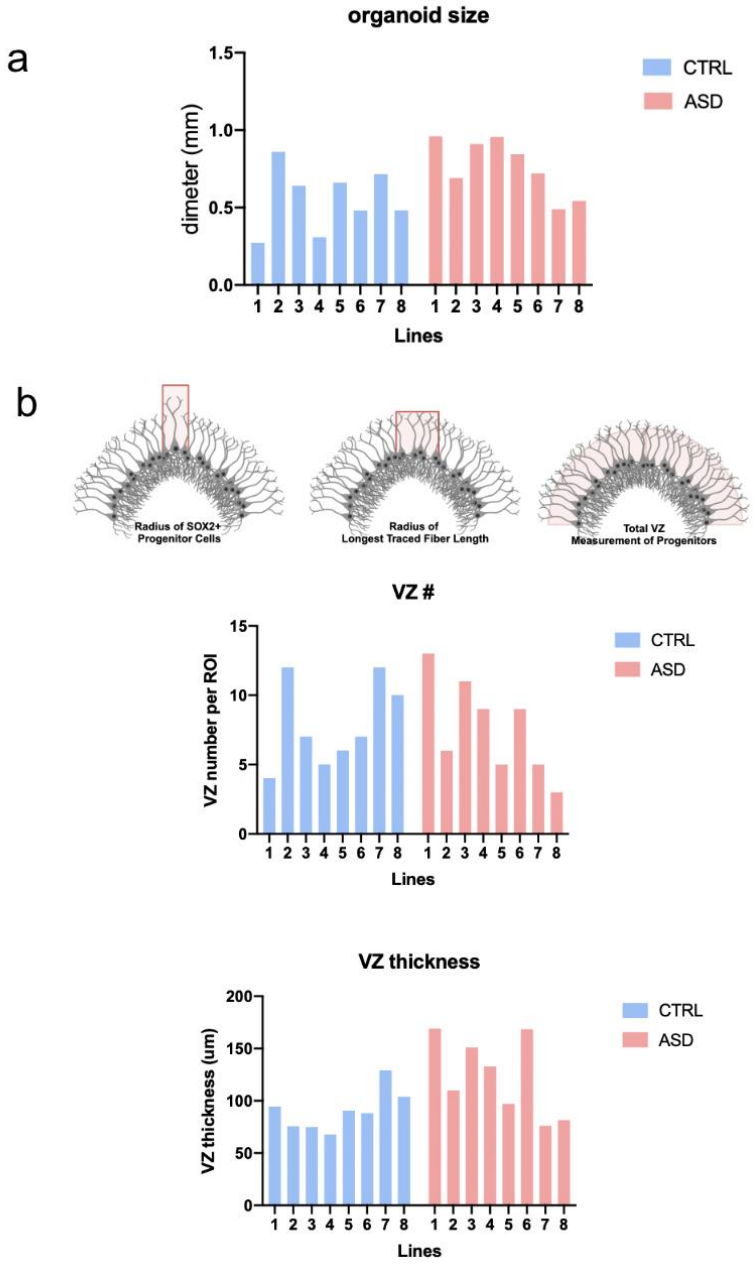
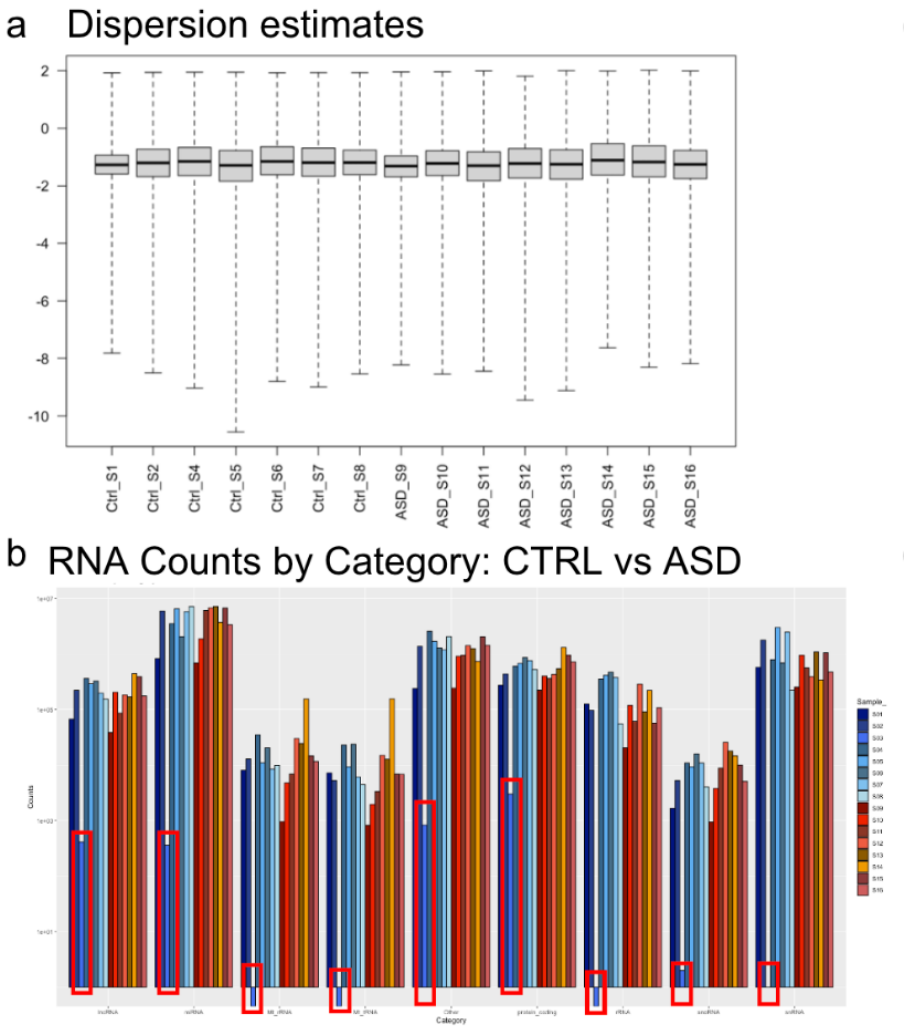


Supplementary Figure 1. CTRL and ASD organoid size
a) Bar graph depicting average organoid size as measured by diameter (mm) in 8 CTRL and 8 ASD lines. Each bar graph represents an average measurement of 6-8 different organoids from the same line. **b)** Top: Schematic of analysis of ventricular zone (VZ) thickness - briefly, ventricular zone, considered as SOX2+ area, thickness was measured by averaging the radius of the longest traced SOX2+ progenitor cells. Bottom: Quantifications depict VZ numbers and VZ thickness across 16 lines used in the study. Each bar graph represents an average measurement of at least 3 organoids (VZ #) or 3 VZ (thickness measurements).



Supplementary Figure 2. EV cargo RNA variability
a) Box plot depicting consistent dispersion estimates across 16 lines used in the study. **b)** Bar graph depicting RNA counts by different RNA types across 16 lines. Each bar represents a different line used in the study. CTRL line 3 (marked by red rectangle) was a significant outlier and was excluded from further bioinformatic analysis.

Top 20 differentially expressed genes by significance

Gene	baseMean	log2FoldChange	lfcSE	stat	p-value	adj p-value	Gene ID
LRRN2	43.11	6.52	1.11	5.87	0.00	0.00	ENSG00000170382
ATP6V1B2	114.86	7.27	1.34	5.41	0.00	0.00	ENSG00000147416
EML4	54.46	5.67	1.12	5.07	0.00	0.00	ENSG00000143924
TTC4	22.73	-7.33	1.47	-4.99	0.00	0.00	ENSG00000243725
PES1	50.31	6.32	1.32	4.78	0.00	0.00	ENSG00000100029
CMTM1	20.78	6.24	1.39	4.50	0.00	0.01	ENSG00000089505
SLC5A11	15.27	4.97	1.12	4.43	0.00	0.01	ENSG00000158865
SP140	11.56	6.40	1.46	4.39	0.00	0.01	ENSG00000079263
DOCK11	13.39	-4.21	0.98	-4.30	0.00	0.01	ENSG00000147251
NARS1	53.78	-5.59	1.32	-4.24	0.00	0.01	NA
LIPM	74.37	-6.19	1.46	-4.25	0.00	0.01	ENSG00000173239
DYRK2	15.80	-6.13	1.46	-4.20	0.00	0.01	ENSG00000127334
KIAA2012	19.16	5.61	1.34	4.18	0.00	0.01	ENSG00000182329
OPRD1	41.06	4.41	1.07	4.13	0.00	0.01	ENSG00000116329
NELFA	48.94	5.30	1.29	4.11	0.00	0.01	ENSG00000185049
MATN3	23.92	5.77	1.40	4.12	0.00	0.01	ENSG00000132031
ANAPC13	12.26	-6.78	1.65	-4.11	0.00	0.01	ENSG00000129055
DNAJA4	14.75	4.76	1.16	4.10	0.00	0.01	ENSG00000140403
PLEKHH1	50.55	-5.86	1.44	-4.07	0.00	0.01	ENSG00000054690
PALD1	32.50	-4.88	1.21	-4.04	0.00	0.01	ENSG00000107719

Top 20 differentially expressed miRNAs by significance

mIcroRNA	baseMean	log2FoldChange	lfcSE	stat	p-value	adj p-value	Gene ID
MIR6724-4	17.67	-7.42	1.65	-4.51	0.00	0.01	ENSG00000275692
MIR6724-3	17.45	-7.16	1.66	-4.31	0.00	0.01	ENSG00000277379
MIR4676	19.34	-7.85	1.88	-4.17	0.00	0.01	ENSG00000266719
MIR6724-2	17.18	-6.94	1.70	-4.09	0.00	0.01	ENSG00000274060
MIR6724-1	16.92	-6.75	1.84	-3.68	0.00	0.03	ENSG00000275950
MIR4440	27.03	-6.07	1.68	-3.61	0.00	0.03	ENSG00000266109
MIR508	15.50	-6.36	1.77	-3.60	0.00	0.03	ENSG00000207589
MIR370	6.07	-6.19	1.74	-3.55	0.00	0.03	ENSG00000199005
MIR2116	4.68	-5.81	1.71	-3.40	0.00	0.04	ENSG00000253030
MIR4286	102.09	-2.94	0.95	-3.10	0.00	0.07	ENSG00000263762
MIR548Q	18.67	-5.50	1.77	-3.10	0.00	0.07	ENSG00000221331
MIR1263	90.31	7.70	2.84	2.71	0.01	0.13	ENSG00000221251
MIR5699	15.76	-2.46	0.96	-2.56	0.01	0.16	ENSG00000263511
MIR4677	4.26	-4.24	1.66	-2.55	0.01	0.16	ENSG00000265201
MIR548J	5.01	4.53	1.82	2.49	0.01	0.18	ENSG00000221760
MIR3943	24.56	3.68	1.50	2.46	0.01	0.19	ENSG00000264069
MIR4443	3.88	-3.03	1.24	-2.45	0.01	0.19	ENSG00000265483
MIR489	54.47	3.39	1.45	2.34	0.02	0.21	ENSG00000207656
MIR5587	8.09	3.78	1.69	2.24	0.02	0.24	ENSG00000266124
MIR3180-4	25.37	2.51	1.12	2.24	0.03	0.24	ENSG00000257391

Supplementary Figure 3. Top 20 differentially regulated coding and miRNAs in ASD EVs compared to CTRL EVs

Top: Table depicting top 20 differentially expressed coding genes (ASD vs CTRL), ordered by their significance ($p < 0.05$). Bottom: Table depicting top 20 differentially expressed miRNAs (ASD vs CTRL), ordered by their significance ($p < 0.05$).

Supplementary Table. Purchase source and clinical notes of CTRL* and ASD iPSC lines used in the study.**

ID	Source	Information
GM23279	Coriell	**CTRL**, female, Hispanic, sample collected at age 29
MH0159019	NIMH	**CTRL**, female, White, sample collected at age 36
MH0159020	NIMH	**CTRL**, male, White, sample collected at age 58
MH0159021	NIMH	**CTRL**, male, Hispanic, sample collected at age 32
MH0174677	NIMH	**CTRL**, male, White, male, white, sample collected at age 9
MH0174679	NIMH	**CTRL**, male, White, male, white, sample collected at age 17
MH0174681	NIMH	**CTRL**, male, White, male, white, sample collected at age 8
MH0174686	NIMH	**CTRL**, male, White, sample collected at age 17
CW20008	CIRM	**ASD**; male, Asian, sample collected at age 5, low IQ (68), communication and social deficits (ADOS score 16) as well as stereotyped behaviors and restricted interests (ADOS score 5)
CW20142	CIRM	**ASD**; male, White, sample collected at age 15, low IQ (40), poor eye contact, communication and social deficits (ADOS score 18) as well as stereotyped behaviors and restricted interests (ADOS score 5)
CW60115	CIRM	**ASD**; male, White, sample collected at age 4, poor eye contact, social deficits,

		stereotyped behaviors and restricted interests (ADOS score 3)
CW20083	CIRM	**ASD**; male, White, sample collected at age 22, poor eye contact, severe communication and social deficits (ADOS score 18) repetitive behavior (ADOS score 8)
MH0148698	NIMH	**ASD**; male, White, low IQ, severely affected (Mariani et al., 2015, Allen et al., 2022)
MH0148713	NIMH	**ASD**; male, White, low IQ, severely affected (Mariani et al., 2015, Allen et al., 2022)
CW20026	CIRM	**ASD**; male, White, sample collected at age 10, low IQ (52), communication and social deficits (ADOS score 19) as well as stereotyped behaviors and restricted interests (ADOS score 3)
CW20044	CIRM	**ASD**; male, White, sample collected at age 10, low IQ (40), spinning, severe communication deficits (ADOS score 16) as well as stereotyped behaviors and restricted interests (ADOS score 7)

* All controls had no history or reported family history of ASD.

** ADOS stands for Autism Diagnostic Observation Schedule. ADOS testing is a standardized assessment tool designed to evaluate and observe social communication, interaction, and behavior in individuals suspected of having ASD. It is considered the “gold standard” for assessment.