Attempted expression and purification of full-length huntingtin Q23 with putative interaction partners from baculoviral expression system production in sf9 insect cells – 2018/07/19

Rationale:

To obtain monodisperse and conformationally constrained huntingtin protein samples for high resolution structural biology, interaction partners are required as highlighted by Guo et al (2018).

Constructs:

Putative interactor	Component	Construct	Molecular Weight (kDa)	Sequence (including tags)
HDAC4		JMC110A07	121.3	MHHHHHHSSGRENLYFQGMSSQSHPDGLSGRDQPVELLNPARVNHMPSTVDVATALPLQVAPSAVPMDLRLDHQFSLPVAEPALREQQLLQQELLALKQKQQIQRQILIAEFQRQHEQLSRQHEAQLHE HIKQQQEMLAMKHQQELLEHQRKLERHRQEQELEKQHREQKLQQLKNKEKGKESAVASTEVKMKLQEFVLNKKKALAHRNLNHCISSDPRYWYGKTQHSSLDQSSPPQSGVSTSYNHPVLGMYDAKDDF PLRKTASEPNLKLRSRLKQKVAERRSSPLLRRKDGPVVTALKKRPLDVTDSACSSAPGSGPSSPNNSSGSVSAENGIAPAVPSIPAETSLAHRLVAREGSAAPLPLYTSPSLPNITLGLPATGPSAGTAGGQDTER LTLPALQQRLSLFPGTHLTPYLSTSPLERDGGAAHSPLLQHMVLLEQPPAQAPLVTGLGALPLHAQSLVGADRVSPSIHKLRQHRPLGRTQSAPLPQNAQALQHLVIQQQHQQFLEKHKQQFQQQQLQMN KIIPKPSEPARQPESHPEETEEELREHQALLDEPYLDRLPGQKEAHAQAGVQVKQEPIESDEEEAEPPREVEPGQRQPSEQELLFRQQALLLEQQRIHQLRNYQASMEAAGIPVSFGGHRPLSRAQSSPASATF PVSVQEPPTKPRFTTGLVYDTLMLKHQCTCGSSSSHPEHAGRIQSIWSRLQETGLRGKCECIRGRKATLEELQTVHSEAHTLLYGTNPLNRQKLDSKKLLGSLASVFVRLPCGGVGVDSDTIWNEVHSAGAARL AVGCVVELVFKVATGELKNGFAVVRPPGHHAEESTPMGFCYFNSVAVAAKLLQQRLSVSKILIVDWDVHHGNGTQQAFYSDPSVLYMSLHRYDDGNFFPGSGAPDEVGTGPGVGFNVNMAFTGGLDPP MGDAEYLAAFRTVVMPIASEFAPDVVLVSSGFDAVEGHPTPLGGYNLSARCFGYLTKQLMGLAGGRIVLALEGGHDLTAICDASEACVSALLGNELDPLPEKVLQQRPNANAVRSMEKVMEIHSKYWRCLQ RTTSTAGRSLIEAQTCENEEAETVTAMASLSVGVKPAEKRPDEEPMEEEPPL
USP9X		YTC039G10	292.7	MHHHHHHSSGRENLYFQGMTATTRGSPVGGNDNQGQAPDGQSQPPLQQNQTSSPDSSNENSPATPPDEQGQGDAPPQLEDEEPAFPHTDLAKLDDMINRPRWVVPVLPKGELEVLLEAAIDLSKKGL DVKSEACQRFFRDGLTISFTKILTDEAVSGWKFEIHRCIINNTHRLVELCVAKLSQDWFPLLELLAMALNPHCKFHIYNGTRPCESVSSSVQLPEDELFARSPDPRSPKGWLVDLLNKFGTLNGFQILHDRFINGS ALNVQIIAALKPFGQCYEFLTLHTVKKYFLPIIEMVPQFLENLTDEELKKEAKNEAKNDALSMIIKSLKNLASRVPGQEETVKNLEIFRLKMILRLLQISSFNGKMNALNEVNKVISSVSYYTHRHGNPEEEEWLTA ERMAEWIQQNNILSIVLRDSLHQPQYVEKLEKILRFVIKEKALTLQDLDNIWAAQAGKHEAIVKNVHDLLAKLAWDFSPEQLDHLFDCFKASWTNASKKQREKLLEIIRRLAEDDKDGVMAHKVLNLLWNLA HSDDVPVDIMDLALSAHIKILDYSCSQDRDTQKIQWIDRFIEELRTNDKWVIPALKQIREICSLFGEAPQNLSQTQRSPHVFYRHDLINQLQHNHALVTLVAENLATYMESMRLYARDHEDYDPQTVRLGSRY SHVQEVQERLNFLRFLLKDGQLWLCAPQAKQIWKCLAENAVYLCDREACFKWYSKLMGDEPDLDPPINKDFFESNVLQLDPSLLTENGMKCFERFFKAVNCREGKLVAKRRAYMMDDLELIGLDYLWRVV IQSNDDIASRAIDLLKEIYTNLGPRLQVNQVVIHEDFIQSCFDRLKASYDTLCVLDGDKDSVNCARQEAVRMVRVLTVLREYINECDSDYHEERTILPMSRAFRGKHLSFVVRFPNQGRQVDDLEVWSHTNDTI GSVRRCILNRIKANVAHTKIELFVGGELIDPADDRKLIGQLNLKDKSLITAKLTQISSNMPSSPDSSSDSSTGSPGNHGNHYSDGPNPEVESCLPGVIMSLHPRYISFLWQVADLGSSLNMPPLRDGARVLMKL MPPDSTTIEKLRAICLDHAKLGESSLSPSLDSLFFGPSASQVLYLTEVVYALLMPAGAPLADDSSDFQFHFLKSGGLPLVLSMLTRNNFLPNADMETRRGAYLNALKIAKLLLTAIGYGHVRAVAEAECQPGVEGV NPMTQINQVTHDQAVVLQSALQSIPNPSSECMLRNVSVRLAQQISDEASRYMPDICVIRAIQKIIWASGCGSLQLVFSPNEEITKIYEKTNAGNEPDLEDEQVCCEALEVMTLCFALIPTALDALSKEKAWQTF IIDLLLHCHSKTVRQVAQEQFFLMCTRCCMGHRPLLFFITLLFTVLGSTARERAKHSGDYFTLLRHLLNYAYNSNINVPNAEVLLNNEIDWLKRIRDDVKRTGETGIEETILEGHLGVTKELLAFQTSEKKFHIGCE KGGANLIKELIDDFIFPASNVYLQYMRNGELPAEQAIPVCGSPPTINAGFELLVALAVGCVRNLKQIVDSLTEMYYIGTAITTCEALTEWEYLPPVGPRPPKGFVGLKNAGATCYMNSVIQQLYMIPSIRNGILAI EGTGSDVDDDMSGDEKQDNESNVDPRDDVFGYPQQFEDKPALSKTEDRKEYNIGVLRHLQVIFGHLAASRLQYYVPRGFWKQFRLWGEPVNLREQHDALEFFNSLVDSLDEALKALGHPAMLSKVLGGSF ADQKICQGCPHRYEYCEESFTTLNVDIRNHQNLLDSLEQYVKGDLLEGANAYHCEKCNKKVDTVKRLLIKKLPPVLAIQLKRFDYDWERECAIKFNDYFEFPRELDMEPYTVAGVAKLEGDNVNPESQLIQQSE QSESETAGSTKYLRVGVLVHSGQASGGHYYSYIIQRNGGDGERNRWYKFDDGDVTECKMDDDEEMKNQCFGGEYMGEVFDHMMKRMSYRRQKRWWNAYILFYERMDTIDQDDELIRYISELAITTRPH QIIMPSAIERSVRKQNVQFMHNRMQYSMEYFQFMKKLLTCNGVYLNPPPGQDHLLPEAEEITMISIQLAARFLFTTGFHTKKVVRGSASDWYDALCILLRHSKNVRFWFAHNVLFNVSNRFSEYLLECPSAE VRGAFAKLIVFIAHFSLQDGPCPSPASGPGSSQAYDNLSLSDHLLRAVLNLLRREVSEHGRHLQQYFNLFVMYANLGVAEKTQLLKLSVPATFMLVSLDEGPGPPIKYQYAELGKLYSVVSQLIRCCNVSSRM QSSINGNPPLPNPFGDPNLSQPIMPIQQNVADILFVRTSYVKKIIEDCSNSEETVKLLRFCCWENPQFSSTVLSELLWQVAYSYTYELRPYLDLLLQILIEDSWQTHRIHNALKGIPDDRDGLFDTIQRSKNHYQ KRAYQCIKCMVALFSNCPVAYQILQGNGDLKRKWTWAVEWLGDELERRPYTGNPQYTYNNWSPPVQSNETSNGYFLERSHSARMTLAKACELCPEEEPDDQDAPDEHESPPPEDAPLYPHSPGSQYQQ NNHVHGQPYTGPAAHHMNNPQRTGQRAQENYEGSEEVSPPQTKDQ
HTATIP		JMC118F04	60.8	MHHHHHHSSGRENLYFQGMAEVGEIIEGCRLPVLRRNQDNEDEWPLAEILSVKDISGRKLFYVHYIDFNKRLDEWVTHERLDLKKIQFPKKEAKTPTKNGLPGSRPGSPEREVPASAQASGKTLPIPVQITLRF NLPKEREAIPGGEPDQPLSSSSCLQPNHRSTKRKVEVVSPATPVPSETAPASVFPQNGAARRAVAAQPGRKRKSNCLGTDEDSQDSSDGIPSAPRMTGSLVSDRSHDDIVTRMKNIECIELGRHRLKPWYFSP YPQELTTLPVLYLCEFCLKYGRSLKCLQRHLTKCDLRHPPGNEIYRKGTISFFEIDGRKNKSYSQNLCLLAKCFLDHKTLYYDTDPFLFYVMTEYDCKGFHIVGYFSKEKESTEDYNVACILTLPPYQRRGYGKLLIEFS YELSKVEGKTGTPEKPLSDLGLLSYRSYWSQTILEILMGLKSESGERPQITINEISEITSIKKEDVISTLQYLNLINYYKGQYILTLSEDIVDGHERAMLKRLLRIDSKCLHFTPKDWSKRGKW
PRMT5 complex	PRMT5	JMC046A09	72.7	MAAMAVGGAGGSRVSSGRDLNCVPEIADTLGAVAKQGFDFLCMPVFHPRFKREFIQEPAKNRPGPQTRSDLLLSGRDWNTLIVGKLSPWIRPDSKVEKIRRNSEAAMLQELNFGAYLGLPAFLLPLNQEDN TNLARVLTNHIHTGHHSSMFWMRVPLVAPEDLRDDIIENAPTTHTEEYSGEEKTWMWWHNFRTLCDYSKRIAVALEIGADLPSNHVIDRWLGEPIKAAILPTSIFLTNKKGFPVLSKMHQRLIFRLLKLEVQFII TGTNHHSEKEFCSYLQYLEYLSQNRPPPNAYELFAKGYEDYLQSPLQPLMDNLESQTYEVFEKDPIKYSQYQQAIYKCLLDRVPEEEKDTNVQVLMVLGAGRGPLVNASLRAAKQADRRIKLYAVEKNPNAVV TLENWQFEEWGSQVTVVSSDMREWVAPEKADIIVSELLGSFADNELSPECLDGAQHFLKDDGVSIPGEYTSFLAPISSSKLYNEVRACREKDRDPEAQFEMPYVVVRLHNFHQLSAPQPCFTFSHPNRDPMID NNRYCTLEFPVEVNTVLHGFAGYFETVLYQDITLSIRPETHSPGMFSWFPILFPIKQPITVREGQTICVRFWRCSNSKKVWYEWAVTAPVCSAIHNPTGRSYTIGL
	MEP50	JMC015F03	38.8	MGSSHHHHHHSSGLVPRGSMRKETPPPLVPPAAREWNLPPNAPACMERQLEAARYRSDGALLLGASSLSGRCWAGSLWLFKDPCAAPNEGFCSAGVQTEAGVADLTWVGERGILVASDSGAVELWEL DENETLIVSKFCKYEHDDIVSTVSVLSSGTQAVSGSKDICIKVWDLAQQVVLSSYRAHAAQVTCVAASPHKDSVFLSCSEDNRILLWDTRCPKPASQIGCSAPGYLPTSLAWHPQQSEVFVFGDENGTVSLVD TKSTSCVLSSAVHSQCVTGLVFSPHSVPFLASLSEDCSLAVLDSSLSELFRSQAHRDFVRDATWSPLNHSLLTTVGWDHQVVHHVVPTEPLPAPGPASVTE
PRC2 trimer complex	EED/EZH2	JMC023TA04	137.3 (80.2/50.1)	MGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQKILERTEILNQEWKQRRIQPVHILTSVSSLRGTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSPLQQNFMVEDETVLHNIP YMGDEVLDQDGTFIEELIKNYDGKVHGDRECGFINDEIFVELVNALGQYNDDDDDDGDDPEERREEKQKDLEDHRDDKESRPPRKFPSDKIFEAISSMFPDKGTAEELKEKYKELTEQQLPGALPPECTPNID GPNAKSVQREQSLHSFHTLFCRRCFKYDCFLHRKCNYSFHATPNTYKRKNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVLESKDTDSDREAGTETGGENN DKEEEEKKDETSSSSEANSRCQTPIKMKPNIEPPENVEWSGAEASMFRVLIGTYYDNFCAIARLIGTKTCRQVYEFRVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHCRKIQLKKDGSSSNHVYNYQPCDHPR QPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQCNTKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLLAPSDVAGWGIFIKDPVQKNEFISEYCGEIISQDEADRRGKVYD KYMCSFLFNLNNDFVVDATRKGNKIRFANHSVNPNCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPHHHHHHHHH MSEREVSTAPAGTDMPAAKKQKLSSDENSNPDLSGDENDDAVSIESGTNTERPDTPTNTPNAPGRKSWGKGKWKSKKCKYSFKCVNSLKEDHNQPLFGVQFNWHSKEGDPLVFATVGSNRVTLYECHSQ GEIRLLQSYVDADADENFYTCAWTYDSNTSHPLLAVAGSRGIIRIINPITMQCIKHYVGHGNAINELKFHPRDPNLLLSVSKDHALRLWNIQTDTLVAIFGGVEGHRDEVLSADYDLLGEKIMSCGMDHSLKL WRINSKRMMNAIKESYDYNPNKTNRPFISQKIHFPDFSTRDIHRNYVDCVRWLGDLILSKSCENAIVCWKPGKMEDDIDKIKPSESNVTILGRFDYSQCDIWYMRFSMDFWQKMLALGNQVGKLYVWDLE VEDPHKAKCTTLTHHKCGAAIRQTSFSRDSSILIAVCDDASIWRWDRLR
	SUZ12	JMC023TB06	85.5	MSYYWSHPQFEKENLYFQGMAPQKHGGGGGGGSGPSAGSGGGGGFGGSAAVAAATASGGKSGGGSCGGGGSYSASSSSSAAAAAGAAVLPVKKPKMEHVQADHELFLQAFEKPTQIYRFLRTRNLIAPIF LHRTLTYMSHRNSRTNIKRKTFKVDDMLSKVEKMKGEQESHSLSAHLQLTFTGFFHKNDKPSPNSENEQNSVTLEVLLVKVCHKKRKDVSCPIRQVPTGKKQVPLNPDLNQTKPGNFPSLAVSSNEFEPSNS HMVKSYSLLFRVTRPGRREFNGMINGETNENIDVNEELPARRKRNREDGEKTFVAQMTVFDKNRRLQLLDGEYEVAMQEMEECPISKKRATWETILDGKRLPPFEFTSQGPTLQFTLRWTGETNDKSTAPIA KPLATRNSESLHQENKPGSVKPTQTIAVKESLTTDLQTRKEKDTPNENRQKLRIFYQFLYNNNTRQQTEARDDLHCPWCTLNCRKLYSLLKHLKLCHSRFIFNYVYHPKGARIDVSINECYDGSYAGNPQDIHR QPGFAFSRNGPVKRTPITHILVCRPKRTKASMSEFLESEDGEVEQQRTYSSGHNRLYFHSDTCLPLRPQEMEVDSEDEKDPEWLRKTITQIEEFSDVNEGEKEVMKLWNLHVMKHGFIADNQMNHACMLF VENYGQKIIKKNLCRNFMLHLVSMHDFNLISIMSIDKAVTKLREMQQKLEKGESASPANEEITEEQNGTANGFSEINSKEKALETDSVSGVSKQSKKQKL
PRC2 pentamer complex (additional components)	AEBP2	MVC001B02	53.9	MEQKLISEEDLAAAITDMADLEELSRLSPLPPGSPGSAARGRAEPPEEEEEEEEEEEAEAEAVAALLLNGGSGGGGGGGGGGVGGGEAETMSEPSPESASQAGEDEDEEEDDEEEEDESSSSGGGEEESSAE SLVGSSGGSSSSDETRSLSPGAASSSSGDGDGKEGLEEPKGPRGSQGGGGGSSSSSVVSSGGDEGYGTGGGGSSATSGGRRGSLEMSSDGEPLSRMDSEDSISSTIMDVDSTISSGRSTPAMMNGQGSTTS SSKNIAYNCCWDQCQACFNSSPDLADHIRSIHVDGQRGGVFVCLWKGCKVYNTPSTQSWLQRHMLTHSGDKPFKCVVGGCNASFASQGGLARHVPTHFSQQNSSKVSSQPKAEESPSKAGMNKRRK LKNKRRRSLPRPHDFFDAQTLDAIRHRAICFNLSAHIESLGKGHSVVFHSTVIAKRKEDSGKIKLLLHWMPEDILPDVWVNESERHQLKTKVVHLSKLPKDTALLLDPNIYRTMPQKRLKR
	RBBP4	APC062D03	49.7	MGSSHHHHHHSSGLVPRGSMADKEAAFDDAVEERVINEEYKIWKKNTPFLYDLVMTHALEWPSLTAQWLPDVTRPEGKDFSIHRLVLGTHTSDEQNHLVIASVQLPNDDAQFDSAHYSDKGEFGGFGS VSGKIEEIKIHNHEGEVNRARYMPQNPCIIATKTPSSDVLVFDYTKHPSKPDPSGECNPDLRLRGHQKEGYGLSWNPNLSGHLLSASDDHTICLWDISAVPKEGKVVDAKTIFTGHTAVVEDVSWHLLHESLFG SVADDQKLMIWDTRSNNTSKPSHSVDAHTAEVNCLSFNPYSEFILATGSADKTVALWDLRNLKLKLHSFESHKDEIFQVQWSPHNETILASSGTDRRLNVWDLSKIGEEQSPEDAEDGPPELLFIHGGHTAKI SDFSWNPNEPWVICSVSEDNIMQVWQMAENIYNDEDPEGSVDPEGQGS
ELP1		JMC035C03	153.4	MHHHHHHSSGRENLYFQGMRNLKLFRTLEFRDIQGPGNPQCFSLRTEQGTVLIGSEHGLIEVDPVSREVKNEVSLVAEGFLPEDGSGRIVGVQDLLDQESVCVATASGDVILCSLSTQQLECVGSVASGISVM SWSPDQELVLLATGQQTLIMMTKDFEPILEQQIHQDDFGESKFITVGWGRKETQFHGSEGRQAAFQMQMHESALPWDDHRPQVTWRGDGQFFAVSVVCPETGARKVRVWNREFALQSTSEPVAGLG PALAWKPSGSLIASTQDKPNQQDIVFFEKNGLLHGHFTLPFLKDEVKVNDLLWNADSSVLAVWLEDLQREESSIPKTCVQLWTVGNYHWYLKQSLSFSTCGKSKIVSLMWDPVTPYRLHVLCQGWHYAYD WHWTTDRSVGDNSSDLSNVAVIDGNRVLVTVFRQTVVPPPMCTYQLLFPHPVNQVTFLAHPQKSNDLAVLDASNQISVYKCGDCPSADPTVKLGAVGGSGFKVCLRTPHLEKRYKIQFENNEDQDVNPLK LGLLTWIEEDVFLAVSHSEFSPRSVIHHLTAASSEMDEEHGQLNVSSSAAVDGVIISLCCNSKTKSVVLQLADGQIFKYLWESPSLAIKPWKNSGGFPVRFPYPCTQTELAMIGEEECVLGLTDRCRFFINDIEVA SNITSFAVYDEFLLLTTHSHTCQCFCLRDASFKTLQAGLSSNHVSHGEVLRKVERGSRIVTVVPQDTKLVLQMPRGNLEVVHHRALVLAQIRKWLDKLMFKEAFECMRKLRINLNLIYDHNPKVFLENVETFIK QIDSVNHINLFFTELKEEDVTKTMYPAPVTSSVYLSRDPDGNKLDLVCDAMRAVMESMNPHKYCLSILTSHVKKTTPELEIVLQKVHELQGNAPSDPDAVSAEEALKYLLHLVDVNELYDHSLGTYDFDLVLM VAEKSQKDPKEYLPFLNTLKKMETNYQRFTIDKYLKRYEKAIGHLSKCGPEYFPECLNLIKDKNLYNEALKLYSPSSQQYQDISIAYGEHLMQEHMYEPAGLMFARCGAHEKALSAFLTCGNWKQALCVAAQL NFTKDQLVGLGRTLAGKLVEQRKHIDAAMVLEECAQDYEEAVLLLEGAAWEEALRLVYKYNRLDIIETNVKPSILEAQKNYMAFLDSQTATFSRHKKRLLVVRELKEQAQQAGLDDEVPHGQESDLFSETSSV VSGSEMSGKYSHSNSRISARSSKNRRKAERKKHSLKEGSPLEDLALLEALSEVVQNTENLKDEVYHILKVLFLFEFDEQGRELQKAFEDTLQLMERSLPEIWTLTYQQNSATPVLGPNSTANSIMASYQQQKTSV PVLDAELFIPPKINRRTQWKLSLLDHHHHHH

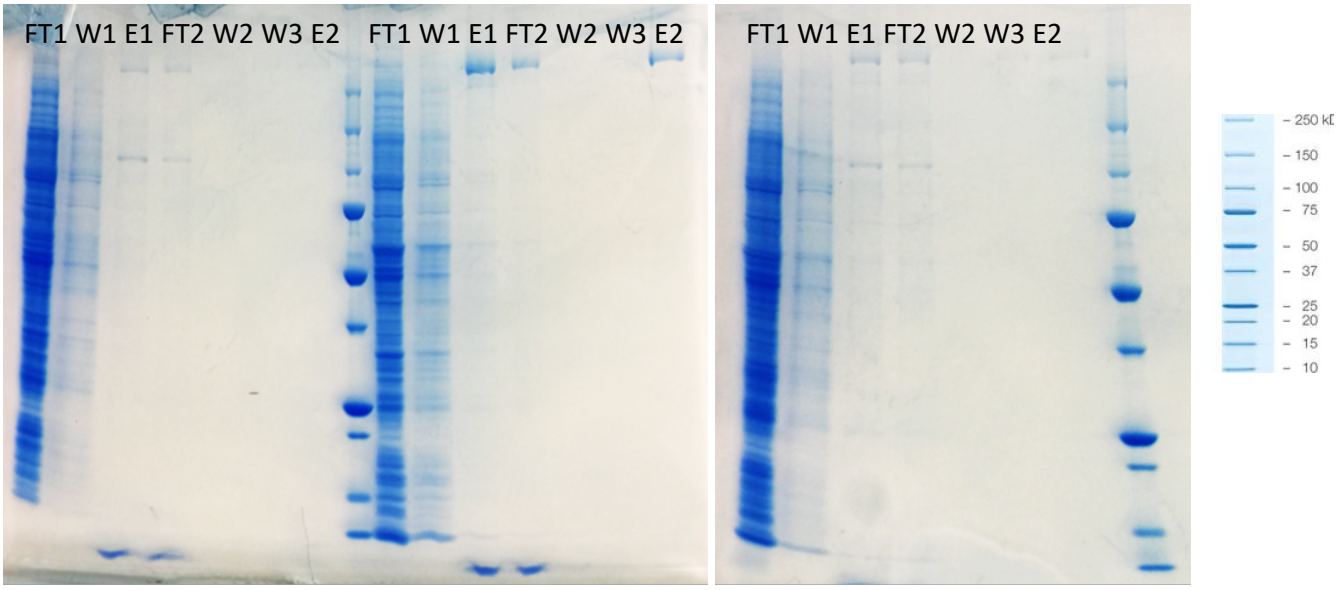
Growth:

3:1 HTT Q23 (FLAG-tagged, TOC009:D01) to binding partner (His-tagged) virus ratios used for 1 L BVES sf9 production. Cells were harvested by centrifugation at 4000 rpm, 10 mins, 4 °C (Beckman JLA 8.1000). HTT cell pellets were resuspended in ~80 mL of 50 mM Tris pH 8, 300 mM NaCl. Cell resuspensions were spiked with protease inhibitor mix and then stored at -80 ˚C prior to purification in 40 mL aliquots. Full for BVES production methods are here: https://zenodo.org/record/154611

Purification round 1 – HDAC4/USP9X/HTATIP:

Cell pastes were thawed and diluted to 250 mL with 50 mM Tris pH 8, 300 mM NaCl and supplemented with benzonase and 1 x protease inhibitors. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation at 15,000 rpm, 1 h, 4 °C (Beckman JLA16.2500) and then bound to 2 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT1). Lysate-resin mix was transferred to an open column (BioRad) and the resins then washed with 250 mL 50 mM Tris pH 8, 300 mM NaCl (wash – W1). HTT protein was eluted with ~5 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide (elution – E1). The sample was then rocked with 1 mL Ni-NTA at 4 ˚C with rocking for 30 mins (flow through – FT2). Ni-NTA beads were washed with 50 mM Tris pH 8, 300 mM NaCl then 50 mM Tris pH 8, 300 mM NaCl supplemented with 15 mM imidazole (wash – W2 and W3) and then eluted with 50 mM Tris pH 8, 300 mM NaCl, 300 mM imidazole (elution – E2). Samples were analysed by 4-20 % tris-glycine SDS-PAGE.

SDS-PAGE:



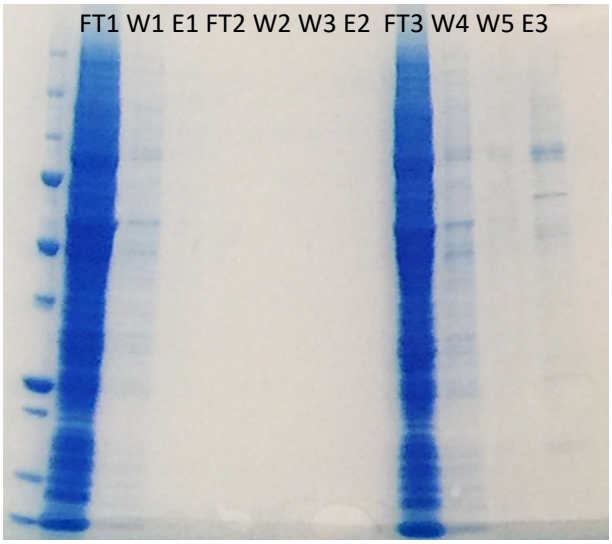
HTT + HDAC4 HTT + HTATIP HTT + USP9X

Purification round 2 – PRC2 3-mer/PRC2 5-mer/PRMT5-MEP50/USP9X:

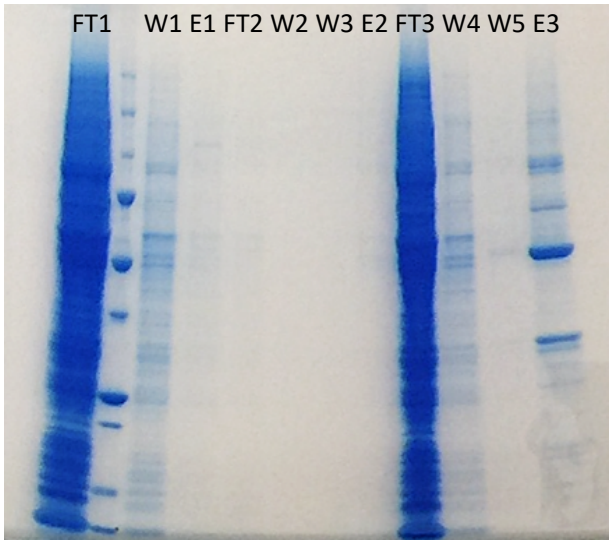
Cell pastes were thawed and diluted to 250 mL with 50 mM Tris pH 8, 300 mM NaCl and supplemented with benzonase and 1 x protease inhibitors. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation at 15,000 rpm, 1 h, 4 °C (Beckman JLA16.2500) and then bound to 2 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT1). Lysate-resin mix was transferred to an open column (BioRad) and the resins then washed with 250 mL

50 mM Tris pH 8, 300 mM NaCl (wash – W1). HTT protein was eluted with ~5 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide (elution – E1). The sample was then rocked with 1 mL Talon at 4 ˚C with rocking for 30 mins (flow through – FT2). Ni-NTA beads were washed with 50 mM Tris pH 8, 300 mM NaCl then 50 mM Tris pH 8, 300 mM NaCl supplemented with 5 mM imidazole (wash – W2 and W3) and then eluted with 50 mM Tris pH 8, 300 mM NaCl, 300 mM imidazole (elution – E2). Samples were analysed by 4-20 % tris-glycine SDS-PAGE. FT1 rocked with 1 mL Talon at 4 ˚C with rocking for 30 mins (flow through – FT3). Ni-NTA beads were washed with 50 mM Tris pH 8, 300 mM NaCl then 50 mM Tris pH 8, 300 mM NaCl supplemented with 5 mM imidazole (wash – W4 and W5) and then eluted with 50 mM Tris pH 8, 300 mM NaCl, 300 mM imidazole (elution – E3).

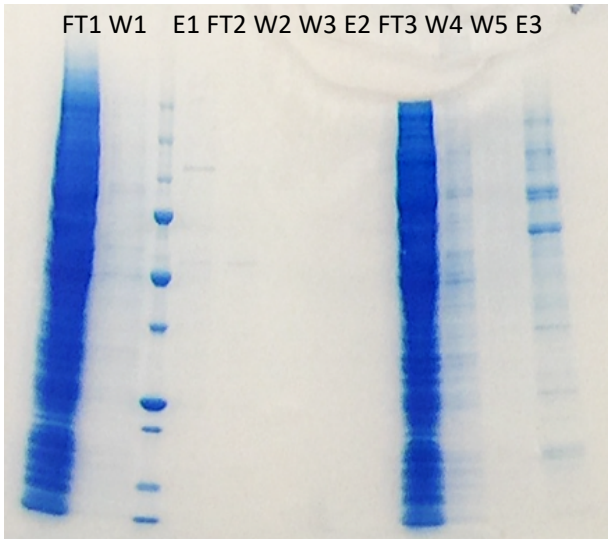
SDS-PAGE:



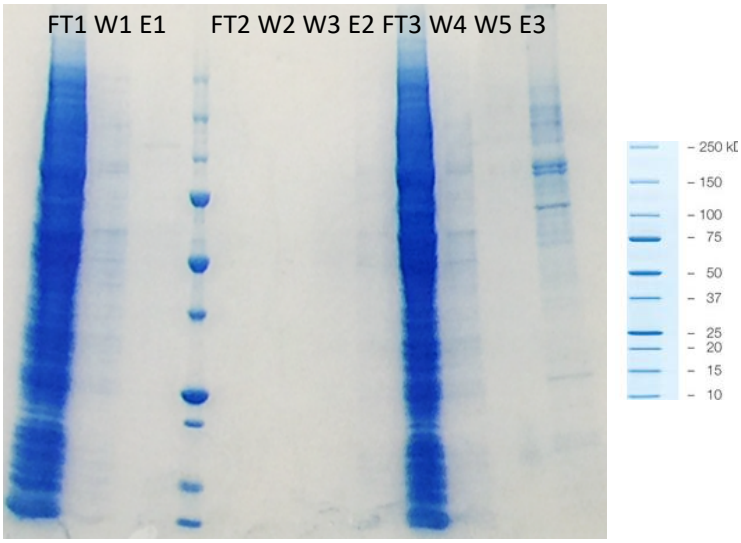
HTT + PRC2 trimer



HTT + PRC2 pentamer



HTT + PRMT5



HTT + ELP1

Comments:

- HTT-HAP40 complex can be readily purified in FLAG affinity chromatography, Ni affinity chromatography, gel filtration 3-step purification protocol. Therefore same protocol was used to explore other putative HTT interaction partners
- Following run #1 with putative binders – fix by using FT1 to bind to Talon to validate expression of binding partner in subsequent pull-down experiments as not visible in other fractions on SDS-PAGE
- Second run of HTT co-expression purifications – cannot see clear band for HTT (~350 kDa) in FLAG elution so initial pull-down has not worked
- How to improve in future experiments
    - New FLAG beads – current beads have been used to purify more than 200 L of BVES production
    - Run small-scale first using 3 mL TestX protocol as per BVES production methods are here: https://zenodo.org/record/154611
    - Plans for 3 mL production of different combos plus controls in 24-well block:

	1	2	3	4	5	6
A	TOC009-D01 + TOC011-C01, 3 to 1 ratio	TOC009-D01 + TOC011-C01, 3 to 1 ratio	TOC009-D01 + (JMC023-T-A04 + JMC023-T-B06), 3 to 1 ratio (HTT:PRC2)	TOC009-D01 + (JMC023-T-A04 + JMC023-T-B06), 3 to 1 ratio (HTT:PRC2)	TOC011-C01	JMC023-T-A04 + JMC023-T-B06
B	TOC009-D01 + JMC110-A07, 3 to 1 ratio	TOC009-D01 + JMC110-A07, 3 to 1 ratio	TOC009-D01 + (JMC023-T-A04 + JMC023-T-B06 + MVC001-B02 + APC062-D03), 3 to 1 ratio (HTT:PRC2)	TOC009-D01 + (JMC023-T-A04 + JMC023-T-B06 + MVC001-B02 + APC062-D03), 3 to 1 ratio (HTT:PRC2)	JMC110-A07	JMC023-T-A04 + JMC023-T-B06 + MVC001-B02 + APC062-D03
C	TOC009-D01 + (JMC046:A09 + JMC015:F03), 3 to 1 ratio (HTT:PRMT5)	TOC009-D01 + (JMC046:A09 + JMC015:F03), 3 to 1 ratio (HTT:PRMT5)	TOC009-D01 + JMC035-C03, 3 to 1 ratio	TOC009-D01 + JMC035-C03, 3 to 1 ratio	JMC046:A09 + JMC015:F03	JMC035-C03
D	TOC009-D01 + YTC039-G10, 3 to 1 ratio	TOC009-D01 + YTC039-G10, 3 to 1 ratio	TOC009-D01 + JMC118-F04, 3 to 1 ratio	TOC009-D01 + JMC118-F04, 3 to 1 ratio	YTC039-G10	JMC118-F04
	HTT + interactor in duplicate		HTT + interactor in duplicate		Interactors without HTT	