Expanded View Figures

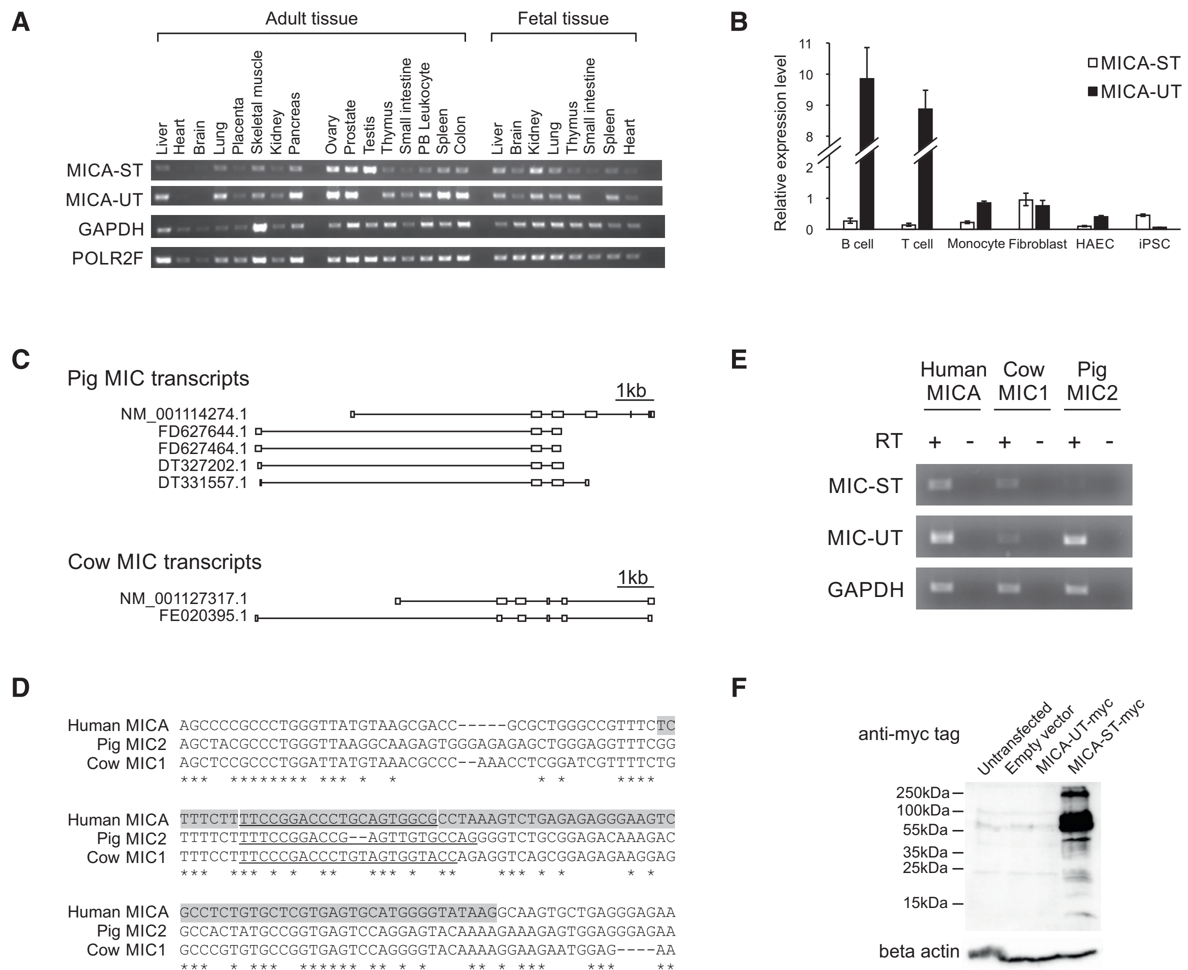

Figure EV1. Properties of the MICA upstream transcript.

A Semi-quantitative RT–PCR analysis of the transcript levels in adult and foetal tissues. GAPDH and POLR2F levels are shown as controls.

B The upstream transcript and standard transcript levels measured by qPCR in different primary human cells. Relative expression levels were normalized to that of the standard transcript in HT1080 cells. Error bars represent standard deviations of three replicates.

C Exon structure of the upstream and standard transcripts of the *MICA* homologs in pig and cow based on EST and RefSeq data. Transcript sequences were aligned to the pig (CT737281.12) or cow (AC_000180.1) genomes for visualization.

D Alignment of the region around the first exon of the upstream transcript (highlighted in grey) demonstrating sequence conservation. The sequence information was used to design species-specific primers (underlined) for detection of *MICA* homolog transcripts in these species.

E RT–PCR demonstrates expression of upstream transcripts and standard transcripts for *MICA* homologs in adult kidney tissue from different species. Reactions without reverse transcriptase (RT) were used as negative controls.

F Western blot analysis of lysates from 293T cells transfected with C-terminal in-frame myc-tagged upstream transcript (MICA-UT-myc) or standard transcript (MICA-ST-myc) expression constructs demonstrate that no protein was detectable for the upstream transcript.

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Figure EV2. Transcription from the upstream promoter represses MICA expression.

A, B Mapping of the core standard promoter (A) or upstream promoter (B) using luciferase reporter assays with constructs carrying serial promoter truncations in HT1080 cells. Error bars represent standard deviations of three biological replicates.

C Sequence of the upstream promoter region subjected to CRISPR-mediated deletion. The core promoter mapped by reporter assays is boxed, the transcription start site mapped by 5′-RACE is in bold, and the upstream first exon is highlighted in grey. The deletions produced with the two pairs of CRISPR guide RNAs are underlined in orange and red, respectively.

D Flow cytometry of cell surface MICA expression in primary human arterial endothelial cells following transfection with CRISPR plasmids targeting deletions of the *MICA* upstream promoter or control genes (*HLA-B* or *PDPN*). Cells were gated for the CRISPR Cas9 nuclease-transfected GFP-positive population.

E qPCR analysis of MICA upstream (MICA-UT) or standard transcript (MICA-ST) expression in primary human fibroblasts transfected with CRISPR plasmids targeting deletions of the MICA upstream promoter or control genes (*HLA-B* or *PDPN*). GFP-positive cells were sorted 3 days post-transfection for analysis. Error bars represent standard deviations of three replicates.

F Diagram of constructs for luciferase reporter assays with deletion of the upstream (red) or standard (blue) core promoters.

G Reporter assays using the above constructs in 293T and HT1080 cells. Deletion of the core standard promoter completely abolished luciferase activity, confirming that the activity measured from the wild-type construct is derived exclusively from the standard promoter. Deletion of the core upstream promoter has no effect. Error bars represent standard deviations of three biological replicates. NS, not significant: Student's *t*-test.

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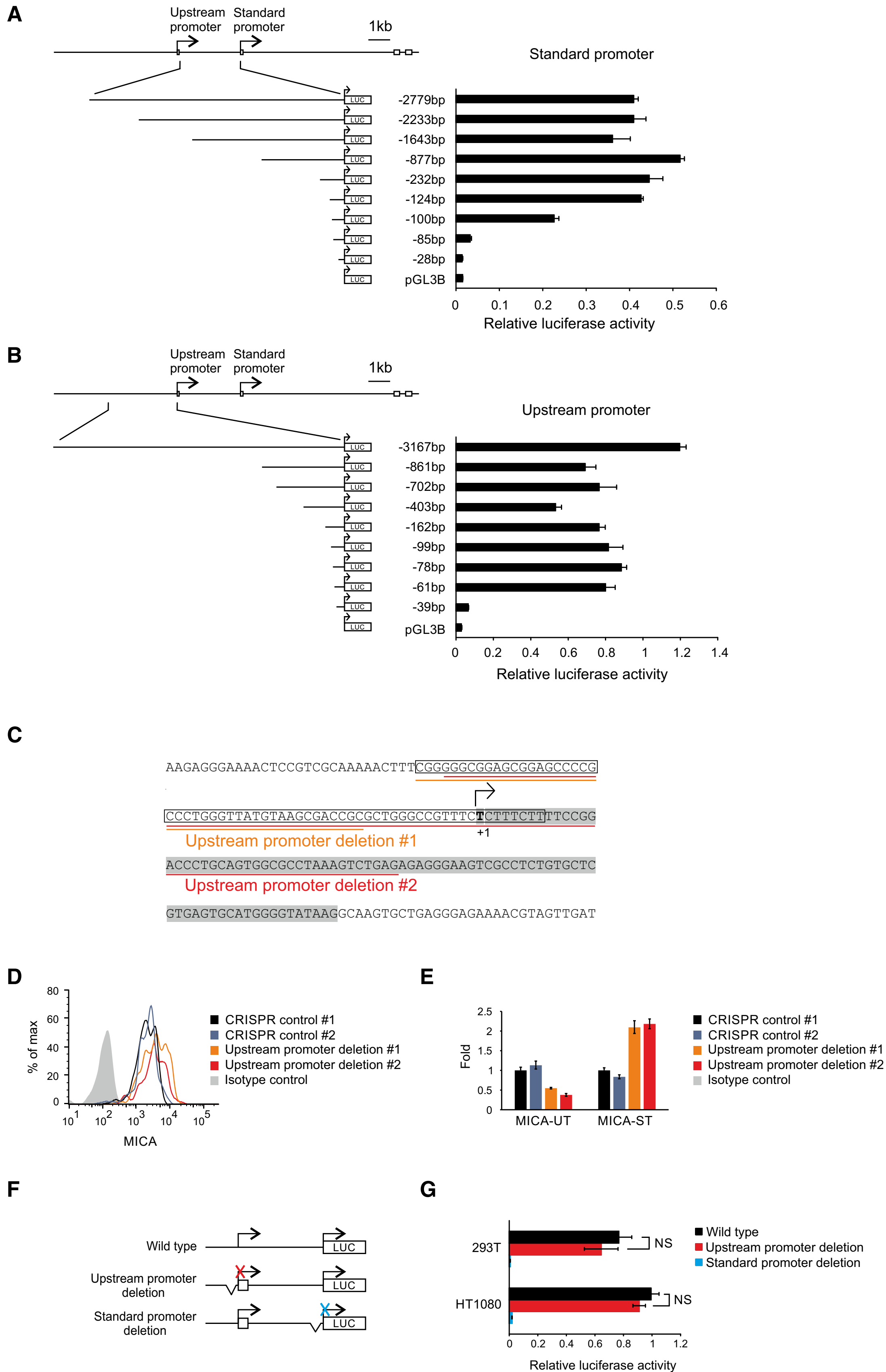

Figure EV2.

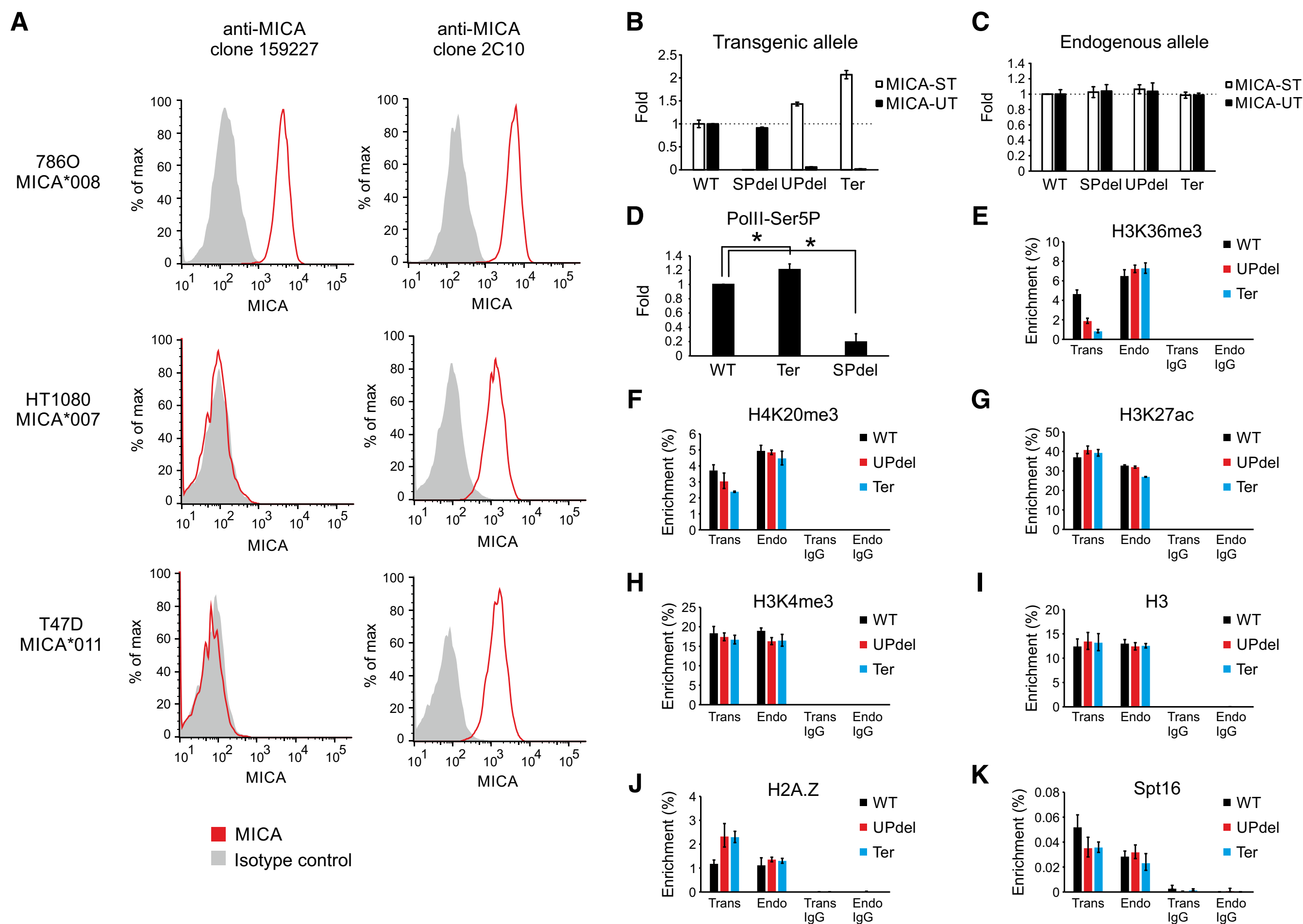

Figure EV3. Transcriptional interference of MICA.

A Flow cytometric analysis of MICA surface expression in cell lines homozygous for MICA alleles demonstrates allelic specificity of the clone 159227 anti-MICA antibody for the MICA*008 allele. In contrast, the clone 2C10 anti-MICA antibody does not display allele-specific activity.

B, C qPCR analysis of transgenic (B) or endogenous (C) MICA upstream and standard transcript expression in isogenic cell lines carrying a transgenic 161-kb *MICA* locus. Deletion of the upstream promoter or insertion of a transcription terminator between the upstream promoter and the standard promoter results in an increase in the level of the transgenic standard transcript. No effect was seen on the endogenous transcripts. Error bars represent standard deviations of multiple independently generated clones (n = 2–3, Appendix Table S1).

D ChIP analysis of Pol II phospho-Ser5 signal at the transgenic standard promoter region. Data are shown as fold change over the wild-type construct and normalized to endogenous standard promoter Pol II phospho-Ser5 signal. Error bars represent standard deviations of three independent experiments. *$P < 0.05$, Student's t-test.

E–K ChIP analysis of H3K36me3 (E), H4K20me3 (F), H3K27ac (G), H3K4me3 (H), H3 (I), H2A.Z (J) and Spt16 (K) at the transgenic (Trans) and endogenous (Endo) standard promoter regions. Error bars represent standard deviations of three replicates.

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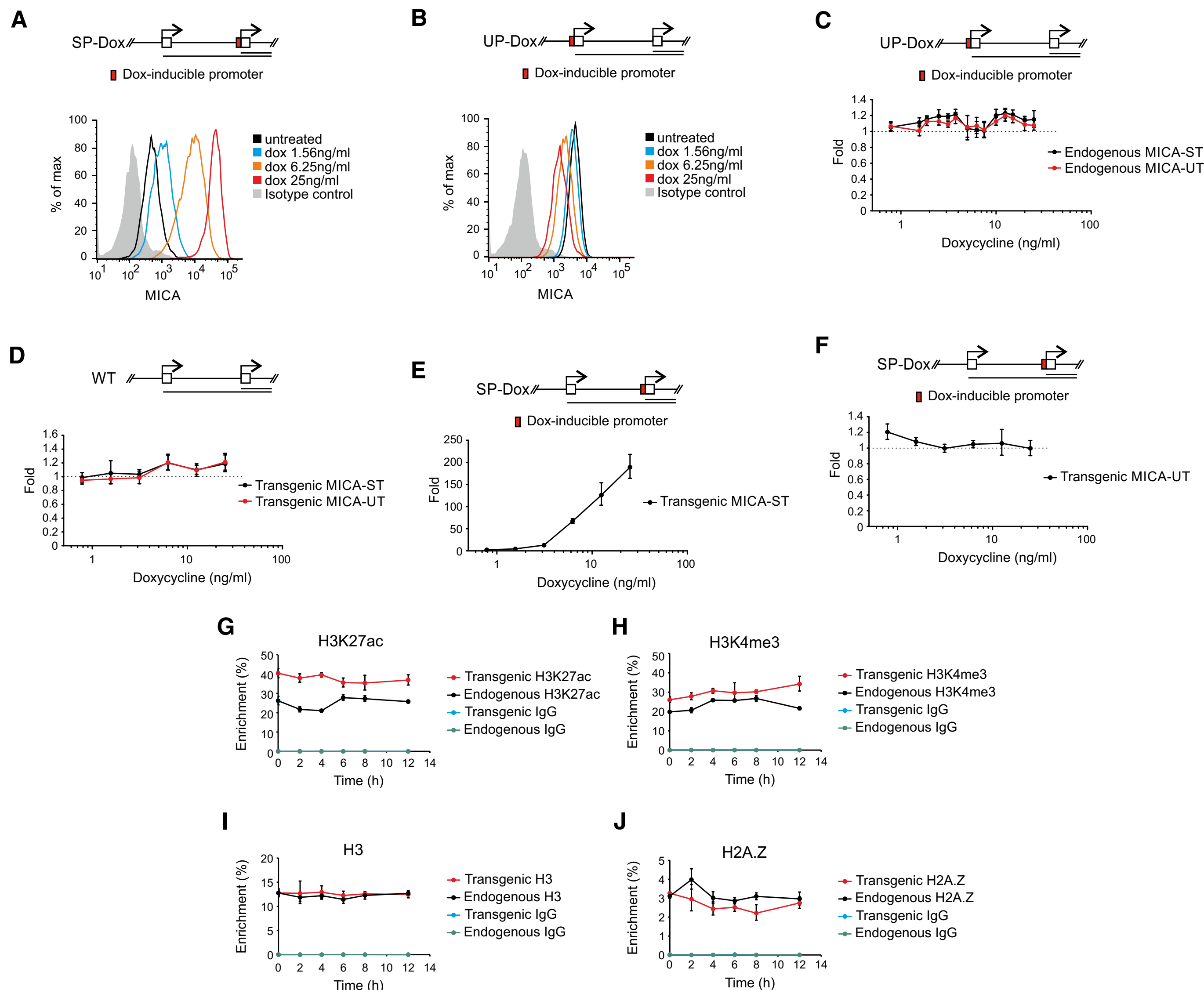

Figure EV4. Transcriptional interference demonstrated with doxycycline-inducible promoters.

A, B Representative flow cytometry histograms showing dose-dependent changes of opposite direction in transgenic MICA surface expression when expression of the standard (A) or upstream (B) transcript was under control of a doxycycline-inducible promoter.

C qPCR analysis showing no change of endogenous upstream and standard transcript expression in modified isogenic cells in which the upstream transcript of the *MICA* transgene is under the direct control of a doxycycline-inducible promoter.

D qPCR analysis showing no response of the transgenic upstream and standard transcript expression to doxycycline in isogenic cells with the transgenic wild-type upstream and standard promoters.

E, F qPCR analysis of dose-dependent changes in expression of the transgenic standard transcript (E) and upstream transcript (F) in modified isogenic cells in which the transgenic standard transcript is under the control of a doxycycline-inducible promoter. The strong induction of the standard transcript is not associated with any change in the upstream transcript which confirms the unidirectional nature of the in *cis* transcriptional interference.

G–J ChIP analysis of H3K27ac (G), H3K4me3 (H), H3 (I) or H2A.Z (J) at the transgenic and endogenous standard promoter over time following induction of the transgenic upstream transcript by doxycycline (7.5 ng/ml).

Data information: Error bars represent standard deviations of three replicates.

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