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2 **Pax3- and Pax7-mediated Dbx1 regulation orchestrates the**
3 **patterning of intermediate spinal interneurons**

4

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23 **Abstract**

24 Transcription factors are key orchestrators of the emergence of neuronal diversity within the
25 developing spinal cord. As such, the two paralogous proteins Pax3 and Pax7 regulate the
26 specification of progenitor cells within the intermediate neural tube, by defining a neat
27 segregation between those fated to form motor circuits and those involved in the integration of
28 sensory inputs. To attain insights into the molecular means by which they control this process,
29 we have performed detailed phenotypic analyses of the intermediate spinal interneurons (IN),
30 namely the dI6, V0_D, V0_{VCG} and V1 populations in compound null mutants for Pax3 and Pax7.
31 This has revealed that the levels of Pax3/7 proteins determine both the dorso-ventral extent and
32 the number of cells produced in each subpopulation; with increasing levels leading to the
33 dorsalisation of their fate. Furthermore, thanks to the examination of mutants in which Pax3
34 transcriptional activity is skewed either towards repression or activation, we demonstrate that
35 this cell diversification process is mainly dictated by Pax3/7 ability to repress gene expression.
36 Consistently, we show that Pax3 and Pax7 inhibit the expression of *Dbx1* and of its repressor
37 *Prdm12*, fate determinants of the V0 and V1 interneurons, respectively. Notably, we provide
38 evidence for the activity of several *cis*-regulatory modules of *Dbx1* to be sensitive to Pax3 and
39 Pax7 transcriptional activity levels. Altogether, our study provides insights into how the
40 redundancy within a TF family, together with discrete dynamics of expression profiles of each
41 member, are exploited to generate cellular diversity. Furthermore, our data supports the model
42 whereby cell fate choices in the neural tube do not rely on binary decisions but rather on
43 inhibition of multiple alternative fates.

44

45 **Highlights**

- 46 • Pax3 and Pax7 control the spatial organisation of spinal differentiation
- 47 • Pax3 and Pax7 repressive activity spatially restricts Dbx1 and Prdm12 expression
- 48 • An intronic *cis*-regulatory module recapitulates *Dbx1* expression
- 49 • The *Dbx1* intronic *cis*-regulatory module is sensitive to Pax3/7 transcriptional activity

50 **Keywords**

51 Neuronal patterning; Pax3/Pax7 transcription factors; Dbx1; repression; spinal cord
52 interneurons; *cis*-regulatory modules.

53 **Introduction**

54 The developing vertebrate neural tube is a classical and well-studied model to address how cell
55 diversity is generated and organised within a tissue. The stereotypical arrangements and
56 numbers of neuronal subtypes composing the adult spinal cord emanate from two rounds of
57 cell-fate choices operating during embryogenesis (Lai et al., 2016; Lu et al., 2015). During the
58 first phase, “naïve” progenitor cells acquire distinct molecular signatures depending on their
59 position along the dorsal-ventral (D-V) axis of the neural tube (Del Corral and Storey, 2004;
60 Lai et al., 2016; Lu et al., 2015). These signatures are then further variegated when cells undergo
61 post-mitotic differentiation. Ultimately, cells will express unique sets of genes imposing
62 stereotypic physiological and morphological traits upon each neuronal subtype. This process of
63 cellular determination is largely controlled by transcription factors (TFs) (Lai et al., 2016; Lu
64 et al., 2015). These proteins operate at the level of non-coding *cis*-regulatory modules (CRMs)
65 where they recruit factors impacting on the activity of the basal transcriptional machinery
66 and/or the chromatin state. Thereby, their activity underpins the overall levels of gene activation
67 or repression within cells (Spitz and Furlong, 2012). Although the extent of TF diversity within
68 the developing vertebrate neural tube can now be estimated (e.g. Armant et al., 2013, see also
69 Supp. Table 4), how their activity at the level of CRMs generates robust gene expression
70 profiles and cellular phenotypes is still poorly defined and hence remains under intensive
71 investigation (e.g. Bailey et al., 2006; Borromeo et al., 2014; Kutejova et al., 2016; Mazzoni et
72 al., 2013).

73

74 To delve into this issue, we focussed on the activity of two paralogous and highly related
75 proteins, Pax3 and Pax7 (Mayran et al., 2015). These TFs are amongst the first to be induced
76 within the dorsal part of the developing spinal cord; in mouse, Pax3 is induced by gestational
77 day (GD) 8, while Pax7 appears 24 hours later (Goulding et al., 1991; Jostes et al., 1990). It has

78 been known for a long time that these factors are essential to spinal cord development and
79 control several key cellular processes ranging from neural tube closure to timing of post-mitotic
80 differentiation (e.g. Epstein et al., 1991; Nakazaki et al., 2008). In addition, several studies have
81 led to the idea that these factors orchestrate cell type specification not only within the dorsal
82 neural tube where they are expressed (Moore et al., 2013; Nakazaki et al., 2008) but also within
83 the intermediate neural tube (Mansouri and Gruss, 1998). In this region, 3 adjacent progenitor
84 pools, dp6, p0 and p1 (from dorsal to ventral), are fated to become dI6, V0 and V1 interneurons
85 (IN), respectively (Lu et al., 2015). The V1 and V0 IN populations are further diversified into
86 discrete neuronal subtypes. For instance, the most dorsal p0 progenitors give rise to two GABA-
87 and glycine-secreting populations, termed V0_D and V0_V IN, while the ventral p0 progenitors
88 give rise to two excitatory neuronal subtypes, the cholinergic V0_C and glutamatergic V0_G IN
89 (Lu et al., 2015). Within these populations, it was previously shown that the position and the
90 number of V1 and V0_{VCG} IN are regulated by Pax3 and Pax7 activity; in their absence, the V1
91 IN are produced in excess and are intermingled with V0 IN (Mansouri and Gruss, 1998).
92 Whether Pax3 and Pax7 regulate the fate of the other intermediate cell types remains to be
93 tested and the molecular mechanisms by which they repress ventral fates needs to be
94 established. Notably, it is still unclear whether they are able to directly repress the expression
95 of ventral specifiers. Indeed, even though both Pax3 and Pax7 possess an octapeptide motif
96 capable of interactions with the Groucho/TLE co-repressors (Muhr et al., 2001), most studies
97 suggest that they act as transcriptional activators. For instance, Pax3 and Pax7 have been shown
98 to be necessary to promote the activity of CRMs regulating the expression of key patterning
99 genes such as Pax3 itself or Neurog2 (Moore et al., 2013; Nakazaki et al., 2008). Further
100 supporting this, the expression of a single functional allele of Pax3 encoding an activator form
101 of Pax3 is sufficient to rescue many of the morphological defects of Pax3 single mutants (Relaix
102 et al., 2003).

103 Altogether these data prompted us to assess the precise contribution of Pax3 and Pax7 to the
104 patterning of the intermediate neural tube and to test whether their active and/or repressive
105 transcriptional activity is involved in this process. To this end, we have analysed the distribution
106 of intermediate interneuron populations in mouse embryos carrying either compound null
107 mutations for these two genes or dominant active or repressive forms of Pax3. These analyses
108 revealed that the modulation of the levels and the type of transcriptional activity determines not
109 only the position but also the number of neurons generated. Furthermore, they led us to identify
110 Prdm12 and Dbx1 as target genes, the latter being capable of sensing the levels of Pax3 and
111 Pax7 transcriptional activity through several *cis*-regulatory modules.

112

113 **Material and Methods**

114 **Mouse lines and *in ovo* electroporation**

115 Mice carrying *Pax3* null, *PAX3A*, *Pax3R* and *Pax7* null alleles were previously described
116 (Bajard et al., 2006; Mansouri et al., 1996; Relaix et al., 2005, 2003). Notably, *PAX3A* and
117 *Pax3R* alleles encode for proteins made of Pax3 DNA-binding domains fused to either the
118 FOXO1 transactivation or Engrailed repressor domain. These are expressed from the *Pax3*
119 locus upon activation of the Cre recombinase driven by the zygote specific *PGK* enhancer
120 (Lallemand et al., 1998). Electroporation constructs expressing Pax3 and Pax7 variants and
121 Dbx1 from the *pCIG* vector engineered to bi-cistronically express nuclear-targeted GFP
122 (Megason and McMahon, 2002) were generated. The *CRM4.8kb*, which contains the *Dbx1*
123 minimal promoter and the *CRM4.8kb::iCRM* were cloned into a *pBluescript* vector, while
124 *CRM1* to *3* and the *iCRM* were inserted into a *bGZA* vector (Yee et al., 1993), upstream of a
125 minimal *HBB* promoter. For detailed cloning strategies see Supp. Material and Methods.
126 Reporter plasmids (0.5µg/µl) and *pCIG* based constructs (1.5 µg/µl) were electroporated in

127 Hamburger and Hamilton (HH) stage 10-11 chick embryos according to described protocols
128 (Briscoe et al., 2000).

129

130 **Immunohistochemistry and *in situ* hybridisation**

131 Mouse and chick embryos were fixed in 4% paraformaldehyde for 45 min to 2 hr at 4°C. Fixed
132 embryos were cryoprotected by equilibration in 15% sucrose, embedded in gelatin,
133 cryosectioned (14 µm), and processed for immunostaining (Briscoe et al., 2000) or *in situ*
134 hybridisation (ISH) (Yamada et al., 1993). Details of the reagents are provided in the Supp.
135 Material and Methods. All quantifications were performed at brachial levels using ImageJ
136 v.1.43 g image analysis software (NIH) on usually more than 3 embryos and on 2 to 4 transverse
137 sections per embryo (Supp. Table 1, Table 2). The number of cells per section was determined
138 using the cell counter plugin. Fluorescence intensity of β-Galactosidase and of GFP in chick
139 embryos and the evaluation of dorsal–ventral boundaries position were determined as described
140 in (Balaskas et al., 2012). The heat maps were generated using MATLAB (Mathworks, Natick,
141 MA) and correspond to the mean of the ratios of β-Galactosidase fluorescence intensity over
142 that of GFP on the number of sections and embryos indicated in Supp. Table 2. The heat maps
143 provide a color-coded scale for individual datasets by associating the lowest value in the data
144 matrix with dark blue and the highest value with dark red. All statistical analyses were
145 performed using a Mann-Whitney U test in GraphPad Prism and all the p-values are given in
146 Supp. Table 3.

147

148 ***In silico* analysis of *Dbx1* locus**

149 The mouse sequence of each CRM was used as the query sequence for cross-species BLAT
150 searches, performed with the Ensembl genome browser (<http://www.ensembl.org/>). The
151 alignments of enhancer sequences across anole lizard, cat, chicken, chimpanzee, Chinese soft-

152 shell turtle, cow, horse, human, mouse, rat, xenopus and zebrafish genomes were produced and
153 analysed using ClustalOmega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) and phylogenetically
154 conserved motifs were defined using MEME (<http://meme.ebi.edu.au/meme/tools/meme>).
155 Putative TF binding sites within the conserved motifs were identified using the Tomtom tool in
156 the MEME suite (<http://meme.ebi.edu.au/meme/tools/tomtom>). The alignments of enhancer
157 sequences were searched for putative Pax3 and Pax7 homeodomain and paired binding sites
158 using position weight matrices (PWMs) for these sites using FIMO ([http://meme-](http://meme-suite.org/tools/fimo)
159 [suite.org/tools/fimo](http://meme-suite.org/tools/fimo)). PWMs for Pax homeodomain binding sites were either taken from work
160 by (Jolma et al., 2013) or created *de novo* from Pax3 and Pax7 ChIP-Sequencing data
161 (Soleimani et al., 2012) using MEME (Supp. Fig. 3A, Supp. Material and Methods). To
162 characterise the functionality of Dbx1 CRMs, H3K27ac, H3K4me1, DNase-seq and enhancer-
163 like predictions from mouse GD11.5 and/or GD12.5 neural tube reference epigenome data were
164 obtained from the ENCODE data center (Encode project consortium, 2012). Data were
165 produced by the B. Ren (UCSD) and Z. Weng (Umass) laboratories and can be retrieved under
166 the ENCODE project accession numbers ENCSR111SYT, ENCSR362MIE, ENCSR003PNR,
167 ENCSR448TTC and ENCSR263CKR. Sox2 and Sox3 binding events in neural progenitors
168 were obtained from Bergsland et al. (2012). Pax7 binding events in muscle were obtained from
169 Soleimani et al. (2012). Processed data in mm9 assembly coordinates were transformed into
170 mm10 using the UCSC LiftOver tool.

171 **RESULTS**

172 **Modulations in the levels of Pax3 and Pax7 transcriptional activities impact on the** 173 **specification of intermediate spinal interneurons**

174 In order to analyse the fate of intermediate dp6 to p1 progenitors upon the modulation of Pax3
175 and Pax7 transcriptional activity, we analysed the expression profiles of several TFs allowing

176 the identification of dI6, V0_D, V0_{VCG} and V1 IN in several mutants for Pax3 and Pax7 (Fig.
177 1)(Lai et al., 2016; Lu et al., 2015). To distinguish dI6 IN we performed immunolabelling for
178 Lbx1 together with AP2 α and/or Brn3a; the dI6 express only Lbx1, while the abutting dorsal
179 dI5 population is Lbx1⁺, AP2 α ⁺, Brn3a⁺ (Supp. Fig. 1A-D, Fig. 1Ai, C). Detecting Pax2 and
180 Lbx1 allowed us to identify V0_D IN, as a Pax2⁺/Lbx1⁻ population ventrally flanking
181 Pax2⁺/Lbx1⁺ dI6 cells (Fig. 1Aviii, C). The identification of this V0_D population can be
182 facilitated by the additional labelling of Evx1, allowing to distinguish between Evx1⁻ V0_D and
183 Evx1⁺ V0_{VCG} IN populations (Fig. 1Aviii). Finally, V1 IN were observed by visualising FoxD3
184 expression (Fig. 1Axv, C).

185 These immunolabellings revealed that the generation of dI6 IN is not dramatically affected by
186 the loss of either Pax3 or Pax7 (Fig. 1Ai, ii, Bi and data not shown). However, their number
187 greatly decreases in Pax3^{-/-};Pax7^{+/-} embryos and even more in double knock-out (dKO) embryos
188 (Fig. 1Aiii, iv, Bi). This indicates that production of dI6 IN requires both Pax3 and Pax7
189 activities and is sensitive to gene dosage. Similar conclusions can be drawn for the V0_D IN. The
190 decrease in their number seen in Pax3^{-/-} single mutants is accentuated by the loss of one allele
191 of Pax7 (Fig. 1Aviii-x, Bii). The dKO embryos display barely any V0_D cells (Fig. 1Axi, Supp.
192 Fig. 1E). However, precise quantification was compromised by the GFP expressed from Pax3
193 mutant alleles and the absence of the Pax2⁻ V0_V population that normally separates the Pax2⁺
194 V0_D from the V0_{VCG}/V1 cells (Fig. 1Axi, Fig. 1C, (Lai et al., 2016; Lu et al., 2015)). In contrast
195 with dI6 and V0_D IN, the number of Evx1⁺ V0_{VCG} IN is increased in Pax3 single null mutants
196 (Fig. 1Axv, xvi, Biii). This expansion is further increased in absence of one allele of Pax7,
197 indicating that the generation of this population is restricted by Pax3 and Pax7 and that the
198 restriction is dependent on Pax3/7 levels (Fig. 1Axvii, Biii). Intriguingly, the V0_{VCG} population
199 is very much reduced in dKO embryos (Fig. 1Axviii, Biii). This can be correlated with the
200 dramatic dorsal expansion of FoxD3⁺ V1 cells observed in absence of both Pax3 and Pax7 (Fig.

201 1Axv, xviii, Biv), which was also previously observed using *in situ* hybridisation labelling of
202 *EN1* mRNA, a V1 determinant (Mansouri and Gruss, 1998). This dorsal expansion of V1 IN
203 was also observed, although to a lesser extent, in Pax3^{-/-};Pax7^{+/-} embryos and is even less
204 pronounced in Pax3^{-/-} embryos (Fig. 1Axvi, xvii, Biv). Taken together these analyses
205 demonstrate that the number and the position of dI6, V0_D, V0_{VCG} and V1 IN are regulated by
206 the levels of Pax3 and Pax7 proteins, with dorsal fates being progressively converted to ventral
207 ones upon loss of Pax3/7 alleles.

208

209 To assess whether Pax3/Pax7-mediated patterning of the intermediate spinal cord involves
210 transcriptional activation or repression, we performed the same immunostainings on brachial
211 sections of embryos expressing a dominant active (PAX3A) or repressive form (Pax3R) of Pax3
212 from the Pax3 locus (Fig. 1). Upon PAX3A expression, the generation of dI6 IN is completely
213 lost, while the expression of Pax3R has little, if any, effect on this population (Fig. 1Av-vii,
214 Bv). Taken together with the progressive loss of dI6 IN when Pax3/Pax7 alleles are successively
215 removed, this indicates that the generation of dI6 IN requires Pax3/7 repressive activity.
216 Conversely, the V0_D population, which is also decreased upon loss of Pax3/7 alleles, expands
217 upon the expression of PAX3A and recedes slightly upon expression of Pax3R (Fig. 1Axii-xiv,
218 Bvi), suggesting that the fate of this population is more dependent on Pax3 positive activity.
219 V0_{VCG} IN are also affected by the expression of the transcriptional active and repressive forms
220 of Pax3. Expression of PAX3A increases the population, while it is reduced in Pax3^{KIPax3R/-}
221 embryos (Fig. 1Axix-xxi, Bvii), suggesting that Pax3 repressive activity prevents V0_{VCG} IN
222 generation. Finally and intriguingly, contrasting with the dKO phenotype, the V1 population is
223 neither affected by the expression of PAX3A nor by that of Pax3R. Several hypotheses can be
224 drawn to explain this phenotype. First, the ventral restrictions of the V1 population by Pax3 and
225 Pax7 depend on a complex regulatory network, implicating both their positive and negative

226 transcriptional potential. Second, the remaining two alleles of Pax7 in embryos carrying the
227 Pax3R and PAX3A alleles may interfere, thus rendering the interpretation of these results
228 difficult. To conclude, our data demonstrate that the levels of Pax3 and Pax7 activity, notably
229 those leading to transcriptional repression, play a key role in the positioning and numbering of
230 four distinct interneuron populations of the intermediate neural tube.

231

232 **Pax3 and Pax7 repressive transcriptional activity restricts the Dbx1 dorsal** 233 **boundary**

234 Amongst the known TFs that regulate the fate of neurons within the intermediate spinal cord
235 and that could be targeted by Pax3 and Pax7, Dbx1 stood out. Dbx1 is mainly expressed by p0
236 progenitors in a domain that just overlaps with that of Pax7 ((Dessaud et al., 2010; Dyck et al.,
237 2012; Lanuza et al., 2004; Pierani et al., 2001) and is able to direct spinal progenitor cells
238 towards a V0 fate by preventing them from adopting either a V1 or a dI6 fate ((Lanuza et al.,
239 2004; Pierani et al., 2001), Fig. 4A, Supp. Fig. 1F). To evaluate the possibility of Pax3/7
240 regulating Dbx1, we first compared the dynamics in the expression profile of these three TFs
241 in mouse embryos in which the stable reporter proteins GFP and β -Galactosidase are expressed
242 from *Pax3* and *Pax7* loci, respectively (Fig. 2A, Supp. Fig. 2A, data not shown). Pax7 is induced
243 in the neural tube concomitantly with Dbx1 around GD9.0 (data not shown; (Jostes et al., 1990;
244 Pierani et al., 1999)) and at all stages analysed their expression only overlaps within the most
245 dorsal row of the Dbx1⁺ domain (Fig. 2Aii', Supp. Fig. 2A). Within this row of cells, Pax3 is
246 also detected (Fig. 2Aii), with Pax3⁺ cells representing less than 20% of Dbx1⁺ cells (Fig.
247 2Aiii). This contrasts with the presence of Pax3 lineage marker GFP in more than 98% of Dbx1⁺
248 cells (Fig. 2Aiii). Dbx1 induction, thus, appears temporally correlated with the dynamic dorsal
249 restriction of Pax3 expression (Fig. 2B). Dbx1 induction may then be stabilised by a Dbx1

250 mediated repression of Pax3, as illustrated by the downregulation of Pax3 and, to a lesser extent
251 of Pax7, upon Dbx1 gain of function in chick embryos (Fig. 4Aii, iii).

252 We then assessed whether *Dbx1* expression is modulated by the loss of function of Pax3 and
253 Pax7 by performing *in situ* hybridisation against *Dbx1* transcripts in *Pax3* and *Pax7* double null
254 mutant mice at GD10.5 and GD11.5 (Fig. 2C, Supp. Fig. 2B). At both stages, the *Dbx1*⁺ domain
255 is dorsally expanded within the neural tube of double mutants and reaches a size double that of
256 control embryos. To further determine the contribution of Pax3 and/or Pax7 to *Dbx1* ventral
257 restriction, we examined Dbx1 protein distribution in several compound null mutants for both
258 genes at GD11.5 (Fig. 2D, Supp. Fig. 2C, Di). In *Pax7* null mutants, the Dbx1 expression profile
259 remains unchanged (Supp. Fig. 2C). Conversely, a slight, yet significant, increase in the number
260 of Dbx1⁺ cells was observed in *Pax3* single mutants (Fig. 2Di, ii, v). This phenotype appears
261 stronger upon removal of one *Pax7* allele (Fig. 2Diii, v). Indeed, in *Pax3*^{-/-}; *Pax7*^{+/-} embryos,
262 not only is the Dbx1⁺ domain dorsally expanded (Supp. Fig. 2Di), but also all of the cells within
263 this domain express Dbx1 protein. By contrast, in control embryos, Dbx1 protein distribution
264 is present in a salt-and-pepper pattern (compare Fig. 2Diii to Fig. 2Di). Intriguingly, despite the
265 large expansion of *Dbx1* transcript distribution in *Pax3*;*Pax7* double mutants (Supp. Fig. 2Di),
266 we observed a reduced number of cells expressing Dbx1 protein compared to control embryos
267 (Fig. 2Div, v); suggesting post-transcriptional down-regulation of Dbx1 in the absence of Pax3
268 and Pax7. This could explain the reduction of V0_{vCG} IN production previously observed in dKO
269 mutants (Fig. 1Axviii, Biii). Taken together these results indicate that both Pax3 and Pax7 are
270 required to position the dorsal border of the *Dbx1* expression domain.

271 We then sought to evaluate whether gain of function for Pax3 and Pax7 impacts on *Dbx1*
272 expression. For this, we electroporated chick embryos at Hamilton and Hamburger stage HH10-
273 11 with constructs driving the bi-cistronic expression of either Pax3 or Pax7 together with GFP
274 (Fig. 2E). At 24 hours post electroporation (hpe), both Pax3 and Pax7 ectopic expression are

275 sufficient to prevent the induction of *Dbx1* transcription, while electroporation of the empty
276 *GFP* containing plasmid (*pCIG*) has no effect.

277 Altogether, these results prompted us to investigate whether Pax3- and Pax7-mediated
278 restriction of *Dbx1* expression occurs by transcriptional activation or repression (Drouin, 2014).
279 First, we analysed the distribution of *Dbx1* mRNA and protein in mouse embryos expressing
280 Pax3R or PAX3A, at GD10.5 and GD11.5. (Fig. 2F-H, Supp. Fig. 2Dii). Embryos carrying the
281 *Pax3R* allele display a reduction in the levels of *Dbx1* transcripts and protein (Fig. 2F, G). Upon
282 quantification, no significant changes in the position of *Dbx1* D-V boundaries were noted in
283 mutants carrying a *Pax3R* allele compared to control embryos (Fig. 2Fv, Supp. Fig. 2Dii). By
284 contrast, in *Pax3^{Pax3A/+}* embryos, the *Dbx1* domain is more than doubled in size at GD10.5 (Fig.
285 2Fiv, v, 2Giv, v) and further expands with time to encompass the entire dorsal region of the
286 spinal cord in GD11.5 embryos (Fig. 2Hi, ii). In addition, this expansion occurs both dorsally
287 and ventrally, unlike the purely dorsal expansion observed in double knockout embryos (Fig.
288 2Fv, Supp. Fig. 2Dii). Taken together these results show that while Pax3 and Pax7 have
289 previously been mainly demonstrated to act as transcriptional activators during embryogenesis
290 (Budry et al., 2012; McKinnell et al., 2008; Relaix et al., 2003), in the neural tube these TFs
291 contribute to neural tube patterning through transcriptional repression. It is also noteworthy that
292 *Dbx1* expression is hardly ever detected and only occurs at very low levels in the somites of
293 wild-type embryos (Fig. 2Hiii) but that it is strongly induced in *Pax3^{PAX3A/+}* embryos (Fig.
294 2Hiv); therefore suggesting that Pax3-mediated repression of *Dbx1* also occurs in Pax3
295 dependent myogenic lineages. This hints that our work may prove relevant in a spectrum of
296 tissue types.

297

298 ***Dbx1* locus harbours several Pax responsive cis-regulatory modules**

299 We next decided to evaluate whether Pax3 and Pax7 could directly control *Dbx1* transcription
300 by acting at the level of *Dbx1* associated cis-regulatory modules (CRM). To identify these
301 CRMs, we first compared the *Dbx1* loci and the surrounding 10kb intergenic regions of several
302 vertebrate species ranging from human to fugu. This approach revealed the existence of 5
303 blocks of conservation: three of them located within 4.2kb upstream of the *Dbx1* transcription
304 start site (CRM1, CRM2, CRM3); the other two within the second intron (iCRM1, iCRM2)
305 (Fig. 3A). In addition, ENCODE epigenetic data on the neural tube of GD11.5 or GD12.5
306 mouse embryos further supports the functionality of these CRMs (see Material and Methods).
307 Notably, DNase hypersensitivity and histone modification H3K27ac, whose combined
308 occurrence defines active enhancers, are both present at the level of the two iCRMs.
309 Additionally, the H3K4me1 epigenetic mark of active and/or poised enhancers is detected at
310 iCRM2, CRM2 and CRM3 (Fig. 3A). Furthermore, in ESC-derived neuroprogenitors, binding
311 events for Sox2 and Sox3, two TFs involved in *Dbx1* regulation (Rogers et al., 2014), have
312 been identified within the *Dbx1* iCRM (Fig. 3A)(Bergsland et al., 2012). Finally, the 3
313 intergenic CRMs are located within a 5.7kb region capable of driving the expression of reporter
314 transgenes within the intermediate spinal cord of mouse and chick embryos (Dyck et al., 2012;
315 Lu et al., 1996; Matisse et al., 1999; Thelié et al., 2015); while CRM3 on its own has also been
316 reported to be active in the intermediate neural tube of chick embryos (Oosterveen et al., 2012).
317 In order to test the ability of the candidate CRMs to respond to Pax3/7 individually or
318 collectively, we generated reporter constructs and tested their activity in the neural tube of chick
319 embryos 24 and 36 hours after their electroporation at stage HH10-11 (Fig. 3B,C, data not
320 shown). Amongst the three intergenic CRMs, only CRM3 displays any activity, as previously
321 described (Oosterveen et al., 2012)(Fig. 3Bi-iii, C), yet, the expression of the reporter protein
322 driven by this element is weak, as it was detected in only 4 embryos out of 14. In addition, it is

323 not restricted to the *Dbx1*⁺ domain (Fig. 3Biii). Likewise, the CRM4.8kb module, which
324 contains CRM1, 2 and 3 (Fig. 3A) is poorly able to induce gene expression in the neural tube
325 of chick embryos (Fig. 3Biv); only 4 embryos out of 21 display any β -Galactosidase expression.
326 When detected, the activity of this module is not restricted to the *Dbx1*⁺ domain but
327 encompasses the entire dorsal neural tube (Fig. 3Biv, C). This is consistent with previously
328 identified ectopic dorsal activity of a 5.7kb CRM in the neural tube of mouse embryos (Lu et
329 al., 1996; Matise et al., 1999) and indicates that the intergenic CRMs may not be the main
330 drivers of the restriction of *Dbx1* expression to the intermediate spinal cord. We next evaluated
331 the ability of the two intronic CRMs to induce gene expression using a construct, containing
332 both modules and the region between them, termed iCRM (Fig. 3A). The iCRM construct
333 systematically promotes β -Galactosidase expression in the neural tube 24hpe and 36hpe. The
334 overall levels of this reporter are weak, but expression is always found exclusively within the
335 *Dbx1* domain (Fig. 3Bv-v'', C and data not shown). Hence the iCRM contains all the necessary
336 information to recapitulate *Dbx1* induction and D-V restriction. Strengthening this argument,
337 adding the iCRM to the CRM4.8kb was sufficient to abolish the CRM4.8kb ectopic dorsal
338 activity and promote strong *LacZ* gene expression in the *Dbx1*⁺ domain alone, as assessed at
339 36hpe (Fig. 3Bvi). It is worth mentioning that *Dbx1 LacZ* KI reporter mice have previously
340 been generated, where *LacZ* replaces the four exons and three introns (including the iCRM) of
341 *Dbx1* (Pierani et al., 2001). Interestingly, β -Galactosidase expression matches with that of *Dbx1*
342 in these animals, thus, the iCRM is unlikely to be the only module responsible for *Dbx1* gene
343 regulation in the neural tube. It is tempting to hypothesise that it behaves as a shadow enhancer
344 providing robustness to *Dbx1* transcription.

345 We next used *in silico* approaches to evaluate whether these CRMs contain putative Pax3/7
346 binding sites. We first identified the most phylogenetically conserved 15 base pair long DNA
347 motifs contained in these CRMs using MEME (cf. Materials and Methods). Then, we searched

348 within the extracted motifs for putative Pax binding sites using the TOMTOM database, or
349 using FIMO and Pax3- and Pax7-specific position weight matrices (PWMs)(Supp. Fig. 3A), as
350 Pax binding domains have some level of family member specificity and Pax3 and Pax7 PWMs
351 are not defined in TOMTOM databases (Jolma et al., 2013; Mayran et al., 2015). These were
352 either previously defined by HT-SELEX (Jolma et al., 2013) or Pax3 and Pax7 ChIP-Seq
353 performed in myoblasts (Soleimani et al., 2012). These two methods identified putative Pax3
354 and Pax7 binding sites in iCRM, CRM2 and CRM3 (Fig. 3Axi). Further supporting this, Pax7
355 has been shown to be able to bind to the 2 sites identified in iCRM and one of the sites located
356 in CRM3 in myoblasts (Soleimani et al., 2012) (Fig. 3Axi). Probing this further, we noted that
357 the electroporation of either Pax3 or Pax7 induces significant changes in the activity of iCRM,
358 CRM4.8kb and CRM3 in the neural tube of chick embryos 24hpe (Fig. 3D, Supp. Fig. 3B, data
359 not shown); promoting the activity of the latter and repressing that of the other two. These
360 results provide one possible explanation for why the intergenic CRM3 or CRM4.8Kb harbour
361 ectopic activity in the dorsal neural tube which expresses Pax3 and Pax7, while the iCRM is
362 restricted to the intermediate domain. Furthermore, electroporation of PAX3A stimulates the
363 activity of all the CRMs (Fig. 3D, Supp. Fig. 3B). Notably, its impact was stronger on that of
364 the iCRM than on all other constructs. Conversely, electroporation of Pax3R inhibits the
365 activity of iCRM, CRM4.8kb and CRM3 (data not shown). Altogether, these results support
366 the idea that Pax3 and Pax7 directly regulate *Dbx1* by modulating the activity of several CRMs,
367 repressing transcription through iCRM, CRM1 and CRM2, and activating it through CRM3.

368

369 **Pax3 and Pax7 mediated *Dbx1* regulation involves an additional genetic interaction**

370 The progressive attenuation of *Dbx1* expression in the dKO embryos observed between GD10.5
371 and GD11.5 (compare Fig. 2C to Supp. Fig. 2B) could not be solely explained by the repression
372 of *Dbx1* locus activity by Pax3 and Pax7. We, thus, looked for candidate TFs that would be

373 regulated by Pax3 and Pax7 and whose activity would impact onto *Dbx1* expression. *Prdm12*,
374 a p1 fate determinant known to repress *Dbx1* expression, as well as the activity of CRM4.8kb
375 in chick embryos (Kinameri et al., 2008; Thelié et al., 2015), appeared an obvious candidate.
376 We analysed its expression by *in situ* hybridisation in GD11.5 Pax3 and Pax7 mutant embryos
377 (Fig. 4B). In single mutants for Pax3 and Pax7, we did not detect any defects in the expression
378 profile of this TF (Fig. 4Bii). In contrast, in dKO embryos, the *Prdm12* expression domain is
379 dorsally enlarged and overlaps with the extended *Dbx1* domain (Fig. 4Biv, Supp. Fig. 2Di).
380 *Prdm12* is also ectopically induced in *Pax3^{-/-};Pax7^{+/-}* embryos, but to a much lesser extent than
381 in the dKO embryos (Fig. 4Biii-iv). These results indicate that Pax3 and Pax7 are both required
382 to position of the dorsal boundary of *Prdm12* domain. To assess whether this is mediated by a
383 repressive activity of the Pax TFs, we assessed for *Prdm12* in embryos carrying Pax3A and
384 Pax3R variants (Fig. 4Bv-vii). Expression of the latter Pax3 variant has no effect on *Prdm12*
385 expression (Fig. 4Bvii), while the dominant active form of Pax3 induces ectopic dorsal
386 expression of *Prdm12* (Fig. 4Bv). Taken together these results indicated that Pax3 and Pax7
387 repress *Prdm12* expression. As *Prdm12* is a repressor of *Dbx1* (Thelié et al., 2015), Pax3/7
388 mediated *Prdm12* repression provides an explanation for why the removal of Pax3 and Pax7
389 results in the attenuation of *Dbx1* expression. This also shows that the regulation of *Dbx1* by
390 Pax3 and Pax7 is complex and includes both positive and negative components. The latter is a
391 direct regulation while the former is indirect. It is tempting to hypothesise that this incoherent
392 regulatory network formed by Pax3/7, *Dbx1* and *Prdm12* explains, in parts, why the levels of
393 Pax3 and Pax7 repression play such a key role during the generation of intermediate neural cell
394 fates (Fig. 4C, Alon, 2007; Balaskas et al., 2012).

395 **Conclusion/Discussion**

396 First of all, this paper provides strong evidence that progenitor cells of the intermediate neural
397 tube are able to sense and interpret the dose and the levels of Pax3 and Pax7 transcriptional
398 activity, as shown by the progressively more dorsal fates seen upon increasing Pax levels (Fig.
399 1). This is highly reminiscent of the mode of interpretation of TF-based morphogen gradients
400 (Rushlow and Shvartsman, 2012). Our data further suggest that in the case of Pax3 and Pax7,
401 graded activity throughout the D-V axis of the intermediate neural tube is likely to be
402 established thanks to the temporal restriction of their expression profiles. It is also tempting to
403 hypothesise that Pax3 and Pax7 diffuse ventrally over a short range and thus form a gradient
404 sufficient to provide robustness to pattern formation, as proven for Pax6, another member of
405 the Pax family (Prochiantz and Di Nardo, 2015; Quiñinao et al., 2015). Second, we have
406 highlighted the importance of the repressive transcriptional activity of Pax3 and Pax7 in this
407 process, especially for the spatial restriction and numbering of the V0 and V1 IN. This contrasts
408 with Pax3/7 mediated cell fate specification in other tissues, which is rather based on
409 transcriptional activation (Budry et al., 2012; McKinnell et al., 2008; Relaix et al., 2003).
410 Whether this is due to differential usage of their DNA binding domains or specific protein
411 partners remains to be studied (Mayran et al., 2015). Finally, our study has identified several of
412 the genetic interactions of the gene network by which Pax3 and Pax7 regulate the organisation
413 of cell fates within the intermediate spinal cord. *Dbx1* displays a central position in this network
414 and we have demonstrated that it receives both a likely direct negative input and an indirect
415 positive input from Pax3 and Pax7 (Fig. 4C). On the one hand, we provide several pieces of
416 evidence for the direct repression of *Dbx1* by Pax3 and Pax7. We have shown that *Dbx1*
417 expression displays a dose response to levels of Pax3 and Pax7 activity and that several *Dbx1*
418 *cis*-regulatory elements are responsive to the modulation of Pax3 and Pax7 transcriptional
419 activity. Not only do these harbour putative Pax3 and Pax7 binding sites, but physical

420 interactions between Pax3 and Pax7 and two of these CRMs have also been observed in
421 myoblast cells (Soleimani et al., 2012). Importantly, our data indicate that the negative input of
422 Pax3 and Pax7 on Dbx1 plays an essential role in positioning the dorsal border of the Dbx1
423 expression domain. As we and others have demonstrated that Dbx1 promotes V0 fate by
424 inhibiting the expression of determinants of the adjacent dI6 and V1 cell populations ((Karaz
425 et al., 2016; Lanuza et al., 2004; Pierani et al., 2001), Fig. 4A), this genetic interaction provides
426 a fairly simple mechanism for how the Pax TFs impact on the size of V0 and dI6 IN populations
427 (Karaz et al., 2016; Lanuza et al., 2004; Pierani et al., 2001), Fig. 1, Fig. 4A, Supp. Fig. 1E,F).
428 On the other hand, we have shown that Pax3 and Pax7 repress Prdm12, a known repressor of
429 Dbx1 (Thelié et al., 2015). Interestingly, this regulatory loop provides a molecular explanation
430 for the massive ectopic dorsal generation of V1 cells in Pax3 and Pax7 double mutants
431 described twenty years ago ((Mansouri and Gruss, 1998), Fig. 1). Furthermore, it supports a
432 model whereby D-V progenitor pool determinants not only repress genes expressed in adjacent
433 pools, but also expressed in pools located further way (e.g. (Kutejova et al., 2016)). Altogether,
434 our paper supports the ideas that (i) the patterning potential of TFs is extended by the ability of
435 progenitors to read and extrapolate discrete levels of TF activity, that (ii) these levels can be
436 modulated thanks to genetic redundancy induced by gene duplication and/or dynamics in their
437 expression profiles and that (iii) the generation of the diversity of progenitor pools in the
438 developing neural tube might not be mediated by multiple binary cell fate choices between
439 adjacent progenitor pools but by the active repression of several alternative fates.

440

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611

612 **Figure legends**

613 **Fig. 1: Pax3 and Pax7 regulate the production of interneuron populations in the**
614 **intermediate part of the spinal cord**

615 **A.** Immunostaining for Lbx1, AP2 α , Brn3a to identify dI6 IN (**i-vii**), Pax2, Lbx1 and Evx1 to
616 reveal V0_D IN (**viii-xiv**) and FoxD3 and Evx1 to label V0_{VCG} and V1 IN populations,

617 respectively (**xv-xxi**) performed on brachial transverse sections of GD11.5 mouse embryos of
618 the indicated genotype. In **ii**, $Ap2\alpha$ is not labelled. In **ix-xi** $Evx1$ is not marked. Scale bars:
619 $50\mu\text{m}$. Dashed lines delineate the pools of interneuron subtypes. Asterisks in **iv** and **vii** mark
620 the lack of dl6 cells. In **xii** and **xiii**, $V0^*$ indicates that the $V0_D$ and $V0_{VCG}$ are intermingled.
621 Arrowheads in **xvii** and **xviii** point at ectopic $V1$ cells and limited $V0$ cells, respectively. **B.**
622 Quantification of the number of each neuronal subtype in GD11.5 mouse embryos with the
623 indicated genotype ($dl6$ IN are $Lbx1^+/Ap2\alpha^-/Brn3a^-$, $V0_D$ IN are $Pax2^+/Lbx1^-/Evx1^-$, $V0_{VCG}$ IN
624 are $Evx1^+$, $V1$ IN are $FoxD3^+$). Red bars denote the mean values. Mann–Whitney U test p-
625 values are represented as follows: *: $p\leq 0.05$; **: $p\leq 0.01$; ***: $p\leq 0.001$; ****: $p\leq 0.0001$. **C.**
626 Representation of the pools of progenitor cells in the spinal cord and the neuronal populations
627 they give rise to. The dl6, $V0$ and $V1$ populations are highlighted and the expression of TFs
628 that allows their determination are shown. The distribution of these populations in the
629 $Pax3/Pax7$ mutant backgrounds that were analysed are summarised.

630

631 **Fig. 2: Pax3 and Pax7 repressive activity positions the dorsal boundary of *Dbx1* expression**

632 **A.** Immunostaining for $Dbx1/\beta\text{-Galactosidase}/\text{GFP}$ in thoracic transverse sections of GD11.5
633 $Pax3^{GFP/+}$; $Pax7^{LacZ/+}$ embryos (**i-i'**) and $Dbx1/Pax3$ at brachial level of GD10.5 wild-type
634 embryo (**ii-ii'**). Quantification of the proportion of $Dbx1^+$ cells expressing Pax3 or Pax3 lineage
635 marker GFP at GD11.5 in thoracic transverse sections (**iii**, $\text{mean}\pm\text{s.e.m}$, $n=4$ embryos). **B.**
636 Schematics showing the temporal dynamics of Pax3, Pax7, $Dbx1$ and $Prdm12$ expression
637 profiles in the dp6, p0 and p1 spinal progenitor pools and the neurons they will generate. **C-H.**
638 *In situ* hybridisation for $Dbx1$ (**Ci-ii**; **Ei-iii**; **Fi-iv**, **Hi-iv**) and immunolabelling of $Dbx1$ (**Di-iv**,
639 **Gi-iv**) in brachial transverse sections of either mouse embryos of the indicated stage and
640 genotype (**C, D, F-H**) or in chick embryos 24hpe with the indicated plasmid (**E**). For the latter
641 samples, nuclei were also labelled with DAPI and the electroporated cells with

642 immunofluorescent labelling of GFP (**Ei'**, **ii'**, **iii'**). Graphs in **Ciii** and **Fv** display the absolute
643 D-V position of the *Dbx1* expression domain from the floor plate in μm (mean \pm s.e.m on 6
644 sections for each embryo subtype). Graphs in **Dv** and **Gv** show the number of *Dbx1*⁺ cells per
645 transverse section at brachial level in GD11.5 embryos of the indicated genotype. The mean
646 value is indicated by the red bar. Mann–Whitney U test p-values are represented as follows: *:
647 $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$. Scale bars: 50 μm .

648

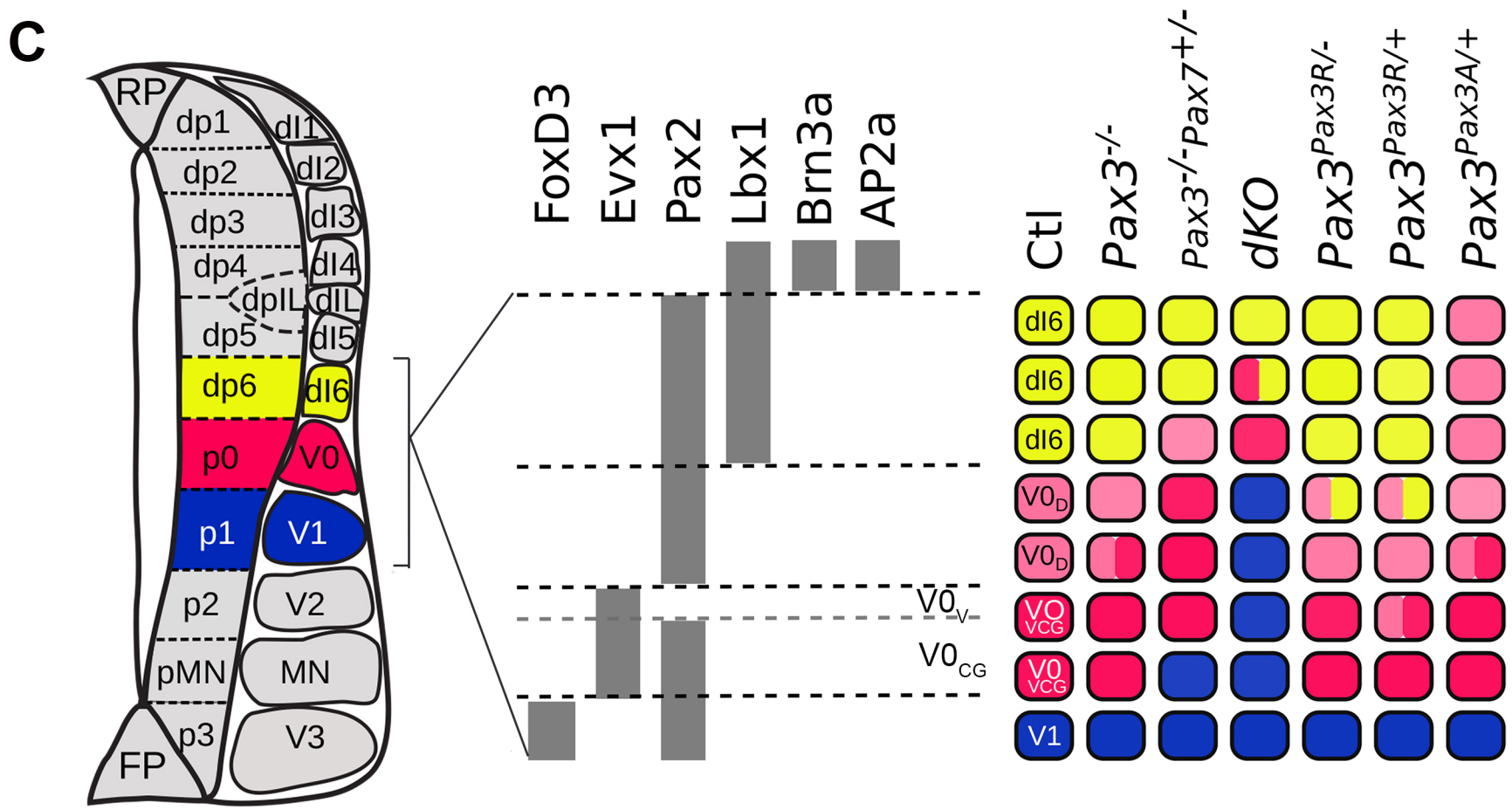
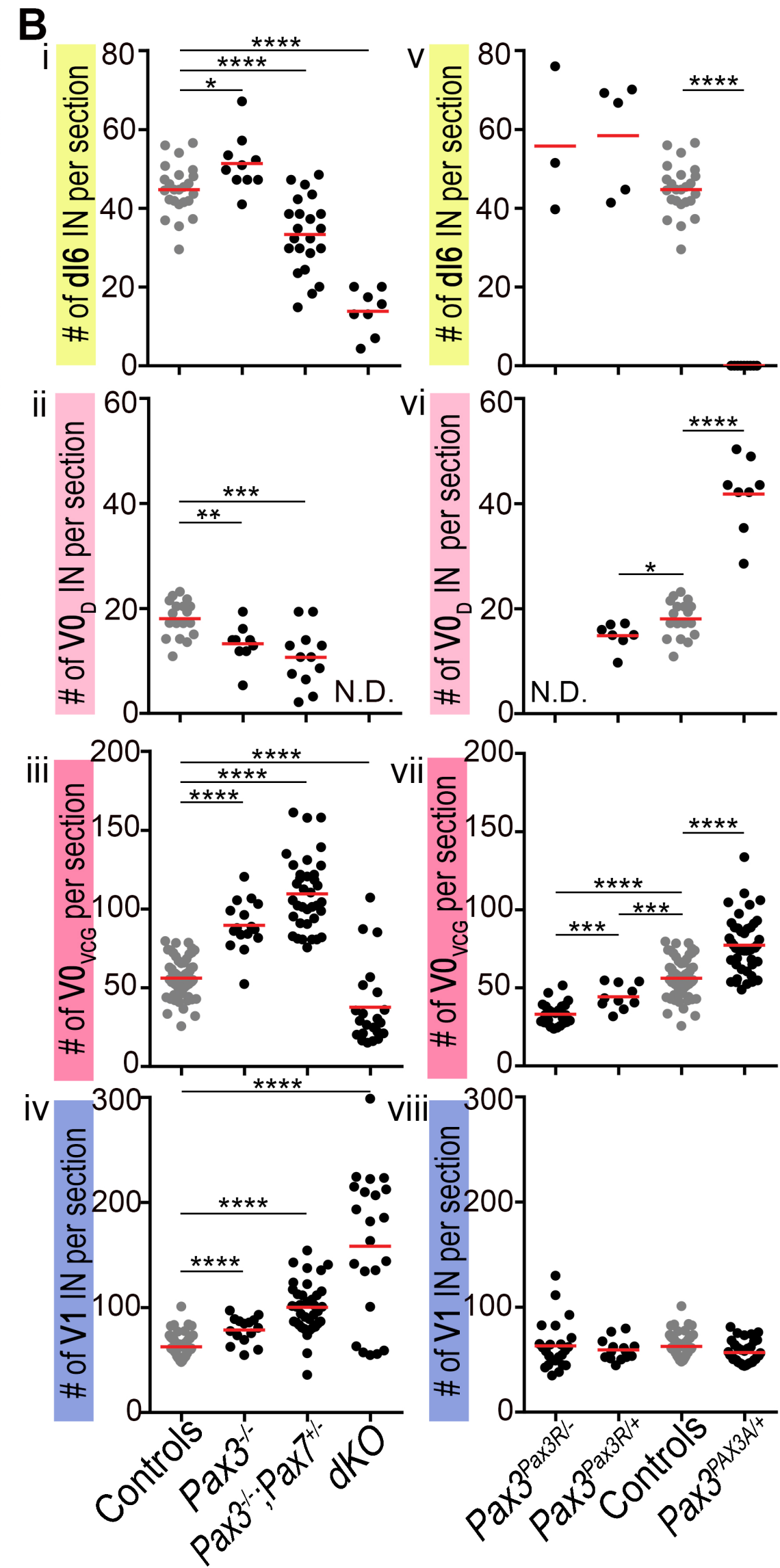
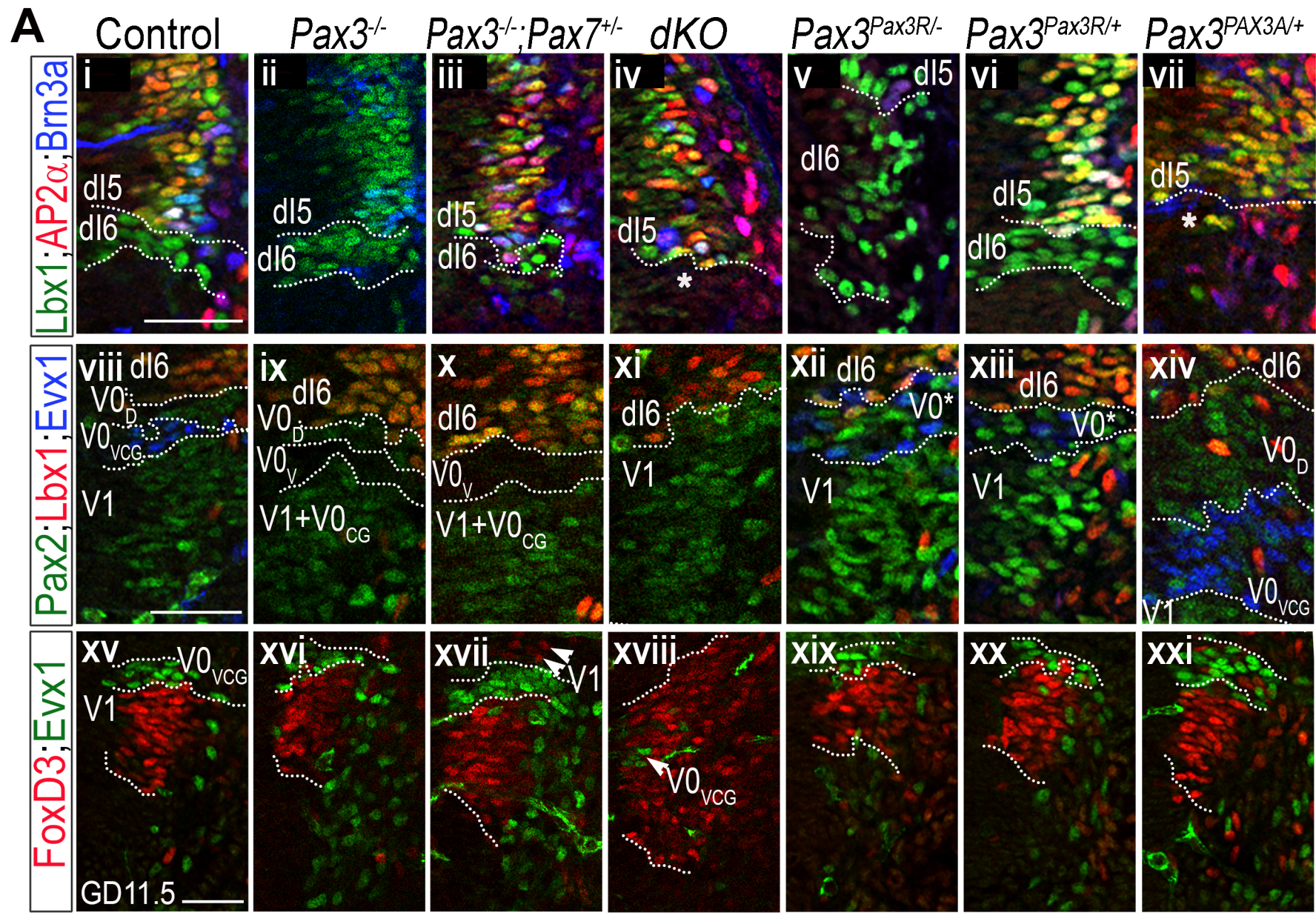
649 **Fig. 3: *Dbx1* CRMs are responsive to *Pax3* and *Pax7* activity**

650 **A.** Schematics displaying the *Dbx1* locus (**i**), regions of conservation across 60 species of
651 vertebrates (**ii**) and putative *cis*-regulatory modules (**iii**). iCRM is located in *Dbx1* intron 2,
652 while CRM1-3 are located in a 4.8kb region upstream the *Dbx1* TSS (CRM4.8kb) and all show
653 high sequence conservation. DNase-seq (**iv**), H3K27ac ChIPseq (**v**) and enhancer predictions
654 (**vi**) in GD11.5 neural tube (NT) support the potential enhancer role of iCRM. H3K4me1
655 ChIPseq signal at GD11.5 (**vii**) and GD12.5 (**viii**) is enriched in iCRM and in CRM2 and CRM3
656 within the CRM4.8kb. Moreover, the iCRM region shows Sox2 (**ix**) and Sox3 (**x**) binding
657 events. Pax7 binding events in muscle cells (**xi**) coincide with the iCRM and CRM4.8kb
658 putative regulatory regions, which also harbour *in silico* predicted Pax3/7 binding sites (**xii**). **B.**
659 Immunostaining for *Dbx1*/ β -Galactosidase/GFP in chick embryos 24hpe with *pCIG* and the
660 indicated reporter constructs carrying *Dbx1* CRMs. Scale bars: 50 μm . **C-D.** Heatmaps display
661 the levels of β -Galactosidase expression normalised to that of GFP along the D-V axis of the
662 neural tube of chick embryos 24hpe with the indicated *Dbx1* CRM reporters and either *pCIG*
663 (C and columns 1 in D), *Pax3-GFP* (columns 2 in D) or *PAX3A-GFP* (columns 3 in D).

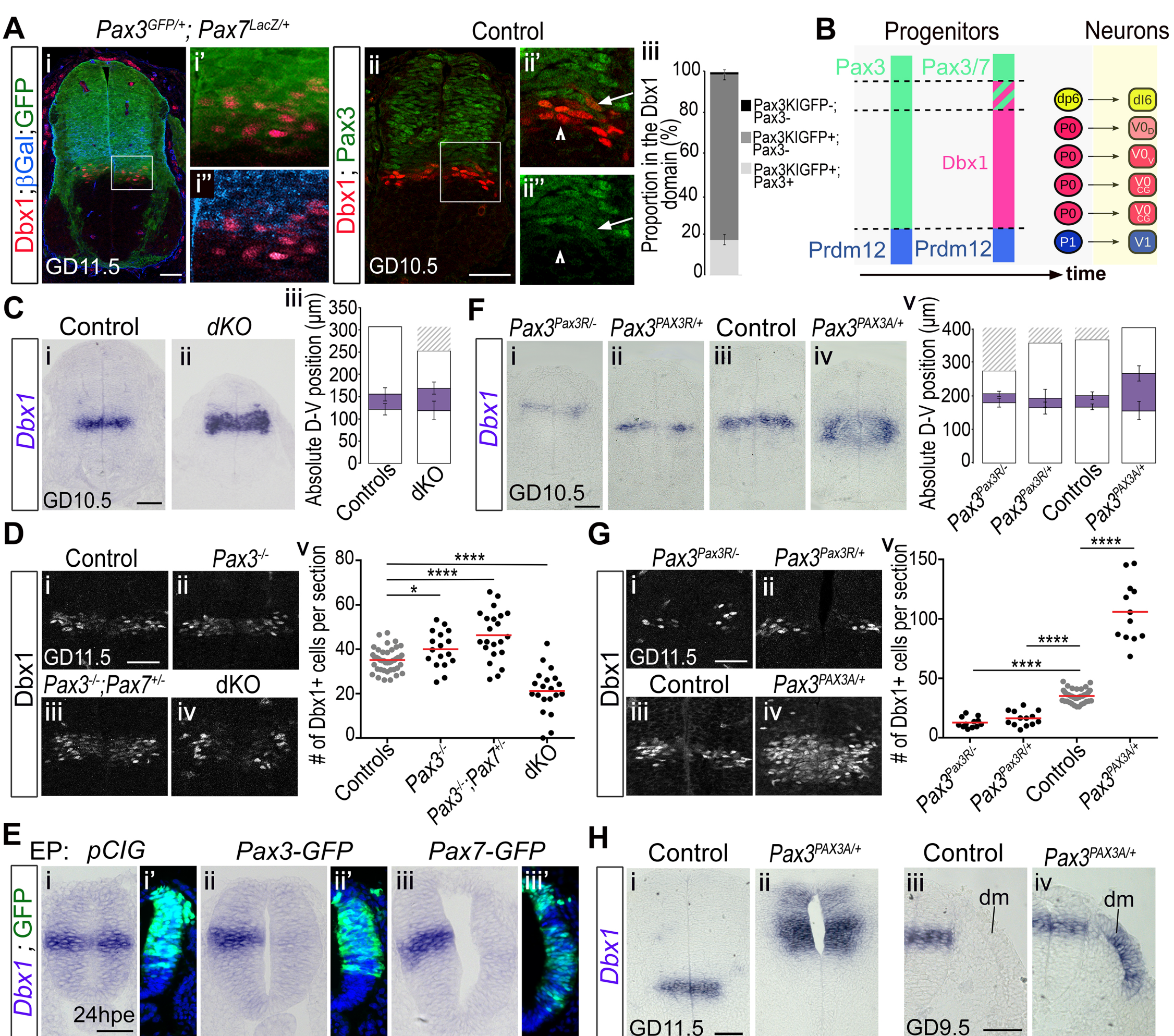
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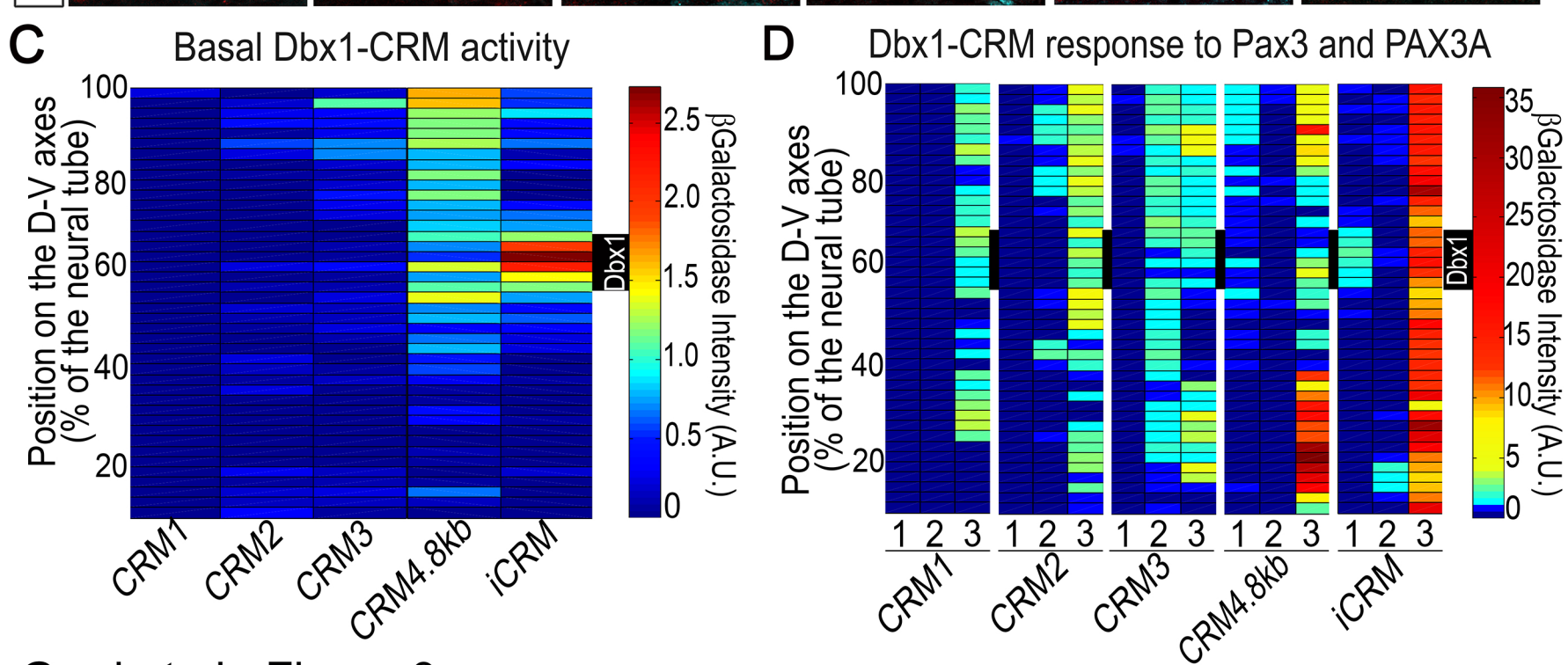
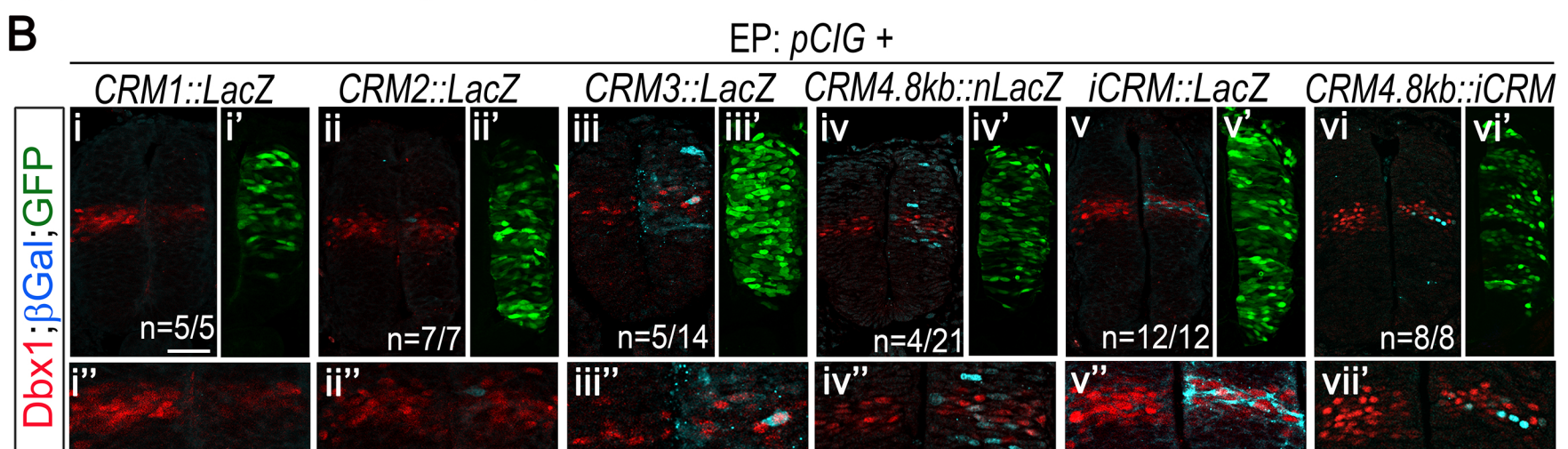
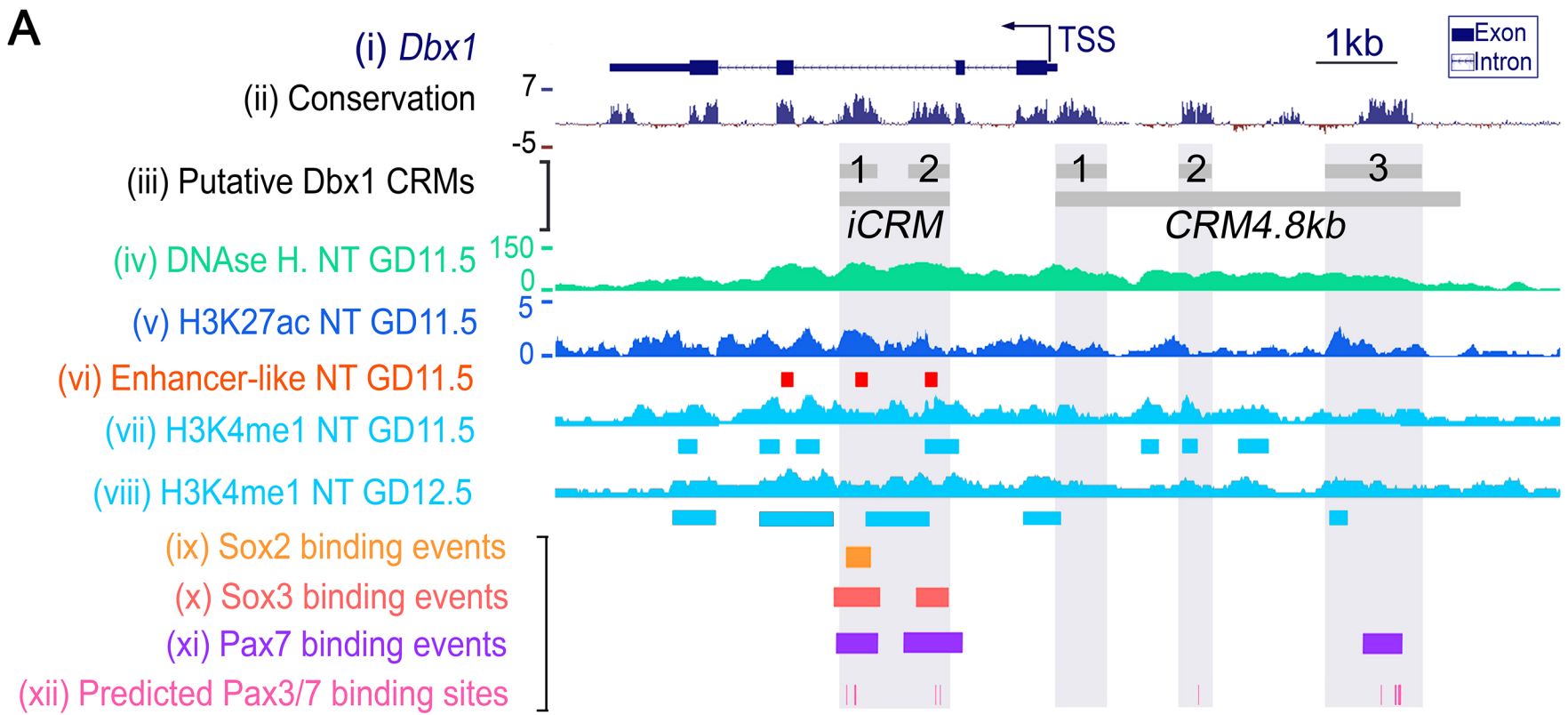
665 **Fig. 4: Characterisation of genetic interactions within the *Pax3* and *Pax7* linked gene**
666 **network**

667 **A.** Immunostaining for Lbx1, Pax3, Pax7 and GFP on chick embryos 40hpe with *mDbx1-IRES-*
668 *GFP* constructs, indicating that Dbx1 overexpression is sufficient to inhibit the generation of
669 Lbx1⁺ neurons (**i, i'**), shut down Pax3 (**ii, ii'**), and decrease the levels of Pax7 expression (**iii,**
670 **iii'**). Scale bars = 50 μ m. **B.** *In situ* hybridisation for *Prdm12* in brachial transverse sections of
671 GD11.5 (**i-v**) and GD10.5 (**vi, vii**) mouse embryos of the indicated genotype. Graphs below the
672 ISH panels display the absolute D-V position of the *Dbx1* expression domain (pink, the striped
673 domain indicates low levels of expression) and the distribution profile of *Prdm12* mRNA (pale
674 blue) along the D-V axis (y axis gives the D-V position from the floor plate in μ m, mean \pm
675 s.e.m on more than 6 sections for each embryo subtype). **C.** Schematics representing the
676 regulatory interactions within the gene network that is responsible for Pax3 and Pax7 mediated
677 Dbx1 gene regulation; (a) refers to Fig 2C & E, (b) repression in dashed line as the repression
678 of Pax7 is partial, see Fig. 4Aii,iii, (c) refers to Fig. 4B, (d) refers to (Thelié et al., 2015).



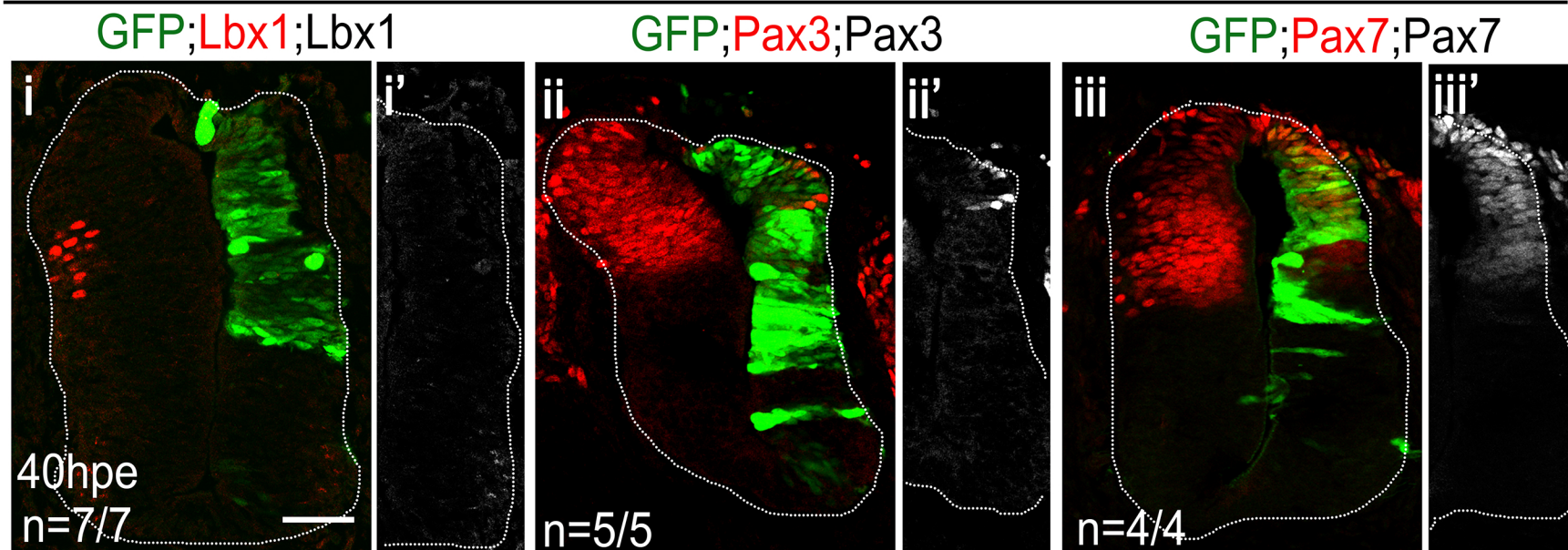
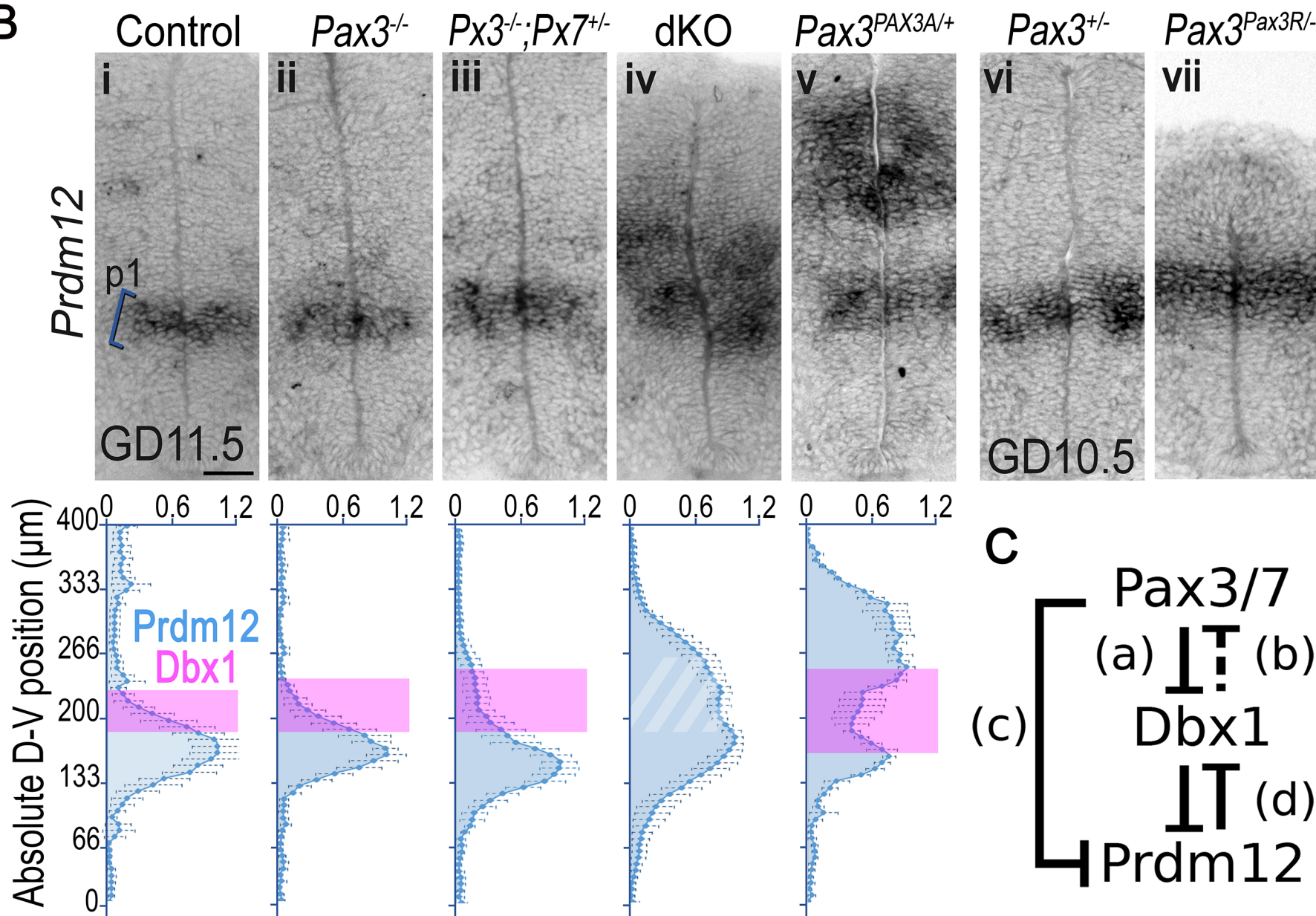
Gard et al., Figure 1





A

EP:mDbx1-IRES-GFP

**B**

SUPPLEMENTARY MATERIAL

Pax3- and Pax7-mediated Dbx1 regulation orchestrates the patterning of intermediate spinal interneurons

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Supplementary Material and Methods

Electroporation constructs

Pax3 and Pax7 variants in pCIG vector and Dbx1 in pCAGGS vector

Pax3R and *Pax7R* were blunt subcloned into a *pCIG* EcoRV site from intermediate plasmids that were respectively cut by EcoRI or XhoI/SpeI. *Pax3* was inserted into a *pCIG* EcoRI site from a plasmid kindly donated by P. Gruss. *Pax7* was taken from the *pPax7cp* plasmid (gift from M.A. Rudnicki) using XhoI and MfeI restriction enzymes and inserted between XhoI and EcoRI sites in *pCIG*. The *PAX3A* construct was previously described (Relaix et al., 2003), while *PAX7A* was amplified by PCR from cDNA provided by F. Barr and cloned into XhoI and XmaI *pCIG* sites. We used a *pCAGG-IRES-EGFP* plasmid containing an HA tagged version of the mouse *Dbx1* gene (Karaz et al., 2016).

Reporter constructs for Dbx1 CRM activity

To generate the 4.8kbCRM reporter plasmid, a 4.8kb BamHI/BstEII fragment corresponding to sequences located immediately 5' to the *Dbx1* TSS (Pierani et al., 2001) was inserted upstream of an *NLS-LacZ* cassette in a *pBluescript* vector. Sequences corresponding to CRM1, and CRM2 were individually amplified using the 4.8kbCRM construct as a template and the following primers:

CRM1-Fwd: AAAGCGGCCGCATCTGCTCTACGAGTCCCAT

CRM1-Rev: AAAACTAGTTACTTGGGCGTCTGCGAGT

CRM2-Fwd: AAAGCGGCCGCCCTAAATAACTTTTTAATTTGA

CRM2-Rev: AAAACTAGTGCTCTGAGCCTCCACTGC

The iCRM sequence was amplified by PCR from mouse genomic DNA using the primers:

iCRM-Fwd: AAAGCGGCCGCTCCCTACCCCGAAGTCGTAT

iCRM-Rev: AAAACTAGTTCGTTTTAAAACTACATATG

PCR products were subsequently cloned into the BGZA vector using NotI/SpeI restriction sites in order to match with the CRM3 reporter plasmid (Oosterveen et al., 2012). For the 4.8kbCRM+iCRM construct, the sequence corresponding to the iCRM was inserted into the 4.8kbCRM reporter plasmid after the *LacZ* cassette, in order to mimic the genomic context.

Dbx1 mutant mouse line

The knock-in mouse line carrying *LacZ* in the *Dbx1* locus was previously described (Pierani et al., 2001).

Immunohistochemistry and *in situ* hybridisation

The following primary antibodies were used: rabbit anti-Dbx1 (Pierani et al., 1999), chick anti-GFP (1/1000, Abcam), mouse anti- β -Galactosidase (1/500, Promega), mouse anti-Evx1 (1/500, DSHB), mouse anti-Pax3 (1/100, DSHB), mouse anti-Pax7 (1/100, DSHB), mouse anti-Brn3a (1/500, gift from C. Parras), guinea-pig anti-FoxD3 (1/10,000, gift from T. Muller/C. Birchmeier), rabbit and guinea-pig anti-Lbx1 (1/10,000, gift from T. Muller/C. Birchmeier), rabbit anti-AP2 α (1/500, Santa-Cruz), goat anti-Lhx2 (1:250, Santa Cruz), rabbit anti-Pax2 (1/500, Thermo Scientific), goat anti-Sox9 (1/500, R&D). Secondary antibodies were all from Thermo Scientific, coupled to Alexa Fluorophores (A488, A568, A647) and diluted 1/500. Immunofluorescence microscopy was carried out using a Leica TCS SP5 confocal microscope. For *in situ* hybridisation experiments, we used probes against mouse *Dbx1* (Lu et al., 1994), chick *Dbx1* (Pierani et al., 1999) and mouse *Prmd12* (Kinameri et al., 2008). Pictures were then taken with an Axio Observer Z1 microscope (Zeiss). All the images were processed with Photoshop 7.0 software (Adobe Systems, San Jose, CA, USA) or Image J v.1.43 g image analysis software (NIH).

Defining Pax3 and Pax7 specific position weight matrices

Position weight matrices (PWMs) for Pax homeodomain binding sites were taken from work by (Jolma et al., 2013) or created *de novo* from Pax3 and Pax7 ChIP-Sequencing data (Soleimani et al., 2012) using MEME (Supp. Fig. 3A). Pax3 and Pax7 peak location data were retrieved for the top 500 ChIP peaks and 100bp sequences either side of their centre were fetched using Galaxy (usegalaxy.org). A Pax7 HD PWM was created using 200bp sequences of the top 500 peaks using MEME. The PWM for the Pax3 paired domain binding site was created by selecting 51 sequences from the ChIP-Seq data that contained at least three consecutive bases of the core GTCAC motif. These sequences were reduced to 100 base pairs in length and used in MEME to establish the PWM.

Defining the transcription factors expressed in GD11.5 mouse neural tube

Genes with an expression level below 0 TPM in the ENCODE RNAseq screen performed on GD11.5 mouse spinal cord (ENCODE project, accession number: ENCSR337FYI) were annotated with gene ontology terms using the Bioconductor packages AnnotationDbi (Pagès H, Carlson M, Falcon S and Li N (2017). *AnnotationDbi: Annotation Database Interface*. R package version 1.36.2.) and Mus.musculus (Team BC (2015). *Mus.musculus: Annotation package for the Mus.musculus object*. R package version 1.3.1.). Genes annotated under the GO term “sequence-specific DNA binding” were selected (Supp. Table 4).

Supplementary Table 1. Number of embryos and sections used for quantifications of cell number per sections at GD11.5

<i>Pax3</i> ^{-/-}		<i>Pax3</i> ^{-/-} ; <i>Pax7</i> ^{+/-}		<i>dKO</i>		<i>Control</i>		<i>Pax3</i> ^{<i>Pax3R</i>-/}		<i>Pax3</i> ^{<i>Pax3R</i>+/}		<i>Pax3</i> ^{<i>PAX3A</i>+/}	
Number of dl6 cells per section at GD11.5 - cf Graphs Fig. 1Bi,v													
4	<i>10</i>	9	<i>21</i>	3	<i>8</i>	13	<i>23</i>	1	<i>3</i>	2	<i>5</i>	3	<i>9</i>
Number of V0 _D cells per section at GD11.5 - cf Graphs Fig. 1Bii, vi													
2	<i>9</i>	6	<i>11</i>	nd	nd	7	<i>15</i>	nd	nd	4	<i>7</i>	4	<i>8</i>
Number of V0 _{VCG} cells per section at GD11.5 - cf Graphs Fig. 1Biii, vii													
7	<i>15</i>	14	<i>35</i>	8	<i>21</i>	31	<i>73</i>	11	<i>24</i>	5	<i>10</i>	18	<i>45</i>
Number of V1 cells per section at GD11.5 - cf Graphs Fig. 1Biv, viii													
7	<i>14</i>	15	<i>36</i>	9	<i>21</i>	27	<i>68</i>	8	<i>23</i>	7	<i>13</i>	12	<i>35</i>
Number of Dbx1 ⁺ cells per section at GD11.5 - cf Graphs Fig. 2Dv, Gv													
5	<i>16</i>	7	<i>21</i>	5	<i>19</i>	17	<i>43</i>	4	<i>11</i>	5	<i>12</i>	6	<i>11</i>

In **bold** are given the numbers of embryos analysed

In *Italics* are given the numbers of sections considered

Supplementary Table 2. Number of embryos and sections used for generating the heatmaps in Fig. 3C and 3D

<i>CRM1</i> + <i>pCIG</i>		<i>CRM1</i> + <i>Pax3</i>		<i>CRM1</i> + <i>PAX3A</i>		<i>CRM2</i> + <i>pCIG</i>		<i>CRM2</i> + <i>Pax3</i>		<i>CRM2</i> + <i>PAX3A</i>	
5	<i>5</i>	5	<i>5</i>	3	<i>4</i>	7	<i>7</i>	5	<i>7</i>	5	<i>7</i>
<i>CRM3</i> + <i>pCIG</i>		<i>CRM3</i> + <i>Pax3</i>		<i>CRM3</i> + <i>PAX3A</i>		<i>CRM4.8kb</i> + <i>pCIG</i>		<i>CRM4.8kb</i> + <i>Pax3</i>		<i>CRM4.8kb</i> + <i>PAX3A</i>	
7	<i>7</i>	5	<i>5</i>	5	<i>5</i>	10	<i>17</i>	4	<i>6</i>	6	<i>6</i>
<i>iCRM</i> + <i>pCIG</i>		<i>iCRM</i> + <i>Pax3</i>		<i>iCRM</i> + <i>PAX3A</i>							
9	<i>9</i>	5	<i>8</i>	6	<i>9</i>						

In **bold** are given the numbers of embryos analysed

In *Italics* are given the numbers of sections considered

Supplementary Table 3. Mann–Whitney *U* test probabilities.

All comparisons are done to control embryos.

<i>Pax3</i> ^{-/-}	<i>Pax3</i> ^{-/-} ; <i>Pax7</i> ^{+/-}	<i>dKO</i>		<i>Pax3</i> ^{<i>Pax3R</i>^{-/-}}	<i>Pax3</i> ^{<i>Pax3R</i>^{+/+}}	<i>Pax3</i> ^{<i>PAX3A</i>^{+/+}}
Number of dl6 cells per section at GD11.5 - cf Graphs Fig. 1Bi,v						
0.0167	< 0.0001	< 0.0001		0.3446	0.0992	< 0.0001
Number of V0_D cells per section at GD11.5 - cf Graphs Fig. 1Bii, vi						
0.0024	0.0002	NA		NA	0.0233	< 0.0001
Number of V0_{VCG} cells per section at GD11.5 - cf Graphs Fig. 1Biii, vii						
< 0.0001	< 0.0001	< 0.0001		< 0.0001	0.0009	< 0.0001
Number of V1 cells per section at GD11.5 - cf Graphs Fig. 1Biv, viii						
< 0.0001	< 0.0001	< 0.0001		0.2658	0.3328	0.0072
Number of Dbx1⁺ cells per section at GD11.5 - cf Graphs Fig. 2Dv, Gv						
0.0223	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001

Significant differences are highlighted in orange

Supplementary Table 4. Transcription factors expressed in GD11.5 mouse spinal cord.

ENSEMBLE gene ID	MGI SYMBOL	Expression (TPM)	ENSEMBLE gene ID	MGI SYMBOL	Expression (TPM)	ENSEMBLE gene ID	MGI SYMBOL	Expression (TPM)	ENSEMBLE gene ID	MGI SYMBOL	Expression (TPM)
ENSMUSG00000000078	Klf6	24.39	ENSMUSG00000022463	Sreb2	103.48	ENSMUSG00000032238	Rora	2.55	ENSMUSG00000048280	Zfp738	11.27
ENSMUSG00000000093	Tbx2	3.17	ENSMUSG00000022479	Vdr	0.13	ENSMUSG00000032292	Nr2e3	0.06	ENSMUSG00000048349	Pou4f1	76.1
ENSMUSG00000000094	Tbx4	0.19	ENSMUSG00000022484	Hoxc10	47.89	ENSMUSG00000032318	Isl2	42.04	ENSMUSG00000048377	Foxi2	0.07
ENSMUSG00000000134	Tfe3	20.25	ENSMUSG00000022485	Hoxc5	29.18	ENSMUSG00000032368	Zic1	14.22	ENSMUSG00000048402	Gli2	9.5
ENSMUSG00000000247	Lhx2	6.91	ENSMUSG00000022508	Bcl6	0.13	ENSMUSG00000032376	Usp3	81.54	ENSMUSG00000048450	Msx1	43.75
ENSMUSG00000000266	Mid2	5.14	ENSMUSG00000022510	Trp63	4.34	ENSMUSG00000032398	Snapc5	42.25	ENSMUSG00000048481	Mypop	4.15
ENSMUSG00000000275	Trim25	1.91	ENSMUSG00000022521	Crebbp	28.31	ENSMUSG00000032402	Smad3	12.05	ENSMUSG00000048482	Bdnf	2.31
ENSMUSG00000000282	Mnt	5.45	ENSMUSG00000022526	Zfp251	29.18	ENSMUSG00000032411	Fdp2	50.68	ENSMUSG00000048490	Nrip1	28.98
ENSMUSG00000000317	Bcl6b	4.95	ENSMUSG00000022528	Hes1	45.03	ENSMUSG00000032419	Tbx18	2.3	ENSMUSG00000048528	Nkx1-2	1.02
ENSMUSG00000000435	Myf5	9.27	ENSMUSG00000022529	Zfp263	21.2	ENSMUSG00000032481	Smarcc1	79.39	ENSMUSG00000048540	Nhlh2	67.01
ENSMUSG00000000440	Pparg	0.13	ENSMUSG00000022556	Hsf1	46.82	ENSMUSG00000032501	Trib1	17.17	ENSMUSG00000048581	E130311K13Rik	2.49
ENSMUSG00000000567	Sox9	127.4	ENSMUSG00000022676	Snai2	20.41	ENSMUSG00000032515	Csrnp1	0.66	ENSMUSG00000048728	Zfp454	12.61
ENSMUSG00000000690	Hoxb6	69.42	ENSMUSG00000022682	Rrn3	96.57	ENSMUSG00000032652	Creb2	1.16	ENSMUSG00000048756	Foxo3	3.08
ENSMUSG00000000708	Kat2b	4.11	ENSMUSG00000022683	Pla2g10	0.06	ENSMUSG00000032691	Nlrp3	0.08	ENSMUSG00000048763	Hoxb3	8
ENSMUSG00000000731	Aire	0.1	ENSMUSG00000022710	Usp7	30.76	ENSMUSG00000032698	Lmo2	21.9	ENSMUSG00000048776	Pthlh	1.22
ENSMUSG00000000782	Tcf7	10.08	ENSMUSG00000022811	Zfp148	32.26	ENSMUSG00000032744	Heyl	26.8	ENSMUSG00000048904	Neurog1	35.39
ENSMUSG00000000861	Bcl11a	57.5	ENSMUSG00000022828	Gtf2e1	43.84	ENSMUSG00000032745	Gbp1	105.63	ENSMUSG00000049086	Bmyc	54.08
ENSMUSG00000000869	Ilf4	3.15	ENSMUSG00000022838	Eaf2	0.97	ENSMUSG00000032815	Fanca	18.31	ENSMUSG00000049164	Zfp518a	11.56
ENSMUSG00000000902	Smad3	233.16	ENSMUSG00000022895	Ets2	18.05	ENSMUSG00000032897	Nfyf	99.8	ENSMUSG00000049295	Zfp219	53.62
ENSMUSG00000000938	Hoxa10	57.75	ENSMUSG00000022952	Runx1	0.93	ENSMUSG00000032998	Foxj3	16.94	ENSMUSG00000049321	Zfp2	27.33
ENSMUSG00000000942	Hoxa4	9.26	ENSMUSG00000022974	Paxbp1	66.12	ENSMUSG00000033006	Sox10	26.04	ENSMUSG00000049421	Zfp260	107.77
ENSMUSG00000001156	Mxd1	17.7	ENSMUSG00000022987	Zfp641	1.53	ENSMUSG00000033016	Nfatc1	2.15	ENSMUSG00000049532	Sall2	43.18
ENSMUSG00000001228	Uhrf1	101.85	ENSMUSG00000022996	Wnt10b	0.6	ENSMUSG00000033080	Vsx1	0.78	ENSMUSG00000049577	Zfpm1	1.12
ENSMUSG00000001280	Sp1	9.38	ENSMUSG00000022997	Wnt1	10.22	ENSMUSG00000033249	Hsf4	0.07	ENSMUSG00000049604	Hoxb13	0.26
ENSMUSG00000001288	Rarg	19.02	ENSMUSG00000023027	Atf1	19.45	ENSMUSG00000033276	Stk36	17.91	ENSMUSG00000049672	Zbtb14	23.48
ENSMUSG00000001418	Glmp	54.87	ENSMUSG00000023034	Nr4a1	3.52	ENSMUSG00000033542	Arhgef5	2.05	ENSMUSG00000049691	Nkx3-2	1.96
ENSMUSG00000001419	Mef2d	14.26	ENSMUSG00000023055	Calcoco1	31.95	ENSMUSG00000033543	Gtf2a2	154.14	ENSMUSG00000049728	Zfp668	21.1
ENSMUSG00000001444	Tbx21	0.16	ENSMUSG00000023079	Gtf2ird1	39.21	ENSMUSG00000033585	Ndn	372.16	ENSMUSG00000049791	Fzd4	3.57
ENSMUSG00000001472	Tcf25	133.57	ENSMUSG00000023110	Prmt5	97.94	ENSMUSG00000033669	Zfp7	9.91	ENSMUSG00000050100	Hmx2	1.39
ENSMUSG00000001493	Meox1	15.8	ENSMUSG00000023391	Dlx2	1.77	ENSMUSG00000033713	Foxn3	69.8	ENSMUSG00000050288	Fzd2	29.57
ENSMUSG00000001497	Pax9	2.92	ENSMUSG00000023411	Nfatc4	15.24	ENSMUSG00000033726	Emx1	0.05	ENSMUSG00000050295	Foxc1	8.7
ENSMUSG00000001504	Irx2	17.73	ENSMUSG00000023826	Park2	0.86	ENSMUSG00000033740	St18	25.57	ENSMUSG00000050328	Hoxc12	3.65
ENSMUSG00000001510	Dlx3	1.75	ENSMUSG00000023882	Zfp54	3.49	ENSMUSG00000033837	Foxh1	0.65	ENSMUSG00000050368	Hoxd10	23.23
ENSMUSG00000001517	Foxm1	43.12	ENSMUSG00000023892	Zfp51	13.39	ENSMUSG00000033863	Klf9	0.99	ENSMUSG00000050397	Foxl2	0.15
ENSMUSG00000001524	Gtf2h4	33.98	ENSMUSG00000023902	Zscan10	0.06	ENSMUSG00000033871	Pparg1b	1.1	ENSMUSG00000050619	Zscan29	11.83
ENSMUSG00000001552	Jup	38.22	ENSMUSG00000023927	Satb1	26.44	ENSMUSG00000033883	D3Ert254e	8.32	ENSMUSG00000050747	Trim15	0.33
ENSMUSG00000001566	Mnx1	6.02	ENSMUSG00000023990	Tfeb	1.44	ENSMUSG00000033943	Mga	28.43	ENSMUSG00000050846	Zfp623	25.47
ENSMUSG00000001627	Ifrd1	128.89	ENSMUSG00000023991	Foxp4	27.39	ENSMUSG00000033961	Zfp446	6.14	ENSMUSG00000050855	Zfp940	9.83
ENSMUSG00000001655	Hoxc13	3.58	ENSMUSG00000023994	Nfyf	21.92	ENSMUSG00000034023	Fancd2	10.67	ENSMUSG00000050945	Zfp438	2.72
ENSMUSG00000001656	Hoxc11	1.62	ENSMUSG00000024014	Pim1	11.6	ENSMUSG00000034041	Lyl1	3.64	ENSMUSG00000051034	Zfp11	6.47

ENSMUSG00000001657	Hoxc8	50.52	ENSMUSG00000024081	Cebpz	23.49	ENSMUSG00000034042	Gbp11	24.96	ENSMUSG000000051159	Cited1	4.74
ENSMUSG00000001661	Hoxc6	75.86	ENSMUSG00000024134	Six2	1.21	ENSMUSG00000034057	Myrf1	0.37	ENSMUSG000000051251	Nhlh1	10.95
ENSMUSG00000001729	Akt1	99.79	ENSMUSG00000024137	E4f1	10.45	ENSMUSG00000034071	Zfp551	4.09	ENSMUSG000000051316	Taf7	18.47
ENSMUSG000000001815	Evx2	0.54	ENSMUSG00000024140	Epas1	6.93	ENSMUSG00000034161	Scx	9.62	ENSMUSG000000051341	Zfp52	1.29
ENSMUSG000000001819	Hoxd13	0.79	ENSMUSG00000024176	Sox8	7.5	ENSMUSG00000034227	Foxj1	2.45	ENSMUSG000000051351	Zfp46	27.06
ENSMUSG000000001823	Hoxd12	1.62	ENSMUSG00000024206	Rfx2	3.29	ENSMUSG00000034266	Batf	1.04	ENSMUSG000000051367	Six1	27.79
ENSMUSG000000001911	Nfix	2.93	ENSMUSG00000024215	Spdef	0.08	ENSMUSG00000034271	Jdp2	0.51	ENSMUSG000000051413	Plagl2	26.99
ENSMUSG000000001988	Npas1	0.34	ENSMUSG00000024238	Zeb1	29.88	ENSMUSG00000034341	Wbp2	83.07	ENSMUSG000000051451	Crebzf	28.63
ENSMUSG000000002028	Kmt2a	8.5	ENSMUSG00000024276	Zfp397	31.63	ENSMUSG00000034384	Barhl2	3.09	ENSMUSG000000051469	Zfp24	80.43
ENSMUSG000000002057	Foxn1	0.02	ENSMUSG00000024401	Tnf	0.12	ENSMUSG00000034429	Zfp707	14.87	ENSMUSG000000051510	Mafg	21.6
ENSMUSG000000002108	Nr1h3	0.88	ENSMUSG00000024431	Nr3c1	5.34	ENSMUSG00000034430	Zxdc	7.68	ENSMUSG000000051675	Trim32	60.96
ENSMUSG000000002111	Spi1	2.22	ENSMUSG00000024454	Hdac3	108	ENSMUSG00000034460	Six4	6.74	ENSMUSG000000051817	Sox12	15.14
ENSMUSG000000002147	Stat6	5.43	ENSMUSG00000024457	Trim26	29.8	ENSMUSG00000034486	Gbx2	36.53	ENSMUSG000000051910	Sox6	15.34
ENSMUSG000000002249	Tead3	3.49	ENSMUSG00000024497	Pou4f3	2.61	ENSMUSG00000034522	Zfp395	11.14	ENSMUSG000000051977	Prdm9	0.79
ENSMUSG000000002250	Ppard	1.7	ENSMUSG00000024498	Tcerg1	59.66	ENSMUSG00000034538	Zfp418	3.01	ENSMUSG000000052040	Klf13	26.74
ENSMUSG000000002266	Zim1	1.8	ENSMUSG00000024513	Mbd2	11.68	ENSMUSG00000034673	Pbx2	13.76	ENSMUSG000000052056	Zfp217	2.6
ENSMUSG000000002325	Irf9	4.68	ENSMUSG00000024515	Smad4	35.84	ENSMUSG00000034701	Neurod1	35.54	ENSMUSG000000052135	Foxo6	0.35
ENSMUSG000000002345	Mef2b	41.38	ENSMUSG00000024560	Cxnc1	30.39	ENSMUSG00000034762	Glis1	1.84	ENSMUSG000000052271	Bhlha15	0.07
ENSMUSG000000002393	Nr2f6	66.45	ENSMUSG00000024563	Smad2	52.99	ENSMUSG00000034777	Vax2	0.1	ENSMUSG000000052435	Cebpe	0.05
ENSMUSG000000002428	Hltf	17.79	ENSMUSG00000024565	Sall3	15.51	ENSMUSG00000034800	Zfp661	7.8	ENSMUSG000000052534	Pbx1	36.29
ENSMUSG000000002578	Ikzf4	3.94	ENSMUSG00000024619	Cdx1	0.2	ENSMUSG00000034957	Cebpa	0.15	ENSMUSG000000052684	Jun	39.32
ENSMUSG000000002617	Zfp40	15.27	ENSMUSG00000024777	Ppp2r5b	47.93	ENSMUSG00000035011	Zbtb7a	6.93	ENSMUSG000000052837	Junb	4.83
ENSMUSG000000002983	Relb	2.96	ENSMUSG00000024789	Jak2	11.31	ENSMUSG00000035033	Tbr1	0.23	ENSMUSG000000052942	Glis3	1.26
ENSMUSG000000003032	Klf4	1.06	ENSMUSG00000024826	Dpf2	162.14	ENSMUSG00000035041	Creb3l3	0.56	ENSMUSG000000053007	Creb5	3.18
ENSMUSG000000003051	Elf3	1.11	ENSMUSG00000024837	Dmrt1	0.12	ENSMUSG00000035125	Gcfc2	9.63	ENSMUSG000000053110	Yap1	21.48
ENSMUSG000000003154	Foxj2	4.04	ENSMUSG00000024912	Fosl1	0.24	ENSMUSG00000035158	Mitf	1.06	ENSMUSG000000053129	Gsx1	8.92
ENSMUSG000000003184	Irf3	45.75	ENSMUSG00000024913	Lrp5	2.99	ENSMUSG00000035187	Nkx6-1	4.62	ENSMUSG000000053175	Bcl3	0.86
ENSMUSG000000003282	Plag1	1.99	ENSMUSG00000024922	Ovol1	0.26	ENSMUSG00000035277	Arx	1.37	ENSMUSG000000053178	Mterf1b	2.91
ENSMUSG000000003283	Hck	1.34	ENSMUSG00000024927	Rela	24.71	ENSMUSG00000035397	Klf16	4.37	ENSMUSG000000053470	Kdm3a	38.87
ENSMUSG000000003308	Keap1	25.51	ENSMUSG00000024947	Men1	53.39	ENSMUSG00000035451	Foxa1	1.3	ENSMUSG000000053477	Tcf4	98.59
ENSMUSG000000003382	Etv3	6.84	ENSMUSG00000024955	Esrra	2.85	ENSMUSG00000035478	Mbd3	215.2	ENSMUSG000000053552	Ebf4	1.62
ENSMUSG000000003423	Pih1d1	85.3	ENSMUSG00000024968	Rcor2	27.83	ENSMUSG00000035576	L3mbtl1	0.05	ENSMUSG000000053747	Sox14	3.33
ENSMUSG000000003662	Ciao1	56.12	ENSMUSG00000024985	Tcf7l2	47.14	ENSMUSG00000035799	Twist1	16.67	ENSMUSG000000053985	Zfp14	5.07
ENSMUSG000000003847	Nfat5	6.94	ENSMUSG00000024986	Hhex	3.51	ENSMUSG00000035868	3110052M02Rik	9.18	ENSMUSG000000054160	Nkx2-4	0.03
ENSMUSG000000003868	Ruvbl2	116.45	ENSMUSG00000025019	Lcor	22.49	ENSMUSG00000035877	Zhx3	8.41	ENSMUSG000000054191	Klf1	10.17
ENSMUSG000000003923	Tfam	62.63	ENSMUSG00000025034	Trim8	24.86	ENSMUSG00000035923	Myf6	2.28	ENSMUSG000000054321	Taf4b	1.39
ENSMUSG000000003949	Hlf	0.73	ENSMUSG00000025049	Taf5	8.18	ENSMUSG00000035934	Pknx2	17.94	ENSMUSG000000054381	Zfp747	6.38
ENSMUSG000000004040	Stat3	34.44	ENSMUSG00000025050	Pcgf6	22.85	ENSMUSG00000035946	Gsx2	5.06	ENSMUSG000000054604	Cggbp1	99.58
ENSMUSG000000004043	Stat5a	1.02	ENSMUSG00000025056	Nr0b1	0.2	ENSMUSG00000036098	Myrf	1.39	ENSMUSG000000054648	Zfp869	29.37
ENSMUSG000000004151	Etv1	10.98	ENSMUSG00000025128	Bhlhe22	1.42	ENSMUSG00000036139	Hoxc9	28.63	ENSMUSG000000054715	Zscan22	15.54
ENSMUSG000000004231	Pax2	6.36	ENSMUSG00000025172	Ankrd2	0.33	ENSMUSG00000036144	Meox2	8.65	ENSMUSG000000054717	Hmgb2	316.93
ENSMUSG000000004264	Phb2	293.52	ENSMUSG00000025215	Tlx1	0.23	ENSMUSG00000036180	Gatad2a	55.26	ENSMUSG000000054737	Zfp182	5.88
ENSMUSG000000004328	Hif3a	5.92	ENSMUSG00000025216	Lbx1	6.14	ENSMUSG00000036192	Rorb	1.99	ENSMUSG000000054808	Actn4	145.03
ENSMUSG000000004359	Spic	0.1	ENSMUSG00000025223	Ldb1	75.41	ENSMUSG00000036461	Elf1	6.85	ENSMUSG000000054892	Tkk	0.05
ENSMUSG000000004661	Arid3b	20.49	ENSMUSG00000025225	Nfkb2	3.04	ENSMUSG00000036602	Alx1	0.7	ENSMUSG000000054931	Zkscan4	3.2
ENSMUSG000000004842	Pou1f1	0.03	ENSMUSG00000025229	Pitx3	1.74	ENSMUSG00000036686	Cc2d1a	8.93	ENSMUSG000000054939	Zfp174	0.84
ENSMUSG000000004872	Pax3	33.97	ENSMUSG00000025255	Zfmx4	33.74	ENSMUSG00000036721	Zscan12	18	ENSMUSG000000054967	Zfp647	21.26

ENSMUSG00000004897	Hdgf	202.43	ENSMUSG00000025323	Sp4	12.92	ENSMUSG00000036867	Smad6	5.41	ENSMUSG00000055024	Ep300	16.16
ENSMUSG00000005148	Klf5	2.61	ENSMUSG00000025364	Pa2g4	219.16	ENSMUSG00000036923	Stox1	0.89	ENSMUSG00000055053	Nfic	12.84
ENSMUSG00000005267	Zfp287	11.39	ENSMUSG00000025369	Smarcc2	85.36	ENSMUSG00000036940	Kdm1a	80.63	ENSMUSG00000055067	Smyd3	15.59
ENSMUSG00000005373	Mlxipl	0.43	ENSMUSG00000025373	Rnf41	22.19	ENSMUSG00000036972	Zic4	45.71	ENSMUSG00000055116	Arntl	6.26
ENSMUSG00000005413	Hmox1	12.38	ENSMUSG00000025407	Gli1	2.86	ENSMUSG00000036980	Taf6	99	ENSMUSG00000055148	Klf2	4.78
ENSMUSG00000005503	Evx1	1.48	ENSMUSG00000025408	Ddit3	17.29	ENSMUSG00000037001	Zfp39	5.73	ENSMUSG00000055210	Foxd2	0.42
ENSMUSG00000005533	Igf1r	12.65	ENSMUSG00000025423	Pias2	64.9	ENSMUSG00000037007	Zfp113	19.75	ENSMUSG00000055228	Zfp935	11.61
ENSMUSG00000005566	Trim28	419.76	ENSMUSG00000025469	Msx3	81.49	ENSMUSG00000037017	Zscan21	102.03	ENSMUSG00000055313	Pgbd1	3.91
ENSMUSG00000005583	Mef2c	14.09	ENSMUSG00000025494	Sigirr	1.33	ENSMUSG00000037025	Foxa2	3.2	ENSMUSG00000055320	Tead1	10.23
ENSMUSG00000005672	Kit	17.71	ENSMUSG00000025498	Irf7	0.69	ENSMUSG00000037029	Zfp146	11.63	ENSMUSG00000055341	Zfp457	0.14
ENSMUSG00000005677	Nr1i3	0.05	ENSMUSG00000025529	Zfp711	21.6	ENSMUSG00000037034	Pax1	13.48	ENSMUSG00000055435	Maf	4.34
ENSMUSG00000005698	Ctcf	85.05	ENSMUSG00000025602	Zfp202	5.49	ENSMUSG00000037138	Aff3	22.77	ENSMUSG00000055491	Pprc1	36.01
ENSMUSG00000005718	Tfap4	13.58	ENSMUSG00000025612	Bach1	18.01	ENSMUSG00000037169	Mycn	84.65	ENSMUSG00000055639	Dach1	17.27
ENSMUSG00000005774	Rfx5	5.61	ENSMUSG00000025782	Taf3	13.31	ENSMUSG00000037171	Nodal	0.03	ENSMUSG00000055799	Tcf7l1	17.28
ENSMUSG00000005836	Gata6	0.45	ENSMUSG00000025880	Smad7	3.73	ENSMUSG00000037174	Elf2	36.64	ENSMUSG00000055817	Mta3	29.62
ENSMUSG00000005886	Ncoa2	23.93	ENSMUSG00000025902	Sox17	8.46	ENSMUSG00000037188	Grhl3	3.33	ENSMUSG00000055835	Zfp1	16.66
ENSMUSG00000005893	Nr2c2	15.7	ENSMUSG00000025912	Mybl1	3.65	ENSMUSG00000037214	Thap1	17.3	ENSMUSG00000055917	Zfp277	32.78
ENSMUSG00000005897	Nr2c1	9.46	ENSMUSG00000025927	Tfap2b	38.23	ENSMUSG00000037279	Ovol2	0.04	ENSMUSG00000055991	Zkscan5	16.7
ENSMUSG00000005917	Otx1	0.17	ENSMUSG00000025930	Mfap	6.26	ENSMUSG00000037335	Hand1	0.43	ENSMUSG00000056216	Cebpg	28.85
ENSMUSG00000006215	Zbtb17	38.39	ENSMUSG00000025958	Creb1	48.57	ENSMUSG00000037343	Taf2	28.59	ENSMUSG00000056493	Foxk1	2.47
ENSMUSG00000006311	Etv2	0.06	ENSMUSG00000025959	Klf7	27.29	ENSMUSG00000037369	Kdm6a	21.29	ENSMUSG00000056501	Cebpb	2.38
ENSMUSG00000006362	Cbfa2t3	7.49	ENSMUSG00000025997	Ikzf2	2.66	ENSMUSG00000037373	Ctbp1	377.15	ENSMUSG00000056537	Rlim	36.78
ENSMUSG00000006498	Ptbp1	169.24	ENSMUSG00000026017	Carf	4.29	ENSMUSG00000037465	Klf10	23.24	ENSMUSG00000056648	Hoxb8	22.18
ENSMUSG00000006586	Runx1t1	37.06	ENSMUSG00000026021	Sumo1	398.52	ENSMUSG00000037523	Mavs	12.08	ENSMUSG00000056749	Nfil3	24.08
ENSMUSG00000006705	Pknox1	36.71	ENSMUSG00000026077	Npas2	0.13	ENSMUSG00000037601	Nme1	202.68	ENSMUSG00000056758	Hmga2	39.39
ENSMUSG00000006720	Zfp184	17.46	ENSMUSG00000026104	Stat1	12.31	ENSMUSG00000037621	Atoh8	5.83	ENSMUSG00000056820	Tsnax	119.82
ENSMUSG00000006932	Ctnnb1	536.21	ENSMUSG00000026234	Ncl	288.57	ENSMUSG00000037640	Zfp60	66.23	ENSMUSG00000056829	Foxb2	0.08
ENSMUSG00000007216	Zfp775	6.42	ENSMUSG00000026313	Hdac4	25.46	ENSMUSG00000037674	Rfx7	17.82	ENSMUSG00000056854	Pou3f4	6.26
ENSMUSG00000007415	Gatad1	57.96	ENSMUSG00000026321	Tnfrsf11a	0.57	ENSMUSG00000037791	Phf12	15.74	ENSMUSG00000057093	C030039L03Rik	3.91
ENSMUSG00000007805	Twist2	12.68	ENSMUSG00000026374	Tsn	295.18	ENSMUSG00000037868	Egr2	0.58	ENSMUSG00000057098	Ebf1	88.51
ENSMUSG00000007812	Zfp655	33.12	ENSMUSG00000026380	Tfcp2l1	0.07	ENSMUSG00000037894	H2afz	1953.87	ENSMUSG00000057156	Homez	16.92
ENSMUSG00000007872	Id3	348.73	ENSMUSG00000026398	Nr5a2	0.44	ENSMUSG00000037935	Smarce1	152.19	ENSMUSG00000057173	Rfx8	0.12
ENSMUSG00000007946	Phox2a	1.48	ENSMUSG00000026436	Elk4	14.72	ENSMUSG00000037984	Neurod6	0.92	ENSMUSG00000057236	Rbbp4	380.44
ENSMUSG00000008398	Elk3	14.1	ENSMUSG00000026459	Myog	38.23	ENSMUSG00000037992	Rara	57.96	ENSMUSG00000057406	Whsc1	87.66
ENSMUSG00000008496	Pou2f2	32.04	ENSMUSG00000026468	Lhx4	10.84	ENSMUSG00000038151	Prdm1	1.15	ENSMUSG00000057409	Zfp53	8.59
ENSMUSG00000008575	Nfib	22.98	ENSMUSG00000026484	Rnf2	120.35	ENSMUSG00000038193	Hand2	0.17	ENSMUSG00000057469	E2f6	50.65
ENSMUSG00000008855	Hdac5	34.55	ENSMUSG00000026491	Ahctf1	28.39	ENSMUSG00000038203	Hoxa13	0.08	ENSMUSG00000057522	Spop	100.85
ENSMUSG00000008976	Gabpa	33.78	ENSMUSG00000026497	Mixl1	0.08	ENSMUSG00000038210	Hoxa11	4.8	ENSMUSG00000057551	Zfp317	22.74
ENSMUSG00000009097	Tbx1	3.05	ENSMUSG00000026536	Mnda	0.4	ENSMUSG00000038227	Hoxa9	79.32	ENSMUSG00000057691	Zfp746	15.71
ENSMUSG00000009248	Ascl2	0.37	ENSMUSG00000026565	Pou2f1	21.84	ENSMUSG00000038236	Hoxa7	55.62	ENSMUSG00000057842	Zfp595	1.4
ENSMUSG00000009406	Elk1	5.45	ENSMUSG00000026586	Prrx1	24.24	ENSMUSG00000038253	Hoxa5	42.33	ENSMUSG00000057895	Zfp105	29.07
ENSMUSG00000009471	Myod1	7.94	ENSMUSG00000026610	Esrrg	10.06	ENSMUSG00000038255	Neurod2	0.93	ENSMUSG00000057982	Zfp809	6.27
ENSMUSG00000009555	Cdk9	67.12	ENSMUSG00000026628	Atf3	2.51	ENSMUSG00000038331	Satb2	1.19	ENSMUSG00000058099	Nfam1	2.38
ENSMUSG00000009569	Mkl2	17.86	ENSMUSG00000026630	Batf3	3.77	ENSMUSG00000038342	Mlxip	17.46	ENSMUSG00000058192	Zfp846	12.1
ENSMUSG00000009733	Tfcp2	16.21	ENSMUSG00000026637	Traf5	4.01	ENSMUSG00000038346	Zfp384	7.59	ENSMUSG00000058230	Arhgap35	45.18
ENSMUSG00000009734	Pou6f2	1.8	ENSMUSG00000026638	Irf6	4.37	ENSMUSG00000038402	Foxf2	4.11	ENSMUSG00000058239	Usf2	23.11
ENSMUSG00000009739	Pou6f1	6.83	ENSMUSG00000026641	Usf1	127.86	ENSMUSG00000038415	Foxq1	2.43	ENSMUSG00000058291	Zfp68	63.47

ENSMUSG00000009741	Ubp1	36.15	ENSMUSG00000026646	Suv39h2	32.21	ENSMUSG00000038418	Egr1	2.44	ENSMUSG00000058318	Phf21a	27
ENSMUSG00000009900	Wnt3a	2.64	ENSMUSG00000026663	Atf6	9.45	ENSMUSG00000038482	Tfdp1	153.66	ENSMUSG00000058331	Zfp85	4.45
ENSMUSG00000010175	Prox1	10.96	ENSMUSG00000026674	Ddr2	28.05	ENSMUSG00000038518	Jarid2	37.9	ENSMUSG00000058402	Zfp420	9.23
ENSMUSG00000010476	Ebf3	8.54	ENSMUSG00000026686	Lmx1a	2.92	ENSMUSG00000038533	Cbfa2t2	50.03	ENSMUSG00000058440	Nrf1	60.91
ENSMUSG00000010505	Myt1	29.98	ENSMUSG00000026700	Tnfsf4	0.03	ENSMUSG00000038539	Atf5	2.04	ENSMUSG00000058638	Zfp110	23.4
ENSMUSG00000010797	Wnt2	2.64	ENSMUSG00000026735	Ptf1a	10.79	ENSMUSG00000038550	Ciart	2.65	ENSMUSG00000058665	En1	4.15
ENSMUSG00000012350	Ehf	0.11	ENSMUSG00000026739	Bmi1	34.63	ENSMUSG00000038615	Nfe211	36.3	ENSMUSG00000058669	Nkx2-9	0.67
ENSMUSG00000012396	Nanog	0.06	ENSMUSG00000026751	Nr5a1	0.06	ENSMUSG00000038630	Zkscan16	1.05	ENSMUSG00000058756	Thra	25.36
ENSMUSG00000012520	Phox2b	1.21	ENSMUSG00000026805	Barhl1	7.4	ENSMUSG00000038648	Creb3l2	11.44	ENSMUSG00000058794	Nfe2	5.13
ENSMUSG00000013089	Etv5	15.51	ENSMUSG00000026826	Nr4a2	4.8	ENSMUSG00000038679	Trps1	17.4	ENSMUSG00000058881	Zfp516	10.08
ENSMUSG00000013663	Pten	6.45	ENSMUSG00000026843	Fubp3	68.74	ENSMUSG00000038692	Hoxb4	3.03	ENSMUSG00000058883	Zfp708	4.34
ENSMUSG00000013787	Ehmt2	50.09	ENSMUSG00000026890	Lhx6	0.57	ENSMUSG00000038700	Hoxb5	38.94	ENSMUSG00000058886	Deaf1	45.89
ENSMUSG00000014030	Pax5	1.87	ENSMUSG00000026923	Notch1	47.94	ENSMUSG00000038705	Gmeb2	16.99	ENSMUSG00000058900	Rsl1	3.46
ENSMUSG00000014039	Prdm15	10.47	ENSMUSG00000026932	Nacc2	7.62	ENSMUSG00000038718	Pbx3	69.66	ENSMUSG00000059213	Ddn	0.38
ENSMUSG00000014303	Glis2	28.68	ENSMUSG00000026934	Lhx3	20.08	ENSMUSG00000038721	Hoxb7	52.22	ENSMUSG00000059246	Foxb1	2.94
ENSMUSG00000014592	Camta1	59.85	ENSMUSG00000026942	Traf2	23.28	ENSMUSG00000038765	Lmx1b	1.31	ENSMUSG00000059291	Rpl11	316.52
ENSMUSG00000014603	Alx3	0.47	ENSMUSG00000026976	Pax8	20.6	ENSMUSG00000038797	Zscan2	3.45	ENSMUSG00000059436	Max	14.77
ENSMUSG00000014704	Hoxa2	30.64	ENSMUSG00000027102	Hoxd8	18.27	ENSMUSG00000038805	Six3	0.11	ENSMUSG00000059552	Trp53	133.82
ENSMUSG00000014767	Tbp	66.37	ENSMUSG00000027104	Atf2	65.37	ENSMUSG00000038845	Phb	178.78	ENSMUSG00000059669	Taf1b	25.01
ENSMUSG00000014859	E2f4	80.34	ENSMUSG00000027109	Sp3	53.47	ENSMUSG00000038872	Zf hx3	29.02	ENSMUSG00000059824	Dbp	2.77
ENSMUSG00000015053	Gata2	4.7	ENSMUSG00000027164	Traf6	17.41	ENSMUSG00000038909	Kat7	70.05	ENSMUSG00000059897	Zfp930	14.06
ENSMUSG00000015092	Edf1	271.47	ENSMUSG00000027168	Pax6	22.32	ENSMUSG00000039005	Tlr4	0.66	ENSMUSG00000060314	Zfp941	6.14
ENSMUSG00000015120	Ube2i	612.01	ENSMUSG00000027186	Elf5	0.1	ENSMUSG00000039086	Ss18l1	16.58	ENSMUSG00000060336	Zfp937	11.11
ENSMUSG00000015461	Atf6b	70.02	ENSMUSG00000027210	Meis2	93.02	ENSMUSG00000039087	Rreb1	4.69	ENSMUSG00000060373	Hnrnpc	347.7
ENSMUSG00000015501	Hivep2	5.78	ENSMUSG00000027230	Creb3l1	1.56	ENSMUSG00000039095	En2	0.02	ENSMUSG00000060397	Zfp128	5.51
ENSMUSG00000015522	Arnt	21.46	ENSMUSG00000027387	Zc3h8	7.23	ENSMUSG00000039117	Taf4	6.11	ENSMUSG00000060477	Irak2	1.32
ENSMUSG00000015579	Nkx2-5	0.04	ENSMUSG00000027433	Xrn2	86.87	ENSMUSG00000039153	Runx2	1.06	ENSMUSG00000060601	Nr1h2	55.15
ENSMUSG00000015605	Srf	3.51	ENSMUSG00000027434	Nkx2-2	5.25	ENSMUSG00000039158	Akna	4.72	ENSMUSG00000060743	H3f3a	2565.29
ENSMUSG00000015619	Gata3	6.85	ENSMUSG00000027439	Gzf1	21.61	ENSMUSG00000039191	Rbpj	45.34	ENSMUSG00000060969	Irx1	1.49
ENSMUSG00000015627	Gata5	0.12	ENSMUSG00000027490	E2f1	11.03	ENSMUSG00000039231	Suv39h1	67.15	ENSMUSG00000061013	Mkx	5.22
ENSMUSG00000015709	Arnt2	20.88	ENSMUSG00000027544	Nfatc2	0.74	ENSMUSG00000039238	Zfp750	0.46	ENSMUSG00000061311	Rag1	0.13
ENSMUSG00000015839	Nfe2l2	21.01	ENSMUSG00000027547	Sall4	6.15	ENSMUSG00000039275	Foxk2	16.32	ENSMUSG00000061360	Phf5a	176.84
ENSMUSG00000015843	Rxrg	6.57	ENSMUSG00000027552	E2f5	54.32	ENSMUSG00000039377	Hlx	7.42	ENSMUSG00000061371	Zfp873	9.47
ENSMUSG00000015846	Rxra	5.71	ENSMUSG00000027582	Zgpat	36.51	ENSMUSG00000039410	Prdm16	8.5	ENSMUSG00000061436	Hipk2	24.16
ENSMUSG00000015937	H2afy	485.52	ENSMUSG00000027660	Skil	47.65	ENSMUSG00000039474	Wfs1	7.07	ENSMUSG00000061517	Sox21	10.14
ENSMUSG00000016087	Fli1	18	ENSMUSG00000027667	Zfp639	38.55	ENSMUSG00000039476	Prrx2	7.13	ENSMUSG00000061524	Zic2	4.97
ENSMUSG00000016458	Wt1	1.04	ENSMUSG00000027671	Actl6a	185.1	ENSMUSG00000039477	Tnrc18	21.12	ENSMUSG00000061544	Zfp229	5.81
ENSMUSG00000016477	E2f3	13.6	ENSMUSG00000027678	Ncoa3	17.73	ENSMUSG00000039521	Foxp3	0.04	ENSMUSG00000061894	Zscan20	3.54
ENSMUSG00000016503	Gtf3a	69.73	ENSMUSG00000027684	Mecom	25.2	ENSMUSG00000039634	Zfp189	11.93	ENSMUSG00000061911	Myl1l	4.89
ENSMUSG00000016559	H3f3b	1424.99	ENSMUSG00000027794	Sohlh2	0.07	ENSMUSG00000039656	Rxbp1	20.31	ENSMUSG00000062012	Zfp13	15.86
ENSMUSG00000016624	Phf21b	25.47	ENSMUSG00000027796	Smad9	0.45	ENSMUSG00000039671	Zmynd8	42.03	ENSMUSG00000062040	Zfp27	11.46
ENSMUSG00000017007	Rbpjl	0.01	ENSMUSG00000027833	Shox2	16.5	ENSMUSG00000039699	Batf2	0.15	ENSMUSG00000062115	Rai1	22.27
ENSMUSG00000017491	Rarb	57.44	ENSMUSG00000027859	Ngf	0.7	ENSMUSG00000039741	Bahcc1	15.58	ENSMUSG00000062116	Zfp954	14.6
ENSMUSG00000017548	Suz12	16.9	ENSMUSG00000027868	Tbx15	1.7	ENSMUSG00000039830	Olig2	1.67	ENSMUSG00000062327	T	1.66
ENSMUSG00000017667	Zfp334	18.25	ENSMUSG00000027938	Creb3l4	2.74	ENSMUSG00000039834	Zfp335	11.03	ENSMUSG00000062329	Cyt11	1.02
ENSMUSG00000017688	Hnf4g	0.08	ENSMUSG00000027967	Neurog2	32.66	ENSMUSG00000039852	Rere	40.36	ENSMUSG00000062563	Cys1	1.23
ENSMUSG00000017724	Etv4	7.41	ENSMUSG00000027985	Lef1	20.37	ENSMUSG00000039853	Trim14	0.71	ENSMUSG00000062713	Sim2	0.14

ENSMUSG00000017801	Mlx	47.87	ENSMUSG00000028023	Pitx2	12.77	ENSMUSG00000039910	Cited2	64.29	ENSMUSG00000062743	Zfp677	3.03
ENSMUSG00000017861	Mybl2	21.54	ENSMUSG00000028042	Zbtb7b	0.2	ENSMUSG00000039997	Ifi203	0.39	ENSMUSG00000062794	Zfp599	5.1
ENSMUSG00000017950	Hnf4a	0.01	ENSMUSG00000028086	Fbxw7	18.16	ENSMUSG00000040007	Bahd1	1.15	ENSMUSG00000062861	Zfp28	7.51
ENSMUSG00000018143	Mafk	6.56	ENSMUSG00000028161	Ppp3ca	55.52	ENSMUSG00000040033	Stat2	10.9	ENSMUSG00000062939	Stat4	0.86
ENSMUSG00000018160	Med1	58.93	ENSMUSG00000028163	Nfkb1	12.23	ENSMUSG00000040054	Baz2a	12.43	ENSMUSG00000062944	9130023H24Rik	10.19
ENSMUSG00000018168	Ikzf3	0.07	ENSMUSG00000028266	Lmo4	52.05	ENSMUSG00000040123	Zmym5	13.64	ENSMUSG00000063047	Zfp780b	3.34
ENSMUSG00000018263	Tbx5	0.36	ENSMUSG00000028341	Nr4a3	0.43	ENSMUSG00000040138	Ndp	6.12	ENSMUSG00000063060	Sox7	7.63
ENSMUSG00000018347	Zkscan6	11.52	ENSMUSG00000028417	Tal2	0.16	ENSMUSG00000040148	Hmx3	1.11	ENSMUSG00000063065	Mapk3	42.69
ENSMUSG00000018476	Kdm6b	33.3	ENSMUSG00000028423	Nfx1	72.44	ENSMUSG00000040167	Ikzf5	26.66	ENSMUSG00000063108	Zfp26	24.18
ENSMUSG00000018501	Ncor1	138.66	ENSMUSG00000028466	Creb3	49.77	ENSMUSG00000040187	Arntl2	0.83	ENSMUSG00000063281	Zfp35	25.53
ENSMUSG00000018548	Trim37	62.32	ENSMUSG00000028517	Plpp3	38.3	ENSMUSG00000040209	Zfp704	4.98	ENSMUSG00000063358	Mapk1	170.96
ENSMUSG00000018604	Tbx3	7.59	ENSMUSG00000028565	Nfia	22.69	ENSMUSG00000040270	Bach2	23.49	ENSMUSG00000063535	Zfp773	5.31
ENSMUSG00000018651	Tada2a	25.98	ENSMUSG00000028582	Cc2d1b	13.99	ENSMUSG00000040289	Hey1	38.85	ENSMUSG00000063632	Sox11	476.41
ENSMUSG00000018654	Ikzf1	2.52	ENSMUSG00000028610	Dmrtb1	4.57	ENSMUSG00000040310	Alx4	1.67	ENSMUSG00000063659	Zbth18	9.04
ENSMUSG00000018678	Sp2	23.52	ENSMUSG00000028634	Hivep3	3.93	ENSMUSG00000040321	Zfp770	4.29	ENSMUSG00000063672	Nkx6-3	0.1
ENSMUSG00000018697	Aatf	43.67	ENSMUSG00000028639	Ybx1	1001.84	ENSMUSG00000040390	Map3k10	7.01	ENSMUSG00000063870	Chd4	98.25
ENSMUSG00000018698	Lhx1	36.7	ENSMUSG00000028640	Tfap2c	1.02	ENSMUSG00000040433	Zbth38	9.97	ENSMUSG00000063889	Crem	14.21
ENSMUSG00000018750	Zbth4	0.79	ENSMUSG00000028654	Mycl	80.26	ENSMUSG00000040463	Mybbp1a	116.89	ENSMUSG00000063894	Zkscan8	8.18
ENSMUSG00000018822	Sfrp5	3.28	ENSMUSG00000028707	Dmbx1	0.61	ENSMUSG00000040481	Bptf	33.22	ENSMUSG00000063972	Nr6a1	4.95
ENSMUSG00000018899	Irf1	3.14	ENSMUSG00000028717	Tal1	5.87	ENSMUSG00000040489	Sox30	0.08	ENSMUSG00000066224	Arid3c	0.94
ENSMUSG00000018973	Hoxb1	13.59	ENSMUSG00000028736	Pax7	3	ENSMUSG00000040610	Tlx3	22.57	ENSMUSG00000066456	Hmgn3	82.36
ENSMUSG00000018983	E2f2	5.7	ENSMUSG00000028756	Pink1	23.16	ENSMUSG00000040629	Mael	0.04	ENSMUSG00000066613	Zfp932	18.32
ENSMUSG00000019230	Lhx9	8.9	ENSMUSG00000028800	Hdac1	204.51	ENSMUSG00000040712	Camta2	8.43	ENSMUSG00000066687	Zbth16	3.82
ENSMUSG00000019256	Ahr	3.86	ENSMUSG00000028820	Sfpq	124.38	ENSMUSG00000040726	Hexx1	0.05	ENSMUSG00000066838	Zfp772	8.79
ENSMUSG00000019564	Arid3a	5.3	ENSMUSG00000028890	Mtf1	12.11	ENSMUSG00000040732	Erg	1.66	ENSMUSG00000066839	Ecsit	30.31
ENSMUSG00000019768	Esr1	0.06	ENSMUSG00000028899	Taf12	46.53	ENSMUSG00000040761	Spen	12.98	ENSMUSG00000067071	Hes6	250.56
ENSMUSG00000019777	Hdac2	355.16	ENSMUSG00000028901	Gmeb1	32.61	ENSMUSG00000040841	Six5	5.86	ENSMUSG00000067261	Foxd3	4.41
ENSMUSG00000019789	Hey2	3.57	ENSMUSG00000028964	Park7	330.24	ENSMUSG00000040857	Erf	13.08	ENSMUSG00000067438	Hmx1	6.85
ENSMUSG00000019803	Nr2e1	0.08	ENSMUSG00000028975	Pex14	34.08	ENSMUSG00000040891	Foxa3	0.03	ENSMUSG00000067724	Gbx1	1.01
ENSMUSG00000019817	Plagl1	118.59	ENSMUSG00000028988	Ctnnbip1	52.18	ENSMUSG00000040929	Rfx3	22.48	ENSMUSG00000067860	Zic3	15.41
ENSMUSG00000019826	Zbth24	13.96	ENSMUSG00000029003	Mad2l2	57.07	ENSMUSG00000041000	Trim62	16.33	ENSMUSG00000067931	Zfp948	16.27
ENSMUSG00000019878	Hsf2	42.52	ENSMUSG00000029026	Trp73	0.24	ENSMUSG00000041168	Lonp1	43.98	ENSMUSG00000067942	Zfp160	11.45
ENSMUSG00000019900	Rfx6	0.12	ENSMUSG00000029075	Tnfrsf4	0.36	ENSMUSG00000041187	Prkd2	8.03	ENSMUSG00000068130	Zfp442	7.17
ENSMUSG00000019913	Sim1	1.54	ENSMUSG00000029127	Zbth49	15.77	ENSMUSG00000041235	Chd7	89.29	ENSMUSG00000068154	Insm1	7.33
ENSMUSG00000019947	Arid5b	10.51	ENSMUSG00000029135	Fosl2	0.43	ENSMUSG00000041309	Nkx6-2	5.61	ENSMUSG00000068327	Tlx2	23.94
ENSMUSG00000019982	Myb	3.51	ENSMUSG00000029167	Ppargc1a	2.4	ENSMUSG00000041343	Ankrd42	7.7	ENSMUSG00000068457	Uty	8.34
ENSMUSG00000020037	Rfx4	49.77	ENSMUSG00000029191	Rfc1	59.92	ENSMUSG00000041420	Meis3	153.05	ENSMUSG00000068551	Zfp467	8.96
ENSMUSG00000020038	Cry1	27.19	ENSMUSG00000029196	Tada2b	3.3	ENSMUSG00000041483	Zfp281	32.16	ENSMUSG00000068742	Cry2	16.94
ENSMUSG00000020052	Ascl1	32.09	ENSMUSG00000029238	Clock	5.94	ENSMUSG00000041515	Irf8	2.79	ENSMUSG00000068959	Zfp619	3.16
ENSMUSG00000020063	Sirt1	10.55	ENSMUSG00000029249	Rest	19.85	ENSMUSG00000041530	Ago1	20.81	ENSMUSG00000069171	Nr2f1	46.38
ENSMUSG00000020086	H2afy2	194.68	ENSMUSG00000029313	Aff1	8.68	ENSMUSG00000041540	Sox5	24.48	ENSMUSG00000069184	Zfp72	4.99
ENSMUSG00000020160	Meis1	150.77	ENSMUSG00000029475	Kdm2b	59.29	ENSMUSG00000041565	L3mbtl4	0.18	ENSMUSG00000069727	Gm5595	7.63
ENSMUSG00000020167	Tcf3	141.82	ENSMUSG00000029478	Ncor2	19.22	ENSMUSG00000041703	Zic5	8.13	ENSMUSG00000070345	Hsf5	0.19
ENSMUSG00000020185	E2f7	23.39	ENSMUSG00000029484	Anxa3	23.28	ENSMUSG00000041730	Prrxl1	12.01	ENSMUSG00000070420	Zscan25	0.35
ENSMUSG00000020230	Prmt2	91	ENSMUSG00000029546	Uncx	41.48	ENSMUSG00000041852	Tcf20	8.17	ENSMUSG00000070493	Chchd2	802.88
ENSMUSG00000020232	Hmg20b	82.34	ENSMUSG00000029556	Hnf1a	0.02	ENSMUSG00000041911	Dlx1	0.93	ENSMUSG00000070495	Ctcf	0.04
ENSMUSG00000020248	Nfyb	58.36	ENSMUSG00000029563	Foxp2	24.46	ENSMUSG00000042002	Foxn4	1.34	ENSMUSG00000070544	Top1	82.94

ENSMUSG00000020275	Rel	0.74	ENSMUSG00000029580	Actb	5603.4	ENSMUSG00000042063	Zfp386	56.05	ENSMUSG00000070632	Btbd8	2.17
ENSMUSG00000020335	Zfp354b	1.29	ENSMUSG00000029587	Zfp12	21.85	ENSMUSG00000042197	Zfp451	37.28	ENSMUSG00000070643	Sox13	9.73
ENSMUSG00000020358	Hnmpab	602.52	ENSMUSG00000029595	Lhx5	24.35	ENSMUSG00000042258	Isl1	109.32	ENSMUSG00000070691	Runt3	2.38
ENSMUSG00000020364	Zfp354a	6.76	ENSMUSG00000029627	Zkscan14	6.74	ENSMUSG00000042292	Mkl1	18.62	ENSMUSG00000070709	1700049G17Rik	10.56
ENSMUSG00000020420	Zfp607	2.2	ENSMUSG00000029687	Ezh2	95.94	ENSMUSG00000042372	Dmrt3	1.87	ENSMUSG00000070822	Zscan18	7.33
ENSMUSG00000020453	Patz1	83.04	ENSMUSG00000029705	Cux1	86.03	ENSMUSG00000042390	Gatad2b	6.94	ENSMUSG00000071054	Safb	91.88
ENSMUSG00000020472	Zkscan17	20.57	ENSMUSG00000029723	Tsc22d4	57.86	ENSMUSG00000042406	Atf4	105.31	ENSMUSG00000071076	Jund	62.12
ENSMUSG00000020473	Aebp1	7.13	ENSMUSG00000029729	Zkscan1	35.28	ENSMUSG00000042448	Hoxd1	7.74	ENSMUSG00000071256	Zfp213	18.73
ENSMUSG00000020484	Xbp1	131.03	ENSMUSG00000029754	Dlx6	0.03	ENSMUSG00000042477	Tfp2e	0.08	ENSMUSG00000071291	Zfp58	14.03
ENSMUSG00000020538	Srebfl1	42.29	ENSMUSG00000029755	Dlx5	0.59	ENSMUSG00000042499	Hoxd11	6.74	ENSMUSG00000071646	Mta2	45.55
ENSMUSG00000020542	Myocd	1.98	ENSMUSG00000029771	Irf5	1.78	ENSMUSG00000042557	Sin3a	43.8	ENSMUSG00000071665	Foxr2	0.11
ENSMUSG00000020601	Trib2	62.72	ENSMUSG00000029832	Nfe2l3	0.34	ENSMUSG00000042589	Cux2	10.52	ENSMUSG00000071757	Zhx2	6.49
ENSMUSG00000020644	Id2	234.2	ENSMUSG00000029833	Trim24	31.64	ENSMUSG00000042596	Tfp2d	0.17	ENSMUSG00000072294	Klf12	15.41
ENSMUSG00000020653	Klf11	13.07	ENSMUSG00000029836	Cbx3	487.86	ENSMUSG00000042622	Maff	0.98	ENSMUSG00000072889	Nflx1	20.6
ENSMUSG00000020656	Grhl1	0.48	ENSMUSG00000029840	Mtpn	120.48	ENSMUSG00000042745	Id1	92.13	ENSMUSG00000073043	Atoh1	13.61
ENSMUSG00000020679	Hnf1b	0.22	ENSMUSG00000029844	Hoxa1	7.36	ENSMUSG00000042750	Bex2	236.45	ENSMUSG00000073176	Zfp449	6.51
ENSMUSG00000020871	Dlx4	0.09	ENSMUSG00000029913	Prdm5	10.56	ENSMUSG00000042812	Foxf1	2.07	ENSMUSG00000073489	Ifi204	0.45
ENSMUSG00000020875	Hoxb9	54.04	ENSMUSG00000030001	Figla	0.08	ENSMUSG00000042821	Snai1	25.58	ENSMUSG00000073490	Al607873	0.44
ENSMUSG00000020888	Dvl2	30.83	ENSMUSG00000030067	Foxp1	91.7	ENSMUSG00000042895	Abra	0.03	ENSMUSG00000074151	Nlrc5	0.03
ENSMUSG00000020889	Nr1d1	1.54	ENSMUSG00000030087	Klf15	2.23	ENSMUSG00000042903	Foxo4	2.11	ENSMUSG00000074158	9830147E19Rik	1.1
ENSMUSG00000020893	Per1	8.39	ENSMUSG00000030103	Bhlhe40	2.77	ENSMUSG00000043013	Onecut1	6.73	ENSMUSG00000074220	Zfp382	8.76
ENSMUSG00000020914	Top2a	268.02	ENSMUSG00000030189	Ybx3	43.07	ENSMUSG00000043099	Hic1	3.26	ENSMUSG00000074221	Zfp568	12.85
ENSMUSG00000020919	Stat5b	14.44	ENSMUSG00000030199	Etv6	13.31	ENSMUSG00000043219	Hoxa6	12.65	ENSMUSG00000074406	Zfp628	3.03
ENSMUSG00000020923	Ubtf	74.19	ENSMUSG00000030201	Lrp6	14.67	ENSMUSG00000043263	Pyhin1	0.02	ENSMUSG00000074519	Etohi1	11.12
ENSMUSG00000020950	Foxg1	0.69	ENSMUSG00000030232	Aebp2	10.15	ENSMUSG00000043342	Hoxd9	11.29	ENSMUSG00000074622	Mafb	10.53
ENSMUSG00000020954	Strn3	38.3	ENSMUSG00000030256	Bhlhe41	0.83	ENSMUSG00000043456	Zfp536	18.1	ENSMUSG00000074637	Sox2	26.22
ENSMUSG00000021010	Npas3	10.09	ENSMUSG00000030353	Tead4	1.55	ENSMUSG00000043535	Setx	9.77	ENSMUSG00000074676	Foxs1	0.37
ENSMUSG00000021055	Esr2	0.09	ENSMUSG00000030380	Mzf1	1.75	ENSMUSG00000043602	Zfp3	8.38	ENSMUSG00000074731	Zfp345	0.02
ENSMUSG00000021067	Sav1	8.84	ENSMUSG00000030386	Zfp606	38.21	ENSMUSG00000043753	Dmrt1	0.29	ENSMUSG00000074824	Rslcn18	0.23
ENSMUSG00000021095	Gsc	0.43	ENSMUSG00000030393	Zik1	37.77	ENSMUSG00000043866	Taf10	152.03	ENSMUSG00000074865	Zfp934	10.12
ENSMUSG00000021098	4930447C04Rik	1.48	ENSMUSG00000030424	Zfp939	4.02	ENSMUSG00000043909	Trp53bp1	116.78	ENSMUSG00000074867	Zfp808	7.51
ENSMUSG00000021109	Hif1a	103.48	ENSMUSG00000030443	Zfp583	6.49	ENSMUSG00000043962	Thrap3	155.51	ENSMUSG00000075304	Sp5	1.81
ENSMUSG00000021144	Mta1	17.03	ENSMUSG00000030446	Zfp273	5.36	ENSMUSG00000043969	Emx2	1.32	ENSMUSG00000075394	Hoxc4	53.38
ENSMUSG00000021156	Zmynd11	80.68	ENSMUSG00000030469	Zfp719	14.58	ENSMUSG00000043991	Pura	1.56	ENSMUSG00000075588	Hoxb2	70.44
ENSMUSG00000021239	Vsx2	5.89	ENSMUSG00000030470	Csrp3	1.95	ENSMUSG00000044167	Foxo1	3.37	ENSMUSG00000076431	Sox4	128.34
ENSMUSG00000021250	Fos	1.99	ENSMUSG00000030507	Dbx1	14.71	ENSMUSG00000044220	Nkx2-3	0.08	ENSMUSG00000078302	Foxd1	8.77
ENSMUSG00000021255	Esrrb	0.75	ENSMUSG00000030543	Mesp2	0.61	ENSMUSG00000044296	Zfp879	0.44	ENSMUSG00000078427	Sarmp	142.22
ENSMUSG00000021264	Yy1	18.89	ENSMUSG00000030551	Nr2f2	49.8	ENSMUSG00000044312	Neurog3	10.01	ENSMUSG00000078496	Gm13152	4.87
ENSMUSG00000021318	Gli3	25.34	ENSMUSG00000030557	Mef2a	22.99	ENSMUSG00000044501	Zfp758	7.04	ENSMUSG00000078502	Gm13212	1.28
ENSMUSG00000021319	Sfrp4	1.28	ENSMUSG00000030604	Zfp626	0.93	ENSMUSG00000044518	Foxe3	0.15	ENSMUSG00000078580	E430018J23Rik	5.55
ENSMUSG00000021326	Trim27	92.64	ENSMUSG00000030678	Maz	66.8	ENSMUSG00000044636	Csrnp2	24.31	ENSMUSG00000078619	Smarcd2	35.28
ENSMUSG00000021327	Zkscan3	57.88	ENSMUSG00000030699	Tbx6	0.31	ENSMUSG00000044646	Zbtb7c	2.17	ENSMUSG00000078671	Chd2	15.89
ENSMUSG00000021356	Irf4	0.01	ENSMUSG00000030757	Zkscan2	5.15	ENSMUSG00000044647	Csrnp3	12.99	ENSMUSG00000078779	Zfp59	9.26
ENSMUSG00000021359	Tfp2a	9.29	ENSMUSG00000030793	Pycard	2.94	ENSMUSG00000044674	Fzd1	15	ENSMUSG00000078866	Gm14420	7.92
ENSMUSG00000021362	Gcm2	0.06	ENSMUSG00000030796	Tead2	205.03	ENSMUSG00000044676	Zfp612	9.51	ENSMUSG00000078896	Gm14436	5.37
ENSMUSG00000021366	Hivep1	8.41	ENSMUSG00000030823	9130019O22Rik	7.02	ENSMUSG00000044807	Zfp354c	38.67	ENSMUSG00000079033	Mef2b	0.46
ENSMUSG00000021381	Barx1	0.04	ENSMUSG00000030966	Trim21	0.1	ENSMUSG00000044876	Zfp444	55.99	ENSMUSG00000079037	Prnp	26.24

ENSMUSG00000021457	Syk	3.05	ENSMUSG00000031079	Zfp300	1.96	ENSMUSG00000044934	Zfp367	11.37	ENSMUSG00000079056	Kcnp3	2.67
ENSMUSG00000021466	Ptch1	15.38	ENSMUSG00000031103	Elf4	1.81	ENSMUSG00000045179	Sox3	8.7	ENSMUSG00000079215	Zfp664	123.24
ENSMUSG00000021469	Msx2	16.02	ENSMUSG00000031162	Gata1	3.89	ENSMUSG00000045268	Zfp691	9	ENSMUSG00000079277	Hoxd3	43.5
ENSMUSG00000021488	Nsd1	25.28	ENSMUSG00000031241	Tbx22	0.07	ENSMUSG00000045302	Preb	81.64	ENSMUSG00000079487	Med12	32.8
ENSMUSG00000021506	Pitx1	2.05	ENSMUSG00000031311	Nono	522.48	ENSMUSG00000045333	Zfp423	31.54	ENSMUSG00000079509	Zfx	19.23
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ENSMUSG00000021546	Hnrnpk	2038.29	ENSMUSG00000031365	Zfp275	14.31	ENSMUSG00000045518	Onecut3	1	ENSMUSG00000087598	Zfp111	9.49
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ENSMUSG00000021690	Jmy	2.86	ENSMUSG00000031451	Gas6	5.05	ENSMUSG00000045757	Zfp764	5.98	ENSMUSG00000090272	Mndal	0.38
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ENSMUSG00000021848	Otx2	0.26	ENSMUSG00000031665	Sall1	27.99	ENSMUSG00000046179	E2f8	13.09	ENSMUSG00000091764	Zfp964	1.23
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ENSMUSG00000021994	Wnt5a	9.97	ENSMUSG00000031706	Rfx1	6.28	ENSMUSG00000046518	Ferd3l	0.57	ENSMUSG00000093668	Pou5f2	0.82
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ENSMUSG00000022361	Zhx1	25.59	ENSMUSG00000032119	Hinfp	13.95	ENSMUSG00000048138	Dmrt2	12.02	ENSMUSG00000100235	Zfp708	3.92
ENSMUSG00000022383	Ppara	0.19	ENSMUSG00000032187	Smarca4	138.4	ENSMUSG00000048249	Crebrf	2.47	ENSMUSG00000101174	Hoxd4	8.89
ENSMUSG00000022389	Tef	10.82	ENSMUSG00000032228	Tcf12	165.62	ENSMUSG00000048251	Bcl11b	7.41			

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Supplementary Figure Legends

Supp. Fig. 1: Characterisation of AP2 α expression profiles in the embryonic dorsal spinal cord and roles of Dbx1 in restricting the generation of dorsal interneurons

A. Immunostaining for AP2 α /Sox9/GFP on thoracic sections of GD11.5 Pax3^{GFP/+} embryos showing that AP2 α is induced in the post-mitotic dorsal GFP⁺, Sox9⁻ neurons of the spinal cord. **B.** Immunostaining for AP2 α /Lbx1/Brn3a on brachial sections of GD11.5 wild-type embryos showing that AP2 α expression largely overlaps with the profile of Brn3a and Lbx1 with two exceptions. First, it is excluded from the most ventral Lbx1⁺ cells and its ventral boundary matches that of Brn3a (arrowheads). Hence its ventral expression includes the Brn3a⁺ dI5 IN and excludes the Lbx1⁺/Brn3a⁻ dI6 IN. Second, it is excluded from the most dorsal cells expressing Brn3a, likely the dI1 IN (arrows). **C.** Immunostaining for AP2 α /Lhx2 on brachial sections of GD10.5 wild-type embryos confirming the exclusion of AP2 α from dI1 IN. **D.** Representation of the spinal cord showing the distribution of AP2 α protein in red, characterised by the markers whose D-V extent are depicted in grey. **E.** Immunostaining for Pax2 and FoxD3, as well as GFP detection, on brachial sections of GD11.5 of Pax3^{K1GFP/+} and Pax3^{K1GFP/GFP}; Pax3^{K1LacZ/LacZ} (dKO) embryos, showing that, in absence of both Pax3 and Pax7 Pax2⁺;GFP⁺ cells express the V1 marker, FoxD3. The white dashed line in **i, i'** marks the border between dI5 and dI6 cells; the light green one in **i-ii'** segregates dorsal GFP⁺ cells from the ventral GFP⁻ cells. **F. i-ii** Immunostaining for AP2 α /Lbx1/ β Galactosidase on brachial sections of GD11.5 Dbx1^{K1LacZ/+} and Dbx1^{K1LacZ/LacZ} embryos showing that, in absence of Dbx1, p0 progenitors give rise to Lbx1⁺; AP2 α ⁻; Pax2⁺ dI6 interneurons (see brackets in **ii, ii'**). **iii** Quantification of Lbx1⁺; AP2 α ⁻ dI6 cells that derive from progenitors that have or have not expressed the β Galactosidase under the control of the Dbx1 locus (mean \pm s.e.m., n=4 Dbx1^{K1LacZ/+} embryos, n=2 Dbx1^{K1LacZ/LacZ} embryos). **iv.** Immunostaining for Pax2/Lbx1/ β Galactosidase on brachial sections of GD11.5 Dbx1^{K1LacZ/LacZ} embryos showing that Lbx1⁺ neurons derived from p0 progenitors express another dI6 marker, Pax2.

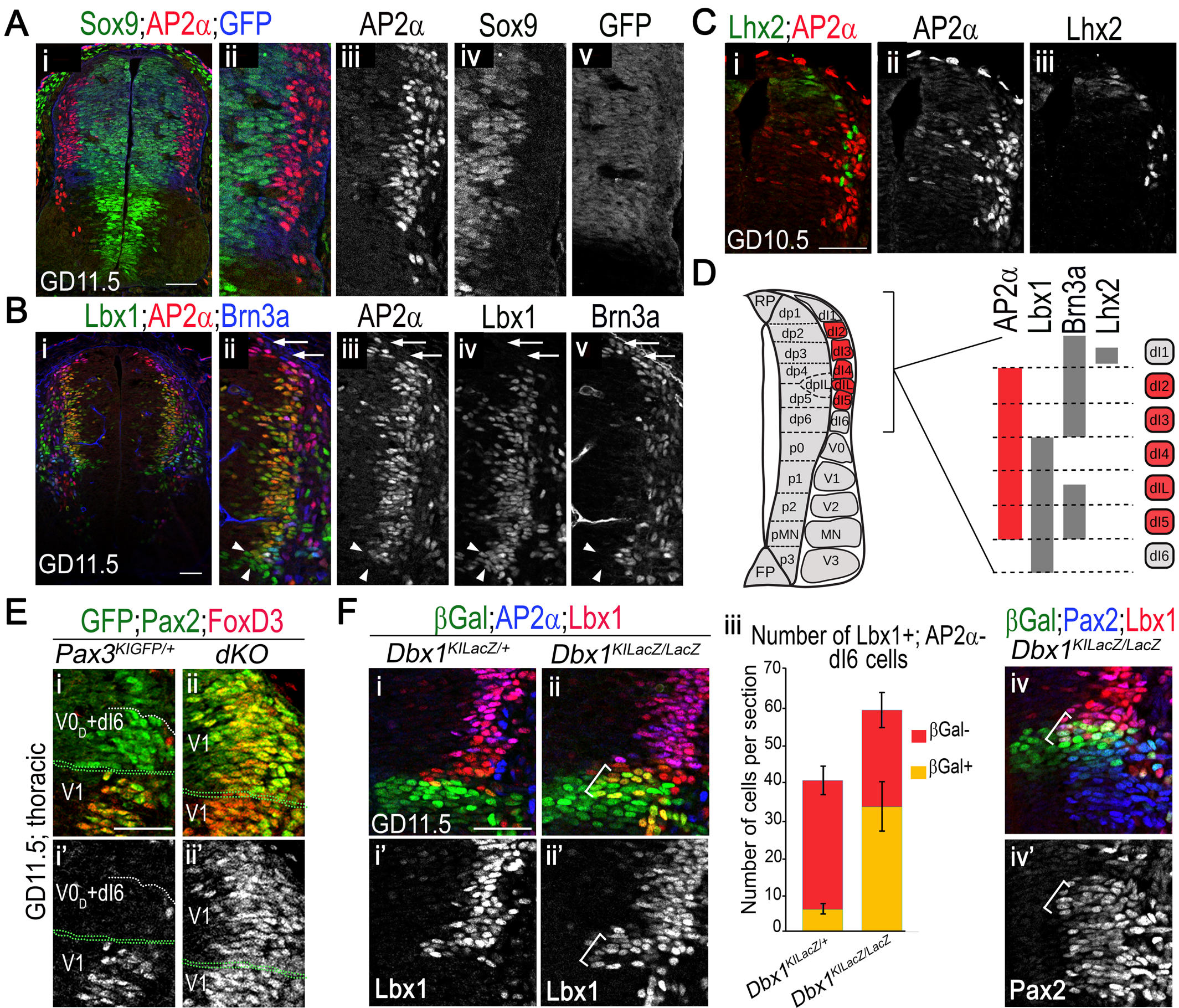
Supp. Fig. 2: Characterisation of Dbx1 profiles in wild-type and Pax3/Pax7 mutant embryos

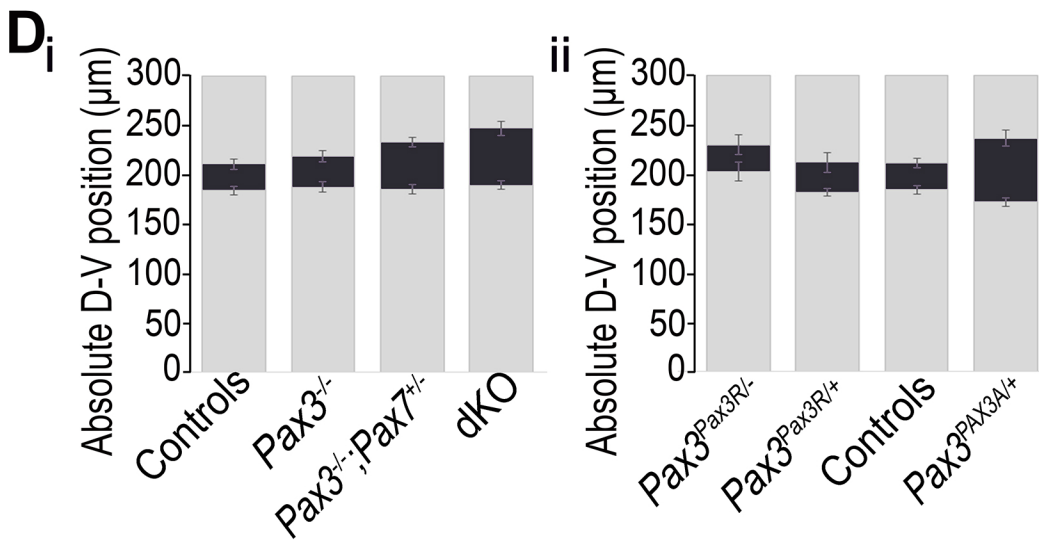
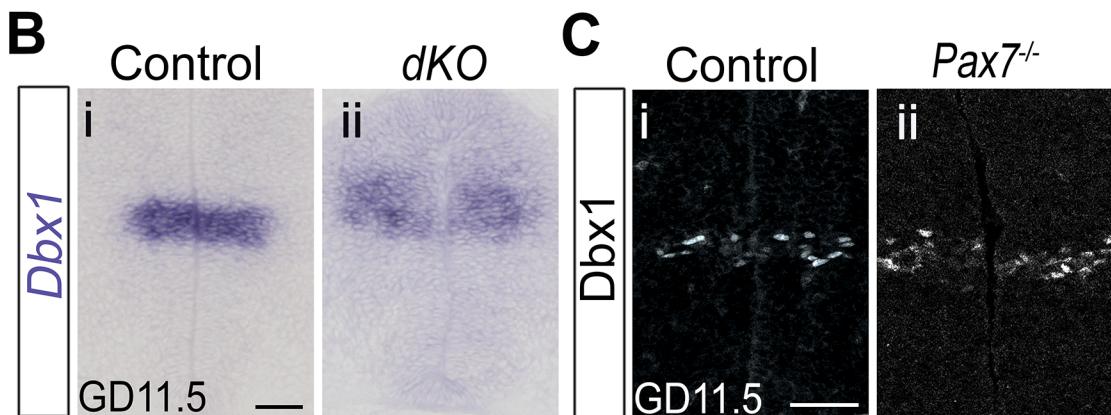
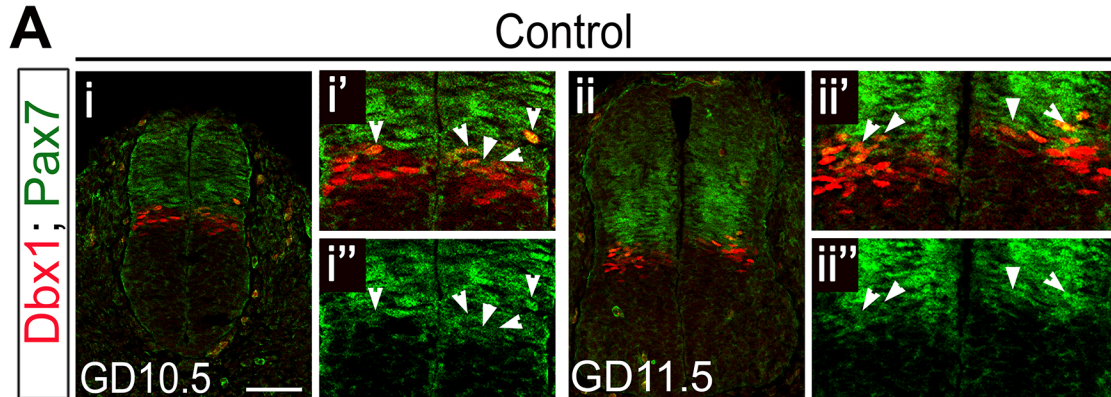
A. Immunostaining for Dbx1 and Pax7 in thoracic transverse sections of wild-type embryos at the indicated stages. **B-C.** *In situ* hybridisation for Dbx1 (**B**) and immunolabelling of Dbx1 (**C**) in brachial transverse sections of GD11.5 mouse embryos of the indicated genotype. **D.** Graphs displaying the absolute D-V position from the floor plate in μ m of Dbx1 protein

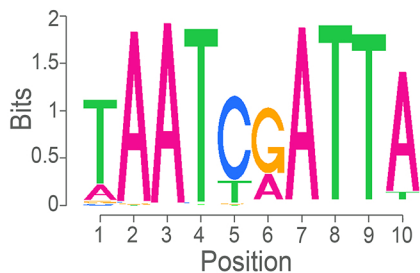
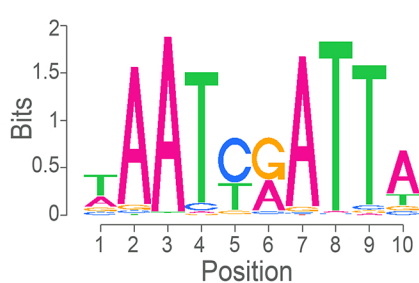
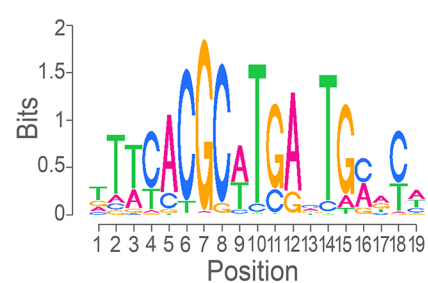
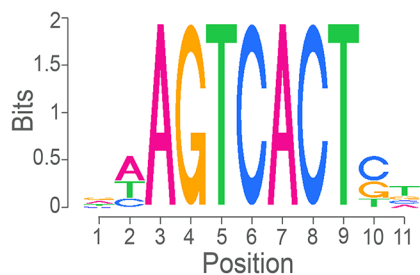
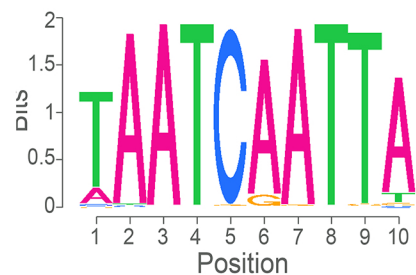
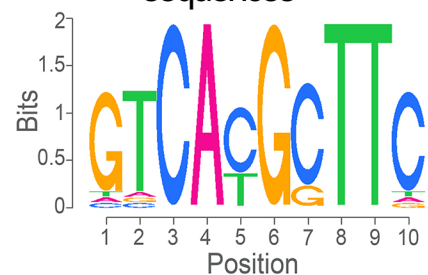
expression in GD11.5 mouse embryos with the indicated genotype (mean \pm s.e.m). Scale bars: 50 μ m.

Supp. Fig. 3: Pax Position Weight Matrices and response of Dbx1 CRMs to Pax3 and PAX3A

A. Position Weight Matrices retrieved from data generated by Jolma et al. (2013) (1), identified by MEME from ChIP-Seq data generated by Soleimani et al. (2012) (2), or created using MEME by compiling sites previously identified by Soleimani et al. (2012), Moore et al. (2013) and Relaix et al. (2003) (3). **B.** Immunostaining for β -Galactosidase and GFP combined with DAPI labelling performed on sections of chick embryos 24hpe with the indicated reporter constructs carrying *Dbx1* CRMs and either Pax3-GFP (**i-v'**) or PAX3A-GFP (**vi-x'**). Scale bars: 50 μ m.





APax7 HD BS from HT Selex⁽¹⁾Pax3/7 HD BS from HT Selex⁽¹⁾Pax6 PrD BS from HT Selex⁽¹⁾PrD BS from Pax3 ChIPseq⁽²⁾HD BS from Pax7 ChIPseq⁽²⁾PrD BS from selected sequences^(2,3,4)**B***CRM1::LacZ**CRM2::LacZ**CRM3::LacZ**CRM4.8kb::nLacZ**iCRM::LacZ*