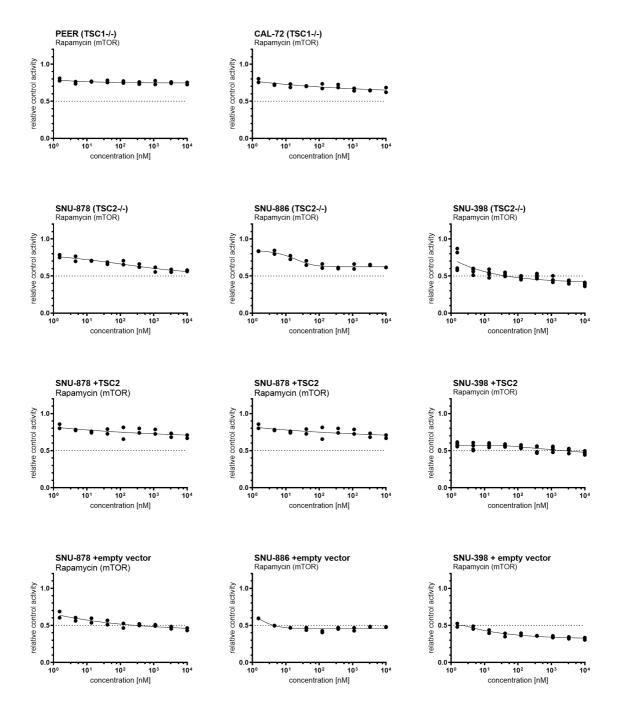
| | | 492 G>A allele ratio | | allele ratio varietion/total |
|---------|-------------------|---|------------------------|---|
| PEER | TSC1 exon6 | | CW2 TSC1 exon3 | J-26 c>t g t c c t g g a g c c a g c a g c g 0.47 |
| control | TSC1 exon6 | | control TSC1 exon3 | g t c c t g g a g c c a g c a c a g c g |
| KM12 | TSC1 exon7 | | CW2 TSC1 exon15 | ATGCCCCCTCCGACGGGCGA TCCGACGGGCGAA 0.47 |
| control | TSC1 exon7 | | control TSC1 exon15 | |
| 23132 | TSC1 exon12 | | CW2 TSC2 exon9 | G T G T T T T T T G T G G G C A T G G G T G T G T T T T G T G G G C A T G G G |
| control | TSC1 exon12 | | control TSC2 exon9 | GTG TTTTTTGTGGGCATGGC |
| DV90 | TSC2 exon18 | G C C A G A G A G A G A G G C T C T G A G A A G G C T C T G C T C T G A G A A G G C T C T G C T C T G A G A A G G C T C T C T G A G A A G G C T C T C T G A G A A G G C T C T C T G A G A A G C T C T C T G A G A G C T C T C T G A G A G C T C T C T C T G A G A A G G C T C T C T C T C T C T C T C T C T | NCIH1651 TSC2 exon2 | |
| control | TSC2 exon18 | | control TSC2 exon2 | |
| OVK18 | TSC2 exon18 | GCCAGAGAGAGAGGCTCTGAGAG GCCAGAGAGAGAGCCTCTGAGAG | HEC151 TSC2 exon18 | G CCA G A G A G A G A G G C T C T G A G A G A G A G A G A G A G A G A G |
| control | TSC2 exon18 | | control TSC2 exon18 | GC CA G A G A G A G G G C T C T G A G A |
| MFE319 | TSC2 exon20 | | HEC151 TSC2 exon41 | 5378 G≥A TG G G C C A G C G G A A G C G C C T C 0.45 |
| control | TSC2 exon20 | GAGCGGCTCCGAGGCGCCCC | control TSC2 exon41 | |
| SNU886 | TSC2 exon26-2 | 7 ATGATGGCTTGATACGTCTT 0.97 | HCC95 TSC2 exon41 | |
| control | TSC2 exon26-2 | 7 AT GAT G G CT CG AT A C G T CT T | control TSC2 exon41 | |
| SNU878 | exon34 | 4541 C>G GC G A C G A G T G A AAC AA G C C A GC G A C G A G T G A AAC AA G C C A GC G A C G A G T G A AAC AA G C C A | | a, c, g, t nucleotides of introns A, C, G, T nucleotides of exsons > substitution |
| contro | ol TSC2 exon34 | | | ins insertion del deletion |

S1 Fig. Confirmation of reported mutations by Sanger sequencing

Sequencing traces with mutation are shown, including a control for each sequenced region. PEER cells showed a homozygous nonsense mutation in TSC1 and SNU-878 and SNU-886 cells a homozygous nonsense mutation in TSC2. All other cell lines showed heterozygous mutations.

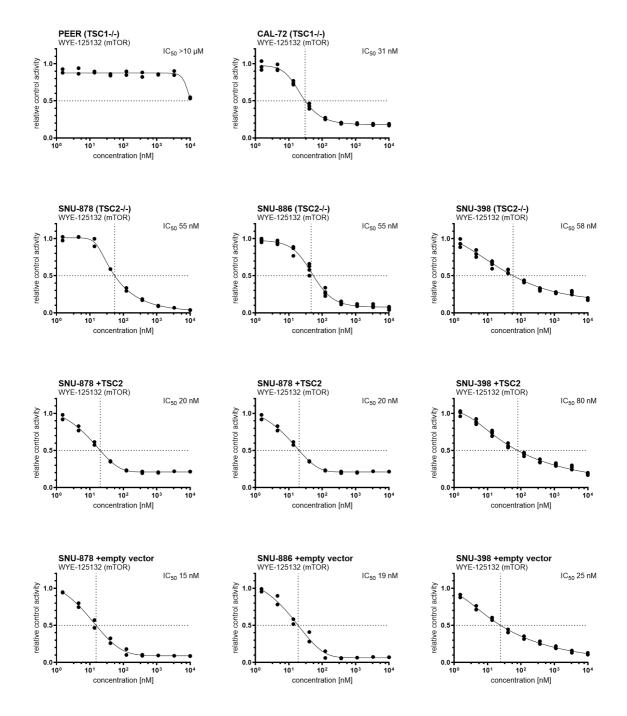
Rapamycin (mTOR)



S2 Fig. IC50 determination for Rapamycin

Rapamycin was serially diluted three-fold from 10 µM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown relative to control, n= 2- 4. Rapamycin did not achieve IC50 over this dose range.

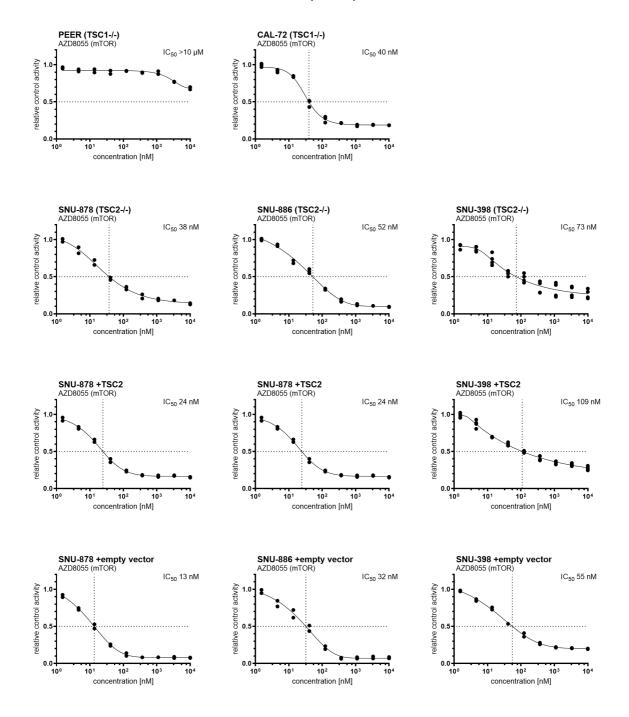
WYE-125132 (mTOR)



S3 Fig. Cell viability after WYE-125132 treatment

WYE-125132 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. WYE-125132 is an mTORC1/2 inhibitor. The SNU cell lines and CAL-72 were very sensitive to all tested mTORC1/2 inhibitors.

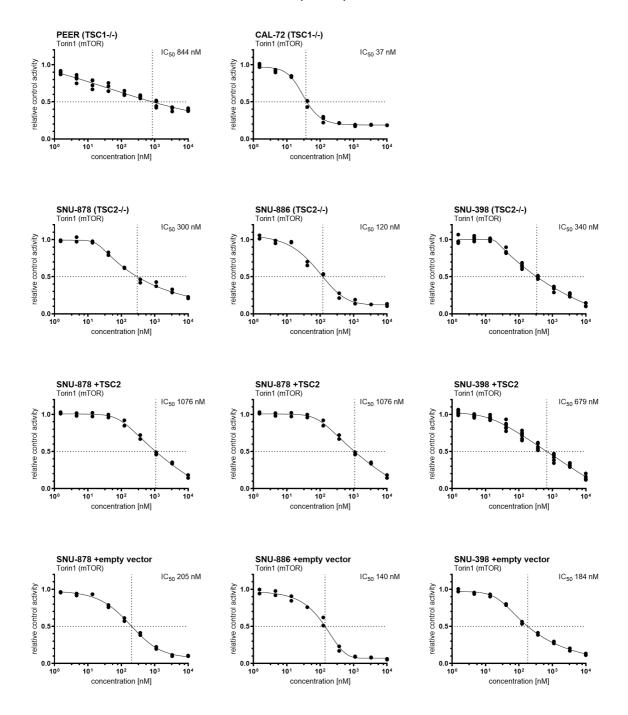
AZD8055 (mTOR)



S4 Fig. Cell viability after AZD8055 treatment

AZD8055 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. AZD8055 is an mTORC1/2 inhibitor. The SNU cell lines and CAL-72 were very sensitive to all tested mTORC1/2 inhibitors.

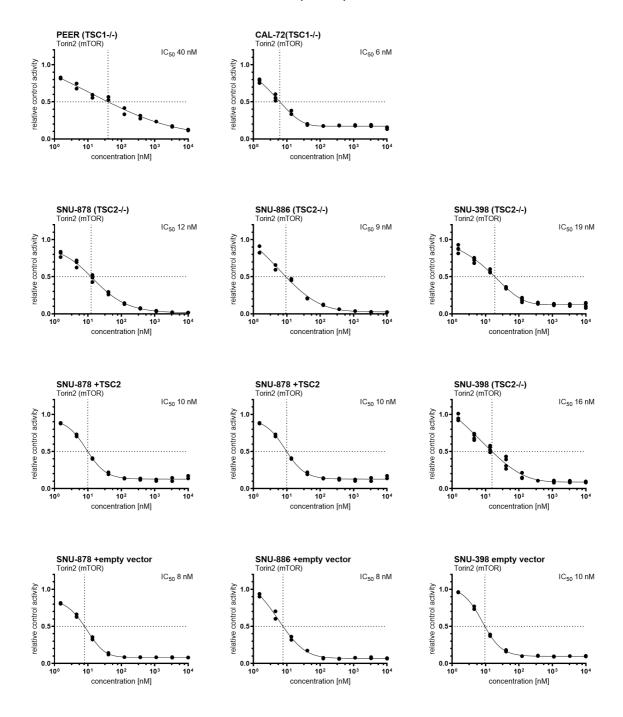
Torin1 (mTOR)

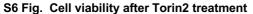


S5 Fig. Cell viability after Torin1 treatment

Torin1 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-6. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. Torin1 is an mTORC1/2 inhibitor. The SNU cell lines and CAL-72 were very sensitive to all tested mTORC1/2 inhibitors.

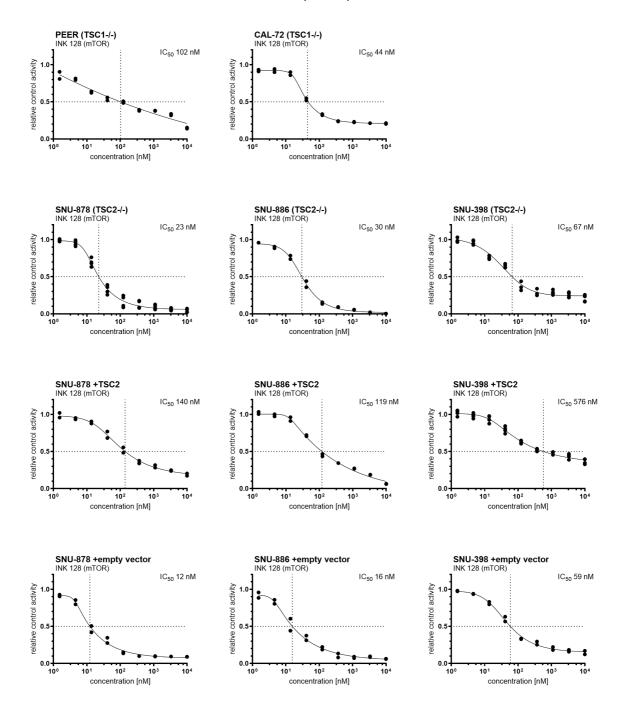
Torin2 (mTOR)





Torin2 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. Torin2 is an mTORC1/2 inhibitor. Among the tested mTOR inhibitors, Torin2 showed the lowest IC50 for each of the 5 cell lines.

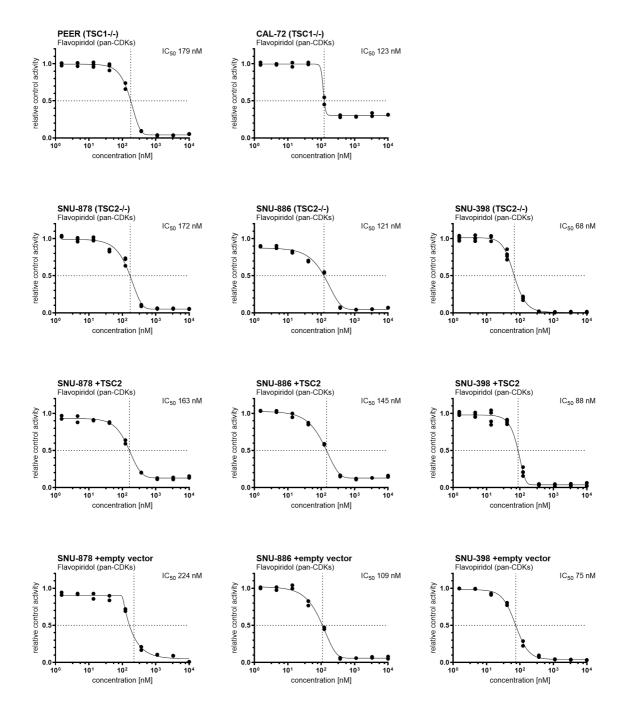
INK 128 (mTOR)



S7 Fig. Cell viability after INK 128 treatment

INK 128 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. INK 128 is an mTORC1/2 inhibitor. All TSC1 or TSC2 deficient tumor cell lines were very sensitive to all tested mTORC1/2 inhibitors.

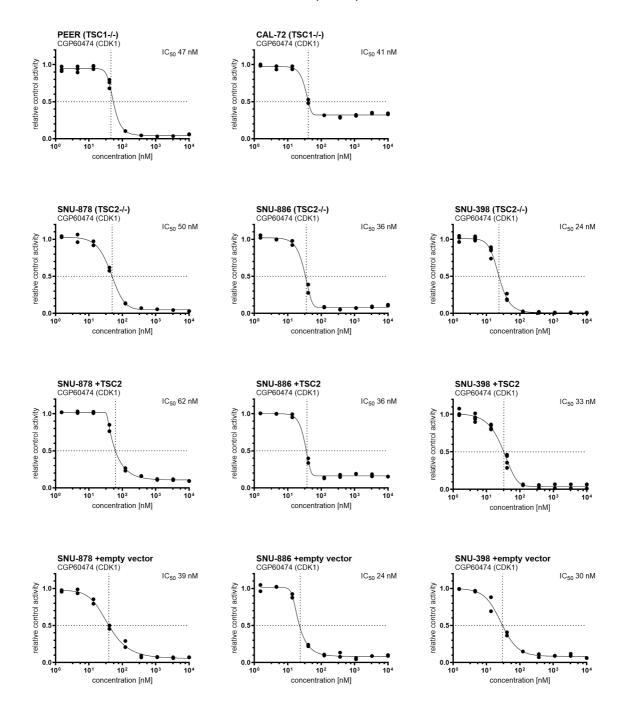
Flavopiridol (pan-CDKs)



S8 Fig. Cell viability after Flavopiridol treatment

Flavopiridol was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. Flavopiridol is a CDKs inhibitor. All cell lines were sensitive to Flavopiridol.

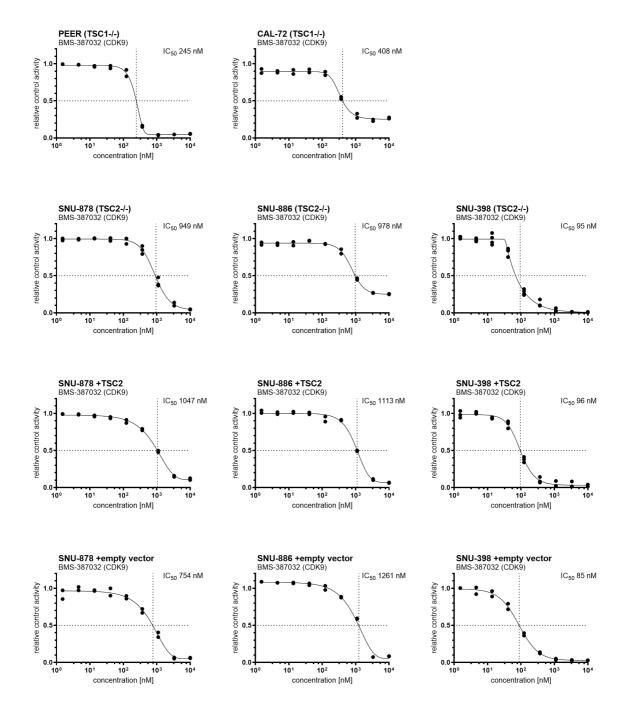
CGP60474 (CDK1)





CGP60474 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. CGP60474 is CDKs and mTOR inhibitor. All cell lines were sensitive to CGP60474.

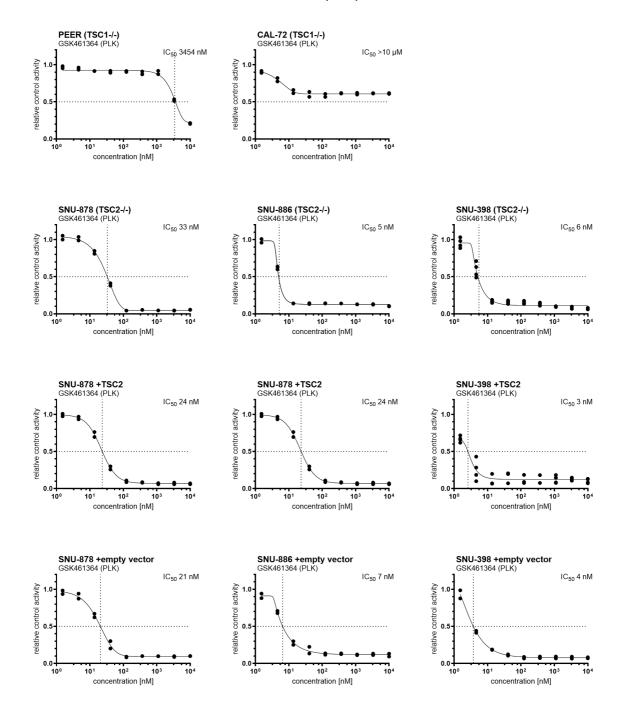
BMS-387032 (CDK9)





BMS-387032 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. BMS-387032 is a CDKs inhibitor. SNU-398 cells were the most sensitive cell line to BMS-387032.

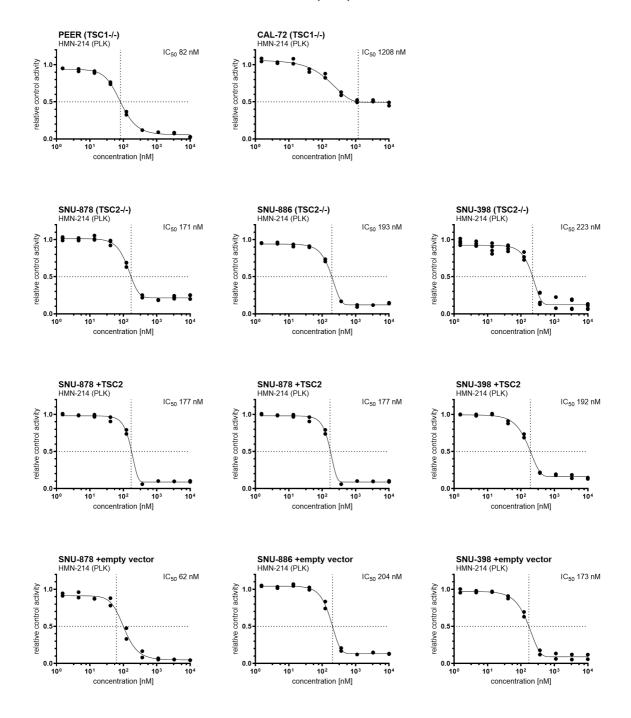
GSK461364 (PLK)

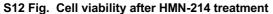




GSK461364 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. GSK461364 is a PLK inhibitor. The cell lines SNU-886, SNU-878, and SNU-398 were sensitive to GSK461364, while in contrast the cell lines PEER and CAL-72 were not sensitive.

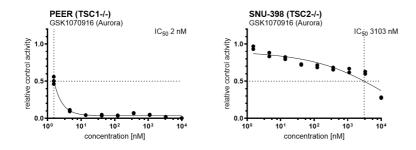
HMN-214 (PLK)





HMN-214 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. HMN-214 is a PLK inhibitor. PEER cells were most sensitive to HMN-214.

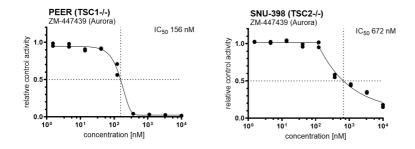
GSK1070916 (Aurora)



S13 Fig. Cell viability after GSK1070916 treatment

GSK1070916 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. GSK1070916 is an Aurora A, B and C inhibitor. TSC1 null PEER cells were very sensitive to all Aurora inhibitors, in contrast to TSC2 null SNU-398 cells, which were much less sensitive to Aurora inhibitors.

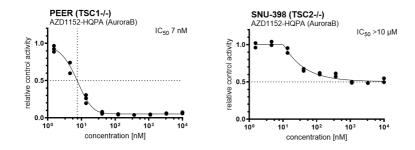
ZM-447439 (Aurora)



S14 Fig. Cell viability after ZM-447439 treatment

ZM-447439 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. ZM-447439 is an Aurora A and B inhibitor. TSC1 null PEER cells were very sensitive to all Aurora inhibitors, in contrast to TSC2 null SNU-398 cells, which were much less sensitive to Aurora inhibitors.

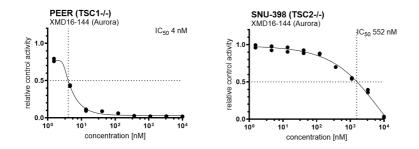
AZD1152-HQPA (AuroraB)



S15 Fig. Cell viability after AZD1152-HQPA treatment

AZD1152-HQPA was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. AZD1152-HQPA is an Aurora A, B and C inhibitor. TSC1 null PEER cells were very sensitive to all Aurora inhibitors, in contrast to TSC2 null SNU-398 cells, which were much less sensitive to Aurora inhibitors.

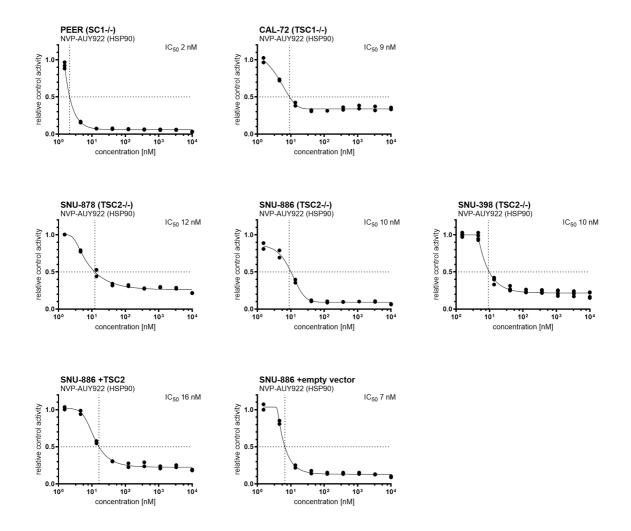
XMD16-144 (Aurora)



S16 Fig. Cell viability after XMD16-144 treatment

XMD16-144 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. XMD16-144 is an Aurora A and B inhibitor. TSC1 null PEER cells were very sensitive to all Aurora inhibitors, in contrast to TSC2 null SNU-398 cells, which were much less sensitive to Aurora inhibitors.

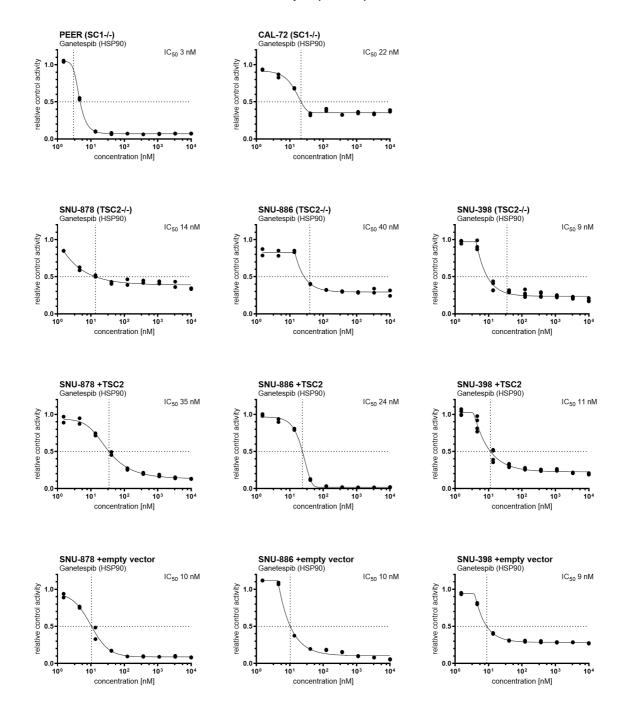
NVP-AUY922 (HSP90)



S17 Fig. Cell viability after NVP-AUY922 treatment

NVP-AUY922 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. NVP-AUY922 is an HSP90 inhibitor. All cell lines were very sensitive to NVP-AUY922.

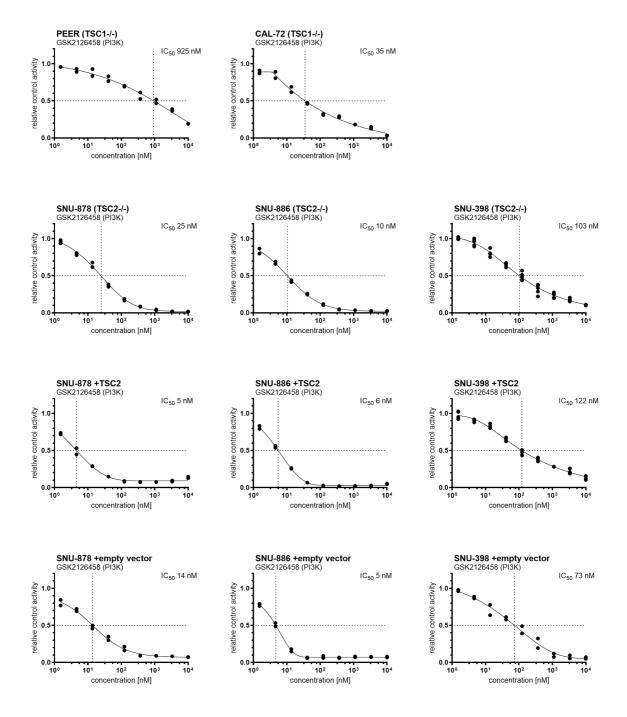
Ganetespib (HSP90)





Ganetespib was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. Ganetespib is an HSP90 inhibitor. All cell lines were very sensitive to ganetespib.

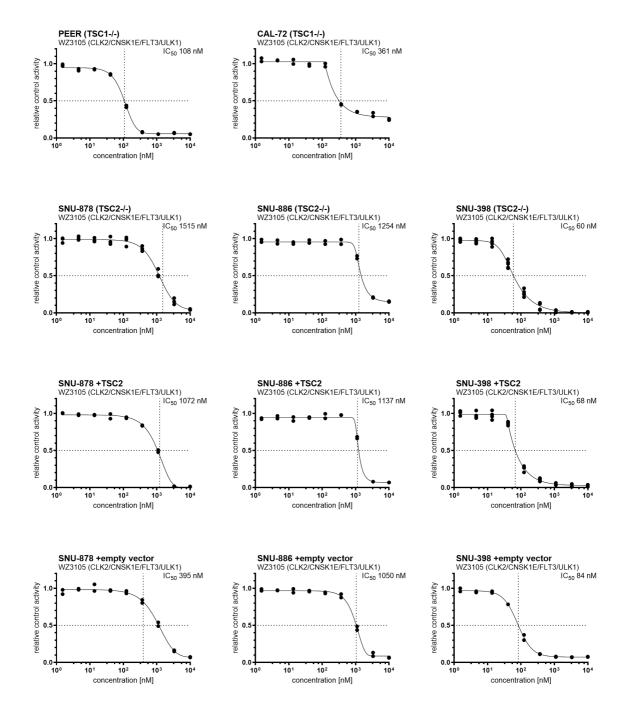
GSK2126458 (PI3K)

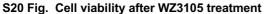




GSK2126458 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. GSK2126458 is a PI3K inhibitor. CAL-72 und the SNU cell lines were sensitive to GSK2126458.

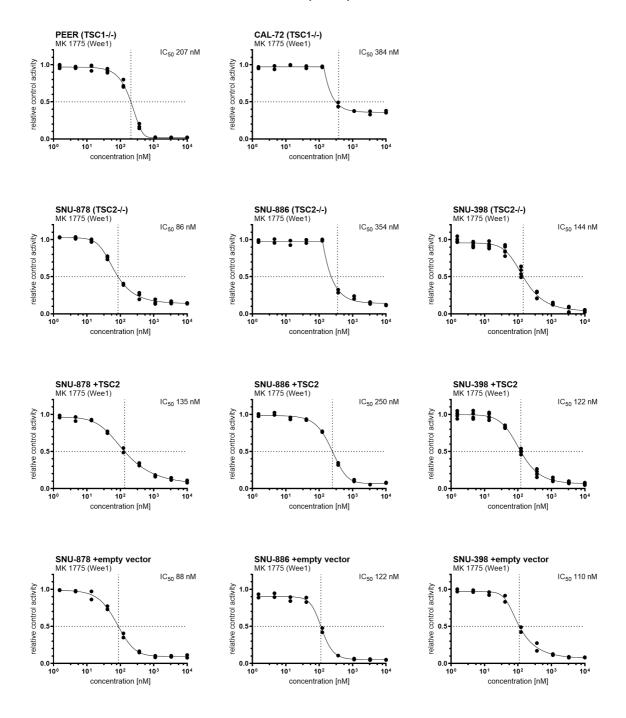
WZ3105 (CLK2/CNSK1E/FLT3/ULK1)

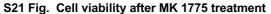




WZ3105 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. WZ3105 is a CLK2, CNSK1E, FLT3 and ULK1 inhibitor. PEER, CAL-72 and SNU-398 cells were sensitive to WZ3105.

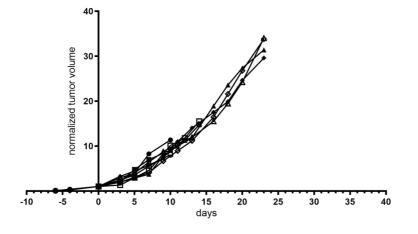
MK 1775 (Wee1)



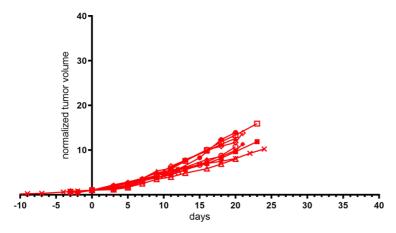


MK 1775 was serially diluted three-fold from 10 μ M to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-5. IC₅₀ was calculated as the drug concentration that reduced cell viability by 50% compared to untreated cells. MK 1775 is a Wee1 inhibitor. All cell lines were sensitive to MK 1775.

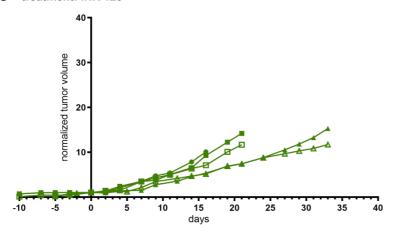
a treatment: vehicle



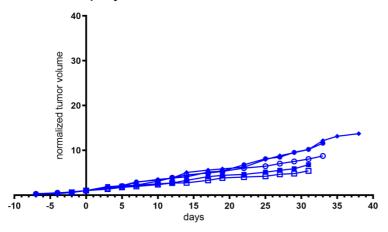
b treatment: Ganetespib



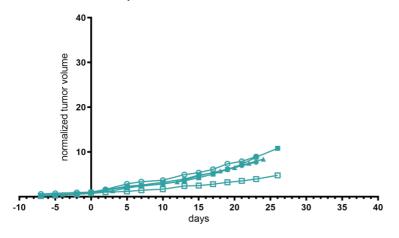




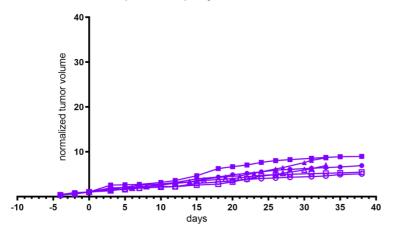
d treatment: Rapamycin



e treatment: Ganetespib and INK 128



f treatment: Ganetespib and Rapamycin





Tumor volume is shown as normalized tumor volume to day 1 of treatment. Tumors were measured every 2-3 days. Each tumor is depicted separately, n= 5- 9 per treatment. Mice were treated with vehicle (a), ganetespib (50mg/kg, 1x/week, i.v.) (b), INK 128 (1mg/kg, 5x/week, i.g.) (c), rapamycin (3 mg/kg, 3x/week, i.p.) (d), or ganetespib and rapamycin combined (same doses) (e) or ganetespib and INK 128 combined (same doses) (f). Tumors under treatment grew less compared to vehicle-treated tumors.