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Supplementary Materials for

Non–cell-autonomous regulation of interneuron specification mediated by extracellular vesicles

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This PDF file includes:

Fig. S1.

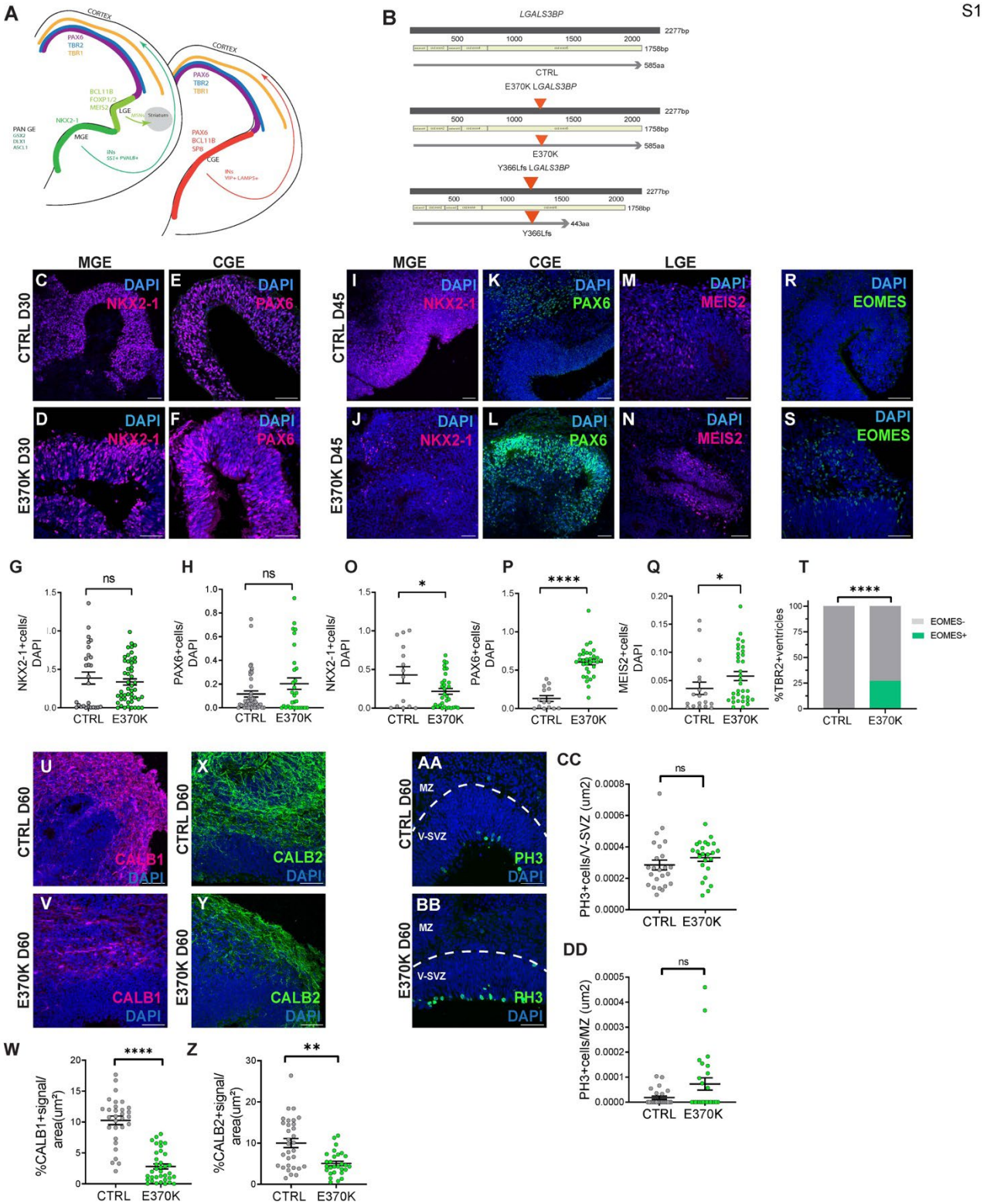

A
CORTEX
PAX6
TBR2
TBR1
BCL11B
FOXP1/2
MEIS2
LGE
Striatum
NKX2-1
MGE
PAN GE
GSX2
DLX1
ASCL1
INs
SST+ PVALB+
CORTEX
PAX6
TBR2
TBR1
PAX6
BCL11B
SP8
CGE
INs
VIP+ LAMP5+
B
LGALS3BP
2277bp
500
1000
1500
2000
1758bp
585aa
CTRL
E370K LGALS3BP
E370K
Y366Lfs LGALS3BP
Y366Lfs
443aa
MGE
CGE
LGE
CTRL D30
E370K D30
CTRL D45
E370K D45
DAPI
NKX2-1
PAX6
MEIS2
EOMES
G
H
O
P
Q
T
ns
*
****
NKX2-1+cells/ DAPI
PAX6+cells/ DAPI
MEIS2+cells/ DAPI
%TBR2+ventricles
EOMES-
EOMES+
CTRL
E370K
U
V
X
Y
CALB1
CALB2
DAPI
CTRL D60
E370K D60
AA
BB
MZ
V-SVZ
PH3
CC
DD
PH3+cells/V-SVZ (um2)
PH3+cells/MZ (um2)
W
Z
**
%CALB1+signal/ area(um²)
%CALB2+signal/ area(um²)

Fig. S1. LGALS3BP E370K-mutant ventral organoids show alterations in cell identity
(**A**) Schematic of tangential migration of interneurons from MGE and CGE to the cortex and markers used for forebrain regional characterization.
(**B**) Schematic representation of the E370K and Y366Lfs mutant iPSC lines generated using the CRISPR/Cas9 genome editing in control iPSCs. The E370K IPSC line carries a point mutation located in LGALS3BP exon 5 found in a neurological patient, while the Y366Lfs IPSC line presents a 2bp deletion resulting in a frameshift and premature stop codon generating a truncated/KO form of LGALS3BP in exon 5.
(**C** to **F**) Micrograph of D30 CTRL and E370K vCOs sections immunostained for MGE marker NKX2-1 antibody (**C** and **D**) and CGE marker PAX6 antibody (**E** and **F**). Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**G**) Quantification of the number of NKX2-1+cells normalized by DAPI showing no difference in NKX2-1+cells in D30 E370K vCOs compared to CTRL vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001. Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**H**) Quantification of the number of PAX6+cells normalized by DAPI showing no difference in PAX6+cells in D30 E370K vCOs compared to CTRL vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001 Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**I** to **N**) Micrograph of D45 CTRL and E370K vCOs sections immunostained for MGE marker NKX2-1 antibody (**I** and **J**), CGE marker PAX6 antibody (K and L), and LGE marker MEIS2 antibody (**M** and **N**). Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**O**) Quantification of the number of NKX2-1+cells normalized by DAPI showing a decrease of NKX2-1+ cells in D45 E370K vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001. Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**P**) Quantification of the number of PAX6+cells normalized by DAPI showing an increase of PAX6+ cells in D45 E370K vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001 Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**Q**) Quantification of the number of MEIS2+cells normalized by DAPI showing an increase of MEIS2+ cells in D45 E370K vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001. Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**R** and **S**) Micrograph of D45 CTRL and E370K vCOs sections immunostained for IP marker EOMES. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**T**) Quantification of the percentage of ventricles with EOMES+cells (>10 cells), showing the unexpected expression of EOMES in D45 E370K vCOs. Statistical significance was based on exact binomial test ****p<0.0001. n of analyzed ventricles: CTRL=54, E30K=63, from 2 different batches.
(**U** and **V**) Micrograph of D60 CTRL and E370K vCOs sections immunostained for the inhibitory neuronal marker CALB1. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**W**) Quantification of the percentage of CALB1+ pixels normalized per area (µm2), showing the decrease of CALB1 in E370K vCOs. Data are shown mean ± SEM. Statistical significance was

based on two-tailed Mann-Whitney U test ****p<0.0001. Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**X** and **Y**) Micrograph of D60 CTRL and E370K vCOs sections immunostained for the inhibitory neuronal marker CALB1. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**Z**) Quantification of the percentage of CALB2+ pixels normalized per area (µm2), showing the decrease of CALB2 in E370K vCOs. Data are shown mean ± SEM. Statistical significance was based on two-tailed Mann-Whitney U test ****p<0.0001. Every dot in the plot refers to analyzed ventricles per vCO from at least 3 different vCOs generated in at least 2 independent batches.
(**AA** and **BB**) Micrograph of D60 CTRL (**AA**) and Y366Lfs (**BB**) vCOs sections immunostained for PH3. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.
(**CC** and **DD**) Quantification of the number of PH3+ cells, showing no difference in their number in V-SVZ (**CC**) and MZ (**DD**) in E370K compared to CTRL vCOs.

Fig. S2.

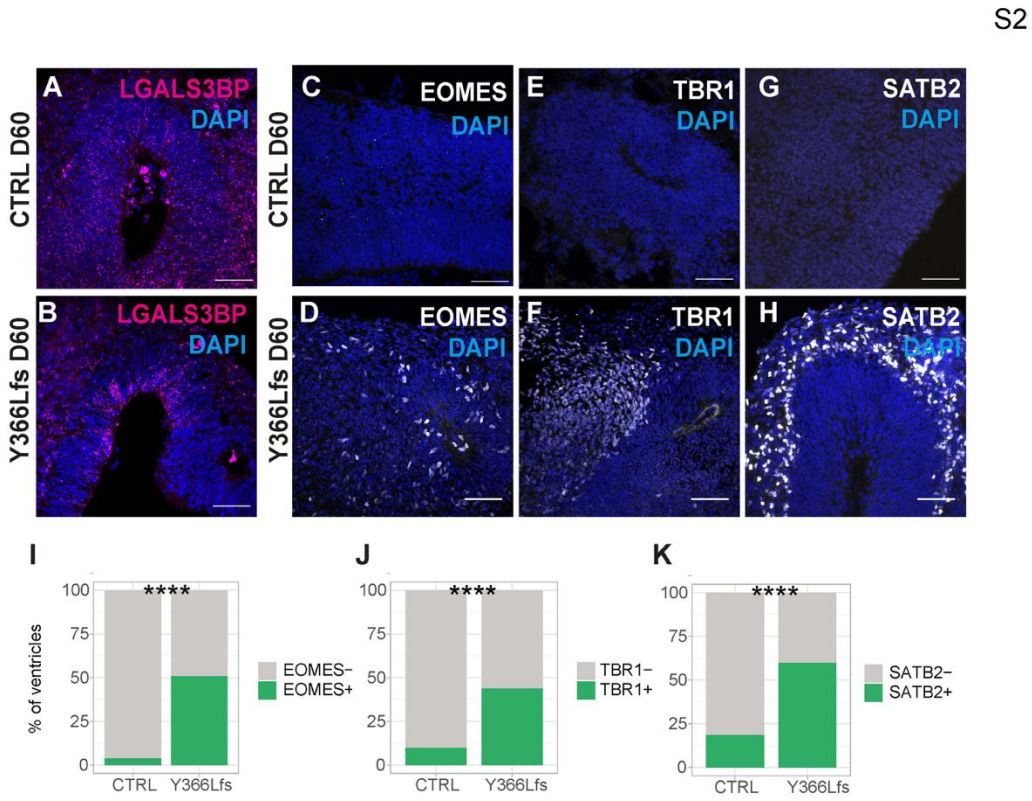


Fig. S2. LGALS3BP E370K-mutant ventral organoids show dorsal identity

(**A** and **B**) Micrograph of D60 CTRL and Y366Lfs vCOs sections immunostained for LGALS3BP. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.

(**C** to **H**) Micrograph of D60 CTRL and Y366Lfs vCOs sections immunostained for IP marker EOMES (**C** and **D**), deep layer cortical neurons TBR1 (**E** and **F**) and upper layer cortical neurons SATB2 (**G** and **H**) Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.

(**I**) Quantification of the percentage of ventricles with EOMES+cells (>10 cells), showing the unexpected expression of EOMES in D60 Y366Lfs vCOs. Statistical significance was based on exact binomial test ****p<0.0001. n of analyzed ventricles: CTRL=96, Y366Lfs =80, from 2 different batches.

(**J**) Quantification of the percentage of ventricles with TBR1+cells (>10 cells), showing the unexpected expression of TBR1 in D60 Y366Lfs vCOs. Statistical significance was based on exact binomial test ****p<0.0001. n of analyzed ventricles: CTRL=100, Y366Lfs =119, from 2 different batches.

(**K**) Quantification of the percentage of ventricles with SATB2+cells (>10 cells), showing the unexpected expression of SATB2 in D60 Y366Lfs vCOs. Statistical significance was based on exact binomial test ****p<0.0001. n of analyzed ventricles: CTRL=48, Y366Lfs =119; from 2 different batches.

Fig. S3.

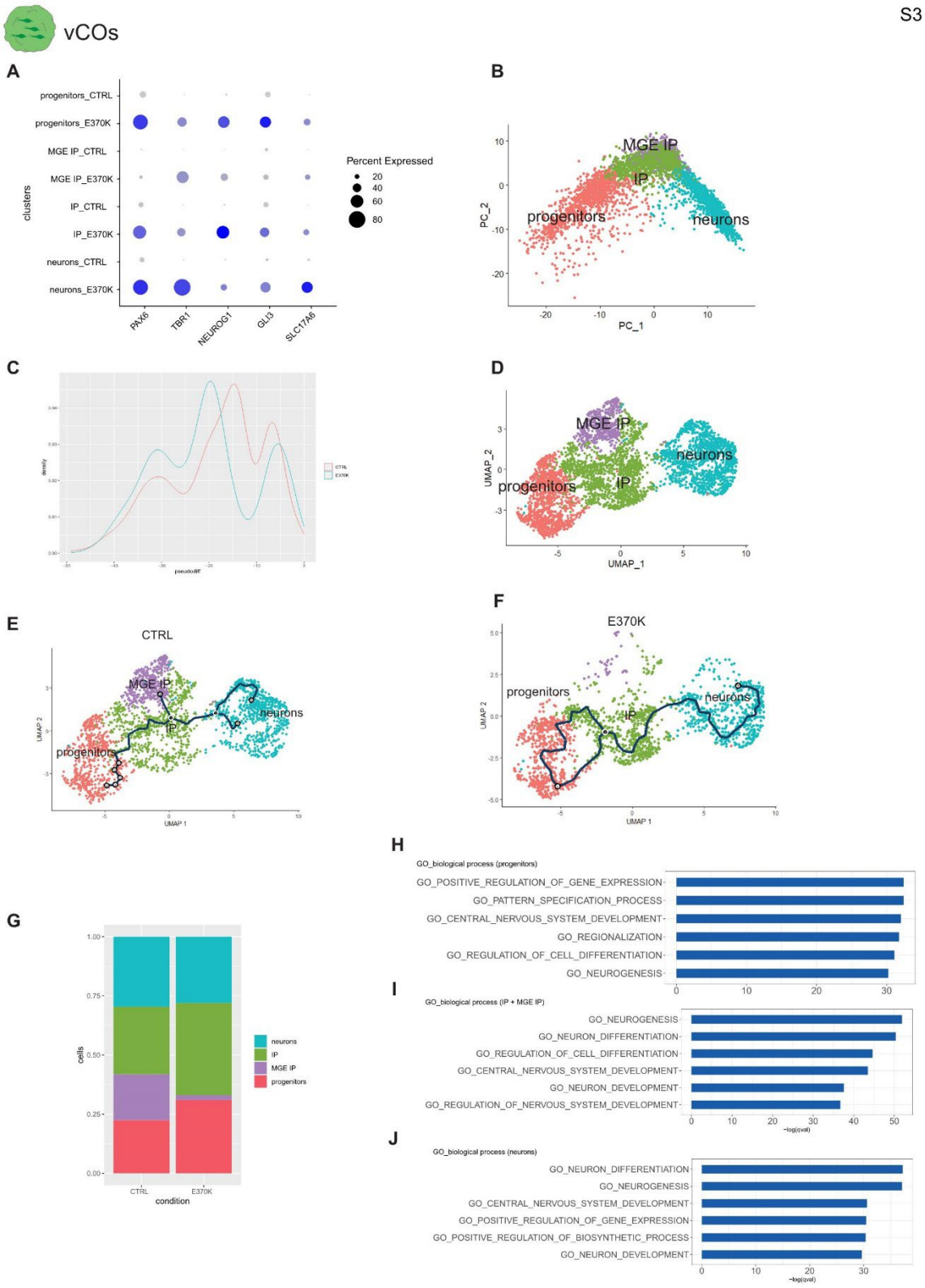

vCOs
S3
A
progenitors_CTRL
progenitors_E370K
MGE IP_CTRL
MGE IP_E370K
IP_CTRL
IP_E370K
neurons_CTRL
neurons_E370K
clusters
PAX6
TBR1
NEUROG1
GLI3
SLC17A6
Percent Expressed
20
40
60
80
B
MGE IP
IP
progenitors
neurons
PC_2
PC_1
C
density
pseudoDiff
CTRL
E370K
D
MGE IP
neurons
progenitors
IP
UMAP_2
UMAP_1
E
CTRL
MGE IP
neurons
IP
progenitors
UMAP 2
UMAP 1
F
E370K
progenitors
neurons
IP
UMAP 2
UMAP 1
G
cells
condition
CTRL
E370K
neurons
IP
MGE IP
progenitors
H
GO_biological process (progenitors)
GO_POSITIVE_REGULATION_OF_GENE_EXPRESSION
GO_PATTERN_SPECIFICATION_PROCESS
GO_CENTRAL_NERVOUS_SYSTEM_DEVELOPMENT
GO_REGIONALIZATION
GO_REGULATION_OF_CELL_DIFFERENTIATION
GO_NEUROGENESIS
I
GO_biological process (IP + MGE IP)
GO_NEUROGENESIS
GO_NEURON_DIFFERENTIATION
GO_REGULATION_OF_CELL_DIFFERENTIATION
GO_CENTRAL_NERVOUS_SYSTEM_DEVELOPMENT
GO_NEURON_DEVELOPMENT
GO_REGULATION_OF_NERVOUS_SYSTEM_DEVELOPMENT
−log(qval)
J
GO_biological process (neurons)
GO_NEURON_DIFFERENTIATION
GO_NEUROGENESIS
GO_CENTRAL_NERVOUS_SYSTEM_DEVELOPMENT
GO_POSITIVE_REGULATION_OF_GENE_EXPRESSION
GO_POSITIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS
GO_NEURON_DEVELOPMENT
−log(qval)

Fig. S3. LGALS3BP variation causes alterations in cell fate and developmental trajectory

(**A**) Dot plot showing the percentage of cells expressed cortical genes in D60 CTRL and E370K-vCO clusters. Progenitors, IP and MGE IP, and neurons from E370K-vCOs show a higher percentage of cells expressing *PAX6*, *TBR1, NEUROG1, GLI3* and *SCL17A6* compared to CTRL cells.

(**B**) Principal component analysis (PCA) visualization of D60 CTRL and E370K vCOs, showing the pseudo-differentiation axis from progenitors to neurons.

(**C**) Density plot showing cell distribution along pseudo-differentiation axis in CTRL and E370K vCOs.

(**D**) UMAP visualization of scRNA-seq data of telencephalic cells in D60 CTRL and E370K vCOs.

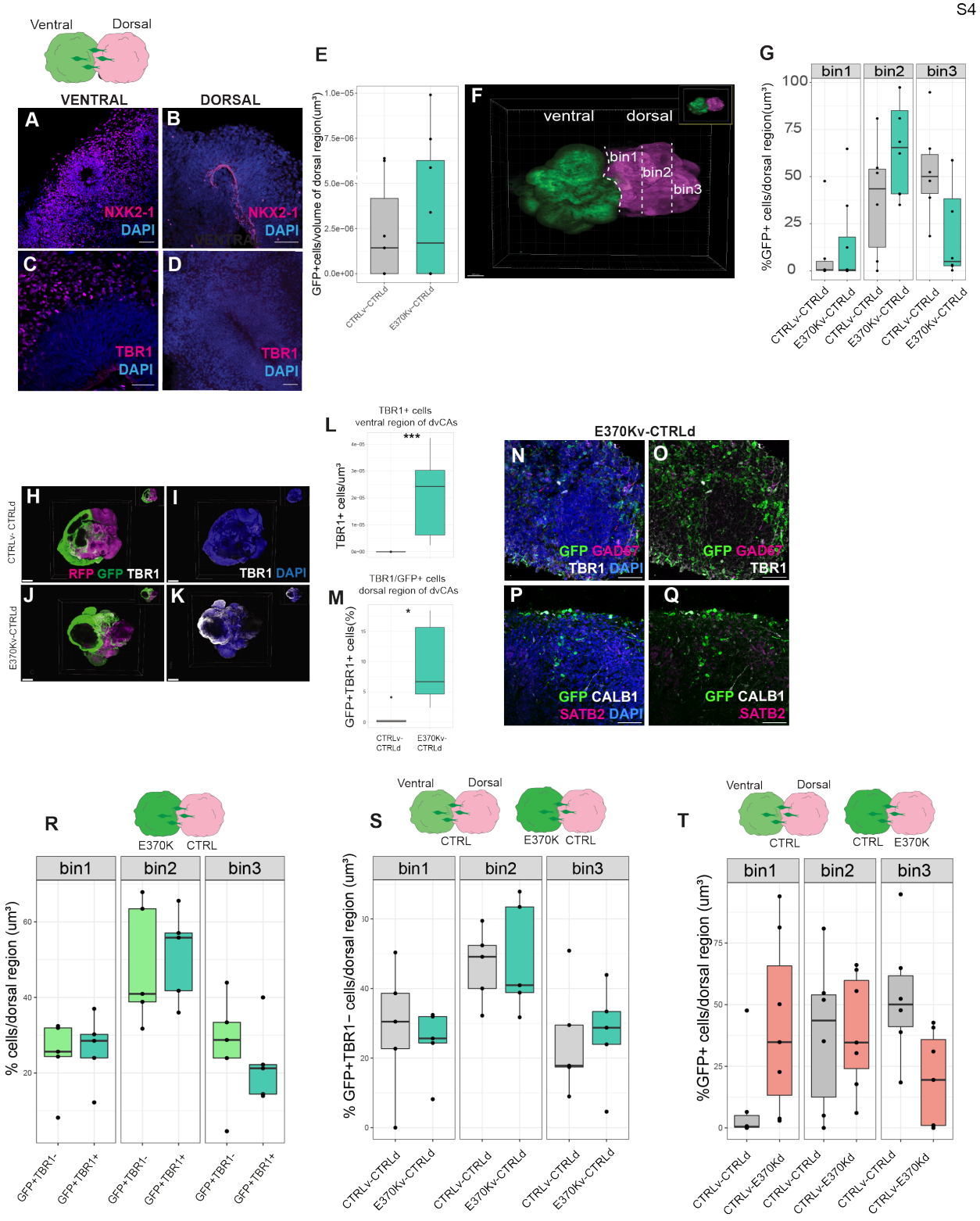
(**E**) UMAP visualization of pseudo-differentiation trajectories in 60 days old CTRL vCOs clusters from progenitors to INs. CTRL vCOs present trajectories from progenitors to IP; from IP to MGE IP, and from IP to neurons.

(**F**) UMAP visualization of pseudo-differentiation trajectories in D60 E370K vCOs clusters from progenitors to INs. E370K vCOs lack in the trajectory from IP to MGE IP.

(**G**) Bar plot showing cell proportion in D60 CTRL and E370K vCOs. E370K vCOs present an increased progenitor population and decreased MGE-IP population compared to CTRL vCOs.

(**H** to **J**) GO enrichment for DE genes in E370K progenitors (**H**), E370K IP and MGE-IP (**I**), and E370K neurons (**J**). Significant GOs for biological processes are reported (FDR<0.05).

Fig. S4.



Lorem ipsum

Fig. S4. Cell fate changes result in migratory dynamics defects

(**A** and **B**) Micrograph of D60 dvCAs, showing the expression of the ventral marker NKX2-1 (magenta) only in the ventral region (A) and not in the dorsal region of dvCAs (B).Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.

(**C** and **D**) Micrograph of D60 dvCAs, showing the expression of the dorsal marker TBR1 (magenta) only in the dorsal region (**D**) and not in the ventral region of dvCAs (**D**). Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.

(**E**) Quantification of the number of GFP+ventral cells migrated from ventral to dorsal in D60 CTRLv-CTRLd and E370Kv-CTRLd Cas, showing no difference in GFP+migrated cells. Box plots show median and interquartile range. Significance was based on the Mann-Withney U test. Every dot in the plots refers to analyzed vCAs generated in at least 2 independent batches.

(**F**) Schematic of binning analysis in cleared D60 dvCAs.

(**G**) Binning analysis in cleared D60 CTRL and E370Kv-CTRLd CAs. Quantification of the distribution of GFP+cells migrated into the dorsal region of CTRL dvCAs, compared to GFP+cells migrated into the dorsal region of E370Kv-CTRLd CAs, showing no difference in their distribution. Box plots show median and interquartile ranges. Statistical significance was based on the Kruskal-Wallis test. Every dot in the plots refers to analyzed dvCAs generated in at least 2 independent batches.

(**H** to **K**) Micrograph of 3D immunostaining of cleared CTRLv-CTRLd and E370Kv-CTRLd CAs for GFP (green) and TBR1 (grey). Scale bar: 500 µm. Nuclei (blue) are stained with DAPI.

(**L**) Quantification of TBR1+cells in the ventral region of CTRLv-CTRLd and E370Kv-CTRLd CAs, normalized by volume (µm3) showing the unexpected expression of TBR1 in the ventral side of E370Kv-CTRLd dvCAs. Box plots show median and interquartile range. Statistical significance was based on Mann-Withney U test*$p<0.05$, ***$p<0.001$. n of organoids: CTRLv-CTRLd =5, LGALS3BPv-CTRLd=9, from at least 2 different batches.

(**M**) Quantification of the percentage of migrated GFP+ventral cells expressing the deep layer cortical marker TBR1 in dorsal region of CTRLv-CTRLd and E370v-CTRLd dvCAs, showing the significant increase of E370K-GFP migrating cells expressing TBR1. Box plots show median and interquartile range. Statistical significance was based on Mann-Withney U test*$p<0.05$, ***$p<0.001$. n of organoids: CTRLv-CTRLd =5, LGALS3BPv-CTRLd=9, from at least 2 different batches.

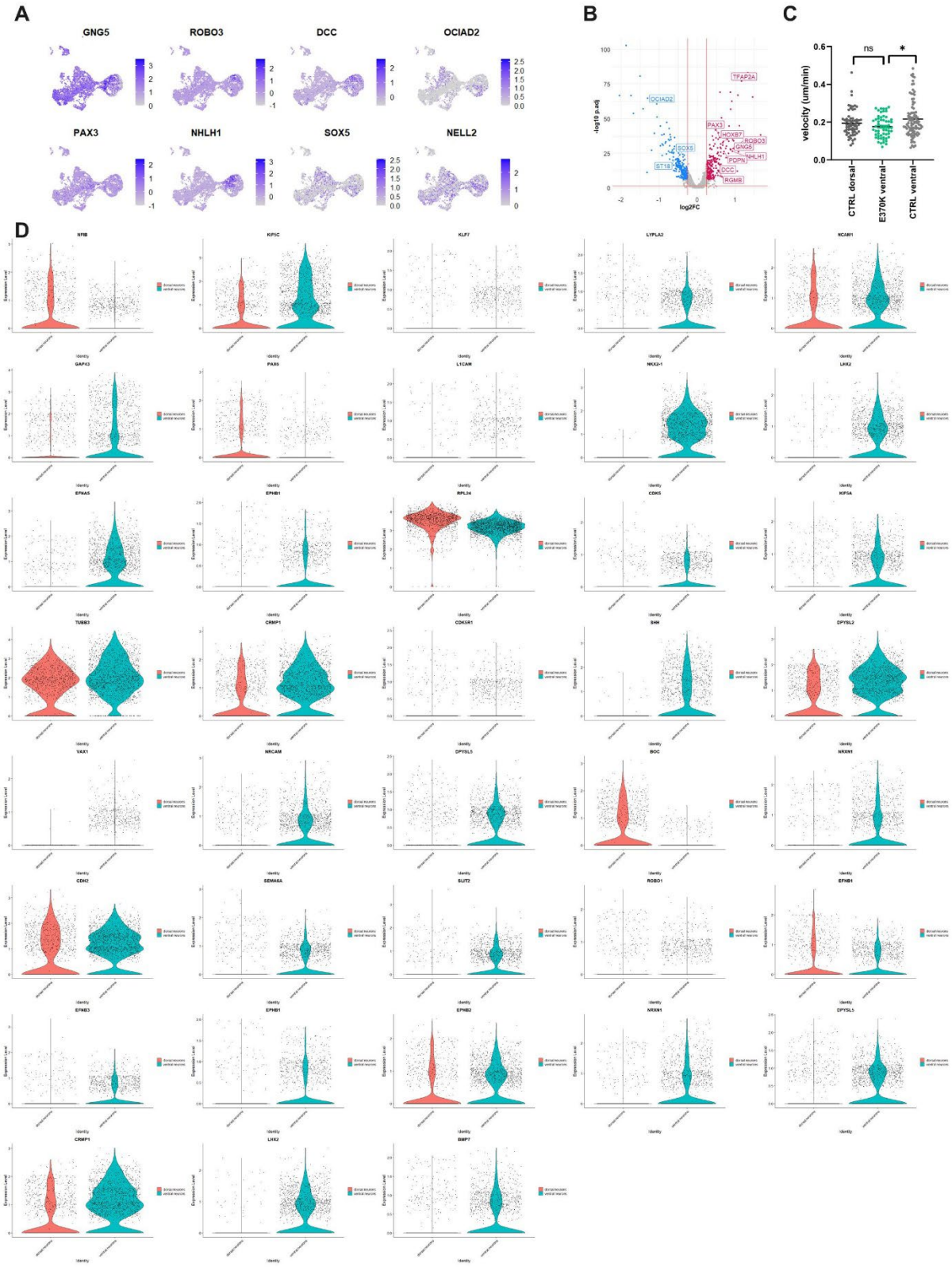
(**N** to **Q**) Micrograph of D60 dorsal regions of E370Kv-CTRLd CAs immunostained for the inhibitory neuronal marker GAD67 (magenta) and the deep layer neuronal cortical marker TBR1 (grey) (**N** and **M**O; and for the inhibitory neuronal marker CALB1 (grey) and the upper layer neuronal cortical marker SATB2 (magenta) (**P** and **Q**), showing that E370K-GFP migrating cells expressing cortical markers do not co-express inhibitory markers. Scale bar: 50 µm. Nuclei (blue) are stained with DAPI.

(**R**) Binning analysis in cleared D60 E370Kv-CTRLd CAs. Quantification of the distribution of E370K-GFP+TBR1- cells migrated into the dorsal region of E370Kv-CTRLd CAs, compared to E370K-GFP+TBR1+ cells, showing no difference in their distribution. Box plots show median and interquartile range. Statistical significance was based on the Kruskal-Wallis test. Every dot in the plots refers to analyzed dvCAs generated in at least 2 independent batches.

(**S**) Binning analysis in cleared D60 CTRL and E370Kv-CTRLd CAs. Quantification of the distribution of GFP+TBR1- cells migrated into the dorsal region of CTRL and E370Kv-CTRLd CAs, showing no difference in their distribution. Box plots show median and interquartile range. Statistical significance was based on the Kruskal-Wallis test.

(**T**) Binning analysis in cleared D60 CTRLv-CTRLd and CTRLv -E370Kd CAs (schematic at the top). Quantification of distribution of GFP+ ventral cells migrated into dorsal of 60 days old CTRLv-CTRLd and CTRLv -E370Kd CAs, showing significant changes in distribution in bin 1 and 2. Box plots show median and interquartile range. Statistical significance was based on the Mann-Withney U test *$p<0.05$. Every dot in the plots refers to analyzed dvCAs generated in at least 2 independent batches.

Fig. S5.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DCC
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PAX3
NHLH1
SOX5
NELL2
B
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OCIAD2
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HOXB7
ROBO3
GNG5
NHLH1
PDPN
DCC
RGMB
SOX5
ST18
-log10 p-adj
log2FC
C
ns
*
velocity (um/min)
CTRL dorsal
E370K ventral
CTRL ventral
D
NFIB
KIF5C
KLF7
LYPLA2
NCAM1
GAP43
PAX6
L1CAM
NKX2-1
LHX2
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VAX1
NRCAM
DPYSL5
BOC
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CDH2
SEMA5A
SLIT2
ROBO1
EFNB1
EFNB3
EPHB1
EPHB2
NRXN1
DPYSL5
CRMP1
LHX2
BMP7

Fig. S5. Genes associated to neuronal migration are dysregulated in LGALS3BP E370K-mutant ventral organoids

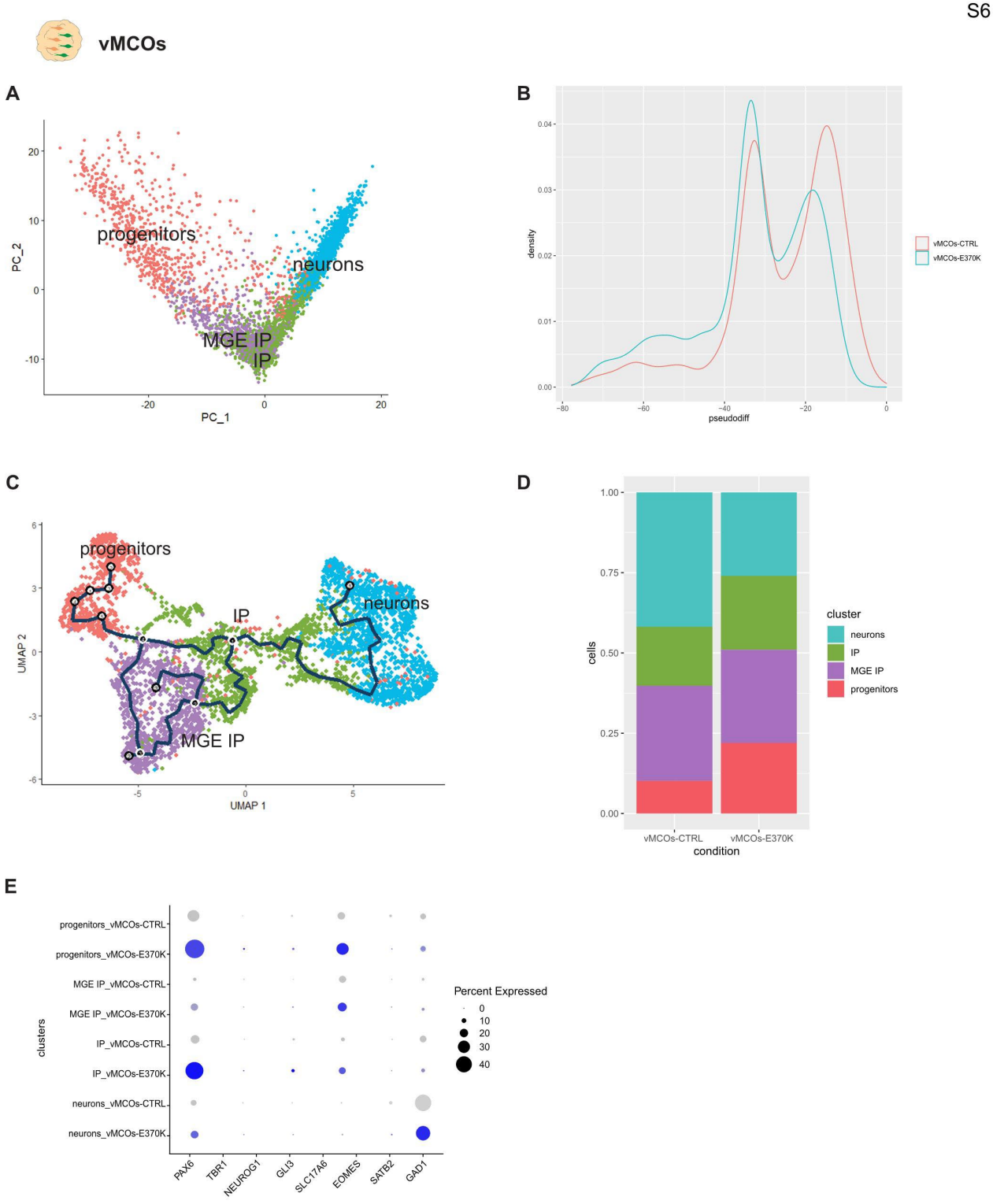
(**A**) Feature plot depicting the expression of genes associated with PH (*GNG5, ROBO3, DCC, OCIAD2, PAX6, NHLH1, SOX5, NELL2*) in CTRL and E370K vCOs.

(**B**) Volcano plot showing the fold change (CTRL vs E370K) of gene expression of PH-associated genes in E370K neurons.

(**C**) Graph showing the comparison between velocity (um/min) of CTRL dorsal neurons (analyzed in Klaus et al., 2019 36), E370K ventral neurons and CTRL ventral neurons migrating within the dorsal region. E370K neurons from E370Kv-CTRLd CAs show a migratory behavior similar to control dorsal neurons. Data are shown mean ± SEM Statistical significance was based on the Mann-Withney U test *$p<0.05$. Every dot in the plots refers to single cells per CO or dvCA.

(**D**) Violin plots depicting the expression of genes associated with neuronal migration and axon guidance in dorsal and ventral organoids, from scRNA-seq data.

Fig. S6.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progenitors
neurons
MGE IP
IP
PC_1
PC_2
B
density
pseudodiff
vMCOs-CTRL
vMCOs-E370K
C
progenitors
IP
neurons
MGE IP
UMAP 1
UMAP 2
D
cells
condition
vMCOs-CTRL
vMCOs-E370K
cluster
neurons
IP
MGE IP
progenitors
E
clusters
progenitors_vMCOs-CTRL
progenitors_vMCOs-E370K
MGE IP_vMCOs-CTRL
MGE IP_vMCOs-E370K
IP_vMCOs-CTRL
IP_vMCOs-E370K
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neurons_vMCOs-E370K
PAX6
TBR1
NEUROG1
GLI3
SLC17A6
EOMES
SATB2
GAD1
Percent Expressed
0
10
20
30
40

Fig. S6. LGALS3BP can revert the molecular identity of mutant ventral progenitors and neurons

(**A**) Principal component analysis (PCA) visualization of D60 vMCOs, showing the pseudo-differentiation axis from progenitors to neurons.

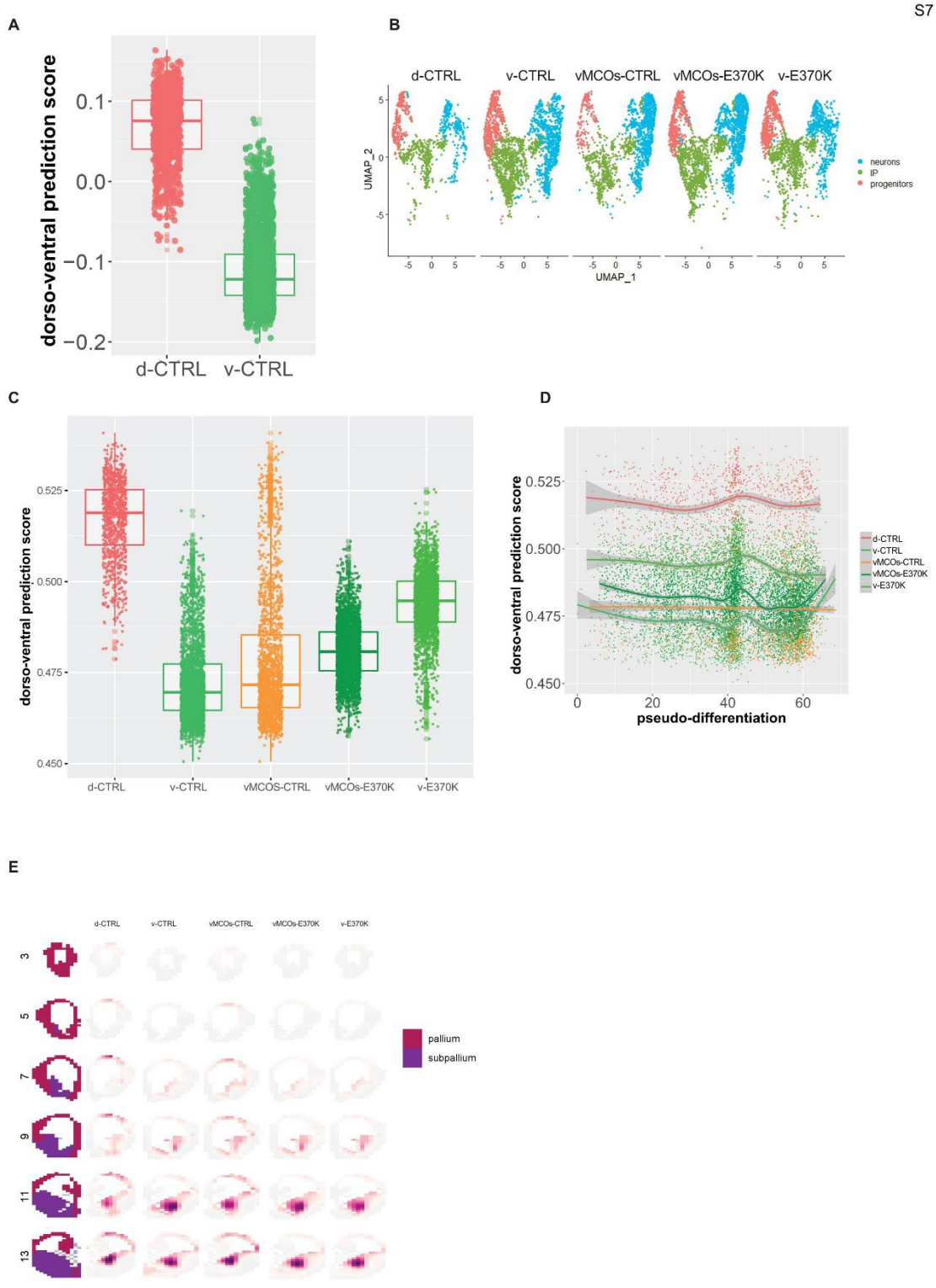
(**B**) Density plot showing cell distribution along pseudo-differentiation axis of vMCOs-CTRL and vMCOs-E370K obtained from D60 vMCOs.

(**C**) UMAP visualization of pseudo-differentiation trajectories in D60 vMCOs clusters from progenitors to neurons, showing trajectories from progenitors to IP; from IP to MGE IP, and from IP to neurons.

(**D**) Bar plot showing cell proportion in D60 vMCOs -CTRLs and vMCOs- vMCOs. E370K vCOs present an increased progenitor population and decreased MGE-IP population compared to CTRL vCOs.

(**E**) Dot plot showing the percentage of cells expressed cortical genes in D60 in vMCOs clusters. E370K progenitors, IP and MGE IP, show a higher percentage of cells expressing *PAX6,* and *EOMES*, compared to vMCOs-CTRL, while E370K and CTRL neurons show comparable percentage of cells expressing *TBR1, NEUROG1, GLI3, SCL17A6, SATB2* and *GAD1*.

Fig. S7.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dorso-ventral prediction score
0.1
0.0
−0.1
−0.2
d-CTRL
v-CTRL
B
d-CTRL
v-CTRL
vMCOs-CTRL
vMCOs-E370K
v-E370K
UMAP_2
UMAP_1
neurons
IP
progenitors
C
dorso-ventral prediction score
0.525
0.500
0.475
0.450
d-CTRL
v-CTRL
vMCOS-CTRL
vMCOs-E370K
v-E370K
D
dorso-ventral prediction score
0.525
0.500
0.475
0.450
0
20
40
60
pseudo-differentiation
d-CTRL
v-CTRL
vMCOs-CTRL
vMCOs-E370K
v-E370K
E
d-CTRL
v-CTRL
vMCOs-CTRL
vMCOs-E370K
v-E370K
3
5
7
9
11
13
pallium
subpallium

Fig. S7. LGALS3BP can revert the molecular identity of mutant ventral progenitors and neuron

(**A**) Box plot showing the DV prediction score based on fold cross validation. The model was trained on a subset of scRNA-seq data from D60 dorsal CTRL COs (d-CTRL) and ventral CTRL COs (v-CTRL).

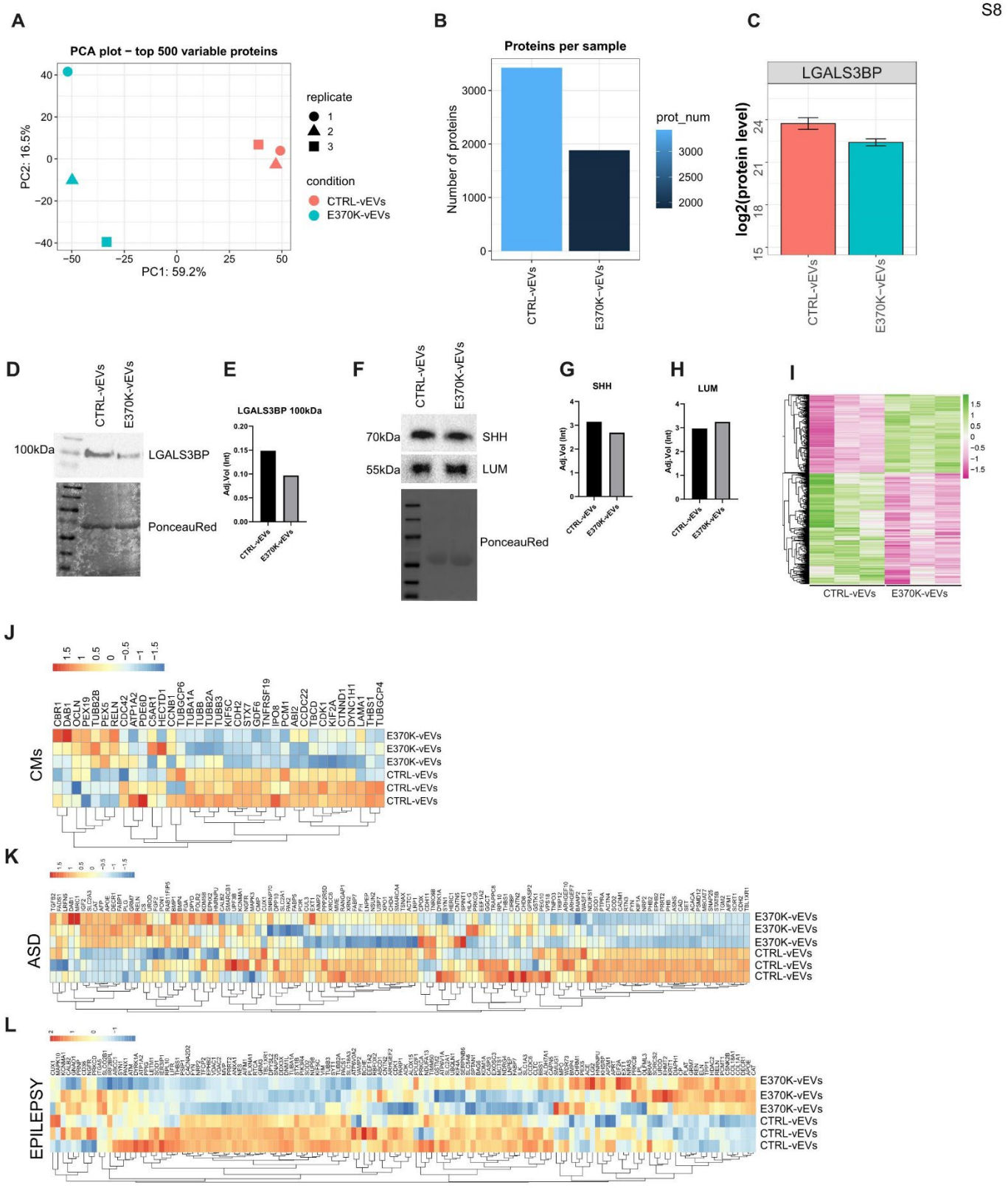
(**B**) UMAP visualization of scRNA-seq data of D60 CTRL dCOs (d-CTRL), CTRL vCOs (v-CTRL), E370K vCOs (v-E370K) and vMCOs (vMCOs-CTRL and -E370K), split by conditions.

(**C**) Boxplot showing the DV score of d-CTRL, v-CTRL, v-E370K and vMCOs-CTRL and vMCOs-E370K. v-E370K cells show a higher DV score compared to v-CTRL cells, and vMCOs-E370K cells show a lower DV score compared to v-E370K cells. Box plots show median and interquartile range. Each dot in the plot represents one cell.

(**D**) Density plot showing the distribution of DV prediction score of each condition along the pseudo-differentiation axis of d-CTRL, v-CTRL, v-E370K and vMCOs-CTRL and vMCOs-E370K.

(**E**) VoxHunt spatial brain mapping of the scRNA-seq from all conditions onto data from E13.5 mouse brains from the Allen Brain Institute.

Fig. S8.


A
PCA plot – top 500 variable proteins
PC2: 16.5%
PC1: 59.2%
replicate
1
2
3
condition
CTRL-vEVs
E370K-vEVs
B
Proteins per sample
Number of proteins
prot_num
CTRL-vEVs
E370K-vEVs
C
LGALS3BP
log2(protein level)
CTRL-vEVs
E370K-vEVs
D
CTRL-vEVs
E370K-vEVs
100kDa
LGALS3BP
PonceauRed
E
LGALS3BP 100kDa
Adj.Vol (Int)
CTRL-vEVs
E370K-vEVs
F
CTRL-vEVs
E370K-vEVs
70kDa
SHH
55kDa
LUM
PonceauRed
G
SHH
Adj.Vol (Int)
CTRL-vEVs
E370K-vEVs
H
LUM
Adj.Vol (Int)
CTRL-vEVs
E370K-vEVs
I
CTRL-vEVs
E370K-vEVs
J
CMs
CBR1
DAB1
OCLN
PEX19
TUBB2B
PEX5
RELN
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SNAP1
CCT3
CCNB1
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TUBB
TUBB2A
TUBB3
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CDH2
STX7
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ABI2
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CDK1
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THBS1
TUBGCP4
E370K-vEVs
E370K-vEVs
E370K-vEVs
CTRL-vEVs
CTRL-vEVs
CTRL-vEVs
K
ASD
E370K-vEVs
E370K-vEVs
E370K-vEVs
CTRL-vEVs
CTRL-vEVs
CTRL-vEVs
L
EPILEPSY
E370K-vEVs
E370K-vEVs
E370K-vEVs
CTRL-vEVs
CTRL-vEVs
CTRL-vEVs

Fig. S8. Extrinsic EV-mediated function of LGALS3BP in progenitor specification and neurodevelopmental disorders

(**A**) Principal component analysis (PCA) of protein samples from EVs collected from CTRL and E370K vCOs, based on LFQ intensity of quantified proteins. All the replicates are represented.

(**B**) Bar plot showing the number of proteins detected in CTRL-vEVs and E370K-vEVs collected from vCOs. E370K-vEVs show decreased number of detected proteins.

(**C**) Bar plot of LGALS3BP protein level detected in CTRL-vEVs and E370K-vEVs, showing a decrease of LGALS3BP expression in E370K-vEVs compared to CTRL-vEVs.

(**D**) WB of protein extract of CTRL-vEVs and E370K-vEVs for LGALS3BP.

(**E**) Volumetric analysis of the expression of LGALS3BP in CTRL-vEVs and E370K-vEVs, normalized by PonceauRed.

(**F**) WB of protein extract of CTRL- vEVs and E370K-vEVs for SHH and LUM.

(**G**) Volumetric analysis of the expression of SHH in CTRL-vEVs and E370K-vEVs, normalized by PonceauRed.

(**H**) Volumetric analysis of the expression of LUM in CTRL-vEVs and E370K-vEVs, normalized by PonceauRed.

(**I**) Heatmap showing transcriptomic profile changes in NPCs after treatment with vEVs and E370K-vEVs.

(**J** to **L**). Heatmap of DE protein in E370K-vEVs compared to CTRL-EVs associated with CM (**J**), ASD (**K**), and epilepsy (**L**).

Table S1. Immunostaining primary antibodies

Antibody	Host	Company	Catalogue No.	Dilution
NKX2.1	Mouse IgG1	Merk Millipore	MAB5460	1:500
TBR1	Rabbit	Abcam	Ab31940	1:500
LGALS3BP	Mouse IgG1	eBioscience	BMS146	1:100
PAX6	Rabbit	Biolegend	PRB-278p	1:500
MEIS2	Mouse IgG1	Santa Cruz Biotechnology	SC 101850	1:300
EOMES	Rabbit	Abcam	ab23345	1:500
SATB2	Mouse IgG1	Abcam	Ab51502	1:500
GAD67	Mouse IgG2b	Millipore	MAB5406	1:1000
CALB1	Rabbit	Swang	CB-38a	1:1000
CALB2	Rabbit	Swang	CRGP7	1:1000
GFP	Chicken	Aves Lab	GFP-1020	1:1000
pH3	Rabbit	Millipore	06-570	1:500

Table S2. Immunostaining secondary antibodies

Antibody	Host	Company	Catalogue No.	Dilution
AlexaFluor647	Rabbit	ThermoFisher Scientific	A21244	1:1000
AlexaFluor488	Chicken	ThermoFisher Scientific	A11039	1:1000
AlexaFluor546	Mouse IgG1	ThermoFisher Scientific	A21123	1:1000
AlexaFluor488	Rabbit	ThermoFisher Scientific	A11008	1:1000
AlexaFluor647	Mouse IgG2a	ThermoFisher Scientific	A21241	1:1000