Table S1. Difference in neural tube, mz and vz size in E11.5 neural tubes from multiple sections from *Cux2* transgenic and *Cux2*$^{neo/neo}$ mutants relative to control littermates

	VZ size	MZ size	Total NT size	NT height	NT width
Control versus *Cux2* transgenic (% difference; *n*=7)	108%	114%	111%	107%	110%
Control versus *Cux2*$^{neo/neo}$ (% difference; *n*=7)	87%*	51%*	72.2%*	98%	79.3%*

VZ, ventricular zone; MZ, marginal zone; NT, neural tube.
*Statistical significance (P<0.05) by Student's *t*-test.

Table S2. Effect of *Cux2* loss- and gain-of-function on neuronal cell fate in the spinal cord

	Control E10.5	*Cux2*$^{neo/neo}$ mutants E10.5	*Cux2* Tg E10.5
Isl1	67.12±14.6*	88.7±14.5	53.7±16.1
P value (*t*-test)†		0.000003	0.053
Lhx1	101.1±21.2	85.2±30.7	144.6±38.1
P value (*t*-test)		0.0007	0.0058
	Cux2$^{neo/neo}$/Control (%)	*Cux2* Tg/Control (%)	*Cux2*$^{neo/neo}$/Cux2 Tg (%)
Isl1	32	–20‡	61
P value (*t*-test)			0.000002
Lhx1	–16	43	–70
P value (*t*-test)			0.0007

Cux2$^{neo/neo}$ mutants display increased numbers of Isl1-positve cells and decreased Lhx1 numbers in the ventral neural tube at E10.5, relative to control littermates. *Cux2* transgenics (Tg), however, display decreased numbers of Isl1-positive cells and increased numbers of Lhx1-positive cells relative to control embryos in E10.5 ventral neural tubes.
*Figures are represented as average values±s.d.
†P values were determined using a one-tailed Student's *t*-test with two samples, unequal variance.
‡Negative sign denotes decrease.

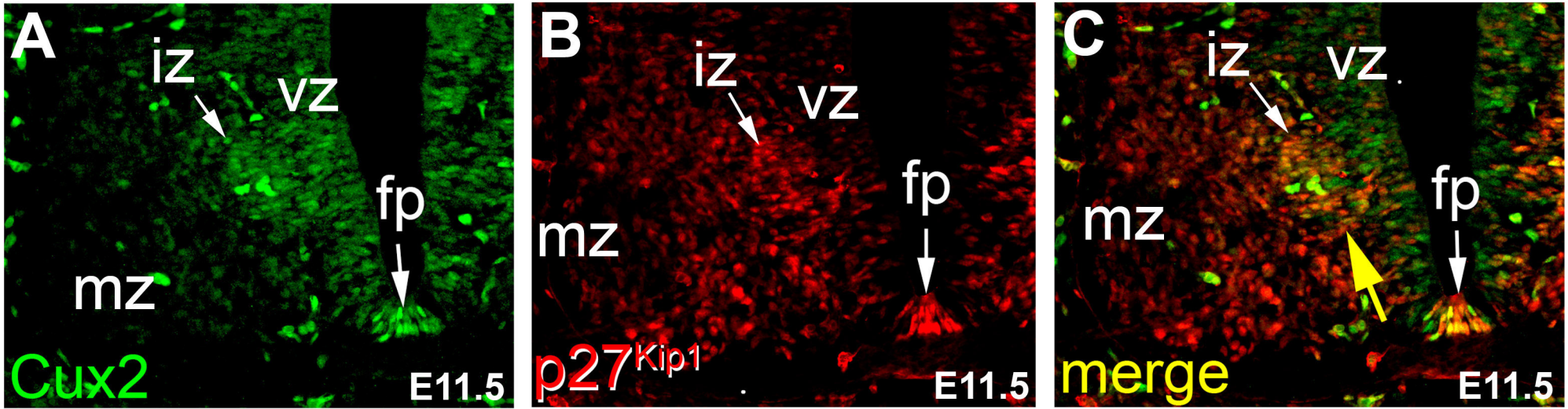
Supplementary Figure S1. Cux2 and $P27^{Kip1}$ protein levels in the developing spinal cord at E11.5. (A-C) Cux2 levels (A), $P27^{Kip1}$ levels (B), and the merged image of Cux2 /$P27^{Kip1}$ (yellow; C) in transverse sections of the ventral neural tube at the forelimb bud level of E11.5 embryos. (A) Little or no Cux2 levels were detected in the motor neuron domain in ventro-lateral mz. (C) Cux2 and $P27^{Kip1}$ are co-expressed in the iz and fp, but not in post-mitotic motor neurons in the mz, which expresses $p27^{Kip1}$ (red) but not Cux2 (green). Abbreviations: cn, commissural neurons; fp, floorplate; iz, intermediate zone; mz, marginal zone; vz, ventricular zone.

Supplementary Figure S2. The growth of the dorsal root ganglia (drg) is dependent on *Cux2* function. (A-C) Immunohistochemistry of NeuN in the drg of E11.5 wild type (A), $Cux2^{neo/neo}$ mutant (B), and *Nestin-Cux2-ires-EGFP* transgenic (C) embryos. (D-F) Immunohistochemistry of $P27^{Kip1}$ in wild type (D), *Cux2* mutant (E) and transgenic (F) drg. (G-I) Immunohistochemistry of NeuroD showing greatly reduced staining in *Cux2* mutant (H), and enhanced staining in *Cux2* transgenics (I) relative to controls (G). All three post-mitotic neuronal markers show robust expression in *Cux2* mutants and transgenics, but the drg is smaller in the mutants and larger in the transgenics.

Supplementary Figure S3. Modulation of Cux2 protein expression in gain-of-function and loss-of-function experiments in the chick and mouse embryonic spinal cord.
(A-C) Electroporation of HH12 chick neural tubes *in ovo* with *Cux2-IRES-nucEGFP* bicistronic vector (A) and analysis of Cux2 expression using a mouse-specific polyclonal antibody 48 hours later (B) reveals GFP-positive cells express mCux2 protein (C). (D-F) Cux2 protein expression in a *Nestin-Cux2-ires-EGFP* transgenic neural tube at E11.5. (D) High GFP expression co-labeling with ectopic Cux2 protein expression (E, F) in the vz. (G-H) Cux2 protein levels in the spinal cord at the forelimb level of control wild type (G) and $Cux2^{neo/neo}$ mutant (H) embryos at E10.5. (H) The *Cux2* mutation is hypomorphic and leads to a near complete loss of Cux2 expression in the ventral spinal cord at E10.5. Abbreviations: fp, floorplate.

Supplementary Figure S4. Dependence of expression of $P27^{Kip1}$ and NeuroD on *Cux2* function in the developing spinal cord. (A-B) $P27^{Kip1}$ (red) and NeuroD (green) immunohistochemistry on E9.5 trunk-level neural tubes from littermate control (A) and $Cux2^{neo/neo}$ mutants (B). $P27^{Kip1}$ is

localized in the maturing populations in the ventral-lateral neural tube, while NeuroD is localized in neuroblasts at lateral half of the vz. The occasional nuclei is positive for both $P27^{Kip1}$ and NeuroD (arrow), but most expression remains mutually exclusion. (B) *Cux2* mutants showed a strong reduction of $P27^{Kip1}$ levels at E9.5 and a less severe loss of NeuroD-positive nuclei at E9.5. (C-D) $P27^{Kip1}$ (red) and NeuroD (green) levels in forelimb bud level spinal cords at E10.5 in a littermate control (C) and a $Cux2^{neo/neo}$ mutant (D). At E10.5, $P27^{Kip1}$ and NeuroD levels are largely non-overlapping except for a 2-4 cell layer thick zone in the ventral half of the neural tube (arrow in C-D), which identifies the iz, and in the drg. (D) *Cux2* mutants show a loss of NeuroD expression in the neural tube at E10.5 (but not drg). $P27^{Kip1}$ levels were reduced in the mz of the medial neural tube, but enhanced in the mns, reflecting an increase in post-mitotic mns at the expense of in (Fig. 7).


A
iz
vz
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Cux2
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B
iz
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E11.5

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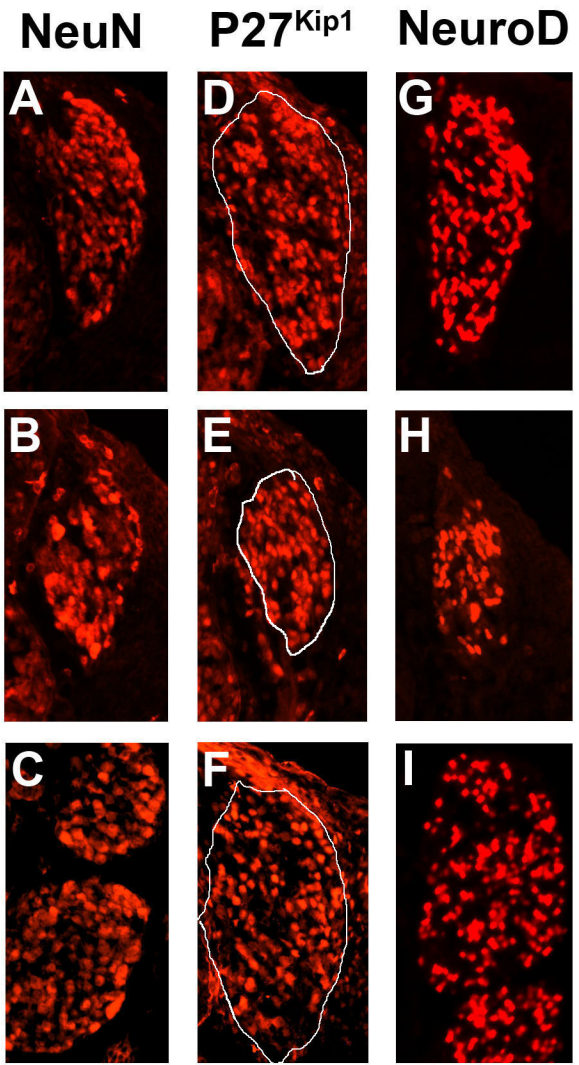

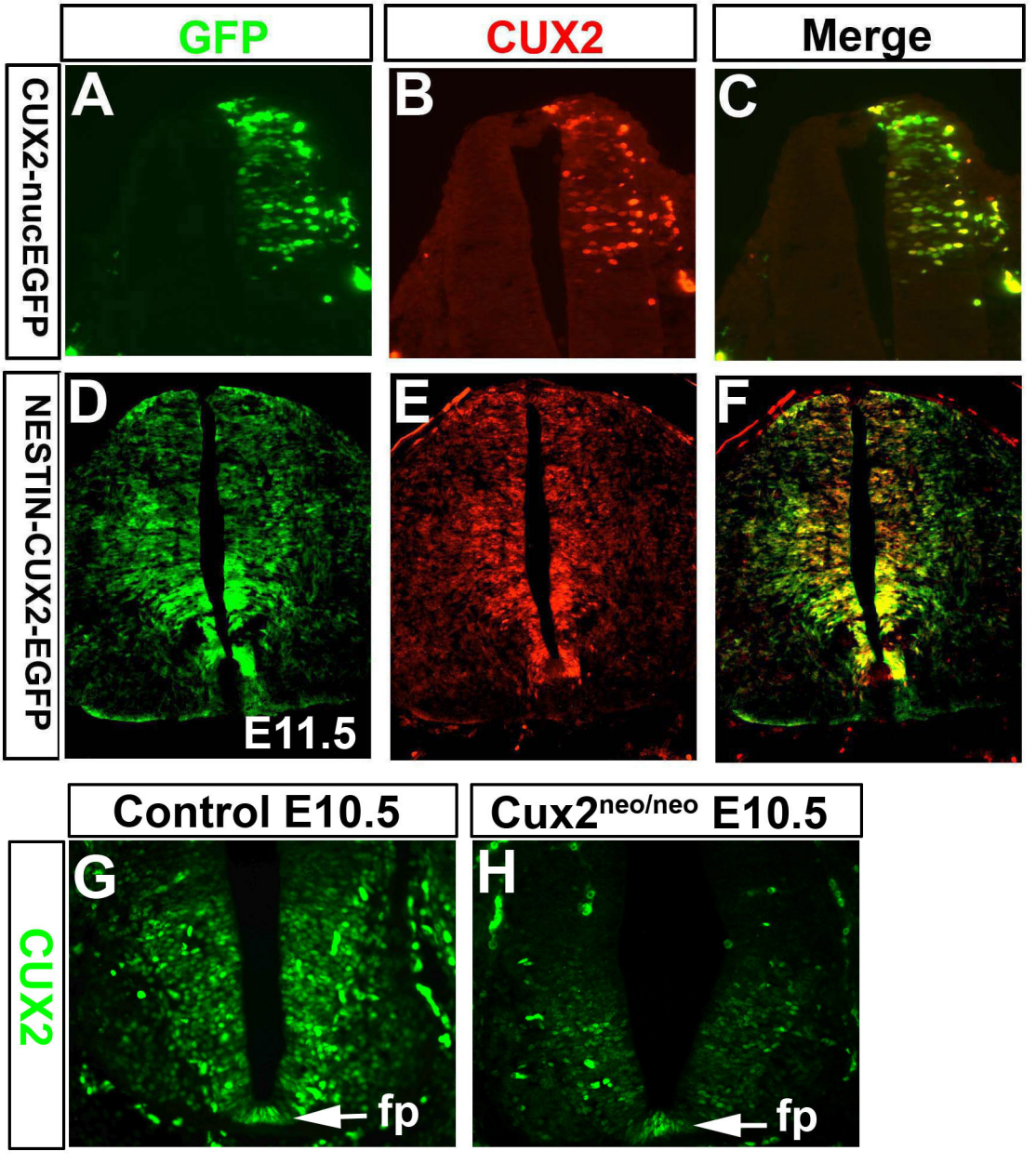
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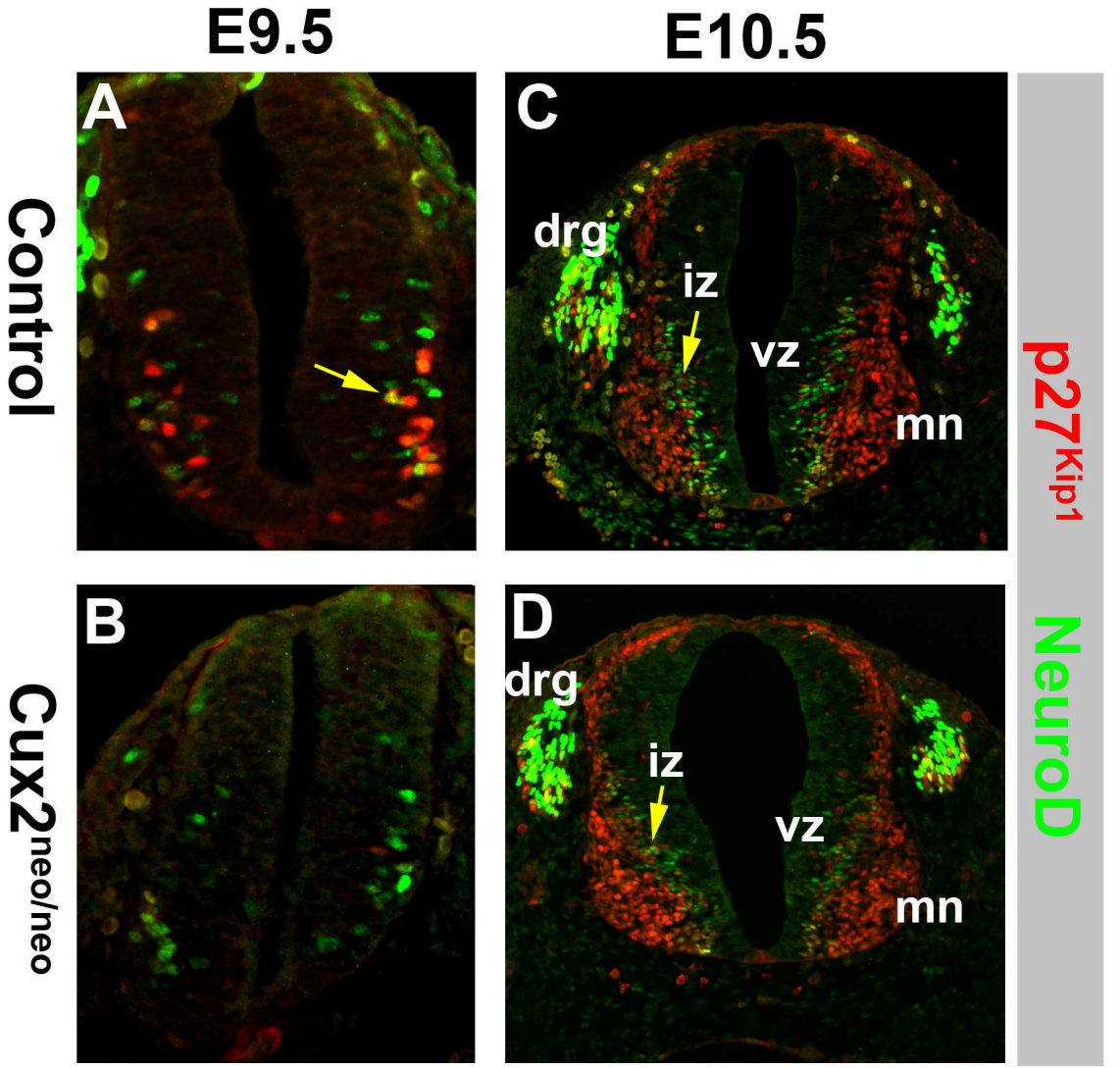
CUX2
Merge
CUX2-nucEGFP
A
B
C
NESTIN-CUX2-EGFP
D
E
F
E11.5
Control E10.5
Cux2neo/neo E10.5
CUX2
G
H
fp
fp


E9.5
E10.5
Control
Cux2neo/neo
A
B
C
D
drg
iz
vz
mn
drg
iz
vz
mn
p27Kip1
NeuroD