Supporting Information

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SI Materials and Methods

Production of hCsp-12L Transgenic Mice. XhoI-digested BAC (AP002004) DNA was resolved on a 1% regular agarose pulsed-field gel electrophoresis. The 107-kb band was isolated and digested with β-agarase, and the solution was dialyzed overnight against the injection buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, and 100 mM NaCl). Fertilized oocytes (1-cell stage) were collected from superovulating F_1 (C57BL/6 × CBA) 4-week-old female mice. A total of 5 ng/mL DNA were microinjected into the pronuclei and surviving zygotes were cultured overnight till the 2-cell stage and then transferred into a 0.5-day pseudopregnant female recipient. A total of 20–30 microinjected zygotes were transferred into each pseudopregnant recipient.

Southern Blot Analysis. To prepare the Southern probe, gDNA was extracted from human blood with the DNeasy Tissue kit (QIAGEN, no. 69504) and a 623-bp fragment was amplified using the primers listed in Table S1. The PCR amplicon was labeled with ^{32}P-dCTP (Perkin−Elmer) and used as a probe. A total of 10 μg gDNA extracted from mouse ear notches or HEK293T cells were digested with 40 units/μL of SpeI at 37 °C overnight. DNA fragments were resolved on 0.7% agarose gel electrophoresis and then transferred on nitrocellulose membrane overnight. The membrane was hybridized with the radioactive probe using a standard protocol.

Mouse Transgenic Mapping Using Sequential G-Banding to FISH. Fibroblast cultures (from mouse ear biopsy) grown in alpha-MEM medium with 15% FBS were exposed for 30 min to Colcemid (final concentration 0.05 μg/mL) and harvested according to standard cytogenetic protocol, using hypotonic 0.075-M KCl and Carnoy's fixative. Metaphase preparations were made by dropping the fixed cell suspension onto precleaned slides in a Thermotron and aged overnight at 55 °C before G-banding using Pancreatin followed by staining with Leishmann/Giemsa stain. Ten metaphase spreads were imaged using bright field microscopy and the coordinates of these were recorded for metaphase relocation following FISH. Before doing FISH, slides were destained with Carnoy's fixative, rehydrated with an ethanol series, pretreated with 2× SSC at 37 °C, and then postfixed with 1% formaldehyde/PBS/$MgCl_2$ and washed in PBS. Metaphases were denatured for ≈30 s in 70% formamide/SSC at 70 °C and dehydrated with ethanol. Genomic DNA was isolated from BAC clones and labeled with Spectrum Green (green, RP23–440M12, mouse chromosome 6 control probe) and SpectrumOrange (red, AP002004, transgene). Probes were denatured for 5 min at 75 °C and hybridized to slides overnight at 37 °C. Following hybridization, slides were washed and incubated with detection solution for 30 min at 37 °C, rinsed, and stained with DAPI. Previously imaged G-banded metaphases were relocated, examined using fluorescence microscopy, and reimaged.

***Listeria monocytogenes* Challenge and Estradiol Treatment of Mice.** For in vivo infections, *Listeria monocytogenes* strain 10403s belonging to serotype 1 and resistant to streptomycin (a generous gift from D. Portnoy, University of California) was grown to midlogarithmic phase in tryptic soy broth supplemented with 50 μg/mL streptomycin (OD_{600} of 0.1 = 4 × 10^8 CFU/mL). Approximately 5 × 10^4 CFU in 200 μL PBS were injected into the lateral tail vein. For in vivo experiments with estradiol (E2), mice received a daily i.p. injection of E2 (50 μg/kg, Sigma no. E8875) diluted in 200 μL corn oil.

Determination of Bacterial Content. On days 1, 2, 3, and 4 pi, spleens and livers were collected and were homogenized in a 10-mL volume of a 0.2% Nonidet P-40 solution, using the homogenizer Power Gen 35 (Fisher Scientific). Serial dilutions (1:10, 1:100, 1:1,000) were prepared in 0.2% Nonidet P-40 solution, plated on tryptic soy agar containing streptomycin (50 μg/mL), and incubated for 24 h in a 37 °C incubator. Bacterial colonies were enumerated and the number of CFUs per milliliter was calculated.

Splenocyte Culture, Treatment, and Infection. Splenocytes derived from male and female *casp-12*$^{+/+}$, *casp-12*$^{-/-}$, and hCsp-12L/*casp-12*$^{-/-}$ mice were isolated by mechanical disruption followed by passage through a 70-μm sieve to remove clumps. Cells were then centrifuged at 1500 rpm for 10 min at 4 °C and were resuspended in RPMI-1640 medium supplemented with 10% FCS, 11.2 units/L penicillin, 11.2 μg/L streptomycin, and 2 mM glutamine. Cells were treated for 1 h with 1 μg/mL *Escherichia coli* LPS (serotype 055:B5; Sigma no. L6529) and RNA was extracted with TRIzol following the manufacturer's instructions. IL-1β, TNFα, and IL-12 levels were quantified by qRT-PCR using primers listed in Table S1. For mouse IL-1β production, cells were primed overnight with 50 ng/mL *E. coli* LPS (serotype 0111:B4; Sigma no. L4391) and then infected for 3 h with *L. monocytogenes* strain 10403s at a multiplicity of infection of 50 (bacteria:cell). Bacteria were cultured standing till an OD_{600} of 0.1, which corresponds to 4 × 10^8 CFU/mL. Ninety minutes pi, 20 μg/mL of gentamicin were added on infected wells to kill extracellular bacteria. Culture supernatants were then collected and assayed for IL-1β by ELISA (Biosource, no. KMC0011).

RNA Extraction, PCR, and Quantitative RT-PCR. Total RNA was extracted from HeLa cells, splenocytes, or different mouse organs using the TRIzol reagent (Invitrogen, no. 15596018) followed by chloroform extraction and isopropanol precipitation. Two micrograms total RNA were reverse transcribed to cDNA using M-MLV reverse transcriptase (Invitrogen, no. 28025013), using random hexamers (Invitrogen, no. 48190011) in a volume of 20 μL according to the manufacturer's protocol. PCR of hCsp-12 and Upf1 was performed in a total volume of 50 μL containing 5 μL 10× TaqPCR reaction buffer (Roche Diagnostics, no. 1418432), 1 μL dNTP mix at 2.5 mM each (Roche Diagnostic, no. 11277049001), 0.5 μL primers at 50 μM each, 2–7.5 μL cDNA, and 0.5 μL TaqDNA Polymerase (Roche Diagnostics, no. 1418432). Amplification was made in a thermocycler with the following conditions: 94 °C for 5 min; 45 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min; and then 72 °C for 7 min. For hCsp-12L genotyping, PCR was performed as described above using 10 μL of cDNA and an annealing temperature of 58 °C. As for the mouse caspase-12 mutation, it was genotyped as described in ref. 1. In all experiments, PCR of mouse glyceraldehyde-3-phosphate dehydrogenase (mGADPH) and human β2-microglobulin was carried out to ensure that equal quality and quantity of cDNA were used. For qRT-PCR, iTaq SYBR green supermix (Bio-Rad, no. N8080247) was used following the manufacturer's instructions. PCR and qRT-PCR primers are described in Table S1.

Assessment of hCsp-12S mRNA Decay. HeLa cells (5×10^4) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 11.2 units/L penicillin, 11.2 μg/L streptomycin, and 2 mM glutamine in 24-well plates and incubated overnight at 37 °C, 5% CO_2 in a humidified chamber. Cells were treated with 10 μg/mL of cycloheximide (CHX; Sigma, no. C4859) for different time points (0–180 min). RNA was extracted from cells and reverse transcribed to cDNA, and semiquantitative RT-PCR was then performed using specific primers for hCsp-12 listed in Table S1.

siRNA-Mediated Knockdown of Human Upf1. HeLa cells (3×10^5) were grown in DMEM containing 10% FBS, 11.2 units/L penicillin, 11.2 μg/L streptomycin, and 2 mM glutamine in 6-well plates and transiently transfected with 100 nM of siRNA [Dharmacon: D-011763–01, 5′-r(UAC UGC ACC AGG UAG GAG CUU) d(TT)-3′; and D-011763–03, 5′-r(UCA AAG GAG GUC GAC UUC CUU) d(TT)-3′] using the oligofectamine reagent (Invitrogen, no. 12252011). Nonsilencing siRNA was used as a negative control (QIAGEN, no. 1022076). Forty-eight hours posttransfection, RNA was extracted from cells and reverse transcribed to cDNA, and semiquantitative RT-PCR was then carried out with primers for UPF1 and hCsp-12 listed in Table S1.

Preparation of Protein Extracts from Organ Homogenates for Western Blot Analysis. Various tissues were collected from a humanized mouse, homogenized using Power Gen 35 (Fisher Scientific) in lysis buffer (2% SDS, 50 mM Tris-HCl, 10 mM EDTA, PBS pH 7.4–7.6), and sonicated 3 times for 30 s on ice using the homogenizer Vibra Cell (Sonics) at 40% intensity. Protein extracts were heat denatured at 95 °C for 20 min and then centrifuged at 17,000 $\times$ *g* for 20 min. Protein concentrations were determined by the BCA protein assay kit (Perkin−Elmer, no. PI23227). A total of 20 μg total protein were used for Western analysis with monoclonal anti-caspase 12 antibodies raised in rat (Sigma, no. C7611). HCsp12-L was in vitro transcribed and translated using TNT-coupled reticulocyte lysates (Promega, no. PRL4610) and was used as a positive control for Western analysis.

Chromatin Immunoprecipitation (ChIP). Spleens from hCsp-12L/*casp-12*$^{-/-}$ male and female mice injected or not with E2 were collected in 5 mL ice-cold PBS, homogenized for 5 s, and centrifuged at 2,000 rpm for 2 min. Pellets were resuspended in 10 mL PBS supplemented with protease inhibitor mixture tablets (Roche Diagnostics) and 1% formaldehyde. Samples were cross-linked for 10 min at room temperature, washed twice with ice-cold PBS, and centrifuged. Pellets were incubated in nucleus lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, supplemented with protease inhibitors) for 15 min and sonicated 6 times for 8 s using a VirSonic 100 (Virtis) sonicator. After centrifugation at 14,000 rpm to remove debris, soluble chromatin was diluted 10-fold in chromatin immunoprecipitation dilution buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1, supplemented with protease inhibitors) and salmon sperm DNA/protein A-agarose (Upstate, no. 16–157) was added. After 1 h rotation at 4 °C, samples were centrifuged at 4,000 rpm and chromatin was immunoprecipitated overnight using 2 μg of specific anti-mouse ERα polyclonal antibodies (Santa Cruz, no. sc-542). Following immunoprecipitation, 40 μL salmon sperm DNA/protein A-agarose was added and incubated for 3 h. The precipitates were washed sequentially for 10 min each with buffer I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), with buffer II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM HCl, pH 8.1, 500 mM NaCl), and with buffer III (0.25 mM LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). Precipitates were then resuspended in 1 mL TE buffer (10 mM Tris, pH 8.5, 1 mM EDTA) and, after a quick centrifugation, eluted with 150 μL elution buffer (1% SDS, 0.1 M NaHCO3). The eluates were incubated at 65 °C for 6 h. The DNA fragments were purified using a QIAquick PCR purification kit (QIAGEN) and qRT-PCR was performed as described above using specific primers for the region containing the ERE in the human *caspase-12* gene. Enrichment of the ERα was normalized against the negative control region located 4 kb upstream of the initiation start site of the *Esrra* gene.

Statistical Analysis. Statistical analysis was performed using ANOVA and an unpaired Student's *t* test.

1. Saleh M, et al. (2006) Enhanced bacterial clearance and sepsis resistance in caspase-12 deficient mice. *Nature* 440:1064–1068.

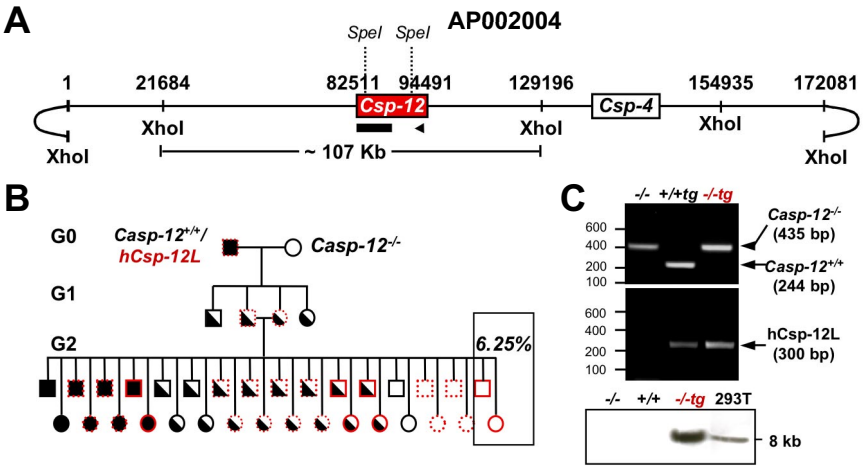


Fig. S1. (*A*) A representation of the BAC clone used to isolate the *Csp-12* transgene. A 107-kb XhoI fragment containing *Csp-12* with the rare polymorphism was used to generate the founder transgenic mouse. (*B*) Schematic diagram of the breeding strategy used to obtain the humanized mouse. Dashed red line, +/− for Csp-12L; solid red line, +/+ Csp-12L; squares, males; circles, females; black, wild type; white, knockout. (*C*) *Top*, genotyping of mouse *caspase-12* by PCR: 435 bp, *casp-12*$^{-/-}$; 244 bp, wild type. *Middle*, genotyping of human *Csp-12L* by PCR: the presence of the human transgene is detected by a 300-bp amplicon. *Bottom*, genotyping of human *Csp-12L* by Southern blot. HEK293T gDNA was used as a positive control.

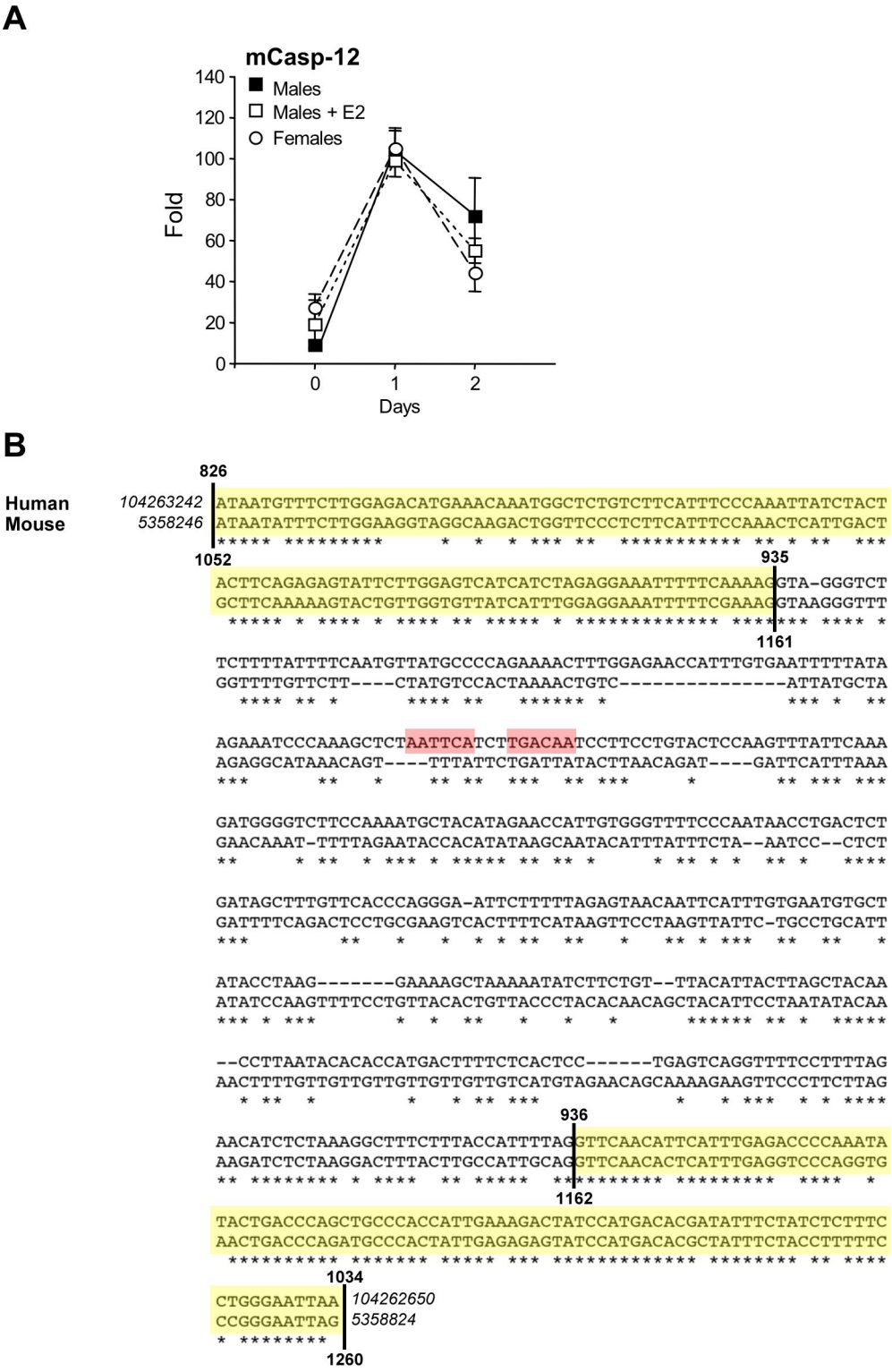
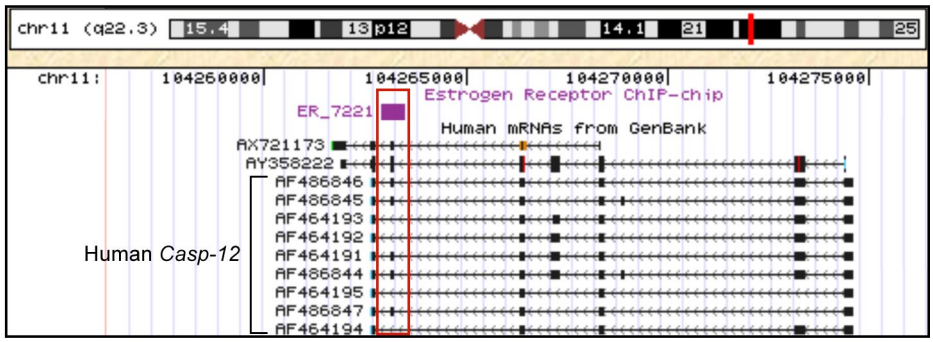


Fig. S2. (*A*) Mouse caspase-12 expression kinetics during infection with *L. monocytogenes.* Expression in the spleen of wild-type males ($n = 4$), females ($n = 4$), or males injected with E2 ($n = 4$) was determined on days 0, 1, and 2 pi. Data represent average expression ± SEM from 4 different mice. (*B*) Sequence alignment of human and mouse *caspase-12* genomic DNA (positions: human chr11, 104,263,242–104,262,650; mouse chr 9, 5,358,246–5,358,824). Exons are shown in yellow. Exons 7 and 8 of human *caspase-12* correspond to exons 9 and 10 of mouse *caspase-12*. The cDNA nucleotide numbers corresponding to the beginning and the end of the exons are shown linked by vertical black lines. The putative ERE site embedded in intron 7 of human *caspase-12* is highlighted in red.

A



B

5’ ATGAATTGTTACTCTAAAAAGAATTCCCTGGGTGAACAAAGCTATCAGAGTCAGGTTATTGGG
AAAACCCACAATGGTTCTATGTAGCATTTTGGAAGACCCCATCTTTGAATAAACTTGGAGTACAG
GAAGGA**TTGTCA**AGA**TGAATT**AGAGCTTTGGGATTTCTTATAAAAATTCACAAATGGTTCTCC
AAAGTTTTCTGGGGCATAACATTGAAAATAAAAGAAGACCCTACCTTTTGAAAAATTTCCTCTAG
ATGATGACTCCAAGAATACTCTCTGAAGTAGTAGATAATTTGGGAAATGAAGACAGAGCCATTTG
TTTCATGTCTCCAAGAAACATTATCTGCAAATAAAAGGCATCAAAATATTGGAGGATGTGATCTT
TTATACATGTGGAAGACTCCTGGAGACATAACTTTGGGAAAAAAAATCTGATTTTGTTTCTTTGG
AGAAGAGAGGGAAACCAATGCTAAATAAAGATGGACCTCCAACTTCCATACCAGGCCCAGAAAA
AGCCATCATGGACCTTCCTCACTCATAAATCACCTTGATTTTCTAGTAGGCTAGACCGAAGTGAT
ATCCTCTGGGTTTGCAAGTAG 3’

Fig. S3. Genomewide analysis of estrogen receptor binding sites identifies an ERE in the human *caspase-12* gene. (*A*) The image was obtained from JS Carroll et al. [Carroll et al. 2006], using the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) with the May 2004 version of the human genome. The position of the human *caspase-12* locus on chromosome 11 is shown. The ERE site is represented by a purple box. (*B*) Sequence of the human *caspase-12* (intron 7 position: chr11, 104,262,898–104,263,498) bound by human ERα. The putative ERE is shown in red.

Table S1. Primers used in the study

	Forward primer	Reverse primer
	Human gene	
L32	TGTCCTGAATGTGGTCACCTGA	CTGCAGTCTCCTTGCACACCT
Csp-12 (tissue expression)	GTGAGCAATGCTGAAAACCTG	CTTGCATGATGATGACCTTGG
Csp-12 (NMD)	GCGGCTCGAGATGGCTGATGAGAAACCATCCAACGG	TCACATCCCCAAAAGGTCAAGTTC
Csp-12L (genotyping)	GTCATTCTGTGTGTATTAAT	CTATAATATCATACATCTT
Csp-12 (Southern blot probe)	GTGAGCAATGCTGAAAACCTG	GGGGATGATCAAGGTATTTTGTG
Csp-12 (ChIP)	GAATTCCCTGGGTGAACAAAGCTATCAG	GTTATGCCCCAGAAAACTTTGGAG
β2-microglobulin	CTCACGTCATCCAGCAGAGA	TCTTTTTCAGTGGGGGTGAA
Upf1	CGACGCGCAGGTTGGGCC	GCAGAAGGGTTTTCCTTCCAC
IL-8	CTGGCCGTGGCTCTCTTG	CTTGGCAAAACTGCACCTTCA
	Mouse gene	
mL32	GAAACTGGCGGAAACCCA	GGATCTGGCCCTTGAACCTT
mGAPDH	TGAAGGTCGGTGTGAACGGATTTGGC	CATGTAGGCCATGAGGTCCACCAC
mTNFα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
mIFNγ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
mIL-1β	CGGCACACCCACCCTG	AAACCGCTTTTCCATCTTCTTCT
mIL-12	GAGCACTCCCCATTCCTACT	CCCTCCTCTGTCTCCTTCAT
mIL-10	AATAACTGCACCCACTTCCCA	GAAGGCAGTCCGCAGCTCT
mERRα (ChIP negative control)	TTGGCATTGATATTGGGGGTGGGAGCAACT	GACTTCTTACTTTGACGCTTTCCTCCATCG
mCasp-12 (expression)	CAGCTCAGGAAATGGAGACA	CCACAGATTCCTTCCAGGAT
mCasp-12 (genotyping)	CAGCTGTTCCTGGGAATTGGCAATG	GCCAGGAGGACACATGAAAGAGATC
mCasp-12 (genotyping)	CAGCTGTTCCTGGGAATTGGCAATG	GGGTGGGATTAGATAAATGCCTGCTCT