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Supplementary Materials for

Engineered binding to erythrocytes induces immunological tolerance to *E. coli* asparaginase

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This PDF file includes:

SUPPLEMENTARY MATERIALS

SUPPLEMENTARY MATERIALS AND METHODS

Antibodies for flow cytometry

The following anti-mouse antibodies were used for flow cytometry: IgD Pacific Blue, CD45R (B220) PerCP-Cy5.5, IgM PE-Cy7, CD21/35 biotin, CD23 PE, CD23 PE, CD3ε PE-Cy7, CD11b PerCP-Cy5.5, CD11c-APC, Gr-1 biotin, CD3ε FITC, CD25 PE, FoxP3 PerCP-Cy5.5 (all from eBioscience), CD8 BV650, CD24 PE, MHCII I-A/I-E FITC, CD4 Pacific Blue, CD19 PE, CD45 APC-Cy7, CD19 APC, MHCII I-A/I-E AlexaFluor647 (all from Biolegend), rabbit anti-asparaginase (polyclonal, Abnova), chicken anti-rabbit IgG-AlexaFluor488, fixable live/dead dye, streptavidin AlexaFluor488 (all from LifeTechnologies), annexin-V Cy5 labeling kit (BioVision). Samples were analyzed on a CyAn ADP Analyzer (Beckman Coulter) and a LSR-II (BD Biosciences).

Plasma asparagine quantification

For pharmacodynamics studies, 15 µg of ERY1-ASNase or WT ASNase in 100 µL of 0.9% saline was injected into the tail vein of anesthetized mice. At predetermined time points, 30 µL of whole blood was collected into EDTA tubes, centrifuged at 1200 g for 10 minutes, and plasma was stored at -80 C for analysis.

A solution containing 2.5 mM asparagine, glutamine, tryptophan and allo-isoleucine in 0.1 N HCl was prepared (solution A). Premixed solutions of 34 amino acids and amino acid metabolite standards at 2.5 mM (Beckman USA, AN+, STD and B standards) were diluted with solution A to yield a final stock solution with a concentration of 0.35 mM for all amino acids. The resulting stock solution was further diluted in 60 mg/mL BSA in PBS to form calibration solutions at final concentrations of 10, 20, 40, 60, 80 and 100 µM. An internal standard mix of 12 stable isotopes (Cambridge Isotope Laboratories, labeled amino acids set A) was used completed with ^{13}C6-histidine, d_4-lysine, ^{13}C4, ^{15}N-threonine, d_7-proline and d_3-serine (Cambridge Isotope Laboratories). The resulting internal standard solution contained labeled amino acids at a concentration of 1.25 mM in 0.1 % (v/v) formic acid in water, except for ^{13}C, ^{15}N-glycine, which were 6.25 mM. Calibration standards or plasma samples (5 µL) were added to 45 µL of 0.1 % (v/v) formic acid and mixed with 250 µL of acetonitrile containing the internal standards at a final concentration of 5 µM (except for ^{13}C, ^{15}N-glycine at 25 µM) in a 96 x 1 mL deep well plate. After shaking for 20 min at 1200 rpm, the plate was incubated for 10 min at 4 C and centrifuged at 4,000 g for 10 min). The supernatant (200 µL) was transferred to a 96-well plate, sealed, and transferred to the LC-MS autosampler for analysis.

Chromatographic analysis was performed using an Accela pump (Thermo Scientific) and a PAL System autosampler (Thermo Scientific, CTC Analysis). Separation of the amino acids was achieved using an Atlantis HILIC silica column (3 µm 2.1 mm x 150 mm, Waters). The mobile phases consisted of water (mobile phase A), acetonitrile (mobile phase B) and ammonium formate (200 mM, pH 3.4 in H_2O, mobile phase C). The flow rate was 300 µL/min throughout and the gradient increased linearly from 5% A, 90% B and 5% C to 50% A, 45% B and 5% C within 20 min.

Detection was performed using an orbitrap Q-Exactive mass spectrometer (Thermo Scientific). The instrument was equipped with an electrospray interface and was controlled by Xcalibur software version 2.2 (Thermo Scientific). The samples were analyzed in the positive-ionization mode in full scan mode with a scan range from 70 to 500 m/z, at a resolution of 70 000.

Calibration curves were computed using the ratio of the peak area of the amino acids and their internal standards with a weighted (1/x) linear regression analysis.

SUPPLEMENTARY FIGURES

fig. S1

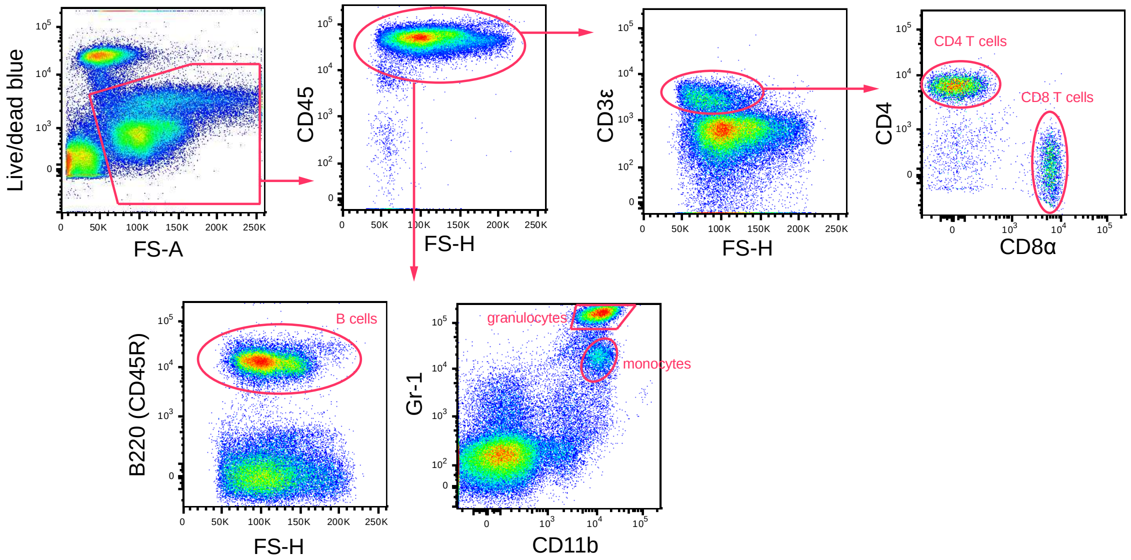

fig S1: Flow cytometry gating strategy for leukocyte populations in blood. Blood leukocytes (CD45$^+$) were further categorized into CD4 T cells (CD3ε$^+$ CD4$^+$), CD8 T cells (CD3ε$^+$ CD8α$^+$), B cells (B220/CD45R$^+$), monocytes (CD11b$^+$ Gr-1^{mid}), and granulocytes (CD11b$^+$ Gr-1^{hi})

fig. S2

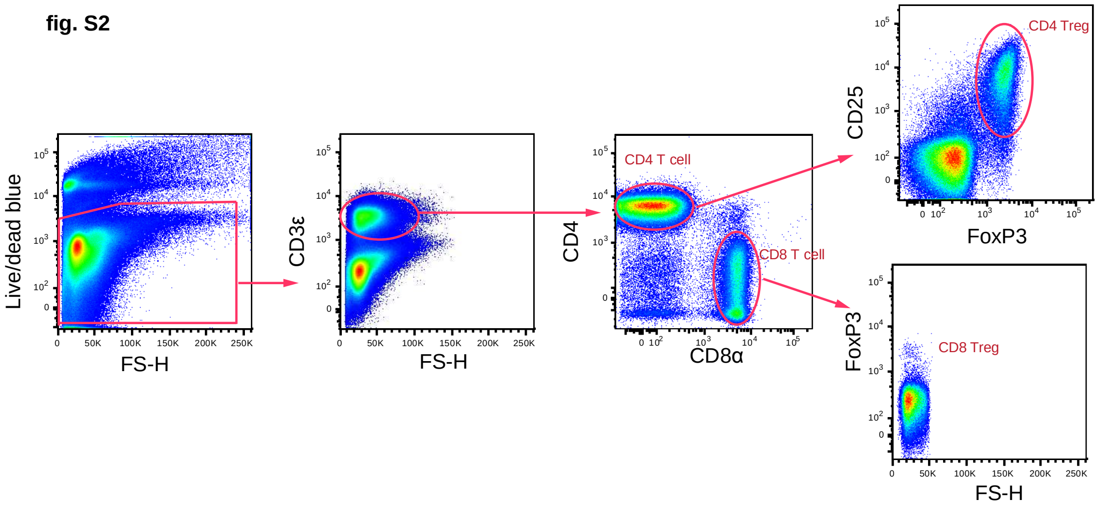

fig S2: Flow cytometry gating strategy for T_{reg} cells in the blood, bone marrow, and spleen. T cells (CD3ε$^+$) were further categorized into CD4 Treg (CD4$^+$ CD25$^+$ FoxP3$^+$) and CD8 Treg (CD8α$^+$ FoxP3$^+$).

fig. S3

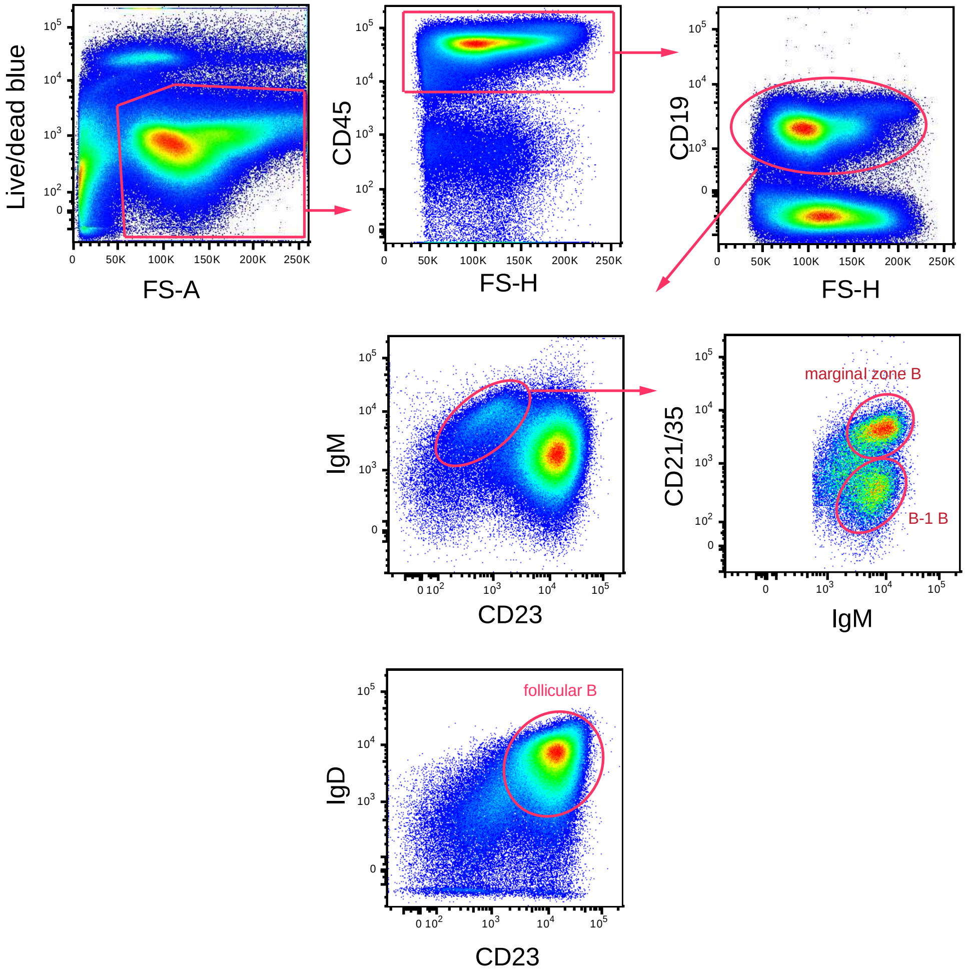

fig S3: Flow cytometry gating strategy for B cell populations in the spleen. B cells (CD45$^+$ CD19$^+$) were further categorized into marginal zone B cells (IgM$^+$ CD23$^-$ CD21/35$^+$), B-1 B cells (IgM$^+$ CD23$^-$ CD21/35$^-$) and follicular B cells (IgD$^+$ CD23$^+$).

fig. S4

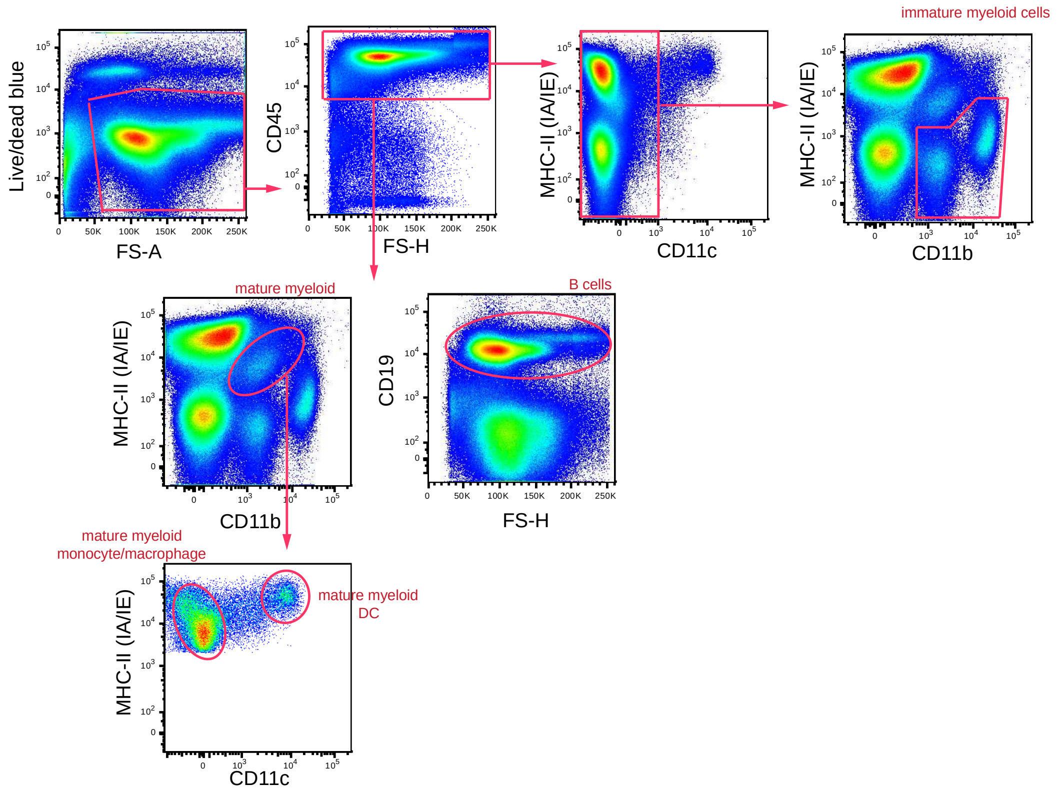

fig S4: Flow cytometry gating strategy for myeloid populations in the spleen and bone marrow. Leukocytes (CD45^{+}) were further differentiated into B cells (CD19^{+}), mature myeloid cells (CD11b^{mid} MHC-II^{hi}), mature myeloid DC (CD11b^{mid} MHC-II^{hi} CD11c^{+}), and mature myeloid monocyte/macrophage (CD11b^{mid} MHC-II^{hi} CD11c^{-}). Non-DC myeloid cells (CD45^{+} CD11c^{-}) were further differentiated into immature myeloid cells (CD11b^{+} MHC-II^{low}).

fig. S5

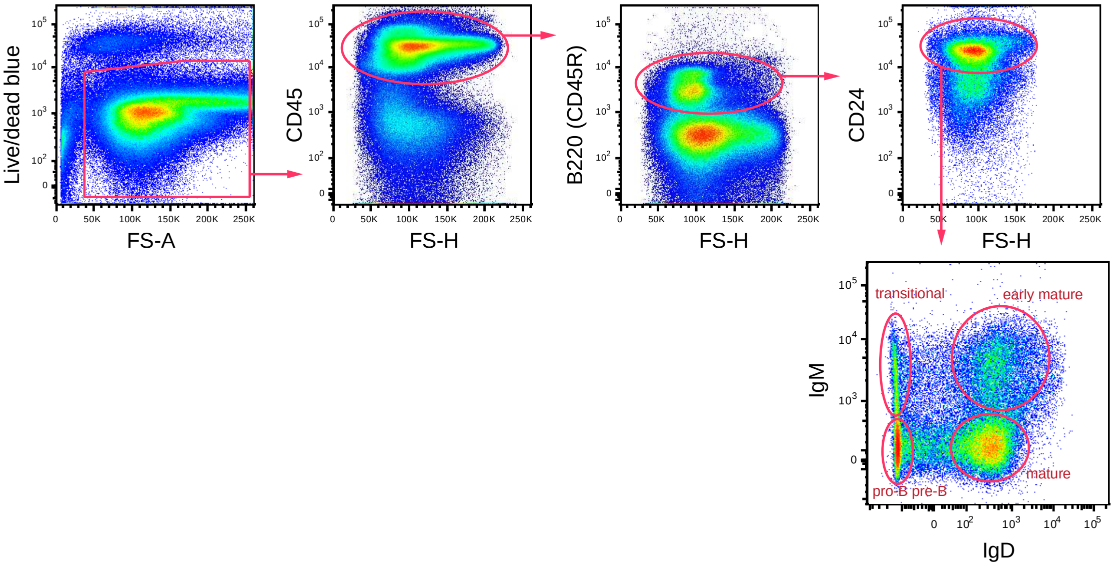

fig S5: Flow cytometry gating strategy for B cell populations in the bone marrow. B cells (CD45^{+} B220/CD45R^{+}) were further differentiated into early mature B cells (CD24^{+} IgD^{+} IgM^{+}), mature B cells (CD24^{+} IgD^{+} IgM^{-}), pro-B/pre-B cells (CD24^{+} IgD^{-} IgM^{-}), and transitional B cells (CD24^{+} IgD^{-} IgM^{+}).

fig. S6

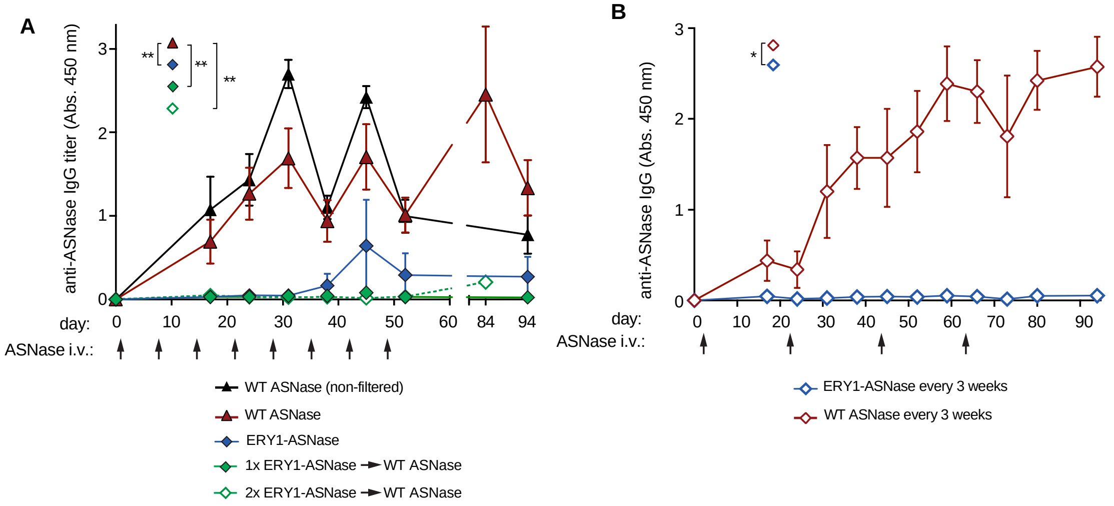

fig. S6: Erythrocyte-binding ASNase is nonimmunogenic and acts as a tolerogen that enables follow-on treatment with wild-type enzyme. (**A**) Time course of anti-ASNase IgG antibody development in plasma of mice administered with 8 weekly 15 µg doses of either WT ASNase, ERY1-ASNase, or 1 or 2 tolerogenic doses of ERY1-ASNase followed by WT ASNase for remaining doses, represented as ELISA absorbance values of 1:100 diluted plasma samples (ANOVA $^{**}p \leq 0.01$ for continuous data until 52 d); (**B**) Time course of anti-ASNase IgG antibody development in plasma of mice administered a dose-sparing regimen of 15 µg of either WT ASNase or ERY1-ASNase every 3 wk for a total of 4 doses, represented as ELISA absorbance values of 1:100 diluted plasma samples (ANOVA $^{*}p \leq 0.05$ for continuous data until 38 d); data represented as mean ± SEM; n = 4-9 per group until 80 d; n = 3-9 from 80 d onwards.

fig. S7

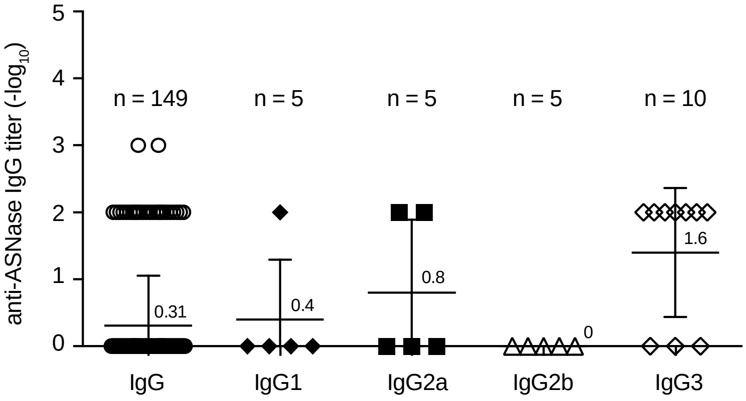

fig. S7: Anti-ASNase seropositivity analysis in naïve BALB/cOlaHsd mice. Plasma from cohorts of naïve BALB/cOlaHsd mice were assayed for total IgG, IgG1, IgG2a, IgG2b, and IgG3 reactivity towards ASNase by direct ELISA; data depicted as mean ± SD, mean values written at mean bars.

fig. S8

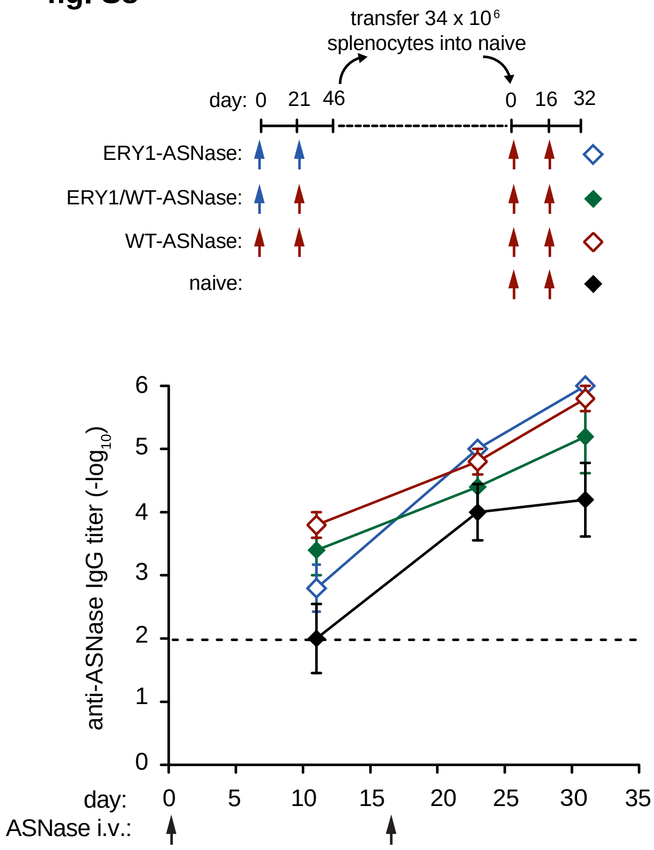

fig S8: Adoptive transfer of splenocytes from ERY1-ASNase tolerized mice does not transfer tolerance. Donor mice were administered with i.v. doses of 15 µg of ERY1-ASNase only, ERY1-ASNase followed by WT ASNase, or WT ASNase only, for a total of 2 administrations. Naïve host mice were adoptively transferred with donor splenocytes, challenged with 2 i.v. doses of 15 µg WT ASNase, and anti-ASNase IgG responses were quantified over time.

Hematology parameters (characterized by automated hematology analyzer)

Hematocrit % (36.7-46.8)	mean ± SD	**Comparison between groups**		p-value
Naive	42.84 ± 3.98	WT : Naive	n.s.	p = 0.075
WT	47.64 ± 1.95	ERY1 : Naive	n.s.	p = 0.691
ERY1	41.52 ± 2.68	ERY1 : WT	**	p = 0.008

Mean corpuscular volume fL/RBC (42.2-59.2)	mean ± SD	**Comparison between groups**		p-value
Naive	48.92 ± 0.97	WT : Naive	n.s.	p = 0.095
WT	51.04 ± 2.15	ERY1 : Naive	*	p = 0.024
ERY1	51.12 ± 1.29	ERY1 : WT	n.s.	p = 0.833

White blood cell count 1000/μL (3.2-12.7)	mean ± SD	**Comparison between groups**		p-value
Naive	4.25 ± 1.34	WT : Naive	n.s.	p = 0.151
WT	5.55 ± 0.67	ERY1 : Naive	n.s.	p = 0.310
ERY1	3.62 ± 0.44	ERY1 : WT	**	p = 0.008

Lymphocyte count 1000/μL (3.8-8.9)	mean ± SD	**Comparison between groups**		p-value
Naive	3.35 ± 1.05	WT : Naive	n.s.	p = 0.528
WT	4.22 ± 0.75	ERY1 : Naive	n.s.	p = 0.402
ERY1	2.72 ± 0.40	ERY1 : WT	*	p = 0.012

Neutrophil count 1000/μL (0.5-2.0)	mean ± SD	**Comparison between groups**		p-value
Naive	0.680 ± 0.312	WT : Naive	n.s.	p = 0.151
WT	0.976 ± 0.129	ERY1 : Naive	n.s.	p = 0.530
ERY1	0.692 ± 0.259	ERY1 : WT	*	p = 0.036

Monocyte count 1000/μL (0.0-0.3)	mean ± SD	**Comparison between groups**		p-value
Naive	0.068 ± 0.018	WT : Naive	n.s.	p = 0.651
WT	0.164 ± 0.210	ERY1 : Naive	n.s.	p = 0.585
ERY1	0.084 ± 0.078	ERY1 : WT	n.s.	p = 0.264

Eosinophil count 1000/μL (0.0-0.4)	mean ± SD	**Comparison between groups**		p-value
Naive	0.132 ± 0.041	WT : Naive	n.s.	p = 0.528
WT	0.172 ± 0.073	ERY1 : Naive	n.s.	p = 0.139
ERY1	0.076 ± 0.048	ERY1 : WT	*	p = 0.044

Lymphocyte Percent % (60.2-95.0)	mean ± SD	**Comparison between groups**		p-value
Naive	78.84 ± 3.27	WT : Naive	n.s.	p = 0.421
WT	75.64 ± 4.87	ERY1 : Naive	n.s.	p = 0.222
ERY1	75.06 ± 6.98	ERY1 : WT	n.s.	p = 0.841

Neutrophil Percent % (6.8-31.1)	mean ± SD	**Comparison between groups**		p-value

Naive	15.76 ± 3.35	WT : Naive	n.s.	p = 0.222
WT	17.76 ± 3.18	ERY1 : Naive	n.s.	p = 0.222
ERY1	19.00 ± 6.70	ERY1 : WT	n.s.	p = 0.310

Monocyte Percent % (0-4.3)	mean ± SD	**Comparison between groups**		p-value
Naive	1.76 ± 0.52	WT : Naive	n.s.	p = 0.421
WT	3.12 ± 4.36	ERY1 : Naive	n.s.	p = 0.841
ERY1	2.34 ± 2.48	ERY1 : WT	n.s.	p = 0.834

Eosinophil Percent % (0.2-5.9)	mean ± SD	**Comparison between groups**		p-value
Naive	3.14 ± 0.73	WT : Naive	n.s.	p = 0.841
WT	3.08 ± 1.20	ERY1 : Naive	n.s.	p = 0.209
ERY1	2.20 ± 1.41	ERY1 : WT	n.s.	p = 0.599

Hematology parameters (characterized by flow cytometry)

Granulocytes % of live cells	mean ± SD	**Comparison between groups**		p-value
Naive	19.10 ± 3.29	WT : Naive	n.s.	p = 0.548
WT	21.08 ± 3.67	ERY1 : Naive	n.s.	p = 0.691
ERY1	19.80 ± 11.75	ERY1 : WT	n.s.	p = 0.691

B cells % of live cells	mean ± SD	**Comparison between groups**		p-value
Naive	38.50 ± 4.07	WT : Naive	n.s.	p = 0.841
WT	39.70 ± 5.64	ERY1 : Naive	**	p = 0.008
ERY1	29.32 ± 3.03	ERY1 : WT	**	p = 0.008

Table S1: **Hematology characterization after short-term treatment regimens**. Mice were administered either two doses of ERY1-ASNase or WT ASNase, and hematological parameters were determined by automated blood analysis and flow cytometry methods; n = 5; *p ≤ 0.05, **p ≤ 0.01, Student’s two-tailed t test.

CD4 T cells (% of live cells)	**blood** mean ± SD	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	11.77 ± 3.00	17.16 ± 1.12	0.495 ± 0.079
WT	10.98 ± 1.41	13.85 ± 4.75	0.582 ± 0.078
ERY1	10.38 ± 2.63	15.72 ± 0.94	0.692 ± 0.043
WT : Naive	n.s. p = 1.000	n.s. p = 0.222	n.s. p = 0.151
ERY1 : Naive	n.s. p = 0.463	n.s. p = 0.116	** p = 0.008
ERY1 : WT	n.s. p = 0.421	n.s. p = 0.690	* p = 0.032
CD8 T cells			
Naive	4.20 ± 0.82	6.06 ± 0.87	0.331 ± 0.078
WT	4.11 ± 0.69	5.41 ± 1.75	0.356 ± 0.153
ERY1	4.74 ± 0.87	5.60 ± 0.40	0.443 ± 0.099
WT : Naive	n.s. p = 0.841	n.s. p = 0.690	n.s. p = 1.000
ERY1 : Naive	n.s. p = 0.421	n.s. p = 0.222	n.s. p = 0.095
ERY1 : WT	n.s. p = 0.095	n.s. p = 1.000	n.s. p = 0.421
CD4 T_{reg}			
Naive	-	2.820 ± 0.308	0.301 ± 0.054
WT	-	2.104 ± 0.768	0.363 ± 0.045
ERY1	-	2.638 ± 0.320	0.399 ± 0.031
WT : Naive	-	n.s. p = 0.095	n.s. p = 0.151
ERY1 : Naive	-	n.s. p = 0.222	* p = 0.016
ERY1 : WT	-	n.s. p = 0.249	n.s. p = 0.151
CD8 T_{reg}			
Naive	-	0.028 ± 0.003	0.003 ± 0.001
WT	-	0.030 ± 0.008	0.004 ± 0.002
ERY1	-	0.033 ± 0.008	0.005 ± 0.001
WT : Naive	-	n.s. p = 0.841	n.s. p = 0.310
ERY1 : Naive	-	n.s. p = 0.346	* p = 0.032
ERY1 : WT	-	n.s. p = 0.548	n.s. p = 0.690

Table S2: **T cell characterization after short-term treatment regimens.** Mice were administered either two doses of ERY1-ASNase or WT ASNase, and T cells were enumerated by flow cytometry. All values represent % of live cells; n = 5; $^*p \leq 0.05$, $^{**}p \leq 0.01$, Student's two-tailed t test.

Table S3: B cell characterization after short-term treatment regimens. Mice were administered either two doses of ERY1-ASNase or WT ASNase, and splenic B cell populations were enumerated by flow cytometry. All values represent % of live cells; n = 5; *$p \leq 0.05$, **$p \leq 0.01$, Student's two-tailed t test.

B cells (CD19)	mean ± SD
Naive	42.78 ± 3.12
WT	36.74 ± 12.21
ERY1	28.50 ± 2.73
WT : Naive	n.s. $p = 0.295$
ERY1 : Naive	* $p = 0.012$
ERY1 : WT	n.s. $p = 0.151$

B1 B cells	mean ± SD
Naive	1.36 ± 0.20
WT	1.61 ± 0.63
ERY1	1.83 ± 0.49
WT : Naive	n.s. $p = 0.841$
ERY1 : Naive	n.s. $p = 0.151$
ERY1 : WT	n.s. $p = 0.548$

Marginal Zone B cells	mean ± SD
Naive	1.83 ± 0.46
WT	1.32 ± 0.80
ERY1	1.67 ± 0.31
WT : Naive	n.s. $p = 0.222$
ERY1 : Naive	n.s. $p = 0.753$
ERY1 : WT	n.s. $p = 0.310$

Follicular B cells	mean ± SD
Naive	36.06 ± 2.43
WT	29.45 ± 12.40
ERY1	20.28 ± 1.77
WT : Naive	n.s. $p = 0.310$
ERY1 : Naive	** $p = 0.008$
ERY1 : WT	n.s. $p = 0.151$

Immature myeloid cells	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	7.40 ± 0.62	38.22 ± 2.09
WT	17.35 ± 14.88	38.30 ± 1.41
ERY1	16.92 ± 1.14	42.68 ± 5.11
WT : Naive	n.s. p = 0.056	n.s. p = 0.675
ERY1 : Naive	** p = 0.008	n.s. p = 0.115
ERY1 : WT	n.s. p = 0.151	n.s. p = 0.151

Mature myeloid cells	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	3.27 ± 0.54	2.51 ± 0.22
WT	4.04 ± 1.14	2.38 ± 0.32
ERY1	3.80 ± 0.28	1.91 ± 0.20
WT : Naive	n.s. p = 0.421	n.s. p = 0.675
ERY1 : Naive	n.s. p = 0.095	* p = 0.016
ERY1 : WT	n.s. p = 1.000	* p = 0.036

Mature myeloid dendritic cells	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	0.456 ± 0.113	0.061 ± 0.004
WT	0.481 ± 0.125	0.072 ± 0.013
ERY1	0.485 ± 0.093	0.067 ± 0.005
WT : Naive	n.s. p = 0.421	n.s. p = 0.094
ERY1 : Naive	n.s. p = 0.548	n.s. p = 0.094
ERY1 : WT	n.s. p = 0.463	n.s. p = 0.691

Mature non-myeloid dendritic cells	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	0.597 ± 0.156	0.090 ± 0.005
WT	0.705 ± 0.250	0.120 ± 0.034
ERY1	0.621 ± 0.072	0.097 ± 0.011
WT : Naive	n.s. p = 0.548	n.s. p = 0.151
ERY1 : Naive	n.s. p = 0.548	n.s. p = 0.310
ERY1 : WT	n.s. p = 0.691	n.s. p = 0.421

Mature myeloid macrophages / monocytes	**spleen** mean ± SD	**bone marrow** mean ± SD
Naive	2.57 ± 0.42	2.27 ± 0.22
WT	3.24 ± 0.94	2.10 ± 0.27
ERY1	2.87 ± 0.30	1.64 ± 0.17
WT : Naive	n.s. p = 0.548	n.s. p = 0.346
ERY1 : Naive	n.s. p = 0.548	** p = 0.008
ERY1 : WT	n.s. p = 0.691	* p = 0.032

Table S4: **Myeloid cell characterization after short-term treatment regimens**. Mice were administered either two doses of ERY1-ASNase or WT ASNase, and myeloid cells in the spleen and bone marrow were enumerated by flow cytometry. All values represent % of live cells; n = 5; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, Student’s two-tailed t test.

Table S5: **B cell characterization after short-term treatment regimens**. Mice were administered either two doses of ERY1-ASNase or WT ASNase, and B cell populations in the bone marrow were enumerated by flow cytometry. All values represent % of live cells; $n = 5$; *$p \leq 0.05$, **$p \leq 0.01$, Student's two-tailed t test.

B cells (B220)	mean ± SD	
Naive	24.38 ± 4.98	
WT	23.68 ± 1.80	
ERY1	24.74 ± 3.78	
WT : Naive	n.s.	$p = 1.000$
ERY1 : Naive	n.s.	$p = 0.691$
ERY1 : WT	n.s.	$p = 0.599$

Early Mature B cells	mean ± SD	
Naive	2.22 ± 0.31	
WT	2.11 ± 0.62	
ERY1	2.73 ± 0.67	
WT : Naive	n.s.	$p = 0.841$
ERY1 : Naive	n.s.	$p = 0.151$
ERY1 : WT	n.s.	$p = 0.222$

Mature B cells	mean ± SD	
Naive	7.82 ± 2.80	
WT	7.56 ± 1.30	
ERY1	8.21 ± 1.84	
WT : Naive	n.s.	$p = 0.917$
ERY1 : Naive	n.s.	$p = 0.841$
ERY1 : WT	n.s.	$p = 0.841$

Pro-B Pre-B cells	mean ± SD	
Naive	2.88 ± 1.09	
WT	2.76 ± 0.41	
ERY1	3.33 ± 0.92	
WT : Naive	n.s.	$p = 0.834$
ERY1 : Naive	n.s.	$p = 0.548$
ERY1 : WT	n.s.	$p = 0.402$

Transitional cells	mean ± SD	
Naive	0.447 ± 0.055	
WT	0.512 ± 0.161	
ERY1	0.690 ± 0.194	
WT : Naive	n.s.	$p = 1.000$
ERY1 : Naive	n.s.	$p = 0.095$
ERY1 : WT	n.s.	$p = 0.210$

Hematology Parameters (characterized by automated blood analyzer)

Hematocrit % (36.7-46.8)	mean ± SD	**Comparison between groups**		p-value
WT	52.93 ± 2.34	ERY1 : WT	n.s.	p = 0.786
ERY	52.56 ± 2.18			

Mean corpuscular volume fL/RBC (42.2-59.2)	mean ± SD	**Comparison between groups**		p-value
WT	51.27 ± 0.94	ERY1 : WT	n.s.	p = 0.250
ERY	52.58 ± 1.56			

White blood cell count 1000/µL (3.2-12.7)	mean ± SD	**Comparison between groups**		p-value
WT	4.38 ± 0.42	ERY1 : WT	n.s.	p = 0.393
ERY	3.60 ± 0.90			

Lymphocyte count 1000/µL (3.8-8.9)	mean ± SD	**Comparison between groups**		p-value
WT	3.25 ± 0.35	ERY1 : WT	n.s.	p = 0.393
ERY	2.68 ± 0.75			

Neutrophil count 1000/µL (0.5-2.0)	mean ± SD	**Comparison between groups**		p-value
WT	0.89 ± 0.10	ERY1 : WT	n.s.	p = 0.071
ERY	0.65 ± 0.18			

Monocyte count 1000/µL (0.0-0.3)	mean ± SD	**Comparison between groups**		p-value
WT	0.05 ± 0.01	ERY1 : WT	n.s.	p = 0.759
ERY	0.05 ± 0.03			

Eosinophil count 1000/µL (0.0-0.4)	mean ± SD	**Comparison between groups**		p-value
WT	0.17 ± 0.05	ERY1 : WT	n.s.	p = 0.763
ERY	0.21 ± 0.08			

Lymphocyte Percent % (60.2-95.0)	mean ± SD	**Comparison between groups**		p-value
WT	74.10 ± 0.92	ERY1 : WT	n.s.	p = 1.000
ERY	74.04 ± 5.15			

Neutrophil Percent % (6.8-31.1)	mean ± SD	**Comparison between groups**		p-value
WT	20.40 ± 0.78	ERY1 : WT	n.s.	p = 0.250
ERY	18.12 ± 2.81			

Monocyte Percent % (0-4.3)	mean ± SD	**Comparison between groups**		p-value
WT	1.27 ± 0.15	ERY1 : WT	n.s.	p = 0.291
ERY	1.50 ± 0.42			

Eosinophil Percent % (0.2-5.9)	mean ± SD	**Comparison between groups**		p-value
WT	4.03 ± 1.40	ERY1 : WT	n.s.	p = 0.294
ERY	6.14 ± 3.10			

Table S6: **Hematology characterization after long-term treatment regimens**. Hematology characterization of mice undergoing chronic administration of ERY1-ASNase or WT ASNase every 3 weeks for a total of 4 injections, as determined by automated blood analysis; n = 3-5; *p ≤ 0.05, **p ≤ 0.01, Student's two-tailed t test.