



Supple Figure 1

Ljpz/LjpzESd+/7+ZDS1

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. Phospho-S6 staining (red) in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and tsc2;p53 compound mutant zebrafish tumors. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance.



Supple Figure 2

Supplementary Fig. 2. Immunoblotting of whole abdominal tumor extracts do not show any changes in mTORC2 signaling in tumors from *tsc2;p53* compound mutant zebrafish compared to $p53^{zdf1/zdf1}$ zebrafish. (A) Quantification of phospho-Akt (Serine 473) levels normalized to actin expression. (B) Quantification of total Akt levels normalized to actin expression. (C) Increased ratio of phospho-Akt to total Akt in *tsc2;p53* compound mutant compared to $p53^{zdf1/zdf1}$ zebrafish. Student's *t*-test was used to assess statistical significance.





LJpz/LJpz ESd

Libz/LibzEZq^{+/24}Zuv222

Supplementary Fig. 3. Phospho-tuberin Ser939 staining (red) in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and tsc2;p53 compound mutant zebrafish. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance.





ιμpz∕ιμpzεςd

Libz/Libz&Zd^{+/24}ZuvZozi

Supplementary Fig. 4. Total Akt staining (red) in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and *tsc2;p53* compound mutant zebrafish. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance.





ιJpz∕ιJpz εςd

Ljpz/LjpzESd+/Z+ZnnZ2SJ

Supple Figure 5

Supplementary Fig. 5. Phospho-Akt Thr308 (red) staining in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and tsc2;p53 compound mutant zebrafish. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance.





IJpz/IJpz€Sd



Supplementary Fig. 6. Hif2a staining (red) in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and *tsc2;p53* compound mutant zebrafish. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance.





Supple Figure 7

ιjpz∕ιjpz εςd

Ljpz/LjpzESd_{+/ZZDA}ZSSI

Supplementary Fig. 7. Vegf-c staining (red) in a series of abdominal tumors from $p53^{zdf1/zdf1}$ and *tsc2;p53* compound mutant zebrafish. (Left) 8 tumor samples per each line were used for comparison. (Right) Mean values of intensity (arbitrary units) were graphed. M, muscle; T, tumor. Scale bar = 100 µm. Student's *t*-test was used to assess statistical significance. fli1:EGFP

H & E



Supple Figure 8

WΤ

Supplementary Fig. 8. Increased size of blood vessels in the liver of *tsc2* homozygous mutant zebrafish. The *tsc2* mutant allele was crossed to transgenic fish expressing EGFP under the *Fli1* promoter that directs expression in endothelial cells. Examples shown are liver from 7 day old larvae. Above images are wildtype (wt) control, below are *tsc2* homozygous mutant sections. Left images are *Fli1:EGFP*, and right images hematoxylin and eosin staining. Asterisks (yellow in *Fli1:EGFP*, black in hematoxylin and eosin stain) denote the blood vessel lumen. Scale bar = 100 μ m.

p53^{zdf1/zdf1}

tsc2^{vu242/+}p53^{zdf1/zdf1}



AMPK (Thr172), DAPI

Supplementary Fig. 9. AMPK Thr172 staining (red) and DAPI (blue) in abdominal tumors from $p53^{zdf1/zdf1}$ and tsc2;p53 compound mutant zebrafish. Merged colors above and AMPK Thr172 alone shown below. M, muscle; T, tumor. Scale bar = 100 µm.