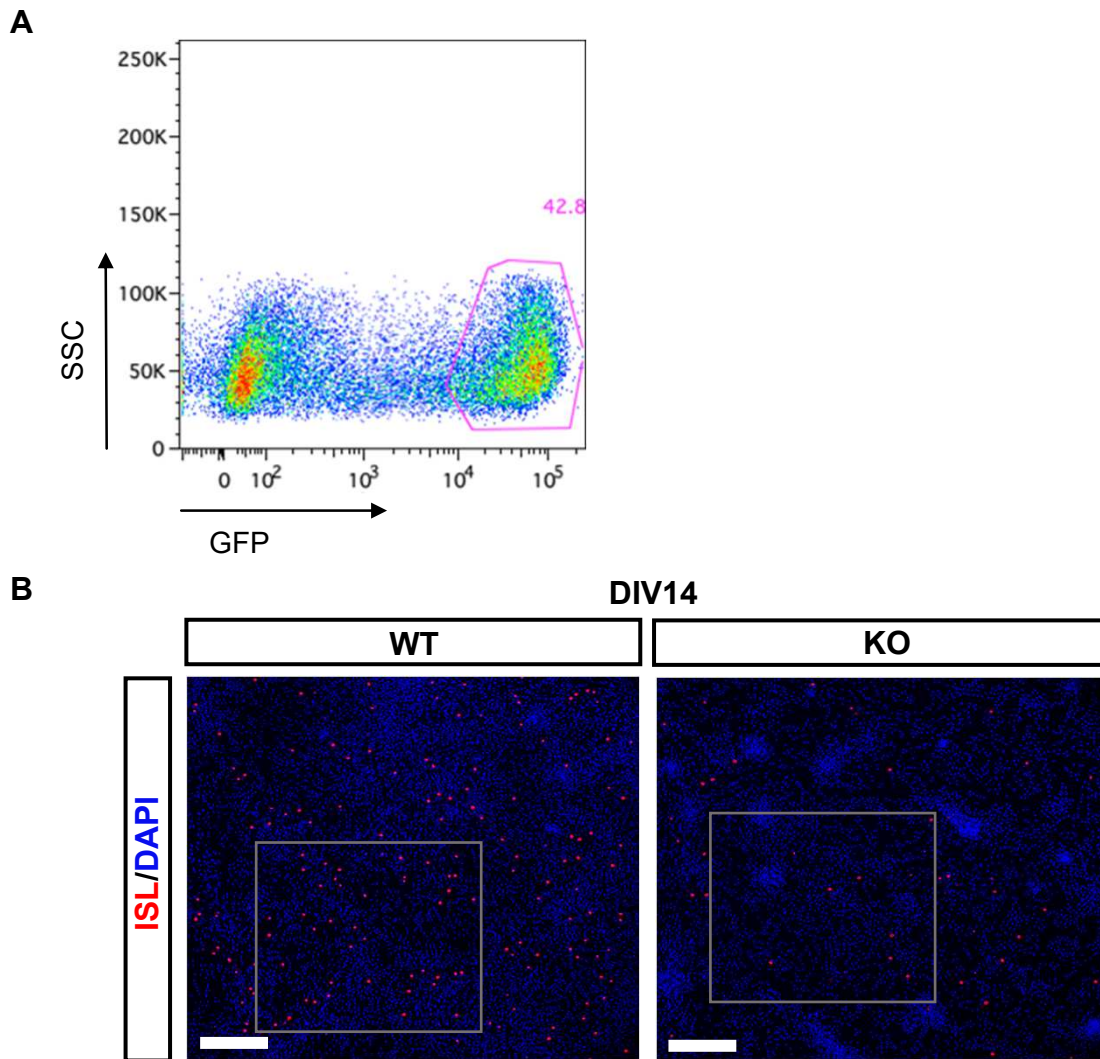


APPENDIX MATERIAL

- **Appendix Figures S1-5 (pages 2-6)**
- **Appendix Figure S1-5 Legends (pages 2-7)**
- **Appendix Tables S1-2 (pages 8-9)**

Appendix Figure S1 (Relative to Figure 1).

2

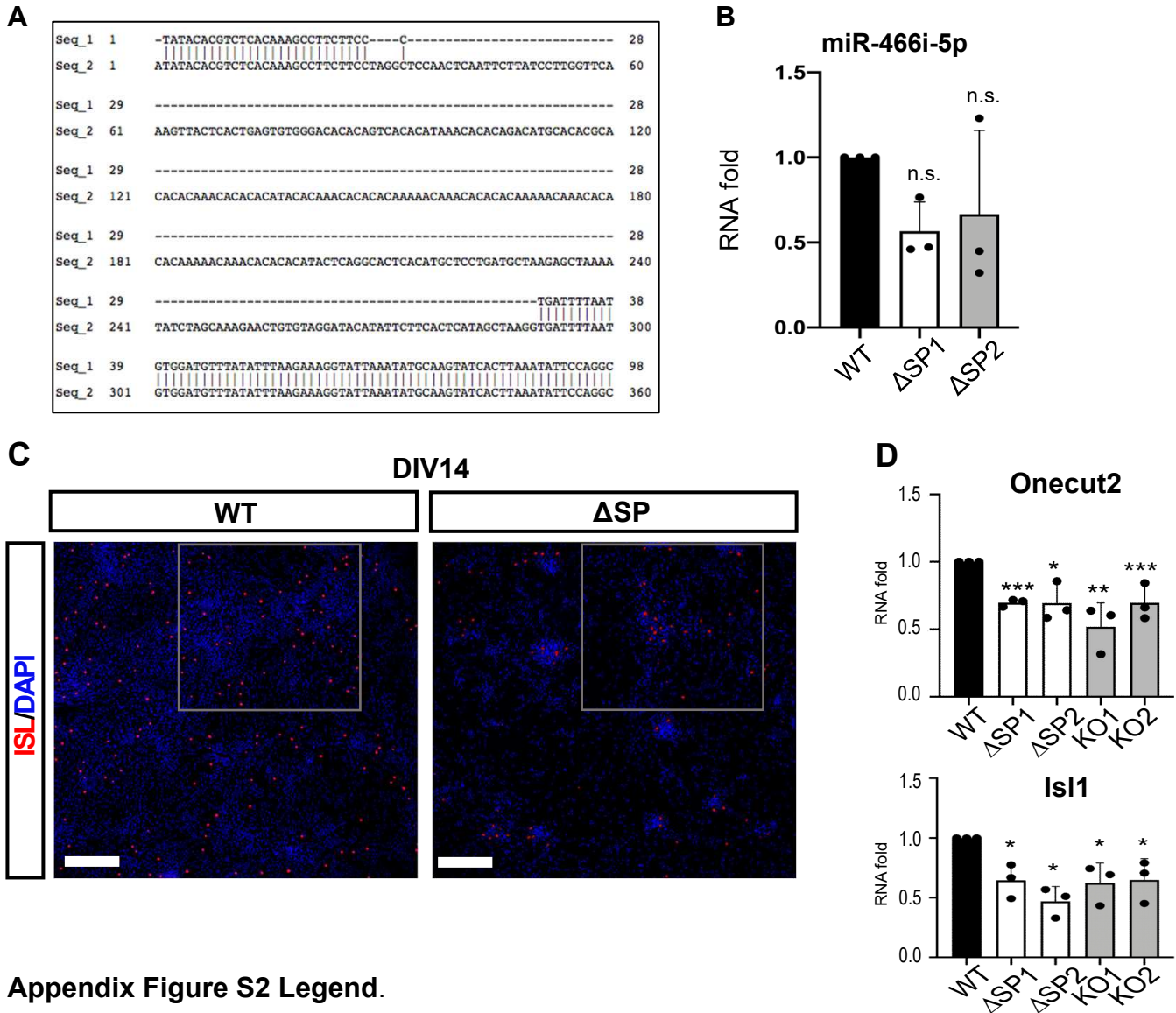


Appendix Figure S1 Legend.

A. Cytofluorimetric determination (side scatter vs GFP detection) of cells derived from WT EB6 dissociation. The MN percentage is indicated in violet.

B. Full size field (1,3x1,3mm) of images of **Figure 1E**. Gray squares indicate the field (0,667x0,667mm) shown in the main figure. DAPI in blue, Isl1/2 in red. Scale bar = 200mm

Appendix Figure S2 (Relative to Figure 3).



Appendix Figure S2 Legend.

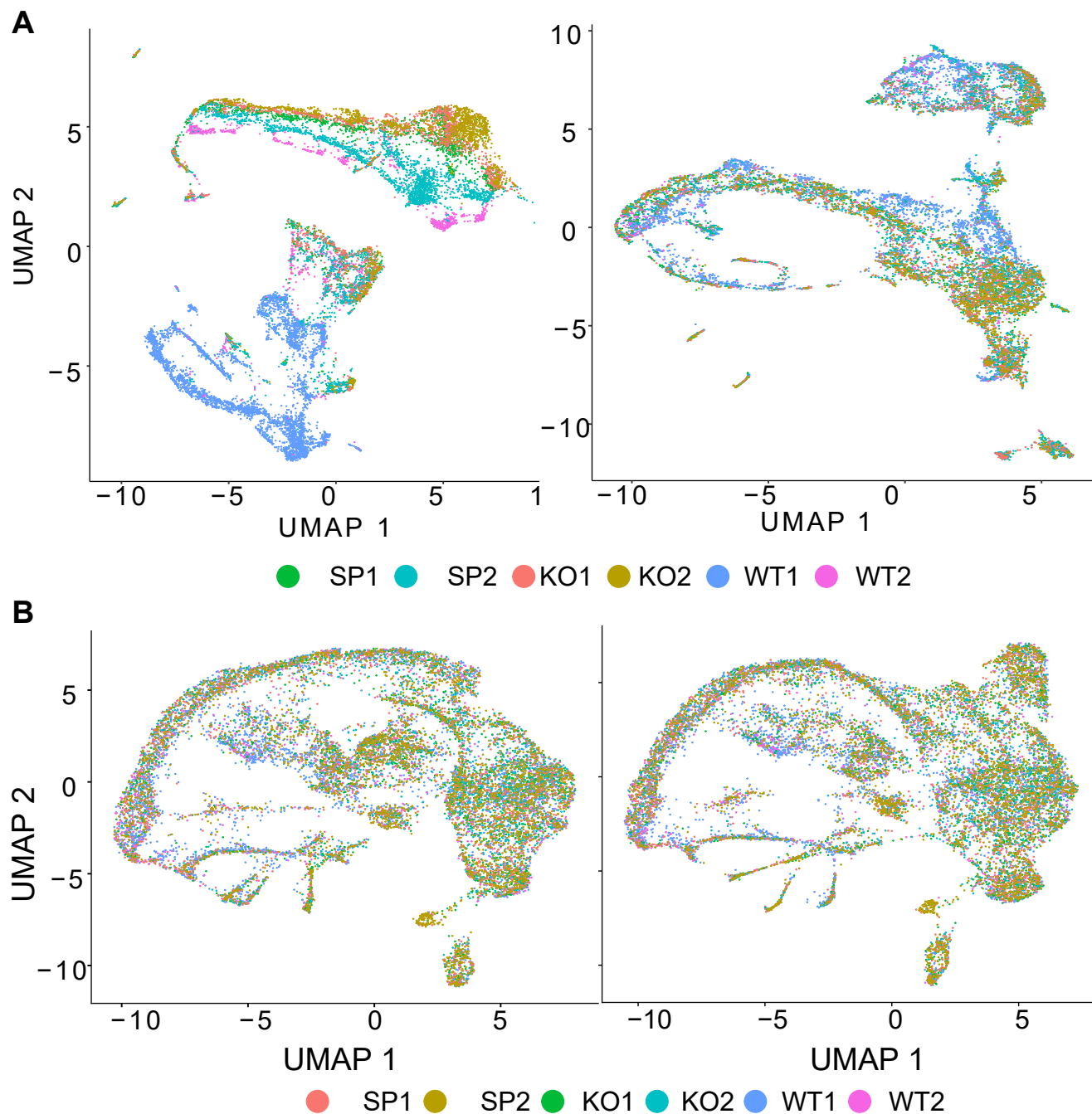
A. Alignments of IncMN2-203 WT (Seq_2) and ΔSP (Seq_1) sequences highlight a 260 bp-long genomic deletion.

B. qRT-PCR expression analysis of miR-466i-5p in WT, ΔSP1 and ΔSP2 EB6. Data, that are expressed as mean (error bars represent SD), are normalized over *snoRNA U25* levels and are expressed as RNA fold over the control (WT EB6), set as 1. N=3. biological replicates.

C. Full size field (1,3x1,3mm) of images of **Figure 3D**. Gray squares indicate the field (0,667x0,667mm) shown in the main figure. DAPI in blue, *Isl1/2* in red. Scale bar = 200μm.

D. qRT-PCR expression analysis of *Onecut2* and *Isl1* in WT and mutant EB6 (KO1, KO2, ΔSP1 and ΔSP2). Data, that are expressed as mean (error bars represent SD), are normalized Data (means ± SD) were normalized over *Atp5o* mRNA levels and expressed as RNA fold over the control (WT), set as 1. N=3 biological replicates.

Data information: histogram dots represent single replicates. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; n.s. > 0.05 (two-tailed, unpaired Student's t-test).

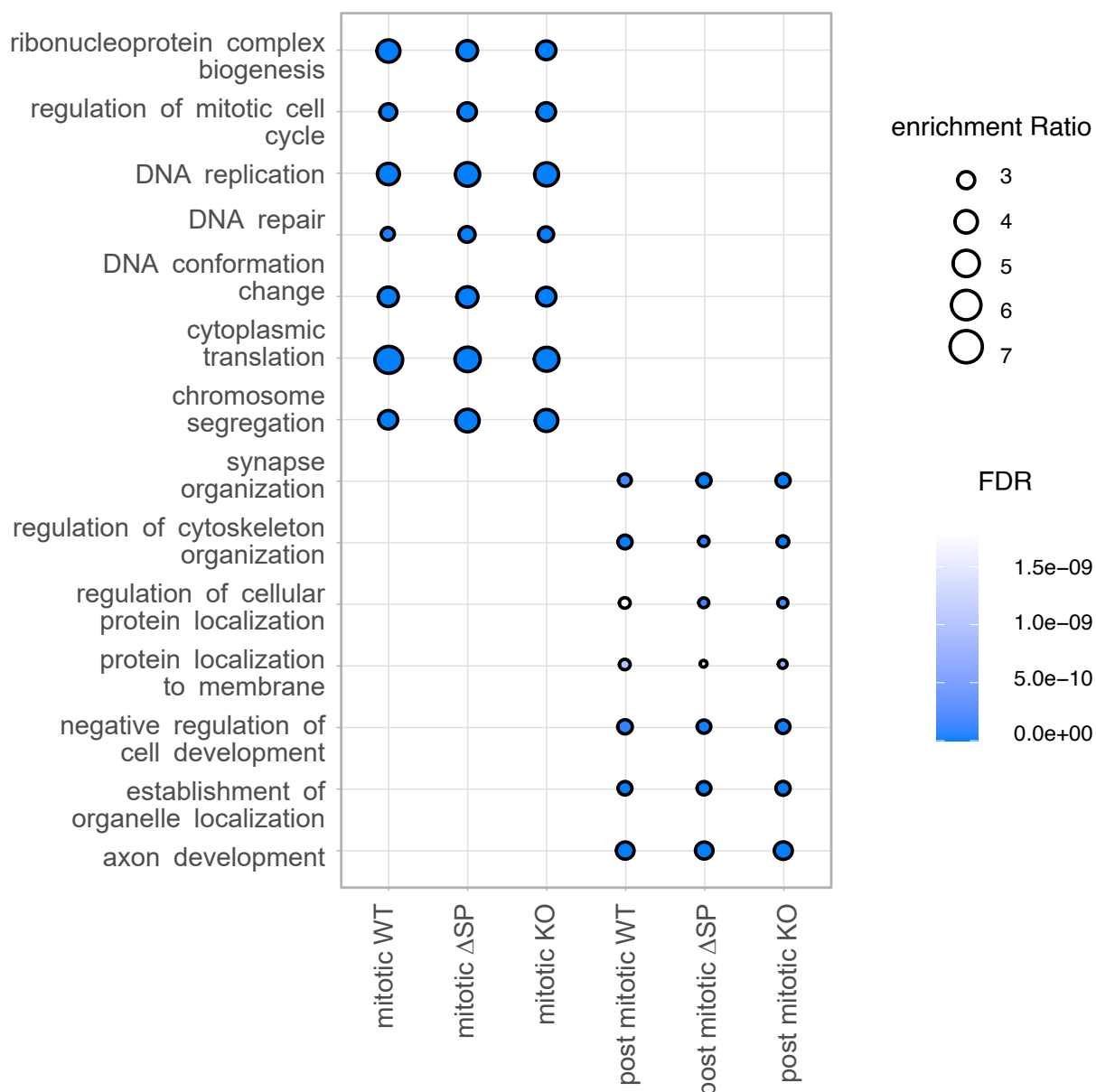
**Appendix Figure S3 Legend.**

A. UMAP plots of the whole dataset before (left) and after (right) dataset integration using anchors were performed by Seurat.

B. UMAP plots of the whole dataset before (left) and after (right) batch correction performed by Monocle (panel A) softwares following the official documentation of Monocle software using the *combine_cds* and *align_cds* functions. The incremented overlap after dataset integration confirms that both algorithms are able to minimize possible batch effects.

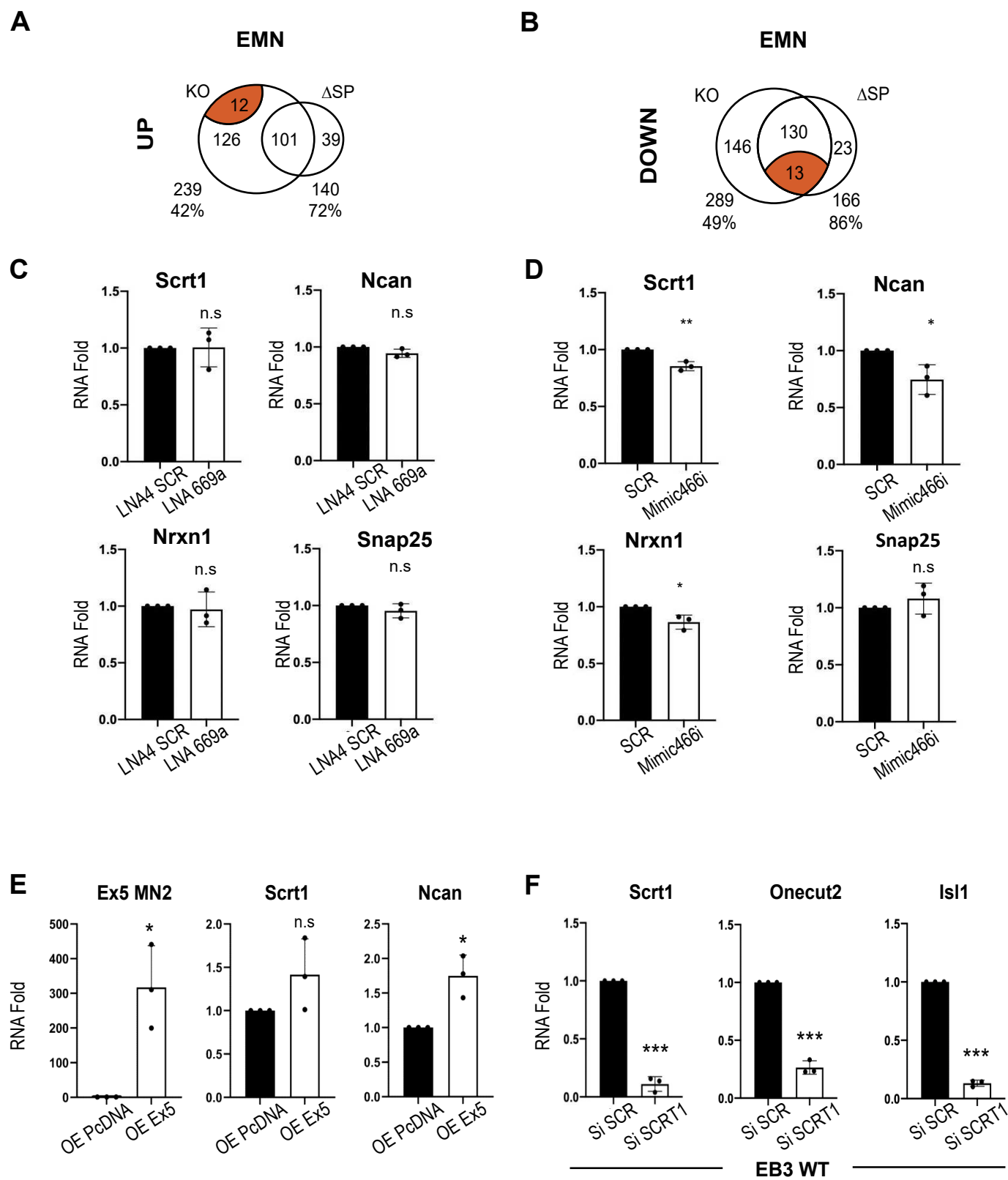
Appendix Figure S4 (Relative to Figure 4).

5



Appendix Figure S4 Legend.

Dotplot representing for each condition (WT, Δ SP and KO) the GO enriched categories related to differentially expressed genes between proliferating and post-mitotic cells (Dataset EV2 and EV3). GO enrichment analysis was performed using WebGestalt software with weighted set cover dimensionality reduction. Categories not in common among conditions were filtered out.



Appendix Figure S5 Legend.

A. Venn diagram showing the relationships between genes up-regulated in KO EMN and Δ SP EMN mutants. Gene numbers and percentages of overlap are shown below diagrams for both groups. The intersection between miR-325-3p and miR-384-5p predicted targets and genes specifically up-regulated in KO mutants are indicated in orange.

B. Venn diagram showing the relationships between genes down-regulated in KO EMN and Δ SP EMN. Gene numbers and percentages of overlap are shown below diagrams for both groups. The intersection between miR-466i-5p predicted targets and genes down-regulated in both mutants are indicated in orange.

C. qRT-PCR expression analysis of *Scrt1*, *Ncan* (up) and *Nrxn1*, *Snap25* (down) in NSC-34 cells transfected with miR-669a-5p LNA. Data, that are expressed as mean (error bars represent SD), are normalized over *Atp5o* mRNA levels and expressed as RNA fold over the control (scrambled LNA, CTRL), set as 1. N=3 biological replicates.

D. qRT-PCR expression analysis of *Scrt1*, *Ncan* (up) and *Nrxn1*, *Snap25* (down) in NSC-34 cells transfected with miR-466i-5p Mimic. Data, that are expressed as mean (error bars represent SD), are normalized over *Atp5o* mRNA levels and expressed as RNA fold over the control (scrambled, CTRL), set as 1. N=3 biological replicates.

E. qRT-PCR analysis of *IncMN2* (left) *Scrt1* (middle) and *Ncan* (right) upon ectopic expression of *IncMN2-Ex5* in NSC-34 cells. Data, that are expressed as mean (error bars represent SD), are normalized over *Atp5o* mRNA levels and are relative to cells transfected with the empty vector, set to 1. N=3 biological replicates.

F. qRT-PCR analysis of *Scrt1* (left), *Onecut2* (middle) *Isl1* (right) upon depletion of *Scrt1* in EB3

Appendix Table S1.

MicroRNAs binding sites predicted by Miranda algorithm on lncMN2-203 isoform

Seq1 is microRNA Id from miRBase 22; Seq2 is the Id of targeted transcript ; Score of the base pair (bp); Energy of the bp; Position of the bp in microRNA sequence; Position of the bp in target sequence; Length of the interaction; Flag of bp localization inside lncMN2-203 accessible region

Seq1	Seq2	Score	Energy	Position1	Position2	LenInt	lncMN2-203 Accessible Region
mmu-miR-466i-5p	lncMN2-203	182	-32.83	2 19	1891 1910	17	YES
mmu-miR-466i-5p	lncMN2-203	178	-31.31	2 19	1907 1926	17	YES
mmu-miR-466i-5p	lncMN2-203	158	-23.71	3 19	1863 1882	16	YES
mmu-miR-466i-5p	lncMN2-203	166	-22.98	2 19	1923 1942	17	YES
mmu-miR-466i-5p	lncMN2-203	166	-22.98	2 19	1939 1958	17	YES
mmu-miR-466i-5p	lncMN2-203	166	-22.98	2 19	1955 1974	17	YES
mmu-miR-466i-5p	lncMN2-203	140	-22.62	2 17	1756 1775	15	NO
mmu-miR-669a-5p	lncMN2-203	174	-30.51	3 23	1880 1903	20	YES
mmu-miR-669a-5p	lncMN2-203	142	-19.29	2 23	1954 1978	22	YES
mmu-miR-669a-5p	lncMN2-203	158	-19.2	3 23	1906 1929	20	YES
mmu-miR-669a-5p	lncMN2-203	158	-18.51	3 23	1922 1945	20	YES
mmu-miR-669a-5p	lncMN2-203	158	-18.51	3 23	1938 1961	20	YES
mmu-miR-669p-5p	lncMN2-203	174	-30.51	3 23	1880 1903	20	YES
mmu-miR-669p-5p	lncMN2-203	142	-19.29	2 23	1954 1978	22	YES
mmu-miR-669p-5p	lncMN2-203	158	-19.2	3 23	1906 1929	20	YES
mmu-miR-669p-5p	lncMN2-203	158	-18.51	3 23	1922 1945	20	YES
mmu-miR-669p-5p	lncMN2-203	158	-18.51	3 23	1938 1961	20	YES
mmu-miR-574-5p	lncMN2-203	165	-29.75	2 22	1958 1980	20	YES
mmu-miR-574-5p	lncMN2-203	153	-29.23	2 22	1888 1910	20	YES
mmu-miR-574-5p	lncMN2-203	153	-28.18	2 22	1904 1926	20	YES
mmu-miR-574-5p	lncMN2-203	149	-25.61	2 22	1872 1894	20	YES
mmu-miR-574-5p	lncMN2-203	141	-23.02	2 22	1920 1942	20	YES
mmu-miR-574-5p	lncMN2-203	141	-23.02	2 22	1936 1958	20	YES
mmu-miR-1187	lncMN2-203	185	-28.62	2 22	1890 1912	20	YES
mmu-miR-1187	lncMN2-203	150	-26.99	2 19	1755 1777	17	NO
mmu-miR-1187	lncMN2-203	176	-25.97	2 22	1952 1976	22	YES
mmu-miR-1187	lncMN2-203	153	-25.53	3 22	1872 1894	19	YES
mmu-miR-1187	lncMN2-203	161	-22.66	3 22	1920 1942	19	YES
mmu-miR-1187	lncMN2-203	161	-22.66	3 22	1936 1958	19	YES
mmu-miR-1187	lncMN2-203	161	-22.27	2 18	1850 1872	16	YES
mmu-miR-466d-5p	lncMN2-203	164	-28.25	2 18	1877 1900	18	YES
mmu-miR-466d-5p	lncMN2-203	148	-16.06	2 18	1903 1926	18	YES
mmu-miR-466d-5p	lncMN2-203	140	-15.44	2 9	1921 1942	7	YES
mmu-miR-466d-5p	lncMN2-203	140	-15.44	2 9	1937 1958	7	YES
mmu-miR-466d-5p	lncMN2-203	140	-15.44	2 9	1953 1974	7	YES
mmu-miR-669n	lncMN2-203	162	-24.97	2 19	1856 1875	17	YES
mmu-miR-669n	lncMN2-203	174	-23.72	2 19	1910 1929	17	YES
mmu-miR-669n	lncMN2-203	170	-23.38	2 19	1884 1903	17	YES
mmu-miR-669n	lncMN2-203	166	-17.44	2 19	1926 1945	17	YES
mmu-miR-669n	lncMN2-203	166	-17.44	2 19	1942 1961	17	YES
mmu-miR-669o-5p	lncMN2-203	145	-24.09	4 22	1882 1904	18	YES
mmu-miR-669o-5p	lncMN2-203	141	-20.26	4 22	1908 1930	18	YES
mmu-miR-669o-5p	lncMN2-203	145	-20.07	2 22	1956 1979	21	YES
mmu-miR-669o-5p	lncMN2-203	141	-19.6	4 22	1924 1946	18	YES
mmu-miR-669o-5p	lncMN2-203	141	-19.6	4 22	1940 1962	18	YES
mmu-miR-466k	lncMN2-203	165	-23.45	2 22	1904 1926	20	YES
mmu-miR-466k	lncMN2-203	166	-22	2 22	1880 1900	20	YES
mmu-miR-466k	lncMN2-203	150	-20.78	2 22	1922 1942	20	YES
mmu-miR-466k	lncMN2-203	150	-20.78	2 22	1938 1958	20	YES
mmu-miR-466k	lncMN2-203	150	-20.78	2 22	1954 1974	20	YES

Appendix Table S2.

List of oligos and antibodies

qRT-PCR and cloning Oligos		RNA Single guides and oligos for IncMN2 locus CRISPR/Cas9 genome editing	
LncMN2-203_F	TAAGAGCCAGCACCTTCACG	Sg1	TTCCTGGATTTACCGACCTC
LncMN2-203_R	GCCTCCAACACAAAGCATCAG	Sg2	TTGCTACTCCGTGAAGGTGC
LncMN2-202/204_F	CTGGTTTCCCTGTTATCCTGCT	HAL_primer_F	GTTCTGCTCCCTCCTTTCC
LncMN2-202/204_R	GTCTTCTCGCTCTGTCTCAAA	HAL_primer_R	CTCAGGCTCCAGTTCTGCAT
LncMN2-203_CLIP_F	ACACACAGACATGCACACG	HAR_primer_F	GTCGGTAAATCCAGGAAGATGGCTG
LncMN2-203_CLIP_R	AGCATCAGGAGCATGTGAGTG	HAR_primer_R	CCACTGTCCCACTGCATCA
Isl1_F	TGTGGACATTACTCCCTCTTACA	RFP_F	ATATGAGAGAGCTGAGGGCA
Isl1_R	TCGTGAATTTGATTGCCGCA	LncMN2 exon2_R	CAGGGTGAAGTCAAGGAAGAAAG
Onecut2_F	AGGATGTGGAAGTGGCTGC	RNA Pull down biotinylated probes	
Onecut2_R	TCTGCCAGTGTGTTCTGTCT	LncMN2. PD1	AACCTCTGGCCATTTTCAACCCATTTGCTCCAGTTCACAG CACTCACGCAGAAAGTATGGCACTGAGGGGCT CAGGATACCTCAGGAATGA
Scrt1_F	GTTGCCAATTTCACCCGTC	LncMN2. PD2	ATCTTCTGGATTTACCGACCTCAGGCTCCAGTTCTGCAT AATTAGCTTAAACTGGCTCAATGATTTTAACTGGTCAG AAATTCATTT
Scrt1_R	AAGAAGGGTGGGACTGCATG	LncMN2. PD3	TCTTGAGCCTTGGTTTTCTCATCATATCACAAGCCTCC AGTCACCA CAGGGCCAGGGTGAAGTCAAGGAAGAAAGTG AAGCTGGAATCC
Ncan_F	GCTTTTCTGGACTGGAGCCT	LncMN2. PD4	ACTCCGTGAAGGTGCTGGCTCTTAGGCCACTTAATAGCT GCATTCTAGGGAGCAGCAGGATAACAGGGAAACCGAGAA GCTGATGACTGGC
Ncan_R	ACCTGTAGCCATTCTCGCAC	LncMN2. PD5	AGAAGGCTCCCAACACAGCATCAGCCTCCTTGAGACTA TATAGATCACTATTGCTGTGATGTTCAAGCATTGGGAATTT CCATAGGCTGA
Nrxn1_F	GGTCGAGTAGTTGATGAATGGC	LncMN2. PD6	AAGAATGATGGAGTTTCTGCTTTTATGGTTCTATTCTTGT GTACAGCAGAGGAAAAGTGTATAAGGCCAGATGGAT ATGGAAGATGC
Nrxn1_R	CCTGCTCTTTCCCGCAATT	LncMN2. PD7	TCATCCAGAGCCAGCAGAACCCACTGGCTCAACTGCAC AACAAATTTTCAACAGTCCACATATTAAGGGCTTTTCAAC AATGTGGTTCT
Snap25_F	TTGACACCCAGAATCGCCAG	LncMN2. PD8	CTATACATCAGTACAATCATGTACTGGCACTGGGCTAAA GACCATGTGACTTTATCTTCTCAATATGGTATTAATTTT CACCAAGAA
Snap25_R	GCACGTTGGTTGGCTTCATC	LncMN2. PD9	GCCAGGCAGAGTGGATGGTGGAGGAAGTCCACAACC CTAGCCTAAAAAGCAGTGTGCTAGTGGTGAGATCAACT CTACATTCTCTATC
Phb2_F	AGTGCTGCCGTCCATTGTTA	LncMN2. PD10	TGATCCCTTTCTTATGGTGAGCTCAGTCTCTGAGGACTT CTGTTGTGCTGTTAATAAGACTGACATAAGGAGACAAAG CAGACATTGA
Phb2_R	CTCTGTCAGCTCTCTTCGGA	LncMN2. PD11	TTATGTGTGACTGTGTGTCCCACTCAGTGAGTAACCTT GAACCAAGGATAAGAATTAGTTGGAGCCTAGGACTTGG GGAAGAAGGCT
Fbxw11_F	TGGTTCTCAGACTCCACGG	LncMN2. PD12	TAAATATAAACATCCACATTAATAACCTTAGTCAGCTAT GAGTGAAGAATATGATCCTACACAGTTCTTTGCTAGATA TTTTAGCTC
Fbxw11_R	ACGGCTTCATTGTGGTGGAT	LncMN2. PD13	CCCTCCAGAAATGATCAACAGATCCTGATTATAAGTTCT AAGAGTGTCTACTTTTGCAACAGCTCTGATCCATTTGTGG TCATAGTCAT
Tdrkh_F	ACAGAAGGAAGTGGCAGCAG	LncMN2. PD14	CTCAGTTGGGACCAATGATGTTAACCTTAGTCTTGTGCT TGTTGATTTCTCCACTATGAGCTGACTTTGCCTCTTATT CTGGACTCCA
Tdrkh_R	CTGATTGGCTGCTTTCGTGG	LncMN2. PD15	AGATAGAGATCCGTTTTCTCTTCTTCCATCATTTTACC ATGTTTTTTCATTGTCTTTAAACTGAAGCAGAATCTTTA TTTTAATG
Sapcd2_F	CTGGCATCCCTTGTCTTGT	U1 Pull down biotinylated probes	
Sapcd2_R	AAAGAGGACGAAGGCAGAGC	mU1a1-1	ACATCCGGAGTGAATGGATAAGCCTCGCCCTGGGAAAA CCACCTTCGTGATCATGGTATCTCCCTGCCAGGTAAGT AT
Suv39h2_F	GGCTGTGGTTGGGGTGAAA	mU1b1-2	CACCCCAAAGTGAATGGGTGAGCCTCGCCCTGGGAAA ACCACCTTCATGATCATGGTATCTCCCTGCCAGGTAAG TAT
Suv39h2_R	CTCGGCTCTTCACTTGTGA	mU1b1-3	GAGAGCGCAACGAGTCCCCCACTACCACAAATTATGC AGTCGAGTTTCCCGCATTGGGGAATCGCAGGGGTCA GCA
H2afz_F	GCCGTATTATCGACACCTG	mU1b6-4	CACCCCAAAGTGAATGGGTGAGCCTCGCCCTGGGAAA ACCACCTTCGTGATCATGGTATCTCCCTGCCAGGTAAG TAT
H2afz_R	CATTTCTGCCAACTCAAGTAC	mU1b6-5	GGGCGCGCAACGAGCTCCCCCACTACCACAAATTATG CAGTCGAGTTTCCCGCATTGGGGAATCGCAGGGGTCA CA
Atp5o_F	CAACCGCCCTGTACTCTGCT	Antibodies	
Atp5o_R	GGATTGAGAACAGCCAGAGACAC	anti-ISLET1/2	DSHB Hybridoma Product 39.4D5
Gapdh_F	AATGGTGAAGGTCGGTGTGAAC	Anti-Ago2	018-22021,Wako
Gapdh_R	CCGTGAGTGGAGTCATACTGGAAC		
Ptbp1_F	CCGTACACCCACCTCTAAG		
Ptbp1_R	CAGCATTGGGGAGCCTATGT		
DSP_F	ACCTGGAGTGC AAAAGAGCA		
DSP_R	TGCTCTTTGCACTCCAGGT		
Infusion LncMN2-203 EX5_F	GGAGACCCAAGCTGGGTTGAGCTATGGAATTC		
Infusion LncMN2-203 EX5_R	AGGCTGATCAGCGGGGTAGATTTTTTCTTTAAAAATT		
Infusion Inverse_F	CCCGCTGATCAGCCTCGA		
Infusion Inverse_R	CCAGCTTGGGTCTCCCTA		
Ex5 IncMN2 in PsciCHECK2_F	AGGCGATCGCTCGAGAAACACACAGACATGCACACG		
Ex5 IncMN2 in PsciCHECK2_R	GCGGCCAGCGGCCCATGTGAGTGCCTGAGTATGTG		
miR-325-3p	MS00011788		
miR-384-5p	MS00011963		
miR-466i-5p	MS00064462		
miR-669 a-5p	MS00026222		
Locked Nucleic Acids (LNA) Codes			
LNA mir-466i-5p	YI04100068-ADA		
LNA mir-669a-5p	YI04101197-ADA		
LNA mir-325-3p	MS00011788		
LNA mir-384-5p	YI04101478-ADA		
LNA scrambled	ID: 1027280		
siRNA & Mimics			
Name	Sequence		
Si-SCR	D-001810-10-05 ON-TARGET plus Control pool Non-Targeting pool		
Si-Scrt1	L-041476-01-005 ON-TARGET plus Mouse Scrt1 SMART pool		
miR-466i-5p Mimic	MSTUD0838 MISSION Synthetic microRNA mouse mmu miR-466i-5p		
RNA Single guides for miRNA binding region CRISPR/Cas9 genome editing			
Sg1_F	AAGTCTAGGCTCCAATC		
Sg1_R	GAGTTGGAGCCTAGGACTT		
Sg2_F	TCTTCACTCATAGCTGACTA		
Sg2_R	TAGTCAGCTATGAGTGAAGA		
RNA Single guides for miR-325 CRISPR/Cas9 genome editing			
Sg1_F	TCTTGCTAACTTAGCTATG		
Sg1_R	CATAGCTAAGTTAGACAAGA		
Sg2_F	CAACACTTGGCCCTAGTCC		
Sg2_R	GGACTAGGGGCCAAGTGTGG		