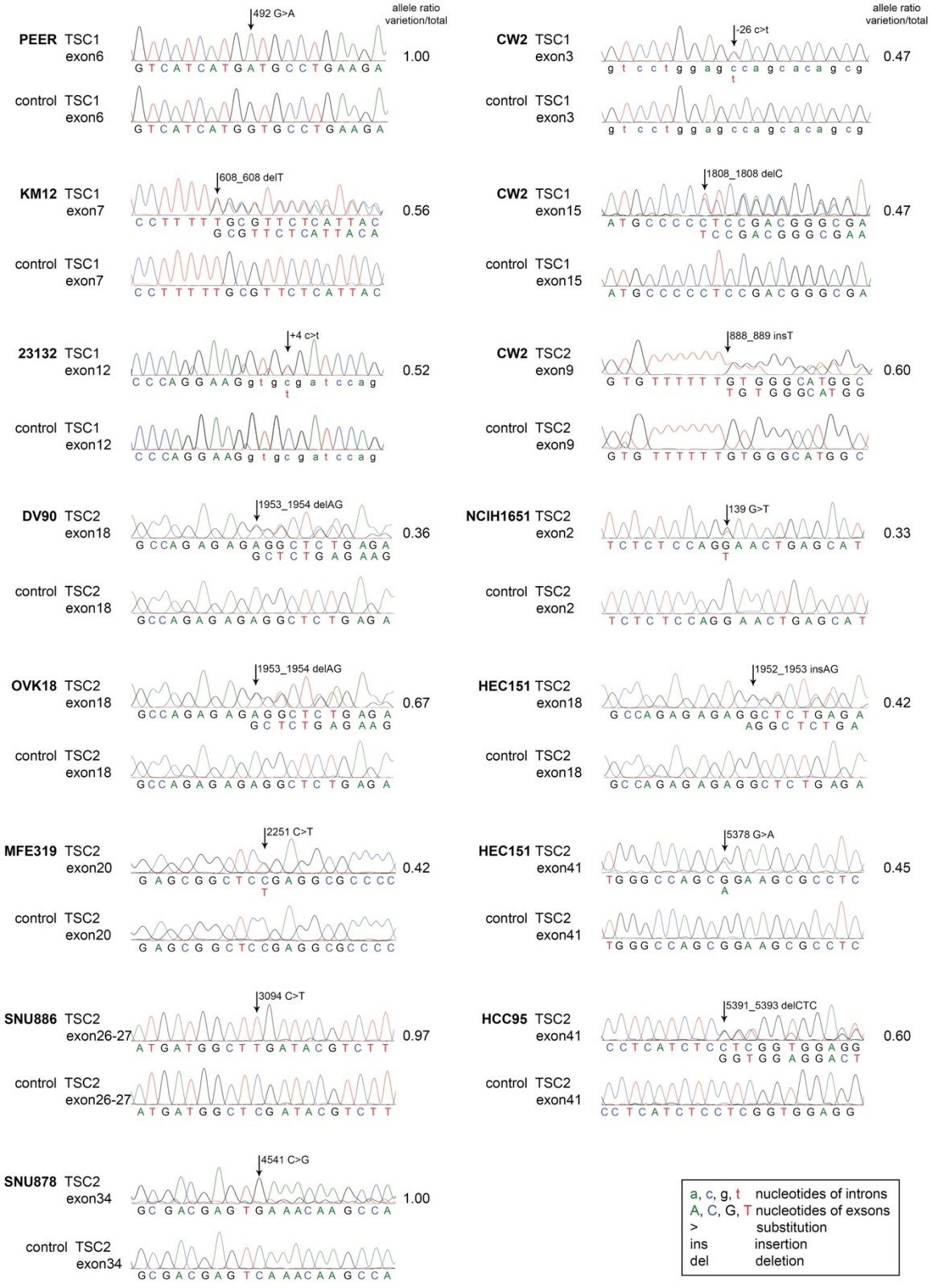
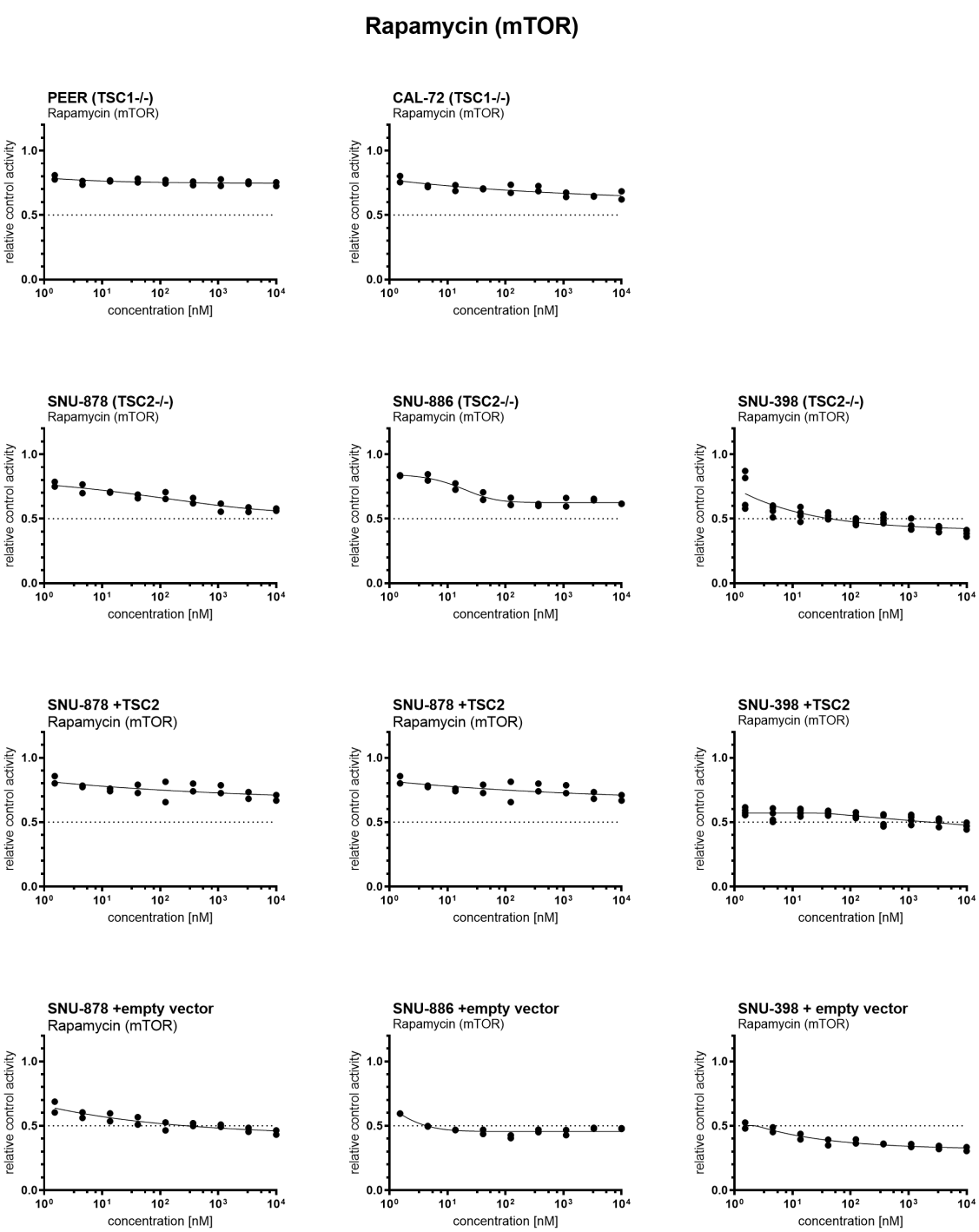


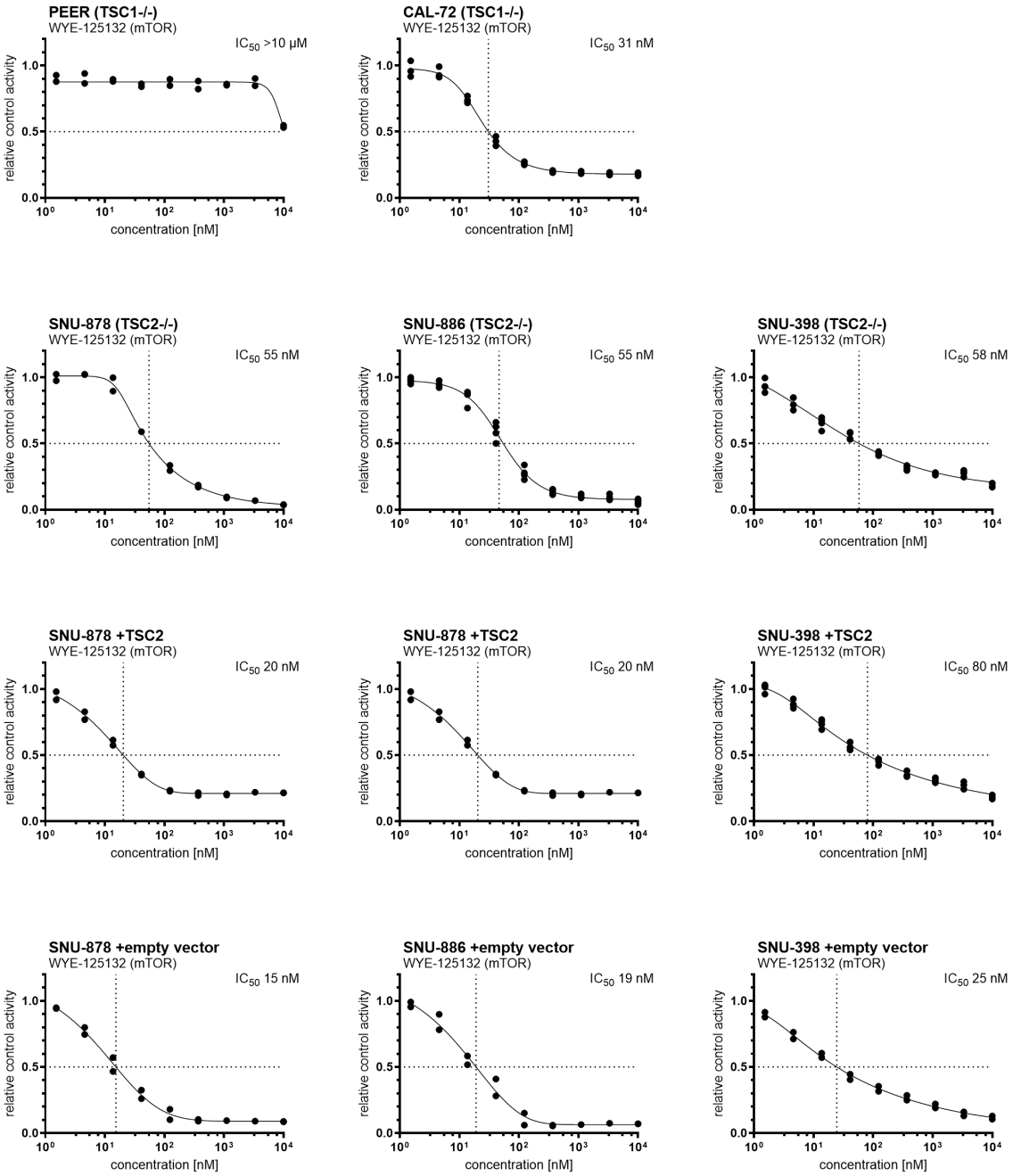
S1 Fig. Confirmation of reported mutations by Sanger sequencing

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.



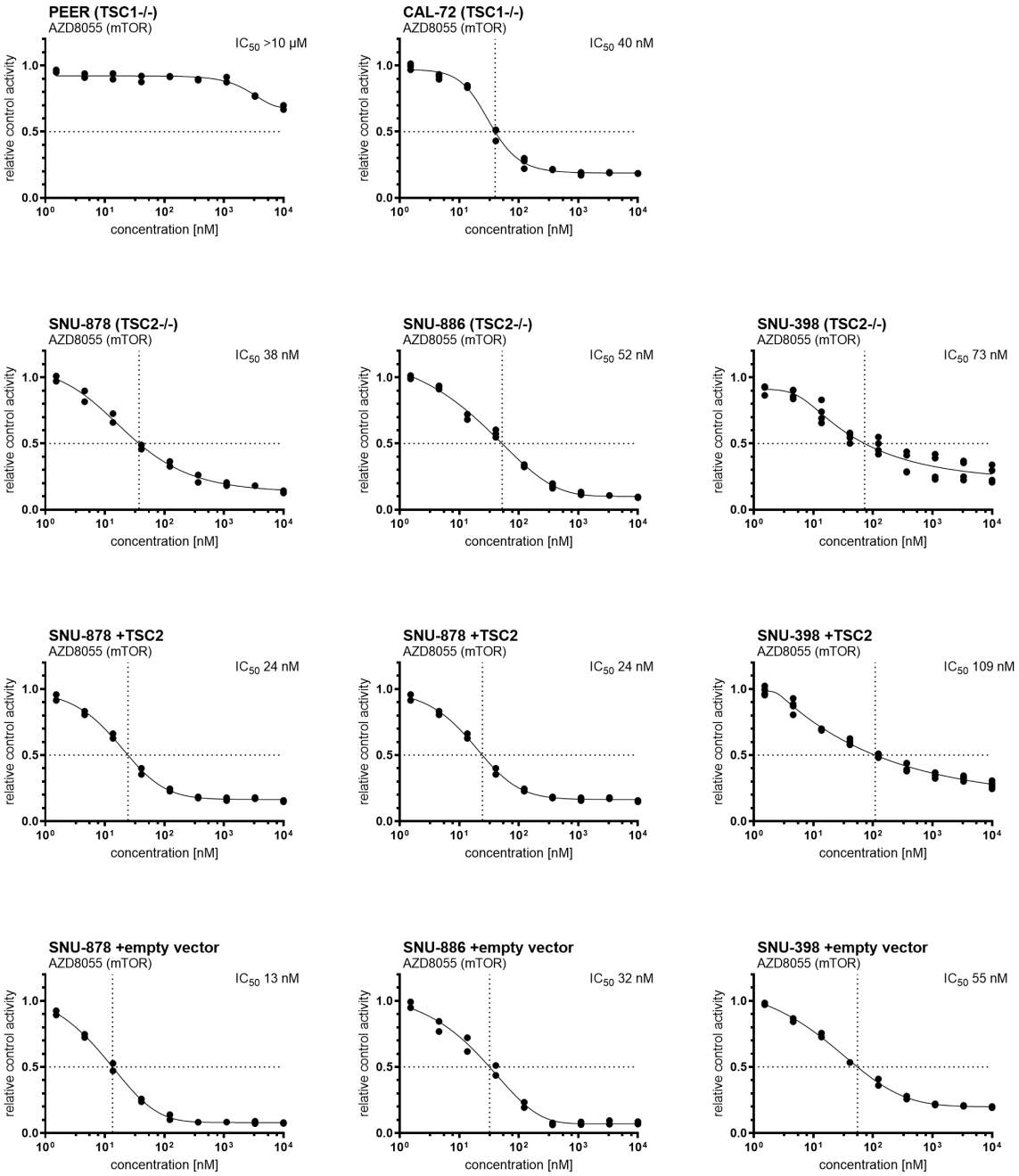
S2 Fig. IC50 determination for Rapamycin

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.



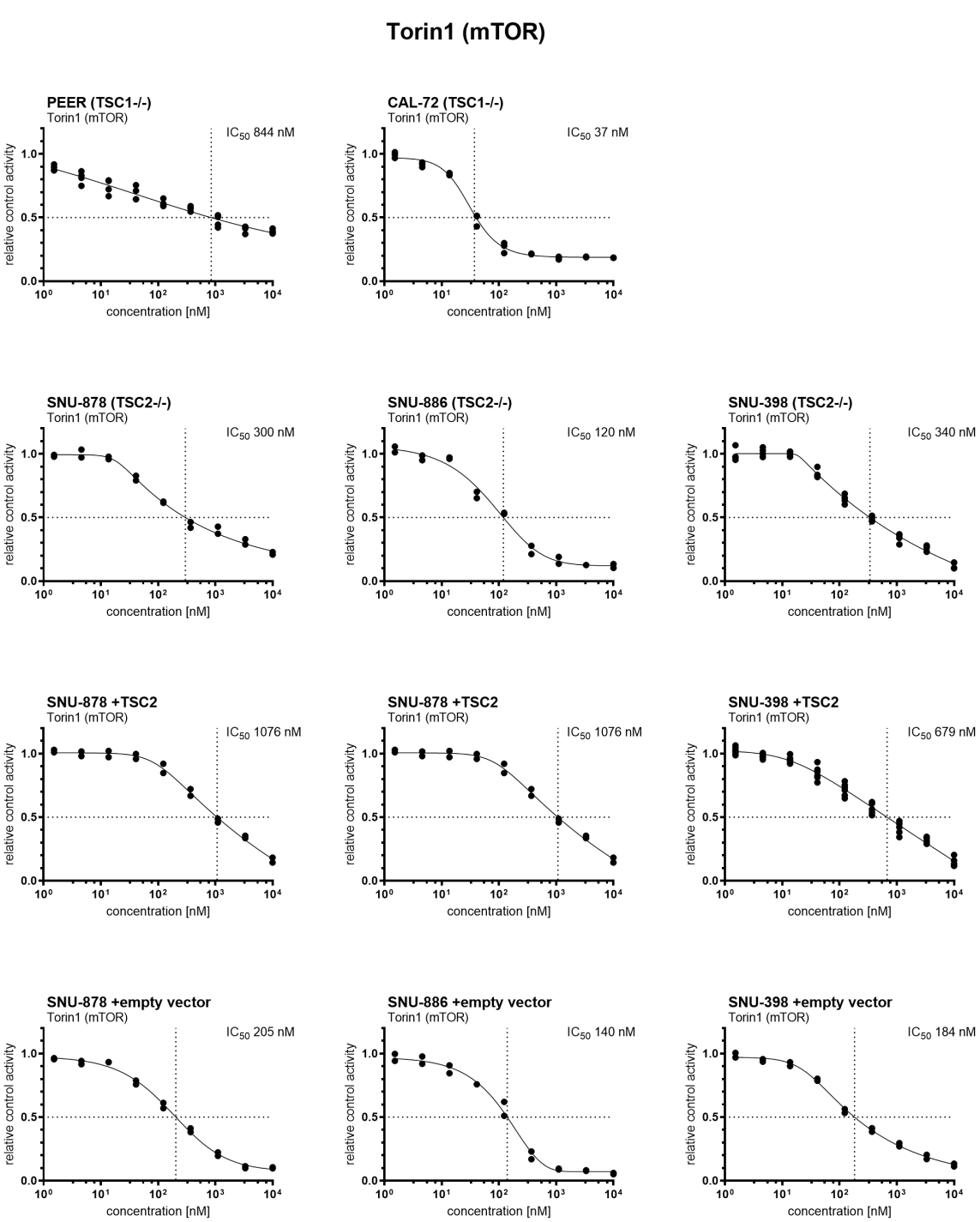
S3 Fig. Cell viability after WYE-125132 treatment

WYE-125132 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 in relative control activity, n= 2-4. IC_{50} 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.



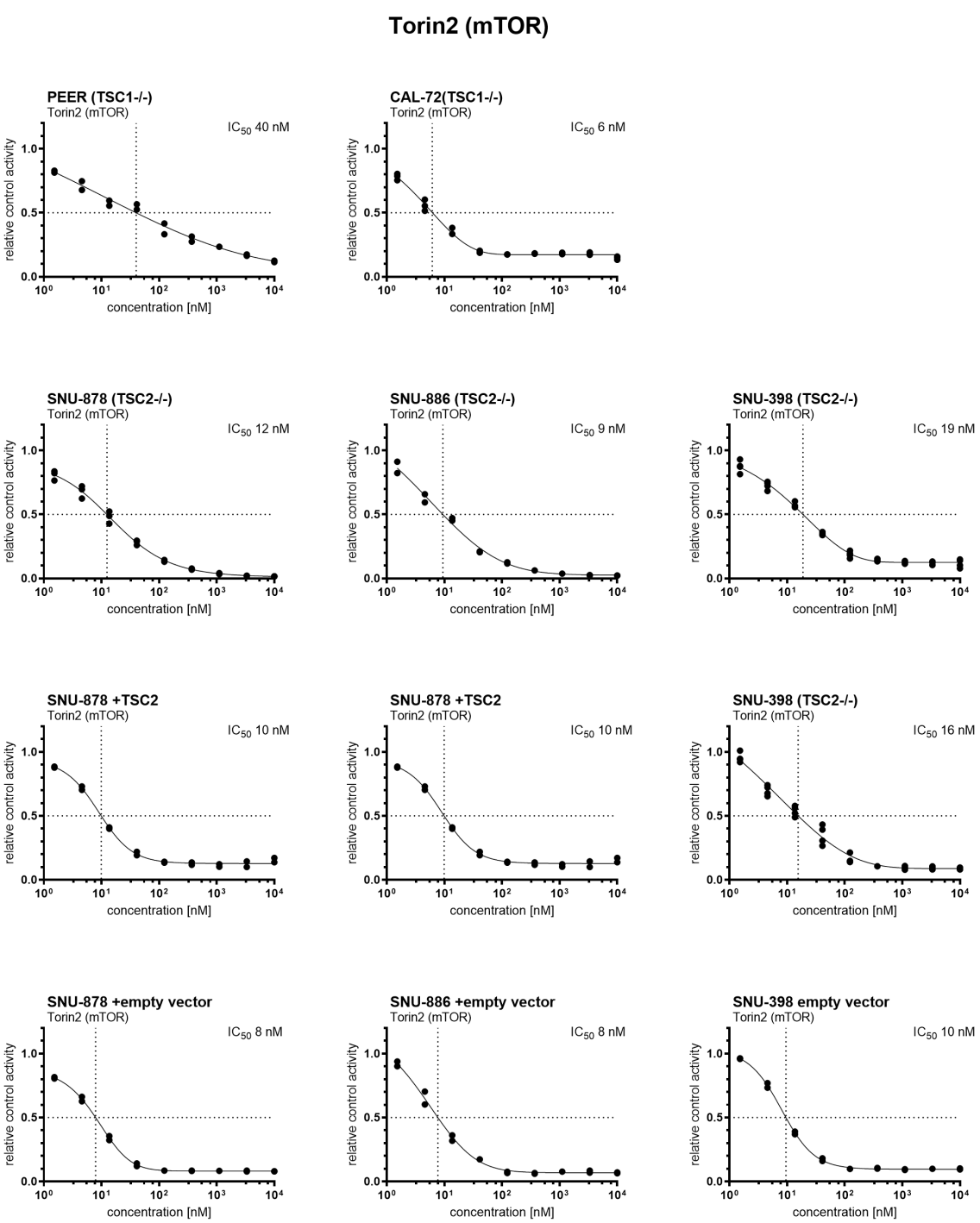
S4 Fig. Cell viability after AZD8055 treatment

AZD8055 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 in relative control activity, n= 2-4. IC_{50} 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.



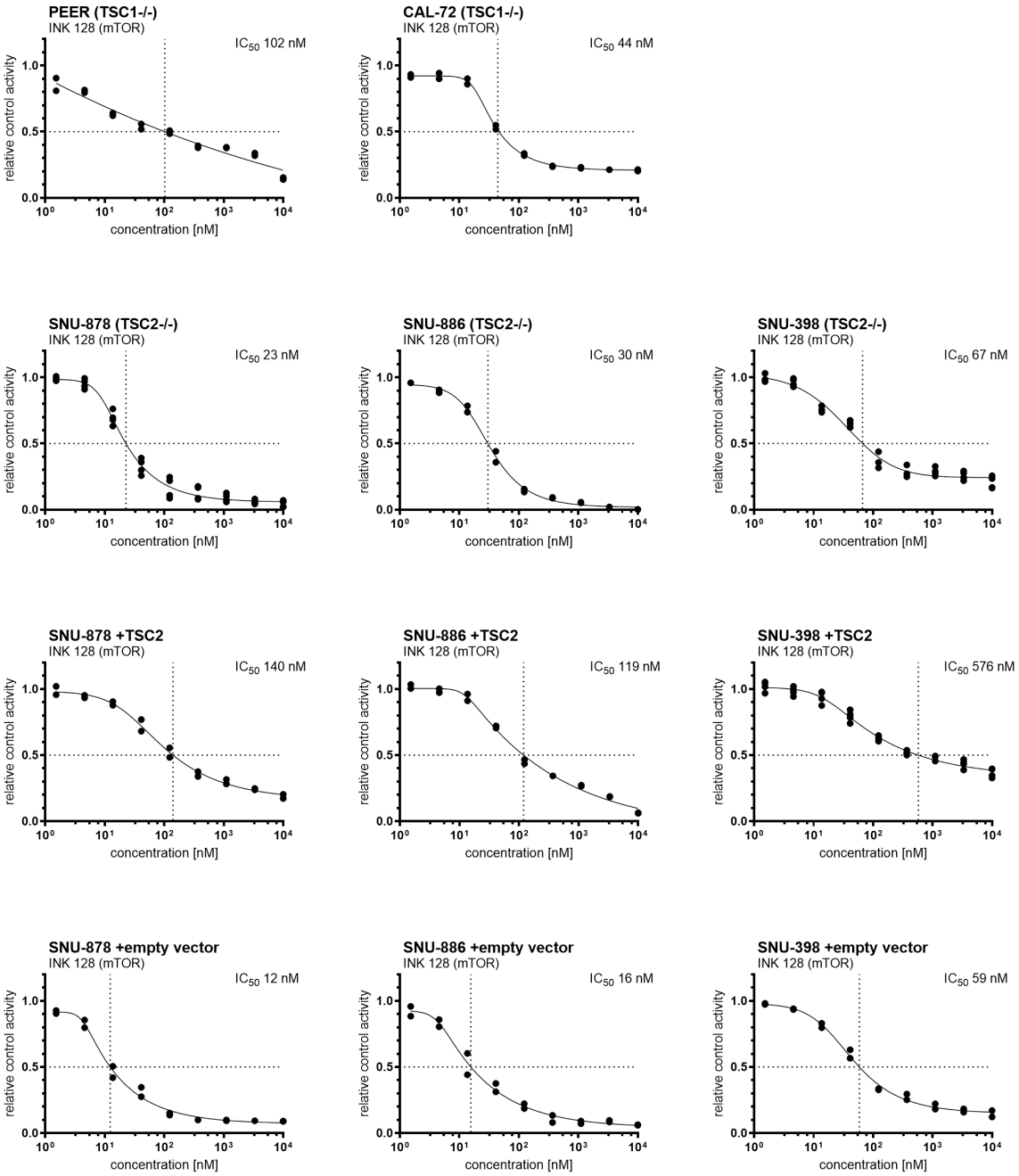
S5 Fig. Cell viability after Torin1 treatment

Torin1 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 in relative control activity, n= 2-6. IC_{50} 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.



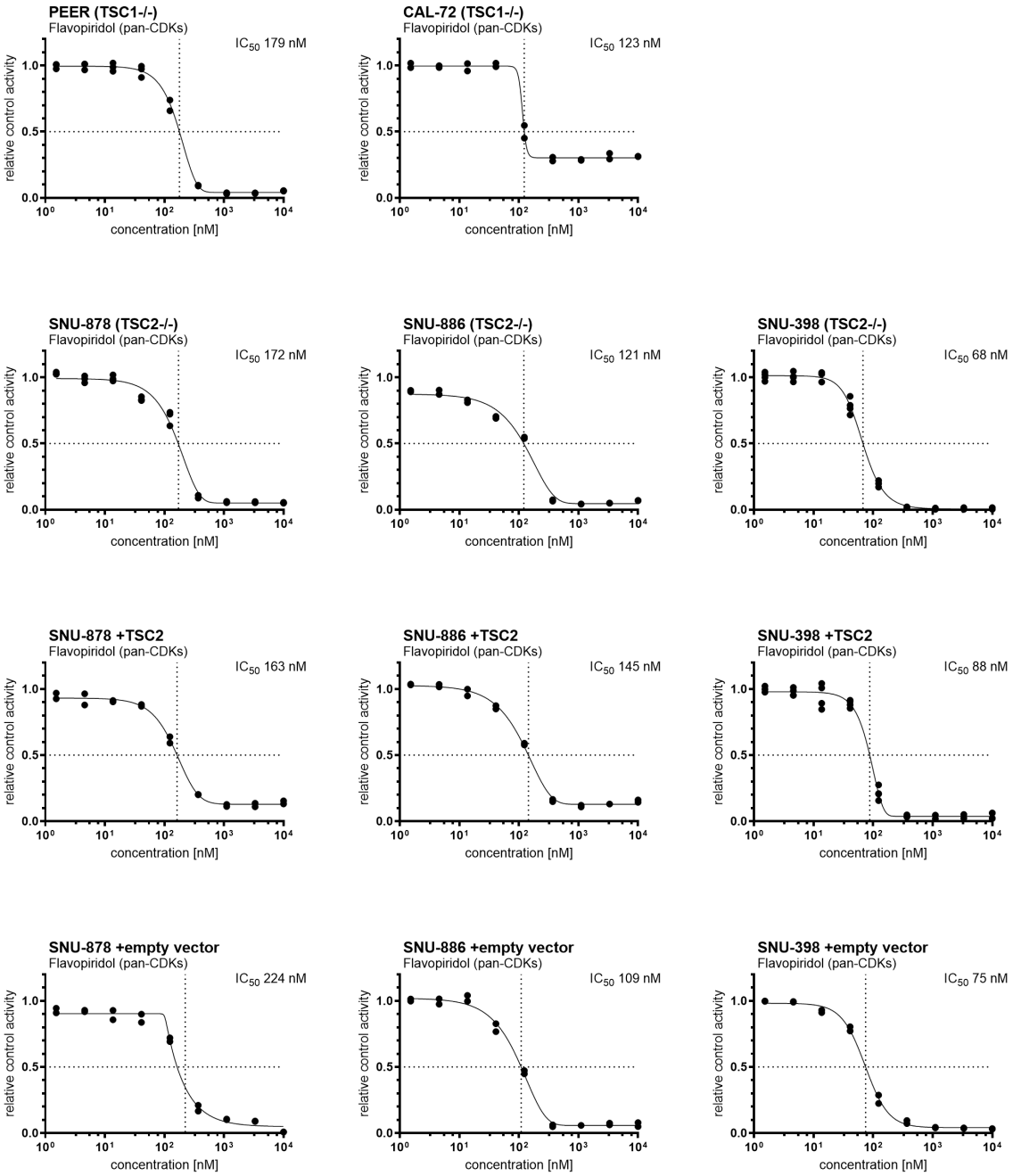
S6 Fig. Cell viability after Torin2 treatment

Torin2 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 in relative control activity, n= 2-4. IC_{50} 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.



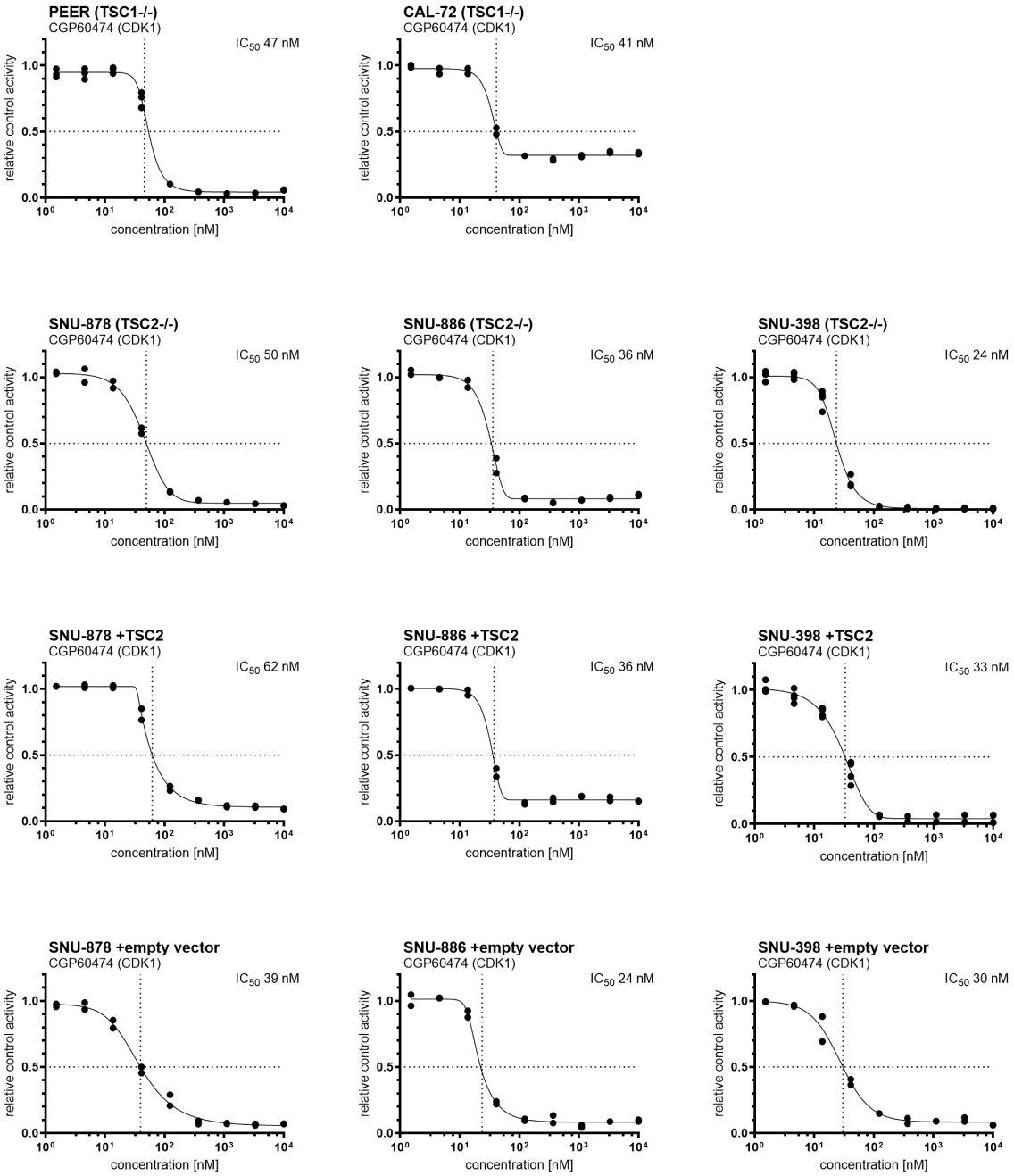
S7 Fig. Cell viability after INK 128 treatment

INK 128 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 in relative control activity, n= 2-4. IC_{50} 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.



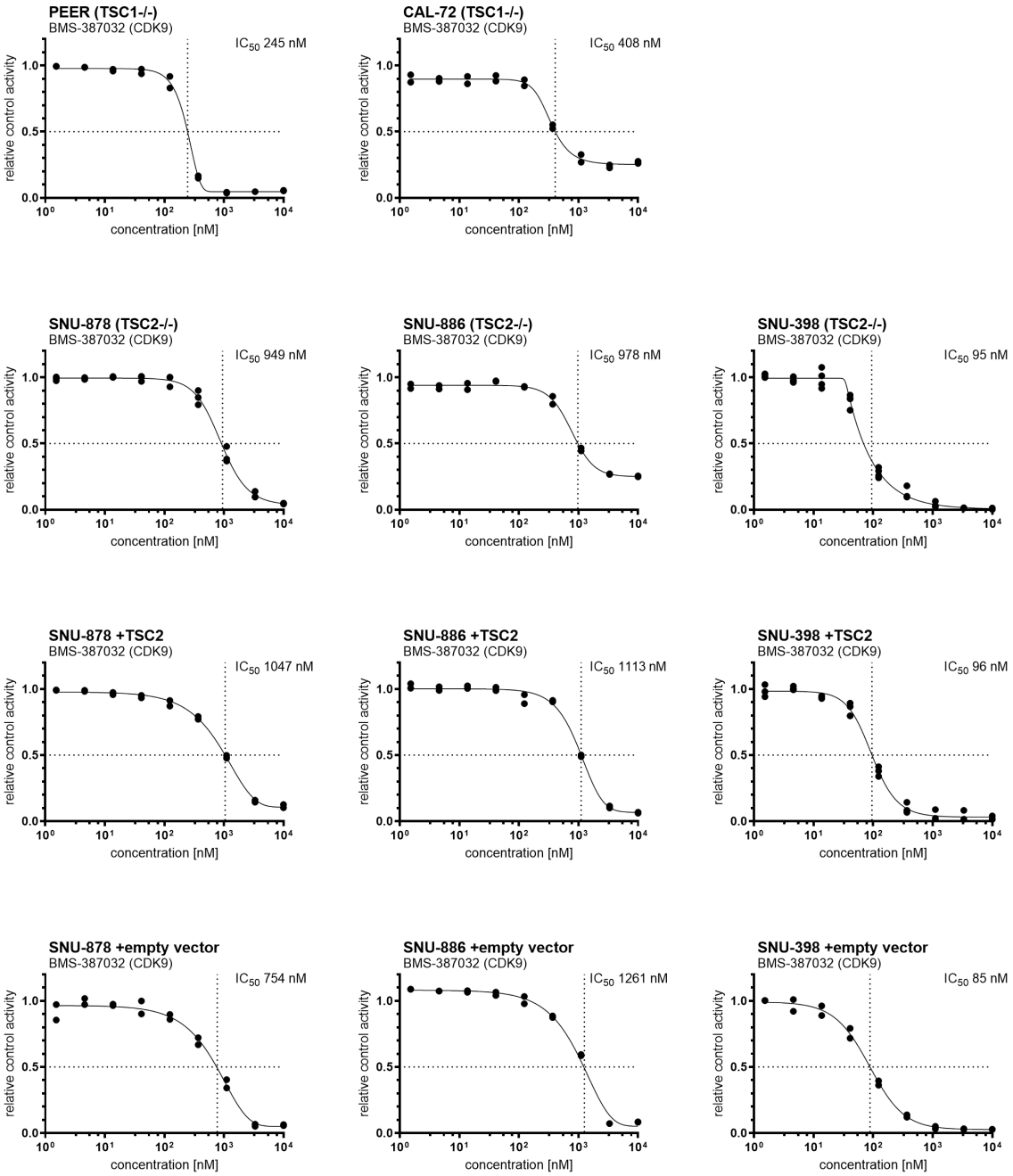
S8 Fig. Cell viability after Flavopiridol treatment

Flavopiridol 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 in relative control activity, n= 2-4. IC_{50} 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.



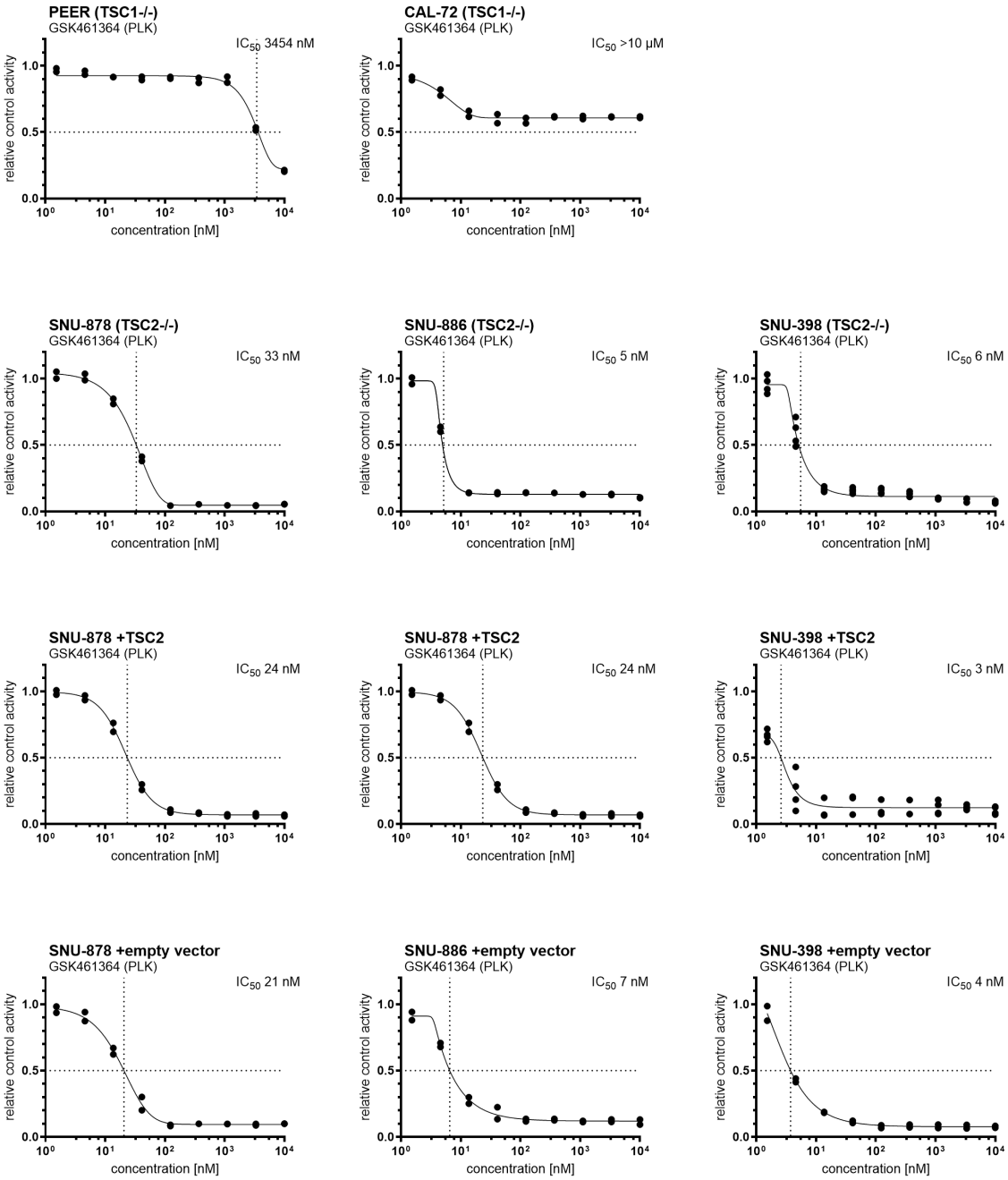
S9 Fig. Cell viability after CGP60474 treatment

CGP60474 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 in relative control activity, n= 2-4. IC_{50} 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.



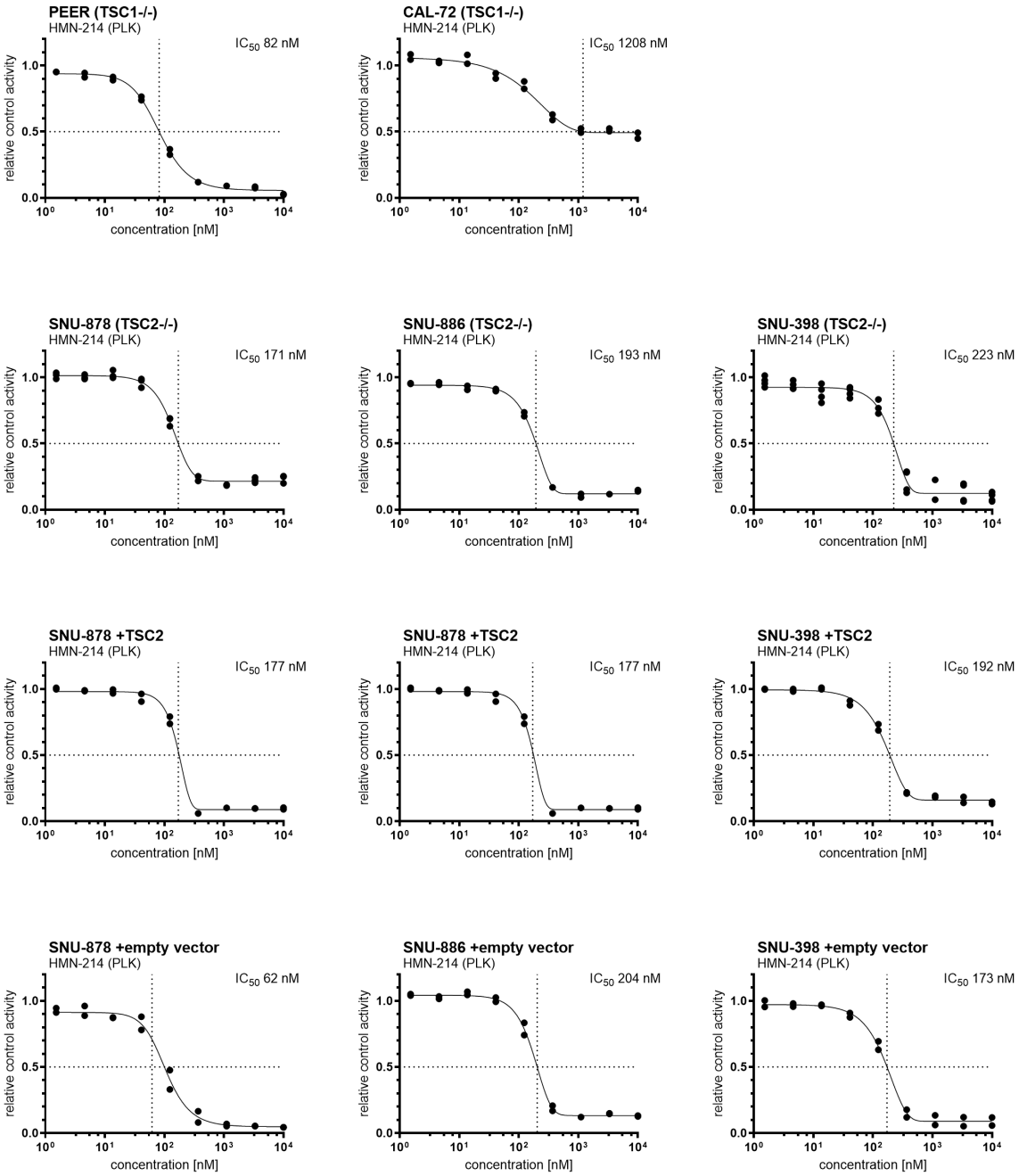
S10 Fig. Cell viability after BMS-387032 treatment

BMS-387032 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 in relative control activity, n= 2-4. IC_{50} 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.



S11 Fig. Cell viability after GSK461364 treatment

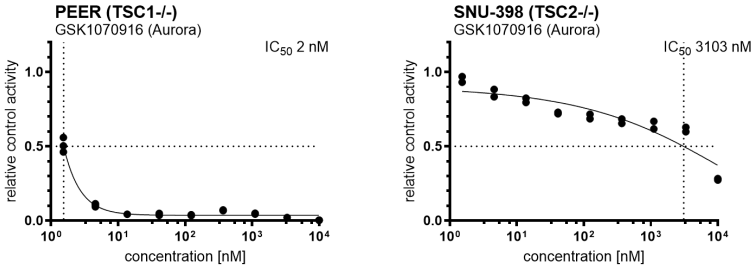
GSK461364 was serially diluted three-fold from 10 µM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% 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.



S12 Fig. Cell viability after HMN-214 treatment

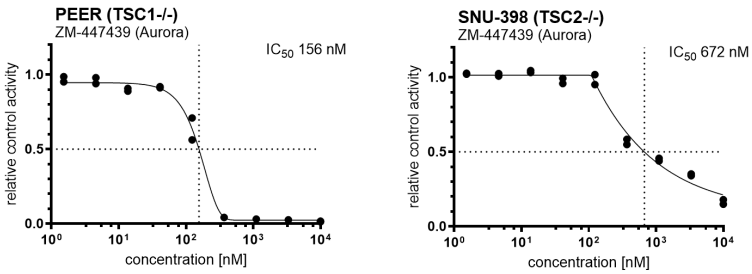
HMN-214 was serially diluted three-fold from 10 μM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% 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

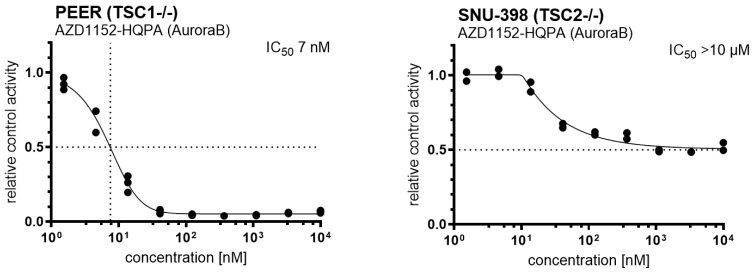
GSK1070916 was serially diluted three-fold from 10 μM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% 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.



S14 Fig. Cell viability after ZM-447439 treatment

ZM-447439 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 in relative control activity, n= 2. IC_{50} 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.

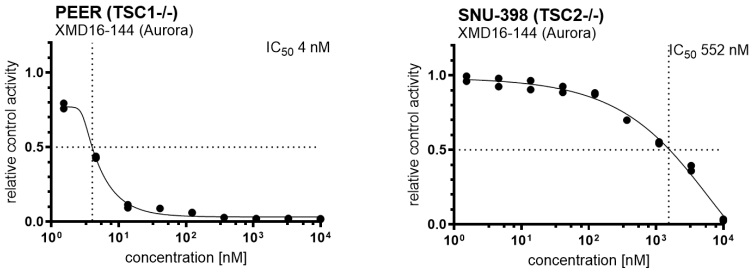
AZD1152-HQPA (AuroraB)



S15 Fig. Cell viability after AZD1152-HQPA treatment

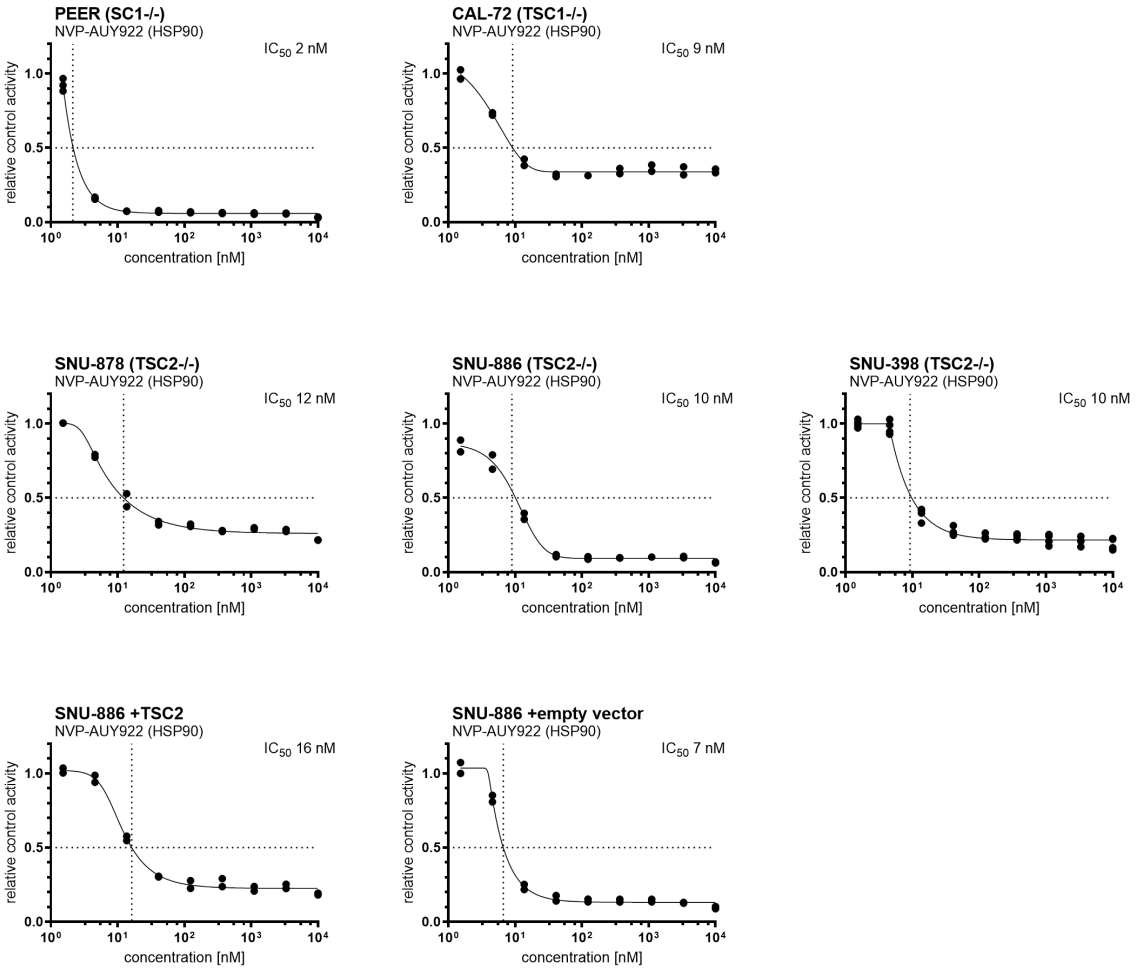
AZD1152-HQPA was serially diluted three-fold from 10 µM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% 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)



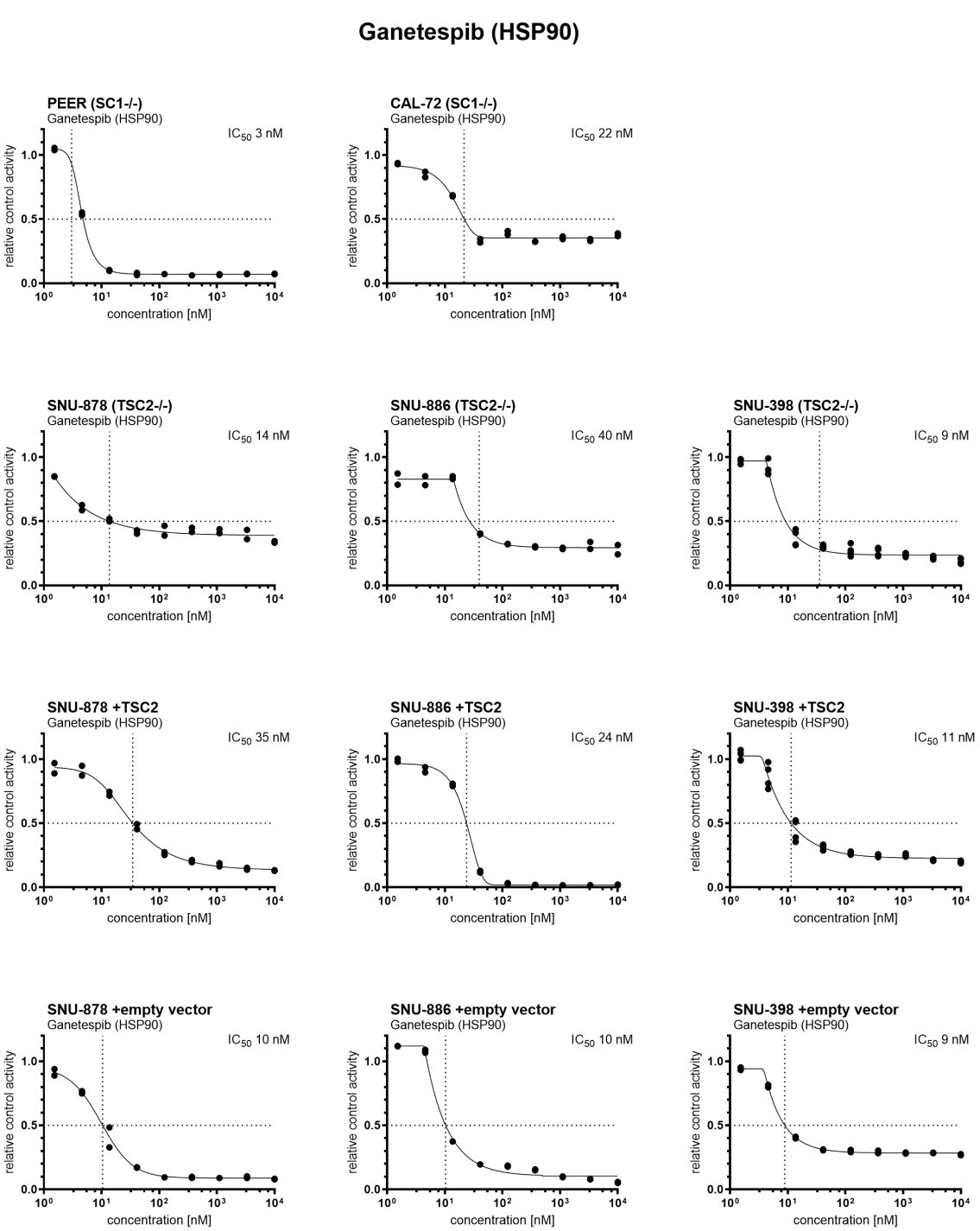
S16 Fig. Cell viability after XMD16-144 treatment

XMD16-144 was serially diluted three-fold from 10 μM 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. IC_{50} 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.



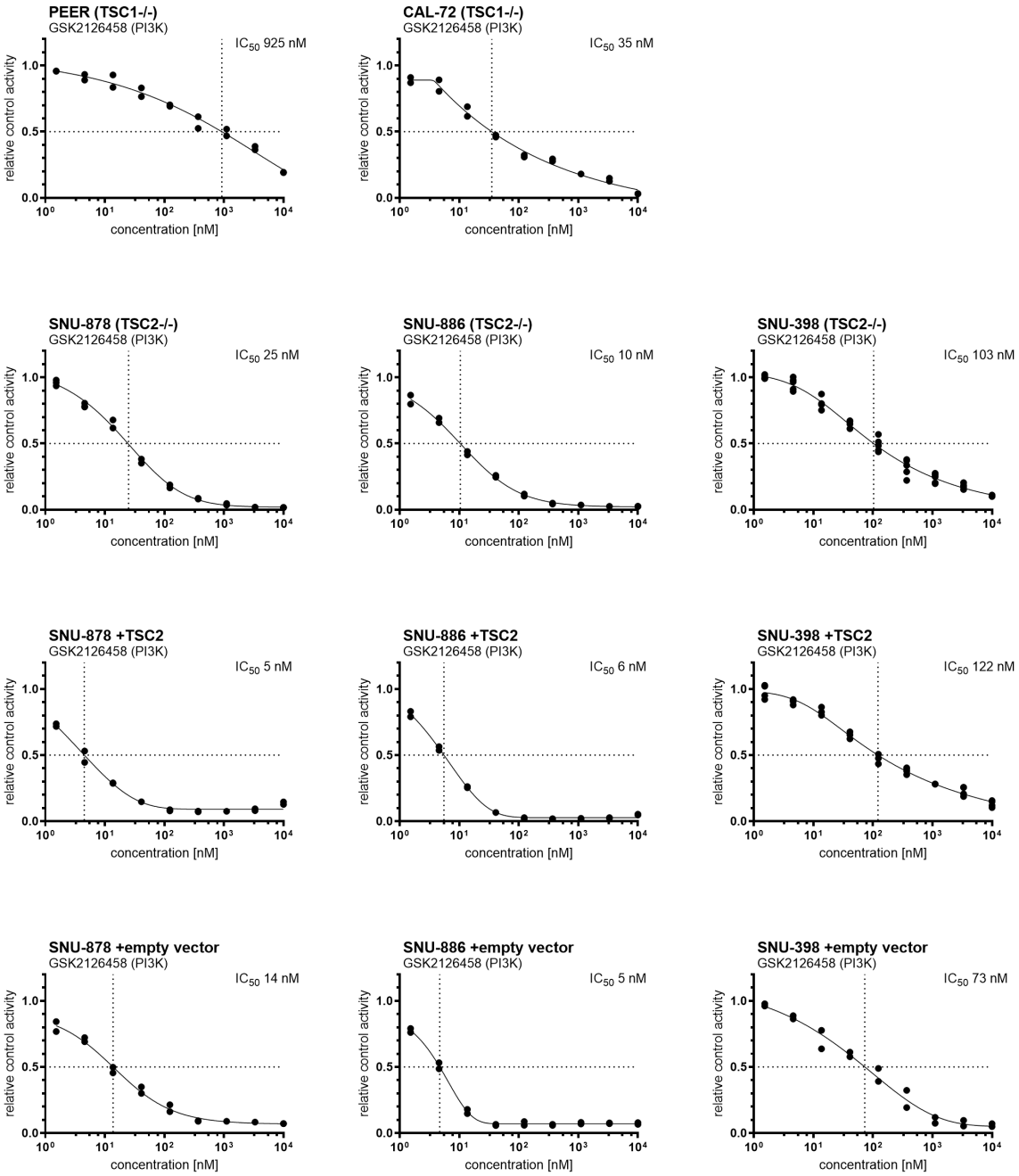
S17 Fig. Cell viability after NVP-AUY922 treatment

NVP-AUY922 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 in relative control activity, n= 2-4. IC_{50} 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.



S18 Fig. Cell viability after ganetespib treatment

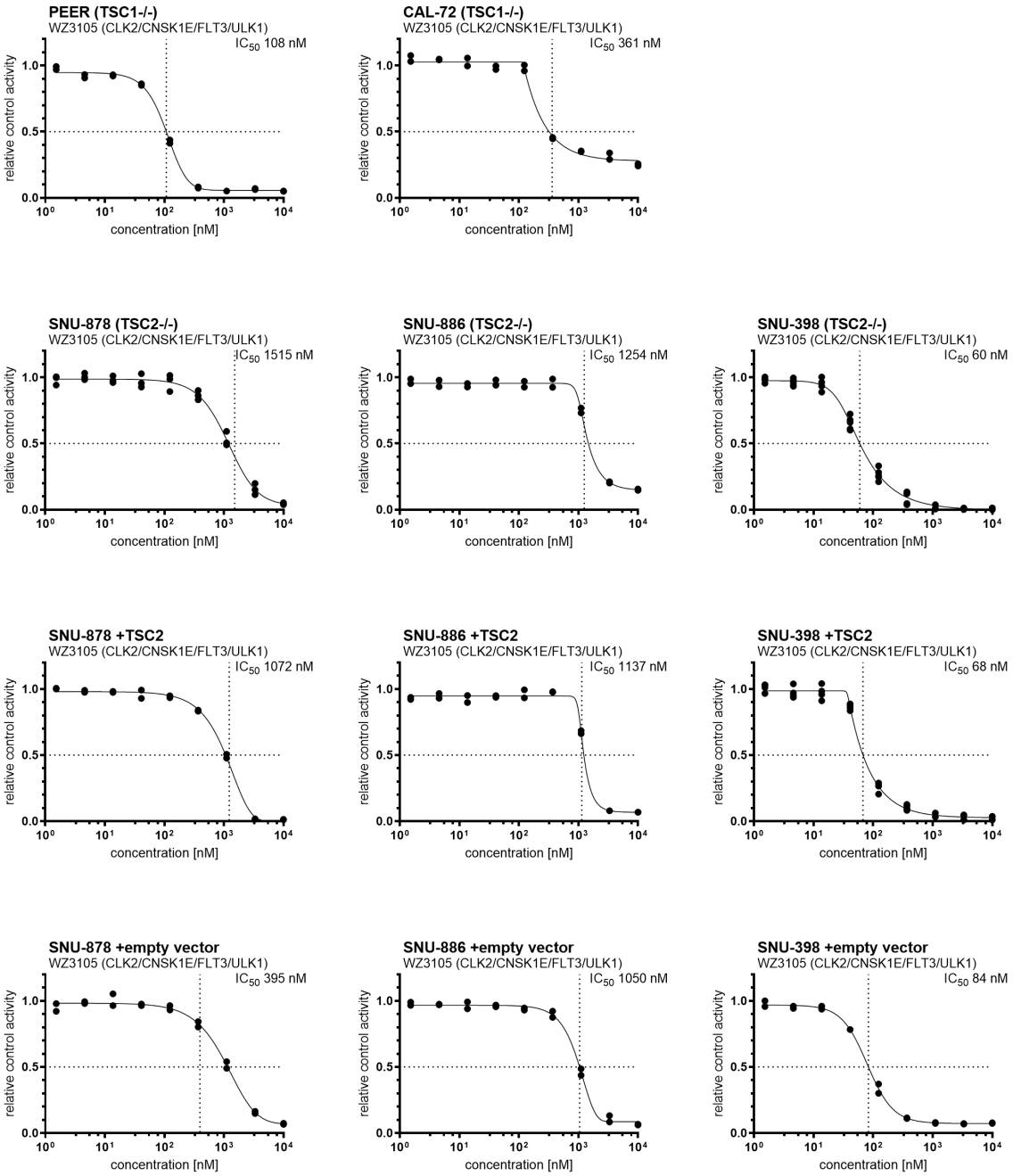
Ganetespib was serially diluted three-fold from 10 μM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2-4. IC_{50} was calculated as the drug concentration that reduced cell viability by 50%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



S19 Fig. Cell viability after GSK2126458 treatment

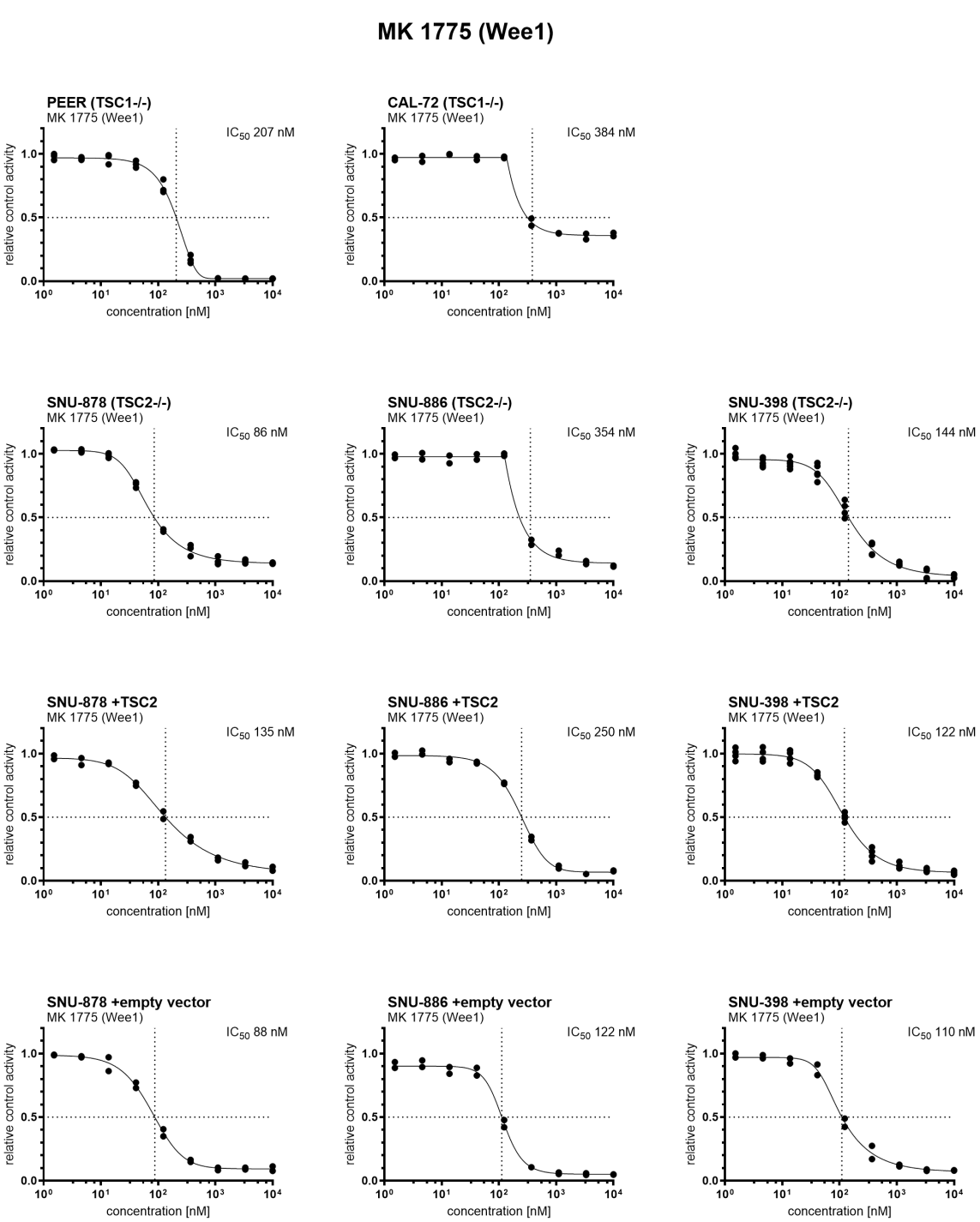
GSK2126458 was serially diluted three-fold from 10 µM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% 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)



S20 Fig. Cell viability after WZ3105 treatment

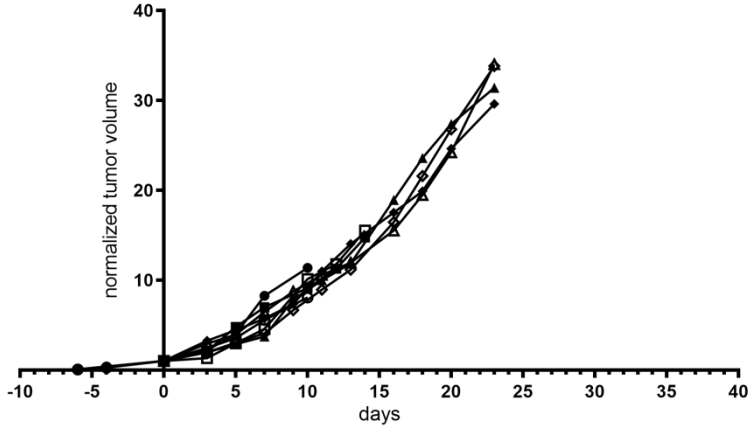
WZ3105 was serially diluted three-fold from 10 μM to 1.5 nM. Cell viability was determined 72h after treatment using CellTiter-Glo and XLfit4.0 software. Cell viability is shown in relative control activity, n= 2. IC_{50} was calculated as the drug concentration that reduced cell viability by 50% compared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



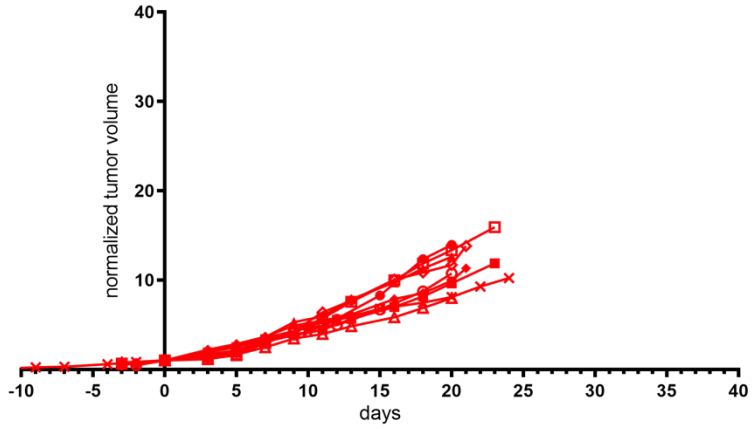
S21 Fig. Cell viability after MK 1775 treatment

MK 1775 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 in relative control activity, n= 2-5. IC_{50} 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.

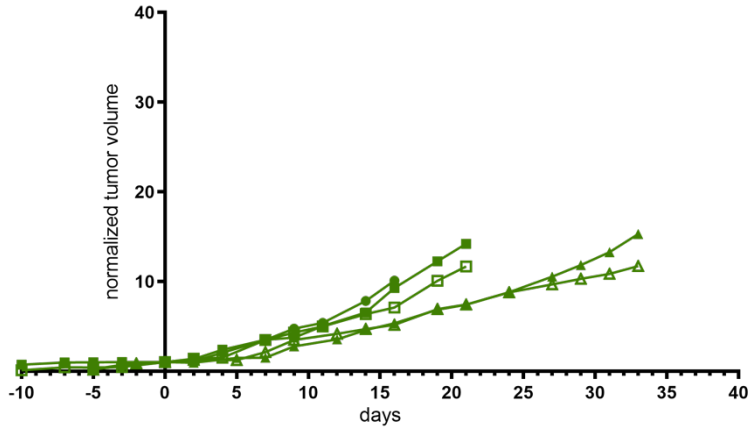
a **treatment: vehicle**

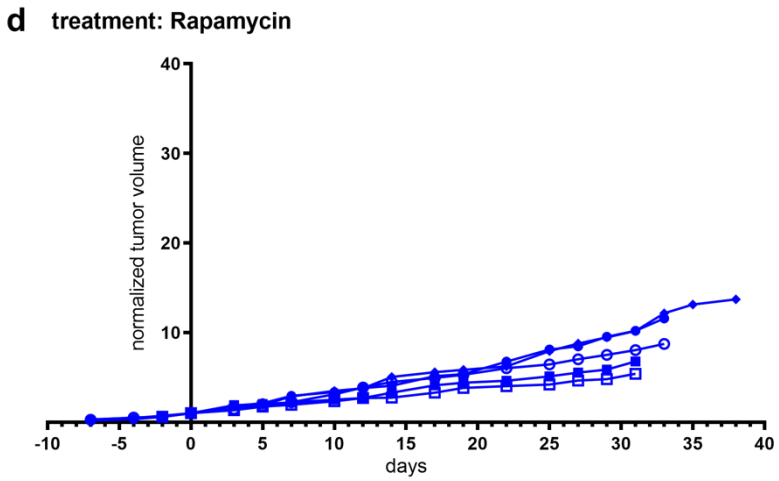


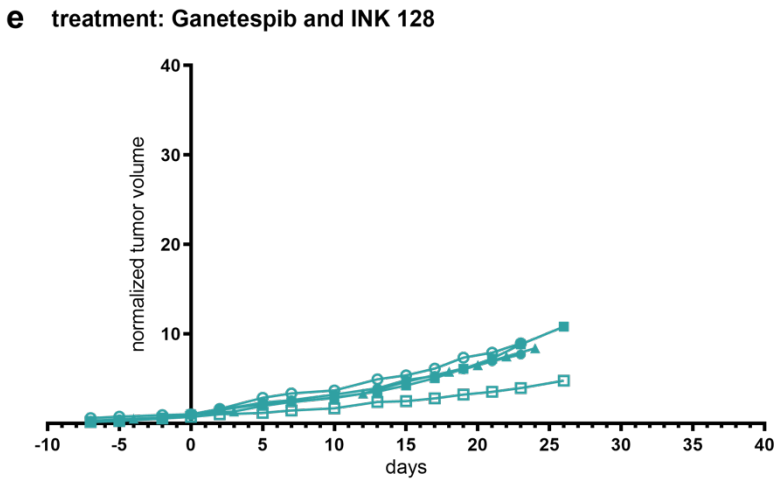
b **treatment: Ganetespib**

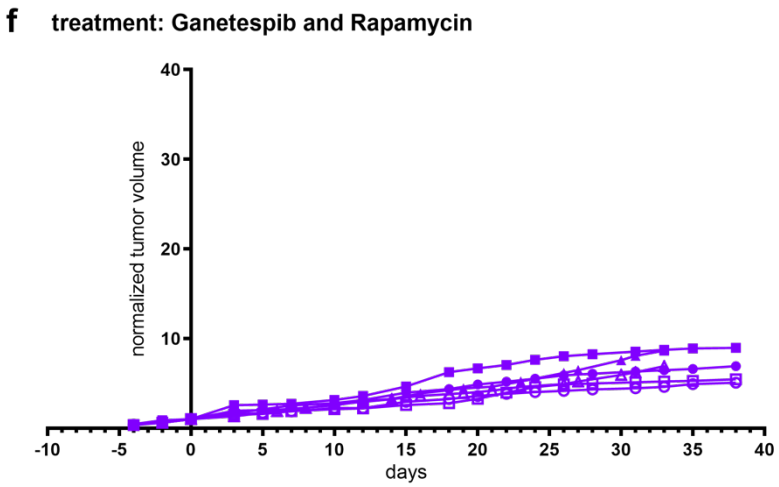


c **treatment: INK 128**









S22 Fig. S-398 tumor xenograft treatment.

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.