SUPPLEMENTAL FIGURE LEGENDS

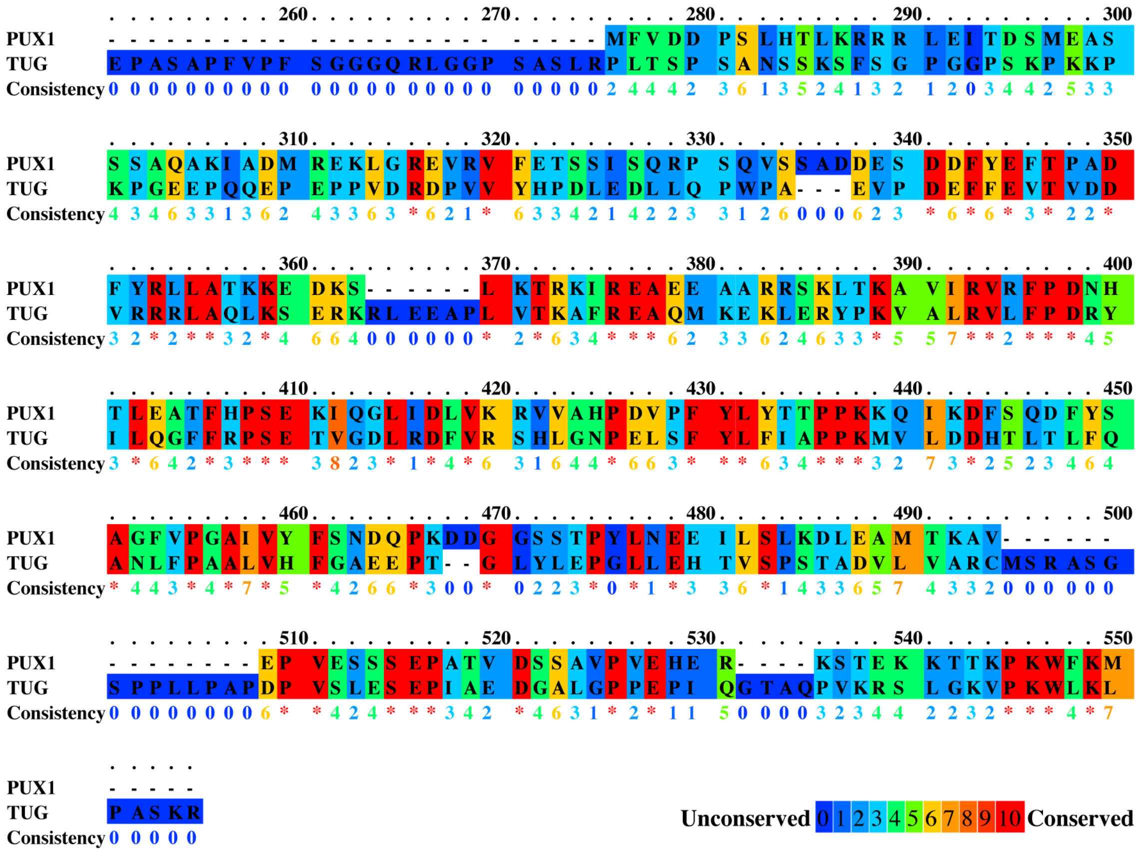
Supplemental Figure 1**. Alignment of TUG and PUX1.** A sequence alignment of TUG residues 250-550 with intact PUX1 (residues 1-251) is shown. Conservation is indicated by progressively warmer colors (yellows, oranges and reds), as shown on the scale at bottom. The UBX domains span positions 385-459, as numbered on the diagram. The important proline residue conserved in both the PUX1 and TUG UBX domains is at position 436.

Supplemental Figure 2. **Specificity of TUG antisera.** TUG antisera were preincubated in the absence or presence of an excess of the peptide to which the antisera were raised, as indicated, then used for immunofluorescence microscopy of HeLa cells. Images were acquired using equal exposure times and camera settings. The brightness of the lower pair of images was adjusted so that the outlines of the cells in the peptide-treated samples are visible.

Supplemental Figure 3. **Localization of TUG at early stages of the secretory pathway.** A-C. HeLa cells were transfected with VSVG-ts045-YFP in HeLa cells. After incubation at 40° C for 6 hours to induce accumulation of VSV-G in the ER, cells were shifted to 32°C for various times (t= 0, 4, 8, 16, 40 min.), then fixed, stained to detect endogenous TUG and to increase the YFP signal, and imaged using confocal microscopy. Representative images from timepoints taken at t= 0 (A), t=4 min. (B), and t=40 min. (C) after release of VSV-G from the ER. At t=0, the VSVG-ts045-YFP construct displays a reticular ER pattern. The TUG staining was closely related to the lacy ER pattern, but the signal appeared concentrated in areas where VSV-G was not present (A). A few minutes after the temperature shift (t=4), TUG and VSVG showed some degree of co-localization (B). This overlap was also observed at later time points (t=8, 16 min.). At 40 minutes, the VSV-G was prominently displayed at the cell surface, with significantly less co-localization with TUG in the perinuclear region (C). These data are consistent with the idea that TUG localizes to early compartments in the secretory pathway taken by VSV-G. D. HeLa cells were transfected with Sec13-GFP, then fixed and stained to detect endogenous TUG. Confocal images are shown. Cells with modest expression of Sec13-GFP displayed a pattern consistent with ERGIC localization, with some additional cytoplasmic and nuclear localization. TUG was highly colocalized with the perinuclear ERGIC accumulation of Sec13-GFP.

Supplemental Figure 4. **TUG siRNA does not affect VSV-G trafficking to the cell surface.** HeLa cells were transfected using scrambled (control) or TUG siRNAs, as indicated. At 48 hours later, VSVG-ts045-YFP was transfected, and the cells were maintained at 40° C for six hours. Cells were shifted to 32° C for the indicated amounts of time, then fixed in 4% paraformaldehyde and permeabilized with 0.1% triton X-100. Indirect immunofluorescence was done using an anti-GFP antibody to increase the fluorescent signal. The brightness of the images at right was increased equally in all images, so as to make clear the VSVG at the plasma membranes (arrowheads). Scale bar, 10 μm.

Suppl. Fig. 1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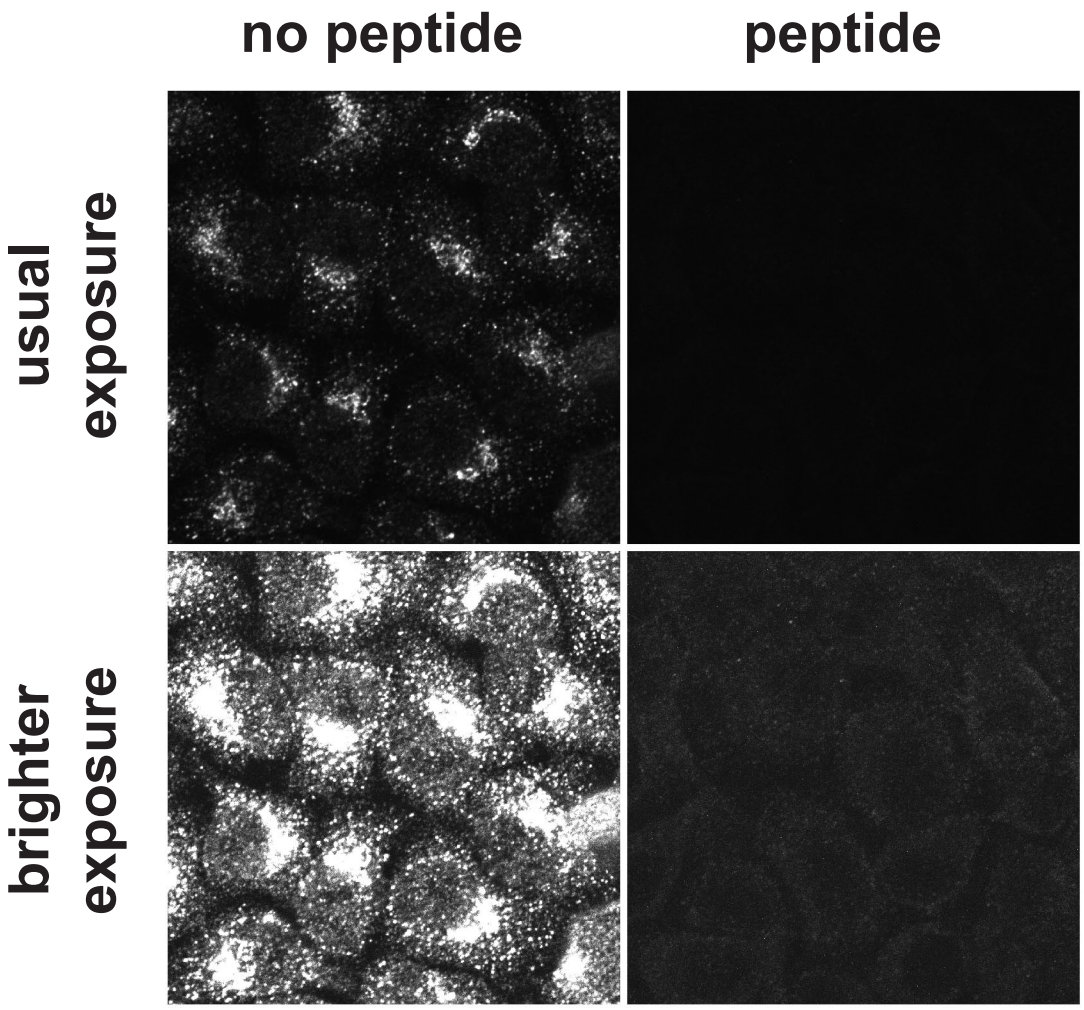
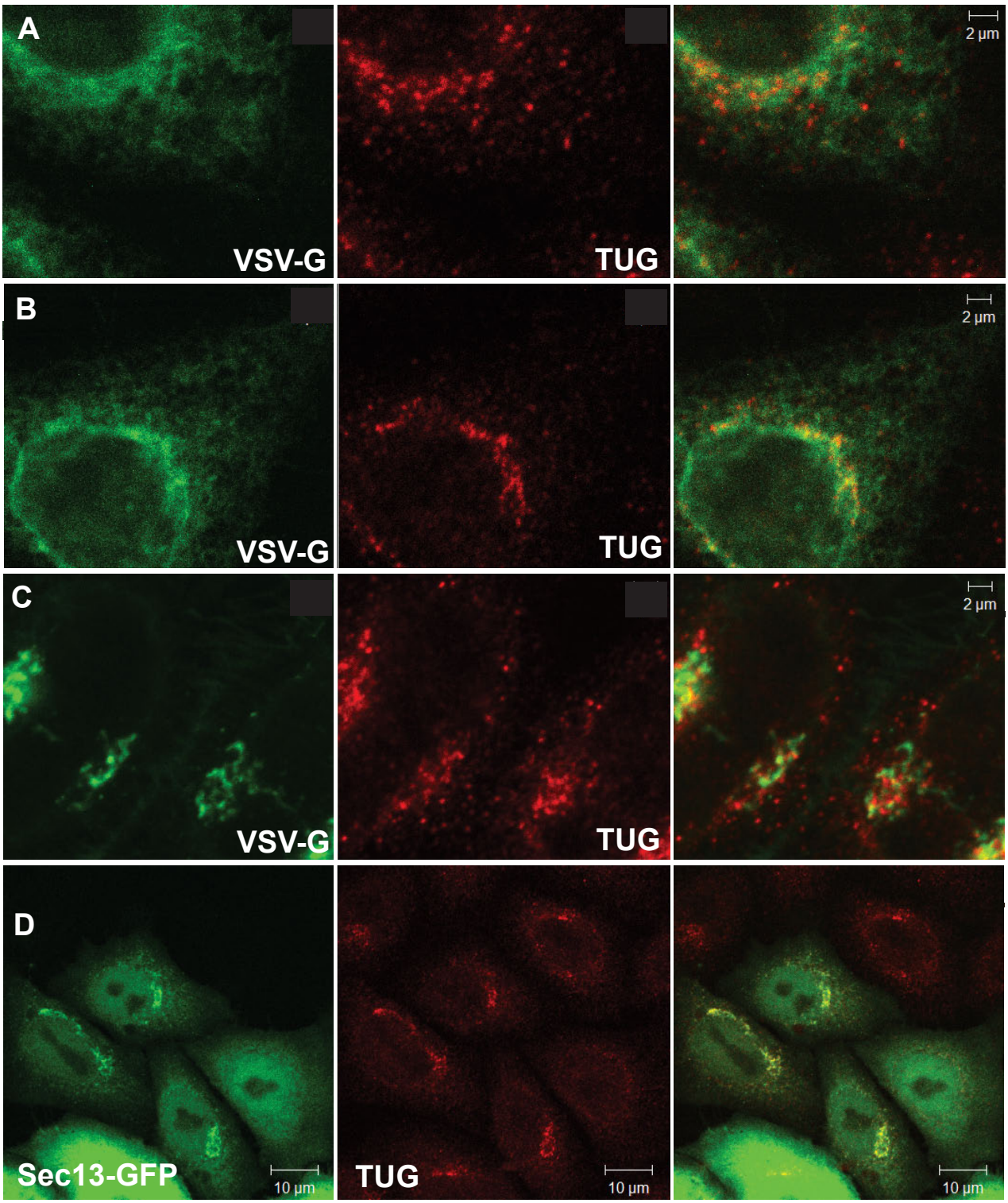
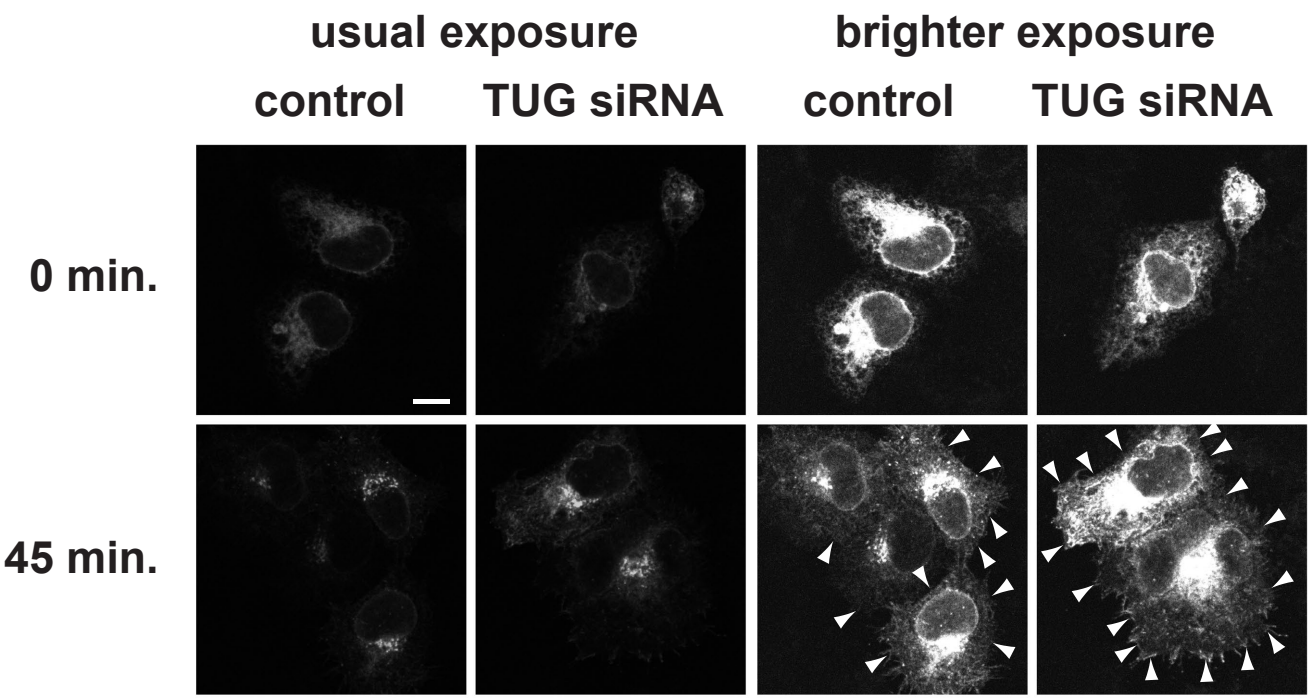
Consistency 0000000000 0000000000 0000024442 3613524132 1203442533
. . . . 310 . . . . 320 . . . . 330 . . . . 340 350
PUX1 SSAQAKIADM REKLGREVRV FETSSISQRP SQVSSADDES DDFYEFTPAD
TUG KPGEEPQQEP EPPVDRDPVV YHPDLEDLLQ PWPA---EVP DEFFEVTVDD
Consistency 4346331362 43363*621* 6334214223 3126000623 *6*6*3*22*
. . . . 360 . . . . 370 . . . . 380 . . . . 390 400
PUX1 FYRLLATKKE DKS------L KTRKIREAEE AARRSKLTKA VIRVRFPDNH
TUG VRRRLAQLKS ERKRLEEAPL VTKAFREAQM KEKLERYPKV ALRVLFPDRY
Consistency 32*2**32*4 664000000* 2*634***62 33624633*5 57**2***45
. . . . 410 . . . . 420 . . . . 430 . . . . 440 450
PUX1 TLEATFHPSE KIQGLIDLVK RVVAHPDVPF YLYTTPPKKQ IKDFSQDFYS
TUG ILQGFFRPSE TVGDLRDFVR SHLGNPELSF YLFIAPPKMV LDDHTLTLFQ
Consistency 3*642*3*** 3823*1*4*6 31644*663* **634***32 73*2523464
. . . . 460 . . . . 470 . . . . 480 . . . . 490 500
PUX1 AGFVPGAIVY FSNDQPKDDG GSSTPYLNEE ILSLKDLEAM TKAV------
TUG ANLFPAALVH FGAEEPT--G LYLEPGLLEH TVSPSTADVL VARCMSRASG
Consistency *443*4*7*5 *4266*300* 0223*0*1*3 36*1433657 4332000000
. . . . 510 . . . . 520 . . . . 530 . . . . 540 550
PUX1 --------EP VESSSEPATV DSSAVPVEHE R----KSTEK KTTKPKWFKM
TUG SPPLLPAPDP VSLESEPIAE DGALGPPEPI QGTAQPVKRS LGKVPKWLKL
Consistency 000000006* *424***342 *4631*2*11 5000032344 2232***4*7
. . . . .
PUX1 -----
TUG PASKR
Consistency 00000
Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved


no peptide
peptide
usual exposure
brighter exposure

Suppl. Fig. 3


A
VSV-G
TUG
2 µm
B
VSV-G
TUG
2 µm
C
VSV-G
TUG
2 µm
D
Sec13-GFP
10 µm
TUG
10 µm
10 µm

Suppl. Fig. 4


usual exposure
brighter exposure
control
TUG siRNA
control
TUG siRNA
0 min.
45 min.