Recent studies indicate that the generation of fully matured and functional insulin-producing beta cells from human stem cell populations is within grasp. We have optimized and extended differentiation protocols to generate mature beta cells from human embryonic stem cell (hESC) through replication of delamination and reaggregation processes that occur normally during embryonic islet formation. These enhanced beta cell clusters (eBC) display physiological features of human beta cells and can be used to interrogate previously intractable aspects of human beta cell biology. Genetic manipulation of critical beta cell factors reveals novel insights into human beta cell development, maturation, and function.