Supplemental Materials

Molecular Biology of the Cell

Cox et al.

Figure S1

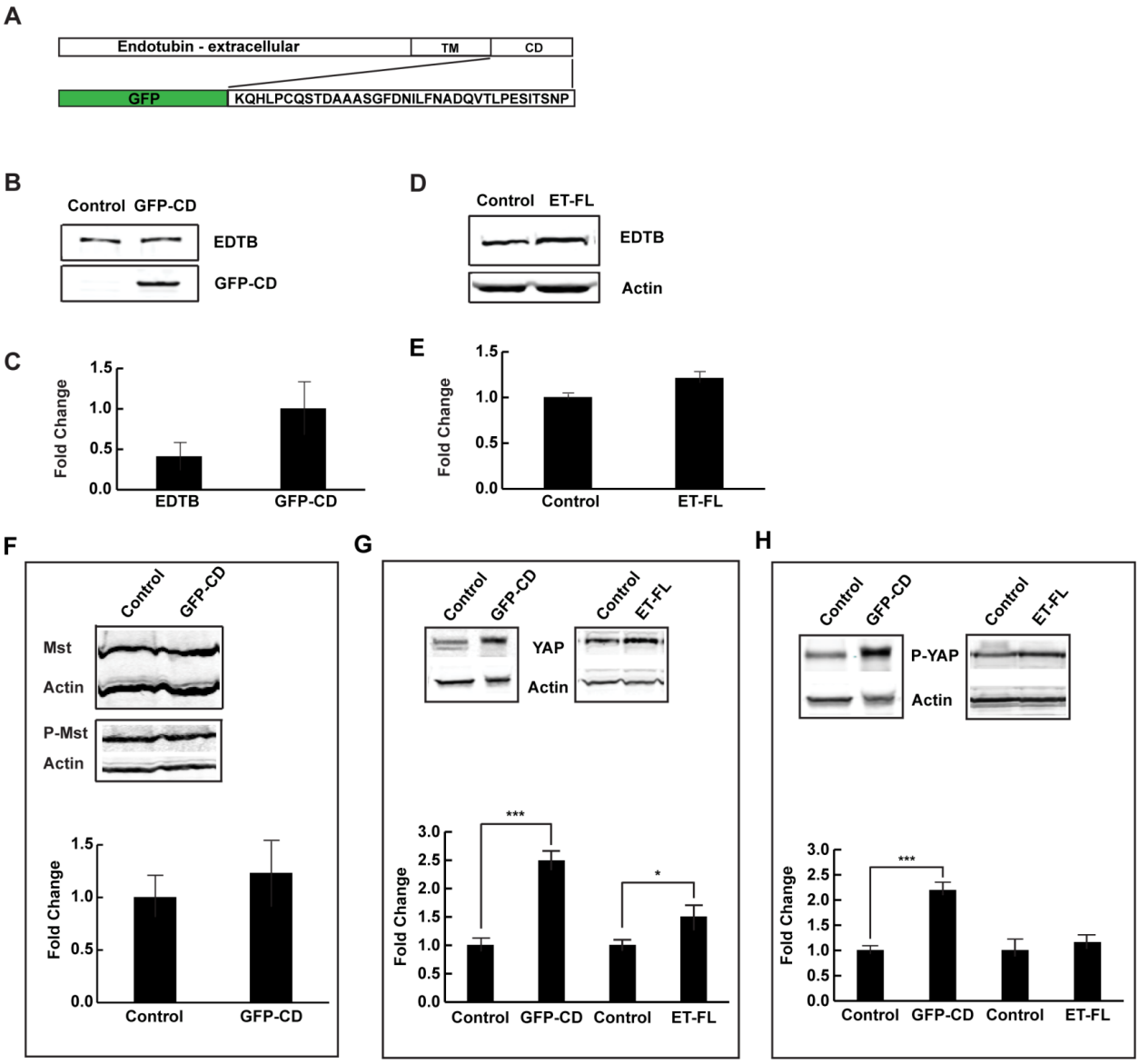

Figure S1. Relative levels of GFP-CD and ET-FL compared to endogenous EDTB. (A) Diagram of GFP-CD construct used in many experiments. GFP was fused to the N-terminal of the EDTB cytoplasmic domain. (B, C) The expression of GFP-CD in stable cell lines was determined by Western blot analysis and quantified using LiCor Odyssey imaging. (D, E) The expression of ET-FL from stable cell lines was determined as above. (F) Expression of Mst and p-Mst in cells expressing GFP-CD (G, H) Expression of YAP and phospho-YAP in cells expressing GFP-CD or ET-FL. Error bars, SEM. ***$p<0.005$, *$p<0.05$ based on t-test analysis.

Figure S2

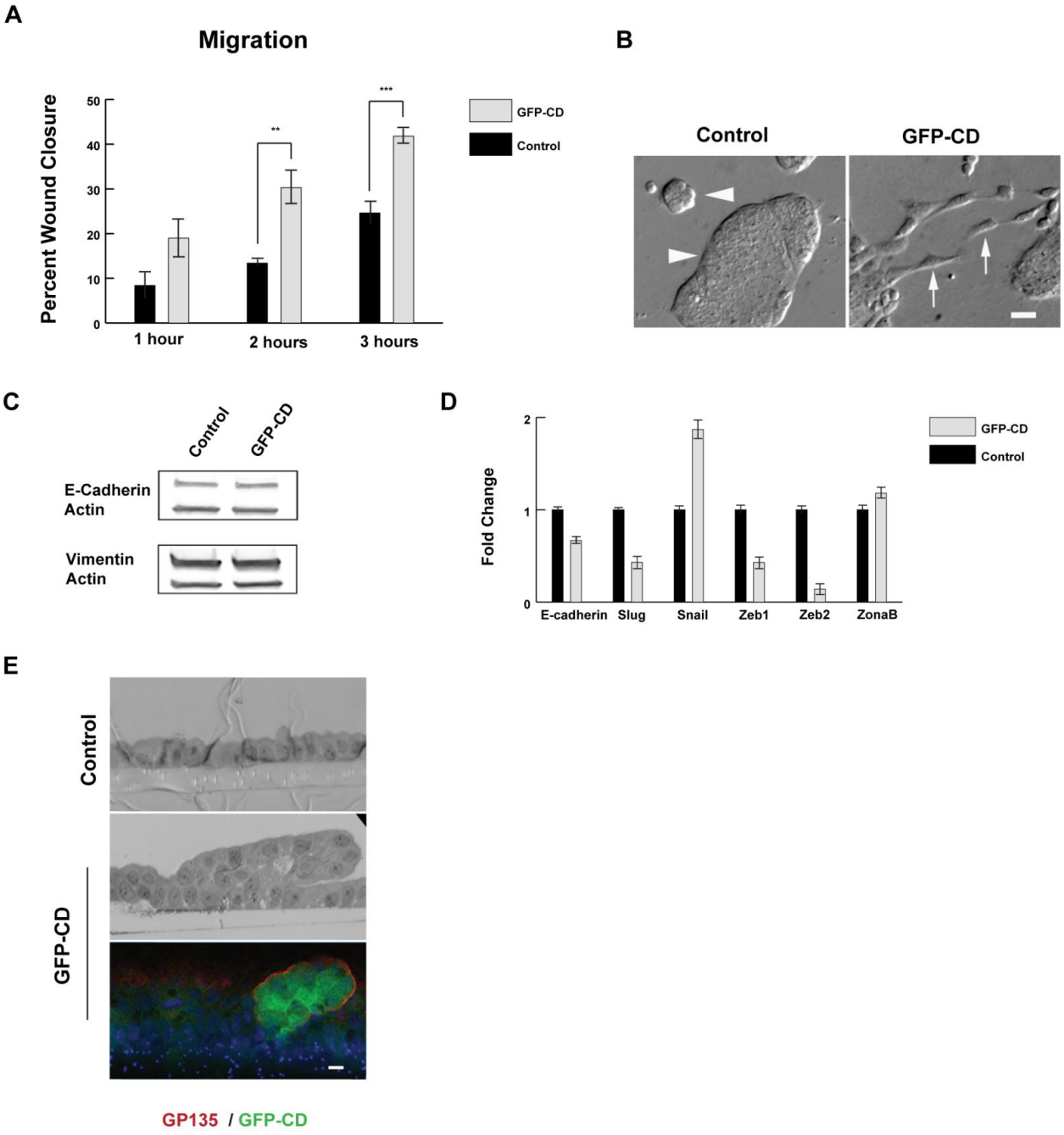

Figure S2. GFP-CD expressing cells migrate more quickly than control cells but this increased migration rate is not due to epithelial to mesenchymal transition. (A) Confluent monolayers of MDCK control or GFP-CD expressing cells were wounded using a pipet tip and the percentage wound closure was measured. GFP-CD expressing cells migrate more quickly than control cells. Error bars, SEM. **p<0.01, ***p<0.005 based on t-test analysis. (B) To test for an epithelial to

mesenchymal transistion, MDCK control and GFP-CD cells were plated on collagen matrix. Control MDCK's remain as compact colonies (arrowheads) while the GFP-CD expressing cells form spindle-shaped cells (arrows). The MDCK control and GFP-CD expressing cells do not invade the matrix. Scale bar, 20μm. (C) MDCK control and GFP-CD lysates were analyzed by western blot for expression of the epithelial marker E-cadherinin and the mesenchymal marker vimentin. There is no change in the expression of the markers when GFP-CD is expressed. (D) Quantitative PCR of control and GFP-CD expressing MDCK cells shows an increase in the transcription factor Snail and no increase in the Zeb transcription factors. Error bars, SEM. (E) GFP-CD cells grown on Transwell filters lose contact inhibition. GFP-CD expressing cells form polyp-like structures with strong GFP expression and maintain expression of the apical maker GP135. Scale bar, 20μm.

Figure S3

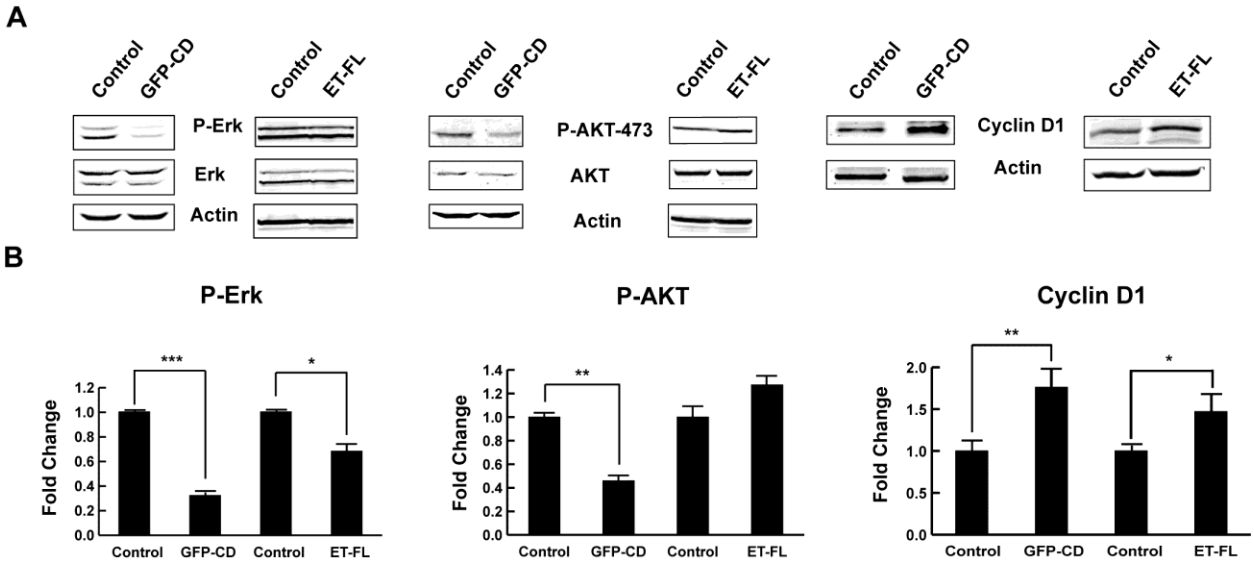

Figure S3. EDTB over-expression results in increased cyclin D1 independent of MAPK and AKT pathway activation. (A, B) MDCK cells expressing full-length or the cytoplasmic domain of EDTB were analyzed by western blot for pathway activation. Error bars, SEM, *p<0.05, **p<0.01, ***p<0.005 based on t-test analysis.

LIHC EDTB rsem values (T/N)

Sample ID	Stage	Tumor	Normal	Fold Change
TCGA-FV-A2QR	Stage I	4187	184	22.76
TCGA-DD-A1EH	Stage III	6206	643	9.65
TCGA-DD-A118	Stage I	3885	436	8.91
TCGA-BD-A2L6	NA	835	134	6.23
TCGA-DD-A3A2	Stage I	4062	676	6.01
TCGA-DD-A11A	Stage I	204	64	3.19
TCGA-ES-A2HT	Stage I	1688	585	2.99
TCGA-DD-A3A3	Stage I	102	51	2.00

Table S1. EDTB is upregulated in a subset of LIHC. RSEM normalized values of tumor and normal adjacent tissue from 48 pairs were analyzed for changes in EDTB expression. There is a 2 fold or greater increase in 8 tumor samples.

Stage distribution of EDTB expression in LIHC samples.

	Low	**Medium**	**High**
Stage I	11	13	19
Stage II	9	10	6
Stage III/IV	15	12	10

Table S2. Binning of EDTB expression values in LIHC tumor samples. Binning of rsem values into 3 equal groups based on expression of EDTB shows an increase in the percentage of Stage I tumors with high expression. 44% of stage I tumors express EDTB at high levels and 26% are low expressors. This trend is reversed for the stage III/IV tumors. In these tumors 27% are high expressors while 41% express EDTB at low levels.