Methods

RDB 2289 with pGEX2-T His7-Rub1 was made by cloning a His7 tag in place of the GST tag in RDB 1436. To make Rub1-conjugated SCF^{Cdc4}, the procedure for isolating SCF^{Cdc4} was modified.[20] Instead of eluting SCF^{Cdc4} from Py conjugated protein A beads after washing, 50 μM Rub1, 10 μM Ubc12, 1 μM Ula1–Uba3, and 2 mM ATP were incubated together overnight. The beads were washed before elution.

For all reactions, gels were dried and exposed to Phosphor screens (Molecular Dynamics). Images were scanned and then quantified in ImageQuant using a rolling ball background subtraction. For each lane, every band was quantified as a percent of the total signal in all bands.

The relationship between η, φ, and λ was mathematically analogous to the probability of the sum of multiple dice throws. However, the probability of throwing each number on the dice was a weighted normalized distribution (analogous to η) and the number of throws was also a weighted normalized distribution (analogous to φ). A distribution that is normalized sums to 1. Thus, λ equaled the weighted sum of multiple discrete convolutions of η with itself as governed by φ, as shown by example in Supplementary Fig. 3. Knowledge of λ and η allowed us to calculate φ by multiple weighted deconvolutions, as shown by example in Supplementary Fig. 4. This was true for calculating η from λ and φ, as shown by example in Supplementary Fig. 5a. If we assigned a distribution to η, we determined φ by deconvolutions with λ, and vice versa. Considering normalized distributions of η that only contain η(1) and η(2), exponential distributions, poisson distributions and normal distributions, we varied parameters over a wide range and performed deconvolutions, as shown by example in Supplementary Fig. 5b. An exponential distribution is described by a single parameter, here called α. A poisson distribution is also described by a single parameter, here called α. The normal distribution is described by two parameters, the mean and the standard deviation (SD). Parameters were varied starting at 0 and increasing by step sizes of 0.1 until parameters equaled 10. For the Normal distribution, each value of the mean was held constant while the SD was varied. We sought the distribution which deviated most from η(1)=100% whose φ did not contain values >1 or <0 and that when convoluted with φ, the sum of λ fell within 0.95 and 1.05, or an error rate of ± 5% was found. This was repeated for φ. These distributions are shown in Supplementary Fig. 6 and 7. Random distributions were also considered (data not shown).

For mass spectrometry analysis, Uba1 (1 μM), Cdc34-Δ270 (10 μM) and ubiquitin or K48 linked di-ubiquitin (15 μM, Boston Biochem) were incubated for 2 minutes in reaction buffer (30 mM Tris, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 2 mM DTT, and 2 mM ATP) in a volume of 10 μl both in the presence and absence of SCF (100 nM). Reactions were quenched by the addition of 90 μl 5 % acetic acid. Quenching was verified by an order of addition reaction where E1 was left out of the initial incubation and was added following quenching. This resulted in 100 % quenching of the thioester charging reaction. Separation of E2 thioesters in the presence of SCF was accomplished by the addition of 100 mM DTT after the 2 minute incubation period. The DTT was incubated with the reaction mixture for 5 minutes followed by the addition of 90 μl of 5 % acetic acid. Detection of proteins was carried out on an Agilent LC-MSD (Agilent, Palo Alto, CA). Mass spectra were acquired in positive-ion mode, scanning

from 500 to 1700 m/z. The electrospray voltage was set to 4 kV and the gas temperature in the spray chamber was maintained at 350 °C. A stationary phase, Zorbax 300SB C3 150×2.1-mm column was used for separation (Agilent; Bodman, Aston, PA). Mobile phase A was 0.2% formic acid and mobile phase B was 0.2% formic acid, 10% methanol, and 90% acetonitrile. The flow rate was 0.200 ml/min. After a 25 min delay, the effluent was directed into the mass spectrometer. Linear gradients started with 5% mobile phase B and finished at 95% from 25 – 50 min. Data were processed using the chemstation software package. The sequence of yeast Cdc34-Δ270 contains the amino acids from positions 1 to 270 of the yeast Cdc34 sequence following by the sequence ARPLHHHHHH, yielding a theoretical molecular mass of 32,245 Daltons. The theoretical mass of Cdc34-Δ270 thioesterified with ubiquitin (40,792) was calculated by summing the masses of Cdc34-Δ270 (32,245) and ubiquitin (8,565) and subtracting the mass of a water molecule, which is lost during formation of the thioester bond.

For CycE global fitting with KinTek Global Kinetic Explorer, the average of two independent experiments was fit to a model with η=1 and the fit for k_1 through k_4 used the normalized option while the rest of the rate constants did not. For β-Cat global fitting, rate constants were fit without normalization. To improve fitting, neighboring rate constants were constrained by the end point.

Supplementary References

[21] Hao, B., S. Oehlmann, et al. (2007). Structure of a Fbw7-Skp1-Cyclin E Complex: Multisite-Phosphorylated Substrate Recognition by SCF Ubiquitin Ligases. Molecular Cell. 26: 131-143.

[22] Wu, G. (2003). Structure of a ?-TrCP1-Skp1-?-Catenin ComplexDestruction Motif Binding and Lysine Specificity of the SCF?-TrCP1 Ubiquitin Ligase. Molecular Cell. 11: 1445-1456.