

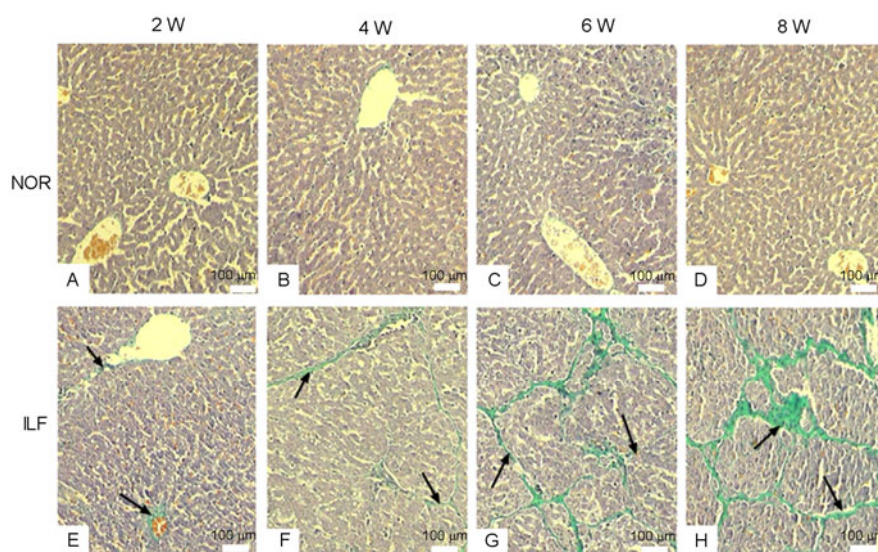


Figure S1 Histopathology of liver fibrosis examined by Masson's trichrome staining. (A–D, 100×) Nor, saline-treated rats livers at 2, 4, 6, and 8 weeks (W); ILF, (E–H, 100×), pig serum-treated rat livers at 2, 4, 6, and 8 W. No obvious difference was found at 2–8 W in normal rats. In the ILF model, the degree of liver fibrosis was increased gradually from S0–1 at 2 W, S2 at 4 W, and S3–4 at 6 W to S4 at 8 W as highlighted in green and indicated by arrow-heads.

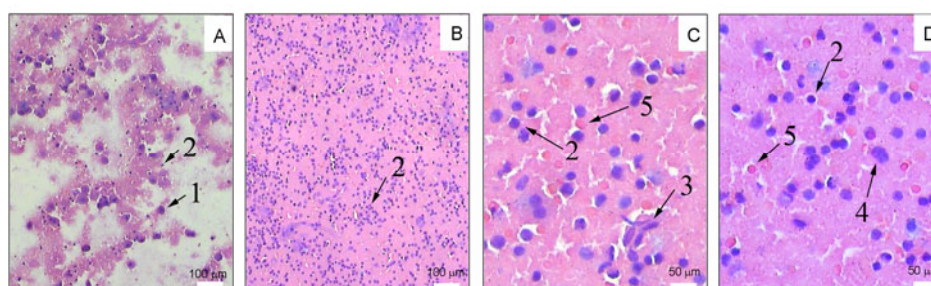


Figure S2 H&E staining to monitor the purity of isolated hepatic NPCs. A, H&E staining of the hepatic parenchymal cell fraction, 100×. B, H&E staining of the NPC fraction purified by Percoll density gradient centrifugation, 100×. C and D, H&E staining of the NPC fraction, 400×. 1, hepatocytes; 2, NPCs (lymphocytes); 3, NPCs (fibrocytes); 4, NPCs (KCs); 5, red blood cells.

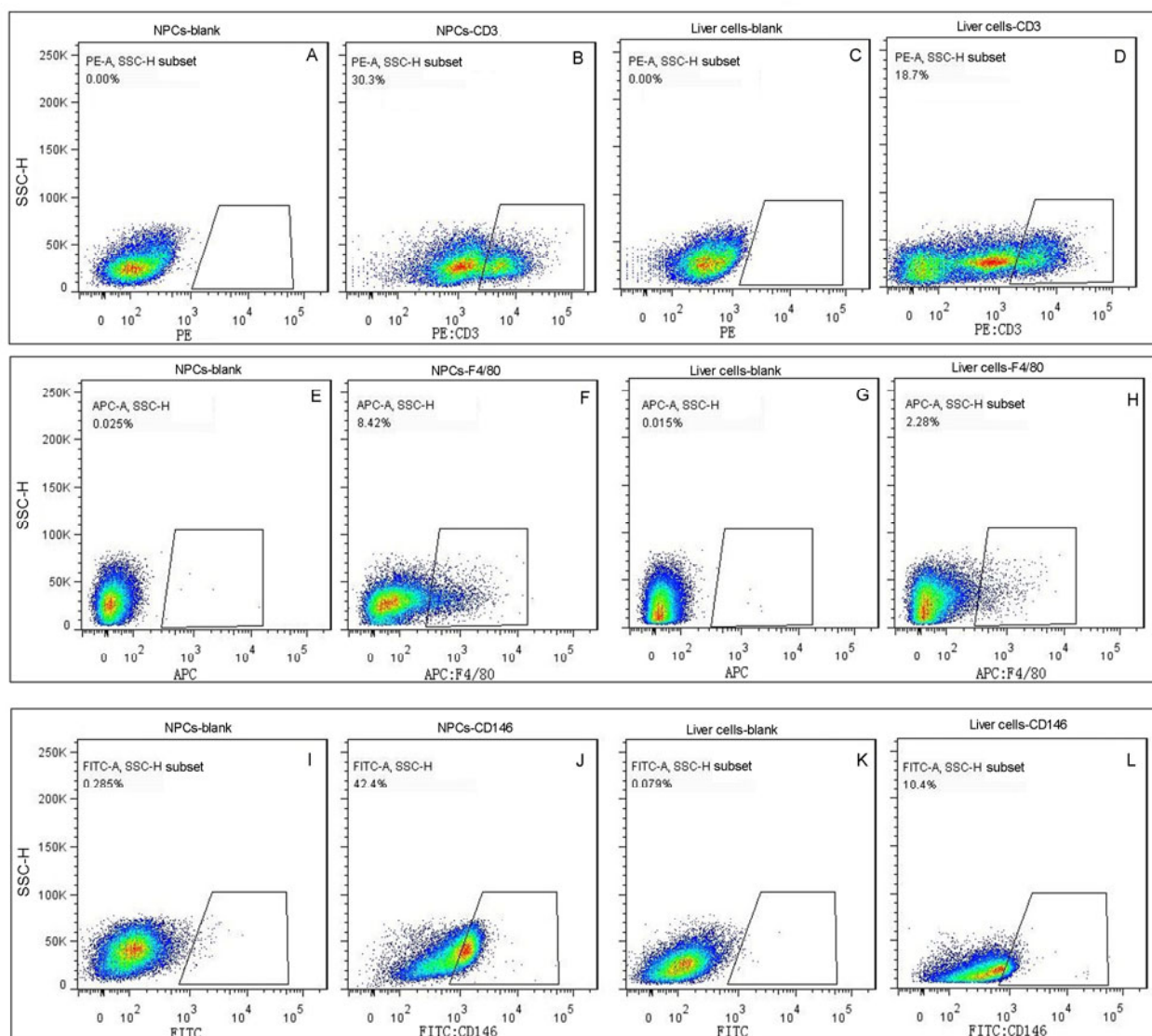


Figure S3 Validation of the enrichment of NPCs by flow cytometry. F4/80, CD146, and CD3 were used to detect LSECs, KCs, and lymphocytes, respectively. CD3-positive cells accounted for 30.3% of NPCs and 18.7% of total liver cells (B and D). F4/80-positive cells accounted for 8.24% of NPCs and 2.28% of total liver cells (F and H). CD146-positive cells accounted for 42.4% of NPCs and 10.4% of total liver cells (J and L). Unstained cells were used as negative controls as shown in A and C for CD3, E and G for F4/80, and I and K for CD146.

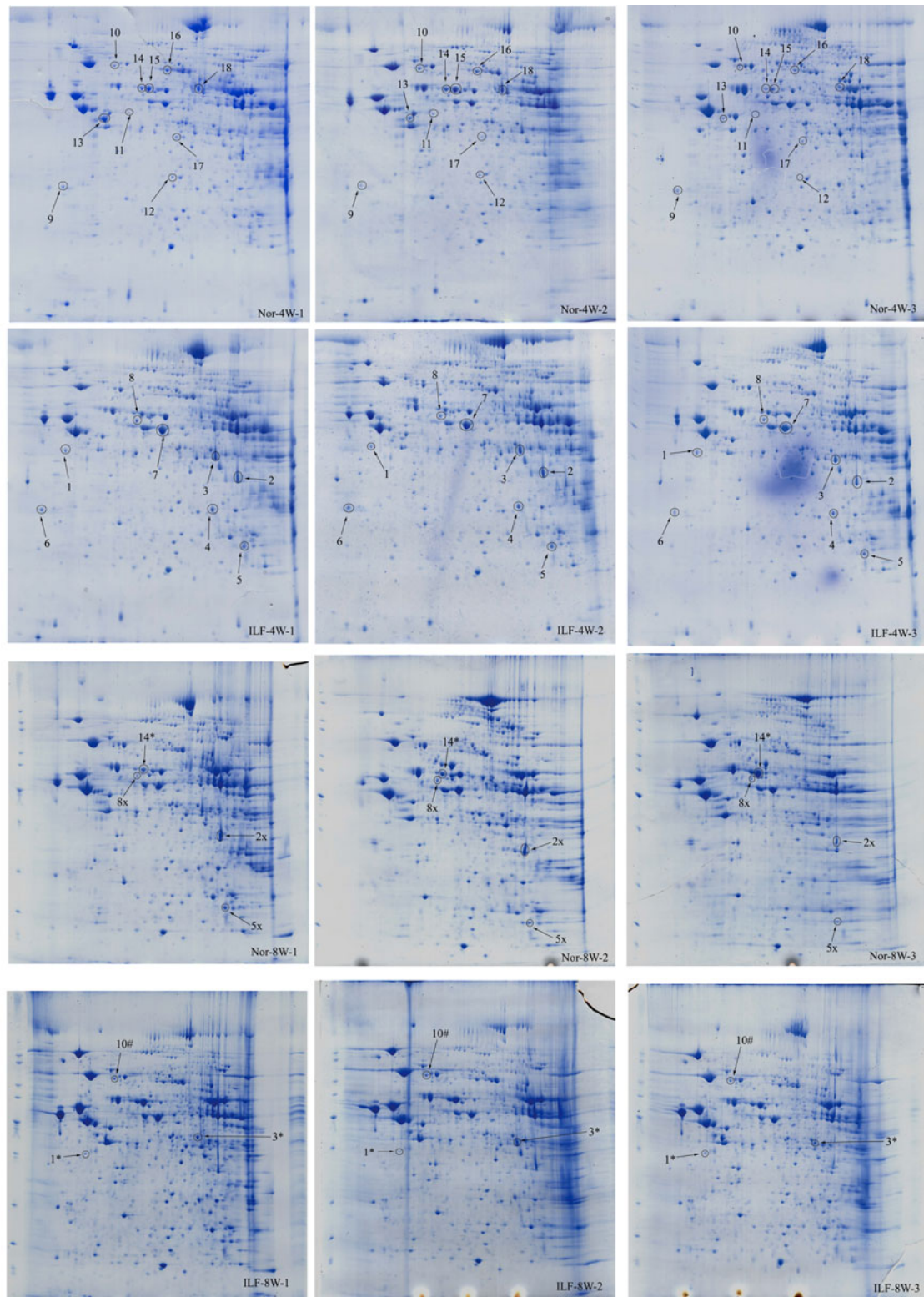


Figure S4 Protein expression profiles of the ILF model and normal control. Twelve raw 2DE gel images labeled with differential spots are shown. ILF, immune liver fibrosis; Nor, normal control. Up-regulated proteins are labeled in ILF gels and down-regulated proteins are labeled in Nor gels.

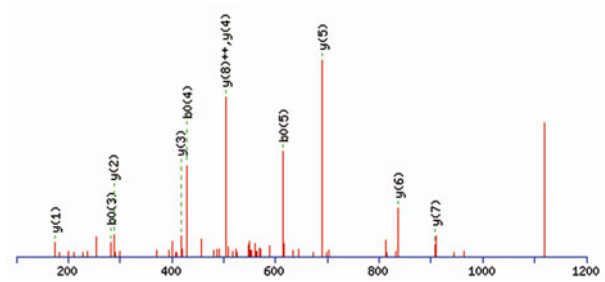


Figure S5 MS/MS spectrum of the protein (carboxylesterase 3) identified by one peptide.

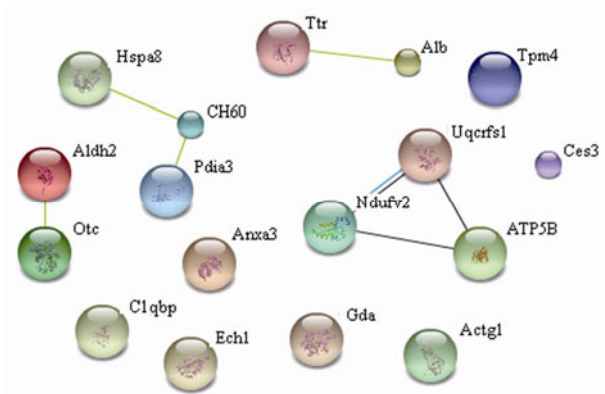


Figure S6 Protein-protein interaction network identified by STRING software using differentially expressed proteins as seeds.

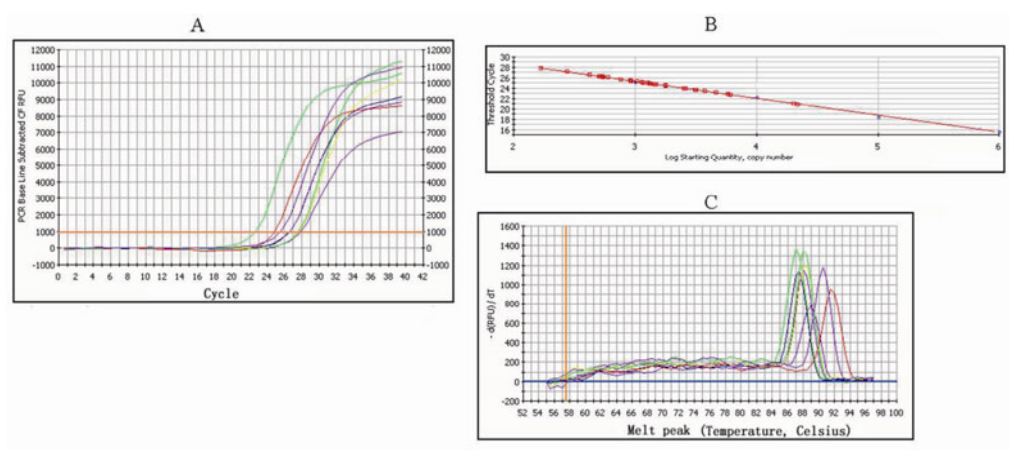


Figure S7 qRT-PCR analysis of the eight selected genes in controls at 8 weeks. GAPDH was used as an internal standard. A, qRT-PCR amplification curve. B, Ct values (threshold cycle). C, Standard curve. D, Melting curve. The eight target genes could be amplified and quantified by qRT-PCR. There was no detection of non-specific PCR products.

Table S1 Raw data of ImageMaster software analysis. Intensity value, volumes, volume% (after normalization), area of identified differential spots, and the numbers of matched protein spots in each gel are shown

4 weeks																										
	ILF-4W-1				ILF-4W-2				ILF-4W-3				Nor-4W-1				Nor-4W-2				Nor-4W-3				Ratio	
N	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	(ILF/ Nor)	
1	56.3	85.8	0.121	3.39	54	98.1	0.163	4.95	52.5	96.8	0.144	4.32	20.3	22.6	0.0341	4.68	21	18.9	0.0374	2.95	28.5	12.1	0.0187	4.65	4.75	
2	1.3	623	0.878	15.9	113	620	1.03	16	100	290	0.431	8.19	81.8	266	0.401	8.19	66.8	225	0.447	10.4	47.2	67.8	0.105	5.47	2.45	
3	102	663	0.935	18.8	122	599	0.994	14.2	100	419	0.622	9.02	36	70.9	0.107	8.18	86.3	127	0.252	4.06	85.5	121	0.187	3.39	4.67	
4	90	218	0.308	5.81	94.5	255	0.422	7.26	86.3	141	0.209	4.31	7.5	3.91	0.00589	1.84	20.3	38.6	0.0766	5.98	23.3	18.1	0.028	2	8.50	
5	69.8	321	0.453	15.8	82.5	274	0.454	11.4	75.8	170	0.253	7.42	29.3	7.78	0.01177	1.61	19.5	11.3	0.0225	3.02	31.5	87	0.135	10.3	6.85	
6	72	117	0.164	4.42	69.8	150	0.249	8	42	92.4	0.137	5.73	31.5	3	0.00452	0.13	24	37.2	0.0738	7.73	143	51	0.0792	0.19	3.49	
7	122	1186	1.67	16.8	123	1109	1.84	18.6	103	1068	1.59	17.3	102	407	0.61	7.05	111	554	1.1	11.5	97.5	334	0.51	8.08	2.30	
8	74.3	117	0.165	3.87	65.3	91.6	0.152	3.74	75.8	92.1	0.137	3.1	49.5	36.4	0.0549	1.92	13.5	9.07	0.018	1.94	52.5	7.26	0.0112	0.32	5.40	
9	29.3	35.1	0.0495	4.71	24	10.4	0.0172	3.15	20.3	21.5	0.0319	4.39	57	68.9	0.104	4.21	38.3	44.9	0.0891	3.84	16.5	36.1	0.0558	6.94	0.40	
10	33.8	14.4	0.0204	1.13	37.5	37.4	0.062	3.39	30.8	37.8	0.0562	3.24	51	68.2	0.103	2.44	29.3	36.4	0.0723	3.95	48.8	68.1	0.105	3.61	0.49	
11	18	16	0.0226	4.29	22.5	9.44	0.0157	1.27	16.5	11.7	0.0173	3.19	33	18.4	0.0277	1.56	51.8	43.9	0.0872	2.71	21	12.6	0.0195	2.63	0.41	
12	16.5	17.2	0.0242	3.44	36	7.24	0.012	0.57	17.3	36.4	0.0541	7.77	54	50.7	0.0764	2.56	48	42.8	0.085	2.63	18.8	27.1	0.0419	4.08	0.44	
13	87.8	177	0.25	4.82	60	82.8	0.137	4.71	61.5	74.5	0.111	3.44	106	720	1.09	14.2	74.3	200	0.397	6.97	102	577	0.892	10.5	0.21	
14	63	141	0.199	5.95	54.8	75.4	0.125	3.23	59.3	99.2	0.147	4.87	86.3	182	0.275	5.81	91.5	26.8	0.533	8.4	46.5	98.8	0.153	5.26	0.49	
15	83.2	194	0.273	4.65	49.5	78.9	0.131	4.02	99.8	298	0.443	8.13	105	427	0.644	8.48	128	574	1.14	10	78	111	0.172	3.02	0.43	
16	30	19.2	0.0271	2.1	62.3	84.3	0.14	3.47	26.3	50.3	0.0747	4.81	84	178	0.268	5.26	92.3	182	0.361	5.66	75.8	235	0.364	7.44	0.24	
17	21	40.8	0.0575	6.19	29.3	14	0.0232	1.32	76.5	7.46	0.0111	0.11	53.3	91.2	0.137	4.97	24	26.5	0.0525	3.5	48	40.6	0.0628	2.23	0.36	
18	18.8	24.9	0.0351	3.4	42	15.2	0.0252	0.89	24.8	34.7	0.0518	2.74	84	320	0.482	9.9	110	361	0.716	7.68	87.8	328	0.507	11.2	0.07	
8 weeks																										
	ILF-4W-1				ILF-4W-2				ILF-4W-3				Nor-4W-1				Nor-4W-2				Nor-4W-3				Ratio	
N	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	Int	Vol	Vol%	Area	(ILF/ Nor)	
1*	65.3	65.7	0.0509	2.73	40.5	53	0.0427	1	48.8	69.9	0.0775	4.08	33.8	33.6	0.0291	2.82	66.8	33.6	0.0256	9.98	50.3	36.1	0.0305	0.15	2.01	
2x	116	630	0.488	12.5	94.5	445	0.358	10.2	63	218	0.242	10.6	117	1278	1.11	23.8	134	1589	1.21	23	102	415	0.35	11.1	0.41	
3*	109	508	0.394	11.6	99	511	0.411	12.5	85.5	372	0.413	12.6	111	197	0.171	19	129	202	0.154	17.9	81	263	0.222	19	2.23	
5x	99.8	282	0.218	7.94	61.5	23.5	0.0189	8.84	71.3	217	0.242	6.63	9.39	361	0.313	9.39	47.2	387	0.281	0.11	97.5	403	0.34	16	0.51	
8x	39.8	46.5	0.0361	3.76	33	44.3	0.0356	4.1	101	105	0.117	15	45.8	94.8	0.0822	5.44	90	242	0.184	6.68	60.8	168	0.142	7.13	0.46	
10#	107	161.2	0.125	2.82	77.3	190	0.153	5.5	71.3	136	0.151	4.76	78	41.3	0.0358	4.85	68.3	86.1	0.0656	3.56	89.3	128	0.108	4.23	2.05	
14*	119	724.9	0.562	26.7	143	887	0.714	22	99.8	561	0.623	6.48	96	1144	0.991	10.6	123	1278	0.973	13.6	123	2228	1.88	27.5	0.49	

Table S3 The relative mRNA expression in ILF models compared with that in normal controls at 4 and 8 weeks as determined by qRT-PCR

Genes down-regulated in ILF compared with the corresponding controls in 4 and 8 weeks						
Genes	PDIA3-4	PDIA3-8	TTHY-4	TTHY-8		
data 1	0.02	0.22	0	0.095		
data 2	0	0.4	0.03	0.32		
data 3	0.01	0.33	0.016	0.14		
average	0.01	0.32	0.02	0.19		
Std	0.01	0.09	0.02	0.08		
Genes up-regulated in ILF compared with the corresponding controls in 4 and 8 weeks						
Genes	NDUV-4	NDUV-8	ATPB-4	ATPB-8	CH60-4	CH60-8
data 1	2.27	1.29	1.31	2.6	2.23	2.06
data 2	2.04	1.45	1.39	3.32	1.8	3.05
Data	2.13	1.35	1.34	2.8	2	2.54
average	2.15	1.36	1.35	2.91	2.02	2.55
Std	0.12	0.08	0.04	0.37	0.22	0.5
Genes with inconsistent changes from 4 to 8 weeks						
Genes	HSP7C-4	HSP7C-8	OTC-4	OTC-8	UCRI-4	UCRI-8
data 1	0.15	4.8	0.04	1.25	0	1.77
data 2	0.11	2.16	0.01	1.17	0.01	2.03
data 3	0.13	3.45	0.03	1.2	0.01	1.8
average	0.13	3.47	0.03	1.21	0.01	1.9
std	0.02	1.32	0.02	0.04	0.01	0.14