1. Supplementary Figures

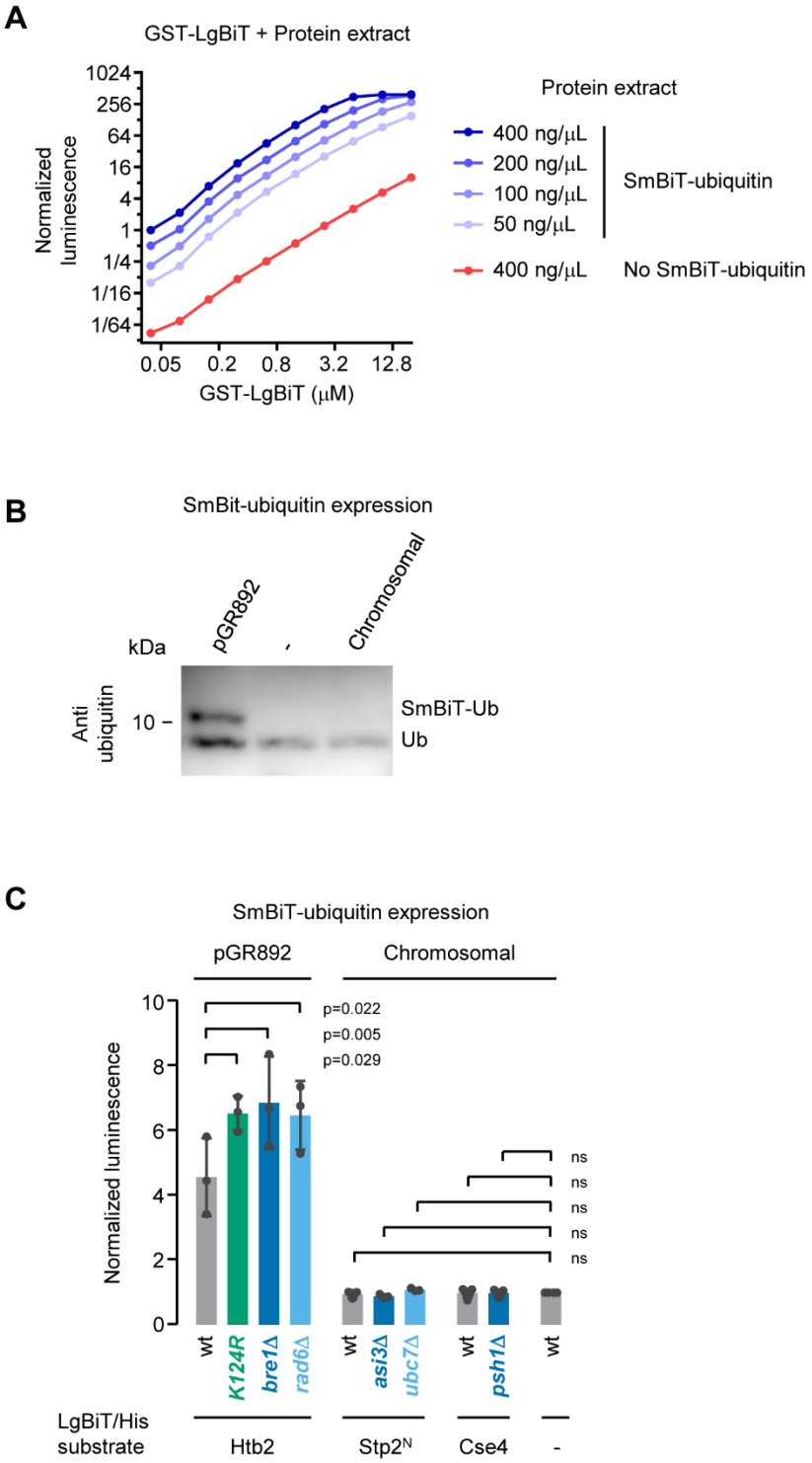

Fig. S1: Analysis of SmBiT-ubiquitin expression.

(A) Quantification of SmBiT-ubiquitin in protein extracts. Protein extracts were prepared from control cells or from cells expressing SmBiT-ubiquitin from the pGR892 plasmid. Extracts were diluted at the indicated concentrations and supplemented with varying amounts of GST-LgBiT before measuring their luminescence. Luminescence values were normalized to 1 in the sample having the highest concentration of SmBiT-ubiquitin and the lowest concentration of GST-LgBiT. A wide range of GST-LgBiT concentrations produce luminescence signals that are linearly related to the concentration of SmBiT-ubiquitin in the extract. Higher GST-LgBiT concentrations yield higher luminescence signals and higher background levels. Detector saturation was observed in the samples with the highest GST-LgBiT concentration. Axis are displayed in log2 scale. Mean of 3 independent experiments.

(B) Comparison of the expression level of SmBiT-ubiquitin and endogenous ubiquitin. Protein extracts were prepared from the indicated strains. The proteins were separated by SDS-PAGE, transferred on a PVDF membraned and revealed with an anti-ubiquitin antibody (P4D1, Santa-Cruz). Representative of 3 experiments.

(C) Relative expression level of SmBiT-ubiquitin in NUbiCA strains. Protein extracts were prepared from the indicated strains and supplemented with 1 μM GST-LgBiT before measuring their luminescence. Htb2 strains express SmBiT-ubiquitin from the pGR892 plasmid, while the other strains express SmBiT-ubiquitin from a chromosomally integrated expression cassette. Background subtracted luminescence values were normalized to 1 in the control strain expressing SmBiT-ubiquitin from the chromosomally integrated expression cassette. Mean ± s.d., n≥3, p-value: One-way ANOVA with Tukey's correction for multiple comparison, ns: not significant.

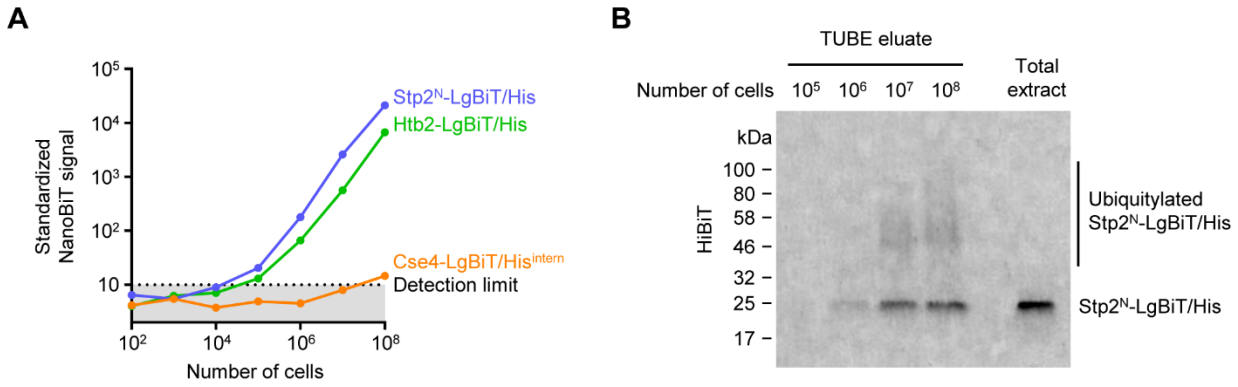

Fig. S2: Sensitivity of NUbiCA.

(**A**) NUbiCA sensitivity for the detection of the ubiquitylation of Htb2, Stp2^{N} and Cse4. LgBiT/His-tagged proteins were purified from extracts prepared from 10^2 to 10^8 cells. The recorded NanoBiT signals were normalized and adjusted so that background measurements have identical standard deviations and means. The dotted line and shaded area delimit a detection limit corresponding to 5 times the standard deviation of background measurements (Detection limit). Axis are displayed in log10 scale.

(**B**) Sensitivity of the detection of the ubiquitylation of Stp2^{N} after TUBE pull-down. Protein extracts were prepared from 10^5 to 10^8 cells expressing Stp2-LgBiT/His. Poly-ubiquitylated proteins were then pulled-down using TUBE2-agarose, separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was imaged in presence of the HiBiT peptide to reveal the migration pattern of Stp2-LgBiT/His. Representative of 2 independent experiments.

2. Supplementary Tables

Table S1: List of strains in the NUbiCA collection

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Table S2: Yeast strains used in this study

Strain	Background	Genotype	Source
BY4741	S288c	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachman et al., 1998
BY4742	S288c	*MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Brachman et al., 1998
BY4745	S288c	*MATalpha his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachman et al., 1998
C-SWAT library	BY4741	*ORF::C-SWAT-acceptor (L3-CYC1term-ScURA3-hphΔN-ALG9term-L4)*	Meurer et al., 2018
NUbiCA library	BY4745	*ORF::LgbiT-HIS-HPH met17::PDC1pr-SmBiT-Ub-NAT can1Δ::STE3pr-LEU2-Gal1pr-NLS-I-SCEI lyp1Δ*	This study
scAB0178	BY4742	*htb2::HTB2-LgBit/His-URA3*	This study
scAB0179	BY4742	*htb2::htb2(K124R)-LgBit/His-URA3*	This study
scAB0180	BY4742	*htb2::HTB2-LgBit/His-URA3 bre1Δ::HPH*	This study
scAB0181	BY4742	*htb2::HTB2-LgBit/His-URA3 rad6Δ::HPH*	This study
scAB0190	BY4741	*stp2::STP2(1-45)-yLgBiT/His-URA lyp1Δ::PDC1pr-SmBiT-Ub-LEU can1Δ::STE2pr-SpHis5*	This study
scAB0191	BY4741	*stp2::STP2(1-45)-yLgBiT/His-URA asi3::HPH lyp1Δ::PDC1pr-SmBiT-Ub-LEU can1Δ::STE2pr-SpHis5*	This study
scAB0195	BY4741	*stp2::STP2(1-45)-yLgBiT/His-URA ubc7::KAN lyp1Δ::PDC1pr-SmBiT-Ub-LEU can1Δ::STE2pr-SpHis5*	This study
scGLD0122	BY4741	*met17::PDC1pr-SmBiT-Ub-NAT*	This study
scGLD0153	BY4741	*cse4::CSE4-LgBit-10xHis-CSE4-URA psh1Δ::HPH lyp1::pPDC1-SmBit-UBQ-Leu*	This study

		can1::STE2pr-Sphis5 *ura3Δ::KAN*	
scMLB0191	BY4741	*cse4::CSE4-LgBit-10xHis-CSE4-URA* *lyp1::pPDC1-SmBit-UBQ-Leu* *can1::STE2pr-Sphis5* *ura3Δ::KAN*	This study
YMaM1205	BY4745	*can1Δ::STE3pr-LEU2-Gal1pr-NLS-I-SCEI* *lyp1Δ*	Meurer et al., 2018

Table S3: Plasmids used in this study

Plasmid	Description	Source
pAB0010	Yeast/*E. coli* shuttle plasmid, KanR, L3-yLgBiT/His-ADH1term-TEF1pr-hphΔC-L4	This study
pGEX6P1_HR23A-TUBE	*E. coli* expression plasmid, GST-HR23A-TUBE	Thimo Kurz lab
pGEX6P1_ubiquilin1-TUBE	*E. coli* expression plasmid, GST-ubiquilin1-TUBE	Thimo Kurz lab
pGR0135	Yeast/*E. coli* shuttle plasmid, LEU2	This study
pGR0313	*E. coli* expression plasmid, GST	Rabut et al., 2011
pGR0691	*E. coli* expression plasmid, GST-Rx3(A7)-TUBE	This study
pGR0890	*E. coli* expression plasmid, GST-yLgBiT	This study
pGR0892	Yeast/*E. coli* shuttle plasmid, LEU2, TEFpr-SmBiT-Ub	This study

Table References

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