Reversible phosphorylation of cyclin T1 promotes assembly and stability of P-TEFb Fig. 1. Critical residues in CycT1 (Thr143 and Thr149) are required for its binding to CDK9





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Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Fig. 1. Critical residues in CycT1 (Thr143 and Thr149) are required for its binding to CDK9





Red marked area was used for the figure. Right lanes are the same proteins with different conditions





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Left lanes are the same proteins with different conditions





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Middle lanes are the same proteins with different conditions Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure. Left lanes are the same proteins with different conditions Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure. Right lanes are the same proteins with different conditions Membrane was cut after transfer, and before probing by Ab Fig. 1. Critical residues in CycT1 (Thr143 and Thr149) are required for its binding to CDK9





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Membrane was cut after transfer, and before probing by Ab Right lanes are the same proteins under different conditions



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab





Red marked area was used for the figure. Right lane are the same protein for the control, CycT1TT143149AA



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Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Fig. 1. Critical residues in CycT1 (Thr143 and Thr149) are required for its binding to CDK9

E	IP:	lgG	CDK9					
ł	n:CycT1	+	+	-	-	-	-	
h:CycT14MUT			-	+	-	-	-	
h:CycT	-	-	-	+	-	-		
h:CycT	-	-	-	-	+	-		
h:CycT	-	-	-	-	-	+		
bortezomib (2µM)		+	+	+	+	+	+	
WB	CycT1		-					
	CDK9		-	-	-	-	-	
		1	2	3	4	5	6	
Input	CycT1			-	-	-	-	
	CDK9		-	-	-	_	_	



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Left lanes are same proteins for other conditions



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Left lanes are same proteins for other conditions



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Fig. 1. Critical residues in CycT1 (Thr143 and Thr149) are required for its binding to CDK9

F	IP:		CDK9				
h:Cyc⁻	+	+	-	-	-		
h:CycT1(280)4MUT			-	+	-	-	
h:CycT1(280)T3A			-	-	+	-	
h:CycT1(280)TT143,149AA			-	-	-	+	
bortezomik	+	+	+	+	+		
	CycT1						
WB	CDK9			_	-	-	
	1	2	3	4	5		
Input	CycT1		-	-	-	-	
input	CDK9		-	-	-	-	



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Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Two input proteins blots are in the same slide Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9





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Membrane was cut after transfer, and before probing by Ab Two antibodies are used in the same time for input proteins Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9









Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9



Source gel-staining for Figure 2D



Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9



Source gel staining for Figure 2E



Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9



Ε

Source gel staining for Figure 2E



Fig. 2. Phosphorylation of Thr143 and Thr149 in CycT1 contributes to its binding to CDK9



Fig.3. Phosphorylation of Thr143 and Thr149 stabilizes the interface between CycT1 and CDK9







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Membrane was cut after transfer, and before probing by Ab Two input proteins bolts are in the slide Fig.3. Phosphorylation of Thr143 and Thr149 stabilizes the interface between CycT1 and CDK9





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Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab



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Fig.3. Phosphorylation of Thr143 and Thr149 stabilizes the interface between CycT1 and CDK9







Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lanes are the same proteins under different conditions



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lanes are the other proteins under different conditions



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Membrane was cut after transfer, and before probing by Ab Two antibodies are used in the same time for input proteins Fig.3. Phosphorylation of Thr143 and Thr149 stabilizes the interface between CycT1 and CDK9





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The beside lane is the same protein with higher concentration of staurosporine



Red marked area was used for the figure.

The beside lane is the same protein with higher concentration of staurosporine other lanes are the same protein under different conditions



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lane is the same protein with higher concentration of staurosporine



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lane is the same protein with higher concentration of staurosporine Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation








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Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation







Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab upper bands are heavy chain



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Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation





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Membrane was cut after transfer, and before probing by Ab Right four lanes are for Figure 4E Others are under different conditions



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right four lanes are for Figure 4E Others are under different conditions



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Right four lanes are for Figure 4E Others are under different conditions Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation





Membrane was cut after transfer, and before probing by Ab Left four lanes are for Figure 4D



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Membrane was cut after transfer, and before probing by Ab Left four lanes are for Figure 4D



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left four lanes are for Figure 4D Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation









Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Two different cut membranes are in the same tray for imaging Fig. 4. PKC inhibitors impair interactions between CycT1 and CDK9, and promote CycT1 degradation

Activated Primary CD4+ T cells









Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Right lanes are other proteins with same tag



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Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





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Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab upper and lower bands are heavy chains and light chains



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Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab other bands are same proteins under different conditions



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab other bands are same proteins under different conditions





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab other bands are same proteins under different conditions

Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab







Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1







Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lanes are the same proteins under different conditions



Fig. 5. PKCα and PKCβ bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





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Source gel-staining for Figure 5G



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Fig. 5. PKC α and PKC β bind to CycT1 for its phosphorylation, also promote interactions between CycT1 and CDK9, and increase the stability of CycT1





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right 2 lanes the same proteins under PMA treatment for 120 h and 144 h



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right 2 lanes the same proteins under PMA treatment for 120 h and 144 h





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Membrane was cut after transfer, and before probing by Ab Right 2 lanes the same proteins under PMA treatment for 120 h and 144 h 3 different membranes are in the same tray for imaging Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells





Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lanes the same proteins from 2nd donor



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lanes the same proteins from 2nd donor



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab









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Membrane was cut after transfer, and before probing by Ab Right lanes the same proteins from 2nd donor Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells





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Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left lane is the same protein without treatment



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Membrane was cut after transfer, and before probing by Ab Left lane is the same protein without treatment Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells

Activated Primary CD4+ T cells


Source blot for Figure 6D



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Source blot for Figure 6D



Red marked area was used for the figure.

Source blot for Figure 6D



Red marked area was used for the figure.

Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells

Mouse anergic T cells

Source data for Figure 6E

Gene Ccnt1 Cdk9

Ensembl_ID ENSMUSG00000011960 ENSMUSG00000009555

Description	Peripheral OT2 T cells			Peripheral W131AOT2		
cyclin T1 [Source:MGI Symbol;Acc:MGI:1328363]	3808	4229.6	4076.9	3608.1	3656.1	4889.9
cyclin-dependent kinase 9 (CDC2-related kinase) [Sour	4060.6	4500	4485.6	4459.2	5112.8	5461.8
CvcT1/OTI	3808	4038 167	0 943002	1		
eyer nem	4229.6	4038 167	1 047406			
	4076.9	4038.167	1.009592			
CycT1/W131AOTII	3608.1	4038.167	0.8935	1.003269	0.17993	
	3656.1	4038.167	0.905386			
	4889.9	4038.167	1.210921			
CDK9/OTII	4060.6	4348.733	0.933743	1		
	4500	4348.733	1.034784			
	4485.6	4348.733	1.031473			
CDK9/W131AOTII	4459.2	4348.733	1.025402	1.152351	0.117035	
	5112.8	4348.733	1.175699			
	5461.8	4348.733	1.255952			

CycT1/OTII	1	0
CycT1/W131AOTII	1.003269	0.17993
CDK9/OTII	1	0
CDK9/W131AOTII	1.152351	0.117035



Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells

Mouse anergic T cells



Source blot for Figure 6F



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lane is the same protein without treatment, same as lane 1

Source blot for Figure 6F



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lane is the same protein without treatment, same as lane 1 Source blot for Figure 6F



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Right lane is the same protein without treatment, same as lane 1 Fig. 6. Depletion of PKCs leads to decreased levels of CycT1 in cell lines and primary cells



Figure supplement 1. Thr143 and T149 are the phosphorylated residues in CycT1



NetPhos 3.1 Server - prediction results



Technical University of Denmark

>:	Sequence 43 amino	acio	ds								
# # #	netphos-3.1b prediction	re	sult	5							
#	Sequence	#	x	Context	Score	Kinase	Answer				
#	Sequence	3	Y	EAYLQQV	0.409	EGFR					
#	Sequence	3	Y	EAYLQQV	0.360	INSR	•				
# #	Sequence	3	Y	EAYLQQV	0.343	unsp SRC	:				
#	bequenee	5	-	21112981	0.010	Ditto					
#	Sequence	15	s	VILESIILQ	0.445	CaM-II	•				
#	Sequence	15	S	VILESIILQ	0.443	PKC	•				
# #	Sequence	15	s	VILESIILQ	0.429	GSK3 CKTT	•				
#	Sequence	15	s	VILESIILQ	0.413	PKA	:				
#	Sequence	15	s	VILESIILQ	0.387	CKI					
#	Sequence	15	S	VILESIILQ	0.363	cdc2	•				
#	Sequence	15	S	VILESIILQ	0.345	DNAPK	•				
#	Sequence	15	s	VILESTILO	0.307	RSK	÷				
#	Sequence	15	s	VILESIILQ	0.266	ATM					
#	Sequence	15	s	VILESIILQ	0.252	PKG					
#	Sequence	15	S	VILESIILQ	0.124	cdk5	·				
#	Sequence	15	s	VILESIILO	0.008	INSD	·				
#	bequence	15	5	VIDBOIIDQ	0.000	unap	•				
#	Sequence	20	т	IILQTLGFE	0.672	PKC	YES				
#	Sequence	20	т	IILQTLGFE	0.463	cdc2	•				
#	Sequence	20	T	IILQTLGFE	0.437	PKA	•				
#	Sequence	20	Ť	TILOTLGFE	0.423	CaM-TT	÷				
#	Sequence	20	т	IILQTLGFE	0.374	CKI					
#	Sequence	20	т	IILQTLGFE	0.371	CKII					
#	Sequence	20	Т	IILQTLGFE	0.350	DNAPK	•				
# #	Sequence	20	T	TILOTLGFE	0.313	р38МАРК аттм	•				
#	Sequence	20	T	IILOTLGFE	0.228	PKG	÷				
#	Sequence	20	т	IILQTLGFE	0.220	RSK					
#	Sequence	20	т	IILQTLGFE	0.161	cdk5	•				
#	Sequence	20	Т	IILQTLGFE	0.089	PKB	•				
#	Sequence	20	т	TIPALPE	0.049	unsp	•				
#	Sequence	26	т	GFELTIDHP	0.680	unsp	YES				
#	Sequence	26	т	GFELTIDHP	0.553	CKI	YES				
#	Sequence	26	Т	GFELTIDHP	0.457	CaM-II	·				
# #	Sequence	26	T	GFELTIDHP	0.434	GSK3	•				
#	Sequence	26	T	GFELTIDHP	0.420	CKII	÷				
#	Sequence	26	т	GFELTIDHP	0.349	DNAPK					
#	Sequence	26	т	GFELTIDHP	0.338	рЗ8МАРК	•				
#	Sequence	26	Т	GFELTIDHP	0.258	ATM	•				
#	Sequence	26	T	GFELTIDHP	0.246	RSK	÷				
#	Sequence	26	т	GFELTIDHP	0.182	cdk5					
#	Sequence	26	т	GFELTIDHP	0.150	PKC					
#	Sequence	26	т	GFELTIDHP	0.130	PKA	•				
# #	sequence	26	т	GFELTIDHP	0.085	PKB	·				
#	Sequence	32	т	DHPHTHVVK	0.468	GSK3					
#	Sequence	32	т	DHPHTHVVK	0.405	cdc2					
#	Sequence	32	т	DHPHTHVVK	0.404	CaM-II	·				
#	Sequence	32	T	DHPHTHVVK	0.400	p38MAPK CKT	·				
#	Sequence	32	Ť	DHPHTHVVK	0.373	CKII	:				
#	Sequence	32	т	DHPHTHVVK	0.343	DNAPK					
#	Sequence	32	т	DHPHTHVVK	0.282	unsp	·				
#	Sequence	32	T	DHPHTHVVK	0.243	ATM	·				
#	Sequence	32	Ť	DHPHTHVVK	0.231	RSK					
#	Sequence	32	т	DHPHTHVVK	0.200	cdk5					
#	Sequence	32	т	DHPHTHVVK	0.195	PKC					
#	Sequence	32	Т	DHPHTHVVK	0.108	PKA	•				
# #	sequence	32	т	DHPHTHVVK	0.080	PKB	·				
#	Sequence	38	т	VVKCTQLVR	0.431	ATM					
#	Sequence	38	т	VVKCTQLVR	0.423	GSK3					
#	Sequence	38	т	VVKCTQLVR	0.423	PKG	•				
#	Sequence	38	T	VVKCTQLVR	0.413	CaM-II	·				
#	Sequence	38	T	VVKCTQLVK	0.402	cdc2	:				
#	Sequence	38	Ť	VVKCTQLVR	0.373	DNAPK					
#	Sequence	38	т	VVKCTQLVR	0.361	CKI					
#	Sequence	38	т	VVKCTQLVR	0.324	CKII	·				
#	Sequence	38	T	VVKCTQLVR	0.263	PKC	·				
#	Sequence	38	Ť	VVKCTOLVR	0.216	PKA					
#	Sequence	38	т	VVKCTQLVR	0.175	cdk5					
#	Sequence	38	т	VVKCTQLVR	0.079	PKB	•				
#	Sequence	38	т	VVKCTQLVR	0.026	unsp	·				
#	# EAYLOOVODLVILESIILOTLGFELTIDHPHTHVVKCTOLVRA # 50										

81T....T.



Source blot for Figure supplement 1B



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Source blot for Figure supplement 1B



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Fig. S1.



Source blot for Figure supplement 1C



Fig. S1.



Source blot for Figure supplement 2A



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Source blot for Figure supplement 2A



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Membrane was cut after transfer, and before probing by Ab Two different cut membranes are in the same tray for imaging Fig. S2. PKC inhibitors promote CycT1 degradation in different cells



Source blot for Figure supplement 2B



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left lanes are the same proteins under different conditions

Source blot for Figure supplement 2B



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left lanes are the same proteins under different conditions Source blot for Figure supplement 2B



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left lanes are the same proteins under different conditions Fig. S2. PKC inhibitors promote CycT1 degradation in different cells



Source blot for Figure supplement 2C



Source blot for Figure supplement 2C





Source blot for Figure supplement 2C



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Two different cut membranes are in the same tray for imaging Fig. S2. PKC inhibitors promote CycT1 degradation in different cells



Source blot for Figure supplement 2D



Source blot for Figure supplement 2D



Source blot for Figure supplement 2D



Fig. S2. PKC inhibitors promote CycT1 degradation in different cells

Activated Primary CD4+ T cells



Source blot for Figure supplement 2E



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Source blot for Figure supplement 2E



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Fig. S2. PKC inhibitors promote CycT1 degradation in different cells

Activated Primary CD4+ T cells



Source blot for Figure supplement 3A



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Fig. S3. PKC δ , PKC γ , and PKC ϵ bind weakly to CycT1





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Membrane was cut after transfer, and before probing by Ab Other lanes are different proteins under different conditions



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Membrane was cut after transfer, and before probing by Ab Other lanes are the same proteins under different conditions



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Membrane was cut after transfer, and before probing by Ab Other lanes are the same proteins under different conditions



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Fig. S3. PKCδ, PKCγ, and PKCε bind weakly to CycT1





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Membrane was cut after transfer, and before probing by Ab Other lanes are different proteins under different conditions



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Membrane was cut after transfer, and before probing by Ab Other lanes are the same proteins under different conditions



Red marked area was used for the figure. Membrane was cut after transfer, and before probing by Ab Fig. S3. PKC δ , PKC γ , and PKC ϵ bind weakly to CycT1





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Membrane was cut after transfer, and before probing by Ab Left lanes the same proteins from 1st donor



Red marked area was used for the figure.

Membrane was cut after transfer, and before probing by Ab Left lanes the same proteins from 1st donor



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Membrane was cut after transfer, and before probing by Ab lower bands are unspecific detection by target antibodies



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Membrane was cut after transfer, and before probing by Ab Left lanes the same proteins from 1st donor Fig. S4. Chronic activation in primary cells decreases levels of endogenous CycT1 protein





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Fig. S4. Chronic activation in primary cells decreases levels of endogenous CycT1 protein

Activated Primary CD4+ T cells

В