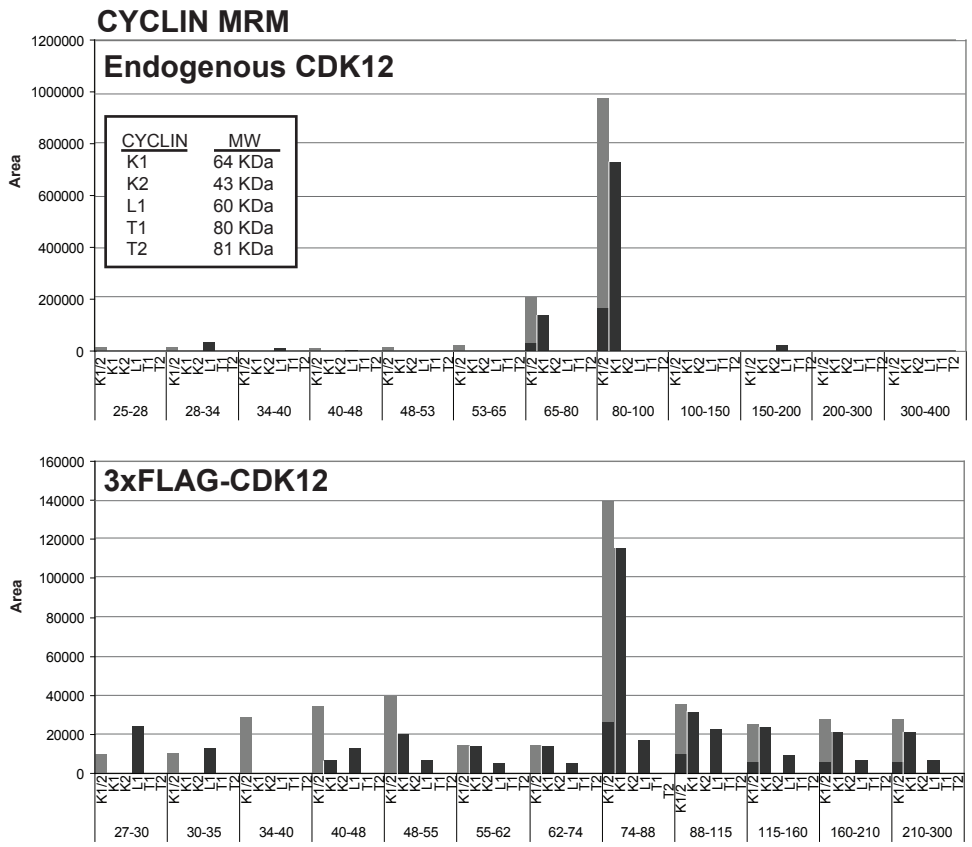
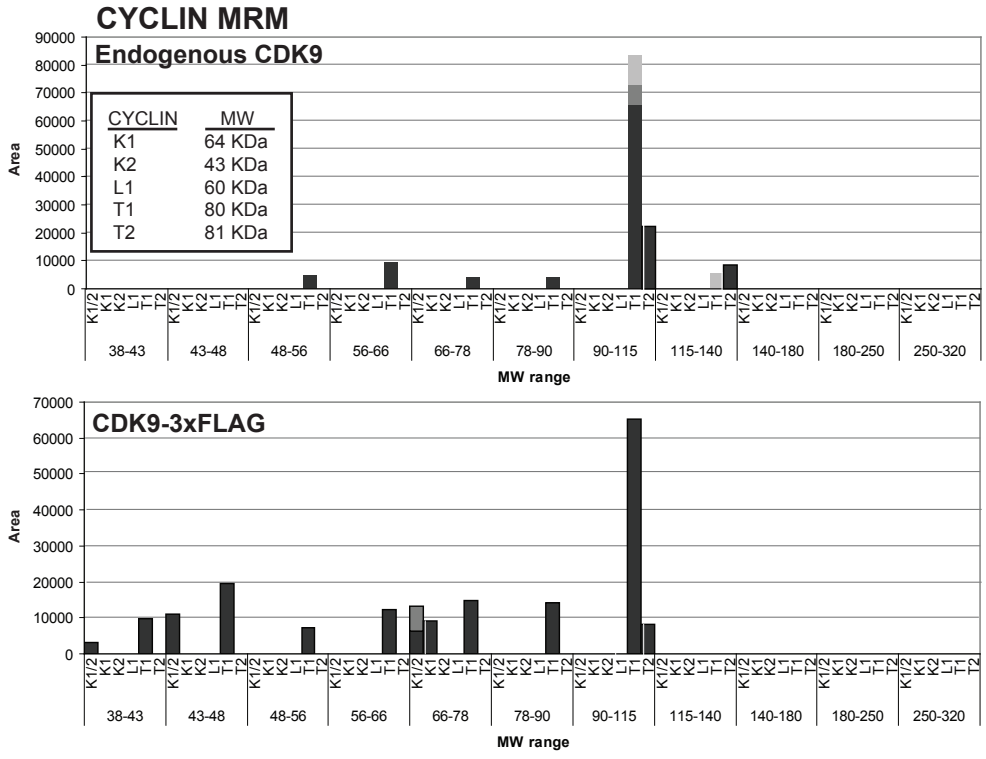
A


CYCLIN MRM
Endogenous CDK12
CYCLIN MW
K1 64 KDa
K2 43 KDa
L1 60 KDa
T1 80 KDa
T2 81 KDa
Area
25-28
28-34
34-40
40-48
48-53
53-65
65-80
80-100
100-150
150-200
200-300
300-400
3xFLAG-CDK12
27-30
30-35
34-40
40-48
48-55
55-62
62-74
74-88
88-115
115-160
160-210
210-300

B


CYCLIN MRM
Endogenous CDK9
CYCLIN MW
K1 64 KDa
K2 43 KDa
L1 60 KDa
T1 80 KDa
T2 81 KDa
Area
38-43
43-48
48-56
56-66
66-78
78-90
90-115
115-140
140-180
180-250
250-320
MW range
CDK9-3xFLAG
MW range

Supplemental Fig S1. Multiple Reaction Monitoring (MRM) analysis of immunoprecipitated complexes.

Immunoprecipitated protein complexes were separated by SDS-PAGE. The gel was cut into approximate molecular weight ranges, subjected to in-gel trypsin digestion and the resulting peptides were analyzed by MRM. Different shades of grey on the stacked columns represent different peptides from the same protein used in the MRM assay. A minimum of two transitions per peptide were summed. **A.** CYCLIN MRM analysis of endogenous CDK12 and 3xFLAG-CDK12 protein complexes showed that CDK12 complexes contained CYCLIN K1 and not CYCLIN K2. CYCLIN K1 and CYCLIN K2 were distinguished in this assay by CYCLIN K1 and CYCLIN K2 specific MRM signatures as well as by molecular weight. CYCLIN L1 appeared to be a minor CDK12 interacting protein using this assay. **B.** CDK MRM analysis of 3xFLAG-CYCLIN K1 and 3xFLAG-CYCLIN L1 protein complexes showed that CYCLIN K1 interacted predominantly with CDK12 and CDK13 and minimally with CDK9, while CYCLIN L1 interacted only with CDK11 in this assay.