Supporting Information

Thai et al. 10.1073/pnas.1317632110

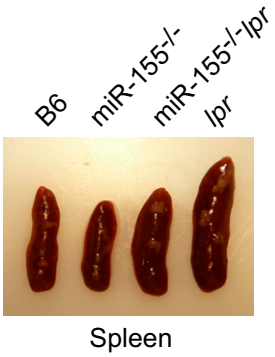

Fig. S1. Ablation of microRNA-155 (miR-155) alleviates splenomegaly in the *Fas*^{lpr} mouse. Representative spleens were obtained from aged mice (10-12 mo old). WT B6 ($n = 5$); miR-155$^{-/-}$ ($n = 5$); miR-155$^{-/-}$-*Fas*^{lpr} ($n = 10$); *Fas*^{lpr} ($n = 10$).

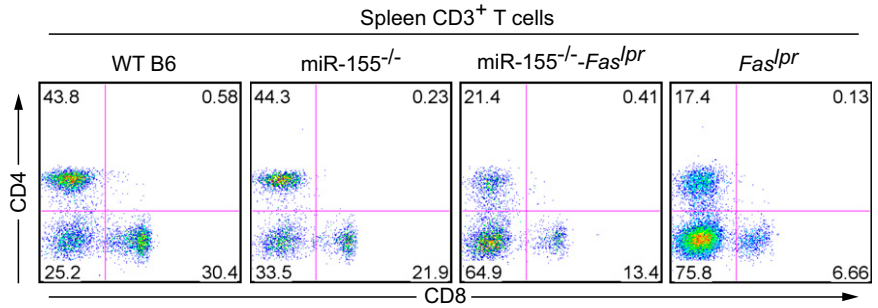

Fig. S2. MiR-155 deficiency does not alter the frequency of peripheral CD4^{-}CD8^{-} T cells in *Fas*^{lpr} mice. The frequency of CD4^{-}CD8^{-} T cells within the CD3 gate was determined by FACS analysis from spleens of 10- to 12-mo-old mice. Plots are representative of 5–10 experiments. B6 ($n = 5$); miR-155$^{-/-}$ ($n = 5$); miR-155$^{-/-}$-*Fas*^{lpr} ($n = 10$); *Fas*^{lpr} ($n = 10$).

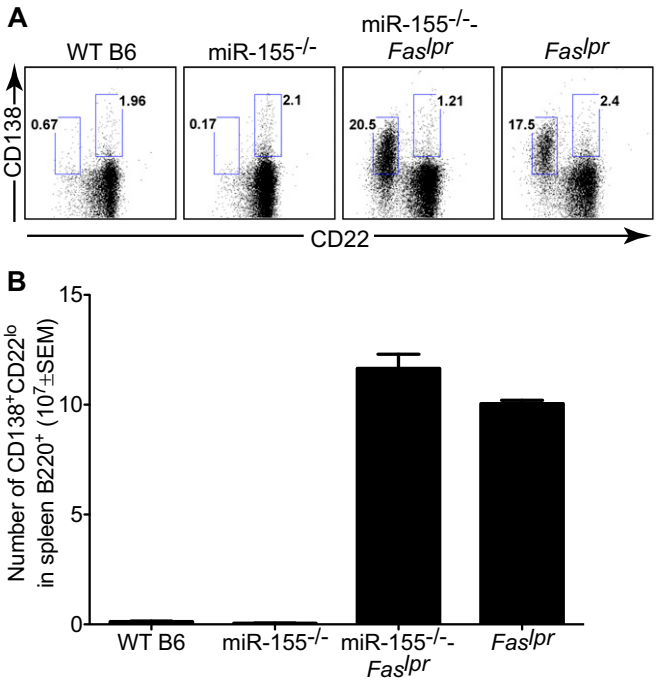

Fig. S3. Spleen B220^{+}CD138^{+}CD22^{lo} short-lived plasmablasts remained unchanged in miR-155$^{-/-}$-*Fas*^{lpr} mice. (*A*) Spleen B220^{+}CD138^{+}CD22^{lo} short-lived plasmablasts were identified by FACS. B6 (n = 5); miR-155$^{-/-}$ (n = 5); miR-155$^{-/-}$-*Fas*^{lpr} (n = 5); *Fas*^{lpr} (n = 10). (*B*) The number of spleen B220^{+}CD138^{+}CD22^{lo} plasmablasts was calculated within the lymphoid and B220 gates. *P* values were determined by Student *t* test (GraphPad Software).

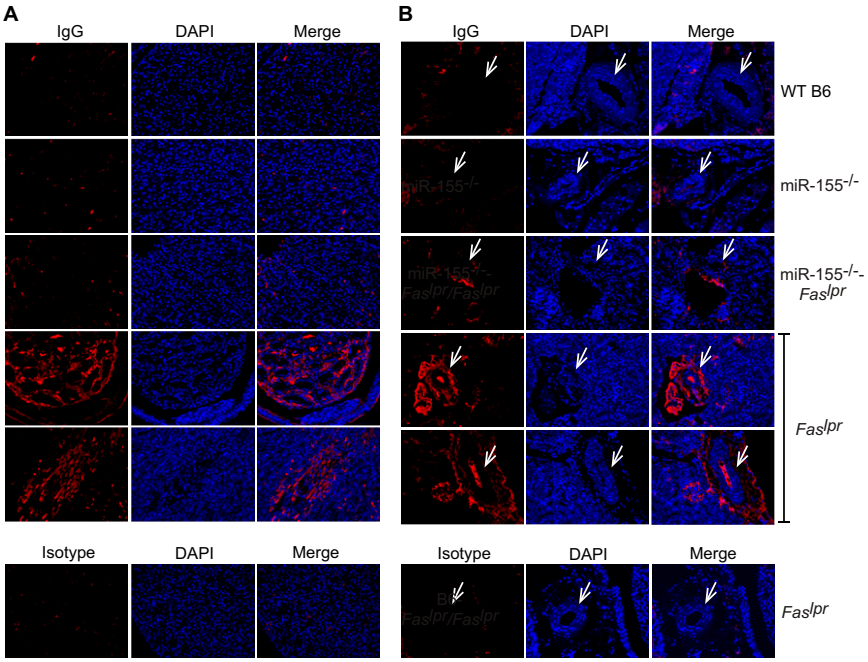

Fig. S4. MiR-155 deficiency alleviates renal pathologies in *fas*-deficient lupus-prone mice. Kidneys were obtained from 10- to 12-mo-old mice. Immunofluorescence staining was done to determine IgG (red) deposition on kidney medullas (*A*) and blood vessels (arrows, *B*). Nuclei were identified by DAPI (blue) staining.

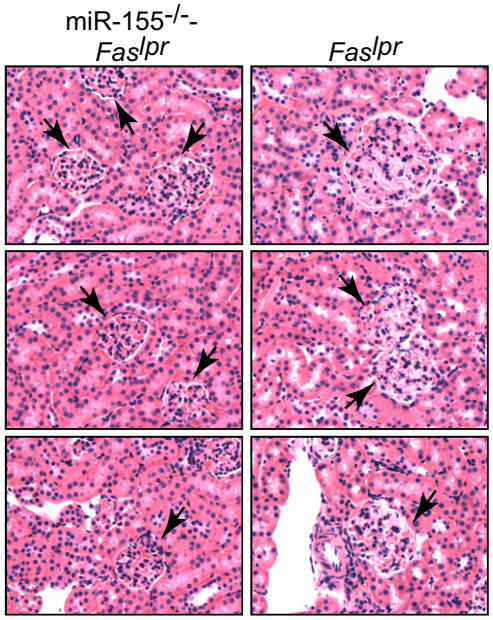

Fig. S5. MiR-155 deficiency restores renal architecture in *fas*-deficient lupus-prone mice. Kidneys were obtained from 10- to 12-mo-old mice. Kidney morphology was assessed by histology with H&E stain. Arrows indicate glomeruli.

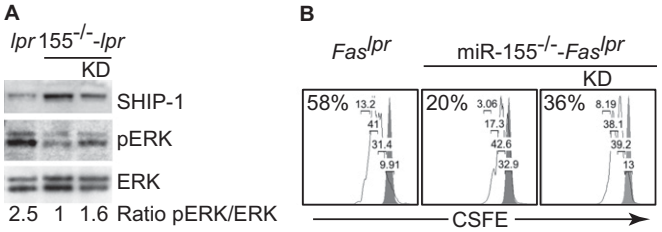

Fig. S6. In vitro knock-down (KD) of mouse *Inpp5d* partially restored ERK phosphorylation and proliferation in miR-155$^{-/-}$-*Fas*^{lpr} B cells. (*A*) SHIP-1, pERK, and total ERK protein levels were detected by Western blots in miR-155$^{-/-}$-*Fas*^{lpr} spleen B cells stimulated with intact anti-IgM (B-cell receptor, BCR). Ratio: pERK/total ERK was determined by Bio-Rad Quantity One software. Knock-down was done using the GIPZ *Inpp5d* Lentiviral shRNA Transduction Starter Kit according to the manufacturer's protocol (Thermo Scientific). The shRNA sequences were AATCCTGGATGGCTTTCAG and TAATGCTGATCAGGATATG. (*B*) Cell division was determined for knock-down B-cell cultures as in Fig. 6 using FlowJo Proliferation Platform software. Results are representative of two independent experiments; $n = 4$ mice.

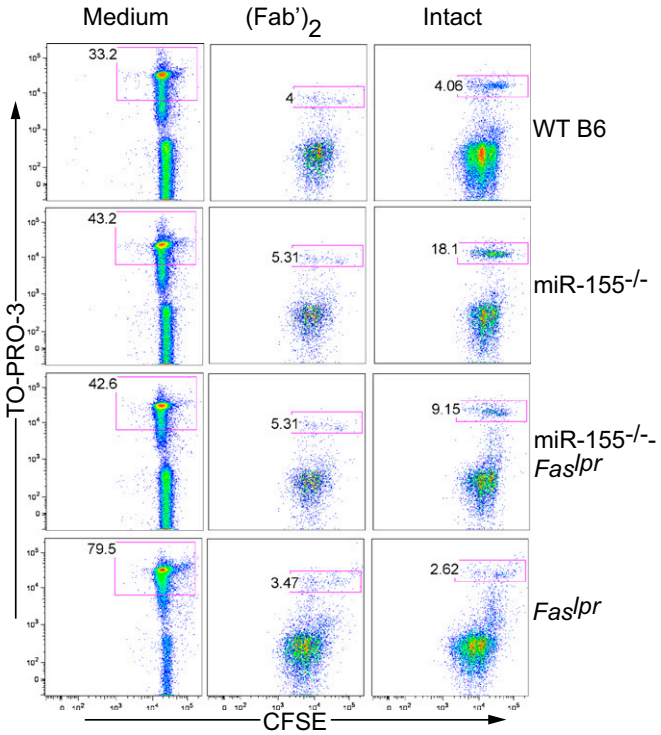

Fig. S7. MiR-155 deficiency leads to increased cell death after BCR and FcγRIIB coligation. Naïve spleen B cells were purified by negative selection using CD43 MACS beads, followed by carboxyfluorescein succinimidyl ester (CFSE) labeling. Labeled cells were stimulated with either intact IgG or $F(ab')_2$ anti-IgM for 3 d. FACS analysis was used to determine CSFE dilution as a function of cell division. Dead cells were detected by the DNA vital dye TO-PRO-3.

Table S1. Urinalysis to determine renal functions

Variable	Leukocyte	Protein (mg/mL ± SD)	Blood
C57BL/6 ($n = 5$)	ND	6 ± 13*	ND-trace
MiR-155$^{-/-}$ ($n = 5$)	ND	0	ND
MiR-155$^{-/-}$-*Fas*^{lpr} ($n = 6$)	ND	53 ± 36*,†	ND-trace
Fas^{lpr} ($n = 5$)	Trace-few	260 ± 89†	Trace-moderate

The presence of leukocyte, proteins, and blood in urine samples was determined using Multistix 10 SG strips and the CLINITEKStatus machine. ND, not detected.
*$P = 0.0223$.
†$P = 0.0006$.