FIG. S1: The $^1$H-$^{15}$N HSQC amide chemical shift changes of $^{15}$N-labeled UBC9 at 30 °C, pH 6.5, calculated as $\{(\Delta\omega_H)^2 + (\Delta\omega_N)^2\}^{1/2}$ at 600 MHz, are shown due to addition of 4-fold molar excess of (A) Ets-1$^{(1-138)}$, (B) Ets-1$^{(1-52)}$, and (C) Ets-1$^{(51-138)}$ (400 μM starting concentration). Combined with results presented within the paper, these data demonstrate fragments of Ets-1 containing Lys15 bind UBC9 near its active site Cys93.
FIG. S2: Overlaid $^1$H-$^{15}$N HSQC spectra of $^{15}$N-Ets-1$^{(1-138)}$ (red) and $^{15}$N-Ets-1$^{(1-138)}$:SUMO-1$_{gg}$ (blue) at pH 6.5 and 30 °C. Main chain amide assignment are provided for the unmodified protein. The * indicates the signal from the $^1$H-$^{15}$N of the new isopeptide between Lys15 of Ets-1$^{(1-138)}$ and Gly97 of SUMO-1$_{gg}$. The $^{15}$NH$_2$ groups are not assigned. Due to cis/trans isomerization, several residues near prolines in the unstructured N-terminal region of Ets-1$^{(1-138)}$ show multiple peaks, identified as "_1" and "_2". At this contour level, the signals from several serine or threonine amides in $^{15}$N-Ets-1$^{(1-138)}$:SUMO-1$_{gg}$ are not observed, possibly due to enhanced hydrogen exchange under slightly more basic conditions than used with the unmodified protein.
**FIG. S3:** Overlaid $^1$H-$^{15}$N HSQC spectra of $^{15}$N-SUMO-1 $_{1-138}$ (red) and $^{15}$N-SUMO-1$_{1-138}$:Ets-1$^{(1-138)}$ (blue) at pH 6.5 and 17 °C. Main chain assignment are provided for the unmodified protein. The arrows indicate the changes in the chemical shifts of Gly96 and Gly97 due to covalent bonding to Lys15. Aliased peaks are underlined and signals from $^{15}$NH$_2$ groups not assigned. At the displayed contour levels, the signals from $^{15}$N-SUMO-1$_{1-138}$:Ets-1$^{(1-138)}$ appear weaker than those from unmodified protein due to lower sample concentration.
FIG. S4: Sumoylation of Ets-1\textsuperscript{(1-52)} does not lead to any spectral, and hence structural, perturbation of this polypeptide corresponding to the N-terminal region of Ets-1. Shown are overlaid $^1$H-$^{15}$N HSQC spectra of $^{15}$N-Ets-1\textsuperscript{(1-52)} (red) and $^{15}$N-Ets-1\textsuperscript{(1-52)}:SUMO-1\textsubscript{88} (blue) at pH 6.5 and 22 °C. Main chain amide assignment are provided for the unmodified protein. The * indicates the signal from the $^1$H-$^{15}$N of the new isopeptide between Lys15 of Ets-1\textsuperscript{(1-52)} and Gly97 of SUMO-1\textsubscript{88}. Several amides exhibit resolved strong and weak signals, denoted as “_1” and “_2”, respectively, likely due to cis/trans isomerization of nearby X-Pro groups. The downfield peak from the indole $^{15}$N-H of Trp49 is not shown.
FIG. S5: Sumoylation of Ets-1\(^{(1-52)}\) does not lead to any changes in its fast timescale backbone dynamics beyond the site of the isopeptide bond. Shown are the steady-state \(^1\)H-\(^{15}\)N NOE values of (A) \(^{15}\)N-Ets-1\(^{(1-52)}\) and (B) \(^{15}\)N-Ets-1\(^{(1-52)}\):SUMO-1\(_{gg}\) at pH 6.5 and 22 °C (500 MHz). Increasing amide mobility on the nsec-psec timescale results in decreasing \(^1\)H-\(^{15}\)N NOE values. (A) Consistent with its random coil amide chemical shifts, Ets-1\(^{(1-52)}\) is predominantly disordered in solution. However, the slightly elevated NOE values for amide near residues 23 and 42 suggests a population of more locally ordered conformers. (B) Upon sumoylation, the fast local dynamic features of \(^{15}\)N-Ets-1\(^{(1-138)}\):SUMO-1\(_{gg}\) do not change significantly, except near the site of sumoylation. The isopeptide \(^{15}\)N\(_{gg}\) has a NOE value of ~0.02 (not shown), indicating that it is also highly mobile. Missing data points correspond to prolines or residues with severe spectral overlap. Errors are ~5%.