

Supplementary Information

The benzyloisoquinoline alkaloids, berberine and coptisine, act against camptothecin-resistant topoisomerase I mutants

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These authors contributed equally to this work.

Supplementary Table 1. Herbal extracts used in this study

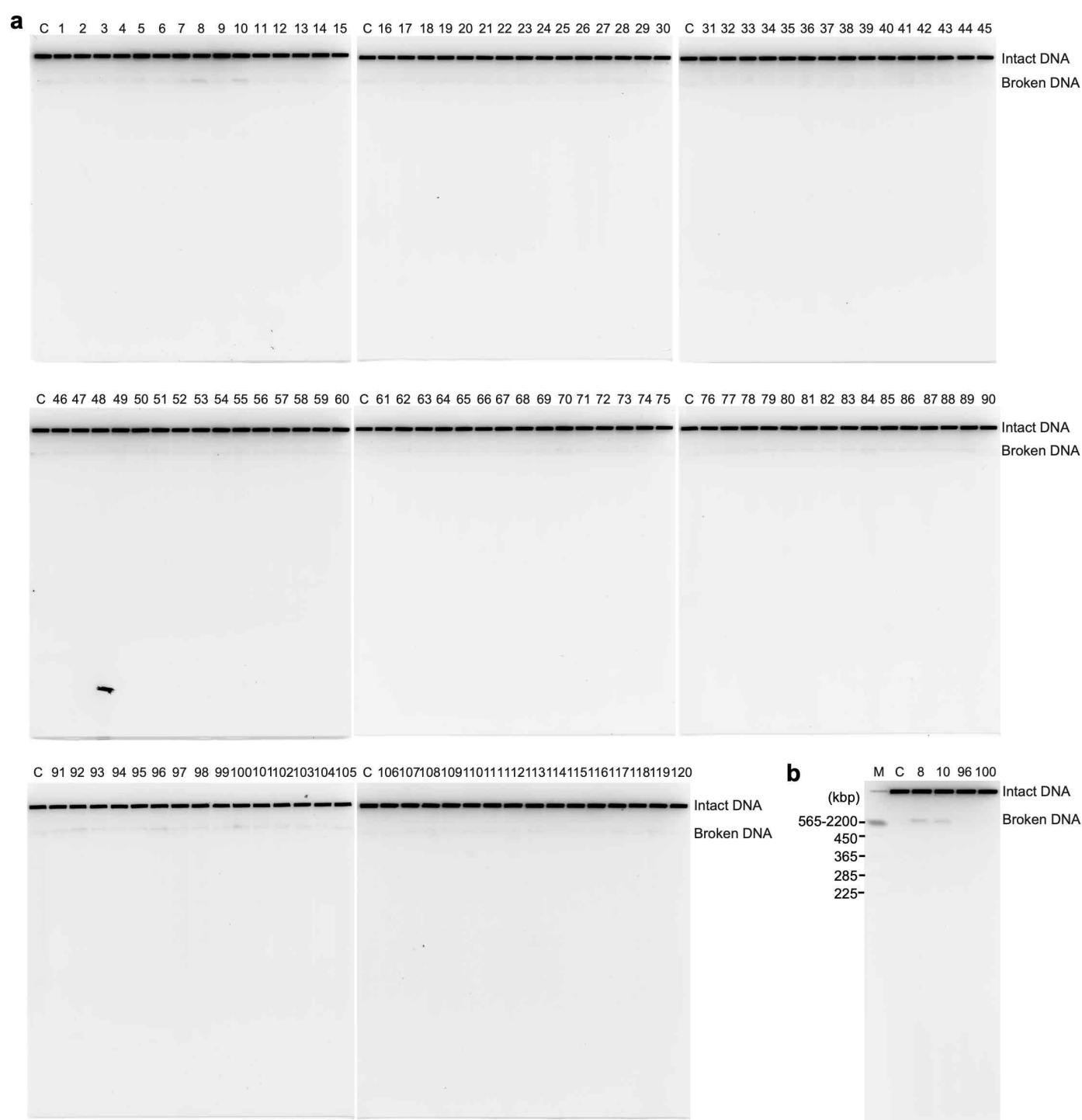
ID #	Name of herbal medicine	Botanical source
#1	Clematis Root	<i>Clematis chinensis</i> Osbeck, <i>Clematis mandshurica</i> Ruprecht , <i>Clematis hexapetala</i> Pallas
#2	Artemisia Capillaris Flower	<i>Artemisia capillaris</i> Thunberg
#3	Turmeric	<i>Curcuma longa</i> L.
#4	Lindera Root	<i>Lindera strychnifolia</i> (Siebold et Zucc.) F. Vill.
#5	Corydalis Tuber	<i>Corydalis turtchaninovii</i> Besser forma <i>yanhusuo</i> Y.H.Chou et C.C.Hsu
#6	Astragalus Root	<i>Astragalus membranaceus</i> Bunge, <i>Astragalus mongholicus</i> Bunge
#7	Scutellaria Root	<i>Scutellaria baicalensis</i> Georgi
#8	Phellodendron Bark	<i>Phellodendron amurense</i> Ruprecht
#9	Cherry Bark	<i>Prunus jamasakura</i> Siebold ex Koidzumi, <i>Prunus verecunda</i> Koehne
#10	Coptis Rhizome	<i>Coptis japonica</i> Makino var. <i>japonica</i> Satake, <i>Coptis japonica</i> Makino var. <i>dissecta</i> Nakai, <i>Coptis japonica</i> Makino var. <i>major</i> Satake, <i>Coptis teeta</i> , <i>Coptis chinensis</i> , <i>Coptis deltoidea</i>
#11	Polygala Root	<i>Polygala tenuifolia</i> Willdenow
#12	Artemisia Leaf	<i>Artemisia princeps</i> Pampanini, <i>Artemisia montana</i> Pampanini
#13	Polygonum Root	<i>Polygonum multiflorum</i> Thunberg
#14	Zedoary	<i>Curcuma zedoaria</i> (Christ.) Roscoe
#15	Pueraria Root	<i>Pueraria lobata</i> (Willd.) Ohwi
#16	Trichosanthes Seed	<i>Trichosanthes bracteata</i> (Lam.) Voigt
#17	Processed Ginger	<i>Zingiber officinale</i> Roscoe
#18	Glycyrrhiza	<i>Glycyrrhiza uralensis</i> Fisher, <i>Glycyrrhiza glabra</i> Linne
#19	Platycodon Root	<i>Platycodon glandiflorum</i> (Jacq.) A. DC.
#20	Chrysanthemum Flower	<i>Chrysanthemum morifolium</i> Ramatulle, <i>Chrysanthemum indicum</i> Linné
#21	Immature Orange	<i>Citrus aurantium</i> Linné var. <i>daidai</i> Makino, <i>Citrus aurantium</i> Linné , <i>Citrus natsudaikai</i> Hayata

#22	Notopterygium	<i>Notopterygium incisum</i> Ting ex H. T. Chang , <i>Notopterygium forbesii</i> Boissieu
#23	Apricot Kernel	<i>Prunus armeniaca</i> Linné, <i>Prunus armeniaca</i> Linné var. <i>ansu</i> Maximowicz, <i>Prunus sibirica</i> Linné
#24	Sophora Root	<i>Sophora flavescens</i> Aiton
#25	Sasa Leaf	<i>Sasa palmata</i> Nakai, <i>Sasa senanensis</i> Rehder, <i>Sasa yahikoensis</i> Makino
#26	Schizonepeta Spike	<i>Schizonepeta tenuifolia</i> Briq.
#27	Suberect Spatholobus Stem	<i>Spatholobus suberectus</i> Dunn, <i>Mucuna birdwoodiana</i> Tutcher
#28	Cinnamon Bark	<i>Cinnamomum cassia</i> Blume
#29	Figwort Root	<i>Scrophularia ningpoensis</i> Hemsley, <i>Scrophularia buergeriana</i> Miquel
#30	Safflower	<i>Carthamus tinctorius</i> L.
#31	Silktree Albizia Bark	<i>Albizia julibrissin</i> Durazzini
#32	Red Ginseng	<i>Panax ginseng</i> C. A. Mey.
#33	Cyperus Rhizome	<i>Cyperus rotundus</i> Linné
#34	Magnolia Bark	<i>Magnolia obovata</i> Thunb.
#35	Achyranthes Root	<i>Achyranthes fauriei</i> Lév. et Vaniot, <i>Achyranthes bidentata</i> Blume
#36	Evodia Fruit	<i>Evodia rutaecarpa</i> (Juss.) Benth.
#37	Burdock Fruit	<i>Arctium lappa</i> Linné
#38	Sesame	<i>Sesamum indicum</i> Linné
#39	Scisandra Fruit	<i>Schisandra chinensis</i> (Turcz.) Baill.
#40	Bupleurum Root	<i>Bupleurum falcatum</i> L.
#41	Asiasarum Root	<i>Asiasarum sieboldii</i> (Miq.) F. Maekawa = <i>Asarum sieboldii</i> Miq. , <i>Asiasarum heterotropoides</i> (F. Schmidt) F. Maekawa var. <i>mandshuricum</i> F. Maekawa = <i>Asarum heterotropoides</i> F. Schmidt var. <i>mandshuricum</i> (Maxim.) Kitagawa
#42	Saffron	<i>Crocus sativus</i> Linne
#43	Smilax Rhizome	<i>Smilax glabra</i> Roxburgh
#44	Gardenia Fruit	<i>Gardenia jasminoides</i> Ellis
#45	Notoginseng Root	<i>Panax notoginseng</i> (Burkill) Feng Hwai Chen
#46	Cornus Fruit	<i>Cornus officinalis</i> Siebold et Zucc.
#47	Zanthoxylum Fruit	<i>Zanthoxylum piperitum</i> (L.) DC.
#48	Jujube Seed	<i>Zizyphus jujuba</i> Miller var. <i>spinosa</i> Hu ex H. F. Chou
#49	Dioscorea Rhizome	<i>Dioscorea japonica</i> Thunb., <i>Dioscorea batatas</i> Decne.

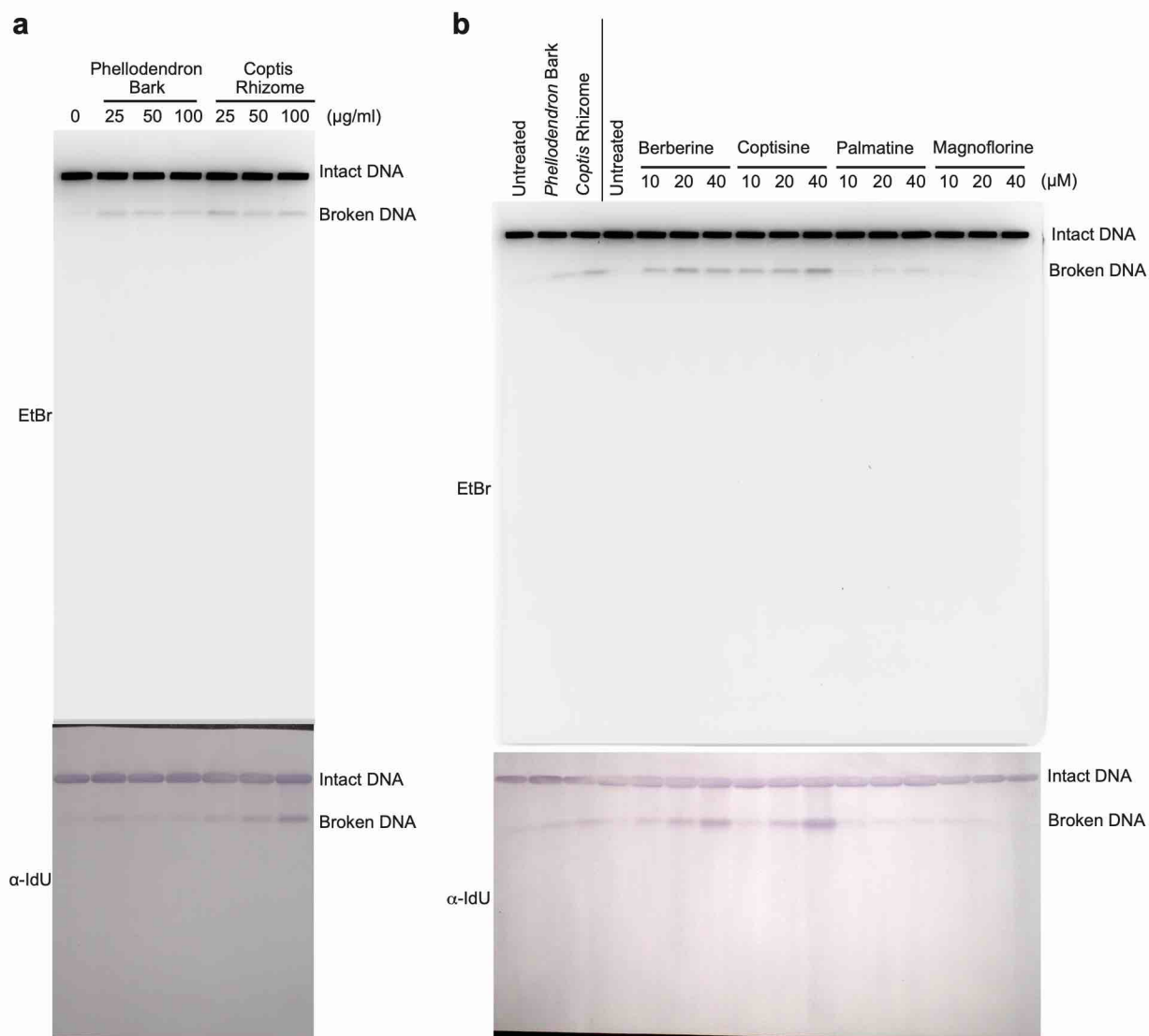
#50	Sparganium Rhizome	<i>Sparganium stoloniferum</i> Buchanan-Hamilton
#51	Rehmannia Root	<i>Rehmannia glutiosa</i> Libosch. var. <i>purpurea</i> Makino, <i>Rehmannia glutiosa</i> Libosch. f. <i>hueichingensis</i> (Chao et Schih) Hsiao
#52	Eleutherococcus Senticosus Rhizome	<i>Eleutherococcus senticosus</i> Maximowicz
#53	Chinese Wolfberry Root-bark	<i>Lycium chinense</i> Miller <i>Lycium barbarum</i> Linné
#54	Tribulus Fruit	<i>Tribulus terrestris</i> Linné
#55	Paony Root	<i>Paeonia lactiflora</i> Pallas
#56	Plantago Seed,	<i>Plantago asiatica</i> L.
#57	Prepared Rehmannia Root	<i>Rehmannia glutinosa</i> Liboschitz var. <i>purpurea</i> Makino <i>Rehmannia glutinosa</i> Liboschitz
#58	Amomum Seed	<i>Amomum xanthioides</i> Wallich
#59	Ginger	<i>Zingiber officinale</i> Roscoe
#60	Cimicifuga Rhizome	<i>Cimicifuga simplex</i> Wormsk.
#61	Magnolia Flower	<i>Magnolia salicifolia</i> Maximowicz, <i>Magnolia biondii</i> Pampanini, <i>Magnolia sprengeri</i> Pampanini, <i>Magnolia heptapeta</i> Dandy (<i>Magnolia denudata</i> Desrousseaux)
#62	Gentiana Macrophylla Root	<i>Gentiana macrophylla</i> Pallas, <i>Gentiana straminea</i> Maximowicz, <i>Gentiana crassicaulis</i> Duthie ex Burkill, <i>Gentiana dahurica</i> Fischer
#63	Red Paeony Root	<i>Paeonia lactiflora</i> Pallas
#64	Cnidium Rhizome	<i>Cnidium officinale</i> Makino
#65	Ginseng Root	<i>Panax ginseng</i> C. A. Meyer (<i>Panax schinseng</i> Nees)
#66	Atractylodes Lancea Rhizoma	<i>Atractylodes lancea</i> De Candole
#67	Mulberry Bark, White Mulberry Root-bark	<i>Morus alba</i> Linné
#68	Mulberry Leaf	<i>Morus alba</i> Linné, <i>Morus bombycis</i> Koidzumi
#69	Himalayan Teasel Root	<i>Dipsacus asperoides</i> C. Y. Cheng et T.M. Ai
#70	Perilla Herb	<i>Perilla frutescens</i> (L.) Britton var. <i>acuta</i> Kudo
#71	Rhubarb	<i>Rheum palmatum</i> Linne
#72	Jujube	<i>Zizyphus jujuba</i> Miller var. <i>inermis</i> Rehder
#73	Alisma Rhizome	<i>Alisma orientale</i> Juzepczuk
#74	Danshen Root	<i>Salvia miltiorhiza</i> Bunge
#75	Bamboo Culm	<i>Bambusa tuldoidea</i> Munro, <i>Phyllostachys nigra</i> Munro var. <i>henonis</i> Stapf ex Rendle, <i>Phyllostachys bambusoides</i> Siebold et Zuccarini

#76	Panax Rhizome	<i>Panax japonicus</i> C. A. Meyer
#77	Anemarrhena Rhizome	<i>Anemarrhena asphodeloides</i> Bunge
#78	Dingxiang, Caryophylli Flos	<i>Syzygium aromaticum</i> Merrill et Perry (<i>Eugenia caryophyllata</i> Thunberg)
#79	Uncaria Thorn	<i>Uncaria rhynchophylla</i> (Miq.) Miq.
#80	Polyporus Sclerotium	<i>Polyporus umbellatus</i> Fries
#81	Citrus Unshiu Peel	<i>Citrus unshiu</i> Marcowicz, <i>Citrus reticulata</i> Blanco
#82	Gastrodia Tuber	<i>Gastrodia elata</i> Blume
#83	Asparagus Tuber	<i>Asparagus cochinchinensis</i> Merrill
#84	Benincasa Seed	<i>Benincasa cerifera</i> Savi <i>Benincasa cerifera</i> Savi forma <i>emarginata</i> K. Kimura et Sugiyama
#85	Japanese Angelica root	<i>Angelica acutiloba</i> Kitagawa, <i>Angelica acutiloba</i> var. <i>sugiyamae</i> Hikino
#86	Codonopsis Root	<i>Codonopsis pilosula</i> (Franchet) Nannfeldt, <i>Codonopsis pilosula</i> Nannfeldt var. <i>modesta</i> (Nannfeldt) L.T. Shen, <i>Codonopsis tangshen</i> Oliver
#87	Peach Kernel	<i>Prunus persica</i> Batsch, <i>Prunus persica</i> Batsch var. <i>davidiana</i> Maximowicz
#88	Aralia Rhizome	<i>Aralia cordata</i> Thunberg
#89	Eucommia Bark	<i>Eucommia ulmoides</i> Oliver
#90	Desertliving Cistanche	<i>Cistanche deserticola</i> Y.S.Ma, <i>Cistanche tubulosa</i> (Schenk) R.Wight
#91	Ginseng Root	<i>Panax ginseng</i> C. A. Meyer (<i>Panax schinseng</i> Nees)
#92	Fritillaria Bulb	<i>Fritillaria verticillata</i> Willd. var. <i>thunbergii</i> Baker
#93	Densefruit Pittany Root-bark	<i>Dictamnus dasycarpus</i> Turczaninow
#94	Ophiopogon Tuber	<i>Ophiopogon japonicus</i> Ker-Gawler
#95	Mentha Herb, Japanese Mint	<i>Mentha arvensis</i> Linné var. <i>piperascens</i> Malinvaud
#96	Pinellia Tuber	<i>Pinellia ternata</i> Breitenb.
#97	Angelica Dahurica Root	<i>Angelica dahurica</i> (Fisch.) Benth. et Hook. fil.
#98	Atractylodes Rhizome	<i>Atractylodes japonica</i> Koidzumi ex Kitamura, <i>Atractylodes ovata</i> DC.
#99	Loquat Leaf	<i>Eriobotrya japonica</i> Lindley
#100	Areca	<i>Areca catechu</i> Linné
#101	Poria Sclerotium	<i>Poria cocos</i> Wolf
#102	Sinomenium Stem	<i>Sinomenium acutum</i> Rehder et Wilson
#103	Imperata Rhizome	<i>Imperata cylindrica</i> Beauvois
#104	Saposhnikovia Root	<i>Saposhnikovia divaricata</i> Schischkin
#105	Aconite, Common Monkshood	<i>Aconitum carmichaeli</i> , <i>Debeaux Aconitum japonicum</i> Thunberg

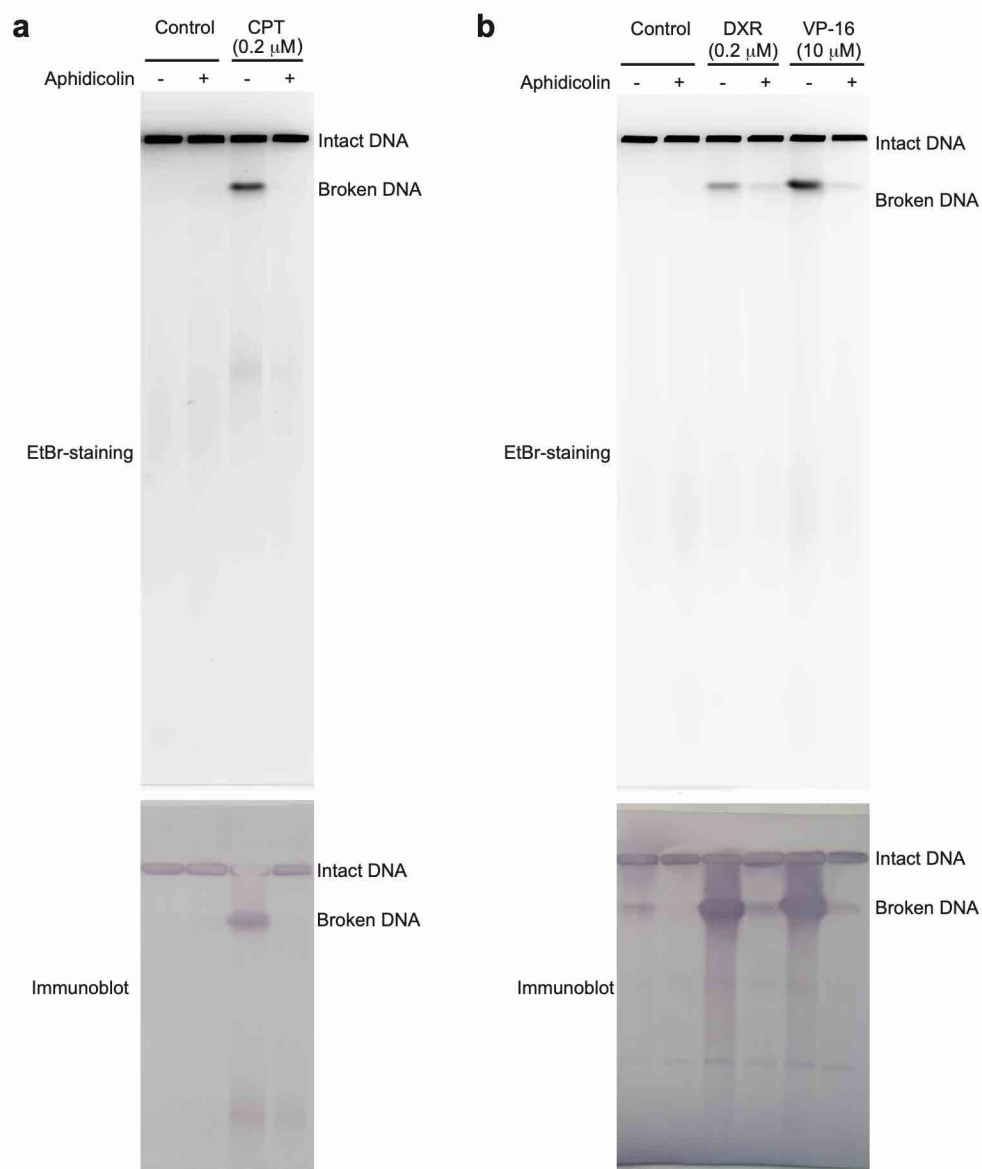
	Dauguter Root	
#106	Quercus Bark	<i>Quercus acutissima</i> Carruthers, <i>Quercus serrata</i> Murray, <i>Quercus mongolica</i> Fischer ex Ledebour var. <i>crispula</i> Ohashi, <i>Quercus variabilis</i> Blume
#107	Malaytea Scurfpea Fruit	<i>Psoralea corylifolia</i> Linné
#108	Moutan Bark	<i>Paeonia suffruticosa</i> Andrews
#109	Ephedra Herb	<i>Ephedra intermedia</i> Schrenk et C.A.May., <i>Ephedra sinica</i> Stapf, <i>Ephedra equisetina</i> Bunge
#110	Cannabis Fruit	<i>Cannabis sativa</i> L.
#111	Shrub Chaste Tree Fruit	<i>Vitex rotundifolia</i> Linné filius, <i>Vitex trifolia</i> Linné
#112	Akebia Stem	<i>Akebia quinata</i> Decne., <i>Akebia trifoliata</i> Koidz.
#113	Myrrha	<i>Commiphora molmol</i> Engler, <i>Balsamodendron ehrenbergianum</i> Otto Karl (Carl) Berg
#114	Bitter Cardamon	<i>Alpinia oxyphylla</i> Miquel
#115	Leonurus Herb	<i>Leonurus japonicus</i> Houttuyn, <i>Leonurus sibiricus</i> Linné
#116	Coix Seed	<i>Coix lacryma-jobi</i> L. var. <i>ma-yuen</i> Stapf
#117	Japanese Gentian	<i>Gentiana scabra</i> Bunge, <i>Gentiana manshurica</i> Kitagawa, <i>Gentiana triflora</i> Pallas
#118	Alpinia Officinarum Rhizome	<i>Alpinia officinarum</i> Hance
#119	Ganoderma	<i>Ganoderma lucidum</i> (Leysser ex Fries) Karsten, <i>Ganoderma sinense</i> Zhao, Xu et Zhang
#120	Forsythia Fruit	<i>Forsythia suspensa</i> Vahl, <i>Forsythia viridissima</i> Lindley



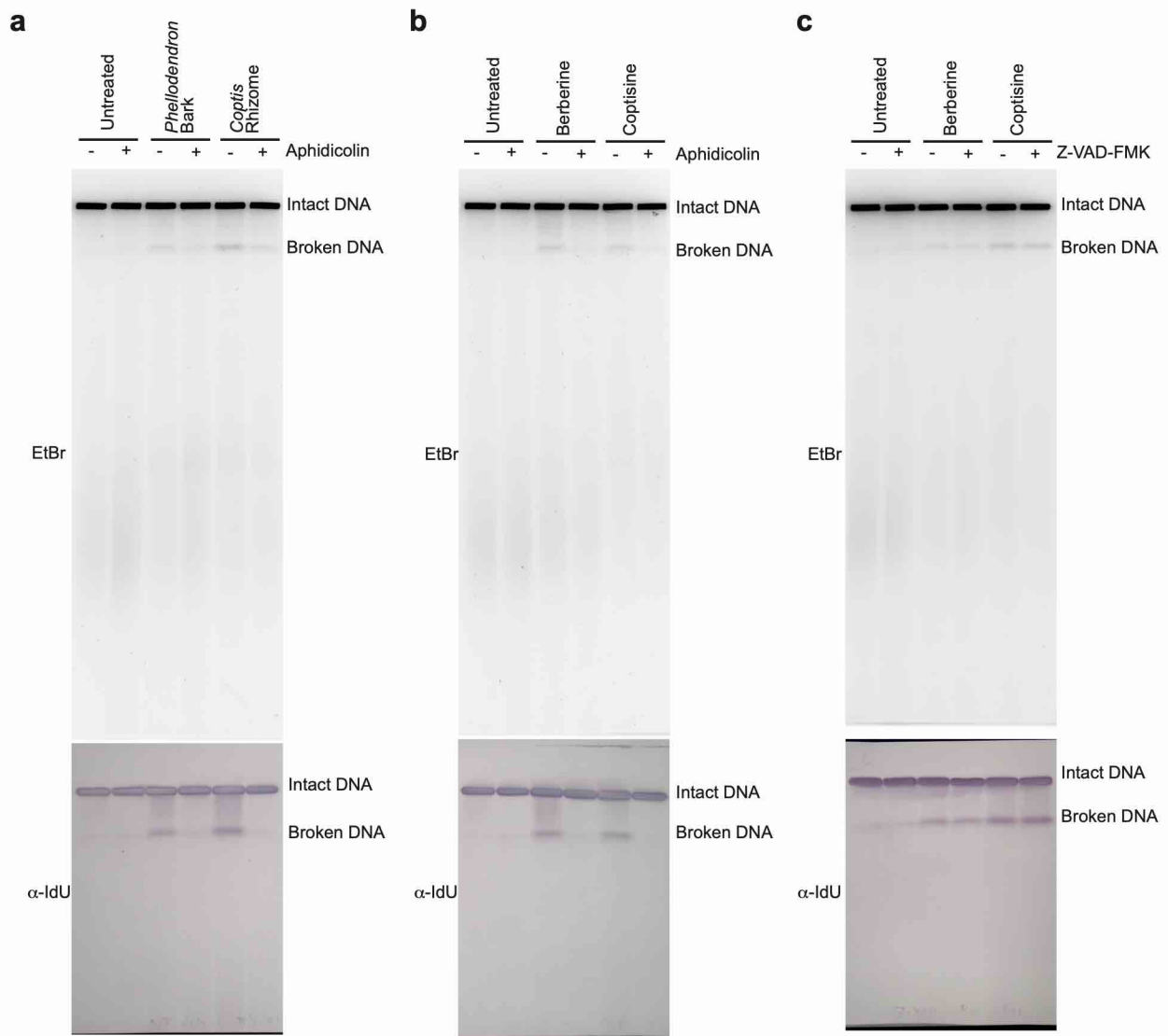
Supplementary Fig. 1. Screening of 120 herbal extracts by pulsed-field gel electrophoresis (PFGE). Cells were treated with each herbal extract at a concentration of 50 $\mu\text{g/mL}$ for 24 h, and the accumulation of DSBs was analysed by PFGE. DNAs were visualized by ethidium bromide (EtBr) staining. **(a)** Results of primary screening. The number corresponds to the herbal medicine described in Table S1. **(b)** Results of secondary screening. M: DNA size marker (*S. cerevisiae* chromosomes). C: Untreated control.



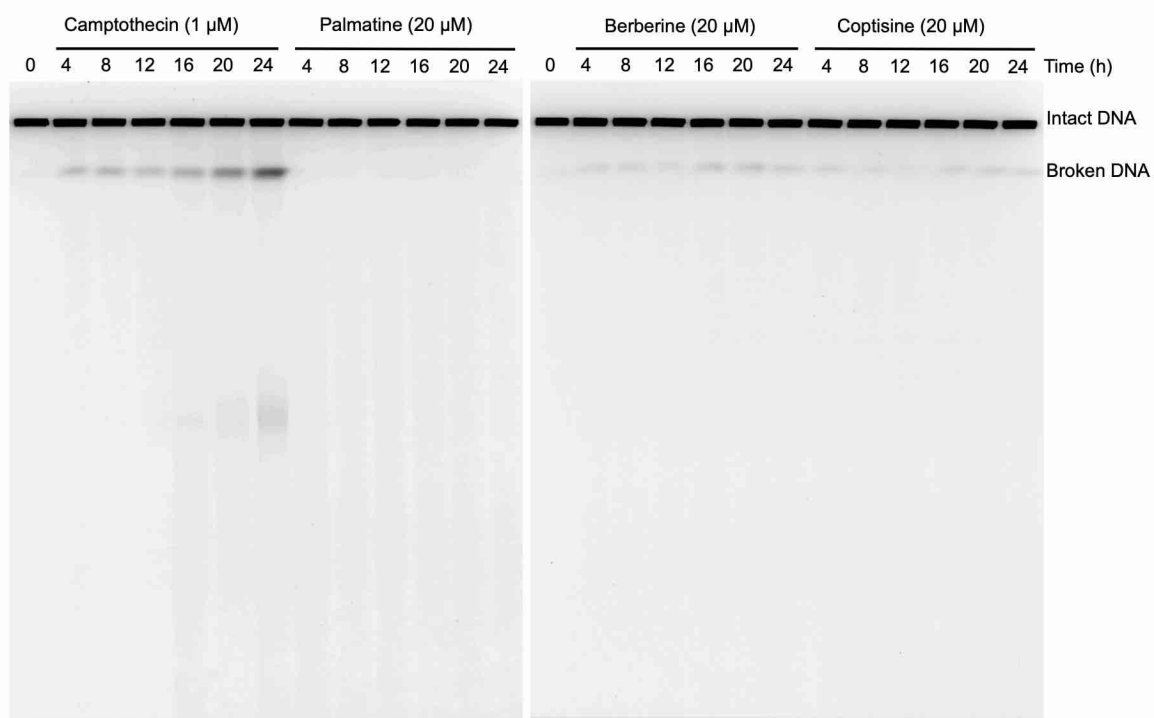
Supplementary Fig. 2. Complete gel images of the data shown in Figs. 1a and 1d. **(a)** Complete image of the EtBr-stained PFGE gel (upper) and the immunostained image (lower) presented in Fig. 1a. PFGE analysis of DSB accumulation after treatment with extracts of Phellodendron Bark and Coptis Rhizome. Total broken DNA was detected by ethidium bromide (EtBr) staining, whereas DSBs at DNA replication sites were detected by immunoblotting with anti-BrdU antibody. **(b)** Complete image of the EtBr-stained PFGE gel (upper) and the immunostained image (lower) presented in Fig. 1d. PFGE analysis of DSB accumulation after treatment with berberine, coptisine, palmatine, and magnoflorine.



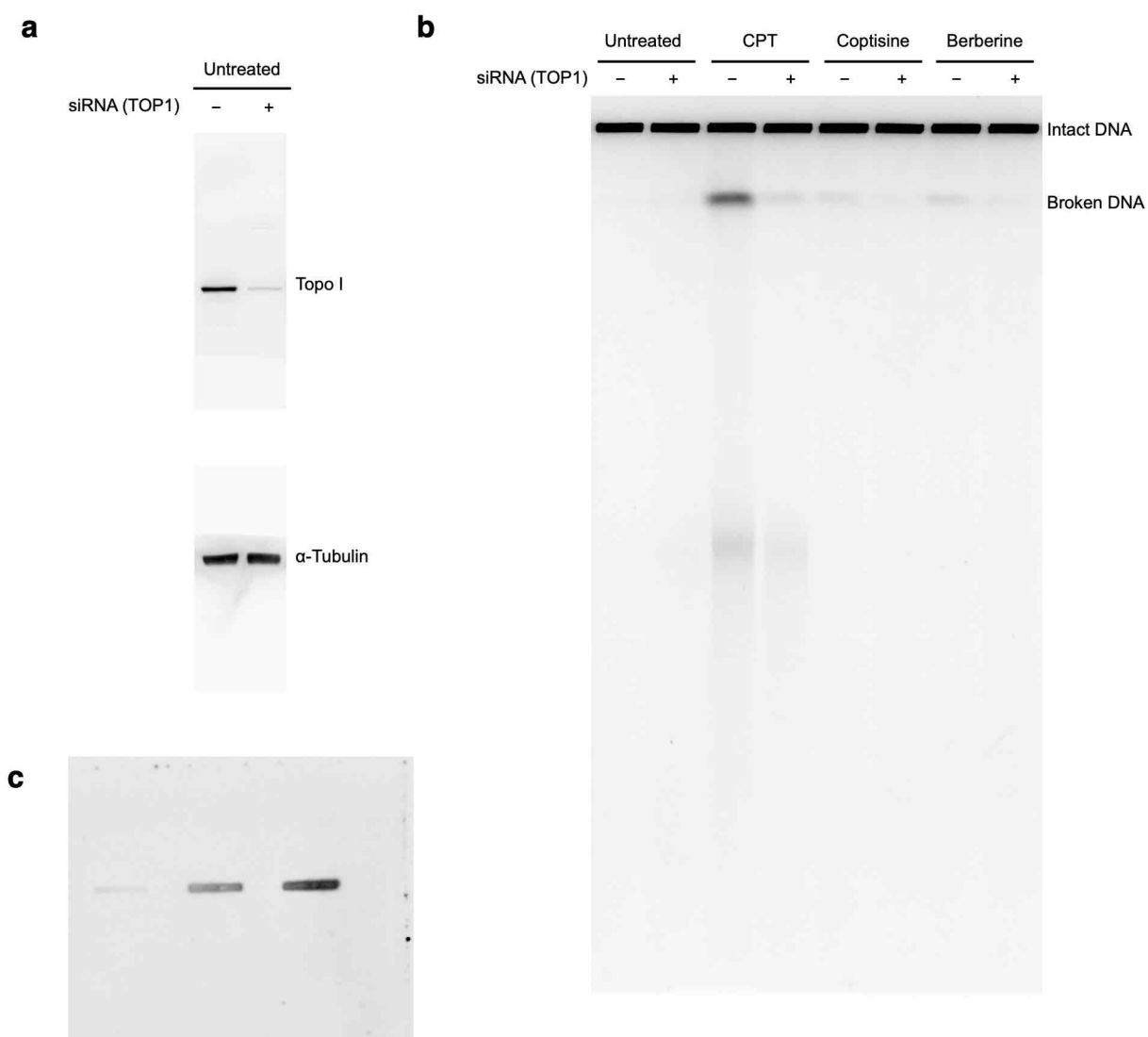
Supplementary Fig. 3. Suppression of DSBs after combined treatment with aphidicolin and topoisomerase poisoning. (a) PFGE analysis of DSB accumulation after treatment with aphidicolin combined with camptothecin (CPT). **(b)** PFGE analysis of DSB accumulation after treatment with aphidicolin combined with doxorubicin (DXR) and etoposide (VP-16).



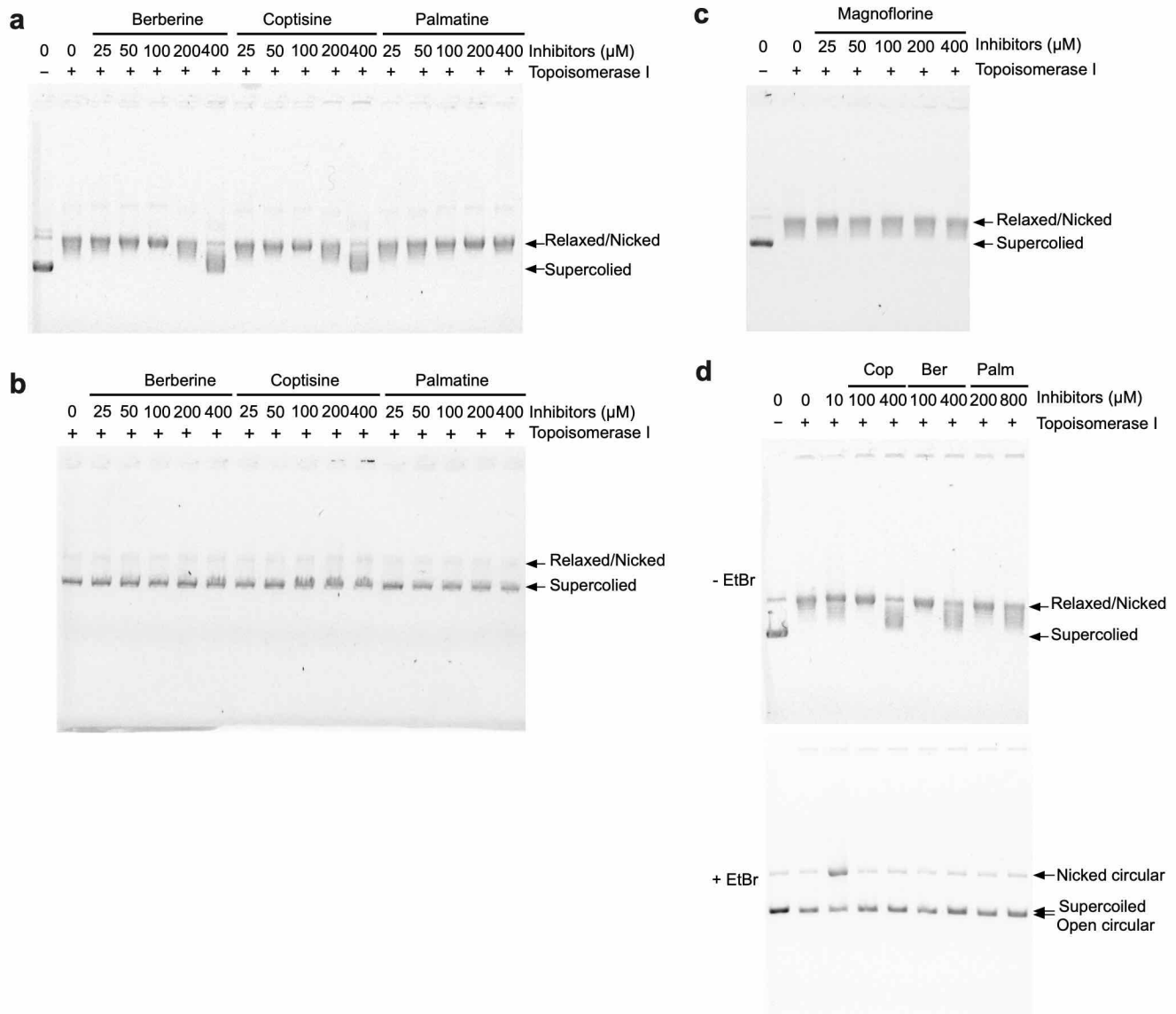
Supplementary Fig. 4. Complete gel images of the data shown in Figs. 2a-2c. **(a)** Complete image of the EtBr-stained PFGE gel (upper) and the immunostained image (lower) presented in Fig. 2a. PFGE analysis of DSB accumulation after treatment with aphidicolin combined with extracts of *Phellodendron Bark* and *Coptis Rhizome*. **(b)** Complete image of the EtBr-stained PFGE gel (upper) and the immunostained image (lower) presented in Fig. 2b. PFGE analysis of DSB accumulation after treatment with aphidicolin combined with extracts of berberine and coptisine. **(c)** Complete image of the EtBr-stained PFGE gel (upper) and the immunostained image (lower) presented in Fig. 2c. PFGE analysis of DSB accumulation after treatment with Z-VAD-FMK combined with extracts of berberine and coptisine.



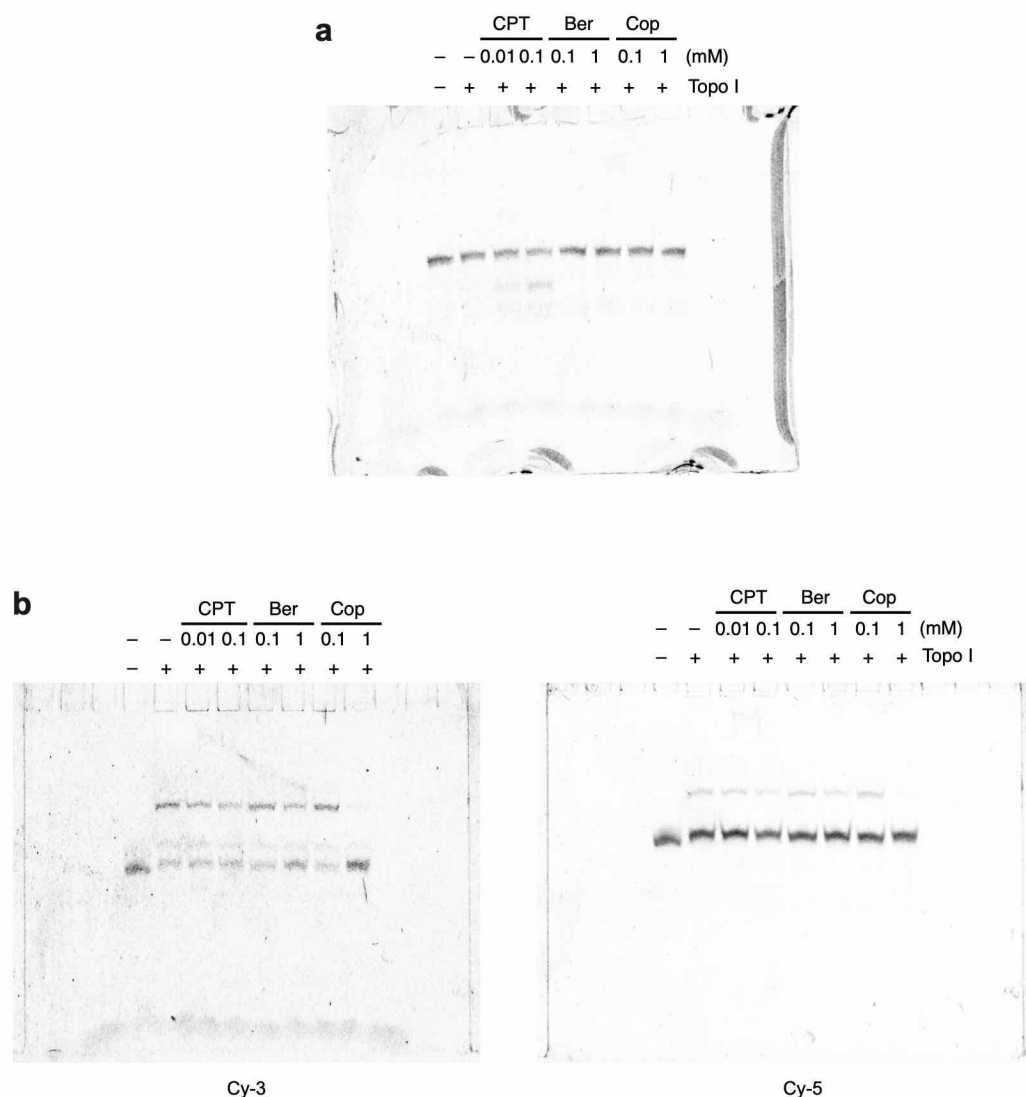
Supplementary Fig. 5. Complete gel images of the data shown in Fig. 2d. Complete image of the EtBr-stained PFGE gel. Time-course PFGE analysis of DSB accumulation after treatment with CPT, palmatine, berberine and coptisine.



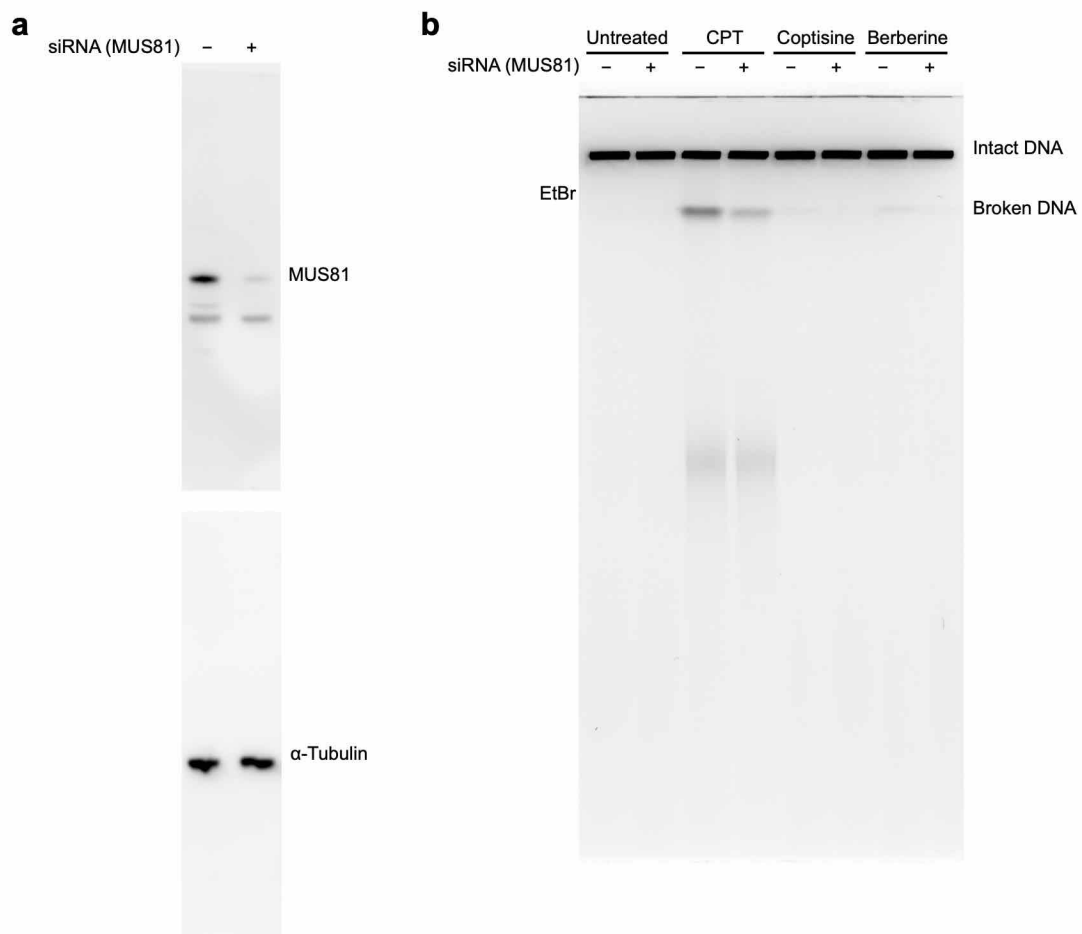
Supplementary Fig. 6. Complete gel images of the data shown in Figs. 3a,3b, and 3d. (a) Complete gel images of the Western blot analysis of Topo I and α -tubulin (Fig. 3a). α -Tubulin was used as a loading control. **(b)** Complete gel image of the PFGE analysis of DSB formation after treatment with CPT, coptisine and berberine in the absence of Topo I presented in Fig. 3b. **(c)** The original image of ICE assay blots.



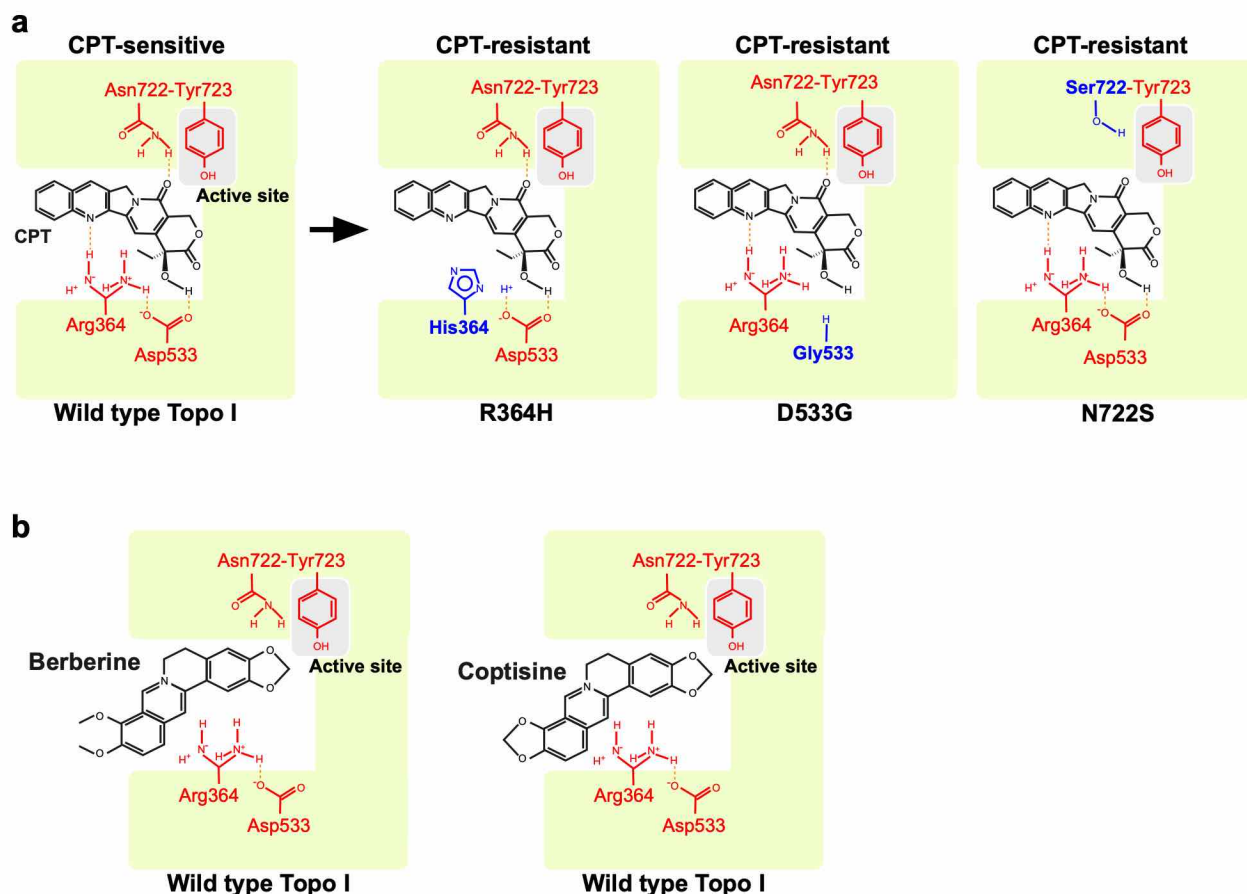
Supplementary Fig. 7. Complete gel images of the data shown in Figs. 4a-4d. (a, b) Complete gel images of the relaxation activity of Topo I presented in Figs. 4a and 4b. Inhibition of the relaxation activity of Topo I by berberine, coptisine, and palmatine. **(c)** Complete gel images of the relaxation activity of Topo I presented in Fig. 4c. Complete gel images of the relaxation activity of Topo I. Relaxation activity of Topo I after treatment with magnoflorine. **(d)** Complete gel images of the relaxation activity of Topo I presented in Fig. 4d. Detection of nicked DNA due to the relaxation activity of Topo I after treatment with CPT, coptisine (Cop), berberine (Ber), and palmatine (Palm).



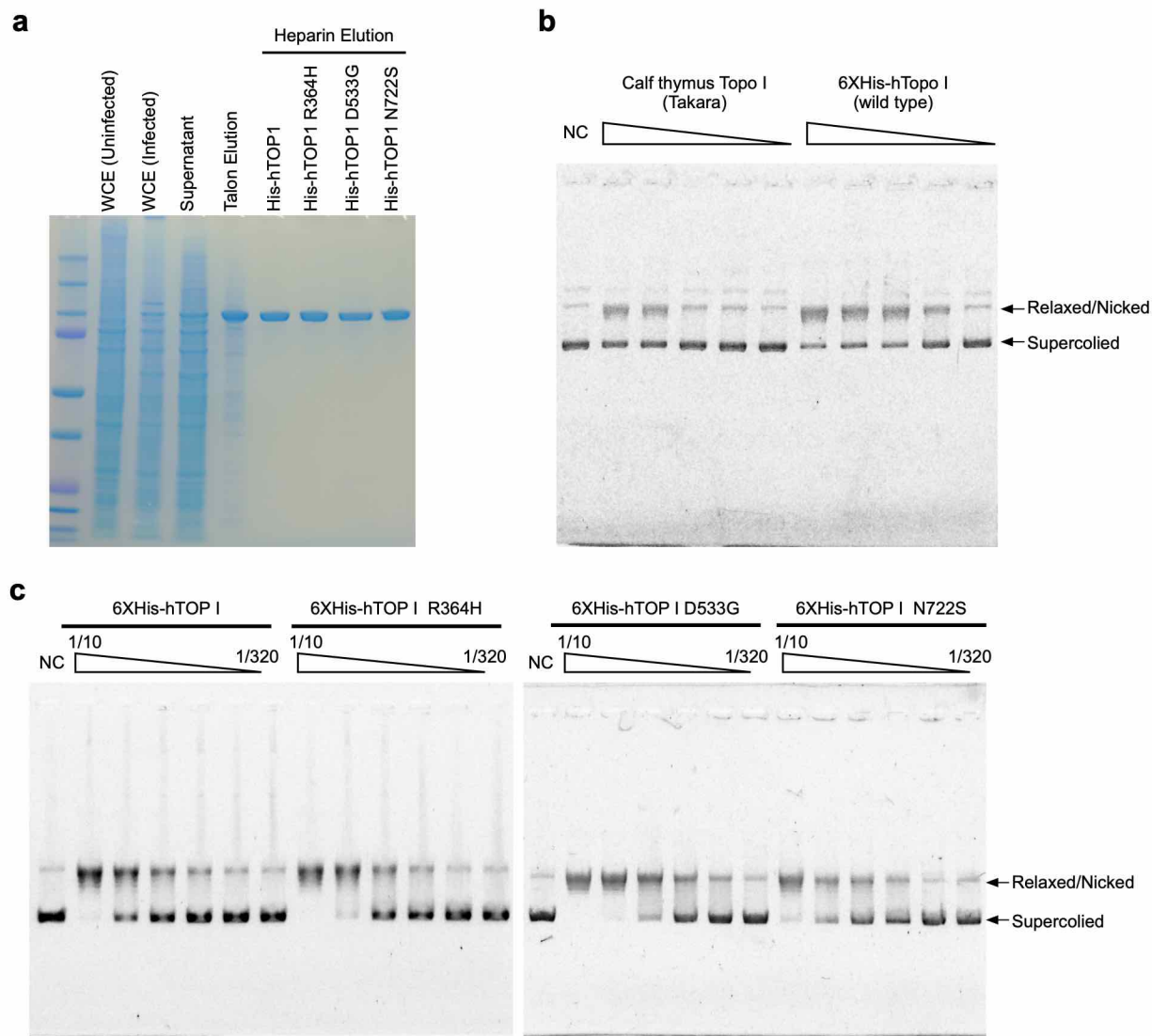
Supplementary Fig. 8. Complete gel images of the data shown in Figs. 5b and 5d. (a) Complete gel images of the nicking activity of Topo I presented in Figs. 5b. Inhibition of the relaxation activity of Topo I by berberine, coptisine, and palmatine. **(b)** Complete gel images of the rejoining activity of Topo I presented in Fig. 5d. Detection of nicking and rejoining activity of Topo I after treatment with CPT, coptisine (Cop), berberine (Ber), and palmatine (Palm). (e) Complete gel image of nicking assay



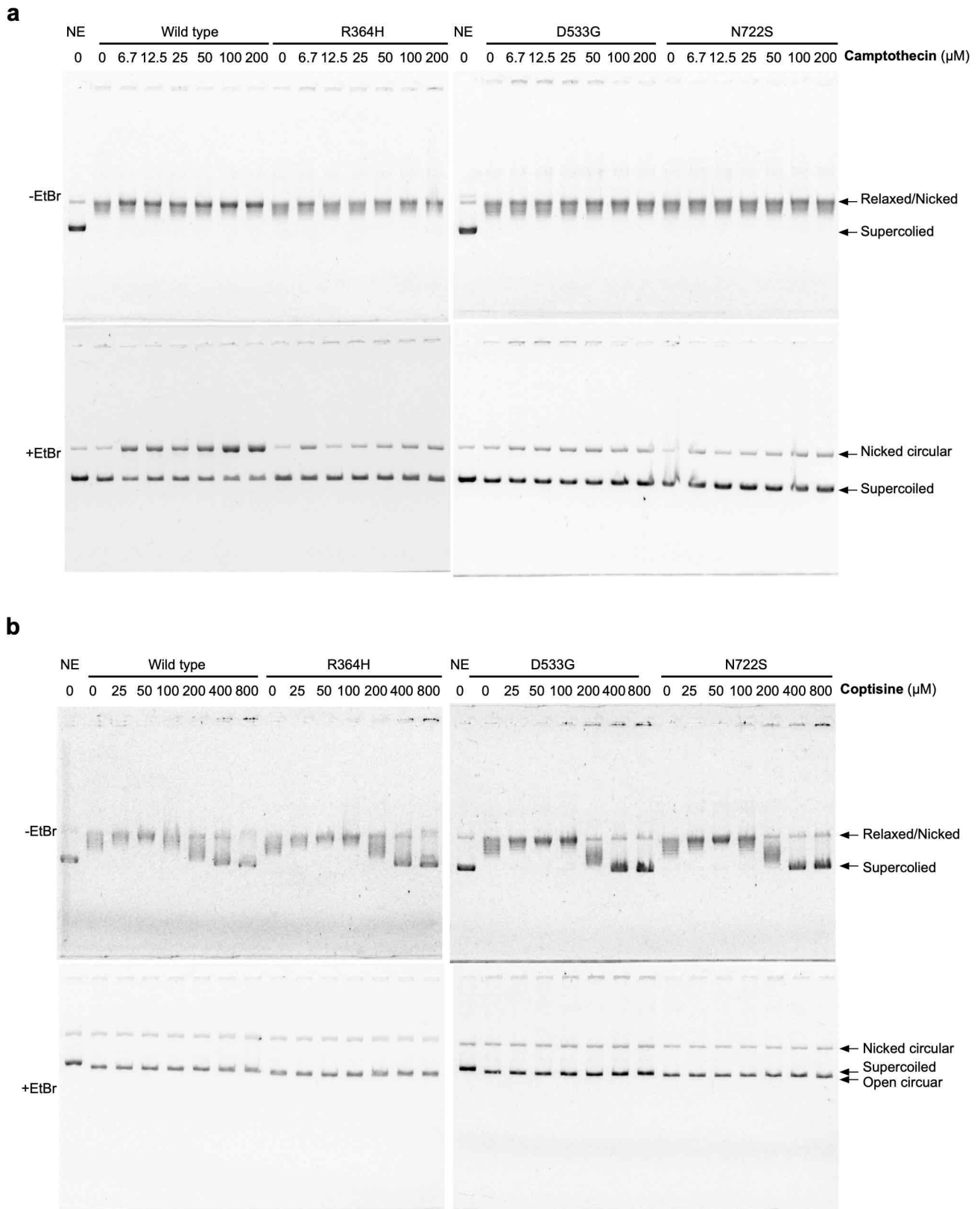
Supplementary Fig. 9. Complete gel images of the data shown in Figs. 6a and 6b. (a) Complete gel images of the Western blot analysis of mMUS81 and α -tubulin (Fig. 6a). α -Tubulin was used as a loading control. **(b)** Complete gel image of the PFGE analysis of DSB formation after treatment with CPT, coptisine and berberine in the absence of MUS81 presented in Fig. 3b.



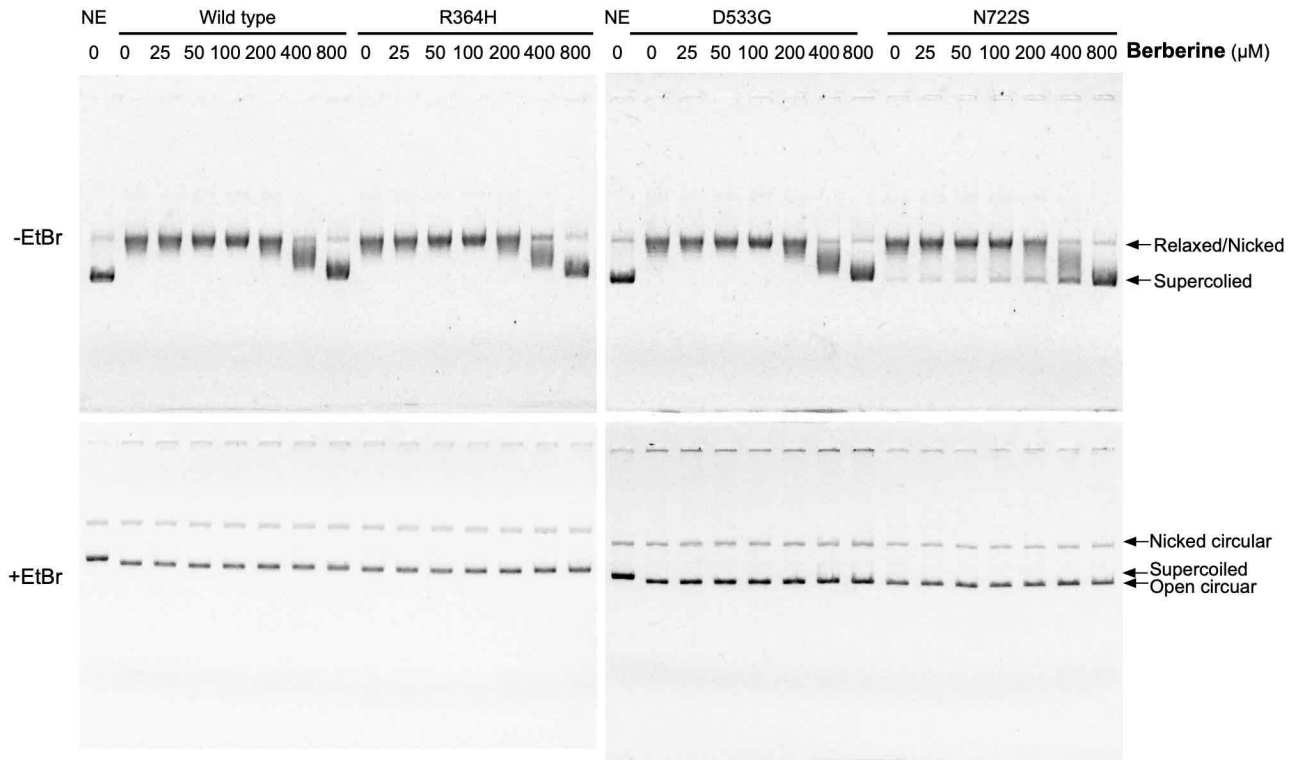
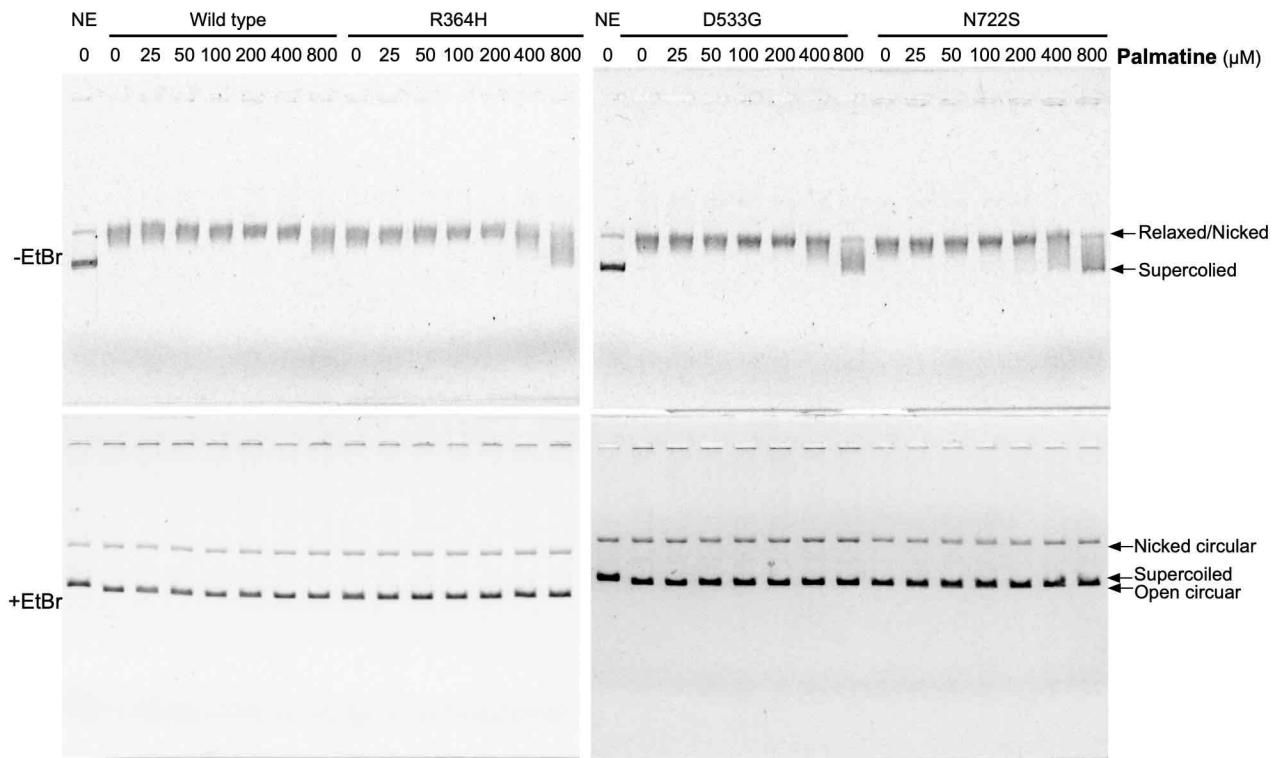
Supplementary Fig. 10. Hypothesized binding modes of Topo I with berberine and coptisine. (a) Interaction domains of CPT with the active site of Topo I. The 364th Arg, 533rd Asp and 722nd Asn residues of Topo I are responsible for the interaction with CPT. Defective interaction of CPT with the active site of CPT-resistant Topo I. (b) Conceptualization of the mechanism of action of berberine and coptisine that enables their activity against CPT-resistant Topo I. These BIAAs act against CPT-resistant mutants because they do not possess the functional groups responsible for interactions with Topo I.



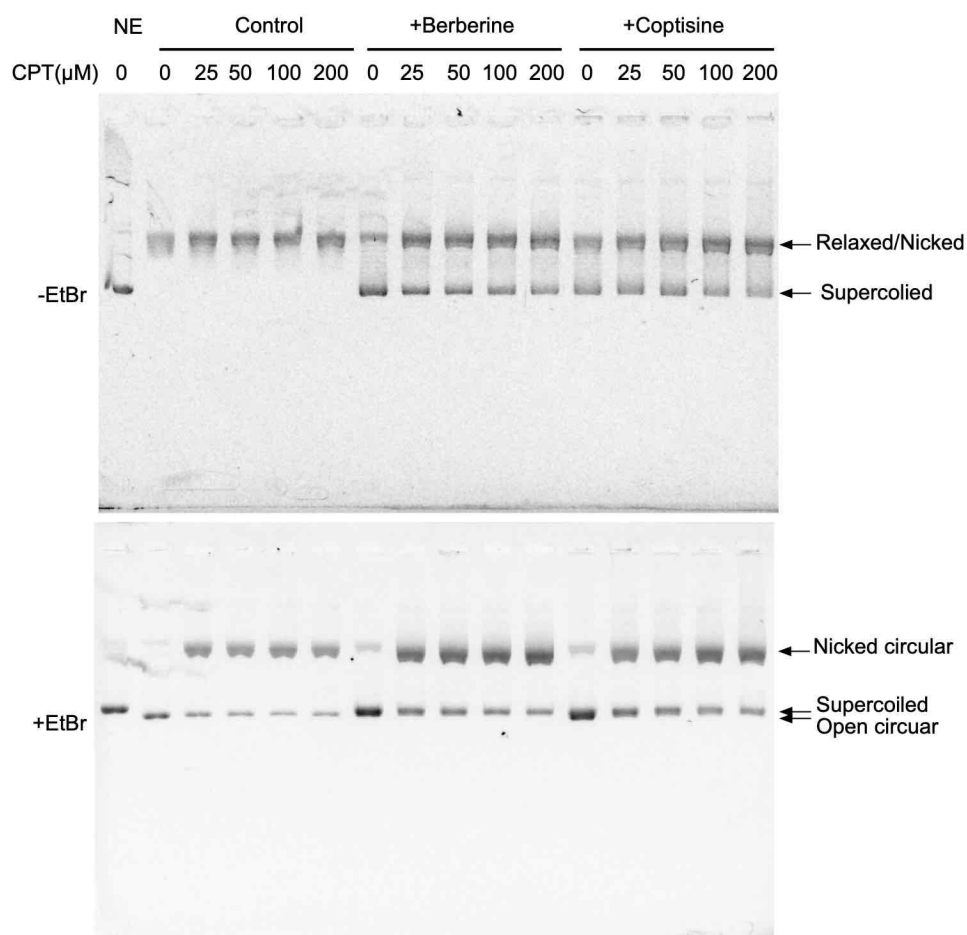
Supplementary Fig. 11. Purification of CPT-resistant Topo I. (a) SDS-PAGE of purified wild-type and CPT-resistant Topo I proteins, such as R364H, D533G or N722S mutants. (b) Comparison of the activities between calf thymus Topo I purchased from Takara and his-tagged Topo I purified in this study. (c) Relaxation activities of various Topo I-mutated proteins.



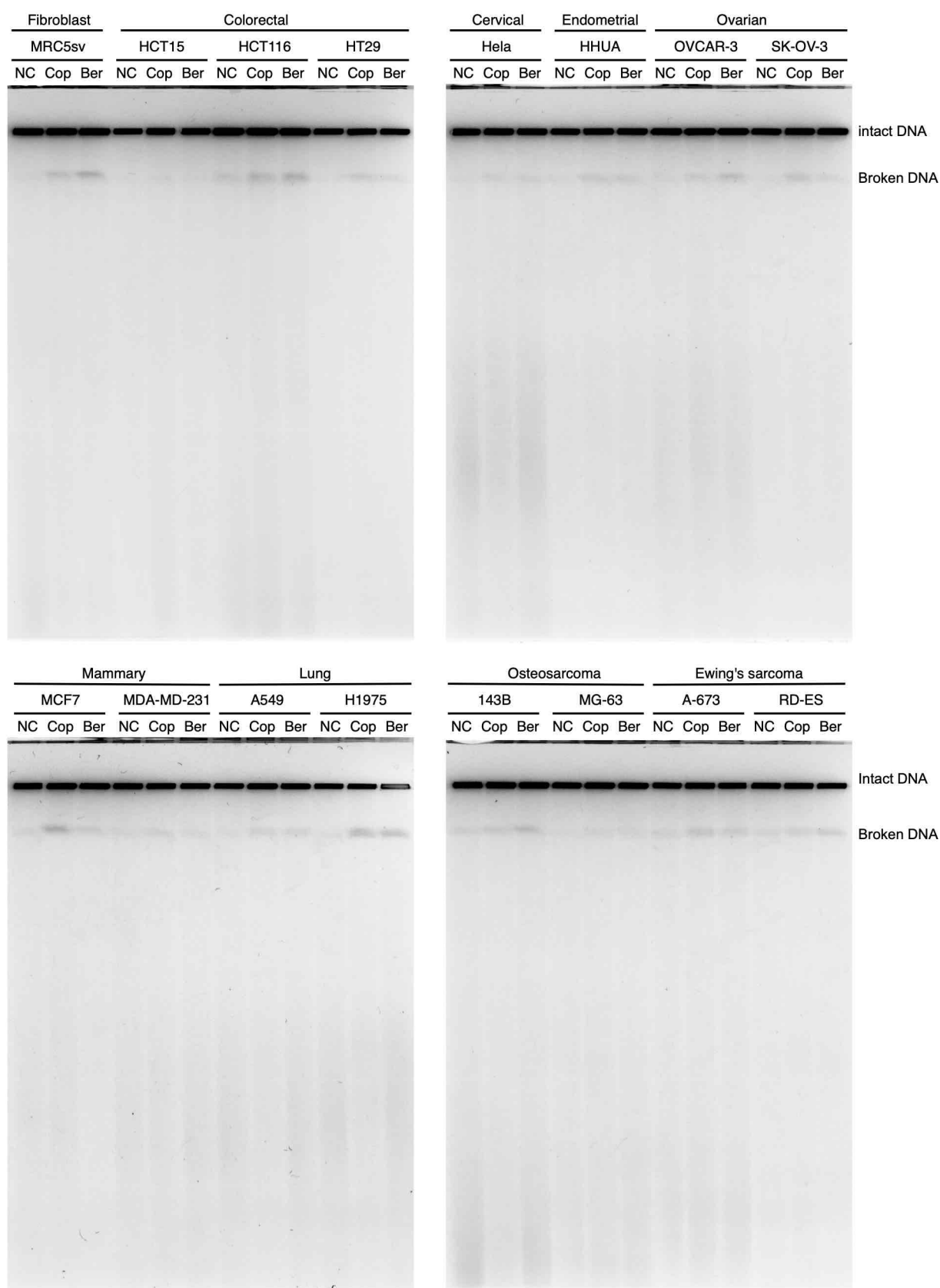
Supplementary Fig. 12. Complete gel images of the data shown in Figs. 7b and 7c. (a) Complete gel images of the relaxation activity of Topo I presented in Fig. 7b. Relaxation activity of CPT-resistant Topo I proteins in the presence of CPT in vitro. (b) Complete gel images of the relaxation activity of Topo I presented in Fig. 7c. Inhibitory effect of coptisine on CPT-resistant Topo I proteins in vitro.

c**d**

Supplementary Fig. 12 (Continued). Complete gel images of the data shown in Figs. 7d and 7e. (c, d) Complete gel images of the relaxation activity of Topo I presented in Figs. 7d and 7e. Inhibitory effect of berberine (c) and palmatine (d) on CPT-resistant Topo I proteins in vitro.



Supplementary Fig. 13. Complete gel images of the data shown in Fig. 9a. Complete gel images of the relaxation activity of Topo I presented in Fig. 9a. Relaxation activity of CPT-resistant Topo I proteins in the presence of CPT in vitro.



Supplementary Fig. 14. Complete gel images of the data shown Fig. 10. Complete image of the EtBr-stained PFGE gel. The cells were incubated with 10 μ M coptisine (Cop) and berberine (Ber) for 24 h, and the accumulation of DSBs was analysed by PFGE.