

Multiplexed Profiling of CDK Kinase Activities in Tumour Biopsies with Fluorescent Peptide Biosensors.

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SUPPLEMENTARY

EXPERIMENTAL PROCEDURES

CDKACT Peptide Biosensors CDKACT1, 2, 4 and 6 biosensors have previously been described^{23–29} Peptide biosensors and peptide substrates were synthesized by GENEPEP, labelled with TAMRA through incorporation of a TAMRA-labelled cysteine within the substrate moiety, and purified to 95%.

CDKACT4: GFARVYMSRSSGWERPSGG–YKFCSSPLRIPG

CDKACT6: GFARVYMSRSSGWERPSGG–GHnLQGGKPSPFDRNFGT

CDKACT2: GFARVYMSRSSGWERPSGG–RAGGPATCLSPTRL

CDKACT1: GFARVYMSRSSGWERPSGG–PEPILVDCSSPSPMET

CDK4 peptide substrate : YKFCSSPLRIPG

CDK6 peptide substrate : GHnLQGGKPSPFDRNFGT

CDK2 peptide substrate: RAGGPATCLSPTRL

CDK1 peptide substrate: PEPILVDCSSPSPMET

Fluorescence kinase assays. Fluorescence kinase assays were performed in 96-well plates in a thermostated chamber (Clariostar spectrofluorimeter, BMG) at 30° C in 200 µL phosphate buffer saline (PBS) purchased from Sigma., supplemented with 5 mM MgCl₂, 0.5 mM ATP, using 200 nM TAMRA-labelled peptide biosensors. Changes in TAMRA fluorescence emission were recorded at 585 nm following excitation at 535 nm. In all experiments, relative fluorescence was calculated following subtraction of CDKACT-TAMRA fluorescence from fluorescence values obtained in the presence of cell extracts. Experiments were performed at least three times, and error bars indicate standard deviation from average. Histograms represent relative fluorescence intensity values after 1800s, unless specified otherwise.

Cell culture and cell extract preparation. Cell culture media, serum and antibiotics were purchased from Thermofisher. A375, A549, PC9, H1299, HeLa, MDA-MB-231, MCF7 and HCT116 cell lines used in this study were cultured in RPMI/DMEM + Gluta-max supplemented with 10% FCS, 100 units/mL penicillin (G sodium salt) and 100 µg/mL streptomycin at 37° C in an atmosphere containing 5% CO₂.

Cell extracts were prepared in PBS lysis buffer (PBS Sigma pH 7.4, 150mM NaCl, 0.2% NP40) complemented with 1 mM EDTA, 2 mM PMSF, Complete protease inhibitors (Roche), and total protein concentration was normalized by spectrophotometric dosage at 280 nm.

Total protein extraction from human biopsies. 150-200mg frozen human biopsies obtained from the CRB-Montpellier hospital tumour bank were cut with a scalpel and placed in an Eppendorf with 500ul PBS lysis buffer complemented with 1 mM EDTA, 2 mM PMSF, Complete protease inhibitors (Roche), together with zirconium beads and processed in a FastPrep for 3X 30s cycles; samples were placed for 5min on ice between each shaking cycle.

Western Blotting

Monoclonal mouse anti-CDK1 (sc-54), CDK2 (sc-6248) and GAPDH (sc-47724) antibody were purchased from Santa Cruz. Monoclonal rabbit anti-CDK4 (#12790) and CDK6 (#13331) antibody were purchased from Cell signalling. SDS-PAGE was performed according to standard protocols and proteins were transferred onto PVDF membranes (Immobilon-P, Merck Millipore) for 2h at 100V in precooled Towbin buffer using a liquid BioRad system. Western blotting was performed according to standard procedures: membranes were first saturated during one hour with 5% BSA in PBS, 0.1% Tween, then incubated overnight with primary antibody diluted 1:500 (CDK1, CDK2, CDK4, CDK6) and 1:2500 (GAPDH) in PBS 0.1% Tween, then washed three times for 10min with PBS 0.1% Tween and incubated for 1h with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare) diluted 1:10000 in PBS 0.1% Tween. After three final 10-minute washes with PBS 0.1% Tween membranes were processed with Prime ECL (GE Healthcare) for signal detection.

Statistical analyses The Wilcoxon unpaired method was used for statistical analyses of cell lines. All p-values were calculated on Rstudio by “wilcox.test” function.

ANOVA or the Kruskal Wallis test, as appropriate, was applied to evaluate markers differences between groups.

Principal Component Analysis (PCA). This unsupervised multidimensional method summarizes all feature information into novel features (obtained from linear combinations of the original parameters) that maximize the variance of the data. PCA creates a number of dimensions equal to the number of features and with decreasing importance.

The first dimensions allow to visualize similarities and dissimilarities between samples and to interpret those characteristics with respect to original features [[ref DOI : 10.1371/journal.pone.0301372](https://doi.org/10.1371/journal.pone.0301372)] .

To this purpose, the CDK1, CDK2, CDK4 and CDK6 activities were analyzed with the R function *prcomp* . Cofactors common to all disease and disease specific were then visualized on PCA representations.

Unsupervised simple and double hierarchical clustering analysis of patients and markers were carried out for samples, using the Euclidian distance to measure the distance between observations, and another one for markers, using 1-abs (correlation) as a distance, as previously described [[ref : DOI: 10.1186/s12979-024-00416-5](https://doi.org/10.1186/s12979-024-00416-5)]. For both of them, Ward’s minimum variance method was used as a mean of linkage.

First these unsupervised analyses were performed on all samples together, then on adenocarcinoma and lymphoma samples, each separately.

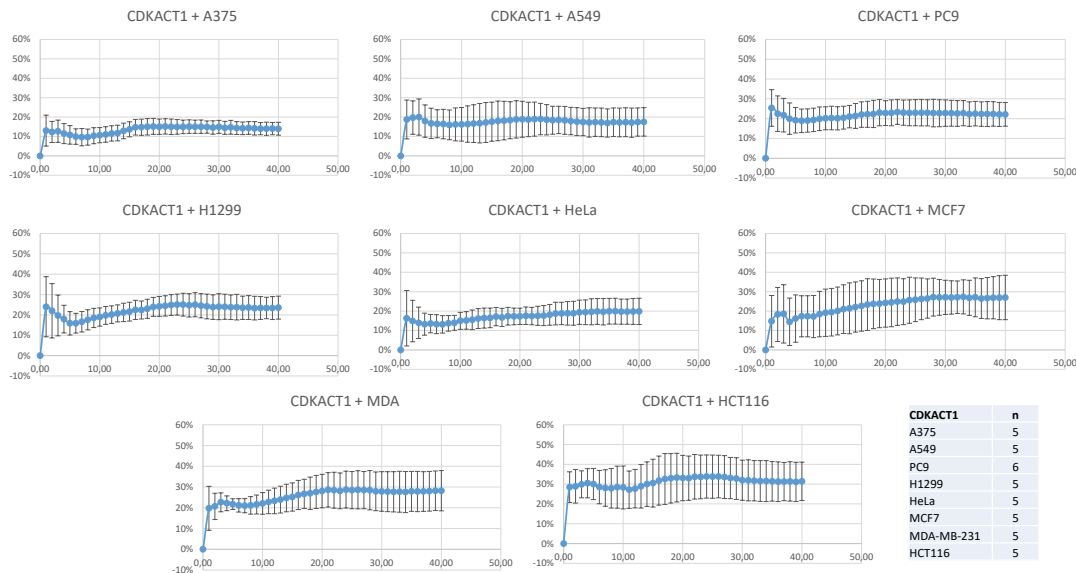
For Principal Component Analysis (PCA) the following parameters were considered:

- 4 quantitative variables : CDK1, CDK2, CDK4 and CDK6 activities
- 2 types of patient/samples : adenocarcinoma / lymphoma
- Cofactors sex and age for all patients
- Specific factors for the 2 type of samples:
 - Adenocarcinoma: size, metastasis, KRAS mutation, EGFR mutation
 - Lymphoma: grade, P16, cyclin D1, cyclin E expression

First and unsupervised analysis was performed on all samples together, then on adenocarcinoma and lymphoma samples, each separately. PCA, hierarchical classification (clustering), heatmap (double classification patient & variables), and colour by common factor (age, sex)

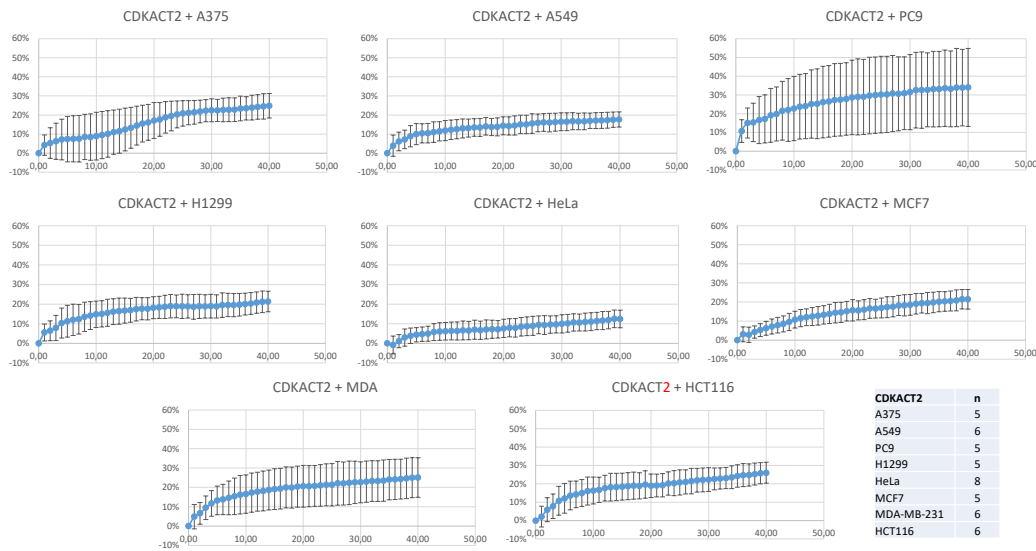
Supplementary Figure S1 - CDK1 kinase activities in lysates from different cell lines

Relative changes in fluorescence of 150nM TAMRA-labelled CDKACT1 incubated with 100µg lysates from different cell lines: A375, A549, PC9, H1299, HCT116, HeLa, MCF7, MDA. Fluorescence activity assays were repeated n=5-6 times



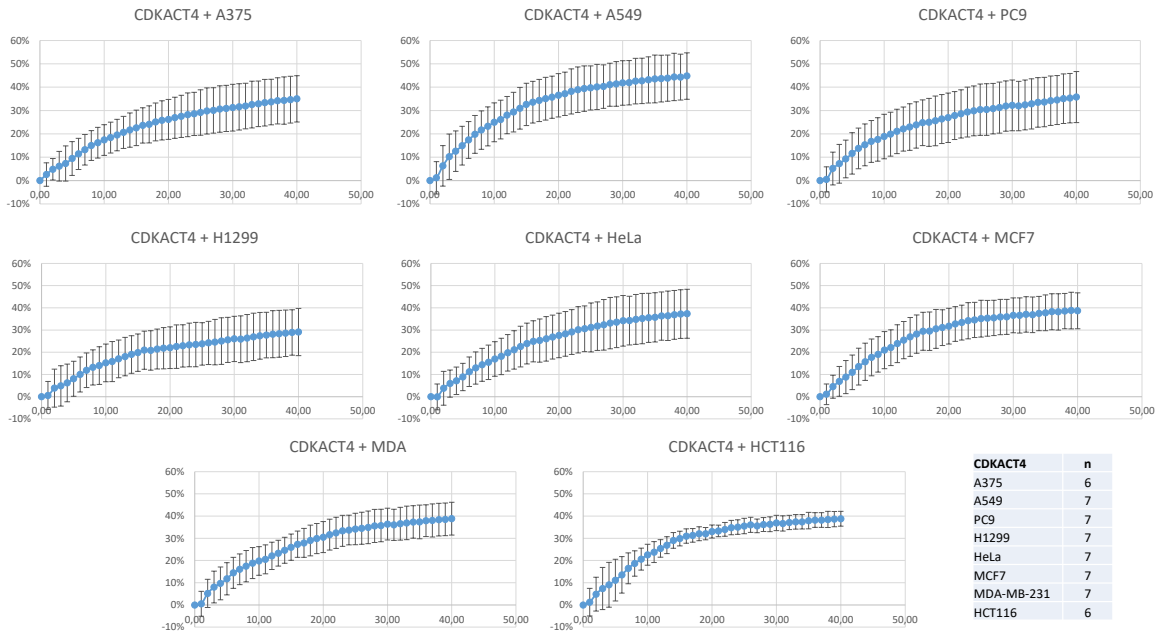
Supplementary Figure S2 – CDK2 kinase activities in lysates from different cell lines

Relative changes in fluorescence of 150nM TAMRA-labelled CDKACT2 incubated with 100µg lysates from different cell lines: A375, A549, PC9, H1299, HCT116, HeLa, MCF7, MDA. Fluorescence activity assays were repeated n=5-8 times



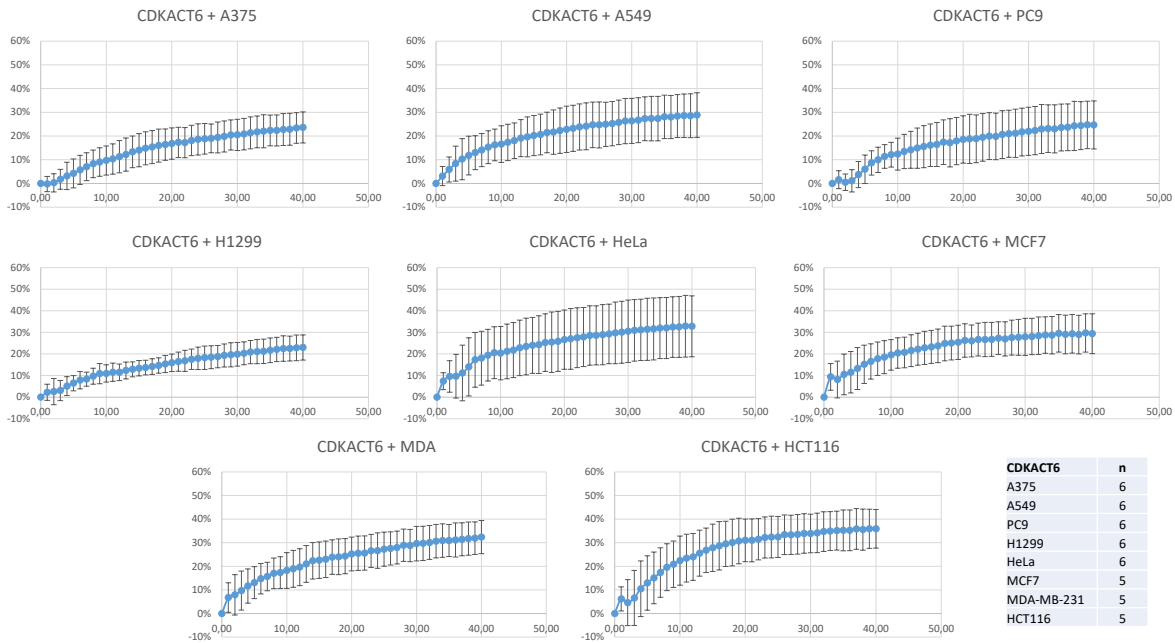
Supplementary Figure S3 – CDK4 kinase activities in lysates from different cell lines

Relative changes in fluorescence of 150nM TAMRA-labelled CDKACT4 incubated with 100ug lysates from different cell lines: A375, A549, PC9, H1299, HCT116, HeLa, MCF7, MDA. Fluorescence activity assays were repeated n=6-7 times



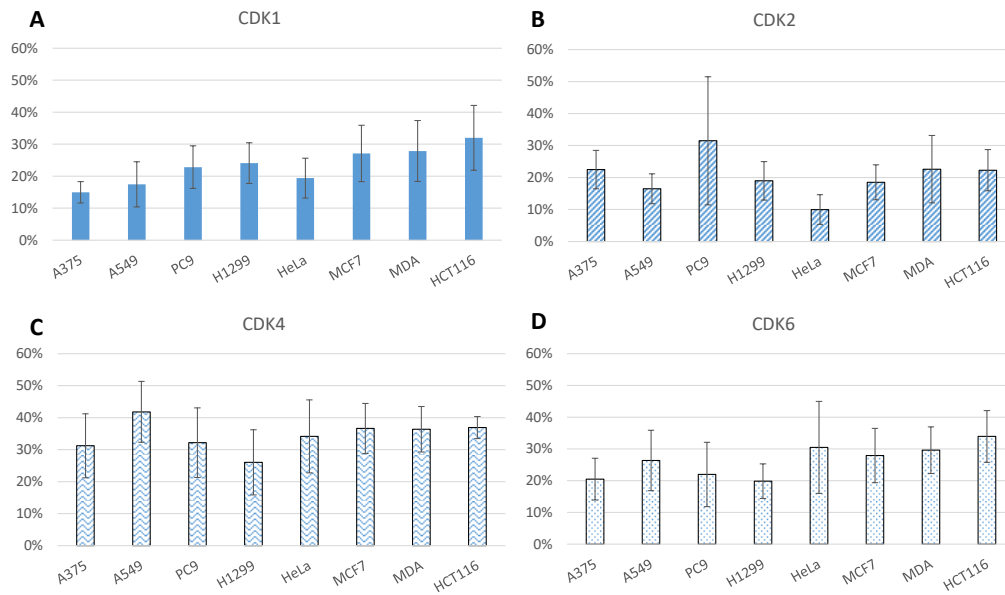
Supplementary Figure S4 – CDK6 kinase activities in lysates from different cell lines

Relative changes in fluorescence of 150 nM TAMRA-labelled CDKACT6 incubated with 100ug lysates from different cell lines: A375, A549, PC9, H1299, HCT116, HeLa, MCF7, MDA. Fluorescence activity assays were repeated n=5-6 times



Supplementary Figure S5 – Relative CDK1,2,4,6 kinase activities in lysates from different cell lines

Bar Plot representation of maximal relative fluorescence enhancement at 30 minutes of 150nM TAMRA-labelled (A) CDKACT1 (B) CDKACT2 (C) CDKACT4 and (D) CDKACT6 incubated with 100µg lysates from different cell lines: A375, A549, PC9, H1299, HCT116, HeLa, MCF7, MDA. Fluorescence activity assays were repeated n=5-8 times



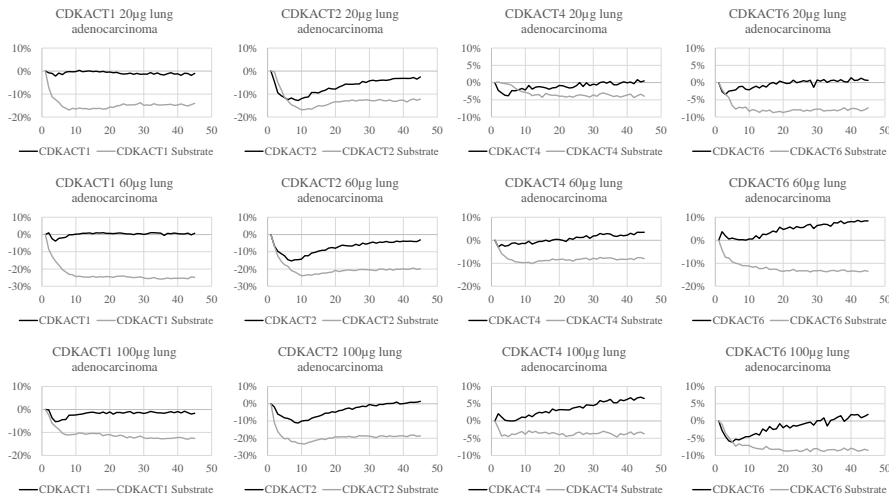
Supplementary Figure S6 – Relative CDK1,2,4,6 kinase activities in lysates from A549 and A375 cells incubated with ATP/MgCl₂, CaCl₂, glucose, lactate and glutamate

Bar plot representation of maximal relative fluorescence enhancement of 150nM TAMRA-labelled (A) CDKACT1 (B) CDKACT2 (C) CDKACT4 and (D) CDKACT6 after 20min incubation with 100µg cell lysate in PBS buffer supplemented with ATP/MgCl₂ (T), with 100uM, 1mM or 10mM CaCl₂ (C, orange), glucose (G, green), lactate (L, magenta) or glutamate (E, red). Fluorescence activity assays were repeated n=2-6 times.

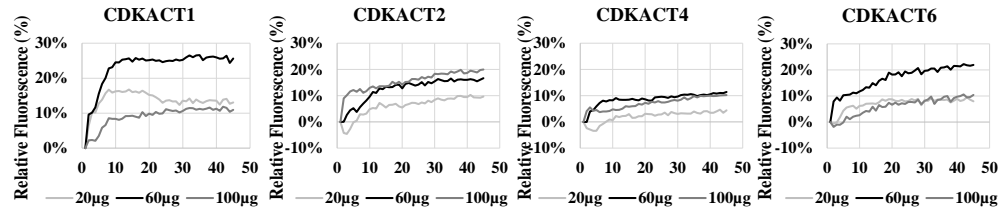


Supplementary Figure S7 - Fluorescence enhancement of CDKACT1,2,4,6 and of cognate substrate peptides in 20, 60, 100µg total lysates from adenocarcinoma biopsy N°8

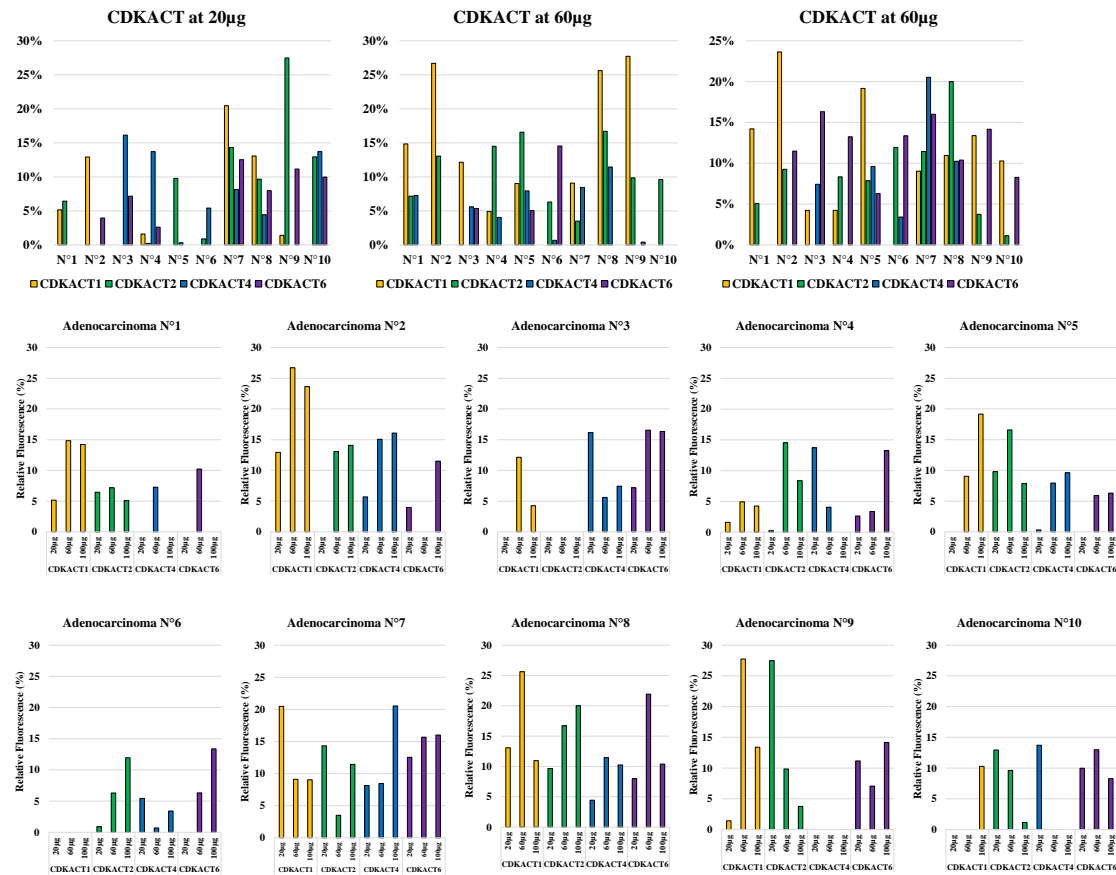
(A) Relative fluorescence emission of CDKACT and substrate peptides



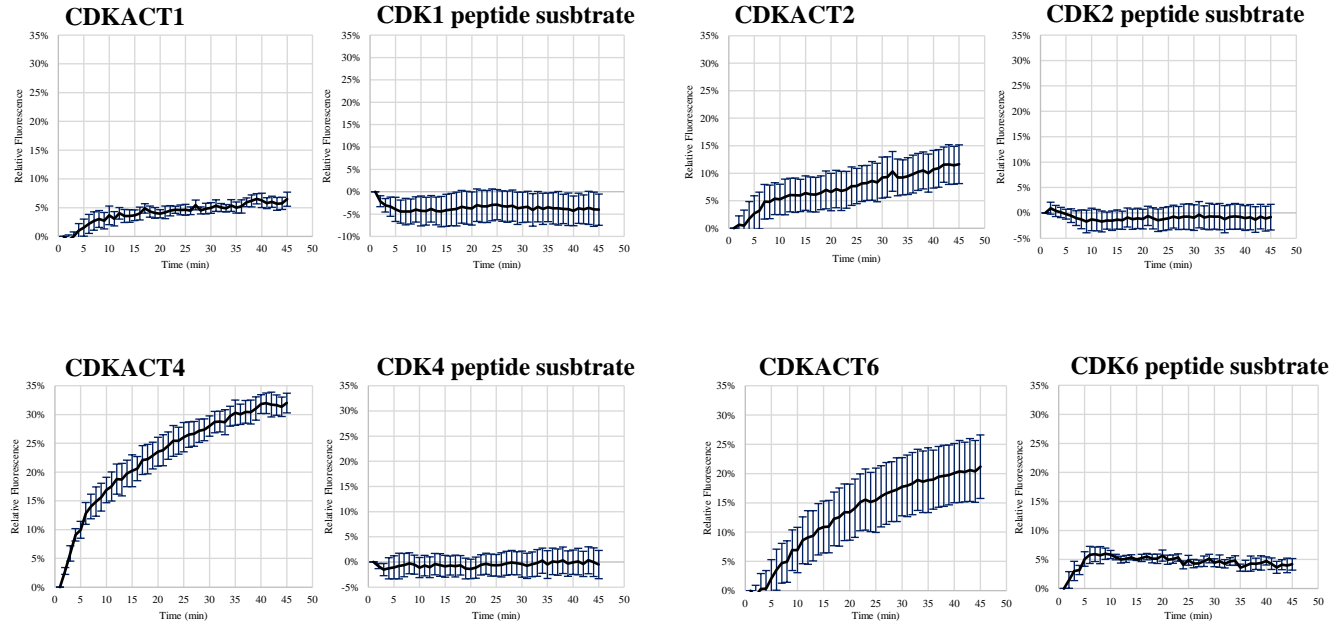
(B) Relative CDKACT1,2,4,6 fluorescence following subtraction of substrate peptide fluorescence



(C) Bar plot representation of CDKACT1,2,4,6 fluorescence enhancement in 20, 60, 100µg total lysates

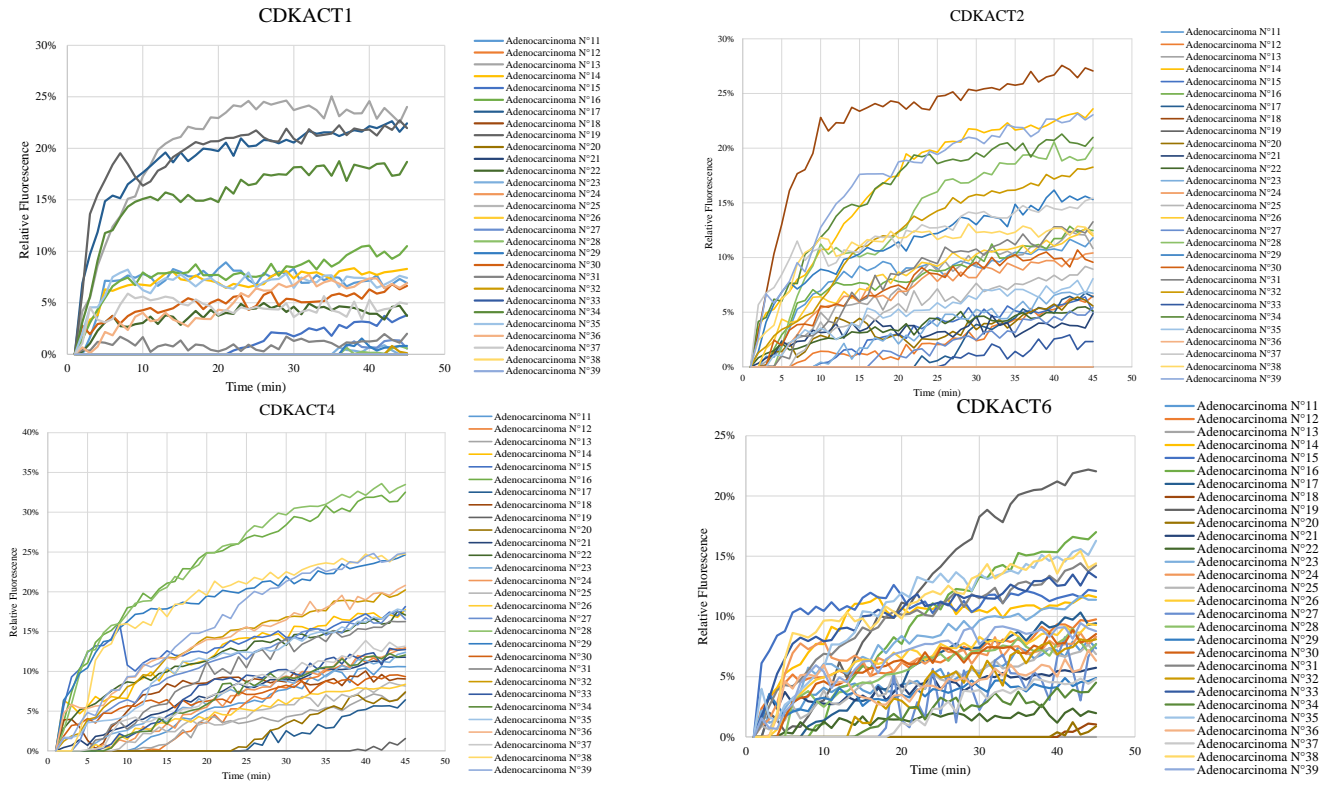


Supplementary Figure S8 - CDKACT1,2,4,6 fluorescence enhancement in 60µg total lysates from adenocarcinoma biopsy N°16
Relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4, CDKACT6 as well as CDK1, CDK2, CDK4 and CDK6 peptide substrates incubated with 60µg lysates from adenocarcinoma biopsy N°16. Fluorescence activity assays were performed in triplicate.



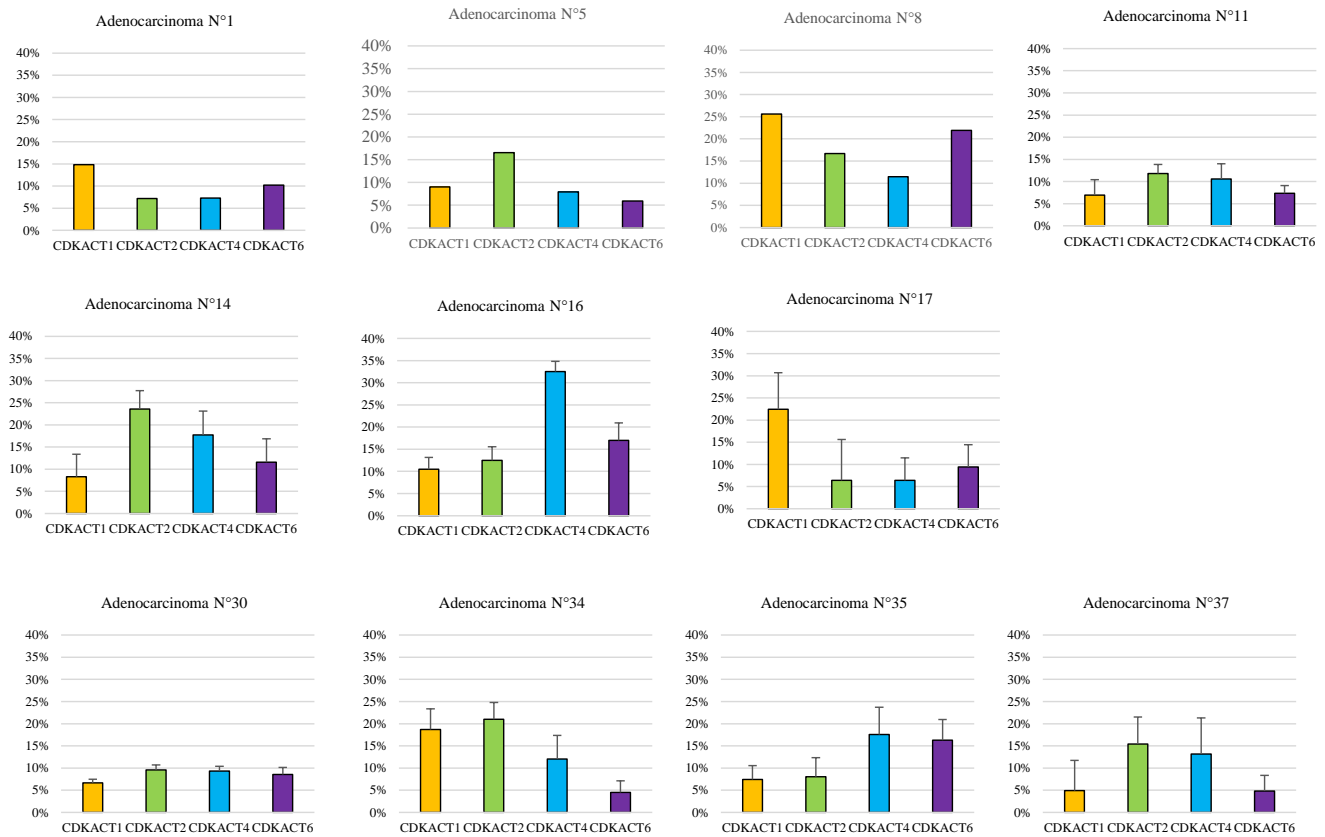
Supplementary Figure S9 - CDKACT1,2,4,6 fluorescence emission curves in 60µg total lysates from adenocarcinoma biopsies (11-39)

Relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from adenocarcinoma biopsies N° 11 to 39. Fluorescence activity assays were performed and each curve represents the average of triplicates; error bars are not shown to avoid overloading the graphs.



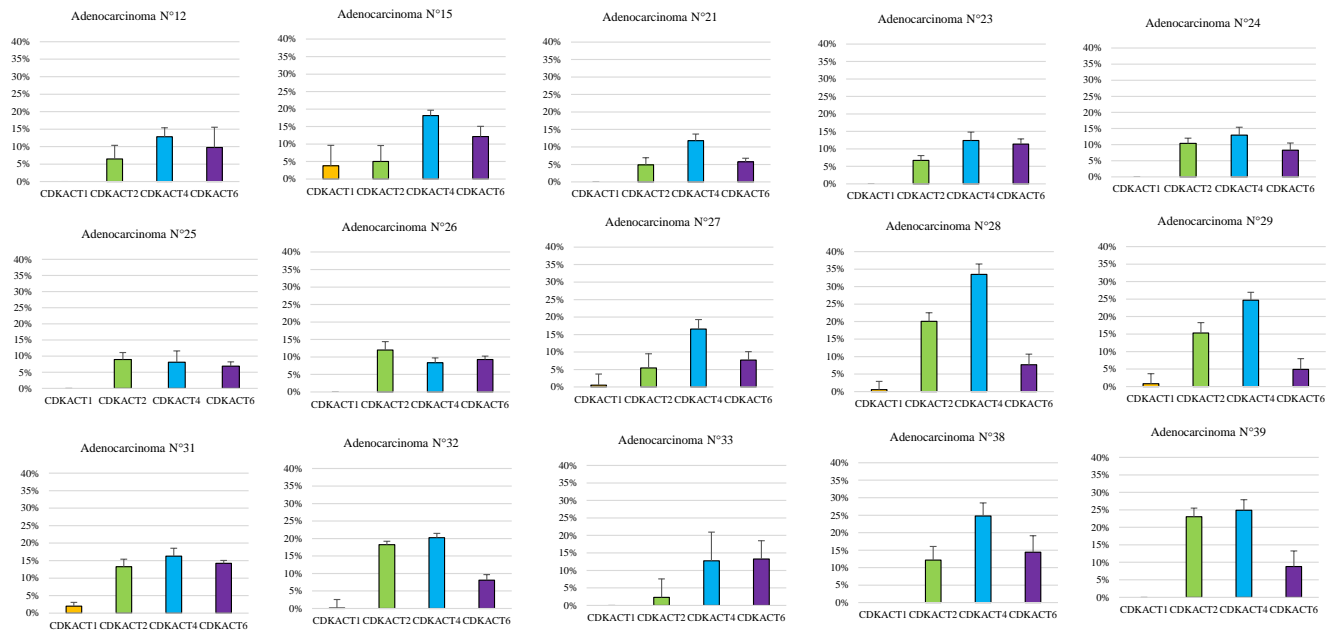
Supplementary Figure S10 –Lung Adenocarcinoma CDKACT1,2,4,6 Activity Profiles: All CDKs

Bar plot representation of all biopsies displaying « all CDK » activities. Activity assays were performed in triplicate (except for biopsies 1-10) and relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from adenocarcinoma biopsies.



Supplementary Figure S11 –Lung Adenocarcinoma CDKACT1,2,4,6 Activity Profiles: no CDK1

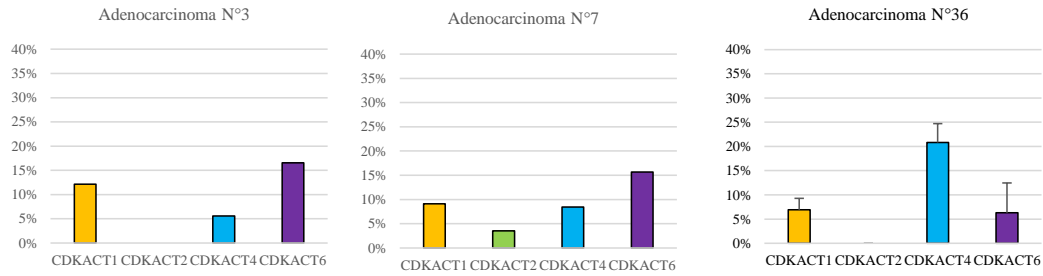
Bar plot representation of all biopsies displaying « no CDK1 » activity. Activity assays were performed in triplicate and relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from adenocarcinoma biopsies.



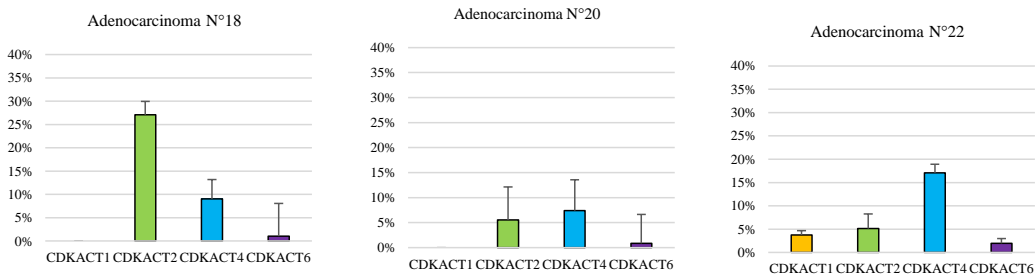
Supplementary Figure S12 –Lung Adenocarcinoma CDKACT1,2,4,6 Activity Profiles

Activity assays were performed in triplicate (except for biopsies 1-10) and relative changes in fluorescence of 200nM TAMRA-labelled CDKACTs were measured following incubation with 60µg lysates from adenocarcinoma biopsies.

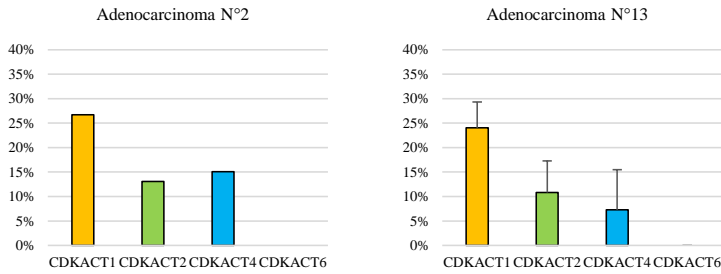
A. Bar plot representation of all biopsies displaying « no or low CDK2 » activity.



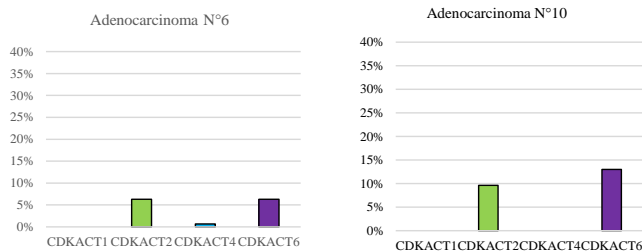
B. Bar plot representation of all biopsies displaying « CDK2 & CDK4 » activity.



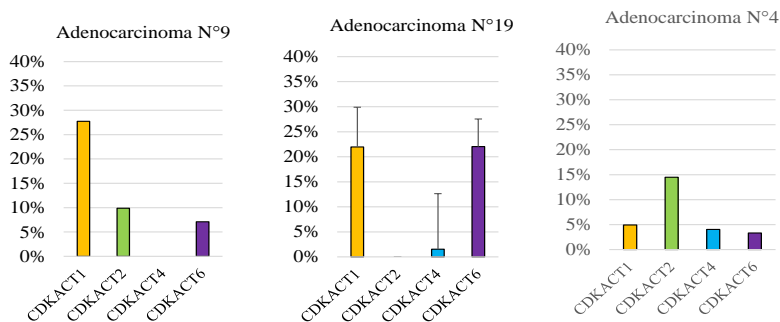
C. Bar plot representation of all biopsies displaying « no or low CDK6 » activity.



D. Bar plot representation of all biopsies displaying « CDK2 & CDK6 » activity.

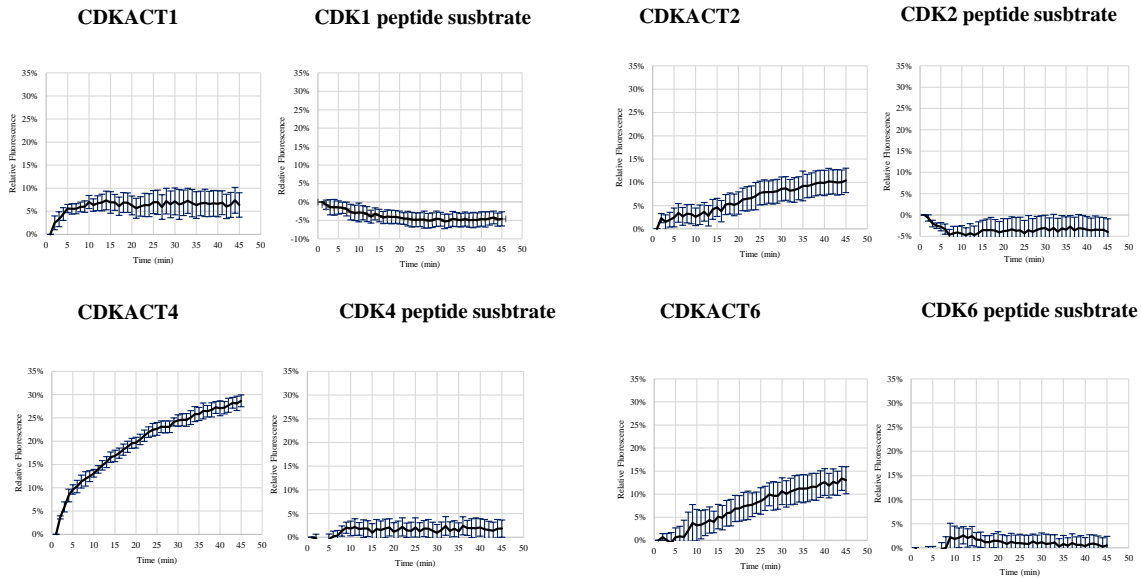


E. Bar plot representation of biopsies displaying unique CDK activity.



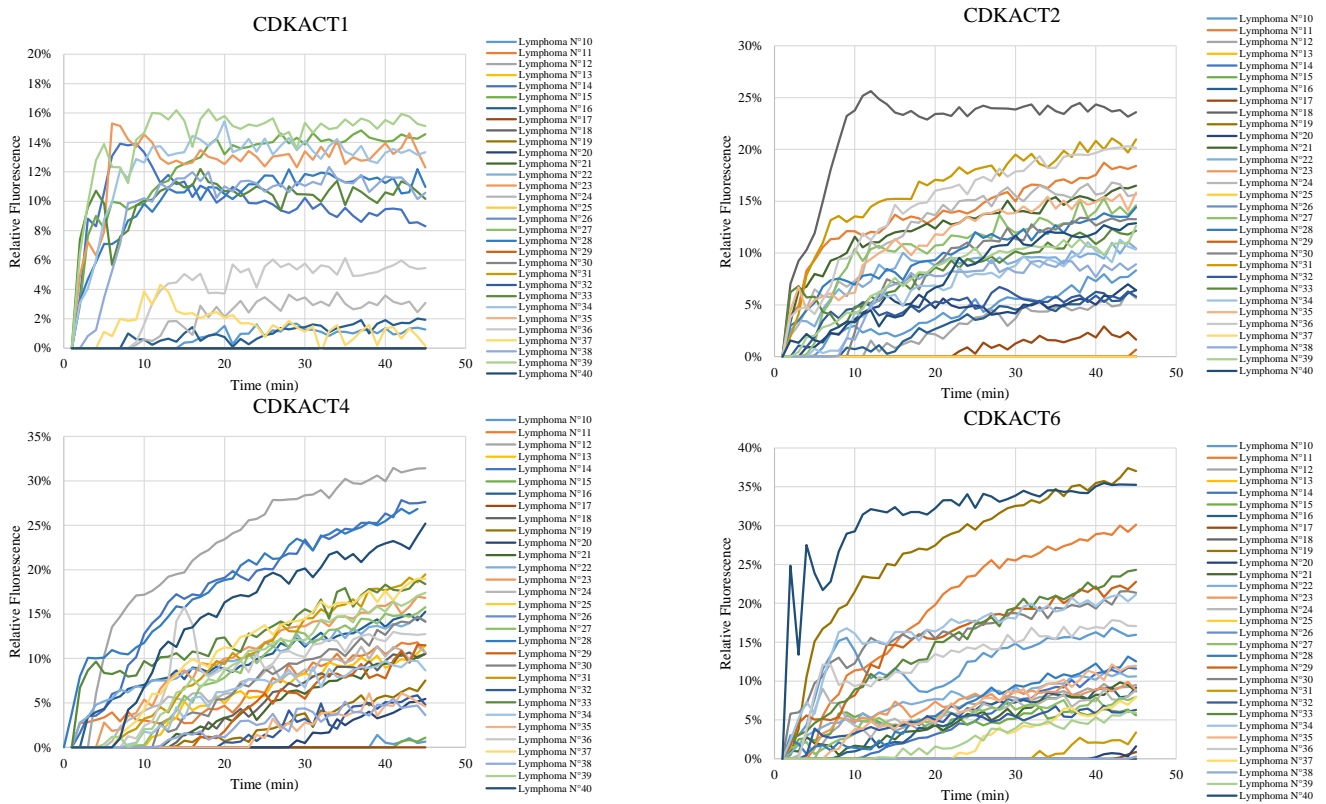
Supplementary Figure S13 - CDKACT1,2,4,6 fluorescence enhancement in 60µg total lysates from lymphoma biopsy 28

Relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4, CDKACT6 as well as CDK1, CDK2, CDK4 and CDK6 peptide substrates incubated with 60µg lysates from lymphoma biopsy N°28. Fluorescence activity assays were performed in triplicate.



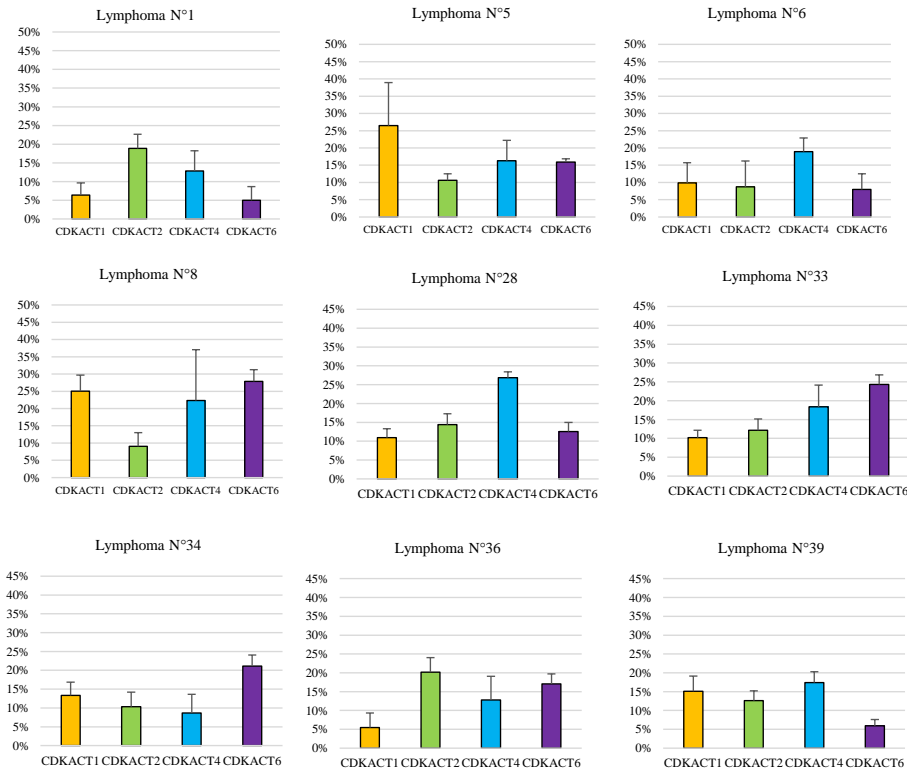
Supplementary Figure S14- CDKACT1,2,4,6 fluorescence enhancement in 60µg total lysates from lymphoma biopsies (10-40)

Relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from lymphoma biopsies N°10 to 40. Fluorescence activity assays were performed and each curve represents the average of triplicates; error bars are not shown to avoid overloading the graphs.



Supplementary Figure S15- Lymphoma CDKACT1,2,4,6 Activity Profiles: All CDKs

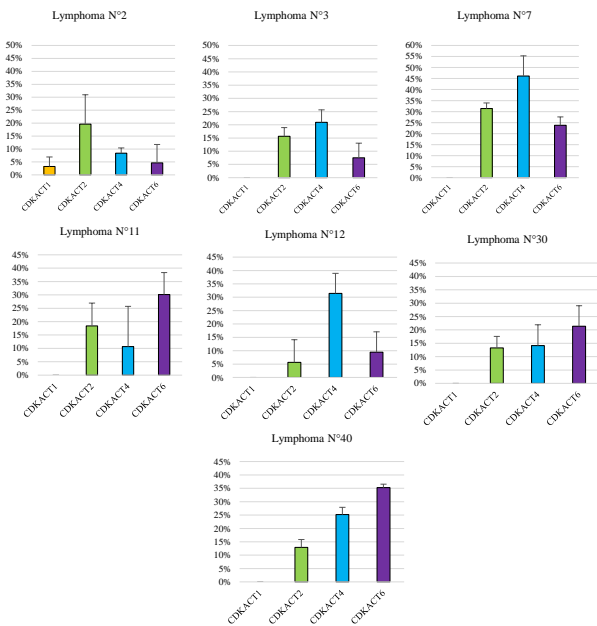
Bar plot representation of all biopsies displaying « All CDK » activities. Activity assays were performed in triplicate and relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from lymphoma biopsies.



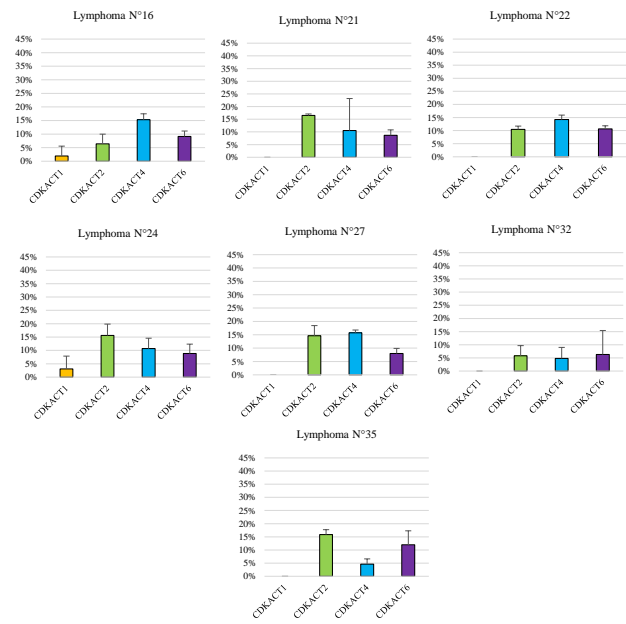
Supplementary Figure S16 - Lymphoma CDKACT1,2,4,6 Activity Profiles: No CDK1

Bar plot representation of all biopsies displaying « No CDK1 » activity. A. « No CDK1, high CDK2, 4, 6 » activity. B. « No CDK1, low CDK2, 4, 6 » activity. Activity assays were performed in triplicate and relative changes in fluorescence of 200nM TAMRA-labelled CDKACT1, CDKACT2, CDKACT4 and CDKACT6 incubated with 60µg lysates from lymphoma biopsies.

A. No CDK1 but high CDK2, CDK4 or CDK6



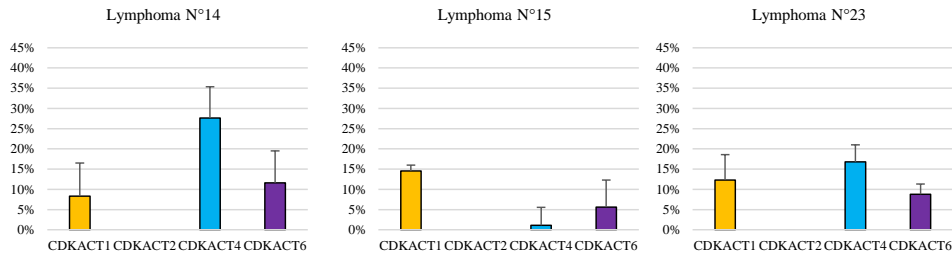
B. No CDK1 and low CDK2, CDK4 or CDK6



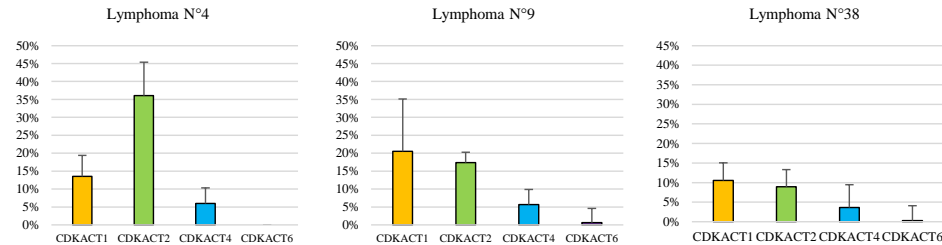
Supplementary Figure S17 – Other Lymphoma CDKACT1,2,4,6 Activity Profiles

Activity assays were performed in triplicate and relative changes in fluorescence of 200nM TAMRA-labelled CDKACT biosensors were measured following incubation with 60µg lysates from lymphoma biopsies.

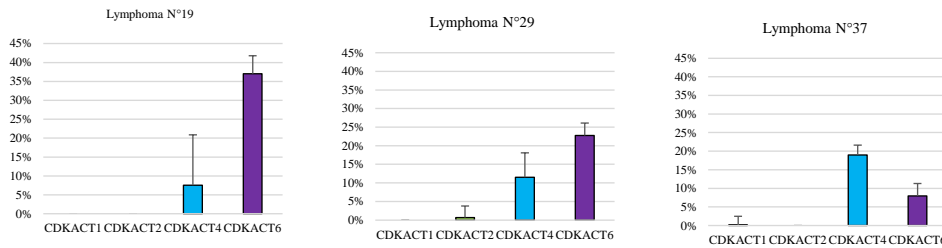
A. Bar plot representation of all biopsies displaying « No CDK2 » activity.



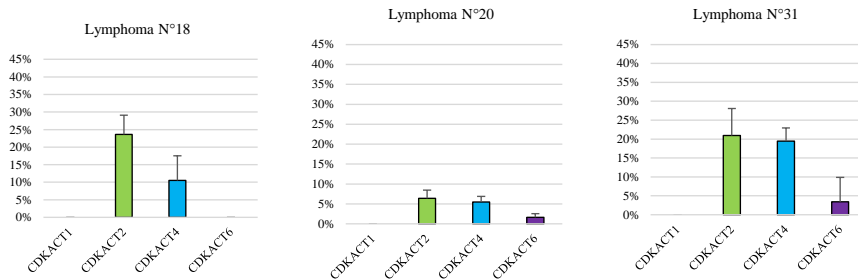
B. Bar plot representation of all biopsies displaying « No CDK6 » activity.



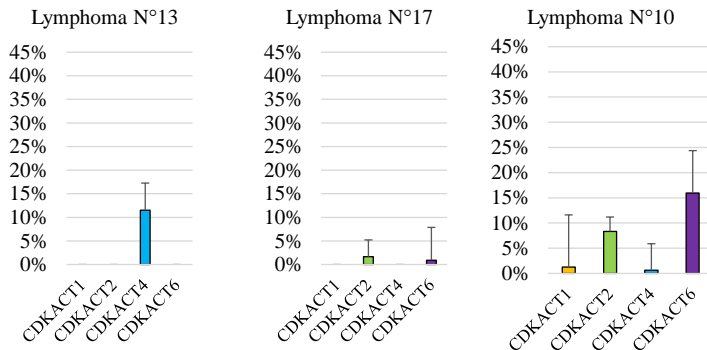
C. Bar plot representation of all biopsies displaying « CDK4 & CDK6 » activity.



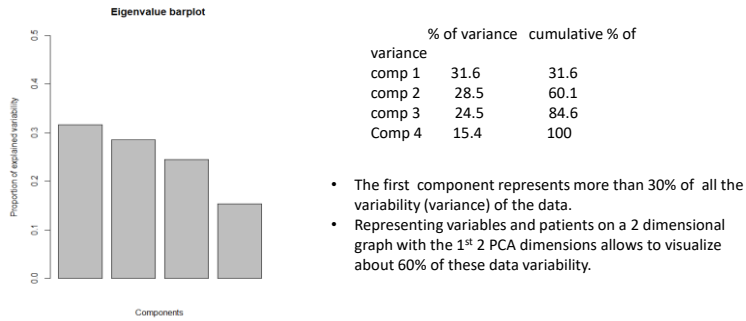
D. Bar plot representation of all biopsies displaying « CDK2 & CDK4 » activity.



E. Bar plot representation of biopsies displaying unique profiles.



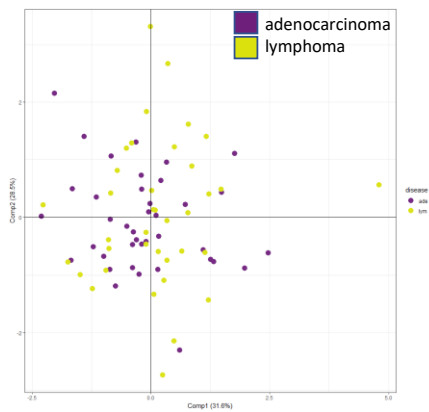
Supplementary Figure S18 - Unsupervised PCA for all samples (lung adenocarcinoma & lymphoma)



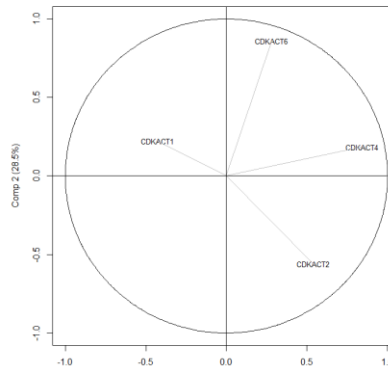
Sample projections in the new main component dimensions (left) and variable correlations (right)
 (A) By tumor type (B) by sex (C) by age group

(A) Components 1&2 – by tumour type

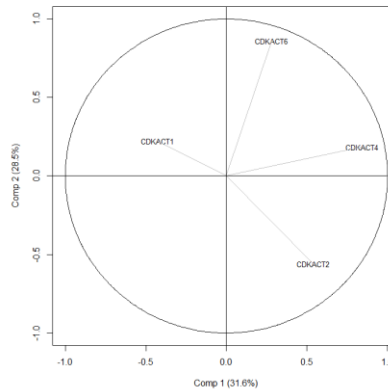
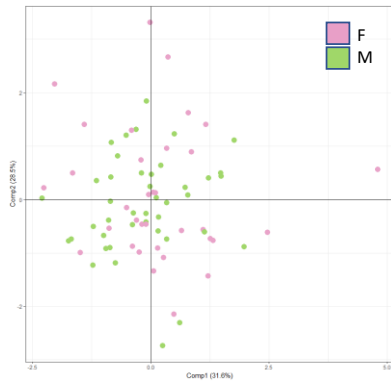
Sample projections in the new component dimensions



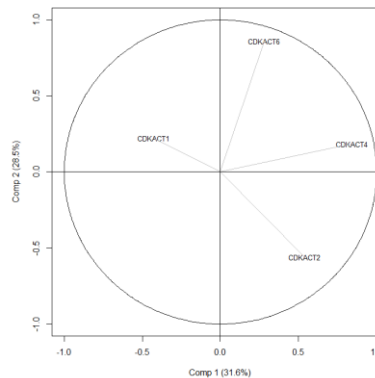
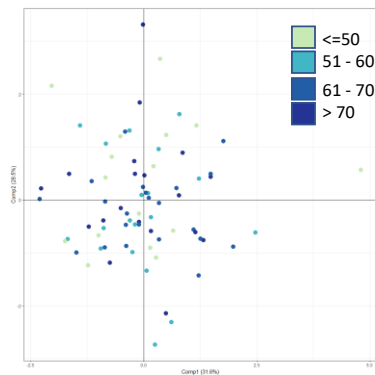
Variable correlations to new component



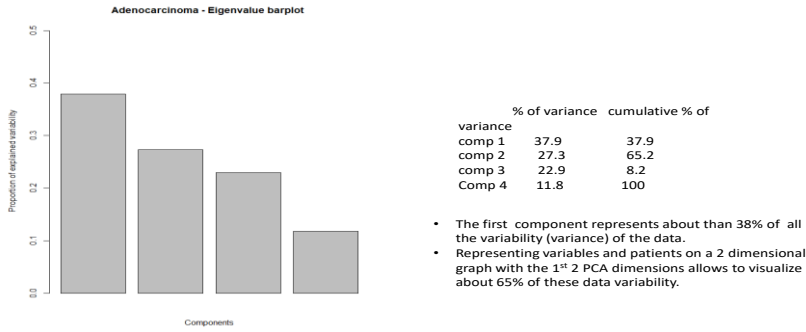
(B) Components 1&2 – by sex



(C) Components 1&2 – by age



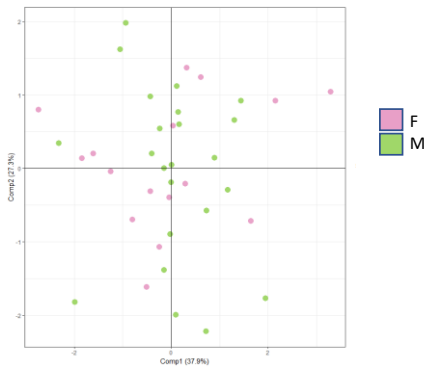
Supplementary Figure S19 – Unsupervised PCA for lung adenocarcinoma samples



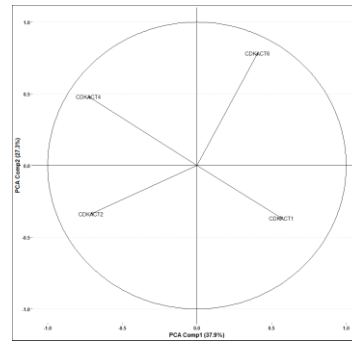
Sample projections in the new main component dimensions (left) and variable correlations (right) (A) by sex (B) by age group (C) by tumour size (D) by metastasis (proximal, distal) (E) by KRAS mutation (F) by EGFR mutation

(A) Components 1&2 – by sex

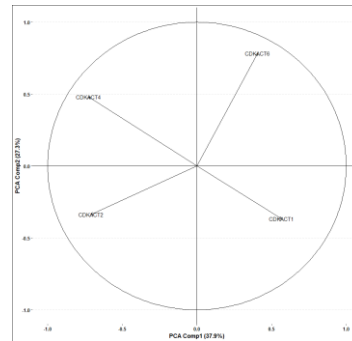
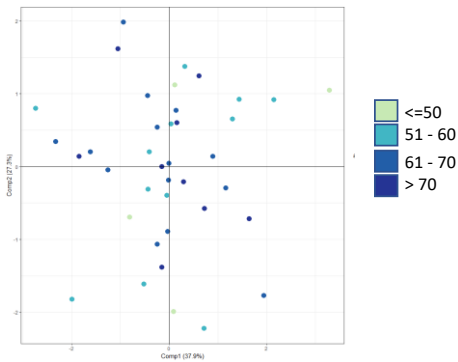
Sample projections in the new component dimensions



Variable correlations to new component

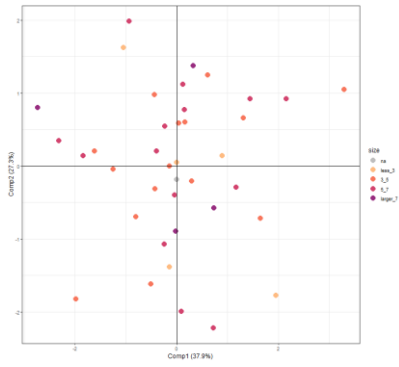


(B) Components 1&2 – by age

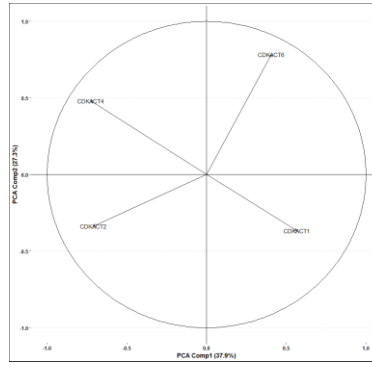


(C) Components 1&2 – by tumour size

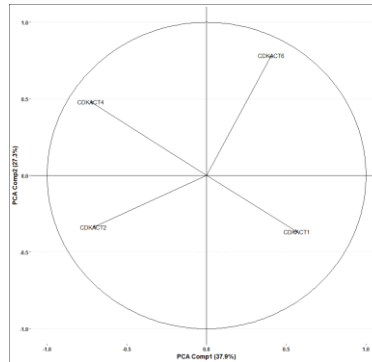
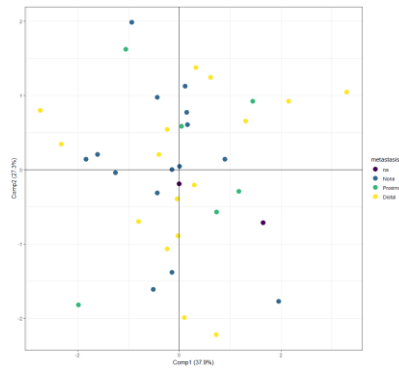
Sample projections in the new component dimensions



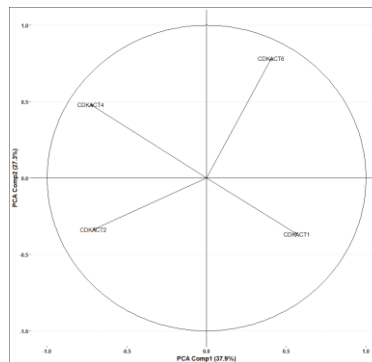
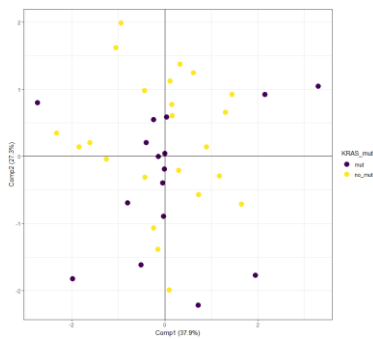
Variable correlations to new component



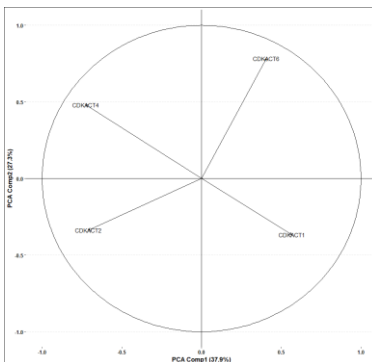
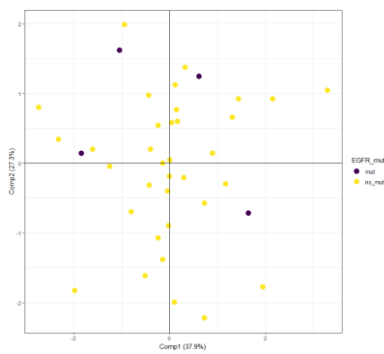
(D) Components 1&2 – by metastasis (proximal, distal)



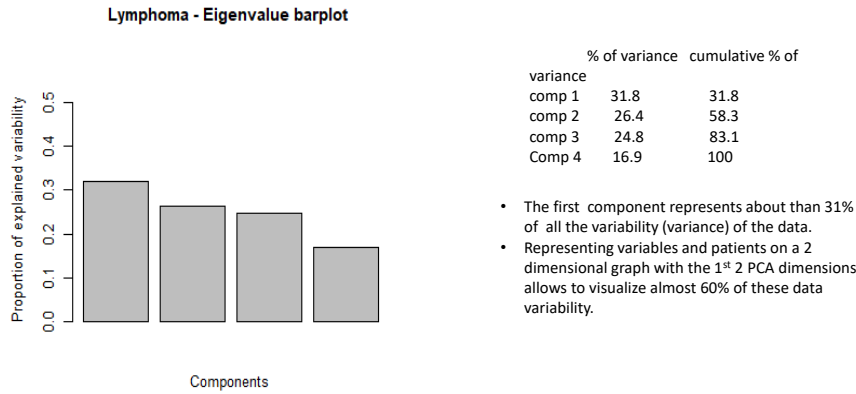
(E) Components 1&2 – by KRAS mutation



(F) Components 1&2 – by EGFR mutation



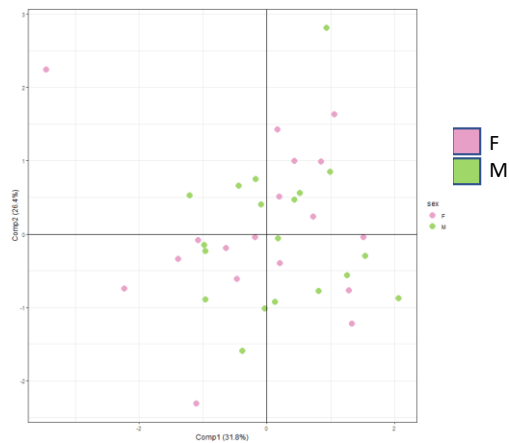
Supplementary Figure S20 – Unsupervised PCA for lymphoma samples



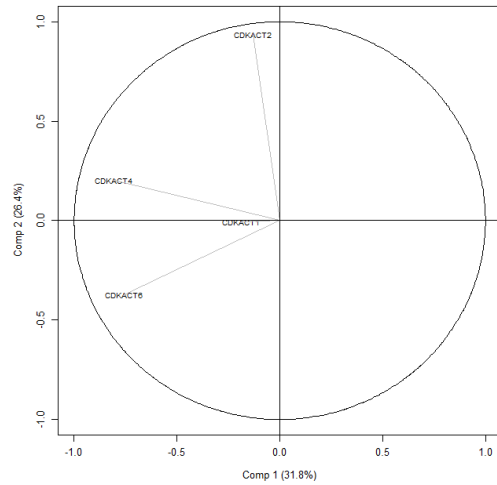
Sample projections in the new main component dimensions (left) and variable correlations (right)
 (A) by sex (B) by age group (C) by grade (D) by p16 expression level (E) by cyclin D expression level (F) by cyclin E expression level

(A) Components 1&2 – by sex

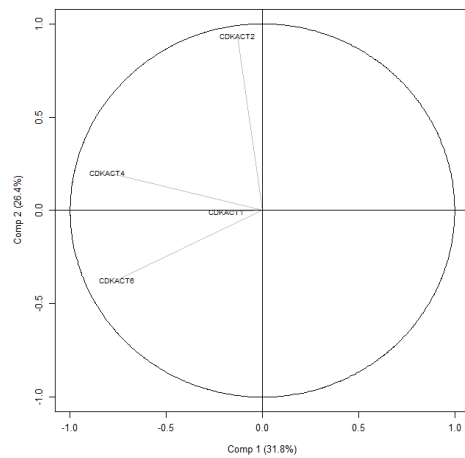
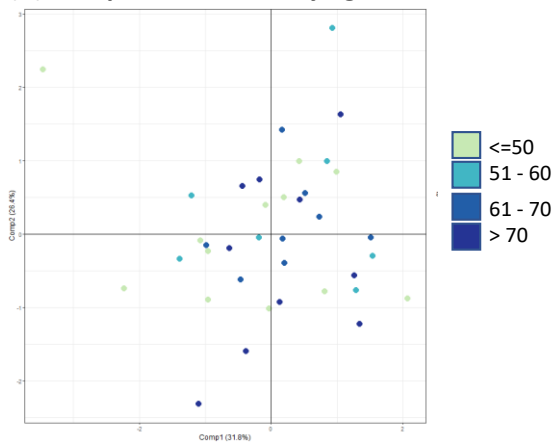
Sample projections in the new component dimensions



Variable correlations to new component

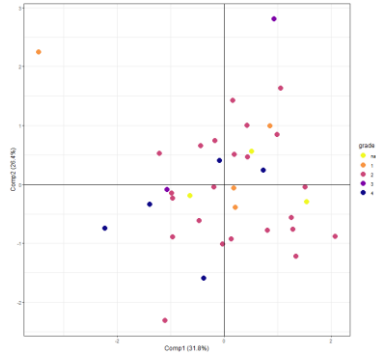


(B) Components 1&2 – by age

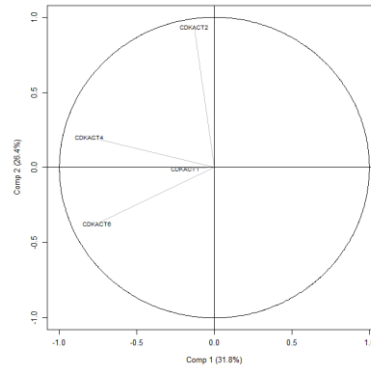


(C) Components 1&2 – by grade

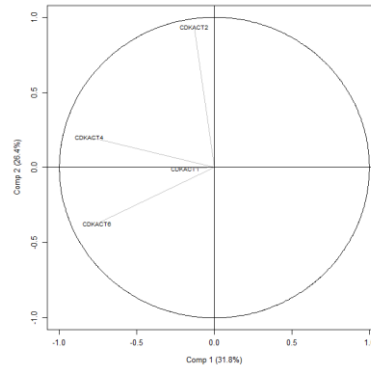
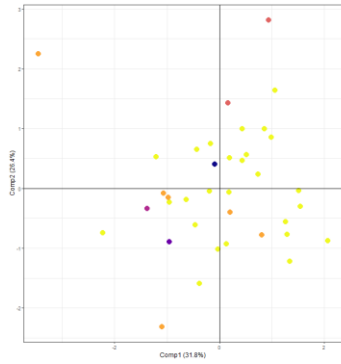
Sample projections in the new component dimensions



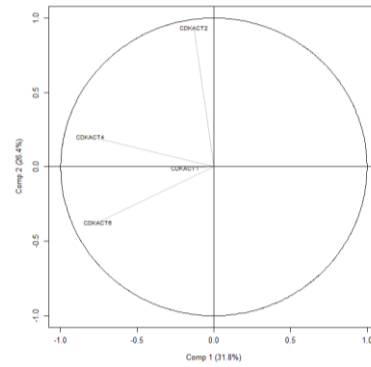
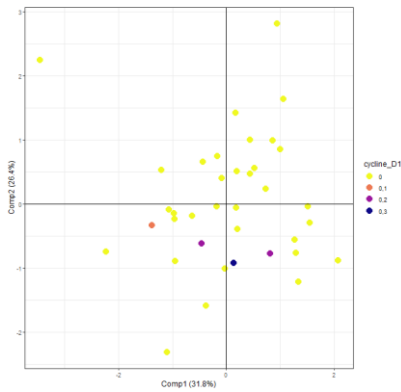
Variable correlations to new component



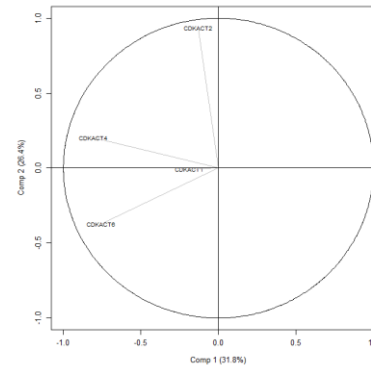
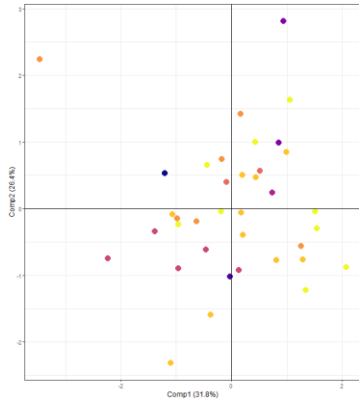
(D) Components 1&2 – by p16



(E) Components 1&2 – by cyclin D

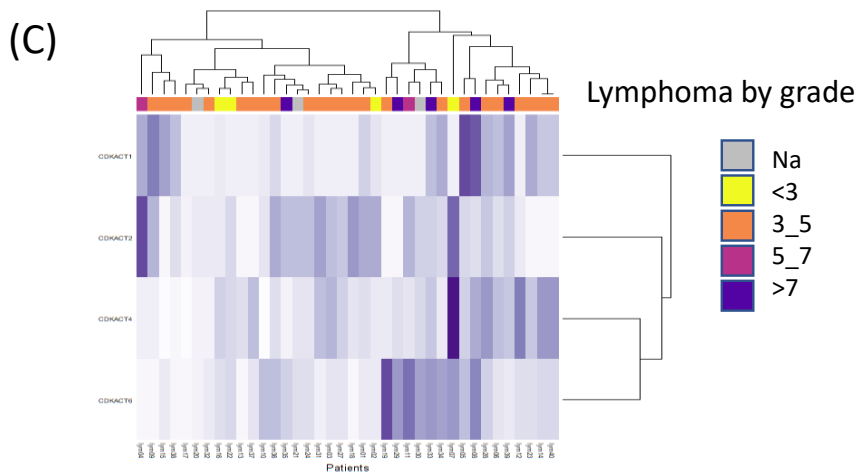
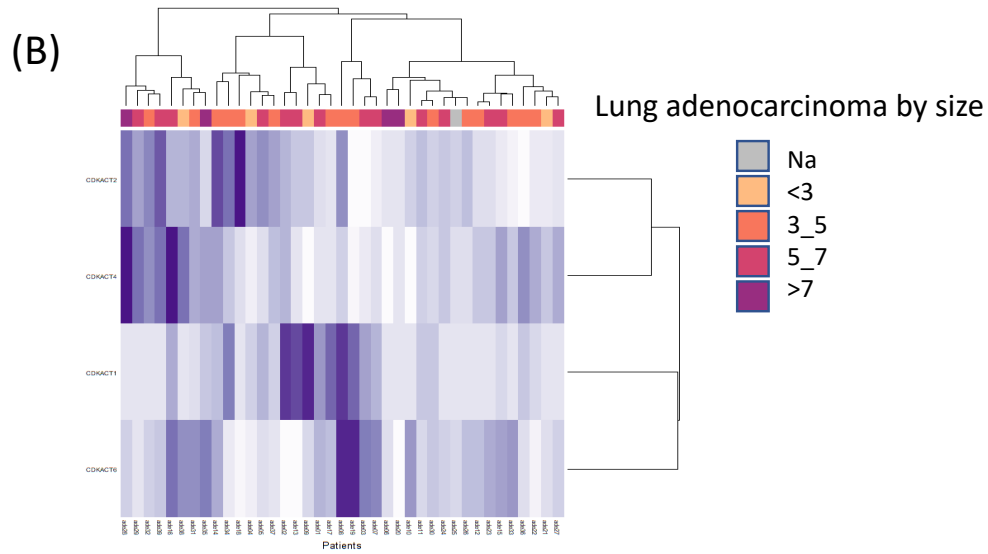
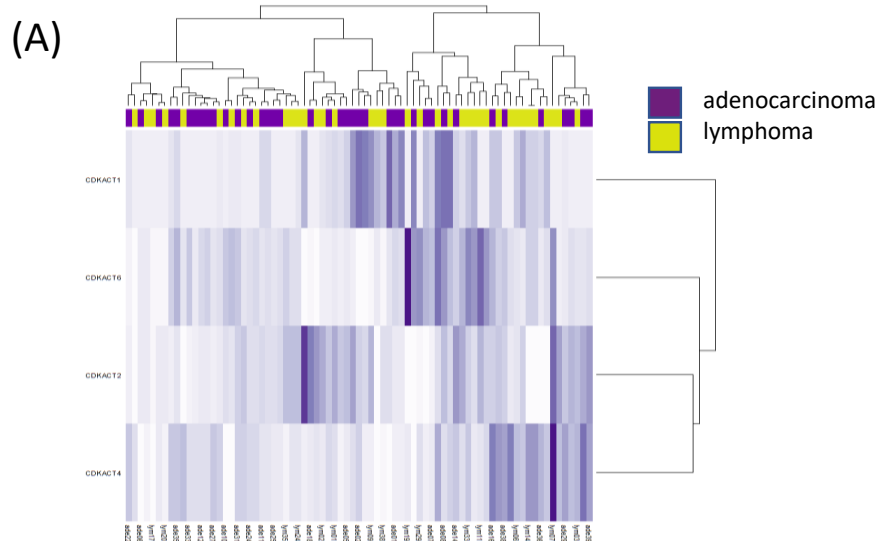


(F) Components 1&2 – by cyclin E



Supplementary Figure 21– Heatmap Plots of CDK activities clustered based on PCA analysis

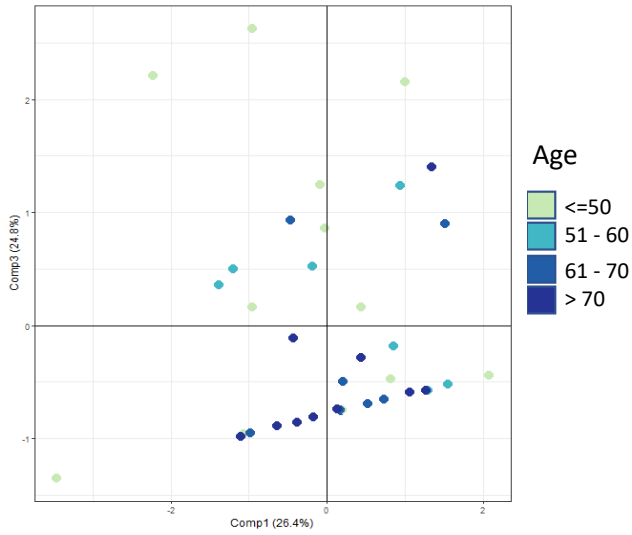
Heatmaps showing the hierarchical clustering of CDK activities (horizontal) and samples (vertical). A) All samples with type of tumor. B) Lung adenocarcinoma samples with tumor size. C) Lymphoma samples with tumor grade.



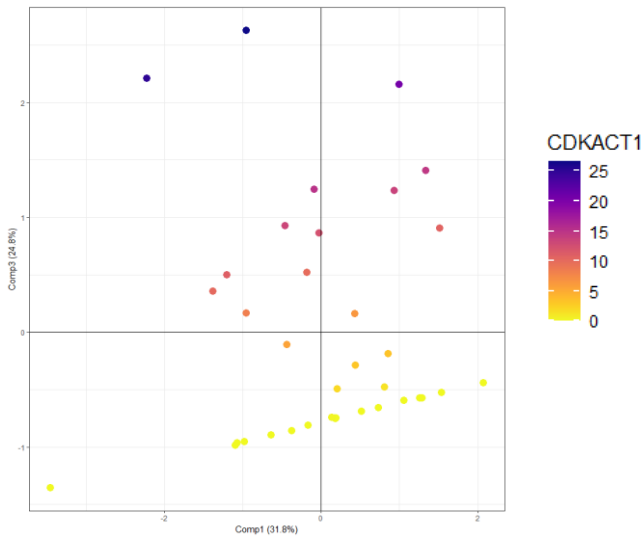
Supplementary Figure S22– Supervised PCA on lymphoma samples: age and CDK1 activity

Projection of the samples onto the 3D space generated by the first three Principal Components. Representations by age and CDK 1 show that oldest patients have the lowest CDK1 values. CDK 6 values increase over the 3rd to 1st quadrant diagonal.

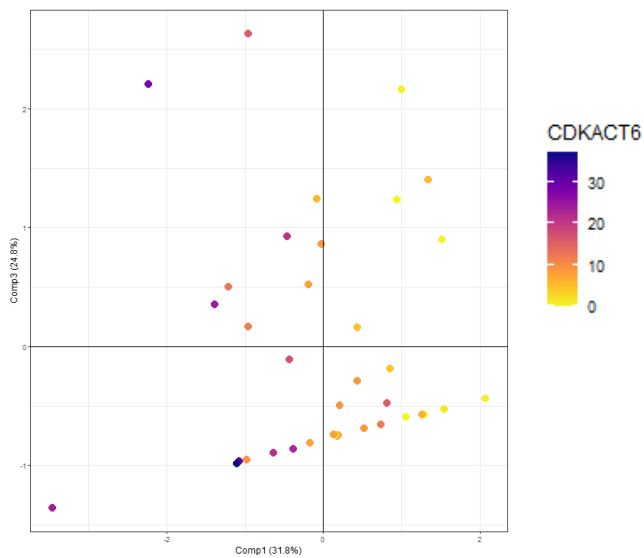
Components 1&3 – by age



Components 1&3 – CDKACT1



Components 1&3 – CDKACT6



Supplementary Table S1 – Age, Sex, TNM stage, Genetic features of lung adenocarcinoma biopsies

		Sex	Age of patient	TNM Stage	EGF-R (exons 18, 19, 20 and 21) mutation	K-Ras (exon 2) mutation
N°1	GC13024012.3	M	64	pT3 N1		
N°2	15.30369-TK.2	M	38	pT3 N2 M1b		
N°3	GC12014684.2	F	51	ypT3 N2		exon 2 c.34G>T; p.G12C
N°4	GC13027666.3	M	84	pT1a N0 R0		
N°5	GC12024148.2	F	65	pT3 N2		
N°6	GC13010170.3	M	75	pT4 N1 R1		
N°7	GC12010747.3	M	53	pT3 N1		
N°8	GC12021659.3	M	60	ypT2b N2		
N°9	GC12007948.1	M	67	pT1a N0		exon 2 c.34G>T; p.G12C
N°10	GC12014581.2	M	69	pT1a N0		
N°11	GC12000840.4	F	57	pT3 N2		exon 2 c.35G>T; p.G12V
N°12	16.08734-01TU TK.2	F	58	pT2a N1		exon 2 c.34G>T; p.G12C
N°13	GC13020427.3	M	51	pT3 N2 M1		exon 12/13 ?
N°14	GC12019931.2	F	64	pT2a N0		
N°15	GC12020922.2	M	48	pT3 N0		
N°16	GC13007716.2	M	68	pT3 N0		
N°17	GC13015232.3	F	77	pT2 R1	exon 18 p.G179	
N°18	GC14031888.2	M	58	pT2a N1		exon 2 c.35G>A; p.G12D
N°19	GC13018905.3	F	42	pT2b N2		exon 2 c.34G>T; p.G12C
N°20	15.17595-TK.4	M	67	pT4 N2 R1		exon 2 c34G>T; p.G12C
N°21	GC12019882.1	M	67	pT1b N0		exon 2 c.34G>T; p.G12C
N°22	GC12027958.2	F	52	pT2a N0		
N°23	GC13009159.3	M	65	pT3 N0		
N°24	GC15000387.1	M	52	pT3 N2		exon 2 c.34G>A; p.G13D
N°25	GC14007109.3	M	62			exon 2 c.34G>T; p. G12C

N°26	GC14018336.3	M	72	ypT2b N0		exon 2 c.38G>A; p.G13D
N°27	GC14032272.3	M	68	pT3 N2 (M1 cerebral and R1 (mediastin))		exon 2 c.34G>T; p. G12C
N°28	GC14003687.3	F	56	pT4 N2 R0		exon 2 c.34G>T; p. G12C
N°29	GC15009877.3	F	76	pT3 N0	exon 19	
N°30	GC13011370.1	F	72	pT2a N2		
N°31	GC13011941.3	M	67	pT2a N0		
N°32	GC15001821.1	F	68	pT2aN0		
N°33	16.07221-05TU TK.1	F	78	pT2a N2 M1a	exon 18 and 21	
N°34	16.07959-01TU TK.1	F	59	ypT2b N0 R0		c.34G>T; p.G12C
N°35	GC12022623.2	F	60	ypT4 N2 R1		
N°36	GC13013572.2	M	73	pT2b N0		
N°37	GC14026165.1	F	48	ypT2a N2		exon 2 c.35G>T; p.G12V
N°38	GC13027604.3	M	71	pT1b N1 R0	exon 19	
N°39	GC12019287.2	M	61	pT3 N2		

Supplementary Table S2 – Age, Sex, TNM stage, Genetic Features of lung adenocarcinoma biopsies with “All CDK” activities

		Sex	Age of patient	TNM	K-Ras	EGF-R
N°1	GC13024012.3	M	64	pT3 N1		
N°5	GC12024148.2	F	65	pT3 N2		
N°8	GC12021659.3	M	60	ypT2b N2		
N°11	GC12000840.4	F	57	pT3 n2		+
N°14	GC12019931.2	F	64	pT2a N0		
N°16	GC13007716.2	M	68	pT3 N0		
N°17	GC13015232.3	F	77	pT2 R1	+	
N°30	GC13011370.1	F	72	pT2a N0		
N°34	16.07959-01TU TK.1	F	59	ypT4 N2 R1		+
N°35	GC12022623.2	F	60	ypT4 N2 R1		
N°37	GC14026165.1	F	48	ypT2a N2		+

Supplementary Table S3 – Age, Sex, TNM stage, Genetic Features of lung adenocarcinoma biopsies with “No CDK1” activities

		Sexe	Age of patient	TNM	K-Ras	EGF-R
N°12	16.08734-01TU TK.2	F	58	pT2a N1		+
N°15	GC12020922.2	M	48	pT3 N0		
N°21	GC12019882.1	M	67	pT1b N0		+
N°23	GC13009159.3	M	65	pT3 n0		
N°24	GC15000387.1	M	52	pT3 N2		+
N°25	GC14007109.3	M	62			+
N°26	GC14018336.3	M	72	ypT2b N0		+
N°27	GC14032272.3	M	68	pT3 N2 M1 R1		+
N°28	GC14003687.3	F	56	pT4 N2 R0		+
N°29	GC15009877.3	F	76	pT3 N0	+	
N°31	GC13011941.3	M	67	pT2a N0		
N°32	GC15001821.1	F	68	pT2a N0		
N°33	16.07221-05TU TK.1	F	78	pT2a N2 M1a	+	
N°38	GC13027604.3	M	71	pT1b N1 R0	+	
N°39	GC12019287.2	M	61	pT3 N2		

Supplementary Table S4 – Age, Sex, TNM stage, immunohistochemical features of lymphoma biopsies

		Sexe	Age of patient	Ki67	Grade	P16	Cycline D1	Cycline E
N°1	GC11010171.2	F	49	30%	1-2			
N°2	GC10003529.5	F	55	15-20%	1			50%
N°3	GC12017540.6	M	76	35%	1-2			10%
N°4	GC11008175.2	M	53	up to 40%	2	1%		50%
N°5	GC13004244.2	M	47	15%	1-2			
N°6	GC13006230.1	F	54	20%	1-2			
N°7	GC09.16397.4	F	38		1	1%		10%
N°8	GC12024886.6	F	32	50-60%	3A			30%
N°9	GC.09.4974.3	M	27	5-50%	1-2			5%
N°10	GC11017015.3	M	48	15-20%	1-2	1%	20%	5%
N°11	GC10007401.2	F	44	20-40%	2	1%		5%
N°12	GC13011521.2	M	64	30%	1-2	1%		10%
N°13	GC12027931.2	F	56	20%	1-2			5%
N°14	GC14014140.3	M	61		1-2	1%		5%
N°15	15.18673-TK.1	F	76	45%	1-2			
N°16	GC11006262.2	F	69	20%	1	1%		5%
N°17	GC120111824.3	M	48	15%	1-2			
N°18	GC13025697	F	73	<15%	1-2			
N°19	GC13020845.2	F	82	<10%	1-2	1%		5%
N°20	GC11017443.1	M	60	15%				
N°21	16.00081-TK.4	M	61	45%				20%
N°22	GC13014027.4	M	66		1			5%
N°23	GC13025051.4	M	45	25-30%	1-2			60%
N°24	GC12010435.1	M	73	20%	1-2			5%
N°25	GC13012266.4	F	72	70%	3	20%	20%	50%
N°26	GC13016579.3	F	61	25%	1-2			5%
N°27	GC13000926.6	F	35	30%	1-2			5%
N°28	16.00555-TK.4	M	56	15%	1-2			80%
N°29	16.21542-01TU TK.4	M	77	60%	3A			5%
N°30	17.06304-02TU TK.8	F	78	70%				10%
N°31	17.17762-01TU TK.4	F	61	20-30%	1-2	5%		10%

N°32	17.23763-01TU TK.4	M	77	40%	1-2			10%
N°33	17.35038-01TU TK.6	F	58	60%	3A	10%	10%	30%
N°34	18.10117-01TU TK.4	F	66	50%	1-2		20%	30%
N°35	18.24505-02TU TK.6	F	67	60-70%	3A			40%
N°36	20.23156-01TU TK.4	M	76	30%	1-2			
N°37	GC10016185.4	M	76	30%	1-2		30%	30%
N°38	GC11018967.2	F	66	15%	1-2			
N°39	GC10.1018.4	M	48	30-50%	3B	70%		20%
N°40	GC11003342.4	M	38	30%	1-2	50%		30%

Supplementary Table S5– Age, Sex, TNM stage, immunohistochemical features of lymphoma biopsies with “All CDK” activities

		Sex	Age of patient	Grade	Ki67	P16	Cyclin D1	Cyclin E
N°1	GC11010171.2	F	49	1-2	30%			
N°5	GC13004244.2	M	47	1-2	15%			
N°6	GC13006230.1	F	54	1-2	20%			
N°8	GC12024886.6	F	32	3A	50-60%			30%
N°28	16.00555-TK.4	M	56	1-2	15%			80%
N°33	17.35038-01TU TK.6	F	58	3A	60%	10%	10%	30%
N°34	18.10117-01TU TK.4	F	66	1-2	50%			30%
N°36	20.23156-01TU TK.4	M	76	1-2	30%			
N°39	GC10.1018.4	M	4	3B	30-50%	70%		20%

Supplementary Table S6– Age, Sex, TNM stage, immunohistochemical features of lymphoma biopsies with “No CDK1” activities

		Sexe	Age of patient	Grade	Ki67	P16	Cyclin D1	Cyclin E
N°2	GC10003529.5	F	55	15-20%	1			50%
N°3	GC12017540.6	M	76	1-2	35%			10%
N°7	GC09.16397.4	F	38	1		1%		10%
N°11	GC10007401.2	F	44	2	20-40%	1%		5%
N°12	GC13011521.2	M	64	1-2	30%	1%		10%
N°16	GC11006262.2	F	69	1	20%	1%		5%
N°21	16.00081-TK.4	M	61		45%			20%
N°22	GC13014027.4	M	66	1				5%
N°24	GC12010435.1	M	73	20%	1-2			5%
N°27	GC13000926.6	F	35	1-2	30%			5%
N°30	17.06304-02TU TK.8	F	78		70%			10%
N°32	17.23763-01TU TK.4	M	77	1-2	40%			10%
N°35	18.24505-02TU TK.6	F	67	3A	60-70%			40%
N°40	GC11003342.4	M	38	1-2	30%	50%		30%