Supplementary Information

Chimeric Fusion between Clostridium ramosum IgA Protease and IgG Fc Provides Long-lasting Clearance of IgA deposits in Mouse Models of IgA Nephropathy

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Table of Contents

Table S1. Baseline characteristics of patients with IgA nephropathy.

ID	Age	Gender	Scr (μmol/l)	UTP(g/d)	SBP	DBP	M	E	S	T	C
PT.1	30	M	167.3	12.37	134	83	1	1	1	1	2
PT.3	26	F	263.2	4.42	130	70	1	1	0	2	2
PT.6	14	F	650.9	4.42	117	68	1	0	0	2	2
PT.7	27	M	180.9	3.73	118	62	1	1	1	2	1
PT.9	20	F	76.5	2.75	120	75	1	1	0	1	2
PT.12	38	M	101	2.12	150	100	1	1	1	1	1
PT.19	44	F	43.7	0.44	110	90	0	0	0	0	1
PT.22	31	F	133	1.78	115	78	1	1	0	0	1
PT.26	47	M	94	1.82	130	82	0	0	0	0	0

Table S2. Baseline characteristics of patients with Henoch-Schönlein purpura nephritis.

ID	Age	Gender	Scr (μmol/l)	UTP(g/d)	SBP	DBP
HSPN.1	22	M	126.95	5.98	154	91
HSPN.2	23	M	111.1	2.67	134	92
HSPN.3	26	M	84.31	1.99	117	76
HSPN.4	74	F	73.7	1.15	128	72

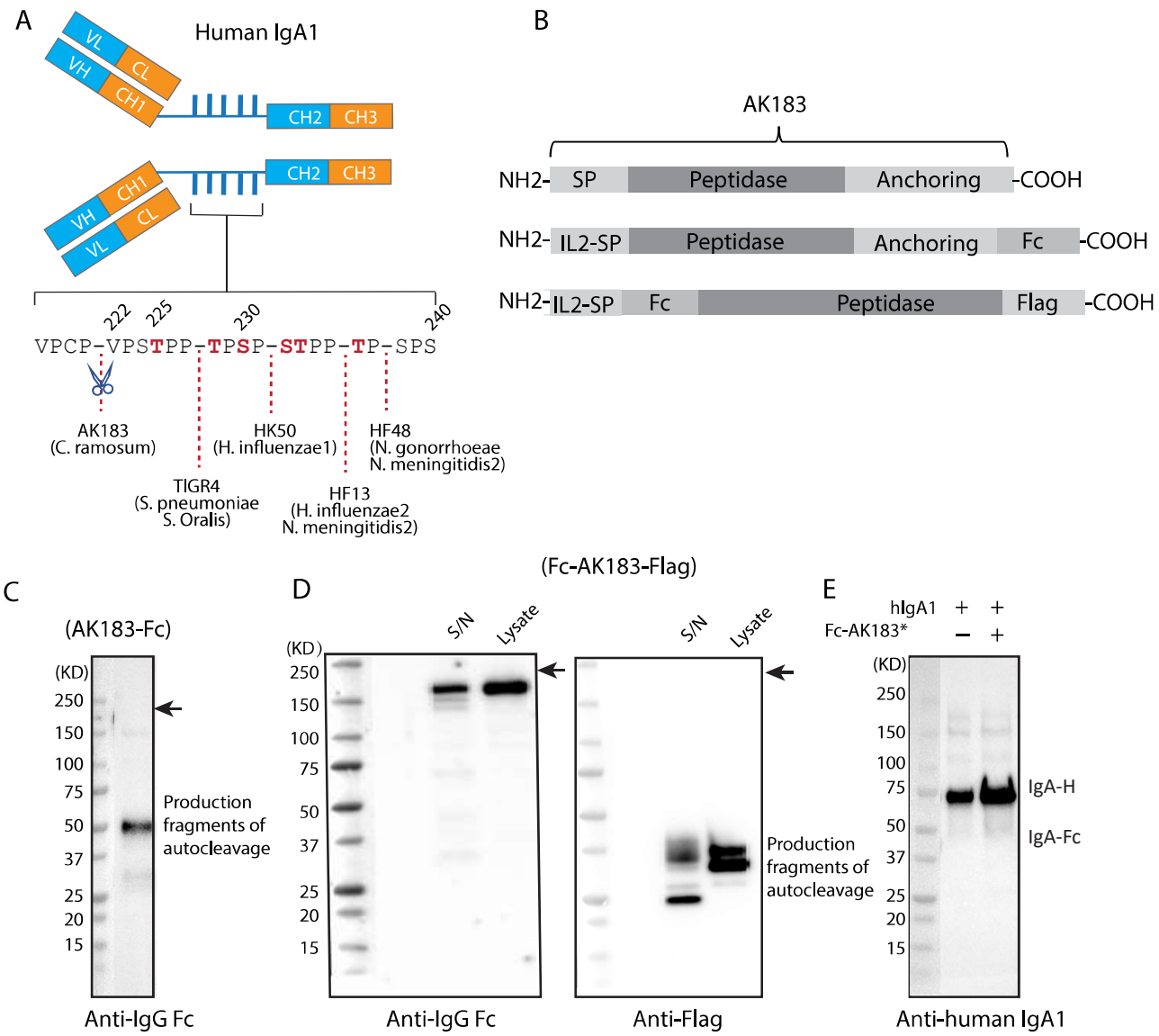


Figure S1. AK183 autocleaves its C-terminus β-domain.

A. Human IgA1 isotype contains a hinge segement between CH1 and CH2 domains of the heavy chain. There are a number of O-linked glycosylation sites in the sequence (red letters). A number of bacteria produce specific IgA1 proteases that cleave hinge amino acids (dotted lines). B. We constructed a number of fusions of Fc and AK183 IgA protease. Schematics of wild-type (top), and AK183-Fc (middle) or Fc-AK183 (bottom) are shown. C and D. Expressions of recombinant AK183-Fc constructs in HEK293 cells showed autocleaved fragments, indicating autocleave sites in the C-terminus β-domain of AK183 (full-length of AK183-Fc fusion is expected to be ~200 kDa: pointed by arrow.). D. Alternative Fc-AK183 fusion also produced autocleaved fragments of 160 kDa (left panel) and 30 kDa (right panel). E. However, these HEK293-expressed recombinant Fc-AK183 had no activity against purified IgA substrate. Intact IgA heavy chain was at its expected molecular weight of ~65 kDa.

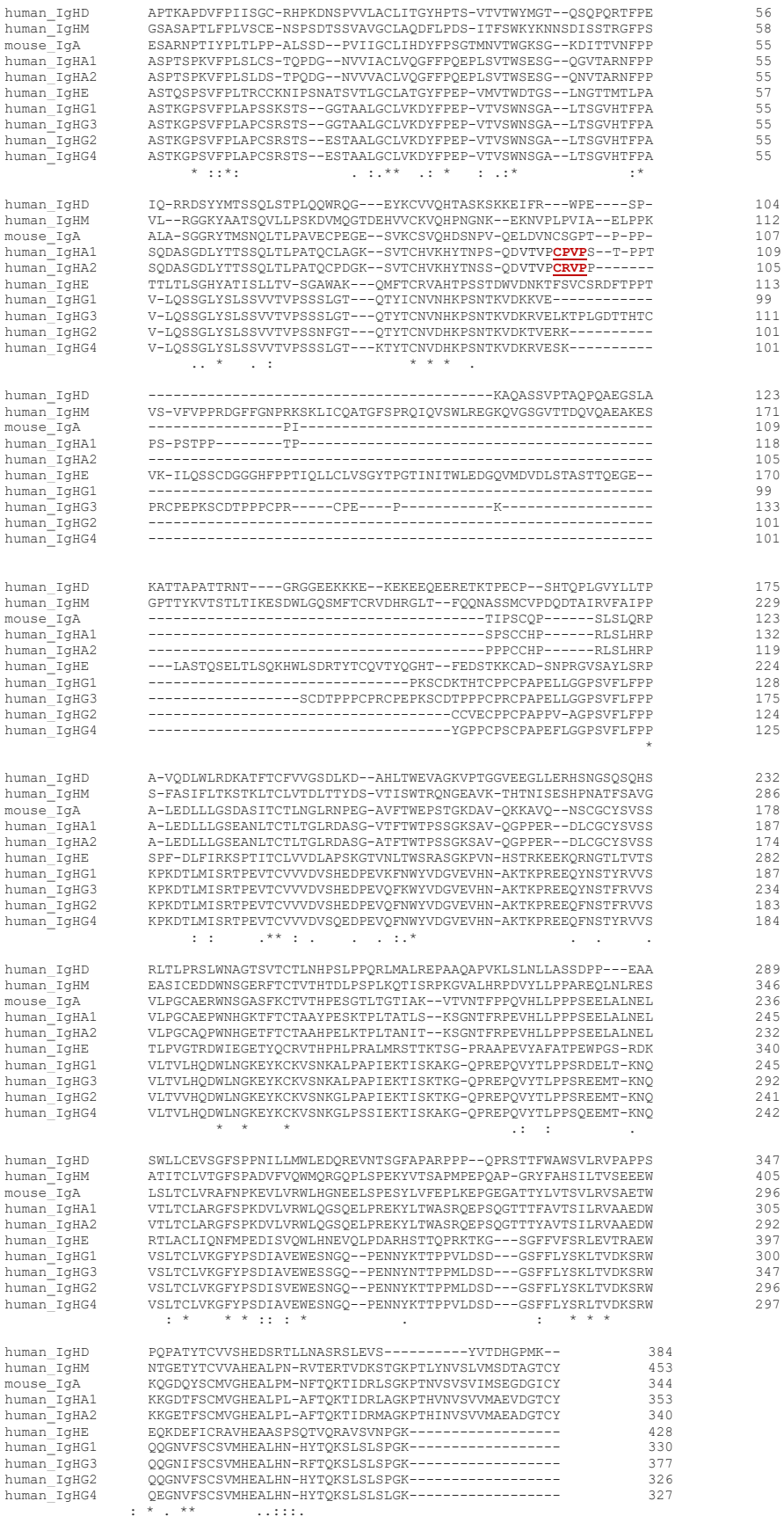


Figure S2. Alignment of CH1-CH3 sequences of murine IgA, human IgA1, IgA2, IgG, IgM, IgD and IgE.

The constant regions (CH) of immunoglobulin heavy chains are aligned. The highlighted “CPVP” motif in IgHA1 and IgHA2 is located immediately upstream of the IgA1 hinge sequence populated with proline, threonine and serine residues. Bacteria IgA protease AK183 cleaves the Pro-Val amino acid bond of the “CPVP” motif of both IgA1 and IgA2.

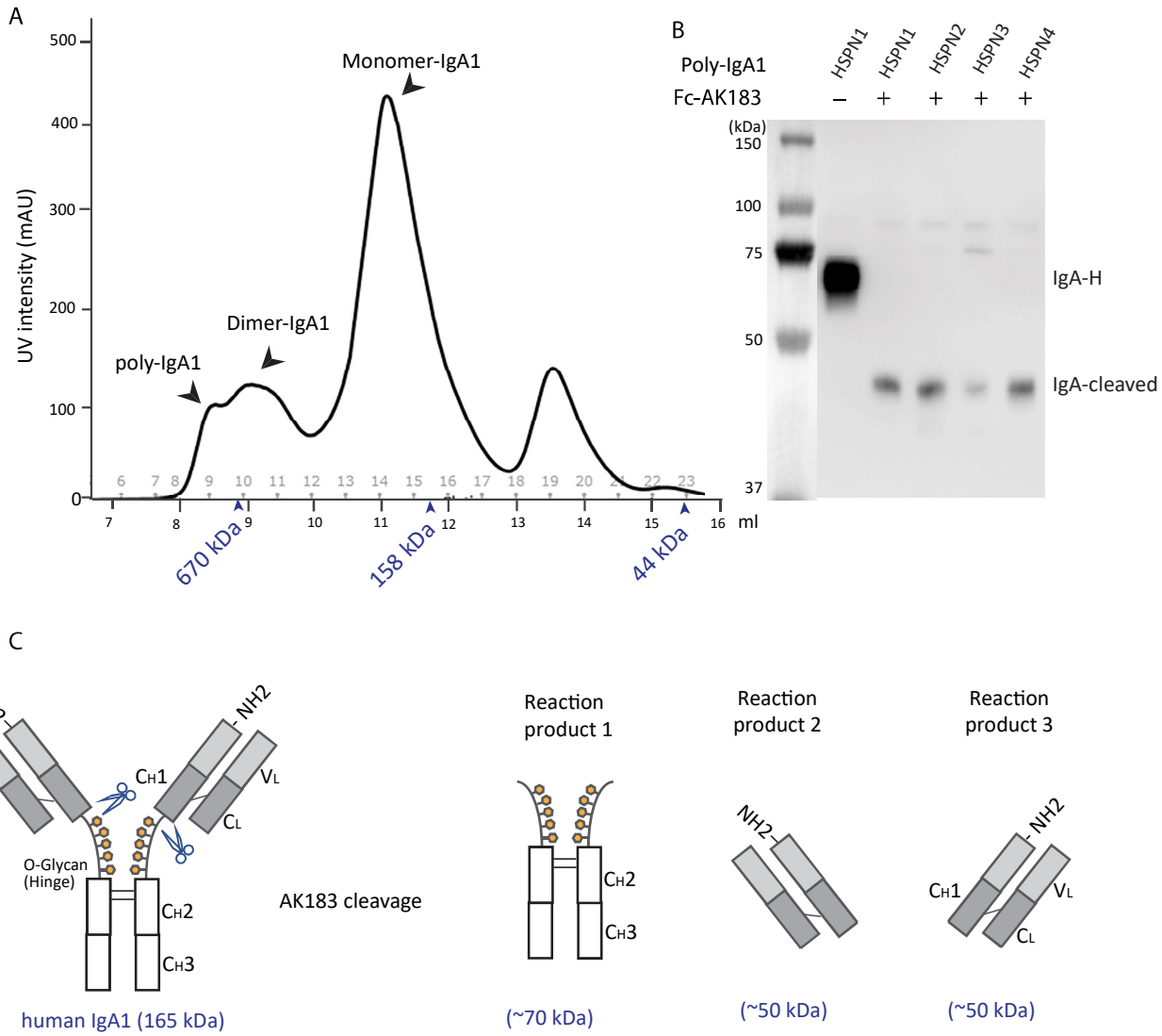


Figure S3. Recombinant Fc-AK183 cleaved poly-IgA1 purified from patients with Henoch-Schonlein purpura nephritis (HSPN).

A. Whole sera from HSPN patients were subjected to Jacalin purification of IgA1 contents, followed by SEC analyses of IgA complex size. A representative example of HSPN #1 is shown with SEC tracing. Protein peaks representing poly-IgA1 complex, normal dimeric and monomeric IgA1 were visible. We specifically collected poly-IgA from all 4 HSPN samples (HSPN1-4). These poly-IgA isolates were subjected to reactions with Fc-AK183 (+), or buffer control (-). Uncleaved IgA heavy chain appeared at 65 kDa, whereas samples in reactions with Fc-AK183 resulted cleaved heavy chain. The production fragments of IgA-H was running at reduced molecular weight of ~37 kDa. C. Schematics of the reaction with Fc-AK183: Intact IgA1 is reduced to three separated pieces of IgA Fc, and two Fab fragments.

(*a1KI*$^{+/-}$ #2 mouse).

(*a1KI*$^{+/-}$ #3 mouse).

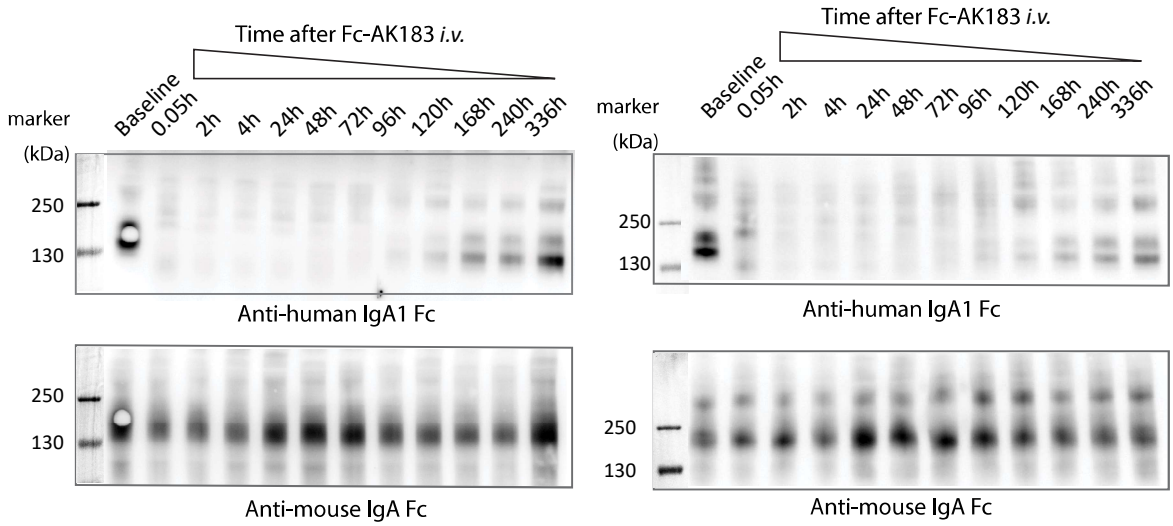


Figure S4. A single injection of Fc-AK183 achieved week-long suppression of circulating IgA1 levels in humanized transgenic models of IgA1 (additional examples to Fig5).

Additional humanized *a1KI*$^{+/-}$ mice (#2 and #3) were each treated with a single dose of 5mg/kg.BW Fc-AK183 at time zero. Tail bleeding following a time series were performed to these mice between 0 and 14 days (336 hrs). Whole sera were analyzed by SDS PAGE followed with immunoblotting of anti-human IgA1 (top panels) or anti-mouse IgA (bottom panels). In both cases, human IgA1 (running at ~165 kDa) quickly disappeared 3 min after injection of treatment. Its levels remained undetectable for another 96 hrs. Then the levels gradually increased but still below pretreatment levels on day 14, indicating the in vivo efficacy of Fc-AK183 lasted longer than 2 weeks, in keeping with expected longivity of Fc-tagged proteins in circulation. Bottom panels showed controls of endogenous mouse IgA being resistant to Fc-AK183 treatment due to the lack of cleave site in its sequence.

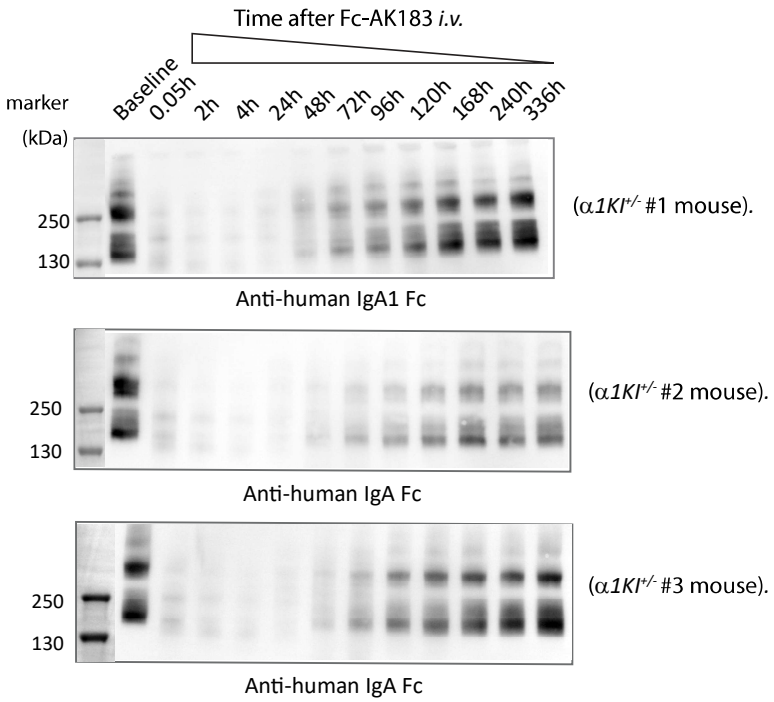


Figure S5. A new cohort of three α*1KI*$^{+/-}$ mice treated with Fc-AK183 (additional cohort to Fig5 and supplementary figure S4).

Similar to the experiment in Fig5 and figure S4, a new group of mice were each treated with a single dose of 5mg/kg.BW Fc-AK183 and longitudinal changes in their blood humanized IgA1 levels were monitored by Western blotting.

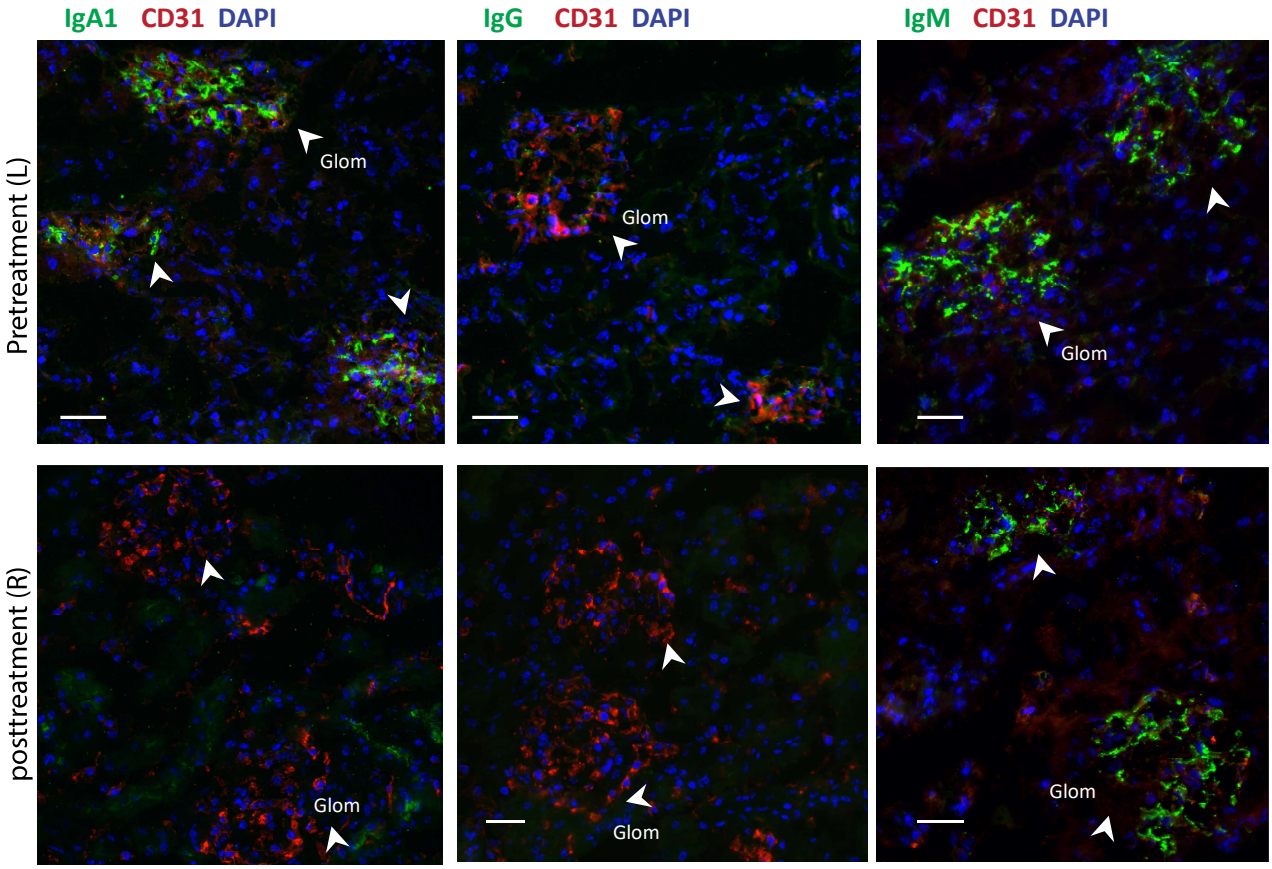
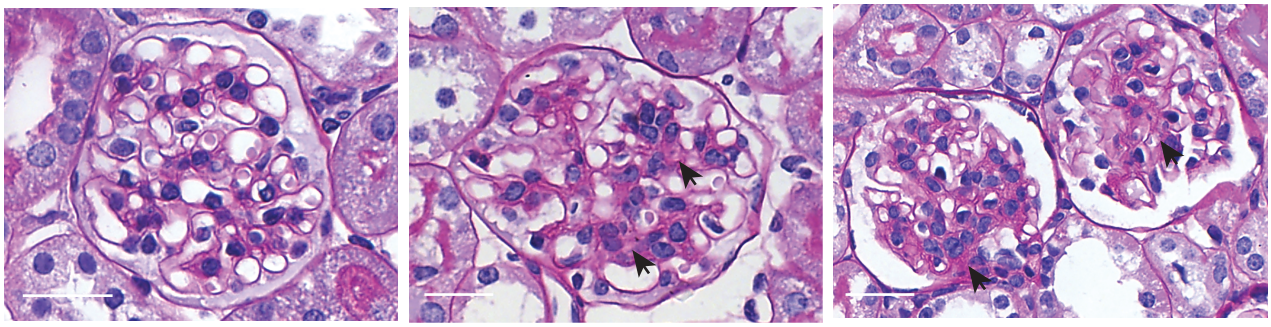


Figure S6. Fc-AK183 treatment of humanized *a1KI*$^{+/-}$ mice did not affect glomerular IgG and IgM levels.
These kidney specimens were from the study in Fig6 of humanized *a1KI*$^{+/-}$ mice induced with FAD. While IgA1 (left panels) and C3 deposits were mostly cleared from glomerular mesangium following treatment with Fc-AK183, IgG and IgM signals were unchanged by the treatment (middle and right panels). It is worth noting the difference between glomerular IgG and IgM staining. IgG deposits were absent in this transgenic IgA1 deposit model, whereas IgM signals were present in pretreatment glomerulus (top right), but their levels remained unchanged following treatment (bottom right). Scale bar: 30 μm.



Humanized *α1KI*$^{+/+}$ without adjuvant (FAD) induction

Humanized *α1KI*$^{+/+}$ following adjuvant (FAD) induction

Humanized *α1KI*$^{+/+}$ following adjuvant (FAD) induction

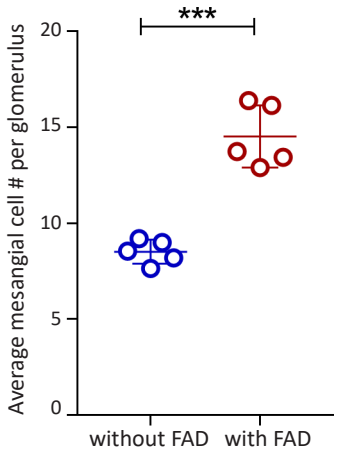


Figure S7. Induction of α1KI$^{+/+}$ mice with adjuvant (FAD) caused IgAN-like glomerular mesangial expansion.
These kidney specimens were from the study in Fig6. Humanized *α1KI*$^{+/+}$ mice(n=5) were injected with 3 doses of adjuvant between 15 and 19 weeks of age. The control specimen was from a mouse not induced with adjuvant. The kidneys were collected at 21 weeks of age. While the uninduced control showed normal PAS staining pattern (representative glomerulus shown in top left panel), mice induced by adjuvant demonstrated significant mesangial expansion, both in terms of hypercellularity (bottom panel, $P<0.001$) and deposition of an excess of matrix materials (representative images in top middle and right panels: purple mass pointed by arrows). Scale bar: 30μm.

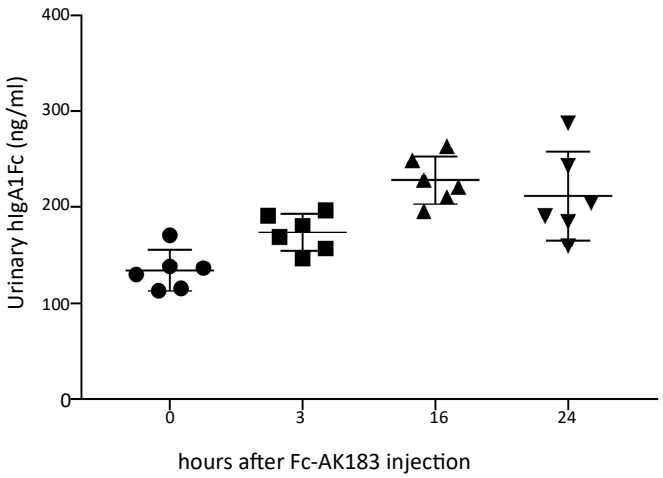


Figure S8. Changes of urinary human IgA1(Fc) levels in α1KI$^{+/-}$ mice treated with Fc-AK183.
Following the injection of Fc-AK183 at 5mg/kg.BW, urine samples were collected for detection of human IgA1(Fc) production fragment by ELISA. There was only a slight change in IgA1(Fc) levels in the urine.

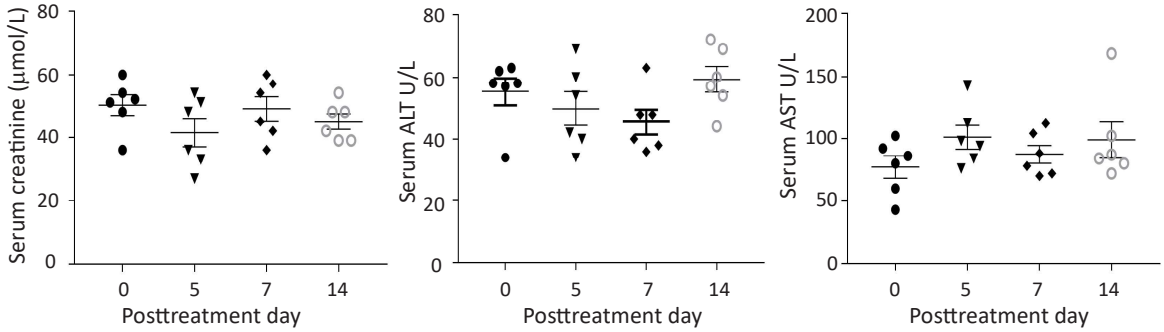


Figure S9. Fc-AK183 did not show kidney and liver toxicity in mice.

Six *a1KI*+/- mice were each injected with a bolus dose of Fc-AK183 and their tail blood samples were collected on days 0, 5, 7 and 14. Serum creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured. The levels all remained relatively constant, indicating normal kidney and liver functions following treatment with Fc-AK183.

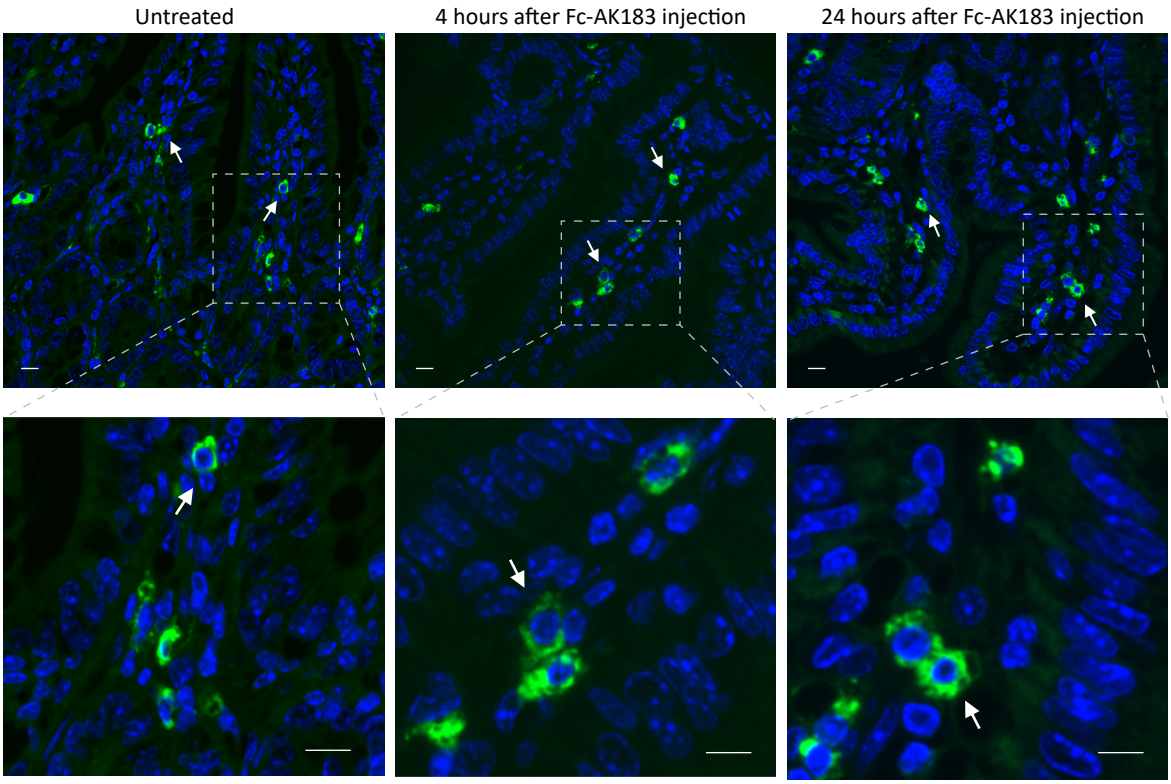


Figure S10. Fc-AK183 did not abrogate IgA1-expressing plasma cells in the small intestine.
Unlike IgA1 levels in the blood and in kidney deposits that are affected by Fc-AK183 treatment, staining of small intestinal specimens of *a1KI*$^{+/-}$ mice did not show changes of the IgA1-expressing plasma cells (white arrow). Scale bar 0.1μm.

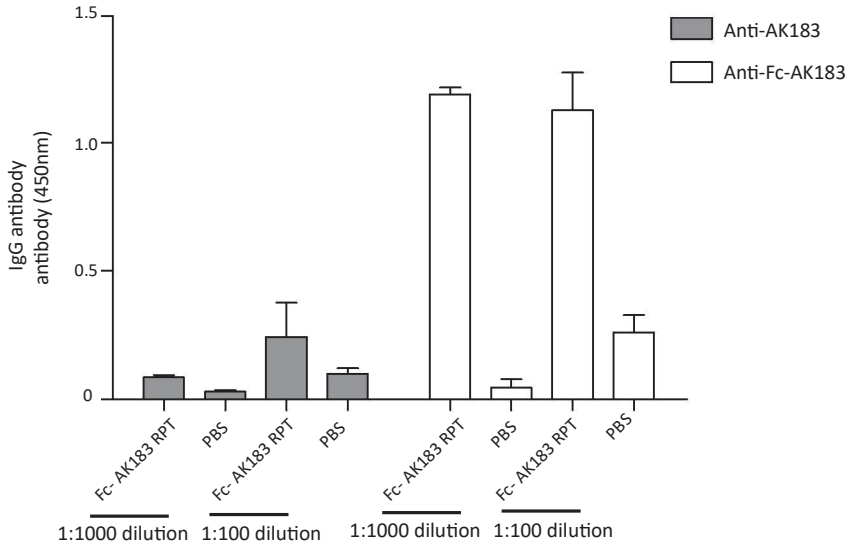


Figure S11. Testing of neutralizing antibodies against AK183 and human Fc in mice 30 days after the initial treatment with Fc-AK183.

Mice (n=3) each received an initial injection of Fc-AK183. 30 days later antisera were collected from these mice for antibody tests. ELISA plates coated with either Fc-AK183 fusion antigen or untagged AK183 alone were used to detect antibodies. Antibody titers were measured with dilutions of the antisera at either 1:100 or 1:1,000. It was evident that higher antibody titers were developed against the Fc-portion of the fusion antigen, as opposed to the AK183 portion of the antigen. Control mice treated with PBS (n=2) had significantly lower readings of antibody signals, as expected.