SUPPLEMENTAL DATA

Experimental determination of the transcription start site of MICA

The transcription start site of MICA used to define the co-ordinates within the MICA gene in this study is underlined in the following sequence: TCTCCCCGGC**C**ACTGCTTGAG. This was determined experimentally by RNA ligase-mediated RACE (RLM-RACE) using total RNA of Hela cells and ExactSTART™ Eukaryotic mRNA 5´- & 3´-RACE Kit (Epicentre, Madison, WI).

Reporter constructs for the NF-κB site in MICA intron1

The MICA intron1 reporter plasmids pOC292 MICA- NF-κB -InF and pOC293 MICA- NF-κB -InR were constructed by cloning a PCR fragment corresponding to +450 to +692bp of MICA intron1 into the KpnI site of the pGL3-Promoter plasmid (pGL3P, Promega, Madison, WI).

Determination of MICA mRNA half-life

Primary human arterial endothelial cells were treated with actinomycin D (5μM) and harvested at different time points. MICA mRNA levels were analyzed by real-time PCR using equal amounts of cDNA from cells treated for 0, 2, 4, or 8h. Primer efficiency was determined using the standard curve, and relative MICA expression level was calculated taking into account the primer efficiency. The half-life of MICA mRNA was calculated by linear regression of the logarithm of the transcript level with respect to the time of actinomycin D treatment (Supplemental Figure 5).

Supplemental Table 1. Real-time PCR primers

Primer Name	**Primer sequence**
MICA forward	TCTTCCTGCTTCTGGCTGGCAT
MICA reverse	CCTGACTGCACAGATCCATCCC
GAPDH forward	TCCATGACAACTTTGGTATCGTGG
GAPDH reverse	AGAGCCCCGCGGCCATCACG

Supplemental Table 2. ChIP assay primers

Primer Name	**Primer sequence**
MICA NF-κB site forward	AATTAAAGCTTCGAAGCTGGTCCCTGCTTTAGGC
MICA NF-κB site reverse	CAGCGGCTCAAGCAGTGGCCGGG
MICA control locus forward	GGGACAGCAGACCTGTGTGTTA
MICA control locus reverse	CTGACTGCACAGATCCATCCCA

Supplemental Figure 1. The intronic NF-κB site does not respond to NF-κB.

A. The location of the putative NF-κB site in the first intron of MICA. B. Lack of response of the putative NF-κB site in MICA intron1 in response to NF-κB activation. A DNA segment carrying the putative NF-κB site from the first intron of MICA was cloned in either orientation upstream of the SV40 promoter in the pGL3P luciferase reporter construct. The indicated reporter constructs were transfected with either an NF-κB p65 expression vector (NF-κB) or empty control vector (Control). Cells were harvested and assayed for luciferase activity 48h post transfection. The error bars represent standard deviations of three replicates. An NF-κB reporter carrying four copies of an HIV NF-κB site was used in parallel as a positive control for NF-κB activation.

Supplemental Figure 2. TNFα –induced binding of NF-κB to the -130bp regulatory element persists for at least 24h.

TNFα -induced binding of NF-κB to the -130bp site was analyzed by EMSA in primary human arterial endothelial cells. Primary human arterial endothelial cells were treated with TNFα for the times indicated. Nuclear extracts were prepared and EMSAs undertaken using a ^{32}P-labeled DNA probe corresponding to the wild-type -130bp regulatory site. The arrow indicates the TNFα-induced band which corresponds in size to the NF-κB p65/p50 heterodimer.

Supplemental Figure 3. TNFα induces NF-κB p65 homodimer and p65/p50 heterodimer binding to the -130bp control site.

A and B. *In vitro* binding of NF-κB to the -130bp MICA NF-κB site demonstrated by EMSA in primary human venous endothelial cells. ^{32}P-labeled DNA probes containing the -130bp site were incubated with nuclear extract from cells treated with TNFα for 1h or from untreated cells. In the competition assay (A), nuclear extracts were preincubated with 100-fold excess of unlabeled probe containing the intact -130bp site (W), a mutated site (M) or a consensus NF-κB site (N); in the supershift assay (B), nuclear extracts were preincubated with antibodies against NF-κB p65, p50 or c-Rel before adding ^{32}P-labeled probe. Protein-probe complexes were resolved by native PAGE. Results are representative of four experiments using different batches of independently purified nuclear extracts.

Supplemental Figure 4. Characterization of species bound to the -130bp site when probe concentrations are limiting.

The identities of the protein-probe complexes formed under limiting probe condition in Figure 6A were determined by supershift assay. Nuclear extracts were preincubated with ant-NF-κB p65 or anti-HSF1 antibody before adding ^{32}P-labeled probe. ^{32}P-labeled DNA probes containing the -130bp site were incubated with nuclear extract from primary arterial endothelial cells transduced with lentivirus expressing treated with TNFα for 1h as indicated. The arrows indicated the endogenous HSF1 trimer and p65/p50 heterodimer, as well as the antibody-supershifted complexes. HSF1 is represented by a doublet as seen in previous studies (1). The mobility of the anti-p65 antibody-supershifted p65/p50 complex is similar to the natural HSF1 trimer complex. There is a clear supershift of the band corresponding to HSF1 by anti-HSF1 antibody and a clear supershift of the TNFα- inducible band corresponding to NF-κB by anti-p65 NF-κB antibody.

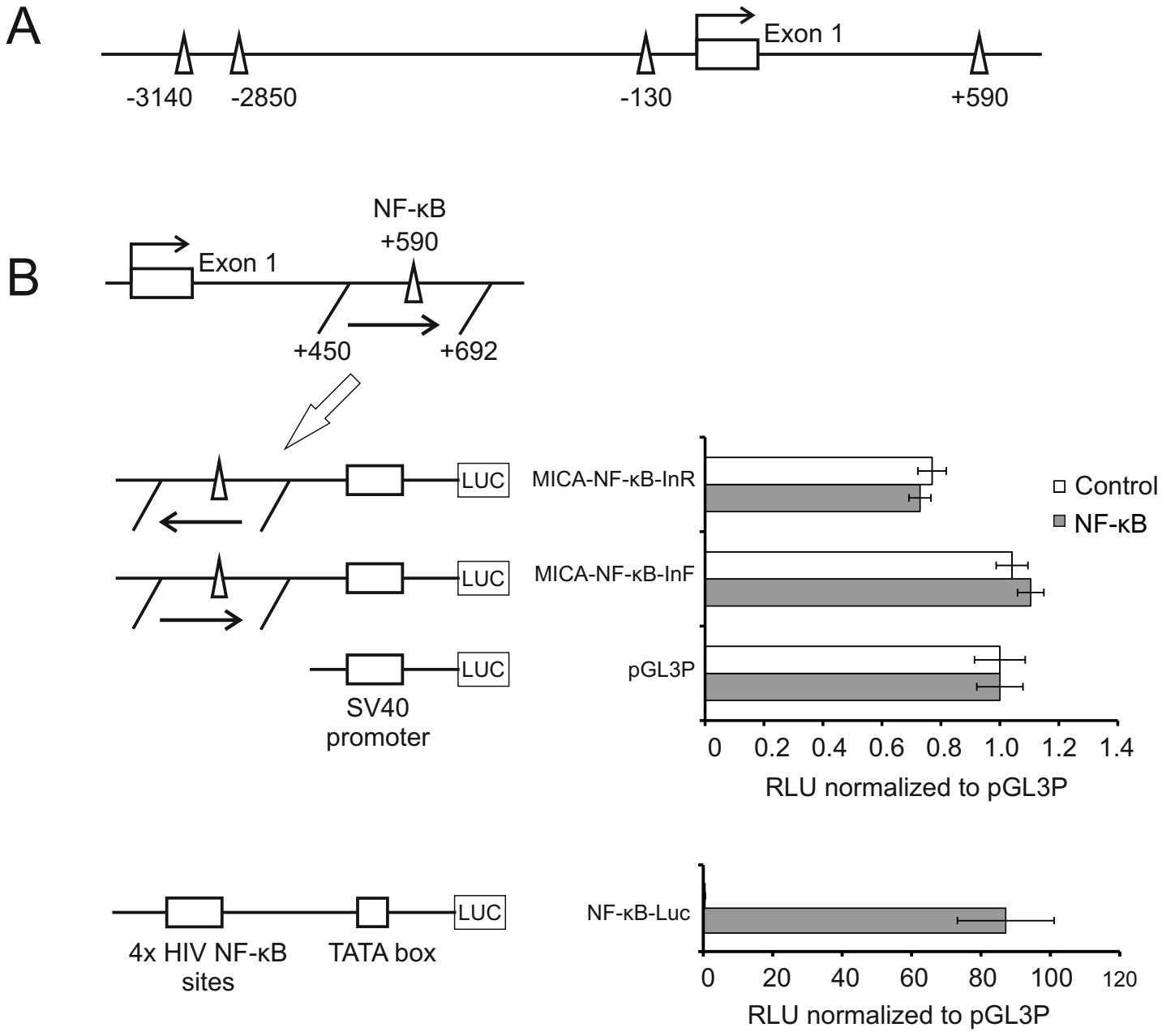
Supplemental Figure 5. The half-life of MICA mRNA is around 18h.

Primary human arterial endothelial cells were treated with actinomycin D (5uM) and harvested at different time points. The MICA expression level relative to an untreated sample was determined by real-time RT-PCR and plotted with a $\log_2$ scale against the time duration of actinomycin D treatment. The half-life calculated by linear regression is: $t_{1/2} = \log_2(0.5)/(-0.0565) = \sim 18h$, $R^2 = 0.82$). The error bars represent standard deviations of three replicates.

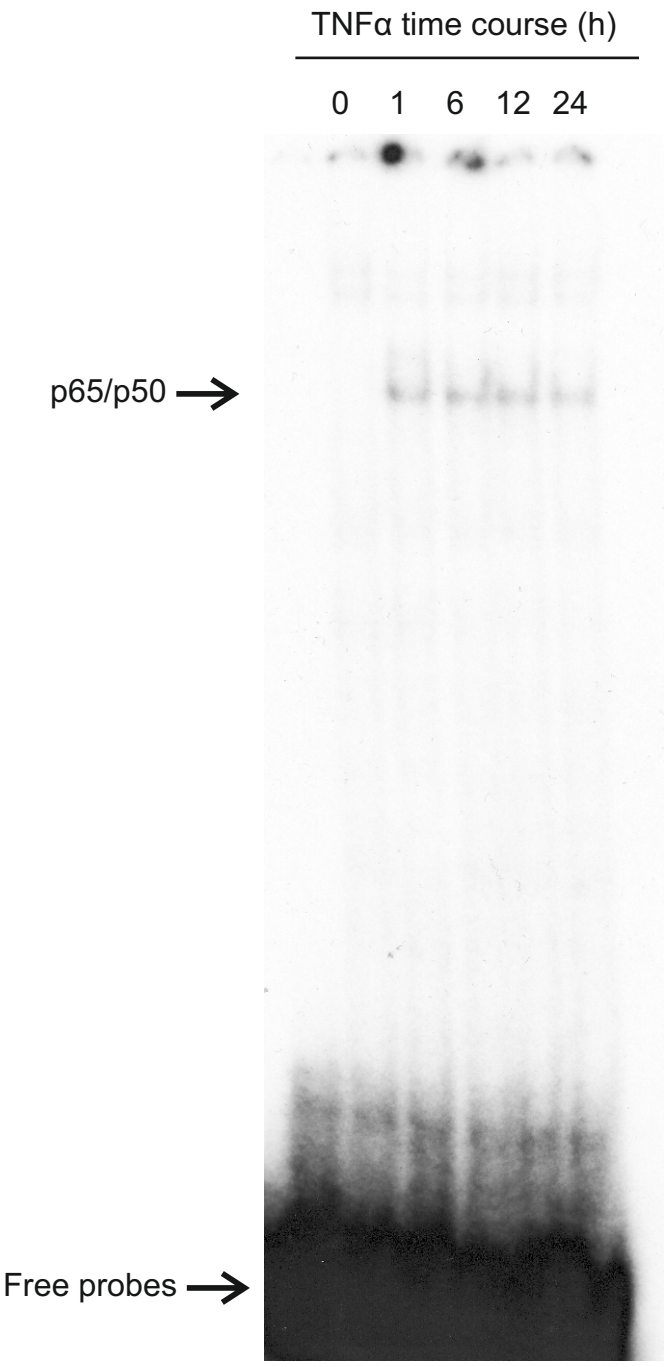
SUPPLEMENTAL REFERENCES

1. Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993) *Molecular and cellular biology* **13**, 1392-1407

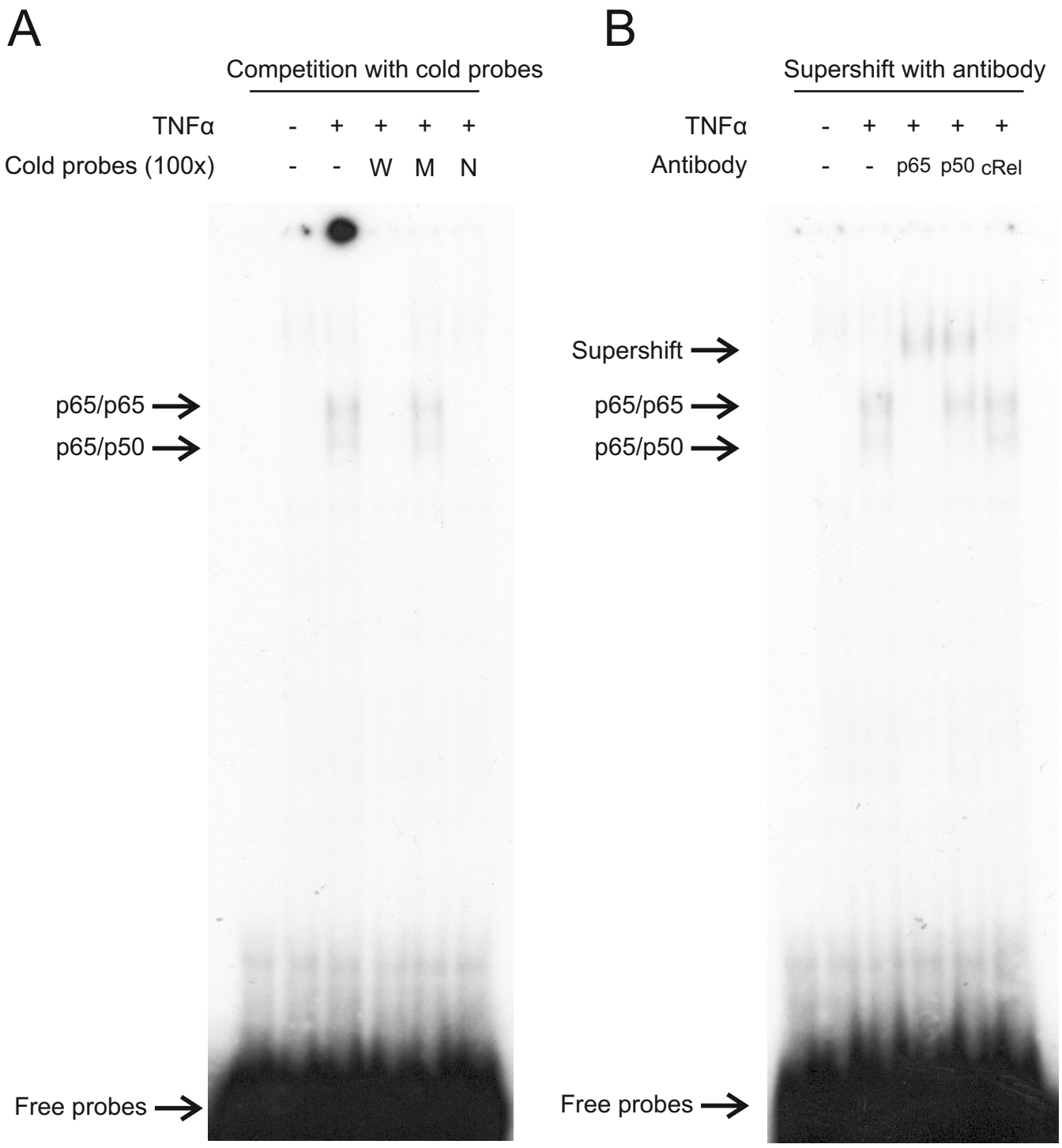
Supplemental Figure 1



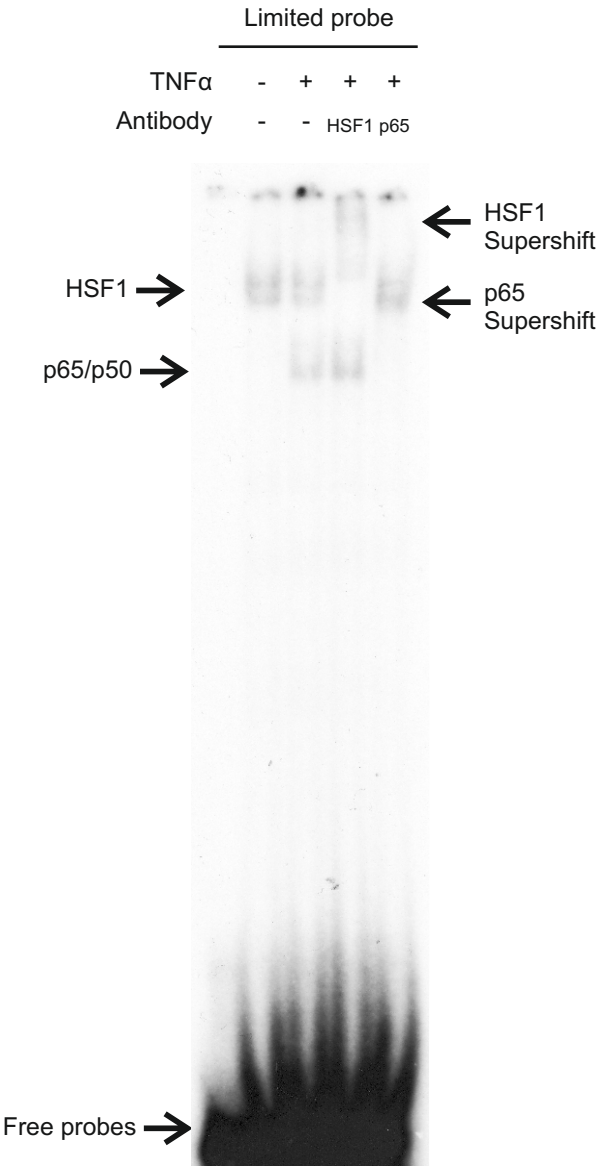
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5