Androgen-deprivation therapy boosts *MX1* expression, a silent effector against COVID-19.

Sanchis P. [1-2], Lavignolle R.[1-2] Sabater A. [1-2], Bizzotto J.[1-2], Lage-Vickers S. [1-2], Abbate M.[1-2], Toro A.[1-2], Cascardo F.[1-2], Olszevicki S.[1-2] Anselmino N.[3], Labanca E.[3], Ortiz E.[1-2], #Vazquez E.[1-2], Cotignola J.[1-2], Gueron G.[1-2] (ggueron@gmail.com)

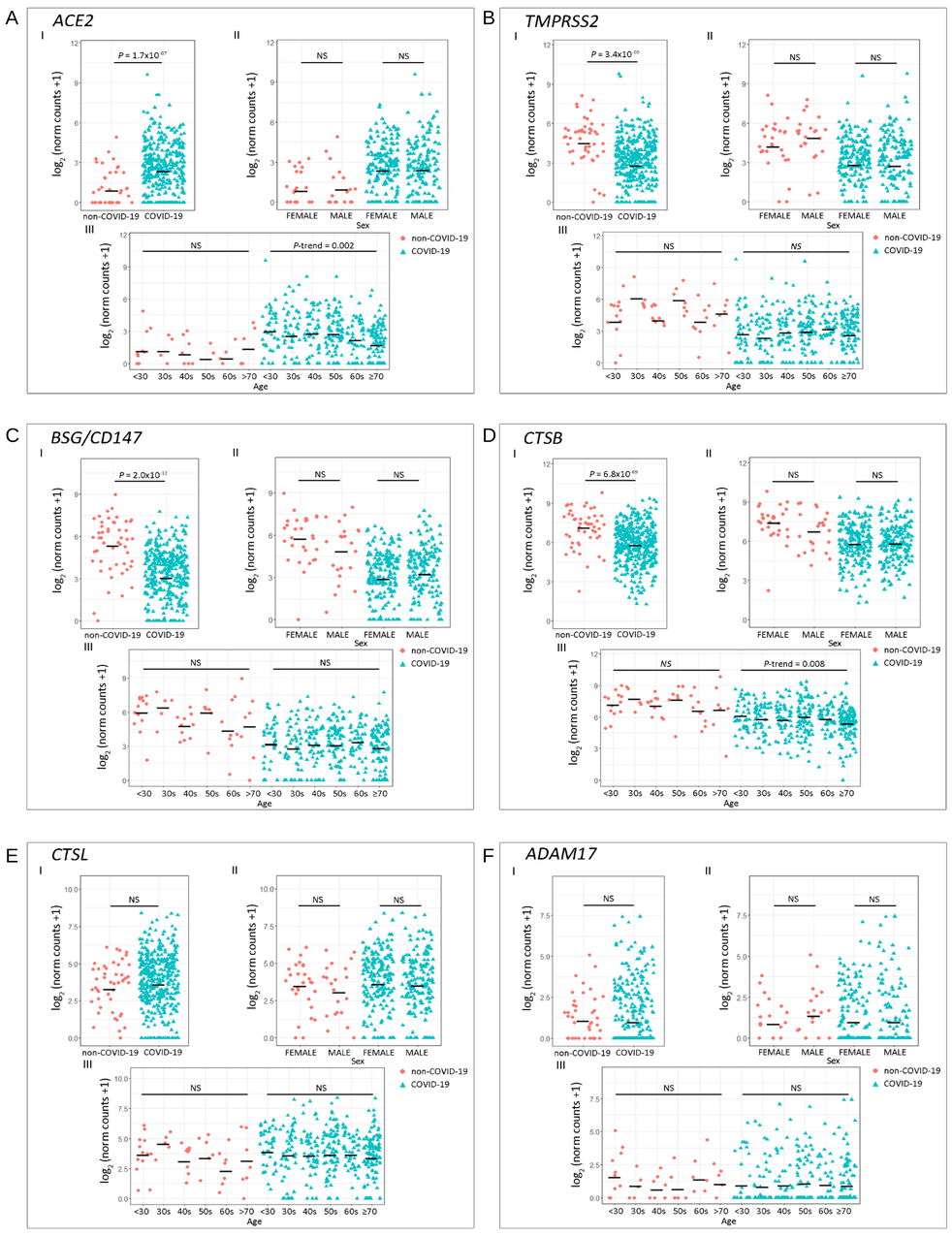
[1] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Buenos Aires C1428EGA, Argentina;
[2] CONICET - Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires C1428EGA, Argentina
[3] Department of Genitourinary Medical Oncology and the David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

SUMMARY

Cancer is a risk factor for SARS-CoV-2 infection. Recent reports have shown that prostate cancer (PCa) patients who underwent androgen-deprivation therapies (ADT) were partially protected from COVID-19. The human myxovirus resistance gene 1 (*MX1)* is expressed in many tissues, including prostate, and we have previously demonstrated its antitumoral activity in PCa, tilting the balance of endoplasmic reticulum stress towards pro-death events. Another key aspect of this protein is its participation in the antiviral response. It is recognized as an IFN-stimulated gene (ISGs), especially during influenza virus infection. Currently, there are several ongoing clinical trials for COVID-19 prevention and/or treatment using type I or III interferons. However, IFN administration could enhance a "cytokine-storm" causing a hyper-inflammatory response and contributing to multiple organ failure. In this work we used a published case-control study (GSE152075) from SARS-CoV-2 positive (n=403) and negative patients (n=50) to analyze the response to infection assessing gene expression profiles of key host cell receptors and antiviral proteins. Additionally, given that *MX1* was differentially expressed between COVID-19 and non-COVID-19 patients, we evaluated *MX1* expression in A549 and Calu3 lung cell lines and ferrets infected with SARS-CoV-2. Since ADT seems to reduce SARS-CoV-2 infection incidence, we aimed to study *MX1* regulation by dihydrotestosterone (DHT). We browsed publicly available ChIP-seq experiments evaluating androgen receptor (AR) binding sites in *MX1* promoter and coding region in different PCa cell lines under DHT stimulation; and we treated LNCaP cells with DHT to assess *MX1* expression under androgen stimulation. Finally, using transcriptomics data from PCa patients under ADT, we studied how androgen ablation regulates *MX1* expression.

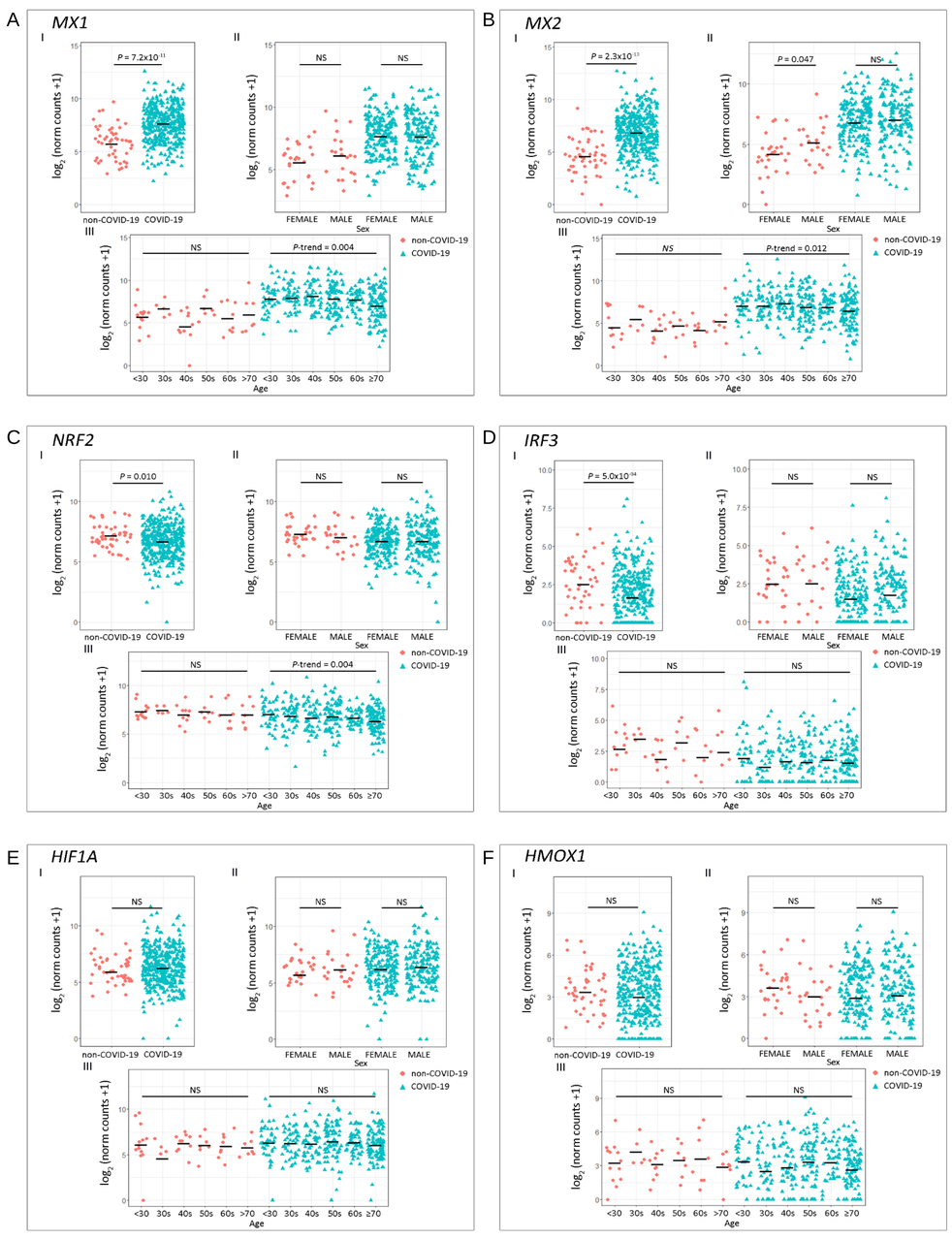
RESULTS

1. Expression of host cell receptor genes in COVID-19 and non-COVID-19 patients



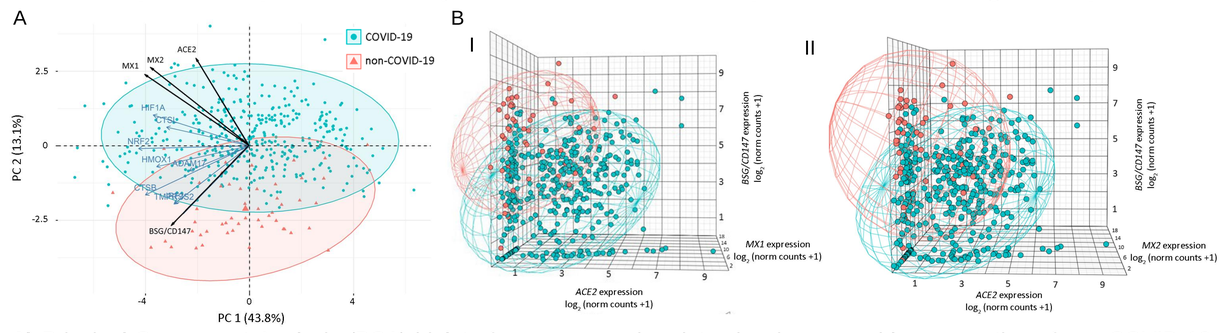
Gene expression analysis for host cell receptor genes: **A)** *ACE2*, **B)** *TMPRSS2*, **C)** *BSG/CD147*, **D)** *CTSB*, **E)** *CTSL* and **F)** *ADAM17.* I) COVID-19 *vs.* non-COVID-19 patients (*P*-values correspond to Wilcoxon rank sum test); II) COVID-19 and non-COVID-19 patients by sex (*P*-values correspond to Wilcoxon rank sum test); III) COVID-19 and non-COVID-19 patients categorized by age groups (*P*-values correspond to decreasing Jonckheere-Terpstrata trend test). Data was obtained from (GSE152075). Statistical significance was set at $P < 0.05$.

2. Expression of host antiviral effector genes in COVID-19 and non-COVID-19 patients.



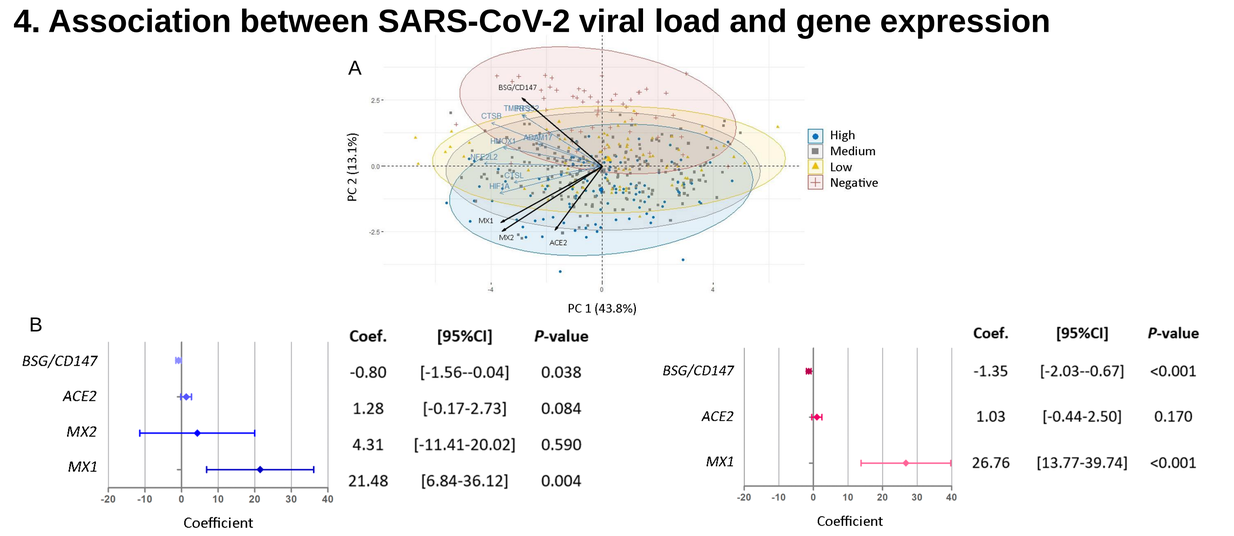
Gene expression analysis for key antiviral genes: **A)** *MX1*, **B)** *MX2*, **C)** *NRF2*, **D)** *IRF3*, **E)** *HIF1A* and **F)** *HMOX1*. I) COVID-19 *vs.* non-COVID-19 patients (*P*-values correspond to Wilcoxon rank sum test), II) COVID-19 and non-COVID-19 patients by sex (*P*-values correspond to Wilcoxon rank sum test), and III) COVID-19 and non-COVID-19 patients categorized by age groups (*P*-values correspond to decreasing Jonckheere-Terpstrata trend test). Data was obtained from (GSE152075). Statistical significance was set at $P < 0.05$.

3. Principal component analysis in SARS-CoV-2 positive and negative patients



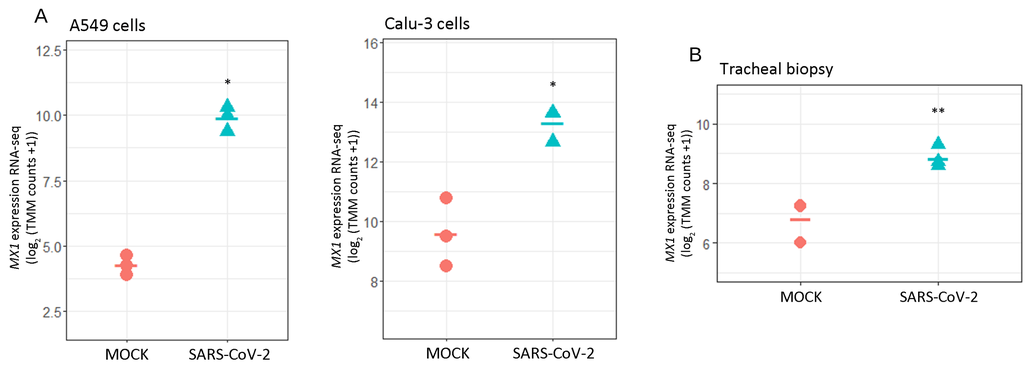
A) Principal Component Analysis (PCA) biplot of gene expression data showing a roughly segregation of non-COVID-19 and COVID-19 samples. Each point represents one individual, and the arrows depict the gene expression profile; black arrows show the 4 genes that have the greatest weight in driving the difference between the groups. **B)** 3D-scatter plots for I) *ACE2*, *MX1*, and *BSG/CD147* and II) *ACE2*, *MX2*, and *BSG/CD147.*

4. Association between SARS-CoV-2 viral load and gene expression



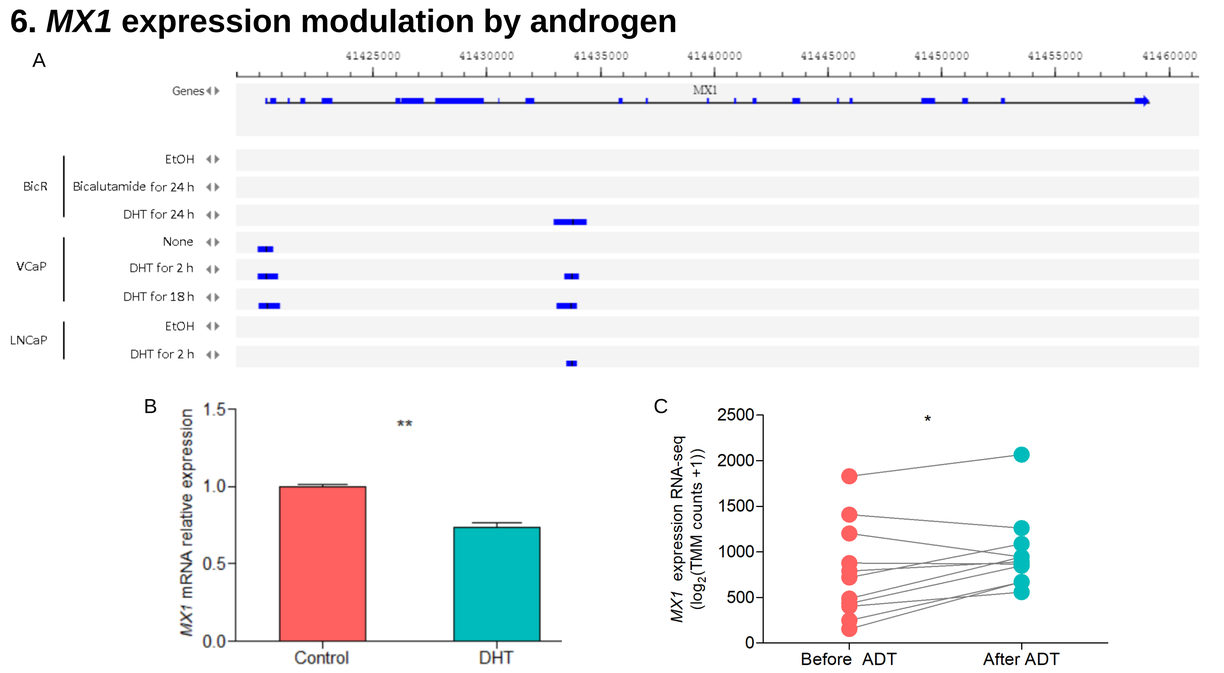
A) Principal Component Analysis biplot of gene expression data showing a roughly segregation of non-COVID-19 and COVID-19 patients stratified by low (Ct>24), medium (Ct=24-19) and high (Ct<19; blue) viral load. Each point represents one individual, and the arrows depict the gene expression profile; black arrows show the 4 genes that have the greatest weight in driving the difference between the groups. **B)** Forest plots representing multivariable regression analysis. Model I) considering as covariates: individual gene expression, viral load and age; and model II) considering as covariates: viral load, age, and all genes.

5. Analysis of *MX1* expression by SARS-CoV2 infection in lung cell lines



A) Dot plots showing *MX1* mRNA expression levels in A549 (n = 6) and Calu-3 (n = 6) human cell lines comparing SARS-CoV-2 infection *vs.* mock infection. **B)** Dot plots depicting *MX1* expression in tracheal biopsy samples (n = 7) from SARS-CoV-2 infected *vs.* mock-treated ferrets. Samples were collected on day 3 after SARS-CoV-2 infection. Expression data was obtained from the GSE147507 dataset. For all graphs, the mean is represented as a horizontal line. Statistical significance *$P < 0.05$, **$P < 0.01$.

6. *MX1* expression modulation by androgen



A) Androgen receptor (AR) binding sites in *MX1* from ChIP-seq datasets GSE66037, GSE28950 and GSE108704. ChIP-seq reads for dihydrotestosterone (DHT), bicalutamide, ethanol (EtOH) or control treatments are represented as blue boxes. **B)** MX1 mRNA expression assessed by RT-qPCR in LNCaP cell line under dihydrotestosterone (DHT) treatment or PBS as control. **C)** Plot comparing *MX1* mRNA expression in locally-advanced/metastatic PCa patients paired samples before (red) and after (green) androgen-deprivation therapy (ADT) from the merged GSE51005 and GSE48403 datasets (n = 11). Statistical significance: *$P < 0.05$, ** $P < 0.01$.

CONCLUSIONS

Our study shows differences in *ACE2, MX1, MX2 and BSG/CD147* expression between COVID-19 and non-COVID-19 patients and point out to *MX1* as a critical responder in SARS-CoV-2 infection. Furthermore, we demonstrated *MX1* modulation by ADT. **Taking into consideration the fact that PCa patients that underwent ADT were less prone to present the infection, we propose this gene as an alternative druggable target for COVID-19 patients, especially those with PCa as a co-morbidity.**

Funding acknowledgments: this work was supported by grants from AGENCIA-PICT 2016-1366 (Argentina), AGENCIA-PICT 2016-0056 (Argentina), UBACyT 2018-20020170100585BA (Argentina), and AGENCIA-PICT-RAICES-2018-02639 (Argentina).

Conflicts of interests: authors declare no conflicts of interests