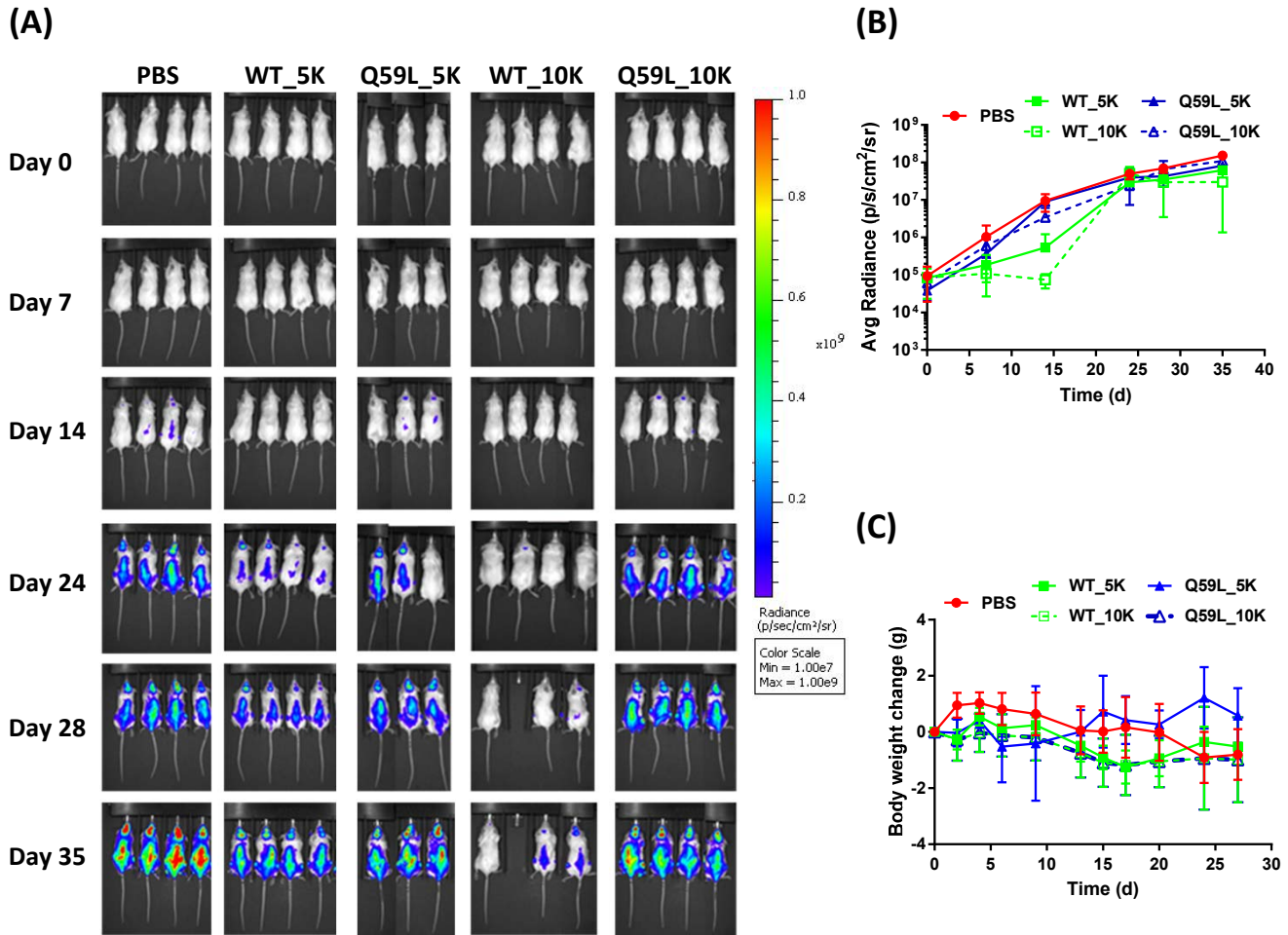
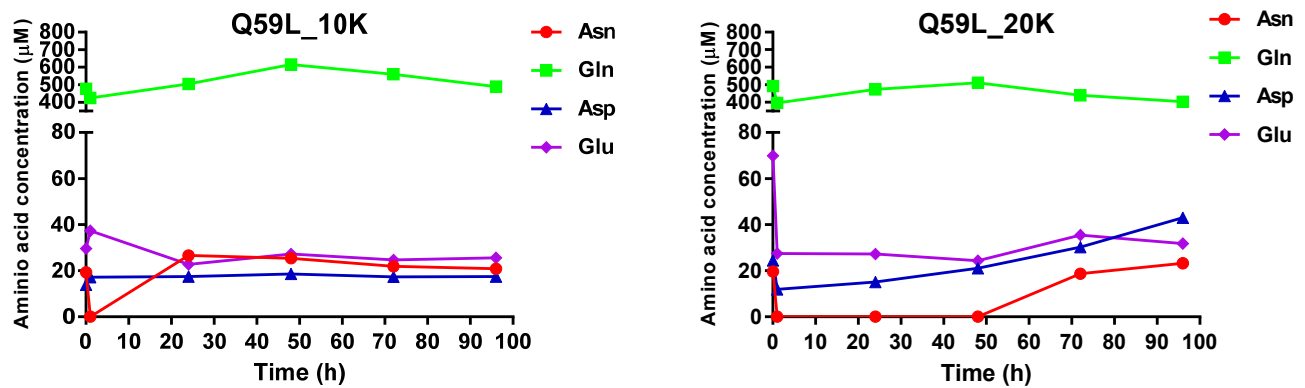


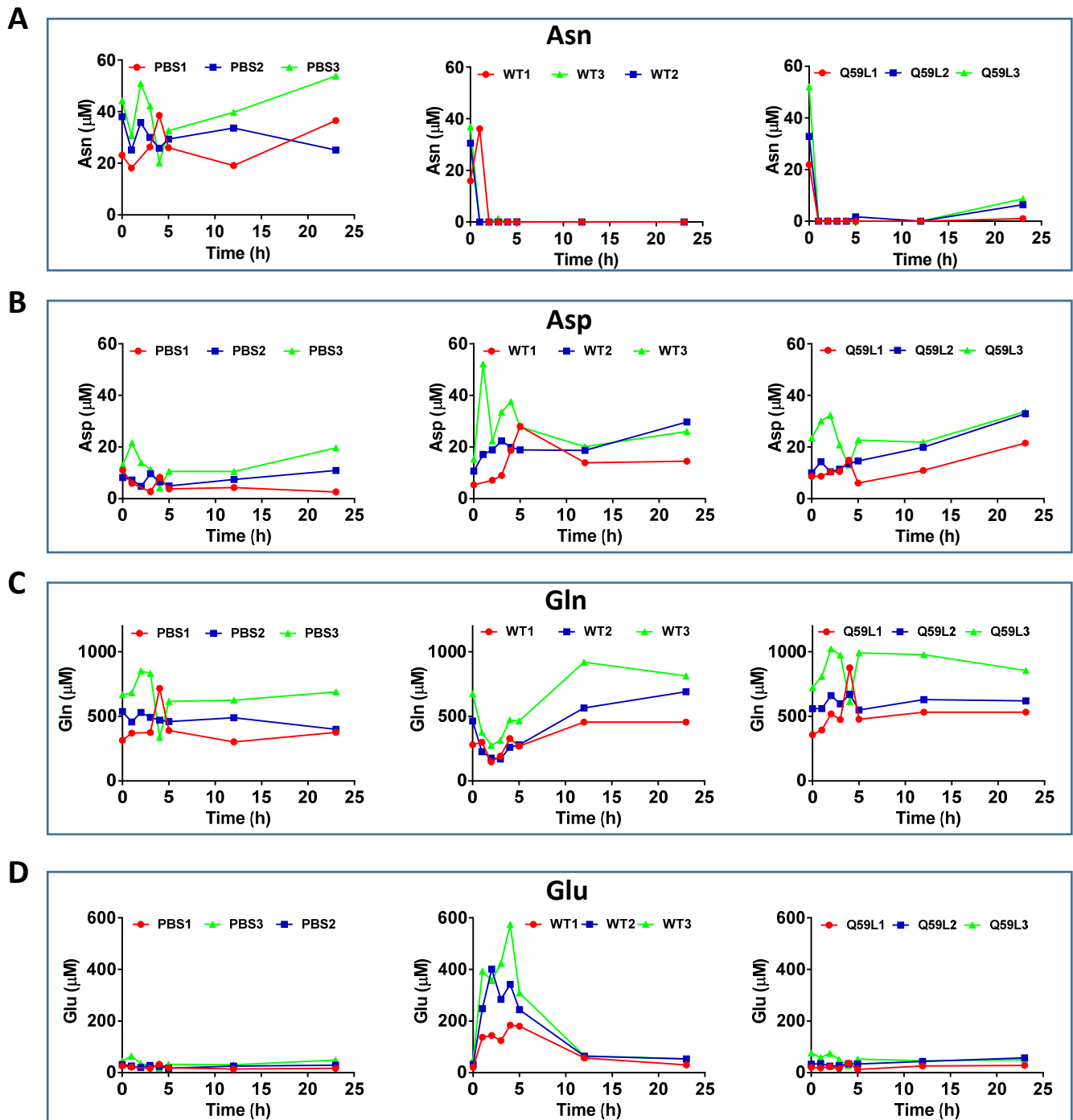
Supplementary Figures and Tables



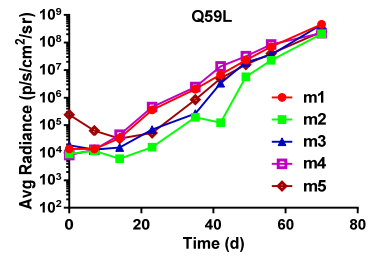
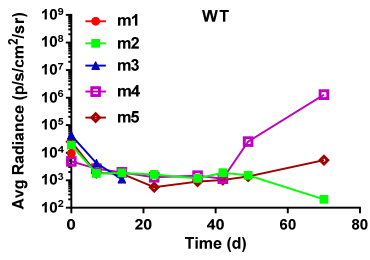
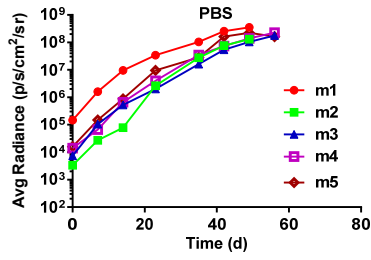
Supplemental Figure S1. Anticancer activity of ASNase^{WT} and ASNase^{Q59L} *in vivo* at the dose of 5,000 U/kg or 10,000 U/kg body weight. **(A)** NSG mice xenografted with the luciferase engineered leukemia cell line Sup-B15 were administered daily with the indicated dose for two weeks by intraperitoneal injection. The bioluminescence signals of tumor burden in each mice were measured at the indicated time point. Day 0 was assigned as one day before the treatment. PBS, phosphate-buffered saline, was a negative control. **(B)** The average of the bioluminescent signal of tumor burden in each treatment group as described in the (A). **(C)** The average of body weight loss of each group in panel A at the indicated time. WT_5K and WT_10K are the ASNase^{WT} treatments of 5,000 U/kg and 10,000 U/kg body weight, respectively. Q59L_5K and Q59L_10K are the ASNase^{Q59L} treatments of 5,000 U/kg and 10,000 U/kg body weight, respectively. Mean and SEM are shown.



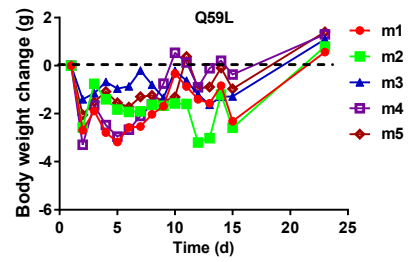
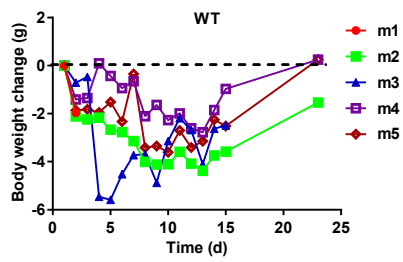
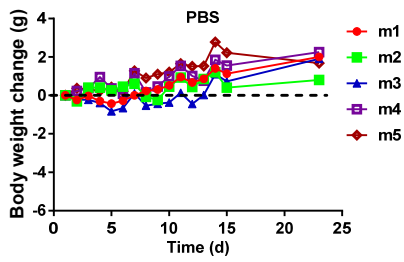
Supplemental Figure S2. Pharmacodynamics of ASNase^{Q59L} *in vivo*. The pharmacodynamics of ASNase^{Q59L} in the plasma of non-tumor bearing NSG mice was determined by LC-MS/MS-based analysis of the amino acids asparagine (Asn), aspartate (Asp), glutamine (Gln) and glutamate (Glu). NSG mice were treated with (A) Q59L 10,000 U/kg body weight (Q59L_10K) or (B) 20,000 U/kg body weight (Q59L_20K) by intraperitoneal injection. Time 0 measurements were made on samples collected immediately before injection.



Supplemental Figure S3. Concentration of the four amino acids (Asn, Asp, Gln and Glu) in the plasma of individual mice in each treatment group during the 14-day ASNase treatment as described in **Figure 1**.



Supplemental Figure S4. The tumor burden of individual mice in each treatment group during the 14-day ASNase treatment as described in **Figure 2**.



Supplemental Figure S5. The daily body weight loss of individual mice in each treatment group during the 14-day ASNase treatment as described in **Figure 2**.

Supplemental Table I. The paired *t*-test *p*-value on the PK/PD studies in **Figure 1**.

(a)

ASN (<i>p</i> value)	Time (h)					
	0	1	2	4	12	23
PBS vs. WT	0.452553	0.371558	0.004475	0.006503	0.007274	0.009951
PBS vs. Q59L	0.965276	0.002473	0.004512	0.006503	0.007274	0.018629
WT vs. Q59L	0.507221	0.379173	0.373901	n.a.	n.a.	0.078111

(b)

GLN (<i>p</i> value)	Time (h)					
	0	1	2	4	12	23
PBS vs. WT	0.839007	0.120649	0.032246	0.357857	0.29162	0.320543
PBS vs. Q59L	0.801083	0.605574	0.86326	0.216129	0.194258	0.264568
WT vs. Q59L	0.662752	0.089075	0.025883	0.750284	0.022105	0.916078

(c)

ASP (<i>p</i> value)	Time (h)					
	0	1	2	4	12	23
PBS vs. WT	0.931419	0.212169	0.395433	0.037025	0.017354	0.140849
PBS vs. Q59L	0.540964	0.495621	0.468065	0.004598	0.056619	0.044178
WT vs. Q59L	0.552668	0.352387	0.868545	0.13096	1	0.379064

(d)

GLU (<i>p</i> value)	Time (h)					
	0	1	2	4	12	23
PBS vs. WT	0.997547	0.042356	0.076252	0.039348	0.002042	0.317667
PBS vs. Q59L	0.632083	0.988859	0.586254	0.177617	0.127171	0.352596
WT vs. Q59L	0.653752	0.041618	0.032932	0.042189	0.026604	0.983032

(e)

ASNase Activity (<i>p</i> value)	Time (h)					
	0	1	2	4	12	23
PBS vs. WT	0.050151	0.013634	4.5E-06	0.000247	0.018981	0.004237
PBS vs. Q59L	0.556883	2.95E-05	0.00015	3.69E-06	0.001816	0.005905
WT vs. Q59L	0.518519	0.235693	0.020594	0.07323	0.635191	0.106166