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Supplementary Information for

Klebsiella oxytoca enterotoxins tilimycin and tilivalline have distinct host DNA damaging and microtubule stabilizing activities

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Table S1. Detection of TM and TV in human colonic fluid and stool samples

patient	diagnosis	*K. oxytoca*	cytotoxicity[a]	*npsA*/*B*[b]	sample	TM	TV
A	AAHC	+	+	+	colonic fluid[c]	+	+
					stool acute	+	+
					stool day 3[d]	~	+
					stool day 5[d]	-	-
B	AAHC	+	+	+	colonic fluid[c,e]	-	-
					stool acute	+	+
					stool day 25[d]	-	+
C	AAHC	+	+	+	colonic fluid[c]	+	+
					stool day 41[d]	-	-

[a] Determined with MTT-assay;
[b] Gene presence verified with PCR;
[c] Obtained by colonoscopy at diagnosis;
[d] Follow up stool samples taken after diagnosis;
[e] Patient received colonic lavage prior to endoscopy;
+/- Above/below limit of detection TM <0.5 nmol g^{-1}, TV <0.5 pmol g^{-1};
~ Traces of TM were detectable;

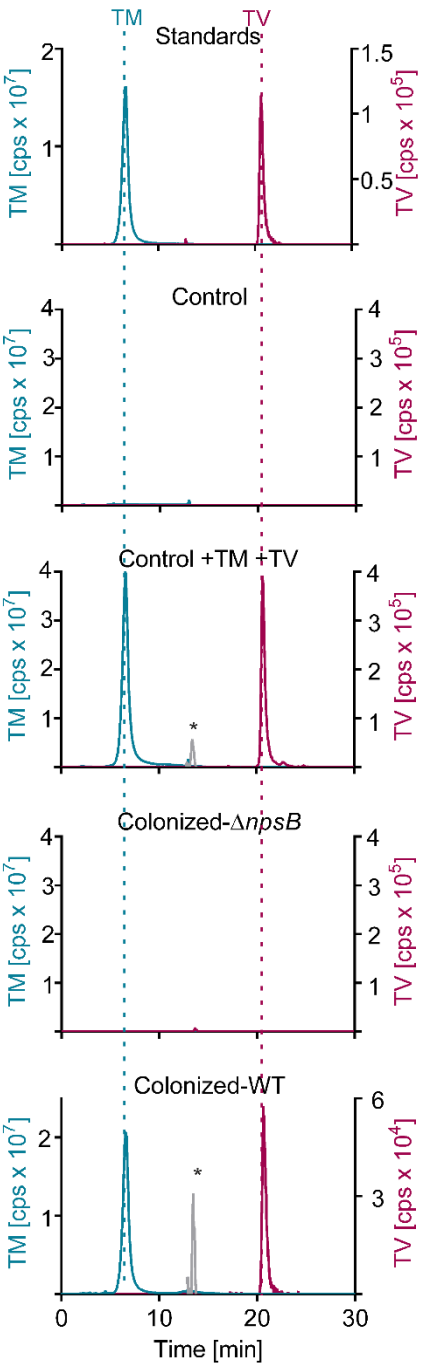

Fig. S1. TM and TV are present in feces of mice during colitis. HPLC-ESMS chromatograms detect TM (m/z 235.1004 ± 1 ppm) and TV (m/z 334.1477 ± 1 ppm) in feces of mice colonized with *K. oxytoca*, but not in medication control animals and mice colonized with the toxin negative Δ*npsB*-mutant. 10 µM TM and 10 nM TV in *n*-butanol were used as standards. (*) Peaks at retention time 13 min (m/z 334.1477, ± 1 ppm) are interferences caused by sample matrix and the applied gradient.

Table S2. Sensitivity of tumor cell lines and non-transformed cells to enterotoxins

		Tilimycin [μM]	Tilivalline [μM]
Cell line	Tissue	IC_{50}[a] ± SD	IC_{50}[a] ± SD
HeLa	Cervix	3.21 ± 0.04	6.57 ± 0.88
HT-29	Colon	1.67 ± 0.03	3.44 ± 0.79
SW48	Colon	1.42 ± 0.12	3.70 ± 0.32
T84	Colon	1.46 ± 0.15	21.65 ± 1.44
A549	Lung	1.88 ± 0.38	7.59 ± 0.88
1A9	Ovary	1.74 ± 0.07	3.63 ± 0.20
LNCaP	Prostate	0.85 ± 0.21	32.81 ± 0.98
MCF7	Breast	0.80 ± 0.06	50.25 ± 14.59
HUVEC	Vein	1.17 ± 0.16	7.21 ± 1.11
Hap1	Leukemia	1.34 ± 0.13	4.60 ± 1.45

[a]50% inhibitory concentration; Values are means ± SD (n=4) normalized to *n*-butanol and DMSO solvents.

Table S3. Bacterial susceptibility to TM and TV

		Inhibition Zone [mm][a]		
Strain	Gram	TM	TV	Phylum
Bifidobacterium longum	-	18 ± 2	0	Actinobacteria
Bifidobacterium bifidum	+	18 ± 3	0	Actinobacteria
Bacteroides fragilis	-	24 ± 3	0	Bacteroidetes
Pediococcus acidilactici	-	12 ± 1	0	Firmicutes
Cutibacterium acnes	-	12 ± 2	0	Firmicutes
Lactobacillus acidophilus	+	24 ± 4	0	Firmicutes
Fusobacterium nucleatum	-	27 ± 1	0	Fusobacteriia
Proteus mirabilis	-	15 ± 1	0	Proteobacteria
Yersinia enterocolitica	-	17 ± 2	0	Proteobacteria
Enterobacter cloacae	-	0	0	Firmicutes
Staphylococcus aureus	+	0	0	Firmicutes
Enterococcus faecalis	+	0	0	Firmicutes
Ruminococcus gnavus	+	0	0	Firmicutes
Klebsiella oxytoca (PAI+)	-	0	0	Proteobacteria
Klebsiella oxytoca (PAI-)	-	0	0	Proteobacteria
Klebsiella pneumoniae	-	0	0	Proteobacteria
Proteus vulgaris	-	0	n.d.	Proteobacteria
Bacillus cereus	+	0	n.d.	Firmicutes
Clostridium difficile	+	0	n.d.	Firmicutes
Bacillus subtilis	-	0	n.d.	Firmicutes
Escherichia coli	-	0	n.d.	Proteobacteria
Campylobacter jejuni	-	0	n.d.	Proteobacteria
Candida albicans		0	n.d.	Ascomycota

[a]Means ±SD are shown (values in upper box TM n=3,TV n=2; lower box n=1);
n.d. not determined;

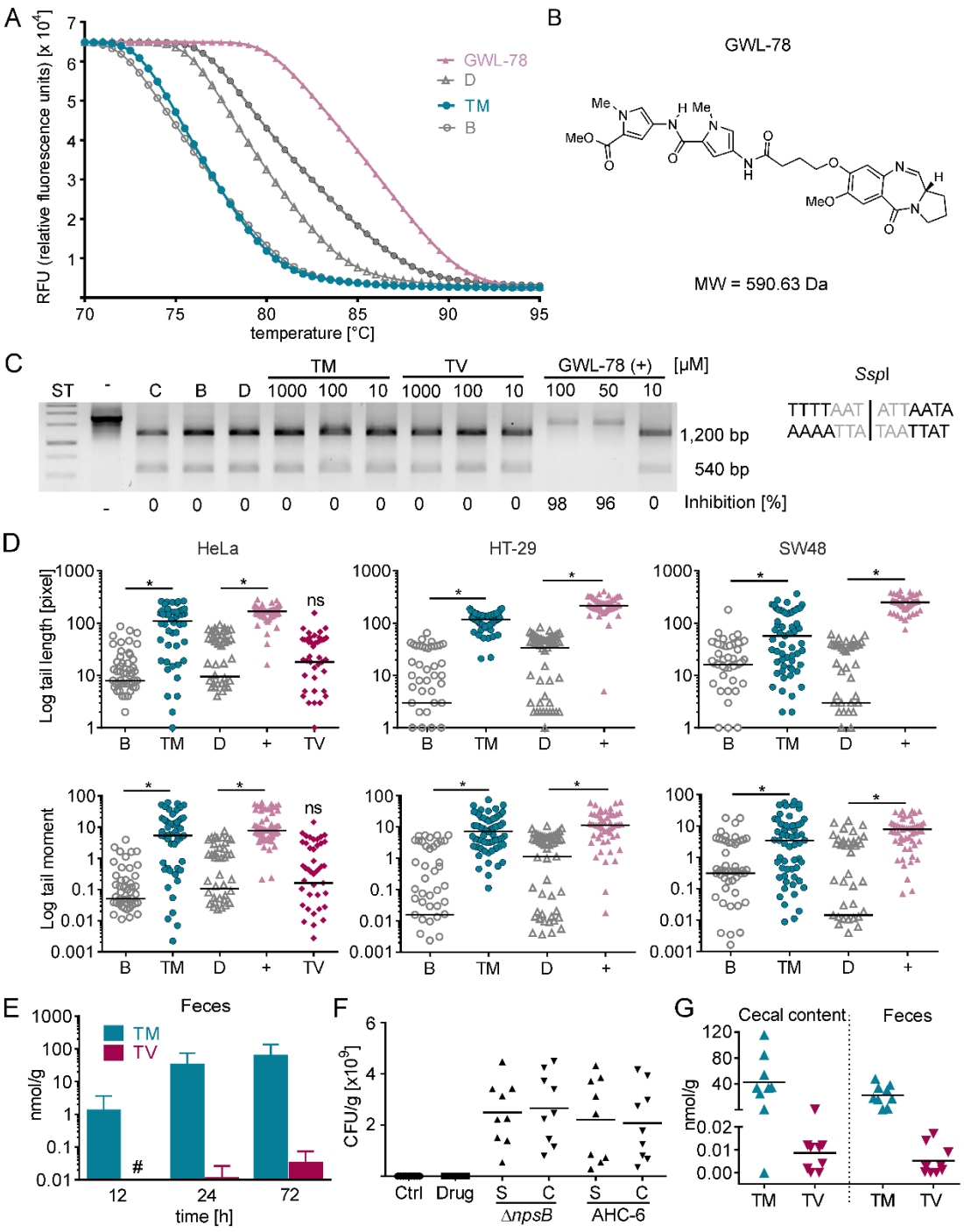

Fig. S2. TM interacts with duplex DNA in vitro and induces cellular DNA damage. (*A*) TM, positive control GWL-78 or solvents DMSO (D) or *n*-butanol (B) were reacted with DNA containing a consensus PBD-binding site (1:1 molar ratio) then denatured thermally. Melting curves show one representative experiment. (*B*) Structure and molecular weight of

control GWL-78. (*C*) DNA substrate carrying an *Ssp*I site (right) was incubated without (C) or with solvents *n*-butanol (B), DMSO (D), different concentrations of enterotoxins, or positive control GWL-78 (+). Inhibition of endonuclease activity was visualized by agarose gel electrophoresis of treated DNA compared to solvent and uncut control (-). (*D*) Tail length and tail moment for COMET of HeLa treated 4 h with 10 µM TM, 10 µM GWL-78 (+), 20 µM TV, or solvents (B, D). HT-29 and SW48 cells were treated with 1 mM TM or controls. Medians of $n \geq 50$ cells per treatment are shown. Kruskal-Wallis test followed by Dunn's multiple comparison (*$P \leq 0.05$). (*E*) TM and TV detected in feces ($n \geq 3$) of *K. oxytoca* colonized mice (# = below limit of detection). (*F*) *K. oxytoca* cfu g^{-1} in cecal content of mice from drug, infection groups *K. oxytoca* AHC-6 and Δ*npsB* (each n=9) determined with indicated selection-agar as means (S=SCAI-agar, C= CASO-agar). (*G*) TM and TV concentrations detected in cecal content and feces (n=9) of *K. oxytoca* colonized mice (24h). Bars indicate means.

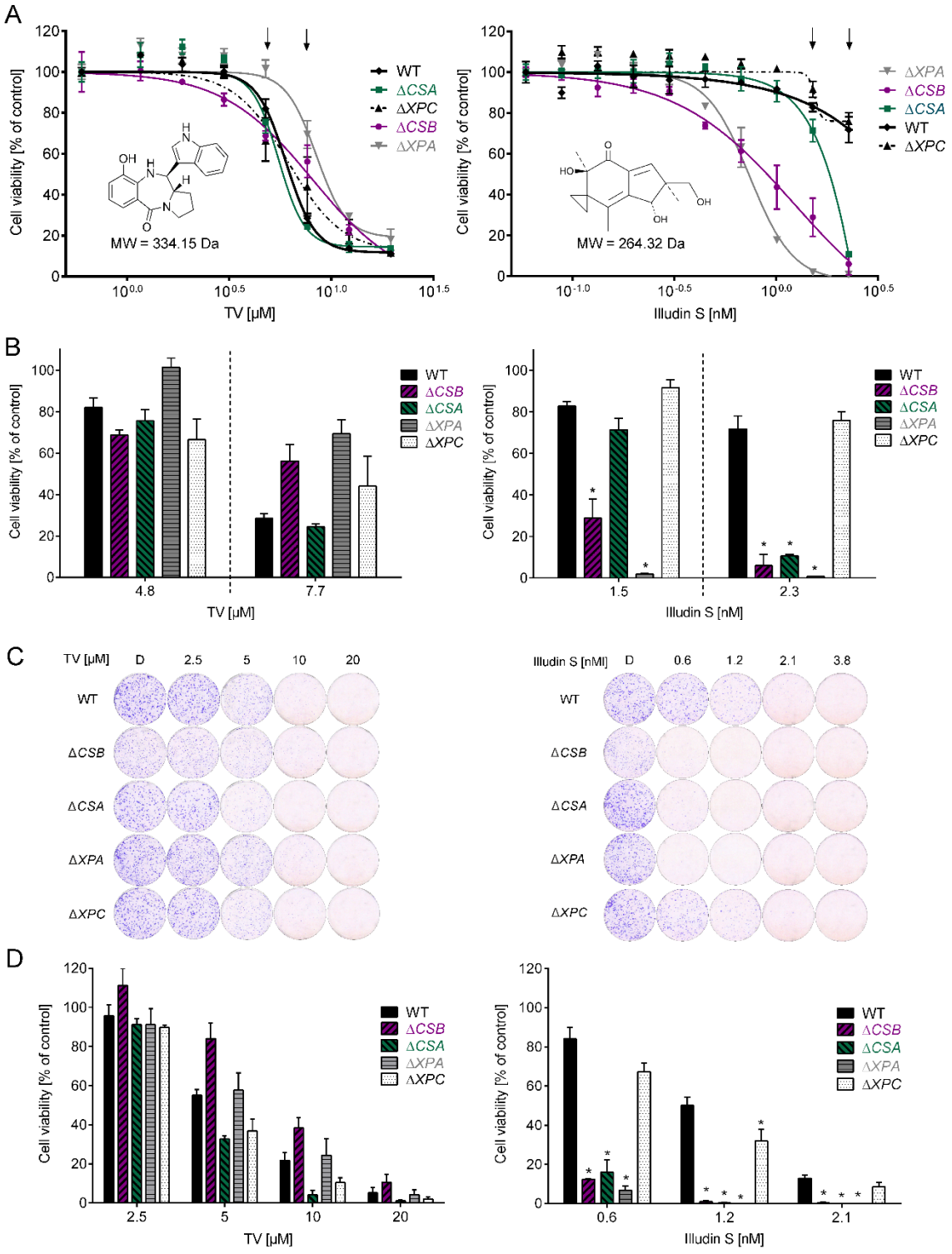

Fig. S3. DNA repair-deficient cell lines are hypersensitive to illudin S but not TV. (*A*) Dose-response survival curves of cells treated with TV (left) and illudin S (right). Values are normalized to solvent controls and represent means ± SEM of three technical replicates. Data from one of three biological replicates is shown. (*B*) Cell viability shown as means ±

SEM at two assay concentrations for each substance (indicated with arrows in A). (*C*) Colony formation of cells treated with solvent DMSO (D) or the indicated concentrations of TV and illudin S followed by recovery in drug-free medium. Macroscopic colonies were stained with crystal violet. (*D*) Values of C normalized to DMSO. Means ± SEM are shown (n=3). Significance of results for DNA repair-deficient cell lines compared to WT was calculated using One-Way Anova followed by Sidak's multiple comparison (*$P \leq 0.05$).

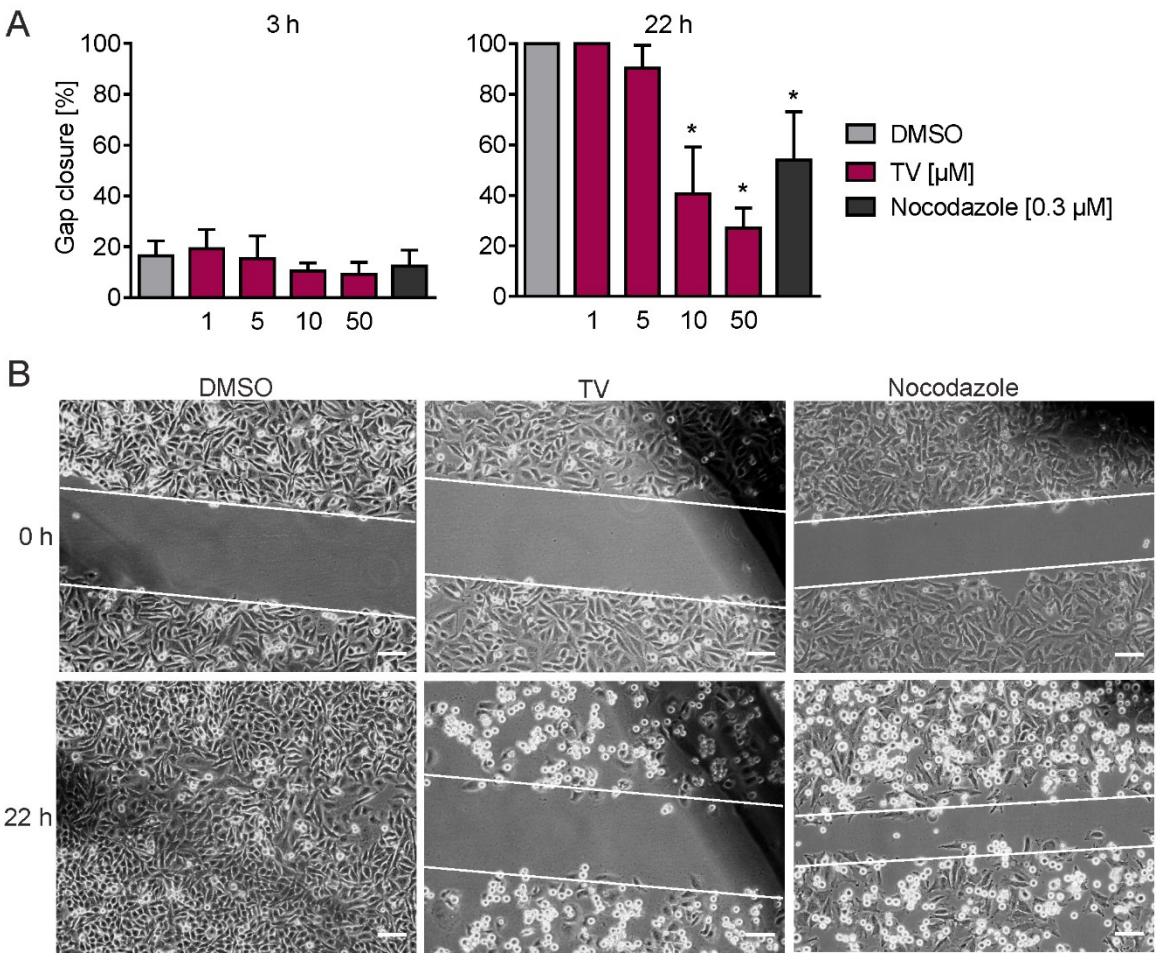

Fig. S4. TV inhibits reconstitution of cell monolayer. (*A*) Percent gap closure of TV- or controls- treated HeLa cell monolayers relative to solvent after 3 and 22 h indicated as means ± SD (n=6). One-way ANOVA followed by Sidak's multiple comparison (*$P \leq 0.05$). (*B*) Inhibition of reconstitution with 50 µM TV and 0.3 µM nocodazole at 22 h compared to solvent. Scale bars = 100 µm.

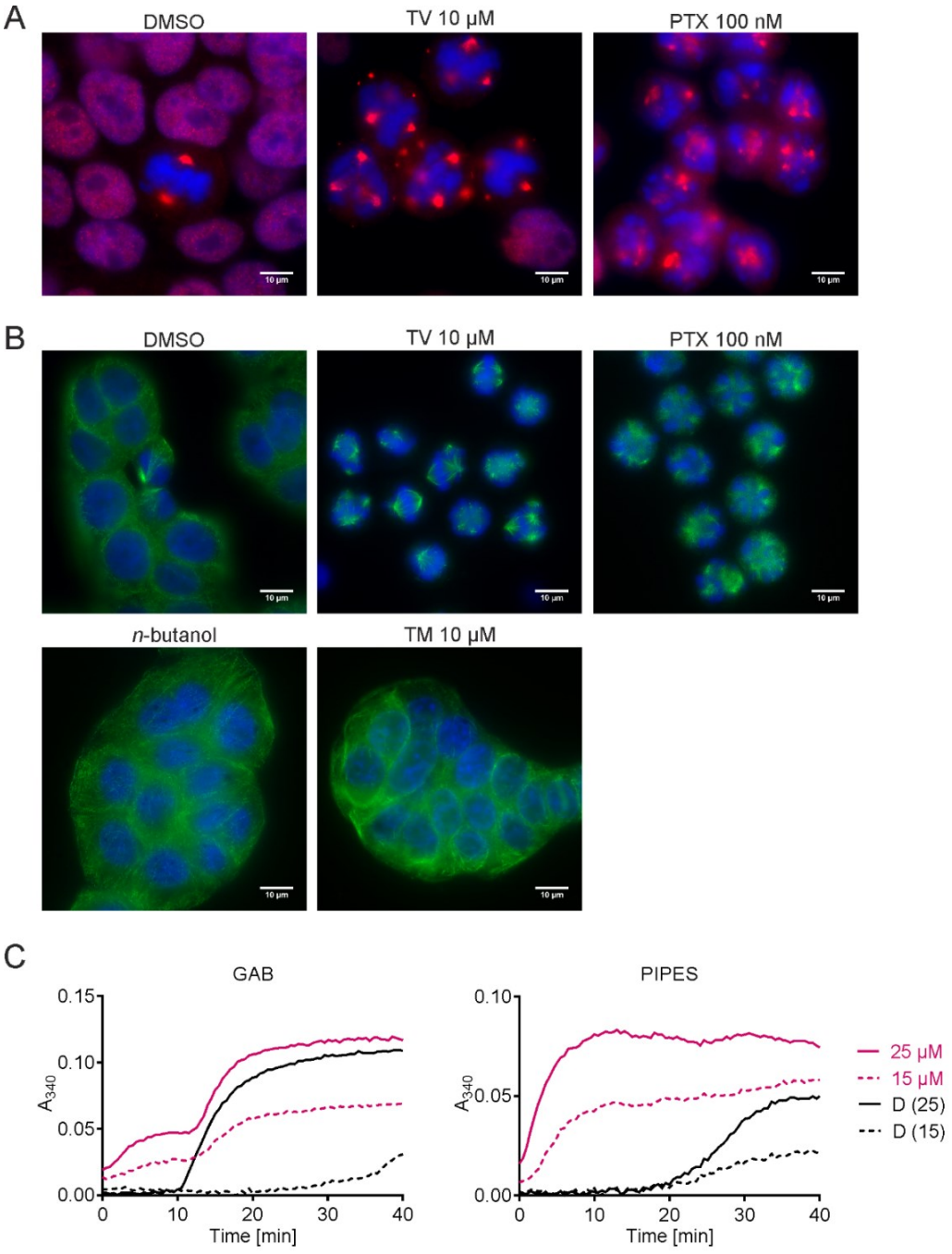

Fig. S5. TV induced tubulin phenotype is cell line and buffer independent. (*A*) Multipolar spindles were detected in HT-29 cells with antibody to nuclear mitotic apparatus protein (NuMA) (channel colour was changed to red). (*B*) ß-tubulin (green) of HT-29 cells treated with DMSO, TV, PTX, *n*-butanol and TM. DNA is stained with DAPI (blue). (*C*) Polymerization of 15 µM and 25 µM tubulin in GAB or PIPES buffer in the presence of 100 µM TV (pink) or DMSO (D, black).