Supplemental Materials

Molecular Biology of the Cell

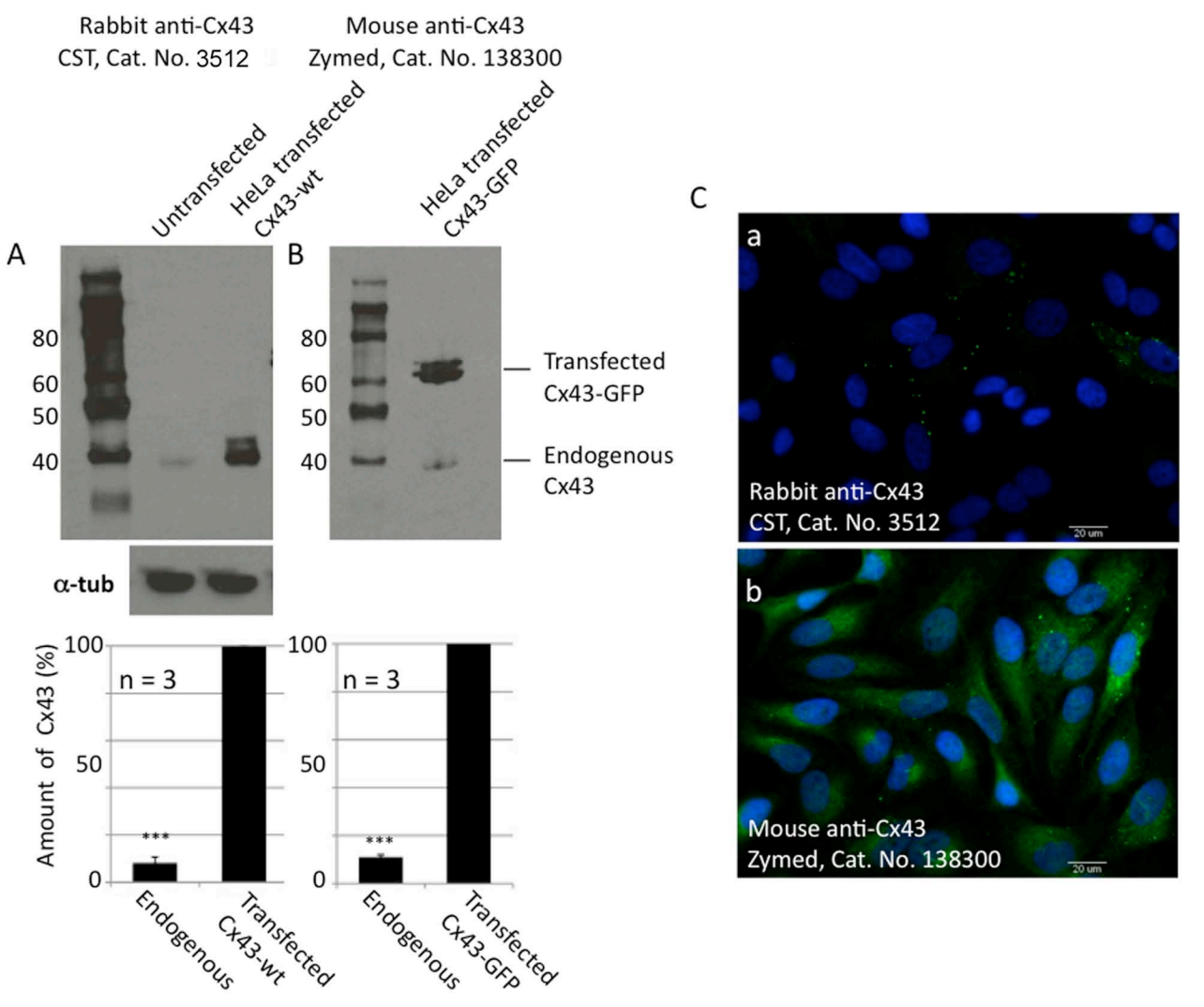
Fong et al.

Table S1: Time in hours required for GJ plaques assembled of wild type and AP-2 binding-impaired Cx43 mutants (S2, S3, S2+3) to constitutively turn over (assemble, mature, and being removed).

wt	S2[a]	S3[b]	S2+3[c]
2[d]	5	8	**21**
2	5	8	24
2	15	**12**	**25**
2	**19**	12	25
4	25	12	25
4	25	**17**	**27**
4	30	**17**	30
4		17	33
5[e]		20	40
5		22	48
6		22	
6		22	
6		22	
8		22	
10		25	
10		25	
		30	
n: 16	7	17	10
Mean: 5	18	19	30
SEM: 1.4	3.7	1.3	2.7

[a]S2 mutant: Cx43-F^{268}A-GFP
[b]S3 mutants: Cx43-Y^{286}H-GFP, Cx43-ΔP^{283}PGYKLV-GFP
[c]S2+3 mutants: Cx43-F^{268}A+Y^{286}H-GFP, Cx43-Δ$L^{254\text{-}290}$-GFP, Cx43-Δ$L^{254\text{-}CT}$-GFP
[d]Regular: GJ plaques already existed at start of imaging
[e]**Bold**: Recordings include GJ plaque formation and internalization

Figure S1: Basal levels of endogenous Cx43 were detected in HeLa cells (ATCC clone CCL-2). **(A)** HeLa cells were cultured in 3.5 cm diameter dishes and transfected with GFP-tagged and untagged wt Cx43 DNA constructs as described in Materials and Methods. Cells were lysed with 500 µl of protein sample buffer/dish. Transfected and endogenous Cx43 proteins from 15 µl of each sample (lysates of approximately 3000-6000 cells) were detected by Western blots using rabbit polyclonal antibodies (Cell Signaling Technology, Cat. No. 3512) **(A)**, or mouse monoclonal antibodies (Zymed, Cat. No. 138300) **(B)** directed against Cx43 as described in Materials and Methods. X-ray films were exposed to ECL in the dark for 5 minutes. Membranes were stripped and re-probed with mouse monoclonal antibodies directed against α-tubulin to control for equal loading. Results indicate that total amount of endogenous Cx43 protein (lane 2) in the pools is ~10% (SEM ±3, $p<0.0001$) of the transfected Cx43 protein (CMV promoter) (lane 2, 3). Transfection efficiency in these experiments was about 20-30%. If transfection efficiency were 100%, the amount of enodogenous Cx43 in the pool would have been 1-2% compared to transfected Cx43. **(C)** Immunofluorecence staining of enodogenous Cx43 in HeLa cells using respective rabbit and mouse Cx43 antibodies performed as described in Materials and Methods. Representative images acquired using a 20x long distance lens are shown. Some GJ-like puncta and intracellular fluorescence and were detected using respective rabbit and mouse antibodies.


Rabbit anti-Cx43
CST, Cat. No. 3512
Mouse anti-Cx43
Zymed, Cat. No. 138300
Untransfected
HeLa transfected
Cx43-wt
HeLa transfected
Cx43-GFP
A
B
80
60
50
40
Transfected
Cx43-GFP
Endogenous
Cx43
α-tub
Amount of Cx43 (%)
100
50
0
n = 3
***
Endogenous
Transfected
Cx43-wt
Cx43-GFP
C
a
Rabbit anti-Cx43
CST, Cat. No. 3512
20 um
b
Mouse anti-Cx43
Zymed, Cat. No. 138300
20 um