



Supplemental Figure 1: Recombinant expression and activity of ASH4. A) Coomassie stained gel image (left) showing expression and purification of recombinant ASH4 (rASH4). Coomassie stained gel (right) showing expression and purification of recombinant catalytic dead ASH4 (rash4^{S124A}). NI indicates not induced sample; I indicates induction sample; FT indicates flow through; BM indicates bead bound material; E indicates elution from beads. Arrow indicates rASH4, and rash4^{S124A}. **B)** Structures of ester substrates and graph of a representative progress curve for rash4^{S124A} cleavage of the substrates. Error bars show the +/- SD. Cleavage of 4MU-Acetate by rASH4 shown as a positive control. Graph is the average of 3 technical replicates. **C)** Substrate structures and graph of a representative progress curve for rASH4 cleavage of 4-methyl-2-oxo-2*H*-chromen-7-yl acetate (4MU-Acetate), and *N*-(4-methyl-2-oxo-2*H*-chromen-7-yl) acetamide (AMC-Acetate) +/- SD. Graph is the average of three technical replicates.