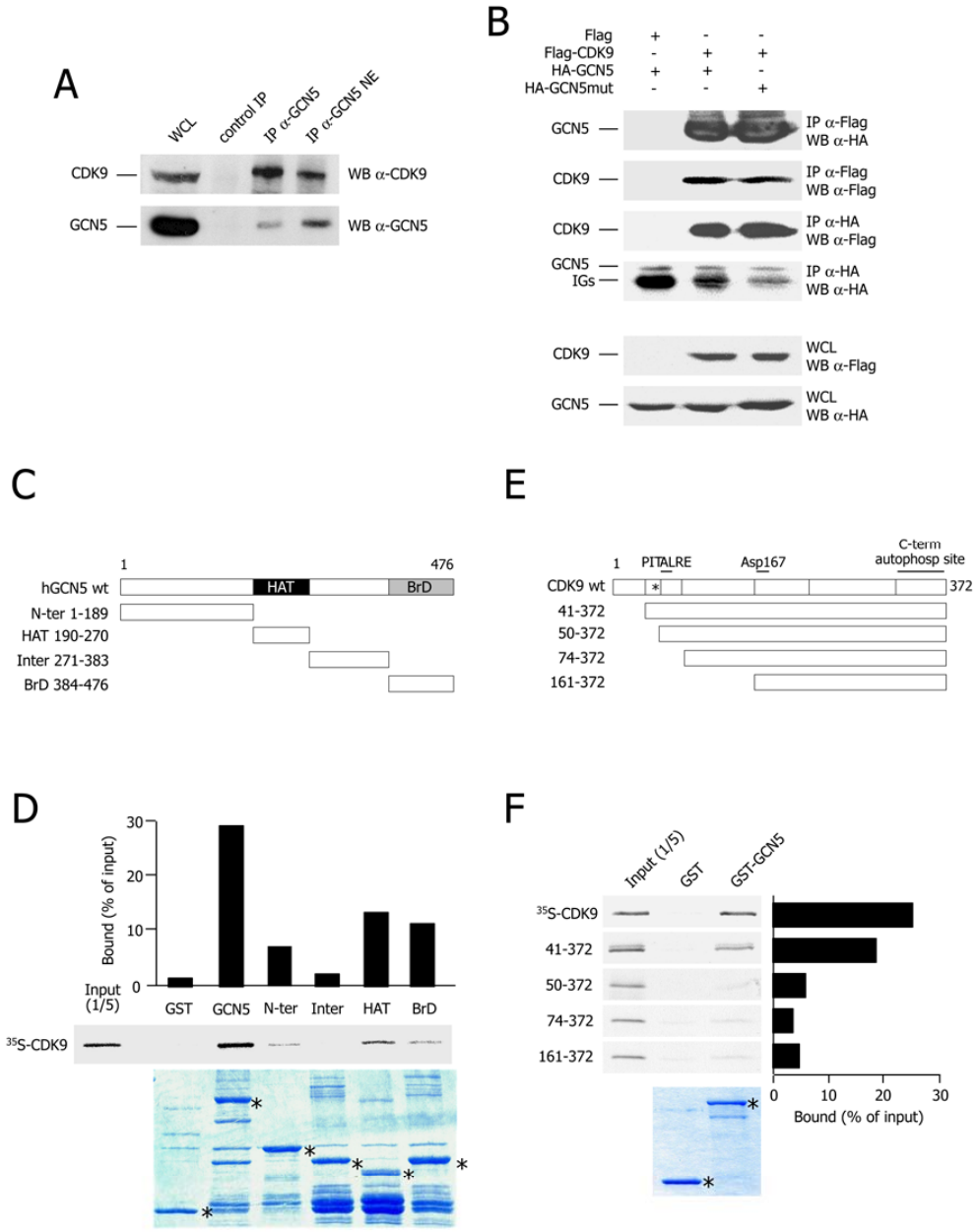
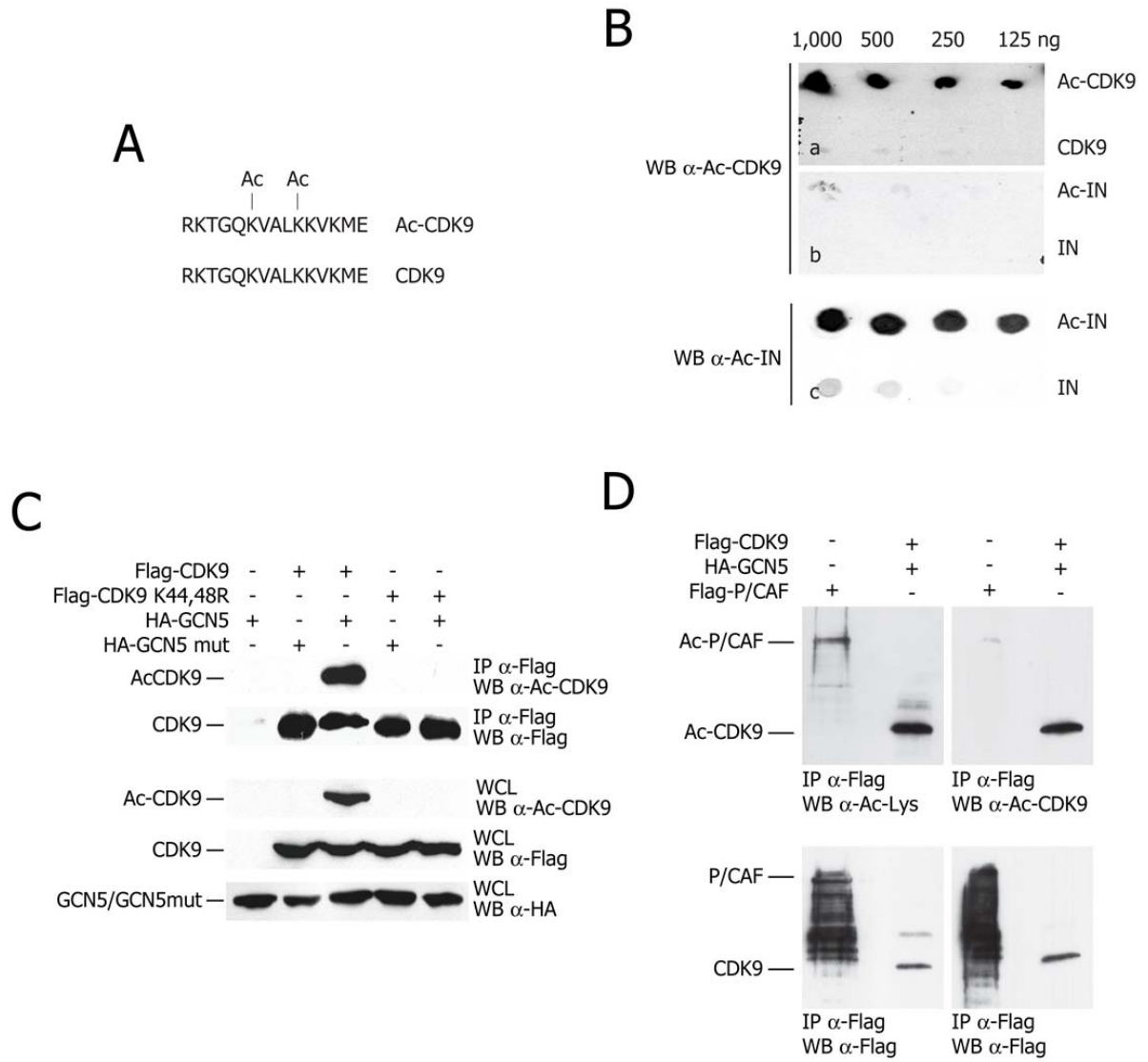
SUPPLEMENTARY FIGURE 1 - CDK9 binds GCN5 in vitro and in vivo



A. CDK9 interacts with GCN5 in vivo. HeLa whole cell lysate (WCL) and nuclear extract (NE) were subject to immunoprecipitation with an anti-GCN5 antibody and then analyzed by western blotting using a specific anti-CDK9 antibody. CDK9 could be detected together with GCN5 in the bound fraction. **B.** CDK9 binds both wt GCN5 and its catalytically inactive mutant. Extracts prepared from cells co-transfected with Flag-CDK9 and wt or mutated HA-GCN5 were immunoprecipitated with an anti-Flag antibody and immunoblotted with anti-HA antibody and vice versa (upper four panels). The same extracts were run on an SDS-PAGE gel and immunoblotted with anti-Flag and anti-HA antibodies to verify protein expression levels (lower two panels). **C.** CDK9 binds the bromo- and HAT-domains of GCN5 in vitro. Schematic representation of the GST-GCN5 fragments used for pull down assays. **D.** GST-GCN5 fragments were incubated with [^{35}S]-CDK9, extensively washed and then analyzed by SDS-PAGE. **E.** GCN5 binds the N-terminus of CDK9 in vitro. Schematic representation of the [^{35}S]-CDK9 deletion mutants used for pull down assays. The position of the lysines that are positive for acetylation is indicated by an asterisk. **F.** GST-GCN5 or GST alone as a control were incubated with [^{35}S]-CDK9 deletion mutant proteins, extensively washed and then analyzed by SDS-PAGE.

The upper panels in (D) and (F) show the gels exposed to a phosphoimager screen, while the lower panels the Coomassie stainings of the same gels. The bands corresponding to the relevant intact GST proteins are indicated by asterisks. The graphs show the amounts of bound proteins as percentages of radiolabeled input.

SUPPLEMENTARY FIGURE 2 - Characterization of anti-acetylated CDK9 antibody



A. Sequence of the 15-mer peptides corresponding to human CDK9 amino acids 39-53, either acetylated or not acetylated on lysines 44 and 48. **B.** Dot blot experiment showing specificity of the anti-Ac-CDK9 antibody. Peptides corresponding to human CDK9 (shown in (A)) were blotted onto nitrocellulose filters in the indicated amounts and challenged with IgGs (1:500) from an animal immunized with the acetylated peptide. The antibody only recognizes the acetylated CDK9 peptide, but not its non-acetylated version (panel a), or an unrelated peptide from HIV-1 integrase (IN), either acetylated or not-acetylated (panel b). We have previously shown that this IN sequence is acetylated inside the cells (Cereseto, A, et al. 2005. EMBO J. 24, 3070); the acetylated IN peptide is recognized by another antiserum specifically raised against this acetylated peptide (shown here as a loading control in panel c). **C.** Anti-Ac-CDK9 antibody specifically reacts with wild type CDK9 and not with the not-acetylable CDK9 K44,48R mutant. Extracts of 293T cells transfected with the indicated plasmids were immunoprecipitated with an anti-Flag antibody and subject to western blot (WB) with the anti-Ac-CDK9 or anti-Flag antibodies (upper two panels). Protein expression levels were verified by western blot on total cell lysates (lower three panels). **D.** Anti-Ac-CDK9 antibody specifically reacts with Ac-CDK9 but not with Ac-P/CAF. Flag immunoprecipitates from cells expressing Flag-CDK9 (plus GCN5) or Flag-P/CAF (another acetylated protein) were probed by western blotting with an antibody against total acetylated lysines (upper left panel) or the anti-Ac-CDK9 antibody (upper right panel). The former antibody detects both acetylated CDK9 and acetylated P/CAF; the anti-Ac-CDK9 antibody only recognizes acetylated CDK9. The lower two panels show loading controls (western blot with anti-Flag antibody).