

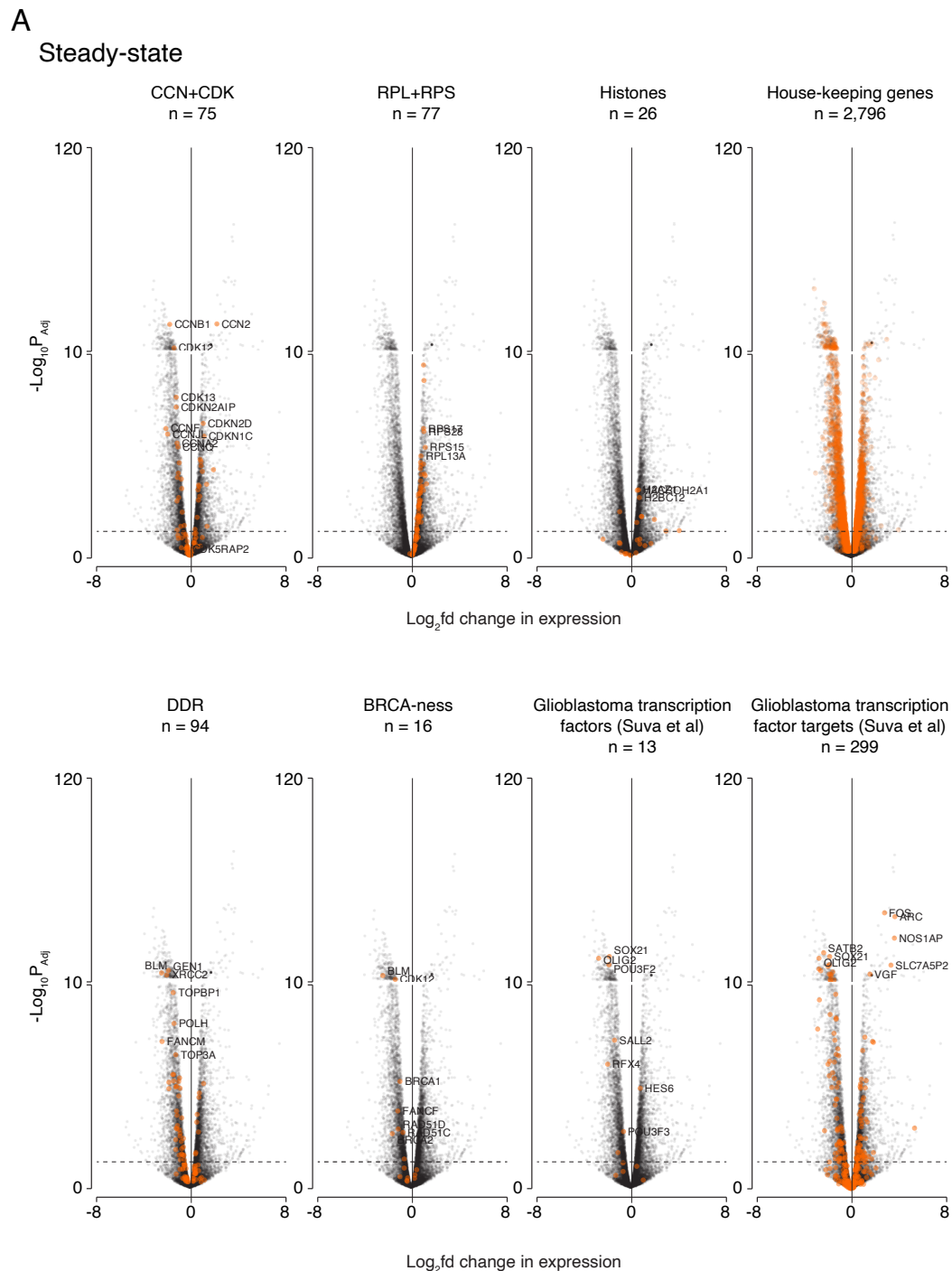
Appendix to *CDK12/13 inhibition disrupts transcriptional elongation and fork progression in glioblastoma*

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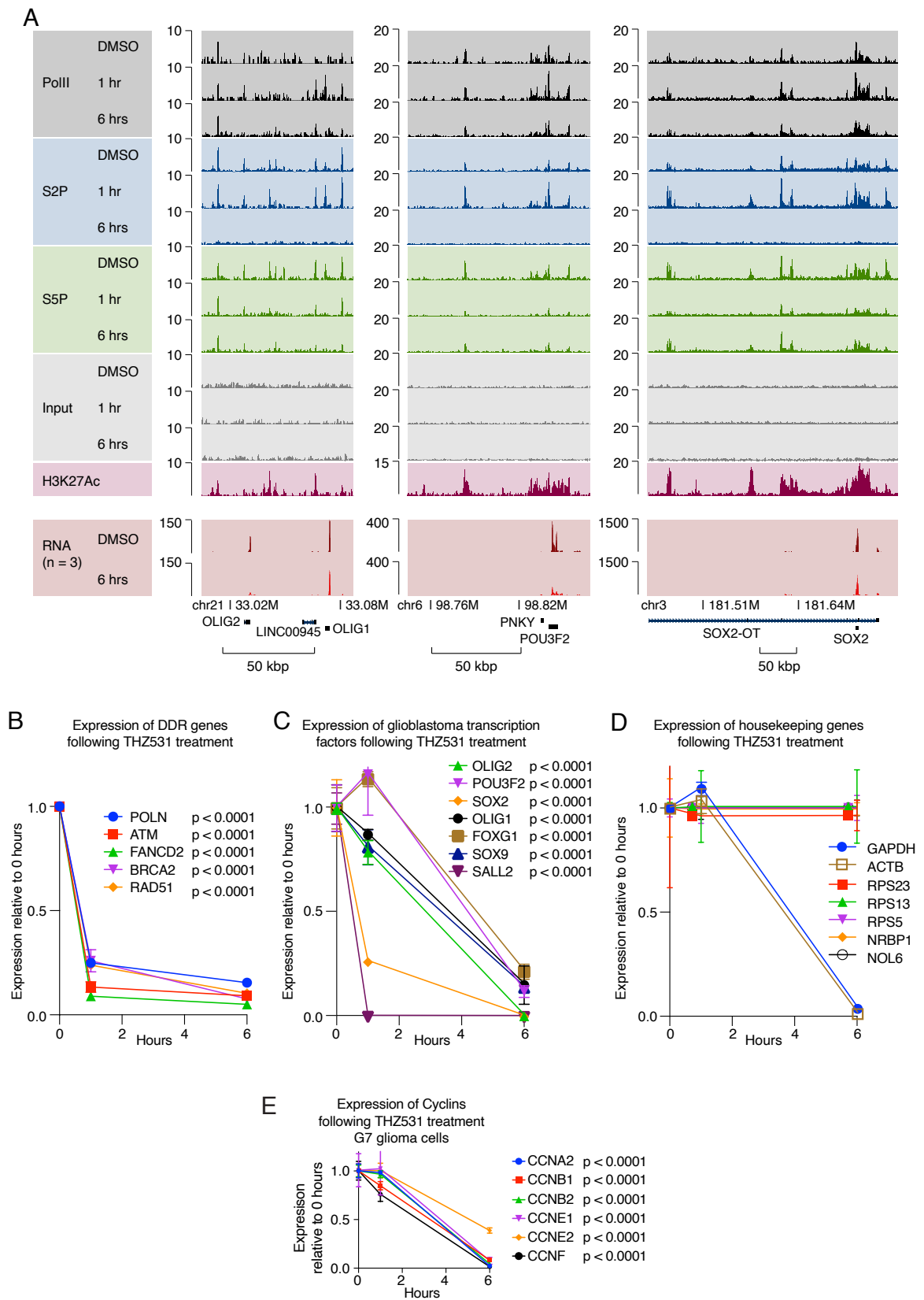
Appendix Figures

Appendix Figure S1



(A) Volcano plots showing the overall transcriptional differences in steady-state transcripts as in Figure 3D, but with certain gene populations highlighted. X-axes show the $\log_2$ fold difference in transcription in G7 cells treated with THZ531 for 6 h compared to DMSO controls. Y-axes show the $-\log_{10}$ transformed p-values Benjamini-Hochberg corrected for multiple testing. Colored dots illustrate the transcriptional changes of the listed gene populations.

Appendix Figure S2



(A) Genome browser tracks of Cut&Run signal density and total transcript-levels (bottom) at the Olig2, Pou3f2 and Sox2 loci at indicated treatments. (B)-(E) RT-qPCR analyses of the mRNA levels of DDR genes (B), glioblastoma transcription factors (C), housekeeping genes

(D) or cell cycle regulators (E) in G7 cells treated with 500 nM THZ531 for 1 h and 6 h. Data represent mean $\pm$ SD of two replicates. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test, significant differences as compared to the starting point (0h).

Appendix Tables

Appendix Table S1: Characteristics of the patients included in the IHC

Patient No.	Slide No.	Age (y)	Sex	Tissue	Diagnosis
1	C12	81	Female	Fascia	Fasciitis
2	C18	40	Male	CNS	Cavernous hemangioma
3	C15	74	Female	CNS	Cerebral amyloid angiopathy (CAA)
4	C14	59	Female	CNS	Glioblastoma, CNS WHO grade 4
5	C16	85	Female	CNS	Glioblastoma, CNS WHO grade 4
6	C17	69	Female	CNS	Glioblastoma, CNS WHO grade 4
7	C19	64	Male	CNS	Glioblastoma, CNS WHO grade 4
8	C21	55	Male	CNS	Recurrent Glioblastoma, CNS WHO grade 4

Appendix Table S2: Brain concentrations of SR-4835 in tolerability studies

Dose (mg/kg)	Regimen	Sample ID	Brain Concentration (ng/mL)	Detection
20	Daily	#1/1	<LLOQ	Not detected
20	Daily	#2/1	<LLOQ	Not detected
20	Daily	#3/1	<LLOQ	Not detected
30	Daily	#4/2	<LLOQ	Not detected
30	Daily	#5/2	7.6	Detected (low)
30	Daily	#6/2	<LLOQ	Not detected
30	Alternate days	#7/3	<LLOQ	Not detected
30	Alternate days	#8/3	<LLOQ	Not detected
30	Alternate days	#9/3	<LLOQ	Not detected

Lower Limit of Quantification. LLOQ = 1.00 ng/mL

Appendix Table S3: Antibody dilution

Antibody	Manufacturer	Catalog number	Dilution for CUT&RUN	Dilution for WB	Dilution for IF
RNAPII total	MBL	MABI0601	1:100	1:10 000	
RNAPII pSer2	MBL	MABI0602	1:100	1:10 000	
RNAPII pSer5	MBL	MABI0603	1:100	1:10 000	
H3K27ac	CST	D5E4	1:100	1:1000	
H3K27me3	CST	C36B11	1:100	1:1000	
RNAPII pThr4	Active Motif	61461	NA	1:1000	
RNAPII pSer7	Active Motif	61087	NA	1:1000	
Vinculin	Sigma	V9131	NA	1:10 000	
PARP	CST	9542	NA	1:1000	
GAPDH	Santa Cruz Biotechnology	sc25778	NA	1:20 000	
IgG	Abcam	ab6721	1:100	NA	
RFP	BioNordika (Rockland)	600-401-379			1:200

Appendix Table S4: P-values**Figure 1C****Two-way ANOVA****Tukey's multiple comparisons test****Summary****P Value****Cell type: G7**

16 days

DMSO vs. 100nM THZ531

0,000807565680004

DMSO vs. 500nM THZ531

0,000806253394743

100nM THZ531 vs. 500nM THZ531	ns	0,999999617121843
Cell type: G144		
16 days		
DMSO vs. 100nM THZ531	****	<0,0000000000000001
DMSO vs. 500nM THZ531	****	<0,0000000000000001
100nM THZ531 vs. 500nM THZ531	ns	0,997777369708783
Cell type: Hela		
14 days		
DMSO vs. 100 nM THZ531	****	0,000000467842452
DMSO vs. 500 nM THZ531	****	0,000000000400941
100 nM THZ531 vs. 500 nM THZ531	****	<0,0000000000000001

Figure 1G

Two-way ANOVA

Tukey's multiple comparisons test	Summary	P Value
NC		
day 0 vs. day 7	ns	0,999976191524814
day 0 vs. day 14	ns	0,970651962495543
day 0 vs. day 21	ns	0,983512179424494
sg_MCM2		
day 0 vs. day 7	ns	0,621972681697333
day 0 vs. day 14	****	0,000001724614945
day 0 vs. day 21	****	0,000000003441056
sg_RPS19		
day 0 vs. day 7	*	0,032946463771858
day 0 vs. day 14	****	0,00000885195386
day 0 vs. day 21	****	0,000000906224435
sg1_CDK9		
day 0 vs. day 7	ns	0,093696896150888
day 0 vs. day 14	****	0,000022282175578
day 0 vs. day 21	****	0,000000152732645
sg2_CDK9		
day 0 vs. day 7	ns	0,781276693650005
day 0 vs. day 14	ns	0,420027399424275
day 0 vs. day 21	*	0,048822205674024

sg3_CDK9		
day 0 vs. day 7	*	0,01495519069165
day 0 vs. day 14	***	0,000221124721562
day 0 vs. day 21	****	0,000018827665707
sg1_CDK12		
day 0 vs. day 7	ns	0,118741854461276
day 0 vs. day 14	*	0,014955190691651
day 0 vs. day 21	**	0,002497995965609
sg2_CDK12		
day 0 vs. day 7	ns	0,075425742134187
day 0 vs. day 14	*	0,011873203341995
day 0 vs. day 21	**	0,003220572882048
sg3_CDK12		
day 0 vs. day 7	ns	0,327886559841536
day 0 vs. day 14	ns	0,226308696537047
day 0 vs. day 21	**	0,004904037932036
sg1_CDK13		
day 0 vs. day 7	ns	0,139901702807128
day 0 vs. day 14	*	0,011444028413037
day 0 vs. day 21	***	0,00057409884113
sg2_CDK13		
day 0 vs. day 7	*	0,034448799757469
day 0 vs. day 14	**	0,002272987932976
day 0 vs. day 21	****	0,000038563662239
sg3_CDK13		
day 0 vs. day 7	ns	0,074672056266458
day 0 vs. day 14	ns	0,451311004268309
day 0 vs. day 21	****	0,000000387327652

Figure 3A

Independent two-sample t-test (SciPy library)

	Summary	P value
G7		
control-gefitinib	**	0,0035407
control-THZ531	****	0,000000003146

Gefitinib-THZ531	****	0,0000021645
G144		
control-gefitinib	****	0,000098938
control-THZ531	****	0,000000005489
Gefitinib-THZ531	****	0,0000011153
G14		
control-gefitinib	**	0,0032866
control-THZ531	***	0,0002912
Gefitinib-THZ531	ns	0,10051
G166		
control-gefitinib	*	0,013192
control-THZ531	****	0,0000031442
Gefitinib-THZ531	***	0,00029259

Figure EV1D

Two-way ANOVA

Tukey's multiple comparisons test	Summary	P Value
THZ531		
Cell line: G14		
Day 10		
DMSO vs. 100nM THZ531	****	0,00000127
DMSO vs. 500nM THZ531	****	0,00000019
100nM THZ531 vs. 500nM THZ531	ns	0,192712
Cell line: G166		
Day 10		
DMSO vs. 100nM THZ531	****	0,0000104
DMSO vs. 500nM THZ531	****	0,000000466
100nM THZ531 vs. 500nM THZ531	*	0,045061
SR-4835		
Cell line: G14		
Day 10		
DMSO vs. 100nM SR-4835	****	0,000000290
DMSO vs. 500nM SR-4835	****	0,000000156
100nM SR-4835 vs. 500nM SR-4835	ns	0,797952

Cell line: G144

Day 9

DMSO vs. 100nM SR-4835	****	0,000000242
DMSO vs. 500nM SR-4835	****	0,00000024
100nM SR-4835 vs. 500nM SR-4835	ns	0,999972

Cell line: G166

Day 10

DMSO vs. 100nM SR-4835	****	0,000000000000417
DMSO vs. 500nM SR-4835	****	0,000000000000395
100nM SR-4835 vs. 500nM SR-4835	ns	0,957067