# Zebrafish Collagen Type I: Molecular and Biochemical Characterization of the Major Structural Protein in Bone and Skin

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# Supplementary Methods
## MALDI TOF Mass Spectrometry Analysis

Coomassie bands and silver stained spots were manually excised from the gel. Coomassie bands were destained in 2.5 mM ammonium bicarbonate and 50% (v/v) acetonitrile, while silver stained spots were destained as previously described [1]. Then, both bands and spots were dehydrated using acetonitrile. Before trypsin digestion, proteins resolved in 1D gels were reduced with 10 mM DTE in 25 mM ammonium bicarbonate, for 1 h at 56°C. Then, they were alkylated with 55 mM IAA in 25 mM ammonium bicarbonate, for 45 min at room temperature in the dark. After 10 min of incubation in 50 mM ammonium bicarbonate, protein bands were dehydrated in acetonitrile. Finally, bands and spots were rehydrated in trypsin solution (Sigma Aldrich, St. Louis, MO) and in-gel protein digestion was performed by an overnight incubation at 37°C. From each band or spot, 1.25 µl of the recovered peptide mixture was prepared for MALDI-TOF MS by spotting them onto the MALDI target, allowed to dry, and then mixed to 0.75 µl of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50% (v/v) acetonitrile and 0.5% (v/v) trifluoroacetic acid). Spectra were analyzed by Flex Analysis software v.3.0 and calibrated using, as internal standard, the 842.509 and 2211.105 m/z peptides arising from trypsin auto-proteolysis. The resulting mass lists were filtered for contaminant removal: mass matrix-related ions, trypsin autolysis and keratin peaks. Then, peptide mass fingerprinting searching was carried out in the NCBInr database, limited to *Danio rerio*, by Mascot (Matrix Science Ltd., London, UK, http://www.matrixscience.com). Protein identification was achieved on the basis of corresponding experimental and theoretical peptide fingerprinting patterns with a Δ mass less than 100 ppm and the number of accepted missed cleavage sites set to one. Alkylation of cysteine by carbamidomethylation was assumed as fixed modification, while oxidation of methionine and oxidation of proline were considered as possible modifications. The criteria used to accept identifications included the extent of sequence coverage, number of matched peptides, and probabilistic score.

## *SRM Analysis*

## Selected Reaction Monitoring (SRM) analysis

First, LC-MS/MS analysis was done using a LTQ-Orbitrap Velos mass spectrometer. Here, a peptide mixture was introduced into an LC-MS/MS system; the Ultimate 3000 RSLC nano (Dionex, Amsterdam, The Netherlands) in-line connected to an LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany). The sample mixture was first loaded on a trapping column (made in-house, 100 μm internal diameter (I.D.) x 20 mm, 5 μm beads C18 Reprosil-HD, Dr. Maisch, Ammerbuch-Entringen, Germany). After flushing from the trapping column, the peptides were loaded on a reverse-phase column (made in-house, 75 μm I.D. x 150 mm , 3 μm beads C18 Reprosil-HD, Dr. Maisch). Peptides were loaded with solvent A and separated with a linear gradient from 98% solvent A' (0.1% formic acid) and 2% solvent B' (0.1% formic acid and 80% acetonitrile) to 55% solvent B' at a flow rate of 300 nl/min followed by a wash reaching 100% solvent B'.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the ten most abundant peaks in a given MS spectrum. In the LTQ-Orbitrap Velos, full scan MS spectra were acquired in the Orbitrap at a target value of 1E6 with a resolution of 60,000. The ten most intense ions were then isolated for fragmentation in the linear ion trap, with a dynamic exclusion of 30 s. Peptides were fragmented after filling the ion trap at a target value of 1E4 ion counts. From the MS/MS data in each LC run, Mascot Generic Files (MGFs, peak lists) were created using the Distiller software (version 2.4.3.3, Matrix Science, www.matrixscience.com/Distiller). These MGFs were then searched using the Mascot search engine (Matrix Science, www.matrixscience.com) in the Mascot Daemon interface (version 2.4, Matrix Science). Spectra were searched against the NCBI database of *zebrafish* proteins. Variable modifications were set to pyro-glutamate formation of N-terminal glutamine, acetylation of the

protein N-terminus and methionine oxidation. The mass tolerance on precursor ions was set to ± 10 ppm (with Mascot's C13 option set to 1), and on fragment ions to ± 0.5 Da. The peptide charge was set to 1+,2+,3+ and the instrument setting was put on ESI-TRAP. Enzyme was set to trypsin, allowing for one missed cleavage, and cleavage was also allowed when arginine or lysine were followed by proline. Only peptides that were ranked one and scored above the threshold score, set at 99% confidence, were withheld. All further data management was done with ms_lims [2].

The nano-LC system was configured with a trapping column (100 µm I.D. x 20 mm, 5 µm beads, C18 Reprosil-HD, Dr. Maisch) and an analytical column (75 µm I.D. x 150 mm, 3 µm beads, C18 Reprosil-HD, Dr. Maisch). Transitions for each peptide were optimized for their collision energy (CE). The 5 most intense transitions were chosen to detect the peptides. In order to approximate the concentration of the peptide in the sample, one sample was run without spike-in of the synthetic peptides. The intensities of the light peptides in the sample were used as an indication for the amount of synthetic peptide that needed to be spiked in. Spike-in concentrations can be found in Supplementary Table S9 Table. For all samples a total volume of 20 µl of synthetic peptides was added, so a final volume of 40 µl was present for analysis. From each sample, 1 µl was injected using a µl pickup method. Injection occurred at 10 µl/min in loading solvent A. After loading, the trapping column was flushed for 3 min in order to pre-concentrate the peptides while removing buffer components, before it was put in-line with the analytical column. Compounds were eluted at 300 nl/min with an acetonitrile gradient of 28 min from 4% to 55% of solvent B'. The column was washed with 90% of solvent B' for 5 min and equilibrated with solvent A' for 14 min before analysis of the next sample. The voltage on the needle in the nano-ESI source was set at 1,300 V and the capillary temperature at 275°C. A dwell time of 41 ms for each transition was applied. Transitions measured for the different peptides are listed in Supplementary Table S9. Peak areas and ratios between the heavy peptide and its light counterpart were calculated with Skyline (v2.6.1.7171, MacCoss Lab Software, University of Washington) [3].

## *In situ* hybridization

For each WISH experiment 2 different probes were used per gene and approximately 20 embryos of the same age were stained per probe. Staining was homogenous for different embryos within one group and expression patterns for different probes detecting the same gene where highly comparable. For each gene 4 representative embryos for one of the probes were selected for embedding and sectioning. Sections of all 4 embryos per gene were assessed to determine the average signal intensity (listed in Table 2), which was comparable at the same site in each embryo hybridized for the same gene. For each gene a slide representative for the mean signal intensity at a certain site was selected for illustration in Figure 4b.


1 Gharahdaghi, F., Weinberg, C. R., Meagher, D. A., Imai, B. S. & Mische, S. M. Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. *Electrophoresis* **20**, 601-605, (1999).

2 Helsens, K. *et al.* ms_lims, a simple yet powerful open source laboratory information management system for MS-driven proteomics. *Proteomics* **10**, 1261-1264, (2010).

3 MacLean, B. *et al.* Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **26**, 966-968, (2010).

**Supplementary Table S1. List of genes flanking human *COL1A1***

| | *Homo sapiens* | | | *Mus musculus* | | | *Danio rerio* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Gene name** | **Ensembl ID** | **Mb from *COL1A1*** | **Gene name** | **Ensembl ID** | **Mb from *Col1a1*** | **Gene name** | **Ensembl ID** | **Mb from *col1a1a*** | **Mb from *col1a1b*** |
| | *B4GALNT2* | ENSG00000167080 | -1,05 | *B4galnt2* | ENSMUSG00000013418 | 0,93 | *b4galnt2.1* | ENSDARG00000094579 | -0,15 | |
| | *GNGT2** | ENSG00000167083 | -0,98 | *Gngt2* | ENSMUSG00000038811 | 0,90 | *gngt2a* | ENSDARG00000010680 | -0,13 | |
| | | | | | | | *gngt2b* | ENSDARG00000089997 | | 0,17 |
| | *ABI3** | ENSG00000108798 | -0,97 | *Abi3* | ENSMUSG00000018381 | 0,89 | *abi3* | ENSDARG00000060072 | 10,02 | |
| | | | | | | | *abi3* | ENSDARG00000063283 | | 2,59 |
| | *PHOSPHO1* | ENSG00000173868 | -0,96 | *Phospho1* | ENSMUSG00000050860 | 0,89 | *phospho1* | ENSDARG00000008403 | -8,10 | |
| | *ZNF652* | ENSG00000198740 | -0,89 | *Zfp652* | ENSMUSG00000075595 | 0,78 | *znf652* | ENSDARG00000062302 | -8,13 | |
| | *PHB* | ENSG00000167085 | -0,78 | *Phb* | ENSMUSG00000038845 | 0,73 | *phb* | ENSDARG00000057414 | -8,17 | |
| | *NGFR** | ENSG00000064300 | -0,69 | *Ngfr* | ENSMUSG00000000120 | 0,63 | *ngfr* | ENSDARG00000088708 | -3,47 | |
| | | | | | | | *ngfrb* | ENSDARG00000032984 | | 7,24 |
| | *NXPH3* | ENSG00000182575 | -0,61 | *Nxph3* | ENSMUSG00000046719 | 0,57 | *nxph3* | ENSDARG00000070694 | -3,22 | |
| | *SPOP* | ENSG00000121067 | -0,58 | *Spop* | ENSMUSG00000057522 | 0,48 | *spop* | ENSDARG00000038215 | -2,96 | |
| | *SLC35B1* | ENSG00000121073 | -0,48 | *Slc35b1* | ENSMUSG00000020873 | 0,45 | *slc35b1* | ENSDARG00000038213 | -2,46 | |
| | *FAM117A** | ENSG00000121104 | -0,47 | *Fam117a* | ENSMUSG00000038893 | 0,40 | *fam117a* | ENSDARG00000078640 | -2,44 | |
| | | | | | | | *fam117a* | ENSDARG00000079252 | | 7,14 |
| | *KAT7** | ENSG00000136504 | -0,39 | *Kat7* | ENSMUSG00000038909 | 0,34 | *kat7* | ENSDARG00000031770 | | 2,98 |
| | | | | | | | *kat7* | ENSDARG00000070371 | 9,51 | |
| | *TAC4* | ENSG00000176358 | -0,34 | *Tac4* | ENSMUSG00000020872 | 0,33 | / | / | / | / |
| | *DLX4** | ENSG00000108813 | -0,21 | *Dlx4* | ENSMUSG00000020871 | 0,20 | *dlx4a* | ENSDARG00000011956 | 11,24 | |
| | | | | | | | *dlx4b* | ENSDARG00000071560 | | 2,37 |
| | *DLX3* | ENSG00000064195 | -0,19 | *Dlx3* | ENSMUSG00000001510 | 0,18 | *dlx3b* | ENSDARG00000014626 | | 2,39 |
| | *ITGA3** | ENSG00000005884 | -0,13 | *Itga3* | ENSMUSG00000001507 | 0,11 | *itga3a* | ENSDARG00000037917 | 11,42 | |
| | | | | | | | *itga3b* | ENSDARG00000012824 | | 2,44 |
| | *PDK2* | ENSG00000005882 | -0,09 | *Pdk2* | ENSMUSG00000038967 | 0,09 | *pdk2* | ENSDARG00000020876 | | 26,42 |
| | *SAMD14* | ENSG00000167100 | -0,07 | *Samd14* | ENSMUSG00000047181 | 0,07 | *samd14* | ENSDARG00000090966 | | 5,17 |
| | *PPP1R9B** | ENSG00000108819 | -0,05 | *Ppp1r9b* | ENSMUSG00000038976 | 0,05 | *ppp1r9b2* | ENSDARG00000076431 | | 6,76 |
| | | | | | | | *ppp1r9b* | ENSDARG00000079366 | 0,03 | |
| | *SGCA* | ENSG00000108823 | -0,02 | *Sgca* | ENSMUSG00000001508 | 0,03 | *sgca* | ENSDARG00000074156 | | -0,02 |
| | ***COL1A1*** | ENSG00000108821 | 0,00 | ***Col1a1*** | ENSMUSG00000001506 | 0,00 | ***col1a1a*** | ENSDARG00000012405 | 0,00 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ***col1a1b*** | ENSDARG00000035809 | | 0,00 |
| | *TMEM92* | ENSG00000167105 | 0,09 | *Tmem92* | ENSMUSG00000075610 | -0,16 | / | / | / | / |
| | *XYLT2* | ENSG00000015532 | 0,16 | *Xylt2* | ENSMUSG00000020868 | -0,27 | *xylt2* | ENSDARG00000059557 | 37,07 | |
| | *MRPL27* | ENSG00000108826 | 0,18 | *Mrpl27* | ENSMUSG00000024414 | -0,28 | *mrpl27* | ENSDARG00000032985 | | 19,35 |
| | *EME1* | ENSG00000154920 | 0,19 | *Eme1* | ENSMUSG00000039055 | -0,29 | *eme1* | ENSDARG00000076913 | | 19,34 |
| | *LRRC59* | ENSG00000108829 | 0,19 | *Lrrc59* | ENSMUSG00000020869 | -0,31 | *lrrc59* | ENSDARG00000071426 | | 29,78 |
| | *ACSF2* | ENSG00000167107 | 0,24 | *Acsf2* | ENSMUSG00000076435 | -0,38 | *acsf2* | ENSDARG00000061201 | | 24,38 |
| | *CHAD* | ENSG00000136457 | 0,28 | *Chad* | ENSMUSG00000039084 | -0,37 | *chad* | ENSDARG00000045071 | | 24,40 |
| | *RSAD1* | ENSG00000136444 | 0,30 | *Rsad1* | ENSMUSG00000039096 | -0,40 | *rsad1* | ENSDARG00000077698 | 24,08 | |
| | *MYCBPAP* | ENSG00000136449 | 0,33 | *Mycbpap* | ENSMUSG00000039110 | -0,43 | *mycbpap* | ENSDARG00000069194 | 1:57700573-57748929 | |
| | *EPN3* | ENSG00000049283 | 0,35 | *Epn3* | ENSMUSG00000010080 | -0,45 | *epn3* | ENSDARG00000078953 | 23,98 | |
| | *SPATA20* | ENSG00000006282 | 0,36 | *Spata20* | ENSMUSG00000020867 | -0,46 | *spata20* | ENSDARG00000013880 | | 25,47 |
| | *CACNA1G* | ENSG00000006283 | 0,38 | *Cacna1g* | ENSMUSG00000020866 | -0,53 | *cacna1g* | ENSDARG00000089913 | | 25,59 |
| | *ABCC3* | ENSG00000108846 | 0,45 | *Abcc3* | ENSMUSG00000020865 | -0,59 | *abcc3* | ENSDARG00000077243 | | -2,79 |
| | *ANKRD40* | ENSG00000154945 | 0,51 | *Ankrd40* | ENSMUSG00000020864 | -0,61 | *ankrd40* | ENSDARG00000076490 | -5,72 | |
| | *LUC7L3* | ENSG00000108848 | 0,54 | *Luc7l3* | ENSMUSG00000020863 | -0,65 | *luc7l3* | ENSDARG00000014366 | 36,16 | |
| | *WFIKKN2** | ENSG00000173714 | 0,65 | *Wfikkn2* | ENSMUSG00000044177 | -0,70 | *wfikkn2a* | ENSDARG00000013460 | -5,69 | |
| | | | | | | | *wfikkn2* | ENSDARG00000059139 | | -2,69 |
| | *TOB1** | ENSG00000141232 | 0,68 | *Tob1* | ENSMUSG00000037573 | -0,72 | *tob1a* | ENSDARG00000032619 | -5,60 | |
| | | | | | | | *tob1b* | ENSDARG00000021372 | | -2,64 |
| | *SPAG9** | ENSG00000008294 | 0,78 | *Spag9* | ENSMUSG00000020859 | -0,94 | *cabz1073118.1* | ENSDARG00000078142 | | -2,57 |
| | | | | | | | *spag9a* | ENSDARG00000074531 | -5,48 | |
| | *NME1* | ENSG00000239672 | 0,97 | *Nme1* | ENSMUSG00000037601 | -0,98 | *nme2b.2* | ENSDARG00000076892 | 19:49813241-49861384 | |
| | *NME2* | ENSG00000011052 | 0,97 | *Gm20390* | ENSMUSG00000091228 | -0,99 | | | | |

*genes flanking *COL1A1* that have orthologs located in both zebrafish chromosome 3 and chromosome 12

**Supplementary Table S2. List of genes flanking human *COL1A2***

| | *Homo sapiens* | | | *Mus musculus* | | | *Danio rerio* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Gene Name** | **Ensembl ID** | **Mb from *COL1A2*** | **Gene name** | **Ensembl ID** | **Mb from *Col1a2*** | **Gene name** | **Ensembl ID** | **Mb from *col1a2*** |
| | *AKAP9 #* | ENSG00000127914 | -2,45 | *Akap9* | ENSMUSG00000040407 | 5:3928054-4080209 | *cabz01010098.1* | ENSDARG00000086145 | -41,47 |
| | | | | | | | *akap9* | ENSDARG00000079610 | -41,41 |
| | | | | | | | *cabz01010103.1* | ENSDARG00000077668 | -41,45 |
| | *CYP51A1 #* | ENSG00000001630 | -2,28 | *Cyp51* | ENSMUSG00000001467 | 5:4081145-4104746 | *cyp51* | ENSDARG00000042641 | -41,37 |
| | *LRRD1* | ENSG00000240720 | -2,28 | *Lrrd1* | ENSMUSG00000040367 | 5:3845173-3866596 | *ascl1b* | ENSDARG00000009702 | 7:51153042-51174183 |
| | *KRIT1 #* | ENSG00000001631 | -2,20 | *Krit1* | ENSMUSG00000000600 | 5:3803165-3844515 | *krit1* | ENSDARG00000036115 | 5,96 |
| | *ANKIB1 #* | ENSG00000001629 | -2,15 | *Ankib1* | ENSMUSG00000040351 | 5:3690000-3803109 | *ankib1a* | ENSDARG00000060768 | 3,47 |
| | *GATAD1 #* | ENSG00000157259 | -1,95 | *Gatad1* | ENSMUSG00000007415 | 5:3632932-3647934 | *gatad1* | ENSDARG00000027612 | 3,53 |
| | *ERVW-1* | ENSG00000242950 | -1,93 | / | / | / | / | / | / |
| | *PEX1 #* | ENSG00000127980 | -1,91 | *Pex1* | ENSMUSG00000005907 | 5:3596066-3637232 | *pex1* | ENSDARG00000076647 | -6,73 |
| | *RBM48 #* | ENSG00000127993 | -1,87 | *Rbm48* | ENSMUSG00000040302 | 5:3583978-3596585 | *rbm48* | ENSDARG00000033754 | -0,81 |
| | *FAM133B #* | ENSG00000234545 | -1,83 | *Fam133b* | ENSMUSG00000058503 | 5:3543844-3569810 | *fam133b* | ENSDARG00000013863 | -0,65 |
| | *CDK6 #* | ENSG00000105810 | -1,79 | *Cdk6* | ENSMUSG00000040274 | 5:3343893-3523218 | *cdk6* | ENSDARG00000070228 | -0,73 |
| | *SAMD9* | ENSG00000205413 | -1,30 | / | / | / | samd9l | ENSDARG00000095065 | 1:40549425-40570211 |
| | *SAMD9L* | ENSG00000177409 | -1,26 | *Samd9l* | ENSMUSG00000047735 | -1,13 | | | |
| | *HEPACAM2 #* | ENSG00000188175 | -1,21 | *Hepacam2* | ENSMUSG00000044156 | -1,05 | *hepacam2* | ENSDARG00000061365 | -0,63 |
| | *CCDC132 #* | ENSG00000004766 | -1,16 | *Ccdc132* | ENSMUSG00000001376 | -1,01 | *ccdc132* | ENSDARG00000096912 | -0,60 |
| | | | | | | | *ccdc132* | ENSDARG00000002880 | -0,60 |
| | *CALCR #* | ENSG00000004948 | -0,97 | *Calcr* | ENSMUSG00000023964 | -0,82 | *calcr* | ENSDARG00000028845 | -0,27 |
| | *GNGT1 #* | ENSG00000127928 | -0,80 | *Gngt1* | ENSMUSG00000029663 | -0,51 | *gngt1* | ENSDARG00000035798 | -0,13 |
| | *TFPI2 #* | ENSG00000105825 | -0,51 | *Tfpi2* | ENSMUSG00000029664 | -0,54 | *tfpi2* | ENSDARG00000061351 | -0,14 |
| | *GNG11 #* | ENSG00000127920 | -0,47 | *Gng11* | ENSMUSG00000032766 | -0,50 | *gngt1* | ENSDARG00000035798 | -0,13 |
| | *BET1 #* | ENSG00000105829 | -0,43 | *Bet1* | ENSMUSG00000032757 | -0,43 | *bet1* | ENSDARG00000034885 | -0,08 |
| | ***COL1A2*** | ENSG00000164692 | 0,00 | ***Col1a2*** | ENSMUSG00000029661 | 0,00 | ***col1a2*** | ENSDARG00000020007 | 0,00 |
| | *CASD1 #* | ENSG00000127995 | 0,11 | *Casd1* | ENSMUSG00000015189 | 0,10 | *casd1* | ENSDARG00000023900 | 0,02 |
| | *SGCE #* | ENSG00000127990 | 0,19 | *Sgce* | ENSMUSG00000004631 | 0,17 | sgce | ENSDARG00000012138 | 0,06 |
| | *PEG10* | ENSG00000242265 | 0,26 | *Peg10* | ENSMUSG00000092035 | 0,24 | / | / | / |
| | *PPP1R9A #* | ENSG00000158528 | 0,51 | *Ppp1r9a* | ENSMUSG00000032827 | 0,40 | *ppp1r9a* | ENSDARG00000061304 | 0,09 |
| | *PON1* | ENSG00000005421 | 0,90 | *Pon1* | ENSMUSG00000002588 | 0,66 | *pon1* | ENSDARG00000032496 | 16:26885893-26895558 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *PON3* | ENSG00000105852 | 0,97 | *Pon3* | ENSMUSG00000029759 | 0,72 | *zgc:174637* | ENSDARG00000040290 | 16:26896251-26907913 |
| | *PON2* | ENSG00000105854 | 1,01 | *Pon2* | ENSMUSG00000032667 | 0,76 | *pon2* | ENSDARG00000016856 | 16:26909176-26912212 |
| | *ASB4 #* | ENSG00000005981 | 1,08 | *Asb4* | ENSMUSG00000042607 | 0,88 | *asb4* | ENSDARG00000034988 | 0,19 |
| | *PDK4 #* | ENSG00000004799 | 1,19 | *Pdk4* | ENSMUSG00000019577 | 0,98 | *pdk4* | ENSDARG00000054848 | 0,21 |
| | *DYNC1I1* | ENSG00000158560 | 1,38 | *Dync1i1* | ENSMUSG00000029757 | 1,22 | *dync1i1* | ENSDARG00000060948 | 16:27556254-27577115 |
| | *SLC25A13 #* | ENSG00000004864 | 1,73 | *Slc25a13* | ENSMUSG00000015112 | 1,54 | *slc25a13* | ENSDARG00000070172 | 0,31 |
| | *SHFM1* | ENSG00000127922 | 2,09 | / | / | / | / | / | / |
| | *DLX6 #* | ENSG00000006377 | 2,61 | *Dlx6* | ENSMUSG00000029754 | 2,36 | *dlx6a* | ENSDARG00000042291 | 0,45 |
| | *DLX5 #* | ENSG00000105880 | 2,63 | *Dlx5* | ENSMUSG00000029755 | 2,37 | *dlx5a* | ENSDARG00000042296 | 0,50 |
| | *ACN9 #* | ENSG00000196636 | 2,72 | *Acn9* | ENSMUSG00000042505 | 2,45 | *acn9* | ENSDARG00000036092 | -15,93 |
| | *TAC1 #* | ENSG00000006128 | 3,34 | *Tac1* | ENSMUSG00000061762 | 3,05 | *tac1* | ENSDARG00000014490 | -15,81 |
| | *ASNS #* | ENSG00000070669 | 3,46 | *Asns* | ENSMUSG00000029752 | 3,17 | *asns* | ENSDARG00000016375 | -15,80 |
| | *OCM2* | ENSG00000135175 | 3,59 | *Ocm* | ENSMUSG00000029618 | 5:144019804-144026670 | *pvalb8* | ENSDARG00000037790 | 3:61763106-61771637 |
| | | | | | | | *pvalb9* | ENSDARG00000071601 | 12:18763656-18766066 |
| | *LMTK2* | ENSG00000164715 | 3,71 | *Lmtk2* | ENSMUSG00000038970 | 5:144100436-144188204 | *lmtk2* | ENSDARG00000076324 | 3:61830081-61897661 |
| | *BHLHA15* | ENSG00000180535 | 3,82 | *Bhlha15* | ENSMUSG00000052271 | 5:144190286-144194441 | *bhlha15* | ENSDARG00000045166 | 12:18822354-18824381 |

# genes flanking *COL1A2* that are located in the same chromosome of zebrafish *col1a2* (chromosome 19)

**Supplementary Table S3. Content of GG+GGG and GPP repeats in human, murine and zebrafish type I collagen α chains.**

| | GG + GGG | GPP |
|---|---|---|
| *H. sapiens* α1(I) | 3 | 41 |
| *H. sapiens* α2(I) | 3 | 26 |
| *M. musculus* α1(I) | 3 | 41 |
| *M. musculus* α2(I) | 4 | 21 |
| *D. rerio* α1(I) | 5 | 31 |
| *D. rerio* α2(I) | 6 | 19 |
| *D. rerio* α3(I) | 12 | 26 |

**Supplementary Table S4**. **Mass spectrometry analysis of pepsin soluble collagen extracted from 48 hpf embryos and separated by 1D electrophoresis.**

| Band ID | Protein description | NCBI ID | Mascot search results | | |
|---|---|---|---|---|---|
| | | | Score | No. of matched peptides | Sequence coverage (%) |
| **A** | Collagen, type I, alpha 1 | gi\|38649122 | 140 | 37/73 | 38 |
| | Collagen alpha-1(II) chain isoform X2 | gi\|528487168 | 65 | 24/73 | 24 |
| **B** | Collagen, type I, alpha 1 | gi\|38649122 | 163 | 25/43 | 24 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 117 | 24/43 | 23 |
| **C** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 189 | 30/37 | 32 |

**Supplementary Table S5. α1-α3 / α2 ratio based on densitometric analysis performed on 1D SDS-PAGE of collagen extracted from adult tissues**

| | **Reducing conditions*** | **Non reducing conditions** |
|---|---|---|
| **Skin ASC** | 1.89 ± 0.20 | 2.18 ± 0.31 |
| **Skin PSC** | 2.09± 0.44 | 2.00 ± 0.29 |
| **Scales ASC** | 1.79 ± 0.45 | 2.21 ± 0.47 |
| **Scales PSC** | 2.24 ± 0.26 | 2.03 ± 0.19 |
| **Bone ASC** | 2.26 ± 0.27 | 2.10 ± 0.21 |
| **Bone PSC** | 2.09 ± 0.43 | 2.16 ± 0.40 |

* In presence of 0.1 mM DTT

**Supplementary Table S6. Mass spectrometry analysis of adult pepsin soluble collagen separated by 1D electrophoresis.**

| Band ID | Protein description | NCBI ID | Mascot search results | | |
|---|---|---|---|---|---|
| | | | Score | No. of matched peptides | Sequence coverage (%) |
| **Bone** | | | | | |
| **D** | Col1a1 protein | gi\|171846460 | 87 | 41/85 | 36 |
| | Collagen alpha-2(I) chain precursor | gi\|48762667 | 84 | 34/85 | 39 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 60 | 33/85 | 30 |
| **E** | Col1a1 protein | gi\|171846460 | 160 | 42/62 | 36 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 79 | 30/63 | 27 |
| **F** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 150 | 19/20 | 29 |
| **Skin** | | | | | |
| **D** | Col1a1 protein | gi\|171846460 | 107 | 41/87 | 39 |
| | Collagen alpha-2(I) chain precursor | gi\|42542708 | 90 | 33/87 | 33 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 78 | 36/87 | 32 |
| **E** | Col1a1 protein | gi\|171846460 | 126 | 36/53 | 34 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 103 | 31/53 | 30 |
| **F** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 157 | 19/20 | 29 |
| **Scales** | | | | | |
| **D** | Col1a1 protein | gi\|171846460 | 86 | 42/97 | 38 |
| | Collagen alpha-2(I) chain precursor | gi\|42542708 | 126 | 42/97 | 44 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 82 | 39/97 | 34 |
| **E** | Col1a1 protein | gi\|171846460 | 131 | 40/66 | 36 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 120 | 37/66 | 38 |
| **F** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 144 | 19/21 | 28 |

**Supplementary Table S7. Mass spectrometry analysis of adult pepsin soluble collagen from scales separated by 2D electrophoresis.**

| Spot number | Protein description | NCBI ID | Mascot search results | | |
|---|---|---|---|---|---|
| | | | Score | No. of matched peptides | Sequence coverage (%) |
| **1** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 69 | 20/46 | 25 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 68 | 21/46 | 21 |
| | Col1a1 protein | gi\|171846460 | 67 | 21/46 | 21 |
| **2** | Collagen, type I, alpha 3 | gi\|42542708 | 75 | 25/62 | 25 |
| | Col1a1 protein | gi\|171846460 | 74 | 24/62 | 28 |
| | Collagen alpha-2(I) chain precursor | gi\|48762667 | 66 | 22/62 | 23 |
| **3** | Col1a1 protein | gi\|171846460 | 102 | 20/31 | 22 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 96 | 19/31 | 19 |
| **4** | Col1a1 protein | gi\|171846460 | 88 | 18/28 | 23 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 88 | 17/28 | 22 |
| **5** | Collagen, type I, alpha 3 | gi\|42542708 | 116 | 22/35 | 28 |
| | Col1a1 protein | gi\|171846460 | 93 | 22/35 | 26 |
| **6** | Collagen, type I, alpha 3 | gi\|42542708 | 114 | 23/35 | 22 |
| | Col1a1 protein | gi\|171846460 | 102 | 22/35 | 27 |
| **7** | Col1a1 protein | gi\|171846460 | 161 | 39/64 | 35 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 121 | 34/64 | 33 |
| **8** | Col1a1 protein | gi\|171846460 | 98 | 22/34 | 22 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 94 | 20/34 | 22 |
| **9** | Col1a1 protein | gi\|171846460 | 111 | 33/53 | 30 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 89 | 27/53 | 27 |
| **10** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 97 | 26/59 | 31 |
| | Col1a1 protein | gi\|171846460 | 74 | 28/59 | 30 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 71 | 26/59 | 27 |
| **11** | Procollagen type I alpha 2 chain | gi\|15209312 | 117 | 16/21 | 18 |
| **12** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 133 | 34/65 | 34 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 89 | 29/65 | 29 |
| **13** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 81 | 35/85 | 35 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 76 | 34/85 | 31 |
| **14** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 152 | 39/72 | 42 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 80 | 30/72 | 29 |
| **15** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 157 | 44/83 | 42 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 81 | 35/83 | 31 |
| | Col1a1 protein | gi\|171846460 | 62 | 32/83 | 31 |
| **16** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 139 | 43/84 | 43 |
| | Col1a1 protein | gi\|171846460 | 77 | 35/84 | 32 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 73 | 33/84 | 30 |
| **17** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 147 | 45/88 | 45 |
| | Col1a1 protein | gi\|171846460 | 74 | 36/88 | 31 |
| **18** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 114 | 26/47 | 35 |
| | Col1a1 protein | gi\|171846460 | 59 | 21/47 | 24 |
| **19** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 103 | 23/41 | 29 |
| | Col1a1 protein | gi\|171846460 | 63 | 20/41 | 20 |
| **20** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 124 | 28/49 | 27 |
| | Col1a1 protein | gi\|171846460 | 63 | 20/49 | 22 |

| | | | | | |
|---|---|---|---|---|---|
| **21** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 153 | 31/51 | 37 |
| **22** | Collagen, type I, alpha 3 | gi\|42542708 | 159 | 38/67 | 38 |
| | Col1a1 protein | gi\|171846460 | 158 | 41/67 | 40 |
| **23** | Col1a1 protein | gi\|171846460 | 158 | 56/94 | 47 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 129 | 51/94 | 38 |
| **24** | Col1a1 protein | gi\|171846460 | 176 | 59/97 | 48 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 121 | 50/97 | 39 |
| **25** | Col1a1 protein | gi\|171846460 | 175 | 57/97 | 48 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 116 | 45/97 | 39 |
| **26** | Col1a1 protein | gi\|171846460 | 181 | 41/57 | 37 |
| | Collagen, type I, alpha 3 | gi\|42542708 | 86 | 27/57 | 27 |
| **27** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 242 | 38/46 | 41 |
| **28** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 285 | 56/73 | 48 |
| **29** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 247 | 51/71 | 43 |
| **30** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 259 | 33/35 | 34 |
| **31** | Collagen alpha-2(I) chain precursor | gi\|48762667 | 249 | 34/40 | 38 |

**Supplementary Table S8**. **Theoretic and experimental values obtained from aminoacid analysis of zebrafish α1, α2 and α3 chains of type I collagen and type I collagen extracted from skin, bone and scales, respectively**.

| | Theoretic Values | | | | | Experimental Values | | |
|---|---|---|---|---|---|---|---|---|
| | **MW** | **α1(I)** | **α2(I)** | **α3(I)** | | **Skin** | **Bone** | **Scales** |
| Asp | 133,103 | 25 | 25 | 29 | Asp + Asn* | 45 ± 2 | 42 ± 1 | 46 ± 1 |
| Glu | 147,130 | 53 | 39 | 48 | Glu + Gln* | 73 ± 2 | 73 ± 3 | 76 ± 2 |
| His | 155,157 | 2 | 5 | 3 | His | 2 | 2 ± 1 | 2 |
| Cys | 162,109 | 0 | 0 | 0 | Cys | 0 | 0 | 0 |
| Lys | 146,190 | 35 | 31 | 32 | Lys | 14 ± 2 | 16 ±2 | 16 ±1 |
| Arg | 174,204 | 53 | 55 | 54 | Arg | 54 | 54 | 54 |
| Hyp | 131,131 | 100 | 84 | 101 | Hyp | 112 ± 11 | 114 ± 22 | 110 ± 9 |
| Ser | 105,093 | 31 | 41 | 43 | Ser | 24 ± 2 | 21 ± 3 | 27 ± 2 |
| Thr | 119,120 | 27 | 21 | 22 | Thr | 24 ± 2 | 29 ± 3 | 30 ± 1 |
| Tyr | 181,191 | 0 | 2 | 0 | Tyr | 2 ± 1 | 2 | 2 ± 1 |
| Hyl | | | | | Hyl | 4 ± 2 | 5 ± 1 | 5 ± 1 |
| Gly | 75,067 | 346 | 347 | 352 | Gly | 346 ± 10 | 344 ± 9 | 338 ± 5 |
| Asn | 132,119 | 15 | 28 | 17 | | | | |
| Gln | 146,146 | 22 | 23 | 18 | | | | |
| Ala | 89,094 | 138 | 113 | 118 | Ala | 126 ± 2 | 129 ± 2 | 126 ± 2 |
| Phe | 165,192 | 13 | 9 | 11 | Phe | 13 ± 1 | 12 ±1 | 13 ±1 |
| Ile | 131,175 | 12 | 14 | 8 | Ile | 13 ± 1 | 12 ± 1 | 13 ± 2 |
| Leu | 131,175 | 13 | 30 | 22 | Leu | 23 ±1 | 22 ± 2 | 24 ± 2 |
| Met | 149,208 | 14 | 12 | 15 | Met | 9 ± 1 | 6 ± 1 | 4 ± 1 |
| Pro | 115,132 | 103 | 112 | 108 | Pro | 113 ± 20 | 117 ± 16 | 111 ± 11 |
| Val | 117,148 | 15 | 26 | 16 | Val | 20 ± 1 | 17 ± 1 | 21 ± 1 |
| | | 1017 | 1017 | 1017 | | 1017 | 1017 | 1017 |

*due to acidic hydrolysis

**Supplementary Table S9. Selective Reaction Monitoring (SRM) synthetic peptides**

| Protein | Synthetic Peptide Sequence | precursor m/z | fragment m/z | Collision energy (eV) | amount of heavy peptide spiked (fmol) |
|---|---|---|---|---|---|
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 232.14 | 29 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 544.284 | 27 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 748.374 | 29 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 849.421 | 27 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 977.48 | 28 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 714.355 | 1.074.533 | 29 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 232.14 | 29 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 544.284 | 27 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 748.374 | 29 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 849.421 | 27 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 977.48 | 28 | 8.333 |
| Type I collagen alpha 1 | GAAGPPGATGFP[+16.0]GAAGR | 717.362 | 1.080.546 | 29 | 8.333 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 544.76 | 400.23 | 24 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 544.76 | 528.289 | 24 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 544.76 | 643.316 | 22 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 544.76 | 714.353 | 23 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 544.76 | 884.422 | 16 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 547.767 | 406.244 | 24 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 547.767 | 534.303 | 24 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 547.767 | 649.33 | 22 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 547.767 | 720.367 | 23 | 50 |
| Type I collagen alpha 1 | GFP[+16.0]GADGAAGPR | 547.767 | 890.436 | 16 | 50 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.066.528 | 417.22 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.066.528 | 982.506 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.066.528 | 1.152.612 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.066.528 | 1.320.702 | 40 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.066.528 | 1.377.723 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.071.532 | 427.229 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.071.532 | 992.515 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.071.532 | 1162.62 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.071.532 | 1330.71 | 40 | 100 |
| Type I collagen alpha 1 | GNPGAEGATGPAGIP[+16.0]GPQGIGGQR | 1.071.532 | 1.387.732 | 40 | 100 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 914.945 | 147.113 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 914.945 | 204.134 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 914.945 | 372.224 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 914.945 | 623.351 | 34 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 914.945 | 906.468 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 918.952 | 155.127 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 918.952 | 212.148 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 918.952 | 380.238 | 35 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 918.952 | 631.365 | 34 | 41.666 |
| Type I collagen alpha 1 | VGPPGP[+16.0]SGNSGP[+16.0]P[+16.0]GPPGPAGK | 918.952 | 914.482 | 35 | 41.666 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 615.809 | 204.134 | 26 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 615.809 | 301.187 | 26 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 615.809 | 671.336 | 19 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 615.809 | 728.357 | 19 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 615.809 | 799.394 | 19 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 619.318 | 204.134 | 26 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 619.318 | 301.187 | 26 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 619.318 | 671.336 | 19 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 619.318 | 728.357 | 19 | 0.5 |
| Type I collagen alpha 3 | GFGGLAGPSGEPGK | 619.318 | 799.394 | 19 | 0.5 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 715.345 | 232.14 | 28 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 715.345 | 544.284 | 28 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 715.345 | 748.374 | 27 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 715.345 | 849.421 | 26 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 715.345 | 1.090.528 | 29 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 718.351 | 232.14 | 28 | 37.6 |

| | | | | | |
|---|---|---|---|---|---|
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 718.351 | 544.284 | 28 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 718.351 | 748.374 | 27 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 718.351 | 849.421 | 26 | 37.6 |
| Type I collagen alpha 3 | GGAGPP[+16.0]GATGFP[+16.0]GAAGR | 718.351 | 1.090.528 | 29 | 37.6 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.275.086 | 147.113 | 45 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.275.086 | 275.171 | 44 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.275.086 | 346.208 | 42 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.275.086 | 573.299 | 45 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.275.086 | 987.489 | 46 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.279.093 | 155.127 | 45 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.279.093 | 283.186 | 44 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.279.093 | 354.223 | 42 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.279.093 | 581.313 | 45 | 1000 |
| Type I collagen alpha 3 | GNDGNTGAAGP[+16.0]PGPTGPAGPP[+16.0]GFP[+16.0]GGAGAK | 1.279.093 | 995.504 | 46 | 1000 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 887.442 | 204.134 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 887.442 | 388.219 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 887.442 | 445.241 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 887.442 | 894.468 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 887.442 | 1.038.521 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 891.449 | 212.148 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 891.449 | 396.233 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 891.449 | 453.255 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 891.449 | 902.482 | 35 | 3.57 |
| Type I collagen alpha 3 | VGP[+16.0]PGPSGASGPPGPTGP[+16.0]AGK | 891.449 | 1.046.536 | 35 | 3.57 |

Peptides selected for spike-in are highlighted in yellow.

[+16.0] in the peptide sequence indicates the hydroxylation of the Proline residue

**Supplementary Figure 1. Expression of collagen type I genes in early zebrafish development.** Relative expression values were normalized to maximum expression values for each gene and depicted in the y-axis. Only low levels of maternal transcripts can be detected for the three collagen type I genes. An expression peak for all three genes is detected at 72-96 hpf.