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Appendix Figure S1

Appendix Figure S1.

Synthetic lethality of *dsl1* mutations with unpaired VN tags and its suppression by specific BiFC pairs

The growth assays were performed with cells that were grown over night on SGal-Leu plates at permissive temperatures (30 °C (A-L) or 25 °C (M)). Synthetic minimal medium and galactose as carbon source were used to select for the *LEU2*-carrying plasmids and to switch on the GAL-regulated chromosomal copy of *DSL1*. The plasmids used were: pRS215, pRS315-*DSL1*, pRS315-*dsl1-5xWA*, pRS315-*dsl1-5xWA*-VN, pRS315-*dsl1-5xWA*-VC or pRS315-*dsl1-22*. Cells were transferred to Eppendorf tubes with glucose-containing rich medium to stop the expression of the chromosomal *DSL1* gene, and the density of the suspensions was adjusted to 1 OD_{600}. After 4 hours incubation, series of 1/10 dilutions was made, and 5 µl aliquots were spotted on either galactose or glucose-containing YEP plates. Images were taken after two days incubation at 30 °C or 37 °C (A-L), or 25 °C and 30 °C (M). Some important results are presented in Fig 1F. A schematic summary or all results is shown in Figure EV2. The first line of each panel demonstrates that all cells carry a GAL-regulated *DSL1* version at the normal *DSL1* locus, since all strains stop growing when they contain the empty vector. The plasmids with *DSL1* (second row) should rescue all kind of GAL-*DSL1* cells. Some cells do not grow at 37 °C when the other modifications in the cell cause themselves temperature-sensitivity. In the third row of most panels, one can see whether the plasmid-encoded mutant versions of *DSL1* reduce the growth rate of cells carrying a wide variety of tags and additional mutations. See 'additional discussion of results from Figure EV2 and Appendix Figure S1' (next page) for an interpretation of the results. Panels are arranged in a way that important growth assays can be compared directly, for instance those in (B), (E) and (H) as well as (C) and (F).

Additional discussion of results from Figure EV2:

During the construction of *dsl1-5xWA* mutant cells expressing various BiFC combinations we observed that *dsl1-5xWA* mutants producing β'-COP^{VN} alone were not obtained, while the *dsl1-5xWA* cells expressing the β'-COP^{VN}•Dsl3^{VC} BiFC pair were viable (Fig 1F). We asked what other combinations of tags and mutations are lethal and which of them can also be suppressed by paired VN•VC combinations. For that we wanted to rule out the chance that additional mutations accumulating in slowly growing colonies would affect the outcome of the analysis. Therefore, we created GAL-*DSL1* strains expressing various tagged genes and BiFC combinations. At least at 30 °C, these cells grow well on galactose-containing plates and the effect of plasmid-encoded *dsl1* mutations can be analyzed conveniently on glucose-containing plates, where the chromosomal *DSL1* gene is repressed (Zink et al., 2009; see Appendix Fig S1 for the controls involving the empty vector). The consistently green color of the "30 °C" and "Gal"-labeled columns in Fig EV2 indicates that the growth of cells on galactose-containing plates was always normal no matter whether they expressed wild type or mutated *DSL1* alleles. The "37 °C" and "Gal"-labeled third column shows that a few strains were temperature-sensitive even on galactose plates indicating that the BiFC tag or the combination of BiFC tags alone had an effect on their growth. Significant differences between the *DSL1* and *dsl1-5xWA*-labeled sectors are often visible within the columns representing the results obtained with glucose-containing plates ("Glc").

Summary: When *DSL1* is mutated, the VN BiFC tag negatively affects the growth of cells when present at COPI subunits or Dsl1p itself, but not at Dsl3p and Sec16p or Sec24p. VN at COPI subunits is in fact lethal in combination with *dsl1* mutations. Almost normal growth is restored in cells carrying this combination when Dsl3p or Dsl1p carry the VC-tag. On the other hand, VC-fragments fused to the ER protein Sec16p, the COPII subunit Sec24p, the cargo receptor Rer1p or other COPI subunits have no beneficial effect. Thus, the genetic effects of the COPI•Dsl BiFC complexes are very specific and indicate that they functionally mimic the normal COPI•Dsl complex interaction. This very strong genetic effect

also illustrates the importance of this interaction all the more as it can be observed with at least four different COPI subunits and two *dsl1* mutant, that are effected in two different dsl1 mutation, one affecting the central lasso domain and one affecting the E-domain at the C-terminus.

Some important minor points:

Efficient suppression of the β'-COP^{VN}/*dsl1-5xWA* growth defect by Dsl3p^{VC} also requires very efficient formation of BiFC complexes. This is suggested by two observations. First, the presence of an untagged *DSL3* allele in addition to VC-tagged version in diploid cells resulted in much less suppression (H.D.S. unpublished results). Secondly, the I152L mutation within the VN tag of β'-COP reduced the positive effects of the COPI•Dsl BiFC complex as seen in this Figure (compare the first and the second row in panel figure EV2 E). Thus, the beneficial effects of the BiFC complex formation are not due to a very small fraction of molecules that is able to undergo binding. Cells only grow well if all Dsl3 molecules are able to bind to β'-COP^{VN}, and only if β'-COP^{VN} binds efficiently.

Figure EV2 L shows that *the dsl1-5xWA* mutation was also synthetically lethal with COPI subunits carrying GFP or RFP tags that are prone to oligomerize. In fact, the more the tags tend to oligomerize, the stronger the growth impairment of the strains was. This indicates that the synthetic lethality caused by the combination of *dsl1* mutations with unpaired BiFC tags may arise from a propensity of the VN-fragment to aggregate (through exposure of hydrophobic regions, as discussed by Isogai et al. (2011). On the vesicles, unpaired VN fragments on COPI subunits of neighboring COPI heptamers may aggregate, linking the coat blocks and making uncoating more difficult. In combination with a mutated Dsl1p that has been implicated in COPI coat removal (Zink et al., 2009), this may lead to uncoating defects.

Appendix Table S1.

Saccharomyces cerevisiae strains used in this study

Strains are listed in order of their appearance in Figure 1 to 7 and Figure EV1 to 4. Note that the names of genes encoding α-COP, β'-COP, δ-COP and ε-COP are *COP1*, *SEC27*, *RET2* and *SEC28*, respectively. Most strains are from this study. The references for strains BY4741 and BY4742, for the six strains used for Fig EV2M / Appendix Fig S1M, as well as four miscellaneous strains listed at the end of this table can be found below. The respective strains are marked by asterisks.

Figure 1

B		
XV-27	*SEC27*^{VN} ::NatMX • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 trp1 lys2*
XIV-40	*SEC27*^{VN} ::KanMX • *DSL3*^{VC}::KanMX *dsl1*-5xWA	"
C		
XVII-11	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
XVII-2	*SEC27*^{VN}::KanMX *DSL3*^{VC}::KanMX	„
XVII-7	„	*MAT*a *ura3 leu2 his3 met15 lys2*
XVII-9	„	*MAT*α *ura3 leu2 his3 trp1*
3 5xWA Klon 5	*SEC27*^{VN}::*TRP1* • *DSL3*^{VC}::KanMX *dsl1*-5xWA	*MAT*a *ura3 leu2 his3 trp1 lys2*
22 5xWA-2A	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *dsl1*-5xWA	*MAT*α *ura3 leu2 his3 trp1*
22 5xWA-4A	„	*MAT*a *ura3 leu2 his3 trp1 lys2*
22 5xWA-6B	„	„
WT^{-I152L}-1A	*SEC27*^{VNI152L}::*TRP1* • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 met15 lys2*
WT^{-I152L}-2C	„	*MAT*α *ura3 leu2 his3*
WT^{-I152L}-3A	„	*MAT*α *ura3 leu2 his3 met15*
3^{-I152L}Klon 2	*SEC27*^{VNI152L}::KanMX • *DSL3*^{VC}::KanMX *dsl1*-5xWA	*MAT*a *ura3 leu2 his3 trp1 lys2*
3^{-I152L}Klon 6	„	„
3^{-I152L}Klon 7	„	„
3^{-I152L}Klon 8	„	„
22$^{-\Delta I}$-1B	*SEC27*^{VN}:: NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 lys2*
22$^{-\Delta I}$-2A	„	*MAT*a *ura3 leu2 his3 lys2*
22$^{-\Delta I}$-4A	„	*MAT*a *ura3 leu2 his3 trp1 lys2*
22$^{-\Delta lasso}$-2B	*SEC27*^{VN}:: NatMX • *DSL3*^{VC}::KanMX *dsl1*::KanMX::pRS306-dsl1$^{\Delta lasso}$	*MAT*α *ura3 leu2 his3 lys2*
22$^{-\Delta lasso}$-3A	„	*MAT*a *ura3 leu2 his3 trp1 lys2*
XVI-6	„	*MAT*α *ura3 leu2 his3 lys2*
22$^{-\Delta lasso}$-8A	„	„
22$^{-\Delta lasso}$-12C	„	„
22$^{-\Delta lasso}$-13A	„	*MAT*a *ura3 leu2 his3 trp1 lys2*

XVI-7 (=XVI-6 FOA^{+})	*SEC27*^{VN}:: NatMX • *DSL3*$^{VC::}$KanMX dsl1$^{\Delta lasso}$ (5-FOA+)	*MAT*α *ura3 leu2 his3 lys2*
D		
D1D3	*DSL1*^{VN}::KanMX *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3*
SB1-77-3A	*SEC16*^{VN}::*TRP1* • *SEC16*^{VC}::*TRP1*	*MAT*α/a *met15/met15 ura3/ura3*
XIII-26	*COP1*^{VN}*::TRP1* • *COP1*^{VC}*::TRP1* GAL^{P}*-DSL1 /GAL-DSL1*	*MATa/MAT*α *ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 MET15/met15 LYS2/lys2*
E		
XVII-2	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX with pRS316 APE1-RFP	*MAT*α *ura3 leu2 his3 trp1*
F		
XVI-52	GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XVI-29	*SEC27*^{NV}::KanMX • GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XIV-25	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL1-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
XVI-29	*SEC27*^{NV}::KanMX GAL-*DSL1* pRS315-dsl1-5xWA	*MAT*a *ura3 leu2 his3 trp1 met15*
XVI-36	*SEC27*^{VN}::KanMX • *SEC16*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15*
XVI-35	*SEC27*^{VN}::KanMX • *SEC24*^{VC}::*TRP1* GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVII-22	*SEC27*^{VN}::NatMX • *rer1::TRP1* GAL-*DSL1* pRS316-^{VC}RER1	*MAT*a *ura3 leu2 his3 trp1 met15*
G		
XVII-11	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
XVII-2	*SEC27*^{VN}::KanMX *DSL3*^{VC}::KanMX	„
XVI-16	*SEC27*^{VN}::NatMX • *DSL1*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
XVI-17	*SEC27*^{VN}::NatMX • *DSL1*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 met15*
XVII-14	*SEC27*^{VN}::NatMX • *DSL1*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 met15*
SB2-131-4B	*SEC27*^{VN}::KanMX • *SEC24*^{VC}::*TRP1*	*MAT*a *ura3 leu2 his3*
SB1-8-1A	*SEC27*^{VN}::KanMX • *SEC16*^{VC}::*TRP1*	*MAT*α *ura3 his3 met15*
XV-67	*SEC27*^{VN}::KanMX • His3MX::RPL7B^{P}::^{VC}Rer1 GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XV-68	*SEC27*^{VN}::KanMX • His3MX::RPL7B^{P}::^{VC}Rer1 GAL-*DSL1*	*MAT*a *ura3 leu2 his3*
H		
XVII-11	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
XVII-2	*SEC27*^{VN}::KanMX *DSL3*^{VC}::KanMX	„
XIV-13	*DSL3*^{VN}::KanMX • *COP1*^{CV}::*TRP1*	*MAT*a *ura3 leu2 his3 lys2*
XIV-7	*DSL3*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*a *ura3 leu2 his3*
XIV-28	*DSL3*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 trp1*
SB1-31-4C	*SEC16*^{VN}::KanMX • Dsl3^{VC}::KanMX	*MAT*α *ura3 his3 met15*
SB1-65-2C	*DSL3*^{VN}::KanMX6 • *SEC16*^{VC}::*TRP1*	*MAT*a *leu2 ura3 his3*
SB2-132-1D	*DSL3*^{VN}::KanMX • *SEC24*^{VC}::*TRP1*	*MAT*α *ura3 leu2*
I		
XV-62	*SEC27*^{VN}::KanMX • *DSL3*^{VC} ::KanMX	MATα *ura3 leu2 his3 trp1*

Figure 2

A		
XIV-20	*DSL1*^{VN}::KanMX/ *DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX/*SEC28*^{VC}::KanMX	*MAT*a/α *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*TRP met15*/*MET*
B & C		
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
D		
XVII-2	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
XVII-7	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 lys2*
E		
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
F		
XV-27	*COPI*^{VN}::*TRP1 SEC27*^{VN}:KanMX •	*MAT*? *ura3 leu2 his3 met15 lys2*

Figure 3

A		
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
B		
XIV-28	*DSL3*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*a *ura3 leu2 his3*
C		
XV-27	*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 trp1 lys2*
D		
XVI-71	*SEC27*^{VN}::KanMX • *DSL3*$^{VC\text{-}A206K}$::*TRP1*	*MAT*α *ura3 leu2 his3 trp1 met15*
E		
XVII-21	*SEC27*^{VN}::NatMX • *rer1*::TRP1 pRS316-^{VC}*RER1*	*MAT*a *ura3 leu2 his3 trp1 met15*
F		
XV-23	*DSL1*^{VN}::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1*	*MAT*α *ura3 leu2 his3*
G		
SB1-8-1A	*SEC27*^{VN}::KanMX • *SEC16*^{VC}::*TRP1*	*MAT*α *ura3 his3 met15*
H		
XIII-57	*COP1*^{VN}*::TRP1* • *COP1*^{VC}*::TRP1*	*MAT*a/*MAT*α *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*trp1 MET15*/*met15*
I		
XVI-38	*SEC27*^{mRFP}::His3MX • GAL-*DSL1*	*MAT*a *ura3 leu2 his3*

Figure 4

A		
XV-45	*SEC27*^{VN}::KanMX/ *SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanM/*DSL3*^{VC}::KanMX with YCplac33-crimson-HDEL	*MAT*α/a *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*TRP1 met15*/MET15 *lys2*/*LYS2*
B		
B'D313	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *SEC13*^{mRFP}::KanMX	*MAT*a *ura3 leu2 his3 trp1 met15 lys2*

C		
XV-27	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX with pRS416 Cherry-Sed5	*MAT*a *ura3 leu2 his3 trp1 lys2*
D		
XVII-12	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX pAG416-with GPD-Cerulean-GOS1	*MAT*α *ura3 leu2 his3 lys2*
E		
B'D37 (-13A)	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *TRP1*::Sec7-DsRedT4	*MAT*α *ura3 leu2 his3 trp1*
F		
XVII-13	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX with pAG416-GPD-SEC7-Cerulean	*MAT*α *ura3 leu2 his3*
G		
XV-45	*SEC27*^{VN}::KanMX/ *SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanM/*DSL3*$^{VC::}$KanMX with pRS316-GPD-CFP-*RER1*	*MAT*α/a *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*TRP1 met15*/*MET15 lys2*/LYS2
H		
XV-45	*SEC27*^{VN}::KanMX/ *SEC27*^{VN}::KanMX • *DSL3*$^{VC::}$KanM/*DSL3*$^{VC::}$KanMX with pAG416-GPD-Cerulean-*SEC4*	*MAT*α/a *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*TRP1 met15*/MET15 *lys2*/*LYS2*

Figure 5

A		
XIV-40	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *dsl1*-5xWA	*MAT*a *ura3 leu2 his3 trp1 lys2*
B		
SB2-150-4A	*SEC16*^{VN}::*TRP1* • *SEC28*^{VC}::KanMX6	*MAT*a *met15 his3 ura3*
SB2-151-4C	*SEC16*^{VN}::*TRP1* • *SEC28*^{VC}::KanMX6 *dsl1*-5xWA::pRS306-dsl1-5WA	*MAT*a *leu2 met15 his3*
SB2-152-5D	*SEC16*^{VN}::*TRP1* • *SEC28*^{VC}::KanMX6	*MAT*α *leu2 lys2 ura3*
SB2-153-7B	*SEC16*^{VN}::*TRP1* • *SEC28*^{VC}::KanMX6 *dsl1*-5xWA::pRS306-*dsl1*-5WA	*MAT*a *leu2 lys2 met15*
XVI-34	*SEC16*^{VN}::KanMX6 • *COP1*^{VC}::*TRP1*	*MAT*α *ura3 leu2 his3 met15 lys2*
XVI-33	*SEC16*^{VN}::KanMX6 • *COP1*^{VC}::*TRP1* *dsl1*-5xWA::pRS306-*dsl1*-5WA	"

Figure 6

A and B,		
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
C		
XIV-54	*DSL1*^{VN}::KanMX6/*DSL1*^{VN}::KanMX6 *SEC28*-VC::KanMX/*SEC28*^{VC}::KanMX *myo2-66*/*myo2-66*	*MAT*α/a *ura3*/*ura3 leu2*/*leu2 his3*/*his3*
D		
VX-14	*SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX *myo4*::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
E		
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
F		
XV-15	*SEC27*^{VN}::KanMX • *DSL3*$^{VC::}$KanMX *myo2-66*	*MAT*a *ura3 leu2 his3 lys2*

Figure 7

XV-27	*SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX	*MAT*a *ura3 leu2 his3 trp1 lys2*

Figure EV1

A		
XV-49	*DSL1*^{2myc}::KanMX *SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX	*MAT*a *ura3 leu2 his3 lys2*
XV-48	*DSL1*^{2myc}::KanMX *SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX	*MAT*α *ura3 leu2 his3 trp1*
XV-50	*dsl1 5xWA*^{2myc}::KanMX *SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
XV-51	*dsl1 5xWA*^{2myc}::KanMX *SEC27*^{VN}::NatMX • *DSL3*$^{VC::}$KanMX	*MAT*a *ura3 leu2 his3 trp1 met15 lys2*
XVI-18	*dsl1-Δlasso*^{2myc}::KanMX	*MAT*α *ura3 leu2 his3 lys2*
XVI-19	*dsl1-Δlasso*^{2myc}::KanMX	“
B and **C**		
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XVII-17	*COP1*^{VN}::*TRP1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XVII-10	*COP1*^{VC}::*TRP1*	*MAT*α *ura3 leu2 his3 trp1 met15*
XVII-1	*SEC27*^{VN}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
XIII-76	*SEC27*^{VC}::KamMX	*MAT*a *ura3 leu2 his3 met15*
XVII-18	*SEC28*^{VN}::KanMX	*MAT*a *ura3 leu2 his3 lys2*
XVII-19	*SEC28*^{VC}::KamMX	*MAT*α *ura3 leu2 his3 lys2*
XVI-48	*RET2*^{VN}::KamMX	*MAT*a *trp1* MET *lys2*
XIII-77	*RET2*^{VC}::KamMX	*MAT*a *ura3 leu2 his3 trp1 lys2*
D		
BY4742		*MAT*α *ura3 leu2 his3 lys2*
XVI-9	*DSL1*^{VN}::KanMX	*MAT*α *leu2 his3 trp1*
XIII-80	*SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1 lys2*
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 met15*
E		
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XVII-1	*SEC27*^{VN}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
XVI-13	*DSL3*^{VC}::KanMX	*MAT*α ura2 *leu2 his3 trp1*
XVII-2	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX	“
F		
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XV-24	*DSL1*^{VN}::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1*	*MAT*a *ura3 leu2 his3 trp1*
XVI-30	*SEC27*^{VN}::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1*	*MAT*α *ura3 leu2 his3*
G		
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XIII-48 ~	*DSL1*^{GFP}::His5MX	*MAT*α *ura3 leu2 his3*
XII-67 ~	*DSL3*^{GFP}::His5MX	*MAT*α *ura3 leu2 his3*

XVII-4	*DSL1*^{VN}::KanMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3*
XVII-5	*DSL3*^{VN}::KanMX • *DSL1*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 lys2*
H		
BY4741 *		*MAT*a *ura3 leu2 his3 met15*
XIII-30	*DSL1*^{VN}::KanMX • *COP1*^{VC}::*TRP1*	"
XIII-31	*COP1*^{VN}::*TRP1* • *DSL1*$^{V'C}$::KanMX	*MAT*α *ura3 leu2 his3 met15 lys2*
XVII-15	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX	*MAT*α *leu2 his3 met15*
XIII-41	*SEC28*^{VN}::KanMX • *DSL1*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 met15 lys2*
BY4742		*MAT*α *ura3 leu2 his3 lys2*
XVII-2	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XV-24	*DSL1*^{VN}::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1*	*MAT*a *ura3 leu2 his3 trp1*
BY4742		*MAT*α *ura3 leu2 his3 lys2*
XIV-67	*SEC27*^{VN} ::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1*	*MAT*α *ura3 leu2 his3 lys2*
XVII-4	*DSL1*^{VN}::KanMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3*
XVII-5	*DSL3*^{VN}::KanMX • *DSL1*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 lys2*
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
XIII-58	*COP1*^{VN}::*TRP1*/*COP1*^{VC}::*TRP1* • *DSL1*/GAL-*DSL1*	*MAT*a/α *ura3*/*ura3 leu2*/*leu2* his/*his3* trp/*trp1 MET15*/*met15 LYS2*/*lys2*
XIII-39	*SEC28*^{VN}::KanMX/*SEC28*^{VC}::KanMX • *DSL1*/GAL-*DSL1*	*MAT*a/α *ura3*/*ura3 leu2*/*leu2* his/*his3* trp/*trp1 MET15*/*met15 LYS2*/*lys2*
BB^{wg}	*SEC27*^{VN}::KanMX/*SEC27*^{VC}::KanMX • *DSL1*/GAL-*DSL1*	*MAT*a/α *ura3*/*ura3 leu2*/*leu2 his*3/*his3 TRP1*/*trp1 met15*/*met15* LYS2/*lys2*
I		
XVI-1	*SEC27*^{VN}::KanMX . *DSL3*^{VC}::KanMX Sec20^{TAP}::His3MX	*MAT*α *ura3 leu2 his3 trp1*
XVI-3	SEC20^{TAP}::His3MX	*MAT*α *ura3 leu2 his3*
XIV-59	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX Sec20^{TAP}::His3MX	*MAT*α *ura3 leu2 his3 trp1*
J		
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
[22]^{cg}-6C	*SEC27*^{NV}::NatMX	*MAT*a *ura3 leu2 his3 trp1*
[22]^{cg}-6A	*SEC27*^{NV}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
13R12-4iii-1D	*SEC27*^{GFP}::His3MX	*MAT*a *ura3 leu2 his3 trp1*
13R12-4ii-6A	*SEC27*^{NV}::NatMX	*MAT*a *ura3 leu2 his3 trp1 met15*
13R12-4i-6B	*SEC27*^{NV}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
13R12-4i-4A	*SEC27*^{GFP}::His3MX	*MAT*α *ura3 leu2 his3 trp1 lys2*
13R12-4i-11D	*SEC27*^{NV}::NatMX	"
13R12-4i-10D	*SEC27*^{NV}::NatMX • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 trp1 met15*
XVII-16	*SEC27*^{NV}::KanMX	*MAT*a *ura3 leu2 his3 met15*
XVII-2	*SEC27*^{NV}::KanMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
[22]^{cg}-10B		*MAT*a *ura3 leu2 his3 trp1*
XVII-1	*SEC27*^{NV}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
XVII-7	*SEC27*^{NV}::KanMX • *DSL3*^{VC}::KanMX	*MAT*a *ura3 leu2 his3 met15 lys2*

β'-7A		*MAT*α *ura3 leu2 his3 met15 lys2*
[22]^{cg}-5B	*SEC27*^{NV}::NatMX	*MAT*a *ura3 leu2 his3 trp1*
XVII-11	*SEC27*^{NV}::NatMX • *DSL3*^{VC}::KanMX	*MAT*α *ura3 leu2 his3 trp1*
K		
XVII-39	*SEC27*^{VN}::NatMX/*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX/*DSL3*	*MAT*a/α *ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 MET15/met15 LYS2/lys2*
XVII-40	*SEC27*^{VN}::NatMX/*SEC27*^{VN}::NatMX • *DSL3*^{VC}::KanMX/*DSL3*^{VC}::KanMX	*MAT*a/α *ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 MET15/met15 LYS2/lys2*

Figure EV2, Appendix Figure S1

A		
XVI-52	GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
B		
XVI-29	*SEC27*^{NV}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XVI-25	*SEC28*^{NV}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 met15*
XVI-31	*COP1*^{NV}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3*
XVI-47	*RET2*^{NV}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
C		
XVI-45	*COP1*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3* met
XVI-44	*SEC28*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 lys2*
XVI-46	*RET2*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 lys2*
D		
XVII-8	GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVI-54	*DSL3*^{VN}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XVI-55	*DSL3*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 lys2*
SB2-173	*SEC16*^{VC}::*TRP1* GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
SB2-168	*SEC16*^{VN}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3 lys2 met15*
XVII-20	*SEC24*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3*
E		
XIV-25	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL1-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
XVI-56	*SEC27*^{VNi152l} ::*TRP1* • *DSL3*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVI-37	*COP1*^{VN}::*TRP1* • *DSL3*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVI-39	*SEC28*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVI-51	*RET2*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL-DSL2	*MAT*a *ura3 leu2 his3*
F		
XIV-12	*DSL3*^{VN}::KanMX • *COP1*^{VC}::*TRP1* GAL-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
XVI-45	*COP1*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15*
XVI-44	*SEC28*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 lys2*
XVI-46	*RET2*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 lys2*
G		
	same as B	

H		
XVI-36	*SEC27*^{VN}::KanMX • *SEC16*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15*
XVI-35	*SEC27*^{VN}::KanMX • *SEC24*^{VC}::*TRP1* GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
SB2-163	*SEC28*^{VN}::KanMX • *SEC16*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3*
SB2-162	*SEC16*^{VN}::KanMX • *COP1*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
SB2-157	*SEC16*^{VN}::KanMX • *SEC28*^{VC}::KanMX GAL-*DSL1*	*MAT*a *leu2 ura3* lys3 met3 *his3*
SB1-64	*SEC16*^{VC}::*TRP1* • *DSL3*^{VN}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15 his3 lys2*
I		
XVI-30	*SEC27*^{VN}::KanMX • His3MX::RPL7B^{P}-^{VC}*RER1* GAL-*DSL1*	*MAT*α *ura3 leu2 his3*
XVII-22	*SEC27*^{VN}::NatMX • *rer1::TRP1* GAL-*DSL1* pRS316-^{VC}RER1	*MAT*a *ura3 leu2 his3 trp1 met15*
J		
XVI-40	*SEC27*^{VN}::KanMX • *COP1*^{VC}::*TRP1* GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15*
XVI-41	*SEC27*^{VN}::NatMX • *SEC28*^{VC}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 met15*
XIII-26	*COP1*^{VN}::*TRP1*/*COP1*^{VC}::*TRP1* GAL-*DSL1*/ GAL-*DSL1*	*MAT*a/*MAT*α *ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 MET15/met15 LYS2/lys2*
XIII-20	*SEC28*^{VN}::KanMX/*SEC28*^{VC}::KanMX GAL-*DSL1*/ GAL-*DSL1*	*MAT*a/*MAT*α *ura3/ura3 leu2/leu2 his3/his3 MET15/met15 LYS2/lys2*
δδ	*RET2*^{VN}::KanMX/*RET2*^{VC}::KanMX GAL-*DSL1*/ GAL-*DSL1*	*MAT*a/ *MAT*α *ura3/ura3 leu2/leu2 his3/his3 trp1/TRP1 lys2/lys2*
K		
XVI-29	*SEC27*^{VN}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3 trp1 met15*
XIV-25	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL1-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
εN1-7A	*SEC28*^{VN}::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 met15*
5-2D	*SEC28*^{VN}::KanMX • *DSL3*^{VC}::KanMX GAL1-*DSL1*	*MAT*α *ura3 leu2 his3*
2-6A	*COP1*^{VN}*::TRP1* • *DSL3*^{VC}::KanMX GAL-*DSL1*	“
2-3B	*COP1*^{VN}*::TRP1* GAL-*DSL1*	“
L		
XI-8	*SEC27*^{TAP}::HygB GAL-*DSL1*	*MAT*α *ura3 leu2 his3 trp1 met15*
XVI-38	*SEC27*^{mRFP}::KanMX GAL-*DSL1*	*MAT*a *ura3 leu2 his3*
X-73	*SEC27*^{GFP} GAL-*DSL1*	*MAT*a *ura3 leu2 his3 met15 lys2*
XI-1	*SEC28*^{TAP}::His3MX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 met15*
XIII-45	*SEC28*^{GFP}::His3MX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
XI-26	*SEC28*$^{RFP(redstar)}$::KanMX GAL-*DSL1*	*MAT*α *ura3 leu2 his3 met15*
M		
XI-53**	*sec27-1* GAL-*DSL1*	MATa *ura3 leu2 his3*
XI-54**	*ret3-1* GAL-*DSL1*	MATa *ura3 leu2 his3 trp1 lys2*
XI-55**	*sec28*Δ GAL-*DSL1*	MATa *ura3 leu2 his3 trp1*
XI-56**	*ret2-1* GAL-*DSL1*	MATα *ura3 leu2 his3 trp1 lys2*
XVI-64**	*sec21-1* GAL-*DSL1*	MATα *ura3 leu2 lys2*

Figure EV3A see Figure 3

Figure EV3B see Figure 4

Figure EV4

A		
XV-45	*SEC27*^{VN}::KanMX/ *SEC27*^{VN}::KanMX • *DSL3*$^{VC::}$KanMX/ *DSL3*$^{VC::}$KanMX with YCplac33-crimson-HDEL	*MAT*α/a *ura3*/*ura3 leu2*/*leu2 his3*/*his3 trp1*/*TRP1 met15*/MET15 *lys2*/*LYS2*
B		
XVI-72	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *SEC13*^{RFP}::KanMX *sec12-4*	*MAT*α *ura3 leu2 his3 trp1 lys2*
XVI-73	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *SEC13*^{RFP}::KanMX	*MAT*a *ura3 leu2 his3 trp1*
C		
B'D313	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *SEC13*^{RFP}::KanMX	*MAT*a *ura3 leu2 his3 trp1 met15 lys2*
XIV-18	*DSL1*^{VN}::KanMX • *SEC28*^{VC}::KanMX *SEC13*^{RFP}::KanMX	*MAT*α *ura3 leu2 his3 trp1 met15*
D		
B'D313	*SEC27*^{VN}::KanMX • *DSL3*^{VC}::KanMX *SEC13*^{RFP}::KanMX	*MAT*a *ura3 leu2 his3 trp1 met15 lys2*

Miscellaneous strains

	Strain used for the construction of the *dsl1*$^{\Delta lasso}$ strain	
IX-45 **	*dsl1*::KanMX with pRS313 GAL^{TAP}*DSL1*	*MAT*α *ura3 leu2 his3 lys2*
	Strains used as source for myc and TAP tags	
YUA-11 ***	*DSL1*$^{6His\text{-}2myc}$:: loxP-KanMX-loxP	*MAT*a *ura3 leu2 his4 lys2 bar1-1*
YSC1178-7502517 ****	*DSL1*^{TAP}::HIS5MX	*MAT*a *ura3 leu2 his3 met15*
YSC1178-7500084 ****	*SEC20* ^{TAP}::HIS5MX	*MAT*a *ura3 leu2 his3 met15*
	for PLA experiments	
YLR440C ~	*DSL3*^{GFP}::HIS5MX	*MAT*a *ura3 leu2 his3 met15*
CUY2925 ~~	HIS3MX-PHO5pr-^{GFP}::SEC20	*MAT*α *ura3 leu2 his3* trp1 met15 *lys2*
YUA-11 ***	*DSL1*$^{6His\text{-}2myc}$:: loxP-KanMX-loxP	*MAT*a *ura3 leu2 his4 lys2 bar1-1*
BY4742 *		*MAT*α *ura3 leu2 his3 lys2*
X-19	*ura3::pSTI22 (*TPIpr-*SEC20-myc)*	*MAT*α *ura3 leu2 his3 lys2*

* (Winzeler et al., 1998)

** (Zink et al., 2009)

*** (Andag et al., 2001)

**** (Ghaemmaghami, et al., 2003)

~ (Huh et al., 2003)

~~ (Meiringer et al., 2011)

Appendix Table S2.

Plasmids used in this study

	Designation	Characteristics	Source
1	p^{VN}TRP	pFA6a Venus-N *TRP1* AmpR	Sung and Huh, 2007
2	p^{VC}TRP	pFA6a Venus-C *TRP1* AmpR	Sung and Huh, 2007
3	p^{VN}kan	pFA6a Venus-N KanMX6 AmpR	Sung and Huh, 2007
4	p^{VC}kan	pFA6a Venus-C KanMX6 AmpR	Sung and Huh, 2007
5	pHIS^{VC}	pFA6a His3MX6 RPL7B^{P}-Venus-C AmpR	Sung and Huh, 2007
6	$p^{VNI152L}$TRP	pFA6a-*TRP1*-VN^{I152L} AmpR	this lab
7	p^{mVC}TRP	pFA6a-*TRP1*-VC^{A206K} AmpR	this lab
8	pTOPO-^{VC}*RER1*	pCR2.1-TOPO-His3MX::CV-*RER1*	this lab
9	pUA73	pRS315-*DSL1 LEU2* CEN6 ARSH4 AmpR	Andag et al., 2001
10	p315-d1	pRS315-dsl1-5WA *LEU2* CEN6 ARSH4 AmpR	Zink et al., 2009
11	p315-D1^{VN}	pRS315-*DSL1*-VN *LEU2* CEN6 ARSH4 AmpR	this lab
12	p315-D1^{VC}	pRS315-*DSL1*-VC *LEU2* CEN6 ARSH4 AmpR	this lab
13	p315-d1^{VN}	pRS315-dsl1-5WA-VN *LEU2* CEN6 ARSH4 AmpR	this lab
14	p315-d1^{VC}	pRS315-dsl1-5WA-VC *LEU2* CEN6 ARSH4 AmpR	this lab
15	pUA86	pRS315-dsl1-22, *LEU2* CEN6 ARSH4 AmpR	Andag et al., 2001
16	p306-d1-5WA	pRS306 dsl1-5WA *URA3* CEN6 ARSH4 AmpR	Zink et al., 2009
17	p^{RFP}HDEL-T	YIplac204 TKC-E2-crimson-HDEL *TRP1*	Strack et al., 2009; a gift from Birka Hein
18	p^{RFP}HDEL-U	YCplac33-TPI^{P}-crimson-HDEL ARS1 CEN4 *URA3* AmpR	this lab

19	pSEC7^{CFP}	pAG416 GPD^{P}-SEC7-Cerulean *URA3* CEN6 ARSH4 AmpR	Gitler et al., 2009
20	p^{CFP}GOS1	pAG416 GPD^{P}-Cerulean-GOS1 *URA3* CEN6 ARSH4 AmpR	Gitler et al., 2009
21	p^{CFP}SEC4	pAG416 GPD^{P}-Cerulean-SEC4 *URA3* CEN6 ARSH4 AmpR	Gitler et al., 2009
22	p^{cherry}SED5	pRS416 MET25^{P}-Cherry-Sed5 *URA3* CEN6 ARSH4 AmpR	Powis et al., 2012
23	pAPE^{mRFP}1	pRS316 *APE1*-mRFP *URA3* CEN6 ARSH4 AmpR	Meiling-Wesse et al., 2005
24	p^{GST}*RER1*	pRS316 CYC1-GST-*RER1 URA3* CEN6 ARSH4 AmpR	this lab
25	p^{CFP}*RER1*	pRS316 GPD^{P}-Cerulean-Rer1 *URA3* CEN6 ARSH4 AmpR	this lab
26	pCR2.1-TOPO	ampR, kanR	Invitrogen
27	pRS408	NatMX AmpR	gift from Fred Cross (Addgene plasmid # 11255)
28	p316-^{VC}RER1	pRS316 RER1^{p}-^{VC}-RER1 *URA3* CEN6 ARSH4 AmpR	this lab
29	pKT212	pFA6a–link–yEmCFP–CaURA3	(Sheff & Thorn, 2004)

Appendix Table S3.

Oligonucleotides used in this study

	designation	Sequence
Oligonucleotides for in-frame integration of BiFC tags		
1	TEF-term-rev	GGATGGCGGCGTTAGTATCGAATCG
2	*COP1*-F2	CTATGATTTCTAAGATCGGTGCACCTGCATCCGGATTAAGAATACGTGTAGGTCGACGGATCCCCGGGTT
3	Dsl3-F2	GTAAGGTAGGCGGCATTGCTAGGGAGATTTTCCACAACGTGACTAATTTCGGTCGACGGATCCCCGGGTT
4	Dsl1-F2	GGAATGCCATTGATGATATTTACGAAATTAGAGGCACTGCTCTAGATGATGGTCGACGGATCCCCGGGTT
5	*SEC28*-F2	AAATTGACGCAAAATTCGATGAATTAGTGAGGAAATATGATACGTCCAACGGTCGACGGATCCCCGGGTT
6	*COP1*-R-bifc	AGATTAAAAGGACTTGTCACATGAAATTTAGGTCGAGCAGAATACAATCCTCGATGAATTCGAGCTCGTT
7	*SEC27*-F2	AACAGCCGGAGCAAGGAGAGGCAGTGCCGGAGCCTGTGGAAGAAGAGAGTGGTCGACGGATCCCCGGGTT
8	*SEC27*-REV	ACCATCCATTAAAGATGTTTTCTCATTCGTTGATTAGTTCTTTATTTGTCTCGATGAATTCGAGCTCGTT
9	*RET2*-F2	TCCCTTACGACGTCATCACCTCTTTGAAATCAGATGAATATCTTGTCCAAGGTCGACGGATCCCCGGGTT
10	*RET2*-REV	CTCGATTTTTATTTATTTAGTTTGTGTATATAACTTAAGTATTTTATATGTCGATGAATTCGAGCTCGTT
11	SEC23-F2	TACAAAATTTCATGACTCACTTACAACAAGTAGCCGTCTCTGGTCAGGCAGGTCGACGGATCCCCGGGTT
12	SEC23-R1	CGCAATGGACTGAACAGGCCTTATATTATACAAAAAGCCTAATAACGATCTCGATGAATTCGAGCTCGTT
13	SEC24-F2	ACGAAAGTTACAGAGAATTCTTACAAATCATGAAAGCCAGAATTAGCAAAGGTCGACGGATCCCCGGGTT
14	SEC24-R1	ACATAATATAATTGGAAAGCAAAAATGCAACCAACAAAAATATACAGTTTTCGATGAATTCGAGCTCGTT

15	*SEC13*-F2	GGAAGGAAAATCTTGAGGGTAAATGGGAACCCGCTGGTGAAGTTCATCAGGGTCGACGGATCCCCGGGTT
16	*SEC13*-R1	TCCCTTCAAATTCGTCGATAGACTCATTTGCATTCTTTTTTCTTTTGAGATCGATGAATTCGAGCTCGTT
17	SEC31-F2	GGCTGACAGGAGTGAAGAGGTTGATTGGCATAGCTGAAGCGACTTTGAATGGTCGACGGATCCCCGGGTT
18	SEC31-R1	ATATAATACTATAAGACATAATAAGAAAAAAACAAGGCCAATACGCCACTTCGATGAATTCGAGCTCGTT
19	SEC16-F2	GGAAGAAGAAAACAGCGAGAGGCTATGTTAATGTAATGGATAACATACAAGGTCGACGGATCCCCGGGTT
20	SEC16-R1	GTTTTTATAAGTATATAATATACATCAAGAAAAGGGAAGGCATAAGGGTATCGATGAATTCGAGCTCGTT
21	^{VC}*RER1*-5' (1st cycle)	CGCAGAGGAAAAAAAAACTTGTCAAAACACGCCTCTGCAGTAGAACACGAGAATTCGAGCTCGTTTAAAC
22	^{VC}ATG-*RER1* (1st cycle)	AAGGGGTTACTTGAACCACCGTTCATTGTATCAGAGCTATCGTAATCCATAGTACCACCAGAACCCTTGTACAGCTCGTCCATG
23	*RER1*-extent-3´ (2nd cycle)	AGGGCTCCTCAACCTCTCTCTGGAAAACCTTACCTCAATGAGGTTAAAAATTGTGGGTTTTTCGTTCGCAGAGGAAAAAAAAACTTGTCA
24	*RER1*-extent-5´ (2nd cycle)	CGTGAGGAGTGACTTTATCCAAATAGTGTTGATATAATAATTTCATTGTATTCATCTTAGTGATTAAGGGGTTACTTGAACCACCGTTCA
Oligonucleotides used to verify the correct integration of the tags		
25	*RET2*-contr-forw	TGGACCAAGAAATGGGAACCTCCA
26	*SEC27*-contr-forw	AAGCTGAAGTTGAGGACAGCGAGT
27	*COP1*-contr-forw	GCTTCAGAGAAGCTATCTACCGTATTA
28	*DSL1*-contr-forw	GATATACAATTATTAGCGAAGGAACCA
29	*DSL3*-contr-forw	GTACATTGAAGCCATCATAGAAAGATT
30	*SEC28*-contr-forw	ACTACTATAGCGTCGAACAAAAAGAAA
31	Dsl3 forw	CACAGCATACGAATTGGGCAAGCA

32	SEC16-F	GAAACGTAAAGATGGCGGCCCAAAGACAAA
33	SEC23-F	AAAGCCGGTTACCAAGACGATCCACAGTAC
34	SEC24-F	CCAGCGCTAGTATTCGATGTGTTTGGAACA
35	*SEC13*-F	CTTAGAAGGTCACAGCGATTGGGTTAGAGA
36	SEC31-F	CCGCTGAGAATGTCAGTCATGAAATTCCAG
37	*DSL3* 3-prime	TAGGCGGCATTGCTAGGGAGATTT
38	*COP1* 3-prime	TCTTCTGGTCCTCGTGCAGAACAA
39	*RER1* upstream	GCCAAGCCCTGCAGAACAATAACA
40	*RER1* RF 5' revers	AACAAACCACCCAATACAGCCCAC
41	TRP1-revers	AATACCCAGCAAGTCAGCATCGGA
42	KanMX-coding-revers	TTAAAAGGACAATTACAAACAGGAATC
Oligonucleotides used to create mutants		
43	mVENUS-forw	CAAGCTGAGCAAAGACCCCAACGAGAAGCG
44	mVENUS-rev	CTTTGCTCAGCTTGGACTGGTAGCTCAGGTAGTGGT
45	VN^{I152L} forw	GTACAACTACAACAGCCACAACGTCTATCTCACCGCCTTAAGCGCGCCAC
46	VN^{I152L} rev	AATCATAAGAAATTCGCTTATTTAGAAGTGGCGCGCCCTA
47	*DSL1*-ADH1-VN-rev	GCATCGGCGGCCGCTGCCGGTAGAGGTGTGGTCAATAAGAGC
48	Dsl1-C-term-VN	GCATCGTCTAGATGATGGTCGACGGATCCCCGGGTTAATTAACAG
49	Dsl1-C-term-VC	GCATCGTCTAGATGATGGTCGACGGATCCCCGGGTTAATTAACCG

51	ΔLASSO-forw	ATGGCGCGCCCCACATTGAAGTTACACAGTTACCAAAATTGTTC
52	ΔLASSO-rev	ATGGGCGCGCCCTCAACTACTTCCAAATTTTGCCAGCTCTTTTT
53	XhoI-CFP	GGAACAAAAGCTGCTCGAGTTCGAGTTTATCATTATCAATACTCG
54	CFP-BglI	CATAGATCTTTTGTTATCTCCTTCGAAGCCTGCTTTTTTGTACAA
55	MYO4 downstr	ACGAGTCCAACGGAACAGAACCTT
56	MYO4 RF 5' revers	AATTTCGCCACAGAGTCCCTAGCA
57	MYO4 RF 3' forw	CTTTCCAGACTTCCCGCATTTGCT
58	*DSL1*^{TAG} ampli5´	ACGCCACCATTGAGAAACTGCTAC
59	*DSL1*^{TAG} ampli3'	TATTGACTGCACCAGAACATCCCG
60	TEF-pro	ATCTGTTTAGCTTGCCTTGTCCCCGCCG
61	TEF-ter	TGGATGGCGGCGTTAGTATCGAAT
62	Dsl1-yEmCFP-5pr	CCCCTGCGAAGGAATGCCATTGATGATATTTACGAAATTAGAGGCACTGCTCTAGATGATggtgacggtgctggttta
63	Dsl1-yEmCFP-3pr	CTTACGCATACGTAATACAAGATGTACATCTATTGCAATGATCATGTAACCTATCCTACGtcgatgaattcgagctcg

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