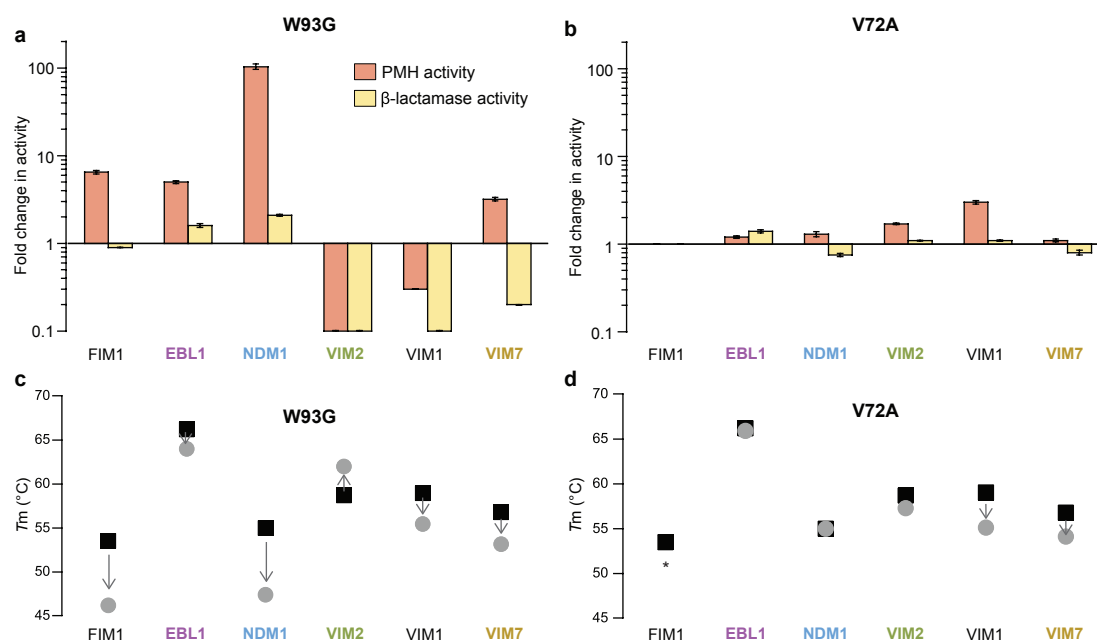


**Figure 2-figure supplement 2**



**Figure 2-figure supplement 2.** Differential effects of mutations (W93G and V72A) on six metallo-β-lactamases. Fold change in PMH and β-lactamase activity of variants. (A) W93G and (B) V72A variants compared to the variants containing a Trp93 and Val72. Activity levels of purified enzymes were measured at single enzyme (5 μM for PMH and 1 nM for β-lactamase activity) and substrate (500 μM for PMH and 100 μM for β-lactamase activity) concentrations. The wild-type NDM1 contains an Ala at position 72; the activity presented is the ratio of the wild-type to the A72V variant. The wild-type EBL1 contains a Pro at position 72; the activity presented is the ratio of the P72A to the P72V variant. Errors bars represent the standard deviation calculated from three measurements. Changes in melting temperature ( $T_m$ ) of (C) W93G and (D) V72A. Black squares denote the wild-type enzyme, and grey circles denote the mutant. Arrows indicate the change of melting temperature by the mutation. The melting temperature was calculated from the midpoint of the thermal denaturation curve of purified proteins. Asterisks indicate that the fold change in activity could not be determined, because one of the variants was not soluble. The catalytic activities and  $T_m$  of each mutant are listed in **Supplementary File 1H**.