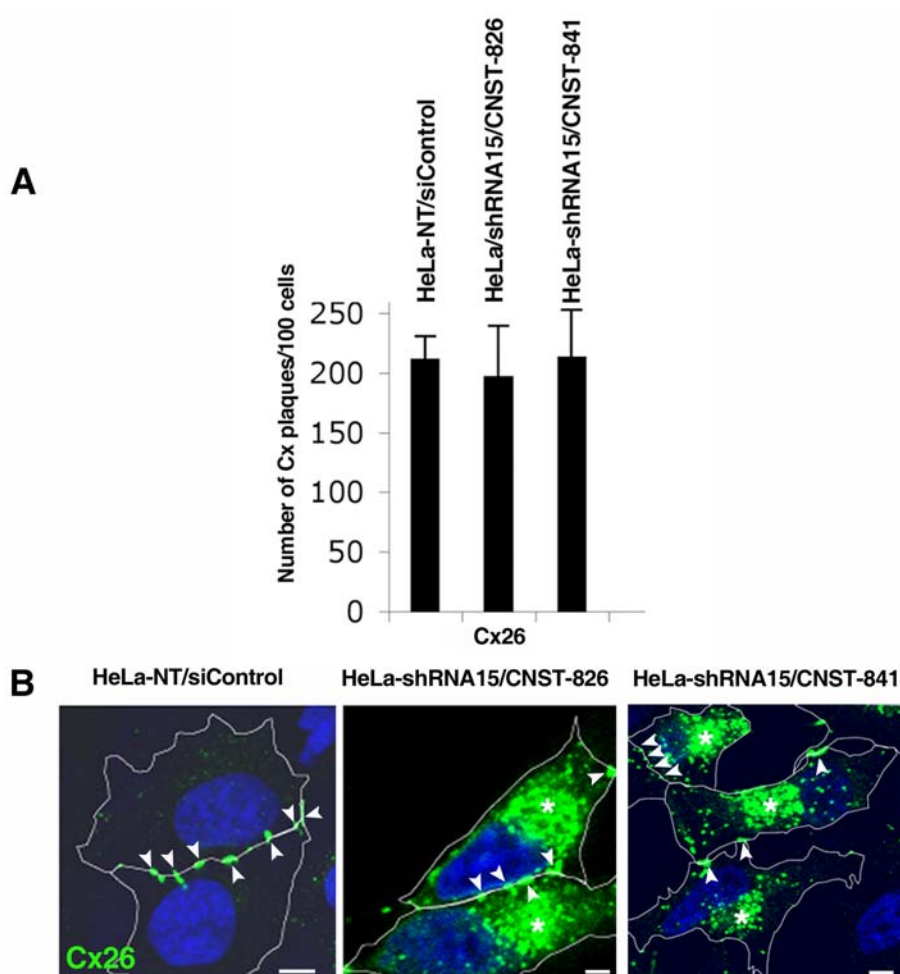
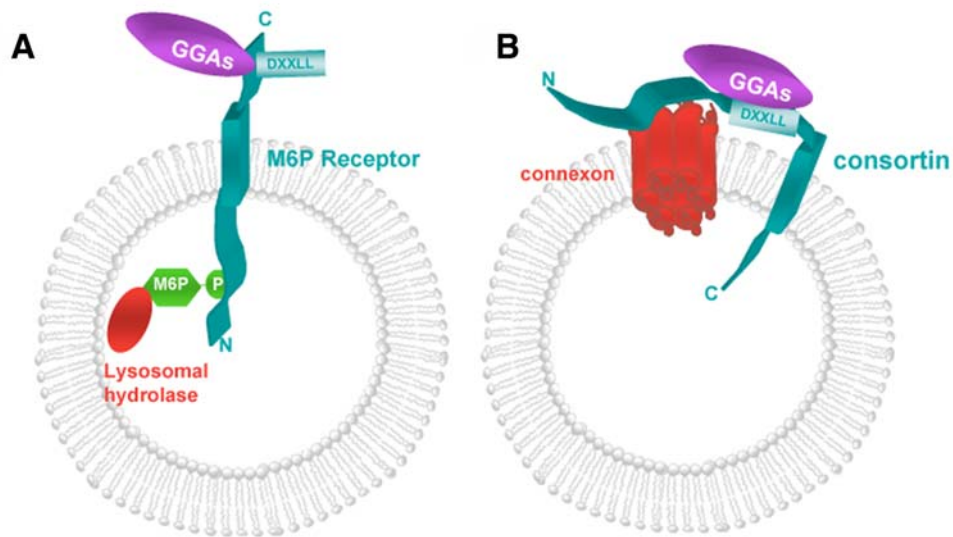


## SUPPLEMENTARY MATERIAL



**Figure S1.** Effect of consorin RNAi-mediated silencing on the membrane targeting of Cx26. **(A)** After 72 hours of siRNA-mediated consorin knockdown, a similar number of Cx26 immunostained plaques at the plasma membrane was detected in control (HeLa-RNA-NT) and consorin-silenced cells (HeLa-shRNA15/*CNST*-826 and HeLa-shRNA15/*CNST*-841) (Supplementary Material, Table S1). **(B)** Cx26 accumulates in the cytoplasm of HeLa cells undergoing consorin knockdown, although connexin plaques (arrowheads) are still visible at the plasma membrane. Cells were stained for Cx26 (green) and counterstained with DAPI (blue). White lines outline cell contours, as observed in differential interference contrast (DIC) confocal images. Bars, 5  $\mu$ m.



**Figure S2.** Models for the interactions of TGN cargo receptors with their cargoes and adaptors. **(a)** The long N-terminal segment of the mannose-6-phosphate (M6P) receptor, a type-I transmembrane protein, binds to the M6P moieties of lysosomal hydrolases on the luminal side of the membrane, while interacting with GGAs on its cytosolic side. **(b)** By contrast, the long N-terminal segment of consortin, a predicted type-II transmembrane protein, binds to both connexin hexamers (connexon) and GGAs on the cytosolic side of the membrane.

**Table S1. Number of connexin immunolabeled plaques per 100 cells undergoing RNAi-mediated consortin silencing**  
**Two-tailed, unpaired t test with Welch's correction**

<b>Connexin</b>	<b>Cells / siRNA treatment</b>	<b>Mean <math>\pm</math> SD</b>	<b>N</b>	<b><i>P</i> value (versus control)</b>
Cx26	<b>Control:</b> HeLa-RNA-NT / siControl	212.09 $\pm$ 18.45	3	-
	HeLa-shRNA15 / <i>CNST</i> -826	197.64 $\pm$ 41.59	3	0.6374
	HeLa-shRNA15 / <i>CNST</i> -841	213.50 $\pm$ 39.25	3	0.9603
Cx32	<b>Control:</b> HeLa-RNA-NT / siControl	320.38 $\pm$ 15.82	3	-
	HeLa-shRNA15 / <i>CNST</i> -826	98.66 $\pm$ 3.21	3	0.0018
	HeLa-shRNA15 / <i>CNST</i> -841	40.28 $\pm$ 1.26	3	0.0011
Cx43	<b>Control:</b> HeLa-RNA-NT / siControl	116.63 $\pm$ 9.52	3	-
	HeLa-shRNA15 / <i>CNST</i> -826	3.24 $\pm$ 2.19	3	0.0025
	HeLa-shRNA15 / <i>CNST</i> -841	1.92 $\pm$ 1.89	3	0.0024
Cx45	<b>Control:</b> HeLa-RNA-NT / siControl	188.11 $\pm$ 25.08	3	-
	HeLa-shRNA15 / <i>CNST</i> -826	14.43 $\pm$ 2.48	3	0.0069
	HeLa-shRNA15 / <i>CNST</i> -841	13.74 $\pm$ 3.73	3	0.0070

SI Table 2. Subcellular distribution of Cx43 and consorin immunolabeling in HeLa cells

type of HeLa cells	# <sup>1</sup> cells scored	number of particles									
		Cx43					Consortin				
		Golgi Apparatus <sup>2</sup>	vesicles <sup>2</sup>	lysosomes <sup>2</sup>	membrane GJ plaques <sup>2</sup>	cytoplasmic annular GJ <sup>2</sup>	Golgi apparatus <sup>2</sup>	vesicles <sup>2</sup>	non junctional membrane <sup>2</sup>	membrane GJ plaques <sup>2</sup>	cytoplasmic annular GJ <sup>2</sup>
Wild type	30/59	0 (0; 64)	0 (0; 326)	0 (0; 128)	0 (0; 0)	0 (0; 0)	8.0 ± 0.7 (19.6; 132)	3.0 ± 0.3 (4.0; 600)	12.4 ± 0.9 (79.7; 59)	0 (0; 0)	0 (0; 0)
NT-transfected	42/40	ND	ND	ND	0 (0; 0)	0 (0; 0)	ND	ND	ND	ND	ND
shRNA15 - transfected	45/41	ND	ND	ND	0 (0; 0)	0 (0; 0)	ND	ND	ND	ND	ND
Cx43 transfected	32/53	3.9 ± 0.5 (17.1; 76)	3.0 ± 0.2 (4.2; 360)	7.0 ± 2.0 (2.0; 100)	20.7 ± 1.0 (100; 26)	0 (0; 0)	ND	ND	10.5 ± 0.8 (83.0; 53)	0 (0; 37)	0 (0; 1)
Cx43 - NT transfected	44/40	4.2 ± 0.4 (18.0; 100)	2.8 ± 0.2 (4.7; 402)	9.0 ± 0.9 (2.8 ; 143 )	19.3 ± 0.7 (100; 32)	15 (100; 1)	ND	ND	ND	0 (0; 27)	0 (0; 0)
Cx43 - shRNA15 transfected	56/62	4.3 ± 0.4* <sup>**</sup> (17.7; 124)	3.0 ± 0.2* <sup>**</sup> (4.9; 610)	16.2 ± 1.5* <sup>**</sup> (5.8; 189)	27.2 ± 1.2* <sup>**</sup> (100; 38)	27.7 ± 1.2* <sup>**</sup> (100; 15)	ND	ND	10.0 ± 1.1 (22.6; 62)	0 (0; 40)	0 (0; 13)

<sup>1</sup>Values are numbers of cell scored after Cx43 immunolabeling/ numbers of cell scored after consorin immunolabeling; <sup>2</sup> Values are mean +/- SE number of gold particles per organelle. Values in parenthesis are % of the organelle labeled; number of organelles scored. GJ = gap junctions. ND = not determined.

\*p < 0.05, \*\* p < 0.03 as compared by the chi square test to the distribution in Cx43 - NT-transfected cells ( $\chi^2 = 10.11$ ; df = 4) and Cx43-transfected cells ( $\chi^2 = 11.23$ ; df = 4), respectively.