

Steps to Validation of Interferon Alpha Inducible Protein 27 (IFI27) Knock Outs in Human Lung Epithelial Cells

INTRODUCTION

Preliminary investigation of the intracellular immune response to SARS-CoV-2 has identified a series of interferon stimulated genes (ISGs) that are highly upregulated, one of which is the gene encoding IFI27(1-2).

We propose:

1. To generate a cellular model lacking expression of IFI27
2. To investigate the behavior of cells lacking IFI27 in response to dsRNA (immune activator)

METHODS

Plasmids encoding Cas9 and guide RNA sequences (sgRNA) to IFI27 were transfected into wild type (WT) A549 cells in order to create an IFI27 knockout cell line.

Real-time PCR was used to establish regulation of IFI27 mRNA expression.

Genetic mutations of IFI27 were probed using western blot analysis of protein expression as compared to the WT A549.

REFERENCES

1. Butler DJ, Mozsary C, Meydan C, et al. Host, Viral, and Environmental Transcriptome Profiles of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *bioRxiv*. 2020. <https://doi.org/10.1101/2020.04.20.048066>
2. Mick E, Kamm J, Pisco AO, et al. Upper airway gene expression differentiates COVID-19 from other acute respiratory illnesses and reveals suppression of innate immune responses by SARS-CoV-2. *medRxiv*. 2020. doi: [10.1101/2020.05.18.20105171](https://doi.org/10.1101/2020.05.18.20105171).

Further work is needed to optimize IFI27 western blotting

We are testing a tagged IFI27 expression plasmid to confirm protein size and antibody binding

It is possible that we are detecting IFI27 protein with a post translational modification unique to A549 respiratory cells

RESULTS

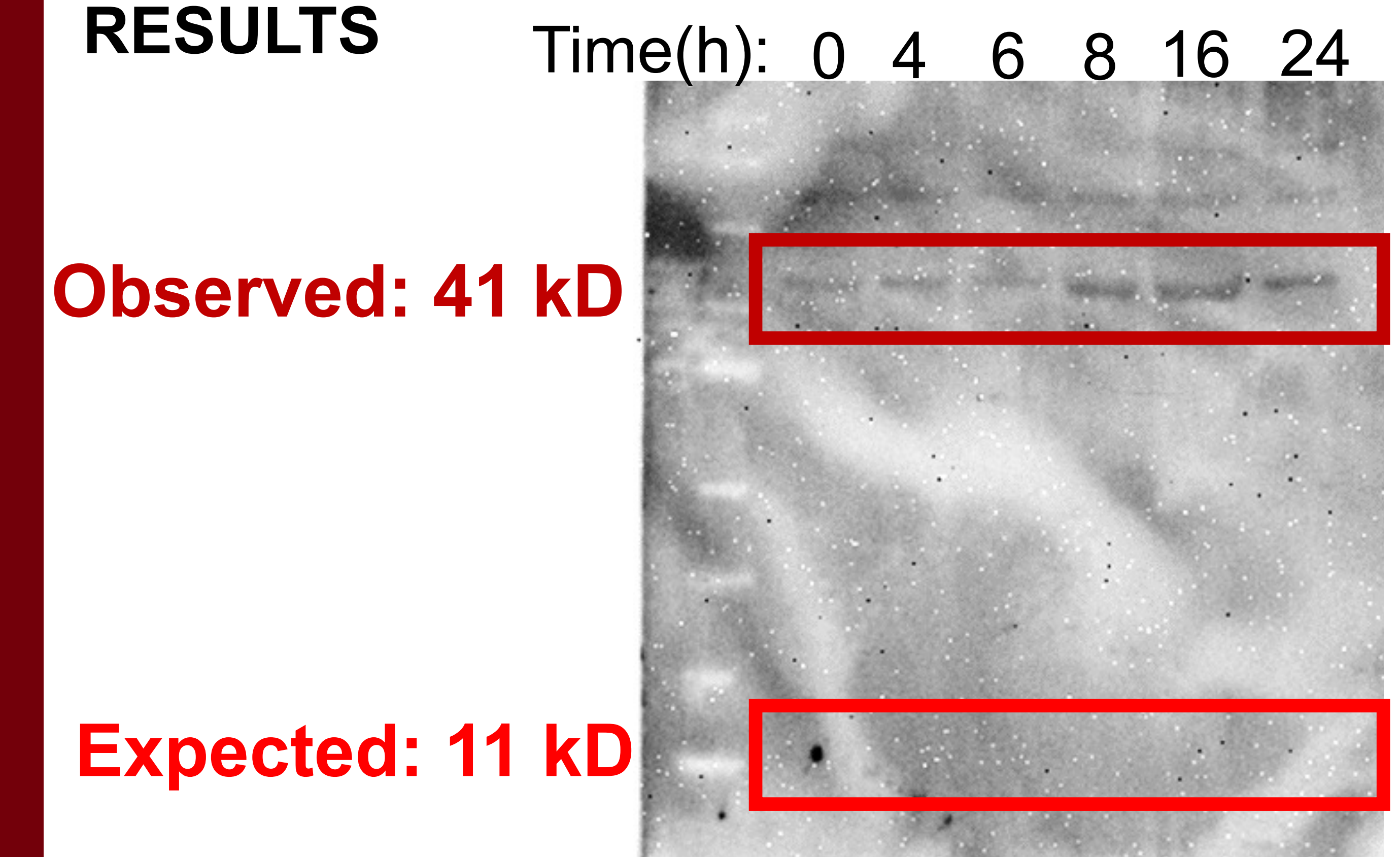


Fig 1. IFI27 Western Blot. Protein detected by IFI27 antibody after Poly I:C exposure.

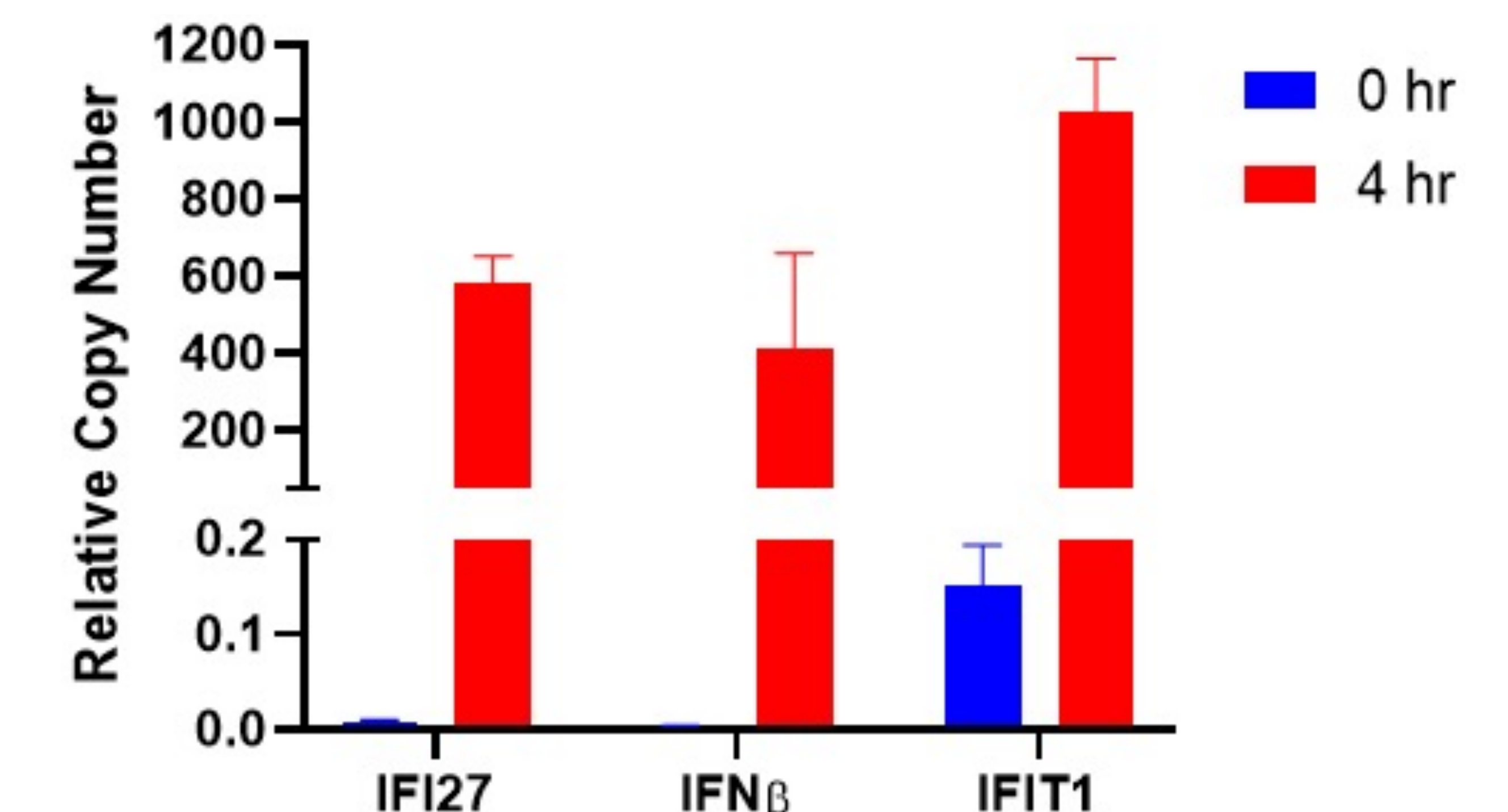


Fig 2. mRNA expression by Real-time PCR. IFI27 mRNA is upregulated during anti-viral response to Poly I:C (4hr).

CONCLUSIONS

Confirmed that the immune response of WT A459 cells includes upregulation of IFI27, validating this model to study IFI27 function.

Unable to confirm IFI27 protein expression due to off target antibody binding.