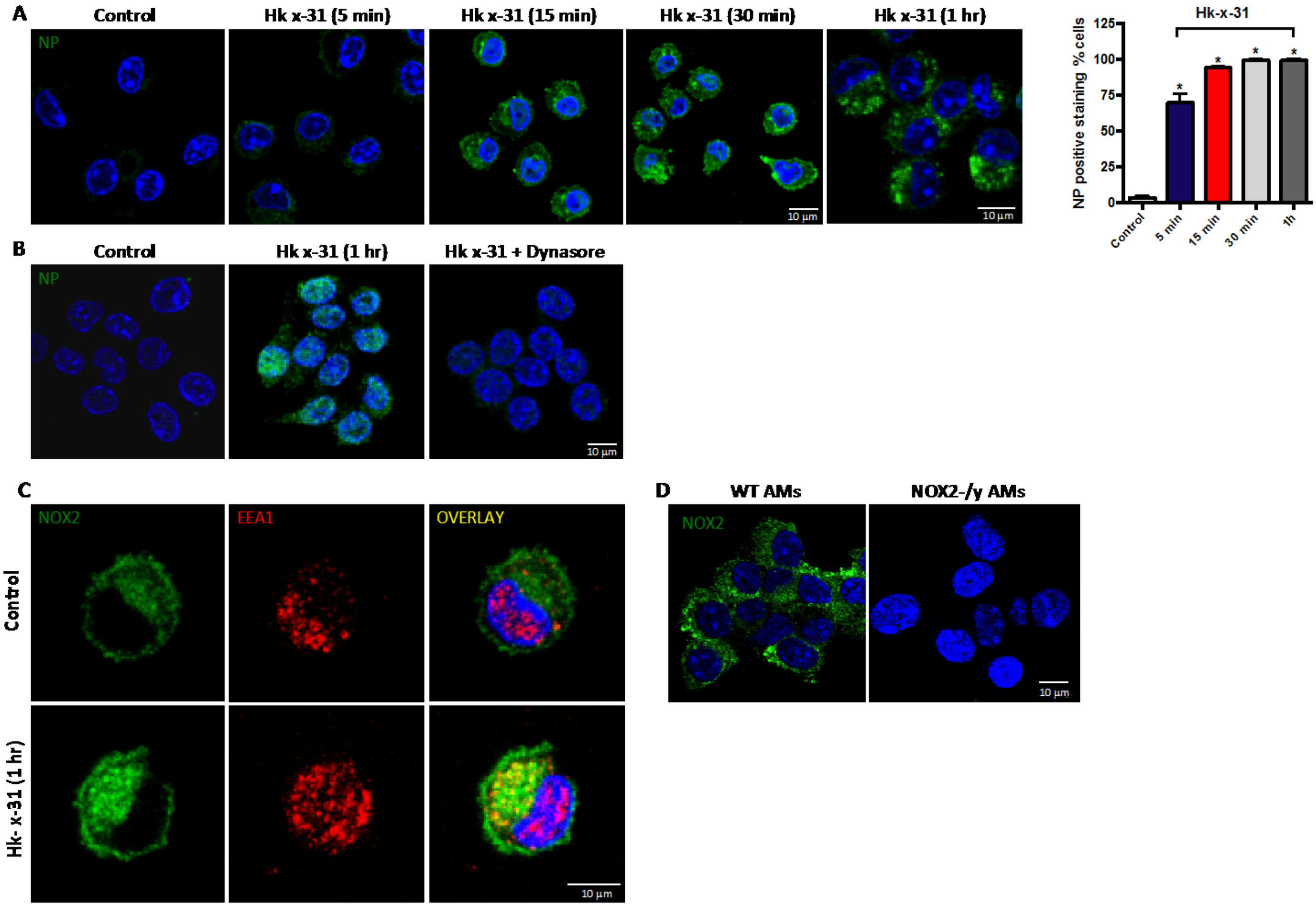
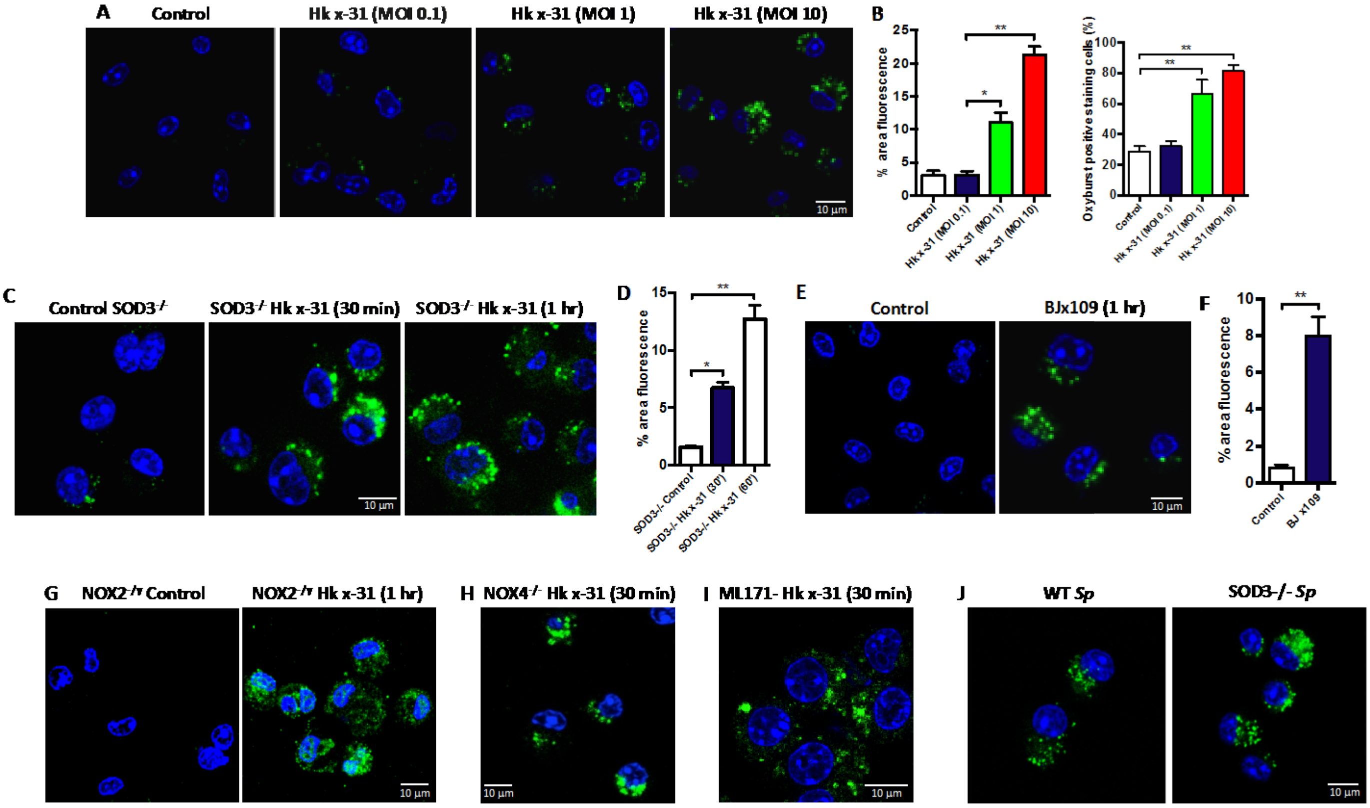
File name: Supplementary Information
Description: Supplementary Figures and Supplementary Tables

File name: Peer Review File
Description:



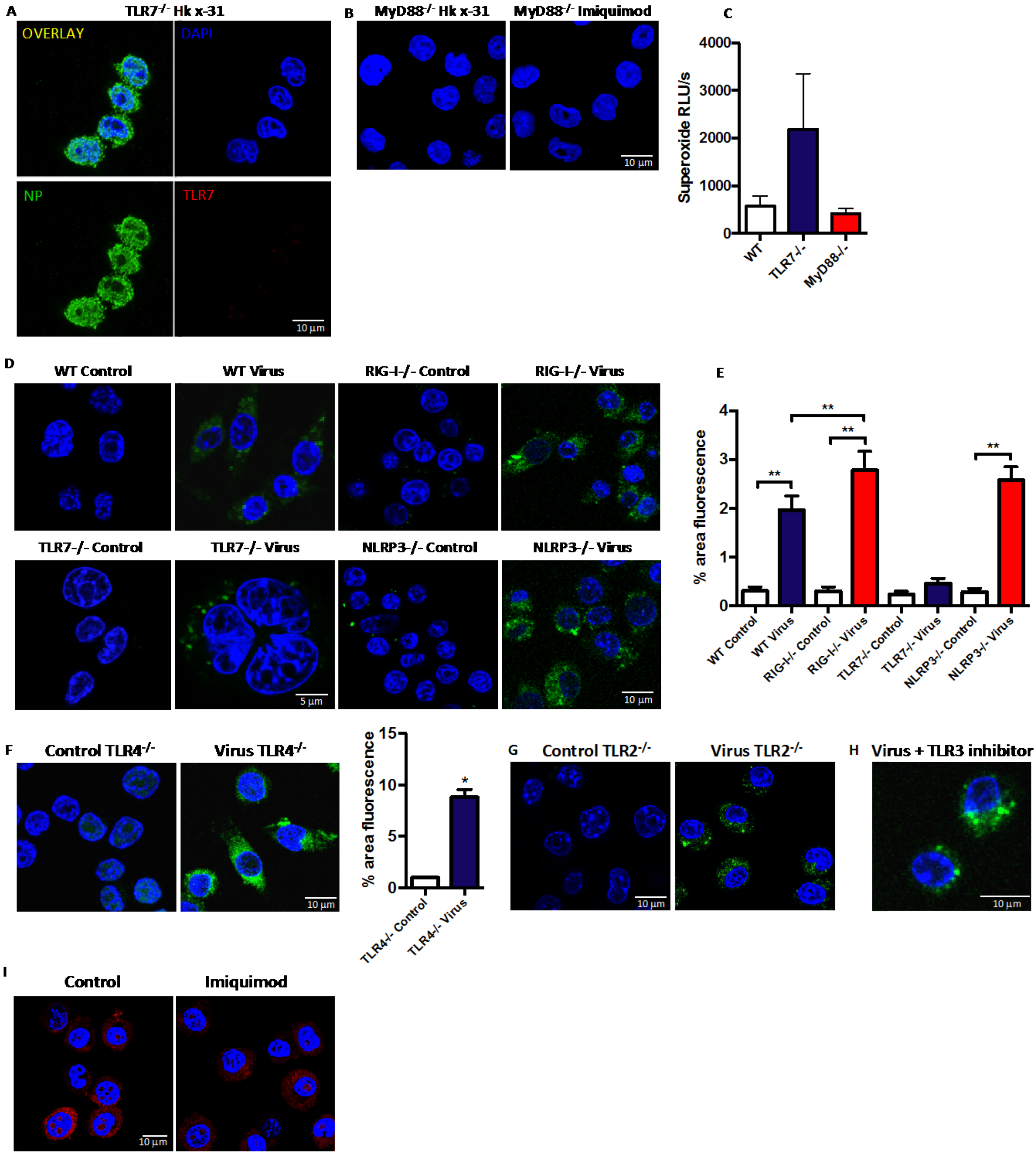
Supplementary Figure 1. Dynamin-dependent endocytosis of influenza A virus and entry into early endosomes expressing NOX2 oxidase.

(A, B) Confocal microscopy of WT mouse primary alveolar macrophages that were infected with the HKx31 strain of influenza A virus (MOI of 10 at various time points) in the absence and presence of the dynamin inhibitor, Dynasore (100 µM), and then labeled as indicated with antibody to influenza A virus nucleoprotein (NP) and 4',6'- diamidino-2-phenylindole (DAPI) (n=5). **(C)** Alveolar macrophages infected with influenza A virus as in **A)**, and then labeled with the early endosome antigen 1 (EEA1) antibody and NOX2 antibody. **(D)** WT and NOX2$^{-/y}$ alveolar macrophages were labeled with the NOX2 antibody and DAPI. **(A-D)** Images are representative of > 100 cells analysed over each experiment. Original magnification X100. Data are shown as mean ± SE. One-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons. * P<0.05 compared to control.



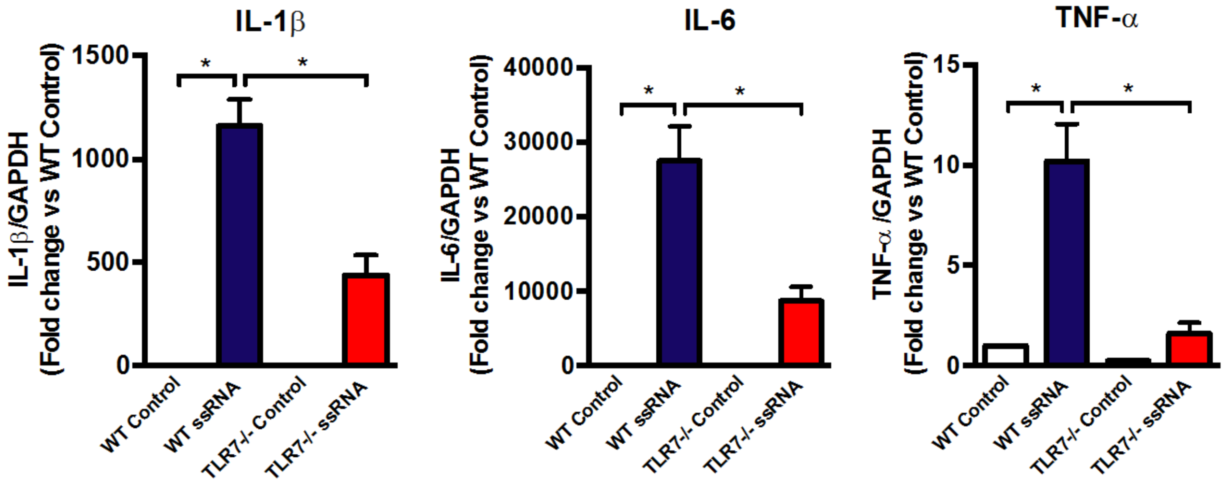
Supplementary Figure 2. Influenza A viruses induce dose-dependent elevations in endosomal NOX2 oxidase-dependent ROS production.

(A, B) Endosomal ROS production in wild-type (WT) mouse primary alveolar macrophages as assessed by OxyBURST (100 µM) confocal fluorescence microscopy in the absence or presence of various doses of HKx31 virus (MOI of 0.1, 1 and 10) and labeled with DAPI (n=5). **(C, D)** Endosomal ROS production following infection with the HKx31 virus in alveolar macrophages taken from superoxide dismutase 3 deficient ($SOD3^{-/-}$) mice (n=5). **(E, F)** Endosomal ROS production in WT alveolar macrophages following infection with the BJx109 virus (n=6). **(G)** Influenza nucleoprotein fluorescence in alveolar macrophages taken from $NOX2^{-/y}$ mice demonstrating that influenza internalization is unaffected by the expression of NOX2 (n=3). **(H and I)** Endosomal ROS production in **H)** $NOX4^{-/-}$ mouse primary alveolar macrophages or **I)** WT mouse primary alveolar macrophages treated with the NOX1 inhibitor ML171 (100nM) as assessed by OxyBURST fluorescence microscopy in the presence of HKx31 virus and labeled with DAPI (n=5). **(J)** Phagosomal ROS production to *Streptococcus pneumoniae (Sp)* as assessed by OxyBURST fluorescence microscopy in WT or $SOD3^{-/-}$ alveolar macrophages (n=3). **(A, C, E, G, H, I, J)** Images are representative of >100 cells analysed over each experiment. Original magnification X100. All data are represented as mean ± SEM. **(B and D)** One-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons. * P<0.05 comparisons indicated by horizontal bars. **P<0.01. **(F)** Unpaired t-test; statistical significance taken when the P<0.05. ** P<0.01

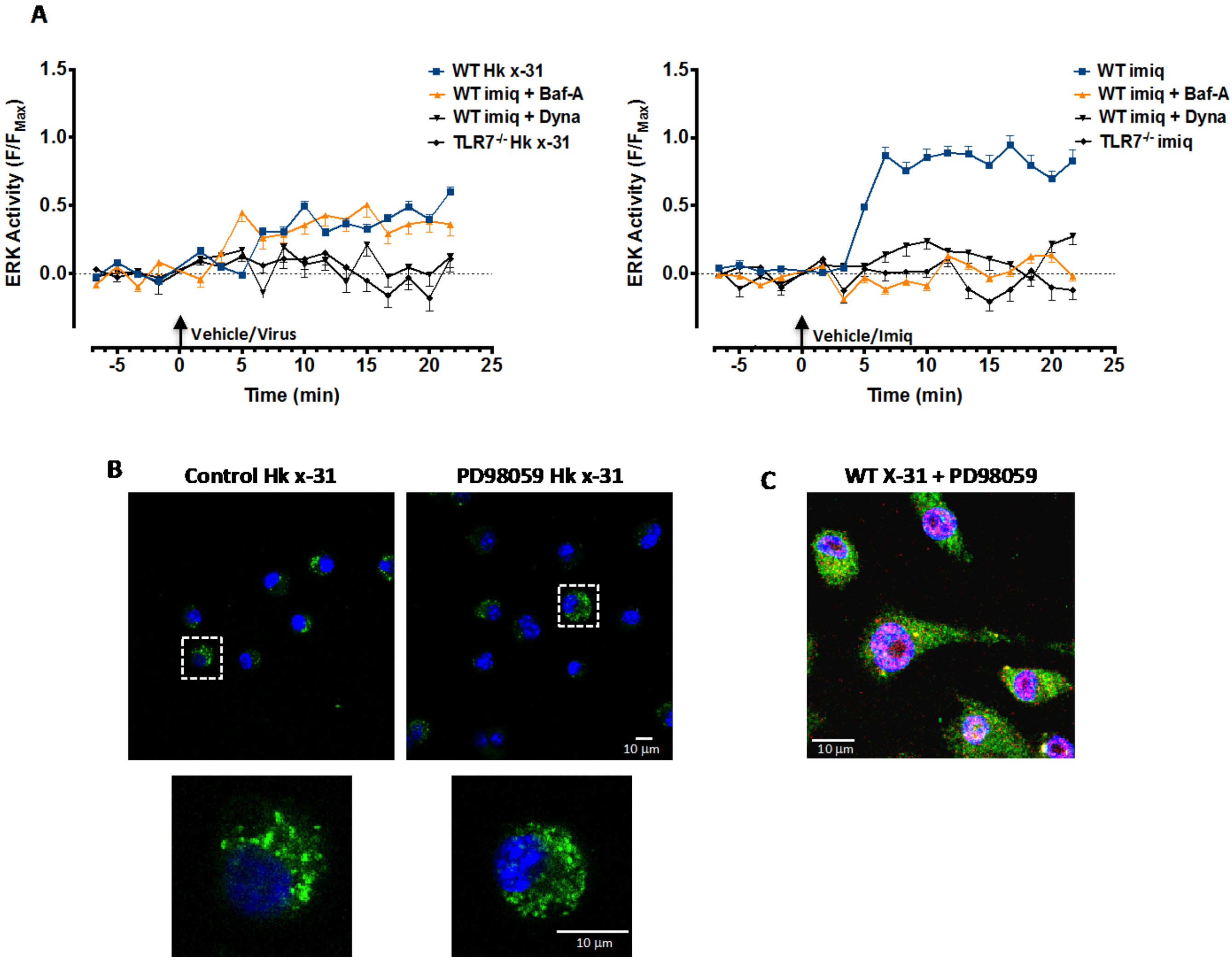


Supplementary Figure 3. Endosomal ROS production to influenza A virus is dependent on MyD88 but independent of RIG-I, NLRP3, TLR2, TLR3, and TLR4.

(A) $TLR7^{-/-}$ immortalized bone marrow-derived macrophages (BMDMs) were infected with HKx31 influenza A virus ((MOI of 10) and then stained with anti-TLR7 antibody and anti nucleoprotein (NP) antibody, and then DAPI (n=3). **(B)** $MyD88^{-/-}$ immortalized BMDMs were infected with HKx31 influenza A virus ((MOI of 10) or exposed to imiquimod (10 µg/mL) and endosomal ROS production was assessed by OxyBURST (100 µM) fluorescence microscopy (n=3). **(C)** Phorbol-dibutyrate-stimulated, NOX2 oxidase-dependent superoxide production in WT, $TLR7^{-/-}$ and $MyD88^{-/-}$ immortalized bone marrow-derived macrophages measured by L-O12 (100 µM)-enhanced chemiluminescence (n=3); RLU/s= relative light units/sec. **(D-G)** Endosomal ROS production in WT, $RIG\text{-}I^{-/-}$, $TLR7^{-/-}$, and $NLRP3^{-/-}$ and **F)** $TLR4^{-/-}$ or **G)** $TLR2^{-/-}$ BMDMs, as assessed by OxyBURST fluorescence microscopy in the absence or presence of HKx31 virus and co-labeled with DAPI (n=3-5). **(H)** WT mouse primary alveolar macrophages were infected with HKx31 virus (MOI of 10) in the presence of the TLR3 inhibitor (50 µM, n=3). **(I)** WT mouse primary alveolar macrophages were treated with imiquimod (10µg/mL) for 60 mins and mitochondrial superoxide measured by MitoSOX confocal fluorescence microscopy and co-labeled with DAPI (n=3). **(A, B, D, F, G, H and I)** Images are representative of >100 cells analyzed over each experiment. Original magnification X100. All data are represented as mean ± SEM. **(E)** One-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons. * $P<0.05$ and ** $P<0.01$ comparisons indicated by horizontal bars. **(F)** Unpaired t-test; statistical significance taken when the $P<0.05$.

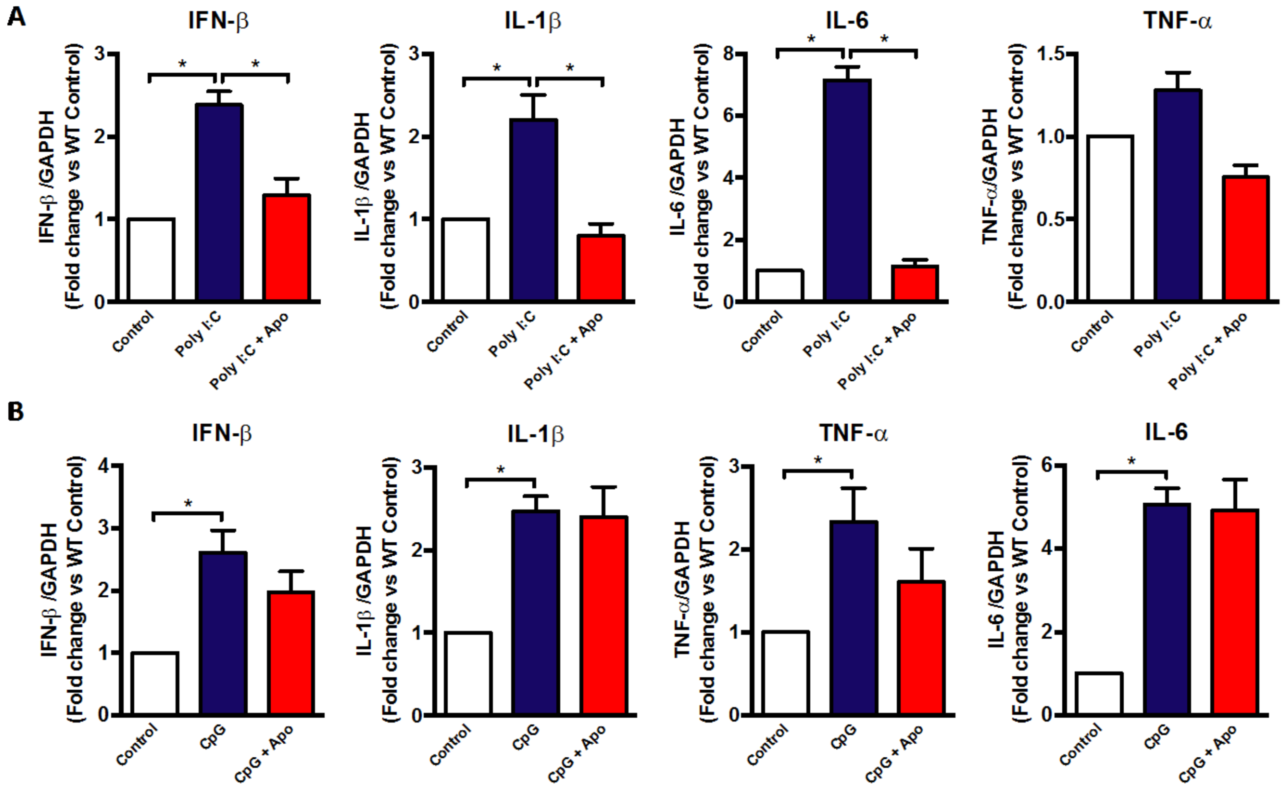


Supplementary Figure 4. Single stranded RNA (ssRNA) drives IL-1β, TNF-α and IL-6 mRNA expression *via* a TLR7-dependent mechanism in macrophages.
WT and $TLR7^{-/-}$ immortalized bone marrow derived macrophages were treated with ssRNA lyoVec (100 μM) and cytokine expression was determined by QPCR after 24 h. Kruskal-Wallis test with Dunn's *post hoc* for multiple comparisons. All data are represented as mean ± SEM. Statistical significance was taken when the P<0.05 (n=6).

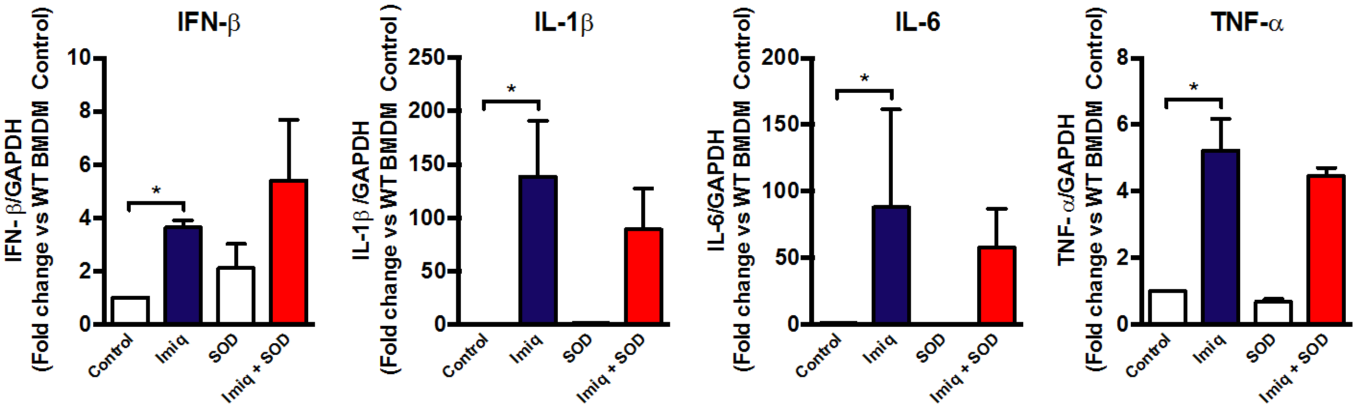


Supplementary Figure 5. Influenza A virus and imiquimod trigger ERK1/2-phosphorylation in a TLR7-dependent manner whereas ERK does not contribute to activation of NOX2 oxidase.

(A) Cytosolic ERK activity as assessed by high content ratiometric FRET imaging analysis in WT and TLR7$^{-/-}$ immortalized bone marrow-derived macrophages (BMDMs). Cells were either treated with vehicle controls or with bafilomycin A (Baf-A; 100 nM) or Dynasore (Dyna; 100 µM) and then exposed for 25 min to HKx31 influenza A virus (MOI of 10) or imiquimod (imiq; 10 µg/ml).All data are represented as mean ± SEM (n=3). **(B)** Endosomal superoxide production to HKx31 influenza A virus in WT alveolar macrophages as assessed by OxyBURST (100 µM) fluorescence microscopy in the absence or presence of the MEK inhibitor PD98059 (30 µM). **(C)** Immunofluorescence microscopy for assessment of NOX2 and p47phox association. WT immortalized BMDMs were infected with HKx31 virus, (MOI of 10) in the presence of PD98059 (30 µM) and then labeled with antibodies to NOX2 and p47phox. All images are representative of >100 cells analyzed over each experiment. Original magnification X100. (n=4).

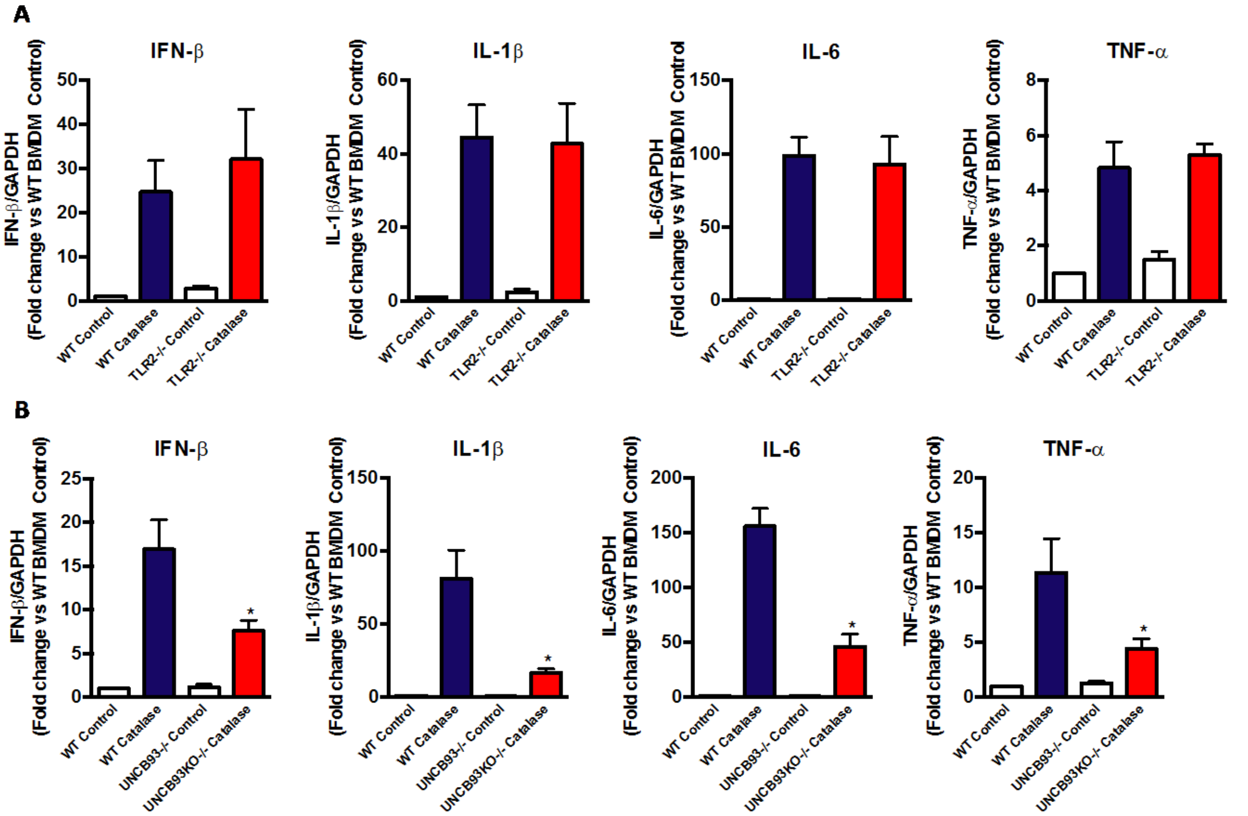


Supplementary Figure 6. Effect of NOX2 oxidase inhibition on cytokine expression by TLR3 agonist, poly I:C. or TLR9 agonist CpG. WT immortalized bone marrow derived macrophages were treated with **A)** poly I:C (25 μg/mL) or **B)** CpG (10μg/mL) in the absence or presence of apocynin (Apo; 300 μM) and cytokine expression was determined by QPCR after 24 h (n=6). Responses are relative to GAPDH and then expressed as a fold-change above controls. All data are represented as mean ± SEM. Kruskal-Wallis test with Dunn's *post hoc* for multiple comparisons. Statistical significance was taken when the $P<0.05$. * $P<0.05$.



Supplementary Figure 7. TLR7-dependent cytokine production to imiquimod is unaffected by endosomal superoxide production.

WT immortalized bone marrow derived macrophages were treated with imiquimod (Imiq; 10 μg/mL) in the absence or presence of superoxide dismutase (SOD; 300 U/mL) and cytokine expression was determined by QPCR after 24 h. Responses are relative to GAPDH and then expressed as a fold-change above WT controls. All data are represented as mean ± SEM. Kruskal-Wallis test with Dunn's *post hoc* for multiple comparisons. Statistical significance was taken when the $P<0.05$. * $P<0.05$ (n=6).



Supplementary Figure 8. Catalase-dependent elevation in cytokine expression is suppressed by deletion of UNCB93 but not by TLR2 deletion.

(A) WT and TLR2$^{-/-}$ immortalized bone marrow derived macrophages (BMDMs) were treated with catalase (1000 U/mL) for 1hr and cytokine mRNA expression determined by QPCR after 24 h (n=6). **(B)** WT and UNCB93$^{-/-}$ immortalized BMDMs were treated with catalase (1000 U/mL) for 1hr and cytokine mRNA expression determined by QPCR after 24 h (n=6). Responses are relative to GAPDH and then expressed as a fold-change above WT controls. All data are represented as mean ± SEM. Kruskal-Wallis test with Dunn's *post hoc* for multiple comparisons. Statistical significance was taken when the $P<0.05$. * $P<0.05$.

```
TLR3     ------------------------MRQTLPCIY---------FWGGLLPF-------GML   20
TLR9     -------MGFCRSA----LHP---LS-----LLVQAIMLAMTLALGTLPA-------FLP   34
TLR7     -----------------MVFPMWTLKRLILILF-NIILISKLLGARWFPK-------TLP   35
TLR8     MKESSLQNSSCSLGKETKKENMFLQSSMLTCIFLLISGSCELCAEENFSR-------SYP   53
TLR5     ----------------------------MGDHLDLL--LGVVLMAGPVFG-------IPS   23
TLR4     ---------------------MMSASRLAGTL---I--PAMAFLSCVRPE------SWEP   28
TLR2     ----------------------------MP----HT--LWMVWVLGVIISLSKEESSNQA   26
TLR10    -------------------------MRLIRNIYIFC--SIVMTAEGDAPE----------   23
TLR1     ----------------------------MTSIFHFA--IIFMLILQIRIQ----------   20
TLR6     ---------------------MTKDKEPIVKSFHFV--CLMIIIVGTRIQ----------   27

         C36             C51
TLR3     CASSTTKCTVSHEVADCSHLKLTQVPD----DLPTNITVLNLTHNQLRRLPAANFTRYSQ   76
TLR9     CEL-QP-----HGLVNCNWLFLKSVPHFSMAAPRGNVTSLSLSSNRIHHLHDSDFAHLPS   88
TLR7     CDV-TLDVPKNHVIVDCTDKHLTEIPG----GIPTNTTNLTLTINHIPDISPASFHRLDH   90
TLR8     CDE-KK--QNDSVIAECSNRRLQEVPQ----TVGKYVTELDLSDNFITHITNESFQGLQN   106
TLR5     CSF-----DGRIAFYR--FCNLTQVPQ----VL-NTTERLLLSFNYIRTVTASSFPFLEQ   71
TLR4     CVE-----VVPNITYQCMELNFYKIPD----NLPFSTKNLDLSFNPLRHLGSYSFFSFPE   79
TLR2     SLS-----CDRNGICKGSSGSLNSIPS----GLTEAVKSLDLSNNRITYISNSDLQRCVN   77
TLR10    -LP-----EERELMTNCSNMSLRKVPA----DLTPATTTLDLSYNLLFQLQSSDFHSVSK   73
TLR1     -LS-----EESEFLVDRSKNGLIHVPK----DLSQKTTILNISQNYISELWTSDILSLSK   70
TLR6     -FS-----DGNEFAVDKSKRGLIHVPK----DLPLKTKVLDMSQNYIAELQVSDMSFLSE   77
                              :  :*             * :: * :  :   .:

                C98 C100        C112
TLR3     LTSLDVGFNTISK---------------LEPELCQKLPMLKVLNLQHNELSQLSDKTFAFC   122
TLR9     LRHLNLKWNCPPVGLSPMH--FPCHMTIEPSTFLAVPTLEELNLSYNNIMTVP----ALP   142
TLR7     LVEIDFRCNCVPIPLGSKNNMCIKRLQIKPRSFSGLTYLKSLYLDGNQLLEIPQ---GLP   147
TLR8     LTKINLNHNPNVQHQNGNPGIQSNGLNITDGAFLNLKNLRELLLEDNQLPQIPS---GLP   163
TLR5     LQLLELGSQYTPL--------------TIDKEAFRNLPNLRILDLGSSKIYFLHPDAFQGL   118
TLR4     LQVLDLSRC-EIQ--------------TIEDGAYQSLSHLSTLILTGNPIQSLALGAFSGL   125
TLR2     LQALVLTSN-GIN--------------TIEEDSFSSLGSLEHLDLSYNYLSNLSSSWFKPL   123
TLR10    LRVLILGHN-RIQ--------------QLDLKTFEFNKELRYLDLSNNRLKSVT---WYLL   116
TLR1     LRILIISHN-RIQ--------------YLDISVFKFNQELEYLDLSHNKLVKIS---CHPT   113
TLR6     LTVLRLSHN-RIQ--------------LLDLSVFKFNQDLEYLDLSHNQLQKIS---CHPI   120
         *   : .                     :          *  * *   . :  :

                                             C183  C189
TLR3     TNLTELHLMSNSIQKIKNNPFVK-------------------------------QKNLI   150
TLR9     KSLISLSLSHTNILMLDSASLAGLHALRFLFMDGNCYYKNPCRQALEVAPGALLGLGNLT   202
TLR7     PSLQLLSLEANNIFSIRKENLTELANIEILYLGQNCYYRNPCYVSYSIEKDAFLNLTKLK   207
TLR8     ESLTELSLIQNNIYNITKEGISRLINLKNLYLAWNCYFNKVCEK-TNIEDGVFETLTNLE   222
TLR5     FHLFELRLYFCGL-----------------------------SDAVLKDGYFRNLKALT   148
TLR4     SSLQKLVAVET-------------------------------NLASLENFPIGHLKTLK   153
TLR2     --------------------------------------------------------SSLT   127
TLR10    --------------------------------------------------------AGLR   120
TLR1     --------------------------------------------------------VNLK   117
TLR6     --------------------------------------------------------VSFR   124
                                                                 :

TLR3     TLDLSHNGLSSTKLGTQVQLENLQELLLSNNKIQALKSEELDIFANSSLKKLELSSNQIK   210
TLR9     HLSLKYNNLTVVP---RNLPSSLEYLLLSYNRIVKLAPEDLANLTA--LRVLDVGG----   253
TLR7     VLSLKDNNVTAVP---TVLPSTLTELYLYNNMIAKIQEDDFNNLNQ--LQILDLSG----   258
TLR8     LLSLSFNSLSHVP---PKLPSSLRKLFLSNTQIKYISEEDFKGLIN--LTLLDLSG----   273
TLR5     RLDLSKNQIRSLY-----------------------LHPSFGKLNS--LKSIDFSSNQIF   183
TLR4     ELNVAHNLIQSFK-----------------------LPEYFSNLTN--LEHLDLSSN---   185
TLR2     FLNLLGNPYKTLG-----------------------ETSLFSHLTK--LQILRVGNMDTF   162
TLR10    YLDLSFNDFDTMP-----------------------ICEEAGNMSH--LEILGLSGA---   152
TLR1     HLDLSFNAFDALP-----------------------ICKEFGNMSQ--LKFLGLSTT---   149
TLR6     HLDLSFNDFKALP-----------------------ICKEFGNLSQ--LNFLGLSAM---   156
          *.:   *                                :    *  : ..
```

```
            C260  C263       C270  C273
TLR3      EESPGCFHAIGRLFGLFLNN-VQLGPSLTEKLCLELANTSIRNLSLSNSQL-STTSNTTF    268
TLR9      -NCRRCDHAPNPCMECPRHFPQ-LHPDT------FSHLSRLEGLVLKDSSL-SWLNASWF    304
TLR7      -NCPRCYNAPFPCAPCKNNSPLQIPVNA------FDALTELKVLRLHSNSL-QHVPPRWF    310
TLR8      -NCPRCFNAPFPCVPCDGGASINIDRFA------FQNLTQLRYLNLSSTSL-RKINAAWF    325
TLR5      LVCE---HELEPLQGK-----------------------TLSFFSLAANSLYSRVSVDWG    217
TLR4      -KIQSIYCTDLRVLHQM----------------------PLLNLSLDLSLNPMNF-IQ-P    220
TLR2      TKIQRKDFAGLTFLE-------------------------EL----EIDASDLQS-YE-P    191
TLR10     -KIQKSDFQKIAHLH-------------------------LNTVFLGFRTL--PH-YE-E    182
TLR1      -HLEKSSVLPIAHLN-------------------------ISKVLLVLGETYGEK-ED-P    181
TLR6      -KLQKLDLLPIAHLH-------------------------LSYILLDLRNYYIKE-NE-T    188

TLR3      LGLKWTNLT----MLDLSYNNLN--VVGNDSFAWLPQLEYFFLEYNNIQHLFSHSL----    318
TLR9      RGL--GNLR----VLDLSENFLYKCITKTKAFQGLTQLRKLNLSFNYQKRVSFAHLSLAP    358
TLR7      KNI--NKLQ----ELDLSQNFLAKEIGDAKFLHFLPSLIQLDLSFNFELQVYRASMNLSQ    364
TLR8      KNM--PHLK----VLDLEFNYLVGEIASGAFLTMLPRLEILDLSFNYIKGSYPQHINISR    379
TLR5      KCMNPFRNMVLEI-LDVSGNGWTVDIT----------------------GNFSNAISKSQ    254
TLR4      GAFKEIRLHKLTL----RNNFDSLNVMKT-CIQGLAGLEVHRL----VLGEFRNE-GNLE    270
TLR2      KSLKSIQNV-SHLILHMKQHILLLEIFVD-VTSSVECLELRDT----DLDT--------F    237
TLR10     GSLPILNTTKLHIVLPMDTNFW--VLLRD-GIKTSKILEMTNI----DGKS---------    226
TLR1      GGLQDFNTESLHIVFPTNKEFH--FILDV-SVKTVANLELSNI----KCVL--ED-SKCS    231
TLR6      ESLQILNAKTLHLVFHPTSLFA--IQVNI-SVNTLGCLQLTNI----K--L--ND-DNCQ    236
            :   .

TLR3      --HGLFNVRYLNLKRSFTKQSISLASLPKIDDFSFQWLKCLEHLN-MEDNDIPGIKSNMF    375
TLR9      SFGSLVALKELDMHGIF------FRSLDETTLRPLARLPMLQTLR-LQMNFINQAQLGIF    411
TLR7      AFSSLKSLKILRIRGYV------FKELKSFNLSPLHNLQNLEVLD-LGTNFIKIANLSMF    417
TLR8      NFSKLLSLRALHLRGYV------FQELREDDFQPLMQLPNLSTIN-LGINFIKQIDFKLF    432
TLR5      AFSLILA------HHIM-GAGFGFHNIKDPDQ----------------------------    279
TLR4      KFDKSA---LEGLCNLT-IEEFRLAYLDYYLDDIIDLFNCLTNVSSFSLVSVTIERVKDF    326
TLR2      HF-SELS---TGETNSL-IKKFTFRNVKITDESLFQVMKLLNQIS---------------    277
TLR10     QFVSYEMQRNLSLEHAK-TSVLLLNKVDLLWDDLFLILQFVWHTS---------------    270
TLR1      YFLSILAK---LQTNPK-LSSLTLNNIETTWNSFIRILQLVWHTT---------------    272
TLR6      VFIKFLSE---LTRGPT-LLNFTLNHIETTWKCLVRVFQFLWPKP---------------    277
                                 :  :

                                C445
TLR3      TGLINLKYLSLSNSFTSLRT-L--------------------------------------    396
TLR9      RAFPGLRYVDLSDNRISGASELTATMGEADGGEKV-WLQPG-------DL----------    453
TLR7      KQFKRLKVIDLSVNKISPSGDSSEVGFCSNARTSVESYEPQVL-EQLHYFRYDKY-----    471
TLR8      QNFSNLEIIYLSENRISPLVKDTRQSYANSSSF-----QRHIRKRRSTDFEFDPH-----    482
TLR5      -------------NTFAGL--------------------------------------ARS    288
TLR4      SYNFGWQHLELVNCKFGQFPTLKL---------------------KS-------------    352
TLR2      ----GLLELEFDDCTLNGVGNFRA---------------------SDNDRVIDPGKVETL    312
TLR10     -----VEHFQIRNVTFGGKAYLDH---------------------NS--FDYSNTVMRTI    302
TLR1      -----VWYSSISNVKLQGQ--LDF---------------------RD--FDYSGTSLKAL    302
TLR6      -----VEYLNIYNLTIIES--IRE---------------------ED--FTYSKTTLKAL    307

                    C475           C491                          C521
TLR3      ------------------TNETFVSLAHSPLHILNLTKNKISKIESDAFSWLGHLEVLDL    438
TLR9      ----------APAPVDTPSSEDFRPNCSTLNFTLDLSRNNLVTVQPEMFAQLSHLQCLRL    503
TLR7      -------ARSCRFKNKEASFMSVNESCYKYGQTLDLSKNSIFFVKSSDFQHLSFLKCLNL    524
TLR8      -------SNFYHF-----TRPLIKPQCAAYGKALDLSLNSIFFIGPNQFENLPDIACLNL    530
TLR5      SVRHLDLSHGFVFSLN-----SRVFETLKDLKVLNLAYNKINKIADEAFYGLDNLQVLNL    343
TLR4      ------------------------------LKRLTFTSNK--GGNAFSEVDLPSLEFLDL    380
TLR2      TIRRLHIPREYLFYDL-----STLYSLTERVKRITVENSKVFLVPCLLSQHLKSLEYLDL    367
TLR10     KLEHVHFRVEYI--QQ-----DKIYILLTKMDIENLTISNAQMPHMLFPNYPTKFQYLNF    355
TLR1      SIHQVVSDVEGF--PQ-----SYIYEIFSNMNIKNFTVSGTRMVHMLCPSKISPFLHLDF    355
TLR6      TIEHITNQVELF--SQ-----TALYTVFSEMNIMMLTISDTPFIHMLCPHAPSTFKFLNF    360
                                               .  .              :  * :
```

```
TLR3     GLNEIGQELTG----QEWRGLENIFEIYLSYNKYLQLTRNSFALVPSLQRLMLRR----V   490
TLR9     SHNCISQAVNG----SQFLPLTGLQVLDLSHNKLDLYHEHSFTELPRLEALDLSYNSQPF   559
TLR7     SGNLISQTLNG----SEFQPLAELRYLDFSNNRLDLLHSTAFEELHKLEVLDISSNSHYF   580
TLR8     SANSNAQVLSG----TEFSAIPHVKYLDLTNNRLDFDNASALTELSDLEVLDLSYNSHYF   586
TLR5     SYNLLGELYSS-----NFYGLPKVAYIDLQKNHIAIIQDQTFKFLEKLQTLDLRDNALTT   398
TLR4     SRNGLS---FKGCCSQSDFGTTSLKYLDLSFNGVIT-MSSNFLGLEQLEHLDFQHSNLKQ   436
TLR2     SENLMVEEYLKNSACED--------------------------AWPSLQTLILRQNHLAS   401
TLR10    ANNILTDELFKRT---L--------------------------QLPHLKTLILNGNKLET   386
TLR1     SNNLLTDTVFENC---G--------------------------HLTELETLILQMNQLKE   386
TLR6     TQNVFTDSIFEKC---S--------------------------TLVKLETLILQKNGLKD   391
           *                                         *:  *  :

TLR3     ALKNVDSSPSPFQPLRNLTILDLSNNNIANIN-DDMLEGLEKLEILDLQHNNLARLWKHA   549
TLR9     GMQGVGHNFSFVAHLRTLRHLSLAHNNIHSQ--VSQQLCSTSLRALDFSGNALGHMWAEG   617
TLR7     QSEGITHMLNFTKNLKVLQKLMMNDNDISSS--TSRTMESESLRTLEFRGNHLDVLWREG   638
TLR8     RIAGVTHHLEFIQNFTNLKVLNLSHNNIYTLT-DKYNLESKSLVELVFSGNRLDILWNDD   645
TLR5     I-HFIPSIP----------DIFLSGNKLVTLPKINLTANLIHLSE---N--------R-L   435
TLR4     M-SE-----------------------------FSVFLSLRNLIYLDISH-------T-H   458
TLR2     L-EK-----------------------------TGET-----------------------   408
TLR10    L-SL-----------------------------VSCF-----------------------   393
TLR1     L-SK-----------------------------IAEM-----------------------   393
TLR6     L-FK-----------------------------VGLM-----------------------   398

TLR3     NPGGPIYFLKGLSHLHILNLESNGFDEIPVEVFKDLF-----------------------   586
TLR9     D--LYLHFFQGLSGLIWLDLSQNRLHTLLPQTLRNLPKSLQVLRLRDNYLA-----FFKW   670
TLR7     DN-RYLQLFKNLLKLEELDISKNSLSFLPSGVFDGMPPNLKNLSLAKNGLK-----SFSW   692
TLR8     DN-RYISIFKGLKNLTRLDLSLNRLKHIPNEAFLNLPASLTELHINDNMLK-----FFNW   699
TLR5     ENLDILYFLLRVPHLQILILNQNRFSSCSGDQTPSENPSLEQLFLGENMLQLAWETELCW   495
TLR4     TRVAFNGIFNGLSSLEVLKMAGNSFQENFLPDIFTEL-----------------------   495
TLR2     --------LLTLKNLTNIDISKNSF--HSMPETCQWPEKMKYLNLSSTRI---------H   449
TLR10    --------ANN-TPLEHLDLSQNLLQ-HKNDENCSWPETVVNMNLSYNKL--------SD   435
TLR1     --------TTQMKSLQQLDISQNSVSYDEKKGDCSWTKSLLSLNMSSNIL--------TD   437
TLR6     --------TKDMPSLEILDVSWNSLESGRHKENCTWVESIVVLNLSSNML--------TD   442
                       *   :  :   *  .

             C697                     C721
TLR3     --------ELKIIDLG------------------------LNNLNTLPASVFNNQVSLKS   614
TLR9     WSLHFL-PKLEVLDLAGNQLKALTNGSLPAGTRLRRLDVSCNSISFVAPGFFSKAKELRE   729
TLR7     KKLQCL-KNLETLDLSHNQLTTVPERLSNCSRSLKNLILKNNQIRSLTKYFLQDAFQLRY   751
TLR8     TLLQQF-PRLELLDLRGNKLLFLTDSLSDFTSSLRTLLLSHNRISHLPSGFLSEVSSLKH   758
TLR5     DVFEGL-SHLQVLYLNHNYLNSLPPGVFSHLTALRGLSLNSNRLTVLSH--NDLPANLEI   552
TLR4     -------RNLTFLDLSQCQLEQLSPTAFNSLSSLQVLNMSHNNFFSLDTFPYKCLNSLQV   548
TLR2     SVTGCIPKTLEILDVSNNNLNLF-S---LNLPQLKELYISRNKLMTLPD--ASLLPMLLV   503
TLR10    SVFRCLPKSIQILDLNNNQIQTV-PKETIHLMALRELNIAFNFLTDLPG--CSHFSRLSV   492
TLR1     TIFRCLPPRIKVLDLHSNKIKSI-PKQVVKLEALQELNVAFNSLTDLPG--CGSFSSLSV   494
TLR6     SVFRCLPPRIKVLDLHSNKIKSV-PKQVVKLEALQELNVAFNSLTDLPG--CGSFSSLSV   499
                :  : :                         * :  :           *

                                                C787 789
TLR3     LNLQKNLITSVEKKVFGP-AFRNLTELDMRFNPFDCTCESIAWFVNWINE-THTNIPELS   672
TLR9     LNLSANALKTVDHSWFGP-LASALQILDVSANPLHCACG-AAFMDFLLE--VQAAVPGLP   785
TLR7     LDLSSNKIQMIQKTSFPENVLNNLKMLLLHHNRFLCTCD-AVWFVWWVNH-TEVTIPYLA   809
TLR8     LDLSSNLLKTINKSALETKTTTKLSMLELHGNPFECTCD-IGDFRRWMDEHLNVKIPR-L   816
TLR5     LDISRNQLLAPNPDVFV-----SLSVLDITHNKFICECE-LSTFINWLNHT-NVTIAGPP   605
TLR4     LDYSLNHIMTSKKQELQH-FPSSLAFLNLTQNDFACTCE-HQSFLQWIKDQRQLL--VEV   604
TLR2     LKISRNAITTFSKEQLDS-FH-TLKTLEAGGNNFICSCE-FLSFTQE-QQALAKVLIDWP   559
TLR10    LNIEMNFILSPSLDFVQS-CQ-EVKTLNAGRNPFRCTCE-LKNFIQ-LETYSEVMMVGWS   548
TLR1     LIIDHNSVSHPSADFFQS-CQ-KMRSIKAGDNPFQCTCE-LGEFVKNIDQVSSEVLEGWP   551
TLR6     LIIDHNSVSHPSADFFQS-CQ-KMRSIKAGDNPFQCTCE-LREFVKNIDQVSSEVLEGWP   556
         *   . * :    .    .        :  :    * : * *      :    .
```

```
      C814                    C833
TLR3   SHYICNTPPHYHGFPVRLFDTS--SCKDSAPFELFFMINTSILLIFIFIVLLIHFEGWRI   730
TLR9   SRVKCGSPGQLQGLSIFAQDLR--LCLDEALSWDCFALSLLAVALGLGVPMLHHLCGWDL   843
TLR7   TDVTCVGPGAHKGQSVISLDLY--TCELDLTNLILFSLSISVSLFLMVMMTASHLYFWDV   867
TLR8   VDVICASPGDQRGKSIVSLELT--TCVSDVTAVILFFFTFFITTMVMLAALAHHLFYWDV   874
TLR5   ADIYCVYPDSFSGVSLFSLS--TEGCDEEEVLKSLKFSLFIVCT---VTLTLFLMTILTV   660
TLR4   ERMECATPSDKQGMPVLSLNI---TCQMNKTIIGVSVLSVLVVS---VVAVLVYKFYFHL   658
TLR2   ANYICDSPSHVRGQQVQDVRLSVSECHRTALVSGMCCALFLL-----ILLTGVLCHRFHG   614
TLR10  DSYTCEYPLNLRGTRLKDVHLHELSCNTALLIVTIVVIMLVL-----GLAVAFCCLHFDL   603
TLR1   DSYKCDYPESYRGTLLKDFHMSELSCNITLLIVTIVATMLVL-----AVTVTSLCIYLDL   606
TLR6   DSYKCDYPESYRGSPLKDFHMSELSCNITLLIVTIGATMLVL-----AVTVTSLCIYLDL   611
           *   *     *   :        *

        C874                        C889 890
TLR3   SFYWNVSVHRVLGFKEID-R------QTEQFEYAAYIIHAYKD---KDWVWEHFSSMEKE   780
TLR9   WYCFHLCLAWLPWRGRQSGR------DEDALPYDAFVVFDKTQSAVADWVYNELRGQLEE   897
TLR7   WYIYHFCKAKIKGYQRLI---------SPDCCYDAFIVYDTKDPAVTEWVLAELVAKLED   918
TLR8   WFIYNVCLAKVKGYRSLS---------TSQTFYDAYISYDTKDASVTDWVINELRYHLEE   925
TLR5   TKFRGFCFICYKTAQRLVFKDHPQGTEPDMYKYDAYLCFSSKD---FTWVQNALLKHLDT   717
TLR4   ----MILAGCIKY--G-----------RGENIYDAFVIYSSQD---EDWVRNELVKNLEE   698
TLR2   LWYMKMMWAWLQA--KRKPRKAP----SRNICYDAFVSYSERD---AYWVENLMVQELEN   665
TLR10  PWYLRMLGQCTQT--WHRVRKTTQEQLKRNVRFHAFISYSEHD---SLWVKNELIPNLEK   658
TLR1   PWYLRMVCQWTQT--RRRARNIPLEELQRNLQFHAFISYSGHD---SFWMKNELLPNLEK   661
TLR6   PWYLRMVCQWTQT--RRRARNIPLEELQRNLQFHAFISYSEHD---SAWVKSELVPYLEK   666
             .                         : *::  .   :     *:   :   .

           C927
TLR3   ----DQSLKFCLEERDFEAGVFELEAIVN-SIKRSRKIIFVITHHLLKDPLCKRFKVHHA   835
TLR9   CR-GRWALRLCLEERDWLPGKTLFENLWA-SVYGSRKTLFVLAHTDRVSGLLR-ASFLLA   954
TLR7   PR-EK-HFNLCLEERDWLPGQPVLENLSQ-SIQLSKKTVFVMTDKYAKTENFK-IAFYLS   974
TLR8   SR-DK-NVLLCLEERDWDPGLAIIDNLMQ-SINQSKKTVFVLTKKYAKSWNFK-TAFYLA   981
TLR5   QYSDQNRFNLCFEERDFVPGENRIANIQD-AIWNSRKIVCLVSRHFLRDGWCL-EAFSYA   775
TLR4   G---VPPFQLCLHYRDFIPGVAIAANIIHEGFHKSRKVIVVVSQHFIQSRWCI-FEYEIA   754
TLR2   F---NPPFKLCLHKRDFIPGKWIIDNIID-SIEKSHKTVFVLSENFVKSEWCK-YELDFS   720
TLR10  E---DGSILICLYESYFDPGKSISENIVS-FIEKSYKSIFVLSPNFVQNEWCH-YEFYFA   713
TLR1   E---G--MQICLHERNFVPGKSIVENIIT-CIEKSYKSIFVLSPNFVQSEWCH-YELYFA   714
TLR6   E---D--IQICLHERNFVPGKSIVENIIN-CIEKSYKSIFVLSPNFVQSEWCH-YELYFA   719
              . :*:    :  *     :    .  * * : :::              :

                                         C1008                 C1028
TLR3   VQQAIEQNLDSIILVFLEEIPDYKLNHALCLRRGMFKSHCILNWPVQKERIGAFRHKLQV   895
TLR9   QQRLLEDRKDVVVLVILSPD--GRRSRYVRLRQRLC-RQSVLLWPHQPSGQRSFWAQLGM   1011
TLR7   HQRLMDEKVDVIILIFLEKP--FQKSKFLQLRKRLC-GSSVLEWPTNPQAHPYFWQCLKN   1031
TLR8   LQRLMDENMDVIIFILLEPV--LQHSQYLRLRQRIC-KSSILQWPDNPKAEGLFWQTLRN   1038
TLR5   QGRCLSDLNSALIMVVVGSLSQYQLMKH-QSIRGFVQKQQYLRWPEDLQDVGWFLHKLSQ   834
TLR4   QTWQFLSSRAGIIFIVLQKVEKTLLRQQ-VELYRLLSRNTYLEWEDSVLGRHIFWRRLRK   813
TLR2   HFRLFDENNDAAILILLEPIEKKAIPQRFCKLRKIMNTKTYLEWPMDEAQREGFWVNLRA   780
TLR10  HHNLFHENSDHIILILLEPIPFYCIPTRYHKLKALLEKKAYLEWPKDRRKCGLFWANLRA   773
TLR1   HHNLFHEGSNSLILILLEPIPQYSIPSSYHKLKSLMARRTYLEWPKEKSKRGLFWANLRA   774
TLR6   HHNLFHEGSNNLILILLEPIPQNSIPNKYHKLKALMTQRTYLQWPKEKSKRGLFWANLRA   779
          :  .     :::.:                  :        * *  .       *   :

TLR3   ALGSKNSVH------------------------------   904
TLR9   ALTRDNHHFYNRNFCQGPTAE------------------   1032
TLR7   ALATDNHVAYSQVFKETV---------------------   1049
TLR8   VVLTENDSRYNNMYVDSIKQY------------------   1059
TLR5   QILKKEKEKKKDNNIPLQT-------VATIS-------   858
TLR4   ALLDGKSWNPEGTV-GTGCNWQE---ATSI--------   839
TLR2   AIKS-----------------------------------   784
TLR10  AINVNVLATREMYELQTFTELNEESRGSTISLMRTDCL   811
TLR1   AINIKLTEQAKK---------------------------   786
TLR6   AFNMKLTLVTENNDVKS----------------------   796
  .
```

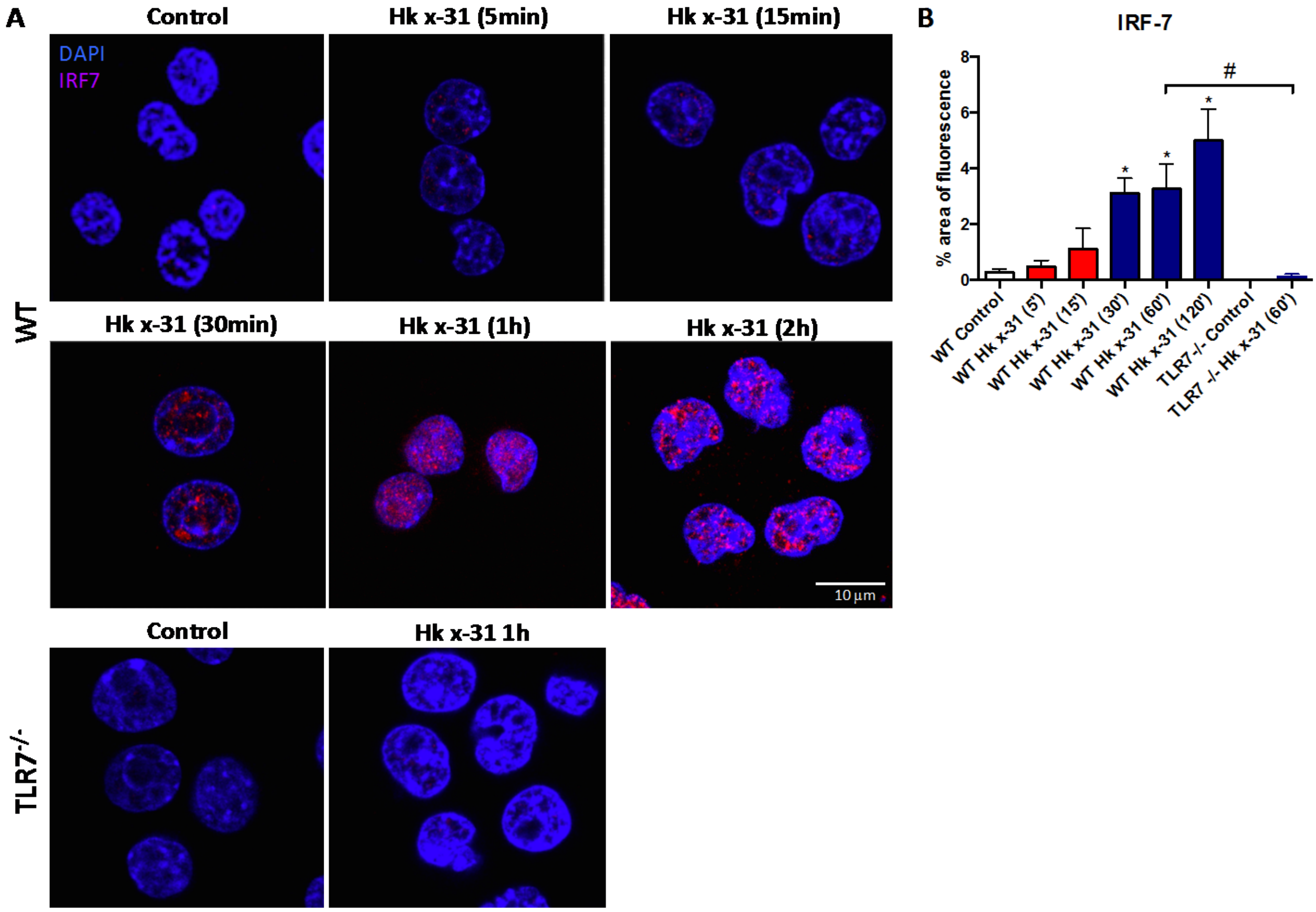
Supplementary Figure 9 Multiple sequence alignment analysis demonstrating the position of all cysteine residues on human TLR7. Individual sequences of human TLRs were obtained from NCBI GenBank protein databases with the following accession numbers TLR1 (CAG38593.1), TLR2 (AAH33756.1), TLR3 (ABC86910.1), TLR4 (AAF07823.1), TLR5 (AAI09119.1), TLR6 (BAA78631.1), TLR7 (AAZ99026.1), TLR8 (AAZ95441.1), TLR9 (AAZ95520.1) and TLR10 (AAY78491.1) and then sequence alignment was performed with CLUSTAL OMEGA (EMBL-EBI). Shown in red dotted rectangular boxes are the cysteines on human TLR7 and the respective position indicated.

```
                                                                      C36
[Salmo      MLSRMT------RSECASFHVCGVILLGLWCSSVLAAGSWYPKTLPCDVTLDSNDTMVNV  54
[Xenopus    MHGKTFKV-----FYFGMRRQLLFFLISILSFSGLLATNWFPKSLPCDVEQNAKGNVIVV  55
[Gallus     -MTNLSEVAAHRKMVHHARTSNALLFVLLFLFPMLLSGRWFPKTLPCDVEA--FESTVRV  57
[Mus        ---------MV--FSMWTRKRQILIFLNMLLVSRVFGFRWFPKTLPCEVKVNIPEAHVIV  49
[Rattus     ---------MV--FPMWTLKRQSFIFLNMILVSRVLGFRWYPKTLPCDVSLDSTNTHVIV  49
[Homo       ---------MV--FPMWTLKRLILILFNIILISKLLGARWFPKTLPCDVTLDVPKNHVIV  49
[Sus        ---------MV--FPVWTLKRQFLILFNIVLISELLGARWFPKTLPCDVSLDAPNAHVIV  49
[Bos        -MGDLFLYFQV--FPMWTLKRQFPILFNMILISGLLGARWFPKTLPCDVTLDAPNTHVIV  57
                                    ::. :      :  .   *:**:***:*       : *
  C51                                                                C98 C100
[Salmo      DCTERGLLEVPKDIPRNTTNLTLTINHIPHINSTSFQGLENLTEIDMRCNCVPIKIGPKD  114
[Xenopus    DCSDRHLTSIPWGIPTNVTNLTLTINHIPRISVDSFAEFTNLVELDFRCNCVPAKVGPKD  115
[Gallus     DCSDRRLKEVPRGIPGNATNLTLTINHIPRISPASFTQLENLVEIDFRCNCVPPRLGPKD  117
[Mus        DCTDKHLTEIPEGIPTNTTNLTLTINHIPSISPDSFRRLNHLEEIDLRCNCVPVLLGSKA  109
[Rattus     DCTDKHLTEIPEGIPTNTTNLTLTINHIPSISPDSFHRLKHLEELDLRCNCVPILLGSKA  109
[Homo       DCTDKHLTEIPGGIPTNTTNLTLTINHIPDISPASFHRLDHLVEIDFRCNCVPIPLGSKN  109
[Sus        DCTDKHLTAIPGGIPTNATNLTLTINHIASITPASFQQLDHLVEIDFRCNCIPVRLGPKD  109
[Bos        DCTDKHLTEIPGGIPANATNLTLTINHIAGISPASFHRLDHLVEIDFRCNCVPVRLGPKD  117
            **.:::  *   :*   ** *.**********  *.   **   :  .* *:*:*****.*   :* *
    C112
[Salmo      RMCTESVTIKTNTFKDLRNLKALYLDGNQLSSIPKGLPPNLILLSLEVNKIYTILKRNLS  174
[Xenopus    HVCTKRLDVEDRSFASLYNLRSLYLDGNQLIEFPKGLPPNLQLLSLEINNIISISRNNLS  175
[Gallus     NVCVTPPSIENGSFAALTRLKSLYLDANQLSKIPRGLPATLRLLSLEANNIFSIKKNTFS  177
[Mus        NVCTKRLQIRPGSFSGLSDLKALYLDGNQLLEIPQDLPSSLHLLSLEANNIFSITKENLT  169
[Rattus     NVCTKRLQIRPGSFSGLSDLKSLYLDGNQLLEIPQDLPSSLQLLSLEANNIFSITKENLS  169
[Homo       NMCIKRLQIKPRSFSGLTYLKSLYLDGNQLLEIPQGLPPSLQLLSLEANNIFSIRKENLT  169
[Sus        NICTRRLQIKPSSFSKLTYLKALYLDGNQLLEIPRDLPPSLQLLSLEANNIFWIMKENLT  169
[Bos        NVCTKRLQIKPNSFSKLTYLKSLYLDGNQLLEIPQDLPPSLQLLSLEANNIFLIMKENLT  177
            .:*       :.   :*   *   *::****.***  .:*: **  .*  ***** *:*   *  :..::
                           C183  C189
[Salmo      DITNVQILYLGQNCYYRNPCNVSYQIEEGAFLQLGNMTLLSLKSNNLSYIPPRLPTSLRE  234
[Xenopus    ELSNIQMLYLGQNCYHRNPCSDSFKIEKDAFKDLKNLSILSMKSNNLSFVPGGLSDSLKE  235
[Gallus     ELRNIELLYLGQNCYYRNPCNVSFEIEETAFLNLKNLTVLSLKSNNLTFIPPNLSSTLKE  237
[Mus        ELVNIETLYLGQNCYYRNPCNVSYSIEKDAFLVMRNLKVLSLKDNNVTAVPTTLPPNLLE  229
[Rattus     ELVNIESLYLGQNCYYRNPCNVSYSIEKDAFLVMKNLKVLSLKDNNVTAVPTILPPNLLE  229
[Homo       ELANIEILYLGQNCYYRNPCYVSYSIEKDAFLNLTKLKVLSLKDNNVTAVPTVLPSTLTE  229
[Sus        ELANLEMLYLGQNCYYRNPCNVSFSIEKDAFLSLRNLKLLSLKDNNISAVPTVLPSTLTE  229
[Bos        ELANLEILYLGQNCYYRNPCNVSFTIEKDAFLNMRNLKLLSLKDNNISAVPTVLPSSLTE  237
            :: *:: ********:****  *: *: **    **  : ::.:**:*.**::  :*   *   .* *
                                           C260 C263     C270 C273
[Salmo      LYLYNNKIEMVTDKDFHNLTQLEILDLCGNCPRCYNAPFHCIHCPNN-SLQIDPSTFKTL  293
[Xenopus    LYLYNNAIQYIEEHDLENLINLEILDLSGNCPRCYNSPFHCTHCPNNAPIQIHPKAFSSL  295
[Gallus     LYIYNNRIQEVQEHDLSNLYNLEILDLSGNCPRCYNAPYHCTHCPNI-SIKIHSKAFYSL  296
[Mus        LYLYNNIIKKIQENDFNNLNELQVLDLSGNCPRCYNVPYHCTHCENNSPLQIHDNAFNSL  289
[Rattus     LYLYNNIIKRIQEHDFNKLSQLQVLDLSGNCPRCYNVPYHCTHCENNSPLQIHDNAFDSL  289
[Homo       LYLYNNMIAKIQEDDFNNLNQLQILDLSGNCPRCYNAPFHCAHCKNNSPLQIPVNAFDAL  289
[Sus        LFLYNNIIAKIQEDDFNNLSQLQVLDLSGNCPRCYNVPFHCTHCENNAPLQIHLHAFDAL  289
[Bos        LYLYNNIITKIQEDDFNNLSQLQVLDLSGNCPRCYNVPFHCTHCENNSPLQIDPNAFDAL  297
            *::*** *   :  :.*:  :*  :*::***.*********  *:**  **  *    ::*    :*  :*

[Salmo      TKLRILRLHSNSLTYVLREWFQNCKELRVLDLSTNFLAREIAITYFPRALPNLEELDLSF  353
[Xenopus    KNLQVLRLHSNSLRSIPEQWFKNNRNLQVLDLSENFLASEISTANFLKYIPSLKSLDLSF  355
[Gallus     KKLRILRLHSNSLQSIPSSWFKNIKNLKNLDLSQNFLIKEIGDAEFLKLIPSLVELDLSF  356
[Mus        TELKVLRLHSNSLQHVPPTWFKNMRNLQELDLSQNYLAREIEEAKFLHFLPNLVELDFSF  349
[Rattus     TELKVLRLHSNSLQHVPAEWFKNMSNLQELDLSQNYLAREIEEAKFLNSLPNLVQLDLSF  349
[Homo       TELKVLRLHSNSLQHVPPRWFKNINKLQELDLSQNFLAKEIGDAKFLHFLPSLIQLDLSF  349
[Sus        TELQVLRLHSNSLQYVPQRWFQNLNKLKELDLSQNFLAKEIGDAKFLHLLPNLVKLDLSF  349
[Bos        TELQVLRLHSNSLQHVPQRWFKNINKLKELDLSQNFLAKEIGDAKFLHLLHNLVNLDLSF  357
            .:*::********  :     **:*  :*:  ****  *:*  **  :  *  .  :  .*  .**:**

[Salmo      NYELQRYPATLHLSPSFSSLKSLKVLRIRAFVFQQLTLEDISPLIHLKNLEVIDLGTNFI  413
[Xenopus    NFELQVYPSDLKLSSIFSSLASLETLRIRGYVFQNLKKNNLMPLVHLPNLTLLDLSTNFI  415
[Gallus     NFELQMYSPFLNLSKTFSCLSNLETLRIKGYVFKELREENLDPLLNLRNLTVLDLGTNFI  416
[Mus        NYELQVYHASITLPHSLSSLENLKILRVKGYVFKELKNSSLSVLHKLPRLEVLDLGTNFI  409
[Rattus     NYELQVYHASITLPHSLSSLTKLKNLYIKGYVFKELKDSSLSVLHNLSNLEVLDLGTNFI  409
[Homo       NFELQVYRASMNLSQAFSSLKSLKILRIRGYVFKELKSFNLSPLHNLQNLEVLDLGTNFI  409
[Sus        NYELQVYHTFMNLSDSFSSLKNLKVLRIKGYVFKELKSLNLSPLRNLPNLEVLDLGTNFI  409
[Bos        NYDLQVYHAVINLSDAFSSLKKLKVLRIKGYVFKELNSLNLFPLHNLPNLEVLDLGTNFI  417
            *::** *    : *    :*.*  .*: *  ::.:**::*     .:   *  .*  .*  ::**.****
```

```
                                                       C445
[Salmo      KITNLSILMELKSFKIINLSDNKISSPSESGQSVAFSGGEAIHGSPMSDAGHNRNGEVRE  473
[Xenopus    KVADFSLFPKFKSLQTIILSNNKISPSSEA--NIDSCSASQV------SSGHYIGRTFQE  467
[Gallus     KIADLRVFKKFRSLKIIDLSMNKISPSSGEGNFYGFCSDHRI------TVEQYSRHVLQE  470
[Mus        KIADLNIFKHFENLKLIDLSVNKISPS-EESREVGFCPNAQT------SVDRHGPQVLEA  462
[Rattus     KIADLNIFQQFENLKFIDLSVNKISPS-EESREVGLCPNAQT------SVDWHGPQVLEA  462
[Homo       KIANLSMFKQFKRLKVIDLSVNKISPS-GDSSEVGFCSNART------SVESYEPQVLEQ  462
[Sus        KIANLSIFKQFKTLKFIDLSVNKISPS-GDSSESGFCSGMRT------SAESHGPQVLES  462
[Bos        KIANLSIFNQFKTLKFIDLSVNKISPS-GDSPEGGFCSNRRT------SVEGHGPQVLET  470
            *:::: :: .      * ** ****                                  ..

                         C475             C491
[Salmo      IHYFRYDEYARSCKYKDKEDGTLNSFVNTQCSKFGKTLDISRNNIFFLHS-RFLNLADLR  532
[Xenopus    VHYFEYDENARKCKAKDKENFTFKLFLNESCQAYGQSLDLSQNNIFFVKATDFTNLSFLK  527
[Gallus     MHYFRYDEYGRSCKSKDKEADSYQPLVNGDCMSYGETLDLSRNNIFFVNSIDFQDLSFLK  530
[Mus        LHYFRYDEYARSCRFKNKEPPSFLPL-NADCHIYGQTLDLSRNNIFFIKPSDFQHLSFLK  521
[Rattus     LHYFRYDKYARSCRFKNKEPPTFLPL-NADCHTYGKTLDLSRNNIFFIKPSDFKHLSFLK  521
[Homo       LHYFRYDKYARSCRFKNKEA-SFMSV-NESCYKYGQTLDLSKNSIFFVKSSDFQHLSFLK  520
[Sus        LHYFRYDEYARSCRFKNKEPSSSLPL-NEDCSMYGQTLDLSRNNIFFIRSSEFQHLTFLK  521
[Bos        LHYFRYDEYARSCRSKSKEPPSFLPL-NEDCYMYGQTLDLSRNNIFFIKPSDFQHLSFLK  529
             ***.**: .*.* *.**  :    . * .*  :*::**:*:*.***:.    * .*: *:

            C521
[Salmo      CLNLSGNAMSQSLNGSEFTFLTKLQYLDFSFNRLDLLYSSAFQELENLVILDISNNNHYF  592
[Xenopus    CLNLSGNAISQTLNGSEFRNLNRLKYLDFSNNRIDLLYSTAFQELTELEVLDISNNDHYF  587
[Gallus     CLNLSGNAISQTLNGSEFYYLSGLKYLDFSNNRIDLLYSTAFKELKFLEILDLSNNKHYF  590
[Mus        CLNLSGNTIGQTLNGSELWPLRELRYLDFSNNRLDLLYSTAFEELQSLEVLDLSSNSHYF  581
[Rattus     CLNLSGNAIGQTLNGSELQPLRELRYLDFSNNRLDLLYSTAFEELQNLEILDLSSNSHYF  581
[Homo       CLNLSGNLISQTLNGSEFQPLAELRYLDFSNNRLDLLHSTAFEELHKLEVLDISSNSHYF  580
[Sus        CLNLSGNSISQALNGSEFQPLVELKYLDFSNNRLDLLHSTAFEELRNLEVLDISSNSHYF  581
[Bos        CLNLSGNSISQTLNGSEFQPLVELKYLDFSNNRLDLLYSTAFEELHNLEVLDISSNSHYF  589
            ******* :.*:******:   *  *:****** **:***:*:**:**   * :**:*.*.***

[Salmo      ESEGLTHMLNFTKNLTKLKILLMNYNKISTSTNTELESRSLEKLEFKGNRLDMLWRDGDT  652
[Xenopus    LAEGITHVFNFTKNLEKLTKLMMNNNQISTSTNRHLVSQSLRILEFKGNYLNILWKDGDT  647
[Gallus     LAEGVSHVLSFMKNLAYLKKLMMNENEISTSISTGMESQSLQTLEFRGNRLDIFWSDGKK  650
[Mus        QAEGITHMLNFTKKLRLLDKLMMNDNDISTSASRTMESDSLRILEFRGNHLDVLWRAGDN  641
[Rattus     QAEGITHMLNFTKKLRHLEKLMMNDNDISTSASRTMESESLRVLEFRGNHLDVLWRDGDN  641
[Homo       QSEGITHMLNFTKNLKVLQKLMMNDNDISSSTSRTMESESLRTLEFRGNHLDVLWREGDN  640
[Sus        QSEGITHMLDFTKNLKVLKKLMMNNNDIATSTSTTMESESLRILEFRGNHLDILWRDGDN  641
[Bos        QSEGITHMLNFTKNLKVLRKLMMNYNDIATSTSRTMESESLQILEFRGNHLDILWRDGDN  649
              :**::*::.* *:*   *   *:** *.*::*  .   :  * **.  ***:** *:::

                                                                 C697
[Salmo      RYIDYFKKLLNLRVLDISHNNLNFIPQQVFQGLPDKLTNLYINNNRLKIFSWEKLICIQY  712
[Xenopus    RYLNFFKNLNKLYKLDISENSLTFVPPGVFEGMPPDLLELYLARNKLKTFSWDKLHCIEK  707
[Gallus     EYLSFFKNLTNLEQLDISSNMLNFLPPDVFEAMPPELKILNLTSNRLHTFNWGKLHCITK  710
[Mus        RYLDFFKNLFNLEVLDISRNSLNSLPPEVFEGMPPNLKNLSLAKNGLKSFFWDRLQCIKH  701
[Rattus     RYLDFFKNLLNLEELDISRNSLNSVPPGVFEGMPPNLTTLSLAKNGLRSFSWGRLQCIKH  701
[Homo       RYLQLFKNLLKLEELDISKNSLSFLPSGVFDGMPPNLKNLSLAKNGLKSFSWKKLQCIKN  700
[Sus        RYLKFFKNLHKLEELDISENSLSFLPSGVFDGMPPNLKTLSLAKNGLKSFNWGKLQCIQN  701
[Bos        RYLKFFKNLLNLEELDISENSLSFLPLGVFDSMPPNLKTLSLAKNGLKSFSWERLQCIKN  709
            .*:. **:* :*   **** * *   :*   **:.:* .*   * :   * *: * * :* *

                                C721
[Salmo      LEVLDLSSNSISTVPPELSNCTKSLKTLLLRRNQISKLSAYFLKDAFSLKYLDLSFNHIQ  772
[Xenopus    LSVLDLSNNYLTTVPRELSNCTSSIKKLILSNNKIKKLTPFFLRGSVSLKYLDLSDNLIQ  767
[Gallus     LITLDLSNNLLTTVPRKLSNCTSTLQELILRNNRITRITKYFLRGAIQLTYLDLSSNKIQ  770
[Mus        LEILDLSHNQLTKVPERLANCSKSLTTLILKHNQIRQLTKYFLEDALQLRYLDISSNKIQ  761
[Rattus     LKNLDLSHNQLTTVPARLANCSKSLTKLILNHNQIRQLTKYFLEDALQLRYLDISSNKIQ  761
[Homo       LETLDLSHNQLTTVPERLSNCSRSLKNLILKNNQIRSLTKYFLQDAFQLRYLDLSSNKIQ  760
[Sus        LETLDLSYNQLKTVPERLSNCSRSLKKLILKNNEIRNLTKYFLQDAFQLRHLDLSSNKIQ  761
[Bos        LETLDLSFNQLKTVPERLSNCSRSLKKLILKNNQIRCLTKYFLQGAFQLRHLDLSSNKIQ  769
            *   **** *  :..** .*:**  :-   *.*   *.*   :: :**. :..* :**.* * **

                                     C787 C789                 C814
[Salmo      NIEQTSIPDDVVDQMDTLVLNNNKFMCNCNALMFVMWLNRTMVNIPRLATAVVCAAPGAQ  832
[Xenopus    NIGHSSFPEDVLDNLTELLLQGNPFKCNCNLVWLVSWINQTKVYIPNLVTGVTCSGPGAH  827
[Gallus     IIKKSSFPENIINNLRMLLLHNNPFKCNCIAVWFVGWINQTQVAIPLLATDVTCAGPGAH  830
[Mus        VIQKTSFPENVLNNLEMLVLHHNRFLCNCIAVWFVWWVNHTDVTIPYLATDVTCVGPGAH  821
[Rattus     VIQKTSFPENVLNNLNMLLLHHNRFLCNCIAVWFVWWVNHTDVTIPYLATDVTCAGPGAH  821
[Homo       MIQKTSFPENVLNNLKMLLLHHNRFLCTCIAVWFVWWVNHTEVTIPYLATDVTCVGPGAH  820
[Sus        VIQKTSFPENVLNNLQILFLHHNRFLCNCIAVWLVWWVNHTEVTIPFLATDVTCMGPGAH  821
[Bos        VIQKTSFPENVLNNLNILFLHHNRFLCNCIAVWFVWWVNHTEVTIPYLATDVTCMGPGAH  829
             * ::*:*::::::::   *.*. * * * .*: : :* *:*:* * ** *.* *.* .***:
```

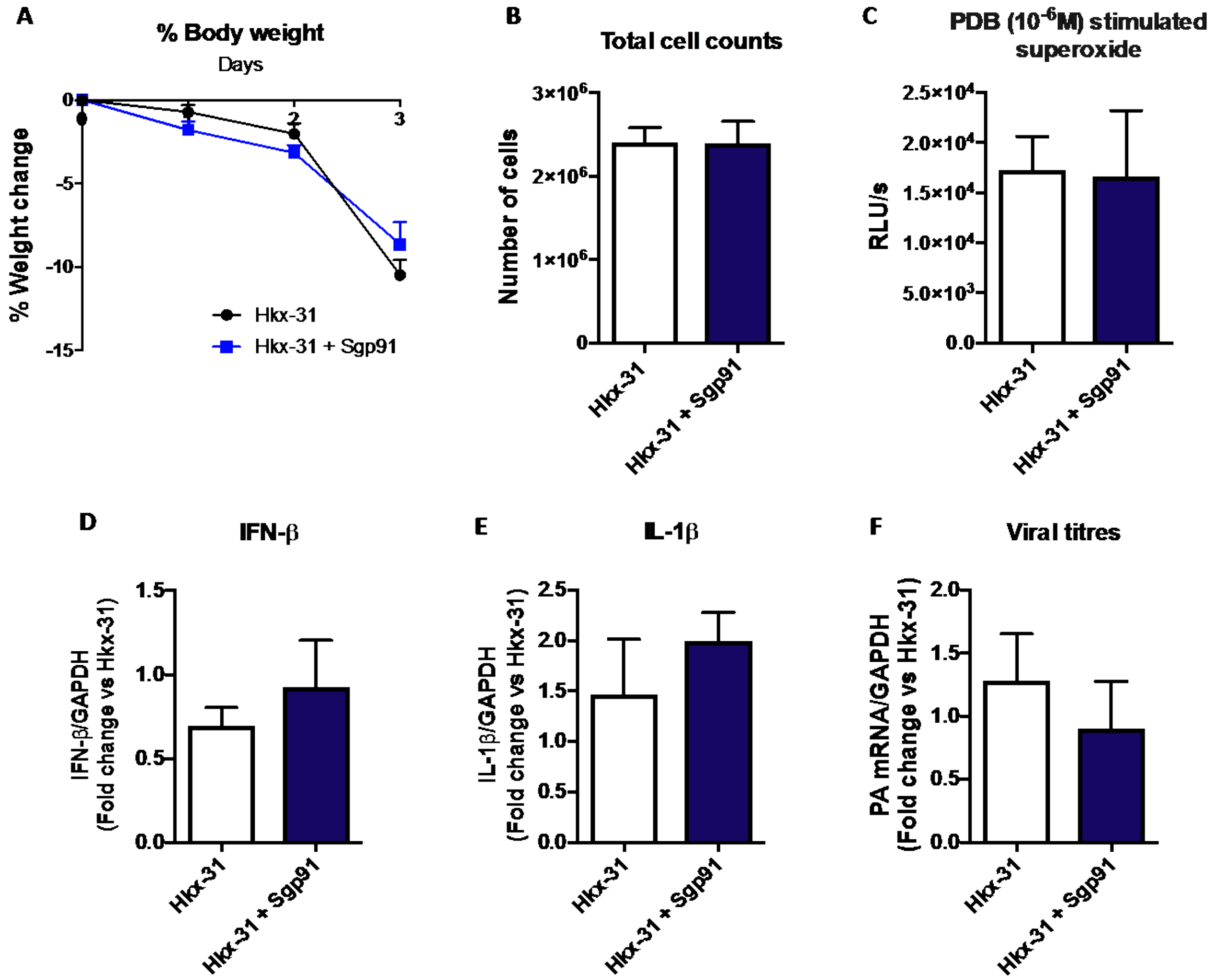
```
[Salmo      RGHPVISLDLELQACQHNYLSIILYILLTSLVLSFVTLPISSHLFLWDVWYLYHFLLAKF 892
[Xenopus    RGQSLVLLDL--YTCEQYHLNLILQALSASFIICLMVVSVSSHLFYWDFWFIYHLFKAKI 885
[Gallus     KGRSLVFLDL--NTCELDTSYFIMYALSTSAVLCLMMFAVMSHLYFWDVWYSYHYCTAKL 888
[Mus        KGQSVISLDL--YTCELDLTNLILFSVSISSVLFLMVVMTTSHLFFWDMWYIYYFWKAKI 879
[Rattus     KGQSVISLDL--YTCELDLTNLILFSVSISSVLFLMIVMTTSHLFFWDMWYIYYFWKAKI 879
[Homo       KGQSVISLDL--YTCELDLTNLILFSLSISVSLFLMVMMTASHLYFWDVWYIYHFCKAKI 878
[Sus        KGQSVVSLDL--YTCELDLTNFVLFSLSLSAVLFLIVITIANHLYFWDVWYSYHFCKAKI 879
[Bos        KGQSVVSLDL--YTCELDLTNFILFSLSISAVLSLMMITIANHLYFWDVWYSYHFCKAKI 887
            :*: ::                  ::: :  *  : :: .   .**:     *:   **:

                      C8898 C890                                C927
[Salmo      KGYRRLSSPSAAYDAFVVYDKKDPEVSEWVLKELLVQLEEHGDHPLQICLEERDWIPGCP 952
[Xenopus    HGYKRF--PKCCYDALIMYDTKDSAVSDWVFNDLVNILEKQGNKMLNICLEERDFLAGQP 943
[Gallus     KGYRRIPLPIACYDAFIAYDNTDLAVNEWVMTELVEKLEDQKARQFNICLEERDWLPGQP 948
[Mus        KGYQHLQSMKSCYDAFIVYDTKNSAVTEWVLQELVAKLEDPREKHFNICLEERDWLPGQP 939
[Rattus     KGYQHLQSMKSCYDAFIVYDTKNSAVTEWVLQELVVKLEDPREKHFNICLEERDWLPGQP 939
[Homo       KGYQRLISPDCCYDAFIVYDTKDPAVTEWVLAELVAKLEDPREKHFNICLEERDWLPGQP 938
[Sus        KGYQRLISPNSCYDAFIVYDTKDPAVTEWVLDELVAKLEDPREKHFNICLEERDWLPGQP 939
[Bos        KGYRRLISPNSCYDAFIVYDTKDPAVTEWVLDELVAKLEDPREKCFNICLEERDWLPGQP 947
            :**:::   . . ***:: **..:  *.:**: :*:  **.    : ::********:: * *

[Salmo      LIDNLSQSIHQSKRTVFILTNKYIKSGDFKTAFYMAHQRLMDERDDVIVLIFLEKVPSHS 1012
[Xenopus    FLDNLSESIQISRKTVFVLTRKYVKKGHFKTAFYMAHQRLIEEKVDVIILILLEKTLQRS 1003
[Gallus     VFDNLSQSIQLSKKTIFVLTNKYIKSGTFKTTFYMAHQRLLDEKIDVIILIFLEKVLQKS 1008
[Mus        VLENLSQSIQLSKKTVFVMTQKYAKTESFKMAFYLSHQRLLDEKVDVIILIFLEKPLQKS 999
[Rattus     VLENLSQSIQLSRKTVFVMTQKYAKTESFKMAFYLSHQRLMDEKVDVIILIFLEKPLQKS 999
[Homo       VLENLSQSIQLSKKTVFVMTDKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPFQKS 998
[Sus        VLENLSQSIQLSKKTVFVMTDKYAKTEKFKIAFYLSHQRLMDEKVDVIILIFLEKPLQKS 999
[Bos        VLENLSQSIQLSKKTVFVMTDKYAKTENFKIAFYLSHQRLMDEKVDVIILIFLEKPLQKS 1007
            .::***:       :*:*::* ** *.       *::****::*: ***:**:***   .:*

                     C1008                C1029
[Salmo      KYLRLRKRLYRRSVIEWPTNPQAQQYFWKSLRSVLVTDSQKQYSNLFKETR      1063
[Xenopus    RYLRLRKRLCANSVLYWPSNPNSQSYFWHCLKSAIATENQMAYDKLFKDHT      1054
[Gallus     RYVQLRKRLCRSSVLEWPTNPRSQPYFWQRLKNAIAMNNTLSYNKLLQETV      1059
[Mus        KFLQLRKRLCRSSVLEWPANPQAHPYFWQCLKNALTTDNHVAYSQMFKETV      1050
[Rattus     KFLQLRKRLCSSSVLEWPTNPQAHPYFWQCLKNALTTDNHVAYSQMFKETV      1050
[Homo       KFLQLRKRLCGSSVLEWPTNPQAHPYFWQCLKNALATDNHVAYSQVFKETV      1049
[Sus        KFFQLRKRLCGSSVLEWPTNPQAHPYFWQCLKNALATDNHVTYSQVFKETA      1050
[Bos        KFLQLRKRLCGSSVLEWPTNPQAHPYFWQCLKNALATDNHVTYSQVFKETA      1058
            ::.:*****    **: **:**.:: ***  *:..:. :.   *.:::::
```

Supplementary Figure 10. Multiple sequence alignment analysis of vertebrate TLR7. Individual sequences of TLRs were obtained from NCBI GenBank protein databases with the following accession numbers *Salmo salar* (**CCX35457.1**), *Xenopus tropicalis* (**AAI66280.1**), *Gallus gallus* (**ACR26243.1**), *Mus musculus* (**AAI32386.1**), *Rattus norvegius* (**NP_001091051.1**), *Homo sapiens* (AAZ99026.1), *Sus scrofa* (**ABQ52583.1**) and *Bos taurus* (**NP_001028933.1**) and then sequence alignment was performed with CLUSTAL OMEGA (EMBL-EBI). Shown in red dotted rectangular boxes are the cysteines on human TLR7 and the respective position indicated.



Supplementary Figure 11. Influenza A virus causes interferon regulatory factor 7 (IRF-7) nuclear translocation *via* a TLR7-dependent mechanism.

(A) Immortalized bone marrow macrophages from WT or TLR7$^{-/-}$ mice were either left untreated or infected with HKx31 influenza A virus (HKx31, MOI of 10) for various times as indicated and then labeled with the IRF-7 antibody and 4',6'- diamidino-2-phenylindole (DAPI). Images are representative of >100 cells analyzed over 4 separate experiments. Original magnification X100. All data are represented as mean ± SEM. One-way ANOVA followed by Dunnett's *post hoc* test for multiple comparisons. *P<0.05 compared to WT controls. #P<0.05 - comparison indicated by horizontal bar.



Supplementary Figure 12. Scrambled cholestanol-conjugated gp91ds-TAT failed to influence influenza A virus dependent pathology in vivo.

Scrambled gp91ds-TAT (Sgp91; 0.02mg/kg/day) was delivered intranasally to WT mice once daily for 4 days. At 24h after the first dose of inhibitor, mice were infected with HKx31 influenza A virus (1×10^5 PFU per mouse). Mice were culled at day 3 post-infection and **(A)** body weight, **(B)** airway inflammation was assessed by BALF cell counts, **(C)** superoxide was measured from BALF inflammatory cells via L-O12 (100μM)-enhanced chemiluminescence, (D and E) lung IFN-β and IL-1β mRNA, respectively, was determined by QPCR and **(F)** viral load was determined byQPCR. All data are represented as mean ± SEM, n=8.

TLR7-H	C36	C51	C98	C100	C112	C183	C189	C260	C263	C270	C273	C445	C475	C491	C521	C697	C721	C787	C789	C814	C833	C874	C889	C890	C927	C1008	C1028	Accession
TLR1-H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes	Yes	Yes	Yes	-	Yes	-	-	Yes	-	-	CAG38593.1
TLR2-H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes	-	Yes	Yes	Yes	-	-	-	Yes	Yes	-	-	AAH33756.1
TLR3-H	-	-	-	-	-	-	-	-	Yes	-	-	-	-	-	-	-	-	Yes	Yes	Yes	yes	-	-	-	Yes	-	-	ABC86910.1
TLR4-H	-	-	-	-	-	-	-	-	Yes	-	-	-	Yes	-	Yes	-	-	Yes	Yes	Yes	Yes	-	-	-	Yes	-	-	AAF07823.1
TLR5-H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	-	-	AAI09119.1
TLR6-H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes	Yes	Yes	Yes	-	Yes	-	-	Yes	-	-	BAA78631.1
TLR8-H	Yes	Yes	-	-	-	Yes	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	-	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	-	AAZ95441.1
TLR9-H	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	-	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	-	AAZ95520.1
TLR10-H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes	-	Yes	Yes	Yes	-	-	-	-	Yes	-	-	AAY78491.1
TLR7 *Mus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	Yes	Yes	AAI32386.1
TLR7 *rattus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-	Yes	Yes	Yes	Yes	NP_001009151.1
TLR7 *Sus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	ABQ52583.1
TLR7 *Bos taurus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	NP_001028933.1
TLR7 *Xenopus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	AAI66280.1
TLR7 *Gallus*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	-	ACR26243.1
TLR7 *Salmo*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-	-	Yes	-	-	CCX35457.1

Supplementary Table 1. Pairwise sequence alignment analysis of human TLR7 vs other members of the human TLR family and vs vertebrate TLR7. Individual sequences of TLRs were obtained from NCBI GenBank protein databases with the indicated accession numbers and then sequence alignment was performed with Pubmed NCBI BLAST.

Gene	Company	Gene expression assay	Catalog no.	Ref Seq
Mouse IL-1β TaqMan Primer	Applied Biosystems	Mm00434228_m1	4331182	NM_008361.3
Mouse CYBB TaqMan Primer	Applied Biosystems	Mm01287743_m1	4331182	NM_007807.5
Mouse IFNB1 TaqMan Primer	Applied Biosystems	Mm00439552_s1	4331182	NM_010510.1
Mouse TNFα TaqMan Primer	Applied Biosystems	Mm00443258_m1	4331182	NM_001278601.1
Mouse IL6 TaqMan Primer	Applied Biosystems	Mm00446190_m1	4331182	NM_031168.1
Mouse TLR7 TaqMan Primer	Applied Biosystems	Mm00446590_m1	4331182	NM_016562.3
Mouse GAPDH (X20)	Applied Biosystems		4352339E	
Influenza polymerase forward primer	Applied Biosystems	5′-CGGTCCAAATTCCTGCTGA-3′		
Influenza polymerase reverse primer	Applied Biosystems	5′-CATTGGGTTCCTTCCATCCA-3-3′		

Supplementary Table 2: List of primers and their sources and reference sequences. The influenza polymerase primers were custom synthesized and the sequences are shown.