Supplementary material

Profiling the circulating miRnome reveals a temporal regulation of the bone injury response

Andreia M. Silva, Maria I. Almeida, José H. Teixeira, Cristina Ivan, Joana Oliveira, Daniel Vasconcelos, Nuno Neves, Cláudia Ribeiro-Machado, Carla Cunha, Mário A. Barbosa, George A. Calin, Susana G. Santos

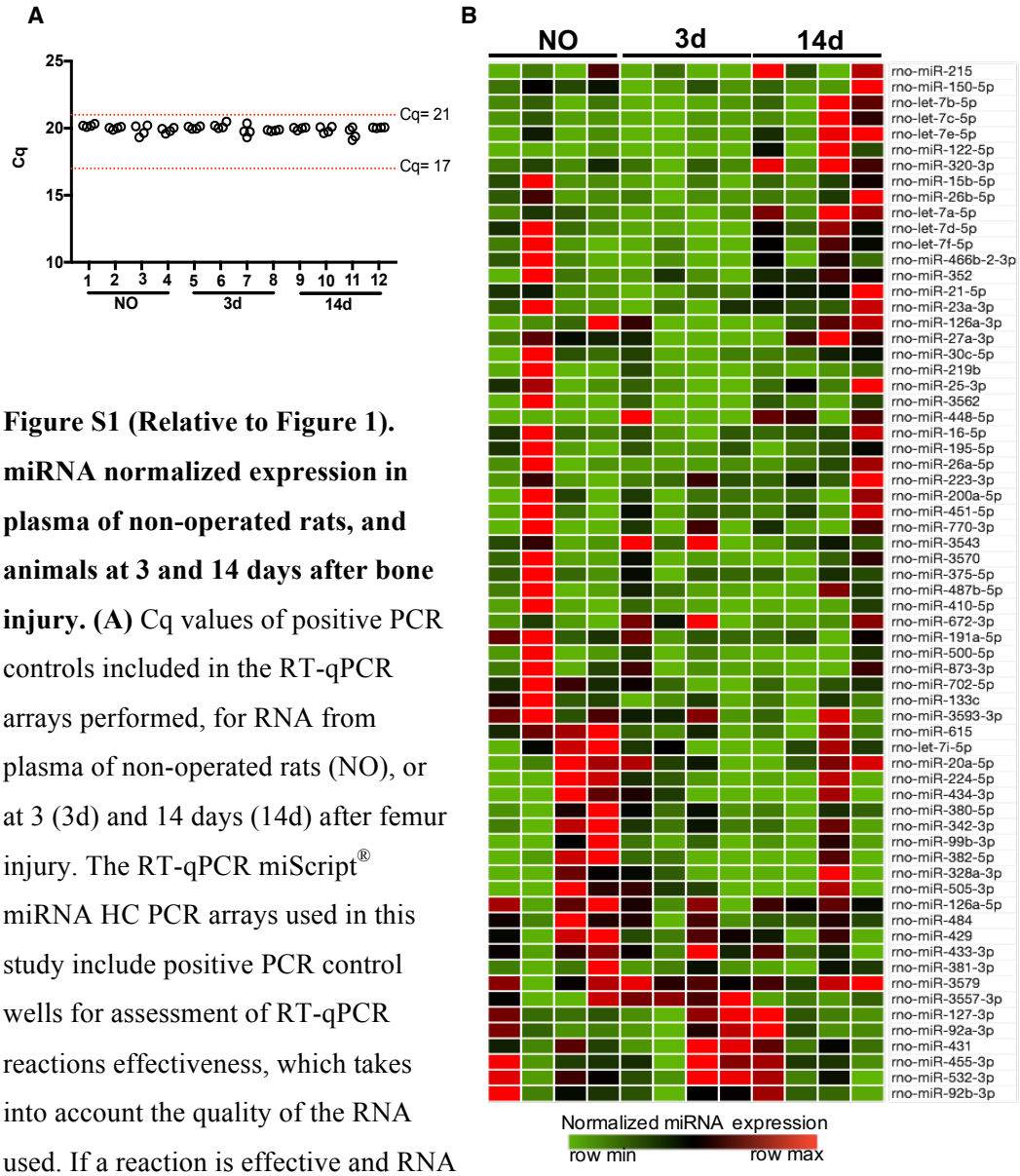

Figure S1 (Relative to Figure 1). miRNA normalized expression in plasma of non-operated rats, and animals at 3 and 14 days after bone injury. (A) Cq values of positive PCR controls included in the RT-qPCR arrays performed, for RNA from plasma of non-operated rats (NO), or at 3 (3d) and 14 days (14d) after femur injury. The RT-qPCR miScript® miRNA HC PCR arrays used in this study include positive PCR control wells for assessment of RT-qPCR reactions effectiveness, which takes into account the quality of the RNA used. If a reaction is effective and RNA is of high quality, the Cq values for these wells should range between 17<Cq<21, indicated by dashed red lines in the graph. Cq values are presented for all the samples included in this study, and each symbol represents a technical replicate. (**B)** Heat map of miRNA expression levels, normalized to endogenous and exogenous miRNA controls, for each animal included in the experimental groups (each column represents a different animal). In each row, light red represents the highest $2^{-\Delta Cq}$ value for that miRNA, and light green the lowest $2^{-\Delta Cq}$.

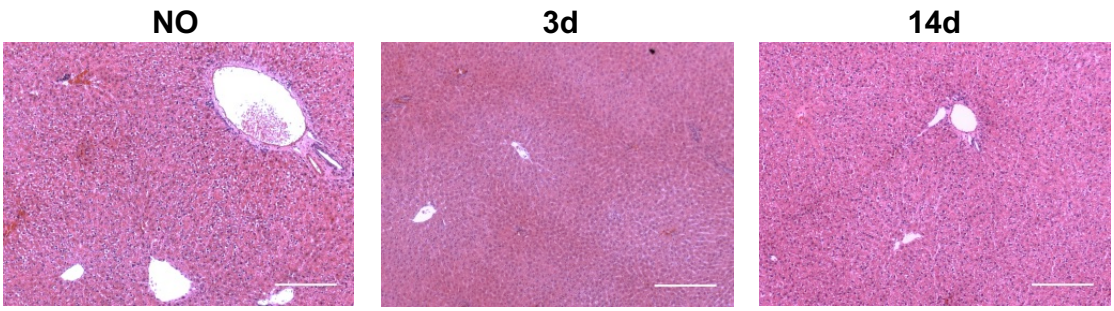

Figure S2 (Relative to Table 1). Histological analysis of rat liver does not reveal any tissue alteration upon bone injury. Representative micrographs of haematoxylin and eosin staining of liver sections from non-operated (NO) rats, and from rats at 3 days (3d) and 14 days (14d) after bone lesion (NO, 3d and 14d: 6 animals per group). Scale bar: 250 µm.

Table S1 (Relative to Figure 2). List of miRNAs up-regulated upon injury as determined by Venn diagram analysis. miRNA expression fold-change at 3 days (3d) and 14 days (14d) after injury was calculated relative to non-operated (NO) control animals, and miRNAs with a fold-change ≥1.5 were compared for both timepoints post-injury (NO, 3d and 14d: 4 animals per group). Specific miRNAs up-regulated only at day 3 (relative to NO), maintained up-regulated both at 3 days and 14 days (relative to NO), and up-regulated only at day 14 (relative to NO) are indicated. Relevant miRNAs, discussed in our analysis are highlighted in bold.

miRNAs up-regulated at 3d versus 14d		
Up-regulated at 3d only (relative to NO)	Up-regulated at 3d and 14d (relative to NO)	Up-regulated at 14d only (relative to NO)
rno-miR-3543	rno-miR-448-5p	**rno-let-7a-5p**
rno-miR-3557-3p	rno-miR-672-3p	**rno-let-7b-5p**
		rno-let-7c-5p
		rno-let-7e-5p
		rno-miR-122-5p
		rno-miR-21-5p
		rno-miR-215
		rno-miR-223-3p
		rno-miR-320-3p
		rno-miR-352

Table S2 (Relative to Figure 2). List of miRNAs down-regulated upon injury as determined by Venn diagram analysis. miRNA expression fold-change at 3 days (3d) and 14 days (14d) after injury was calculated relative to non-operated (NO) control animals, and miRNAs with a fold-change ≤-1.5 were compared for both timepoints post-injury (NO, 3d and 14d: 4 animals per group). Specific miRNAs down-regulated only at 3d (relative to NO), maintained down-regulated both at 3 days and 14 days (relative to NO), and down-regulated only at 14 days (relative to NO) are indicated. Relevant miRNAs, discussed in our analysis are highlighted in bold.

miRNAs down-regulated at 3d versus 14d		
Down-regulated at 3d only (relative to NO)	Down-regulated at 3d and 14d (relative to NO)	Down-regulated at 14d only (relative to NO)
rno-let-7a-5p	rno-let-7i-5p	rno-miR-3557-3p
rno-let-7b-5p	rno-miR-133c	rno-miR-3593-3p
rno-let-7c-5p	rno-miR-191a-5p	rno-miR-380-5p
rno-let-7d-5p	**rno-miR-195-5p**	rno-miR-429
rno-let-7e-5p	rno-miR-219b	rno-miR-505-3p
rno-let-7f-5p	rno-miR-224-5p	
rno-miR-122-5p	rno-miR-342-3p	
rno-miR-126a-3p	rno-miR-375-5p	
rno-miR-150-5p	rno-miR-382-5p	
rno-miR-15b-5p	rno-miR-410-5p	
rno-miR-16-5p	rno-miR-434-3p	
rno-miR-200a-5p	rno-miR-484	
rno-miR-21-5p	rno-miR-500-5p	
rno-miR-215	rno-miR-615	
rno-miR-23a-3p	rno-miR-702-5p	
rno-miR-25-3p	rno-miR-873-3p	
rno-miR-26a-5p	rno-miR-99b-3p	
rno-miR-26b-5p		
rno-miR-27a-3p		
rno-miR-30c-5p		
rno-miR-320-3p		
rno-miR-328a-3p		
rno-miR-352		
rno-miR-3562		
rno-miR-3570		
rno-miR-381-3p		
rno-miR-466b-2-3p		
rno-miR-487b-5p		

Table S3 (Relative to Figure 2). List of miRNAs with opposite expression from day 3 to day 14 after injury. miRNA expression fold-change at 3 days (3d) and 14 days (14d) after injury was calculated relative to non-operated (NO) control animals, and miRNAs up-regulated (fold-chane ≥1.5) and down-regulated (fold-change ≤-1.5) compared for both timepoints post-injury (NO, 3d and 14d: 4 animals per group). Specific miRNAs down-regulated only at 3 days (relative to NO), down-regulated at 3 days but then up-regulated at 14 days (relative to NO), and up-regulated only at 14 days (relative to NO) are indicated. Relevant miRNAs, discussed in our analysis are highlighted in bold.

miRNAs down-regulated at 3d versus up-regulated at 14d

miRNAs down-regulated at 3d (relative to NO)	miRNAs down-regulated at 3d and up-regulated at 14d (relative to NO)	miRNAs up-regulated at 14d (relative to NO)
rno-let-7d-5p	**rno-let-7a-5p**	rno-miR-223-3p
rno-let-7f-5p	**rno-let-7b-5p**	rno-miR-448-5p
rno-let-7i-5p	**rno-let-7c-5p**	rno-miR-672-3p
rno-miR-126a-3p	**rno-let-7e-5p**	
rno-miR-133c	rno-miR-122-5p	
rno-miR-150-5p	**rno-miR-21-5p**	
rno-miR-15b-5p	rno-miR-215	
rno-miR-16-5p	rno-miR-320-3p	
rno-miR-191a-5p	rno-miR-352	
rno-miR-195-5p		
rno-miR-200a-5p		
rno-miR-219b		
rno-miR-224-5p		
rno-miR-23a-3p		
rno-miR-25-3p		
rno-miR-26a-5p		
rno-miR-26b-5p		
rno-miR-27a-3p		
rno-miR-30c-5p		
rno-miR-328a-3p		
rno-miR-342-3p		
rno-miR-3562		
rno-miR-3570		
rno-miR-375-5p		
rno-miR-381-3p		
rno-miR-382-5p		
rno-miR-410-5p		
rno-miR-434-3p		
rno-miR-466b-2-3p		
rno-miR-484		
rno-miR-487b-5p		
rno-miR-500-5p		
rno-miR-615		
rno-miR-702-5p		
rno-miR-873-3p		
rno-miR-99b-3p		