



UNIVERSITÀ
DEGLI STUDI
DI MILANO



FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO

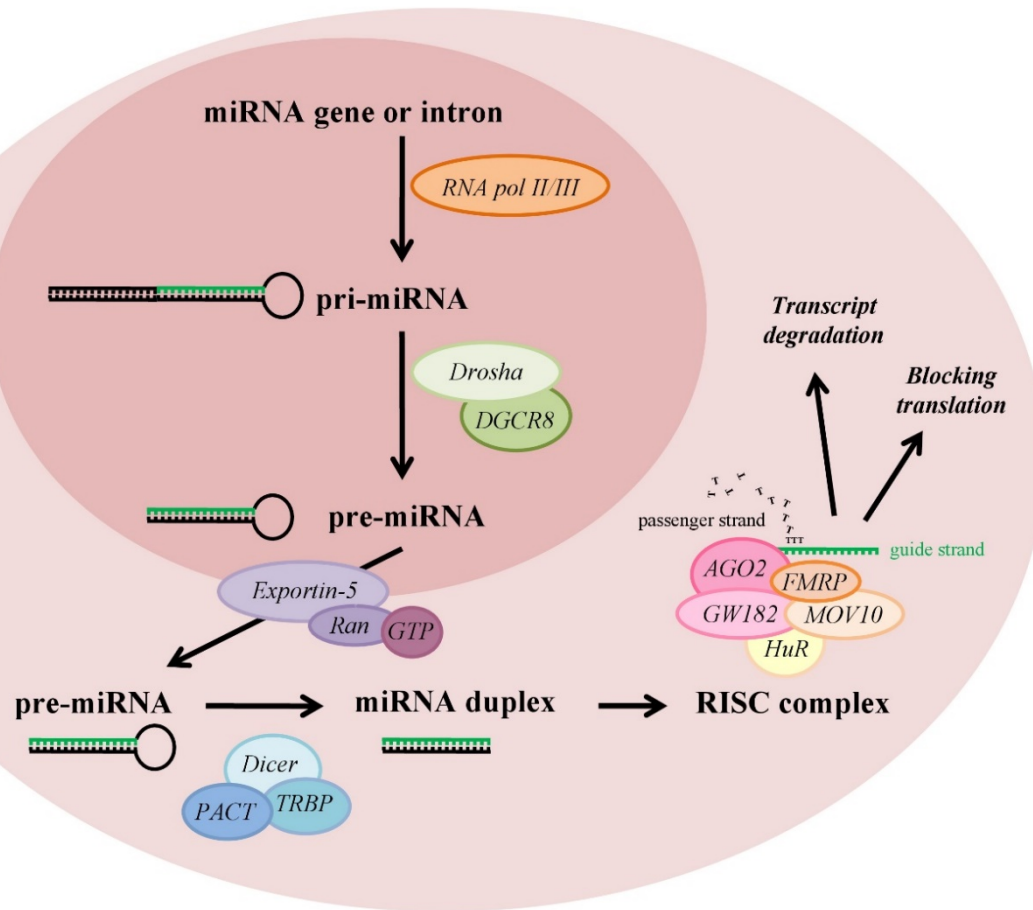
Sistema Sanitario  Regione
Lombardia

MicroRNAs analysis of patient-derived iPSCs and MNs as molecular therapy for ALS

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DePT, University of Milan
IRCCS Ca' Granda Foundation
Ospedale Maggiore Policlinico

MicroRNAs (miRNAs)

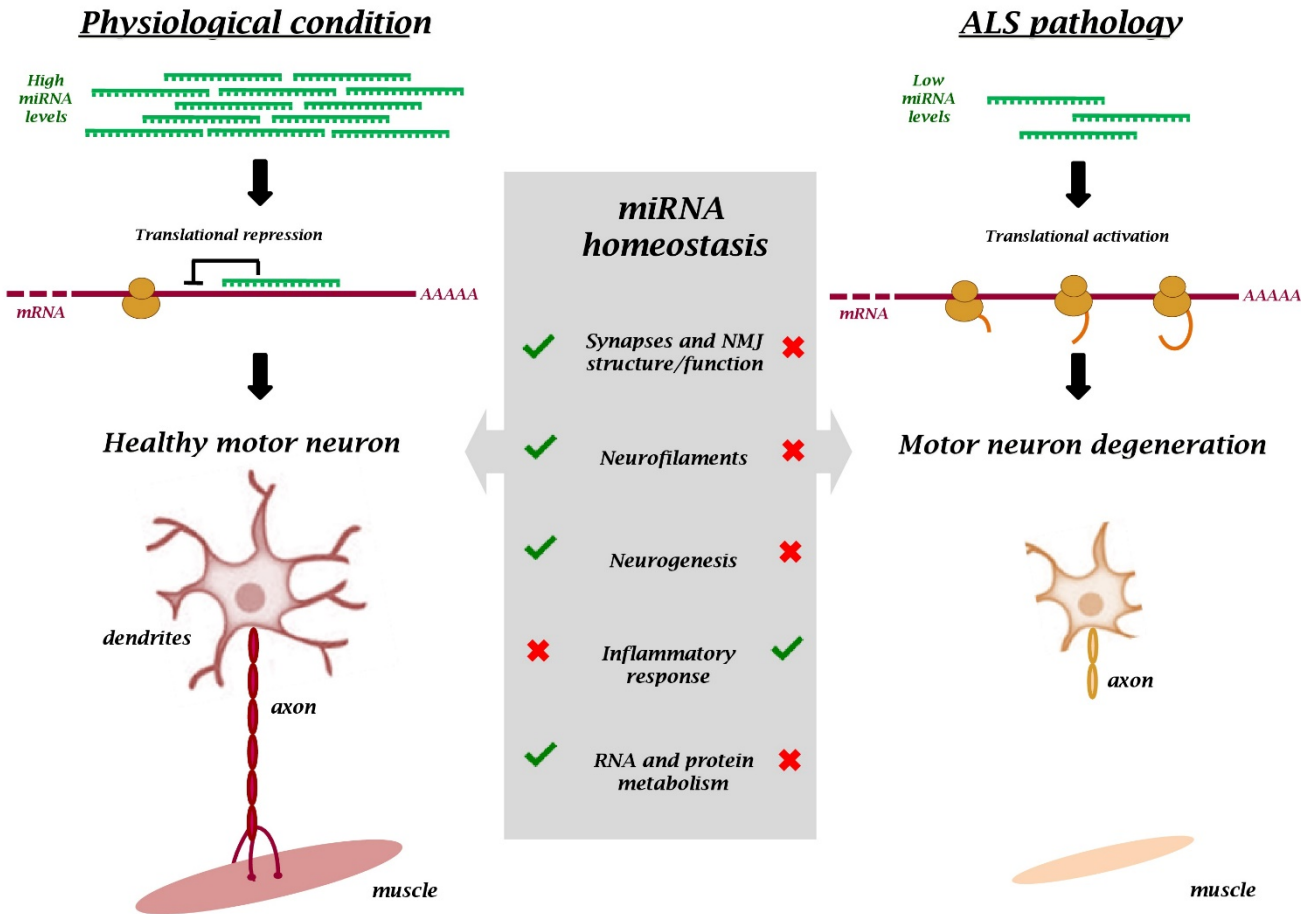
MiRNAs biogenesis process



- miRNAs regulate mRNA: transcript degradation, translation blocking or post-transcriptional regulation
- miRNAs are highly expressed in CNS and are required for neuronal differentiation and survival
- A deregulation of miRNAs expression may be involved in aetiology or progression of neurodegenerative disorders, including ALS

miRNA and ALS

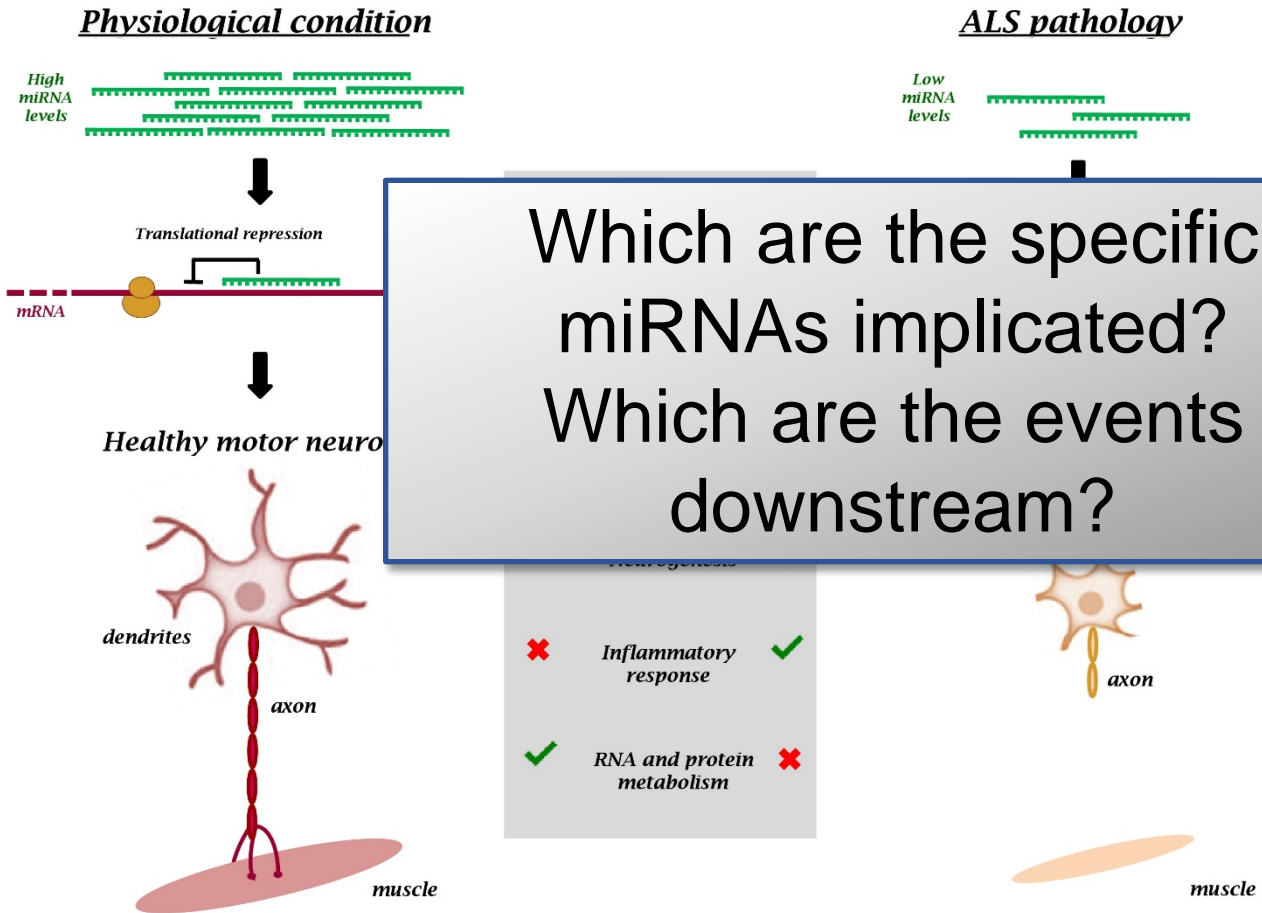
Role of miRNAs in motor neuron physiology and degeneration



- ALS-linked genes can affect miRNA biogenesis
- TDP-43 and FUS have been found implicated in several steps of RNA metabolism, including miRNA processing
- A deregulation of miRNA expression has been showed in human s/fALS biological samples and in murine models of ALS

miRNA and ALS

Role of miRNAs in motor neuron physiology and degeneration



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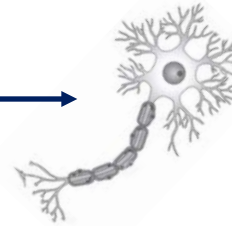
ALS patient



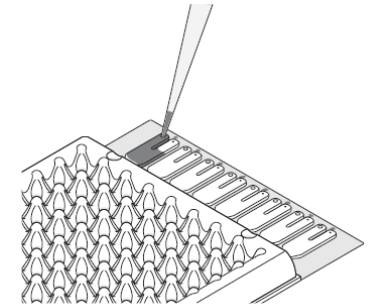
ALS iPSCs



MNs

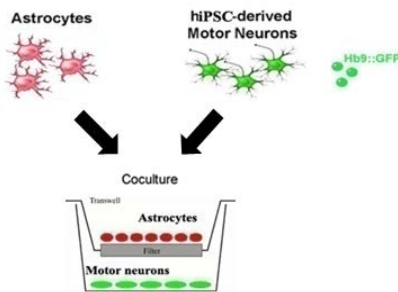


miRNA expression analysis



1. Investigation of miRNAs dysregulation in ALS iPSCs and ALS iPSC-derived motor neurons compared with the control lines

MNs-astrocytes co-culture



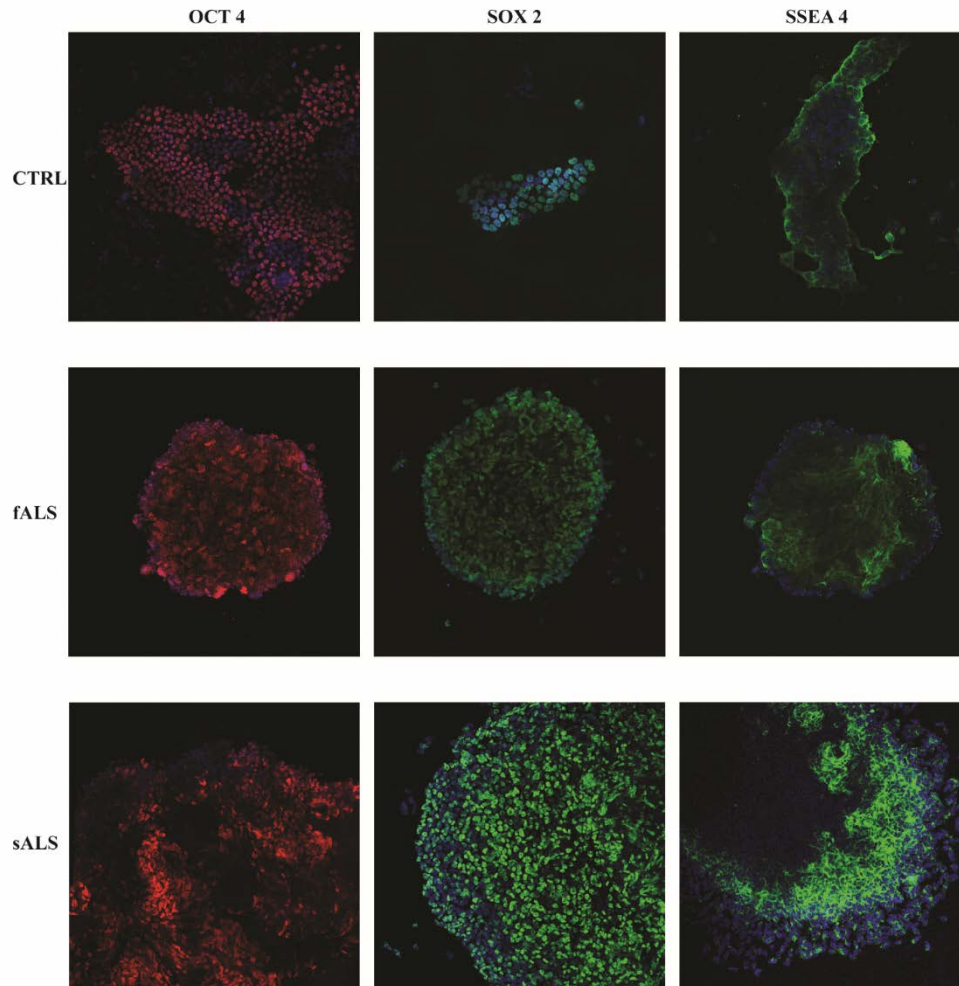
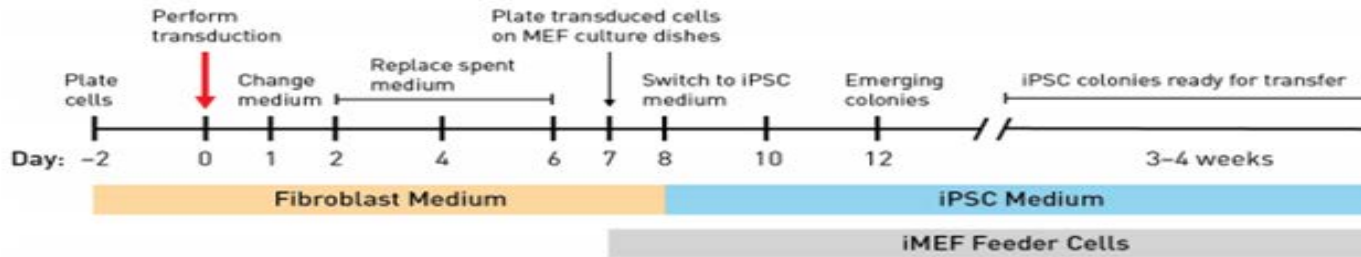
SOD1G93A



Identification of targets

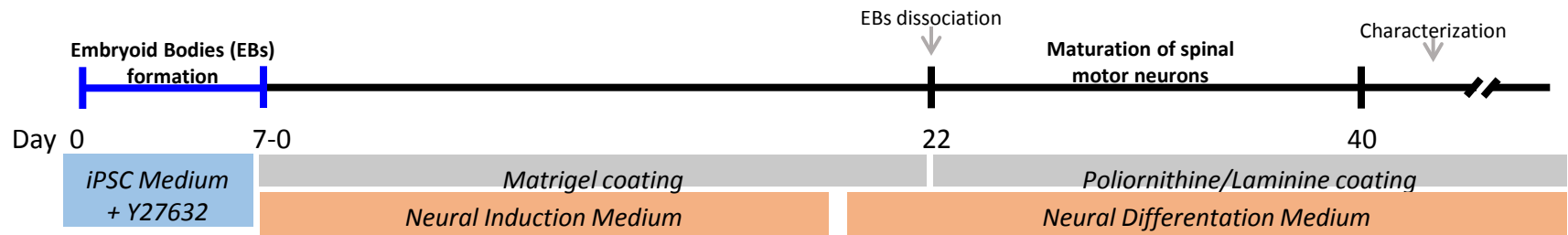
2. Modulation of identified miRNA targets evaluating MN protection from degeneration in ALS *in vitro* co-culture and in the SOD1G93A animal model

iPSCs reprogramming

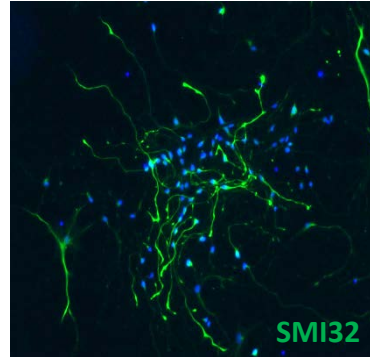
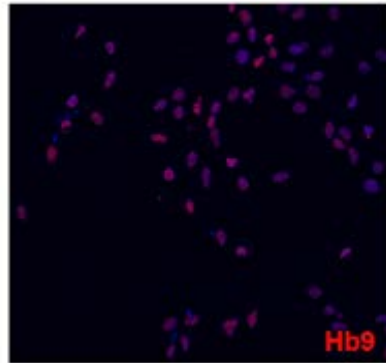
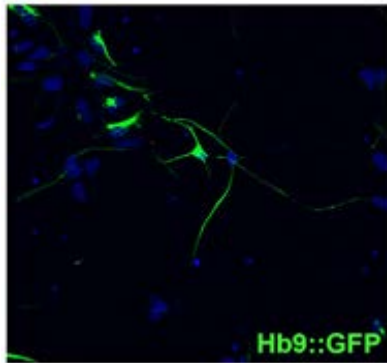


iPSCs	Number of lines
WILD-TYPE	3
C9ORF72	2
TDP43	2
SOD1	2

Motor neurons (MNs) differentiation

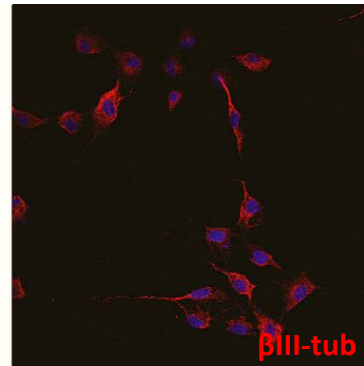
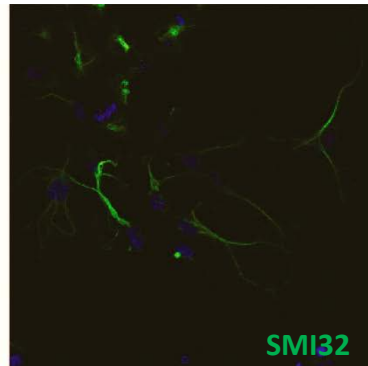
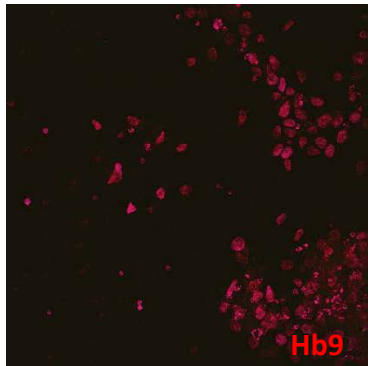


Ng et al., 2015



CTRL

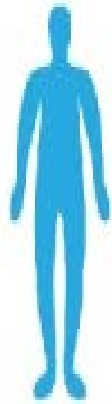
Obtained
MNs express
specific
motoneuronal
markers



ALS

miRNAs expression analysis

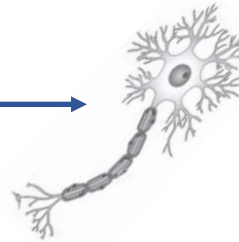
Human fibroblasts



iPSCs



MNs



- wild-type iPSCs
- ALS iPSCs
- wild-type MNs
- ALS MNs



Total RNAs

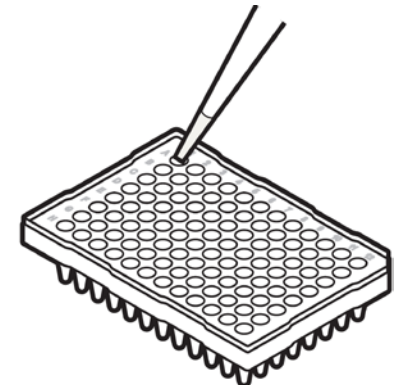
1



Total RNA



Human Array MicroRNA Cards



miRNAs isolation

Taqman array miRNA cards

TaqMan® Array Human MicroRNA Card A v2

A	hsa-let-7a	hsa-let-7c	hsa-let-7d	hsa-let-7e	hsa-let-7f	hsa-let-7g	hsa-miR-1	hsa-miR-9	hsa-miR-10a	hsa-miR-10b	UG snRNA	UG snRNA	hsa-miR-15a	hsa-miR-15b	hsa-miR-16	hsa-miR-17	hsa-miR-18a	hsa-miR-18b	hsa-miR-19a	hsa-miR-19b	hsa-miR-20a	hsa-miR-20b	hsa-miR-21	hsa-miR-22
B	hsa-miR-23a	hsa-miR-23b	hsa-miR-24	hsa-miR-25	hsa-miR-26a	hsa-miR-26b	hsa-miR-27a	hsa-miR-27b	hsa-miR-28-3p	hsa-miR-29-5p	UG snRNA	UG snRNA	hsa-miR-29a	hsa-miR-29b	hsa-miR-29c	hsa-miR-30b	hsa-miR-30c	hsa-miR-31	hsa-miR-32	hsa-miR-33b	hsa-miR-34a	hsa-miR-34c-5p	hsa-miR-92a	hsa-miR-93
C	hsa-miR-95	hsa-miR-96	hsa-miR-98	hsa-miR-99a	hsa-miR-99b	hsa-miR-100	hsa-miR-101	hsa-miR-102	hsa-miR-105	hsa-miR-106a	hsa-miR-106b	hsa-miR-107	hsa-miR-122	hsa-miR-124	hsa-miR-125a-3p	hsa-miR-125a-5p	hsa-miR-125b	hsa-miR-126	hsa-miR-127-3p	hsa-miR-127-5p	hsa-miR-128	hsa-miR-129-3p	hsa-miR-129-5p	hsa-miR-139-5p
D	hsa-miR-130a	hsa-miR-130b	hsa-miR-132	hsa-miR-133a	hsa-miR-133b	hsa-miR-134	hsa-miR-135a	hsa-miR-135b	hsa-miR-136	hsa-miR-137	hsa-miR-138	hsa-miR-139-3p	hsa-miR-139-5p	hsa-miR-140-3p	hsa-miR-140-5p	hsa-miR-141	hsa-miR-142-3p	hsa-miR-143	hsa-miR-145	hsa-miR-146a	hsa-miR-146b-3p	hsa-miR-146b-5p	hsa-miR-147b	hsa-miR-147c
E	hsa-miR-148a	hsa-miR-148b	hsa-miR-149	hsa-miR-150	hsa-miR-152	hsa-miR-153	hsa-miR-154	hsa-miR-181a	hsa-miR-181c	hsa-miR-182	hsa-miR-183	hsa-miR-184	hsa-miR-185	hsa-miR-186	hsa-miR-187	hsa-miR-188-3p	hsa-miR-189	hsa-miR-191	hsa-miR-192	hsa-miR-193a-3p	hsa-miR-193a-5p	hsa-miR-193b	hsa-miR-194	hsa-miR-194a
F	hsa-miR-195	hsa-miR-196b	hsa-miR-197	hsa-miR-198	hsa-miR-199a-5p	hsa-miR-199a-3p	hsa-miR-199b-5p	hsa-miR-200a	hsa-miR-200b	hsa-miR-200c	hsa-miR-202	hsa-miR-203	hsa-miR-204	hsa-miR-205	hsa-miR-209b	hsa-miR-210	hsa-miR-214	hsa-miR-215	hsa-miR-216a	hsa-miR-216b	hsa-miR-217	hsa-miR-218	hsa-miR-219-5p	hsa-miR-221
G	hsa-miR-222	hsa-miR-223	hsa-miR-224	hsa-miR-296-3p	hsa-miR-296-5p	hsa-miR-299-3p	hsa-miR-299-5p	hsa-miR-301a	hsa-miR-301b	hsa-miR-302a	hsa-miR-159a	hsa-miR-302b	hsa-miR-302c	hsa-miR-320	hsa-miR-323-3p	hsa-miR-324-3p	hsa-miR-324-5p	hsa-miR-326	hsa-miR-328	hsa-miR-329	hsa-miR-330-3p	hsa-miR-330-5p	hsa-miR-331-3p	hsa-miR-331-5p
H	hsa-miR-335	hsa-miR-337-5p	hsa-miR-338-3p	hsa-miR-339-3p	hsa-miR-339-5p	hsa-miR-340	hsa-miR-155	hsa-let-7b	hsa-miR-342-3p	hsa-miR-342-5p	hsa-miR-345	hsa-miR-361-5p	hsa-miR-362-3p	hsa-miR-362-5p	hsa-miR-363	hsa-miR-365	hsa-miR-367	hsa-miR-369-3p	hsa-miR-369-5p	hsa-miR-370	hsa-miR-371-3p	hsa-miR-372	hsa-miR-373	hsa-miR-374a
I	hsa-miR-374b	hsa-miR-375	hsa-miR-376a	hsa-miR-376b	hsa-miR-377	hsa-miR-379	hsa-miR-380	hsa-miR-381	hsa-miR-382	hsa-miR-383	hsa-miR-409-5p	hsa-miR-410	hsa-miR-411	hsa-miR-422a	hsa-miR-423-5p	hsa-miR-424	hsa-miR-425	hsa-miR-429	hsa-miR-431	hsa-miR-433	hsa-miR-440a	hsa-miR-440b	hsa-miR-450a	hsa-miR-450b-3p
J	hsa-miR-450b-5p	hsa-miR-451	hsa-miR-452	hsa-miR-453	hsa-miR-454	hsa-miR-455-3p	hsa-miR-455-5p	hsa-miR-483-5p	hsa-miR-484	hsa-miR-485-3p	hsa-miR-485-5p	hsa-miR-486-3p	hsa-miR-486-5p	hsa-miR-487a	hsa-miR-487b	hsa-miR-488	hsa-miR-489	hsa-miR-490-3p	hsa-miR-491-3p	hsa-miR-491-5p	hsa-miR-493	hsa-miR-494	hsa-miR-495	hsa-miR-496
K	hsa-miR-499-3p	hsa-miR-499-5p	hsa-miR-500	hsa-miR-501-3p	hsa-miR-501-5p	hsa-miR-502-3p	hsa-miR-502-5p	hsa-miR-503	hsa-miR-504	hsa-miR-505	hsa-miR-507	hsa-miR-508-3p	hsa-miR-508-5p	hsa-miR-509-5p	hsa-miR-510	hsa-miR-512-3p	hsa-miR-512-5p	hsa-miR-513-5p	hsa-miR-515-3p	hsa-miR-515-5p	hsa-miR-516a-5p	hsa-miR-516b	hsa-miR-517a	hsa-miR-517c
L	hsa-miR-518a-3p	hsa-miR-518a-5p	hsa-miR-518b	hsa-miR-518c	hsa-miR-518d-3p	hsa-miR-518d-5p	hsa-miR-519a	hsa-miR-519b	hsa-miR-519c	hsa-miR-519e	hsa-miR-520a-3p	hsa-miR-520a-5p	hsa-miR-520b	hsa-miR-520c	hsa-miR-521	hsa-miR-522	hsa-miR-523	hsa-miR-524-5p	hsa-miR-525-3p	hsa-miR-525-5p	hsa-miR-528b	hsa-miR-532-3p	hsa-miR-532-5p	hsa-miR-533
M	hsa-miR-539	hsa-miR-541	hsa-miR-542-3p	hsa-miR-542-5p	hsa-miR-544	hsa-miR-545	hsa-miR-548a-3p	hsa-miR-548a-5p	hsa-miR-548b-3p	hsa-miR-548b-5p	hsa-miR-548c-3p	hsa-miR-548c-5p	hsa-miR-548d-3p	hsa-miR-548d-5p	hsa-miR-551b	hsa-miR-556-3p	hsa-miR-556-5p	hsa-miR-561	hsa-miR-570	hsa-miR-574-3p	hsa-miR-576-3p	hsa-miR-576-5p	hsa-miR-579	hsa-miR-582-3p
N	hsa-miR-582-5p	hsa-miR-589	hsa-miR-590-5p	hsa-miR-597	hsa-miR-598	hsa-miR-615-3p	hsa-miR-615-5p	hsa-miR-616	hsa-miR-618	hsa-miR-624	hsa-miR-625	hsa-miR-627	hsa-miR-629-5p	hsa-miR-629	hsa-miR-636	hsa-miR-642	hsa-miR-651	hsa-miR-652	hsa-miR-653	hsa-miR-654-3p	hsa-miR-654-5p	hsa-miR-655	hsa-miR-660	hsa-miR-671-3p
O	hsa-miR-672	hsa-miR-674	hsa-miR-708	hsa-miR-744	hsa-miR-758	hsa-miR-871	hsa-miR-872	hsa-miR-873	hsa-miR-874	hsa-miR-875-3p	hsa-miR-878-3p	hsa-miR-878-5p	hsa-miR-885-3p	hsa-miR-885-5p	hsa-miR-886-3p	hsa-miR-886-5p	hsa-miR-887	hsa-miR-888	hsa-miR-889	hsa-miR-890	hsa-miR-891a	hsa-miR-891b	hsa-miR-892a	hsa-miR-147
P	hsa-miR-208	hsa-miR-211	hsa-miR-212	hsa-miR-219-1-3p	hsa-miR-219-2-3p	hsa-miR-220	hsa-miR-220c	hsa-miR-298	hsa-miR-325	hsa-miR-346	hsa-miR-370c	hsa-miR-384	hsa-miR-412	hsa-miR-448	hsa-miR-492	hsa-miR-506	hsa-miR-509-3-5p	hsa-miR-511	hsa-miR-517b	hsa-miR-519c-3p	hsa-miR-520b	hsa-miR-520c	hsa-miR-520f	hsa-miR-520g

➤ 754 unique assays specific to human miRNAs

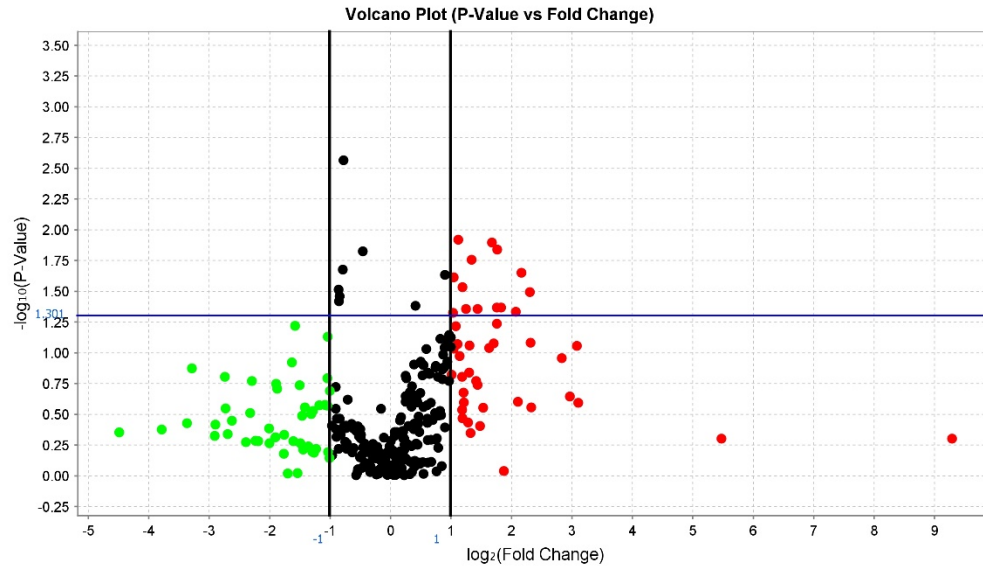
➤ Card A focuses on the most highly characterized miRNAs

TaqMan® Array Human MicroRNA Card B v3

A	dmiR-7	hsa-miR-548l	hsa-miR-30a-3p	hsa-miR-30a-5p	hsa-miR-50f	hsa-miR-30e-3p	hsa-miR-126*	hsa-miR-154*	hsa-miR-182*	UG snRNA	UG snRNA	hsa-miR-706	hsa-miR-213	hsa-miR-300c*	hsa-miR-303d	hsa-miR-378	hsa-miR-380-5p	hsa-miR-1257	hsa-miR-200a*	hsa-miR-432	hsa-miR-432*	hsa-miR-487	hsa-miR-500
B	hsa-miR-1238	hsa-miR-488	hsa-miR-517*	hsa-miR-516-3p	hsa-miR-516-5p	hsa-miR-519c*	hsa-miR-520b	hsa-miR-524	hsa-miR-7d*	UG snRNA	UG snRNA	hsa-miR-7*	hsa-miR-656	hsa-miR-549	hsa-miR-457	hsa-miR-658	hsa-miR-659	hsa-miR-551a	hsa-miR-552	hsa-miR-553	hsa-miR-554	hsa-miR-555	hsa-miR-557
C	hsa-miR-558	hsa-miR-559	hsa-miR-562	hsa-miR-563	hsa-miR-564	hsa-miR-566	hsa-miR-567	hsa-miR-569	hsa-miR-586	hsa-miR-587	hsa-miR-588	hsa-miR-590	hsa-miR-591	hsa-miR-607	hsa-miR-603	hsa-miR-622	hsa-miR-626	hsa-miR-627	hsa-miR-628	hsa-miR-629	hsa-miR-633	hsa-miR-634	hsa-miR-635
D	hsa-miR-625	hsa-miR-629	hsa-miR-630	hsa-miR-631	hsa-miR-603	hsa-miR-604	hsa-miR-605	hsa-miR-606	hsa-miR-607	hsa-miR-609	hsa-miR-609	hsa-miR-633	hsa-miR-634	hsa-miR-635	hsa-miR-637	hsa-miR-638	hsa-miR-639	hsa-miR-640	hsa-miR-641	hsa-miR-613	hsa-miR-614	hsa-miR-616	hsa-miR-617
E	hsa-miR-644	hsa-miR-645	hsa-miR-621	hsa-miR-646	hsa-miR-647	hsa-miR-648	hsa-miR-649	hsa-miR-650	hsa-miR-661	hsa-miR-662	hsa-miR-663	hsa-miR-664	hsa-miR-665	hsa-miR-666	hsa-miR-667	hsa-miR-668	hsa-miR-669	hsa-miR-670	hsa-miR-671	hsa-miR-672	hsa-miR-673	hsa-miR-674	hsa-miR-675
F	hsa-miR-668	hsa-miR-767-5p	hsa-miR-767-3p	hsa-miR-454*	hsa-miR-769-5p	hsa-miR-769-3p	hsa-miR-802	hsa-miR-675	hsa-miR-505*	hsa-miR-216-1*	hsa-miR-221*	hsa-miR-222*	hsa-miR-223*	hsa-miR-136*	hsa-miR-34b	hsa-miR-185*	hsa-miR-186*	hsa-miR-195*	hsa-miR-30c-1*	hsa-miR-30c-2*	hsa-miR-32*	hsa-miR-31*	hsa-miR-130a*
G	hsa-miR-25a-2*	hsa-miR-361-3p	hsa-miR-7a-let	hsa-miR-302a*	hsa-miR-302b*	hsa-miR-367*	hsa-miR-374a*	hsa-miR-29b*	hsa-miR-375a*	hsa-miR-377*	hsa-miR-378*	hsa-miR-379*	hsa-miR-380*	hsa-miR-381*	hsa-miR-382*	hsa-miR-383*	hsa-miR-384*	hsa-miR-385*	hsa-miR-386*	hsa-miR-387*	hsa-miR-388*	hsa-miR-389*	hsa-miR-390*
H	hsa-miR-101*	hsa-miR-139-2*	hsa-miR-141*	hsa-miR-143*	hsa-miR-144*	hsa-miR-145*	hsa-miR-820	hsa-miR-801	hsa-miR-822	hsa-miR-824	hsa-miR-337-3p	hsa-miR-129b-2*	hsa-miR-135b*	hsa-miR-148a*	hsa-miR-146a*	hsa-miR-149*	hsa-miR-270b-1*	hsa-miR-270b-2*	hsa-miR-105*	hsa-miR-106a*	hsa-miR-16-2*	hsa-miR-156*	hsa-miR-27b*
I	hsa-miR-833	hsa-miR-934	hsa-miR-935	hsa-miR-936	hsa-miR-937	hsa-miR-938	hsa-miR-939	hsa-miR-941	hsa-miR-935*	hsa-miR-942	hsa-miR-943	hsa-miR-944	hsa-miR-996*	hsa-miR-124*	hsa-miR-541*	hsa-miR-875-5p	hsa-miR-888*	hsa-miR-892b	hsa-miR-91*	hsa-miR-411*	hsa-miR-378	hsa-miR-340*	hsa-miR-190b
J	hsa-miR-545*	hsa-miR-183*	hsa-miR-192*	hsa-miR-200b*	hsa-miR-200c*	hsa-miR-155*	hsa-miR-10a*	hsa-miR-214*	hsa-miR-218-2*	hsa-miR-129*	hsa-miR-72*	hsa-miR-425*	hsa-miR-304*	hsa-miR-7a*	hsa-miR-424*	hsa-miR-108*	hsa-miR-206*	hsa-miR-431*	hsa-miR-7-2*	hsa-miR-106*	hsa-miR-34a*	hsa-miR-191a-2*	hsa-miR-744*
K	hsa-miR-489-3p	hsa-miR-181c*	hsa-miR-196a*	hsa-miR-483-3p	hsa-miR-708*	hsa-miR-62b*	hsa-miR-651a*	hsa-miR-202*	hsa-miR-193b*	hsa-miR-487*	hsa-miR-518a*	hsa-miR-543	hsa-miR-125b-1*	hsa-miR-194*	hsa-miR-108b*	hsa-miR-302a*	hsa-miR-519b-3p	hsa-miR-5181*	hsa-miR-520c-3p	hsa-miR-7e*	hsa-miR-7c*	hsa-miR-7a-let	hsa-miR-550
L	hsa-miR-593	hsa-miR-571*	hsa-miR-144	hsa-miR-15a*	hsa-miR-16-1*	hsa-miR-17*	hsa-miR-18a*	hsa-miR-19a*	hsa-miR-19b-1*	hsa-miR-625*	hsa-miR-626-3p	hsa-miR-20a*	hsa-miR-21*	hsa-miR-23a*	hsa-miR-24-1*	hsa-miR-24-2*	hsa-miR-25*	hsa-miR-26a-1*	hsa-miR-26a-2*	hsa-miR-27a*	hsa-miR-29a*	hsa-miR-151-5p	hsa-miR-765
M	hsa-miR-623	hsa-miR-577	hsa-miR-144	hsa-miR-580-3p	hsa-miR-181*	hsa-miR-665	hsa-miR-5200-3p	hsa-miR-1224-3p	hsa-miR-1305	hsa-miR-613c	hsa-miR-5138	hsa-miR-1226*	hsa-miR-1228	hsa-miR-1229-3p	hsa-miR-1233	hsa-miR-1227	hsa-miR-1286	hsa-miR-5488a	hsa-miR-1179	hsa-miR-1178	hsa-miR-1205	hsa-miR-1271	hsa-miR-1201
N	hsa-miR-548j	hsa-miR-1283	hsa-miR-1794	hsa-miR-1799	hsa-miR-1795	hsa-miR-1244	hsa-miR-1303	hsa-miR-1799	hsa-miR-548f	hsa-miR-1264	hsa-miR-1258b	hsa-miR-1282	hsa-miR-1170	hsa-miR-1197	hsa-miR-1374	hsa-miR-5490	hsa-miR-1254	hsa-miR-5490c	hsa-miR-1251	hsa-miR-1285	hsa-miR-1245	hsa-miR-1292	hsa-miR-1301
O	hsa-miR-1200	hsa-miR-1182	hsa-miR-1298	hsa-miR-1291	hsa-miR-1275	hsa-miR-1183	hsa-miR-1184	hsa-miR-1276	hsa-miR-3208	hsa-miR-1272	hsa-miR-1180	hsa-miR-1278	hsa-miR-1282	hsa-miR-1243	hsa-miR-4638	hsa-miR-1252	hsa-miR-1298	hsa-miR-1290	hsa-miR-1249	hsa-miR-1248	hsa-miR-1289	hsa-miR-1204	hsa-miR-1626
P	hsa-miR-1204	hsa-miR-1203	hsa-miR-1206	hsa-miR-548k	hsa-miR-1208	hsa-miR-548e	hsa-miR-1274a	hsa-miR-1274b	hsa-miR-1267	hsa													

miRNA cards on iPSCs

miRNA cards on iPSCs

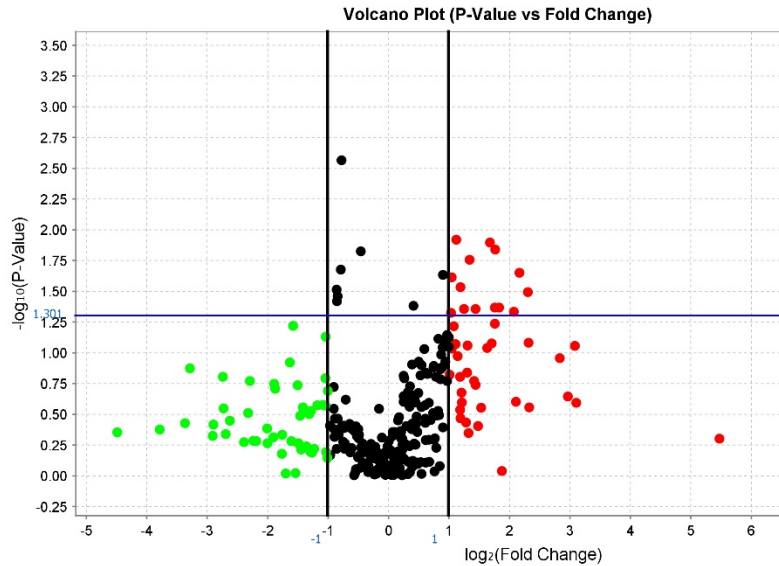


Legend

Study: SLA 2017, IPS vs IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

Data analysis encloses ALS subjects in a unique biological group versus controls

miRNA cards on iPSCs

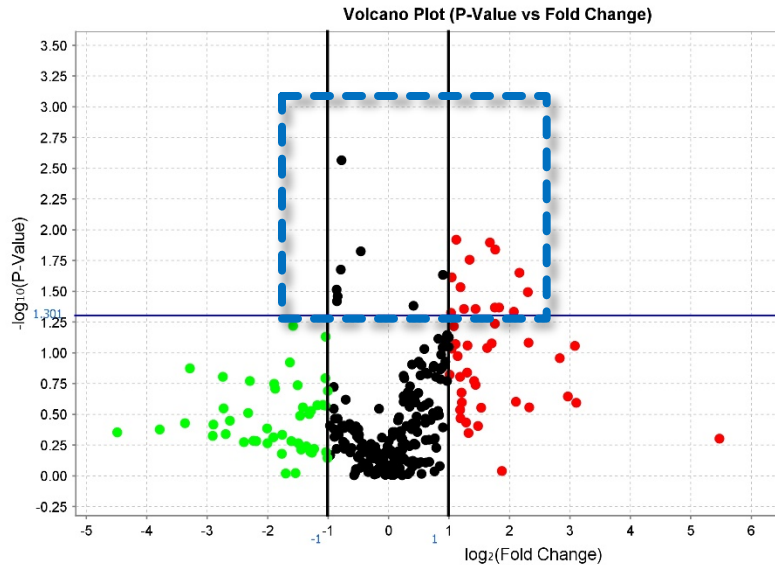


Legend
Study: SLA 2017, IPS vs IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

miRNA	Fold Change	P value	Expression
hsa-miR-1233-002768	0,583	0,003	Downregulated
hsa-miR-331-000545	2,171	0,012	Upregulated
hsa-miR-1180-002847	3,196	0,013	Upregulated
hsa-miR-128a-002216	3,398	0,014	Upregulated
hsa-miR-659-001514	0,727	0,015	Downregulated
hsa-miR-340-002258	2,536	0,017	Upregulated
hsa-miR-206-000510	0,578	0,021	Downregulated
hsa-miR-488-002357	4,477	0,022	Upregulated
hsa-miR-186-002285	1,868	0,023	Upregulated
hsa-miR-101-002253	2,063	0,024	Upregulated
hsa-miR-103-000439	2,284	0,029	Upregulated
hsa-miR-571-001613	0,550	0,031	Downregulated
hsa-miR-138-002284	4,932	0,032	Upregulated
hsa-miR-1225-3P-002766	0,557	0,035	Downregulated
hsa-miR-432#-001027	0,553	0,038	Downregulated
hsa-miR-1208-002880	1,332	0,042	Upregulated
hsa-miR-301-000528	3,378	0,043	Upregulated
hsa-miR-301b-002392	3,549	0,043	Upregulated
hsa-miR-590-5p-001984	2,376	0,044	Upregulated
hsa-miR-340#-002259	2,708	0,044	Upregulated
hsa-miR-422a-002297	4,206	0,046	Upregulated
hsa-miR-19a-000395	2,048	0,047	Upregulated

Data analysis encloses ALS subjects
a unique biological group
controls

miRNA cards on iPSCs



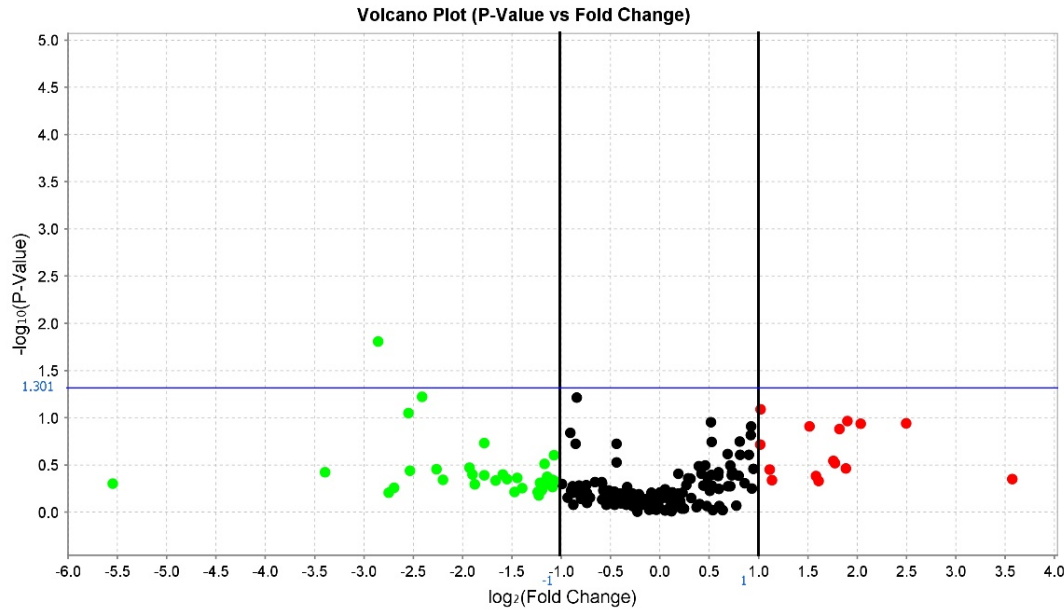
Legend
Study: SLA 2017, IPS vs IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

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hsa-miR-422a-002297	4,206	0,046	Upregulated
hsa-miR-19a-000395	2,048	0,047	Upregulated

Data analysis encloses ALS subjects
a unique biological group
controls

List of the most significant deregulated miRNAs

miRNA cards on iPSC-derived MNs



Legend

Study: SLA 2017, MN vs MN CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

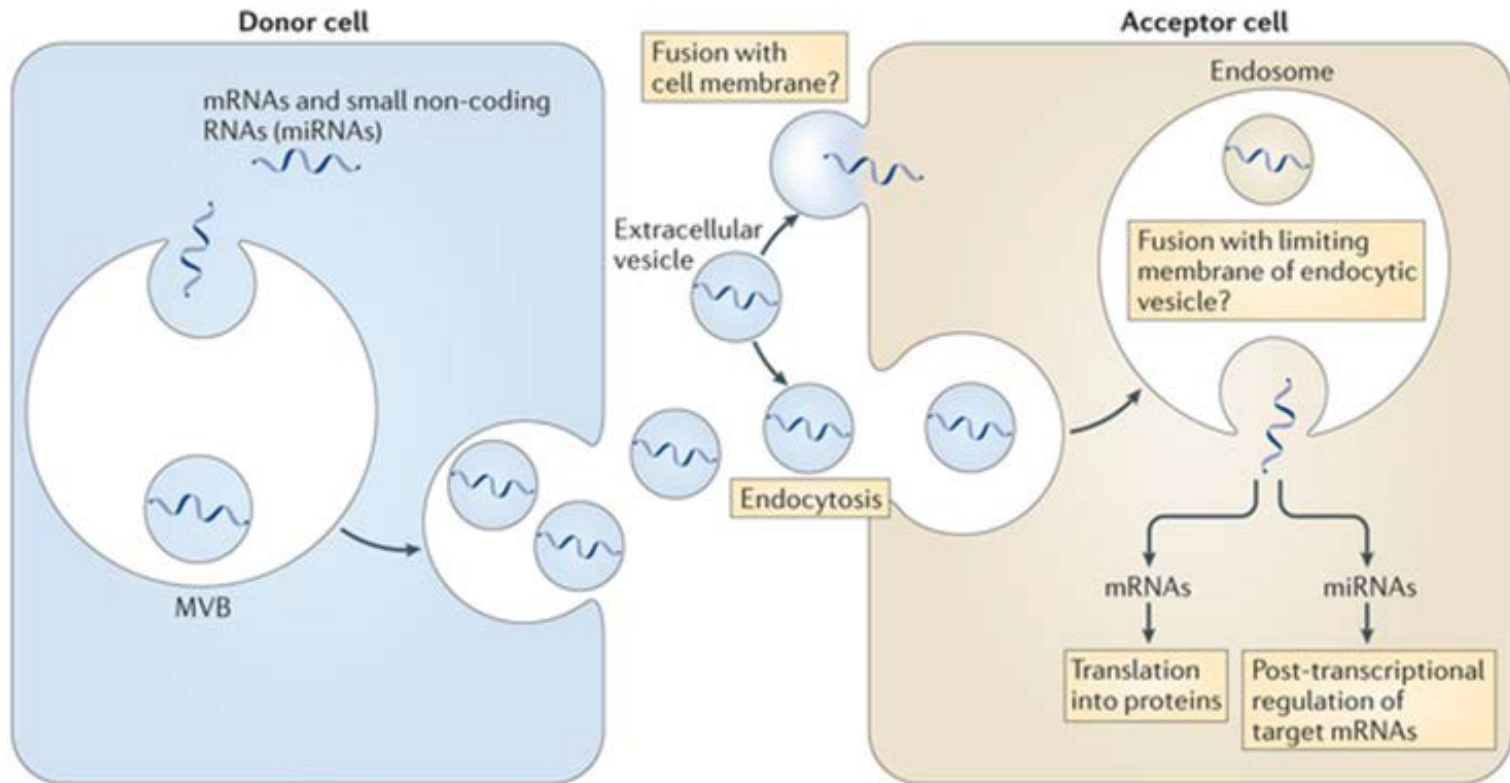
Vulcano plot

miRNA	Fold Change	P value	Expression
Let 7C	0.138	0.016	Downregulated

List of the most significant deregulated miRNAs

Extracellular vesicles (EVs) miRNAs

Robbins and Morelli, 2014



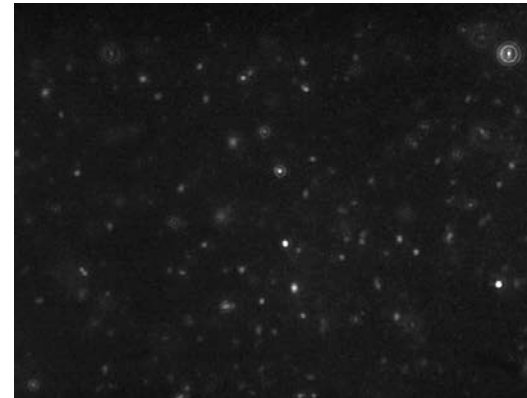
- extracellular vesicles (EVs) are small membrane vesicles containing proteins, lipids and RNA species and can regulate intercellular communication
- many cells in the nervous system release EVs
- miRNAs are one of the EV cargos

Are EV-miRNAs
implicated in ALS
spread?

EVs and miRNAs isolation

EVs miRNAs isolation from:

- iPSC culture medium
- MN culture medium

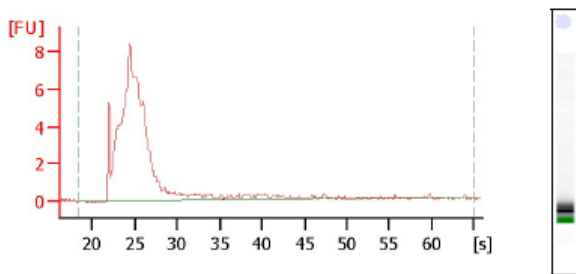


NANOSIGHT:
particle size 100 nm

NANOSIGHT

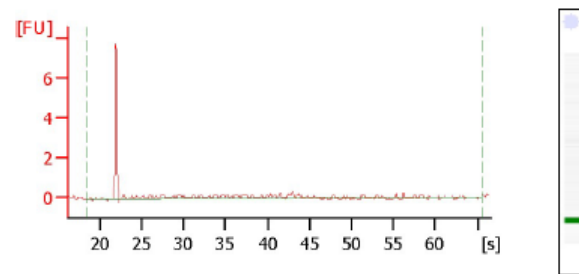
miRNA sample

Blank



Overall Results for sample 8 :

RNA Area:	110,8
RNA Concentration:	530 pg/μl
rRNA Ratio [28s / 18s]:	0,0
RNA Integrity Number (RIN):	2.5 (B.02.07)
Result Flagging Color:	
Result Flagging Label:	RIN: 2.50



Overall Results for sample 9 :

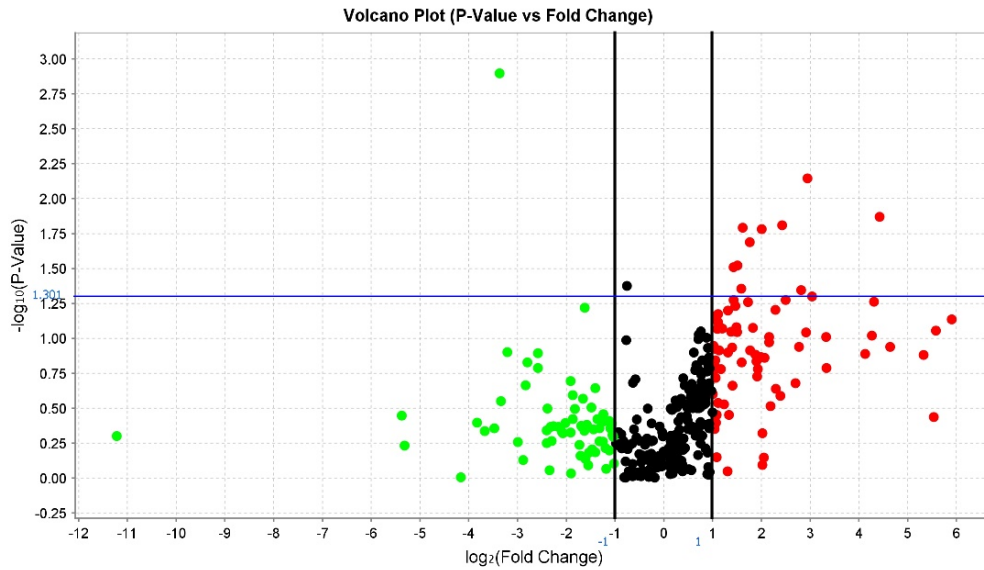
RNA Area:	5,4
RNA Concentration:	26 pg/μl
rRNA Ratio [28s / 18s]:	0,0
RNA Integrity Number (RIN):	1 (B.02.07)
Result Flagging Color:	
Result Flagging Label:	RIN:1

BIOANALYZER



**TaqMan® Array
miRNA cards**

miRNA cards on iPSC-EVs

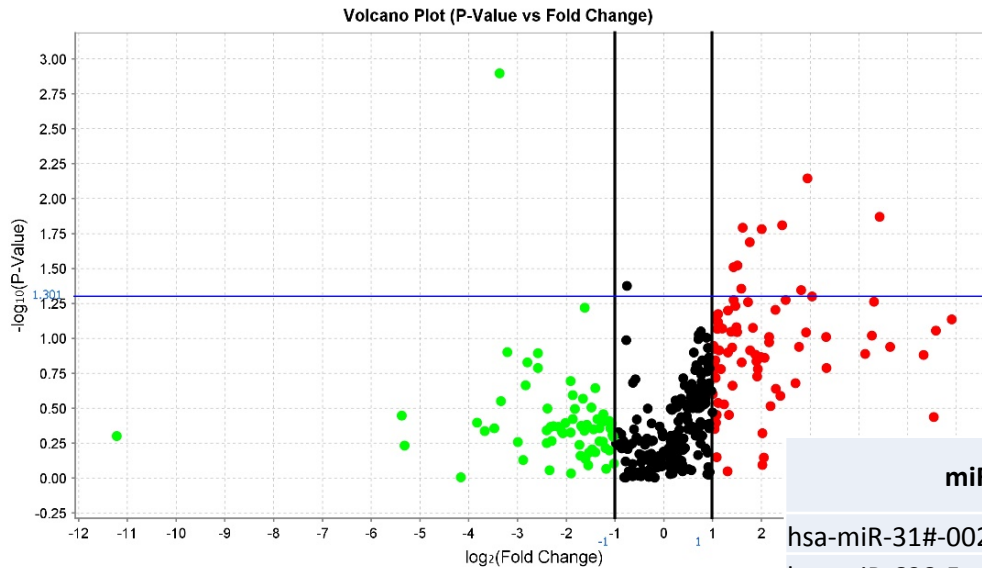


Legend

Study: SLA 2017, EX IPS vs EX IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

Vulcano plot

miRNA cards on iPSC-EVs

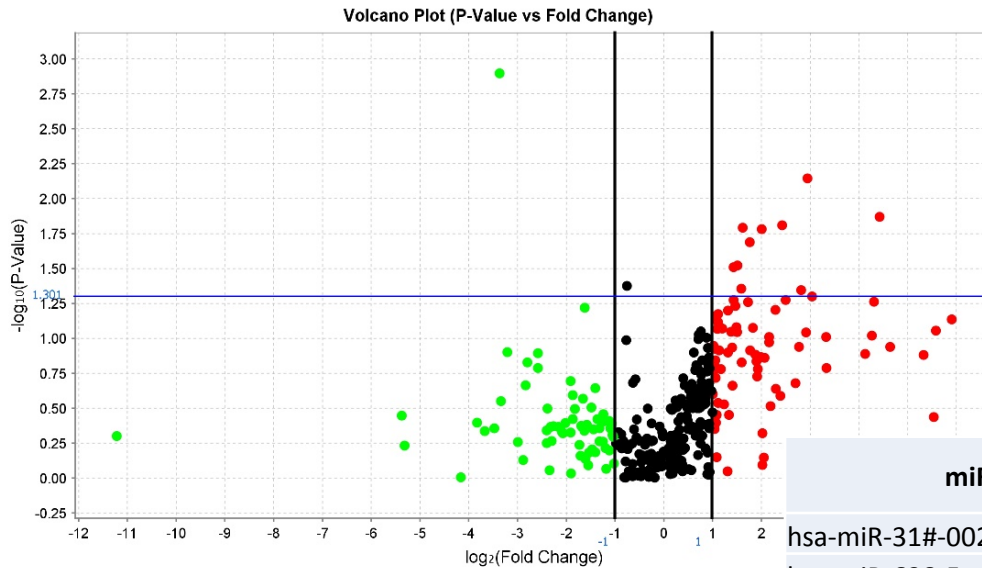


Legend
Study: SLA 2017, EX IPS vs EX IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

Vulcano plot

miRNA	Fold Change	P value	Expression
hsa-miR-31#-002113	0,097	0,001	Downregulated
hsa-miR-628-5p-002433	7,683	0,007	Upregulated
hsa-miR-10b#-002315	21,518	0,014	Upregulated
hsa-miR-19b-1#-002425	5,367	0,015	Upregulated
hsa-miR-671-3p-002322	3,076	0,016	Upregulated
hsa-miR-211-000514	4,019	0,016	Upregulated
hsa-miR-331-000545	3,391	0,020	Upregulated
hsa-miR-95-000433	2,850	0,030	Upregulated
hsa-miR-103-000439	2,700	0,031	Upregulated
hsa-miR-28-3p-002446	0,591	0,042	Downregulated
hsa-miR-454-002323	3,010	0,044	Upregulated
hsa-miR-196b-002215	7,044	0,045	Upregulated
hsa-miR-485-3p-001277	8,222	0,050	Upregulated

miRNA cards on iPSC-EVs



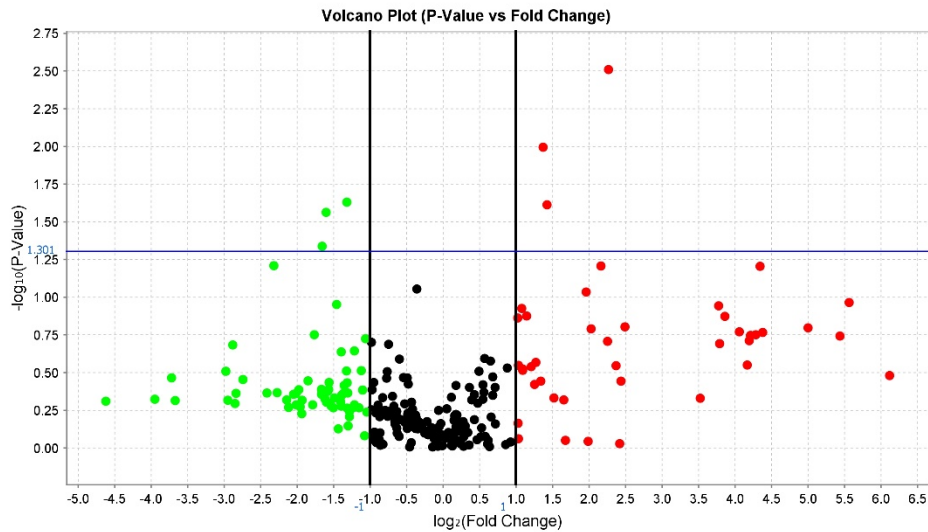
Legend
Study: SLA 2017, EX IPS vs EX IPS CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

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List of the most significant deregulated miRNAs

miRNA cards on MNs-EVs



Legend
Study: SLA 2017, EX MN vs EX MN CT, Fold Change Boundary: 2.0, P-Value Boundary: 0.05

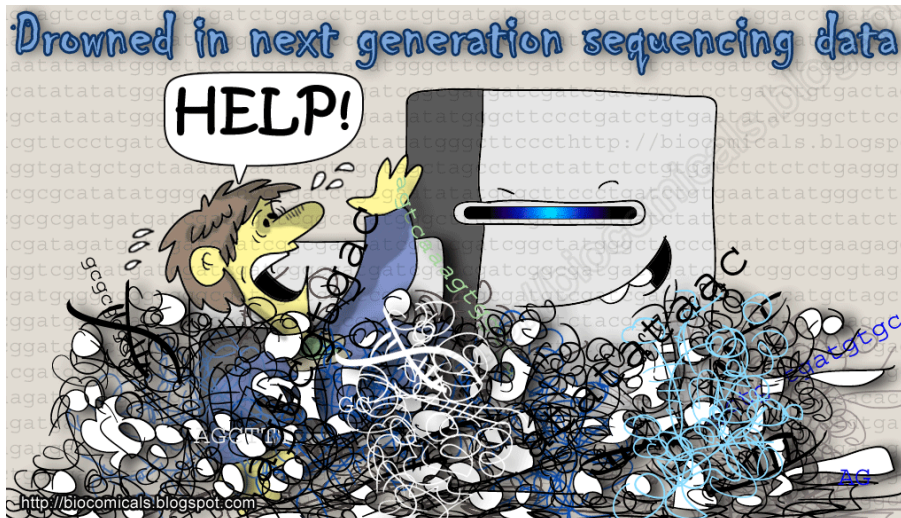
Vulcano plot

miRNA	Fold Change	P value	Expression
hsa-miR-629-002436	4,807	0,003	Upregulated
hsa-miR-194-000493	2,587	0,010	Upregulated
hsa-miR-625#-002432	0,401	0,023	Downregulated
hsa-miR-192-000491	2,687	0,024	Upregulated
hsa-miR-9-000583	0,329	0,027	Downregulated
hsa-miR-1267-002885	0,318	0,046	Downregulated

List of the most significant deregulated miRNAs

Bioinformatic analysis

- ❖ mirGate and mirtarbase database to screen for predicted genes targeted by identified miRNAs;
- ❖ Gene Ontology Enrichment analysis to identify biological processes affected;
- ❖ Reactome analysis to identify molecular pathways (and related genes) regulated by identified miRNAs.



Giuseppe Filosa

miR-129-5p is upregulated in ALS

Spinal cord of SOD1G93A mice and PBMCs of sALS patients

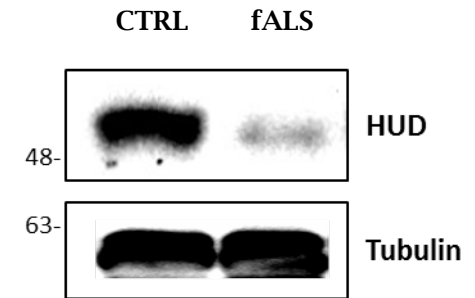
3' cguucgggucuggcGUUUUUc 5'

miR-129-5p



5' agaaaaacaaaaaCAAAAa 3'

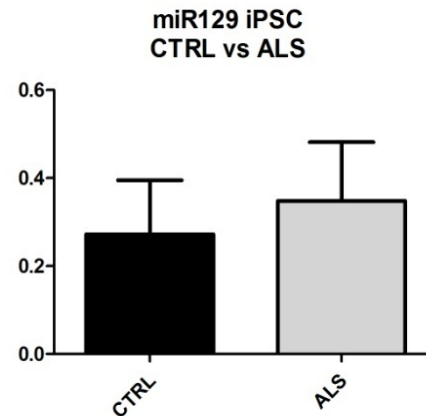
ELAVL4/HuD



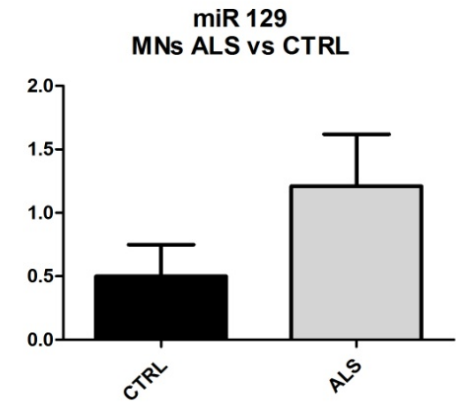
MiR-129-5p target gene HuD is downregulated in ALS

Pathways regulated by HuD

- ✓ Neuronal cell identity
- ✓ Neuronal maturation
- ✓ Neuronal survival



qPCR miR129-5p in iPSCs



qPCR miR129-5p in MNs

Next steps

TARGET IDENTIFICATION

- ❖ To complete the analysis with the last four MNs lines and to compare results among the different groups
- ❖ To define miRNAs target genes and pathways by the bioinformatical analysis

TARGET MODULATION

- ❖ To modulate targets *in vitro* on ALS iPSC-derived MNs to evaluate their potential therapeutic effect
- ❖ To modulate targets *in vivo* by AAV9-mediated over-expression or by Morpholino-mediated silencing into the SOD1G93A mouse model to study their neuropathological or neuroprotective role

**Elucidate ALS pathogenesis and
find therapeutic targets**

Acknowledgments

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Giulia Forotti

Sabrina Salani



University of Milan

Laura Dioni

Valentina Bollati

University of Milano-Bicocca

Giuseppe Filosa

Silvia Barabino



Thank you!