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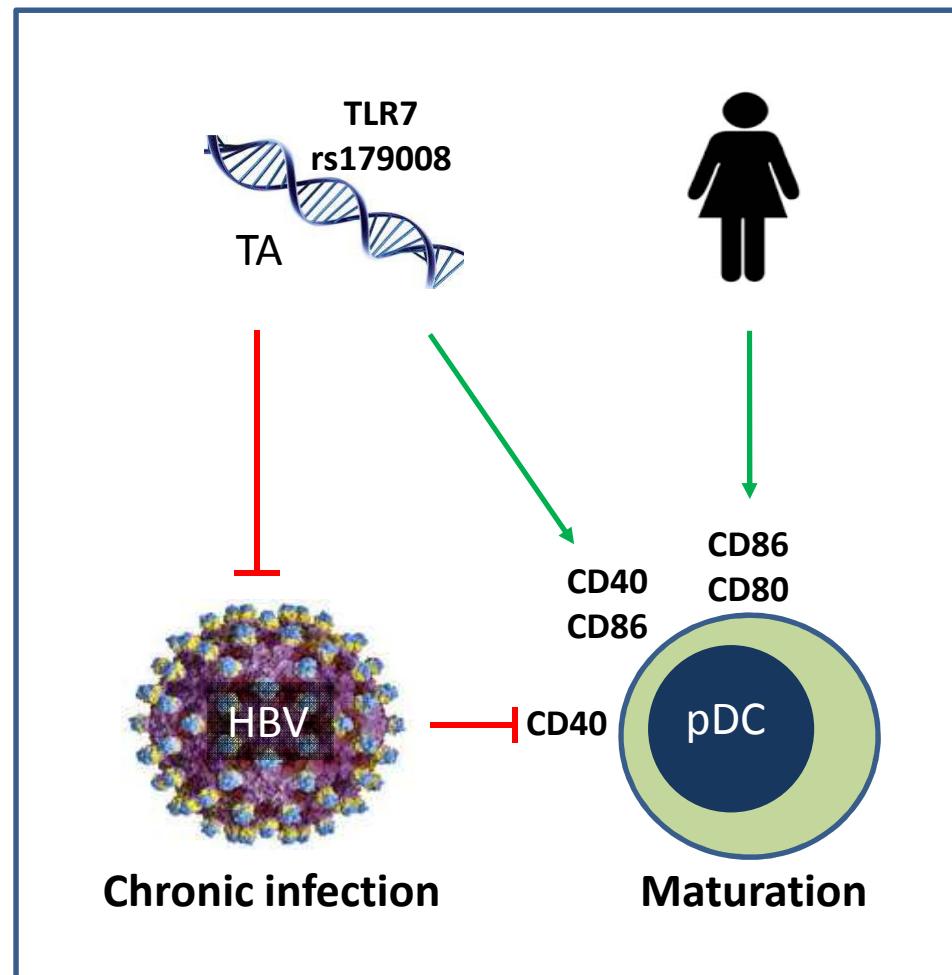
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## Graphical abstract



**TLR7 polymorphism, sex and chronic HBV infection influence plasmacytoid DC maturation by TLR7****ligands**

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**Abstract**

TLR7 agonists are of high interest for the treatment of cancer, auto-immunity and chronic viral infections. They are known to activate plasmacytoid dendritic cells (pDCs) to produce high amounts of Type I Interferon (IFN) and to facilitate T and B cell responses, the latter with the help of maturation markers such as CD40, CD80 and CD86. The TLR7 single nucleotide polymorphism (SNP) rs179008 (Gln11Leu), sex and chronic viral infection have all been reported to influence pDC IFN production. It is unknown, however, whether these factors also influence pDC phenotypic maturation and thereby IFN-independent pDC functions. Furthermore, it is unclear whether SNP rs179008 influences HBV susceptibility and/or clearance.

Here we investigated whether the SNP rs179008, sex and HBV infection affected phenotypic maturation of pDCs from 38 healthy individuals and 28 chronic HBV patients. In addition, we assessed SNP prevalence in a large cohort of healthy individuals (n=231) and chronic HBV patients (n=1054). Consistent with previous reports, the rs179008 variant allele was largely absent in Asians and more prevalent in Caucasians. Among Caucasians, the SNP was equally prevalent in healthy and chronically infected males. The SNP was, however, significantly more prevalent in healthy females than in those with chronic HBV infection (42 versus 28%), suggesting that in females it may offer protection from chronic infection. *Ex vivo* experiments demonstrated that induction of the co-stimulatory molecules CD40 and CD86 by TLR7 ligands, but not TLR9 ligands, was augmented in pDCs from healthy SNP-carrying females. Furthermore, CD80 and CD86 upregulation was more pronounced in females independent of the SNP. Lastly, our data suggested that chronic HBV infection impairs pDC maturation. These findings provide insight into factors determining TLR7 responses, which is important for further clinical development of TLR7-based therapies.

**Keywords:** plasmacytoid dendritic cell; TLR7; rs179008; sex; hepatitis B virus; Immunotherapy

## 1. Introduction

Toll Like Receptor 7 (TLR7) is of interest as a therapeutic target for chronic viral infections, including those with Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV), and also for other non-infectious diseases such as cancer, asthma and autoimmunity (1–3). The TLR7 gene is located on the X-chromosome. The receptor is expressed intracellularly on plasmacytoid dendritic cells (pDCs) and recognizes viral single stranded RNA (4). Besides TLR7, human pDCs also express autosomal TLR9 recognizing unmethylated CpG DNA (5). Upon TLR ligation, pDCs secrete high amounts of Interferon  $\alpha$  (IFN $\alpha$ ) inducing a potent anti-viral response in neighboring cells (2). In addition, TLR ligation induces pDC phenotypic maturation, characterized by upregulation of co-stimulatory molecules such as CD40, CD80 and CD86 and by the secretion of pro-inflammatory cytokines (4, 5). Via these and other receptors and cytokines, pDCs communicate with other immune cells and exert also IFN-independent immune functions, such as antigen presentation to T cells or facilitating B cell differentiation (6). Both the induction of IFN $\alpha$  and adaptive immunity by pDCs are considered important for TLR7 agonists to combat viral infection (2, 7). To optimize and personalize treatment and to select patients that benefit most from TLR7 agonist treatment, it is important to understand how genetic and environmental factors influence the response of pDCs to TLR7 agonists.

Previously, the TLR7 SNP rs179008 (A/T; Gln/Leu) has been shown to alter TLR7 function. PBMCs from males carrying the variant T allele secreted less IFN $\alpha$  upon TLR7 ligation than those from males with the more common A (i.e. WT) allele (8). Furthermore, in HIV, the variant allele associated with higher infection rates, viral load and disease progression (8, 9). In HCV, the variant was more prevalent among chronic patients as compared to healthy controls and related to a poor IFN $\alpha$  treatment response (10, 11). For HBV, the prevalence of the variant allele among chronic patients has not yet been explored.

pDC function is also affected by sex: male pDCs, as compared to female pDCs, produce less IFN $\alpha$  upon TLR7 but not TLR9 ligation (12, 13). This difference is believed to derive from expression differences of X-chromosomal genes downstream of TLR7, combined with hormonal effects (14–16). In addition, chronic HBV (CHB) infection itself can also impair pDC function; HBV impairs IFN $\alpha$  secretion and other pDC functions in response to TLR9 ligation (17–19). Whether rs179008, sex or CHB affect pDC phenotypic maturation in response to TLR7 ligands is not known.

Here we performed an elaborate screen of healthy individuals and CHB patients for their rs179008 genotype. In addition, on PBMCs from healthy and CHB patients, we assessed how this SNP, sex and CHB influence TLR-ligand induced pDC phenotypic maturation. Our results indicate that for CHB the rs179008 variant is not a risk factor and is even underrepresented in Caucasian female patients. On pDCs, both CD40 and CD86 were more induced on variant carrying females. Upregulation of co-stimulatory molecules on pDCs in response to TLR7, but not TLR9, was significantly higher in females than in males. Lastly, our results suggest that in CHB patients TLR7 ligand-induced pDC maturation may be suppressed. Together, these data demonstrate that rs179008 genotype, sex and chronic viral infection influence the response to TLR7 agonist therapy or pathogens that act via TLR7.

## 2. Methods

### 2.1 Sample collection and rs179008 genotyping

Blood samples were collected in EDTA or SST tubes. Patients were either CHB patients attending the outpatient clinic of Erasmus MC (Rotterdam, The Netherlands), participants in the 99-01 or PARC study (20, 21), or members of the Chinese community participating in viral hepatitis outreach screening events (Antwerp, Belgium). The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Written informed consent was obtained from all individuals. The ethical review board of Erasmus MC (Rotterdam, the Netherlands) and Antwerp University Hospital (Belgium) approved use of archived serum for this study.

Competitive allele-specific PCR assays (KASP, LGC genomics, Huddleston, UK) were employed for the detection of the reference SNP TLR7 rs179008. Whole blood or serum samples stored at -20°C or -80°C were used for DNA extraction and genotyping procedures, which were carried out centrally at LGC genomics as before (22, 23). Purified genomic DNA was used for genotyping. The genotype sequence was derived from NCBI.

### 2.2 Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by Ficoll density centrifugation (Ficoll-Paque™ plus, Amersham) and frozen at -150°C. PBMC were thawed and washed with RPMI Glutamax medium (Life Technologies) with 10% fetal calf serum (FCS; Sigma). 1,000,000 PBMC were stimulated in a 96-well plate in 250 µl RPMI glutamax medium containing 10% FCS, 100 U/ml penicillin/streptavidin (Gibco) and 10 mM HEPES (Lonza) using various stimuli. The cells were incubated with 20 ng/ml IL-3 (Miltenyi) alone or in combination with either 10 µg/ml CpG-A (Invivogen),

0.4 mM Loxoribin (Invivogen) or 0.5  $\mu\text{g/ml}$  R848 (Invivogen). For all conditions, cells were stimulated for 24 hours at 37°C, 5% CO<sub>2</sub>. Cells were stained with anti-CD123-APC (clone AC145; Miltenyi), anti-CD304-PerCP-Cy-5.5 (clone 12C2; Biolegend), anti-CD11c-Pe-Cy7 (clone 3.9; eBioscience), anti-CD80-FITC (clone MAB104; Beckman Coulter), anti-CD86-V450 (clone 2331; BD) and anti-CD40-PE (MAB89; Beckman Coulter). Data was acquired on a Canto II (BD) and analyzed using Flowjo (version 10.1 Tree Star Inc). pDCs were defined as CD11c-CD123+CD304+ within the lymphocyte gate as depicted in Figure S1.

### *2.3 Statistical analysis*

For statistical analysis flow cytometry-derived mean fluorescence intensities (MFI) were first log<sub>10</sub>-transformed and then fitted in linear mixed models in the R programming environment. Models included interaction terms between TLR effect and SNP, disease status (HBV or healthy) or sex and separate terms for sex, age and ethnicity. To test if the effect of TLR ligation was different between SNP genotypes, healthy individuals and HBV patients or between sexes, the model was fitted with and without these interaction terms. The effect on model fit was assessed using a likelihood ratio test (LRT). In case inclusion of interaction terms significantly improved model fit, regression coefficients and corresponding standard errors, p-values and adjusted p-values (corrected for multiple testing using the Holms procedure) were calculated from the model with that interaction term (24).



### 3. Results

#### 3.1 Population prevalence of rs179008 genotypes

To assess the prevalence of the rs179008 variant across different ethnicities we genotyped a large number of healthy donors and CHB patients. The SNP was successfully genotyped in 176 (out of 231) healthy donors and 994 (out of 1054) CHB patients. First, we compared our data to that of a published large genome-wide screen on genetic variation across many different ethnic backgrounds (25). We assessed SNP prevalence between cohorts for the various ethnicities separately to exclude that the ethnical composition of the cohorts would influence SNP prevalence. The prevalence of the SNP variant (T) in our own healthy cohort (from Rotterdam and Antwerp; Healthy EA) was nearly identical to that in the published database (Healthy DB) which contained rs179008 information of over 2000 individuals (Table 1; Figure 1). In both cohorts the variant was most present in Caucasians (21-22% in males and 42-43 % in females) and almost absent from Asians. The higher prevalence in females can be attributed to the X-chromosomal location of the TLR7 gene. (Table 1; Figure 1). Overall, male CHB patients carried the variant with a comparable frequency as healthy males. Interestingly, the variant was significantly more prevalent in healthy Caucasian females (~42% in both cohorts) compared to Caucasian female CHB patients (28%). Heterozygous females were almost twofold more prevalent in the healthy cohorts (TA: 37% in healthy versus 21 % in CHB). Thus, in Caucasian females the variant (T) allele may offer protection from CHB development.

#### 3.2 Effect of rs179008 genotype on pDC phenotypic maturation

Next, we studied the effect of rs179008 genotype on pDC phenotypic maturation. Based on rs179008 genotype, sex, age and sample availability, we selected PBMCs for *in vitro* stimulation. We analyzed

PBMC samples from 20 healthy individuals (10F/ 10M) carrying the WT allele only (A or AA), and 10 heterozygous (TA) females. Due to the low prevalence of the variant, we only had available PBMCs of 8 healthy individuals (2F/ 6M) homo- or hemizygous for the variant (T or TT; Table S1). WT individuals were chosen to match the age of those carrying the variant.

After thawing, PBMCs were cultured in the presence or absence of TLR-ligands Loxoribin (Lox; TLR7), R848 (TLR7 & TLR8) and CpG (TLR9) and after 24 hours the levels of CD40, CD80 and CD86 were assessed by flow cytometry (Figure S1). Lox, R848 and CpG all significantly increased expression levels of one or more surface markers (Figure 2A). Percentages of positive cells were also increased, but skewed towards “all positive” (Figure S2). Therefore we used expression levels for further statistical analysis. Lox and R848 potentially induced CD40 and CD86, while CpG hardly induced CD86, but upregulated CD40 and CD80. Lox, but not R848, also induced expression of CD80. Plotting samples from the two sexes separately indicated that CD40 and CD80 expression levels were significantly higher in females and this was most clear after TLR7/8 ligation (Figure 2B). In contrast, expression levels of CD86 were higher in males, both at baseline and after TLR9 ligation. Next, to examine the effect of rs179008 genotype on TLR-ligand induced pDC maturation we fitted linear mixed models including and excluding SNP genotype information and determined the effect on model fit using likelihood ratio tests (LRT). In this analysis samples from one individual were matched and age and sex were included in the models to reduce confounding effects. LRTs indicated that both CD40 and CD86, but not CD80, upregulation (i.e. expression change relative to subject-matched cells cultured without TLR ligands) was influenced by rs179008 genotype (Table 2). Closer inspection revealed that pDCs from heterozygous (TA) females significantly more upregulated CD40 and CD86 upon TLR 7/8 ligation, compared to WT individuals (A and AA; Table 2, Figure 3). Homo- or hemizygous presence of the variant allele (TT or T) did not appear to affect CD40 and CD86 upregulation compared to WT, but for these genotypes samples were limited. CpG-induced pDC maturation was less influenced by the SNP (Table 2; Figure 3). Together these data

indicate that TLR7/8 induced maturation of pDCs from females in general, but especially of rs179008 heterozygous females, was augmented.

### *3.3 Effect of sex on pDC phenotypic maturation*

Next, we investigated the effect of sex on pDC phenotypic maturation. Regression analysis demonstrated for all three co-stimulatory receptors that model fit was significantly improved by including the interaction between sex and TLR-effect (Table 3). All receptors were upregulated more on female pDCs, but only upon TLR7/8 ligation (Table 3 and Figure 4). Because the rs179008 heterozygous genotype (TA) only occurs in females, SNP genotype will contribute to, or could even explain, this effect of sex. Directly comparing pDCs from homo- and heterozygous females (AA vs TA; Figure 3 red symbols only) as well as comparing males and females carrying only the WT allele (A vs AA; Figure 3 black versus red) suggested that the higher upregulation of CD40 in females may be fully attributed to the SNP, while for CD86 the SNP may not be fully responsible. For CD80, as previously indicated, the SNP does not seem to contribute at all (Table 2). To further isolate the sex from the SNP effect, we performed regression analysis on males and females carrying the WT allele only (A versus AA; blue symbols in Figure 4). For CD80 and CD86 regression analysis indicated a remaining effect of sex on model fit within SNP WT samples. Closer inspection revealed that an interaction between sex and TLR7/8 ligand treatment remained also in this patient sub-group, but was reduced to a trend ( $p < 0.1$ ) after applying multiple testing correction (Table 4). CD40 upregulation within the SNP WT samples was not affected by sex. In summary, pDCs from females more profoundly upregulated all maturation markers upon TLR7/8 ligation, which for CD40, and to a lesser extend for CD86, could be attributed to the rs179008 TA genotype.

### *3.4 Effect of CHB on pDC phenotypic maturation*

Lastly, we assessed pDC maturation in CHB patients. We retrieved PBMC samples from 28 CHB patients, 19 (10F/ 9M) carrying the WT genotype only, 6 heterozygous females and 3 carrying the variant only (1F/ 2M). While the healthy cohort consisted mostly of Caucasians, approximately half of our CHB cohort was of Turkish decent, including most females (Table S1). The imbalanced distribution of ethnicities over sexes did not allow proper evaluation of the effect of sex or rs179008 genotype within this cohort (Table S1). In CHB patients, however, we observed a rather low induction of maturation markers, especially of CD40 (Figure 5A). Direct comparison of the healthy and CHB cohorts by regression analysis, taking into account sex and ethnicity, suggested that CD40 upregulation by TLR7 ligands may indeed be impaired in CHB patients (Figure 5B and Table 5). Inspection of viral and patient parameters (HBsAg, HBV DNA, ALT) did not reveal any causal relation between these parameters and this impairment (data not shown).

#### 4. Discussion

Our results demonstrate that rs179008 may reduce the risk of heterozygous Caucasian females to develop CHB, and that the heterozygous SNP genotype and female sex together positively influence pDC phenotypic maturation, while CHB impairs pDC maturation.

Our data adds to previous reports on the influence of sex on production of IFN $\alpha$  by pDCs, demonstrating that also surface maturation markers are upregulated more in female pDCs (12–15). The effect of sex on upregulation of especially CD40 but also CD86 was respectively mostly or partially explained by the rs179008 variant allele. For CD80 only an effect of sex was observed that was independent of rs179008 genotype.

Although we applied stringent statistical criteria to reach our conclusions, the limited number of samples in our study may have caused us to miss less pronounced effects. Furthermore, analysis of the effect of sex and rs179008 in CHB patients was hampered by differences in ethnicities between the sexes in this cohort. For more definitive conclusions on TLR7 function in CHB patients, therefore, additional studies are needed.

Our observations that the rs179008 variant may protect against CHB in Caucasian females is contrasting previous findings for HCV and HIV. In both infections, the variant allele associated with higher disease prevalence and augmented disease progression, and with reduced response to IFN $\alpha$ -therapy for HCV (8, 9, 11, 26, 27). In all these studies, effects were most prominent in (mostly rs179008 heterozygous) females and all studies predominantly contained Caucasian individuals. The fact that HCV and HIV, but not HBV, have been reported to activate TLR7 and pDCs, could contribute to the contrasting observations for these diseases (17, 28, 29). To confirm that rs179008 SNP is truly protective for CHB, our study needs to be repeated in a larger cohort. This cohort preferably should also contain patients

spontaneously clearing the disease, similar to a recent study performed on TLR9 SNPs in HCV disease progression (30).

In HCV, the rs179008 variant associated with lower levels of IL-10 and Type III IFNs in infected liver tissue, while healthy donor pDCs carrying the variant displayed impaired IFN $\alpha$  production (8, 11). These are the only reported effects of the SNP at the protein/cellular level and these data were mostly (for livers), or completely (for pDCs), derived from hemizygous males. As stated above, however, most reported clinical effects of the SNP are observed in females (8, 26). We also identified effects of the SNP only in females. Unfortunately, we could not assess IFN $\alpha$  secretion to reunite these studies, because we relied on pDCs from a biobank and IFN $\alpha$  secretion is hampered by cryopreservation (31).

Incomplete X-inactivation of TLR7 may contribute to the effects of sex. Although previous studies found no evidence for incomplete or skewed X-inactivation this was recently challenged by Souyris and colleagues who reported that pDC express more TLR7 in females due to escape of X-inactivation (10, 12, 32). Besides, TLR7 function may be enhanced by female hormones and/or enhanced expression of molecules downstream of TLR7 (14–16). It remains unclear why possession of a heterozygous genotype offers an advantage when it comes to pDC maturation and reducing CHB risk. Possibly, pDC heterogeneity may facilitate their diverse functions in innate and adaptive immunity.

Because we stimulated PBMCs rather than pDCs, also other immune cells contributed to pDC maturation via cytokines or membrane receptors, as would occur *in vivo*. Previously, we reported on indirect effects of other cells in culture (17, 33). TLR7/8 ligand R848 also activates monocytes and other DC-subsets expressing TLR8 (or TLR7 upon activation) which indirectly contributes to the activation of pDCs (34–36). Dissecting direct and indirect effects requires additional experiments with sorted pDCs, which are not feasible due to insufficient fresh PBMC of rs179008 genotyped individuals.

Recently, two studies reported that pDCs sorted based on the absence of CD11c and expression of CD123 and BDCA4, as was also our strategy and common practice until recently, are contaminated by a population of pre-cDCs expressing AXL that highly expresses co-stimulatory molecules (37, 38) . Although it can be deduced from these studies that *bona fide* pDCs still outnumber contaminating cDCs using this gating strategy, it is not known whether the extent of pre-cDC contamination varies between males and females or whether it is affected by infection. Therefore, we do not know if pre-cDCs have influenced our results. Future studies should address this issue.

Recently, oral TLR7 ligands successfully induced viral control in Woodchuck and chimpanzee HBV-animal models (39, 40). Disappointingly in humans, oral TLR7 ligands thus far have not achieved any beneficial effect, despite that treatment was well tolerated (41, 42). It is not clear why clinical benefit in CHB patients was lacking, but impaired patient TLR7 responses could have contributed. Of note, these studies mainly included Asians and males and therefore only very few rs179008 variant carrying individuals and even less to no SNP heterozygous females were treated.

Future studies are needed to determine the functional consequences of the variation in pDC maturation. Effects were most prominent for CD40, which is mostly expressed on antigen presenting cells. Its ligand is expressed on activated T cells, endothelial cells and platelets (reviewed in (43)). On pDCs, CD40 ligation has been described to augment the production of IL-6, thereby facilitating generation of antibody producing plasma cells (44). Furthermore, CD40 signaling can aid effective activation of T cells or prevent T cell exhaustion (5, 45–48). In CHB patients low and exhausted T cells are consistently observed and held responsible for the inability to clear HBV (49–53). In CHB patients and individuals homozygous for rs179008 less induction of CD40 may thus affect the ability of pDCs to drive adaptive immune responses and could facilitate T cell exhaustion. Simultaneous low induction of CD86 and CD80 may further limit adaptive responses.

Taken together, our study indicates that rs179008 genotype may be relevant for the immune response against HBV and highlights that TLR7 induced pDC maturation is affected by sex, TLR7 genotypic variation and chronic viral infection. These findings provide important insight in the variation in TLR7 responses in healthy and diseased individuals which is of relevance for the further clinical development and evaluation of TLR7-based therapies

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## 6. Conflicts of interest

AB has been consulting or in advisory boards for Gilead Sciences and Bristol-Myers Squibb and has received research grants from Roche, Gilead Sciences, Fujirebio, and Janssen. AW has received research grants from Roche.



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## 8. Tables

Table 1: TLR7 rs179008 Genotype Distribution in Healthy individuals and HBV patients.

Gender	Ethnicity	Dataset	# A/AA (%)	AT	T/TT (%)	T (%)	vs HBV (RA) p-value <sup>§</sup>	vs Healthy (RA) p-value <sup>§</sup>
Male	Combined	Healthy (DB)*	1100 (89.2)	-	133 (10.8)	10.8	-	-
		Healthy (RA) <sup>#</sup>	50 (89.3)	-	6 (10.7)	10.7	-	-
		HBV (RA)	566 (85.8)	-	94 (14.2)	14.2	-	-
	Caucasian	Healthy (DB)	189 (78.8)	-	51 (21.3)	21.3	0.5192	1
		Healthy (RA)	21 (77.8)	-	6 (22.2)	22.2	0.6149	-
		HBV (RA)	256 (81.3)	-	59 (18.7)	18.7	-	0.6149
	Turkish	Healthy (DB)	-	-	-	-	-	-
		Healthy (RA)	-	-	-	-	-	-
		HBV (RA)	63 (86.3)	-	10 (13.7)	13.7	-	-
	Asian	Healthy (DB)	489 (97)	-	15 (3.0)	3.0	-	-
		Healthy (RA)	27 (100)	-	0 (0.0)	0.0	-	-
		HBV (RA)	133 (100)	-	0 (0.0)	0.0	-	-
	Other	Healthy (DB)	-	-	-	-	-	-
		Healthy (RA)	2 (100)	-	-	0.0	-	-
		HBV (RA)	114 (82.0)	-	25 (18.0)	18.0	-	-
Female	Combined	Healthy (DB)	989 (77.8)	251	31 (2.4)	22.2	-	-
		Healthy (RA)	95 (79.2)	22 (18.3)	3 (2.5)	20.8	-	-
		HBV (RA)	277 (82.9)	47 (14.1)	10 (3.0)	17.1	-	-
	Caucasian	Healthy (DB)	151 (57.4)	98 (37.3)	14 (5.3)	42.6	<b>0.0198</b>	1
		Healthy (RA)	33 (57.9)	21 (36.8)	3 (5.3)	42.1	0.101	-
		HBV (RA)	60 (72.3)	17 (20.5)	6 (7.2)	27.7	-	<b>0.0198</b>
	Turkish	Healthy (DB)	-	-	-	-	-	-
		Healthy (RA)	4 (80.0)	1 (20.0)	-	20.0	-	-
		HBV (RA)	31 (68.9)	11 (24.4)	3 (6.7)	31.1	-	-
	Asian	Healthy (DB)	476 (95.6)	21 (4.2)	1(0.2)	4.4	-	-
		Healthy (RA)	55 (100.0)	0 (0.0)	0 (0.0)	0.0	-	-
		HBV (RA)	121 (98.4)	2 (1.6)	0 (0.0)	1.6	-	-
	Other & Unknown	Healthy (DB)	-	-	-	-	-	-
		Healthy (RA)	3 (100)	-	-	0.0	-	-
		HBV (RA)	65 (78.3)	17 (20.5)	1 (1.2)	21.7	-	-

\*Data from the "1000 genomes project" database (DB). Caucasians are all Europeans from this DB. East and south Asia were pooled. <sup>#</sup> Data from healthy controls and HBV patients collected in Rotterdam and Antwerp (RA). <sup>§</sup>Prevalence of variant allele carrying individuals in the two cohorts being compared by Fisher exact test.

**Table 2: Interaction of TLR7 SNP rs179008 with TLR-ligand induced pDC maturation in healthy individuals.**

		CD40			CD80			CD86		
rs179008 LRT p- value:		0.0003			0.2465			0.0082		
Type	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
<b>Main effects</b>	Intercept	3.38			2.98			3.46		
	CpG	0.40	0	0	0.17	0	0	0.04	0.2702	1
	Lox	0.42	0	0	0.12	0.0001	0.0005	0.26	0	0
	R848	0.34	0	0	0.00	0.8678	1	0.15	0.0002	0.0027
	rs179008 TT	0.03	0.7221	1	-0.03	0.4586	1	-0.06	0.3897	1
	rs179008 TA	-0.34	0.0009	0.0083	-0.05	0.2159	0.8635	-0.11	0.0784	0.6268
	SexM	-0.20	0.0096	0.0671	-0.08	0.0366	0.1830	0.09	0.0460	0.4137
	Age	0.00	0.0785	0.3926	0.00	0.4712	1	0.00	0.7196	1
<b>Interactions</b>	CpG: rs179008 TT	-0.03	0.7356	1	-	-	-	0.06	0.3835	1
	Lox: rs179008 TT	0.09	0.2728	1	-	-	-	0.05	0.4535	1
	R848: rs179008 TT	-0.08	0.3737	1	-	-	-	0.09	0.1932	1
	CpG: rs179008 TA	0.20	0.0129	0.0773	-	-	-	0.10	0.1369	0.9582
	Lox: rs179008 TA	0.26	0.0013	0.0103	-	-	-	0.23	0.0010	0.0101
	R848: rs179008 TA	0.32	0.0001	0.0010	-	-	-	0.23	0.0007	0.0082

**Table 3: Interaction of sex with TLR-ligand induced pDC maturation in healthy individuals**

		CD40			CD80			CD86		
Sex LRT p- value:		0.0039			0.0001			0		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
<b>Main effects</b>	Intercept	3.25			2.93			3.37		
	CpG	0.47	0	0	0.15	0	0.0002	0.11	0.0026	0.0077
	Lox	0.56	0	0	0.20	0	0	0.43	0	0
	R848	0.51	0	0	0.06	0.0838	0.3353	0.32	0	0
	SexM	-0.02	0.8177	0.8818	0.01	0.7935	1	0.21	0.0001	0.0004
	Age	0.00	0.1291	0.3872	0.00	0.8484	1	0.00	0.6595	0.6595
<b>Interactions</b>	CpG: Sex M	-0.05	0.4409	0.8818	0.03	0.6066	1	-0.06	0.2919	0.5838
	Lox: Sex M	-0.13	0.0569	0.2276	-0.18	0.0014	0.0082	-0.25	0	0
	R848: Sex M	-0.24	0.0008	0.0041	-0.16	0.0045	0.0225	-0.22	0.0001	0.0003

**Table 4: Interaction of Sex with TLR-ligand induced pDC maturation in healthy individuals carrying the WT A(A) rs179008 allele only.**

		CD40			CD80			CD86		
<b>Sex LRT p-value in A &amp; AA only:</b>		0.2352			<b>0.0073</b>			<b>0.0152</b>		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
<b>Main effects</b>	Intercept	3.49			2.94			3.46		
	CpG	0.40	0	<b>0</b>	0.18	0.0019	<b>0.0133</b>	0.07	0.2182	0.6545
	Lox	0.42	0	<b>0</b>	0.20	0.0005	<b>0.0040</b>	0.36	0	<b>0</b>
	R848	0.34	0	<b>0</b>	0.08	0.1746	0.6983	0.23	0.0001	<b>0.0004</b>
	SexM	-0.18	0.0831	0.1663	0.04	0.6106	1	0.18	0.0098	0.0500
	Age	0.00	0.7225	0.7225	0.00	0.6928	1	-0.00	0.7572	1
<b>Interactions</b>	CpG: Sex M	-	-	-	0.03	0.7258	1	-0.05	0.5318	1
	Lox: Sex M	-	-	-	-0.20	0.0118	0.0692	-0.21	0.0083	0.0500
	R848: Sex M	-	-	-	-0.20	0.0115	0.0692	-0.17	0.0263	0.1051

**Table 5: Interaction between chronic HBV infection and TLR-ligand induced pDC maturation**

		CD40			CD80			CD86		
<b>HBV LRT p-value:</b>		<b>0.027</b>			0.7405			0.2298		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
<b>Main effects</b>	Intercept	3.41			2.96			3.46		
	CpG	0.44	0	<b>0</b>	0.17	0	<b>0</b>	0.08	0.0013	<b>0.0064</b>
	Lox	0.50	0	<b>0</b>	0.10	0	<b>0.0001</b>	0.30	0	<b>0</b>
	R848	0.41	0	<b>0</b>	0.00	0.9490	1	0.19	0	<b>0</b>
	CHB	-0.06	0.4737	1	0.00	0.9168	1	0.07	0.0430	0.1720
	Sex M	-0.12	0.0423	0.2115	-0.08	0.0020	<b>0.0098</b>	0.05	0.1204	0.3613
	Age	0.00	0.7110	1	0.00	0.1975	0.7901	0.00	0.7040	0.7040
	Ethnicity Turkish	0.05	0.5726	1	-0.05	0.2085	0.7901	-0.06	0.1520	0.3613
	<b>Interactions</b>	CpG: CHB	-0.05	0.3765	1	-	-	-	-	-
Lox: CHB		-0.14	0.0071	<b>0.0498</b>	-	-	-	-	-	-
R848: CHB		-0.11	0.0312	0.1873	-	-	-	-	-	-

## 9. Figure legends

**Figure 1: TLR7 rs179008 Genotype Distribution in Healthy individuals and HBV patients.** Visualization of the most important findings in table 1.

**Figure 2: Effect of the rs179008 SNP on the expression level of maturation markers on pDCs from healthy donors.** (A) Mean fluorescent intensities (MFI) of CD40, CD80 and CD86 after 24 hours in the presence of absence of indicated TLR-ligands or medium alone (B) Data as in A but for males (M) and females separately (F) derived pDCs and color coded for the rs179008 TLR7 SNP. P-values \*\*\*<0.001, \*\*<0.01 and \*< 0.05 by paired t-tests comparing TLR-ligand activated pDCs to medium cultured pDCs (A) or by unpaired t-tests comparing pDCs from males to those of females for each condition (B), all test were performed on log transformed data. Non-significant results are not shown.

**Figure 3: Interaction of SNP rs179008 with TLR induced upregulation of maturation markers on pDCs from healthy donors.** (A & B) Fold change (FC) in log mean fluorescence intensity of CD40 (A) and CD86 (B) on pDCs from healthy donors upon TLR ligation compared to incubation with medium only (i.e.  $\log(\text{TLR}) - \log(\text{medium})$ ). Data was grouped according to TLR rs179008 genotype and colored according to sex of the donor (males in black, females in red). P-values displayed represent the (multiple testing corrected) adjusted p-values displayed in Table 2 for the interaction between the genotypes and TLR-ligand induced upregulation of each surface marker. \*\*  $p < 0.01$ , \*  $p < 0.05$ . Non-significant results are not shown.

**Figure 4: Interaction of sex with TLR-ligand induced pDC surface marker upregulation.** Fold change (FC) in log mean fluorescence intensity of CD40, CD80 and CD86 on pDCs from healthy donors upon TLR ligation compared to incubation with medium only (i.e.  $\log(\text{TLR}) - \log(\text{medium})$ ). Data are grouped according to sex and colored according to rs179008 SNP (Legend). P-values displayed represent the (multiple testing corrected) adjusted p-values displayed in table 3 for the interaction between sex and



TLR-ligand induced upregulation of each surface marker. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  
 $p < 0.0001$ . Non-significant results are not shown.

**Figure 5: Effect of TLR ligation on maturation markers on pDCs from HBV patients and healthy individuals.** A) Mean fluorescent intensities (MFI) of CD40, CD80 and CD86 after 24 hours in the presence of absence of indicated TLR-ligands or medium alone for healthy individual and HBV patient derived pDCs. P-values comparing healthy and HBV for all conditions by unpaired t-tests on log transformed data. \*  $p < 0.05$ . Non-significant p-values are not shown (B) Fold change (FC) in log mean fluorescence intensity of CD40 on pDCs from healthy donors and HBV patient upon TLR ligation compared to incubation with medium only (i.e.  $\log(\text{TLR}) - \log(\text{medium})$ ). \*  $p < 0.05$  represents adjusted p-values displayed in table 4 for the interaction between HBV status and induced upregulation of CD40.

Figure 1

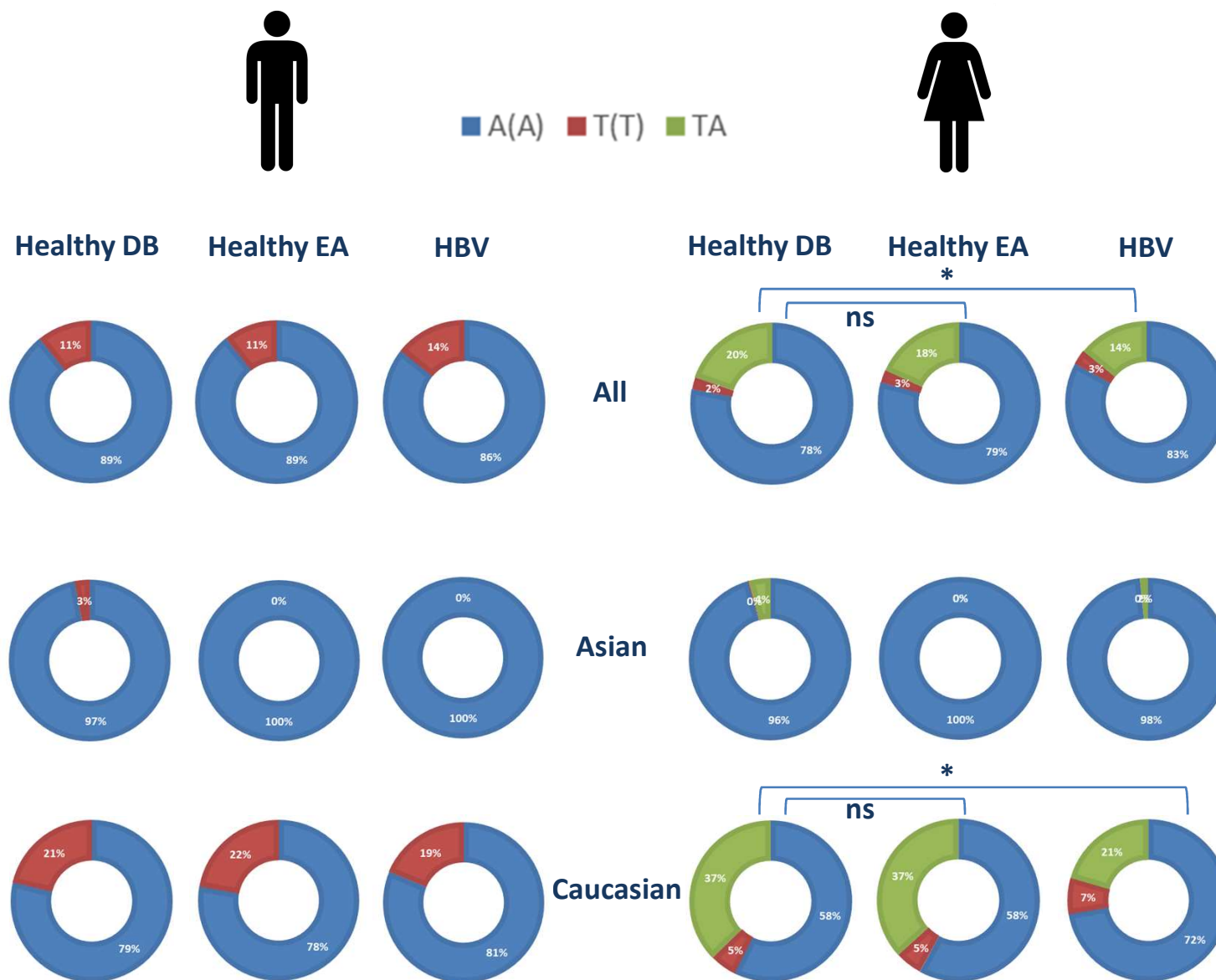


Figure 2

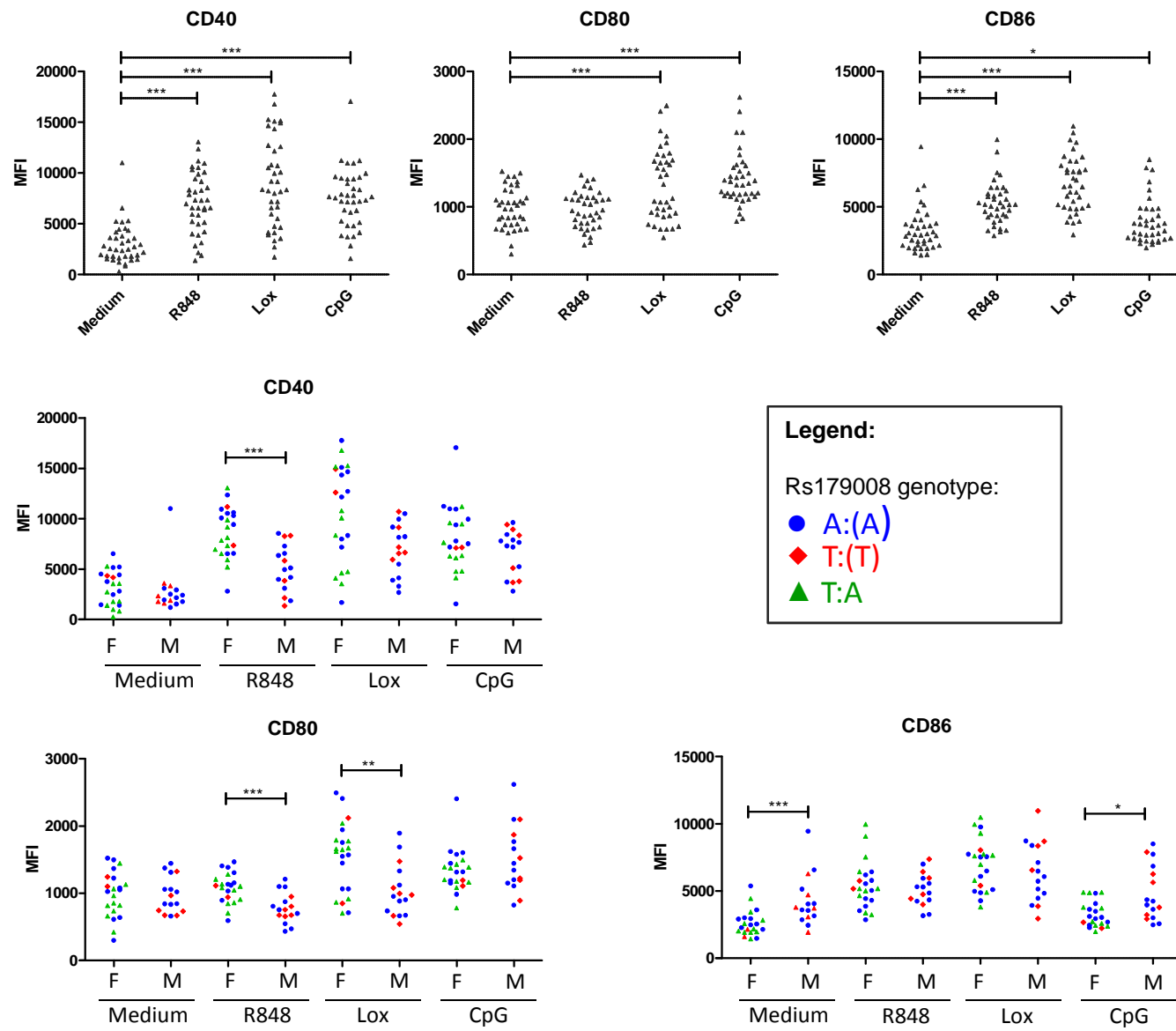


Figure 3

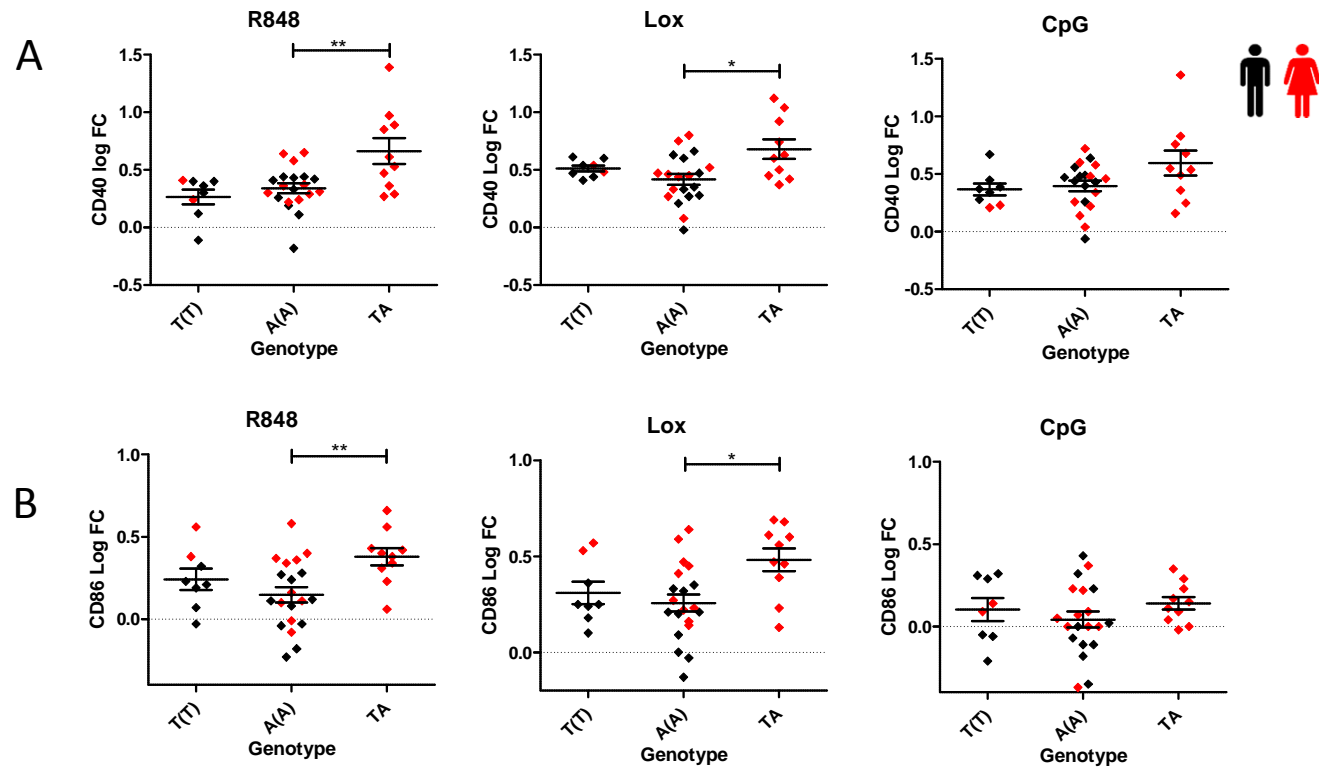


Figure 4

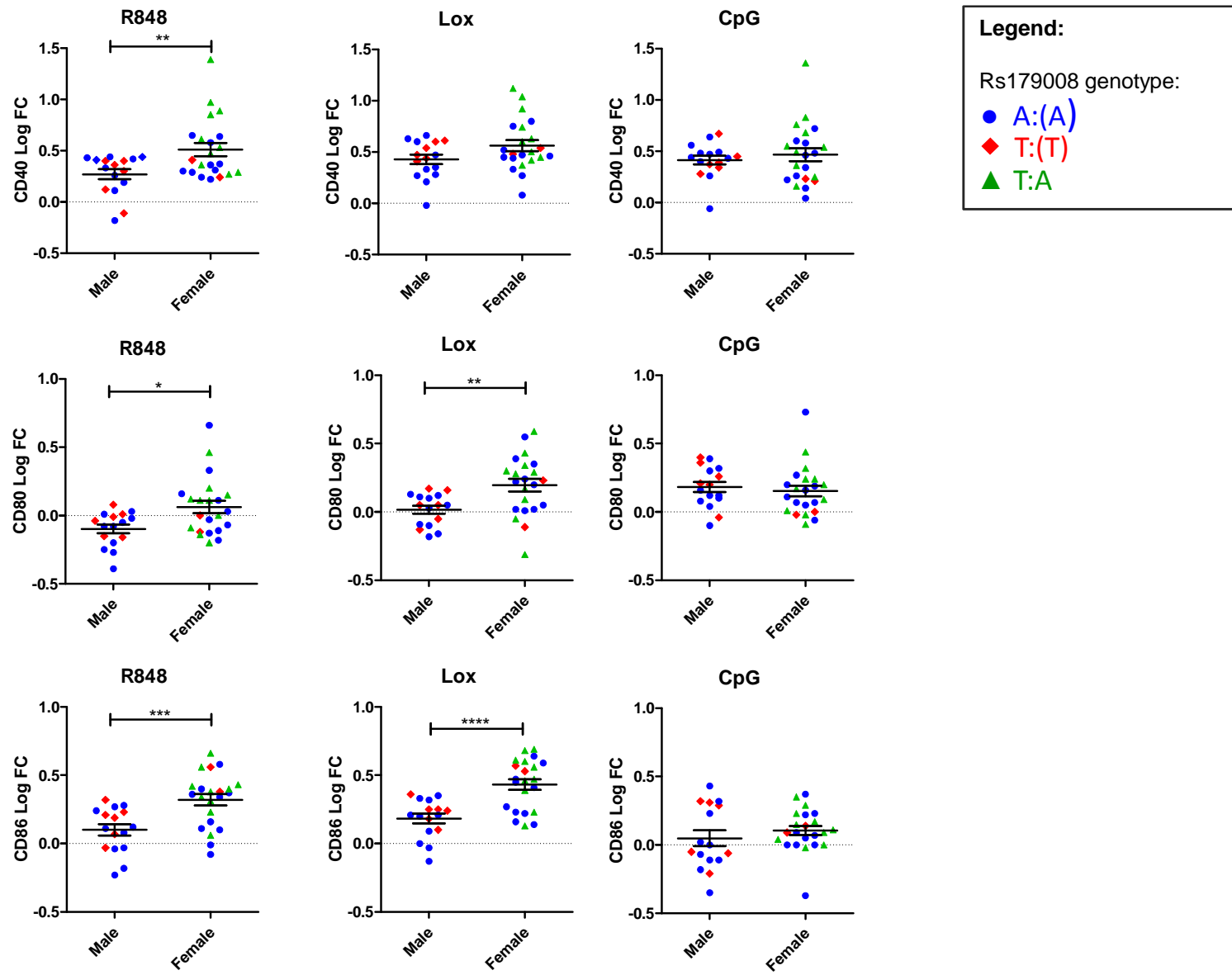
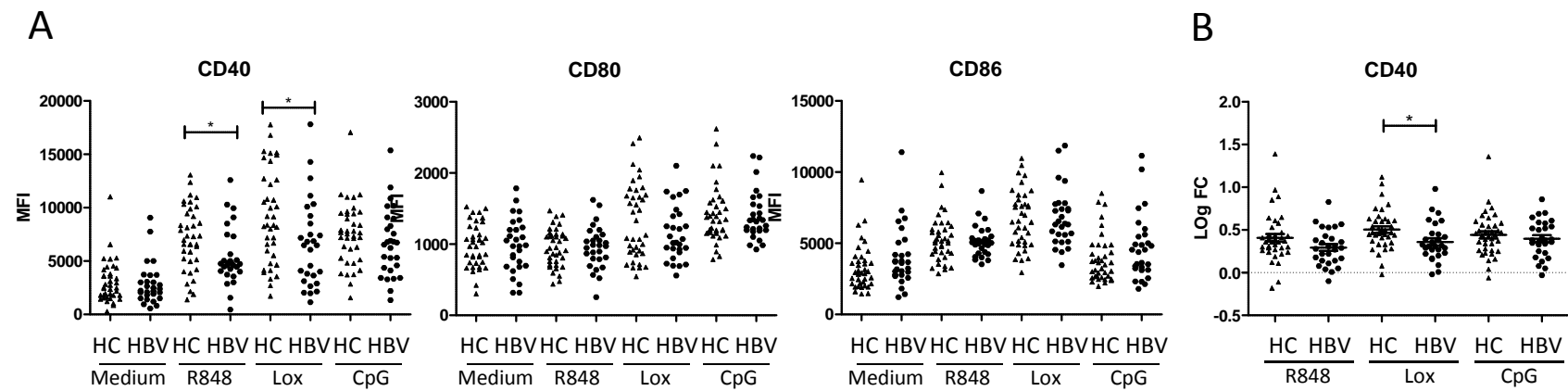


Figure 5



**Highlights**

- The TLR7 SNP rs179008 variant is more prevalent in healthy than in chronic HBV-infected females.
- TLR7 SNP rs179008 and sex both affect pDC phenotypic maturation in response to TLR7 ligation.
- Chronic HBV infection suppresses upregulation of CD40 on pDC upon TLR7 ligation.
- TLR7 SNP rs179008 genotype, sex and HBV infection status may influence the response to TLR7 targeted Immunotherapy.