Supporting Information

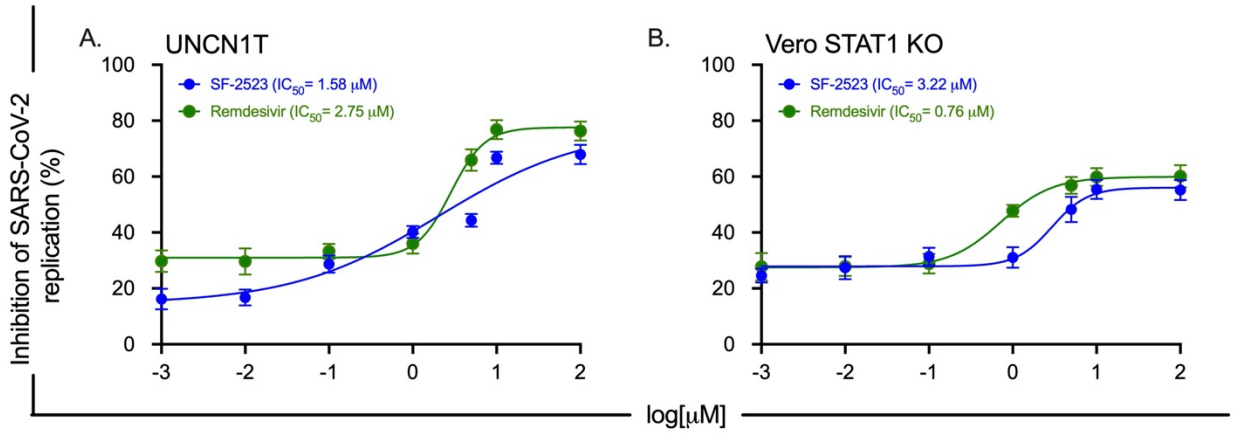

Fig S1. Wild type SARS-CoV-2 replication inhibition dose-response curves of SF2523 in UNCN1T and Vero STAT1 KO cells. (A, B). UNCN1T and Vero STAT1 knockout cells (20,000 cells/well) were seeded in 96-well plates 24 hours before infection. Different concentrations of SF2523 and remdesivir (100 μM, 10 μM, 5 μM, 1 μM, 0.1 μM, 0.01 μM and 0.001 μM) were added to the cells 2 hours before infection. The cells were infected with 0.1 MOI of SARS-CoV-2 isolate USA-WI1/2020. Culture supernatant was collected at 48 hrs post-infection. The SARS-CoV-2 viral load was quantified in the culture supernatant using RT-QPCR with primer probes targeting E gene of SARS-CoV-2 using PrimeDirect Probe RT-qPCR Mix (TaKaRa Bio USA, Inc) and Applied Biosystems QuantStudio3 real-time PCR system (Applied Biosystems, Waltham, MA, USA) per the manufacturer's instructions. The SARS-CoV-2 genome equivalent copies were calculated using quantitative PCR (qPCR) control RNA from heat-inactivated SARS-CoV-2, isolate USA-WA1/2020 (BEI, Catalog# NR-52347). The percentage inhibition of SARS-CoV-2 replication in SF2523 and remdesivir treated wells was calculated with respect to virus concentration in positive control wells treated with DMSO (considered 0% inhibition) and negative control wells (uninfected cells). SF2523 (in blue) and remdesivir (in green) IC_{50} values were calculated using four-parameter variable slope sigmoidal dose-response models using Graph Pad Prism 8.0 software; [CC_{50}: Drug concentration that required to reduces cell viability by 50%; IC_{50}: Drug concentration that required to reduce the viral replication by 50%].

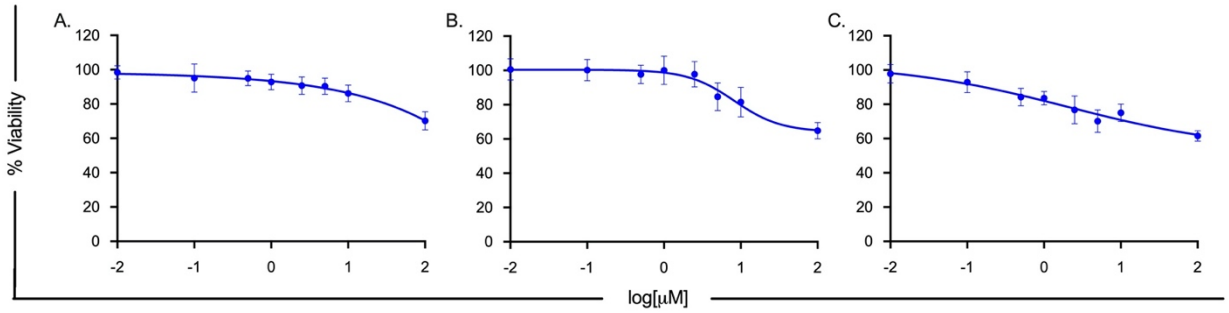

Fig S2. Cytotoxicity of SF2523 in Calu-3 cells (A) and combination of SF2523/RDV and SF2523/MU-UNMC-2 in UNCN1T cells (B and C) cells was measured by MTT assay. The cells were treated with increasing concentration of SF2523, SF2523/RDV and SF2523/MU-UNMC-2 (0.001 to 100 μM) and incubated at 37º C in a humidified 5% CO_2 incubator. After 72-hour post-treatment, 20 μL of MTT substrate (5 mg/mL) was added to each well and incubated for 4 additional hours at 37º C in the dark. Then the culture media was carefully removed, and blue formazan crystals were dissolved in 200 μl of DMSO, and the purple color was read at 595 nm with a reference filter of 620 nm. In respective cells, SF2523, SF2523/RDV and SF2523/MU-UNMC-2 has a CC_{50} value above 100 μM.

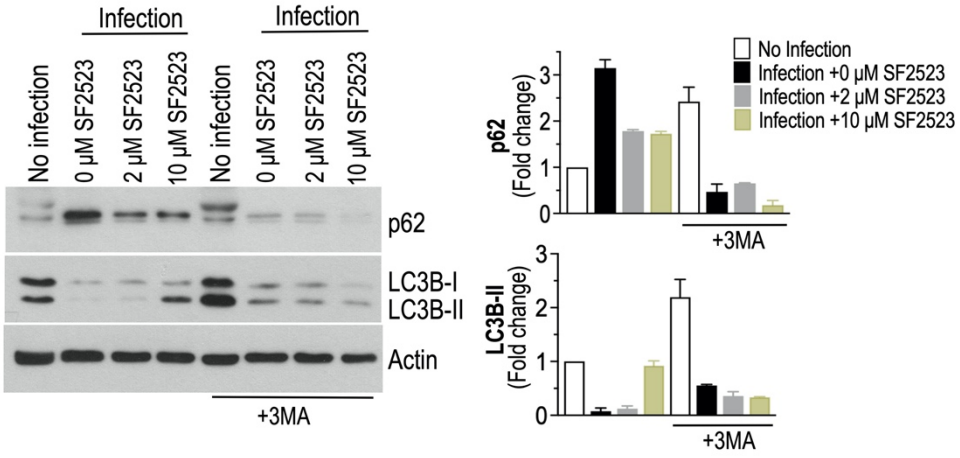

Fig S3. SF2523 may induces autophagy in SARS-CoV-2 infected Vero cells. The cells were treated with the indicated concentration of SF2523 and 5 mM 3-MA and infected with SARS-CoV-2. After 24h, cells were harvested, cell lysate prepared, total protein content quantified, and immunoblotting was performed. The upper and lower panels are the representative immunoblots of p62 and LC3B-I//II. Actin was used as a loading control. The upper and lower bar graphs show densitometric analysis of p62 and LC3B-II immunoblots, respectively normalized with actin. Data is expressed as mean ± SEM derived from two experiments.