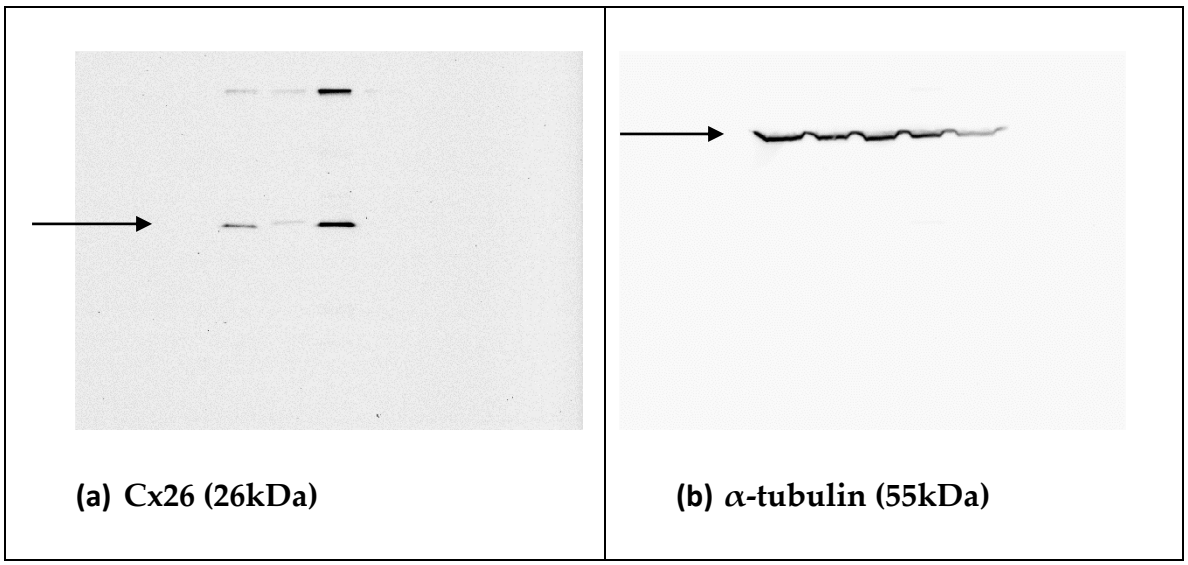

Figure S1. The results of Western blotting analysis (original Western blotting for Figure 3). Representative immunoblotting for: (a) Cx26 and (b) α-tubulin.

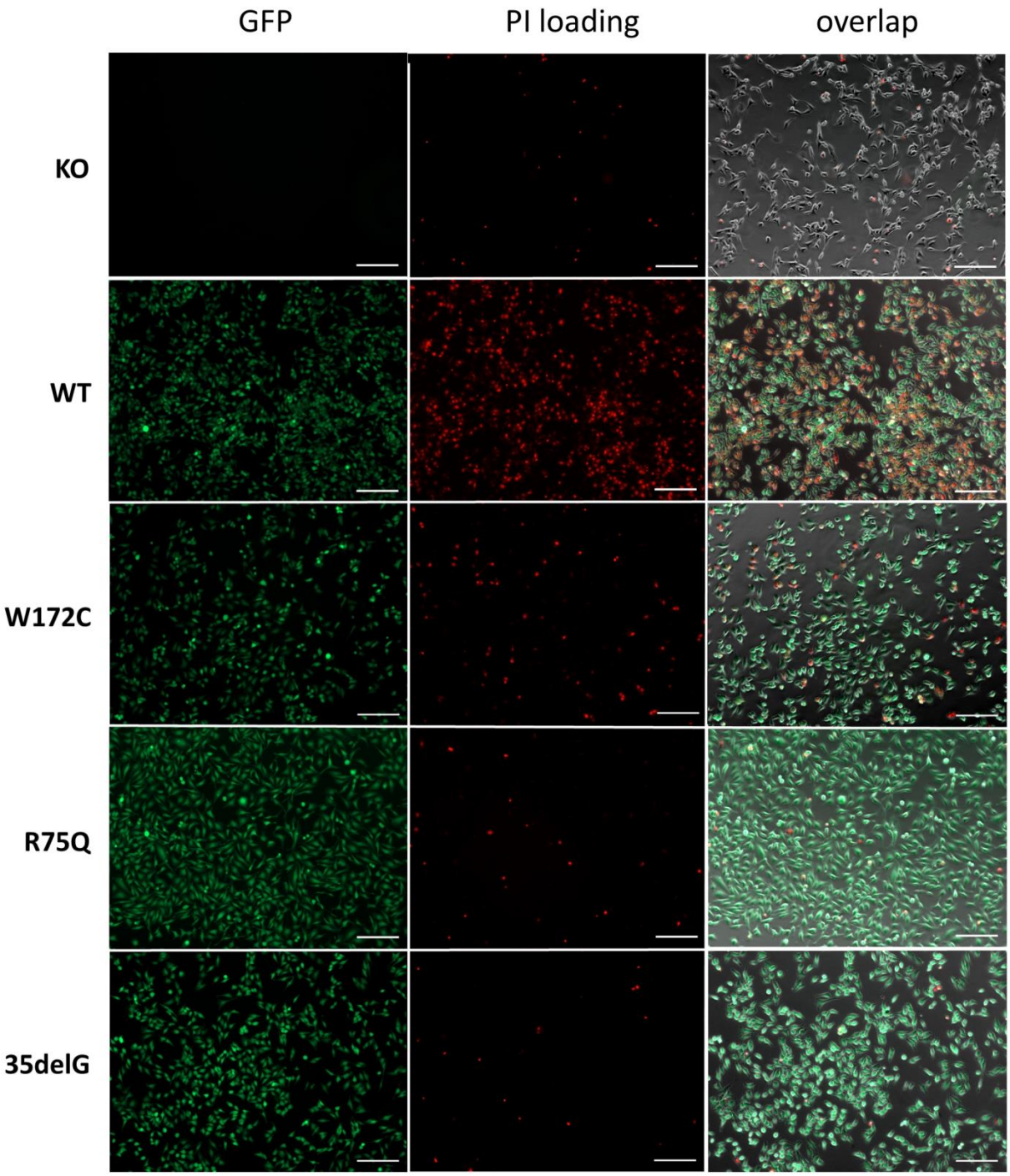

Figure S2. PI loading through Cx26-hemichannels in examined HeLa cell lines (fluorescent microscopy). KO, WT, W172C, R75Q, and 35delG denote lines HeLa Cx26-KO, HeLa-Cx26wt, HeLa-p.W172C, HeLa-p.R75Q, and HeLa-c.35delG, respectively. Green color corresponds to GFP signal, red color corresponds to PI signal. Scale bar = 200 μm.

Table S1. Missense variants of the *GJB2* gene in the extracellular loop 2 (E2) of Connexin 26 defined as 'pathogenic' and 'likely pathogenic' in the Deafness Variation Database (http://deafnessvariationdatabase.org/).

Amino acid position	Nucleotide change	Missense mutation	Pathogenic / Likely pathogenic	Phenotype	Functional studies
158	c.473A>G	**p.Tyr158Cys**	pathogenic	Sensorineural hearing loss	
159	c.475G>T	**p.Asp159Tyr**	likely pathogenic	Deafness	
	c.476A>T	**p.Asp159Val**	pathogenic	Deafness	
	c.475G>A	**p.Asp159Asn**	likely pathogenic	Deafness	
161	c.482T>C	**p.Phe161Ser**	pathogenic	Deafness	Thönnissen et al., 2002
163	c.487A>C	**p.Met163Leu**	pathogenic	Deafness, autosomal dominant 3	Matos et al., 2008
	c.487A>G	**p.Met163Val**	pathogenic	Deafness	Brussone et al., 2003; Press et al., 2017
	c.488T>C	**p.Met163Thr**	pathogenic	Sensorineural hearing loss	
169	c.505T>C	**p.Cys169Arg**	likely pathogenic	Hearing loss, non-syndromic	
	c.506G>A	**p.Cys169Tyr**	pathogenic	Deafness	Zonta et al., 2015
170	c.509A>C	**p.Asn170Thr**	likely pathogenic	Sensorineural hearing loss	
171	c.511G>T	**p.Ala171Ser**	likely pathogenic	Sensorineural hearing loss	
172	c.514T>A	**p.Trp172Arg**	pathogenic	Deafness	Mani et al., 2009
	c.516G>C	**p.Trp172Cys**	pathogenic	Deafness, nonsyndromic sensorineural	**this study**
173	c.517C>T	**p.Pro173Ser**	pathogenic	Deafness, autosomal recessive 1	
	c.518C>G	**p.Pro173Arg**	pathogenic	Deafness, autosomal recessive 1	Thönnissen et al., 2002
174	c.520T>C	**p.Cys174Arg**	pathogenic	Deafness, autosomal recessive 1	
	c.521G>C	**p.Cys174Ser**	likely pathogenic	Hearing loss	
175	c.523C>A	**p.Pro175Thr**	pathogenic	Deafness, autosomal recessive 1	
178	c.533T>C	**p.Val178Ala**	pathogenic	Deafness, autosomal recessive 1	
179	c.535G>A	**p.Asp179Asn**	pathogenic	Deafness	Yum et al., 2010; Zhang et al., 2011
	c.535G>C	**p.Asp179His**	likely pathogenic	Sensorineural hearing loss	
183	c.548C>T	**p.Ser183Phe**	pathogenic	Focal palmoplantar keratoderma with Deafness	de Zwart-Storm et al., 2008; Shuja et al, 2016; Press et al., 2017; Beach et al., 2020
184	c.550C>G	**p.Arg184Gly**	pathogenic	Deafness	
	c.550C>T	**p.Arg184Trp**	pathogenic	Deafness, autosomal recessive 1	
	c.551G>A	**p.Arg184Gln**	pathogenic	Deafness, autosomal dominant 3	Su et al., 2010; Yum et al., 2010; Zhang et al., 2011
	c.551G>C	**p.Arg184Pro**	pathogenic	Deafness, autosomal recessive 1	Thönnissen et al., 2002; Brussone et al., 2003;

					Mani et al., 2009; Beach et al., 2020
186	c.557C>A	**p.Thr186Lys**	pathogenic	Sensorineural hearing loss	
188	c.563A>G	**p.Lys188Arg**	pathogenic	Sensorineural hearing loss	
190	c.569T>A	**p.Val190Asp**	pathogenic	Deafness, nonsyndromic	

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