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Supplementary material

Vector constructs

Cloning of discoidin RNAi construct

The discoidin RNAi construct was cloned by amplification of a 509 bp discoidin fragment with primers:
Forward: AAACTGCAGTCTACCCAAGGTTTAGTTC
Reverse: AAAGTCGACCAGTGTAAATATCTGCACC.
This was cloned into pDneo2 downstream of the actin6 promoter in sense orientation via PstI and HincII. A second discoidin fragment (768 bp) was amplified by PCR using primers:
Forward: AAGGTACCTCGAGAAGATCTGTAGCAATGTAATCAGCTC
Reverse: AACTCGAGCTAGCCAAGGTTTAGTTCAAC
and fused to the first one in antisense orientation via Acc65I and XhoI.

Cloning of coronin RNAi construct

The coronin sense fragment (642 bp) was generated by PCR using primers carrying the endogenous PstI site and SmaI site:
Cor sense 5' PstI : CTGCAGGTGGTGGTTCATTCGCTGT
Cor sense 3' SmaI : CCCGGGAAGAGACCAGAGGCAGAAT.
The second fragment of the coronin gene (903 bp) was generated by PCR using primers carrying BamHI and SmaI sites:
Cor as 5' Bam HI: GGATCCCTGCAGGTGGTGGTTCATT
Cor as 3' EcoRV: GATATCAGATTTTCTTGGTACACGGAA
The coronin sense PCR product was subcloned in sense orientation into pDneo2 via PstI at the 5' end and blunt ligation via SmaI (primer designed) and HincII (vector restriction site) at the 3' end. pDneo2 vector was linearized with EcoRI, the resulting ends were blunt-ended using Klenow polymerase and then digested with BglII thus generating one blunt and one sticky end. The second PCR fragment was ligated to the first one in antisense orientation via BamHI/BglII compatible overhangs at the 5' end and blunt ligation at the 3' end.

Cloning of sp96 RNAi construct

The Sp96 fragment of 405 bp was amplified with primers:
Forward: AACTGCAGAGATGAACCAACTCATAGACCA
Reverse: TTGGATCCAGCAATTGAAGCGATGGTTGAG,
and cloned into pDneo2 downstream of the Actin6 promoter in sense orientation via PstI and BamHI. A second Sp96 fragment (656 bp) was amplified by PCR using primers:
Forward: AAGAATTCAGATGAACCAACTCATAGACCA
Reverse: TTGGATCCATGATGATGGTGAAGATGAAGC
and fused to the first one in antisense orientation via EcoRI and BamHI.

Cloning of Trxi

Trxi construct was generated by amplification of the full length thioredoxin cDNA (trxA) of 321 bp with primers:
Forward: GAACGAGCTCCATGGCCAATAGAGTAATTCATG
Reverse: CGCGGATCCTTATTTGTTTGCTTCTAGAGTACTTC.
The fragment was cloned via BamHI and NcoI in antisense orientation downstream of the actin6 promoter into pDneo2. A discoidin fragment of 393 bp was fused to the first one, thus

forming the loop of the hairpin construct. A second thioredoxin fragment (330 bp) was amplified by PCR using primers:
Forward: AAAGTCGACCCAATAGAGTAATTC
Reverse: CTAGCATGCCCTTATTTGTTTGC
and fused downstream in sense orientation via SalI and SphI to the discoidin loop sequence.

Figure Legends:

Fig. S1 HelF disruption enhances gene silencing by RNAi
Comparison of gene silencing in wild type (WT, left) and HelF$^-$ background (HelF$^-$, right).
A. Colony blots using a monoclonal anti discoidin antibody (blue staining) were counterstained with Ponceau (red) to visualize the entire colonies. Western blots on proteins isolated from wild type cells and four representative clones transformed with a discoidin RNAi (Disci) construct. A second monoclonal antibody against coronin was used as an internal control. Northern blots with RNA from the same cell lines hybridized with a discoidin specific probe are shown below. Ethidiumbromide staining of the large rRNA was used as a loading control.
B. Transformation with a coronin RNAi construct in the wild type (cori/WT) and the HelF$^-$ (cori/ HelF$^-$) background. Colony blots stained with a monoclonal anti coronin antibody are shown in the upper part, Western blots using the anti coronin mAb and the anti discoidin mAb as an internal control are displayed in the lower part.
C. Transformation with a thioredoxin RNAi construct in the wild type (trxi/WT) and the HelF$^-$ (trxi/HelF$^-$) background. Northern blots with RNA from independent clones were hybridized with a thioredoxin specific probe. Ethidiumbromide staining of the large rRNA was used as a loading control.
D. Transformation with a sp96 RNAi construct in the wild type (sp96i/WT) and the HelF$^-$ (sp96i/HelF$^-$) background. Northern blots with RNA from cells developed for 16 hours on filters were hybridized with a sp96 specific probe. RNA from vegetative wild type and HelF$^-$ cells that do not express sp96 are shown for comparison. Ethidiumbromide staining of the large rRNA was used as a loading control.

Fig. S2 Antisense RNA mediated gene silencing is not enhanced in HelF$^-$ cells
Transformation with discoidin antisense (discas), coronin antisense (coras) and thioredoxin antisense (trxas) constructs in the wild type and the HelF$^-$ background.
A. Colony blots stained with an anti discoidin mAb (top) and an anti coronin mAb (bottom) are shown. Untransformed wild type and HelF$^-$ cells are displayed as controls (right).
B. Western blots of clonal isolates from wild type and HelF$^-$ cells transformed with the discoidin antisense construct were probed with the discoidin mAb and the coronin mAb as a loading control.
C. Northern blots of RNA prepared from clonal isolates of cells transformed with the thioredoxin antisense construct were hybridized with a labelled trx antisense probe (detecting the mRNA). Ethidiumbromide staining of the large rRNA was used as a loading control.
D. Northern blot of RNA from the same strains hybridized with a labelled trx sense probe (detecting antisense transcripts) to demonstrate expression of the silencing construct.

Fig. S3 Run-on assays of discoidin
Individual filters were hybridized to labeled run-on transcripts from four completely silenced clones (Disci1/WT, Disci2/WT, Disci9/HelF$^-$ and Disci10/HelF$^-$), one partially silenced clone (Disci9/WT) and one non-silenced clone (Disci10/WT). Discoidin, GFP (NC - negative control) and Actin15 in vitro transcripts were applied on the filters by slot blotting. The same

filters were stripped and re-hybridized with nuclear run-on transcripts from wild type cells (WT). Signals were quantitated and are presented in Fig. 9.

Fig. S4 Steady-state level of hairpin transcripts
Northern blot of different clones, transformed with the discoidin hairpin construct and showing different silencing levels (completely silenced: Disci8/HelF$^-$1, Disci8/HelF$^-$4, Disci1/WT, Disci2/WT, Disci9/HelF$^-$2; partially silenced clones: Disci3/WT and Disci9/WT), probed with a discoidin sense transcript for detection of steady-state hairpin expression levels. As a positive control, 0,1ng of *in vitro* transcribed discoidin dsRNA was applied. Ethidiumbromide staining of the large rRNA is shown as a loading control.