Lieu et al., http://www.jcb.org/cgi/content/full/jcb.201304055/DC1

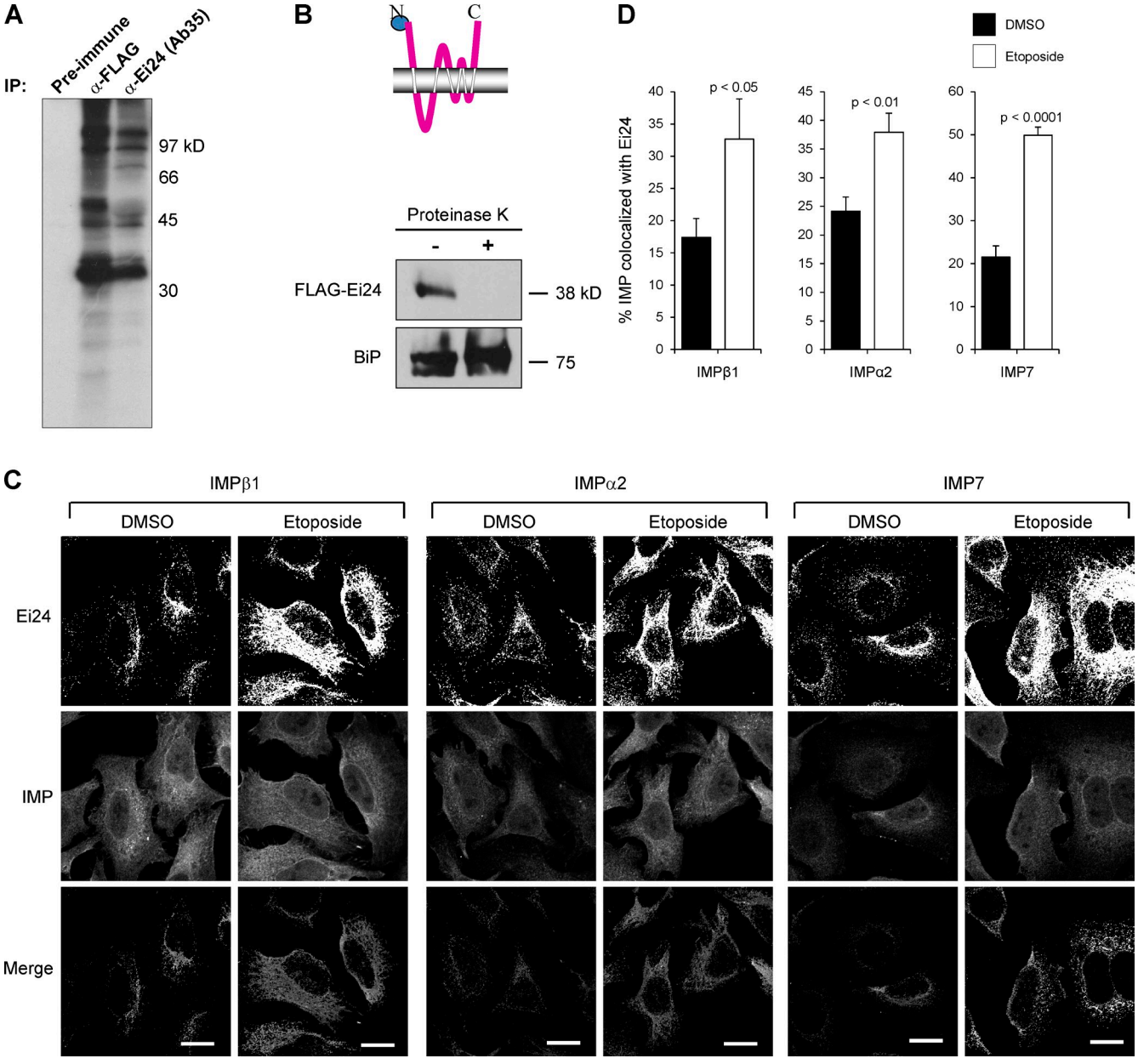

Figure S1. **Proteomic analysis, membrane topology of Ei24, and colocalization with IMPs.** (A) [^{35}S]Met-labeled cell extracts from HEK293T cells transfected to express FLAG-Ei24 were subjected to immunoprecipitation using anti-FLAG or Ei24 (Ab35) antibodies, or preimmune serum as a control. (B) A schematic of predicted topology of Ei24 within the ER membrane (top), with the N terminus residing at the cytosolic face of the ER. Support for this was obtained from subcellular fractionation approaches to extract intact ER-enriched fractions from cells expressing N-terminally FLAG-tagged Ei24, which were subsequently treated with proteinase K for 30 min to cleave exposed FLAG tag within the cytosol. Protein was separated by SDS-PAGE and Western analysis performed using antibodies against FLAG or the ER marker and unfolded protein response sensor protein BiP (bottom). (C) Representative CLSM images for colocalization of IMPβ1, IMPα2, or IMP7 with Ei24 in HeLa cells treated with etoposide or DMSO vehicle control as per the legend to Fig. 1 D. For Ei24, a defined threshold signal above background levels (top) was overlaid onto that of IMPβ1, IMPα2, or IMP7 (middle) to produce a merged image of colocalized Ei24 and IMP pixels (bottom). Bars, 20 µm. (D) Quantitative analysis of images such as those in C to determine the percentage of total IMP colocalized with Ei24. Results represent the mean ± SEM (error bars; $n > 30$ cells). P-values (Student's t test) denote significant differences between DMSO- and etoposide-treated cells for each IMP.

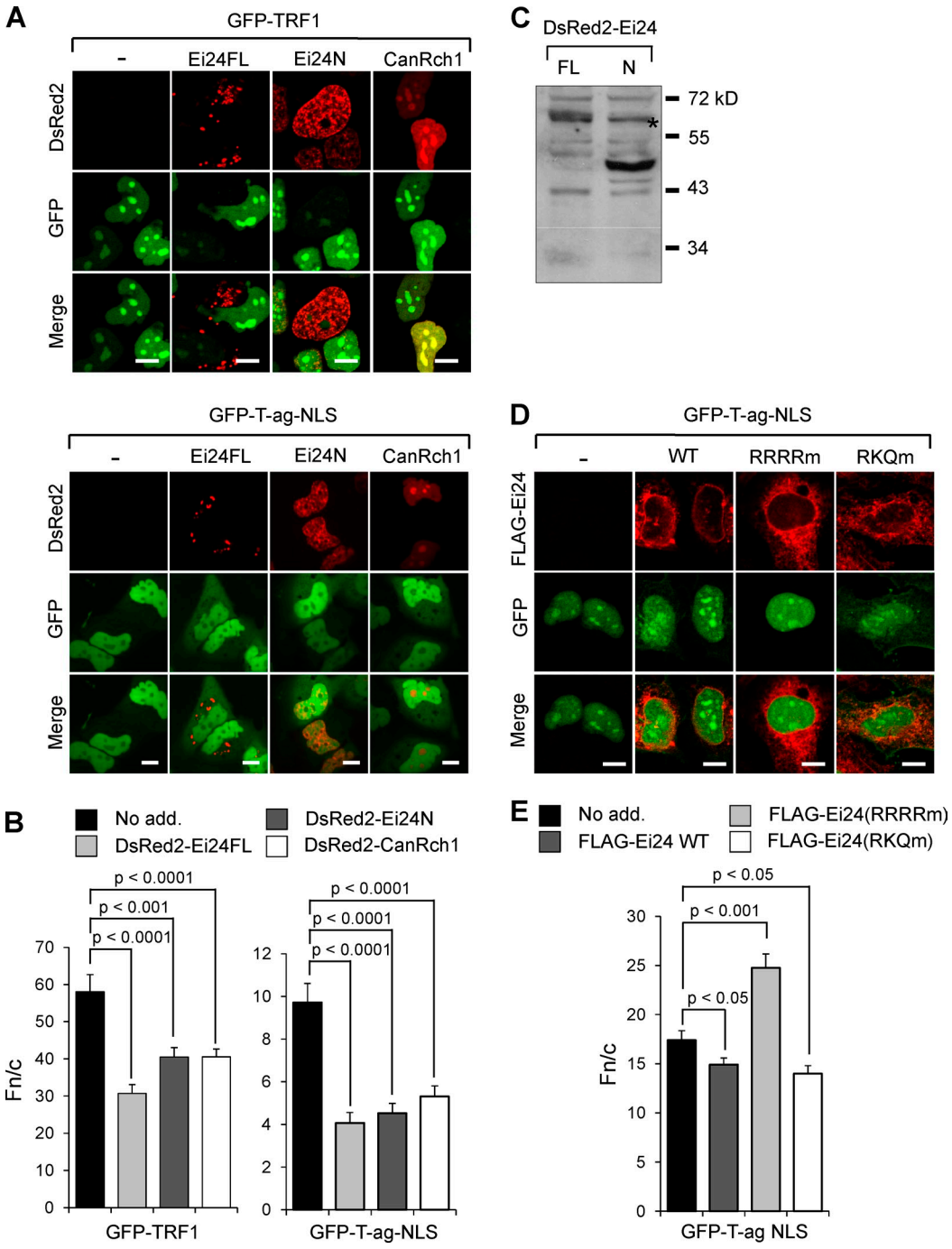

Figure S2. **Ei24 and CanRch1 inhibit TRF1 and T-ag NLS IMP-mediated nuclear translocation.** (A) Live-cell CLSM images of HeLa-Bcl$_{XL}$ cells transfected to coexpress the indicated GFP and DsRed2 fusion proteins 20 h after transfection are shown. Bars, 10 µm. (B) Quantitative analysis of the extent of nuclear accumulation for various GFP fusion proteins. Results are the mean ± SEM (error bars; $n > 40$) from a single assay, representative of three separate experiments. Significant differences between samples in the absence or presence of ectopically expressed Ei24/CanRch1 are denoted by p-values. (C) Whole-cell extracts of HeLa cells expressing DsRed2-Ei24FL (FL) or DsRed2-Ei24N (N) were subjected to Western analysis using an anti-mCherry antibody (which also detects DsRed2-fusion proteins) to verify the integrity of the DsRed2 fusion proteins. The asterisk indicates a nonspecific band. (D) CLSM images of HeLa-Bcl$_{XL}$ cells transfected to coexpress GFP-T-ag NLS in the absence or presence of FLAG-Ei24 derivatives were fixed and immunostained as per the legend to Fig. 5. Bars, 10 µm. (E) Quantitative analysis for the extent of nuclear accumulation of GFP-T-ag NLS. Results are for the mean ± SEM (error bars; $n \geq 63$) for a single assay, representative of three independent experiments; statistically significant differences are denoted by p-values.

Table S1. **Ei24 mass spectrometric MASCOT scores**

Protein name	Protein score	Total ion score
IMPβ1	516 (100%)	385 (100%)
IMP7	679 (100%)	507 (100%)
Ei24	437 (100%)	341 (100%)

Number in parentheses indicate the confidence interval (%).