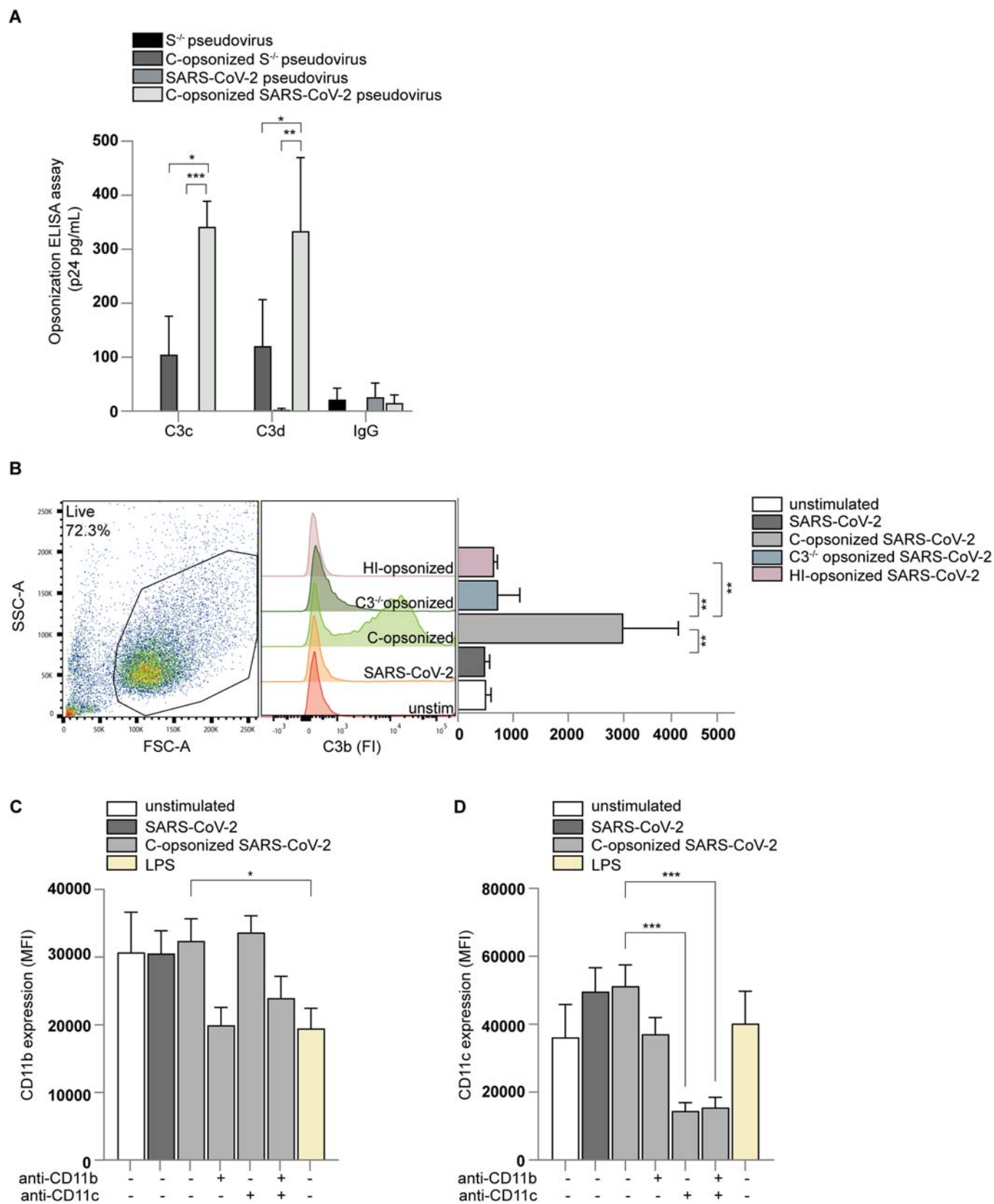


Expanded View Figures

Figure EV1. Spike-dependent opsonization requires C3 deposition for complement opsonization.

(A) Particles lacking SARS-CoV-2 Spike glycoprotein and SARS-CoV-2 pseudovirus opsonisation were determined by ELISA (p24 pg/mL) ($n = 3$ donors). (B) Deposition of C3b on DC-internalized SARS-CoV-2 after incubation with pre-pandemic NHS, C3-depleted sera and heat-inactivated sera ($n = 3$ donors). (C, D) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) in presence or absence of anti-CD11b and anti-CD11c. LPS stimulation was used as positive control for DC maturation, which was measured after 24 h by flow cytometry. Cumulative flow cytometry data of CD11b and CD11c ($n = 12$ donors). Data show the mean values and error bars are the SEM. Statistical analysis was performed using (A) two-way ANOVA with Tukey multiple-comparison test. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ ($n = 3$ donors). (B) ordinary one-way ANOVA with Dunnett's multiple-comparison test $**P \leq 0.01$ ($n = 3$ donors). (C, D) Two-way ANOVA with Tukey multiple-comparison test. $*P \leq 0.05$, $***P \leq 0.001$ ($n = 12$ donors). Source data are available online for this figure.



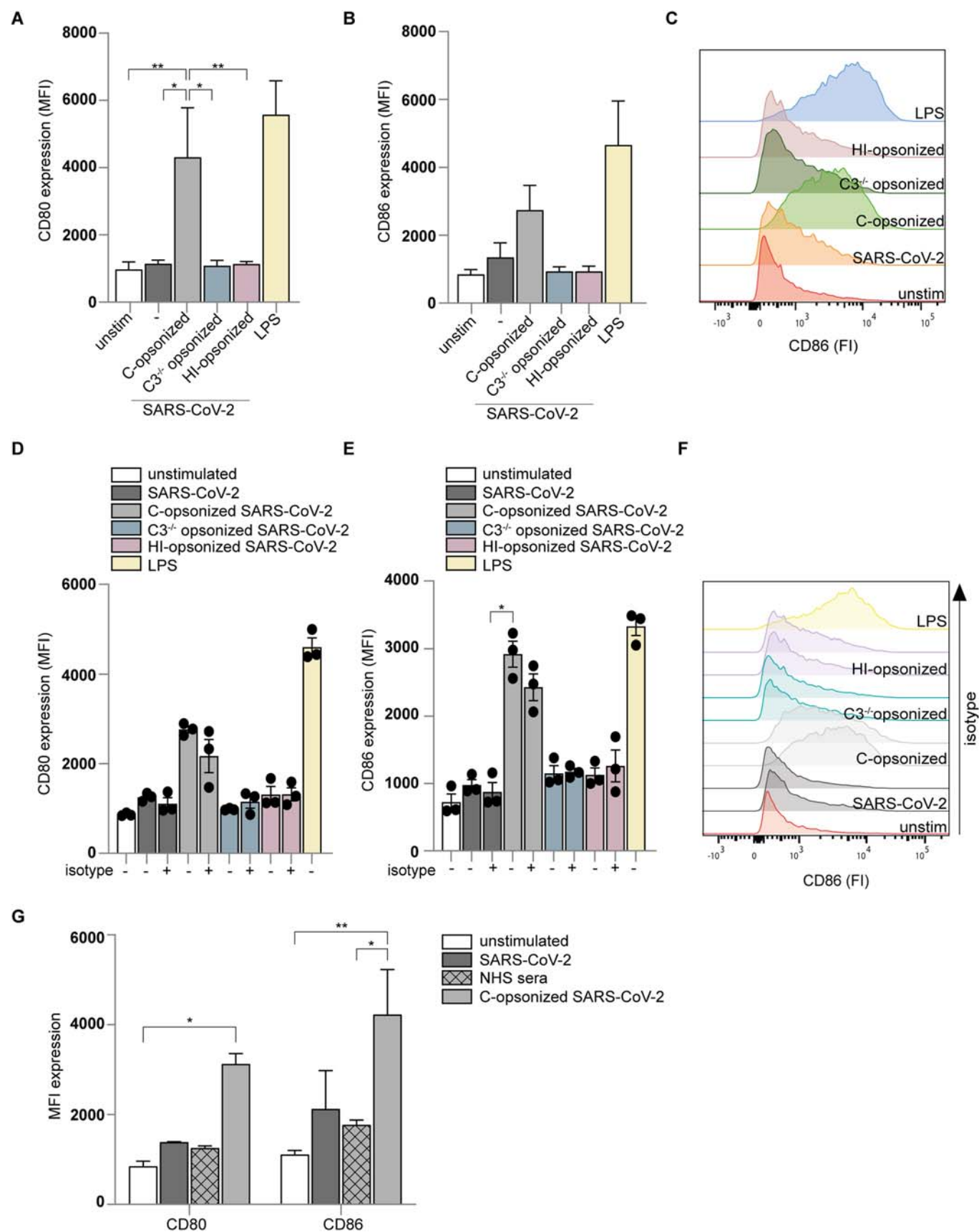
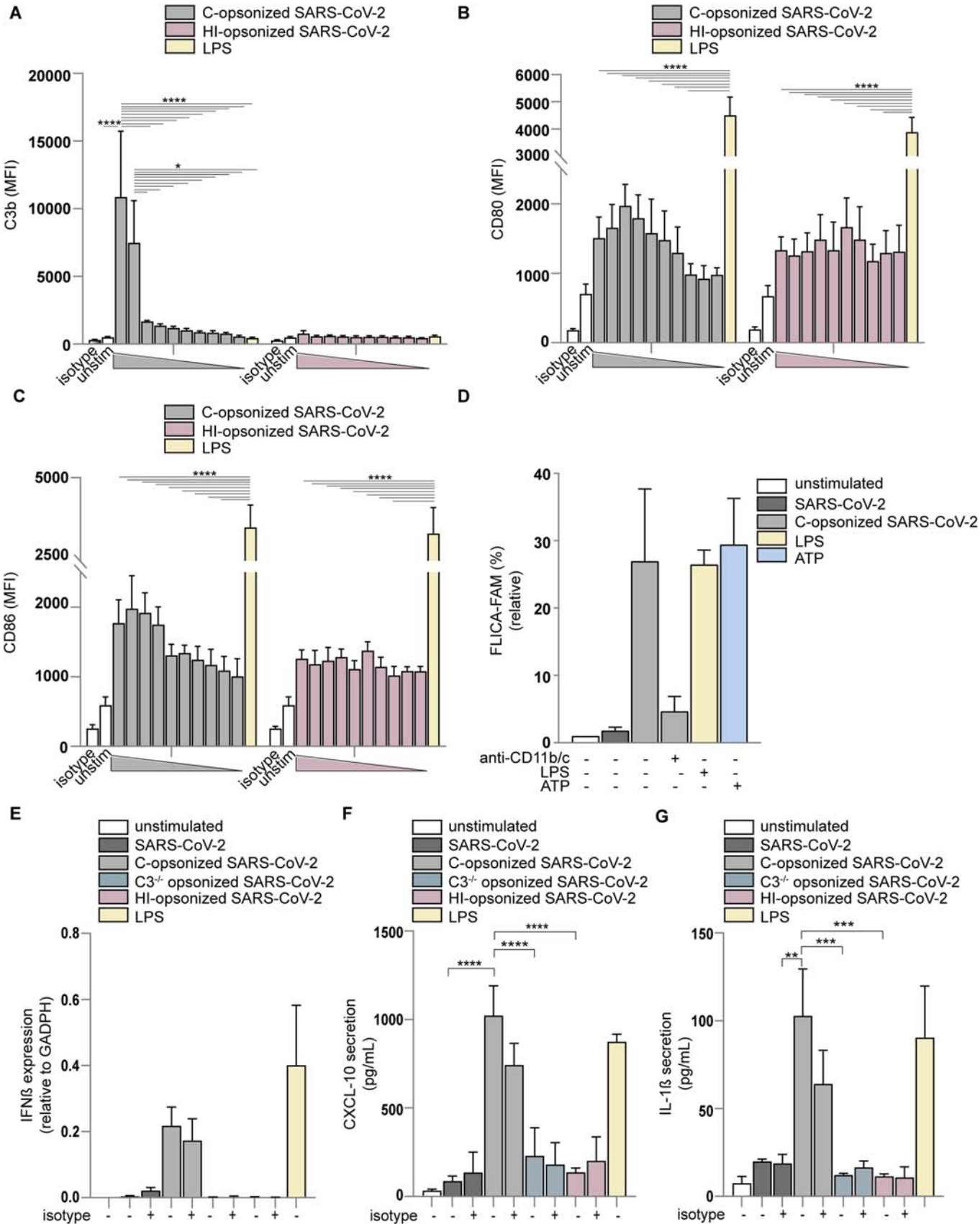


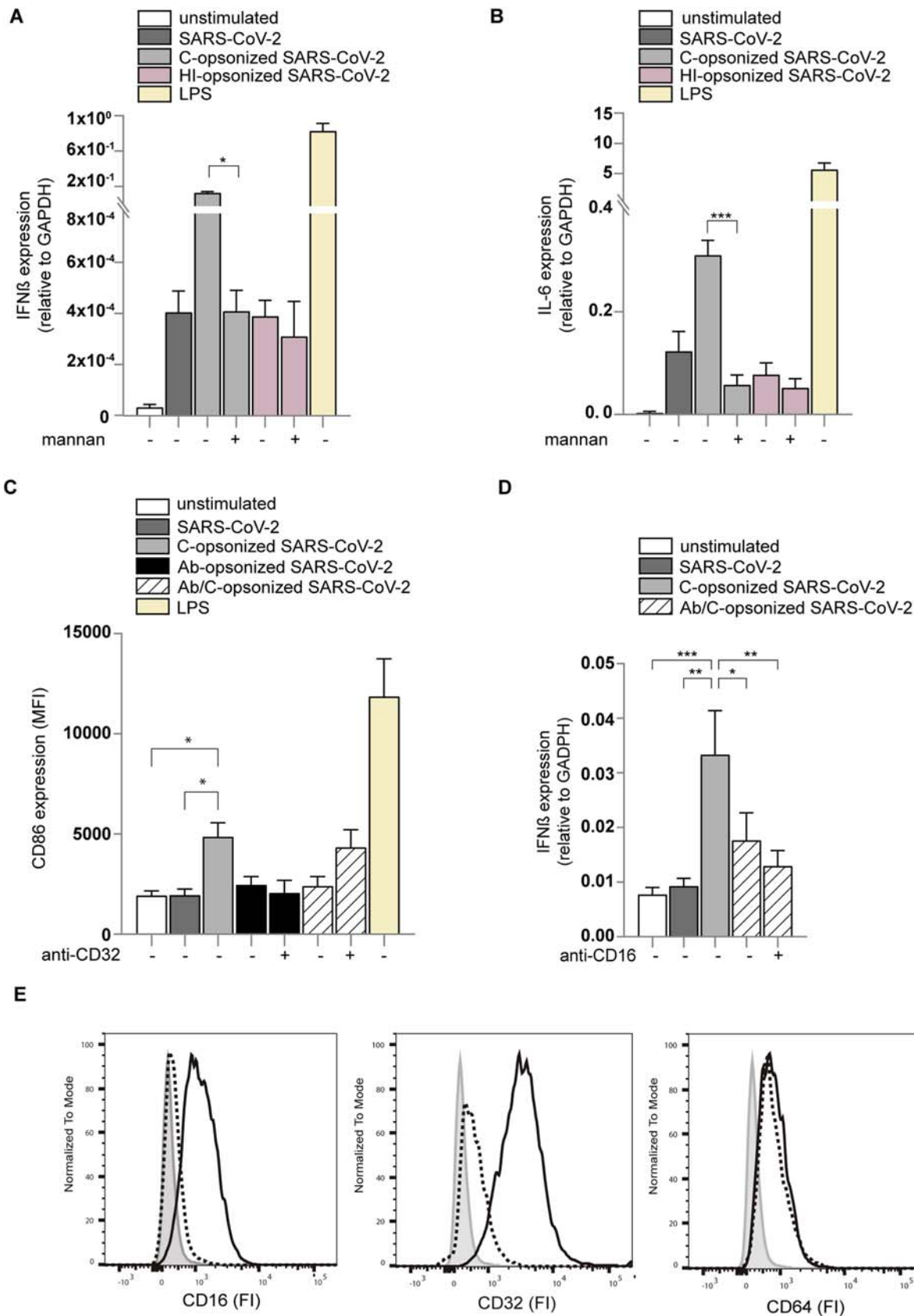

Figure EV2. C3 deposition is required for DC maturation.

(A–F) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), C3-depleted-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and heat-inactivated-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) in absence (A–C) or presence (D–F) of an isotype. LPS stimulation was used as positive control for DC maturation, which was measured after 24 h by flow cytometry. Cumulative flow cytometry data of CD80 (A–D) and CD86 (B–E) ($n = 5$ donors). (C, D) Representative histograms of CD86 expression. (G) DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), pre-pandemic NHS sera and complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and the expression of CD80 and CD86 markers were measured ($n = 4$ donors). Data show the mean values and error bars are the SEM. Statistical analysis was performed using (A) ordinary one-way ANOVA with Dunnett's multiple-comparison test. $*P \leq 0.05$, $**P \leq 0.01$ ($n = 5$ donors). (F) ordinary one-way ANOVA with Tukey multiple-comparison test. $*P \leq 0.05$ ($n = 5$ donors). (G) ordinary one-way ANOVA with Tukey multiple-comparison test. $*P \leq 0.05$, $**P \leq 0.01$ ($n = 4$ donors). Source data are available online for this figure.



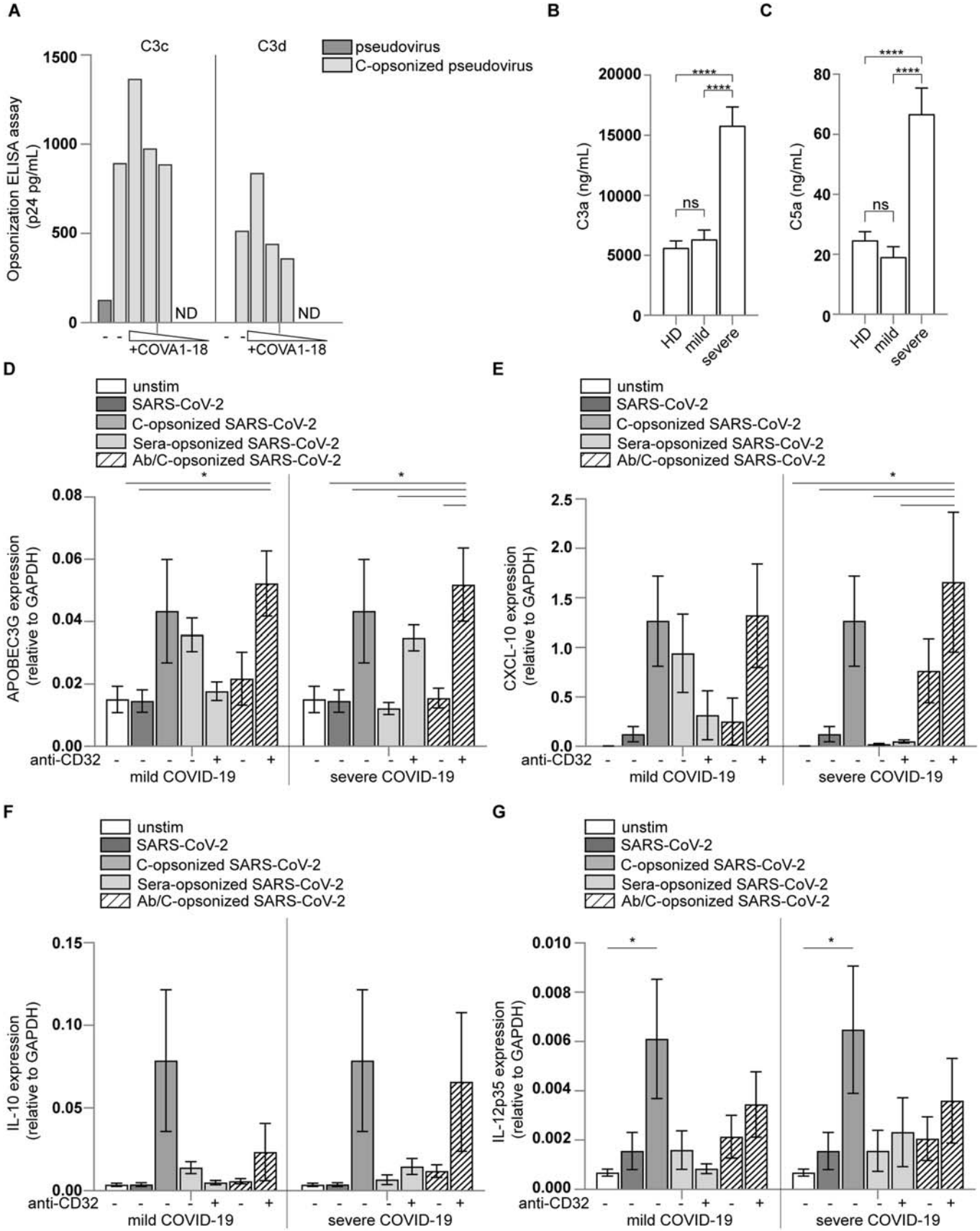
◀ **Figure EV3. SARS-CoV-2 opsonization is concentration-dependent and requires C3.**

(A) Mean fluorescence index (MFI) of serum concentration-dependent C3b deposition on DC-internalized complement-opsonized SARS-CoV-2 and heat-inactivated-opsonized SARS-CoV-2 after 24 h ($n = 4$ donors). (B, C) Mean fluorescence index (MFI) of serum concentration-dependent co-stimulatory markers, CD80 (B) and CD86 (C) on DC activated cells after exposure to complement-opsonized SARS-CoV-2 and heat-inactivated-opsonized SARS-CoV-2 after 24 h ($n = 4$ donors). LPS stimulation was used as positive control for DC maturation, which was measured after 24 h by flow cytometry. (D) Percentages of FLICA⁺ from different stimulated DC ($n = 3$ donors). (E–G) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), C3-depleted-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and heat-inactivated-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), as well as LPS (100 ng/mL) in presence or absence of an isotype for 2 h (E) and 24 h (F, G). mRNA levels of IFN β after 2 h were measured by qPCR ($n = 7$ donors) (E). CXCL10 and IL-1 β secretion (pg/mL) in the supernatant were measured after 24 h by ELISA ($n = 4$ donors). Data show the mean values and error bars are the SEM. Statistical analysis was performed using (A–C), two-way ANOVA with Tukey multiple-comparison test. * $P \leq 0.05$, **** $P \leq 0.0001$ ($n = 4$ donors) (A–C). (E–G) Two-way ANOVA with Tukey multiple-comparison test. ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ ($n = 7$ donors) (E) and ($n = 4$ donors) (F, G). Source data are available online for this figure.



◀ **Figure EV4. Complement-mediated DC activation and antiviral response is dependent on lectin pathway.**

(A, B) NHS and HIS were incubated with mannan (100 µg/mL), prior SARS-CoV-2 opsonization. DCs were exposed to non-, complement-opsonized SARS-CoV-2 and heat-inactivated-opsonized SARS-CoV-2 in presence or absence of mannan, and mRNA levels of IFN β after 2 h ($n = 3$ donors) (A) and IL-6 after 6 h ($n = 3$ donors) (B) were determined by qPCR. (C) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), to complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), to antibody-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and to antibody/complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) in presence or absence of anti-CD32 for 24 h. LPS stimulation was used as positive control for DC maturation, which was measured after 24 h by flow cytometry. Cumulative flow cytometry data of CD86 ($n = 12$ donors). (D) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), to complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) and to antibody/complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) in presence or absence of anti-CD16 for 2 h, and mRNA levels of IFN β ($n = 6$ donors) were determined by qPCR. (E) DCs were stained with antibodies against the surface markers CD16, CD32 and CD64 and analyzed by flow cytometry. Representative histograms for an experiment repeated more than three times with similar results ($n = 3$ donors). Data show the mean values and error bars are the SEM. Statistical analysis was performed using (A, B) two-way ANOVA with Tukey multiple-comparison test. * $P \leq 0.05$, *** $P \leq 0.001$ ($n = 3$ donors). (C) ordinary one-way ANOVA with Tukey multiple-comparison test. * $P \leq 0.05$ ($n = 6$ donors). (D) Two-way ANOVA with Tukey's multiple-comparison test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ ($n = 6$ donors). Source data are available online for this figure.



◀ **Figure EV5. Increased complement activation is a distinctive feature of severe COVID-19 patients.**

(A) NHS concentration-dependent SARS-CoV-2 pseudovirus opsonization by C3c and C3d were determined by (A) ELISA (p24 pg/mL) ($n = 2$ donors) (B, C) C3a and C5a level were determined in healthy donors, mild and severe COVID-19 patients ($n = 7$ donors per group). Plasma samples were harvested and C3a (ng/mL) and C5a (ng/mL) levels were analyzed using a BD Biosciences OptEIA Human C3a and C5a ELISA kit. (D–G) Human monocyte-derived DCs were exposed to SARS-CoV-2 isolate (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), to complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL), COVID-19 patient serum (mild or severe) and antibody/complement-opsonized SARS-CoV-2 (hCoV-19/Italy-WT, 1000 TCID₅₀/mL) in presence or absence of anti-CD32 for 6 h. mRNA levels of APOBEC3G (D) CXCL10 (E), IL-10 (F) and IL-12p35 (G) after 6 h were determined by qPCR ($n = 8$ donors). Data show the mean values and error bars are the SEM. Statistical analysis was performed using (B, C) ordinary one-way ANOVA with Tukey multiple-comparison test. **** $P \leq 0.0001$ ($n = 7$ donors). (D, E, G) Two-way ANOVA with Tukey's multiple-comparison test. * $P \leq 0.05$ ($n = 8$ donors). Source data are available online for this figure.