

Supplementary Figure S1

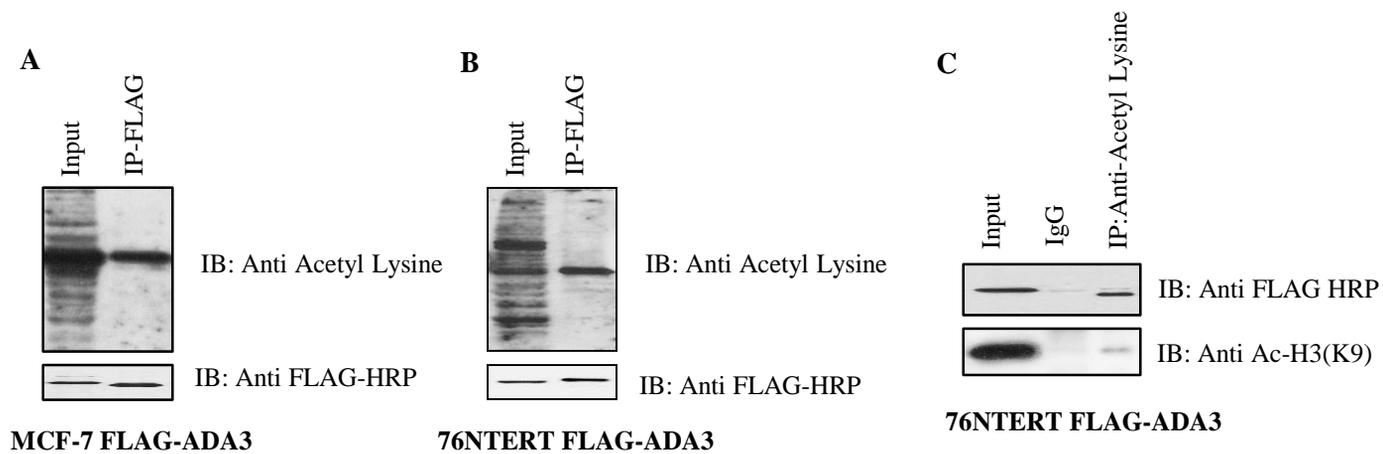
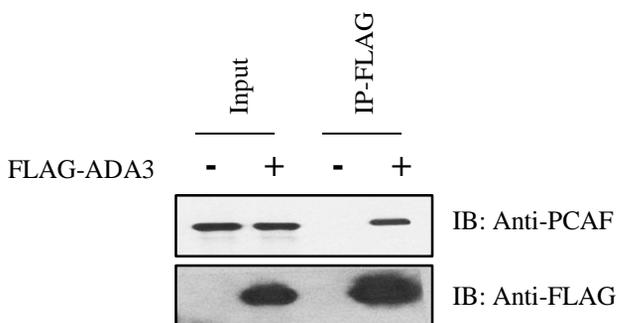


Figure S1: ADA3 is acetylated in various cell lines upon inhibition of HDACs . (A, B & C) MCF7 (A) and 76NTERT (B & C) cells stably overexpressing FLAG-ADA3 were treated with TSA+NAM and whole cell lysate were immunoprecipitated with M2 agarose FLAG beads (A & B) or pan acetyl lysine antibodies (C) followed by immunoblotting with indicated antibodies.

Supplementary Figure S2

A



B

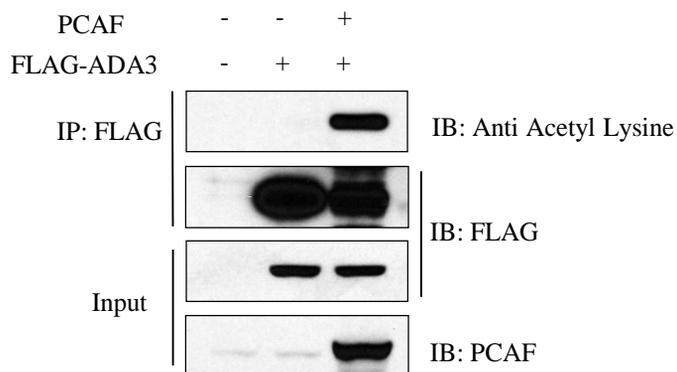


Figure S2: ADA3 interacts with and acetylated by PCAF. (A & B) HEK 293T cells were transfected with empty vector or FLAG-ADA3 (A) and with or without PCAF (B). Whole cell extracts were subjected to immunoprecipitation with M2 FLAG agarose beads, eluted with 3X FLAG peptide and immunoblotted with indicated antibodies.

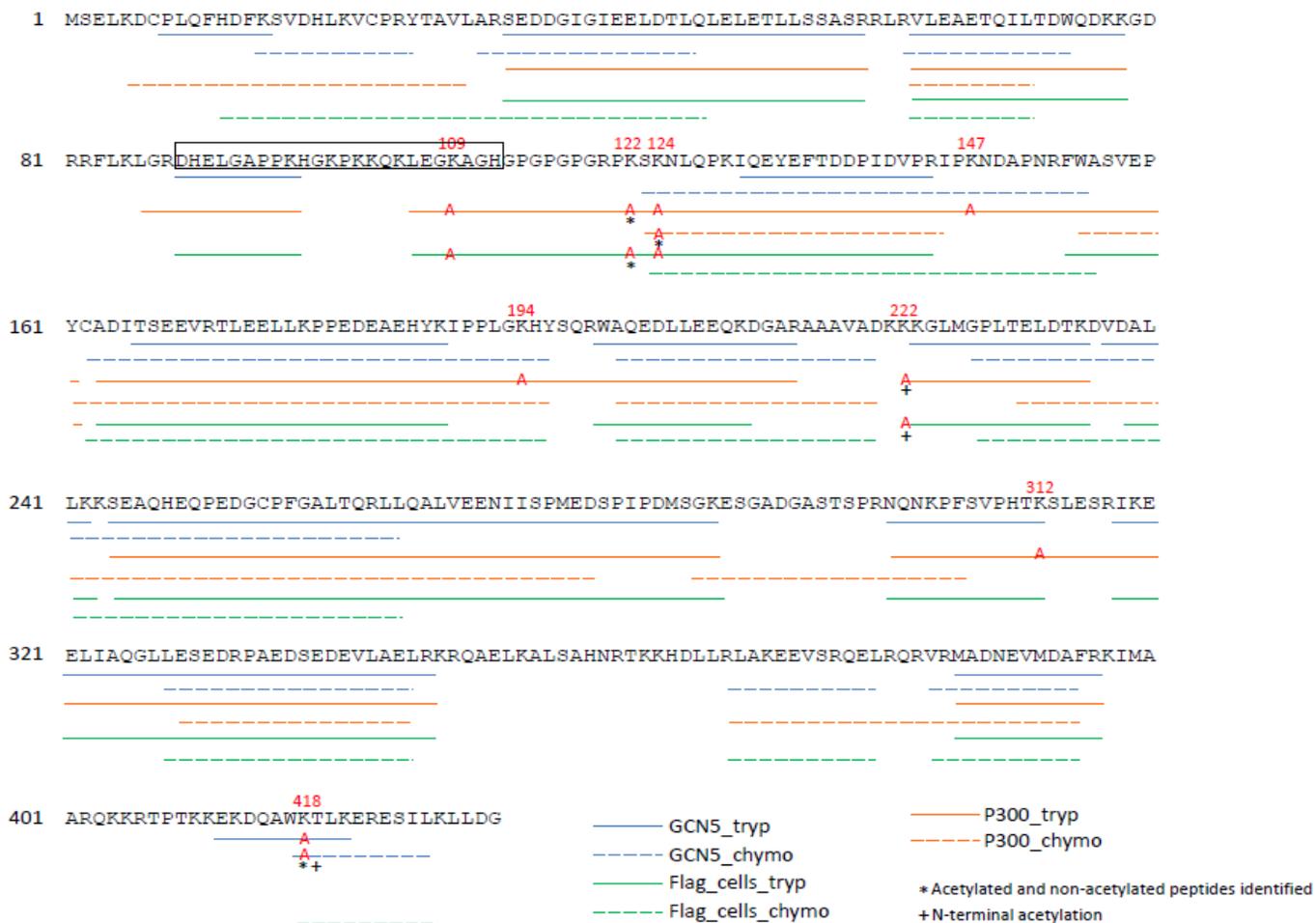
Supplementary Figure S3

A Lysines colored in **red** were identified as acetylated lysines and the underlined sequences indicate the regions of ADA3 covered by mass spectrometry

(i) Trypsin, TripleTOF: 67.1% sequence coverage
 MSELKDCPLOFHDFKSVDHLKVCPRYTAVLARSEDDGIGIEELDTLQLELETLSSASRR
 LRVLEAETQILTDWQDKKGRDRFLKLGRDHELGAPPKHGKPKKQKLEGKAGHGPGPGPGR
PKSKNLQPKIQEYEFTDDPIDVPRIPKNDAPNRFWASVEPYCADITSEEVRTLEELLKPP
EDEAEHYKIPPLGKHYSQRWAQEDLLEEQKDGARAAAVADKKKGLMGPLTELDTKDVDAL
 LKKSEAQHEQPEDGCPFGALTQRLLOALVEENIISPMEDSPIPDMSGKESGADGASTSPR
NONKPFVPHTKSLESRIKEELIAOGLLESEDRPAEDSEDEVLAELRKRQAEKALSAHN
 RTKKHDLRLAKEEVSQELRQRVRMADNEVMDAFRKIMAARQKKRTPTKKEKDQAWKTL
KERESILKLLDG

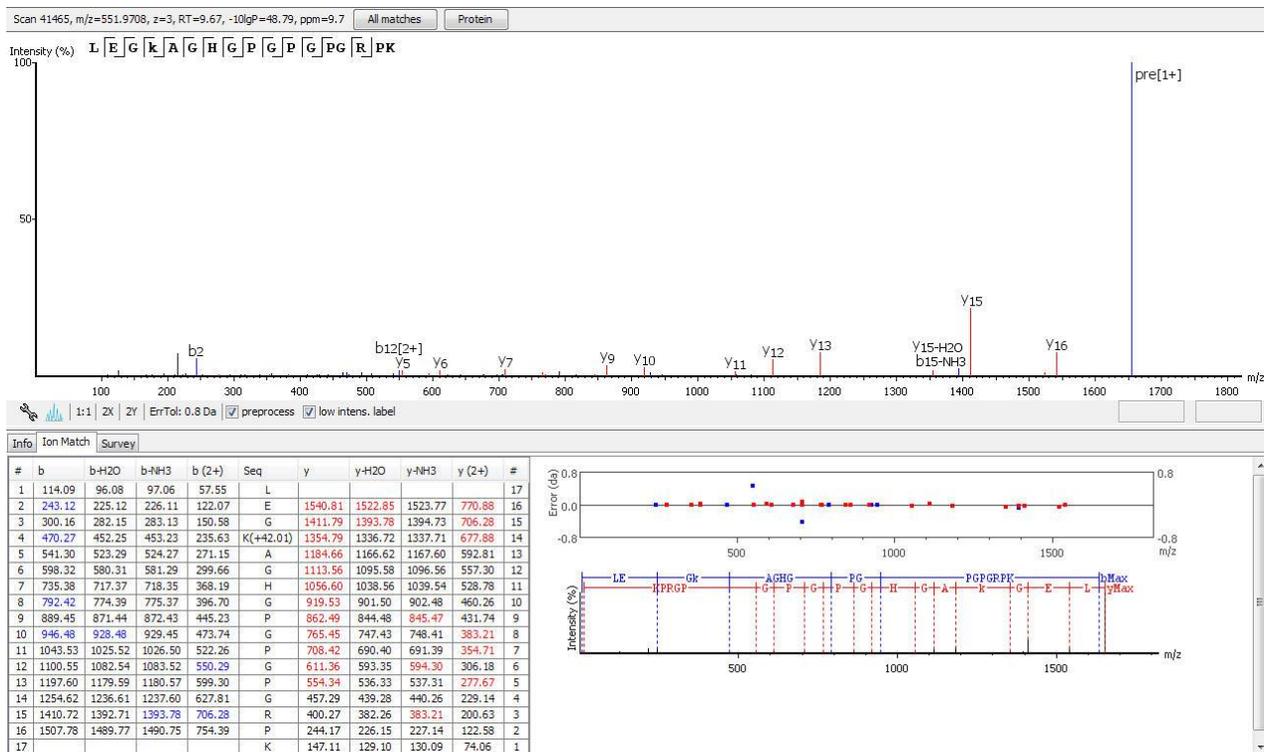
(ii) Chymotrypsin, TripleTOF: 73% sequence coverage
 MSELKDCPLOFHDFKSVDHLKVCPRYTAVLARSEDDGIGIEELDTLQLELETLSSASRR
 LRVLEAETQILTDWQDKKGRDRFLKLGRDHELGAPPKHGKPKKQKLEGKAGHGPGPGPGR
PKSKNLQPKIQEYEFTDDPIDVPRIPKNDAPNRFWASVEPYCADITSEEVRTLEELLKPP
EDEAEHYKIPPLGKHYSQRWAQEDLLEEQKDGARAAAVADKKKGLMGPLTELDTKDVDAL
 LKKSEAQHEQPEDGCPFGALTQRLLOALVEENIISPMEDSPIPDMSGKESGADGASTSPR
NONKPFVPHTKSLESRIKEELIAOGLLESEDRPAEDSEDEVLAELRKRQAEKALSAHN
 RTKKHDLRLAKEEVSQELRQRVRMADNEVMDAFRKIMAARQKKRTPTKKEKDQAWKTL
KERESILKLLDG

B

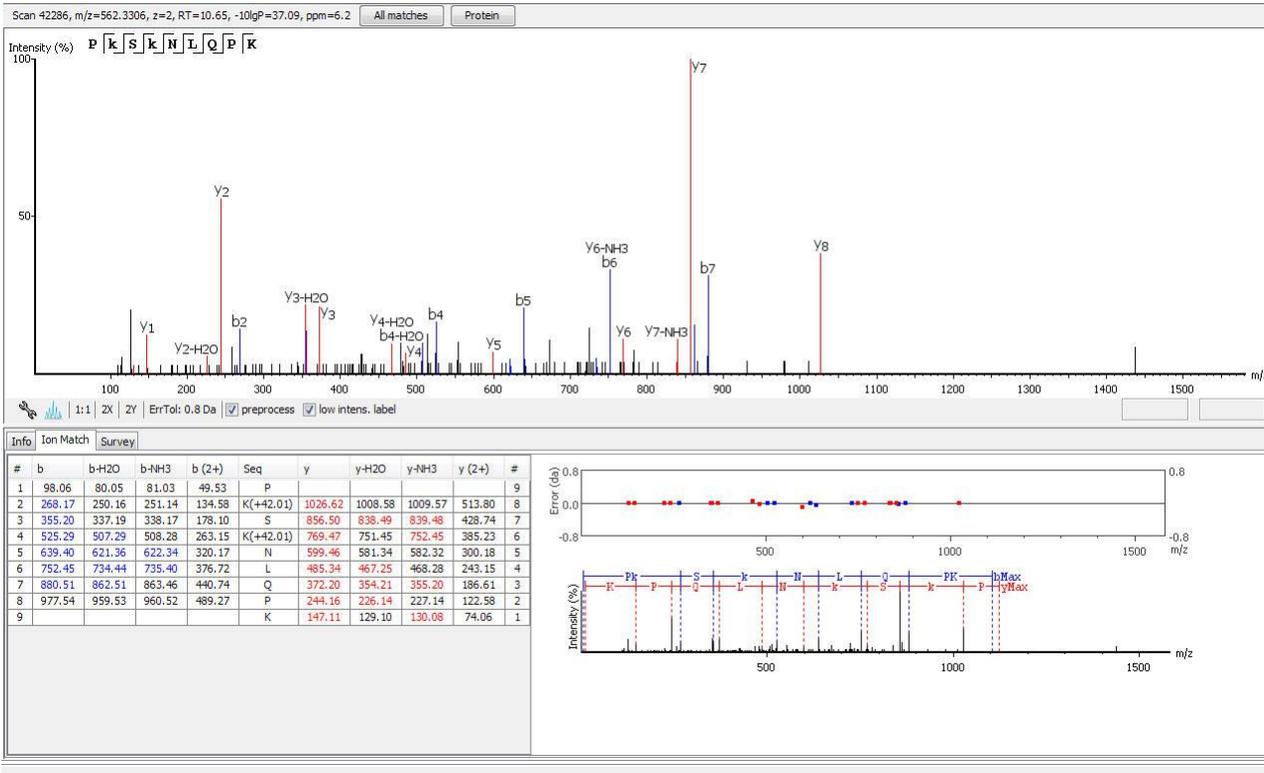


Supplementary Figure S3 (Continued)

C

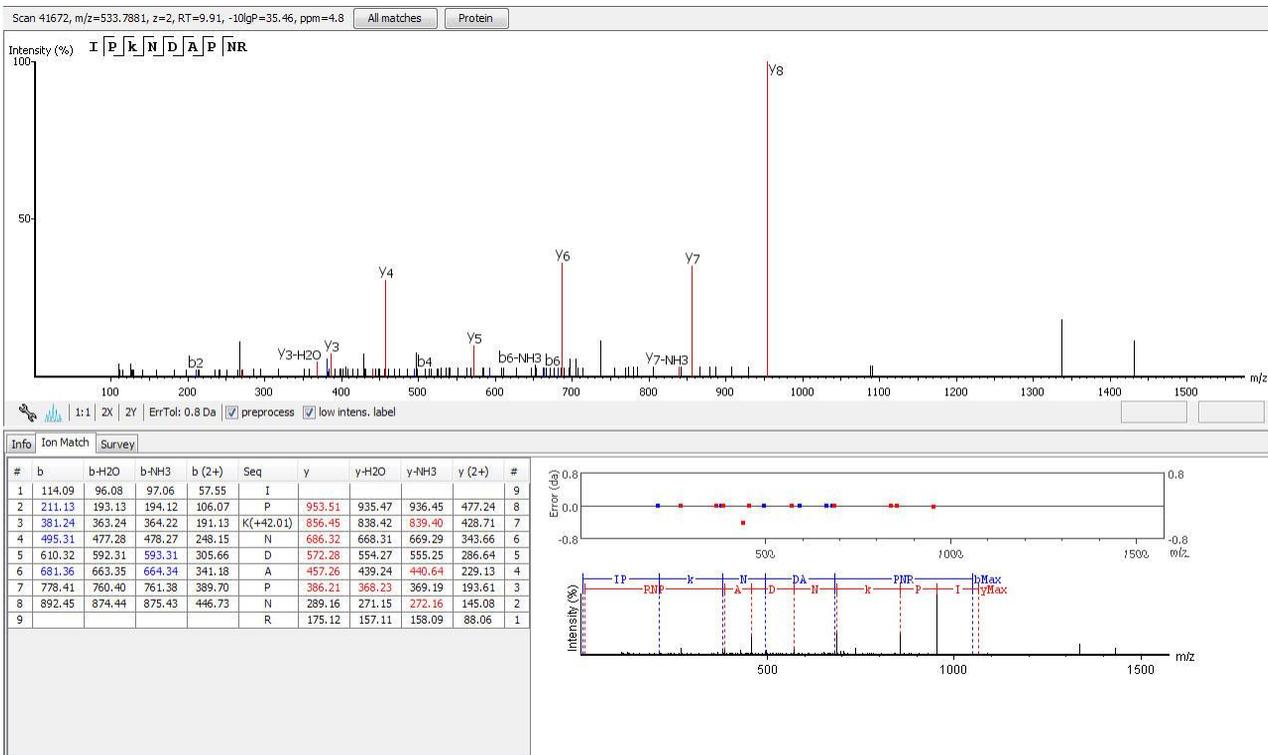


D

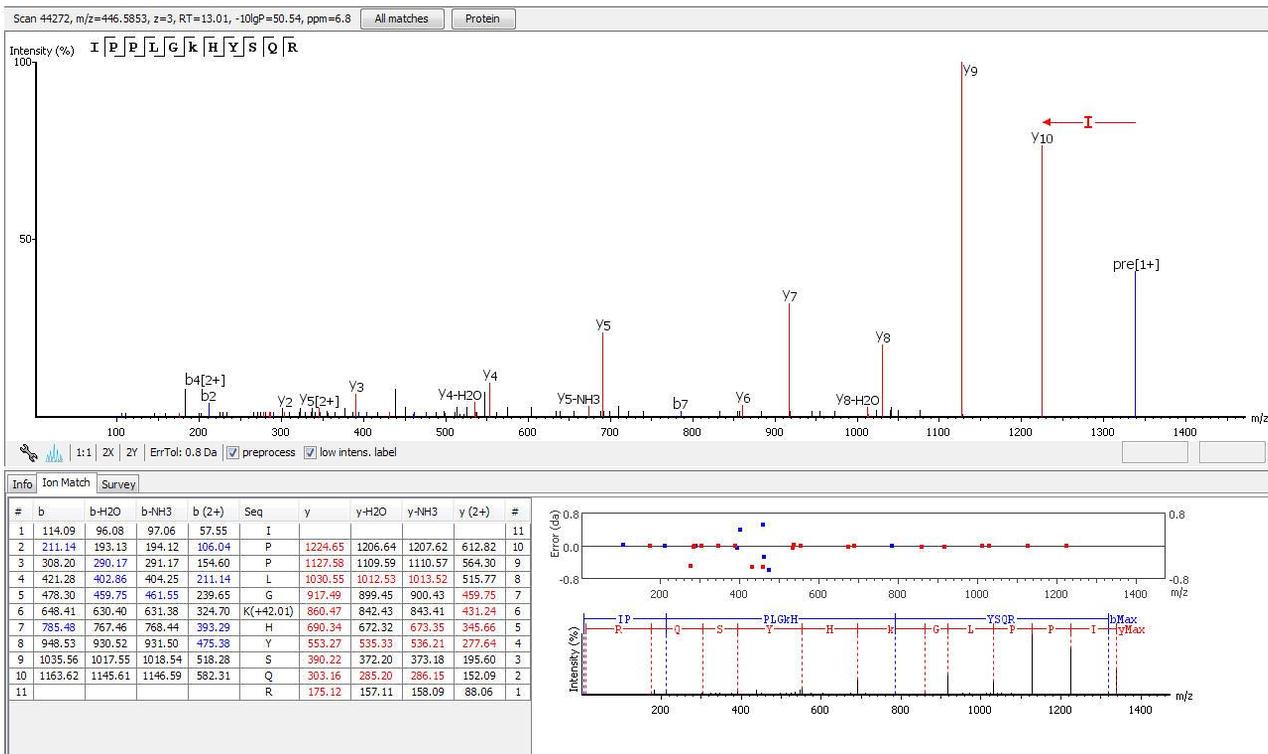


Supplementary Figure S3 (Continued)

E

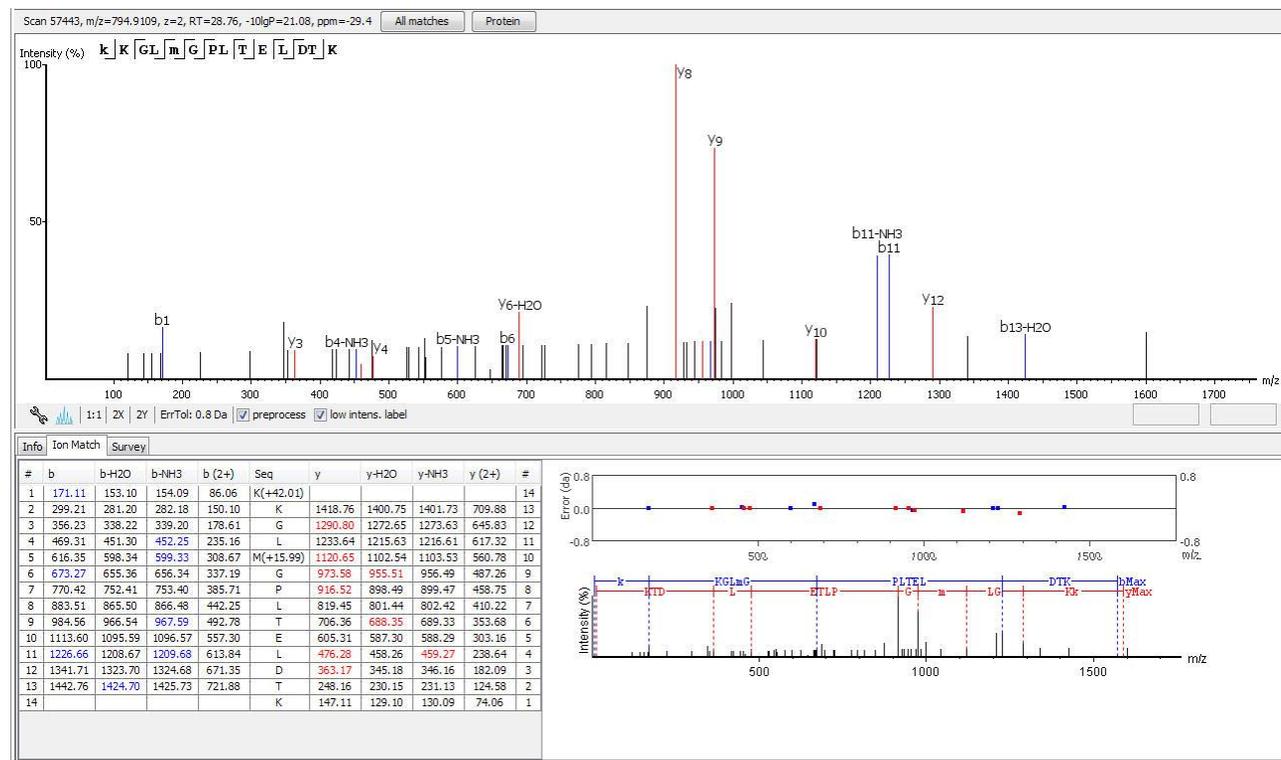


F

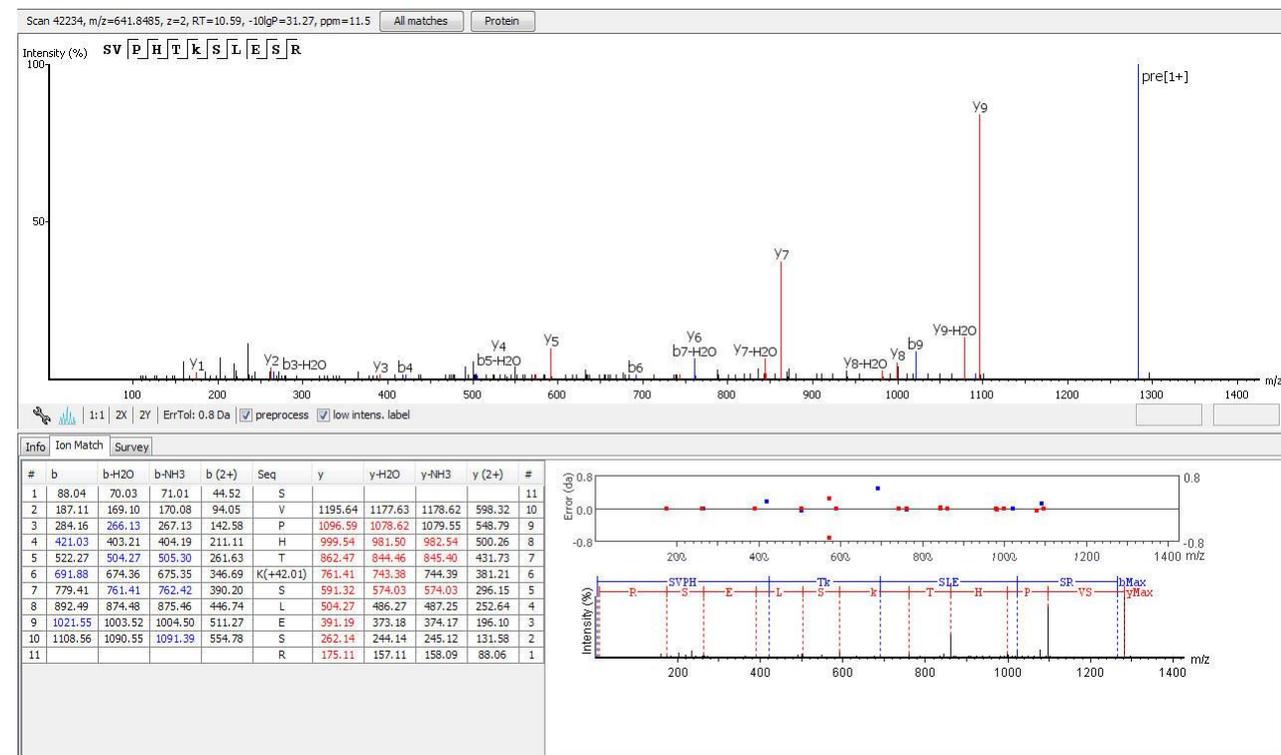


Supplementary Figure S3 (Continued)

G



H



I

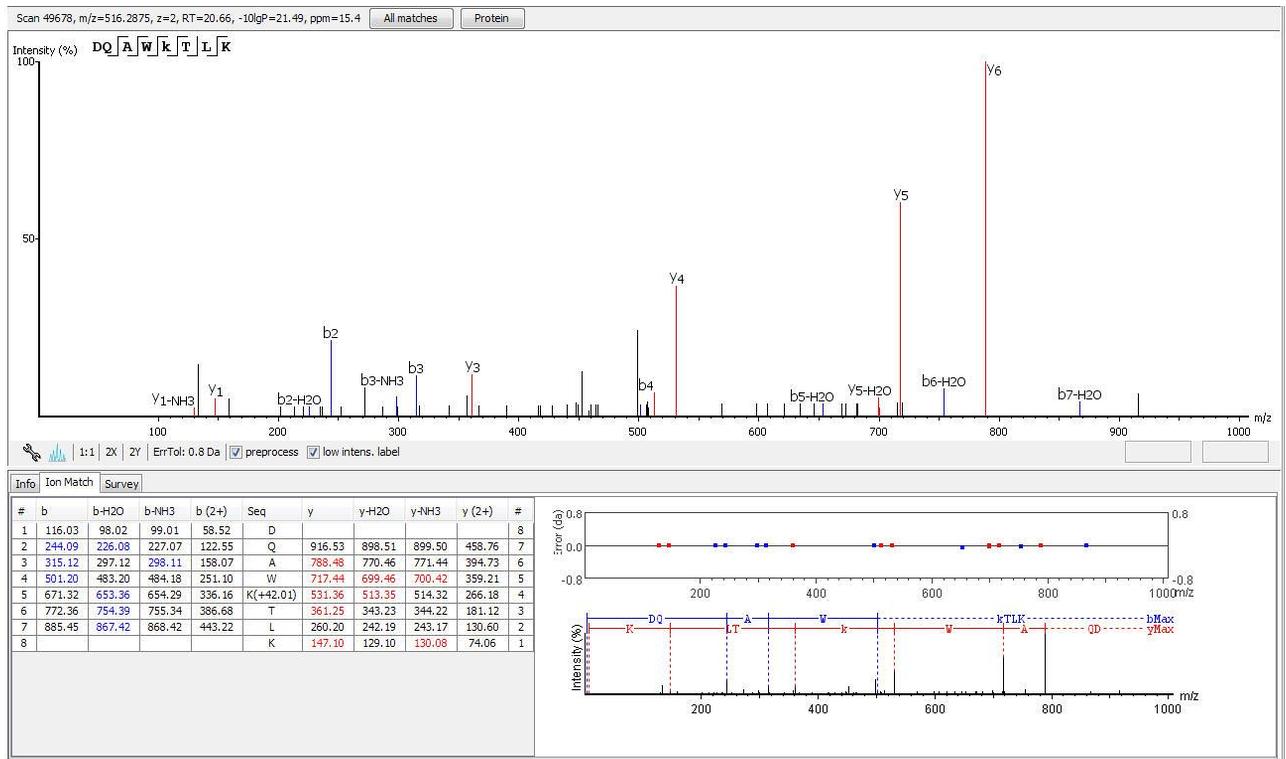


Figure S3: Coverage of ADA3 and detection of acetylated lysines by mass spectrometric analysis of *in vitro* or *in vivo* acetylated ADA3. (A and B) Summary of coverage by mass spectrometry and acetylated lysines identified in ADA3 by mass spectrometry of both trypsin and chymotrypsin digested samples immunoprecipitated from (A) HEK293T, (B) 76NTERT or (B) *in vitro* acetylated by GCN5 or p300. (C-I) Identification of various acetylated lysine residues on ADA3 by mass spectrometry - (C) K109 (D) K122, K124 (E) K147 (F) K194 (G) K222 (H) K312 and (I) K418

Supplementary Figure S4:

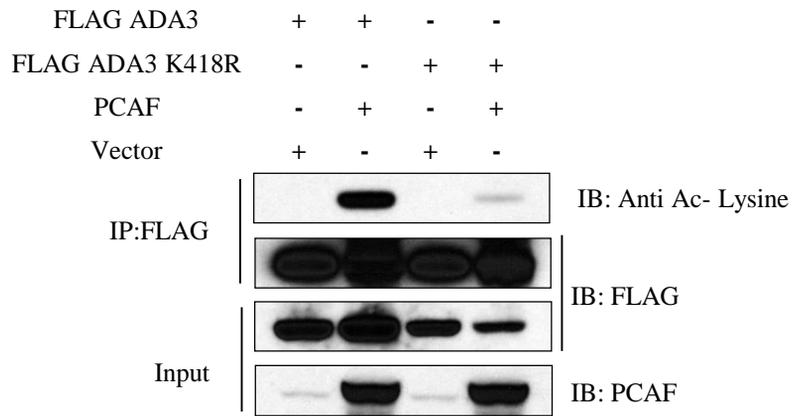


Figure S4: PCAF acetylates ADA3 at K418. HEK 293T cells were transfected with FLAG ADA3 WT or K418R along with empty vector or PCAF. Whole cell extracts were subjected to immunoprecipitation with M2 FLAG agarose beads, eluted with 3X FLAG peptide and immunoblotted with indicated antibodies.

Supplementary Figure S5

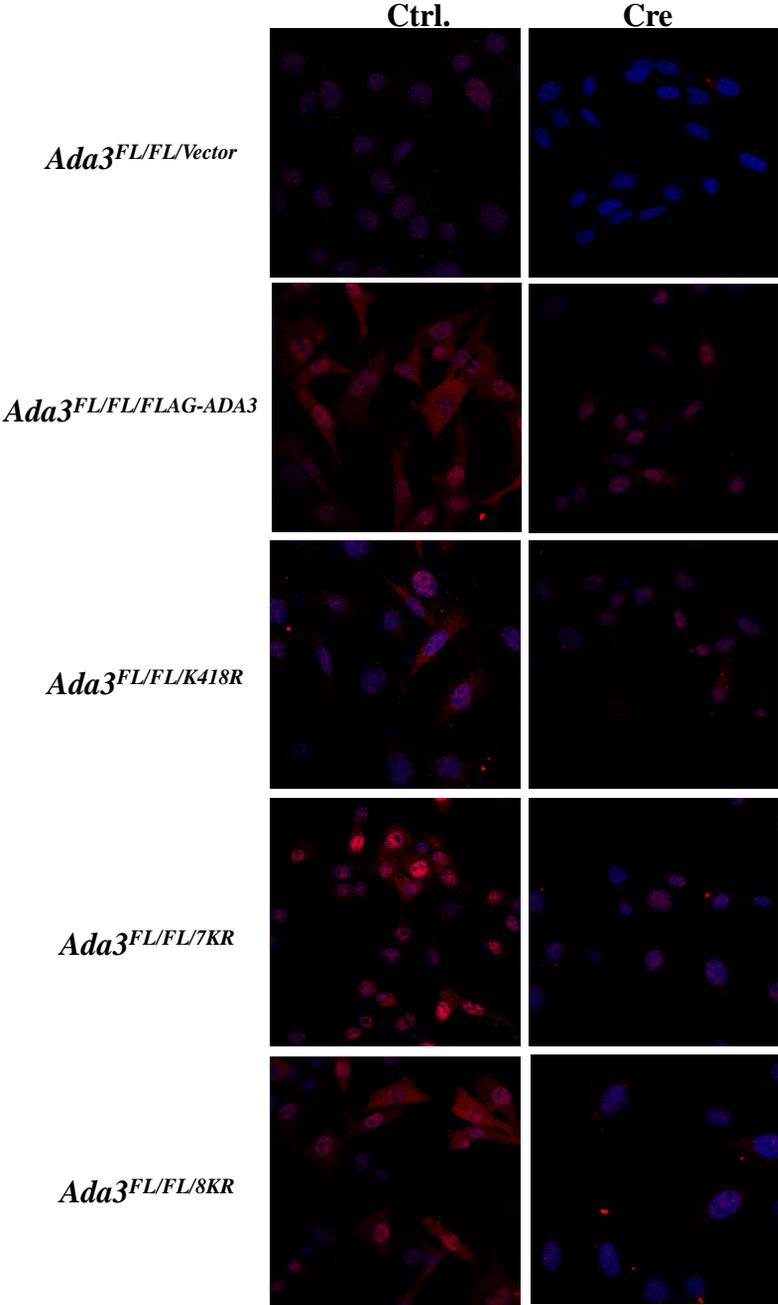


Figure S5: Defects ADA3 acetylation does not alter its nuclear localization. *Ada3* deleted MEFs stably expressing ADA3 WT or various acetylation defective mutants were fixed in 4% PFA and immunostained with DAPI and anti-ADA3.

Supplementary Figure S6

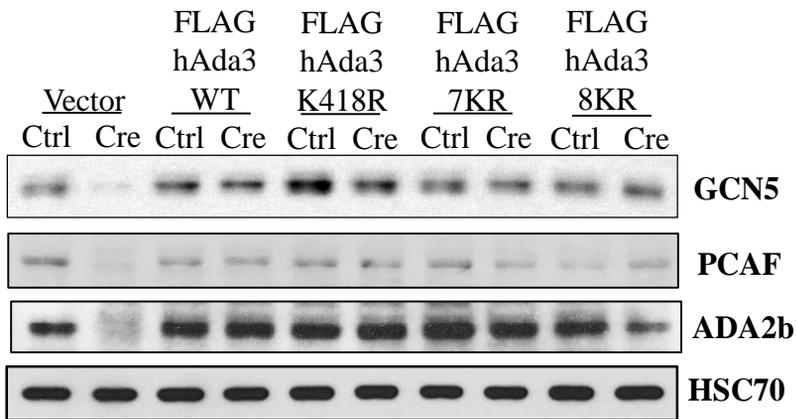


Figure S6: Whole cell lysates of Day 5 from cell cycle rescue experiment were immunoblotted anti-GCN5, -PCAF, -ADA2b or HSC70 antibodies.