

## **Supporting Information**

Human Concentrative Nucleoside Transporter 3 (hCNT3, SLC28A3) Forms a Cyclic Homotrimer

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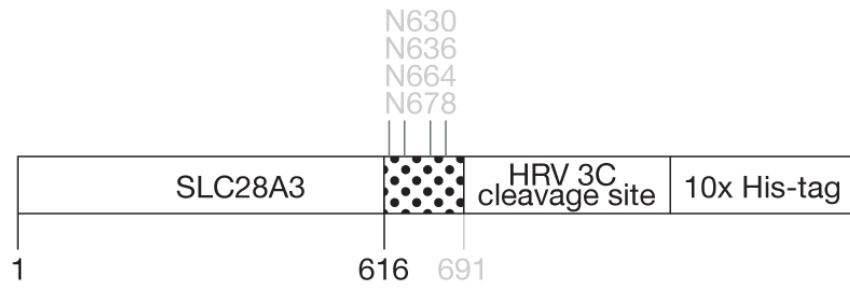
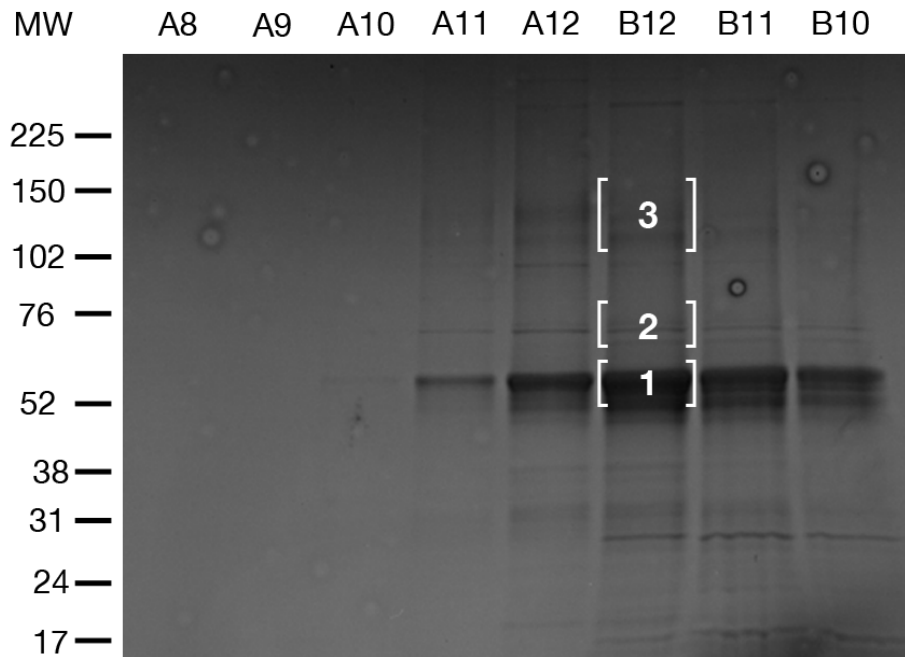
**A****B**

Figure S1 Expression and purification of hCNT3 from High Five insect cells. A) Schematic of the expression construct. B) Coomassie stained gel of the selected fractions, indicating the areas that were cut out for mass spectrometry analysis.

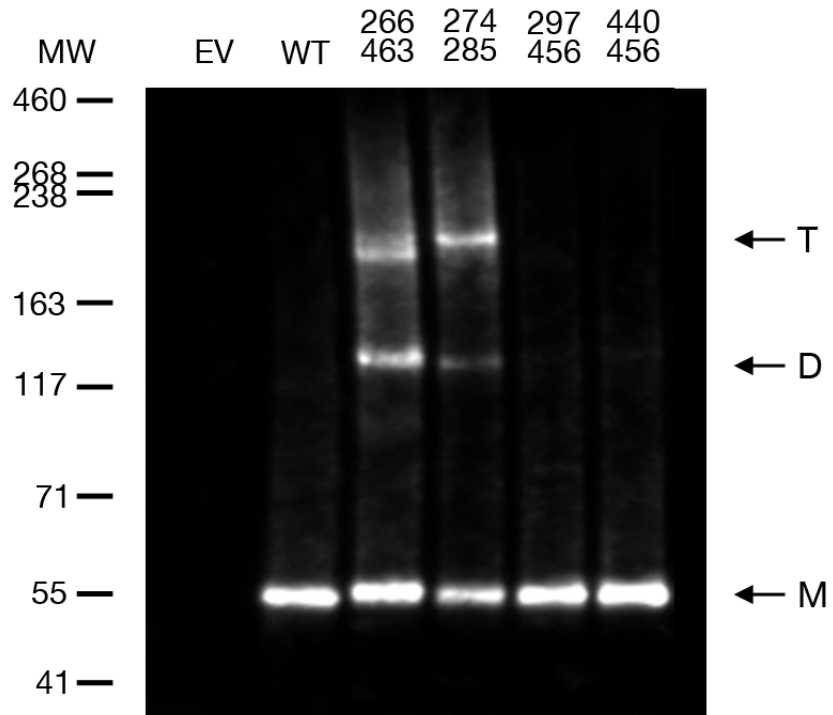


Figure S2 Western blot of the cysteine I<sub>2</sub> cross-linking experiment involving V274C/A285C, Y266C/F463C, T297C/A456C, and A440C/A456C double mutants.

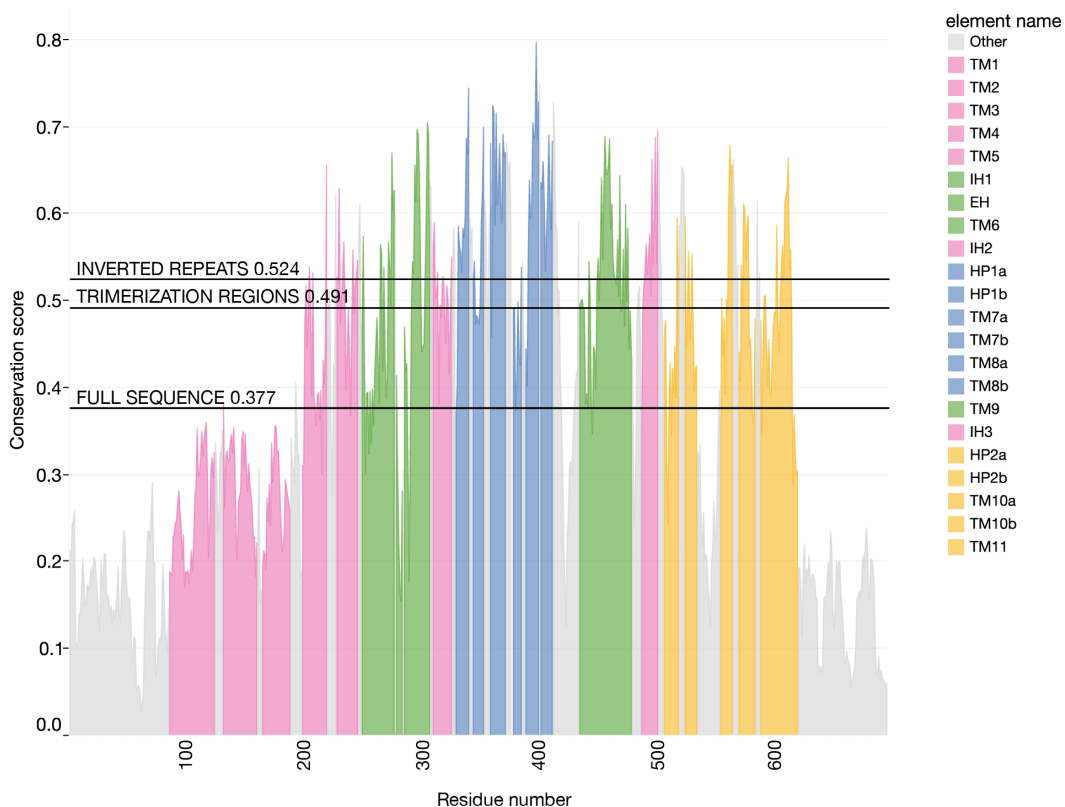


Figure S3 Graph of the sequence conservation score plotted for each residue. Colors follow the domains outlined in Figure 2A. Averages represent the full sequence, trimerization domain (IH1, EH, TM6, TM9), and inverted repeats (HP1a, HP1b, TM7a, TM7b, TM8a, TM8b, HP2a, HP2b, TM10a, TM10b, TM11). Secondary structure definitions: TM1 86-123, TM2 131-159, TM3 164-187, TM4 198-218, TM5 227-244, IH1 248-275, EH 277-282, TM6 284-305, IH2 308-323, HP1a 327-338, HP1b 342-350, TM7a 356-369, TM7b 375-382, TM8a 386-397, TM8b 399-408, TM9 432-475, IH3 484-497, HP2a 503-515, HP2b 520-530, TM10a 550-560, TM10b 566-579, TM11 584-615.



Sample ID	UNIPROT ID	Number Unique	Peptide Count	% Sample	% Coverage	Protein MW	Species	Source	Protein Name
1	Q9HAS3	16	114	97%	20.8	76931	HUMAN	Target	Solute carrier family 28 member 3
1	Q2F6C3	2	3	3%	7	57745.4	BOMMO	Insect	Chaperonin subunit 6a zeta
2	Q9HAS3	12	35	41%	17.9	76931	HUMAN	Target	Solute carrier family 28 member 3
2	Q9U639	13	18	21%	23.8	71432.3	MANSE	Insect	Heat shock 70 kDa protein cognate 4
2	Q00704	9	12	14%	14.9	79075.9	NPVAC	Virus	Occlusion-derived virus envelope protein E66
2	A9LST1	7	7	8%	25.1	35944	SPOEX	Insect	Activated C kinase 1 receptor
2	P41473	4	4	5%	23	30567.3	NPVAC	Virus	Uncharacterized 30.6 kDa protein in IAP2-VLF1 intergenic region
2	Q0MUU6	4	4	5%	8	73138.6	TRINI	Insect	Heat shock cognate 70 protein
2	G6C119	3	3	3%	2.7	166194.3	DANPL	Insect	Eukaryotic translation initiation factor 3 subunit A
2	D6WLZ3	2	3	3%	3.9	83204.3	TRICA	Insect	Putative uncharacterized protein
3	Q9HAS3	10	23	51%	18.2	76931	HUMAN	Target	Solute carrier family 28 member 3
3	A9LST1	9	9	20%	32.3	35944	SPOEX	Insect	Activated C kinase 1 receptor
3	P41473	4	6	13%	23	30567.3	NPVAC	Virus	Uncharacterized 30.6 kDa protein in IAP2-VLF1 intergenic region
3	G6D0K3	2	4	9%	3.1	142316.5	DANPL	Insect	Putative ubiquitin specific protease 7
3	A7LAG1	2	3	7%	3.1	112072.5	FHV	Virus	Protein A

Table S1 Table containing the identity of peptides identified in each of the samples (shown in Figure S1B) by mass spectrometry.