# Figures and figure supplements

SMA-miRs (miR-181a-5p, -324-5p, and -451a) are overexpressed in spinal muscular atrophy skeletal muscle and serum samples

**Emanuela Abiusi *et al***

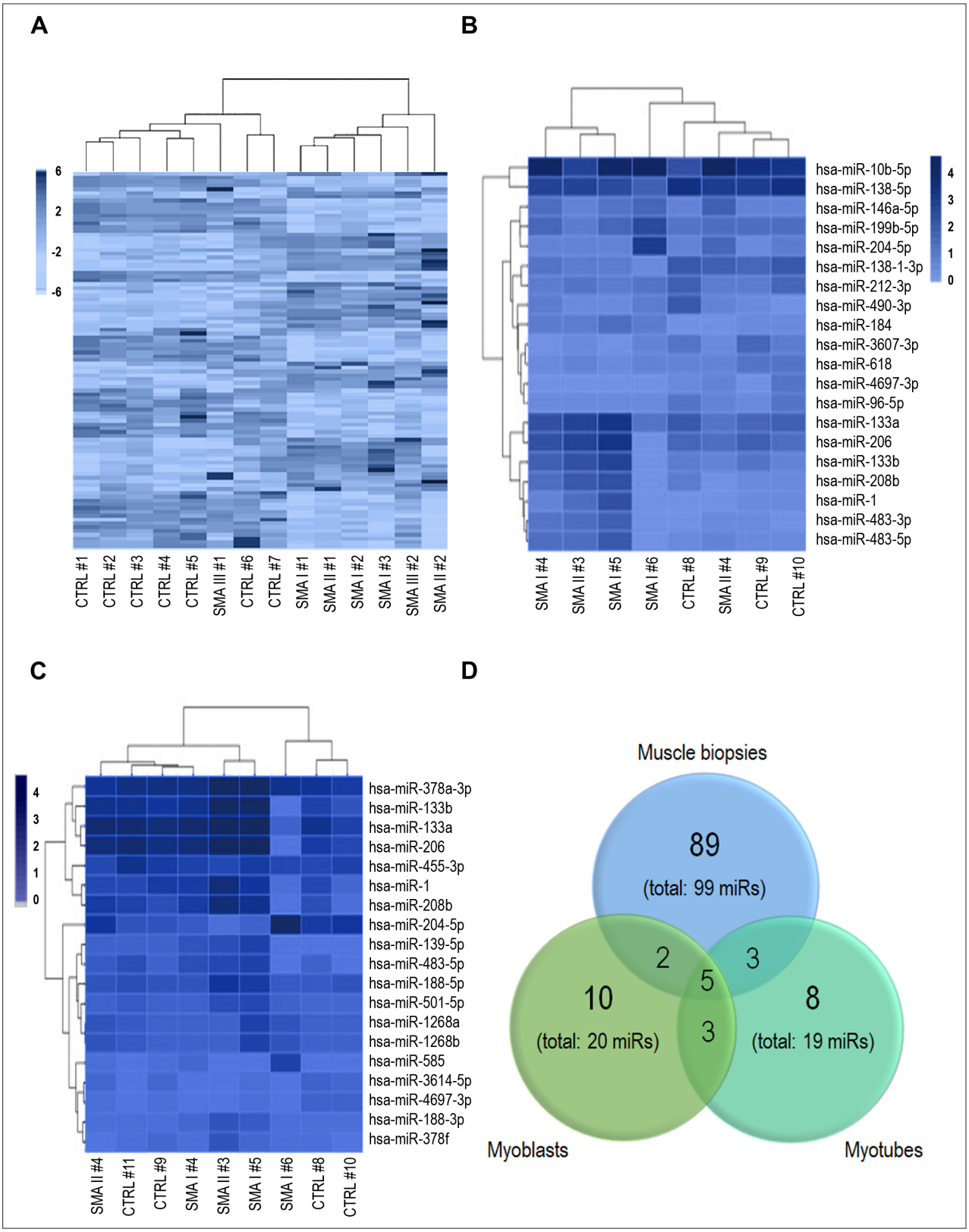



**Figure 1.** Heatmaps obtained by the whole miRNome analysis of muscle biopsies (**A**), myoblasts (**B**), and myotubes (**C**) of spinal muscular atrophy (SMA) patients and controls; patient and control samples display a separate clusterization. 99, 20, and 19 miRs were found deregulated in SMA in muscle biopsies, myoblasts, and myotubes, respectively; (**D**) Venn's diagram showing the five miRNAs shared among the three groups, three between myoblasts and myotubes, two between myoblasts and biopsies, and three between myotubes and biopsies.

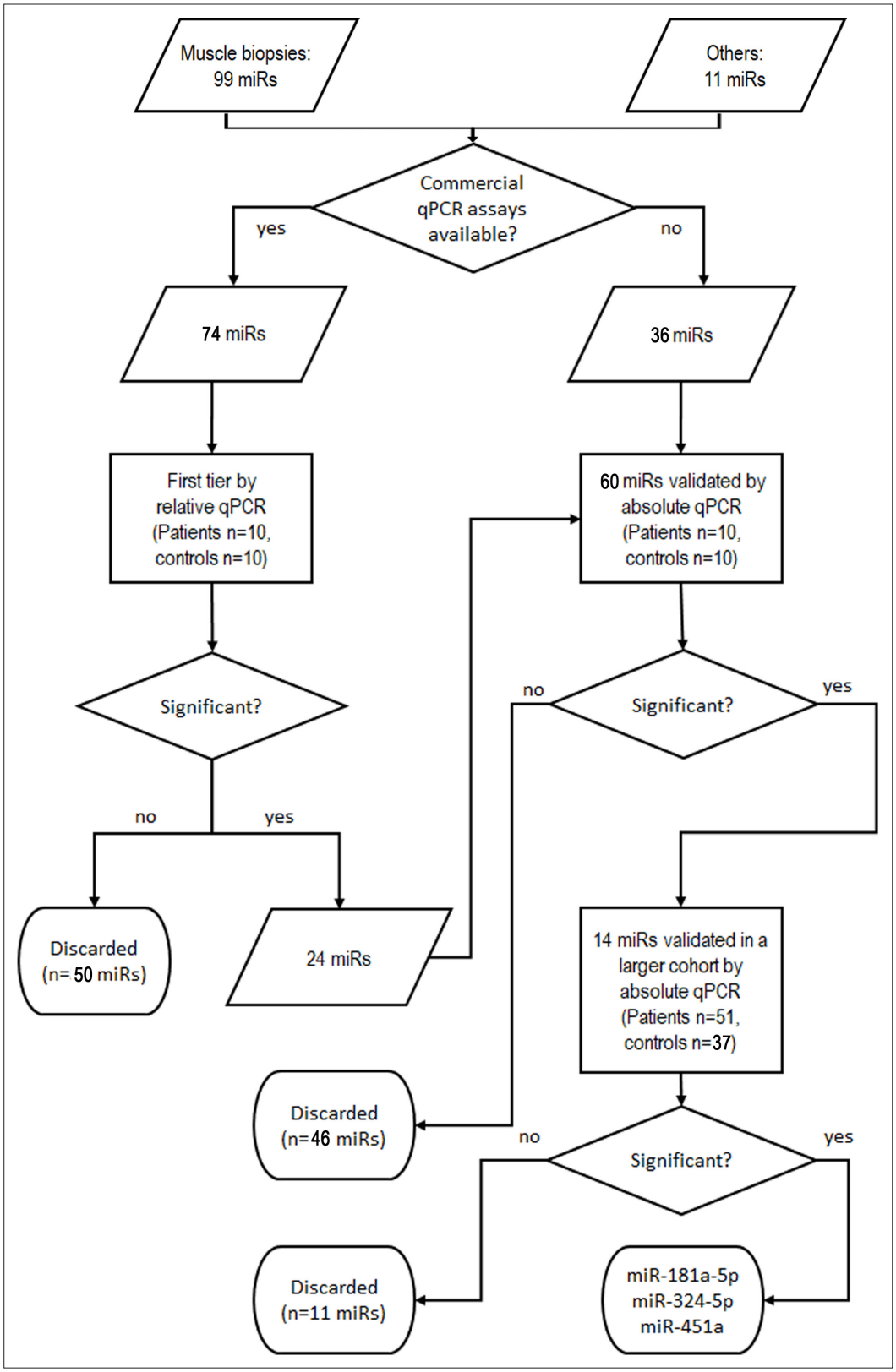



**Figure 2.** Validation pipeline of miRNAs identified by whole miRNome analysis in serum samples of patients and controls. 'Others' indicates miRs that were identified in other studies or with key function in skeletal muscle.

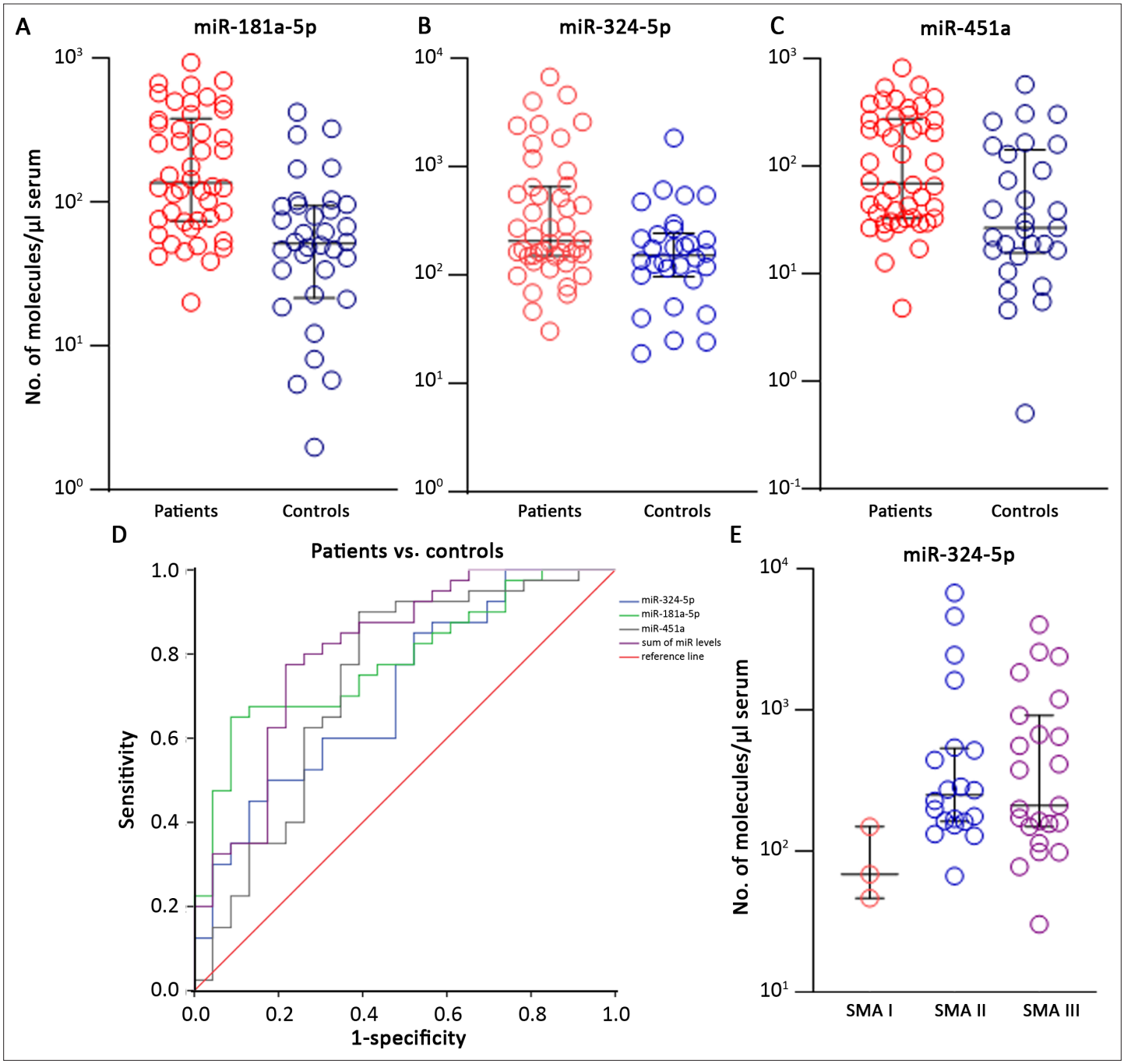
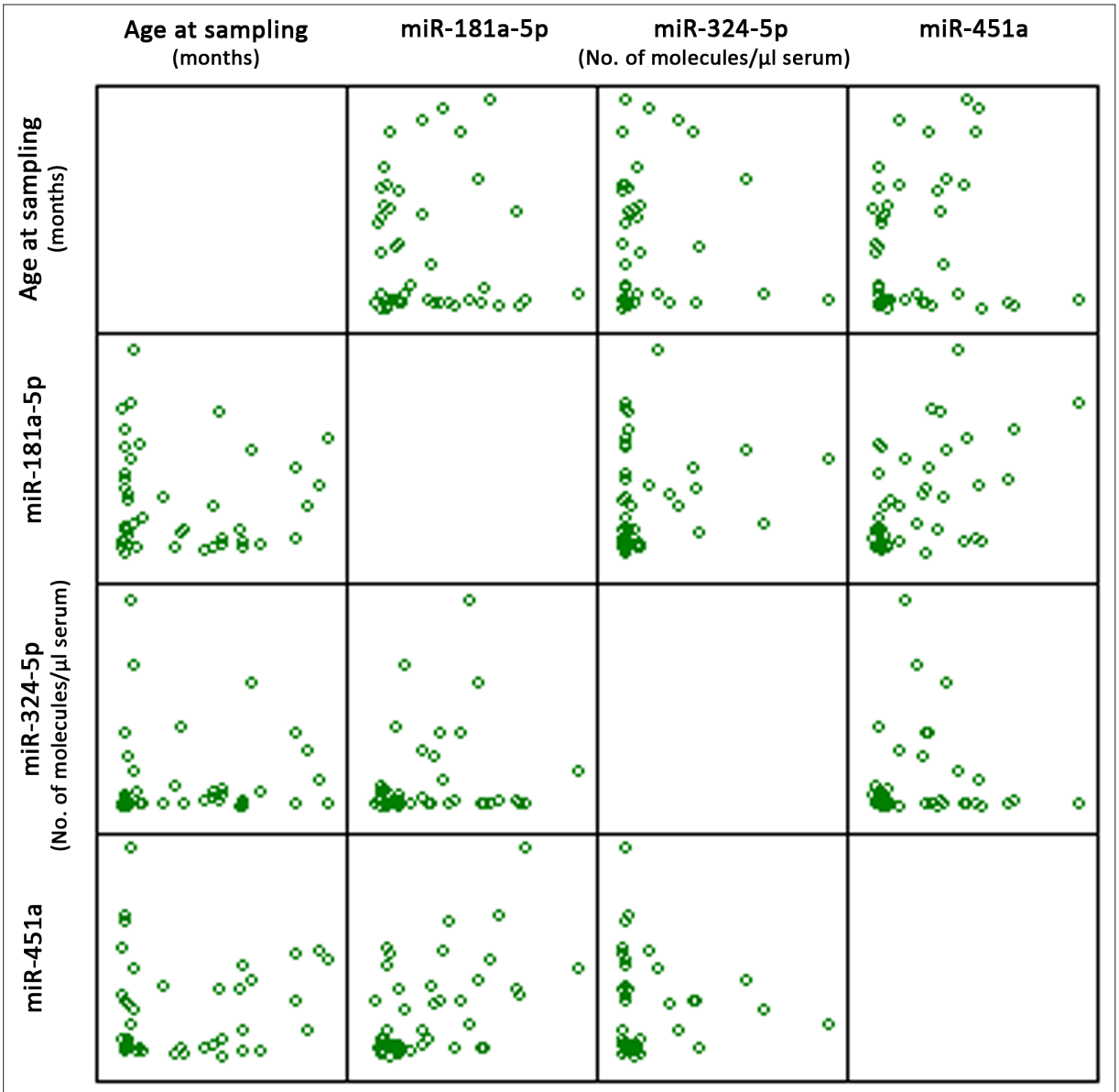



**Figure 3.** The SMA-miRs (miR-181a-5p [**A**], miR-324-5p [**B**] and miR-451a [**C**]) were significantly upregulated in serum samples of spinal muscular atrophy (SMA) patients ($p=4.3 * 10^{-4}$; 0.02; 0.004, respectively). Receiver operating characteristic (ROC) curves showed that the quantification of SMA-miRs has 80% sensitivity and 75% specificity in distinguishing patients from controls (**D**). Correlation of miR-324-5p with SMA type (**E**): the levels in SMA II and SMA III patients were significantly increased compared to those of SMA I patients (p=0.03 and 0.04, respectively).




| | | Age at sampling | miR-181a-5p | miR-324-5p | miR-451a |
|---|---|---|---|---|---|
| Correlation coefficient | Age at sampling | | -0,0697 | 0,0372 | 0,1095 |
| Sample size | | | 46 | 46 | 46 |
| *p-value* | | | 0,6452 | 0,8059 | 0,4688 |
| Correlation coefficient | miR-181a-5p | -0,0697 | | 0,1749 | 0,5247 |
| Sample size | | 46 | | 46 | 46 |
| *p-value* | | 0,6452 | | 0,2450 | 0,0002 |
| Correlation coefficient | miR-324-5p | 0,0372 | 0,1749 | | 0,0011 |
| Sample size | | 46 | 46 | | 46 |
| *p-value* | | 0,8059 | 0,2450 | | 0,9942 |
| Correlation coefficient | miR-451a | 0,1095 | 0,5247 | 0,0011 | |
| Sample size | | 46 | 46 | 46 | |
| *p-value* | | 0,4688 | 0,0002 | 0,9942 | |

**Figure 3—figure supplement 1.** Multiple variable correlation of miR-181a-5p, -324-5p, and -415a levels and age at sampling. In red, the only significant correlation, between miR-181a-5p and miR-451a (p=0.0002).

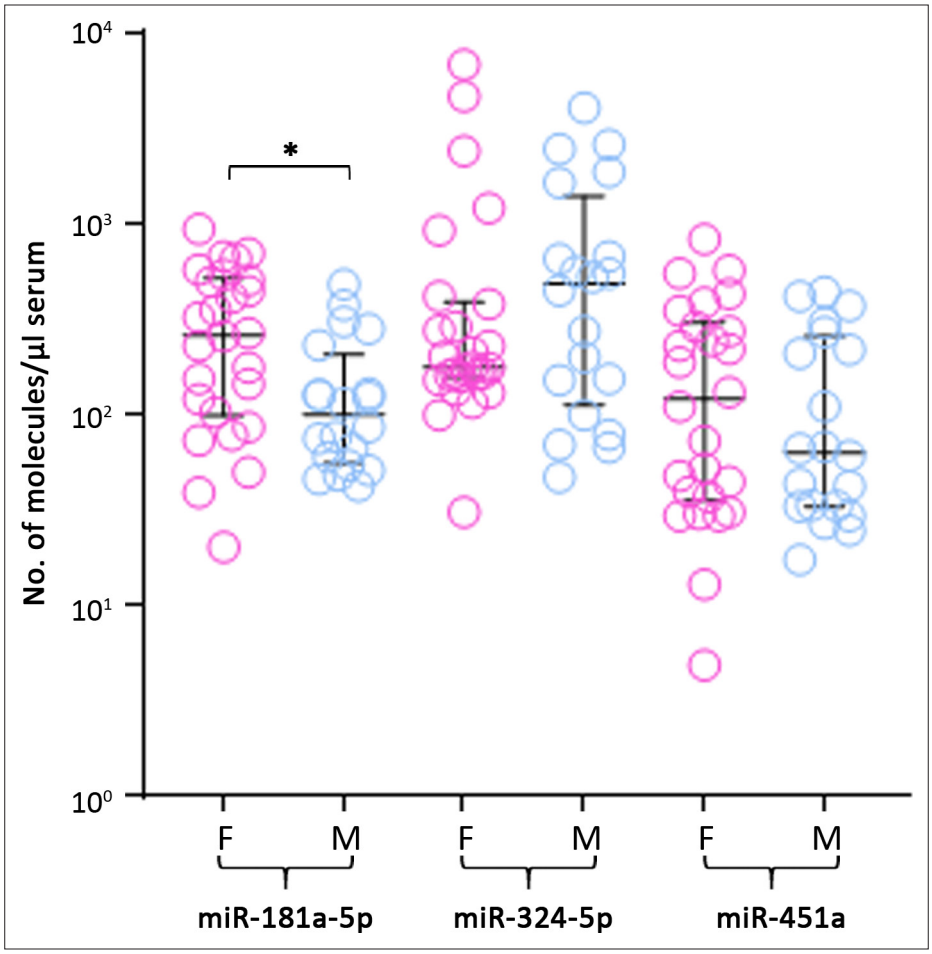



**Figure 3—figure supplement 2.** Comparison of levels of miR-181a-5p, -324-5p, and -451a in male and female patients; only miR-181a-5p showed a significant difference in females compared to males (*p=0.024).

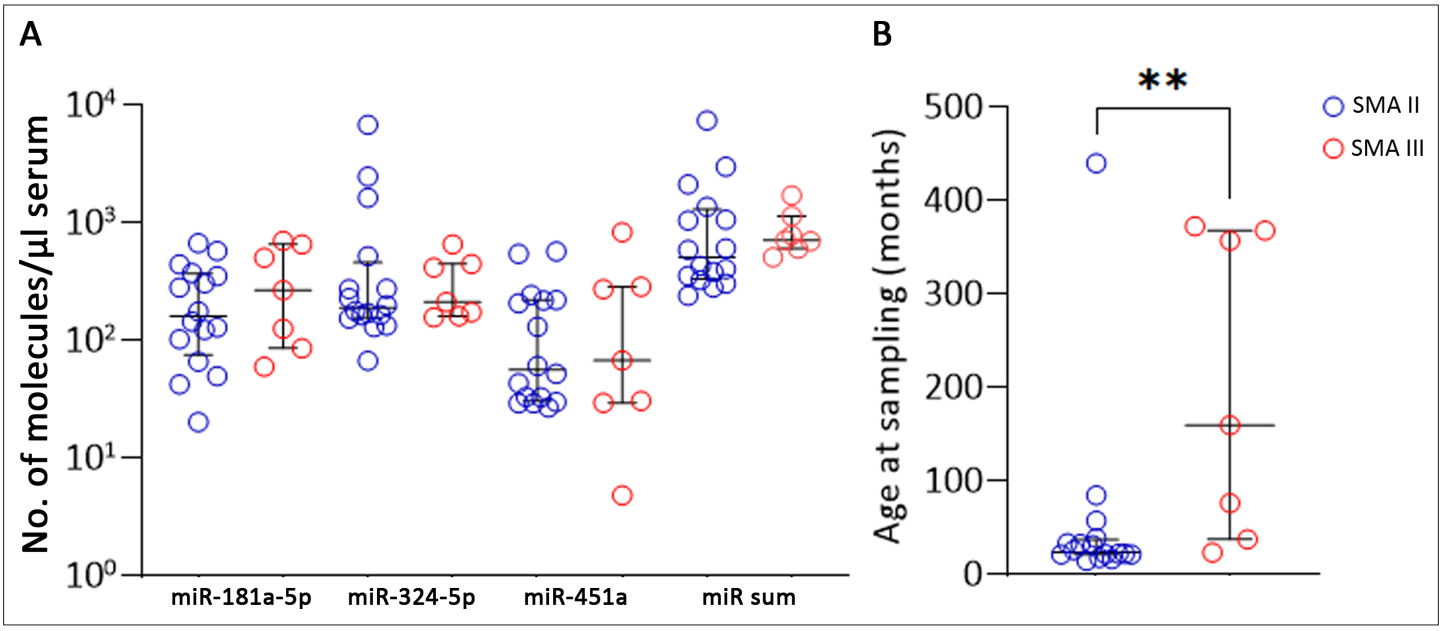



**Figure 3—figure supplement 3.** Analysis of type II and III patients with three *SMN2* copies; the two groups were not different for miRs levels (p>0.05, **A**) but showed a significant difference in age (**p=0.0092, **B**).

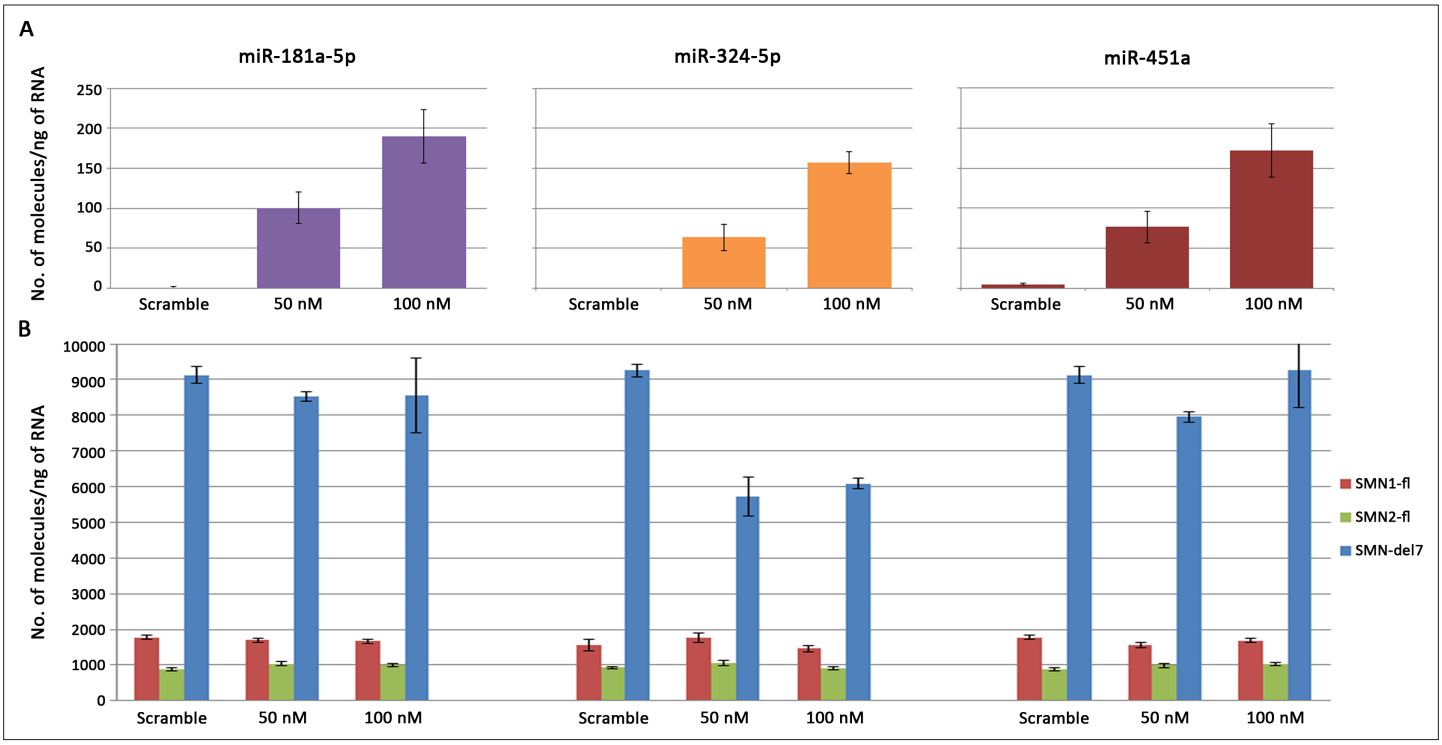



**Figure 3—figure supplement 4.** Transfections of SH-SY5Y neuroblastoma cells with SMA-miR mimics (final concentration: 50 or 100 nM). In spite of the huge increase in SMA-miR levels (**A**), *SMN1*/*SMN2* transcripts remained unchanged, except for the *SMNΔ7* isoform in cells treated with miR-324-5p , which was reduced by 50%, independently of the mimic concentration (**B**).

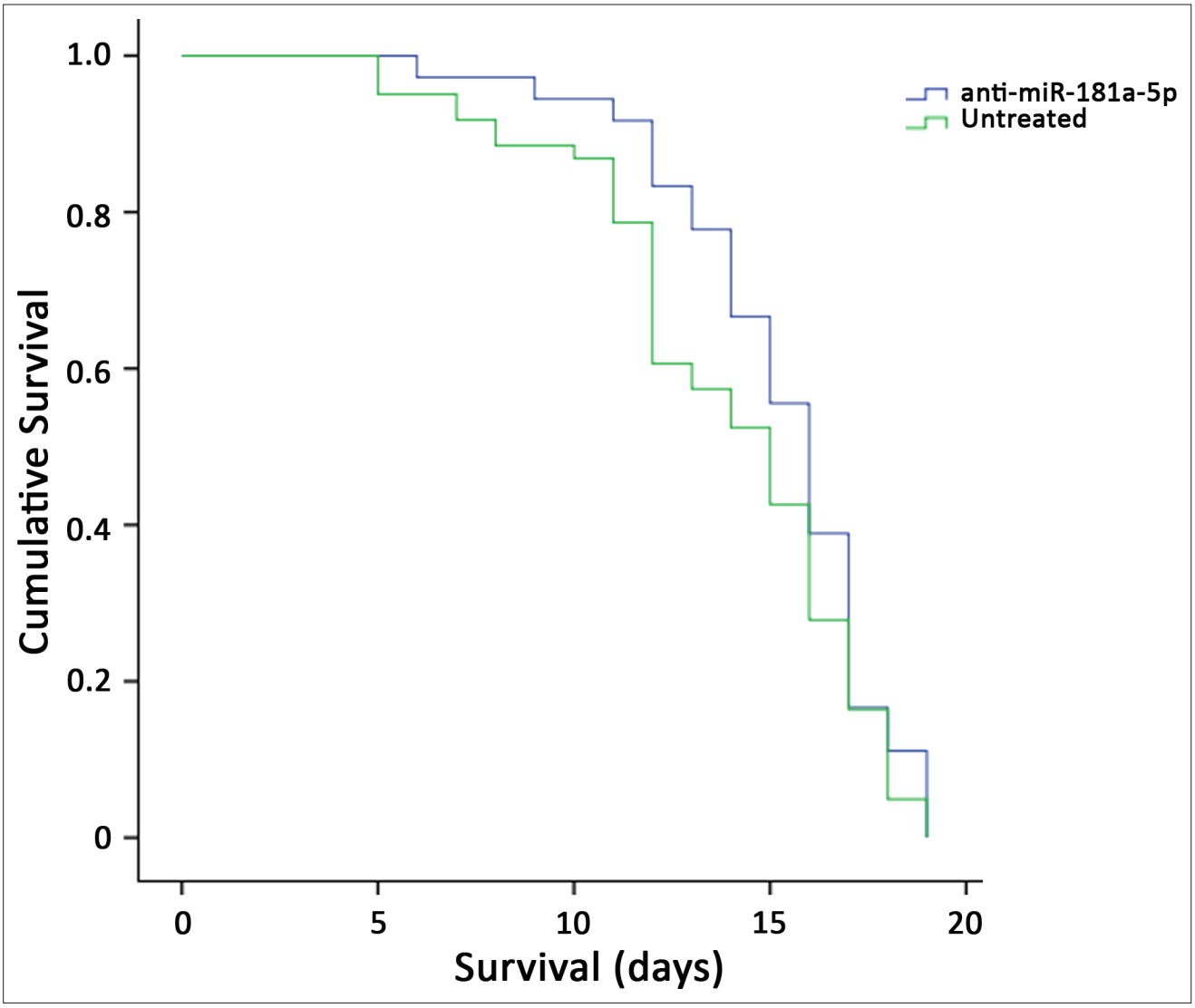



**Figure 4.** Survival curves of SMNΔ7-mice treated with intrathecal injection of anti-miR-181a-5p (n = 36) and untreated (n = 71); the overall survival remained unchanged (p>0.05).

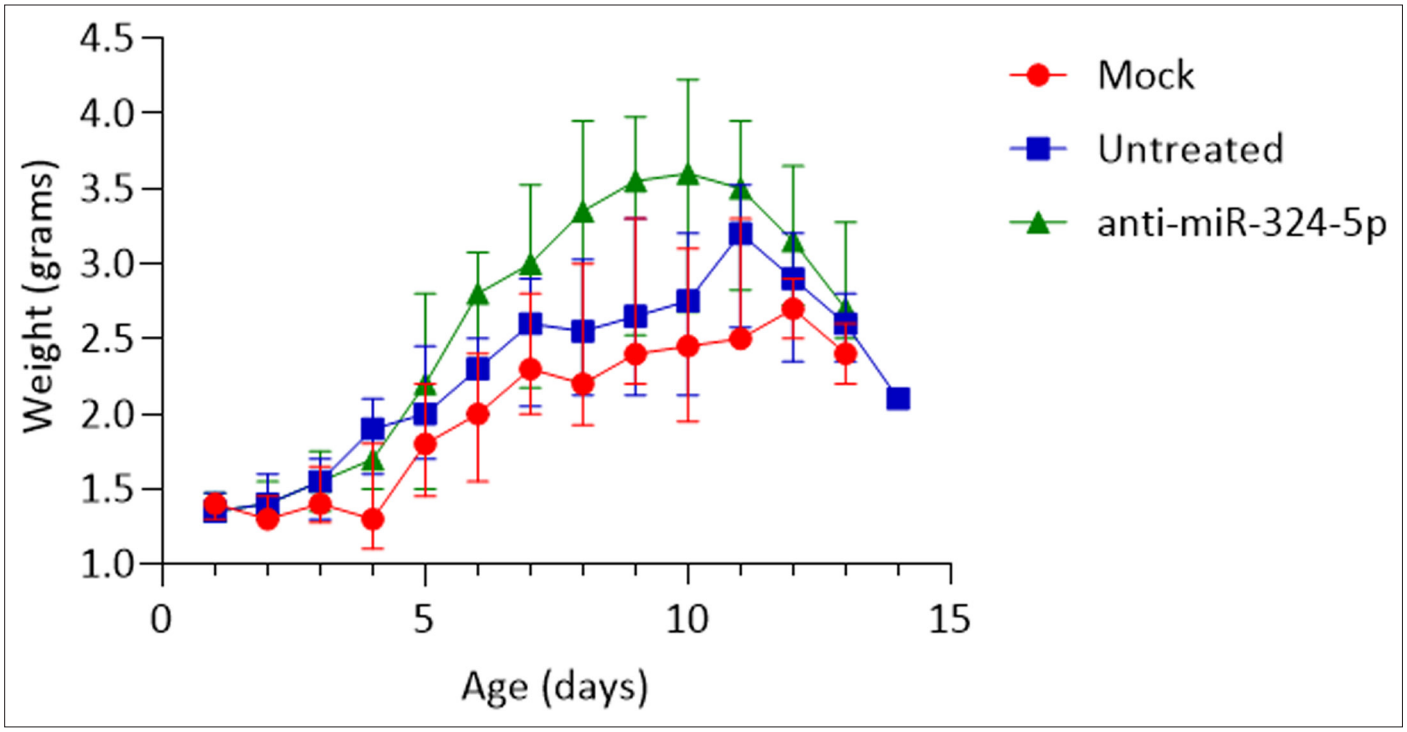



**Figure 4—figure supplement 1.** SMNΔ7 mice treated with anti-miR-324-5p showed a significant transient increase in body weight, between P7 and P10 (p=0.002).

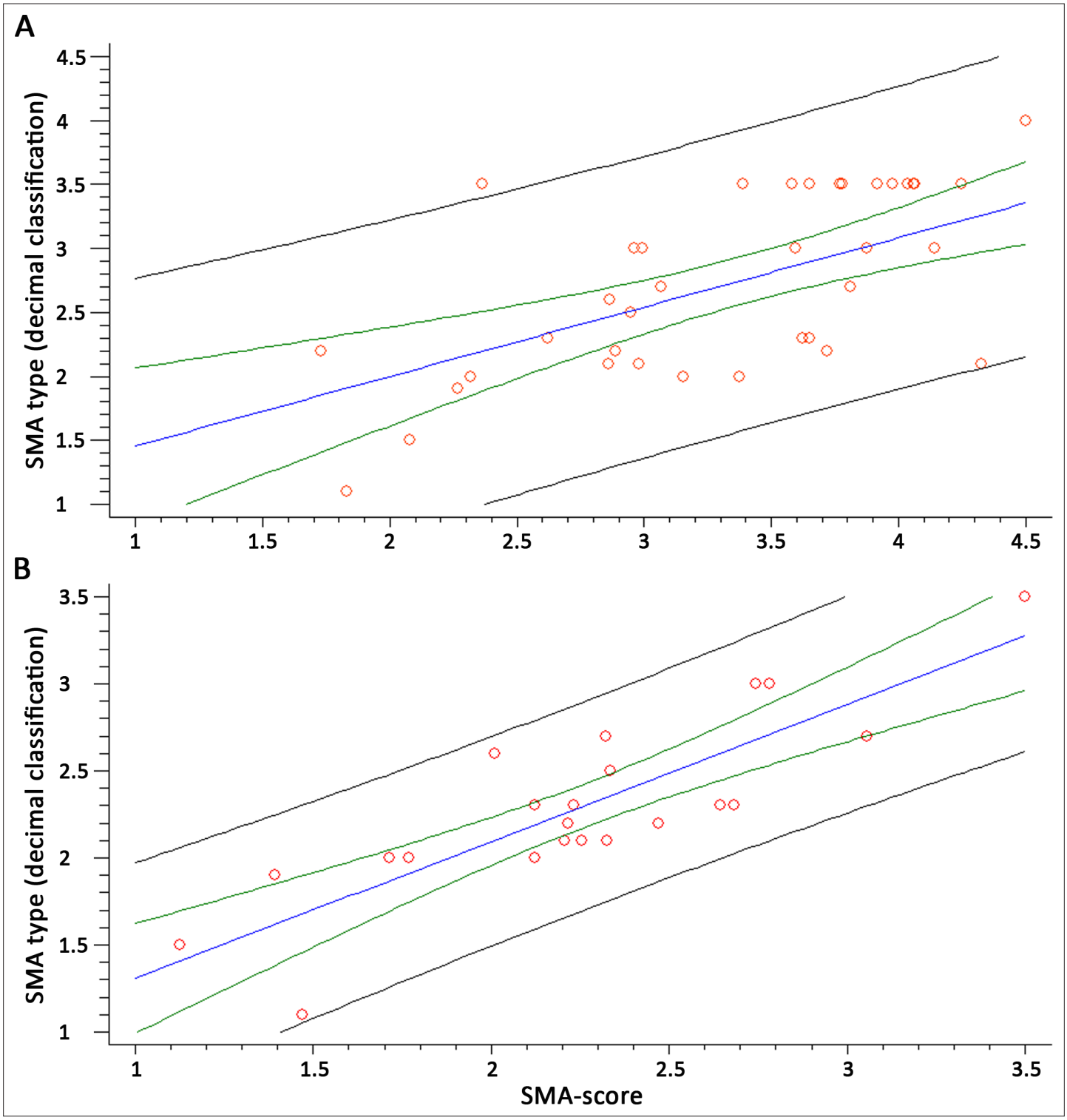



**Figure 5.** The spinal muscular atrophy score (SMA-score) predicts the phenotypic severity in SMA patients. Correlation between the SMA-score and the clinical decimal SMA subtype in the whole cohort (**A**) and aged ≤6 years (**B**). Red circles are individual samples, the blue line indicates the expected distribution, the green line indicates the 95% confidence interval, and the black lines are the prediction interval.

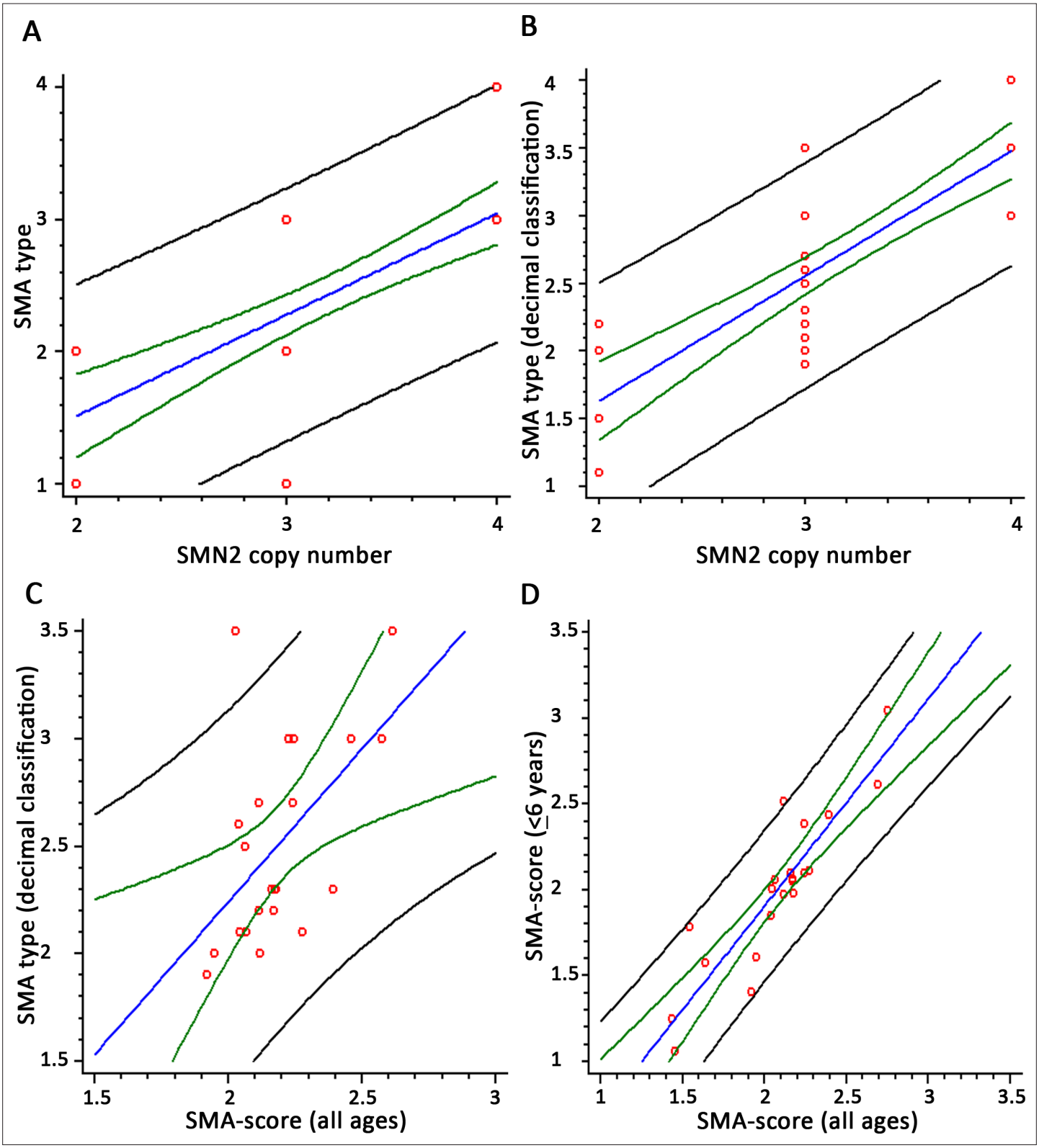



**Figure 5—figure supplement 1.** Linear correlation analysis among *SMN2* copy number and spinal muscular atrophy (SMA) type, estimated by the standard classification (**A**; $R^2$ = 52.45%, n = 41, $p<10^{-5}$) and the decimal classification (**B**; $R^2$ = 67.04%, n = 39, $p<10^{-5}$). Correlation with SMA-score and SMA type estimated by the decimal classification in patients with three *SMN2* copies (**C**; $R^2$ = 30.04, n = 21, p=0.008). Linear correlation analysis among SMA-scores obtained with the two equations (all ages vs. <6 years) (**D**; $R^2$ = 80.31, n = 21, $p<10^{-5}$). Red circles are individual samples, the blue line indicates the expected distribution, the green line indicate the 95% confidence interval, and the black lines are the prediction interval.