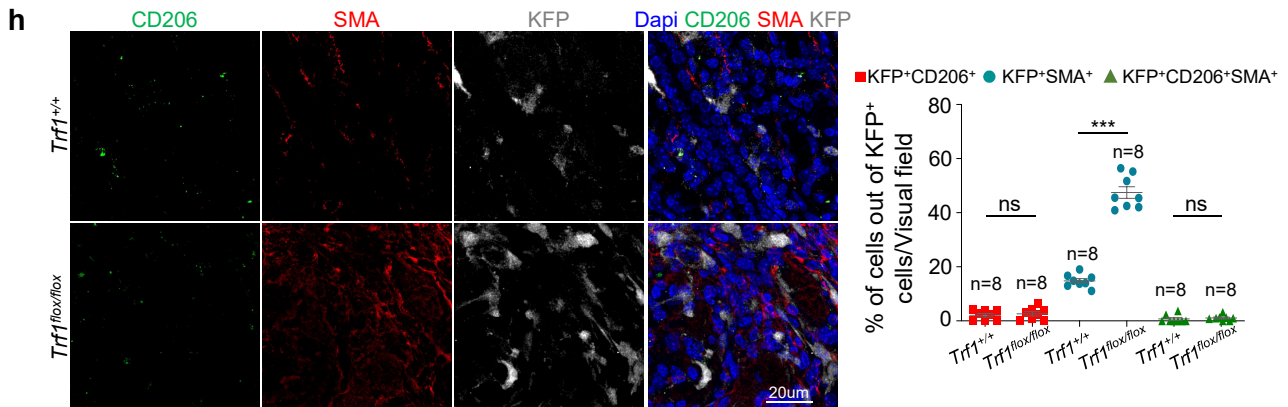
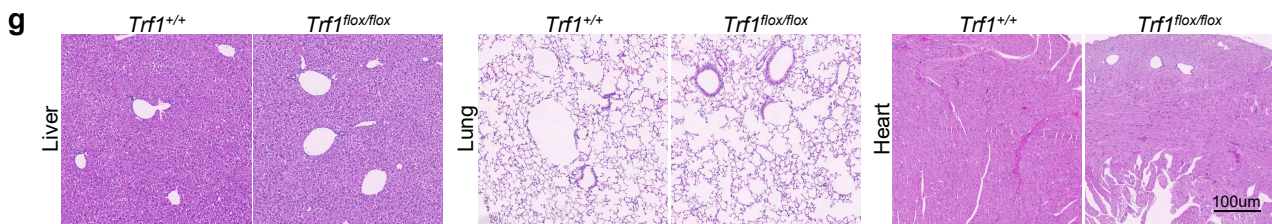
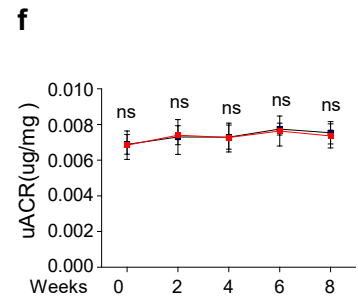
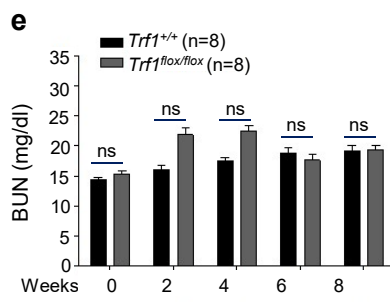
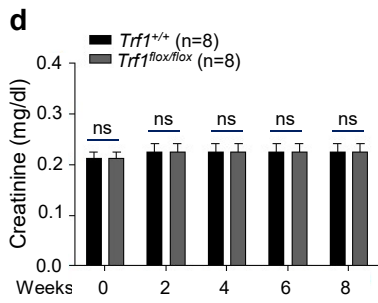
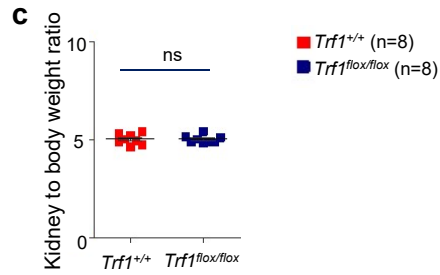
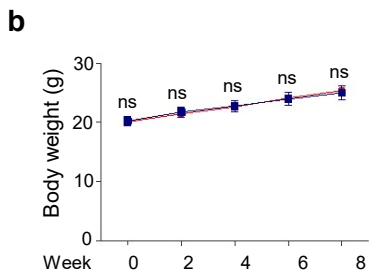
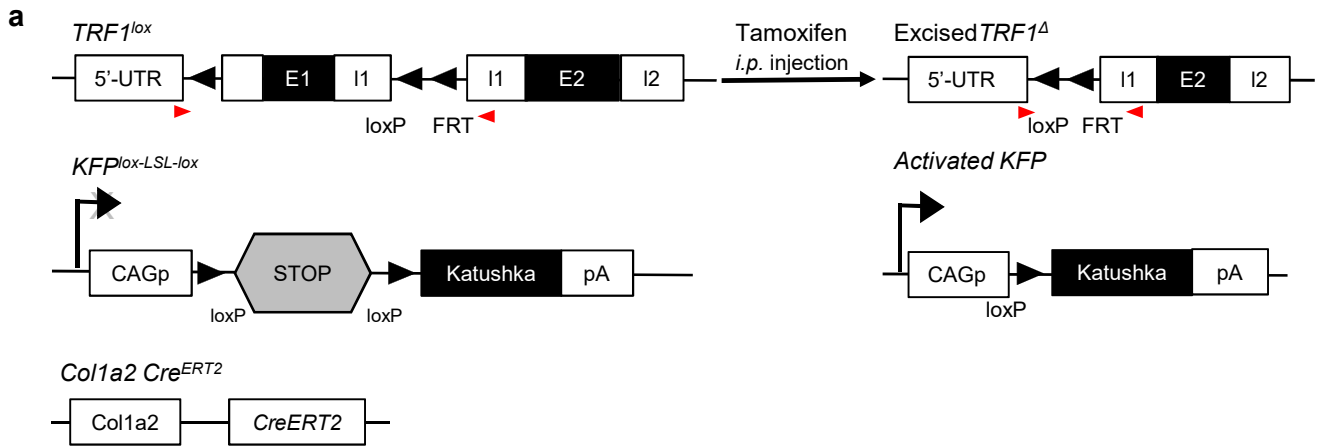
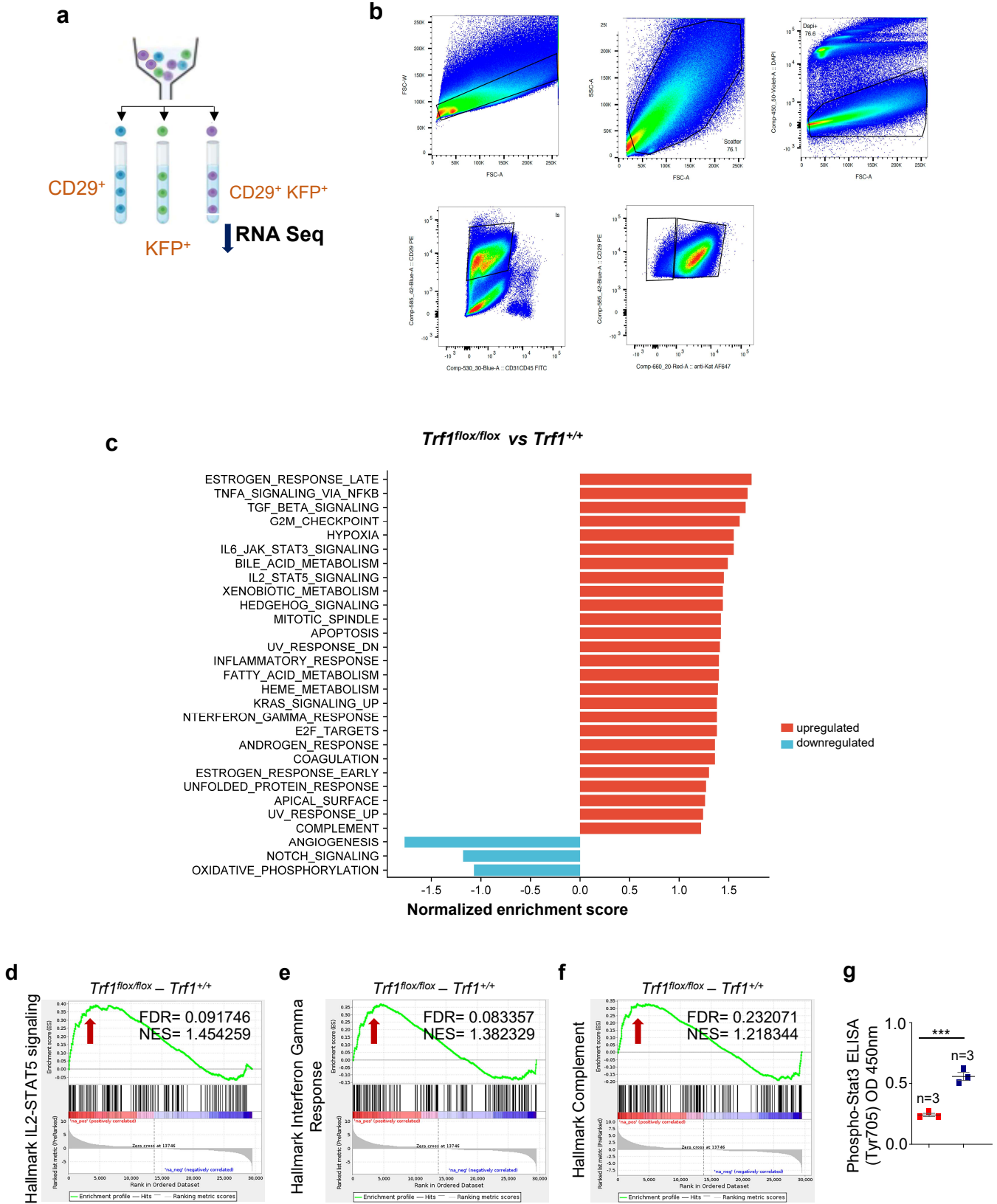


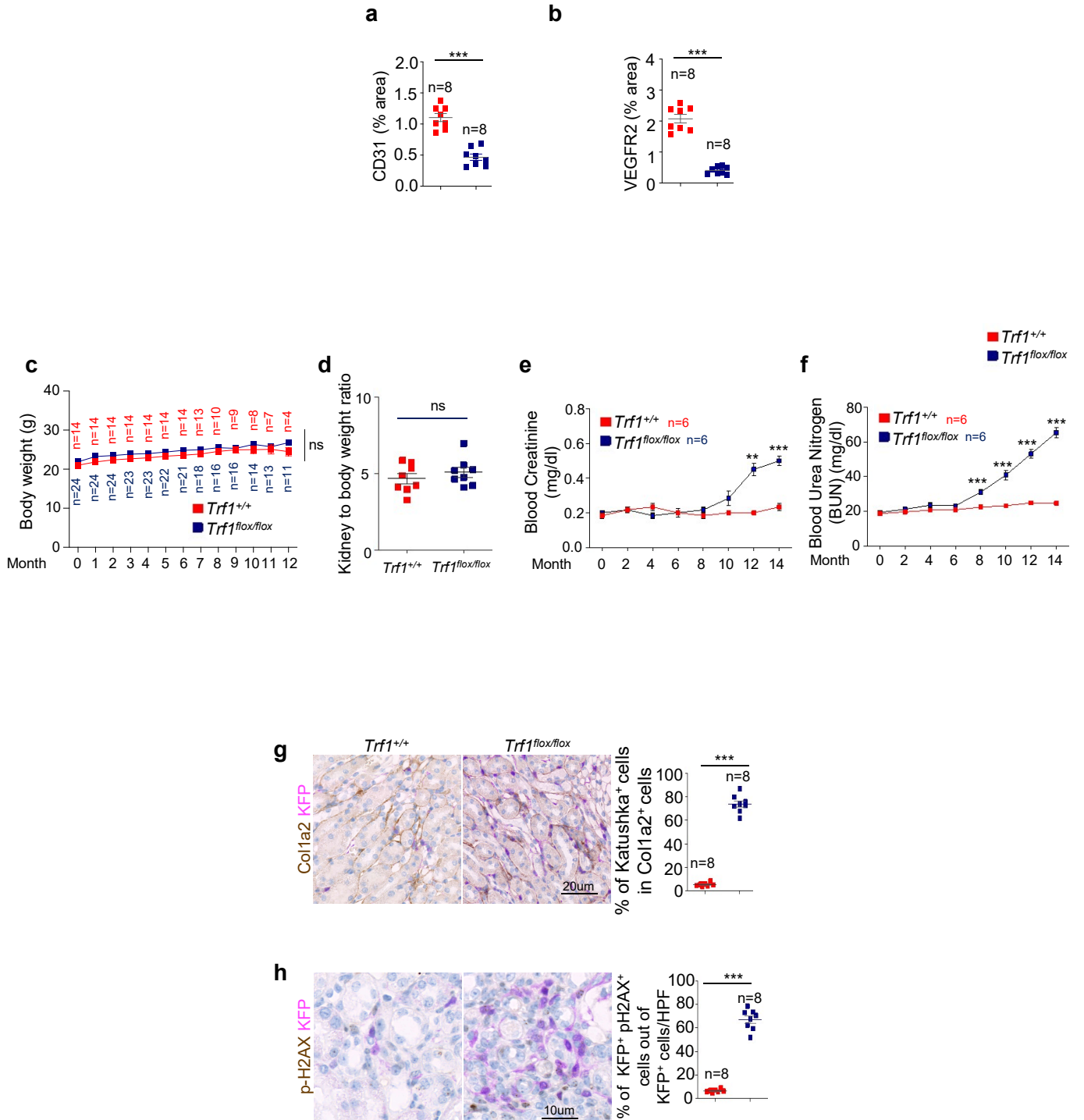
# Supplementary Fig. 1



Supplementary Fig. 2

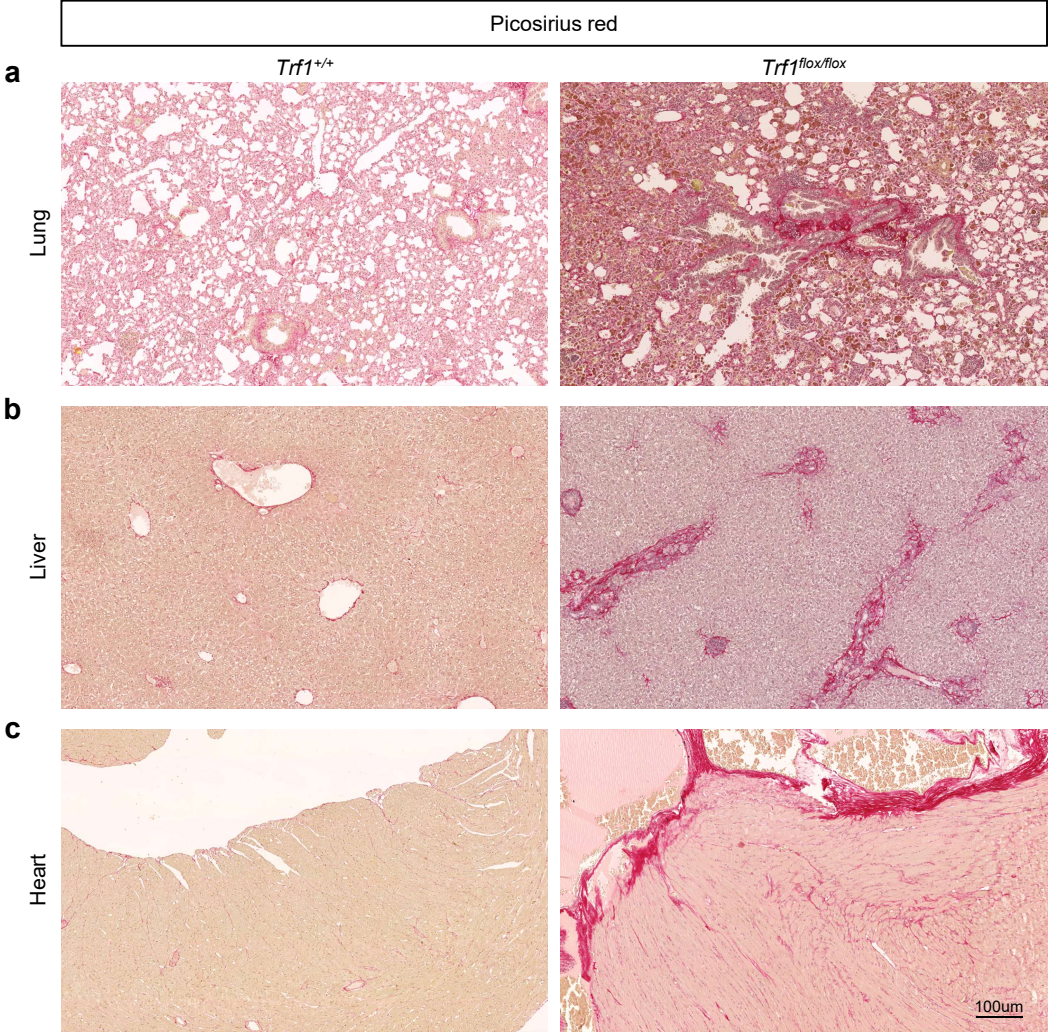


**Supplementary Fig. 3**

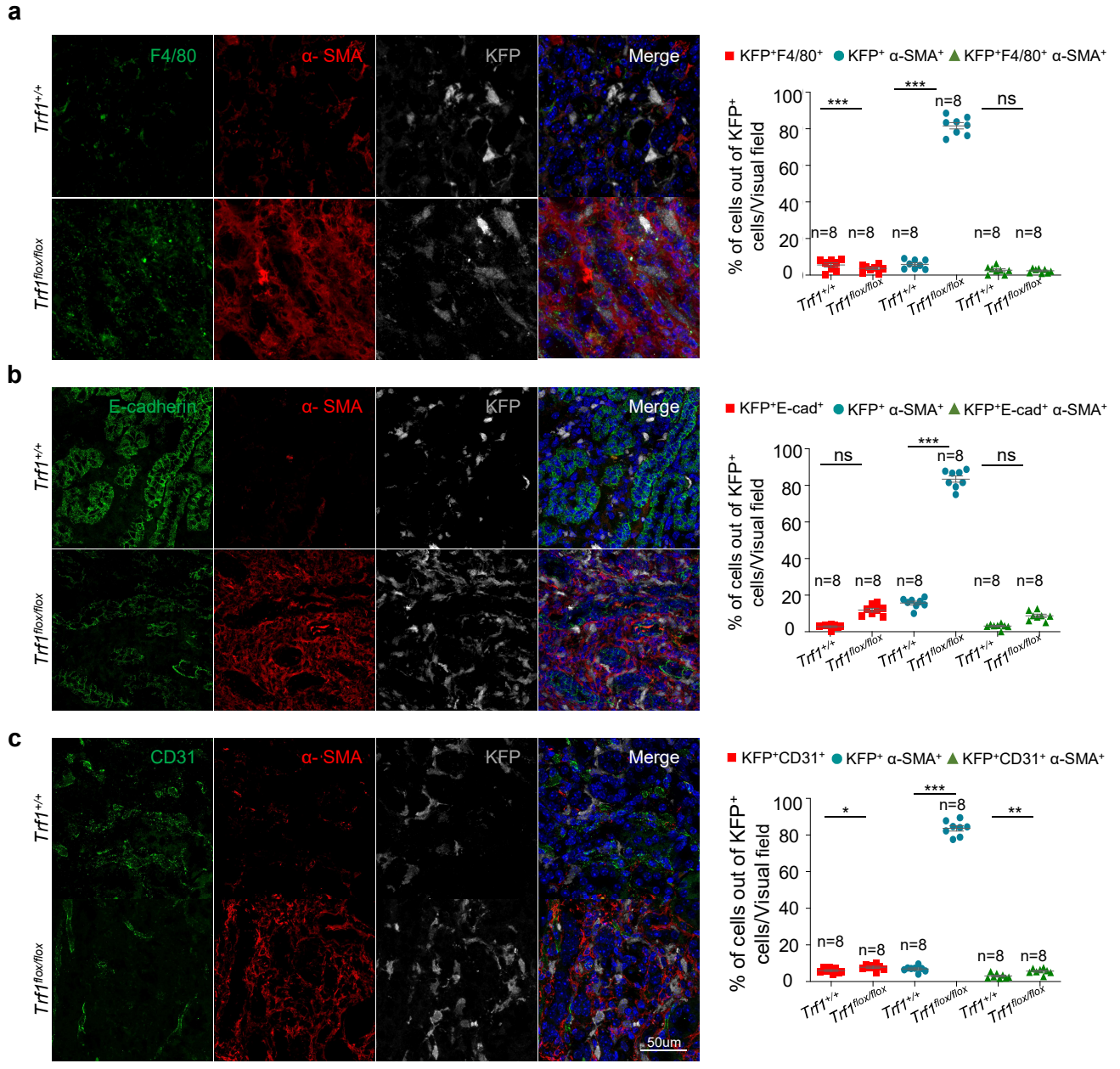




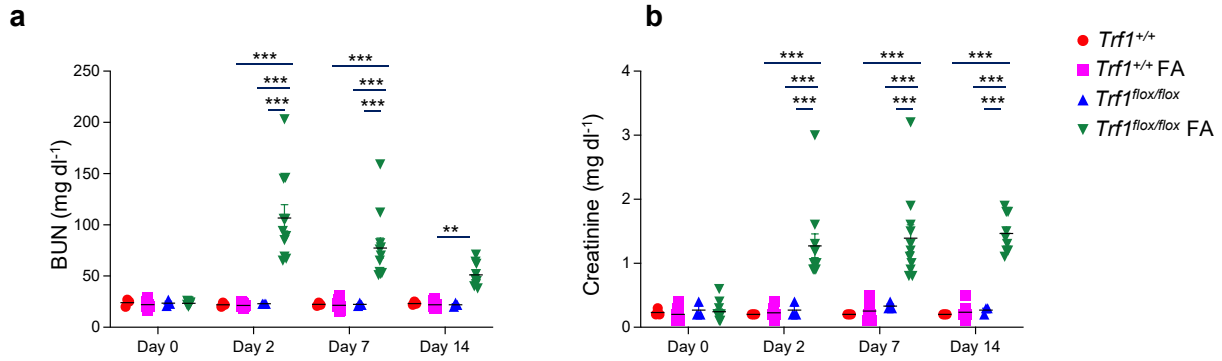
Supplementary Fig. 5



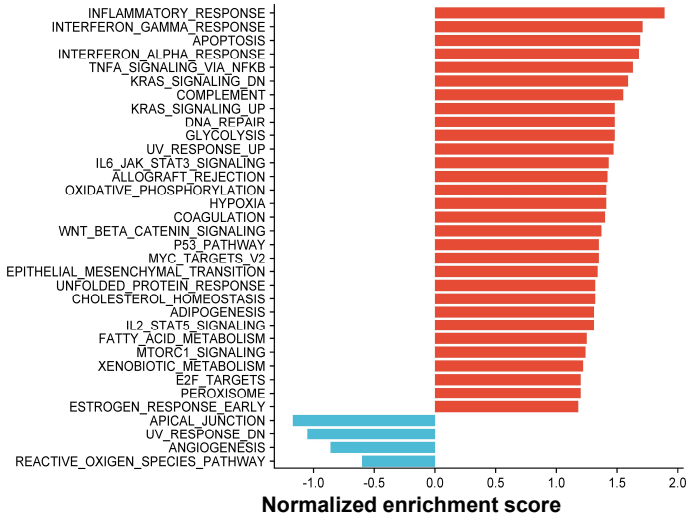
# Supplementary Fig. 6



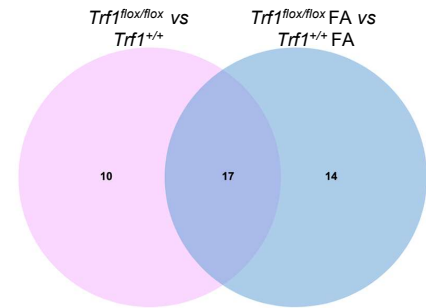
# Supplementary Fig. 7



**c** *Trf1<sup>flox/flox</sup> FA vs Trf1<sup>+/+</sup> FA*



**d** Venn diagram: number of enriched canonical pathways



**e** KNOWN PATHWAYS UPREGULATED IN KIDNEY FIBROSIS

- HALLMARK\_ALLOGRAFT\_REJECTION
- HALLMARK\_INTERFERON\_GAMMA\_RESPONSE
- HALLMARK\_INFLAMMATORY\_RESPONSE
- HALLMARK\_TNFA\_SIGNALING\_VIA\_NFKB
- HALLMARK\_INTERFERON\_ALPHA\_RESPONSE
- HALLMARK\_IL6\_JAK\_STAT3\_SIGNALING
- HALLMARK\_EPITHELIAL\_MESENCHYMAL\_TRANSITION
- HALLMARK\_COMPLEMENT
- HALLMARK\_KRAS\_SIGNALING\_UP
- HALLMARK\_IL2\_STAT5\_SIGNALING
- HALLMARK\_E2F\_TARGETS
- HALLMARK\_G2M\_CHECKPOINT
- HALLMARK\_MITOTIC\_SPINDLE
- HALLMARK\_UV\_RESPONSE\_DN
- HALLMARK\_COAGULATION
- HALLMARK\_HYPOXIA
- HALLMARK\_TGF\_BETA\_SIGNALING
- HALLMARK\_P53\_PATHWAY
- HALLMARK\_HEME\_METABOLISM
- HALLMARK\_FATTY\_ACID\_METABOLISM
- HALLMARK\_UV\_RESPONSE\_UP
- HALLMARK\_CELLULAR\_SENESCENCE

## Supplementary Information

### Supplementary Figures

#### Supplementary Fig. 1. Conditional deletion of *Trf1* in fibroblasts

**a** Generation of the conditional knockout mouse model in which *Trf1* was deleted in fibroblasts using the Cre recombinase driven by the *Col1a2* promoter. **b** Body weight and **c** kidney to body weight ratio in *Trf1<sup>flox/flox</sup>* compared to *Trf1<sup>+/+</sup>* mice. **d** blood creatinine **e** blood urea nitrogen (BUN) **f** urinary albumin-to-creatinine ratio (uACR) in *Trf1<sup>flox/flox</sup>* compared to *Trf1<sup>+/+</sup>* mice. **g** Representative H & E of liver, lung and heart in *Trf1<sup>flox/flox</sup>* compared to *Trf1<sup>+/+</sup>* mice. **h** Representative images (six to eight visual fields) and quantifications of immunolabeling for CD206,  $\alpha$ -SMA and KFP in *Trf1<sup>flox/flox</sup>* compared to *Trf1<sup>+/+</sup>* mice. Nuclei are stained with DAPI (blue). Data are represented as mean  $\pm$  SEM. An unpaired, two-tailed Student's *t*-test was used. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . The number of mice analyzed per genotype is  $n=8$ , if not indicated.

#### Supplementary Fig. 2. Flow cytometry cell isolation

**a** Schematic summary of the experimental protocol used to isolate CD29<sup>+</sup> or CD29<sup>+</sup> KFP<sup>+</sup> or or KFP<sup>+</sup> cells from *Trf1<sup>flox/flox</sup>* and *Trf1<sup>+/+</sup>* kidneys. **b** Gating strategy for isolating fibroblasts from *Trf1<sup>flox/flox</sup>* and *Trf1<sup>+/+</sup>* kidneys. **c** Results of GSEA Hallmark pathways (FDR < 25%) in *Trf1<sup>flox/flox</sup>* compared to *Trf1<sup>+/+</sup>* mice. GSEA enrichment plots of hallmark gene dataset showed the enrichment in **d** IL2-STAT5 signaling **e** Interferon Gamma Response **f** Complement system in the kidneys of *Trf1<sup>+/+</sup>* and *Trf1<sup>flox/flox</sup>* mice. **g** ELISA of pSTAT3 in the kidneys of *Trf1<sup>+/+</sup>* and *Trf1<sup>flox/flox</sup>* mice. All groups,  $n = 3$  mice for each group. Data are represented as mean  $\pm$  SEM. An unpaired, two-tailed Student's *t*-test was used. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

#### Supplementary Fig. 3. Effects of short term and long-term fibroblast-specific deletion of *Trf1*

Representative immunostainings (15-20 visual fields) and quantifications **a** CD31<sup>+</sup> **b** VEGFR2<sup>+</sup> in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> kidneys **c** Body weight **d** kidney to body weight ratio **e** Blood creatinine **f** blood urea nitrogen (BUN) in *Trf1*<sup>flox/flox</sup> compared to *Trf1*<sup>+/+</sup> mice at HEP. Representative immunostainings (15-20 visual fields) and quantifications **g** Col1a2<sup>+</sup>KFP<sup>+</sup> **h** p-H2AX<sup>+</sup>KFP<sup>+</sup> in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> kidneys. Data are represented as mean ± SEM. An unpaired, two-tailed Student's *t*-test was used. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. The number of mice analyzed per genotype is n=8, if not indicated.

#### **Supplementary Fig. 4. Fibroblast-specific deletion of *Trf1* exacerbates inflammatory response, hypoxia, and vascular rarefaction at HEP**

Representative images (15-20 visual fields) and quantifications of **a** PAS+D **b** Collagen I **c** Vimentin **d** CD206<sup>+</sup> KFP<sup>+</sup> **e** IL6<sup>+</sup> KFP<sup>+</sup> **f** p-JAK2<sup>+</sup> KFP<sup>+</sup> **g** p-STAT3<sup>+</sup> KFP<sup>+</sup> **h** HIF-1α<sup>+</sup> KFP<sup>+</sup> **i** Glut1<sup>+</sup> KFP<sup>+</sup> **j** CD31 in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> kidneys. Data are represented as mean ± SEM. An unpaired, two-tailed Student's *t*-test was used. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. The number of mice analyzed per genotype is n=8, if not indicated.

#### **Supplementary Fig. 5. Long-term and persistent fibroblast-specific deletion of *Trf1* leads to signs of fibrosis in different organs at HEP**

Representative images and quantifications of Picosirius red stainings in **a** Lung **b** Liver **c** Heart in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> mice. Scale bars, 100μm. The number of mice analyzed per genotype is n=8, if not indicated.

#### **Supplementary Fig. 6. Persistent long-term fibroblast-specific deletion of *Trf1* leads to acquisition of a mesenchymal phenotype via MMT, partial EMT and EndMT at HEP**

**a** Representative image (six to eight visual fields) and quantifications of immunolabeling for F4/80, α-SMA and KFP in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> kidneys. **b** Representative images (six to eight visual fields) and quantifications of immunolabeling for E-cadherin, α-SMA and KFP in *Trf1*<sup>+/+</sup> and *Trf1*<sup>flox/flox</sup> kidneys. **c** Representative images (six to eight visual fields) and quantifications

of immunolabeling for CD31,  $\alpha$ -SMA and KFP in *Trf1*<sup>+/+</sup> and *Trf1*<sup>fllox/fllox</sup> kidneys. Nuclei are stained with DAPI (blue). Data are represented as mean  $\pm$  SEM. An unpaired, two-tailed Student's *t*-test was used. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. The number of mice analyzed per genotype is n=8, if not indicated.

**Supplementary Fig. 7. Effect of *Trf1* deletion on Folic acid-induced fibrosis model.**

**a** Blood urea nitrogen (BUN) **b** blood creatinine in the kidneys of *Trf1*<sup>+/+</sup> and *Trf1*<sup>fllox/fllox</sup> mice untreated or treated with FA at a dose of 125 mg kg<sup>-1</sup>. Representative images (10 to 15 visual fields) **c** Results of GSEA Hallmark pathways (FDR < 25%) **d** Venn diagram representing number of enriched canonical pathways in the kidneys of *Trf1*<sup>+/+</sup> and *Trf1*<sup>fllox/fllox</sup> mice untreated or treated with FA at a dose of 125 mg kg<sup>-1</sup>. **e** Known pathways set. Data are represented as mean  $\pm$  SEM. An unpaired, two-tailed Student's *t*-test was used. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. The number of mice analyzed per genotype is n=8, if not indicated.

**Supplementary Table 1: RT-qPCR primers**

<b>Gene</b>	<b>Sequence (5'- 3')</b>
Acta2 F	ATCACCAACTGGGACGACAT
Acta2 R	AGTGTCGGATGCTCTTCAGG
Ca9 F	TACGAACAGTTGCTGTCCCA
Ca9 R	GGTAGTAGCGGCTGAGGTCC
Cd4 F	ACCAGAGGAAGATTCTGGGG
Cd4 R	TCAAAACGATCAAACCTGCGA
Cd8a F	GATTGGACTTCGCCTGTGAT
Cd8a R	GCCTTCCTGTCTGACTAGCG
Cd8b F	ATGTGAAGCCAGAGGACAGTG
Cd8b R	GGGGCAGTTGTAGGAAGGAC
Cdh1 F	<i>GCAGAACTGTCCCTGTCC CAG</i>
Cdh1 R	<i>GAACAGCACGTACACAGC CCT</i>
Cdkn1a F	TGTCCGTCAGAACCCATGC
Cdkn1a R	AAAGTCGAAGTTCCATCGCTC
Cdkn1b F	AACGTGCGAGTGTCTAACGG
Cdkn1b R	CCCTCTAGGGGTTTGTGATTCT
Cxcl10 F	AGGGTCGTGACTGAAAACCA
Cxcl10 R	AGCGTGGTGACCATTTCAA
Emr1 F	GCACCATCTTGCTGGAGACT
Emr1 R	TTCATTGATGACTTTGCTTTTCG
GAPDH F	AGGTCGGTGTGAACGGATTTG
GAPDH R	TGTAGACCATGTAGTTGAGGTCA
Hif1 $\alpha$ F	TAGTAAGCGGAGATCGGGTG
Hif1 $\alpha$ R	CTGAGGGGTTATGGATGCAA

IL15 $\alpha$ - F	TTAAGCGGAAAGCTGGAACA
IL15 $\alpha$ R	GTCATTTTGGAACTGTGGGG
IL17rb F	GTGTGGCAGCAGAGGAGTTT
IL17rb R	TGTTGTTTTTGCCACTCACG
IL18r1 F	TAGTCTCACTTGCTGGGCG
IL18r1 R	GATGCATGTTTAGGCTTCCACT
IL1 $\beta$ F	GCTGAAAGCTCTCCACCTCA
IL1 $\beta$ R	CCAAGGCCACAGGTATTTTG
IL1r1 F	CTCATTGTCTCATGGTGCCT
IL1r1 R	TGGAGTAAGAGGACACTTGCG
IL6st F	TTGATTGCAAACGTGGATTG
IL6st R	TGAATCGGAAACAAGTTCGC
Kdr F	GCGACCCCAAATTCCATTAT
Kdr R	CCGGCTCTTTCGCTTACTG
Lnc2 F	CAATGTCACCTCCATCCTGG
Lnc2 R	CCTGGAGCTTGAACAAATG
Nfk $\beta$ 1 F	TCCGGGAGCCTCTAGTGAG
Nfk $\beta$ 1 R	CTTTGCAGGCCCCACATAGT
Nfk $\beta$ 2 F	GGCCGGAAGACCTATCCTACT
Nfk $\beta$ 2 R	CTACAGACACAGCGCACACT
Pecam F	AAACCGTATCTCCAAAGCCAG
Pecam R	ACAGGTCCAACAACCTCCCCT
Smad3 F	TGAACACCAAGTGCATTACCA
Smad3 R	AGGCGGCAGTAGATAACGTG
Snai1 F	ACCCTGCTGGTATCTCTCCC
Snai1 R	TCTTCACATCCGAGTGGGTT
Snai2 F	GGCTGCTTCAAGGACACATT

Snai2 R	GTGCCCTCAGGTTTGATCTG
Terf1 F	TGGAAAATGAGAAAGCGAGG
Terf1 R	TTTCTGTAGACTGCTGGCCATT
Tgfb1 F	GGAGAGCCCTGGATACCAAC
Tgfb1 R	ATCCTACTTCCAACCCAGGTC
Tnfaip3 F	AGAAGTGTCCAGGCTTCCCT
Tnfaip3 R	ATGCATGAGGCAGTTTCCAT
Traf1 F	ATGCAGAGCAGACAACCTCC
Traf1 R	TGAAGGAACAGCCAACACCT
Twist F	CACGCTGCCCTCGGACAA
Twist R	GGGACGCGGACATGGACC
Zeb1 F	CCAAGAAGCTGCTGGCAAGA
Zeb1 R	TTCGGATCATGGTTTTGCTC
Zeb2 F	AAACAAGCCAATCCCAGGAG
Zeb2 R	GAGGGTTTGCAAGGCTATCA

**Supplementary Table 2: Blood parameters of mice at day 0, 2, 7 and 14 post-FA.**

Parameters		<i>Trf1</i> <sup>+/+</sup>	<i>Trf1</i> <sup>+/+</sup> FA	<i>Trf1</i> <sup>flox/flox</sup>	<i>Trf1</i> <sup>flox/flox</sup> FA
Calcium(mg/dL)	Day 0	10.3 ± 0.1	10.32 ± 0.200	10.43 ± 0.305	10.35 ± 0.206
	Day 2	10.06 ± 0.057	10.23 ± 0.170	10.3 ± 0.173	11.748 ± 0.787 <sup>a,b,c</sup>
	Day 7	10.4 ± 0.173	10.5 ± 0.260	10.46 ± 0.305	12.25 ± 0.738 <sup>a,b,c</sup>
	Day 14	10.36 ± 0.115	10.46 ± 0.224	10.23 ± 1.527	12.64 ± 0.320 <sup>a,b,c</sup>
Phosphorus (mg/dL)	Day 0	5.96 ± 0.208	5.85 ± 1.073	6.03 ± 0.378	5.85.87 ± 0.452
	Day 2	5.6 ± 0.529	5.32 ± 0.827	5.3 ± 0.953	8.12 ± 1.920 <sup>b,c,d</sup>
	Day 7	5.9 ± 0.264	5.66 ± 0.812	5.96 ± 0.776	9.44 ± 1.999 <sup>a,b,c</sup>
	Day 14	6.33 ± 0.321	6.23 ± 0.320	6.43 ± 0.208	10.17 ± 1.992 <sup>a,b,c</sup>
Sodium (mMol/L)	Day 0	147 ± 1.732	145.45 ± 2.018	144.66 ± 1.527	145.09 ± 1.578
	Day 2	145 ± 3	143.5 ± 3.439	145.66 ± 0.577	146.54 ± 2.207 <sup>a,b,f</sup>
	Day 7	143.33 ± 4.041	141.18 ± 2.60	144.33 ± 2.081	147.09 ± 2.913 <sup>a,b</sup>
	Day 14	143.66 ± 0.577	140 ± 2.449	143 ± 3.605	149.77 ± 2.728 <sup>a,b,c,g,h</sup>
Albumin (g/dL)	Day 0	4.26 ± 0.115	4.24 ± 0.191	3.76 ± 1.265	4.17 ± 0.110
	Day 2	3.73 ± 0.208	3.8 ± 0.2	3.55 ± 1.208	3.53 ± 0.374
	Day 7	3.73 ± 0.057	3.97 ± 0.261	3.45 ± 1.140	3.72 ± 0.228 <sup>a,b,f</sup>
	Day 14	3.76 ± 0.152	3.99 ± 0.273	3.52 ± 1.159	4.26 ± 0.187 <sup>a,b,c</sup>
Total Protein (g/dL)	Day 0	5.73 ± 0.152	5.62 ± 0.148	5.66 ± 0.152	5.52 ± 0.100
	Day 2	5.5 ± 0.1	5.5 ± 0.189	5.66 ± 0.251	6.07 ± 0.462 <sup>e,f</sup>
	Day 7	5.46 ± 0.208	5.56 ± 0.287	5.66 ± 0.057	6.50 ± 0.717 <sup>a,b,g</sup>
	Day 14	5.56 ± 0.404	5.62 ± 0.319	5.6 ± 0.2	6.85 ± 0.461 <sup>a,b,c</sup>
Globulin(g/dL)	Day 0	1.46 ± 0.057	1.43 ± 0.220	1.43 ± 0.057	1.40 ± 0.943
	Day 2	1.56 ± 0.057	1.609 ± 0.157	1.46 ± 0.152	2.26 ± 0.576 <sup>a,b,c</sup>
	Day 7	1.73 ± 0.057	1.67 ± 0.110	1.66 ± 0.115	2.62 ± 0.431 <sup>a,b,c</sup>
	Day 14	1.56 ± 0.110	1.64 ± 0.150	1.6 ± 0.3	2.83 ± 0.269 <sup>a,b,c</sup>

<sup>a</sup>p < 0.001 *Trf1*<sup>+/+</sup> vs. *Trf1*<sup>flox/flox</sup> FA; <sup>b</sup>p < 0.001 *Trf1*<sup>+/+</sup>FA vs *Trf1*<sup>flox/flox</sup> FA; <sup>c</sup>p < 0.001 *Trf1*<sup>flox/flox</sup> vs.

*Trf1*<sup>flox/flox</sup> FA

<sup>d</sup>p < 0.01 *Trf1*<sup>+/+</sup> vs. *Trf1*<sup>flox/flox</sup> FA; <sup>e</sup>p < 0.001 *Trf1*<sup>flox/flox</sup> vs. *Trf1*<sup>flox/flox</sup> FA

<sup>f</sup>p < 0.05 *Trf1*<sup>flox/flox</sup> FA vs. *Trf1*<sup>+/+</sup> FA; <sup>g</sup>p < 0.01 *Trf1*<sup>flox/flox</sup> vs. *Trf1*<sup>flox/flox</sup> FA; <sup>h</sup>p < 0.01 *Trf1*<sup>+/+</sup> vs. *Trf1*<sup>flox/flox</sup>

FA

Data are presented as mean ± standard deviation of the mean.