

Table S1. Genes up or down regulated by Top3 β overexpression.

Number	Annotation	Orf number	Fold change (pPTop3 β /5' Δ 5N-Pac)*
1	VSP	113304	65.66
2	VSP	113163	40.28
3	Hypothetical protein	31366	16.43
4	Cyst wall protein 1	5638	11.69
5	Hypothetical protein	5206	9.48
6	Hypothetical protein	3731	9.29
7	Cyst wall protein 2	5435	9.10
8	Hypothetical protein	16622	8.65
9	Ceramide glucosyltransferase	11642	8.40
10	VSP	41472	8.33
11	VSP with INR	113450	8.01
12	Glucose 6-phosphate N-acetyltransferase	14259	8.01
13	High cysteine membrane protein Group 6	113512	7.50
14	VSP with INR	16501	7.30
15	VSP	114162	6.81
16	Hypothetical protein	27652	6.34
17	Hypothetical protein	14690	6.19
18	Hypothetical protein	4984	5.98
19	VSP, putative	114286	5.54
20	Hypothetical protein	11120	5.35
21	VSP	112867	5.25
22	Hypothetical protein	7374	5.08
23	Hypothetical protein	135270	5.07
24	Hypothetical protein	35638	4.64
25	Hypothetical protein	115669	4.55
26	Hypothetical protein	8505	4.49
27	Hypothetical protein	112575	4.45
28	Hypothetical protein	9605	4.40
29	VSP	11521	4.37
30	VSP presumed INR	137714	4.32
31	VSP with INR	11470	4.19

32	Retinoic acid induced 17-like protein	11930	4.01
33	VSP	137708	3.89
34	Sugar transport family protein	9046	3.88
35	Hypothetical protein	32657	3.88
36	Hypothetical protein	28112	3.83
37	CEGP1 protein	17120	3.82
38	Hypothetical protein	116201	3.51
39	Adenylate cyclase	14367	3.46
40	Hypothetical protein	119703	3.45
41	Hypothetical protein	16078	3.37
42	Zinc finger domain	2116	3.35
43	Hypothetical protein	113303	3.31
44	Hypothetical protein	13878	3.20
45	VSP	115796	3.13
46	VSP, putative	118133	3.12
47	High cysteine membrane protein Group 1	32607	3.12
48	VSP	101765	3.11
59	Hypothetical protein	8960	2.99
50	Hypothetical protein	23934	2.96
51	Hypothetical protein	119224	2.92
52	Cathepsin B-like cysteine proteinase 3 precursor	114165	2.92
53	Hypothetical protein	36883	2.90
54	High cysteine membrane protein Group 6	114470	2.84
55	Protein 21.1	4846	2.73
56	Nicotinamide-nucleotide adenylyltransferase	92618	2.71
57	Hypothetical protein	101768	2.59
58	VSP	122566	2.58
59	Hypothetical protein	2860	2.55
60	Hypothetical protein	11050	2.52
61	Hypothetical protein	2605	2.51
62	Hypothetical protein	8325	2.51

63	VSP	112801	2.49
64	VSP with INR	113439	2.48
65	Glucosamine-6-phosphate deaminase	8245	2.44
66	VSP with INR	115797	2.43
67	Hypothetical protein	2692	2.41
68	VSP	26894	2.39
69	Chorein	87358	2.38
70	Furin precursor putative serine protease	2897	2.38
71	UDP-glucose 4-epimerase	7982	2.37
72	High cysteine membrane protein Group 1	11309	2.36
73	Hypothetical protein	33672	2.33
74	Hypothetical protein	114210	2.30
75	Hypothetical protein	29757	2.29
76	Protein Kinase	4033	2.25
77	Protein 21.1	92983	2.21
78	Hypothetical protein	2404	2.21
79	Hypothetical protein	7598	2.17
80	Hypothetical protein	10425	2.14
81	Fatty acid elongase 1	92729	2.14
82	Hypothetical protein	117068	2.12
83	Hypothetical protein	11148	2.11
84	Dolichol-phosphate mannosyltransferase, putative	3180	2.10
85	High cysteine protein	94003	2.08
86	Variant-specific surface protein	11690	2.08
87	UDP-N-acetylglucosamine pyrophosphorylase	16217	2.07
88	DNA topoisomerase III	15190	2.06
89	Hypothetical protein	119599	2.06
90	Hypothetical protein	10552	2.06

91	Variant-specific surface protein	9276	2.05
92	Hypothetical protein	15532	2.04
93	Hypothetical protein	14833	2.00
94	DNA topoisomerase III	7615	0.02
95	VSP	137617	0.20
96	VSP	40571	0.24
97	VSP with INR	119707	0.24
98	VSP	34357	0.24
99	VSP	90215	0.24
100	VSP with INR	40592	0.32
101	VSP	41539	0.34
102	VSP	115047	0.35
103	Hypothetical protein	92625	0.37
104	VSP	114065	0.38
105	High cysteine membrane protein Group 3	112126	0.38
106	High cysteine protein	87706	0.39
107	VSP	111903	0.39
108	VSP	113093	0.39
109	VSP	99743	0.40
110	Protein 21.1	15965	0.40
111	VSP	137612	0.40
112	VSP	121070	0.41
113	Cytosine deaminase, putative	2486	0.42
114	VSP	116477	0.42
115	DNA-damage inducible protein DDII-like	7718	0.43
116	VSP	34196	0.43
117	VSP	41401	0.44
118	Hypothetical protein	19870	0.44
119	VSP	112678	0.44
120	High cysteine membrane protein Group 3	114891	0.45
121	VSP	137611	0.45
122	Hypothetical protein	125106	0.45

123	VSP	41349	0.45
124	VSP	118900	0.45
125	Hypothetical protein	123980	0.46
126	Hypothetical protein	17241	0.47
127	High cysteine membrane protein Group 1	10659	0.47
128	VSP	111874	0.48
129	VSP	137607	0.48
130	VSP	101410	0.49
131	Kinase, NEK	9870	0.49
132	Hypothetical protein	31420	0.49
133	Hypothetical protein	99726	0.50

**p* values were determined to be <0.05 for groups in which the average means changed by a factor of ≥ 2.0 or ≤ 0.5 .

Table S2. Oligonucleotides used for construction of plasmids and PCR.

Name	Sequence (5'--->3')
top3 β F (PCR1F)	CACCATGATCCTTCTCATTGCA
top3 β R (PCR1R)	TCTGTGTTTGCGACCCCT
top3 β HAF	CAGACAGTATCTGAGTAC
HAR	AGCGTAATCTGGAACATCGTATGGGTA
cwp1F	ATGATGCTCGCTCTCCTT
cwp1R	TCAAGGCGGGGTGAGGCA
cwp2F	ATGATCGCAGCCCTTGTCTA
cwp2R	CCTTCTGCGGACAATAGGCTT
cwp3F	ATGTTTTCTCTGCTTCTTCT
cwp3R	TCTGTAGTAGGGCGGCTGTA
myb2F	ATGTTACCGGTACCTTCTCAGC
myb2R	GGGTAGCTTCTCACGGGGAAG
ranF	ATGTCTGACCCAATCAGC
ranR	TCAATCATCGTCGGGAAG
top3 β realF	GGAGGGGGACAGACCAAG
top3 β realR	CGTGAGGGATTAGCCCAGT
cwp1realF	AACGCTCTCACAGGCTCCAT
cwp1realR	AGGTGGAGCTCCTTGAGAAATTG
cwp2realF	TAGGCTGCTTCCCACTTTTGAG
cwp2realR	CGGGCCCGCAAGGT
cwp3realF	GCAAATTGGATGCCAAACAA
cwp3realR	GACTCCGATCCAGTCGCAGTA
myb2realF	TCCCTAATGACGCCAAACG
myb2realR	AGCACGCAGAGGCCAAGT
ranrealF	TCGTCCTCGTCGAAACAA
ranrealR	AACTGTCTGGGTGCGGATCT
18SrealF	AAGACCGCCTCTGTCAATCAA
18SrealR	GTTTACGGCCGGGAATACG
top3 β NF	GGCCGGCTAGCTCGGTGTGCGATGTGCCAAAGC

top3 β MR	GGCCGACGCGTTCGGTGTTCGATGTGCCAAAGC
top3 β 865F	CGCCCCTTGCCAATGA
top3 β 926R	GAGAGACCGAGCACCGTTGA
top3 β m1F	CTGGATACATTTCTTTCCCAAGAACTGAGTC
top3 β m1R	GACTCAGTTCTTGGGAAAGAAATGTATCCAG
top3 β m2MR	GGCCGACGCGTAATAGAGGTAAACTTCGCGT
top3 β m3MR	GGCCGACGCGTTGTTGCCAGGAAGTGCTTAG
top3 β 5HF	GGCGGAAGCTTTACTAACTATGACTCTAGGGC
top3 β 5NR	GGCGGCCATGGGAATTATTTTTAGCATCCCAG
top3 β 3XF	GGCGGCTCGAGTGACCATCAAGTGCTTGCTATTAT
top3 β 3KR	GGCGGGGTACCCTGGCTATCCTTGTAGCATCC
top3 β -guide	<p>GAGAGCGGGTACCCTAGCTTATTGAAAAAGCGAGAGGCCATTGAGATGACTCGCCT</p> <p>GATTGCAATAGCAAACAGTGTCTATAGTCTAATTGTGGACAACAGAGGGCTTATTG</p> <p>CAACGTTGATGACCAAGTTCAACAAGGGCGTCGCCTCCTATGAGCAGGTCATTCCG</p> <p>AATTTGACGACCGGTAGCGTCCCCAGAGTAAACCATTTTAAATTGAAATAGGCGG</p> <p>TTGGAAATAAAAAGCGCGCCGACCTTCATGTGTACTTCTGTTTTAGAGCTAGAAAT</p> <p>AGCAAGTTAAAAAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGG</p> <p>TGCTTTTTTTGAATTCGAGAGCG, underlined region is U6 promoter, bold region is for annealing, underlined and bold region is upstream 3nt of PAM, other region is scaffold RNA</p>
top3 β m2R	AATAGAGGTAAACTTCGCGT
top3 β m3R	TGTTGCCAGGAAGTGCTTAG
PCR2F	AAGGACCGCGCGACCTGG
PCR2R	CGTCTCCACATGGTATGACCG
top3 β 5F	TTGGGGGATGATGGTATCTTC
top3 β 5R	GCATCCCAGCCCTCAGCC
18S5F	CCAAAAAAGTGTGGTGCAGG
18S5R	GCCGGGCGCGGGCGCCGCGG
cwp15F	CAACGGCTTACTAAATCATTCTCTTG
cwp15R	TTCTGTGTTTCTTGATCTGAGAGTTGT
cwp25F	CACTTTGATGAGAGCATGGG
cwp25R	TTAGTTCATATCTTAAGTTA
cwp35F	TGGGGGAGATAGGAGAATAC

cwp35R	ATCAGTAGTAACTTATTTTTTGGGAAAGAC
myb25F	TGGCTATGTATTTTTTCTTCTTCTACAGCT
myb25R	TAGCAGTACAGAGTAATTATTATTTTAGTA
U65F	TTGAGATGACTCGCCTGATTG
U65R	GAAATTCCGAATGACCTGCTC
iscsF	CACCATGATTTACCTGGACAAC
iscsR	GTCATGCTTCCACTCTAT
bipF	CACCATGACGTCTAGTCACGTTAA
bipR	GAGTTCATCTTTTTCTGCAT

Figure S1

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G115190 1 -----MILLIAEKPSIAEMISRN YEG---A
G17615 1 -----MNRVILCVTEKNSVAAEVSNVLSKGSYSK
HsTOP3b 1 -----MKTVLMVAEKPSLAQSTAKILSRGSLSS
MmTOP3b 1 -----MKTVLMVAEKPSLAQSTAKILSRGNMSS
HsTOP3a 1 MIFPVARYALRWLRPEDRAFSRAAMEMALRGVRKVL CVAEKNDAAKGIADLLSNGRMRR
MmTOP3a 1 MIFPVTLALFQWHRPPGGRALSRAAMEVAFRGVRKVL CVAEKNDAAKGIADLLSNGRMRR
ScTOP3 1 -----MKVLCVAEKNSIAKAVSQILGGGRSTS
EcTOP1 1 -----MGKALVIVESPAKAKTINKYLGSDYVVK
TmTOP1 1 -----MSKKVKYIVVESPAKAKTIKSYLGNEYEVF
EcTOP3 1 -----MKLFIAEKPAVANDIVKALGGNFRH

G115190 24 KKLDNVSFPTYTFVQSFE---GSKETFMCTSVAGHVFEIDFDAELN-GSSVPOERLFE
G17615 30 KQLVQYFNEYKFTTHSEEE---HCPAQQYVVVHAQGHMLELE-PDEGYEWGKCSFSDLFT
HsTOP3b 29 HKGLNGACSVHEYTGTF A---GQPVRFKMTSVCGHVMTLDELGKYNKWDKVPDAELFS
MmTOP3b 29 HKGLNGACSVHKYTGTF A---GQPVHFKMTSVCGHVMTLDELGKYNKWDKVPDAELFS
HsTOP3a 61 REGLSKFNKIYEFDYHLY---GQNVMTMVMTSVSGHLLAHDFQMQRKWKQSCNPLVLFE
MmTOP3a 61 REGLSKFNKIYEFDYHLY---GQNVMTMIMTSVSGHLLAHDFQMQRKWKQSCNPLVLFE
ScTOP3 28 RDSGYMYVKNYDFMFSGFPFARNANCEVTMTSVAGHLTGIDFSDHSHGKGKCAIQELFD
EcTOP1 29 SSVGHIIDLPTSGSAAK-----SADSTSTKTAKKPKKDERGALVNRMGVDFWHNWE
TmTOP1 32 ASMGHIIDLPKS-----KFGVDLEKDFE
EcTOP3 27 DGWFESDNTIVTNCFGHI-----IESQPPENYNPEYKEWKIETLELRLLYP

G115190 78 RGHVHYSFTDSGSTVAKHLKKSIGGKAMQLILCLDNDREGENICFEVLKVLKPTLRSDCR-
G17615 85 CGVHFKANAVF---KKHVLRLPNAETNVLVLMLDADREGENICYDIEIFCSVL PADTLL
HsTOP3b 84 QAPTEKKEANPKLNMVKFLQVEGRGCDYIVLWLDLDCDKEGENICFEVLDAVLPMNKAHGG
MmTOP3b 84 QAPTEKKEANPKLNMVKFLQVEGRGCDYIVLWLDLDCDKEGENICFEVLDAVLPMNNAHNG
HsTOP3a 116 AEIEKYCPENF-VDIKKTLERETROCCALVIWTD CDREGENICFEIILHVCK---AVKPNL
MmTOP3a 116 AEIEKYCPENF-IDIKKTLERETHHCALVIWTD CDREGENICFEIILHVCK---AVKPNL
ScTOP3 88 APLNEIMNNQ-KKIASNIKREARNADYLMIWTD CDREGEYIGWEIWEAKRGNRLIQND
EcTOP1 81 AHYEVLPGKEK---VVSELKQLAEKADHIYLATDLDREGEATAWHLREVIGDDARYSR-
TmTOP1 55 PEFAVIKGKEK---VVEKLKDLAKKGE-LLIASMDREGEATAWHTARVTN-TLGRKNR-
EcTOP3 72 VKYQPVESA EKQVKTIEELIR-RADVTEIIHAGDPDDEGQLLVDEEVLEYAGNTKPVKRVL

G115190 137 -----VRRARFSAVTKAEIQNAFRNL D
G17615 142 DIRPARLPKIRSPTEHTLRHILAADHVPSQTSVKRTIIVKRARFEGLYPELTSAVYNAG
HsTOP3b 144 EK-----TVFRARFSSITDTDLCNAMACLG
MmTOP3b 144 EK-----TVFRARFSSITDTDLCNAMTRL S
HsTOP3a 172 Q-----VLRARFSEITPHAVRTACENLT
MmTOP3a 172 R-----VLRARFSEITPHAVRTACENLT
ScTOP3 147 Q-----VYRAVFSHLERQHTLNAARNPS
EcTOP1 137 -----VVENEITKNATROAFNKP G
TmTOP1 109 -----IVESEITPRVIREAVKNPR
EcTOP3 131 IN-----DNTLPVKKALANPK

G115190 159 KPD--QNLSDAVEARQELDLKIGVAFTRFOTAE LHAQFA-----DLQSS
G17615 202 PID--MNVVDADVVRQFTDLRLGYSITRLQTDKLRSSFIDYRTG-----AFKAKK
HsTOP3b 169 EPD--HNEALSVDARQELDLRIGCAFTRFQTKYFQGKYG-----DLDS
MmTOP3b 169 EPD--HNEALSVDARQELDLRIGCAFTRFQTKYFQGKYG-----DLDS
HsTOP3a 195 EPD--QRVSDADVVRQELDLRIGAAFTRFQTLRLQRIFF-----EVLAEQ
MmTOP3a 195 EPD--QRVSDADVVRQELDLRIGAAFTRFQTLRLQRIFF-----EVLAEQ
ScTOP3 170 RLD--MKSVAHVGTREHDLRAGVFTFRLLTETLRNKLNRQATMTKDGAKHRGGNKDSQ
EcTOP1 156 ELN--IDRVNAQQARFMDRVVGYMVSPLLWKKIARGLS-----
TmTOP1 128 EID--MKKVRAQLARRILDRIVGYSLSPVLWRNFKSNLS-----
EcTOP3 148 NNRDFRGLYLKALARSVADAIYGLSMTRAYTIPAKTKGYG-----

G115190 201 VISYGPCQPTTLSCFVS-----RHDEIQRFREKQIFSL
G17615 250 PISFGPCQVPTLGLVHSNDLLSLKNHQSSSFTLHLVCDPRPLQTHSGSVFKGLADALKRW
HsTOP3b 211 LISFGPCQPTTLGLFCVE-----RHDKIQSFKPETYWVL
MmTOP3b 211 LISFGPCQPTTLGLFCVE-----RHDKIQSFKPETYWVL
HsTOP3a 238 LISYGSQCFPTLGFVVE-----RFKAIQAFVPEIFHRI
MmTOP3a 238 LISYGSQCFPTLGFVVE-----RFKAIQAFVPEVFHKI
ScTOP3 228 VVSYGTCQFPTLGFVVD-----RFERIRNFVPEEFWYI
EcTOP1 193 ---AGRVQSVAVRLVVE-----REREIKAFVPEEFWEV
TmTOP1 165 ---AGRVQSATLKLVC D-----REREILRFVPEKKYHRI
EcTOP3 189 VLSVGRVQTEFVLGLTVN-----RTRANKNHKSSFYYT M

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G115190 234 DVICALIG--DQPHSLRWQGVPSSESRVIE-----EKRTGLFGKSATKCLKVLSFKSSKST
 G17615 310 DSKHRNREKDDQLQGLVFSPIDTSHFKSYLGSLPV LKLVFDLTQNKQKRVVSDVEEKPYT
 HsTOP3b 244 QAKVNTDK-DRSLLLDWDRVRVFDREIAQ-----MFLN---MTKLEKEAQVEATSRKKA
 MmTOP3b 244 QAKVHTDK-EESLLLDWDRVRVFDWEIAQ-----MFLN---MTKLEKEAQVEATSRKKA
 HsTOP3a 271 KVTHDHKD--GIVEFNWKRHRLFNHTACL-----VLYQL-CVEDP--MATVVEVRSKPKS
 MmTOP3a 271 KVTHDHKD--GTVEFNWKRHRLFNHTACL-----VLYQL-CMEDP--MATVVEVRSKPKS
 ScTOP3 261 QLVVENKDNNGGTTTTFQWDRGHLEDRLSVL-----TFYET-CIETAGNVAQVVDLKSKEPTT
 EcTOP1 223 DASTTTPSGEALALQVTHQNDKPFPRPVNK-----EQTQAASVSLLEKARYSVLEREDKPTT
 TmTOP1 195 TVN-----FDGLTAEIDVKEKKFDDAETL-----KEIQSIDELVVEEKVSVKKFAP--
 EcTOP3 222 TGHFQRGADVIRANWKPGEFAPLTDKRL LDK---TWANGTATSLAGKPAATVVEAAATDDK

G115190 287 LPRPLPMNTVALLKAAS TVLGLSPNSAMALAERLYI-----AGYISYPRTESTTYPES
 G17615 370 VPRPHPVSTLDMQRAISESLSS--QSVMTIAESLYT-----RGLISYPRTETKSYBN
 HsTOP3b 295 KQRPLALNTVEMLRVASSSLGMSGPOHAMQTAERLYT-----QGYISYPRTETTHYPE
 MmTOP3b 295 KQRPLALNTVEMLRVASSSLGMSGPOHAMQTAERLYT-----QGYISYPRTETTHYPE
 HsTOP3a 321 KWRPQALD TVELEKLASRKLRINAKETMRIAEKLYT-----QGYISYPRTETNIPFR
 MmTOP3a 321 KWRPQALD TVELEKLASRKLRINAKETMRIAEKLYT-----QGYISYPRTETNIPFR
 ScTOP3 315 KYRPLPLT TVELOKNCARYLRINAKESQLDAERLYQ-----KGFISYPRTETDTPBH
 EcTOP1 278 SKPGAPFITSTLQQAASSTRLGFGVKKTMMMAQRLYE-----AGYITVMRTDSTNLSQ
 TmTOP1 243 ---EPFKTSTLQQAASSTRLGFGVKKTMMMAQRLYE-----AGYITVMRTDSTNLSQ
 EcTOP3 279 TAAPLPFNLVRLQOQYMNKKFKMTAQKTLDTITQOLREK-----YKAITYNRSDCSYLSQ

G115190 339 GYNLREIVLALKESS-----STRISQCAONLLPSSKKSSDESVRYNPRRGTDQGD
 G17615 420 TYNLSYFKQLVDKLAHMPPNVSP LCALLADYAQVLLDDSPGNPYTLQLPRSNSASTDNAH
 HsTOP3b 347 NFDLKGSLRQOAN-----HPYWADTVKRLLAEG-----INRPRKGHDAGD
 MmTOP3b 347 NFDLKGSLRQOAN-----HPYWADSVKQLLAEG-----INRPRKGHDAGD
 HsTOP3a 373 DLNLTVLVEQQT-----DPRWGAFQSI LERGGPT-----PRNGNKSQOAH
 MmTOP3a 373 DLNLTVLVEQQT-----DPHWGAFQSI LERGGPT-----PRNGSKSDOAH
 ScTOP3 367 AMDLKSLEKQAQLDQLAA--GGRTAWASYAASLLOPENTSNNNKFKFPRSGSHDDKAH
 EcTOP1 330 DAVNMVRGYISDNFG-----KKYLPESPNOYASKE-----NSQEAHEAIR
 TmTOP1 299 YAKEEARNLITEVFG-----EYVGSKRERRKSNA-----KIQDAHEAIR
 EcTOP3 332 EQFSEAPQIIDALKS-----VFDQPMIDITTRKS-----KAFNSAKVTAH

G115190 390 HPIIPTG-LIPHVDSSNELSLYNLT TKHFLATVSPDAVYSHSSLTLOSVDMPSETFLLS
 G17615 480 LPIHPLACPSR--PLDKDEELVYGYITRSFLASVSPDARGTRMTMRTR---LDKCEFVAT
 HsTOP3b 387 HPIITPMKSATEAELGGDAWRLYEYITRHFLATVSHDCKYLQSTISFR---IGPELFTCS
 MmTOP3b 387 HPIITPMKSATEAELGGDAWRLYEYITRHFLATVSHDCKYLQSTISFR---IGPEHFTCM
 HsTOP3a 415 PPIHPTKYTN---NLQGDQRRLYEYIVRHFLACCSQDAQGGETTVEID---IAQERFVAH
 MmTOP3a 415 PPIHPTKYTS---GLQGDQRRLYEYIVRHFLACCSQDAQGGETTVEID---IAQERFVAH
 ScTOP3 424 PPIHPIVSLGPEANVSPVERRVYEVVARHFLACCSEDAKGQSM TLVLD---WAVERESAS
 EcTOP1 370 PSDVNMAESL-KDMEADAQKLYQLTWRFVACQMTPAKYDSTLTVTG-AGDFRLKARGR
 TmTOP1 339 PTNVFMTPEEAGKYLSNDQKKLYELTWKRFLASQMKPSQYEETRFVLR-TKDGYRFRGT
 EcTOP3 372 TAIIPTVSVPDVNALSTDERNVYLAIAQHVLVQFMPEKAYQEVSVAIQ---CGDESEVYAR

G115190 449 ITDVVDQGWKALYSATEFSSMRDNDLSDDLADESISLSKETLQAISATGATLTIASAAIK
 G17615 535 QNSLTTFDGRKRI LRKSLEEAG-----HLSSSASSPASIESSSSIVVGSKLL
 HsTOP3b 444 GKTVLSPGETEVMFWQSVF-----LEESLPTCQRGDAFPVGEVKML
 MmTOP3b 444 GKTVISPGETEIMPWQSVF-----LEESLPTCQRGDTFTVGEVKML
 HsTOP3a 469 GLMILARNYLDVYPYDHS-----DKILPVYEQGSHFQFPSTVEMV
 MmTOP3a 469 GLIILARNYLDVYPYDHS-----DKLLPVYEQGSHFQFPSTVEMV
 ScTOP3 481 GLVVLERNELDVYPWARWET-----TKQLPRLEMNALVDIAKAEK
 EcTOP1 428 ILR--FDGWTQVMPALRKGD-----EDRILPAVNKGDALTLVELTPA
 TmTOP1 398 VLKKIFDGYEKVWKTERNTG-----EFPFE---EGESVKPVVVKIE
 EcTOP3 429 ARKTTDSGEAFLGVENAGDDE-----AEVENDSDSFDLCKKIRTGETVTTKEVVVN

G115190 509 AGMTKPPGYLSESDLLGLME-----KHGIGTDASMAATHIG
 G17615 581 VLQAPYKQFFGTQDLHIINP-----TANDESELLASSIVW
 HsTOP3b 485 EKQTNPPDYLTAEELITLME-----KHGIGTDASIPVHIN
 MmTOP3b 485 EKQTSPPDYLTAEELITLME-----KHGIGTDASIPVHIN
 HsTOP3a 509 DGETSPPKLLTEADLIALME-----KHGIGTDATHAEHIE
 MmTOP3a 509 DGETSPPQLLTEADLIALME-----KHGIGTDATHAEHIE
 ScTOP3 522 AGTTAPPKPMTESELILMD-----TNGIGTDATIAEHID
 EcTOP1 468 QHFTKPPARFSEASLVKELE-----KRGICRFPSTYASTIS
 TmTOP1 436 EQETKPKPRYTEGSLVKEME-----RLGICRFPSTYASTIK
 EcTOP3 481 EKKTTTPEPLFTEATLAAALVRVADFVTDPIVKLLKEKDRDKKDEHGGIGTPATRASILE

G115190 544 NIVTRAYVELRVGTGRRRC[●]LVPTSMGISLIHGYQLIDGDLSS--SPQLRASIERDVT[●]RTAE[●]G
 G17615 616 DGDAQSLVIDIDSSDESEQIHAEENVHPPPEFVMVNDREDP---SNSTSQFSTELVPLPMK
 HsTOP3b 520 NICOENYVTVESGRR--LKPTNLGIVLVHGYKKIDAE[●]LV--LPTIRSAVEKQLNLIAQG
 MmTOP3b 520 NICOENYVTVESGRR--LKPTNLGIVLVHGYKKIDAE[●]LV--LPTIRSAVEKQLNLIAQG
 HsTOP3a 544 TIKARMYVG---LTPDKRFLPGHLGMLVEGYDSMGYEMS--KPD[●]LRAELEADLK[●]LICD[●]G
 MmTOP3a 544 TIKARMYVG---LTPDKRFLPGHLGMLVEGYDSMGYEMS--KPD[●]LRAELEADLK[●]LICD[●]G
 ScTOP3 557 KIQVRNYVRSEKVGKETYLQPTTLGVSLVHGFEATIGLED[●]SFAKPFQREMEQDLKKLICG
 EcTOP1 503 TIQDRGYVR---VENRRFYAEKMG[●]EIVTDRLEENFR[●]ELMN--YDFTAQMENSLDQVANH
 TmTOP1 471 LLLNKG[●]YIK---KIRGYLYPTIVGVSVVMDYLEKKYSDVVS--VSFTAE[●]MEKDLDEVEQG
 EcTOP3 541 TLKKRNYIT---LEK[●]GKLIPTDTGYALIDALPDIAVNP[●]D----MTALWAEKQTL[●]ENG

G115190 602 KIRKDVLVNQVLSK[●]ELTKFLHFKQNIGKLE-----ALLNAKFTSIKKAG[●]RPFIL[●]C
 G17615 673 ESALLLAMDQHGIGTDATMSD[●]HALITDR-----EYINSKKQVVS[●]LGAE[●]LLQFY
 HsTOP3b 575 KADYRQVLGHTLDVFKRK[●]FHYFVDSIAGMD-----ELMEVSFSPLAAT[●]GKPLSR[●]C
 MmTOP3b 575 KADYRQVLGHTLDVFKRK[●]FHYFVDSIAGMD-----ELMEVSFSPLAAT[●]GKPLSR[●]C
 HsTOP3a 599 KKD[●]KFVVL[●]RQQVQ[●]KYKQV[●]FI[●]EAVAKAK[●]KLDE[●]ALAQYFGNGTE[●]LAQQE[●]DIY[●]PAMPE[●]PIR[●]KC
 MmTOP3a 599 KKD[●]KFVVL[●]RQQVQ[●]KYKQV[●]FI[●]EAVAKAK[●]KLDE[●]ALAQYFGNGTE[●]LAQQE[●]DIY[●]PAMPE[●]PIR[●]KC
 ScTOP3 617 HAS[●]KTDV[●]VKDI[●]VEK[●]YRKY[●]WH[●]KT[●]NACKN-----TLLQVYD[●]RVK[●]ASM-----
 EcTOP1 557 EA[●]E[●]WKAV[●]LDH[●]FFSD[●]ETQ[●]QLD[●]KA[●]EK[●]DPEEG[●]GM-----RPNQ[●]MLV[●]TS[●]DCPT[●]CG[●]KMG[●]IR
 TmTOP1 525 KKT[●]DKI[●]VL[●]REFY[●]ES[●]SSV[●]ED[●]RND[●]R-----IVVD[●]FPTN-----
 EcTOP3 592 EMTIEQFVDELYNDLIPMISNANS-----AEIKVSPSPAPS[●]CQSQR[●]LS

G115190 652 GECHRYTD[●]LIEQYPPRVYCVTDKLYTIPMRGTFIEIPSRKCPYDGWFLILHMAEATQ[●]KR
 G17615 722 KQTPMGVQALSCSYRSMLEHGMHLICEGQIDCKSVHDDCIRWGMKLYDSIDRLQVIKVG-
 HsTOP3b 625 GKCHRFMKYIQA[●]KPSRLHCSHCDETYTLPQNGTIKLYKELRCPLDDFELVLWSSGSRG[●]KS
 MmTOP3b 625 GKCHRFMKYIQA[●]KPSRLHCSHCDETYTLPQNGTIKLYKELRCPLDDFELVLWSSGSRG[●]KS
 HsTOP3a 659 PQCKND[●]MLV[●]LKTKKSGGFYLS[●]CMGFPECRSAVWLPDSVLEASRDSSVCVPQPHPVYRLKL
 MmTOP3a 659 PQCKND[●]MLV[●]LKTKKSGGFYLS[●]CMGFPECRSAVWLPDSVLEASRDSSVCVPQPHPVYRLKL
 ScTOP3 -----
 EcTOP1 610 TASTGVFLGCSGYALPPKERCKTTINLVPENEVLNVLEGEDAETNALRAKRRCPKCGTAM
 TmTOP1 557 -----QKCS-----
 EcTOP3 634 SPCP-----

G115190 712 TFFCLHCYTFGLNK[●]EEALTQLQTVAADGSTDIEQT[●]KAITCGMCLNSLC[●]MS[●]FLKTQV[●]GAC
 G17615 781 -----GYGGYRMLNEKPKKTTIKRK
 HsTOP3b 685 YPLCPYCYNHPPFR-----DMKKGMGCNECTHPS[●]CHSL[●]SMLGIG[●]QC
 MmTOP3b 685 YPLCPYCYNHPPFR-----DMKKGMGCNECTHPT[●]CHSL[●]SMLGIG[●]QC
 HsTOP3a 719 KFKRGS[●]LPP[●]TMPLE[●]FEV[●]CCIGG[●]DDTL[●]REIL[●]DLRFSGGPPRASQPSGRLOANQSLNRMDNS
 MmTOP3a 719 KFKRGS[●]LPP[●]TMPLE[●]FEV[●]CCIGG[●]DDTL[●]KEIFGLRFPRALPRASQPSGHLQASQALNRMDSS
 ScTOP3 -----
 EcTOP1 670 DSYLIDPKRKLHVC[●]GNNPTCDGYEIEEGEFRIKGYD[●]G-PIVECEKCGSEMHLKMG[●]RFCKY
 TmTOP1 561 -----CGKEMRLSFGKYG[●]FFY
 EcTOP3 638 -----SCGKQIVIRPK-----

G115190 772 WHCNADGRPMLS[●]VNRDGPDS[●]SPGATNDTLAVARSPEAQEQTP[●]EKSAELASAPVQPKKISI
 G17615 801 YHSATDGTALVSKRR-----QKEAQFVRVRGTF
 HsTOP3b 727 VE[●]CSG-----VLVD[●]LPTSGPKW[●]KV
 MmTOP3b 727 VE[●]CENG-----VLVD[●]LPTSGPKW[●]KV
 HsTOP3a 779 QH--PQPADS[●]RQTGSSKALAQTLPPTAAGESNSVTCNCGQEA[●]VLLTVRKEGPNRGRQFF
 MmTOP3a 779 QHNL[●]SQPLVNRHTRPSKTVAQALLPPTTAGESNSVTCNCGREAVLLTVR[●]KQGP[●]NQGR[●]HFY
 ScTOP3 -----
 EcTOP1 729 MAC[●]TNEECKNTRKILRNGEVAP-----PKEDPVPLPELPCEKSDAYF
 TmTOP1 576 LK[●]C---ECGKTRSVK-NDEIA-----
 EcTOP3 649 -----SY

G115190 832 TIKPQAKISLNIPQANKSSGSASAVPSPNK-----ASSLAELVG[●]CYLCLDVGTARRLP
 G17615 829 TCCGERCSRNSLAGSCAACGKAQFI[●]PRTDG-----VIVVLNEECDNCGLS
 HsTOP3b 747 ACNKC[●]NVVAHCFENAHRRVRSADTCSVCEA-----ALLDVDFNKA[●]KSP[●]LPGDETQ[●]HMG
 MmTOP3b 747 ACNKC[●]NVVAHCFENAHRRVRSADTCSVCEA-----ALLDVDFNKA[●]KSP[●]LPGDETQ[●]HMG
 HsTOP3a 837 KCNGGSCNFFLWADSPNPGAGGPPALAYRPLGASLGCPGPGGIHLG[●]FGNPGD[●]SGSG[●]TS
 MmTOP3a 839 KCSNGDCNFFLWADSS[●]HSTGGTPTASAGPPGSSVGCPSVGD[●]SHMD[●]FGSLGSDSDGGT[●]P
 ScTOP3 -----
 EcTOP1 771 VLRDGAAGVFLAANTFPK[●]SRETRAPLVEELYRFRDRLPEK[●]LRYLADAPQ[●]QDPE[●]GK[●]NTM[●]VR
 TmTOP1 593 VIDDGK--IFLGR---KDS[●]ESGSP-----DGRSVEGK[●]N-----
 EcTOP3 651 SCTGCEFKIWN[●]EFSGKKITQAQAEKLIKSG-----KTDLIK[●]GFKKKSGCTYDAVL

G115190	885	GIYCNRCNFQIAFQECKLSLSSDFCACGRIKLRATDLLDKKSASSEVF	GCVCNDEL	FQT
G17615	874	LATITQSRSEIRICVRCRTDPL		
HsTOP3b	800	CVFCDPVFQELVELKHAASCHPMHRGG		P
MmTOP3b	800	CIFCDPVFQELVELKHAASCHPMHRGG		P
HsTOP3a	897	CLCSQPSVTRTVQKDGPNKGRQFHTCAKPREQQCGFFQWVDENTAPGTSGAPSWTGDGRGR		
MmTOP3a	899	CLCGQPAVTRTVQKDGPNKGRQFHTCAKPREQQCGFFQWVDENVAPGSFAAPAWPGGRGK		
ScTOP3				
EcTOP1	831	PSRRTKQQYVSSEKDGKATGWSAFYVDGKWVEGKK		
TmTOP1	622	-----LSEKRRKGKKGS		
EcTOP3	701	VLEDKKTGKLGFPARAK		
G115190	945	VSEYKDGSGRHPRRAAQPKRHGPRGRKHR		
G17615				
HsTOP3b	828	GRRQGRGRGRARRPPGKPNRRPKDKMSALAAFYV		
MmTOP3b	828	GRRQGRGRGRARRPPGKPNRRPKDKMSALAAFYV		
HsTOP3a	957	TLESEARSKRPRASSSDMGSTAKKPRKCSLCHQPGHTRPFCPQNR		
MmTOP3a	959	AQRPEAASKRPRAGSSDAGSTVKKPRKCSLCHQPGHTRPFCPQNR		
ScTOP3				
EcTOP1				
TmTOP1				
EcTOP3				

Fig. S1. Alignment of the type IA topoisomerases. The type IA topoisomerases from different organisms, including *Mus musculus* (Mm), *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *Saccharomyces cerevisiae* (Sc), *G. lamblia* (Gl), *Escherichia coli* (Ec), *Thermotoga Maritima* (Tm), and *Leishmania donovani* (Ld), are analyzed by ClustalW 1.83 with all default settings. GenBank *accession* numbers for MmTOP3 α , MmTOP3 β , HsTOP3 α , HsTOP3 β , DmTOP3 α , DmTOP3 β , ScTOP3, LdTOP3 β , EcTOP1, EcTOP3, TmTOP1, G17615, and G115190 are [NP_033436.1](#), [NP_035754.1](#), [NP_004609.1](#), [NP_003926.1](#), [NP_523602.2](#), [NP_511059.2](#), [NP_013335.1](#), [ACX31684.1](#), [WP_097426177.1](#), [WP_024221956.1](#), [WP_004082962.1](#), [XP_001709812.1](#), and [XM_001709742.1](#), respectively. Black boxes, gray boxes and hyphens indicate identical amino acids, conserved amino acids and gaps in the respective proteins, respectively. The catalytic important Tyrosine 328 is pointed by a red arrow. The decatenation loop of EcTOP3 (residues 502–519) is indicated by a purple line. The zinc ribbon domains are indicated by red lines. The cysteine residues in the zinc ribbon domains are indicated by red filled circles.

Figure S2

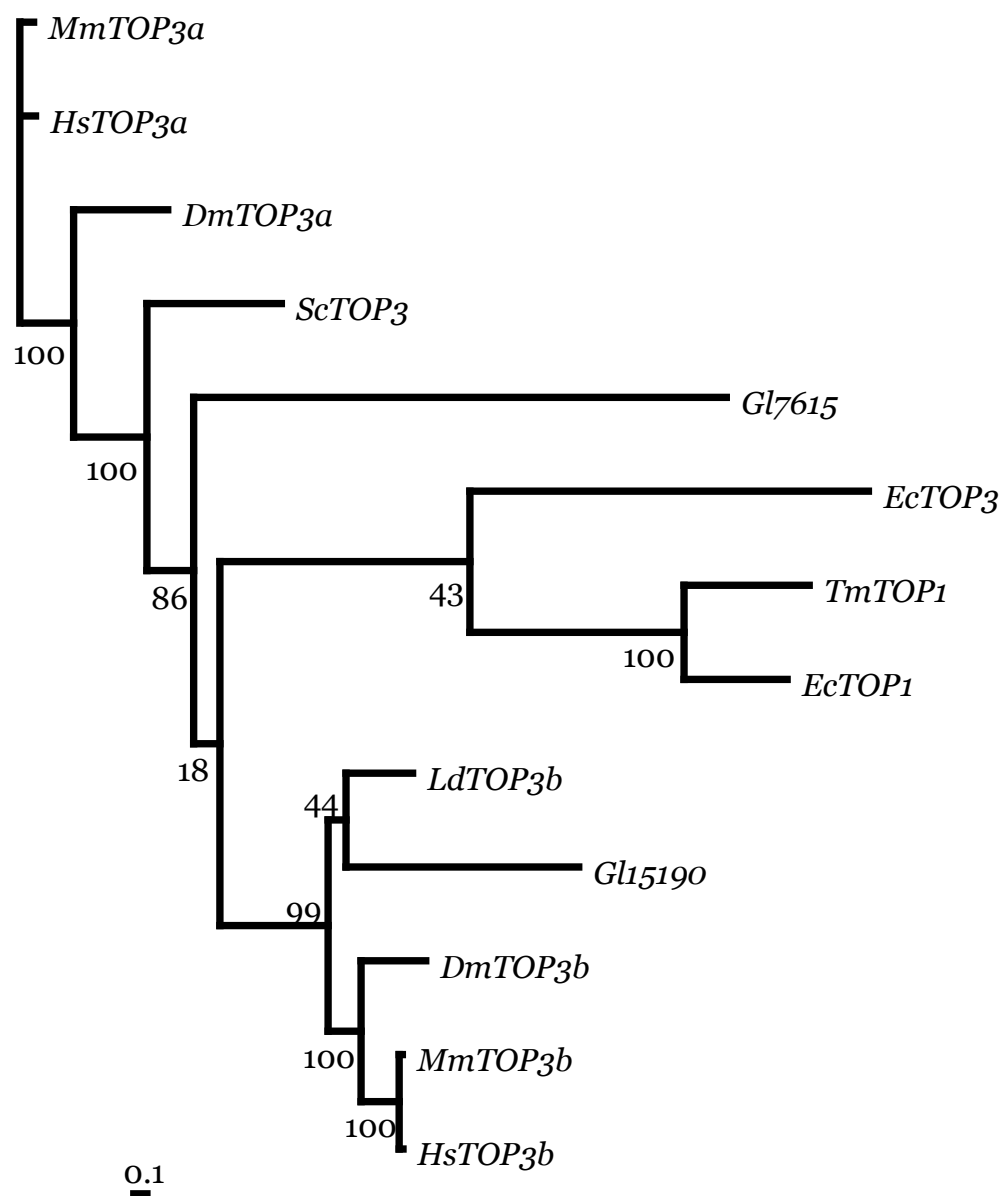
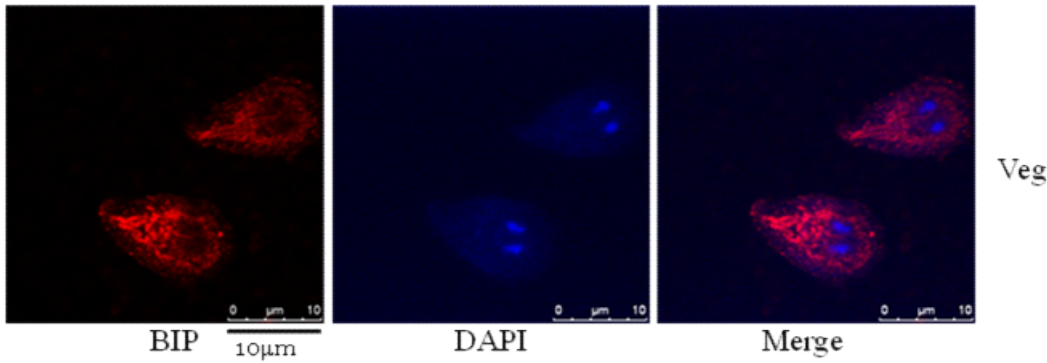


Fig. S2. Phylogenetic analysis of the type IA topoisomerases. A neighbor-joining phylogenetic tree was obtained from alignment of type IA topoisomerases from

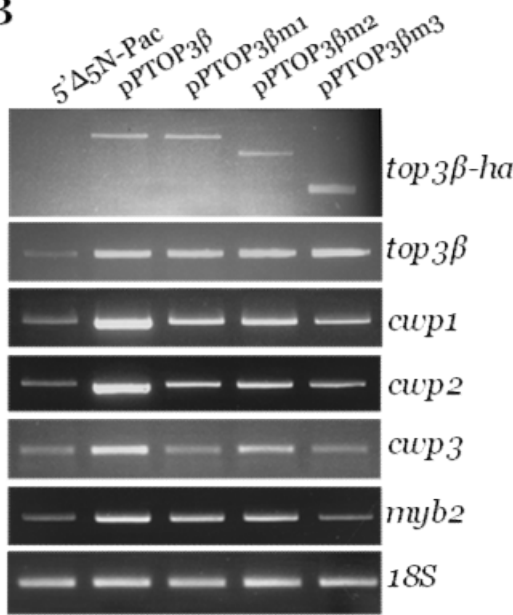
various organisms as described. The bootstrap values determined from 1000 trees are not shown. Values are higher than 400.

Figure S3

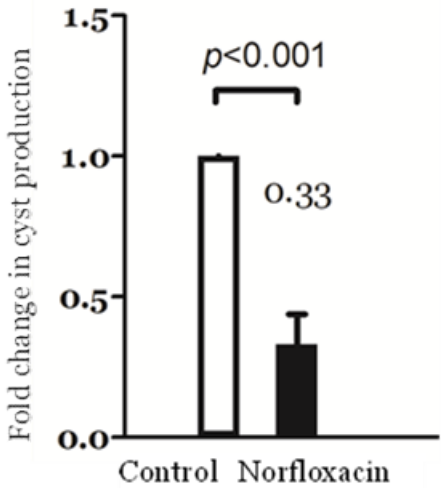
A



B



D



C

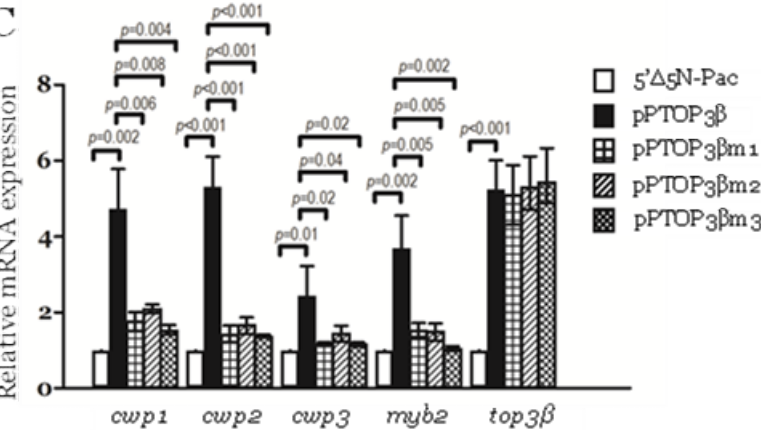
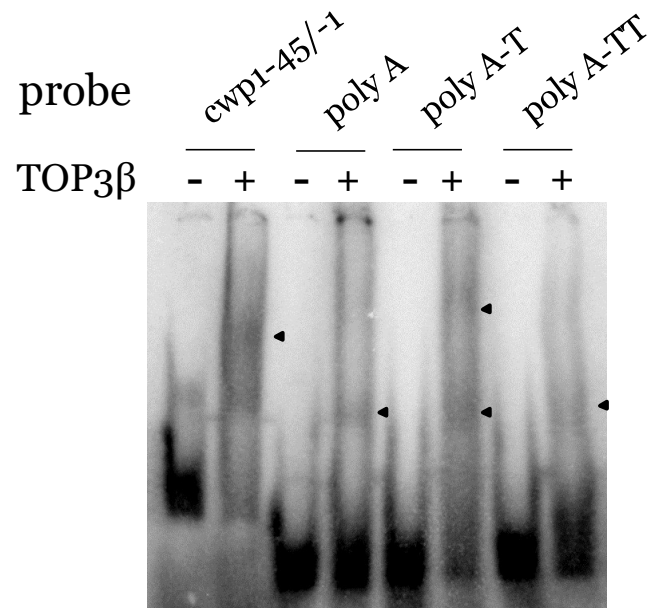


Fig. S3. Induction of *cwp1-3* and *myb2* gene expression in the TOP3 β -overexpressing cell line during encystation and inhibition of cyst formation by norfloxacin. (A) ER staining as determined by BIP localization. The wild-type WB trophozoites were cultured in growth medium and then subjected to immunofluorescence assays. The endogenous BIP protein was detected by anti-BIP antibody. The left panel shows that the BIP protein is localized to ER. The middle panel shows that the DAPI staining of cell nuclei. The right panel shows the merged image. The BIP staining did not overlap with DAPI. (B) RT-PCR analysis of gene expression in the TOP3 β - and TOP3 β mutants-expressing cell lines during encystation. The 5' Δ 5N-Pac, pPTOP3 β , pPTOP3 β m1-3 stable transfectants were cultured in encystation medium and then subjected to RT-PCR analysis using primers specific for *top3 β -ha*, *top3 β* , *cwp1*, *cwp2*, *cwp3*, *myb2*, and 18S ribosomal RNA genes, respectively. Representative results are shown. (C) The intensity of bands from three RT-PCR assays was quantified using Image J. The ratio of each target gene over the loading control (18S ribosomal RNA gene) is calculated. Fold change is calculated as the ratio of the difference between the pPTOP3 β /m1-3 sample and control sample, to which a value of 1 was assigned. Results are expressed as means \pm 95% confidence intervals (error bars) of at least three separate experiments. $p < 0.05$ was considered significant and the value was shown. (D) The addition of norfloxacin decreased cyst formation. The wild-type nontransfected WB cells were cultured in growth medium containing 497 μ M norfloxacin, or the same volume of Me2SO for 24h and then subjected to cyst count as described under “Materials and Methods” and Fig. 3D.

Figure S4



<i>cwp1-45/-1</i>	GTTTACAACCTCTCAGATCAAGAAACACAGAA <u>ATA[*]AAATAT</u> CAGGG
poly A	AAAAAAAAAAAAAAAAAAAAAAAAA
poly A-T	AAAAAAAAAAATAAAAAAAAAAAA
poly A-TT	AAAAAAAAAAATTAAAAAAAAAAAA

Fig. S4. TOP3β may bind to AT-rich sequence. Electrophoretic mobility shift assays were performed using purified TOP3β and various ³²P-end-labeled oligonucleotide probes *cwp1-45/-1*, poly A, poly A-T, and poly A-TT as described. Components in the binding reaction mixtures are indicated above the lanes. The arrowhead indicates the shifted complex.

Figure S5

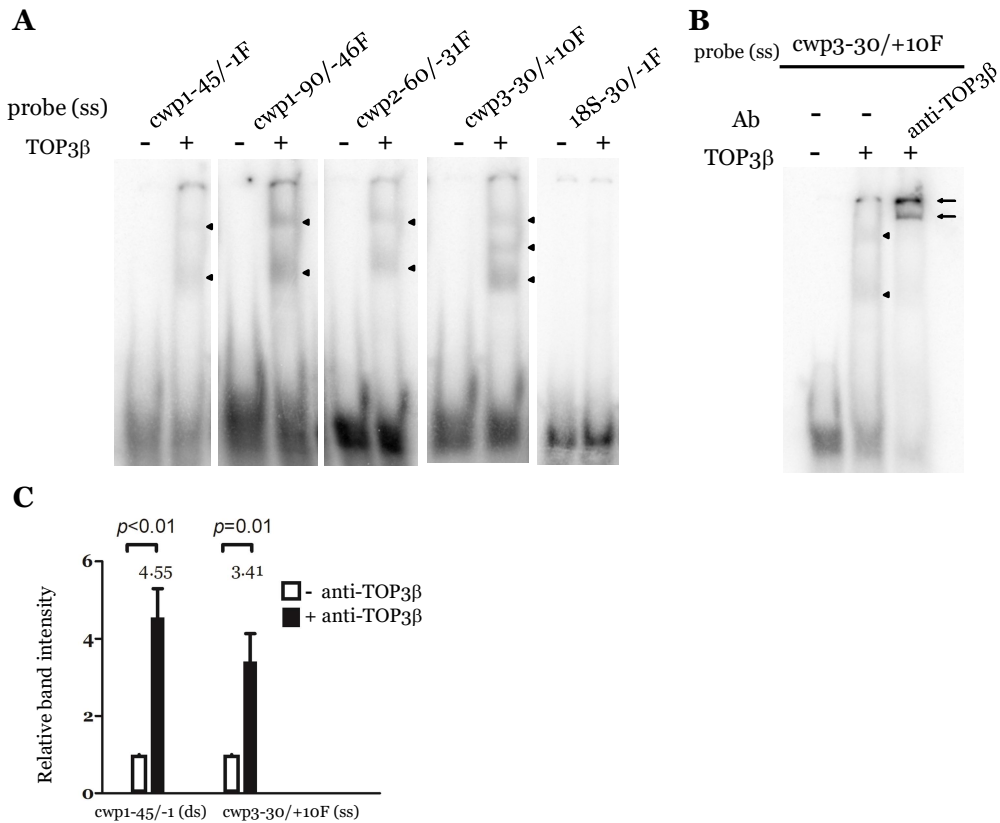


Fig. S5. Single-stranded DNA-binding and cleavage ability of TOP3 β . (A)

Single-stranded DNA-binding ability of TOP3 β . Electrophoretic mobility shift assays were performed using purified TOP3 β and 32 P labeled single-stranded (ss) oligonucleotide probes, such as cwp1-45/-1F (the forward single strand of the cwp1-45/-1). Components in the binding reaction mixtures are indicated above the lanes. The arrowheads indicate the shifted complexes. (B) The single-stranded DNA-binding activity of TOP3 β was validated by supershift assays. Some reaction

mixtures contained 0.8 μ g of anti-TOP3 β antibody as indicated above the lanes. (C) Quantitation of the intensity of anti-TOP3 β supershift bands as indicated by arrows in Fig. 5C and Fig. S5B. The intensity of bands from three assays with double-stranded (ds) cwp1-45/-1 probe and single-stranded (ss) cwp3-40/+10F probe was quantified using Image J. Fold change is calculated as the ratio of the difference between the + anti-TOP3 β sample and - anti-TOP3 β sample, to which a value of 1 was assigned. Results are expressed as means \pm 95% confidence intervals (error bars) of at least three separate experiments. $p < 0.05$ was considered significant and the value was shown.

Figure S6

ATGGGCACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCCGGGCGG
TACGCACCCTCGCCGCCGCGTTTCGCCGACTACCCCGCCACGCGCCACACCGTCGACCCGGA
CCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCGCGTCGGGCTCGAC
ATCGGCAAGGTGTGGGTTCGCGGACGACGGCGCCGCGGTGGCGGTCTGGACCACGCCGGAGA
GCGTCGAAGCGGGGGCGGTGTTTCGCCGAGATCGGCCCCGCGCATGGCCGAGTTGAGCGGTTC
CCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCACCGGCCCAAGGAGCCC
GCGTGGTTCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGCAAGGGTCTGGGCAGCG
CCGTCGTGCTCCCCGAGTGGAGGCGGCCGAGCGCGCCGGGGTGGCCGCCTTCTGGAGAC
CTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTCACCGTCACCGCCGACGTC
GAGTGCCCCG**AAGGACCGCGCGACCTGG**TGCATGACCCGCAAGCCCGGTGCCCTCGAGTGAc
catcaagtgccttgctattatgtaaactacttcctactccattaaatatttagacgcgcagt
tctggctatgggaccgaaacttttgaagagcgagtcagctatgagtttatggatgaaaacg
cagttcaatcagatcgtgcggtatataagtataagatacataaccgtagagtgctaatac
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cagtccttctgatcagccttgcgctccaactggcgacagaggagtgcacaaccgtctctgg
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cagtgcaacggcggtgggtctcaatctgccccaacggacggagtggtgctcagcagacaaca
acgaatgcccctaaaagaacaacggagtatgtacacagtgcgctcatgagtccttcatgta
caagagcggatgctacaaggatagccaggcacctggcaacacgatgtgtgaaacagcaact
gatggagtggtgcacactaactaaggctggatacttcgtgcccggcgggcgagacgcctctc
accagt**cggtcataccatgtggagacg**

Fig. S6. Replacement of the *top3 β* gene with the *pac* gene in the TOP3 β td cell line confirmed by PCR and sequencing. Genomic DNA was isolated from the TOP3 β td and control cell lines cultured in growth medium. PCR was performed using primers specific for *pac* (PCR2 in Fig. 8A), which are PCR2F for bold region 1 and PCR2R for bold region 2, to verify the integration of the *pac* gene into the correct region in genomic DNA. The sequence results obtained from the PCR2 product are shown as underlined letters. Capital letters indicate the coding sequence for the *pac* gene, which

starts at ATG and stops at TGA. This indicates the replacement of the *top3β* gene with the *pac* gene. The region used to clone the *top3β* 3' region into the pTOP3βtd plasmid for HR is shown in red, which is also between the sequence of top3β3XF and top3β3KR. The underlined and lower case letters, which are downstream and outside of the red region of top3β3XF and top3β3KR, indicate that HR occurred in the sequence of *top3β* 3' region and that the *pac* gene was integrated in the genomic DNA.

Figure S7

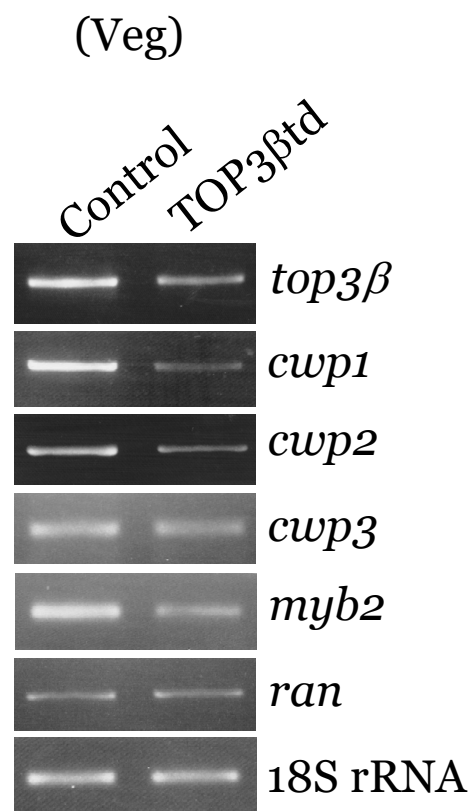


Fig. S7. RT-PCR analysis of gene expression in the TOP3 β td cell line. The control and TOP3 β td cell lines were cultured in growth medium and then subjected to RT-PCR analysis using primers specific for *top3 β* , *cwp1*, *cwp2*, *cwp3*, *myb2*, *ran*, and 18S ribosomal RNA genes, respectively. The *ran* mRNA levels did not significantly change.

Figure S8

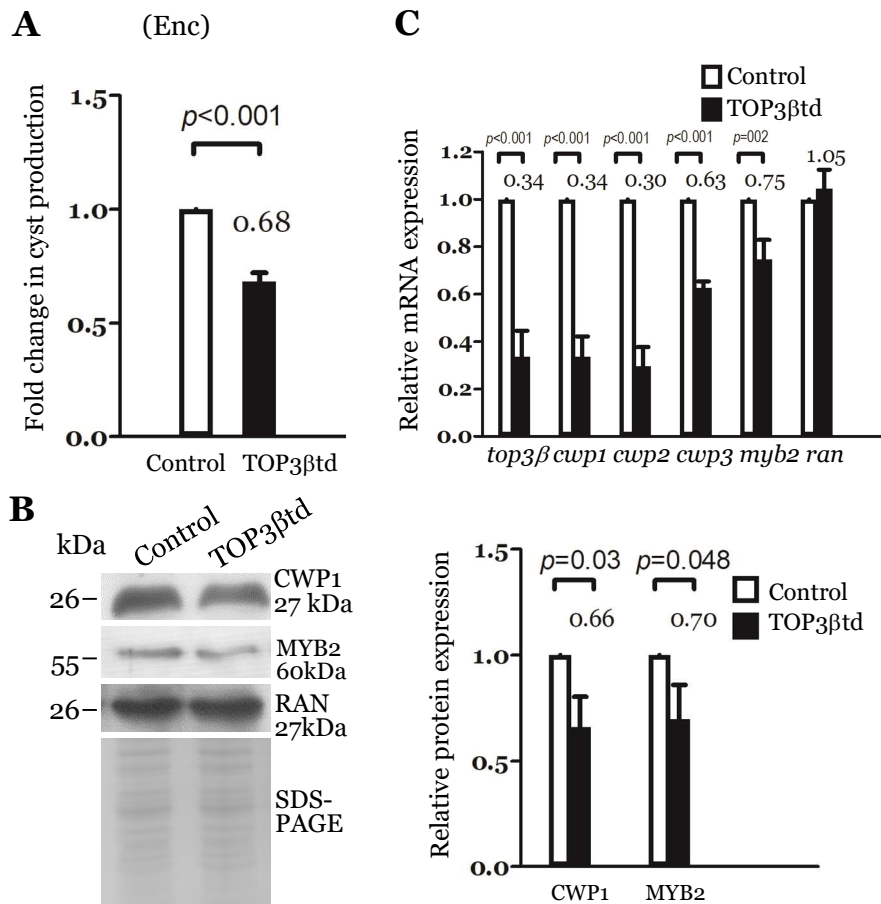


Fig. S8. Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3β* gene during encystation. (A) Cyst formation decreased by targeted disruption of the *top3β* gene in the TOP3βtd cell line during encystation. The control and TOP3βtd cell lines were cultured in encystation medium for 24h (Enc) and then subjected to cyst count as described under “Materials and Methods” and Fig. 3D. (B) Targeted disruption of the *top3β* gene decreased the CWP1 and MYB2 levels in the TOP3βtd

cell line during encystation. The control and TOP3 β td cell lines were cultured in encystation medium for 24h and then subjected to SDS-PAGE and Western blot analysis as described in Fig. 3A. The blot was probed with anti-CWP1, anti-MYB2, and anti-RAN antibodies, respectively. The intensity of bands from three Western blot assays was quantified as described in Fig. 3A. (C) Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3 β* gene in the TOP3 β td cell line during encystation. The control and TOP3 β td cell lines were cultured in encystation medium for 24h and then subjected to quantitative real-time RT-PCR analysis using primers specific for *top3 β* , *cwp1*, *cwp2*, *cwp3*, *myb2*, *ran*, and 18S ribosomal RNA genes, respectively, as described in Fig. 1B.

Figure S9

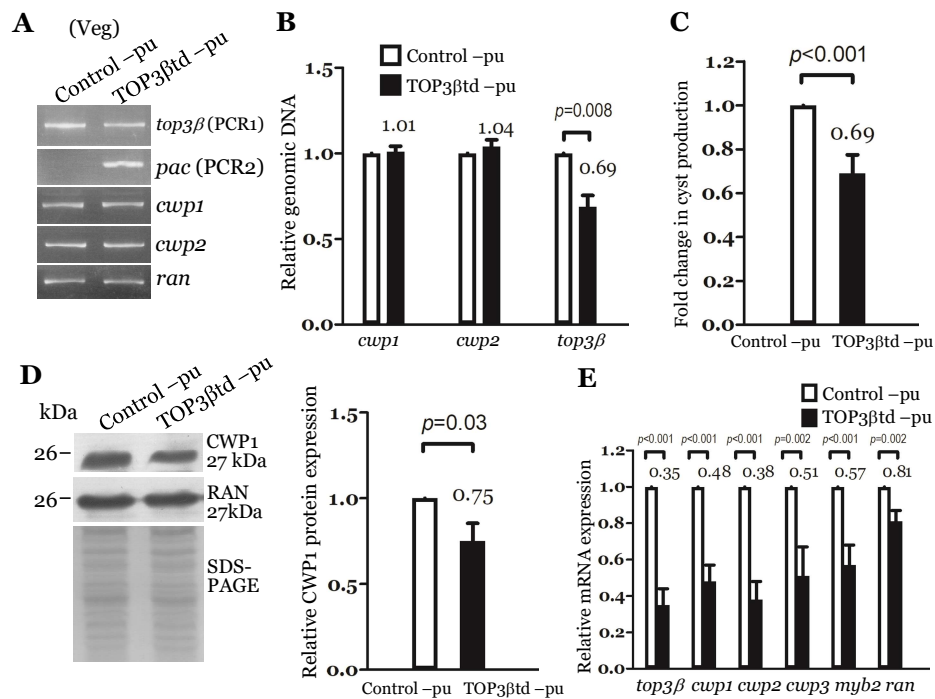


Fig. S9. Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3β* gene after the removal of puromycin during vegetative growth. (A) Partial replacement of the *top3β* gene with the *pac* gene in the TOP3β -pu cell line confirmed by PCR. Puromycin was removed from the TOP3βtd and control cell lines to obtain the TOP3βtd -pu and control -pu cell lines, respectively. Genomic DNA was isolated from the TOP3βtd -pu and control -pu cell lines cultured in growth medium (vegetative growth, Veg). PCR was performed using primers specific for *top3β* (PCR1), *pac* (PCR2), *cwp1*, *cwp2*, and *ran* genes, respectively, as described in Fig. 8B. (B) Partial disruption of the *top3β* gene in the TOP3βtd -pu cell line confirmed by real-time PCR. Real-time PCR was performed using genomic DNA and primers specific for *top3β*, *cwp1*, *cwp2*, and *ran* genes, respectively, as described in

Fig. 8C. (C) Cyst formation decreased by targeted disruption of the *top3 β* gene in the TOP3 β td –pu cell line during vegetative growth. The control –pu and TOP3 β td –pu cell lines were cultured in growth medium and then subjected to cyst count as described under “Materials and Methods” and Fig. 3D. (D) Targeted disruption of the *top3 β* gene decreased the CWP1 and MYB2 levels in the TOP3 β td –pu cell line during vegetative growth. The control –pu and TOP3 β td –pu cell lines were cultured in growth medium and then subjected to SDS-PAGE and Western blot analysis as described in Fig. 3A. The blot was probed with anti-CWP1, anti-MYB2, and anti-RAN antibodies, respectively. The intensity of bands from three Western blot assays was quantified as described in Fig. 3A. (E) Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3 β* gene in the TOP3 β td –pu cell line during vegetative growth. The control –pu and TOP3 β td –pu cell lines were cultured in growth medium and then subjected to quantitative real-time RT-PCR analysis using primers specific for *top3 β* , *cwp1*, *cwp2*, *cwp3*, *myb2*, *ran*, and 18S ribosomal RNA genes, respectively, as described in Fig. 1B.

Figure S10

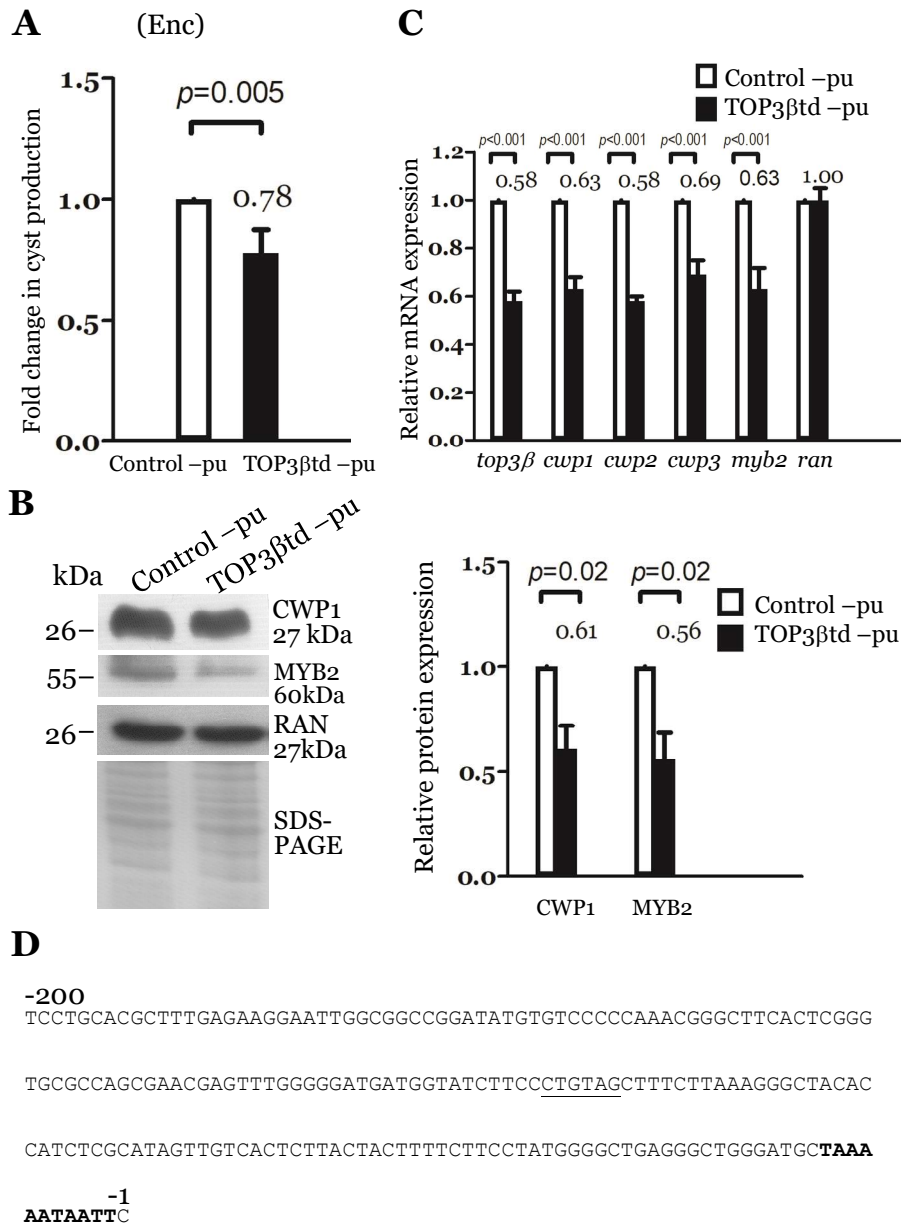


Fig. S10. Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3β* gene after the removal of puromycin during encystation. (A) Cyst formation decreased by targeted disruption of the *top3β* gene in the TOP3βtd -pu cell line during encystation. The control -pu and TOP3βtd -pu cell lines were cultured in

encystation medium for 24h (Enc) and then subjected to cyst count as described under “Materials and Methods”, as described in Fig. 3D. (B) Targeted disruption of the *top3 β* gene decreased the CWP1 and MYB2 levels in the TOP3 β td –pu cell line during encystation. The control –pu and TOP3 β td –pu cell lines were cultured in encystation medium and then subjected to SDS-PAGE and Western blot analysis as described in Fig. 3A. The blot was probed with anti-CWP1, anti-MYB2, and anti-RAN antibodies, respectively. The intensity of bands from three Western blot assays was quantified as described in Fig. 3A. (C) Decrease in expression of *cwp1-3* and *myb2* by targeted disruption of the *top3 β* gene in the TOP3 β td –pu cell line during encystation. The control –pu and TOP3 β td –pu cell lines were cultured in encystation medium and then subjected to quantitative real-time RT-PCR analysis using primers specific for *top3 β* , *cwp1*, *cwp2*, *cwp3*, *myb2*, *ran*, and 18S ribosomal RNA genes, respectively, as described in Fig. 1B. (D) The MYB2 binding site in the *top3 β* promoter. The 200-bp 5’ untranslated region of the *top3 β* promoter is shown. The AT-rich promoter element is in bold. The underlined sequence, CTGTAG, is the reverse sequence of the MYB2 binding sequence, CTACAG.