Supporting Information

Tyrrell et al. 10.1073/pnas.1010648107

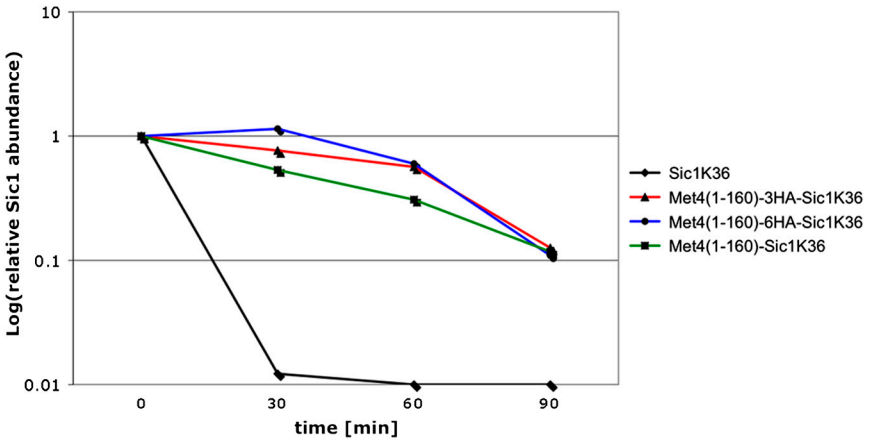

Fig. S1. Cells expressing *GAL1*-controlled RGS6His tagged $^{Met4(1-160)}Sic1^{K36}$ with either 0, 3, or 6 copies of the HA-tag inserted between Sic1 and the Met4 N-terminus to increase the distance between ubiquitin acceptor lysine and the ubiquitin-binding region were synchronized in G1 using α-factor. Cells were released from the G1 arrest and expression from the *GAL1* promoter was blocked by shifting cells to dextrose media. Protein levels were monitored at indicated time points.

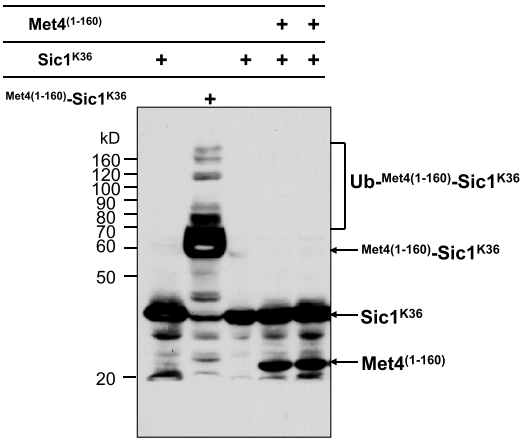

Fig. S2. Effect of expression of the Met4 N-terminus on $Sic1^{K36}$ ubiquitination. Cells expressing *GAL1*-controlled RGS6His tagged $Sic1^{K36}$ and *GAL1-RGS6His-MET4(1-160)* as indicated were grown under induced conditions for 4 hours. Total cell lysates were analyzed by immunoblotting using antibodies directed against the RGS6His tag. Cells expressing $^{Met4(1-160)}Sic1^{K36}$ were analyzed in parallel as a positive control.

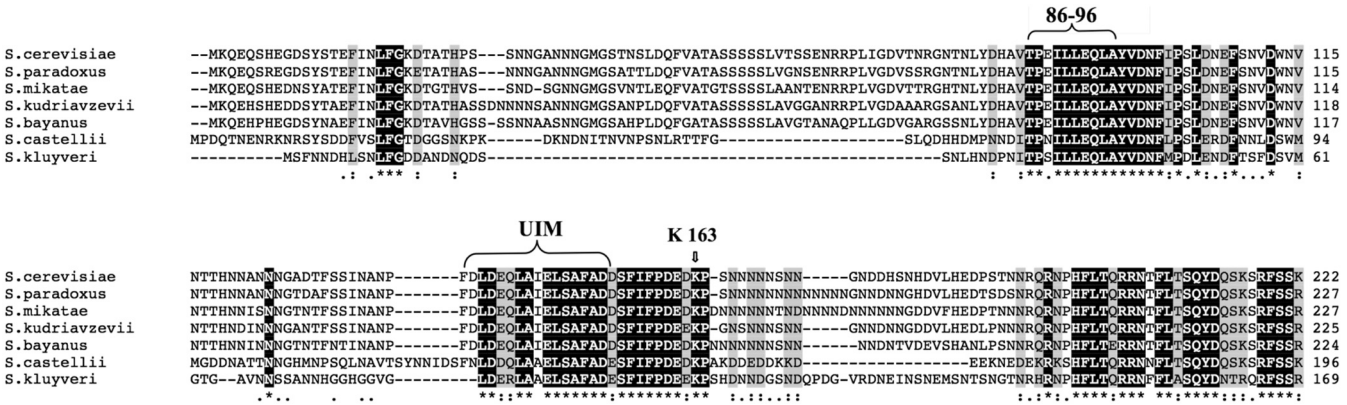

Fig. S3. Alignment of the first 222 residues of Met4 from different yeast species. Lysine at position 163 is the ubiquitin acceptor site. The UIM domain and the region starting at residue 86 are indicated as regions with a high degree of conservation.

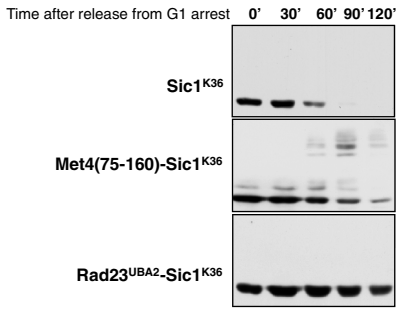

Fig. S4. The UBA2 domain of Rad23 stabilizes Sic1 in its deubiquitinated form. Cells expressing *GAL1*-controlled RGS6His tagged $SicI^{K36}$, $^{Met4(75\text{-}160)}SicI^{K36}$, $^{Rad23(UBA2)}SicI^{K36}$ were analyzed. The C-terminal 350 residues of yeast Rad23 were used for fusion to Sic1 to generate $^{Rad23(UBA2)}SicI^{K36}$. Cells were synchronized in G1 using α-factor, released from the G1 arrest and expression from the *GAL1* promoter was blocked by shifting cells to dextrose media. Protein levels were monitored at indicated time points.