SUPPLEMENTARY INFORMATION

TOPOISOMERASE IIα MEDIATES TCF-DEPENDENT EPITHELIAL-MESENCHYMAL TRANSITION IN COLON CANCER

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S1
SI MATERIALS AND METHODS

**Antibodies:** Monoclonal mouse anti-TCF4 antibody was purchased from EMD Millipore (catalog# 05-511), a 1:1000 dilution was used for Western blot and 2 µg antibody per 300 µg of protein was used for IP. Polyclonal rabbit anti-c-Myc (catalog# sc-788) and goat anti-zeb1 (catalog# sc-10572) antibodies were purchased from Santa Cruz and a 1:500 dilution was used for Western blot. Monoclonal rabbit anti-Vimentin (catalog# 5741), anti-slug (catalog# 9585), anti-E-cadherin (catalog# 3195), anti-ZO-1 (catalog# 8193) and mouse anti-β-actin (catalog# 3700) were purchased from Cell Signaling and a 1:1000 dilution was used for Western blot. Monoclonal rabbit anti-β-catenin (catalog# 9582), polyclonal rabbit anti-TopoIIα (catalog# 4733) were purchased from Cell Signaling, a 1:1000 dilution was used for Western blot and 2 µg antibody per 300 µg of protein was used for IP. Monoclonal rabbit anti-TCF4 (catalog# 2569) and anti-Histone H3 (catalog# 4620) were purchased from Cell Signaling and 2 µg antibody per 1 mg of protein was used for ChIP. Anti-rabbit IgG HRP-linked secondary antibody (catalog#7074) was purchased from Cell Signaling and a 1:3000 dilution was used for Western blot. Anti-goat and anti-mouse IgG HRP-linked secondary antibodies (catalog# 805-035-180 and 115-035-003) were purchased from Jackson ImmunoResearch and a 1:10,000 dilution was used for Western blot. FITC conjugated polyclonal goat anti-rabbit IgG secondary antibody (catalog# PI31573) was purchased from ThermoScientific and a 1:1000 dilution was used in spheroid staining.

**Plasmid construction:** TOPflash-luc plasmids (Millipore, Billerica, MA, USA) were digested with *Pvu*II to create a blunt 5’ end and *Not*I to create sticky 3’ end. pCDH-CMV-MCS-EF1-puro was digested with *BspDI* upstream of the CMV promoter, blunt ended, then digested with *Not*I to remove the MCS. TOPflash-luc segment was ligated to the backbone to create pCDH-TOPflash-luc-EF1-puro plasmids. The pCDH-VimPro-Fluc-EF1-puro plasmid was previously reported (see reference 25 in the main article). The firefly luciferase ORF was replaced with GFP flanked by *BamHI* and *NotI* sites to generate the pCDH-VimPro-GFP-EF1-puro plasmid.

**shRNA Topollα knockdown:** Mission shRNA (scrambled) and TRCN00000492-78/79 (sh78 and sh79) specific for Topollα were purchased from Sigma-Aldrich. Virus was produced in HEK293T cells using TransIT®-293 reagent (Mirus, Madison, WI, USA), the plasmids delta 8.9, and pVSVG. CRC cells were transduced and selected with 4 µg/ml puromycin for 7 days.

**Detailed Chromatin Immunoprecipitation (ChIP):** Cells were treated with DMSO or 10 µM neo for 6 h followed by cross-linking with 1.42% formaldehyde for 15 min and quenching with 125 mM glycine for 5 min. Cells were lysed with Szak’s RIPA buffer and sonicated using a Brandson Sonifier. The IP steps were conducted at 4 °C as follows: 50 µl of protein A/G aragose beads were prewashed with cold Szak’s RIPA buffer and incubated with 1 mg of lysate for 2 h. 0.3 mg/ml of salmon sperm DNA was added and incubated for 2 h. 100 µl of the lysate was set aside as the input control. 2 µg of anti-Topollα or anti-TCF4 antibody was added to the remainder and incubated overnight. An
unconjugated normal rabbit IgG polyclonal antibody was used as a negative control and anti-Histone H3 rabbit monoclonal antibody formulated for ChIP was used as a positive control. Beads were washed 2 × with Szak’s RIPA buffer, 4 × with Szak’s IP wash buffer, 2 × with Szak’s RIPA buffer, and 2 × with 1X-Tris EDTA. The supernatant was aspirated down to 100 µl and 200 µl of 1.5X-Talianidis elution buffer was added to both the input and IP samples. To elute immunocomplexes and reverse crosslink, 12 µl of 5M NaCl was added and the mixture was incubated at 65 °C for 16 h. The supernatant was mixed with 20 µg of proteinase K and incubated for 30 min at 37 °C. DNA were extracted with phenol/chloroform and precipitated with ethanol. The IP product was amplified with the All-in-One™ qPCR Mix (GeneCopoeia™, Rockville, MD, USA) using known primers as follows:

- **c-Myc WRE:** sense: 5’-AAATCAAGGGCAGGGACCACAG-3’
  antisense: 5’-CAGAATGGCAGAGTGAAGACAT-3’
- **Axin2 WRE:** sense: 5’-CTGGAGCCGGCTGCGCTTTGATAA-3’
  antisense: 5’-CGGCCGAAATCCATCGC-TCTGA-3’
- **LEF1 WRE:** sense: 5’-TCGACCCGGGAACAAAGAGG-3’
  antisense: 5’-GCCGAGGAGGGGAAGAG-3’
- **Vim Promoter:** sense: 5’-CTGAAGTAACGGGACCATGC-3’
  antisense: 5’-CTCGAGCTACCTCCACAT-3’
- **N-cad Promoter:** sense: 5’-ACCAGGATCAAGGACGTG-3’
  antisense: 5’-CTCCACTTACCCTCCACAT-3’
- **GAPDH (Control):** sense: 5’-CGACCACCTT-GTCAAGCTCA-3’
  antisense: 5’-AGGGTCTACATGGCAACTG-3’

**In vitro metabolic stability and in vivo pharmacokinetic studies with of neo**

Neo (1 µM) was tested for metabolic stability in human and various animal liver microsomes and hepatocytes as well as for its ability to inhibit cytochrome P450 enzymes (Figures S6a-c), using previously reported methodologies.  

Neo’s pharmacokinetics were assessed using Sprague-Dawley rats after i.v. administration (1.4 mg/kg). Rat plasma samples (50 µL) were prepared via protein precipitation with acetonitrile (50 µL) and supernatants were analyzed via LC/MS/MS analysis. Positive ion electrospray ionization (ESI) mass spectra were obtained with a MDS Sciex 3200 Q-TRAP triple quadrupole mass spectrometer (Applied Biosystems, Inc., Foster City, CA, USA) with a turbo ionspray source interfaced to a Shimadzu LC-20AD HPLC system (Shimadzu Corporation, Kyoto, Japan). Samples were quantified in the MRM mode monitoring ion transitions m/z 314.0 → 229.2, 285.2, and 296.2 for neo with unknown samples quantified from linear standard curves (2.5–1,000 ng/mL). Pharmacokinetic parameters were calculated from plasma concentration data over time with standard noncompartmental methods using Phoenix® WinNonlin® software, version 1.3 (Pharsight Corp., Sunnyvale, CA, USA) (see Figure S6d and e).
Chemical reagents and general procedures for the synthesis of BAP-1 and neo

All reagents were purchased from commercial sources and used as received, unless otherwise indicated. All solvents were dried and distilled using standard protocols. All reactions were carried out under a nitrogen atmosphere unless otherwise noted. All organic extracts were dried over sodium sulfate. Thin layer chromatography (TLC) was performed using aluminum-backed plates coated with 60Å Silica gel F254 (Sorbent Technologies). Plates were visualized using a UV lamp ($\lambda_{max} = 254\text{ nm}$) and/or by staining with phosphomolybdic acid solution (20 wt% in ethanol). Column chromatography was carried out using 230-400 mesh 60Å silica Gel (Silycyle). Proton ($\delta_H$) and carbon ($\delta_C$) nuclear magnetic resonances were recorded on a Varian INOVA 500 MHz spectrometer (500 MHz proton, 125.7 MHz carbon). High-resolution mass spectra (HRMS) were recorded on a Bruker Q-TOF-2 Micromass spectrometer equipped with lock spray, using ESI with methanol as the carrier solvent. Accurate mass measurements were performed using leucine enkephalin as a lock mass and the data were processed using MassLynx 4.1. Exact m/z values are reported in Daltons. Infrared (IR) spectra were recorded on a Bruker ALPHA FT-IR fitted with a Platium ATR diamond sampler (oils and solids were examined neat). Absorption maxima ($\nu_{max}$) are recorded in wavenumbers (cm$^{-1}$).

The synthesis of neo was done exactly as previously described (See reference 30 in the main article).

The Synthesis of BAP-1

BAP-1 was previously published (see reference 31 and 32 in the main article), however, the synthetic details were not available. Thus, we have synthesized BAP-1 as follows:

\[ \text{N}^6-\text{6-benzothiazolyl-}N^\beta\text{-tert-butyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-2,6-diamine (2).} \]

To a stirred solution of N-(tert-butyl)-2-chloro-8-ethyl-9H-purin-6-amine (1) (182.0 mg, 0.588 mmol) (see reference 31 in the main article), 6-aminobenzothazole (97.2 mg, 0.647 mmol), and Cs$_2$CO$_3$ (957.9 mg, 2.94 mmol) in 1,4-dioxane (6 mL) were added Pd(OAc)$_2$ (13.2 mg, 58.8 mol) and 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP, 73.2 mg, 118 $\mu$mol). The reaction mixture was heated to reflux for 2 h. After cooling to room temperature, reaction mixture was filtered through celite and concentrated, followed by purification on silica gel (80% ethyl acetate in hexane) provided 2 (228.2 mg, 0.540 mmol, 92%) as light yellow oil; IR (neat) $\nu_{max}$ 3282, 2960, 2858, 1600, 1472, 1379, 1210 cm$^{-1}$; TLC (ethyl acetate) $R_f = 0.50$; $^1$H NMR (500 MHz, CDCl$_3$) d 8.83 (s, 1H), 8.73 (d, J = 1.5 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.74 (s, 1H), 7.48-7.46 (dd, J =
2.0, 9.0 Hz, 1H), 7.25 (s, 1H), 5.65 (s, 1H), 5.58-5.55 (dd, J = 2.0, 10.0 Hz, 1H), 4.18-4.15 (m, 1H), 3.76 (d, J = 12.0 Hz, 1H), 2.15-2.04 (m, 3H), 1.77-1.74 (m, 2H), 1.67-1.63 (m, 1H), 1.55 (s, 9H); $^{13}$C NMR (125.7 MHz, CDCl$_3$) δ 156.0, 154.8, 151.4, 149.3, 148.3, 138.8, 135.3, 134.9, 123.2, 119.0, 115.9, 110.6, 81.9, 68.7, 52.1, 31.4, 29.3, 25.1, 23.1; ESI-HRMS calcd. for C$_{21}$H$_{26}$N$_7$OS [M + H]$^+$ 424.1914, found 424.1908.

$N^2$-6-benzothiazolyl-$N^6$-tert-butyl-9H-purine-2,6-diamine (BAP-1). A solution of 2 (115.2 mg, 0.272 mmol) in TFA/MeOH (1 ml TFA, 3 ml MeOH) was stirred at room temperature for 2 h and concentrated. The residue was diluted with ethyl acetate and washed with 2N NaOH. The organic extracts were dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. Chromatographic purification on silica gel (80% acetone in hexane) provided BAP-1 (74.2 mg, 0.219 mmol, 80%) as yellow oil. IR (neat) $\nu_{\text{max}}$ 3307, 2961, 2928, 2859, 1601, 1520, 1387, 1200 cm$^{-1}$; TLC (ethyl acetate) R$_f$ = 0.15; $^1$H NMR (500 MHz, acetone-$d_6$) δ 8.97 (s, 1H), 8.91 (d, J = 2.0 Hz, 1H), 8.66 (s, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.81 (s, 1H), 7.73-7.70 (dd, J = 2.0, 9.0 Hz, 1H), 6.04 (s, 1H), 1.50 (s, 9H); $^{13}$C NMR (125.7 MHz, acetone-$d_6$) δ 157.0, 155.4, 152.3, 151.9, 148.7, 140.7, 136.8, 135.5, 123.5, 119.4, 110.7, 110.3, 52.2, 29.3; ESI-HRMS calcd. for C$_{16}$H$_{18}$N$_7$S [M + H]$^+$ 340.1339, found 340.1333.
N²-6-benzothiazolyl-N⁶-tert-butyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-2,6-diamine (2)

¹H NMR
$N^2$-6-benzothiazolyl-$N^6$-tert-butyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-2,6-diamine (2)

$^{13}$C NMR

$\text{13C NMR, } 125 \text{ MHz, CDCl}_3$

$\delta_{\text{ppm}}$

- 23.08
- 25.12
- 29.28
- 31.43

- 52.08

- 68.72
- 76.91 cdcd
- 77.16 cdcd
- 77.41 cdcd
- 81.93

- 110.55
- 115.95
- 119.01
- 122.37

- 134.77
- 135.32
- 138.85
- 148.22
- 149.29
- 154.43
- 154.76
- 155.99
$N^2$-6-benzothiazolyl-$N^6$-tert-butyl-9H-purin-2,6-diamine (BAP-1)

$^1$H NMR

1H NMR, 500 MHz, Acetone-d6

8.98
8.49
8.44
8.39
8.34
8.08
7.94
7.87
7.82
7.77
7.72
7.39
6.98
5.06
4.24
3.61
3.22
2.01
1.20
0.81
0.54
0.43
0.34
0.25
$N^2$-6-benzothiazolyl-$N^6$-tert-butyl-9H-purin-2,6-diamine (BAP-1)

$^{13}$C NMR
HIGH RESOLUTION MASS SPECTROMETRY (HRMS) ANALYSIS OF COMPOUND 2 AND BAP-1

**Compound 2**

- Calculated Mass: 424.1914
- Observed Mass: 424.1908
- Error: 0.14 ppm

**BAP-1**

- Calculated Mass: 340.1339
- Observed Mass: 340.1333
- Error: 0.17 ppm
Supplementary Figure S1. (a) the chemical structure of BAP-1. (b) 3D Western blot analysis using SW620 MCTS treated with 20 μM BAP-1 or vehicle control (DMSO) for 72 h. BAP-1 treatment induced the downregulation of key mesenchymal genes, including vimentin, Slug, ZEB1, and c-Myc. β-Actin was used as a loading control.
Supplementary Figure S2. KinaseSeeker™ Screen. Neo was screened against 156 kinases implicated in cancer. The results are tabulated as a heat map and indicate that neo is not a potent protein kinase inhibitor. None of the 156 kinases tested showed greater than 30% inhibition when treated with 10 µM neo.
Supplementary Figure S3. Human cell line STR profiling. Fifteen short tandem repeat (STR) loci plus the gender determining locus, Amelogenin, were amplified using the AmpFLSTR® Identifiler® PCR Amplification Kit (Applied Biosystems). Samples were processed using the ABI Prism® 3100-Avant Genetic Analyzer. Data were analyzed using GeneMapper® v3.5 Software (Applied Biosystems).

| Sample Name | Marker | Allele 1 | Allele 2 | Additional Allele | Additional Allele |
|-------------|--------|----------|----------|-------------------|-------------------|
| DLD1        | Amel   | X        | Y        |                   |                   |
|             | CSF1PO | 11       | 12       |                   |                   |
|             | D13S317| 8        | 11       |                   |                   |
|             | D16S539| 12       | 13       |                   |                   |
|             | D18S51 | 11       | 17       |                   |                   |
|             | D19S433| 14       | 16       |                   |                   |
|             | D21S11 | 29       | 32.2     |                   |                   |
|             | D2S1338| 17       | 25       |                   |                   |
|             | D3S1358| 17       | 17       |                   |                   |
|             | D5S818 | 13       | 13       |                   |                   |
|             | D7S820 | 10       | 12       |                   |                   |
|             | D6S1179| 15       | 15       |                   |                   |
|             | FGA    | 22       | 22       |                   |                   |
|             | THO1   | 7        | 9.3      |                   |                   |
|             | TPOX   | 8        | 11       |                   |                   |
|             | vWA    | 18       | 19       |                   |                   |
| HCT116      | Amel   | X        | Y        |                   |                   |
|             | CSF1PO | 7        | 10       |                   |                   |
|             | D13S317| 10       | 12       |                   |                   |
|             | D16S539| 11       | 13       |                   |                   |
|             | D18S51 | 16       | 17       |                   |                   |
|             | D19S433| 12       | ?        |                   |                   |
|             | D21S11 | 29       | 30       |                   |                   |
|             | D2S1338| 16       | 16       |                   |                   |
|             | D3S1358| 12       | 18       |                   |                   |
|             | D5S818 | 10       | 11       |                   |                   |
|             | D7S820 | 11       | 12       |                   |                   |
|             | D6S1179| 10       | 11       | 12                | 14                |
|             | FGA    | 18       | 23       |                   |                   |
|             | THO1   | 8        | 9        |                   |                   |
|             | TPOX   | 8        | 9        |                   |                   |
|             | vWA    | 17       | 22       | 23                |                   |
| SW480       | Amel   | R        | X        |                   |                   |
|             | CSF1PO | B        | 13       | 14                |                   |
|             | D13S317| G        | 12       |                   |                   |
|             | D16S539| G        | 13       |                   |                   |
|             | D18S51 | Y        | 13       |                   |                   |
|             | D19S433| Y        | 13       |                   |                   |
|             | D21S11 | B        | 30       | 30.2              |                   |
|             | D2S1338| G        | 17       | 24                |                   |
|             | D3S1358| G        | 15       |                   |                   |
|             | D5S818 | R        | 13       |                   |                   |
|             | D7S820 | B        | 8        |                   |                   |
|             | D6S1179| B        | 13       |                   |                   |
|             | FGA    | R        | 24       |                   |                   |
|             | THO1   | G        | 8        |                   |                   |
|             | TPOX   | Y        | 11       |                   |                   |
|             | vWA    | Y        | 16       |                   |                   |
| SW620       | Amel   | R        | X        |                   |                   |
|             | CSF1PO | B        | 13       | 14                |                   |
|             | D13S317| G        | 12       |                   |                   |
|             | D16S539| G        | 9        | 13                |                   |
|             | D18S51 | Y        | 13       |                   |                   |
|             | D19S433| Y        | 13       |                   |                   |
|             | D21S11 | B        | 30       | 30.2              |                   |
|             | D2S1338| G        | 17       | 24                |                   |
|             | D3S1358| G        | 16       |                   |                   |
|             | D5S818 | R        | 13       |                   |                   |
|             | D7S820 | B        | 8        | 9                 |                   |
|             | D6S1179| B        | 13       |                   |                   |
|             | FGA    | R        | 24       |                   |                   |
|             | THO1   | G        | 8        |                   |                   |
|             | TPOX   | Y        | 11       |                   |                   |
|             | vWA    | Y        | 16       |                   |                   |
Supplementary Figure S4. The SRB cytotoxicity results of 32 different colorectal cancer cell lines after 72 h treatment with neo, tabulated with mutation status (MUT). Neo displays nM IC\(_{50}\) values for all cell lines tested. In particular, SW48, SKCO1, and SW620 cell lines (highlighted in red) were the most sensitive to neo.

| Cell Line | Neo IC\(_{50}\) (µM) | KRAS | NRAS | BRAF | PIK3CA | APC | β-Catenin | p53 |
|-----------|-----------------|------|------|------|--------|-----|-----------|-----|
| SW48      | 0.007           |      |      |      |        |     |           |     |
| SKCO1     | 0.013           | MUT  |      |      |        |     |           | MUT |
| SW620     | 0.059           | MUT  | MUT  |      |        |     |           | MUT |
| LS180     | 0.093           | MUT  | MUT  |      |        |     |           | MUT |
| RKO       | 0.118           |      | MUT  |      |        |     |           |     |
| CL34      | 0.195           |      | MUT  |      |        |     |           |     |
| MIP101    | 0.199           | MUT  |      |      |        |     |           |     |
| HT15      | 0.204           | MUT  |      |      |        |     |           |     |
| SW1463    | 0.204           | MUT  |      |      |        |     |           | MUT |
| HCT116    | 0.229           | MUT  |      |      |        |     |           | MUT |
| HT55      | 0.257           |      |      |      |        |     |           | MUT |
| GP2D      | 0.282           | MUT  |      |      |        |     |           |     |
| LS123     | 0.301           | MUT  |      |      |        |     |           | MUT |
| HCT8      | 0.302           | MUT  |      |      |        |     |           |     |
| DLD1      | 0.307           | MUT  |      |      |        |     |           |     |
| LS513     | 0.324           | MUT  |      |      |        |     |           | MUT |
| HT29      | 0.327           | MUT  | MUT  | MUT  |        |     |           | MUT |
| LS174T    | 0.339           | MUT  | MUT  |      |        |     |           | MUT |
| GEO       | 0.350           | MUT  |      |      |        |     |           |     |
| Colo320   | 0.360           | MUT  |      |      |        |     |           | MUT |
| LOVO      | 0.365           | MUT  |      |      |        |     |           | MUT |
| SW480     | 0.383           | MUT  |      |      |        |     |           |     |
| GP5D      | 0.388           | MUT  |      |      |        |     |           |     |
| COLO678   | 0.428           | MUT  |      |      |        |     |           |     |
| WiDr      | 0.435           |      |      |      |        |     |           | MUT |
| SNU1684   | 0.478           | MUT  |      |      |        |     |           |     |
| HCA24     | 0.549           |      |      |      |        |     |           |     |
| COLO201   | 0.643           | MUT  |      |      |        |     |           | MUT |
| NCIH747   | 0.684           | MUT  |      |      |        |     |           |     |
| KM20      | 0.687           |      |      |      |        |     |           |     |
| LS1034    | 0.695           |      | MUT  |      |        |     |           | MUT |
| MDST8     | 0.733           |      |      |      |        |     |           | MUT |
| HCA7      | 0.746           |      |      |      |        |     |           | MUT |
Supplementary Figure S5. Co-immunoprecipitation Western blot analysis using DLD1 and HCT116 cells treated with 10 µM neo for 6 h or vehicle control (DMSO). Topollα immuno-precipitated with β-catenin and TCF4 in the nuclear lysate. Neo does not prevent any protein-protein interactions observed.
Supplementary Figure S6. (a) Neo was exposed to liver microsomes indicating that it is only stable in human liver microsomes. (b) Neo displayed stability in rat and human hepatocytes. (c) Neo inhibited CYP1A2 but was not a potent pan cytochrome P450 inhibitor. (d) and (e) Pharmacokinetics of Neo in rats following 1.4 mg/kg i.v. administration. Plasma levels were measured by LC/MS/MS and PK parameters were calculated using sparse sampling by non-compartmental modeling (Phoenix WinNonlin (v6.3)). Three samples were collected at each time point from six rats.
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