Supplementary Table 1. Oligonucleotides and plasmids

Oligonucleotides (5′➔3′)	**Lab Stock #**
DNA-RNA release assay non-template DNA GGCGTACCCTTTTTATTCACGGCGAATACCCATCGATCGATCG	15217
DNA-RNA release assay template DNA CGATCGATCGATGGGTATTCGCCGTGAATAAAAAGGGTACGCC	15218
DNA-RNA release assay RNA ACGCGUCGCAGGCUUUUUAU	15131
DNA-RNA release assay asRNA GGCCUGCGACGA	15132
Scaffold-based termination assay non-template DNA CGCTGTACTGGTAATCGCAGGCCTTTTTATTTGGGAGAGGGAAGT CGCTTCCAGTGG	14380
Scaffold-based termination assay template DNA CCACTGGAAGCGACTTCCCTCTCCCAAATAAAAAGGCCTGCGATT ACCAGTACAGCG	14379
Scaffold-based termination assay RNA UUUUUUAGGCCUGCUGGUAAUCG	14375
Mapping termination HP stem position that triggers termination asDNA CCATACAACCTCCTTACTACAT	14483
Mapping termination HP stem position that triggers termination asRNA (-8) GGCCAGGCAUUACC	14751
Mapping termination HP stem position that triggers termination asRNA (-9) GCCAGGCAUUACC	15160
Mapping termination HP stem position that triggers termination asRNA (-10) CCAGGCAUUACC	15127
Mapping termination HP stem position that triggers termination asRNA (-11) CAGGCAUUACC	15128
Mapping termination HP stem position that triggers termination asRNA (-12) AGGCAUUACCU	15161
Mapping termination HP stem position that triggers termination asRNA (-13) GGCAUUACCUA	15162
Mapping termination HP stem position that triggers termination asRNA (-14) GCAUUACCUAU	15163
Mapping termination HP stem position that triggers termination asRNA (-15) CAUUACCUAUU	15164
non-template DNA for cryo-EM structures of TTC-pause and TTC-hairpin GGCGTACGGAAAAATAACACGGCGAATACCC	
non-template DNA for cryo-EM structure of TTC-release GGCGTACCCTTTTTATTCACGGCGAATACCC	
template DNA for cryo-EM structures of TTC-pause, TTC-hairpin, and TTC-release GGGTATTCGCCGTGAATAAAAAGGGTACGCC	
RNA for cryo-EM structures of TTC-pause and TTC-hairpin GCGUCGCAGGCCUUUUUAUU	
RNA for cryo-EM structure of TTC-release Cy5-ACGCGUCGCAGGCCUUUUUAUU	

antisense RNA10 (asRNA10) for cryo-EM structure of TTC-hairpin CCUGCGACGC	
antisense RNA12 (asRNA12) for cryo-EM structure of TTC-release GGCCUGCGACGA	
Plasmids	**Reference**
pRL706 – β–overexpression plasmid. His6-tag on *rpoB* C terminus	1
pRL706 *rpoB* D446A (pEO9) – β derivative–overexpression plasmid	this work
pRL706 *rpoB* R465A (pEO10) – β derivative–overexpression plasmid	this work
pRL706 *rpoB* R180A-V469A – β derivative–overexpression plasmid	this work
pRM1235 – λP_R WT λt_{R2} terminator sequence	this work.
pRM1065 – Harbors a variant of λt_{R2} terminator with DS DNA changed	2
pRM1066 – Harbors WT ϕt_{500} terminator	2
pRM1234 – Harbors a variant of λt_{R2} terminator lacking the upstream half of the terminator hairpin	this work
pET28a-TEV-*Ec*σ^{70} – σ^{70}–overexpression plasmid	this work
p*Ec*ABC – αββ′–overexpression plasmid. His6-tag on *rpoC* C terminus	3
pCDF-*Ec*rpoZ – ω–overexpression plasmid	3
Strains	
RL1204 *E. coli* K-12 λ^- F^- *trpR tnaA*2 *rpoB5201*(SPA) Δ(*recA*–*srl*)306 *srl301*::Tn10-84. A $recA^-$ derivative of RL1120[4].	This work.

Supplementary references

1 Severinov, K., Mooney, R., Darst, S. A. & Landick, R. Tethering of the large subunits of Escherichia coli RNA polymerase. *J Biol Chem* **272**, 24137-24140, doi:DOI 10.1074/jbc.272.39.24137 (1997).

2 Bellecourt, M. J., Ray-Soni, A., Harwig, A., Mooney, R. A. & Landick, R. RNA Polymerase Clamp Movement Aids Dissociation from DNA but Is Not Required for RNA Release at Intrinsic Terminators. *J Mol Biol* **431**, 696-713, doi:10.1016/j.jmb.2019.01.003 (2019).

3 Hudson, B. P. *et al.* Three-dimensional EM structure of an intact activator-dependent transcription initiation complex. *P Natl Acad Sci USA* **106**, 19830-19835, doi:10.1073/pnas.0908782106 (2009).

4 Opalka, N. *et al.* Direct localization of a beta-subunit domain on the three-dimensional structure of Escherichia coli RNA polymerase. *Proc Natl Acad Sci U S A* **97**, 617-622, doi:10.1073/pnas.97.2.617 (2000).

Legends for supplementary videos

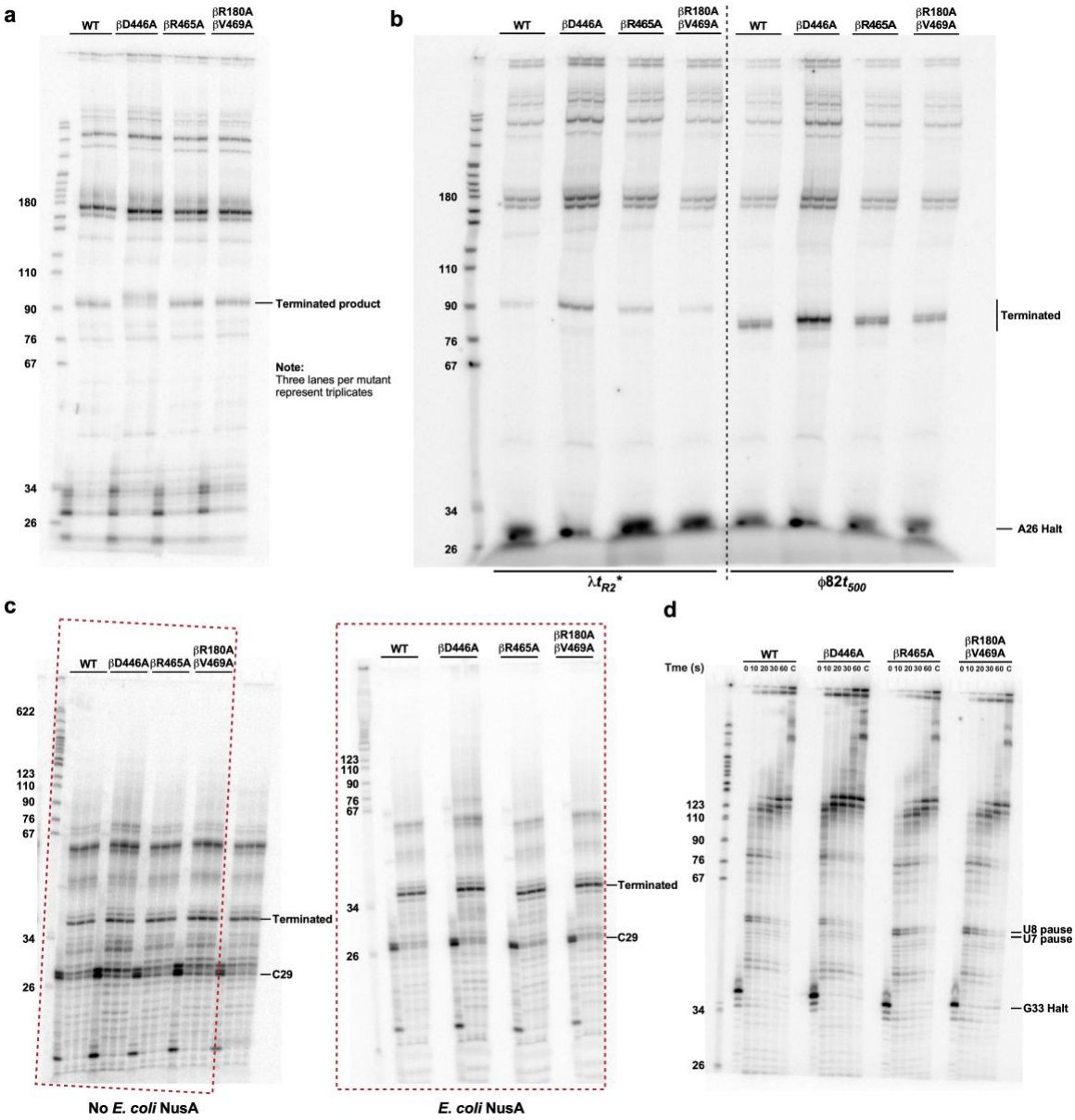
Supplementary Video 1. Cryo-EM map and structural analysis of TTC-pause.

Supplementary Video 2. Cryo-EM map and structural analysis of TTC-hairpin.

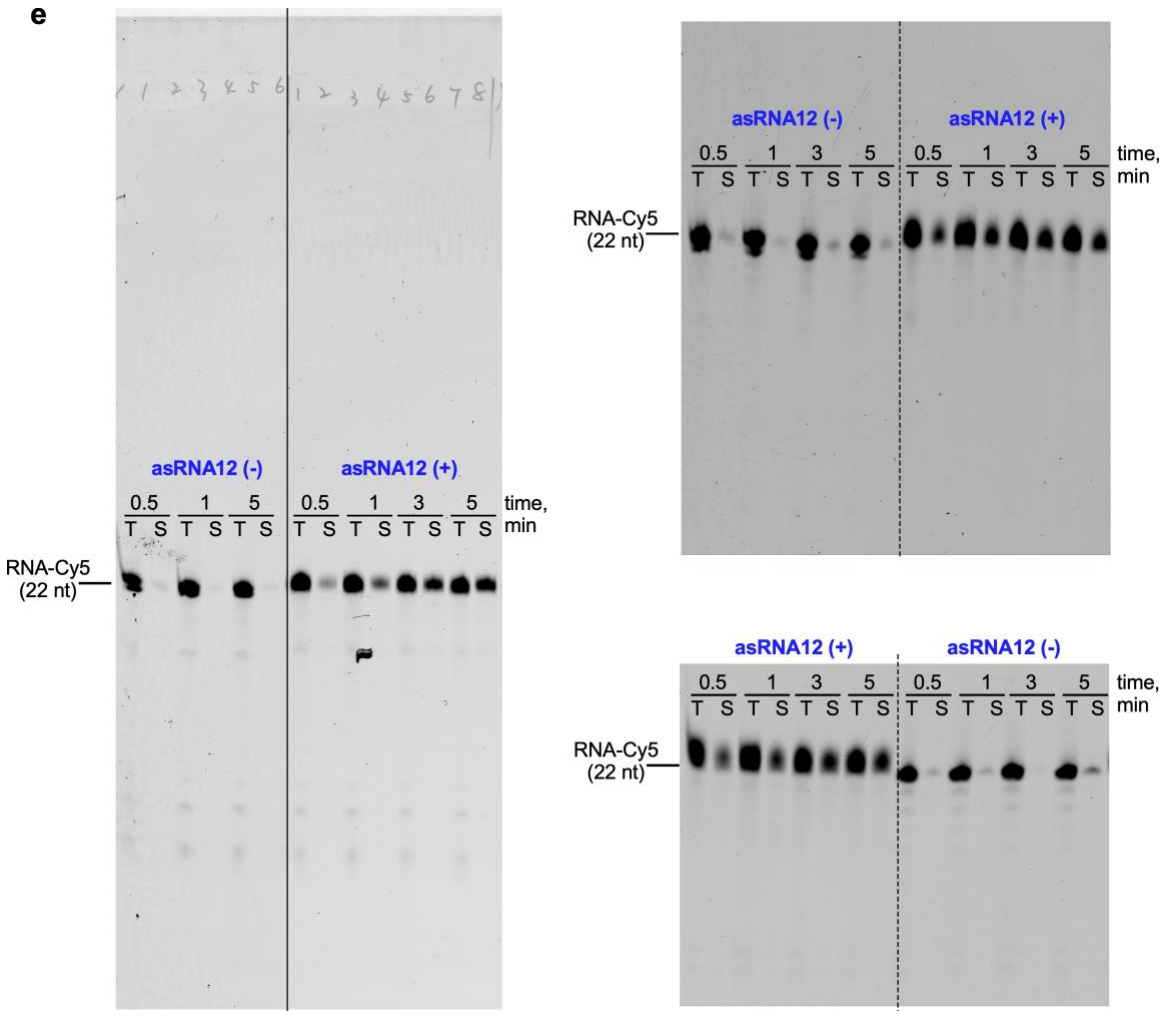
Supplementary Video 3. A proposed route for rewinding of the -10 nucleotides.

Supplementary Video 4. Cryo-EM map and structural analysis of TTC-release.

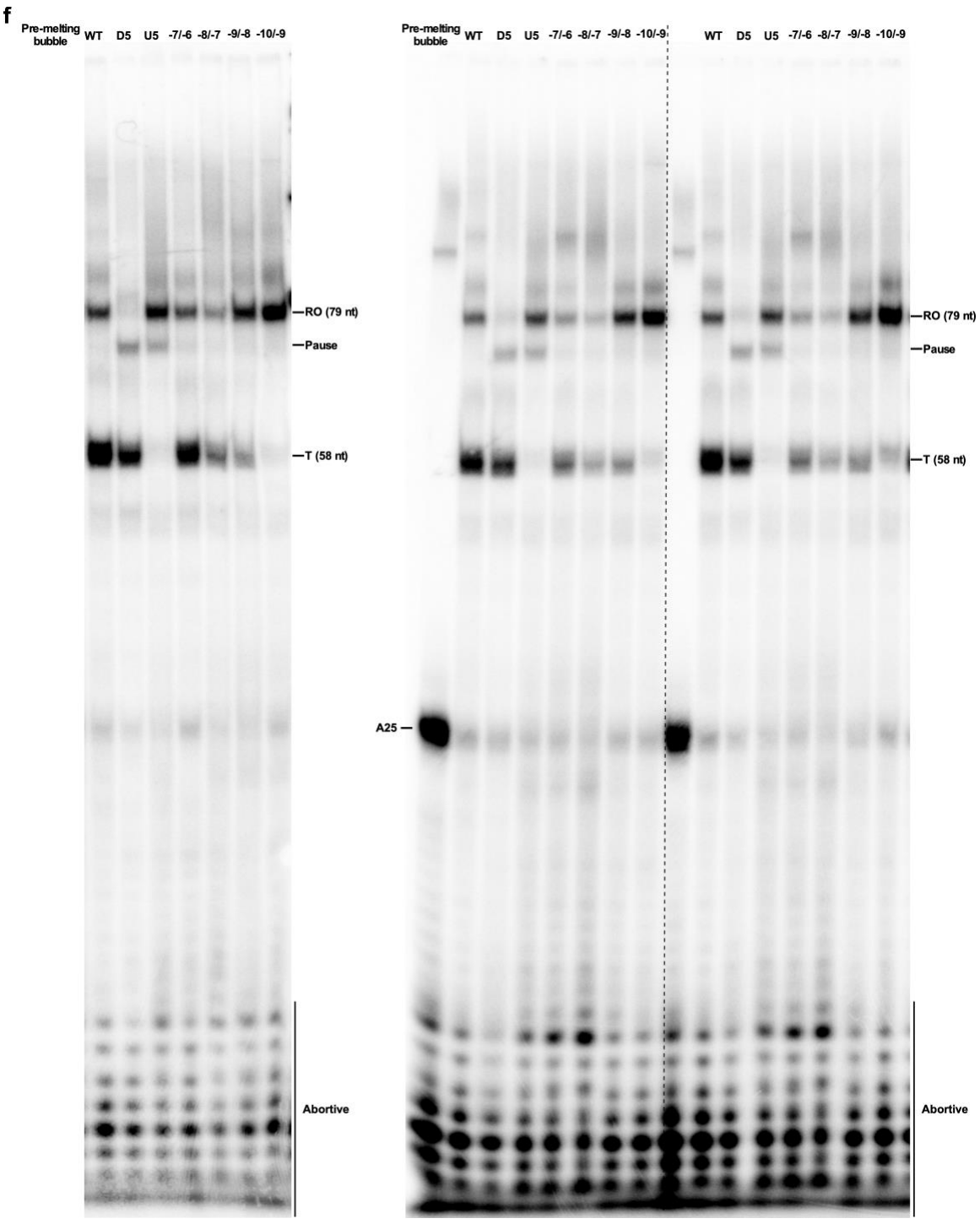
Supplementary Figure 1



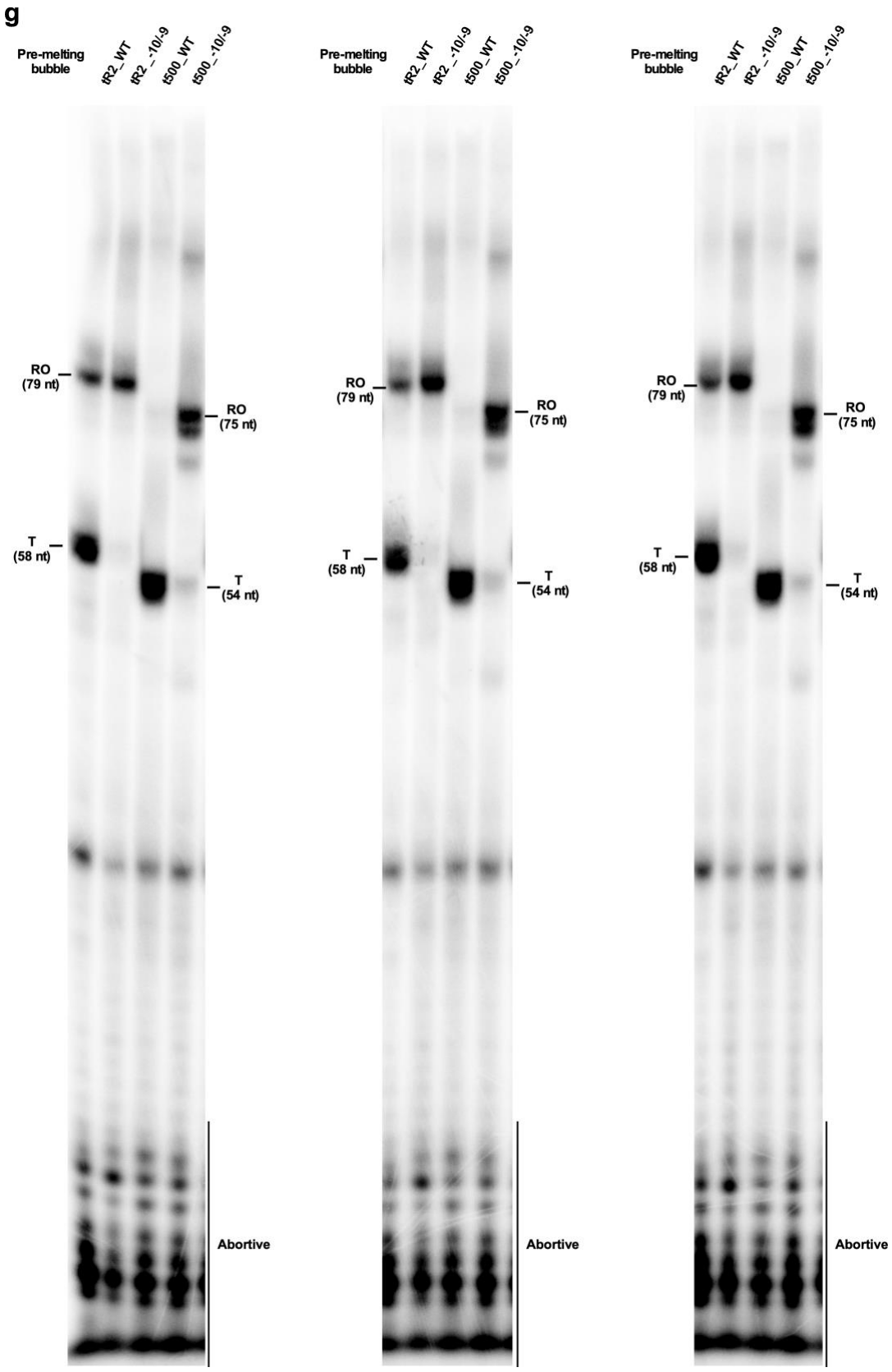
Supplementary Figure 1a-d. The raw data of three replicates for Extended Data Figure 3c (a), 3d-3e (b), 3f (c), and 3g (d).



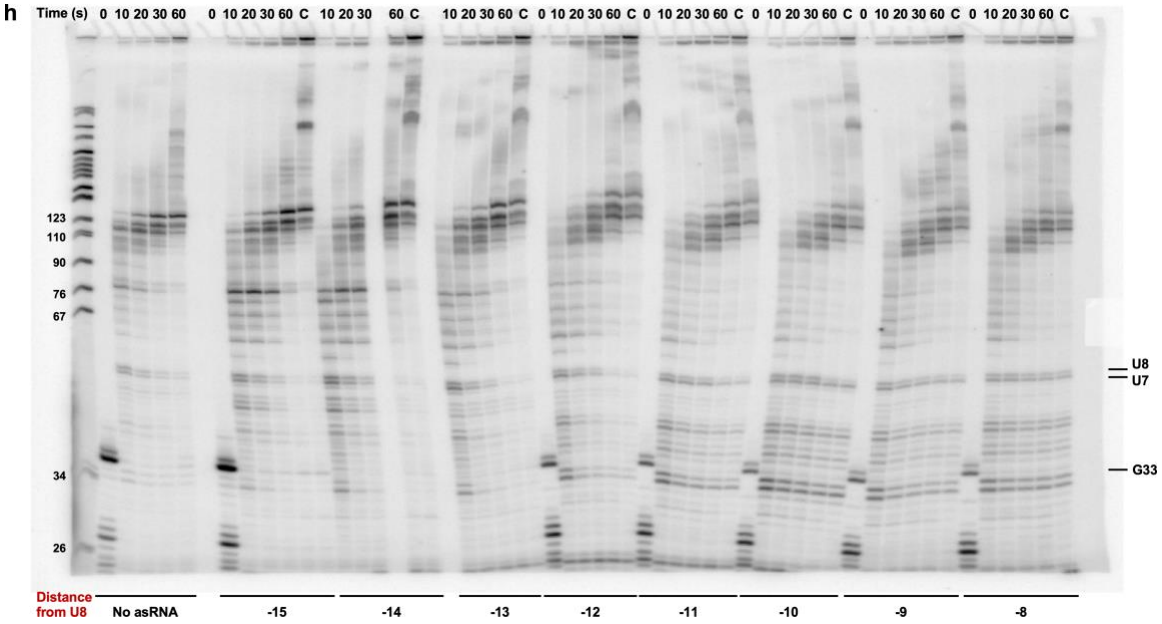
Supplementary Figure 1e. The raw data for Figure 3b. The left panel shows the uncropped gel for Figure 3b and the right panels show two additional replicates.



Supplementary Figure 1f. The raw data for Figure 3g. The left panel shows the uncropped gel for Figure 3g and the right panels show uncropped gel for two additional replicates.

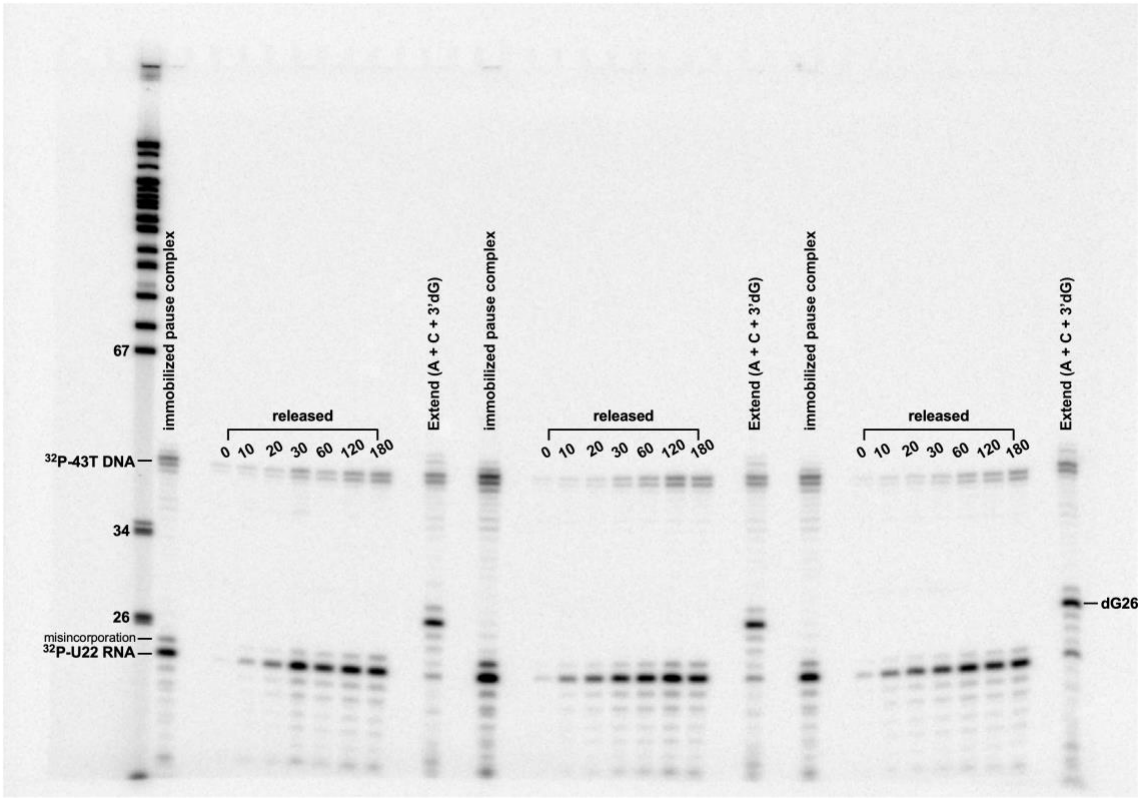


Supplementary Figure 1g. The raw data of three replicates for Extended Data Figure 8b.



Supplementary Figure 1h. The raw data for Extended Data Figure 8c.

i



Supplementary Figure 1i. The raw data of three replicates for Figure 3h.