

**Supplemental Figure 1.** Schematic representation of the domain structure of the CX30.2/CX31.3 protein with indication of known variants. The arrows indicate the mutations of CX30.2/CX31.3. TM1-4: transmembrane domains; E1-2: extracellular domains; CL: cytoplasmic linking domain

| A<br>CX26<br>CX30<br>CX32<br>CX30.3<br>CX31<br>CX43<br>CX30.2/CX31.3  | CKNVC<br>CKNVC<br>CNSVC<br>CPNVC<br>CTNVC<br>CENVC<br>CENVC | IDH FFP<br>IDH FFP<br>IDH FFP<br>IDH FFP<br>IDH FFP<br>IDK SFP<br>IDA FHP | ISHIR<br>ISHIR<br>ISHIR<br>ISHIR<br>ISHIR<br>ISHIR | P.W77<br>LWALQ<br>LWSLQ<br>LWALQ<br>FWLQ<br>FWLQ<br>FWLQ |
|---|---|---|--|--|
| mCx23<br>consensus  | INLF  | NQER<br>7d ffP  | ishir  | IWalo  |
| _   |   |   |  | ~  |
| B ConSeq results  |   |   |  |  |
|   |   |   |  |  |
| 1<br>MCCPET PRIT  | 11  | 21  | 31   | 41   |
| eeeebbeebb  | eebeeebebb  | bebbbbbbbbb   | bbbbbbbbbb   | eebbeeeeee   |
| f s s   | fsf   | sf s  | SS   | ffff   |
| 51  | 61  | 71 p.W77S   | 81   | 91   |
| FVCH1QQPGC<br>bebeeeeeb   | eeebeeebbe  | bbbbbbbbbb  | bbbbbbbbbb   | YMGFTLYHVI<br>bbbbbbbbbe                                 |
| s sff fffs  | ffsff f   | SSS S S S   | S SS   |  |
| 101   | 111   | 121   | 131  | 141  |
| WHWELSGKGK  | EEETLIQGRE  | GNTDVPGAGS  | LRLLWAYVAQ   | LGARLVLEGA   |
| CECEECEEE   | ecceccecce  | ecceccecce  | s  | f f  |
| 151   | 161   | 171   | 181  | 191  |
| ALGLQY <mark>H</mark> LYG   | FOMPSSFACE  | REPCLGSITC  | NLSRPSEKTI   | FLKTMFGVSG   |
|   | bebeebbebe  | eeeeeebeb   | bbeeeeebb  |  |
| 201   | 211   | 221   | 231  | 241  |
| F <mark>CLL</mark> FTFLEL   | VLIGLGRWWR  | TWKHKSSSSK  | YFLTSESTRR   | HKKATDSLPV   |
| bbbbbebbbb  | bbbbbeebee  | ebeeeeeee   | eeeeeeeee  | eeeeeeebe  |
| 251 S   | 261   | 271   |  | T  |
| VETREQFQEA  | VPGRSLAQEK  | QRPVGPRDA   |  |  |
| eeeeeeeee   | eeeebeeeee  | eeeeeeeee   |  |  |
|   |   | f   |  |  |
| 1 2   | 3 4 5 6   | 7 8 9   |  |  |
| Variable Average conserved  |   |   |  |  |
| <ul> <li>An exposed residue according to the neural-network algorithm.</li> <li>A buried residue according to the neural-network algorithm.</li> <li>A predicted functional residue (highly conserved and exposed).</li> <li>A predicted structural residue (highly conserved and huriod).</li> </ul> |   |   |  |  |

Insufficient data- the calculation for this site was performed on less than 10% of the sequences.

Supplemental Figure 2. Multiple alignments of amino acid sequence in connexin proteins by bioinformatics. (A) Alignment of the amino acid sequences of the average domain of human CX30.2/CX31.3 and members of human CX family using Biology WorkBench Clustal W (1.81). The p.W77S are indicated by the frame. (B) ConSeq predictions demonstrated on human CX30.2/CX31.3 [NP\_853516; SWISS-PROT: Q8NFK1 (CXG3\_Human)], using 50 homologues obtained from the Pfam database (family code: PF00029). The sequence of the CX30.2/CX31.3 protein is displayed with the evolutionary rates at each site colour-coded onto it (see legend). The residues of the CX30.2/CX31.3 sequence are numbered starting from 1. The first row below the sequence lists the predicted burial status of the site (i.e. "b"—buried versus "e"—exposed). The second row indicates residues predicted to be structurally and functionally important: "s" and "f", respectively. Vertical arrows indicate amino acid codons (p.W77).



**Supplemental Figure 3.** Expression analysis of *GJC3* mRNA in the CX30.2/CX31.3WT and CX30.2/CX31.3 W77S transfected HeLa cells by RT-PCR (A) and Quantitative-PCR (B). Total RNA from HeLa cells expressing CX30.2/CX31.3WT and CX30.2/CX31.3W77S confirms expression of the corresponding mRNAs in transfected HeLa cell.  $\beta$ -actin served as reference of the amount of total RNA for each sample. Data represent average  $\pm$  SD. Results are representative of three separate experiments.