SUPPLEMENTAL MATERIAL

for Müller et al.

Mass determination by light scattering analysis

Ribosomes, ribosomal subunits, and Sec62N were characterized by asymmetric-flow field-flow fractionation (Eclipse 2, Wyatt Technology) and online multi-angle light scattering analysis (miniDAWN Tristar and QELS, Wyatt Technology), 280 nm UV absorbance- (Agilent 1100 series), and refractive index-measurements (Shodex RI-101). Samples were applied by autosampler (Agilent 1100 series). Ribosomes were fractionated in 20 mM HEPES-KOH, pH 7.6, 50 mM KCl, 2 mM $MgCl_2$. Ribosomal subunits were fractionated in 20 mM HEPES-KOH, pH 7.6, 1 M KCl, 2 mM $MgCl_2$, 1 mM DTT. Sec62N was fractionated in 50 mM Tris-HCl, pH 8, 300 mM NaCl. The data were analyzed with ASTRA software version 5.3.2.16 (Wyatt Technology) and a value for *dn*/*dc* for protein of 0.185 ml/g. Molecular mass and hydrodynamic radius of the purified 80S ribosomes were determined as 3.62 $\pm$ 0.15 MDa and 18.25 nm $\pm$ 1.71, of 60S as subunits 2.5 $\pm$ 0.03 MDa and 14.45 $\pm$ 0.36 nm, and of 40S subunits as 1.4 $\pm$ 0.01 MDa and 7.54 $\pm$ 0.43 nm; molecular mass of purified Sec62N was determined as 35 kDa (Supplemental Figures 1 and 2).

Interaction of human Sec62 and Sec63 involves the carboxyterminus of Sec63 and the central region of Sec62N

Peptide spots that corresponded to Sec62 and Sec63C, respectively, were synthesized on cellulose membranes according to established procedure (Frank, 1992). Subsequently, the membranes were incubated with 14C-labeled Sec63C, Sec63C26, or Sec62N under the conditions of the pull down assay. The bound proteins were visualized by phosphorimaging and plotted against the respective protein sequence (Supplemental Figures 3 and 4). Upon incubation of the Sec62 membrane with Sec63C or Sec63C26, a highly polar and overall

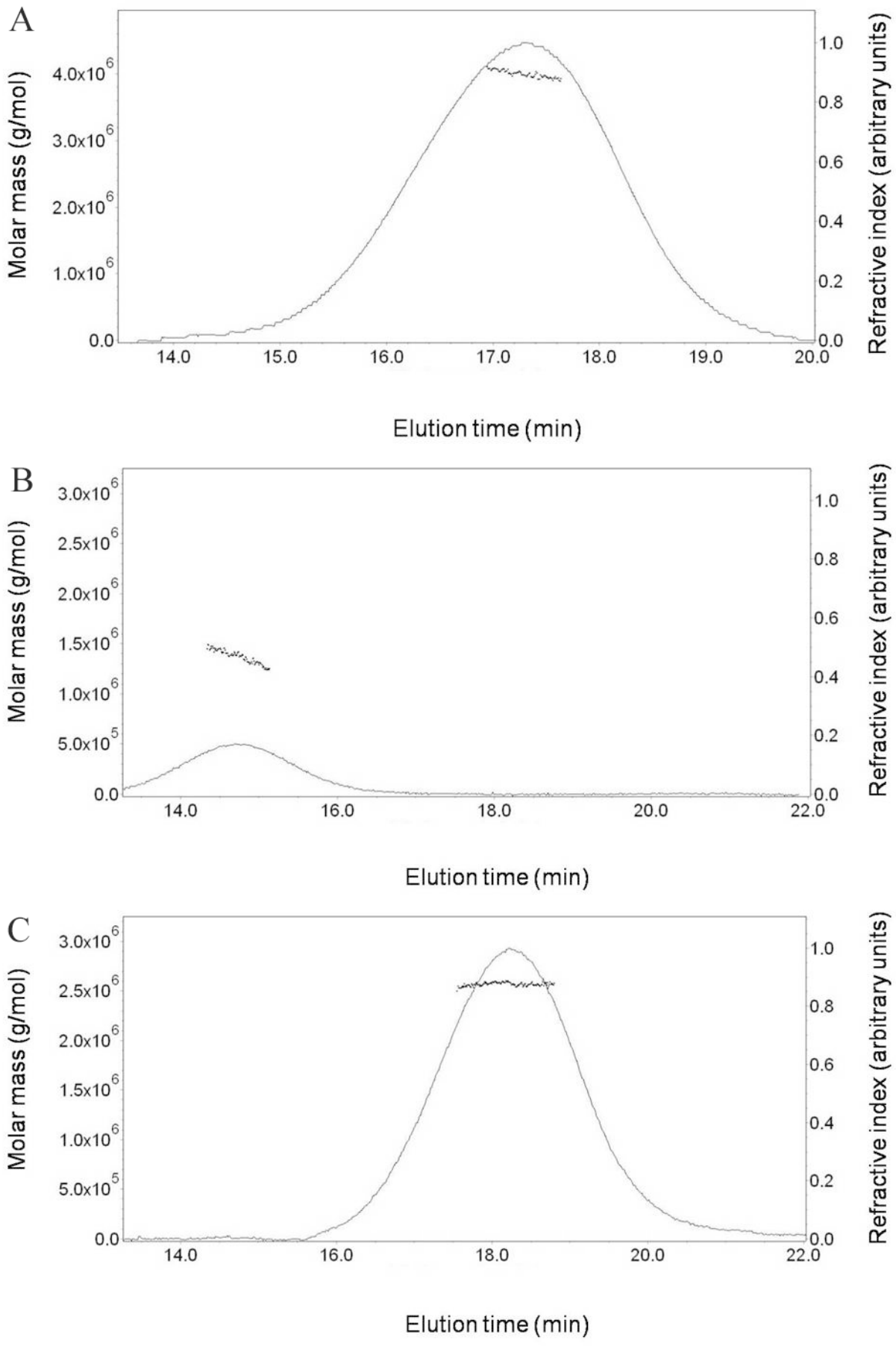
positively charged section within Sec62N was identified as dominant interaction site (amino acid residues 96-145). We note that the aminoterminal oligopeptide, involved in ribosome binding of Sec62N, did not significantly interact with Sec63C or Sec63C26. When the Sec63C membrane was probed with Sec62N, the carboxyterminal region of Sec63C was identified as the predominant binding site. Thus these results confirmed the pull down and peptide competition experiments that are shown in Figure 2.

Mammalian Sec62 inhibits translation in the presence of microsomes

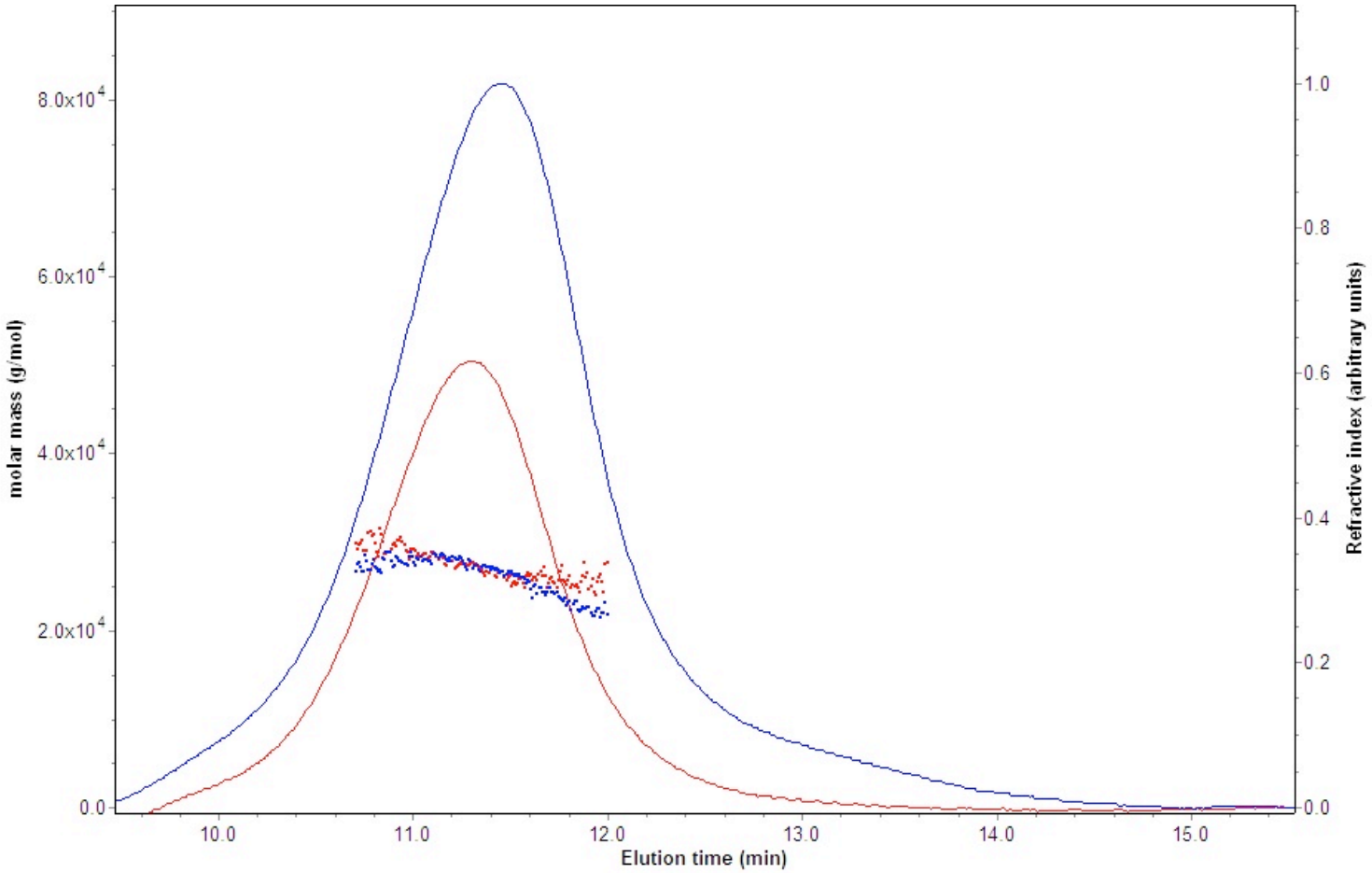
Sec62N was also tested for its ability to inhibit synthesis of firefly luciferase or bovine preprolactin in the presence of canine pancreatic microsomes (Supplemental Figure 5, A and B). In this assay, Sec62N was active in inhibiting protein synthesis, too. We note that preprolactin was not affected by Sec62N with respect to its transport into microsomes.

Sequence comparison

The sequences of canine Sec62, Se63, and ERj1, respectively, were used for BLAST database searches. The identified orthologs in *Homo sapiens, Mus musculus, Gallus gallus, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Trypanosoma brucei, Saccharomyces cerevisiae, Arabidopsis thaliana, and Oryza sativa* are shown in Supplemental Figures 6 through 8.

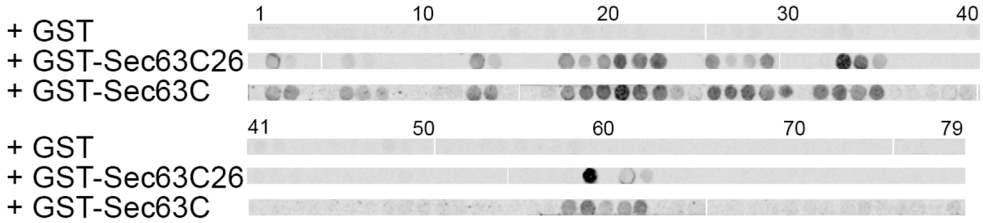

A
Molar mass (g/mol)
4.0x10^6
3.0x10^6
2.0x10^6
1.0x10^6
0.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
Refractive index (arbitrary units)
1.0
0.8
0.6
0.4
0.2
0.0
Elution time (min)
B
Molar mass (g/mol)
3.0x10^6
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
14.0
16.0
18.0
20.0
22.0
Refractive index (arbitrary units)
1.0
0.8
0.6
0.4
0.2
0.0
Elution time (min)
C
Molar mass (g/mol)
3.0x10^6
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
14.0
16.0
18.0
20.0
22.0
Refractive index (arbitrary units)
1.0
0.8
0.6
0.4
0.2
0.0
Elution time (min)

Supplemental Figure 1. Molecular mass of canine ribosomes and their subunits. Ribosomes were purified from canine pancreas and washed with high salt and puromycin. Ribosomal subunits were prepared by sucrose gradient centrifugation. Ribosomes (270 μg, A), 40S-subunits (36 μg, B), and 60S-subunits (169 μg, C) were fractionated in 20 mM HEPES-KOH, pH 7.6, 50 mM KCl, 2 mM $MgCl_2$ (80S) and 20 mM HEPES-KOH, pH 7.6, 1 M KCl, 2 mM $MgCl_2$, 5 mM DTT (60S, 40S), respectively, on a cellulose membrane (cut off: 5 kDa, spacer 350 μm) with a channel flow of 1 ml/min and a cross flow of 1 ml/min. The continuous trace refers to the refractive index. In the peak area, molar mass was calculated for each individual data set from the multi-angle light scattering monitor. We note that a horizontal line for the molar mass values indicates that a homogeneous/monodispers sample was analyzed.

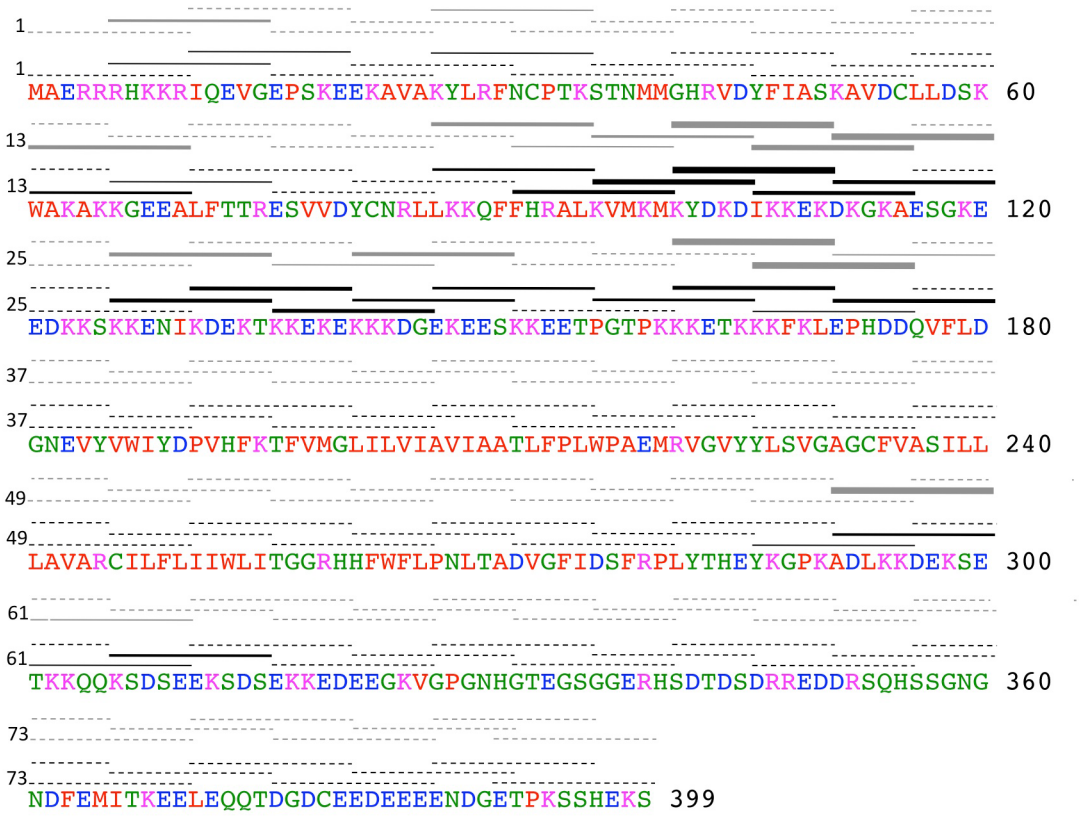

molar mass (g/mol)
8.0x10^4
6.0x10^4
4.0x10^4
2.0x10^4
0.0
Refractive index (arbitrary units)
1.0
0.8
0.6
0.4
0.2
0.0
10.0
11.0
12.0
13.0
14.0
15.0
Elution time (min)

Supplemental Figure 2. Molecular mass of purified human Sec62N. Sec62N was purified from E. coli cells. Sec62N (10 and 20 μg, respectively) was fractionated in 50 mM Tris-HCl, pH 8, 300 mM NaCl on a cellulose membrane (cut off: 5 kDa, spacer 350 μm) with a channel flow of 1 ml/min and a cross flow of 3 ml/min. The two analyses were compiled in a single graph and are differentiated by colours (10 μg, red; 20 μg, blue). The continuous traces refer to the refractive index. In the peak areas, molar masses were calculated for each individual data set from the multi-angle light scattering monitor. We note that a horizontal line for the molar mass values indicates that a homogeneous/monodispers sample was analyzed.

A)

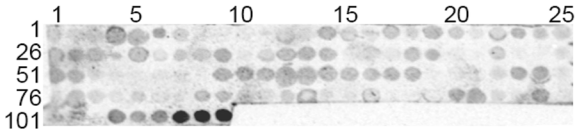

1
10
20
30
40
+ GST
+ GST-Sec63C26
+ GST-Sec63C
41
50
60
70
79
+ GST
+ GST-Sec63C26
+ GST-Sec63C

B)

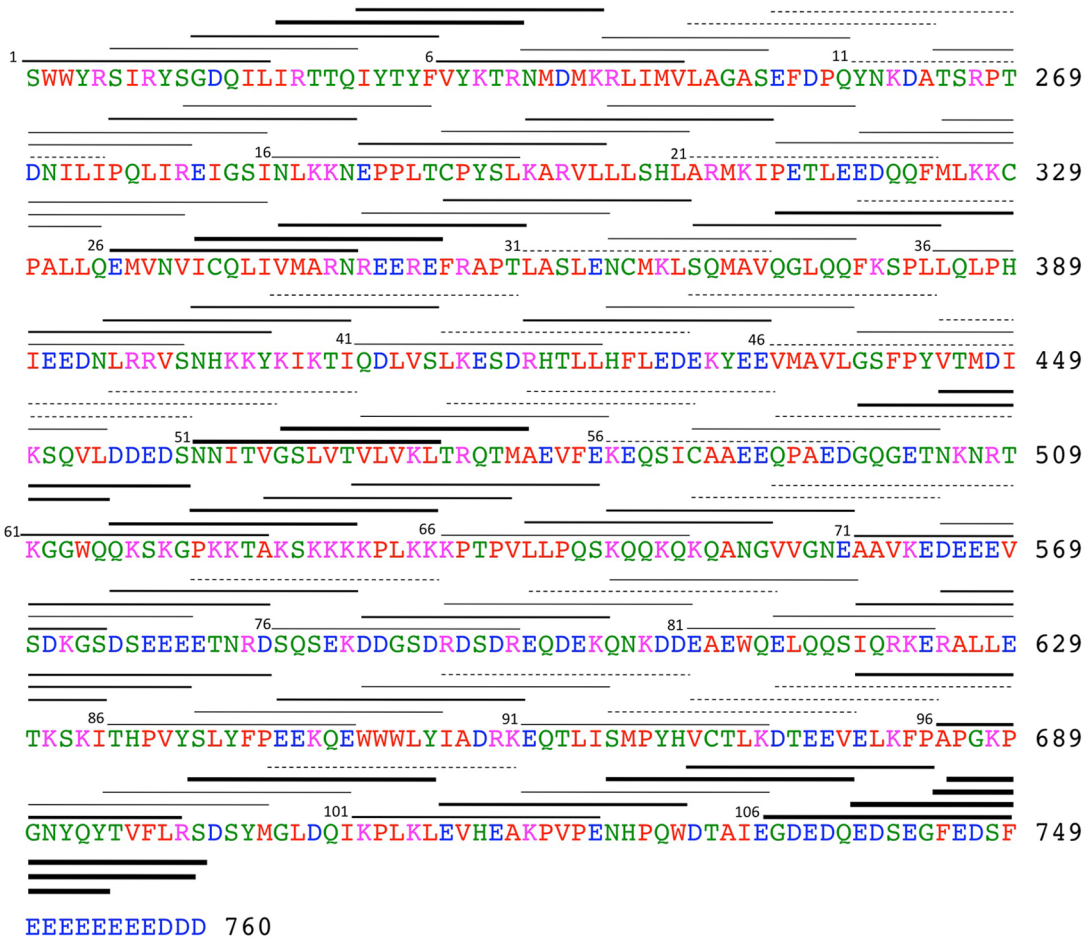

1
1
MAERRRHKKRIQEVGEPSKEEKAVAKYLRFNCPTKSTNMMGHRVDYFIASKAVDCLLDSK 60
13
13
WAKAKKGEEALFTTRESVVDYCNRLLKKQFFHRALKVMKMKYDKDIKKEKDKGKAESGKE 120
25
25
EDKKSKKENIKDEKTKKEKEKKKDGEKEESKKEETPGTPKKKETKKKFKLEPHDDQVFLD 180
37
37
GNEVYVWIYDPVHFKTFVMGLILVIAVIAATLFPLWPAEMRVGVYYLSVGAGCFVASILL 240
49
49
LAVARCILFLIIWLITGGRHHFWFLPNLTADVGFIDSFRPLYTHEYKGPKADLKKDEKSE 300
61
61
TKKQQKSDSEEKSDSEKKEDEEGKVGPGNHGTEGSGGERHSDTDSDRREDDRSQHSSGNG 360
73
73
NDFEMITKEELEQQTDGDCEEDEEEENDGETPKSSHEKS 399

Supplemental Figure 3. Interaction of human Sec62 and Sec63 involves the central region of Sec62N. Peptide spots (10-mers, overlapping by 5 amino acid residues) that corresponded to Sec62 were synthesized on cellulose membranes according to established procedure (Frank, 1992). Subsequently, the membranes were incubated with 14C-labeled GST, GST-Sec63C, or GST-Sec63C26. The bound proteins were visualized by phosphorimaging (A) and plotted against the Sec62 sequence (B). The lines refer to spots (grey, GST-Sec63C26; black, GST-Sec63C). The line thickness refers to signal intensity, the clolours of amino acid residues refer to polarity. Small numbers indicate the respective peptide spot, large numbers indicate the amino acid residue in the sequence (B).

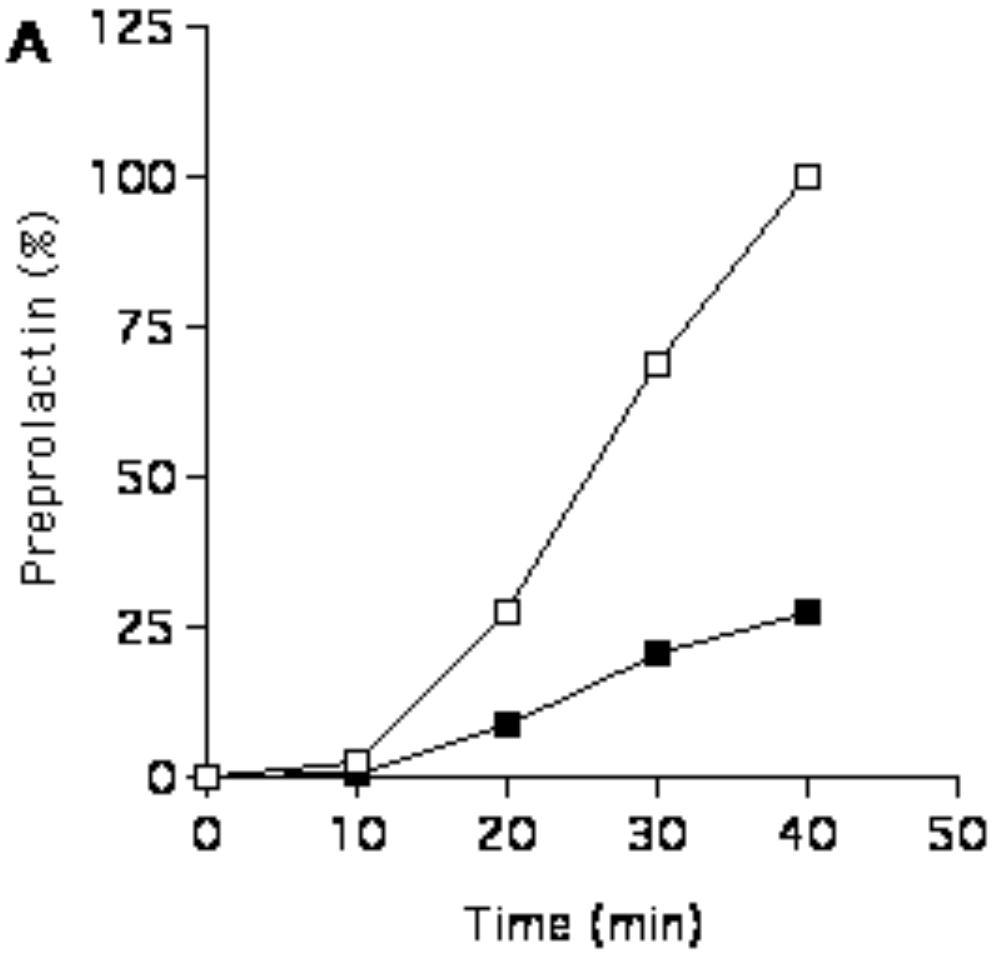
A)

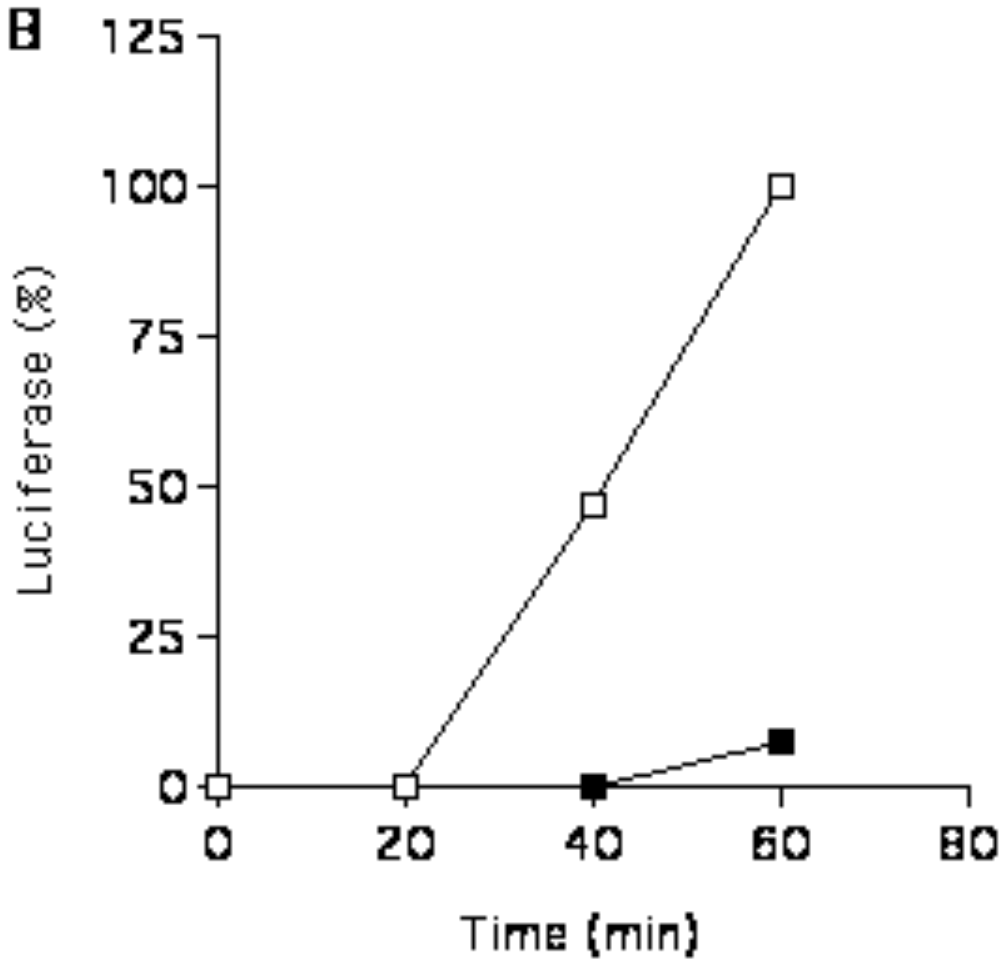

1 5 10 15 20 25
1
26
51
76
101

B)

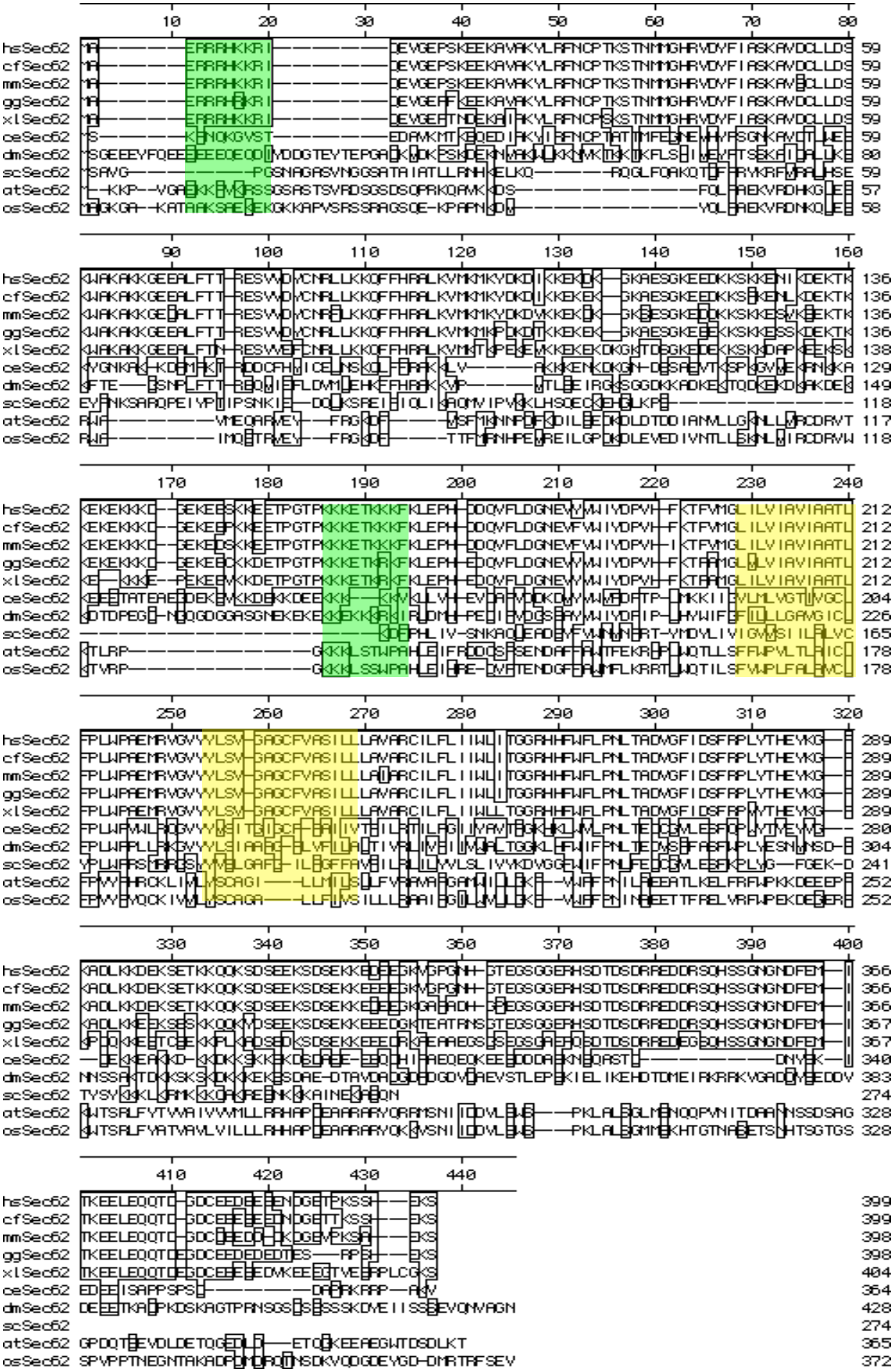

SWWYRSIRYSGDQILIRTTQIYTYFVYKTRNMDMKRLIMVLAGASEFDPQYNKDATSRPT 269
DNILIPQLIREIGSINLKKNEPPLTCPYSLKARVLLLSHLARMKIPETLEEDQQFMLKKC 329
PALLQEMVNVICQLIVMARNREEREFRAPTLASLENCMKLSQMAVQGLQQFKSPLLQLPH 389
IEEDNLRRVSNHKKYKIKTIQDLVSLKESDRHTLLHFLEDEKYEEVMAVLGSFPYVTMDI 449
KSQVLDDEDSNNITVGSLVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRT 509
KGGWQQKSKGPKKTAKSKKKKPLKKKPTPVLLPQSKQQKQKQANGVVGNEAAVKEDEEEV 569
SDKGSDSEEEETNRDSQSEKDDGSDRDSDREQDEKQNKDDEAEWQELQQSIQRKERALLE 629
TKSKITHPVYSLYFPEEKQEWWWLYIADRKEQTLISMPYHVCTLKDTEEVELKFPAPGKP 689
GNYQYTVFLRSDSYMGLDQIKPLKLEVHEAKPVPENHPQWDTAIEGDEDQEDSEGFEDSF 749
EEEEEEEEDDD 760

Supplemental Figure 4. Interaction of human Sec62 and Sec63 involves the carboxyterminus of Sec63**.** Peptide spots (15-mers, overlapping by 10 amino acid residues) that corresponded to Sec63C were synthesized on cellulose membranes according to established procedure (Frank, 1992). Subsequently, the membranes were incubated with 14C-labeled Sec62N. The bound proteins were visualized by phosphorimaging (A) and plotted against the Sec63C sequence (B). The lines refer to spots, the clolours of amino acid residues refer to polarity. The line thickness refers to signal intensity. Small numbers indicate the respective peptide spot, large numbers indicate the amino acid residue in the sequence (B).

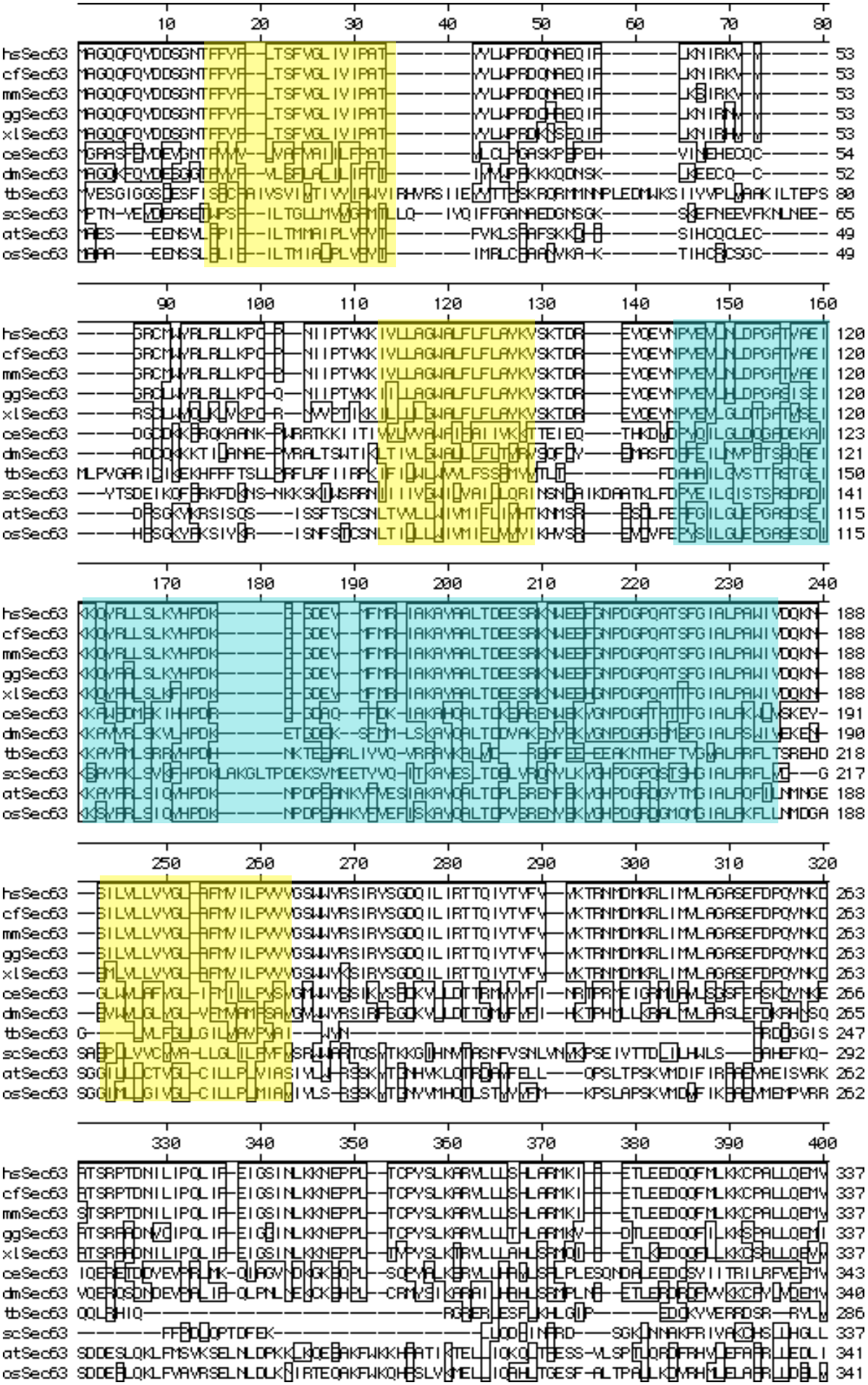

A
125
100
75
50
25
0
Preprolactin (%)
0
10
20
30
40
50
Time (min)


B
125
100
75
50
25
0
Luciferase (%)
0
20
40
60
80
Time (min)

Supplemental Figure 5. Mammalian Sec62 inhibits translation in the presence of microsomes. Preprolactin (A) or luciferase (B) were synthesized in reticulocyte lysate in the presence of [^{35}S]methionine and canine pancreatic microsomes. The translation reactions were supplemented with buffer (open squares) or recombinant protein (filled squares; 1 μM) in the same buffer. After the indicated incubation times, aliquots of the complete translation reactions were subjected to SDS-PAGE and phosphorimaging. The amount of preprolactin or luciferase that was present at the end of the buffer control reaction was set to 100 percent.



Supplemental Figure 6. Sequence alignment for Sec62 proteins. The alignment was created with the MegAlign option of the DNASTAR sequence analysis software, version 7.1.1 (Clustal V, PAM 250). The most conserved regions were boxed. The basic oligopeptides (green shading) and the predicted transmembrane domains (yellow shading) are indicated. The sequences were taken from the following accession numbers: *Homo sapiens* (AAB51391), *Canis lupus familiaris* (XP_851757), *Mus musculus* (AAH67202), *Gallus gallus* (NP_001012620), *Xenopus laevis* (NP_001086825), *Caenorhabditis elegans* (NP_495908), *Drosophila melanogaster* isoform B (AAO39524), *Saccharomyces cerevisiae* (CAB56541), *Arabidopsis thaliana* (NP_001030734), *and Oryza sativa* (EEE56884). We note that there is no Sec62 ortholog in *Trypanosoma brucei* (also see reference 10). Furthermore, we note that there also is a isoform A in *Drosophila melanogaster* that differs from isoform B (AAO39524) in the amino terminal peptide (MSEKKRARRRKD instead of MSGEEEYFGE EEEEEGEGDIYDDGT).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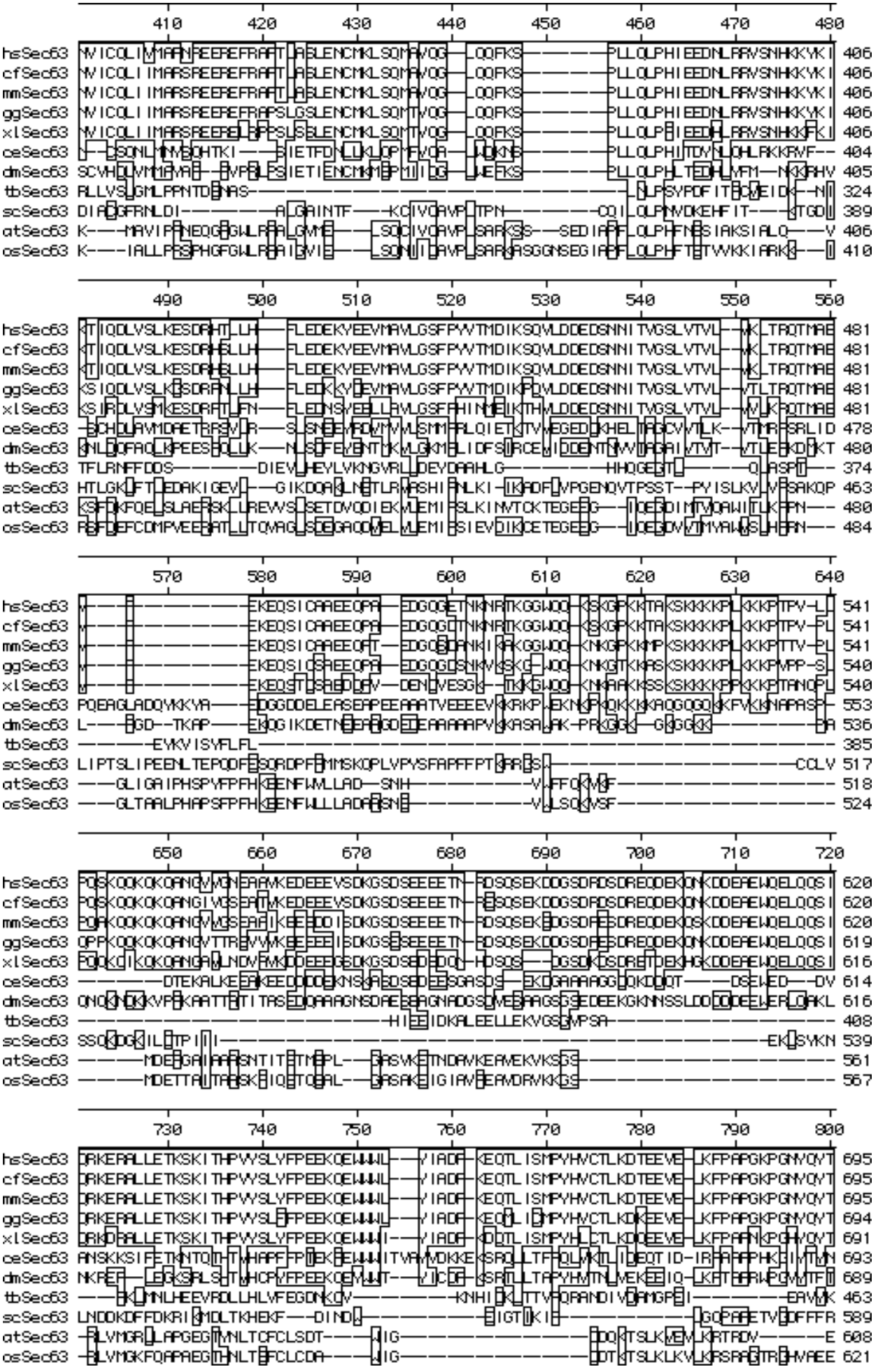
mmSec63
ggSec63
xlSec63
ceSec63
dmSec63
tbSec63
scSec63
atSec63
osSec63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cfSec63 406
mmSec63 406
ggSec63 406
xlSec63 406
ceSec63 404
dmSec63 405
tbSec63 324
scSec63 389
atSec63 406
osSec63 410
490 500 510 520 530 540 550 560
hsSec63 481
cfSec63 481
mmSec63 481
ggSec63 481
xlSec63 481
ceSec63 478
dmSec63 480
tbSec63 374
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atSec63 480
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mmSec63 541
ggSec63 540
xlSec63 540
ceSec63 553
dmSec63 536
tbSec63 385
scSec63 517
atSec63 518
osSec63 524
650 660 670 680 690 700 710 720
hsSec63 620
cfSec63 620
mmSec63 620
ggSec63 619
xlSec63 616
ceSec63 614
dmSec63 616
tbSec63 408
scSec63 539
atSec63 561
osSec63 567
730 740 750 760 770 780 790 800
hsSec63 695
cfSec63 695
mmSec63 695
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xlSec63 691
ceSec63 693
dmSec63 689
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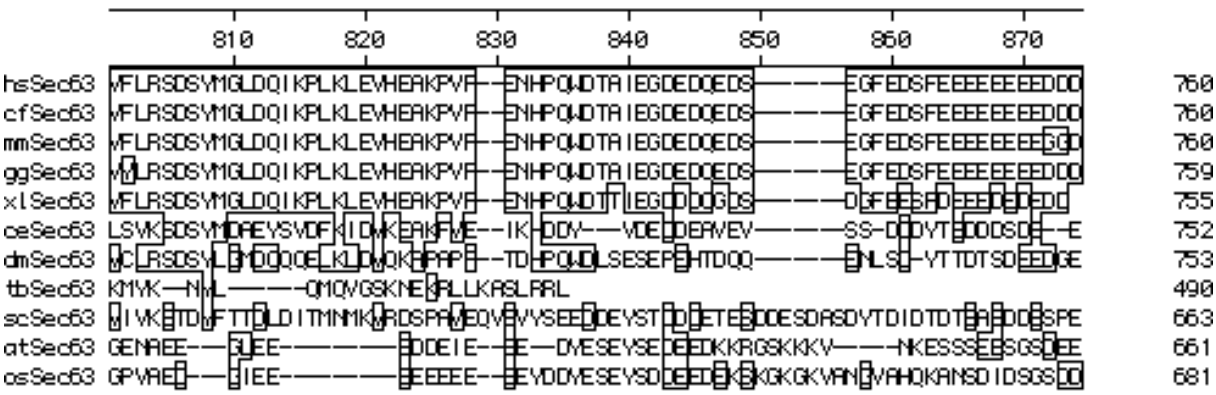
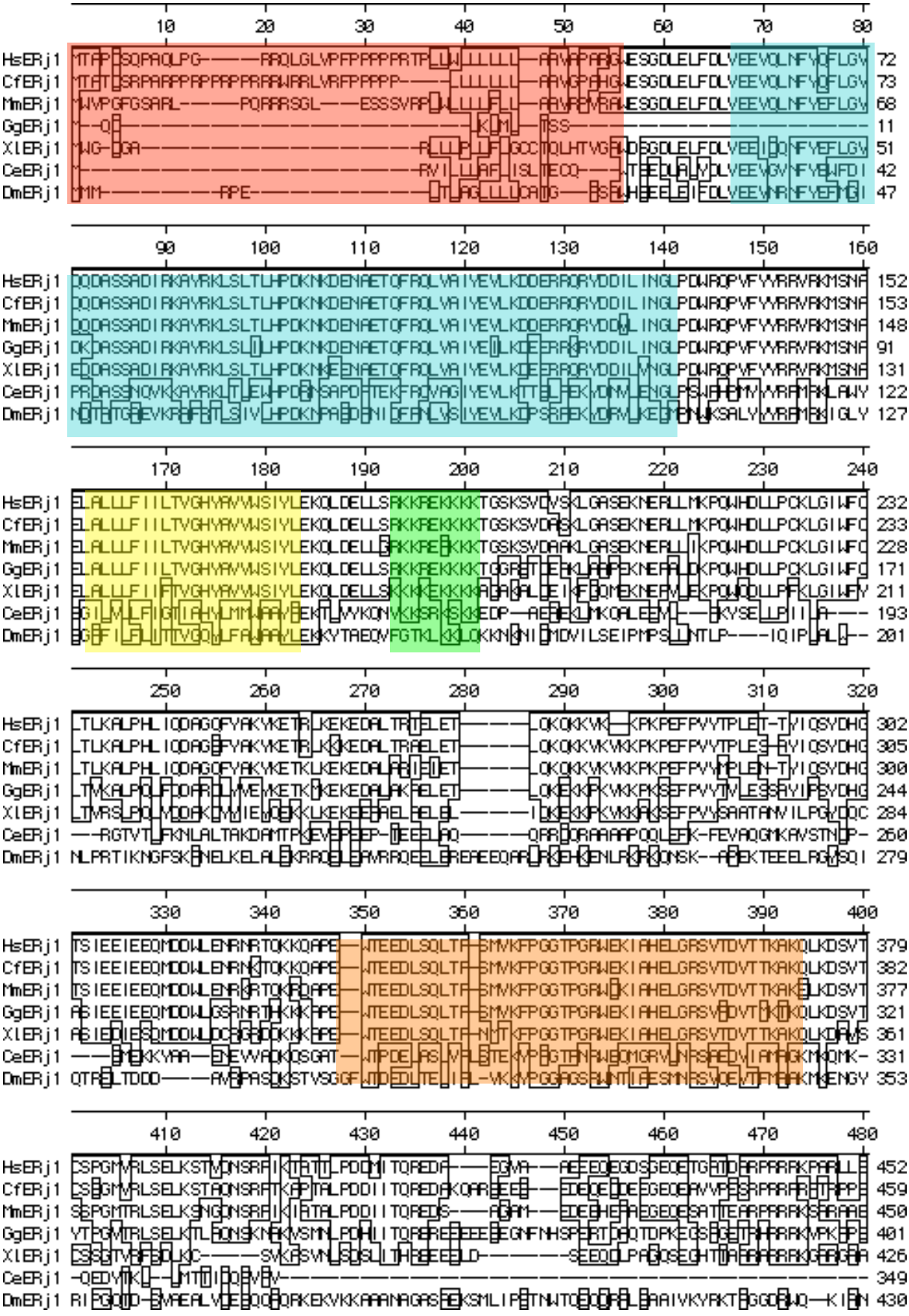
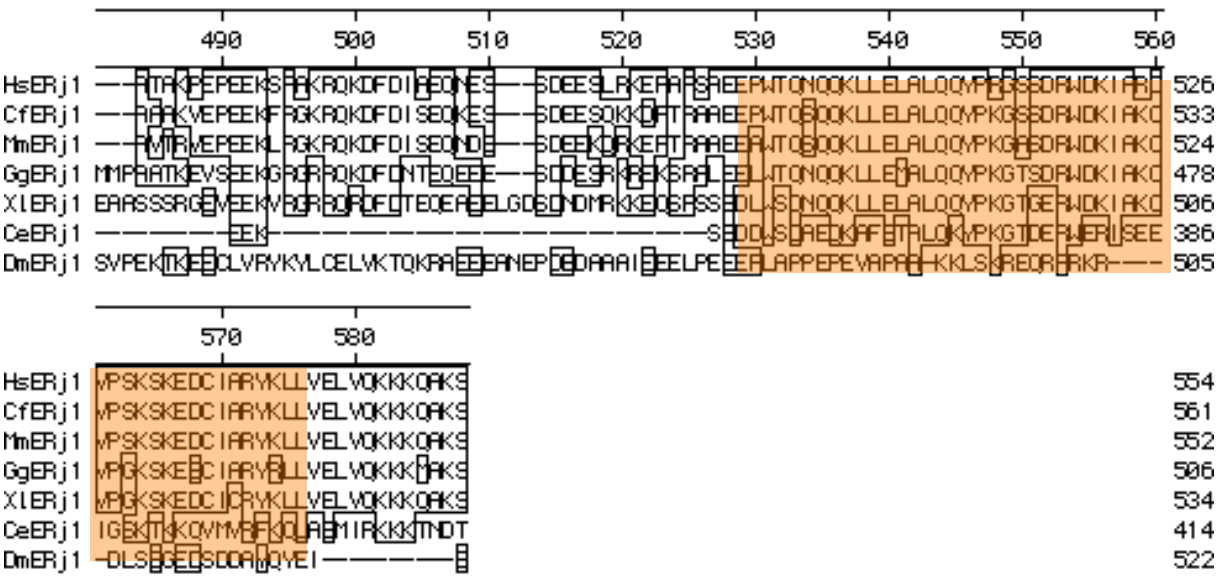
scSec63 589
atSec63 608
osSec63 621


810
820
830
840
850
860
870
hsSec63
cfSec63
mmSec63
ggSec63
xlSec63
ceSec63
dmSec63
tbSec63
scSec63
atSec63
osSec63
760
760
760
759
755
752
753
490
663
661
681

Supplemental Figure 7. Sequence alignment for Sec63 proteins. The alignment was created with the MegAlign option of the DNASTAR sequence analysis software, version 7.1.1 (Clustal V, PAM 250). The most conserved regions were boxed. The J-domain (blue shading) and the predicted transmembrane domains (yellow shading) are indicated. The sequences were taken from the following accession numbers: *Homo sapiens* (AAC83375), *Canis lupus familiaris* (XM_532252), *Mus musculus* (AAK00580), *Gallus gallus* (XP_419802), *Xenopus laevis* (AAI10928), *Caenorhabditis elegans* (NM_061062), *Drosophila melanogaster* (AAF50571), *Trypanosoma brucei* (XM_822206), *Saccharomyces cerevisiae* (CAA99476), *Arabidopsis thaliana* (NP_001117623), *and Oryza sativa* (NM_001058956).




490
500
510
520
530
540
550
560
HsERj1
CfERj1
MmERj1
GgERj1
XlERj1
CeERj1
DmERj1
526
533
524
478
506
386
505
570
580
554
561
552
506
534
414
522

Supplemental Figure 8. Sequence alignment for ERj1/DnaJC1 proteins. The alignment was created with the MegAlign option of the DNASTAR sequence analysis software, version 7.1.1 (Clustal V, PAM 250). The most conserved regions were boxed. The signal peptide (red shading), the J-domain (blue shading), the predicted transmembrane domain (yellow shading), the basic oligopeptide (green shading), and the predicted Myb-domains (orange shading) are indicated. The sequences were taken from the following accession numbers: *Homo sapiens* (NP_071760), *Canis lupus familiaris* (XM_844389), *Mus musculus* (NP_031895), *Gallus gallus* (XM_418609), *Xenopus laevis* (AAH73404), *Caenorhabditis elegans* (NM_066548), *Drosophila melanogaster* (NM_133126). We failed to identify any orthologs of ERj1 in *Trypanosoma brucei, Saccharomyces cerevisiae, Arabidopsis thaliana, and Oryza sativa*.