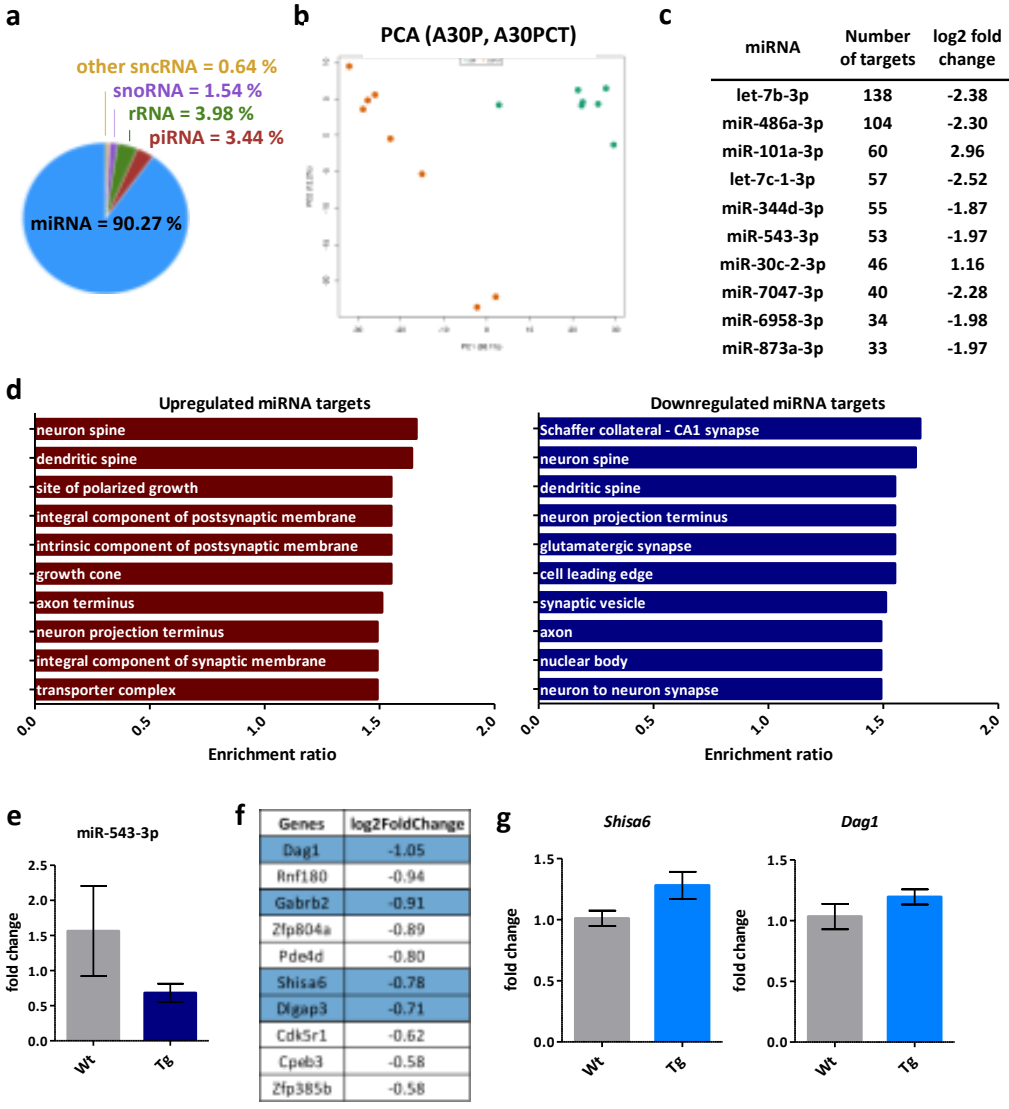
Supplementary Material

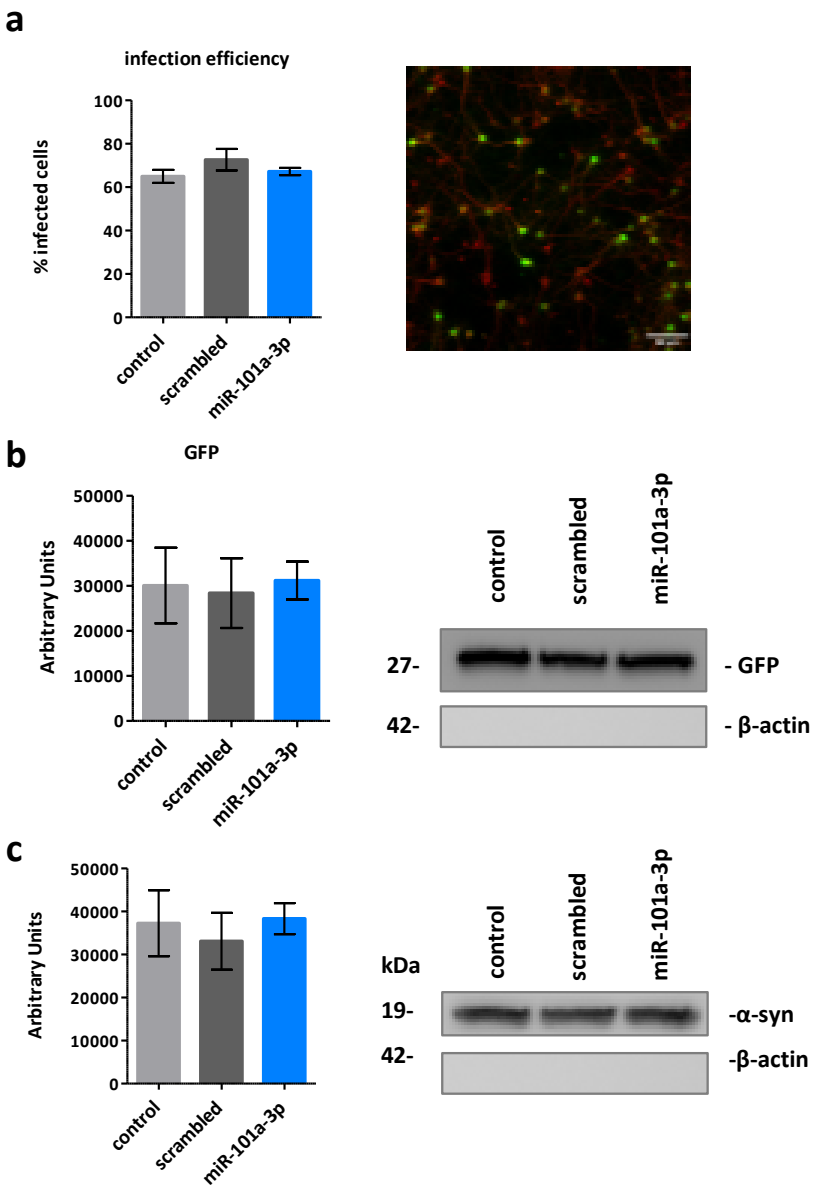
miR-101a-3p Impairs Synaptic Plasticity and Contributes to Synucleinopathy



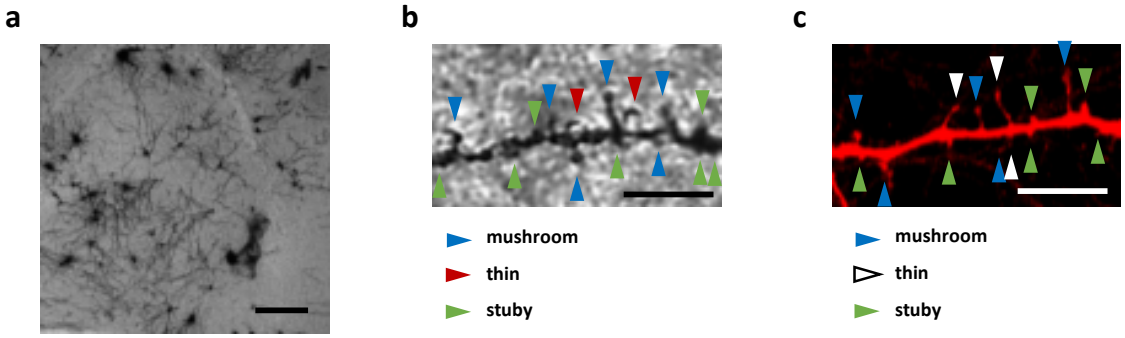
miRNA	Number of targets	log2 fold change	Number of reads
let-7b-3p	138	-2.38	23.08
miR-486a-3p	104	-2.30	13.74
miR-101a-3p	60	2.96	1424.29
let-7c-1-3p	57	-2.52	13.13
miR-344d-3p	55	-1.87	315.66
miR-543-3p	53	-1.97	2133.82
miR-30c-2-3p	46	1.16	479.59
miR-7047-3p	40	-2.28	4.13
miR-6958-3p	34	-1.98	3.79
miR-873a-3p	33	-1.97	101.72

Genes	log2FoldChange
Dag1	-1.05
Rnf180	-0.94
Gabrb2	-0.91
Zfp804a	-0.89
Pde4d	-0.80
Shisa6	-0.78
Dlgap3	-0.71
Cdk5r1	-0.62
Cpeb3	-0.58
Zfp385b	-0.58

Supplementary Figure 1 miRNAome changes revealed in midbrain of [A30P] αsyn mice by small-RNA-seq. a) Pie chart depicting average percentages of the different RNA classes detected in the small RNA libraries as a readout for quality of the sequencing technique; b) Principal component analysis (PCA) plots showing the clustering of Wt (orange) and Tg (green); c) Table of the top interacting miRNAs according to number of confirmed target genes, including log2 fold change and number of reads; d) Enrichment ratio of overrepresented Gene Ontology terms of upregulated and downregulated miRNA targets; e) Real time qPCR validation of miR-543-3p levels in Wt (n = 8) and Tg (n = 7) mouse midbrain; f) List of the top 10 downregulated miR-101a-3p target genes, genes relative to post-synapses are marked; g) Real time qPCR assessment of miR-101a-3p target genes Shisa6 and Dag1 levels in Wt (n = 8) and Tg (n = 7) mouse midbrain



Supplementary Figure 2. Lentiviral induction of miR-101a-3p *in vitro*. a) Quantification of infected neurons in mass primary cultures expressed as the percentage of GFP positive cells to total neuron number and a representative image of infected mass cultures (infection with control vector is depicted); GFP - green; MAP2 - red; scale bar = 50 µm. b) Quantification of GFP levels in infected neurons by immunoblotting analysis and representative immunoblots. c) Quantification of α-syn levels in infected neurons by immunoblotting analysis and representative immunoblots. All data are expressed as mean ± SEM; Student's t-test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)



Supplementary Figure 3. Dendritic spine classification. a) Representative Golgi-Cox staining image of the analyzed brain region in Tg mouse; brightfield; scale bar = 200 µm; b) Magnification of dendritic spine segment with classification to mushroom, thin and stubby; brightfield; scale bar= 10 µm; c) Magnification of dendritic spine segment *in vitro* with classification to mushroom, thin and stubby; MAP2 - red; scale bar= 10 µm

Supplementary Table 1. Human samples

Case Number	NBTR Number	Diagnosis	age	gender	PMD	pH	Braak NFT	LBs	Other pathologies
26383	19830263	Control	76	F	24	6.11	0	No	-
30783	19830307	Control	93	F	10	6.27	III	No	Primary visual infarct
98089	19890980	Control	85	M	23	#	I	-	-
87887	19870878	Control	81	F	32	6.23	-	-	-
96288	19880962	Control	34	F	103	#	-	-	-
103688	19881036	Control	79	F	26	6.4	-	-	-
103689	19891036	Control	79	F	26	6.4	II	-	-
5690	19900056	Control	51	M	25	#	-	-	-
1991	19910019	Control	54	M	12	#	-	-	-
22991	19910229	Control	53	M	12	#	-	-	-
29391	19910293	Control	65	F	17	#	0	-	-
31991	19910319	Control	67	M	36	6.56	-	-	-
2793	19930027	Control	84	F	7	6.55	IV	-	-
5095	19950050	DLB	76	F	23	6.03	II	Neocortical	-
20292	19920202	DLB	66	M	31	6.54	0	Limbic	-
10498	19980104	DLB	86	M	41	6.08	II	Neocortical	-
13798	19980137	XCBD	85	M	39	6.23	II	No	-
131 96	19960131	DLB	77	F	23	6.24	IV	Neocortical	-
703	20030007	DLB	88	F	16	5.92	III	Neocortical	Right cerebellar infarct
10504	20040105	PDD/DLB	68	M	11	6.15	V	Neocortical	-

Supplementary Table 2. Primer List

Primers	5'-3' Sequence/ID	Company
***Actb* forward**	GCG AGA AGA TGA CCC AGA TC	Metabion
***Actb* reverse**	CCA GTG GTA CGG CCA GAG G	Metabion
***Gabrb2* forward**	GCC TGC ATG ATG GAC CTA AG	Metabion
***Gabrb2* reverse**	CCT GTG GAG AAA ACA ACT TTC TTG	Metabion
***Dlgap3* forward**	GCT CCT CCT TCA ACT TCA GA	Metabion
***Dlgap3* reverse**	GGA CTG GCT CGG GGT GG	Metabion
***Dag1* forward**	TTG ACA GGG TAG ATG CCT GG	Metabion
***Dag1* reverse**	ATA CAT GAG CTG GCT GTT GG	Metabion
***Shisa6* forward**	AGT TCG AGT GCA ACA ACA GC	Metabion
***Shisa6* reverse**	AGT TGG TCT TGT CCT TCT CC	Metabion
Hs_RNU6-2_11 miScript Primer Assay	MS00033740	Qiagen
Mm_miR-101a_3 miScript Primer Assay	MS00011011	Qiagen