Supplementary Figure Legends

Figure S1. Expression of endogenous TMPRSS2 and Cathepsin-L in human macrophages

(A-B) Western blot analysis for total TMPRSS2 (A) and Cathepsin-L (B) expression in wildtype and transduced HT-29 cells (control), HEK293T, THP-1/PMA, and primary MDMs from multiple donors. β-actin was probed as a loading control.

Figure S2. Infection of primary MDMs by S-pseudotyped lentivirus is blocked by anti-SARS-CoV-2 NTD antibodies.

SARS-CoV-2 S-pseudotyped lentivirus (20 ng) was pre-incubated with indicated anti-Spike neutralizing antibodies for 30 mins at 37°C, followed by infection of primary MDMs for 3 days. Relative infection quantified by luciferase activity from whole cell lysates. NT: no-treatment with neutralizing antibody. Data are representative of 2 independent experiments, from 3 different donors each. Mock: no virus added, PBS: no-pre-incubation of virus with antibody. The means ± SEM are shown. P-values: one-way ANOVA followed by the Dunnett's post-test comparing to untreated (PBS) control, *: p < 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

Figure S3. Expression profiles of human CD169 and ACE2 in THP1 monocytes and primary MDMs.
Representative flow cytometry profiles of different THP1 cell lines and primary MDMs stained for surface expression of CD169 and ACE2. (A) Transduced THP1 cell lines stably expressing wild type (wt) CD169, mutant (R116A) CD169, ACE2, or both wt CD169 and ACE2. (B) Untransduced primary MDMs from multiple donors showing differential expression of endogenous CD169. After 5-6 days of macrophage differentiation, cells were either unstained or stained with anti-human CD169 antibody, and surface expression analyzed by flow cytometry. (C-D) Representative flow cytometry profiles of primary MDMs transduced with wt CD169 (C) or ACE2 (D) lentiviruses compared to negative (vector only) control.

Figure S4. Exogenous expression of ACE2 in primary MDMs rescues SARS-CoV-2 replication.

Representative immunofluorescence images (20x) of primary MDMs infected with SARS-CoV-2 (MOI=1) and stained for nucleus (DAPI, blue), and SARS-CoV-2 nucleocapsid protein (red), at 24 hpi. Images from primary MDMs overexpressing either CD169 or ACE2 compared to vector-only control were captured and represent at least 3 independent donors. Bar = 25 µm.

Figure S5. CD169 and ACE2-dependent temporal enhancement of SARS-CoV-2 RNAs in THP1/PMA macrophages.

Single molecule FISH analysis of viral +gRNA using high fidelity probes in SARS-CoV-2 (MOI=10) infected THP1/PMA macrophages at the indicated timepoints. Representative fields of cells were hybridized at indicated times with 7 sets of smFISH probes labeled...
with Quasar670 targeting the + strand of SARS-CoV-2 ORF1a (NSP1-3) and N transcripts. Data are representative of 2 independent experiments. Bar = 50µM

**Figure S6. Lack of pro-inflammatory cytokine expression in THP1/PMA macrophages infected with SARS-CoV-2 S-pseudotyped lentiviruses.**

PMA-differentiated THP1 cell lines infected with SARS-CoV-2 S-pseudotyped lentivirus (20 ng) and total RNA harvested at 2 dpi, followed by qRT-PCR analysis. Fold expression of indicated cytokines normalized to mock (uninfected) condition in each group. Data are representative of at least 3 independent experiments. The means ± SEM from 3 independent experiments are shown.