# Supplementary material

*for*

# Kidney injury molecule-1 is a potential receptor for SARS-CoV-2

*Short title: KIM1 mediates SARS-CoV-2 invasion*

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**Supplementary Table S1 MM-GBSA binding free energy of SARS-CoV-2-RBD and receptors.**

| Ligand | Receptor | Binding free energy (kcal/mol) |
|---|---|---|
| SARS-CoV-RBD | KIM1 Ig V domain | -21.59 |
| SARS-CoV-2-RBD | KIM1 Ig V domain | -35.64 |
| SARS-CoV-2-RBD (V367F) | KIM1 Ig V domain | -37.26 |
| SARS-CoV-2-RBD | ACE2 | -50.60 |
| MERS-COV-RBD | KIM1 Ig V domain | -10.12 |
| SARS-CoV-2-RBD | AP1 | -7.13 |
| SARS-CoV-2-RBD | AP2 | -6.65 |

MM-GBSA binding free energy of the protein complex was cauclated by the accumulation of the binding free energy of all involved residues. The binding free energy of SARS-CoV-2-RBD and ACE2 was also cauclated and provided as a reference.

**Supplementary Table S2 Top 20 clinically identified mutations in SARS-CoV-2 spike protein.**

| Rank | Mutation | Occurrence (Log10) | Rank | Mutation | Occurrence (Log10) |
|---|---|---|---|---|---|
| 1 | D614G | 5.016 | 11 | D294D | 3.004 |
| 2 | Q616Q | 3.906 | 12 | P25P | 2.961 |
| 3 | S477N | 3.886 | 13 | D936Y | 2.940 |
| 4 | T723T | 3.710 | 14 | N824N | 2.929 |
| 5 | A222V | 3.322 | 15 | F275F | 2.892 |
| 6 | Y789Y | 3.163 | 16 | P1263L | 2.866 |
| 7 | F306F | 3.155 | 17 | P715P | 2.779 |
| 8 | R21I | 3.050 | 18 | L54F | 2.676 |
| 9 | L5F | 3.037 | 19 | D253G | 2.646 |
| 10 | N439K | 3.004 | 20 | T1100T | 2.598 |

Synonymous mutations are underlined. Data were acquired on October 5, 2020 from http://giorgilab.dyndns.org/coronapp/.

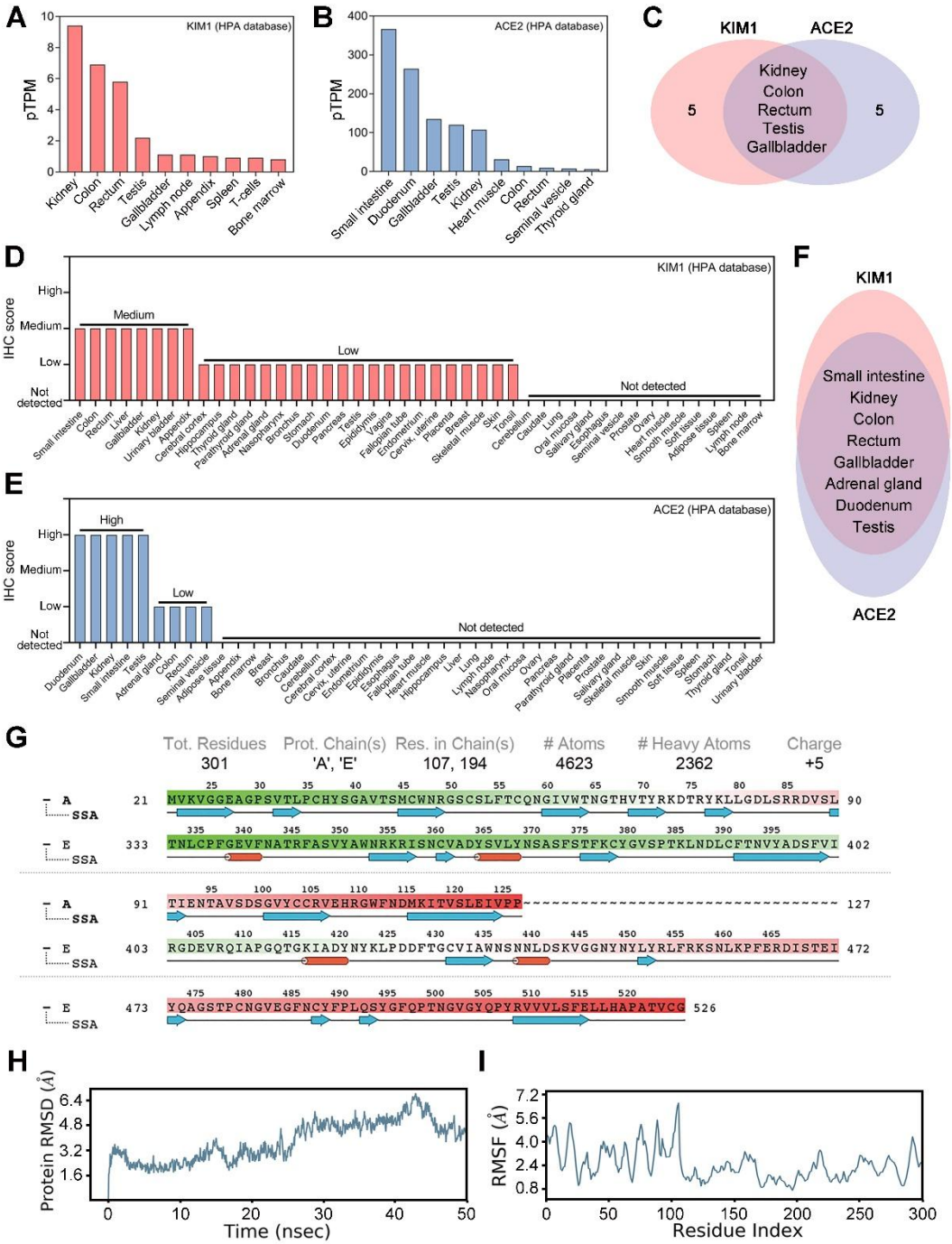
**Supplementary Table S3 MM-GBSA binding free energy of residues in SARS-CoV-RBD and KIM1 Ig V domain complex.**

| Rank | SARS-CoV-RBD | | KIM1 Ig V | |
|---|---|---|---|---|
| | Residue | Binding free energy (kcal/mol) | Residue | Binding free energy (kcal/mol) |
| 1 | Phe360 | -3.67 | Trp112 | -4.87 |
| 2 | Val354 | -1.93 | Phe55 | -4.33 |
| 3 | Asn424 | -1.34 | Leu54 | -4.17 |
| 4 | Trp423 | -1.12 | Phe113 | -3.62 |
| 5 | Asn427 | -0.71 | Gln58 | -1.46 |
| 6 | Thr359 | -0.69 | Asn114 | -1.42 |
| 7 | Ser358 | -0.68 | Asn59 | -0.54 |
| 8 | Phe361 | -0.59 | Asp115 | -0.37 |
| 9 | Leu355 | -0.45 | Asp74 | -0.20 |
| 10 | Asn357 | -0.41 | Asp99 | -0.20 |

Top 10 ranked residues that involved in the binding of SARS-CoV-RBD and KIM1 Ig V domain were listed.

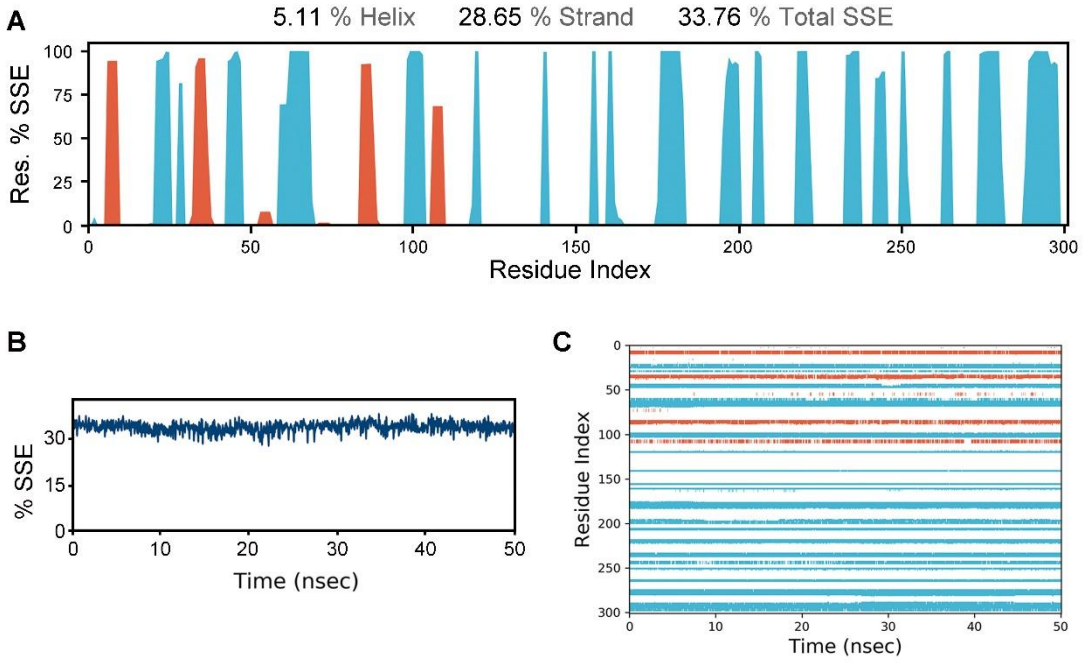
**Supplementary Table S4 Primers used in the study.**

| Primer | Sequence: 5' to 3' |
|---|---|
| **Primers for qPCR** | |
| M-*Ace2*-F | CAAGTGTTGGCTTCGGTGTG |
| M-*Ace2*-R | ATTCAAGTGACCAGCGAGCA |
| M-*Kim1*-F | TCCACACATGTACCAACATCAA |
| M-*Kim1*-R | GTCACAGTGCCATTCCAGTC |
| M-*Actinb*-F | GGCTGTATTCCCCTCCATCG |
| M-*Actinb*-R | CCAGTTGGTAACAATGCCATGT |
| **Primers for CRISPR-Cas9 system** | |
| H-*KIM1*-KO-1-F | CACCGCTGACGGCCAATACCACTAA |
| H-*KIM1*-KO-1-R | AAACTTAGTGGTATTGGCCGTCAGC |
| H-*KIM1*-KO-2-F | CACCGGTTCGAACAGTCGTGACGGT |
| H-*KIM1*-KO-2-R | AAACACCGTCACGACTGTTCGAACC |
| H-*KIM1*-KO-3-F | CACCGGTCGTTGGAACAGTCGTCAT |
| H-*KIM1*-KO-3-R | AAACATGACGACTGTTCCAACGACC |

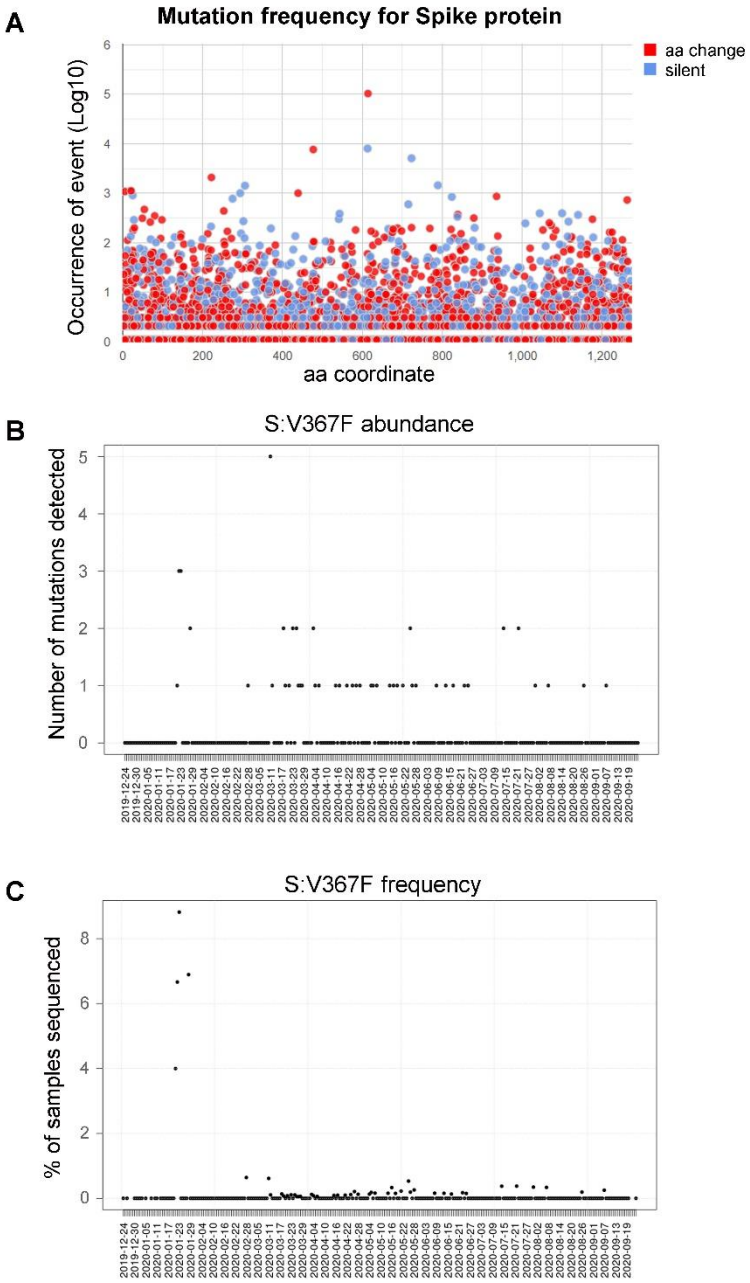



**Supplementary Figure S1** Expression profiles of KIM1, ACE2 and molecular dynamics docking information of SARS-CoV-2-RBD and KIM1. (**A**) Tissue transcriptional expression of *KIM1* from The Human Protein ATLAS dataset (HPA). pTPM, protein-coding transcripts per million. (**B**) Tissue transcriptional expression of *ACE2* from HPA. (**C**) Transcriptional expression intersection of *KIM1* and *ACE2* form HPA. (**D**) Histology-based protein expression levels of KIM1. (**E**) Histology-based protein expression levels of ACE2. (**F**) Histology-based protein expression intersection of KIM1 and

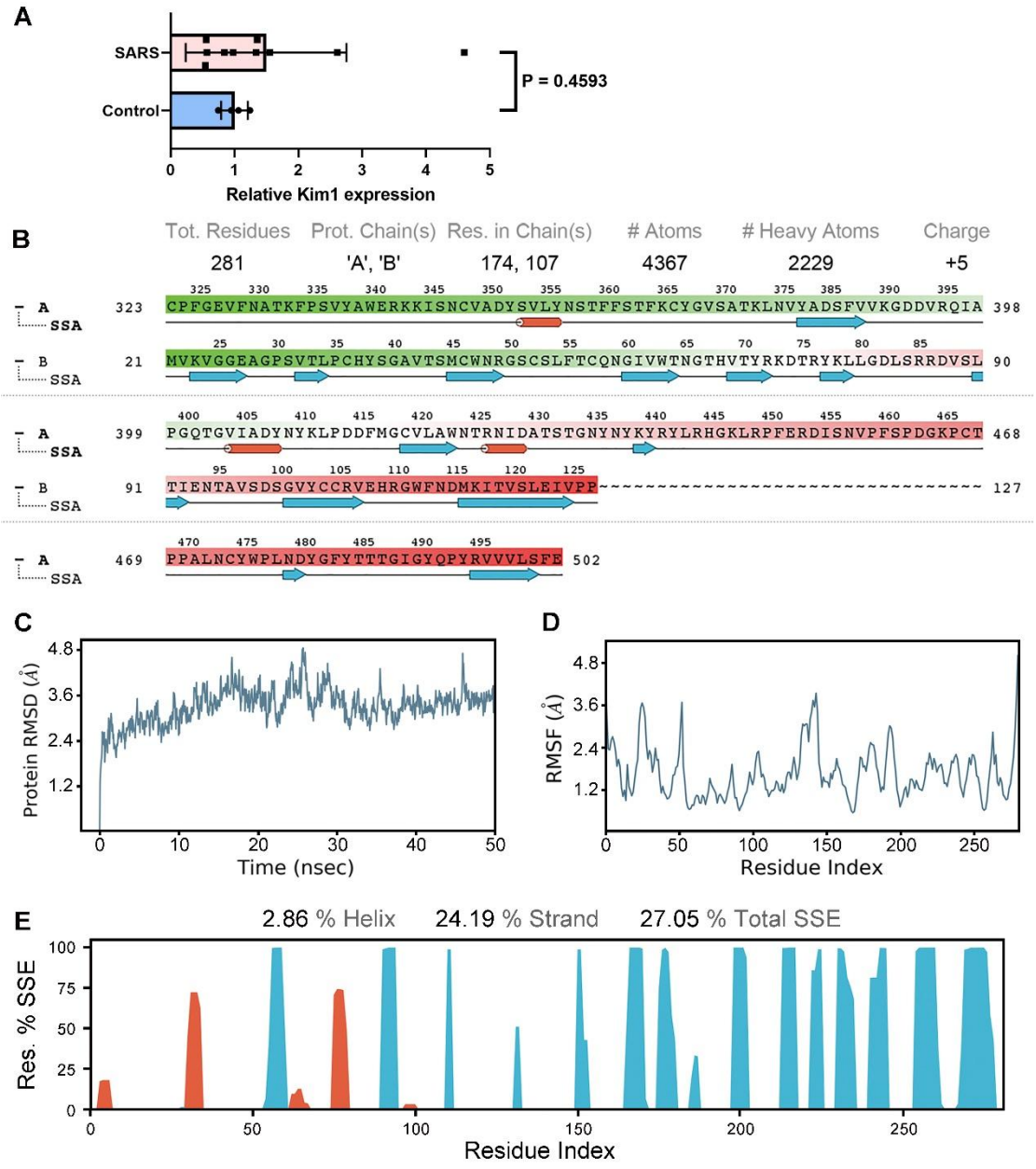
ACE2. (**G**) Basic protein information of SARS-CoV-2-RBD and KIM1 Ig V domain. Red cylinder represents α-Helix structure. Blue arrow indicates β-Sheet structure. (**H**) RMSD values of the SARS-CoV-2-RBD and KIM1 Ig V domain binding models. (**I**) RMSF values of the SARS-CoV-2-RBD and KIM1 Ig V domain binding models.



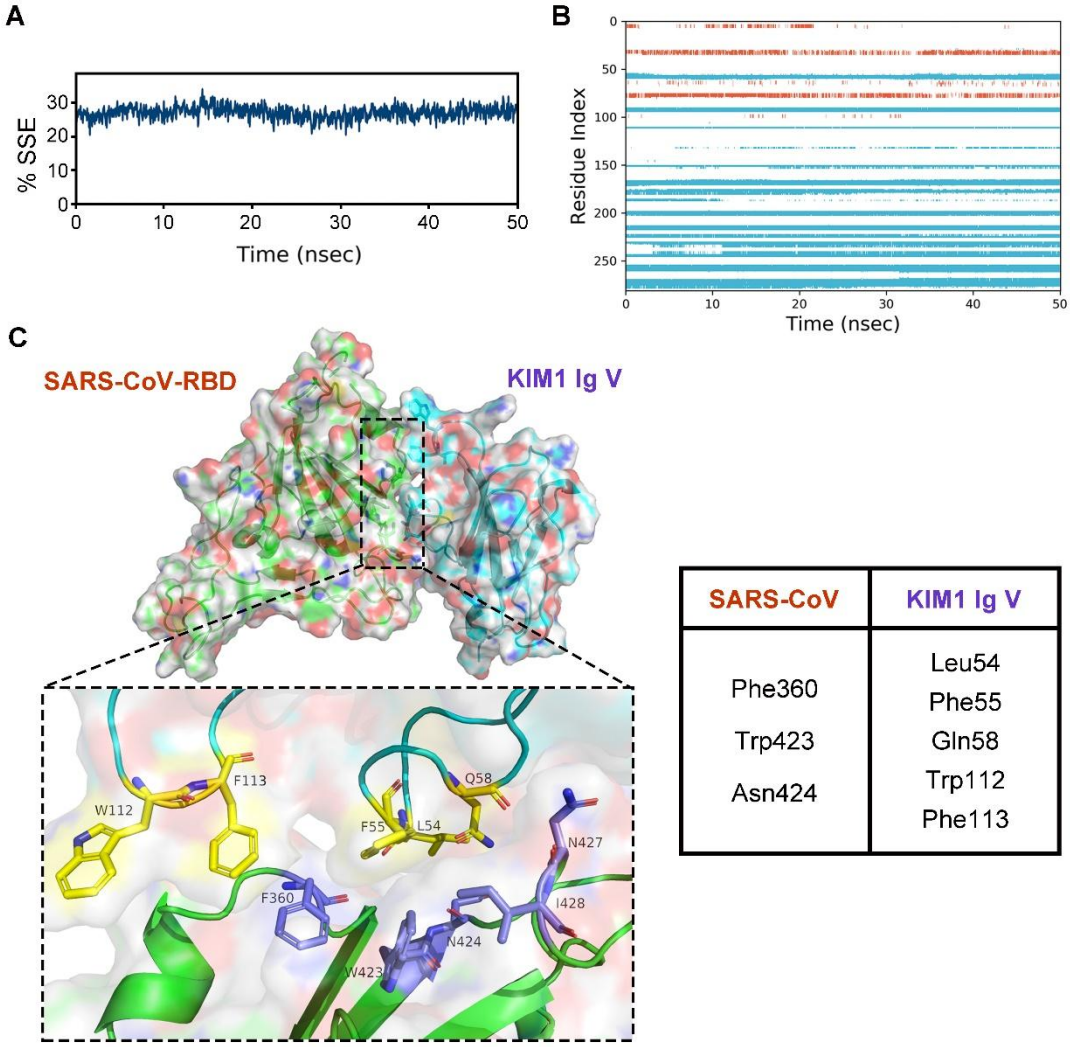

**Supplementary Figure S2** Molecular dynamics simulations information of SARS-CoV-2-RBD and KIM1. (**A**) Residue SSE distribution by residue index throughout the protein structure. Red column suggests α-Helix structure and blue colunm indicates β-strand structure. (**B**) SSE composition for trajectory frames in the dynamics simulation process of SARS-CoV-2-RBD and KIM1 Ig V domain, the curve indicates SSE composition for each trajectory frame over the course of the simulation. (**C**) SSE assignment of the residues over the simulation time, red dots indicates α-Helix structures and blue dots indicate β-strand structures. SSE, sencondary structure elements.




**Supplementary Figure S3** Clinically identified mutations in SARS-CoV-2 spike protein. (**A**) Mutation frequency for SARS-CoV-2 spike protein worldwide. Blue dots indicate synonymous mutation and red dots refer to amino acid substitution. (**B**) The number of clinically detected V367F mutation in COVID-19 patients. (**C**) Percentage of COVID-19 cases carrying V367F mutation. Data were acquired on October 5, 2020 from http://giorgilab.dyndns.org/coronapp/.

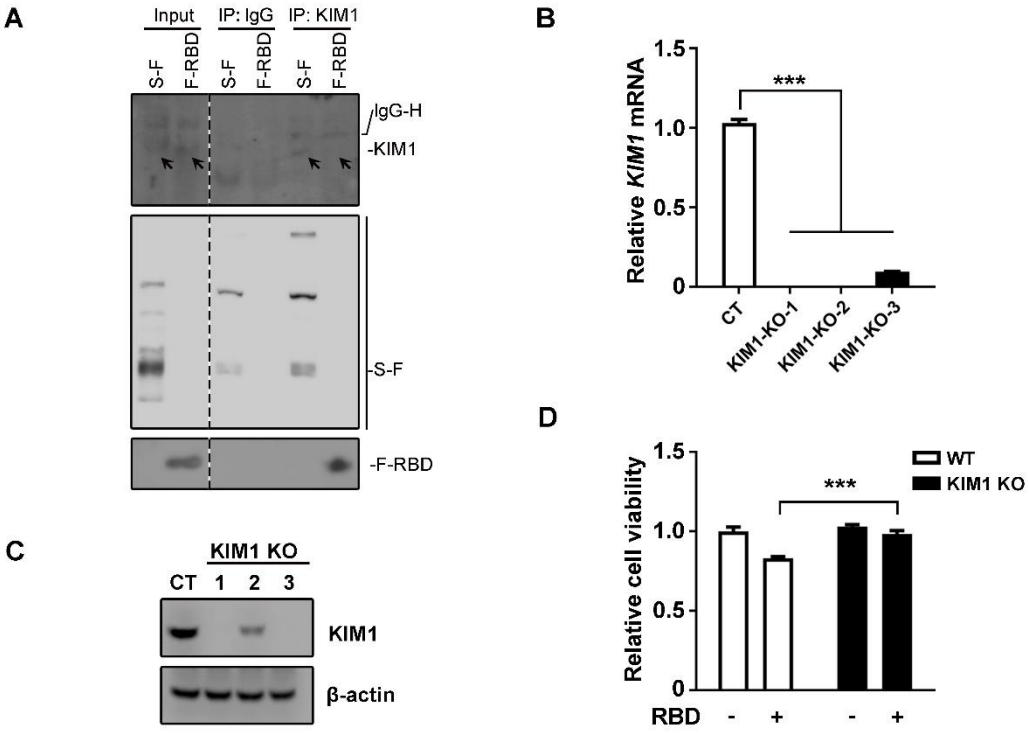



**Supplementary Figure S4** Molecular dynamics simulations information of SARS-CoV-RBD and KIM1. (**A**) *KIM1* mRNA expression in 10 SARS patients-derived peripheral blood mononuclear cells and 4 control samples (GSE1739). (**B**) Basic protein information of SARS-CoV-RBD and KIM1 Ig V domain. Red cylinder represents α-Helix structure of, blue arrow indicates β-Sheet structure. (**C**) RMSD values of the SARS-CoV-RBD and KIM1 Ig V domain binding models. (**D**) RMSF values of SARS-CoV-RBD and KIM1 Ig V domain. (**E**) Residue SSE distribution by residue index throughout the protein structure. Red column suggests α-Helix structure and blue columm indicates β-strand structure. SSE, secondary structure elements.




| SARS-CoV | KIM1 Ig V |
|---|---|
| Phe360 | Leu54 |
| Trp423 | Phe55 |
| Asn424 | Gln58 |
| | Trp112 |
| | Phe113 |

**Supplementary Figure S5** Binding model of SARS-CoV-RBD and KIM1. (**A**) SSE composition for trajectory frames in the dynamics simulation process of SARS-CoV-RBD and KIM1 Ig V domain, the curve indicates SSE composition for each trajectory frame over the course of the simulation. (**B**) SSE assignment of the residues over the simulation time, red dots indicate α-Helix structures and blue dots indicate β-strand structures. (**C**) Low-energy binding conformations of SARS-CoV-RBD binds to KIM1 Ig V domain. SSE, secondary structure elements.




**Supplementary Figure S6** Identification of KIM1 knockout HK-2 cell line and the protective effects of antagonist peptide on SARS-CoV-2-RBD induced cytotoxicity. (**A**) The interaction between endogenous KIM1 and overexpressed Flag-tagged Spike/RBD proteins in HK-2 cells. Mammalian expression plasmids encoding Flag-tagged Spike/RBD were transfected to HK-2 cells ($1 \times 10^7$). 36 hours later, cells were lysed and subjected to co-immunoprecipitation and followed by immunoblotting with indicated antibodies. IgG-H indicates the heavy chain of IgG. (**B**) *KIM1* mRNA expression in WT and KIM1 knockout HK-2 cell line. (**C**) KIM1 protein level in WT and KIM1 knockout HK-2 cell line. (**D**) Cytotoxicity of SARS-CoV-2-RBD in WT and KIM1 knockout HK-2 cell line. $^{***}P < 0.001$.