SUPPLEMENTAL FIGURES

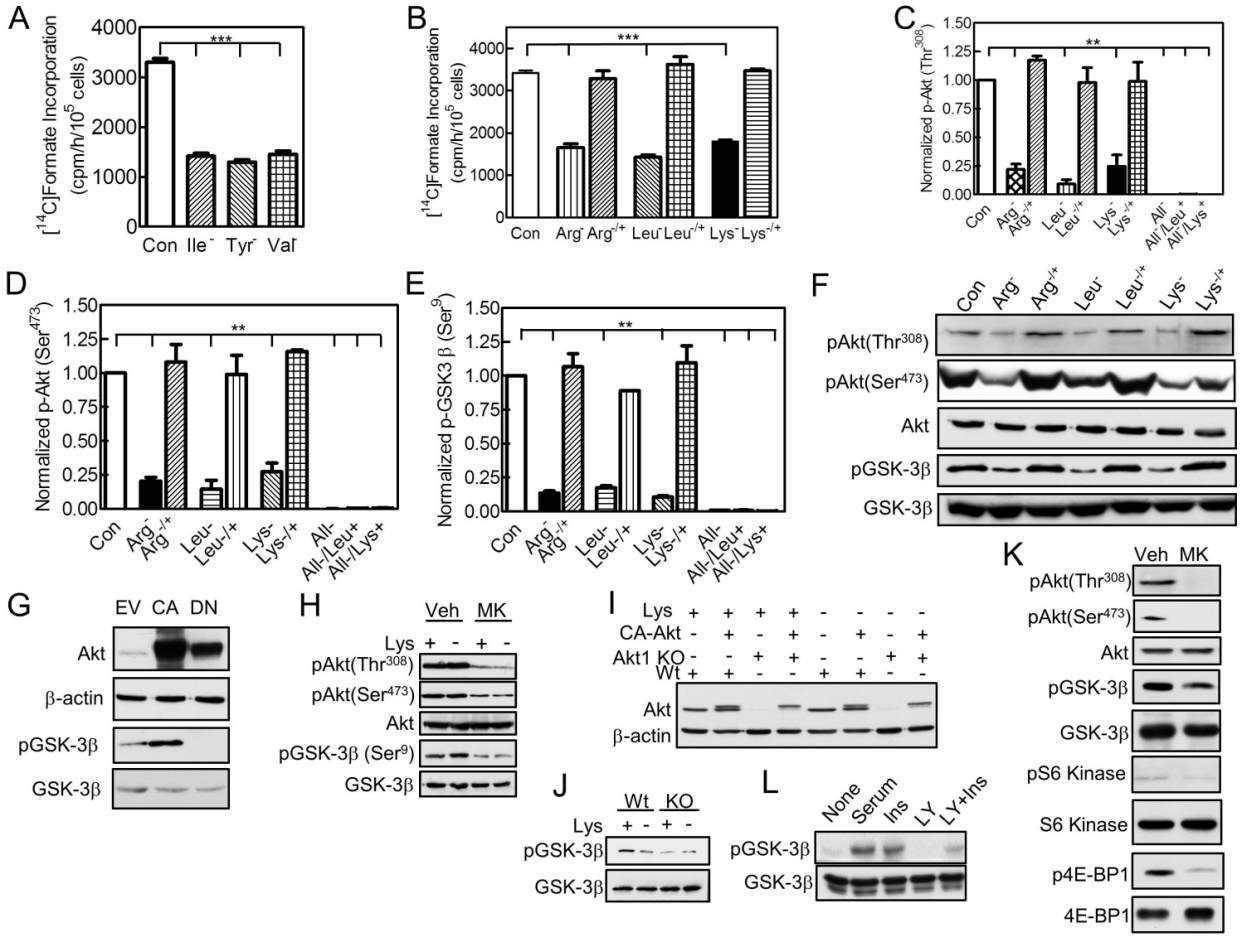


Figure S1, Related to Figure 1. Amino Acids Regulate *de novo* Purine Synthesis via Akt
(A) HeLa or **(B)** HCT116 cells were incubated for 3 h in full medium (Con) or medium lacking **(A)** isoleucine-, tyrosine-, or valine, or **(B)** arginine, leucine, or lysine; in the latter case, the deficient amino acid was added back for 1 h. Rates of *de novo* purine synthesis were measured as described in Figure 1A; data are the means ± S.E. of three independent experiments performed in duplicate. **(C-E)** Blots from three independent experiments described in Figure 1B were analyzed by densitometric scanning; **(C)** p-Akt(Thr^{308}), **(D)** p-Akt(Ser^{473}) and **(E)** p-GSK-3β. **(F)** HCT116 cells were incubated as described in B, and Akt Thr^{308} and Ser^{473} phosphorylation, and GSK-3β Ser^{9} phosphorylation were assessed by immunoblotting. **(G)** Expression of CA- and DN-Akt in HeLa cells was assessed by immunoblotting, and Akt activity was assessed by GSK-3β Ser^{9} phosphorylation. **(H)** HeLa cells were transfected with CA-Akt for 24 h, followed by a 3 h incubation in full (Lys +) or lysine-deficient (Lys -) medium containing vehicle (0.1% DMSO, Veh) or 1 μM MK2206 (MK). Akt Thr^{308}, Akt Ser^{473}, and GSK-3β phosphorylation and total GSK-3β protein were assessed by immunoblotting. **(I,J)** Wild type and Akt1 knock out (KO) MEFs were incubated for 3 h in lysine-deficient or sufficient media, and **(I)** Akt and β-actin protein, and **(J)** GSK-3β Ser^{9} phosphorylation and total GSK-3β were assessed by immunobloting; some of the cells in **I** had been transfected 24 h earlier with CA-Akt. **(K)** HeLa cells were treated with vehicle (0.1% DMSO, Veh) or 1 μM MK2206 (MK) for 3 h, and Akt Thr^{308}, Akt Ser^{473}, GSK-3β, S6 kinase, and 4EBP1 phosphorylation or total protein were assessed by immunoblotting. **(L)** HeLa cells were incubated in serum-deficient medium, and then treated with serum, insulin, LY294002, or LY294002 plus insulin as described in Figure 1F. GSK-3β Ser^{9} phosphorylation and total GSK-3β were assessed by immunobloting. *, **, and *** indicate $p < 0.05$, 0.01, and 0.001, respectively.

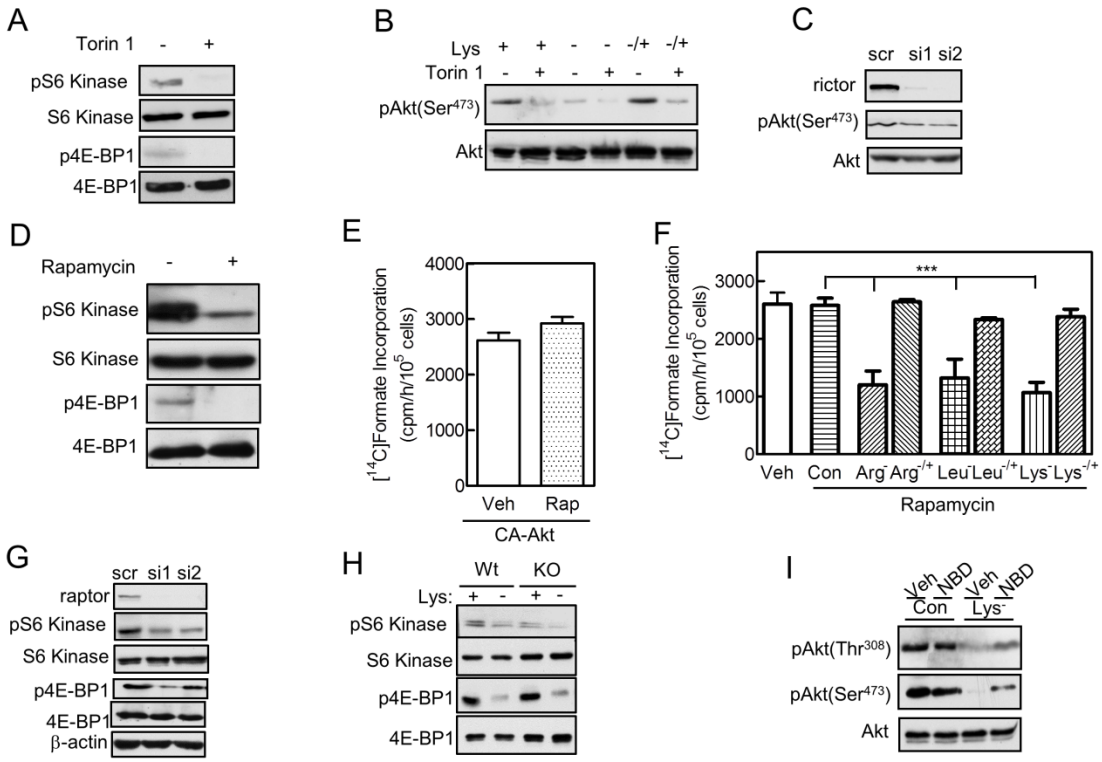
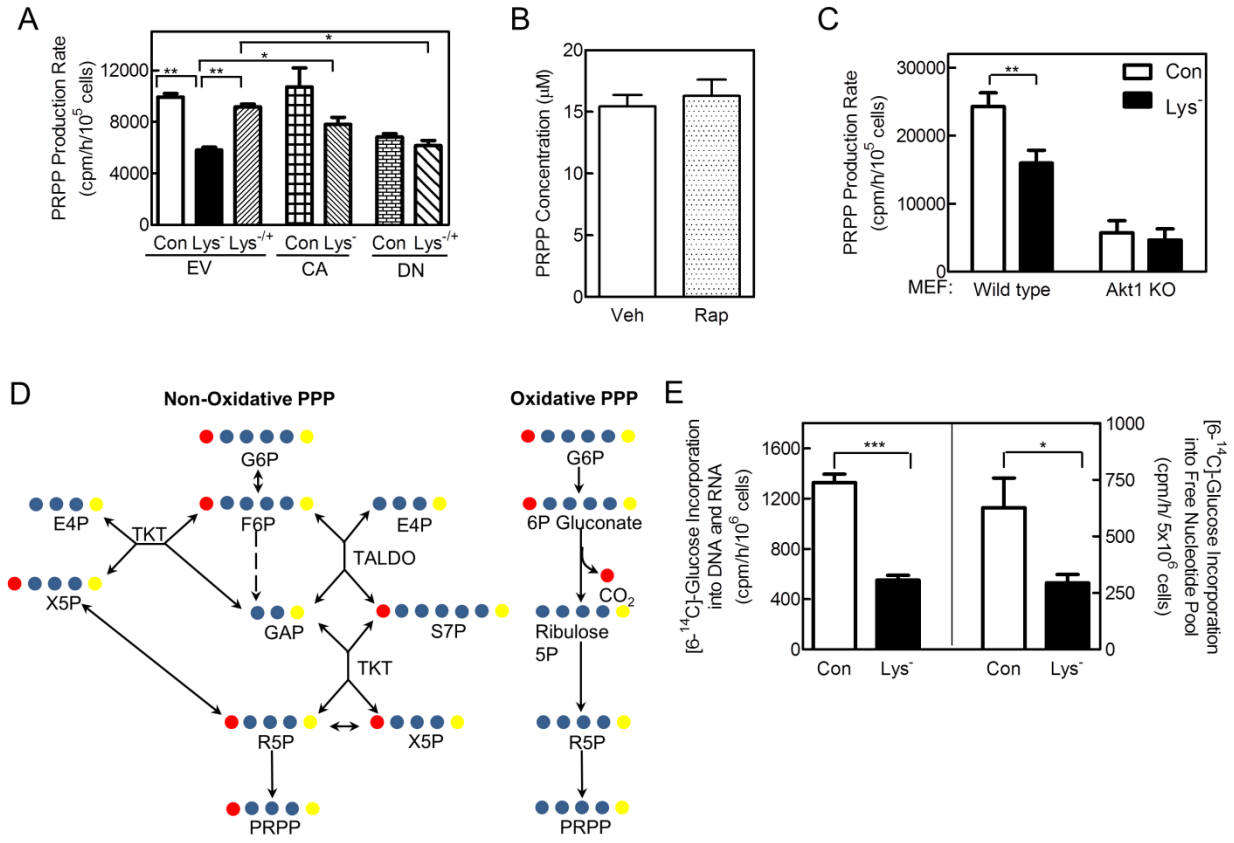


Figure S2. Related to Figure 2. Amino Acids Regulate Akt and Purine Synthesis via mTORC2 and IκB Kinase

(A,B) HeLa cells were treated with 250 nM Torin-1 as described in Figure 2A, and assessed for **(A)** S6 kinase and 4E-BP1, and **(B)** Akt Ser^{473} phosphorylation by immunoblotting. In **(B)**, some cells were deprived of lysine and then some were reconstituted with lysine. **(C)** HeLa cells were transfected with two different rictor siRNAs (si1 and si2) or a scrambled siRNA (scr) as described in Figure 2B, and rictor and total Akt expression and Akt Ser^{473} phosphorylation were assessed by immunoblotting. **(D)** HeLa cells were treated with rapamycin as described in Figure 2C, and assessed for S6 kinase and 4E-BP1 expression and phosphorylation by immunoblotting. **(E)** HeLa cells were transfected with CA-Akt, and 24 h later received vehicle (Veh, 0.1% DMSO) or 10 nM rapamycin (Rap); rates of purine synthesis were then measured over 3 h. **(F)** The experiment described in Figure 2C with HeLa cells was performed with HCT 116 cells. **(G)** HeLa cells were transfected with two different raptor siRNAs (si1 and si2) or a scrambled siRNA (scr) as described in Figure 2D, and assessed for raptor, S6 kinase, 4E-BP1, and β-actin expression, and S6 kinase and 4E-BP1 phosphorylation by immunoblotting. **(H)** Wild type and Akt1 knock-out (KO) cells were deprived of lysine for 3 h, and S6 kinase and 4EBP-1 expression and phosphorylation were assessed by immunoblotting. **(I)** HeLa cells were treated with vehicle (Veh, 0.1% DMSO) or 100 μM NBD peptide (NBD) as described in Figure 2F, and assessed for total Akt and Akt Thr^{308} and Ser^{473} phosphorylation by immunoblottting. *** indicate $p < 0.001$.



<u>Figure S3, Related to Figure 3.</u> Amino Acids Regulate PRPP Production by the Non-oxidative PPP via Akt

(A) HeLa cells were transfected and incubated as described in Figure 3A. Cellular PRPP production in intact cells was measured over 1 h by following [8-^{14}C]adenine incorporation into adenylates. Con, control; EV, empty vector; CA, CA-Akt; DN, DN-Akt. **(B)** HeLa cells were treated with vehicle (Veh, 0.1% DMSO) or 10 nM rapamycin (Rap) for 3 h, and then the intracellular PRPP concentration was measured as described in Figure 3A. **(C)** Wild type and Akt1 knock out (KO) cells were incubated in full (Con) or lysine-deficient (Lys$^-$) medium for 3 h, and then cellular PRPP production was measured as described in A. **(D)** Carbon flow through the oxidative and non-oxidative pentose phosphate pathway is depicted diagrammatically; the fate of carbon one (red circles) and six (yellow circles) of glucose 6-phosphate (G6P) is shown. F6P, fructose 6-phosphate; E4P, erythrose 4-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; GAP, glyceraldehyde 3-phosphate; R5P, ribose 5-phosphate; PRPP, phosphoribosylpyrophosphate; TKT, transketolase; TALDO, transaldolase. **(E)** Carbon flow through both the oxidative and non-oxidative pentose phosphate pathways was measured by following [6-^{14}C]glucose incorporation into DNA and RNA (left half) and the free purine nucleotide pool (right half). *, **, and *** indicate $p< 0.05$, 0.01, and 0.001, respectively.

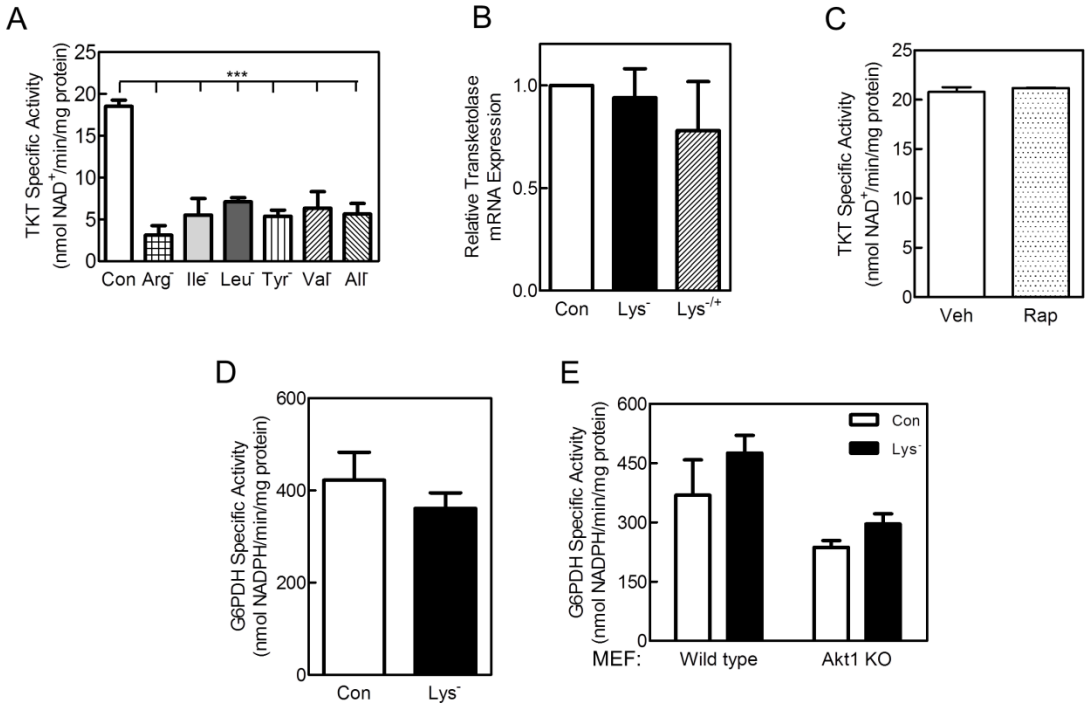


Figure S4, Related to Figure 4. TKT Activity and mRNA Expression, and Glucose 6-phosphate Dehydrogenase Activity During Amino Acid Deprivation

(A) HeLa cells were incubated for 3 h in full medium (Con) or in medium lacking arginine, isoleucine, leucine, tyrosine, valine, or all essential amino acids, and TKT activity was measured in cell extracts as described in Figure 4A. **(B)** HeLa cells were incubated in full medium (Con) or lysine-deficient medium (Lys^-), with some of the latter cells reconstituted with lysine ($Lys^{-/+}$). TKT mRNA was measured by RT-qPCR using glyceraldehyde 3-phosphate dehydrogenase mRNA as an internal control. **(C)** HeLa cells were treated for 3 h with vehicle (Veh, 0.1% DMSO) or 10 nM rapamycin (Rap), and then TKT activity was measured as described in Figure 4A. **(D,E)** HeLa **(D)** or MEF **(E)** (wild type or Akt1 knock out) cells were incubated in full (Con) or lysine-deficient (Lys^-) or medium for 3 h. Glucose 6-phosphate dehydrogenase (G6PDH) activity was measured in cell lysates based on a coupled reaction of NADP conversion to NADPH. In panel A, *** indicates $p< 0.001$ between control and the amino acid-deficient conditions; in the other four panels, no significant differences were found between control and lysine-deficient or rapamycin-treated conditions.

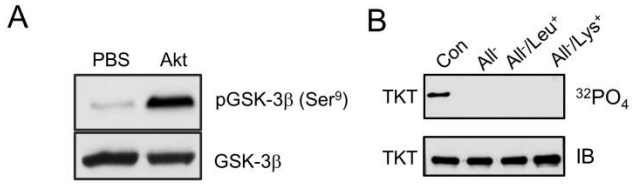
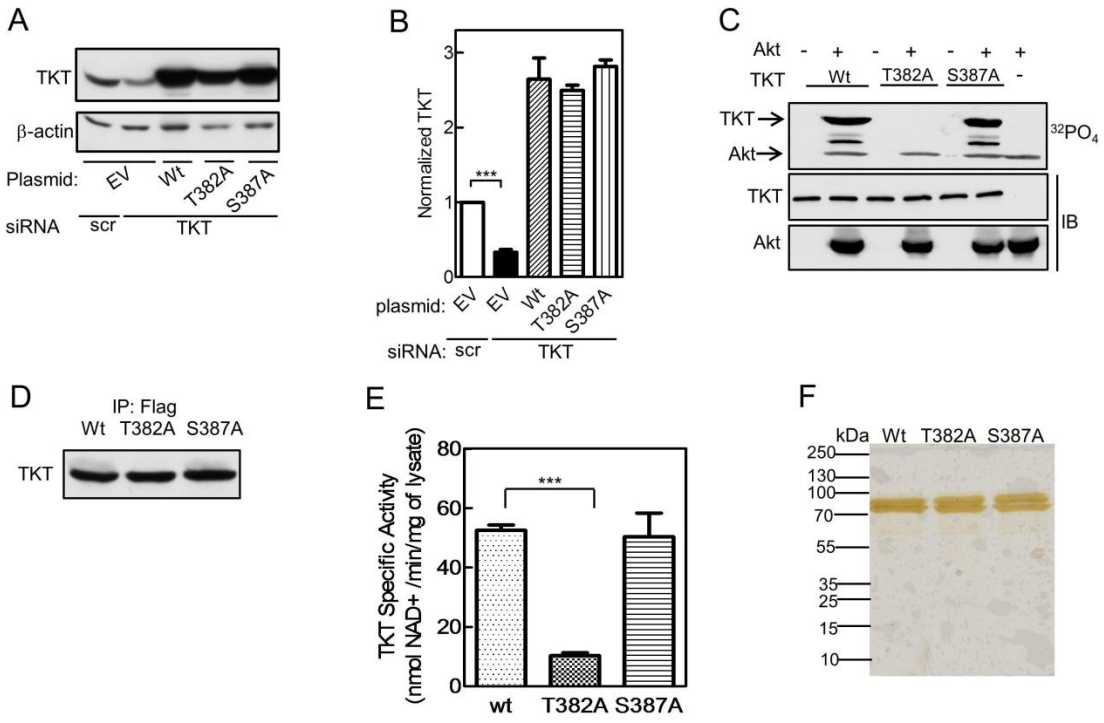


Figure S5, Related to Figure 5. Phosphorylation of GSK-β by Constitutively-Active Akt and *in vivo* TKT Phosphorylation

(A) Phosphate-buffered saline (PBS) or constitutively-active Akt (Akt) and 1 mM ATP were added to HeLa cell lysates at room temperature, and 30 min later SDS sample buffer was added. Phosphorylated GSK-3β(Ser^9) (upper blot) or total GSK-3β (lower blot) were assessed by immunoblotting. **(B)** *In vivo* phosphorylation of TKT was assessed as described in Figure 5E in cells that had been cultured either in full medium (Con) or in medium lacking all essential amino acids; leucine or lysine were added to some of the amino acid-deprived cells.



<u>Figure S6, Related to Figure 6.</u> TKT Thr^{382} Is an Akt Phosphorylation Site Critical to Enzyme Activity and Purine Synthesis

(A,B) HeLa cells were transfected with scrambled (scr) or a TKT-specific siRNA, and 48 h later they were transfected with empty vector (EV) or Flag-tagged wild type (Wt) or T382A or S387A mutant TKT. TKT and β-actin were assessed in cell lysates by immunoblotting. **A** shows a representative blot, and **B** shows a summary of three blots, with TKT expression in cells transfected with the scr siRNA and EV plasmid adjusted to a value of one (all conditions were normalized to the β-actin loading control). **(C)** Experiment was performed similarly to Figure 6E, except we included the S387A mutant TKT and used purified wild type Akt instead of CA-Akt, the latter to show that wild type Akt, as well as CA-Akt, phosphorylated wild type TKT. The two bands between TKT and Akt could be TKT break-down products or bacterial proteins phosphorylated by Akt. **(D,E)** Same experiment as in A,B, except anti-Flag antibody was added to the lysates, and TKT **(D)** protein and **(E)** activity in the immunoprecipitates was assessed. Wt, wild type. **(F)** His-tagged wild type and T382A and S387A mutant TKT were purified from *E.Coli*, and analyzed by SDS-PAGE and silver staining of the gels. TKT migrates on SDS gels with an apparent molecular weight of 78 kDa. *** indicates $p<0.001$.

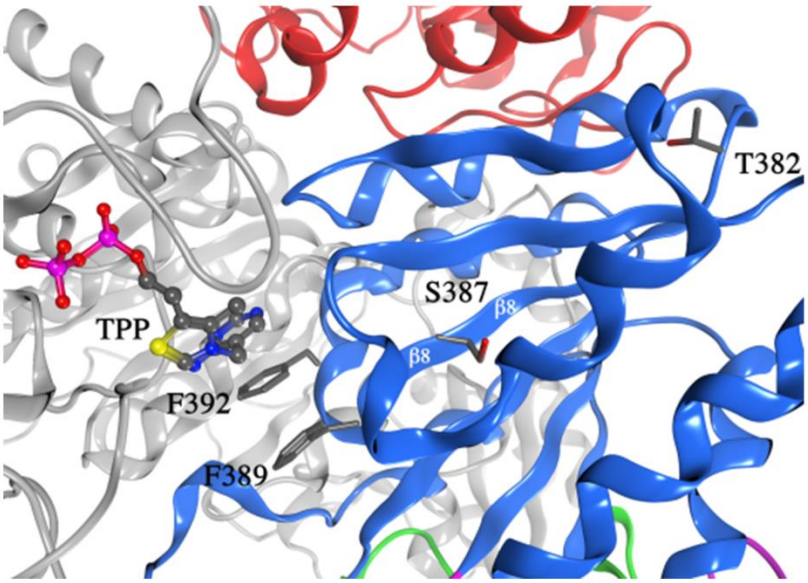


Figure S7, Related to Figure 6. Close-up view of the TKT dimer interface between the aminopyrimidine-binding (Pyr) and pyrophosphate-binding (PP) domains, which form the TPP cofactor and substrate-binding sites (ribbon representation; pdb 3MOS). One monomer is shown in grey, while the other monomer is colored by domain: PP domain in red and Pyr domain in blue. The Pyr domain comprises a parallel six-stranded β-sheet and three helices on each side, with Thr^{382} on the turn preceding the β8 strand and Ser^{387} on the strand. At the end of the β8 strand is a connecting 3/10 helix containing the aromatic residues Phe^{389} and Phe^{392}. These residues are critically important in binding the aminopyrimidine ring of thiamine pyrophosphate (TPP), the latter through a π-stacking arrangement (Mitschke,.et al, 2010).

SUPPLEMENTAL TABLES

Table S1, Related to Figure 3. Metabolite Analysis fom HeLa Cells

HeLa cells were cultured for 3 h in full medium (Con) or medium lacking lysine (Lys^-), with some lysine-deficient cells reconstituted with lysine for 1 h ($Lys^{-/+}$). Cell extracts were analyzed by LC/MS-MS, with 150 identified and 384 unidentified metabolites analyzed (Budczies et al, 2013)]. The data are expressed as the ratio of the value for the indicated metabolite from cells in the lysine-deficient medium (or lysine-reconstituted medium) to that in full medium, and were analyzed by a paired one-tailed Student t test. The nine metabolites that yielded a significant difference ($p < 0.05$) between the full and lysine-deficient medium are shown (first nine entries), while only representative examples of metabolites yielding a non-significant difference are shown (no unidentified metabolites yielded a significant difference between control and lysine-deprived cells). None of the metabolites from cells reconstituted with lysine showed a significant difference from cells in full medium.

Metabolite	$\frac{Lys^-}{Con}$	*p* value	$\frac{Lys^{-/+}}{Con}$	*p* value
Inosine	0.18	<0.05	1.08	>0.05
Guanosine	0.27	<0.05	1.24	>0.05
Hypoxanthine	0.19	<0.05	0.90	>0.05
Lysine	0.12	<0.001	0.85	>0.05
Nicotinamide	0.10	<0.05	.85	>0.05
Ribose	0.14	<0.05	1.26	>0.05
Uridine	0.24	<0.05	1.13	>0.05
Uracil	0.27	<0.05	0.92	>0.05
Xanthine	0.43	<0.05	1.15	>0.05
Adenosine	0.39	>0.05	1.24	>0.05
Aspartic Acid	0.97	>0.05	.845	>0.05
Cholesterol	1.49	>0.05	.97	>0.05
Glucose	1.69	>0.05	1	>0.05
Glutamine	1.37	>0.05	1.25	>0.05
Glycine	0.95	>0.05	0.9	>0.05
Pyruvic Acid	1	>0.05	1.29	>0.05
Serine	1.13	>0.05	0.93	>0.05
Tryptophan	1.12	>0.05	1.03	>0.05
Urea	1.26	>0.05	0.9	>0.05
Thymine	1.32	>0.05	1.22	>0.05
Tyrosine	1.07	>0.05	1.01	>0.05
Valine	1.17	>0.05	0.99	>0.05
3-phosphoglycerate	1.40	>0.05	1.05	>0.05

Table S2. **Akt Substrates Lacking an Arginine at -5 Position**

The table lists five human proteins (other than TKT described in this work) documented to be Akt substrates, both *in vitro* and *in vivo*. They all have the invariant arginine at the -3 position, but do not have an arginine at the -5 position.

Gene name	**Accession Number**	**Phosphorylated Sequence**	**Reference**
TKT (human)	AAH 08615.1	GCATRNR**pT**VPF (pThr382)	this manuscript
Beta1-catenin (human)	NP_001895.1	QDTQRRT**pS**MGG (pSer552)	Fang et al, 2007
Yes-associated protein (human YAP-1)	NP_001123617	PQHVRAH**pS**SPA (pSer127)	Basu et al, 2003
p47-Phox (human)	AAF 34737.1	GAPPRRS**pS**IRN (Ser 304) QDAYRRN**pS**VRF (Ser 328)	Hoyal et al, 2003
ATP-citrate synthase (human isoform-1)	NP_001087.2	PAPSRTA**pS**FSE (pSer454)	Berwick et al, 2002
cAMP-response element-binding protein (human CREB1)	NP_604391.1	EILSRRP**pS**YRK (pSer133)	Du and Montmini, 1998

Table S3, Related to Figure 7. Mouse Organ Weights

Mice were fed either normal chow (Con) or the same chow lacking lysine (Lys^-) for two days, and then were euthanized, and their livers, kidneys, and spleens were removed and weighed immediately. Data represent the mean ± SE from six mice in each group.

	Liver	Kidney	Spleen
	(mg)	(mg)	(mg)
Con	960±81	342±17	81±5
Lys^-	842±54	345±14	86±8

Table S4, Related to Figure 7. Plasma Amino Acid Concentrations in Mice

Mice were fed either normal chow (Con) or the same chow lacking lysine (Lys^-) for two days, and then were euthanized, and blood was removed by cardiac puncture. Plasma was analyzed for amino acids on an Hitachi amino acid analyzer. Data represent the mean ± SE from six mice in each group. Lysine was the only amino acid that showed a significant difference between control and lysine-deprived animals.

Amino acid	Con	Lys^-	P value
	(μM)	(μM)	
Alanine	504 ± 58.2	400 ± 73.3	P > 0.05
Arginine	73.9 ± 14.4	92.5 ± 12.8	P > 0.05
Asparagine	55.3 ± 8.51	42 ± 5.41	P > 0.05
Aspartate	15.8 + 0.735	17 ± 2.87	P > 0.05
Cysteine	3.09 ± 0.576	1.48 ± 0.258	P > 0.05
Glutamate	41.3 ± 9.08	35.7 ± 4.23	P > 0.05
Glycine	617 ± 67.9	525 ± 87.9	P > 0.05
Isoleucine	79.1 ± 11.4	74.7 ± 11.9	P > 0.05
Leucine	112 ± 20.45	119 ± 16	P > 0.05
Lysine	**414 ± 44.6**	**194 ± 28.8**	**P<0.001**
Methionine	90.8 ± 12.2	71.9 ± 23.6	P > 0.05
Phenyl-alanine	70.5 ± 12.5	72.5 ± 9.11	P > 0.05
Proline	91.6 ± 13.7	75 ± 5.1	P > 0.05
Serine	191 ± 15	182 ± 25.9	P > 0.05
Threonine	301 ± 25	251 ± 53.5	P > 0.05
Tryptophan	79.6 ± 7.52	68.5 ± 3.59	P > 0.05
Tyrosine	92.7 ± 13.4	58.6 ± 8.38	P > 0.05
Valine	208 ± 26.8	180 ± 31.9	P > 0.05