

Supplementary data for assessment by the referees.

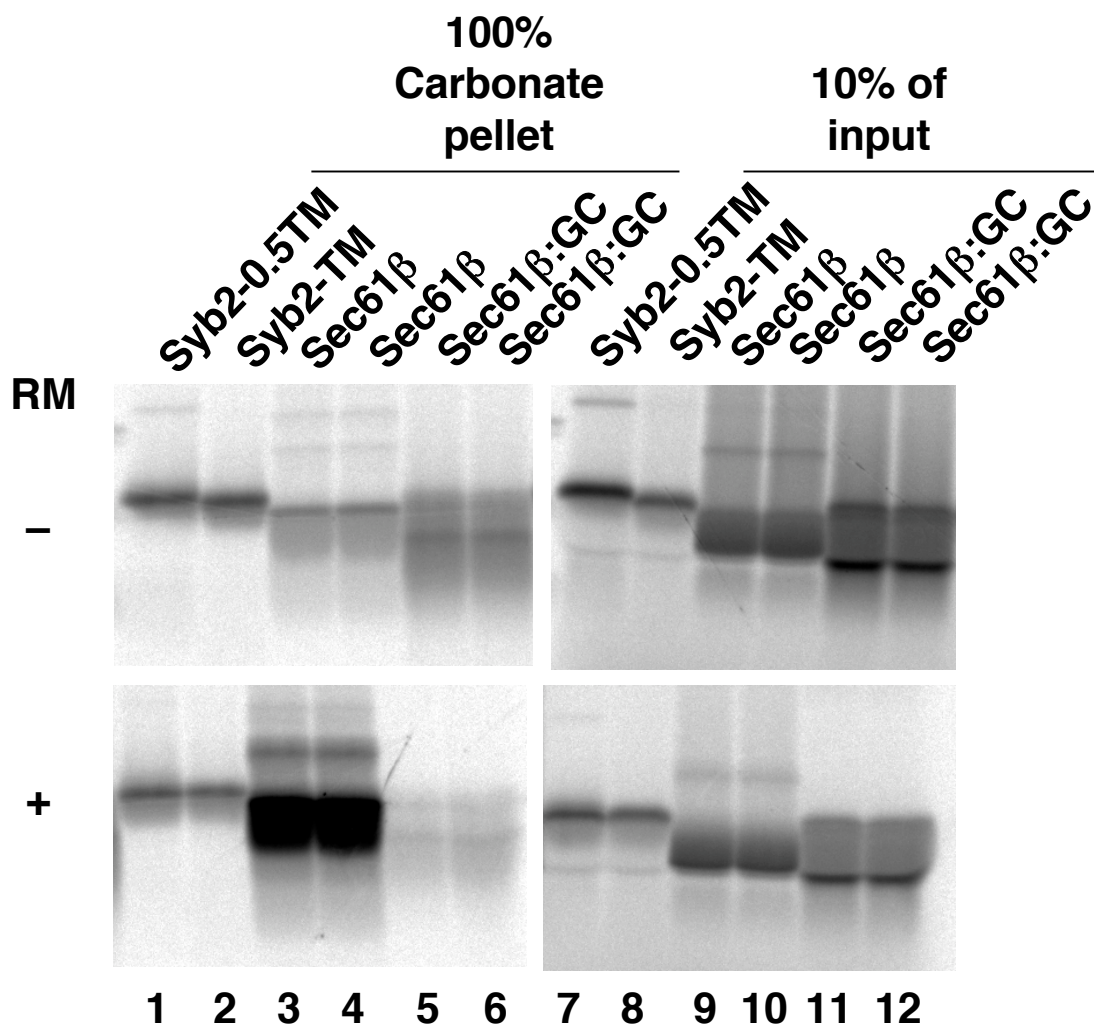
Supplementary Figure 1: Calculating the efficiency of TA protein membrane integration.

(A) Stop codon minus RNA encoding Syb2-0.5TM, Syb2-TM, Sec61 β and Sec61 β :GC as indicated was translated for 20 min in the presence (+) or absence (-) of rough microsomes (RM), and then incubated with puromycin for a further 20 min. A fraction of the total translation products was removed, and the membranes were then recovered by sequential centrifugation through a high salt sucrose cushion and then from an alkaline sodium carbonate solution. The resulting samples equivalent to 10% of the total protein synthesised (input) and 100% of the protein recovered in the final pellet fraction were then separated by SDS-PAGE.

(B) The efficiency of membrane integration for each precursor was assessed by quantifying the amount of each protein that was recovered in the pellet fraction from the incubation containing rough microsomes (Carb pel (RM+)), and this was then expressed as a proportion of the total amount of protein synthesised in that reaction (Pel/Tot (RM+)). This figure was then corrected for any background by subtracting the value obtained when the same calculation was performed using samples prepared in the absence of rough microsomes (Pel/Tot (RM-)). The final values for percentage RM integration indicate the amount of each precursor that was specifically recovered in the membrane fraction. The values shown in Figure 1D are the mean of two or more independent experiments, and standard errors are indicated. Similar experiments were performed for all of the precursors studied to generate the data shown in Figure 1D.

Supplementary Figure 1

A



B

Construct	Name	Carb pel (RM-)	10% Tot (RM-)	Pel/Tot (RM-)	Carb pel (RM+)	10% Tot (RM+)	Pel/Tot (RM+)	rel RM integ	% RM integ
1	Syb2-0.5TM	6694	11305	0.059	5818	6710	0.087	0.027	2.7
2	Syb2-TM	6446	5858	0.110	5435	4852	0.112	0.002	0.2
3	Sec61beta	3205	12390	0.026	61580	11654	0.528	0.503	50.3
4	Sec61beta	3408	15558	0.022	59392	12378	0.480	0.458	45.8
5	Sec61beta:GC	5181	19759	0.026	1251	8887	0.014	-0.012	-1.2
6	Sec61beta:GC	4735	15793	0.030	1277	8216	0.016	-0.014	-1.4