# Prion-like Properties of Short Isoforms of Human Chromatin Modifier PHC3

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## Supplementary Information

**Table S1.** Amyloidogenic and aligned regions of PHC proteins

| Protein | Motifs predicted by ArchCandy | Motifs predicted by AmylPred2 | Aligned regions |
|---|---|---|---|
| PHC1 | 49-80; 180-229; 242-275; 331-353; 386-438; 528-545 | 55-58; 130-134; 173-178; 397-403; 618-628; 733-743; 946-951;972-975; 996-999 | 16-76; 102-154; 720-758 (HD1); 940-1004 (SAM) |
| PHC2 | 70-131; 149-187; 523-548 | 60-62; 190-196; 451-458; 496-497; 524-528; 568-578; 800-803 | 32-112; 137-195; 555-593 (HD1); 794-858 (SAM) |
| PHC3* | 48-61; 73-112 | 59-64; 189-195; 200-204; 254-259; 423-427; 549-553; 599-600; 713-723; 755-759; 931-933; 963-964 | 40-114; 138-194; 701-738 (HD1); 931-995 (SAM) |

Numbers indicate amino acid positions for each protein.

* Numbering corresponds to the maximal length isoform PHC3(7).

**Table S2.** Detection of aggregates in yeast cells by fluorescence microscopy (Data for Fig. 3C).

| Construct | Cells with aggregates | | | Total number of cells with fluorescence |
|---|---|---|---|---|
| | Number | % | SE*, % | |
| Aβ42-YFP | 273 | 98.9 | ± 0.6 | 276 |
| Aβ42$^{***}$-YFP | 0 | 0 | + 0.2 | 523 |
| PHC3(1)-CFP | 201 | 94.8 | ± 1.5 | 212 |
| PHC3(5-1)-YFP | 324 | 59.0 | ± 2.1 | 549 |
| PHC3(6)-YFP | 147 | 53.1 | ± 3.0 | 277 |

Data are combined from 4 independent cultures in each case.
*SE – standardized error of proportion. (Same designation is in Table S3.)

**Table S3.** Colocalization of aggregates formed by short and full-length isoforms of PHC3 in yeast cells. (Data for Fig. 3D.)

| Constructs | Cells with aggregates | | | Total number of cells with both types of aggregates |
|---|---|---|---|---|
| | Number | % | SE*, % | |
| YFP-PHC3(5-1) + PHC3(1)-CFP | 99 | 75.6 | ± 3.8 | 131 |
| YFP-PHC3(6) + PHC3(1)-CFP | 69 | 57.5 | ± 4.5 | 120 |

**Table S4.** Localization of full-length PHC3 in cells with overexpression of truncated PHC3 isoforms. (Data for Fig. 5D.)

| Constructs | Cells with cytosolic full-length PHC3 | | | Total number of cells analyzed |
|---|---|---|---|---|
| | Number | % | SE*, % | |
| No vector | 13 | 7.0 | ± 1.9 | 187 |
| CMV-EGFP | 8 | 7.6 | ± 2.6 | 106 |
| CMV-PHC3(5-1)-EGFP | 7 | 10.5 | ± 3.7 | 67 |
| CMV-PHC3(6)-EGFP | 10 | 12.1 | ± 3.6 | 83 |

**Table S5.** Plasmid and viral vectors used in this study.

| Vector | Promoter/Gene | Marker(s) | Source |
|---|---|---|---|
| pCUP1-YFP(-2)-INTctd | $P_{CUP1}$-YFP-INTctd | *2μ LEU2* $Amp^R$ | This study |
| pCUP1-Sup35(NM)Om-CFP | $P_{CUP1}$-*SUP35(NM)*$_{Om}$-*CFP* | *2μ URA3* $Amp^R$ | This study |
| pCUP1-Sup35N-PHC3(5-1) | $P_{CUP1}$-*SUP35N-PHC3(5-1)* | *CEN URA3* $Amp^R$ | This study |
| pCUP1-Sup35N-PHC3(5-2) | $P_{CUP1}$-*SUP35N-PHC3(5-2)* | *CEN URA3* $Amp^R$ | This study |
| pCUP1-Sup35N-PHC3(6) | $P_{CUP1}$-*SUP35N-PHC3(6)* | *CEN URA3* $Amp^R$ | This study |
| pCUP1-Sup35N-Aβ42 | $P_{CUP1}$-*SUP35N-Aβ42* | *CEN URA3* $Amp^R$ | [41] |
| pCUP1-Sup35N- Aβ42$^{***}$ | $P_{CUP1}$-*SUP35N-Aβ42*$^{***}$ | *CEN URA3* $Amp^R$ | [41] |
| pCUP1-YFP-PHC3(5-1) | $P_{CUP1}$-*YFP-PHC3(5-1)* | *2μ LEU2* $Amp^R$ | This study |
| pCUP1-YFP-PHC3(6) | $P_{CUP1}$-*YFP-PHC3(6)* | *2μ LEU2* $Amp^R$ | This study |
| pCUP1-PHC3(1)-CFP | $P_{CUP1}$-*PHC3(1)-CFP* | *2μ URA3* $Amp^R$ | This study |
| pCUP1-Aβ42-YFP | $P_{CUP1}$-*Aβ42-YFP* | *2μ LEU2* $Amp^R$ | This study |
| pCUP1-Aβ42$^{***}$-YFP | $P_{CUP1}$-*Aβ42*$^{***}$*-YFP* | *2μ LEU2* $Amp^R$ | This study |
| pGPD-PrP-YFP | $P_{GPD}$-*Prnp-YFP* | *2μ URA3* $Amp^R$ | [54] |
| pC-DAG-CsgAss-PHC3(5-1) | $P_{BAD}$-*CsgAss-PHC3(5-1)* | $Amp^R$ | This study |
| pC-DAG-CsgAss-PHC3(6) | $P_{BAD}$-*CsgAss-PHC3(6)* | $Amp^R$ | This study |
| pVS72 | $P_{BAD}$-*CsgAss-Sup35NM* | $Amp^R$ | [24] |
| pVS105 | $P_{BAD}$-*CsgAss-Sup35M* | $Amp^R$ | [24] |
| pLenti-CMV-GFP Hygro (656-4) | $P_{CMV}$-*EGFP* | $Hyg^R$ $Amp^R$ | [55] |
| LV-CMV-EGFP Hygro (656–4) | $P_{CMV}$-*EGFP* | $Hyg^R$ | This study |
| pMD2.G | $P_{CMV}$-*VSV-G* | $Amp^R$ | Didier Trono (Addgene #12259) |
| psPAX2 | $P_{CAG}$-*GAG, Pol, Rev, Tat* | $Amp^R$ | Didier Trono (Addgene #12260) |
| pLenti-CMV-PHC3(5-1)-EGFP | $P_{CMV}$-*PHC3(5-1)-EGFP* | $Hyg^R$ $Amp^R$ | This study |
| pLenti-CMV-PHC3(6)-EGFP | $P_{CMV}$-*PHC3(6)-EGFP* | $Hyg^R$ $Amp^R$ | This study |

**Table S6.** Primers used in this study.

| Primer | Sequence 5′-3′ |
|---|---|
| PHC3(5)-SfiI-For | TAGGCCATTATGGCCGCGGAAGCGGAATTTAAG |
| PHC3(5,6)-SfiI-Rev | GGCCGAGGCGGCCTTAAAATTTTAAATCCAG |
| PHC3(6)-SfiI-For | TAGGCCATTATGGCCGATACTGAACCAAACCC |
| PHC3(5)-NotI+1-For | ATGCGGCCGCAATGGCGGAAGCG |
| PHC3(5,6)-XbaI-Rev | GGACTCTAGATTAAAATTTTAAATCCAGAAAAAAAAAAATTTAGGG |
| PHC3(6)-NotI-For | ATGCGGCCGCAATGGATACTGAACC |
| PHC3(5)-XbaI-For | CATCTAGAATGGCGGAAGCGGAATTTAAGG |
| PHC3(5,6)-BamHI+2-Rev | GTGGATCCGCAAATTTTAAATCCAGAAAAAAAAAATTTAG |
| PHC3(6)-XbaI-For | ACTCTAGAATGGATACTGAACCAAACCCG |
| PHC3(For)983 | AAGGATCCATGGATACTGAACCAAACCCGGGAACATCTTCTGTG |
| PHC3(Rev) | AATCTAGAAGATTCCTTCAGAGAGTTGATGCGTGCACAGATCTT |