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FREQUENT HOMOZYGOUS DELETIONS OF TYPE I INTERFERON GENES IN PLEURAL MESOTHELIOMA CONFER SENSITIVITY TO ONCOLYTIC MEASLES VIRUS

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Abstract

Oncolytic immunotherapy is based on the use of non-pathogenic replicative oncolytic viruses (OV) that infect and kill exclusively tumor cells. Recently, we showed that the spontaneous oncolytic activity of the Schwarz strain of measles virus (MV) against human malignant pleural mesothelioma (MPM) depends on defects in the antiviral type I interferon (IFN I) response in tumor cells. In this study, we identify the most frequent defect as the homozygous deletions (HD) of all fourteen IFN I genes (IFN- α and IFN- β) that we found in more than half of MV-sensitive MPM cell lines. These HD occur together with the HD of the tumor suppressor gene *CDKN2A* also located in the 9p21.3 chromosome region. Therefore, the IFN I^{-/-} MPM cell lines develop a partial and weak IFN I response when they are exposed to the virus compared to normal cells and MV-resistant MPM cell lines. This response consists in the expression of a restricted number of IFN-stimulated genes that do not depend on the presence of IFN I. In addition, the IFN I^{-/-} MPM cell lines infected by MV also develop a pro-inflammatory response associated with a stress of the endoplasmic reticulum. Our study emphasizes the link between HD of IFN I encoding genes and *CDKN2A* gene in MPM and sensitivity to MV oncolytic immunotherapy.

Introduction

Oncolytic immunotherapy is a developing strategy to treat cancer that is based on the use of non-pathogenic replicative oncolytic viruses (OV). OV exclusively replicate in and kill tumor cells, and stimulate the antitumor immune response ¹. Indeed, viral replication is often favored in tumors due to the presence of an immunosuppressive environment and defects in several pathways in tumor cells such as apoptotic or antiviral pathways that are normally used to prevent viral replication ².

Attenuated vaccine strains of measles virus (MV) such as Edmonston or Schwarz strains display a spontaneous oncolytic activity against numerous tumor cell types ^{3,4}. These strains use the CD46 molecule as a second receptor to infect human cells, unlike the pathogenic strains that mainly use the CD150 molecule ^{5,6}. Tumor cells often overexpress CD46 to escape complement-mediated cytotoxicity ^{7,8}. This expression at high density favors the infection of tumor cells by attenuated MV ⁹. We and others have shown that the oncolytic activity of MV also depends on deficiencies of the antiviral type I interferon (IFN I) response in tumor cells ¹⁰⁻¹². All nucleated cells are able to detect viral infection using intracytoplasmic pattern recognition receptors ¹³. In the case of MV, cytosolic helicases such as RIG-I and MDA5 detect the viral RNA and send a signal to the nucleus via IRF3, NF-KB. This signal induces the secretion of IFN I that protects infected and neighboring cells from further viral replication. Indeed, exposure to IFN I induces the expression of hundreds of IFN-stimulated genes (ISG) in cells that express the IFN- α / β receptors (IFNAR). These ISG are directly or indirectly responsible for the antiviral activity. Some of these ISG, such as ISG15 or ISG54 (IFIT1), can be directly activated in response to the virus via IRF3 without the need of IFN I, whereas others require IFN I and IFNAR signaling¹³⁻¹⁵.

Malignant pleural mesothelioma (MPM) is a cancer of the pleura often due to chronic asbestos exposure ¹⁶. Treatments of MPM include chemotherapy, radiotherapy and surgery, and are of limited efficacy, urging the development of new therapeutic approaches, such as oncolytic immunotherapy. In a recent study, we reported that fifteen out of twenty-two human malignant pleural mesothelioma (MPM) cell lines were sensitive to Schwarz MV oncolytic activity due to defects of their antiviral IFN I response ¹⁰. Eleven out of these fifteen MPM cell lines were unable to secrete IFN I (IFN- β and IFN- α) in response to MV making them permissive to the viral replication that ended up killing them. However, these cell lines were able to control viral replication and to resist MV oncolytic activity if they were previously exposed to IFN I, suggesting that the defects of the IFN I response were located upstream of IFNAR. The seven others MPM cell lines as well as four types of healthy cells were able to produce type I IFN when exposed to MV and thus block viral replication and cell lysis.

In this study, we aimed at further identifying the defects of IFN I response present in MPM tumor cells, that make them sensitive to MV oncolytic activity. We also wanted to characterize their impacts on the cellular response to the virus. We show that the most frequent defect in MPM is the homozygous deletions (HD) of all genes encoding IFN I (IFN- α and IFN- β) that were found in eight out of the fifteen MV-sensitive MPM cell lines. These frequent HD of IFN I encoding genes in MPM that occur together with the HD of *CDKN2A* gene represents a therapeutic target that can be exploited with OV such as Schwarz MV to induce immunogenic death of tumor cells.

Materials and methods

Cell culture

Human MPM cell lines (from Meso 4 to Meso 225) were established in our laboratory from pleural effusions collected by thoracocentesis, and genetically characterized¹⁷. All patients gave their informed consent. All cell lines were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100U/mL penicillin, 100µg/mL streptomycin and 2mM L-glutamine (all reagents from Gibco-Invitrogen) and cultured at 37°C in a 5% CO₂ atmosphere. Normal peritoneal mesothelial cells MES-F were purchased from Tebu-bio, pulmonary fibroblasts CCD-19Lu from the ATCC-LGC Standards, and pulmonary endothelial cells HMVEC-L from Lonza. These cells were cultured in their specific media according to the manufacturers' recommendations. The bronchial epithelial cells were obtained and cultured as previously described¹⁸. Cells were routinely checked for Mycoplasma contamination using the Plasmotest™ from InvivoGen.

The eighty MPM cell lines used to confirm the HD of *IFNB1* gene were early passages of primary tumor cells established at INSERM U1162 laboratory, Paris, from surgical resection, pleural biopsies, or malignant pleural fluid of confirmed MPM cases, obtained from several French hospitals with patient's consents^{19, 20}.

MV infection, IFN and inhibitor treatments

Live-attenuated Schwarz vaccine strain of measles virus (MV), MV recombinant for the enhanced green fluorescent protein (MV-eGFP) and MV recombinant for the cherry protein (MV-ch) were produced and purified as previously described²¹. Infection of cells with MV lasted 2h at 37°C. Viral inoculum was then replaced by fresh culture medium, unless otherwise indicated. Type I IFN treatment was performed by adding

rhIFN- α 2a and rhIFN- β 1a (ImmunoTools) at 1,000IU/mL and type III treatment was done by adding rhIL-29 at 10ng/mL during 48h. The IFN I pathway was inhibited by ruxolitinib, a chemical inhibitor of janus-associated kinases (JAK1, JAK2 and JAK3), at 1 μ M three days before infection and added every days during the time of the experiment.

MV replication assay

Three days before infection, cells were seeded in 6-well plates at a density of 0.5×10^6 cells/well for the MPM cell lines with or without ruxolitinib. A day before infection, cells were seeded in 96-well plates, at a density of 10,000 cells/well. Infection was performed at multiplicity of infection of 1 (MOI 1) and fluorescence at 610nm was analyzed every day during 9 days using a ChemiDoc™ MP imaging system (Bio-Rad). Quantification was done with the Image Lab 4.1 Software (Bio-Rad) with the relative fluorescence corresponding to the ratio between the fluorescence measured in treated and non-treated cells.

Confocal microscopy

MPM cell lines were seeded in 8-well silicone cultivation chamber (IBIDI ®) at a density of 0.5×10^5 cells/well and then infected with MV at MOI 10 during 12h. Cell membrane cells were labeled with WGA, a lectin marker, at 5 μ g/mL for 10min at room temperature. Cell were fixed with 4% paraformaldehyde for 20min at room temperature, cell and nuclear membranes were permeabilized for 5min at 20°C with absolute methanol and then blocked in PBS 0.1% BSA (Sigma Aldrich) at 4°C overnight. Cells were incubated 5min in PBS 0.1% BSA 0.1% saponin (Sigma Aldrich) and then with anti-IRF3 and NF- κ B primary antibodies (Cell Signaling Technology) for

1h, followed by incubation with DyLight 488-coupled secondary antibodies (Life Technologies™) for 20min. Both were diluted in PBS 0.1% BSA 0.1% saponin and incubated at room temperature. Finally, nucleus were labeled by Hoescht 5min at room temperature. Labeled cells were directly viewed with a confocal microscope (Nikon A1RSi). The images were recorded with NIS Element software (Version 3.6, Nikon) and processed with the software Fiji²². Ratio of the nuclei/cytoplasm fluorescence intensity was measured with the software Volocity software (Perkin-Elmer).

Real-time RT-qPCR

MPM cell lines and healthy cells were seeded in 6, 12 or 24-well plates at a density of 0.5, 0.25 or 0.1×10⁶cells/well respectively. Then, cells were treated with Type I or III IFNs or ruxolitinib or/and infected with MV at MOI 1 during 48h or at MOI 10 during 12h. Total cell RNA was extracted using the Nucleospin® RNA II kit (Macherey-Nagel) and 0.5µg or 0.25µg total RNA was reverse transcribed using MMLV reverse transcriptase (Invitrogen). PCR reactions were conducted using QuantiTect primer assays (Qiagen) and Maxima SYBR Green/ROX qPCR Master Mix (Fisher Scientific) according to the manufacturer's instructions. Gene expression was analyzed in treated or non-treated cells using QuantiTect primers pairs for *Mx1*, *TLR3*, *IRF7*, *STAT1*, *XAF1*, *IFIT1*, *DDX58 (RIG-I)*, *OAS1*, *IRF3*, *RELA (p65)* and *CXCL10*. Gene expression was expressed as relative expression compared to the expression of a housekeeping gene that encodes the human large ribosomal protein (*RPLPO*) and in fold change ($\Delta\Delta Ct = (Ct \text{ gene non-treated} - Ct \text{ RPLPO non-treated}) - (Ct \text{ gene treated} - Ct \text{ RPLPO treated})$).

Genomic PCR

Genomic DNA was extracted with the Nucleospin® Blood kit (Macherey-Nagel) according to the manufacturer's instructions. 250ng of genomic DNA was amplified according Phusion hot start II high fidelity DNA polymerase protocol (thermo scientific). The following primers were used: 5'-GGTCGTTTGCTTTCCTTTGC-3' (forward) and 5'-AGCAATTGTCCAGTCCCAGA-3' (reverse) for *IFNB1* gene. A two steps cycling protocol was used with 1 cycle during 30s at 98°C, then 30 cycles of amplification 10s at 98 °C and 1min at 72 °C, following by a final extension 7min at 72°C. The Genomic PCR on the eighty MPM cell lines of the validation series was performed as described in supplemental material and methods.

Genomic array analysis

Genomic DNA was extracted with Nucleospin® Blood kit (Macherey-Nagel) according to the manufacturer's instructions. DNA (500 ng) was processed and hybridized to Affymetrix CytoScanHD Array according to the manufacturer's instructions (Affymetrix, Santa Clara, CA). The detection, determination and visualization of gains, losses were performed using Affymetrix Chromosome Analysis Suite software (ChAS v3.1.1.27). All obtained data have been uploaded on GEO Omnibus site (GSE134349).

Analysis of The Cancer Genome Atlas (TCGA) database

Available alteration data (copy number variation, mutation and fusion) for *CDKN2A*, *IFNA2*, *IFNB* and several genes that encodes protein implicated in the IFN I response were retrieved from cBioPortal, an online portal for accessing data from TCGA project (<http://www.cbioportal.org>).

Transcriptomic study

MPM cell lines and healthy cells were seeded in 6-well plates at a density of 0.5×10^6 cells/well. Then, cells were treated with Type I IFN or infected with MV at MOI 1 during 48h. Total cell RNA was extracted using the Nucleospin® RNA II kit (Macherey-Nagel). For each sample, a mix of an equal amount of RNA from three separate experiments was done. Transcriptome analysis was performed with Affymetrix human Gene 2.0 ST arrays according to the manufacturer's protocol. Briefly, total RNA (300 ng) was labeled and cRNA (antisens RNA) was synthesized using the Affymetrix WT cDNA Synthesis and Amplification Kit. After cleanup protocol, ssDNA (sense single stranded DNA) was synthesized, fragmented and labeled with biotin. Biotin-ssDNA was hybridized onto microarrays according to the manufacturer's instructions. After 16 h at 45 °C, microarrays were washed and stained using Affymetrix fluidics station 450 and scanned with an Affymetrix GeneArray scanner 3000 7G. Raw data were normalized using the Robust Multichip Algorithm (RMA) in Bioconductor R. Then all quality controls and statistics were performed using Partek GS® (version 6.6 Copyright© 2012 Partek Inc., St. Louis, MO, USA). To find differentially expressed genes, classical analysis of variance (ANOVA) was performed for each gene and pair wise Tukey's post hoc tests between groups. P-values and fold changes were used to filter and select differentially expressed genes. Interaction, pathway and functional enrichment analyses were carried out with IPA (Ingenuity® Systems, www.ingenuity.com, USA). All data obtained by microarray analysis have been uploaded on GEO Omnibus site (GSE117668).

Western-blotting

MPM cell lines were seeded in 6-well plates at a density of 0.5×10^6 cells/well. Then, cells were treated with IFN I or infected with MV at MOI 1 during 48h. Cells were lysed in RIPA buffer containing a Protease Inhibitor Cocktail (Sigma) and denatured at 95°C for 5min in Laemmli buffer with 10% β -mercaptoethanol. 20 μ g of proteins for cellular lysate were separated by SDS-polyacrylamide gel electrophoresis on 8% gels and transferred to PVDF membranes. Blots were incubated with anti-MX1 (clone M143, Dr. Georg Kochs, University Medical Center Freiburg, Germany), TLR3, STAT1, XAF-1, RIG-I, RSAD2, OAS1, IFIT-1 or actin primary monoclonal antibodies (Cell Signaling Technology), followed by incubation with HRP-coupled secondary antibodies (Jackson Immuno research). Proteins were revealed using Enhanced Chemiluminescence Detection ECL (BioRad).

Results

The IFN I response controls MV replication and oncolytic activity.

In a previous study, we demonstrated that the sensitivity of MPM cell lines to MV oncolytic activity depends on defects of the antiviral IFN I response in tumor cells¹⁰. To confirm the role of the IFN I response on MV oncolytic activity we performed a first experiment where we exposed four MV-resistant MPM cell lines and two types of healthy cells (MESF mesothelial cells and CCD-19Lu fibroblast) to MV encoding the cherry fluorescent protein (MV-ch) in presence of the JAK1 and JAK2 inhibitor ruxolitinib that blocks the IFNAR signaling. We observed that healthy and MPM cells were resistant to MV-ch replication in absence of ruxolitinib (Figure 1A and 1B). This was probably due to the expression of ISG, as MV induce the expression of *MX1*, *TLR3* and *IRF7* in healthy and MV-resistant MPM cells (Figure 1C). However, adding ruxolitinib prevented ISG expression, releasing therefore the break on MV replication.

These results prove that MV replication and lytic activity are highly sensitive to a functional IFN I response and that defects in this signaling pathway in MPM tumor cells make them sensitive to the MV oncolytic activity.

IRF3 and NF-KB signaling is functional in MV-resistant and MV-sensitive MPM cell lines.

We then sought to identify the defects in the IFN I response affecting MPM cell lines that are sensitive to MV replication. We determined if the IRF3, IRF7 and NF-KB proteins were functional in MV-sensitive MPM cell lines and allowed the signal transduction from pattern recognition receptors (PRR) to the nucleus.

First, we assessed the expression of *IRF3*, *IRF7* and *RELA* genes that encode IRF3, IRF7 and p65, a sub-unit of NF-KB respectively (Figure 2A). *IRF3* and *RELA*

were constitutively expressed in all cell groups. *IRF7* was expressed at low basal level in MV-resistant MPM cell lines and barely detectable in healthy cells and MV-sensitive MPM cell lines. 48h after exposure to MV, we observed that *IRF3* and *IRF7* expression was increased in healthy cells and MV-resistant MPM cell lines but was not induced in MV-sensitive MPM cell lines. On the opposite, *RELA* expression increased after MV infection in the three groups. We tried to measure earlier at 12h or 24h the induction of *IRF3*, *IRF7* and *RELA* expression in response to MV without success (data not shown).

We then studied IRF3 and NF-KBp65 nuclear relocation 12h after MV exposure at high MOI by confocal microscopy. Without virus, IRF3 and p65 were not activated and were located mainly in the cytoplasm. After infection, we observed that both transcription factors relocated to the nucleus of MV-resistant MPM cells, as well as to the nucleus of MV-sensitive MPM cells (Figure 2B and 2C). No significant difference in the intensity of nuclear relocation was observed between the two groups (Figure 2D).

Altogether these results show that MV is well detected by MPM cell lines, even in cells that failed to produce IFN I. Detection of MV is then followed by a signal that reaches the nucleus via the relocation of IRF3 and p65 transcription factors.

The most frequent defect of the IFN I response in MV-sensitive MPM cell lines is HD of the genes encoding IFN- α and IFN- β .

Since the PRR signaling is functional in MV-sensitive MPM cell lines, the defects are probably located in the nucleus at the level of genes encoding IFN I. We thus performed a genomic PCR on six MV-resistant and the fifteen MV-sensitive MPM cell lines to assess the presence of the *IFNB1* gene that encodes IFN- β (Figure 3A).

Whereas six MV-resistant and seven MV-sensitive MPM cell lines had at least one copy of the *IFNB1* gene, eight out of the fifteen MV-sensitive MPM cell lines had a HD of the *IFNB1* gene: Meso 11, Meso 13, Meso 31, Meso 47, Meso76, Meso 96, Meso 163 and Meso 225.

The *IFNB1* gene is located on the chromosome 9 next to a cluster of thirteen genes encoding the IFN- α cytokines and close to the tumor suppressor gene *CDKN2A* that encodes p16^{INK4A} and p14^{ARF} (Figure 3B). In the next experiment, we measured the size of the deletions of the chromosome 9 region where the *IFNB1* gene is located. We performed a cytogenetic study by Cytoscan™ that allowed analyzing the genome of the eight MPM cell lines that have lost the *IFNB1* gene and 4 MV-resistant MPM cell lines (Figure 3B). This technique covers the entire human genome with specific probes spaced by 1Kb in average. We observed in the MV-sensitive MPM cell lines that the bi-allelic deletion of *IFNB1* genes located at the 21.08Mb position encompassed all the genes encoding the IFN- α cytokines between the 21.17 and 21.44Mb positions and extend after the *CDKN2A* gene located at the 21.97Mb position. We also found that the four MV-resistant MPM cell lines had at least one copy of the gene encoding the IFN I cytokines. However, in three out of the four MV resistant tumor cell lines the two alleles of the *CDKN2A* gene were deleted. Only the cell line Meso61 conserved one copy of the *CDKN2A* gene.

Then we sought to confirm the frequent HD of the *CDKN2A* and *IFNB1* genes on another bio-collection of 78 short term-cultured MPM cell lines (Figure 3C). We found the HD of the *CDKN2A* and *IFNB1* genes in 57 (73%) and 18 (23%) out of the 78 MPM cell lines respectively. 17 (30%) out of the 57 MPM cell lines that have lost the *CDKN2A* gene have also lost the *IFNB1* gene and 17 (94%) out of 18 MPM cell lines that have lost the *IFNB1* gene have also lost the *CDKN2A* gene (94%). One MPM

cell lines, MPM_51, lost only the two copies of the *IFNB1* gene while keeping the *CDKN2A* gene. Interestingly, the *IFNB1* gene HD was significantly more frequent in MPM with a sarcomatoid component, the most aggressive form of the disease (Fisher's exact test, p-value=0.0085), i.e. 17% of epithelioid MPM, 45% of biphasic MPM and 50% of sarcomatoid/desmoplastic MPM.

Then we wanted to determine if other alterations of the IFN I pathway exist in MPM and to estimate their frequencies. Thus, we looked by the cbiportal.org website (<http://www.cbiportal.org>) in the TCGA database on 87 MPM patients for alterations of *CDKN2A*, *IFNA2* and *IFNB1* genes, but also at several other genes that encodes protein implicated in the IFN I response (Figure 4A). We observed 43.8%, 18.4% and 9.2% of *CDKN2A*, *IFNA2* and *IFNB1* genes HD respectively. We found no other alteration on these genes except one fusion for *CDKN2A*. The genes encoding the other proteins of IFN I response pathway were rarely altered with two patients out of eighty-seven having a *STAT1* alteration and several patients showing a unique alteration either on *DDX58* (RIG I), *IFIH1* (MDA5), *STAT1*, *STAT2*, *JAK1* or *JAK2*. The alteration of *DDX58* and *IFIH1* were found in patients with IFN I genes HD, whereas alterations of *STAT1*, *STAT2*, *JAK1* or *JAK2* were found in patients without.

To determine if the IFN I gene HD have a role in MV-replication permissivity, we used CRISPR-cas9 technology and lentiviral transduction to knock out the *IFNB1* genes of the four MV-resistant MPM cell lines in order to determine if it was sufficient to make them permissive to MV replication (Supplemental Figure 1). We found that *IFNB1* inactivation was sufficient to make Meso45 permissive to MV replication (Supplemental Figure 1A and 1B). For the three other cell lines, it was not sufficient and the inactivation of some of the 13 genes encoding IFN- α may be required (Supplemental Figure 1C). We also restored the *IFNB1* gene and its promoter in 5 of

the 8 IFN I^{-/-} MPM cell lines by lentiviral transduction. Replication of MV was strongly reduced in 4 MPM cell lines (Meso11, Meso13, Meso47 and Meso225) and moderately in the last one (Meso163) (Supplemental Figure 2). Altogether these results show that IFNB1 expression is often sufficient to block MV replication, but *IFNA* genes may also play a role.

We then sought to determine if these HD are also frequent in other cancers. In the TCGA, we looked at copy number alterations of *CDKN2A*, *IFNA2* and *IFNB1* genes. Mesothelioma is the second cancer with the most frequent *CDKN2A* HD after glioblastoma multiforme (Figure 4B and 4C). Nine types of cancer, including lung squamous cell carcinoma, have a frequency of *CDKN2A* HD superior to 20% that always correlate with lower frequencies of *IFNA2* and *IFNB1* HD. Mutations of the IFN I genes, *IFNA2* and *IFNB1*, are rare in all cancers and absent in MPM meaning that alteration of IFN I genes in cancer occurs mainly by HD (Figure 4C).

Since IFN I gene HD are also frequent in lung cancer, we used the canSAR database <https://cansar.icr.ac.uk/cansar> to randomly select eleven lung cancer cell lines with four of them having IFN I genes HD to determine their permissivity to MV replication (Supplemental Figure 3). First, we confirmed by genomic PCR that IFN I genes were absent in the four cell lines (Supplemental Figure 3A). Then, we exposed the eleven lung tumor cell lines to MV-cherry and measured cherry fluorescence during 9 days. We observed that the four IFN I^{-/-} lung cancer cell lines were permissive to MV replication and that in presence of IFN I this permissivity was lost for three of them (Supplemental Figure 3B). five of the seven other lung cancer cell lines with at least one copy of the IFN I genes were also permissive suggesting that, like in MPM, other defects beside IFN I gene HD affect the IFN I pathway in lung cancer.

Altogether these results show that IFN I genes HD are frequent in cancers where *CDKN2A* gene HD is a malignancy driver.

MPM cell lines that have lost IFN I genes develop a partial IFN I response and a proinflammatory program after exposure to MV.

We then wanted to characterize how the MPM cell lines that have lost IFN I encoding genes (IFN I^{-/-} MPM cell lines) respond to MV or exogenous IFN I (IFN- α 2a and IFN- β 1a). Thus we performed a transcriptomic analysis on four types of healthy primary cells (G1: CCD19Lu fibroblasts, HMVEC endothelial cells, MESF peritoneal mesothelial cells and CEB lung epithelial cells), on four MV-resistant MPM cell lines (G2: Meso4, Meso45, Meso61 and Meso173) and on the eight IFN I^{-/-} MPM cell lines (G3: Meso11, Meso13, Meso31, Meso47, Meso76, Meso96, Meso163 and Meso225) (Figure 5, Supplemental Figure 4).

First we analyzed the expression of genes encoding type I, II and III IFN and their receptors (Figure 5A-B). Constitutive expression of these genes was comparable in the three groups (Figure 5B). After 48h of MV exposure, expression of *IFNB1* gene was induced only in some healthy cells (G1) and in resistant MPM lines (G2) and absent from IFN I^{-/-} MPM cell lines (G3), whereas no expression of the IFNAs genes was observed (Figure 5A). Expression of type III IFN genes (*IFNL1*, *IFNL2* and *IFNL3*) was strongly increased after exposition to MV in MPM cell lines (G2 and G3), but not in healthy cells (G1). We also observed that MV had no effect on expression of all IFN receptors. Furthermore, exposure of the different groups to exogenous IFN I had no effects on the expression of genes encoding type I, II and III IFN and their receptors.

Then, we investigated the expression of ISG by the three groups of cells in response to MV or type I IFN (Figure 5C-5D). When healthy cells (G1) and resistant

MPM lines (G2) were exposed to MV, we observed the overexpression of a large panels of ISG (Figure 5C) which encode proteins with varied antiviral functions²³. In response to IFN I, G1 and G2 expressed a similar set of ISG, although at a lower level. The MV resistant MPM cell lines (G2) developed a weaker response to MV and IFN I compared to healthy mesothelial cells, but the basal expression of these genes was often higher in G2 compared to G1 (Figure 5, Supplemental Figure 4), especially in Meso4 and to a lower extent Meso45 and Meso173 (Figure 5D). In contrast, the constitutive expression of these genes was lower in IFN I^{-/-} MPM cell lines (G3) compared to healthy mesothelial cells. In response to MV, IFN I^{-/-} MPM cell lines (G3) expressed a limited set of genes, such as *RSAD2*, *IFIT1*, *OAS1* and *DDX58* compared to G1 and G2 cells (Figure 5C). Other genes such as *IFI35*, *IFIT1M*, *XAF1* and *TLR3* expressed in response to MV in G1 and G2, failed to be expressed in G3 cells. However G3 cell lines were able to express these genes after exposure to IFN I. Altogether these results show that IFN I^{-/-} MPM cell lines (G3) develop a partial IFN I response characterized by the expression of a limited set of ISG independently of IFN I expression that does not succeed in controlling MV replication. The other set of ISG that requires IFN I is not expressed in response to MV by IFN I^{-/-} MPM cell lines resulting in absence of MV replication control.

We also detected in G3 a particular set of ISG that were expressed mainly in response to MV, but that were not or weakly expressed in the two other groups of cells, such as *TNFAIP3*, *ATF3*, *IRF1* and *CCL2* (Figure 5C and 5D, lower panels). This result suggests that in absence of IFN I, IFN I^{-/-} MPM cell lines develop an alternative response to MV compared to the two other groups of cells. Thus, we analyzed the expression of genes other than ISG in the three groups in response to MV or exogenous IFN I (Supplemental Figure 5) and using Ingenuity Pathway Analysis

(Ingenuity® Systems) we identified canonical cellular pathways that are induced by the virus in the three groups (Figure 5E). In G1 and G2, the virus mainly induced pathways that are typical of the IFN I response such as “Activation of IRF by Cytosolic Pattern Recognition Receptors” and “Interferon Signaling” pathways. In the G3, the IFN I response was also induced by MV, but we observed that numerous other pathways were activated contrary to G1 and G2. These pathways can be summarized in two main types: an inflammatory response with activated pathways such as “TNFR2 Signaling”, “IL-17A Signaling in Gastric Cells”, “IL-6 Signaling”, “iNOS Signaling”, “Death Receptor Signaling” and “TNFR1 Signaling” and an endoplasmic reticulum stress response characterized by activated pathway such as “Unfolded protein response”, “Protein Ubiquitination Pathway” and “Endoplasmic Reticulum Stress Pathway”.

Altogether these results show that before being lysed by MV, IFN I^{-/-} MPM cell lines engage transcriptionally in a partial, dysfunctional IFN I response characterized by the expression of a limited set of ISG. In addition, these cells develop an inflammatory response associated with an endoplasmic reticulum stress response.

Partial and weak expression of proteins encoded by ISG in IFN I^{-/-} MPM cell lines exposed to MV.

The transcriptomic study pointed out two types of ISG: the ISG that are transcribed in response to MV without the presence of IFN I, and the ISG that are transcribed only in presence of IFN I. Thus, in the next experiment we wanted to confirm the transcriptomic study by qRT-PCR and to measure by western-blot the level of proteins encoded by these two types of ISG (Figure 6).

First, we analyzed four ISG expressed in response to MV without the presence of IFN I: *RSAD2* (Viperin), *IFIT1* (ISG56), *OAS1* and *DDX58* (RIG I). The expression of these ISG measured by RT-qPCR was increased by both, MV and IFN I, in IFN I^{-/-} MPM cell lines, confirming results from the transcriptomic study (Figure 6A). By western-blot, we observed a stronger basal expression of these proteins in MV-resistant MPM cell lines, especially Meso4 and Meso45, compared to IFN I^{-/-} MPM cell lines thereby suggesting a constitutively activated IFN I response in MV-resistant cells (Figure 6B). The expression of these proteins was upregulated after exposure to MV or IFN I in all MV-resistant MPM cell lines. In contrast, in IFN I^{-/-} MPM cell lines, MV induced only low levels of these proteins, whereas IFN I induced a higher expression similar to what was observed in MV-resistant cells.

We then confirmed by RT-qPCR that the expression of the ISG *XAF1*, *TLR3* and *STAT1* was only induced by IFN I in IFN I^{-/-} MPM cell lines and not by exposure to MV (Figure 6C). The proteins encoded by this IFN I-dependent ISG are also more constitutively expressed in MV-resistant MPM cell lines compared to IFN I^{-/-} MPM cell lines (Figure 6D). In MV-resistant MPM cell lines, MV as well as IFN I induced a strong expression of these proteins. In contrast, except for Meso163, MV did not induce these proteins in IFN I^{-/-} MPM cell lines, whereas they were expressed at high level in response to IFN I.

Altogether these results show that IFN I^{-/-} MPM cell lines develop a weak and partial IFN I response at the protein level that is not sufficient to control MV replication. They also point out that two of the four MV-resistant MPM cell lines display a high constitutive level of proteins involved in the antiviral IFN I response.

Discussion

In this study, we demonstrated that the sensitivity of human MPM cells to the attenuated MV oncolytic activity is often due to IFN I genes HD. These frequent deletions are associated with the simultaneous HD of the tumor suppressor gene *CDKN2A* also located in the 9p21.3 chromosome region. These co-deletions are frequent in other cancers such as glioblastoma multiforme, melanoma or lung squamous cell carcinoma. We also showed that IFN I^{-/-} tumor cells that are sensitive to MV oncolytic activity are able to detect the virus and respond by relocating IRF3 and NF- κ B in the nucleus. Thus, before being lysed by MV, the IFN I^{-/-} tumor cells engage in a partial IFN I response characterized by the weak expression of a limited number of ISG compared to the large panel of ISG that are expressed by healthy cells and MV-resistant MPM exposed to MV. The IFN I^{-/-} tumor cells also develop a pro-inflammatory response and endure a stress of the endoplasmic reticulum that are hallmarks of immunogenic cell death. Our work provides a better understanding of MV oncolytic activity against MPM.

Inactivation of the tumor suppressor gene *CDKN2A* is a key driver of mesothelioma^{16, 24}. HD of *CDKN2A* and also of the IFN I genes have been described in MPM as soon as the nineties, but these studies mainly focused on the *CDKN2A* locus and failed to estimate the frequency of IFN I gene HD^{25, 26}. In our study, we showed that 36% of the MPM cell lines (8/22) from our collection have lost both copies of the *IFNB1* gene while they all lost the two copies of *CDKN2A* except Meso61 (data not shown). However, a majority of these MPM cell lines have more than ten passages *in vitro* and there could be a culture bias that makes *CDKN2A*^{-/-} MPM cell lines easier to establish. To get a more accurate estimation of the IFN I genes HD frequency in MPM, we measured this frequency in a series of eighty short term MPM cell lines. We

found that 71% and 23% of these cell lines have lost the two copies of *CDKN2A* and *IFNB1* genes respectively. These percentages probably better reflect the reality as they correspond to the 60% to 74% of *CDKN2A* HD found by fluorescence in situ hybridization²⁷⁻³⁰. Interestingly *IFNB1* HD are more frequent in mesothelioma cell lines with a sarcomatoid component (biphasic, sarcomatoid and desmoplastic MPM) with 47% of them harboring these deletions. In the TCGA public database, we found that 43.8% and 9.2% of MPM frozen tumor samples lost the two copies of *CDKN2A* and *IFNB1* genes respectively. However these results are likely underestimated, since they are obtained by high-throughput sequencing of tumor biopsies that contains healthy cells which can hide HD only present in tumor cells as it was demonstrated for *BAP1* gene, also frequently deleted in MPM³¹.

IFN I genes HD is not the only defect of the IFN I response in MPM. Indeed, seven out of the fifteen MV-sensitive cell lines have at least one copy of the *IFNB1* gene. Three of them behave like IFN I^{-/-} MPM cell lines by failing to produce IFN I in response to MV, whereas the other four produce IFN I in response to MV, but failed to control viral replication¹⁰. This converging selection of tumor cells toward inactivation of the IFN I response strongly suggests that the IFN I genes HD are not just a collateral damage of the loss of the *CDKN2A* locus, but probably bring additional advantages for tumor development in MPM.

Among cancers that have lost IFN I genes, glioblastoma multiforme displays the highest frequency of these HD (16.4% for *IFNB1* and 25.7% for *IFNA2*). This cancer is also one of the most studied for oncolytic immunotherapy with different viruses such as herpes simplex, adenovirus and parvovirus^{32, 33}. Recently, the group of Evanthia Galanis reported in human tumor xenograft models in mice and by monitoring ten glioblastoma patients receiving MV oncolytic immunotherapy that the main

transcriptional signature associated with resistance was the presence of a constitutive activation of the IFN I response in tumor cells³⁴. This gene signature can be used to predict which patients are likely to respond to MV oncolytic immunotherapy. We also found a constitutive activation of the type I IFN pathway in several MV-resistant MPM cell lines compared to healthy cells, whereas none of the eight IFN I^{-/-} MPM cell lines present a constitutive activation of the IFN I pathway.

In four out of five IFN I^{-/-} MPM cell lines, restoration of the *IFNB1* genes led to the blocking of MV replication without the need to restore *IFNA* genes, whereas in the last one, blocking was partial and may require restoration of some *IFNA* genes. We focused on the *IFNB1* gene since after 48h of MV exposure, it was the most expressed IFN I genes in resistant MPM cell lines. We also inactivated the *IFNB1* genes in the four MV-resistant MPM cell lines and only one tumor cell line became permissive to MV replication. Inactivation of *IFNB1* gene was not sufficient for the other three cell lines. Altogether these results show a major contribution of the *IFNB1* gene in controlling MV replication, but *IFNA* genes may also play a role.

In absence of IFN I genes, MPM cell lines exposed to MV develop a weak and partial IFN I response characterized by the expression of a limited number of ISG, such as *RSAD2*, *IFIT1*, *OAS1* and *DDX58*. They fail to express other ISG, such as *XAF1*, *TLR3*, *STAT1* and *MX1* that need the presence of IFN I to be expressed. This group of ISG that can be directly activated by IRF3 independently of IFN I as a part of the early antiviral response is not sufficient to control MV replication. In addition to this weak and partial IFN I response, IFN I^{-/-} MPM cell lines also develop a pro-inflammatory response such as TNFR1/TNFR2, IL-17A, IL-6, iNOS signaling and a stress of the endoplasmic reticulum. This corroborates the *in vivo* model described by the group of Michael Diamond where *IFNAR*^{-/-} myeloid cells that are equivalent to the IFN I^{-/-} MPM

cell lines in our study develop an exacerbated pro-inflammatory TNF- α response when infected by the West Nile virus ³⁵. Our and their studies show that in absence of the IFN I signaling, the pro-inflammatory response is amplified. This pro-inflammatory response and the stress of the endoplasmic reticulum are probably part of the immunogenic cell death induced by MV replication. We and other showed that tumor cells lysed by MV are immunogenic by releasing tumor-associated antigens and danger signals from cellular or viral origins ³⁶⁻⁴³. These signals induce the maturation of myeloid and plasmacytoid dendritic cells that are in turn able to cross-present tumor antigens to T cells. Thus our study identify the frequent IFN I genes HD as a therapeutic targets to induce cell death of tumor cells with oncolytic MV for the treatment of MPM.

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References

1. Achard C, Surendran A, Wedge ME, et al. Lighting a Fire in the Tumor Microenvironment Using Oncolytic Immunotherapy. *EBioMedicine* 2018;31:17-24.
2. Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. *Nat Biotechnol* 2012;30:658-670.
3. Guillerme JB, Gregoire M, Tangy F, et al. Antitumor virotherapy by attenuated measles virus (MV). *Biology (Basel)* 2013;2:587-602.
4. Robinson S, Galanis E. Potential and clinical translation of oncolytic measles viruses. *Expert Opin Biol Ther* 2017;17:353-363.
5. Dorig RE, Marcil A, Chopra A, et al. The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* 1993;75:295-305.
6. Nanche D, Varior-Krishnan G, Cervoni F, et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J Virol* 1993;67:6025-6032.
7. Fishelson Z, Donin N, Zell S, et al. Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors. *Mol Immunol* 2003;40:109-123.
8. Ravindranath NM, Shuler C. Expression of complement restriction factors (CD46, CD55 & CD59) in head and neck squamous cell carcinomas. *J Oral Pathol Med* 2006;35:560-567.
9. Anderson BD, Nakamura T, Russell SJ, et al. High CD46 receptor density determines preferential killing of tumor cells by oncolytic measles virus. *Cancer Res* 2004;64:4919-4926.
10. Achard C, Boisgerault N, Delaunay T, et al. Sensitivity of pleural mesothelioma to oncolytic measles virus depends on defects of the type I interferon response. *Oncotarget* 2015;Nov 2.
11. Berchtold S, Lampe J, Weiland T, et al. Innate immune defense defines susceptibility of sarcoma cells to measles vaccine virus-based oncolysis. *J Virol* 2013;87:3484-3501.
12. Noll M, Berchtold S, Lampe J, et al. Primary resistance phenomena to oncolytic measles vaccine viruses. *Int J Oncol* 2013;43:103-112.
13. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014;32:513-545.
14. Grandvaux N, Servant MJ, tenOever B, et al. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. *J Virol* 2002;76:5532-5539.
15. Nakaya T, Sato M, Hata N, et al. Gene induction pathways mediated by distinct IRFs during viral infection. *Biochem Biophys Res Commun* 2001;283:1150-1156.
16. Yap TA, Aerts JG, Popat S, et al. Novel insights into mesothelioma biology and implications for therapy. *Nat Rev Cancer* 2017;17:475-488.
17. Gueugnon F, Leclercq S, Blanquart C, et al. Identification of novel markers for the diagnosis of malignant pleural mesothelioma. *Am J Pathol* 2011;178:1033-1042.
18. Hackett TL, Warner SM, Stefanowicz D, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. *Am J Respir Crit Care Med* 2009;180:122-133.
19. de Reynies A, Jaurand MC, Renier A, et al. Molecular classification of malignant pleural mesothelioma: identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. *Clin Cancer Res* 2014;20:1323-1334.

20. Tranchant R, Quétel L, Tallet A, et al. Co-occurring Mutations of Tumor Suppressor Genes, LATS2 and NF2, in Malignant Pleural Mesothelioma. *Clin Cancer Res* 2017;23:3191-3202.
21. Combredet C, Labrousse V, Mollet L, et al. A molecularly cloned Schwarz strain of measles virus vaccine induces strong immune responses in macaques and transgenic mice. *J Virol* 2003;77:11546-11554.
22. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nature methods* 2012;9:676-682.
23. Schoggins JW, Wilson SJ, Panis M, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 2011;472:481-485.
24. Jean D, Daubriac J, Le Pimpec-Barthes F, et al. Molecular changes in mesothelioma with an impact on prognosis and treatment. *Arch Pathol Lab Med* 2012;136(3):277-293.
25. Cheng JQ, Jhanwar SC, Klein WM, et al. p16 alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. *Cancer Res* 1994;54:5547-5551.
26. Xio S, Li D, Vijg J, et al. Codeletion of p15 and p16 in primary malignant mesothelioma. *Oncogene* 1995;11:511-515.
27. Monaco SE, Shuai Y, Bansal M, et al. The diagnostic utility of p16 FISH and GLUT-1 immunohistochemical analysis in mesothelial proliferations. *Am J Clin Pathol* 2011;135:619-627.
28. Chiosea S, Krasinskas A, Cagle PT, et al. Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2008;21:742-747.
29. Dacic S, Kothmaier H, Land S, et al. Prognostic significance of p16/cdkn2a loss in pleural malignant mesotheliomas. *Virchows Arch* 2008;453:627-635.
30. Illei PB, Rusch VW, Zakowski MF, et al. Homozygous deletion of CDKN2A and codeletion of the methylthioadenosine phosphorylase gene in the majority of pleural mesotheliomas. *Clin Cancer Res* 2003;9:2108-2113.
31. Yoshikawa Y, Emi M, Hashimoto-Tamaoki T, et al. High-density array-CGH with targeted NGS unmask multiple noncontiguous minute deletions on chromosome 3p21 in mesothelioma. *Proc Natl Acad Sci U S A* 2016;Nov 22;113(47):13432-13437.
32. Kaufmann JK, Chiocca EA. Glioma virus therapies between bench and bedside. *Neuro Oncol* 2014;16:334-351.
33. Marchini A, Bonifati S, Scott EM, et al. Oncolytic parvoviruses: from basic virology to clinical applications. *Virology journal* 2015;12:6.
34. Kurokawa C, Iankov ID, Anderson SK, et al. Constitutive Interferon Pathway Activation in Tumors as an Efficacy Determinant Following Oncolytic Virotherapy. *Journal of the National Cancer Institute* 2018.
35. Pinto AK, Ramos HJ, Wu X, et al. Deficient IFN signaling by myeloid cells leads to MAVS-dependent virus-induced sepsis. *PLoS Pathog* 2014;10:e1004086.
36. Delaunay T, Violland M, Boisgerault N, et al. Oncolytic viruses sensitize tumor cells for NYESO-1 tumor antigen recognition by CD4+ effector T cells. *in revision* 2017;Dec 26;7(3):e1407897.
37. Achard C, Boisgerault N, Delaunay T, et al. Induction of immunogenic tumor cell death by attenuated oncolytic measles virus. *J Clin Cell Immunol* 2015;in press.
38. Achard C, Guillerme JB, Bruni D, et al. Oncolytic measles virus induces Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity by human myeloid and plasmacytoid dendritic cells. *Oncoimmunology* 2016;6:e1261240.

39. Donnelly OG, Errington-Mais F, Steele L, et al. Measles virus causes immunogenic cell death in human melanoma. *Gene Ther* 2011.
40. Gauthier A, Brandler S, Sapède-Peroz C, et al. Measles virus induces oncolysis of mesothelioma cells and allows dendritic cells to cross-prime tumor-specific CD8 response. *Cancer Res* 2008;68:4882-4892.
41. Guillerme JB, Boisgerault N, Roulois D, et al. Measles virus vaccine-infected tumor cells induce tumor antigen cross-presentation by human plasmacytoid dendritic cells. *Clin Cancer Res* 2013;19:1147-1158.
42. Fonteneau JF, Guillerme JB, Tangy F, et al. Attenuated measles virus used as an oncolytic virus activates myeloid and plasmacytoid dendritic cells. *Oncol Immunology* 2013;2:e24212.
43. Galanis E, Atherton PJ, Maurer MJ, et al. Oncolytic measles virus expressing the sodium iodide symporter to treat drug-resistant ovarian cancer. *Cancer Res* 2015;75:22-30.

Figure legends

Figure 1: The IFN I response controls MV replication and oncolytic activity. Cells were treated 3 days before infection with ruxolitinib (1 μ M) and infected with MV encoding cherry fluorescent protein (MV-ch) (A and B) or with MV (C-D) at MOI=1. Viral replication of MV-ch was followed by measuring cherry fluorescence at 610 nm during 9 days in healthy cells (A) or in MV-resistant MPM cell lines (B). Relative RNA expression of three ISG, *Mx1*, *TLR3* and *IRF7*, was studied 48h after infection by RT-qPCR in healthy cells (C) or in resistant MPM cell lines (D). *RPLP0* gene expression was used as reference and indicated values are means \pm SEM of relative expression of three independent experiments. NI = non-infected, ruxo = ruxolitinib.

Figure 2: IRF3 and NF-KB signaling is functional in MV-sensitive cells. (A) Cells were infected at MOI=1 during 48h and *IRF3*, *IRF7* and *RELA* (NF-KB p65) genes expression was studied by RT-qPCR with *RPLP0* gene expression used as reference. Indicated values are means \pm SEM of relative expression of three independent experiments. NI = non-infected. ns=non-significative, * $p<0.05$; ** $p<0.01$; *** $p<0.001$, (Wilcoxon-Mann-Whitney test). (B) Cells were infected at MOI=10 during 12h. Then localization of IRF3 and NF-kBp65 was determined using IRF3 and NF-kBp65 specific monoclonal antibodies and a Dylight 488-conjugated secondary antibodies (green). Membranes were labelled with a lectin marker WGA (red) and nucleus with Hoescht (blue). Fluorescence was analyzed by confocal microscopy. (C) Ratio of the nuclei/cytoplasm fluorescence intensity was measured with the software Volocity software on more than 100 cells.

*Figure 3: Frequent bi-allelic deletion of the genes encoding IFN- α and IFN- β in MV-sensitive MPM cell lines. (A) Genomic DNA of MPM cell lines was extracted and presence of the *IFNB1* gene was analyzed by PCR with *IFNB1* primers (chr9:21077267-21078217). (B) Analysis of the copy number of the chromosome 9p21.3 chromosome region containing IFN I and *CDKN2A* genes was performed on extracted DNA from MPM cell lines. Each blue dot represents a genomic probe. Red lines represent location of *IFNB1*, *IFNA2* and *CDKN2A* genes. (C) Analysis of the copy numbers of the *IFNB1* and the *CDKN2A* genes was determined on genomic DNA of 78 short term-cultured MPM cell lines. Genomic DNA was amplified by PCR using *IFNB1* primers (chr9:21077267-21078217) and the deletion status was determined based on the presence of an amplification by capillary electrophoresis. *IFNB1* and *CDKN2A* deletion status, and histologic types are indicated in the heat map (DEL: bi-allelic deletion; WT: wild-type; MME: epithelioid MPM; MMB: biphasic MPM; MMS: sarcomatoid MPM; MMD: desmoplastic MPM; ND: not determined).*

*Figure 4: Bi-allelic deletion of IFN I encoding genes are frequent in mesothelioma and other cancers. (A) Frequency of alteration of genes coding for protein implicated in the IFN I response in mesothelioma. (B) Frequency of *CDKN2A*, *IFNA2* and *IFNB1* genes bi-allelic deletions in different types of cancer. (C) Frequency of alteration of *CDKN2A*, *IFNA2* and *IFNB1* genes in different types of cancer. These results were obtained from TCGA database using cbiportal.org website. GBM=Glioblastoma multiforme, DLBC=diffuse large B-cell lymphoma, LGG=Low grade glioma, ACC=adenoid cystic carcinoma, pRCC=papillary renal cell carcinoma, ccRCC=clear renal cell carcinoma, cRCC= chromophobe renal cell carcinoma, AML=acute myeloid leukemia.*

Figure 5: Partial IFN I and proinflammatory response from IFN I^{-/-} MPM cell lines exposed to MV. Cells were infected with MV at MOI=1 or treated with IFN I (IFN- α and IFN- β) during 48h. Equal amounts of RNA from three separate experiments were mixed. Transcriptome analysis was performed with Affymetrix human Gene 2.0 ST arrays. (A) Differential expression of the IFN genes and their receptors were analyzed comparing the MV infected or IFN I treated cells with untreated cells. (B) Basal expression of the IFN genes and their receptors were analyzed comparing untreated cells with untreated healthy mesothelial cells MESF. (C) Differential expression of the interferon stimulated genes (ISG) were analyzed comparing the MV infected or IFN I treated cells with untreated cells. (D) Basal expression of the ISG were analyzed comparing untreated cells with untreated healthy mesothelial cells MESF. (E) Canonical pathway study was carried out with Ingenuity Pathway Analysis (Ingenuity® Systems) for each group and binary logarithm of fold change was defined.

Figure 6. MV exposure induce a partial and weak expression of proteins encoded by ISG in IFN I^{-/-} MPM cell lines. Cells were infected with MV at MOI=1 or treated with IFN I (IFN- α and IFN- β) during 48h. (A) RNA expression of four ISG induced by MV in IFN I^{-/-} MPM cells (*RSAD2*, *IFIT1*, *OAS1* and *DDX58*) analyzed by RT-qPCR with *RPLPO* gene expression used as reference. Results are expressed as means \pm SEM of relative expression of three independent experiments and are also expressed as $\Delta\Delta Ct$ between conditions MV and NI (MV), and conditions IFN I and NI (IFN I). ns=non-significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, one-way ANOVA (Kruskal-Wallis test) (B) Protein expression of ISG induced by MV in IFN I^{-/-} MPM cells (*RSAD2*, *IFIT1*, *OAS1* and *RIG I*) analyzed by western-blot with actin protein expression used as a reference. (C) RNA expression of four ISG that are not induced by MV in IFN I^{-/-} MPM cells (*XAF-*

1, TLR3, STAT1 and Mx1) analyzed by RT-qPCR with *RPLPO* gene expression used as reference. Results are expressed as means \pm SEM of relative expression of three independent experiments. (D) Protein expression of ISG that are not induced by MV in IFN I^{-/-} MPM cells (XAF-1, TLR3, STAT1 and Mx1) analyzed by western-blot with actin protein expression used as a reference.

Figure 1

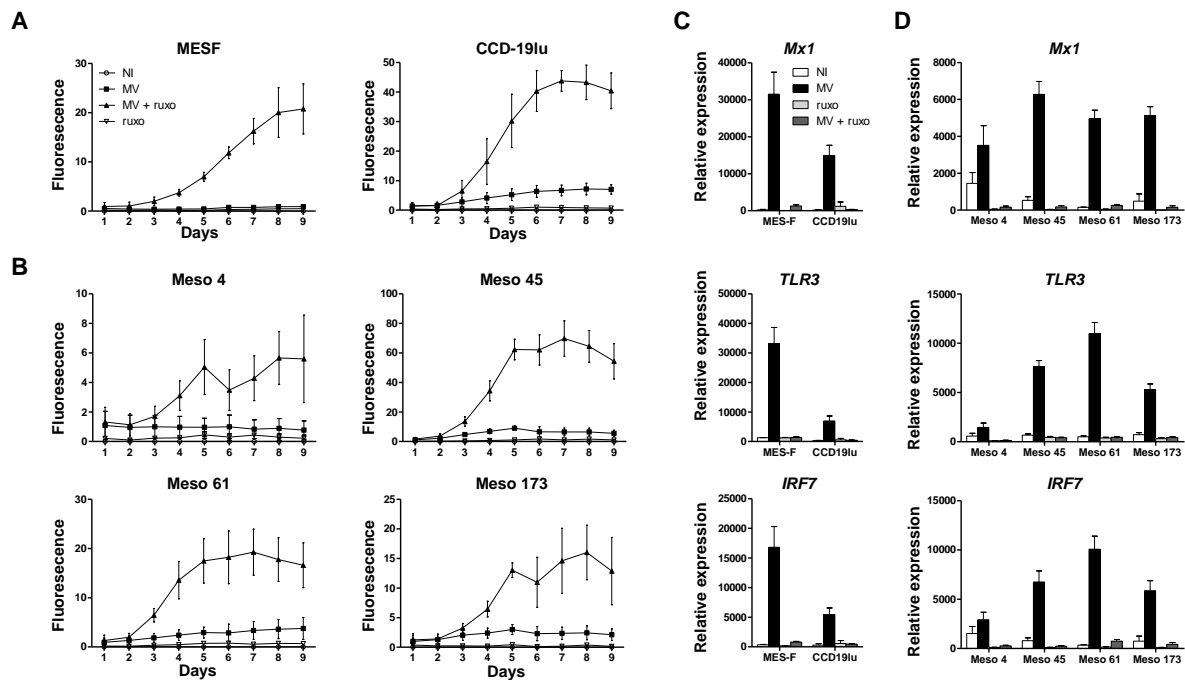


Figure 2

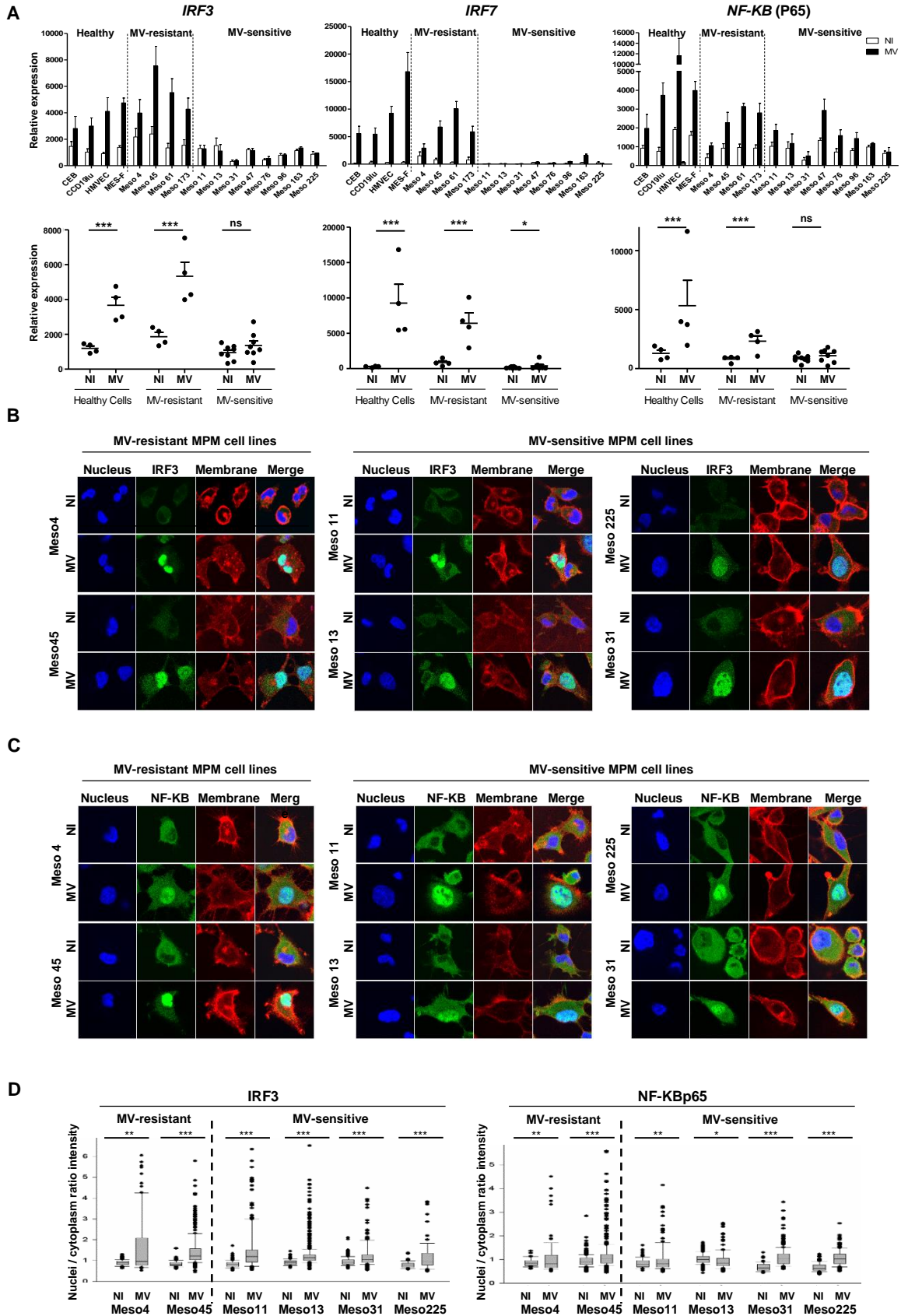


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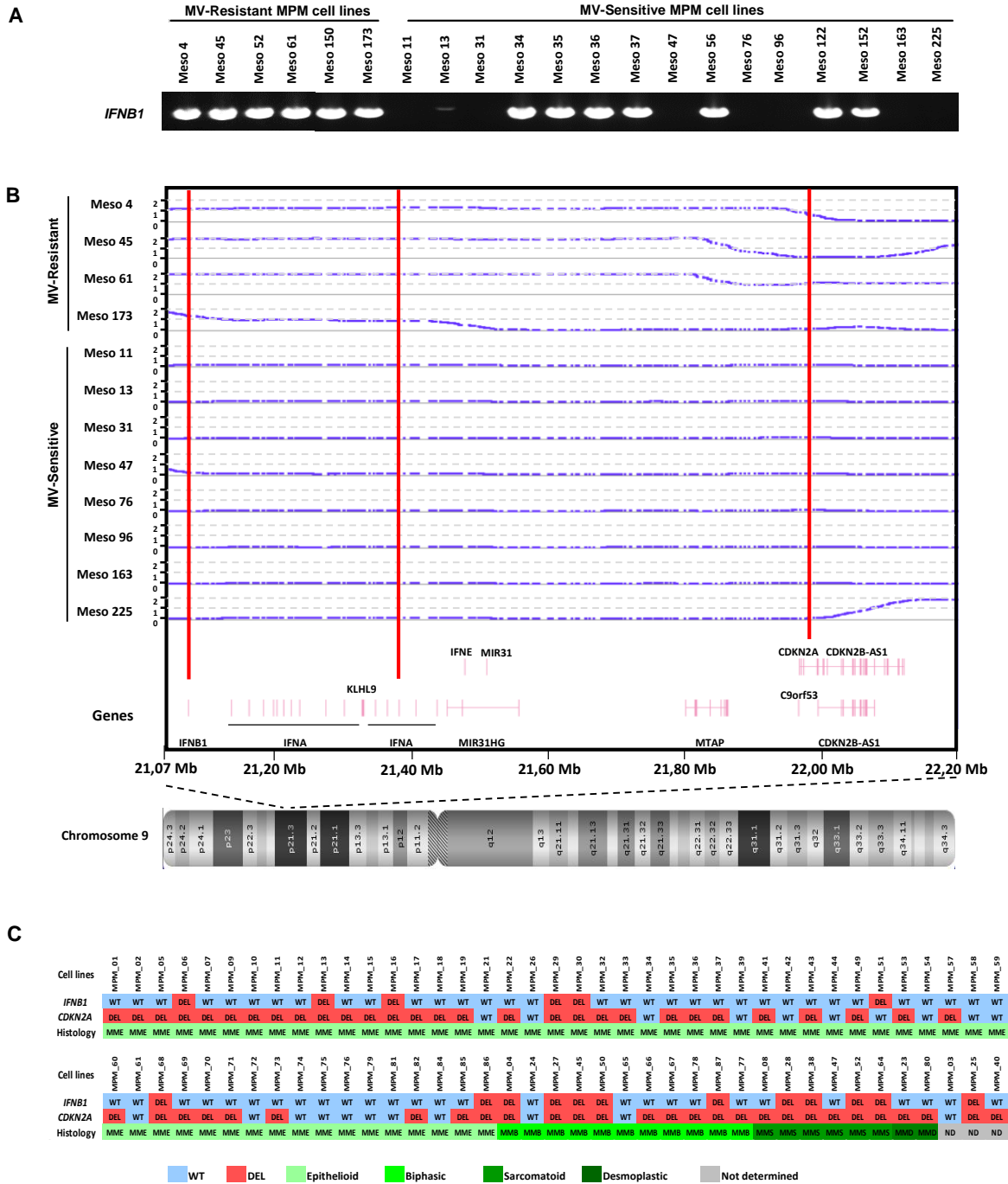


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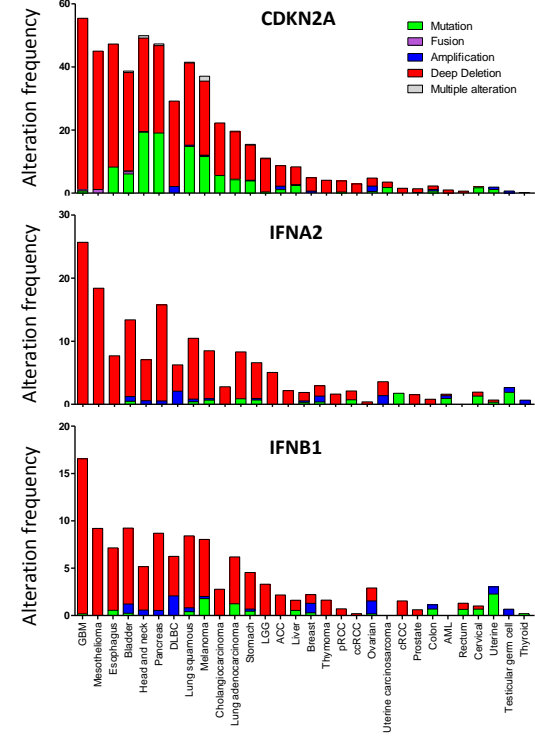
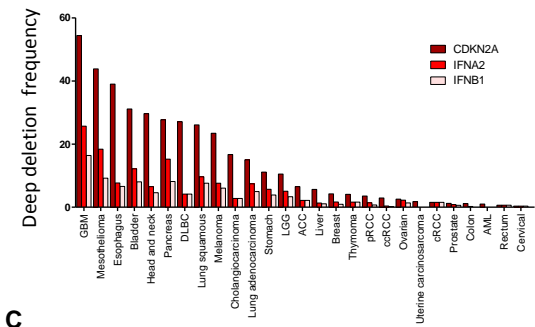
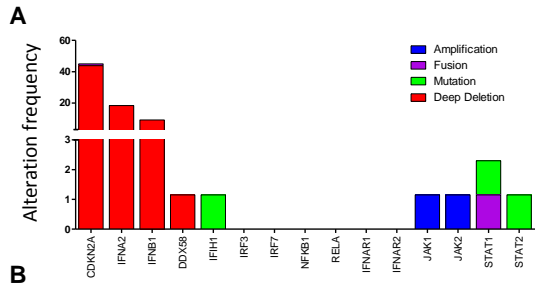


Figure 5

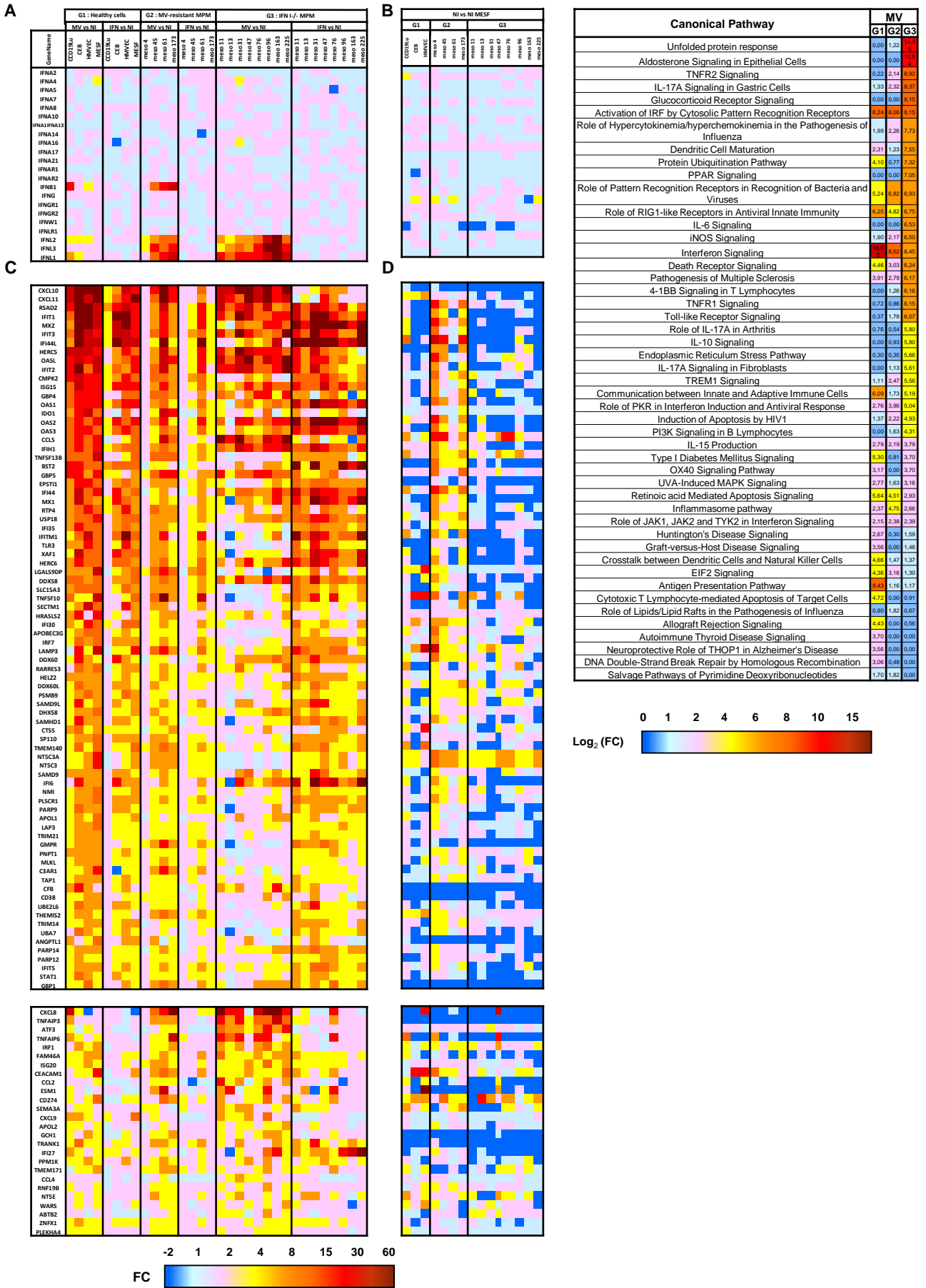
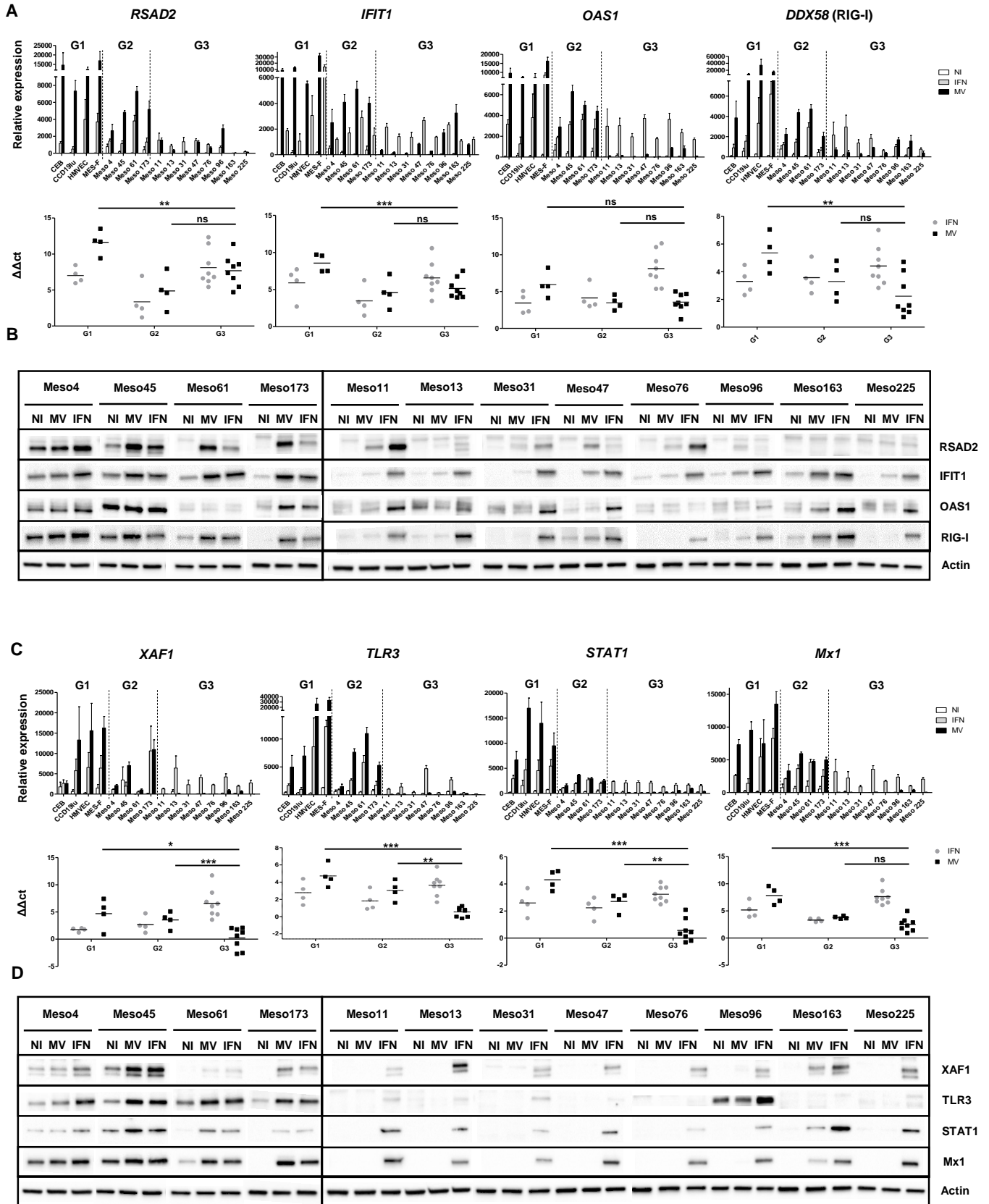
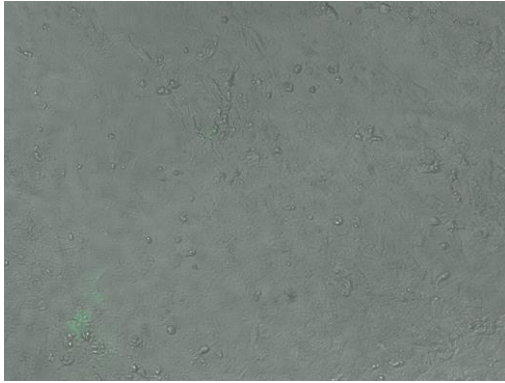
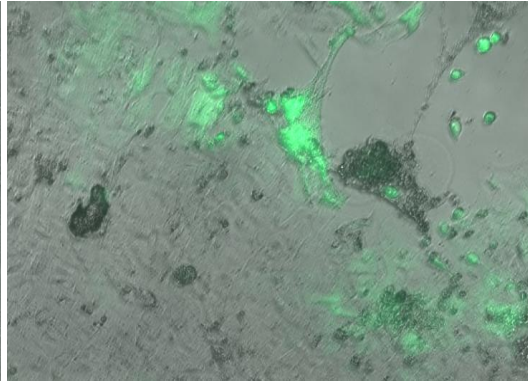
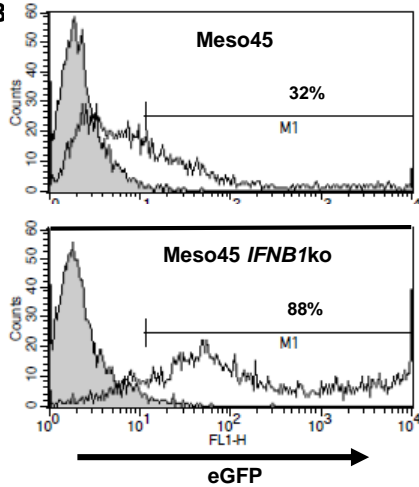
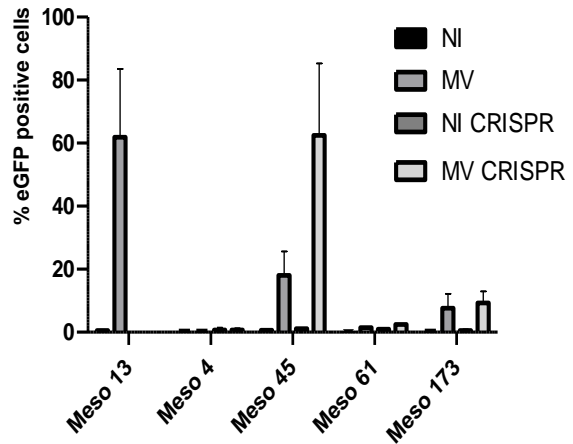
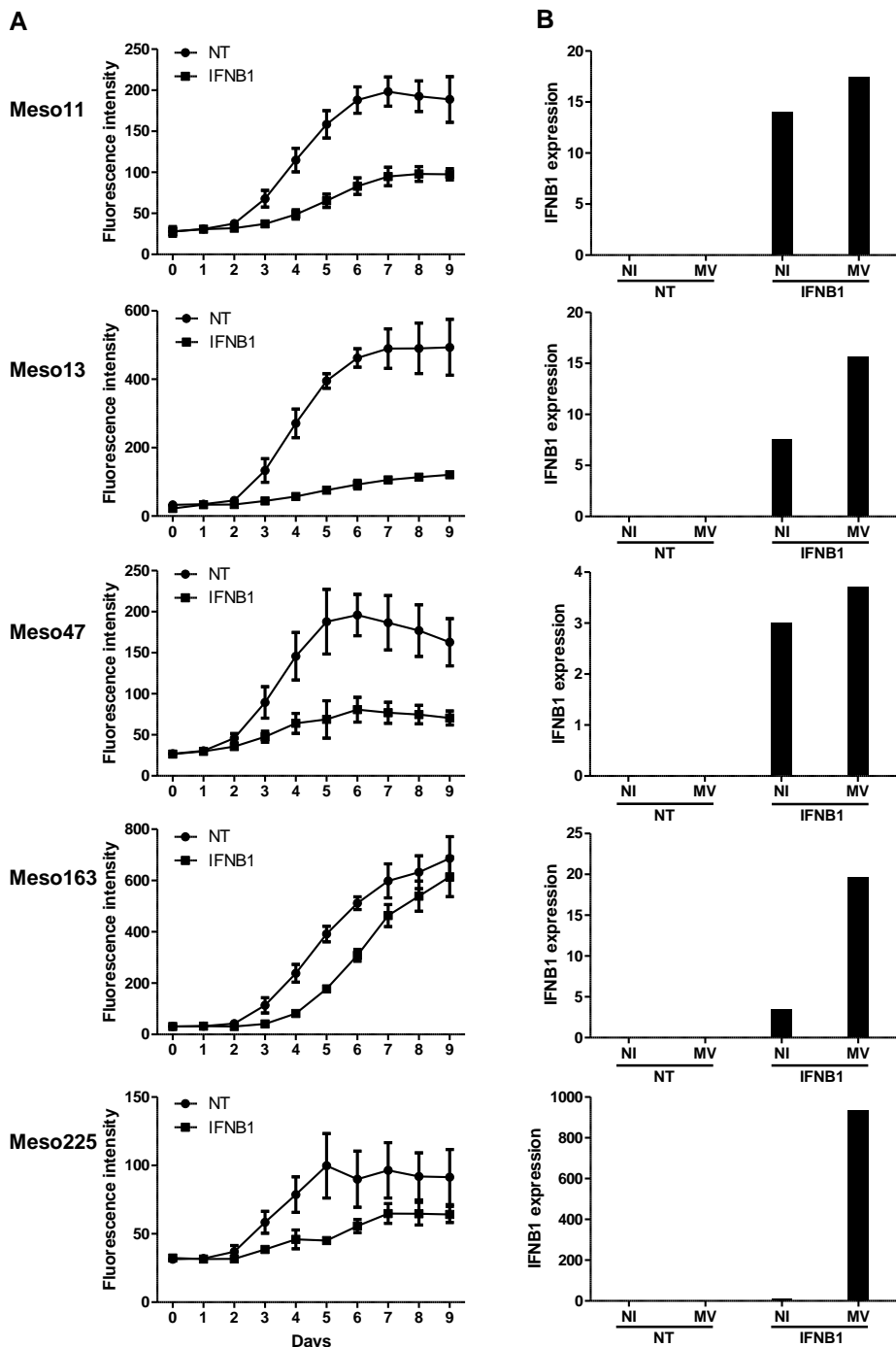


Figure 6

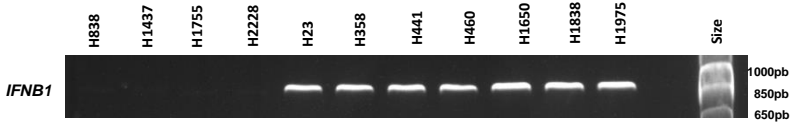
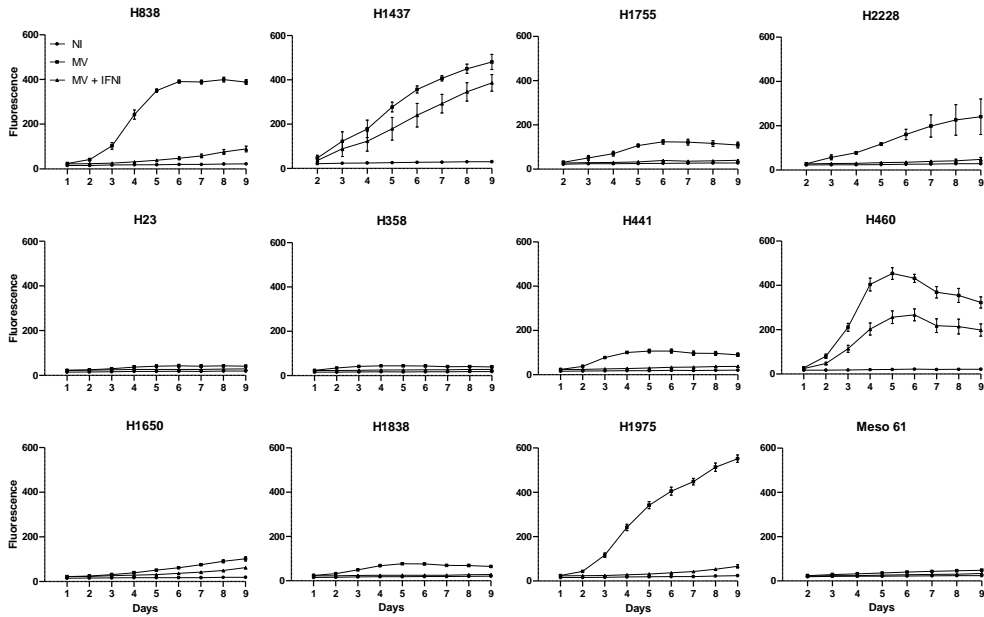


A**Meso45 + MVeGFP****Meso45 *IFNB1*ko + MVeGFP****B****C**

Supplemental Figure 1. Knock out of the *IFNB1* genes in MV-resistant MPM cell lines is not sufficient to make them permissive to MV replication. The four MV-resistant cell lines Meso4, Meso45, Meso61 and Meso173 were transduced using the lentiviral vector plentiCrisprV2 encoding the RNA guide 5'-GCCTCCCATTC AATTGCCAC-3' (Genscript, Netherlands) to break the *IFNB1* gene at the location encoding the Leucine 42. (A) Meso45 or Meso45 *IFNB1*ko were exposed to MV-eGFP. After four days, eGFP fluorescence was analyzed by microscopy (A) or cytometry (B). (C) the four original or *IFNB1* Ko MV-resistant MPM cell lines were exposed to MV-eGFP. Meso13 MPM cell line was used as a positive control. After four days, eGFP fluorescence was analyzed by flow cytometry. Results represent the mean with standard deviation of three independent experiments.



Supplemental Figure 2. Transduction of the IFNB1 gene and its promoter in IFN I^{-/-} MPM cell lines blocks MV replication. The five IFN I^{-/-} MPM cell lines Meso11, Meso13, Meso47, Meso163 and Meso225 were transduced using the lentiviral vector pLx307 (Addgene, #98377) with insertion between XhoI (3) - MluI of the IFNB1 gene and its promoter (NC_000009.12: 21,077,051-21,079,050; Genscript, Netherlands). (A) non-transduced (NT) and *IFNB1* transduced (IFNB1) MPM cell lines were plated at 10,000 cells/wells in 96 well plates. One day later, they were exposed to MV-Cherry at MOI=1 and Cherry fluorescence was measured every day during 9 days. Results represent the mean with standard deviation of three independent experiments. (B) non-transduced (NT) and *IFNB1* transduced (IFNB1) MPM cell lines were plated at 500,000 cells/wells in 6-well plates. One day later, they were cultured alone (NI) or with MV-Cherry at MOI=1 (MV). Two days later, expression of IFNB1 was measured by RT-PCR relative to RPLPO gene expression.

A**B**

Supplemental Figure 3: Lung cancer cell lines with IFN I genes HD are permissive to MV replication. Lung cancer cell lines NCHI-H23, NCHI-H358, NCHI-H441, NCHI-H460, NCHI-H838, NCHI-H1437, NCHI-H1650, NCHI-H1755, NCHI-H1838, NCHI-H1975 and NCI-H2228 were purchased from Igcstandards-atcc.org. They were cultured in the same conditions as MPM cell lines as described in material and methods (A) Genomic DNA of lung cancer cell lines was extracted and the presence of the *IFNB1* gene was analyzed by PCR with *IFNB1*-specific primers (chr9:21077267-21078217) as described in material and methods. (B) lung cancer cell lines and the MPM cell line Meso61 used as negative control were plated at 10,000 cells/wells in 96 well plates and were treated or not with 1,000 u/ml of IFN- α 2 and IFN- β . One day latter, they were exposed to MV-Cherry at MOI=1 and Cherry fluorescence was measured every day during 9 days. Results represent the mean with standard deviation of three independent experiments.

Genomic PCR

For the eighty MPM cell lines of the validation series, genomic DNA was extracted using a standard isopropanol precipitation procedure. PCR was performed on genomic DNA (40 ng) using Phusion U Multiplex PCR according to the manufacturer's protocol (Thermo Scientific). The same couple of primers as above was used for the *IFNB1* gene and the following couple was used for the *IFNB1* promoter 5'-GCCTCCACAGATACCAAATCA-3' (forward) and 5'-AAGCCTCCCATTCAATTGCC-3' (reverse). The following primers were used for *CDKN2A* : 5'-CGGTAGGGACGGCAAGAGAG-3' (forward) and 5'-CCTGTAGGACCTTCGGTGA-3' (reverse), and for *ARID1A* 5'-TTCGCAACTGGACTTTCTCTC-3' (forward) and 5'-TCAAATTAGCTAACTTCCAACC-3' (reverse). *IFNB1* was amplified by PCR including an initial denaturation step at 98°C for 30 s; 40 cycles including denaturation at 98°C for 10s, annealing at 60°C for 30s, extension at 72 °C for 30s; and a final extension at 72°C for 7min. *CDKN2A* and *ARID1A* genes were amplified by touchdown PCR including an initial denaturation step at 94°C for 15min; 40 cycles including denaturation at 94°C for 30s, annealing at different temperatures for 30s (2 cycles at 62°C, 2 at 61°C, 2 at 60°C, 3 at 59°C, 3 at 58°C, 4 at 57°C, 4 at 56°C, 5 at 55°C and 15 at 54°C), extension at 72°C for 1min; and a final extension at 72°C for 5min. PCR products were analyzed on a QIAxcel capillary electrophoresis system (Qiagen). *ARID1A* was used as a control to verify the quality of the amplification. *CDKN2A* status was checked based on previous available data; gene expression and mutational status on this gene (21). For association study with histologic types, the Fisher's exact test was performed using GraphPad Prism version 6 software.