TBCE Mutations Cause Early-Onset Progressive Encephalopathy with Distal Spinal Muscular Atrophy

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Abstract

We report on an early-onset neurodegenerative encephalopathy with distal spinal muscular atrophy caused by TBCE mutations, with Ile155Asn occurring at the heterozygous/homozygous state in all affected subjects. Clinical features resemble the phenotype of *pmn/pmn* mice ($Tbce^{Trp524Gly}$) and are associated with reduced TBCE levels and altered tubulin polymerization. While truncating *TBCE* mutations have been described to perturb developmental processes, we document altered TBCE function as a cause of neurodegeneration in humans.

Tubulinopathiesincludeafamilyofsevereneurodevelopmentaldisorderscausedby mutationsinmultiplegenesencodingtubulinisoforms(*i.e.*,*TUBA1A*,*TUBA8*,*TUBB2B*, *TUBB3*and*TUBB5*),withdefectiveneuronalmigrationandmalformationofthecerebral cortexrepresentingmajorfeatures[1].Truncatingmutationsin*TBCE*(tubulin-specific chaperoneE),oneofthefivetubulinbindingco-factorsinvolvedintubulinfoldingand polymerization,areknowntocausetworareneurodevelopmentaldiseases, hypoparathyroidism-retardation-dysmorphism(HRD)andKenny-Caffeysyndrome(KCS)[2]. Morerecently,microtubule-disruptingmutationsintubulingeneshavebeenshownto triggerneurodegeneration,asillustratedby*TUBA4A*and*TUBB4A*mutationsunderlying amyotrophiclateralsclerosis[3]andhypomyelinatingleukodystrophyassociatedwith progressivespasticdystonictetraplegia[4],respectively.Similarly,amissensemutationin *Tbce*hasbeenassociatedwithprogressivedistalmotorneuronopathyinmice[5],butno similarphenotypehassofarbeenreportedinhumans.

Weusedatrio-basedwholeexomesequencing(WES)strategytodelineatethe molecularbasisofanovelrecessiveencephalopathyaffectingtwoapparentlyunrelated youngsubjectsoriginatingfromtheislandofIschia,SouthernItaly(**Fig.1a**). Both individualsshoweddevelopmentaldelaywithoutdysmorphiaorgrowthdefects,and manifestedearly-onset,slowlyprogressivedistalmotorneuropathyresemblingdistal spinalmuscularatrophy,withbilateralfootdropassociatedtospasticityandcerebellar ataxia.WESdatawereanalyzedtoidentifygenessharedbytheprobandshaving functionallyrelevantrare/privatevariants(**On-linemethods**),takingintoaccount recessiveinheritancemodels(**SupplementaryTableS1**). Weidentifiedonlyonegene, *TBCE*,satisfyingthesefilteringcriteria.Subject1544334(Pt1)washomozygousforthe c.464T>Amissensechange(p.Ile155Asn),whichwasinlinewiththedocumented consanguinityascertainedinthefamily;subject2518864(Pt2)wasacompound heterozygoteforthesameaminoacidsubstitutionandthetruncatingc.1076delCchange

(p.Ser359fs*1). Sanger sequencing confirmed both mutations and segregation in both families (**Supplementary Fig. S1**). The shared genomic region encompassing the c.464T>A change covered 2.5 Mb (chr1:234529552-237060433). Mutations can of the *TBCE* coding sequence performed on 14 subjects with similar clinical features allowed to identify a third family from the same geographical area having two monozygotic twins homozygous for the c.464T>A change. All subjects exhibited early-onset, slowly progressive spastic ataxia, distal spinal muscular atrophy, cerebellar atrophy, and developmental delay (**Supplementary Table S2**). This phenotype is reminiscent of the mutant *pmn*/*pmn* mouse with progressive motor neuronopathy, hindlimb atrophy and reduced brain size[5]. In these subjects, the neurodegenerative signs, normal calcium/phosphate metabolism, and absence of repeated infections (**Supplementary Information**) clearly differ from HRD and KCS.

TCBE is characterized by a CAP-Gly domain at the *N*-terminus, followed by nine tandemly arranged leucine-rich repeats (LRRs), and a *C*-terminal ubiquitin-like domain (**Supplementary Fig. S1**). Ile^{155} is a highly conserved residue across orthologs (**Supplementary Fig. S1**) located within the LRR domain. A homology model of the TBCE regions spanning the nine LRRs (residues 123-348) was generated, which fitted well with the established structure of these motifs[6], and placed Ile^{155} into the hydrophobic core stabilizing these second LRR motif (**Fig. 1b**). The introduction of the hydrophilic asparagine is predicted to perturb dramatically the structure of the motif and the overall folding of the LRR domain. Since LRR domains are functional modules that mediate protein-protein interaction, this amino acid substitution is expected to affect TBCE function or the overall TBCE structure and protein instability. To verify the latter hypothesis, the level of the TBCE protein was evaluated in primary fibroblasts obtained from Pt1 and Pt2. Western blot analysis documented a strongly reduced amount of TBCE compared to control cells (**Fig. 1c**), suggestive of rapid degradation of the TBCE mutant. Of note, reduction in TBCE

levelswasmorepronouncedinfibroblastsfromPt2,whowascompoundheterozygotefor theIle155AsnandSer359fs*1changes.Inthesecells,*TBCE*mRNApoolwasreducedto halfofnormal(asshownbyreal-timeqRT-PCR)(**Fig.1d**),andthemutantallelecarrying theframeshiftvariantwasundetectablebySangersequencingofretro-transcribedRNA (datanotshown),indicatingnonsense-mediatedmRNAdecay.A similarreductioninTbcelevelswasdocumentedinfibroblasts(**Fig.1e**)andmotoneurons[7]ofthe*pmn*/*pmn*mouse.Remarkably,TBCElevelshadbeenreportedtobevirtuallyabsentinHRDandKCS[8].

TBCEisrequiredforthefoldingofα-tubulin,α/β-tubulindimerizationand subsequentheterodimerpolymerizationintomicrotubules,whicharemajorcomponentsoftheneuronalcytoskeleton[9].Toexploretheimpactofthetwo*TBCE*mutationson microtubulepolymerization,wefirstdeterminedthelevelsoftotal,solubleandpolymerized α-tubulininpatientandcontrolfibroblasts,usingbiochemicalfractionation.Thelevelof totalα-tubulinwasnotaffected.Bycontrast,theamountofpolymerizedα-tubulinwas significantlyreducedcomparedtocontrolcells(**Fig.2a**),inlinewithwhatoccurredintail fibroblastsof*pmn*/*pmn*mice(**Fig.2b**). Wethendeterminedthenucleationofmicrotubules atthecentrosomeandatextracentrosomalsites. Centrosomesorganizethespindle microtubulesduringmitosis,andconfocalmicroscopyanalysisofsynchronizedfibroblasts frompatientswithmutated*TBCE*allelesdocumentedabnormalmitoticmorphologyand disorganizedmitoticmicrotubules;spindlesappearedasymmetricandunrefined,with irregularasterstructure(**SupplementaryFig.S2**). Defectivenucleationof extracentrosomalmicrotubuleswasalsonoticed. Specifically,weobservedsignificantly reducedlevelsofGolgi-associateddetyrosinatedα-tubulininpatientfibroblasts(**Fig.2c**), inlinewiththereportedlossofGolgi-derivedmicrotubulesinmotorneuronsof*pmn*/*pmn* mice[7].

Todemonstratealteredmicrotubuledynamics,wetreatedthefibroblastcultureswith themicrotubule-disruptingdrugnocodazole,andanalyzedmicrotubuleregrowthafterdrug

washout.Incontrolfibroblasts,microtubulebegantopolymerizeefficientlyasearlyas10 minutesafternocodazolewashout,andextendedpolymerizedstructureswerewell-formed at30min(**Fig.2d**).Differently,microtubulere-polymerizationappearedmarkedlydelayed inpatientfibroblasts;microtubuleswerealsolessabundantandstronglydisorganizedin bothearlyandlatestagesofre-polymerization.Suchaltereddynamicsappeared particularlypronouncedinfibroblastswithcompoundheterozygosityfortheIle155Asnand Ser359fs*1changes,consistentwiththemoreprofoundreductionofTBCEexpression levels.

Overall,wedescribeanewdistalneuronopathyandspasticataxiacausedbybiallelic mutationsin*TBCE*overlappingthephenotypeof*pmn/pmn*mouse,awell-knownmodelof motorneurondisease.Onthebasisofthepreviouslydocumentedimpactofabolished TBCEfunctionondevelopmentalprocessesandgrowth,ourfindingsdemonstratea stringentrequirementofTBCEfunctionspecificallyforneuronalsurvivalandfunction.

URLs

GenomeAnalysisToolkit,https://www.broadinstitute.org/gatk/;Gene, http://www.ncbi.nlm.nih.gov/gene/;RCSBProteinDataBank, http://www.rcsb.org/pdb/home/home.do;Gromacs,http://www.gromacs.org;SWISS-MODEL,http://swissmodel.expasy.org;vmd,http://www.ks.uiuc.edu/Research/vmd/; MODELLER,https://salilab.org/modeller/.

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Authorcontributions

A.S.,G.B.,T.R.andE.P.designedandperformedthebiochemicalandfunctionalstudies, analyzedthedata;S.B.performedmutationanalyses;E.F.,C.C.,andS.C.carriedoutthe confocallaserscanningmicroscopyanalysisofculturedhumanandmicefibroblasts;A.C., M.N.andV.C.performedWESdataprocessing,analysisandvalidation.E.Bell.performed thestructuralanalyses.G.T,A.D'A.,B.D.,G.Z.,D.D.,D.M.andC.D.-V.,clinically characterizedtheaffectedsubjects,collectedbiologicalspecimensandstudied histopathologyofmusclebiopsies.M.T.,G.H.andE.B.conceivedtheproject,designed andsupervisedtheexperiments,analyzedandinterpretedthedata,andwrotethe manuscript.Allauthorscontributedtothefinalmanuscript.

Competingfinancialintereststatement

Allauthorsstatethattheyhavenocompetingfinancialinterests.

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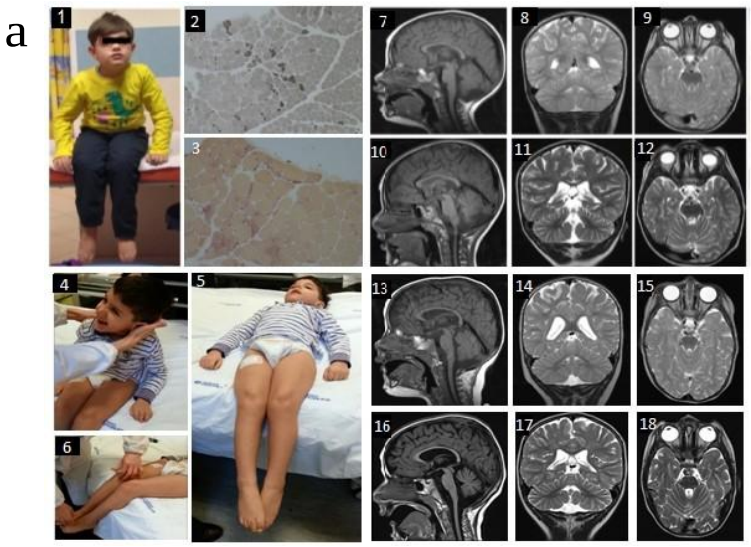
LegendstoFigures

Figure1.*TBCE*mutationscauseearly-onsetencephalopathywithdistalspinal muscularatrophy.(**a**)ClinicalfeaturesofPt1(1)andPt2(4,5,6);musclebiopsyofPt1 stainedwithATPase4.3(2)andesterase(3)showingneurogenicabnormalities;brain MRIsofPt1(7,8,9,at2years;10,11,12,at3years)andPt2 (13,14,15,at1year;16, 17,18,at2years).Notethehypoplasiaofcorpuscallosum,andcerebellaratrophywithprominentf olia(12,17).(**b**)HomologymodeloftheTBCELRRdomain.$Ile^{155}$with surroundinghydrophobicresiduescontributetothehydrophobiccorerequiredforproper foldingofthedomain.(**c**)Westernblot(WB)analysisdocumentingreducedTBCElevels inpatientfibroblasts.Dataarepresentedasmeans±s.d.,*indicates*P*<0.05. (**d**)Real-timeqRT-PCRshowingmarkedlyreducedexpressionlevelof*TBCE*mRNAinfibroblasts fromPt2.*indicates*P*<0.05.(**e**)WBanalysisdemonstratingreducedlevelsofTbceinthe *pmn*/*pmn*mouse($Tbce^{Trp524Gly}$);recombinantprotein(TBCE)isalsoreported.

Figure2.Defectivemicrotubulepolymerizationinsubjectswith homozygous/heterozygousTBCEp.Ile155Asnsubstitution,and*pmn*/*pmn*mice.(**a**) Westernblot(WB)analysesshowingtotal(T),soluble(S)andpolymerized(P)α-tubulin (above),detyrosinatedtubulin(middle),andβ-actin(below)inpatientfibroblastsand controlcells(Ctrl1,Ctrl2).Diagrams(right)showreducedfractionsofpolymerizedα-tubulinanddetyrosinatedtubulininpatientfibroblasts.(**b**)WBanalysesshowingreduced amountofpolymerizedα-tubulin(P)infibroblastsfrom*pmn*/*pmn*micecomparedto fibroblastsfromunaffectedmice.(**c**)Immunofluorescenceanalysisdocumentinglossof microtobulescontainingdetyrosinatedtubulin(detyr-Tub)inpatientfibroblasts.Notethat detyrosinatedmicrotubules(red)incontrolfibroblastsarecloselyassociatedwithGM130-

stainedGolgimembranes(green).NucleiareDAPI-stained.Scalebar,10μm. (**d**) Immunofluorescenceanalysesshowingreducedre-polymerizationofmicrotubules followingnocodazoletreatmentinfibroblastsfromPt1andPt2,andcontrolcells(Ctrl). Scalebar,20μm.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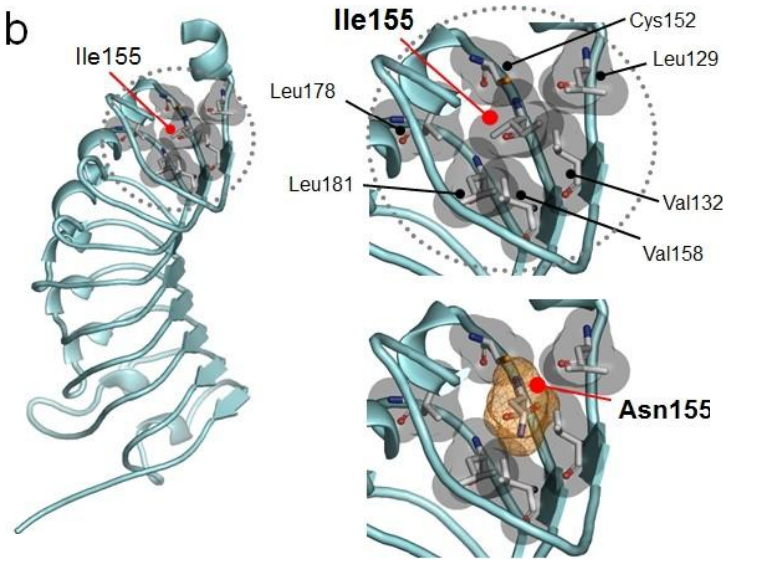
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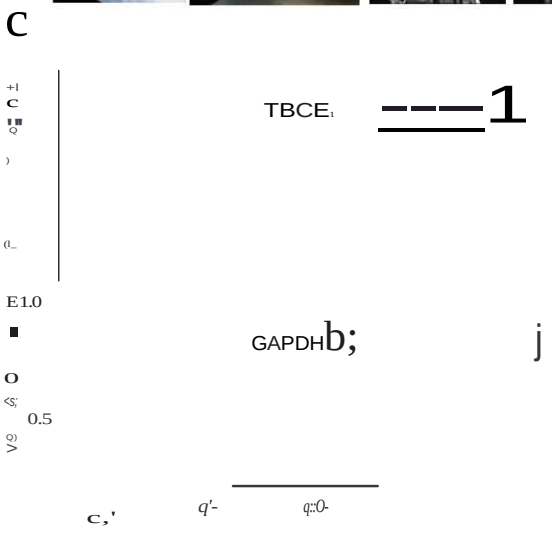
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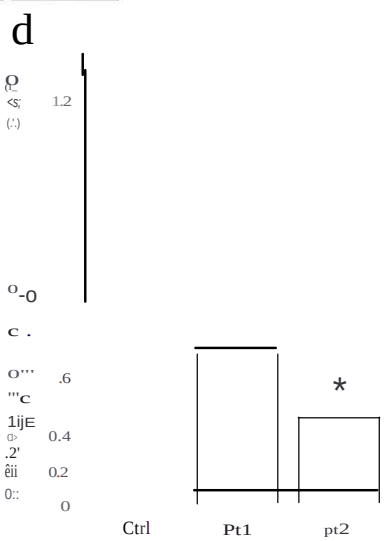
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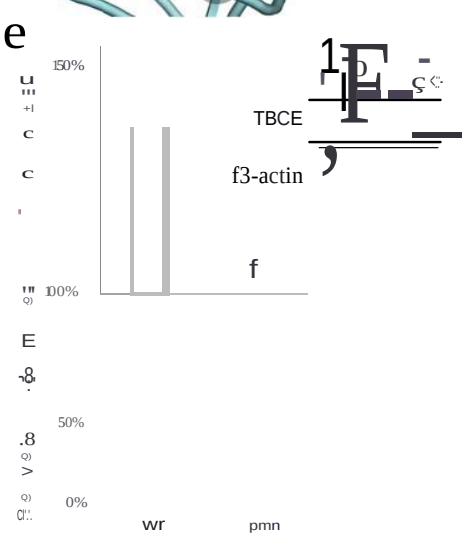
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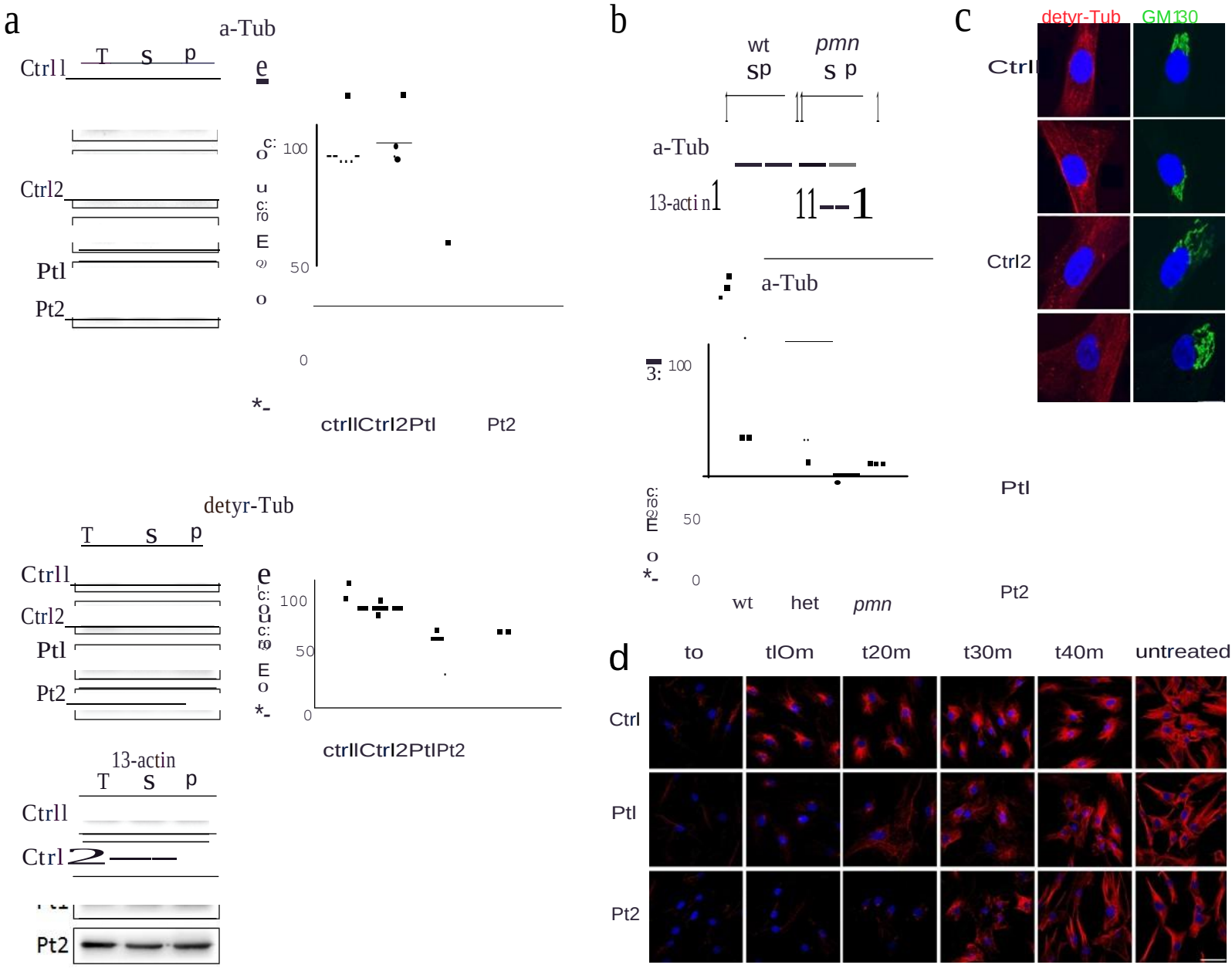
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