Supporting Information

PLX038: a long-acting topoisomerase I inhibitor with robust anti-tumor activity in ATM deficient tumors and potent synergy with PARP inhibitors

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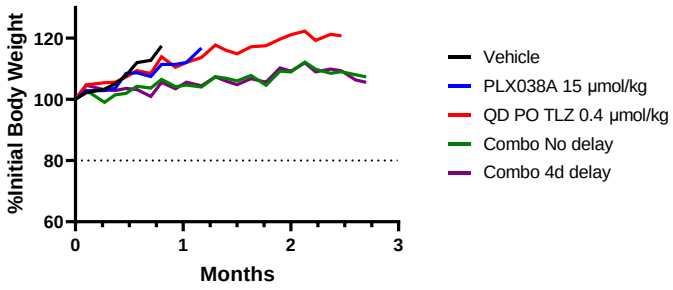

Fig S1. Normalized body weight of mice bearing MX-1 xenografts post-administration of PLX038A TLZ or the combination. Groups of mice (n=4/group) received a single IP dose of vehicle (black), PLX038A at 15 µmol/kg (blue), or started QD PO dosing of TLZ at 0.4 µmol. Two groups received a combination of both agents with a single IP dose of PLX038A (15 µmol/kg) on day 0 and starting QD PO TLZ on day 0 (No delay, green) or day 4 (4d delay, purple). Horizontal dashed line represents threshold for 20% bodyweight loss.

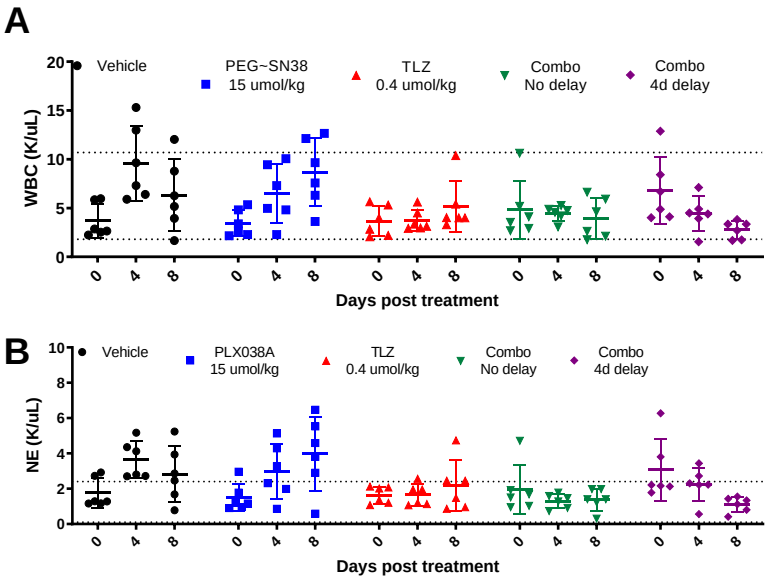

Fig S2. A) White blood cell count of CD-1 mice after treatment with PLX038A TLZ or the combination. B) Neutrophil counts (NE) of CD-1 mice after treatment. Groups of mice (n=6/group) received a single IP dose of vehicle (●), PLX038A at 15 µmol/kg (■), or started QD PO dosing of TLZ at 0.4 µmol/kg (▲). Two groups received a combination of both agents with a single IP dose

of PLX038A (15 µmol/kg) on day 0 and starting QD PO TLZ on day 0 (No delay, ▼) or day 4 (4d delay, ◆). Horizontal lines bracket generic normal ranges for WBC and NE.

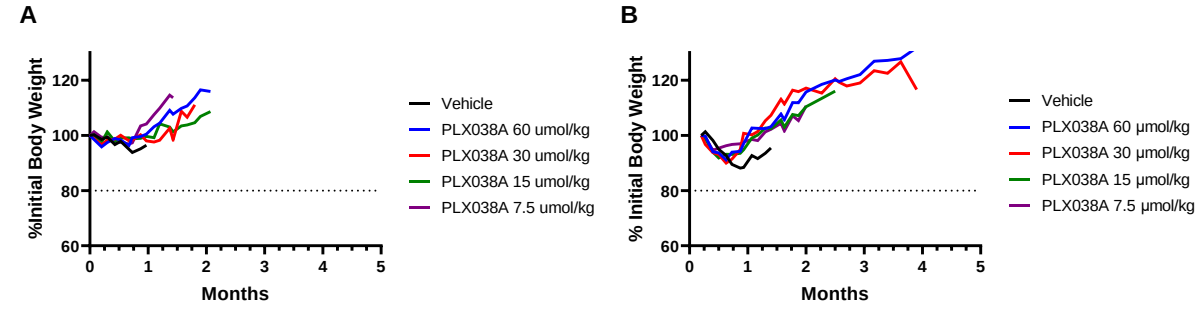

Fig S3. Normalized body weight of mice (n=8/group) bearing A) 22Rv1 ATM WT or B) 22Rv1 ATM KO tumors. Mice received a single IP dose of PLX038A at 60 µmol/kg (blue), 30 µmol/kg (red), 15 µmol/kg (green), 7.5 µmol/kg (purple). Horizontal dashed line represents threshold for 20% bodyweight loss

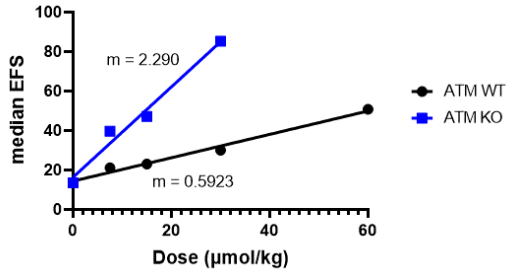

Fig S4. Plot of median EFS versus dose for mice bearing 22Rv1 ATM WT or ATM KO tumors. The slopes indicate a ~4-fold difference in sensitivity between the ATM WT and KO tumors.

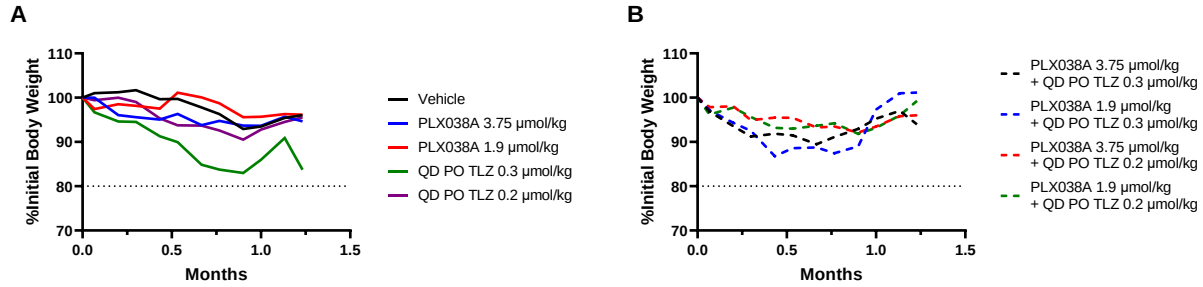

Fig S5. Normalized body weights of mice bearing 22Rv1 ATM KO xenografts post treatment with PLX038A TLZ or the combinatio. Groups of mice (n=5/group) received either A) single agent treatments of vehicle, single dose of PLX038A at 3.75 µmol/kg (blue, solid) or 1.9 µmol/kg (red, solid), QD PO TLZ at 0.3 µmol/kg (green solid) or 0.2 µmol/kg (purple solid); or a B) combination of PLX038A and QD PO TLZ: PLX038A at 3.75 µmol/kg and QD PO TLZ at 0.3 µmol/kg (black, dotted), PLX038A at 1.9 µmol/kg and QD PO TLZ at 0.3 µmol/kg (blue, dotted), PLX038A at 3.75 µmol/kg and QD PO TLZ at 0.2 µmol/kg (red, dotted), PLX038A at 1.9 µmol/kg and QD PO TLZ at 0.2 µmol/kg (green, dotted). Horizontal dashed line represents threshold for 20% bodyweight loss

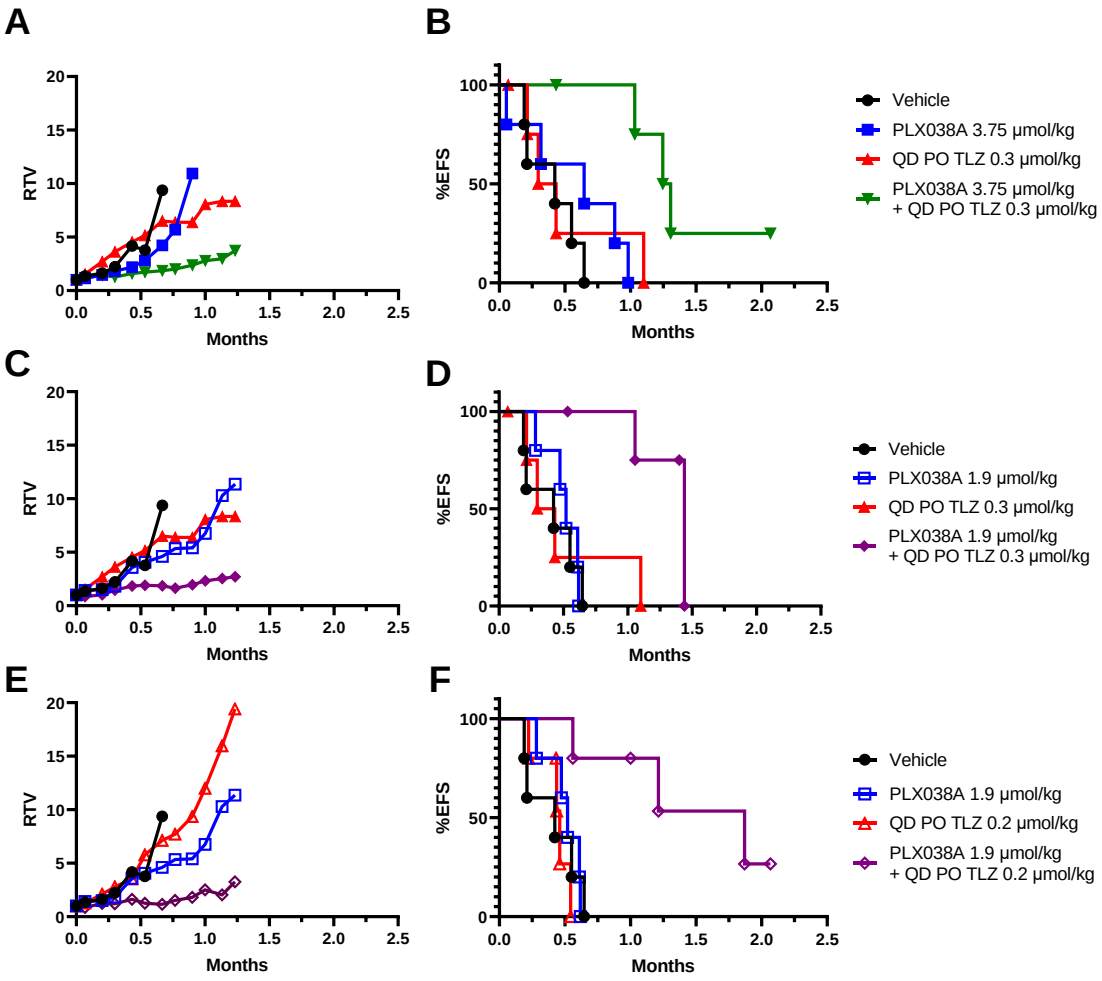

Fig S6. Tumor growth of 22Rv1 KO tumors after single IP doses of PLX038A, QD TLZ or the combination. A, C, E) Relative tumor volume versus time post-treatment. B,D,F) Event-free survival where an event is defined as a 4-fold increase in tumor volume from day 0. Mice (n=5/group) received a single IP dose of vehicle (●), PLX038A at 3.75 µmol/kg (■) or 1.9 µmol/kg (□), QD PO TLZ at 0.3 µmol/kg (▲) or 0.2 µmol/kg (△), or combinations of PLX038A at 3.75 and QD PO TLZ at 0.3 µmol/kg (▼), PLX038A at 1.9 µmol/kg and QD PO TLZ at 0.3 µmol/kg (◆), or PLX038A at 1.9 µmol/kg and QD PO TLZ at 0.2 µmol/kg (◇).

Table S1. Synergy of varying doses of PLX038 and TLZ in MX-1 and 22Rv1 ATM KO xenografts

	MX-1	22RV1 ATM KO	22RV1 ATM KO	22RV1 ATM KO	22RV1 ATM KO	22RV1 ATM KO
T/C_{3wk}	PLX038A: 15 µmol/kg TLZ: 0.4 µmol/kg/day	PLX038A: 7.5 µmol/kg TLZ: 0.4 µmol/kg/day	PLX038A: 3.75 µmol/kg TLZ: 0.3 µmol/kg/day	PLX038A: 3.75 µmol/kg TLZ: 0.2 µmol/kg/day	PLX038A: 1.95 µmol/kg TLZ: 0.3 µmol/kg/day	PLX038A: 1.9 µmol/kg TLZ: 0.2 µmol/kg/day
Vehicle	1.0	1.0	1.0	1.0	1.0	1.0
PLX038A	0.30	0.40	0.45	0.45	0.49	0.49
TLZ	0.21	0.72	0.69	0.76	0.69	0.76
Combination observed	0.038	0.17	0.20	0.20	0.20	0.12
Combination predicted	0.062	0.29	0.31	0.34	0.34	0.37
predicted/observed >1 = synergy	1.6	1.6	1.6	1.7	1.7	3.0

T/C_{3wk} is defined as (RTV after treatment for 3 weeks)/(RTV vehicle at 3 weeks).
Combination predicted = $T/C_{PLX038A,3wk} * T/C_{TLZ,3wk}$; combination observed = $T/C_{combo,3wk}$