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, GGTCGA 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 (GGTCGA), 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.