SUPPLEMENTARY DATA

accompanying the main manuscript entitled

Molecular basis for the autonomous promotion of cell proliferation by angiogenin

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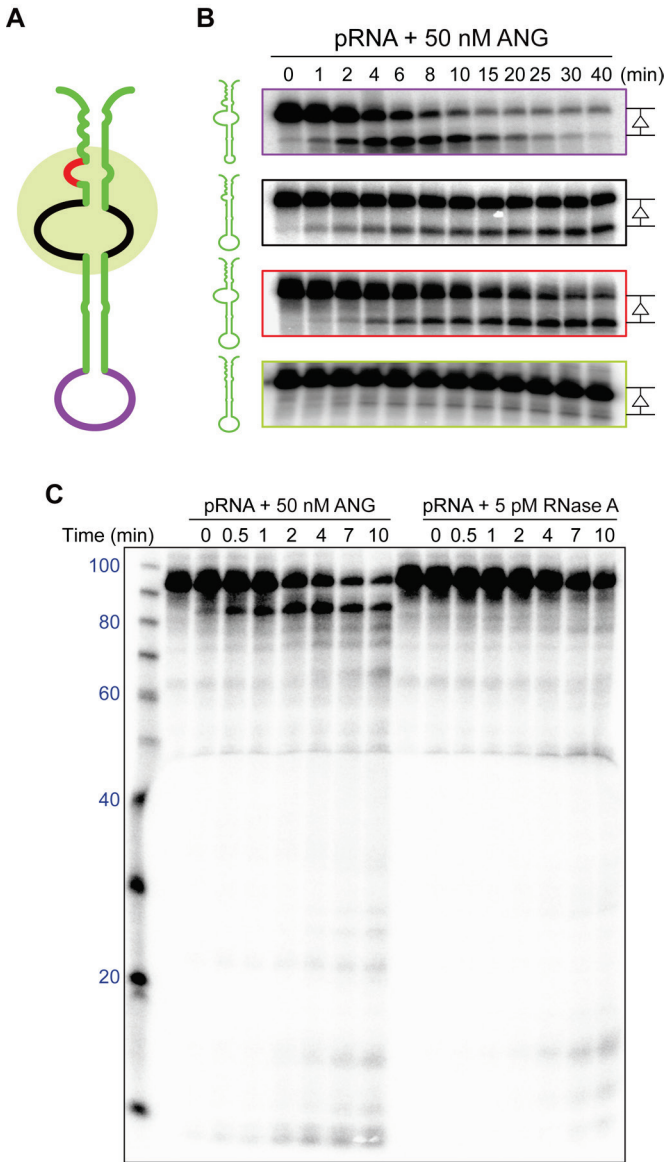

Supplementary Figure S1: related to Figure 1. ANG possesses substrate specificity toward pRNA unlike RNase A.

(A) The lower loop (purple), upper loop (black), and series of uridine residues (red) of pRNA were deleted individually to identify regions of RNA important for cleavage by ANG.

(B) Only deletion of the upper loop and series of uridine residues attenuated catalysis by ANG, and deleting both regions (light green in panel A) made pRNA resistant to cleavage by ANG. Notably, the size of the substrate and product is constant (Δ) regardless of the pRNA substrate, indicative of the high specificity of ANG for a particular C–G phosphodiester bond near the 3′ end of pRNA (Figure 1B of the main text).

(C) Unlike ANG, RNase A cleaves pRNA nonspecifically (Figure 1C of the main text).

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Human −88 GGCGGCGTGGTCGGTGACGCG−ACCTCCC−GG−−CCCCGGGG−AGGTATATCTTTCG−−CT
Mouse −83 TCT−CTGTGGCCTGT−−CACTTTCCTCC CTGTCTCTTTT−−−−−−−−ATGCTTGTGAT−CT
Rat   −83 TCC−GTGTGGCCTGT−−CATGTTTATCC CTGTGTCTTTT−−−−−−−−ACACTTTTCAT−CT
Pig   −85 ACCCCCACGTCCTGTG−−−−GGTGGGCC CCG−−−CTGCTGGGCATGGACATTTTTCG−−C−
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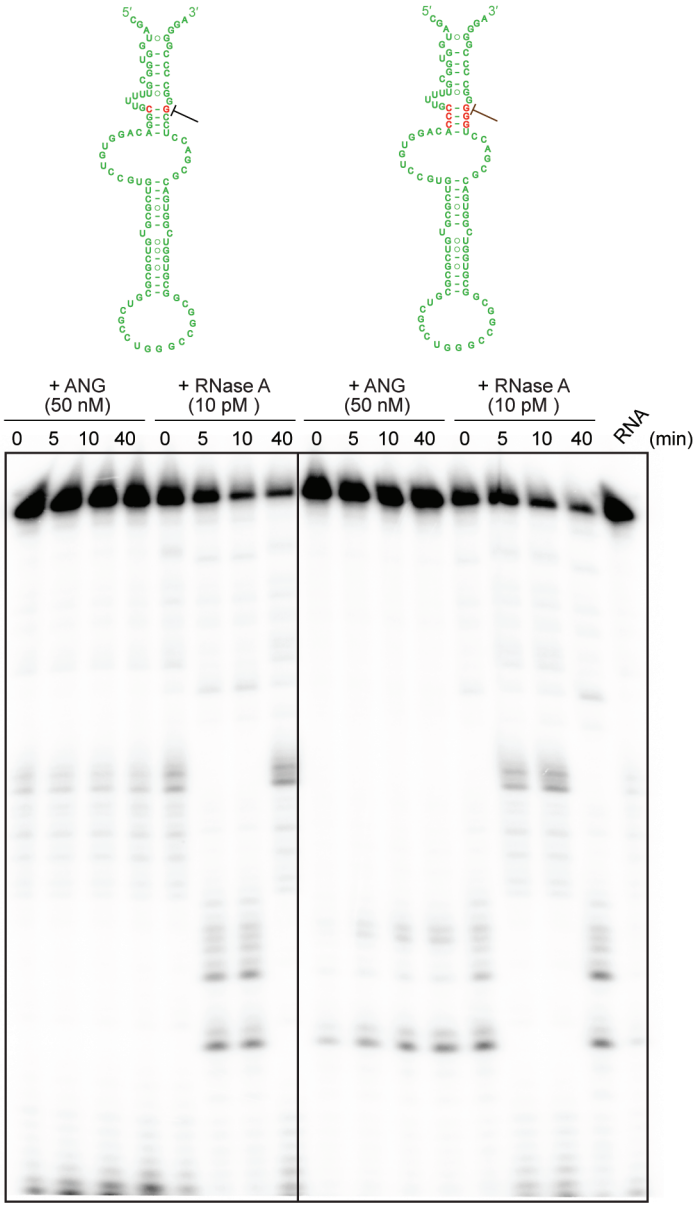
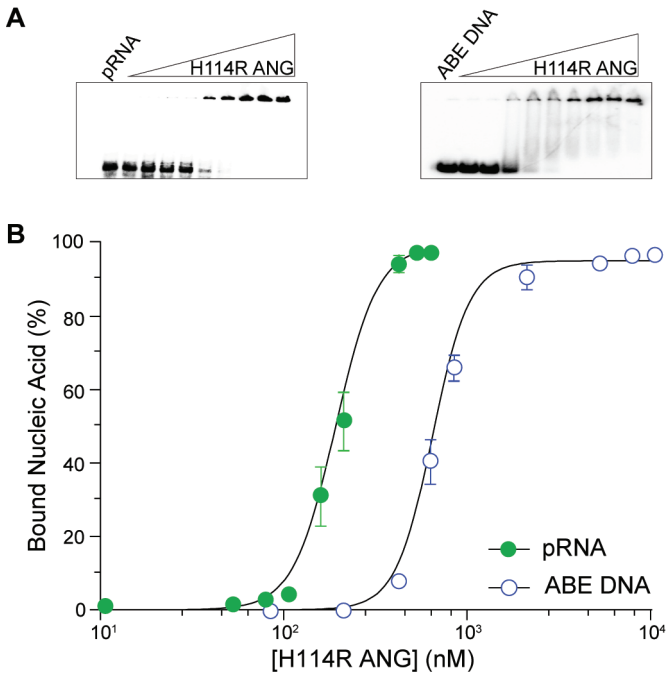
Supplementary Figure S2: related to Figure 1. Conservation of the ANG-cleavage site in pRNA

pRNA sequences were aligned with CLUSTALW software to examine the conservation of the ANG-cleavage site in pRNA. Fully conserved nucleotides are highlighted (black box), and numbers refer to their position with respect to the polymerase I transcription start site. The results indicate that cleavage sites (red) are conserved in human, mouse, rat, and pig. Accession numbers: *Homo sapiens* (X01547), *Mus musculus* (BK000964), *Rattus norvegicus* (X00677), *Sus scrofa* (L31782).



Supplementary Figure S3: related to Figure 1. Swapping G·C base pairs makes pRNA resistant to ANG cleavage

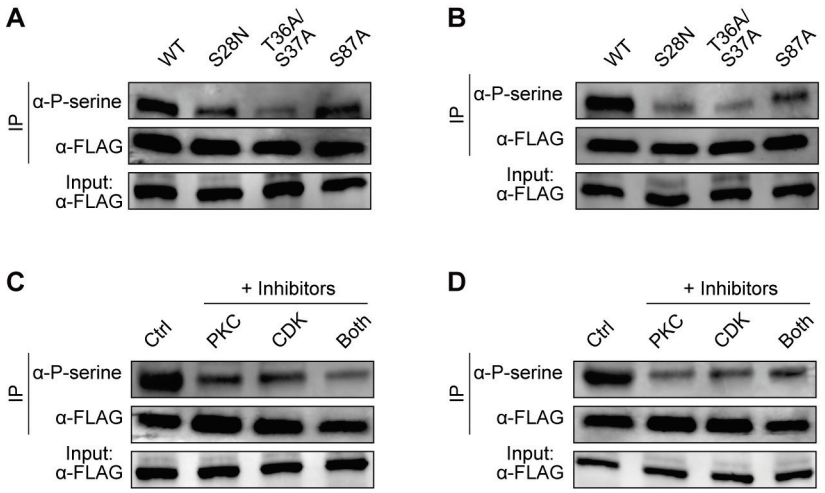
After identifying the cleavage site of pRNA by ANG, the three G·C base pairs (red) were replaced with C·G (right). The swap prevented hydrolysis by ANG but not by RNase A. The replacement of G18–C86 in ^{32}P-labeled pRNA with C18–G86 eliminates cleavage by ANG but not RNase A (left) (Figure 1D of the main text).



Supplementary Figure S4: related to Figures 1 and 3. ANG has higher affinity for pRNA than for ABE DNA

(A) pRNA and ABE DNA were labeled on their 5′ end with [γ-^{32}P]ATP. pRNA was heated and allowed to refold prior to conducting the assay. Nucleic acid (0.2 nM) was incubated with increasing concentrations of H114R ANG, and binding was assessed with a gel-shift assay.

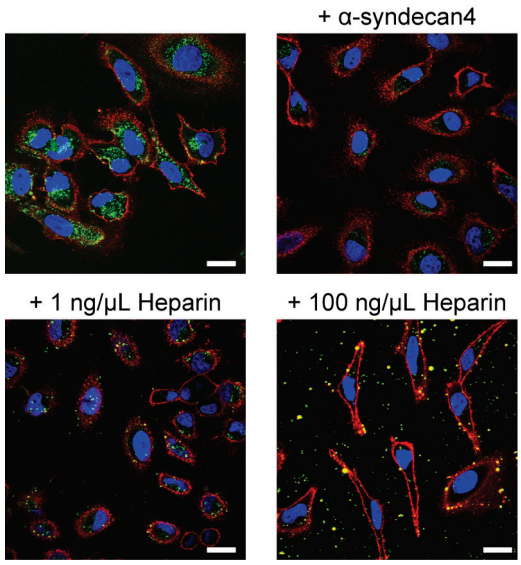
(B) Values of K_d were determined to be (192 ± 8) and (651 ± 12) nM for the H114R ANG·pRNA and H114R ANG·ABE DNA complexes, respectively. Values represent the mean ± SD (n = 3, technical replicates).



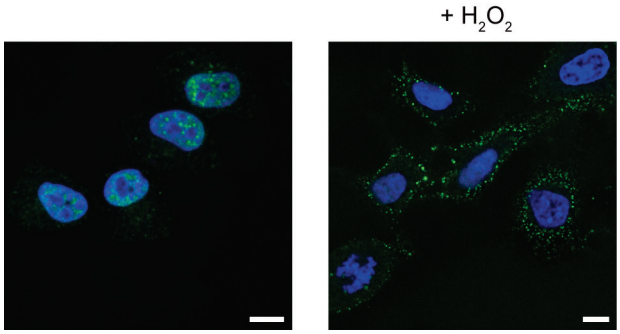
Supplementary Figure S5: related to Figure 4. ANG phosphorylation occurs at specific sites and is regulated by PCK and CDK

(A,B) Two independent replicates of Figure 4C

(C,D) Two independent replicates of Figure 4D



Supplementary Figure S6: related to Figure 4. ANG uptake in HeLa cells occurs via the syndecan-4 receptor
BODIPY-labeled ANG was internalized into HeLa cells after a 3-h incubation. Pre-incubating cells with α-syndecan-4, an antibody that binds to syndecan-4, blocked ANG internalization. Internalization was also impaired by adding heparin, which leads to the formation of extracellular heparin·ANG complexes. Green: BODIPY-labeled ANG. Red: Alexa Fluor 594-labeled wheat germ agglutinin, which is a cell-surface stain. Blue: Hoechst 33342, which is a nuclear stain. Scale bar: 20 μm.



Supplementary Figure S7: related to Figure 6. Oxidative stress alters ANG localization
Immunofluorescence images of FLAG–ANG (green) in HUVEC cells without or with oxidative stress. Cells were incubated for 3-h with FLAG–ANG (1 μg/mL) in EBM-2 medium or EBM-2 medium containing H_2O_2 (0.1 mM). ANG localizes in the nucleolus, but not in cells suffering oxidative stress. Blue: Hoechst 33342, which is a nuclear stain. Scale bar: 10 μm.

Supplementary Table S1. Oligonucleotide primers used in this work

Primer	Sequence (5′→3′)	Usage
pRNA-For (P1)	GTGTCCTGGGGTTGACCAG	qPCR & PCR (RIP)
pRNA-Rev (P1)	GGACACCTGTCCCCAAAAAC	qPCR & PCR (RIP)
GAPDH-For	GTGACTAACCCTGCGCTCC	qPCR
GAPDH-Rev	ATCACCCGGAGGAGAAATCG	qPCR
RT	GCCTTGGCACCCGAGAATTCCATTTTTTTTTTTTTTV	RT & PCR (RNA sequencing)
For-P2	CGATGGTGGCG	PCR (RNA sequencing & RIP)
Rev-P2	TCCCCGGGGCCGGGAGGTC	PCR (RIP)

Supplementary Table S2. Thermal stability of wild-type ANG, its variants and FLAG fusions as determined by differential scanning fluorimetry

ANG	T_m (°C)[a]	ANG	T_m (°C)
Wild-type	61.3 ± 0.2	FLAG–wild-type	65.4 ± 0.9
S28N	61.4 ± 0.2	FLAG–S28N	64.5 ± 0.2
T36A/S37A	58.7 ± 0.1	FLAG–S28D	65.5 ± 0.5
C39W	44.4 ± 0.3	FLAG–T36A/S37A	62.1 ± 0.2
S87A	61.4 ± 0.4	FLAG–T36D/S37D	59.6 ± 1.1
H114R	62.5 ± 0.2	FLAG–S87A	63.5 ± 0.6
Q117G	56.0 ± 0.4	FLAG–S87D	61.7 ± 1.1
S28N/T36A/S37A/S87A	56.8 ± 0.1	FLAG–H114R	65.5 ± 1.4
		FLAG–S28N/T36A/S37A/S87A	58.7 ± 0.1
		FLAG–Ser free	51.7 ± 0.1

[a]Values represent the mean ± SD (n = 3, technical replicates).