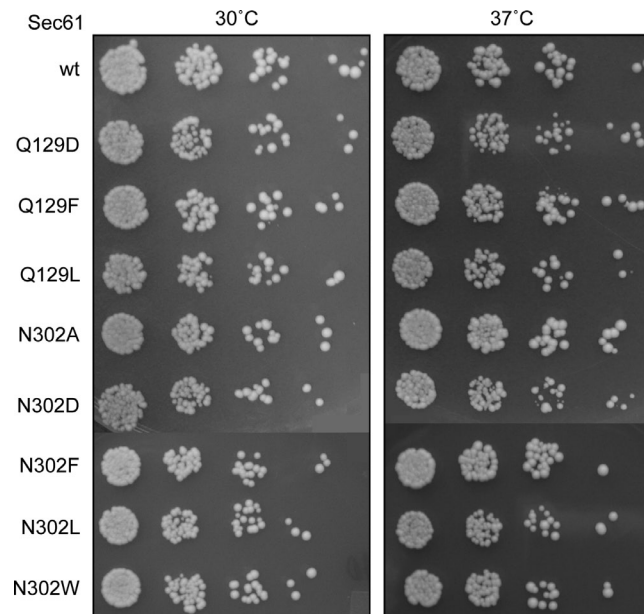Figure S1.   **Growth rates of selected *sec61* lateral gate mutants.** Yeast strains were grown in YPAEG media at 30°C to mid-log phase. After dilution of cells to 0.1 OD at 600 nm, 5-μl aliquots of fivefold serial dilutions were spotted onto YPAD plates that were incubated at 30 or 37°C for 2 d.
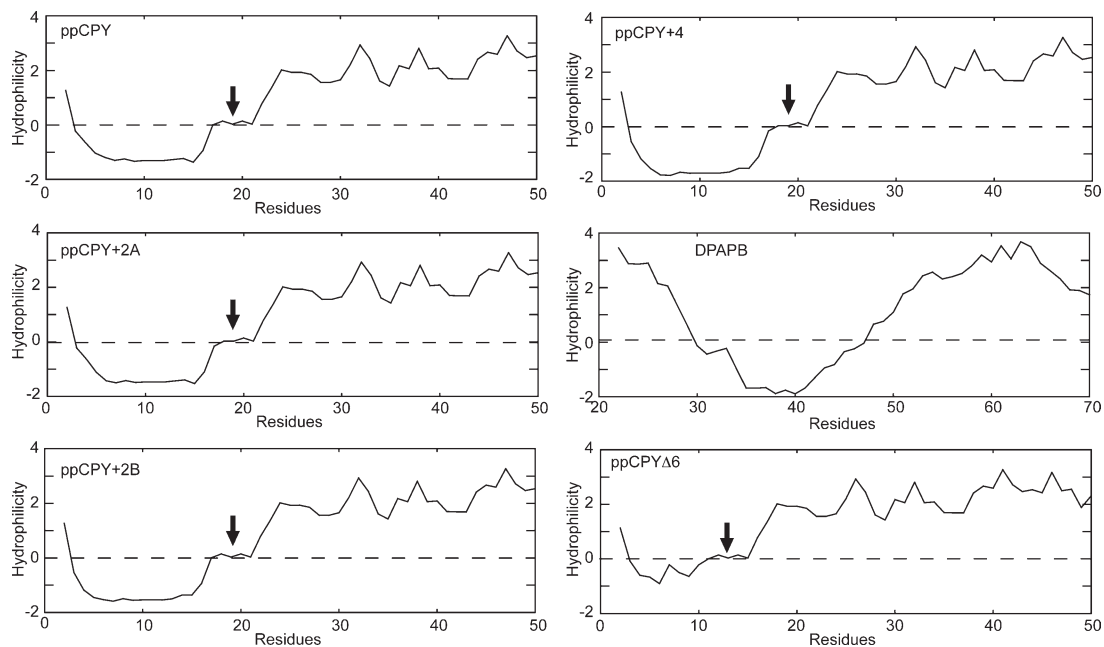

Figure S2.   **Hydropathy plots for CPY, CPY derivatives, and DPAPB.** Hydropathy plots of the first 50 residues of ppCPY+2A, ppCPY+2B, ppCPY+4, and ppCPYΔ6 are compared with hydropathy plots of ppCPY and DPAPB. Hydropathy plots were prepared using MacVector, Inc. software using the Goldman-Engelman-Steitz algorithm. Arrows designate the signal sequence cleavage site for CPY and the predicted signal sequence cleavage sites for the CPY mutants as calculated by SignalP 4.0.
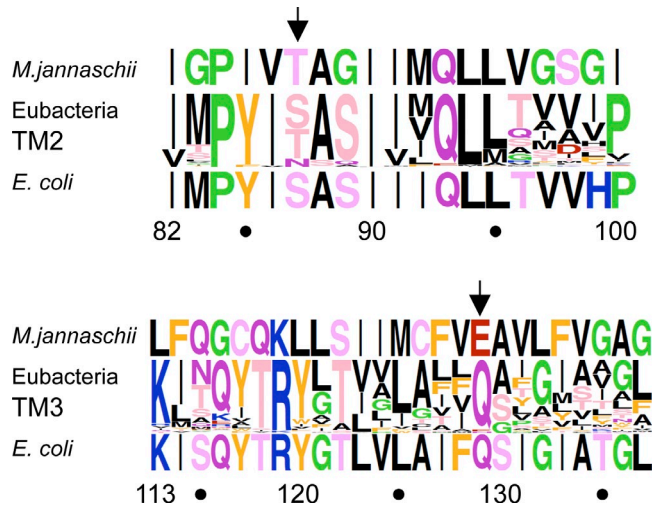
Figure S3. **Conservation of the lateral gate polar cluster.** Sequence logos of eubacterial TM2 and TM3 derived from 1573 SecY sequences. Residues are color coded by side chain property; letter height is proportional to frequency. The *M. jannaschii* and *E. coli* sequences flank the eubacterial logo. Numbers correspond to the *S. cerevisiae* Sec61 sequence. Arrows designate the residues that align with T87 and Q129 of *S. cerevisiae* Sec61.

Table S1. **Translocation assays of the *sec61* apolar patch mutants**

Allele	DPAPB	CPY	CPYΔ2	CPYΔ4	Σp1CPY	Δ Translocation
	%[a]	%	%	%	%[b]	%[c]
Wild type	88 ± 2	82 ± 3	64 ± 5	24 ± 3	170 ± 11	0 ± 11
L63A	84 ± 3	78 ± 4	76 ± 10	71 ± 9	225 ± 23	55 ± 23
L63D	88 ± 2	84 ± 1	90 ± 1	88 ± 1	262 ± 3	92 ± 3
L63N	83 ± 3	82 ± 3	89 ± 1	84 ± 2	255 ± 6	84 ± 6
L63S	87 ± 1	84 ± 1	89 ± 2	87 ± 1	260 ± 4	90 ± 4
L63W	84 ± 6	86 ± 1	71 ± 4	28 ± 3	185 ± 8	15 ± 8
W65A	90 ± 5	88 ± 1	65 ± 2	26 ± 3	179 ± 6	9 ± 6
W65D	86 ± 2	84 ± 1	83 ± 1	62 ± 3	229 ± 5	60 ± 5
W65L	86 ± 2	78 ± 2	67 ± 3	25 ± 2	170 ± 7	0 ± 7
W65N	88 ± 1	84 ± 1	80 ± 1	53 ± 1	217 ± 3	47 ± 3
W65S	86 ± 1	83 ± 1	74 ± 1	39 ± 1	196 ± 3	25 ± 3
W65Y	87 ± 1	85 ± 1	73 ± 3	32 ± 1	190 ± 5	20 ± 5
L66A	85 ± 3	80 ± 1	76 ± 4	57 ± 8	214 ± 15	44 ± 15
L66E	86 ± 1	80 ± 3	79 ± 7	77 ± 5	236 ± 15	66 ± 15
L66F	86 ± 2	76 ± 5	74 ± 1	38 ± 1	187 ± 6	17 ± 6
L66N	87 ± 2	81 ± 3	82 ± 4	65 ± 1	228 ± 8	58 ± 8
L66S	86 ± 4	80 ± 1	80 ± 3	75 ± 4	235 ± 8	65 ± 8
L66W	85 ± 3	80 ± 2	64 ± 6	27 ± 3	171 ± 12	1 ± 12
L66Y	88 ± 4	83 ± 4	78 ± 1	46 ± 7	207 ± 12	37 ± 12

[a]Values for percentage of translocation of DPAPB, CPY, CPYΔ2, and CPYΔ4 are the mean of two or more determinations. Error bars are standard deviations or, when two assays were done, the difference between the mean and an assay value. Errors <1% were rounded up.
[b]The sum of the percentage of translocation (p1CPY) for the CPY, CPYΔ2, and CPYΔ4 reporters.
[c]The value for Δ translocation is obtained by subtracting Σ p1CPY_{wt} from Σ p1CPY_{mt}.

Table S2. **Translocation assays of *sec61* lateral gate polar cluster mutants**

Allele	DPAPB	CPY	CPYΔ2	CPYΔ4	Σp1CPY	Δ Translocation
	%[a]	%	%	%	%[b]	%[c]
Wild type	88 ± 3	82 ± 3	64 ± 5	24 ± 3	170 ± 11	0 ± 11
T87A	88 ± 3	85 ± 3	70 ± 3	31 ± 2	185 ± 8	15 ± 8
T87D	88 ± 4	86 ± 2	86 ± 1	71 ± 3	243 ± 6	73 ± 6
T87I	91 ± 1	84 ± 3	64 ± 8	28 ± 6	176 ± 17	6 ± 17
T87F	87 ± 6	80 ± 5	41 ± 3	17 ± 3	138 ± 11	−33 ± 11
T87V	8 ± 3	82 ± 5	67 ± 2	19 ± 3	168 ± 10	−3 ± 10
T87Y	94 ± 2	87 ± 1	80 ± 4	46 ± 6	213 ± 11	43 ± 11
Q129A	87 ± 7	75 ± 3	25 ± 10	16 ± 4	117 ± 17	−53 ± 17
Q129D	85 ± 5	82 ± 2	81 ± 5	72 ± 2	236 ± 9	65 ± 9
Q129F	92 ± 1	58 ± 3	22 ± 6	14 ± 4	94 ± 13	−76 ± 13
Q129H	85 ± 4	80 ± 5	73 ± 7	42 ± 9	194 ± 21	24 ± 21
Q129L	93 ± 3	46 ± 15	11 ± 2	6 ± 1	63 ± 18	−107 ± 18
Q129M	89 ± 2	77 ± 5	48 ± 7	20 ± 7	145 ± 19	−25 ± 19
Q129N	84 ± 1	71 ± 9	82 ± 2	60 ± 2	213 ± 13	43 ± 13
Q129W	82 ± 5	77 ± 5	69 ± 3	49 ± 2	194 ± 7	24 ± 7
Q129Y	84 ± 5	72 ± 6	30 ± 2	16 ± 6	118 ± 14	−52 ± 14
N302A	87 ± 3	70 ± 5	20 ± 2	7 ± 1	97 ± 8	−73 ± 8
N302D	90 ± 1	85 ± 2	83 ± 4	78 ± 1	246 ± 7	76 ± 7
N302E	89 ± 1	83 ± 2	76 ± 1	41 ± 1	200 ± 4	30 ± 4
N302F	88 ± 2	61 ± 1	15 ± 1	9 ± 1	85 ± 3	−85 ± 3
N302L	90 ± 1	41 ± 3	13 ± 2	10 ± 2	64 ± 7	−106 ± 7
N302Q	88 ± 1	79 ± 2	82 ± 2	35 ± 1	196 ± 5	26 ± 5
N302W	91 ± 1	77 ± 2	43 ± 8	19 ± 7	139 ± 17	−31 ± 17
N302Y	88 ± 1	87 ± 2	52 ± 4	24 ± 2	163 ± 8	−7 ± 8
T136D	88 ± 3	77 ± 5	76 ± 5	63 ± 1	215 ± 11	45 ± 11
T136F	88 ± 1	60 ± 4	16 ± 2	11 ± 2	87 ± 8	−83 ± 8
T136L	89 ± 1	80 ± 1	37 ± 1	13 ± 1	129 ± 3	−41 ± 3
T136N	87 ± 1	82 ± 1	82 ± 2	63 ± 1	226 ± 4	56 ± 4
Q308D	90 ± 1	83 ± 3	39 ± 3	17 ± 1	138 ± 7	−33 ± 7
Q308F	87 ± 1	76 ± 4	42 ± 2	16 ± 2	133 ± 8	−37 ± 8
Q308L	89 ± 1	82 ± 4	67 ± 1	24 ± 2	173 ± 7	2 ± 7
Q308N	86 ± 1	79 ± 3	46 ± 1	24 ± 4	148 ± 8	−23 ± 8

[a]Values for percentage of translocation of DPAPB, CPY, CPYΔ2, and CPYΔ4 are the means of two or more determinations. Error bars are standard deviations or, when two assays were done, the difference between the mean and an assay value. Errors <1% were rounded up.
[b]The sum of the percentage of translocation (p1CPY) for the CPY, CPYΔ2, and CPYΔ4 reporters.
[c]The value for Δ translocation is obtained by subtracting Σ p1CPY$_{wt}$ from Σ p1CPY$_{mt}$.

Table S3. **Translocation assays of *sec61* double mutants**

Allele	DPAPB	CPY	CPYΔ2	CPYΔ4	Σp1CPY	Δ Translocation
	%[a]	%	%	%	%[b]	%[c]
Wild type	88 ± 3	82 ± 3	64 ± 5	24 ± 3	170 ± 6	0 ± 6
Q129L-N302L	87 ± 1	44 ± 1	13 ± 2	7 ± 1	64 ± 4	−107 ± 4
L66S-Q129L	90 ± 1	81 ± 4	69 ± 1	34 ± 5	184 ± 10	13 ± 10
L66N-Q129L	91 ± 1	84 ± 1	86 ± 1	78 ± 1	248 ± 3	76 ± 3
L66N-N302L	91 ± 1	84 ± 1	83 ± 1	62 ± 1	229 ± 3	58 ± 3
Q129E-N302D	90 ± 1	83 ± 1	63 ± 3	25 ± 1	171 ± 5	0 ± 5
Q129N-N302D	89 ± 1	85 ± 3	81 ± 2	83 ± 6	249 ± 11	78 ± 11
L63N-Q129D	90 ± 1	81 ± 1	88 ± 2	83 ± 1	252 ± 4	81 ± 4
Q129D-T136N	85 ± 1	76 ± 3	80 ± 1	72 ± 1	228 ± 5	57 ± 5
L66N-N302D	91 ± 1	84 ± 1	87 ± 1	89 ± 1	260 ± 3	90 ± 3

[a]Values for percentage of translocation of DPAPB, CPY, CPYΔ2, and CPYΔ4 are the means of two or more determinations. Error bars are standard deviations or, when two assays were done, the difference between the mean and an assay value. Errors <1% were rounded up.
[b]The sum of the percentage of translocation (p1CPY) for the CPY, CPYΔ2, and CPYΔ4 reporters.
[c]The value for Δ translocation is obtained by subtracting Σ p1CPY$_{wt}$ from Σ p1CPY$_{mt}$.