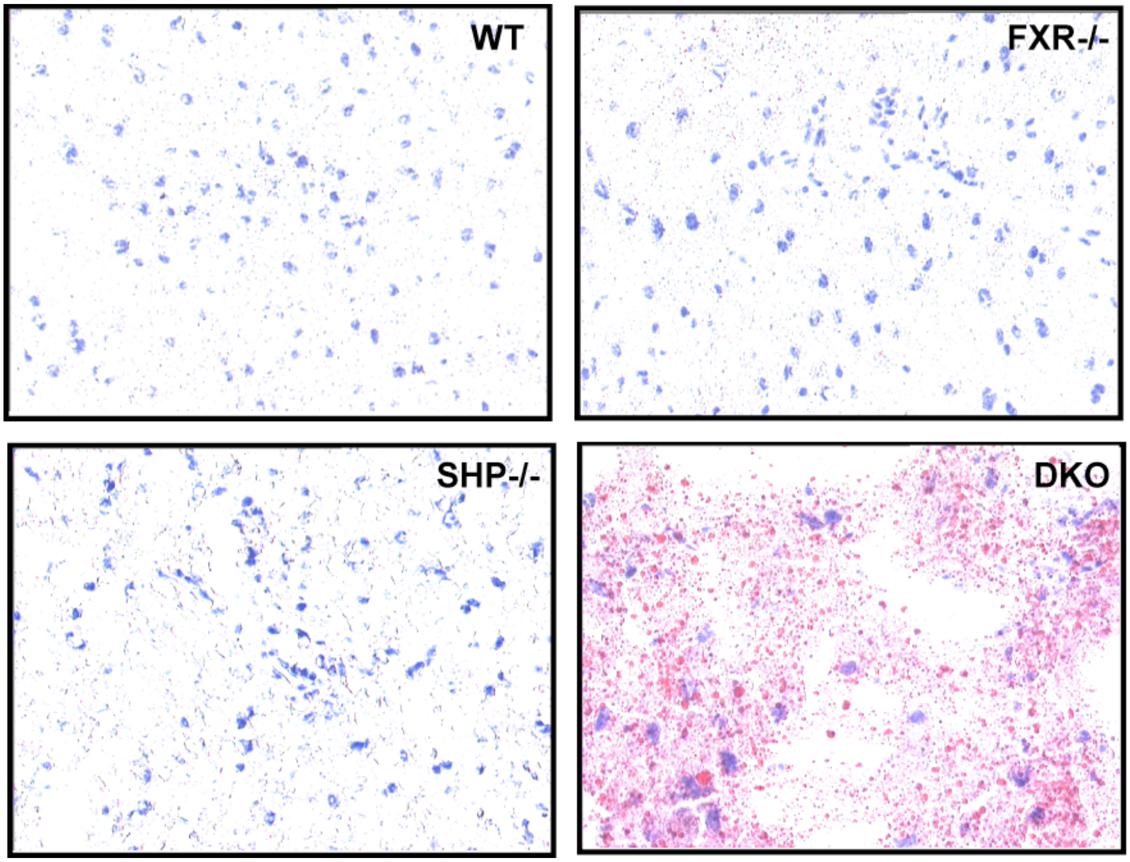
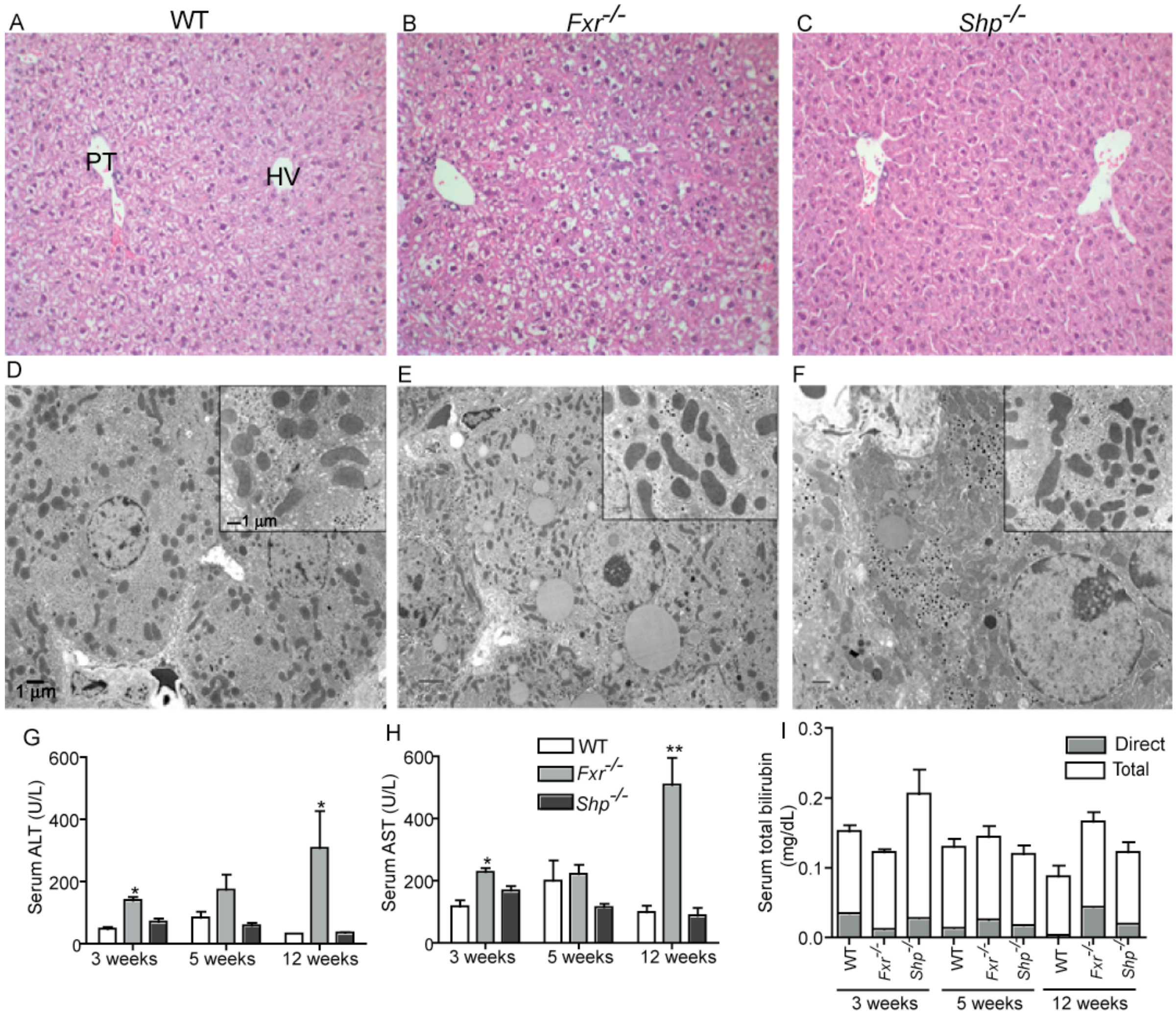


Supplementary Figure 1: OCT embedded frozen liver sections were stained with Oil Red O. Increased Oil Red O staining is clearly visible in DKO but not in WT, $Fxr^{-/-}$ and $Shp^{-/-}$ indicating increased lipid accumulation in 8-10 week old DKO liver.

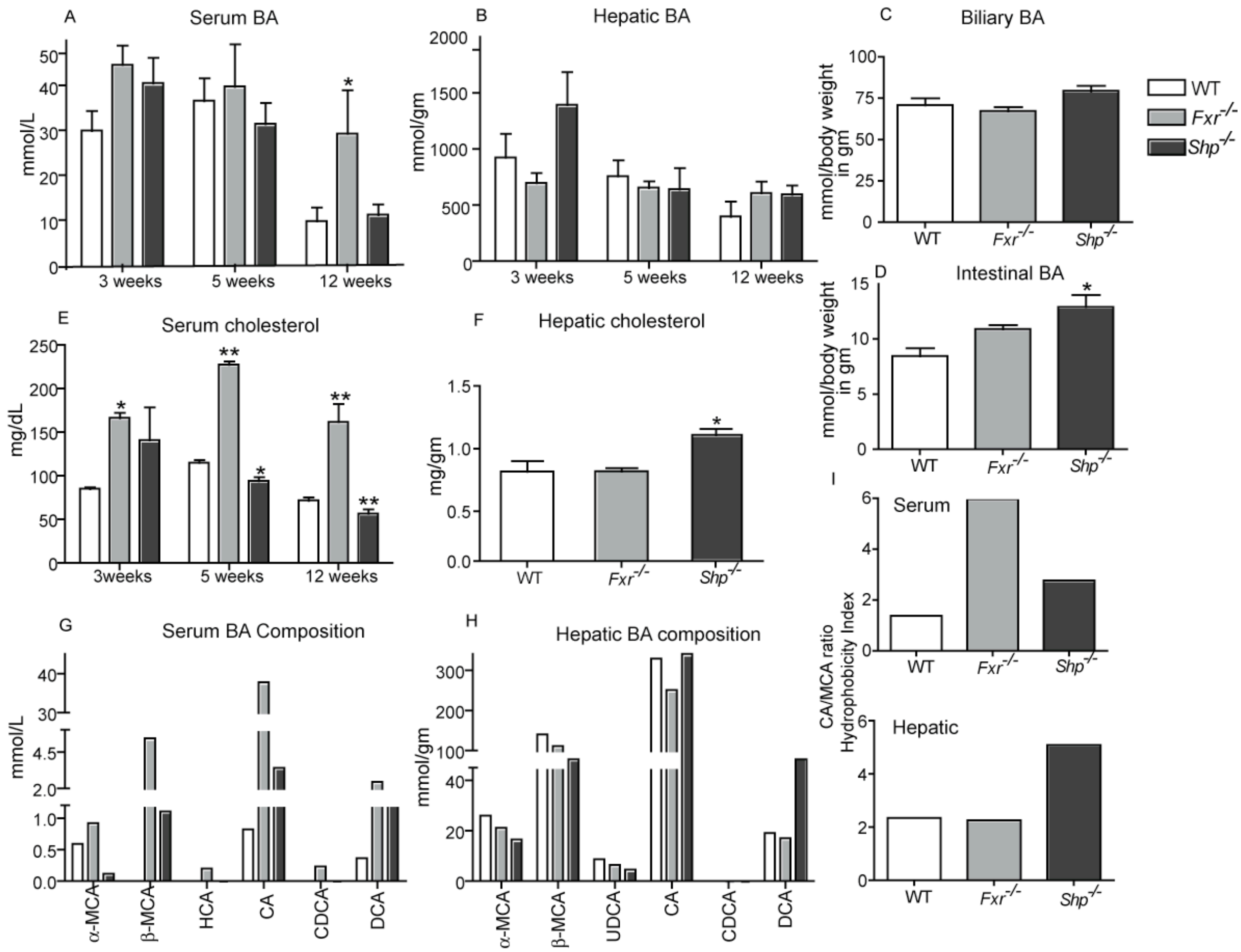


Supplementary Figure 2: Minimal liver injury in individual Fxr$^{-/-}$ and Shp$^{-/-}$ mice.

Hematoxylin-eosin stain, x200 of 5 week old mouse liver. (A) Histology of the WT liver shows hepatocytes between portal tract, PT (left) and terminal hepatic vein, HV (right). (B) *Fxr* $^{-/-}$ - hepatocyte nuclei are enlarged, cytoplasm is focally vacuolated due to lipid and there is focal hepatitis (C) *Shp*$^{-/-}$- depletion of glycogen leaves cytoplasm more eosinophilic. Electron micrographs of the corresponding samples show small lipid droplets in the cytoplasm of a normal WT hepatocyte in (D). E. *Fxr* $^{-/-}$ displayed moderate hepatocellular microsteatosis; small lipid droplets marked by arrows.

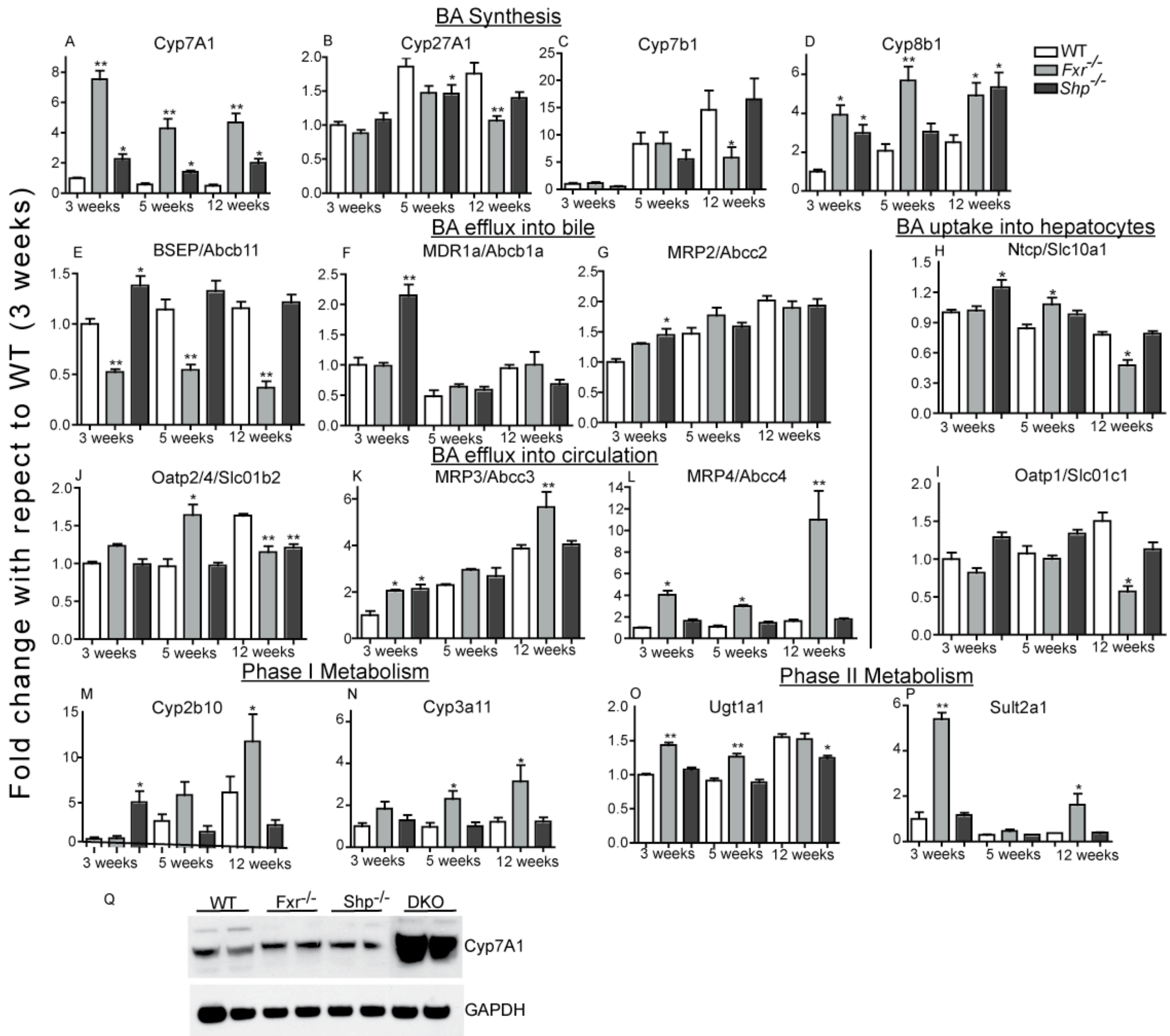
(F) *Shp*$^{-/-}$ displayed mild mitochondrial pleomorphism (see inset). The original magnification is x200 for light microscopy; x1500 for EM and x3000 for EM insets.

(G-I) Serum ALT (G), AST (H) and bilirubin (I) levels suggest modest liver injury in *Fxr* $^{-/-}$ mice. Data are presented as mean ± SEM, n=8-10. *p<0.05 and **p<0.001 when compared to WT.



Supplementary Figure 3: Individual FXR and SHP null mice display modest biliary dysfunction.

BA levels in serum (A) and liver (B) remain unchanged in *Shp*$^{-/-}$ whereas *Fxr*$^{-/-}$ mice show increased serum levels (A) only at 12 weeks of age. (C) Biliary BA remains unaffected but intestinal BA is slightly induced only in the absence of SHP (D). (E-F) Cholesterol, a precursor for BA production is increased in serum of *Fxr*$^{-/-}$ and liver of *Shp*$^{-/-}$ but not WT mice. (G-I) BA composition reveals increased hydrophobic pool size in serum of *Fxr*$^{-/-}$ and in liver of *Shp*$^{-/-}$ mice. Data are presented as mean ± SEM, n=6. *p<0.05 and **p<0.001 when compared to WT.



Supplementary Figure 4: Dysfunction in BA synthesis and transport pathways in *Fxr*$^{-/-}$ and *Shp*$^{-/-}$.

Total RNA (n=4) per time point was prepared from WT, *Fxr*$^{-/-}$, *Shp*$^{-/-}$ and DKO mice and gene expression was analyzed in duplicates using nanostring technology.

(A-D) Genes involved in the synthesis of neutral (A) and hydrophobic (D) BA is increased but acidic BA (B&C) is decreased only in *Fxr*$^{-/-}$ not *Shp*$^{-/-}$; (E-G) Aberrant expression of genes involved in BA efflux into bile. (H-I) Genes involved in BA efflux into circulation is increased in *Fxr*$^{-/-}$ but decreased in *Shp*$^{-/-}$. (J-L) Genes involved in BA uptake into liver is decreased in *Fxr*$^{-/-}$ but not changed in *Shp*$^{-/-}$ at 12 weeks. (M-P) genes involved in BA detoxification is increased only in *Fxr*$^{-/-}$. CYP7A1 protein is modestly induced in *Fxr*$^{-/-}$ and *Shp*$^{-/-}$ (Q). *p<0.05 and **p<0.001 when compared to their respective age matched WT.

Gene	Accession	Targeted Region	Target Sequence
Cyp7a1	NM_007824.2	480-580	CTCTCTGAAGCCATGATGCAAAACCTCCAATCTGTCATGAGACCTCCGGGCCTTCCTAAATCAAAGAGCGCTGTCTGGGTCACGGAAGGGATGTATGCCT
Cyp7b1	NM_007825.2	1165-1265	TTGGTTTGCCTGGAAAGCACTATTCTTGAGGTTCTGAGGCTGTGCTCATACTCCAGCATCATCCGAGAAGTGCAGGAGGATATGAATCTCAGCTTAGAGA
Cyp8b1	NM_010012.2	1825-1925	CATCCAGCCTGCCTTACTCGATGCCCTTACTCCAAATCCTACCAGCTCAGACTCCAGGGATGTTGCTCAATGGAATCGAGCCATGTGTGCGACTGTTAAT
Cyp27a1	NM_024264.3	1725-1825	GTCTCATGTCACATGTCACGATGTCAGATTCAACAGGAGAACTCTGTGCCCTTCCTATAGACACCAAATGTCTGGCACAATCTCTACTGAGCAGCACCCA
Abcb11	NM_021022.2	1660-1760	AGAGGCGACAATGGAAGACATAGTCCAAGCTGCCAAGGATGCTAATGCATACAACTTCATTATGGCCCTGCCACAGCAATTTGACACCCTAGTTGGAGAA
Abcb1a	NM_011076.1	2600-2700	AAGGGGCTACAGGGTCTAGGCTTGCTGTGATTTTCCAGAACATAGCAAATCTTGGGACAGGAATCATCATATCCCTAATCTATGGCTGGCAACTAACACT
Abcc2	NM_013806.2	4600-4700	TAATGGTCCTAGACAGCGGCAAGATTGTTGAATACGGCAGTCCTGAAGAACTGCTGTCCAATATGGGTCCCTTCTACTTGATGGCCAAGGAAGCCGGCAT
Slc10a1	NM_011387.1	130-230	CTGGTAGTTATGTTGCTGCTCATCATGCTCTCGCTTGGCTGCACCATGGAGTTCAGCAAGATCAAGGCTCACTTCTGGAAGCCCAAAGGGGTGATCATCG
Slco1c1	NM_021471.1	320-420	CGCGGCCATGCTGTGGAAAACTCAAGGTGTTCTTGGGTGCCCTGTCGTTTGTTTACTTTGCCAAAGCATTGGCAGAAGGCTATCTGAAGAGCACCGTGAC
Slco1b2	NM_020495.1	635-735	GGAACCTCACCTGAGATAATGGAGAAAGGTTGTGAAAAGGGGTCCAACTCATACACCTGGATTTATGTCTTGATGGGGAACATGCTTCGTGGGATAGGGG
Abcc3	NM_029600.3	2730-2830	CTACGAGGTCCGCAAGCAGTTCATGAGAGAGATGAGCTCCTTGTCTTCTGAAGGGGAGGTCCAGAACCGGACTATGCCCAAGAAACACACAAATTCATTG
Abcc4	NM_001033336.1	2305-2405	CACTGGGCGAACAAGCAAGGTGCACTGAACAACACCAGAAATGCGAATGGAAATATAACGGAGACCCTAGACCTCAGCTGGTACTTAGGAATTTACGCAG
Cyp2b10	NM_009999.3	1535-1635	AATGACTCTATCTTTGAGCCTCTGAGAGACCTGCTGGAAATCAGTACTCCTATTGCATGTCTCCAAATCTCCAGGGCTCCAAGGCATGTTCTTCTTCCCT
Cyp3a11	NM_007818.3	315-415	CCTCTCCTTGCTGTCACAGACCCAGAGACGATTAAGAATGTGCTAGTGAAGGAATGTTTTTCTGTCTTCACAAACCGGCGGGATTTTGGCCCAGTGGGGA
Ugt1a1	NM_201645.1	480-580	CTTTGATGCTCTGCTGACAGACCCTTTCCTTCCCTGTGGCTCCATTGTGGCCCAGTACCTGACTGTGCCCACTGTGTACTTCTTGAATAAATTGCCATGC
Sult2a1	NM_001111296.1	778-878	GCTTGAAGCTCATGAGAAAAGGCACAATTGGGGACTGGAAGAATCACTTCACAGTAGCCCAAGCTGAAGCCTTCGATAAAGTTTTCCAGGAGAAAATGGC
Actb	NM_007393.1	815-915	CAGGTCATCACTATTGGCAACGAGCGGTTCCGATGCCCTGAGGCTCTTTTCCAGCCTTCCTTCTTGGGTATGGAATCCTGTGGCATCCATGAAACTACAT
Hmgcr	NM_008255.1	1870-1970	GTAAAGACCTGGCTTGAAACACCTGAAGGGTTTGCAGTGATAAAGGAGGCCTTTGATAGCACCAGCAGATTTGCTCGTCTACAGAAACTCCACGTGACGA
Cyp17a1	NM_007809.2	530-630	CTGTTCAGGGATGACCAGAAACTGGAGAAGATGATATGTCAGGAAGCCAACTCACTGTGTGACTTGATACTTACATACGACGGGGAGTCCCGAGATCTGT
Srd5a1	NM_175283.3	610-710	GCCAGTTTGCGGTGTATGCTGAAGACTGGGTAACCCATCCCTGTTTCCTGACAGGCTTTGCCCTGTGGTTAGTGGGCATGGTGATAAATATCCACTCAGA
Hsd3b5	NM_008295.2	1405-1505	TTGCCCAGTAATGCACAGGCTGTTGTGACTCAGCTGCTGTGACCCAAGAATCTGTGGCTGGTACTGAATTGCCTGGAACCTCTTGTAGGTAGAATTTCCT
Mmp13	NM_008607.1	190-290	ACAGTGACCTCCACAGTTGACAGGCTCCGAGAAATGCAATCTTTCTTTGGCTTAGAGGTGACTGGCAAACTTGATGATCCCACCTTAGACATCATGAGAA
Aqp1	NM_007472.1	2255-2355	ACACTCGTCTCCGTTCCCTAGCAGACACTGTACCCACTTAACAGATAAGGGCACTGAGGACCCATATGCTCAACTGTATCATATTGGATCAAGCTTAGCA
S100a8	NM_013650.2	280-380	ATAAAAGTGGGTGTGGCATCTCACAAAGACAGCCACAAGGAGTAGCAGAGCTTCTGGCCTAGGGCTGGGTCCCTGGATATGTCTACAGAATAAAGTCATC
Col1a1	NM_007742.3	215-315	CAATGGTGAGACGTGGAAACCCGAGGTATGCTTGATCTGTATCTGCCACAATGGCACGGCTGTGTGCGATGACGTGCAATGCAATGAAGAACTGGACTGT
Col6a1	NM_009933.2	2435-2535	AATGTCATTTCCTGCCAAGGCTTATCGCAAGGTCGGCCAGGTATCTCCCTGGTGAAGGAGAACTATGCAGAGCTTCTCGATGACGGCTTTCTGAAGAACA

Supplementary Table 1: Nanostring probe sets.

100 bp nanostring probes were custom synthesized from Nanostring Inc and utilized for quantification of gene expression.