Table S1: Sequence of qPCR primers

Gene name	5’ primer	3’ primer
ABCB1	ACAGAGGGGATGGTCAGTGT	TCACGGCCATAGCGAATGTT
ABCB6	GAAAGGAGCCAGGTTCGGTC	ACATTGAGTGCCCGTTCCAA
ABCG2	TCCCCAGGCCTCTATAGCTC	AACACTGGTTGGTCGTCAGG
GAPDH	ATCTTCTTTTGCGTCGCCAG	ACGACCAAATCCGTTGACTCC

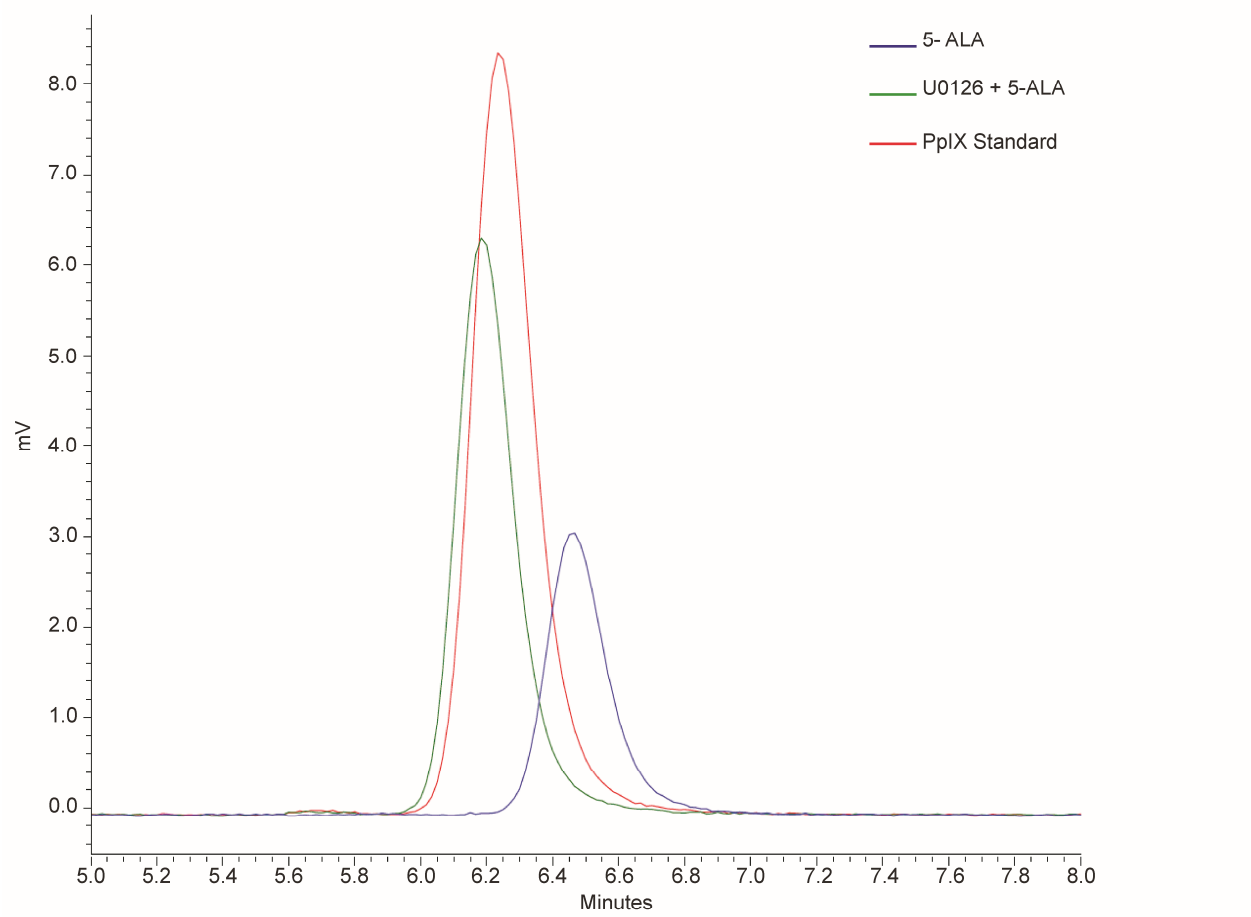

Figure S1: HPLC analysis on 5-ALA-induced PpIX accumulation by MEK inhibition. DLD-1 cells were treated with control vehicle (DMSO) or U0126 (20 μM) for 20 hours and 5-ALA (5 mM) for 5 hours. The peaks of PpIX in the cell lysates were measured by HPLC. (red: PpIX standard, blue: DMSO and 5-ALA treated, green: U0126 and 5-ALA treated)

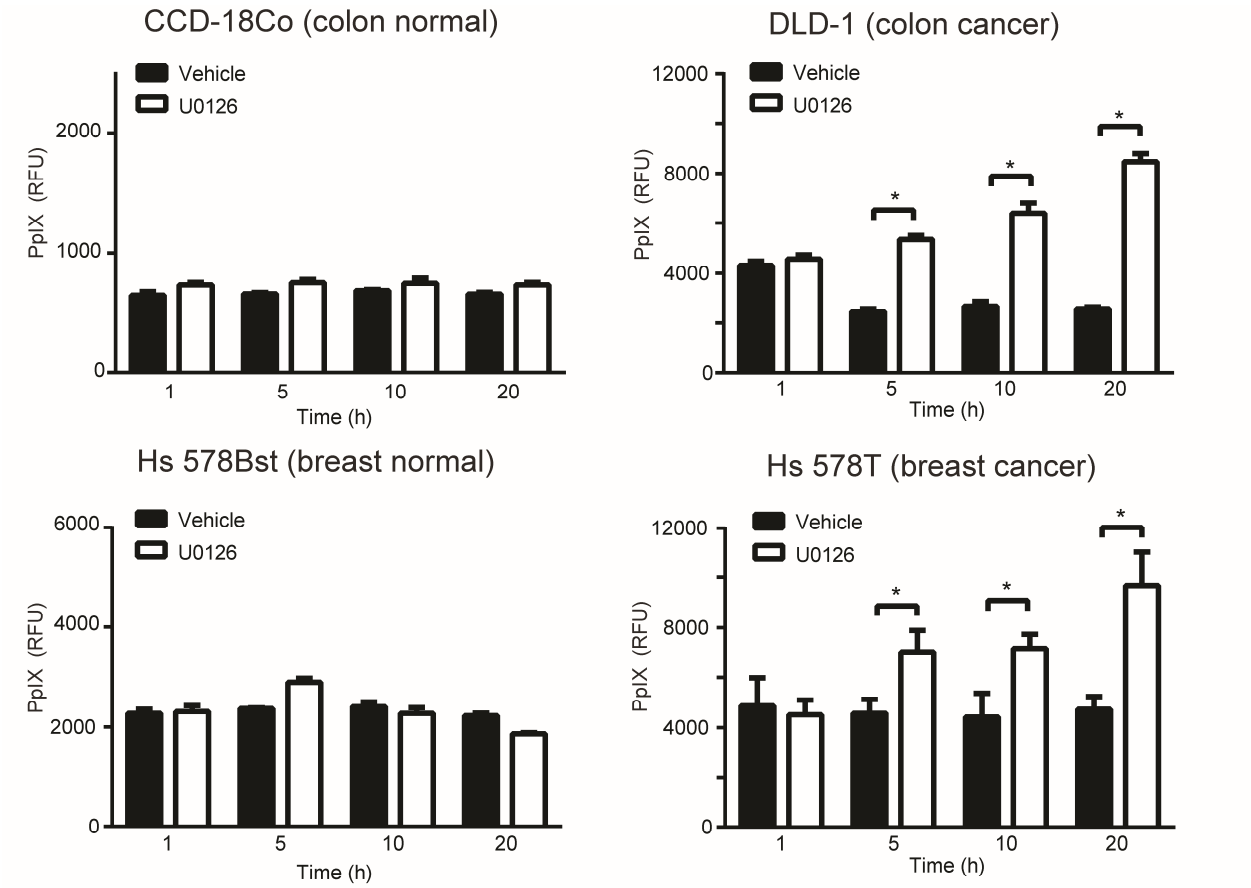

Figure S2: PpIX accumulation in normal and cancer cells.
Normal colon cells (CCD-18Co) and colon cancer cells (DLD-1) (top), and normal breast cells (Hs 578Bst) and breast cancer cells (Hs 578T) (bottom) were treated with or without U0126 (20 μM) for 1, 5, 10 or 20 hours and with 5-ALA (5 mM) for 5 hours. Cellular PpIX was measured using a fluorescence plate reader with a 405 nm excitation/630 nm emission filter. Data are expressed as the mean ± SE of 3 independent samples. Statistical analysis was conducted using two-way ANOVA, *$p<0.01$

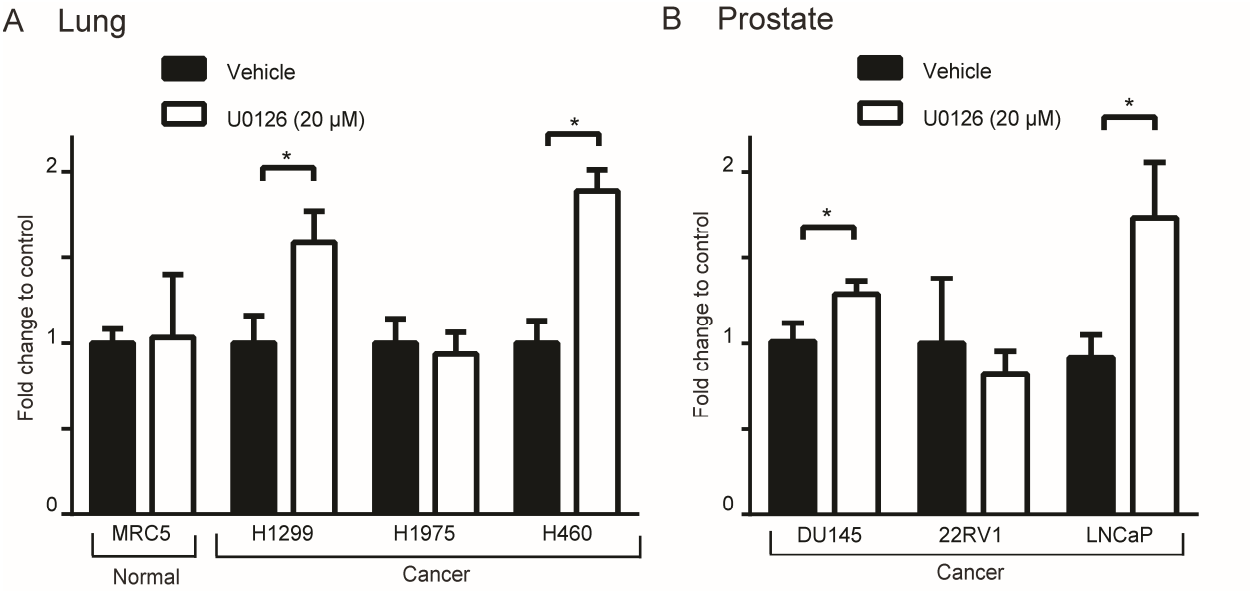

Figure S3. Increase of PpIX accumulation in human lung and prostate cell lines. (A) Normal lung cells (MRC5) and lung cancer cells (H1299, H1975 and H460), and (B) prostate cancer cells (DU145, 22RV1 and LNCaP) were treated with DMSO (Vehicle) or U0126 (20 μM) and with 5-ALA (5 mM). Cellular PpIX is reported as fold change compared to vehicle control. Data are expressed as the mean ± SE of 3 independent samples. Statistical analysis was conducted using student t Test *$p<0.01$.

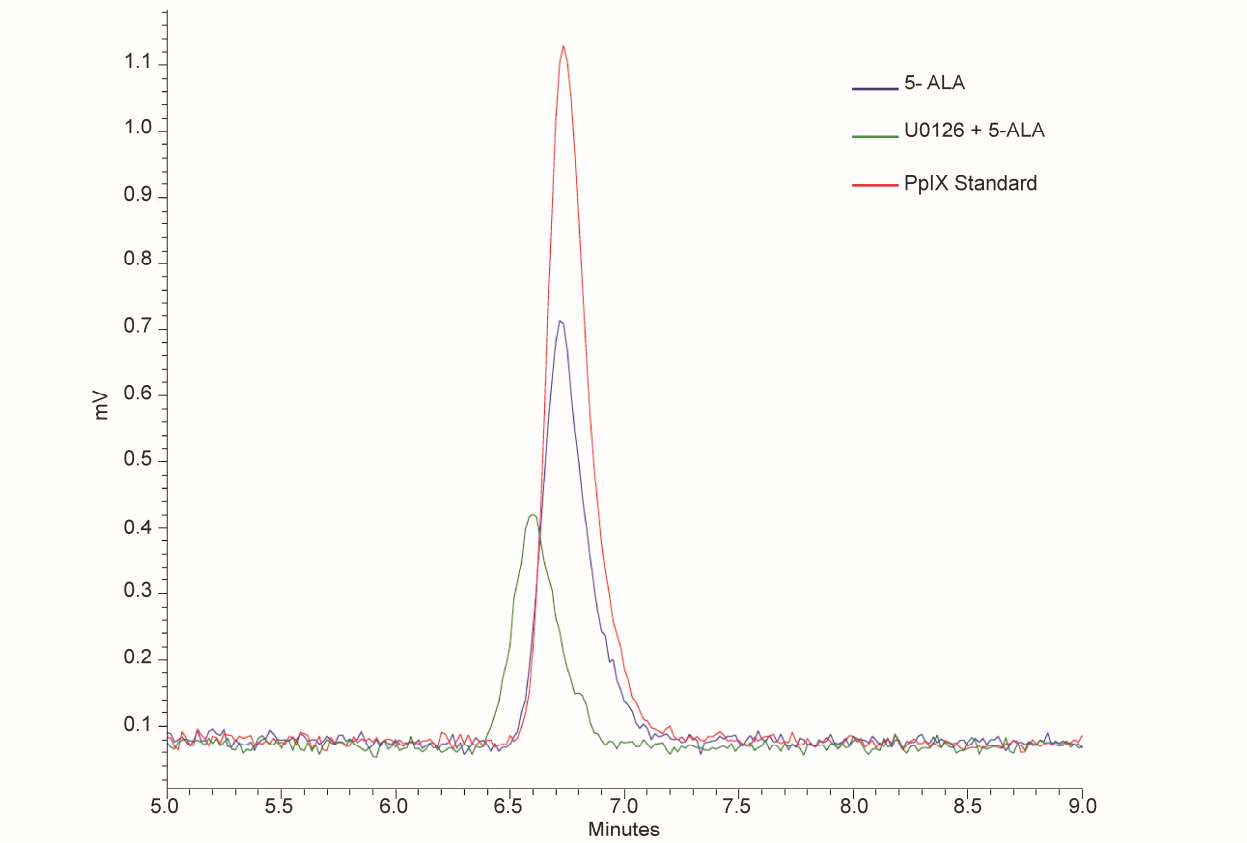

Figure S4: HPLC analysis on PpIX efflux suppressed by MEK inhibition.
DLD-1 cells were treated with DMSO or U0126 (20 μM) for 19 hours and 5-ALA (5 mM) for 4 hours. The cells were then washed and incubated with PBS for 1 hour. The peaks of PpIX in the culture supernatant were measured by HPLC (red: PpIX standard, blue: DMSO and 5-ALA treated, green: U0126 and 5-ALA treated).

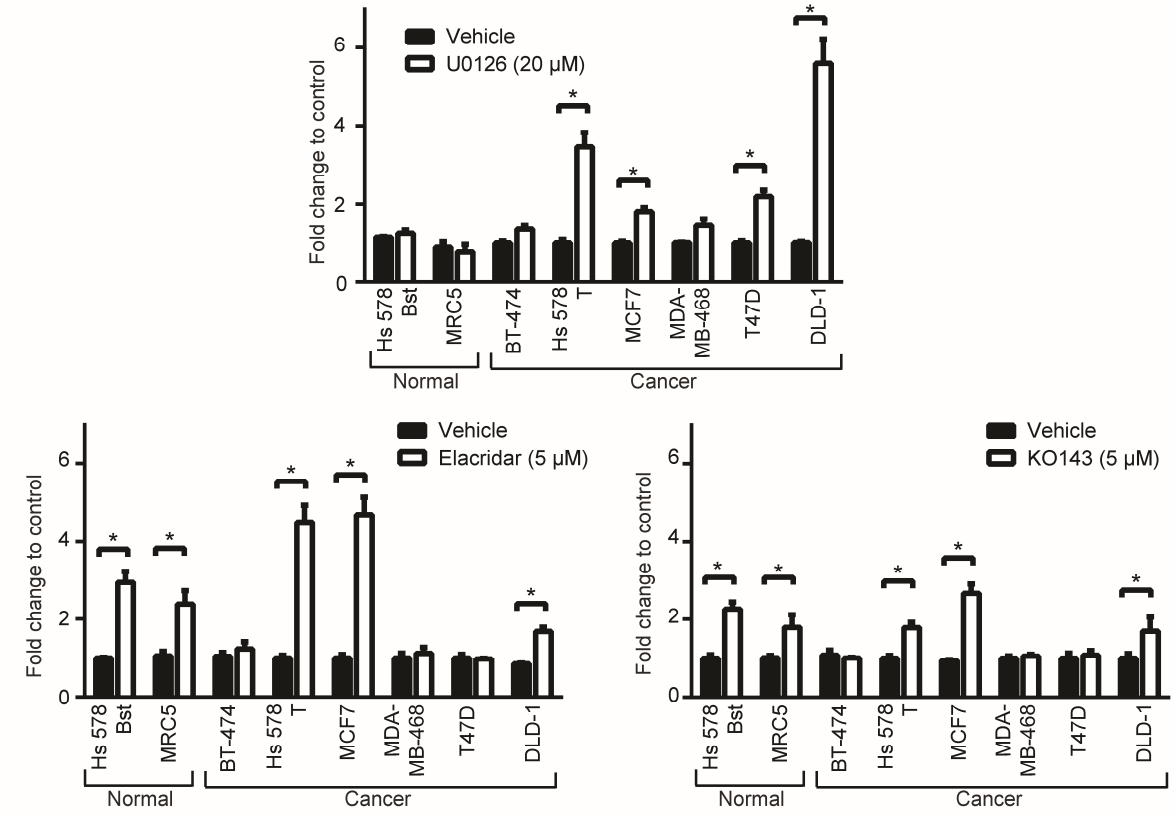

Figure S5: Effects of MEK and ABCG2 inhibition on 5-ALA induced PpIX accumulation. Human normal cells (Hs 578Bst and MRC5) and human cancer cells (BT-474, Hs 578T, MCF7, MDA MB468, T47D and DLD-1) were treated with DMSO (vehicle) or U0126 (20 μM) for 20 hours or with DMSO (vehicle), Elacridar (5 μM) or KO143 (5 μM) for 5 hours and with 5-ALA (5 mM) for the last 5 hours. Cellular PpIX fluorescence is reported as fold change compared to vehicle control. Data are expressed as the mean ± SE of 3 independent samples. Statistical analysis was conducted using two-way ANOVA, *p<0.01

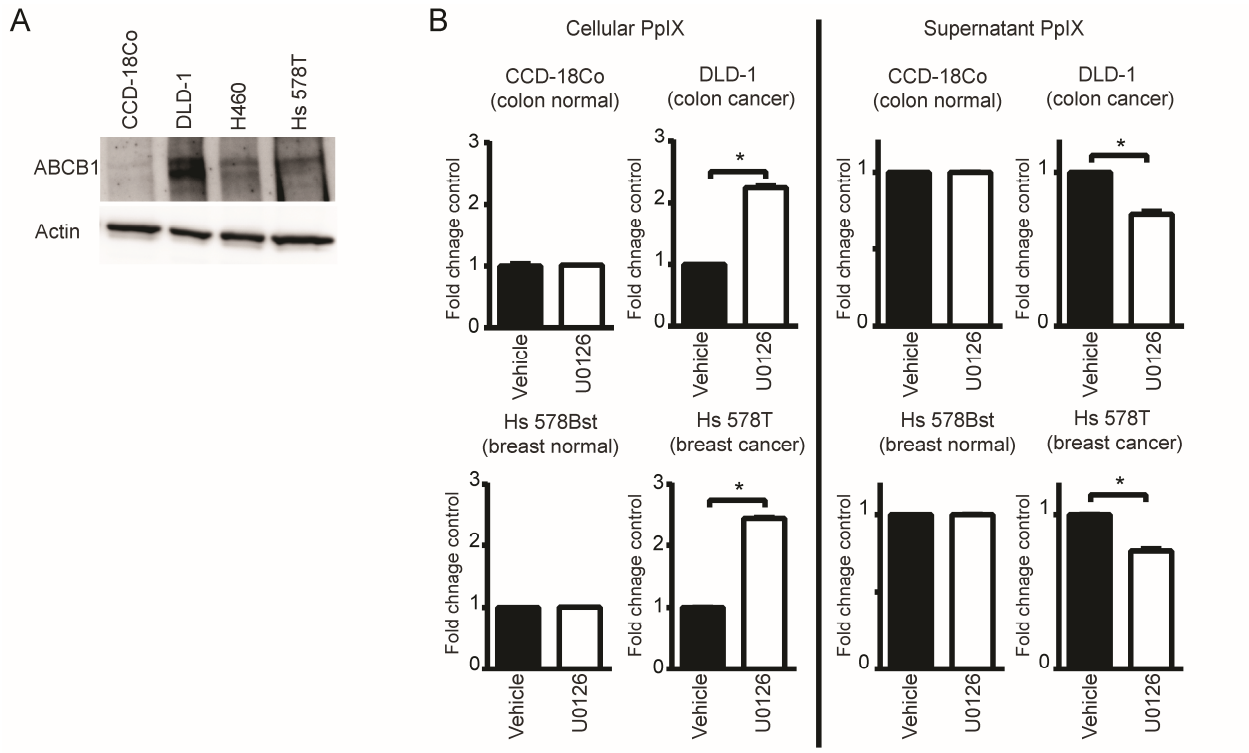

Figure S6: ABCB1 expression and PpIX efflux in normal and cancer cells.
(A) Western blot analysis using antibodies against ABCB1 and actin on cell lysates obtained from CCD-18Co (colon normal), DLD-1 (colon cancer), H460 (lung cancer) and Hs578T (breast cancer). ABCB1 expression was expressed higher in the cancer cells than in the normal cells. (B) CCD-18Co, DLD-1, Hs 578Bst (breast normal) and Hs 578T (breast cancer) cells were treated with DMSO (Vehicle) or U0126 (20 μM) for 24 hours and with 5-ALA (5 mM) for thelast 5 hours. At one hour before the time points, the cells were washed with PBS and incubated with PBS, and then PpIX amount in the cell lysate and the culture supernatant was measured. Data are expressed as the mean ±SE of 3 independent samples. Statistical analysis was conducted using Unpaired –t Test, *p<0.01. MEK inhibition increased PpIX accumulation in the cell lysate and decreased PpIX efflux into the culture supernatant of the cancer cells while it did not have any effects on PpIX amount in cell lysate and culture supernatant of the normal cells.

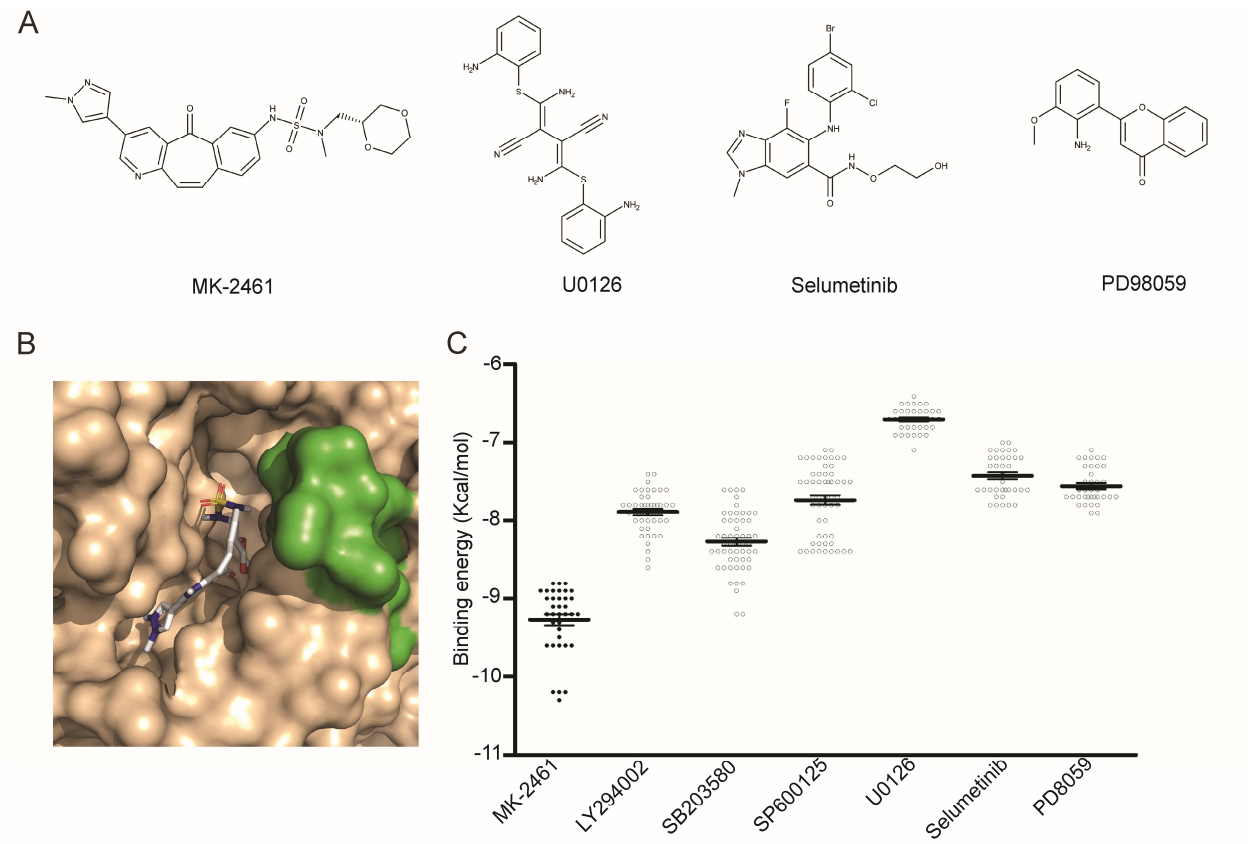

Figure S7. Docking analysis of the MEK inhibitors to FECH.
(A) Chemical structures of MK-2461 (FECH inhibitor), U0126 (MEK inhibitor), Selumetinib (MEK inhibitor) and PD98059 (MEK inhibitor). (B) Molecular docking of MK-2461 (stick representation) with FECH (PDB: 2QD4) (colored cream) using Autodock VINA (http://vina.scripps.edu/). Each low-energy binding cluster of MK-2461 bound deep in the PPIX binding site of FECH, adjacent to the conserved hydrophobic lip (colored green). (C) The binding energies of MK-2461, U0126, Selumetinib, PD98059, LY294002 (PI3K inhibitor), SB203580 (p38MAPK inhibitor) and SP600125 (JNK inhibitor) to FECH. The binding energies of the MEK inhibitors (U0126, Selumetinib and PD98059) was significantly higher than that of MK-2461, showing that the MEK inhibitors have a lower affinity to FECH. The MEK inhibitors had binding energies similar to the inhibitors against other downstream elements of Ras (LY294002, SB203580 and SP600125), which did not show an increase in 5-ALA-induced PpIX accumulation.