Supplementary Figure S2. Quantitation of Sec61 complex depletion and reconstitution.

(A) The extent of Sec61 complex removal during the two-step depletion procedure (performed as described in Gorlich and Rapoport, 1993) was monitored by immunoblotting for Sec61β. One equivalent of material at each step was compared to a serial dilution of RM. Note that after the first depletion step (based on ribosome association), ~95% reduction of the Sec61 complex was achieved. The remaining Sec61 complex was removed to undetectable levels by the second step (which used an immunodepletion approach). Together, more than 98% of the Sec61 complex was depleted from the extract.

(B) The amounts of various membrane proteins recovered in the reconstituted proteoliposomes were quantified relative to their starting amounts in RM. One equivalent of the Sec61-depleted ('Depl.') or depleted and replenished ('Repl.') proteoliposomes were compared to a serial dilution of RM by immunoblotting against the indicated components. Note that in general, the amount of each component found in one equivalent of proteoliposomes corresponds to the amount found in ~0.25 equivalent of RM. Thus, recovery of membrane proteins after solubilization, fractionation, and reconstitution was ~20-25%. For this reason, the experiments testing the translocation activities of proteoliposomes utilized ~5 times more equivalents of proteoliposomes compared to RM (see Materials and Methods).