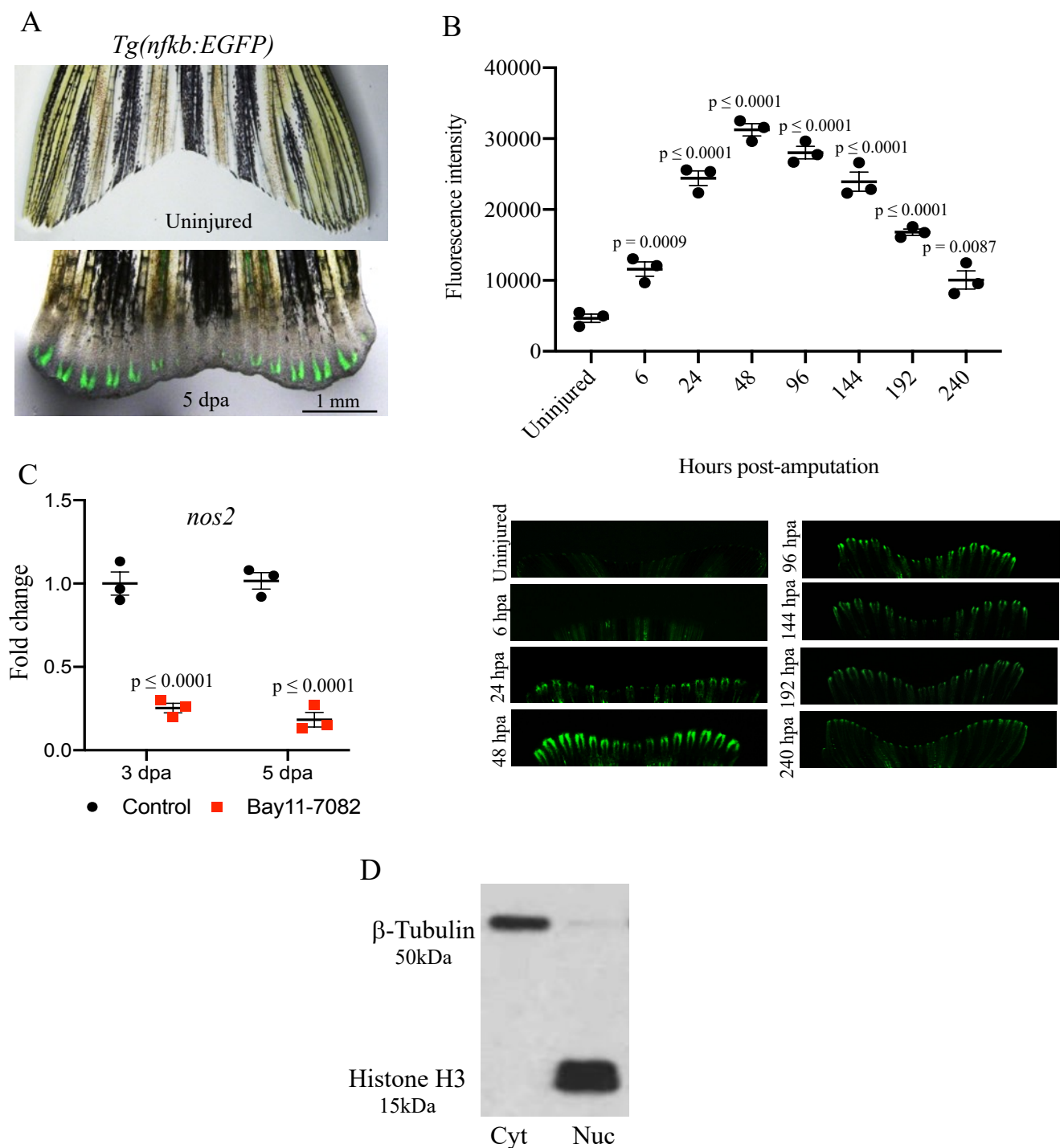


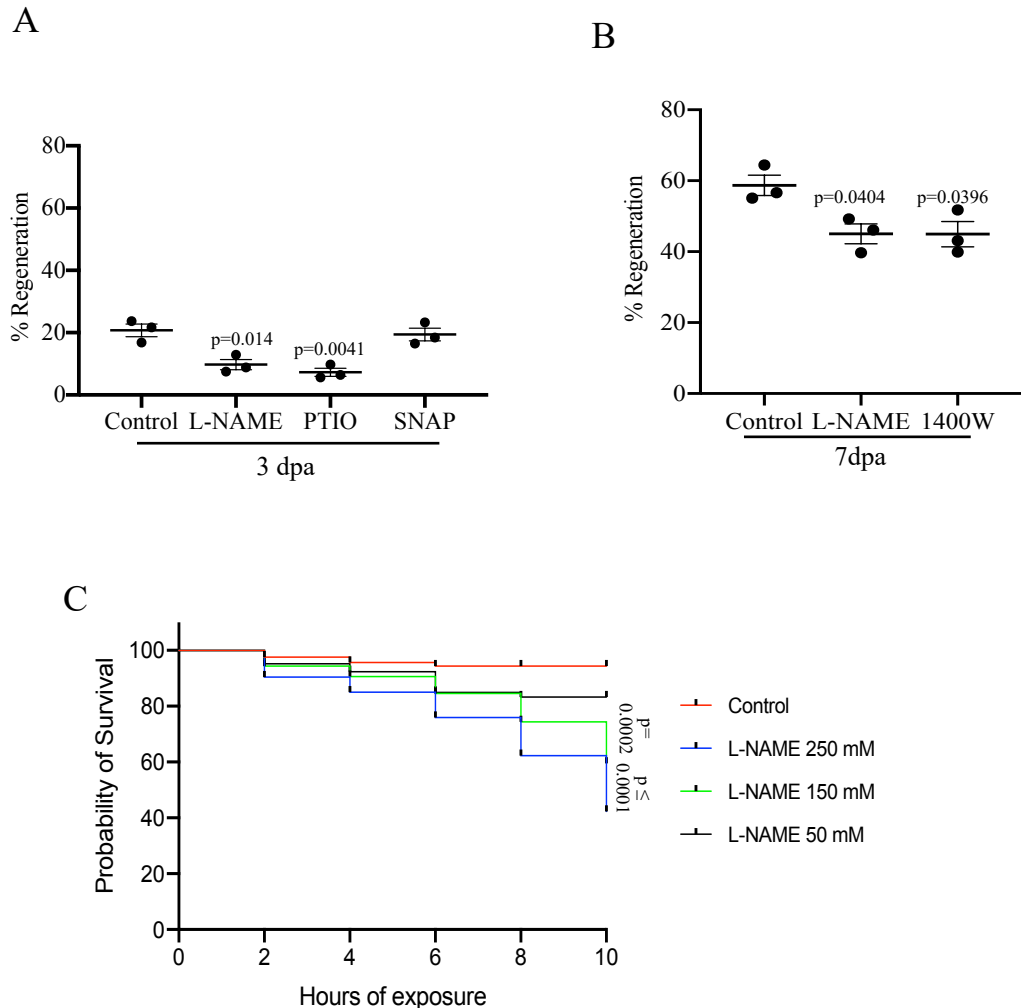
# **Supplementary Material for**

## **Nuclear S-nitrosylation impacts tissue regeneration in zebrafish**

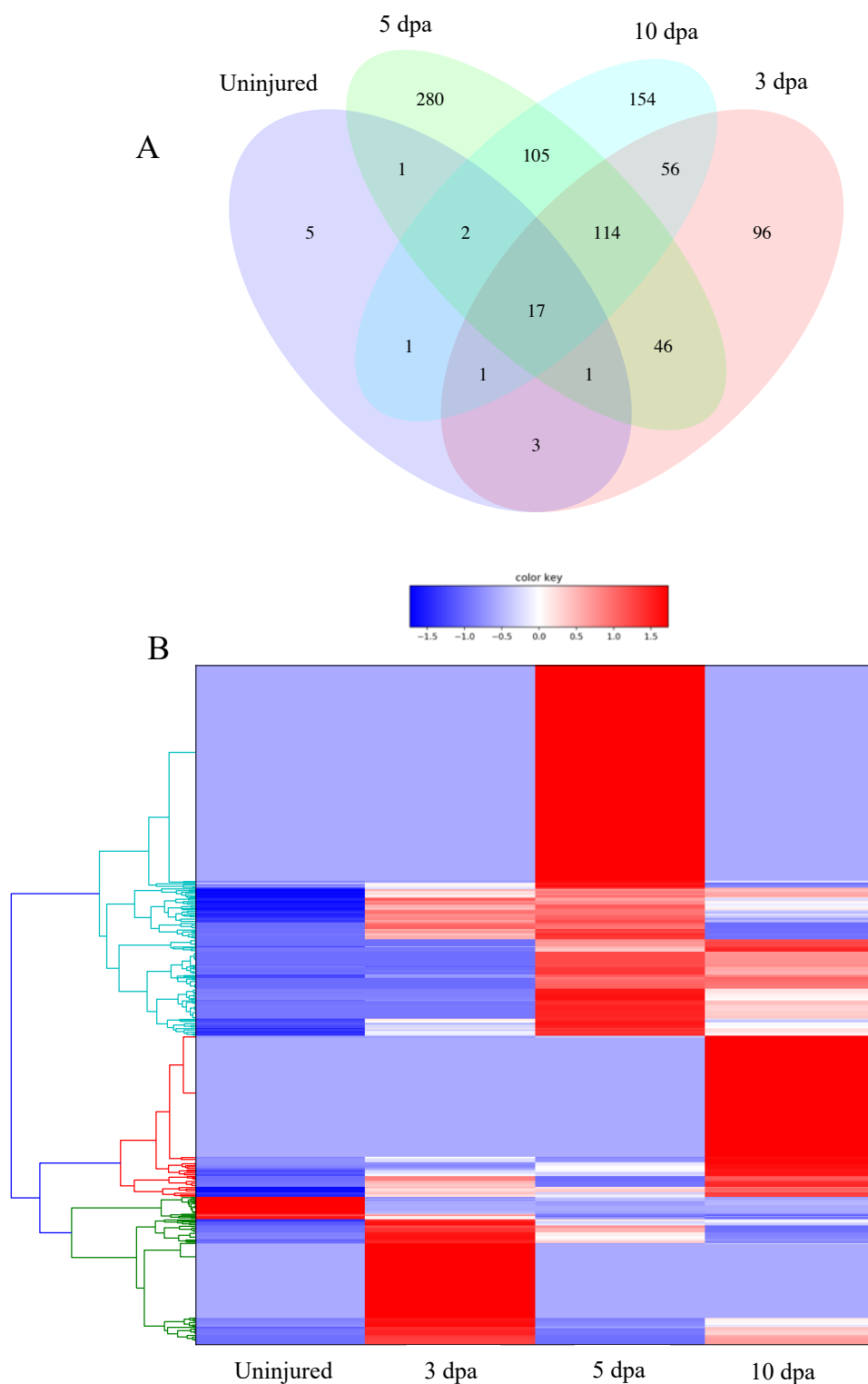
Gianfranco Matrone, Sung Yun Jung, Jong Min Choi, Antrix Jain, Hon-Chiu Eastwood Leung, Kimal Rajapakshe, Cristian Coarfa, Julie Rodor, Martin A Denvir, Andrew H Baker and John P Cooke



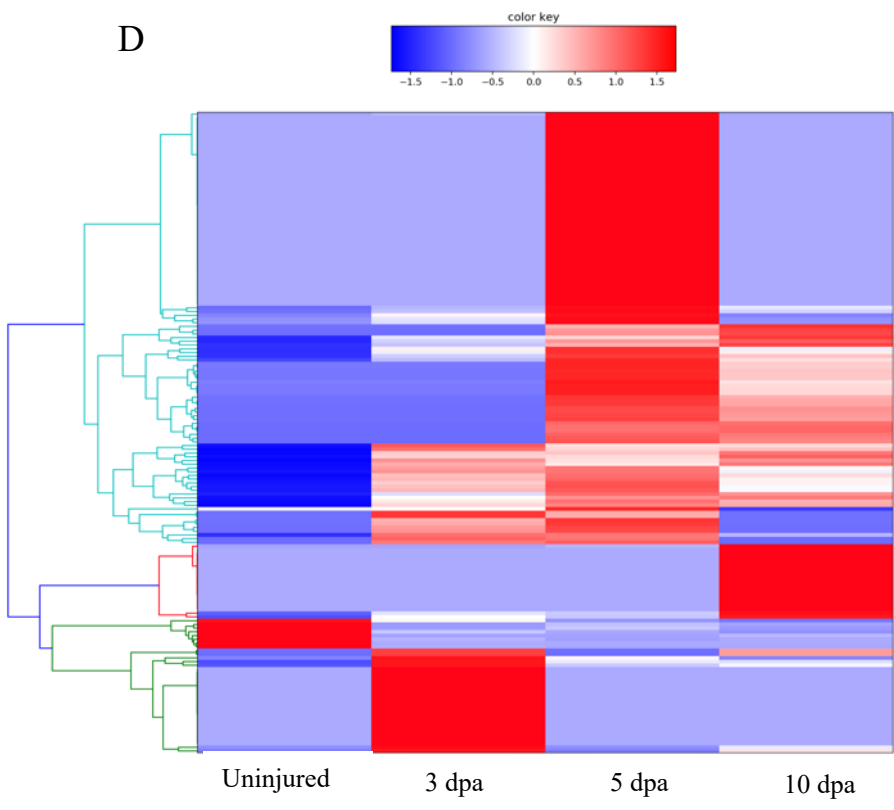
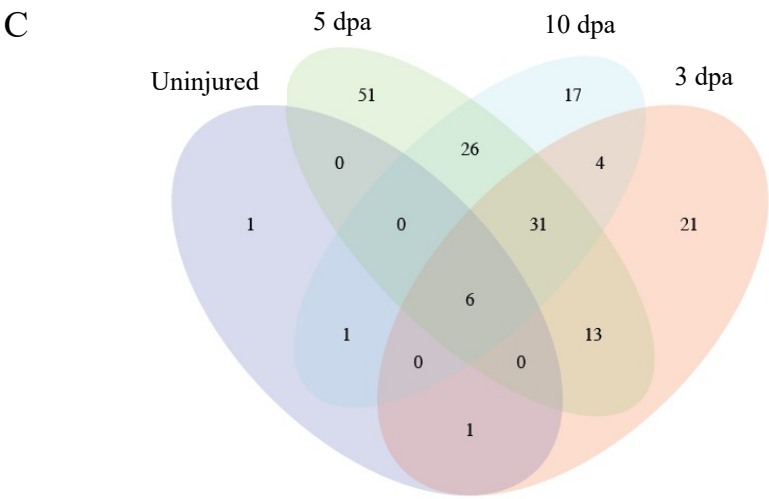
**Figure S1 – Activation of the innate immune system following tailfin injury.** **A.** Images (fluorescence and brightfield merged) of the *Tg(nfkb:EGFP)<sup>nc1</sup>* zebrafish tailfins showing the increase of Nf-kb activity (green fluorescence) following tailfin injury, here shown at 2 days post-amputation (dpa), compared to the uninjured. **B.** Dot plot graph showing the quantification of the fluorescent GFP signal at different time-points after injury. Fluorescent images at the respective time-points are shown in the lower panels. One-way ANOVA test followed by Bonferroni's multiple comparisons test, p values vs uninjured. **C.** Real-time PCR for *nos2* in zebrafish tailfin at 3 and 5 dpa following treatment with the Nf-kb inhibitor Bay11 30 mM and control vehicle. Two-way ANOVA test followed by Bonferroni's multiple comparisons test, p values vs control. **D.** Western blotting showing the effective fractionation of cytoplasmic and nuclear proteins from zebrafish tailfin and blotted with antibodies for  $\beta$ -tubulin and Histone H3, respectively cytoplasmic and nuclear markers. N=3 biological replicates. Data are presented as mean values  $\pm$  SEM.



**Figure S2– Effects of total Nos and Nos2 inhibition on tailfin regeneration. A.** Dot plot showing changes in tailfin regeneration rate at 3 dpa following treatments with L-NAME 50 mM, 2-Phenyl-4,4,5,5-tetramethyl imidazoline-1-oxyl 3-oxide (PTIO) 10 mM, S-Nitroso-N-acetyl-DL-penicillamine (SNAP) 30 mM, compared to control (PBS). **B.** Treatment with 1400W (specific Nos2 inhibitor) 50 mM reduced tailfin regeneration similarly to L-Name (general Nos inhibitor) 50mM, showing that Nos2 is the main Nos isoenzyme involved in the regenerative process. **C.** Kaplan-Meier survival curve following treatment of adult zebrafish with different doses of L-NAME 50 mM. Log-rank test and Gehan-Breslow-Wilcoxon test were used for statistical analysis of Kaplan-Meier curve. One-way ANOVA followed by Bonferroni's multiple comparisons test was used for **B** and **C**. p values vs control. N=3 biological replicates. Data are presented as mean values +/- SEM.



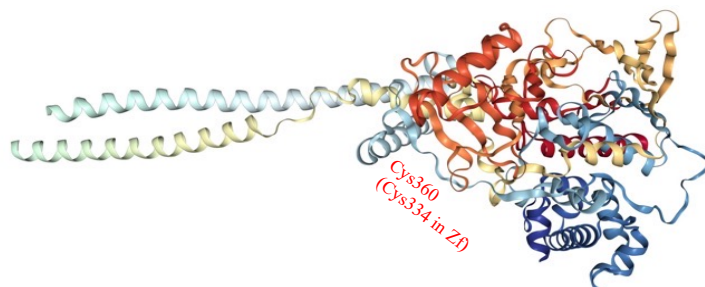
**Figure S3 – Bioinformatic analysis of the S-nitrosylome in tailfin regeneration in zebrafish. A-B.** Venn diagram and Hierarchical clustering heat map showing changes in the number of S-nitrosylated nuclear peptides, analysed by LC/MS/MS, during the regeneration process compared to uninjured. **C-D.** Venn diagram and Hierarchical clustering heat map showing changes in the number of S-nitrosylated nuclear proteins with a human orthologue during the regeneration process compared to uninjured. Key: dpa – days post-amputation.



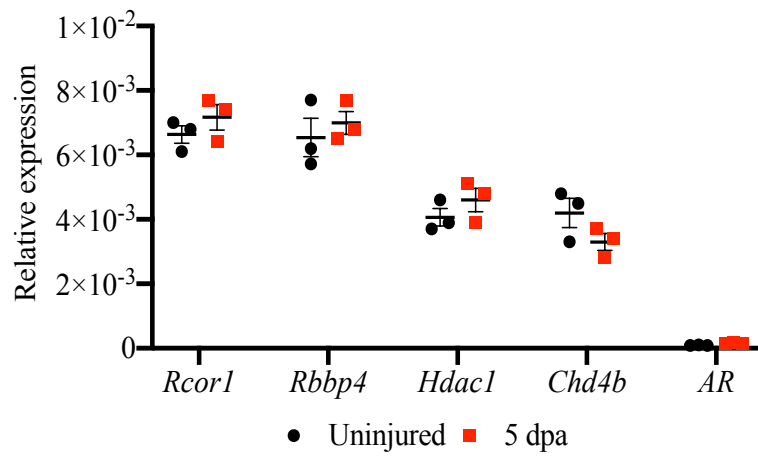
A

| Score           | Expect                                                        | Method                       | Identities   | Positives    | Gaps       |
|-----------------|---------------------------------------------------------------|------------------------------|--------------|--------------|------------|
| 1397 bits(3615) | 0.0                                                           | Compositional matrix adjust. | 716/847(85%) | 751/847(88%) | 38/847(4%) |
| Query 10        | GSSSSSGGNGGDRAPETLAAPQAGPAGPS--GPISADVK---KKE--RASPSGEPGGPPL  |                              |              |              | 62         |
| Sbjct 20        | G+ + G GG +AA AG +GP+ GP + + KKE RASP PGG L                   |                              |              |              | 74         |
|                 | GTEAGPGTAGGSENGSEVAAQPAGLSGPAEVGPGAVGERTPRKKEPPRASP---PGG--L  |                              |              |              |            |
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| Sbjct 75        | PPG G ++ EGRRTSRRKRAKVEYREMDSELANLSEDE                        |                              |              |              | 134        |
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| Query 106       | YYSEEERNAKAERKQVIPPAPPIEEENDSEPEEPSGINGVEGAAFQSRPLPHDRMTSQ    |                              |              |              | 165        |
| Sbjct 135       | YYSEEERNAKAKE+K PP + EP +GVEGAAFQSRPLPHDRMTSQ                 |                              |              |              | 191        |
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| Sbjct 192       | EAACFPDII+GPQ TQKVFL+IRNRTLQWLNDNPK+QLTFEAT+QQLEAPYNSD VLVHR  |                              |              |              | 251        |
|                 | EAACFPDIIISGPQQTQKVFLFIRNRTLQWLNDNPKIQLTFEATLQQLEAPYNSDVLVHR  |                              |              |              |            |
| Query 226       | IHSYLERHGFINFGIYKRVKPLPTKKTGKVVIVIGAGVSGLAAARQLQSFQMDVTVLESRD |                              |              |              | 285        |
| Sbjct 252       | +HSYLERHG INFGIYKR+KPLPTKKTGKVI+IG+GVSGLAAARQLQSFQMDVT+LE+RD  |                              |              |              | 311        |
|                 | VHSYLERHGLINFGIYKRIKPLPTKKTGKVIIIGSGVSGLAAARQLQSFQMDVTVLLEARD |                              |              |              |            |
| Query 286       | RVGGRVATFRKGNYVADLGAMVVTGLGGNPMNAVSKQVNMELAKIKQKCPLYEANGQAGE  |                              |              |              | 345        |
| Sbjct 312       | RVGGRVATFRKGNYVADLGAMVVTGLGGNPMNAVSKQVNMELAKIKQKCPLYEANGQA    |                              |              |              | 369        |
|                 | RVGGRVATFRKGNYVADLGAMVVTGLGGNPMNAVSKQVNMELAKIKQKCPLYEANGQA--  |                              |              |              |            |
| Query 346       | RCTSVPEKDEKMEVQEFNRLLEATSYLSHQLDNFNLNNKPVSLGQALEVVIQLQEKHVKD  |                              |              |              | 405        |
| Sbjct 370       | VPKEKDEKMEVQEFNRLLEATSYLSHQLDNFNLNNKPVSLGQALEVVIQLQEKHVKD     |                              |              |              | 425        |
|                 | ----VPKEKDEKMEVQEFNRLLEATSYLSHQLDNFNLNNKPVSLGQALEVVIQLQEKHVKD |                              |              |              |            |
| Query 406       | EQIEHWKKIVKTQEELKDLLNKMVTTKEKVKELHQYKEASEVKPPRDITAEFLVKSKHR   |                              |              |              | 465        |
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| Sbjct 546       | TLSLKHWQDDDDFEFTGSHLTVRNGYSCVPVALAEGLDIKLNTAVRQVRYT+SGCEVIAV  |                              |              |              | 605        |
|                 | TLSLKHWQDDDDFEFTGSHLTVRNGYSCVPVALAEGLDIKLNTAVRQVRYTASGCEVIAV  |                              |              |              |            |
| Query 586       | NTRSTTQTFIYKCDAVLCTPLPLGV+KQPPPAVQFVPPLEWKTAAIQRMGFGNLNKVVLC  |                              |              |              | 645        |
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|                 | RCLAILKGIFGSSAVPQPKETVVSRRADPWARGSYSYVAAGSSGNDYDLMAQPIITPGPS  |                              |              |              |            |
| Query 766       | IPGASQVPRLFFAGEHTIRNYPATVHGALLSGLREAGRIADQFLGAMYTMPRQATANPN   |                              |              |              | 825        |
| Sbjct 786       | IPGA QP+PRLFFAGEHTIRNYPATVHGALLSGLREAGRIADQFLGAMYT+PRQAT      |                              |              |              | 845        |
|                 | IPGAPQPIPRLFFAGEHTIRNYPATVHGALLSGLREAGRIADQFLGAMYTLPQATPGVP   |                              |              |              |            |
| Query 826       | PQPSPSI 832                                                   |                              |              |              |            |
| Sbjct 846       | Q SPS+ AQQSPSM 852                                            |                              |              |              |            |

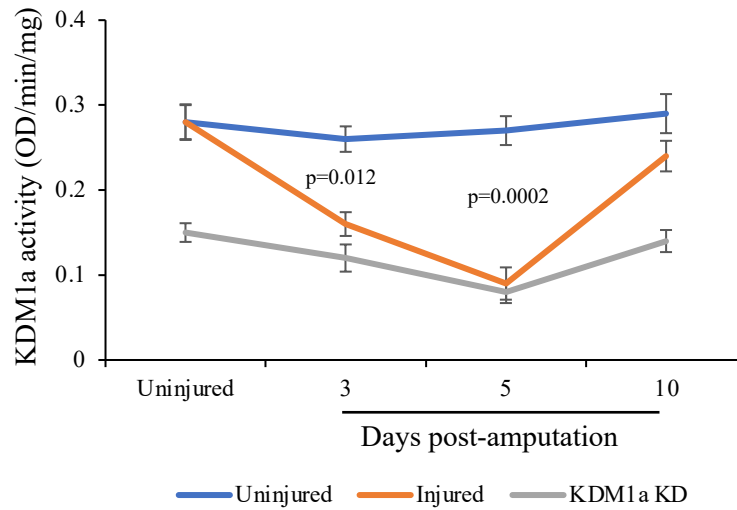
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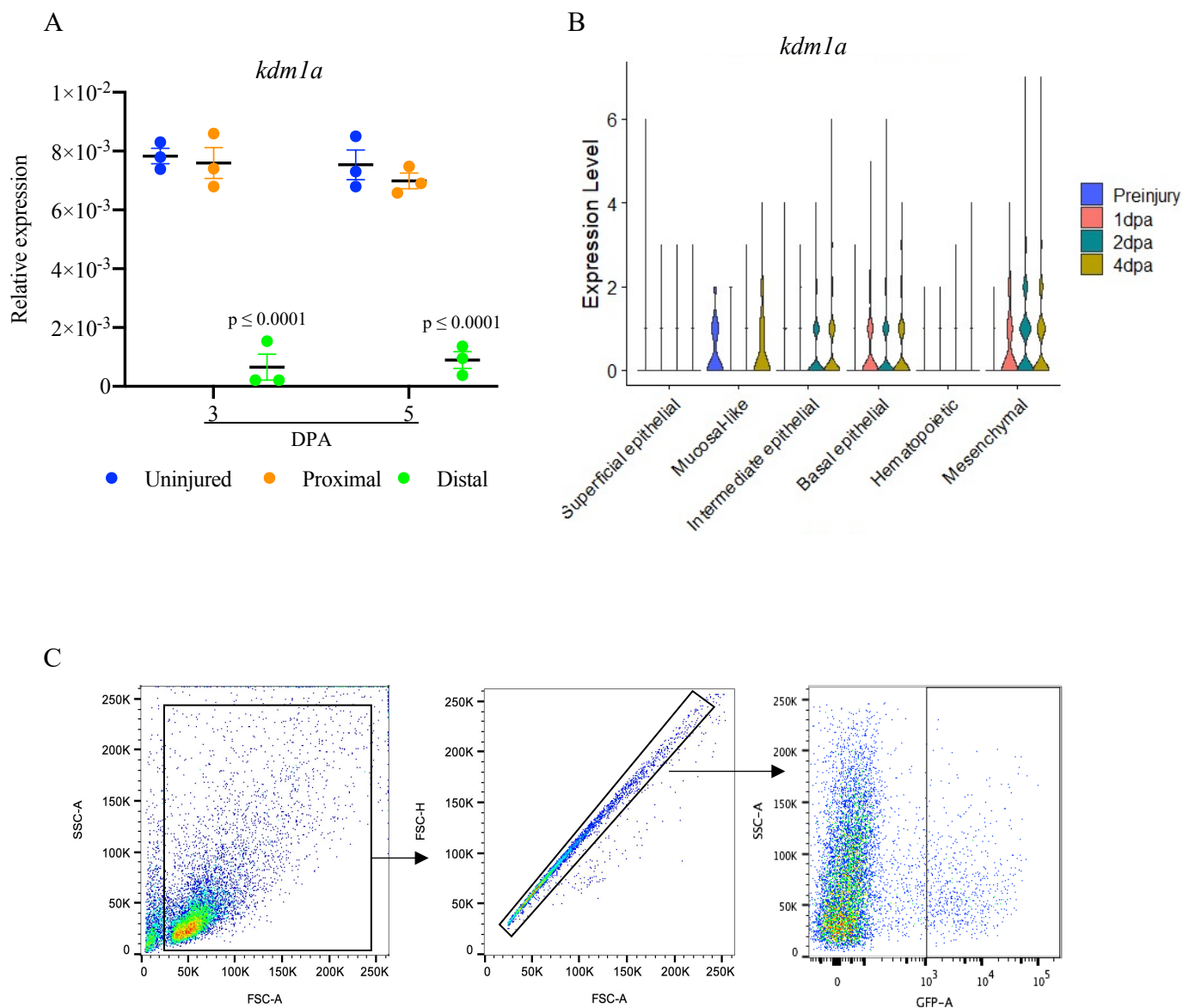
**Figure S4 – Similarities between zebrafish and human Kdm1a.** **A.** Comparison of Kdm1a (also known as Lsd1) protein in Zebrafish (*Danio rerio*, Uniprot F6NIA2) and Human (*Homo sapiens*, Uniprot O60341), analysed using BLAST (<https://blast.ncbi.nlm.nih.gov>). The red rectangle encloses the cysteine (position 334 in zebrafish and 360 in human). **B.** Crystal structure of Human Kdm1a (Protein Data Bank no. 6NQM), showing the position of cysteine 360.



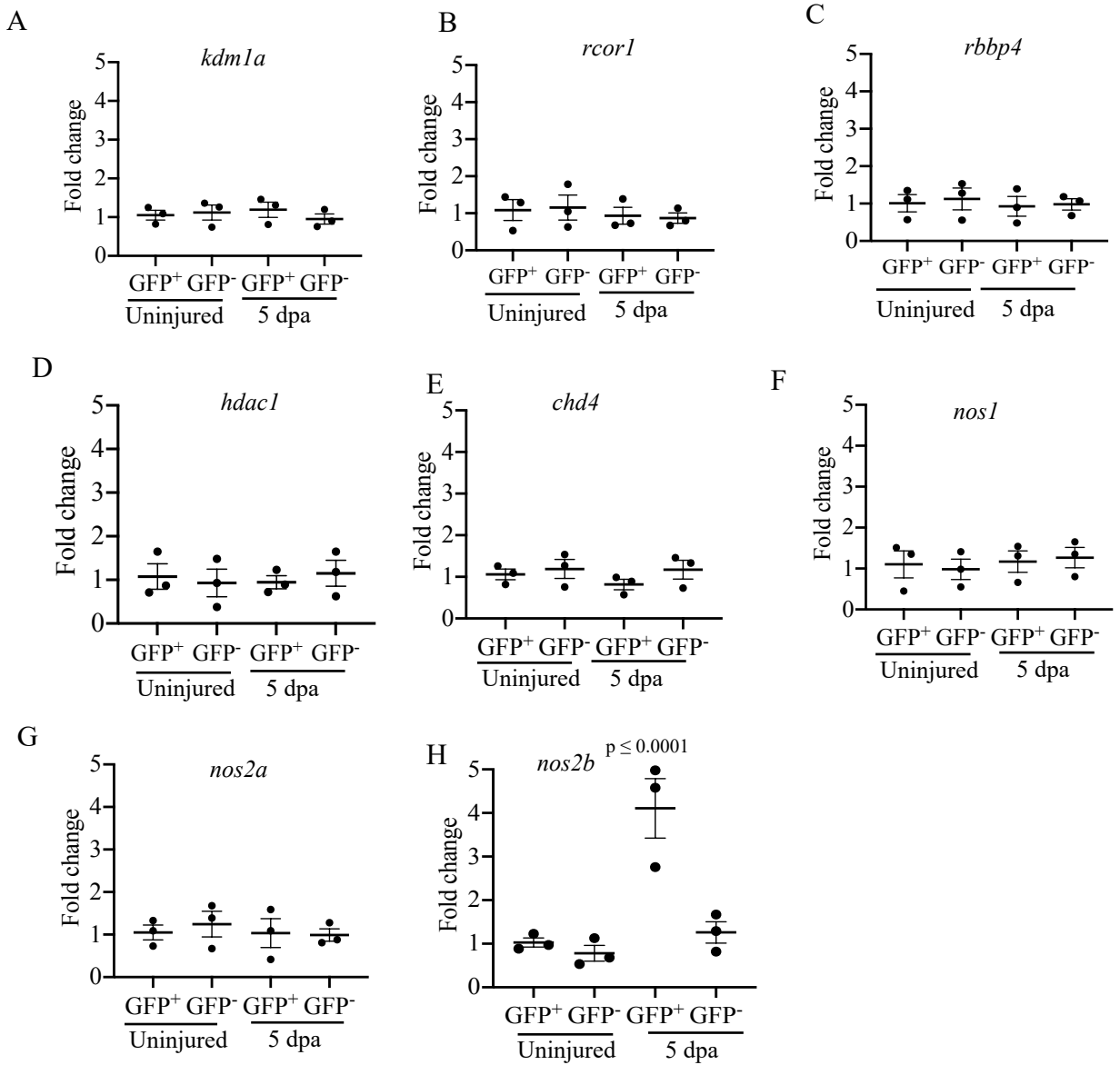
**Figure S5 – Expression of *Rcor1*, *Rbbp4*, *Hdac1*, *Chd4b* and *Androgen receptor (ar)* genes.** Bar graph showing real time PCR for the expression of these genes in zebrafish tailfin at 5 days post-amputation (dpa). N=3 biological replicates. Two-tailed t-test was used to compare the means. No statistical significance was observed. N=3 biological replicates. Data are presented as mean values +/- SEM.



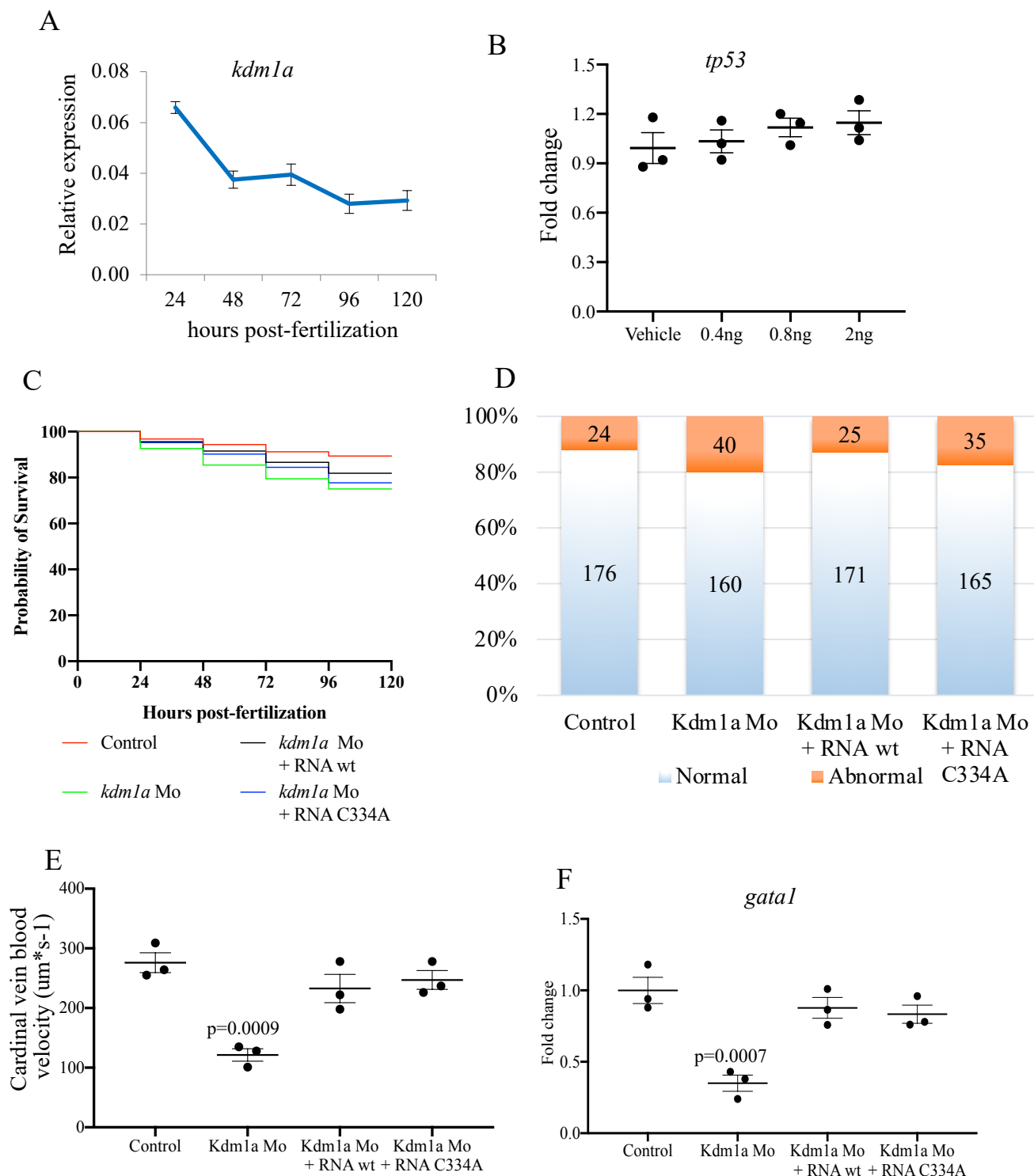
**Figure S6 – Kdm1a activity.** Analysis of Kdm1a demethylase enzymatic activity, expressed as optical density (OD)/min/mg, during the regeneration and following *kdm1a* KD. N=3 biological replicates. Two-way ANOVA test followed by Bonferroni's multiple comparisons test. N=3 biological replicates. Data are presented as mean values +/- SEM.



**Figure S7 – A.** Bar graph showing real-time PCR for the *kdm1a* in the whole, proximal and distal area of the zebrafish tailfin at 3 and 5 days post-amputation (dpa). Two-way ANOVA test followed by Bonferroni's multiple comparisons test. N=3 biological replicates. Data are presented as mean values  $\pm$  SEM. **B.** Violin plot of *kdm1a* expression across cell types during regeneration of zebrafish tailfin, obtained from single-cell datasets by Hou et al. (22). \*\*\* $P < 0.001$ . ANOVA test followed by Bonferroni post hoc test was used to compare the means. **C.** Gating strategy for detection of GFP<sup>+</sup> (endothelial) cells from Tg(fli1:EGFP)y1 zebrafish embryos. The same gating strategy was applied on all FACS analyses.



**Figure S8 – Expression of gene implicated in Kdm1a function.** Real time PCR was used to perform the analysis in GFP<sup>+</sup> (endothelial) and GFP<sup>-</sup> (non-endothelial) cells isolated from zebrafish *Tg(fli1:EGFP)<sup>y1</sup>* uninjured (control) and injured tailfin at 5 days post-amputation (dpa). Among the *nos* genes, the expression of *nos2b* was significantly increased in GFP<sup>+</sup> of injured tailfin compared to control. The expression of other Nos and Kdm1a and its interacting proteins Rcor1 Rbbp4, Hdac1 and Chd4 was unchanged among the groups. Two-way ANOVA test followed by Bonferroni's multiple comparisons test. N=3 biological replicates. Data are presented as mean values +/- SEM.



**Figure S9 – Effects of *kdm1a* knockdown in zebrafish embryos and rescue by wild-type and C334A (mutant) mRNA.** **A.** Real time PCR analysis for *kdm1a* gene expression during zebrafish development. **B.** Real time PCR for of *tp53* gene expression in zebrafish embryos at increasing dose of *kdm1a* morpholino. **C.** Kaplan-Meier survival curve showing the survival rate following injection of *kdm1a* Mo and mutant or wild type mRNA. **D.** Bar graph showing the analysis of phenotypic and functional features. Normal - embryos not showing any abnormal features, Abnormal - embryos showing one or more of the features: reduced length, curved body, chorionated, edema mild, edema severe, reduced swim. **E.** Analysis of blood velocity in the caudal vein following injection of *kdm1a* Mo and mutant or wild type mRNA. **F.** Real time PCR for of *gatal*. PCR data were ormalized to  $\beta$ -actin. One-way ANOVA followed by Bonferroni's multiple comparisons test was used in **B**, **E** and **F**, p values vs control. N=3 biological replicates. Data are presented as mean values +/- SEM.

**Table S1 – List of S-nitrosylated proteins selected for deep mass-spec analysis.**

Kdm1a, marked in red text, has been selected for further studies.

| Gene              | Gene full name                                                 | Source                        |
|-------------------|----------------------------------------------------------------|-------------------------------|
| <i>aldoa</i>      | aldolase a, fructose-bisphosphate, a                           | ZFIN;Acc:ZDB-GENE-030131-8369 |
| <i>atrx</i>       | alpha thalassemia/mental retardation syndrome X-linked homolog | ZFIN;Acc:ZDB-GENE-030912-11   |
| <i>Bub3</i>       | BUB3 mitotic checkpoint protein                                | ZFIN;Acc:ZDB-GENE-041010-210  |
| <i>zcchc13</i>    | CCHC-type zinc finger, nucleic acid binding protein b          | ZFIN;Acc:ZDB-GENE-030131-7782 |
| <i>cpsf2</i>      | cleavage and polyadenylation specific factor 2                 | ZFIN;Acc:ZDB-GENE-040718-79   |
| <i>ddx5</i>       | DEAD (Asp-Glu-Ala-Asp) box helicase 5                          | ZFIN;Acc:ZDB-GENE-030131-925  |
| <i>dhx9</i>       | DEAH (Asp-Glu-Ala-His) box helicase 9                          | ZFIN;Acc:ZDB-GENE-070912-171  |
| <i>eef2</i>       | eukaryotic translation elongation factor 2, like 2             | ZFIN;Acc:ZDB-GENE-030131-8112 |
| <i>huwe1</i>      | HECT, UBA and WWE domain containing 1                          | ZFIN;Acc:ZDB-GENE-081104-387  |
| <i>hnrnab</i>     | heterogeneous nuclear ribonucleoprotein A/Bb                   | ZFIN;Acc:ZDB-GENE-040426-2516 |
| <i>hnrnkp</i>     | heterogeneous nuclear ribonucleoprotein K                      | ZFIN;Acc:ZDB-GENE-040426-1926 |
| <i>Ac008982.1</i> | heterogeneous nuclear ribonucleoprotein L2                     | ZFIN;Acc:ZDB-GENE-040426-2707 |
| <i>hnrnpm</i>     | heterogeneous nuclear ribonucleoprotein M                      | ZFIN;Acc:ZDB-GENE-030131-6898 |
| <i>hexim1</i>     | hexamethylene bis-acetamide inducible 1                        | ZFIN;Acc:ZDB-GENE-030131-4637 |
| <i>kdm1a</i>      | lysine (K)-specific demethylase 1a                             | ZFIN;Acc:ZDB-GENE-030131-7828 |
| <i>mphosph8</i>   | M-phase phosphoprotein 8                                       | ZFIN;Acc:ZDB-GENE-050309-191  |
| <i>nsun2</i>      | NOP2/Sun RNA methyltransferase family, member 2                | ZFIN;Acc:ZDB-GENE-030131-4017 |
| <i>nop56</i>      | NOP56 ribonucleoprotein homolog                                | ZFIN;Acc:ZDB-GENE-040109-1    |
| <i>nop58</i>      | NOP58 ribonucleoprotein homolog                                | ZFIN;Acc:ZDB-GENE-040426-2140 |
| <i>ncl</i>        | nucleolin                                                      | ZFIN;Acc:ZDB-GENE-030131-6986 |
| <i>parp1</i>      | poly (ADP-ribose) polymerase 1                                 | ZFIN;Acc:ZDB-GENE-030131-3955 |
| <i>pa2g4</i>      | proliferation-associated 2G4, b                                | ZFIN;Acc:ZDB-GENE-030131-2182 |
| <i>rif1</i>       | replication timing regulatory factor 1                         | ZFIN;Acc:ZDB-GENE-030131-3406 |
| <i>rbm4</i>       | RNA binding motif protein 4.1                                  | ZFIN;Acc:ZDB-GENE-030131-3902 |
| <i>snrnp200</i>   | small nuclear ribonucleoprotein 200                            | ZFIN;Acc:ZDB-GENE-081105-64   |
| <i>sod1</i>       | superoxide dismutase 1, soluble                                | ZFIN;Acc:ZDB-GENE-990415-258  |
| <i>thoc1</i>      | THO complex 1                                                  | ZFIN;Acc:ZDB-GENE-030826-9    |
| <i>tceal</i>      | transcription elongation factor A (SII), 1                     | ZFIN;Acc:ZDB-GENE-030131-8049 |
| <i>tpt1</i>       | tumor protein, translationally-controlled 1                    | ZFIN;Acc:ZDB-GENE-990603-10   |
| <i>znf346</i>     | zinc finger protein 346                                        | ZFIN;Acc:ZDB-GENE-070209-152  |
| <i>znf865</i>     | zinc finger protein 865                                        | ZFIN;Acc:ZDB-GENE-060503-896  |

**Table S2 – List of primers.**

| Gene                                                      | Primers                                                           |
|-----------------------------------------------------------|-------------------------------------------------------------------|
| <i>kdm1a</i> (NM_001242995.1)                             | Forward GAAGTAAAGCCACCCAGAGAC<br>Reverse CATCTCCACCAGCTCATCATAC   |
| <i>nos1</i> (NM_131660.1)                                 | Forward GAACGGAAGTCCCTCCAAAT<br>Reverse TTA CTGGAGCTGTGGTGTAAAG   |
| <i>nos2a</i> (NM_001104937.1)                             | Forward CACTGTCTGCTCTTCACTCTAAA<br>Reverse TTCAGCTCCACCAGGATAGTA  |
| <i>nos2b</i> (NM_001113501.1)                             | Forward CCAGGCTCTCACCTACTATCT<br>Reverse TCCTCCTGTCTGGCTATCTT     |
| <i>hdac1</i> (NM_173236.1)                                | Forward GTCCTGTCTTTGACGGCTTAT<br>Reverse GCAATGTCTGTCTGCTGTTTG    |
| <i>rcor1</i> (NM_001080041.1)                             | Forward CACTGCCAACAGTAGAGTTTCT<br>Reverse TGGCTTTAGGGTGGGTTTATG   |
| <i>rbbp4</i> (NM_212595.1)                                | Forward CTGGGACACTCGTTCCAATAA<br>Reverse ACTCGCTGTAAGGGTTGAAG     |
| <i>chd4b</i> (XM_680607.9), ortholog to human <i>Chd4</i> | Forward CTCCTGGCTGGGATCATATTC<br>Reverse CCCTCTGCTCATTCTCCTTTA    |
| <i>gata1</i> (NM_131234.2)                                | Forward GATGAGCATGTAGGAGCGTATT<br>Reverse CAAAGTGGTAGAGGAGTGTAAAG |
| <i>ar</i> - Androgen receptor (NM_001083123.1)            | Forward AGCGACGAGACTGAACAATAC<br>Reverse GGGAGAGACGAGACTGAGATAG   |
| <i>relA</i> (P65) (NM_001001839.2)                        | Forward CTGAAGATCTGCCGTGTCAATA<br>Reverse GCACCTCAATGTCCTCTTCT    |
| <i>kdr</i> (NM_001024653.2)                               | Forward CTGAAACCCGGCTCACTATATC<br>Reverse GCTATCCCAGATCACCTCTTTC  |
| <i>mmp2</i> (NM_198067.1)                                 | Forward CCAGTACCCTGGAAGAGAATATC<br>Reverse TTGGTCTTGTGGAAGGAGTAAG |
| <i>angpt2</i> (NM_131814.1)                               | Forward CGTCAGGAGAACTAGGTTAG<br>Reverse CTGGTGTTCCTCTGCATCTT      |
| <i>fgf2</i> (NM_212823.2)                                 | Forward ACAGACGGCGGGTTTATTT<br>Reverse AGATCATATCGGTGGGATTTGG     |
| <i>tek</i> (NM_131461.1)                                  | Forward CTGTGGGAAGTGGTCAGTTTAG<br>Reverse TCGTCACAGTTCAGTGGTTTC   |
| <i>cdh5</i> (NM_001003983.1)                              | Forward CTCAGTTCTCTCCTCAACATAC<br>Reverse TGAAGAGGTCTGTGCCATTAG   |
| <i>cd31</i> (NM_001113799.1)                              | Forward GCGTGCAGAAGAAGGTAGAA<br>Reverse ATCTGATGGAGGTGATGGTAAAG   |
| <i>vegfaa</i> (NM_131408.3)                               | Forward GAGCTGCTGGTAGACATCATC<br>Reverse TTCGAGCGCCTCATCATTAC     |
| <i>tie1</i> (NM_001346150.1)                              | Forward CCTGAAGCCCTCAACCAAATA<br>Reverse CTTGCTGAGTGGTGGGAATAA    |
| <i>tbx20</i> (NM_131506.2)                                | Forward CCTGACACTGCAGAGTGATAC<br>Reverse GGTCGTCCGGTACTGTTATTT    |
| <i>actb1</i> (NM_131031.2)                                | Forward GATCTGGCATCACACCTTCTAC<br>Reverse CACCAGAGTCCATCACAATACC  |