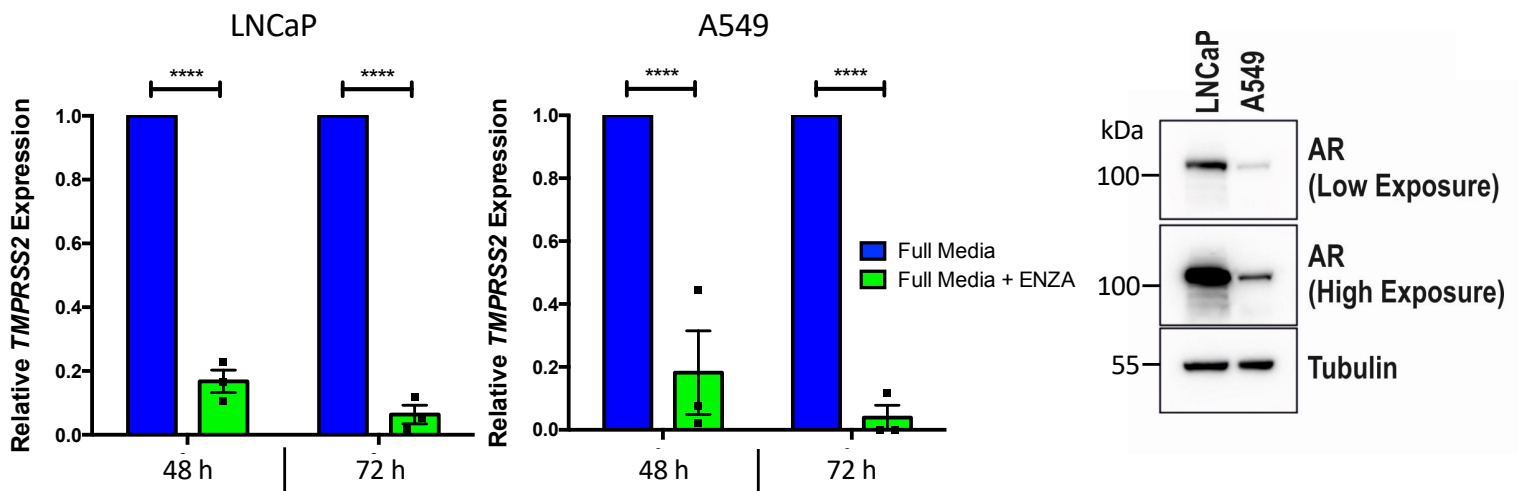


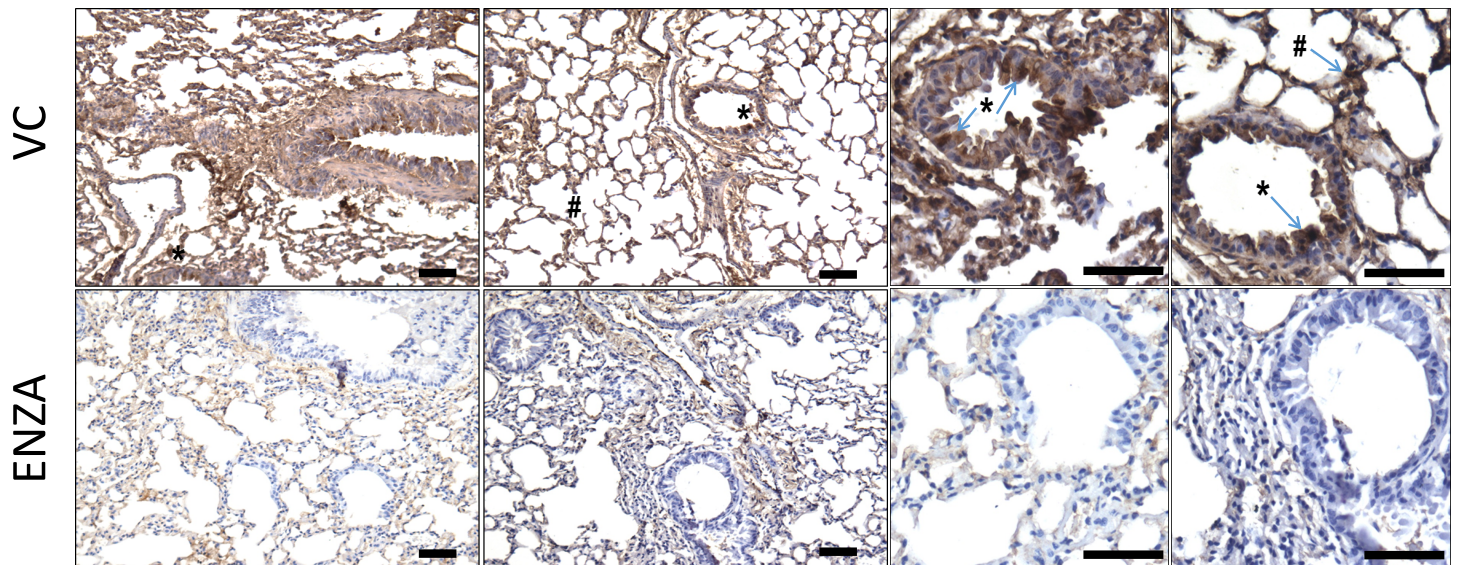
# Leach et al. Supplemental Figure 1



**Supplemental Figure 1. *TMPRSS2* expression is reduced by antiandrogens.** LNCaP and A549 were incubated in full media for 48 or 72 h + 10 nM mibolerone ± 10 µM enzalutamide (ENZA). RNA was harvested, reverse transcribed and qPCR performed to quantify the expression of *TMPRSS2*. Mean of 3 independent repeats (± 1SEM). Two-way ANOVA with Sidak's multiple comparison test. LNCaP 48 h  $p=1.1 \times 10^{-8}$ , 72 h  $p=4.3 \times 10^{-9}$ ; A549 48 h  $p=6.4 \times 10^{-5}$ , 72 h  $p=2.0 \times 10^{-5}$ . A549 and LNCaP cells were lysed and proteins separated using SDS-PAGE. Immunoblotting was performed to visualise AR expression levels and  $\alpha$ -tubulin was used as a loading control. Source data are provided as a Source Data file.

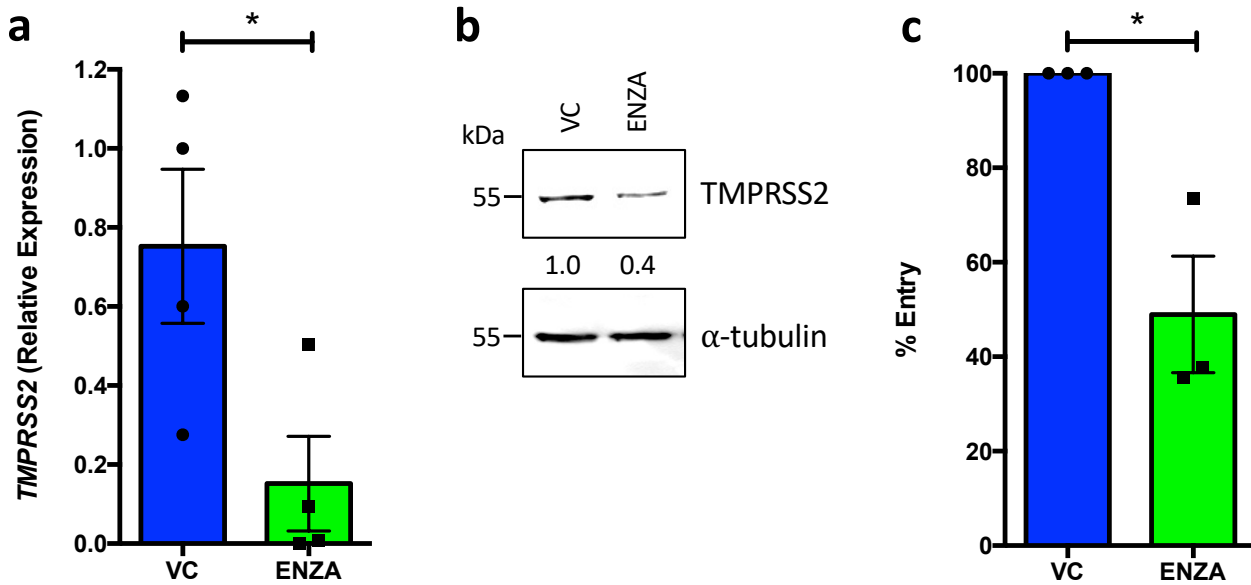
10X magnification

20X magnification



**Supplemental Figure 2. Enzalutamide downregulates Ar expression in the mouse lung.**  $Pten^{loxP/loxP};Pb-Cre4$  mice were treated with 50 mg/kg enzalutamide (ENZA, n=9) or vehicle control (VC, n=8) once daily for 3 days. Lungs were harvested, formalin fixed and wax embedded. Immunohistochemistry was performed using an antibody specific for the Ar. Representative immunostaining of epithelial cells (\*) and parenchyma/AT cells (#). Bar = 50  $\mu$ m.

# Leach et al. Supplemental Figure 3



**Supplemental Figure 3. Enzalutamide reduces TMPRSS2 expression and SARS-CoV-2 cell entry.** A549 were grown in full media  $\pm$  10  $\mu$ M enzalutamide (ENZA) for 72 hrs. **a**) RNA was harvested and qPCR performed to quantify *TMPRSS2* expression in response to vehicle control (VC) or ENZA. One-tailed t-test  $p=0.0197$  **b**) Cells were lysed and proteins separated using SDS-PAGE. *TMPRSS2* levels were visualized using immunoblotting (representative image,  $n=3$ ). Densitometry was performed and *TMPRSS2* expression normalised to  $\alpha$ -tubulin levels. **c**) Cells were transduced with SARS-CoV-2 Spike protein lentiviral particles expressing luciferase. Cells were left for an additional 48 h and luciferase assays performed. Luciferase data were normalised to total protein content. Mean of 3 independent repeats  $\pm$  1SE. One-tailed t-test,  $p=0.0072$ . Source data are provided as a Source Data file.

# Leach et al. Supplemental Table 1

**Supplemental Table 1. Table of the chromatin areas associated with the MA0007.2 AR motif.** Tabled are the 17 highest scoring motif matches in the both regulatory regions indicated in Figure 6. First column is the identifier attributed to each motif site, followed by the motif name, the chromosomal region of the motif match, statistical measures of how exact a match there is between the DNA region and the motif, and the matched sequence indicates the DNA sequence the motif matches. The p-value (two-sided) was taken from log-likelihood ratio score and assumes a zero-order null model. This value represents the probability of a random part of the motif scoring similarly to the observed match. The q-value was calculated using a Benjamini & Hochberg modification of the P-value.

## Regulatory region 1 Chr21:41490000-41580500

|             |          |       | Start    | End      | Strand | p-value   | q-value | Matched Sequence |
|-------------|----------|-------|----------|----------|--------|-----------|---------|------------------|
| <b>RG1a</b> | MA0007.2 | Chr21 | 41498078 | 41498092 | -      | 0.0000367 | 0.359   | AAGAACTGTAAGTAC  |
| <b>RG1b</b> | MA0007.2 | Chr21 | 41521454 | 41521468 | +      | 0.0000198 | 0.359   | GGGCACAGCCTGAGC  |
| <b>RG1c</b> | MA0007.2 | Chr21 | 41521553 | 41521567 | -      | 0.0000737 | 0.392   | CAGAACAGCCTGTGG  |
| <b>RG1d</b> | MA0007.2 | Chr21 | 41528878 | 41528892 | -      | 0.0000652 | 0.392   | CAGAACATTATGGAC  |
| <b>RG1e</b> | MA0007.2 | Chr21 | 41529170 | 41529184 | -      | 0.0000354 | 0.359   | GGGCACACGCTGTTA  |
| <b>RG1f</b> | MA0007.2 | Chr21 | 41532582 | 41532596 | +      | 0.0000215 | 0.359   | GAGAACAGTGGGTTC  |
| <b>RG1g</b> | MA0007.2 | Chr21 | 41569350 | 41569364 | -      | 0.000068  | 0.392   | AGGAACACCGGGAGC  |
| <b>RG1h</b> | MA0007.2 | Chr21 | 41578975 | 41578989 | +      | 9.64E-07  | 0.171   | GAGAACATCGTGTGC  |
| <b>RG1i</b> | MA0007.2 | Chr21 | 41578977 | 41578991 | -      | 3.27E-06  | 0.29    | AGGCACACGATGTTC  |

## Regulatory region 2 Chr21:4160000-41690500

|             |          |       | Start    | End      | Strand | p-value   | q-value | Matched Sequence |
|-------------|----------|-------|----------|----------|--------|-----------|---------|------------------|
| <b>RG2a</b> | MA0007.2 | Chr21 | 41605770 | 41605784 | -      | 0.0000931 | 0.514   | AGGTACAAAATGAAA  |
| <b>RG2b</b> | MA0007.2 | Chr21 | 41611959 | 41611973 | -      | 0.0000994 | 0.514   | AGGTACACACTGATG  |
| <b>RG2c</b> | MA0007.2 | Chr21 | 41630148 | 41630162 | -      | 4.91E-06  | 0.263   | AAGTACAGAGTGCGC  |
| <b>RG2d</b> | MA0007.2 | Chr21 | 41642129 | 41642143 | -      | 0.0000122 | 0.269   | GGGAACAGGCAGTGT  |
| <b>RG2e</b> | MA0007.2 | Chr21 | 41656029 | 41656043 | -      | 0.0000922 | 0.514   | AGGTACAAAGGGTAC  |
| <b>RG2f</b> | MA0007.2 | Chr21 | 41676130 | 41676144 | +      | 0.000068  | 0.498   | AAGAACACTGTGAGG  |
| <b>RG2g</b> | MA0007.2 | Chr21 | 41683746 | 41683760 | +      | 0.0000209 | 0.367   | GGGAACAGAAAGGGC  |
| <b>RG2h</b> | MA0007.2 | Chr21 | 41687334 | 41687348 | +      | 0.0000314 | 0.367   | CAGCACAGAGTGAAC  |

# Leach et al. Supplemental Table 2

## Supplemental Table 2. Primer sequences used in the study

| <b>Human qPCR Primers</b> | <b>Forward (5'-3')</b> | <b>Reverse (5'-3')</b>   |
|---------------------------|------------------------|--------------------------|
| <i>TMPRSS2</i>            | CCTGTGTGCCAAGACGACTG   | TTATAGCCCATGTCCCTGCAG    |
| <i>FKBP5</i>              | ATTATCCGGAGAACCAAACG   | CAAACATCCTTCCACCACAG     |
| <i>L19</i>                | GCGGAAGGGTACAGCCAAT    | GCAGCCGGCGCAAA           |
| <i>GAPDH</i>              | ATGGGGAAGGTGAAGGTTCG   | GGGGTCATTGATGGCAACAATA   |
| <b>Mouse qPCR Primers</b> | <b>Forward (5'-3')</b> | <b>Reverse (5'-3')</b>   |
| <i>Tmprss2</i>            | GAGAACCGTTGTGTTTCGTCTC | GCTCTGGTCTGGTATCCCTTG    |
| <i>Ace2</i>               | TGATGAATCAGGGCTGGGATG  | ATTCTGAAGTCTCCGTGTCCC    |
| <i>Ar</i>                 | TGGGACCTTGGATGGAGAAC   | CTCCGTAGTGACAGCCAGAAL19  |
| <i>L19</i>                | GAAATCGCCAATGCCAAC     | TCTTAGACCTGCGAGCCTCA     |
| <i>Gapdh</i>              | GCAAAGTGGAGATTGTTGCCAT | CCTTGACTGTGCCGTTGAATTT   |
| <i>β-actin</i>            | CCTCTATGCCAACACAGTGC   | CCTGCTTGCTGATCCACATC     |
| <b>ChIP-qPCR Primers</b>  | <b>Forward (5'-3')</b> | <b>Reverse (5'-3')</b>   |
| RG1a                      | CCTTGCAATTGCTGACCCCA   | ACAGCAAGATGGCTTTGAACT    |
| RG1d                      | CATGAGGGCAGTGAGAGTGC   | TTTCTCTGGTCCCAGCCATC     |
| RG1e                      | CTGCTGTGTTACAAACGACG   | CTCCCCGCTCTAGGATGTC      |
| RG1f                      | TTACACCACTGGCTATTGGCTC | CTTTTCAGCCTTGGACATCGG    |
| RG1g                      | GGCTATTGGCTCCTTCTTCTT  | CCTGTTTCTAAACCTTTTCAGCCT |
| RG1h                      | CCAAAAGTGTGCTCGGCTT    | AGAAGTGCAGCTGGCATCG      |
| RG2a                      | GCAACCTGAGCCTGTTGACT   | CTCAGGTCAGGCTTCCACAC     |
| RG2b                      | TCACATCTGGAGGCACTCTG   | CATTGCACCTTCCGCCTTAC     |
| RG2d                      | ACAGAACGGAAAGGAAGGCA   | CAACTGGGAAACTGTCTGCAT    |
| RG2e                      | AGTTACAACCACTGGGTGGG   | AGATTCAAAGCGCCTTACAG     |
| RG2f                      | ACACCCCTAGAGTATTTTCCCC | GGTGATGGTTACACTACAGCTAC  |
| RG2g                      | GTAGCTCTGAGGGCAGTTCA   | ATCGGATGCTGGGAATGACG     |
| RG2h                      | AAGCTCCGGGGAAACCTTATT  | CATGATGGCTTTTGACGCCTG    |