Supplementary Information to the manuscript:

A role for human DNA polymerase λ in alternative lengthening of telomeres

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Contents:

Supplementary Figure Legends for Figures S1-S8

Supplementary Figures S1-S8

Supplementary Figure Legends

Supplementary Figure S1. A. MMST DNA synthesis by Pol λ in the presence of different combinations of dNTPs and in the presence of both the donor and acceptors strands (lanes 3 - 9) or with the donor strand alone (lanes 10-14). Lane 1, labelled 25 mer donor alone; lane 2, control reaction in the absence of dNTPs. **B.** Pol λ was incubated in the presence of dNTPs, (either single or in combination) with the 5'-labelled dideoxy-terminated16/48 acceptor template. Lane 1, 16 mer labelled oligonucleotide alone; lane 2, control reaction in the absence of dNTPs. **C**. MMST DNA synthesis by Pol β in the absence (lane 1) or in the presence (lanes 2-4) of different concentrations of RP-A and in the presence of the 5'-labelled donor strand and the dideoxy terminated 16/48mer acceptor template. Lane 5, control reaction with Pol λ. **D.** Titration of the five telomeric repeats 5xtel (lanes 1-5) or the single telomeric repeat (lanes 6-10) dideoxy terminated 16/48mer acceptor templates, in the presence of 50 nM Pol λ, RP-A and dGTP.

Supplementary Figure S2. A. Confocal microscopy imaging of Pol λ colocalization with TERRA RNA in Saos-2 cells ectopically expressing c-Myc Pol λ and the corresponding statistical analysis. The white arrow shows a representative colocalization event. **B**. Confocal microscopy imaging of Pol λ and TERRA foci in Saos-2 cells either transfected with the control empty vector (top and mid lane) or with the vector ectopically expressing c-Myc Pol λ

(bottom lane), untreated (top lane) or treated with RNase A before staining (mid and bottom lanes).

Supplementary Figure S3. Original uncropped images of the western blots shown in Fig.1A, lanes 1-4.

Supplementary Figure S4. Original uncropped images of the western blots shown in Fig.1A, lanes 5-6.

Supplementary Figure S5. Original image of the western blots shown in Fig.2H, lanes 1-2. area shown in Figure 2H is boxed.

Supplementary Figure S6. Original uncropped image of the gel shown in Fig. 4G, lanes 1-6. The area between the solid lines was cropped out in Fig. 4G. The omitted lanes represent another Pot1/TTP1 titration with a lower Pol λ concentration.

Supplementary Figure S7. Original uncropped image of the gel shown in Fig.4 H, lanes 1-8.

Supplementary Figure S8. Original uncropped image of the gel shown in Fig.4 E.

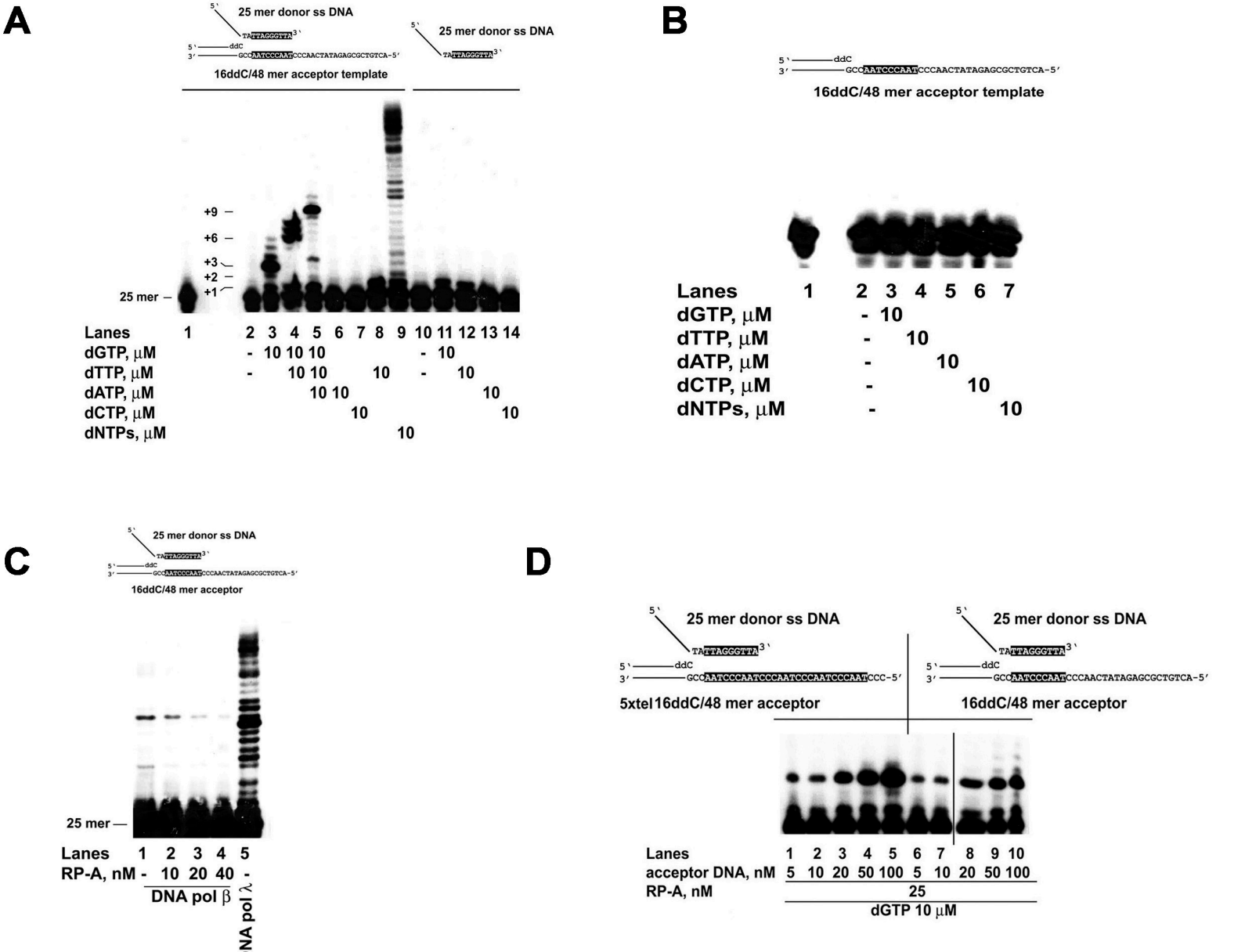
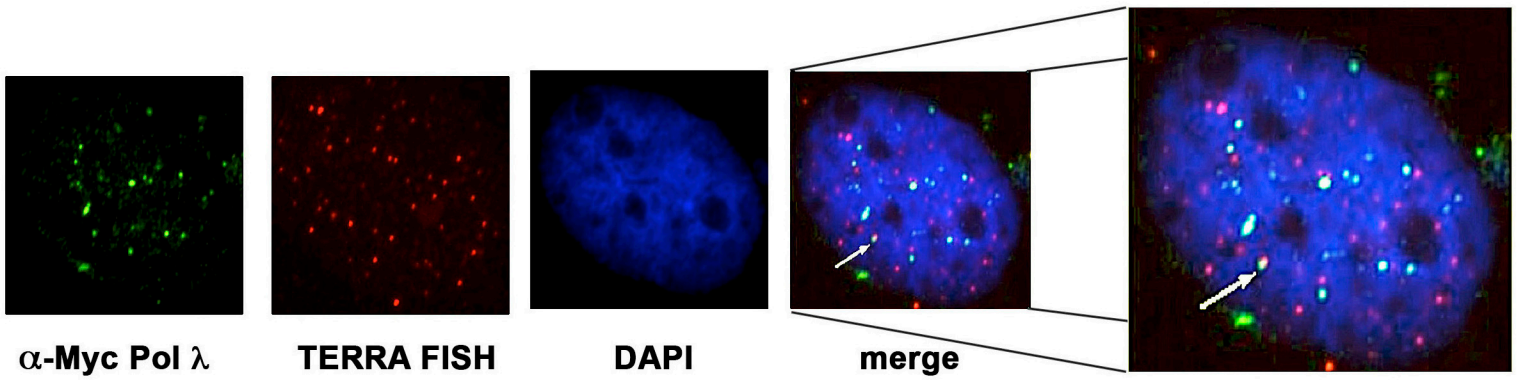

A
25 mer donor ss DNA
16ddC/48 mer acceptor template
+9
+6
+3
+2
+1
25 mer
Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13 14
dGTP, μM - 10 10 10 - 10
dTTP, μM - 10 10 10 - 10
dATP, μM 10 10 10
dCTP, μM 10 10
dNTPs, μM 10
B
16ddC/48 mer acceptor template
Lanes 1 2 3 4 5 6 7
dGTP, μM - 10
dTTP, μM - 10
dATP, μM - 10
dCTP, μM - 10
dNTPs, μM - 10
C
25 mer donor ss DNA
16ddC/48 mer acceptor
25 mer
Lanes 1 2 3 4 5
RP-A, nM - 10 20 40 -
DNA pol β
DNA pol λ
D
25 mer donor ss DNA
5xtel16ddC/48 mer acceptor
25 mer donor ss DNA
16ddC/48 mer acceptor
Lanes 1 2 3 4 5 6 7 8 9 10
acceptor DNA, nM 5 10 20 50 100 5 10 20 50 100
RP-A, nM 25
dGTP 10 μM

Figure S1

A



Cells	Pol λ foci (tot)	TPP1 foci (tot)	Colocalizations expected	Colocalizations observed	χ^2 *p*-value
21	404	1056	4.2	7	p=0.134

B

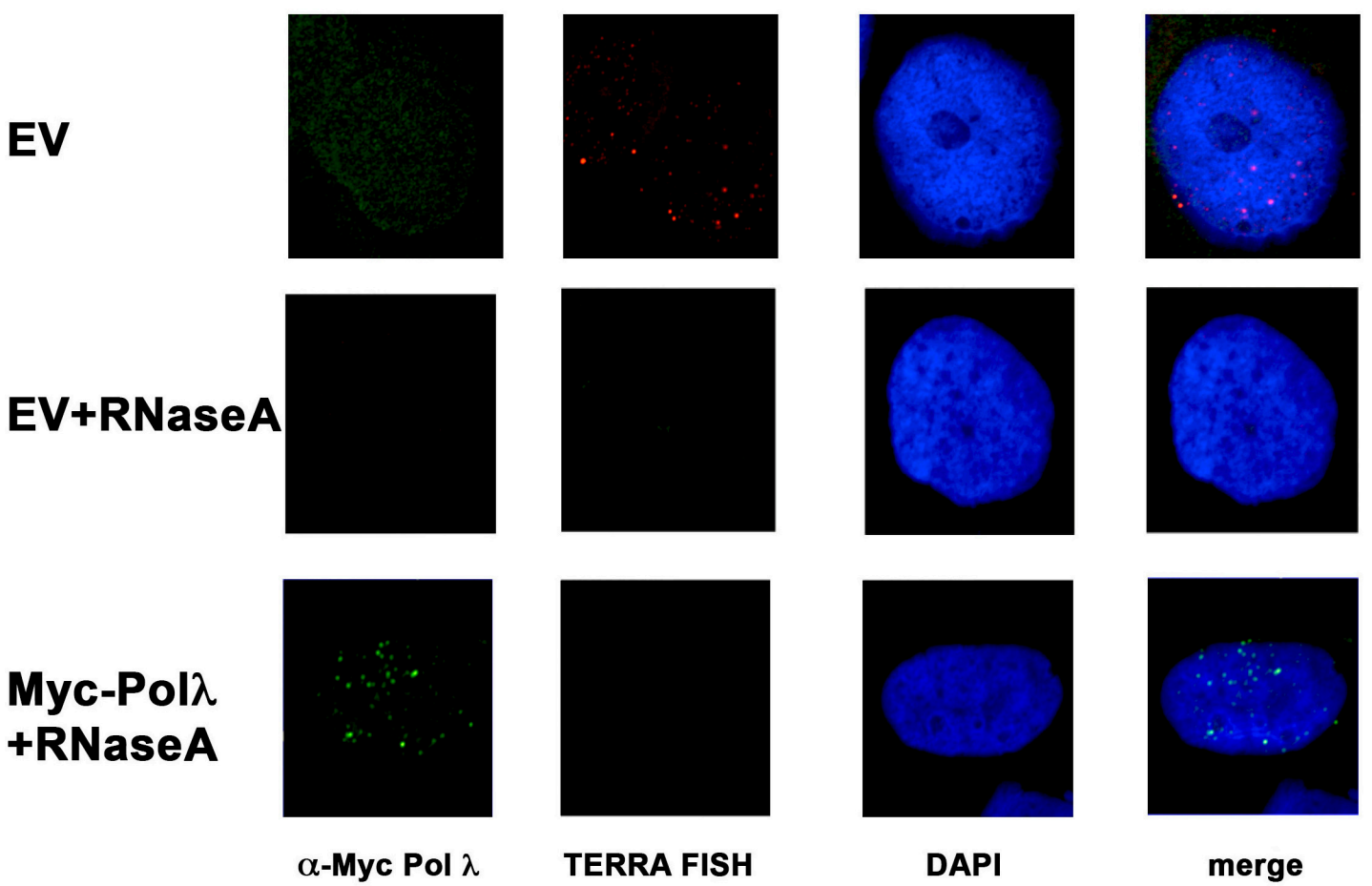


Figure S2

Fig 1 A, lanes 1-4

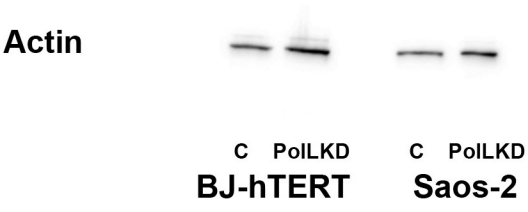
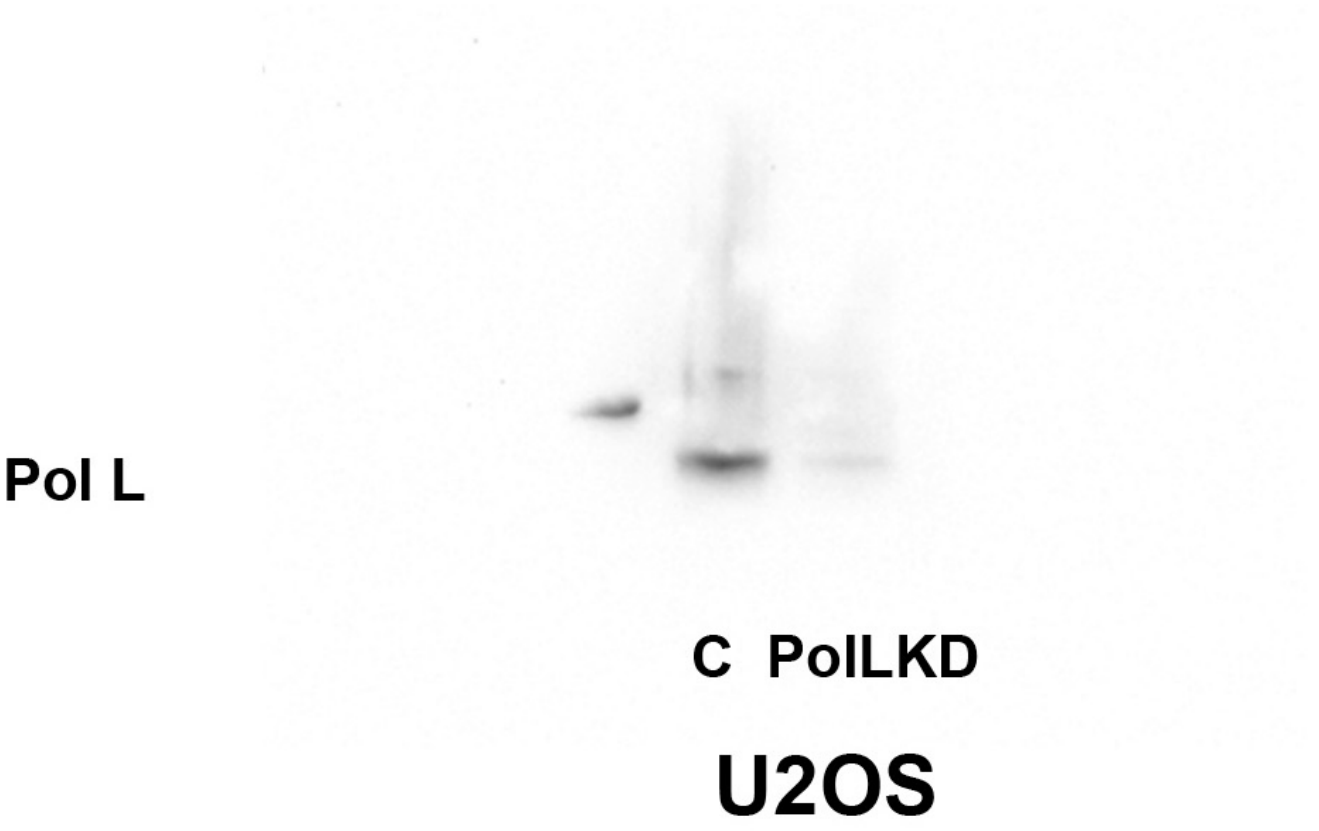


Figure S3

Fig.1A, lanes 5-6



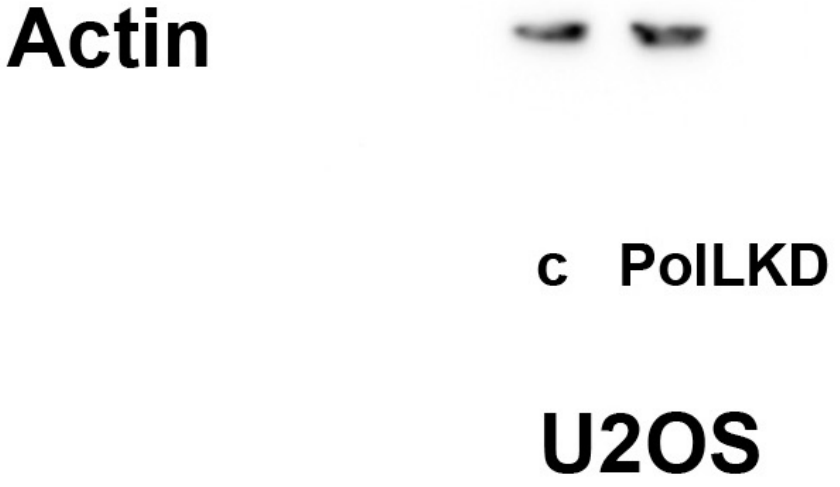


Figure S4

Fig.2H, lanes 1,2

22-01-19 chemidoc

U2OS

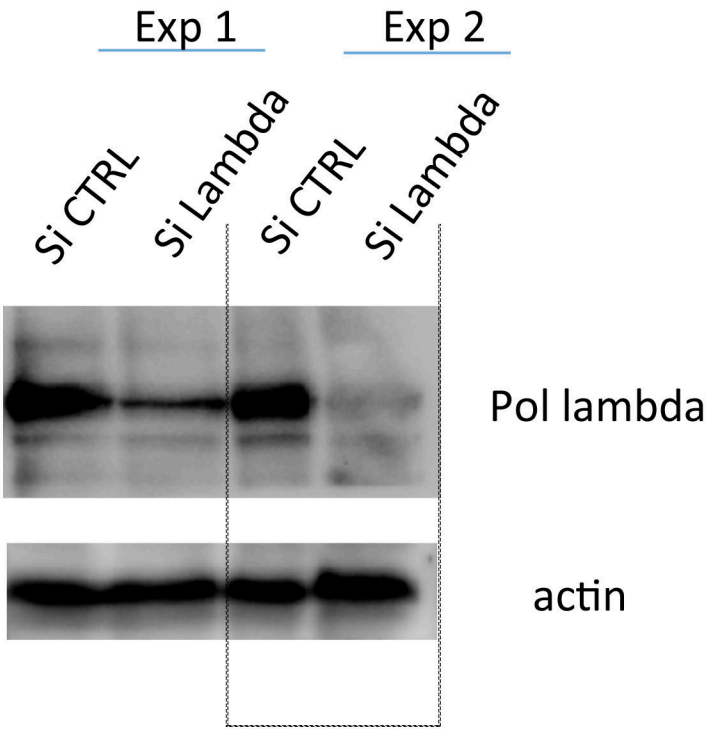


Figure S5

Fig. 4 G lanes 1, 2-6

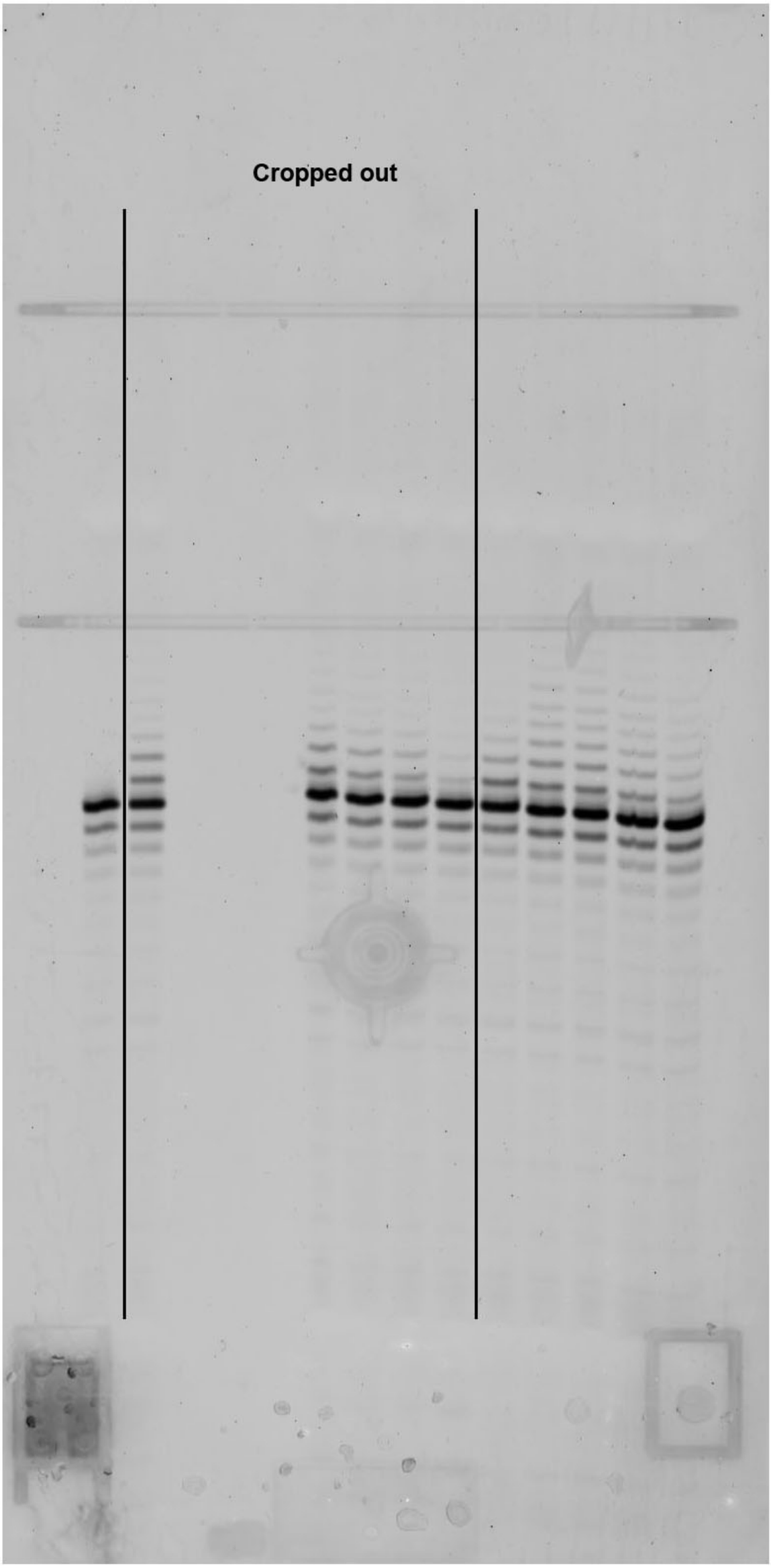


Figure S6

Fig. 4H, lanes 1-8

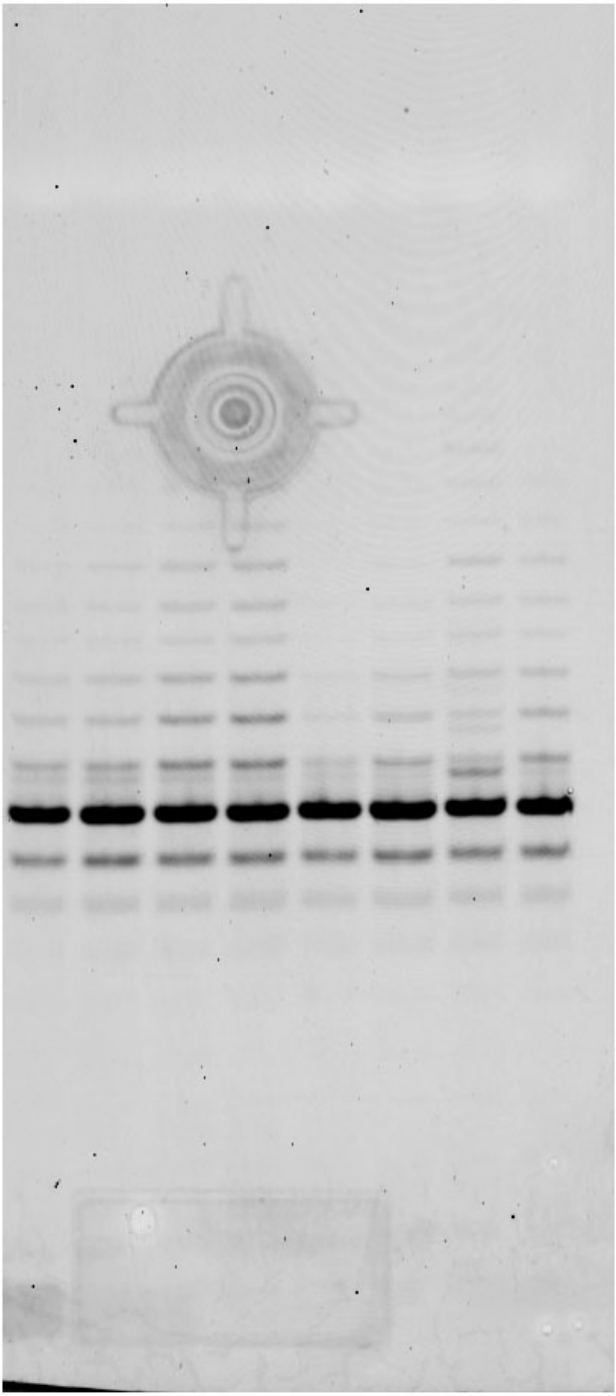


Figure S7

Fig 4 E

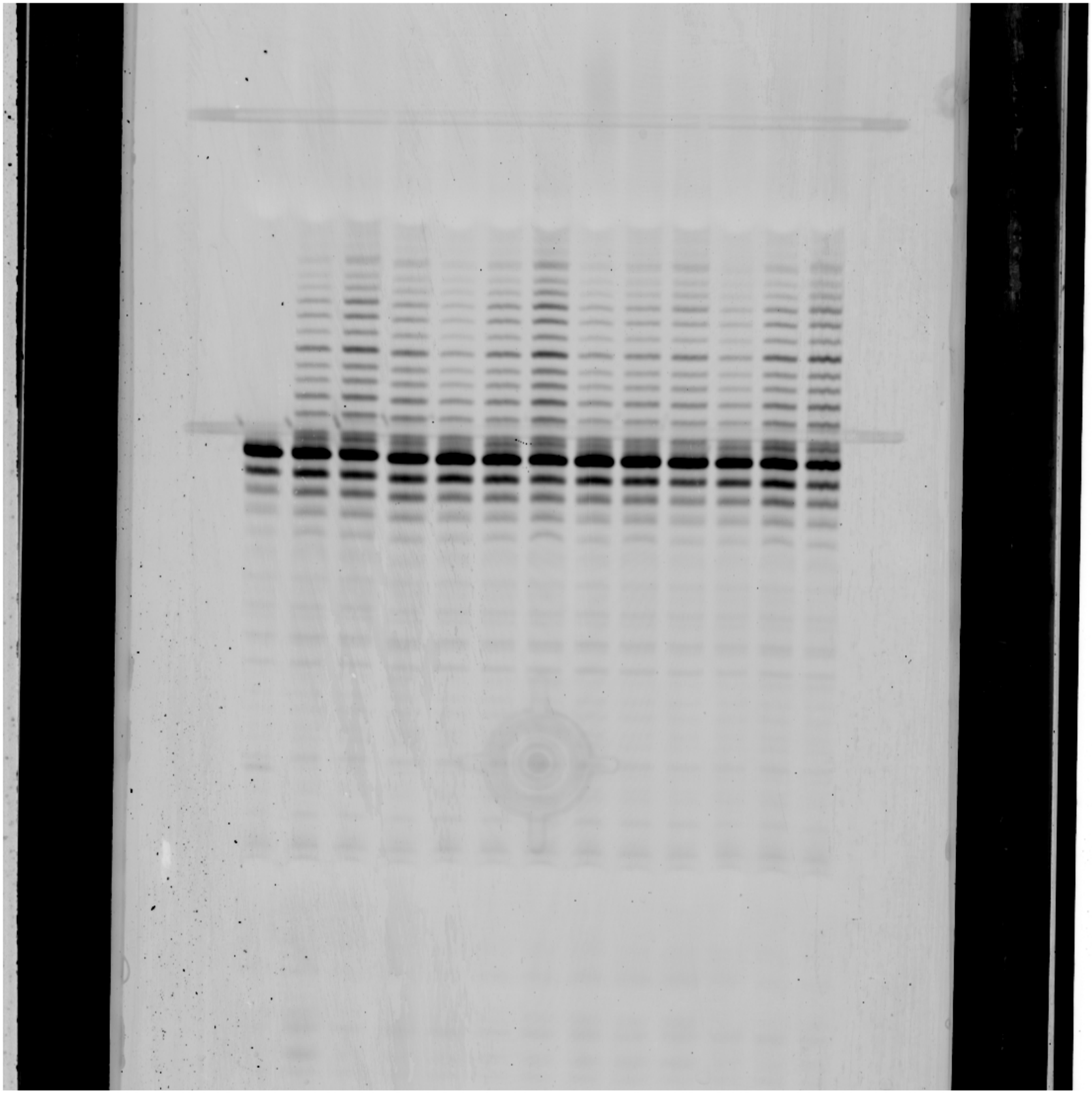


Figure S8