SARS-CoV-1-NSP14 and MERS-CoV-NSP2 block anti-viral IFN-α-mediated JAK/STAT signalling

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INTRODUCITON

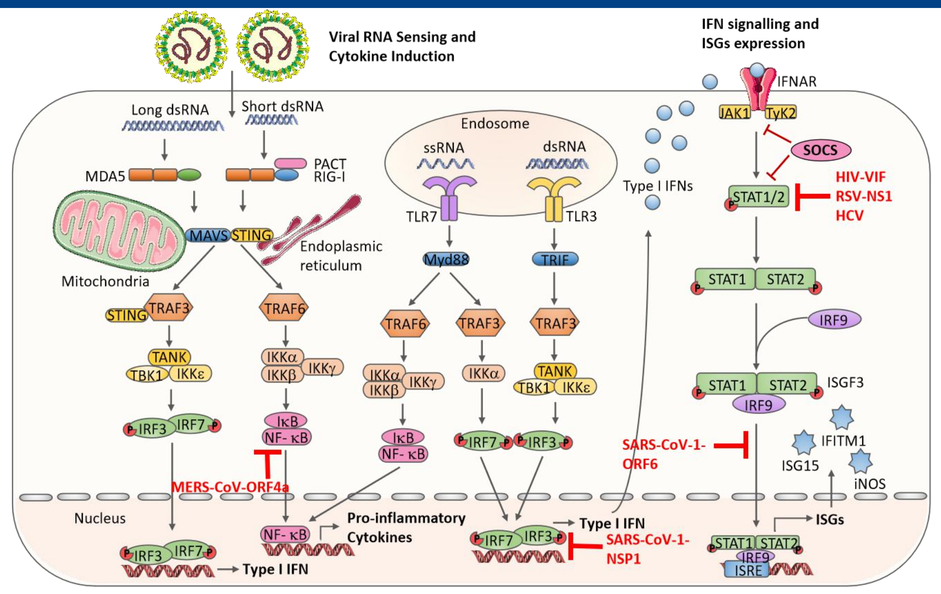


Figure 1. Viral Detection and Immune Response

- Upon CoVs infection, the Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors, have been shown to quickly detect the their cellular presence
- Viral activated PRRs and triggered downstream production of proinflammatory cytokines and Type I Interferon. Type I Interferons then activate JAK-STAT signalling pathways, leading >300 ISGs expression.
- The effective antiviral role of type I IFNs, especially IFN-α, have been harnessed in the treatment of several viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), Human papillomavirus (HPV) and Human herpes virus (HHV).
- HIV, RSV and HCV were demonstrated all target STAT proteins for proteasomal degradation, in order to block antiviral responses to IFN-α
- Trials with therapeutic IFN-α have shown MERS-CoV to have disappointingly weak clinical responses
- Others have shown that IFN-α did not effectively inhibit SARS-CoV-1 replication in vitro

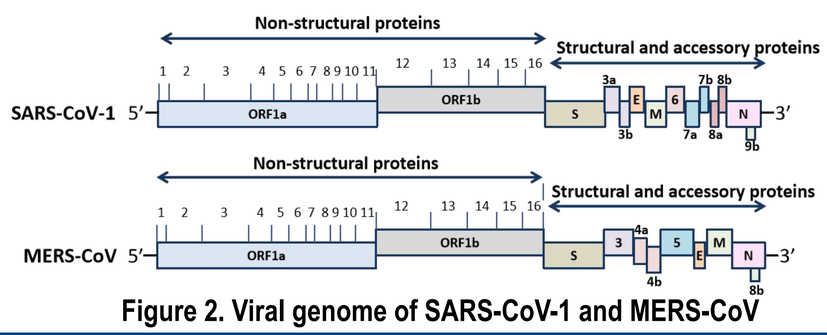


Figure 2. Viral genome of SARS-CoV-1 and MERS-CoV

HYPOTHESIS

Hypothesis: 1. CoVs block molecular signalling responses of IFN-α

AIM OF THIS STUDY

- To investigate whether SARS-CoV-1-NSP14 and MERS-CoV-NSP2 *directly block the molecular signalling responses of IFN-α*
- To investigate which components of JAK/STAT pathway are targeted by CoVs-NSPs
- To investigate the mechanisms used by CoVs to block the signalling

RESULTS

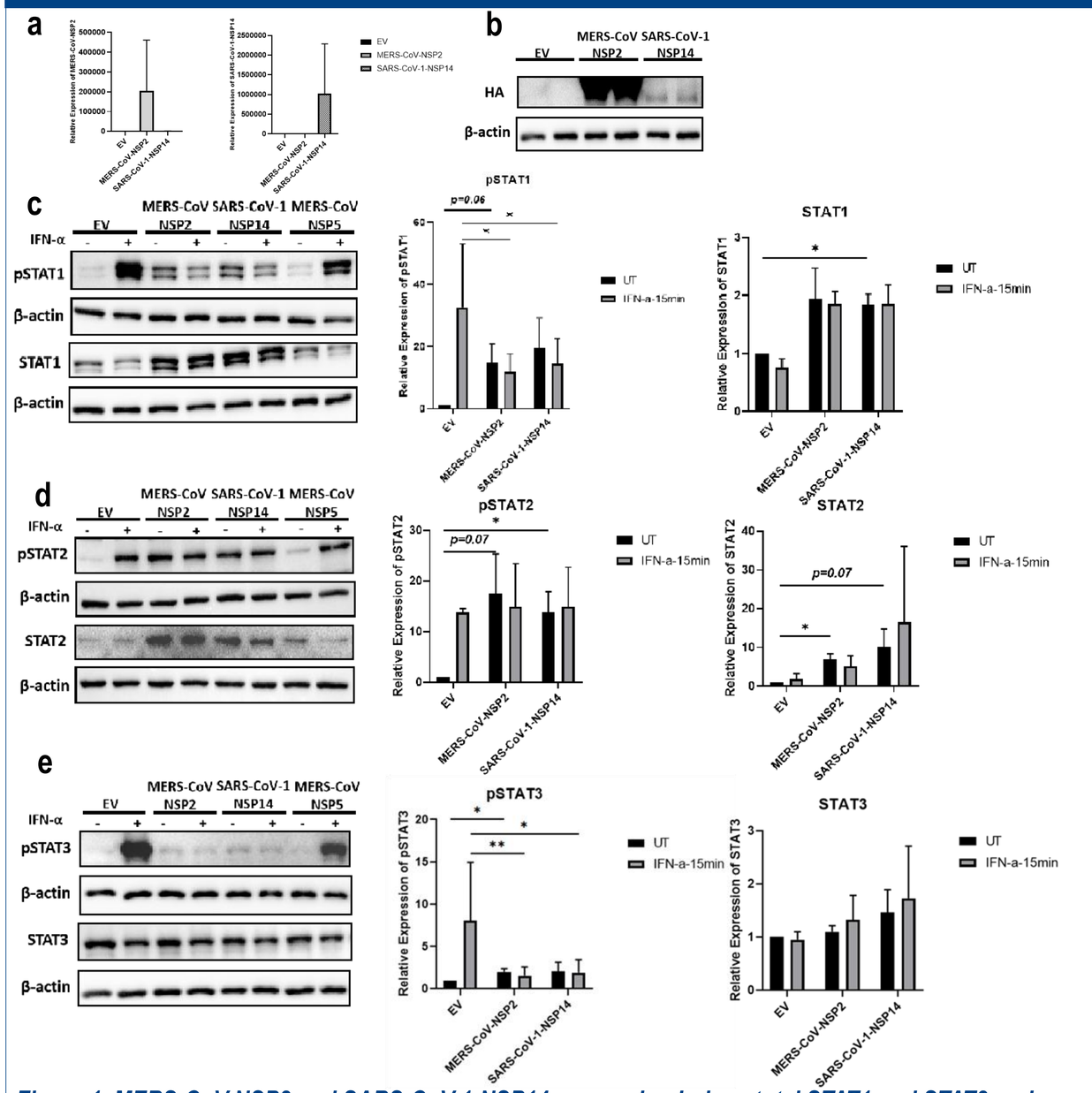


Figure 1. MERS-CoV-NSP2 and SARS-CoV-1-NSP14 expression induce total STAT1 and STAT2 and reduce IFN-α-mediated phosphorylation of STAT1, STAT2 and STAT3. A549 epithelial cells were transfected with EV or HA-tagged MERS-CoV-NSP2, SARS-CoV-1-NSP14 or MERS-CoV-NSP5. After 24, A549 cells were rested in serum free medium for 2h, prior to 15min of IFN-α (1000U/ml) treatment, before analyzing (a) basal levels of MERS-CoV-NSP2 and SARS-CoV-1-NSP14 mRNAs by qRT-PCR. Lysates were also generated and subjected into immunoblotting with antibodies for (b) HA (to detect either MERS-CoV-NSP2 or SARS-CoV-1-NSP14) (c) pSTAT1 and STAT1, (d) pSTAT2 and STAT2 or (e) pSTAT3 and STAT3. All blots were also probed with β-actin antibody. (N.B STAT1 and STAT2 were probed in one membrane and therefore share the same β-actin, and the pSTAT2 and STAT3 were probed in one membrane and therefore share the same β-actin). Densitometric analysis was performed using Image Lab software and values for STATs or phosphorylated STATs were calculated relative to β-actin and compared to the EV transfected UT (untreated) control, which was normalized to 1. All graphs are the mean ± SEM of three independent experiments. *P <0.05, **P <0.01 (Paired t-test).

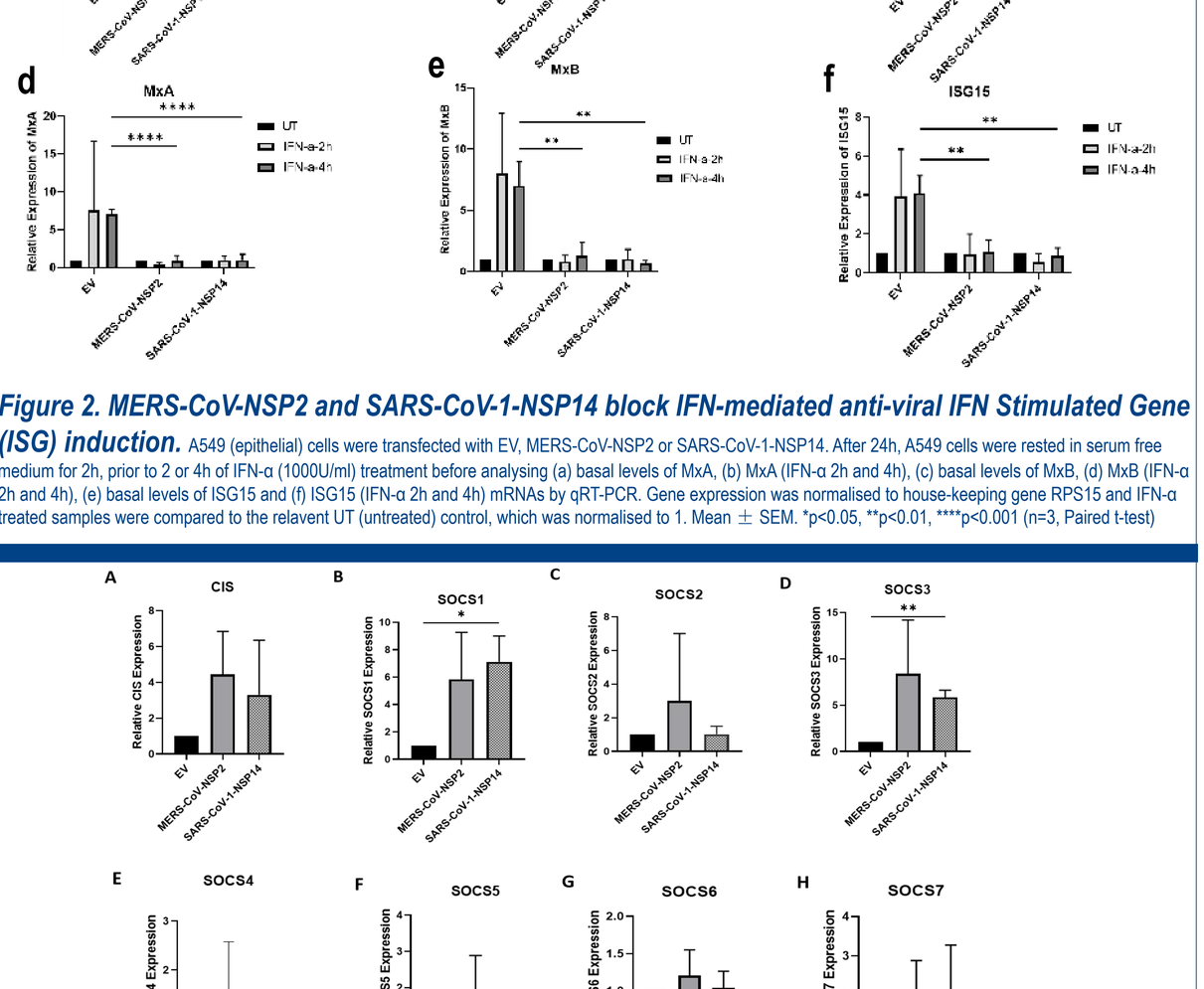


Figure 2. MERS-CoV-NSP2 and SARS-CoV-1-NSP14 block IFN-mediated anti-viral IFN Stimulated Gene (ISG) induction. A549 (epithelial) cells were transfected with EV, MERS-CoV-NSP2 or SARS-CoV-1-NSP14. After 24h, A549 cells were rested in serum free medium for 2h, prior to 2 or 4h of IFN-α (1000U/ml) treatment before analysing (a) basal levels of MxA, (b) MxA (IFN-α 2h and 4h), (c) basal levels of MxB, (d) MxB (IFN-α 2h and 4h), (e) basal levels of ISG15 and (f) ISG15 (IFN-α 2h and 4h) mRNAs by qRT-PCR. Gene expression was normalised to house-keeping gene RPS15 and IFN-α treated samples were compared to the relavent UT (untreated) control, which was normalised to 1. Mean ± SEM. *p<0.05, **p<0.01, ****p<0.001 (n=3, Paired t-test)

Figure 3. SARS-CoV-1-NSP14 expression induced SOCS1 and SOCS3, but reduced SOCS4 mRNAs expression. A549 (epithelial) cells were transfected with EV, MERS-CoV-NSP2 or SARS-CoV-1-NSP14. After 24 hours, (a)CIS, (b) SOCS1, (c) SOCS2 (d) SOCS3, (e) SOCS4 (f) SOCS5, (g) SOCS6 and (h) SOCS7mRNAs were analysed by qRT-PCR. Gene expression was normalised to house-keeping gene RPS15 and IFN-α treated samples were compared to the relavent UT (untreated) control, which was normalised to 1. Mean ± SEM. *p<0.05, **p<0.01, ****p<0.001 (n=3, Paired t-test)
SOCS family gene expression was normalised to house-keeping gene RPS15 and then compared to the relavent control, which was normalised to 1. Mean ± SEM. *p<0.05, **p<0.01 (n=3, Paired t-test)

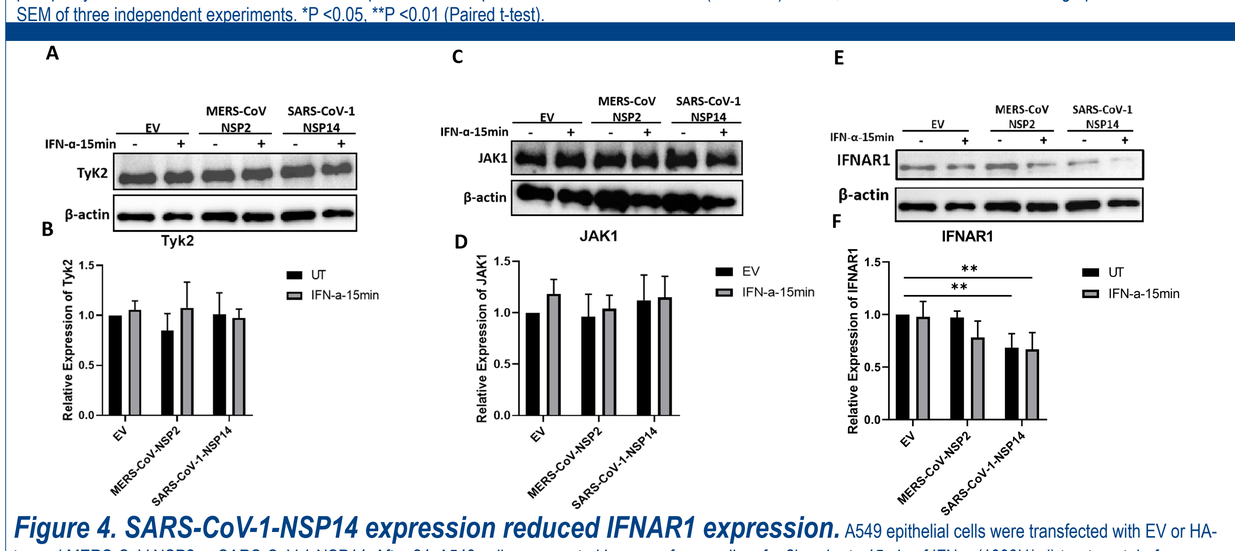
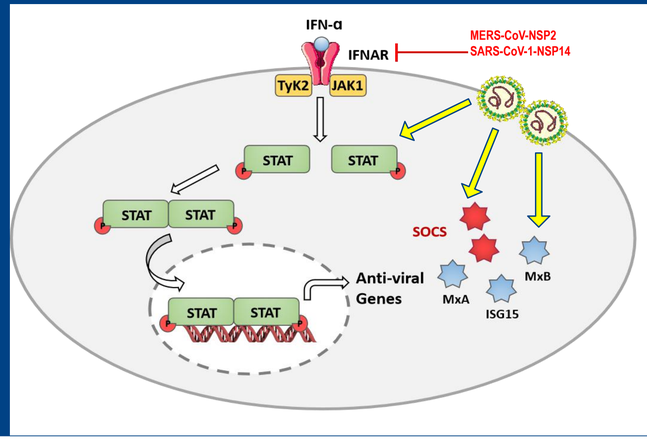


Figure 4. SARS-CoV-1-NSP14 expression reduced IFNAR1 expression. A549 epithelial cells were transfected with EV or HA-tagged MERS-CoV-NSP2 or SARS-CoV-1-NSP14. After 24, A549 cells were rested in serum free medium for 2h, prior to 15min of IFN-α (1000U/ml) treatment, before generating cell lysate for immunoblotting with antibodies for (a) Tyk2, (c) JAK1 and (e) INFAR1. All blots were also probed with β-actin antibody. Densitometric analysis was performed using Image Lab software and values for Tyk2, JAK1 or IFNAR1 were calculated relative to β-actin and compared to the EV transfected UT (untreated) control, which was normalized to 1. All graphs are the mean ± SEM of three independent experiments. **P <0.05, **P <0.01* (*Paired t-test*).

SUMMARY



- MERS-CoV-NSP2 and SARS-CoV-1-NSP14 induce basal levels of STAT1&2 and their phosphorylation, but inhibit IFN-α-induced STAT1, 2&3 phosphorylation.
- MERS-CoV-NSP2 and SARS-CoV-1-NSP14 induce basal mRNA levels of MxA, MxB and ISG15, but reduce IFN-α-mediated ISG induction.
- MERS-CoV-NSP2 and SARS-CoV-1-NSP14 induce mRNA levels of SOCS1 and SOCS3
- SARS-CoV-1-NSP14 slightly reduces basal levels of IFNAR1 expression