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Supplementary Materials: A Kidney-Targeted Nanoparticle to Augment Renal Lymphatic Density Decreases Blood Pressure in Hypertensive Mice

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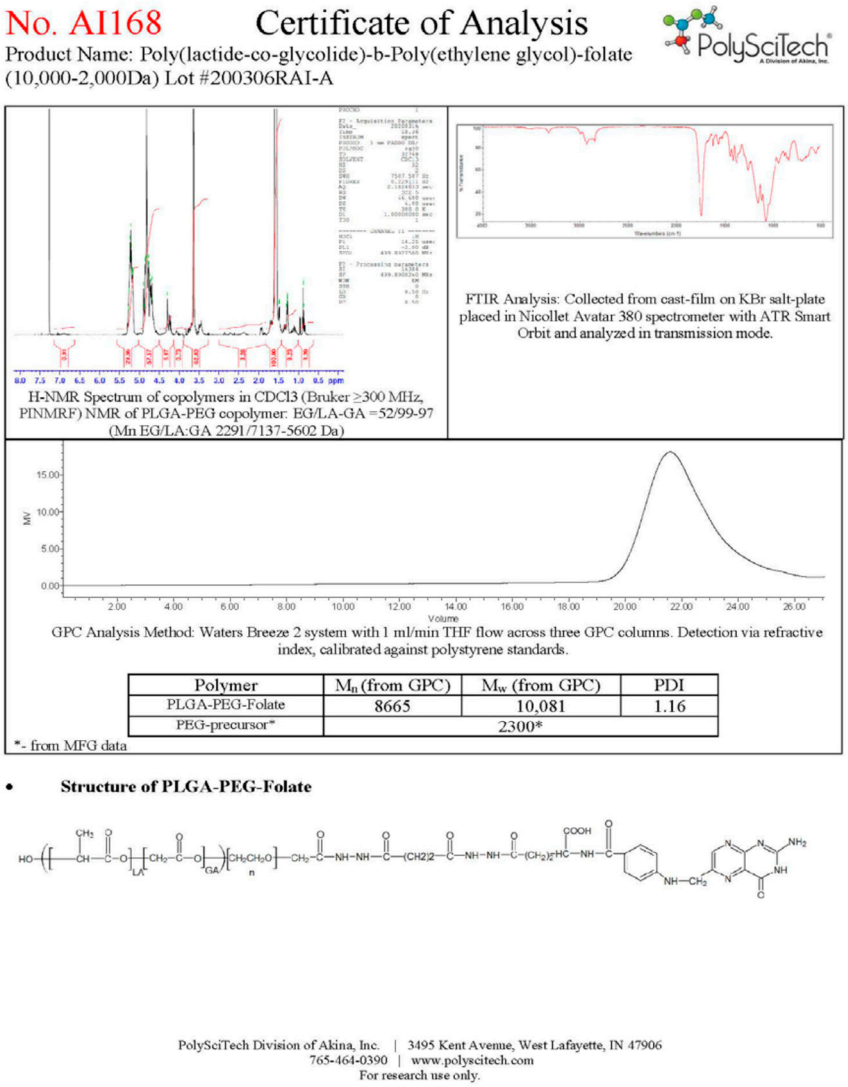

Figure S1. Certificate of analysis provided by PolySciTech. The NMR, FTIR analysis, and the GPC for the PLGA-PEG-folate polymer.

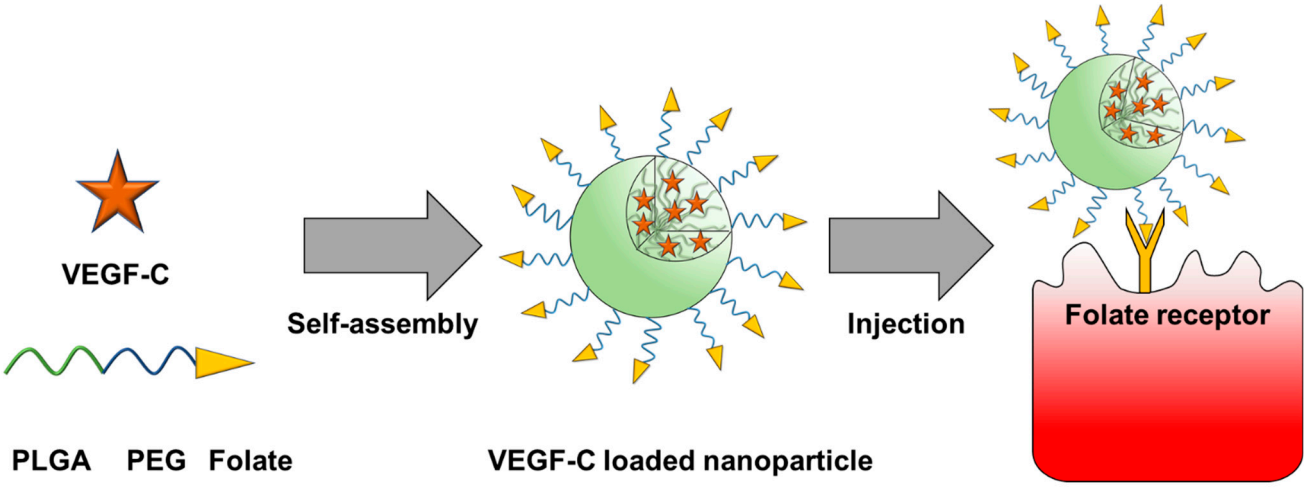

Figure S2. Schematic for nanoparticle assembly. PLGA, PEG, folate, and VEGF-C were utilized for the nanoparticle assembly followed by injection into the mouse.

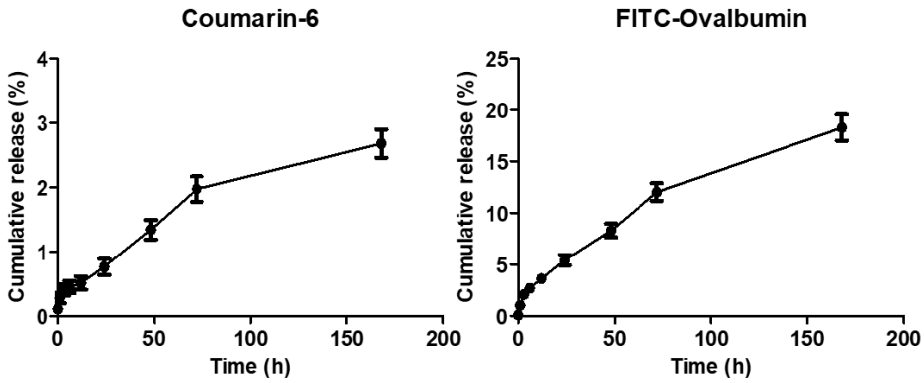

Figure S3. Cumulative release of Coumarin 6 and FITC-ovalbumin from the folate nanoparticles. The % of cumulative release over hours for both Coumarin 6 and FITC-ovalbumin.

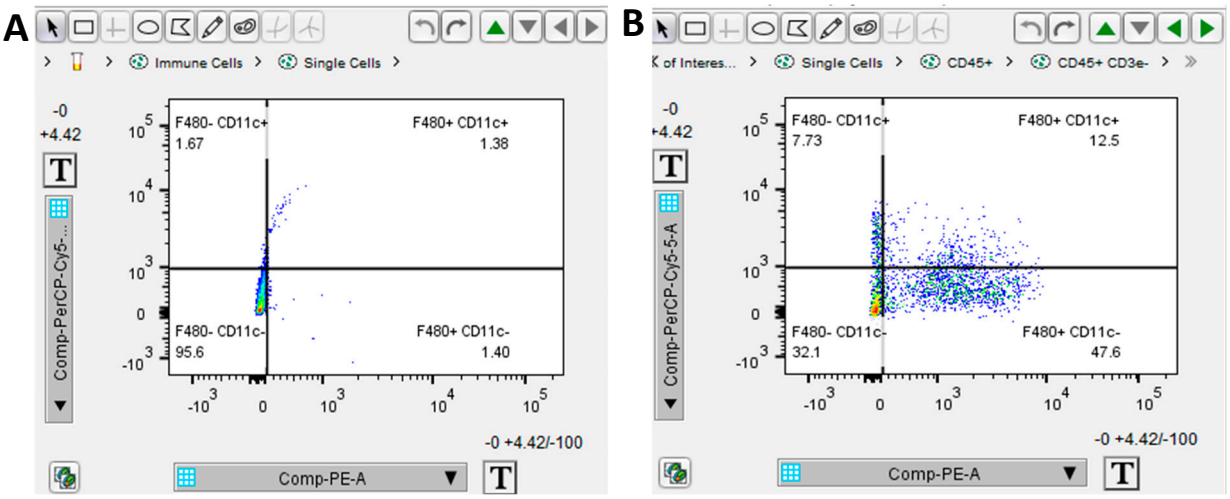

Figure S4. Gating strategy for renal immune cell analysis. (A) An unstained control kidney and (B) a fully stained kidney sample. Data was imported to FlowJo and gated for single, live cells. Then, a quadrant gate was used to determine the presence or absence of F4/80+ and CD11c+ cells. A similar approach was used for CD3+ T cells.

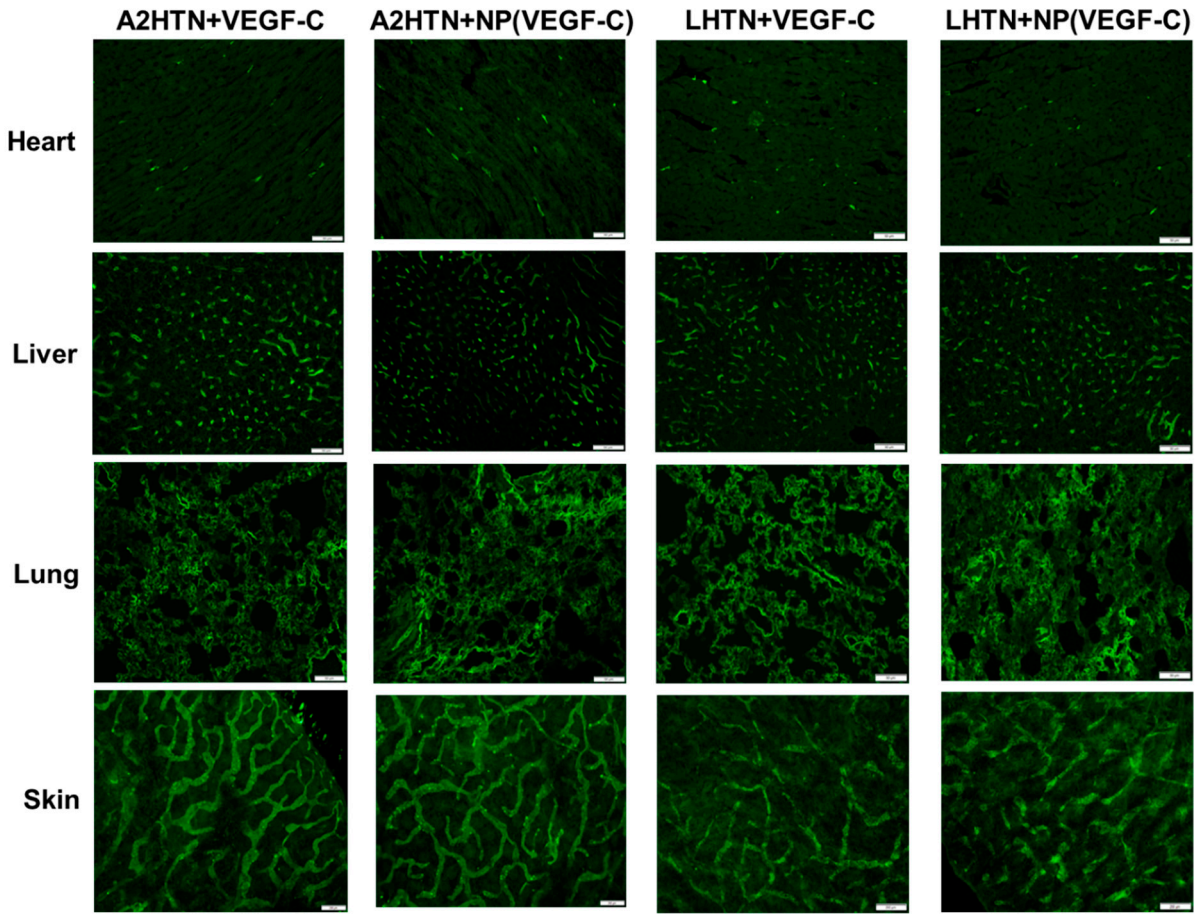

Figure S5. No increase in extra-renal lymphatic vessel density in hypertensive mice treated with VEGF-C in nanoparticles. LYVE-1 staining (green) in tissues from A2HTN and LHTN mice treated with VEGF-C in NPs or VEGF-C alone. Scale bars = 100 μm for heart, liver, and lung, and 200 μm for ear skin.

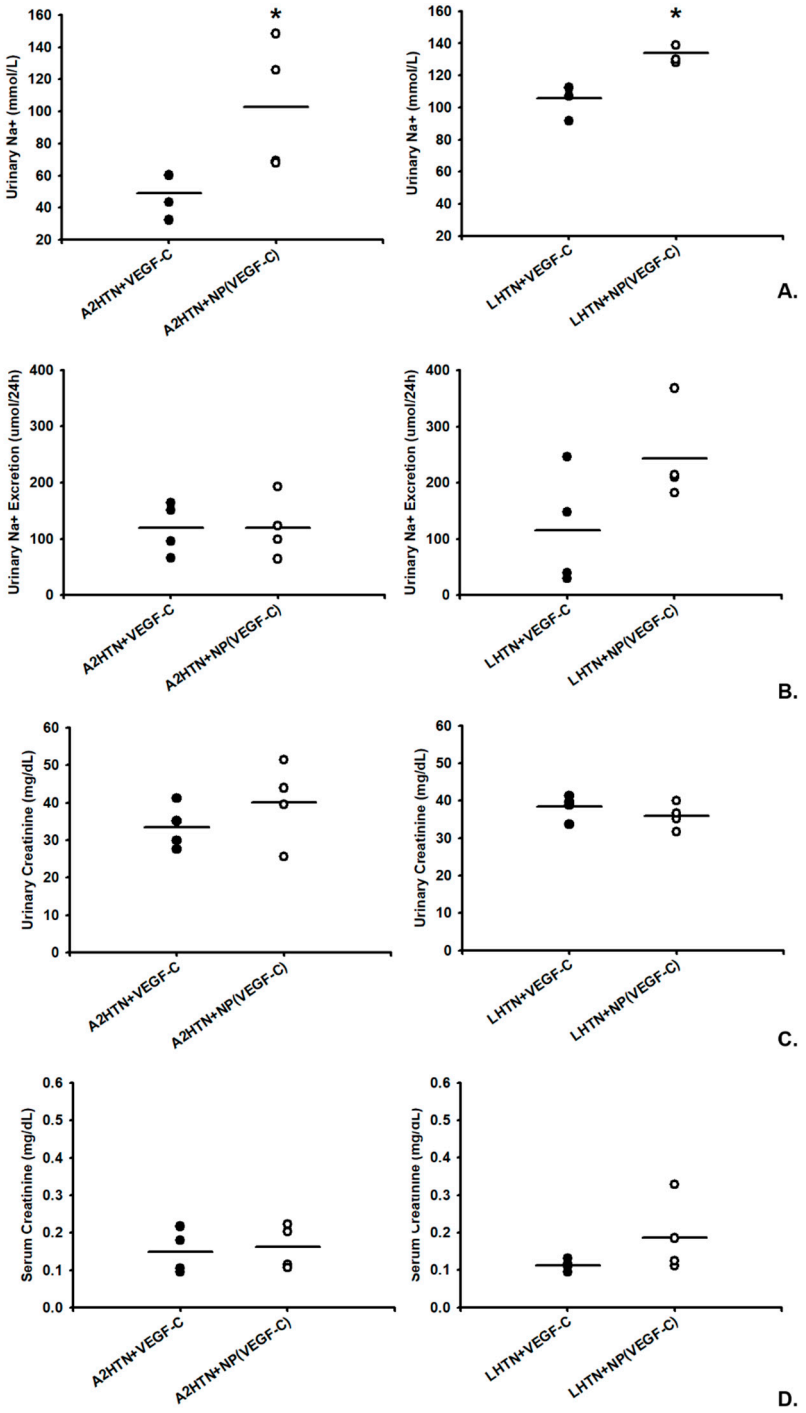

Figure S6. Treatment with VEGF-C in NPs significantly increased urinary sodium. (A) Urinary sodium, (B) urinary sodium excretion, (C) urinary creatinine, and (D) serum creatinine in male and female A2HTN and LHTN mice treated every 3 days with VEGF-C in NPs or VEGF-C alone. Urine samples were collected over a 24-hour period. Results are expressed as mean (n= 4-5 per group) and statistical analyses were performed with Student's *t* test. **P*<0.05 vs VEGF-C alone treated mice.

Table S1. Flow cytometry panel description for mouse kidneys.

Fluorochrome	FITC	PacBlue	PE	PerCP-Cy5.5
Antigen	CD45.2	CD3e	F4/80	CD11c
Final Conc (μg/mL)	2.5	0.1	0.25	0.5
Dilution Factor	1:200	1:2,000	1:400	1:400
Clone	104	500A2	T45-2342	HL3

Abbreviations: FITC = fluorescein isothiocyanate; PacBlue = pacific blue; PE = phycoerythrin; PerCP-Cy5.5 = peridinin chlorophyll protein complex cyanine 5.5.

Table S2. Body and tissue weights for the A2HTN group.

	Body Weight (BW) (g)		R Kidney (mg)/BW		L Kidney (mg)/BW		Spleen (mg)/BW	
	M	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**
A2HTN+VEGF-C	28.5	1.9	6.3	0.4	5.9	0.4	3.2	0.5
A2HTN+NP(VEGF-C)	27.1	3.1	6.1	0.4	5.4	0.4	3.6	0.4

Table S3. Body and tissue weights for the LHTN group.

	Body Weight (BW) (g)		R Kidney (mg)/BW		L Kidney (mg)/BW		Spleen (mg)/BW	
	M	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**
LHTN+VEGF-C	27.1	2.5	6.7	0.3	7.1	0.2	3.4	0.5
LHTN+NP(VEGF-C)	27.5	3.5	6.3	0.2	7.4	0.4	2.6	0.5