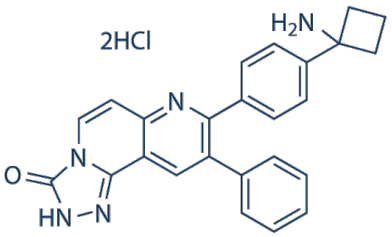
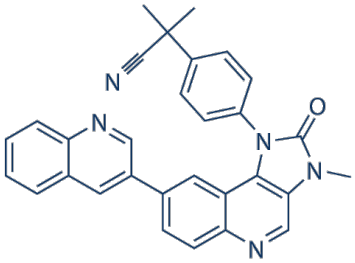
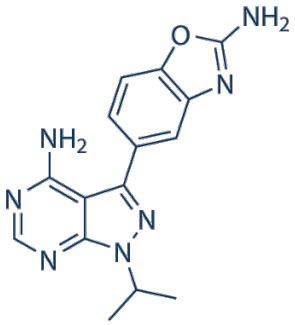


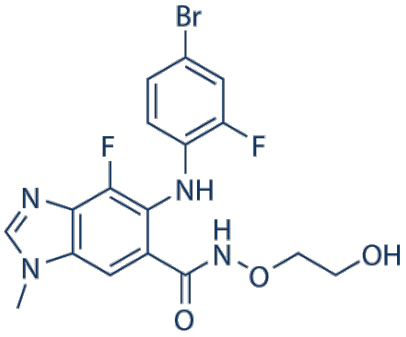
MK-2206



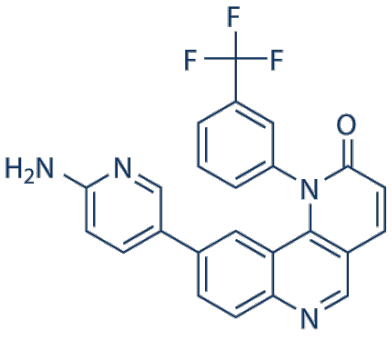
BEZ235



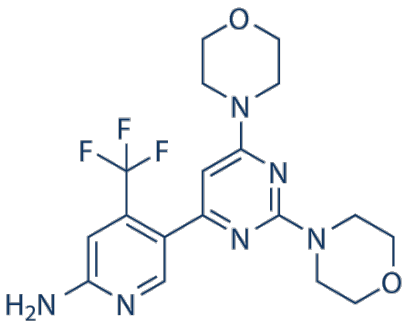
MLN0128



MEK162

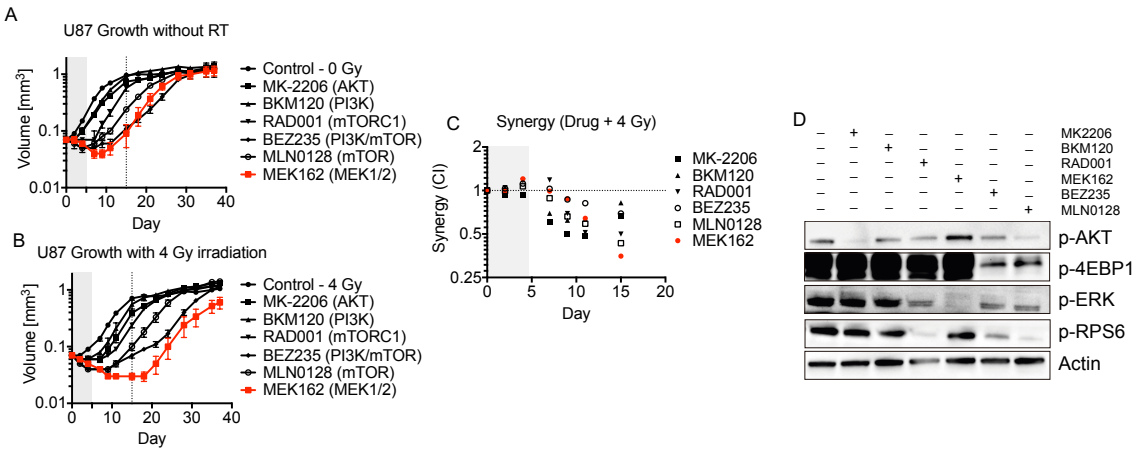


Torin2

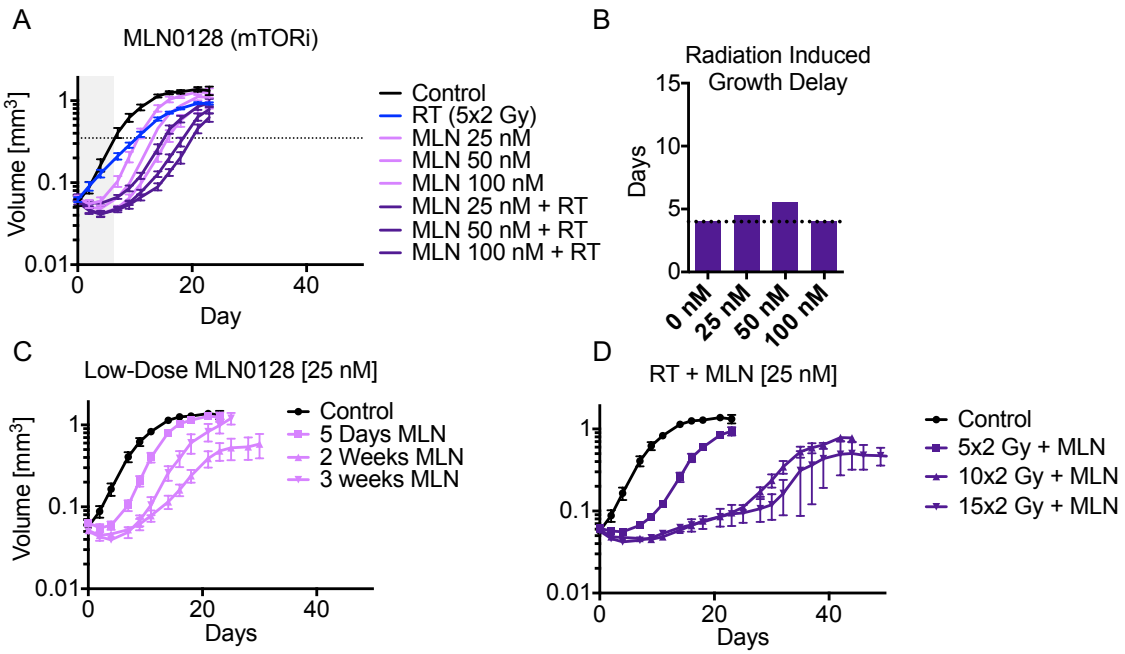


BKM120

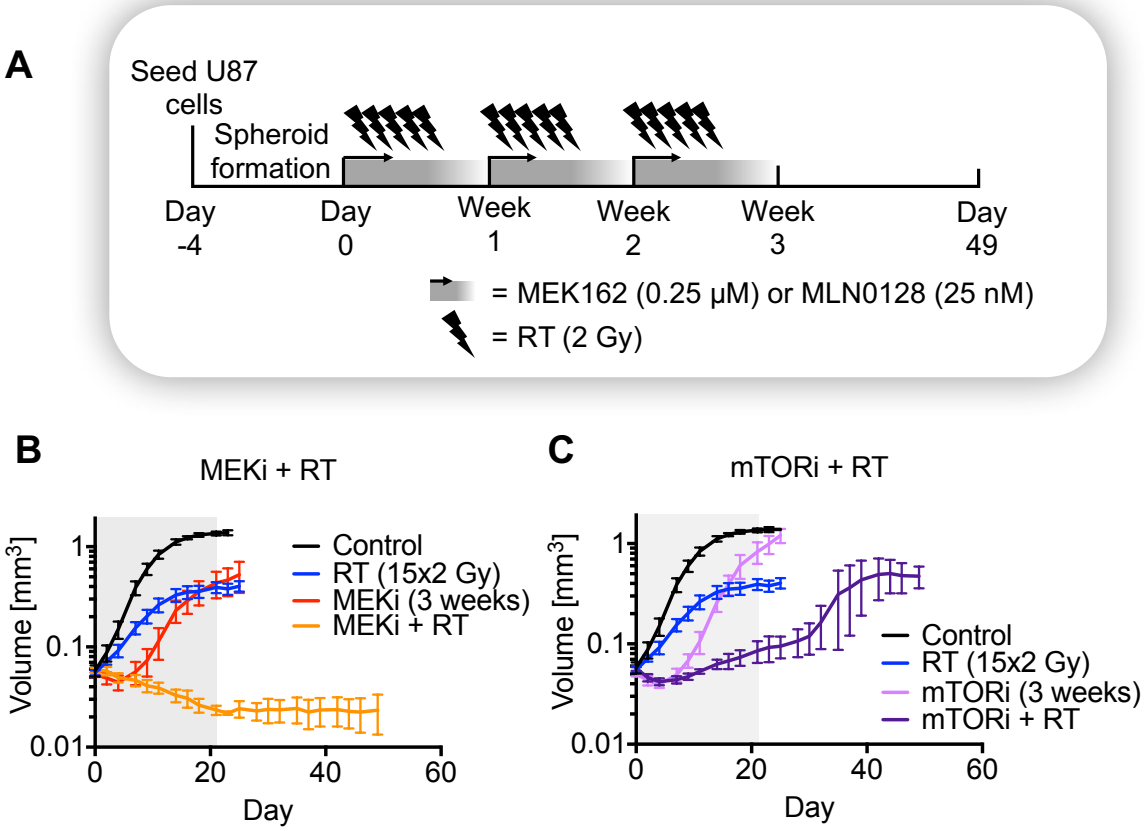
Supplemental Figure 1. Chemical structures of non-FDA approved compounds used in the study.



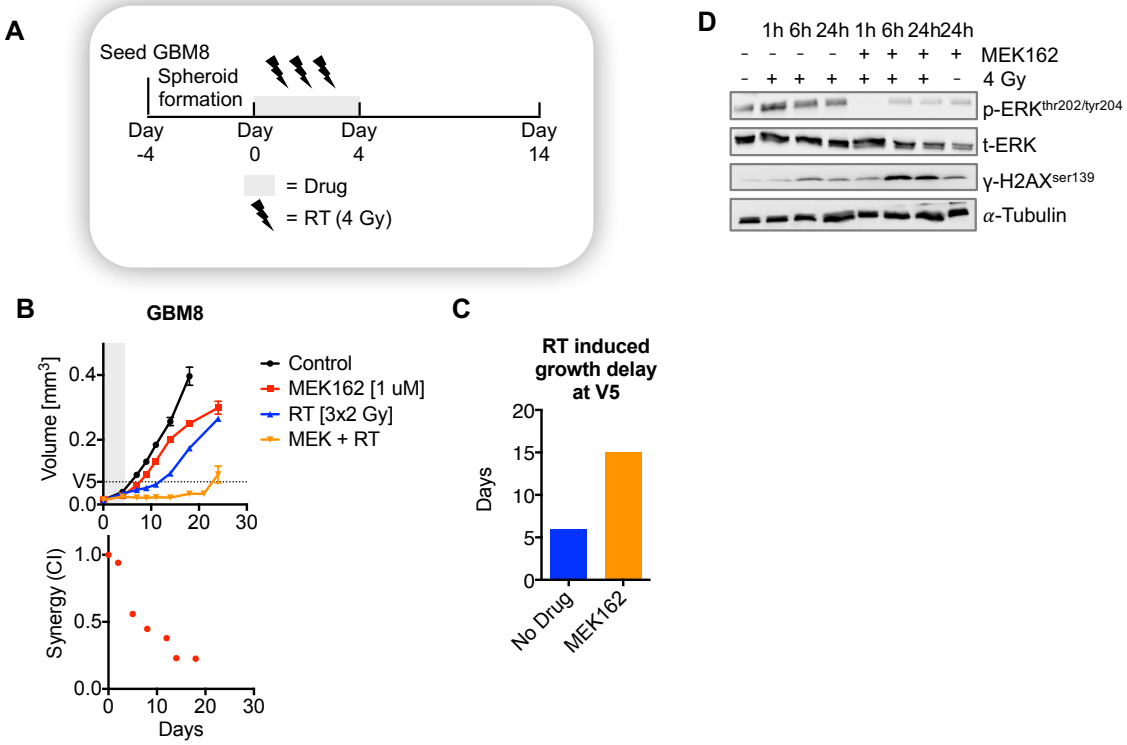
Supplemental Figure 2. Effect of drugs on growth and target phosphorylation. A & B) U87 cells were plated and allowed to form spheroids for 4 days, on day 0 spheres were given 4 Gy irradiation and treated with indicated drugs for 4 days (marked by grayed bar), medium was refreshed after 4 days. Dotted vertical lines shows the day when control untreated spheroids start to plateau. Spheroid size was assessed 2-3 times a week. Averages are shown, error bars indicate SD (n=6). C) Synergy calculations up to 15 days. D) U87 spheroids were treated for 1h with indicated drugs and harvested. Concentrations used; 1 uM MK- 2206; 1 uM BKM120, 100 nM RAD001, 1 uM MEK162, 100 nM BEZ235, 100 nM MLN0128.



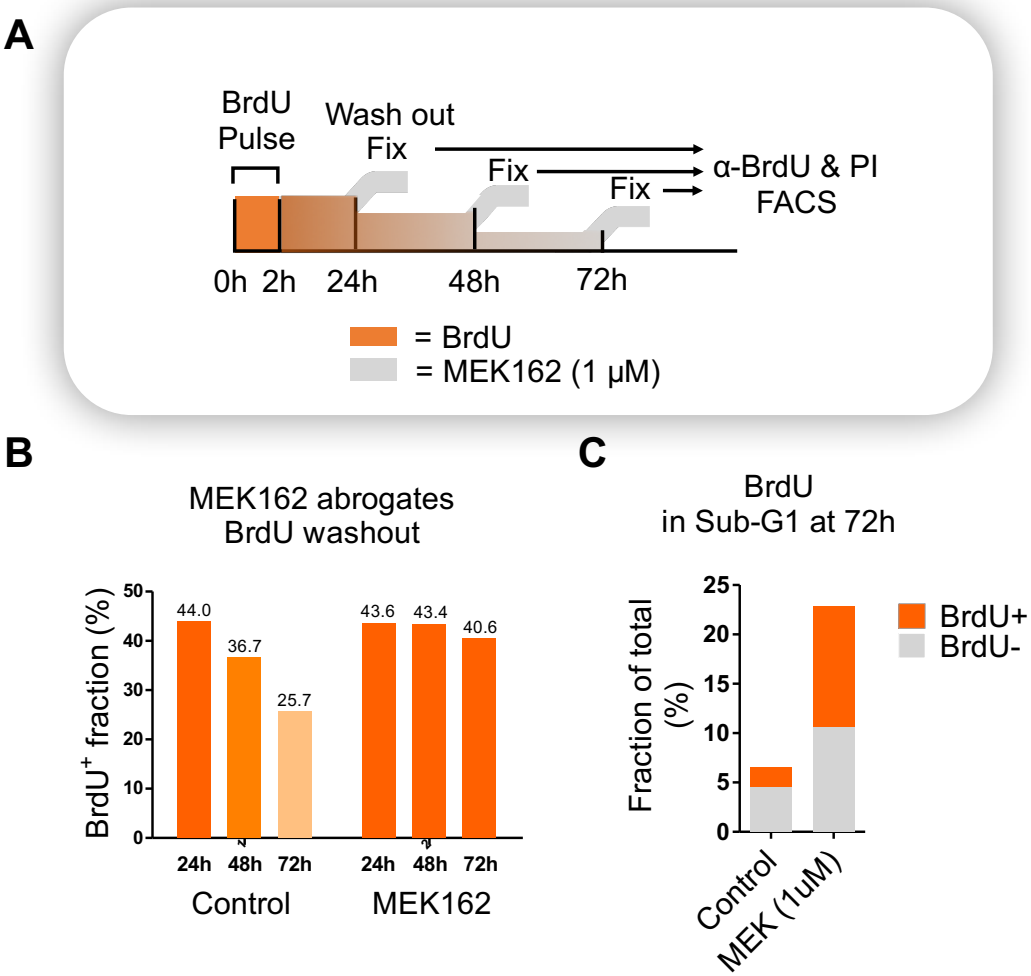
Supplemental Figure 3. mTOR inhibition does not show dose dependent radiosensitization. (A,C, D) Points are averages of 12 spheroids at each point, ±SD. B) Radiation induced growth delay for each concentration of MLN0128.



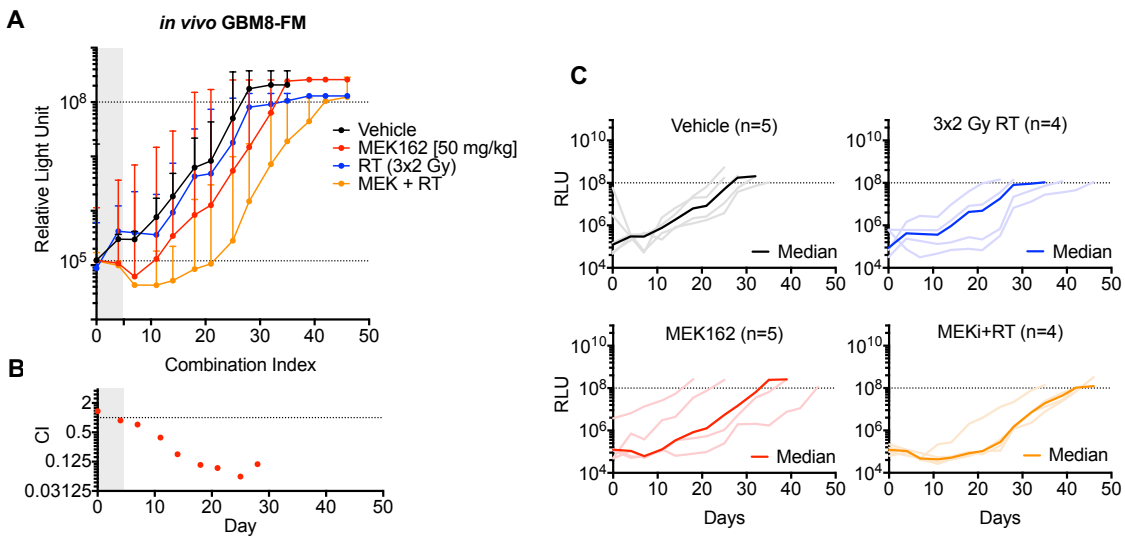
Supplemental Figure 4. MEK162 + RT abrogate spheroid regrowth. A) Schematic overview of experimental setup. Drug was refreshed at the beginning of each week B) Growth of U87 spheroids with low-dose MEK162 (0.25 μM) and 15x2 Gy irradiation. Error bars show SD (n=12). C) Growth of U87 spheroids with low-dose MLN0128 (0.25 μM) and 15x2 Gy irradiation. Error bars show SD (n=12). D) Control graph, Spheroids treated with MEK162 alone for different time periods.



Supplemental Figure 5. MEK162 radiosensitizes GBM8 primary spheroid culture and increases γH2AX levels. A) Schematic overview experimental setup. GBM8 cells were treated for 4 days with 1 μM MEK162 and 3 x 2 Gy irradiation and size was measured up to 14 days after start of treatment. B) Growth of GBM8 spheroids with combination index at each measurement. Error bars show SD (n=12). D) Combination index of MEK+RT at each time point. D) GBM8 cells were treated with 1 μM MEK162 and/or 4 Gy and cells were lysed at indicated time-points.



Supplemental Figure 6. MEK162 abrogates BrdU washout and increases sub-G1 accumulation of BrdU+ cells. A) Schematic overview of BrdU washout setup. B) Fraction of BrdU+ cells of the total amount of cells. C) Fraction of BrdU+ cells in sub-G1.



Supplemental Figure 7. MEK162 + RT in vivo. A) Bioluminescence of GBM8-FM cells in vivo. Medians over 4-5 mice are shown for each time point, error bars indicate interquartile range. B) Combination index for the combinatorial treatment at each time point. C) Tumor growth of each tumor separate with median growth.

Supplemental Table 1: Antibodies used

Target	Cell Signaling#
total-ERK	#4695
phospho-p44/42- ERK	#4370
phospho-H2AX	#9718
total-CDK1	#9116
phospho-CDK1	#9111
total-CDK2	#2546
phospho-CDK2	#2561
WEE1	#4936
total-ATM	#2873
phospho-ATM	#5883
total-CHK2	#6334
phospho-CHK2	#2197
with loading control β-Actir	#3700

Supplemental Table 2: Gene signature MEK+RT response

Positive Correlating		
Gene	R-value	pvalue
RHEB	0.936	0.34921308
PTGDR	0.918	0.40075257
TAGLN2	0.889	0.52291603
CES3	0.872	0.55621899
LRP10	0.862	0.6051217
ETV7	0.858	0.62805255
ATG9A	0.85	0.61698983
TAX1BP1	0.848	0.56671343
LEPROT	0.845	0.56060762
MON2	0.845	0.56550334
EEA1	0.842	0.56923133
SLC37A1	0.835	0.58243946
VPS52	0.834	0.56822245
DFNA5	0.828	0.59456916
TNNI3	0.824	0.59025847
ACACB	0.824	0.5957514
ZNF81	0.823	0.59182894
ADCK2	0.819	0.61006708
ACSM3	0.818	0.6054525
CASP9	0.816	0.61038858

Negative Correlating		
Gene	R-value	pvalue
LINC00472	-0.971	0.13857833
MYO16	-0.969	0.08397032
SRCAP	-0.958	0.16498223
HESX1	-0.951	0.20313991
ROBO1	-0.941	0.30857065
EYA2	-0.932	0.3603438
CTNND2	-0.93	0.35971613
SYNCRIP	-0.926	0.38649705
COTL1	-0.925	0.35911979
C9orf114	-0.919	0.41489475
CAP2	-0.917	0.38034072
NUFIP1	-0.914	0.408534
LGI2	-0.91	0.44793719
FANCC	-0.906	0.47986416
NAA40	-0.905	0.47022926
MPPED2	-0.896	0.60306144
HRK	-0.895	0.58628067
IPO5	-0.892	0.58956067
ZC3H13	-0.892	0.61637212
PATZ1	-0.891	0.57701091