SUPPLEMENTARY DATA

Human CCR4 deadenylase homolog Angel1 is a Non-Stop mRNA Decay factor

Tim Nicholson-Shaw[1], Megan E. Dowdle[1], Yasmeen Ajaj[1], Mark Perelis[2,3,4], Amit Fulzele[1], Gene W. Yeo[2,3,4], Eric J. Bennett[1] and Jens Lykke-Andersen[1,5,*]

Correspondence authors: Jens Lykke-Andersen

Email: jlykkeandersen@ucsd.edu

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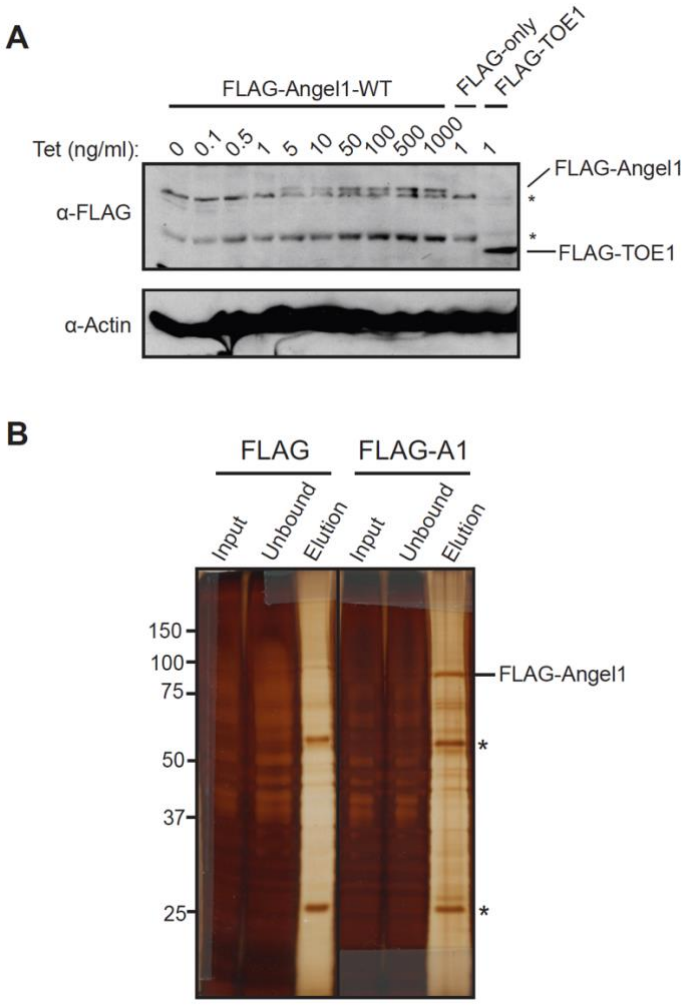
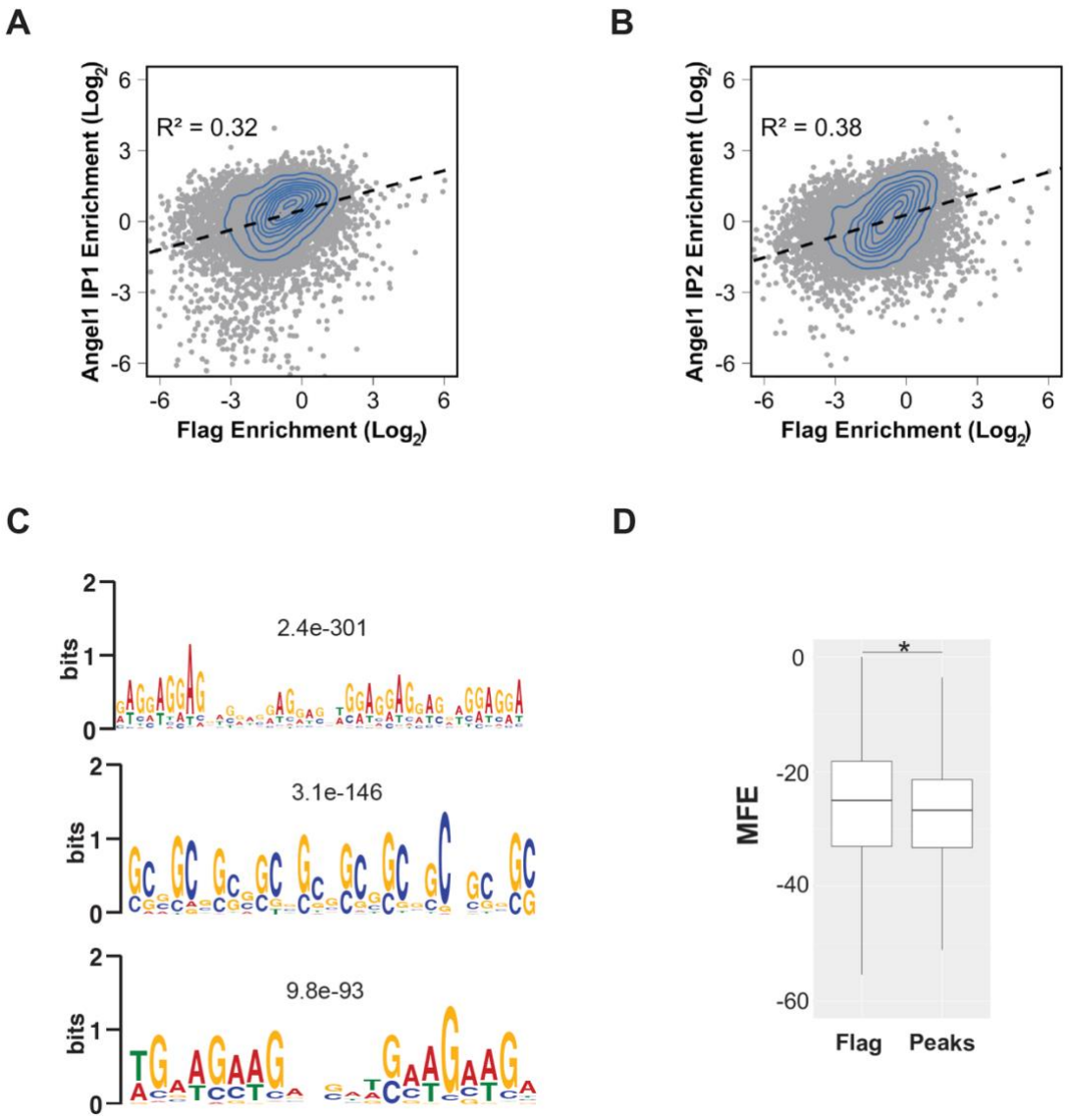


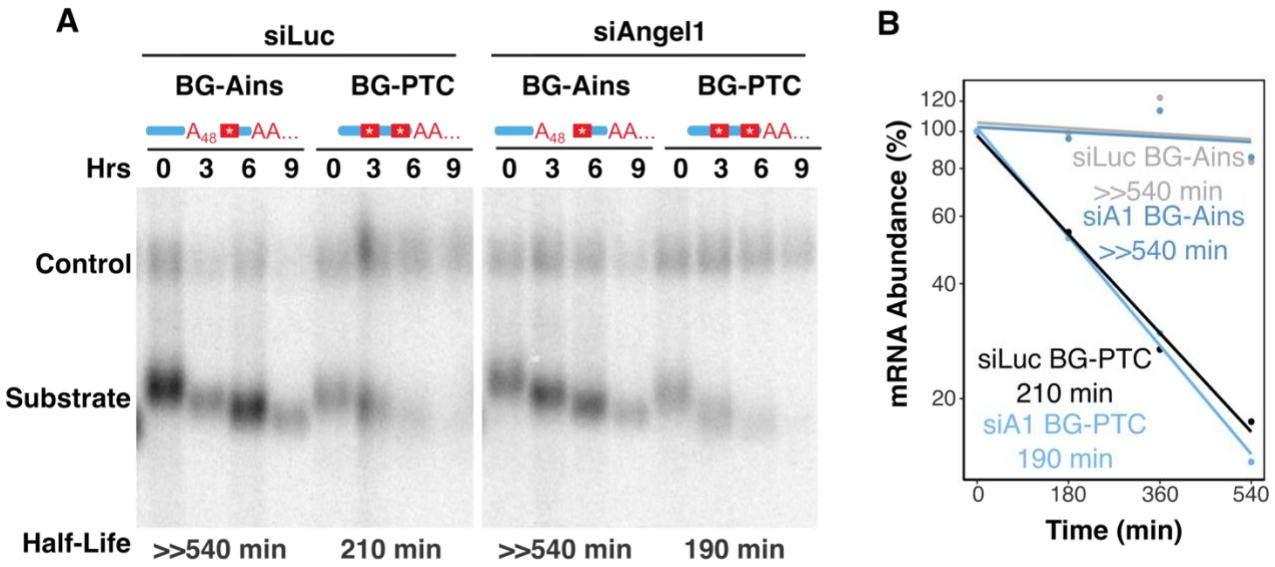
SUPPLEMENTARY FIGURE S1. FLAG-Angel1 HEK293 Flp-In T-REx cell line validation. (A) Western blot showing expression of FLAG-Angel1 WT with titration of tetracycline (Tet) as indicated above lanes. The parental Flp-In T-REx line expressing no fusion protein serves as a negative control (FLAG-only), and Flp-In T-REx expressing FLAG-TOE1 at near endogenous levels was included as a reference. β-Actin was used as a loading control. *: non-specific bands. (B) Silver-stained SDS-PAGE gel examining protein from FLAG-Angel1-WT IPs. An input sample of 10% of the total cell extract used for the IP (Input), a sample from the remaining lysate after IP

containing unbound proteins (Unbound), and a sample of the pooled elutions (Elution) were run. A control IP using a Flp-In T-REx line expressing no fusion protein (FLAG) was run alongside. *: denotes the antibody heavy and light chain.

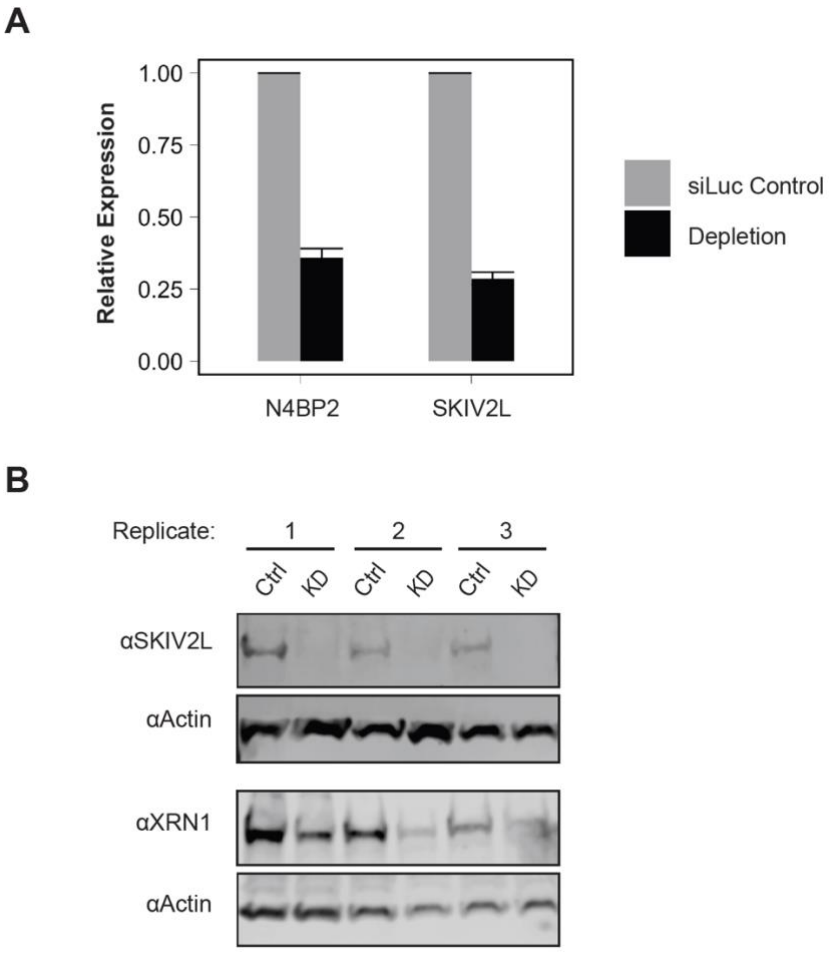


SUPPLEMENTARY FIGURE S2. Extended eCLIP analysis. (A and B) Log_2 fold enrichment of IP reads over input compared between either Flag-tagged Angel1 eCLIP replicate and the parental Flag control. Grey dots represent individual genes and the blue contour lines represent a 2D kernel density estimate. Pearson correlations are shown. (C) Logo plots of top three enriched nucleotide motifs identified by MEME analysis of sequences within 50 nucleotides upstream or downstream of Angel1 CLIP peaks as compared to sequences within 50 nucleotides of mapped reads in the FLAG input sample. E-values are displayed above each plot. (D) Box plots

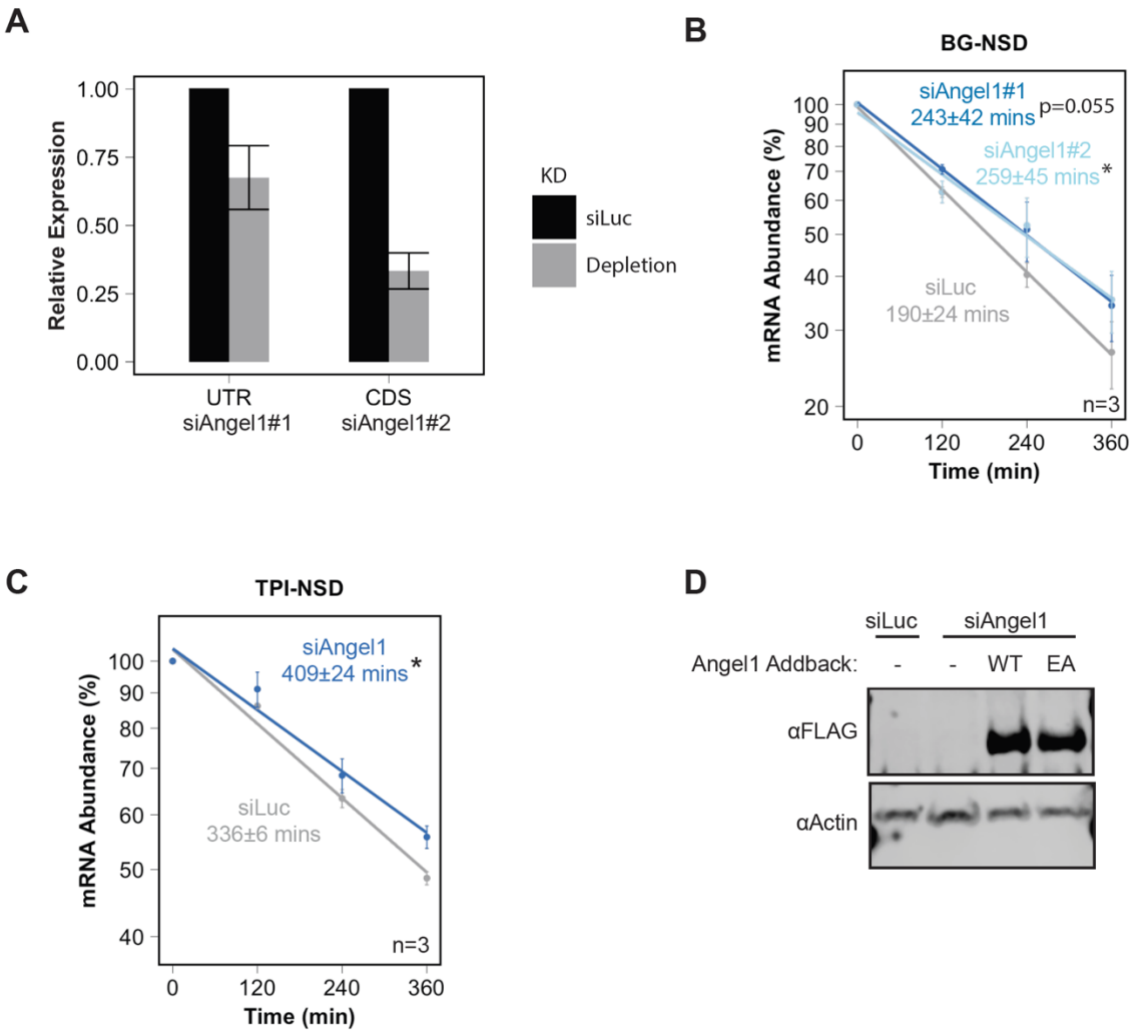
comparing predicted mean free energy (MFE) calculated by RNAfold for regions within 50 nucleotides upstream or downstream of Angel1 CLIP peaks and within 50 nucleotides of mapped reads in the FLAG input sample. Lower MFE scores are associated with stronger predicted secondary structure. *: p-value < 2.2e-22 (KS-test).



SUPPLEMENTARY FIGURE S3. A β-globin NGD reporter mRNA with a coding region A_{48} insertion is stable and not detectably impacted by Angel1 depletion. (A) Northern blot of a pulse-chase mRNA decay assay in HeLa Tet-off cells monitoring degradation of BG-Ains and BG-NMD mRNAs performed as described in Figure 3, in the absence or presence of Angel1 depletion (siAngel1#1). (B) Exponential decay plots of the experiment in panel *A*.

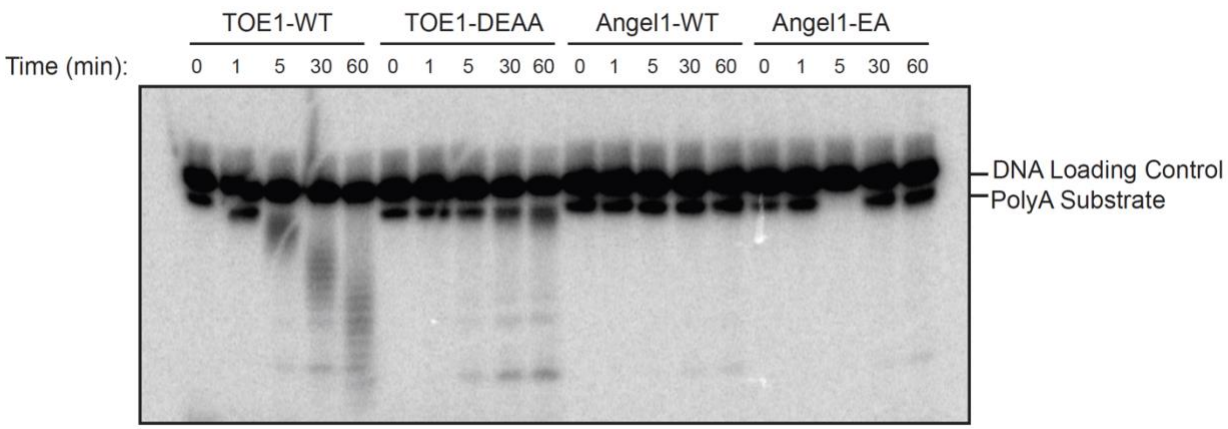


SUPPLEMENTARY FIGURE S4. Validation of siRNA-mediated depletions. (A) Relative expression of knockdown targets compared to a control knockdown (siLuc) obtained from RT-qPCR. Error bars represent standard error of the mean (n=3). Comparisons between each knockdown and its control had a p-value < 0.05 as calculated by a one-tailed Student's t-test. (B) Western blots examining levels of SKIV2L and XRN1 proteins after siRNA-mediated knockdown (KD) in triplicate or knockdown with a non-targeting control siRNA (Ctrl). β-Actin is shown as a loading control.



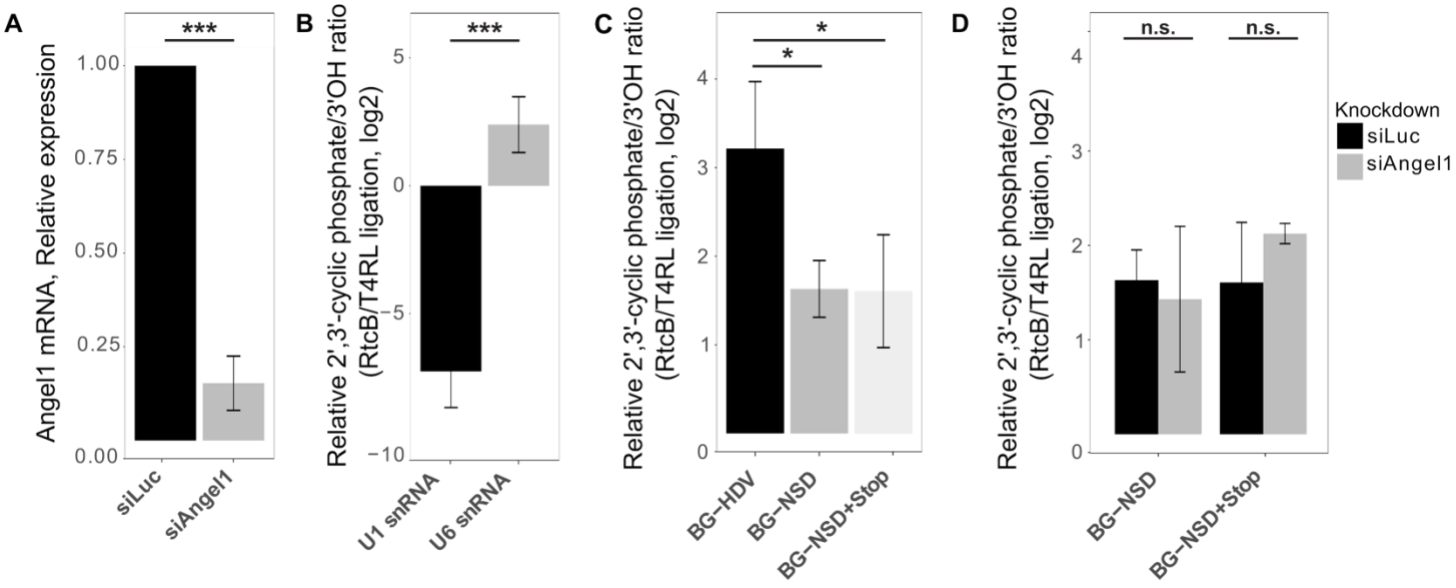
SUPPLEMENTARY FIGURE S5. Additional BG-NSD validation. (A) Relative expression of Angel1 mRNA levels determined by RT-qPCR for two independent Angel1 siRNAs compared to a non-targeting control (siLuc). Comparisons between each knockdown and its control had a p-value of < 0.05 as calculated by a one-tailed Student's t-test. Error bars represent standard error of the mean (n=3). siRNA#1, which targets endogenous but not exogenous Angel1, was used for all other depletions. (B) Exponential decay graphs of pulse-chase mRNA decay experiments using the BG-NSD substrate with depletion using the Angel1-targeting siRNAs shown in panel *A* and siLuc siRNA. (C) Exponential decay graphs of pulse-chase mRNA decay experiments using

the TPI-NSD substrate with depletion of Angel1 or a non-targeting control. Error bars represent standard error of the mean. *: p-value < 0.05. n=3. (D) A Western blot examining total protein from Hela Tet-off cells either depleted by a non-targeting siRNA (siLuc) or an siRNA targeting endogenous Angel1 (siAngel1). Lanes 3 and 4 represent cells that were transfected with constructs expressing siRNA-resistant active (WT) or catalytically dead (EA) FLAG-Angel1 to complement depletion. β-Actin is shown as a loading control.



SUPPLEMENTARY FIGURE S6. Angel1 shows no activity in a deadenylation assay.

Phosphorimager scan of a 5' ^{32}P-labelled poly-A RNA substrate incubated with the indicated active (WT) or catalytically dead (DEAA, EA) proteins (≈50 nM) for the indicated amounts of time and subsequently separated in a denaturing gel. A 5' ^{32}P-labelled DNA substrate was included in each reaction as an internal loading control.



SUPPLEMENTARY FIGURE S7. Angel1 depletion does not cause detectable accumulation of a 2',3' cyclic phosphate on the BG-NSD Reporter mRNA. (A) Relative levels of Angel1 mRNA determined by RT-qPCR of four independent samples after knockdown with siRNA for Angel1 (siAngel1#1) compared to non-targeting control siLuc. Error bars represent standard error of the mean (n=4). ***: $p < 0.001$, two-tailed Student's t-test. (B) Relative 2',3' cyclic phosphate over 3'OH ratios of U1 (negative control) and U6 (positive control) snRNAs as measured by RNA adapter ligation by 2',3' cyclic phosphate-specific RtcB ligase as compared to 3'OH-specific T4 RNA ligase, followed by RT-qPCR. Error bars represent standard error of the mean (n=9). ***: $p < 0.001$, two-tailed Student's t-test. (C) Relative 2',3' cyclic phosphate over 3'OH ratios of indicated β-globin reporter mRNAs measured as in panel *B*. Error bars represent standard error of the mean (n=3). *: $p < 0.05$, two-tailed Student's t-test. (D) Relative 2',3' cyclic phosphate over 3'OH ratios of BG-NSD+Stop and BG-NSD reporter mRNAs in siLuc control depletion versus

siAngel1 depletion conditions, measured as in panel *B* (n=3). n.s.: no significant difference by two-tailed Student's t-test.