

## Supplementary Materials

**Figure S1:** Calculated IC<sub>50</sub> values of different human cancer cell lines incubated with palytoxin at various concentrations between 2 and 8 hours. Data are the mean of SD ± of three independent experiments.

### Leukemia cell lines:

K562	IC <sub>50</sub> (pM)
2h	52.1 ± 5.9
4h	32.8 ± 7.8
6h	28.5 ± 7.3
8h	24.9 ± 5.0

Jurkat	IC <sub>50</sub> (pM)
2h	12.8 ± 6.0
4h	13.5 ± 2.1
6h	8.8 ± 1.0
8h	8.7 ± 1.0

U937	IC <sub>50</sub> (pM)
2h	24.8 ± 1.7
4h	27.3 ± 8.1
6h	14.0 ± 6.1
8h	9.0 ± 0.7

### Solid tumor cell lines:

A549	IC <sub>50</sub> (pM)
2h	93 ± 9.8
4h	46.5 ± 5.8
6h	30.8 ± 3.7
8h	31.5 ± 2.7

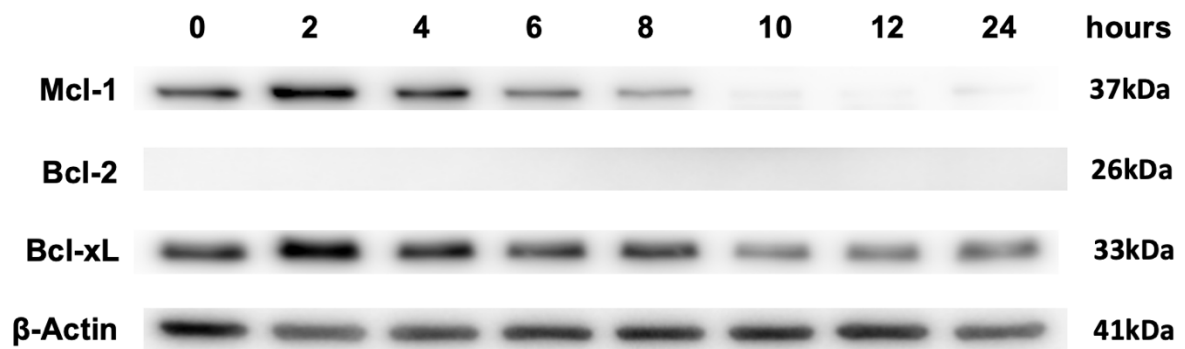
PC3	IC <sub>50</sub> (pM)
2h	93.2 ± 20.4
4h	72.1 ± 9.9
6h	31.5 ± 2.8
8h	35.9 ± 4.6

MCF7	IC <sub>50</sub> (pM)
2h	136.4 ± 30.9
4h	98.9 ± 9.2
6h	88.6 ± 7.8
8h	72.1 ± 8.8

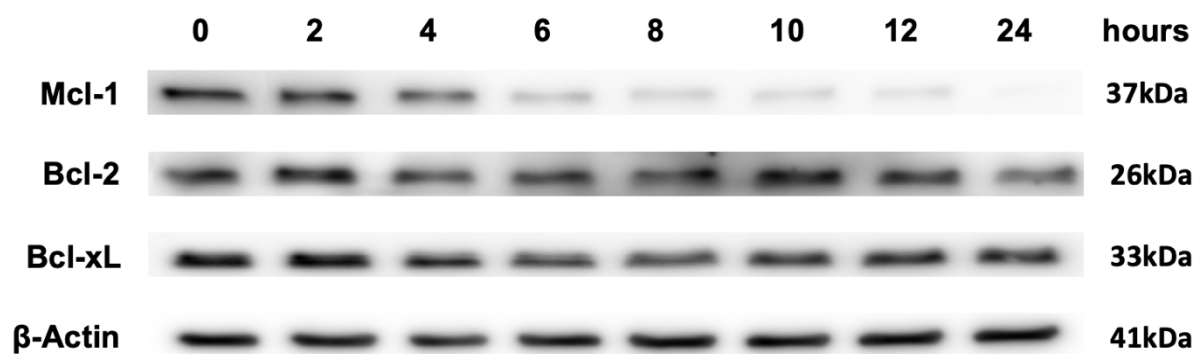
SH-SY5Y	IC <sub>50</sub> (pM)
2h	62.4 ± 7.3
4h	48.6 ± 5.9
6h	32.1 ± 3.6
8h	23.1 ± 3.9

**Figure S2:** Palytoxin down-regulates the expression of anti-apoptotic Bcl-2 family proteins in solid tumor cell lines. Western blot analysis of anti-apoptotic Bcl-2 family protein expression levels in A549 (lung cancer), PC3 (prostate cancer), MCF7 (breast cancer), and SH-SY5Y (neuroblastoma) cells.

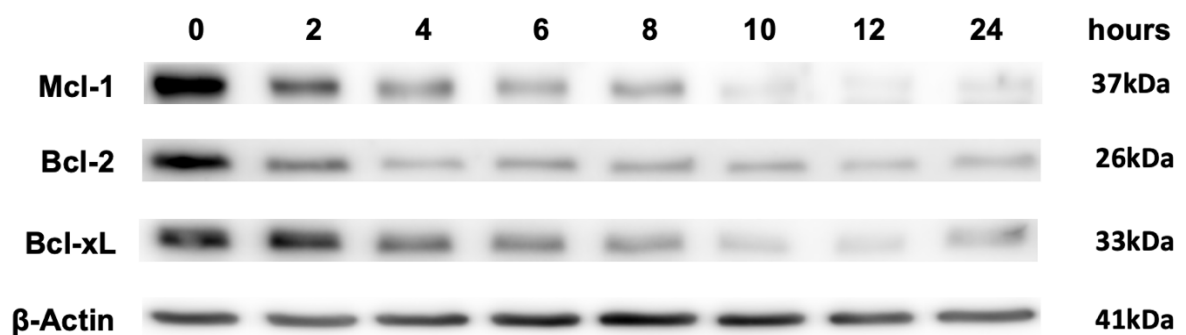
#### A549



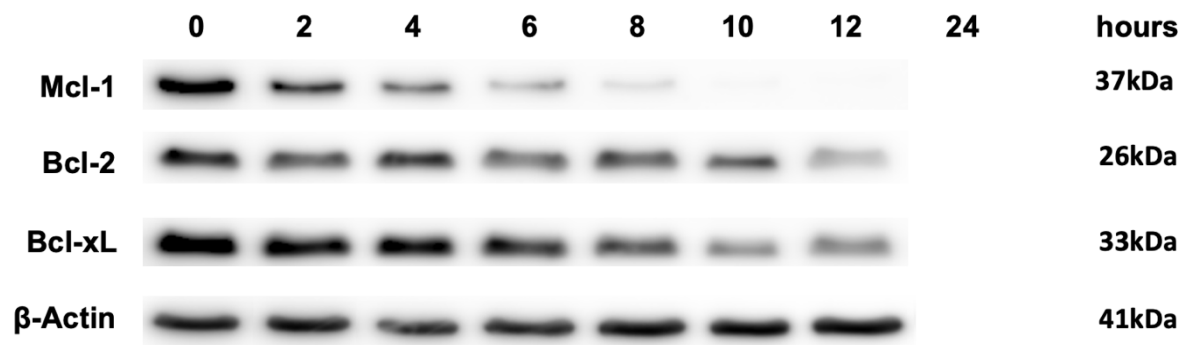
#### PC3



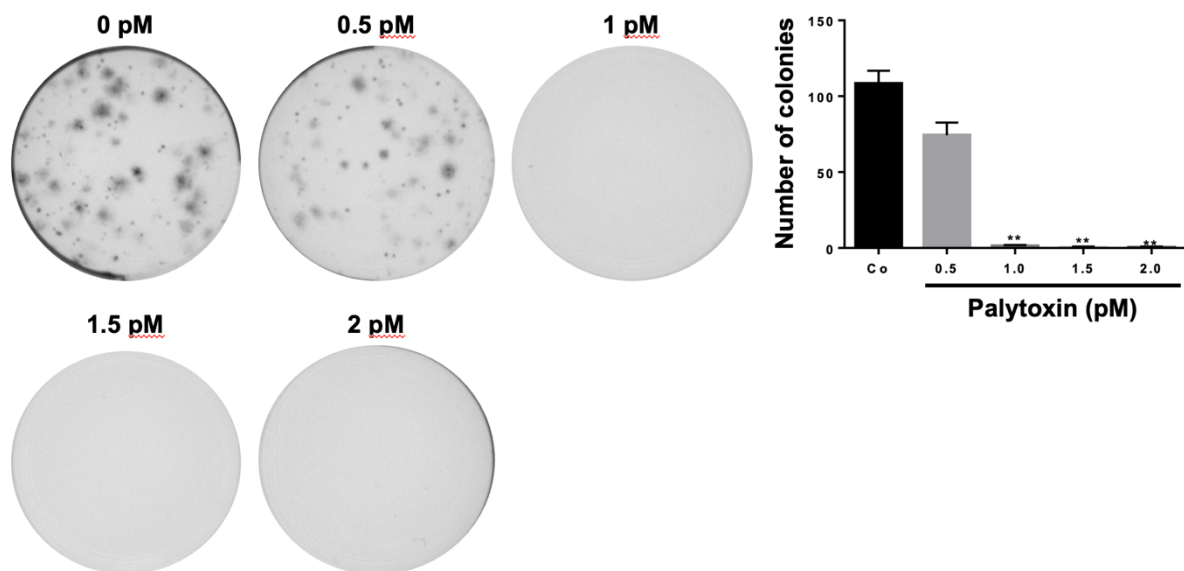
#### MCF7



SH-SY5Y

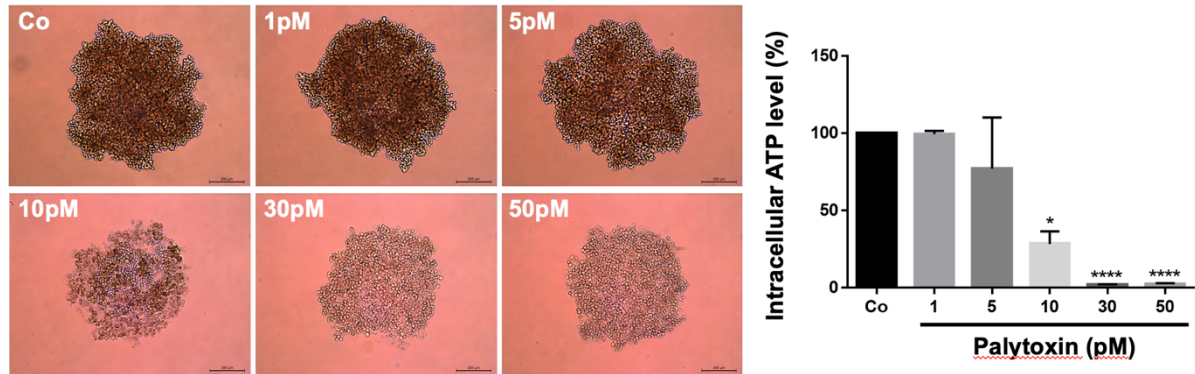


**Figure S3:** Palytoxin reduces the colony-forming ability in PC3 prostate cancer cells. Palytoxin showed an inhibitory effect on colony formation in a dose-dependent manner. All data represent the mean  $\pm$  SD. of three independent experiments. Statistical significance was assessed as \*\* $p < 0.01$  versus control.



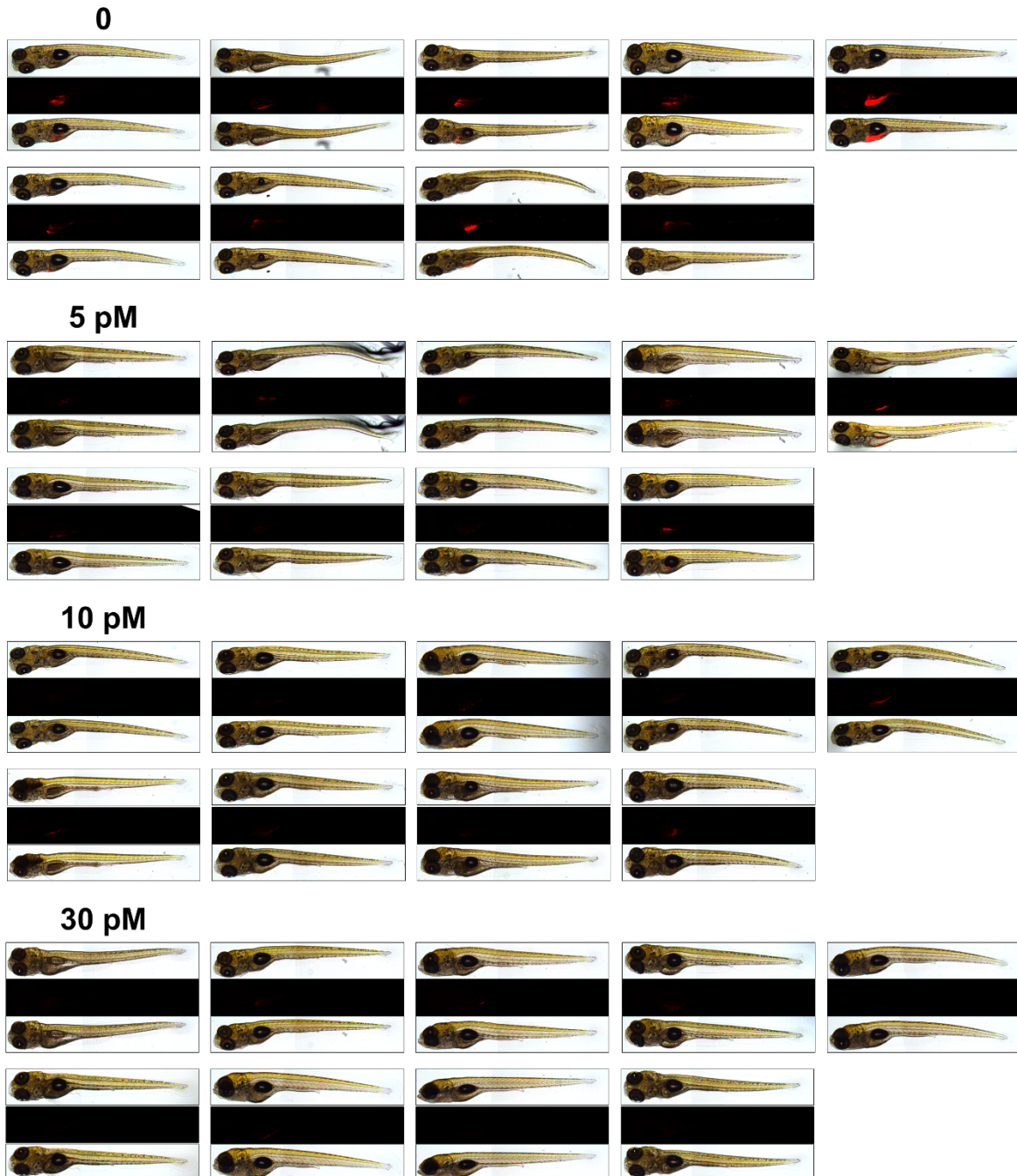


**Figure S4:** Palytoxin inhibited PC3 prostate cancer spheroid formation in a dose-dependent manner. Statistical significance was assessed as \* $p < 0.05$  and \*\*\* $p < 0.0001$  versus control.



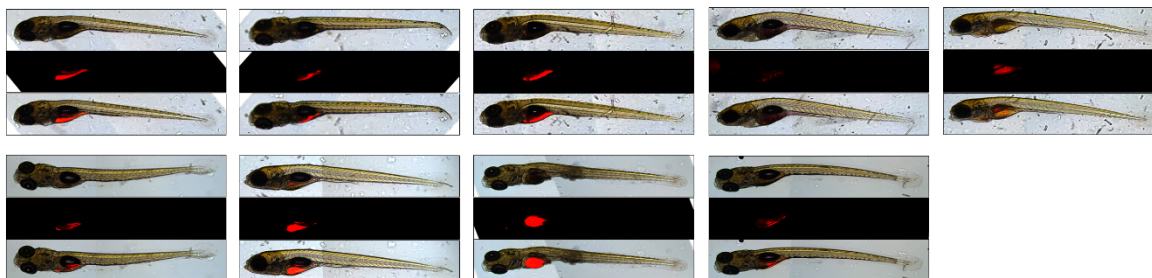
**Figure S5:** Palytoxin inhibits tumor-forming ability in *in vivo* zebrafish xenografts. Fluorescently-labeled leukemia cells were treated with palytoxin at 5, 10, and 30 pM for 6 hrs and then injected into the zebrafish yolk sac. A total of nine fish were obtained per condition. Top: bright field, middle: CM-Dil, bottom: merge. PBS injection was used as a control for injection toxicity.

#### A. U937

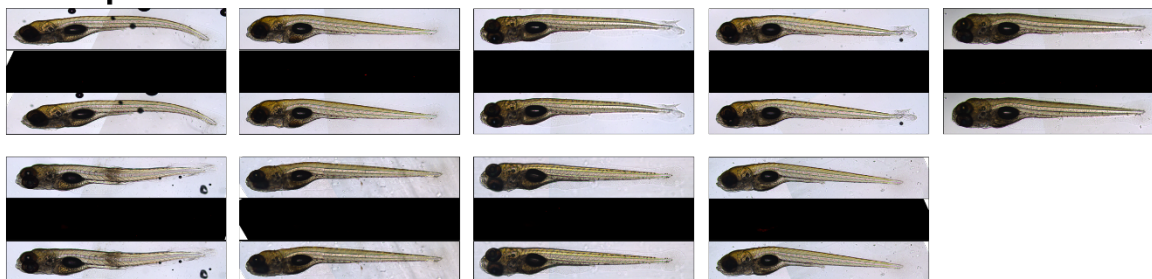


## B. HL60

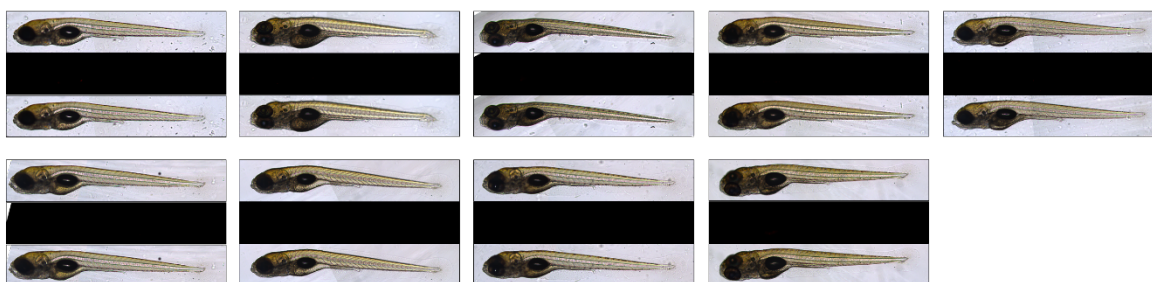
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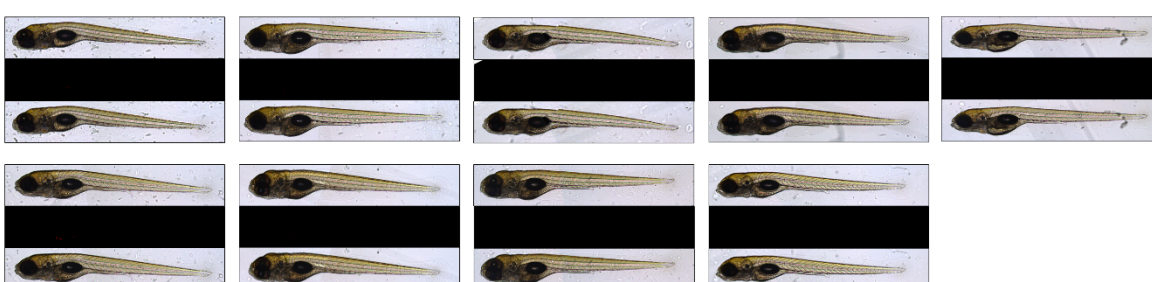
5 pM



10 pM



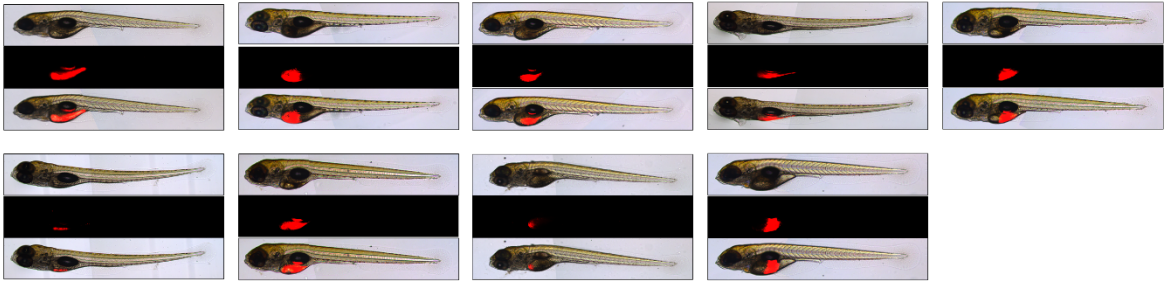
30 pM



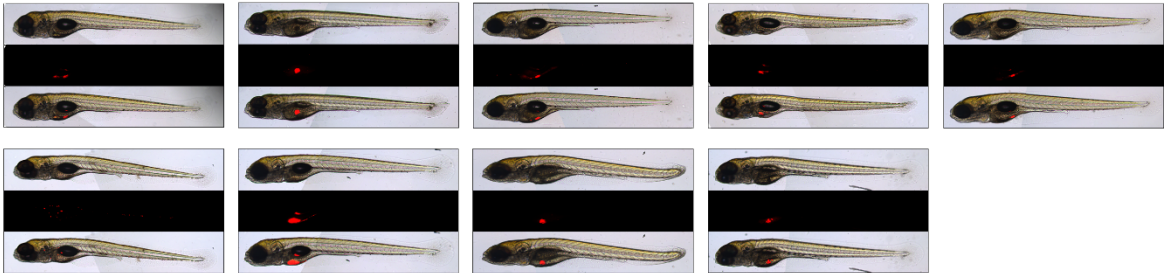


C. KG-1

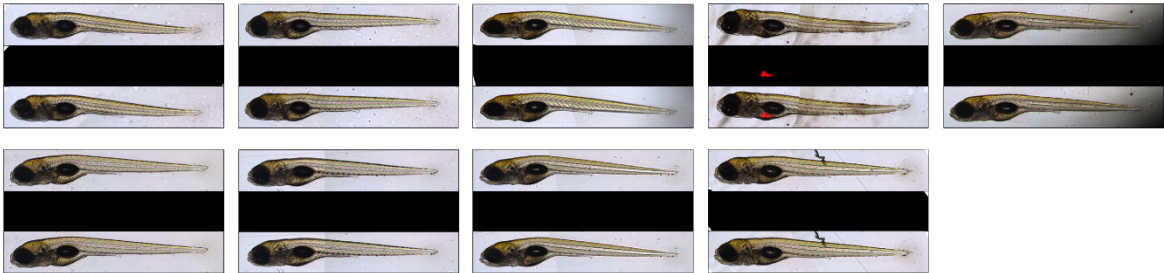
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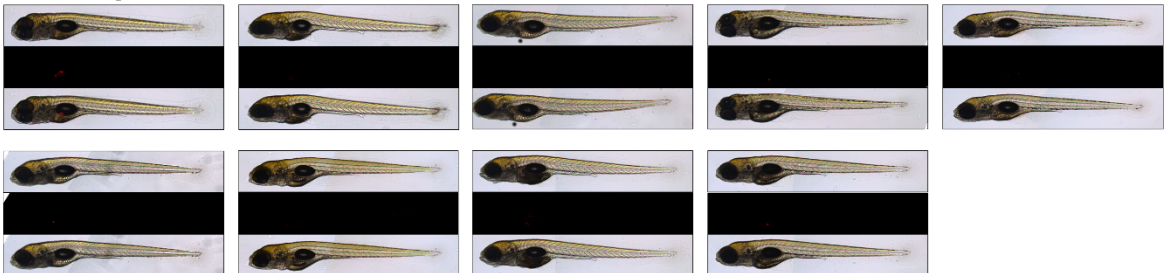
5 pM



10 pM

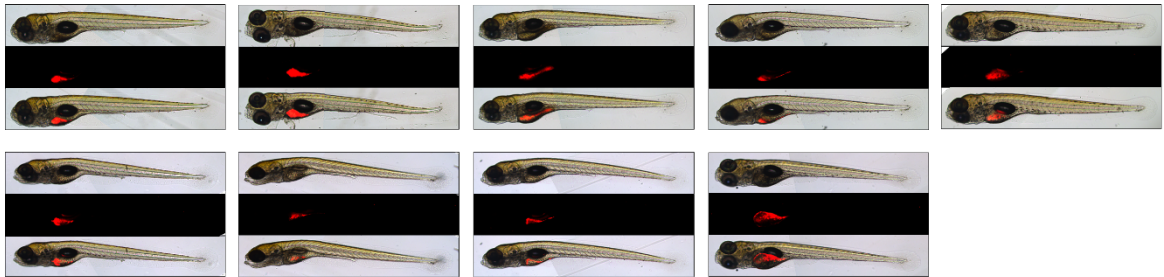


30 pM

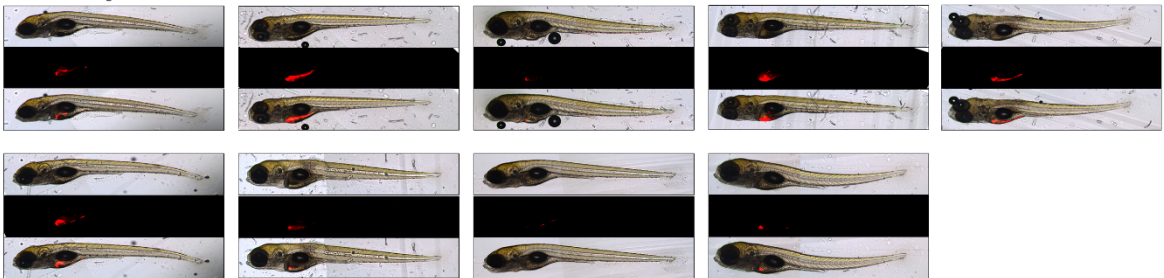


D. THP1

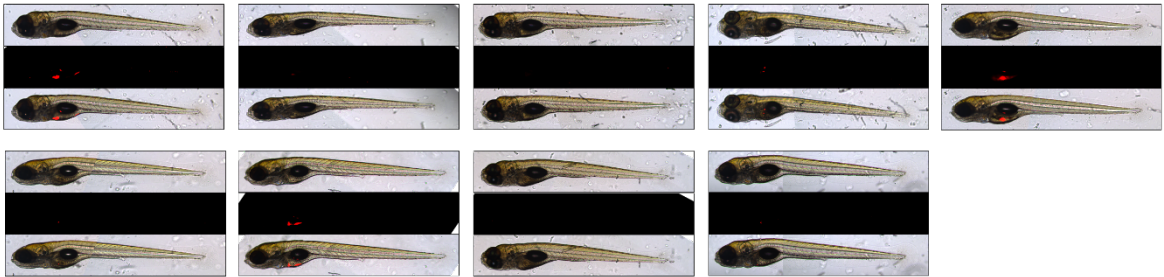
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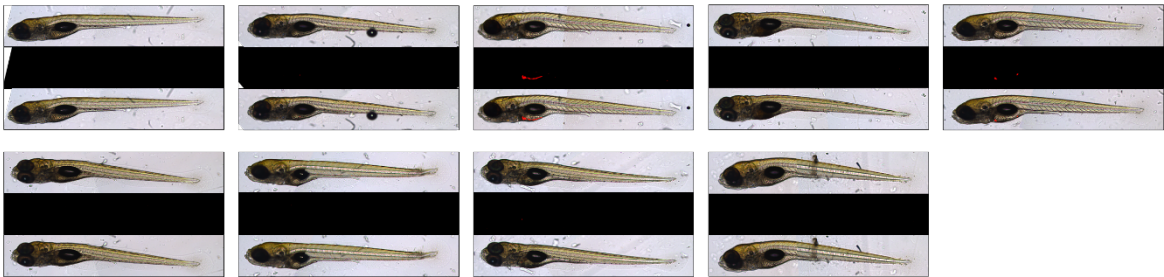
5 pM



10 pM

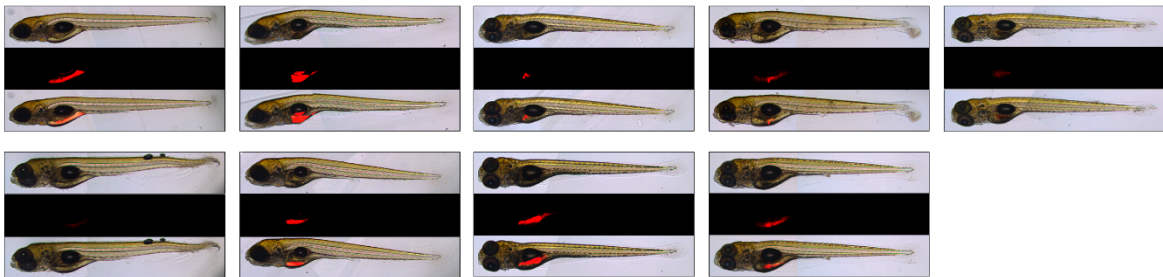


30 pM

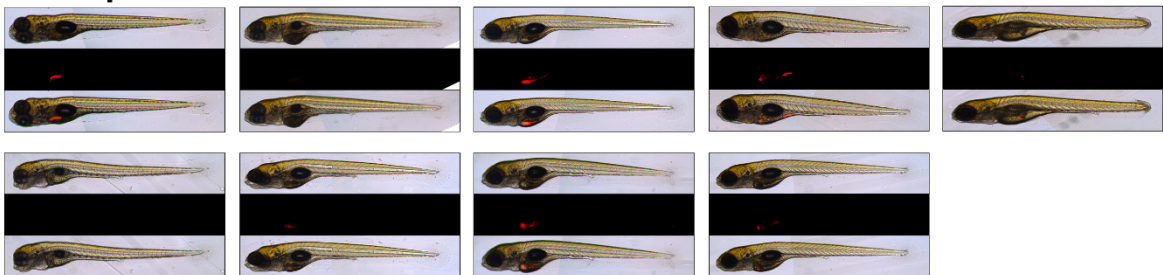


## E. K562

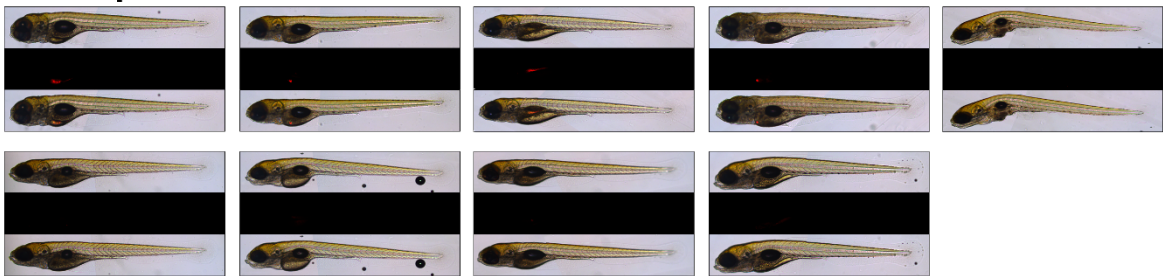
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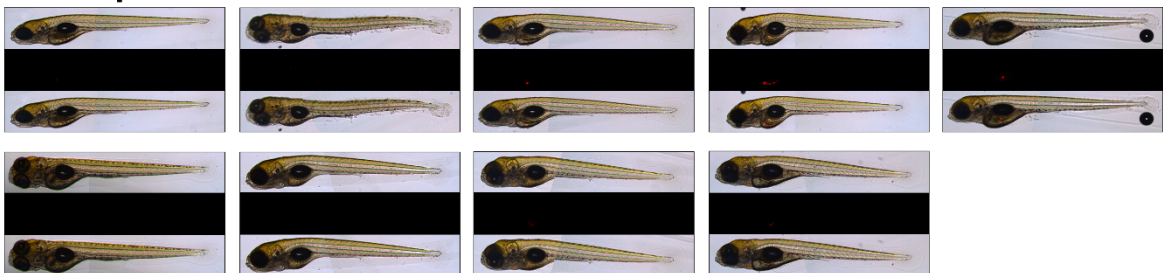
5 pM



10 pM



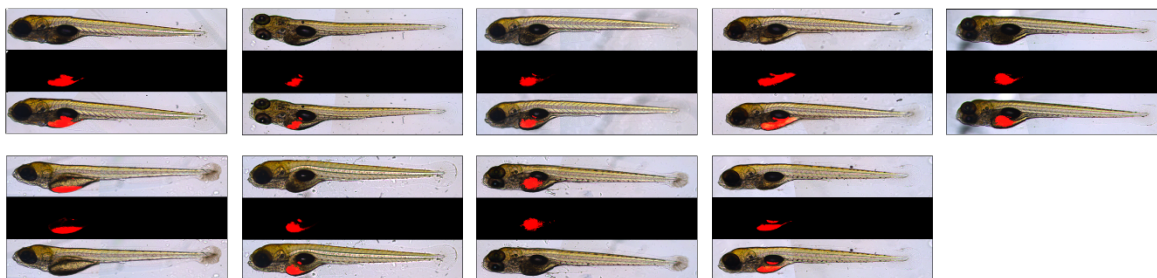
30 pM



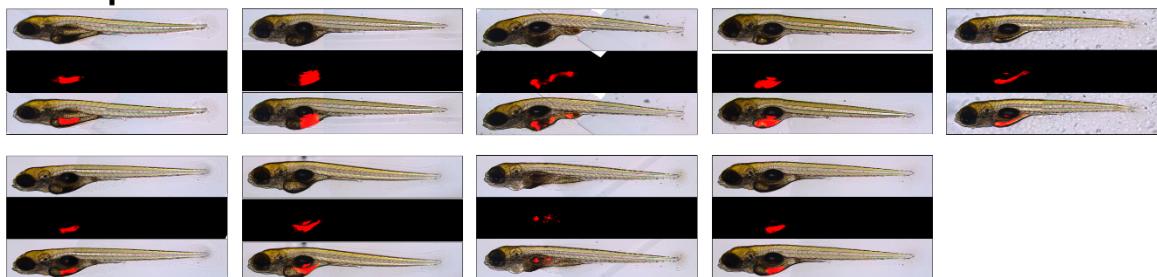


## F. K562IR

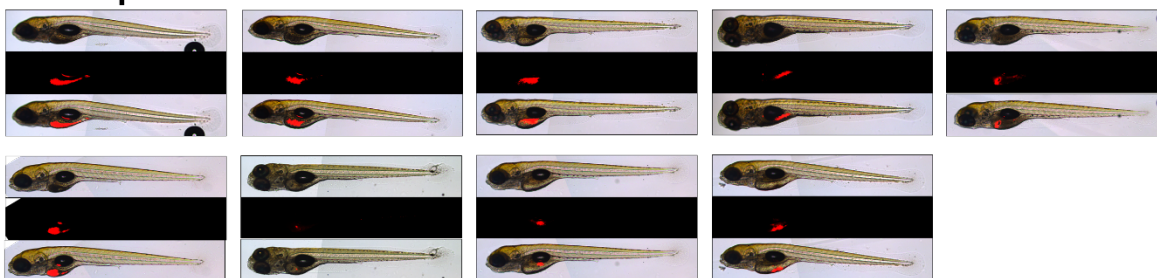
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5 pM



10 pM



30 pM

