Epigenetic Priming of Enhancers Predicts Developmental Competence of hESC-Derived Endodermal Lineage Intermediates Wang et al., 2015

1. Why do you think it was important that they differentiate cells to pancreatic endoderm in a step-wise manner? How are enhancer states defined? What is the importance of quantitating the enhancer transitions? How might chromatin states and transitions contribute to developmental competence? How do you think PE-active enhancers are poised in the gut tube (GT) prior to pancreatic induction signaling? Figures 1 - 2.

2. Why do some poised enhancers never become active in their study? What is the evidence presented supporting this explanation? Why might gut tube (GT) be more enriched for poised enhancers than definitive endoderm (DE) - does this mean GT has more "developmental competence"? How definite is Figure 4 in showing that P3 and P4 poised enhancers become active in other tissues? What functional experiment might you do or additional evidence would you want to see to prove P3 and P4 enhancers can be active in endodermal tissues? Figures 3 - 4.

3. What are pioneering transcription factors (TFs)? Do you think they are transcriptional activators? Does the *FOXA1* shRNA data (Fig. 5F) fit with the poised enhancer model? Are FOXAs required for pancreas development? Figure 5. Is FOXA required for enhancer activation, or do you think it vacates the enhancer upon localization of other TFs?

4. Based on evidence in Figure 6, do you think PE cells might go on to form functional islets either *in vitro* or *in vivo*? Based on data presented in the paper do you think developmental competence in stem/progenitors is conferred by poised enhancer states? How else might developmental competence be conferred?

At times you may need to refer to supplemental figures that accompany the main figures.