SUPPLEMENTARY TEXT

Parallel MD simulations for $E2^{Ubc9}$-$E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$ and $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$

A second set of simulation is performed for $E2^{Ubc9}$-$E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$ and $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$ complexes to validate the allosteric mechanisms proposed. The control set is 28 ns long for each complex, with 2.5 ns and 5 ns equilibration times for $E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$ and $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$, respectively. In this second set of MD simulations, we mainly focused on the network of the correlated fluctuations of residues for the proposed allosteric mechanisms and investigated the dynamic behavior of $E2^{Ubc9}$'s Loop2 and Gly68 of SUMO.

The comparative analysis starts with the evaluation of the complexes' and their individual chains' RMSD behavior. For both complexes the general RMSD trend is unchanged: $E2^{Ubc9}$-$E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$ and its individual chains reflect a rather stable behavior (Supplementary Figure 3A); on the other hand, this is not the case for the overall RMSD of the $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$ complex (Supplementary Figure 3B). The snapshots taken from the trajectory of $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$ revealed that this instability largely emanates from the rotation of SUMO (as also previously indicated in Figure 5). This quaternary change is consistently observed for both simulation sets, which supports our finding that the presence of $E3^{RanBP2}$ causes a reduction in the configurational space of SUMO. This is in line with the finding of our recent study (Tozluoğlu *et al.*[30]).

As done with the first simulation sets (described in the main text), the clustering analysis around 2.5 Å was performed on the second sets. This allowed us to divide the simulation into specific time windows. The clustering provided two time windows for $E2^{Ubc9}$-$E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$, where the first cluster resides during 77.6% of the simulation time and appears to be the dominantly sampled conformation throughout the whole trajectory (Supplementary Figure 4A). Removal of $E3^{RanBP2}$ provided more conformational freedom to the system, as the number of clusters

increased from two to three for this case (where cluster 1 resides for 53%; cluster 2 for 31% and cluster 3 for 16% of the simulation time) (Supplementary Figure 4B).

After the simulations are divided into time windows, for each time window the network of correlated fluctuations are illustrated as a correlation map. The focus of this analysis is to spot the most important coupled fluctuations which could support the proposed allosteric mechanisms: (i) in the presence of $E3^{RanBP2}$, the correlations between $Target^{RanGAP1}$ binding sites of $E2^{Ubc9}$; the $E2^{Ubc9}$'s Loop2 and the E2 binding sites of $Target^{RanGAP1}$ (ii) in the absence of $E3^{RanBP2}$, the correlations between $Target^{RanGAP1}$ binding sites of $E2^{Ubc9}$ the anchoring imposed by Gly68 of SUMO on E2 (Figure 1A, Supplementary Figure 2A). In the case of $E2^{Ubc9}$-$E3^{RanBP2}$-SUMO-$Target^{RanGAP1}$ complex, the dominant cluster (cluster 1) of the second simulation set agrees with the first simulation set: The fluctuations of $E2^{Ubc9}$'s Loop2 demonstrate strong correlations with the fluctuations of one of the explicit $E2^{Ubc9}$ binding sites (Leu555-Pro566) and SIM motif of $Target^{RanGAP1}$ for most (77.6%) of the simulation time (Supplementary Figure 5).

During the time course of the $E2^{Ubc9}$-SUMO-$Target^{RanGAP1}$ simulation, there exists a strong correlation between $E2^{Ubc9}$'s Loop2 and Leu555-Pro566 region of $Target^{RanGAP1}$ in the first two clusters, which lasts for 84% of the simulation time. Yet, the correlations with the $E2^{Ubc9}$'s Loop2 and SIM motif of $Target^{RanGAP1}$ are missing here, which might indicate an efficiency loss [15; 22; 28] as described in the Discussion section of the main text. As a next step, the MSF correlations are further anlyzed to see whether the anchoring behavior of SUMO's Gly68 on SUMO is coupled to the existence of the latter correlation observed between $E2^{Ubc9}$'s Loop2 and Leu555-Pro566 region of $Target^{RanGAP1}$. This anchoring behavior of SUMO's Gly68 coexists with the latter correlation during the time period indicated by the two clusters. Strikingly, only in these two clusters this new interface between $E2^{Ubc9}$ and SUMO presents a correlated motion with part of the $E2^{Ubc9}$'s catalytic pocket (Asp127-Ala129) as observed in the first simulation set (Supplementary Figure 6).

To further validate our allosteric hypotheses, the mean square fluctuations of $E2^{Ubc9}$'s Loop2 within different time windows are investigated. This analysis nicely pointed out the fact that, for both of the complexes, Loop2 is more mobile when it

demonstrates strong correlations with the fluctuations of one of the explicit $E2^{Ubc9}$ binding sites (Leu555-Pro566) of $Target^{RanGAP1}$, as described in the results of the first simulation set (Supplementary Table 1).

Combining these results, the recurrence of the correlations related with Loop2 of $E2^{Ubc9}$ in the presence of E3 and Gly68 of SUMO in the absence of E3 strengthens the statistical significance of the allosteric mechanisms observed. This however does not neccessarily point out that these mechanisms might be consistenly observed in a larger time window; but it already provides a clue about the allosteric correlations within the observed period of time.