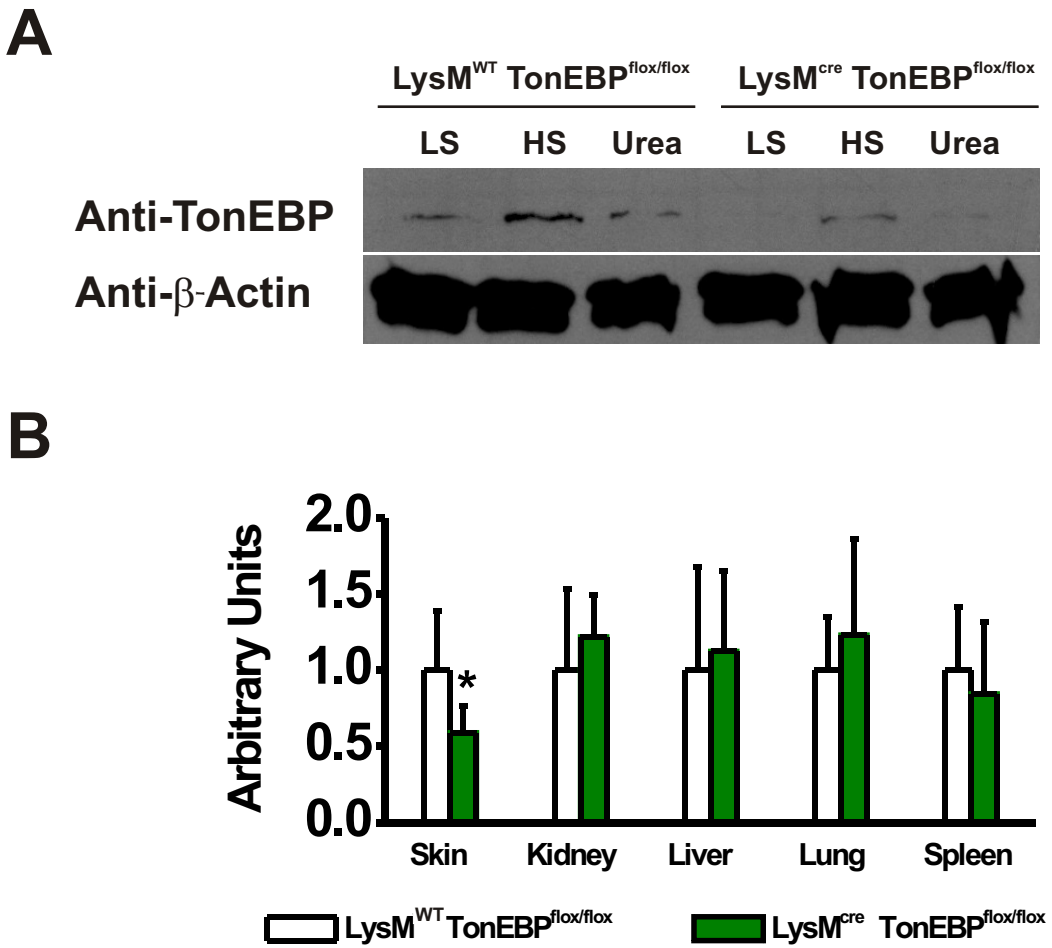
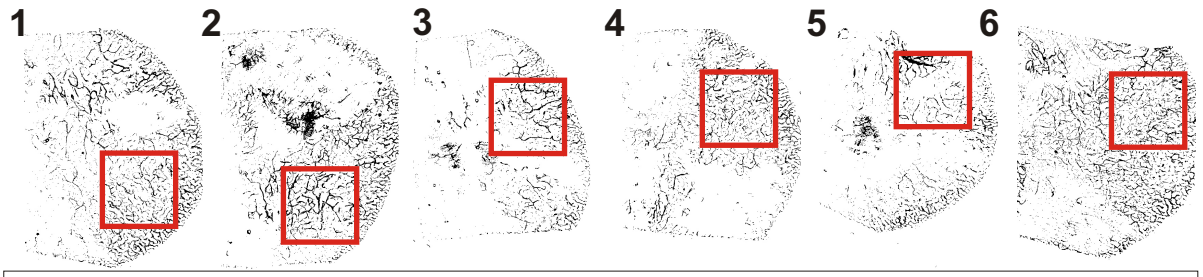


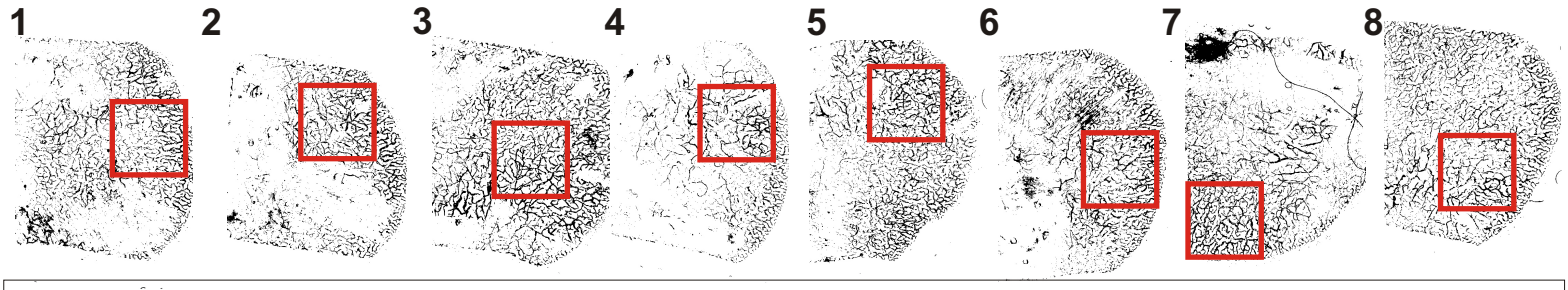
Supplemental Figure 1. Conditional TonEBP knockout in MPS cells reduces TonEBP response to osmotic stress in-vitro and in-vivo. Conditional deficiency of TonEBP in MPS cells reduced TonEBP message selectively in the skin, but not in other tissues, including the kidney. **(A)** TonEBP protein expression in primary bone marrow cells from mice without ($LysM^{WT}$ $TonEBP^{flox/flox}$ mice) and with MPS-specific conditional deficiency of the TonEBP gene ($LysM^{cre}$ $TonEBP^{flox/flox}$ mice.) We cultured cells with standard culture medium (LS), with increased medium NaCl concentration (190 mmol/L), and with increased urea concentration (380 mmol/L). **(B)** TonEBP mRNA expression in various organs of $LysM^{WT}$ $TonEBP^{flox/flox}$ and $LysM^{cre}$ $TonEBP^{flox/flox}$ are shown in mice fed HSD. * $P_{(genotype)}$ <0.05.

A Low salt diet



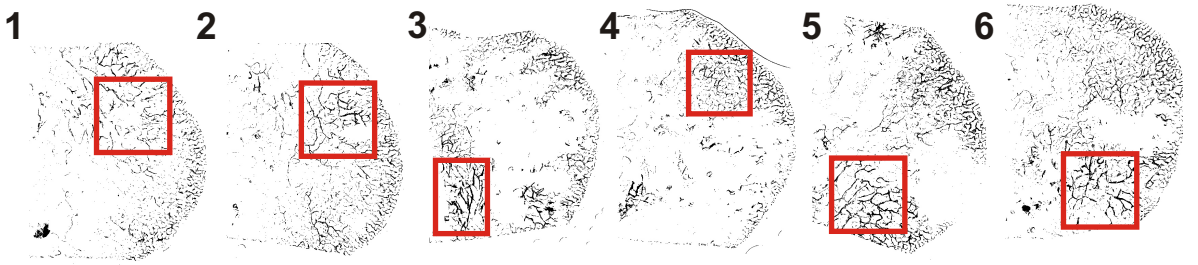
	1	2	3	4	5	6
GV [AU]:	26.4	40.2	25.2	31.4	24.3	34.3
MAP [mmHg]:	113	115	123	109	119	117

B High salt diet



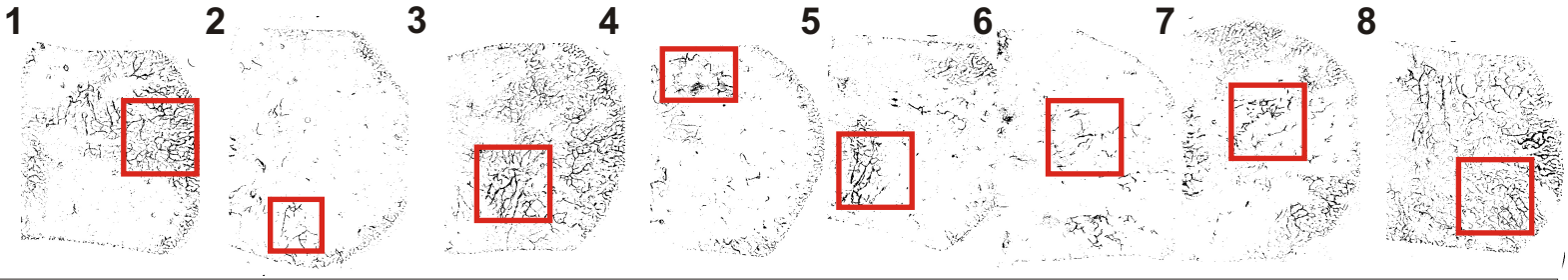
	1	2	3	4	5	6	7	8
GV [AU]:	45.3	51.3	62.1	41.0	52.3	57.8	68.6	44.3
MAP [mmHg]:	125	126	126	120	124	126	128	117

C Low salt diet + mF4-31c1



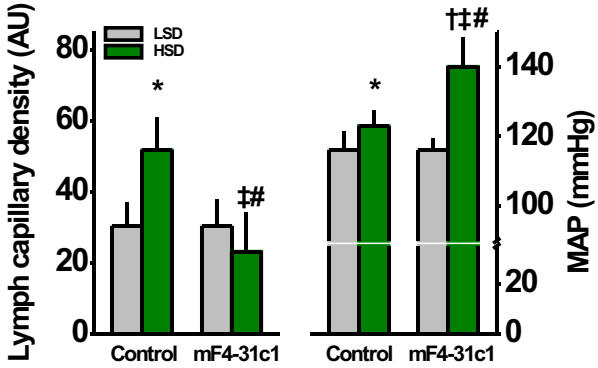
	1	2	3	4	5	6
GV [AU]:	21.2	25.0	41.9	28.7	32.7	32.5
MAP [mmHg]:	111	117	118	114	120	114

D High salt diet + mF4-31c1



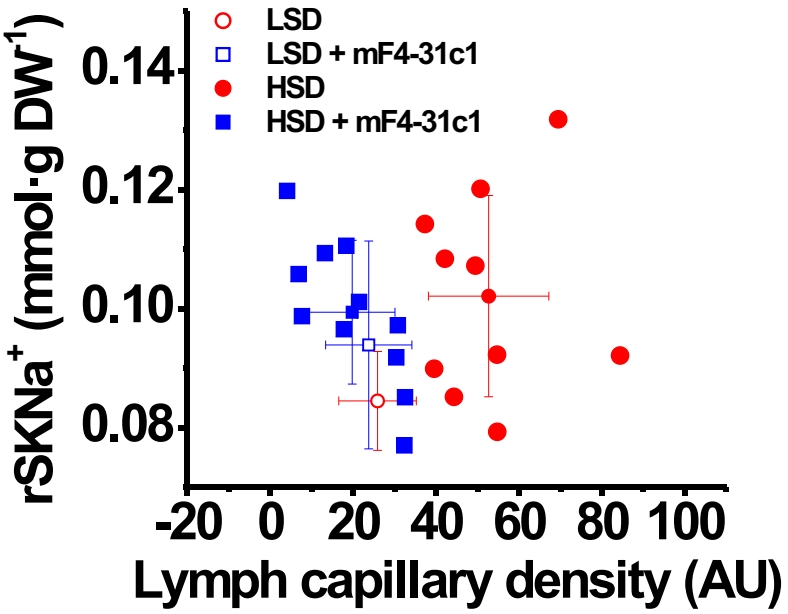
	1	2	3	4	5	6	7	8
GV [AU]:	39.3	9.9	33.4	17.4	21.8	9.1	15.2	25.2
MAP [mmHg]:	129	142	130	152	146	151	142	134

E

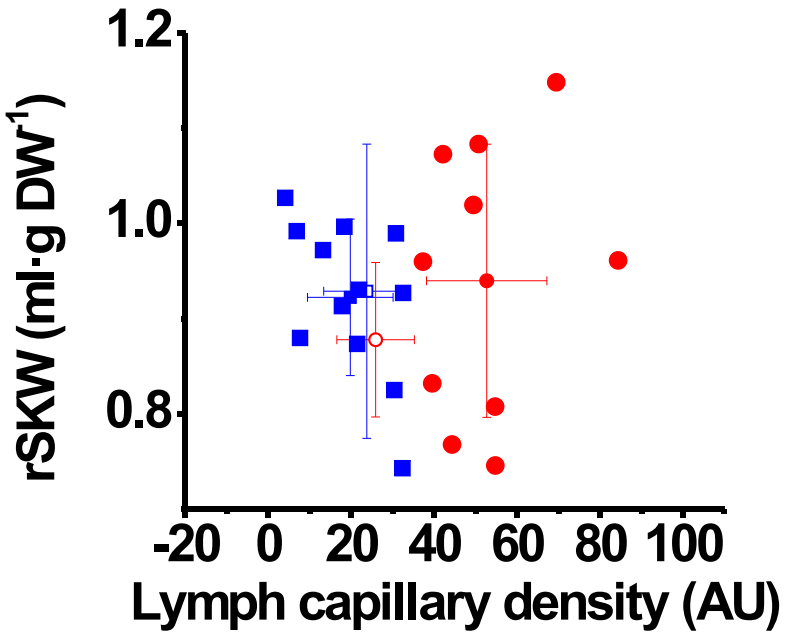


Supplemental Figure 2: Panels (A-D) Whole mount stainings of lymph capillaries (anti-Lyve-1 antibody) in ears of FVB mice fed LSD or HSD, with and without mF4-31c1 treatment. Red square is the computerized quantitated area; numerical value is lymph-capillary density (arbitrary units) which is given together with mean arterial blood pressure (MAP; mmHg) for each individual animal. (E) Lymph-capillary density in ear and mean arterial blood pressure (MAP) in the mice.

A

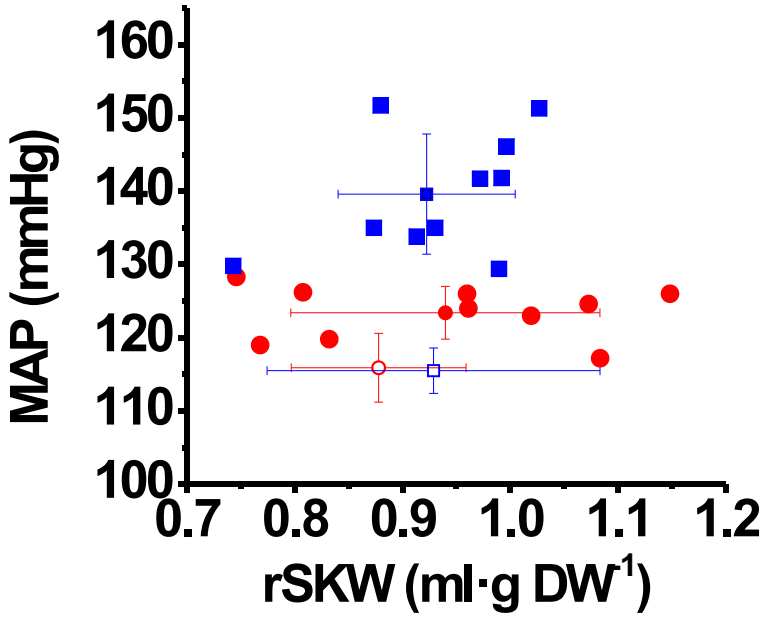


B

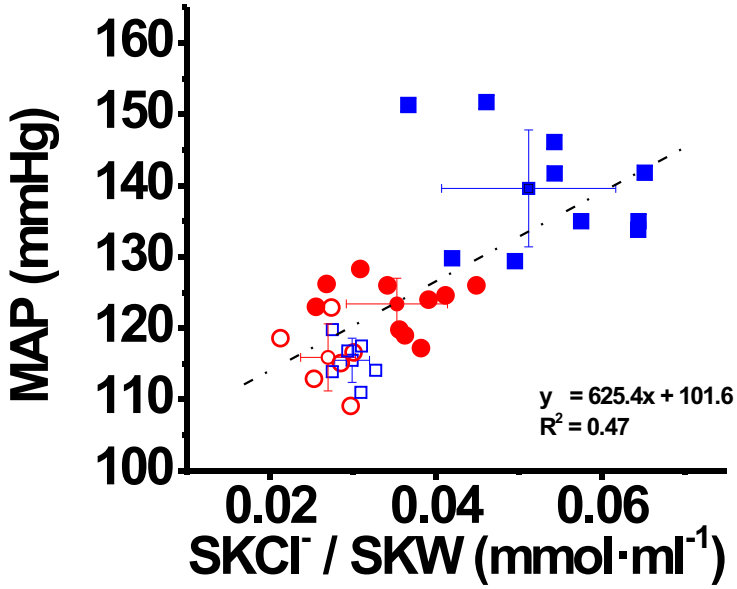


Supplemental Figure 3. Relationship between lymph capillary density in ear and (A) skin Na^+ content ($rSKNa^+$; mmol/g dry weight, and relationship between lymph capillary density and (B) skin water content (rSKW; ml/g dry weight) in mice fed a low-salt diet (LSD) or a high-salt diet (HSD) with and without additional anti-VEGFR3 treatment with mF4-31c1. AU: arbitrary units; DW: dry weight.

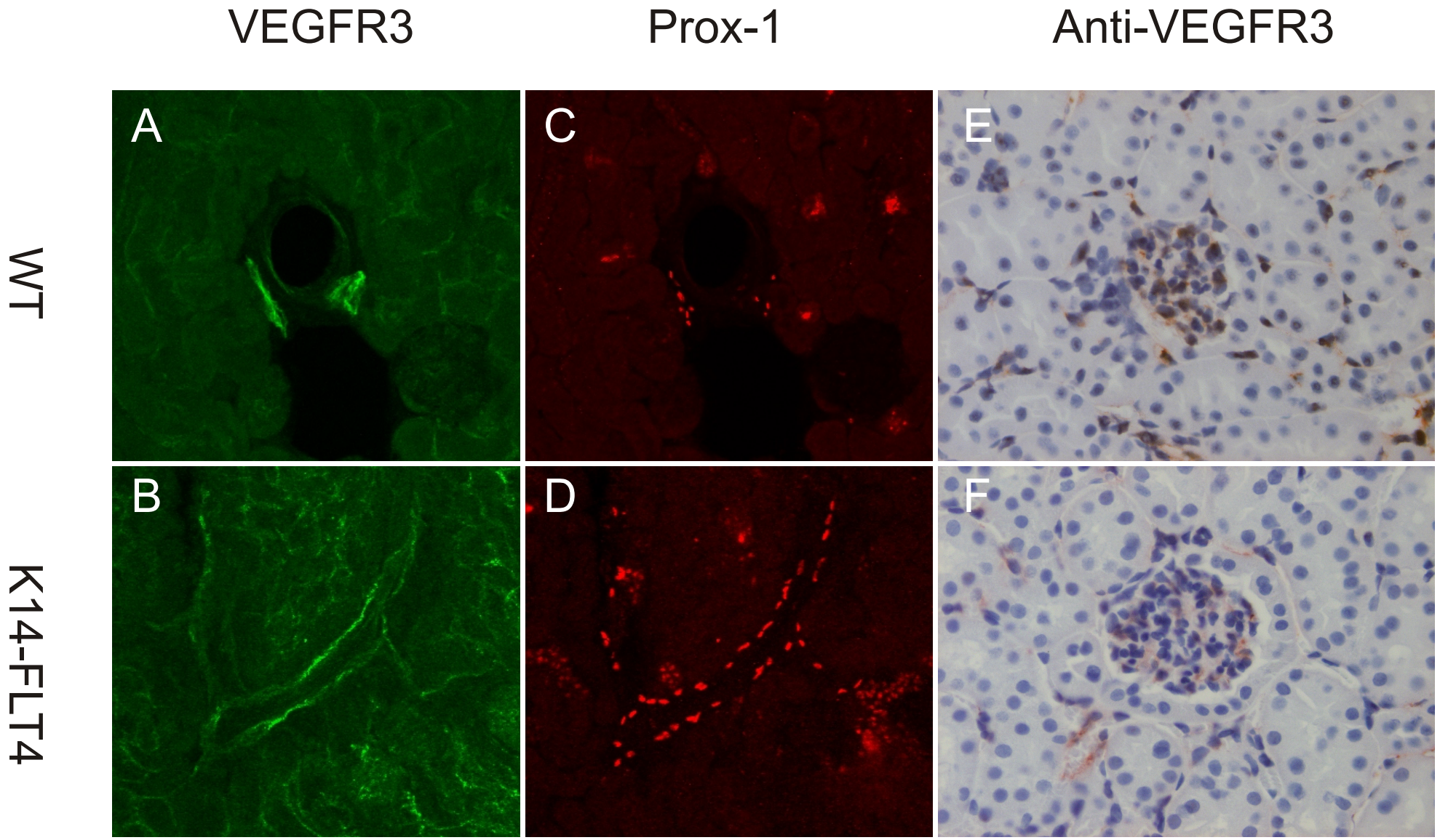
A



B



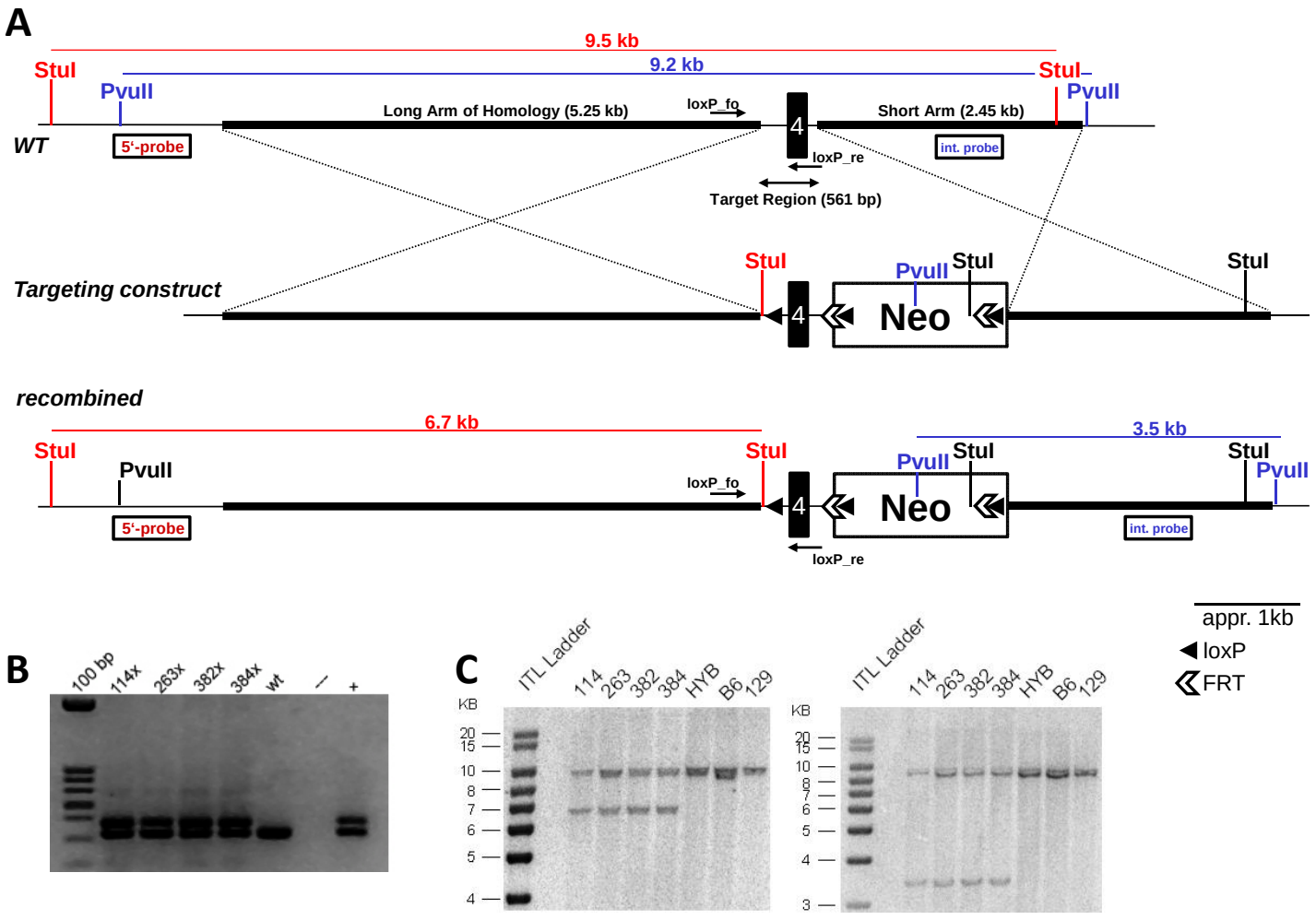
Supplemental Figure 4. Relationship between (A) skin water content (rSKW; ml/g dry weight) and mean arterial blood pressure (MAP; mmHg), and between (B) skin Cl^- concentration (mmol/ml tissue water) in mice fed a low-salt diet (LSD) or a high-salt diet (HSD) with and without additional anti-VEGFR3 treatment with mF4-31c1.



Supplemental Figure 5: Lymph capillaries in kidneys in wild type mice (WT) and in mice with expression of soluble VEGFR3 under the control of the keratinocyte receptor (K14-FLT4 mice). Green: VEGFR3 reporter fluorescence in wt and K14 FLT4 mice (Panels A and B); red: Prox-1 reporter fluorescence expression in WT and in K14-FLT4 (Panels C and D). Panels E and F: anti-VEGFR3 staining (brown) of renal lymph vessels in the same mice. In contrast to the hypoplastic lymph vessels in the skin, K14-FLT4 mice showed normal lymph vessels in the kidney.

Supplemental Table 1: Differences in Na^+ concentration and osmolality between plasma and microdialysate from skin interstitium in rats. LSD: low-salt diet, HSD: high-salt diet.

	LSD (n=10)	**HSD (n=10)**	P value (plasma versus microdialysate)
a) Na^+ concentration (mmol/L)			
Plasma	140.8±8.5	138.5±12.6	LSD: 0.01 HSD: <0.001
Microdialysate	149.6±8.1	149.2±9.0	
P value (LSD versus HSD)	plasma: 0.65; microdialysate: 0.92		
b) Osmolality (mosmol/kg)			
Plasma	309.5±7.9	304.6±10.1	LSD: 0.45 HSD: 0.03
Microdialysate	312.2±12.7	313.7±8.8	
P value (LSD versus HSD)	plasma: 0.24; microdialysate: 0.76		



Supplemental Figure 6. Generation of TonEBP-floxed mice.
Panel A. Targeting Strategy. TonEBP-floxed mice were generated by "inGenious Targeting Laboratory, Inc." (2200 Smithtown Avenue, Ronkonkoma, NY 11779) using standard gene-targeting techniques. The targeting construct was designed such that the short homology arm extends about 2.45 kb 3' to exon 4, whereas the long homology arm extends about 5.25 kb 5' to exon 4. A single loxP-site, containing an engineered StuI-site for Southern Blot analysis, was inserted 166bp upstream of exon 4 and a loxP/FRT-flanked Neo cassette was inserted 202 bp downstream of exon 4. Thus the target region is 561 bp long and includes exon 4. The figure is not exactly drawn to scale! BA1 (C57BL/6 x 129/SvEv) hybrid embryonic stem cells were electroporated with 10µg of linearized targeting vector and selected with G418 antibiotic.
Panel B. Screening and analysis of the retention of the third loxP-Site. (Initial Screening was carried out by PCR, using reverse primer 5'-ACGCCAGTGTCATGTTGTTG-3' downstream of the short arm and forward primer 5'-GCATAAGCTTGGATCCGTTCTTCGGAC-3' within the Neo cassette generating a product of 2.73 kb in case of homologous recombination; data not shown). Four PCR-positive clones were expanded (indicated by an "x)" and retention of the third loxP-site upstream of exon 4 was proven by PCR with primers 5'-GTAACCATGATTAGTCTTTTAGCTTTATG-3' and 5'-GTTCTGAGAATCCAAAGCACAAC-3' generating a 334 bp long fragment from the WT-allele and an additional 392 bp long fragment from the floxed allele.
Panel C. Southern Blot analysis. Further confirmation of the 4 expanded homologous recombinant clones was performed by two different Southern Blot experiments. **Left panel:** StuI-digested DNA was hybridized with a 755 bp long probe (probe primers: 5'-TTTTGTGGCTAAGCACAGTCCC-3' and 5'-CATACTGCAGCTCTGCTCAGATTC-3'), which was targeted against the 5' external region, detecting a 9.5 kb long WT-fragment and the 6.7 kb long floxed fragment. **Right panel:** PvuII-digested DNA was hybridized with a 613 bp long probe (probe primers: 5'-TGACTGCCCTCAACAGTTCATTTG-3' and 5'-ATTCAGGATCTGCTACCACCACTG-3') targeted against the 3' internal region, detecting a 9.2 kb long WT- and the 3.5 kb long floxed fragment. In both Southern Blot experiments, DNA from C57Bl/6 (B6), 129/SvEv (129), and BA1 (C57Bl/6 x 129/SvEv; Hyb) mouse strains were used as wild type controls.

Confirmation of Neo-deletion and standard Genotyping procedures.
Targeted iTL BA1 (C57BL/6N x 129/SvEv) hybrid embryonic stem cells were microinjected into C57BL/6 blastocysts. Resulting chimeras with a high percentage agouti coat color were mated to C57BL/6 homozygous FLP mice to remove the Neo cassette. Primers Ndel1 (5'-GTTGTGCTTTGGATTCTCAGAAC-3') and Ndel2 (5'-CTTCTACCCTTCTATTTCAGGAAGC-3') were used to confirm Neo-deletion indicated by a 675 bp fragment. DNA still containing Neo is not amplified since the fragment would be too long. The same primer pair also generates a 497 bp long WT-fragment. Thus it can be routinely applied for standard genotyping. Genotyping primers are not depicted within the figure.