

## Supplementary materials and methods

### Vector construction and lentiviral vector preparation and infection

The dual-promoter lentiviral vector system was described elsewhere<sup>1</sup>. Destabilized ZsGreen ( $t_{1/2}$ =8-12hr) was amplified from plasmid pZsGreen1-DR (Clontech Laboratories Inc., Mountain View, CA) using primers AgeI-ZsGD-for:

ACAACAACCGGTATCCGCCACCATGGCCCAGT and ZsGD-SPA-XbaI-

rev:ACAACACTCTAGACACACAAAAACCAACACACAGATCTAATGAAAATAAAGA

TCTTTTATTGCTCGCGGCCGCC TACACATTGAT, and replaced SPA\_d2EGFP in vector

pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_d2EGFP to generate

pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD through restriction sites AgeI

and XbaI. Oligo nucleotides containing multiple cloning sites (MCS) RsrII, HpaI, AfeI, AsiSI,

EcoRI was further inserted upstream of ZsGD using EcoRI and ClaI restriction sites, resulting in

vector pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD\_MCS. NFκB response

element together with a minimum CMV promoter was obtained from vector pRE-

NFκB\_CMVmini\_d2EGFP (a gift from Dr. Arul Jayaraman, Texas A&M University,<sup>2-3</sup>) and

was cloned into pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD\_MCS through

restriction sites ClaI and AgeI and yielded vector

pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD\_RE-NFκB\_MCS. Vector

pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD\_MCS was used as a parental

vector to clone promoters and

pCS\_sMAR8\_HSVpA\_DsRed\_PGK\_cHS4\_Tactb\_SPA\_ZsGD\_RE-NFκB\_MCS was used as a

parental vector to clone response elements. The cloning sites and primers/oligos were listed in

**Table 1.**

All PCR reactions were carried out with Phusion™ High-Fidelity DNA Polymerase following manufacturer's protocols. For oligonucleotide annealing, sense and antisense oligonucleotides were mixed at 1:1 ratio to a final concentration of 50 µM and the mixture was incubated at 95°C for 30 sec, followed by stepwise cooling at 72°C for 2 min, 37°C for 2 min and 25°C for 2 min. All cloning products were confirmed by sequencing with ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA). All restriction enzymes and polymerase were ordered from New England Biolabs (Ipswich, MA).

Transfection was performed as described previously<sup>1</sup>. Virus was harvested 24hr post transfection, filtered through 0.45µm filter (Millipore, Bedford, MA), pelleted by ultracentrifugation (50,000g at 4°C for 2 hr) and resuspended in fresh medium or array buffer (array buffer as in <sup>4</sup>: 0.4 M HEPES, pH adjusted to 7.4 with KOH, 12.5 mg/ml of Trehalose (Calbiochem, Gibbstown, NJ), 6 µg/ml of protamine sulfate (Sigma, MO) and 1.23 M KCL (Sigma)). Titers of lentiviral preparations were determined using 293T/17 cells and ranged between 10<sup>7</sup>-10<sup>8</sup> IFU/ml.

To infect cells using lentiviral vectors, cells were placed in a 6-well plate at a density of 0.1 million cells/well the day before infection. The next day medium was removed and 800µl lentivirus (40x) in cell culture medium was added in the presence of 8µg/ml polybrene to initiate infection. Viruses were removed 6hr after infection and fresh cell culture medium was added.

### **Flow cytometry**

Infected HeLa cells were expanded in T-75 tissue culture flask and were passaged into 24-well plates (0.2 million cells/well) for cytokine treatment. After culturing in 24-well plates for two days, HeLa cells were treated with different cytokines (see Cell Culture and treatment section)

and were harvested for flow cytometry (FACSCalibur; Becton Dickinson, San Jose, California) at the indicated time points.

### Quantitative real-time PCR

Infected HeLa cells were expanded in T-75 tissue culture flask and passaged into 6-well plates (0.5 million cells/well). After culturing in 6-well plates for two days, HeLa cells were treated with different cytokines (see Cell culture and treatment section). Total RNA was isolated with RNeasy Mini Kit (Qiagen, Valencia, CA) from the treated cells at indicated time points. cDNA was obtained using Superscript III cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). cDNA aliquots were subjected to real-time PCR reactions using SYBR Green Kit (Bio-Rad, Hercules, CA). Primers for real-time PCR were listed in **Table 2**.

**Table 1: Cloning of promoter and transcriptional response elements**

Promoters/ REs	Cloning sites	Primers/Oligos	Source of inserts	References
pIL8	ClaI, AgeI	<b>ClaI-IL8F</b> :ACAACAATCGATGAAGTGTGATGACTCAGG; <b>AgeI-IL8R</b> :ACAACAACCGGTGAAGCTTGTGTGCTCTGC	Amplified from human gDNA	5
pIKBA	EcoRI, AgeI	<b>EcoRI-IKBAF</b> : ACAACAGAATTCCATCAGGTCGGCGTCCTTGG; <b>AgeI-IKBAR</b> : ACAACAACCGGTACGGACTGCTGTGGGCTCTG	Amplified from human gDNA	6
pTNFAIP3	ClaI, AgeI	<b>ClaI-TNFAIP3F</b> :ACAACAATCGATGGGGCGAGGGAGTTTCTCCG; <b>AgeI-TNFAIP3R</b> :ACAACAACCGGTGCCACGAAGACTGCAGACT	Amplified from human gDNA	7
pSAA1	ClaI, AgeI	<b>ClaI-SAA1F</b> :ACAACAATCGATGGACTGCAGGTGCACACTAC; <b>AgeI-SAA1R</b> :ACAACAACCGGTGTGCTGTAGCTGAGCTGCGG	Amplified from human gDNA	8
AP1	HpaI, BstBI	<b>APIF</b> :TGAGTCAGTGAGTCAGTGAGTCAGTGAGTCAGTGAGTCA GTGAGTCAGTGAGTCAG; <b>APIR</b> :CGCTGACTCACTGACTCACTGACTCACTGACTCACTGACT CACTGACTCACTGACTCA	oligos	SABiosciences Inc
RE-STAT1(2)	HpaI, BstBI	<b>STAT1F</b> :CATGTTATGCATATTCCTGTAAGTGCATGTTATGCATA TTCCTGTAAGTGCATGTTATGCATATTCCTGTAAGTG; <b>STAT1R</b> :CGCACTTACAGGAATATGCATAACATGCACTTACAGG AATATGCATAACATGCACTTACAGGAATATGCATAACATG	oligos	Panomics Inc

RE-STAT4	HpaI, BstBI	<b>STAT4F</b> :CTAGAGCCTGATTTCCCCGAAATGATGAGCTAGCTAG AGCCTGATTTCCCCGAAATGATGAGCTAG; <b>STAT4R</b> :CGCTAGCTCATCATTTTCGGGGAAATCAGGCTCTAGCT AGCTCATCATTTTCGGGGAAATCAGGCTCTAG	oligos	Panomics Inc
RE-GAS	HpaI, BstBI	<b>GASF</b> :AGGTTTCCGGGAAAGCAGTAGGTTTAGGTTTCCGGGAAA GCAGTAGGTTT; <b>GASR</b> :CGAAACCTACTGCTTTCCCGAAACCTAAACCTACTGCT TTCCCGAAACCT	oligos	Panomics Inc
RE-TGFB	HpaI, BstBI	<b>TGFBF</b> :AGCCAGACAAGCCAGACAAGCCAGACAAGCCAGACAA GCCAGACAAGCCAGACA; <b>TGFBR</b> :CGTGTCTGGCTTGTCTGGCTTGTCTGGCTTGTCTGGCTT GTCTGGCTTGTCTGGCT	oligos	SABiosciences Inc

**Table 2:** Primers for qRT-PCR

Accession number	Gene Name	primers
NM_006290	TNFAIP3	For: CAGGACTGCTTCATCGTCTT
		Rev: CTGAGCTTCCTTGAGCTTCT
NM_000331	SAA1	For: AGTGATCAGCGATGCCAGAG
		Rev: CTCCTGAGAGCAGAGTGAAG
NM_000584	IL8	For: GGCCAAGAGAATATCCGAAC
		Rev: CAAGGCACAGTGAACAAGG
NM_020529	IKBA	For: CTGGTGTCACTCCTGTTGAA
		Rev: ACTCCGTGAACTCTGACTCT

### Supplementary references

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