The Transcription Factor-microRNA Regulatory Network during hESC-chondrogenesis

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Supplementary Data Contents

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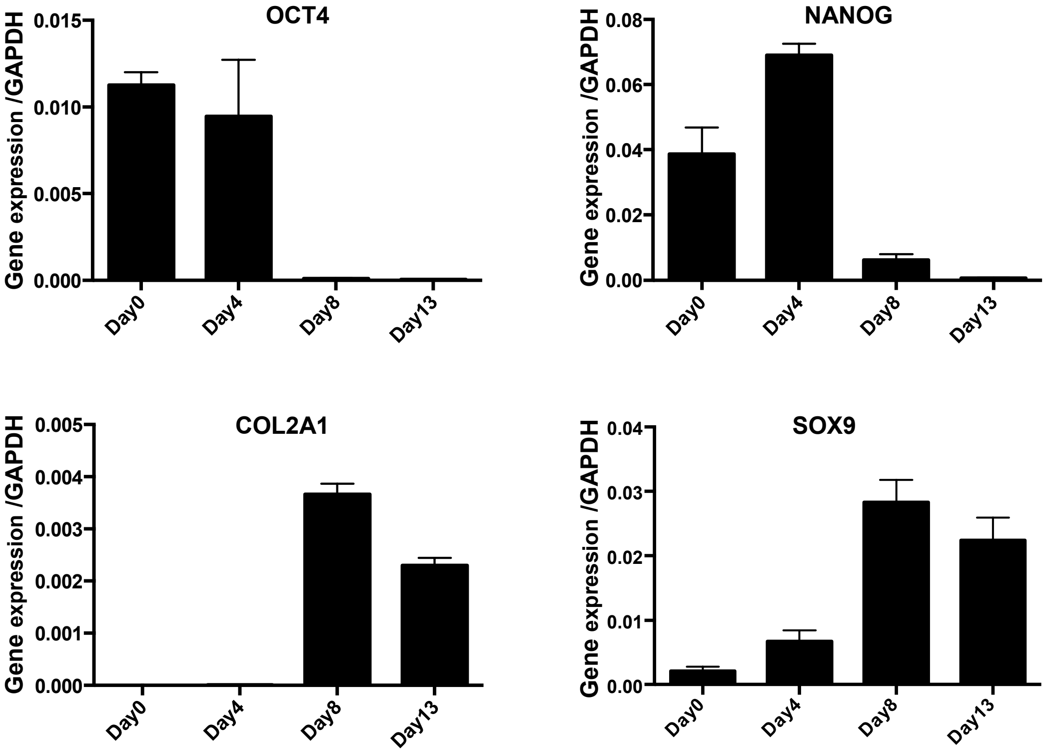
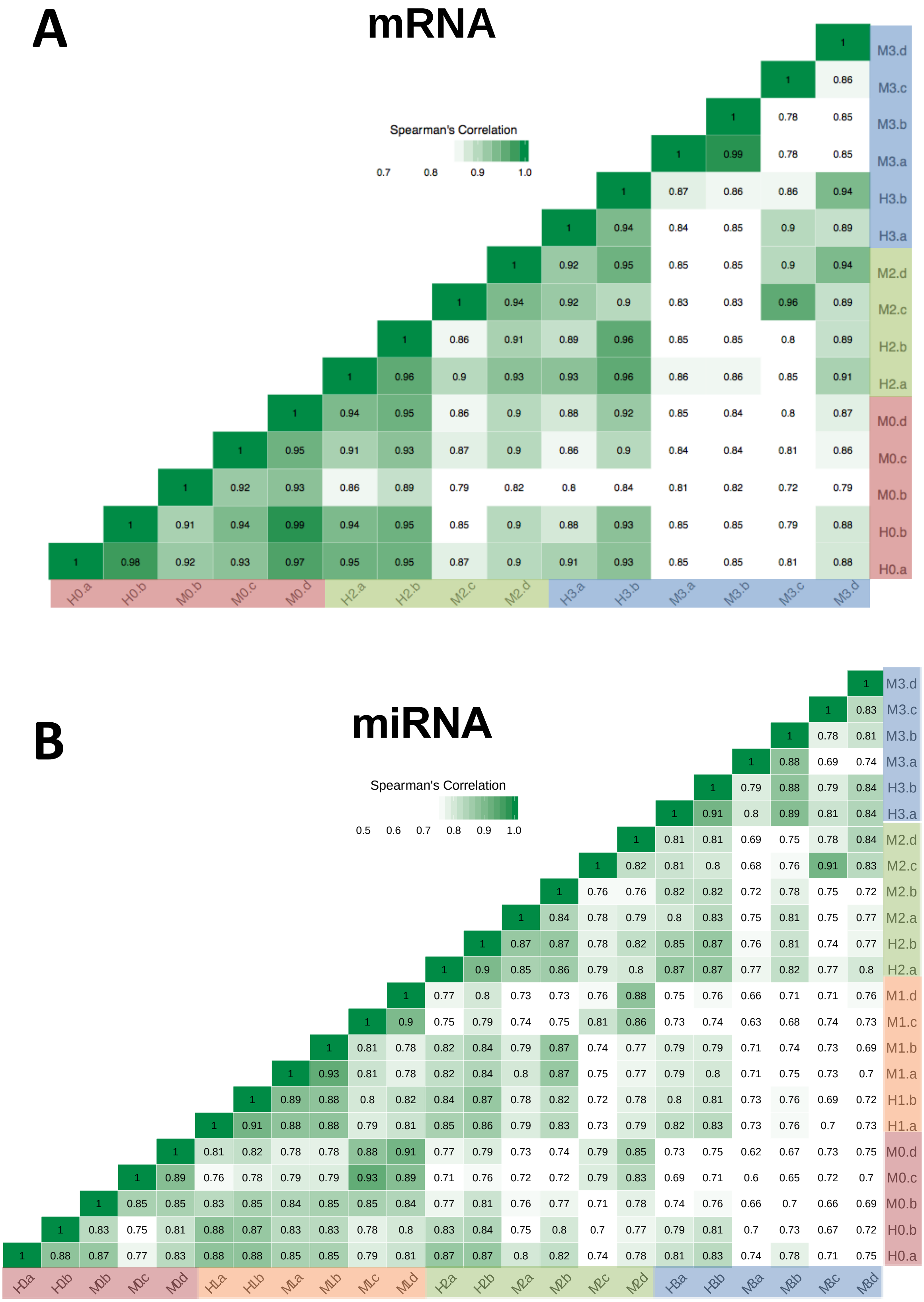


Figure S1 Validation of transcript expression for 2 pluripotency associated and 2 chondrogenic genes for RNAseq runs. Q-PCR analysis of transcript extracted from Man13 cells at 4 stages of chondrogenesis (Oldershaw et al 2010) to validate progression of the protocol. N=3



H0.a	H0.b	M0.b	M0.c	M0.d	H2.a	H2.b	M2.c	M2.d	H3.a	H3.b	M3.a	M3.b	M3.c	M3.d	
														1	M3.d
													1	0.86	M3.c
												1	0.78	0.85	M3.b
											1	0.99	0.78	0.85	M3.a
										1	0.87	0.86	0.86	0.94	H3.b
									1	0.94	0.84	0.85	0.9	0.89	H3.a
								1	0.92	0.95	0.85	0.85	0.9	0.94	M2.d
							1	0.94	0.92	0.9	0.83	0.83	0.96	0.89	M2.c
						1	0.86	0.91	0.89	0.96	0.85	0.85	0.8	0.89	H2.b
					1	0.96	0.9	0.93	0.93	0.96	0.86	0.86	0.85	0.91	H2.a
				1	0.94	0.95	0.86	0.9	0.88	0.92	0.85	0.84	0.8	0.87	M0.d
			1	0.95	0.91	0.93	0.87	0.9	0.86	0.9	0.84	0.84	0.81	0.86	M0.c
		1	0.92	0.93	0.86	0.89	0.79	0.82	0.8	0.84	0.81	0.82	0.72	0.79	M0.b
	1	0.91	0.94	0.99	0.94	0.95	0.85	0.9	0.88	0.93	0.85	0.85	0.79	0.88	H0.b
1	0.98	0.92	0.93	0.97	0.95	0.95	0.87	0.9	0.91	0.93	0.85	0.85	0.81	0.88	H0.a

H0a	H0b	M0b	M0c	M0d	H1a	H1b	M1a	M1b	M1c	M1d	H2a	H2b	M2a	M2b	M2c	M2d	H3a	H3b	M3a	M3b	M3c	M3d	
																						1	M3.d
																					1	0.83	M3.c
																				1	0.78	0.81	M3.b
																			1	0.88	0.69	0.74	M3.a
																		1	0.79	0.88	0.79	0.84	H3.b
																	1	0.91	0.8	0.89	0.81	0.84	H3.a
																1	0.81	0.81	0.69	0.75	0.78	0.84	M2.d
															1	0.82	0.81	0.8	0.68	0.76	0.91	0.83	M2.c
														1	0.76	0.76	0.82	0.82	0.72	0.78	0.75	0.72	M2.b
													1	0.84	0.78	0.79	0.8	0.83	0.75	0.81	0.75	0.77	M2.a
												1	0.87	0.87	0.78	0.82	0.85	0.87	0.76	0.81	0.74	0.77	H2.b
											1	0.9	0.85	0.86	0.79	0.8	0.87	0.87	0.77	0.82	0.77	0.8	H2.a
										1	0.77	0.8	0.73	0.73	0.76	0.88	0.75	0.76	0.66	0.71	0.71	0.76	M1.d
									1	0.9	0.75	0.79	0.74	0.75	0.81	0.86	0.73	0.74	0.63	0.68	0.74	0.73	M1.c
								1	0.81	0.78	0.82	0.84	0.79	0.87	0.74	0.77	0.79	0.79	0.71	0.74	0.73	0.69	M1.b
							1	0.93	0.81	0.78	0.82	0.84	0.8	0.87	0.75	0.77	0.79	0.8	0.71	0.75	0.73	0.7	M1.a
						1	0.89	0.88	0.8	0.82	0.84	0.87	0.78	0.82	0.72	0.78	0.8	0.81	0.73	0.76	0.69	0.72	H1.b
					1	0.91	0.88	0.88	0.79	0.81	0.85	0.86	0.79	0.83	0.73	0.79	0.82	0.83	0.73	0.76	0.7	0.73	H1.a
				1	0.81	0.82	0.78	0.78	0.88	0.91	0.77	0.79	0.73	0.74	0.79	0.85	0.73	0.75	0.62	0.67	0.73	0.75	M0.d
			1	0.89	0.76	0.78	0.79	0.79	0.93	0.89	0.71	0.76	0.72	0.72	0.79	0.83	0.69	0.71	0.6	0.65	0.72	0.7	M0.c
		1	0.85	0.85	0.83	0.85	0.84	0.85	0.85	0.84	0.77	0.81	0.76	0.77	0.71	0.78	0.74	0.76	0.66	0.7	0.66	0.69	M0.b
	1	0.83	0.75	0.81	0.88	0.87	0.83	0.83	0.78	0.8	0.83	0.84	0.75	0.8	0.7	0.77	0.79	0.81	0.7	0.73	0.67	0.72	H0.b
1	0.88	0.87	0.77	0.83	0.88	0.88	0.85	0.85	0.79	0.81	0.87	0.87	0.8	0.82	0.74	0.78	0.81	0.83	0.74	0.78	0.71	0.75	H0.a

Figure S2 Spearmans correlation matrix of hESC directed chondrogenesis. Spearman's correlation matrix comparing the read counts per million (cpm) of each gene for all mRNA libraries (A) and miRNA libraries (B). Each value refers to the Spearman's correlation coefficient (rho) between the two corresponding samples.

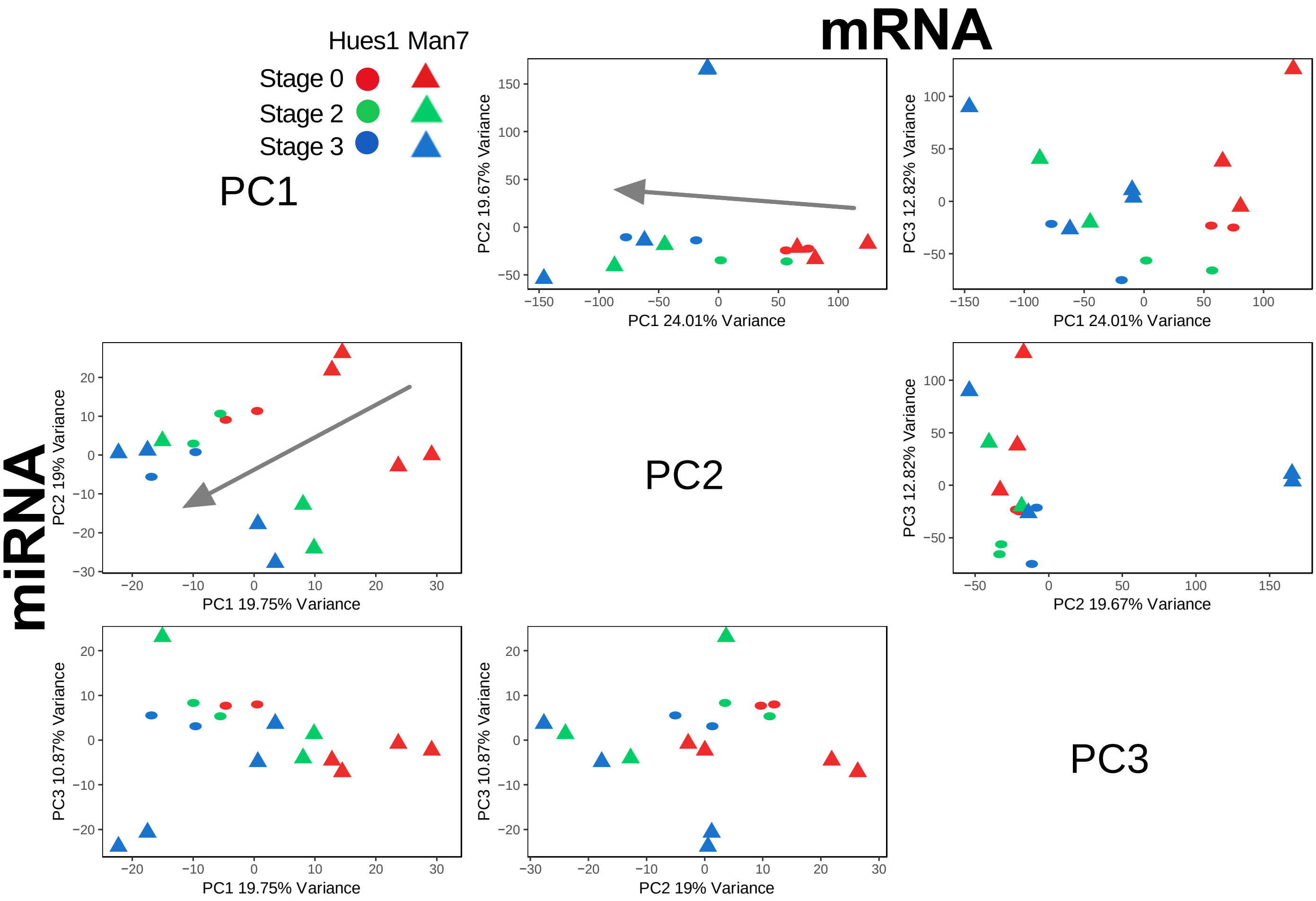


Figure S3 X-Y scatter graphs of PCA scores for top three components (PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3) for microRNA (bottom, left) and mRNA (top, right) log transformed read counts. Samples separate out by stage of the protocol as indicated by the arrows. Points are colour coded by stage of the DDP. PC, principal component.

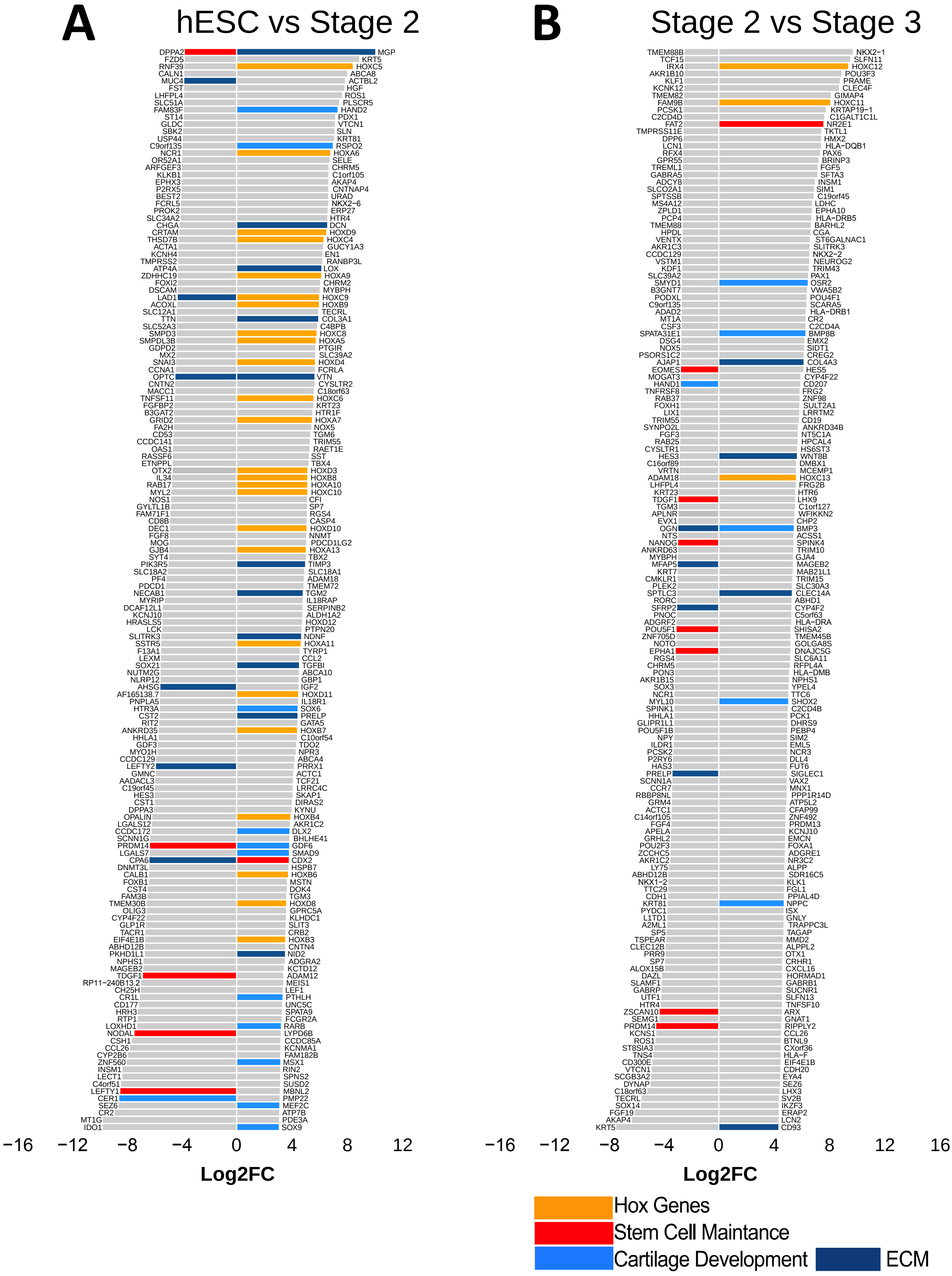


Figure S4 Differential expression analysis between stage 2 and hESCs (A) or Stage 3 (B). Top 150 up-regulated and 150 down-regulated transcripts (Pval<0.05). Some bars are highlighted by function identified from literature.

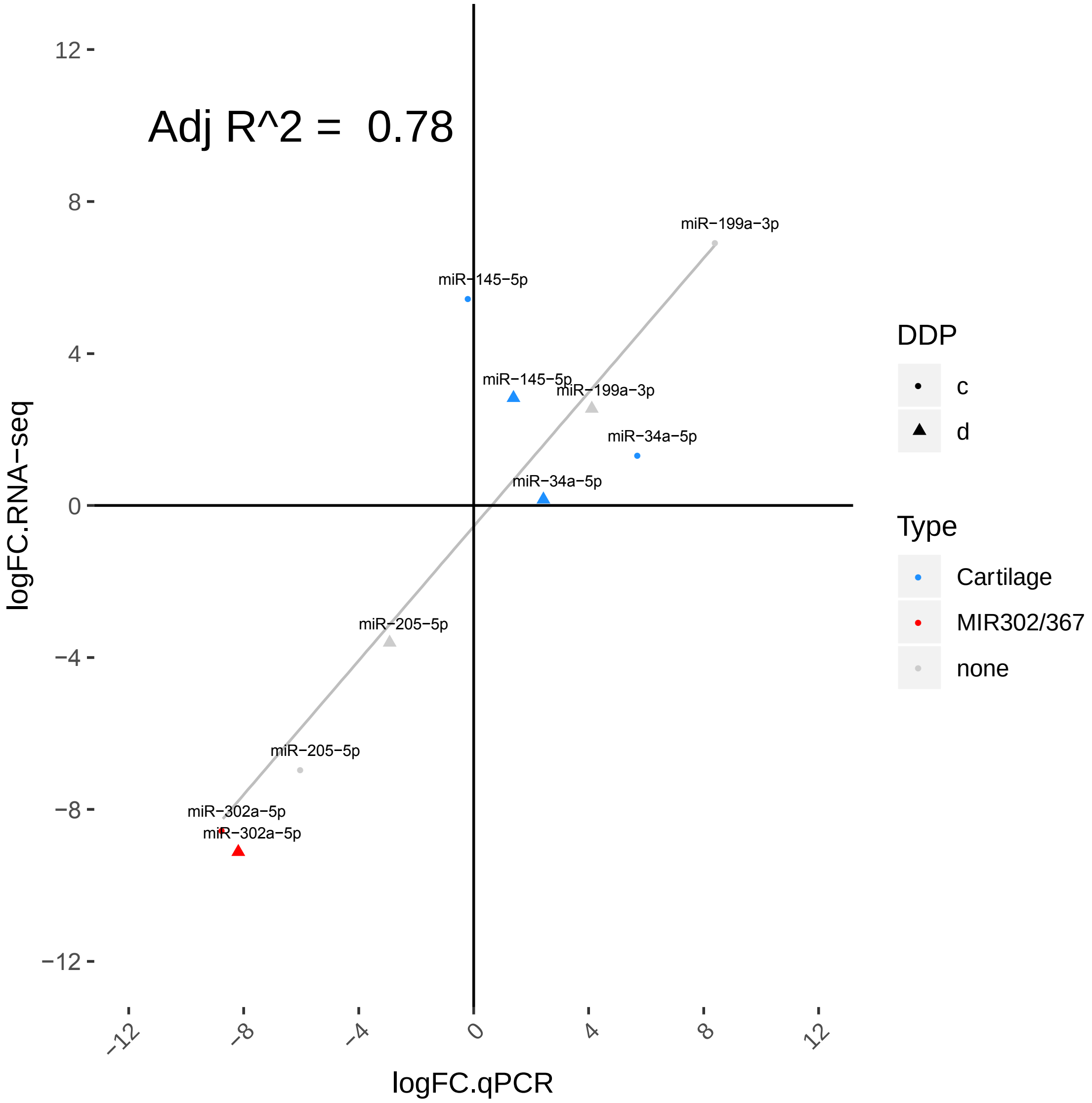


Figure S5 Comparison of qPCR and RNAseq. log2FC values of miRNAs between stage 0 and stage 3 determined by qPCR and RNAseq.

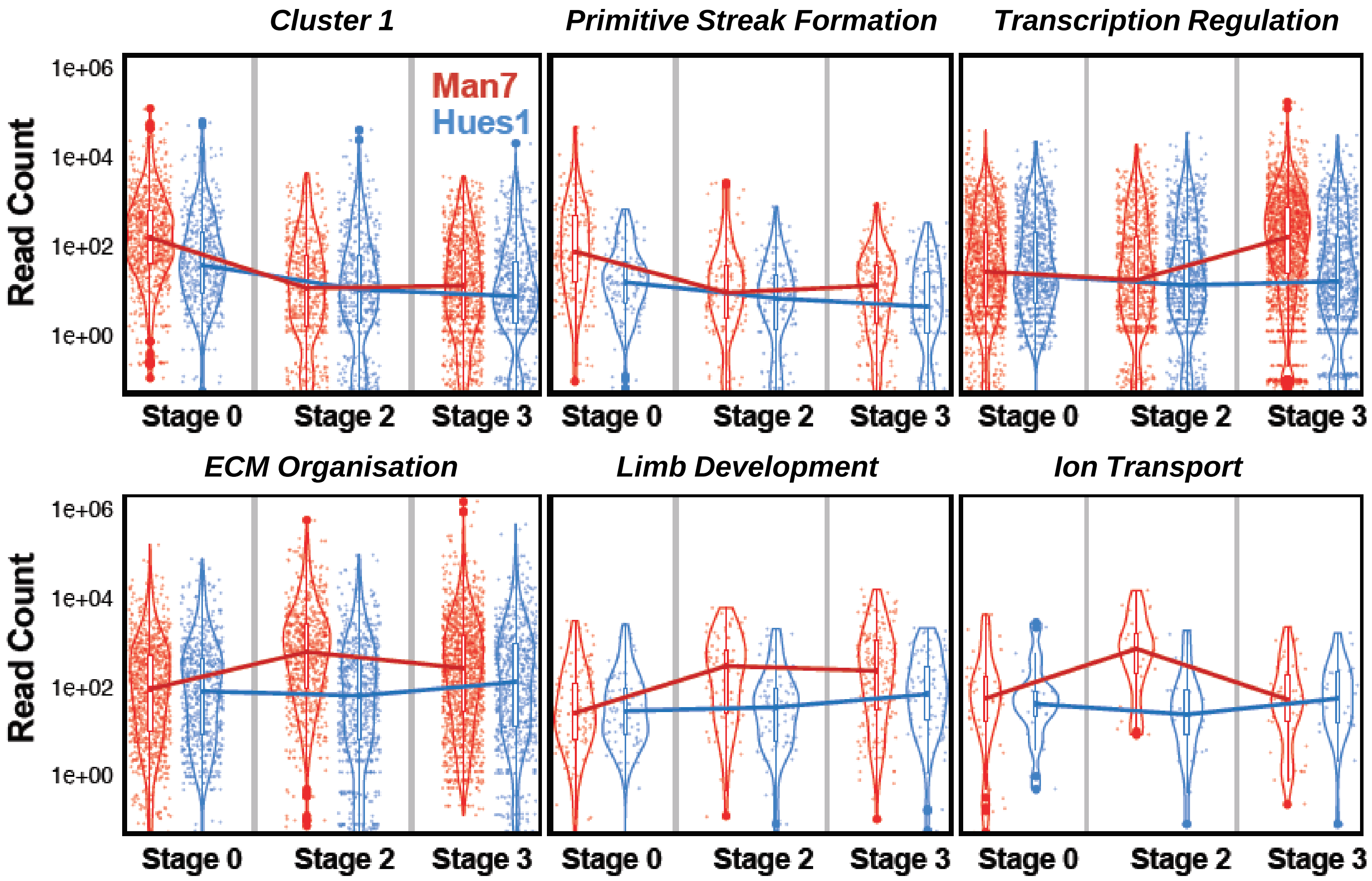


Figure S6 Expression profiles of co-expression clusters generated by BioLayout (Figure 5A). Violin plots of the average read count of each gene/microRNA for all biological replicates (y-axis) in each stage (x-axis). Each cluster is named according to Gene Ontology analysis of genes within the cluster.