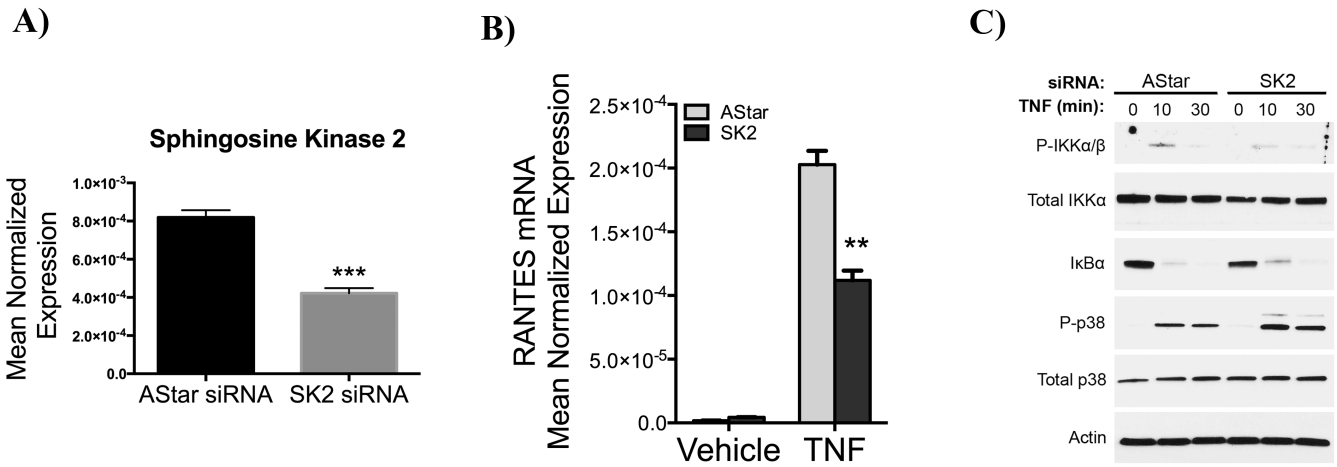

FIGURE S1

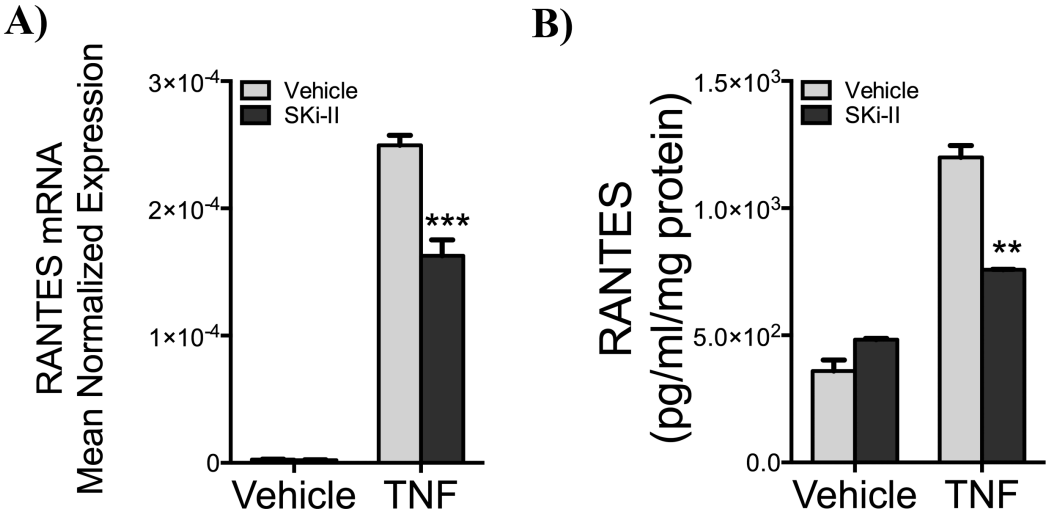

FIGURE S2

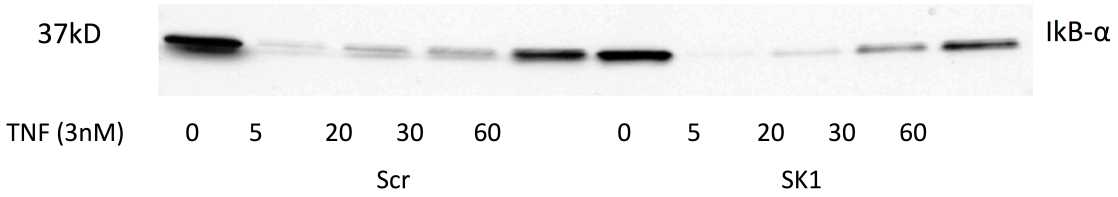

FIGURE S3

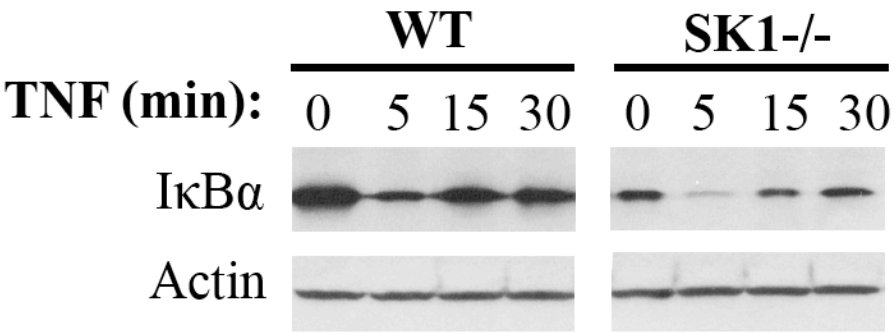

FIGURE S4

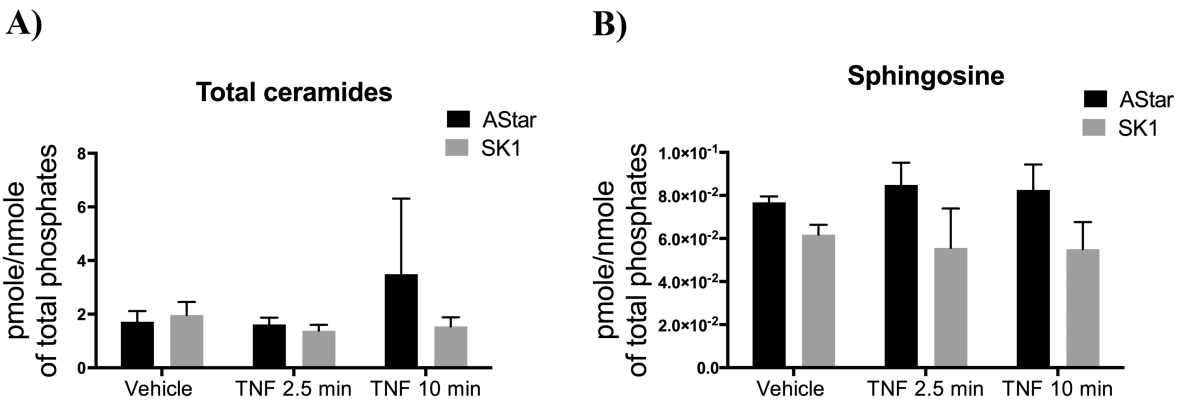

FIGURE S5

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Effect of SK2 on RANTES induction, acute NF-kB activation and p38 MAPK phosphorylation. A) HeLa cells were treated with 20nM Astar or SK1 siRNA for 48 h and SK1 mRNA levels were assessed by qRT-PCR. Data are presented as mean ± SEM from 3 independent experiments performed in duplicate. B) HeLa cells were treated with 20nM Astar or SK2 siRNA for 48h, treated with vehicle (PBS) or TNF (20ng/ml) for 24h and the levels of RANTES mRNA assessed by qRT-PCR. Data represent mean ± SEM from 3 independent experiments. C) HeLa cells were treated with 20nM Astar or SK2 siRNA for 48h, treated with vehicle (PBS) or TNF (20ng/ml) for 0, 10 or 30 minutes. Early downstream signals of TNF including IKKα/β phosphorylation, IκBα degradation, phospho-p38 and total p38 MAPK were assessed by immunoblot with actin as loading control. Each blot is representative of 2 independent experiments (** $p<0.01$, *** $p<0.001$)

Figure S2. Effect of the sphingosine kinase inhibitor Ski-II on RANTES mRNA and protein levels. Hela cells were incubated with DMSO or Ski-II (10uM) overnight prior to treatment with vehicle (PBS) or TNF (20ng/ml) for 24h. **A**) RANTES mRNA levels were assessed by qRT-PCR. Data shown are mean ± SEM of 3 independent experiments. **B**) Cells were stimulated in serum-free media and RANTES levels in the media were determined by ELISA. Data represent mean ± SEM of 2 independent experiments performed in duplicate (** $p<0.01$, *** $p<0.001$)

Figure S3: Loss of SK1 does not prevent IκB degradation in A549 cells. A549 cells were treated with 20nM negative control (Scr) or SK1 siRNA for 48h prior to stimulation with TNF (50ng/ml) as indicated. Protein lysates were prepared and equal amounts of protein analyzed for IκBα levels by immunoblot as described in 'Experimental Procedures."

Figure S4. The lack of requirement of SK1 for TNF-mediated activation of NF-κB is not due to clone specific effects. MEFs derived from WT or $SK1^{-/-}$ mice were treated with TNF (50ng/ml) as shown, and IκBα levels were assessed by immunoblot with actin as a loading control. The blot shown is representative of 3 independent experiments**.**

Figure S5. Effect of TNF on total ceramides and sphingosine in HeLa cells lacking SK1. HeLa cells were treated with 20nM Astar or SK1 siRNA for 48h prior to treatment with vehicle (PBS) or TNF (20ng/ml) for 2.5 or 10 min. Cellular lipids were directly extracted and **A**) Total ceramide levels and **B**) sphingosine levels were analyzed by tandem LC/MS mass spectrometry. Lipids were normalized to nmol of total lipid phosphate as described in Experimental Procedures. Data represent mean ± SEM of 2 independent experiments performed in duplicate.

	Fold increase upon TNF treatment with respect to Astar non-treated cells	
Cytokine/Chemokine	**Astar**	**SK1 siRNA**
ADIPOQ	1.52	1.75
BMP2	1.52	-1.13
BMP4	-1.33	-2.28
BMP6	1.51	1.74
BMP7	-1.33	3.55
C5	-1.26	-2.21
CCL1	-1.32	3.38
CCL11	3.04	7.05
CCL13	3.00	3.45
CCL17	-2.63	1.76
CCL18	3.00	6.95
CCL19	-1.33	1.75
CCL2	3.00	3.50
CCL20	11.99	27.63
CCL21	1.52	1.71
CCL22	6.08	6.91
CCL24	3.00	3.48
CCL3	1.50	1.74
CCL5	49.32	113.61
CCL7	1.51	3.45
CCL8	1.51	3.48
CD40LG	-1.33	1.75
CNTF	3.02	1.75
CSF1	3.04	7.05
CSF2	3.02	7.00
CSF3	5.91	3.45
CX3CL1	1.51	1.70
CXCL1	48.30	110.51
CXCL10	193.21	890.21
CXCL11	96.60	442.03
CXCL12	-2.69	1.74
CXCL13	1.52	-1.14
CXCL16	3.02	13.72
CXCL2	23.98	55.64
CXCL5	1.49	-1.15
CXCL9	1.52	1.74
FASLG	23.98	3.48
GPI	1.51	-1.17
IFNA2	1.51	1.76
IFNG	1.55	1.78
IL10	1.52	1.73
IL11	3.02	3.48
IL12A	3.00	3.45
IL12B	3.02	-1.16
IL13	3.04	1.76

IL15	1.50	1.71
IL16	1.50	1.74
IL17A	-1.33	-1.15
IL17F	2.98	1.73
IL18	1.51	1.74
IL1A	12.08	27.82
IL1B	6.00	13.81
IL1RN	6.04	13.72
IL2	1.49	1.71
IL21	-2.60	1.73
IL22	1.54	7.20
IL23A	3.04	3.45
IL24	1.52	3.53
IL27	-1.34	1.71
IL3	1.51	1.75
IL4	-1.32	-1.14
IL5	1.51	1.73
IL6	12.16	14.10
IL7	3.06	7.00
IL8	97.95	225.66
IL9	-1.33	1.71
LIF	3.04	3.50
LTA	1.52	-1.13
LTB	3.00	6.86
MIF	1.50	1.71
MSTN	3.30	3.50
NODAL	1.52	-4.93
OSM	1.50	-1.16
PPBP	-1.33	-1.13
SPP1	-1.33	1.73
TGFB2	1.50	1.74
THPO	1.49	1.74
TNF	12.08	27.82
TNFRSF11B	2.98	3.48
TNFSF10	3.02	13.81
TNFSF11	3.06	3.48
TNFSF13B	5.95	27.44
VEGFA	1.51	1.75
XCL1	5.87	-1.25
ACTB	-1.29	-1.13
B2M	1.53	1.75
GAPDH	-1.35	-2.33
HPRT1	1.50	1.73
RPLP0	-1.32	-1.15
HGDC	2.90	-1.18

Supplementary Table 1: Selected Cytokines and Chemokines fold changes upon TNF treatment of the array done on AStar or SK1 siRNA transfected cells.