



Supplementary information, Fig. S6 LDHA increases lactylation of α-MHC K1897.

a Lactylation of α-MHC was determined by IP analysis. HEK293T cells were transfected with plasmids expressing Myc-α-MHC and Myc-LDHA. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of Pan Kla. **b** HEK293T cells were transfected with plasmids expressing Myc-α-MHC and transfected with negative control or LDHA siRNAs. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection

of Pan Kla. **c** IP analysis of lactylation of α -MHC with LDHA inhibitor. HEK293T cells were transfected with plasmids expressing Myc- α -MHC and treated with or without LDHA inhibitor. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of Pan Kla. **d** IP analysis of lactylation of α -MHC K1897. HEK293T cells were transfected with plasmids expressing Myc- α -MHC and Myc-LDHA. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of α -MHC K1897 Lactyl Lysine. **e** HEK293T cells were transfected with plasmids expressing Myc- α -MHC WT, Myc- α -MHC K1897R and Myc-LDHA. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of Pan Kla. **f** HEK293T cells were transfected with plasmids expressing Myc- α -MHC WT, Myc- α -MHC K1897R, and transfected with negative control or LDHA siRNAs. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of Pan Kla. **g** HEK293T cells were transfected with plasmids expressing Myc- α -MHC and treated with LDHA inhibitor or LDHA siRNAs. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of α -MHC K1897 Lactyl Lysine. **(a-g)** Anti-Myc magnetic beads were added in per immunoprecipitated sample.