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26 Abstract: The detyrosination-tyrosination cycle, removal and re-ligation of the C-terminal 27 tyrosine of α -tubulin, is implicated in cognitive, cardiac and mitotic defects. The enzymatic 28 origin of detyrosination is only partially explained by the Vasohibin-SVBP complex. Here, we 29 used haploid genetic screens to identify an unannotated protein, MATCAP, as a remaining 30 detyrosinating enzyme. X-ray crystallography and cryo-EM structures established MATCAP's 31 cleaving mechanism, substrate specificity, and microtubule recognition, all distinct from 32 Vasohibins. Paradoxically, while abrogation of tyrosine re-ligation is lethal in mice, codeletion of MATCAP and SVBP was not. Although viable, defective detyrosination caused 33 34 microcephaly associated with proliferative defects during neurogenesis, and abnormal 35 behavior. Thus, MATCAP is a missing component of the detyrosination-tyrosination cycle, 36 revealing the importance of this modification in brain formation. 37

38 **One-Sentence Summary:**

39 Identification and characterization of MATCAP as a missing component of the tubulin40 detyrosination-tyrosination cycle.

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42 **Main Text:** Microtubules, composed of polymerized α - and β -tubulin heterodimers, are core 43 components of the cytoskeleton, and are essential for cargo trafficking, cellular organization 44 and force generation. Microtubule heterogeneity is created by multiple α/β tubulin genes 45 (isotypes) in combination with an array of posttranslational modifications which in particular affect the disordered C-terminal tail of α -tubulin (1, 2). Most α -tubulin isotypes are translated 46 47 with a C-terminal tyrosine, which can be enzymatically removed and re-ligated (3, 4). This 48 dynamic modification cycle has been implicated in cell division (5-8), neurogenesis (9-11)49 and heart failure (12-15). Detyrosinated tubulin also serves as the sole precursor for the

50 irreversible generation of $\Delta 2$ -tubulin which, when stimulated through chemotherapeutic agents

51 leads to peripheral neuropathy (*16*). The removal of tyrosine can be catalyzed by the 52 Vasohibins (two paralogs, VASH1 and VASH2) in complex with the essential co-factor Small 53 Vasohibin-Binding Protein (SVBP) (*10*, *17*) and reversed by the tubulin tyrosine ligase (TTL) 54 (*18*) (Fig. 1A). Loss of VASH-SVBP in cell lines (Fig. 1B and C) (*17*) or mice (*11*) does only 55 partially impair tubulin detyrosination, indicating the existence of yet-to be discovered 56 enzyme(s) which complicates functional studies on the role of detyrosination.

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58 The tubulin detyrosination-tyrosination cycle is regulated by MATCAP

59 To identify remaining tubulin carboxypeptidases, we carried out genome-wide mutagenesis 60 screens in human haploid HAP1 cells lacking both Vasohibins (Fig. 1D). For the first genetic screen we treated the cells with the microtubule stabilizing agent paclitaxel (known to increase 61 62 detyrosination) and we searched for factors that upon gene-trap inactivation reduced tubulin 63 detyrosination (Fig. 1E). In a second genetic screen we knocked out TTL using CRISPR/Cas9 64 in the Vasohibin-deficient cells (to make detyrosination irreversible) and used the amount of tyrosinated α -tubulin as read-out (Fig. 1F). Combined analysis revealed the unstudied gene 65 KIAA0895L as the strongest regulator that diminished detyrosination upon genetrap 66 67 inactivation in the first screen, and stimulated tubulin tyrosination upon inactivation in the 68 second screen (Fig. 1G and fig. S1A and B). In addition, KIAA0895L was also distinguished 69 from most other hits by having no significant effect on 22 phenotypes unrelated to tubulin 70 biology that were previously examined using haploid genetic screens (fig. S1C). As 71 KIAA0895L also scored in Δ TTL cells (Fig. 1F), it likely participates in tyrosine removal rather than re-ligation. Thus, KIAA0895L affects tubulin detyrosination and for reasons 72 73 described below we renamed it as MATCAP (Microtubule Associated Tyrosine 74 CArboxyPeptidase).

Expectedly, tubulin detyrosination is only moderately decreased upon the loss of both 75 76 Vasohibins (Fig. 1H). Combined ablation of MATCAP and Vasohibins resulted in 77 undetectable levels of detyrosination in different cell lines, even in the presence of paclitaxel 78 (Fig. 1H). Furthermore, overexpression of MATCAP in HeLa cells, which normally have low 79 levels of tubulin detyrosination, efficiently stimulated tubulin detyrosination (Fig. 1I). 80 Moreover, expression of MATCAP re-established tubulin detyrosination in ΔVASH1 81 Δ VASH2 Δ MATCAP knockout cells (Fig. 1J). Next, we asked if the C-termini of different α -82 tubulin isotypes are differentially affected by MATCAP. For that we fused different tubulin 83 tails to the C-terminus of GFP. Co-expression with MATCAP removes the terminal residue of all GFP-fused α -tubulin tails, including those corresponding to TUBA8 (which possesses a 84 85 phenylalanine as terminal amino acid), and to TUBA4A (which is encoded without C-terminal 86 tyrosine but can be re-tyrosinated by TTL) (fig. S2A). Thus, MATCAP acts on the C-terminus 87 of α -tubulin and affects detyrosination independently of the known (de)tyrosination enzymes.

89 MATCAP is a metalloprotease with an atypical zinc-binding motif

90 As MATCAP was not annotated as a protease, we expressed and purified both full-length MATCAP, and MATCAP without its disordered N-terminal region (MATCAP¹³⁷⁻⁴⁷¹) (Fig. 91 2A). Next, a crystal structures of MATCAP¹³⁷⁻⁴⁷¹ was determined using experimental phasing 92 93 (Fig. 2B and Table S1). The structure reveals that MATCAP belongs to the gluzincin metallopeptidase clan (19), which is characterized by a Zinc ion (Zn^{2+}) . Consistently, EDTA 94 95 abolished enzymatic activity (Fig. 2C). The metal ion is coordinated by a HExxH motif 96 incorporated in an α -helix, and resembles the active site and the fold of the SARS-CoV-2 spike-97 protein receptor ACE2 and the anthrax lethal factor (Fig. 2D). The HExxH motif is degenerate in MATCAP (²⁸⁰HExxxH²⁸⁵), as has been observed in other rare metallo-exopeptidases (20, 98 99 21). The extra residue destroys the canonical Ni-Oi+4 hydrogen bonding helical pattern 100 (formally breaking the α 6 helix in two), but forms an active site similar to its structural

101 homologues, positioning the presumed nucleophile Glu281 for catalysis. Because the original crystallization conditions contained EDTA, Zinc ions were not visible. Therefore we 102 determined a structure of MATCAP in the presence of Zn^{2+} , and refined both the apo and the 103 104 Zn²⁺-bound structures to 2.1 and 2.5 Å respectively. The Zinc ion is coordinated by His280, His285 and Glu316 (Fig. 2E). The structure of the zinc binding site is remarkably well-105 106 maintained in the apo structure, with the exception of Glu316 that is further away from the 107 Zinc ion. As expected, MATCAP activity was metal-dependent in vitro (Fig. 2B) and mutating 108 the Glu281 nucleophile to glutamine (E281Q) or the zinc-binding residues to alanine abolishes activity in cells (Fig. 2F) and in vitro (fig. S2B and C). Notably, the structure predicted by 109 110 AlphaFold (22) (fig. S2D) is similar to both experimental structures. Thus, MATCAP is a peptidase that relies on an atypical catalytic domain for its optimal enzymatic efficiency. 111

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113 Substrate recognition by MATCAP and VASH1-SVBP

114 To elucidate how MATCAP recognizes its substrate, the negatively charged glutamate-rich C-115 terminal α -tubulin tail, we first visualized the electrostatic potential of MATCAP's protein 116 surface (Fig. 3A). Mutation of residues in the positively charged patch close to the catalytic 117 site, decreased or abolished the activity of MATCAP (Fig. 3B). Further experimental mutant analysis combined with *in silico* docking using HADDOCK (23) yielded two plausible models 118 119 of the α -tubulin tail bound to MATCAP (Fig. 3C and fig. S3A, B). Further mutagenesis 120 experiments were equally supportive for both docking models (fig. S3C-F). The C-terminal 121 part of the tail is firmly positioned in a pocket for catalysis, by two lysine (389, 417) and four 122 arginine (334, 373, 376, 393) residues in both models. According to these models, the tubulin 123 tail could enter the pocket via two routes. In sharp contrast with the MATCAP tail-binding 124 pocket, VASH1-SVBP binds its substrate in an open groove (Fig. 3D and fig. S4A) (5, 24–28). 125 Furthermore, VASH1-SVBP engages with the second-last glutamate of the α -tubulin tail, while 126 MATCAP forms an extensive network of interactions with many glutamates, and in particular with the last one, positioned before the scissile bond (fig. S4B and C). Consequently, the two 127 128 enzymes have different dependencies on the amino acids of the tubulin tail: in contrast to 129 VASH1, MATCAP's activity is affected by most amino acid alterations along the tubulin tail except the terminal residue, in vitro (Fig. 3E and fig. S4D and E) and in cells (Fig. 3F). We 130 131 conclude that MATCAP removes the terminal residue independently of its amino acid identity 132 and strongly relies on the penultimate residue to be a glutamate for proper substrate cleavage. Contrary, VASH1 largely tolerates amino acid changes in the α -tubulin tail as long as the 133 134 terminal residue is aromatic and the second last glutamate is intact (fig. S4D-F). Thus, the 135 unique structural architectures of MATCAP and Vasohibins represent two convergent 136 solutions for tubulin detyrosination, with divergent substrate recognition and catalytic modes.

137

138 A cryo-EM strycture of MATCAP on microtubules reveals multiple binding interfases

139 Consistent with the reported preferential activity of brain detyrosinases on microtubules (29– 140 31), recombinant MATCAP is more efficient in processing α -tubulin tails on microtubules than

- 141 free tubulin dimers (Fig. 4A). Recombinant MATCAP co-pellets with microtubules in an 142 ultracentrifugation sedimentation assay in vitro (Fig. 4B). Consistently, MATCAP
- 143 preferentially localizes to microtubules in cells, especially when the catalytic nucleophile is 144 mutated (E281Q) (Fig. 4C).
- 145 To understand how MATCAP engages with microtubules, we prepared fully tyrosinated 146 microtubules from triple knockout (Δ VASH1 Δ VASH2 Δ MATCAP) cells and incubated these
- 147 with recombinant catalytically inactive (E281Q) MATCAP to form a complex on cryo-EM
- 148 grids. Electron micrographs showed MATCAP-decorated microtubules, further confirmed in
- 149 2D and 3D class averages (Fig. 4D and E and fig. S5A and B). The cryo-EM reconstruction
- 150 based on particles extracted from microtubules with 14 protofilaments has a nominal resolution

- 151 of 2.9Å, with the binding interface being resolved to ~4.5Å (Fig. 4F and fig. S6). The cryo-
- EM density allows a clear and unique placement of atomic models of the tubulin dimer, and of MATCAP (Fig. 4G and fig. S5C-E), and shows low-resolution density that can be attributed
- to the α -tubulin tail (Figure 4F inlet), supporting only one of the computational models for the α -tubulin tail entering the catalytic MATCAP pocket (Fig. 3C).
- 156 The cryo-EM structure shows that the binding interface between α -tubulin and MATCAP has
- 157 three principal constituents (Fig. 4G): (1) the α -tubulin tail binding pocket; (2) an interface
- between the last helix of α -tubulin and the α -helix²¹⁸⁻²³² of MATCAP; and (3) the loop²⁵⁷⁻²⁵⁹
- 159 that contacts an adjacent tubulin dimer along the microtubule protofilament. The structure also
- 160 points out two "regions of interest": (4) a positively charged patch at the "backside" of
- 161 MATCAP, covered by density which could possibly be attributed to binding of the negatively 162 charged β -tubulin tail; and (5) the positively charged (pI 11.3), proline-rich N-terminus of
- 163 MATCAP, for which there is no clear experimental density.
- 164 To study the interfaces, we designed mutations in each of them and monitored their effect on 165 microtubule localization of MATCAP in cells. All five interfaces individually contribute to
- 166 microtubule localization (fig. S7). Mutations in the helix-helix interface (2) combined with
- 167 removal of the N-terminal region (5) decreases the binding of MATCAP to microtubules (Fig.
- 168 4H and I). This effect is stronger than the effect of the separate mutants (Fig. 4G and H and
- 169 fig. S7C). This combinatorial effect further demonstrates that multiple binding interfaces are
- 170 involved in recruitment of MATCAP to the polymer. Because the N-terminal disordered region 171 contributes to microtubule binding (fig. S7L), but its positioning in the context of
- 172 protofilaments is unclear, it could influence MATCAP's enzymatic activity on microtubules. 173 To study this we incubated Δ N-MATCAP with either tubulin dimers or assembled
- 174 microtubules. In this assay Δ N-MATCAP displayed enhanced activity on microtubules than
- tubulin dimers (fig. S7M), compared to wild-type (Fig. 4A). Thus, whereas the disordered region of MATCAP enhances its anchoring on microtubules, it dampens processivity on the
- 177 polymerized substrate.
- 178 In summary, multiple specific interactions between MATCAP and microtubules ensure 179 efficient binding to particularly the microtubule polymer and regulate detyrosination activity.
- 180 Whereas MATCAP recognizes adjacent tubulin dimers along the same protofilament with the
- 181 interdimeric loop interface, Vasohibins recognize tubulin dimers in juxtaposed protofilaments
- 182 (Fig. 4J) (32), further illustrating the differences between these two tyrosine carboxypeptidases.
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- 185

185 Combined loss of MATCAP and SVBP perturbs detyrosination in vivo

186 To address the physiological role of detyrosination, we disrupted MATCAP in mice. 187 Homozygous mice were viable and fertile and displayed no gross alterations. We subsequently crossed these mice with a mouse strain deficient for SVBP, which is required for the 188 189 Vasohibin's enzymatic activity. The resulting double knock out mice were viable, born at 190 mendelian ratio, and showed comparable body weights to wildtype mice (fig. S8A and B). 191 Immunohistochemical analysis of brain tissue indicated a strong decrease of detyrosinated 192 tubulin (Fig. 5A). Similarly, immunoblot analysis on brain tissue showed a reduction of detyrosinated tubulin and a concomitant increase in tyrosinated tubulin (Fig. 5B). Whereas $\Delta 2$ 193 194 tubulin was undetectable, a faint signal was detected with detvrosination specific antibodies. 195 which could either be unspecific background signal, detection of TUBA4A (the tubulin isotype 196 which is translated without C-terminal tyrosine), or remaining detyrosination. Quantitative 197 proteomics on brain tissue from double knockout mice detected exclusively tyrosinated, but 198 not detyrosinated TUBA1A/B (Fig. 5C), in agreement with our observations in human cell

199 lines (Fig. 1H) and in line with the undetectable levels of $\Delta 2$ -tubulin in mice, whose production

relies on detyrosinated tubulin as precursor. Thus, combined inactivation of SVBP and
 MATCAP enables the generation of viable adult mice with impaired tubulin detyrosination.

202

203 Impaired detyrosination affects brain volume

SVBP knockout mice (11), albeit having substantial amounts of detyrosinated tubulin, have 204 smaller brains, resembling human patients with cognitive impairments and microcephaly. Mice 205 206 lacking MATCAP showed a moderate but significant reduction of total brain volume, 207 cerebellum and olfactory bulb (Fig. 5D and E) as quantified using MRI brain scans. The 208 combined removal of MATCAP and SVBP led further to a more drastic decrease in brain size 209 compared to single knockouts (Fig. 5E) and a reduction in total brain weight and total cell 210 number compared to wild-type mice (Fig. 5F and G). To address the origin of the small brain 211 phenotype we first measured apoptotic cells. Only very infrequent and similar amounts of 212 apoptotic cells were detected in adult or embryonic brains of either genotype (fig. S8C-E). Next, we examined cell proliferation during neurogenesis in embryos at day E14.5. 213 214 Immunostainings showed a significant decrease in mitotic phospho-histone H3 positive cells 215 (Fig. 5H, I). Of note, this decrease was observed in the ventricular and subventricular zone 216 (Fig. 5J), the proliferative zones of the embryonic brain (Fig. 5J), suggesting a defect in proliferation of the progenitor cells. To examine if this brain phenotype led to behavioral 217 phenotypes we examined nest building, a behavior that is sensitive to brain lesions in mice. 218 219 When housed with nesting material overnight, double knockout mice completely failed to 220 construct nests, whereas MATCAP or SVBP single knockout mice were largely able to build 221 nests (Fig. 5K). Thus, these distinct detyrosinating enzymes have important and overlapping 222 roles in brain development and behavior. 223

224 Conclusion

While most post-translational modifications of the α -tubulin tail are generated by large families 225 226 of related enzymes (2), VASH-SVBP and MATCAP constitute isofunctional yet evolutionary 227 unrelated enzymes. Their identification now enables functional studies of detyrosination, 228 further facilitated by the survival of detyrosination-impaired mice. As detyrosination precedes 229 re-tyrosination in this biochemical cascade, their viability is in surprising contrast with the 230 postnatal lethality of TTL deficient mice (33). As such, the absence of SVBP/MATCAP 231 interferes with the generation of (1) detyrosinated tubulin, (2) subsequent tubulin modifications 232 preceded by detyrosination (Δ 2-tubulin formation), (3) microtubule heterogeneity through 233 gradients of tyrosination levels and (4) the presence of a modification cycle affected by tubulin polymerization. Thus, the lethality of TTL mice may not be caused by disturbance of these 234 235 processes but rather by the low levels of tyrosinated tubulin due to non-reversable 236 detyrosination. Collectively, our work shows similar activities for these two distinct classes of 237 tubulin detyrosinases, but at the same time highlights mechanistic differences between them. 238 These differences lay a foundation to understand how distinct enzymatic properties may 239 facilitate a more nuanced impact on the pool of heterogenous microtubules.

240 Materials and methods

241

242 <u>Reagents, constructs and treatments</u>

243 To enhance detyrosination cells were incubated with 2 µM Paclitaxel (Santa Cruz Biotechnology) for 3 hours. MATCAP (UniProt identifier Q68EN5-1) was cloned with or 244 245 without 2xHA-tag into the pcDNA3.1(-) vector and also into the pLEX 305. VASH1, SVBP 246 and GFP-tubulin tail constructs were generated in a previous study (17). Mutant variants of 247 MATCAP were derived through site-directed mutagenesis. All generated constructs were 248 confirmed by sanger sequencing. A rabbit anti-MATCAP antibody was generated by 249 immunizing rabbits with full-length recombinant human MATCAP protein and obtained sera 250 were affinity purified (David Biotechnologie Gmbh).

- 251
- 252 <u>Cell culture</u>
- 253 Used cell lines include HAP1 (34), HCT116 (RRID:CVCL_0291), U2OS
- 254 (RRID:CVCL_0042), HEK293T (RRID:CVCL_0063) and HeLa H1 (RRID:CVCL_3334).

255 Cells were cultured in IMDM (HAP1) and DMEM (HCT116, U2OS, HEK293T, HeLa H1)

supplemented with 10% FBS, L-glutamine, penicillin and streptomycin at 37°C in 5% CO₂.

- 257 Cell lines were monitored for mycoplasma contamination.
- 258
- 259 Generation of cell lines
- 260 Knockout cell lines were generated either by lentiviral transduction of Cas9/gRNA cloned into
- the pLentiCrispr vector (including a blasticidin or puromycin resistance cassette) or by cotransfection of a vector with a blasticidin-resistance cassette and the pX330 vector (Addgene
- 263 42230) including the Cas9/gRNA. gRNA sequences were:
- 264 TTL (5'- GTTCTCATCGCGTACCACGA-3'),
- 265 VASH1 (5'-ACGGCTTCCAGGCATTTGAT-3'),
- 266 VASH2 (5'-TTTGATAGGCAAGGACTCTC-3') and
- 267 MATCAP (5'-TGCGCACAATCGACCATATC-3').

268 After selection with antibiotics, individual clones for each cell line were derived. Genome-

editing was monitored by amplifying genomic loci and subsequent sanger sequencing of PCR
 products. Primers used were:

- 271 VASH1 (5'-GTGGGAGGTGGAAGTGAAAGAAGG-3', 5'-GCCAAGCAGATGACTGCATATGG-3'),
- 272 VASH2 (5'-TGCTATGGAGTACTGGCACTAACTCC-3', 5-GTCCAAGAAGCCAAGCATTGATTTGG-3'),
- 273 MATCAP (5'-GAGTACAGGGCCTGCTGGTCGTTTG-3', 5'-CGCCCACAAGACACCCTGATTTG-3').
- 274 Compound heterozygote clones were identified with the TIDE web tool (35) and further
- confirmed by subcloning of the PCR amplicons and sequencing. TTL knockout was confirmed
- by immunoblotting using the rabbit anti-TTL (Proteintech, 13618-1-AP, RRID:AB_2256858).
- 278 FACS-based phenotypic screens in HAP1 cells

279 Screens were carried out as described previously (17, 36). Retrovirus was produced by transfection of HEK293T cells with gene-trap vector, packaging plasmids Gag-pol, VSVg, and 280 pAdv. After 48 hours the viral supernatant was harvested and filtered using Amicon filters and 281 282 applied together with protamine sulfate $(8\mu g/ml)$ to $40x10^{6}$ HAP1 cells. This procedure was 283 repeated the next day. After expansion mutagenized HAP1 cells were dissociated using 284 Trypsin-EDTA and washed with PBS. Cells were fixed with ice-cold methanol for 10 min on 285 ice, resuspended in FACS buffer (PBS with 10% FBS) and single cells were ensured by 286 applying cells to a 40 µm strainer. After incubation with the primary antibody (rabbit antidetvrosinated tubulin (5), rat anti-tyrosinated α -tubulin clone YL1/2 (Thermo Fisher Scientific, 287 288 MA1-80017, RRID:AB 2210201) for 1.5 hours at room temperature cells were washed three 289 times with PBS and then incubated for 1 hour with the secondary antibody (Alexa 488,

290 Invitrogen) and DAPI for 1 hour at room temperature. After three washes with FACS buffer, G1 cells (identified by DAPI) within the highest or lowest 1% of staining signal were isolated 291 292 using FACS. DNA was isolated and sequencing libraries were generated as described before 293 (36). Deep sequencing reads from each sample (low and high) were aligned to HG38 allowing 294 a single mismatch and assigned to non-overlapping protein-coding gene regions (Refseq). 295 Unique disruptive integrations per gene body (intron and exon) of a gene were normalized to 296 the total number of sense integrations of each sorted cell population. In this analysis the 5'UTR 297 region was excluded in the analysis in contrast to previous analysis (36). Then the mutational 298 index (MI) was calculated by comparing normalized sense integrations of the high and low cell 299 population using a two-sided Fisher's exact test. Fishtail blots were visualized using Python. 300 Data used to plot the uniqueness across published haploid screens is available on Phenosaurus 301 (https://phenosaurus.nki.nl).

302

303 Immunofluorescence

304 Cells were seeded on cover slips and fixed with ice-cold methanol for 4 min at -20°C. After 1 hour in blocking buffer (5% bovine albumin serum in PBS) at room temperature, cells were 305 306 incubated with primary antibodies in blocking buffer overnight. After three washes with PBS cells were incubated for 1 hour with secondary antibodies (Alexa Invitrogen) in blocking 307 buffer. Cell nuclei were counterstained with DAPI (1 µg/ml) for 10 min. Cells were mounted 308 309 with Aqua-Poly Mount (Fisher Scientific) and imaged on a confocal microscope. Primary 310 antibodies used were mouse anti-HA (BioLegend, #901503, RRID AB:2565005), rabbit antidetyrosinated α -tubulin (by Marin Barisic) (5), rabbit anti- α -tubulin (Abcam, ab18251, 311 312 RRID:AB 2210057). Images were processed in Fiji (37). Colocalization was measured as 313 pearson's correlation coefficient with the Fiji plugin EzColocalization (38). Colocalization was 314 determined for cells within the same range of MATCAP intensity.

- 315
- 316 <u>Immunoblotting</u>

317 Cells were lysed directly in sample buffer containing 100 mM DTT. Proteins were separated 318 on 4-12% Bis-Tris gels and transferred to nitrocellulose membranes. After blocking in TBS 319 with 5% milk and 0.1% Tween20, membranes were incubated with primary and corresponding 320 HRP-conjugated secondary antibodies (Thermo Fisher Scientific). Primary antibodies were 321 anti-a-tubulin (Santa Cruz Biotechnology, sc-32293, RRID:AB 628412), rabbit anti-322 MATCAP (this study), rabbit anti-detyrosinated α -tubulin (Merck Millipore, AB3201, 323 RRID:AB 177350), rabbit anti- $\Delta 2 \alpha$ -tubulin (Merck Millipore, AB3203, RRID:AB 177351), 324 rat anti-tyrosinated a-tubulin clone YL1/2 (Thermo Fisher Scientific, MA1-80017, 325 RRID:AB 2210201), HRP-conjugated mouse anti-GAPDH (GeneTex, GTX627408, 326 RRID:AB 2888014).

327

328 MATCAP expression and purification

329 Sequence analysis based on the Protein-CCD² application (40) was used to identify the 330 catalytic domain of MATCAP. Synthetic sequences of the catalytic domain of MATCAP (137-471) and full-length MATCAP (1-471) were used for cloning. The wildtype constructs were 331 332 used to introduce the nucleophilic E281Q mutation. All proteins were expressed in Spodoptera 333 frugiperda (Sf9) insect cells. In brief, a pFastBac1 plasmid with a his10-twinstrepII-3C-334 MATCAP genetic construct was created through ligation independent cloning (LIC) to custom expression vectors (41). Bacmid virus was produced with the Invitrogen Bac-to-Bac method 335 and expressed in Sf9 insect cells using Insect-Xpress medium (LONZA). The cells were 336 337 cultured in a shaker incubator at 28°C at 80 rpm and infected with the bacmid virus 8 hours after seeding a culture at 1 x 10⁶ cells mL⁻¹. The insect cells were harvested 72 hours after 338 339 the infection, snap-frozen in liquid nitrogen and kept at -20°C until usage.

340 The cell pellet was thawed on ice, and lysed with 4 min sonication (10s on / 30s off) in ice cold lysis buffer (40 mM HEPES/HCl, pH 7.5, 500 mM NaCl, 1 mM Tris(2-carboxyethyl) 341 342 phosphine (TCEP), 1 tablet Ethylenediaminetetraacetic acid (EDTA) free protease inhibitor 343 (Thermo Fisher Scientific)). To clear the lysate, it was centrifuged (30 min, 54.000 g using a JA 25.50 rotor, Beckman Coulter). The supernatant was incubated with 500 µL pre-equilibrated 344 Chelating Sepharose Fast Flow beads (GE healthcare) to capture the his-tagged MATCAP. 345 346 After 1 hour the beads were washed with an excess of washing buffer (20 mM HEPES/HCl, 347 pH 7.5, 500 mM NaCl, 1 mM TCEP) and MATCAP was eluted using an imidazole step-348 gradient (30-500 mM imidazole). The elution fractions that contained MATCAP were 349 supplemented with 3C protease during a 4-hour-dialysis (20 mM HEPES/HCl, pH 7.5, 150 350 mM NaCl, 1 mM TCEP). Finally, cleaved MATCAP was gel-filtered using a s200 10/300 351 column (GE Healthcare) on a FPLC system (Bio-Rad) into 20 mM HEPES, pH 7.5, 140 mM 352 NaCl (protein used for cryo-EM contained 100mM NaCl), 1 mM TCEP, 1 µM ZnCl₂ to remove remaining impurities. The purity of the fractions was assessed with an 8% Coomassie SDS 353 354 page gel (Invitrogen) and the protein was concentrated with a 0.5 mL 10.000 MW Amicon-355 Ultra concentrator (Amicon) to a concentration above 5 mg mL⁻¹. The concentrated protein 356 was snap-frozen in liquid nitrogen and kept at -80°C until usage.

357

358 Crystallization and data collection

359 Purified MATCAP (137-471) was concentrated at about 15-20 mg mL⁻¹ for crystallization. Initial hits were obtained in a sitting drop format using 96-well two-drop MRC plates and the 360 commercial screens PACT, JCSG+, Classics-I & II, ProComplex, ComPAS, pH-Clear and 361 362 Morpheus. The drops consisted of 100 nL protein with 100 nL reservoir solution. After a period 363 of one week, initial hits were identified in the Classics-I screen and further optimized using 364 hanging drops in Limbro plates. Diffracting crystals of MATCAP were obtained in 4.3 M NaCl 365 and 0.1M HEPES/NaOH pH 7. Crystals appeared as clusters, which were further optimized by using the additive screen from Hampton. Crystals grew to maximum dimensions over a period 366 of two weeks and the most promising additive agent for growing single crystals was EDTA. 367 368 We were unable to grow single crystals by varying the concentration of EDTA, but we 369 succeeded in reducing the number of needles that grew in each cluster. Crystals were harvested 370 and cryo-protected in a solution containing 30% ethylene glycol and vitrified in liquid nitrogen. 371 All data collection and refinement statistics are shown in Table S1. A native crystal diffracted 372 to 2.1 Å resolution at PSI X06DA (PXIII, Villingen Switzerland) at the Swiss Light Source (SLS) synchrotron, using a PILATUS 2M-F detector. Crystal content analysis has indicated 373 374 four molecules in the asymmetric unit, with a solvent content 52.6%. As there was no structure 375 of a close homologue of MATCAP and a reliable homology model could not be constructed at 376 the time, the structure had to be determined by experimental phasing. We therefore soaked crystals in mother liquor containing methylmercury chloride solution (10 mM) for ~3 min, 377 378 prior to cryo-protection and vitrification. A highly-redundant Hg derivative dataset was 379 collected also in PSI X06DA at 1.00 Å wavelength at the Hg L-III edge. Crystals belong to the same space group with very similar unit cell dimensions as the native. Following structure 380 381 determination, after we confirmed that MATCAP is a zinc-dependent metalloprotease, we removed EDTA from the crystallization conditions and managed to grow reasonably sized (but 382 383 smaller and weaker diffracting) crystals of MATCAP (137-471) in the presence of Zn^{2+} ions; these crystals diffracted to 2.5 Å resolution. All data were also collected at PSI X06DA. All 384 diffraction data were integrated automatically by XDS (18). Final scaling was performed in 385 AIMLESS (42) within the CCP4 suite (43). Structures were visualized for figure panels with 386 387 ChimeraX (44).

388

389 Crystallographic structure determination and refinement

390 The MATCAP structure was determined using the SIRAS phasing method, with the EDTA 391 structure as native and the mercury soak as a derivative with anomalous scattering. Solvent content analysis identified that this crystal form had four molecules in the asymmetric unit, 392 393 related by non-crystallographic symmetry. The HKL2MAP interface (45) was used to 394 determine that the anomalous signal extended to about 4.0Å, and that dataset was used to run 395 SHELXD (46) using all low-resolution reflections. Most solutions from the different semi-396 random trials of the direct methods were considered reasonable based on internal software 397 statistics (CC vs CCweak). The best solution identified 11 mercury atoms, four with occupancy 398 between 0.85 and 1.0. These sites were virtually identical to those detected by the same 399 software in the context of the CRANK2 pipeline (47). Phasing within CRANK2 resulted to 400 phases with a reasonable overall figure of merit (FOM) of 0.328. Following density 401 modification, the correct hand was determined and the overall FOM improved to 0.668. 402 Automated model building using PARROT (48) within the CRANK2 pipeline, resulted in a model containing 1,369 residues in ten fragments; 90% of the residues were docked in 403 404 sequence.

405 The structure was manually completed and refined with a few iterations of REFMAC (49) and 406 rebuilding in COOT (50). The molecules are arranged by a rotation operator as a dimer, and 407 that dimer is related to another dimer by pure translation, making up the four molecules in the asymmetric unit. At that point, we identified that MATCAP is a metalloprotease, and as that 408 409 dataset clearly did not have zinc in the active site, owing to the presence of EDTA in the 410 crystallization buffer. We thus focused our attention to the lower-resolution dataset that we collected with crystals grown with Zn⁺² in the absence of EDTA. We determined that crystal 411 412 structure by simply refining the model built for the apo form against this new dataset. The 413 electron density map (both the 2mFo-DFc and the phased anomalous difference Fourier 414 synthesis) confirmed the presence of a single zinc ion at the active site. Remarkably, the zinc-415 bound structure had minor differences with the apo structure, even at the vicinity of the active 416 site. Following several iterations of REFMAC, COOT and using PDB-REDO (51) the structure 417 refined to an Rfree of 23%. It has no Ramachandran plot outliers, the RamaZ score is -1.46 418 (52), and the Molprobity score of 1.14 ranking in the 100th percentile (53), and has been 419 deposited to the PDB (PDBID: 7Z5H). The apo structure, has subsequently also been refined to an Rfree of 22%, with no Ramachandran plot outliers, RamaZ score of -0.91 (52), and a 420 421 Molprobity score of 1.17 ranking in the 100th percentile (53), and has been deposited to the 422 PDB (PDBID: 7Z5G). We note that the AlphaFold model from the EBI database (22) shows an ordered structure only for this MATCAP region, and rms difference of 0.888 from the 423 424 crystallographic model.

425

426 HADDOCK modelling

427 A work-in progress model of MATCAP was used to predict the binding site of the C-terminal 428 peptide into the active site of MATCAP using high ambiguity driven docking 429 (HADDOCK)(23, 54). As a substrate we used a peptide with sequence GEEEGEEY. Before docking, the crystal structure of MATCAP (137-471) was truncated digitally to 147-471, as 430 431 the conformation of residues 137-147 looked like a crystallographic artefact and could be 432 blocking the entrance of the catalytic pocket. In our initial docking model, we used three types 433 of restraints. Firstly, we defined active residues of the positive patch directly opposed of the 434 active site in MATCAP (residues 334, 373, 376, 389). Second, we defined active residues in the peptide ⁻¹EY⁰ as confirmed by mass spectrometry. Thirdly, analogous to the DPPIII crystal 435 structure (PDB: 3T6J) (55) we restrained the distance of the zinc catalytic ion to the attacked 436 437 carbonyl to 1.7-3.7 Å. Furthermore, we specified that the C-terminus of the 8-meric peptide 438 should be negatively charged. HADDOCK grouped 197 structures in three clusters (Table S2). 439 This initial docking model was confirmed by mutation studies of the active residues, and also

suggested additional residues that could interact with the peptide. In a follow-up docking experiment, we used the original active residues: ${}^{-1}EY^{0}$, 334, 373, 376, 389, and additionally also: 179, 312, 249, 393, 417. As in the first model, the scissile bond was placed very close to the zinc ion, we decided to redefine the distance restraints between the zinc and the scissile bond to 1.2-3.7 Å. All other parameters were kept the same. This run of HADDOCK resulted to similar docking models as the first run; models were now grouped in two clusters (Table S3).

- 447
- 448 <u>Microtubule isolation</u>

449 Microtubules were prepared from an HCT116 triple knock-out cell line (AMATCAP 450 $\Delta VASH1\Delta VASH2$) or fresh mouse brains. Cells were harvested with PBS-EDTA using a cell 451 scraper and resuspended in 1:1 v/v isolation buffer (100 mM PIPES/KOH pH 6.9, 2 mM 452 EGTA, 1 mM MgCl₂, 1 mM PMSF, complete ultra-protease inhibitor tablet (Fisher Scientific)). Brains were homogenized in isolation buffer (1 µl/mg) on ice. Samples were 453 454 sonicated on ice and cleared by centrifugation (1 hour, 100000 xg, 4°C). Paclitaxel (final 455 concentration 20 µM) and GTP (final concentration 1 mM) were added to the supernatant and 456 incubated at 37°C for 30 min. Polymerized microtubules were then pelleted over a cushion (60% v/v glycerol, 20 μM paclitaxel in isolation buffer) for 40 min at 100000 xg at 37°C. The 457 pellet was resuspended in warm isolation buffer (fig. S6A). 458

459

460 Cryo-EM grid preparation and data collection

Purified full-length, E281Q inactive MATCAP was thawed and kept on ice (fig. S6B, C). To 461 462 increase microtubule binding, MATCAP (5.5 mg mL⁻¹) was diluted 1:1 v/v with low salt buffer 463 (100 mM PIPES/KOH, pH 6.9, 2 mM K-EGTA, 1 mM MgCl₂). 3.5 µL of the freshly prepared 464 HCT116-derived microtubules were applied to a glow discharged (30 mA, 60 s, GloQube, 465 Quorum) 300 mesh quantifoil holey carbon copper grid R1.2/1.3 (Quantifoil micro tools GmbH), which was blotted for 2 s, force 10 in a humidified (30°C, 100% humidification) 466 Vitrobot chamber (ThermoFisher Scientific) Subsequently, 3.5 µL diluted MATCAP was 467 468 applied to the grid and allowed to adhere to the microtubules for 10 s. After 6.5 s of blotting 469 (Whatman, cat no. 47000-100) the grid was plunge-frozen in liquid ethane and transferred to liquid nitrogen until imaging. Grids were screened in JEM-F200 "F2" 200 kV (JEOL) with a 470 471 K2 camera (Gatan). A dataset of 8400 micrographs was collected with EPU-2.10.0 on a 300 472 kV Titan Krios1 (ThermoFisher Scientific) with a K3 camera (Gatan) in super-resolution mode. We used a calibrated pixel size of 0.53 Å/pixel with a nominal magnification of 81000x. 473 474 A total dose of 50 electrons per micrograph divided over 50 frames with a total exposure time 475 of 2.14s was used. The defocus range was -0.6 and -2.1 µm.

476

477 Cryo-EM image processing and modelling

478 Data analysis and processing was done in cryoSPARC (56) in the Oncode and RHPC GPU 479 facility at the NKI, unless mentioned otherwise. First, micrographs were preprocessed: 480 Imported micrographs were corrected for gain, patch-motion (57), and CTF (58) with an F-481 crop of 0.5, resulting in a working pixel size of 1.06 Å/pixel. CTF-corrected micrographs were then used to pick particles on. First, five micrographs were used to manually pick particles to 482 483 create picking templates. Using these templates, particles could then be reliably picked with 484 optimized parameters using the cryoSPARC filament picker job. Particles were subsequently extracted with a box size of 512x512 pixels and F-cropped to 128x128 pixels. The 4x binned 485 particle stack was iteratively classified in 2D to remove broken, contaminated and clearly 486 487 undecorated microtubule particles. To sort the particles to different microtubules classes, we 488 created initial microtubule models (11-16 protofilaments) using the helical refinement option 489 with parameters as listed in Table S4. The particles were then sorted over the different 490 microtubule types with a heterogeneous refinement job. The 14 protofilament containing 491 microtubule particles showed the best signal-to-noise ratio and contained most particles. The 492 particles of this class were selected, un-binned to an F-crop of 416x416, and used for a 493 subsequent helical refinement job, creating a clean 14 protofilament microtubule model and 494 allowing good alignment of the particles. The helical refinement job, however, is unable to 495 align particles to the characteristic microtubule seam. Consequently, these maps did not 496 differentiate between α and β monomers, and thus the density for MATCAP was blurred along 497 the protofilament axis, preventing further analysis. To overcome this, particles were 14x 498 symmetry expanded and used for local refinement with a mask spanning a 2x2 tubulin lattice 499 (fig. S6D). This mask was created using Chimera (59) as follows: we first docked the crystal 500 structure of a tubulin dimer to two protofilaments; then we placed one MATCAP catalytic 501 domain per tubulin dimer in the (blurred) density; finally, the *molmap* command was used to 502 create an asymmetric simulated map of four MATCAPs bound to four tubulin dimers belonging to two adjacent protofilaments. After local refinement with this big mask, the 503 504 characteristic loop differentiating α and β tubulin was clearly visible (fig. S5C). At this point the crystal structure of MATCAP could be fitted in the map reliably, in a unique orientation. 505 506 Then, a smaller mask was created, containing only one protofilament and the bound MATCAP. 507 A final local refinement with this smaller mask resulted in the final map. No preferential orientation was observed. (fig. S6E) at a nominal resolution of 2.9 Å and a median resolution 508 509 of 5.3 Å. (fig. S6F and Table S5). This map and the model obtained by rigid body fitting 510 MATCAP (PDB: 7Z5H) and tubulin (PDB: 7SJ7) were deposited to the PDB and EMDB (PDB: 7Z6S, EMDB: EMD-14529). To ease visualization, the local resolution of the map was 511 512 estimated (fig. S6G-J) (60) and locally filtered using cryoSPARC. The HADDOCK cluster 2 513 model agreed well with the cryo-EM density and was therefore connected to the last modelled 514 residue of tubulin for a more intuitive interpretation, using Coot (50) (fig. S7A). Buried surface 515 areas between the contacting interfaces were calculated using PDBePISA (61). Maps were 516 visualized and figures were created using ChimeraX (44).

- 517
- 518 In vitro peptide assay

Peptides incubated with MATCAP were analyzed as decribed previously (24). Briefly, 500 µM 519 520 peptides were incubated with 500 nM MATCAP for 4 hours at 37°C. Reactions were frozen 521 until analysis. Samples were passed through a Microcon-10 spin filter (Millipore), diluted with 2% formic acid and analyzed by LC-MS/MS on a Thermo Orbitrap Fusion hybrid mass 522 spectrometer (Q-OT-qIT, Thermo Scientific) equipped with a Proxeon nLC 1000 system 523 524 (Thermo Scientific, Bremen), using a 30min gradient. RAW files were processed using 525 Proteome Discoverer (version 2.5.400, Thermo Fisher Scientific). MS2 spectra were searched 526 against a custom database containing the mutagenized synthetic versions of tubulin-like C-527 terminal peptides, using SequestHT. No enzyme and miscleavages were allowed and data were 528 filtered with Xcorr score >1. Determination of the extent of peptide cleavage by MATCAP and subsequent assessment of VASH1 substrate specificity- was performed based on the 529 530 number of peptide spectrum matches (PSM) for the intact and cleaved/detyrosinated version 531 of each peptide.

- 532
- 533 <u>Analysis of TUBA1A/B C-termini from mouse brain microtubules</u>

534 Mouse brain tubulin pellets were heated for 5 min. at 95°C in 1x S-Trap lysis buffer (50mM

535 TEAB pH 8.5, 5% SDS), after which 30 µg protein amounts (as determined by BCA Protein

536 Assay Kit, Thermo Fisher Scientific) were digested on S-Trap Micro spin columns according

537 to the manufacturer's instructions (ProtiFi, NY, United States), using 1:15 (enzyme:protein)

- 538 Lys-N (ImmunoPrecise Antibodies Europe BV, Utrecht, The Netherlands). Digests were
- analyzed by LC-MS/MS on the system described above, using a 210-min gradient. RAW files

540 were analyzed by MaxQuant (version 2.0.1.0) (62) using standard settings. Spectra were

- 541 searched against the reviewed Swissprot M. musculus database (2021 04, 17073 sequences) 542 with semi-specific LysN as cleavage specificity, allowing the identification of full-length and
- 543 truncated versions of the tubulin C-terminus. The amount of C-terminal detyrosination was
- 544 calculated for each sample, based on the LFQ abundances of (truncated) terminal peptides.
- 545

546 In vitro detyrosination assays

547 The detyrosination activity on tubulin dimers was assessed by incubating 50 nM Hela α/β -548 tubulin dimers (Cytoskeleton) with 25 nM (unless stated otherwise in the figure legend) 549 recombinant MATCAP protein in 50 mM Tris-HCl, pH 8, 10% glycerol, 1 mM DTT and 1 550 mM PMSF for 45 min at 37°C. To inhibit MATCAP 25 µM EDTA was used. For testing 551 MATCAP's activity on microtubules versus α/β -tubulin dimers, 3 mg/ml HeLa α/β -tubulin 552 (Cytoskeleton) were polymerized into microtubules by the addition of 1 mM GTP, 10% glycerol and stepwise addition of paclitaxel (0.3 to 3 to 30 µM every 10 min). The reaction was 553 554 incubated at 37°C for 60 min. Microtubules equivalent of 500 nM tubulin dimers and 10 nM MATCAP protein were incubated in 50 mM Tris-HCl, pH 8, 10% glycerol, 1 mM DTT and 1 555 556 mM PMSF for the indicated times at 37°C. Detyrosination levels were quantified by 557 immunoblot analysis. To estimate the intensity of each band, the sum of pixel values in a square 558 block, larger than the band, were measured and corrected by the subtraction of a background 559 value using Image Lab software (Bio-Rad). To account for differences in the efficacy of 560 blotting between experiments, a linear scale was applied between equivalent datasets from different experiments. Scale factors were calculated by linear regression to minimize the least 561 squared difference between the points of all five datasets. For each dataset, the detyrosination 562 563 activity was normalized by the measured intensity for a-tubulin. A linear model was fit to 564 estimate the rates of hydrolysis for the two reactions.

565

Co-sedimentation assay 566

567 Microtubules were assembled by incubating porcine tubulin dimers (Cytoskeleton) with GTP 568 (1 mM), glycerol (10%) and paclitaxel (30 µM) at 37°C for 30 min. MATCAP protein (0.5 569 µM) and in vitro assembled microtubules (corresponding to 1 µM tubulin dimers) were incubated at room temperature for 20 min and then pelleted (100000 xg, 35°C, 40 min) over a 570 paclitaxel-glycerol cushion. The microtubule containing pellet was then analyzed by 571 572 immunoblotting.

573 Use, generation and maintenance of mouse strains

574 Mouse experiments were approved by the Animal Ethics Committee of the Netherlands Cancer 575 Institute (AVD301002017840 authorized by the national agency Centrale Commissie 576 Dierproeven) and implemented in accordance with institutional, national and European 577 guidelines for Animal Care and Use. SVBP knockout mice were described before (11). 578 MATCAP KO mice were generated on FVB background (Janvier, FVB/NRj) using two 579 gRNAs (5'-CTGCATGTATTTGCGCACGG-3', 5'-AGATGATTGGCTCGGGCATT-3') to create a 1348 bp 580 deletion, which destroys the catalytic HEIGHT motif. SVBP and MATCAP genomic loci were 581 genotyped by PCR amplification using the following primers:

- 582 MATCAP 5'-GCATCATTGAGGCTGTCCTGGAG-3',
 - 5'-CACCATCATCACAGCCTGGGAC-3',
- 583 584 5'-GCAGCCTGTGAGAAGGATAACCA-3'
- 585 **SVBP** 5'-GATCCACCTGCCCGGAAA-3',
 - 5'-CAAACCATGGATCCACGAAA,
- 586 587 5'-TTTCTTCCAGCACCCTCTCC-3'.
- For genotyping of MATCAP locus an annealing temperature of 67 °C and 45 s extension time 588 589 and for the SVBP locus 50 °C for 30 s, respectively. Amplicons were analyzed on a 1% agarose

- 590 gel. For in vivo studies randomization and statistical methods to predetermine the sample size
- 591 were not performed. All recorded measurements were included.
- 592

593 *In vivo* MRI scanning of mouse brains

594 Mice were injected intraperitoneally with 0.4 mmol/kg dose of 30 mM Manganese Chloride (MnCl₂) 24 hours before scanning them individually with ParaVision 6.0.1 software in a 7 595 596 Tesla BioSpec 70/20 USR MRI system with a ø23 mm¹H transmit-receive mouse brain 597 volume coil (RF RES 300 1H 023 M.BR QSN TR 2.4M), all from Bruker BioSpin. During the 598 acquisition, mice were anaesthetized with 1-2% isoflurane in a mix of air and oxygen. 599 Acquisition parameters are T1-weighted, 3D gradient echo sequence, TR = 31 ms, TE = 7.7600 ms, flip angle = 37° , field-of-view = $20 \times 15 \times 17$ mm, matrix size = $200 \times 150 \times 170$, number 601 of averages and repetitions = 1, total acquisition time = 20 minutes with respiratory trigger, 602 isotropic resolution = $100 \mu m$. For quantifications, DICOM files were loaded into 3D Slicer (63). Brain structures were manually outlined on every third (substructures) or tenth (total 603 604 brain) slice. Then slices in-between were automatically filled using the segmentation editor tool, the selection was visually inspected and if needed manually refined. Measurements were 605 606 made using the quantification tool in 3D Slicer. 3D reconstructions are shown as orthographic 607 renderings. Measurements were done blinded. Statistical tests were performed using GraphPad 608 Prism.

609

610 <u>Counting of brain cells</u>

611 The total number of brain cells per adult mouse brain was counted with the isotropic 612 fractionator method (64). Briefly, intact nuclei were isolated from PFA-fixed adult brains by 613 homogenization in a 40 mM sodium citrate, 1% Triton X-100 solution. Washes from rinsing 614 the glass homogenizer are collected and centrifuged (15 min at 4000 xg) together with the 615 homogenate. Pelleted nuclei are resuspended in PBS with 0.5 μ g/ml DAPI. A fraction of the 616 nuclei solution was mixed with 50 μ l CountBright Absolute Counting Beads (Thermo Fisher)

- and technical duplicates for each sample were analyzed on a flow cytometer. The flow
- 617 and technical duplicates for each sample were analyzed on a flow cytometer. The flow 618 cytometer operator was blinded. Acquired measurements of cells and beads were used to 610 calculate the cell concentration of each comple according to the menufacture's menual
- 619 calculate the cell concentration of each sample according to the manufacturer's manual.620
- 621 Nest building test
- 622 Nest building behaviour was assessed as previously described (65). Briefly, adult mice were
- 623 moved to individual cages with one flat enrichment sheets (7g) per cage. The next morning,
- 624 nests were scored using the following scale: 1) nests with wall higher than mouse body height, 625 2) flat past 3) no alear past but >50% shreddad past material 4) no past but >50% shreddad
- $(625 \quad 2)$ flat nest, 3) no clear nest but >50% shredded nest material, 4) no nest but >50% shredded $(626 \quad nest material, 5)$ no visible engagement with the nesting material at all. The experimenter
- 626 nest material, 5) no visible engagement with the nesting material at all. The experimenter 627 scored the nests blinded.
- 628
- 629 <u>Immunohistochemistry</u>
- Sections of formalin-fixed tissues were deparaffinized and rinsed three times with wash buffer
 (0.05% Tween20 in PBS). After inactivation of endogenous peroxidases with 3% H2O2 in
 Methanol, again rinsed three times and blocked in 4% BSA 5% normal goat serum in PBS
 (deY-Tubulin) or 10% ELK in normal goat serum. Primary antibodies were applied overnight
 at 4°C. After three washes sections were incubated with Envision+ System-HRP labelled
 antibodies. After another three washes with wash buffer, sections were visualized with DAB+
- 636 (Dako, K3468, 1 mL substrate buffer + 20 μl Chromogen) for 3 min, rinsed with demiH₂O,
- 637 counterstained with Haematoxylin, dehydrated and mounted with Entellan. Primary antibodies
- 638 were rabbit anti-detyrosinated tubulin (Barisic lab) and cleaved caspase 3 (Cell signaling,
- 639 #9661, RRID:AB_2341188). Antigen retrieval was performed for the deY-tubulin (Citric

- buffer from Scytek, CBB999, 20 min, 98°C) and cleaved caspase 3 (Tris/EDTA pH9, 30 min,
 96 °C) staining. Apoptotic cells were quantified using QuPath (66).
- 642 For mouse brain stainings, embryonic brains dissected at E14.5 were fixed for 7 hours with 4%
- 643 paraformaldehyde (PFA) in PBS. After three PBS washes brains were cryopreserved using a
- 644 30% sucrose solution, and then frozen in 7.5% gelatin plus 15% sucrose solution. Coronal
- 645 cryosections were obtained at 12 μm thickness and postfixed with 4% PFA for 10 min, washed
- 646 with 1x PBS, then treated with 0.3% Triton X-100 for 5 min, and incubated in blocking solution
- 647 containing 5% FBS, 3% BSA, 0.1% Triton X-100 in 1× PBS, for 2 hours. Samples were
- 648 incubated with primary antibodies diluted in blocking solution at 4°C overnight, as previously
- 649 described (39). The primary antibodies used were: rabbit anti-phospho-Histone H3 (Merck-
- Millipore, 06-570, RRID:AB_310177), mouse anti-Tuj1 (Sigma, T8660, RRID:AB_477590)
 and rabbit anti-cleaved Caspase3 (R&D Systems, AF835, RRID:AB_2243952).
- Next, after three washes with 0.1% Tween-20 in 1x PBS, sections were incubated with
- 653 secondary antibodies (goat anti-rabbit Alexa Fluor 488, goat anti-mouse Alexa Fluor 568)
- diluted in blocking solution for 1 hour. Draq-5 (Biostatus) was used to stain DNA. Sections
- 655 were mounted in Mowiol 4-88 (Calbiochem). Images were recorded on a confocal fluorescence
- 656 microscopy Leica TCS SP5 with a Leica DMI6000B microscope.
- 657 658

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660 **References and Notes:**

- 661
- 662 1. A. Roll-Mecak, The Tubulin Code in Microtubule Dynamics and Information Encoding.
 663 Dev Cell. 54, 7–20 (2020).
- 664 2. C. Janke, M. M. Magiera, The tubulin code and its role in controlling microtubule properties 665 and functions. *Nat Rev Mol Cell Bio*. 21, 307–326 (2020).
- 666 3. J. Nieuwenhuis, T. R. Brummelkamp, The Tubulin Detyrosination Cycle: Function and 667 Enzymes. *Trends Cell Biol.* 29, 80–92 (2018).
- 4. C. Sanyal, N. Pietsch, S. R. Rios, L. Peris, L. Carrier, M.-J. Moutin, The detyrosination/re tyrosination cycle of tubulin and its role and dysfunction in neurons and cardiomyocytes. *Semin Cell Dev Biol* (2021), doi:10.1016/j.semcdb.2021.12.006.
- 5. S. Liao, G. Rajendraprasad, N. Wang, S. Eibes, J. Gao, H. Yu, G. Wu, X. Tu, H. Huang, M.
- Barisic, C. Xu, Molecular basis of vasohibins-mediated detyrosination and its impact on spindle function and mitosis. *Cell Res.* 29, 533–547 (2019).
- 6. M. Barisic, R. S. e Sousa, S. K. Tripathy, M. M. Magiera, A. V. Zaytsev, A. L. Pereira, C.
 Janke, E. L. Grishchuk, H. Maiato, Microtubule detyrosination guides chromosomes during
 mitosis. *Science*. 348, 799–803 (2015).
- 7. L. T. Ferreira, B. Orr, G. Rajendraprasad, A. J. Pereira, C. Lemos, J. T. Lima, C. G. Boldú,
 J. G. Ferreira, M. Barisic, H. Maiato, α-Tubulin detyrosination impairs mitotic error correction
 by suppressing MCAK centromeric activity. *J Cell Biol.* 219, e201910064 (2020).
- 8. T. Akera, L. Chmátal, E. Trimm, K. Yang, C. Aonbangkhen, D. M. Chenoweth, C. Janke,
 R. M. Schultz, M. A. Lampson, Spindle asymmetry drives non-Mendelian chromosome
 segregation. *Science*. 358, 668–672 (2017).
- 9. Z. Iqbal, H. Tawamie, W. Ba, A. Reis, B. A. Halak, H. Sticht, S. Uebe, N. N. Kasri, S.
 Riazuddin, H. van Bokhoven, R. A. Jamra, Loss of function of SVBP leads to autosomal
 recessive intellectual disability, microcephaly, ataxia, and hypotonia. *Genet Med.* 21, 1790–
 1796 (2019).
- 10. C. Aillaud, C. Bosc, L. Peris, A. Bosson, P. Heemeryck, J. V. Dijk, J. L. Friec, B. Boulan,
 F. Vossier, L. E. Sanman, S. Syed, N. Amara, Y. Couté, L. Lafanechère, E. Denarier, C.
 Delphin, L. Pelletier, S. Humbert, M. Bogyo, A. Andrieux, K. Rogowski, M.-J. Moutin,
 Vasohibins/SVBP are tubulin carboxypeptidases (TCPs) that regulate neuron differentiation. *Science*. 358, 1448–1453 (2017).
- 11. A. T. Pagnamenta, P. Heemeryck, H. C. Martin, C. Bosc, L. Peris, I. Uszynski, S. GoryFauré, S. Couly, C. Deshpande, A. Siddiqui, A. A. Elmonairy, W. Consortium, G. E. R.
 Consortium, S. Jayawant, S. Murthy, I. Walker, L. Loong, P. Bauer, F. Vossier, E. Denarier,
 T. Maurice, E. L. Barbier, J.-C. Deloulme, J. C. Taylor, E. M. Blair, A. Andrieux, M.-J. Moutin,
 Defective tubulin detyrosination causes structural brain abnormalities with cognitive
 deficiency in humans and mice. *Hum Mol Genet.* 28, 3391–3405 (2019).

12. J. P. Kerr, P. Robison, G. Shi, A. I. Bogush, A. M. Kempema, J. K. Hexum, N. Becerra, D.
A. Harki, S. S. Martin, R. Raiteri, B. L. Prosser, C. W. Ward, Detyrosinated microtubules
modulate mechanotransduction in heart and skeletal muscle. *Nat Commun.* 6, 8526 (2015).

13. C. Y. Chen, M. A. Caporizzo, K. Bedi, A. Vite, A. I. Bogush, P. Robison, J. G. Heffler, A.
K. Salomon, N. A. Kelly, A. Babu, M. P. Morley, K. B. Margulies, B. L. Prosser, Suppression
of detyrosinated microtubules improves cardiomyocyte function in human heart failure. *Nat Med.* 24, 1225–1233 (2018).

- 14. X. Yu, X. Chen, M. Amrute-Nayak, E. Allgeyer, A. Zhao, H. Chenoweth, M. Clement, J.
 Harrison, C. Doreth, G. Sirinakis, T. Krieg, H. Zhou, H. Huang, K. Tokuraku, D. S. Johnston,
 Z. Mallat, X. Li, MARK4 controls ischaemic heart failure through microtubule detyrosination. *Nature*. 594, 560–565 (2021).
- 709 15. P. Robison, M. A. Caporizzo, H. Ahmadzadeh, A. I. Bogush, C. Y. Chen, K. B. Margulies,
 710 V. B. Shenoy, B. L. Prosser, Detyrosinated microtubules buckle and bear load in contracting
 711 cardiomyocytes. *Science*. 352, aaf0659 (2016).
- 712 16. M. E. Pero, C. Meregalli, X. Qu, G. J. Shin, A. Kumar, M. Shorey, M. M. Rolls, K. Tanji,
- T. H. Brannagan, P. Alberti, G. Fumagalli, L. Monza, W. B. Grueber, G. Cavaletti, F. Bartolini,
 Pathogenic role of delta 2 tubulin in bortezomib-induced peripheral neuropathy. *Proc National Acad Sci.* 118, e2012685118 (2021).
- 716 17. J. Nieuwenhuis, A. Adamopoulos, O. B. Bleijerveld, A. Mazouzi, E. Stickel, P. Celie, M.
 717 Altelaar, P. Knipscheer, A. Perrakis, V. A. Blomen, T. R. Brummelkamp, Vasohibins encode
- tubulin detyrosinating activity. *Science*. 358, 1453–1456 (2017).
- 18. K. Ersfeld, J. Wehland, U. Plessmann, H. Dodemont, V. Gerke, K. Weber, Characterization
 of the tubulin-tyrosine ligase. *J Cell Biol.* 120, 725–732 (1993).
- 19. N. Cerdà-Costa, F. X. Gomis-Rüth, Architecture and function of metallopeptidase catalytic
 domains: Metallopeptidase Catalytic Domains. *Protein Sci.* 23, 123–144 (2013).
- 20. D. Ghilarov, M. Serebryakova, C. E. M. Stevenson, S. J. Hearnshaw, D. S. Volkov, A.
 Maxwell, D. M. Lawson, K. Severinov, The Origins of Specificity in the Microcin-Processing
 Protease TldD/E. *Structure*. 25, 1549-1561.e5 (2017).
- 726 21. K. Fukasawa, K. M. Fukasawa, M. Kanai, S. Fujii, J. Hirose, M. Harada, Dipeptidyl
 727 peptidase III is a zinc metallo-exopeptidase. Molecular cloning and expression. *Biochem J.*728 329, 275–282 (1998).
- 22. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K.
 Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl,
 A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen,
 D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S.
 Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis,
 Highly accurate protein structure prediction with AlphaFold. *Nature*. 596, 583–589 (2021).
- 735 23. G. C. P. van Zundert, J. P. G. L. M. Rodrigues, M. Trellet, C. Schmitz, P. L. Kastritis, E.
 736 Karaca, A. S. J. Melquiond, M. van Dijk, S. J. de Vries, A. M. J. J. Bonvin, The HADDOCK2.2

- Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. *J Mol Biol.* 428,
 720–725 (2016).
- 24. A. Adamopoulos, L. Landskron, T. Heidebrecht, F. Tsakou, O. B. Bleijerveld, M. Altelaar,
 J. Nieuwenhuis, P. H. N. Celie, T. R. Brummelkamp, A. Perrakis, Crystal structure of the
 tubulin tyrosine carboxypeptidase complex VASH1–SVBP. *Nat Struct Mol Biol.* 26, 567–570
 (2019).
- 25. N. Wang, C. Bosc, S. R. Choi, B. Boulan, L. Peris, N. Olieric, H. Bao, F. Krichen, L. Chen,
 A. Andrieux, V. Olieric, M.-J. Moutin, M. O. Steinmetz, H. Huang, Structural basis of tubulin
 detyrosination by the vasohibin–SVBP enzyme complex. *Nat Struct Mol Biol.* 26, 571–582
 (2019).
- 747 26. C. Zhou, L. Yan, W. Zhang, Z. Liu, Structural basis of tubulin detyrosination by
 748 VASH2/SVBP heterodimer. *Nat Commun.* 10, 3212 (2019).
- 749 27. F. Li, Y. Hu, S. Qi, X. Luo, H. Yu, Structural basis of tubulin detyrosination by vasohibins.
 750 *Nat Struct Mol Biol.* 26, 583–591 (2019).
- 28. X. Liu, H. Wang, J. Zhu, Y. Xie, X. Liang, Z. Chen, Y. Feng, Y. Zhang, Structural insights
 into tubulin detyrosination by vasohibins-SVBP complex. *Cell Discov.* 5, 65 (2019).
- 29. H. S. Barra, C. A. Arce, C. E. Argaraña, Posttranslational tyrosination/detyrosination of
 tubulin. *Mol Neurobiol.* 2, 133–153 (1988).
- 30. G. G. Deanin, S. F. Preston, R. K. Hanson, M. W. Gordon, On the Mechanism of Turnover
 of the Carboxy-Terminal Tyrosine of the Alpha Chain of Tubulin. *Eur J Biochem.* 109, 207–
 216 (1980).
- 31. N. Kumar, M. Flavin, Preferential action of a brain detyrosinolating carboxypeptidase on
 polymerized tubulin. *J Biol Chem.* 256, 7678–7686 (1981).
- 760 32. F. Li, Y. Li, X. Ye, H. Gao, Z. Shi, X. Luo, L. M. Rice, H. Yu, Cryo-EM structure of 761 VASH1-SVBP bound to microtubules. *eLife*. 9, e58157 (2020).
- 33. C. Erck, L. Peris, A. Andrieux, C. Meissirel, A. D. Gruber, M. Vernet, A. Schweitzer, Y.
 Saoudi, H. Pointu, C. Bosc, P. A. Salin, D. Job, J. Wehland, A vital role of tubulin-tyrosineligase for neuronal organization. *Proc National Acad Sci.* 102, 7853–7858 (2005).
- 34. J. E. Carette, J. Pruszak, M. Varadarajan, V. A. Blomen, S. Gokhale, F. D. Camargo, M.
 Wernig, R. Jaenisch, T. R. Brummelkamp, Generation of iPSCs from cultured human
 malignant cells. *Blood*. 115, 4039–4042 (2010).
- 35. E. K. Brinkman, T. Chen, M. Amendola, B. van Steensel, Easy quantitative assessment of
 genome editing by sequence trace decomposition. *Nucleic Acids Res.* 42, e168–e168 (2014).
- 36. M. Brockmann, V. A. Blomen, J. Nieuwenhuis, E. Stickel, M. Raaben, O. B. Bleijerveld,
- A. F. M. Altelaar, L. T. Jae, T. R. Brummelkamp, Genetic wiring maps of single-cell protein
- states reveal an off-switch for GPCR signalling. *Nature*. 546, 307–311 (2017).

- 37. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S.
 Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K.
 Eliceiri, P. Tomancak, A. Cardona, Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 9, 676–682 (2012).
- 38. W. Stauffer, H. Sheng, H. N. Lim, EzColocalization: An ImageJ plugin for visualizing and
 measuring colocalization in cells and organisms. *Sci Rep.* 8, 15764 (2018).
- 39. M. Lalioti, K. Kaplani, G. Lokka, T. Georgomanolis, C. Kyrousi, W. Dong, A. Dunbar, E.
 Parlapani, E. Damianidou, N. Spassky, K. T. Kahle, A. Papantonis, Z. Lygerou, S. Taraviras,
- 781 GemC1 is a critical switch for neural stem cell generation in the postnatal brain. *Glia*. 67, 2360–
- 782 2373 (2019).
- 40. W. T. M. Mooij, E. Mitsiki, A. Perrakis, ProteinCCD: enabling the design of protein
 truncation constructs for expression and crystallization experiments. *Nucleic Acids Res.* 37,
 W402–W405 (2009).
- 786 41. M. P. A. Luna-Vargas, E. Christodoulou, A. Alfieri, W. J. van Dijk, M. Stadnik, R. G.
- 787 Hibbert, D. D. Sahtoe, M. Clerici, V. D. Marco, D. Littler, P. H. N. Celie, T. K. Sixma, A.
- 788 Perrakis, Enabling high-throughput ligation-independent cloning and protein expression for the
- family of ubiquitin specific proteases. *J Struct Biol*. 175, 113–119 (2011).
- 42. P. R. Evans, G. N. Murshudov, How good are my data and what is the resolution? *Acta Crystallogr Sect D Biological Crystallogr*. 69, 1204–14 (2013).
- 43. L. Potterton, J. Agirre, C. Ballard, K. Cowtan, E. Dodson, P. R. Evans, H. T. Jenkins, R.
 Keegan, E. Krissinel, K. Stevenson, A. Lebedev, S. J. McNicholas, R. A. Nicholls, M. Noble,
 N. S. Pannu, C. Roth, G. Sheldrick, P. Skubak, J. Turkenburg, V. Uski, F. von Delft, D.
 Waterman, K. Wilson, M. Winn, M. Wojdyr, CCP 4 i 2: the new graphical user interface to the
 CCP 4 program suite. *Acta Crystallogr Sect D Struct Biology*. 74, 68–84 (2018).
- 44. E. F. Pettersen, T. D. Goddard, C. C. Huang, E. C. Meng, G. S. Couch, T. I. Croll, J. H.
 Morris, T. E. Ferrin, UCSF ChimeraX : Structure visualization for researchers, educators, and
 developers. *Protein Sci.* 30, 70–82 (2020).
- 45. T. Pape, T. R. Schneider, HKL2MAP: a graphical user interface for macromolecular
 phasing with SHELX programs. *J Appl Crystallogr*. 37, 843–844 (2004).
- 46. G. M. Sheldrick, Experimental phasing with SHELXC / D / E : combining chain tracing
 with density modification. *Acta Crystallogr Sect D Biological Crystallogr.* 66, 479–485
 (2010).
- 47. N. S. Pannu, W.-J. Waterreus, P. Skubák, I. Sikharulidze, J. P. Abrahams, R. A. G. de
 Graaff, Recent advances in the CRANK software suite for experimental phasing. *Acta Crystallogr Sect D Biological Crystallogr.* 67, 331–337 (2011).
- 48. K. Cowtan, Recent developments in classical density modification. *Acta Crystallogr Sect D Biological Crystallogr*. 66, 470–478 (2010).

- 810 49. G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls,
- 811 M. D. Winn, F. Long, A. A. Vagin, REFMAC 5 for the refinement of macromolecular crystal
- 812 structures. Acta Crystallogr Sect D Biological Crystallogr. 67, 355–367 (2011).
- 50. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr Sect D Biological Crystallogr.* 66, 486–501 (2010).
- 51. R. P. Joosten, K. Joosten, S. X. Cohen, G. Vriend, A. Perrakis, Automatic rebuilding and
 optimization of crystallographic structures in the Protein Data Bank. *Bioinformatics*. 27, 3392–
 3398 (2011).
- 52. O. V. Sobolev, P. V. Afonine, N. W. Moriarty, M. L. Hekkelman, R. P. Joosten, A. Perrakis,
 P. D. Adams, A Global Ramachandran Score Identifies Protein Structures with Unlikely
 Stereochemistry. *Structure*. 28, 1249–1258 (2020).
- 53. C. J. Williams, J. J. Headd, N. W. Moriarty, M. G. Prisant, L. L. Videau, L. N. Deis, V.
 Verma, D. A. Keedy, B. J. Hintze, V. B. Chen, S. Jain, S. M. Lewis, W. B. Arendall, J.
 Snoeyink, P. D. Adams, S. C. Lovell, J. S. Richardson, D. C. Richardson, MolProbity: More
 and better reference data for improved all-atom structure validation. *Protein Sci Publ Protein Soc.* 27, 293–315 (2017).
- 54. C. Dominguez, R. Boelens, A. M. J. J. Bonvin, HADDOCK: A Protein–Protein Docking
 Approach Based on Biochemical or Biophysical Information. *J Am Chem Soc.* 125, 1731–1737
 (2003).
- 55. G. A. Bezerra, E. Dobrovetsky, R. Viertlmayr, A. Dong, A. Binter, M. Abramić, P.
 Macheroux, S. Dhe-Paganon, K. Gruber, Entropy-driven binding of opioid peptides induces a
 large domain motion in human dipeptidyl peptidase III. *Proc National Acad Sci.* 109, 6525–
 6530 (2012).
- 56. A. Punjani, J. L. Rubinstein, D. J. Fleet, M. A. Brubaker, cryoSPARC: algorithms for rapid
 unsupervised cryo-EM structure determination. *Nat Methods*. 14, 290–296 (2017).
- 57. J. L. Rubinstein, M. A. Brubaker, Alignment of cryo-EM movies of individual particles by
 optimization of image translations. *J Struct Biol.* 192, 188–195 (2015).
- 58. J. Zivanov, T. Nakane, S. H. W. Scheres, Estimation of high-order aberrations and anisotropic magnification from cryo-EM data sets in RELION-3.1. *IUCrJ*. 7, 253–267 (2020).
- 59. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng,
 T. E. Ferrin, UCSF Chimera—A visualization system for exploratory research and analysis. J *Comput Chem.* 25, 1605–1612 (2004).
- 842 60. G. Cardone, J. B. Heymann, A. C. Steven, One number does not fit all: Mapping local 843 variations in resolution in cryo-EM reconstructions. *J Struct Biol*. 184, 226–236 (2013).
- 844 61. E. Krissinel, K. Henrick, Inference of macromolecular assemblies from crystalline state. J
 845 *Mol Biol.* 372, 774–97 (2007).

62. J. Cox, M. Y. Hein, C. A. Luber, I. Paron, N. Nagaraj, M. Mann, Accurate Proteome-wide
Label-free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction,
Termed MaxLFQ. *Mol Cell Proteomics*. 13, 2513–2526 (2014).

63. A. Fedorov, R. Beichel, J. Kalpathy-Cramer, J. Finet, J.-C. Fillion-Robin, S. Pujol, C.
Bauer, D. Jennings, F. Fennessy, M. Sonka, J. Buatti, S. Aylward, J. V. Miller, S. Pieper, R.
Kikinis, 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging*. 30, 1323–1341 (2012).

- 853 64. N. A. Young, D. K. Flaherty, D. C. Airey, P. Varlan, F. Aworunse, J. H. Kaas, C. E. Collins,
- 854 Use of flow cytometry for high-throughput cell population estimates in brain tissue. Front
- 855 *Neuroanat.* 6, 27 (2012).
- 856 65. R. M. Deacon, Assessing nest building in mice. Nat Protoc. 1, 1117–1119 (2006).

857 66. P. Bankhead, M. B. Loughrey, J. A. Fernández, Y. Dombrowski, D. G. McArt, P. D. Dunne,

- 858 S. McQuaid, R. T. Gray, L. J. Murray, H. G. Coleman, J. A. James, M. Salto-Tellez, P. W.
- Hamilton, QuPath: Open source software for digital pathology image analysis. Sci Rep. 7,
- 860 16878 (2017).
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891 Author contributions:

892 L.L., J.B., A.P. and T.R.B. wrote the manuscript and visualized the data; A.A. and T.H. established protein purification and crystallised MATCAP; A.A. and A.P. determined and J.B. 893 894 refined the crystal structure; J.B. performed the cryo-EM structure determination and analysis, and computational modelling; K.K performed the experiment on embryonic proliferation under 895 the supervision of S.T.; HADDOCK-related mutagenesis experiments were performed by 896 897 M.M.; O.B. performed mass-spectrometry and analysis; L.G.vd.H. organized mouse 898 experiments and genotyping; JY.S. performed and interpreted brain stainings; MJ.M provided 899 the SVBP KO mouse line, M.B. shared the deY-tubulin antibody; J.N. performed the Y-tubulin 900 screen; L.H. generated the MATCAP KO mice; L.L. performed all other experiments. L.L. 901 A.P. and T.R.B conceptualized and coordinated research.

- 902
- 903 **Competing interests:** T.R.B. is a co-founder and scientific advisor at Scenic Biotech. 904

Data and material availability: Cell lines, MATCAP antibody and MATCAP knockout mice
are available from T.R.B. under a material transfer agreement with the Netherlands Cancer
Institute. Structures and data is available via the corresponding databases (PDB: 7Z5G, 7Z5H,
7Z6S; EMDB: EMD-14529) and screen data is available in the NCBI Sequence Read Archive
(www.ncbi.nlm.nih.gov/sra, BioProject ID PRJNA809639) and on an interactive visualization
platform (https://phenosaurus.nki.nl).

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913 Supplementary Materials

- 914 Fig. S1 to S8
- 915 Table S1-5
- 916 Data S1-2
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Figure 1. Haploid genetics identify MATCAP as a regulator of α-tubulin detyrosination. 922 923 (A) Illustration of the tubulin detyrosination cycle; Vasohibins and a yet unknown enzyme 924 remove the C-terminal residue (tyrosine) from α -tubulin. U2OS cells proficient and deficient 925 for VASH1 and VASH2, with and without paclitaxel treatment were subjected to (B) 926 immunoblot analysis (short and long exposures are indicated) and (C) immunostaining for 927 detyrosinated α -tubulin. Scale bar 20 μ m. (**D**) Schematic representation of a haploid genetic 928 screen. Haploid genetic screens for (E) detyrosinated α -tubulin in HAP1 Δ VASH1 Δ VASH2 929 knockout cells and (F) tyrosinated α -tubulin in HAP1 Δ VASH1 Δ VASH2 Δ TTL knockout 930 cells were performed. The antibody symbol indicates which α -tubulin state was detected as 931 read-out for the genetic screen. In the fishtail plot genes enriched for gene-trap mutations in 932 the population with 'high' or 'low' antibody staining are colored with orange or blue 933 respectively (for both: two-sided Fisher's exact test, FDR corrected $p \le 0.05$). (G) For each gene 934 the difference in mutation index (MI) between the two screens is depicted as delta mutation 935 index (MI), which is calculated as MIdeY-tubulin screen – MIY-tubulin screen. (H) Lysates of indicated 936 knockout cell lines analyzed for detyrosinated α -tubulin by immunoblotting. (I) Lysates of 937 HeLa H1 cells upon ectopic expression of HA-tagged and untagged MATCAP were subjected 938 to immunoblot analysis. (J) Immunoblot analysis of lysates from triple knockout HAP1 cells 939 (ΔVASH1 ΔVASH2 ΔMATCAP) expressing HA-tagged MATCAP or GFP as control. 940

941

Figure 2







942 Figure 2. The metallo-carboxypeptidase MATCAP detyrosinates α-tubulin.

943 (A) Schematic representation MATCAP and its conservation across vertebrates. Amino acid 944 conservation of MATCAP across 39 vertebrate orthologous is shown as AMAS conservation 945 score (calculated in Jalview). The probability of disordered regions as predicted by PrDOS is 946 shown in the bottom. (B) Ribbon representation of MATCAP (blue) with the overlayed surface (grey). (C) Recombinant MATCAP protein was incubated with tubulin heterodimers in the 947 presence or absence of EDTA. Tubulin modifications were analyzed by immunoblotting. (**D**) 948 949 Comparison of MATCAP and two of the related members of the gluzincin clan, highlighting 950 the zinc ion (yellow) and the binding residues. Arrows indicate the two separate α -helices. (E) 951 Close-up of the MATCAP active site pocket (F) Quantification of immunoblots of lysates from 952 HeLa cells expressing single-point mutants of the active site residues. The detyrosination activity (detyrosinated α -tubulin normalized to α -tubulin) was quantified in three independent 953 954 experiments (top). Data shown as mean +/- SD. An example immunoblot is shown (bottom). 955

956

Figure 3



957 Figure 3. The substrate binding mode of MATCAP

958 (A) Electrostatic surface potential of MATCAP (scale bar -10 to +10 ϕ); locations of positively 959 charged residues selected for mutagenesis are indicated. (B) Quantification of the activity of 960 indicated MATCAP mutants in Hela cells. Data shown as mean +/- SD. n=3 independent 961 experiments. (C) Docking models of the α -tubulin tail (pink and magenta ribbon with side-962 chains) binding to MATCAP (grey) from two different views. (**D**) MATCAP binds α -tubulin in a pocket while the VASH-SVBP contains an open groove, both indicated by red arrows. (E) 963 C-terminal α -tubulin peptides were incubated with either recombinant VASH1-SVBP or 964 965 MATCAP and analyzed by nanoLC-MS/MS. Peptide counts were normalized to the wild-type peptide. (F) GFP fused to α -tubulin tails with varying terminal amino acids were co-expressed 966 967 together with the indicated detyrosinating enzymes in HeLa cells. 968

969

Figure 4



970 Figure 4. Binding of MATCAP to microtubules.

(A) In vitro incubation of wild-type purified MATCAP with HeLa tubulin heterodimers or 971 972 microtubules for the indicated times. n=5 independent experiments. Data is shown as mean \pm 973 SEM and the 95% confidence interval is shown as blue/grey shades. The levels of deY-tubulin 974 were normalized to α -tubulin levels. (B) Co-sedimentation assay of full-length MATCAP and 975 paclitaxel-stabilized microtubules using a glycerol cushion. Pellet (sedimented microtubules) 976 and supernatant (non-sedimented proteins) were subjected to immunoblot analysis. (C) 977 Immunostaining of U2OS cells transfected with HA-tagged MATCAP constructs. Scale bar 20 978 um. (**D**) Representative cut-outs of electron micrographs showing microtubules (see Figure 979 S5A, B) and two-dimensional classes of data, with and without MATCAP; arrows indicate 980 bound MATCAP. (E) Cryo-EM density of a microtubule (grey) decorated with MATCAP 981 (blue). (F) Cryo-EM map of MATCAP (blue) bound to the α - β -tubulin heterodimer (grey); 982 density corresponding to the C-terminal end of α -tubulin is shown in salmon pink; MATCAP 983 density interacting with tubulin is shown in cyan. The displayed map is shown at isocontour 984 0.5 and 0.05 for the density belonging to tubulin and MATCAP, respectively, to better visualize 985 the map features at each region. The inlet highlights density that is attributed to the α -tubulin 986 tail. (G) Pseudo-atomic model from rigid-body fitting of the MATCAP crystal structure and 987 tubulin (PDB: 7SJ7). The docking model of the α -tubulin tail (ribbon with the C-terminal 988 tyrosine as ball-and-stick) is highlighted in the inlet, and connected to the last residue of the 989 crystal structure with a dashed line. Five interfaces are indicated, interfaces observed from the 990 structure (pink) and contact sites suggested by mutations (grey). (H) Immunofluorescent 991 staining of U2OS cells expressing the indicated MATCAP mutants. Scale bar 10 µm. (I) 992 Quantification of the colocalization of MATCAP mutants from immunofluorescent stainings. 993 Data is shown as mean. Significance was calculated as one-way ANOVA and Tukey's multiple 994 comparisons test. **** <0.0001, *** <0.001. (J) Composite molecular model of MATCAP 995 and VASH1-SVBP (PDB: 6WSL) bound to a microtubule. Arrows indicate interactions with 996 the protofilaments.

997 998

Figure 5



999 Figure 5. Characterisation of detyrosination-impaired mice.

1000 (A) Immunohistochemical staining of detyrosinated tubulin in adult mouse brains. (B) 1001 Immunoblot analysis of adult mouse brain lysates. (C) Microtubules isolated from adult mouse 1002 brains were subjected to nanoLC-MS-MS. Percentage of microtubules with different Cterminal modifications are shown. (D) MRI analysis of brain size of mice with indicated 1003 1004 genotypes. 3D reconstructions (top) and an exemplary raw data MRI slices (bottom) are shown. 1005 Scale bar 2 mm.(E) Volume quantifications of indicated regions. WT n=14 mice, for all other 1006 genotypes n=13 mice. Significance was calculated as one-way ANOVA and Tukey's multiple 1007 comparisons test. (F) Weights of five adults mouse brains per genotype. (G) Total number of 1008 brain cells counted by flow cytometry using the isotropic fractionator method. n=5 mouse 1009 brains per genotype. Unpaired two-tailed t-test was performed for (F) and (G). (H) 1010 Immunofluorescence for DNA (Draq-5, blue), phospho-histone H3 (PH3, proliferation marker, 1011 green) and Tuj1 (neuron marker, red) on coronal brain sections from E14.5 mouse embryos. Scale 1012 bar 20 µm. (I) Quantification of mitotic PH3+ cells in the dorsal cortex at E14.5. Wilcoxon 1013 signed-rank test. (J) Quantification of mitotic PH3+ cells in subregions of the dorsal cortex at 1014 E14.5. VZ = ventricular zone, SVZ = subventricular zone. Wilcoxon signed-rank test. (I, J) 1015 n=6 mouse embryos per genotype. For each animal 8 sections throughout the rostro-caudal axis 1016 of the embryonic brain were analyzed (K) Nest building tests of mice with the indicated 1017 genotypes. Per genotype n= 10 mice. (E-G, I, J) Significance is depicted as **** <0.0001, *** 1018 <0.001, ** <0.01, * <0.05, non-significant (n.s.) >0.05. Data shown as mean (E-G) or median

1019 (I, J).



Supplementary Materials for

Posttranslational modification of microtubules by the MATCAP detyrosinase

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This PDF file includes:

Figs. S1 to S8 Tables S1 to S5 Captions for Data S1 to S2

Other Supplementary Materials for this manuscript include the following:

Data S1 to S2 (.xlsx)

Figure S1

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С



Figure S1. Gene-trap insertions and expression of MATCAP.

(A) Genomic MATCAP locus (top) and detected gene-trap insertions inside (orange/blue) and outside (grey) the coding sequence for high and low populations of each screen. (B) Ranked gene expression of genes in HAP1 cells involved in the tubulin detyrosination cycle based on published RNA-Seq data (57) (C) The mutation index (MI) of MATCAP, SVBP and an example of a pleiotropic regulator (CNOT10) across screens from this study and previously published.

Figure S2





Figure S2. The conserved C-terminal part of MACTAP possesses catalytic activity.

(A) Scheme representing different tubulin tails of α -tubulin isotypes fused to GFP (top). Isotype specific C-terminal tails fused to GFP were expressed in HeLa H1 cells. Detyrosination was monitored by immunoblotting (bottom). Immunoblots monitoring detyrosination of in vitro reactions using tubulin dimers and MATCAP¹³⁷⁻⁴⁷¹ (**B**) or full length MATCAP (**C**). (**D**) Comparison of the catalytic side between MATCAP, apo-MATCAP and AlphaFold MATCAP prediction (AF-Q68EN5).



Figure S3. An interaction model between MATCAP and the α -tubulin.

(A, B) Ribbon representation of MATCAP bound to the 8mer C-terminal α -tubulin tail. Two high confidence models obtained from HADDOCK modeling are shown. Alanine (C, D) or reversal-of-charge (E, F) mutants of MATCAP were expressed in HeLa H1 cells. Representative immunoblots are shown. (C, E). (D, F) Detyrosination activity was quantified by normalizing detyrosinated tubulin levels to total α -tubulin from 3 independent experiments. Data is shown as mean +/- SD.

В

SVBP - VASH1

front view







C-terminal a-tubulin peptide

С



West of the state

3^{HE3ESHEAEHESHEAMER}





SVBP-VASH1

D



cleavage activity

1.0

VSHEEDER VSHEEDER



Ε





F



MATCAP

Figure S4. MATCAP and VASH1 substrate recognition differs.

(A) Surface representation of the VASH1-SVBP complex (PDB: 6NVQ) bound to a 8mer Cterminal α -tubulin tail. Zoom-in on the interaction of the α -tubulin tail with the experimentally validated residues for substrate recognition of MATCAP (B) or VASH1-SVBP (22) (C). (D, E) nanoLC-MS/MS analysis on C-terminal α -tubulin peptides (TUBA1A/B) incubated with recombinant VASH1-SVBP or MATCAP. Peptide counts were normalized to the wildtype peptide. (F) Summary of the substrate recognition of MATCAP and VASH1.

Figure S5

С В Α luminal side β α D MATCAP β 180° view 2 \rightarrow 0 <†**≈** 180°<mark>,</mark> view 2 view 1 2.9 Å 5.0 Å 7.0 Å view 1 Е β α

Figure S5. The binding of MATCAP to microtubules revealed by Cryo-EM.

(A, B) Example electron micrographs taken at 1.65 Å/px with a K2 detector (left) and 0.53 Å/px with a K3 detector (right) corresponding to Fig. 4D of microtubules only (A) or bound by MATCAP (B). Scale bar for both 100 nm. (C) Long and short loops (green) on the luminal microtubule side distinguish α - and β -tubulin. (D) Different views of the density map colored by resolution. Density outside of the asymmetric unit is shaded. (E) Different views of the transparent cryo-EM structure displaying the fit of tubulin (PDB: 7SJ7) and the MATCAP crystal structure.

Figure S6



Figure S6. Cryo-EM sample purity, data analysis pipeline and map quality assessment.

(A) Coomassie gel assessment of HCT116 microtubule purification. S1,2 and P1,2 are soluble and pellet fractions, respectively. Resuspended P2 is used on the cryo-EM grids. Gel filtration Coomassie (**B**) and chromatogram (**C**) of purified full-length E281Q MATCAP. (**D**) Cryo-EM processing pipeline. (**E**) Euler angle distribution plot showing the viewing direction distribution of the picked microtubule particles contributing to the final cryo-EM map. (**F**) Fourier Shell Correlation (FSC) curves of the final cryo-EM map. (**G**) Local resolution values histogram. (**H-J**). Representative densities of prominent places in the structure, color-coded according to the local resolution are shown. Density of (**H**) the α -tubulin helix of the helixhelix interface (left) and the corresponding β -tubulin helix (right), (**I**) MATCAP corresponding to (left) active site helix and (right) the helix binding α -tubulin and (**J**) α -tubulin tail (top) and the interdimeric interface (bottom).

Figure S7



Figure S7. Multiple interfaces recruit MATCAP to microtubules.

(A) Modelled α -tubulin tail in MATCAP's pocket. (B) Zoom-in on the helix-helix interface between MATCAP (cyan) and α -tubulin (salmon pink) helices. Interacting residues are shown as sticks. (C) Colocalization measurements of helix-helix mutations in the catalytic dead MATCAP. (A-C) The buried surface areas are indicated in brackets. (D) Surface representation of MATCAP (blue) and a protofilament. α -tubulin and β -tubulin are depicted in light and dark grey, respectively. Red circle highlights the interdimeric interface. (E) Immunofluorescent of MATCAP variants expressed in U2OS cells. Scale bar 10 µm. (F) Colocalization quantification of interdimeric interface mutants to microtubules. (G) Electrostatic surface potential of the molecular model highlighting the positively charged patch on the backside of MATCAP (left). Recorded density at the positively charged patch (right). Scale bar -10 to +10 φ . (H) Immunofluorescent of MATCAP variants expressed in U2OS cells. Scale bar 10 μ m. (I) Quantification of colocalization of positively charged patch mutants and microtubules. (J) Conservation of the disordered N-terminus of MATCAP. (K) AlphaFold model of Homo sapiens MATCAP (AF-Q68EN5-F1). Color scale indicates the per-residue confidence score (pLDDT). (L) Colocalization measured for N-terminal MATCAP truncations to microtubules. (C, F, I, L) Data shown as mean (pink line). Significance was calculated as one-way ANOVA and Tukey's multiple comparisons test. Significance is depicted as **** <0.0001, *** <0.001, ** <0.01, * < 0.05, non-significant (n.s.) >0.05. (M) In vitro detyrosination assay of MATCAP 137-471 on free and assembled tubulin. Data is shown as mean \pm SEM and the 95% confidence interval is shown as pink/grey shades (3 independent experiments).











В



Figure S8. Characterization of MATCAP SVBP double knockout mice.

(A) Genotype distribution of 409 progenies from 42 heterozygous (MATCAP^{-/+}, SVBP^{-/+}) breeding pairs. (B) Weight of mice with indicated genotypes. (C) Immunohistochemistry stainings showing cleaved caspase 3 (apoptotic marker) positive cells (arrows) in the adult mouse cortex. Scale bar 50 μ m. (D) Quantification of apoptotic cells (cleaved caspase 3 positive cells) in the indicated brain regions. Two-way ANOVA and Tukey's multiple comparisons test. (E) Immunofluorescence for cleaved caspase positive cells on dorsal cortices from mouse embryos at E14.5. Inlets show higher magnifications of the boxed regions. Scale bar 20 μ m. (B, D) Data is shown as mean. Non-significant (n.s.) > 0.05.

Dataset	Zn	Аро	Hg soak	
Snacegroup	P 1 2, 1	P 1 2, 1	P 1 2, 1	
spacegroup	55.65.88.07.	55.85, 88.03.	55.73.87.73.	
a, b, c (A)	165.586	165.61	165.23	
β (°)	90.8	90.7	90.8	
Wavelength (Å)	1.2809	0.9999	1.0068	
Desclution (Å)	47.0 - 2.50	47.0 - 2.11	47.0 - 2.90	
Resolution (A)	(2.57 - 2.50)	(2.15 - 2.11)	(3.04 - 2.90)	
R _{merge}	0.173 (1.73)	0.065 (0.71)	0.165 (0.78)	
R _{pim}	0.108 (1.07)	0.062 (0.71)	0.039 (0.18)	
<i σ(i)=""></i>	9.1 (1.1)	9.5 (1.4)	19.2 (4.5)	
CC _{1/2}	0.99 (0.55)	0.99 (0.67)	0.99 (0.94)	
Completeness (%)	99.8 (99.8)	98.6 (93.6)	100 (100)	
Redundancy	6.8 (7.0)	3.4 (3.2)	20.6 (21.2)	
Wilson B factor	42.2	34.9	30.5	
Unique reflections	55,542	90,835	35,545	
Refinement	Zn	Аро	Structure determination (S	SIRAS)
Resolution (Å)	2.50	2.11	<u>Phasing</u>	
Reflections (work, test)	52753, 2762	86245, 4578	Anomalous signal >1	4 Å
Rwork / Rfree	0.232 / 0.26	0.217 / 0.25	CFOM substructure	70.3
<u>No. atoms</u>			Hg sites used	11
Protein (chain A B C D)	5333, 5302,	5333, 5321,	FOM Phasing	0 3 2 8
Trotein (chain A,b,c,b)	5302, 5314	5302, 5314	rowritasing	0.520
Zinc. Water	4, 161	4,202	FOM Density	0.668
	-,	-,	Modification	
$\frac{B \text{ factors } (A^2)}{B \text{ factors } (A^2)}$			Automated Model Building	
Protein (chain A,B,C,D)	54, 77, 63, 50	51, 75, 61, 47	R _{free}	0.33
Zinc, Water	51, 36	n.a., 44	Built Residues	1369
<u>R.m.s. deviations (RMSZ sc</u>	<u>orej</u>		Built Fragments	10
Bond lengths	0.006 (0.29)	0.012 (0.60)	Docked Residues (%)	98.7
Bond angles	0.983 (0.48)	1.460 (0.71)		
Ramachandran				
Ramachandran Z-score	-1.46	-0.91		
Favourable	1279	1287		
Allowed	36	29		
Outliers	0	0		
Molprobity score (%ile)	1.14 (100)	1.17 (100)		

 Table S1. Data collection and refinement statistics – Crystal structure of MATCAP.

Table S2.	HADDOCK	docking	results	(first round	l).
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Clusters and scores of 1st round of HADDOCK			
	Cluster 1	Cluster 2	Cluster 3
HADDOCK score	-140.8 ±1.4	-116.4 ± 11.2	-104.3 ±5.9
Cluster size	185	8	4
RMSD from lowest-energy structure	0.9 ± 0.1	1.0 ±0.3	1.7 ± 0.1
Van der Waals energy	-40.0 ± 3.5	-19.7 ± 2.0	-27.1 ± 4.9
Electrostatic energy	-524.9 ±15.9	-524.6 ±66.2	-400.6 ± 51.4
Desolvation energy	4.1 ± 2.0	8.2 ± 1.7	2.8 ± 2.3
Restraints violation energy	0.7 ± 0.4	0.3 ± 0.4	0.8 ±0.3
Buried Surface Area	1348.5 ±52.4	1268.5 ± 122.5	1422.3 ± 61.4
Z-Score	-1.3	0.3	1.1

Table S3. HADDOCK docking results (second round).

Clusters and scores of 2nd round of HADDOCK		
	Cluster 1	Cluster 2
HADDOCK score	-148.7 ± 3.2	-129.6 ± 1.8
Cluster size	166	31
RMSD from lowest-energy structure	0.2 ± 0.1	1.4 ± 0.2
Van der Waals energy	-24.5 ± 2.1	-35.9 ± 4.4
Electrostatic energy	-665.5 ± 21.0	-482.9 ± 20.8
Desolvation energy	8.7 ± 1.4	2.7 ±1.3
Restraints violation energy	2.6 ±1.6	1.1 ± 0.6
Buried Surface Area	1418.9 ± 31.0	1431.5 ± 71.6
Z-Score	-1.0	1.0

Table S4. Cryo-EM parameters and helical parameters.

# protofilaments	Twist (°)	Rise (Å)
11	-32.7	10.9
12	-30.0	10.0
13	-27.7	9.2
14	-25.7	8.6
15	-24.0	8.0
16	22.5	7.5

	PDB ID: 7765
	FMDB ID: FMD-14529
Data collection and Processing	
Microscope	Krios1
Voltage (keV)	300
Camera	K3
Magnification	81 000
Pixel size at detector (Å/nixel)	0 53
Total electron exposure $(e^{-}/Å^2)$	50
Exposure rate (e-/nixel/sec)	28.78
Number of frames collected during exposure	50
Defocus range (um)	[-0.62.1]
Automation software	EPU-2.10.0
Energy filter slit width (eV)	20
Micrographs collected (no.)	8,400
Micrographs used (no.)	6.969
Total extracted particles (no.)	1.690.210
Selected particles after 3 rounds of 2D classification (no.)	718.501
Selected 14 PF microtubule particles after heterogeneous refinement (no.)	158.653
Point-group or helical symmetry parameters	14x expanded, twist=-25.7°, rise=8.7Å
Final particles (no.)	158.653*14=2.221.142
Resolution (global, Å)	
FSC 0.5 (unmasked/loose mask/tight mask/corrected)	4.2, 3.2, 3.1, 3.1
FSC 0.143 (unmasked/loose mask/tight mask/corrected)	3.5, 2.9, 2.8, 2.8
Resolution range ((min, 25th percentile, median, 75th percentile, max (Å)	2.7, 3.1, 3.7, 7.4, 20.1
Map sharpening B factor (Å ²)	-63.5
Map sharpening methods	cryoSPARC job: local filtering
Model composition	
Tubulin (PDB)	7SI7 (chain A. B. H. K)
MATCAP (PDB)	7Z5H (2x chain A)
Protein (aa.)	2,390
Ligands (no.)	2x Zn, 2x Mg, 2x GTP, 2x GDP
Rigid body fit	
Fitting software	ChimeraX
Cross correlation score MATCAP	0.8258 at isocontour 0.05 with a
	simulated map at res 6.0Å
Cross correlation score Tubulin	0.6715 at isocontour 0.05 with a
	simulated map at res 3.0Å

Table S5. Cryo-EM map - Data collection and refinement statistics

Data S1. (separate file)

Mutation frequencies of deY-tubulin screen in VASH1 VASH2 KO HAP1 cells.

Data S2. (separate file)

Mutation frequencies of Y-tubulin screen in VASH1 VASH2 TTL KO HAP1 cells.