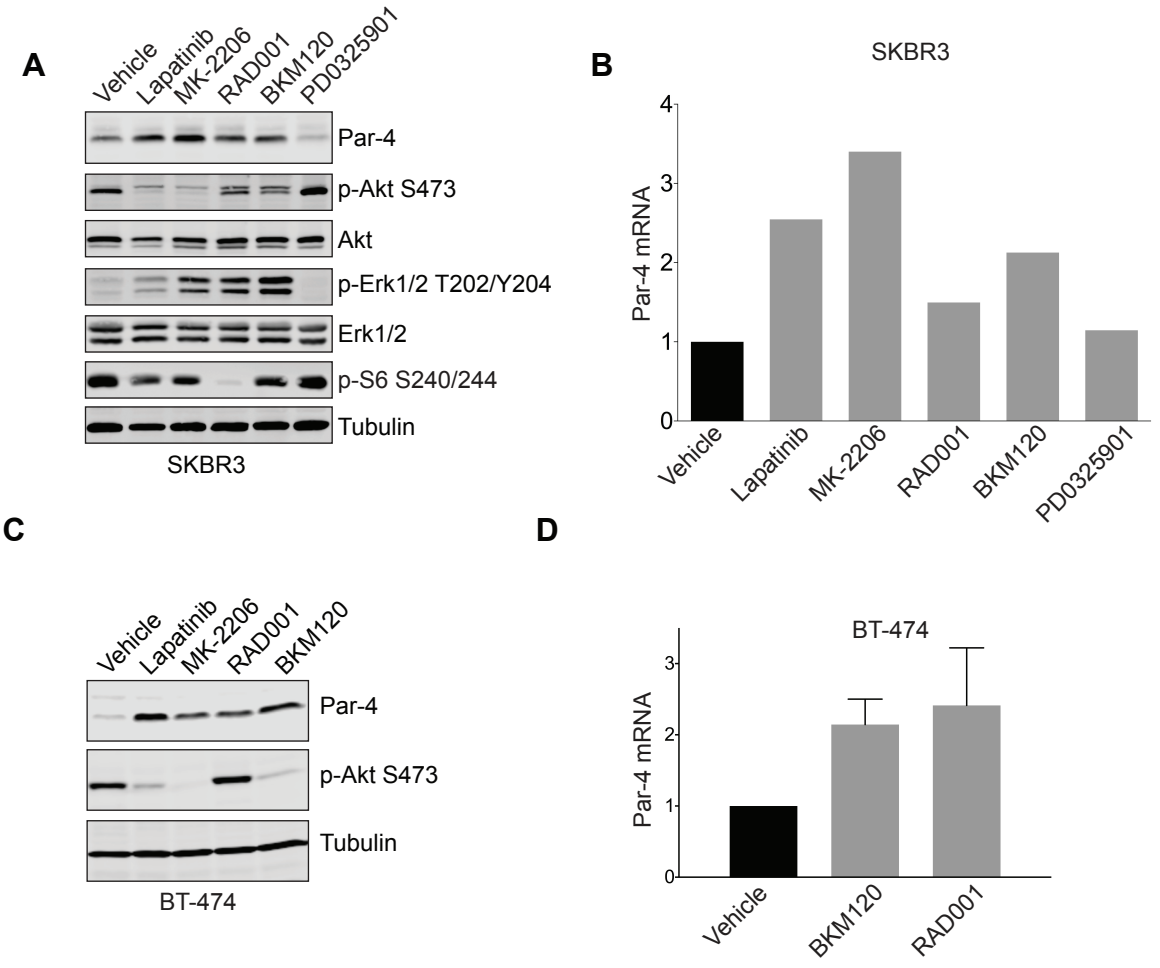
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RAD001
BKM120
PD0325901
Par-4
p-Akt S473
Akt
p-Erk1/2 T202/Y204
Erk1/2
p-S6 S240/244
Tubulin
SKBR3
B
SKBR3
Par-4 mRNA
0
1
2
3
4
Vehicle
Lapatinib
MK-2206
RAD001
BKM120
PD0325901
C
Vehicle
Lapatinib
MK-2206
RAD001
BKM120
Par-4
p-Akt S473
Tubulin
BT-474
D
BT-474
Par-4 mRNA
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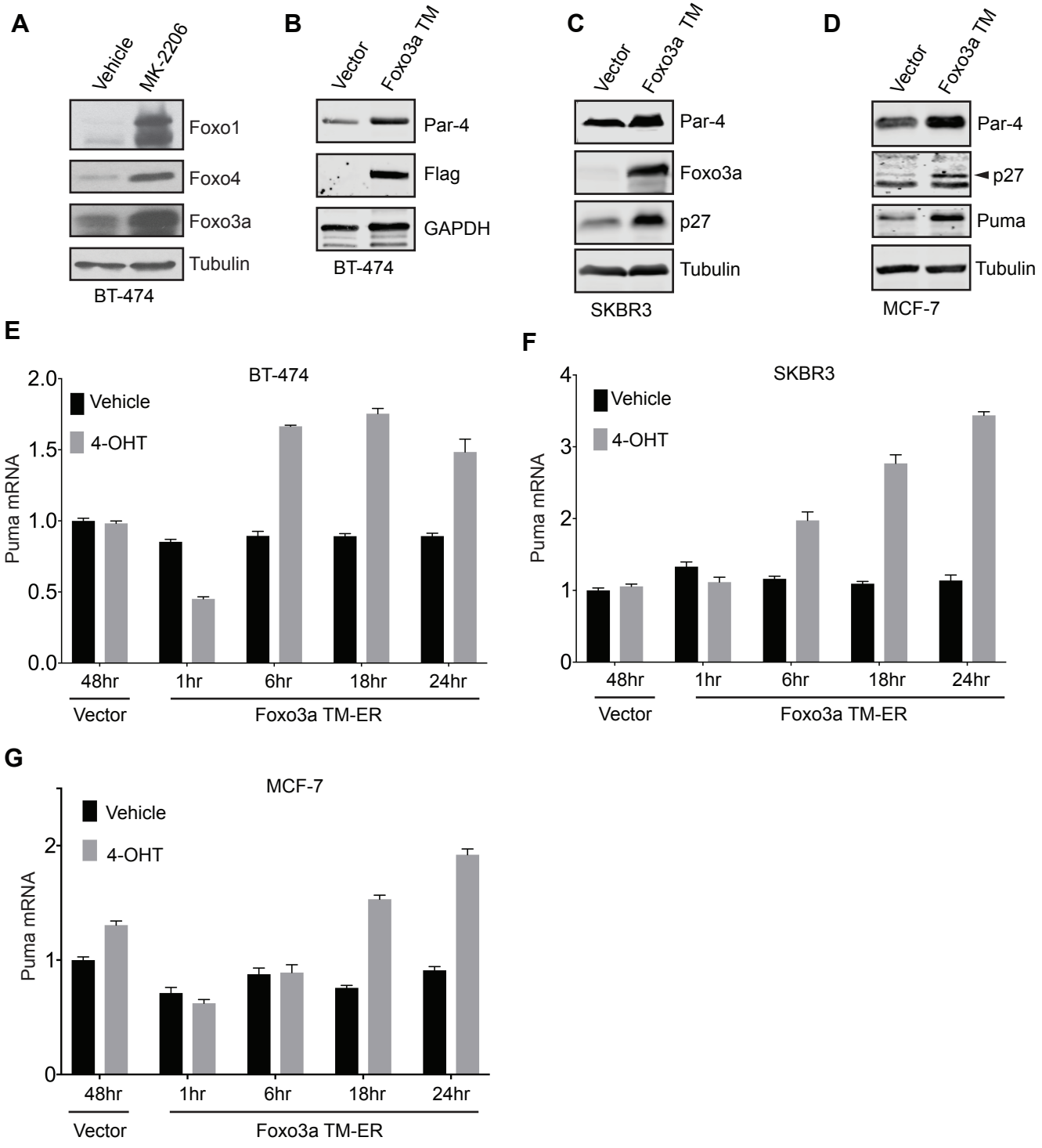
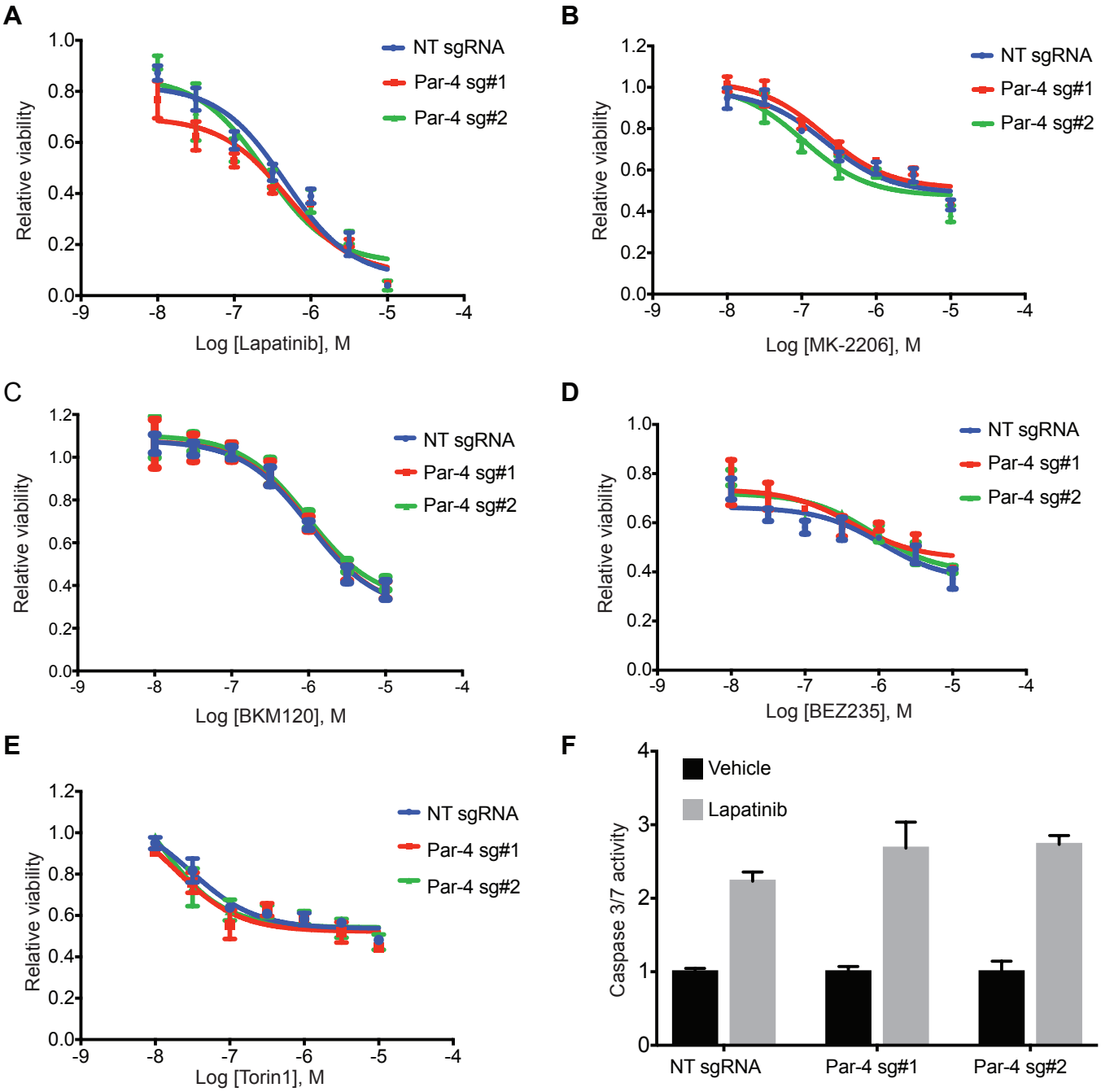
1
2
3
Vehicle
BKM120
RAD001


A
Vehicle
MK-2206
Foxo1
Foxo4
Foxo3a
Tubulin
BT-474
B
Vector
Foxo3a TM
Par-4
Flag
GAPDH
BT-474
C
Vector
Foxo3a TM
Par-4
Foxo3a
p27
Tubulin
SKBR3
D
Vector
Foxo3a TM
Par-4
p27
Puma
Tubulin
MCF-7
E
BT-474
Vehicle
4-OHT
Puma mRNA
2.0
1.5
1.0
0.5
0.0
48hr
1hr
6hr
18hr
24hr
Vector
Foxo3a TM-ER
F
SKBR3
Vehicle
4-OHT
Puma mRNA
4
3
2
1
0
48hr
1hr
6hr
18hr
24hr
Vector
Foxo3a TM-ER
G
MCF-7
Vehicle
4-OHT
Puma mRNA
2
1
0
48hr
1hr
6hr
18hr
24hr
Vector
Foxo3a TM-ER


A
Relative viability
Log [Lapatinib], M
NT sgRNA
Par-4 sg#1
Par-4 sg#2
B
Relative viability
Log [MK-2206], M
NT sgRNA
Par-4 sg#1
Par-4 sg#2
C
Relative viability
Log [BKM120], M
NT sgRNA
Par-4 sg#1
Par-4 sg#2
D
Relative viability
Log [BEZ235], M
NT sgRNA
Par-4 sg#1
Par-4 sg#2
E
Relative viability
Log [Torin1], M
NT sgRNA
Par-4 sg#1
Par-4 sg#2
F
Caspase 3/7 activity
Vehicle
Lapatinib
NT sgRNA
Par-4 sg#1
Par-4 sg#2

Supplemental Figure Legends

Supplemental Figure 1. Par-4 is upregulated following inhibition of the PI3K-Akt-mTOR pathway but not the MEK pathway. A, Western blot analysis showing Par-4 expression in SKBR3 cells treated with the indicated drugs for 2 days. B, qPCR analysis showing Par-4 mRNA expression in SKBR3 cells treated with the indicated drugs for 2 days. C, Western blot analysis showing Par-4 expression in BT-474 cells treated with the indicated drugs for 2 days. D, qPCR analysis showing Par-4 mRNA expression in BT-474 cells treated with the indicated drugs for 2 days.

Supplemental Figure 2. Par-4 is a direct target of Foxo transcription factors. A, Western blot analysis of Foxo family members following Akt pathway inhibition in BT-474 cells. B-D, Western blot analysis of BT-474 (B), SKBR3 (C), and MCF-7 (D) cells transfected with Foxo3a TM. E-G, Time-course of Puma mRNA upregulation following 4-OH Tamoxifen treatment in BT-474 (E), SKBR3 (F), or MCF-7 (G) cells stably expressing Foxo3a TM-ER fusion.

Supplemental Figure 3. Par-4 expression does not affect the acute survival of cells following Her2-PI3K-Akt pathway inhibition. A-E, Dose-response curves showing cell viability of control and Par-4 knockout cells following treatment with the indicated drugs for three days. F, Caspase-Glo assay showing caspase-3/7 activity following three days of Lapatinib treatment in control and Par-4 knockout cells.

Supplemental Table 1.

Primers for cloning par-4 promoter		
	Forward	Reverse
Region #1	5'-TAACGGTACCGGAGCAGCGACTAGGAAAAG-3'	5'-TAACAAGCTTCAGGCAATCCCAGGACAG-3'
Region #2	5'-TAACGGTACCTGTAGCCTCCCACAATGATG-3'	5'-TAACAAGCTTAGAAAGTCTCGAACGCTGCT-3'
Region #3	5'-TAACGGTACCTGCCCAAGATCTCTCAGCTA-3'	5'-TAACCTCGAGCTGACGCACCTAAGACTGACC-3'
Region #4	5'-TAACGGTACCGCAGCCAAAATTCCTGACTCA-3'	5'-TAACAAGCTTTAAGGGAGGCTGGGACATTA-3'
Region #5	5'-TAACGGTACCTTCTTCTCCCTCCCTCCTGT-3'	5'-TAACAAGCTTGGGCTGAGGTGAAAGACAAA-3'
Region #6	5'-TAACGGTACCGGGGTCAGTCCTCTCTCCTC-3'	5'-TAACAAGCTTGCTTCCTCTTCTCGATCTGC-3'
Region #7	5'-TAACGGTACCCAACGAGCTCAACAACAACCT-3'	5'-TAACAAGCTTGAGCCATAAGTTGTCTTTCTACCA-3'
Region #8	5'-TAACGGTACCACACGTGCTTTTCAGGCTTC-3'	5'-TAACAAGCTTAACGTTTCGAAAATGCTCCA-3'
Primers for ChIP		
	Forward	Reverse
Region #3	5’-ATGTGCTACCTGGGCAAAAC-3’	5’-GAGGCTACAAATGCCAGGTC-3’
Region #6	5’-CAGGAAGGAAGAGGAGGCA-3’	5’-CTCCTCCAGGAAGTCTGTGG-3’
Region #8	5’-GGGATGATTTAGTAACCCACAA-3’	5’-TGCAAACAACCCAAAACAAA-3’
Distal region (~10 kb upstream)	5’-TTCTAAGCAGTGCAGGAGCA-3’	5’-TCTGATGACTCGCCCTCTTT-3’