Supplementary Materials:

Functional Therapeutic Target Validation Using Pediatric Zebrafish Xenograft Models

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All drugs detected in at least one of the culture models of the rhabdoid tumor sample INF_R_1288_r1 as a TOP25 hit are listed in **Table S1.** For each model it is noted, whether the drug was within the TOP25 hits (yes) or not (no). For the INF_R_1288_r1-derived models, 13 drugs overlapped in all three models.

Table S1. TOP25 Drug Hits for INF_R_1288_r1

Drug name	TOP25 FTC	TOP25 LTC	TOP25 mPDX-C
AMG-232	yes	yes	yes
bortezomib	yes	yes	yes
cytarabine	yes	yes	yes
dactinomycin	yes	yes	yes
daunorubicin	yes	yes	yes
idasanutlin	yes	yes	yes
panobinostat	yes	yes	yes
ponatinib	yes	yes	yes
selinexor	yes	yes	yes
staurosporine_drug[1]	yes	yes	yes
topotecan	yes	yes	yes
trametinib	yes	yes	yes
vinorelbine	yes	yes	yes
A-1155463	yes	yes	no

A-1210477	yes	yes	no
ceritinib	yes	yes	no
entinostat	yes	yes	no
navitoclax	yes	yes	no
A-1331852	no	yes	yes
doxorubicin	no	yes	yes
gemcitabine	no	yes	yes
mitoxantrone	no	yes	yes
crizotinib	yes	no	yes
vinblastine	yes	no	yes
vincristine	yes	no	yes
cisplatin	yes	no	no
sunitinib	yes	no	no
temsirolimus	yes	no	no
vorinostat	yes	no	no
cobimetinib	no	yes	no
everolimus	1no	yes	no
thioguanine	no	yes	no
axitinib	no	no	yes
etoposide	no	no	yes
methotrexate	no	no	yes
paclitaxel	no	no	yes
rapamycin (sirolimus)	no	no	yes

[1] assay control

All drugs detected in at least one of the culture models of the eRMS tumor sample INF_R_1467_r1 as a TOP25 hit are listed in **Table S2.** For each model it is noted, whether the drug was within the TOP25 hits (yes) or not (no). For the INF_R_1467_r1-derived models, 16 drugs overlapped in all three models.

Table S2. TOP25 Drug Hits for INF_R_1467_r1

Drug name	TOP25 FTC	TOP25 LTC	TOP25 mPDX-C
A-1155463	yes	yes	yes
A-1210477	yes	yes	yes
A-1331852	yes	yes	yes
AMG-232	yes	yes	yes
bortezomib	yes	yes	yes
dactinomycin	yes	yes	yes
everolimus	yes	yes	yes
I-BET151	yes	yes	yes
idasanutlin	yes	yes	yes
navitoclax	yes	yes	yes
panobinostat	yes	yes	yes
selinexor	yes	yes	yes
staurosporine_drug [1]	yes	yes	yes
topotecan	yes	yes	yes
vincristine	yes	yes	yes
vorinostat	yes	yes	yes
cobimetinib	yes	yes	no
trametinib	yes	yes	no
rapamycin (sirolimus)	no	yes	yes
temsirolimus	no	yes	yes
vinorelbine	no	yes	yes
crizotinib	yes	no	yes
daunorubicin	yes	no	yes
entinostat	yes	no	yes
doxorubicin	yes	no	no

imatinib	yes	no	no
ponatinib	yes	no	no
thioguanine	yes	no	no
cytarabine	no	yes	no
gemcitabine	no	yes	no
mitoxantrone	no	yes	no
paclitaxel	no	yes	no
ceritinib	no	no	yes
vinblastine	no	no	yes
volasertib	no	no	yes

[1] assay control

All drugs detected in at least one of the culture models of the neuroblastoma tumor sample INF_R_359_r3 as a TOP25 hit are listed in **Table S3.** For each model it is noted, whether the drug was within the TOP25 hits (yes) or not (no). For the INF_R_359_r3-derived models, 21 drugs overlapped in all three models.

Table S3. TOP25 Drug Hits for INF_R_359_r3

Drug name	TOP25 FTC	TOP25 LTC	TOP25 mPDX-C
AMG-232	yes	yes	yes
navitoclax	yes	yes	yes
vinorelbine	yes	yes	yes
bortezomib	yes	yes	yes
topotecan	yes	yes	yes
idasanutlin	yes	yes	yes
talazoparib	yes	yes	yes
selinexor	yes	yes	yes
mitoxantrone	yes	yes	yes
etoposide	yes	yes	yes
A-1155463	yes	yes	yes
paclitaxel	yes	yes	yes
daunorubicin	yes	yes	yes
doxorubicin	yes	yes	yes
staurosporine_drug [1]	yes	yes	yes
A-1210477	yes	yes	yes
gemcitabine	yes	yes	yes
vincristine	yes	yes	yes
cytarabine	yes	yes	yes
ceritinib	yes	yes	yes
panobinostat	yes	yes	yes
dactinomycin	yes	yes	no
irinotecan	yes	yes	no
A-1331852	no	yes	yes
cisplatin	yes	no	yes
ponatinib	yes	no	yes
volasertib	no	yes	no
dasatinib	no	no	yes

[1] assay control

The comparison of the DNA methylation profile and copy number variations of the neuroblastoma tumor sample INF_R_359_r3 is depicted in **Figure S1.**

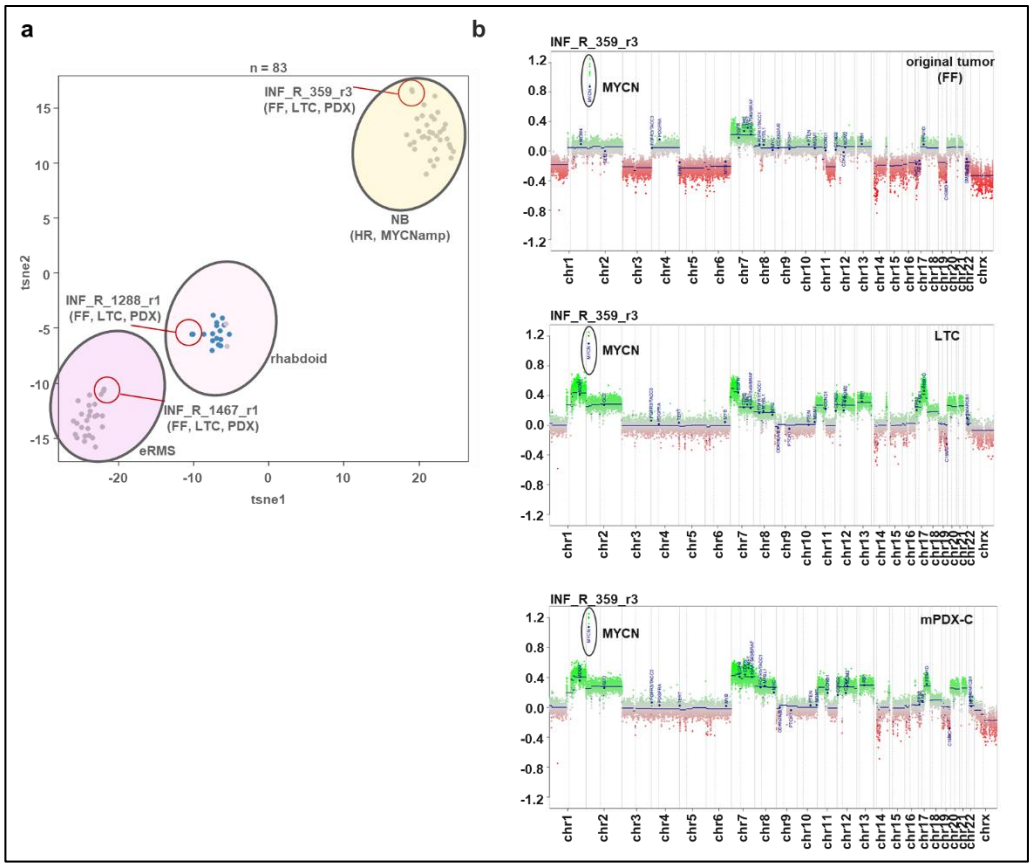

Figure S1: Comparison of the original tumor with matched culture models. (a) t-SNE analysis of DNA methylation profiles for comparison of the original tumors and their tumor-derived culture models LTC and mPDX-C with already existing well-characterized reference tumors (rhabdoid tumors; eRMS tumors and high-risk MYCNamp neuroblastomas). **(b)** Copy-number profiles of the original neuroblastoma tumor INF_R_359_r3, and its LTC and mPDX-C models reveal similar genome-wide methylation patterns and recurrent *MYCN* amplification, characteristic for high-risk neuroblastomas. FF: fresh frozen material of the original tumor; LTC: long-term culture; mPDX-C: mouse-PDX-derived culture; NB: neuroblastoma; HR: high-risk.

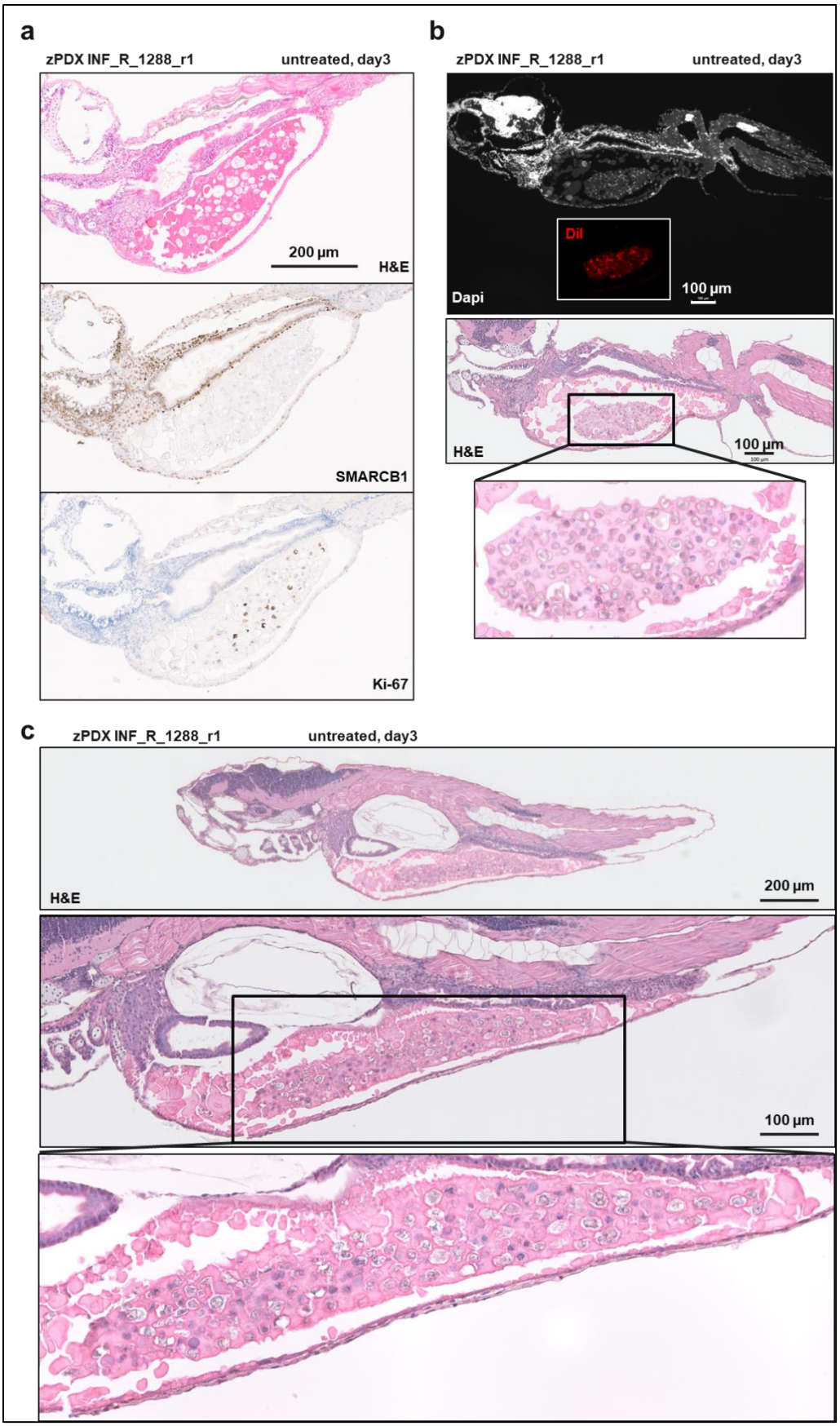

Figure S2: Representative images of immunohistochemistry (IHC) staining. **(a)** Zebrafish early larvae engrafted with INF_R_1288_r1 cells (3 days post implantation) were stained with H&E, with α-SMARCB1, confirming SMARCB1 deletion in engrafted human tumor cells, and with Ki-67 for the detection of mitotic cells. Scale bar = 200 µm. **(b)** Zebrafish early larvae engrafted with INF_R_1288_r1 cells (3 days post implantation) were stained with DAPI and H&E. Scale bar: 100 µm. **(c)** H&E staining of

zebrafish early larvae engrafted with INF_R_1288_r1 cells (3 days post implantation). Scale bar upper panel: 200 µm, middle panel: 100µm.

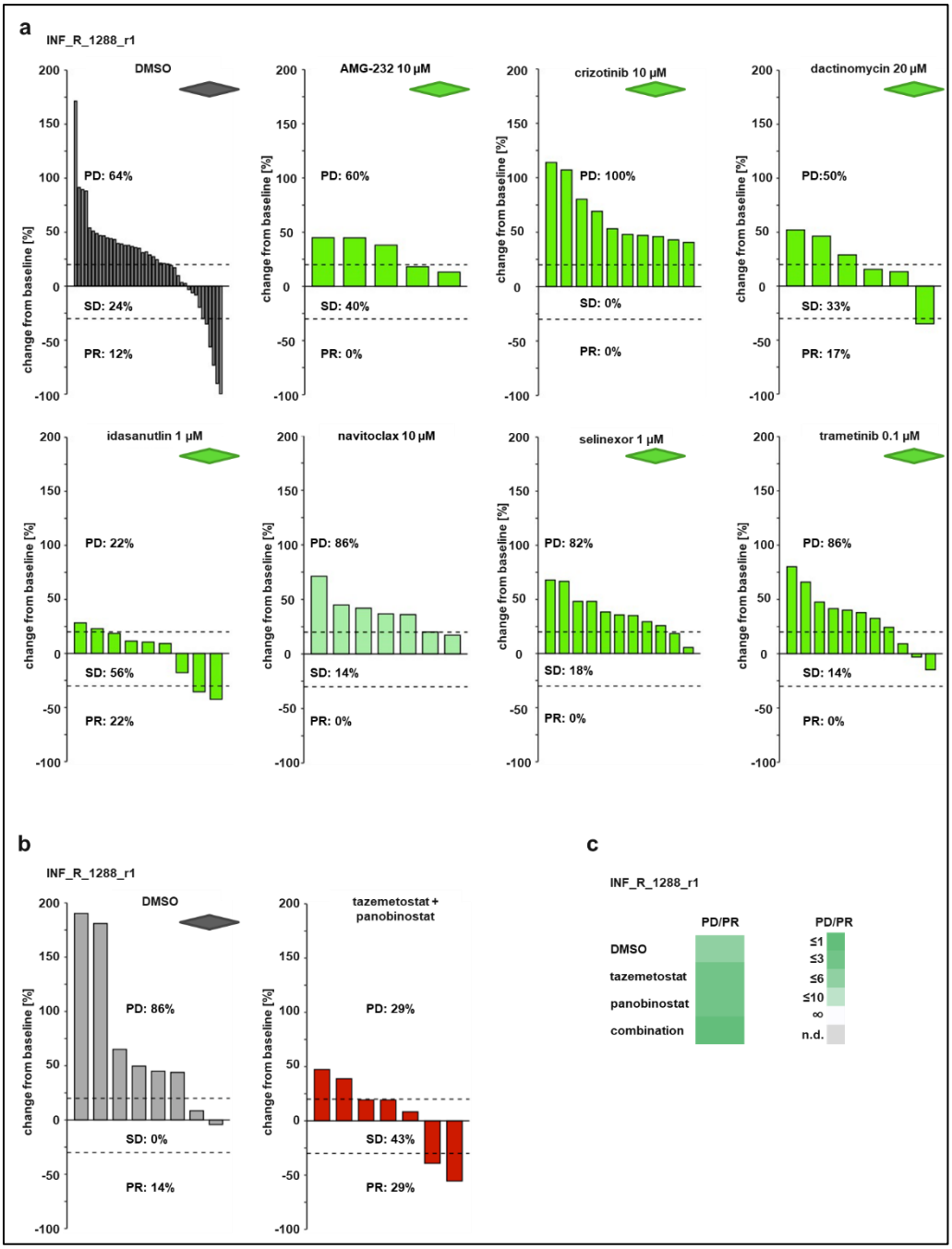

Figure S3: Waterfall plots demonstrating change in tumor volume for the INF_R_1288_r1 rhabdoid tumor zPDX model. (a—b) Depicted is the change in tumor volume [%] for each individual zebrafish early larvae engrafted with tumor cells, from baseline (day 1 = start of the treatment) to day 3 after tumor implantation. Numbers indicate the percentage of early larvae with progressive disease (PD), stable disease (SD) and partial response (PR) in each treatment group on day 3. **(c)** Heatmap reflecting the ratio of PD to PR (green shading). PD: progressive disease, tumor volume must have increased at least 20%; PR: partial response, tumor volume must have decreased by more than 30%. n.d.: not detected; ∞: the percentage of PR was 0%.

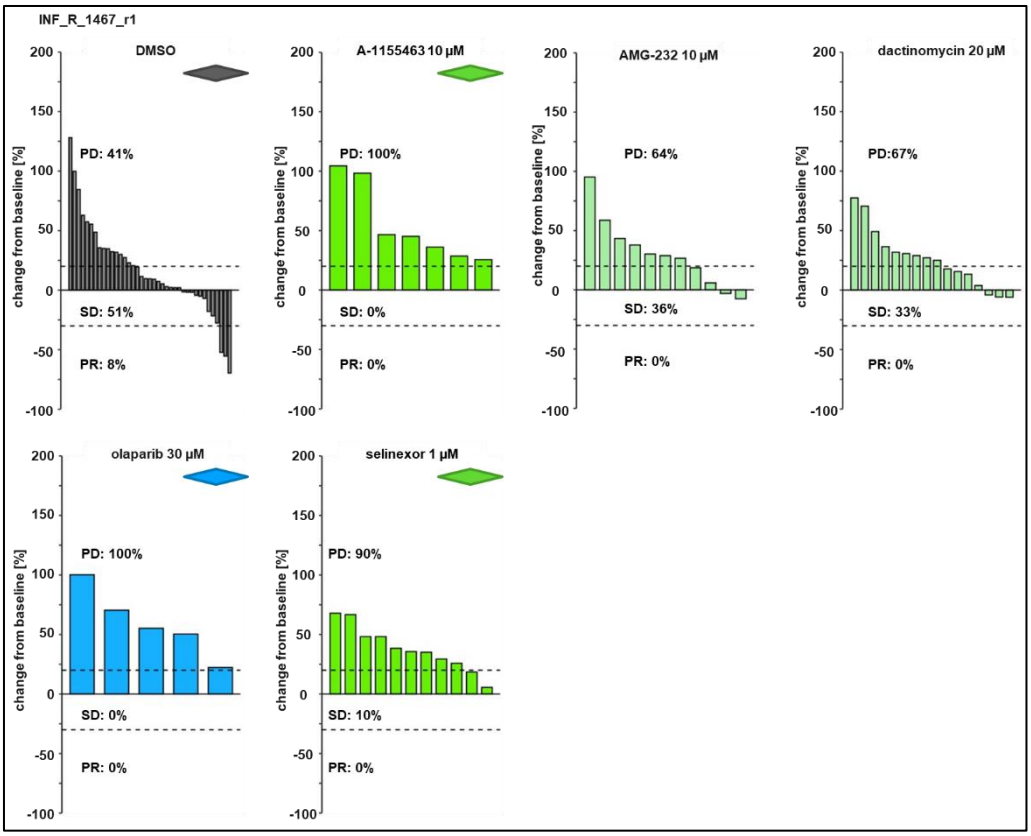

Figure S4: Waterfall plots demonstrating change in tumor volume for the INF_R_1467_r1 eRMS zPDX model. Depicted is the change in tumor volume [%] for each individual zebrafish early larvae engrafted with tumor cells, from baseline (day 1 = start of the treatment) to day 3 after tumor implantation. Numbers indicate the percentage of early larvae with progressive disease (PD), stable disease (SD) and partial response (PR) in each treatment group on day 3.

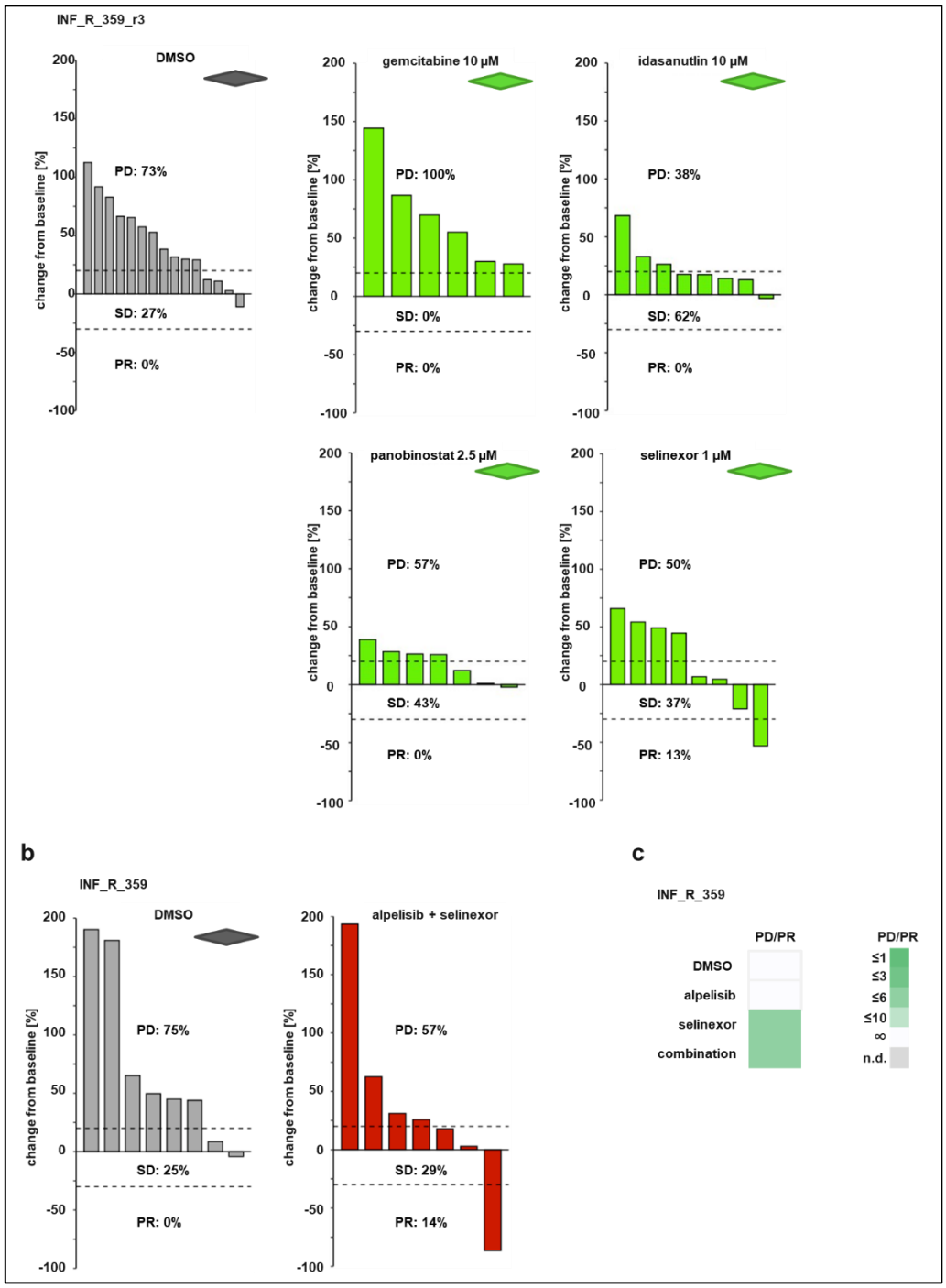

Figure S5: Waterfall plots demonstrating change in tumor volume for the INF_R_359_r3 neuroblastoma zPDX model. a-b) Depicted is the change in tumor volume [%] for each individual zebrafish early larvae engrafted with tumor cells, from baseline (day 1 = start of the treatment) to day 3 after tumor implantation. Numbers indicate the percentage of early larvae with progressive disease (PD), stable disease (SD) and partial response (PR) in each treatment group on day 3. **(c)** Heatmap reflecting the ratio of PD to PR (green shading). PD: progressive disease, tumor volume must have increased at least 20%; PR: partial response, tumor volume must have decreased by more than 30%. n.d.: not detected; ∞: the percentage of PR was 0%.