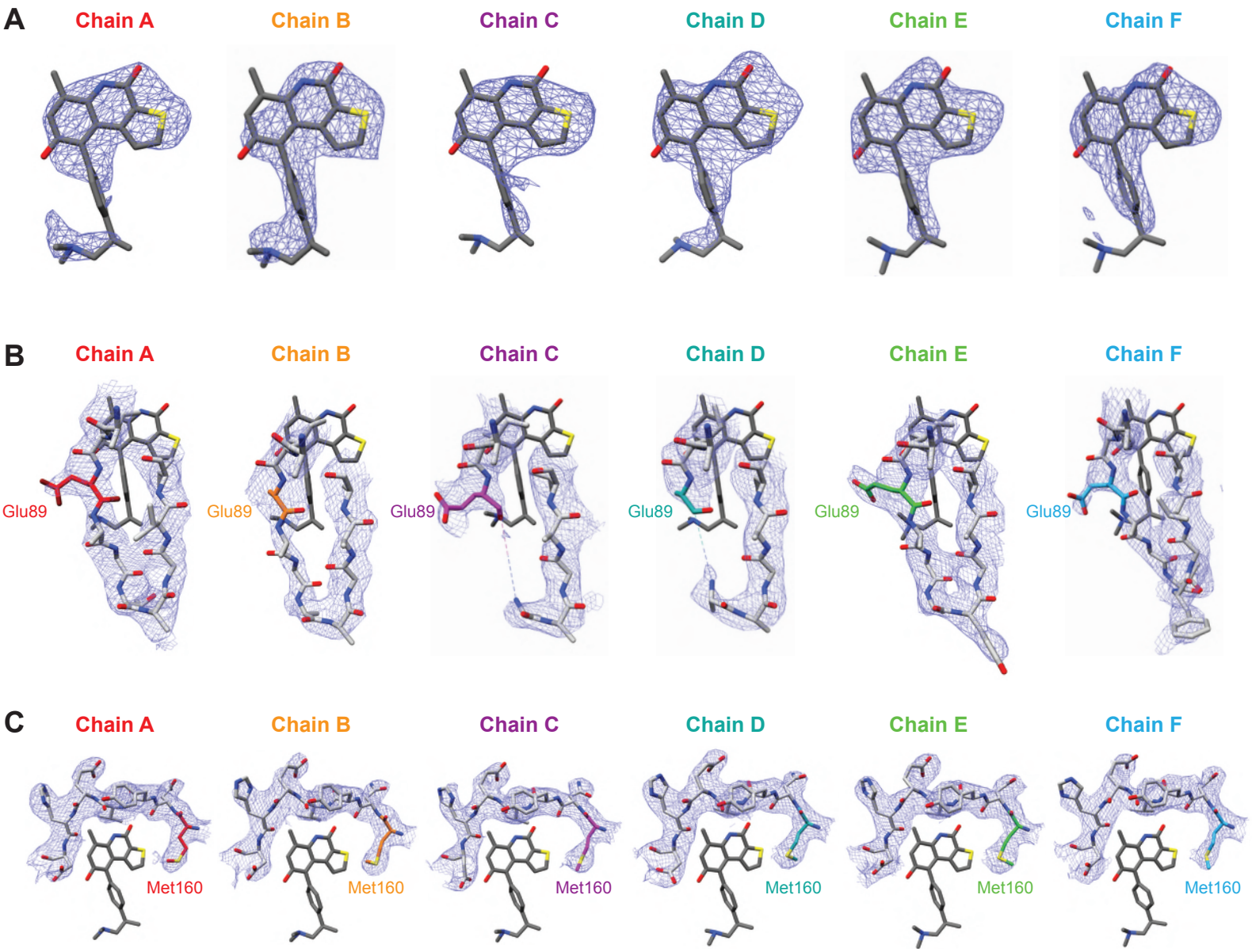
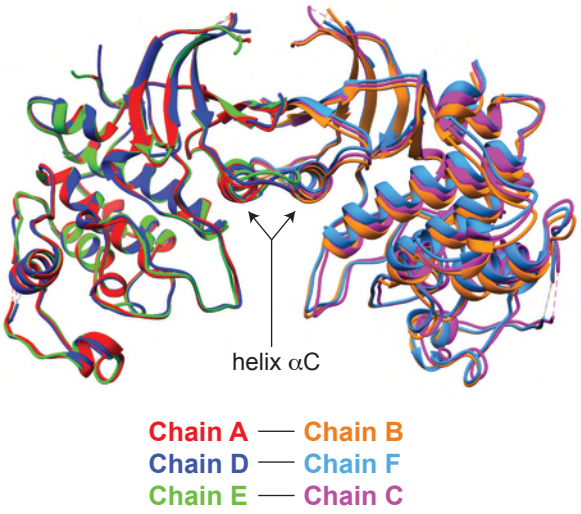
Supplementary Figure 1



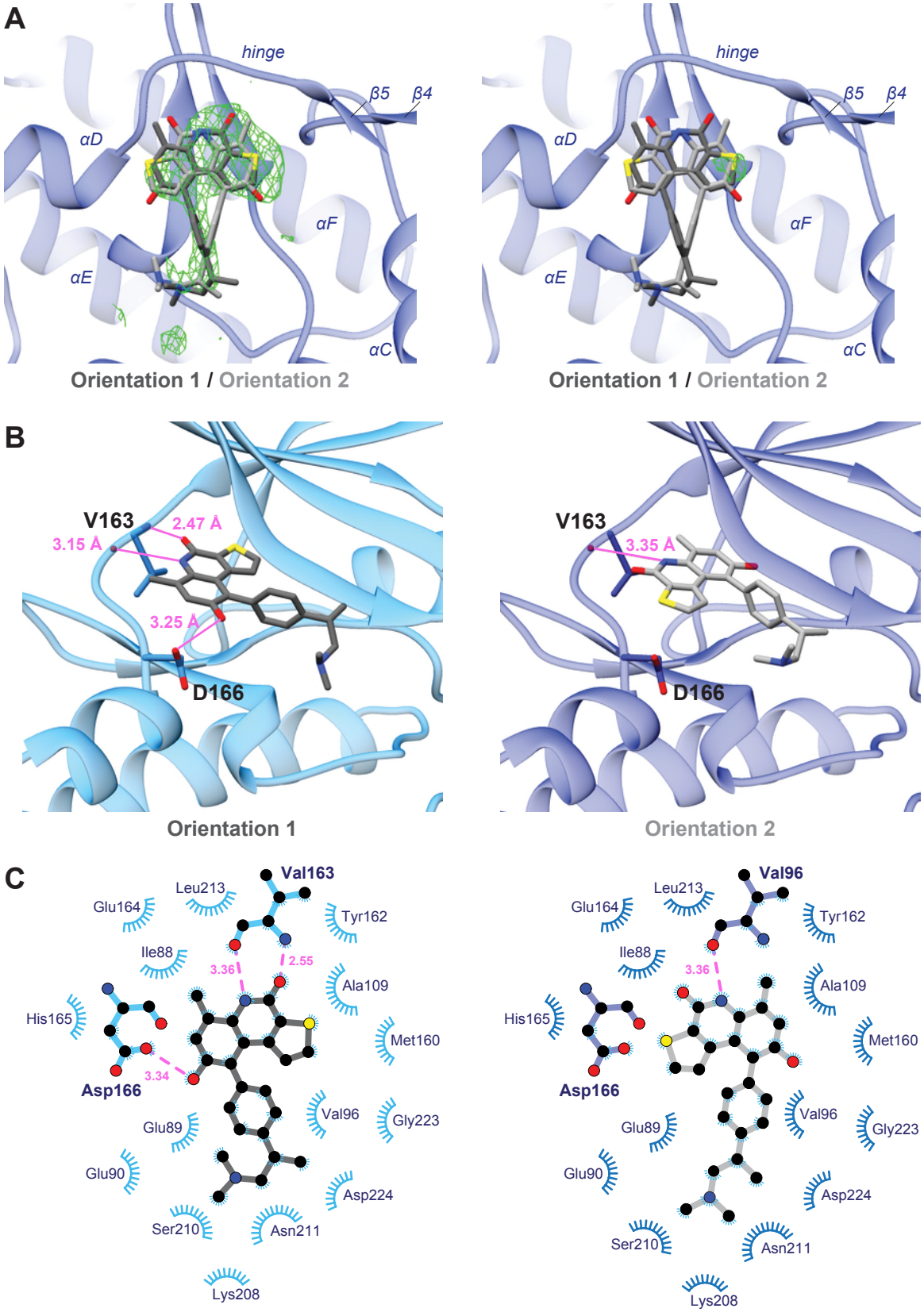
Supp. Figure 1. Features of the OTS964 binding pocket of CDK11, related to Figure 3.
(A) View of simulated annealing omit map (mFo-Fc electron density contoured to 2.5σ) for OTS964.
(B) View of the G-rich loop (including Glu89) of each chain of CDK11 in the asymmetric unit showing 2mFo-DFc electron density contoured to 1σ.
(C) View of the kinase hinge (including Met160) of each chain of CDK11 in the asymmetric unit showing 2mFo-DFc electron density contoured to 1σ.

Supplementary Figure 2



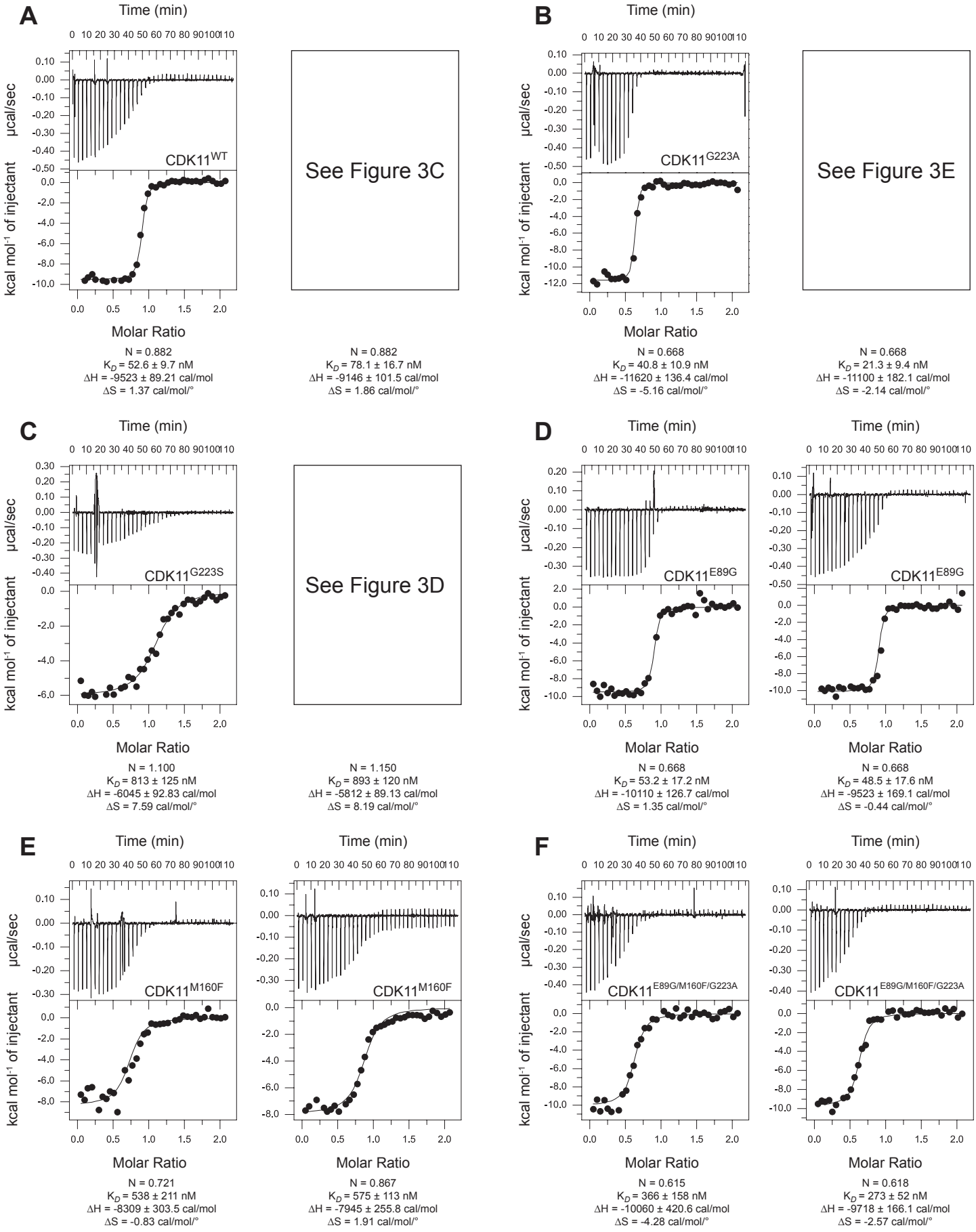
Supp. Figure 2. Crystal packing interactions between CDK11 chains along the surface containing helix αC, related to Figure 2.

Supplementary Figure 3



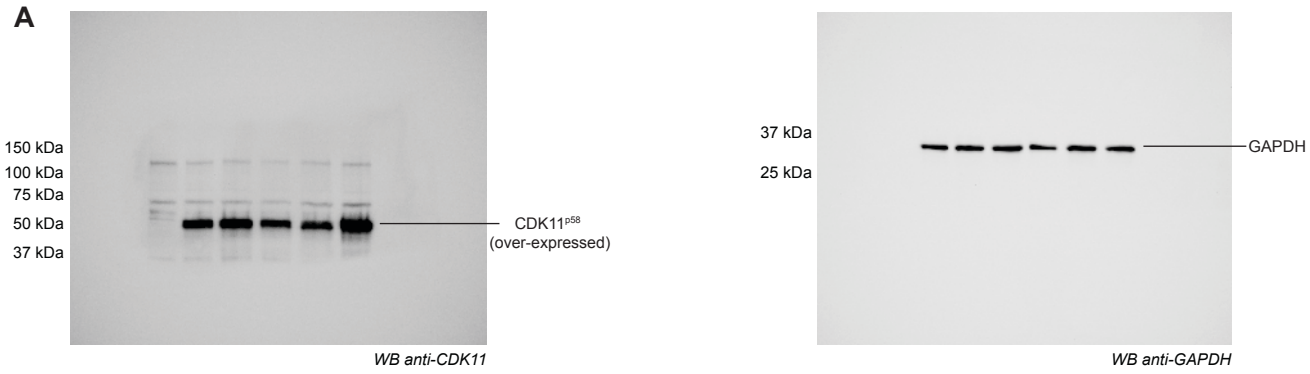
Supp. Figure 3. Determining the orientation of OTS964 in the active site of CDK11, related to Figure 3.
(A) View of |Fo-Fc| electron density prior to docking and refinement of OTS964 coordinates in the ATP binding pocket of CDK11 contoured to 2σ (left) and 5.5σ (right). Strong electron density corresponds to the position of the sulfur atom in Orientation 1.
(B) Binding mode of OTS964 in orientation 1 (left) and orientation 2 (right).
(C) Ligplot view of CDK11 bound to OTS964 in orientation 1 (left) and orientation 2 (right).

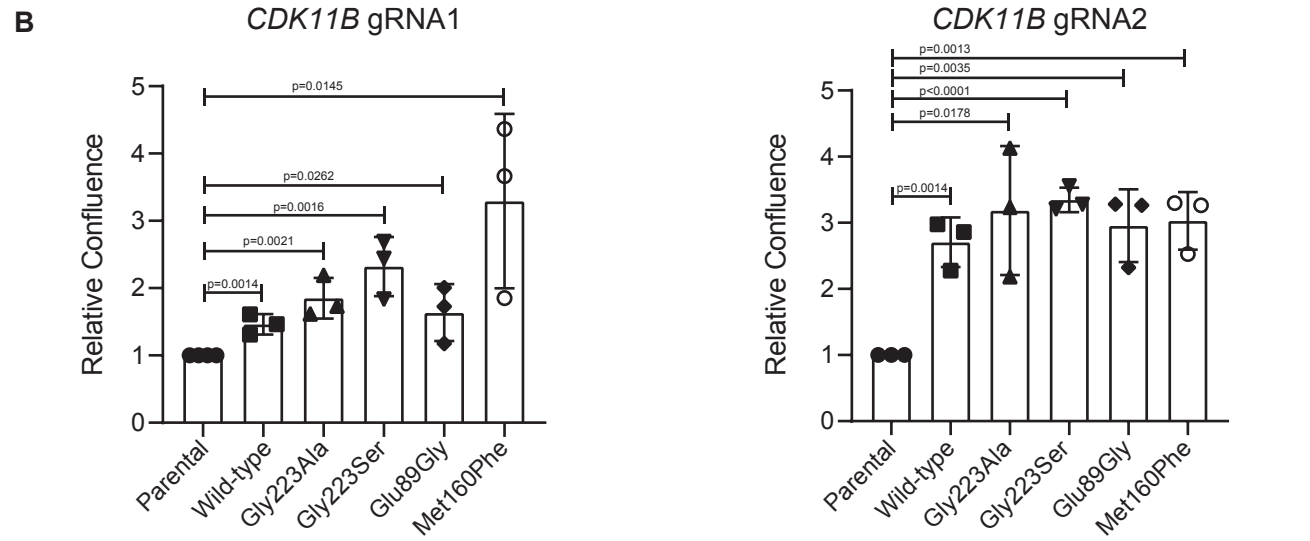
Supplementary Figure 4

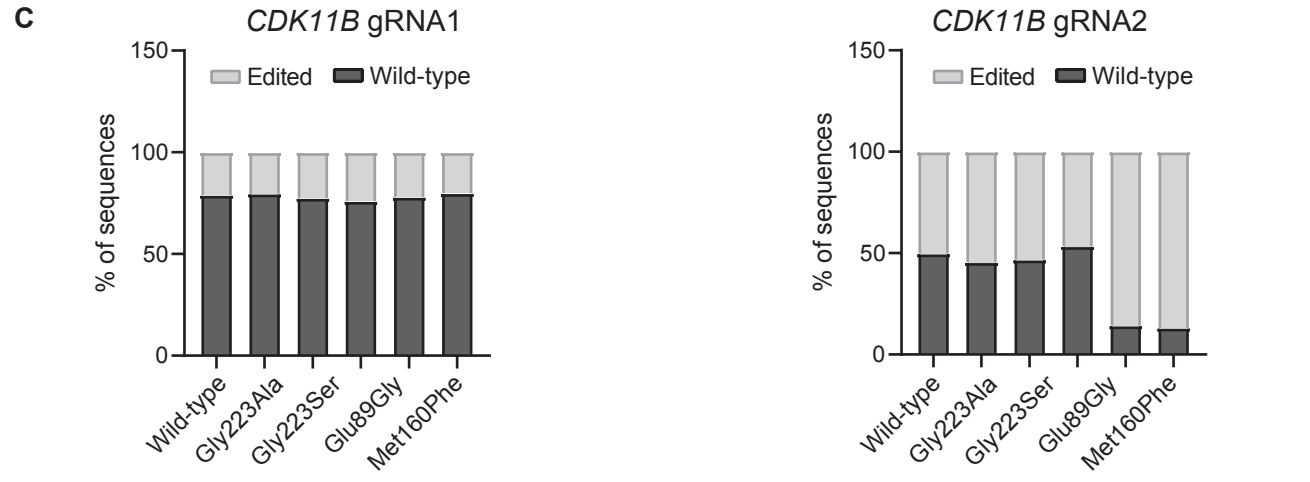


Supp. Figure 4. ITC thermograms for individual OTS964-CDK11 binding experiments, related to Table 2.
ITC thermograms and binding constants for OTS964 binding CDK11 wild-type **(A)** or mutants Gly223Ala **(B)**, Gly223S **(C)**, Glu89Gly **(D)**, Met160Phe **(E)**, or Glu89Gly/Met160Phe/Gly223Ala **(F).**

Supplementary Figure 5

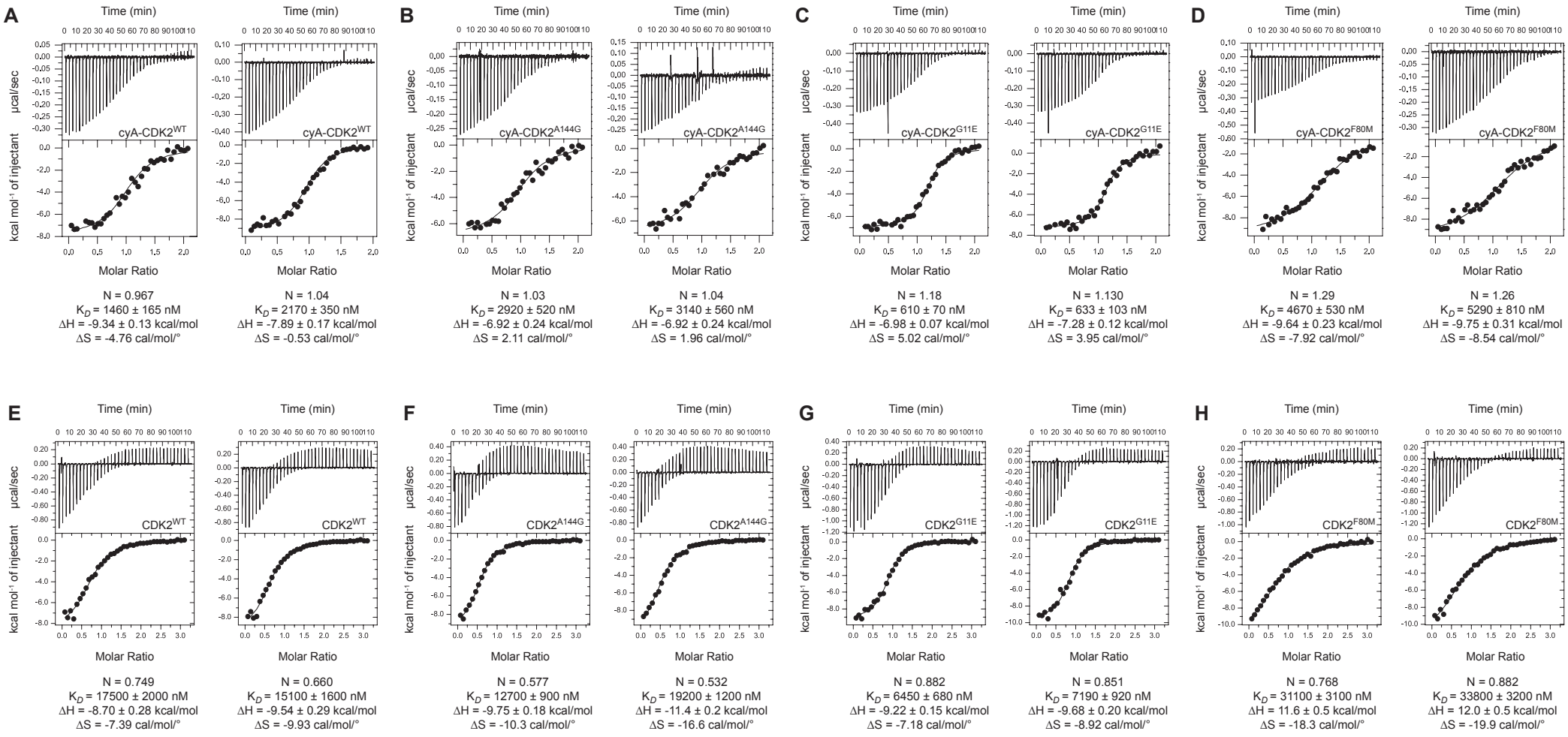






Supp. Figure 5. Survival of A375 cells expressing CDK11p58 mutants after endogenous *CDK11B* CRISPR knock-out , related to Figure 4.
(A) Complete western blot images from Figure 4A, anti-CDK11 (left) and anti-GAPDH (right)
(B) Relative confluence of A375 cell lines stably expressing either CDK11 wild-type or mutants 6 days after treatment to knock-out endogenous *CDK11B*. Data represents three independent replicates, mean ± SEM.
(C) TIDE analysis performed to quantify the editing efficiency of each gRNA targetting *CDK11B*.

Supplementary Figure 6



Supp. Figure 6. ITC thermograms for individual OTS964-CDK2 binding experiments, related to Table 2.
ITC thermograms and binding constants for OTS964 and CDK2-cyclin A complexes containing either CDK2 wild-type **(A)** or mutants Ala144Gly **(B)**, Gly11Glu **(C)**, or Phe80Met **(D)**.
ITC thermograms and binding constants for OTS964 and CDK2 (no cyclin) wild-type **(E)** or mutants Ala144Gly **(F)**, Gly11Glu **(G)**, or Phe80Met **(H)**.

Table S1. Additional oligonucleotides, related to STAR Methods

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Primer CDK11B N1 NcoI forward: TCAGCCATGGGGATGAGTGAAGATGAAGAACGAGAAAATGA	ThermoFisher	N/A
Primer CDK11B N72 NcoI forward: GTCAGCCATGGGGGCCCTGCAGGGCTGC	ThermoFisher	N/A
Primer CDK11B C439 HindIII reverse: TCAGAAGCTTTCAGAACTTGAGGCTGAAGCCG	ThermoFisher	N/A
Primer CDK11B C380 HindIII reverse: TCAGAAGCTTGGGGAACATGGAGGGGTCG	ThermoFisher	N/A
Primer CDK11 M516F forward: GACAAGATCTACATCGTGTTTAACTATGTGGAGC	Millipore Sigma	N/A
Primer CDK11 M516F reverse: GTGCTCCACATAGTTAAACACGATGTAGATCTTG	Millipore Sigma	N/A
Primer CDK11 G579A forward: CATCCTCAAGGTGGCGGACTTCGGGCTGG	Millipore Sigma	N/A
Primer CDK11 G579A reverse: CAGCCCGAAGTCCGCCACCTTGAGGATGC	Millipore Sigma	N/A
Primer CDK11 G579S forward: CATCCTCAAGGTGAGCGACTTCGGGCTGG	Millipore Sigma	N/A
Primer CDK11 G579S reverse: CAGCCCGAAGTCGCTCACCTTGAGGATGC	Millipore Sigma	N/A
Primer CDK11 E445G forward: CCTGAACAGGATCGGAGAGGGCACCTATGG	Millipore Sigma	N/A
Primer CDK11 E445G reverse: CTCCATAGGTGCCCTCTCCGATCCTGTTCAG	Millipore Sigma	N/A
Primer CDK2 F80M forward: CAGCCCGAAGTCGCTCACCTTGAGGATGC	Millipore Sigma	N/A
Primer CDK2 F80M reverse: GTGCAGAAATTCCATAACCAGGTAGAGTTTATTTTCTG	Millipore Sigma	N/A
Primer CDK2 A144G forward: GGCCATCAAGCTAGGCGACTTTGGACTAG	Millipore Sigma	N/A
Primer CDK2 A144G reverse: GGCTAGTCCAAAGTCGCCTAGCTTGATGG	Millipore Sigma	N/A
Primer CDK2 G11E forward: GGAAAAGATCGAAGAGGGCACGTACG	Millipore Sigma	N/A
Primer CDK2 G11E reverse: GTACGTGCCCTCTTCGATCTTTTCCAC	Millipore Sigma	N/A
Primer Cyclin A N173 SfoI forward: GCCAATGAAGTACCAGACTACCATGAGG	Millipore Sigma	N/A
Primer Cyclin A C432 XhoI forward: GCGCTCGAGTTACAGATTTAGTGTCTCTGG	Millipore Sigma	N/A