ZNF403 is an evolutionarily conserved member of the zinc finger protein family containing a zinc finger motif and a nuclear receptor binding domain. To investigate its function, Znf403 mutant mice in the C57BL/6 genetic background were generated using a gene-trap embryonic stem cell clone. The Znf403 null mutant embryos died in utero during E13.5 to E15.5 with dysmorphic placentae, characterized by excessive non-vascular cell nests consisting of proliferative trophoblastic tissue and abundant trophoblast stem cells (TSCs) in the null mutant labyrinth. Immunohistochemistry, morphometric analyses and rhodamine dye perfusion tests revealed that lethality of Znf403 null embryos was likely caused by a defect in placental perfusion due to remarkable decreases in both fetal and maternal blood vessels in the labyrinth. A marked increase in TSC proliferation and accumulation in the Znf403 null E15.5 labyrinth were accompanied by a significant elevation of c-Met expression and phosphorylation and its downstream effector Stat3 activation. In wild type placentae, ZNF403 was localized in both the cytoplasm and nuclei of trophoblasts. Knockdown of Znf403 in TSCs by siRNA in vitro provoked the proliferation but delayed the differentiation, which were also associated with an upregulation of c-Met expression and an enhancement in c-Met and Stat3 phosphorylation. Notably, over-expression of Znf403 exhibited completely opposite effects compared to Znf403 deficient TSCs. These results suggest that loss of ZNF403 in the placenta over-activates the c-Met-Stat3 signaling, alters TSC proliferation and differentiation and ultimately compromises the structure of placental vascular labyrinth. Data indicate for the first time that ZNF403 is an essential factor for pregnancy success through its role in maintenance of a balance of TSC proliferation and differentiation during placental development.