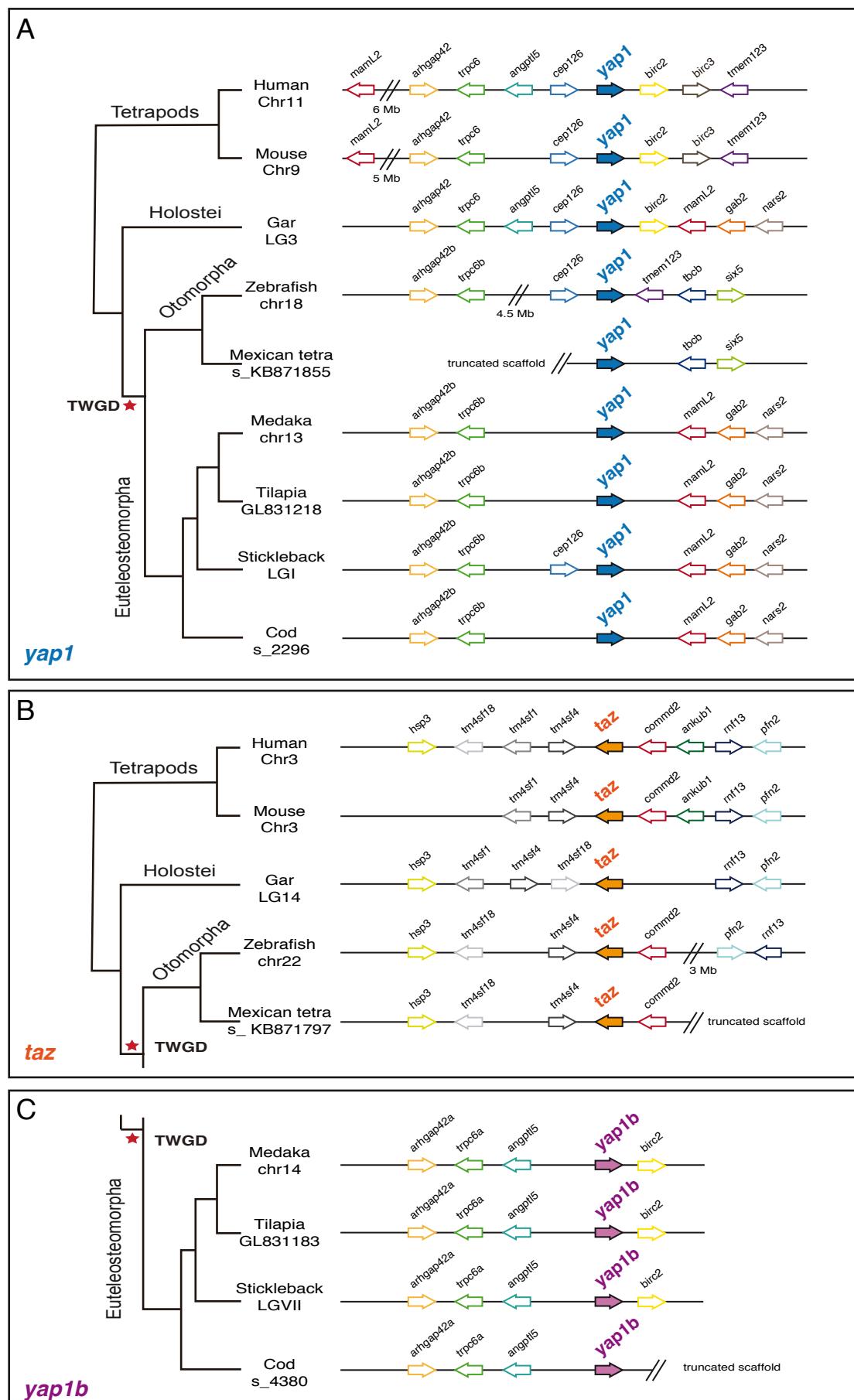
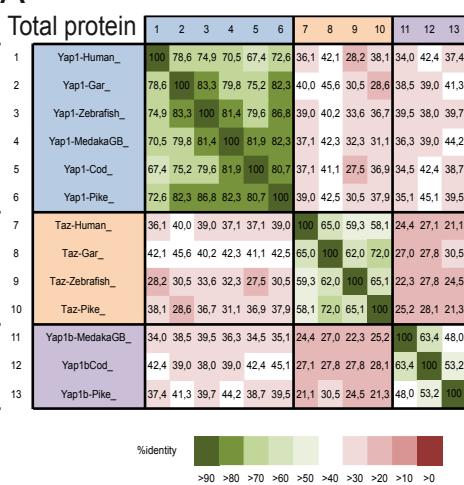
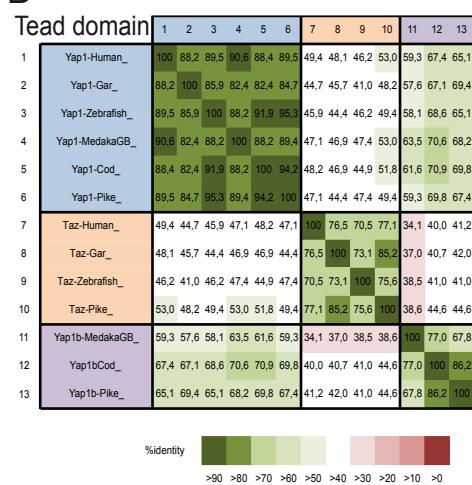
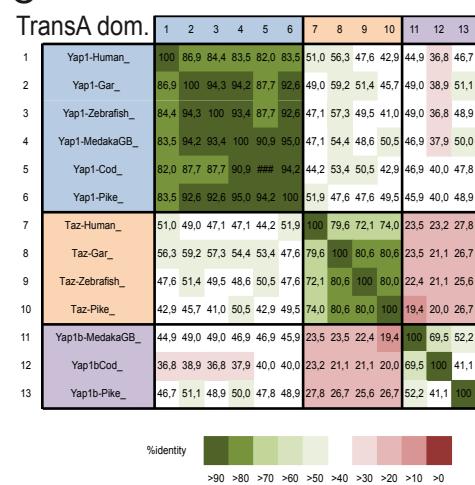


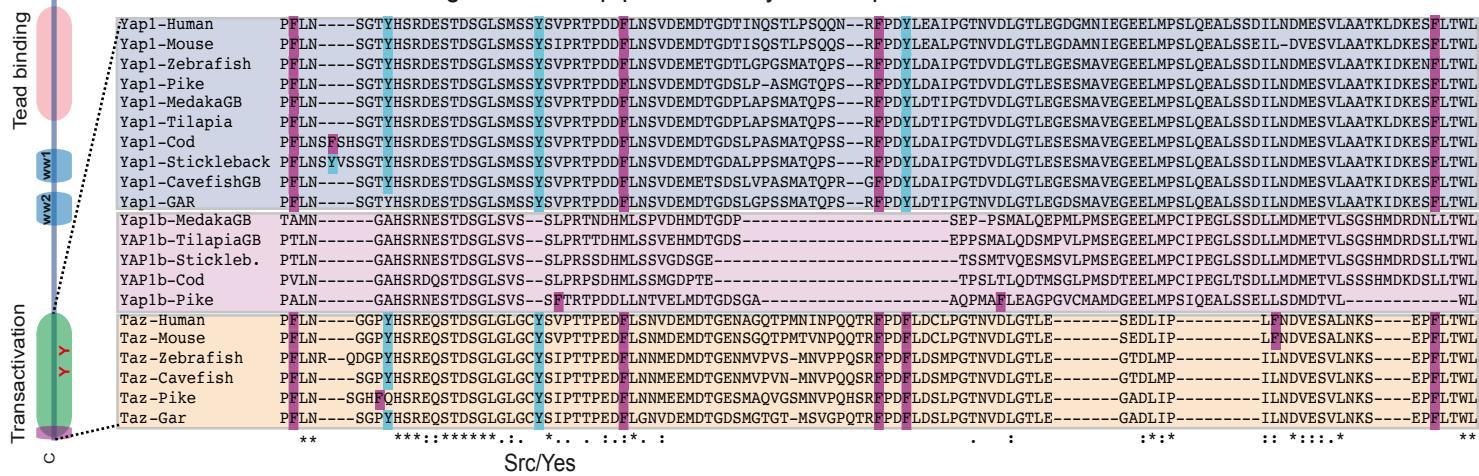
**Figure S1:** Generation of *yap1* mutant alleles by CRISPR/Cas9 genome editing. (A) Schematic representation of the 7 bp and 11 bp deletions generated in the exon 2 of *yap1* in medaka. The sequence in bold corresponds to the region targeted by two partially overlapping sgRNAs (see Methods). The boxes in red and orange correspond to the 7 and 11 bp deletions generated. (B) The predicted *yap1* protein structure is depicted for the wild type and mutant proteins. In both cases, the deletions resulted in frame shift mutations and premature stop codons. (C-H) Phenotypic analysis of wild type (C, E, G) and *yap1* mutant embryos (D, F, H) at stages 22 (E-F) and 25 (C-D, G-H) shows characteristic malformations, such as optic cup flattening and lens misalignment (arrows in H). oc = optic cup. Magnification bars are included in the figure.



**Figure S2:** Micro-synteny arrangement around *yap* family paralogs. (A) Phylogenetic tree displaying gene arrangement around *yap1* for the representative 7 teleost and two tetrapod species analyzed in Figure 1. Note the conserved linkage between *yap1* and the *arhgap42/trpc6* block. (B) Phylogenetic tree and gene arrangement around *taz* in different vertebrate branches. Note the conserved linkage between *hsp3*, *taz*, and *comm2*. (C) Linkage of *arhgap42a* and *trpc6a* to *yap1b* shows that this gene emerged by duplication of and ancestral *yap1*. Color-coded arrows indicate both gene orientation and equivalent orthologs in different species. Chromosomes or gene scaffolds are also indicated. TWGD= teleost-specific genome duplication.

**A****B****C****D**

## Alignment of Yap protein family transcriptional activation domain



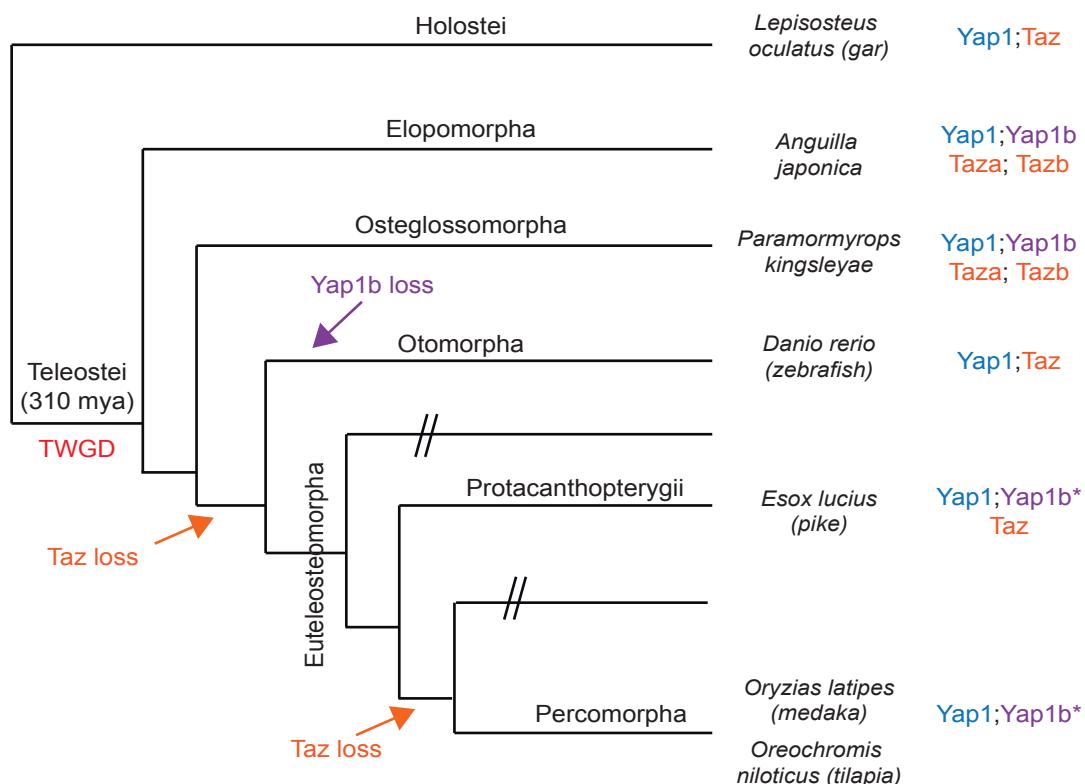
**Figure S3:** Yap1b Divergent transcriptional activation domain. Heat map representations of sequence homology (aa Identity in %) between Yap family proteins from different species are shown for: (A) the entire protein, (B) TEAD binding domains, and (C) transcriptional activation domains. Note that whereas the Tead binding domain of Yap1b is relatively well conserved with respect to the equivalent domain in Yap1, the transcriptional activation domain shows lower amino acid identity (average aa Identity 65.5 % vs. 44.8 %, respectively). (D) Multiple sequence alignment of the entire transactivation domain of Yap family proteins from different species. Note the elimination or substitution of conserved tyrosine (Blue) and phenylalanine (red) residues in Yap1b proteins. The main tyrosine phosphorylated by Scr-family kinases is indicated.

**A**

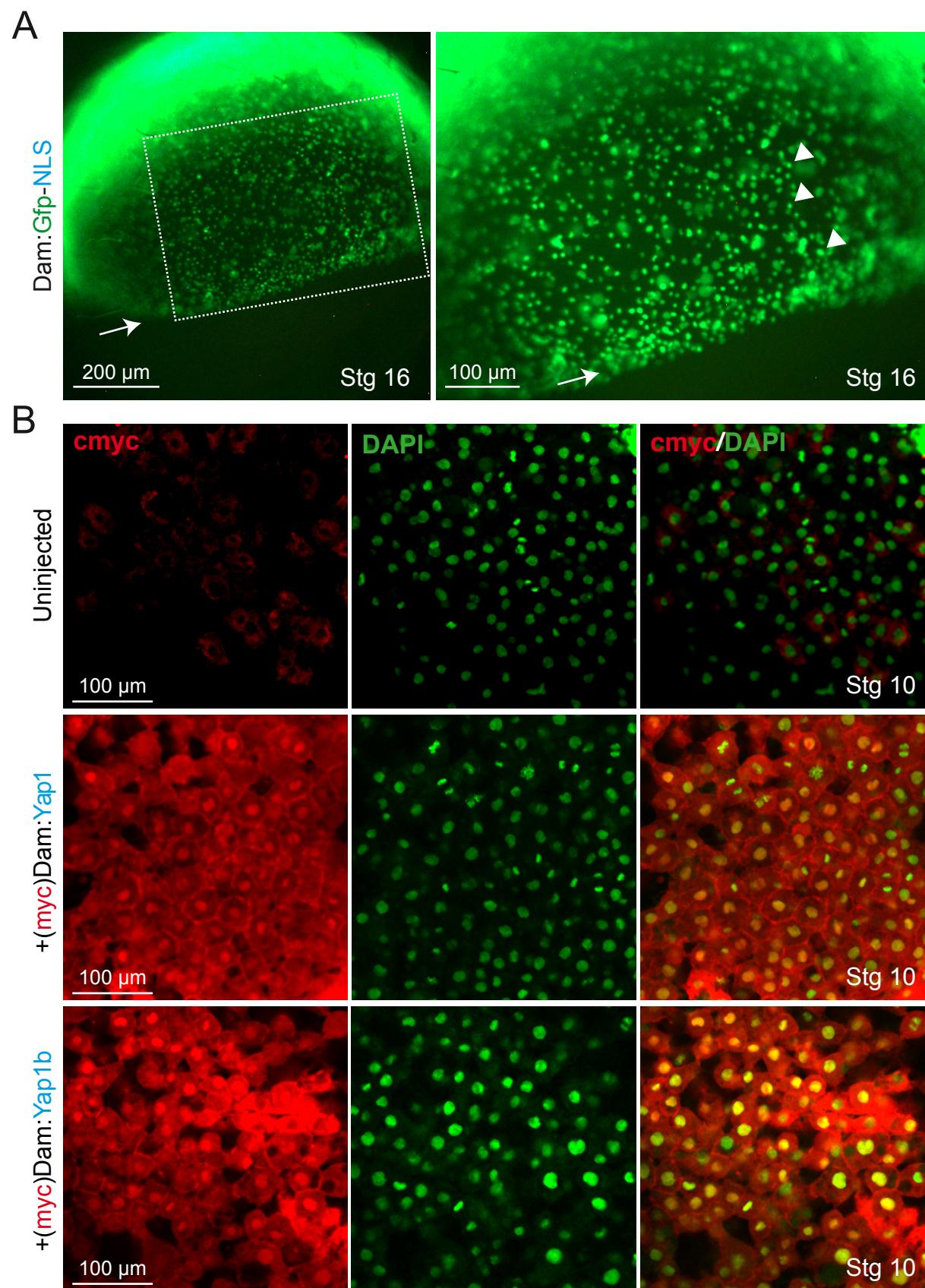
## Alignment of Yap family transactivation domain (exon 7)

|               | Src/Yes                                |
|---------------|--|
| Taza_AnJa     | -GPFHSREQSTDGLGLGCY SIPTTPEDFLNNVDEMD  |
| Taz_Gar       | -GPYHSREQSTDGLGLGCY SIPTTPEDFLGNVDEMD  |
| Taz_Zebraf    | -GPYHSREQSTDGLGLGCY SIPTTPEDFLNNMEDMD  |
| Taz_Pike      | GHQHSREQSTDGLGLGCY SIPTTPEDFLNNMEEMD   |
| Tazb_AnJa     | -GPYHSREQSTDGLGLGCY SIPTTPEDILNNVEEMD  |
| Taza_PaKi     | -GPYHSREQSTDGLGLGCY SVPTTPEDFLNNLEEMD  |
| Tazb_PaKi     | -GHYHSRDKSTDGLGLGCCSHPTTPEGFLS-MDEMD   |
| Yap1_AnJa     | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_PaKi     | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_Gar      | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_Tilapia  | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_Pike     | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_Medaka   | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEMD |
| Yap1_Zebraf   | -GTYHSRDESTDSGLSMSSY SVPRTPDDFLNSVDEME |
| Yap1b_PaKi    | -GTYHSRDESTDSGLSVSSY SVPRTPDDLNSVEEME  |
| Yap1b_AnJa    | -GNFHSRDESTDSGLSVSSY SIPRTPDDFLNSVDEMD |
| Yap1b_Medaka  | -NGAHSRNESTDSGLSVS--SLPRTNDHMLSPVDHMD  |
| Yap1b_Tilapia | -NGAHSRNESTDSGLSVS--SLPRTTDHMLSSVEHMD  |
| Yap1b_Pike    | -NGAHSRNESTDSGLSVS--SFTRTPDDLNTVELMD   |

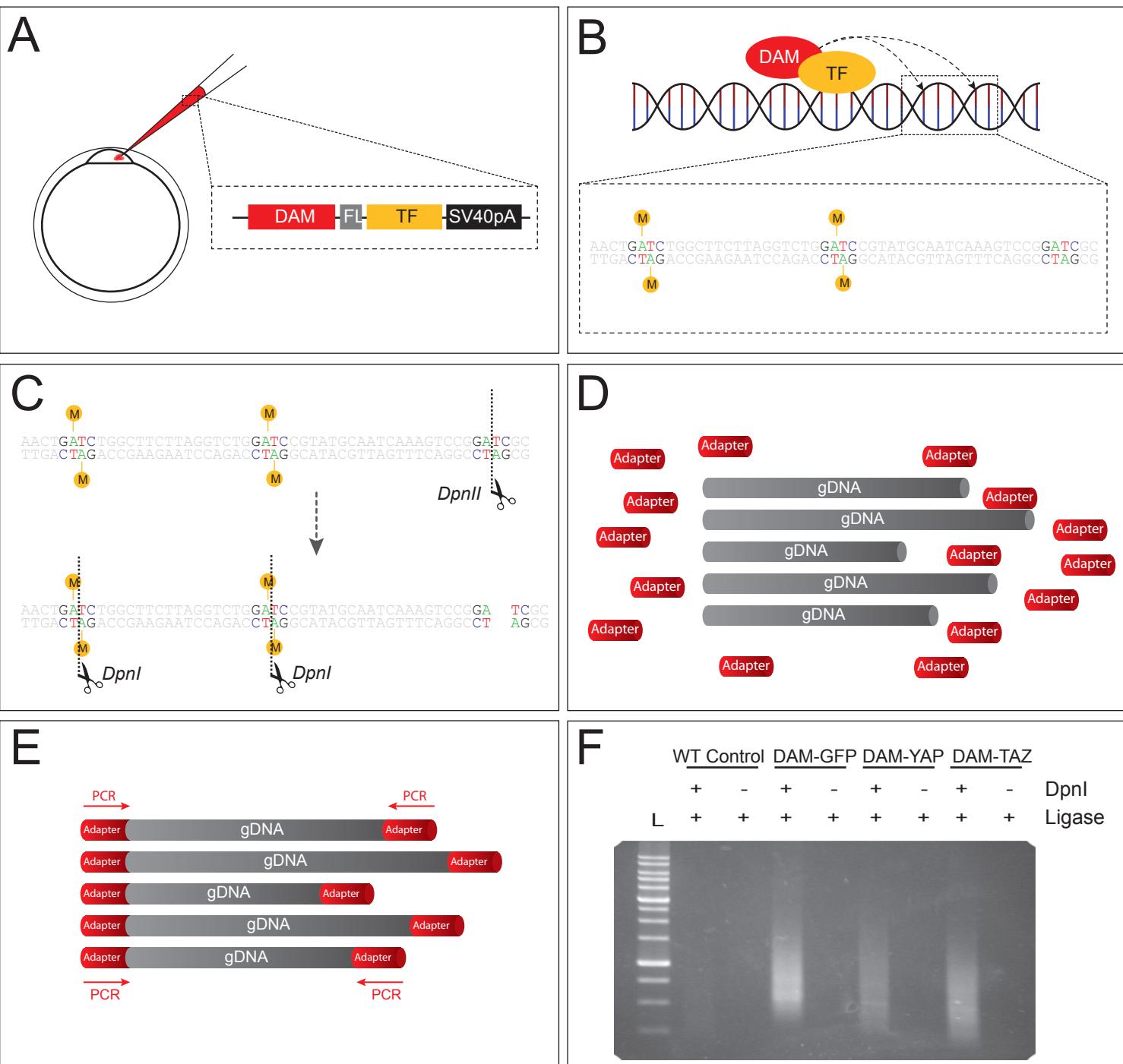
\*\*\*: :\*\*\*\*\*.. . \* . \* : :\* . : : \* :

**B**

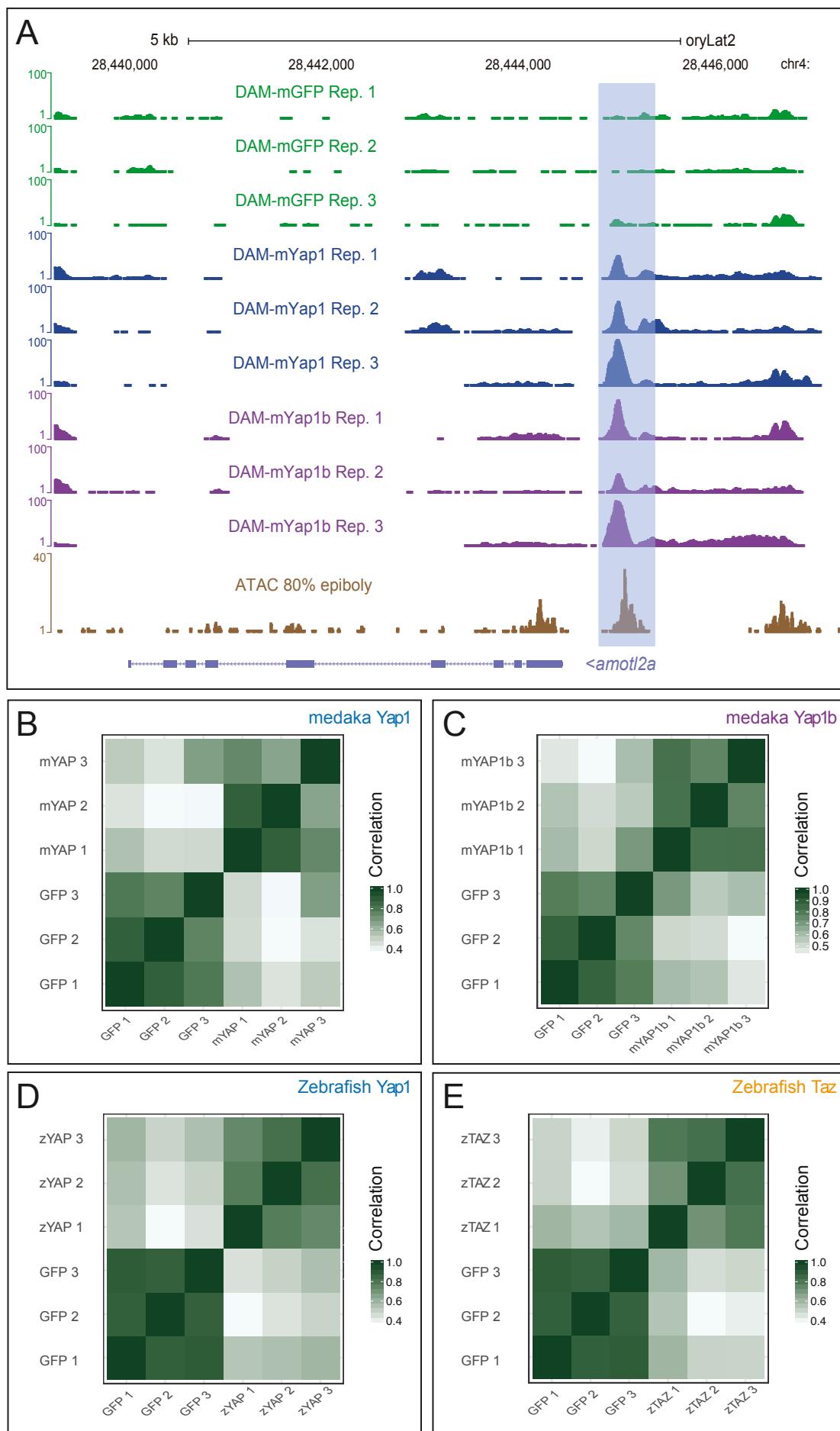
**Figure S4:** Evolutionary history of Yap family in teleost. (A) Multiple sequence alignment of the N-terminal segment of the transactivation domain in different teleost species. Note that the elimination/substitution, in Yap1b proteins of conserved tyrosine (red) and phenylalanine (light blue) residues occurs in Euteleostemorpha, but not in Elopomorpha or Osteoglossomorpha species. The main tyrosine residue that can be phosphorylated by Src-family kinases is also indicated. (B) Phylogenetic tree showing the main teleost branches and their evolutionary relationship. The position of the different species considered for the sequence alignment, the different paralogs (color-coded) present in each species, and the evolutionary gene losses are indicated. TWGD= teleost-specific genome duplication.



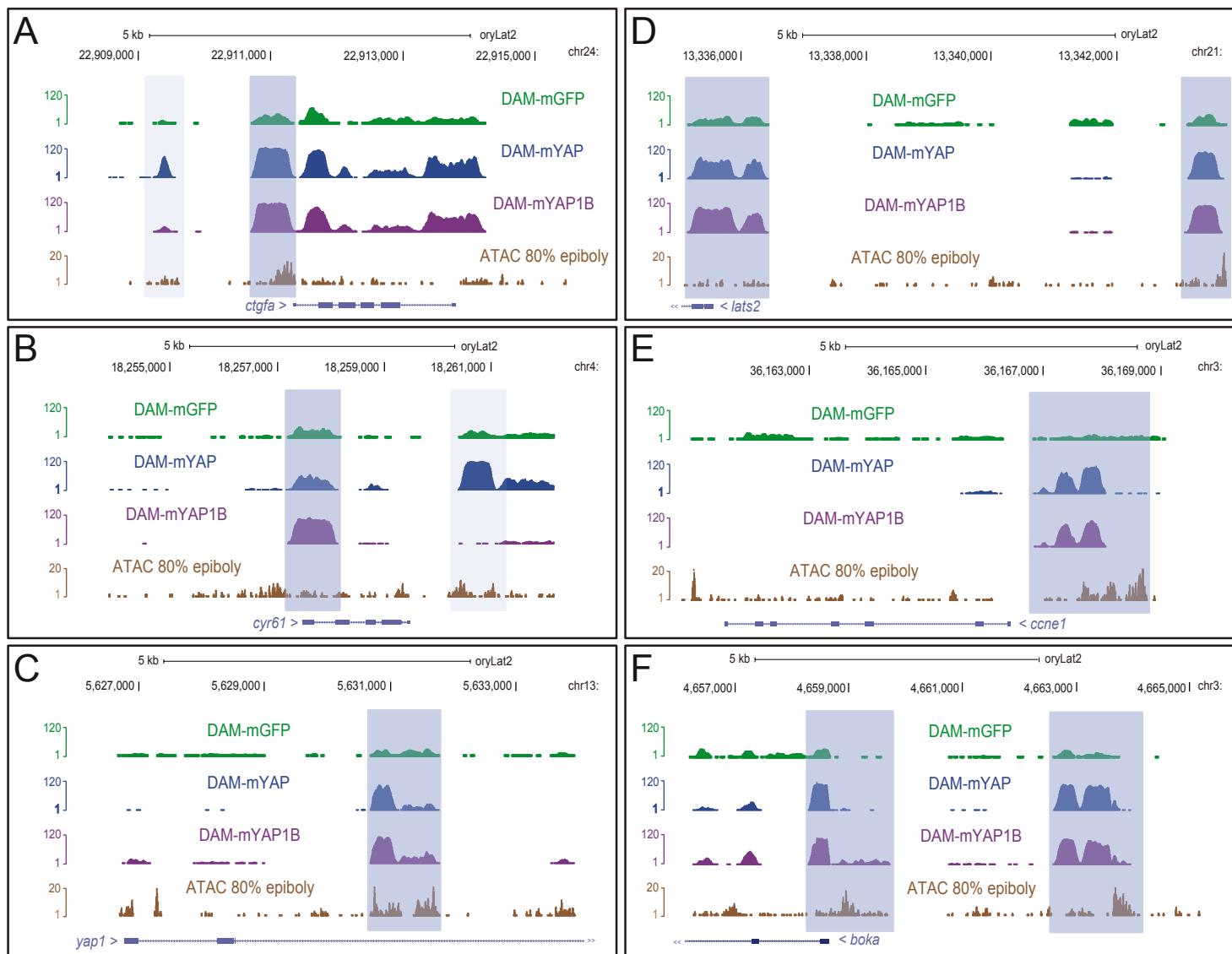
**Figure S5:** Nuclear localization of DamID fusions. After injection into one-cell stage medaka embryos, the nuclear localization of the DamID fusion proteins was evaluated (A) Fluorescent stereo-microscope images show DAM:GFP-NLS signal at the nuclei (arrowheads) in stage 16 embryos. The epiboly front is indicated (white arrow) (B) Confocal images show the nuclear localization of Dam:Yap1 and Dam:Yap1b, as revealed by immunostaining using anti-myc antibodies in stage 10 embryos. Un-injected embryos were included as a control, and samples were counterstained with DAPI to reveal nuclei position. Bar= 100  $\mu\text{m}$ .



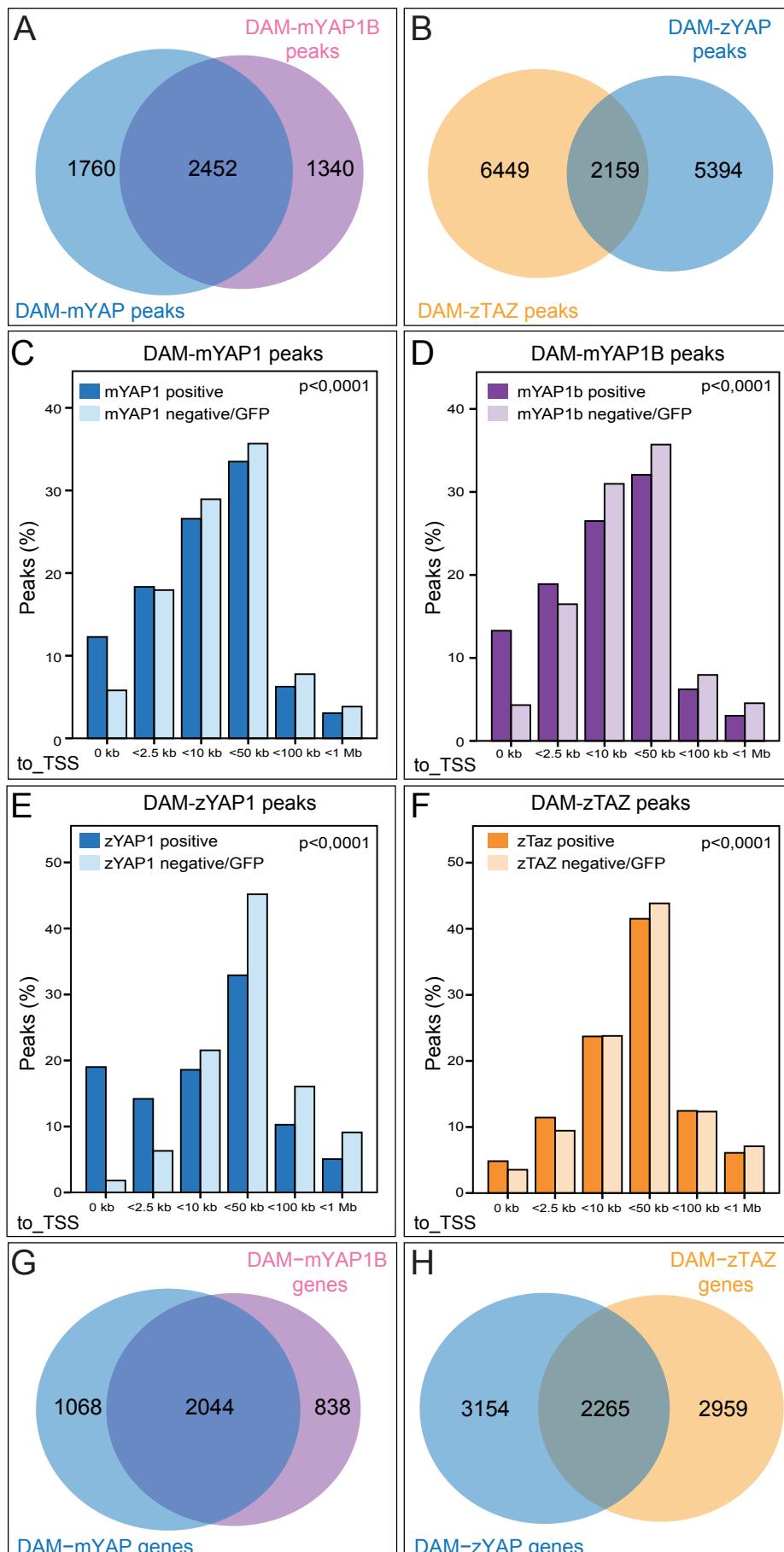
**Figure S6:** Outline of the DamID-seq protocol. (A) mRNA from the different Dam-TF fusions were injected into medaka or zebrafish embryos at single cell stage. (B) Upon binding of the TF to its transcriptional targets, the DAM protein methylates the adjacent GATC sites. (C) After extracting the gDNA fraction from the injected embryos, the genome is fragmented with *DpnII* and the methylated GATC sites are further cut using *DpnI*. (D) Adapters are incorporated at both ends of the resulting fragments. (E) Fragments are amplified using the adapters' sequence to generate the corresponding libraries. (F) Resulting libraries are then visualized as DNA smears on an electrophoresis gel.



**Figure S7:** Correlation between DamID-seq experiments. (A) DamID-seq tracks for three independent replicates are shown in the locus of *amotl2a*. Dam-yap1, Dam-yap1b and Dam-GFP peaks, generated from 80% epiboly medaka embryos, are shown in relation to available ATAC-seq marks (see also figure 3). (B-E) Heatmap matrixes showing the correlation coefficient between mapped reads for each of the samples: medaka DAM:yap1 (B), medaka DAM:yap1b (C), zebrafish DAM:yap1 (D), and zebrafish DAM:taz (E), with its replicates (n=3) and its corresponding DAM:GFP controls.



**Figure S8:** DamID-seq profiling of Yap1 and Yap1b in medaka. Dam-yap1, Dam-yap1b and Dam-GFP tracks from 80% epiboly medaka embryos are shown for several loci harboring known Yap target genes such as: *ctgfa* (A), *cyr61* (B), *yap1* (C), *lats2* (D), *ccne1* (E), and *boka* (F). Peaks are shown in relation to available ATAC-seq marks. Some of the differentially methylated peaks in enhancers, promoters, and introns are highlighted by shadow boxes.



**Figure S9:** Peaks overlap and distance to the closest TSS. Diagrams show the overlap between medaka Dam-yap1 and Dam-yap1b peaks (A) and zebrafish Dam-yap1 and Dam-taz peaks (B). The distribution of the peaks according to their distance to the neighboring TSS is shown for positive and negative peaks associated to medaka Dam-yap1 (C) and Dam-yap1b (D), and zebrafish Dam-yap1 (E) and Dam-taz (F). Statistical differences in the distribution of positive vs. negative peaks were determined using Chi-square (p values are indicated). The overlap between medaka Dam-yap1 and Dam-yap1b genes (G) and zebrafish Dam-yap1 and Dam-taz genes (H) is also represented.

**A**

## Enriched motifs in zYap1 peaks

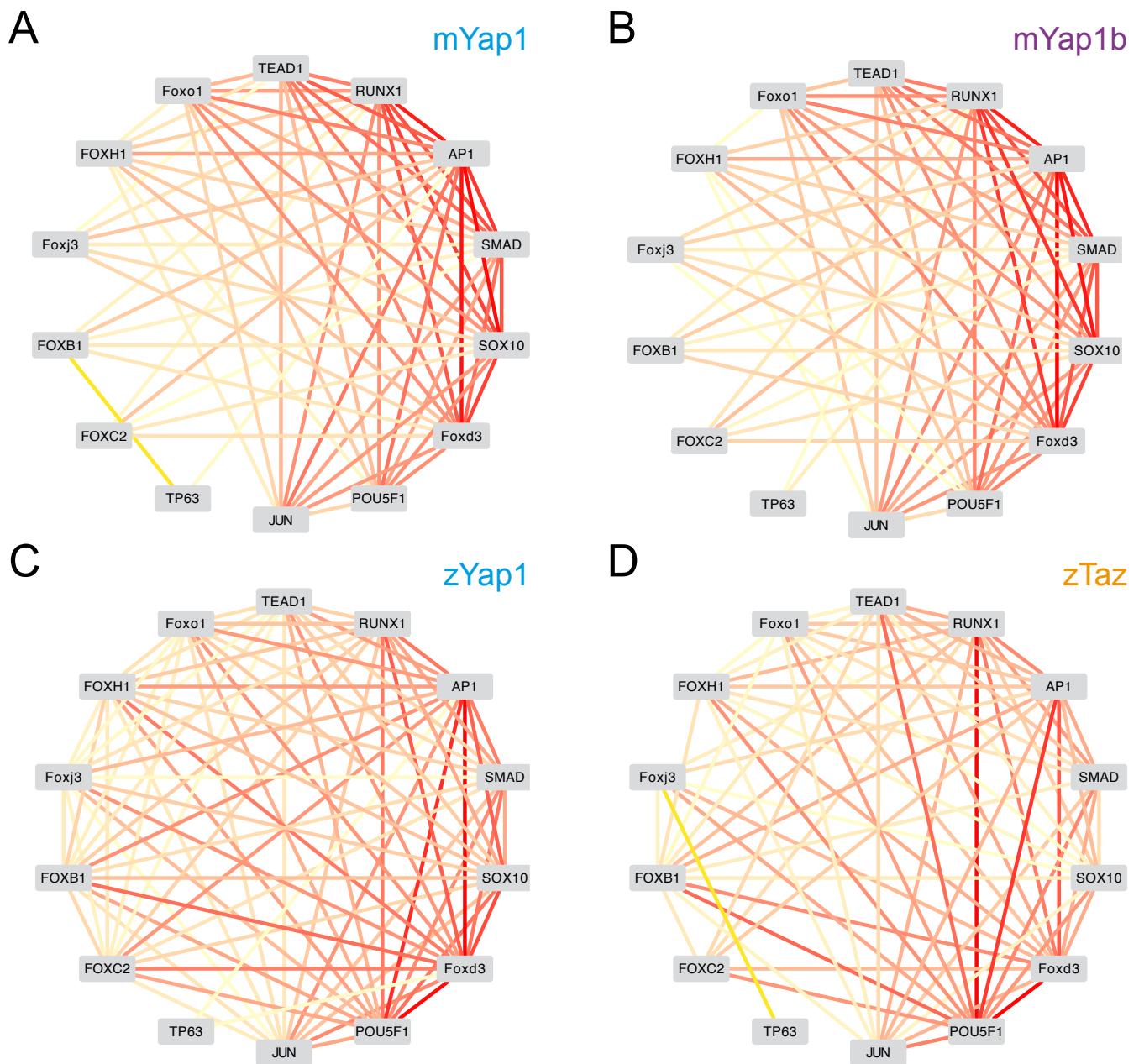
| Identified motif     | Most similar motif                   | Name   | E-value               | q-value              |
|----------------------|--------------------------------------|--------|-----------------------|----------------------|
| TAAA <sub>T</sub> A  | GTAAA <sub>T</sub> AAAC <sub>A</sub> | FOX    | $1.9 \cdot 10^{-373}$ | $5.08 \cdot 10^{-2}$ |
| CATT <sub>T</sub> C  | CAT <sub>T</sub> CC                  | TEAD   | $7.4 \cdot 10^{-147}$ | $2.84 \cdot 10^{-1}$ |
| ACAA <sub>AAG</sub>  | ACAA <sub>T</sub>                    | SOX    | $1.1 \cdot 10^{-145}$ | $3.18 \cdot 10^{-2}$ |
| CGGGG <sub>G</sub> C | GGGGC                                | ZFP161 | $7.6 \cdot 10^{-59}$  | $1.04 \cdot 10^{-2}$ |
| AGGT <sub>TTC</sub>  | GGTGTCGTAAACA                        | ZNF435 | $7.4 \cdot 10^{-53}$  | $9.67 \cdot 10^{-2}$ |
| GTCA <sub>CA</sub>   | TcAGC                                | MABF   | $9.6 \cdot 10^{-47}$  | $2.43 \cdot 10^{-2}$ |
| AA <sub>CG</sub> GGG | ATGGCG                               | YY2    | $5 \cdot 10^{-37}$    | $2.58 \cdot 10^{-2}$ |
| ACATGTCC             | ACATGT <sub>C</sub> ACATGT           | TP63   | $7.2 \cdot 10^{-30}$  | $9.72 \cdot 10^{-2}$ |
| TAT <sub>CAA</sub>   | TATGCCAAAT                           | POU    | $3.6 \cdot 10^{-26}$  | $7.39 \cdot 10^{-2}$ |
| ATTGCT <sub>TG</sub> | TTAATTG                              | HMX2   | $5.3 \cdot 10^{-16}$  | $3.10 \cdot 10^{-2}$ |

**B**

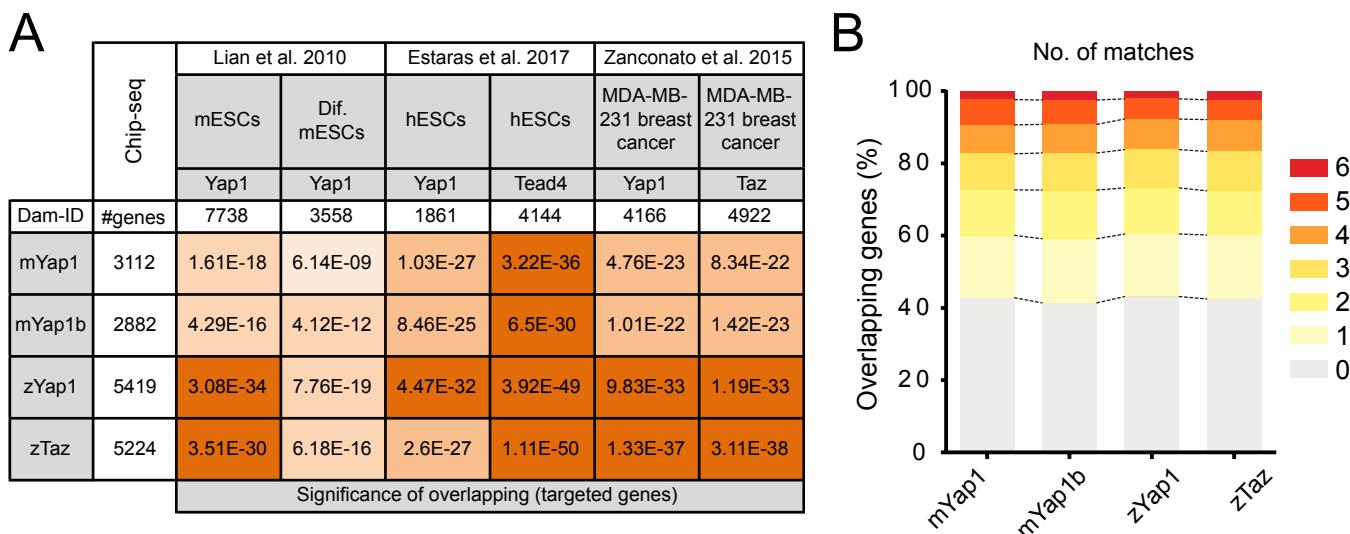
## Enriched motifs in zTaz peaks

| Identified motif     | Most similar motif                   | Name               | E-value               | q-value              |
|----------------------|--------------------------------------|--------------------|-----------------------|----------------------|
| CCTAGGGA             | A <sub>T</sub> CCC <sub>G</sub> GGGA | EBF1               | $3.9 \cdot 10^{-379}$ | $9.29 \cdot 10^{-2}$ |
| GGTCATGA             | GGTCATGACC                           | RXRA               | $1.3 \cdot 10^{-144}$ | $1.30 \cdot 10^{-3}$ |
| CC <sub>T</sub> AGGC | CC <sub>T</sub> GGC                  | TFAP2              | $5.5 \cdot 10^{-122}$ | $1.57 \cdot 10^{-2}$ |
| AACATATG             | AA <sub>A</sub> CATATG               | NEUROG2<br>BHLHE22 | $2.7 \cdot 10^{-98}$  | $2.45 \cdot 10^{-2}$ |
| ATTCATTC             | CATTTCATTCA <sub>T</sub> TC          | ZNF24              | $9.6 \cdot 10^{-66}$  | $5.18 \cdot 10^{-3}$ |
| GTCTAGAC             | GTCTAGAC                             | SMAD3              | $1.7 \cdot 10^{-52}$  | $8.40 \cdot 10^{-4}$ |
| CAGAGGA              | C CAGCAGG                            | ZIC                | $3.3 \cdot 10^{-45}$  | $7.44 \cdot 10^{-2}$ |
| ATGCCAAC             | TGCCAAC                              | HIC1               | $7.4 \cdot 10^{-31}$  | $3.58 \cdot 10^{-2}$ |
| CCCC <sub>A</sub> CG | CGCCCC <sub>A</sub> CG               | EGR1               | $3.3 \cdot 10^{-24}$  | $8.67 \cdot 10^{-4}$ |
| CATTCTC              | cAT <sub>T</sub> Cc                  | TEAD               | $9.5 \cdot 10^{-3}$   | $2.10 \cdot 10^{-1}$ |

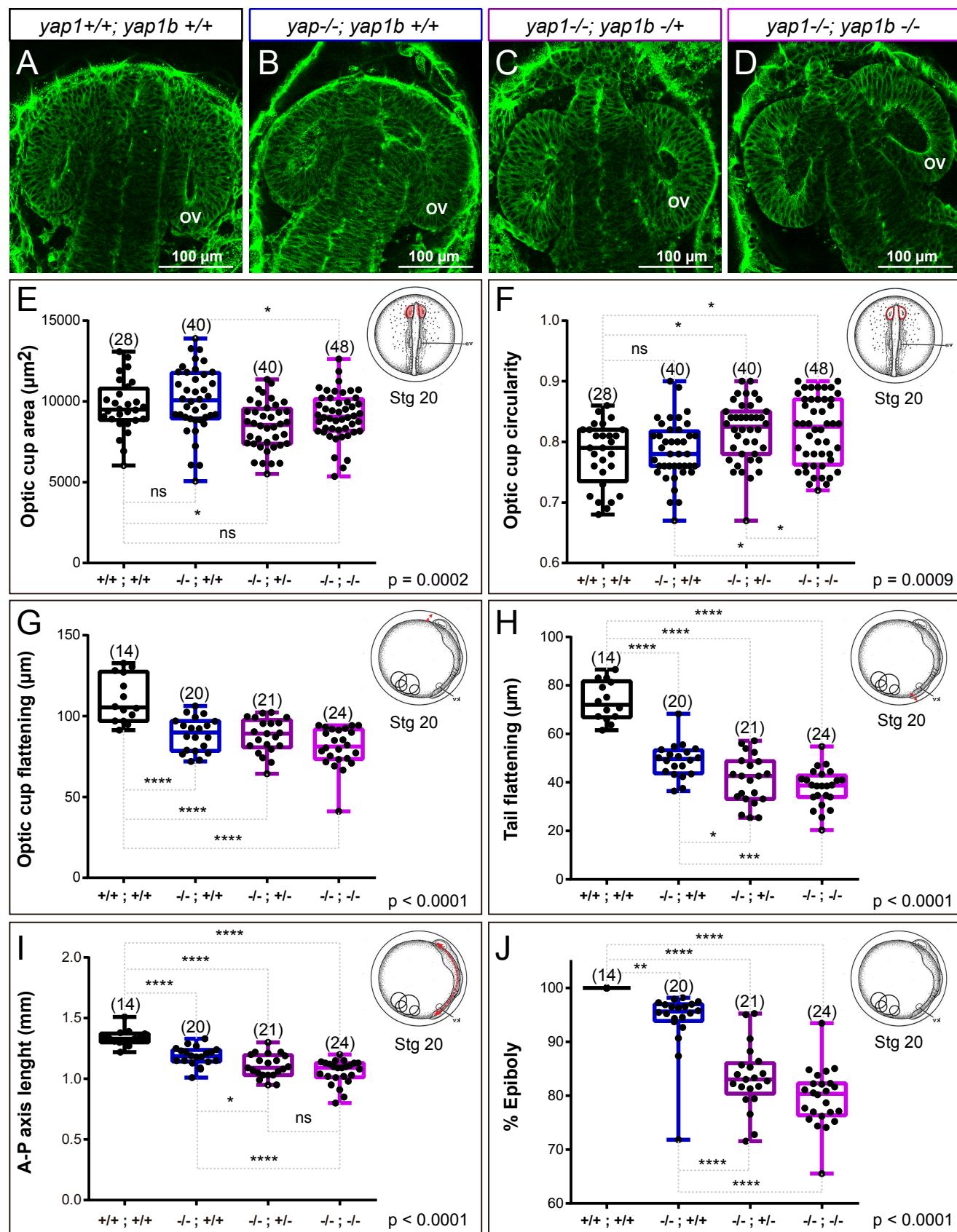
**Figure S10:** Analysis of motifs enriched in zebrafish DamID-seq peaks. List of enriched motifs associated to zebrafish Yap1 (A) and Taz (B) peaks, as identified by DREME and recognized by Tomtom. Both DREME E-value and Tomtom q-values are shown. TEAD motif is highlighted with red boxes. TEAD was only the 22nd best-ranked motif in the taz dataset (11 to 21 are not shown).



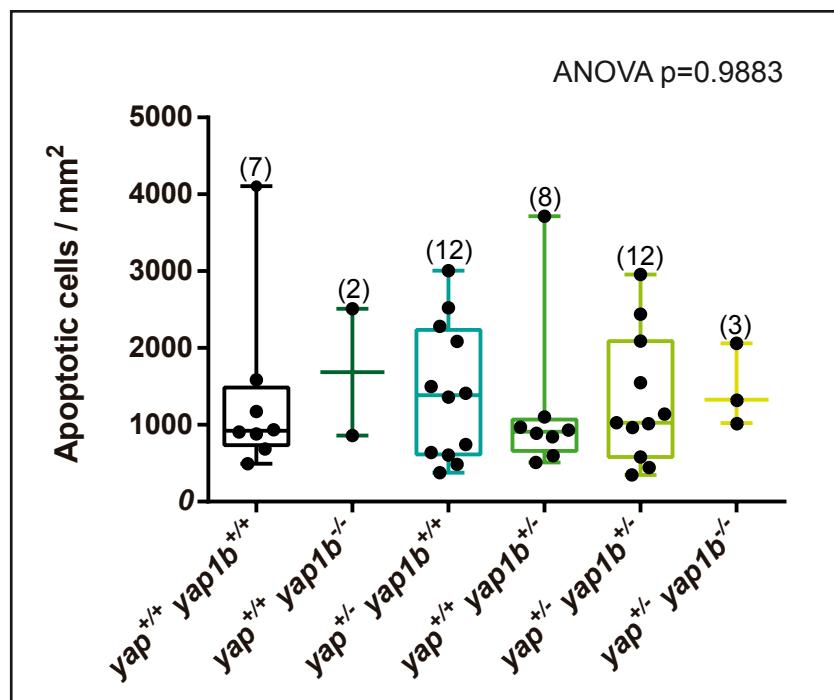
**Figure S11:** Analysis of co-occurring motifs. Cytoscape network visualization of the significant interaction among identified motifs (see Table S2) within medaka Yap1 (A), medaka Yap1b (B), zebrafish Yap1 (C), and zebrafish Taz (D) DamID peaks. For each edge of the network, the percentage (%) of peaks containing at least one match of both motifs was used to infer the intensity of the interactions between motifs. The color of the edges indicates the strength of these interactions, from 5% (pale yellow) to 16% of the peaks (red).



**Figure S12:** Comparative analysis of targeted genes in Chip-seq and DamID studies. (A) Values included in the table show how significant (hypergeometric test) is the overlap between genes identified as Yap1, Yap1b or Taz targets in our DamID study and identified targeted genes in 6 available Chip-seq datasets. Reference papers, source material and antibodies used are indicated. The number of genes associated either to DamID or Chip-seq peaks is also indicated. Significance levels are highlighted with a color code. (B) The graphics shows the % of medaka Yap1, Yap1b, and zebrafish Yap1 and Taz associated genes overlapping with genes identified in 0 to 6 of the Chip-seq datasets.



**Figure S13:** Quantitative analysis of morphogenetic defects in *yap1/yap1b* single and double mutants. (A-D) Confocal sections of phalloidin stained embryos showing optic cup morphology in wild type, *yap1* mutants, and *yap1/yap1b* double mutants. (E-J) Quantitative analysis of optic cup area (E), circularity (F), and flattening (G); as well as tail flattening (H), embryo axis lenght (I), and % of epiboly (J) in wild type and mutant embryos reveal enhanced tissue malformations in *yap1/yap1b* double mutants. One Way ANOVA analysis followed by Fisher's LSD test was used to evaluate statistical significance. ov = optic vesicle. Magnification bars are included in the figure.



**Figure S14:** Apoptosis levels in embryos harboring at least one wild type *yap1* allele. Quantification of caspase-3 positive cells per area shows no significant differences as long as a functional copy of *yap1* is present in the genome. One Way ANOVA analysis followed by Fisher's LSD test was used to evaluate statistical significance.

**Table S1:** (A) List of gene and protein IDs for Yap family members from different species.  
(B) Chromosomes/scaffolds and syntenic arrangements are also indicated

**A**

| Species                         |                   | Gene ID                    | Protein ID           | Genebank protein ID | Chromosome/scaffold |
|---------------------------------|-------------------|----------------------------|----------------------|---------------------|---------------------|
|                                 | <b>Yap1</b>       | <b>Yap1</b>                |                      |                     |                     |
| <i>Homo sapiens</i>             | Human             | ENSG00000137693            | ENSP00000478927.1    |                     | chr11               |
| <i>Mus musculus</i>             | Mouse             | ENSMUSG0000053110          | ENSMUSP0000069554    |                     | chr9                |
| <i>Lepisosteus oculatus</i>     | Gar               | ENSLOC00000005660          | ENSLOCP0000006836    |                     | LG3                 |
| <i>Clupea harengus</i>          | Herring           | XM_012830954.1             |                      | XP_012686408.1,     |                     |
| <i>Danio rerio</i>              | Zebrafish         | ENSDARG00000068401         | ENSDARP0000089684    |                     | chr18               |
| <i>Astyanax mexicanus</i>       | Mexican tetra     | ENSAMXG0000015263          | ENSAMXP0000015710    | XP_022520645.1      | Scaffold KB871855.1 |
| <i>Oryzias latipes</i>          | Medaka            | ENSORLG00000002708         | ENSORLP0000003377*   | XP_004075324.1      | ch13                |
| <i>Oreochromis niloticus</i>    | Tilapia           | ENSONIG00000005729         | ENSONIP0000007219.1  |                     | GL831218.           |
| <i>Gasterosteus aculeatus</i>   | Stickleback       | ENSGACG00000012139         | ENSGACP0000016062.1  |                     | LGI                 |
| <i>Gadus morhua</i>             | Cod               | ENSGM0G00000002826         | ENSGMOP00000002984.1 |                     | Scaffold_2296       |
| <i>Esox lucius</i>              | N. Pike           | XM_013135880               |                      | XP_012991334        | LG1                 |
|                                 |                   |                            |                      |                     |                     |
|                                 | <b>Yap1b</b>      | <b>Yap1b</b>               |                      |                     |                     |
| <i>Oryzias latipes</i>          | Medaka            | ENSORLG00000005573         | ENSORLP0000007010*   | XP_004076495.1      | chr14               |
| <i>Oreochromis niloticus</i>    | Tilapia           | ENSONIG00000004889         | ENSONIP0000006147*   | XP_005472252.1      | Scaffold GL831183   |
| <i>Gasterosteus aculeatus</i>   | Stickleback       | ENSGACG00000020627         | ENSGACP0000027285    |                     | LGVII               |
| <i>Gadus morhua</i>             | Cod               | ENSGM0G00000002165         | ENSGMOP00000002308   |                     | Scaffold_4380       |
| <i>Esox lucius</i>              | N. Pike           | XM_013131740.2             |                      | XP_012987194        | LG07                |
|                                 | <i>birc2</i>      | <i>birc2</i>               |                      |                     |                     |
| <i>Clupea harengus</i>          | Herring           | 105895386 ( <i>birc2</i> ) |                      |                     | Scaffold_244        |
| <i>Danio rerio</i>              | Zebrafish         | ENSDARG00000044619         |                      |                     | chr21               |
| <i>Astyanax mexicanus</i>       | Mexican tetra     | ENSAMXG0000004822          |                      |                     | Scaffold_KB882129   |
|                                 | <b>Taz(wwtr1)</b> | <b>Taz(wwtr1)</b>          |                      |                     |                     |
| <i>Homo sapiens</i>             | Human             | ENSG0000018408             | ENSP00000419465.1    |                     | chr3                |
| <i>Mus musculus</i>             | Mouse             | ENSMUSG0000027803          | ENSMUSP0000029380    |                     | chr3                |
| <i>Lepisosteus oculatus</i>     | Gar               | ENSLOC0000001759           | ENSLOCP0000002036    |                     | LG14                |
| <i>Clupea harengus</i>          | Herring           | 105898312 (wwtr1)          |                      | XP_012680789.1      | Scaffold_328        |
| <i>Danio rerio</i>              | Zebrafish         | ENSDARG00000067719         | ENSDARP00000139152   |                     | chr22               |
| <i>Astyanax mexicanus</i>       | Mexican tetra     | ENSAMXG0000004716          | ENSAMXP0000004825.1  |                     | Scaffold KB871797.1 |
| <i>Esox lucius</i>              | N. Pike           | XM_020044305.1             |                      | XP_019899864.1      | Scaffold_0183       |
| <i>Gadus morhua</i>             | Cod               | na                         |                      | na                  | Scaffold_2167       |
|                                 | <i>commd2</i>     | <i>commd2</i>              |                      |                     |                     |
| <i>Oryzias latipes</i>          | Medaka            | ENSORLG0000002784          |                      |                     | chr4                |
| <i>Oreochromis niloticus</i>    | Tilapia           | ENSONIG0000007638          |                      |                     | Scaffold GL831201.1 |
| <i>Gasterosteus aculeatus</i>   | Stickleback       | ENSGACG0000002117          |                      |                     | scaffold_56         |
|                                 |                   |                            |                      |                     |                     |
| <b>Basal Teleost Species</b>    |                   | Gene ID                    | Protein ID           | Genebank protein ID | Chromosome/scaffold |
|                                 | <b>Yap1</b>       | <b>Yap1</b>                |                      |                     |                     |
| <i>Anguilla japonica</i>        | Japanese eel      | na                         | na                   |                     | ANJA007541          |
| <i>Paramormyrops kingsleyae</i> | Elephantfish      | na                         | na                   |                     | Scaffold84          |
|                                 | <b>Yap1b</b>      | <b>Yap1b</b>               |                      |                     |                     |
| <i>Anguilla japonica</i>        | Japanese eel      | na                         | na                   |                     | ANJA007098          |
| <i>Paramormyrops kingsleyae</i> | Elephantfish      | na                         | na                   |                     | Scaffold63          |
|                                 | <b>Taz(wwtr1)</b> | <b>Taz(wwtr1)</b>          |                      |                     |                     |
| <i>Anguilla japonica</i>        | Japanese eel      | na                         | na                   |                     | ANJA001635          |
| <i>Anguilla japonica</i>        | Japanese eel      | na                         | na                   |                     | ANJA000083          |
| <i>Paramormyrops kingsleyae</i> | Elephantfish      | na                         | na                   |                     | Scaffold797         |
| <i>Paramormyrops kingsleyae</i> | Elephantfish      | na                         | na                   |                     | Scaffold40          |

**B**

| Species                       | Yap1          | Chromosomal arrangement |      |            |          |          |                    |          |         |                    |          |         |                    |        |
|-------------------------------|---------------|-------------------------|------|------------|----------|----------|--------------------|----------|---------|--------------------|----------|---------|--------------------|--------|
|                               |               | <Maml2                  | 6 Mb | arhgap42>  | <trpc6   | <angptl5 | Cep126>            | Yap1>    | Birc3>  | Birc2>             | <tmem123 |         |                    |        |
| <i>Homo sapiens</i>           | Human         | <Maml2                  | 6 Mb | arhgap42>  | <trpc6   | <angptl5 | Cep126>            | Yap1>    | Birc3>  | Birc2>             | <tmem123 |         |                    |        |
| <i>Mus musculus</i>           | Mouse         | <Maml2                  | 5 Mb | arhgap42>  | <trpc6   |          | Cep126>            | Yap1>    | Birc3>  | Birc2>             | <tmem123 |         |                    |        |
| <i>Lepisosteus oculatus</i>   | Gar           |                         |      | arhgap42>  | <trpc6   | <angptl5 | cep126>            | Yap1>    |         | Birc2>             |          | <Maml2  | <gab2              | <nars2 |
| <i>Clupea harengus</i>        | Herring       |                         |      |            |          |          | cep126>            | Yap1a>   |         |                    |          |         | <tbc6              |        |
| <i>Danio rerio</i>            | Zebrafish     |                         |      | arhgap42b> | <trpc6b  | 4.5 Mb   | cep126>            | Yap1>    |         |                    | <tmem123 | <tbc6   | six5>              |        |
| <i>Astyanax mexicanus</i>     | Mexican tetra |                         |      |            |          |          | truncated sca      | Yap1>    |         |                    |          | <tbc6   | six5>              |        |
| <i>Oryzias latipes</i>        | Medaka        |                         |      | arhgap42b> | <trpc6b  |          |                    | Yap1a>   |         |                    |          | <Maml2  | <gab2              | <nars2 |
| <i>Oreochromis niloticus</i>  | Tilapia       |                         |      | arhgap42b> | <trpc6b  |          |                    | Yap1a>   |         |                    |          | <Maml2  | <gab2              | <nars2 |
| <i>Gasterosteus aculeatus</i> | Stickleback   |                         |      | arhgap42b> | <trpc6b  |          | cep126>            | Yap1a>   |         |                    |          | <Maml2  | <gab2              | <nars2 |
| <i>Gadus morhua</i>           | Cod           |                         |      | arhgap42b> | <trpc6b  |          |                    | Yap1a>   |         |                    |          | <Maml2  | <gab2              | <nars2 |
| <i>Esox lucius</i>            | N. Pike       |                         |      | arhgap42b> | <trpc6b  |          | cep126>            | Yap1a>   |         |                    |          | <Maml2  | <gab2              | <nars2 |
|                               |               |                         |      |            |          |          |                    |          |         |                    |          |         |                    |        |
|                               | Yap1b         |                         |      |            |          |          |                    |          |         |                    |          |         |                    |        |
| <i>Oryzias latipes</i>        | Medaka        |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    | Yap1b>   |         | Birc2>             |          |         |                    |        |
| <i>Oreochromis niloticus</i>  | Tilapia       |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    | Yap1b>   |         | Birc2>             |          |         |                    |        |
| <i>Gasterosteus aculeatus</i> | Stickleback   |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    | Yap1b>   |         | Birc2>             |          |         |                    |        |
| <i>Gadus morhua</i>           | Cod           |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    | Yap1b>   |         |                    |          |         | truncated scaffold |        |
| <i>Esox lucius</i>            | N. Pike       |                         |      |            | na       | na       | na                 | Yap1b>   |         | Birc2>             |          |         |                    |        |
|                               | birc2         |                         |      |            |          |          |                    |          |         |                    |          |         |                    |        |
| <i>Clupea harengus</i>        | Herring       |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    |          |         | Birc2>             |          |         |                    |        |
| <i>Danio rerio</i>            | Zebrafish     |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    |          |         | Birc2>             |          |         |                    |        |
| <i>Astyanax mexicanus</i>     | Mexican tetra |                         |      | arhgap42a> | <trpc6a  | <angptl5 |                    |          |         | Birc2>             |          |         |                    |        |
|                               | Taz(wwtr1)    |                         |      |            |          |          |                    |          |         |                    |          |         |                    |        |
| <i>Homo sapiens</i>           | Human         |                         |      | hps3>      | <tm4sf18 | <tm4sf1  | tm4sf4>            | <taz     | <commd2 | <ankub1            | rnf13>   | <pfn2   |                    |        |
| <i>Mus musculus</i>           | Mouse         |                         |      |            |          |          | <tm4sf1            | tm4sf4>  | <taz    | <commd2            | <ankub1  | rnf13>  | <pfn2              |        |
| <i>Lepisosteus oculatus</i>   | Gar           |                         |      | hps3>      | <tm4sf1  | tm4sf4>  | tm4sf18>           | <taz     |         |                    | rnf13>   | <pfn2   |                    |        |
| <i>Clupea harengus</i>        | Herring       |                         |      | hps3>      |          |          | tm4sf4>            | <taz     | <commd2 | <ankub1            | rnf13>   | <pfn2   |                    |        |
| <i>Danio rerio</i>            | Zebrafish     |                         |      | hps3>      | <tm4sf18 |          | tm4sf4>            | <taz     | <commd2 | 3 Mb               | pfn2>    | <rnf132 |                    |        |
| <i>Astyanax mexicanus</i>     | Mexican tetra |                         |      | hps3>      | <tm4sf18 |          | tm4sf4>            | <taz     | <commd2 | truncated scaffold |          |         |                    |        |
| <i>Esox lucius</i>            | N. Pike       |                         |      |            | na       | na       | <taz?              | <commd2  | na      |                    |          |         |                    |        |
| <i>Gadus morhua</i>           | Cod           |                         |      |            |          |          | truncated scaffold | <taz?    | <commd2 |                    |          |         |                    |        |
|                               | commd2        |                         |      |            |          |          |                    |          |         |                    |          |         |                    |        |
| <i>Oryzias latipes</i>        | Medaka        |                         |      |            |          |          | ctns>              |          | <commd2 |                    |          |         |                    |        |
| <i>Oreochromis niloticus</i>  | Tilapia       |                         |      |            |          |          | ctns>              | <trappc8 | <commd2 |                    |          |         |                    |        |
| <i>Gasterosteus aculeatus</i> | Stickleback   |                         |      |            |          |          | ctns>              | <trappc8 | <commd2 |                    |          |         |                    |        |

|                     |               |
|---------------------|---------------|
| deletion            |               |
| insertion           |               |
| re-arrangement      |               |
| inversion           |               |
| truncated scaffolds |               |
| na                  | not annotated |

**Table S2:** List of motifs used to evaluate motifs cooperation

| ID       | NAME                |
|----------|---------------------|
| MA0002.2 | RUNX1               |
| MA0030.1 | FOXF2               |
| MA0041.1 | Foxd3               |
| MA0047.2 | Foxa2               |
| MA0077.1 | SOX9                |
| MA0078.1 | Sox17               |
| MA0090.1 | TEAD1               |
| MA0143.3 | Sox2                |
| MA0157.2 | FOXO3               |
| MA0442.2 | SOX10               |
| MA0479.1 | FOXH1               |
| MA0480.1 | Foxo1               |
| MA0481.2 | FOXP1               |
| MA0488.1 | JUN                 |
| MA0490.1 | JUNB                |
| MA0491.1 | JUND                |
| MA0513.1 | SMAD2::SMAD3::SMAD4 |
| MA0525.2 | TP63                |
| MA0593.1 | FOXP2               |
| MA0628.1 | POU6F1              |
| MA0785.1 | POU2F1              |
| MA0786.1 | POU3F1              |
| MA0787.1 | POU3F2              |
| MA0795.1 | SMAD3               |
| MA0845.1 | FOXB1               |
| MA0846.1 | FOXC2               |
| MA0850.1 | FOXP3               |
| MA0851.1 | Foxj3               |
| MA0852.2 | FOXK1               |
| MA0866.1 | SOX21               |
| MA0870.1 | Sox1                |
| MA0940.1 | AP1                 |
| MA1115.1 | POU5F1              |
| MA1120.1 | SOX13               |
| MA1153.1 | Smad4               |

**Table S3:** Overrepresented GO-slim terms associated to genes targeted by Yap1 or Yap1b

FDR&lt;0.05; Fold enrichment &gt;1.3

Fisher's Exact test with FDR multiple test correction.

| Yap1 medaka   |      | Oryzias latipes (REF) |          | Client Text Box Input ( Hierarchy ) NEW! ) |     |             |          |  |
|---|------|-----------------------|----------|--|-----|-------------|----------|--|
| PANTHER GO-Slim Molecular Function  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| transmembrane receptor protein tyrosine kinase activity                       | 74   | 27                    | 11.38    | 2.37                                       | +   | 3.19E-04    | 9.93E-03 |  |
| sequence-specific DNA binding RNA polymerase II transcription factor activity | 258  | 71                    | 39.69    | 1.79                                       | +   | 3.78E-05    | 2.36E-03 |  |
| sequence-specific DNA binding transcription factor activity                   | 806  | 191                   | 124.00   | 1.54                                       | +   | 1.42E-07    | 2.65E-05 |  |
| binding   | 4505 | 785                   | 693.06   | 1.13                                       | +   | 2.63E-04    | 9.83E-03 |  |
| DNA binding   | 1027 | 229                   | 158.00   | 1.45                                       | +   | 4.37E-07    | 4.09E-05 |  |
| PANTHER GO-Slim Biological Process  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| negative regulation of apoptotic process                                      | 119  | 40                    | 18.31    | 2.18                                       | +   | 5.36E-05    | 3.20E-03 |  |
| developmental process   | 1479 | 316                   | 227.53   | 1.39                                       | +   | 8.11E-08    | 1.94E-05 |  |
| ectoderm development  | 230  | 69                    | 35.38    | 1.95                                       | +   | 4.37E-06    | 5.22E-04 |  |
| embryo development  | 104  | 31                    | 16.00    | 1.94                                       | +   | 2.08E-03    | 4.97E-02 |  |
| cell differentiation  | 488  | 117                   | 75.08    | 1.56                                       | +   | 2.69E-05    | 2.14E-03 |  |
| anion transport   | 272  | 65                    | 41.85    | 1.55                                       | +   | 2.06E-03    | 5.47E-02 |  |
| regulation of phosphate metabolic process                                     | 535  | 115                   | 82.31    | 1.40                                       | +   | 1.53E-03    | 5.21E-02 |  |
| PANTHER Protein Class   | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| transcription factor  | 1088 | 253                   | 167.38   | 1.51                                       | +   | 4.58E-09    | 9.85E-07 |  |
| Yap1b medaka  |      | Oryzias latipes (REF) |          | Client Text Box Input ( Hierarchy ) NEW! ) |     |             |          |  |
| PANTHER GO-Slim Molecular Function  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| transmembrane receptor protein tyrosine kinase activity                       | 74   | 30                    | 10.55    | 2.84                                       | +   | 7.54E-06    | 1.41E-03 |  |
| sequence-specific DNA binding RNA polymerase II transcription factor activity | 258  | 63                    | 36.78    | 1.71                                       | +   | 2.33E-04    | 1.09E-02 |  |
| sequence-specific DNA binding transcription factor activity                   | 806  | 166                   | 114.90   | 1.44                                       | +   | 2.33E-05    | 2.17E-03 |  |
| PANTHER GO-Slim Biological Process  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| negative regulation of apoptotic process                                      | 119  | 39                    | 16.96    | 2.30                                       | +   | 2.72E-05    | 1.63E-03 |  |
| developmental process   | 1479 | 314                   | 210.83   | 1.49                                       | +   | 1.27E-10    | 3.04E-08 |  |
| embryo development  | 104  | 32                    | 14.83    | 2.16                                       | +   | 3.52E-04    | 1.05E-02 |  |
| ectoderm development  | 230  | 65                    | 32.79    | 1.98                                       | +   | 4.62E-06    | 3.68E-04 |  |
| transmembrane receptor protein tyrosine kinase signaling pathway              | 157  | 43                    | 22.38    | 1.92                                       | +   | 3.34E-04    | 1.14E-02 |  |
| MAPK cascade  | 314  | 75                    | 44.76    | 1.68                                       | +   | 1.35E-04    | 6.43E-03 |  |
| cell differentiation  | 488  | 114                   | 69.57    | 1.64                                       | +   | 4.46E-06    | 5.33E-04 |  |
| cell adhesion   | 334  | 72                    | 47.61    | 1.51                                       | +   | 1.85E-03    | 4.91E-02 |  |
| biological adhesion   | 334  | 72                    | 47.61    | 1.51                                       | +   | 1.85E-03    | 4.42E-02 |  |
| regulation of phosphate metabolic process                                     | 535  | 112                   | 76.27    | 1.47                                       | +   | 2.83E-04    | 1.13E-02 |  |
| PANTHER Protein Class   | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| transcription factor  | 1088 | 222                   | 155.10   | 1.43                                       | +   | 1.31E-06    | 2.81E-04 |  |
| Common Yap1 & Yap1b   |      | Oryzias latipes (REF) |          | Client Text Box Input ( Hierarchy ) NEW! ) |     |             |          |  |
| PANTHER GO-Slim Molecular Function  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| sequence-specific DNA binding transcription factor activity                   | 806  | 154                   | 113.34   | 1.36                                       | +   | 5.26E-04    | 4.92E-02 |  |
| DNA binding   | 1027 | 196                   | 144.42   | 1.36                                       | +   | 9.64E-05    | 1.80E-02 |  |
| PANTHER GO-Slim Biological Process  | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| negative regulation of apoptotic process                                      | 119  | 32                    | 12.05    | 2.66                                       | +   | 6.35E-06    | 7.59E-04 |  |
| developmental process   | 1479 | 226                   | 149.76   | 1.51                                       | +   | 9.28E-09    | 2.22E-06 |  |
| transmembrane receptor protein serine/threonine kinase signaling pathway      | 69   | 18                    | 6.99     | 2.58                                       | +   | 1.05E-03    | 2.79E-02 |  |
| embryo development  | 104  | 24                    | 10.53    | 2.28                                       | +   | 6.34E-04    | 2.16E-02 |  |
| ectoderm development  | 230  | 47                    | 23.29    | 2.02                                       | +   | 3.64E-05    | 2.17E-03 |  |
| transmembrane receptor protein tyrosine kinase signaling pathway              | 157  | 31                    | 15.90    | 1.95                                       | +   | 1.34E-03    | 3.21E-02 |  |
| regulation of cell cycle  | 170  | 32                    | 17.21    | 1.86                                       | +   | 2.09E-03    | 4.55E-02 |  |
| MAPK cascade  | 314  | 56                    | 31.79    | 1.76                                       | +   | 1.84E-04    | 7.34E-03 |  |
| cell differentiation  | 488  | 86                    | 49.41    | 1.74                                       | +   | 6.73E-06    | 5.36E-04 |  |
| regulation of phosphate metabolic process                                     | 535  | 86                    | 54.17    | 1.59                                       | +   | 1.30E-04    | 6.23E-03 |  |
| PANTHER Protein Class   | #    | #                     | expected | Fold Enrichment                            | +/- | raw P value | FDR      |  |
| transcription factor  | 1088 | 167                   | 110.17   | 1.52                                       | +   | 7.52E-07    | 1.62E-04 |  |

**Table S4:** List of primers used in this work. Their name, sequence, and application are indicated.

| Nº | Name             | Sequence (5' - 3')                                 | Application                                |
|----|------------------|--|--|
| 1  | mYapKOsg#3_TAGG  | TAGGCACGTCGTGGGAATCTCGT                            | Generation of <i>yap1</i> mutants (sgRNA)  |
| 2  | mYapKOsg#3_AAAC  | AAACACGAGATTCCCGACGACGTC                           | Generation of <i>yap1</i> mutants (sgRNA)  |
| 3  | mYapKOsg#4_TAGG  | TAGGATCTCGTAGGACGACTGC                             | Generation of <i>yap1</i> mutants (sgRNA)  |
| 4  | mYapKOsg#4_AAAC  | AAACGCAGTCGCTTACAGAGAT                             | Generation of <i>yap1</i> mutants (sgRNA)  |
| 5  | mYapKOsg#5_TAGG  | TAGGGACGAGTGTGCGCGTACG                             | Generation of <i>yap1</i> mutants (sgRNA)  |
| 6  | mYapKOsg#5_AAAC  | AAACCGTACGCGCACACTCGTC                             | Generation of <i>yap1</i> mutants (sgRNA)  |
| 7  | Crispr mYAP Fw   | CAGCCCACAAACATACTACGTTA                            | Genotyping of <i>yap1</i> mutants          |
| 8  | Crispr mYAP Rv   | AAGATCTGCTGGGAGAACTCTTT                            | Genotyping of <i>yap1</i> mutants          |
| 9  | sgRNA mYAP1B #1  | taatacgactcaataGGGGGGAGTGGCACATCGTCgttttagagctagaa | Generation of <i>yap1b</i> mutants (sgRNA) |
| 10 | sgRNA mYAP1B #2  | taatacgactcaataGGCGGGGAGCGCAGCGGGGGgttttagagctagaa | Generation of <i>yap1b</i> mutants (sgRNA) |
| 11 | CRISPR mYAP1B Fw | TTTAAAGAAACCTGCAGAGCAAC                            | Genotyping of <i>yap1b</i> mutants         |
| 12 | CRISPR mYAP1B Rv | AGCAACATGTCCTAACAAAAACC                            | Genotyping of <i>yap1b</i> mutants         |
| 13 | OL-YAP Fw-Spel   | AATT TactagtATGGATCCGAGCCAGCACAAACCTC              | Generation of Dam-TF fusions               |
| 14 | OL-YAP Rv-XbaI   | AATT TtctagaTTATAACCATGTGAGGAAGCTCTC               | Generation of Dam-TF fusions               |
| 15 | OL-YAP1B Fw-Spel | AATT TactagtATGGACGCGCACCGAGCGGACG                 | Generation of Dam-TF fusions               |
| 16 | OL-YAP1B Rv-NheI | AATT TgctagcCTATAGCCAGGTGAGCAGATTGTC               | Generation of Dam-TF fusions               |
| 17 | DR-YAP Fw-Spel   | AATT TactagtATGGATCCGAACCAGCACAAAC                 | Generation of Dam-TF fusions               |
| 18 | DR-YAP Rv-NheI   | AATT TgctagcCTATAGCCAGGTAGAAAGTT                   | Generation of Dam-TF fusions               |
| 19 | DR-TAZ Fw-Spel   | AATT TactagtATGAGCGGTATCCTCTCCAGC                  | Generation of Dam-TF fusions               |
| 20 | DR-TAZ Rv-XbaI   | AATT TtctagaTTAGAGCCAGGTGAGGAAGGGCTC               | Generation of Dam-TF fusions               |
| 21 | AdRt             | CTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGA         | Adaptor primers for DamID                  |
| 22 | AdRb             | TCCTCGGCCG   | Adaptor primers for DamID                  |
| 23 | AdR_PCRprimer    | G*G*T*C*G*C*GGCGAGGATC                             | phosphorothioate primer for DamID          |
| 24 | ISH yap MDK Fw   | ACTCCAGATGACTTCCTCAACAG                            | Generation of templates for ISH            |
| 25 | ISH yap MDK Rv   | AGCCTTGAAGACACAGACACAAT                            | Generation of templates for ISH            |
| 26 | ISH yap1b MDK Fw | CCCTGTTCACTAGTAGGGTGTGATGA                         | Generation of templates for ISH            |
| 27 | ISH yap1b MDK Rv | TACATCTCCCTCTGGAGTCAC                              | Generation of templates for ISH            |
| 28 | qPCR zEF1A Fw    | TCCACCGGTACCTGTACCTACA                             | Primers for qPCR y ddPCR                   |
| 29 | qPCR zEF1A Rv    | CAACACCCAGCGTACTTGA                                | Primers for qPCR y ddPCR                   |
| 30 | qPCR zYAP Fw     | ATGAACCCAGCCTCAGGTCC                               | Primers for qPCR y ddPCR                   |
| 31 | qPCR zYAP Rv     | TCCAGCCAAGAGGTGGTTTT                               | Primers for qPCR y ddPCR                   |
| 32 | qPCR zTAZ Fw     | TTCTCTAACAGTGGGCCGTA                               | Primers for qPCR y ddPCR                   |
| 33 | qPCR zTAZ Rv     | AGTGGTGGGATGCTGTAAAC                               | Primers for qPCR y ddPCR                   |
| 34 | qPCR mEF1A Fw    | AAACCCAGAAACACCGAAACAT                             | Primers for qPCR y ddPCR                   |
| 35 | qPCR mEF1A Rv    | CCTCCGCACCTGTAGATCAG                               | Primers for qPCR y ddPCR                   |
| 36 | qPCR mYAP Fw     | GTTGAACCAGGCTACCCCTC                               | Primers for qPCR y ddPCR                   |
| 37 | qPCR mYAP Rv     | AGGTCCACTAGCTGGGCTTA                               | Primers for qPCR y ddPCR                   |
| 38 | qPCR mYAP1B Fw   | GTCAACCCGGAGTCAGGTCC                               | Primers for qPCR y ddPCR                   |
| 39 | qPCR mYAP1B Rv   | AGTACATCTCCCTCTGGAGTC                              | Primers for qPCR y ddPCR                   |
| 40 | cyr61 Fw         | GAGCTCTCCCTGCCATT                                  | Primers for qPCR                           |
| 41 | cyr61 Rv         | CTGTATGCAGGCAGGGCTT                                | Primers for qPCR                           |
| 42 | marcks1b Fw      | AAGACCAACGGACAGGAGAAC                              | Primers for qPCR                           |
| 43 | marcks1b Rv      | CTTCGGGTTGGTGGCTTCC                                | Primers for qPCR                           |
| 44 | amotl2a Fw       | GGGGCGCAAAGAGATACAAC                               | Primers for qPCR                           |
| 45 | amotl2a Rv       | TGTGGATGGTAGGGACGGAT                               | Primers for qPCR                           |
| 46 | lats2 Fw         | AACGAGCAACTTTTCCGC                                 | Primers for qPCR                           |
| 47 | lats2 Rv         | CATCTGCCATGTGTGATG                                 | Primers for qPCR                           |
| 48 | ctgfa Fw         | GCCGACAGGAGATCCACTTG                               | Primers for qPCR                           |
| 49 | ctgfa Rv         | CCTGCAGCCGCGTATGAGTA                               | Primers for qPCR                           |
| 50 | boka Fw          | ACATCTTCTCCACTGGTATCAGC                            | Primers for qPCR                           |
| 51 | boka Rv          | TCAACCACACGGTCAGACTC                               | Primers for qPCR                           |
| 52 | yap1 Fw          | GTTGAACCAGGCTACCCCTC                               | Primers for qPCR                           |
| 53 | yap1 Rv          | AGGTCCACTAGCTGGGCTTA                               | Primers for qPCR                           |
| 54 | yap1b Fw         | GTCAACCCGGAGTCAGGTCC                               | Primers for qPCR                           |
| 55 | yap1b Rv         | AGTACATCTCCCTCTGGAGTC                              | Primers for qPCR                           |
| 56 | ef1a Fw          | AAACCCAGAAACACCGAAACAT                             | Primers for qPCR                           |
| 57 | ef1a Rv          | CCTCCGCACCTGTAGATCAG                               | Primers for qPCR                           |

**Table S5:** List of genomic coordinates for peaks associated to Yap family members

[Click here to download Table S5](#)

**Table S6:** List of genes identified by DamID as Yap1, Yap1b or Taz targets identified also in previous ChIP-seq analyses

[Click here to download Table S6](#)