Supplementary Materials and Methods:

Reagents

Immune complexes (ICs) were prepared as described previously (31). In short, FITC-labeled human serum albumin (HSA, 1 mg/ml) (Abcam) was incubated with polyclonal rabbit anti-FITC IgG (1 mg/ml) (Acris) at 1:6 for 1 h at 4°C. Working solutions of this IC stock were prepared by 1:20 dilution in PBS. Purified human-like ICs were prepared by incubating purified human IgG subtypes (IgG1, IgG2 (Sigma), IgG3, IgG4 (Fitzgerald)) at a molecular ratio of 1 to 4.5 with Cy3-conjugated F(ab)2 anti-human F(ab)2 (Jackson Immunoresearch) for 1 h at 4°C. Working concentrations were adjusted to 50 μg/ml. Heat-aggregated human IgG-ICs were prepared by incubation of 50 mg/ml human IgG (Sigma-Aldrich) in PBS for 60 min at 63°C. Large protein aggregates were removed after heat aggregation by centrifugation at 10000g for 15 min. Specific unconjugated blocking antibodies for human FcγRs were clone 3G8 for CD16 (mouse IgG1; BioLegend), clone AT10 for CD32 (mouse IgG1, Abcam), clone MOPC-21 (mouse IgG1, Biolegend) as nonbinding, and clone DX17 against HLA-ABC (mouse IgG1, BD Biosciences) as binding isotype control. Therapeutic IVIG (Octagam) contains 50 mg/ml IgG, 100mg/ml maltose, Triton X100 not more than 5μg/ml, TNBP not more than 1μg/ml and 0.3 M NaCl (osmolality 310-380 mOsmol). For this study IVIG was dialyzed against large volumes of PBS and concentrated to 100mg/ml before use in the flow chamber assay. Additional antibodies used are CD16 BD Biosciences) and CD105 (Abcam). Additional reagents were HSA (Baxter), the Syk inhibitor BAY-61-3606 (Enzo Life Sciences), and CMFDA (Life Technologies).

Human IgG1, human IgG2, human IgG3, human IgG4, human IgA1, human IgA2 (Fitzgerald,), Cy3-conjugated F(ab)2 anti-human F(ab)2 (Jackson Immunoresearch); CD45-APC-Cy7, CD32-

PE, CD64-PE , CD89(FcαR)-PE (Biolegend), CD56-PE (Immunotools), CD16-PE-Cy7, CD16-PE, mouse IgG1-PE isotype, mouse IgG2b-PE isotype (BD Biosciences), PEN5, goat F(ab)2 anti-mouse IgM FITC (Beckman Coulter), CD144 (Santa Cruz), goat IgG isotype, chicken F(ab)2 anti-goat IgG Alexa Fluor 488 (Invitrogen), CD105 (Abcam), rabbit IgG isotype (Dianova), goat anti-rabbit IgG Alexa Fluor 488 (Life Technologies), propidium iodide (BD Biosciences), DAPI (Sigma-Aldrich).

Cells separation

pDCs and CD4+ T cells were isolated using commercially available kits according to the manufacturer's instructions (Miltenyi Biotec). CD1c+ DCs were isolated with a commercially available kit according to the manufacturer's instructions (Miltenyi Biotec) but without using the Fc receptor block.

Preparation of endothelial cell monolayers

Proliferating primary human dermal microvascular endothelial cells (HDMECs, Promocell) were cultured in EGM-MV2 (Promocell). Monolayers of HDMECs for perfusion assays were generated by harvesting and washing the cells, followed by adjustment of the concentration to 7 x 10^5 cells/mL in EGM-MV2 and injection of 200 µL cell suspension into µ-slide I0.8 Luer chamber slides (IBIDI). After attachment of HDMECs (2 h of cell culture), 60 µL of additional medium was added to the two reservoirs of the chamber slides followed by cell culture for 1 day at 37°C HDMECs. Confluence of HDMECs was verified by staining of VE-Cadherin (CD144, goat IgG; Santa Cruz) expression at the cellular junctions. Expression of CD105 was verified by surface staining of the monolayer.

Production of CX3CL1 by HDMECs was investigated by treating monolayers of HDMEC with supernatants from slanMo previously incubated with IC, for 20h in the presence or absence of the TNF-α mAb inhibitor adalimumab (10µg/ml) (Abbvie). As isotype control we used Ultra-LEAF purified human IgG1 (Biolegend). CX3CL1 secretion was quantified by ELISA (R&D Systems).

Transfection of Jurkat T Cells

For transfection of Jurkat cells 5×10^5 cells/well in 2 mL complete RPMI + 10% fetal calf serum (FCS) were seeded in 6-well plates. 4 µg/well Plasmid DNA in Opti-MEM (Life Technolgies) was prepared using Lipofectamin LTX (Life Technologies) according to the manufacturer's recommendations. Plasmids were pcDNA3.2-V5Dest-CD32a, pcDNA3.2-V5Dest-CD32b, pcDNA3.2-V5Dest-CD16b, and pVITRA1-FceRI-gamma + CD16a (Life Technologies). Jurkat cells were incubated for 24 h at 37°C with the transfection reagent. To positively select plasmid DNA-expressing Jurkat cells G418 (Applichem) was then added at an initial concentration of 250 µg/mL which was later increased to 500 µg/mL.

After reaching a sufficient level of FcγR expression 5×10^5 cells were specifically stained using CD16-PE or CD16-PE-Cy7 (clone 3G8, BD Biosciences) and CD32-PE (clone FUN-2, Biolegend) and then highly FcγR-positive cells were single-cell sorted using a FACSAria (BD Biosciences) into 96-well round bottom plates containing 100 µL of complete RPMI with 20% FCS. Established colonies were later tested for homogeneous and high expression of FcγRs and then used in the experiments or were kept frozen in liquid nitrogen and again cultured at later time points.

Maturation of slanMo

Freshly isolated slanMo were cultured for 8 h in RPMI 1640 supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin, and 100 mg/mL streptomycin (all Biochrom GmbH), and 10% heat-inactivated pooled human serum (CCPro) without any additional stimulation. Non-cultured control cells were stored on ice. Expression level of CD16 on mature and immature slanMo was analyzed by staining with CD16-PE (clone 3G8, BD Biosciences) or a mouse IgG1-PE isotype control (MOPC-21, BD) and acquisition on a FACSCanto flow cytometer (BD Biosciences).

Intra-slide bicinchoninic acid assay

To estimate the efficacy of the coating procedure for immunoglobulins an intra-slide bicinchoninic acid assay (Thermo Fisher Scientific) was applied. As for the perfusion assays the slides were prepared by incubation with 200 µL of a 50 µg/mL protein solution over night at 4°C. The slides were then washed 5 times with 1 mL PBS. Thereafter the liquid was completely removed followed by addition of 200 µL of the assay's working reagent and incubation for 2 h at 37°C. After incubation the working reagent was removed from the slides and transferred to a 96-well flat bottom plate. The quantification of the color reaction was done at 540 nm with the Multiskan EX photometer (Thermo Fisher Scientific).

Immunofluorescence staining of patient samples

Paraffin-embedded tissue sections (0.5 µm) of human kidney were deparaffinized in Roti-Histol and ethanol (both from Carl Roth). Antigen retrieval was performed with citrate buffer at a pH of 6.0. TNF-α was detected by incubation with a 1:50 dilution of an unconjugated TNF-specific antibody (Abcam) followed by detection with Alexa Fluor 488 goat anti-mouse IgG (Invitrogen). For CD68 staining was used anti-CD68 mAb (Agilent) 1:50 dilution followed by detection with Alexa Flour 488 goat anti-mouse IgG (Invitrogen). The slan antigen was detected by incubation

with a 1:10 dilution of the slan-specific DD2 antibody followed by incubation with a biotinylated anti-mouse IgM antibody, and detection with Cy3-conjugated streptavidin. 10% Earle's balanced salt solution (Biochrom GmbH) supplemented with 1% HEPES and 0,1% saponin at pH 7,4 was used as washing solution. Sections were stained with DAPI-containing solution (Sigma) and mounted with anti-fade reagent (Invitrogen) and examined using a Leica DM 5500 B microscope (Leica Microsystems) and the MetaVue software (Molecular Devices).

Immunocytochemistry of endothelial cells

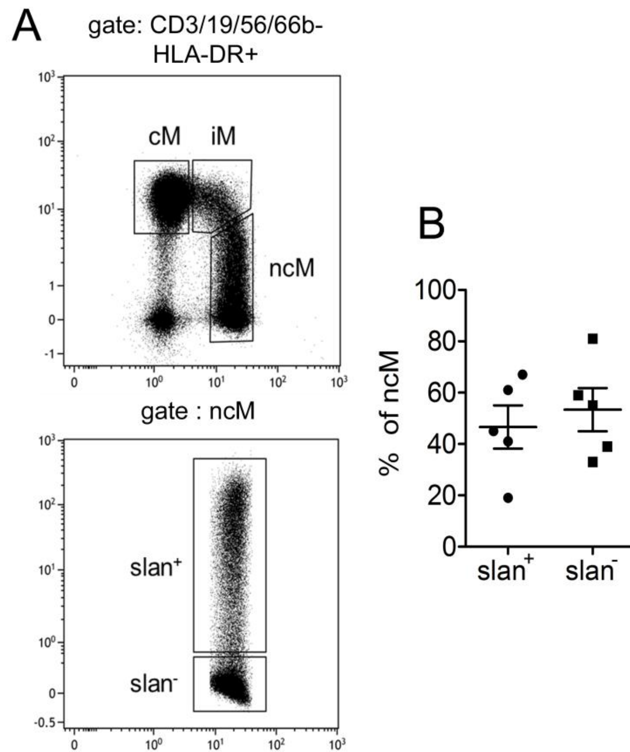
HDMECs were seeded at a concentration of 7 x 10^5 HDMECs/mL into µ-slide I0.8 Luer chamber slides (IBIDI) an incubated in EGM-MV2 (Promocell) at 37°C for approximately one day. After reaching confluency the cells were washed with medium and fixed for 30 min with ice-cold fixation buffer (eBioscience). The fixed cells were washed twice with permeabilization buffer (eBioscience) followed by blocking for additional 30 min with permeabilization buffer. Then the HDMECs were incubated with an antibody against VE-Cadherin (CD144, goat IgG; Santa Cruz) at a concentration of 4 µg/mL diluted in permeabilization buffer or an appropriate isotype control (goat IgG, Invitrogen) for 45 min at RT. After two times washing with permeablization buffer the HDMECs were incubated with a secondary alexa fluor 488-conjugated chicken F(ab)2 anti-goat IgG (Invitrogen) at a concentration of 20 µg/mL for 45 min at RT. After two times washing with permeablization buffer the HDMECs were additionally incubated with a 1:20000 dilution of DAPI (Sigma-Aldrich) for 3 min at RT followed by three times washing with permeabilization buffer. The slides were then analyzed using the Leica DM-IRB microscope (Leica), a 18.2 Color Mosai digital camera (Diagnostic Instruments), and the MetaVue software (Molecular Devices).

To represent binding of CD105 to the surface of confluent HDMECs under experimental conditions as in Figure 5 the cells were incubated with 12,5 µg/mL anti-CD105 (Abcam) or an appropriate isotype control (rabbit-IgG, Dianova) at the same concentration for 8 min at 37°C. The cells were then washed twice with medium and fixed for 30 min with ice-cold fixation buffer (eBioscience). The fixed cells were washed again with non-permeabilizing FACS buffer and then incubated with 20 µg/mL alexa fluor 488-conjugated goat anti-rabbit IgG (Life Technologies) for 15 min at RT. After two times washing with FACS buffer the HDMECs were incubated with a 1:20000 dilution of DAPI (Sigma-Aldrich) for 3 min at RT followed by three times washing with FACS buffer. The slides were then analyzed using the Leica DM-IRB microscope (Leica), a 18.2 Color Mosai digital camera (Diagnostic Instruments), and the MetaVue software (Molecular Devices).

Staining of slanMo in whole blood

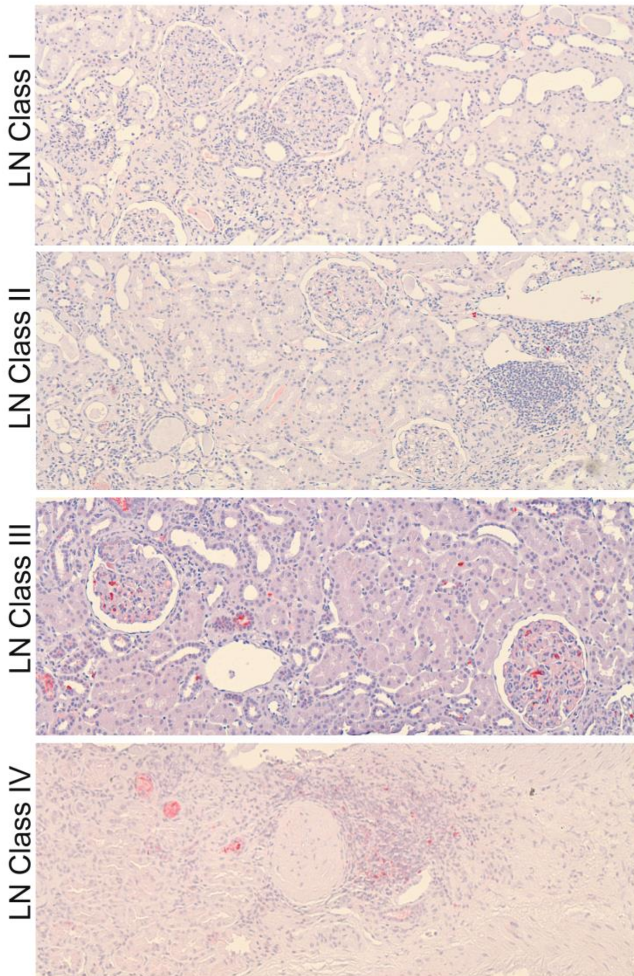
300 µl of whole blood was incubated with anti-slan (M-DC8 hybridoma supernatant raised in our laboratory), washed and stained with the following antibodies at optimal dilutions: goat F(ab')2 anti-mouse IgM, CD16 (clone: 3G8; Beckmann Coulter), CD14 (clone: MphiP9; BD), CD56 (clone: HCD56), CD3 (clone: SK7), CD19 (clone: HIB19), CD66b (clone: 610F5) and HLA-DR (clone: L243; all Biolegend). Erythrocytes were lysed in FACS lysis solution according to the manufacturer's instructions (BD) and cells were analyzed by flow cytometry (Gallios, Beckman Coulter). By gating on the CD56/CD3/CD19/CD66b negative and HLA-DR positive fraction, monocyte subsets were identified by plotting CD14 vs. CD16.

Supplementary Figure 1



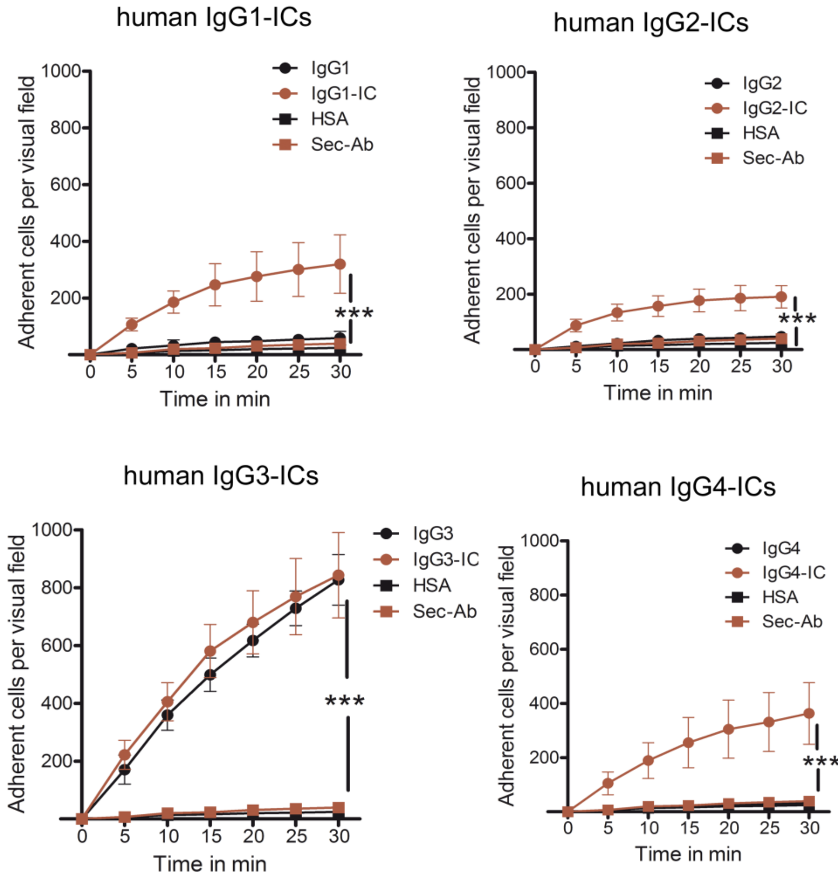
Supplementary Figure 1. Percentage of slan+ cells within ncM. (A) Gating strategy for identification of the different monocyte subsets (CD56/CD3/CD19/CD66b- and HLA-DR+). Percentage of slan+ cells was determined within the gate of ncM**. (B)** Percentage of slan+ and slan- ncM of 5 donors (mean±SEM) are shown.

Supplementary Figure 2



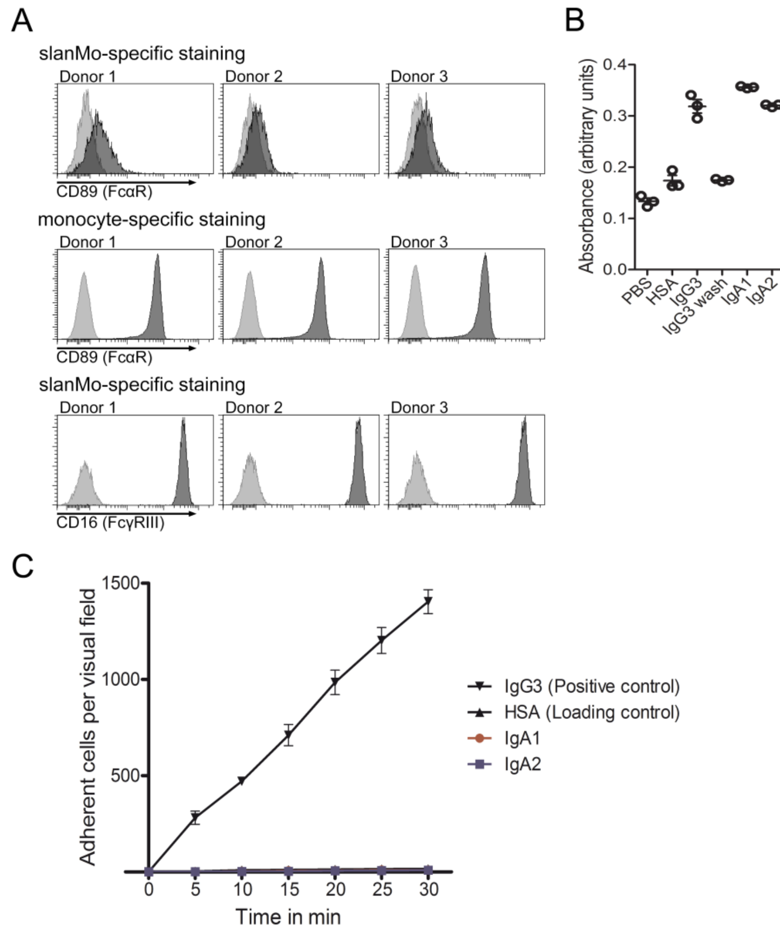
Supplementary Figure 2. SlanMo distribution in class I-IV lupus nephritis. The presence of slanMo was shown using the slan-specific antibody DD2 (original magnification ×10).

Supplementary Figure 3.



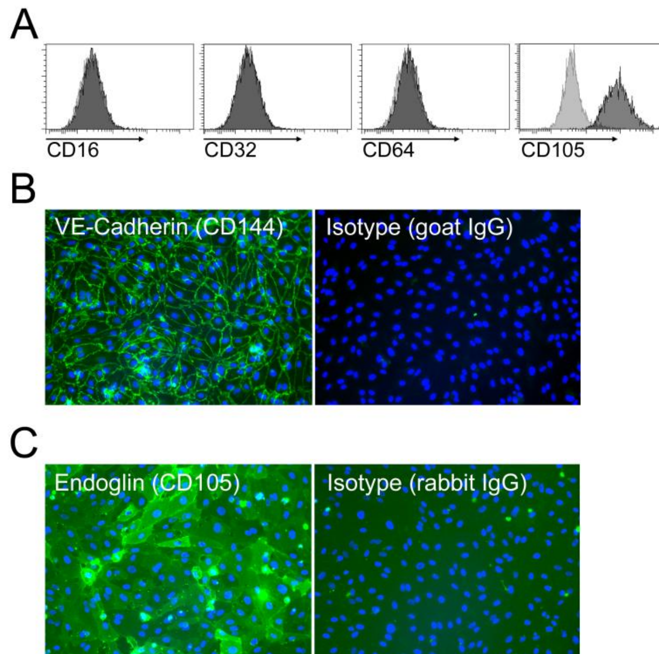
Supplementary Figure 3. Immobilized human-like ICs recruit slanMo from physiologically relevant flow. Freshly isolated slanMo were labeled for fluorescence microscopic detection and subsequently ran over immobilized human-like ICs for 30 min at a shear stress of 0.5 dyn/cm². Human-like ICs consisted of purified human IgG subtypes and a F(ab)2 fragment specific for the F(ab)2 portion of human IgG (n = 3). *p<0.05, **p<0.01, ***p<0.001, ns, not significant

Supplementary Figure 4



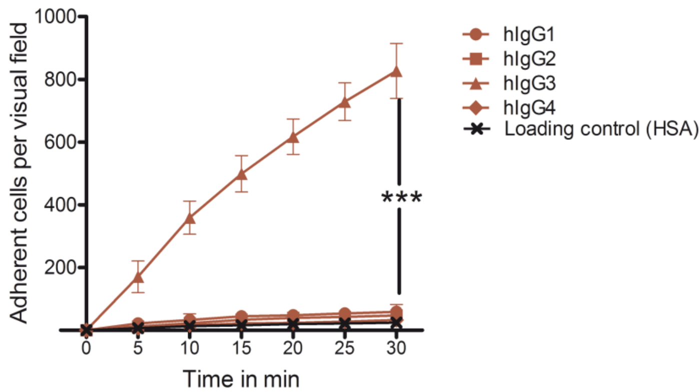
Supplementary Figure 4. Immobilized human IgA does not recruit slanMo from physiologically relevant flow. (A) Expression of the IgA receptor (FcαR, CD89) on slanMo compared to monocytes of the same donors. The expression of CD16 on slanMo of the same donors is additionally shown for comparison. Three representative stainings are shown. **(B)** Detection of coated IgA by an intra-slide bicinchoninic acid assay. 'IgG3 wash' refers to a control where the IgG3 solution of 50µg/mL was added to the slides and removed again after a few seconds (n = 3). **(C)** Freshly isolated slanMo were labeled for fluorescence microscopic detection and subsequently ran over immobilized human-like IgA1 or IgA2 for 30 min at a shear stress of 0.5 dyn/cm² (n = 3).

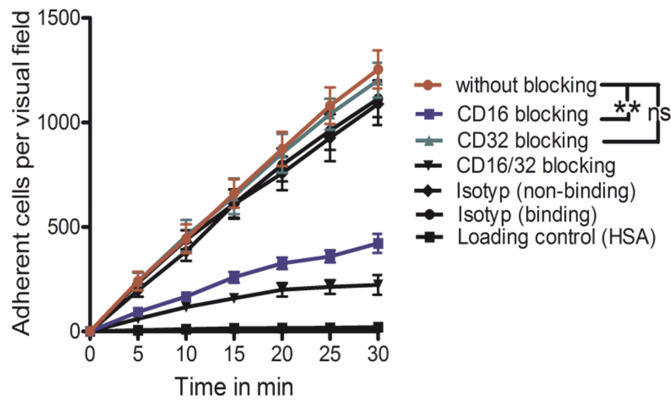
Supplementary Figure 5



Supplementary Figure 5. Control staining of monolayers of human dermal microvascular endothelial cells (HDMECs) (A) HDMECs do not express FcγR. HDMECs were stained for CD16, CD32, CD64, and the endothelial cell marker CD105 (dark gray histograms) or appropriate isotype controls (light gray histograms). **(B)** HDMECs were grown to confluency under the same conditions as for the experiments depicted in Figure 5. To visualize the confluency HDMECs were then stained with an antibody specific for VE-Cadherin (CD144) that stains the cellular junctions or an appropriate isotype control. The nuclei were stained with DAPI (blue) (n = 3). **(C)** To visualize the deposition of antibodies on the surface of the HDMECs monolayers of HDMECs were treated for 8 min with 12.5 µg/mL anti-CD105 or an appropriate isotype control, followed by fixation and detection of the deposited antibody with a secondary antibody. The nuclei were stained with DAPI (blue) (n = 3).

Supplementary Figure 6





Supplementary Figure 6. Human IgG subtype-specific recruitment of slanMo from the flow **(A)** Immobilized monomeric human IgG3 mediates the capture of slanMo from the flow. Freshly isolated slanMo were labeled for fluorescence microscopic detection and subsequently perfused over immobilized human IgG subtypes for 30 min at a shear stress of 0.5 dyn/cm² (n = 3). **(B)** Capturing of slanMo by immobilized human IgG3 largely depends on CD16. SlanMo were treated immediately before the experiment with specific blocking mAbs for CD16 (3G8), CD32 (AT10) or both as well as a binding (DX17) and a non-binding (MOPC-21) isotype control. Subsequently the treated slanMo were perfused for 30 min at a shear stress of 0.5 dyn/cm² over immobilized human IgG3 (n = 4). *p<0.05, **p<0.01, ***p<0.001, ns, not significant.