Supplementary Materials:
Transcriptional dynamics of microRNAs and their targets during Drosophila neurogenesis

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Table 1: GEO Accession Numbers and number of reads for all samples. The GEO BioProject Accession is GSE117245.

GEO accession	time point	marker	fraction	replicate	reads $\times 10^6$
GSM3289833	6-8h	repo	pos	1	37.3
GSM3289834	6-8h	repo	pos	2	32.6
GSM3289835	6-8h	repo	neg	1	34.9
GSM3289836	6-8h	repo	neg	2	26.7
GSM3289837	6-8h	elav	pos	1	27.7
GSM3289838	6-8h	elav	pos	2	25.5
GSM3289839	6-8h	elav	neg	1	18.6
GSM3289840	6-8h	elav	neg	2	26.7
GSM3289841	6-8h	prospero	pos	1	45.4
GSM3289842	6-8h	prospero	pos	2	37.9
GSM3289843	6-8h	prospero	neg	1	22.8
GSM3289844	6-8h	prospero	neg	2	36.9
GSM3289845	18-22h	repo	pos	1	33.6
GSM3289846	18-22h	repo	pos	2	31.4
GSM3289847	18-22h	repo	neg	1	37.6
GSM3289848	18-22h	repo	neg	2	44.4
GSM3289849	18-22h	elav	pos	1	30.7
GSM3289850	18-22h	elav	pos	2	33.1
GSM3289851	18-22h	elav	neg	1	44.5
GSM3289852	18-22h	elav	neg	2	38.6

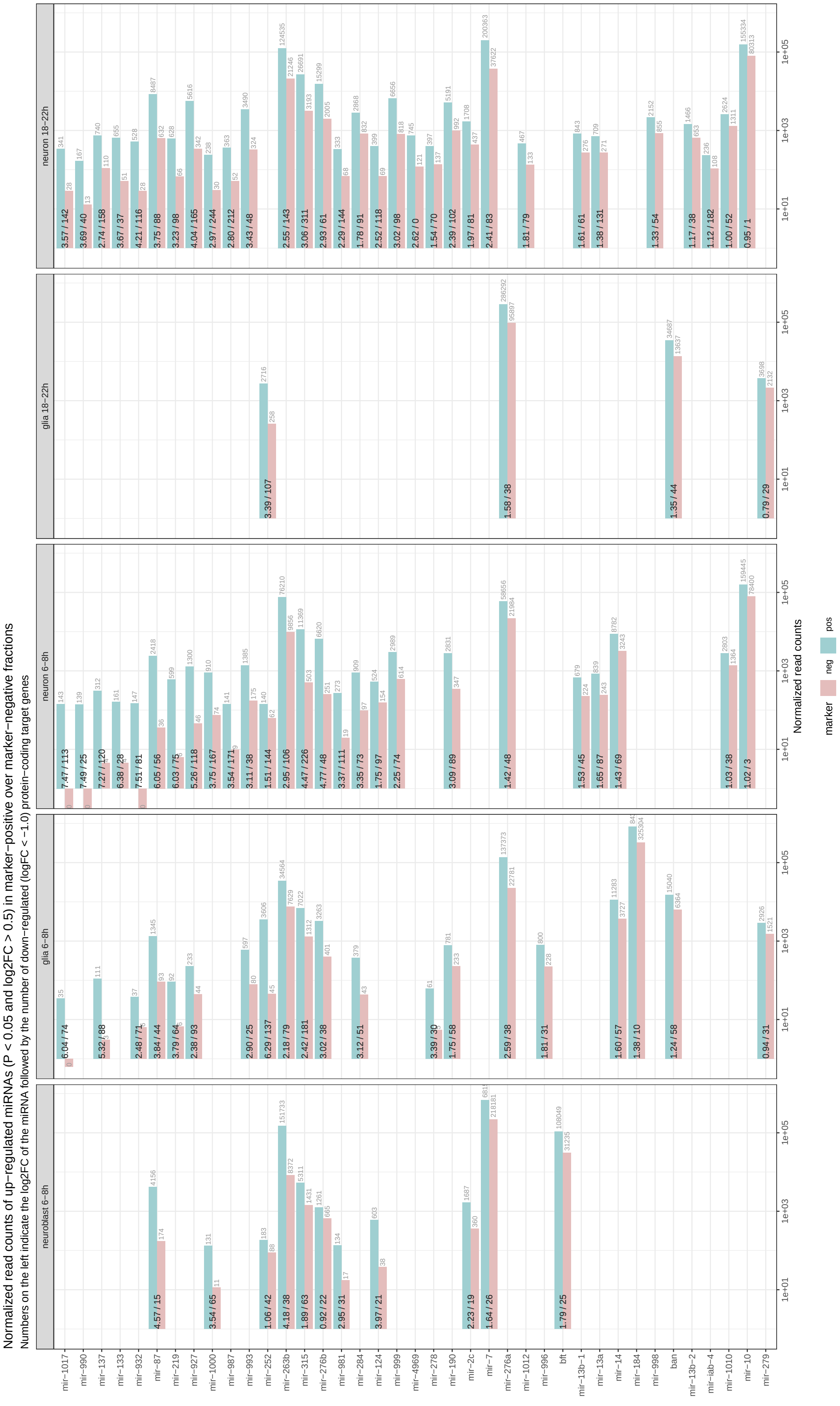


Figure 1: Up-regulated miRNAs in marker-positive over marker-negative fractions

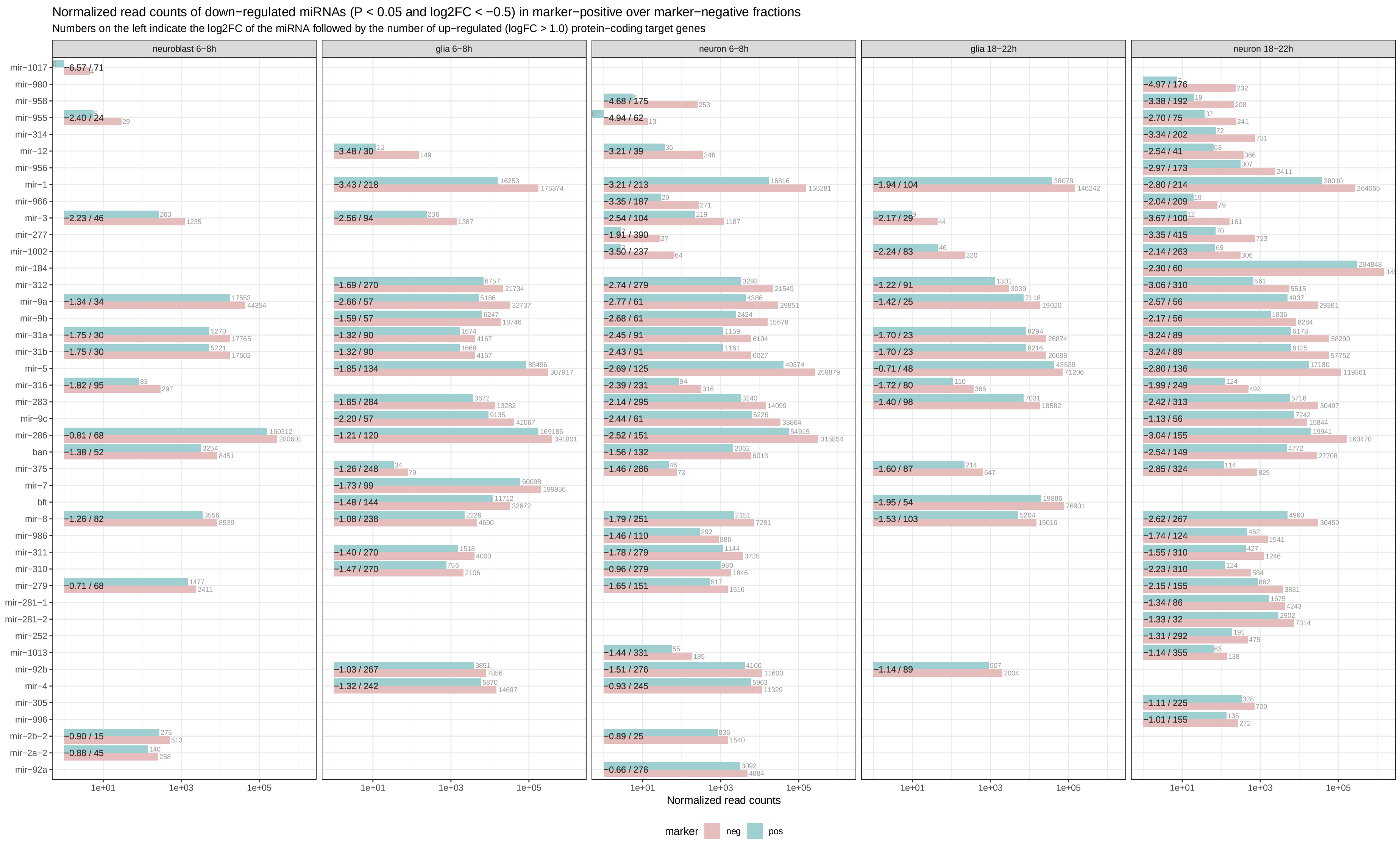


Figure 2: Down-regulated miRNAs in marker-positive over marker-negative fractions

Figure 3: Differential gene expression between marker-positive and marker-negative FACS fractions. Shown are only protein-coding genes that have target sites of differentially expressed microRNAs in the same sample and are associated with the GO term *nervous system development, GO:0007399* or its descendants in the GO tree. Colored points denote significant differential expression.

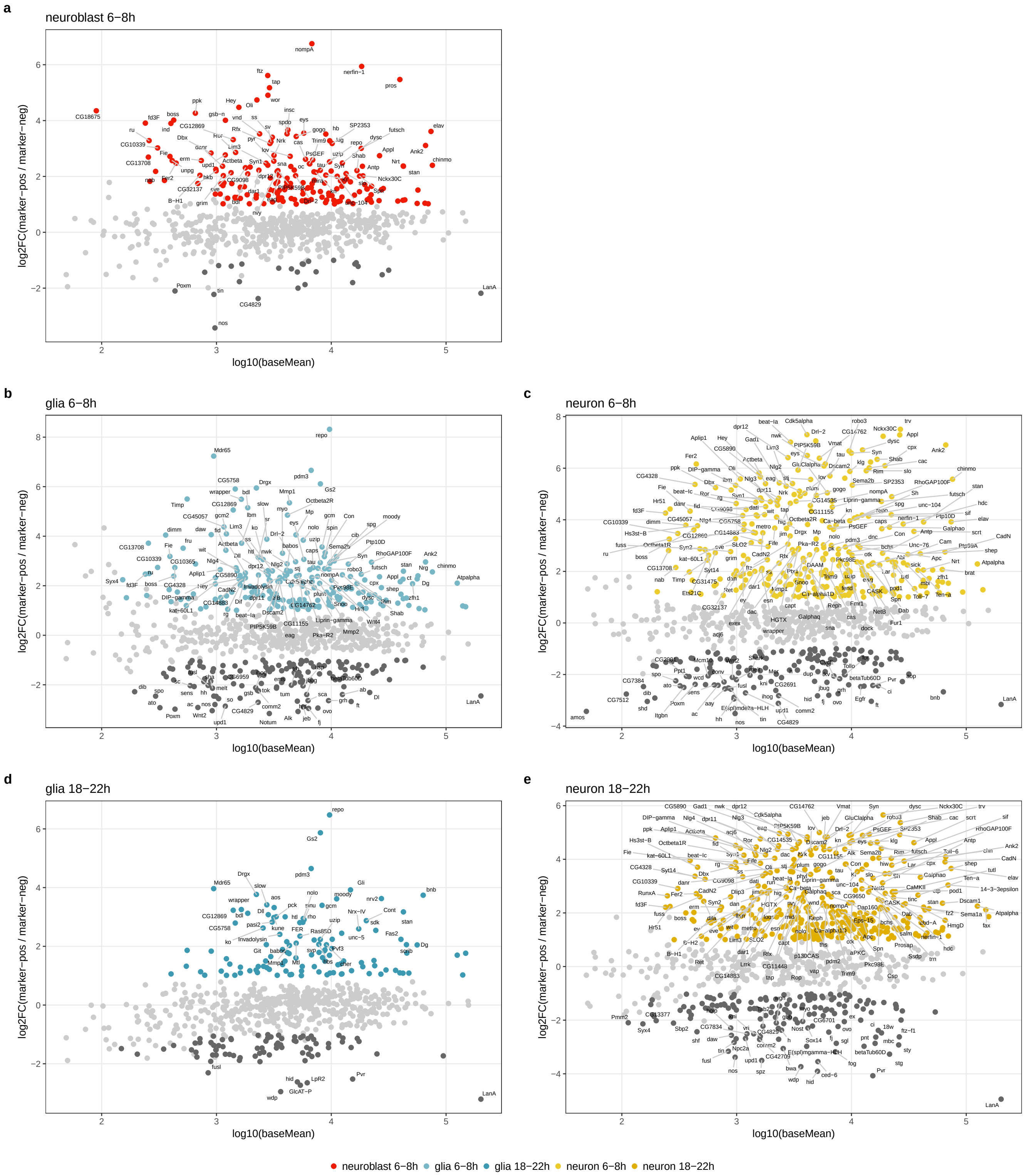


Figure 4: **(a)**: Overlap between up-regulated target genes of down-regulated microRNAs. **(b)**: Overlap between down-regulated target genes of up-regulated microRNAs. Shown are only genes that are associated with the GO term *nervous system development, GO:0007399* or its descendants in the GO tree.

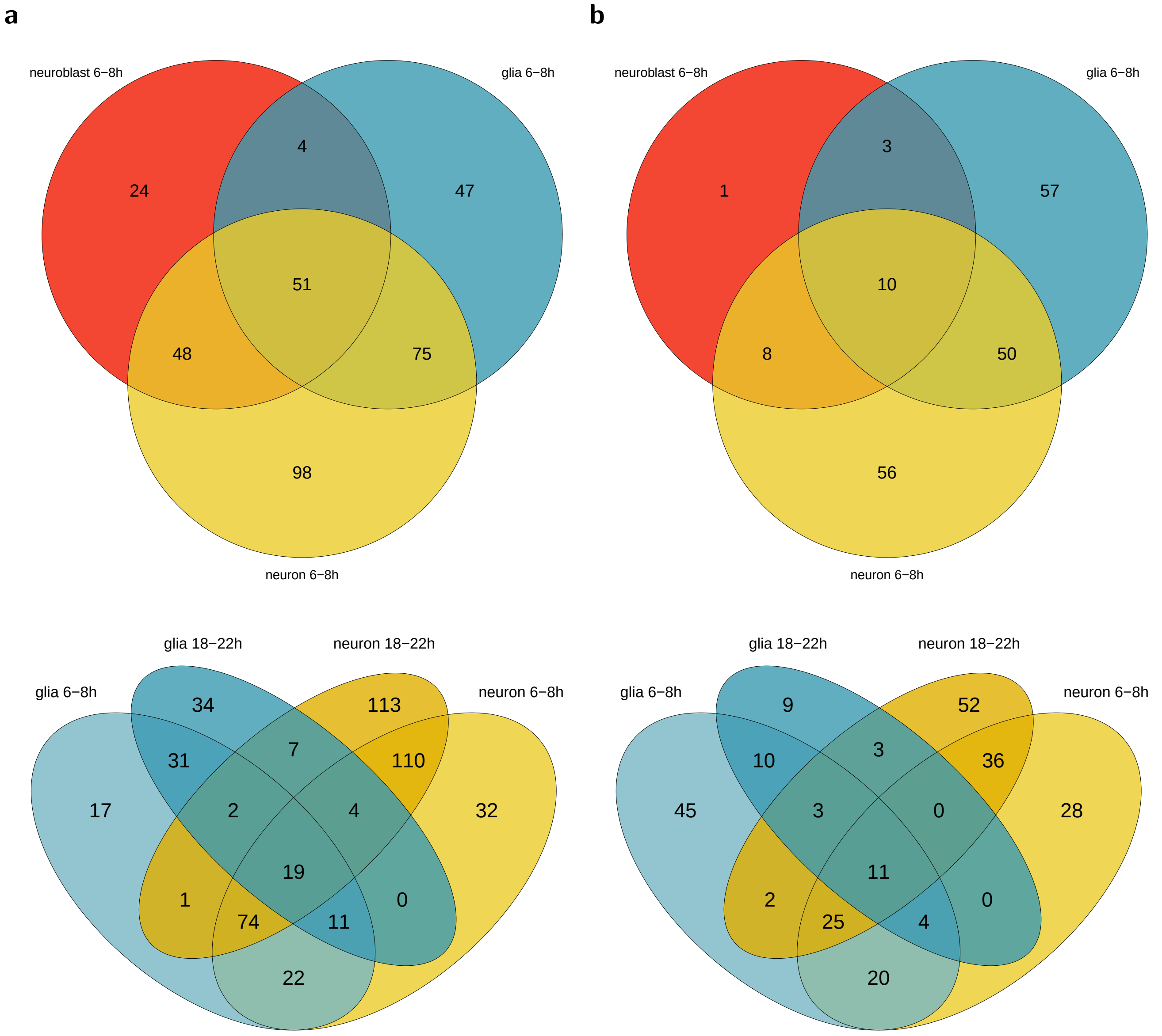


Figure 5: Differential gene expression between between cell types and time points. Shown are only protein-coding genes that have target sites of differentially expressed microRNAs in the same comparison and are associated with the GO term *nervous system development, GO:0007399* or its descendants in the GO tree. Colored points denote significant differential expression.

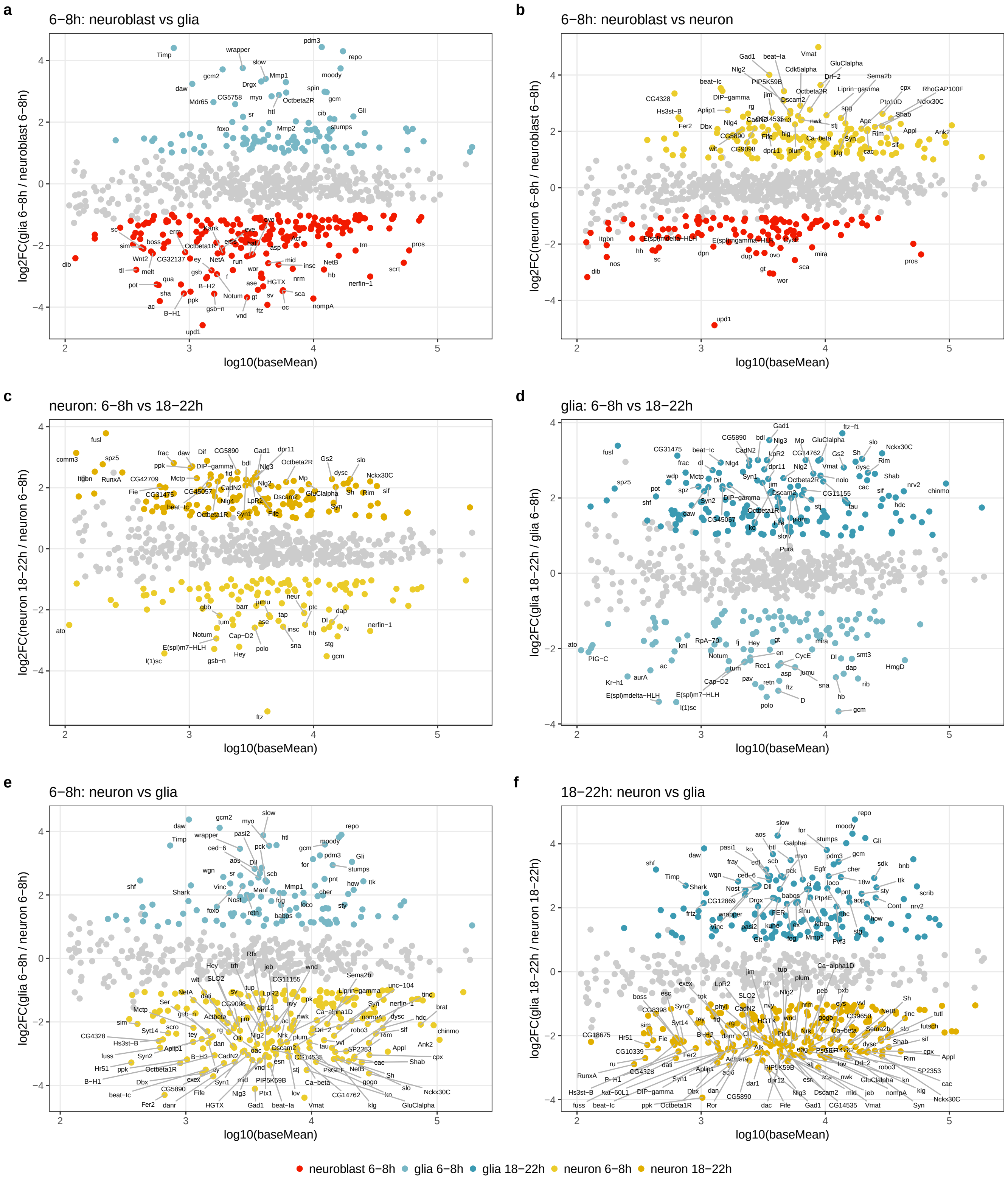


Figure 6: Clustering of microRNAs by their target genes

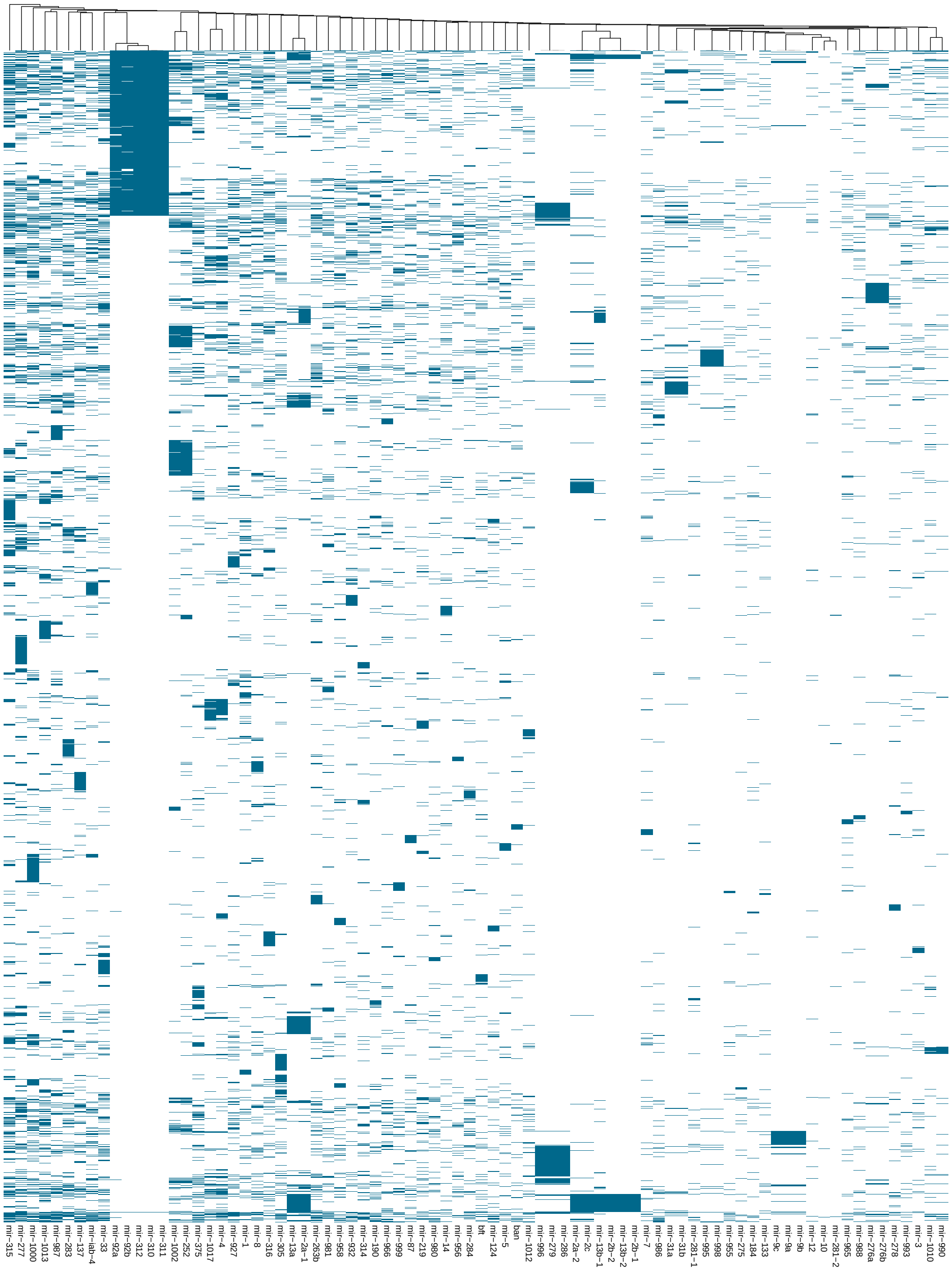
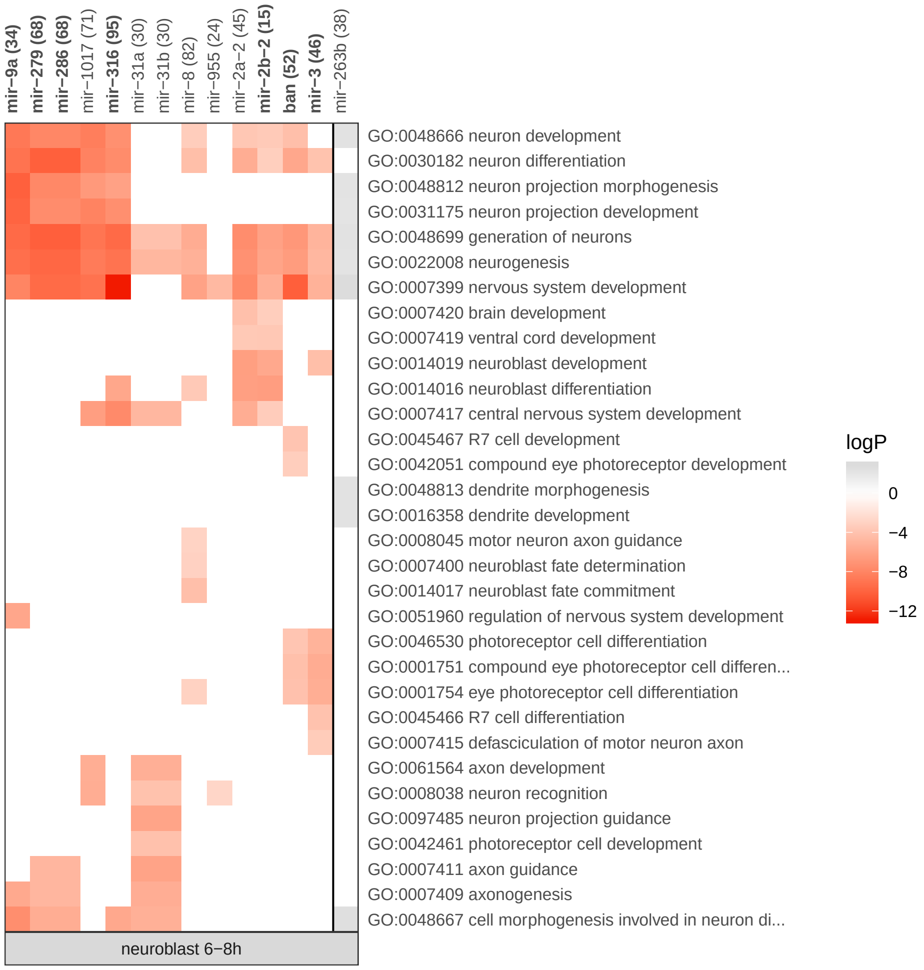
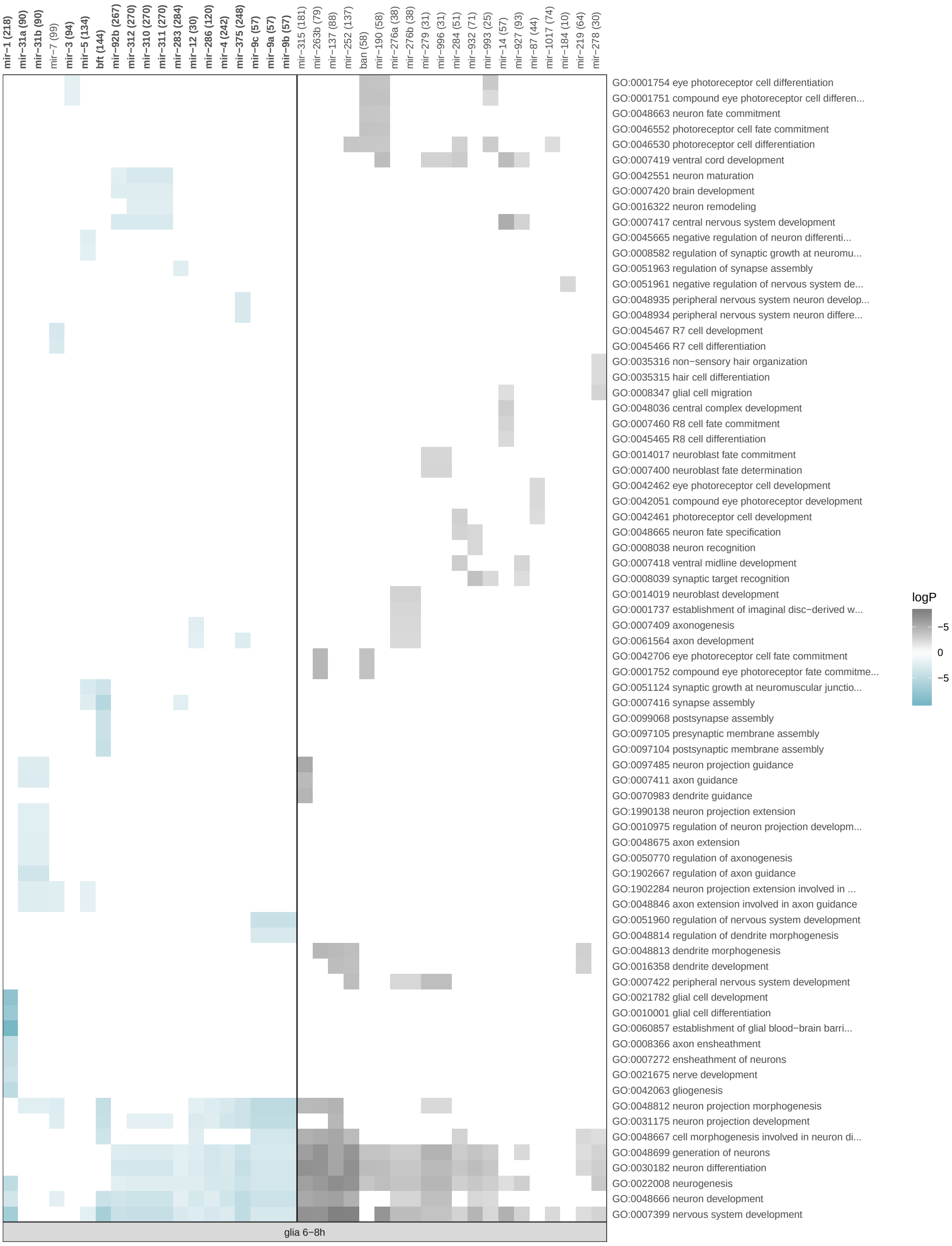


Figure 7: GO *Biological Process* terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the set of up-regulated protein-coding genes that are targets of down-regulated microRNAs in the marker-positive vs. marker-negative FACS fractions and vice versa. MicroRNAs having a significant overlap (number in brackets) of their target genes with genes regulated in the opposite direction are printed in bold. Grey colors denote the GO term enrichment P-value of down-regulated target genes of up-regulated microRNAs in the marker-positive fractions, whereas non-grey colors denote the GO term enrichment P-value of up-regulated target genes of down-regulated microRNAs in the marker-positive fractions, respectively.

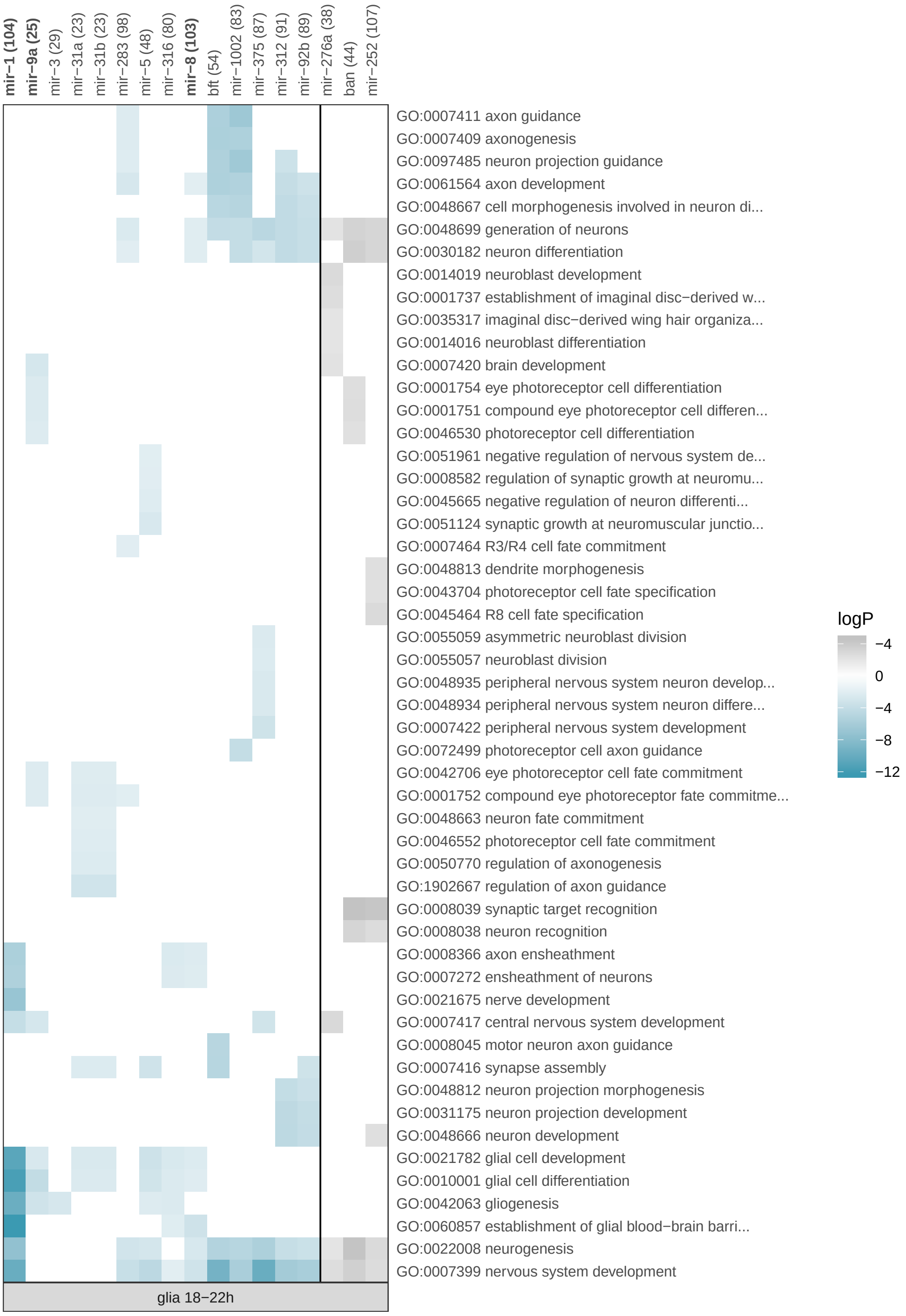
7a neuroblast 6-8h



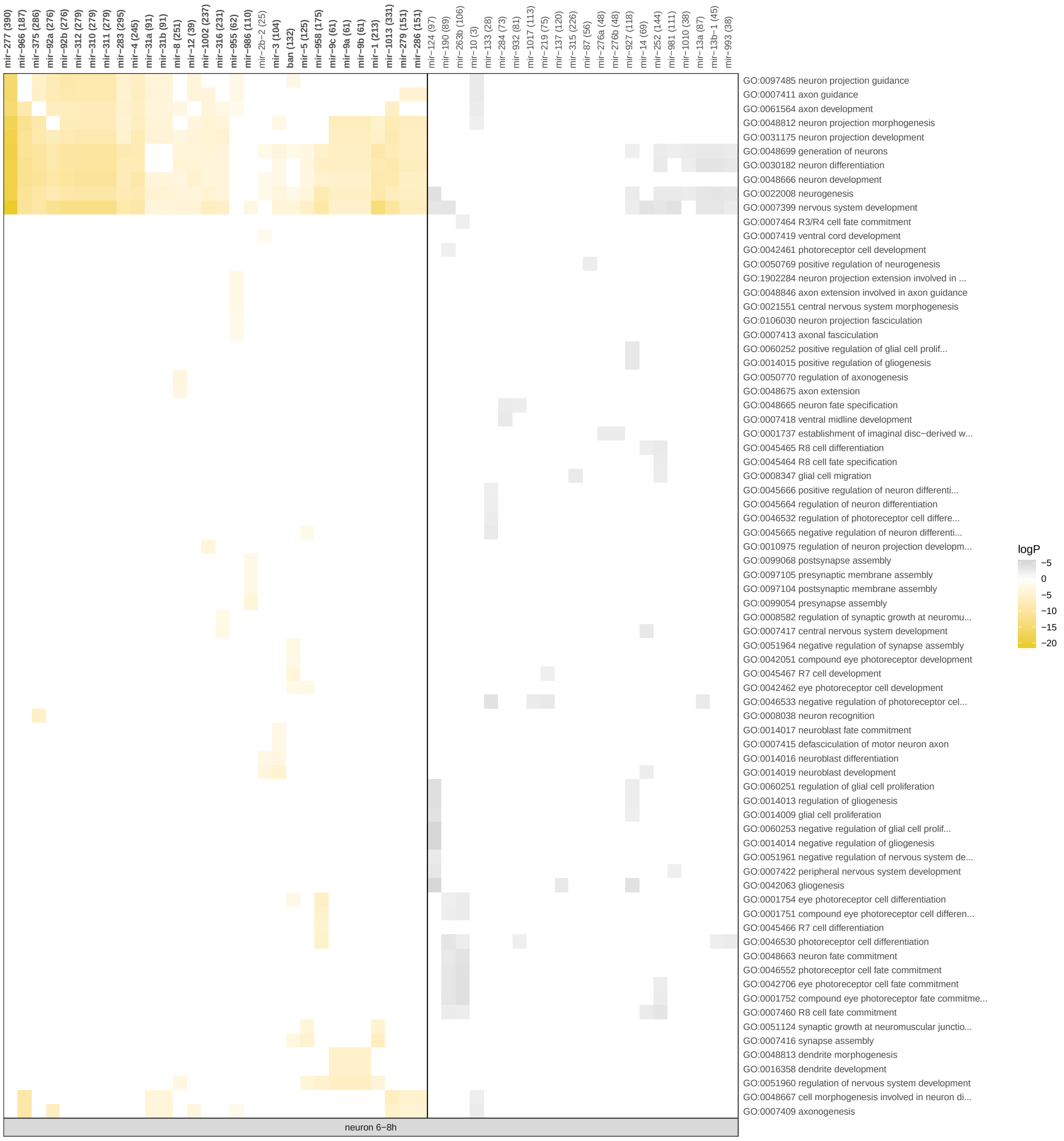
7b glia 6-8h


mir−1 (218)
mir−31a (90)
mir−31b (90)
mir−7 (99)
mir−3 (94)
mir−5 (134)
bft (144)
mir−92b (267)
mir−312 (270)
mir−310 (270)
mir−311 (270)
mir−283 (284)
mir−12 (30)
mir−286 (120)
mir−4 (242)
mir−375 (248)
mir−9c (57)
mir−9a (57)
mir−9b (57)
mir−315 (181)
mir−263b (79)
mir−137 (88)
mir−252 (137)
ban (58)
mir−190 (58)
mir−276a (38)
mir−276b (38)
mir−279 (31)
mir−996 (31)
mir−284 (51)
mir−932 (71)
mir−993 (25)
mir−14 (57)
mir−927 (93)
mir−87 (44)
mir−1017 (74)
mir−184 (10)
mir−219 (64)
mir−278 (30)
GO:0001754 eye photoreceptor cell differentiation
GO:0001751 compound eye photoreceptor cell differen...
GO:0048663 neuron fate commitment
GO:0046552 photoreceptor cell fate commitment
GO:0046530 photoreceptor cell differentiation
GO:0007419 ventral cord development
GO:0042551 neuron maturation
GO:0007420 brain development
GO:0016322 neuron remodeling
GO:0007417 central nervous system development
GO:0045665 negative regulation of neuron differenti...
GO:0008582 regulation of synaptic growth at neuromu...
GO:0051963 regulation of synapse assembly
GO:0051961 negative regulation of nervous system de...
GO:0048935 peripheral nervous system neuron develop...
GO:0048934 peripheral nervous system neuron differe...
GO:0045467 R7 cell development
GO:0045466 R7 cell differentiation
GO:0035316 non−sensory hair organization
GO:0035315 hair cell differentiation
GO:0008347 glial cell migration
GO:0048036 central complex development
GO:0007460 R8 cell fate commitment
GO:0045465 R8 cell differentiation
GO:0014017 neuroblast fate commitment
GO:0007400 neuroblast fate determination
GO:0042462 eye photoreceptor cell development
GO:0042051 compound eye photoreceptor development
GO:0042461 photoreceptor cell development
GO:0048665 neuron fate specification
GO:0008038 neuron recognition
GO:0007418 ventral midline development
GO:0008039 synaptic target recognition
GO:0014019 neuroblast development
GO:0001737 establishment of imaginal disc−derived w...
GO:0007409 axonogenesis
GO:0061564 axon development
GO:0042706 eye photoreceptor cell fate commitment
GO:0001752 compound eye photoreceptor fate commitme...
GO:0051124 synaptic growth at neuromuscular junctio...
GO:0007416 synapse assembly
GO:0099068 postsynapse assembly
GO:0097105 presynaptic membrane assembly
GO:0097104 postsynaptic membrane assembly
GO:0097485 neuron projection guidance
GO:0007411 axon guidance
GO:0070983 dendrite guidance
GO:1990138 neuron projection extension
GO:0010975 regulation of neuron projection developm...
GO:0048675 axon extension
GO:0050770 regulation of axonogenesis
GO:1902667 regulation of axon guidance
GO:1902284 neuron projection extension involved in ...
GO:0048846 axon extension involved in axon guidance
GO:0051960 regulation of nervous system development
GO:0048814 regulation of dendrite morphogenesis
GO:0048813 dendrite morphogenesis
GO:0016358 dendrite development
GO:0007422 peripheral nervous system development
GO:0021782 glial cell development
GO:0010001 glial cell differentiation
GO:0060857 establishment of glial blood−brain barri...
GO:0008366 axon ensheathment
GO:0007272 ensheathment of neurons
GO:0021675 nerve development
GO:0042063 gliogenesis
GO:0048812 neuron projection morphogenesis
GO:0031175 neuron projection development
GO:0048667 cell morphogenesis involved in neuron di...
GO:0048699 generation of neurons
GO:0030182 neuron differentiation
GO:0022008 neurogenesis
GO:0048666 neuron development
GO:0007399 nervous system development
glia 6−8h
logP
−5
0
−5

7c glia 18-22h



7d neuron 6-8h



7e neuron 18-22h

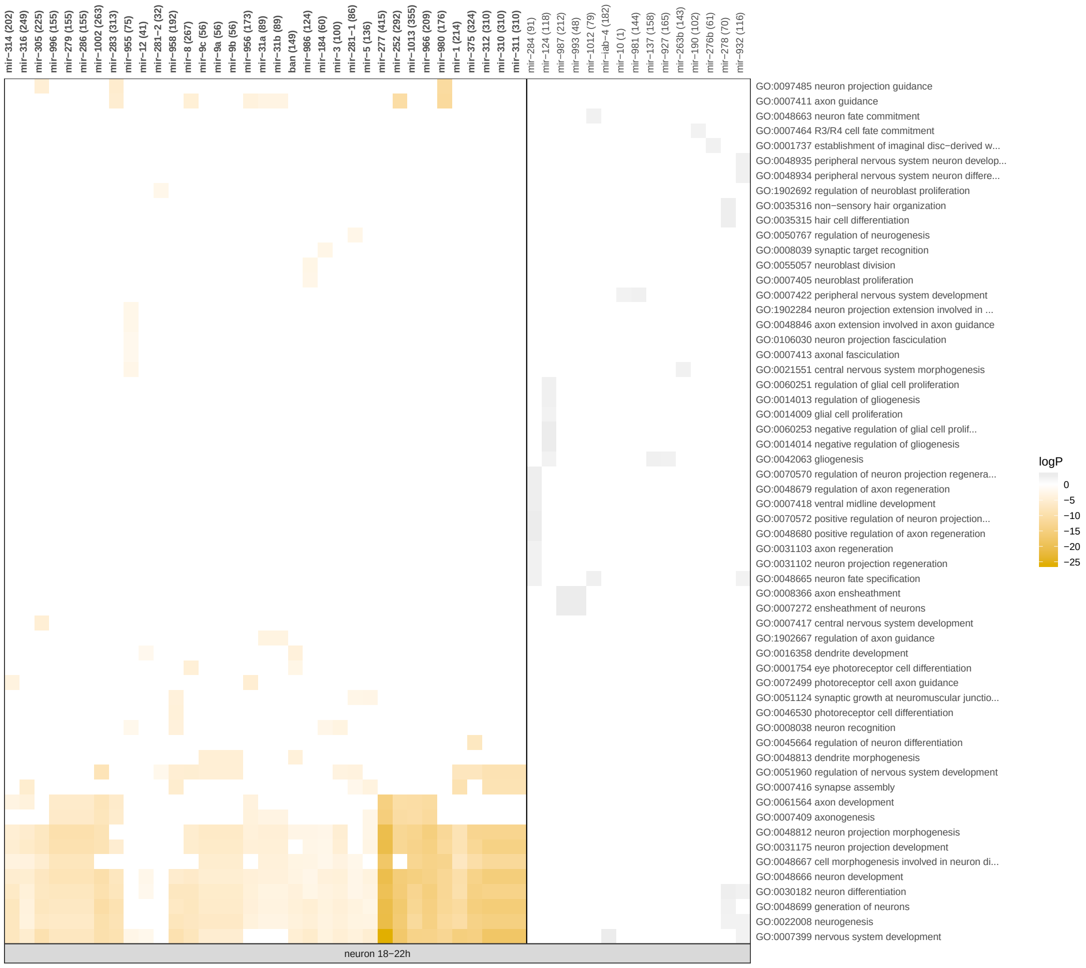
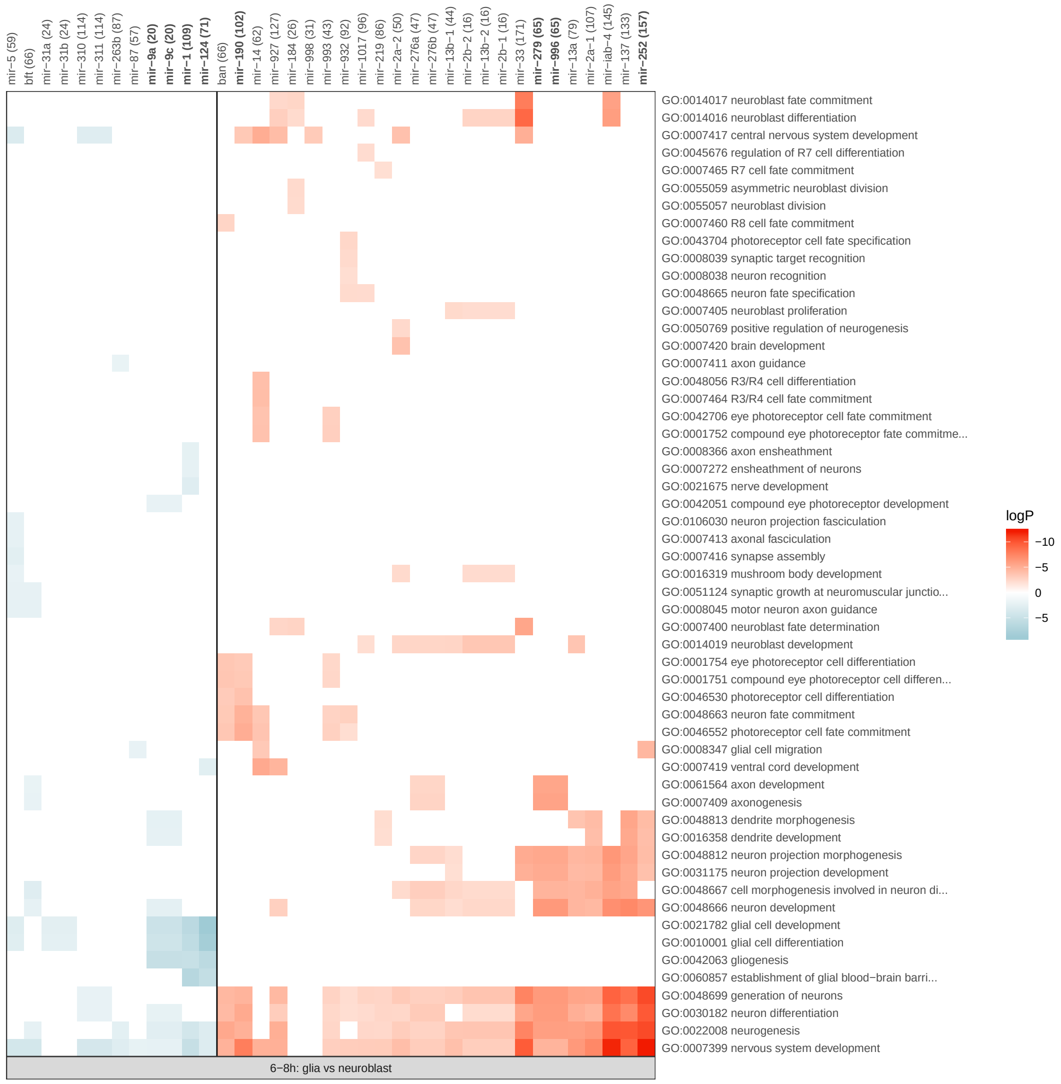


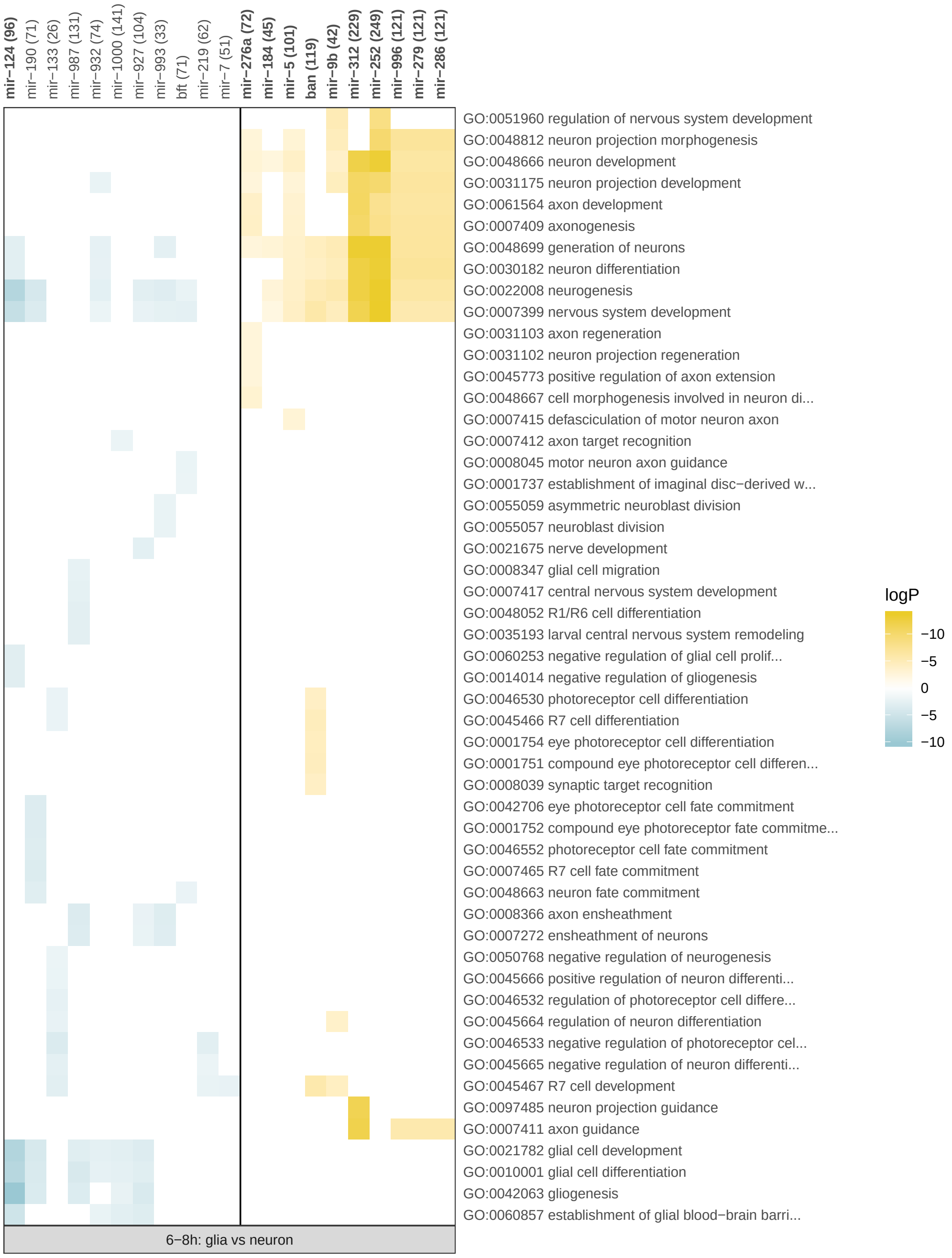
Figure 8: GO *Biological Process* terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the sets of differentially expressed protein-coding genes that are targets of differentially expressed microRNAs in the comparisons between marker-positive fractions. MicroRNAs having a significant overlap (number in brackets) of their target genes with genes regulated in the opposite direction are printed in bold.

8a 6-8h: neuroblast vs. glia



8b 6-8h: neuron vs. neuroblast

8c 6-8h: glia vs. neuron



8d 18-22h: glia vs. neuron

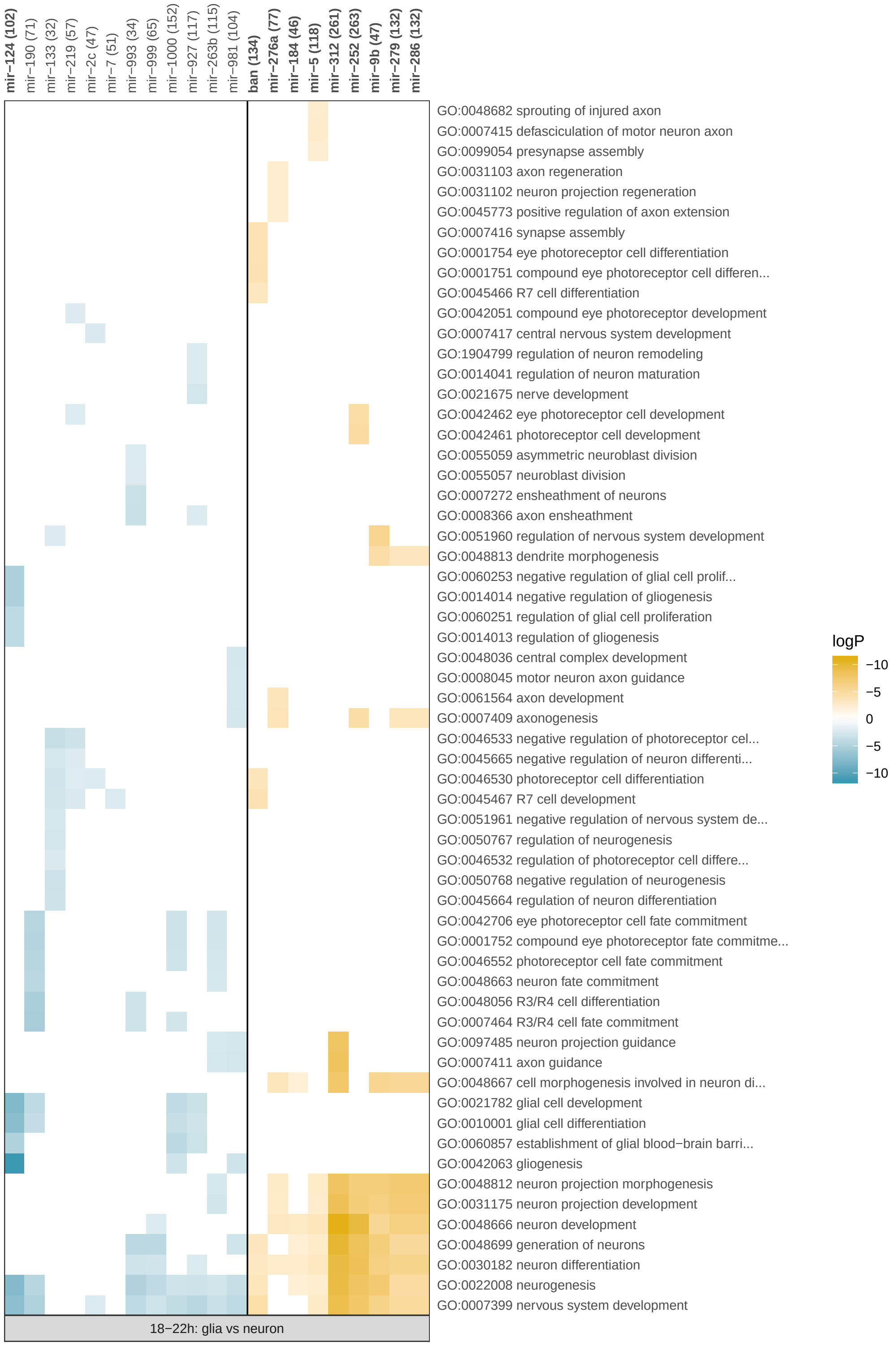


Figure 9: Differential **transcription factor** (TF) expression between marker-positive and marker-negative FACS fractions. Colored points denote significantly differentially expressed TFs and asterisks denote TFs with significantly enriched target genes among genes that are regulated in the same direction.

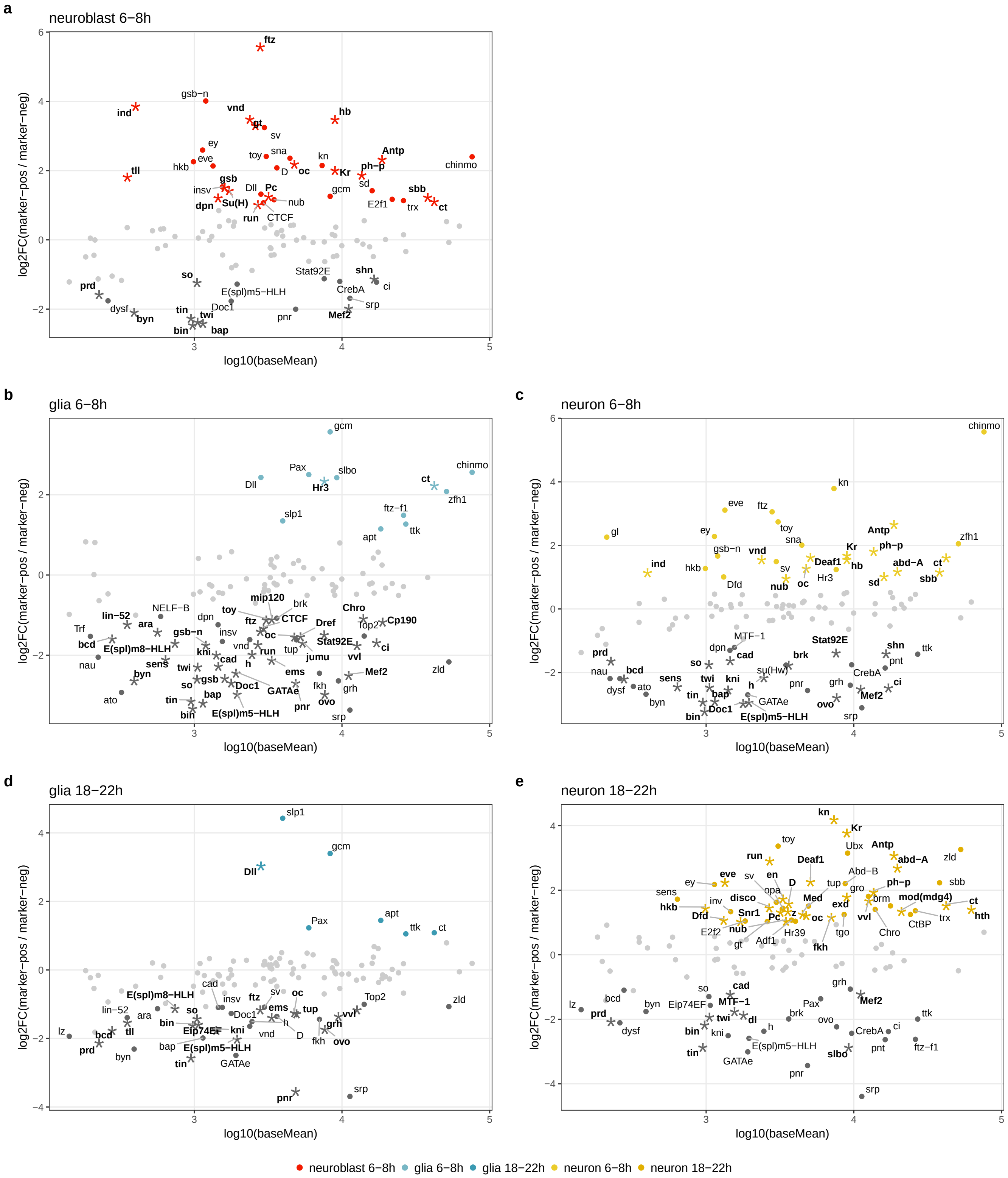


Figure 10: Differential **transcription factor** (TF) expression between time points and cell types. Colored points denote significantly differentially expressed TFs. Asterisks denote TFs with significantly enriched target genes among genes that are up-regulated in the same direction.

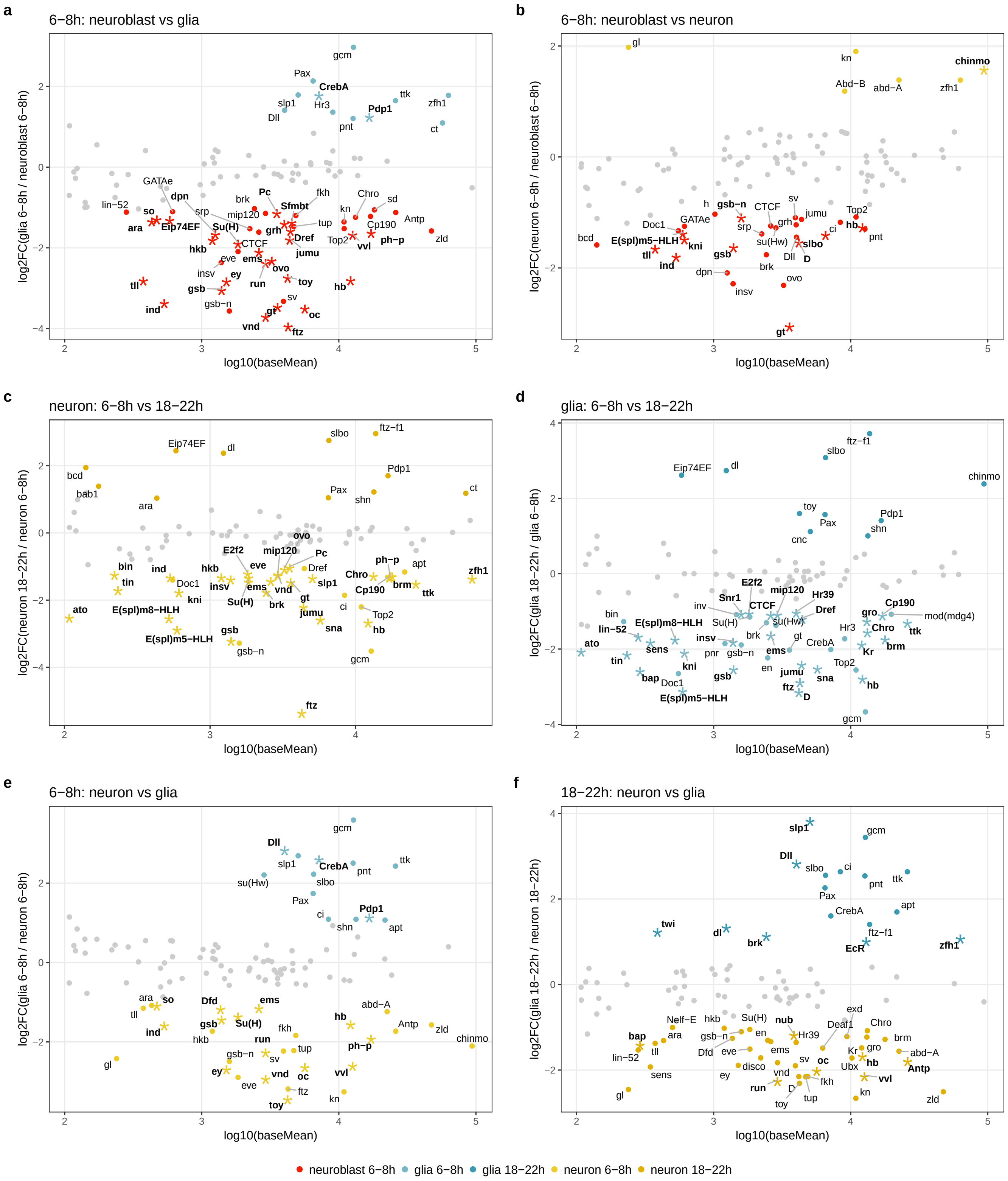


Figure 11: GO *Biological Process* terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the set of up-regulated protein-coding genes that are targets of up-regulated transcription factors (TF) in the positive vs negative FACS fractions and vice versa for neuroblasts at 6-8h AEL and neuron/glia at 18-22h AEL. Shown are only those transcription factors having a significant overlap (numbers in brackets) of their target genes with genes that are differentially expressed in the same direction. Grey colors denote the enrichment P-value of down-regulated target genes of down-regulated TFs in the marker-positive fractions, whereas non-grey colors denote the enrichment P-value of up-regulated target genes of up-regulated TFs in the marker-positive fractions, respectively.

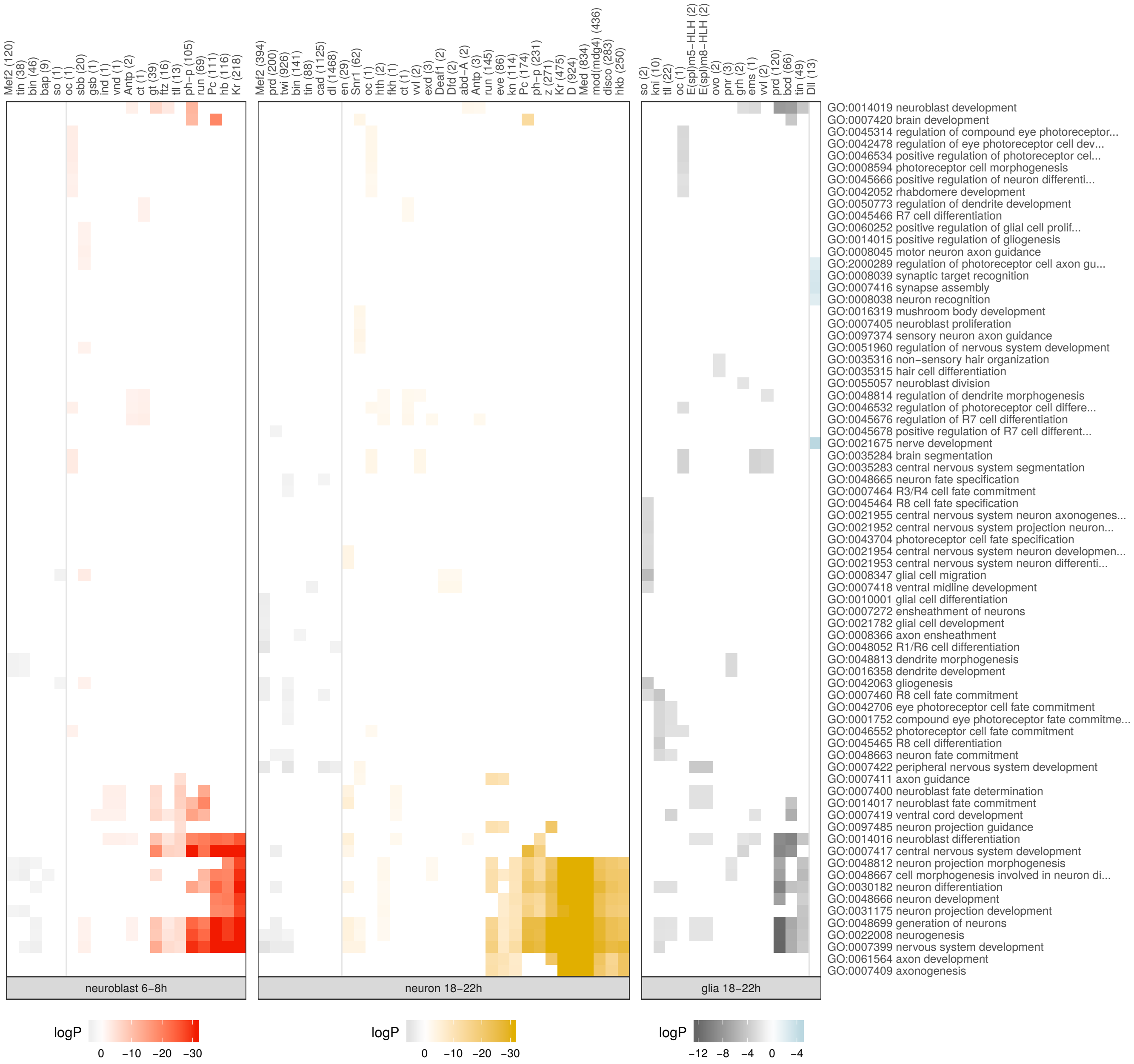


Figure 12: GO *Biological Process* terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the sets of differentially expressed targets of transcription factors that are regulated in the same direction in the comparisons between marker-positive fractions. Shown are only those TFs having a significant overlap (numbers in brackets) of their target genes with genes that are differentially expressed in the same direction.

Figure 13: GO Biological Process terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the set of differentially expressed protein-coding genes that are targets of transcription factors that are differentially expressed in the same direction that in turn are targets of reversely expressed microRNAs. Numbers in brackets denote the number of differentially expressed transcription factors that are targeted by the microRNA.

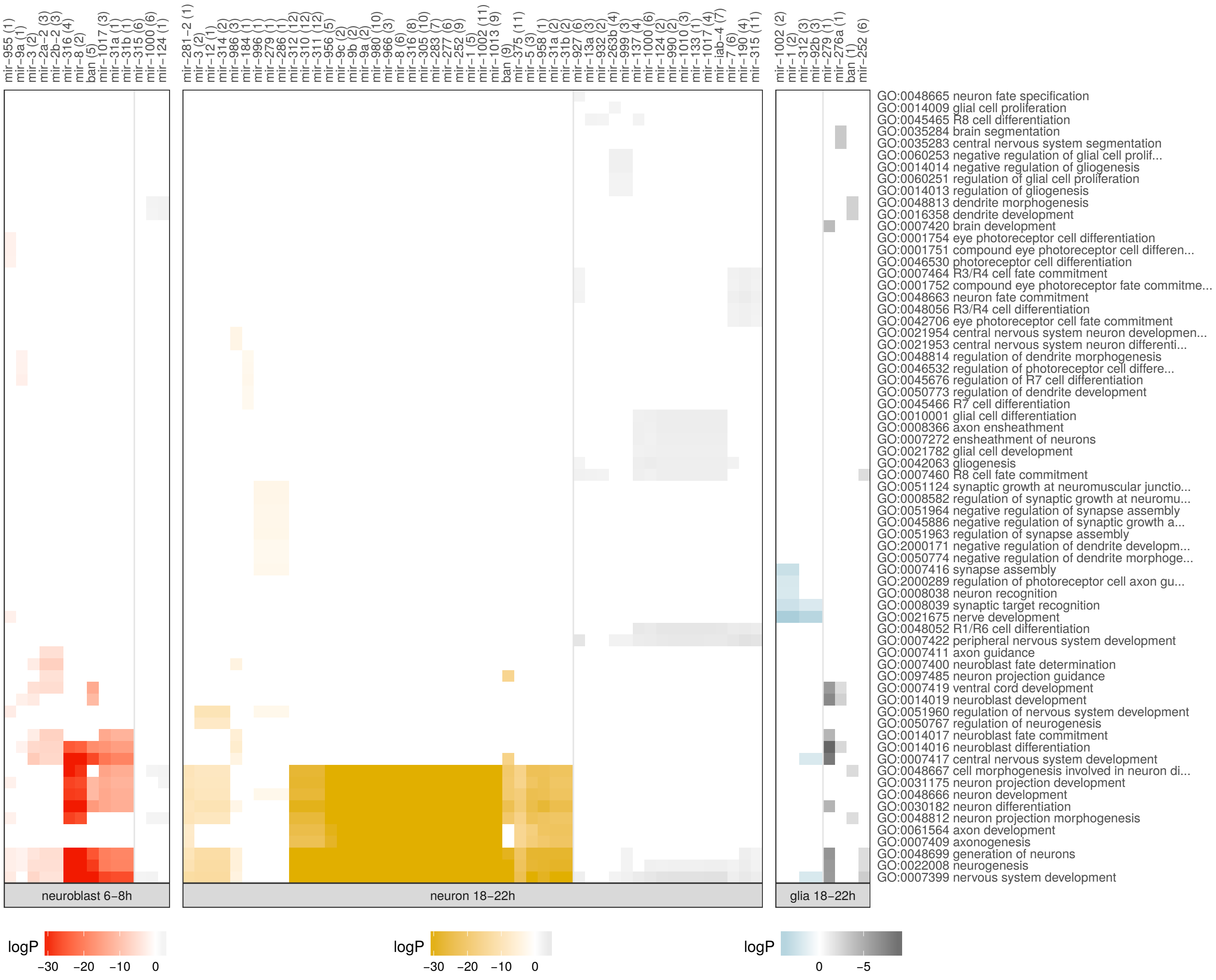


Figure 14: GO Biological Process terms in the sub-tree *nervous system development, GO:0007399* that are significantly enriched in the sets of differentially expressed protein-coding genes that are targets of differentially expressed transcription factors that in turn are targeted by reversely expressed microRNAs. Numbers in brackets denote the number of differentially expressed transcription factors that are targeted by the microRNA.