

Supporting Information

Tyrrell et al. 10.1073/pnas.1010648107

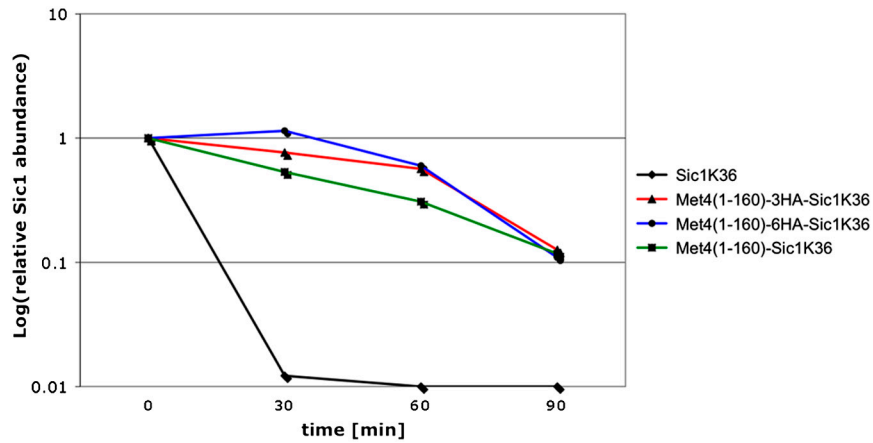


Fig. S1. Cells expressing *GAL1*-controlled RGS6His tagged $\text{Met4}^{(1-160)}\text{Sic1}^{\text{K36}}$ with either 0, 3, or 6 copies of the HA-tag inserted between Sic1 and the Met4 N-terminus to increase the distance between ubiquitin acceptor lysine and the ubiquitin-binding region were synchronized 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.

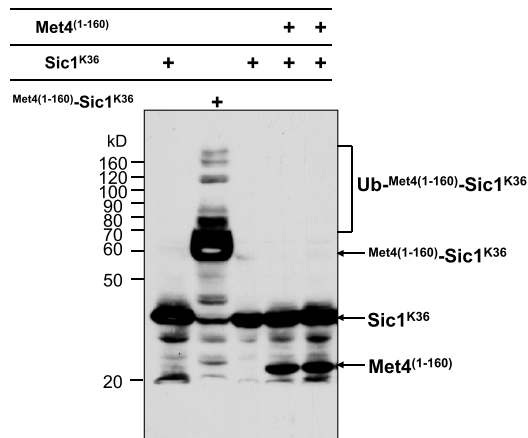


Fig. S2. Effect of expression of the Met4 N-terminus on Sic1^{K36} ubiquitination. Cells expressing *GAL1*-controlled RGS6His tagged Sic1^{K36} and *GAL1-RGS6His-MET4(1-160)* as indicated were grown under induced conditions for 4 hours. Total cell lysates were analyzed by immunoblotting using antibodies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

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                                     86-96
S.cerevisiae  --MKQEQSHREGDSYSTEFINLFGKDTATHPS---SNNGANNGMGSTNSLDQFVATASSSSSLVTSSENRRPLIGDVTNRGNTNLYDHAVTPEILLEQLAYVDNFIPLSDNBEISNVDWNV 115
S.paradoxus  --MKQEQSHREGDSYSTEFINLFGKDTATHAS---NNGANNGMGSATLTDQFVATASSSSSLVGNSENRRPLVGDVSSRGNTNLYDHAVTPEILLEQLAYVDNFIPLSDNBEISNVDWNV 115
S.mikatae    --MKQEQSHEDNSYATEFINLFGKDTGTHVS---SND-SGNNMGMSVNTLEQFVATGTSSSSSLAANTENRRPLVGDVTRGHTNLYDHAVTPEILLEQLAYVDNFIPLSDNBEISNVDWNV 114
S.kudriavzevii --MKQEHSHEDDSYTAEFINLFGKDTATHASSDNNNSANNMGMSANPLDQFVATASSSSSLAVGGANRRPLVGDAAARGSANLYDHAVTPEILLEQLAYVDNFIPLSDNBEISNVDWNV 118
S.bayanus    --MKQEHFHEDGSYNAEFINLFGKDTAVHGSS--SSNNAASNNGMGSAHPLDQFVATASSSSSLAVGTANAQPLLDVGVARGSSNLYDHAVTPEILLEQLAYVDNFIPLSDNBEISNVDWNV 117
S.castellii  MPDQTNENRKRNSYDDFVSLFCFDGGSNKKPK-----DKNDNITVNVPSNLRTTFG-----SLQDHHMDPNNDIIFPEILLEQLAYVDNFIPLSDNBEISNVDWNV 94
S.kluyveri   -----MSFNNDHLSNLGDDANDNQDS-----SNLHNDPNIFPEILLEQLAYVDNFIPLSDNBEISNVDWNV 61
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          UIM          K 163
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S.cerevisiae  NTTHNNANNNGADTFSSINANP-----FDLDEQLALELSAFADDSFIFPDEDKP-SNNNNNSNN-----GNDDHSNHDVLEHEDPSTNNRQNPHEFLTORRNFELTSQYDQSKSRFSSK 222
S.paradoxus  NTTHNNANNNGTDAFSSINANP-----FDLDEQLALELSAFADDSFIFPDEDKP-SNNNNNNNNNNNGNNDNNGHDVLEHEDTSDSNRQNPHEFLTORRNFELTSQYDQSKSRFSSR 227
S.mikatae    NTTHNNISNNGTNTFSSINANP-----FDLDEQLALELSAFADDSFIFPDEDKPDNNNNNNNTDNNNDNNDNNDNNGDDVFHEDPTNNRQNPHEFLTORRNFELTSQYDQSKSRFSSR 227
S.kudriavzevii NTTHNDINNGANTFSSINANP-----FDLDEQLALELSAFADDSFIFPDEDKP-GNSNNNSNN-----GNNDNNGDDVLEHEDLPNNRQNPHEFLTORRNFELTSQYDQSKSRFSSR 225
S.bayanus    NTTHNNINNGTNTFNTINANP-----FDLDEQLALELSAFADDSFIFPDEDKPNNNNNNNSNN-----NNDNTVDEVSHANLPSNNRQNPHEFLTORRNFELTSQYDQSKSRFSSK 224
S.castellii  MGDNATINNGHMNPSQLNAVTSYNNIDSFNLDDEQLALELSAFADDSFIFPDEDKPAKDDDDKDK-----EKNEDKRSKSHFLTORRNFELTSQYDQSKSRFSSK 196
S.kluyveri   GTG--AVNNSANNHGGHGGVG-----LDERLALALELSAFADDSFIFPDEDKPSHDNNDGSDNQPDG-VRDNEINSNEMSNSTNGTNRHRNPHEFLTORRNFELTSQYDQSKSRFSSR 169
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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.

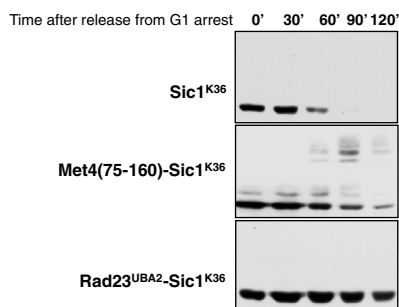


Fig. S4. The UBA2 domain of Rad23 stabilizes Sic1 in its deubiquitinated form. Cells expressing *GAL1*-controlled RGS6His tagged Sic1^{K36}, Met4(75-160)Sic1^{K36}, Rad23(UBA2)Sic1^{K36} were analyzed. The C-terminal 350 residues of yeast Rad23 were used for fusion to Sic1 to generate Rad23(UBA2)Sic1^{K36}. 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.