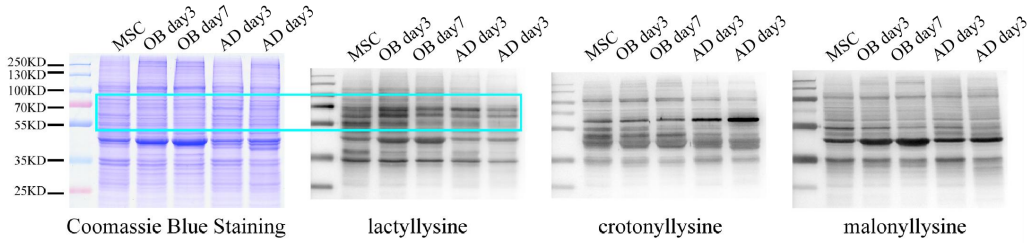
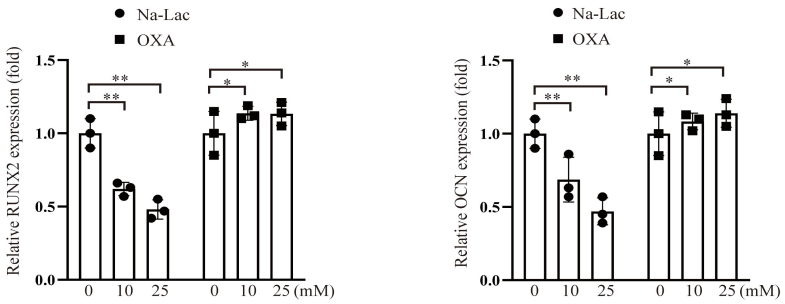
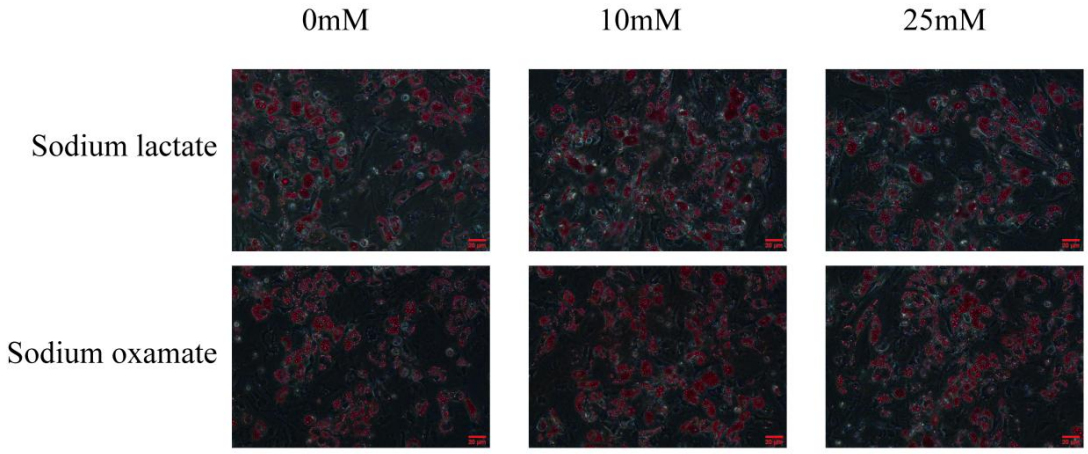
SUPPLEMENTARY FIGURES



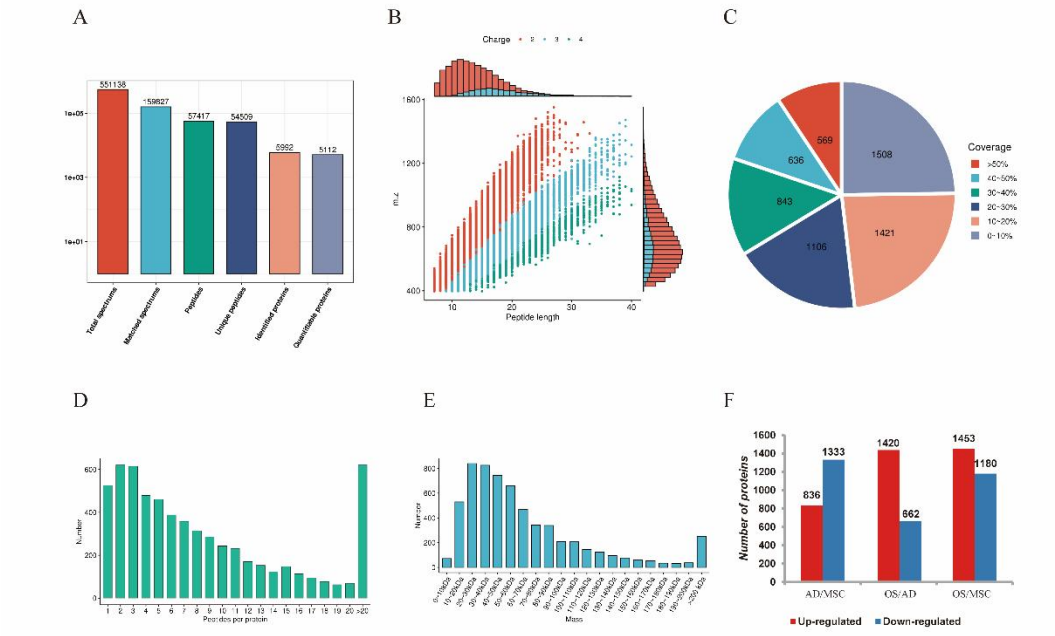
Supplementary Figure 1: Western blotting and Coomassie blue staining were carried out to comparatively assess the levels of global lysine lactylation, crotonylation, and malonylation in primary MSCs at days 3 and 7 of early adipogenic (AD) and osteogenic (OB) differentiation using pan-acylation modification antibodies. Coomassie staining was employed to normalize total protein loading.



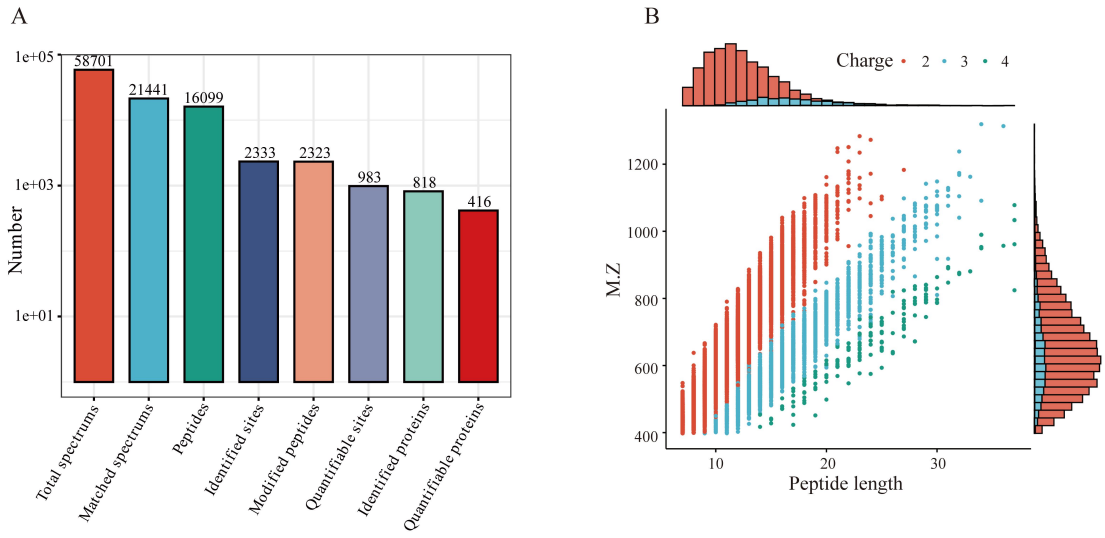
Supplementary Figure 2: Quantitative bar charts indicating the modulatory effects of exogenous sodium oxamate (OXA) and sodium lactate (Na-Lac) on the abundance of osteogenic protein markers, RUNX2 and OCN, after 7 days of MSC osteogenic induction. Data are indicated as mean ± SD, n = 3. $**p < 0.01$ versus the control group.



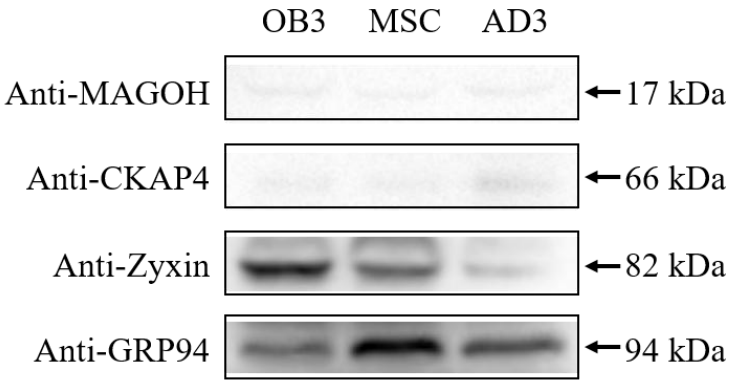
Supplementary Figure 3: Oil Red O staining analysis revealed that gradient concentrations of Na-Lac and OXA had minimal effect on the adipogenic differentiation capacity of MSCs.



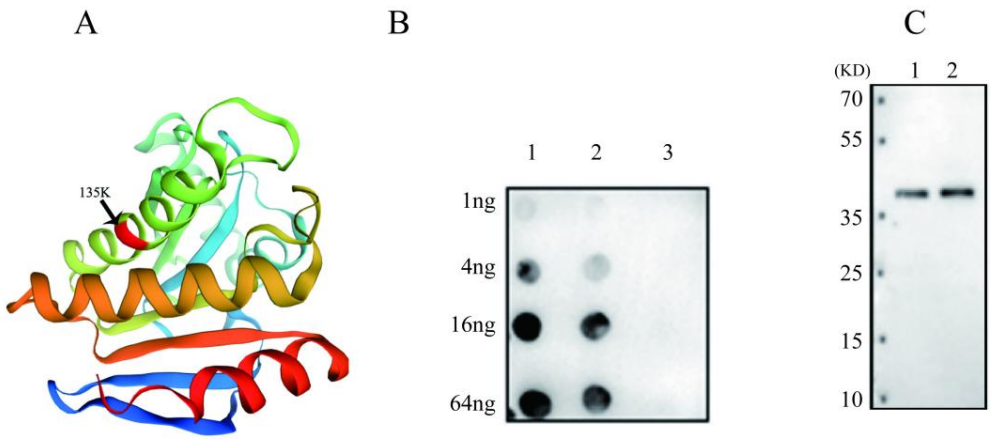
Supplementary Figure 4: LC-MS/MS proteomic raw data summary statistics, including protein molecular weight distribution, precursor ion mass error distribution, total and unique peptide counts, peptide length distribution, protein sequence coverage distribution, and differentially expressed protein statistics across undifferentiated control and MSC groups undergoing osteogenic (OS) and adipogenic (AD) differentiations.



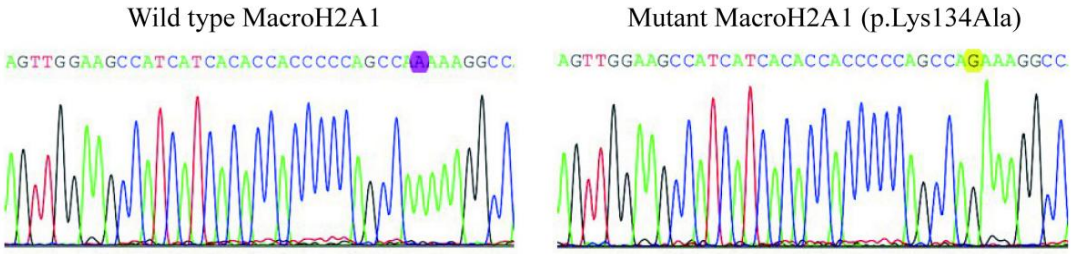
Supplementary Figure 5: Overview statistics of lysine lactylation enrichment proteome dataset, including identified/quantifiable proteins, quantified lactylation sites, modified peptides, total/matched spectral numbers, and the corresponding peptide length distribution.



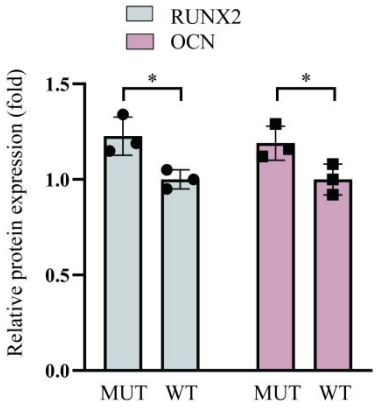
Supplementary Figure 6: Immunoprecipitation (IP) and immunoblotting verification of endogenous lactylation modification of candidate target proteins (MAGOH, CKAP4, Zyxin, GRP94) screened from lactylome profiling at day 3 MSCs osteogenic or adipogenic differentiation.



Supplementary Figure 7: (A) SWISS-MODEL structural simulation revealed that Lys135 of MacroH2A1 is located within an essential α-helix domain. (B) Dot blot assay of serially diluted WT and K135-lactylated synthetic peptides validated high antibody specificity. The custom antibody minimally bound unmodified peptides but showed robust attachment with lactylated positive peptides. (C) Immunoblot of nuclear extracts from untreated and Na-Lac-treated HepG2 cells. Lane 1 indicates untreated HepG2 nuclear lysate blots; Lane 2 denotes Na-Lac-exposed HepG2 nuclear lysate blots. A specific band around 40 kDa indicates the customized antibody.



Supplementary Figure 8: Sanger sequencing chromatograms confirmed successful site-directed mutagenesis of MacroH2A1 with substitution from lysine to alanine at residue 134 (p.Lys134Ala).



Supplementary Figure 9: Quantitative bar graphs of Western blot analysis indicated that the MacroH2A1 p.Lys134Ala mutation upregulates osteogenic transcription factor (RUNX20 and terminal marker (OCN) during MSC osteogenic differentiation. Data are denoted as mean ± SD, n = 3. *$p < 0.05$ versus wild-type group.