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TCL1A SNPs and estrogen-mediated toll-like receptor-MYD88-dependent NF-κB activation: SNP and SERM-dependent modification of inflammation and immune response

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Non-standard abbreviations

4-OH-TAM, 4-hydroxytamxifen

AI, aromatase inhibitor

E2, estradiol

ER, estrogen receptor

ERE, estrogen response element

IRB, Institutional Review Board

MSAEs, Musculoskeletal adverse events

PgRs, progesterone receptors

SNPs, single nucleotide polymorphisms

LCLs, lymphoblastoid cell lines

TCL1A, T-Cell Leukemia/Lymphoma 1A

TLRs, Toll like receptors

Abstract

In a previous genome-wide association study (GWAS) for musculoskeletal adverse events during aromatase inhibitor therapy of breast cancer, we reported that single nucleotide polymorphisms (SNPs) near the *TCL1A* gene were associated with this adverse drug reaction. Functional genomic studies showed that TCL1A expression was induced by estradiol (E2), but only in cells with the variant sequence for the top GWAS SNP (rs11849538), a SNP that created a functional estrogen response element. In addition, *TCL1A* genotype influenced the “downstream” expression of a series of cytokines and chemokines and had a striking effect on NF-κB transcriptional activity. Furthermore, this SNP-dependent regulation could be “reversed” by selective estrogen receptor modulators (SERMs). The present study was designed to pursue mechanisms underlying *TCL1A* SNP-mediated, estrogen-dependent NF-κB activation. Functional genomic studies were performed with a panel of 300 lymphoblastoid cell lines for which we had generated genome-wide SNP and gene expression data. It is known that toll-like receptors (TLRs) can regulate NF-kB signaling by a process that requires the adaptor protein MYD88. We found that TLR2, TLR7, TLR9 and TLR10 expression, as well as that of MYD88, could be modulated by *TCL1A* in a SNP and estrogen-dependent fashion and that these effects were reversed in the presence of SERMs. Furthermore, MYD88 inhibition blocked the *TCL1A* SNP and estrogen-dependent NF-κB activation as well as protein-protein interaction between TCL1A and MYD88. These observations greatly expand the range of pathways influenced by *TCL1A* genotype and raise the possibility that this estrogen and SNP-dependent regulation might be altered pharmacologically by SERMs.

Introduction

In a previous genome-wide association study (GWAS) addressing musculoskeletal adverse events in breast cancer patients treated with aromatase inhibitors, we identified single nucleotide polymorphisms (SNPs) 3' of the T-Cell Leukemia/Lymphoma 1A gene (*TCL1A)* that were associated with this adverse drug reaction (Ingle et al., 2010). The minor allele frequency for the TCL1A SNP (rs11849538) is ~19% in European, African, Asian and Caucasian American according to the 1000 Genome Project (Ingle et al., 2010). We subsequently performed a series of functional genomic studies using as a model system lymphoblastoid cell lines (LCLs) for which we had generated dense genome-wide genomic data. Those studies showed that TCL1A expression was up-regulated by estradiol (E2), but only in cell lines homozygous for the variant *TCL1A* genotype for the top GWAS SNP (rs11849538) (**Figure 1A**), a SNP that created a functional estrogen response element (ERE) (Ingle et al., 2010). We subsequently found that there were at least three SNPs 3' of *TCLIA*, rs11849538, rs7259033, and rs7160302 (see **Figure 1A**)—all in tight linkage disequilibrium (LD)—that appeared to act in concert to influence the E2-dependent induction of TCL1A expression, in part because SNPs near but not in an ERE can affect estrogen receptor (ER) binding to the ERE and subsequent transcription (Ho et al., 2016). In addition, induction of *TCL1A* by E2 was associated "downstream" with variation in the expression of a series of pro-inflammatory cytokines (Liu et al., 2012) and chemokines (Ho et al., 2016) as well as their receptors (see **Figures 1 D-1G**), and with striking variation in NF-κB transcriptional activity (Liu et al., 2012). For example, we found that the expression of immune mediators such as the C-C motif chemokine receptor 6 (CCR6) and its only known ligand, CCL20 as well as the cytokine receptor IL17RA and its ligand IL17A could be modulated by *TCL1A* in a SNP-estrogen-dependent fashion (Ho et al., 2016). In addition, we found that the

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SNP and estrogen-dependent induction of TCL1A expression could be **“reversed”** after estrogen receptor (ER) blockade, i.e. when cells were exposed to 4-hydroxytamoxifen (4-OH-TAM), an active metabolite of tamoxifen, or to fulvestrant (formerly ICI 182,780) (**Figure 1B-1C**) an estrogen receptor antagonist (Ho et al., 2016; Liu et al., 2012). Specifically, cells homozygous for the wild-type (WT) genotype for the *TCL1A* SNPs displayed increased TCL1A expression whereas cells homozygous for the variant genotype showed decreased expression in the presence of these drugs—the opposite of the situation seen after estrogen exposure alone (**Figures 1B-1C**). Even more striking, downstream receptors for inflammatory mediators (CCR6 and IL17RA) responded in a parallel fashion—indicating that TCL1A was “upstream” of the cytokine and chemokine receptors in terms of these pathways (Ho et al., 2016). It should be emphasized that the SNPs involved were those 3’ of *TCLIA*, not SNPs that mapped to the *IL17RA* or *CCR6* genes (Ho et al., 2016). Finally, SNP and estrogen-dependent TCL1A induction resulted in significantly increased NF-κB transcriptional activity after ER blockade, but only for the variant genotype (Liu et al., 2012). Taken as a whole, these observations suggest that *TCL1A* genotype is capable of influencing the expression of a series of genes that play important roles in inflammation and immunity—and they also raise the possibility of pharmacological approaches, i.e. exposure to SERMs, that could be used to modify these effects.

Although we have studied the transcriptional regulation of cytokines and chemokines and their receptors in relation to *TCL1A* SNPs and estrogens (Ho et al., 2016; Liu et al., 2012), significant gaps remain in our knowledge, particularly with regard to mechanisms that might be involved in the effect of *TCL1A* on NF-κB signaling. NF-κB activation is a multifactorial, complex process involving many biological pathways such as DNA damage (McCool and Miyamoto, 2012), proteasome mediated degradation (Hayden et al., 2006), signal transduction via stimulation of T cells or exposure to inflammatory molecules (Takeda et al., 2008). The expression of NF-κB varies among cell-types and can be "event-specific" depending on the stimulus (Tak and Firestein, 2001). Toll like receptors (TLRs) are crucial immune mediators and can have a profound effect on inflammation as a result, in part, of NF-κB activation (Kawasaki and Kawai, 2014; Tak and Firestein, 2001). In the present experiments, we set out to study mechanisms that might be involved in the *TCL1A* SNP-mediated, estrogen-dependent activation of NF-κB (3). Specifically, we set out to determine whether the expression of TLRs and their adaptor molecules might be altered in a *TCL1A* SNP-dependent fashion, and, if so, their possible contribution to the *TCL1A* SNP-mediated, estrogen-dependent activation of NF-κB. In addition, we tested the hypothesis that SERMs might be able to modulate these effects. In summary, the experiments described subsequently involved the application of functional genomic studies to identify mechanisms underlying the effect of *TCL1A* SNPs on the regulation of inflammatory mediators.

Materials and Methods

Ethics statement

The Mayo Clinic Institutional Review Board (IRB) determined that this work did not require IRB review or approval.

"Human Variation Panel" lymphoblastoid cell lines

A "Human Variation Panel" lymphoblastoid cell line (LCL) model system consisting of 300 LCLs from healthy subjects of three ethnicities (100 European-American, 100 African-American and 100 Han Chinese-American) was utilized to perform these studies. This cell line model system provides genome-wide mRNA expression as determined by Affymetrix U133 2.0 Plus GeneChip expression array as well as genome-wide SNP data generated with Illumina 550K and 510S SNP BeadChip SNP arrays (Illumina, San Diego, CA, USA). The genotype data were used to impute approximately seven million SNPs per cell line.

RNA interference and transfection

siRNA (TCL1A and MYD88) and negative control were purchased from Dharmacon (Chicago, IL, USA). LCLs were transfected with siRNA by electroporation using the nucleofector kit (Lonza, Allendale, NJ, USA). Briefly, the electroporation reaction contained 2×10^6 cells, 100µl of nucleofector solution and 1µM of siRNA. After electroporation, cells were transferred into 12-well plates containing pre-equilibrated RPMI medium.

Drug treatment

LCLs with known genotypes were cultured in RPMI 1640 media (Cellgro, Manassas, VA, USA) supplemented with 15% FBS (Atlanta Biologicals, Flowery Branch, GA, USA). Before estrogen treatment, cells were cultured in RPMI media containing 5% (V/V) charcoal stripped FBS for 24 hours. The cells were then cultured in FBS free RPMI media for another 24 hours and were then treated with 0.1nM estradiol (E2) (Sigma, St. Louis, MO, USA) for 24 hours. In some experiments, cells were treated with 10^{-7}µM 4-hydroxytamxifen (4-OH-TAM) (Sigma, St. Louis, MO, USA) or fulvestrant (formerly ICI 182,780) (Sigma, St. Louis, MO, USA) followed by E2 treatment. MYD88 inhibitor peptide was purchased from Novus Biologicals (Littleton, CO, USA).

Real time PCR

RNA was isolated from the cells (Zymo, Irvine, CA, USA). The PCR mixture contained 50ng of total RNA, 5µl of 2X SYBR Green RT-PCR Mix (Affymetrix, Santa Clara, CA, USA), 0.1µl of DNA Polymerase enzyme, 1µl of gene specific primer (Qiagen, Valencia, CA, USA) and distilled water to achieve 10µl per reaction. Real time PCR reactions were performed in duplicate using Applied Biosystems ViiA 7™ Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The $2^{-\Delta\Delta Ct}$ method was employed for statistical data analysis.

Western blot analysis

Protein samples were used to perform electrophoresis followed by transfer to a PVDF membrane. The membranes were incubated overnight with primary antibodies: TCL1A, TLR2, TLR7, TLR9, TLR10, MYD88, UNC93B1 (Novus Biologicals, Littleton, CO, USA), and ACTB at a 1:500 dilution at 4^{o}C. The washed membranes were then incubated with goat anti-rabbit or anti-mouse secondary antibody (Santa Cruz Biotechnology, Dallas, Texas, USA) at a 1:20000 dilution. The washed membranes were subsequently incubated in Pierce$^{®}$ ECL Western blotting substrate (Thermo Scientific, Madison, WI, USA) and were visualized using Geldoc (Biorad, Hercules, CA, USA).

NF-κB reporter assay

Lymphoblastoid cell lines with known *TCL1A* SNP genotypes were transfected by electroporation with 2µg of the pGL4.32[luc2P/NF-κB-RE.Hydro] vector (Promega, Madison, WI, USA). A Renilla construct, pRL-TK (Promega, Madison, WI, USA) was used to determine transfection efficiency. The cells were plated in RPMI medium for 24 hours to allow them to recover from electroporation. Cells were then treated with 0.1nM E2 for 24 hours, followed by 10^{-7}µM 4-OH-TAM or ICI for an additional 24 hours. In some experiments, cells were treated with MYD88 (100µM) for 24 hours before E2 treatment. Luciferase assays were performed with the Dual-Luciferase Reporter assay system (Promega, Madison, WI, USA) Relative NF-kB/luciferase activities were normalized to Renilla luciferase activities.

Co-Immunoprecipitation (Co-IP) of TCL1A and MYD88

LCLs ($1x10^7$) were resuspended in 650µl IP lysis buffer containing 2.2µl protease inhibitor cocktail (Qiagen, Valencia, CA, USA) and were incubated on ice for 30 min. Cells were then centrifuged at 12,000 g at 4°C for 15 min. Supernatant was collected. Protein A agarose (Thermo Scientific, Madison, WI, USA) was prepared and washed 3 times with IP lysis buffer. A precleaning step was performed in order to clean the background. Cell lysates containing protein A agarose beads were rotated at 4°C for an hour. After centrifugation, supernatant was collected. At this point, input (50µl) was collected and stored at -80°C. Anti-TCL1A (1:50) antibodies (Cell Signaling Technology, Danvers, MA, USA) were used to perform IP. IgG (Cell Signaling Technology, Danvers, MA, USA), used as negative control. Specifically, IP Samples containing protein A agarose beads were rotated at 4°C overnight. Immunoprecipitates were washed three times with ice cold lysis buffer, and proteins were eluted with 50µl 1X Laemmli loading buffer. Proteins were separated on 4-12% SDS-PAGE gels and transferred onto PDVF membranes. After blocking, membranes were incubated with primary antibodies against TCL1A at 4°C overnight. The washed membrane was then incubated with secondary antibody (1:15000 dilution) for an hour at room temperature. The membrane was visualized using super signal ECL substrate (Thermo Scientific, Madison, WI, USA).

Statistics

Data were analyzed using GraphPad Prism Software (San Diego, CA, USA) and an R package. Data are presented as mean ± S.E.M unless otherwise stated. Gene expression and NF-κB activities were analyzed using Multivariate Analysis of Variance (MANOVA), followed by planed comparisons with appropriate post hoc tests. We also conducted t-tests for all meaningful

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combinations (effect of treatments on WT and VT, effect of genotype on treatments vs vehicle). To correct for the inflated type I error due to multiple comparisons, we have obtained an false discover rate (FDR) for each comparison using the R package (the default p.adjust function with "BH" method).

Results

Correlation between the expression of TCL1A and that of toll like receptors

Excessive production of cytokines and chemokines mediated by toll like receptor (TLR) pathways is often associated with an inflammatory response mediated though NF-κB signaling (Zeytun et al., 2010). The human TLR family consists of ten isoforms (TLR1-TLR10). TLRs are synthesized in the endoplasmic reticulum (Kawasaki and Kawai, 2014). Most TLRs are located on the cell membrane, but TLR3, TLR7, TLR8 and TLR9 are located on the membrane of intracellular compartments such as the endoplasmic reticulum, endosomes and lysosomes (**Figure 2**) (Joosten et al., 2016). The myeloid differentiation primary response gene 88 (*MYD88*) encodes one of the functional adapter molecules that have been shown to interact with all TLRs except TLR3. MYD88 recruits IL-1R-activating kinase (IRAK) 1, IRAK2, IRAK4 and TNF-receptor associated factor 6 (TRAF6), leading ultimately to NF-κB activation, pro-inflammatory cytokine secretion and an inflammatory response (**Figure 2)**. In the present study, in an attempt to extend our observations with regard to *TCL1A* SNP and estrogen-dependent expression of immune mediators, we set out to determine whether TLR expression might also be regulated by the *TCL1A* SNPs observed in our GWAS in an estrogen-dependent fashion and, if so, whether these observations might cast light on the role of *TCL1A* SNPs in NF-κB activation.

We once again turned to the "Human Variation Panel" of LCLs, a cell line model system that has repeatedly demonstrated its power to generate pharmacogenomic hypotheses and to test hypotheses arising from clinical GWA studies (Ho et al., 2016; Ingle et al., 2013; Ingle et al.,

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2010; Ingle et al., 2016; Li et al., 2008; Liu et al., 2014; Liu et al., 2013; Niu et al., 2016; Niu et al., 2010). This model system consists of LCLs from 300 healthy subjects for which we have generated seven million SNPs after imputation as well as microarray data for basal levels of gene expression for each cell line. We began by asking whether TLR expression might be associated with TCL1A gene expression. Basal levels of expression, in the absence of E2, showed a significant correlation in these 300 cell lines of TCL1A expression with that of both TLR7 and TLR9, with p values of 6.43E-15 and 1.05E-09, respectively (**Table 1**). We also examined the correlation between the expression of MYD88, a critical adapter protein for inflammation signaling pathways downstream of TLRs (Takeuchi and Akira, 2010) and that of Unc93 Homolog B1 (UNC93B1), a molecule that plays a critical role in trafficking TLR7 and TLR9 from the endoplasmic reticulum to endosomes (**Figure 2**) (Lee and Barton, 2014; Sasai and Iwasaki, 2011). Very significant correlations were also observed between basal expression levels of TLR9 and TLR7 with the expression of MYD88, with p values 1.71E-18 and 7.55E-09 respectively (**Table 2**). We also found that expression levels of MYD88 and UNC93B1 were themselves significantly correlated (r=0.41, p=7.66E-13) (**Table 2**). However, in the absence of E2, there was not a significant SNP-dependent difference in the expression of the genes listed in **Table 1** in these 300 LCLs (data not shown). Therefore, we also asked whether the expression of these genes might be influenced by the *TCL1A* SNPs in the presence of E2, as we had observed for pro-inflammatory cytokines, chemokines and their receptors (**Figure 1D-G**) (Ho et al., 2016).

TLR expression can be regulated by *TCL1A* SNPs in an estrogen-dependent fashion

The first set of experiments utilized eight LCLs that were homozygous for wild-type and eight LCLs homozygous for variant genotypes for the *TCL1A* SNPs that we had shown to be associated with AI-induced musculoskeletal adverse events (Ingle et al., 2010) and which we had also shown to be associated with alterations in the expression of a series of cytokines and chemokines in a SNP and estrogen-dependent fashion (**Figures 1D-G**) (Ho et al.). All of the cell lines used were homozygous for either wild-type or variant genotype for all three *TCL1A* SNPs as shown graphically in **Figure 1A**. TCL1A and all of the immune mediators listed in **Table 1** are highly expressed in LCLs—which are EB virus transformed B cells—which made the functional genomic studies described subsequently possible.

The initial experiment represented an attempt to determine whether the expression of TLRs and MYD88 could be modulated by *TCL1A* in a SNP-dependent fashion in response to E2 treatment, and to determine whether the direction of that change in expression could be “reversed” in response to 4-OH-TAM treatment—implying that the expression might be regulated by *TCLIA* genotype. Concentrations of E2 and 4-OH-TAM used to perform these experiments were those that were found to be optimal in previous studies of TCL1A induction by E2 and the “reversal” of that effect by 4-OH-TAM (Ho et al., 2016; Liu et al., 2012) . TCL1A expression was induced by 0.1nM E2 treatment, but only in cells homozygous for variant genotypes for the *TCL1A* SNPs (**Figure 3A**)—confirming our previous results (Liu et al., 2012). However, this genotype-dependent gene expression pattern was “reversed” when 4-OH-TAM (10^{-7}µM) was added to E2—also confirming our previous results (**Figure 3A**) (Ho et al., 2016). In parallel, the expression of TLR2, TLR7, TLR9 and TLR10 were up-regulated (~2-3 fold) in the presence of E2, but only in cells homozygous for variant genotypes for the *TCL1A* SNPs. In addition, as anticipated, in the presence of 4-OH-TAM, expression levels for these four TLRs increased

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significantly in cells homozygous for wild-type genotypes for the *TCL1A* SNPs (**Figure 3B-E**). Furthermore, other TLRs (TLR1, TLR3, TLR4, TLR5, TLR6 and TLR8) did not display alternation in their expression in a *TCL1A* SNP-dependent fashion in the presence of E2 (data not shown). In a similar fashion, we also observed that the expression of MYD88 and UNC93B1 were significantly up-regulated in parallel with TCL1A in a SNP-estrogen-dependent fashion (**Figure 3F-G**). In addition, expression of those immune genes was significantly changed at the protein level as determined by Western blot analysis (**Figure 3H**). It should be pointed out once again that these SNPs were in the *TCL1A* gene, as shown in **Figure 1A**, not in genes encoding the immune mediators, i.e., not in the *TLR2*, *TLR7, TLR9, TLR10*, *MYD88 and UNC93B1* genes (**Figure 3**).

Knockdown of TCL1A resulted in decreased expression of TLRs

We next determined whether TCL1A itself was involved in the differences in expression for TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1 shown in **Figure 3** using cell lines with differing genotypes for the *TCL1A* SNPs. Specifically, siRNA knockdown studies were performed using four independent siRNAs as well as one pooled siRNA (Dharmacon Chicago, IL, USA) and the results for all of these approaches were consistent. Knockdown of TCL1A to 25% of its baseline level resulted in significant down-regulated of the expression of TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1. We observed no SNP-dependent effect when TCL1A was knocked down (**Figure 4A-4B**). In a similar fashion, knockdown of MYD88 resulted in significantly decreased TLR2, TLR7, TLR9 and TLR10 expression in LCLs, but TCL1A expression did not change (**Figure 4D-4E**). Finally, protein expression was altered in parallel with the changes seen for mRNA after TCLIA or MYD88 knock down (**Figure 4C and 4F**). These results indicated that the expression of TLR2, TLR7, TLR9 and TLR10 could be modulated by TCL1A and MYD88. It should be pointed out that knockdown of MYD88 did not alter the expression of TCL1A, suggesting that TCL1A is "upstream" of MYD88 in this signaling pathway. At this point, we had determined that TCL1A can regulate the expression of TLRs as well as that of MYD88, an important adaptor molecule that can trigger downstream signaling, including signaling through the NF-κB pathway.

TCL1A **SNP and SERM-mediated MYD88-dependent NF-κB activation**

We had previously reported that changes in TCL1A expression after ER blockade using fulvestrant (ICI) could influence NF-κB transcriptional activity in a striking SNP genotype-dependent fashion (Liu et al., 2012). Specifically, we treated the same cells that were used to generate the results shown in **Figure 3**, LCLs with differing genotypes for the *TCL1A* genotypes, with ICI or 4-OH-TAM. Cells homozygous for the variant genotype for the *TCL1A* SNPs increased NF-κB transcriptional activity approximately 3-fold in the presence of E2 plus either ICI or 4-OH-TAM (**Figure 5A-B**). MYD88 is the adaptor protein for all TLRs except for TLR3 and is capable of activating the NF-κB signaling pathway and of inducing the production of inflammatory cytokines (Broad et al., 2007; Kawai and Akira, 2007). We had observed that MYD88 expression could be regulated by *TCL1A* in a SNP and estrogen-dependent fashion (**Figure 3F**) and that it is downstream of *TCL1A* (**Figure 4A-B**). The next series of experiment was designed to study the possible contribution of MYD88, a known adaptor molecule for TLR-mediated NF-κB signaling, to *TCL1A* SNP and SERM-dependent NF-κB activation. We found that, after knock down of MYD88 to ~30% of its baseline level, *TCL1A* SNP-dependent NF-κB

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transcriptional activation in the presence of either 4-OH-TAM or ICI was lost (**Figure 5C**). In addition, when MYD88 was silenced by the application of MYD88 inhibitory peptide, the effects of the *TCL1A* SNP-dependent and ER blocker-dependent NF-κB activation were also lost, as anticipated (**Figure 5D**). We also determined by co-immunoprecipitation that TCL1A could interact directly with MYD88 (**Figure 5E**). These results further confirmed that *TCL1A* SNP and estrogen-dependent NF-κB activation may occur, in part, through a TLR-MYD88-dependent pathway. They also greatly extended our original observations and served to highlight a novel pharmacogenomic mechanism by which *TCL1A* can influence and modulate the immune response and inflammation.

Discussion

We previously reported that SNPs 3’ of the *TCL1A* gene that we identified during a GWAS for musculoskeletal adverse events that occur during aromatase inhibitor therapy of breast cancer could have significant effects on the expression of a series of genes encoding inflammatory mediators in a SNP and estrogen-dependent fashion. Even more striking, those effects could be reversed in a *TCL1A* SNP-dependent fashion by exposure to 4-OH-TAM or ICI (Ho et al., 2016). We also reported that variant *TCL1A* SNP genotypes were associated with increased NF-κB transcriptional activity after estrogen receptor blockade, but that was not true for the wild-type genotype (Liu et al., 2012). NF-κB activation is known to play an important role in chronic inflammation (Hayden et al., 2006). As a result, there is great interest in developing specific molecular inhibitors of NF-κB activation. Therefore, the present study represents a step toward a better understanding of molecular and genetic mechanisms responsible for variation in NF-κB activation—understanding that could potentially have implications for the management of NF-kB related inflammation.

In the present study, we pursued mechanisms associated with the *TCL1A* SNP-mediated, SERM-dependent activation of NF-κB. It has been reported that TCL1A can function as a transcriptional regulator which interacts with many transcription factors including AP1 and CREB/p300 and, as a result, that it can influence NF-κB activity in B cell chronic lymphocytic leukemia (Pekarsky et al., 2008). Since TLR signaling, signaling that occurs—in part—through MYD88, can influence NF-kB activity (23), MYD88 has been suggested as a potential therapeutic target for regulating the immune response (Olson et al., 2015). When TCL1A was knocked down in our cell line model system, MYD88 was significantly down-regulated and, in parallel, expression levels of

TLR2, TLR7, TLR9, and TLR10 were also decreased significantly (**Figure 4C-D**). Several TLRs can interact with MYD88 and NF-kB, as determined by STRING protein interaction network analysis (Szklarczyk et al., 2015). We also observed by immunoprecipitation studies that TCL1A protein could interact with MYD88 (**Figure 5E**). We have reported previously that ER blockade by fulvestrant (ICI) can result in the activation of NF-κB transcriptional activity, but only in cells with variant genotypes for the *TCL1A* SNPs (3) (**Figure 5A**). In the present study, we extended that finding by observing a similar response after exposure to 4-OH-TAM (**Figure 5B**). We also demonstrated that the *TCL1A* SNP and SERM-dependent activation of NF-κB through TLRs could be blocked by MYD88 siRNA or MYD88 inhibitory peptide (**Figure 5C-D**). Therefore, the present study has raised the possibility that TLR-MYD88-dependent NF-κB signaling could be involved in *TCL1A* SNP dependent NF-kB activation and—as a result—in the regulation of inflammation and immune response. The fact that expression of a single gene, *TCL1A* can influence signaling through this pathway represents an important advance in our understanding of the regulation of the pathway and suggests novel ways to influence signaling through this pathway.

Obviously, further studies will be required to determine the possible therapeutic implications of this series of observations. Specifically, we found that TCL1A expression was positively correlated with the “basal” expression of toll like receptors TLR7 and TLR9 in 300 LCLs (r=0.443, p=6.43E-15 and r=0.354, p=1.05E-09 respectively), as listed in **Table 1**. Both TLR7 and TLR9 are therapeutic targets for the treatment of lupus erythematosus, an autoimmune disorder for which more than 90% of patients are women. We also found that TCL1A knockdown could influence the expression of TLR2 and TLR10, both of which can play a role in cytokine production, inflammation and NF-κB activation via MYD88 (Joosten et al., 2016). Crosstalk between estrogens and the immune response is well-documented (Hughes and Choubey, 2014; Kovats, 2015; Seillet et al., 2011). In the present study, we have demonstrated the differential expression of a series of genes encoding immune mediators in response to estrogen treatment that was dependent on *TCL1A* SNP genotypes. Even more striking, we showed that 4-OH-TAM, an active metabolite of tamoxifen, an FDA-approved treatment for breast cancer and for breast cancer chemopreventive that has been suggested for the treatment of millions of women at high risk for breast cancer (Maximov et al., 2013), can reverse the *TCL1A* SNP-dependent expression of those immune mediators—all of which play a critical role in inflammatory disease (Bartlett and Million, 2015; Iwakura et al., 2011).

We should emphasize that the LCL model system used in our studies has both strengths and weaknesses, but it has repeatedly demonstrated that it can be a powerful tool for both generating and testing genomic and pharmacogenomics hypotheses (Ingle et al., 2013; Ingle et al., 2010; Li et al., 2008; Liu et al., 2014; Liu et al., 2013; Liu et al., 2012; Niu et al., 2010; Wheeler and Dolan, 2012). The availability of comprehensive genotype and gene expression data for this model system makes it possible to study the functional implications of genetic variants found to be associated with clinical phenotypes, as demonstrated by the present studies. We have previously used this model system successfully to demonstrate novel SNP and estrogen-dependent mechanisms underlying variation in the regulation of the expression of cytokines and chemokines by TCL1A (Liu et al., 2012). In the present study, we have extended those observations with regard to the transcriptional regulation of immune mediators (cytokines and chemokines) to include TLRs and NF-κB transcriptional activity. Obviously, these results will have to be verified using both additional cell-based systems and clinical samples, but the present results represent an important step in the process of providing functional and mechanistic explanations for the association of SNPs in the *TCL1A* gene with inflammation and the immune response.

In summary**,** the series of functional genomic studies described here confirm that TCL1A expression is E2 inducible in a SNP-dependent manner and have demonstrated that TCL1A can influence the downstream expression of a series of immune mediators, including TLR2, TLR7, TLR9, TLR10 and MYD88. Furthermore, inhibition of MYD88 resulted in the blockade of *TCL1A* SNP-dependent NF-κB activation, indicating that the TLR-MYD88-dependent NF-κB signalling pathway might contribute, at least in part, to the *TCL1A* SNP and estrogen-dependent effects that we had observed. *TCL1A* SNP and estrogen-dependent variation in NF-κB transcriptional activity, and the transcriptional regulation of other immune mediators suggests that this pathway may play a role in the complex interactions that are known to exist between the endocrine and immune systems. Of equal importance is the fact that SERMs are able to modulate this pathway in a striking fashion—opening the way for the pharmacologic regulation of this pathway in clinical setting.

Authorship contributions

Participated in research design: Ho, Bongartz, Ingle, Goss, Shepherd, Mushiroda, Kubo, Wang, and Weinshilboum.

Conducted experiments: Ho

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Performed data analysis and interpretation: Ho, Bongartz, Ingle, Wang and Weinshilboum.

Wrote or contributed to the writing of the manuscript: Ho, Bongartz, Ingle, Kalari, Goss, Shepherd, Mushiroda, Kubo, Wang, and Weinshilboum.

All authors have given final approval of the version to be published.

References

Bartlett HS and Million RP (2015) Targeting the IL-17-TH17 pathway. *Nat Rev Drug Discov* **14**(1): 11-12.

Broad A, Kirby JA, Jones DEJ, Applied I and Transplantation Research G (2007) Toll-like receptor interactions: tolerance of MyD88-dependent cytokines but enhancement of MyD88-independent interferon-β production. *Immunology* **120**(1): 103-111.

Hayden MS, West AP and Ghosh S (2006) NF-kappa B and the immune response. *Oncogene* **25**(51): 6758-6780.

Ho M-F, Bongartz T, Liu M, Kalari KR, Goss PE, Shepherd LE, Goetz MP, Kubo M, Ingle JN, Wang L and Weinshilboum RM (2016) Estrogen, SNP-Dependent Chemokine Expression and Selective Estrogen Receptor Modulator Regulation. *Molecular Endocrinology* **30**(3): 382-398.

Hughes GC and Choubey D (2014) Modulation of autoimmune rheumatic diseases by oestrogen and progesterone. *Nat Rev Rheumatol* **advance online publication**.

Ingle JN, Liu M, Wickerham DL, Schaid DJ, Wang L, Mushiroda T, Kubo M, Costantino JP, Vogel VG, Paik S, Goetz MP, Ames MM, Jenkins GD, Batzler A, Carlson EE, Flockhart DA, Wolmark N, Nakamura Y and Weinshilboum RM (2013) Selective Estrogen Receptor Modulators and Pharmacogenomic Variation in ZNF423 Regulation of BRCA1 Expression: Individualized Breast Cancer Prevention. *Cancer Discovery* **3**(7): 812-825.

Ingle JN, Schaid DJ, Goss PE, Liu M, Mushiroda T, Chapman J-AW, Kubo M, Jenkins GD, Batzler A, Shepherd L, Pater J, Wang L, Ellis MJ, Stearns V, Rohrer DC, Goetz MP, Pritchard KI, Flockhart DA, Nakamura Y and Weinshilboum RM (2010) Genome-Wide Associations and Functional Genomic Studies of Musculoskeletal Adverse Events in Women Receiving Aromatase Inhibitors. *Journal of Clinical Oncology* **28**(31): 4674-4682.

Ingle JN, Xie F, Ellis MJ, Goss PE, Shepherd LE, Chapman J-AW, Chen BE, Kubo M, Furukawa Y, Momozawa Y, Stearns V, Pritchard KI, Barman P, Carlson EE, Goetz MP, Weinshilboum RM, Kalari KR and Wang L (2016) Genetic polymorphisms in the long noncoding RNA MIR2052HG offer a pharmacogenomic basis for the response of breast cancer patients to aromatase inhibitor therapy. *Cancer Research* **76**(23): 7012–7023.

Iwakura Y, Ishigame H, Saijo S and Nakae S (2011) Functional Specialization of Interleukin-17 Family Members. *Immunity* **34**(2): 149-162.

Joosten LAB, Abdollahi-Roodsaz S, Dinarello CA, O'Neill L and Netea MG (2016) Toll-like receptors and chronic inflammation in rheumatic diseases: new developments. *Nat Rev Rheumatol* **12**(6): 344-357.

Kawai T and Akira S (2007) Signaling to NF-κB by Toll-like receptors. *Trends in Molecular Medicine* **13**(11): 460-469.

Kawasaki T and Kawai T (2014) Toll-Like Receptor Signaling Pathways. *Frontiers in Immunology* **5**: 461.

Kim Y-M, Brinkmann MM, Paquet M-E and Ploegh HL (2008) UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. *Nature* **452**(7184): 234-238.

Kovats S (2015) Estrogen receptors regulate innate immune cells and signaling pathways. *Cellular Immunology* **294**(2): 63-69.

Lee BL and Barton GM (2014) Trafficking of endosomal Toll-like receptors. *Trends in Cell Biology* **24**(6): 360-369.

Li L, Fridley B, Kalari K, Jenkins G, Batzler A, Safgren S, Hildebrandt M, Ames M, Schaid D and Wang L (2008) Gemcitabine and Cytosine Arabinoside Cytotoxicity: Association with Lymphoblastoid Cell Expression. *Cancer Research* **68**(17): 7050-7058.

Liu M, Goss PE, Ingle JN, Kubo M, Furukawa Y, Batzler A, Jenkins GD, Carlson EE, Nakamura Y, Schaid DJ, Chapman J-AW, Shepherd LE, Ellis MJ, Khosla S, Wang L and Weinshilboum RM (2014)

Aromatase Inhibitor-Associated Bone Fractures: A Case-Cohort GWAS and Functional Genomics. *Molecular Endocrinology* **28**(10): 1740-1751.

Liu M, Ingle JN, Fridley BL, Buzdar AU, Robson ME, Kubo M, Wang L, Batzler A, Jenkins GD, Pietrzak TL, Carlson EE, Goetz MP, Northfelt DW, Perez EA, Williard CV, Schaid DJ, Nakamura Y and Weinshilboum RM (2013) TSPYL5 SNPs: association with plasma estradiol concentrations and aromatase expression. *Mol Endocrinol* **27**(4): 657-670.

Liu M, Wang L, Bongartz T, Hawse J, Markovic S, Schaid D, Mushiroda T, Kubo M, Nakamura Y, Kamatani N, Goss P, Ingle J and Weinshilboum R (2012) Aromatase inhibitors, estrogens and musculoskeletal pain: estrogen-dependent T-cell leukemia 1A (TCL1A) gene-mediated regulation of cytokine expression. *Breast Cancer Research* **14**(2): R41.

Maximov PY, Lee TM and Jordan VC (2013) The Discovery and Development of Selective Estrogen Receptor Modulators (SERMs) for Clinical Practice. *Current Clinical Pharmacology* **8**(2): 135-155.

McCool KW and Miyamoto S (2012) DNA damage-dependent NF-κB activation: NEMO turns nuclear signaling inside out. *Immunological Reviews* **246**(1): 311-326.

Niu N, Liu T, Cairns J, Ly RC, Tan X, Deng M, Fridley BL, Kalari KR, Abo RP, Jenkins G, Batzler A, Carlson EE, Barman P, Moran S, Heyn H, Esteller M and Wang L (2016) Metformin pharmacogenomics: a genome-wide association study to identify genetic and epigenetic biomarkers involved in metformin anticancer response using human lymphoblastoid cell lines. *Human Molecular Genetics*.

Niu N, Qin Y, Fridley BL, Hou J, Kalari KR, Zhu M, Wu T-Y, Jenkins GD, Batzler A and Wang L (2010) Radiation pharmacogenomics: A genome-wide association approach to identify radiation response biomarkers using human lymphoblastoid cell lines. *Genome Research* **20**(11): 1482-1492.

Olson MA, Lee MS, Kissner TL, Alam S, Waugh DS and Saikh KU (2015) Discovery of small molecule inhibitors of MyD88-dependent signaling pathways using a computational screen. *Scientific Reports* **5**: 14246.

Pekarsky Y, Palamarchuk A, Maximov V, Efanov A, Nazaryan N, Santanam U, Rassenti L, Kipps T and Croce CM (2008) Tcl1 functions as a transcriptional regulator and is directly involved in the pathogenesis of CLL. *Proceedings of the National Academy of Sciences* **105**(50): 19643-19648.

Sasai M and Iwasaki A (2011) Love Triangle between Unc93B1, TLR7, and TLR9 Prevents Fatal Attraction. *Immunity* **35**(1): 3-5.

Seillet C, Laffont S, Trémollières F, Rouquié N, Ribot C, Arnal J-F, Douin-Echinard V, Gourdy P and Guéry J-C (2011) The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. *Blood* **119**(2): 454-464.

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C (2015) STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research* **43**(Database issue): D447-D452.

Tak PP and Firestein GS (2001) NF-κB: a key role in inflammatory diseases. *The Journal of Clinical Investigation* **107**(1): 7-11.

Takeda K, Harada Y, Watanabe R, Inutake Y, Ogawa S, Onuki K, Kagaya S, Tanabe K, Kishimoto H and Abe R (2008) CD28 stimulation triggers NF-κB activation through the CARMA1–PKCθ–Grb2/Gads axis. *International Immunology* **20**(12): 1507-1515.

Takeuchi O and Akira S (2010) Pattern Recognition Receptors and Inflammation. *Cell* **140**(6): 805-820.

Wheeler HE and Dolan ME (2012) Lymphoblastoid cell lines in pharmacogenomic discovery and clinical translation. *Pharmacogenomics* **13**(1): 55-70.

Zeytun A, Chaudhary A, Pardington P, Cary RB and Gupta G (2010) Induction of Cytokines and Chemokines by Toll-like Receptor Signaling: Strategies for Control of Infammation. **30**(1): 53-67.

Footnotes:

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Figure Legends

Figure 1. The SNP and estrogen-dependent effects on mRNA expression of TCL1A, cytokines and chemokines in LCLs. **(A),** Schematic diagrams of the two *TCL1A* SNPs, rs7359033 and rs7160302, in tight LD with rs11849538, the top hit signal from the MA.27 GWAS. Locations of EREs are shown as boxes for these three SNPs that map between the 3′-termini of *TCL1A* and *TCL1B*. Estrogen receptor blockade by 4-hydroxytamoxifen (4OH) or fulvestrant (ICI) treatment resulted in the "reversal" of *TCL1A* SNP and estrogen-dependent TCL1A expression patterns (**B-C**), and downstream effects on the expression of CCR6, CCL20, IL17RA and IL17A (**D-G**) Values are mean ± SEM of three assays. * p <0.0001. Adapted from **Figure 2 and Figure 3** in Ho et al. Molecular Endocrinology 2016 (Ho et al., 2016).

Figure 2. Toll like receptor (TLR) intracellular localization and signaling. TLR3, TLR7, TLR8 and TLR9 are located on the membranes of intracellular compartments such as the endoplasmic reticulum and endosomes. The myeloid differentiation primary response gene 88 (*MYD88*) is the one of the functional adapter molecules that have been reported to interact with all TLRs except TLR3. MYD88 recruits IL-1R-activating kinase (IRAK) 1, IRAK2, IRAK4 and TNF-receptor associated factor 6 (TRAF6), leading ultimately to NF-κB activation and pro-inflammatory cytokine secretion and an inflammatory response. UNC93B1 is a multi-transmembrane-domain-containing protein and plays a critical role in trafficking TLR7 and TLR9 from the endoplasmic reticulum to endosomes where TLR7 and TLR9 transmit signals via MYD88/TRIF-dependent pathways (Kim et al., 2008).

Figure 3. SNP and estrogen-dependent mRNA expression of TCL1A **(A),** TLR2 **(B),** TLR7 **(C),** TLR9 **(D),** TLR10 **(E),** MYD88 **(F),** and UNC93B1 **(G)** in LCLs. (**H**), Western blot analysis was performed for TCL1A, TLR2, TLR7, TLR9 TLR10, MYD88, UNC93B1 and ACTB in LCLs with known *TCL1A* SNP genotypes. The cells were treated with 0.1nM estradiol (E2) or with 0.1nM E2 plus 10^{-7}µM 4-hydroxytamoxifen (4OH-TAM) for an additional 24 hours. Eight cell lines homozygous for the variant (V) genotypes for all three of the *TCL1A* SNPs and eight cell lines homozygous for wild-type (WT) genotypes were used in these experiments, ***P <0.0001.

Figure 4. TCL1A could modulate TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1 expression in LCLs. Relative mRNA expression **(A and B)** of TCL1A, TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1 after knockdown of TCL1A in LCLs with known *TCL1A* SNP genotypes using pooled siRNA. Eight cell lines of each genotype were used in these experiments. *p<0.05. A Student's *t* test was performed to compare gene expression in LCLs with differing *TCL1A* SNP genotypes before and after gene knockdown, *A p value ≤0.05 was considered statistically significant. All values are mean +/- S.E.M for three separate independent assays. Protein expression was determined by Western blot analysis (**C**). Relative mRNA expression **(D and E)** of TCL1A, TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1 after knockdown of MYD88 in LCLs with known *TCL1A* SNP genotypes using pooled siRNA. (**F**), Western blot analysis was performed for TCL1A, TLR2, TLR7, TLR9, TLR10, MYD88 and UNC93B1 after knockdown of MYD88.

Figure 5. *TCL1A* SNP and estrogen-dependent NF-κB activation as determined by NF-κB reporter assays could be altered by the knock down or inhibition of MYD88. Estrogen receptor

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blockade by fulvestrant (ICI) or 4-hydroxytamoxifen (4OH) treatment resulted in *TCL1A* SNP -dependent NF-κB activation (**A-B**). *TCL1A* SNP and estrogen-dependent NF-κB activation could be blocked by MYD88 siRNA knockdown **(C)** or by exposure to MYD88 inhibitory peptide (100μM) (**D**). Specifically, LCLs were co-transfected with an NF-κB reporter construct and siRNA (MYD88 or control siRNA). 24 hours after transfection, cells were treated with either vehicle or 0.1nM E2 for 24 hours, followed by 10^{-7}μM 4-hydroxytamoxifen (4OH) or fulvestrant (ICI) for an additional 24 hours. In some experiments, cells were exposed to MYD88 inhibitory peptide for 24 hours before E2 treatment. Luciferase activity was measured 72 hours after transfection. The firefly luciferase activity derived from the NF-κB responsive reporter was normalized by the use of Renilla luciferase activity as a control to correct for possible variation in transfection efficiency. All experiments were repeated three times in triplicate. $^{**}p < 0.001$. (**E**), Co-Immunoprecipitation was used to determine whether TCL1A protein could interact with MYD88 in LCLs. Whole cell lysates from 1×10^7 LCLs were immunoprecipitated with Anti-TCL1A (1:50) antibodies or anti-IgG antibodies. Whole cell lysate (input, left panel) and immunoprecipitated samples (middle and right panel) were immunoblotted and probed with antibodies against TCL1A and MYD88.

Table 1. Correlations of TCL1A mRNA expression and those of toll like receptors in the Human Variation Panel of 300 LCLs.

Gene	Gene	r	p value
TCL1A	TLR1	-0.089	1.36E-01
TCL1A	TLR2	0.276	2.54E-06
TCL1A	TLR3	-0.051	3.98E-01
TCL1A	TLR4	0.158	8.02E-03
TCL1A	TLR5	0.027	6.47E-01
TCL1A	TLR6	-0.073	2.21E-01
TCL1A	**TLR7**	**0.443**	**6.43E-15**
TCL1A	TLR8	0.052	3.89E-01
TCL1A	**TLR9**	**0.354**	**1.05E-09**
TCL1A	TLR10	-0.200	7.29E-04
TCL1A	MYD88	0.273	3.50E-06

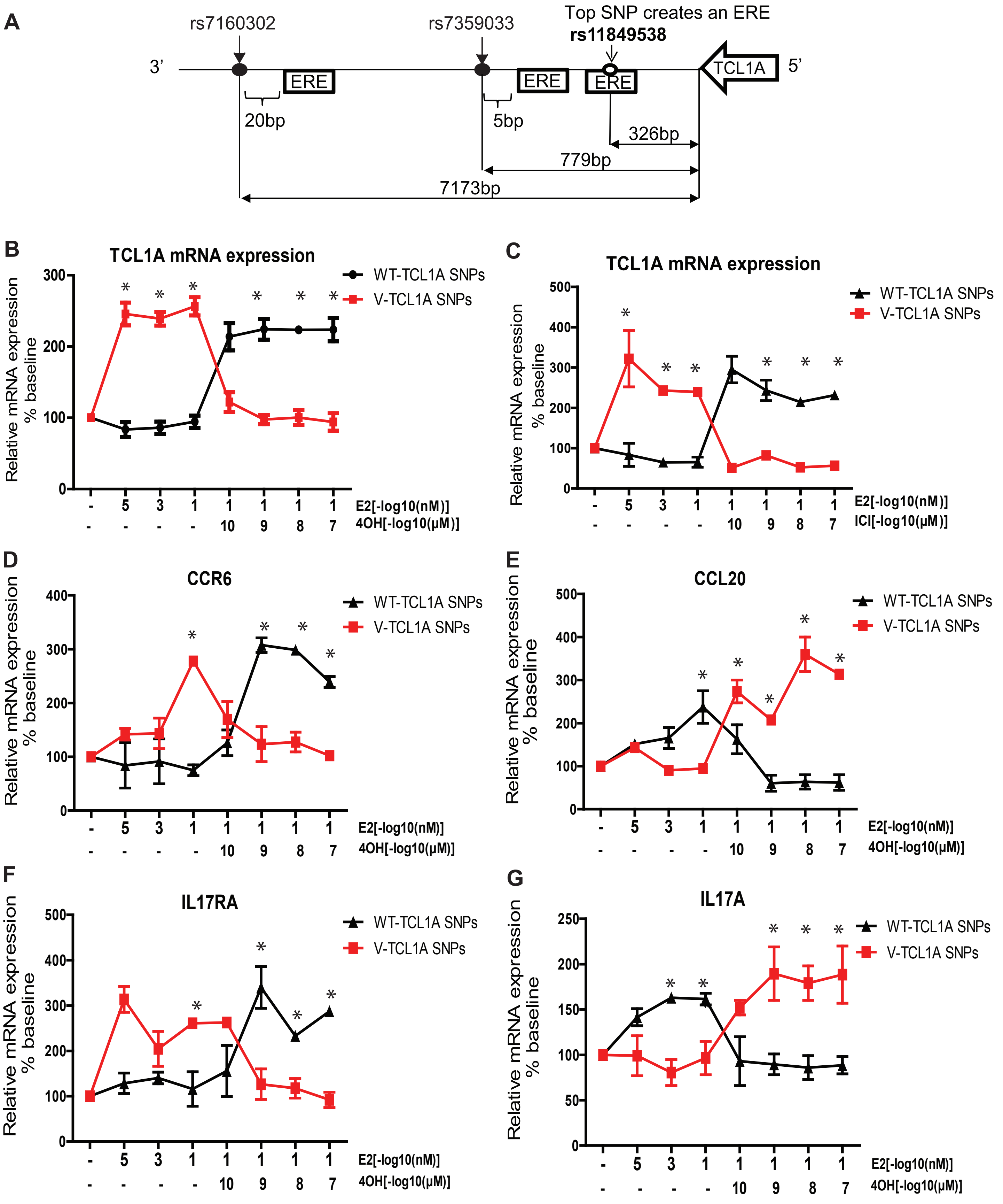
Statistical significance was considered p <1.8E-07.

Table 2. Correlations of MYD88 mRNA expression with those of toll like receptors in the Human Variation Panel of 300 LCLs.

Gene	Gene	r	p value
MYD88	TLR1	0.19	1.36E-03
MYD88	TLR2	0.227	1.26E-04
MYD88	TLR3	0.061	3.10E-01
MYD88	TLR4	0.244	3.50E-05
MYD88	TLR5	0.057	3.42E-01
MYD88	TLR6	0.02	7.34E-01
MYD88	**TLR7**	**0.491**	**1.71E-18**
MYD88	TLR8	-0.097	1.03E-01
MYD88	**TLR9**	**0.336**	**7.55E-09**
MYD88	TLR10	0.145	1.50E-02
MYD88	**UNC93B1**	**0.41**	**7.66E-13**

Statistical significance was considered p <1.8E-07.

Figure 1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Top SNP creates an ERE
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779bp
7173bp
B
TCL1A mRNA expression
Relative mRNA expression % baseline
WT-TCL1A SNPs
V-TCL1A SNPs
E2[-log10(nM)]
4OH[-log10(μM)]
C
TCL1A mRNA expression
E2[-log10(nM)]
ICI[-log10(μM)]
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Figure 2

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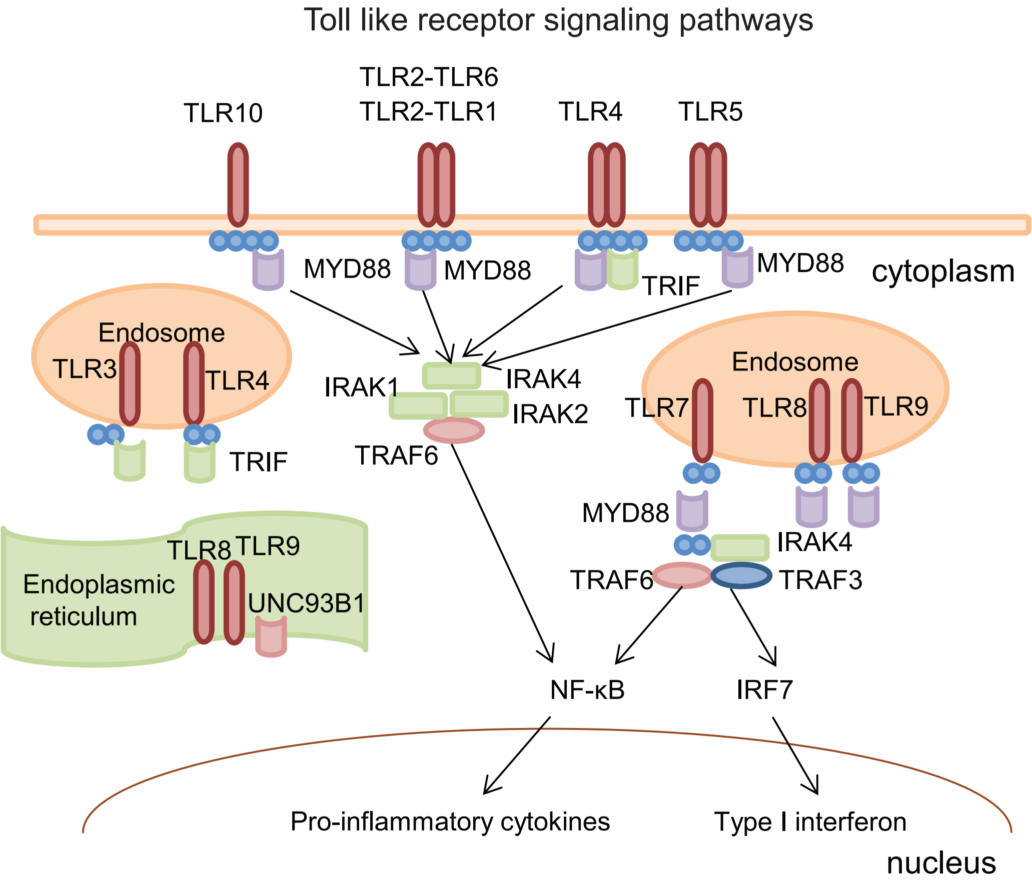

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Figure 3

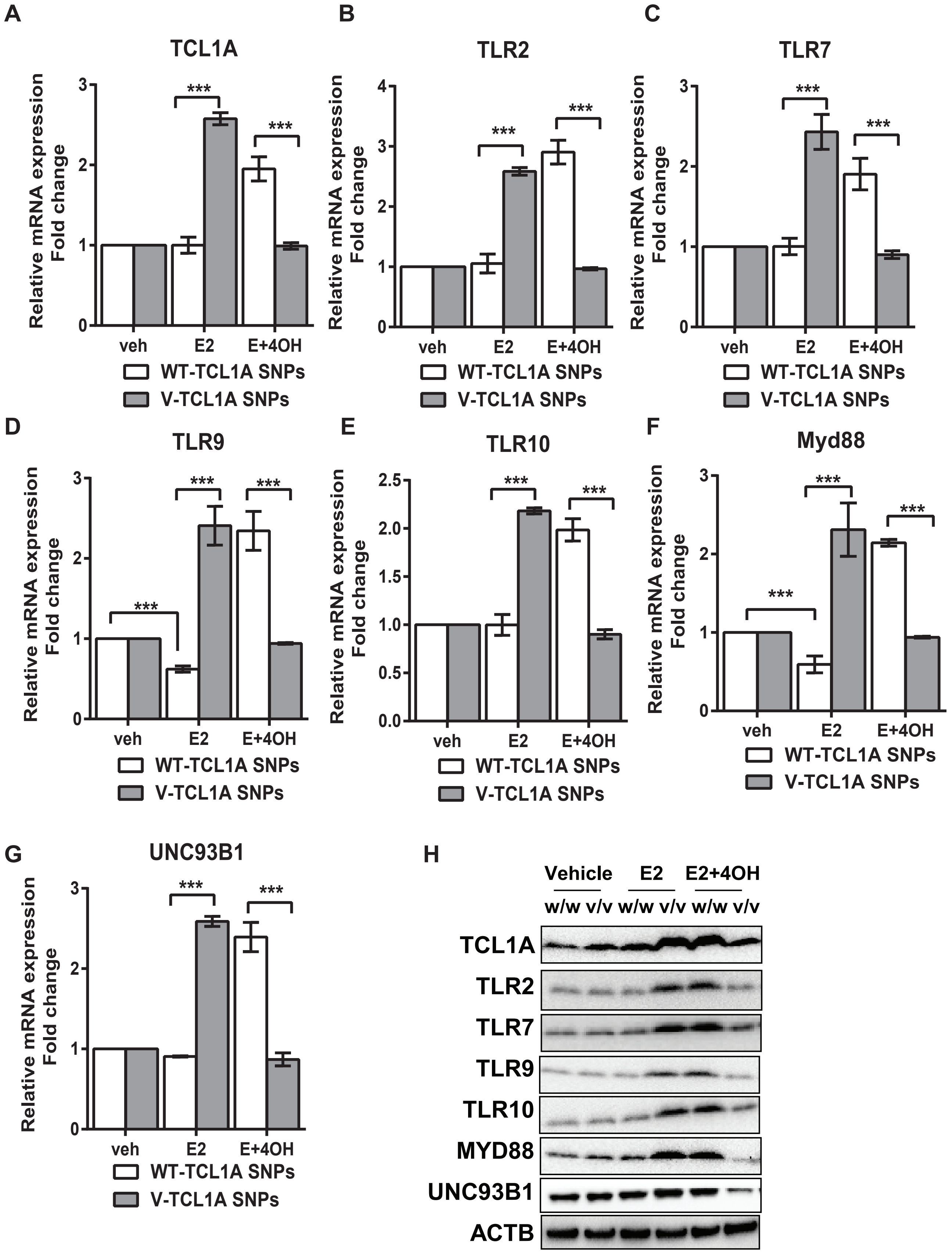

Figure 4

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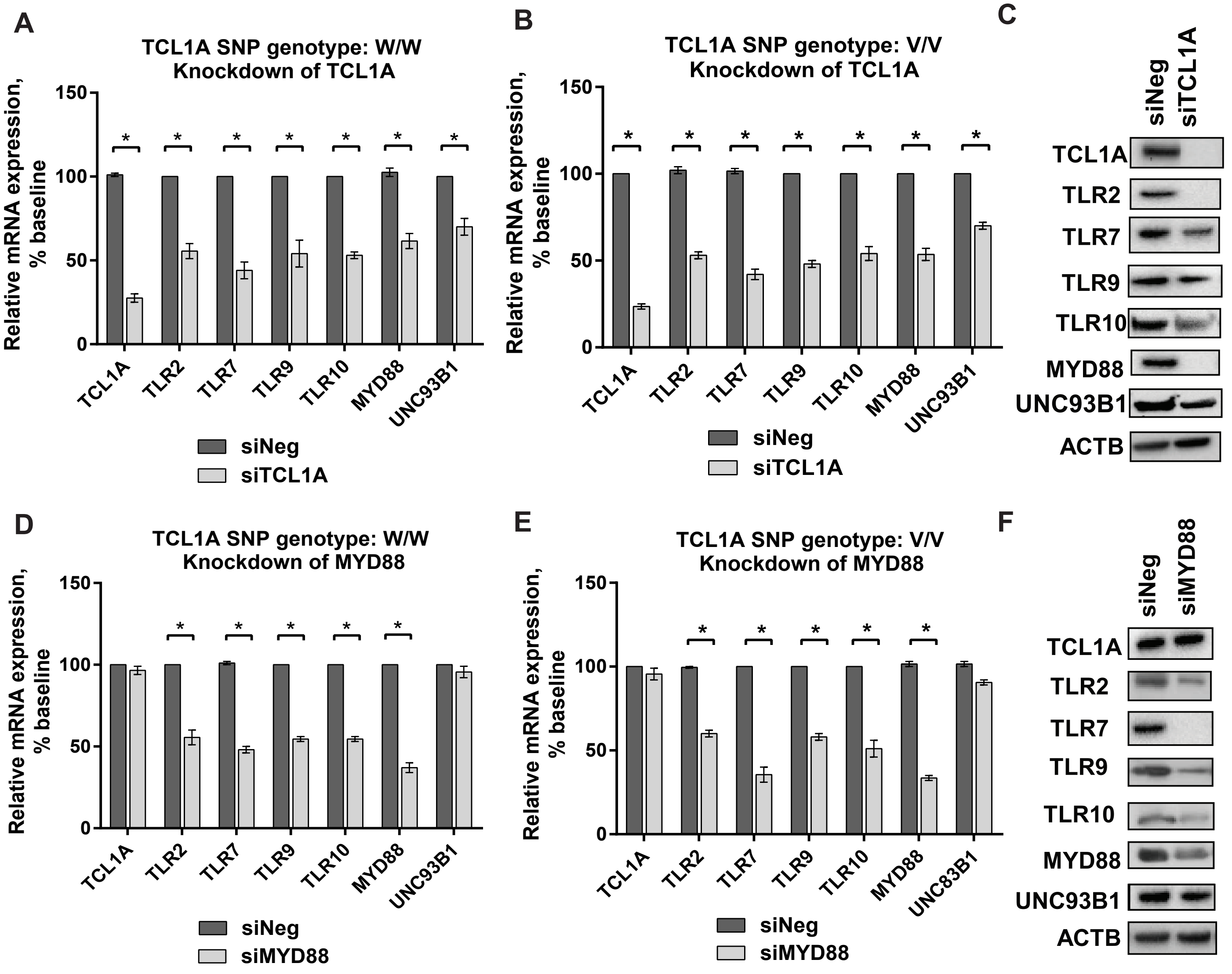
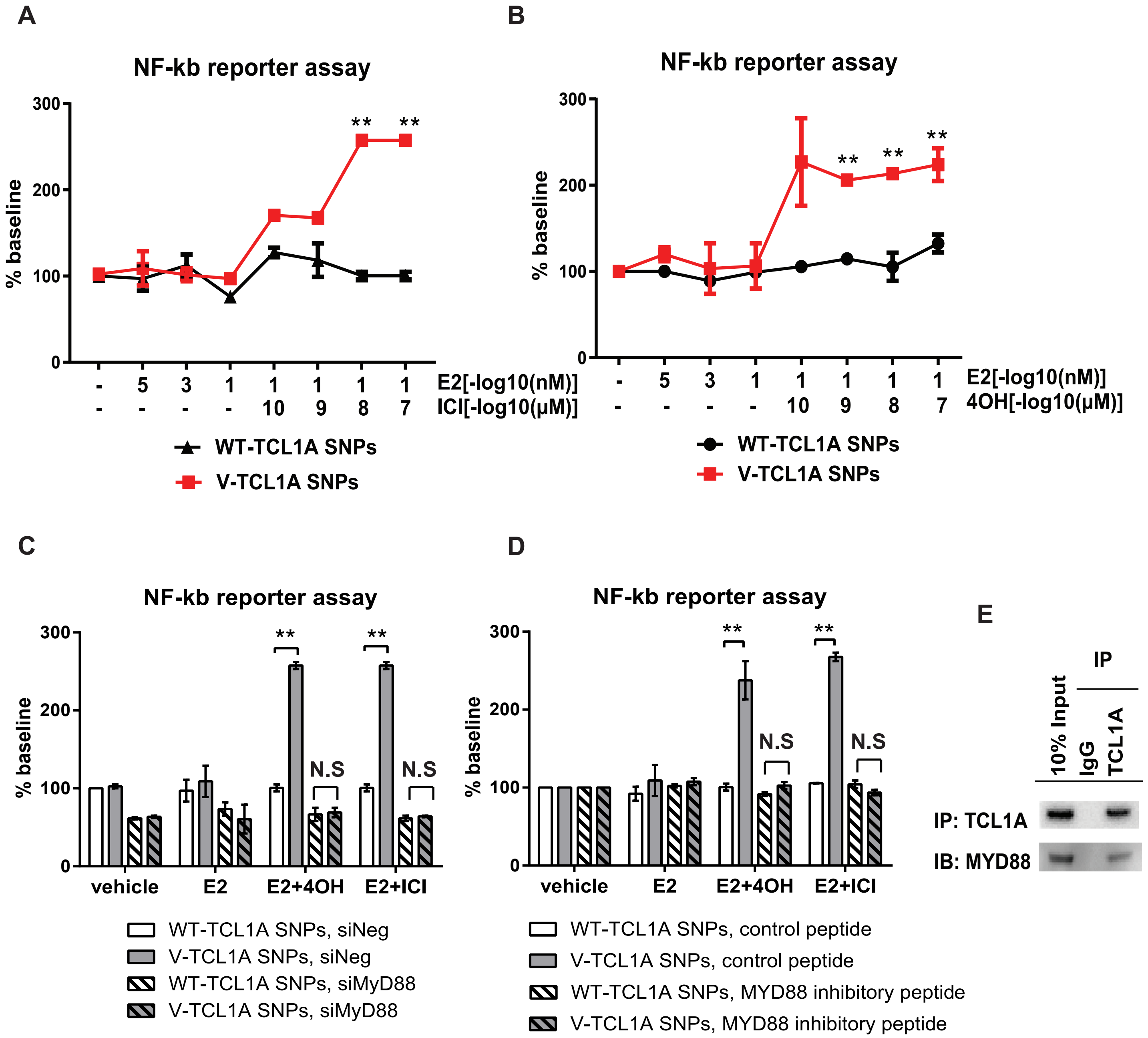


Figure 5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E2+ICI
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V-TCL1A SNPs, siNeg
WT-TCL1A SNPs, siMyD88
V-TCL1A SNPs, siMyD88
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E2+4OH
E2+ICI
WT-TCL1A SNPs, control peptide
V-TCL1A SNPs, control peptide
WT-TCL1A SNPs, MYD88 inhibitory peptide
V-TCL1A SNPs, MYD88 inhibitory peptide
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IB: MYD88