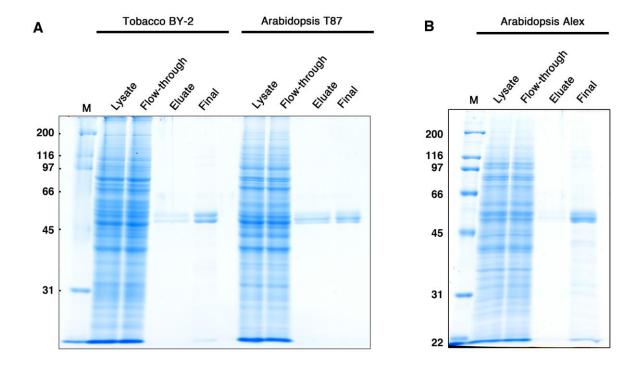


Supplemental Figure S1. A TOG^{MOR1} column does not bind tubulin. A, Experiment flow chart. Crude proteins extracted from Arabidopsis seedlings (fraction 1) were first loaded onto a TOG^{MOR1} column and then eluted (fraction 3). The flow-through fraction (fraction 2) was applied to the TOG column, and its flow-through fraction (fraction 4) and the eluate (fraction 5) were analyzed. B, Protein samples were separated by SDS-PAGE, and analyzed by Coomassie staining (left) and immnoblotting with anti-α-tubulin antibody (right). M, molecular mass marker; Fraction 1, 5 μ l of crude cell extract containing 12.4 μ g of protein; fraction 2, 5 μ l of the flow-through fraction from the TOG^{MOR1} column; fraction 3, eluted, desalted, and concentrated fraction (200 ng protein) after separation on the TOG^{MOR1} column; fraction 4, 5 μ l of the flow-through fraction from the TOG column; fraction 5, eluted, desalted, and concentrated fraction (200 ng protein) after separation on the TOG column. Protein amounts were estimated by the Bradford assay.



Supplemental Figure S2. Purification of tubulin from various plant cell cultures. Protein samples were separated by SDS-PAGE and analyzed by Coomassie staining. A, Purification of tubulin from tobacco BY-2 cells (left) and Arabidopsis T87 cells (right). Lysates, 15 μg protein for BY-2 and 17 μg for T87; flow-through fractions, 5 μl; eluates from the TOG column, 10 μl; and desalted and concentrated tubulin, 0.5 μg. B, Purification of tubulin from Arabidopsis Alex cells. Lysate, 12 μg protein; flow-through fraction, 3.5 μl; eluate, 10 μl; and desalted and concentrated fraction, 705 ng tubulin. M, molecular mass makers.

α -tubulin

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PigTUA
AtTUA1
         1 MRECISIHVGQAGVQIGNACWELYCLEHGIQPDGQMPSDKTIGGGDDSFNTFFSETGAGKHVPRAVFVDLEPTVIDEVRTGTYRQLFHPEQLITGKEDAANNYARGHYTIGKEIIDLVLD
         AtTUA2
         AtTUA4
AtTUA6
         121 RIRKLADQCTGLQGFSVFHSFGGGTGSGFTSLLMERLSVDYGKKSKLEFSIYPAPQVSTAVVEPYNSILTTHTTLEHSDCAFMVDNEAIYDICRRNLDIERPTYTNLNRLIGQIVSSITA
AtTUA1
AtTUA2
       121 L. N. L. NAV. LG. L. F. G.T. S. V.S. SL. T.VVVLL. S. S. S.TI.L.T.
121 ... N. L. NAV. LG. L. G.TV.S. S. V.S.SL. T.VSILL. S.S. ... VS.VI.L.
       121 .V. N. L. NAV. LG L. G.T. S. V.S. SL. T.V.VLL. S. S.I. L.T.
121 ... N. L. NAV. LG L. G.TV.S. S. V.S. SL. T.VSILL. S.S. VS.VI.L.
AtTUA3
AtTUA4
          .V. N. L. NAV. LG. L. G.T. S. V.S.SL. T.V.VLL. S. S.I.L.T.
N. L. NAV. LG. L. G.TV.S. S. V.S.SL. T.VSILL. S.N. VS.VI.L.
AtTUA5
AtTUA6
PigTUA
AtTUA1
       241 SLRFDGALNVDLTEFOTNLVPYPRAHFPLATYAPVISAEKAYHEQLSVAEITNACFEPANOMVKCDPRHGKYMACCLLYRGDVVPKDVNAAIATIKTKRTIQFVDWCPTGFKVGINYEPP
       241 SLRFDGALNVDLTEFOTNLVPYPRAHFPLATTAPVISAEKAYHBOLSVAEITNACFEPANGWVRCDPRHGKYMACCLLYRGDVPKDVRAAIATIKTKRTIOFVDWCPTGFKVGINYEP
241 I. I. I. M.SS SA F.P. TSV S.M.A M. T.V.A A C.Q.
241 V. I. M.SS F. SA SM.A M. VG C.Q.
241 I. I. I. M.SS A P.V.SM.A M. VG V.C.Q.
241 V. I. M.SS F. SA SM.A M. VG V.C.Q.
241 V. I. M.SS F. SA SM.A M. VG C.Q.
241 V. I. M.SS P. SA SM.A M. VG V. C.Q.
241 V. I. M.SS A P. V. SM.A M. VG V. C.Q.
241 V. I. M.SS A P. V. SM.A M. VG V. C.Q.
241 V. I. M.SS A P. V. SM.A M. VG V. C.Q.
AtTUA2
AtTUA3
AtTUA4
AtTUA6
       241 .........V.....I..M.SS......F.....SA...SM.A.....M....VG.....VG......C...Q..
PigTUA
       361 TVVPGGDLAKVQRAVCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWYVGEGMEEGFSEAREDMAALEKDYEEVGVDSVEGEGEEEGEEY
       AtTUA1
AtTUA2
AtTUA3
AtTUA4
AtTUA5
AtTUA6
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β-tubulin

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1 MREIVHIQAGQCGNQIGAKFWEVISDEHGIDPTGSYHGDS-DLQLERINVYYNEAAGNKYVPRAILVDLEPGTMDSVRSGPFGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDSVLDV
PiqTUB
       AtTUB2
AtTUB4
AtTUB5
AtTUB6
AtTUB8
        PigTUB
AtTUB1
      120 VRKESESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEYPDRIMNTFSVVPSPKVSDTVVEPYNATLSVHQLVENTDETYCIDNEALYDICFRTLKLTTPTYGDLNHLVSATMSGVTTC
      121 ...A.N. ...VC. ...M.L. .F. ....A. .CMVL. ....S. .SF. ...I. ....CS
      121 A.N. VC M.L. F. A. CMVL SF. I C.
120 A.N. VC M.L. F. A. CMVL SF. I C.
120 A.N. VC M.L. F. A. CMVL SF. I C.
120 A.NS VC M.M. F. A. CMVL AN. F. I C.
121 A.N. VC M.L. F. A. CMVL S. SF. I CS
AtTUB2
AtTUB4
AtTUB5
AtTUB6
AtTUB7
      120 ...A.N....VC.....M.L...F....A..CMVL.....SF...I.....C
      120 ...A.N. ... VC. ... M.M. F. ... A. CMVL. ... S. SF. ... I ... ... C.
      AtTUB8
AtTUB9
PigTUB
AtTUB1
      240\ LRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPELTQQMFDAKNMMAACDPRHGRYLTVAAVFRGRMSMKEVDEQMLNVQNKNSSYFVEWIPNNVKTAVCDIPPR
      AtTUB3
AtTUB5

    241

    240
    S.
    LI.
    V.
    W.S.
    C.A.
    AS.M.
    K.T.
    I.
    SS.
    AI.

    240
    S.
    LI.
    V.
    N.
    W.
    C.A.
    AS.M.
    K.T.
    T.
    ST.
    T.

    240
    S.
    LI.
    V.
    W.
    C.A.
    AS.M.
    K.T.
    I.
    ST.
    T.

    240
    S.
    LI.
    V.
    S.S.
    W.
    C.A.
    AS.
    K.T.
    M.
    SS.
    A.T.

AtTUB7
AtTUB9
      360 GLKMSATFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLVSEYQQYQDATADEQGEFEEEGEEDEA-----
AtTUB1
AtTUB2
      361 .I.AS..V. S. M.R.V. .ED.YD.E.QVYES----
360 .AS. S. M.R.V. .E.DY.-DE..G.YQQEEEY
      360 AS S M.R.V E.DY.-DE G.YQQEEEY
360 AS S M.R.V A G.E-.Y.E.Y.T-----
361 A.V.S M.R.V A E.YDV.E.EGDYET---
AtTUB3
AtTUB4
AtTUB5
AtTUB6
      AtTUB7
      360 ...AS....S..M.R.V.....E.Y.--E.A.YEQEETY
AtTUB8
At.TUB9
         ....AS.....S...M.R.V..........A....VG.E-.Y..DE..E..-
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Supplemental Figure S3. Sequence alignments of α -tubulin and β -tubulin proteins from pig and Arabidopsis. Asterisks indicate amino acid residues that interact with the TOG1 domain of Stu2. The acetylatable Lys40 residue in α -tubulin is indicated by blue highting. The hexa-histidine insertion is close to this Lys40 residue. All α -tubulin sequences end with Tyr at their C-termini.

	Loop 1	Loop 2
Stu2p TOG1	17 RLTYKLWKA 25	66 DSN V VAQ 72
MOR1 TOG1	20 RLGHKNWKV 28	64 DSNAPVQ 70
Stu2p TOG2	335 RITSSKWKD 343	383 DANIQAV 389
MOR1 TOG2	294 GVKATKWSE 302	337 DVNLAVA 343
	Loop 3	Loop 5
Stu2p TOG1	112 LTSSRATT 119	192 AGHGDRNVRS 201
MOR1 TOG1	109NT 110	178 FDHQDQNVRA 187
Stu2p TOG2	424 TKEKKPSV 431	511 VNDTOPAIRT 520

Supplemental figure S4. Comparison of the TOG1 and TOG2 domains of Stu2 and Arabidopsis MOR1. Amino acid residues that contribute to tubulin-binding in Stu2 TOG1 are shown in bold. The corresponding residues, if conserved, are also highlighted in bold in Stu2 TOG2, as well as in TOG1 and TOG2 of MOR1.

Supplemental Movie S1. Time-lapse movie of MT dynamics for porcine tubulin at 7.5 μ M, corresponding to Fig. 2A, upper panel. Acquisition, every 3 sec for 30 min; frame rate, 100 fps; scale bar, 5 μ m.

Supplemental Movie S2. Time-lapse movie of MT dynamics for porcine tubulin at 15 μ M, corresponding to Fig. 2A, lower panel. Acquisition, every 3 s for 30 min; frame rate, 100 fps; scale bar, 5 μ m.

Supplemental Movie S3. Time-lapse movie of MT dynamics for Arabidopsis tubulin at 7.5 μ M, corresponding to Fig. 2B, upper panel. Acquisition, every 3 s for 30 min; frame rate, 100 fps; scale bar, 5 μ m.

Supplemental Movie S4. Time-lapse movie of MT dynamics for Arabidopsis tubulin at 15 μ M, corresponding to Fig. 2B, lower panel. Acquisition, every 3 s for 30 min; frame rate, 100 fps; scale bar, 5 μ m.