7 Future Work

7.1 Human IFITM3

As discussed, LCLs may not be the most appropriate cell type for investigating alternative splicing, since they are not naturally infected by influenza. One proposal would be to de-differentiate the LCLs used in this study into induced pluripotent stem cells (iPSCs) and then differentiate them into a more relevant cell type such as motile multiciliated cells. Both of these methods have been published and established[281,282]. Cell type may have an impact on the control of splicing by this allele, which we may be able to detect by using these techniques.

Scott *et al.* showed that IFNα alone can cause demethylation of the promoter region of IFITM3. The methylation status of the panel of LCLs used in this thesis could be assessed by using DNase I to digest open regions of chromatin[283]. Alternatively a novel technique called transposase-accessible chromatin using sequencing (ATAC-seq) could be used, which uses a hyperactive Tn5 transposase loaded with sequencing adapters to simultaneously identify and sequence open regions of DNA[284]. However, there are millions of genetic differences between each LCL, therefore if a difference in methylation was seen it could not be directly attributed to rs12252. To confirm this zinc finger nucleases or clustered regularly interspaced short palindromic repeats (CRISPR) could be used to change the allele at this point to a 'T' or a 'C' and test whether or not the methylation status is changed[285,286]. This would allow isolated editing of the allele, and no others, therefore providing a way of determining if the allele controls the methylation status, or the degree of alternative splicing, of IFITM3.

To identify if other SNPs are in LD with rs12252, a different sequencing method could be employed in tandem with Illumina sequencing. This technique is called single-molecule real-time (SMRT) sequencing, which utilises DNA polymerase as the 'sequencing engine'[287]. SMRT sequencing gives much longer reads, reliably up to 4 kb, which may cover the gaps in the current human reference, whilst the shorter more accurate Illumina MiSeq reads would ensure the accuracy of base calling.

7.2 Chicken IFITM Proteins

Characterising the antiviral activity of chIFITM1, 2, and 3 proteins against more relevant avian viruses such as the coronavirus, Infectious Bronchitis Virus (IBV) and

the birnavirus, Infectious Bursal Disease Virus (IBDV) would be an important development of this work. Both these viruses also enter cells by the acidic endosome pathway so it would be interesting to see whether or not they were IFITM3 sensitive. Furthermore, embryos of inbred chickens could be transfected with the RCAS avian retroviral expression system vector (Replication-Competent ASLV long terminal repeat (LTR) with a Splice acceptor) expressing either a) the biologically relevant IFITM or b) the siRNA to knock down expression of each of the IFITMs to characterise their activity *in vivo*.

Our current understanding of genetic variation in the chicken genome is limited to an inbred Red Jungle Fowl genome. The *IFITM* locus in chickens had not been previously characterised, and may contain copy number variations and re-arrangements in different breeds of bird. Comparisons with the genomes of broiler, layer, and Silkie breeds could reveal more about IFITM variation in poultry. This locus could be sequenced in a large number of individuals of each breed to give more insight into the genetic variation at this site.

Mutagenesis studies of human IFITM3 shows that defined mutations in the NTD and the CIL domain can confer decreased restriction to IAV whilst preserving strong restriction of Dengue Virus[5]. It is therefore likely that polymorphisms in the chIFITM locus will alter the restriction of avian viruses with some alleles being more effective than others. Following comprehensive characterisation of IFITM allelic diversity in a defined population, naturally occurring alleles that are protective against one or more avian-infectious-disease could be identified and used to breed into the commercial lines, therefore reducing the risk of coop epidemics.

7.3 IFITM3 Protein-Protein Interactions

In chapter 4, we presented evidence for cleavage of the HA tag in some circumstances, therefore using an anti-HA antibody for the co-immunoprecipitation may have biased our results against those interactions where the HA tag has been lost. Stable cells lines could be generated using an IFITM3 FLAG-tagged protein, which may not be cleaved in the same way, or use the N-terminal anti-IFITM3 antibody, since A549s do not express IFITM1 or IFITM2. This would also remove any chance of cross-reactivity with influenza HA protein. However, both of these approaches would require re-optimisation of the co-immunoprecipitation protocol.

7.4 Conclusions

This thesis furthers our understanding of IFITM3 in both humans and chickens, a biologically relevant species for many zoonotic viral infections. There is more still to be learned regarding how IFITM3 co-ordinates its antiviral activity and which other proteins are involved in this process. The minority C allele of rs12252 in IFITM3 is clearly associated with a severe response to influenza infection, but the reasons behind this remain uncertain. Improving our knowledge of this protein could potentially improve chicken-breeding strategies and human vaccination programs against a host of deadly viruses.