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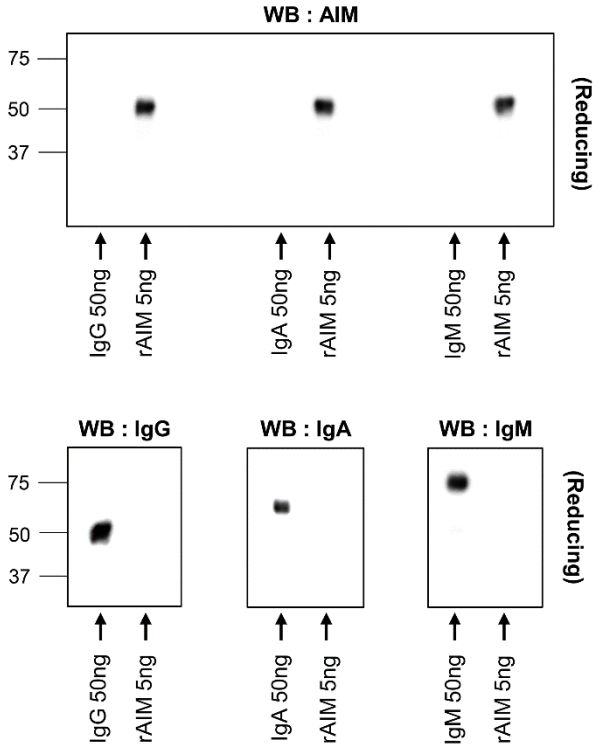

Figure S1. No crossreactivity of anti-AIM with immunoglobulins

Commercial murine IgG, IgA, and IgM were loaded for SDS-PAGE and immunoblotting with anti-AIM, anti-IgG, anti-IgA, and anti-IgM under reducing conditions was performed. No immunoglobulin was detected by anti-AIM.

IgG from mouse serum (15381-1MG; SIGMA), IgA, Kappa from murine myeloma (M1421; SIGMA), and rAIM (purified as previously described[22]) were used. Monoclonal IgM protein was purified using the HiTrap IgM purification column (17511001; GE Healthcare Life Sciences) from the culture supernatant of B-cell hybridoma (clone TC-31, CRL-2494; American Type Culture Collection, Manassas, VA, USA) according to the manufacturer's protocol.

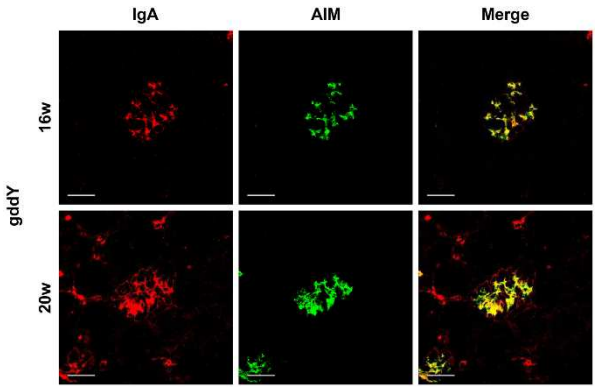

Figure S2. AIM expression in gddY mice

Glomerular AIM co-deposition with IgA and expression of AIM in mesangial cells were examined at different ages. Representative immunostaining images of IgA (red) and AIM (green) in the renal tissue of 16-and 20-week-old gddY and *AIM*$^{-/-}$gddY (n = 5). All samples were stained and observed with the samples shown in Figure 1A. Scale bars: 50 μm.

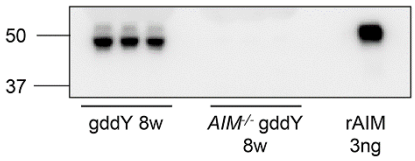

Figure S3. *AIM*$^{-/-}$gddY mouse model establishment using the CRISPR/Cas9 system

To confirm the knockout of AIM in *AIM*$^{-/-}$gddY mice, we performed immunoblotting.

Representative image of immunoblotting for AIM, using reducing conditions, in serum from 8-week-old gddY and *AIM*$^{-/-}$gddY mice.

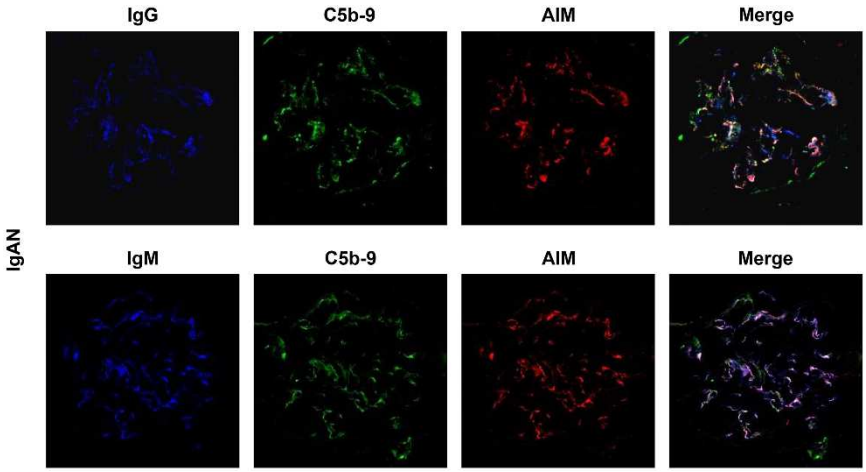

Figure S4. C5b-9 co-deposits with IgG/IgM and AIM in IgAN

Representative immunostaining images of C5b-9 (green) co-deposition with IgG (blue)/AIM (red) and IgM (blue)/AIM (red) (n = 4). Scale bars: 50 μm.

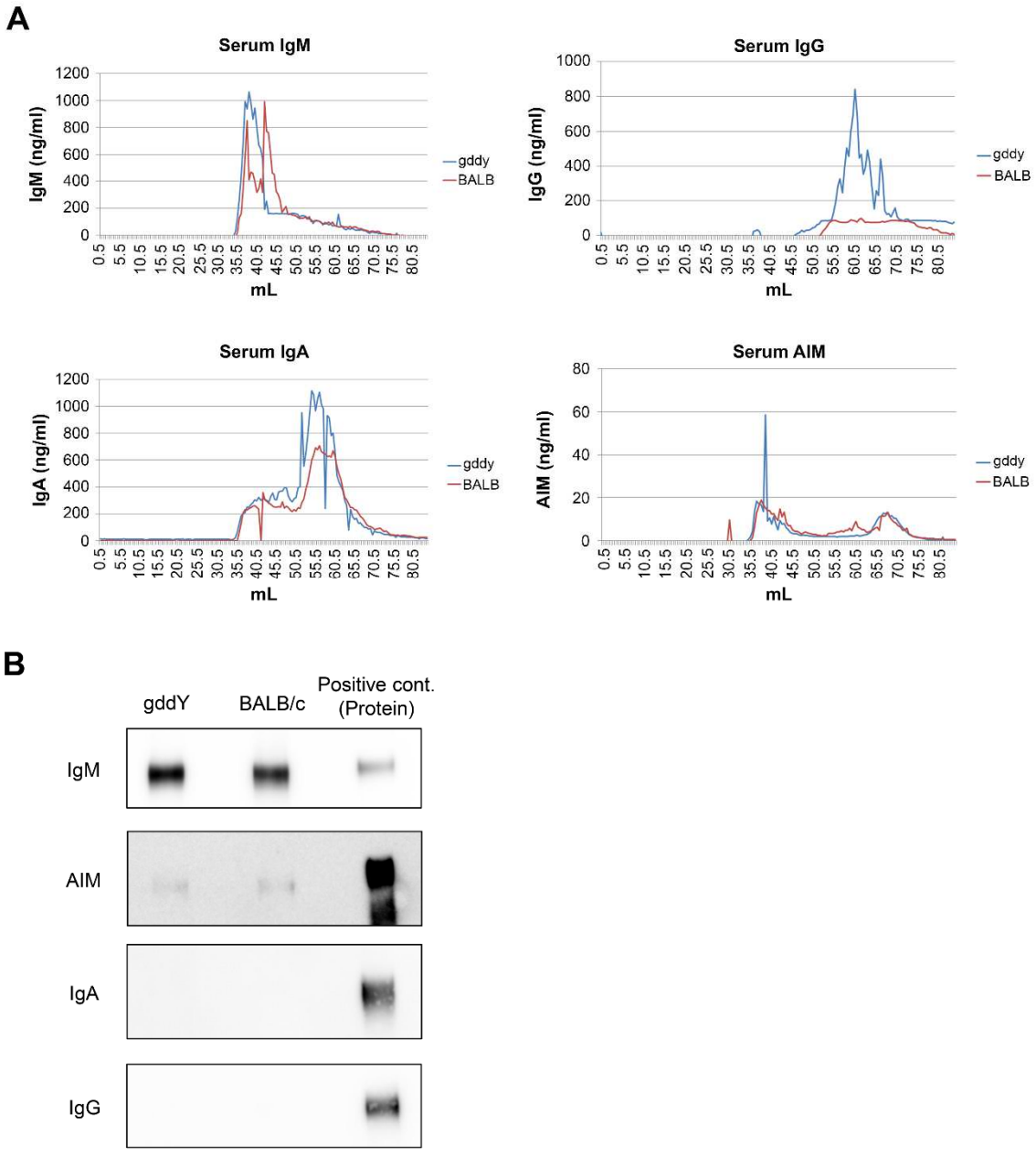

Figure S5. No associations of IgA and IgM containing AIM were observed in serum fractionation samples from gddY or BALB/c mice.

(A) Size fractionation on serum from gddY and BALB/c mice was performed, and each fraction was examined for the presence of IgM, IgA, IgG, and AIM using ELISA. Sera were subjected to

size-exclusion chromatography using the HiPrep 16/60 Sephacryl S-300 HR column (17116701, GE Healthcare Life Sciences) with Dulbecco's PBS. AIM was measured by ELISA using two different rat anti-mouse AIM monoclonal antibodies (rat IgG, clones #36 and #35; generated in our laboratory). Regarding IgM, IgA and IgG, we used an ELISA Quantification Set (IgM: E90-101, IgA: E90–103, IgG: E90-131; Bethyl Laboratories), and we performed assays according to the manufacturer's protocol.

(B) The IgM peak fractions at 39–39.5 ml (10 μl each) were immunoblotted for IgM, IgA, IgG, and AIM under reducing conditions. We used antibodies for immunoblotting as described in the Methods section.

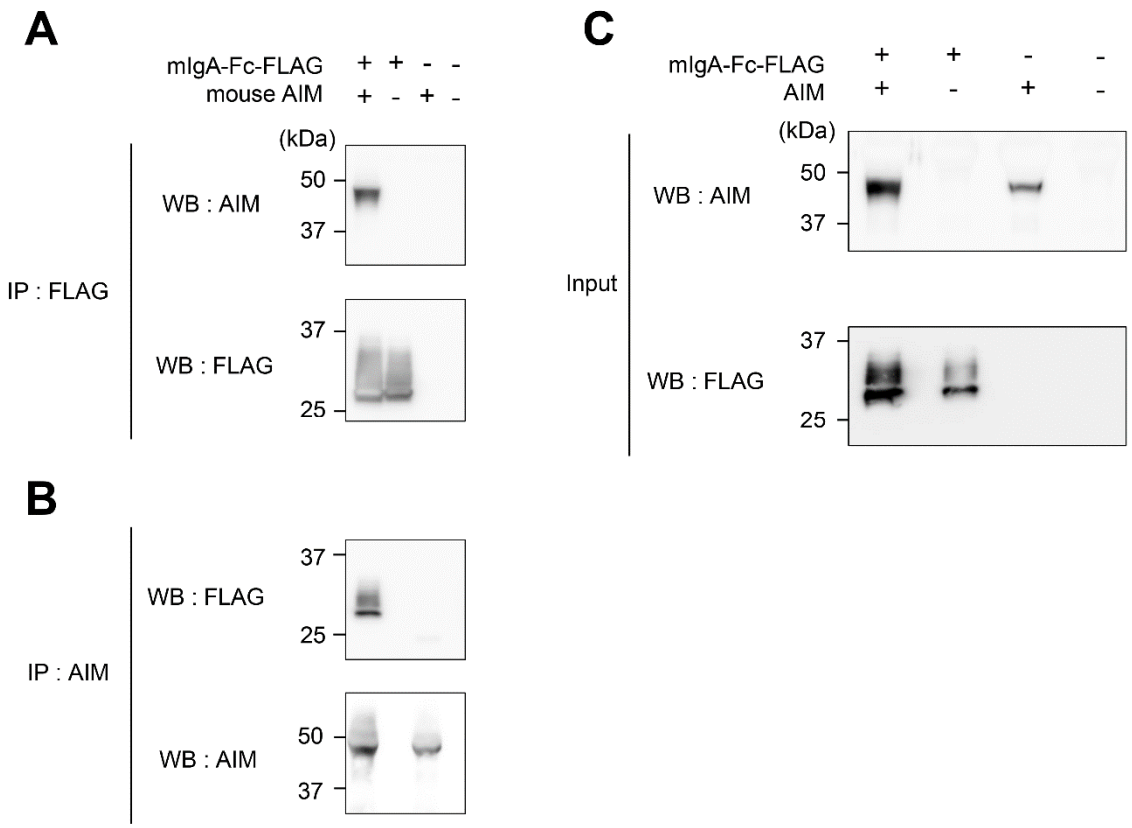

Figure S6. AIM associates with IgA-Fc in mice and humans.

HEK293T cells overexpressing mouse AIM and those overexpressing FLAG-tagged mouse IgA-Fc were co-cultured for 48 h, and supernatants were prepared from co-cultures. Immunoblotting of the supernatant for anti-AIM and IgA were performed (C). The supernatants were incubated with an anti-AIM antibody conjugated with protein G Sepharose (Sigma-Aldrich, P3296) or anti-FLAG M2 affinity gel (Sigma-Aldrich, A2220) at 4°C overnight. The precipitates were washed five times with a wash buffer (TBS plus 0.1%

Tween-20) and eluted by heating at 95°C for 5 min in SDS sample buffer containing 2-mercaptoethanol. Association was assessed by immunoblotting for IgA-Fc (A) and AIM (B).

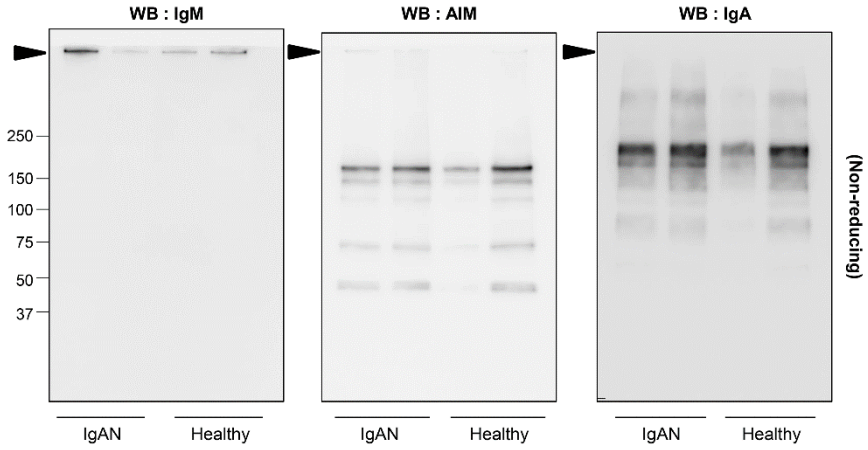

Figure S7. No IgA/AIM/IgM immune complex was observed in serum samples from patients with IgAN.

Immunoprecipitation was performed on sera from human IgAN and healthy control using anti-IgA and the immune complex was analyzed by immunoblotting with anti-IgM (left), AIM (middle), and IgA (right) under nonreducing conditions (n = 2 for each group). Methods and materials are described in the Methods section.