Primer Table

Primer ID	Sequence
372	ATCAAGACTGAGTTGATTTCTGTGTCTGAAGTTCACCCTTCTAG
374	GAAATCAACTCAGTCTTGATATATCCAGTTC
393	CGGATCCCGGGTCGACCCGATGTCTCAGTGGTACGAACTTCA
394	TCCTCTAGTGCGGCCGCCTACTTGTCGTCATCGTCTTTGTAGTCTACTGTGTTCATCATACTGTCGAAT
395	GAGGATATCAAGATCTAGAGGGTATATAATGGAAGCTCGACTTCCAGAAGCTTCCCGGGCAATCCGGTACTGTTGGTAAAGCC
397	TCGAAGCGGCCGGCCAAGCTTCCGACTCTAGAGTCGCGGC
402	ACGCCGGCCTGGACGCCATGAAAG
403	CGTCCAGGCCGGCGTCGTGG
405	CGGATCCCGGGTCGACCCACTAGTCCACCATGGCCTC
406	TCCTCTAGTGCGGCCGCGCTCGAGATCTCAGGAACAGGTG
418	CGGATCCCGGGTCGACTCCGCGCCATGTTCCAGGC
419	TCGTCTTTGTAGTCGCCTAACGTCAGACGCTGGCCTCC
420	CGGATCCCGGGTCGACTCCGCGCCATGTTCCAGGC
421	TCCTCTAGTGCGGCCGCCTACTTGTCGTCATCGTCTTTGTAGTCGCGACCGCCATACATCTGCTTGAACTGAAAGC
422	CGGATCCCGGGTCGACCCCAAATGGCGCAGTGGGAAATG
423	TCCTCTAGTGCGGCCGCTGGTTCCTAGAAGTCAGAAGGCATC
424	CGGATCCCGGGTCGACGGATGGCATCAGGCAGGGCA
425	TCCTCTAGTGCGGCCGCCCCAGGCTCTACACCAGGG
442	TGGCAAGCTAGGATCCTAACTGGCCGGTACCTGAG
443	TCGTCTTTGTAGTCGCGACTCTAGAGTCGCGGC
444	CGACTACAAAGACGATGACGACAAGTAG
445	GATCCTAGCTTGCCAAACCTACAGGTG

All clones were created using In-fusion HD Cloning Kit (Clontech, Madison WI) and were verified by sequencing (UAB Heflin Center Genomics Core Lab, Birmingham, AL). Primers were designed according to the In-Fusion cloning system recommendations (IDT, Coralville, IA and Sigma-Aldrich, St. Louis, MO).

Construction of STAT1 lentivector

To overexpress STAT1 via a lentivector, pCK2134 was created and encodes the MND promoter driven wildtype STAT1α linked to IRES-driven hygromycin expression. In brief, STAT1α-WT was originally repaired from the STAT1 alpha Y701F pRc/CMV (Addgene, Plasmid #8701). This was accomplished by PCR-based site-directed mutagenesis using the primers 393 and 374 which amplified the large fragment 5' (encompassing the Y701F mutation). The small fragment, 3' of the mutation, was amplified with the primers 372 and 394 and incorporated the sequence encoding the FLAG epitope in the reverse primer (394). Next, the large and small fragments were combined to create the full-length STAT1α-FLAG by overlap extension of gel-purified small and large fragments, followed by PCR amplification with primers 393 and 394. The full-length fragment was inserted into the EcoRI/SalI cut pCK2125 (an intermediate cloning vector containing the IRES-Hygro cassette) to create pCK2127. Next, the STAT1-IRES-Hygro cassette in pCK2127 was PCR amplified using the primers 416 and 417 and cloned with the large fragment of DraIII/NotI digested vector pLV.mnd.CXCR4.2A.GFP to create the final STAT1-IRES-Hygromycin lentivector pCK2134.

Construction of STAT2, IRF9, and DsRed lentivectors

Plasmids containing human cDNA sequences were obtained for STAT2 (Transomics, Huntsville, AL) and IRF9 (Transomics, Huntsville, AL), as well as the expression vector for DsRed2 (Clontech, Mountain View,

CA). Cloning fragments were obtained via PCR amplification with the respective primer pairs: STAT2 (422 and 423), IRF9 (424 and 425), and DsRed2 (405 and 406). Each fragment was individually Infusion cloned with the large fragment of SalI/NotI digested pCK2134 to create the following lentivectors: pCK2140 (DsRed), pCK2145 (STAT2), and pCK2146 (IRF9).

Construction of ISRE and NFκB nanoluciferase reporter lentivectors

To create the ISRE and NFκB nanoluciferase reporter lentivectors, we first obtained the plasmid pNL1.2 expressing a PEST-linked nanoluciferase (Promega, Madison, WI) as well as the plasmids pGL4.45 and pGL4.32 (Promega, Madison, WI) which express firefly luciferase under the control of ISRE and NFκB promoter elements respectively. To replace firefly luciferase with nanoluciferase under the control of ISRE or NFκB promoter elements, the nanoluciferase gene was PCR amplified from pNL1.2 using the primers 395 and 397 and Infusion cloned with either the large fragment of FseI/BglII digested pGL4.45 or pGL4.32 plasmids to create pCK2129 (ISRE.Nluc) and pCK2132 (NFkB.Nluc), respectively. Finally, to place the reporter constructs within a lentivector, we obtained a promoterless lentivector-backbone using primers 444 and 445 and pCK2134 as a template. Finally, using primers 442 and 443, the promoter-gene cassettes (ISRE.Nluc and NFκB.Nluc) were PCR amplified from pCK2129 and pCK2132 and Infusion cloned individually with the lentivector-backbone to create the lentivectors pCK2138 (ISRE.Nluc) and pCK2139 (NFkB.Nluc).

Construction of IκBα-SR lentivector

To create the IκBα-SR lentivector, we first obtained a vector containing the cDNA for the human *NFKBIA* gene (GE Dharmacon, Lafayette, CO). Site-directed mutagenesis of S32A and S36A was accomplished by using PCR amplification of the whole vector with the primers 402 and 403 which incorporate the necessary base changes. The whole-vector PCR fragment was then subjected to Infusion cloning treatment to re-circularize the vector (pCK2142). The IκBα-SR gene fragment was PCR amplified with the primers 418 and 419.

The destination vector was based on an intermediate vector containing the gene *TNFAIP3* which was created by PCR amplification of the *TNFAIP3* gene from pUNO1-hTNFAIP3 (Invivogen) using the primers 420 and 421 (which incorporated a FLAG sequence with a unique 5' NruI restriction site) and Infusion cloning the fragment into the large fragment of SalI/NotI digested pCK2134 to create the vector pCK2141. To create the IκBα-SR lentivector, the IκBα-SR (with FLAG tag) gene fragment was Infusion cloned into the large fragment of SalI/NruI digested pCK2141 to create the vector pCK2143.