LEGENDS TO SUPPLEMENTAL FIGURES AND TABLES

Supplemental Figure 1: scRNAseq expression profiles of NDD risk genes

a). Expression of 28 microcephaly genes, catalogued in a recent review[1], across 11 subsets of apparent aPs, bPs, and NBs identified by our scRNAseq analysis of WT (light half circles) and *LgDel* (dark half circles) E14.5 cortical cells. Half circle sizes reflect transcript abundance (scale, lower right). **b**). Bubble plot of 50 highest confidence ASD risk genes, based upon the curated Simons SFARI Gene database[2]. A majority of these genes are expressed at substantial, consistent levels across all aP, bP and NB subsets. **c**). Bubble plot of 50 highest confidence Scz candidate genes, based upon a comprehensive review[3]. Several of these genes are minimally expressed or absent in WT and *LgDel* aP, bP and NB subsets.

Supplemental Figure 2: qPCR validation of DE genes in FACS-*Tbr2*$^{eGFP+}$ cells

Cdc45, Col5a2, Dlk1, Rian, Meg3, Ranbp1, and *Bcan* expression levels quantified by qPCR in RNA samples from pooled fluorescence-activated cell sorted (FACS)-isolated *Tbr2*$^{eGFP+}$ cells from WT (black) and *LgDel* (magenta) samples parallel to those used for bulk RNA sequencing (n=6, from 25 WT and 26 *LgDel* E14.5 fetuses). Results are presented as ΔCT values normalized to GAPDH; lower ΔCT indicates higher gene expression. Each data point represents a biological replicate corresponding to a single pooled sample. Individual data points reflect average values of three independent qPCR runs to ensure robustness. Statistical significance between WT and *LgDel* groups assessed using a paired two-tailed Student's t-test to account for batch effect.

Supplemental Figure 3: Expression of bulk RNAseq DE genes across aP, bP, and NB subsets

Expression of 36/50 differentially expressed (DE) non-22q11 genes, identified from bulk RNA-seq and detected in scRNAseq datasets at a mean threshold of > 0.1 transcripts per cell. Each bubble corresponds to gene expression within a specific cell subsets aP, bP, and NB as defined

by scRNAseq analysis. Light half-circles represent WT cells, and dark half-circles represent *LgDel* cells, highlighting heterogenous expression patterns across all cell subsets.

Supplemental Figure 4: Spatial expression of 22q11 and DE genes in the E14.5 mouse cortex **a**). Ventricular zone (VZ), subventricular zone (SVZ), intermediate zone (IZ), cortical plate (CP) and marginal zone (MZ) expression of DE genes in *Tbr2*$^{eGFP+}$cells based upon colorimetric *in situ* hybridization in midline sagittal sections from the Genepaint (https://genepaint.org[4]) E14.5 fetal mouse dataset. Dark to light shading represents visually assessed labeling intensity in each cortical zone from ventricle to pial surface. All genes are localized to at least one developing cortical zone where *Tbr2*$^{eGFP+}$ cells are found[5]; asterisks indicate genes with rostral-caudal expression gradients. **b**). Five DE genes expressed in anterior low-posterior high (*Slc26a1*, *Bcan*), anterior high-posterior low (*Gfra1*), anterior low-middle high-posterior low (Meg3) or anterior high-middle low-posterior high gradient (*Col5a2*). **c**). 22q11 gene expression in the E14.5 VZ, SVZ, IZ, CP and MZ based upon Genepaint *in situ* localization of 16 22q11 genes: asterisks indicate genes with rostro-caudal expression gradients. **d**). Anterior low – posterior high gradients of *Dgcr14*, *Gsc2* (excluding high olfactory bulb), and *Slc25a1*. **e**). Distribution of DE genes across murine chromosomes based upon random permutation. DEGs counted within non-overlapping 300 kb windows along all 19 autosomes, X and Y. X-axis: genomic position; y-axis: n. DE genes. Peaks indicate locally elevated DE gene density or chromosomal domains of coordinated gene expression changes. Significant enrichment of DE genes on mouse chromosome 12 includes *Dlk1* locus genes (**$p < 0.0001$); significance assessed by simulating DE gene hits to a null distribution of random permutations of DE gene chromosome positions, indicating that enrichment is unlikely to occur by chance.

Supplemental Figure 5: Hallmark GSEA of bulk RNAseq data

GSEA of the bulk RNA sequencing dataset. **a**). Two-side barplot showing hallmark gene sets with significant enrichment (adjusted $p < 0.05$). Gene sets upregulated in *LgDel* bPs with positive normalized enrichment score (NES) are shown on the top right, while downregulated gene sets with negative (NES) are on the bottom left. **b**). A concept network plot highlights functional relationships and overlaps among key proliferative pathways, including E2F targets, G2/M checkpoint, and Mitotic spindle, indicating shared gene components and potential regulatory coordination. **c**). Individual GSEA enrichment plots are shown for five representative hallmark pathways with significant enrichment, illustrating the ranked gene list metric and the running enrichment score for each gene set.

Supplemental Figure 6: Transcriptional dysregulation in *LgDel* Tbr2^{-} cells

a). Significantly altered distribution/variance of *LgDel Cdc45* RNAscope puncta in individual Tbr2^{-} VZ/SVZ cells (Levene's and Brown-Forsythe's tests). *inset*: Significantly reduced (per cell/per animal) mean *Cdc45* puncta frequency in *LgDel* DAPI^{+}/Tbr2^{-} VZ/SVZ cells (unpaired t-test). **b**). Significantly altered distribution/variance of *LgDel Dgcr8* RNAscope puncta in individual Tbr2^{-} VZ/SVZ cells (Levene's and Brown-Forsythe's tests). *inset*: Reduced (per cell/per animal) mean *Dgcr8* puncta frequency in *LgDel* DAPI+Tbr2^{-} VZ/SVZ cells (unpaired t-test) is not statistically significant, possibly because *LgDel Dgcr8* expression, based upon bulk RNAseq and qPCR, is higher than the 50% average of other 22q11 genes. **c**). *Meg3* puncta distribution/variance in individual *LgDel* DAPI^{+}/Tbr2^{-} VZ/SVZ cells are not altered (Levene's and Brown-Forsythe's tests). *inset*: Mean (per cell, per animal) WT vs. *LgDel Meg3* puncta frequency does not differ significantly. **d**). *Rian* puncta distribution/variance in individual *LgDel* Tbr2^{-} VZ/SVZ cells is not altered (Levene's and Brown-Forsythe's tests). *inset*: Mean (per cell, per

animal) WT vs. *LgDel Rian* puncta frequency does not differ significantly. **e**) Very weak (WT) to weak (*LgDel*) correlation between *Cdc45* (*C45*) and *Dgcr8* (*D8*) puncta frequency per $Tbr2^+$ cell. **f**). Weak (*LgDel*) to very weak (WT) correlation between *Cdc45* and *Dgcr8* puncta frequency per $Tbr2^-$ cell. **g**) Very weak correlation between *Dgcr8* puncta frequency and $Tbr2^+$ cell size ($Tbr2^+$ nuclear area) in WT and *LgDel*. **h**). Very weak correlation between *Dgcr8* puncta frequency and $Tbr2^-$ cell size ($DAPI^+$ nuclear area) in WT and *LgDel*.

Supplemental Figure 7: scRNAseq confirmation of *Dlk1* locus expression across aP, bP, and NB subsets

Expression *Dlk1* locus gene across aP, bP, and NB subsets, based upon scRNAseq analysis. Each bubble represents gene expression within a specific subset; light half-circles indicate WT cells and dark half-circles, *LgDel*. The bubble plot scale for *Dlk1* and *Rtl1* is magnified by 5X due to detection of these two genes beneath the > 0.1 transcripts per cell cutoff. Consistent with bulk RNAseq data, expression levels of *Meg3*, *Dlk1*, Rian, and *Rtl1* are apparently increased in scRNAseq-defined bP1 as well as NB5 and NB6 subsets.

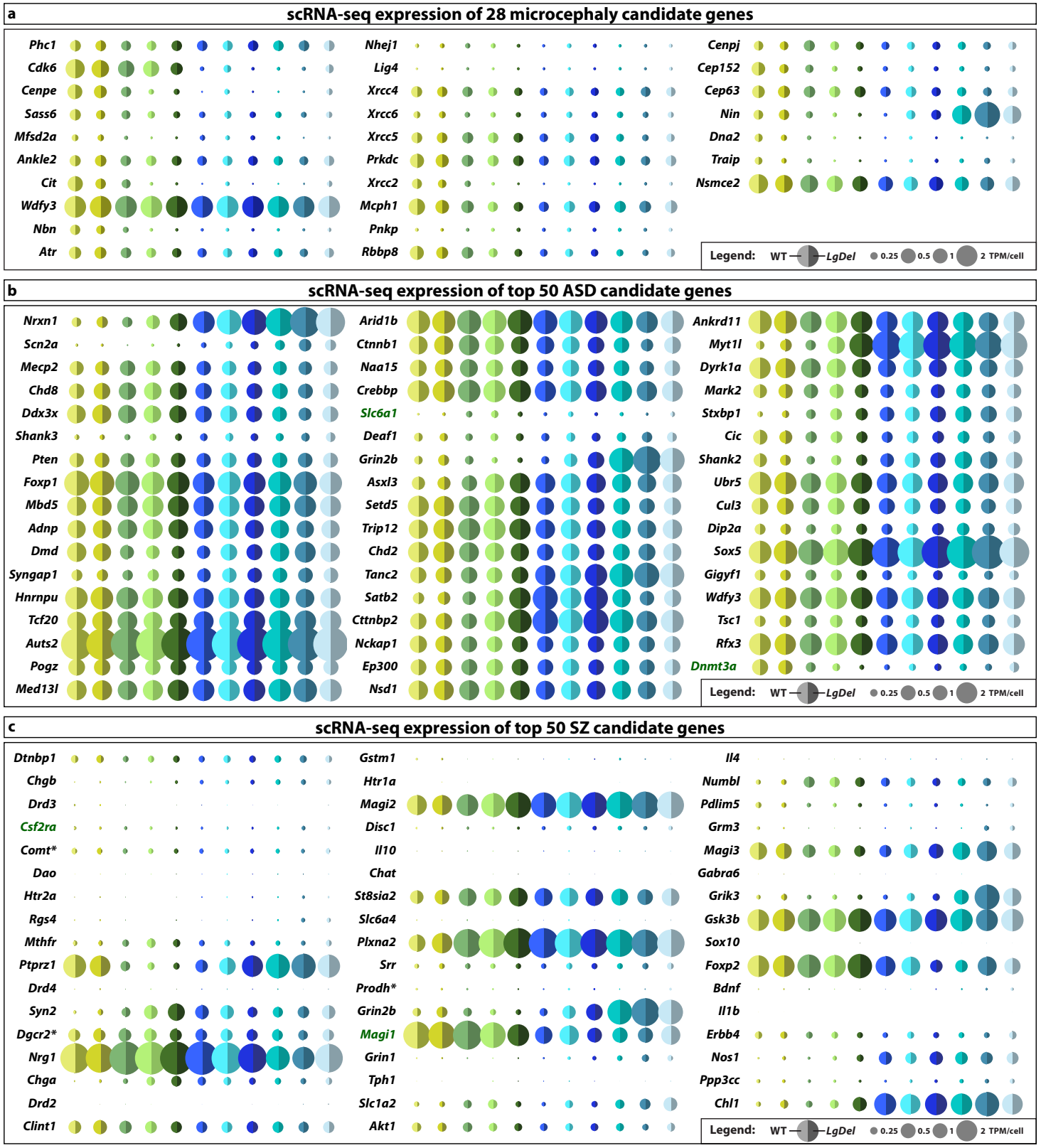
Supplemental Figure 8: P5 expression analysis of Brn2 in WT and *LgDel* Layer 2/3 PNs.

Similar distributions of $Brn2^+$ cells, presumed Layer 2/3 PNs, across 20 equivalent bins from ventricle to pia in the frontal association cortex of P5 WT and *LgDel* mice. *inset*: The frequency of $Brn2^+$ cells in WT and *LgDel* is statistically indistinguishable.

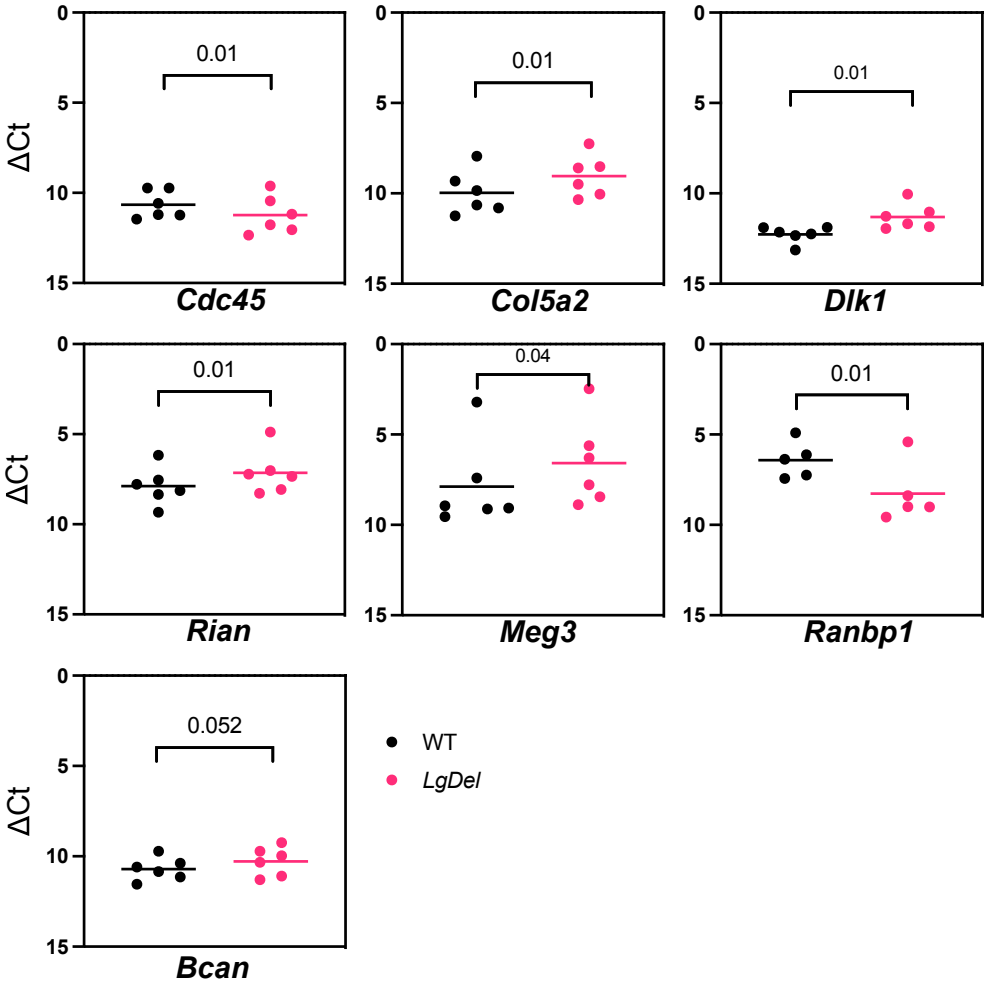
References for Supplemental Material

1. Jayaraman, D., Bae, B.I. & Walsh, C.A. The Genetics of Primary Microcephaly. *Annu Rev Genomics Hum Genet* **19**, 177-200 (2018).
2. Abrahams, B.S. *et al.* SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). *Mol Autism* **4**, 36 (2013).
3. Sun, J., Kuo, P.H., Riley, B.P., Kendler, K.S. & Zhao, Z. Candidate genes for schizophrenia: a survey of association studies and gene ranking. *Am J Med Genet B Neuropsychiatr Genet* **147B**, 1173-81 (2008).
4. Visel, A., Thaller, C. & Eichele, G. GenePaint.org: an atlas of gene expression patterns in the mouse embryo. *Nucleic Acids Res* **32**, D552-6 (2004).
5. Kwon, G.S. & Hadjantonakis, A.K. Eomes::GFP-a tool for live imaging cells of the trophoblast, primitive streak, and telencephalon in the mouse embryo. *Genesis* **45**, 208-17 (2007).

Supplemental Figure S1



Supplemental Figure S2



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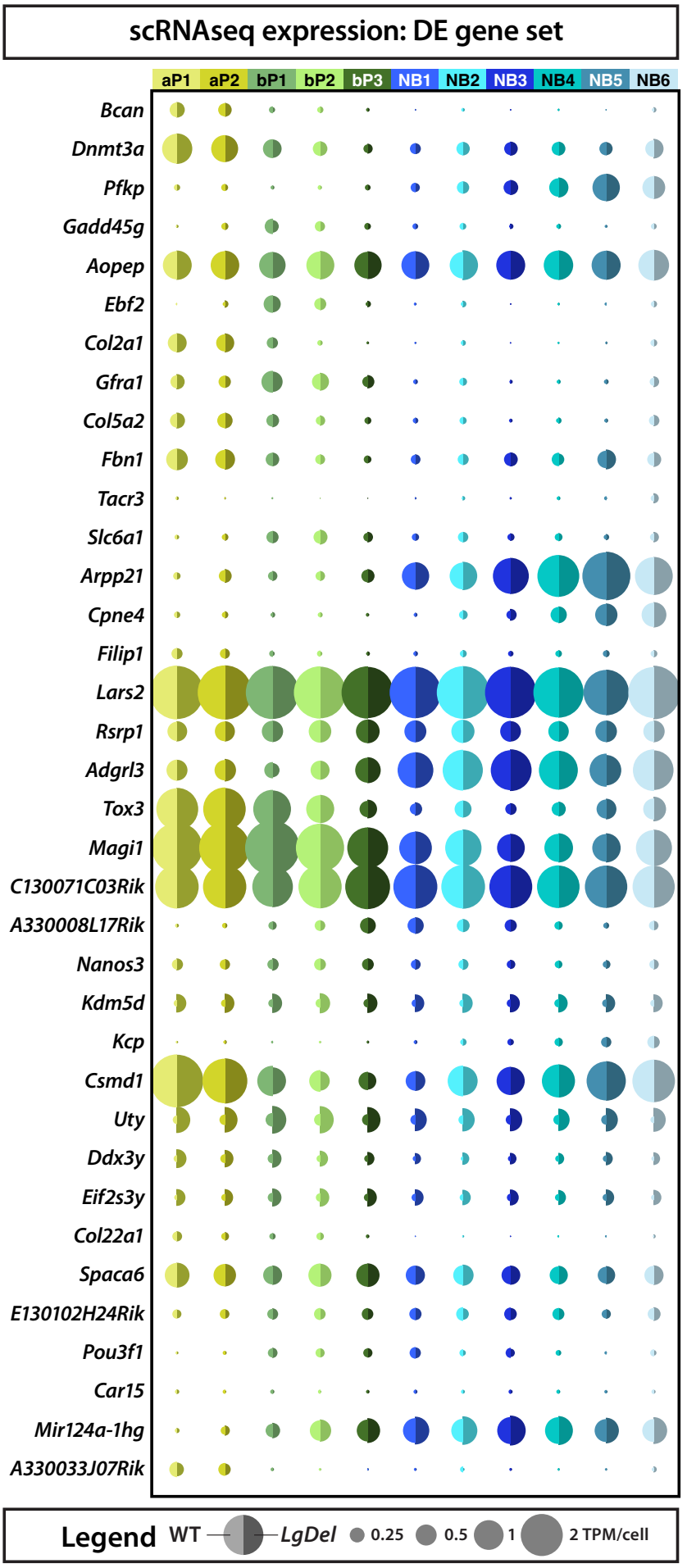

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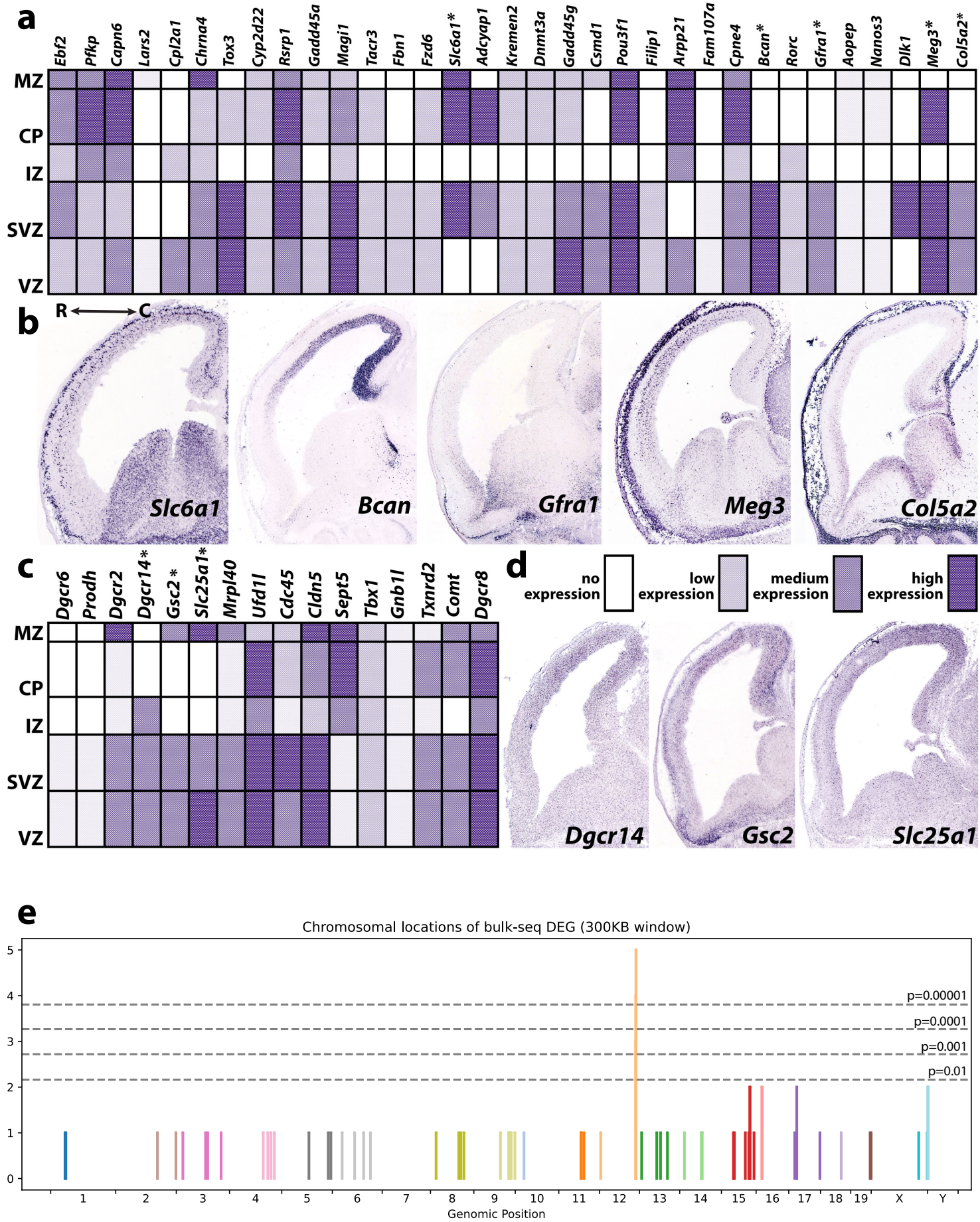

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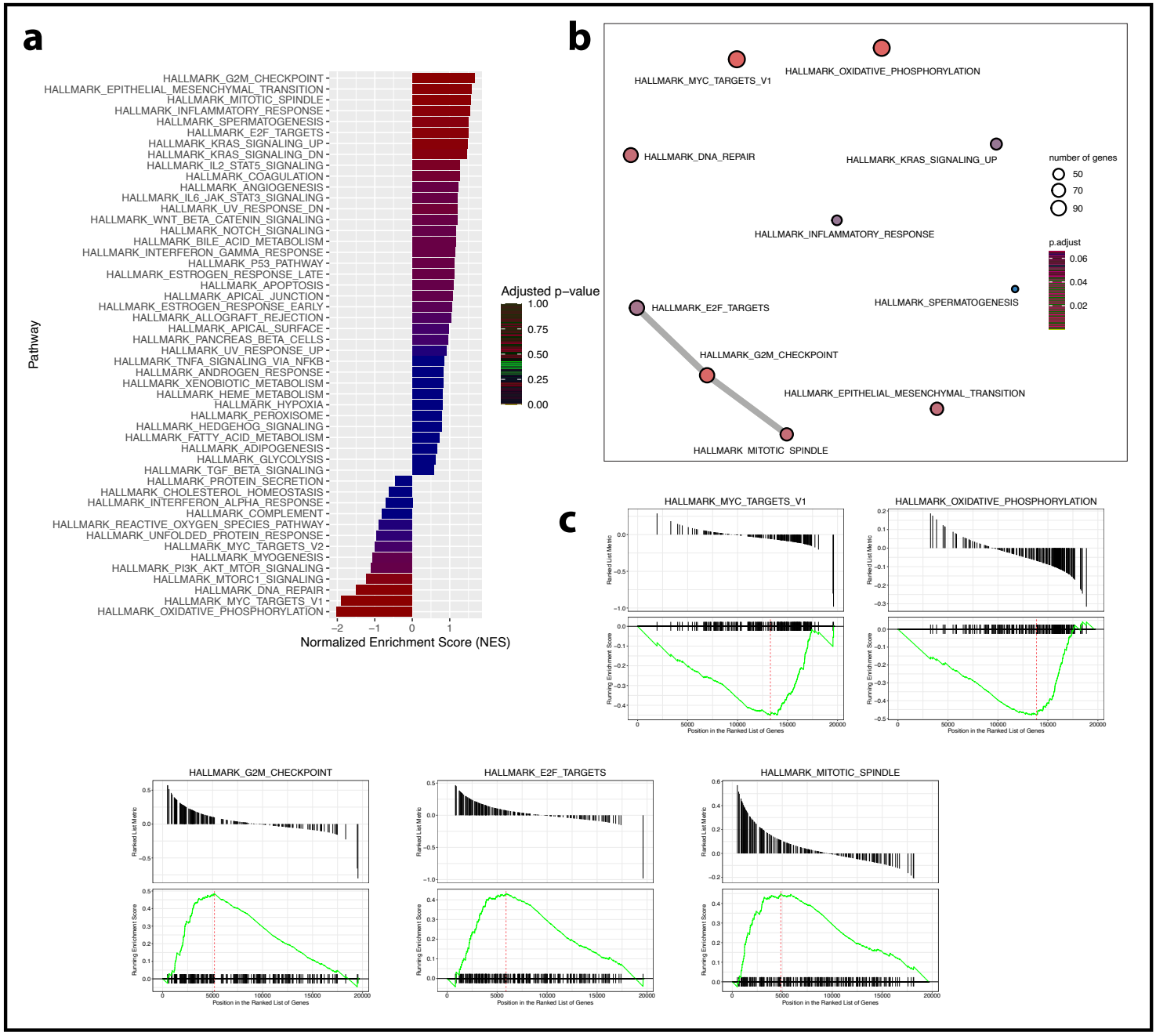

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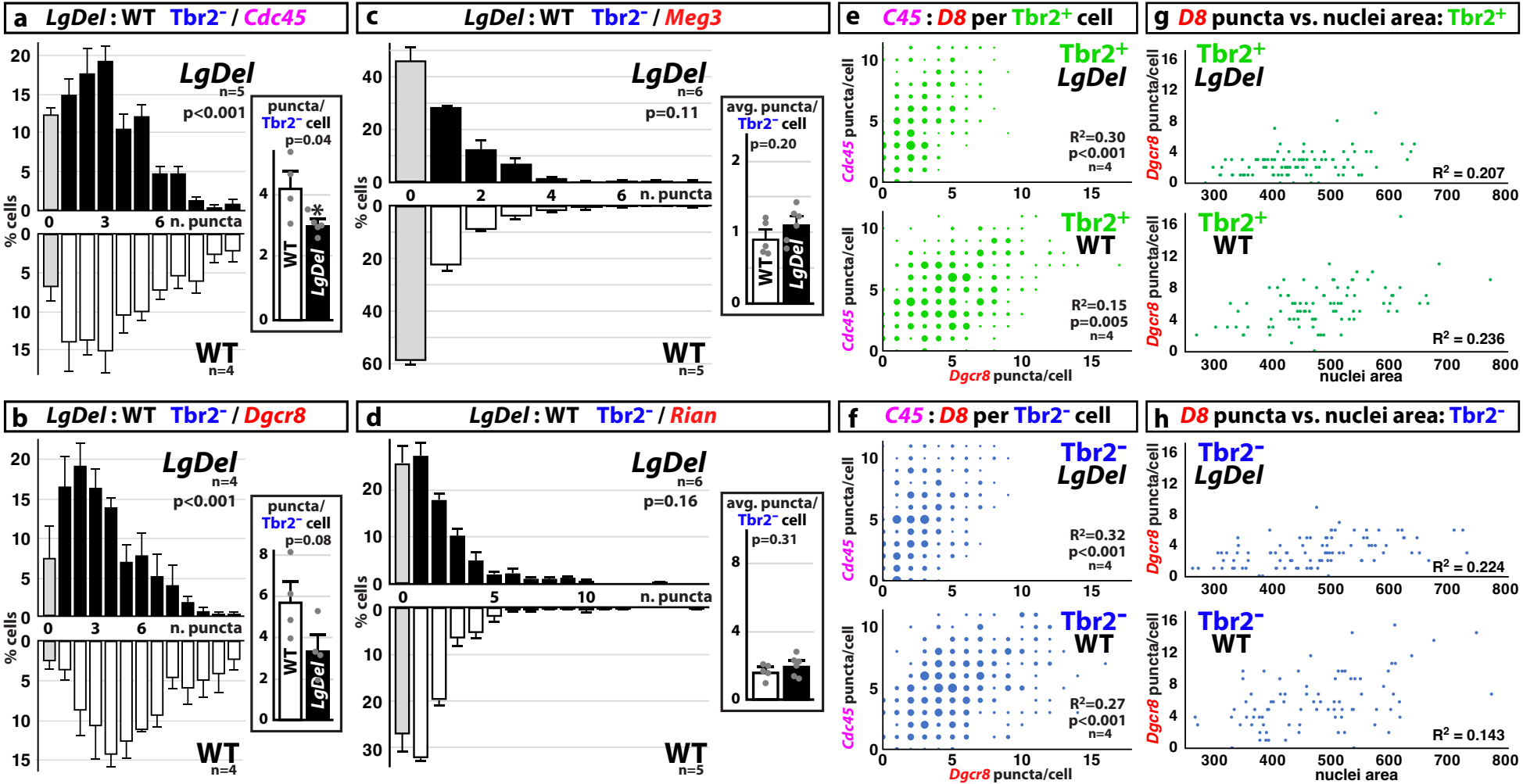

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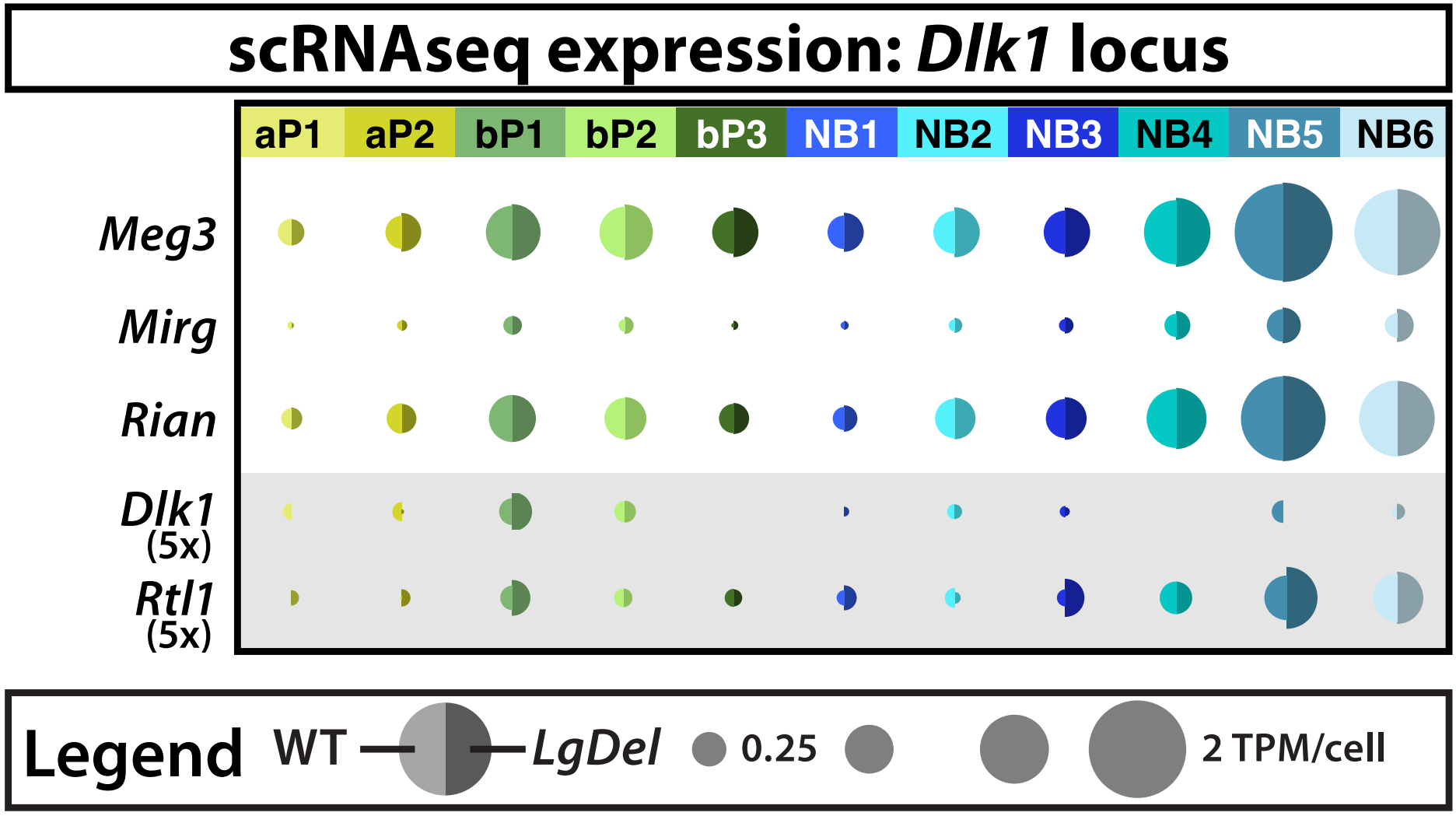

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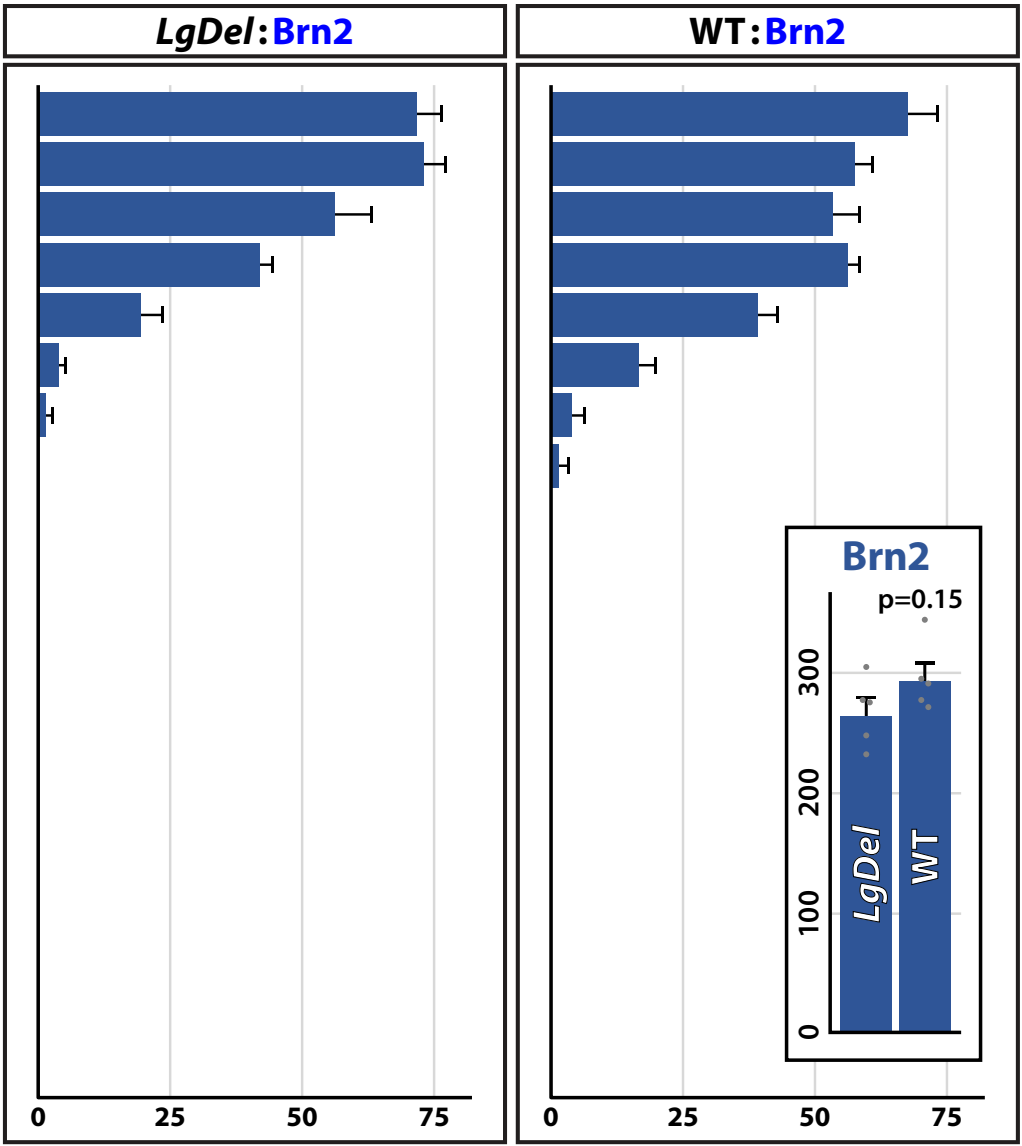

Supplemental Table 1: PCR primers used for quantitative (qPCR) validation of differentially expressed genes (top) as well as genotyping of *LgDel* vs. WT fetuses and postnatal mice.

Supplemental Table 2: Primary (top) and secondary (bottom) antibodies used to identify progenitor and projection neuron cell types as well as S-phase labeled cells. The species and type is provided for each antibody, as well as source (with catalog number), concentration used, and antigen retrieval conditions, when used.

Supplemental Table 3: RNAscope probes used for cellular validation of 22q11 and differentially expressed gene expression levels. All probes from ACD/Biotechne; catalogue numbers and targeted base pairs are provided as available.

Supplemental Table 4: PCR primers for amplicons to tile the Meg3 differentially methylated region (DMR) as well as the *Dlk1* locus intergenic DMR (IG-DMR), two locus control regions whose methylation influences expression of 5 genes within the *Dlk1* locus.

Supplemental Table 1

qPCR primers		
Primer	**Forward**	**Reverse**
Cdc45	CAG AGA AGC GCA CAC GGT TAG AAG AG	CCC ATG TCT GCA AGG AAC TCC TGG AG
Ranbp1	GAC CCC CAG TTC GAG CCA ATA GTT TC	CAT TTA GGA AGC GGA TGG CGA GCA G
Dlk1	CCTGGCTGTGTCAATGGAGT	TGGCAGTCCTTTCCAGAGAA
Meg3	CGAGGACTTCACGCACAA	ATTCCAGATGATGGCTTTG
Rian	TGTCGGAGGATCGTGTCAT	AATCTTCTAGAGCCCCAGATCC
Col5a2	GGAGAAGGAAACATCAGATTCA	CCAAATTCCTGGTCTGCATT
Bcan1	CTATGTTTGCCAGGCTATGGGGG	TGCCTCCTCCCAACTCCTTCGTG
Gapdh	CTG ACG TGC CGC CTG GAG AAA	GTT GGG GGC CGA GTT GGG ATA GG
genotyping primers		
Primer	**Forward**	**Reverse**
LgDel	GGCCAGCTCATTCCTCCCACTC	CACCAATCACAGGGCTGGAGGACTAC
C45	GGCCTCGGACGTGGATGCCTTGTG	CGGGGCTCCTGTATTTCCTGCACTC

Supplemental Table 2

	Antibody Name	Clonality Species	Source	Catalog No.	Concentration	Antigen Retrieval
Primary Antibodies	Tbr2	guinea pig polyclonal antibody	Synaptic Systems	483005	IHC (1:1000)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Satb2	guinea pig polyclonal antibody	Synaptic Systems	327004	IHC (1:500)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	BrdU (+ IdU)	chicken polyclonal antibody	Innovative research	43421	IHC (1:500)	Treated with sodium citrate pH 7.0 at 95°C for 15 min
	BrdU	rat monoclonal antibody	Abcam	Ab6326	IHC (1:100)	Treated with sodium citrate pH 7.0 at 95°C for 15 min
	Brn2	mouse monoclonal antibody	Santa Cruz Biotechnology	393324	IHC (1:100)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Pax6	rabbit polycolnal antibody	Biolegend	9013901	IHC & Pair cell assay (1:1000)	IHC: Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Tbr2	rabbit polycolnal antibody	Abcam	Ab23345	IHC & Pair cell assay (1:1000)	Not required
	Neurod1	mouse monoclonal antibody	Abcam	Ab60704-1001	IHC (1:350)	Required RNAScope manual assay protocol
	Tbr2	chicken polycolnal antibody	Millipore	A615894	IHC (1:200)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Ki67	mouse monoclonal antibody	BD pharminogen	556003	IHC (1:400)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Ki67	rat monoclonal antibody	Biolegend	652402	IHC (1:200)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Cux1/2	rabbit monoclonal antibody	Abcam	Ab309139	IHC (1:50)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
	Cux1	rabbit polycolnal antibody	Proteintech	11733-1-AP	IHC (1:1000)	Treated with sodium citrate pH 7.0 at 95°C for 10 min
Secondary Antibodies	Alexa Fluor 488 anti-Chicken	Goat polyclonal antibody	Invitrogen	A11039	IHC (1:4000)	1-hour incubation at RT
	Alexa Fluor 546 goat anti-Chicken	Goat polyclonal antibody	Invitrogen	A11040	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 647 goat anti-Chicken	Goat polyclonal antibody	Invitrogen	A32933	IHC & Pair cell assay (1:2000)	1-hour incubation at RT
	Alexa Fluor 633 goat anti-Rat	Goat polyclonal antibody	Invitrogen	A21094	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 647 goat anti-Rat	Goat polyclonal antibody	Invitrogen	A21248	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 488 goat anti-Rabbit	Goat polyclonal antibody	Invitrogen	A11034	IHC (1:4000)	1-hour incubation at RT
	Alexa Fluor plus 405 goat anti-Rabbit	Goat polyclonal antibody	Invitrogen	A48254	IHC (1:500)	1-hour incubation at RT
	Alexa Fluor 647 goat anti-Rabbit	Goat polyclonal antibody	Invitrogen	A21244	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 546 goat anti-Rabbit	Goat polyclonal antibody	Invitrogen	A11035	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 680 goat anti-Mouse	Goat polyclonal antibody	Invitrogen	A21057	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 546 goat anti-Mouse	Goat polyclonal antibody	Invitrogen	A11030	IHC & Pair cell assay (1:2000)	1-hour incubation at RT
	Alexa Fluor 647 goat anti-Mouse	Goat polyclonal antibody	Invitrogen	A21235	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 546 goat anti-Guinea pig	Goat polyclonal antibody	Invitrogen	A11074	IHC (1:2000)	1-hour incubation at RT
	Alexa Fluor 647 goat anti-Guinea pig	Goat polyclonal antibody	Invitrogen	A21450	IHC (1:2000)	1-hour incubation at RT

Supplemental Table 3

Gene Name	Catalog No.	Target Region Base Pairs (bp)	RNAscope Assay Platform
Dlk1	405971-C3	1477 - 2387	Manual Assay RNAscope
Meg3	527201	2043 - 3118	Manual Assay RNAscope
Rian	510531	388 - 1387	Manual Assay RNAscope
Dgcr8	1243221-C3	664 - 1667	Manual Assay RNAscope
Cdc45	832311	129-1042	Manual Assay RNAscope

Supplemental Table 4

Bisulphite specific PCR primers						
Name of the target	**Forward Primer Sequence**	**Reverse Primer Sequence**	**Start with primer**	**End with primer**	**Chromosome**	**Strand**
Meg3_DRM_1	AYGTTTTTATTTTTGAAAAGGTTTGAGTTATTT	CCCCCTATAACCAACAAACCTAAAATAC	109539619	109539764	chr12	+
Meg3_DRM_2	GGTTTTTTTAGTTTTATGTTTTTTATAGGGTTTTTG	CCRACTACTCTACCTACCCTAAATAA	109539824	109540055	chr12	+
Meg3_DRM_3	GAGAGGATATTTGATGGATATGTTGTTTTTT	CCCTATAATAATACTTAAAATACACCCCCAA	109539995	109540228	chr12	+
Meg3_DRM_4	YGTTTTTTAAAGTGTGGGGAATTAGTT	AAAACCTTAATTTTAAATCCCAAACTTTACAAA	109540166	109540417	chr12	+
Meg3_DRM_5	GGTGTATAGGTTGTATTTTTTGTAAAGTTTGG	CTAAATTTCTCAAAACATCAATCAAAAACTCC	109540366	109540623	chr12	+
Meg3_DRM_6	GTTTTTGATTGATGTTTTGAGAAATTTAGGTAAG	CCAAATTCTATAACAAATTACTATAACCRCAC	109540594	109540878	chr12	+
Meg3_DRM_7	GTGYGGTTATAGTAATTTGTTATAGAATTTGG	CAAAAAATAACTAACCACTCACCAAATAAAAAATTC	109540846	109541121	chr12	+
Meg3_DRM_8	GGTTAGTTATTTTTTGTTTGAAAGGATGTG	CTCATTATTACTACTTTTCTTAATCAAAATCTCTACC	109541105	109541392	chr12	+
Meg3_DRM_9	GGTTTTGGTGGTTGAAAGTTTTTTTTAG	CAAAATTACTAATCAACATAAACCTCTAAATCTTAC	109541443	109541640	chr12	+
Meg3_DRM_10	GTAAGATTTAGAGGTTTATGTTGATTAGTAATTTTG	TAAAATTTTTTCTACCCTAACCCACCC	109541604	109541866	chr12	+
Meg3_DRM_11	GAGAGGGYGTTAAAGGTAAATTTAGT	CTCCAAATTTCCAACACTCAAATCAC	109541750	109541958	chr12	+
Meg3_DRM_12	GTGATTTGAGTGTTGGAAATTTGGAG	CCATCAATAAAACATTCTAAAAACCTACCA	109541932	109542169	chr12	+
IG_DMR_1	GTGTTTTAGGTTTGTGTTGTGAGGTATTA	ACCATAATATACTACTCCTCAAAACCCA	109526409	109526587	chr12	+
IG_DMR_2	GGGTTTTGAGGAGTAGTATATTATGGT	ACTATTATATCACACTTAACTTTACTACTACACATC	109526560	109526773	chr12	+
IG_DMR_3	GTAAAGTTAAGTGTGATATAATAGTTTTGATTTGGT	CATAAAAACATTACTCCATAATACATCCTATTCC	109526748	109527009	chr12	+
IG_DMR_4	GGAATAGGATGTATTATGGAGTAATGTTTTTATG	CCACATATCTATACTACAAAACACCAAAC	109526975	109527188	chr12	+
IG_DMR_4-1	GTGGGTGTATTATGTATGGTATAAGATAGATG	ACACCTAACCTATACTACAAAACAATCA	109527184	109527350	chr12	+
IG_DMR_5	GGTATAAGATTGAATGTTTTTGTGGTAAAGG	CATCCATATATACCAAACCAATACTATAATACAC	109527287	109527451	chr12	+
IG_DMR_6	GGTATATATGGATGTATTGTAATATAGGTTAGGTG	CCATACACTTTTACTATAAAACACTTAACTC	109527437	109527712	chr12	+
IG_DMR_7	GGTATATTTTGGTATAGATTTGGTGTTTTATGG	TATACCATAACACAACTACACAAAATACTACA	109527752	109527959	chr12	+
IG_DMR_8	GGTGTATTTTGTATTATTGTTTTGTGTTAGAGTAG	CCTTCCTAACACTAACATAATATACCTTAACAC	109527851	109528120	chr12	+
IG_DMR_8-1	GTGTTAAGGTATATTATGTTAGTGTTAGGAAGG	CATAAAACTTACCCATAACAAACCACAA	109528087	109528231	chr12	+
IG_DMR_9	GTTAYGGTTTATAGTGGATAGTTAGTGT	TTCTCCATTAACAAAATAATACAACCCTTC	109528270	109528547	chr12	+
IG_DMR_10	TTTGTTAATGGAGAATGTTTTGAGTATAGG	AAACTCTACAAATACCAAATTCCATCAAA	109528532	109528694	chr12	+
IG_DMR_11	GGTAGTTTGTGAGTTGATTTTTTTTAGTTATAGT	CTATATAATCCACAATCTAACAAAACTACAACAAA	109528781	109528924	chr12	+