



**Fig 4.** (left) In a cell-free enzymatic reaction, 1-MNA is produced by NNMT in a time-dependent manner. P6A is used to monitor production of 1-MNA in this system. (right) P6A is also useful to detect 1-MNA in a crude biological sample. In animals, the concentration of urinary 1-MNA is highly dependent on the activity of NNMT and the total uptake of nicotinamide from foods. Therefore, intake of nicotinamide leads to an increase in the urinary excretion of 1-MNA in wild-type mice, while no 1-MNA excretion is detected in the *Nnmt* deficient mice. The fluorescence intensity of P6A is significantly quenched by wild-type derived urine samples. In addition, the fluorescence intensity was quenched by 1-MNA in a dose-dependent manner. (LC-MS/MS: quantification data using LC-MS/MS, P6A: data from fluorescence spectra with P6A, Wt: wild-type mice, Wt + NA: nicotinamide treatment group, *Nnmt* KO: NNMT deficient mice)