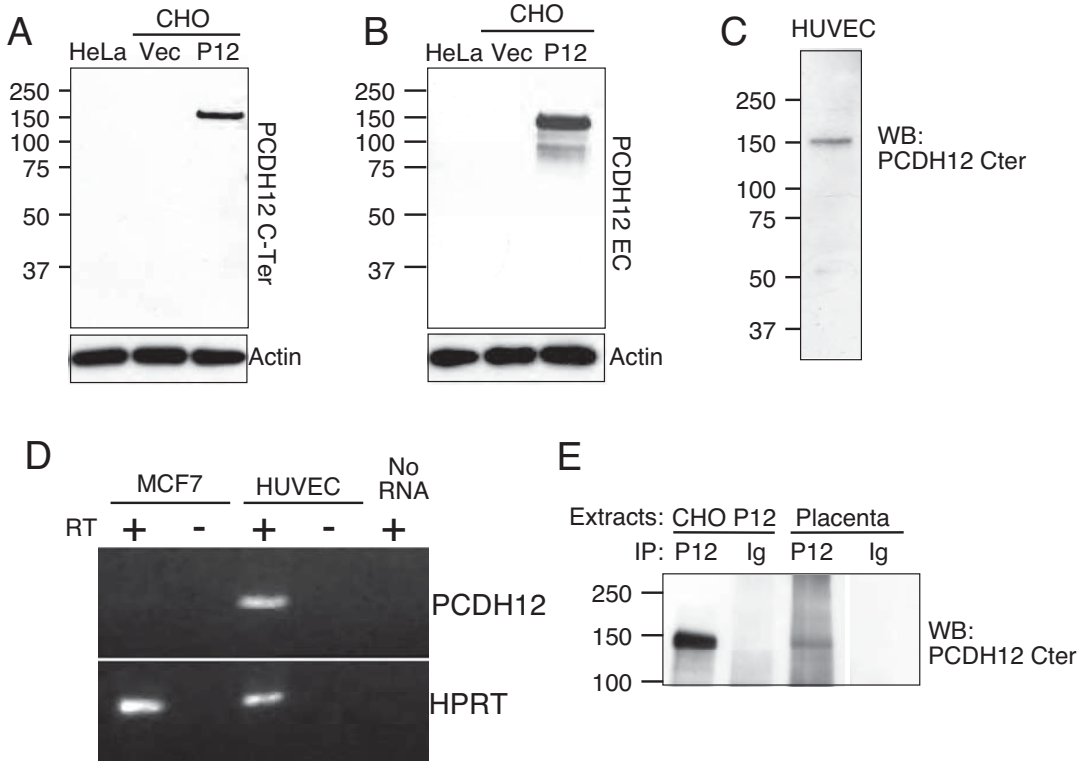

Supplemental Figure 2: Pcdh12 expression in human endothelial cells and placenta

Methods- Antibodies directed against the C-terminus (C-ter) tail (A,C) or the extracellular (EC) domain (B) were tested in Western blot analysis, using protein extracts (20 μg) from HeLa cells or from CHO cells transfected either with Pcdh12 (P12) or empty (Vec) vectors, or protein extracts (80 μg) from HUVECs (C). Actin was revealed as loading control. RNAs were prepared from HUVEC and MCF7 cell line using the Nucleospin kit from Macherey-Nagel (Hoerdt, France). cDNAs were obtained using Superscript II reverse transcriptase (RT; Invitrogen, Cergy Pontoise, France). Primers used for PCR amplification were: Hprt 5'-GACCAGTCAACAGGGGACAT and 5'-AAGCAGATGGCCACAGAACT; Pcdh12 5'-AGGAGTGCAATCCCAGACAC and 5'-CAAAGAGAGTCTCGCCATCC. Amplification products were visualized by ethidium bromide staining. Controls are: PCR amplification with no RNA, or without RT step. (E) Protein extracts (1 mg) from human term placenta (Plac; 300 μg) or from CHO-PCDH12 (CHO P12; 300 μg) were immunoprecipitated with anti-PCDH12 Cter antibody or with non-immune IgG (Ig), electrophoresed and immunoblotted with PCDH12 EC antibody.

Results- (A,B) Both antibodies revealed a single band at the expected size in CHO-PCDH12. (C) PCDH12 could be detected by Western blotting of HUVEC lysates when high amounts of proteins were loaded. (D) PCDH12 mRNA was detected in endothelial and not in epithelial cells. (E) PCDH12 is present in placental extracts.