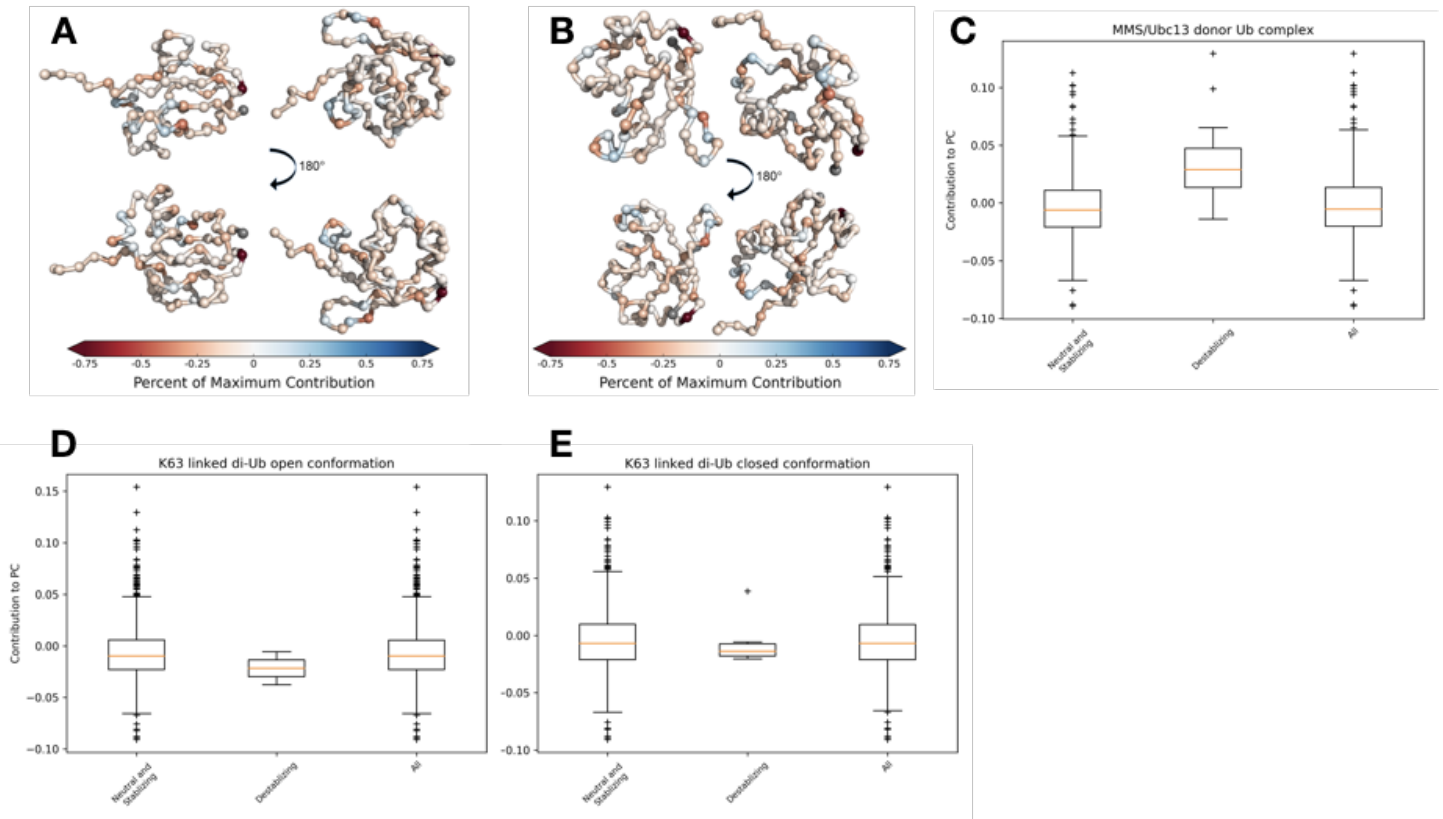


Supplemental Figure 1. Variance explained by the each of first 16 PCs of the difference fitness data.



Supplemental Figure 2. Association between PC3 and K63-related structures. (A) K63 linked di-Ub open conformation (3H7P) colored by average contribution to PC3. (B) K63 linked di-Ub closed conformation (2N2K) colored by average contribution to PC3. (C) Rosetta calculations reveal a potential association between destabilizing the MMS/Ubc13 donor complex (2GM1) and PC3. Binning mutations as either neutral and stabilizing mutations (Rosetta Energy Units (REU) < 0.75) or destabilizing (REU >= 0.75) reveals a significant difference in PC3 contribution magnitude (Student's t-test, $p = 0.000215$) for this complex. (D) In contrast, Rosetta calculations reveal no significant difference for the K63 linked di-Ub open conformation (3H7P, Student's t-test, $p = 0.887$) or (E) for the K63 linked di-Ub closed conformation (2N2K, Student's t-test, $p = 0.887$)