The E3 ubiquitin ligase RNF220 maintains hindbrain *Hox* expression patterns through regulation of WDR5 stability

Huishan Wang[1,#], Xingyan Liu[2,3,#], Yamin Liu[1,4], Chencheng Yang[1,4], Yaxin Ye[1,4], Xiaomei Yu[1,5], Nengyin Sheng[1,6,8], Shihua Zhang[2,3,6,7,*], Bingyu Mao[1,5,6,*], Pengcheng Ma[1,9,*]

[1]Key Laboratory of Genetic Evolution and Animal Models, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650201, China

[2]Academy of Mathematics and Systems Science, Chinese Academy of Science, Beijing 100190, China

[3]School of Mathematical Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

[4]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming 650223, China

[5]National Resource Center for Non-Human Primates, Kunming Primate Research Center and National Research Facility for Phenotypic & Genetic Analysis of Model Animals (Primate Facility), Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650107, China

[6]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China

[7]Key Laboratory of Systems Biology, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Hangzhou 310024, China

[8]Key Laboratory of Animal Models and Human Disease Mechanisms of Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

[9]Lead contact

[#]Contributed equally

*Correspondence: Pengcheng Ma, kunmapch@mail.kiz.ac.cn; Bingyu Mao, mao@mail.kiz.ac.cn; or Shihua Zhang, zsh@amss.ac.cn

Abstract

The spatial and temporal linear expression of *Hox* genes establishes a regional *Hox* code, which is crucial for the antero-posterior (A-P) patterning, segmentation, and neuronal circuit development of the hindbrain. RNF220, an E3 ubiquitin ligase, is widely involved in neural development *via* targeting of multiple substrates. Here, we found that the expression of *Hox* genes in the pons was markedly up-regulated at the late developmental stage (post-embryonic day E15.5) in *Rnf220*$^{-/-}$ and *Rnf220*$^{+/-}$ mouse embryos. Single-nucleus RNA-seq analysis revealed different *Hox* de-repression profiles in different groups of neurons, including the pontine nuclei (PN). The *Hox* pattern was disrupted and the neural circuits were affected in the PN of *Rnf220*$^{+/-}$ mice. We showed that this phenomenon was mediated by WDR5, a key component of the TrxG complex, which can be polyubiquitinated and degraded by RNF220. Intrauterine injection of WDR5 inhibitor (WDR5-IN-4) and genetic ablation of *Wdr5* in *Rnf220*$^{+/-}$ mice largely recovered the de-repressed *Hox* expression pattern in the hindbrain. In P19 embryonal carcinoma cells, the retinoic acid induced *Hox* expression was further stimulated by *Rnf220* knockdown, which can also be rescued by *Wdr5* knockdown. In short, our data suggest a new role of RNF220/WDR5 in *Hox* pattern maintenance and pons development in mice.

Introduction

The coordinated expression of *Hox* genes clusters is critical for axial patterning to determine the positional identities during the development of many tissues, including the hindbrain **(Frank *et al.*, 2019; Parker *et al.*, 2014; Parker *et al.*, 2020; Pöpperl *et al.*, 1993)**. In mammals, there are 39 *Hox* genes organized in four clusters (*HoxA*, *HoxB*, *HoxC*, and *HoxD*) and divided into 1-13 paralog groups (PG). During vertebrate development, the hindbrain forms eight metameric segmented units along the antero-posterior (A-P) axis known as rhombomeres (r), which give rise to the cerebellum, pons and medulla later on **(Ghosh *et al.*, 2018; Parker *et al.*, 2020)**. In the embryonic hindbrain, *Hox*1-5 genes are expressed in a nested overlapping pattern with rhombomere-specific boundaries. This *Hox* code is critical for the establishment of rhombomeric territories and the determination of cell fates **(Barsh *et al.*, 2017; Parker *et al.*, 2016; Parker *et al.*, 2020)**.

At later embryonic stages, *Hox* genes also play vital roles in neuronal migration and circuit formation **(Feng *et al.*, 2021; Hockman *et al.*, 2019; Kratochwil *et al.*, 2017; Wang *et al.*, 2013)**. For example, neurons in the pontine nuclei (PN), which acts as an information integration station between the cortex and cerebellum **(Kratochwil *et al.*, 2017; Maheshwari *et al.*, 2020)**, originate at the posterior rhombic lip of r6-r8, migrate tangentially to the rostral ventral hindbrain, and then receive different inputs from the cortex, in which the specific expression pattern of *Hox3-5* genes determines the migration routines, cellular organization, and neuronal circuits **(Geisen *et al.*, 2008; Kratochwil *et al.*, 2017; Maheshwari *et al.*, 2020; Di Meglio *et al.*, 2013; Lizen *et al.*, 2017)**. Note that *Hox* gene expression is maintained up to postnatal and even adult stages in the hindbrain derivatives **(Farago *et al.*, 2006; Feng *et al.*, 2021; Kratochwil *et al.*, 2017)**.

Hox pattern maintenance is generally governed by epigenetic regulators, especially the polycomb-group (PcG) and trithorax-group (trxG) complexes, which repress and activate *Hox* genes, respectively **(Bahrampour *et al.*, 2019; Chopra *et al.*, 2009; Kang *et al.*, 2022; Papp *et al.*, 2006)**. In mammals, different SET/MLL proteins, and a common multi-subunit core module consisting of WDR5, RbBP5, Ash2L, and DPY-30 (WRAD) constitute the COMPASS (complex of proteins associated with Set1) family of TrxG complexes, which acts as a methyltransferase generally **(Cenik *et al.*, 2021; Jambhekar *et al.*, 2019; Schuettengruber *et al.*, 2017)**. Among them, WDR5 plays a central scaffolding role and has been shown to act as a key regulator of

Hox maintenance **(Wang *et al.*, 2011; Wysocka *et al.*, 2005)**. In acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), WDR5 is responsible for MLL-mediated *HoxA9* activation and is a potential drug target **(Chen *et al.*, 2021; Yu *et al.*, 2021)**. In addition, WDR5 facilitates *HOTTIP*, a long noncoding RNA (lncRNA), to recruit the MLL complex and thus stimulate *HoxA9* and *HoxA13* expression during the progression of prostate and liver tumors **(Fu *et al.*, 2017; Malek *et al.*, 2017; Quagliata *et al.*, 2014; Wong *et al.*, 2020)**.

The E3 ubiquitin ligase RNF220 is widely involved in neural development through targeting a range of proteins **(Kim *et al.*, 2018; Kong *et al.*, 2010; Ma *et al.*, 2021; Ma *et al.*, 2022; Wang *et al.*, 2022)**. Although our previous reports have shown that *Rnf220*$^{-/-}$ mice is neonatal lethal and the survived *Rnf220*$^{+/-}$ mice develop severe motor impairments; the underlying mechanisms remain incompletely understood **(Ma *et al.*, 2021; Ma *et al.*, 2019)**. In the present study, a markedly up-regulation of *Hox* genes is observed in the pons of both *Rnf220*$^{+/-}$ and *Rnf220*$^{-/-}$ mice during late embryonic development. In consequence, the topographic input connectivity of the PN neurons with cortex is disturbed in *Rnf220*$^{+/-}$ mice. Furthermore, we identified WDR5 as a direct target of RNF220 for K48-linked ubiquitination and thus degradation, a mechanism critical for *Hox* de-repression by *Rnf220* knockout. Together, these findings reveal a novel role for RNF220 in the regulation of *Hox* genes and pons development in mice.

Results

Rnf220 insufficiency leads to dysregulation of *Hox* expression in late embryonic pons

Rnf220 is strongly expressed in the embryonic mouse mid/hind brain **(Ma *et al.*, 2019; Wang *et al.*, 2022)**. Based on microarray analysis, we explored changes in expression profiles in E18.5 *Rnf220*$^{+/-}$ and *Rnf220*$^{-/-}$ mouse brains, which breed floxed *Rnf220* to Vasa-Cre mice. Interestingly, several *Hox* genes were up-regulated in the mouse brain of both genotypes **(*Tables 1 and 2*)**. Considering that *Hox* expression in forebrain is too low (data not shown), we focus on the hindbrain and found that this phenomenon was observed only in late embryonic stages after E15.5 **(*Figure 1-figure supplement 1*)**. Since *Rnf220*$^{-/-}$ mice is neonatal lethal and there is not significant diference in the upregulation of *Hox* genes between *Rnf220*$^{+/-}$ and *Rnf220*$^{-/-}$ mice, *Rnf220*$^{+/-}$ mice were

used in subsequent assays in this study. To determine the specific brain regions with aberrant *Hox* expression, we examined the expression levels of *HoxA9* and *HoxB9*, two of the most significantly up-regulated genes, in different brain and spinal regions (***Figure 1-figure supplement 2A-D***). Notably, both *HoxA9* and *HoxB9* were exclusively up-regulated in the brainstem of *Rnf220*$^{+/-}$ mice (***Figure 1-figure supplement 2C and D***). The brainstem comprises the midbrain, pons, and medulla (***Figure 1-figure supplement 2E***). Further, we confirmed that the upregulation of *Hox* genes was restricted to the pons in the *Rnf220*$^{+/-}$ mice (***Figure 1-figure supplement 2F***). In addition, RNA sequencing (RNA-seq) analysis of the pons also revealed an overall increase in *Hox* gene expression in adult *Rnf220*$^{+/-}$ mice (***Figure 1A***).

To identify the specific cell populations in which *Hox* genes were up-regulated, we conducted single-nucleus RNA-seq (snRNA-seq) analysis of the pons of adult wild-type (WT) and *Rnf220*$^{+/-}$ mice. In total, 125956 and 122266 cell transcriptomes with an average of approximately ≥700 genes for the WT and *Rnf220*$^{+/-}$ mice respectively were further analyzed. 15 cell clusters were identified by uniform manifold approximation and projection (UMAP) in both the WT and *Rnf220*$^{+/-}$ groups (***Figure 1B and C***). When we annotate these cell clusters by their uniquely and highly expressed markers (**Table 3**), we found that most of the clusters were identified as distinct neuronal groups (cluster_1, 2, 4, 6, 7, 8, 10, 11, 12, and 13), in addition to astrocyte (cluster_5), oligodendrocyte (cluster_3), oligodendrocyte precursor (cluster_14), ependymal cell (cluster_9), and a group not corresponding to any known cell types (cluster_0) (***Figure 1B***). Then the *Hox* gene expression levels for each cell clusters were analyzed and found that up-regulation of *Hox* genes was most pronounced in three of these clusters (cluster_2, 7, and 10) (***Figure 1D***), albeit with distinct profiles. *Hox7–10* genes were up-regulated genes in cluster_2, while *Hox3–5* and *Hox1–3* genes were activated in cluster_7 and 10, respectively (***Figure 1D***). These findings indicate that *Hox* genes were specifically up-regulated in the pons of *Rnf220*$^{+/-}$ mice from late developmental stages on.

P19 embryonal carcinoma cells can be induced to differentiate and express *Hox* genes upon retinoic acid (RA) administration and have been used to study *Hox* regulation (**Heyden *et al.*, 2003; Kondo *et al.*, 1992; Pöpperl *et al.*, 1993; *Figure 1-figure supplement 3A***). Here, we tested the effects of *Rnf220* knockdown on RA-induced *Hox* gene activation in P19 cells. *Rnf220* knockdown further enhanced the activation of many *Hox* genes, including *HoxA1*, *HoxB1*, *HoxA9*, and *HoxB9*, by

RA, (***Figure 1E***), suggesting a general role of *Rnf220* in *Hox* regulation. Note that *Rnf220* knockdown had no clear effect on *Hox* expression in the absence of RA (***Figure 1-figure supplement 3B***).

***Rnf220* insufficiency disturbs *Hox* expression pattern and the neuronal circuit formation in the PN**

When we refer to the transcriptomic profiles of each cluster in the pons, we found that the cluster_10 likely represents cells derived from rhombomeres 2–5, given their expression of endogenous anterior *Hox* genes (*Hox1–3*), while the cluster_7 likely represents cells derived from rhombomeres 6–8, as evidenced by the expression of *Hox3–5* (***Figure 1D***). After mapping known neuron-specific markers to each cluster, PN-specific markers, including *Nfib*, *Pax6*, and *Barhl*, were enriched in the cluster_7 (***Figure 2-figure supplement 1A and B***). Originating at the posterior rhombic lip and migrating tangentially to their final position in the ventral part of the pons, PN neurons serve as relay cells between the cerebral cortex and cerebellum **(Di Meglio *et al.*, 2013; Kratochwil *et al.*, 2017; Maheshwari *et al.*, 2020)**. The expression pattern of *Hox* genes is crucial for the migration and the final neuronal circuit formation of the PN neurons **(Kratochwil *et al.*, 2017; Maheshwari *et al.*, 2020)**. We analyzed the expression pattern of the endogenous *Hox* genes, including *Hox3-5*, in the PN neurons and the connection between the cortex and the PN neurons in *Rnf220*$^{+/-}$ mice. The results of realtime RT-PCR assays indicated an up-regulation in *Hox4–5* expression levels in the *Rnf220*$^{+/-}$ PN (***Figure 2-figure supplement 1C***). In the PN, endogenous *Hox3–5* genes exhibit a nested and unique expression pattern along the rostral-caudal axis, with *Hox3* ubiquitously expressed throughout the PN, *Hox4* localized to the middle and posterior regions, and *Hox5* confined to the posterior segment **(Kratochwil *et al.*, 2017)**. Nest, the PN was evenly dissected into the rostral, middle, and caudal segments alone the rostral-caudal axis and the endogenous expression pattern of *Hox* genes was examined in the mice. The results showed that all the *Hox3-5* paralogs were uniformly up-regulated along the rostral-caudal axis in in the *Rnf220*$^{+/-}$ PN (***Figure 2A***).

There is no difference of structure in PN or even pons between WT and *Rnf220*$^{+/-}$ mice (***Figure 2-figure supplement 2A and B; Figure 2C-E***). Considering the motor deficits of *Rnf220*$^{+/-}$ mice **(Ma, *et al.*, 2021; Ma *et al.*, 2019)**, the neuronal circuit between the motor cortex and the PN neurons was traced anterogradely by a

non-transneuronal tracing virus, (rAAV-hSyn-EGFP-WPRE-hGH-polyA) (***Figure 2B***). The results showed that fewer PN neurons were targeted by axons from the motor cortex in the *Rnf220*$^{+/-}$ mice (***Figure 2C and F-G; Figure 2-figure supplement 2C***). In addition, the projection from the motor cortex was centralized in the PN of *Rnf220*$^{+/-}$ mice (***Figure 2C; Figure 2-figure supplement 2B***).

Taken together, both the expression level/pattern of the endogenous *Hox* genes and the neuronal projection pattern from the motor cortex were disturbed by *Rnf220* insufficiency in the mouse PN.

RNF220 targets WDR5 for K48-linked polyubiquitination and degradation

The up-regulation of *Hox* genes in the *Rnf220*$^{+/-}$ mice suggested a role for RNF220 in the maintenance of *Hox* expression pattern. Generally, the *Hox* expression pattern is maintained epigenetically by the PcG and trxG complexes, which regulate the silencing and activation of *Hox* genes, respectively (**Bahrampour *et al.*, 2019; Chopra *et al.*, 2009; Kang *et al.*, 2022; Papp *et al.*, 2006**). Indeed, we did observe the local epigenetic modification changes, *i.e.* the downregulated H3K27me3 signals and upregulated H3K4me3 signals, in some *Hox* cluster in hindbrain of *Rnf220*$^{+/-}$ mice by ChIP-qPCR assays (***Figure 3-figure supplement 1A and B***). Therefore, protein levels of the core components of PcG and TrxG complexes in the mouse hindbrain were examined and the results showed that although the protein levels of the core components of PcG we examined were comparable between WT and *Rnf220*$^{+/-}$, a clear increase in the protein level of WDR5, a key component of the trxG complex, was observed in the hindbrain of both *Rnf220*$^{+/-}$*and Rnf220*$^{-/-}$ mouse embryos at E18.5 (***Figure 3A; Figure 3-figure supplement 2A and B***), the phenomenon was also observed at E16.5 (***Figure 3B***). We next tested the expression of WDR5 in the mouse pons, cortex, and cerebellum at adult and found that the increase in the protein level of WDR5 was only observed in the pons, but not in the cortex or cerebellum, in *Rnf220*$^{+/-}$ mice (***Figure 3C; Figure 3-figure supplement 2C and D***), which is consistent with the pons-specific up-regulation in *Hox* genes expression. In addition, the protein level of WDR5 was also enhanced in the PN of *Rnf220*$^{+/-}$ mice (***Figure 3D***) and *Rnf220* knockdown P19 cells in the presence of RA (***Figure 3E***).

The above data suggest that WDR5 might be a direct target of RNF220 for polyubiquitination and thus degradation. Indeed, in HEK293 cells transiently

co-transfected with Flag-tagged RNF220 and myc-tagged WDR5, WDR5 coimmunoprecipitated with RNF220 (***Figure 4A***). In the reverse experiment, RNF220 also coimmunoprecipitated with WDR5 (***Figure 4B***). Furthermore, when WDR5 was immunoprecipitated from the mouse brainstem using an anti-WDR5 antibody, endogenous RNF220 was also detected in the immunoprecipitated (***Figure 4C***). Then we examined if the WDR5 protein level was regulated by RNF220. The results showed that co-expression of wild-type RNF220, but not the ligase-dead mutant (W539A) or the RING domain deletion (△Ring) form, clearly reduced the protein level of WDR5 in HEK293 cells (***Figure 4D***). In addition, the reduction in the protein level of WDR5 by RNF220 overexpression was blocked by MG132, suggesting a role for the proteasome in this regulation (***Figure 4E***).

We further carried out polyubiquitination assays to examine if WDR5 may be a target for RNF220. It was found that co-expression of RNF220 strongly enhanced the polyubiquitination of WDR5 protein, and this regulation depends on the E3 ubiquitin ligase activity of RNF220 because RNF220△Ring failed to promote the polyubiquitinaion of WDR5 (***Figure 4F***). Moreover, when the endogenous WDR5 proteins were immunoprecipitated from the mouse pons, its polyubiquitination level in *Rnf220*$^{+/-}$ mice was markedly decreased compared to WT mice (***Figure 4G***).

Different types of ubiquitination linkages have distinct regulatory effects on the stability or activity of protein targets, and K48-linked polyubiquitination usually leads to proteasomal degradation of protein targets (**Grice *et al.*, 2016.**). Indeed, WDR5 were only ubiquitinated by RNF220 when K48-type ubiquitin was present (***Figure 4H***). Consistent with this, when the K48R mutated ubiquitin was co-expressed, the RNF220 induced polyubiquitination level of WDR5 protein was fully diminished (***Figure 4H***). To determine the exact lysines ubiquitinated by RNF220, we first tested the effects of RNF220 on the stability of different WDR5 truncates and found that the truncate remaining 1-127aa was enough to be degraded by RNF220, suggesting the corresponding lysines were included in this region (***Figure 4-figure supplement 1A***). We individually mutated all these conserved lysines into arginines and then examined RNF220-mediated ubiquitination of each mutant. It was found that K109, K112, and K120 were required for the polyubiquitination of WDR5 (***Figure 4-figure supplement 1B***). Furthermore, when these lysine residues were simultaneously mutated into arginines, RNF220 failed to enhance the polyubiquitination levels of the

resulted WDR5-3KR mutants (***Figure 4I***), suggesting that these lysine residues are direct ubiquitination sites. Together, these results indicate that RNF220 regulates WDR5 ubiquitination by adding K48-linked polyubiquitin chains at the lysine sites of K109, K112, and K120.

The maintenance of *Hox* expression by RNF220 depends on its regulation to WDR5

To investigate the requirement of the RNF220-mediated regulation to WDR5 in *Hox* expression maintenance by RNF220, we first examined whether *Wdr5* knockdown could mitigate the impact of *Rnf220* knockdown on *Hox* expression in P19 cells in the presence of RA. It is observed that the stimulation of *Hox* genes by *Rnf220* knockdown was significantly reduced when *Wdr5* was knocked-down simultaneously by siRNAs in the presence of RA (***Figure 5A and B***). Note that the expression levels of *Hox* genes showed no clear changes when without RA or only *Wdr5 was* knocked-down in P19 cells (***Figure 5-figure supplement 1A and B***).

Using *in-utero* microinjection, we tested the effects of WDR5 inhibition by WDR5-IN-4, an established WDR5 inhibitor (**Aho *et al.*, 2019**), on the upregulation of *Hox* genes in hindbrains of the $Rnf220^{+/-}$ mice at late embryonic stages (***Figure 6A***). It was found that the up-regulation of *Hox3-5* genes in the hindbrain of the $Rnf220^{+/-}$ embryos was largely reversed by WDR5-IN-4 (***Figure 6C***). Notably, we found that WDR5-IN-4 injection had no effect on the endogenous *Rnf220* expression in the hindbrain (***Figure 6B***). Last, the effect of genetic ablation of *Wdr5* in neural system on the upregulation of *Hox* genes in RNF220 hypoinsufficient mice was examined. Considering $Wdr5^{+/-}$ mice is embryonic lethal, and the up-regulation of *Hox* genes could be also observed in the hindbrains of $Rnf220^{fl/wt}$;*Nestin-Cre* mice, we used $Rnf220^{fl/wt}$;*Nestin-Cre* and $Wdr5^{fl/wt}$ mice to generate WT, *Rnf220* knockdown*; Wdr5* knockdown and *Rnf220* and*Wdr5* double knockdown mice (***Figure 6D***). It was found that the upregulation of *Hox3-5* genes in the pons of *Rnf220* hypoinsufficient mice were markedly recovered by *Wdr5* genetic ablation (***Figure 6*E**). ChIP-qPCR also showed the local epigenetic modification changes in some *Hox* cluster, with H3K27me3 upregulated and H3K4me3 downregulated in *Rnf220* and *Wdr5* double knockdown P19 cell line related to *Rnf220* single knock down P19 cell line in the presence of RA (***Figure 6-figure supplement 1***).

Taken together, the above findings support the involvement of WDR5 regulation in

RNF220 mediated the maintenance of *Hox* expression pattern in the mouse hindbrain.

Discussion

During early embryonic development, the *Hox* genes are collinearly expressed in the hindbrain and spinal cord along the A-P axis to guide regional neuronal identity. Later on, the segmental *Hox* gene expression pattern in the hindbrain is maintained till at least early postnatal stages. The *Hox* genes are also expressed in adult hindbrains with restricted anterior boundaries. **(Philippidou *et al.*, 2013; Miller *et al.*, 2024; Bonito *et al.*, 2013)**. In addition to their roles in progenitor cell specification, cell survival, neuronal migration, axon guidance, and dendrite morphogenesis during early development stages **(Smith *et al.*, 2024)**, *Hox* genes also play key roles in the regulation of synapse formation, neuronal terminal identity and neural circuit assembly at late stages **(Feng *et al.*, 2020; Feng *et al.*, 2021; Philippidou *et al.*, 2013)**. Our study revealed a dose-dependent role of RNF220 and WDR5 in the maintenance of *Hox* expression in the hindbrain, which might have a functional role in the neural circuit organization of the pons in mice.

In mammals, WDR5, a key component of the COMPASS-related complexes, has been reported to be absolutely required for the regulation of *Hox* genes expression during mammalian embryo development and cancer progression *via* different mechanisms **(Wysocka *et al.*, 2005;Chen *et al.*, 2021; Yu *et al.*, 2021)**. Here, we report a WDR5 protein stability controlling mechanism by RNF220-mediated polyubiquitination and illustrate the role of this regulation on the maintenance of *Hox* genes expression in the mouse hindbrain. The stabilization of WDR5 and stimulation of *Hox* expression upon RNF220 knockdown was also recapitulated in RA treated P19 cells, a cellular model for *Hox* regulation study.

Interestingly, when examined at single cell level, different *Hox* stimulation profiles were detected in different neuronal groups, so that *Hox 1-3* were up-regulated in cluster 10, while *Hox3-5* and *Hox7-10* upregulation appeared in cluster 7 and cluster 2 respectively **(*Figure 1D*)**. Cluster 10 and 7 express endogenous *Hox1-3* and *Hox3-5*, respectively. It is reasonable that the expression of these *Hox* genes are stimulated upon WDR5 elevation due to RNF220 insufficiency. Cluster 2 is most interesting in that, *Hox7-10* were up-regulated in these cells which were not expressed in wild type hindbrain. This cluster was defined as glycinergic neuron cells with Slc6a5 expression. The nature of this group of cells and the phenotypic effect of *Hox*

up-regulation await further analysis. The de-regulation of *Hox* genes might have wide effect on the development of the many nuclei in the pons, including cell differentiation and neural circuit formation. For the PN, we showed here that the patterning and projection pattern from the motor cortex were affected in the *Rnf220*$^{+/-}$ mice.

The regulation of WDR5 and *Hox* expression by RNF220 seems to be context dependent and precisely controlled in vivo, depending on the molecular and epigenetic status of the cell. For example, although WDR5 and RNF220 are widely co-expressed in the brain, WDR5 levels are not affected in most regions in the *Rnf220*$^{+/-}$ brain, including the cortex, cerebellum etc. (***Figure 3-figure supplement 2C and D***). This is also true for EED, another epigenetic regulator, which is targeted by RNF220 in the cerebellum, but not in the pons **(Ma *et al*.,2020)**. In untreated P19 cells, knockdown of RNF220 also has no effect on the stability of endogenous WDR5, although overexpression of RNF220 did reduce WDR5 level in 293T cells. We suggest that RA treatment of P19 cells helps to set up the molecular environment that facilitates *Hox* expression as well as RNF220-WDR5 interaction.

In summary, our data support WDR5 as a RNF220 target involved in the maintenance of Hox expression and thus development of the pons.

Materials and Methods

Mouse strains and genotyping

All procedures involving mice were conducted in accordance with the guidelines of the Animal Care and Use Committee of the Kunming Institute of Zoology, Chinese Academy of Sciences (IACUC-PA-2021-07-018). Mice were housed under standard conditions at a temperature range of 20–22 °C, humidity of 30%–45%, and 12-h light/dark cycle. *Rnf220* floxed mice (*Rnf220*$^{fl/fl}$) were originally 129Sz/SvPasCrl background and mated with C57BL/6 then. Other mice used were maintained on a C57BL/6 background. *Vasa-Cre* mice were used to mate with *Rnf220* floxed mice (*Rnf220*$^{fl/fl}$) to generate *Rnf220* germ cell knockout (*Rnf220*$^{-/-}$) or heterozygote (*Rnf220*$^{+/-}$) mice. *Nestin-Cre* mice were used to mate with *Rnf220*$^{fl/fl}$ and *Wdr5* floxed mice (*Wdr5*$^{fl/fl}$) to generate conditional neural specific knockout or heterozygote mice. *Rnf220*$^{fl/wt}$;*Wdr5*$^{fl/wt}$;*Nestin-Cre* mice were obtained by crossing *Rnf220*$^{fl/wt}$;*Nestin-Cre*

with *Wdr5*$^{fl/wt}$ mice.

Genotypes were determined by PCR using genome DNA from tail tips as templates. PCR primers were listed as follows: 5'-CTG TTG ATG AAG GTC CTG GTT-3' and 5'-CAG GAA AAT CAA TAG ACA ACT T-3' were used to detect *Rnf220* floxP carrying. 5'-CTG TTG ATG AAG GTC CTG GTT-3' and 5'-CTG ATT TCC AGC AAC CTA AA-3' were used to detect *Rnf220* knockout. 5'-GCC TGC ATT ACC GGT CGA TGC-3' and 5'-CAG GGT GTT ATA AGC AAT CCC-3' were used for *Cre* positive detection. 5'-GAA TAA CTA CTT TCC CTC AGA CC-3' and 5'-CAG GCC AAG TAA CAG GAG GTA G-3' were used to detect *Wdr5* floxP carrying. 5'-GAA TAA CTA CTT TCC CTC AGA CC-3' and 5'-AGA CCC TGA GTG AGG ATA CAT AA-3' were used to detect *Wdr5* knockout.

Cell culture

HEK293 and P19 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, C11995500BT) supplementary with 10% fetal bovine serum (FBS) (Biological Industries, 04-001-1A), 100 units/mL penicillin and 100 mg/ml streptomycin (Biological Industries, 03-031-1B). Cell cultures were maintained at 37°C in a humidified incubator with 5% CO_2. 0.5μM RA (Sigma, R2625) was used to induce *Hox* expression in P19 cells.

To achieve gene overexpression or knockdown, HEK293 and P19 cells were transfected by Lipo2000 (Invitrogen, 11668500) according to the manufacturer's instructions. The following small interfering RNAs (siRNAs) (RiboBio) were used for *Rnf220* or *Wdr5* knockdown in P19 cells: siG2010160325456075, siG2010160325457167 and siG2010160325458259 were used for *Rnf220* knockdown; siB09924171210, siG131113135429 and siG131113135419 were used for *Wdr5* knockdown.

Total RNA isolation and qRT–PCR

Tissue and cells were homogenized with 1 mL TRIZOL (TIANGEN, DP424), after which 200 μL of chloroform was added to the lysates for phase separation. After centrifugation at 12□000 *g* for 15 min at 4 °C, the aqueous phase (500 μL) was transferred to a new tube and mixed with equal volumes of isopropanol for RNA precipitation. After 30 min, RNA pellets were harvested by centrifugation at 12□000 *g* for 15 min at 4 °C, twice washed with 75% ethanol, and dissolved in DNase/RNase-free water.

cDNA was synthesized with Strand cDNA Synthesis Kit (ThermoScientific, K1632) according to the manufacturer's instructions. All reactions were performed at least triplicates with Light Cycler 480 SYBR Green Master (Roche, 04707516001). Primers used for real-time polymerase chain reaction list in **Table 4**.

ChIP-qPCR

P19 cells or grinded mouse PN tissues were cross-linked with 1% formaldehyde for 25 min, and stopped with addition of glycine. After gentle centrifuging, samples were collected and lysed with ChIP lysis buffer (150 mM NaCl, 25 mM Tris, pH 7.5, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, and complete protease inhibitor cocktail) for 30 min on ice. Genomic DNA was sheared into 200-500 bp fragments by sonication. After centrifugation, the supernatants containing chromatin fragments were collected and immunoprecipitated with anti-IgG (2 μg, ProteinTech, B900610), anti-H3K27me3 (2 μg, Abcam, ab192985), or anti-H3K4me3 antibodies (2 μg, Abcam, ab8580) at 4 overnight. Then the immunoprecipitates coupled with protein A/G agarose beads (Santa Cruz, sc-2003) were washed sequentially by a low salt washing buffer, high salt washing buffer, LiCl washing buffer, and TE buffer. The immunoprecipitated chromatin fragments were eluted by 500 μL elution buffer for reversal of cross-linking at 65 °C overnight. Input or immunoprecipitated genomic DNA was purified by the QIAquick PCR Purification Kit (Qiagen, 28104) and used as a template for quantification PCR. The primers we used were listed in **Table 5**.

***In utero* microinjection**

Pregnant mice were administered isoflurane for deep anesthesia. Following this, a laparotomy was performed to carefully extract the embryos, which were then placed on a sterile surgical drape. WDR5-IN-4 (100 μg, MedChemExpress, HY-111753A), containing 0.05% Malachite Green reagent for tracing, was injected into the hindbrain of E15.5 embryos using a finely-tapered borosilicate needle. To minimize the risk of spontaneous abortion, injections were spaced for the selected embryos. Following the injections, the uterus was returned to the abdominal cavity and infused with 2 mL of 37 °C, 0.9% saline solution. The peritoneum and abdominal skin were then sutured. Finally, the mice were placed on a heating pad to facilitate recovery from anesthesia.

Ubiquitination assay, immunoprecipitation and western blot analysis

In vivo ubiquitination, immunoprecipitation and Western blot assays were carried out

as previously described **(Ma *et al.*, 2023.)**. HEK293 cells were transfected in 6-well plates, and at 48 h post transfection cells were lysed in 500 μl of lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM EDTA [pH 8.0], and 1% Triton X-100) that contained a protease inhibitor mixture (Roche Applied Science) for 30 min on ice; mouse tissues were fully grinded and lysed with RIPA Strong Lysis (Beyotime, P0013B) containing a protease inhibitor mixture for 30 min on ice. Following centrifugation at 12,000 rpm for 10 min at 4°C, 50μl supernatant was mixed with loading buffer and incubate boiled at 95°C for 5 min, the remaining supernatant was coating with antibody and A/G agarose beads (or FLAG beads) overnight. After washing 5 times, the bound proteins were eluted with SDS loading buffer at 95°C for 5 min. Final total lysates and immunoprecipitates were subjected to SDS-PAGE and Western blot analysis. The following primary antibodies were used for botting: anti-RNF220 (Sigma-Aldrich, HPA027578), anti-WDR5 (D9E11) (Cell Signaling Technology, 13105), anti-Ring1B (D22F2) (Cell Signaling Technology, 5694S), anti-Sin3B (AK-12) (Santa Cruz, sc-768), anti-EZH2 (ProteinTech, 21800-1-AP), anti-SUZ12 (Bethyl, A302-407A), anti-RYBP (A-1) (Santa Cruz, sc-374235), anti-CBX6 (H-1) (Santa Cruz, sc-393040), anti-CBX7 (G-3) (Santa Cruz; sc-376274), anti-CBX8 (C-3) (Santa Cruz, sc-374332), anti-PHC1 (D-10) (Santa Cruz, sc-390880), anti-α-Tubulin (ProteinTech, 66031-1-Ig), anti-FLAG (Sigma-Aldrich; F-7425), anti-myc (ProteinTech; 16286-1-AP), and anti-Ub (P4D1) (Santa Cruz, sc-2007)

snRNA-seq library preparation, sequencing, and data analysis

snRNA-seq libraries were prepared using the Split-seq platform **(Butler *et al.*, 2018; Rosenberg *et al.*, 2018)**. Freshly harvested mouse pons tissues underwent nuclear extraction following previously described protocols **(Butler *et al.*, 2018; Rosenberg *et al.*, 2018.)**. In brief, mouse brain tissues were transferred into a 2 mL Dounce homogenizer containing 1mL homogenizing buffer (250 mM sucrose, 25 mM KCl, 5 mM $MgCl_2$, and 10 mM Tris-HCl [pH = 8.0], 1 mM DTT, RNase Inhibitor and 0.1% Triton X-100). The mixture was subjected to five strokes with a loose pestle, followed by 10 strokes with a tight pestle. The resulting homogenates were filtered through a 40-μm strainer into 5-mL Eppendorf tubes and subsequently centrifuged for 4 min at 600 *g* and 4 °C. The pellet was re-suspended and washed in 1 mL of PBS containing RNase inhibitors and 0.1% BSA. The nuclei were again filtered through a 40-μm strainer and quantified. These nuclei were partitioned into 48 wells, each of which

contained a barcoded, well-specified reverse transcription primer, to enable in-nuclear reverse transcription. Subsequent second and third barcoding steps were carried out through ligation reactions. After the third round of barcoding, the nuclei were divided into 16 aliquots and lysed prior to cDNA purification. The resulting purified cDNA was subjected to template switching and qRT-PCR amplification, which was halted at the beginning of the plateau stage. Finally, the purified PCR products (600 pg) were used to generate Illumina-compatible sequencing libraries. Distinct, indexed PCR primer pairs were employed to label each library, serving as the fourth barcode.

The libraries underwent sequencing on the NextSeq system (Illumina) using 150-nucleotide kits and paired-end sequencing protocols. In this arrangement, Read 1 covered the transcript sequences and Read 2 contained the UMI and UBC barcode combinations. Initially, a six-nucleotide sequencing index, serving as the fourth barcode, was appended to the ends of Read 2. Subsequently, reads with more than one mismatching base in the third barcode were excluded from the dataset. Furthermore, reads containing more than one low-quality base (Phred score ≤ 10) within the UMI region were also discarded. The sequencing results were aligned to exons and introns in the reference genome (Genome assembly GRCm38, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001635.20/) and aggregated intron and exon counts at the gene level were calculated by kallisto and bustools software as described (https://bustools.github.io/BUS_notebooks_R).

Following export of the matrix, quality control measures were performed to remove low-quality cells and potential doublets, as described previously **(Rosenberg *et al.*, 2018.)**. After filtering, a total of 125,956 and 122,266 cells for wild type and *Rnf220*$^{+/-}$ pons respectively remained for subsequent analysis. Seurat v2 **(Butler *et al.*, 2018.)** was used to identify HVGs within each group. Principal component analysis (PCA) and UMAP were performed to embed each cell from the reduced principal component space on a 2D map. Then clustering of cell populations and identification of differentially expressed genes were carried out as previously described **(Ma *et al.*, 2022.)**. We annotated the embryonic cell populations and lineages based on their DEGs (Table 3).

Mouse brain stereotactic injection and neuronal tracing

Mouse brain stereotactic injection and neuronal tracing were carried out as previously described with minor modifications **(Xu *et al.*, 2021.)**. Adult mice (2–3 months old)

were used for anterograde monosynaptic neuronal tracing. The mice were first deeply anesthetized with isoflurane and placed into a stereotactic apparatus with the front teeth latched onto the anterior clamp. The mouth and nose of the mice were covered with a mask to provide isoflurane and keep them in an anesthetic state during the operation. The head was adjusted and maintained in horizontal by inserting ear bars into the ear canal. The hair of the head was shaved with an electric razor and cleaned using a cotton swab which was dipped in 75% alcohol. Cut the scalp along the midline with surgical scissors and make sure the bregma, lambda and the interaural line were exposed. The intersection between the lambda and interaural line was set to zero. The coordinates ±1.75, −4.9, −1.65) were applied for motor cortex localization. Each mouse received a single injection of 500 nL of rAAV-hSyn-EGFP-WPRE-hGH-poly A (Brainvta, PT-1990) viral fluid in the hemisphere. After the injection, mice were bred for another 3 weeks for neuronal tracing before their brains were harvested for analysis.

Slice preparation

Mice subjected to stereotactic injection were euthanized, and whole brains were collected for the preparation of frozen sections. Following fixation in 4% paraformaldehyde (diluted in PBS) for 48 h, the brains were successively treated with 20% sucrose (diluted in PBS) for 48 h and 30% sucrose for 24 h. Brain tissues were then embedded in Optimal Cutting Temperature (OTC) compound (SAKURA, 4583) at −20 □ and sliced along the sagittal plane at a thickness of 40 μm.

Quantification and statistical analysis

PN and fluorescence length and area were statistically analyzed by Image J software (National Institute of Health). Statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Comparisons were performed using the two-tailed Student's *t*-test. *p*-values of less than 0.05 were considered statistically significant (*), 0.01 were considered statistically significant (**) and 0.001 were considered statistically significant (***).

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Author ORCIDs

Huishan Wang: https://orcid.org/0000-0001-5755-6606
Bingyu Mao: https://orcid.org/0000-0002-7993-3158
Pengcheng Ma: https://orcid.org/0000-0002-1067-8021

Additional flies

Data availability

All the snRNA-seq and RNA-seq raw data have been deposited in the GSA (https://ngdc.cncb.ac.cn/gsa/) with accession number is CRA013111.

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Table1: Differently expressed genes identified using microarray between WT and *Rnf220*$^{+/-}$ mice.

ProbeName	WT_4B_NS	WT_7B_NS	HE_1B_NS	HE_2B_NS	HE_9B_NS	foldchange	GeneSymbol
A_51_P106443	3.9610484	3.8312478	3.911649	3.5465846	6.781389	0.3269886	Ttll4
A_51_P107039	5.9857764	6.293115	7.3528614	7.603712	8.187723	0.3274328	4930555G01
A_51_P145453	1.1030455	1.8843594	13.218153	13.073017	12.635066	0.0003574	Xist
A_51_P225484	3.8190854	4.0877843	2.6871324	1.7105145	2.3572092	3.146021	Epyc
A_51_P232207	8.870988	6.4653354	9.361305	9.746093	10.04952	0.3240584	Hoxb6
A_51_P232617	1.6583239	2.9412262	4.149042	3.8376906	4.293471	0.314739	Spag17
A_51_P246677	10.885349	8.35602	8.185716	7.150078	7.7772675	5.100983	Rec8
A_51_P250590	9.816977	12.201618	9.834278	9.617521	9.242044	3.6539768	Sbf2
A_51_P283044	7.210637	7.1668043	1.0895243	0.916043	1.0501599	71.924886	Kdm5d
A_51_P303160	3.7078912	2.96213	3.7240596	7.054022	3.409316	0.1996523	Arg1
A_51_P305532	9.002047	9.349363	7.1329226	7.1292458	7.182117	4.1062864	Eif2s3x
A_51_P307944	5.1118865	4.8116364	1.2979898	0.8410812	0.8931325	15.390296	S1pr4
A_51_P353232	8.54968	5.1697693	4.654748	5.1941752	5.279355	6.1218148	Tnnc2
A_51_P355122	9.005632	6.4512677	6.390123	6.4971147	6.698944	3.2444464	Tnni2
A_51_P398491	3.57301	3.2548206	0.7871613	2.121579	1.9185144	3.2634084	Trem1
A_51_P401659	8.49288	13.804942	8.294904	8.26872	8.293301	23.508588	Sspn
A_51_P402686	5.390537	0.7771567	6.5473924	7.4789324	8.404096	0.1072456	Hoxb8
A_51_P402994	7.67957	7.889223	0.889564	0.9211274	1.5682712	98.803163	Ddx3y
A_51_P413147	2.9268467	3.3636706	4.4126472	4.0533743	5.4956694	0.3233795	Klk1b3
A_51_P416858	7.626576	3.8670025	4.1174607	4.1858187	4.177822	5.9325753	Myl1
A_51_P447189	7.250779	4.738179	5.3955793	3.4247038	1.976046	4.7295439	LOC1005032
A_51_P479715	8.826565	9.364602	0.9919275	0.9381926	1.0508884	279.38628	Eif2s3y
A_51_P496245	3.5971034	0.8649594	4.750976	5.8702064	8.07007	0.0589677	Hoxc6
A_51_P509263	5.1390595	4.7569666	5.2049723	6.028042	8.534603	0.1975087	Hoxa7
A_52_P106285	7.450177	5.0501213	4.8360424	4.7674747	5.396261	3.1865628	Rnf157
A_52_P113537	0.7702979	0.7451581	9.380402	9.468647	8.730086	0.0028187	Xist
A_52_P129547	2.2627034	2.8756747	4.0872307	3.994681	4.994796	0.2808802	Cdh10
A_52_P194971	7.003126	2.4153306	7.9176655	8.676689	9.467014	0.1475055	Hoxb7
A_52_P245806	2.0121722	2.2222986	8.024733	8.075076	7.757375	0.0174827	Tsix
A_52_P247537	5.6644816	5.820669	1.1234794	1.0404364	1.3392673	23.775287	
A_52_P275069	5.1697693	5.7891135	3.00036	3.540889	4.4741592	3.2708292	Gm6792
A_52_P300376	0.8213641	0.7698782	7.833779	7.7910423	7.6257606	0.0080483	Xist
A_52_P315942	2.6382132	1.1713203	3.8614466	3.9763458	3.6737823	0.2954844	Ptpn6
A_52_P320156	1.7486724	4.2580724	4.245352	5.562554	5.2181706	0.326164	E430024C06
A_52_P328825	9.731535	9.426588	11.306289	11.323352	11.139886	0.3138731	Wdfy1
A_52_P362360	0.9219009	0.8588854	11.556221	11.669298	11.221105	0.0006426	Xist
A_52_P369840	4.6016273	3.4662702	3.311216	1.8128297	0.9486678	3.4481878	2900060N12
A_52_P411296	9.834575	12.287046	9.773543	9.764503	9.759869	3.3943711	Sh3kbp1
A_52_P415996	6.9113474	5.664976	5.3968697	4.112598	4.4212303	3.1742972	Gstm6

A_52_P420563	6.4848566	12.048418	6.416327	6.674661	6.382933	23.939071	Ncor2
A_52_P424550	1.298509	0.9006791	4.036758	2.8420594	3.6169257	0.1810222	
A_52_P459732	8.02701	6.3407764	5.81404	5.444841	5.9562135	3.1675232	Rec8
A_52_P620944	3.8066573	3.6602871	1.5122881	1.4530756	0.9071637	5.3516229	
A_52_P755632	3.5135806	4.4207397	4.49904	4.160672	8.204504	0.1468263	
A_52_P763332	1.06057	5.5879946	6.3257084	7.2547207	6.8896036	0.2141694	
A_52_P909122	9.128128	12.63704	10.234246	9.10839	9.897138	3.8354481	
A_52_P940338	0.8494085	2.08987	4.3664184	2.8508143	5.45008	0.1270092	LOC382202
A_52_P980199	2.3094199	2.2522495	4.359635	4.6852355	3.6413543	0.2482597	Cdh10
A_52_P98462	0.6979232	0.7272494	2.9216394	3.5460274	7.518462	0.0242659	Hoxb9

Table2 : Differently expressed genes identified using microarray between WT and *Rnf220*$^{-/-}$ mice.

ProbeName	WT_4B_NS	WT_7B_NS	KO_3B_NS	KO_5B_NS	KO_6B_NS	foldchange	GeneSymbo
A_51_P125351	6.313856	6.601757	6.4720597	6.4195724	10.480064	0.1653608	Lce1b
A_51_P132185	6.5295115	6.335476	7.368577	6.2388043	11.309154	0.0934783	Stfa1
A_51_P135944	4.8749924	4.083627	5.2269435	4.498599	9.086301	0.1150532	Stfa2
A_51_P170059	3.6045246	4.311549	3.71578	3.9532177	10.690251	0.0285741	Lce1m
A_51_P183197	4.167489	4.6221843	4.5278687	4.208487	8.697207	0.1399295	2310002J15
A_51_P214127	3.6033967	2.9677386	3.4971817	4.097985	8.025378	0.1037036	Cpa3
A_51_P232207	8.870988	6.4653354	9.991578	10.203091	9.469767	0.2872938	Hoxb6
A_51_P250590	9.816977	12.201618	9.5322485	9.307759	9.114188	4.3656323	Sbf2
A_51_P260265	6.1705537	5.226247	7.260891	7.727149	7.3651867	0.3097302	Hoxd4
A_51_P287198	4.01975	4.0344496	3.9391453	3.7721603	7.729372	0.2027683	Krt23
A_51_P296109	4.4744844	3.92833	4.659961	4.587028	7.9215555	0.1925611	Dmkn
A_51_P303784	4.194939	3.565834	1.1152956	1.1867241	2.18678	5.0285685	Olfr218
A_51_P307944	5.1118865	4.8116364	0.8316174	0.7184657	0.8546457	17.960621	S1pr4
A_51_P321011	6.994348	7.199608	3.8395464	4.032716	3.4444318	9.9049398	
A_51_P336830	4.502313	2.8254704	3.985986	3.6439693	8.376842	0.1237058	Fabp4
A_51_P336833	4.593224	3.1738102	4.121704	3.8008437	8.382238	0.1362804	Fabp4
A_51_P384894	7.477057	7.303433	8.060354	7.1584826	12.393815	0.0870583	Csta
A_51_P393968	5.735548	5.217275	2.2287815	1.0561386	1.0544479	15.346941	Vsx2
A_51_P401659	8.49288	13.804942	8.375488	8.372072	8.301443	22.48262	Sspn
A_51_P402686	5.390537	0.7771567	8.055451	8.566343	6.7811	0.0867356	Hoxb8
A_51_P444543	5.1029267	5.1083794	3.17601	2.371889	2.719416	4.9660082	Dbh
A_51_P461040	6.6832285	6.5802245	6.843542	6.9395475	10.69976	0.1566059	Crct1
A_51_P474752	3.7151783	3.267671	0.8849869	0.8739997	0.8531357	6.2244189	Ucn2
A_51_P496245	3.5971034	0.8649594	6.764801	8.603234	4.844242	0.0396761	Hoxc6
A_51_P499698	5.7629433	5.356135	5.2179017	5.541067	10.220467	0.1119105	Asprv1
A_51_P504815	5.939404	5.932974	6.521724	5.831172	12.178275	0.0384009	Stfa3
A_51_P509263	5.1390595	4.7569666	7.245264	8.561127	5.753749	0.1601265	Hoxa7
A_51_P514035	5.6431694	4.7971907	5.6459165	6.3477407	10.138423	0.0926953	Cma1
A_52_P117920	5.120014	3.3515055	3.6814542	3.3344727	8.7837105	0.1455336	
A_52_P117922	4.337855	4.06896	4.056633	3.9852946	9.184422	0.0903605	
A_52_P171692	5.2034197	4.5558033	5.1785574	4.851259	8.4255705	0.2214345	1190003J15
A_52_P194971	7.003126	2.4153306	9.013775	9.578628	8.266773	0.1260852	Hoxb7
A_52_P240842	4.914063	1.2371906	5.9930615	6.191735	5.1821957	0.2816936	9430077A04
A_52_P347956	0.7941967	0.9586504	4.051279	4.083244	3.9578302	0.1124228	Cyp2j13
A_52_P411296	9.834575	12.287046	9.918812	9.747645	9.758842	3.2910543	Sh3kbp1
A_52_P420563	6.4848566	12.048418	6.4241385	6.346026	6.5670357	24.760186	Ncor2
A_52_P436122	2.9213138	3.0083542	5.30703	5.467043	5.6950293	0.1727731	
A_52_P445239	3.697067	5.3639936	3.4139938	3.297052	2.1909828	3.2421623	Plp1
A_52_P487686	6.869822	6.442713	7.520336	6.6244674	11.930332	0.0730948	BC100530

A_52_P523368	4.5968947	4.545647	4.597747	4.583084	7.504041	0.3105278	Psapl1
A_52_P531325	5.7284603	5.8114233	1.0499647	1.0681556	2.071632	19.56396	Rps6
A_52_P620944	3.8066573	3.6602871	0.8701608	0.7867022	0.8770287	7.4133046	
A_52_P673122	3.6899672	3.9134355	2.7354527	0.9638019	2.2010896	3.1768653	
A_52_P909122	9.128128	12.63704	10.752602	9.08155	9.953282	3.1899222	
A_52_P98614	6.431283	5.7046127	1.0239266	1.010514	0.9768893	34.518845	Slc6a2

Table3: Uniquely and highly expressed markers of each cluster in snRNA-seq.

	p_val	avg_logFC	pct.1	pct.2	cluster
Snhg11	0	0.9540666	0.993	0.814	0
Zfp101	0	0.7367873	0.832	0.746	0
Ube3a	0	0.5335392	0.613	0.483	0
D10Wsu102e	0	0.4966353	0.707	0.571	0
Srrm2	0	0.4596324	0.663	0.549	0
AC149090.1	0	0.4474371	0.441	0.301	0
Mmachc	0	0.4081537	0.411	0.296	0
Vmn2r53	0	0.375206	0.426	0.302	0
Pnisr	0	0.3638224	0.487	0.389	0
Rbm25	0	0.3391598	0.515	0.454	0
Nktr	0	0.3348808	0.401	0.305	0
Fus	0	0.3149181	0.424	0.352	0
Syne1	0	0.3130419	0.5	0.447	0
Gm17132	0	0.3126096	0.349	0.257	0
Usp19	0	0.2997827	0.335	0.265	0
Cenpc1	0	0.2940808	0.284	0.199	0
Gm48119	0	0.2927253	0.306	0.21	0
Vmn2r76	0	0.2875166	0.301	0.208	0
Gria2	0	0.2844672	0.458	0.404	0
Luc7l2	0	0.2652363	0.33	0.271	0
Dennd1b	0	0.2634844	0.282	0.212	0
Tmem72	0	0.257478	0.34	0.287	0
Epha5	0	0.2546523	0.332	0.291	0
Snrnp70	0	0.2524077	0.35	0.296	0
Nap1l5	0	0.6473373	0.758	0.393	1
Gad2	0	0.4701617	0.375	0.121	1
Scg2	0	0.4498357	0.503	0.249	1
Ahi1	0	0.4384739	0.506	0.197	1
Gprasp1	0	0.3767198	0.609	0.364	1
6330403K07Rik	0	0.3761973	0.358	0.077	1
Tmem130	0	0.3663431	0.466	0.22	1
Celf4	0	0.3487743	0.501	0.241	1
Peg3	0	0.3147951	0.775	0.588	1
Impact	0	0.3073325	0.433	0.22	1
Gap43	0	0.2998809	0.325	0.107	1
Pnmal2	0	0.297587	0.433	0.22	1
Zwint	0	0.2867501	0.526	0.333	1
Resp18	0	0.2630204	0.322	0.167	1
Nefm	0	0.7882714	0.875	0.396	2
Snap25	0	0.6622256	0.93	0.556	2

Nefh	0	0.6536582	0.736	0.249	2
Map1b	0	0.6242659	0.958	0.646	2
Atp1b1	0	0.6146502	0.954	0.669	2
Cplx1	0	0.6064388	0.795	0.319	2
Sparcl1	0	0.5704161	0.825	0.366	2
Calm1	0	0.5449357	0.902	0.611	2
Atp1a3	0	0.5418078	0.765	0.448	2
Nefl	0	0.5120509	0.704	0.297	2
Pkm	0	0.4929712	0.82	0.423	2
Vsnl1	0	0.4829055	0.733	0.305	2
Map1a	0	0.4735071	0.773	0.383	2
Lars2	0	0.4709724	0.969	0.834	2
Syt2	0	0.4691961	0.684	0.247	2
Kcnc3	0	0.4638185	0.599	0.199	2
Slc25a4	0	0.4524251	0.791	0.461	2
Kcna2	0	0.4155329	0.699	0.288	2
Vamp1	0	0.4013327	0.687	0.27	2
Lamp5	0	0.3926798	0.501	0.15	2
Slc6a5	0	0.3915305	0.373	0.108	2
Ndrg4	0	0.3745433	0.75	0.378	2
Ckb	0	0.3706504	0.725	0.374	2
Map7d2	0	0.3696816	0.65	0.294	2
Hsp90aa1	0	0.3623147	0.815	0.496	2
Stmn3	0	0.36053	0.67	0.334	2
Kcna1	0	0.3599023	0.601	0.222	2
Gm20594	0	0.3511162	0.862	0.561	2
Mdh1	0	0.3501233	0.685	0.325	2
Calm3	0	0.3483847	0.663	0.301	2
Gapdh	0	0.3429265	0.598	0.371	2
Calm2	0	0.342898	0.8	0.465	2
Ldhb	0	0.3426175	0.638	0.264	2
Ghitm	0	0.3340682	0.647	0.273	2
Ina	0	0.3311065	0.563	0.21	2
Atp5b	0	0.3296494	0.76	0.429	2
Ywhag	0	0.3289253	0.674	0.305	2
Hspa8	0	0.3254792	0.695	0.394	2
Scn1a	0	0.3190687	0.688	0.33	2
mt-Nd1	0	0.3189891	0.65	0.327	2
mt-Cytb	0	0.3017935	0.723	0.395	2
Napb	0	0.2963015	0.71	0.386	2
Nars	0	0.295612	0.617	0.287	2
Hsp90ab1	0	0.294607	0.843	0.561	2
Nat8l	0	0.2926316	0.591	0.204	2

Rtn3	0	0.2911262	0.705	0.371	2
Aldoc	0	0.2879719	0.629	0.286	2
Atp2b2	0	0.282642	0.651	0.311	2
Dnm1	0	0.2807882	0.604	0.289	2
Eno2	0	0.2807822	0.647	0.297	2
Glra1	0	0.2801859	0.558	0.213	2
Sv2c	0	0.2795559	0.472	0.151	2
Scn8a	0	0.277919	0.621	0.277	2
Rph3a	0	0.2774697	0.519	0.173	2
Atp2a2	0	0.2686874	0.642	0.309	2
Nsf	0	0.2676745	0.696	0.369	2
Slc24a2	0	0.26494	0.594	0.254	2
Atp6v1a	0	0.2648543	0.658	0.341	2
Cltc	0	0.2623385	0.617	0.293	2
mt-Nd2	0	0.252835	0.532	0.269	2
Clstn1	0	0.250687	0.632	0.3	2
Plp1	0	3.4437013	0.991	0.211	3
Trf	0	1.8707847	0.803	0.058	3
Mag	0	1.7196174	0.799	0.033	3
Apod	0	1.6560469	0.607	0.039	3
Fth1	0	1.6308212	0.792	0.436	3
Ptgds	0	1.5264231	0.343	0.098	3
Scd2	0	1.4443092	0.764	0.222	3
Mbp	0	1.4301111	0.782	0.14	3
Cnp	0	1.4203155	0.663	0.036	3
Mobp	0	1.3381085	0.696	0.087	3
Car2	0	1.2150987	0.543	0.046	3
Mal	0	1.2013232	0.523	0.02	3
Qk	0	1.1079033	0.594	0.064	3
Gpm6b	0	1.0702732	0.564	0.071	3
Aplp1	0	1.0540449	0.636	0.222	3
Ermn	0	0.987124	0.471	0.012	3
Glul	0	0.9430473	0.453	0.064	3
Slc12a2	0	0.8725496	0.479	0.075	3
Ugt8a	0	0.8402525	0.427	0.038	3
Ptprd	0	0.820107	0.602	0.295	3
Enpp2	0	0.7993549	0.648	0.088	3
S100b	0	0.7940206	0.373	0.13	3
Tubb4a	0	0.7869016	0.431	0.212	3
Mog	0	0.7455389	0.362	0.009	3
Cryab	0	0.6964547	0.316	0.018	3
Gjc3	0	0.6954245	0.353	0.023	3
Abca2	0	0.6672388	0.462	0.246	3

Cntn2	0	0.6642329	0.376	0.115	3
Cldn11	0	0.6627474	0.303	0.008	3
Zeb2	0	0.6306229	0.347	0.047	3
Stmn4	0	0.6265405	0.339	0.059	3
Map7	0	0.6238302	0.331	0.042	3
Gsn	0	0.602966	0.279	0.007	3
Ptma	0	0.5983608	0.353	0.133	3
Klk6	0	0.596435	0.236	0.003	3
Syt11	0	0.5895886	0.669	0.548	3
Fnbp1	0	0.5812498	0.383	0.168	3
Actb	0	0.5724746	0.628	0.52	3
Qdpr	0	0.5626731	0.286	0.072	3
Cmtm5	0	0.5616848	0.263	0.009	3
Kcnj10	0	0.5520853	0.288	0.024	3
Lamp1	0	0.531557	0.335	0.153	3
Hapln2	0	0.5287375	0.247	0.006	3
Pex5l	0	0.5259814	0.304	0.091	3
Ndrg1	0	0.5249046	0.256	0.012	3
Tmem88b	0	0.5231319	0.254	0.022	3
Efcab14	0	0.5167435	0.279	0.077	3
Dock9	0	0.5114567	0.318	0.137	3
Kif1b	0	0.5100938	0.607	0.504	3
Tmem151a	0	0.5016586	0.289	0.116	3
Prr5l	0	0.4861403	0.23	0.007	3
Plxnb3	0	0.4751939	0.211	0.007	3
Rtn4	0	0.4746112	0.425	0.323	3
Tspan2	0	0.4740951	0.218	0.017	3
Adamts4	0	0.4727044	0.231	0.009	3
Dock10	0	0.4721793	0.255	0.042	3
Cd81	0	0.4688141	0.309	0.174	3
Sez6l2	0	0.4638343	0.326	0.206	3
Selenop	0	0.4599166	0.215	0.018	3
Aatk	0	0.4584498	0.264	0.106	3
Hmgcs1	0	0.4515978	0.299	0.18	3
Pcdh9	0	0.4486376	0.445	0.323	3
Phldb1	0	0.4436813	0.204	0.017	3
Tuba1a	0	0.4378003	0.347	0.26	3
Tmem63a	0	0.4376406	0.207	0.005	3
Kndc1	0	0.4354788	0.289	0.165	3
Slc48a1	0	0.4345389	0.241	0.071	3
Plekhh1	0	0.4289592	0.2	0.015	3
Ywhaq	0	0.4219728	0.318	0.223	3
Edil3	0	0.4199897	0.285	0.145	3

Fgfr2	0	0.4143634	0.2	0.02	3
Otud7b	0	0.4113574	0.231	0.059	3
Efnb3	0	0.4088633	0.202	0.009	3
Anln	0	0.400709	0.194	0.015	3
Rnf13	0	0.3953748	0.238	0.088	3
Agpat4	0	0.3866612	0.207	0.036	3
Cdc37l1	0	0.3860095	0.285	0.171	3
Gjc2	0	0.3806491	0.179	0.004	3
Lpar1	0	0.3783473	0.185	0.007	3
Adipor2	0	0.3777323	0.187	0.035	3
Secisbp2l	0	0.3754237	0.212	0.06	3
Fez1	0	0.3746373	0.249	0.142	3
Myrf	0	0.3745537	0.185	0.021	3
Nfasc	0	0.371303	0.303	0.265	3
Pmp22	0	0.3701979	0.164	0.012	3
Sirt2	0	0.3635241	0.21	0.071	3
Shisa4	0	0.3625061	0.215	0.09	3
Tmeff2	0	0.3618407	0.214	0.075	3
Kif13b	0	0.3591705	0.195	0.047	3
Sec11c	0	0.3536522	0.207	0.079	3
Daam2	0	0.3526074	0.182	0.037	3
Slc44a1	0	0.3515341	0.189	0.04	3
Arhgap23	0	0.3510998	0.18	0.04	3
Itm2b	0	0.3506868	0.356	0.302	3
Cpd	0	0.3491617	0.188	0.052	3
Zdhhc20	0	0.3481961	0.257	0.161	3
Pacs2	0	0.347316	0.198	0.063	3
Wscd1	0	0.341744	0.175	0.037	3
Tppp3	0	0.336165	0.191	0.088	3
Ttyh2	0	0.3342392	0.159	0.008	3
Atp1b3	0	0.329504	0.189	0.07	3
Aspa	0	0.3264428	0.158	0.014	3
Stmn1	0	0.3257563	0.228	0.123	3
Pea15a	0	0.3252495	0.203	0.09	3
Etv1	0	0.32521	0.152	0.014	3
Plin3	0	0.3230782	0.148	0.01	3
Gjb1	0	0.3226736	0.148	0.002	3
Taldo1	0	0.322439	0.168	0.04	3
Serpinb1a	0	0.3173902	0.143	0.003	3
Arhgef10	0	0.3167752	0.151	0.013	3
Usp54	0	0.3166988	0.171	0.044	3
Dbndd2	0	0.3153281	0.172	0.05	3
Enpp4	0	0.3150774	0.162	0.035	3

Elovl1	0	0.3143994	0.15	0.009	3
Sec14l5	0	0.314212	0.147	0.014	3
Wnk1	0	0.3140146	0.257	0.188	3
Magt1	0	0.3065059	0.155	0.026	3
Npc1	0	0.3014844	0.184	0.074	3
Psat1	0	0.3003193	0.147	0.022	3
Cpox	0	0.299684	0.161	0.048	3
Slc38a2	0	0.2976823	0.226	0.164	3
Tppp	0	0.2973396	0.181	0.086	3
Ptn	0	0.2962332	0.179	0.099	3
Pik3c2b	0	0.2950909	0.164	0.047	3
Gltp	0	0.2946176	0.14	0.009	3
Kctd3	0	0.2930424	0.206	0.115	3
Cers2	0	0.2929419	0.151	0.018	3
Kctd13	0	0.2920035	0.146	0.025	3
Phlpp1	0	0.291286	0.154	0.031	3
Eef1a1	0	0.2910657	0.259	0.19	3
Mast4	0	0.2900613	0.213	0.133	3
Myo6	0	0.2893583	0.175	0.061	3
Ankrd28	0	0.2875955	0.175	0.066	3
Fa2h	0	0.2862184	0.135	0.005	3
Sort1	0	0.284055	0.194	0.121	3
Nrbp2	0	0.2810097	0.158	0.047	3
Bin1	0	0.2803092	0.254	0.219	3
Eml1	0	0.2798412	0.145	0.022	3
Ppp1r16b	0	0.2772155	0.162	0.061	3
Csrp1	0	0.2769179	0.129	0.013	3
Plekhb1	0	0.2768113	0.136	0.012	3
Olig1	0	0.2754197	0.127	0.006	3
Foxn3	0	0.2744346	0.185	0.107	3
Sccpdh	0	0.2725627	0.164	0.073	3
Dpy19l1	0	0.2709104	0.146	0.036	3
Gnao1	0	0.2688801	0.284	0.253	3
Elovl7	0	0.2688145	0.134	0.019	3
Sypl	0	0.2686733	0.147	0.034	3
Inf2	0	0.2684947	0.129	0.008	3
S100a16	0	0.2631298	0.119	0.005	3
Arrdc3	0	0.2628438	0.135	0.028	3
Tmbim1	0	0.2586548	0.157	0.054	3
Arhgef2	0	0.2572418	0.151	0.047	3
Car14	0	0.2563609	0.126	0.008	3
Josd2	0	0.2548116	0.135	0.028	3
Arsg	0	0.2546638	0.122	0.013	3

Ccp110	0	0.2527608	0.139	0.05	3
Ckb1	0	0.4836905	0.724	0.433	4
Pvalb	0	0.4661873	0.413	0.109	4
Sphkap	0	0.438224	0.399	0.08	4
Ppp3ca	0	0.4227978	0.525	0.304	4
Sparcl11	0	0.4128678	0.744	0.447	4
Necab2	0	0.3931824	0.31	0.025	4
Scn4b	0	0.3793392	0.361	0.137	4
Map1a1	0	0.3768442	0.704	0.452	4
Nefm1	0	0.3686965	0.809	0.479	4
Nefl1	0	0.3585811	0.663	0.367	4
Glra11	0	0.3544084	0.505	0.273	4
Slc17a6	0	0.3484613	0.504	0.209	4
AI593442	0	0.3483056	0.371	0.138	4
Vsnl11	0	0.343443	0.618	0.381	4
Snap251	0	0.341713	0.862	0.622	4
Atp1b11	0	0.3404215	0.922	0.718	4
Cplx11	0	0.3299008	0.644	0.405	4
Atp5b1	0	0.3276665	0.695	0.487	4
Ebf1	0	0.3085761	0.297	0.08	4
Arpp21	0	0.304836	0.389	0.139	4
Calm21	0	0.3026335	0.723	0.524	4
Ntng1	0	0.3000027	0.285	0.063	4
Sptb	0	0.2974729	0.308	0.133	4
Tanc1	0	0.2920494	0.295	0.078	4
Cplx2	0	0.2859935	0.31	0.131	4
Chgb	0	0.2855212	0.485	0.303	4
Ldhb1	0	0.2806631	0.53	0.331	4
Vamp11	0	0.2794603	0.546	0.346	4
Rgs7bp	0	0.2751283	0.381	0.198	4
Pitpnc1	0	0.2680408	0.283	0.094	4
Ina1	0	0.2599847	0.441	0.274	4
Plcb4	0	0.2589942	0.389	0.219	4
Smarca2	0	0.2534591	0.459	0.295	4
Stmn31	1.78E-266	0.2545976	0.552	0.396	4
Atp1a31	6.78E-265	0.3353992	0.668	0.505	4
Apoe	0	1.6086973	0.577	0.055	5
Atp1a2	0	1.5247581	0.674	0.031	5
Sparc	0	1.0838668	0.476	0.03	5
Slc1a2	0	1.0801745	0.466	0.047	5
Ptgds1	0	1.0527407	0.236	0.113	5
Cst3	0	0.9195035	0.414	0.152	5
Atp1b2	0	0.7810754	0.351	0.044	5

Aqp4	0	0.6686322	0.258	0.005	5
Slc4a4	0	0.6680274	0.297	0.028	5
Rgs5	0	0.6562534	0.187	0.007	5
Gm205941	0	0.6442261	0.675	0.621	5
Mbp1	0	0.6325801	0.391	0.181	5
Sparcl12	0	0.631754	0.618	0.454	5
Ndrg2	0	0.6043657	0.3	0.057	5
Flt1	0	0.5983766	0.194	0.007	5
Gpr37l1	0	0.589757	0.257	0.008	5
Slc6a11	0	0.583096	0.247	0.009	5
Aldoc1	0	0.5646454	0.446	0.353	5
Bcan	0	0.5470201	0.265	0.121	5
Gja1	0	0.5269379	0.231	0.01	5
Bsg	0	0.5178038	0.325	0.315	5
Cd811	0	0.4869174	0.331	0.179	5
Ttyh1	0	0.4798814	0.311	0.149	5
Fgfr3	0	0.4471816	0.187	0.003	5
Vtn	0	0.4422136	0.121	0.002	5
Mobp1	0	0.4404429	0.297	0.127	5
Ly6c1	0	0.4356071	0.124	0.002	5
Pla2g7	0	0.4336423	0.215	0.067	5
Htra1	0	0.4315661	0.179	0.03	5
Zbtb20	0	0.4251622	0.461	0.352	5
Mt1	0	0.4185149	0.184	0.03	5
Kcnj101	0	0.4136619	0.217	0.038	5
Slc7a10	0	0.4054401	0.161	0.006	5
Clu	0	0.3963422	0.271	0.229	5
Itih3	0	0.3954393	0.149	0.004	5
Glul1	0	0.3948758	0.261	0.088	5
Macf1	0	0.389849	0.47	0.415	5
Scd21	0	0.3857611	0.387	0.258	5
Qk1	0	0.3819904	0.29	0.098	5
Pbxip1	0	0.3818055	0.18	0.021	5
mt-Cytb1	0	0.3775106	0.488	0.461	5
Ntsr2	0	0.3774527	0.153	0.004	5
Son	0	0.3771165	0.412	0.339	5
Fth11	0	0.3770047	0.508	0.461	5
Ptprz1	0	0.3698997	0.192	0.043	5
Slc1a3	0	0.3642971	0.152	0.01	5
mt-Co1	0	0.3642065	0.42	0.417	5
Agt	0	0.362874	0.137	0.003	5
Gpm6b1	0	0.3461048	0.258	0.103	5
Slc6a1	0	0.3452291	0.178	0.053	5

Syne11	0	0.3415591	0.468	0.462	5
Acsl3	0	0.3359276	0.203	0.105	5
Lrp1	0	0.3327339	0.311	0.282	5
Slco1a4	0	0.3311611	0.121	0.003	5
Plpp3	0	0.3203941	0.14	0.009	5
Tril	0	0.3178307	0.144	0.01	5
Serpine2	0	0.314655	0.176	0.056	5
Epas1	0	0.3016294	0.138	0.02	5
Nnat	0	0.2996618	0.108	0.013	5
Rapgef3	0	0.2987869	0.139	0.014	5
Slc38a3	0	0.2909174	0.13	0.006	5
Utrn	0	0.2904571	0.183	0.077	5
Ptn1	0	0.2788613	0.188	0.103	5
Nwd1	0	0.2756162	0.139	0.049	5
Itm2a	0	0.2745194	0.106	0.01	5
mt-Nd5	0	0.2720926	0.393	0.406	5
Slc27a1	0	0.2688832	0.135	0.051	5
Sdc4	0	0.2600682	0.113	0.006	5
Slc2a1	0	0.2588932	0.116	0.015	5
Dbi	0	0.2564351	0.123	0.049	5
Trpm3	8.53350183497001e-320	0.3061331	0.192	0.073	5
Daam21	3.97E-303	0.2576742	0.14	0.045	5
Fn1	0	0.7072006	0.266	0.012	6
Sparcl13	0	0.6895945	0.797	0.45	6
Maf	0	0.5596038	0.514	0.051	6
Hcn1	0	0.5393843	0.518	0.134	6
Ndrg41	0	0.5139276	0.737	0.445	6
Nefh1	0	0.5119571	0.654	0.339	6
Nefm2	0	0.4980926	0.745	0.486	6
Lgi2	0	0.4844967	0.48	0.156	6
Ldhb2	0	0.4824331	0.651	0.33	6
Mafb	0	0.465526	0.401	0.014	6
Ckb2	0	0.4379359	0.713	0.437	6
Map1a2	0	0.4194278	0.711	0.455	6
Lpgat1	0	0.4122993	0.481	0.23	6
Hhip	0	0.4004197	0.216	0.009	6
Atp1b12	0	0.3793687	0.908	0.721	6
Spp1	0	0.3702855	0.351	0.073	6
Pcp4l1	0	0.3627434	0.436	0.194	6
Rap1gds1	0	0.3358917	0.323	0.112	6
Cd24a	0	0.3063732	0.247	0.028	6
Pvalb1	0	0.2977513	0.38	0.115	6
Sema7a	0	0.2931878	0.333	0.076	6

Lifr	0	0.2900475	0.338	0.089	6
Tcf4	0	0.2879863	0.429	0.154	6
Sphkap1	0	0.2814172	0.359	0.086	6
Rcan2	0	0.2679705	0.488	0.228	6
Gm205942	1.88E-254	0.3430948	0.789	0.618	6
Atp1a32	1.33E-253	0.3597125	0.721	0.506	6
mt-Nd11	4.26E-241	0.2693782	0.592	0.386	6
Nefl2	2.18E-227	0.3137003	0.573	0.374	6
Chgb1	0	0.7663805	0.7	0.3	7
Map1b1	0	0.5038042	0.855	0.706	7
Slc24a21	0	0.5022044	0.605	0.316	7
Snap252	0	0.4525786	0.806	0.627	7
Cplx21	0	0.4433372	0.47	0.131	7
Slc17a7	0	0.4349322	0.398	0.017	7
Pcp4	0	0.3590712	0.333	0.092	7
Gls	0	0.3527068	0.515	0.219	7
Sv2b	0	0.3437068	0.409	0.105	7
Spp11	0	0.3404474	0.342	0.074	7
Chd7	0	0.3316383	0.271	0.052	7
Syt1	0	0.3221465	0.462	0.178	7
Igfbp5	0	0.3151454	0.278	0.023	7
Zic1	0	0.2792836	0.206	0.016	7
Cpe	1.80E-266	0.2703337	0.543	0.315	7
Syne12	2.09E-265	0.3894454	0.669	0.456	7
Atp1b13	1.77E-261	0.3342662	0.864	0.723	7
Ndrg42	5.55E-236	0.3163572	0.644	0.448	7
Nefh2	1.86E-228	0.2723553	0.561	0.343	7
Dnm11	6.44E-223	0.3008523	0.552	0.347	7
Calm11	1.15E-216	0.3708707	0.794	0.667	7
Napb1	2.85E-207	0.2796105	0.631	0.447	7
Atp1a33	3.42E-125	0.2994903	0.661	0.509	7
Nefm3	0	1.7615498	0.976	0.485	8
Nefl3	0	1.680043	0.944	0.369	8
Map1b2	0	1.4496842	0.994	0.704	8
Nefh3	0	1.3304945	0.913	0.338	8
Slc5a7	0	0.9078166	0.65	0.011	8
Stmn2	0	0.9042521	0.808	0.399	8
Acly	0	0.8692583	0.702	0.146	8
Prune2	0	0.8525777	0.658	0.172	8
Calm12	0	0.8293291	0.946	0.665	8
S100b1	0	0.7708839	0.662	0.139	8
Sncg	0	0.7306761	0.582	0.061	8
Caln1	0	0.704289	0.649	0.18	8

Fgf1	0	0.6903476	0.619	0.087	8
Tppp31	0	0.6638242	0.554	0.088	8
Prph	0	0.6044724	0.522	0.007	8
Tubb3	0	0.5901334	0.642	0.245	8
Calm22	0	0.5212103	0.824	0.528	8
Pcp4l11	0	0.5067066	0.451	0.196	8
Vamp12	0	0.4938005	0.691	0.349	8
Atp1a1	0	0.4863912	0.422	0.067	8
mt-Nd12	0	0.4760841	0.697	0.387	8
Ahnak2	0	0.4678602	0.395	0.017	8
Ywhag1	0	0.4623602	0.693	0.374	8
Actg1	0	0.4526164	0.621	0.294	8
Epb41l3	0	0.4479075	0.651	0.376	8
Kif5c	0	0.447675	0.63	0.307	8
mt-Cytb2	0	0.4439124	0.764	0.456	8
Dffa	0	0.4280913	0.413	0.075	8
mt-Nd51	0	0.4186614	0.712	0.4	8
Rgs7bp1	0	0.4116456	0.514	0.2	8
Cplx12	0	0.4108131	0.737	0.41	8
Tns1	0	0.4064747	0.438	0.032	8
Fxyd7	0	0.4029779	0.326	0.021	8
Map2	0	0.4013043	0.633	0.336	8
Uchl1	0	0.3851554	0.546	0.253	8
Slc18a3	0	0.3789193	0.398	0.003	8
Sv2c1	0	0.3731882	0.504	0.211	8
Tuba1a1	0	0.3690301	0.575	0.261	8
Chodl	0	0.366018	0.296	0.012	8
Calca	0	0.3606921	0.244	0.004	8
Lgals1	0	0.3576738	0.297	0.011	8
Clu1	0	0.3558484	0.53	0.225	8
Slc7a14	0	0.3557184	0.459	0.125	8
Dkk3	0	0.3495818	0.366	0.053	8
Plec	0	0.348697	0.475	0.192	8
Fam135b	0	0.3239991	0.442	0.129	8
Ret	0	0.308943	0.366	0.046	8
Htra11	0	0.3034084	0.371	0.029	8
Fstl1	0	0.2988288	0.417	0.119	8
Rgs4	0	0.2960419	0.371	0.081	8
Capns1	0	0.2954707	0.395	0.118	8
Anxa6	0	0.2950323	0.412	0.111	8
Susd2	0	0.2771392	0.334	0.032	8
Fbxo2	0	0.2735682	0.363	0.108	8
Lifr1	0	0.2688005	0.359	0.091	8

Nfia	0	0.2673963	0.332	0.079	8
Stk32a	0	0.2630793	0.267	0.009	8
Kcnk9	0	0.2618339	0.409	0.129	8
Cyb5r3	0	0.2597222	0.327	0.046	8
Calcb	0	0.2563324	0.263	0.002	8
Endod1	0.00E+00	0.2885554	0.46	0.19	8
Rab6b	5.57E-307	0.3266993	0.532	0.248	8
Tuba1b	5.64E-302	0.3465961	0.576	0.295	8
Sptbn1	5.84E-296	0.3690503	0.662	0.395	8
mt-Co11	1.60E-295	0.3758191	0.707	0.412	8
Disp2	1.46E-285	0.4065246	0.573	0.323	8
Sorl1	2.44E-284	0.3597243	0.563	0.301	8
Hspa12a	1.09E-278	0.2626278	0.465	0.203	8
Klc1	7.90E-266	0.289986	0.532	0.263	8
mt-Nd6	2.09E-261	0.4164883	0.556	0.296	8
mt-Nd4	1.05E-258	0.3396292	0.524	0.261	8
Nars1	3.10E-256	0.3403952	0.616	0.349	8
mt-Nd21	5.33E-252	0.3968357	0.582	0.317	8
1110008P14Rik	1.02E-250	0.2538771	0.266	0.087	8
Hsp90aa11	5.63E-243	0.3770925	0.765	0.557	8
Map7d21	5.14E-231	0.3180183	0.623	0.362	8
Gm205943	8.43E-230	0.3632201	0.849	0.618	8
Kif21a	1.18E-229	0.3265865	0.641	0.403	8
Calm31	2.57E-227	0.3245775	0.625	0.37	8
Glra12	2.15E-217	0.2504803	0.528	0.279	8
Hsp90ab11	1.30E-192	0.2963025	0.801	0.615	8
Syt111	2.54E-185	0.3116977	0.759	0.553	8
Pkm1	1.46E-180	0.2980566	0.707	0.5	8
Pja2	1.51E-172	0.2607769	0.59	0.366	8
Stmn32	1.01E-169	0.3034142	0.623	0.399	8
Sptan1	1.01E-169	0.2879788	0.641	0.439	8
Ubb	3.79E-167	0.2748471	0.597	0.377	8
Kif5a	4.44E-167	0.2540981	0.617	0.396	8
Dync1h1	1.64E-140	0.2842837	0.633	0.444	8
Slc25a41	2.83E-138	0.2611627	0.726	0.524	8
Lars21	1.17E-80	0.3756277	0.948	0.86	8
Ttr	0	5.5248098	0.987	0.136	9
Enpp21	0	3.2458434	0.985	0.116	9
Trpm31	0	1.4798515	0.901	0.063	9
Apoe1	0	1.1665227	0.814	0.06	9
Arl6ip1	0	0.998859	0.823	0.342	9
Kl	0	0.9728218	0.7	0.017	9
mt-Nd52	0	0.8574512	0.865	0.398	9

Clu2	0	0.8504709	0.785	0.221	9
1500015O10Rik	0	0.8440248	0.621	0.001	9
Bsg1	0	0.8281743	0.813	0.307	9
Igfbp2	0	0.7614576	0.583	0.003	9
Prlr	0	0.7344926	0.574	0.034	9
Hspa5	0	0.7204849	0.817	0.365	9
mt-Nd61	0	0.6708455	0.722	0.293	9
Folr1	0	0.6515169	0.524	0.001	9
Clic6	0	0.6493874	0.496	0.002	9
Pbxip11	0	0.6342494	0.552	0.017	9
Cst31	0	0.5943275	0.595	0.154	9
Hsp90b1	0	0.5700656	0.72	0.34	9
Gm205944	0	0.5534777	0.909	0.618	9
Col9a3	0	0.5530513	0.451	0.005	9
F5	0	0.5422409	0.448	0.001	9
Kcnj13	0	0.5318642	0.444	0.001	9
Abhd2	0	0.5180168	0.367	0.032	9
Cd63	0	0.5174269	0.468	0.025	9
Calr	0	0.5089094	0.628	0.276	9
Slc4a2	0	0.5041416	0.495	0.071	9
Kcne2	0	0.499141	0.424	0	9
Sostdc1	0	0.4843245	0.4	0.001	9
Slc12a21	0	0.4646736	0.535	0.098	9
mt-Co12	0	0.4639086	0.739	0.411	9
Ptgds2	0	0.4631587	0.617	0.108	9
mt-Cytb3	0	0.4605701	0.783	0.456	9
Ace	0	0.4370415	0.303	0.021	9
Slc16a2	0	0.4338758	0.402	0.006	9
Car12	0	0.4242717	0.356	0.004	9
Slc4a10	0	0.4239848	0.445	0.117	9
Htr2c	0	0.4238085	0.431	0.086	9
Ctsd	0	0.4174925	0.462	0.117	9
Ezr	0	0.3879193	0.339	0.003	9
Lamb1	0	0.3734095	0.329	0.017	9
Cab39l	0	0.366835	0.374	0.048	9
Itpr1	0	0.3662083	0.366	0.067	9
Slc4a5	0	0.3541353	0.305	0.001	9
Col8a1	0	0.3474446	0.324	0.009	9
Cgnl1	0	0.3466997	0.325	0.083	9
Otx2	0	0.3452171	0.318	0.001	9
Slco1c1	0	0.3436034	0.312	0.005	9
Ifi27	0	0.3364907	0.333	0.032	9
Sparc1	0	0.3354434	0.378	0.039	9

Sod3	0	0.3312557	0.297	0.003	9
Wls	0	0.325979	0.326	0.022	9
Tbc1d9	0	0.3118417	0.311	0.068	9
Atp1a11	0	0.3046613	0.29	0.07	9
Rdh5	0	0.3028931	0.279	0.002	9
Slc13a4	0	0.3025639	0.282	0.013	9
Ccnd2	0	0.2963421	0.288	0.029	9
Ppp1r1b	0	0.2934323	0.262	0.005	9
Fxyd1	0	0.2914018	0.285	0.004	9
Dnajc3	0	0.2905242	0.35	0.091	9
Msx1	0	0.2867925	0.264	0.001	9
Cdkn1c	0	0.2862008	0.258	0.004	9
Slc31a1	0	0.2846267	0.271	0.016	9
Car21	0	0.279256	0.433	0.077	9
Mfrp	0	0.2777927	0.178	0.002	9
Sulf1	0	0.274706	0.243	0.006	9
Abca4	0	0.2708458	0.225	0.003	9
Stk39	0	0.2640549	0.306	0.088	9
Strip2	0	0.2632678	0.272	0.059	9
Syne2	0	0.2618109	0.258	0.019	9
Plxnb2	0	0.2608731	0.238	0.029	9
Col8a2	0	0.2550985	0.229	0.002	9
Cd59a	1.55595662130222e-314	0.2833584	0.392	0.136	9
mt-Nd41	4.38E-294	0.3260103	0.547	0.261	9
mt-Nd22	9.43E-292	0.3695885	0.61	0.317	9
Tmem721	6.24E-286	0.3403095	0.588	0.297	9
Zbtb201	2.44E-268	0.3166631	0.643	0.35	9
Eef1a11	5.14E-265	0.2800504	0.454	0.19	9
Psap	1.67E-257	0.3603301	0.621	0.358	9
Ahcyl2	3.56E-254	0.2552917	0.315	0.111	9
Slc22a17	6.91E-246	0.3033416	0.538	0.283	9
Atp2b3	4.32E-244	0.298423	0.34	0.152	9
Lars22	2.06E-80	0.4367687	0.956	0.86	9
Nrcam	0	1.2774311	0.788	0.224	10
Scn3a	0	1.101072	0.745	0.08	10
Slc1a21	0	0.730126	0.545	0.054	10
Rph3a1	0	0.6743113	0.589	0.239	10
Cacna1e	0	0.6706936	0.575	0.143	10
Calb1	0	0.6062498	0.466	0.031	10
Arpp211	0	0.5819031	0.547	0.145	10
Syt12	0	0.5563635	0.526	0.18	10
Crh	0	0.528979	0.354	0.009	10
Nell2	0	0.5176584	0.398	0.056	10

Msi2	0	0.5121358	0.514	0.189	10
Foxp2	0	0.5040948	0.425	0.029	10
Gpr88	0	0.4977039	0.372	0.002	10
Cplx22	0	0.4607197	0.449	0.135	10
Gpr26	0	0.4283786	0.347	0.027	10
Pex5l1	0	0.4144912	0.422	0.103	10
Htr5b	0	0.4015871	0.326	0.001	10
Plcxd2	0	0.3858854	0.362	0.089	10
Kit	0	0.3382917	0.293	0.016	10
Gabrg1	0	0.3347289	0.304	0.024	10
Pou4f1	0	0.3278475	0.292	0.006	10
Foxp1	0	0.3243898	0.308	0.054	10
Cacna1g	0	0.2996582	0.276	0.045	10
Kcng1	0	0.2746611	0.257	0.008	10
Cnih3	0	0.2632164	0.258	0.011	10
Ajap1	0	0.2603678	0.269	0.042	10
Lmo7	0	0.2591114	0.203	0.008	10
Dnah11	0	0.2583439	0.19	0.002	10
C1ql1	0	0.2538234	0.244	0.004	10
Smpd3	0	0.251076	0.26	0.038	10
Calb2	1.68970450877706e-321	0.3260941	0.349	0.081	10
Nav3	2.41207098104162e-314	0.3245279	0.334	0.079	10
Adgra1	2.52E-268	0.2809944	0.306	0.081	10
Kcnh7	5.64E-253	0.2745858	0.3	0.077	10
Zfp462	8.68E-233	0.2779432	0.313	0.091	10
Ptprd1	8.32E-229	0.4075489	0.598	0.315	10
Cpne6	6.30E-221	0.2523094	0.269	0.068	10
Fbxw7	4.04E-214	0.2960523	0.364	0.143	10
Pcp4l12	1.81E-213	0.3971873	0.41	0.198	10
Snhg111	5.51E-206	0.496118	0.978	0.863	10
Kmt2c	1.72E-190	0.3067879	0.396	0.161	10
Gpm6a	5.23E-187	0.2938516	0.395	0.164	10
Tsc22d1	2.12E-182	0.2741192	0.358	0.157	10
Lgi21	6.29E-168	0.2698586	0.357	0.163	10
Cntnap2	1.25E-139	0.2832811	0.435	0.251	10
Akap6	1.42E-131	0.2875968	0.414	0.24	10
Ntrk3	1.88E-125	0.2500144	0.399	0.204	10
Rab3c	5.80E-123	0.2532833	0.422	0.231	10
Zbtb202	1.67E-73	0.2667237	0.503	0.353	10
Nat8l1	0	1.7055516	0.764	0.277	11
Stx1b	0	1.0309443	0.694	0.299	11
Lars23	0	0.9530447	0.951	0.86	11
Vmn1r206	0	0.8928626	0.557	0.024	11

Spock2	0	0.797972	0.691	0.39	11
Fyco1	0	0.7464235	0.556	0.129	11
Dcbld2	0	0.6233987	0.43	0.052	11
Mtch1	0	0.6186533	0.497	0.223	11
Spop	0	0.5920649	0.5	0.191	11
Dynll2	0	0.579814	0.543	0.339	11
Srgap3	0	0.5705221	0.511	0.219	11
Ctnnd2	0	0.488252	0.413	0.129	11
Cd22	0	0.4447912	0.27	0.01	11
Tgfbi	0	0.3447108	0.229	0.025	11
Abcc4	0	0.3030108	0.197	0.012	11
Rgs51	0	0.2544776	0.191	0.011	11
Mdga2	7.34132143155508e-319	0.4749736	0.429	0.165	11
Usp53	1.51E-303	0.3861844	0.298	0.065	11
Shq1	5.82E-281	0.3338673	0.241	0.045	11
Lonrf1	1.02E-274	0.3610977	0.287	0.072	11
Fam219a	1.57E-273	0.357584	0.324	0.111	11
Slc16a10	9.07E-271	0.3423832	0.247	0.05	11
Shld1	1.83E-248	0.2934787	0.204	0.035	11
Tmem132d	1.55E-246	0.281562	0.199	0.03	11
Arf3	1.18E-244	0.3719308	0.389	0.182	11
Lhfpl4	7.28E-242	0.3507863	0.32	0.117	11
Slc9b2	4.73E-232	0.26379	0.192	0.029	11
Mrgpre	1.63E-229	0.264325	0.197	0.034	11
Pik3c2a	3.39E-210	0.3078433	0.265	0.072	11
Madd	2.06E-202	0.3710589	0.391	0.204	11
Fam160b2	3.69E-199	0.3052518	0.265	0.082	11
Mroh1	2.08E-188	0.3731423	0.375	0.183	11
Dzank1	2.94E-147	0.3014214	0.406	0.259	11
Celf41	6.04E-135	0.3726442	0.456	0.3	11
Gdi1	4.88E-116	0.2742928	0.465	0.343	11
Atp1a34	3.48E-99	0.3178588	0.663	0.511	11
Pkd1	1.79E-89	0.2681207	0.362	0.205	11
Slc6a51	0	1.0958838	0.891	0.156	12
Sparcl14	0	1.0323294	0.97	0.455	12
Spp12	0	0.9663713	0.864	0.074	12
Ndrg43	0	0.8950686	0.942	0.449	12
Map1a3	0	0.8822419	0.947	0.458	12
Nefh4	0	0.8305947	0.928	0.343	12
Ckb3	0	0.8289576	0.939	0.441	12
Calb11	0	0.7896467	0.714	0.031	12
Lamp51	0	0.7547427	0.878	0.216	12
Gm205945	0	0.6930772	0.954	0.62	12

Atp1b14	0	0.6395438	0.99	0.725	12
mt-Cytb4	0	0.6235564	0.925	0.458	12
Nefm4	0	0.6133711	0.947	0.49	12
Ldhb3	0	0.6117885	0.872	0.335	12
mt-Co13	0	0.6095623	0.901	0.413	12
mt-Nd13	0	0.6000307	0.862	0.389	12
Cd24a1	0	0.5902107	0.63	0.029	12
mt-Nd53	0	0.5291358	0.872	0.402	12
Pcp4l13	0	0.517397	0.702	0.197	12
Cplx13	0	0.5102504	0.908	0.411	12
Doc2g	0	0.5027164	0.436	0.007	12
Kcnc31	0	0.4906735	0.805	0.276	12
Ttn	0	0.4816575	0.447	0.025	12
Sv2c2	0	0.4757905	0.744	0.212	12
Pvalb2	0	0.423102	0.605	0.118	12
Fgf11	0	0.4163892	0.618	0.092	12
Kcna11	0	0.4152854	0.804	0.295	12
Ina2	0	0.4123801	0.75	0.278	12
Vamp13	0	0.4072803	0.847	0.351	12
Rcan21	0	0.4012657	0.736	0.231	12
Nrxn3	0	0.3821606	0.872	0.376	12
Dner	0	0.3754797	0.638	0.129	12
Lpgat11	0	0.3600748	0.691	0.234	12
Lgi22	0	0.3555206	0.638	0.162	12
Ghitm1	0	0.3490615	0.812	0.345	12
Syt21	0	0.3391313	0.82	0.332	12
Tenm4	0	0.333517	0.638	0.194	12
Pacsin2	0	0.3125246	0.59	0.113	12
Elovl5	0	0.3066045	0.52	0.07	12
Rgs7bp2	0	0.2964391	0.644	0.202	12
Hspg2	0	0.2742651	0.314	0.005	12
Ttll7	0	0.2647731	0.674	0.235	12
Mdh11	2.27270197086973e-322	0.4502565	0.846	0.394	12
Scn8a1	3.4090529563046e-322	0.2820236	0.788	0.343	12
mt-Nd42	9.78249978765668e-322	0.3718868	0.713	0.262	12
Atp2a21	1.82557256138341e-320	0.3863988	0.829	0.372	12
Scn4b1	4.67703923514476e-315	0.2729264	0.532	0.143	12
Atp1a35	6.29094088921395e-314	0.7133905	0.916	0.509	12
Psap1	1.12E-307	0.2925393	0.797	0.359	12
Clstn11	5.22E-305	0.3281631	0.806	0.364	12
Cend1	5.78E-305	0.2533888	0.665	0.244	12
Pkm2	4.74E-304	0.4148134	0.919	0.5	12
Atp5b2	6.34E-300	0.4577356	0.9	0.493	12

Myo5a	2.58E-298	0.2777	0.73	0.299	12
mt-Nd23	1.55E-297	0.5236348	0.75	0.319	12
Ywhag2	1.97E-292	0.2905228	0.805	0.376	12
Map1b3	1.72E-290	0.445745	0.982	0.707	12
Ctsb	2.54E-289	0.2507843	0.811	0.393	12
Glra13	4.36E-281	0.3094168	0.701	0.28	12
Aplp2	2.13E-271	0.2744142	0.828	0.42	12
Kcnc1	2.02E-268	0.3417431	0.754	0.335	12
Nars2	3.36E-263	0.2620816	0.753	0.351	12
Dnm12	5.51E-262	0.2891962	0.75	0.349	12
Snap253	2.92E-260	0.398959	0.968	0.629	12
Map7d22	2.46E-258	0.2653492	0.764	0.364	12
mt-Nd62	1.05E-251	0.4035462	0.701	0.297	12
Spock21	9.19E-251	0.337828	0.791	0.39	12
Slc25a42	8.21E-242	0.4874889	0.89	0.525	12
Hspa81	3.03E-235	0.4084137	0.837	0.452	12
Stmn33	1.58E-198	0.2987345	0.759	0.4	12
Dst	1.34E-193	0.2957473	0.888	0.553	12
Hsp90ab12	3.86E-187	0.2540817	0.926	0.616	12
Calm13	7.61E-138	0.2601454	0.925	0.668	12
Lars24	2.81E-90	0.3582159	0.991	0.86	12
Gapdh1	4.79E-87	0.3209065	0.657	0.415	12
Atp2b1	0	1.1125109	0.625	0.332	13
Grid2	0	1.0873093	0.651	0.074	13
Prkcd	0	0.8157546	0.392	0.01	13
Anks1b	0	0.7601955	0.502	0.111	13
Cdh6	0	0.7054873	0.442	0.042	13
Bod1l	0	0.7023377	0.473	0.203	13
Car8	0	0.6920567	0.313	0.015	13
Dgkh	0	0.6657198	0.447	0.054	13
Ryr1	0	0.6467085	0.304	0.007	13
Adarb2	0	0.5900202	0.381	0.051	13
Gria21	2.31173315689119e-319	0.7093644	0.654	0.418	13
Itpr11	9.17E-304	0.5869735	0.341	0.07	13
Grm1	3.61E-232	0.465593	0.358	0.128	13
Tnr	3.05E-228	0.552817	0.388	0.127	13
Ogfrl1	8.27E-190	0.408877	0.286	0.087	13
Cttnbp2	3.02E-181	0.4664438	0.376	0.118	13
Trim9	8.27E-173	0.4336005	0.343	0.124	13
Sptbn2	2.22E-171	0.4270163	0.328	0.135	13
Map4	4.48E-164	0.4223111	0.389	0.218	13
Kalrn	6.16E-147	0.4103535	0.361	0.127	13
Vav3	8.41E-141	0.2847696	0.196	0.033	13

Shank2	8.55E-141	0.3175443	0.227	0.045	13
Rora	7.23E-140	0.3847959	0.317	0.126	13
Snhg112	8.93E-140	0.5868579	0.973	0.863	13
Pcp41	1.67E-131	0.4350893	0.254	0.097	13
Thsd7a	1.75E-129	0.3622019	0.315	0.129	13
Rreb1	5.88E-129	0.2867385	0.178	0.025	13
Grin2b	5.23E-124	0.36892	0.32	0.149	13
Rasa4	8.78E-103	0.2659244	0.194	0.041	13
Tshz2	1.56E-99	0.3142172	0.249	0.099	13
Sorl11	1.05E-95	0.3516879	0.445	0.305	13
Inpp5j	1.31E-94	0.2978166	0.244	0.088	13
Ywhah	4.16E-94	0.2638047	0.327	0.289	13
Exoc6b	5.67E-94	0.302147	0.26	0.114	13
Sipa1l3	1.03E-88	0.2577282	0.22	0.066	13
Frmd4a	1.33E-85	0.3317603	0.307	0.144	13
Cdc42bpa	7.50E-85	0.3280913	0.363	0.204	13
Nsg1	2.60E-83	0.2624021	0.323	0.236	13
Auts2	2.50E-79	0.3059808	0.284	0.128	13
Kcnma1	1.95E-74	0.2800633	0.303	0.157	13
Cpe1	5.51E-68	0.2531741	0.381	0.321	13
Shank1	1.09E-65	0.3032743	0.388	0.269	13
Arhgef12	2.15E-60	0.258207	0.299	0.213	13
Unc80	4.36E-57	0.2639798	0.442	0.349	13
Ccdc88a	9.44E-56	0.2786791	0.314	0.199	13
Ndrg44	2.94E-49	0.260818	0.516	0.453	13
Tnr1	0	1.3532412	0.605	0.125	14
Nfasc1	0	1.2924187	0.454	0.267	14
Gjc31	0	1.0037385	0.424	0.046	14
Vcan	0	0.9512637	0.39	0.017	14
Frmd4a1	0	0.9455795	0.38	0.144	14
Sirt21	0	0.912696	0.386	0.08	14
Ptprz11	0	0.8346418	0.349	0.046	14
Gpr17	0	0.8178256	0.272	0.002	14
Marcks	0	0.7985201	0.325	0.045	14
Bcas1	0	0.7610374	0.284	0.016	14
Pdgfra	0	0.7543389	0.215	0.005	14
Ppfibp1	0	0.6943152	0.309	0.019	14
Fyn	0	0.6609994	0.283	0.053	14
Tns3	0	0.6198661	0.267	0.014	14
Itpr2	0	0.5977648	0.265	0.03	14
Cspg4	0	0.5137608	0.172	0.003	14
Bcan1	1.97626258336499e-323	0.7251053	0.354	0.125	14
Kank1	1.90E-293	0.4255857	0.187	0.009	14

Qk2	9.25E-287	0.8312374	0.447	0.102	14
Mbp2	2.92E-278	1.0087408	0.477	0.186	14
Enpp6	1.38E-270	0.5392537	0.217	0.013	14
Neu4	2.51E-259	0.3235842	0.12	0.001	14
Cd812	4.36E-259	0.6383522	0.38	0.183	14
Lrp11	1.81E-244	0.5703849	0.29	0.283	14
Tcf7l2	1.84E-238	0.3672304	0.155	0.006	14
Gpr37l11	1.03E-235	0.4903116	0.221	0.015	14
Plp11	2.14E-211	0.9779335	0.606	0.268	14
Plxnb31	1.15E-209	0.5915398	0.22	0.021	14
Mical3	2.62E-203	0.4841772	0.276	0.146	14
Cnp1	5.46E-203	0.8255272	0.363	0.081	14
Slc44a11	5.84E-199	0.48689	0.229	0.05	14
Cntn1	7.03E-194	0.3477394	0.316	0.286	14
Serpine21	1.04E-189	0.463918	0.22	0.059	14
Mpzl1	1.41E-189	0.3449105	0.15	0.01	14
Olig11	4.60E-189	0.4088429	0.187	0.014	14
Epn2	9.90E-189	0.4566033	0.219	0.044	14
Cemip2	2.33E-185	0.3978554	0.134	0.03	14
Sema4d	1.45E-180	0.41474	0.179	0.036	14
Sulf2	4.26E-180	0.3866972	0.185	0.042	14
Dock91	4.39E-177	0.510572	0.319	0.15	14
Cacng4	5.23E-176	0.3660899	0.146	0.022	14
Col9a31	1.29E-170	0.3352737	0.147	0.012	14
Epb41l2	1.18E-160	0.3857107	0.159	0.033	14
Tuba1a2	1.85E-158	0.366908	0.283	0.267	14
Xylt1	4.04E-158	0.3290188	0.132	0.024	14
Pik3r3	1.40E-156	0.3150863	0.127	0.039	14
Nav1	1.36E-155	0.3990551	0.293	0.201	14
Acsl31	1.17E-154	0.3782013	0.203	0.107	14
Trio	9.01E-153	0.3681115	0.271	0.19	14
Cd9	1.13E-148	0.2927605	0.117	0.009	14
Pcdh17	8.08E-146	0.3445803	0.202	0.141	14
Sema5a	1.58E-144	0.3585588	0.206	0.089	14
Ankrd281	1.10E-143	0.3999173	0.217	0.074	14
Olig2	1.82E-141	0.2793114	0.115	0.006	14
Mag1	7.97E-140	0.842908	0.325	0.09	14
Hip1r	1.93E-138	0.3327983	0.168	0.063	14
Sema6a	1.83E-137	0.3602354	0.171	0.049	14
Map4k4	1.36E-136	0.3920935	0.228	0.085	14
Son1	1.45E-134	0.332694	0.365	0.341	14
Arhgap31	4.74E-134	0.3162263	0.146	0.037	14
Col11a2	5.33E-134	0.3590702	0.139	0.017	14

Zeb21	5.40E-131	0.4512218	0.258	0.069	14
Rictor	1.18E-130	0.3471	0.194	0.09	14
Dock4	6.96E-129	0.3447094	0.194	0.092	14
Shisal1	7.16E-129	0.2685024	0.133	0.029	14
Ddx5	5.10E-126	0.3043107	0.369	0.365	14
Spon1	8.68E-120	0.2981022	0.145	0.043	14
Adamts41	1.20E-119	0.3744926	0.165	0.025	14
Hnrnpa2b1	2.40E-118	0.2546692	0.285	0.308	14
Ctsl	1.73E-116	0.2567533	0.146	0.082	14
Pcdh15	4.87E-116	0.2923265	0.137	0.059	14
Lcorl	6.19E-116	0.2605945	0.131	0.024	14
Fryl	3.37E-115	0.3081083	0.219	0.154	14
Nckap5	1.19E-114	0.2552841	0.13	0.025	14
Actb1	3.81E-109	0.3534303	0.524	0.528	14
Phldb11	2.18E-108	0.3369088	0.176	0.03	14
Chn2	1.57E-105	0.2714967	0.12	0.024	14
Man2a2	2.15E-104	0.285966	0.187	0.127	14
Sep-71	1.19E-102	0.2532509	0.287	0.292	14
Ncor2	2.04E-97	0.2801186	0.18	0.115	14
Ncam1	3.84E-96	0.2623187	0.334	0.331	14
Cldn111	1.45E-87	0.3507074	0.161	0.029	14
Kndc11	1.86E-84	0.2644373	0.19	0.174	14
Ugt8a1	1.01E-83	0.359458	0.206	0.066	14
Zbtb203	2.13E-82	0.253466	0.341	0.355	14
Utp14b	4.49E-82	0.2556812	0.131	0.048	14
Secisbp2l1	7.37E-82	0.2839502	0.153	0.071	14
Scd22	1.10E-73	0.3693815	0.382	0.262	14
Myrf1	1.12E-73	0.2602219	0.139	0.033	14
Tmeff21	1.98E-71	0.2624986	0.172	0.085	14
AC149090.11	2.41E-70	0.2727262	0.331	0.34	14
S100b2	6.01E-68	0.3718325	0.253	0.147	14
Aatk1	1.27E-65	0.2534311	0.175	0.117	14
Ptma1	1.10E-62	0.2533219	0.202	0.149	14
Sparc2	1.64E-57	0.3038959	0.15	0.044	14
Slc12a22	3.45E-47	0.277781	0.199	0.105	14
Mobp2	3.04E-30	0.3282238	0.22	0.132	14

Table 4: Primers used for quantitative real time polymerase chain reaction (*qRT-PCR*)

Genes	Forward Primers	Reverse Primers
actin	GCCAACCGTGAAAAGATGAC	GAGGCATACAGGGACAGCAC
gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Rnf220	GTCTCAGTAGACAAGGACGTTCACA	GGGGTGGAGGTGTAGTAAGGAAG
Wdr5	CGTGACAGGCGGGAAGTGGA	CGGGTGACAAGCCGTGGAAAT
HoxA1	AGCTCTGTGAGCTGCTTGGT	AAAAGAAACCCTCCCAAAACA
HoxA2	TGCCATCAGCTATTTCCAGG	GATGAAGGAGAAGAAGGCGG
HoxA3	TCTTAACATGGAGGGAGCCA	TCTGAAGGCTACGTGTGCTG
HoxA4	ACGCTGTGCCCCAGTATAAG	ACCTTGATGGTAGGTGTGGC

HoxA5	CAGGGTCTGGTAGCGAGTGT	CTCAGCCCCAGATCTACCC
HoxA6	GTCTGGTAGCGCGTGTAGGT	CCCTGTTTACCCCTGGATG
HoxA7	CTTCTCCAGTTCCAGCGTCT	AAGCCAGTTTCCGCATCTAC
HoxA9	GTAAGGGCATCGCTTCTTCC	ACAATGCCGAGAATGAGAGC
HoxA10	TCTTTGCTGTGAGCCAGTTG	CTCCAGCCCCTTCAGAAAAC
HoxA11	CCTTTTCCAAGTCGCAATGT	AGGCTCCAGCCTACTGGAAT
HoxA13	CGGTGTCCATGTACTTGTCG	AGCGGCTACTACCCGTGC
HoxB1	GGTGAAGTTTGTGCGGAGAC	TTCGACTGGATGAAGGTCAA
HoxB2	GAACCAGACTTTGACCTGCC	GAGCTGGAGAAGGAGTTCCA
HoxB3	ATCTGTTTGGTGAGGGTGGA	CCGCACCTACCAGTACCACT
HoxB4	GACCTGCTGGCGAGTGTAG	CTGGATGCGCAAAGTTCAC
HoxB5	CTGGTAGCGAGTATAGGCGG	AGGGGCAGACTCCACAGATA
HoxB6	TAGCGTGTGTAGGTCTGGCG	AGCAGAAGTGCTCCACGC
HoxB7	CTTTCTCCAGCTCCAGGGTC	AACTTCCGGATCTACCCCTG
HoxB8	GAACTCCTTCTCCAGCTCCA	CACAGCTCTTTCCCTGGATG
HoxB9	TCCAGCGTCTGGTATTTGGT	GAAGCGAGGACAAAGAGAGG
HoxB13	TGCCCCTTGCTATAGGGAAT	ATTCTGGAAAGCAGCGTTTG
HoxC4	CTAATTCCAGGACCTGCTGC	AAAAATTCACGTTAGCACGGT
HoxC5	TTCTCGAGTTCCAGGGTCTG	ATTTACCCGTGGATGACCAA
HoxC6	CAGGGTCTGGTACCGAGAGTA	TCCAGATTTACCCCTGGATG
HoxC8	CAAGGTCTGATACCGGCTGT	ATCAGAACTCGTCTCCCAGC
HoxC9	AATCTGTCTCTGTCGGCTCC	AGTCTGGGCTCCAAAGTCAC
HoxC10	ACCTCTTCTTCCTTCCGCTC	ACTCCAGTCCAGACACCTCG
HoxC11	AAATGAAGGCTCCTACGGCG	TGTCGAAGAAGCGGTCGAAA
HoxC12	AATACGGCTTGCGCTTCTT	GACCCTGGCTCTCTGGTTTC
HoxC13	CTCACTTCGGGCTGTAGAGG	TCAGGTGTACTGCTCCAAGG
HoxD1	TCTGTCAGTTGCTTGGTGCT	TGAAAGTGAAGAGGAACGCC
HoxD3	ACCAGCTGAGCACTCGTGTA	AGAACAGCTGTGCCACTTCA
HoxD4	CTCCCTGGGCTGAGACTGT	CCCTGGGAACCACTGTTCT
HoxD8	GCCCGCGAAGTTTTACGGAT	TAAGTGGTCTGGGTCCTCGC
HoxD9	TTGTTTGGGTCAAGTTGCTG	CTCAGCTTGCAGCGATCA
HoxD10	TCTCCTGCACTTCGGGAC	GGAGCCCACTAAAGTCTCCC
HoxD11	AGTGAGGTTGAGCATCCGAG	ACACCAAGTACCAGATCCGC
HoxD12	TGCTTTGTGTAGGGTTTCCTCT	CTTCACTGCCCGACGGTA
HoxD13	TGGTGTAAGGCACCCTTTTC	CCCATTTTTGGAAATCATCC
Unc5b	CGTGACAGGCGGGAAGTGGA	CGGGTGACAAGCCGTGGAAAT
Pax6	AGACAACAACAAAGCGGACT	CTTCGCAAATGACAACTGAC
Barhl1	TACCAGAACCGCAGGACTAAAT	AGAAATAAGGCGACGGGAACAT

Nfib	AGAAGCCCGAAATCAAGCAG	CCAGTCACGGTAAGCACAAA
Neph2	ACAAGGTTCGGAAATGAAGTCG	GTTGCCATTAGGACGAGGAA

Table 5: Primers used for ChIP-qPCR

Genes	Forward Primers	Reverse Primers
HoxA1	GCAGGACAAGGTTGATGGG	GCAGGTGGGAGGGACAGAT
HoxA3	GGACAGACTCGGTGGTAAGA	AGTTCATGTTCACGGTTCCTAT
HoxA9	GCAGGAAACACTTTGCCAGA	GCCCGAGTTAGGACCCGTA
HoxA10	CTCCTTGCCTCCTTCTTCC	CCTGGGTATCTGAGCATCTAA
HoxB3	CCGAGGACGGACCGAAGAT	CCCTGAACTGGACCACCAT
HoxB4	GAAGAACGCACGGAAAGTAAG	GGGAAAGAATATGAGCGGAGT
HoxB7	CCTTAGGGACGCCTTGGTC	ACGCAGGGATTGAATGTTCG

HoxB8	GCCATTGAATTTCTATCCCAC	GGTGAGGCAAGCTAAAGCAG
HoxC6	CTTCTCCTCTGCCCTCTTC	GTTAGTTAATACATGGACCTCT
HoxC8	GTCGTGGATTGATGAACGCG	TCTGCTCACTGTCGGTAGG
HoxC9	TGTGCCTTGAGTCACTTTGC	CTTGCTCCACTTCTCCAGAT
HoxC10	TTTTCTTTGGGTCCTCGTAAA	AGTCTAGGGAGCCATTTGTC
HoxD3	TTTTCCGAGTCCTATTGCTTG	CTGTATCATCTGCCCTCTATC
HoxD8	AGGACTTTGATTCGCTTTGATA	CGAGGTTGACGGATTGATTG
HoxD9	AACCTACCCTCGGAGATGC	GCACTGGAGTCCCAAGGAG
HoxD10	GGAGGGATGTTTCCGAACT	CACATACCCAGGCAGAACG

Figure 1. *Hox* genes up-regulated in pons of *Rnf220*$^{+/-}$ mice. (**A**) The heatmap of RNA-seq data showing *Hox* genes expression in pons of WT or *Rnf220*$^{+/-}$ mice (n=2 mice per group). (**B-C**) UMAP diagram showing 15 identified cell clusters annotated by snRNA-seq analysis of pons. Each dot represents a single cell, and cells are laid out to show similarities (n=3 mice per group). (**D**) Heatmap of snRNA-seq data showing *Hox* expression changes in each cell cluster. (**E**) Quantitative real time polymerase chain reaction (*qRT-PCR*) analysis showing mRNA levels of indicated *Hox* genes in P19 cells when endogenous *Rnf220* was knocked down by siRNAs in the presence of RA. WT, wild-type. HE, heterozygote. RA, retinoic acid. $^{**}p < 0.01$,

***$p < 0.001$.

Figure 2. *Hox* gene expression was dysregulated and motor cortex projections were disorganized in PN of *Rnf220*$^{+/-}$ mice. (**A**) qRT-PCR analysis of relative expression levels of *Hox3*, *Hox4*, and *Hox5* in rostral, middle, and caudal sections of PN in WT and *RNF220*$^{+/-}$ mice. Expression level of each gene in rostral section of WT PN was set to 1. (n=5 mice per group). Bar graphs show the relative levels normalized against rostral group in the respective wild-type mice. (**B**) Diagram of experimental stereotactic injections. (**C**) Green fluoresce showed the projection from motor cortex to PNs in adult (2 months) WT (n=10 mice) and *Rnf220*$^{+/-}$ mice (n=9 mice). (**D-E**)

The diameter (D) and area (E) sizes of PN in WT and *Rnf220*$^{+/-}$ mice. Each data presents the average diameter and area sizes of PN from four consecutive slices for completely presenting circular fluorescence projections. (**F**) The area of fluorescence projection from motor cortex to PN in WT and *Rnf220*$^{+/-}$ mice. The sample used for statistics is consistent with the one selected in Figure D-E. Each data represents the average fluorescence area of four consecutive slices. (**G**) The proportion of projected-fluorescence area from motor cortex to PN area. The sample used for statistics is consistent with the one selected in Figure D-E. WT, wild-type. HE, heterozygote. n.s., not significant. $^{**}p < 0.01$.

Figure 3. RNF220 mediates WDR5 degradation. (**A-D**) Western blots analysis showing the protein level of WDR5 in the indicated brain tissues of mice with different genotypes at different ages. (**E**) Western blots showed WDR5 levels of in the pons of adult mice with indicated genotypes. **(F)** Western blot analysis of protein levels of WDR5 in P19 cells with *Rnf220* knockdown or not in the presence or absence of RA. IB, immunoblot. WT, wild-type. HE, heterozygote. KO, knockout. PN, pontine nuclei. NC, negative control. RA, retinoic acid.

Figure 4. RNF220 interacts with and targets WDR5 for K48-linked polyubiquitination. (**A-B**) Co-immunoprecipitation (co-IP) analysis of interactions between RNF220 and WDR5 in HEK293 cells. HEK293 cells were transfected with indicated plasmids and harvested after 48 h. Cell lysates were immunoprecipitated with anti-FLAG beads. Whole-cell lysate and immunoprecipitates were subjected to western blot analysis using indicated antibodies. (**C**) Endogenous co-immunoprecipitation analysis showing the interaction between RNF220 and WDR5 in hindbrains of WT mice. (**D**) Western blots analysis shows the protein level

of WDR5 when co-expressed with wild-type or mutated RNF220 in HEK293 cells. (**E**) Western blots analysis shows the protein level of WDR5 when co-expressed with RNF220 in HEK293 cells in the presence of MG132 (10 mM) or not. (**F**) *In vivo* ubiquitination assays showing the ubiquitination status of WDR5 when co-expressed with WT or mutated RNF220 in HEK293 cells. (**G**) *In vivo* ubiquitination assays showing the ubiquitination status of WDR5 in hindbrains of WT and *Rnf220*$^{+/-}$ mice. (**H**) *In vivo* ubiquitination assays showing RNF220-induced polyubiquitination of WDR5 when the indicated ubiquitin mutations were used in HEK293 cells. (**I**) *In vivo* ubiquitination assays showing the ubiquitination status of the indicated WDR5 mutants when co-expressed with WT or ligase dead RNF220 in HEK293 cells. WT, wild-type. HE, heterozygote. KO, knockout. IB, immunoblot. IP, immunoprecipitation. UB, ubiquitin. WCL, whole cell lysate. △Ring, RNF220 Ring domain deletion. W539R, RNF220 ligase dead mutation. K48, ubiquitin with all lysines except the K48 mutated to arginine. K48R, ubiquitin with the K48 was substituted by an arginine. 3KR, substitution of lysines at the positions of 109, 112, and 120 in WDR5 with arginines simultaneously.

Figure 5. WDR5 recovered *Rnf220* deficiency-induced up-regulation of *Hox* genes in P19 cell line. (**A-B**) qRT-PCR analysis showing the expression levels of *Rnf220* (A) and *Wdr5* (B) when transfected the indicated combinations of siRNAs against *Rnf220* or *Wdr5* in the presence or absence of RA. Bar graphs show the relative levels normalized against control group without siRNA or RA treatment. **(C)** qRT-PCR analysis showing the expression levels of *HoxA1*, *HoxB1*, *HoxA9*, *HoxB9* when transfected siRnf220 or both siRnf220 and siWdr5 when treated with RA. RA:

retinoic acid. n.s., not significant. ***$p < 0.001$.

Figure 6. Genetic and pharmacological ablation of WDR5 recovered *Rnf220* deficiency-induced up-regulation of *Hox* genes. (**A**) Diagram of experimental strategy for *in utero* local injection of WDR5 inhibitors. (**B-C**) qRT-PCR analysis of expression levels of *Rnf220* (B), *Hox3-Hox5* (C) in hindbrains of WT and *Rnf220*$^{+/-}$ mouse embryos treated with WDR5 inhibitors or not at E18.5 (n=3 mice per group). Actin was used as the internal controls. Heatmap of *Hox* expression showed the relative levels normalized against WT group. (**D-E**) qRT-PCR analysis of expression

levels of *Rnf220* (D), *Wdr5* (D), *Hox3-Hox5* (E) in pons of P15 mice with indicated genotypes (n=2 mice per group). GAPDH was used as the internal controls. Heatmap of *Hox* expression showed the relative levels normalized against WT group. WT, wild-type. HE, heterozygote.

Figure 1-figure supplement 1. *Hox* genes exhibited de-repression in hindbrain of *Rnf220*$^{-/-}$ embryos at late developmental stages. Heatmap of *Hox* expression in hindbrain of *Rnf220*$^{-/-}$ mouse embryos at E16.5 and E15.5. Expression of each *Hox* gene was analyzed by qRT-PCR against GAPDH. Each *Hox* gene in wild-type controls was set to 1 (n=2 mice per group). Blank color represents the expression of that *Hox* is low and exceed detection range. KO, knockout.

Figure 1-figure supplement 2. *Hox* genes were up-regulated in brainstem of

Rnf220$^{+/-}$ mice. (**A**) Diagram of central neural system (CNS) in mice. (**B**) qRT-PCR analysis of *Rnf220* expression in CNS of *Rnf220*$^{+/-}$ and control mice. (**C-D**) qRT-PCR analysis of *HoxA9* (C) and *HoxB9* (D) expression in each CNS section of *Rnf220*$^{+/-}$ and control mouse (n=2 mice per group). (**E**) Diagram of mouse brain stem. (**F**) qRT-PCR analysis of mRNA levels of *HoxA9* and *HoxB9* in indicated sections of WT and *Rnf220*$^{+/-}$ mouse brainstems (n=2 mice per group). WT, wild-type. HE, heterozygote. SC, spinal cord. n.s., not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 1-figure supplement 3. Expression levels of *Hox* genes were not affected by *Rnf220* knockdown in P19 cell line without RA induction. (**A**) qRT-PCR analysis of *Rnf220* and indicated *Hox* genes expression after RA treatment in P19 cells. (**B**) qRT-PCR analysis the expression levels of *HoxA1*, *HoxB1*, *HoxA9*, and *HoxB9* in *Rnf220* knockdown P19 cells without RA induction. RA: retinoic acid. n.s., not significant. ***$p < 0.001$.

Figure 2-figure supplement 1. *Hox* genes were up-regulated in PN of *Rnf220*$^{+/-}$ mice. (**A**) Heatmap of snRNA-seq data showing expression levels of PN markers (*Pax6, Barhl1, Unc5b*, and *Nfib*) among 15 identified cell clusters (n= 3 mice per group). (**B-C**) qRT-PCR analysis of expression levels of indicated PN markers (B) and *Hox* genes (C) in pons of *Rnf220*$^{+/-}$ and WT mice (n= 3 mice per group). WT, wild-type. HE, heterozygote. n.s., not significant. *$p < 0.05$, ***$p < 0.001$.

Figure 2-figure supplement 2. PN showed no structure difference but disorganized projection pattern from motor cortex between WT and *Rnf220*$^{+/-}$ mice. (**A**) Nissl staining showed the pons structure in adult (2 months) WT and *Rnf220*$^{+/-}$ mice. Scale bars, 500 μm. (**B**) DAPI labeling PN structure in adult (2 months) WT and *Rnf220*$^{+/-}$ mice. Scale bars, 500 μm. (**C**) Continuous slice of projection from motor cortex to PN in adult (2 months) WT and *Rnf220*$^{+/-}$ mice. Scale bars, 200 μm. WT, wild-type. HE, heterozygote. PN, pontine nuclei.

Figure 3-figure supplement 1. Repressive epigenetic modification was down-regulated while activated epigenetic modification was up-regulated in promoter regions of indicated *Hox* genes in hindbrains of *Rnf220*$^{+/-}$ mice. **(A-B)** ChIP-qRT-PCR analysis of repressive epigenetic modification (H3K27me3) (A) and activated epigenetic modification (H3K4me3) (B) levels in promoter regions of indicated *Hox*

genes in hindbrains of *Rnf220*$^{+/-}$ and WT mice (n=2 mice per group). WT, wild-type. HE, heterozygote. n.s., not significant. *p< 0.05, **p < 0.01, ***p < 0.001.

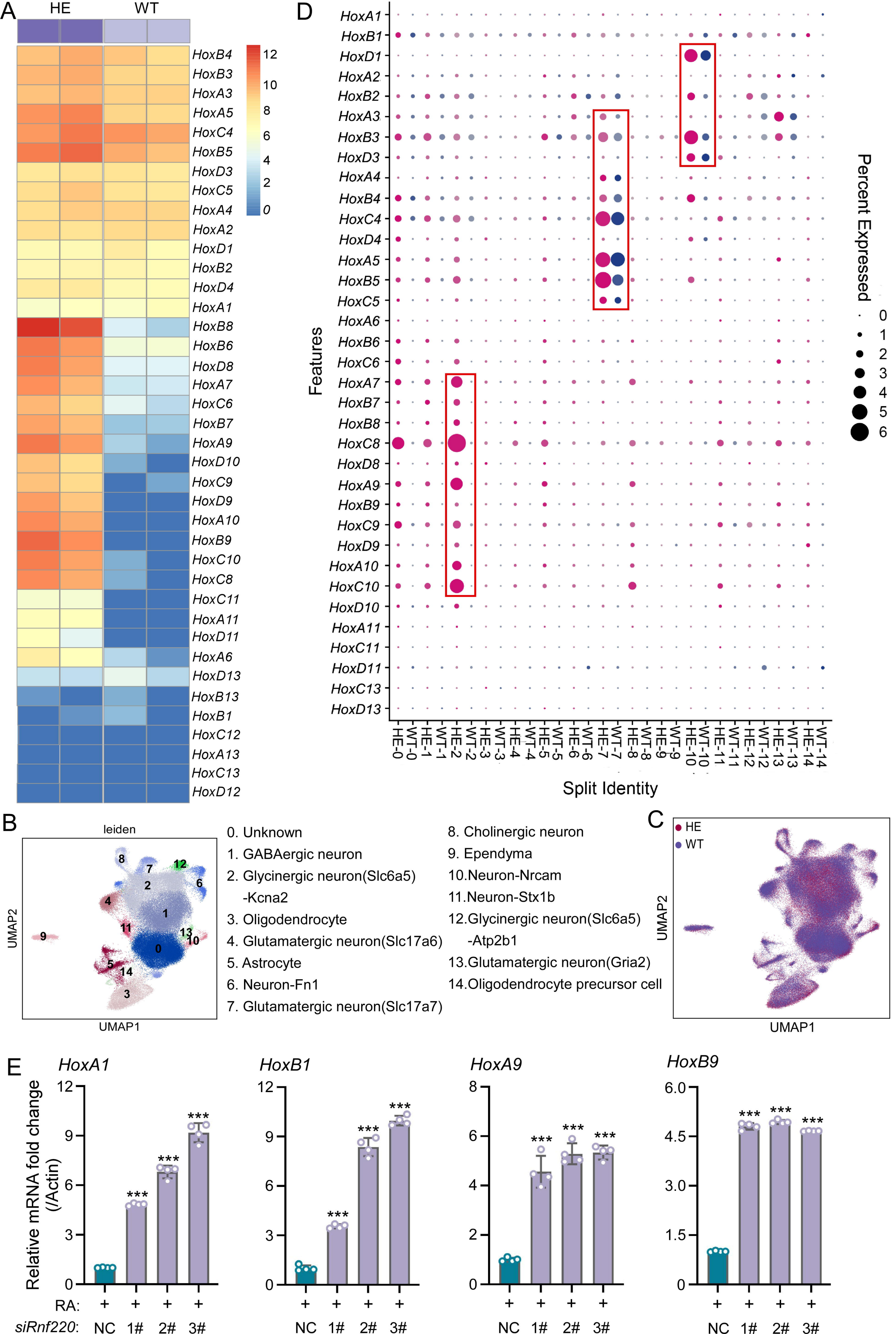
Figure 3-figure supplement 2. Protein levels of the indicated core components of PRC1 and PRC2 complex in indicated mouse brain tissues of different genotypes. (**A**) Western blot analysis of protein levels of core components of PRC1 and PRC2 complexes in hindbrain of WT, *Rnf220*$^{+/-}$, and *Rnf220*$^{-/-}$ mouse embryos at E18.5. (**B**) Western blot analysis of protein levels of core components of PRC1 and PRC2 complexes in pons of adult *Rnf220*$^{+/-}$ and WT mice. (**C, D**) Western blot analysis of protein levels of WDR5 in cerebellum and cortex of adult *Rnf220* $^{+/-}$ and WT mice. WT, wild-type; HE, heterozygote; KO, knockout; IB, immunoblot.

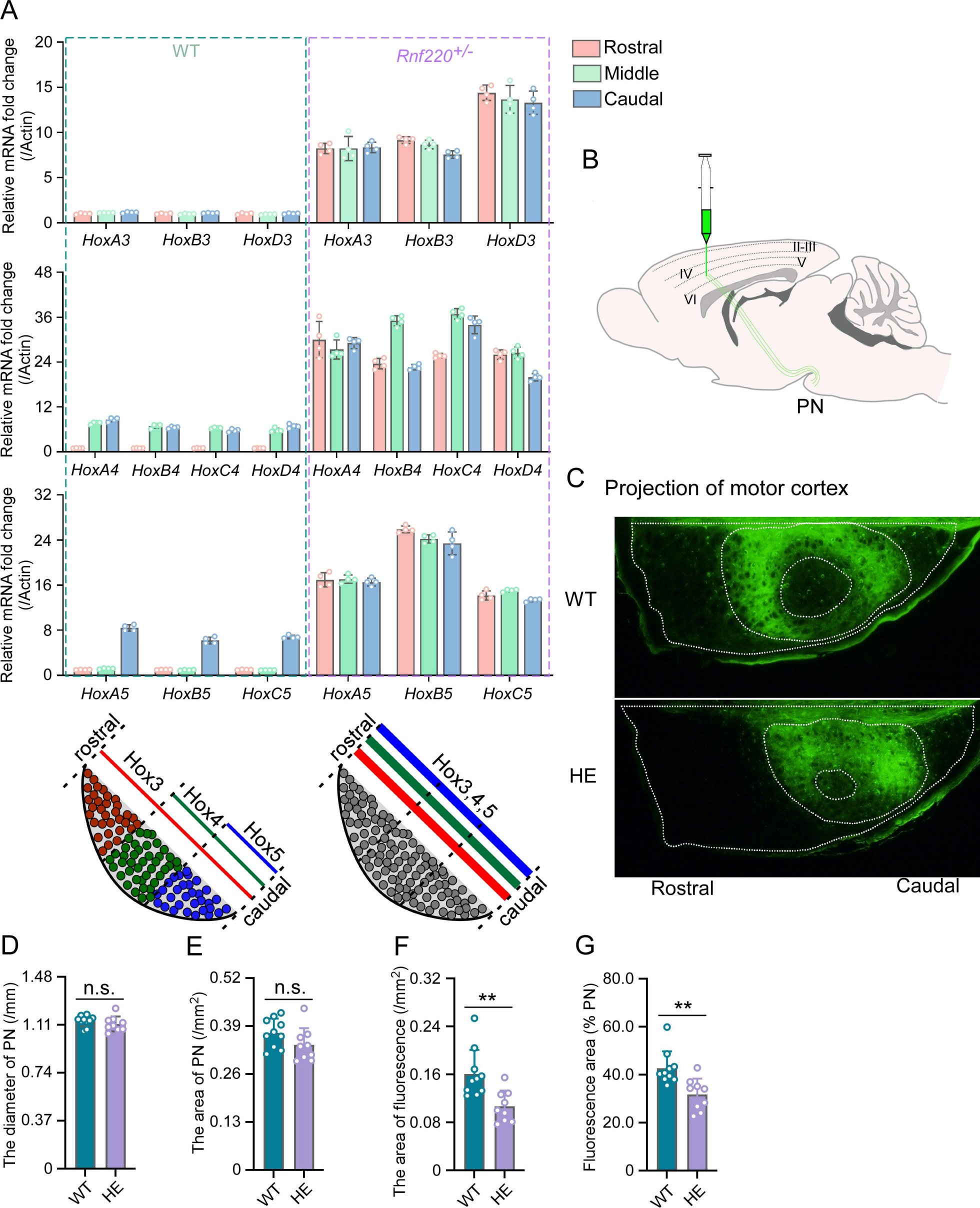
Figure 4-figure supplement 1. RNF220 interacted with and targeted WDR5 for polyubiquitination at multiple lysine sites. (**A**) Western blot analysis the levels of three WDR5 truncated proteins in HEK293 cells when co-transfected with RNF220 or not. (**B**) *In vivo* ubiquitination analysis of ubiquitination status of indicated WDR5 KR mutants when co-expressed with RNF220 or not in HEK293 cells. IB, immunoblot. IP, immunoprecipitation. WCL, whole cell lysate. K31R, K52R, K109R, K112R, K120R, K123R, or K126R, substitution of lysine with arginine in WDR5 at indicated positions.

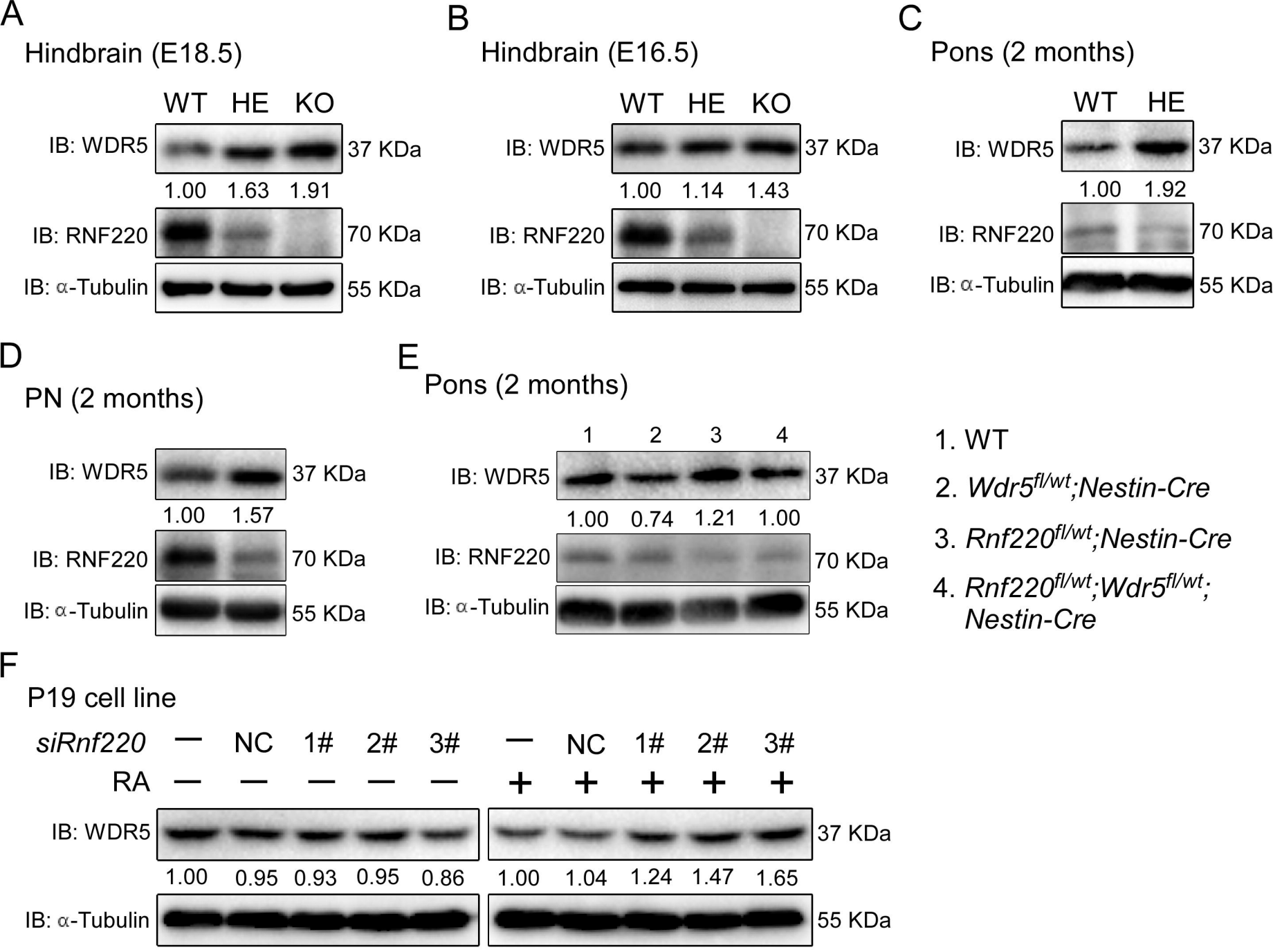
Figure 5-figure supplement 1. WDR5 knockdown or without RA treatment had no effect on *Hox* genes in P19 cell line. (**A**) qRT-PCR analysis showing the expression levels of *HoxA1*, *HoxB1*, *HoxA9*, *HoxB9* when transfected siRnf220 or both siRnf220 and siWdr5 without RA treatment. (**B**) qRT-PCR analysis of mRNA levels of *Wdr5*, *HoxA1*, *HoxB1*, *HoxA9*, and *HoxB9* when *Wdr5* was knocked down by siRNA transfection in P19 cells with or without RA treatment. Bar graphs show the relative levels normalized against control group without siRNA or RA treatment. RA: retinoic acid. n.s., not significant. *p< 0.05.

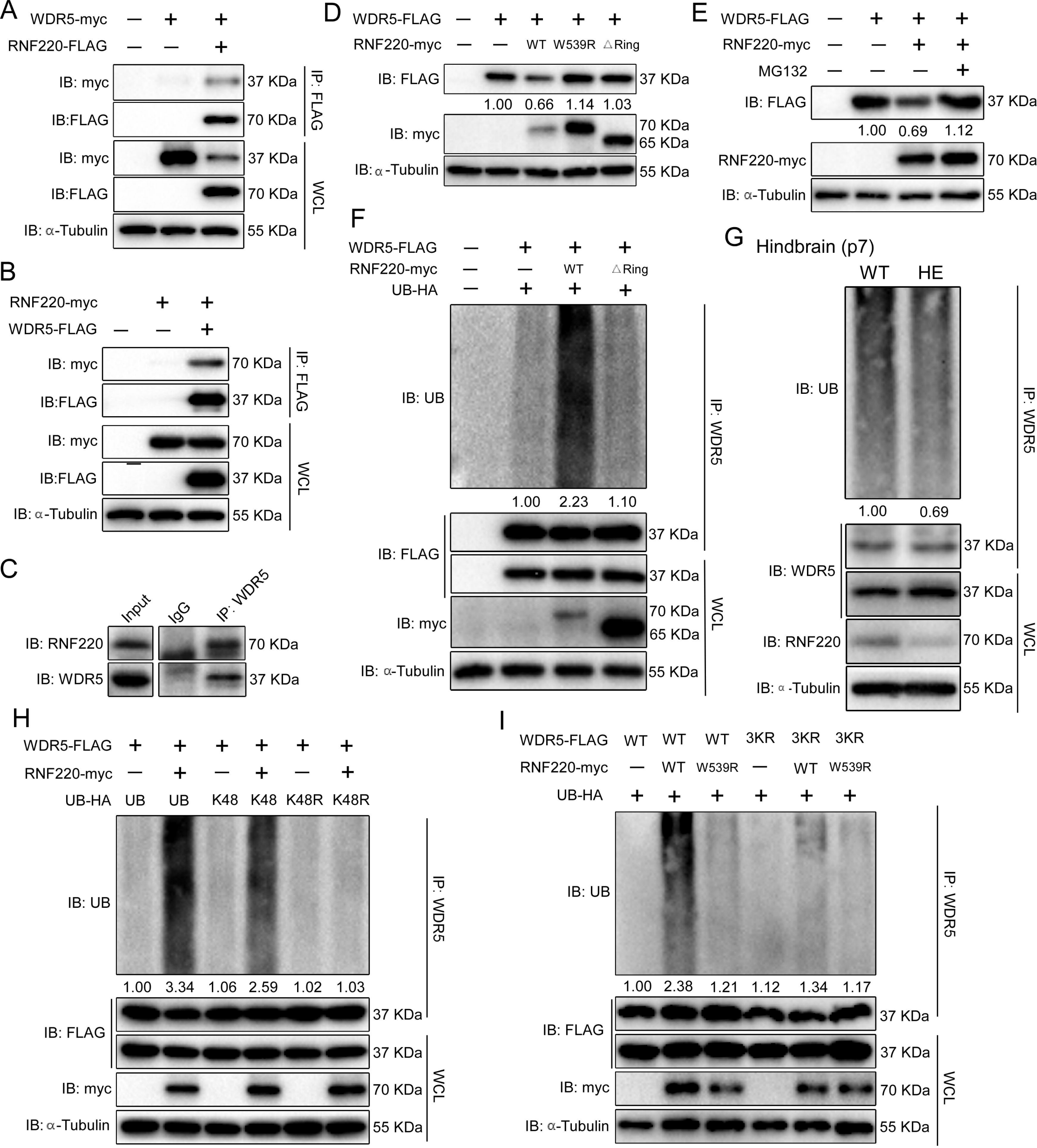
Figure 6-figure supplement 1. *Rnf220* and *Wdr5* co-suppression recovered *Hox* epigenetic modification to a certain degree. (**A-B**) ChIP-qRT-PCR analysis of repressive epigenetic modification (H3K27me3) (A) and activated epigenetic modification (H3K4me3) (B) levels in promoter regions of indicated *Hox* genes in P19 cell line transfected with siRnf220 or both siRnf220 and siWdr5. n.s., not

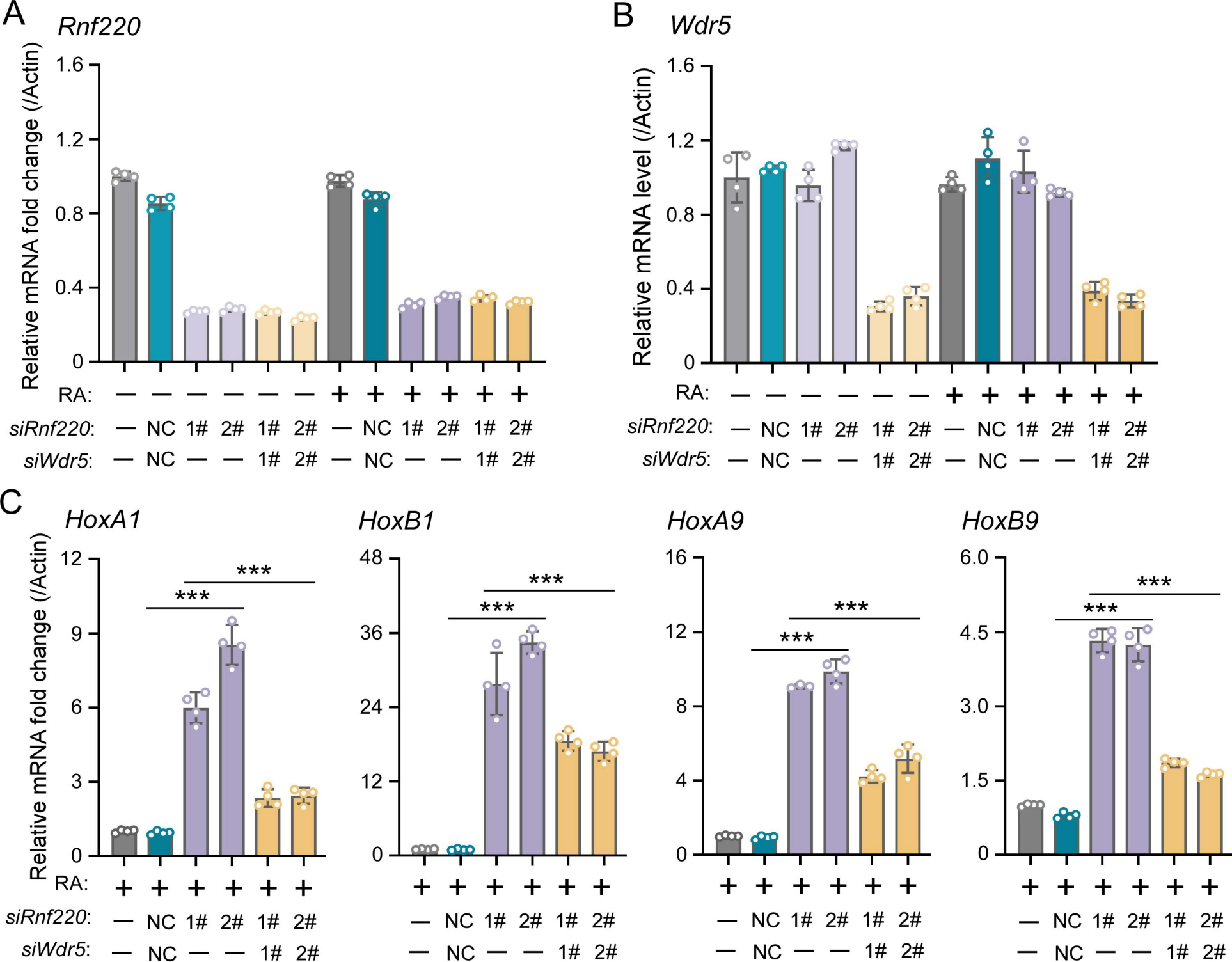
significant. *$p < 0.05$, **$p < 0.01$.

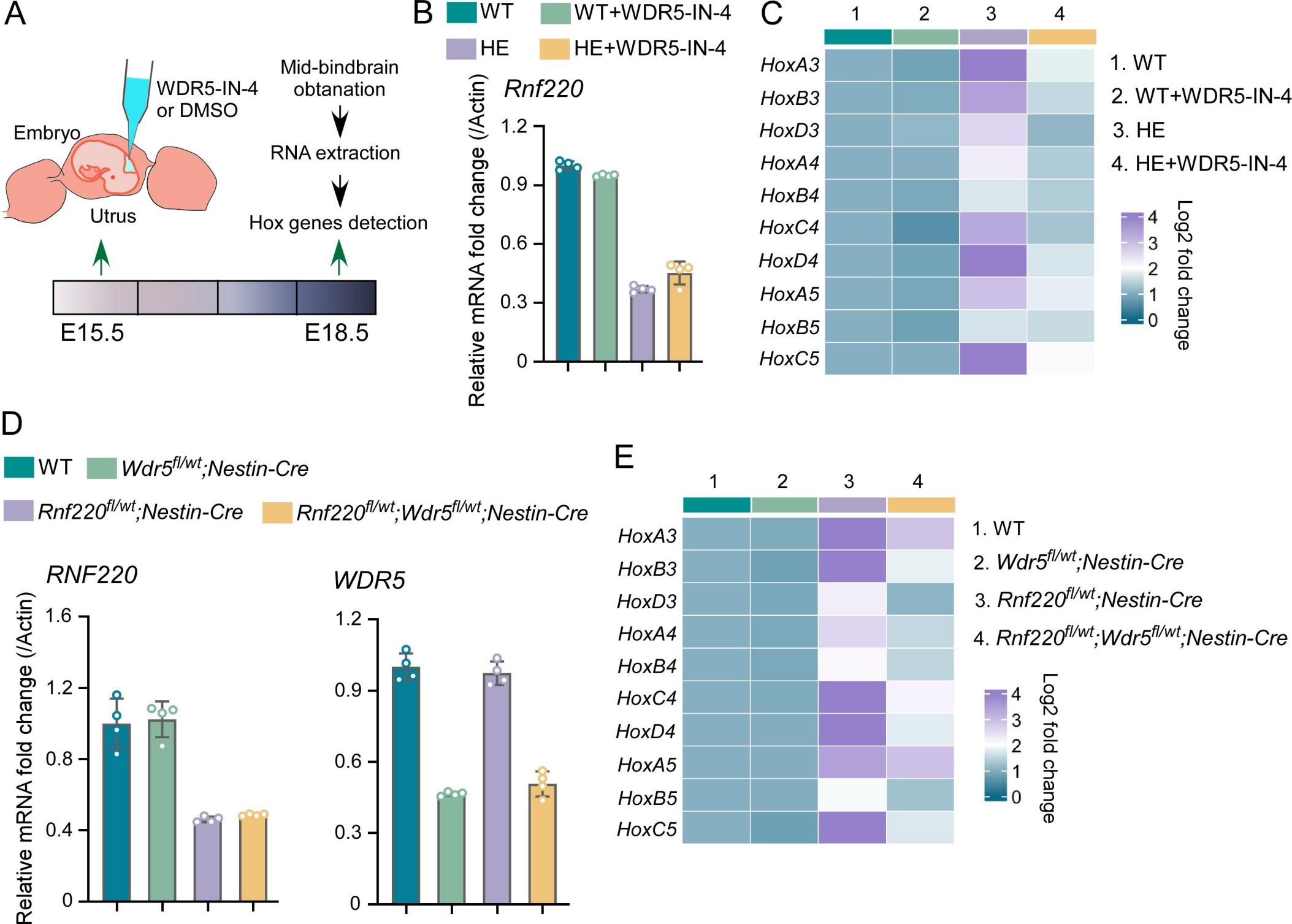

A
HE
WT
HoxB4
HoxB3
HoxA3
HoxA5
HoxC4
HoxB5
HoxD3
HoxC5
HoxA4
HoxA2
HoxD1
HoxB2
HoxD4
HoxA1
HoxB8
HoxB6
HoxD8
HoxA7
HoxC6
HoxB7
HoxA9
HoxD10
HoxC9
HoxD9
HoxA10
HoxB9
HoxC10
HoxC8
HoxC11
HoxA11
HoxD11
HoxA6
HoxD13
HoxB13
HoxB1
HoxC12
HoxA13
HoxC13
HoxD12
12
10
8
6
4
2
0
D
Features
Split Identity
Percent Expressed
0
1
2
3
4
5
6
B
leiden
UMAP2
UMAP1
0. Unknown
1. GABAergic neuron
2. Glycinergic neuron(Slc6a5)
-Kcna2
3. Oligodendrocyte
4. Glutamatergic neuron(Slc17a6)
5. Astrocyte
6. Neuron-Fn1
7. Glutamatergic neuron(Slc17a7)
8. Cholinergic neuron
9. Ependyma
10.Neuron-Nrcam
11.Neuron-Stx1b
12.Glycinergic neuron(Slc6a5)
-Atp2b1
13.Glutamatergic neuron(Gria2)
14.Oligodendrocyte precursor cell
C
HE
WT
UMAP2
UMAP1
E
HoxA1
HoxB1
HoxA9
HoxB9
Relative mRNA fold change
(/Actin)
RA:
siRnf220:
NC
1#
2#
3#
***


A
WT
Rnf220+/-
Rostral
Middle
Caudal
Relative mRNA fold change (/Actin)
HoxA3
HoxB3
HoxD3
HoxA4
HoxB4
HoxC4
HoxD4
HoxA5
HoxB5
HoxC5
B
II-III
V
IV
VI
PN
C
Projection of motor cortex
WT
HE
Rostral
Caudal
rostral
Hox3
Hox4
Hox5
caudal
Hox3,4,5
D
The diameter of PN (/mm)
n.s.
E
The area of PN (/mm2)
n.s.
F
The area of fluorescence (/mm2)
**
G
Fluorescence area (% PN)
**
WT
HE


A
Hindbrain (E18.5)
WT HE KO
IB: WDR5
37 KDa
1.00 1.63 1.91
IB: RNF220
70 KDa
IB: α-Tubulin
55 KDa
B
Hindbrain (E16.5)
WT HE KO
IB: WDR5
37 KDa
1.00 1.14 1.43
IB: RNF220
70 KDa
IB: α-Tubulin
55 KDa
C
Pons (2 months)
WT HE
IB: WDR5
37 KDa
1.00 1.92
IB: RNF220
70 KDa
IB: α-Tubulin
55 KDa
D
PN (2 months)
IB: WDR5
37 KDa
1.00 1.57
IB: RNF220
70 KDa
IB: α-Tubulin
55 KDa
E
Pons (2 months)
1 2 3 4
IB: WDR5
37 KDa
1.00 0.74 1.21 1.00
IB: RNF220
70 KDa
IB: α-Tubulin
55 KDa
1. WT
2. Wdr5fl/wt;Nestin-Cre
3. Rnf220fl/wt;Nestin-Cre
4. Rnf220fl/wt;Wdr5fl/wt;Nestin-Cre
F
P19 cell line
siRnf220 — NC 1# 2# 3# — NC 1# 2# 3#
RA — — — — — + + + + +
IB: WDR5
37 KDa
1.00 0.95 0.93 0.95 0.86 1.00 1.04 1.24 1.47 1.65
IB: α-Tubulin
55 KDa


A
WDR5-myc
RNF220-FLAG
IB: myc
IB:FLAG
IB: α-Tubulin
37 KDa
70 KDa
55 KDa
IP: FLAG
WCL
B
RNF220-myc
WDR5-FLAG
IB: myc
IB:FLAG
IB: α-Tubulin
70 KDa
37 KDa
55 KDa
IP: FLAG
WCL
C
Input
IgG
IP: WDR5
IB: RNF220
IB: WDR5
70 KDa
37 KDa
D
WDR5-FLAG
RNF220-myc
WT
W539R
△Ring
IB: FLAG
IB: myc
IB: α-Tubulin
37 KDa
1.00 0.66 1.14 1.03
70 KDa
65 KDa
55 KDa
E
WDR5-FLAG
RNF220-myc
MG132
IB: FLAG
RNF220-myc
IB: α-Tubulin
37 KDa
1.00 0.69 1.12
70 KDa
55 KDa
F
WDR5-FLAG
RNF220-myc
WT
△Ring
UB-HA
IB: UB
IB: FLAG
IB: myc
IB: α-Tubulin
1.00 2.23 1.10
37 KDa
70 KDa
65 KDa
55 KDa
IP: WDR5
WCL
G
Hindbrain (p7)
WT
HE
IB: UB
IB: WDR5
IB: RNF220
IB: α-Tubulin
1.00 0.69
37 KDa
70 KDa
55 KDa
IP: WDR5
WCL
H
WDR5-FLAG
RNF220-myc
UB-HA
UB
K48
K48R
IB: UB
IB: FLAG
IB: myc
IB: α-Tubulin
1.00 3.34 1.06 2.59 1.02 1.03
37 KDa
70 KDa
55 KDa
IP: WDR5
WCL
I
WDR5-FLAG
RNF220-myc
UB-HA
WT
3KR
W539R
IB: UB
IB: FLAG
IB: myc
IB: α-Tubulin
1.00 2.38 1.21 1.12 1.34 1.17
37 KDa
70 KDa
55 KDa
IP: WDR5
WCL


A
Rnf220
Relative mRNA fold change (/Actin)
1.6
1.2
0.8
0.4
0
RA: — — — — — — + + + + + +
siRnf220: — NC 1# 2# 1# 2# — NC 1# 2# 1# 2#
siWdr5: — NC — — 1# 2# — NC — — 1# 2#
B
Wdr5
Relative mRNA level (/Actin)
1.6
1.2
0.8
0.4
0
RA: — — — — — — + + + + + +
siRnf220: — NC 1# 2# 1# 2# — NC 1# 2# 1# 2#
siWdr5: — NC — — 1# 2# — NC — — 1# 2#
C
HoxA1
HoxB1
HoxA9
HoxB9
Relative mRNA fold change (/Actin)
12
9
6
3
0
48
36
24
12
0
16
12
8
4
0
6.0
4.5
3.0
1.5
0
***
RA: + + + + + +
siRnf220: — NC 1# 2# 1# 2#
siWdr5: — NC — — 1# 2#


A
WDR5-IN-4
or DMSO
Embryo
Utrus
Mid-bindbrain
obtanation
RNA extraction
Hox genes detection
E15.5
E18.5
B
WT
WT+WDR5-IN-4
HE
HE+WDR5-IN-4
Rnf220
Relative mRNA fold change (/Actin)
1.2
0.9
0.6
0.3
0
C
1
2
3
4
HoxA3
HoxB3
HoxD3
HoxA4
HoxB4
HoxC4
HoxD4
HoxA5
HoxB5
HoxC5
1. WT
2. WT+WDR5-IN-4
3. HE
4. HE+WDR5-IN-4
Log2 fold change
4
3
2
1
0
D
WT
Wdr5fl/wt;Nestin-Cre
Rnf220fl/wt;Nestin-Cre
Rnf220fl/wt;Wdr5fl/wt;Nestin-Cre
RNF220
WDR5
Relative mRNA fold change (/Actin)
1.6
1.2
0.8
0.4
0
1.2
0.9
0.6
0.3
0
E
1
2
3
4
HoxA3
HoxB3
HoxD3
HoxA4
HoxB4
HoxC4
HoxD4
HoxA5
HoxB5
HoxC5
1. WT
2. Wdr5fl/wt;Nestin-Cre
3. Rnf220fl/wt;Nestin-Cre
4. Rnf220fl/wt;Wdr5fl/wt;Nestin-Cre
Log2 fold change
4
3
2
1
0