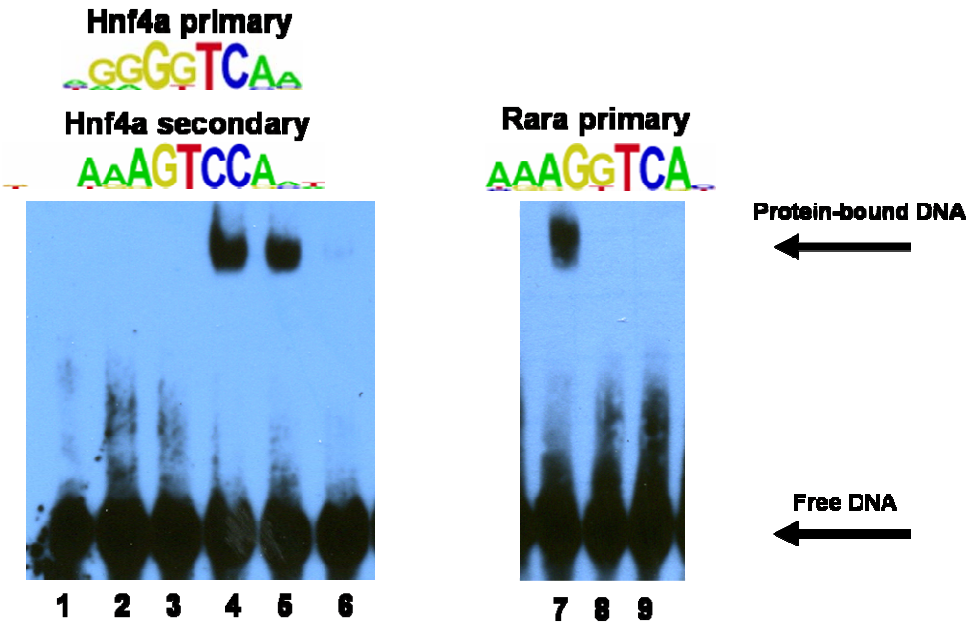

Figure S9. EMSA confirmation of secondary motifs. EMSAs were performed to validate binding to secondary motifs, as determined by the Seed-and-Wobble algorithm (Berger *et al.*, *Nature Biotechnology*, 2006) for Hnf4a. Lane 1: Hnf4a primary probe alone; lane 2: Hnf4a secondary probe alone; lane 3: GGTCCCA probe; lane 4: Hnf4a protein + Hnf4a primary probe; lane 5: Hnf4a protein + Hnf4a secondary probe; lane 6: Hnf4a protein + GGTCCCA probe; lane 7: Rara protein + Hnf4a primary probe; lane 8: Rara protein + Hnf4a secondary probe; lane 9: Rara protein + GGTCCCA probe. Lanes 1-6 show that Hnf4a binds to both the primary and secondary motifs derived by PBM, and very weakly to a third probe containing the sequence GGTCCCA; see **Materials and Methods** for the complete probe sequences. Hnf4a is the only C4 class of zinc finger proteins assayed in this study which showed a preference for this secondary motif (GGTCCA secondary, GGTCA primary). To validate that this secondary motif is specific to Hnf4a, we ran the same probes against another C4 zinc finger protein, Rara (lanes 7-9). Rara can bind to the Hnf4a primary motif sequence (GGTCA), but not the secondary motif of Hnf4a (GGTCCA), or to a probe containing the sequence (GGTCCCA); Rara did not yield a significant secondary Seed-and-Wobble PBM motif. All probe sequences are provided in the **Materials and Methods**.

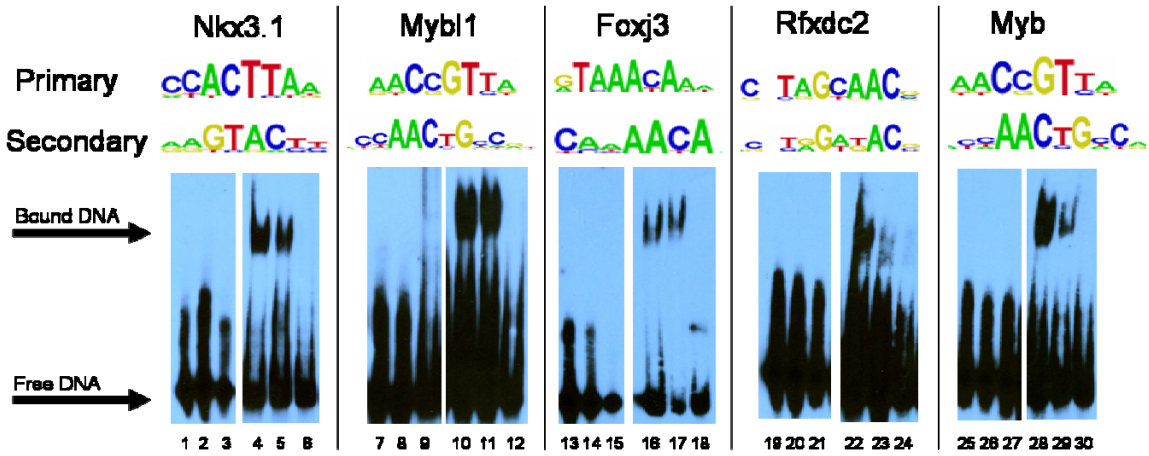

Figure S9 (continued). EMSA confirmation of secondary motifs. EMSAs were performed to validate binding to secondary motifs, as determined by the Seed-and-Wobble algorithm (Berger *et al.*, *Nature Biotechnology*, 2006) Lane 1: Nkx3.1 primary probe alone; lane 2: Nkx3.1 secondary probe alone; lane 3: Foxj3 primary probe alone; lane 4: Nkx3.1 protein + Nkx3.1 primary probe; lane 5: Nkx3.1 protein + Nkx3.1 secondary probe; lane 6: Nkx3.1 protein + Foxj3 primary probe; lane 7: Mybl1 primary probe alone; lane 8: Mybl1 secondary probe alone; lane 9: Foxj3 primary probe alone; lane 10: Mybl1 protein + Mybl1 primary probe; lane 11: Mybl1 protein + Mybl1 secondary probe; lane 12: Mybl1 protein + Foxj3 primary probe; lane 13: Foxj3 primary probe alone; lane 14: Foxj3 secondary probe alone; lane 15: Nkx3.1 primary probe alone; lane 16: Foxj3 protein + Foxj3 primary probe; lane 17: Foxj3 protein + Foxj3 secondary probe; lane 18: Foxj3 protein + Nkx3.1 primary probe; lane 19: Rfxdc2 primary probe alone; lane 20: Rfxdc2 secondary probe alone; lane 21: Mybl1 primary probe alone; lane 22: Rfxdc2 protein + Rfxdc2 primary probe; lane 23: Rfxdc2 protein + Rfxdc2 secondary probe; lane 24: Rfxdc2 protein + Mybl1 primary probe; lane 25: Myb primary probe alone; lane 26: Myb secondary probe alone; lane 27: Rfxdc2 secondary probe alone; lane 28: Myb protein + Myb primary probe; lane 29: Myb protein + Myb secondary probe; lane 30: Myb protein + Rfxdc2 secondary probe. All probe sequences are provided in the **Materials and Methods**.