Ubiquitination is one of the most important protein post-translational modification pathways in human cells, in which a small protein ubiquitin (Ub) is added to substrates and can be elongated on seven conserved lysine residues. Ubiquitination can be reversed by the action of deubiquitinases (DUBs) that have been implicated in numerous diseases including cancer, inflammation and neurodegenerative disorders. OTUD1 belongs to the OTU (ovarian tumor) subfamily of DUBs and has been involved in autoimmune disorders, antiviral responses and tumor metastasis. Therefore, selective targeting of OTUD1 for inhibition promises significant therapeutic potentials for various human diseases. However, developing potent and selective DUB inhibitors is complicated by the convoluted, diverse and dynamic catalysis process. To deal with this problem, our lab developed a protein engineering strategy to systematically develop Ub variant (UbV) inhibitors targeting enzymes in the Ub signalling cascade. Here, the UbV technology will be applied to develop the high-affinity inhibitors of OTUD1 through selecting high-affinity binding UbVs form a phage-displayed UbV library (diversity: $10^{10}$). This is followed by in vitro and cellular characterization to assess the inhibitors’ specificity, affinity and inhibitory effect on OTUD1. This project could provide a better understanding of the biochemical mechanism of OTUD1 deubiquitination and new insights on developing therapeutic inhibitors of OTU family of DUBs.