Supplemental Information

D-Serine mediates cellular proliferation for kidney remodeling

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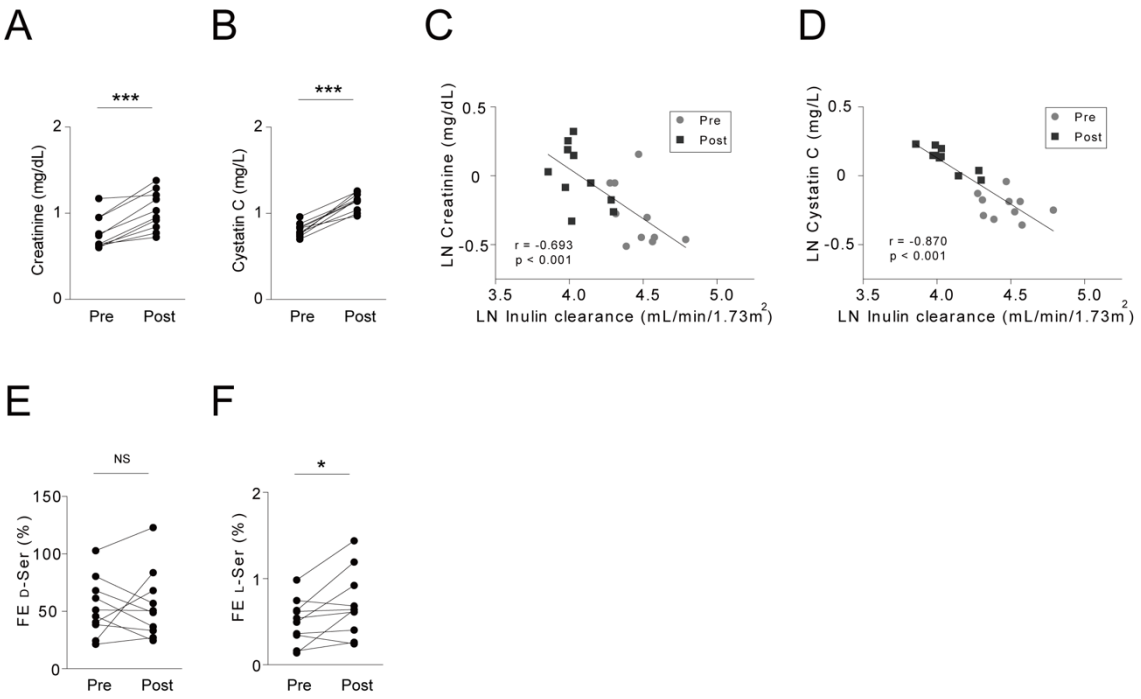
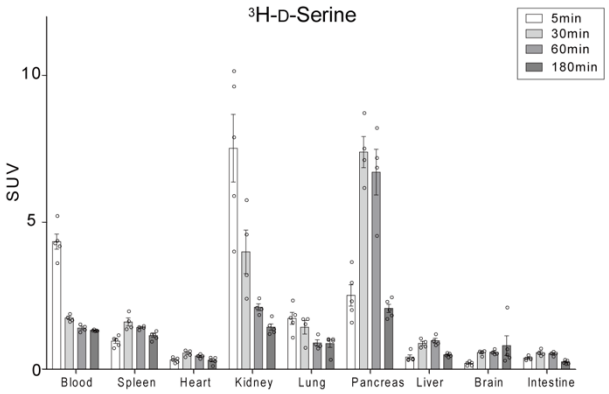
Supplemental Table 1. Characteristics of the participants.

	Before nephrectomy (n = 10)	After nephrectomy (n = 10)	*P*
Age, yr	60 (52 - 70)	62 (54 - 73)	0.002
Male gender, %	40 (4)	40 (4)	
Height, m	1.61 (1.53 - 1.68)	1.60 (1.53 - 1.67)	0.037
Weight, kg	59.2 (51.3 - 64.2)	57.8 (51.0 - 66.6)	0.281
BSA, m^2	1.61 (1.49 - 1.75)	1.60 (1.49 - 1.76)	0.591
BMI, kg/m^2	22.6 (19.8 - 24.5)	23.6 (20.2 - 24.1)	0.105
Serum creatinine, mg/dL	0.69 (0.63 - 0.90)	0.99 (0.86 - 1.20)	< 0.001
Serum cystatin C, mg/L	0.81 (0.76 - 0.84)	1.16 (1.07 - 1.21)	< 0.001
Inulin clearance, $mL/min/1.73m^2$	88.1 (76.1 - 95.0)	55.8 (54.0 - 61.4)	< 0.001

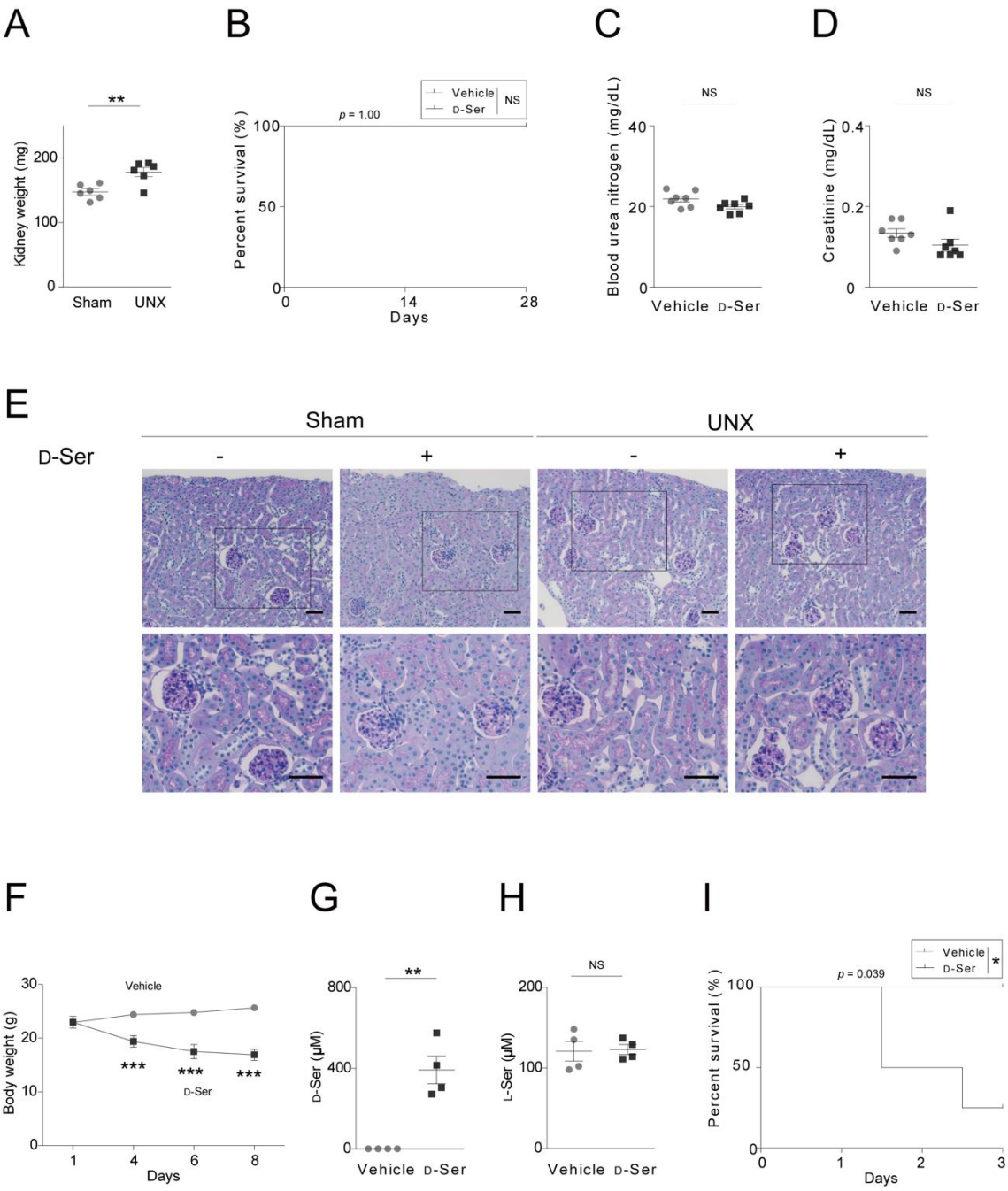
Values are described as median (IQR) or % (count). BSA, body surface area; BMI, body mass index. *P* values, paired two-tailed Student's *t*-test.



Supplemental Figure 1. Clinical parameters and serine enantiomer levels in living kidney donors before and after nephrectomy. (A and B) Serum levels of either creatinine (A) or cystatin C (B). *n* = 10; statistics, paired two-tailed Student's *t*-test. (C and D) Blood levels of creatinine (C) and cystatin C (D) were plotted with Inulin clearance. *n* = 20; statistics, Pearson's correlation. LN, log-natural transformed. (F and G) Fractional excretions (FE) of D- (F) and L-serine (G). *n* = 10; statistics, paired two-tailed Student's *t*-test. NS, not significant. **p* < 0.05, ****p* < 0.001.

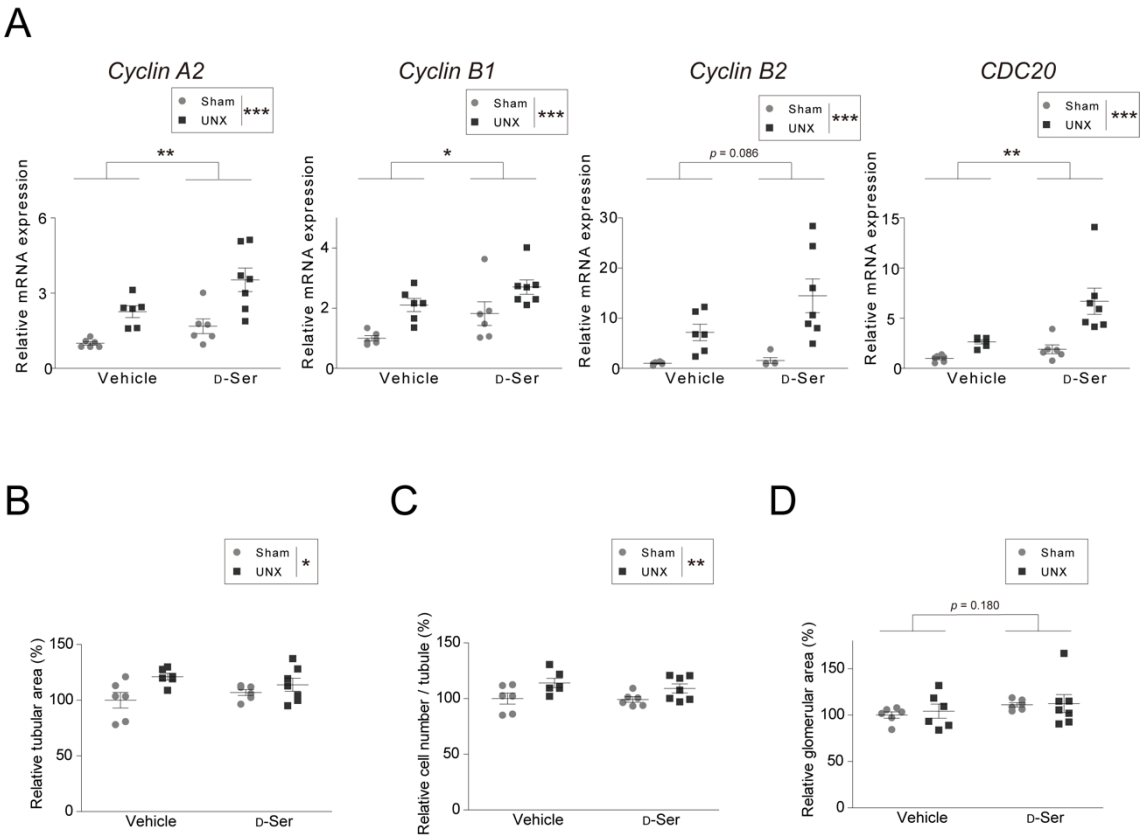


Supplemental Figure 2. D-Serine accumulates in the kidney. Standardized uptake values (SUV) were measured in each organ of 12-week-old Balb/c male mice at the indicated time points after intravenous injection with ^{3}H-labelled D-serine. Original data of Fig. 2A, including those of brain and intestine, were shown. n = 4–5. Data, mean ± SEM.



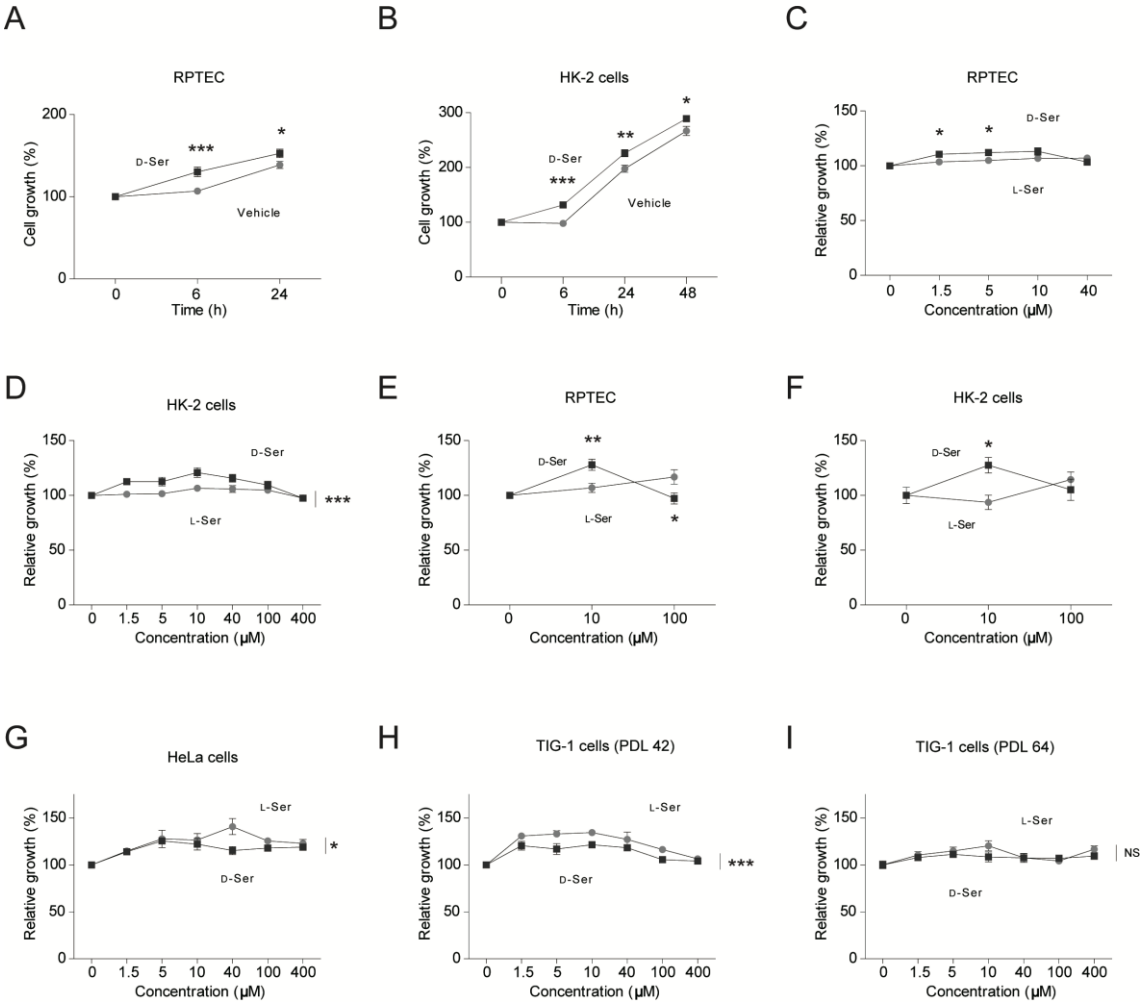
Supplemental Figure 3. Effects of D-serine on unilateral nephrectomy in mice. (A) Kidney weight was measured in 10-week-old C57 BL/6 male mice that had been fed with a serine-free diet for one week, subjected to either unilateral nephrectomy (UNX) or sham operation, and then sacrificed two days after operation. *n* = 6; statistics, unpaired two-tailed Student's *t*-test. (B) Survival analysis of mice treated with D-serine at low-dose (free access to water with 0.1%

D-serine.) $n = 7$; statistics, log-rank test. (C and D) Plasma levels of urea nitrogen (C) and creatinine (D) of mice treated with either vehicle or D-serine at low-dose (with free access to water) with 0.1% D-serine for 28 days. $n = 7$; statistics, unpaired two-tailed Student's *t*-test. (E) Representative images of kidney cortex of mice treated with either vehicle or D-serine at low-dose (free access to water with 0.1% D-serine) for 28 days. Bars, 50 μm. (F to H) Mice were fed with a serine-free diet and with either vehicle or D-serine at high-dose (with free access to water with 1% D-serine) for 8 days, then sacrificed. (F) Time course of body weight. $n = 4$; statistics, two-way repeated-measures ANOVA ($p < 0.001$ for interaction effect). Plasma levels of D- (G) and L-serine (H). $n = 4$; statistics, unpaired two-tailed Student's *t*-test. (I) Survival analysis of mice treated with D-serine at high-dose (75 μmol/g) intraperitoneally. $n = 4$; statistics, log-rank test. NS, not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data, mean ± SEM.



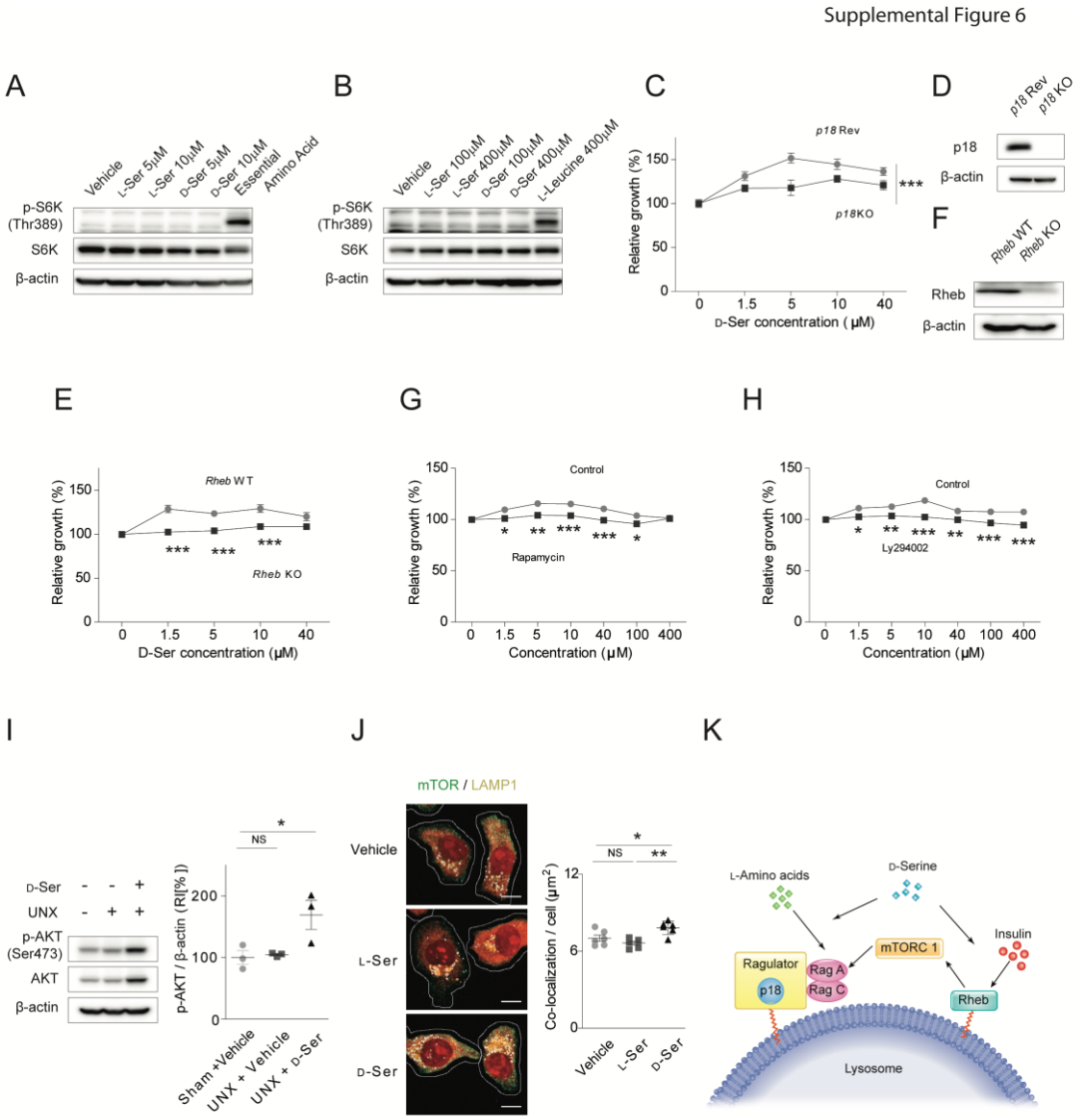
Supplemental Figure 4. D-Serine activates cell cycle in kidney. (A to D) Mice were fed with a serine-free diet and water with or without 0.1% D-serine for one week, subjected to either UNX or sham operation, and then sacrificed two days after operation. (A) Relative expressions of cell cycle-related genes. n = 6–7; statistics, two-way ANOVA (*p < 0.05, and **p < 0.01 for main effect of D-serine; ***p < 0.001 for main effect of operation). Relative (B) tubular area and (C) cell number per tubule, and (D) glomerular area. n = 6–7; statistics, two-way ANOVA ((B) *p < 0.05, and (C) **p < 0.01 for main effect of operation). Data, mean ± SEM.

Supplemental Figure 5



Supplemental Figure 5. D-Serine activates cellular proliferation. (A and B) Time course of relative growth of normal human primary renal proximal tubular cells (RPTEC; A) and HK-2 cells (B) treated with or without 10 μM of D-serine in a serine-free medium. n = 12 (A) and n = 6 (B); statistics, two-way ANOVA (p < 0.05 for interaction effect). (C and D) Relative growth of RPTEC (C) and HK-2 cells (D) treated with either D- or L- serine at indicated concentrations in a serine-free medium for 6 h. n = 12; statistics, two-way ANOVA ((C) p < 0.05 for interaction effect; (D) ***p < 0.001 for main effect of chirality; p < 0.001 for main effect of concentration). (E and F) Relative growth of RPTEC (E) and HK-2 cells (F) treated with either D- or L- serine at indicated concentrations in a serine-free medium for 6 h. Cell number was counted manually. n = 12 (E) and n = 6 (F); statistics, two-way ANOVA ((E) p < 0.05 for interaction effect; (F) p < 0.001 for interaction effect). (G) Relative growth of HeLa cells treated with either D- or L-

serine at indicated concentrations in a serine-free medium for 6 h. *n* = 6; statistics, two-way ANOVA (*$p < 0.05$ for main effect of chirality; $p < 0.001$ for main effect of concentration). (H and I) Relative growth of TIG-1 cells at earlier (population doubling level [PDL], 42; H) and later passages (PDL, 64; I) treated with either D- or L- serine at indicated concentrations in a serine-free medium for 6 h. *n* = 6; statistics, two-way ANOVA ((H) ***$p < 0.001$ for main effect of chirality; $p < 0.001$ for main effect of concentration; (I) $p < 0.01$ for main effect of concentration). NS, not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data, mean ± SEM.



Supplemental Figure 6. D-Serine activates mTOR-related pathway. (A and B) Immunoblots for phospho-S6K (p-S6K) at Thr389 from HK-2 cells that were treated with either D- or L-serine at various concentrations for 10 min in an amino acids-free medium. Either essential amino acids or L-leucine were used as positive control. Representative images of 2 independent experiments. (C) Relative growth rates of *p18*-deficient mouse embryonic fibroblasts (MEF) and their revertants (Rev) treated with D-serine at indicated concentrations in a serine-free medium for 6 h to those of vehicle-treated corresponding cell lines. $n = 6$; statistics, two-way ANOVA (***$p < 0.001$ for main effect of genotype; $p < 0.001$ for main effect of concentration). (D) Relative growth rates of *Rheb*-deficient or –wild type MEF treated with D-serine at indicated concentrations in a serine-free medium for 6 h to those of vehicle-treated corresponding cell lines. $n = 12$;

statistics, two-way ANOVA ($p < 0.01$ for interaction effect). (E) Immunoblots for p18 proteins of *p18*-deficient MEF and their revertants. (F) Immunoblots for Rheb of *Rheb*-deficient and –wild type MEF. (G) Relative growth rates of HK-2 cells that had been treated with or without rapamycin for 24 h and were treated with D-serine at indicated concentrations in a serine-free medium for 6 h to those of D-serine-untreated cells for corresponding treatment. n = 21-24; statistics, two-way ANOVA ($p < 0.01$ for interaction effect). (H) Relative growth rates of HK-2 cells that were treated with D-serine at indicated concentrations and with or without Ly294002 simultaneously in a serine-free medium for 6 h to those of D-serine-untreated cells for corresponding treatment. n = 21-24; statistics, two-way ANOVA ($p < 0.01$ for interaction effect). (I) Immunoblots for phospho-AKT (p-AKT) at Ser473 from the kidney cortexes of 10-week-old C57 BL/6 male mice that had been fed with a serine-free diet and water with or without 0.1% D-serine for one week, subjected to unilateral nephrectomy (UNX) or not, and then sacrificed two days after operation. Representative images of 3 independent experiments and their quantification. RI, relative index. Statistics, one-way ANOVA with Dunnett's post-hoc test. (J) High content microscopy quantification of mTOR and LAMP1 colocalization in revertants mouse embryonic fibroblasts (MEF) that were incubated in a culture medium, then starved for amino acids in the presence of 5 μM of either D- or L-serine for 10min. Mask overlay, primary objects (Algorithm-defined cellular boundaries based on CellMask (pseudo-color in red)), and internal secondary objects (computationally-defined colocalization between mTOR (green) and LAMP1 (yellow). Scale bars, 10 μm. n = 6; statistics, one-way ANOVA with Bonferroni's post-hoc test. (K) Schematic summary of D-serine on activation of mTORC1 signal. D-Serine augments signals from L-amino acids to the activation of mTORC1. D-Serine also activates mTROC1 through the phosphoinositide 3-kinase (PI3K)/Rheb pathway. NS, not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data, mean ± SEM.