Chaperonin containing TCP-1 (CCT/TRiC) is a novel therapeutic and diagnostic target for neuroblastoma

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### Table S1: RT-qPCR Primers

| Gene     | Forward                      | Reverse                      |
|----------|------------------------------|------------------------------|
| GAPDH    | GAA GGT GAA GGT CGG AGTCAA C | TGG AAG ATG GTG ATG GGA TTT C |
| CCT3     | TCAGTCCGGTGGT GCATCTTTGG     | CCTCCAGGTATCTTTTCCACTCT     |
| CCT2-FLAG| CAG AGG TGA TCC TTC TGC GTG TG | TGT CGT CGT CCT TGT AG       |
| CCT2     | GGT AGA AAG CCA CGA CGA AG   | GGT GCC AGA GCC TTT CAG GC  |

### Table S2: CCT2 Sequences

**Sequence:**

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MASLSLAVNIFKAGADEERAAETARLTSFIGAIAIGDLVKSTLGPKMDKILLSSGRDASLMVTNDGATILKINGVDNPAAKVLVDVMSVQDEDVGDDGTTVSLAAELREAESLIAKHHIPQTIAGWREATKAAAREALLSSAVDHGSDEVKFRQDLMNAGTTLSSHDLTHDHFTKLAVEAVRLKGSNGLEAIHIKIKLGGLSLDSYLEDGFLDDKKGVNPQKRKENAKILANTGMDTDFKIFGSRVFVDSTAKVAEIIEAEKMEKVERILKHIGNCFNPHEQYLPFGAAVMAEAPDFAVERALVTGGEIABSTDFDPELVKLGSCLKLEEMDKHPSFGALGACTVLRGATQQILDEAERSHLADVCVLAQKDSRTYVGCGSMELDHAIVOLRNPTEKAEVAMYESYAKALRMLPTIIADGNYDSADLVAQLRRAHSEGNNTAGMTDREGTIGDMAILGITESFQVRQVLLSAEAAEVLRVDNIIKAAPRKRVPDHPGGGGGCENLYFQSS
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**Sequence:**

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CATATGGCGAGCCCTAGCCTGGCCGCGGTGAACATCTTCAAAGCGGGGTGCAGGACGAGAGGC
CTGGCGGAAACCCTGCCTGACCCAGCTTTCATCGGCGACATCGGTATTTGTTGAGCCTGTTGGAAG
AGCAAGCCTTCGGCCGGAAGGTATTTCTGAGACGCCGTTGCTGAGTCCGACCCGCACC
TGATGGTGACCAACGATGCGCGACCACCTCTGAGAAGACATTTGGGTGTCAGCACCCCGGCCG
GAAAGTCTCTGCGTATGAGCGCGTTGAGTTTGCGCGTTGCGTGGAGTGTGCGTCGCTCGAC
CTGGCGGTCTTCCGCGCGGGAACCTGCCGCTGTGAGGCGGAAAGTGGATGGGTCTCCTGAC
TCCCGGGATACCCCGGGAAGAAGAAAATGCAAGAGAAATGGTACCCCGGACGTGGATTTGG
ACAAAAAAAAGAGAGAGAAGAAAAAAAATCAGGAAAGGAAGTAACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
TGGCGAGACATCGAACCCGGAAGAAGAAAAATGCAAGAGAAATGGTACCCCGGAGGACAC
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This table provides the primers used for RT-qPCR experiments. The primers are designed to amplify specific genes, with sequences provided for each primer pair. The Table S2 sequences provide the full-length sequence of the CCT2 gene, which can be used for further molecular biology studies such as cloning or sequencing.
Table S3. Number of tumor tissue types

| Figure 1A | # of tissue samples |
|-----------|---------------------|
| GTEx      | 7862                |
| TCGA      | 734                 |
| TARGET    | 10535               |

| Figure 1B and 1E | |
|------------------|---|
| Solid Tissue Normal | 11 |
| Primary Solid Tumor | 286 |

| Figure 1C | |
|-----------|---|
| Ganglioneuroblastoma | 3 |
| Non-germinomatous germ cell tumor | 1 |
| Hemangioblastoma | 3 |
| Teratoma | 9 |
| Neurofibroma/Plexiform | 21 |
| Oligodendrogliaoma | 2 |
| Malignant peripheral nerve sheath tumor (MPNST) | 4 |
| Langerhans Cell histiocytosis | 4 |
| Glial-neuronal tumor NOS | 5 |
| Sarcoma | 5 |
| Chordoma | 6 |
| Not Reported | 2 |
| Dysplasia/Gliosis | 15 |
| Meningioma | 29 |
| Other | 34 |
| Schwannoma | 17 |
| Cavernoma | 1 |
| Dysembryoplastic neuroepithelial tumor (DNET) | 25 |
| Ganglioglioma | 49 |
| Germinoma | 5 |
| Gliomatosis Cerebri | 2 |
| Craniopharyngioma | 36 |
| Brainstem glioma- Diffuse intrinsic pontine glioma | 14 |
| Tumor Type                                           | Count |
|-----------------------------------------------------|-------|
| Ependymoma                                          | 93    |
| Low-grade glioma/astrocytoma (WHO grade I/II)       | 254   |
| Adenoma                                             | 3     |
| Metastatic secondary tumors                         | 7     |
| Neurocytoma                                         | 3     |
| Neuroblastoma                                       | 5     |
| Atypical Teratoid Rhabdoid Tumor (ATRT)             | 30    |
| Ewing's Sarcoma                                     | 8     |
| High-grade glioma/astrocytoma (WHO grade III/IV)    | 103   |
| Medulloblastoma                                     | 119   |
| Pineoblastoma                                       | 3     |
| Choroid plexus papilloma                            | 15    |
| Subependymal Giant Cell Astrocytoma (SEGA)          | 3     |
| Supratentorial or Spinal Cord PNET                   | 16    |
| Choroid plexus carcinoma                            | 4     |
| Primary CNS lymphoma                                | 1     |
| Rhabdomyosarcoma                                    | 2     |

**Figure 1D**

| Subproject                                           | Count |
|------------------------------------------------------|-------|
| Acute Myeloid Leukemia, Induction Failure Subproject | 32    |
| ALL                                                  | 194   |
| AML                                                  | 196   |
| Clear cell sarcoma of Kidney                         | 13    |
| Neuroblastoma                                        | 162   |
| Wilms Tumor                                          | 126   |

### Table S4. Percent of neuroblastoma tumors with CCT2 score (NB641c, US Biomax)

| CCT2 Score | % CCT2 Score |
|------------|--------------|
| 0          | 13%          |
| 1          | 5.6%         |
| 2          | 11.1%        |
| 3          | 53.7%        |
| 4          | 13%          |
| NR         | 3.7%         |

NR, not readable
Table S5. CCT2 score for Neuroblastoma tissue microarray (TMA), NB641c (US Biomax), with INSS stage and IHC marker (CD56/CgA)

| Number | CD56 | CgA | INSS stage | CCT2 score |
|--------|------|-----|------------|------------|
| 45     | -    | -   | I          | 0          |
| 46     | -    | -   | I          | 0          |
| 1      | +    | -   | I          | 0          |
| 2      | +    | -   | I          | 1          |
| 3      | ++   | ++  | I          | 0          |
| 4      | ++   | ++  | I          | 0          |
| 39     | ++   | +   | I          | 0          |
| 40     | ++   | +   | I          | 0          |
| 43     | ++   | -   | I          | 1          |
| 44     | ++   | -   | I          | 1          |
| 32     | ++   | ++  | I          | 2          |
| 31     | ++   | ++  | I          | 3          |
| 9      | ++   | -   | I          | 4          |
| 10     | ++   | -   | I          | 4          |
| 8      | +++  | ++  | I          | 2          |
| 15     | +++  | +++ | I          | 2          |
| 17     | +++  | +++ | I          | 2          |
| 49     | +++  | +++ | I          | 2          |
| 50     | +++  | +++ | I          | 2          |
| 6      | +++  | ++  | IV         | 3          |
| 7      | +++  | ++  | I          | 3          |
| 11     | +++  | +++ | I          | 3          |
| 12     | +++  | +++ | I          | 3          |
| 13     | +++  | +++ | I          | 3          |
| 14     | +++  | +++ | I          | 3          |
| 16     | +++  | +++ | I          | 3          |
| 18     | +++  | +++ | I          | 3          |
| 19     | +++  | +++ | I          | 3          |
| 20     | +++  | +++ | I          | 3          |
| 21     | +++  | +++ | IIB        | 3          |
| 22     | +++  | +++ | IIB        | 3          |
| 23     | +++  | +++ | IV         | 3          |
| 24     | +++  | +++ | IV         | 3          |
| 29     | +++  | +++ | I          | 3          |
| 30     | +++  | +++ | I          | 3          |
| 35     | +++  | +   | I          | 3          |
|   |     |   |   |   |   |
|---|-----|---|---|---|---|
| 36| +++| + | I | 3 |
| 37| +++| + | I | 3 |
| 38| +++| + | I | 3 |
| 41| +++| +++| I | 3 |
| 42| +++| +++| I | 3 |
| 47| +++| ++ | I | 3 |
| 48| +++| ++ | I | 3 |
| 51| +++| -  | I | 3 |
| 52| +++| -  | I | 3 |
| 53| +++| +  | I | 3 |
| 54| +++| +  | I | 3 |
| 5 | +++| ++ | IV| 4 |
| 25| +++| ++ | I | 4 |
| 26| +++| ++ | I | 4 |
| 33| +++| ++ | I | 4 |
| 34| +++| ++ | I | 4 |
Figure S1. HiLoad 16 600 S200 CCTb60mM. (A) Purified protein from SEC column shows a single peak. (B) Homology modeling using MOE was done. Simulations of CT20p docking were performed with the atomic model of bovine CCT2 subunit derived from a 4.0 Angstrom cryo-EM map. Binding energies of CT20p on CCT2 are shown and most energetically feasible site for CT20p binding on CCT2 is shown by arrows.
Figure S2. Pediatric malignant tumor tissue microarray (TMA) with normal tissue as control, PC701 (US Biomax), processed by IHC for CCT2. (A) Full image of TMA for stained for CCT2. (B) TMA map (n=70), duplicate cores.
Figure S3. Neuroblastoma and peripheral nerve tissue microarray (TMA), with INSS stage, IHC marker (CD56/CgA), NB641c (US Biomax), processed by IHC for CCT2. (A) Full image of TMA stained for CCT2. (B) TMA map, (n=27), duplicate cores. Legend: Adr- Adrenal gland, Med- Mediastinum/left posterior, Pel- Pelvic cavity, Per- Peripheral nerve, Ret- Retroperitoneum.
Figure S4. Representative images from Analyzer II for manually stained IMR-32 cells. CCT2-PE antibody concentration was 12 ug/mL (A) and 24 ug/mL (B). Column one is overlay of columns 2 and 3 which are cytokeratin and DAPI respectively, column 4 is CD45 marker for leukocytes and column 5 is for CCT2-PE antibody.