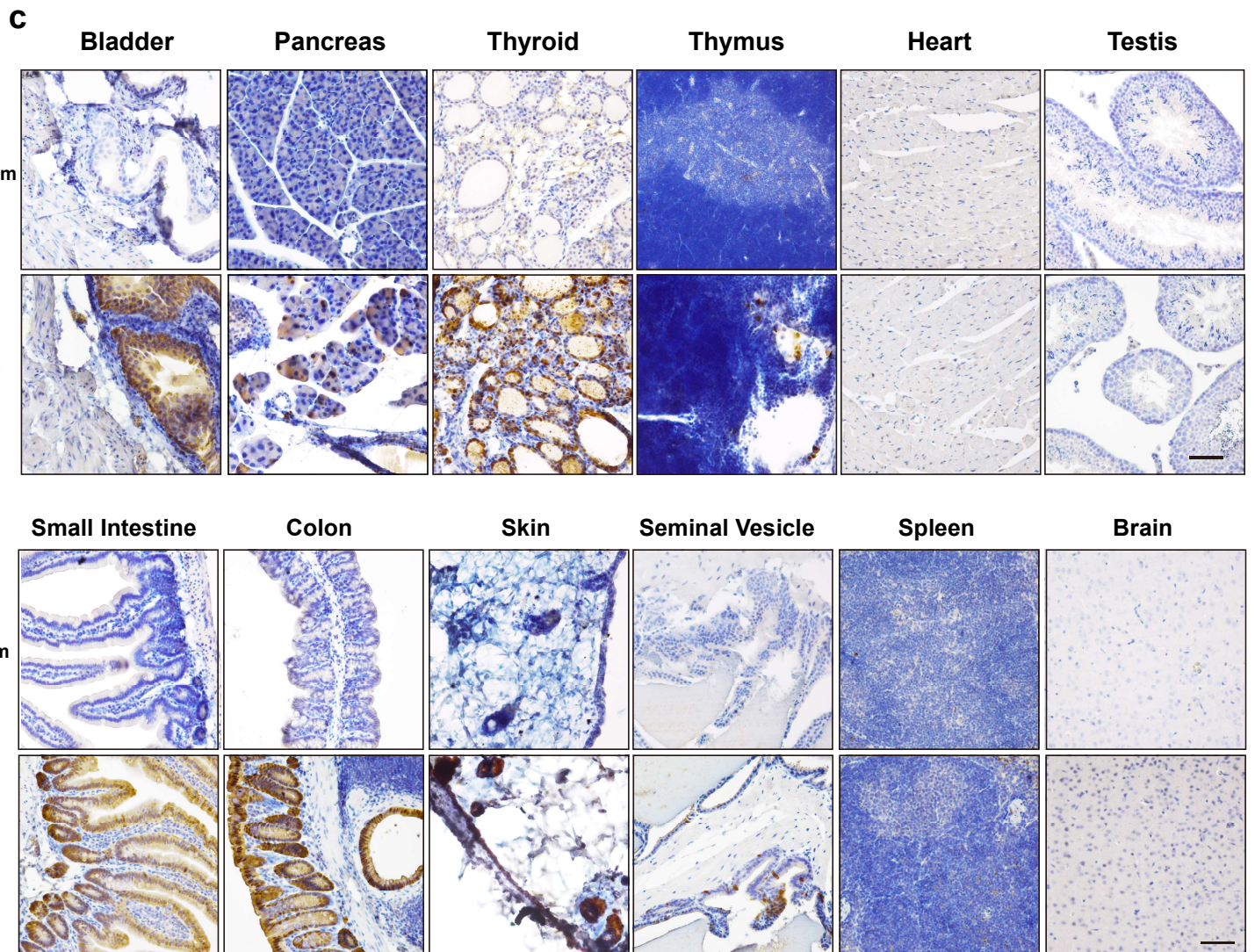
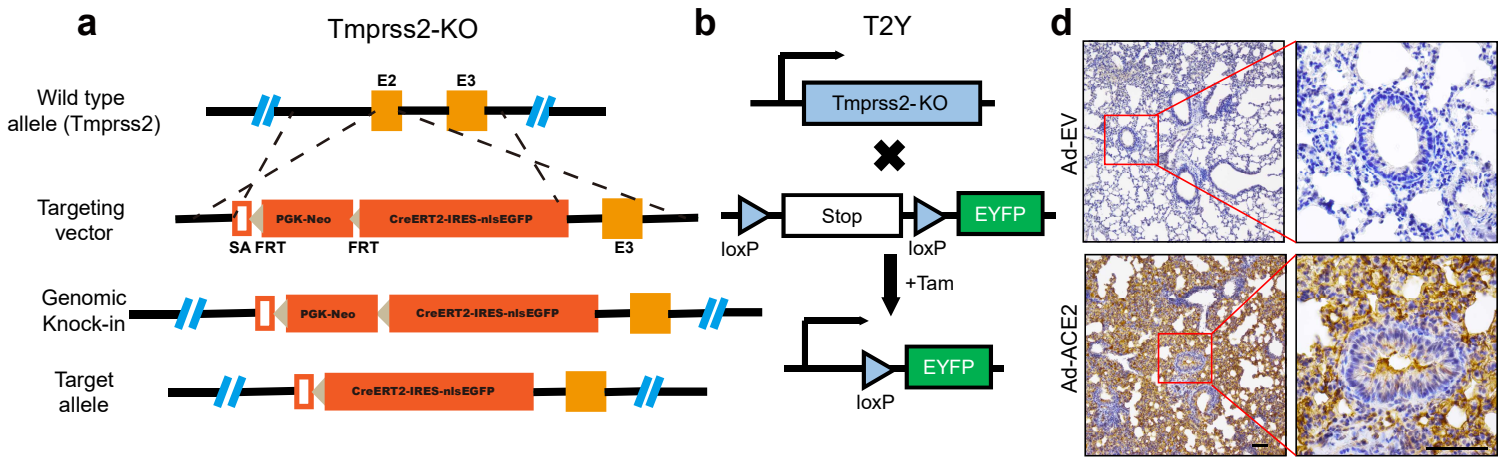


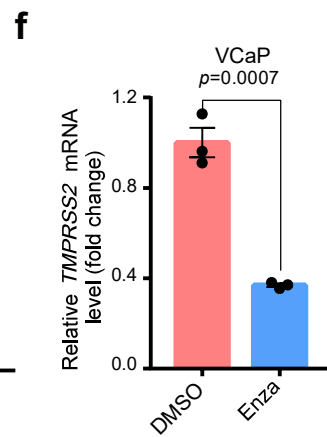
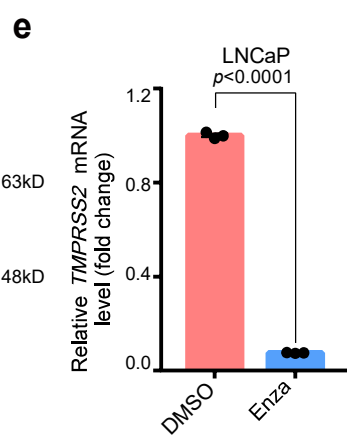
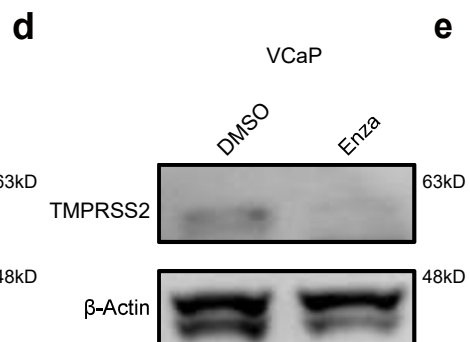
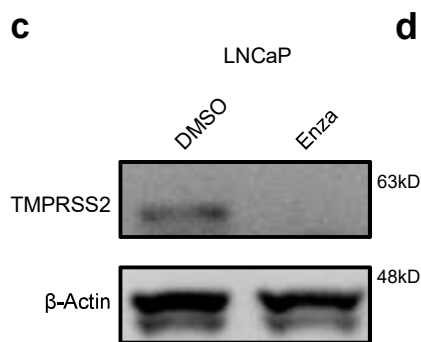
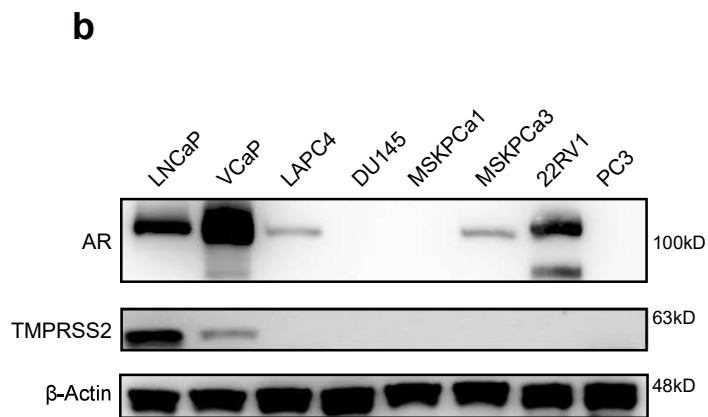
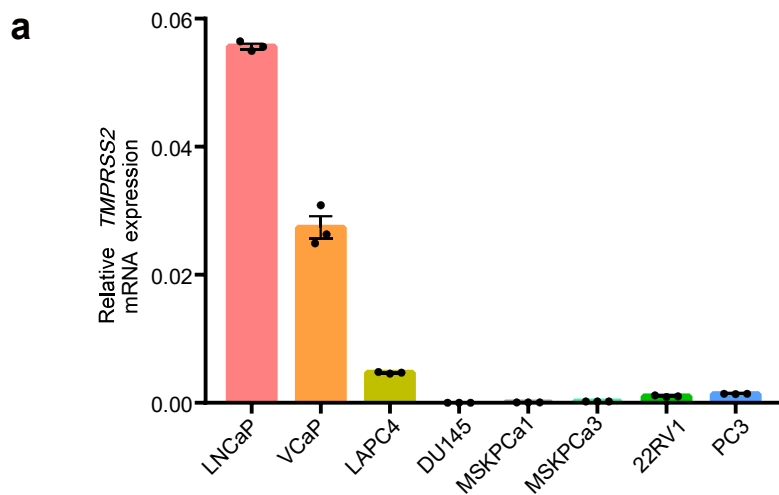
Supplementary Information

Distinct mechanisms for TMPRSS2 expression explain organ-specific inhibition of SARS-CoV-2 infection by enzalutamide

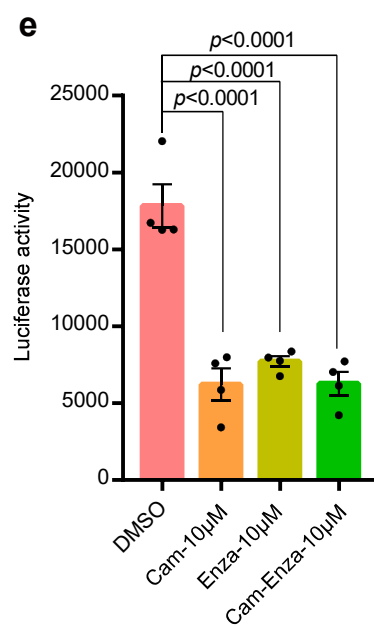
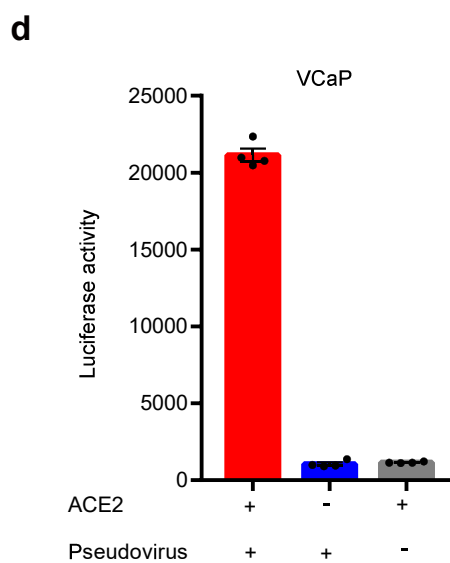
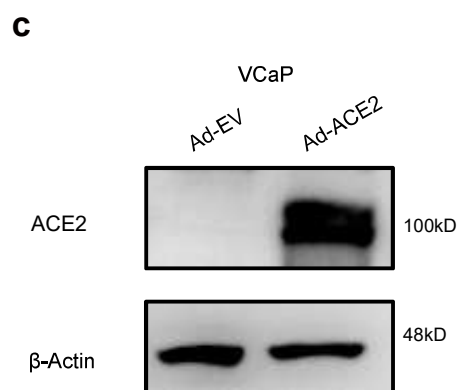
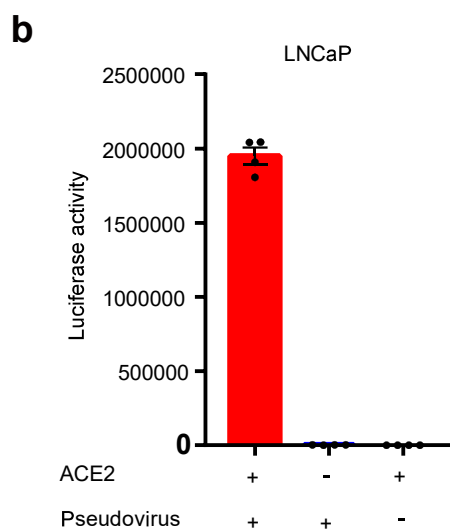
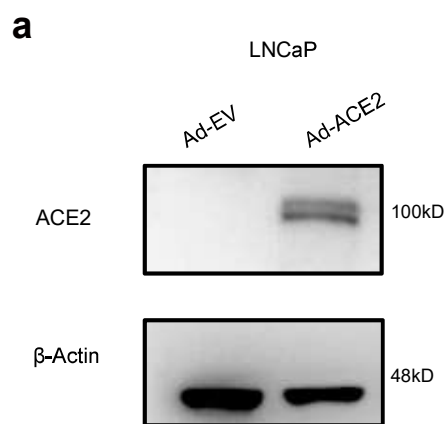
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Supplementary Figure 1 Identification of Tmprss2-positive cells in multiple mouse organs. (a) Construction strategy for Tmprss2-KO mice. **(b)** Breeding strategy for the generation of T2Y mice. **(c)** YFP IHC staining for multiple organs of T2Y mice with (bottom) or without (top) tamoxifen gavage. **(d)** FLAG IHC staining in the lungs of WT transduced with Ad-EV (top) or Ad-ACE2 transduction (bottom), samples were collected 5 days post transduction. Scale bars represent 50 μ m.

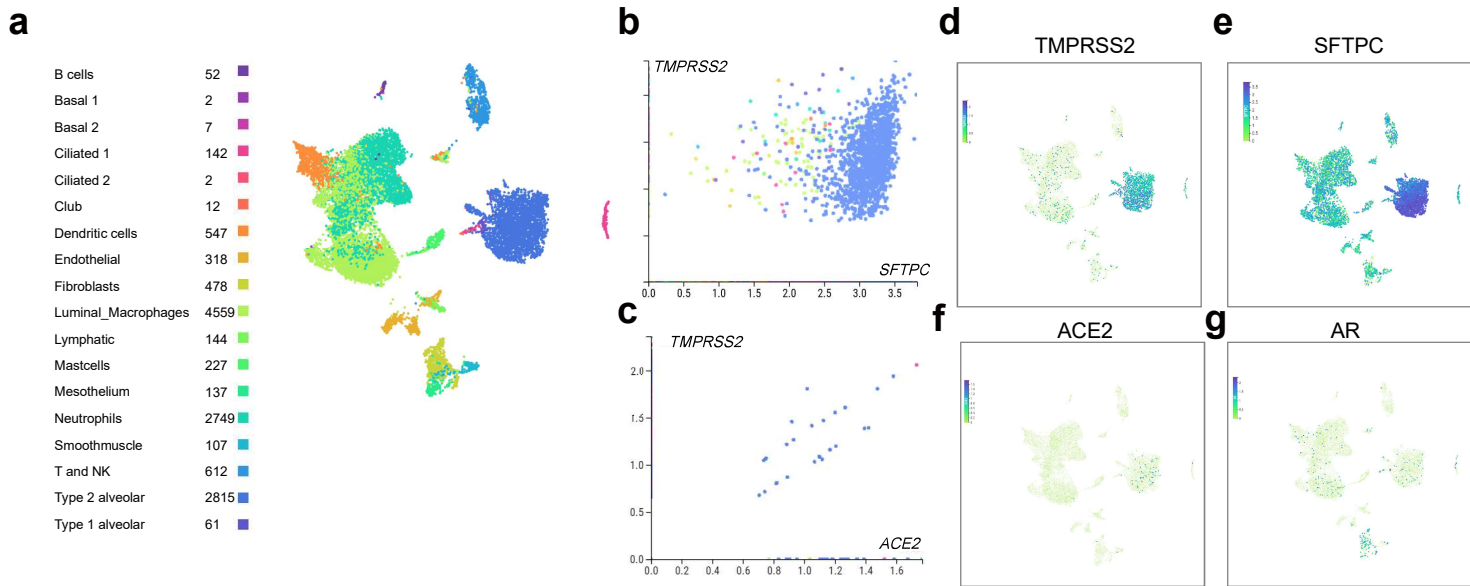


Supplementary Figure 2 TMPRSS2 expression is reduced by enzalutamide in LNCaP and VCaP cells. **(a)** qRT-PCR analysis of *TMPRSS2* mRNA expression in multiple prostate cancer cell lines (LNCaP, VCaP, LAPC4, DU145, 22RV1 and PC3) and two prostate cancer organoid lines (MSKPCa1 and MSKPCa3) (mean $\pm$ SEM, n=3 biologically independent samples). **(b)** Western blotting analysis of TMPRSS2 and AR expression in multiple prostate cancer cell lines and two prostate cancer organoid lines. Source data are provided as a Source Data file. **(c)** Western blotting analysis of TMPRSS2 expression in LNCaP cells treated with DMSO under normal FBS condition or enzalutamide treatment under charcoal stripped FBS condition. Source data are provided as a Source Data file. **(d)** Western blotting analysis of TMPRSS2 expression in VCaP cells treated with DMSO under normal FBS condition or enzalutamide treatment under charcoal stripped FBS condition. Source data are provided as a Source Data file. **(e)** qRT-PCR analysis of *TMPRSS2* mRNA expression in LNCaP cells treated with DMSO under normal FBS condition or enzalutamide treatment under charcoal stripped FBS condition (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent experiments). **(f)** qRT-PCR analysis of *TMPRSS2* mRNA expression in VCaP cells treated with DMSO under normal FBS condition or enzalutamide treatment under charcoal stripped FBS condition (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent experiments).

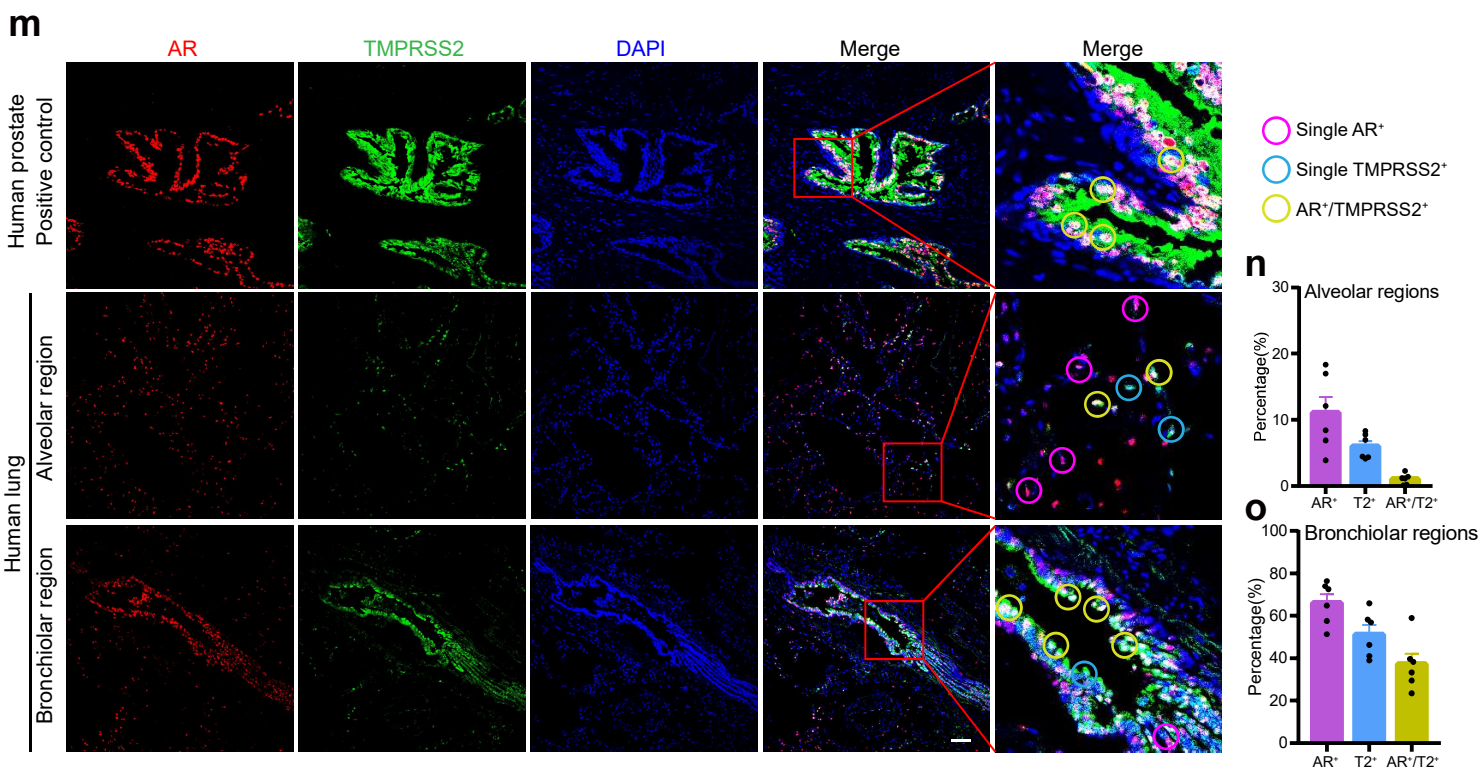
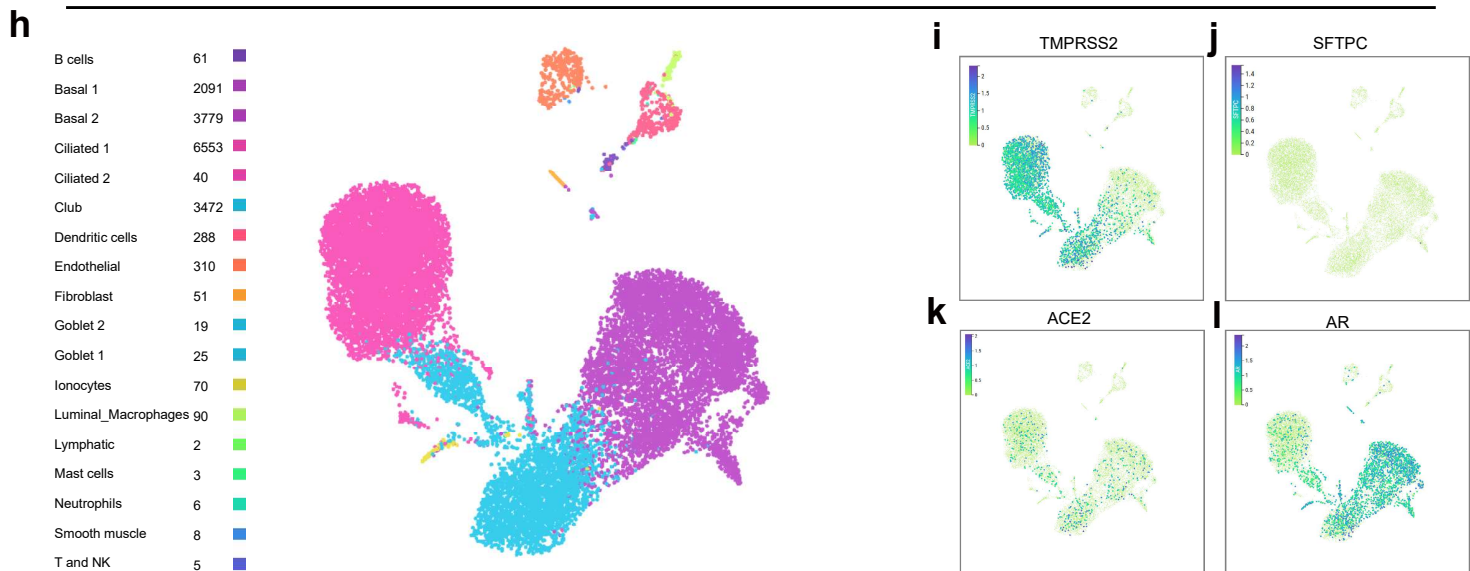


Supplementary Figure 3 Enzalutamide significantly reduces infection of VCaP cell with SARS-CoV-2-S. **(a)** Western blotting analysis of ACE2 and β -Actin expression in LNCaP cells with empty vector adenovirus transduction or ACE2 adenovirus transduction. Source data are provided as a Source Data file. **(b)** SARS-CoV-2-S-driven entry into LNCaP cells with or without Ad-ACE2 transduction. Luciferase activity was measured 48 hours post SARS-CoV-2-S infection (mean $\pm$ SEM, n=4 biologically independent samples). **(c)** Western blotting analysis of ACE2 and β -Actin expression in VCaP cells with empty vector adenovirus transduction or ACE2 adenovirus transduction. Source data are provided as a Source Data file. **(d)** SARS-CoV-2-S-driven entry into VCaP cells with or without Ad-ACE2 transduction, luciferase activity was measured 48 hours post SARS-CoV-2-S infection (mean $\pm$ SEM, n=4 biologically independent samples). **(e)** SARS-CoV-2-S-driven entry into VCaP cells with Ad-ACE2 transduction treated with DMSO, 10 μ M camostat mesylate, 10 μ M enzalutamide and 10 μ M camostat mesylate/enzalutamide. Luciferase activity was measured 48 hours post SARS-CoV-2-S infection (one-way ANOVA and Tukey's test, mean $\pm$ SEM, n=4 biologically independent samples).

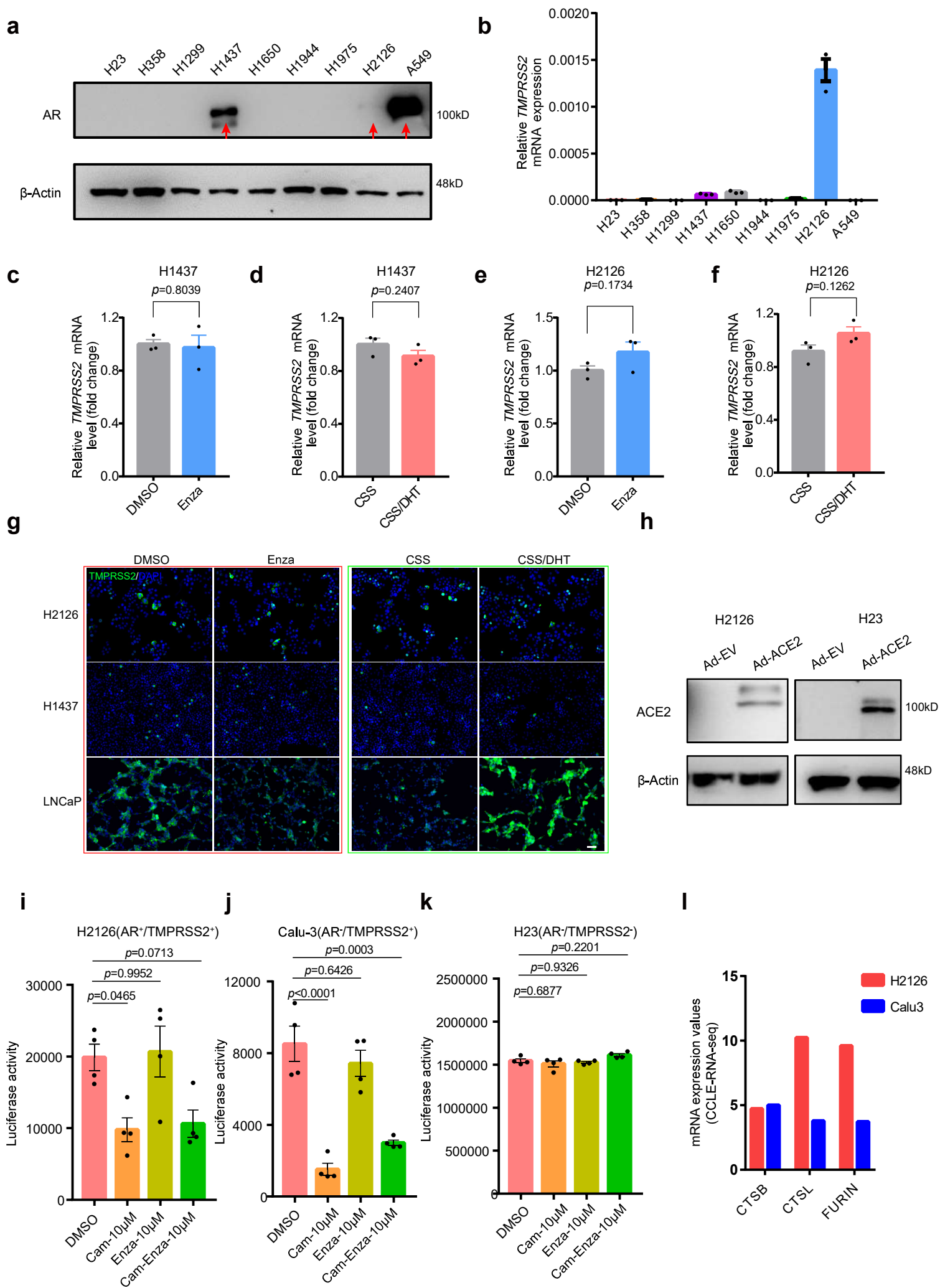
Single cell RNA-sequencing for cells in alveolar regions



Single cell RNA-sequencing for cells in bronchiolar regions

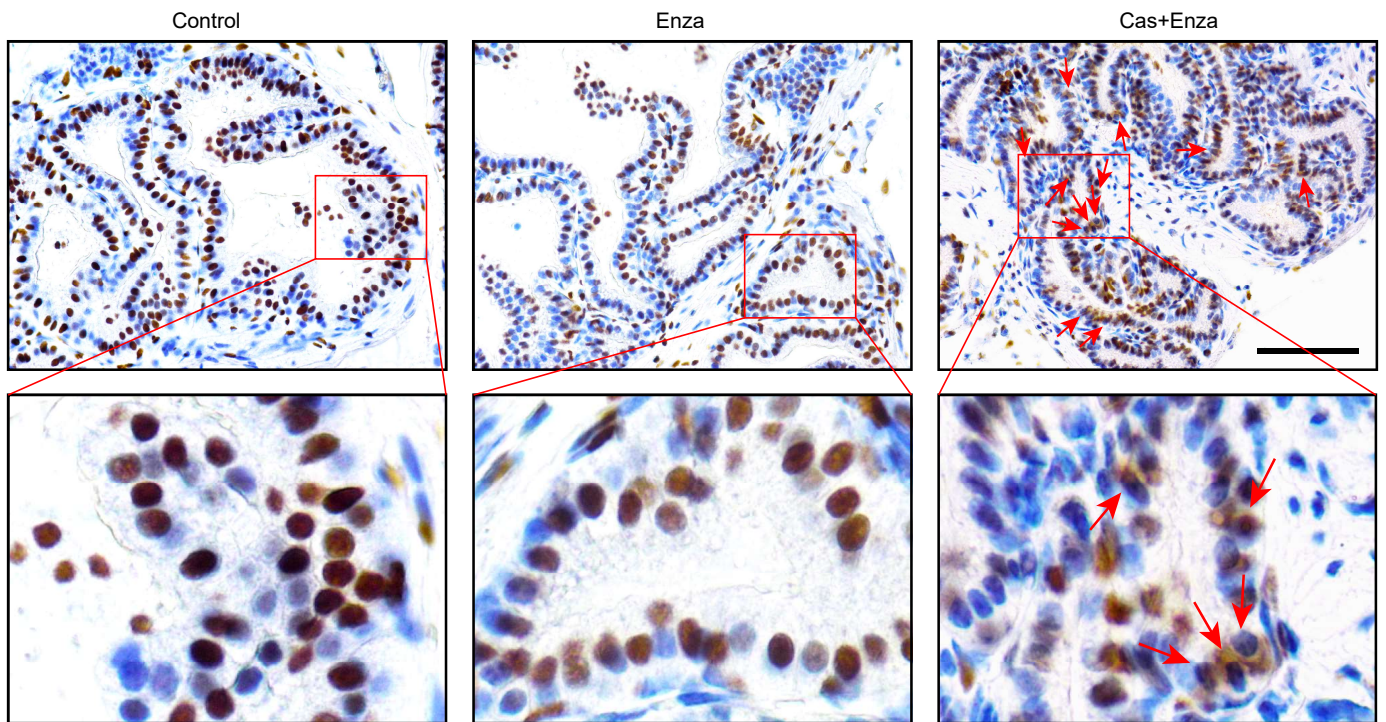
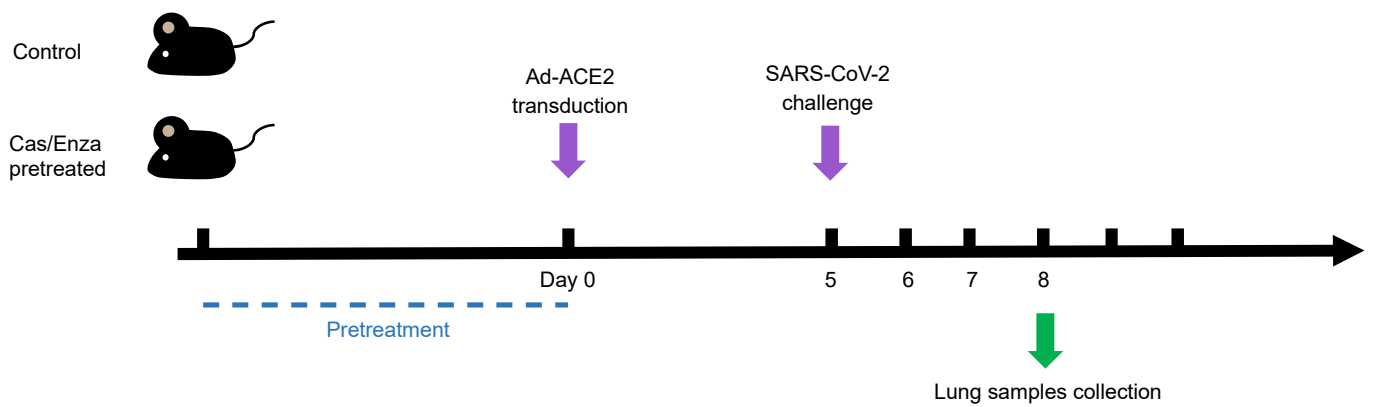


Supplementary Figure 4 AR/TMPRSS2 double positive cells are verified in human lungs. (a) UMAP plot displaying cell clusters of cells from human lung alveolar regions in a public dataset using COVID-19 Cell Atlas. **(b)** *TMPRSS2* and *SFTPC* mRNA expression across multiple cell types. **(c)** *TMPRSS2* and *ACE2* mRNA expression across multiple cell types. **(d-g)** *TMPRSS2* **(d)**, *SFTPC* **(e)**, *ACE2* **(f)** and *AR* **(g)** mRNA expression levels on UMAP plots across multiple cell types in **(a)**. **(h)** UMAP plot displaying cell clusters of cells from human lung bronchiolar regions in a public dataset using COVID-19 Cell Atlas. **(i-l)** *TMPRSS2* **(i)**, *SFTPC* **(j)**, *ACE2* **(k)** and *AR* **(l)** mRNA expression levels on UMAP plots across multiple cell types in **(h)**. **(m)** Immunofluorescence staining of AR and TMPRSS2 in human prostates (top) and lungs (middle and bottom). **(n)** Quantification for AR-positive, TMPRSS2-positive and AR/TMPRSS2 double positive cells in human lung alveolar regions (mean $\pm$ SEM, n=6 biologically independent samples). **(o)** Quantification for AR-positive, TMPRSS2-positive and AR/TMPRSS2 double positive cells in human lung bronchiolar regions (mean $\pm$ SEM, n=6 biologically independent samples). Scale bars represent 50 μ m.

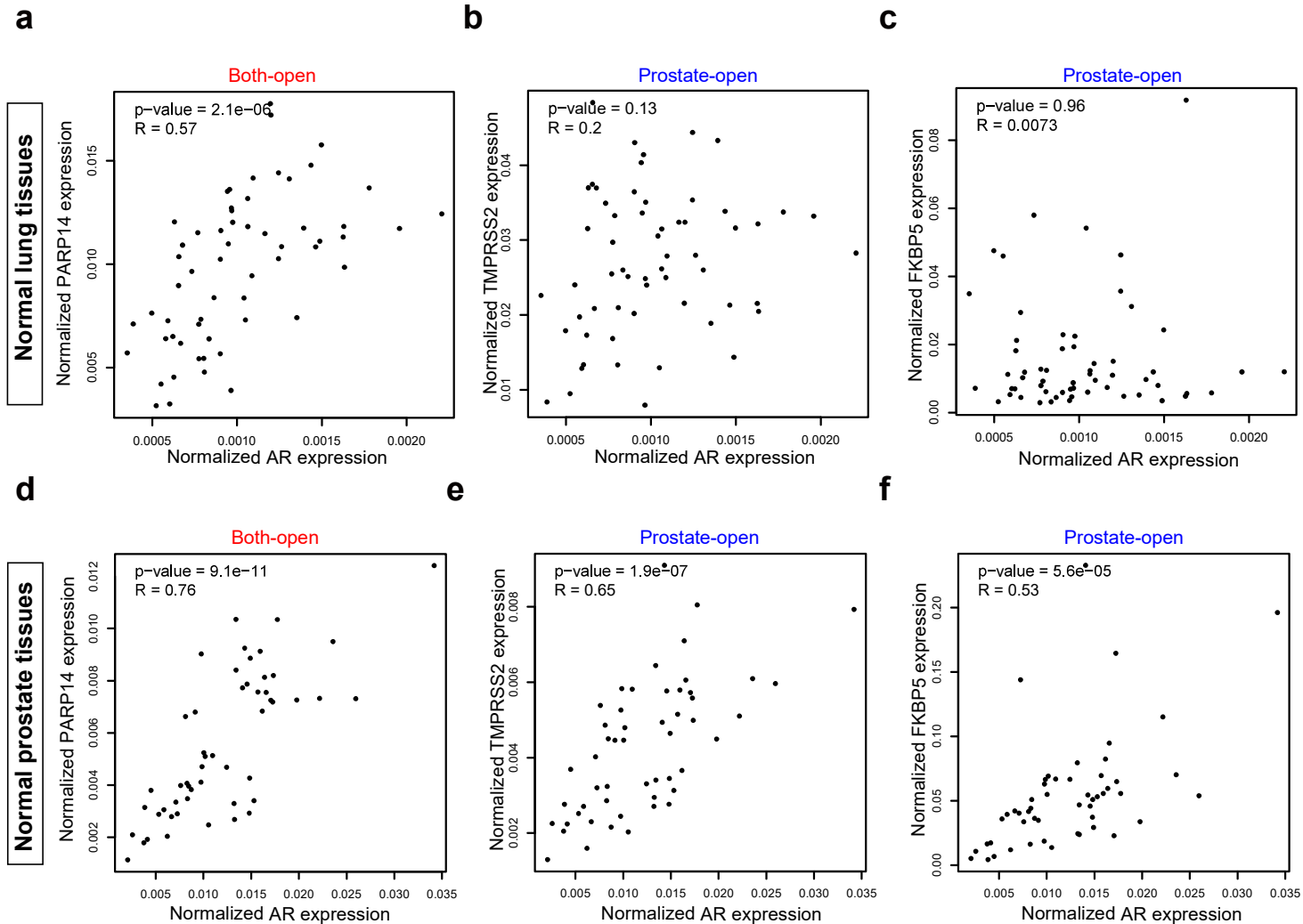


Supplementary Figure 5 Enzalutamide does not prevent SARS-CoV-2-S-driven entry into lung cells.

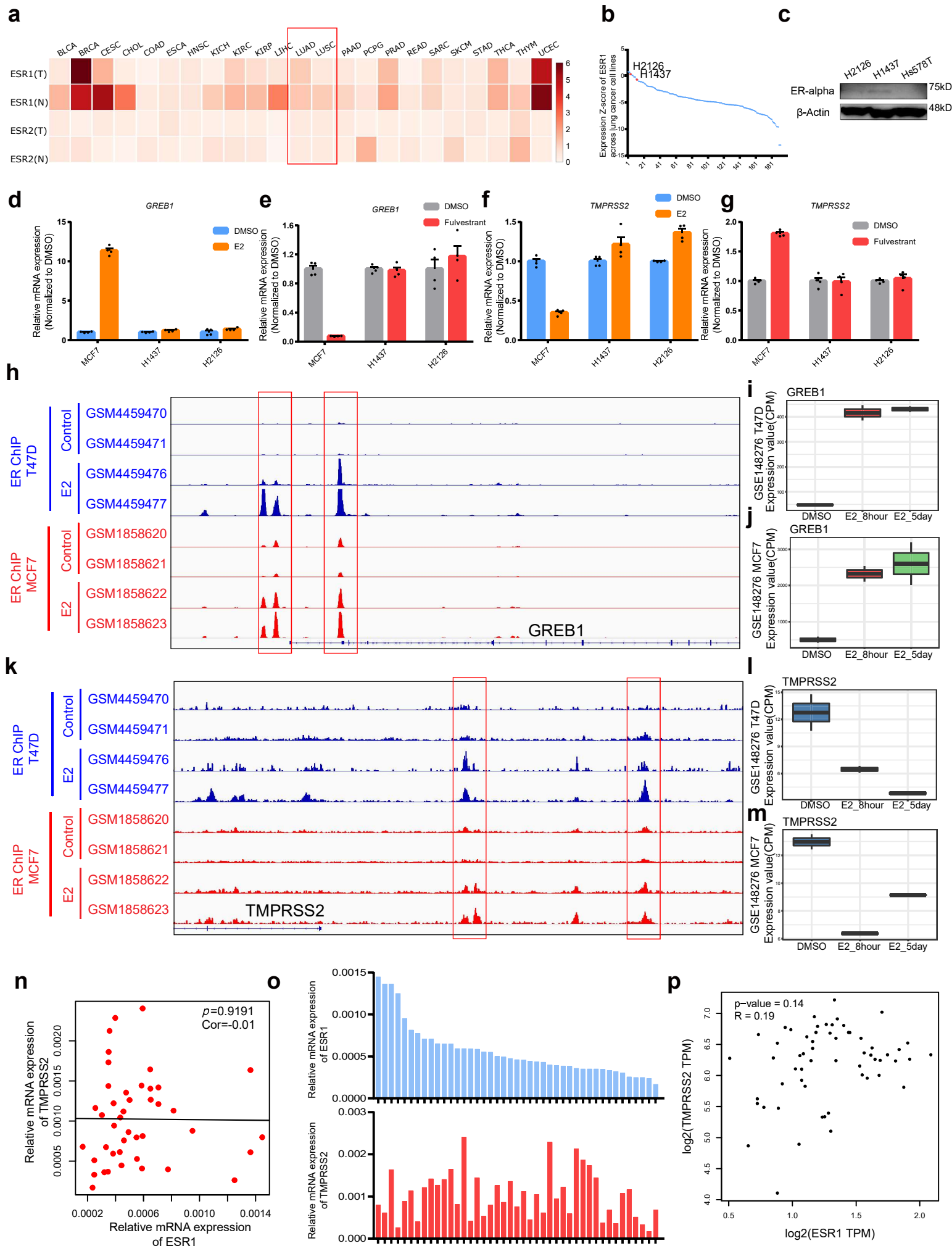
(a) Western blotting analysis of AR expression in multiple lung cancer cells. Source data are provided as a Source Data file. **(b)** qRT-PCR analysis of *TMPRSS2* mRNA expression in multiple lung cancer cells (mean $\pm$ SEM, n=3 biologically independent samples). **(c)** qRT-PCR analysis of *TMPRSS2* mRNA expression in H1437 cells with vehicle or enzalutamide treatment (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent samples). **(d)** qRT-PCR analysis of *TMPRSS2* mRNA expression in H1437 cells with or without DHT treatment under charcoal stripped FBS condition (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent samples). **(e)** qRT-PCR analysis of *TMPRSS2* mRNA expression in H2126 cells with vehicle or enzalutamide treatment (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent samples). **(f)** qRT-PCR analysis of *TMPRSS2* mRNA expression in H2126 cells with or without DHT treatment under charcoal stripped FBS condition (two-tailed t-test, mean $\pm$ SEM, n=3 biologically independent samples). **(g)** Immunofluorescence staining of TMPRSS2 in H2126 (top), H1437 (middle) and LNCaP (bottom) cells under multiple treatments condition. **(h)** Western blotting analysis of ACE2 and β -Actin expression in H2126 (left) and H23 (right) cells with empty vector adenovirus transduction or ACE2 adenovirus transduction respectively. Source data are provided as a Source Data file. **(i-k)** SARS-CoV-2-S-driven entry into H2126 **(i)**, Calu-3 **(j)** and H23 **(k)** cells transduced with Ad-ACE2 and treated with DMSO, 10 μ M camostat mesylate, 10 μ M enzalutamide and 10 μ M camostat mesylate/enzalutamide. Luciferase activity was measured 48 hours post SARS-CoV-2-S infection (one-way ANOVA and Tukey's test, mean $\pm$ SEM, n=4 biologically independent samples). **(l)** CCLE mRNA expression levels of *CTSB*, *CTSL* and *FURIN* in H2126 and Calu3 cells. Scale bars represent 50 μ m.

a**b**

Supplementary Figure 6 Ad-ACE2-transduced mouse models are employed to assess the efficacy of enzalutamide *in vivo*. (a) IHC staining for AR in the prostates of wild type mice, enzalutamide-treated mice and enzalutamide-treated castrated mice respectively. Red arrows indicate blocking of nuclear translocation of AR in the prostates of enzalutamide treated castrated mice. (b) Schematic strategy for Ad-ACE2-transduced mouse to evaluate the therapeutic efficacy of enzalutamide. Scale bars represent 50 μ m.



Supplementary Figure 7 Correlation analysis of mRNA levels of AR and TMPRSS2 in human lung tissues and prostate tissues. (a-c) Correlation analysis for normalized expression of *AR* and a both-open gene *PARP14* (left) (a), two prostate-open genes *TMPRSS2* (middle) (b) and *FKBP5* (right) respectively in normal lung tissues using TCGA datasets (c) (Pearson correlation test). (d-f) Correlation analysis for normalized expression of *AR* and a both-open gene *PARP14* (left) (d), two prostate-open genes *TMPRSS2* (middle) (e) and *FKBP5* (right) respectively in normal prostate tissues using TCGA datasets (f) (Pearson correlation test).



Supplementary Figure 8 Organ-specific regulation of TMPRSS2 by ER. (a) Heatmap for mRNA expression levels of *ESR1* and *ESR2* across multiple tissue or organ types (T: Tumor tissue, N: Normal tissue) (b) mRNA Expression Z-score of *ESR1* across lung cancer cell lines. (c) Western blotting for ER-alpha and β -Actin in H2126, H1437 and Hs578T (negative control) cells. Source data are provided as a Source Data file. (d) qRT-PCR analysis of *GREB1* mRNA expression in MCF7, H1437 and H2126 cells 24 hours post DMSO or E2 treatment in medium containing charcoal-stripped serum (mean $\pm$ SEM, n=4 biologically independent samples). (e) qRT-PCR analysis of *GREB1* mRNA expression in MCF7, H1437 and H2126 cells 24 hours post DMSO or fulvestrant treatment in medium containing normal serum (mean $\pm$ SEM, n=4 biologically independent samples). (f) qRT-PCR analysis of *TMPRSS2* mRNA expression in MCF7, H1437 and H2126 cells 24 hours post DMSO or E2 treatment in medium containing charcoal-stripped serum (mean $\pm$ SEM, n=4 biologically independent samples). (g) qRT-PCR analysis of *TMPRSS2* mRNA expression in MCF7, H1437 and H2126 cells 24 hours post DMSO or fulvestrant treatment in medium containing normal serum (mean $\pm$ SEM, n=4 biologically independent samples). (h) IGV snapshot representing ER binding peaks in *GREB1* locus. (i-j) CPM value for mRNA expression of *GREB1* in T47D (i) and MCF7 cells (j) with or without E2 treatment (upper whisker=largest observation less than or equal to upper hinge+1.5*IQR, upper hinge=75% quantile, median, lower hinge=25% quantile and lower whisker=smallest observation greater than or equal to lower hinge-1.5*IQR, n=2 biologically independent samples). (k) IGV snapshot representing ER binding peaks in *TMPRSS2* locus in T47D and MCF7 cells with or without E2 treatment. (l-m) CPM value for mRNA expression of *TMPRSS2* in T47D (l) and MCF7 cells (m) with or without E2 treatment (upper whisker=largest observation less than or equal to upper hinge+1.5*IQR, upper hinge=75% quantile, median, lower hinge=25% quantile and lower whisker=smallest observation greater than or equal to lower hinge-1.5*IQR, n=2 biologically independent samples). (n) Correlation analysis for relative mRNA expression of *ESR1* and *TMPRSS2* in freshly dissociated human lung cells from 43 normal lung tissues using qRT-PCR (Pearson correlation test). (o) Relative mRNA expression of *ESR1* (top) and *TMPRSS2* (bottom) in freshly dissociated lung cells from 43 normal lung tissue samples. (p) Correlation analysis for normalized mRNA expression (TPM) of *ESR1* and *TMPRSS2* in normal lung tissues using TCGA datasets (Pearson correlation test).