitro* PK activity assay was performed using dialyzed methylation reaction products, as indicated. (**C**) Arginine methylation does not promote PK activity of dimeric mutant PKM2 (R399E). Wild type (WT) and dimeric mutant (R399E) PKM2 were subjected to *in vitro* methylation by CARM1. The reaction products were dialyzed and subjected to *in vitro* PK activity assays. (**D**) Mutations of amino acids involved in the intramolecular interactions of PKM2 does not affect basal PK activity. An *in vitro* PK activity assay was performed using recombinant WT and mutant (L392A, F421A, and L392A/F421A) PKM2 proteins.

Figure S5

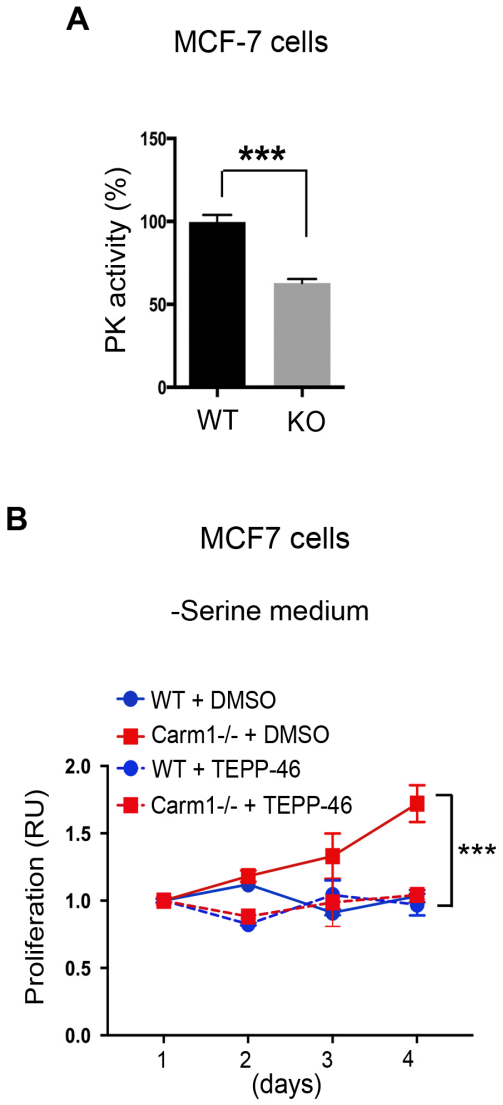

Figure S5. CARM1 knockout MCF7 cells exhibit reduced PK activity and are resistant to serine deprivation. (**A**) Knockout (KO) of CARM1 in MCF7 cells results in lower PK activity. An *in vitro* PK activity assay was performed using total cell lysates from wild type (WT) and CARM1 KO MCF7 cells. (**B**) CARM1 KO MCF7 cells are more resistant to inhibition of serine deprivation and activating PK activity using TEPP-46 abolishes its resistance. WT and CARM1 KO MCF7 cells were treated with DMSO or TEPP-46 (25 μM) and subjected to culture in serine-free medium over a four-day period. The cell proliferation was monitored as described in **(Figure 6)**.