SUPPLEMENTARY MATERIAL Title: Synergistic recruitment of transcription factors to cell type specific enhancers programs motor neuron identity

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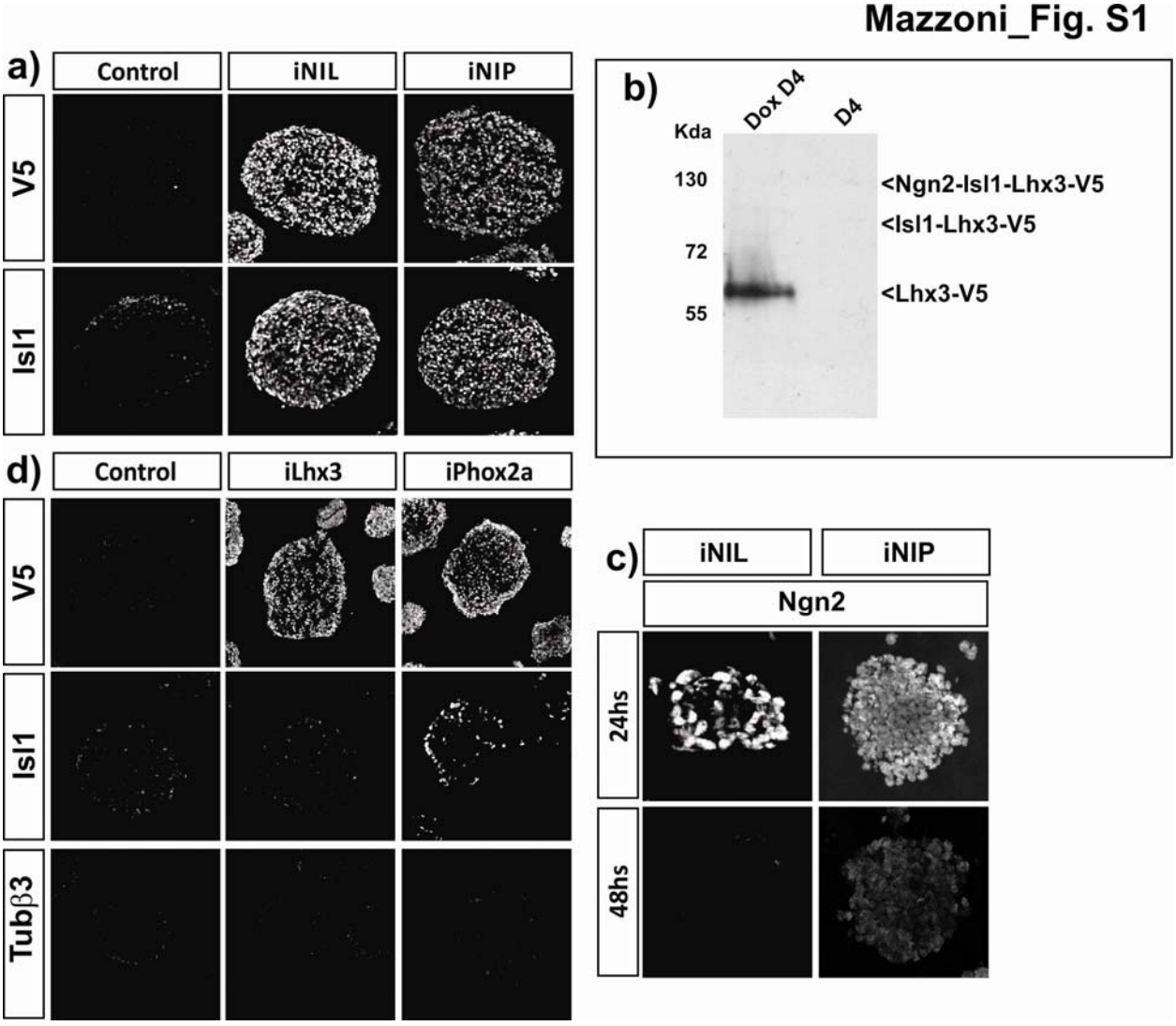
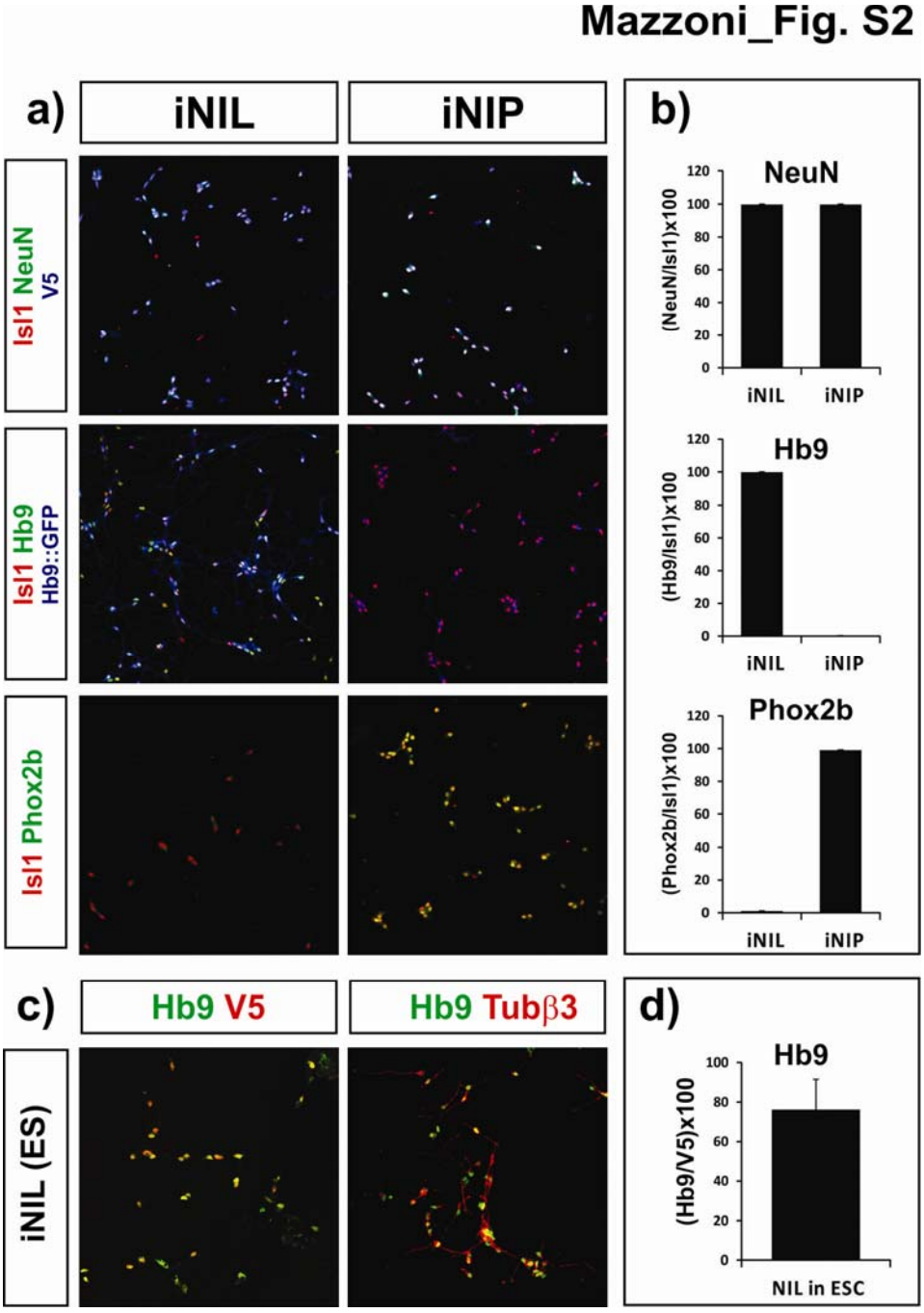


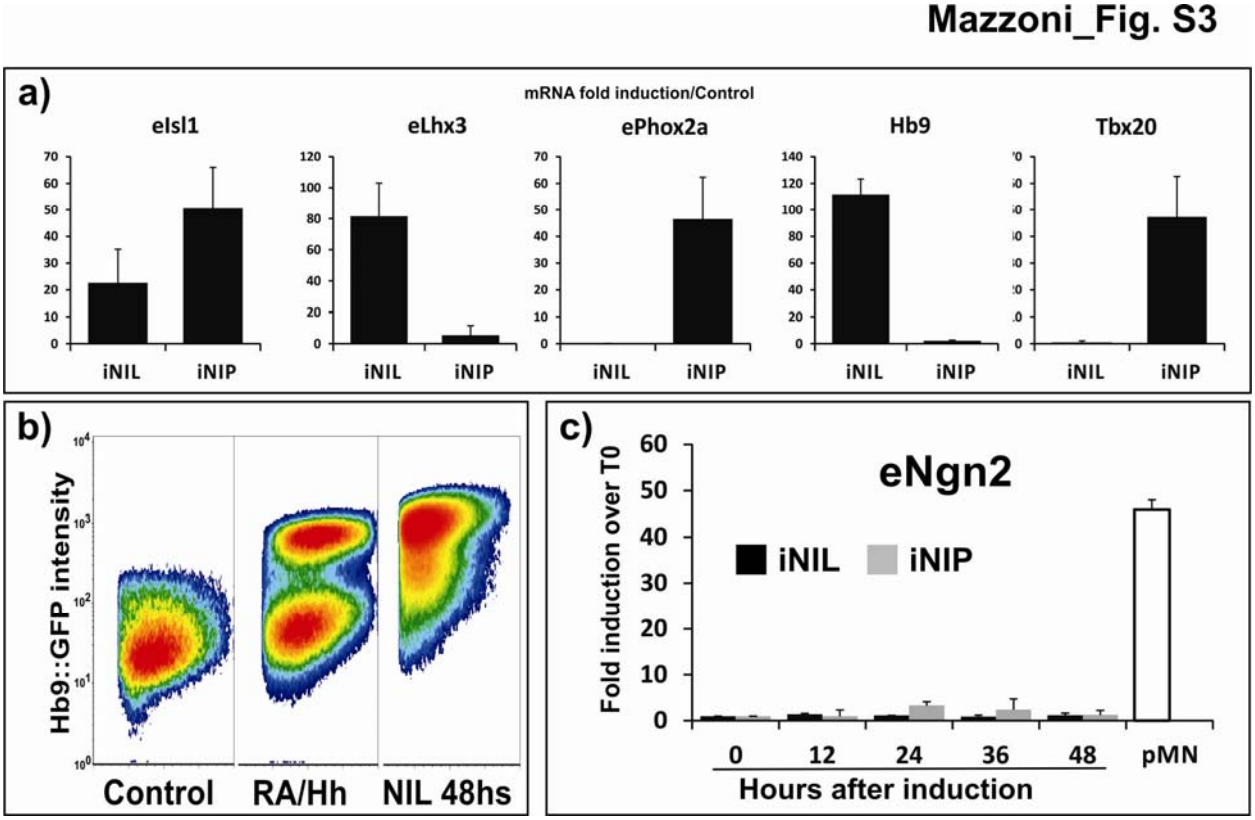
Supplementary Fig.1: Characterization of inducible ESC lines

- **a)** After 48 hours of induction, both iNIL and iNIP cells express Isl1 and V5 tagged Lhx3 and Phox2a respectively. Immunostained cryosections of Dox treated embryoid bodies.
- **b)** Western blot of NIL cells after 48 hours of induction stained with a V5 antibody revealed efficient processing of the tricistronic construct into individual transcription factors.
- **c)** Ngn2 protein is detected 24 hours after Dox treatment, but is largely lost by 48 hours of Dox treatment. Immunostained cryosections of Dox treated embryoid bodies.
- **d)** Neither Lhx3 (iLhx3) nor Phox2a (iPhox2a) alone is sufficient to induce high levels of Isl1 or tubulin beta III. Immunostained cryosections of Dox treated embryoid bodies.



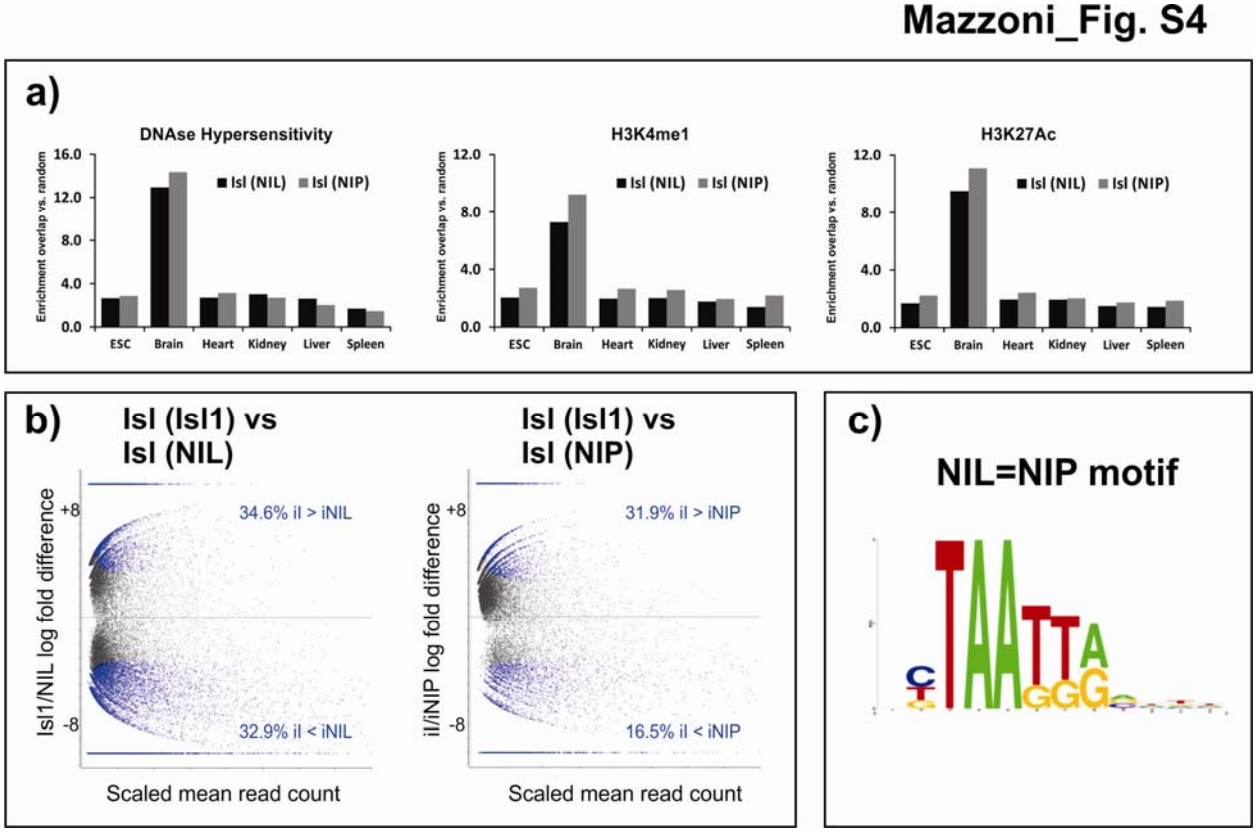
Supplementary Fig. 2: Quantification of motor neuron induction

- **a)** NIL and NIP programmed neurons were plated at low density and stained with Isl1 to reveal induction of transgenes and NeuN (neuralization), Hb9 (spinal motor neuron fate) and Phox2b (cranial motor neuron fate).
- **b)** Quantification of a). n=3 biological replicates; mean ± SEM.
- **c)** NIL expression directly in ESC induces Hb9 and tubulin beta III. ESC were treated with Dox and stained for Hb9 and V5 (inducible Lhx3) or Tubulin beta III.
- **d)** Quantification of c). n=3; mean ± SEM.



Supplementary Fig. 3: Regulation of endogenous transcription factors by NIL and NIP

- **a)** mRNA levels of selected endogenous genes 48 hours after NIL or NIP induction analyzed by qPCR. n=3; mean ± SEM.
- **b)** Efficient induction of motor neuron reporter *Hb9::GFP* by NIL induction. FACS analysis of GFP intensity of control cells, RA/Hh induced cultures and NIL induced cells (X axis represents side scatter - SSC). RA/Hh treated cells were analyzed at day 5 of differentiation. Heat map represents low (blue) to high (red) density of cells.
- **c)** Neither NIL nor NIP programming induces Ngn2. Time series of Ngn2 mRNA levels after the induction of NIL and NIP. Motor neuron progenitors (pMN – 48 hours after RA/Hh treatment) from RA/Hh induced differentiation serve as a positive control. n=2; mean ± SEM.

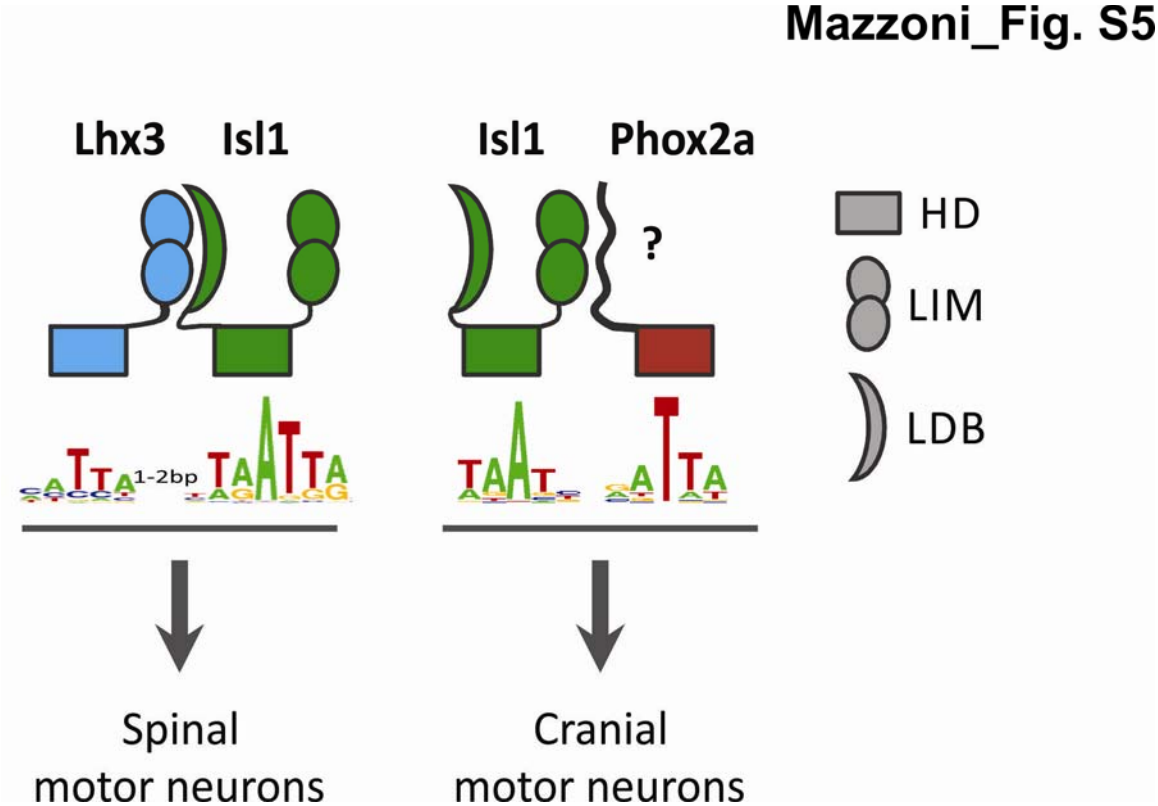


Supplementary Fig. 4: Preferential recruitment of factors to brain specific regulatory regions

a) Isl transcription factors are preferentially recruited to brain specific putative regulatory regions. Quantification of the overlap between Isl ChIP-seq peaks and chromatin features associated with active *cis*-regulatory regions in ESCs and selected tissues reported by ENCODE Project.

b) Isl genomic binding is highly dependent on programming modules. Comparison of read enrichment from Isl when expressed in isolation (Isl1) or within NIL and NIP modules. Blue represents peaks significantly differentially enriched in one experiment over the other.

c) Primary motif over-represented at sites occupied by Isl in both iNIL and iNIP.



Supplementary Fig. 5: Model of transcriptional regulation of spinal and cranial motor neuron identity

We propose that homeodomains (HDs) of Lhx3/Isl and Phox2a/Isl heterodimers preferentially bind pairs of HD motifs with NIL and NIP specific grammar. Interaction between the LIM domain of Lhx3 and LDB (LIM domain binding) domain of Isl transcription factors has been documented. In contrast, Phox2a factor does not contain a typical LIM interacting domain and therefore nature of interaction between Phox2a and Isl transcription factors remains unknown.