TMPRSS13 zymogen activation, surface localization, and shedding is regulated by proteolytic cleavage within the non-catalytic stem region

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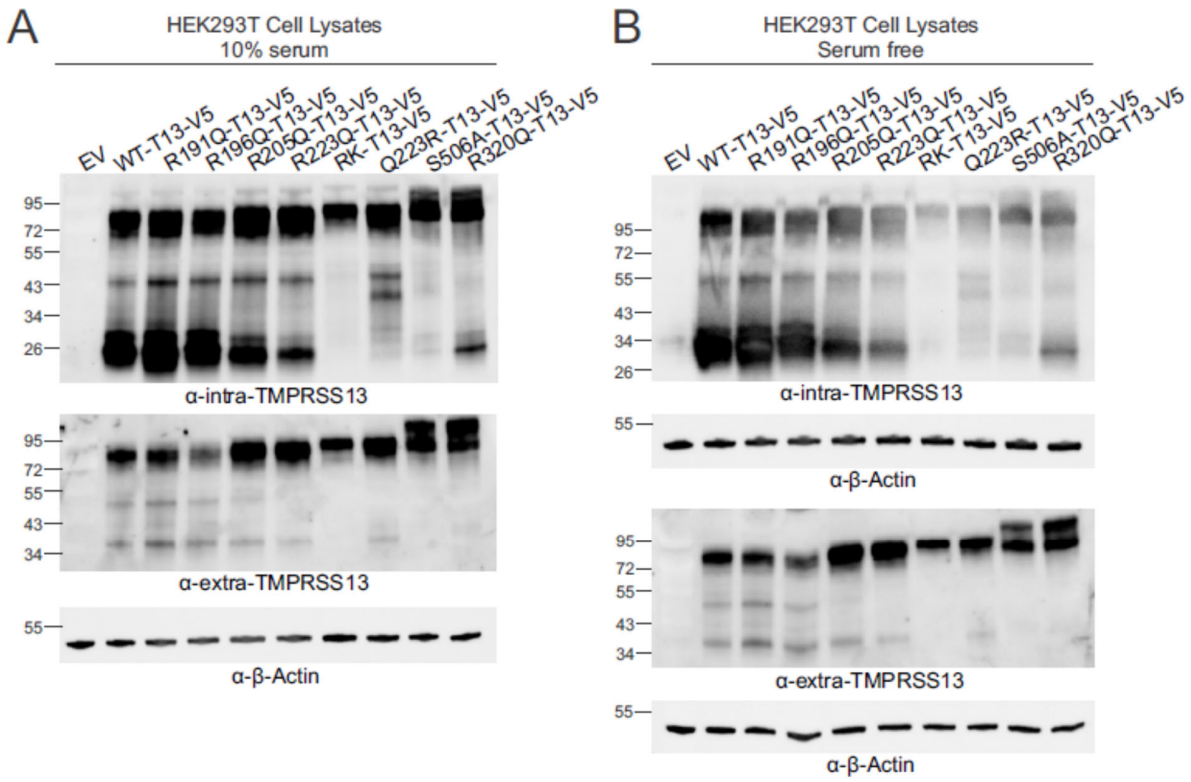
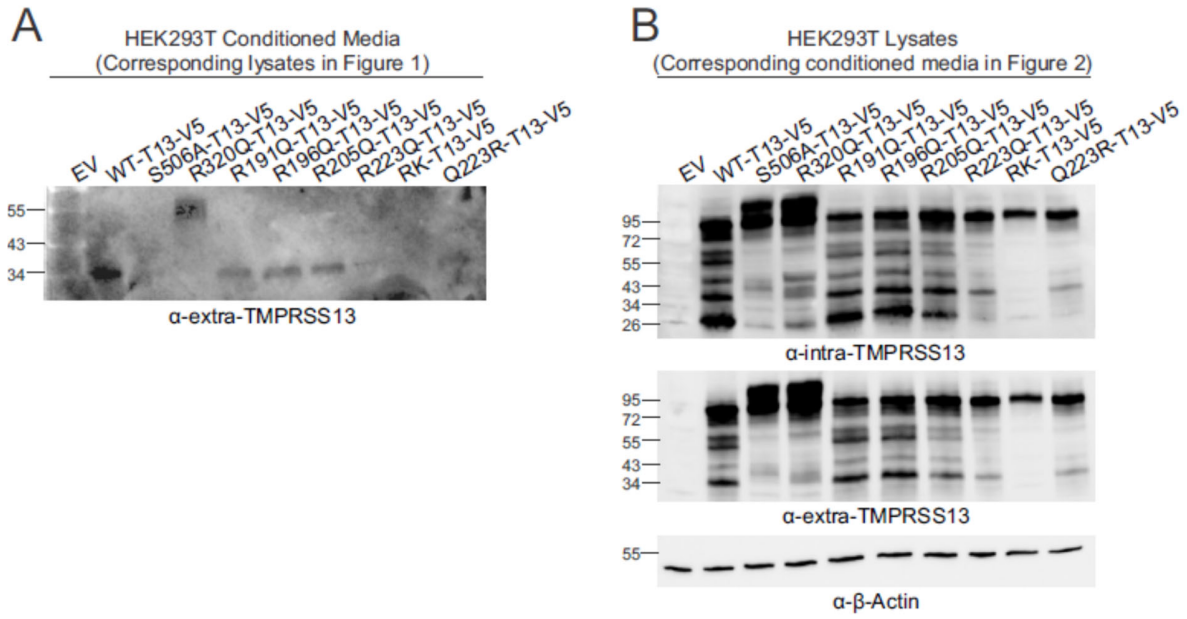
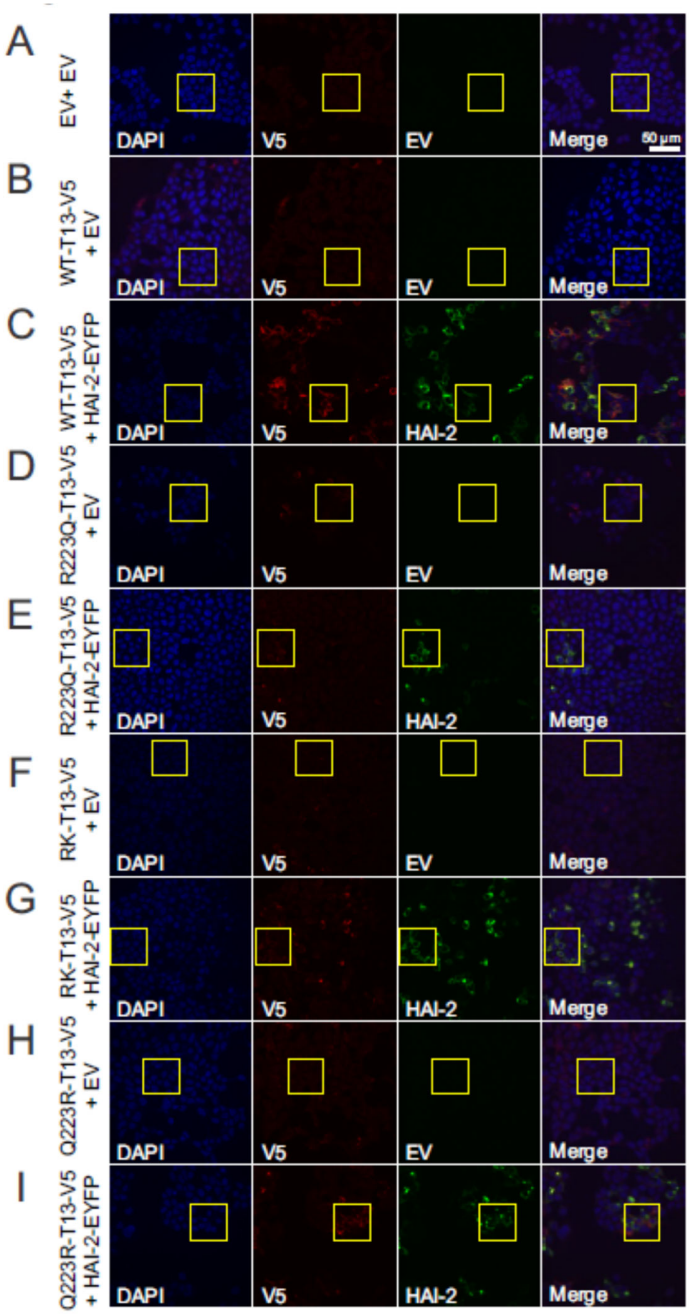
Supplementary material



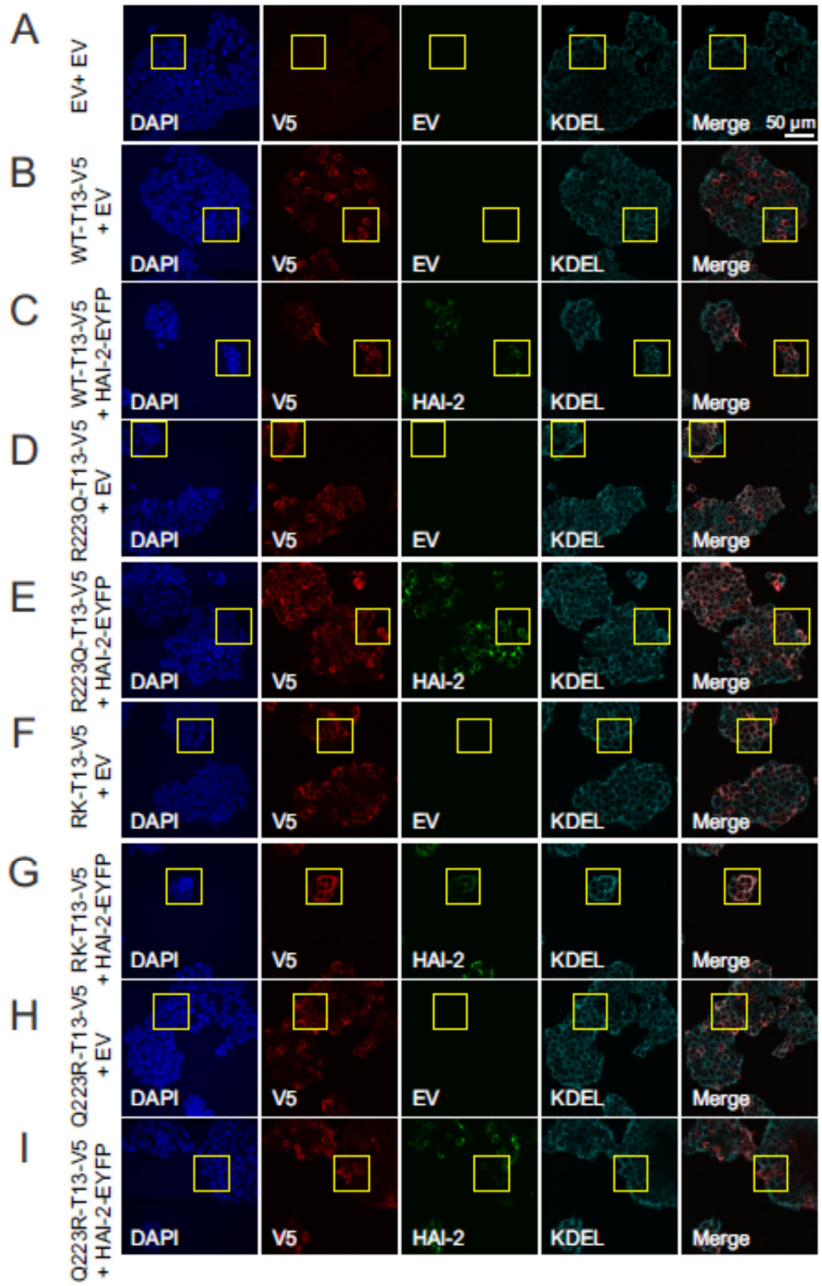
Supplementary Figure S1. HEK293T cells were transfected with empty vector (EV), WT-TMPRSS13 (T13)-V5, S506A-T13-V5, R320Q-T13-V5, R191Q-T13-V5, R196Q-T13-V5, R205Q-T13-V5, R223Q-T13-V5, RK-T13-V5 and Q223R-T13-V5. Transfected cells were either (A) grown in media containing 10% serum for 48 hours or (B) washed and serum-starved 24 hours post-transfection and collected the following day. Lysates were harvested and proteins were separated by SDS-PAGE under reducing conditions on 10% gels and analyzed by western blotting. Proteins were detected using anti-intra-TMPRSS13, anti-extra-TMPRSS13, or anti-beta-actin antibodies as indicated.



Supplementary Figure S2. HEK293T cells were transfected with empty vector (EV), WT-TMPRSS13 (T13)-V5, S506A-T13-V5, R320Q-T13-V5, R191Q-T13-V5, R196Q-T13-V5, R205Q-T13-V5, R223Q-T13-V5, RK-T13-V5 and Q223R-T13-V5. (A) Corresponding conditioned media samples shown in Figure 1 were harvested and proteins were separated by SDS-PAGE under reducing conditions on a 10% gel and analyzed by western blotting. Proteins were detected using anti-extra-TMPRSS13 antibody. (B) Corresponding cell lysate samples shown in Figure 2. Proteins in lysates were separated by SDS-PAGE under reducing conditions on a 4-15% gel and analyzed by western blotting. Proteins were detected using anti-intra-TMPRSS13, anti-extra-TMPRSS13, or anti-beta-actin antibodies.



Supplementary Figure S3. Representative uncropped images from Figure 5. HEK293T cells were plated onto glass coverslips and 24 hours after plating were transfected for 48 hours with (A) EV + EV, (B) WT-T13-V5 + EV, (C) WT-T13-V5 + HAI-2-EYFP, (D) R223Q-T13-V5 + EV, (E) R223Q-T13-V5 + HAI-2-EYFP, (F) RK-T13-V5 + EV, (G) RK-T13-V5 + HAI-2-EYFP, (H) Q223R-T13-V5 + EV, (I) Q223R-T13-V5 + HAI-2-EYFP. Cells were fixed onto coverslips and incubated with anti-V5 to detect TMPRSS13. Coverslips were mounted to slides with DAPI-containing mounting media to detect nuclei. Nuclei=blue, panels A-I. TMPRSS13-V5=red, panels A-I. HAI-2-EYFP=green, panels C, E, G, and I. Merged images are shown in panels on the right. Scale bar measures 50 µm. Yellow boxes indicate cropped areas shown in Figure 5.



Supplementary Figure S4. Representative uncropped images from Figure 6. HEK293T cells were plated onto glass coverslips and 24 hours after plating were transfected for 48 hours with (A) EV + EV, (B) WT-T13-V5 + EV, (C) WT-T13-V5 + HAI-2-EYFP, (D) R223Q-T13-V5 + EV, (E) R223Q-T13-V5 + HAI-2-EYFP, (F) RK-T13-V5 + EV, (G) RK-T13-V5 + HAI-2-EYFP, (H) Q223R-T13-V5 + EV, (I) Q223R-T13-V5 + HAI-2-EYFP. Cells were fixed onto coverslips, permeabilized, and incubated with anti-V5 to detect TMPRSS13 and anti-KDEL to detect the endoplasmic reticulum (ER). Coverslips were mounted to slides with DAPI-containing mounting media to detect nuclei. Nuclei=blue, panels A-I. TMPRSS13-V5=red, panels A-I. HAI-2-EYFP=green, panels C, E, G, and I. ER (KDEL)=cyan, panels A-I. Merged images of TMPRSS13/KDEL are shown in panels on the right. Scale bar measures 50 µm. Yellow boxes indicate cropped areas shown in Figure 6.