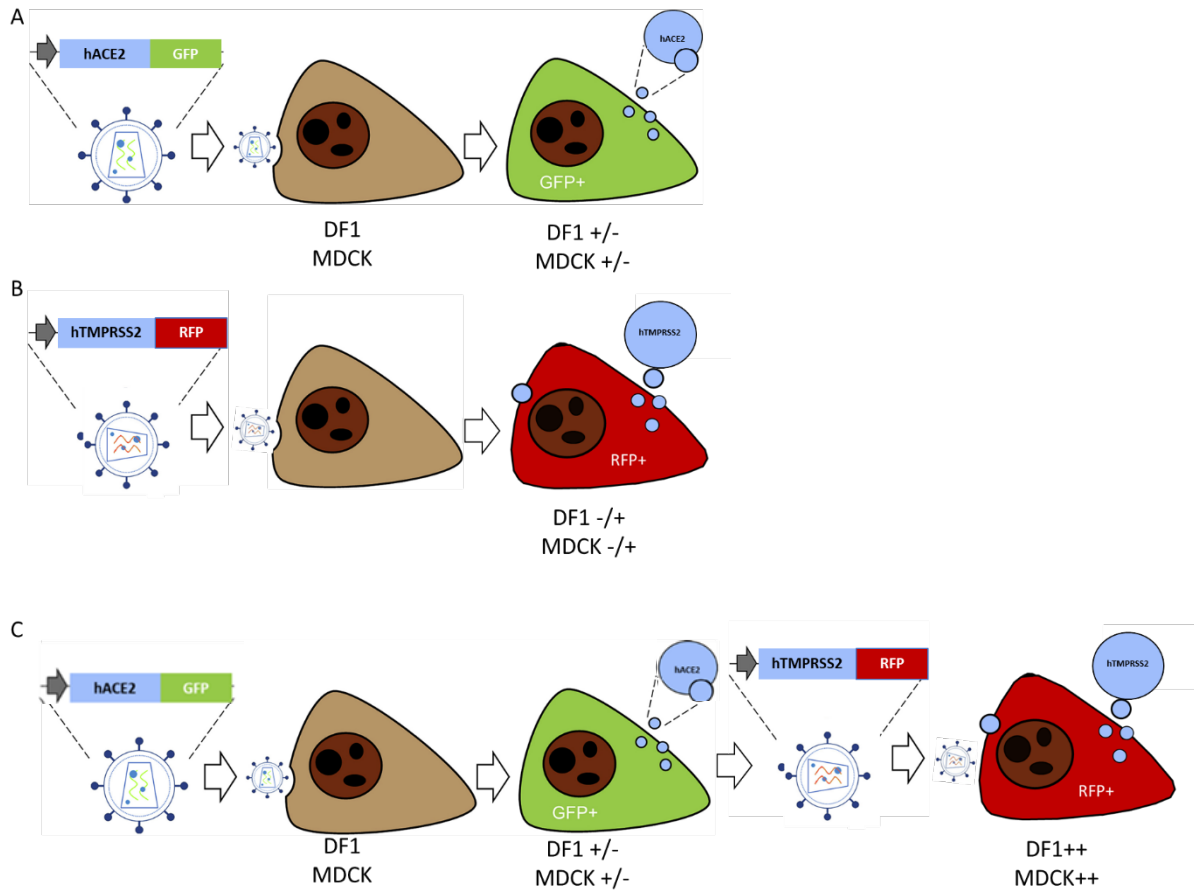
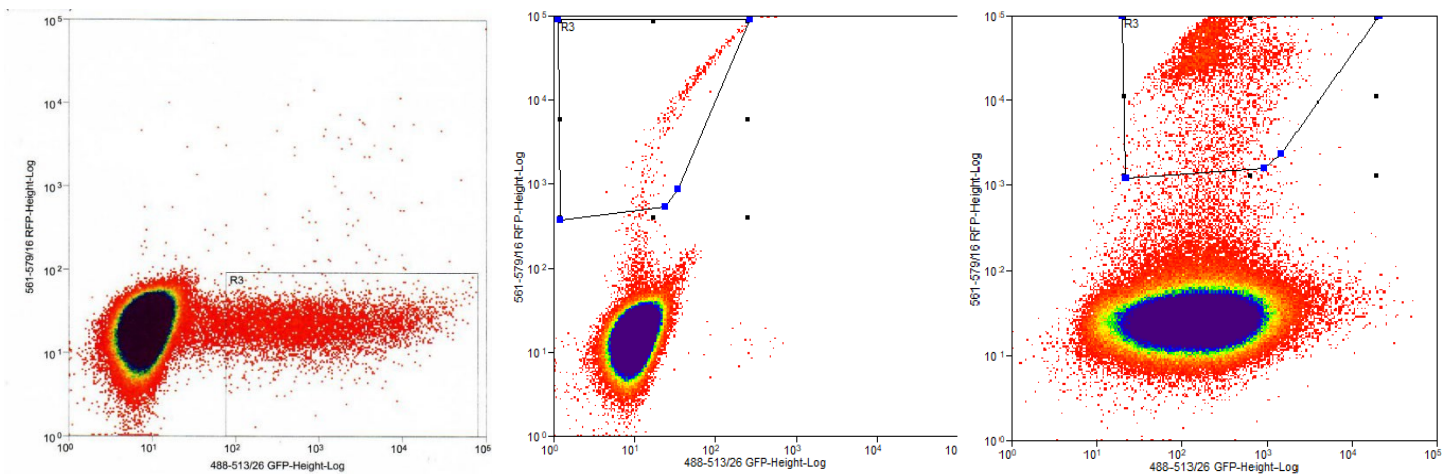


Supplemental Figure 1. Schematic representation of approach to generate transgenic cell lines expressing human or animal ACE2 (A) or TMPRSS2 (B), or both (C).



Supplemental Figure 2. FACS purification scheme for transgenic cells expressing ACE2 only (+/-) with GFP gating, TMPRSS2 only (-/+) with RFP gating, or both genes (+/+). Shown are results of purifications of DF1 cells expressing human ACE2 and TMPRSS2 genes. Similar results were obtained with all cell lines constructed.



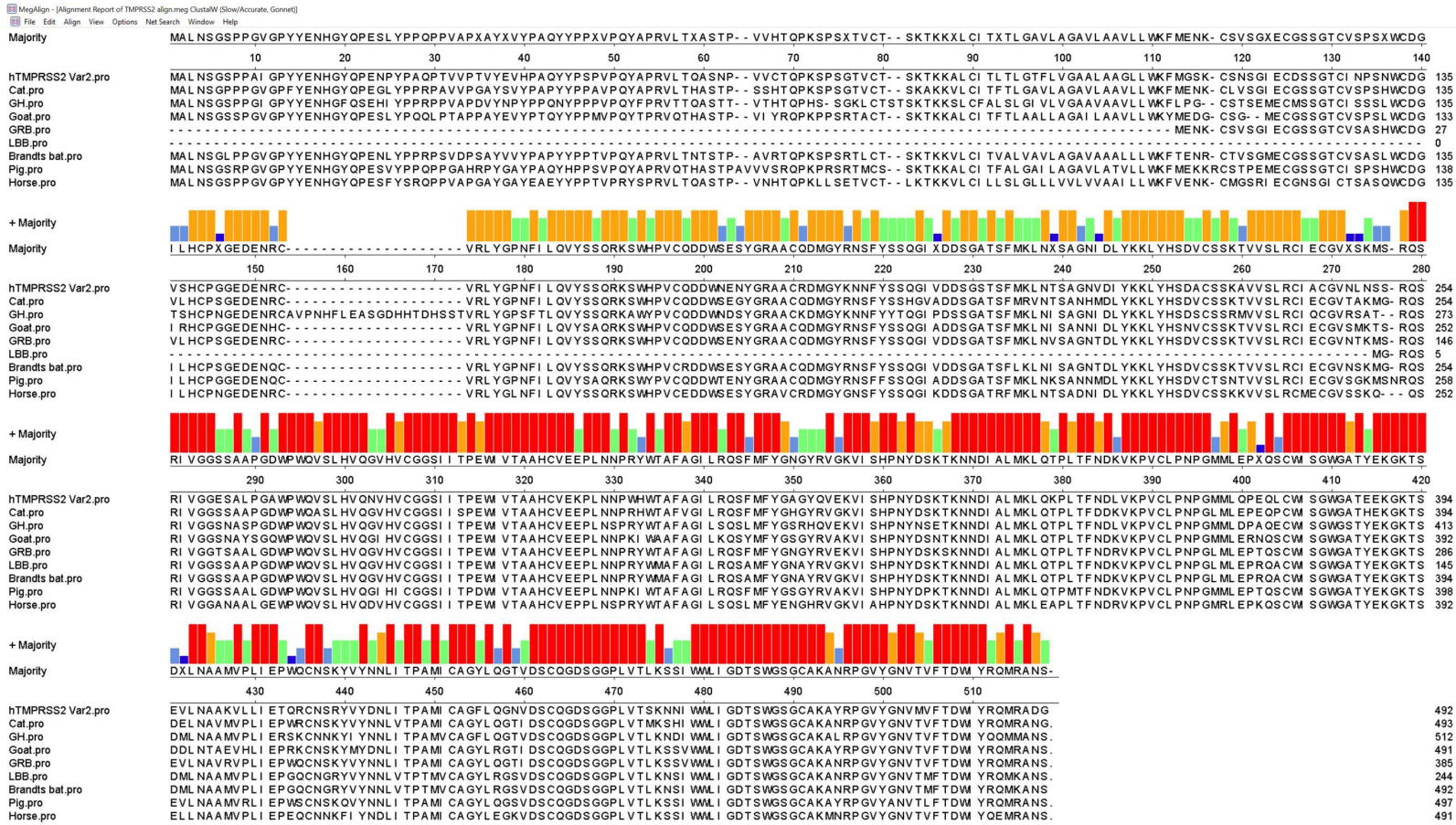
ACE2 + / TMPRSS2 -

ACE2 - / TMPRSS2 +

ACE2 +/- / TMPRSS2 +

Supplemental Figure 3. Amino acid (A) and architectural comparison (B) of TMPRSS2 between human and bat species demonstrating differences at N-terminal section.

A.



B.

| Species   | Gene  | Architecture | aa  |
|---|---|--------------|-----|
| <input type="checkbox"/> <i>Homo sapiens</i><br>human                       | TMPRSS2<br>transmembrane<br>serine protease 2 |              | 492 |
| <input type="checkbox"/> <i>Hipposideros armiger</i><br>great roundleaf bat | TMPRSS2<br>transmembrane<br>serine protease 2 |              | 384 |
| <input type="checkbox"/> <i>Myotis lucifugus</i><br>little brown bat        | TMPRSS2<br>transmembrane<br>serine protease 2 |              | 243 |

Supplemental Table 1. List of genes and accession numbers used in this study.

| <u>Species:</u>     | <u>ACE2</u>    | <u>TMPRSS2</u> |
|---------------------|----------------|----------------|
| Human               | NM_021804.1    | NM_005656.4    |
| Cat                 | NM_001039456.1 | XM_023238709.1 |
| Goat                | NM_001290107.1 | XM_005675629.3 |
| Golden Hamster      | XM_005074209.3 | XM_013116227.3 |
| Great Roundleaf Bat | XM_019667391.1 | XM_019626040.1 |
| Horse               | XM_001490191.5 | XM_005606160.3 |
| Little Brown Bat    | XM_023753670.1 | XM_006104440.3 |
| Pig                 | NM_001123070.1 | XM_021071009.1 |

## 867 Supplemental Table 2. List of primers used in this study

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| <u>Primers used in this study:</u> | <u>Forward</u>            | <u>Reverse</u>                 |
|------------------------------------|---------------------------|--------------------------------|
| ACE2 Universal                     | CTCTTTCTGGCTCCTTCTCAGCTT  | TCCAAGAGCTGATTTTAGGCTTATCC     |
| Human ACE2                         | CTAGCTGTCAAGCTCTTCCTGGCT  | GGATCCTAAAAGGAGGTCTGAACATCATCA |
| Chicken ACE2                       | ACGCTAGCCGCTTCTCACTAGC    | AGCCAATGGATCTGCCAGAA           |
| TMPRSS2 Universal                  | ATGGCTTTGAACTCAGGGTC      | CTGTTTGCCCTCATTGTGCGATA        |
| Human TMPRSS2                      | GGAAAACCCCTATCCCGCAC      | GAATCGACGTTCCCCTGCAG           |
| Chicken TMPRSS2                    | TGTTACCAGAGGACCTCCGC      | TCTGCCAGGCCACAAGTAGG           |
| Bat TMPRSS2                        | CAGGGATTTTGAGACAATCTTTCAT | CAAAGTGACCAGAGGACCG            |

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