

**SUPPLEMENTARY FIGURES**

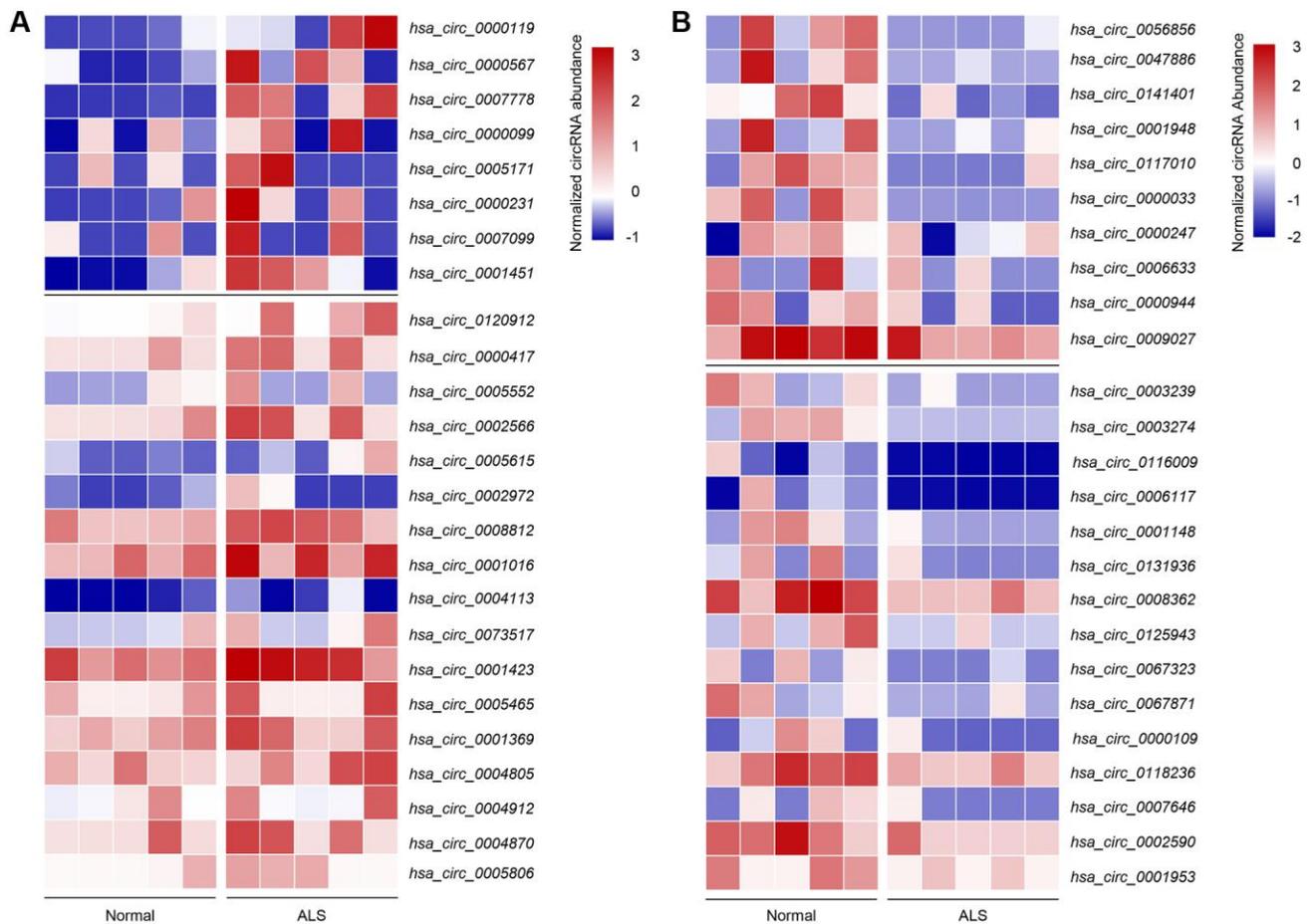
**A**

circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change
hsa_circ_0007778	5.179	hsa_circ_0001369	1.983	hsa_circ_0001900	1.455	hsa_circ_0096442	1.087	hsa_circ_0008732	0.827	hsa_circ_0134651	0.559	hsa_circ_0003253	0.193
hsa_circ_0000119	4.824	hsa_circ_0000099	1.943	hsa_circ_0084615	1.427	hsa_circ_0001246	1.063	hsa_circ_0001788	0.810	hsa_circ_0006717	0.555	hsa_circ_0001727	0.188
hsa_circ_0120912	4.497	chr2:233597263-233599401	1.912	hsa_circ_0000835	1.414	hsa_circ_0024605	1.050	hsa_circ_0000437	0.805	hsa_circ_0008648	0.554	hsa_circ_0000024	0.180
hsa_circ_0000417	3.788	hsa_circ_0004805	1.904	hsa_circ_0004471	1.403	chr4:169432573-169433563	1.035	hsa_circ_0001982	0.774	hsa_circ_0000880	0.544	hsa_circ_0006434	0.179
hsa_circ_0005552	3.532	hsa_circ_0004912	1.852	hsa_circ_0007385	1.398	hsa_circ_0008319	1.032	chr12:112757028-112757546	0.763	hsa_circ_0000745	0.530	hsa_circ_0008114	0.158
hsa_circ_0000567	3.485	chr12:57637893-57637870	1.812	hsa_circ_0000896	1.389	hsa_circ_0105987	1.015	hsa_circ_0001772	0.759	hsa_circ_0007444	0.522	chr6:54001512-54067031	0.140
hsa_circ_0001451	3.334	hsa_circ_0004870	1.808	hsa_circ_0002484	1.301	hsa_circ_0004276	1.011	hsa_circ_0007364	0.731	hsa_circ_0000591	0.483	hsa_circ_00080420	0.138
hsa_circ_0002566	3.058	hsa_circ_0005806	1.807	hsa_circ_0001236	1.278	hsa_circ_0004276	1.009	hsa_circ_0117628	0.726	hsa_circ_0003810	0.461	hsa_circ_0002457	0.136
hsa_circ_0005615	2.955	hsa_circ_0002538	1.762	hsa_circ_0001020	1.271	chr3:114069120-114070725	1.004	hsa_circ_0004058	0.723	chr16:85667519-85667738	0.455	hsa_circ_0000378	0.132
hsa_circ_0002972	2.908	hsa_circ_0007099	1.669	hsa_circ_0000711	1.271	hsa_circ_0006114	1.001	hsa_circ_0000754	0.661	hsa_circ_0001178	0.401	chr3:134327490-134346660	0.110
chr4:114376881-114421667	2.802	hsa_circ_0002245	1.635	hsa_circ_0007883	1.235	hsa_circ_0003624	0.999	hsa_circ_0001359	0.647	hsa_circ_0004212	0.398	hsa_circ_0001159	0.096
hsa_circ_0008812	2.525	chr6:123696749-123714822	1.597	hsa_circ_0001053	1.213	hsa_circ_0001017	0.979	hsa_circ_0001073	0.624	chr2:152403942-152410539	0.397	hsa_circ_0001173	0.076
hsa_circ_0001016	2.474	hsa_circ_0005018	1.566	chr15:63988322-64008672	1.202	hsa_circ_0001445	0.979	hsa_circ_0103069	0.623	hsa_circ_0006916	0.363	hsa_circ_0008832	0.076
hsa_circ_0004113	2.345	hsa_circ_0001439	1.562	hsa_circ_0005328	1.170	hsa_circ_0006665	0.975	hsa_circ_0103891	0.611	hsa_circ_0084606	0.360	hsa_circ_0001801	0.071
hsa_circ_0000231	2.311	hsa_circ_0000605	1.545	hsa_circ_0006629	1.126	hsa_circ_0000906	0.939	hsa_circ_0003922	0.602	hsa_circ_0001367	0.352	hsa_circ_0007039	0.054
hsa_circ_0073517	2.289	hsa_circ_0003270	1.524	hsa_circ_0000296	1.121	hsa_circ_0001329	0.923	hsa_circ_0011536	0.598	hsa_circ_0008103	0.343	hsa_circ_0001684	0.026
hsa_circ_0001423	2.214	hsa_circ_0008501	1.523	hsa_circ_0006501	1.098	chr10:126370175-126370948	0.887	hsa_circ_0000643	0.596	hsa_circ_0000384	0.275	hsa_circ_00018493	0.020
hsa_circ_0005465	2.041	hsa_circ_0001368	1.522	chr3:37132957-37138151	1.095	hsa_circ_0056280	0.853	hsa_circ_0132246	0.583	hsa_circ_0008494	0.197	hsa_circ_0000586	0.014
hsa_circ_0005171	1.994	hsa_circ_0002158	1.477	hsa_circ_0000441	1.093	hsa_circ_0009043	0.845	hsa_circ_0002468	0.572				

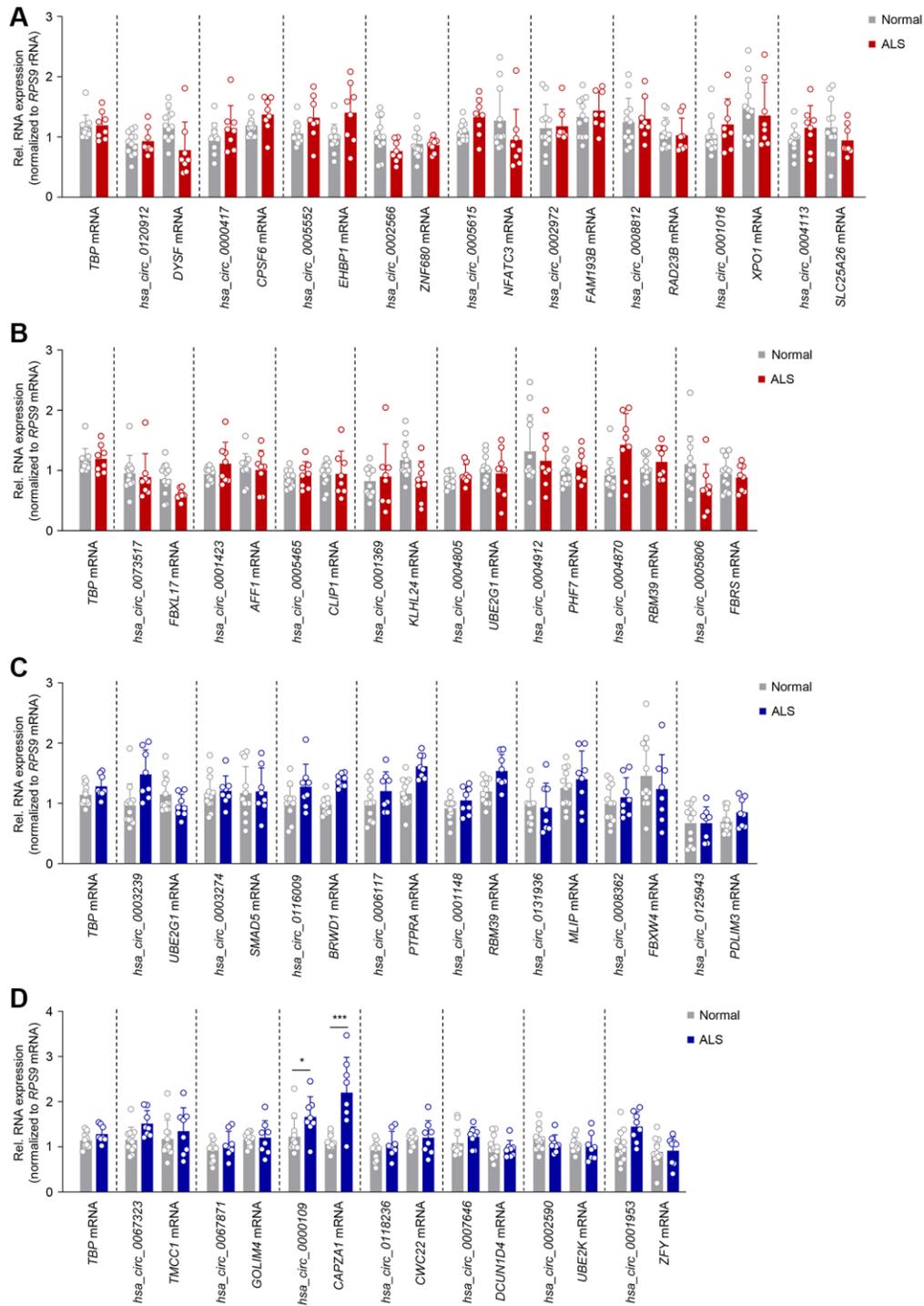
**B**

circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change	circRNA	Log2 fold change
hsa_circ_0000033	-5.490	hsa_circ_0007646	-2.740	hsa_circ_0000002	-1.542	hsa_circ_0008604	-1.067	hsa_circ_0000734	-0.778	hsa_circ_0001092	-0.5035	hsa_circ_0000075	-0.196
hsa_circ_0056856	-4.939	hsa_circ_0002590	-2.734	chr7:152007050-152012423	-1.523	hsa_circ_0000524	-1.051	hsa_circ_0007552	-0.765	hsa_circ_0006404	-0.44229	chr10:69902696-69918384	-0.189
hsa_circ_0003239	-4.637	hsa_circ_0000247	-2.718	hsa_circ_0004524	-1.515	hsa_circ_0000551	-1.035	hsa_circ_0016866	-0.727	chr13:42439871-42461497	-0.42862	hsa_circ_0005946	-0.185
hsa_circ_0003274	-4.559	hsa_circ_0001953	-2.524	chr13:42385360-42393522	-1.502	hsa_circ_0008521	-1.034	hsa_circ_0001333	-0.706	hsa_circ_0002163	-0.38156	hsa_circ_00072547	-0.175
hsa_circ_0116009	-4.433	hsa_circ_0006354	-2.476	hsa_circ_0001792	-1.483	hsa_circ_0001074	-1.018	hsa_circ_0000246	-0.677	hsa_circ_0000471	-0.37706	hsa_circ_0007695	-0.168
hsa_circ_0006117	-4.123	hsa_circ_0001948	-2.269	hsa_circ_0003441	-1.480	hsa_circ_0002387	-0.975	hsa_circ_0008297	-0.615	hsa_circ_0001030	-0.32747	hsa_circ_0003218	-0.162
hsa_circ_0047886	-4.088	hsa_circ_0040823	-2.149	hsa_circ_0078784	-1.473	hsa_circ_0003865	-0.957	chr3:157839891-157841780	-0.611	chr5:137219072-137219280	-0.29999	hsa_circ_0000569	-0.158
hsa_circ_0001148	-4.026	hsa_circ_0009027	-2.055	chr11:64525250-64526176	-1.448	hsa_circ_0001851	-0.941	hsa_circ_0001819	-0.594	hsa_circ_0008285	-0.29369	chr11:22856452-22859910	-0.155
hsa_circ_0131936	-3.981	hsa_circ_0000914	-2.039	hsa_circ_0000061	-1.422	hsa_circ_0003261	-0.928	hsa_circ_0009061	-0.585	hsa_circ_0002301	-0.29046	hsa_circ_0086414	-0.126
hsa_circ_0008362	-3.790	hsa_circ_0006633	-2.035	hsa_circ_0002490	-1.395	hsa_circ_0002398	-0.923	hsa_circ_0001654	-0.582	hsa_circ_0001360	-0.28341	hsa_circ_0004502	-0.118
hsa_circ_0125943	-3.545	chr11:1862138-1862238	-1.960	chr7:65592690-65599361	-1.366	hsa_circ_0001400	-0.907	hsa_circ_0117627	-0.542	hsa_circ_0063050	-0.27398	hsa_circ_0006156	-0.095
hsa_circ_0067323	-3.516	hsa_circ_0018168	-1.872	hsa_circ_0007904	-1.344	hsa_circ_0007367	-0.850	hsa_circ_0008193	-0.542	hsa_circ_0135761	-0.26594	hsa_circ_0002100	-0.061
hsa_circ_0067871	-3.487	hsa_circ_0001756	-1.828	hsa_circ_0002058	-1.218	hsa_circ_0001771	-0.847	hsa_circ_0003713	-0.539	hsa_circ_0105377	-0.26561	hsa_circ_0005087	-0.052
hsa_circ_0000109	-3.336	hsa_circ_0000944	-1.709	hsa_circ_0128535	-1.211	hsa_circ_0001776	-0.846	hsa_circ_0108763	-0.526	hsa_circ_0001380	-0.25838	hsa_circ_00050851	-0.049
hsa_circ_0141401	-3.269	hsa_circ_0000076	-1.680	chr6:123637601-123703292	-1.210	hsa_circ_0001498	-0.843	hsa_circ_0004846	-0.517	hsa_circ_0083220	-0.24715	chr5:149610867-149624764	-0.041
hsa_circ_0118236	-2.926	hsa_circ_0056019	-1.606	hsa_circ_0001136	-1.186	hsa_circ_0000043	-0.827	hsa_circ_0005993	-0.511	hsa_circ_0006107	-0.23091	hsa_circ_0000284	-0.018
hsa_circ_0011710	-2.849	hsa_circ_0008086	-1.568	hsa_circ_0005600	-1.161	hsa_circ_0001861	-0.814	hsa_circ_0008368	-0.509	hsa_circ_0004823	-0.20443	hsa_circ_0008602	-0.013

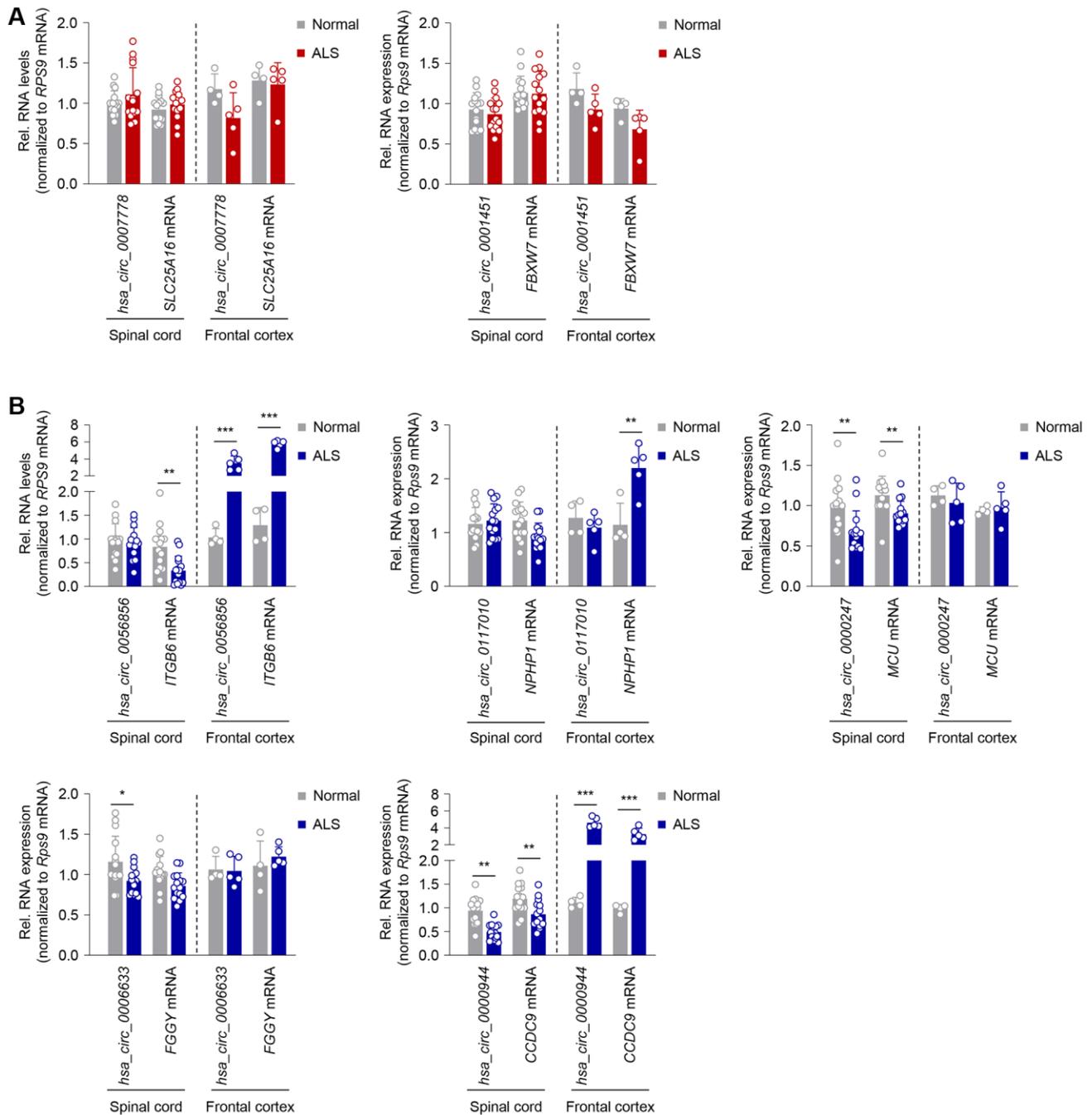
**Supplementary Figure 1. List of 250 circRNAs expressed in at least 40% of the human normal and ALS skeletal muscle biopsy samples. Upregulated (A) and downregulated (B) circRNAs in ALS based on log2 fold change, predicted from our circRNA-enriched RNA-seq analysis of normal (n = 5) and ALS (n = 5) biopsies. CircRNA nomenclature is based on the CircInteractome or circBase databases.**



**Supplementary Figure 2. Expression of select circRNAs based on RNA-seq analysis chosen for RT-qPCR analysis.** Heatmap of circRNAs [upregulated (A) and downregulated (B)] predicted to change based on the fold change of the circRNA-enriched RNA-seq analysis in the ALS cohort. The circRNAs whose fold change was later validated by RT-qPCR analysis (*top* part of heatmaps; Figure 2) are separated with a line from circRNAs whose fold change was not validated by RT-qPCR analysis (*bottom* part of heatmaps; Supplementary Figure 3).



**Supplementary Figure 3. RT-qPCR analysis of circRNAs predicted to change by RNA-seq analysis but not validated.** Differential expression of circRNAs predicted to increase (A, B) or decrease (C, D) based on their fold change and their linear counterpart in normal ( $n = 12$ ) and ALS ( $n = 8$ ) muscle biopsies, as quantified by RT-qPCR analysis. Data were normalized to *RPS9* mRNA levels, and *TBP* mRNA expression levels were included as a control;  $p$ -values \* $p < 0.05$ , \*\*\* $p < 0.001$ .



**Supplementary Figure 4. Differential expression in ALS CNS of circRNAs differentially abundant in human ALS muscle.** Differential expression of upregulated (A) and downregulated (B) circRNAs and their linear counterparts, as validated in human ALS skeletal muscle biopsies, from human spinal cord (cervical, thoracic, lumbar regions;  $n = 5$  for each region in both normal and ALS) and frontal cortex ( $n = 4$  for normal and  $n = 5$  for ALS) biopsies. Data were normalized to *RPS9* mRNA levels;  $p$ -values \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

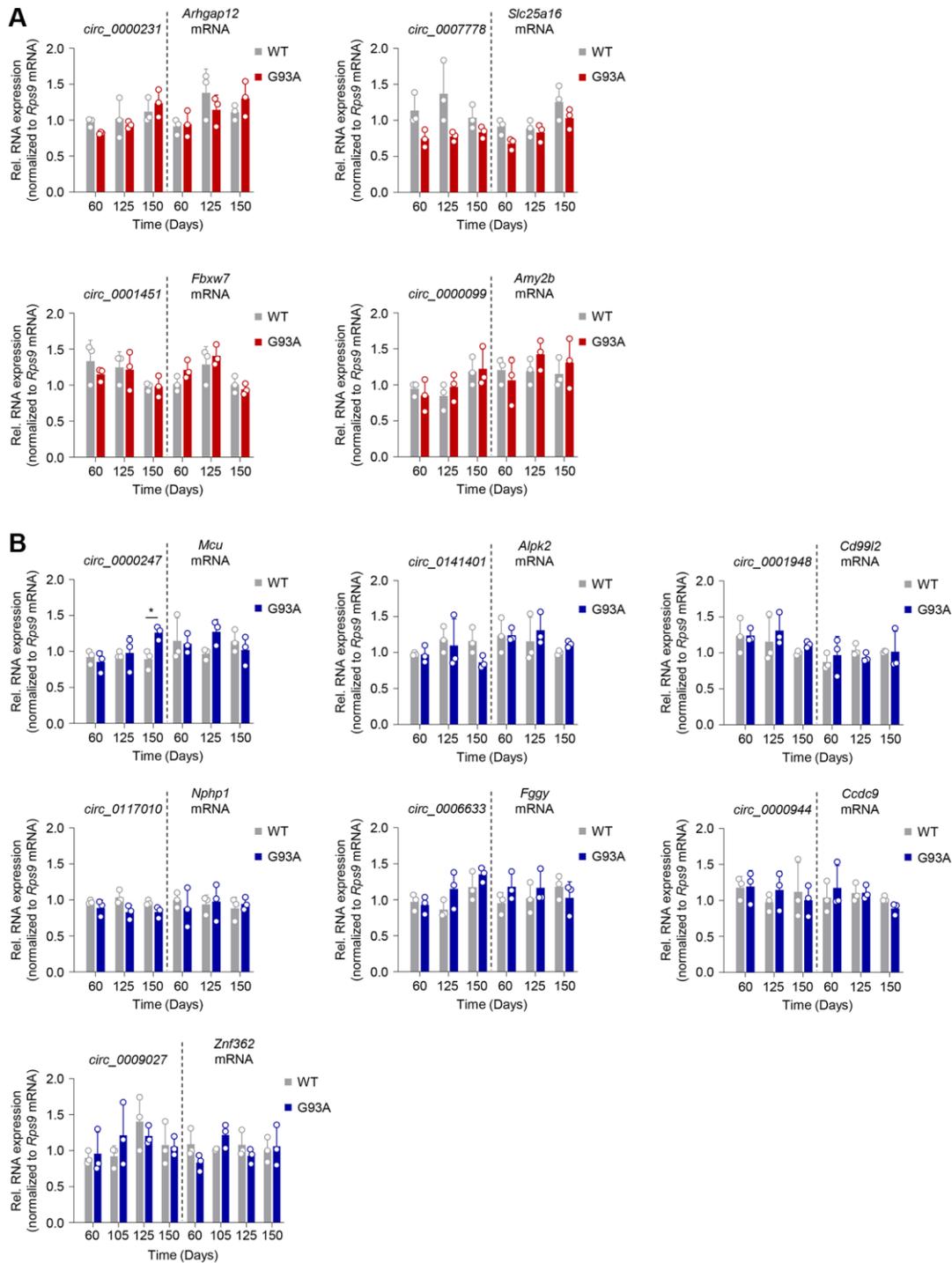
**A**

circRNA	Host Gene (species)	Exons	Sequence overlap
hsa_circ_0000119	NM_006699.5 ( <i>H. sapiens</i> )	2-6	88%
	NM_010763.2 ( <i>M. musculus</i> )	2-6	
hsa_circ_0005171	NM_018112.3 ( <i>H. sapiens</i> )	2-4	85%
	NM_028053.2 ( <i>M. musculus</i> )	2-4	
hsa_circ_0007099	NM_152924.5 ( <i>H. sapiens</i> )	2-3	88%
	NM_018811.6 ( <i>M. musculus</i> )	2-3	
hsa_circ_0000567	NM_032233.3 ( <i>H. sapiens</i> )	2-6	92%
	NM_001364266.1 ( <i>M. musculus</i> )	3-7	
hsa_circ_0000231	NM_018287.7 ( <i>H. sapiens</i> )	2-3	87%
	NM_001039692.1 ( <i>M. musculus</i> )	2-3	
hsa_circ_0007778	NM_001324312.2 ( <i>H. sapiens</i> )	2-8	88%
	NM_175194.2 ( <i>M. musculus</i> )	2-8	
hsa_circ_0001451	NM_033632.3 ( <i>H. sapiens</i> )	2-3	91%
	NM_001177773.1 ( <i>M. musculus</i> )	4-5	
hsa_circ_0000099	NM_001387437.1 ( <i>H. sapiens</i> )	2-9	73%
	NM_001190403.1 ( <i>M. musculus</i> )	2-9	

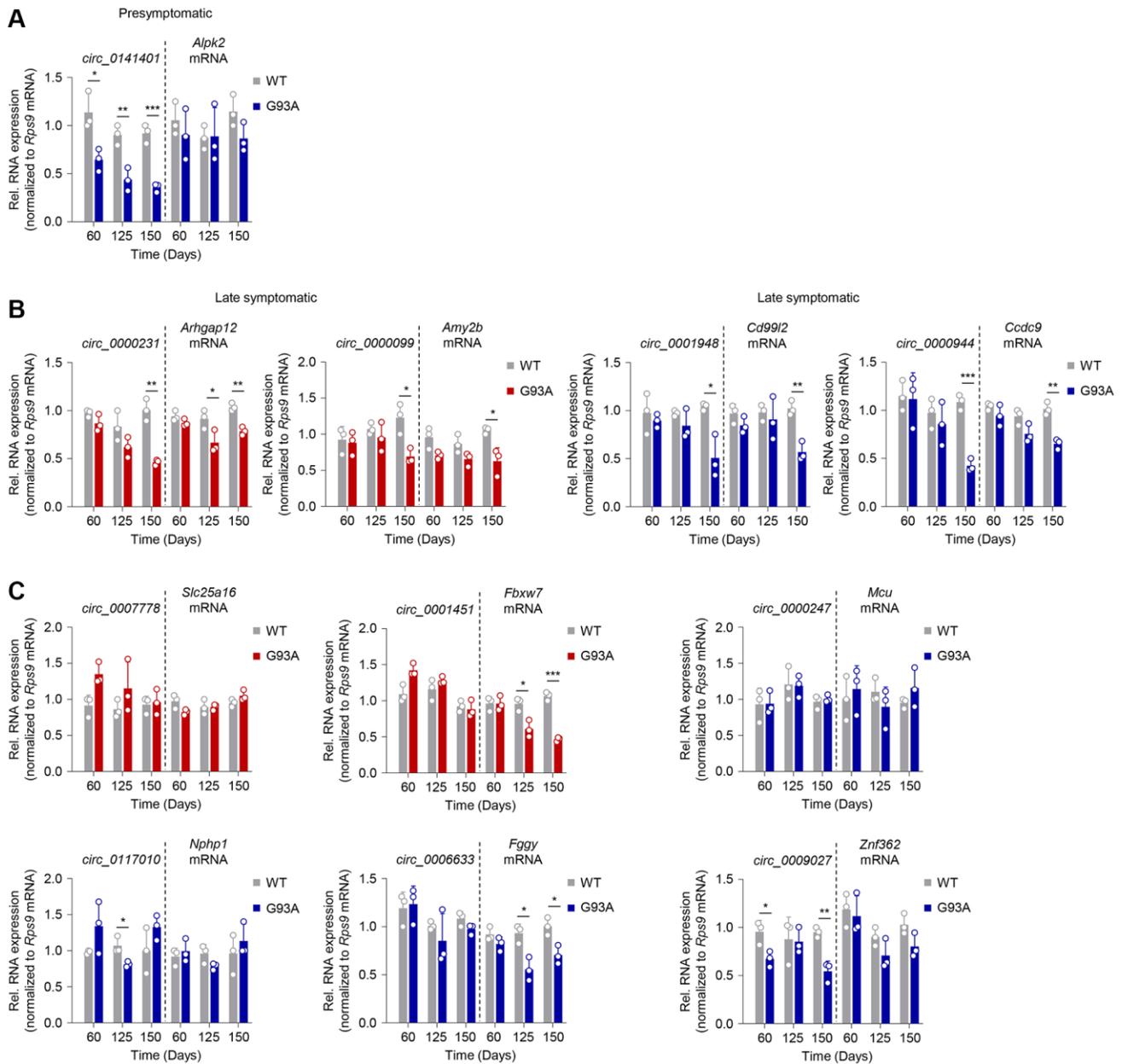
**B**

circRNA	Host Gene (species)	Exons	Sequence overlap
hsa_circ_0056856	NM_000888.5 ( <i>H. sapiens</i> )	11-12	83%
	NM_001159564.1 ( <i>M. musculus</i> )	14-15	
hsa_circ_0000033	NM_022778.5 ( <i>H. sapiens</i> )	4-6	85%
	NM_144527.3 ( <i>M. musculus</i> )	4-6	
hsa_circ_0047886	NM_017742.6 ( <i>H. sapiens</i> )	4-6	86%
	NM_001122675.1 ( <i>M. musculus</i> )	4-6	
hsa_circ_0000247	NM_138357.3 ( <i>H. sapiens</i> )	2-3	85%
	NM_001033259.4 ( <i>M. musculus</i> )	2-3	
hsa_circ_0141401	NM_052947.4 ( <i>H. sapiens</i> )	4	70%
	NM_001037294.1 ( <i>M. musculus</i> )	4	
hsa_circ_0001948	NM_001242614.2 ( <i>H. sapiens</i> )	4-8	69%
	NM_001199349.1 ( <i>M. musculus</i> )	3-7	
hsa_circ_0117010	NM_000272.5 ( <i>H. sapiens</i> )	10-16	84%
	NM_016902.4 ( <i>M. musculus</i> )	10-16	
hsa_circ_0006633	NM_001113411.2 ( <i>H. sapiens</i> )	3-5	88%
	NM_001113412.1 ( <i>M. musculus</i> )	3-5	
hsa_circ_0000944	NM_015603.3 ( <i>H. sapiens</i> )	6-7	86%
	NM_001136471.2 ( <i>M. musculus</i> )	6-7	
hsa_circ_0009027	NM_152493.3 ( <i>H. sapiens</i> )	7-8	94%
	NM_001081098.1 ( <i>M. musculus</i> )	7-8	

**Supplementary Figure 5. Information on upregulated or downregulated circRNAs overlapping between human and mouse.** Tables summarizing the aliases of human and mouse transcripts, the exons predicted to comprise the circRNA body, and the potential sequence overlap which we used to generate primers spanning the predicted junction; shown are upregulated (**A**) and downregulated (**B**) circRNAs, validated in human ALS muscle by RT-qPCR analysis.



**Supplementary Figure 6. Analysis of expression in muscle of ALS mice ( $SOD1^{G93A}$ ) of circRNAs differentially abundant in human ALS muscle.** CircRNAs that were validated in human ALS muscle and predicted to be upregulated (A) or downregulated (B), were quantified in skeletal muscle from  $SOD1^{G93A}$  mice by RT-qPCR analysis and found to not be significantly altered. Upregulated or downregulated circRNAs are designated by red or blue bars, respectively, based on the validation in human ALS muscle biopsies in Figure 2. Data were normalized to *Rps9* mRNA levels;  $p$ -values \* $p < 0.05$ .



**Supplementary Figure 7. Analysis of expression in spinal cord of ALS mice ( $SOD1^{G93A}$ ) of circRNAs differentially abundant in human ALS muscle.** CircRNAs that were validated in human ALS muscle and predicted to be upregulated (A) or downregulated (B), were quantified in spinal cord from ALS mice ( $SOD1^{G93A}$ ) by RT-qPCR analysis at the presymptomatic stage (A), late symptomatic stage (B), and (C) not significantly changed. Upregulated (red) or downregulated (blue) designations are based on the circRNA pattern observed in human ALS muscle biopsies in Figure 2. Data were normalized to *Rps9* mRNA;  $p$ -values \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .