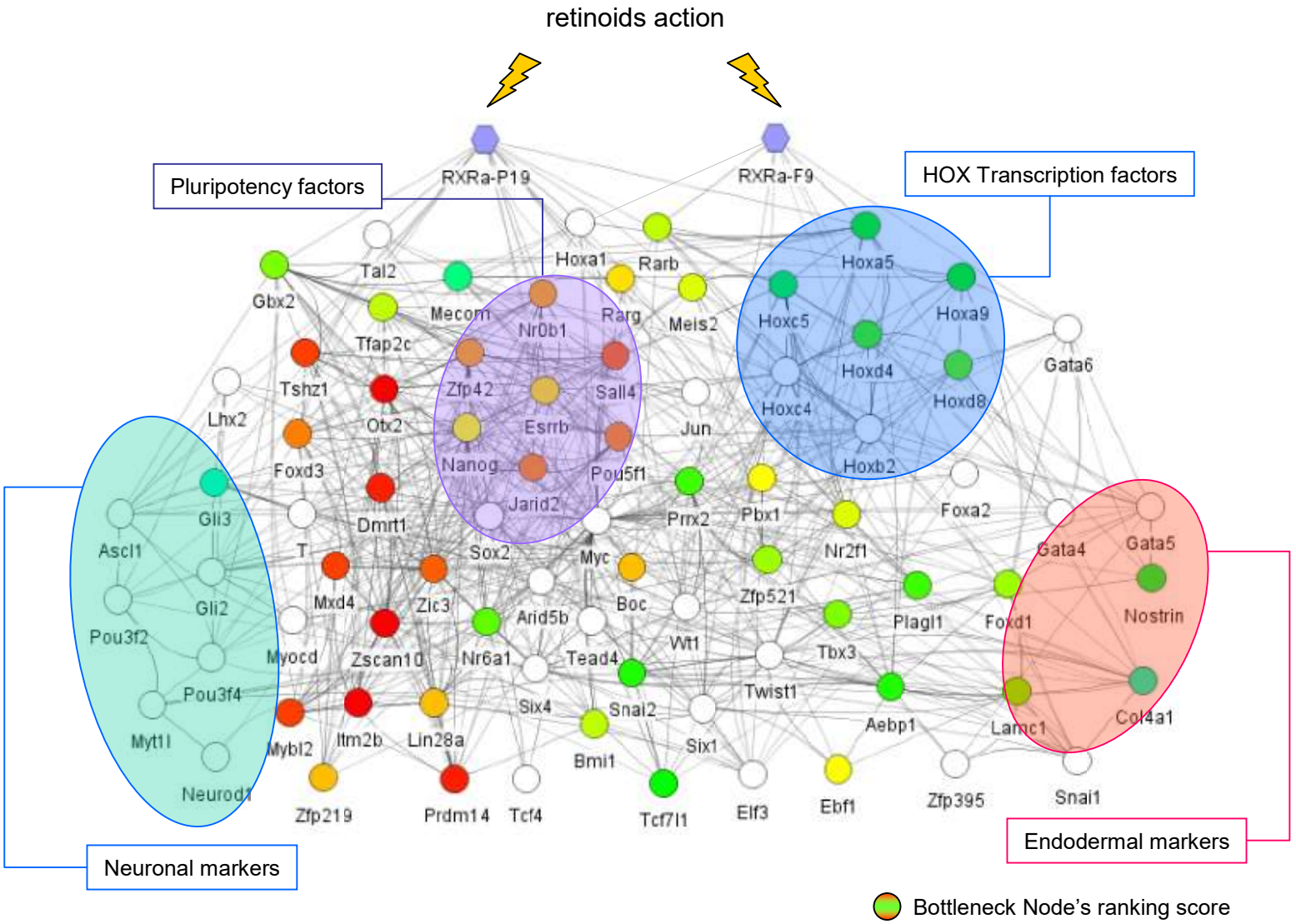
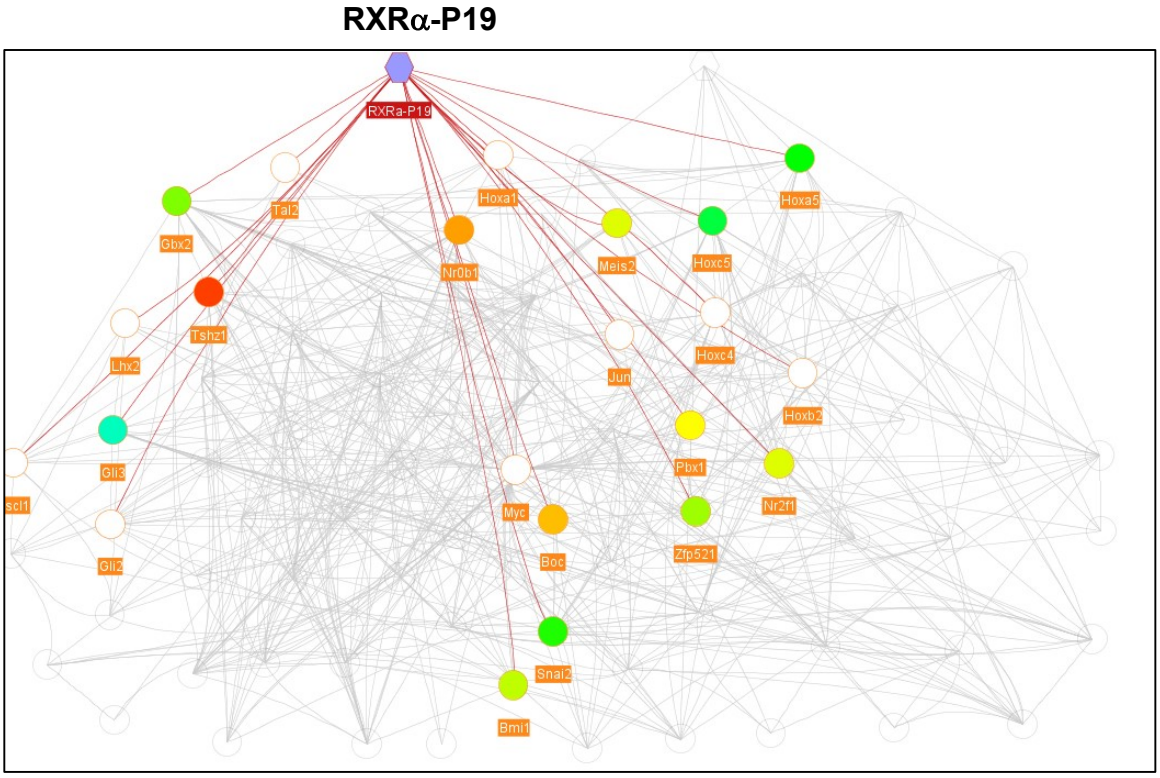
**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**



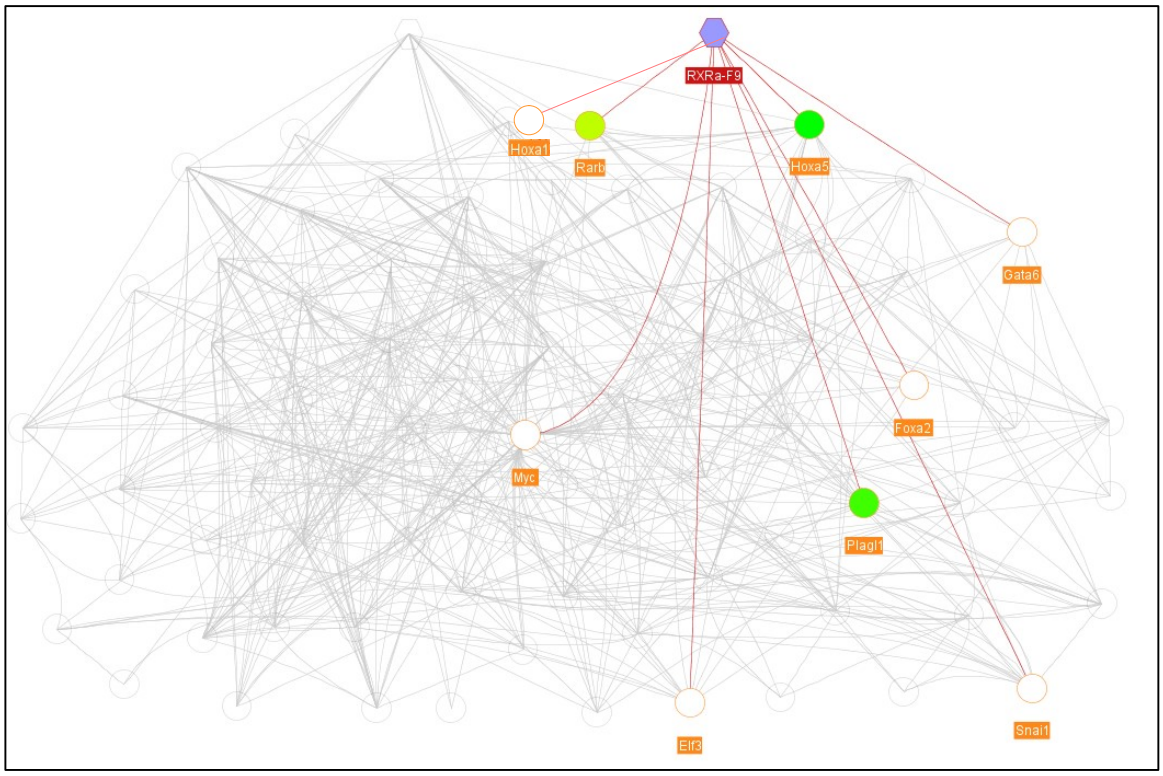

The reduced GRN comprising 80 nodes and 626 edges was reconstructed by applying topological ranking to identify key connector nodes (bottleneck node's ranking score; displayed as heatmap). Four major sub-networks associated with cell pluripotency, HOX factors and neuronal or endodermal cell fate markers are highlighted. A detailed view of the connectivity between these major sub-networks is provided below.

**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

A




B



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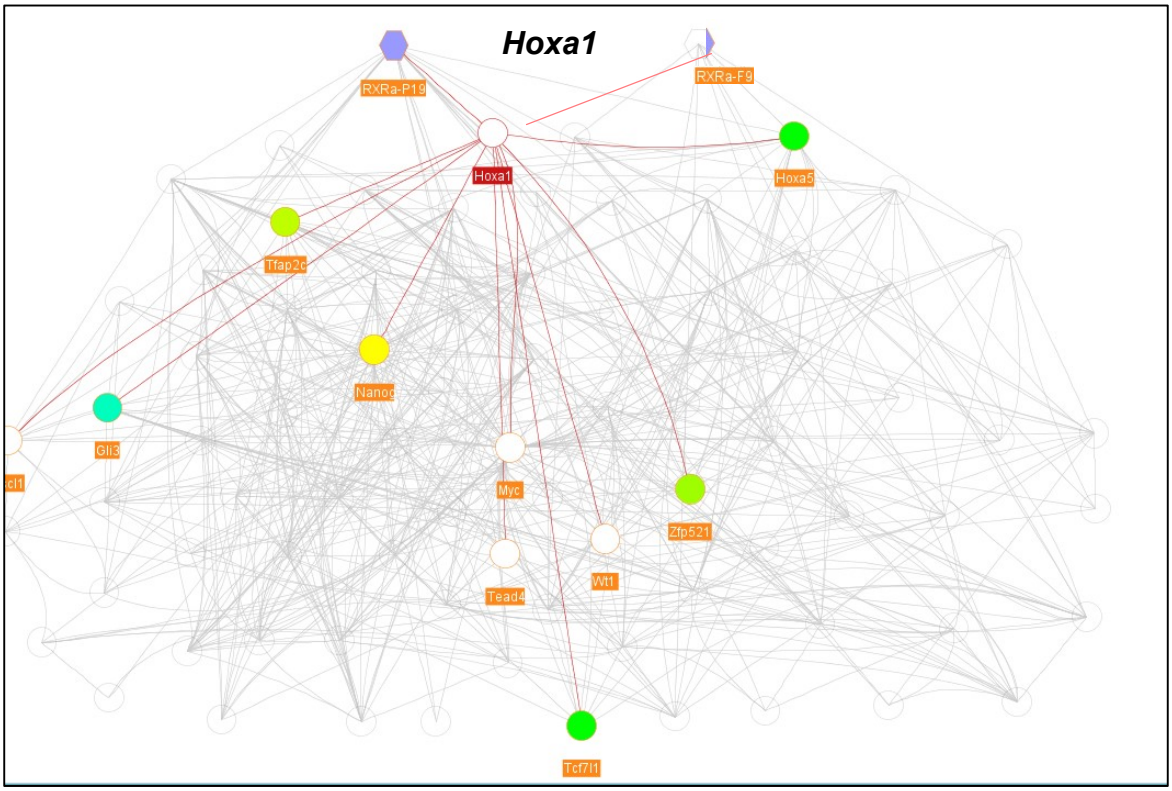
C

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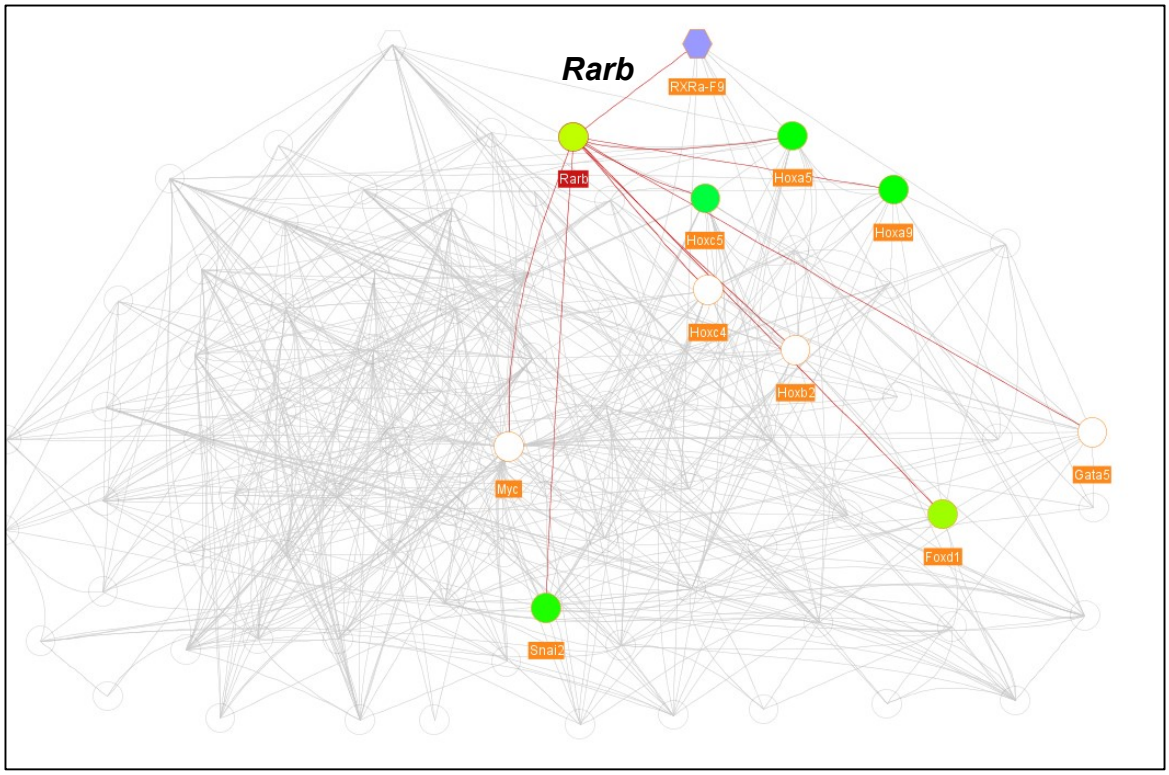
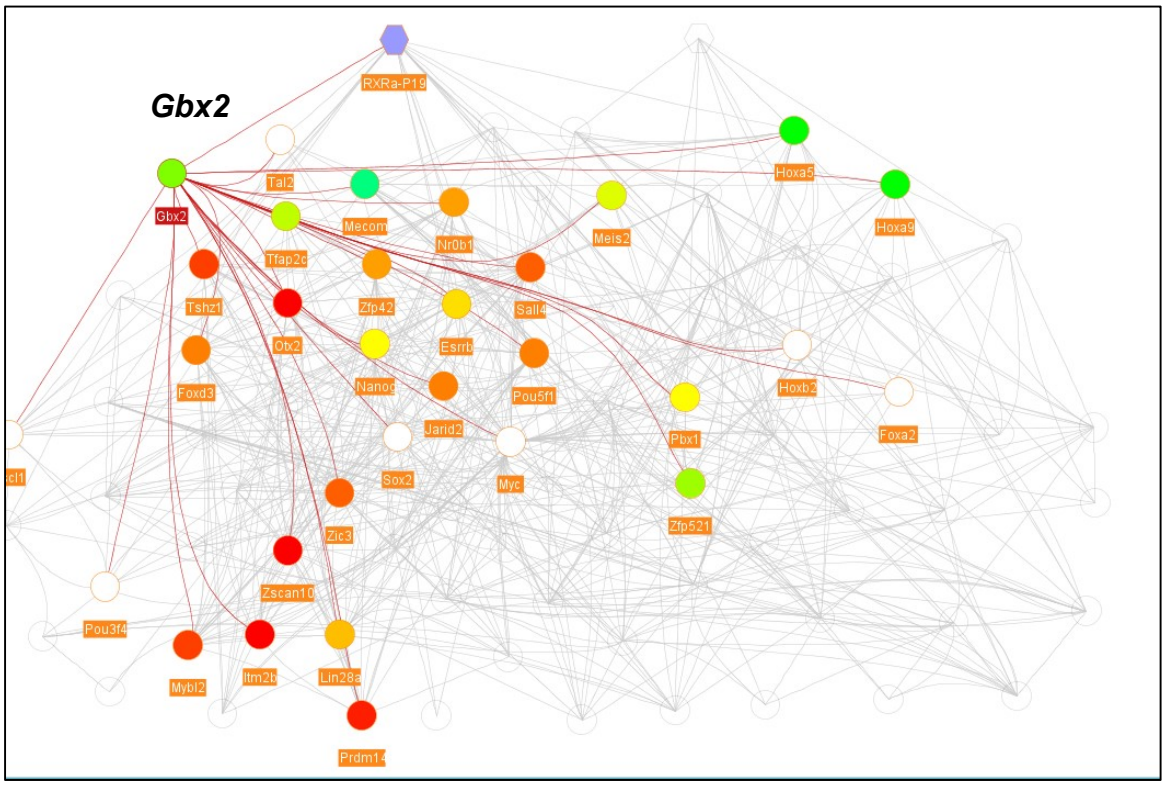


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

E

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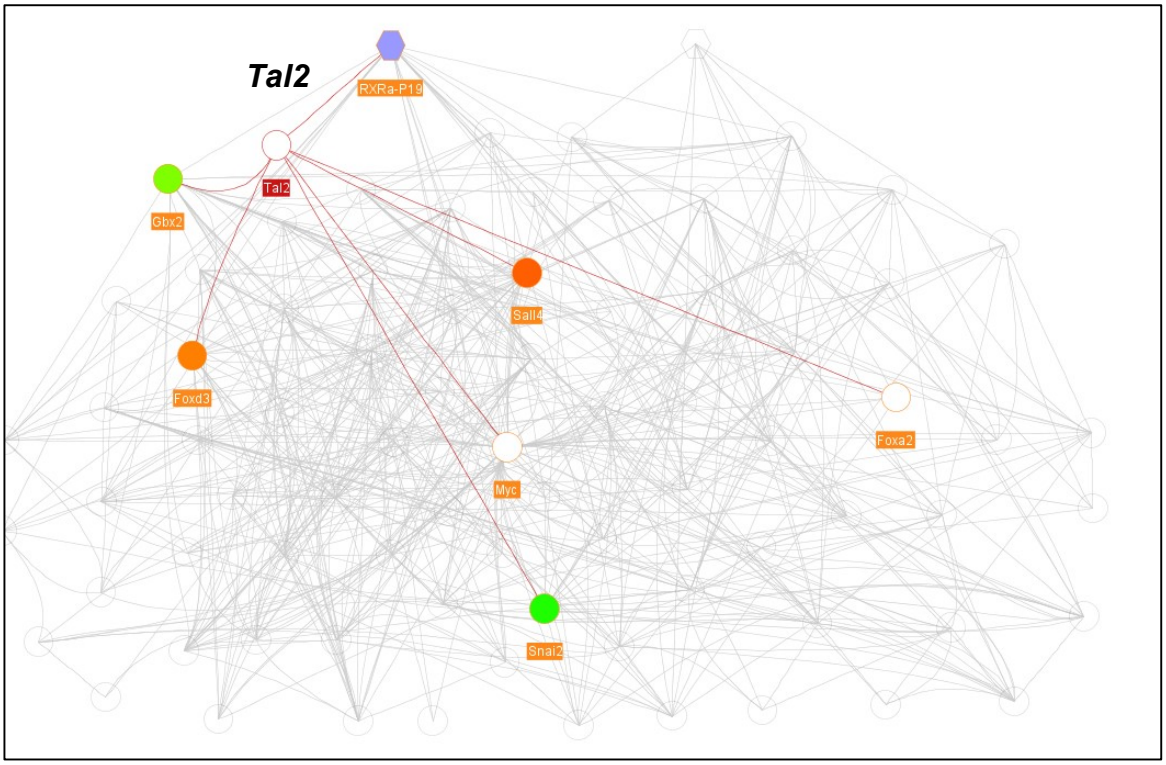
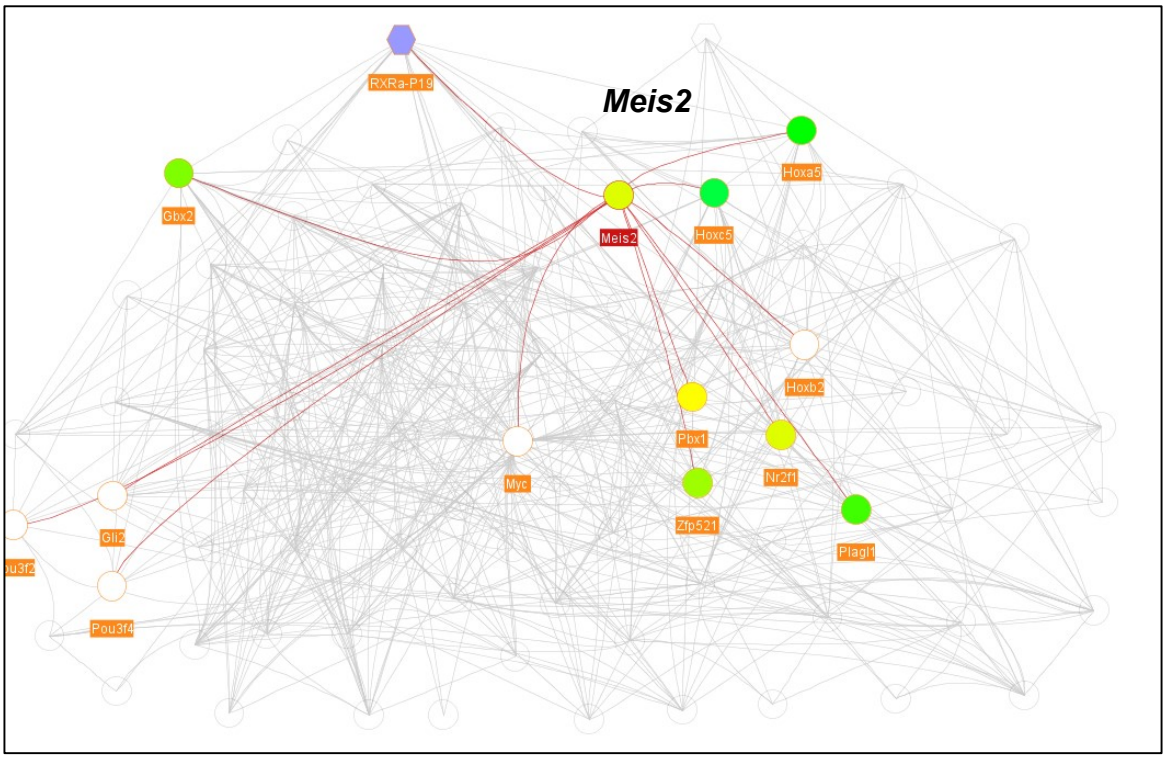
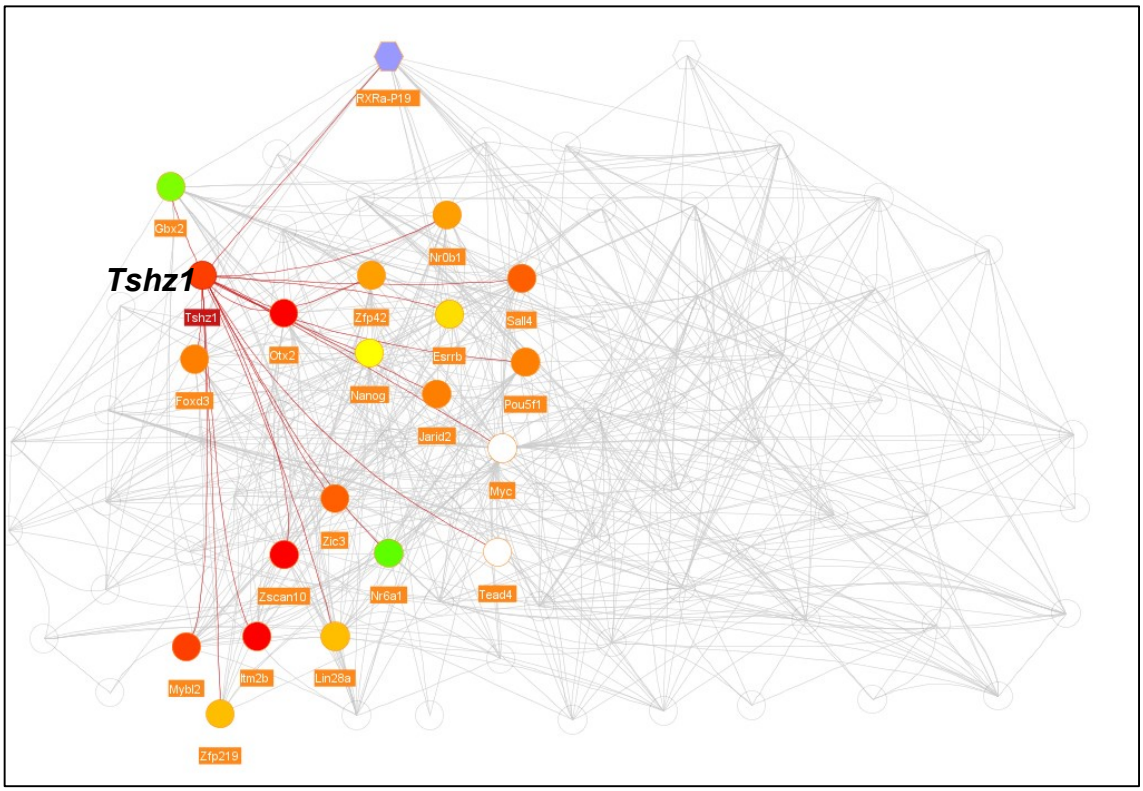


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

G

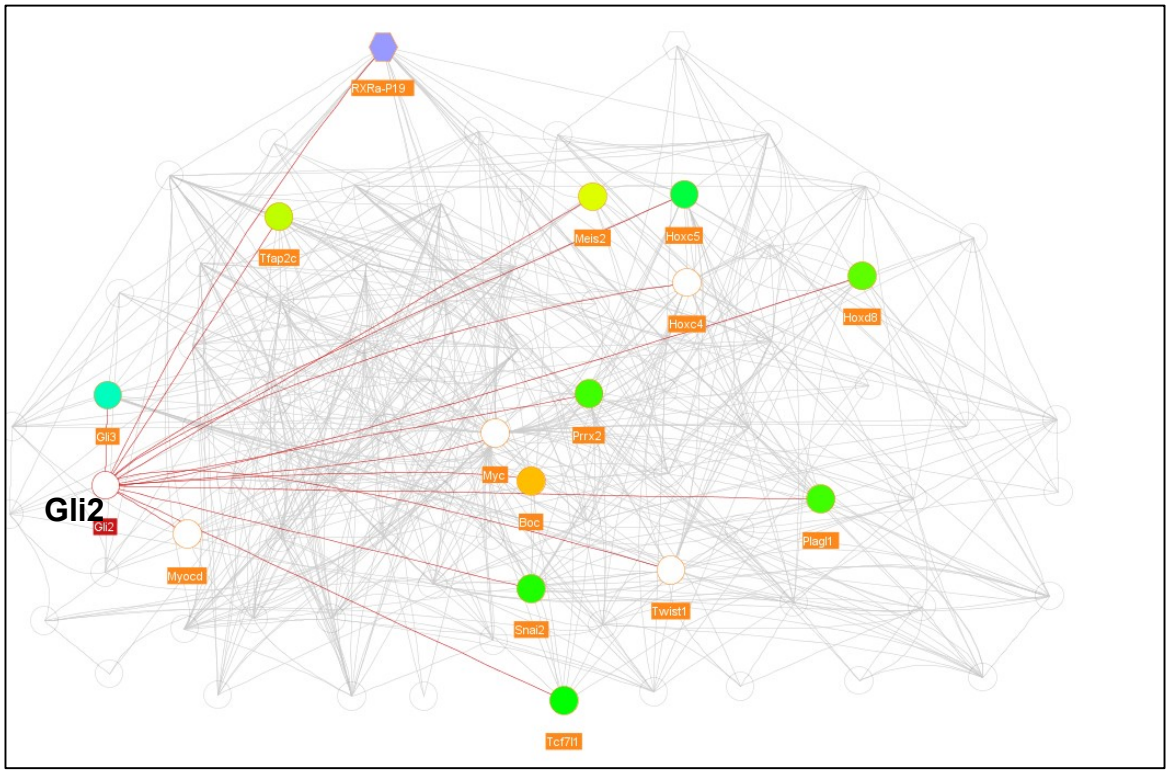



H



**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

I

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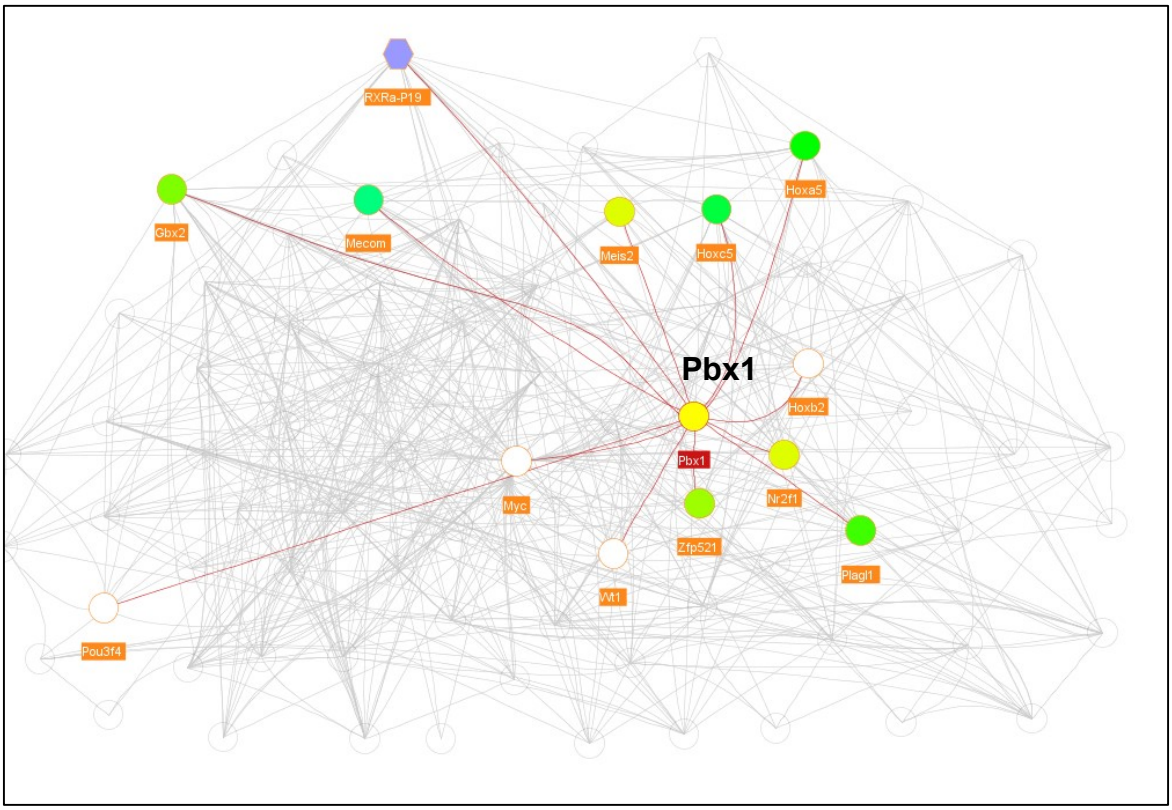
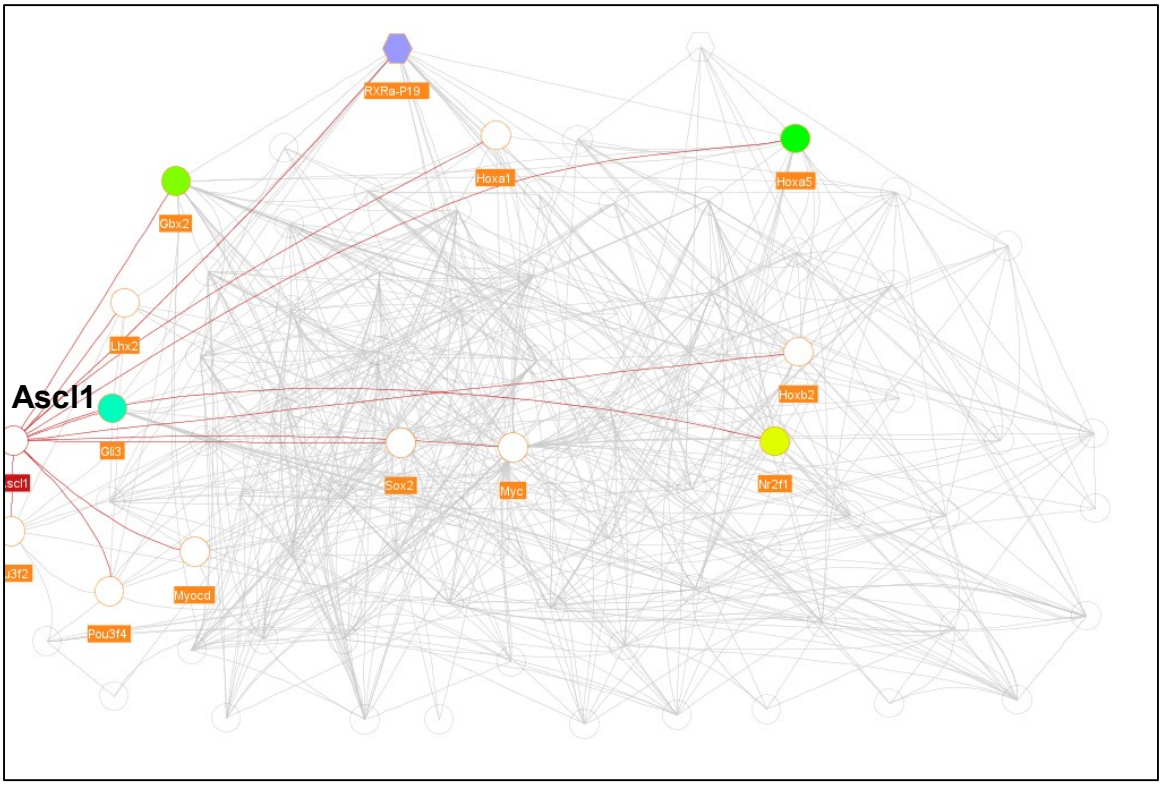
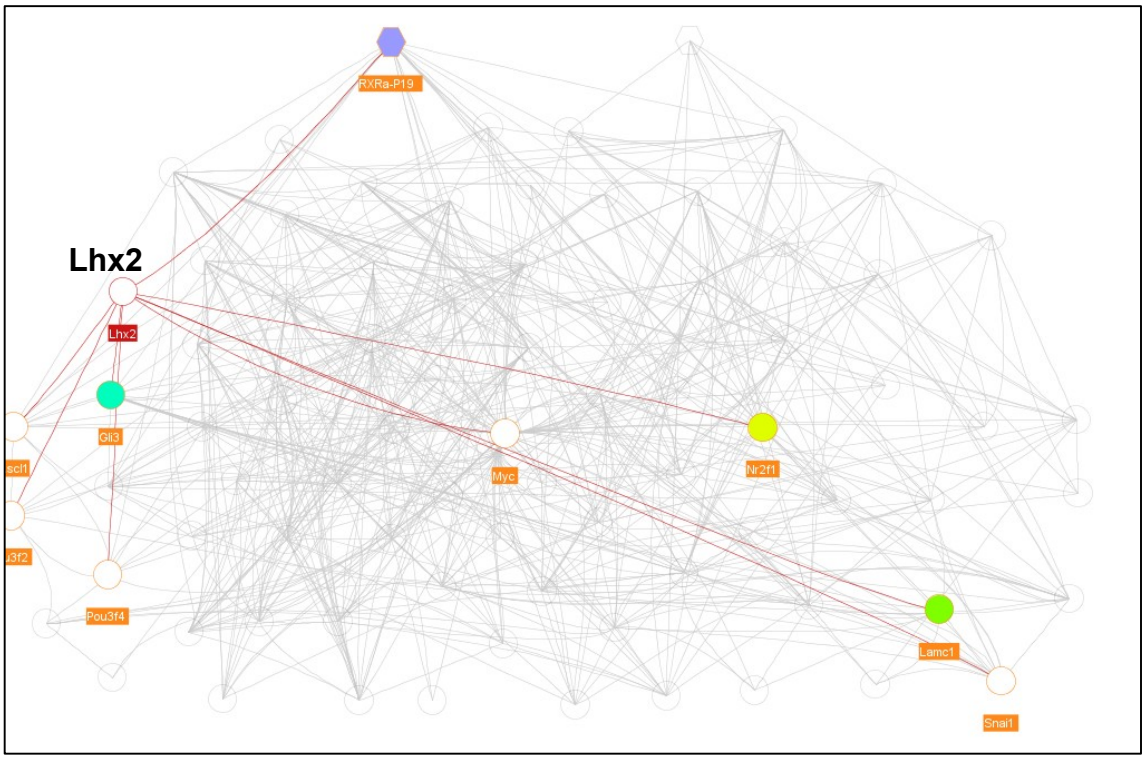


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

K

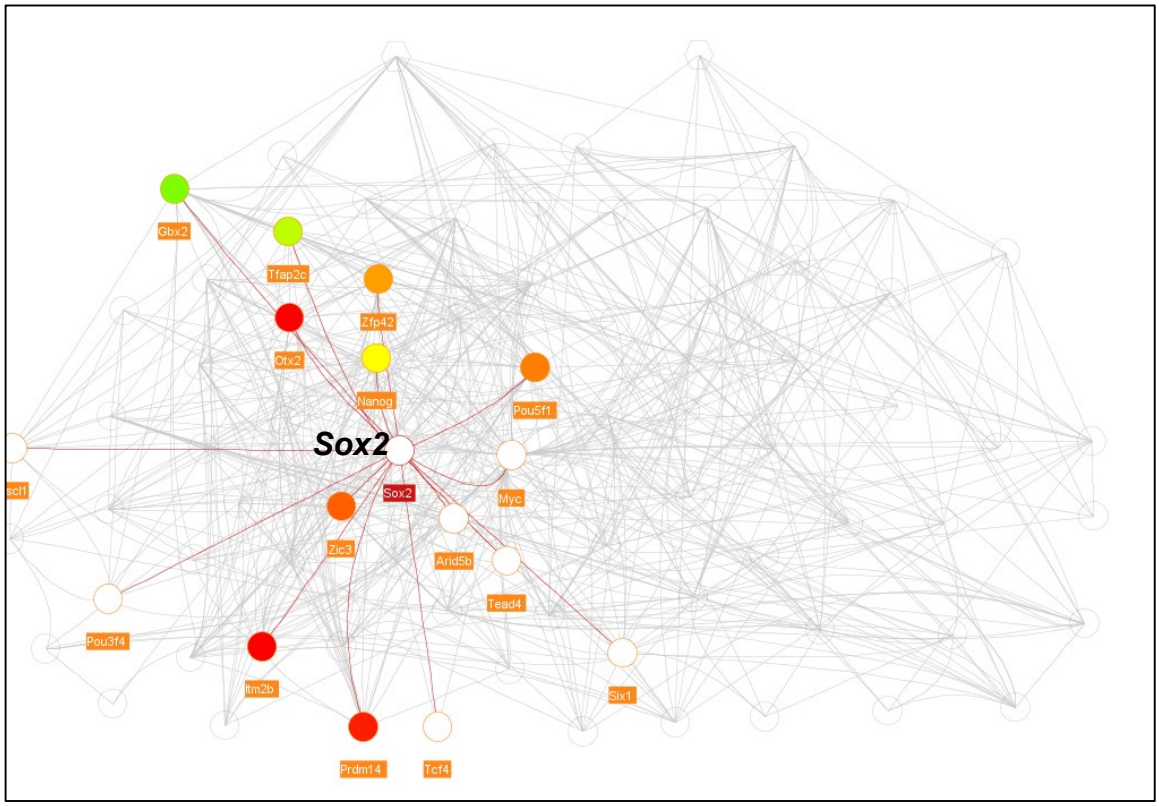



L



**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

M

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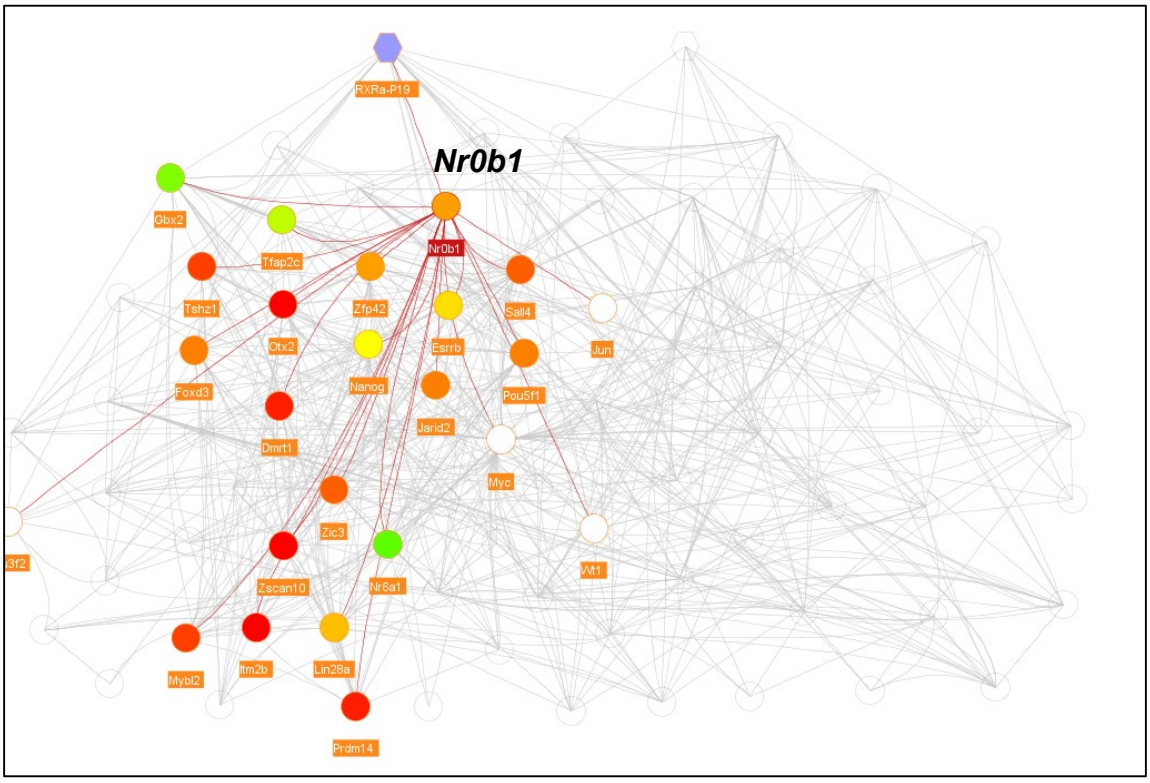
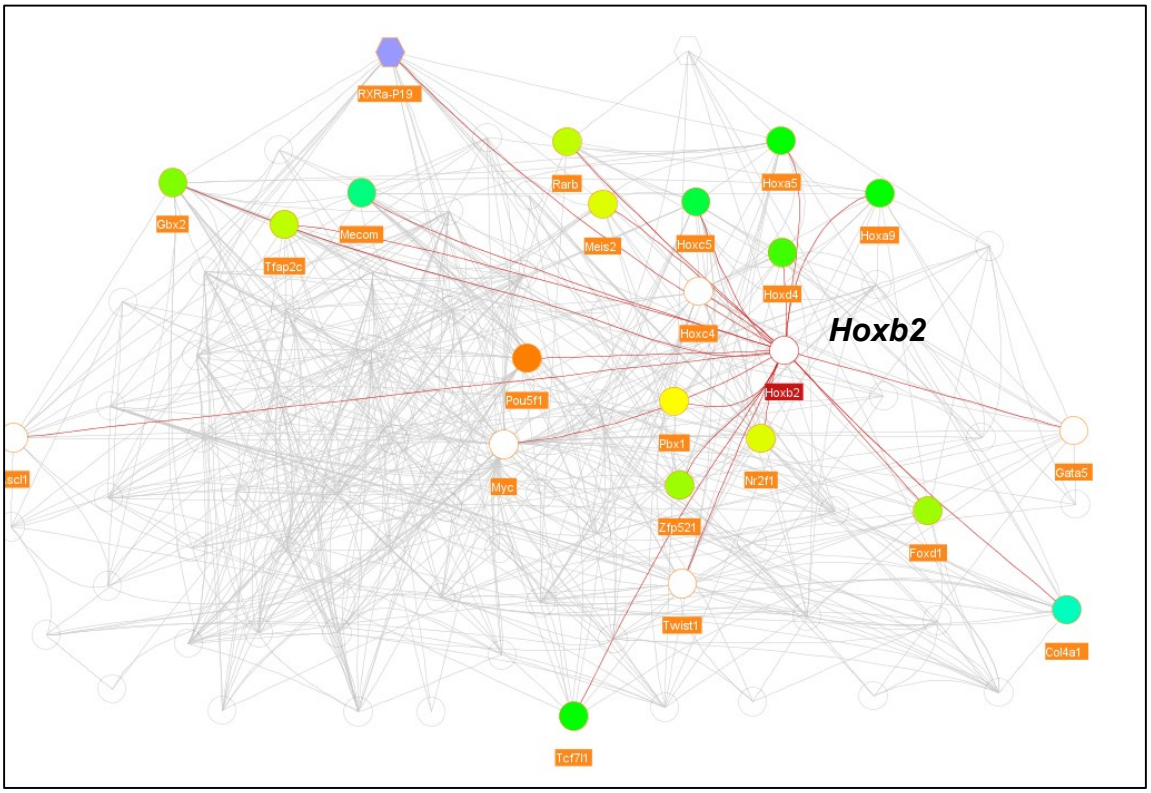


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

O

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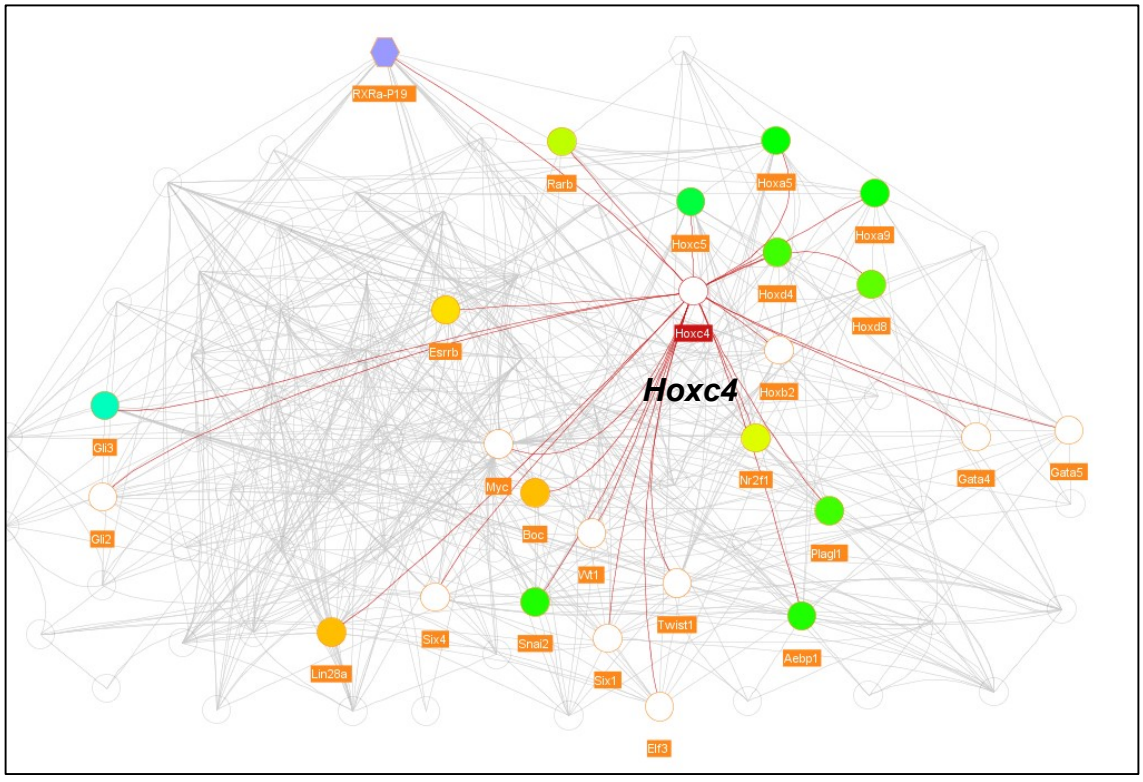
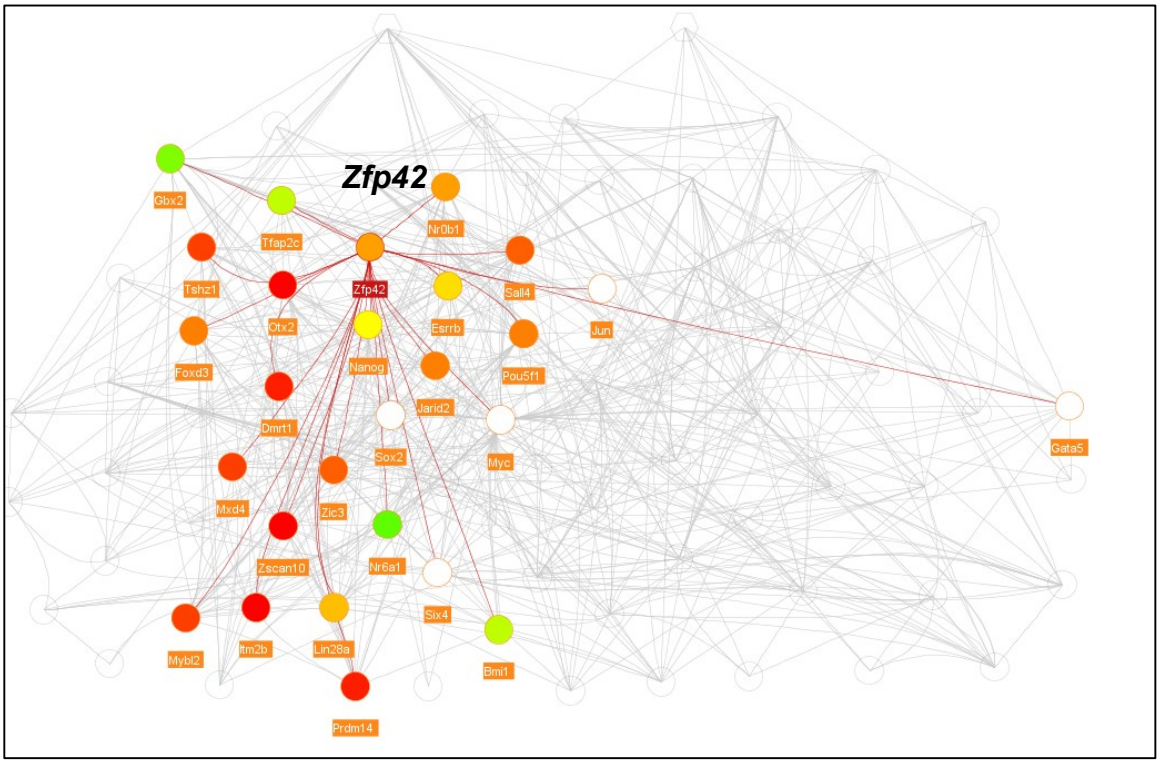


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

Q

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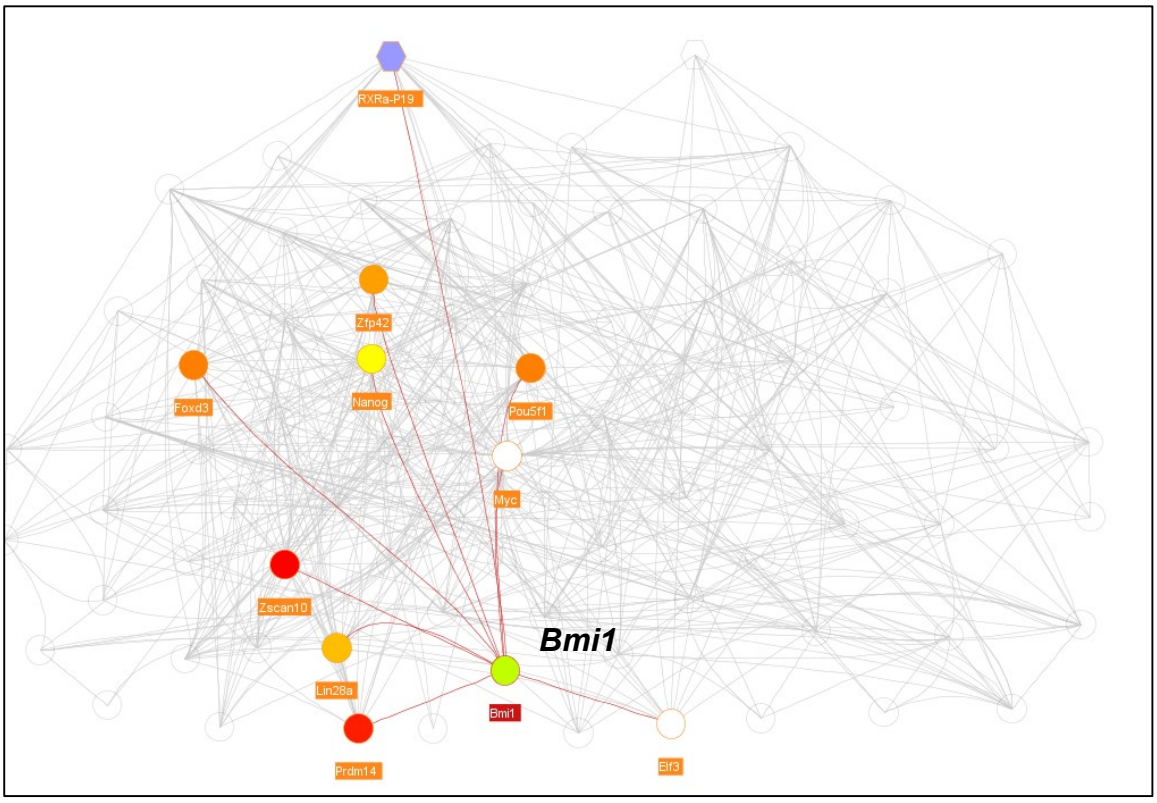
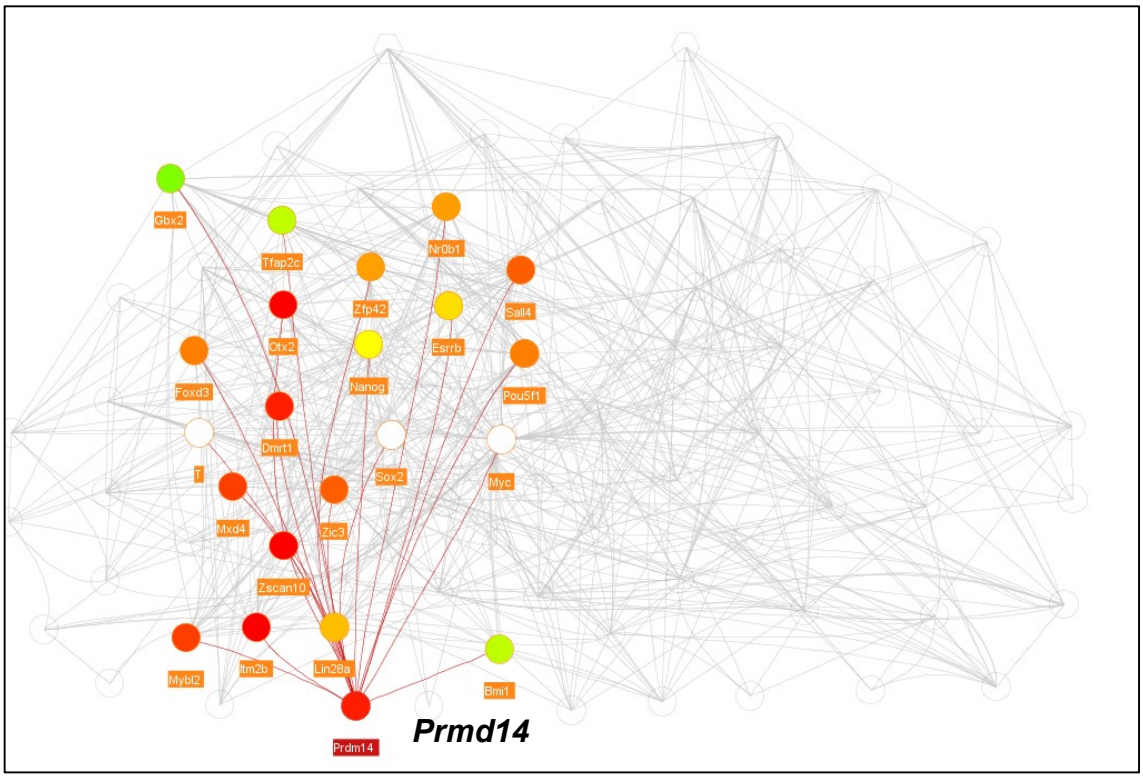
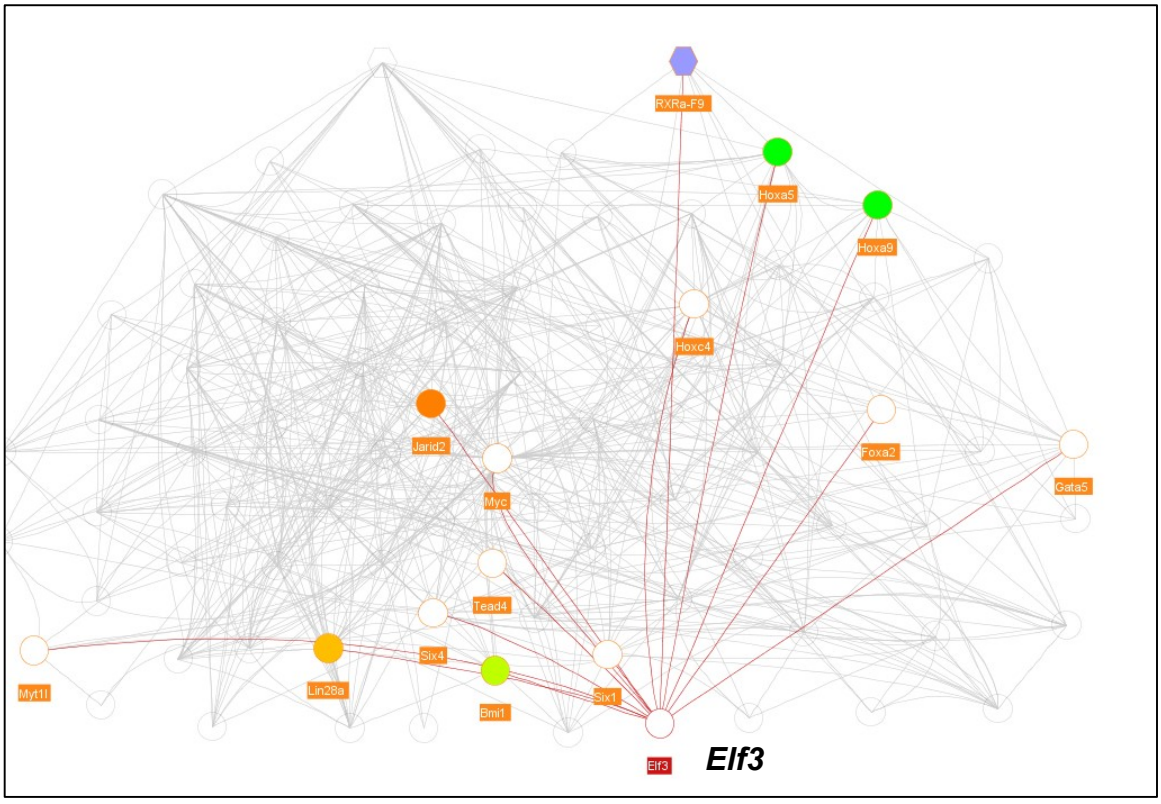


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

S

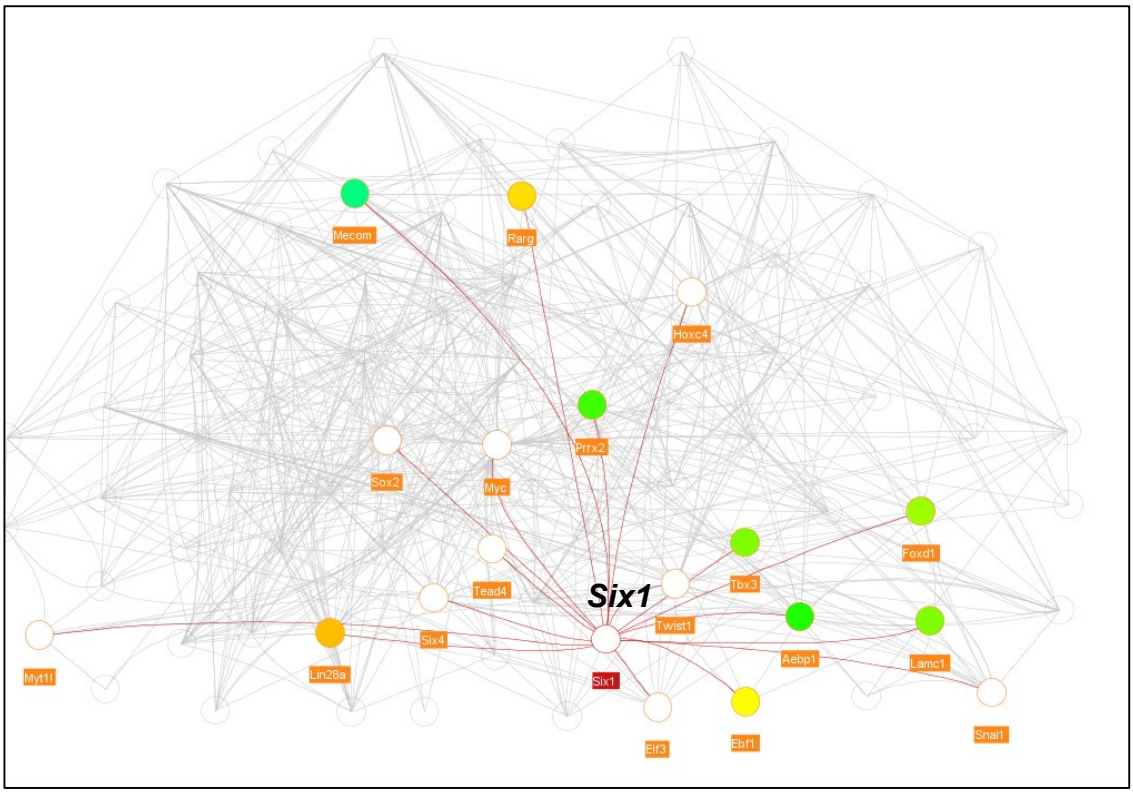



T

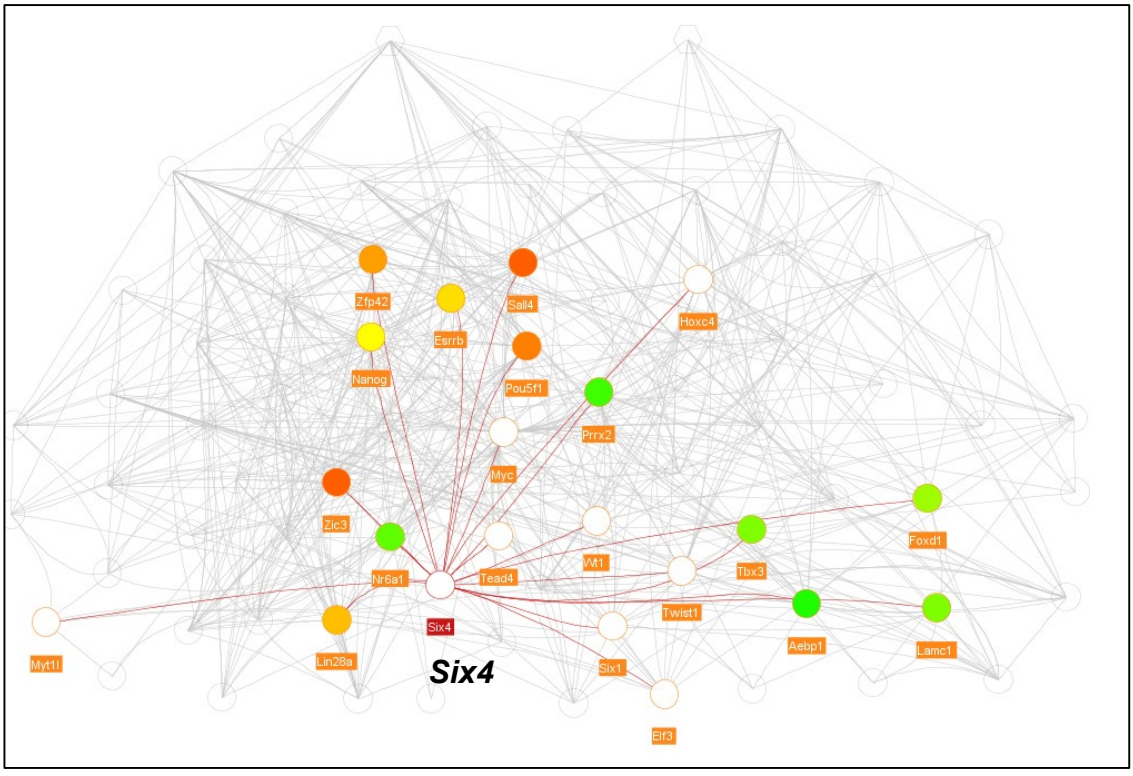


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

U

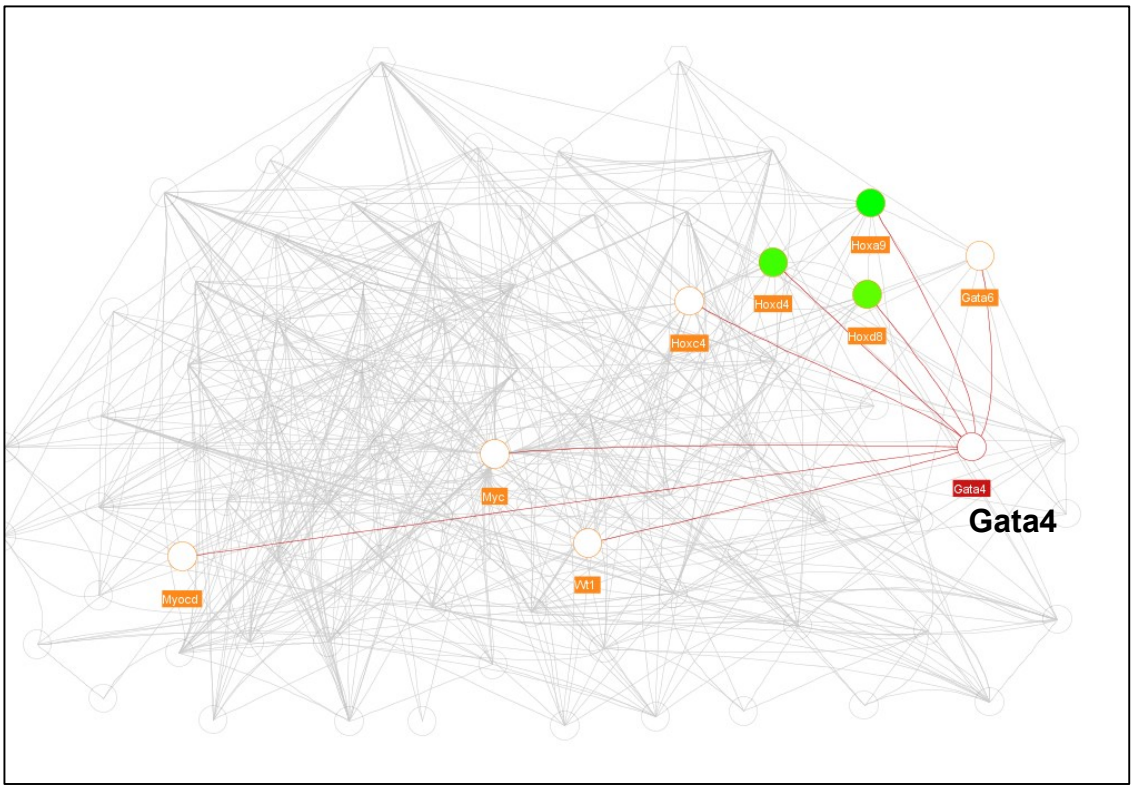



V

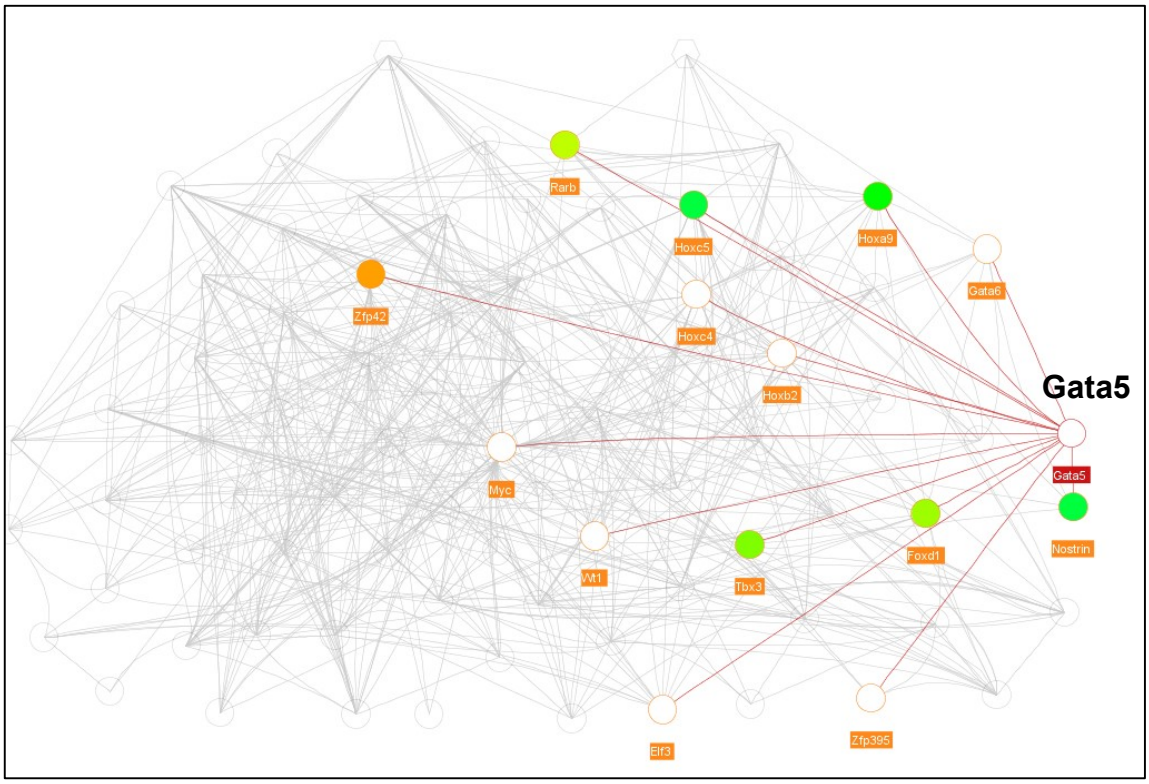


**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.**

W




X



**Supplemental Figure S17. Gene regulatory wiring reconstructed from the reduced Gene regulatory network governing the RA-driven cell-fate decisions in F9 and P19 cells.** (A to X) Illustration of connectivity of RXRα (A, B) and downstream key regulatory genes (C, *Hoxa1*; D, *Rarb*; E, *Gbx2*; F, *Tal2*; G, *Meis2*; H, *Tshz1*; I, *Gli2*; J, *Pbx1*; K, *Ascl1*; L, *Lhx2; M, Sox2; N, Nr0b1; O, Hoxb2; P, Hoxc4; Q, Zfp42; R, Bmi1; S, Prmd14; T, Elf3; U, Six1; V, Six4; W, Gata4; X, Gata5*) with the neuronal, endodermal and common programs. Note that early RA-induced factors like RARβ and HOXA1 are each interconnected with nodes projecting to endodermal and neuronal factors revealing their impact on the development of both the endodermal and the neuronal lineages (**Supplemental Fig. S17C, D**).

In the neuronal lineage, the early-induced transcription factors GBX2 [1] and TAL2 are depicted by edges linking these factors specifically to the RXRα/RAR node of P19 cells. Further downstream, GBX2 is connected to pluripotency factors (SOX2, NANOG, SALL4 or POU5F1/OCT4), which correlates with its repressive activity depicted as part of the co-expression path 10 derived from DREM (**Figure 4D**); it is also linked to the neuronal fate determination factors POU3F4/BRN4 and ASCL1, as well as to the PBX-related homeobox factor MEIS2/STRA10 [2]. Notably, an essential role of MEIS2 in neurogenesis has been recently reported [3], in addition to its implication in cell cycle regulation [4] and cell fate specification [5,6]. The broad functionality of MEIS2 is also apparent from the number of its interactions in the reduced GRN (**Supplemental Fig. S17G**), which includes multiple HOX factors, PBX1 or the zinc finger protein 521(ZFP521) - all of which are expressed in both P19 and F9 cells - and with other neurogenesis-related TFs like GLI2, BRN2 or BRN4. GBX2 is also connected to TSHZ1, a homeodomain factor previously shown to regulate the development and maturation of the olfactory bulb [7], and which we describe here as a new potential factor that promotes neuronal commitment (**Figure 6**). Indeed, TSHZ1 is connected to pluripotency regulators (SALL4, POU5F1, NANOG, NR0B1), but also to ZIC3 – a factor that plays a critical role in forebrain development [8] - and to the neural crest marker FOXD3 [9]. With longer exposure to RA, P19 cells gain in expression of additional neuronal factors, among them ASCL1/MASH1, a factor that is essential for neuronal differentiation of P19 cells [10,11]. Importantly, ASCL1 is connected to several other neuronal-specific factors, such as GLI3, POU3F2/BRN2 or POU3F4/BRN4, as well as to LHX2, a transcriptional factor previously shown to play an essential role for retinal gliogenesis [12]. Note that we demonstrated that LHX2 is able to promote neuronal differentiation (**Figure 6**), which is in keeping with its connection to the downstream acting neuronal factors ASCL1, GLI3, POU3F2/BRN2 and POU3F4/BRN4 (**Supplemental Fig. S17L**). Among the factors involved in endodermal specification is ZFP42/REX1, which is expressed selectively in undifferentiated F9 cells. Notably, RA represses its expression in mouse stem cells [13,14] and its ablation impaired RA-induced primitive endodermal differentiation [15]. Furthermore, previous studies demonstrated that ZFP42/REX1 is regulated by NANOG and SOX2 [16,17]. Notably, this described regulatory relationship with the self-renewal components NANOG, SOX2, KLF4 and OCT4 is recapitulated in the reduced GRN, as is its direct role in endodermal differentiation through interaction with BMI1 and the transcription factor PRDM14 (**Supplemental Fig. S17Q**). In fact, the Polycomb factor BMI1 is known to facilitate primitive endoderm formation through GATA6 stabilisation [18], while PRDM14 is involved in the maintenance of the pluripotency and inhibits ES cells from adopting endodermal fates [19]. Another F9 cell-specific endodermal differentiation-related factor is the Ets domain TF ELF3 [20], which is a direct RA target gene that is also connected to the Homeobox TFs SIX1 and SIX4 (**Supplemental Fig. S17Q**); the latter are involved in pharyngeal endoderm formation [21]. Finally, the GRN contains the nodes GATA4 and GATA6, which are critical for endodermal differentiation [22,23].

In summary, the reconstructed gene regulatory network properly reconstitutes a scenario in which cascades of TF-driven common regulatory programs - preferentially active during the first hours of treatment – in concert with temporally evolving, similarly TF-driven endodermal and neuronal programs are responsible for the acquisition of these two cell fates in distinct germ layers, such that cell fate specification is pre-defined by a given cellular context even when the same trigger is used for program initiation.

**(1).** Bouillet, P., Chazaud, C., Oulad-Abdelghani, M., Dolle, P. & Chambon, P. Sequence and expression pattern of the Stra7 (Gbx-2) homeobox-containing gene induced by retinoic acid in P19 embryonal carcinoma cells. *Dev Dyn* **204**, 372-382 (1995).

**(2).** Oulad-Abdelghani, M. *et al.* Meis2, a novel mouse Pbx-related homeobox gene induced by retinoic acid during differentiation of P19 embryonal carcinoma cells. *Dev Dyn* **210**, 173-183 (1997).

**(3).** Agoston, Z. *et al.* Meis2 is a Pax6 co-factor in neurogenesis and dopaminergic periglomerular fate specification in the adult olfactory bulb. *Development* **141**, 28-38 (2014).
**(4).** Zha, Y. *et al.* MEIS2 is essential for neuroblastoma cell survival and proliferation by transcriptional control of M-phase progression. *Cell death & disease* **5**, e1417 (2014).
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**(6).** Agoston, Z. & Schulte, D. Meis2 competes with the Groucho co-repressor Tle4 for binding to Otx2 and specifies tectal fate without induction of a secondary midbrain-hindbrain boundary organizer. *Development* **136**, 3311-3322 (2009).
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**(8).** Inoue, T., Ota, M., Ogawa, M., Mikoshiba, K. & Aruga, J. Zic1 and Zic3 regulate medial forebrain development through expansion of neuronal progenitors. *J Neurosci* **27**, 5461-5473 (2007).
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**(10).** Huang, H. S. *et al.* Transcriptional Regulatory Events Initiated by Ascl1 and Neurog2 During Neuronal Differentiation of P19 Embryonic Carcinoma Cells. *J Mol Neurosci* (2014).
**(11).** Huang, H. S., Turner, D. L., Thompson, R. C. & Uhler, M. D. Ascl1-induced neuronal differentiation of P19 cells requires expression of a specific inhibitor protein of cyclic AMP-dependent protein kinase. *J Neurochem* **120**, 667-683 (2012).
**(12).** de Melo, J. *et al.* Lhx2 Is an Essential Factor for Retinal Gliogenesis and Notch Signaling. *J Neurosci* **36**, 2391-2405 (2016).
**(13).** Hosler, B. A., Rogers, M. B., Kozak, C. A. & Gudas, L. J. An octamer motif contributes to the expression of the retinoic acid-regulated zinc finger gene Rex-1 (Zfp-42) in F9 teratocarcinoma cells. *Mol Cell Biol* **13**, 2919-2928 (1993).
**(14).** Hosler, B. A., LaRosa, G. J., Grippo, J. F. & Gudas, L. J. Expression of REX-1, a gene containing zinc finger motifs, is rapidly reduced by retinoic acid in F9 teratocarcinoma cells. *Mol Cell Biol* **9**, 5623-5629 (1989).
**(15).** Thompson, J. R. & Gudas, L. J. Retinoic acid induces parietal endoderm but not primitive endoderm and visceral endoderm differentiation in F9 teratocarcinoma stem cells with a targeted deletion of the Rex-1 (Zfp-42) gene. *Mol Cell Endocrinol* **195**, 119-133 (2002).
**(16).** Shi, W. *et al.* Regulation of the pluripotency marker Rex-1 by Nanog and Sox2. *The Journal of biological chemistry* **281**, 23319-23325 (2006).
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