1   **Mx1 in hematopoietic cells protects against Thogotovirus infection**

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3   **Running title: Mx1 in hematopoietic cells restricts Thogotovirus**

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17   Key words: Mx1, bone marrow chimeras, Thogotovirus, myeloid cells

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19   Abstract word count: 229

20   Text word count: 6117

21

22 **Abstract**

23 *Myxovirus resistance 1* (*Mx1*) is an interferon-induced gene that encodes a GTPase that plays an

24 important role in the defense of mammalian cells against influenza A and other viruses. The Mx1

25 protein can restrict a number of viruses, independently of the expression of other interferon-induced

26 genes. *Mx* genes are therefore considered to be an important part of the innate antiviral immune

27 response. However, the possible impact of *Mx* expression in the hematopoietic cellular compartment

28 has not been investigated in detail in the course of a viral infection. To address this, we performed

29 bone marrow chimera experiments using congenic B6.A2G $Mx1^{+/+}$ and B6.A2G $Mx1^{-/-}$ mice to study

30 the effect of *Mx1* expression in cells of hematopoietic versus non-hematopoietic origin. $Mx1^{+/+}$ mice

31 were protected and $Mx1^{-/-}$ mice were susceptible to influenza A virus challenge infection, regardless

32 of the type of bone marrow cells ($Mx1^{+/+}$ or $Mx1^{-/-}$) the animals had received. Infection with

33 Thogotovirus, however, revealed that $Mx1^{-/-}$ mice with a functional *Mx1* gene in the bone marrow

34 compartment showed reduced liver pathology compared with $Mx1^{-/-}$ mice that had been grafted with

35 $Mx1^{-/-}$ bone marrow. The reduced pathology in these mice was associated with a reduction in

36 Thogotovirus titers in the spleen, lung and serum. Moreover, $Mx1^{+/+}$ with $Mx1^{-/-}$ bone marrow failed

37 to control Thogotovirus replication in the spleen. *Mx1* in the hematopoietic cellular compartment

38 thus contributes to protection against Thogotovirus infection.

39 **Importance**

40 Mx proteins are evolutionarily conserved in vertebrates and can restrict a wide range of viruses in a

41 cell autonomous way. The contribution to antiviral defense of Mx1 expression in hematopoietic cells

42 remains largely unknown. We show that protection against influenza virus infection requires *Mx1*

43 expression in the nonhematopoietic cellular compartment. In contrast, *Mx1* in bone marrow-derived

44 cells is sufficient to control disease and virus replication following infection with a Thogotovirus. This

45 indicates that next to its well established antiviral activity in nonhematopoietic cells, Mx1 in

46 hematopoietic cells can also play an important antiviral function. In addition, cells of hematopoietic

47 origin that lack a functional *Mx1* gene, contribute to Thogotovirus dissemination and associated

48 disease.

49   **<u>Introduction</u>**

50   Myxovirus resistance proteins are dynamin-like large GTPases that can inhibit a wide array of viruses,

51   including members of the *Orthomyxoviridae*, *Rhabdoviridae* and *Bunyaviridae* (1). *Mx* genes are

52   evolutionary conserved in vertebrates and their expression is induced by type I and type III interferon

53   (2-4). How Mx1 proteins inhibit viral replication is still largely undetermined. It has been shown that

54   mouse Mx1 can suppress primary transcription of influenza A virus (IAV) genes in the nucleus (5).

55   Furthermore, we previously reported that murine Mx1 can interact with the polymerase basic 2

56   (PB2) protein and nucleoprotein (NP) in IAV ribonucleoproteins (vRNPs), and disturb the PB2-NP

57   interaction (6). Human MxA, the orthologue of mouse Mx1, can also interact with IAV NP (7).

58   Moreover NP has been shown to be a determinant of the sensitivity of IAVs for Mx1 and MxA (8, 9).

59   Based on these studies, and on the observation that human MxA – like dynamins – can form ring-like

60   structures (10-14), we hypothesized that the interaction with IAV PB2 and NP might be mediated by

61   a ring structure comprised of oligomerized Mx1, which then actively disrupts the PB2-NP interaction

62   (6). Indirect support for this hypothesis was obtained from the observation that an Mx1 construct

63   that was only active in the presence of an artificial small compound drug, could disrupt pre-existing

64   IAV vRNPs (15).

65

66   The GTPase activity of Mx1 and MxA is required for the suppression of IAV replication. Presumably

67   the GTPase function combined with the 2 hinges that flank the central bundle signaling element that

68   separates the globular head domain from the extended helical stalk domain, allow Mx proteins to

69   function as molecular machines that exert a kind of 'power stroke'. This mechano-chemical transition

70   might generate latitudinal shear forces between neighboring Mx ring structures that destroy the

71   functional vRNP structure (16). Next to their antiviral effect against IAV, Mx proteins can also restrict

72   Thogoto virus (THOV, a member of the *Orthomyxoviridae* family) replication. Mouse Mx1, which is

73   only active in the cell nucleus, inhibits THOV multiplication (17). It has also been shown that human

74   MxA can interact with the NP molecules of the THOV vRNPs. This interaction prevents THOV vRNPs

75   from entering the nucleus (18, 19).

76

77   The *Orthomyxoviridae* family currently comprises seven genera: *Influenza A*, *B*, *C* and *D*,

78   *Thogotovirus*, *Quaranjavirus* and *Isavirus* (20-22). Influenza A and B viruses are important human

79   respiratory pathogens. THOV is a tick-borne virus that has small rodents as natural hosts and very

80   rarely causes zoonotic infection (23, 24). When people become infected with IAV, the first cells that

81   are targeted, are the airway epithelial cells. After binding, endocytosis and membrane fusion, the

82   viral vRNPs are released into the cytoplasm. These then enter the nucleus, where transcription and

83   replication will take place (reviewed in (25)). The incoming vRNPs first direct the synthesis of viral

84   mRNA (primary transcription), which is transported to the cytosol and translated. Newly produced

85   PB1, PB2, Polymerase acidic (PA) and NP migrate to the nucleus to start initiate replication of the

86   viral genome and boost transcription. The resulting progeny viral RNA molecules form vRNPs, and

87   leave the nucleus, ready for packaging and budding (26). Although THOV has not been studied as

88   elaborately as IAV, it has been shown that both viruses are structurally and genetically similar (27-

89   33). Several studies have also pointed out that their replication cycles are comparable (17-19, 34-40).

90   When a mouse becomes infected with THOV, the virus replicates and spreads rapidly to different

91   sites in the mouse body to eventually kill the mouse (41). A similar pathogenesis in mice following

92   infection with the related Dhori virus has been reported: the virus could be detected in multiple

93   organs such as the brain, lungs, thymus, spleen, adrenal glands, and liver (42). However, the main

94   target organ of THOV and Dhori virus is the liver, where these viruses can replicate to very high titers

95   and cause severe coagulative zonal necrosis leading to the rapid death of the infected mouse (17,

96   42).

97

98   Mx proteins exert their antiviral activity in a cell autonomous way. Haller and coworkers reported

99   that athymic (nude) mice, which carry a functional *Mx1* gene, survived intracerebral infection with a

100   neurotropic IAV strain, demonstrating that *Mx1*-positive mice do not require a functional T cell

101   system to survive the infection (43). Later, the same lab reported that *in vivo* resistance to a

102   pneumotropic, neurotropic or hepatotropic strain of IAV was largely independent of whether

103   macrophages carried a functional *Mx1* gene or not (44). These studies showed that *Mx1* expression

104   in hematopoietic cells does not play a major role in the resistance against IAV infection. It is also

105   important to note that almost all mouse genetic studies on the contribution of interferon-induced

106   gene products to antiviral defense in the immune cell compartment have been carried out in

107   laboratory mouse strains that lack a functional *Mx1* gene (45).

108

109   The aim of the present study is to examine the role of *Mx1* expression in immune cells for the

110   antiviral host defense. We show that in bone marrow chimeric mice the protection by Mx1 against

111   IAV infection depends solely on the genotype of the stromal (nonhematopoietic) cells as previously

112   demonstrated by Haller *et al*. (43). In striking contrast, challenge infection with THOV showed that

113   *Mx1* expression in bone marrow-derived cells is sufficient to markedly reduce virus replication and

114   dissemination, and delay morbidity in mice.

115 **Materials and methods**

116 **Ethics statement.** All animal experiments described in this study were conducted according to the

117 national (Belgian Law 14/08/1986 and 22/12/2003, Belgian Royal Decree 06/04/2010) and European

118 legislation (EU Directives 2010/63/EU, 86/609/EEC). All experiments on mice and animal protocols

119 were approved by the ethics committee of Ghent University (permit numbers LA1400091 and

120 EC2015-027).

121

122 **Mice.** Mice were bred in-house under Specific Pathogen Free (SPF) conditions. Mice were housed in

123 individually ventilated cages, in a temperature-controlled environment with 12h light/dark cycles,

124 with food and water *ad libitum*. Congenic B6.A2G-*Mx1* ($Mx1^{+/+}$) mice with a functional A2G *Mx1*

125 allele were kindly provided by Peter Stäheli (University of Freiburg, Germany). Congenic B6.A2G-*Mx1*

126 ($Mx1^{-/-}$) carrying the defective C57BL/6J *Mx1* allele were generated in our laboratory by crossing

127 B6.A2G-*Mx1* ($Mx1^{+/+}$) with C57BL/6J ($Mx1^{-/-}$) mice, and subsequent crossing of the heterozygous

128 offspring. Mouse genomic DNA was isolated from tail biopsies following digestion at 55°C in buffer

129 containing 50 mM Tris-HCl (pH8.0), 10 mM EDTA, 100 mM NaCl, 0.1% SDS and 1 mg/ml proteinase K.

130 A PCR was performed using the following primers: 5'-GGAGCTCACCTCCCACATCT-3', 5'-

131 AGCATGGCTGTGTCACAAGCA-3', and 5'-CGAAGGCAGTTTGGACCATCT-3'. PCR consisted of a 1 min

132 denaturation step at 94°C, a 1 min annealing step at 61°C, and a 1 min polymerization step at 72 °C

133 for 1min (40 cycles). The resulting PCR products were visualized by agarose gel electrophoresis.

134

135 **Bone marrow chimera mice.** Starting one week before and until three weeks after irradiation, mice

136 were given water containing 0.2% neomycin *ad libitum*. Mice were subjected to lethal total body

137 irradiation (10 Grey) with an X-Rad 320 Biological Irradiator (Precision X-Ray (PXi), North Brandford,

138 Connecticut, USA), and 24h later they were reconstituted with syngeneic or allogeneic bone marrow

139 cells (8-10 x 10^6) that were harvested from femurs of age-matched mice. Experimental transfers were

140 as follows: B6.A2G $Mx1^{-/-}$ donors into B6.A2G $Mx1^{-/-}$ recipients ($Mx1^{-/-} \rightarrow Mx1^{-/-}$), B6.A2G $Mx1^{-/-}$

141 donors into B6.A2G $Mx1^{+/+}$ recipients ($Mx1^{-/-} \rightarrow Mx1^{+/+}$), B6.A2G $Mx1^{+/+}$ donors into B6.A2G $Mx1^{+/+}$

142 recipients ($Mx1^{+/+} \rightarrow Mx1^{+/+}$), and B6.A2G $Mx1^{+/+}$ donors into B6.A2G $Mx1^{-/-}$ recipients ($Mx1^{+/+} \rightarrow$

143 $Mx1^{-/-}$). Animals were allowed to recover and reconstitute their hematopoietic cellular compartment

144 for eight weeks. Only healthy mice without obvious signs of graft-versus-host disease were used in

145 experiments.

146

147 **Virus challenge.** Mice were challenged with 10 lethal dose 50% (LD_{50}) (approximately 170 PFU) of

148 mouse adapted (ma) influenza A/Puerto Rico/8/34 (PR8) (H1N1) or with 10^3 PFU of THOV SiAr 126

149   (17). The challenge dose was administered intranasally in a volume of 50 μl (maPR8) or

150   intraperitoneally in a volume of 100 μl (THOV) to mice that were anesthetized with a mixture of

151   ketamine (10 mg/kg) and xylazine (60 mg/kg). Morbidity was monitored during six (maPR8) or four

152   (THOV) days post infection. Mice that had lost 25% or more of their bodyweight were euthanized by

153   cervical dislocation.

154

155   **Determination of influenza lung virus titers.** Mice were sacrificed at different time points after

156   infection by intraperitoneal injection of pentobarbital (125 μg/g). The mouse lungs were removed

157   aseptically, and the left lobe was snap-frozen in liquid nitrogen. Lung extracts were prepared by

158   homogenizing the lungs in PBS using metal beads. Cell debris was cleared by centrifugation for 10

159   min at 400g and 4°C. Cleared lung extracts were stored at -80°C. Influenza virus titers were

160   determined in triplicate by titration on MDCK cells. Briefly, MDCK monolayers were infected for 1h

161   with 500 μl of serial 1:10 dilutions of the lung homogenates in a 12-well plate in serum-free DMEM

162   medium supplemented with penicillin and streptomycin. Following inoculation, the supernatant was

163   replaced by medium containing 2 μg/ml trypsin and 0.6% avicel RC-851 (FMC Biopolymers). Two days

164   after infection, the cells were fixed with 4% paraformaldehyde, and permeabilized with PBS

165   containing 0.2% Triton X-100. Plaques were stained using a mouse monoclonal antibody against the

166   ectodomain of the influenza M2 protein, and an HRP-conjugated anti-mouse IgG antibody (Sheep

167   anti-mouse IgG HRP, GE Healthcare, UK). Plaques were then visualized by using TrueBlue peroxidase

168   substrate (Seracare, Gaithersburg, MD, USA).

169

170   **Determination of Thogoto liver virus titers.** Mice were killed by cervical dislocation, the liver was

171   removed aseptically, and one of the lobes was used for histochemistry. Liver extracts were made by

172   homogenizing the livers in PBS using metal beads. Cell debris was cleared by centrifugation for 10

173   min at 400g and 4°C. Cleared liver extracts were stored at -80°C before use. THOV titers were

174   determined in triplicate by titration on Vero cells. Monolayers of Vero cells were infected for 1h with

175   1 ml of serial 1:10 dilutions of the liver homogenates in a 6-well plate in DMEM medium

176   supplemented with 2% fetal calf serum and 20 mM HEPES pH 7.3. Following inoculation, the

177   supernatant was replaced by medium containing 0.6% avicel RC-951 (FMC Biopolymers). Four days

178   after infection, the cells were fixed with 4% paraformaldehyde. The cell monolayers were stained

179   with a crystal violet solution (1% crystal violet + 1% methanol + 20% ethanol) for approximately 15

180   minutes at room temperature. The crystal violet solution was then removed and the wells were

181   washed with water to reveal the plaques.

182

183 **Histopathological examination of livers.** Livers of bone marrow chimeric mice were excised at 0, 2 or

184 4 dpi. After fixation in 4% paraformaldehyde (PFA) and embedding in paraffin, livers were sectioned

185 at 5 μm. Sections were used for haematoxylin/eosin staining and immunohistochemical analysis.

186 After incubation with primary and secondary biotin-conjugated antibodies immunoreactivity was

187 revealed using the ABC-HRP Kit (Vector Laboratories, Burlingame, California USA) and the sections

188 were counterstained with hematoxylin. Images were obtained with an Axioscan.Z1 slide scanner

189 (Zeiss, Oberkochen, Germany), and were analyzed with ZEN Lite software (Zeiss, Oberkochen,

190 Germany).

191

192 **ALT/AST assay.** Blood was taken by retro-orbital bleeding after sedation of the mice with

193 pentobarbital (125 μg/g). To prepare mouse serum, the blood samples were allowed to clot

194 overnight at 4°C. The next day the clot was removed and samples were centrifuged at 14000 rpm for

195 3 minutes. Serum samples were stored at -20°C before use. Levels of aspartate aminotransferase

196 (AST) and alanine aminotransferase (ALT) were measured using a Hitachi kit and apparatus in the

197 Clinical Biology Laboratory of Ghent University Hospital.

198

199 **Real-time quantitative PCR (RT-qPCR).** Mice of each group were sacrificed just prior to and on day 3

200 and 6 after IAV infection by intraperitoneal injection of pentobarbital (125 μg/g). The mouse lungs

201 were removed aseptically, and the left lobe was snap-frozen in liquid nitrogen. Lung extracts were

202 made by homogenizing the lungs in PBS using metal beads. Cell debris was cleared by centrifugation

203 for 10 min at 400g and 4°C. Cleared lung extracts were stored at -80°C until use. RNA was isolated

204 with the High Pure RNA Isolation Kit (11828665001, Roche) as indicated by the manufacturer. Total

205 mRNA was converted to cDNA by RT-PCR using oligo-dT reaction (Transcriptor First Strand cDNA

206 Synthesis Kit, 04897030001, Roche). Ten nanogram of cDNA was used for each quantitative PCR

207 (qPCR) reaction, and triplicate reactions were setup in 384-well plates. qPCR reactions based on SYBR

208 green detection, were performed using a LightCycler (Roche). qPCR-data were analyzed using the

209 qbase+ software packet (Biogazelle, Zwijnaarde, Belgium).

210 The primers used in this study are as follows. M1/2 forward: 5'-GGGAAGAACACCGATCTTGA-3'; M1/2

211 reverse: 5'-CGGTGAGCGTGAACACAAAT-3'; NA forward: 5'-CATCTCTTTGTCCCATCCGT-3'; NA reverse:

212 5'-GTCCTGCATTCCAAGTGAGA-3'; HA forward: 5'-GAGGAGCTGAGGGAGCAAT-3'; HA reverse: 5'-

213 GCCGTTACTCCGTTTGTGTT-3'; PB1 forward: 5'-CCTCCTTACAGCCATGGGA-3'; PB1 reverse: 5'-

214 GTGCTCCAGTTTCGGTGTTT-3'; PB2 forward: 5'-GGATCAGACCGAGTGATGGT-3'; PB2 reverse: 5'-

215 CCATGCTTTAGCCTTTCGACT-3'; PA forward: 5'-CATCAATGAGCAAGGCGAGT-3'; PA reverse: 5'-

216 GCCCCTGTAGTGTTGCAAAT-3'; NP forward: 5'-CAGCCTAATCAGACCAAATG-3'; NP reverse: 5'-

217 TACCTGCTTCTCAGTTCAAG-3'; NS1 forward: 5'-TTCACCATTGCCTTCTCTTC-3'; NS1 reverse: 5'-

218   CCCATTCTCATTACTGCTTC-3'; HPRT1 forward: 5'-AGTGTTGGATACAGGCCAGAC-3'; HPRT1 reverse: 5'-

219   CGTGATTCAAATCCCTGAAGT-3'; UBC forward: 5'-AGGTCAAACAGGAAGACAGACGTA-3'; UBC reverse:

220   5'-TCACACCCAAGAACAAGCACA-3'; GAPDH   forward: 5'-TGAAGCAGGCATCTGAGGG-3';   GAPDH

221   reverse: 5'-CGAAGGTGGAAGAGTGGGAG-3'; TBP forward: 5'-TCTACCGTGAATCTTGGCTGTAAA-3'; TBP

222   reverse: 5'-TTCTCATGATGACTGCAGCAAA-3'; RPL13A forward: 5'-CCTGCTGCTCTCAAGGTT-3'; RPL13A

223   reverse: 5'-TGGTTGTCACTGCCTGGTACTT-3';   actin   forward: 5'-GCTTCTAGGCGGACTGTTACTGA-3';

224   actin reverse: 5'-GCCATGCCAATGTTGTCTCTTAT-3'.

225

226   **Antibodies.** A polyclonal antiserum against mouse Mx1 was generated by immunizing New Zealand

227   White   rabbits   with   a   synthetic,   high-performance   liquid   chromatography-purified   peptide

228   CKKFLKRRLLRLDEARQKLAKFSD (C terminus of the Mx1 protein) and purified as described (6). M2e-

229   specific monoclonal antibody was produced in our laboratory. Briefly, hybridomas that produce M2e-

230   specific monoclonal antibodies were isolated as described (46). After subcloning, these hybridoma

231   cultures were scaled up and monoclonal antibodies were purified from the culture supernatant with

232   a protein A column (GE Healthcare). Polyclonal anti-Thogoto virus NP antibody, (antiserum, rabbit),

233   was generated in the laboratory of Georg Kochs (University of Freiburg, Germany) (32). Polyclonal

234   anti-CD45 antibody (rabbit) was obtained from Abcam (ab10558). Biotinylated anti-rabbit antibody

235   (goat) was obtained from Vector Laboratories (BA-1000).

236

237   **Statistical analysis.** The obtained data were analyzed using Graphpad Prism 7 or Genstat software.

238   Methods used in Genstat are described below. Statistical tests were performed in Graphpad Prism 7

239   software   and   are   mentioned   in   the   figure   legends.   Relative   bodyweight   data   were   analyzed   as

240   repeated measurements using the residual maximum likelihood (REML) approach as implemented in

241   Genstat v19 (47). Briefly, a linear mixed model with replicate, genotype, time and genotype x time

242   interaction as fixed terms, and subject time used as residual term, was fitted to the data. Times of

243   measurement were set at equal intervals and an autoregressive correlation structure of order 1 with

244   equal variances (*i.e.* homogeneity across time) was selected as best model fit in all cases, based on

245   the Aikake Information Coefficient. Significances of the fixed terms and significances of changes in

246   differences between genotype effects over time were assessed by an F-test. Viral titers were

247   analyzed with a Hierarchical Generalized Linear Mixed Model (HGLMM; fixed model: poisson

248   distribution, log link; random model: gamma distribution, log link) as implemented in Genstat v19

249   (47). Titers below the detection limit have been imputed with values generated as a random sample

250   from a skewed left tailed beta distribution Beta (5,1). Fixed terms include GENOTYPE, DPI, TISSUE and

251   their two-way and three-way interaction, while REPLICATE was set as random term. T- statistics were

252   used   to   assess   the   significance   of   tissue-specific   genotype   effects   at   dpi = 2   and   4   (on   the

253   transformed scale). Estimated mean values were obtained as predictions from the HGLMM, formed

254   on the scale of the response variable. RT-qPCR data were compared with a Generalized Linear Mixed

255   Model (GLMM) (fixed model: Poisson distribution, log link; random model: gamma distribution, log

256   link) as implemented in Genstat v19 (47) fitted to RT-qPCR expression data of PB1, PB2, PA, NP, HA,

257   NA, M and NS genes simultaneously. The linear predictor vector of the values can be written as

258   follows: $\log(\mu) = \eta = X\beta + Zv$, where the matrix X is the design matrix for the fixed terms genotype,

259   time and genotype x time, β is their vector of regression coefficients, Z is the design matrix for the

260   random term (i.e. gene, replicate and gene x replicate), and v is the corresponding vector of random

261   effect having a gamma distribution. The significance of the fixed interaction term genotype x time

262   was assessed by a Wald test. Significance of the regression coefficients were assessed by a t-test.

263   Estimated mean values and their standard errors were obtained as predictions from the GLMM,

264   formed on the scale of the response variable. A Hierarchical Generalized Linear Mixed Model

265   (HGLMM; fixed model: Poisson distribution, log link; random model: gamma distribution, log link) as

266   implemented in Genstat v19 (47) has been fitted to the "ALT" and "AST" data. Fixed terms include

267   GENOTYPE, DPI and their interaction, while REPLICATE was set as random term. T statistics were

268   used to assess the significance of time-specific genotype effects (on the transformed scale).

269   Estimated mean values were obtained as predictions from the HGLMM, formed on the scale of the

270   response variable.

271   **Results**

272   **Resistance to influenza A virus infection primarily depends on the *Mx1* genotype of the recipient**

273   Most immune cells originate from multipotent hematopoietic stem cells in the bone marrow. To

274   address the possible role of *Mx1* as a virus restriction factor in this compartment, we generated all

275   four possible bone marrow chimeric mice between B6.A2G *Mx1$^{-/-}$* and B6.A2G *Mx1$^{+/+}$* mice (Fig 1A).

276   Eight weeks after bone marrow transfer, the chimeric mice were infected with 10 LD$_{50}$ of maPR8

277   virus, and bodyweight was monitored during 6 days post infection (dpi). B6.A2G *Mx1$^{-/-}$* recipient mice

278   displayed significantly more bodyweight loss than B6.A2G *Mx1$^{+/+}$* recipient mice regardless of the

279   donor genotype (Fig 1B). In addition, infection with maPR8 virus of mice with a functional *Mx1* gene

280   in the stromal cells did not result in bodyweight loss regardless of the donor genotype (Fig 1B). Lung

281   virus loads were significantly lower in the B6.A2G *Mx1$^{+/+}$* recipients than in B6.A2G *Mx1$^{-/-}$* recipients

282   on day 3 and 6 after infection. We observed no significant difference in viral loads between *Mx1$^{-/-}$*

283   mice that had been reconstituted with bone marrow from either donor and neither between the

284   *Mx1$^{+/+}$* recipients that were reconstituted with *Mx1$^{-/-}$* or *Mx1$^{+/+}$* donor bone marrow (Fig 1C). It has

285   been reported that in cells that stably express Mx1, primary transcription of IAV genes is reduced and

286   this reduction is more pronounced for the larger genes encoding the polymerase subunits compared

287   with the shorter viral transcripts (5). To ascertain that such a differential effect might also be

288   observed *in vivo*, we quantified the individual viral mRNA levels in the mouse lung on day 3 and 6

289   after infection by RT-qPCR. Three and six days after infection, the viral mRNA levels were much lower

290   in the lungs of B6.A2G *Mx1$^{+/+}$* compared to those in lungs of B6.A2G *Mx1$^{-/-}$* recipient mice (Fig 1D). In

291   contrast to what was previously reported by Pavlovic *et al.* (5), we noticed that the inhibiting effect

292   of Mx1 was equally strong for the shorter and longer viral RNA segments (Fig 1E). Together, these

293   data show that the *Mx1* genotype of the recipient rather than the donor determines the outcome of

294   maPR8 virus infection, both in terms of controlling disease and viral replication.

295

296   **Mx1 in hematopoietic cells contributes to the control of Thogotovirus infection**

297   We next addressed the possible contribution of Mx1 in the stromal versus hematopoietic cells for

298   control of THOV infection. The rationale for choosing this virus is threefold: (i) THOV, like IAV, is a

299   member of the *Orthomyxoviridae*, (ii) the virus is also sensitive to murine Mx1 (17), and (iii) small

300   rodents are natural hosts of this virus (24). Bone marrow chimeric mice were infected

301   intraperitoneally with 1000 plaque forming units (PFU) of THOV (Sicilian SiAr 126 isolate), and

302   bodyweight and morbidity were monitored daily during four days after infection. *Mx1$^{+/+}$* mice that

303   received *Mx1$^{+/+}$* bone marrow showed no signs of morbidity or bodyweight loss, and *Mx1$^{-/-}$* recipients

304   that had been reconstituted with *Mx1$^{-/-}$* bone marrow lost significantly more bodyweight than all

305   other chimeric groups and became moribund by day 4 post infection (Fig 2A). Interestingly, B6.A2G

306     $Mx1^{+/+}$ mice that had received $Mx1^{-/-}$ bone marrow lost significantly more bodyweight than B6.A2G

307     $Mx1^{-/-}$ mice that had received $Mx1^{+/+}$ bone marrow, indicating a protective role for $Mx1$ expression in

308   hematopoietic cells in this infection model. Surprisingly, liver viral titers did not reflect these findings.

309   The THOV titers in the liver of the B6.A2G $Mx1^{-/-}$ recipient mice were very high (approximately 10^6 to

310   10^7 PFU/g; the two fold lower virus load in the $Mx1^{+/+}$ recipients did not reach statistical significance)

311   (Fig 2B). In contrast, THOV virus could not be detected in liver extracts from infected B6.A2G $Mx1^{+/+}$

312   recipient mice on 4 dpi independent of the donor genotype (Fig 2B). These data suggest that $Mx1$

313   expression in hematopoietic cells contributes to the control of THOV-associated morbidity and, to a

314   limited extent, viral replication in the liver of B6.A2G $Mx1^{-/-}$ recipient mice.

315

316   **$Mx1^{+/+}$ expression in hematopoietic cells reduces THOV-associated liver pathology in B6.A2G $Mx1^{-/-}$**

317   **recipients**

318   THOV infection was previously shown to cause severe liver pathology in $Mx1^{-/-}$ mice, but not in

319     $Mx1^{+/+}$ mice (17). To examine the possible contribution of donor-derived Mx1 on THOV-associated

320   liver damage, we performed histological analysis on the bone marrow chimeric mice sacrificed on

321   day 4 after THOV infection. Livers isolated from $Mx1^{-/-}$ mice that had been reconstituted with $Mx1^{-/-}$

322   bone marrow appeared very pale and friable in comparison to the livers from mice in the three other

323   groups, which had a normal brown-red color and firm tissue. Liver sections were prepared, stained

324   with hematoxylin and eosin (H&E) and analyzed microscopically. H&E-stained liver tissue from THOV

325   infected $Mx1^{-/-}$ mice reconstituted with $Mx1^{-/-}$ bone marrow showed lesions with focal to widespread

326   liver cell necrosis (Fig 2C). Interestingly, liver tissue from $Mx1^{+/+}$ recipient mice reconstituted with

327     $Mx1^{-/-}$ bone marrow also showed lesions, although these lesions appeared to be in an earlier stage of

328   necrosis compared to those in mice that are $Mx1^{-/-}$ in both the stromal and immune cell

329   compartment. This is manifested as foci in the liver tissue where structure and cell architecture are

330   lost, but cell nuclei are still observed. B6.A2G $Mx1^{+/+}$ recipient's liver tissue appeared normal and

331   showed no lesions, irrespective of the donor genotype (Fig 2C). In summary, these results show that

332     $Mx1$ in cells with a hematopoietic origin can reduce or at least delay liver pathology associated with

333   THOV infection in B6.A2G $Mx1^{-/-}$ recipient mice.

334

335   To quantify the degree of liver damage resulting from the THOV infection, alanine aminotransferase

336   (ALT) and aspartate aminotransferase (AST) levels were determined in serum of the bone marrow

337   chimeric mice at 4 dpi. The serum levels of both ALT and AST were markedly increased in THOV-

338   infected $Mx1^{-/-}$ mice reconstituted with $Mx1^{-/-}$ bone marrow compared with the two $Mx1^{+/+}$ recipient

339   groups (Fig 2D and 2E). Interestingly, THOV infection of irradiated $Mx1^{-/-}$ mice reconstituted with

340 $Mx1^{+/+}$ bone marrow was associated with intermediate levels of both enzymes. This is in accordance

341 with the histological scoring of the liver sections, further substantiating that $Mx1^{+/+}$ hematopoietic

342 cells can contribute to protection against THOV infection in mice that lack $Mx1$ in the stromal

343 compartment.

344

345 **Delayed THOV-associated morbidity in $Mx1^{-/-}$ mice grafted with $Mx1^{+/+}$ bone marrow**

346 The previous results led to the hypothesis that $Mx1$ expression in the hematopoietic compartment

347 can delay the course of the THOV infection-associated pathology in $Mx1^{-/-}$ recipient mice. Therefore,

348 we performed additional THOV experiments in the chimeric mice to compare virus replication and

349 pathology at day 2 and 4 after infection. Before infection, all mice had healthy liver tissue (Fig 3A).

350 Two days after infection $Mx1^{-/-}$ recipient mice already showed clear zones of cellular influx (Fig 3A).

351 These zones were markedly larger and more numerous in the $Mx1^{-/-}$ mice reconstituted with $Mx1^{-/-}$

352 bone marrow than in the $Mx1^{+/+}$ mice reconstituted with $Mx1^{-/-}$ donor cells. Amongst the $Mx1^{+/+}$

353 recipient mice that had received $Mx1^{-/-}$ bone marrow, a few small zones of cellular influx were

354 observed after 2 days of infection. Four days after infection, $Mx1^{+/+}$ recipient mice showed no zones

355 of cellular influx or liver cell necrosis. In contrast, all $Mx1^{-/-}$ recipients showed clear zones of cellular

356 influx and/or liver cell necrosis. Livers from mice that lack $Mx1$ in both the stromal and immune cell

357 compartment showed clear zones of liver cell necrosis and only a few small zones of cellular influx.

358 Interestingly, livers from $Mx1^{-/-}$ mice that had received $Mx1^{+/+}$ donor cells, showed large zones of

359 cellular influx. In the largest influx zones a center of necrotic cells could be observed (Fig 3A, arrows).

360 This indicates that the THOV infection-related pathology is at an earlier state in $Mx1^{-/-}$ mice that

361 received $Mx1^{+/+}$ bone marrow than in $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow.

362

363 Four days after THOV infection it was clear that $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow show

364 the highest ALT and AST levels with an average of approximately 4000 and 10 000 U/L, respectively.

365 $Mx1^{+/+}$ recipients showed only background levels of ALT and AST. In $Mx1^{-/-}$ mice reconstituted with

366 $Mx1^{+/+}$ bone marrow, intermediate ALT and AST levels were detected in the serum (approximately

367 100 and 500 U/L, respectively) (Fig 3B and 3C). The differences between the ALT and AST levels

368 between the four groups are most pronounced at 4 dpi. At two dpi the ALT and AST levels of the

369 $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow are comparable to the AST and ALT levels of the $Mx1^{-/-}$

370 mice reconstituted with $Mx1^{+/+}$ bone marrow at four dpi. This shows that the presence of a functional

371 $Mx1$ gene in hematopoietic cells can delay the THOV infection-related liver pathology in mice.

372

373 We also assessed the presence of THOV in the spleen, lung and serum next to the liver. Similar to the

374 experiment shown in Figure 2, liver viral titers at 4 dpi were highest in the $Mx1^{-/-}$ mice that received

375    $Mx1^{-/-}$ bone marrow, 1.5 to 2 logs lower in $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone marrow, and were

376   undetectable in both $Mx1^{+/+}$ recipient groups (Fig 4A). At 2 dpi, liver viral titers in both $Mx1^{-/-}$

377   recipient groups are lower compared to the viral titers at 4 dpi. $Mx1^{+/+}$ recipient mice had

378   undetectable liver viral titers at 2 dpi. THOV was detectable in lung and serum sampled from $Mx1^{-/-}$

379   mice that received $Mx1^{-/-}$ bone marrow but in none of the other groups (Fig 4B and C). Strikingly, the

380   spleen viral titers show a remarkable difference compared to the titers in the liver, lung and serum.

381   Spleen THOV titers were highest in the $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow group at 2 and

382   4 dpi (Fig 4D). However, spleen viral titers are below the detection limit in $Mx1^{-/-}$ mice that received

383    $Mx1^{+/+}$ bone marrow. Surprisingly, $Mx1^{+/+}$ mice that received $Mx1^{-/-}$ bone marrow show viral titers

384   that are only 4 fold lower than $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone marrow at 2 dpi and 9 fold

385   lower at 4 dpi. Thus a functional $Mx1$ gene in the hematopoietic cellular compartment is sufficient to

386   control THOV spread to the lungs, spleen and serum.

387

388   ***Mx1* expression negatively correlates with THOV NP expression in stromal and bone marrow-**

389   **derived cells**

390   The above data show that dissemination of THOV infection depends on the $Mx1$ genotype of the

391   hematopoietic and/or stromal compartment. To demonstrate the cellular import of THOV infection,

392   liver tissue slides of bone marrow chimeras were stained with antibodies specific for CD45 (myeloid

393   cell marker), mouse Mx1, or THOV NP two and four dpi. Accumulation of myeloid cells could be

394   detected in all chimeric groups except in $Mx1^{+/+}$ mice that received $Mx1^{+/+}$ bone marrow (Fig 5). In

395   livers of $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow none of the cells stained positive for Mx1.

396   Surprisingly, the only cells that stained positive for THOV NP – these cells are productively infected by

397   THOV – are cells in the zones of cellular infiltrates. By contrast, cells in the cellular infiltrate zones in

398   livers of $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone marrow stained positive for CD45 and Mx1, but not

399   for THOV NP. In liver tissue of $Mx1^{+/+}$ mice that received $Mx1^{-/-}$ bone marrow the opposite was

400   observed. Here, the cells in the cellular infiltrate zone stained positive for CD45 and THOV NP, but

401   not for Mx1. As expected, there are no zones of cellular infiltration in livers of $Mx1^{+/+}$ mice that

402   received $Mx1^{+/+}$ bone marrow, and liver cells only stained positive for mouse Mx1. Four days after

403   infection, liver tissue of $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone marrow displayed ample liver cell

404   necrosis (Figure 5). This loss of structure in the liver tissue also influenced the background staining.

405   Except for the large zones of cell infiltration in liver tissue of $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone

406   marrow, staining for CD45, mouse Mx1 and THOV NP at 4 dpi was comparable with that at 2 dpi.

407   Together, these data suggest that cells that make up the zones of cellular infiltration are CD45$^+$, that

408   these CD45$^+$ cells first encounter THOV then infiltrate the peripheral organs and thus disseminate the

409   infection.

410   **Discussion**

411   In mice, it is well established that expression of a functional Mx1 protein can protect against a

412   challenge dose of IAV or THOV that otherwise causes severe morbidity and mortality in mice without

413   a functional *Mx1* gene. However, whether *Mx1* expression is induced in every IFN-responsive cell

414   upon infection, and whether *Mx1* is needed for their proper functioning, remains an open question.

415   Several studies have shown the importance of an IFN response in cell types involved in the adaptive

416   immune response after IAV infection (48-50). These studies make it tempting to hypothesize that

417   Mx1 can also play a role in the protection against viral infection of cell types that are involved in

418   adaptive immunity. Here, we generated *Mx1* bone marrow chimeras, allowing us to investigate the

419   possible effect of *Mx1* when it is primarily expressed by bone marrow-derived cells or stromal cells.

420   However, some bone marrow-derived cell types, such as Langerhans cells (51) and mesenchymal

421   stromal cells (reviewed in (52)), are resistant to lethal total body irradiation. Consequently, these cell

422   types will have the genotype of the bone marrow recipient.

423

424   Because of the dominant effect of the presence or lack of *Mx1* expression in epithelial cells, the data

425   obtained in this IAV infection model do not answer the question if *Mx1* can play a role in bone

426   marrow-derived immune cells. Conceivably, the maPR8 virus infection model, characterized by a

427   preferred tropism for epithelial cells, is not the best suited for answering the question. It was

428   recently reported that internal genes of highly pathogenic H5N1 viruses can facilitate replication in

429   myeloid cells and lead to severe disease in *Mx1*-deficient mice (53). It is therefore possible that

430   challenge infections of *Mx1* bone marrow chimeras with such influenza viruses could have revealed a

431   more pronounced effect on the infection outcome in animals that received $Mx1^{+/+}$ bone marrow.

432

433   Instead of exploring the outcome of infections with a highly pathogenic influenza virus, we turned to

434   THOV challenge infections. Bone marrow chimeras were infected intraperitoneally with a high dose

435   of THOV. Morbidity was somewhat different from that seen in the IAV infection model, in that $Mx1^{-/-}$

436   mice that received $Mx1^{+/+}$ bone marrow showed less body weight loss than $Mx1^{+/+}$ mice that received

437    $Mx1^{-/-}$ bone marrow. Remarkably, liver viral titers at 4 dpi were very high for all the $Mx1^{-/-}$ recipients,

438   and below the detection limit for $Mx1^{+/+}$ recipients. In order to find an explanation for the seemingly

439   discrepant data, we examined the liver tissue of bone marrow chimeras at microscopic level 4 days

440   after THOV infection. Liver tissue from THOV infected $Mx1^{-/-}$ recipient mice showed lesions. For the

441    $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone marrow, the cells in these lesions were in an earlier stage of

442   cell necrosis compared to $Mx1^{-/-}$ mice that had been grafted with $Mx1^{-/-}$ bone marrow cells as cell

443   nuclei were still visible. The lesions in these mice were also characterized by a clear cellular influx. As

444   a more objective measure for liver damage, the ALT and AST serum levels were determined following

445   THOV infection. The obtained data reflected the results of the histological analysis of the liver tissue.

446   This suggests that the expression of a functional Mx1 protein in hematopoietic-derived cells cannot

447   protect against productive THOV infection in $Mx1^{-/-}$ recipients, but it can delay disease progression.

448   This was in line with the THOV infection kinetics data. Histological analysis of liver tissue, as well as

449   ALT and AST levels in blood serum, showed that $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone marrow cells

450   have a delayed progression of liver damage compared with $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone

451   marrow cells. Another argument for this theory is that the situation in livers of $Mx1^{-/-}$ mice that

452   received $Mx1^{-/-}$ bone marrow at 2 dpi is comparable with that at 4 dpi in livers of $Mx1^{-/-}$ mice that

453   received $Mx1^{+/+}$ bone marrow. In these two situations we showed comparable liver morbidity and

454   cellular influx in the liver. Immunohistological analysis made it clear that the cellular influx in the

455   livers is mainly composed of CD45$^+$ immune cells. It was apparent that cells that express a functional

456   Mx1 protein (CD45$^+$ or CD45$^-$) do not express the viral protein NP, which suggests that these cells

457   were not productively infected. Interestingly, in livers of $Mx1^{-/-}$ mice that received $Mx1^{-/-}$ bone

458   marrow the CD45$^+$ cells appeared to be the first cells that express the THOV NP.

459

460   Recently, Kochs *et al.* postulated that THOV has a tropism for CD11b$^+$ cells with a clear

461   myeloid/macrophage phenotype (double positive for surface markers CD11b and F4/80) in the

462   peritoneum (54). Therefore, it is conceivable that these cells could be partially protected against

463   THOV infection by *Mx1* expression. When these cells become infected with THOV, they likely

464   transport the virus to the liver. This is a credible theory given that Ghosn *et al.* identified a population

465   of large peritoneal macrophages (LPMs) which seem to have a similar phenotype as the CD11b$^+$

466   myeloid cells described by Kochs *et al.* (54, 55). These LPMs can migrate to the omentum – a fat

467   tissue that connects the abdominal organs – upon inflammation (56), which is in agreement with the

468   disappearance of the CD11b$^+$ myeloid cell population from the peritoneal cavity after THOV infection

469   (54). From the omentum, the LPMs can reach the liver of infected mice. There, the virus can infect

470   hepatocytes, unless these hepatocytes express a functional Mx1 protein, in which case, viral

471   replication would be suppressed in the hepatocytes. In $Mx1^{-/-}$ mice that received $Mx1^{+/+}$ bone

472   marrow we observed high viral titers and necrotic cell lesions in the liver, indicating that THOV can

473   still reach the liver. However, THOV NP expression in the liver was low to nonexistent. It is plausible

474   that myeloid cells are only partially protected against THOV infection, or that the viral inoculum

475   (1000 PFU) used overcomes the Mx1 restriction in the $Mx1^{+/+}$ myeloid cells. Nonetheless, the

476   obtained results indicate that *Mx1* expression in myeloid cells can delay the progression of THOV

477   infection. $Mx1^{+/+}$ mice that received $Mx1^{-/-}$ bone marrow showed no detectable liver viral titers and

478   no liver injury. However, these mice showed clear viral titers in the spleen, which could be explained

479   by the high abundance of myeloid cells in this organ. If THOV could reach the liver tissue via THOV-

480   susceptible $Mx1^{-/-}$ myeloid cells, the virus would still be inhibited by the presence of a functional Mx1

481   protein in the hepatocytes. However, this does not explain the higher weight loss for these mice in

482   comparison with the $Mx1^{-/-}$ and $Mx1^{+/+}$ mice that received $Mx1^{+/+}$ bone marrow. Conceivably, since

483   THOV can still reach the liver and spleen quite easily in $Mx1^{+/+}$ mice that received $Mx1^{-/-}$ bone

484   marrow, an inflammatory response will be triggered. This response can cause the production of

485   inflammatory cytokines (57, 58), and possibly is the reason for the more severe weight loss in $Mx1^{+/+}$

486   mice that received $Mx1^{-/-}$ bone marrow. Replication in myeloid cells could lead to high type I IFN

487   levels and lead to a cytokine storm which would explain the severe weight loss in $Mx1^{+/+}$ mice that

488   received $Mx1^{-/-}$ bone marrow (53).

489

490   In conclusion, to confer resistance against Mx1-susceptible viruses that do not have a tropism for

491   myeloid cells, such as influenza A/Puerto Rico/8/34, $Mx1$ expression is primarily important in the

492   stromal cells. However, for resistance against Mx1-susceptible viruses, like THOV, that can infect

493   myeloid cells and disseminate through these cells, $Mx1$ expression in bone marrow-derived cells is of

494   major importance.

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661

662   **Figure legends**

663

664   **Figure 1**

665

666   **Mx1-mediated resistance to influenza A virus infection primarily depends on the genotype of the**

667   **recipient bone marrow chimeric mice. (A)** Schematic overview of the generation of the bone

668   marrow chimeras. The PCR-based genotyping of the donor and acceptor mice is also depicted. **(B)**

669   Mice (n = 14 per group) were infected intranasally with 10 LD_{50} of maPR8 virus and the body weight

670   change over time after infection was monitored. Data points represent the average of 14 mice from 0

671   dpi until 3 dpi, and the average of 7 mice from 4 dpi until 6 dpi. Error bars represent the standard

672   error of the mean. Asterisks indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$ group

673   and all other groups over time. ***, $p < 0.001$. Circles indicate the significant difference with the

674     $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group over time. °°, $p < 0.01$; °°°, $p < 0.001$. **(C)** Mice were sacrificed on 3 and 6 days

675   post infection and lung viral loads were determined. Each data point represents the lung viral titer of

676   a single animal. Asterisks indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$ group and

677   all other groups. **, $p < 0.01$; ***, $p < 0.001$. Circles indicate the significant difference between the

678     $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group and all other groups. °, $p < 0,05$; °°, $p < 0,01$; °°°°, $p < 0.0001$. **(D)** Viral mRNA

679   load in lung homogenates from mice sacrificed on day 3 and 6 after infection as determined by RT-

680   qPCR. Data points represent the average levels of total viral mRNA relative to household genes. Error

681   bars represent standard error of the mean. Asterisks indicate the significant difference between the

682     $Mx1^{-/-} \rightarrow Mx1^{-/-}$ group and all other groups. ***, $p < 0.001$. Circles indicate the significant difference

683   between the $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group and all other groups. °°°, $p < 0.001$. **(E)** Viral mRNA load of the

684   eight separate influenza virus genome segments in lung homogenates from the respective bone

685   marrow chimeric mice sacrificed on day 6 after infection as determined by RT-qPCR. Bars represent

686   the average levels of viral mRNA relative to household genes. Error bars represent standard error of

687   the mean. The data are pooled from 2 independently performed experiments (first experiment n = 6

688   and second experiment n = 8).

689   **Figure 2**

690

691     $Mx1^{+/+}$ **hematopoietic cells contribute to protection against THOV-associated pathology.** Bone

692   marrow chimeric mice were infected intraperitoneally with 1000 PFU of THOV on day 0. **(A)** Body

693   weight change over time after infection. Data points represent the average of eight mice. Error bars

694   represent the standard error of the mean. Statistical analysis was done using a two-way ANOVA with

695   post hoc Tukey's HSD test. Asterisks indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$

696   group and all other groups. *, p < 0.05; **, p < 0.01; ****, p < 0.0001. Circles indicate the significant

697   difference between the $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group and all other groups. °°, p < 0.01; °°°, p < 0.001; °°°°, p

698   < 0.0001. Caps indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{+/+}$ group and all other

699   groups. ^, p < 0.05; ^^^^, p < 0.0001. **(B)** Viral titers determined by plaque assay in the liver on day 4

700   after infection. Each data point represents the liver viral titer of a single animal. Statistical analysis

701   was performed using Kruskal-Wallis test with post hoc Dunn's multiple comparison test. Asterisks

702   indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$ group and all other groups. ***, p <

703   0.001. Circles indicate the significant difference between the $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group and all other

704   groups. °, p < 0.05. Data are pooled from 2 independently performed experiments (first experiment n

705   = 4, second experiment n = 4). **(C)** Histological analysis of liver tissue section (5 μm slides) after 4 days

706   of infection stained with hematoxylin and eosin. Arrows indicate focal zones of liver cell necrosis.

707   Scale bar = 100 μm. Pictures are representative for n = 8. Serum concentrations of ALT **(D)** and AST

708   **(E)** determined on day 4 after infection. Each data point represents the ALT or AST concentration of a

709   single animal (n = 4). Asterisks indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$ group

710   and all other groups. *, p < 0.05; **, p < 0.01; Kruskal-Wallis test with post hoc Dunn's multiple

711   comparison test.

712

713   **Figure 3**

714

715   *$Mx1^{+/+}$ immune cells delay THOV-associated liver damage in B6.A2G $Mx1^{-/-}$ recipient mice.*

716   Radiation chimeric mice (n = 19 per group) were generated and infected intraperitoneally with 1000

717   PFU of THOV. Before infection (n = 5), and on day 2 (n = 6) and 4 (n = 8) after infection, mice were

718   sacrificed and liver and serum samples were prepared. **(A)** Representative photo micrographs of liver

719   section stained with hematoxylin and eosin. Arrows indicate focal zones of liver cell necrosis. Scale

720   bar = 100 μm. Serum concentrations of ALT **(B)** and AST **(C)**. Each data point represents the ALT or

721   AST concentration of a single animal. Asterisks indicate the significant difference between the $Mx1^{-/-}$

722     $\rightarrow Mx1^{-/-}$ group and all other groups. ****, p < 0.0001. Data are pooled from 2 independently

723   performed experiments (first experiment n = 9, second experiment n = 10).

724

725   **Figure 4**

726

727   **Mx1 in hematopoietic cells differentially controls THOV replication in different parts of the body.**

728   Bone marrow chimeric mice (n = 14 per group) were generated and infected intraperitoneally with

729   1000 PFU of THOV. On day 2 (n = 6) and 4 (n = 8) after infection, mice were sacrificed and viral titers

730   in liver **(A)**, lung **(B)**, spleen **(C)**, and serum **(D)** were determined. Each data point represents the viral

731   titer of a single animal. Asterisks indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{-/-}$

732   group and all other groups. **, p < 0,01; ***, p < 0.001; ****, p < 0,0001. Circles indicate the

733   significant difference between the $Mx1^{+/+} \rightarrow Mx1^{-/-}$ group and all other groups. °, p < 0.05. Caps

734   indicate the significant difference between the $Mx1^{-/-} \rightarrow Mx1^{+/+}$ group and all other groups. ^, p <

735   0.05. Data are pooled from 2 independently performed experiments (first experiment n = 7, second
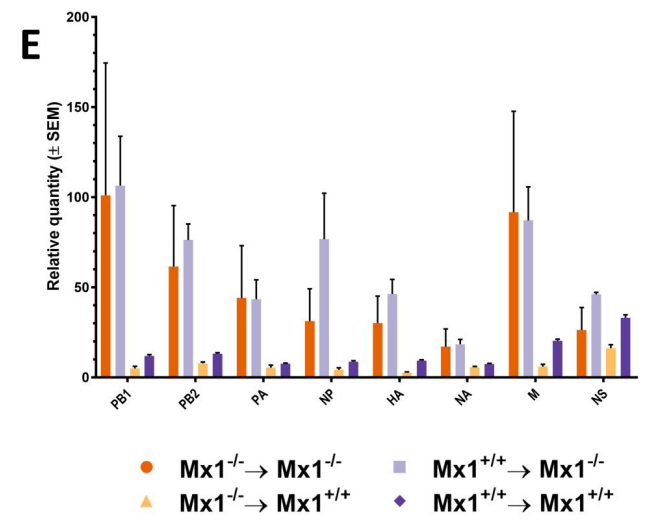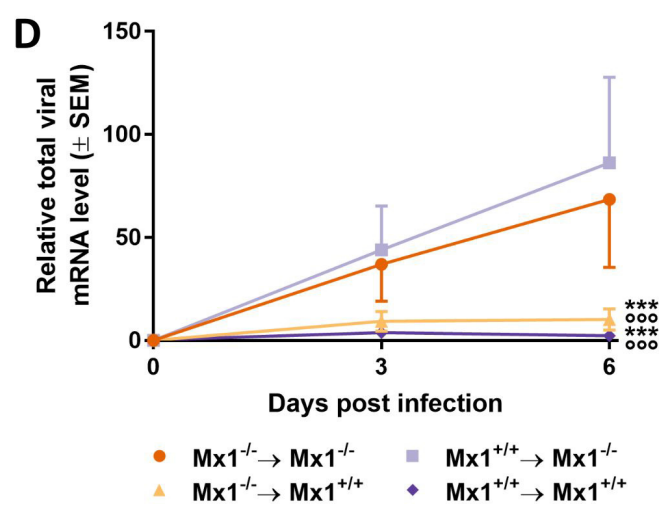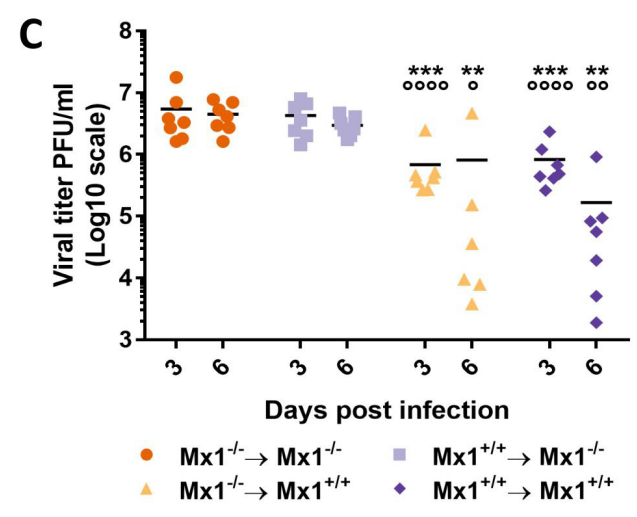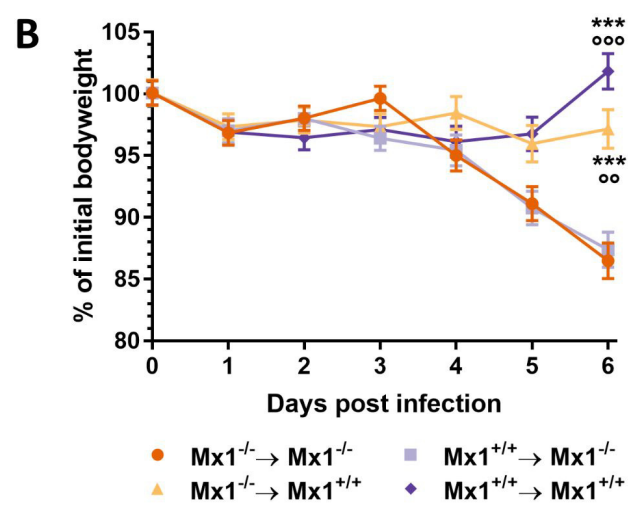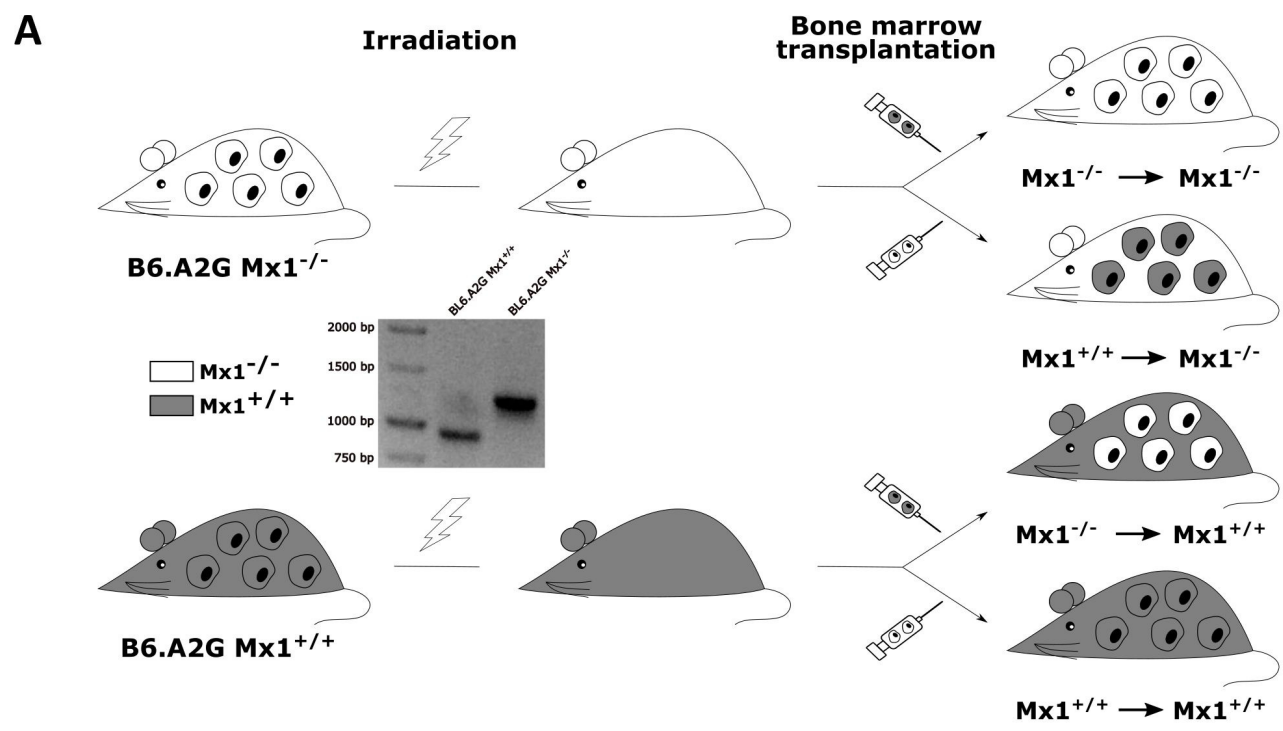
736   experiment n =7).

737

738   **Figure 5**

739

740   *$Mx1^{+/+}$* **hematopoietic cells delay THOV infection-associated liver damage in B6.A2G** *$Mx1^{-/-}$*

741   **recipient mice.** Bone marrow chimeric mice (n = 19 per group) were infected intraperitoneally with

742   1000 PFU of THOV. Before infection (0 dpi) (n = 5), 2 (n = 6) and 4 (n = 8) days after infection mice

743   were sacrificed, livers were isolated, and prepared for histological analysis by staining tissue slides

744   with hematoxylin and immunostaining with polyclonal antiserum directed against THOV NP and Mx1,

745   or a monoclonal antibody against CD45. Scale bar = 100 µm.

A

| | Mx1⁻/⁻ → Mx1⁻/⁻ | Mx1⁺/⁺ → Mx1⁻/⁻ | Mx1⁻/⁻ → Mx1⁺/⁺ | Mx1⁺/⁺ → Mx1⁺/⁺ |

0 dpi

2 dpi

4 dpi

B

ALT (U/L)

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺ → Mx1⁻/⁻
▲ Mx1⁻/⁻ → Mx1⁺/⁺   ◆ Mx1⁺/⁺ → Mx1⁺/⁺

C

AST (U/L)

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺ → Mx1⁻/⁻
▲ Mx1⁻/⁻ → Mx1⁺/⁺   ◆ Mx1⁺/⁺ → Mx1⁺/⁺

A **Liver**

Viral titer PFU/g (Log10 scale)

**** ****
**** **** ** ****

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺→ Mx1⁻/⁻
▲ Mx1⁻/⁻→ Mx1⁺/⁺   ◆ Mx1⁺/⁺→ Mx1⁺/⁺

B **Lung**

Viral titer PFU/g (Log10 scale)

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺→ Mx1⁻/⁻
▲ Mx1⁻/⁻→ Mx1⁺/⁺   ◆ Mx1⁺/⁺→ Mx1⁺/⁺

C **Serum**

Viral titer PFU/ml (Log10 scale)

** ** **

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺→ Mx1⁻/⁻
▲ Mx1⁻/⁻→ Mx1⁺/⁺   ◆ Mx1⁺/⁺→ Mx1⁺/⁺

D **Spleen**

Viral titer PFU/g (Log10 scale)

** ****
° °
** *** ** **
^

Days post infection

● Mx1⁻/⁻→ Mx1⁻/⁻   ■ Mx1⁺/⁺→ Mx1⁻/⁻
▲ Mx1⁻/⁻→ Mx1⁺/⁺   ◆ Mx1⁺/⁺→ Mx1⁺/⁺

0 dpi

	Negative Control	NP	Mx1	CD45

$Mx1^{-/-} \rightarrow Mx1^{-/-}$

$Mx1^{+/+} \rightarrow Mx1^{-/-}$

$Mx1^{-/-} \rightarrow Mx1^{+/+}$

$Mx1^{+/+} \rightarrow Mx1^{+/+}$

2 dpi

	Negative Control	NP	Mx1	CD45

$Mx1^{-/-} \rightarrow Mx1^{-/-}$

$Mx1^{+/+} \rightarrow Mx1^{-/-}$

$Mx1^{-/-} \rightarrow Mx1^{+/+}$

$Mx1^{+/+} \rightarrow Mx1^{+/+}$

4 dpi

	Negative Control	NP	Mx1	CD45

$Mx1^{-/-} \rightarrow Mx1^{-/-}$

$Mx1^{+/+} \rightarrow Mx1^{-/-}$

$Mx1^{-/-} \rightarrow Mx1^{+/+}$

$Mx1^{+/+} \rightarrow Mx1^{+/+}$