Legends to supplemental Figures:

Fig. S1:

(a) Stable ZEB1 knockdown enhances epithelial and represses mesenchymal gene expression in colon and breast cancer cells and (b) results in a reduced migratory potential of MDA-MB231 cells in a wounding assay.

(c) A similar change as for SW480 shZEB clones towards a polarised, epithelial phenotype is also seen in the undifferentiated breast cancer cell line MDA-MB231 and in the colorectal cancer cell line HCT116. Note the almost complete loss of the mesenchymal marker vimentin and the invasive growth of control transfectants in 3D Matrigel for MDA-MB231 shZEB cells.

(d-g) *Increasing malignancy is associated with loss of cellular polarity:*(d) Immunohistochemical staining for E-cadherin in normal colorectal mucosa shows polarized epithelial cells forming a central tubule, characterized by basal localisation of the nuclei, basolateral expression of E-cadherin and apical localisation and secretion of mucins. (e) Well-differentiated colorectal carcinomas (CRCs) (grade 1) with still preserved basal-apical polarity of E-cadherin expressing tumour cells (insert), forming tubular structures, although with atypical architecture. (f) Moderately-differentiated CRC (grade 2) with reduced cellular polarity: Some tumour cells show polar orientation and build up tubules which are fused to each other (cribriform pattern) (insert and arrows), whereas in other regions polarity is lost, although E-cadherin is still expressed (arrowhead). (g) Poorly-differentiated CRC (grade 3), where cellular polarity is completely lost. E-cadherin is reduced at the membrane but expressed in the cytoplasm and tumour cells form an unstructured mass. (Bar size is 50µm and 20µm for the inserts).

Fig. S2:

(a) Promoter regions of human polarity factor genes affected by ZEB1: vertical bars show positions of potential ZEB1 binding sequences (CAGGTG/A) and horizontal bars indicate regions amplified by PCR for ChIPs.

(b) Chromatin IP of the regions indicated in (a) using extracts from indicated cell lines shows specific binding of endogenous ZEB1 to the Lgl2-, Crumbs3- and INADL-promoter (not done with MDA-MB231 extracts for lgl2.2, Crumbs3 2., Gapdh).

(c) Recombinant DNA-binding domain of ZEB1 binds simultaneously to both E-boxes of the Lgl2-promoter indicated by a supershifted band (**). Mutation of either E-box 1 (E1M) or E-box 2 (E2M) results in binding only to one remaining wildtype site, indicated by single band

shift (*). Mutation of both sites (E1M/E2M) inhibits binding of ZEB1. GST alone did not bind. The known ZEB1 binding element of the human interleukin 2 promoter (NRE) was used as positive control.

(d) Nuclear extracts from SW480 colorectal cancer cells contain ZEB1, which binds to the interleukin 2 promoter E-box (NRE) and the Lgl2 E-boxes (E1/E2). ZEB1 is identified by a supershift (*) with a ZEB1-specific antiserum. Mutation of both Lgl2 E-boxes (E1M/E2M) abolished only binding of ZEB1, but not of the other factors, indicating that these are unspecific and none of the other known EMT-repressors are binding to the Lgl2 E-boxes in colorectal cancer cells.

(e) Human Lgl2 promoter activity is suppressed by the EMT-associated transcriptional repressors ZEB1, ZEB2, Slug and Snail in HCT116 colorectal carcinoma cells and HEK293 epithelial cells.

(f) ZEB1 knockdown increases Lgl2 promoter activity.

(g) Transient siRNA mediated knockdown of ZEB1 (mRNA levels in striped columns) leads to increased mRNA expression of Lgl2 (black columns) in colorectal and breast cancer cell lines, depending on absolute levels of endogenous ZEB1 and Lgl2: Stronger increase in high ZEB1- and low Lgl2-expressing SW480, no significant increase in low ZEB1- and high Lgl2-expressing DLD1, intermediate effect in HCT116 cells. Also in the undifferentiated breast cancer line MDA-MB231, ZEB1 knockdown results in 6.8 fold increase in Lgl2 expression. NCM460, a normal colon epithelial cell line, is used as reference for normal expression levels of ZEB1 and Lgl2.

Fig. S3:

(a) *Aberrant expression of ZEB1 in carcinoma cell lines correlates with low Lgl2 expression and unpolar growth pattern of xenograft tumours:*

Expression of ZEB1 (black column) and Lgl2 (striped column) was analysed in eight colorectal cancer cell lines and two breast cancer cell lines (low differentiated MDA-MB231 and differentiated MCF7). Relative expression of ZEB1 in the undifferentiated line SW620 and of Lgl2 in the differentiated line T84 was set to 100%. Normal human foreskin fibroblasts (HFF) were used as control for maximal ZEB1 expression in mesenchymal cells. Aberrant expression of ZEB1 was associated with low expression of Lgl2, with highest ZEB1 in HFF and lowest ZEB1 coupled to highest Lgl2 in the line T84. Xenograft tumours after subcutaneous injection in nude mice supported the role of ZEB1 in suppressing both Lgl2 and subsequent cellular polarity: SW620 form an unstructured tumour mass, whereas T84 grow in differentiated

tubular structures built up by polarized tumour cells. Caco-2, with intermediate expression of ZEB1 and Lgl2, formed mixed tumours with beginning signs of polarity and mucinous differentiation (arrows) as well as undifferentiated tumour cell clusters.

(b) *Reduced mRNA expression of Lgl2 in breast cancers compared to normal breast epithelium* (normal = mean expression value of 3 normal breast epithelia).

(c) Overview of a typical well-to moderately differentiated colorectal adenocarcinoma doublestained for Lgl2 (red) and ZEB1 (brown). The bold arrow indicates the direction of invasion, the squares the magnified central and invasive areas below. (Lower panel) Note that ZEB1 is lacking in differentiated, tubular areas (asterics), built up by polar tumour cells with preserved membranous expression of Lgl2 (insert), whereas ZEB1 is expressed in mesenchyme-derived stromal fibroblasts (arrowheads). Only few single tumour cells embedded in differentiated tumour areas express ZEB1 (arrow). (Upper panel) In contrast ZEB1 is expressed in invasive tumour cells (arrows and insert), which lack any signs of polarity, show reduced levels of LGL2 and detach from the tumour mass. Adjacent more differentiated tumour cells form tubule-like structures (asterics), express more LGL2 and lack nuclear ZEB1.

(d) Transient knockdown of Lgl2 on top of a stable shZEB1 knockdown reverts the epithelial phenotype induced by ZEB1 knockdown MDA-MB231 breast cancer cells as shown by confocal staining: Cells loose polarity, show reduced expression of Lgl2 and translocate β-catenin to the cytoplasm and nucleus. As shown in the z-axis mode for β-catenin staining Lgl2 knockdown leads to flattening of the cells and reduced apical-basal polarity.