Supplemental Data

Methods:

**Immunocytochemistry:** Cells were seeded on coverslips in 35 mm dishes, treated with 0.5 µM or 0.3 µM DAT1 for 12 hours. After 12 hours, the medium containing DAT1 was removed and fresh medium was added. The cells were fixed using methanol-EDTA at different time intervals following drug removal. Fixed cells were blocked with 3% BSA in PBS and stained with anti-tubulin antibody followed by secondary antibody conjugated with alexa 488. They were then visualized in a Leica TCS SP2 confocal microscope.

Figure legends:

**Supplemental Fig S1: Reversible action of DAT1 on mitotic block and cell morphology in HCT 116 cells.** HCT116 cells were treated with either DMSO or 0.5 µM DAT1 for a period of 12 hours, fixed, stained with anti tubulin antibody, followed by secondary antibody conjugated with Alexa 488. Nuclei were stained with propidium iodide. A: DMSO control, B: DAT1 treated. Then the drug containing medium was removed and fresh medium was added. The cells were fixed and stained after C: 6 hours, D: 12 hours and E: 24 hours of drug removal and visualized in a Leica TCS SP2 confocal microscope.

**Supplemental Fig S2: Reversible action of DAT1 on mitotic block and cell morphology in HeLa cells.** HeLa cells were treated with either DMSO or 0.3 µM DAT1 for a period of 12 hours, stained with anti tubulin antibody, followed by secondary
antibody conjugated with Alexa 488. Nuclei were stained with propidium iodide. A: DMSO control, B: DAT1 treated. Then the drug containing medium was removed and fresh medium was added. The cells were fixed and stained after C: 6 hours, D: 12 hours and E: 24 hours of drug removal and visualized in a Leica TCS SP2 confocal microscope.

**Supplemental Fig S3: Effect of pH on colchicine binding to tubulin.** Tubulin colchicine complex in different pH was incubated at 37°C and the fluorescence spectra were taken. The fluorescence values for different pH were plotted against the corresponding pH.

**Supplemental Fig S4: Number of Hydrogen bonds as a function of time.** The molecular dynamic simulations of the docked complexes of DAT1 and colchicine with tubulin were performed in different pH conditions for 10 ns using GROMACS force field. The simulations were analyzed by plotting the number of hydrogen bonds as a function of simulation time during the last 1 ns. A: Colchicine and DAT1 at pH 7.0 B: Colchicine and DAT1 at pH 6.0

**Supplemental Movie SM1: Molecular simulation of DAT1 bound to tubulin at higher pH**

**Supplemental Movie SM2: Molecular simulation of DAT1 bound to tubulin at lower pH**

**Supplemental Movie SM3: Molecular simulation of colchicine bound to tubulin at higher pH**
Supplemental Movie SM4: Molecular simulation of colchicine bound to tubulin at lower pH