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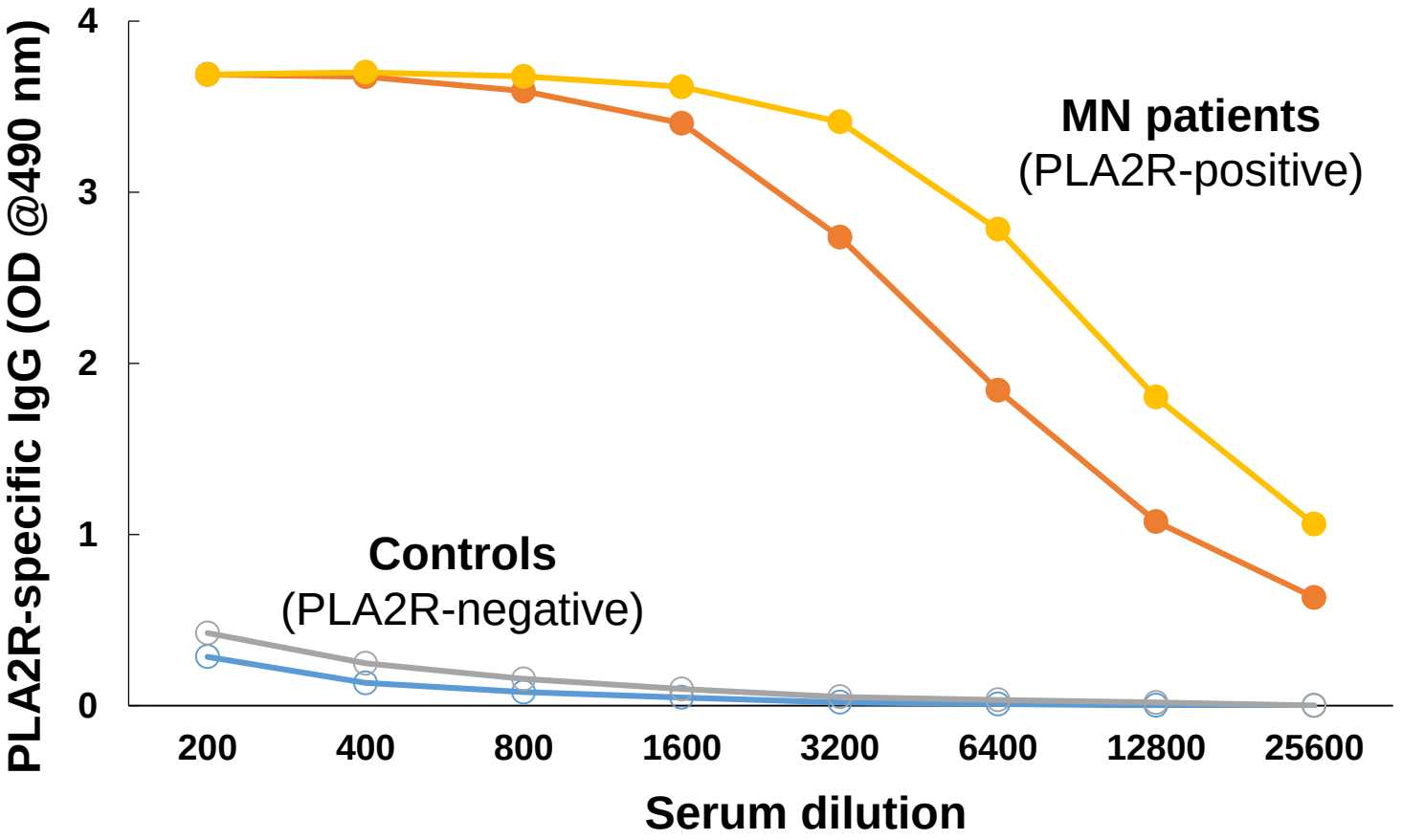
samples from two patients with primary MN were tested for IgG autoantibody. Sera from two controls (negative for PLA2R antibodies) served as negative controls.

Supplemental Figure 2. Confocal-microscopy image of glomerular immunoglobulin in membranous nephropathy (MN).

A) A section from a remnant frozen renal-biopsy specimen from a patient with MN was stained with biotin-labeled nanobody specific for CH3 domain of human IgG (detected with streptavidin-Alexa 555, red) and goat antibody specific for human IgA (detected with Cy2, green). Nuclei were stained with DAPI (blue). Endogenous tissue biotin was blocked with avidin/biotin blocking kit. Staining for IgG is detected. IgA staining is absent. Magnification 200x. Inset: Enlarged portion of a glomerulus shows granular staining for IgG in glomerular basement membranes. B) Another section of the specimen was prepared by using only streptavidin-Alexa 555 (red), i.e., the biotin-labeled nanobody specific for CH3 domain of human IgG was omitted. Goat antibody specific for human IgA was also used. Nuclei were stained with DAPI (blue). No staining for IgG or IgA is detected. Magnification 200x. This negative control confirms the specificity of the IgG staining with nanobody specific for CH3 of human IgG shown in panel A.

Supplemental Figure 3. Analyses of glomerular deposits in a remnant frozen RIF-IgG kidney-biopsy specimen of an IgAN patient show co-localization of IgG and IgA detected by confocal microscopy. **A-C.** A 4-µm-thick section of the renal-biopsy specimen was stained with biotin-labeled nanobody specific for CH3 of human IgG (detected with streptavidin-Alexa 555, red) and goat antibody specific for human IgA (detected with Cy2, green). Nuclei were stained with DAPI (blue). Endogenous tissue biotin was blocked with avidin/biotin blocking kit. Panels **A-C** show single-layer confocal microscopy images of a portion of a glomerulus (magnification 600x) with three combined colors (**A**), red (**B**), and green (**C**). Panels **D1** and **D2** show two line-intensity profiles 1 and 2 respectively, at a single-optical layer, magnification 600x, with arrows in panels **A-C** indicating directions of the two lines.

Supplemental Figure 1. Detection of IgG autoantibodies specific for PLA2R.



Assay for PLAR2-specific IgG autoantibodies from patients with primary MN. Extracellular part of PLA2R was expressed as a soluble protein in FreeStyle 293-F cells and isolated from serum-free medium using affinity chromatography. Purified PLA2R was coated onto the wells of 96-well ELISA as an antigen and dilutions of serum samples from two patients with primary MN were tested for IgG autoantibody. Sera from two controls (negative for PLA2R antibodies) served as negative controls.

Supplemental Table 1. IgG isolated from immunodeposits of a patient with membranous nephropathy is enriched for autoantibodies specific for PLA2R

Sample	IgG (ng / 10 µl)	PLA2R-specific IgG autoantibody (OD @ 490 nm)	PLA2R-specific IgG autoantibody (U / µg IgG)*
MN wash	75.9	0.20	2.6
MN extract	31.2	1.48	31.2

* One Unit (U) of PLA2R-specific IgG was defined as the amount resulting in optical density (OD) @ 490 nm equal to 1.0.

Supplemental Table 2. IgG isolated from immunodeposits of five pooled IgAN biopsy specimens IgG-negative by routine immunofluorescence (pool 4) contained IgG autoantibody with specificity for Gd-IgA1.

Sample	IgG (ng / 1 µl)	Gd-IgA1-specific IgG autoantibody (OD @ 490 nm)	Gd-IgA1-specific IgG autoantibody (U / µg IgG)
IgAN wash	7.83	0.01	1.40
IgAN extract	4.55	0.37	81.88

IgG autoantibody assay was used; One Unit (U) of Gd-IgA1-specific IgG was defined as the amount resulting in optical density (OD) @ 490 nm equal to 1.0.

Supplemental Table 3. Confocal-microscopy analysis of intensity of IgG staining and IgG-IgA co-localization in glomerular immunodeposits in remnant frozen renal-biopsy specimens of IgAN patients.

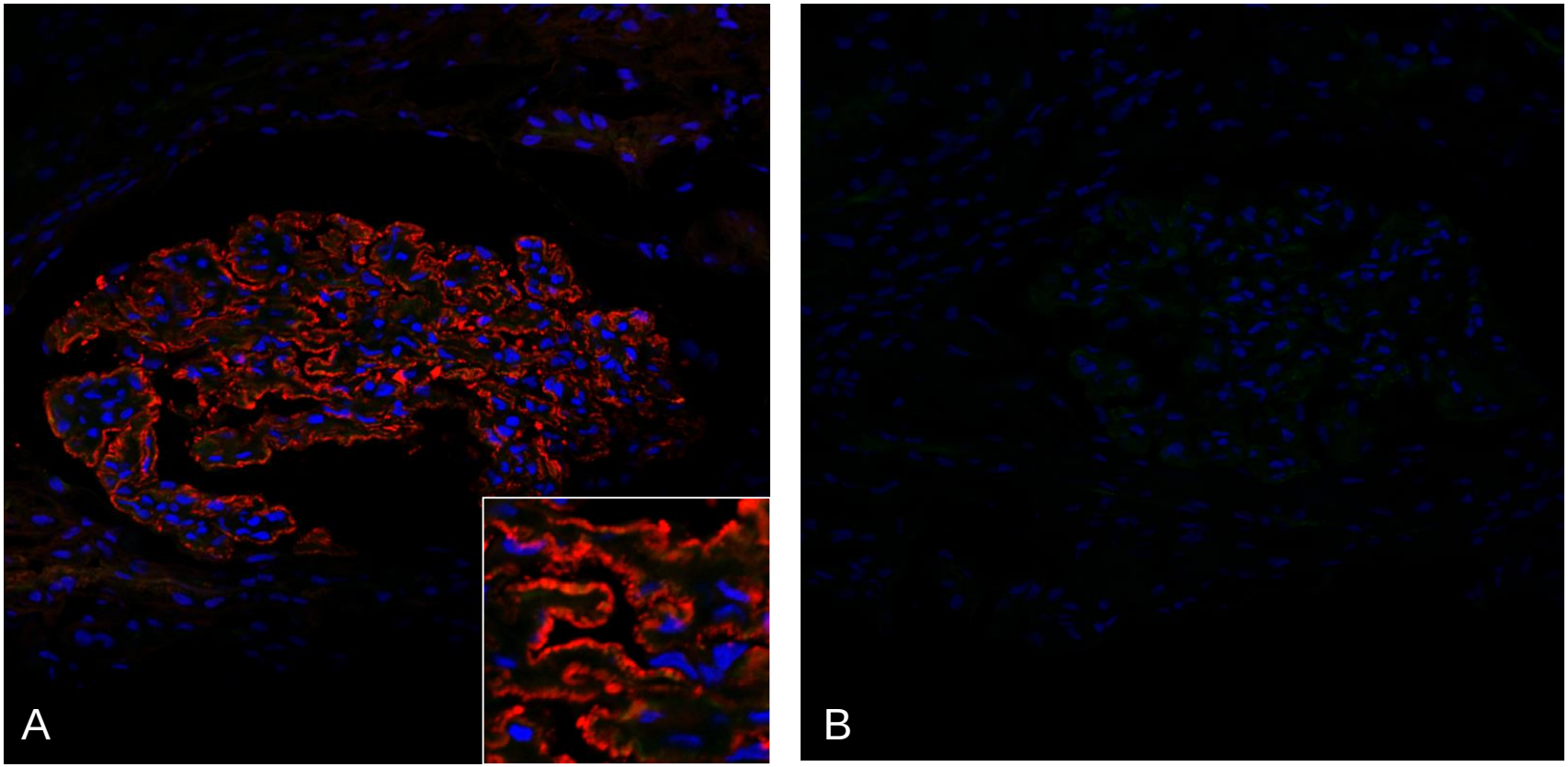
	IgG-positive by RIF * (n=6)	IgG-negative by RIF* (n=3)
IgG intensity # (equal laser settings)	661 ± 162	408 ± 174
Co-localization (Pearson's coefficient)**	0.60 ± 0.10	0.72 ± 0.04

* RIF - Routine Immunofluorescence

** Pearson's coefficient, value of 1 indicates 100% co-localization

The intensity of IgG staining and IgG-IgA co-localization were measured in entire glomerular area using equal laser settings for all samples.

Supplemental Figure 2. Confocal-microscopic image of glomerular immunoglobulin in membranous nephropathy (MN).



A) A section from a remnant frozen renal-biopsy specimen from a patient with MN was stained with biotin-labeled nanobody specific for CH3 domain of human IgG (detected with streptavidin-Alexa 555, red) and goat antibody specific for human IgA (detected with Cy2, green). Nuclei were stained with DAPI (blue). Endogenous tissue biotin was blocked with avidin/biotin blocking kit. Staining for IgG is detected. IgA staining is absent. Magnification 200x. Inset: Enlarged portion of a glomerulus shows granular staining for IgG in glomerular basement membranes. **B)** Another section of the specimen was prepared by using only streptavidin-Alexa 555 (red), i.e., the biotin-labeled nanobody specific for CH3 domain of human IgG was omitted. Goat antibody specific for human IgA was also used. Nuclei were stained with DAPI (blue). No staining for IgG or IgA is detected. Magnification 200x. This negative control confirms the specificity of the IgG staining with nanobody specific for CH3 of human IgG shown in panel A.

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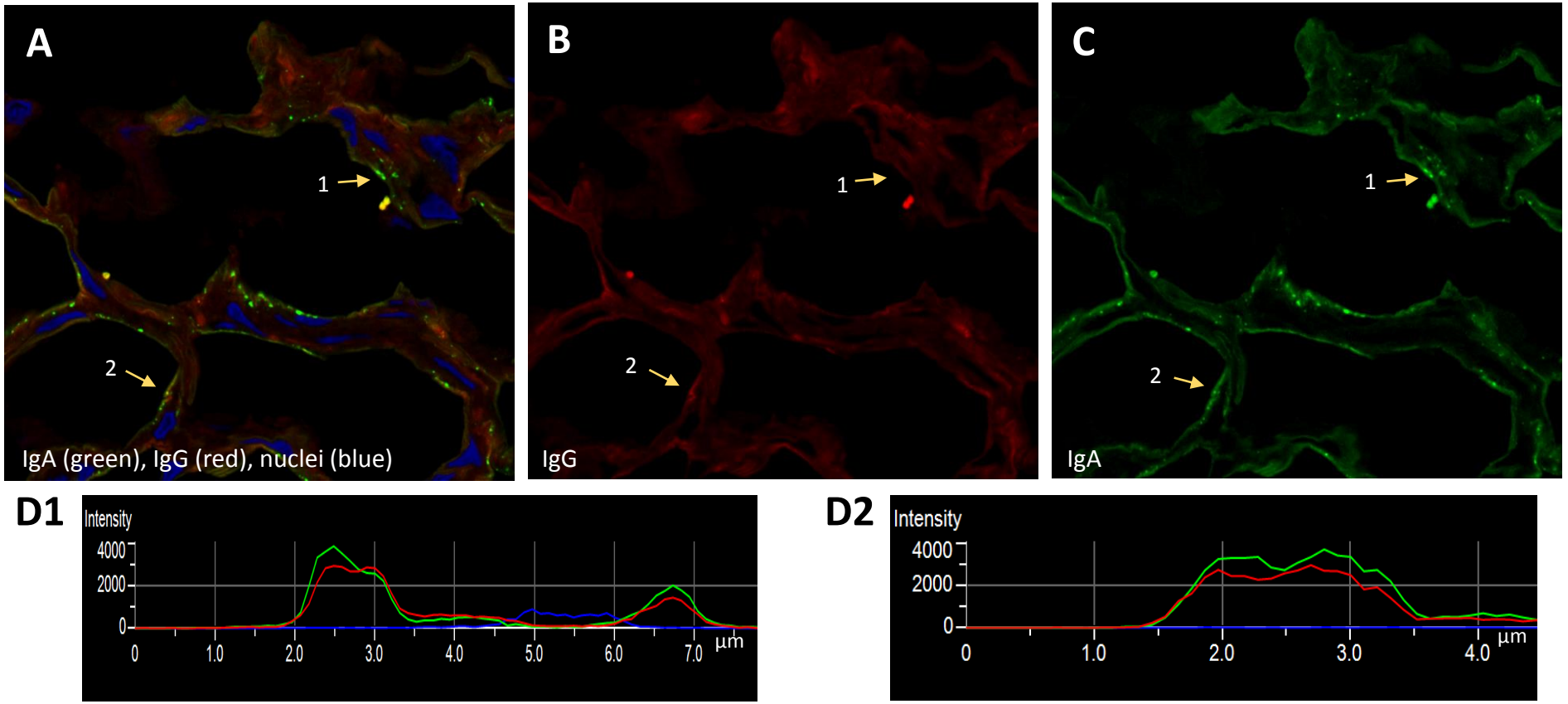

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