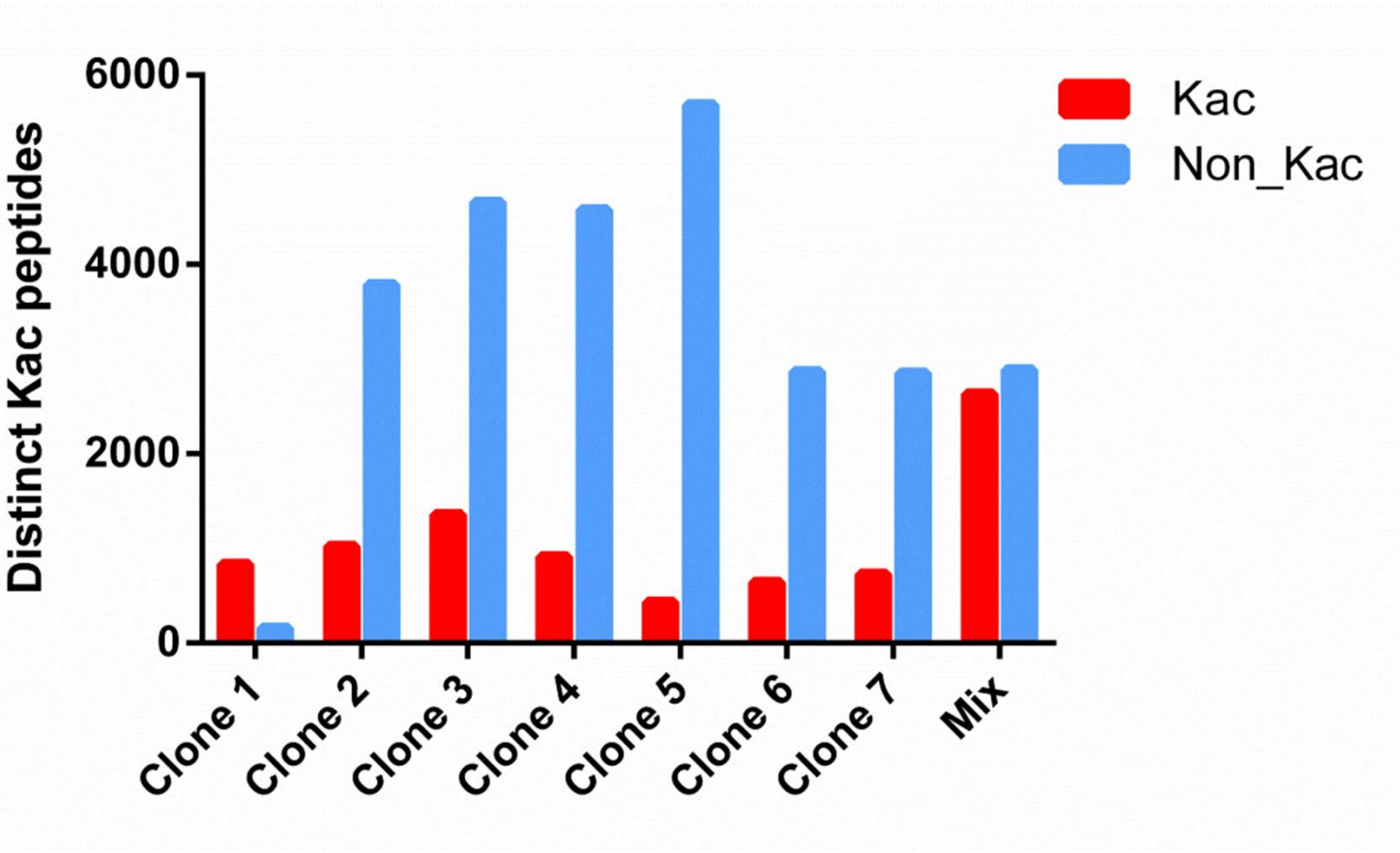
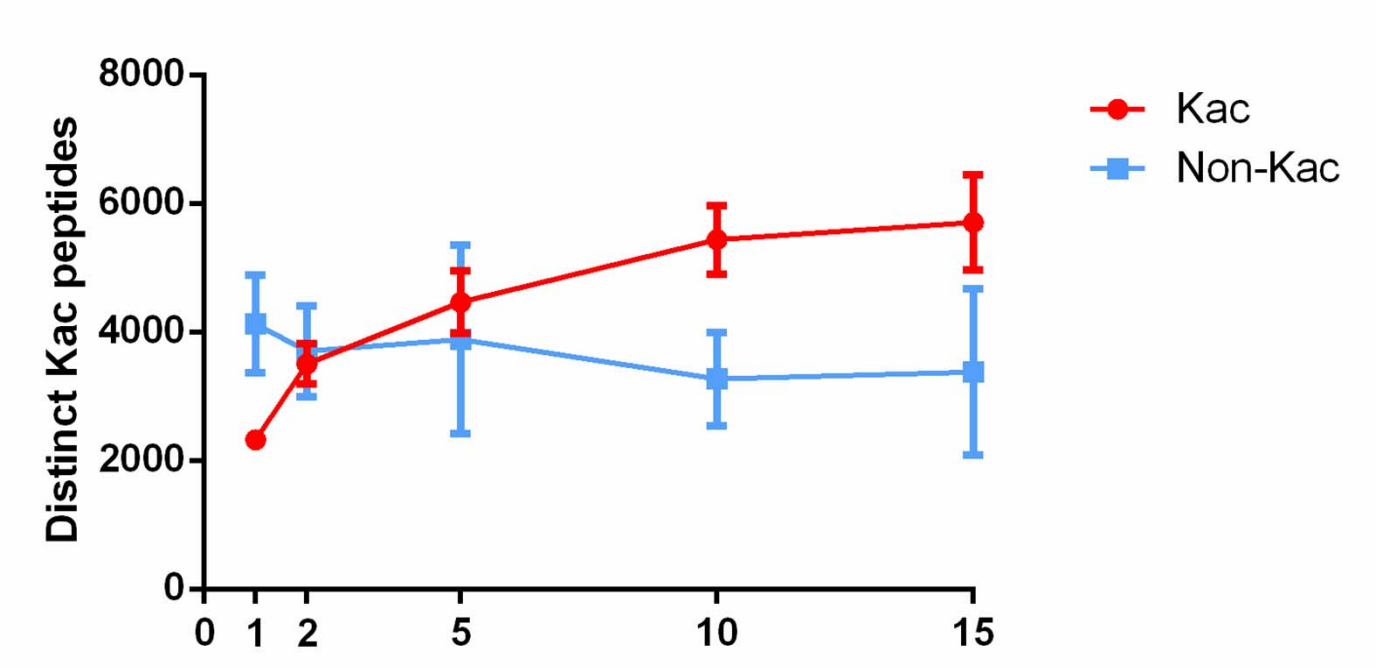
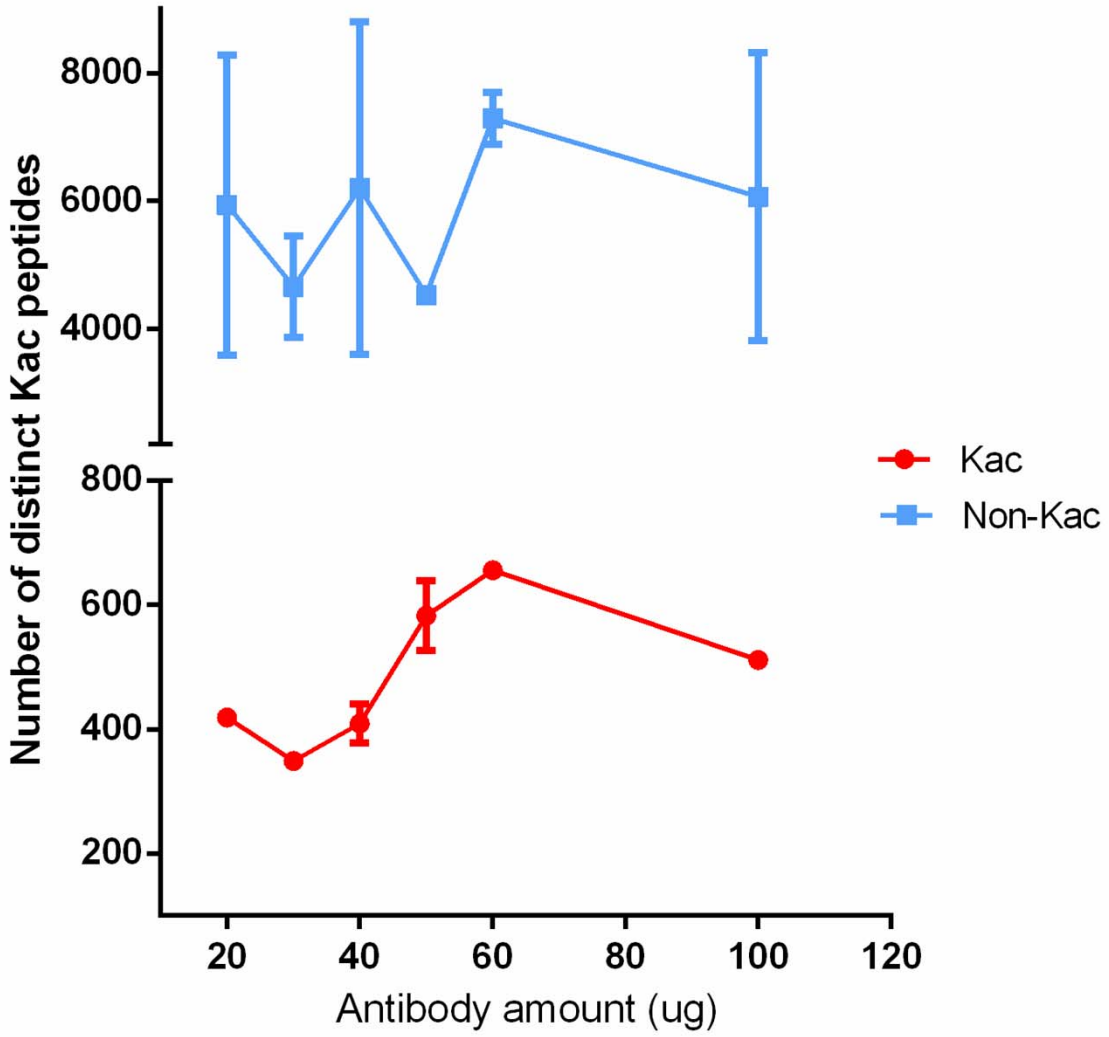


Supplemental Figure 1: Number of distinct Kac and Non-Kac peptides enriched with either individual anti-Kac antibody clones or a mix of all clones (CST).

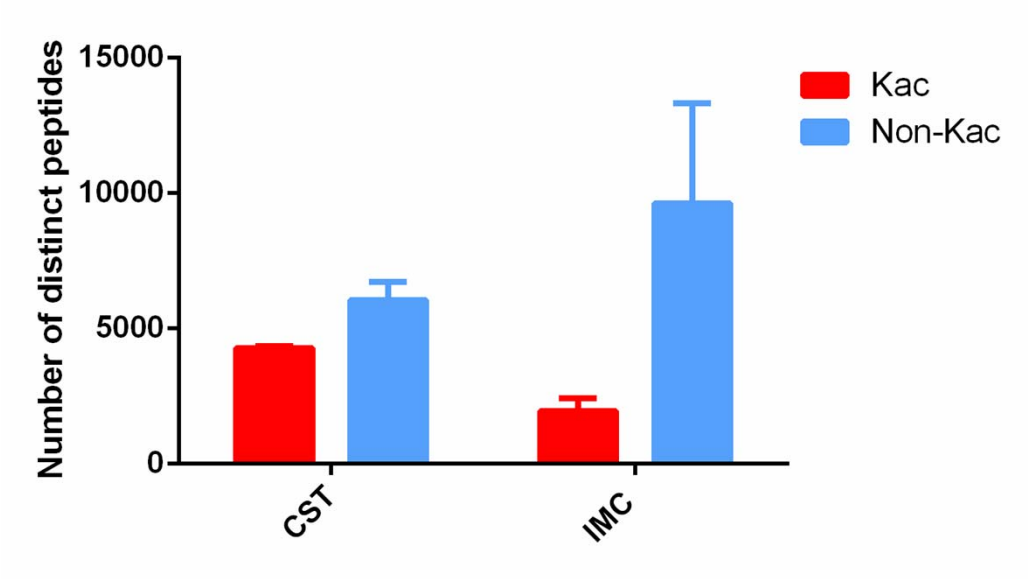


Supplemental Figure 2: Titration curve data for K-ac and non-K-ac peptides identified after enrichment of indicated peptide input using 50 ug of anti-Kac antibody (CST). Each data point is the average of 3 IP process replicates and error bars represent standard deviation.

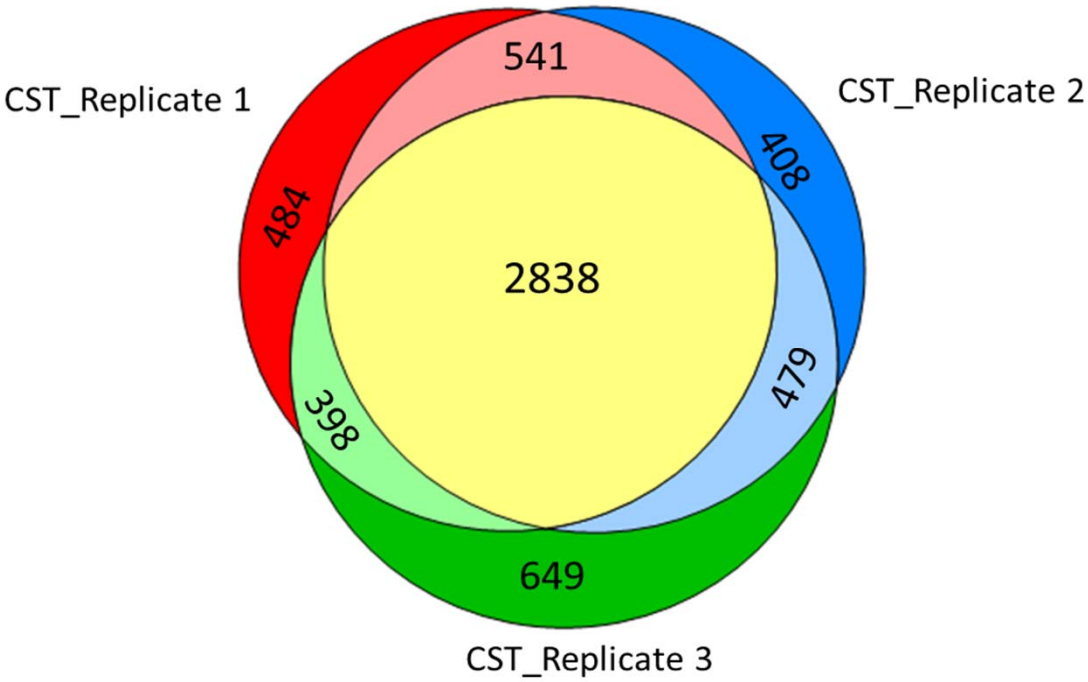


Supplemental Figure 3: Titration curve data for K-ac and non-K-ac peptides identified after enrichment of 1 mg of peptide input using indicated amounts of anti-Kac antibody (IMC). Each data point is the average of 2 IP process replicates and error bars represent range.

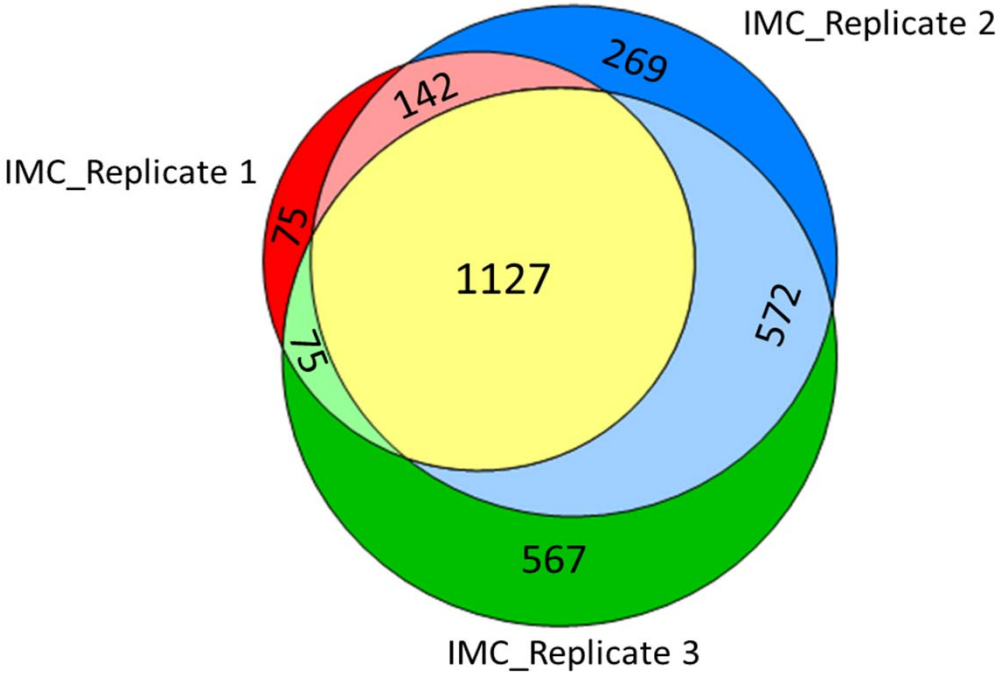
A


Number of distinct peptides
15000
10000
5000
0
Kac
Non-Kac
CST
IMC

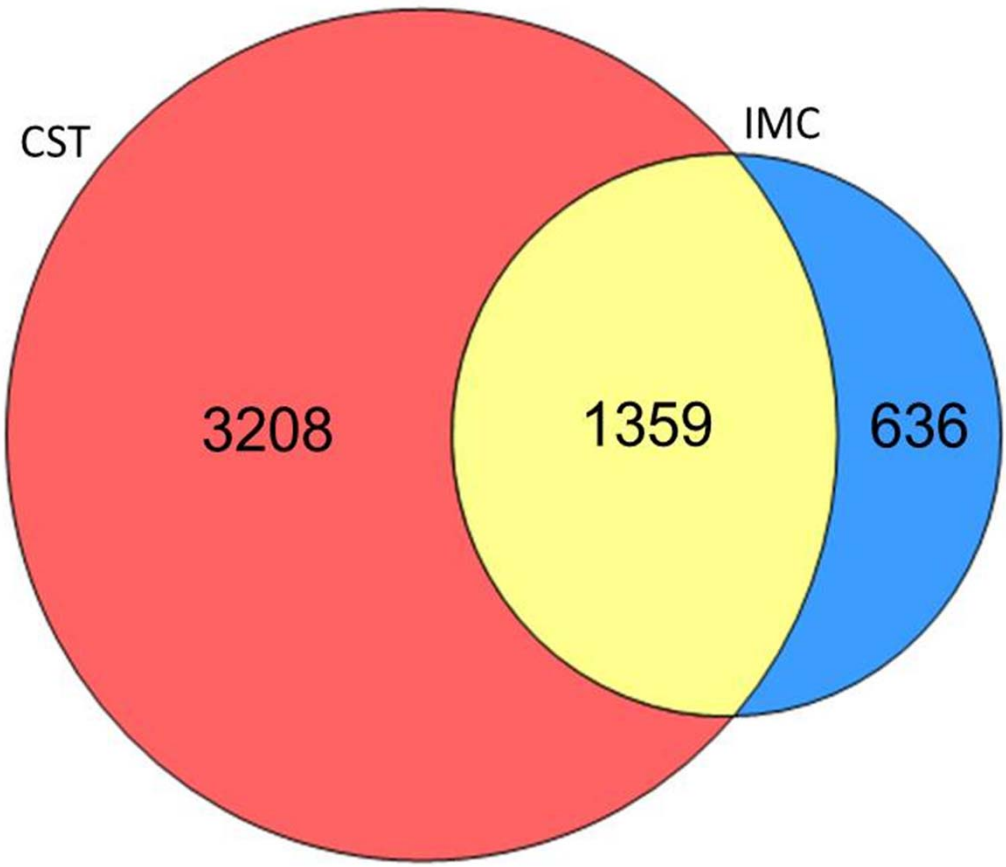
B


CST_Replicate 1
CST_Replicate 2
CST_Replicate 3
541
408
484
2838
479
398
649

C

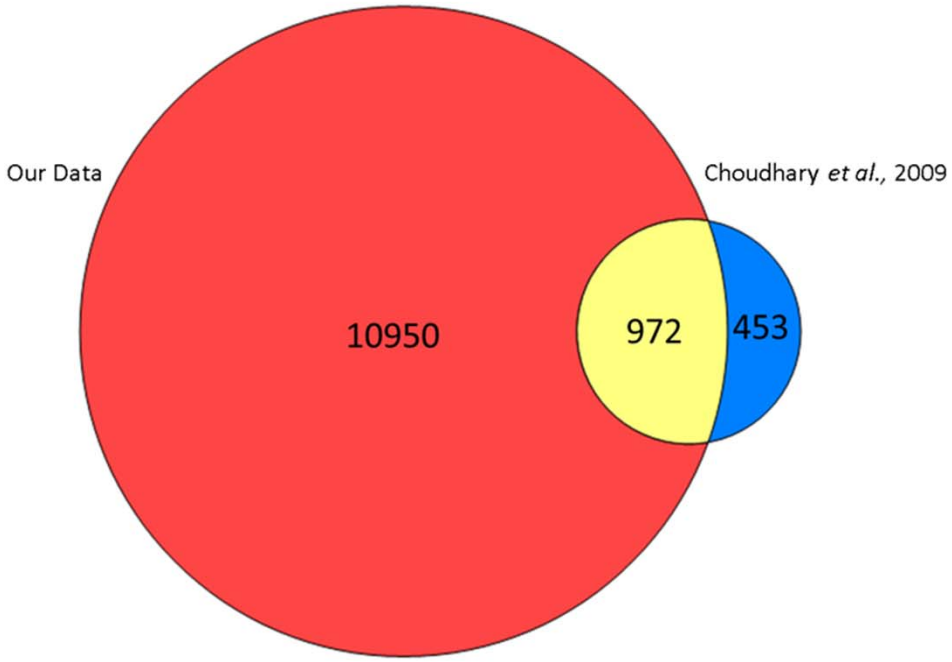

IMC_Replicate 1
IMC_Replicate 2
IMC_Replicate 3
269
142
75
1127
572
75
567

D

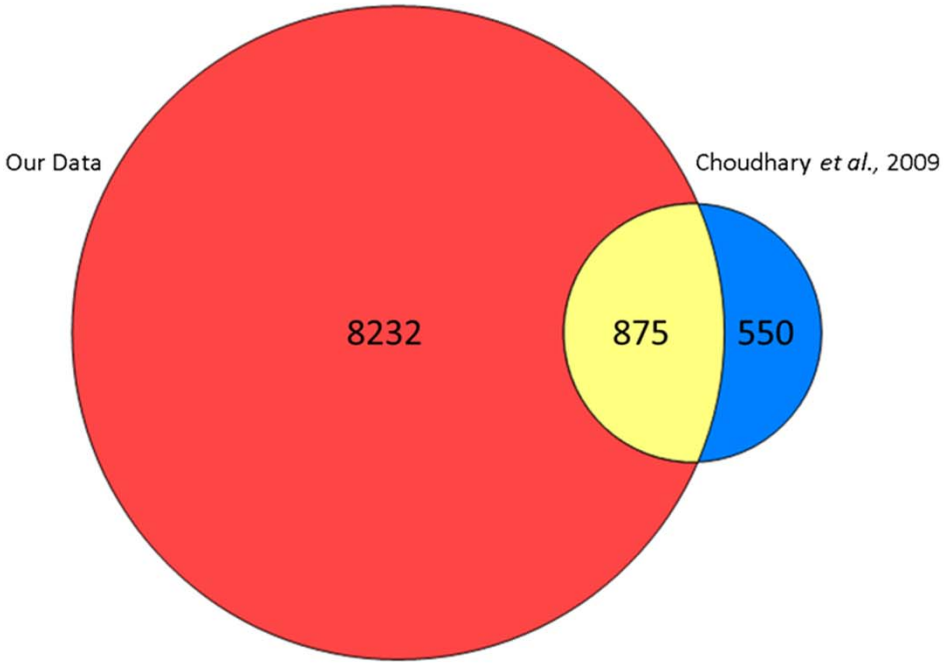


Supplemental Figure 4: (A)Number of distinct Kac and non-Kac peptides identified in with either the anti-Kac antibody from CST or IMC. Error bars represent the standard deviation for 3 replicates. (B) Overlap of distinct Kac peptides identified for three IP process replicates using the CST anti-Kac antibody. (C) Overlap of distinct Kac peptides identified for three IP process replicates using the IMC anti-Kac antibody. (D) Overlap of distinct Kac peptides identified across 3 IP process replicates using the anti-KAC antibody from CST and IMC.

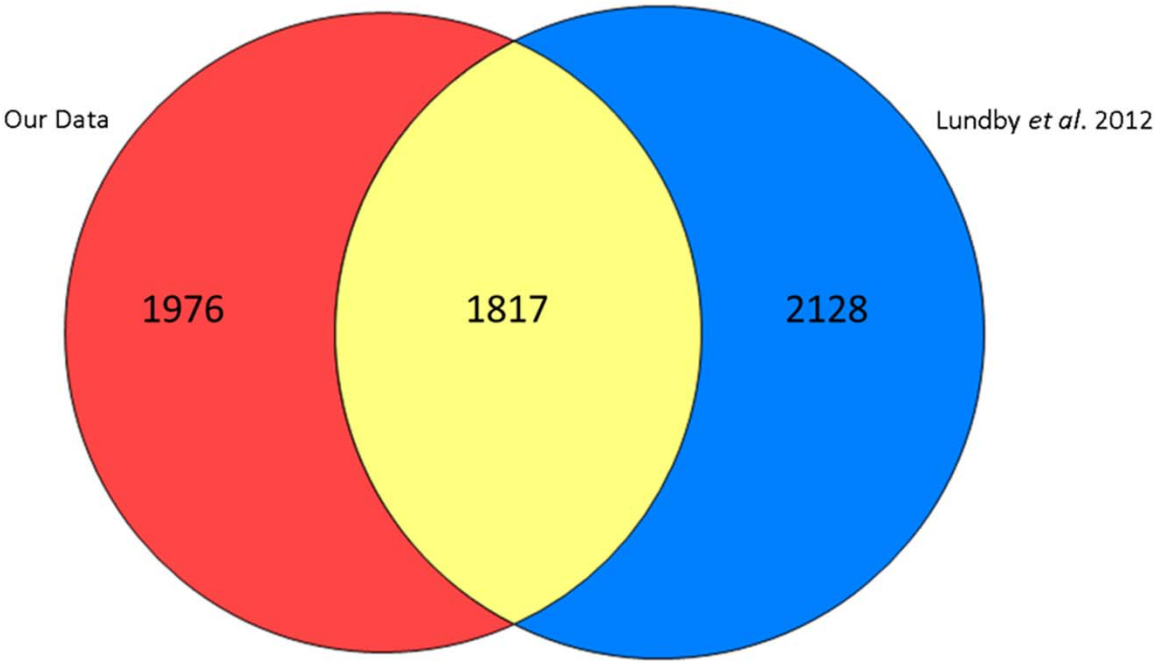
A


Our Data
Choudhary *et al.*, 2009
10950
972
453

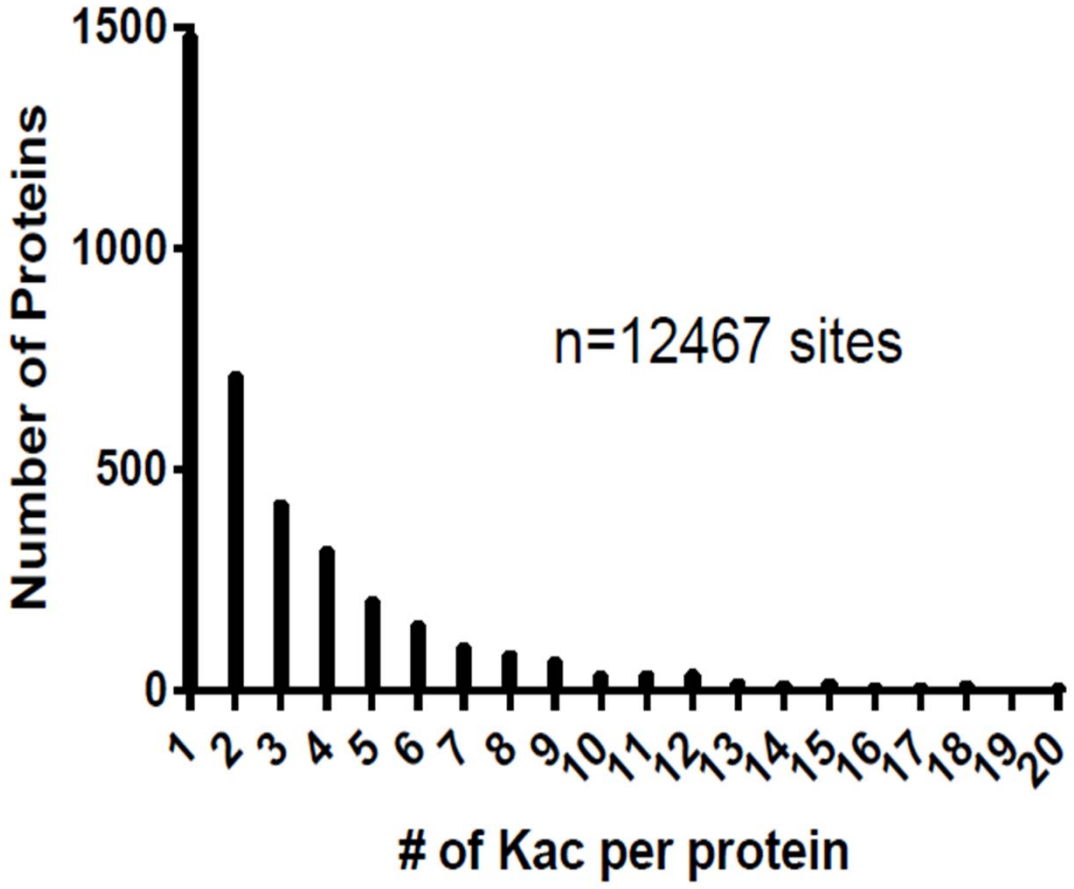
B


Our Data
Choudhary *et al.*, 2009
8232
875
550

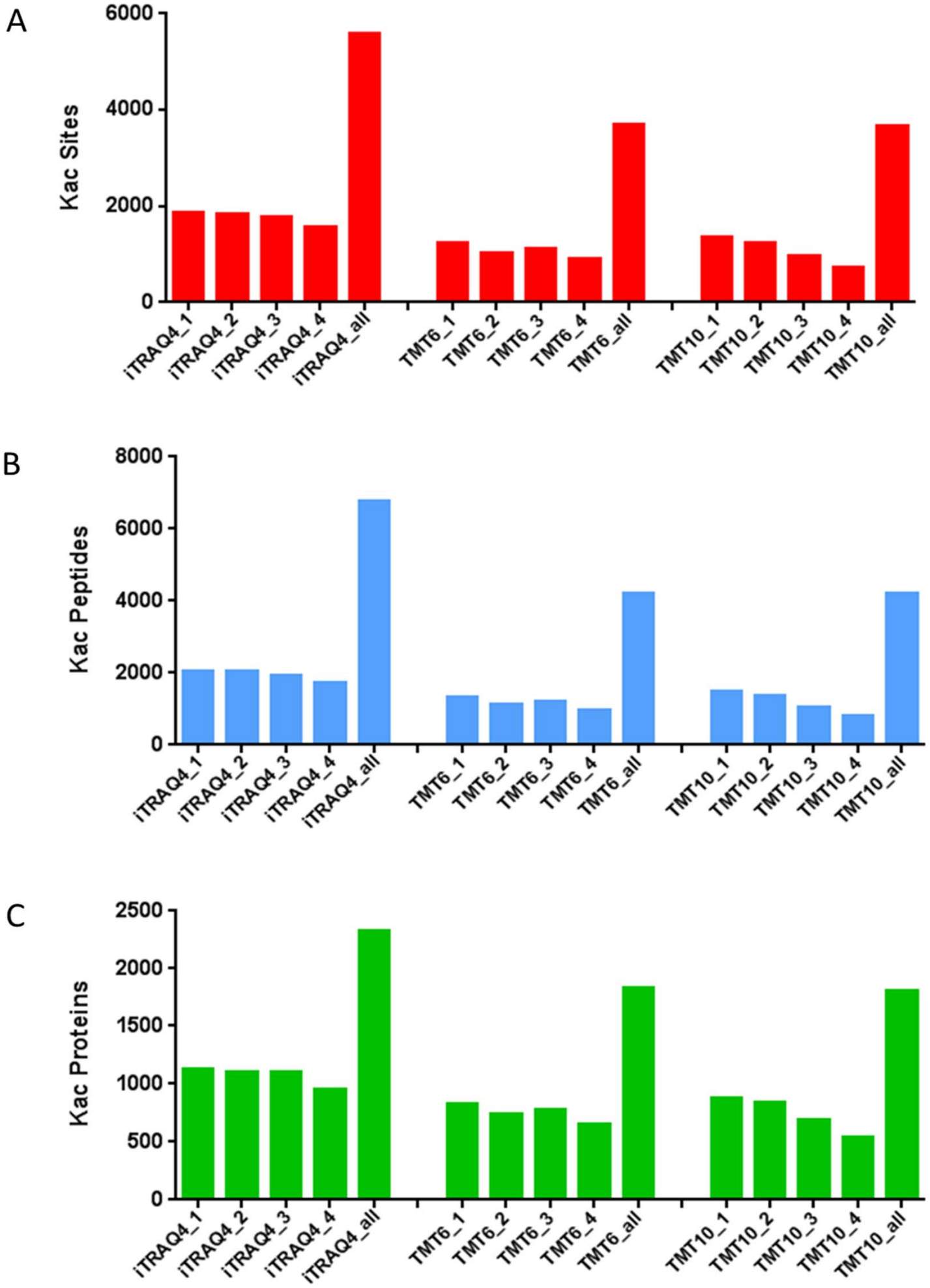
C


Our Data
Lundby *et al*. 2012
1976
1817
2128

Supplemental Figure 5: (A) Overlap of sites identified in 3 replicates of our SILAC–labeled SAHA Jurkat dataset with the Choudhary *et al*. SAHA Jurkat dataset (10). (B) Overlap of sites identified in 1 replicate of our SILAC–labeled SAHA Jurkat dataset with the Choudhary *et al.* SAHA Jurkat dataset (10). (C) Overlap of Kac proteins identified in our dataset and the dataset of Lundby *et al*. (12) .



Supplemental Figure 6: Frequency plot of the number of acetylation sites identified per protein for 2uM SAHA cells vs. DMSO treated Jurkat cells.


A
Kac Sites
6000
4000
2000
0
iTRAQ4_1
iTRAQ4_2
iTRAQ4_3
iTRAQ4_4
iTRAQ4_all
TMT6_1
TMT6_2
TMT6_3
TMT6_4
TMT6_all
TMT10_1
TMT10_2
TMT10_3
TMT10_4
TMT10_all
B
Kac Peptides
8000
6000
4000
2000
0
iTRAQ4_1
iTRAQ4_2
iTRAQ4_3
iTRAQ4_4
iTRAQ4_all
TMT6_1
TMT6_2
TMT6_3
TMT6_4
TMT6_all
TMT10_1
TMT10_2
TMT10_3
TMT10_4
TMT10_all
C
Kac Proteins
2500
2000
1500
1000
500
0
iTRAQ4_1
iTRAQ4_2
iTRAQ4_3
iTRAQ4_4
iTRAQ4_all
TMT6_1
TMT6_2
TMT6_3
TMT6_4
TMT6_all
TMT10_1
TMT10_2
TMT10_3
TMT10_4
TMT10_all

Supplemental Figure 7: Bar graphs illustrate the total number of distinct lysine-acetylated sites (A), peptides (B), and proteins (C) quantified in iTRAQ4-, TMT6-, and TMT10-labeled experiments from luminal and basal breast tumor mouse xenograft samples with 4 mg peptide input per plex. Numbers for individual fractions ("_1" to "_4") and the complete experiment ("_all") are shown.

Supplemental Table 1:

CST anti-Kac antibody development

Tab 1: “ELISA analysis summary”- Absorbance values for ELISA analysis of seven clones in the CST anti-Kac lysine reagent.

Tab 2-Tab 8: “Unique Kac Peptides Clone 1-7”-Distinct Kac peptides identified for each of the seven clones comprising CST anti-Kac antibody.

Tab 9: “Unique Kac Peptides Combined Ab”-Distinct Kac peptides identified in the combined CST anti-Kac antibody.

Supplemental Table 2:

Titration Curves Analysis and CST vs IMC comparison

Tab 1: “Summary CST Ab Input Titration”- Summary of distinct Kac and Non-Kac peptides identified for variable amounts of CST anti-Kac antibody with 2 mg of Jurkat peptide input.

Tab 2: “CST Ab Titration Curve”-MaxQuant MS/MS “evidence” table of distinct peptides for all replicates of CST anti-Kac antibody titration curve data.

Tab 3: “Summary Protein Input Titration”- Summary of distinct Kac and Non-Kac peptides identified across all titration experiments where protein input amount was varied and CST anti-Kac antibody amount was kept constant.

Tab 4: “Protein Input Titration”-MaxQuant MS/MS “evidence” table of distinct peptides for all replicates of protein titration curve data.

Tab 5: “Summary IMC Titration”- Summary of distinct Kac and Non-Kac peptides identified for variable amounts of IMC anti-Kac antibody with 1 mg of Jurkat peptide input.

Tab 6: “IMC Titration Curve”- MaxQuant MS/MS “evidence” table of distinct peptides for all replicates of IMC anti-Kac antibody titration curve data.

Tab 7: “Summary CST Ab vs IMC Ab”-Summary of distinct Kac and non-Kac peptides identified in the comparison study between CST anti-Kac antibody and IMC anti-Kac antibody.

Tab 8: “CST Ab vs IMC Ab”- MaxQuant MS/MS “evidence” table of distinct peptides for all replicates of CST anti-Kac antibody and IMC anti-Kac antibody comparison study.

Supplemental Table 3:

SAHA Treated Jurkat Experiments

Tab 1: “Summary_Jurkat_SAHA”-Table summarizes the number of distinct Kac and Non-Kac peptides found in bRP fractionated and non-fractionated SAHA treated Jurkat samples.

Tab 2: “All_Jurkat_SAHA_KacPeptides”-MaxQuant MS/MS “Evidence” table for all replicates of bRP fractionated and non-fractionated SAHA treated Jurkat samples.

Tab 3: “bRPFxn_Jurkat_SAHA_Kac_Sites”-Kac site data for all replicates of bRP fractionated SAHA treated Jurkat samples.

Tab 4: “NonFxn_Jurkat_SAHA_Kac_Sites”-Kac side data for all replicated of non-fractionated SAHA treated Jurkat samples.

Supplemental Table 4:

iTRAQ and TMT data

Tab 1: “Summary_iTRAQ_TMT”-Summary of Kac proteins and quantified and localized Kac peptides and sites identified in the iTRAQ and TMT experimental dataset.

Tab 2: “Kac_iTRAQ”-Kac sites for the luminal and basal breast cancer samples labeled with iTRAQ4.

Tab 3: “Kac-TMT6”-Kac sites for the luminal and basal breast cancer samples labeled with TMT6.

Tab 4: “Kac_TMT10”- Kac sites for the luminal and basal breast cancer samples labeled with TMT10.