

Hepatic interferon-stimulated genes are differentially regulated in the liver of chronic hepatitis C patients with different interleukin-28B genotypes

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Hepatic interferon-stimulated genes are differentially regulated in the liver of chronic hepatitis C patients with different interleukin 28B genotypes

Running title: Regulation of hepatic ISGs in different IL28B genotypes

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Abbreviations: CH-C, Chronic Hepatitis C; HCV, Hepatitis C Virus; IFN, Interferon; IL, Interleukin; ISGs, Interferon Stimulated Genes; RBV, Ribavirin; SNP, Single Nucleotide Polymorphism, IFI44: Interferon-induced Protein 44, IFIT1; Interferon-induced Protein with Tetratricopeptide Repeats 1, Mx; Myxovirus (Influenza Virus) Resistance, OAS2; 2'-5'-Oligoadenylate Synthetase 2, NK; Natural Killer Cell, ALT; Alanine Aminotransferase, WNT5A; Wngless-related MMTV Integration Site 5A, FZD5; Frizzled Family Receptor 5,

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Abstract

Background: A previous report showed that pretreatment up-regulation of hepatic ISGs had a stronger association with the treatment-resistant IL28B minor genotype (MI) (TG/GG at rs8099917) than with the treatment-sensitive IL28B major genotype (MA) (TT at rs8099917).

Methods: We compared the expression of ISGs in the liver and blood of 146 patients with chronic hepatitis C who received PEGylated-IFN and ribavirin combination therapy. Gene expression profiles in the liver and blood of 85 patients were analyzed using an Affymetrix GeneChip.

Results: ISG expression was correlated between the liver and blood of the MA patients, while no correlation was observed in MI patients. This loss of correlation was due to impaired infiltration of immune cells into the liver lobules of MI patients, as demonstrated by regional gene expression analysis in liver lobules and portal areas using laser capture microdissection and immunohistochemical staining. Despite having lower levels of immune cells, hepatic ISGs were up-regulated in the liver of MI patients and they were found to be regulated by multiple factors; IL28 A/B, IFN- λ 4, and WNT5A. Interestingly, WNT5A induced the expression of ISGs, but also increased HCV replication by inducing the expression of the stress granule protein, G3BP1 in Huh-7 cell line. In the liver, the expression of WNT5A and its FZD5 was significantly correlated with G3BP1.

Conclusions: Immune cells were lost and induced the expression of other inflammatory mediators such as WNT5A, in the liver of IL28B minor genotype patients. This might be related to the high level of hepatic ISG expression and the treatment-resistant phenotype of the IL28B minor genotype.

Keywords: Gene expression, ISGs, Immune cells, IL28B genotype

Introduction

Interferon (IFN) and ribavirin (RBV) combination therapy has been a popular modality for treating patients with chronic hepatitis C (CH-C), but approximately 50% of patients usually relapse, particularly those with hepatitis C virus (HCV) genotype 1b and a high viral load.(1) The recently developed direct anti-viral drug (DAA) telaprevir, combined with PEGylated (PEG)-IFN plus RBV, significantly improved sustained virologic response (SVR) rates, however, the SVR rate was not satisfactory (29–33%) for patients who had no response to previous therapy.(2) Therefore, IFN responsiveness is still an essential clinical determinant for treatment response to triple (PEG-IFN+RBV+DAA) therapy.

A recent landmark genome wide association study identified a polymorphism in the interleukin 28B (IL28B) gene that was associated with the treatment response to PEG-IFN and RBV combination therapy.(3-5) However, the underlying mechanism for the association of this IL28B polymorphism and treatment response has not been clarified. Previously, we showed that the up-regulation of the pretreatment expression of hepatic interferon-stimulated genes (ISGs) was associated with an unfavorable treatment outcome and was closely related to the treatment-resistant IL28B genotype (TG or GG at rs8099917).(6) It could be speculated that the pretreatment activation of ISGs would repress the additional induction of ISGs following treatment with exogenous IFN. However, it is unknown how hepatic ISGs are up-regulated in treatment-resistant CH-C patients and why patients with high levels of ISG expression cannot eliminate HCV. Therefore, other mechanisms should be involved in the unfavorable treatment outcome in patients with the treatment-resistant IL28B genotype.

In the present study, we performed gene expression profiling in the liver and blood and compared the expression of ISGs between them. Furthermore, ISG expression in liver lobules and portal areas was analyzed separately using a laser capture microdissection (LCM) method. Finally, we identified the immune factor that is up-regulated in patients with the treatment-resistant IL28B genotype and mediates favorable signaling for HCV replication.

Materials and Methods

Patients

We analyzed 168 patients with CH-C who had received PEG-IFN α -2b (Schering-Plough K.K., Tokyo, Japan) and RBV combination therapy for 48 weeks at the Graduate School of Medicine, Kanazawa University Hospital, Japan and its related hospitals, as reported previously (Table 1, Supplemental Table 1).⁽⁶⁾ All patients had HCV genotype 1b and high viral loads (≥ 100 KIU/mL). Treatment response was defined as follows: SVR, clearance of HCV viremia at 24 weeks after the cessation of therapy; and transient response (TR), no detectable HCV viremia at the cessation of therapy, but relapse during the follow-up period; and no response,

Preparation of liver tissue and blood samples

A liver biopsy was performed on samples from on 168 patients, and blood samples were obtained from 146 of these patients before starting therapy (Table 1, Supplemental Table 1). Detailed procedures are described in the Supplemental Material and Methods.

Affymetrix GeneChip analysis

Liver tissue samples from 91 patients and blood samples from 85 patients were analyzed using an Affymetrix GeneChip. LCM analysis was performed in 5 MAu, MAd, and MI patients. Affymetrix GeneChip analysis and LCM were performed as described previously.^(6, 7) The microarray data have been submitted to the Gene Expression Omnibus (GEO) public database at NCBI (Accession No. GSE41804). Detailed procedures are described in the Supplemental Material and Methods.

Hierarchical clustering and pathway analysis of GeneChip data

GeneChip data analysis was performed using BRB-Array Tools (<http://linus.nci.nih.gov/BRB-ArrayTools.htm>) as described previously.⁽⁷⁾ Pathway analysis was performed using MetaCore (Thomson Reuters, New York, NY, USA). Detailed procedures are described in the Supplemental Material and Methods.

Quantitative real-time detection PCR, Cell lines, cells migration assay, vector preparation, and HCV replication analysis

These procedures are described in detail in the Supplemental Material and Methods.

Statistical analysis

The Tukey-Kramer Honestly Significant Difference test was used to analyze continuous variables. Chi-squared and Fisher's exact tests were used for the analysis of categorical data. A p-value of less than 0.05 was considered significant. Statistical analyses were performed using JMP9 for Windows (SAS Institute, Cary, NC, USA)

Results

Different ISG expression in the liver and blood in patients with different IL28B genotypes

Previously, we showed that the pretreatment up-regulation of hepatic ISGs was associated with an unfavorable treatment outcome and was closely related to the treatment-resistant IL28B minor genotype (TG or GG at rs8099917).⁽⁶⁾ To examine whether the expression of hepatic ISGs would reflect the expression of blood ISGs, we compared ISG expression between the liver and blood. The mean values of 3 ISGs (IFI44, IFIT1, and Mx1) detected by RTD-PCR in 168 liver tissues samples (Supplemental Table 1) showed a significant up-regulation of ISG expression in non-responder or treatment-resistant IL28B minor genotype (TG/GG; rs8099917) patients compared to responder (SVR+TR) or treatment-sensitive IL28B major genotype (TT; rs8099917) patients, as reported previously (Fig. 1A, Supplemental Fig. 1A).⁽⁶⁾ However, ISG expression in 146 blood samples (Table 1) showed no difference between responders and non-responders or the IL28B major and minor genotypes (Fig. 1B, Supplemental Fig. 1B). To explore these findings further, gene expression profiling using Affymetrix GeneChips was performed on liver and blood samples from 85 patients (Supplemental Tables 2 and 3), and the expression of 37 representative ISGs⁽⁶⁾ was compared (Fig. 1C–E). The patients with the IL28B major genotype (MA patients) were divided into 2 groups according to their ISG expression pattern in the liver: patients with up-regulated ISGs (MAu patients) and patients with down-regulated ISGs (MA_d patients). Patients with the IL28B minor genotype (MI patients) expressed the ISGs at a higher level than the MAu patients. Interestingly, ISG expression in the MA patients showed a similar expression pattern in the liver and blood, and ISGs were up-regulated in the MAu patients and down-regulated in the MA_d patients. However, the MI patients showed a different ISG expression pattern in the liver and blood, and ISGs were up-regulated in the liver but down-regulated in the blood (Fig. 1C). The correlation of the mean values of 3 ISGs (IFI44, IFIT1, and Mx1) between the liver and blood from 146 patients demonstrated a significant correlation between the values in the MA patients (Fig. 1D), while no correlation was observed

in the MI patients (Fig. 1E). Interestingly, ISG expression correlated significantly between the liver and blood in responders, but not in non-responders, in both the MA and MI patients (Supplemental Fig. 1C–F). These results indicate that the association of ISG expression in the liver and blood is an important predictor for treatment response.

Clinical characteristics of IL28B major genotype patients with up-regulated and down-regulated ISGs and IL28B minor genotype patients

From the expression pattern of ISGs and the mean values of 3 ISGs (IFI44, IFIT1, and Mx1), we could use analysis of the receiver operating characteristic curve to set a threshold of 2.1-fold to differentiate MAu and MAd patients. Following this criterion, 42 MAu, 68 MAd, and 36 MI patients (total 146) were grouped (Table 1). The hepatic ISGs were highest in MI patients, while blood ISGs were highest in MAu patients. On the other hand, the hepatic IL28A/B expression was highest in MAu patients, while blood IL28A/B expression showed no differences among three groups. Serum ALT, AST, and γ -GTP levels were significantly higher in the MAu patients compared with the MAd patients. Interestingly, serum ALT levels were significantly correlated with ISG expression in the MA patients, while no correlation was observed in the MI patients (Supplemental Fig. 2E–F).

Gene expression profiling in peripheral immune cells showed the presence of active inflammation in the MAu patients, while the inactive or remissive phase of inflammation was observed in the MAd patients. In contrast, monophasic and intermediate inflammation existed in the MI patients (Supplemental Fig. 3).

Reduced number of immune cells in the liver lobules of IL28B minor genotype patients

To examine the discordant expression of ISGs in the liver and blood of MI patients, we performed LCM to collect cells in liver lobules (CLL) and cells in portal areas (CPA) separately from each of 5 liver biopsied samples from MAu, MAd, and MI patients (Fig. 2A). Interestingly, the ISG expression pattern in CLL from the MA patients was similar to that of CPA, and ISGs were up-regulated in the MAu patients and down-regulated in the MAd

patients. ISG expression in CLL from the MI patients was different to that of CPA, and ISGs were up-regulated in CLL but down-regulated in CPA (Fig. 2A). We hypothesized that the discordance of ISG expression between CLL and CPA in the MI patients might be due to the lower number of immune cells that infiltrated the liver lobules of these patients. To prove this hypothesis, immunohistochemical (IHC) staining was performed (Fig. 2B). IHC staining showed that IFI44 was strongly expressed in the cytoplasm and nucleus of CLL from the MI patients, while it was intermediately expressed in the MAu patients and weakly expressed in the MAd patients. Interestingly, IFI44 was strongly expressed in CPA of the MAu patients and weakly expressed in CPA of the MAd patients, showing the correlation between expression in CLL and CPA of the MA patients, while ILI44 expression was relatively weak in CPA compared with CLL in the MI patients (Fig. 2B). In the same section of the specimens, there were less CD163-positive monocytes and macrophages in the MI patients than in the MAu and MAd patients. Similarly, there were less CD8-positive T cells in the MI patients than in the MAu and MAd patients (Fig. 2B). Semi-quantitative evaluation of CD163 positive and CD8 positive lymphocytes in liver lobules showed the significant lower number of cells in MI patients compared to the MAu and MAd patients (Supplemental Fig. 4A and 4B). To support these findings, we examined the expression of 24 surface markers of immune cells in CLL, including DCs, natural killer (NK) cells, macrophages, T cells, B cells, and granulocytes (Supplemental Fig. 5A). The expression of immune cell surface markers was repressed in the MI patients compared to the MAu and MAd patients. Furthermore, whole liver expression profiling in 85 patients showed reduced expression of these surface markers in the MI patients compared with the MAu and MAd patients (Supplemental Fig. 5B). These results indicated that fewer immune cells had infiltrated the liver lobules of the MI patients.

In addition to these findings, various chemokines such as CCL19, CCL21, CCL5 and CXCL13, which are important regulators for the recruitment of DCs, NK cells, T cells, and B cells in the liver, were significantly down-regulated in the MI patients compared with the MAd and MAu patients (Supplemental Fig. 4C–F).

Hepatic ISG expression is significantly correlated with IL28A/B, but not IFN- α or IFN- β

The lower number of immune cells in the liver lobules of MI patients implies that reduced levels of IFN are produced from DCs and macrophages, etc. These findings prompted us to examine the relationship between hepatic ISGs and IFN- α , IFN- β , and IL28A/B in CH-C patients. Hepatic ISG expression was significantly correlated with IL28A/B, but not IFN- β (Fig. 3A–C) or IFN- α (data not shown) in the MAu, MAd, and MI patients. These results indicate that hepatic ISGs would be mainly induced by IL28A/B in CH-C patients. Interestingly, the correlation between hepatic ISGs and IL28A/B was strongest in the MA patients ($p < 0.0001$ in MAu, $p = 0.0006$ in MAd), while rather a weak correlation was observed in the MI patients ($p = 0.015$). Moreover, the ratio of hepatic ISGs to IL28 A/B was larger in the MI patients than in the MA patients ($S = 0.061$ in MI, $S = 0.028$ in MAu, $S = 0.020$ in MAd), suggesting the presence of factors other than IL28A/B that can induce the expression of ISGs in the MI patients. Therefore, we evaluated the expression of the recently discovered IFN- $\lambda 4$ in the MI patients. Interestingly, there was a significant correlation between hepatic ISG and IFN- $\lambda 4$ expression ($p = 0.0003$) (Fig. 3C).

WNT5A and its receptor FZD5 are significantly up-regulated in the liver of patients with the IL28B minor genotype

IFN- $\lambda 4$ is a promising factor to induce ISG expression in MI patients (8) and the functional relevance of IFN- $\lambda 4$ for the pathogenesis of CH-C has been now under investigation. We searched for other factors that could induce ISGs in MI patients. A closer observation of gene expression profiling in CLL obtained by LCM demonstrated that WNT signaling was specifically up-regulated in the MI patients (Supplemental Fig. 6). Further observation enabled us to identify that the WNT ligand, WNT5A, and its receptor, frizzled receptor 5 (FZD5), were up-regulated in the MI patients. The RTD-PCR results on 168 liver biopsied samples confirmed the significant up-regulation of WNT5A and FZD5 in the MI patients compared with the MAu and MAd patients (Fig. 4A and 4B). Interestingly, WNT5A expression was negatively correlated with chemokine expression (Supplemental Fig. 7). IHC

staining showed the up-regulation of FZD5 in the liver lobules of the MI patients, but not in the MAu or MAd patients (Fig. 4C). WNT5A expression was significantly correlated with hepatic ISG expression in the MI and MAd patients (Fig. 4D). Interestingly, we found a weak but significant correlation between WNT5A and IFN- λ 4 expression in the MI patients (Fig. 4E).

WNT5A induces OAS2 expression but stimulates HCV replication in Huh-7 cells

To examine the functional relevance of up-regulated WNT5A expression in the MI patients, we first evaluated the expression levels of WNT5A and OAS2 in 2 immortalized human hepatocyte cell lines, THLE-5b(9) and TTNT cells,(10) and 1 human hepatoma cell line, Huh-7 cells (Supplemental Fig. 8A and 8B). WNT5A was moderately expressed in THLE-5b and TTNT cells, while its expression in Huh-7 cells was minimal. Interestingly, OAS2 expression in these cells was well correlated with the expression of WNT5A (Supplemental Fig. 8B). Small interfering (si) RNA to WNT5A efficiently repressed WNT5A expression to approximately 20% of the control in THLE-5b cells, and in this condition, OAS2 expression was significantly decreased to approximately 40% of the control (Supplemental Fig. 8C). On the other hand, transduction of WNT5A by a lentivirus expression system in Huh-7 cells significantly increased OAS2 expression in the presence and absence of HCV infection (Supplemental Fig. 8D). Surprisingly, HCV replication, as determined using *Gaussia* luciferase activity, increased in WNT5A-transduced cells (Supplemental Fig. 8E). Furthermore, WNT5A-transduced cells supported more HCV replication than non-transduced cells under IFN treatment (Supplemental Fig. 8F).

WNT5A-FZD5 signaling induces the expression of the stress granule protein G3BP1, which supports HCV replication

These findings were further confirmed by using Huh-7 cells continuously infected with JFH-1 (Huh7-JFH1), which is a genotype 2a HCV isolate.(11) Interestingly, the expression of WNT5A in Huh7-JFH1 cells was significantly up-regulated compared with uninfected Huh-7 cells and showed an equivalent expression level with THLE-5b cells (Fig.

5A). siRNA to WNT5A efficiently repressed WNT5A expression to approximately 20% of the control, and in this condition, OAS2 expression and HCV-RNA levels were repressed to 25% and 60% of the control, respectively (Fig. 5B). We evaluated the expression of GTPase activating protein (SH3 domain) binding protein 1 (G3BP1), a recently recognized stress granule (SG) protein that supports HCV infection and replication.(12) The expression of G3BP1 was repressed to 60% of the control by knocking down WNT5A. Conversely, the over-expression of WNT5A in Huh7-JFH1 cells significantly increased HCV-RNA levels and G3BP1 expression (Fig. 5C). A recent report demonstrated that G3BP1 is a Dishevelled-associated protein that regulates WNT signaling downstream of the FZD receptor.(13) Interestingly, G3BP1 expression was significantly up-regulated in the liver of the MI patients (Fig. 5D). Furthermore, G3BP1 expression significantly correlated with WNT5A expression in the liver of the CH-C patients (Fig. 75E). More dramatically, a strong correlation was observed between the expression of FZD5 and G3BP1 in the liver of the CH-C patients (Fig. 5F).

Discussion

The underlying mechanism for the association of the IL28B genotype with treatment responses to IFN-based therapy for HCV has not been clarified yet. We and others have shown that the pretreatment up-regulation of hepatic ISGs was associated with an unfavorable treatment outcome(7, 14, 15) and was closely related to the treatment-resistant IL28B minor genotype (MI) compared with the treatment-sensitive IL28B major genotype (MA).(6) It could be speculated that the pretreatment activation of ISGs would repress the additional induction of ISGs following the administration of exogenous IFN.(8, 16) However, other mechanisms should be involved in the resistance to treatment.

By comparing ISG expression in the liver and blood, we found that their expression was correlated in the MA patients, but not in the MI patients. LCM analysis of ISG expression in CLL and CPA showed the loss of the correlation between CLL and CPL in the MI patients (Fig. 2A). This might be due to the impaired migration of immune cells into liver lobules that was demonstrated by the decreased expression of immune cell surface markers in CLL by LCM (Supplemental Fig. 5A) and IHC staining (Fig. 2B). To date, the precise dynamics of immune cells between liver lobules and lymphocyte accumulation in the portal area (portal tract associated lymphoid tissue; PALT) has not been clarified. PALT might be involved in the extravasation of lymphocytes from vessels in the portal area, but others demonstrated that DCs appeared in the sinusoidal wall and passed through the space of Disse to PALT, where the draining lymphatic duct is located.(17) There should be an active movement of immune cells between the liver lobules and PALT, as reflected by the correlation of ISG expression in CLL and CPA in the MA patients of this study.

Chen et al. reported that ISGs were up-regulation in hepatocytes in treatment-resistant IL28B genotype patients, but they were up-regulated in Kupffer cells in treatment-sensitive genotype patients.(18) Our results confirmed their findings. However, we also showed that the expression of various immune cell surface markers, such as those on DCs, NK cells, macrophages, T cells, B cells, and granulocytes, was lower in the MI patients than in the MA patients (Supplemental Fig. 5). In addition, we showed that the expression of

various chemokines was also repressed in the MI patients compared to MA patients (Supplemental Fig. 4C–F).

The lower number of immune cells in the liver lobules of the MI patients would imply the reduced production of IFN from DCs and macrophages, etc. Correlation analysis of hepatic ISGs and IL28A/B suggested that factors other than IL28A/B might regulate ISG expression in the MI patients. During the preparation of this study, IFN- λ 4 was newly identified to be expressed in hepatocytes from patients with the treatment-resistant IL28B genotype.(8) Interestingly, we found a significant correlation between hepatic ISGs and IFN- λ 4 in the MI patients ($p = 0.0003$) (Fig. 3C). Moreover, a closer examination of gene expression profiling in the MI patients enabled us to detect the up-regulation of the non-canonical WNT ligand WNT5A. RTD-PCR analysis of 168 patients confirmed the up-regulation of WNT5A and its receptor FZD5 in the MI patients. Importantly, WNT5A expression was significantly correlated with hepatic ISG expression in the MI patients ($p = 0.0003$). A recent report showed that WNT5A induces the expression of ISGs, increases the sensitivity of IFN- α in keratinocytes,(19) and might be involved in the immune response to influenza virus infection.(20) Therefore, we examined the role of WNT5A in hepatocytes. Interestingly, the expression of WNT5A and OAS2 was well correlated and knocking down WNT5A using siRNA reduced the expression of OAS2 in THLE-5b cells (Supplemental Fig. 8). Conversely, the transduction of Huh-7 cells with WNT5A using a lentivirus system increased the expression of OAS2. Despite the increase in ISG expression, WNT5A did not suppress HCV replication, but rather increased it in Huh-7 cells (Supplemental Fig. 8). These results were also confirmed by using Huh-7 cells continuously infected with JFH-1 (Huh7-JFH1). By knocking down or over-expressing WNT5A in Huh7-JFH1 cells, we showed that HCV-RNA was positively regulated by WNT5A (Fig. 5B and 5C).

WNT5A and its receptor FZD5 mediate non-canonical WNT signaling, such as planar cell polarity and the WNT-Ca²⁺ signaling pathway through G proteins. It is reported that WNT5A inhibits both B- and T cell development by counteracting canonical WNT signaling and inhibits the production of IL-12 and the subsequent induction of IFN- γ -producing T helper

1 cells.(21) We found that G3BP1, the SG assembly factor, was up-regulated by WNT5A (Fig. 5C). It was reported that SGs were formed by endoplasmic reticulum stress followed by HCV infection and localized around lipid droplets with HCV replication complexes.(12) G3BP1 contributes to SG formation and increases HCV replication and infection in Huh-7 cells.(12) Moreover, a recent report demonstrated that G3BP1 is a Dishevelled-associated protein that regulates WNT signaling downstream of the FZD receptor.(13) Importantly, we found a significant correlation between WNT5A and G3BP1 expression in liver tissue samples (Fig. 5E). We also found a striking significant correlation between FZD5 and G3BP1 expression in liver tissues samples (Fig. 5F). Thus, up-regulated non-canonical WNT5A-FZD5 signaling participates in the induction of ISG expression, but preserves HCV replication and infection in hepatocytes by increasing the levels of the SG protein G3BP1. These findings may explain the pathophysiological state of the treatment-resistant phenotype in MI patients and, moreover, the results may imply the further clinical usefulness of the IL28B genotype for determining treatment response to IFN-free therapy in terms of the efficacy of HCV replication and infection.

In this study, we demonstrated impaired immune cell infiltration of the liver in patients with the treatment-resistant IL28B genotype, and we also demonstrated that the up-regulation of hepatic ISGs in patients with the treatment-resistant IL28B genotype was mediated by multiple factors such as IL28A/B, IFN- λ 4, and WNT5A. We found a significant negative correlation between WNT5A and various chemokines in the liver of CH-C patients (Supplemental Fig. 7). These results indicate that the loss of immune cells from the liver may induce the expression of other inflammatory factors such as WNT5A in MI patients, although we did not define which cells express WNT5A. Further studies are needed to explore their functional relevance in the pathogenesis of CH-C.

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Figure Legends

Figure 1. Comparison of ISG expression in the liver and blood of patients with different IL28B genotypes

A, B: RTD-PCR results of mean ISG expression (IFI44+IFIT1+Mx1) in the liver (A) and blood (B) of IL28B major and minor genotype patients.

C: One-way hierarchical clustering analysis of 85 patients using 37 representative ISGs derived from the liver (upper) and blood (lower).

D, E: Correlation of mean ISG expression (IFI44+IFIT1+Mx1) in the liver and blood of IL28B major (D) and minor (E) genotype patients.

Figure 2. LCM and IHC staining of biopsied liver specimens

A: Comparison of the ISG expression pattern of whole liver (upper), cells in liver lobules (CLL) (upper-middle), cells in portal areas (CPA) (lower-middle), and blood (bottom). CLL and CPA were obtained from 5 MI, MAu, and MAd patients, who are indicated by small black bars.

B: IHC staining of IFI44, CD163, and CD8 in MI, MAu, and MAd patients.

Figure 3. Correlation analysis of hepatic ISGs and IL28A/B, IFN- β , and IFN- λ 4

Correlation of mean ISG (IFI44+IFIT1+Mx1) and IL28A/B, IFN- β , and IFN- λ 4 expression was evaluated in MAu (A), MAd (B), and MI (C) patients.

Figure 4. WNT5A and FZD5 are up-regulated in IL28B minor genotype patients

A: RTD-PCR results of WNT5A expression in the liver of MAu, MAd, and MI patients.

B: RTD-PCR results of FZD5 expression in the liver of MAu, MAd, and MI patients.

C: IHC staining of IFI44 and FZD5 expression in the liver of MAu, MAd, and MI patients

D: Correlation of mean ISG (IFI44+IFIT1+Mx1) and WNT5A expression in the liver of MAu, MAd, and MI patients.

E: Correlation of WNT5A and IFN- λ 4 expression in the liver of MI patients.

Figure 5. Relationship between WNT5A and FZD5 signaling and the SG protein G3BP1

A: WNT5A expression in THLE-5b, Huh-7, and Huh7-JFH1 cells.

B: Knocking down WNT5A and changes of OAS2 expression, HCV-RNA levels, and G3BP1 expression in Huh7-JFH1 cells.

C: Over-expression of WNT5A following transfection with pCMV-WNT5A and increase in HCV-RNA levels and G3BP1 expression.

A, B, C: Experiments were performed in duplicate and repeated 3 times (n = 6). Values are the means \pm SE. * p < 0.05 ** p < 0.01 *** p < 0.005.

D: RTD-PCR results for G3BP1 expression in the liver of MAu, MAd, and MI patients.

E: Correlation of WNT5A and G3BP1 expression in the liver.

F: Correlation of FZD5 and G3BP1 expression in the liver.

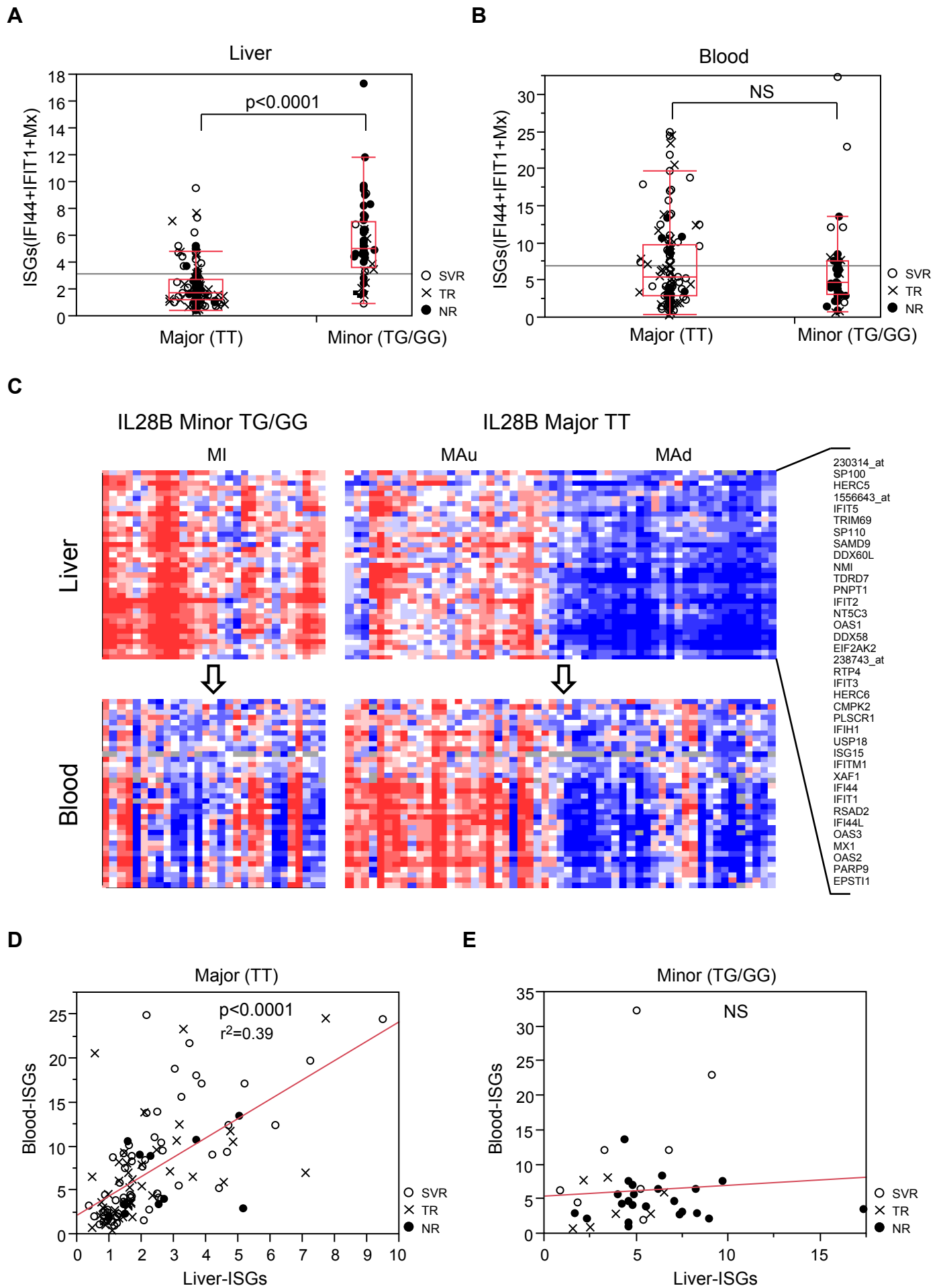
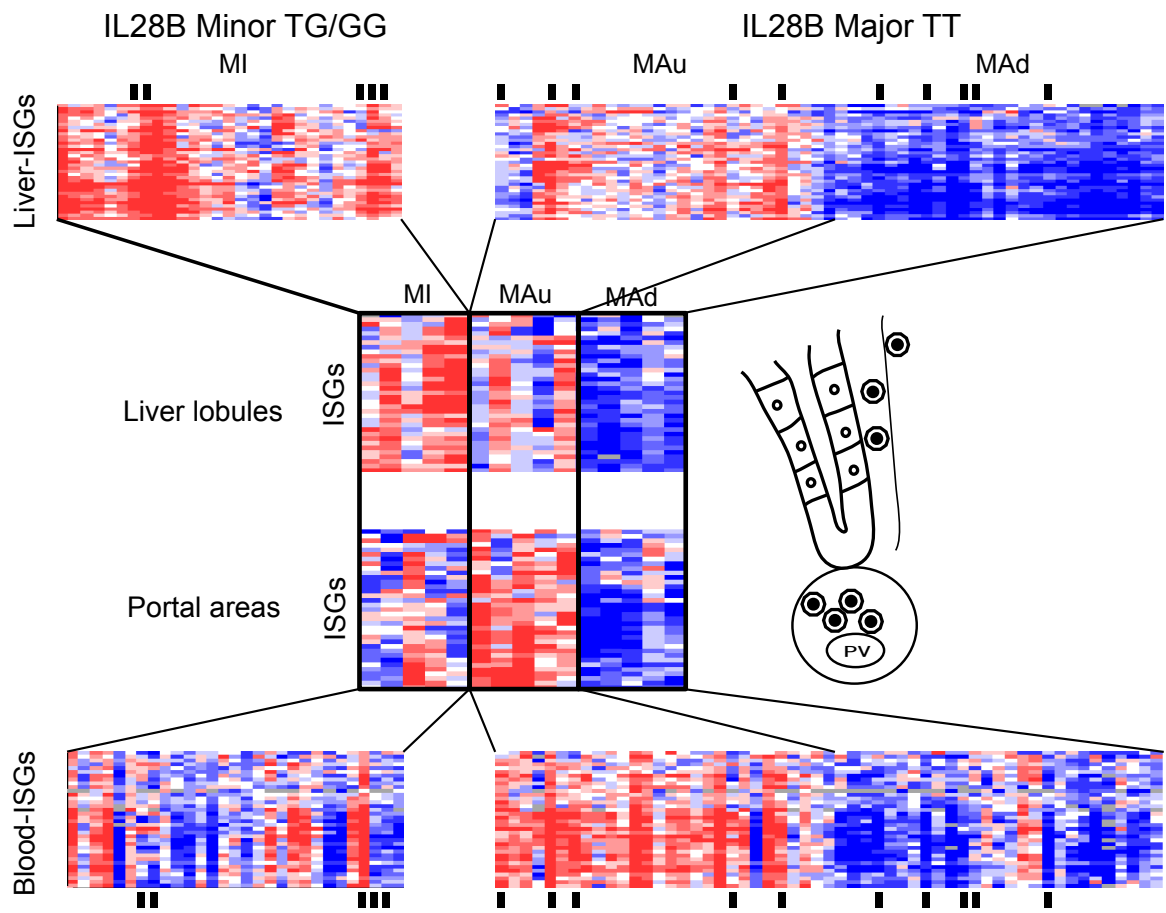
Fig.1

Fig.2

A



B

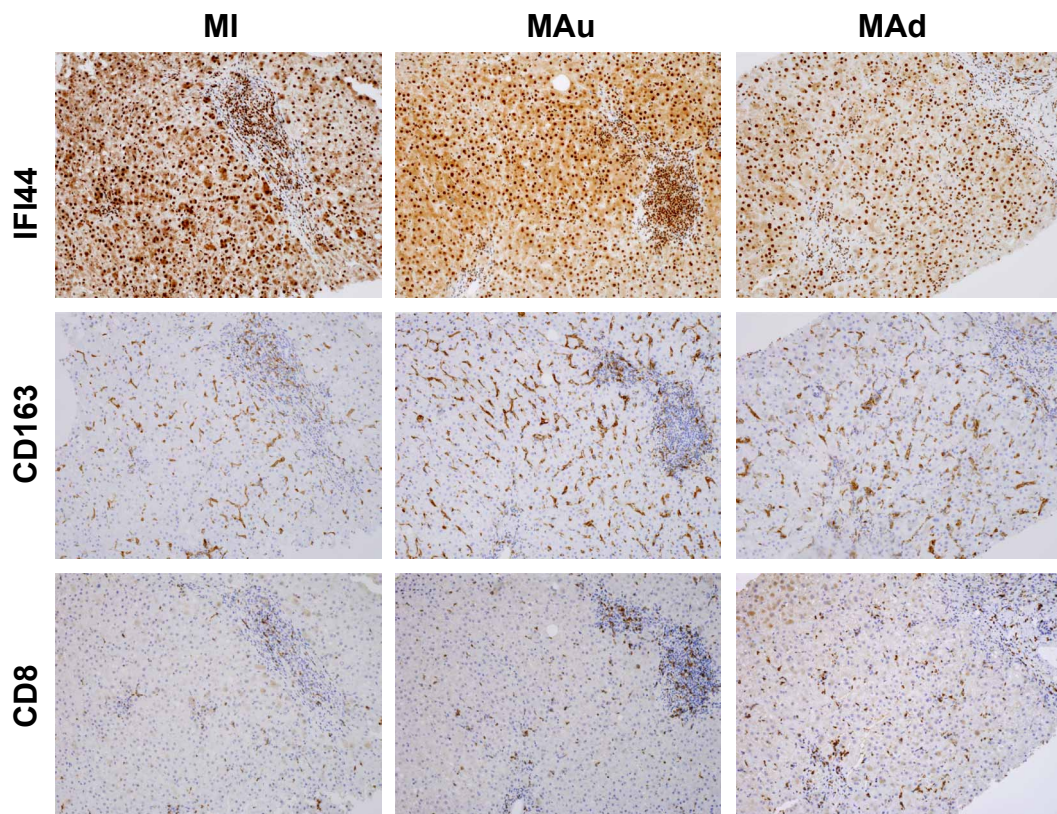


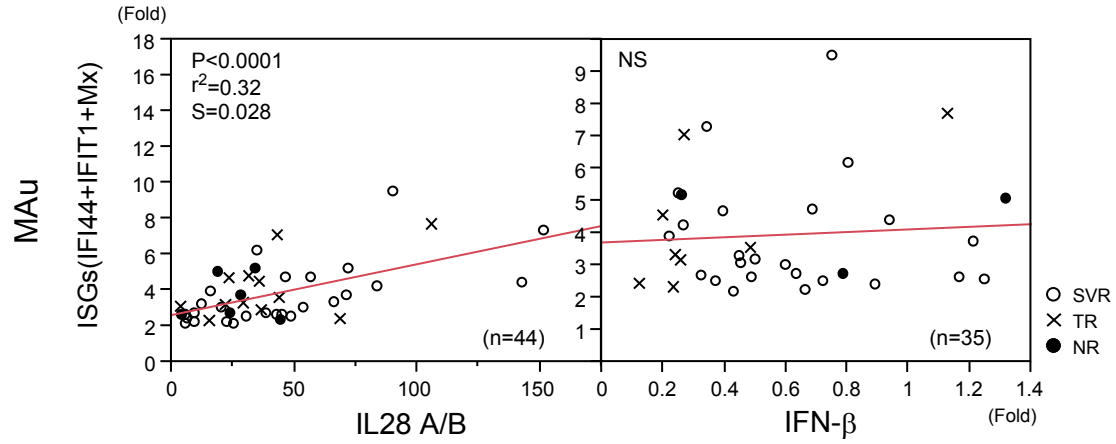
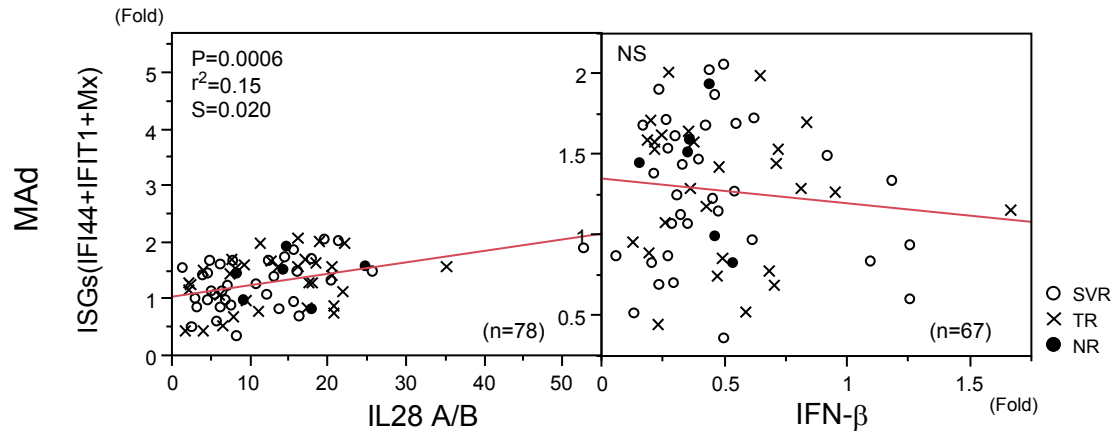
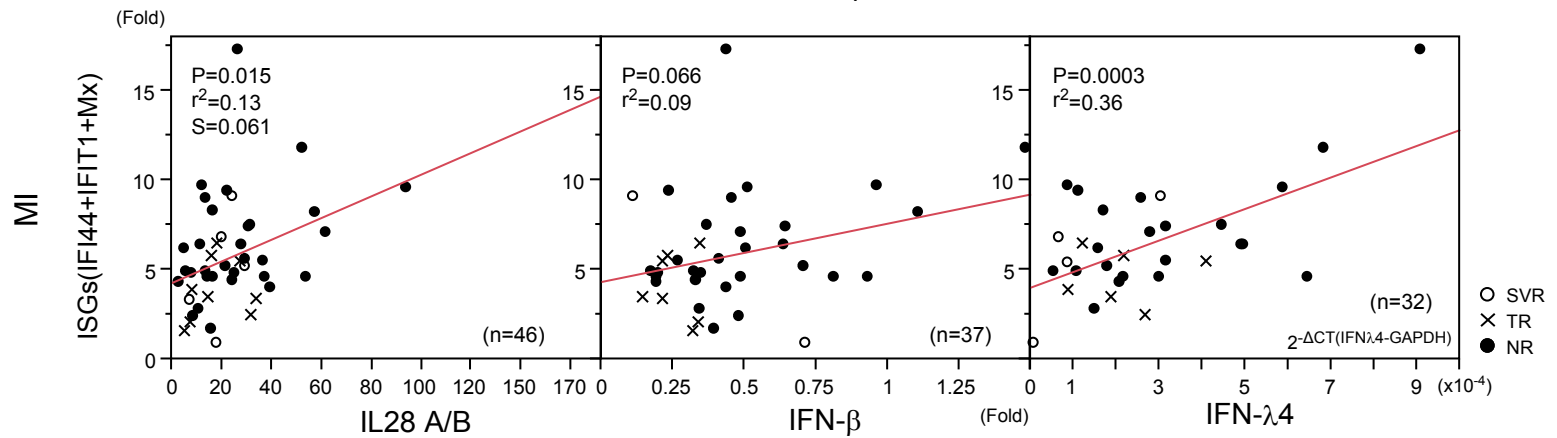
Fig.3**A****B****C**

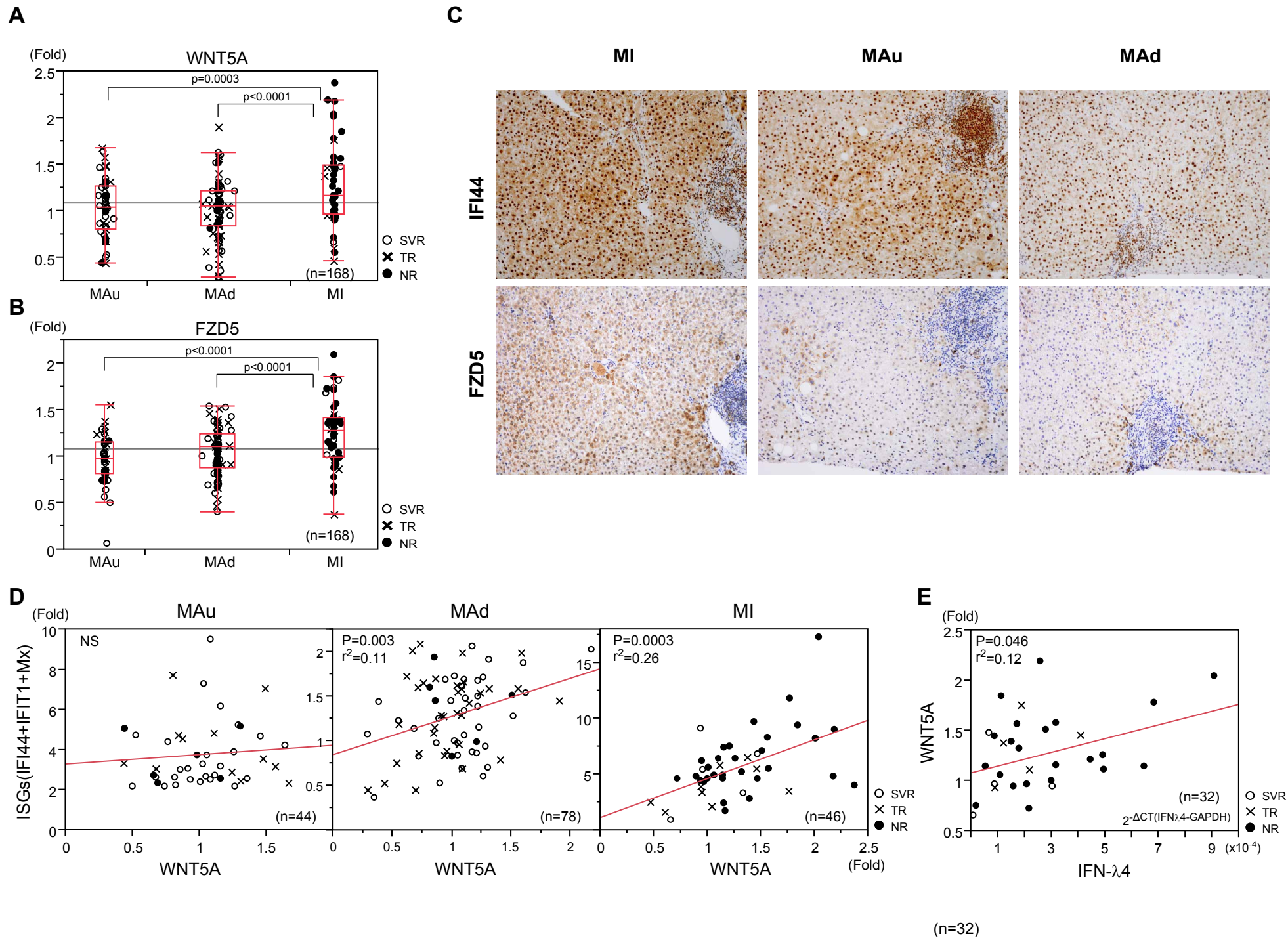
Fig.4

Fig.5