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## Supplemental information

# SARS-CoV-2 N protein mediates intercellular nucleic acid dispersion, a feature reduced in Omicron

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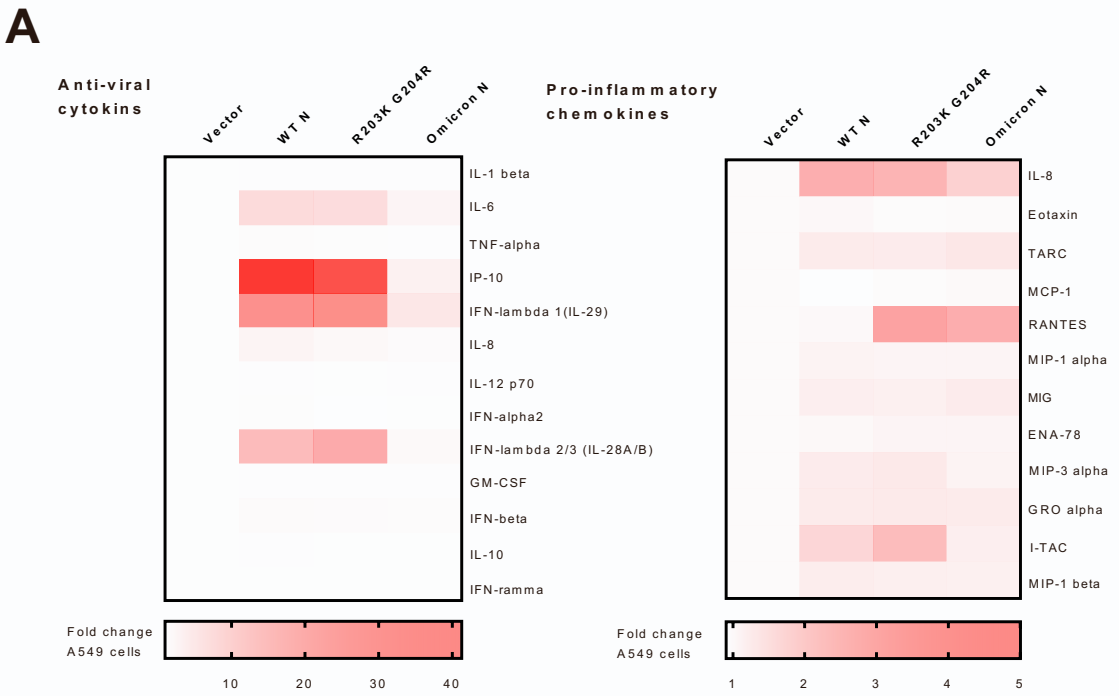
# Supplementary information



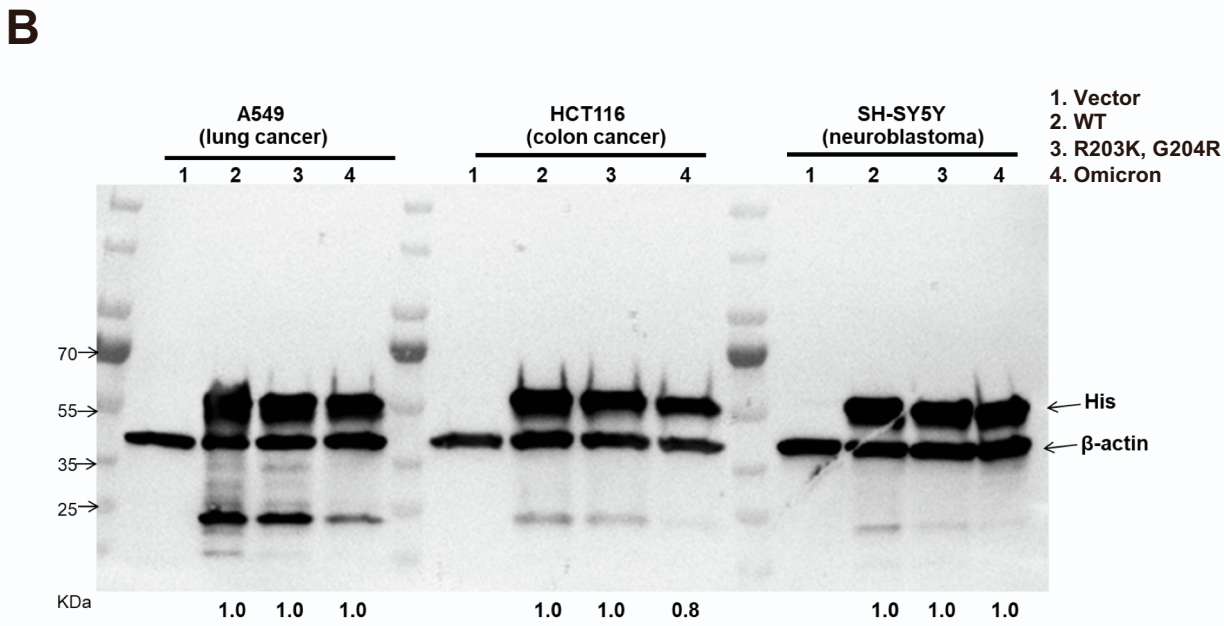




**Figure S1. N protein induce cytokines and chemokines in lung cells**

(Related to STAR Methods and Figure 4)

(A) Secretome analysis of antiviral cytokines and proinflammatory chemokines induced by SARS-CoV-2 N protein and its variants. Supernatant collected from A549 lung carcinoma cells 48 hours after ectopically transfected with pcDNA3.1 (vector control) or plasmids encoding SARS-CoV-2 N proteins and two of its variants found in GISAID database (R203K G204R and Omicron). Fold of cytokine or chemokine levels relative to vector control were shown, and color coded as defined in the bar (red: increase). (B) The expression of N on different cell types. A549, HCT-116, and SH-SY5Y cells were seeded on 12 well plates. Cells were transfected with wild type SARS-CoV-2 N protein (WT N), R203K G204R N variant (R203K G204R) or Omicron N variant (Omicron). The ratio of each cell type indicated the protein level was first normalized by β-actin level and then divided by the level of WT N group of each cell type, respectively.

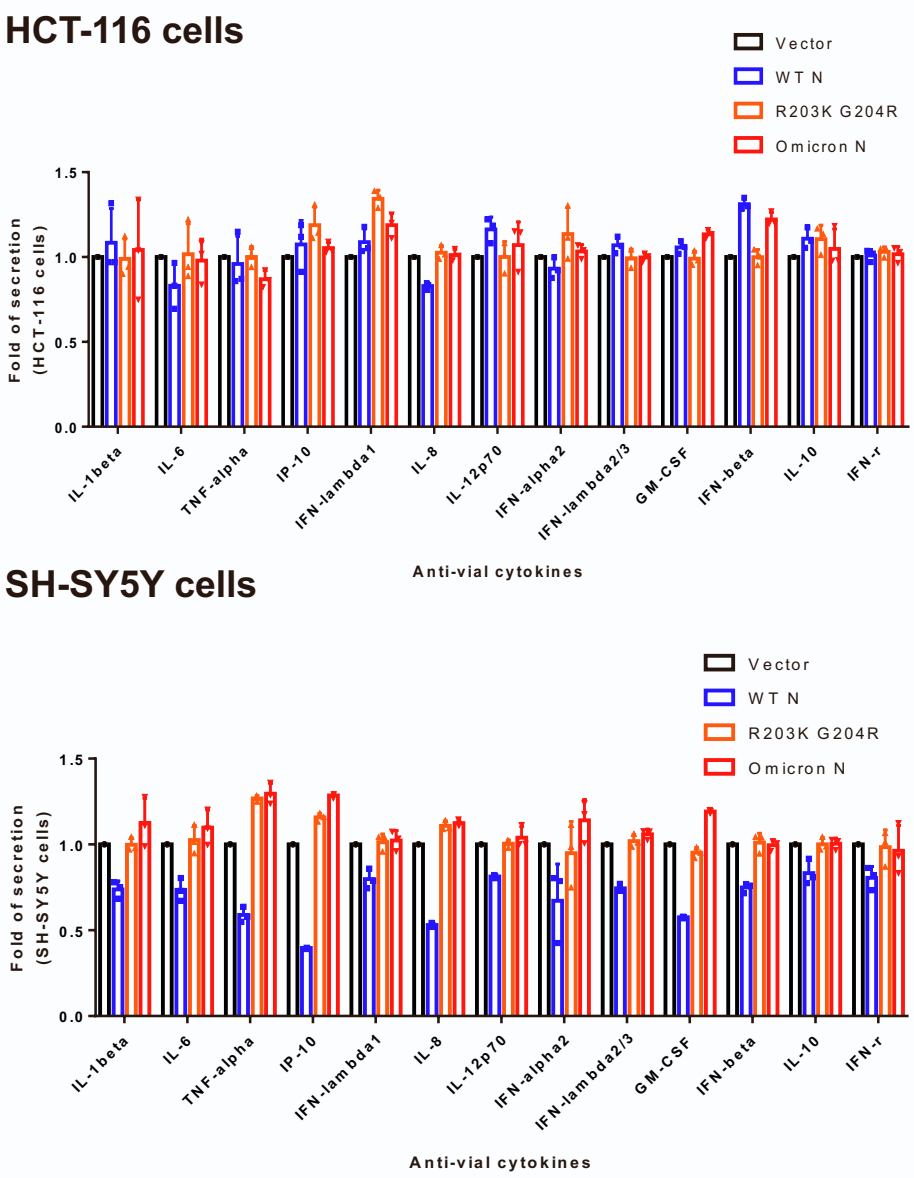



**Figure S2. Anti-viral cytokines secretome analysis of the effect of N protein to HCT-116 (up) and SH-SY5Y (bottom) cells** (Related to STAR Methods and Figure 4)
HCT-116 or SH-SY5Y cells are transfected by vector control (Vector), wild type N (WT N), R203K G204R N variant (R203K G204R) or Omicron N protein (Omicron). After 48hrs transfection, supernatant are collected and perform anti-viral cytokines secretome analysis by flow cytometry.

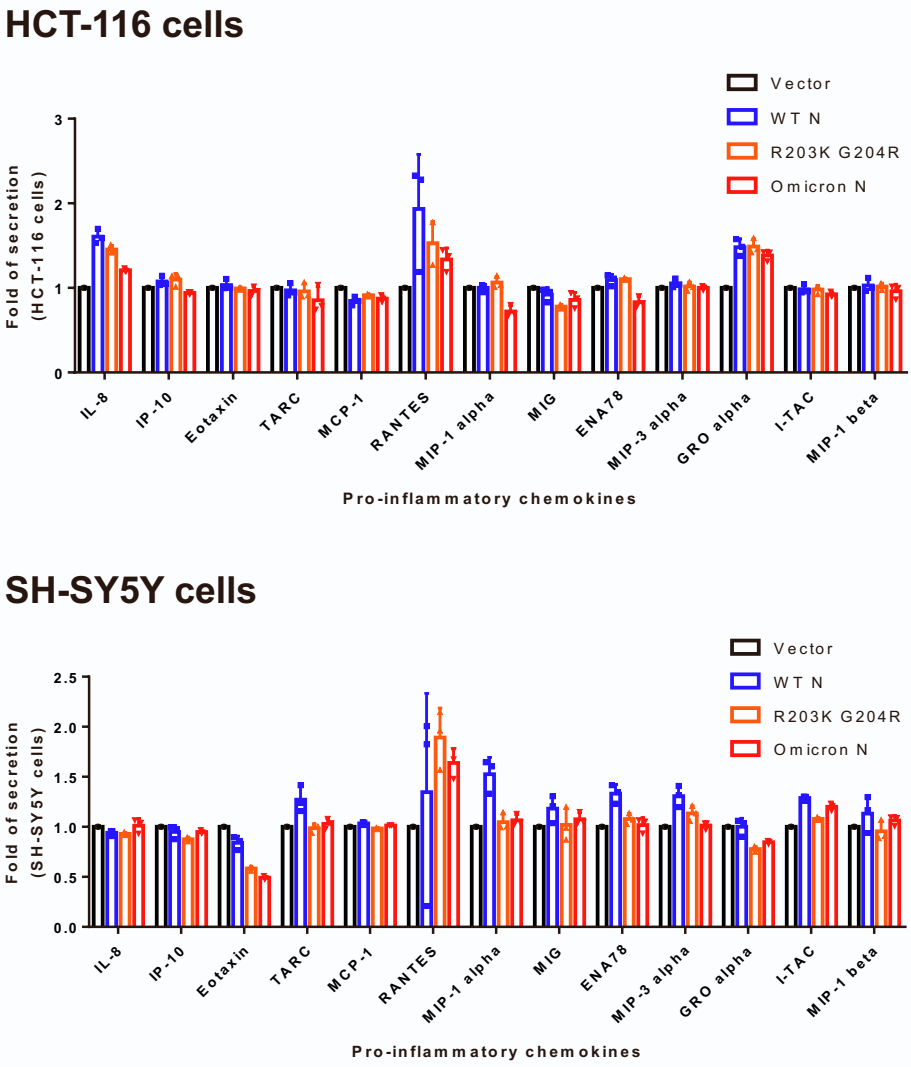



**Figure S3. Pro-inflammatory chemokine secretome analysis of the effect of N protein to HCT-116 (up) and SH-SY5Y (bottom) cells**

(Related to STAR Methods and Figure 4)

HCT-116 or SH-SY5Y cells are transfected by vector control (Vector), wild type N (WT N), R203K G204R N variant (R203K G204R) or Omicron N protein (Omicron). After 48hrs transfection, supernatant are collected and perform pro-inflammatory chemokine secretome analysis by flow cytometry.

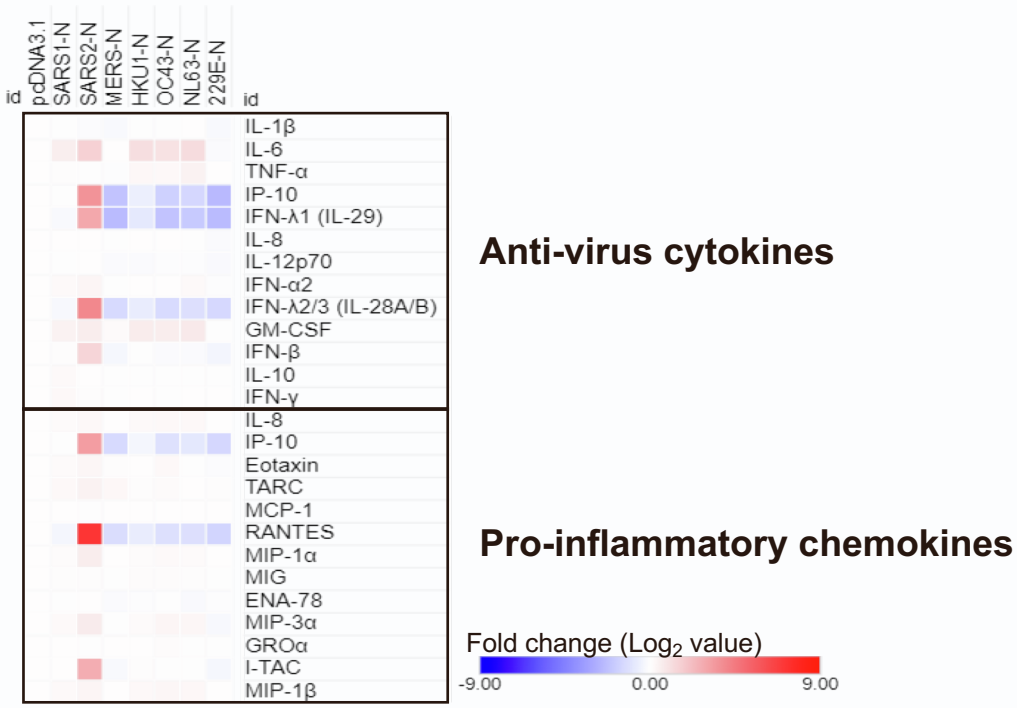



**Figure S4. Secretome screening of the effect of N protein to cells from different coronaviruses** (Related to STAR Methods)

Secretome screening of 7 different coronavirus N protein to cells. Seven different human coronavirus N proteins were overexpressed in A549 cells. After 48 hours transfection, supernatant were collected and the level of cytokines or chemokines were analyzed. (red: increase; blue: decrease by $2^9$ fold).

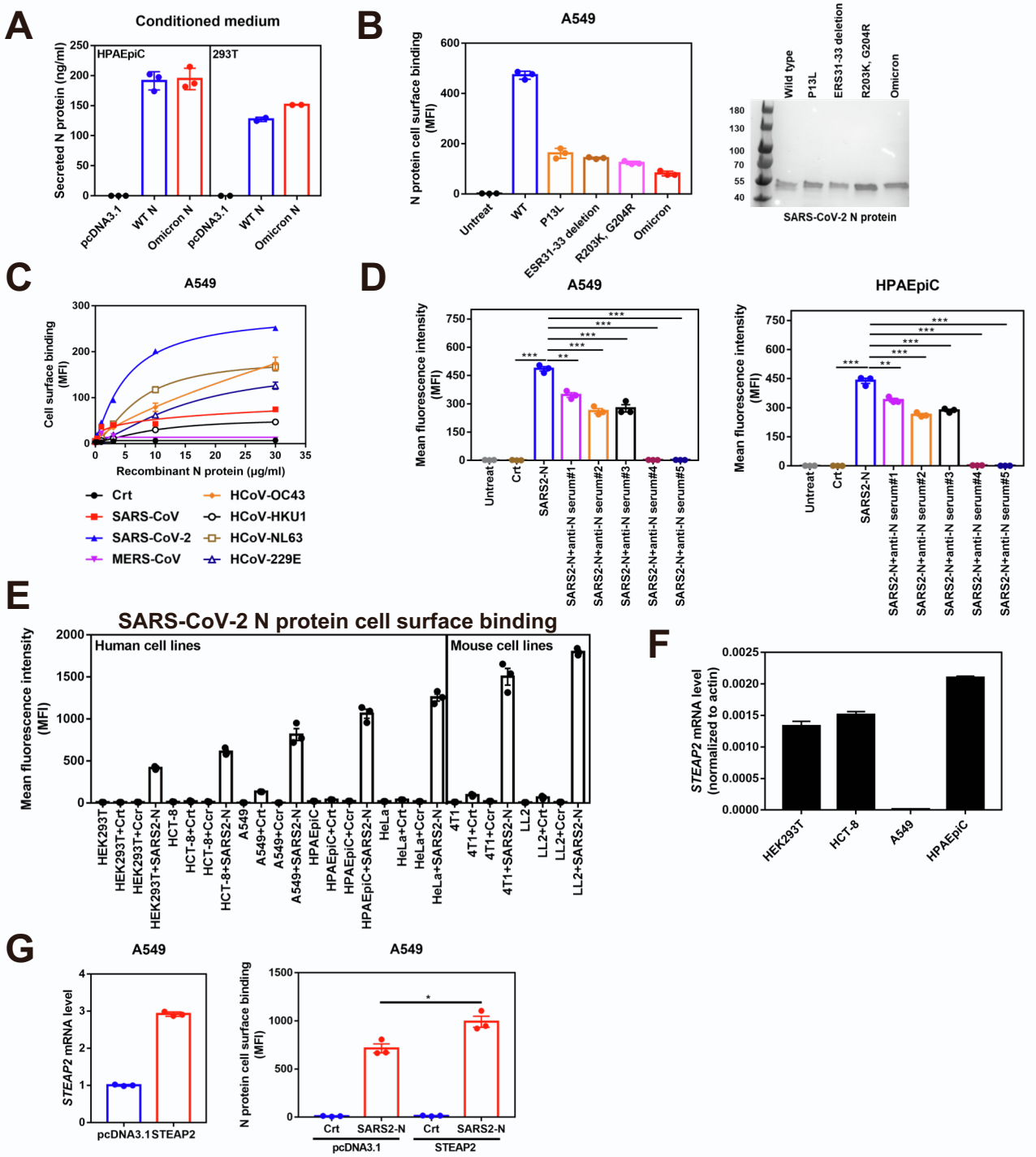



**Figure S5**. **SARS-CoV-2 N protein is secreted and directly binds to cells** (Related to Figure 1)
(A) 293T or HPAEpiC cells were transfected with pcDNA3.1, pcDNA3.1-SARS-CoV-2 wild type N, pcDNA3.1-Omicron N. After 48 hours, secreted N protein was evaluated by ELISA assay. (B) The cell surface binding of omicron-derived mutated N protein in A549 cells was assessed by flow cytometry analysis and shown as mean fluorescence intensity (MFI) (left panel). The loading amount of recombinant N proteins was shown in coomassie blue-stained gel (right panel). (C) A549 cells binding with different amounts of His-tagged purified different Coronavirus N proteins or a His-tagged control protein (crotonase, Crt) of bacterial origin. One hour after protein addition, allophycocyanin (APC) conjugated anti-His antibody was used to detect cell binding capacity of each viral N protein. The samples were analyzed by flow cytometry and data shown as mean fluorescence intensity (MFI). (D) 1 μl of mouse anti-N sera was mixed with 30 μg N protein and incubated at 4°C overnight. Cell binding of the sera-mixed N protein in A549 (left pannel) and HPAEpiC (right panel) was assessed by flow cytometry analysis. Here, Crt was used as control protein. (E) 10 μg N protein was mixed with $1x10^5$ indicated cells and incubated for 1 hour binding on ice. The cell surface binding of N protein on different cell lines was assessed by flow cytometry analysis and shown as mean fluorescence intensity (MFI). (F) qRT-PCR of STEAP2 in HEK293T, HCT-8, A549 and HPAEpiC cells. (G) qRT-PCR of STEAP2 in STEAP2 overexpressed A549 cells was shown in left panel. The cell surface binding of N protein on STEAP2 overexpressed A549 cells was assessed by flow cytometry analysis and shown as mean fluorescence intensity (MFI) (right panel). Data are shown as mean ± SEM. **p<0.01; ***p<0.001; t test.

**Table. S1 Amino acid changes in SARS-CoV-2 N protein** (Related to Figure 1)

| Variants | Amino acid changes in SARS-CoV-2 N protein | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alpha | D3L | | | | | | R203K | G204R | | S235F | |
| Beta | | | | | | | | | T205I | | |
| Gamma | | | | | P80R | | R203K | G204R | | | |
| Delta | | | | D63G | | | R203M | | G215C | D377Y | |
| Omicron BA.1 | | P13L | ERS31-33 deletion | | | | R203K | G204R | | | |
| Omicron BA.2 | | P13L | ERS31-33 deletion | | | | R203K | G204R | | | S413R |
| Omicron BA.4 | | P13L | ERS31-33 deletion | | | P151S | R203K | G204R | | | S413R |
| Omicron BA.5 | | P13L | ERS31-33 deletion | | | | R203K | G204R | | | S413R |

**Source:** Variants of concern (VOC) profiles of amino acid changes from World Health Organization (WHO) website (https://www.who.int/activities/tracking-SARS-CoV-2-variants)

**Table S2. N protein-interacting plasma membrane proteins enriched in A549 and HPAEpiC membrane fraction without crosslinking** (Related to Figure 1)

| A549 mem (no crosslinking) | | | HPAEpiC mem (no crosslinking) | | |
|---|---|---|---|---|---|
| Protein accession | Gene name | -log P | Protein accession | Gene name | -log P |
| P19367 | HK1 | 3.97 | Q9H4G0 | EPB41L1 | 4.31 |
| O00422 | SAP18 | 3.97 | Q14008 | CKAP5 | 4.01 |
| O60437 | PPL | 3.87 | O00422 | SAP18 | 3.81 |
| Q8NFT2 | STEAP2 | 2.99 | O60437 | PPL | 3.72 |
| Q9H4G0 | EPB41L1 | 2.38 | P19367 | HK1 | 2.86 |
| P09496 | CLTA | 1.93 | P68400 | CSNK2A1 | 2.83 |
| P30520 | ADSS | 1.89 | Q13492 | PICALM | 2.62 |
| Q13620 | CUL4B | 1.58 | Q7KZI7 | MARK2 | 2.62 |
| P49755 | TMED10 | 1.56 | Q14247 | CTTN | 2.51 |
| P30530 | AXL | 1.41 | P30101 | PDIA3 | 2.33 |
| P06702 | S100A9 | 1.35 | P17987 | TCP1 | 2.27 |
| | | | Q8NFT2 | STEAP2 | 2.27 |
| | | | Q8IVF2 | AHNAK2 | 2.23 |
| | | | P07858 | CTSB | 2.23 |
| | | | P15559 | NQO1 | 2.20 |
| | | | Q9Y446 | PKP3 | 2.06 |
| | | | Q9UHR4 | BAIAP2L1 | 2.05 |
| | | | Q08722 | CD47 | 2.03 |
| | | | P38606 | ATP6V1A | 1.81 |
| | | | P25490 | YY1 | 1.79 |
| | | | P09496 | CLTA | 1.72 |
| | | | P34932 | HSPA4 | 1.66 |
| | | | Q9C037 | TRIM4 | 1.56 |
| | | | Q96PU8 | QKI | 1.54 |
| | | | P39687 | ANP32A | 1.48 |
| | | | Q9P0L0 | VAPA | 1.48 |
| | | | P60903 | S100A10 | 1.47 |
| | | | P50552 | VASP | 1.46 |
| | | | Q14344 | GNA13 | 1.45 |
| | | | Q9P2D0 | IBTK | 1.37 |
| | | | O60762 | DPM1 | 1.37 |
| | | | O15143 | ARPC1B | 1.34 |
| | | | O00116 | AGPS | 1.34 |
| | | | P21926 | CD9 | 1.32 |
| | | | P49189 | ALDH9A1 | 1.31 |

The plasma membrane proteins enriched from A549 and HPAEpiC membrane fraction with their statistically significant enrichment scores (-log P) were shown in the table.

**Table S3. N protein-interacting plasma membrane proteins enriched in crosslinked A549 and HPAEpiC cells** (Related to Figure 1)

| A549 cell (crosslinking) | | | HPAEpiC cell (crosslinking) | | |
|---|---|---|---|---|---|
| Protein accession | Gene name | -log P | Protein accession | Gene name | -log P |
| P04899 | GNAI2 | 2.84 | Q8NFT2 | STEAP2 | 3.82 |
| P49755 | TMED10 | 2.40 | Q03135 | CAV1 | 2.97 |
| P31641 | SLC6A6 | 2.39 | P01889 | HLA-B | 2.90 |
| O43707 | ACTN4 | 2.11 | P54709 | ATP1B3 | 2.86 |
| P55011 | SLC12A2 | 1.91 | Q01105 | SET | 1.93 |
| O00422 | SAP18 | 1.79 | P02774 | GC | 1.78 |
| Q86SJ6 | DSG4 | 1.52 | P10301 | RRAS | 1.68 |
| Q8NBS9 | TXNDC5 | 1.52 | | | |
| P05026 | ATP1B1 | 1.51 | | | |
| P37802 | TAGLN2 | 1.47 | | | |
| Q9C037 | TRIM4 | 1.38 | | | |
| P17655 | CAPN2 | 1.37 | | | |

The plasma membrane proteins enriched from crosslinked A549 and HPAEpiC cells with their enrichment scores (-log P) were shown in the table.

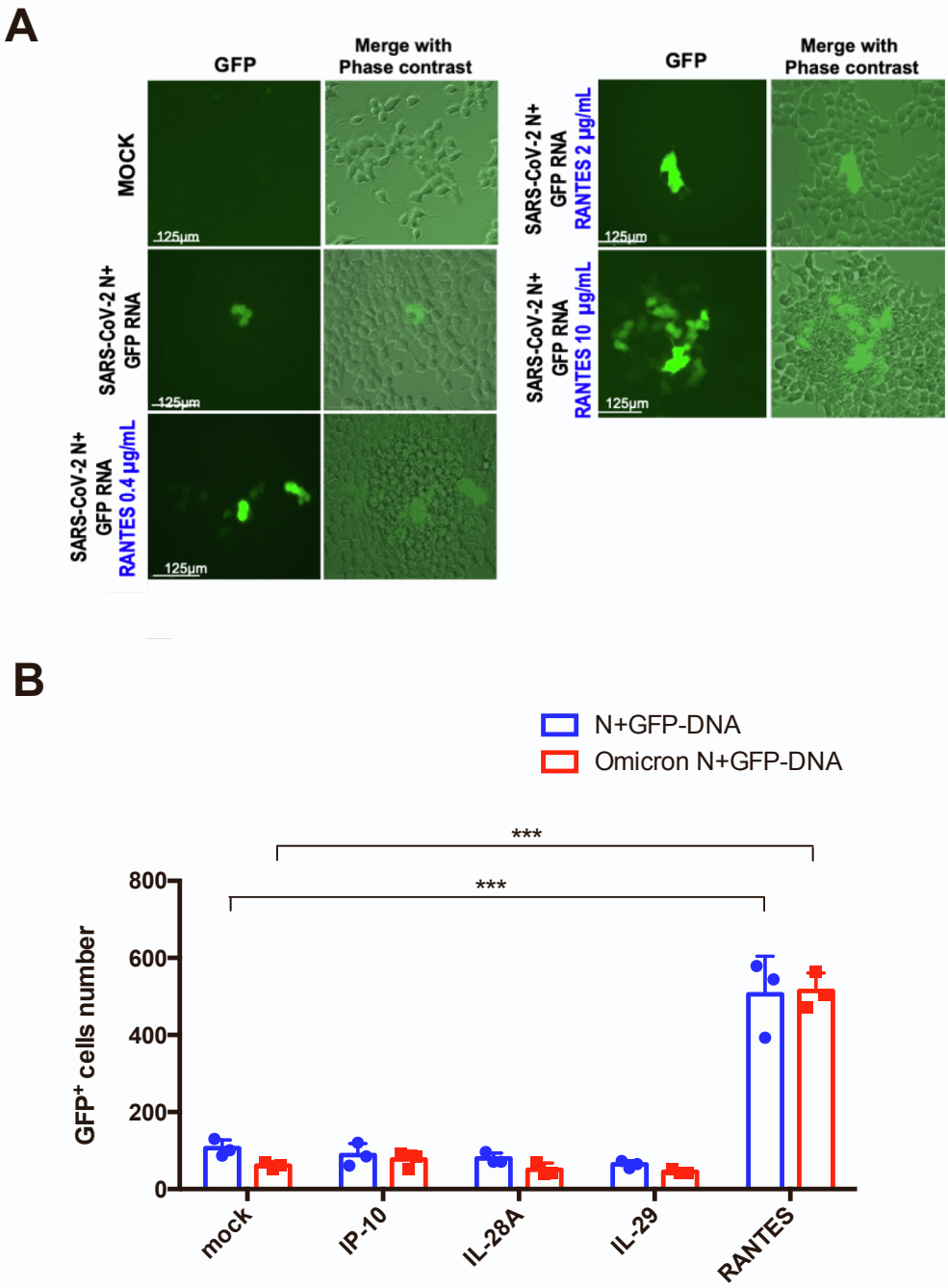



**Figure S6. RANTES enhanced SARS-CoV-2 N protein delivered and nucleic acid expressed in cells** (Related to Figure 4)

(A) SARS-CoV-2 N protein delivered and expressed GFP-RNA in cells and RANTES showed a dosage-dependent effect in enhancing N assisted GFP-RNA expression. 293T cells were pretreated by indicated dosage of RANTES overnight. After treatment, N protein (10 μg, mammalian cell produced) complexed with GFP DNA (40 μg) mixture was added to cells. After 48 hours, the GFP expression was detected by florescence microscopy. Scale bar: 125 μm. (B) RANTES enhanced WT N or Omicron N-mediated GFP expression in 293T cells. 293T cells were seeded onto 8-well glass slides (55000 cells/well). The cells were pretreated with RANTES or other indicated effectors at indicated concentration overnight. SARS-CoV-2 N or Omicron N protein were pre-mixed with GFP-DNA (pmax-GFP) for one hour at 4˚C. The N protein-GFP DNA mixtures were added to 293T cells. After 48 hours, the GFP expression was observed by fluorescence microscopy. The number of $GFP^+$ cells were counted. Data are shown as mean ± SEM. ***$p<0.001$; t test.

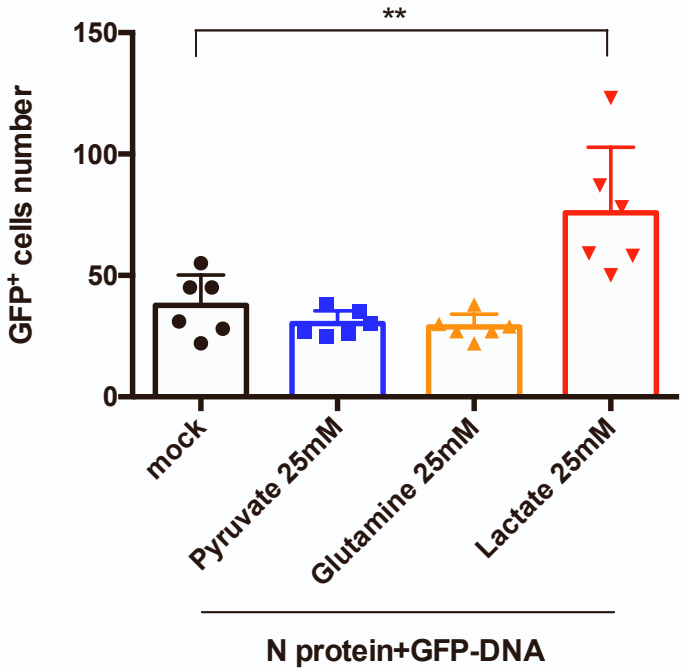



**Figure S7. Metabolite effects on N protein-mediated GFP-DNA expression** (Related to Figure 4)

293T cells were seeded onto 8-well glass slides (55000 cells/well). The cells were pretreated with indicated metabolites at indicated concentration overnight. SARS-CoV-2 N protein were pre-mixed with GFP-DNA (pmax-GFP) for one hour at 4°C. The N protein-GFP DNA mixtures were added to 293T cells. After 48 hours, the GFP expression was observed by fluorescence microscopy. The number of $GFP^+$ cells were counted. Data are shown as mean ± SEM. **p<0.01; t test.

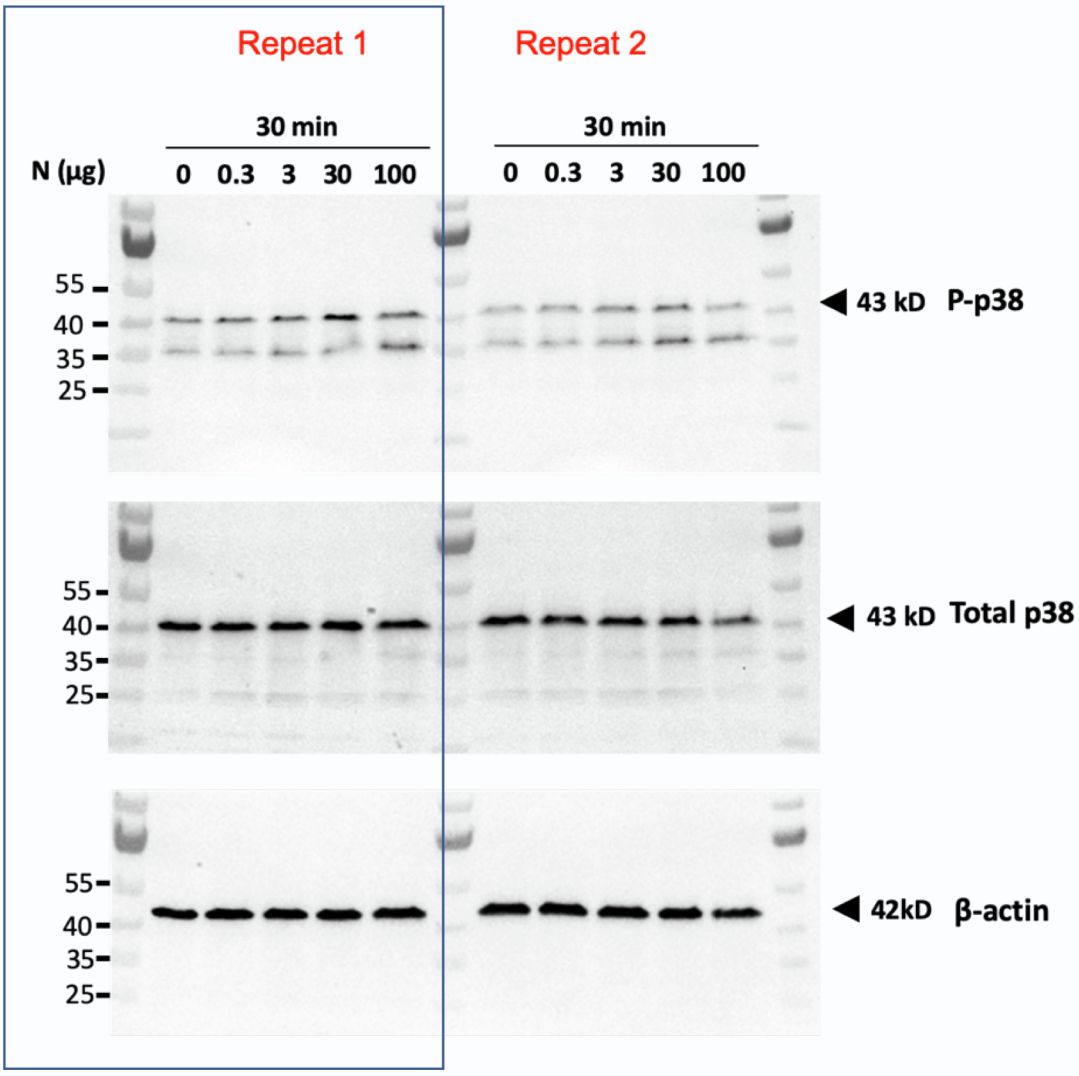



**Figure S8. Original Images of Figure 4D** (Related to Figure 4)
Original image of independent result which demonstrates exogenous adding SARS-CoV-2 N protein to HPAEpiC cells induces p38 MAPK kinase phosphorylation. (image in blue rectangle: the original data shown in Figure 4D)

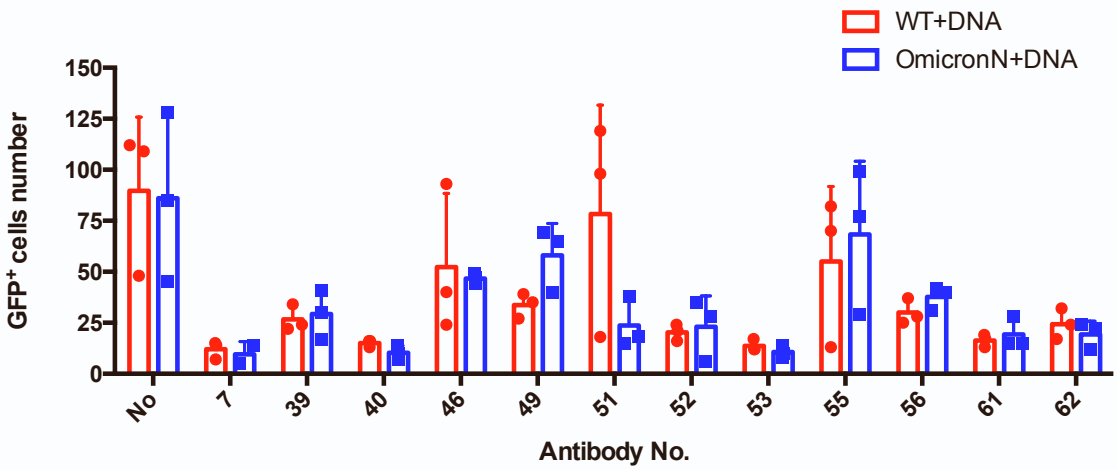



**Figure S9. Anti-N antibodies inhibited N assisted GFP-DNA expression**
(Related to Figure 4)
Mammalian cells expressed 3 μg WT N or Omicron N protein were pre-bound with 9 μg different anti-N antibody, then mixed with 12 μg GFP-DNA. Then these complex were added to $5x10^4$ 293T cells. The $GFP^+$ cells were counted after 48 hours incubation. Data are shown as mean ± SEM.

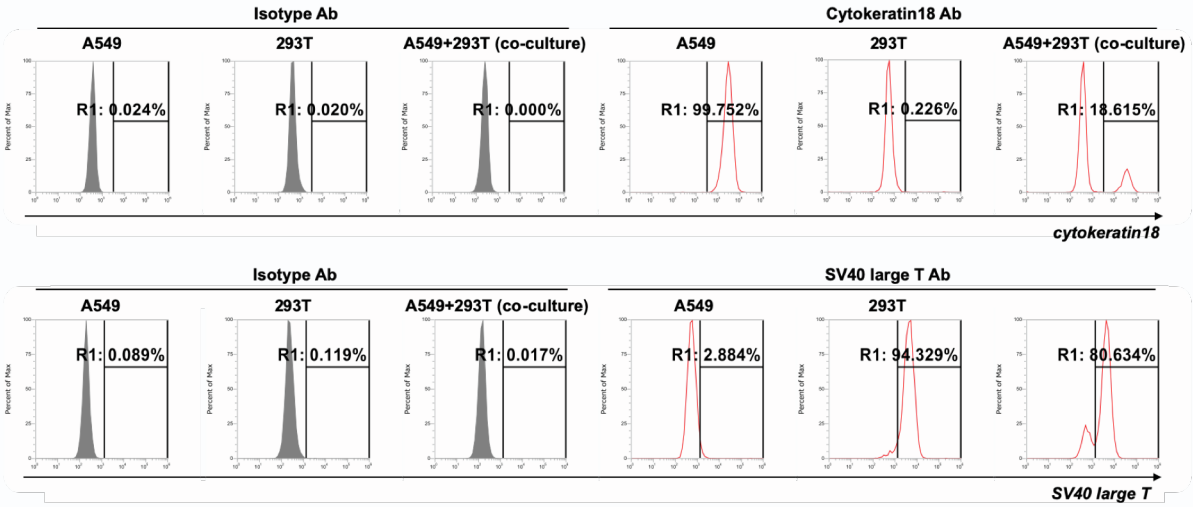



**Figure S10. Cell markers gating** (Related Figure 5)
A549, 293T and co-culture cells were stained with anti-cytokeratin 18 and anti-SV40 large T antibodies.

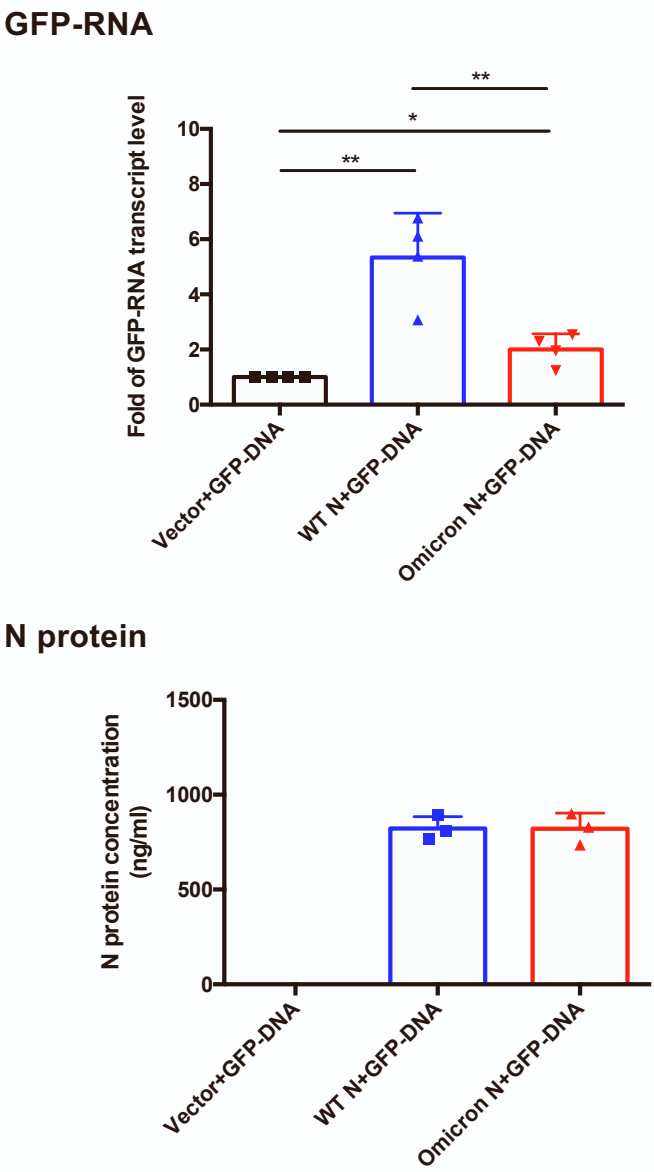



**Figure S11. GFP-RNA and N protein are existed in supernatant after Covid-19 N protein ectopic expression** (Related Figure 5)
293T cells were seeded on 12 well plates. Cells were transfected with SARS-CoV-2 wild type or Omicron N protein and GFP-DNA (pmax-GFP). After ectopically expression, supernatant was harvested and the GFP-RNA and N protein were detected by q-PCR and ELISA.