Supplementary information

Functional consequences of rare *TLR9* gene mutation R892W

Authors

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Supplementary information 1: Patient medical history

Carrier of TLR9 R892W allele

In childhood the R892W carrier had mumps. Occasionally she had suppurative throat anginas. Since her tonsils are enlarged and „destroyed“ even as an adult she has been suffering from recurring anginas. In childhood, she was hospitalized once at the Department of Infectiology due to „food poisoning“ with severe symptoms of vomiting and diarrhea. Her gallbladder was removed due to gallstones and inflammation. From June 2009 until March 2010, the carrier was on therapy for tuberculosis of the right outer and middle ear, as diagnosed intraoperatively (PH). In 2010, she was diagnosed with invasive ductal carcinoma of the right breast, and complete mastectomy suggested.

Supplementary figure S1: Sequencing data in the original carriers. Electrophoretograms of the non-synonymous variants detected by DNA sequencing with indicated nucleotide changes. Genomic DNA was isolated from peripheral blood by standard salting-out procedures. The entire coding region of TLR9 was amplified by sequential amplification of

various fragments sizes using the different primer combinations. Primer sets were designed from the human TLR9 referent coding sequence (NM_017442, Isoform A), by using *Primer Express, Version 2.0* (Applied Biosystems, Foster City, USA). Direct sequencing was performed using a BigDye Terminator Cycle Sequencing Kit 3.1v (Applied Biosystems, Foster City, USA), according to the manufacturer's instructions, followed by analysis on ABI PRISM 3100 DNA Sequencer (Applied Biosystems, Foster City, USA).

Supplementary figure S2: Multiple sequence alignment of TLR9 sequences from different species. Sections for SNP residues analyzed in this study shown. Sequences were retrieved from SwissProt and aligned and color-formatted to reflect residue physico-chemical properties using Clustal W (see Methods). Unusual sequence abbreviations in brackets: chimpanzee (Pantr), bonobo (Panpa), orangutan (Ponpy), gorilla (9prim), rhesus macaque and crab-eating macaque (Macmu and Macfa), Bos taurus (Bovin), Bos indicus (Bosin), Boselaphus tragocamelus (Bostr), Cercocebus torquatus (Certo), Aotus nancymaae (Aotna), Capra hircus (Caphi), dog (Canfa).

Supplementary figure S3: Structural changes induced by the R892W mutation. (A) TLR9 TIR domain model highlighting the position of residue 892 (red). (B) Close-up on structural context of arginine 892 (left) of its tryptophane 892 counterpart (right) with putative hydrogen bonds (green). Some of these bonds would likely be altered by the change to tryptophane (right). (C) and (D) surface charge, or (E) and (F) surface hydrophobicity prediction for R892 (C, E) and R892W (D, F) TIR domain. Negative charges red, positive charges blue, hydrophobic surface green, hydrophilic surface blue.

Figure S1

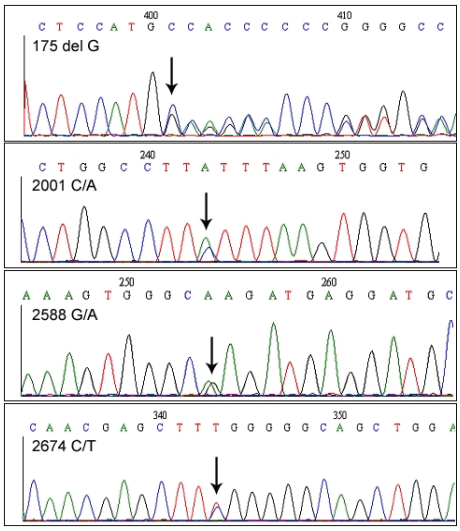
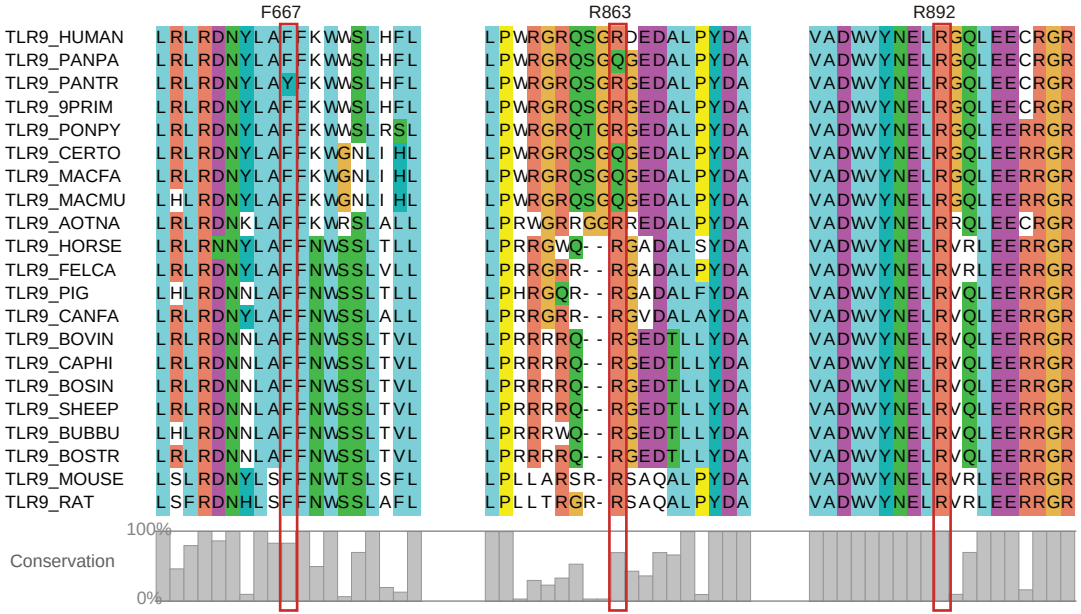


Figure S2


F667
R863
R892
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TLR9_9PRIM LRLRDNYLAFFKWWSLHFL LPWRGRQSGRGEDALPYDA VADWVYNELRGQLEECRGR
TLR9_PONPY LRLRDNYLAFFKWWSLRSL LPWRGRQTGRGEDALPYDA VADWVYNELRGQLEERRGR
TLR9_CERTO LRLRDNYLAFFKWGNLIHL LPWRGRQSGQGEDALPYDA VADWVYNELRGQLEERRGR
TLR9_MACFA LRLRDNYLAFFKWGNLIHL LPWRGRQSGQGEDALPYDA VADWVYNELRGQLEERRGR
TLR9_MACMU LHLRDNYLAFFKWGNLIHL LPWRGRQSGQGEDALPYDA VADWVYNELRGQLEERRGR
TLR9_AOTNA LRLRDNKLAFFKWRSLALL LPRWGRRGGRREDALPYDA VADWVYNELRRQLEECRGR
TLR9_HORSE LRLRNNYLAFFNWSSLTLL LPRRGWQ--RGADALSYDA VADWVYNELRVRLEERRGR
TLR9_FELCA LRLRDNYLAFFNWSSLVLL LPRRGRR--RGADALPYDA VADWVYNELRVRLEERRGR
TLR9_PIG LHLRDNNLAFFNWSSLTLL LPHRGQR--RGADALFYDA VADWVYNELRVQLEERRGR
TLR9_CANFA LRLRDNYLAFFNWSSLALL LPRRGRR--RGVDALAYDA VADWVYNELRVQLEERRGR
TLR9_BOVIN LRLRDNNLAFFNWSSLTVL LPRRRRQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_CAPHI LRLRDNNLAFFNWSSLTVL LPRRRRQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_BOSIN LRLRDNNLAFFNWSSLTVL LPRRRRQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_SHEEP LRLRDNNLAFFNWSSLTVL LPRRRRQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_BUBBU LHLRDNNLAFFNWSSLTVL LPRRRWQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_BOSTR LRLRDNNLAFFNWSSLTVL LPRRRRQ--RGEDTLLYDA VADWVYNELRVQLEERRGR
TLR9_MOUSE LSLRDNYLSFFNWTSLSFL LPLLARSR-RSAQALPYDA VADWVYNELRVRLEERRGR
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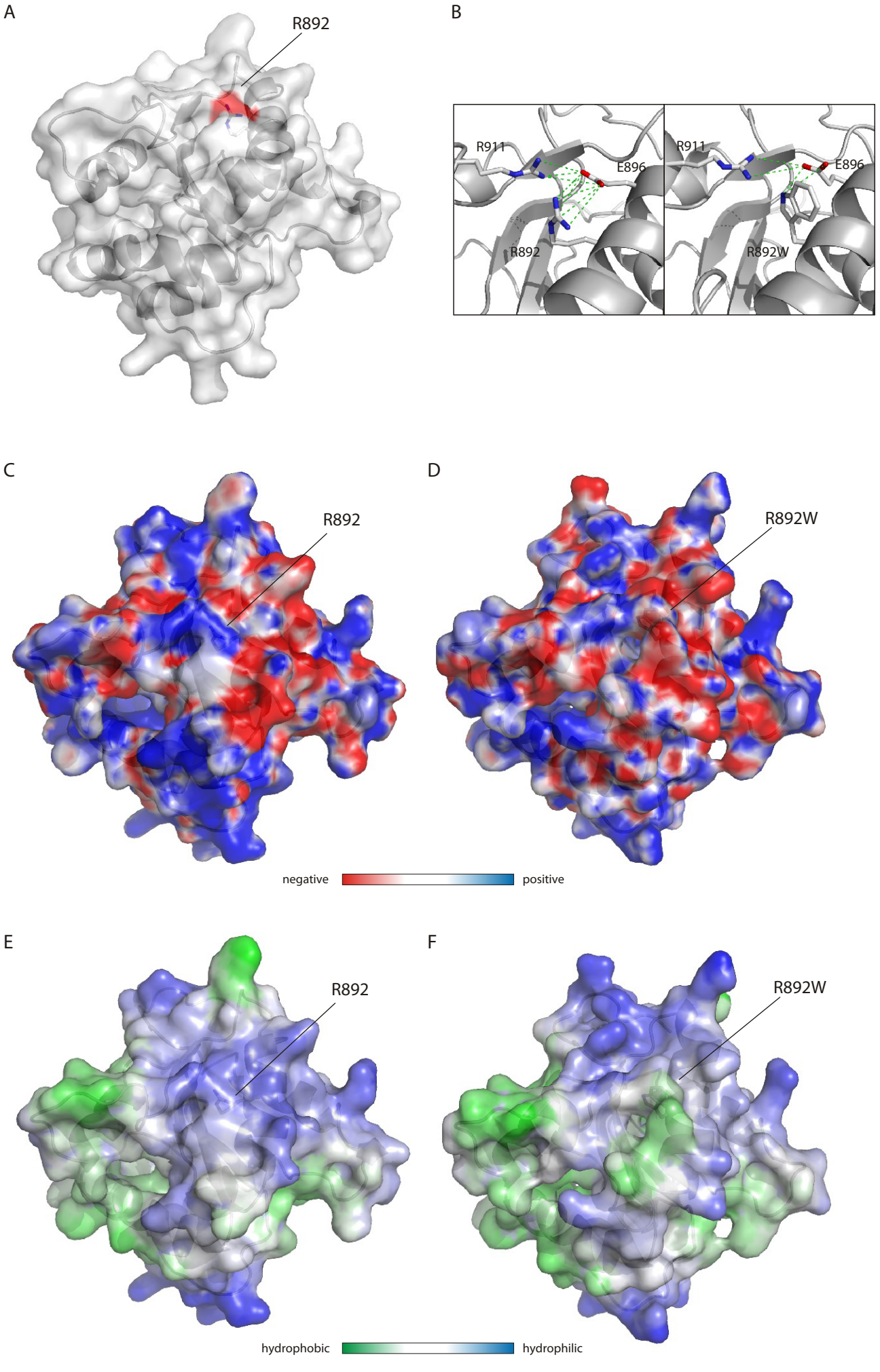
100%
Conservation
0%


A
R892
B
R911
E896
R892
R911
E896
R892W
C
R892
D
R892W
negative
positive
E
R892
F
R892W
hydrophobic
hydrophilic