SUPPLEMENTARY FIGURE LEGENDS.

**Supplementary figure 1.** EphB2 is frequently downregulated in colorectal cancer metastasis. Example of a human colorectal metastasis (CM and black arrowheads) in the liver (L and white arrowheads) stained with anti β−catenin antibodies (a,b) or anti-EphB2 antibodies (c,d). Dashed line depicts the boundary between the metastatic node and the normal hepatic tissue. Note the strong accumulation of nuclear β−catenin in metastatic colorectal tumour cells compared with the subtle membrane staining in normal hepatic tissue. EphB2 is not expressed in the liver and it is also completely absent in metastatic colorectal cancer cells despite prominent β−catenin accumulation.

**Supplementary figure 2.** Examples of tumour classification according to the percentage of EphB2 positive cells. Hematoxylin-eosin staining (e-h) or immunostaining with anti-EphB2 antibodies (a-d) of 4 representative human colorectal cancer lesions. Each of them was classified into one of four groups depending on the numbers of EphB2 positive and negative cells. Red arrows indicate EphB2 positive areas while green arrows point to EphB2 negative tumour cells. a,e, Early dysplastic crypts belonging to group I where virtually all precancerous cells show EphB2 staining. b,f, Carcinoma classified as type-II showing between 80 and 50 percent of EphB2 positive cells. c,g, Carcinoma classified as type-III composed of less than 50 percent of EphB2 positive cells. d,h, Carcinoma completely negative for EphB2 staining. Note that the adjacent normal epithelium stained with anti-EphB2 antibodies at the crypt bottom (arrowheads). Dotted line depicts the boundary between normal and tumour tissues.

**Supplementary Figure 3.** Statistical analysis of EphB2 expression in colorectal lesions. a, Analysis of individual groups of lesions. The mean score of EphB2 expression in dACFs is significantly different to that of Small Adenomas (SA): p=0.001 (t-test), p=0.001 (Mann-Whitney). Mean expression of EphB2 is highly similar in all carcinoma stages p=0.859 (Oneway test) and p=0.625 (Kruskal-Wallis test) and therefore carcinomas can be considered as a single entity regarding EphB2 expression. Mean expression of EphB2 in Large Adenomas (LA) is different to that of carcinomas p=0.048 (t-test), p=0.067 (Mann-Whitney test). b, Analysis of clustered groups of lesions. Carcinomas at different Duke's stage were combined in a group and compared to dACFs, Small Adenomas and Large Adenomas. The 4 groups significantly differed regarding EphB2 levels: p<0.001 (Onway test) and p<0.001 (Kruskal-Wallis test). Abbreviations are as follows: N, number of cases in each group; 95% CI, 95% confidence interval; Sd, standard deviation; Se, standard error.
**Supplementary Figure 4.** Statistical analysis of EphB4 expression in colorectal lesions. a, Analysis of individual groups of lesions. The mean score of EphB4 expression in dACFs is significantly different to that of Small Adenomas (SA): p=0.004 (t-test), p=0.002 (Mann-Whitney). Mean expression of EphB4 is similar in all carcinoma stages p=0.268 (One-way test) and p=0.389 (Kruskal-Wallis test) and therefore they can be considered as a single entity regarding EphB4 expression. Mean expression of EphB4 in Large Adenomas (LA) is not different to that of carcinomas: p=0.270 (t-test), p=0.610 (Mann-Whitney test). b, Analysis of clustered groups of lesions. Carcinomas at different Duke’s stage were combined in a group and compared to dACFs, Small Adenomas and Large Adenomas. The 4 groups significantly differ regarding EphB4 levels: p<0.001 (One-way test) and p<0.001 (Kruskal-Wallis test). Abbreviations are as follows: N, number of cases in each group; 95% CI, 95% confidence interval; Sd, standard deviation; Se, standard error.

**Supplementary figure 5.** Statistical analysis of the correlation between EphB2 and EphB4 expression in colorectal lesions. Correlation coefficients (r) according to Pearson test and Spearman tests are shown for the global series (independently of tumour classification) and for each type of lesion. Correlation coefficients could not be determined (ND) for dACF lesions because variable EphB4 expression was constant. However, as shown in Fig.2c, the correlation between EphB2 and EphB4 expression is almost perfect in this type of lesions.

**Supplementary figure 6.** EphB2 mRNA is not downregulated in a subset of CR tumours. Example of an invasive colorectal carcinoma showing protein downregulation (a-c) yet high levels of EphB2 mRNA expression (d-f). Dashed line depicts the border between normal (N) and tumour tissue (T). Arrowheads point to staining at the bottom of the crypts. Note that EphB2 mRNA expression in tumour cells (f) is as strong as in crypts (e) despite the substantial reduction in protein levels (c) compared to normal cells (b).

**Supplementary figure 7.** EphB3 mRNA expression pattern overlaps with that of EphB2 in CR carcinomas. Representative examples of in situ hybridization using EphB2 (a-c) or EphB3 (d-f) specific cRNA probes on consecutive sections of 3 human carcinomas. a,d, low grade carcinoma classified as Group-II. b,e low-medium grade
carcinoma classified as Group-III. c,f, high grade carcinoma classified as Group-IV. Black arrowheads depict hybridization at the bottom of normal crypts (N). Red arrows indicate examples of areas with exact correspondence between EphB2 and EphB3 mRNA expression. Note the low levels of EphB2 and EphB3 mRNA expression present in the high grade carcinoma (c,f) compared to low (a,d) and medium (b,e) grade tumours.

**Supplementary figure 8.** EphB4 is a β-catenin/Tcf target gene. a-f, anti-EphB2 (a,c,e) and anti-EphB4 (b,d,f) immunostainings in the small intestine of wild-type (a,b), EphB2-EphB3 double knock out (c,d) or Min mice (e,f). EphB4 outlines the membranes of proliferative crypt cells with identical pattern to that of EphB2. EphB4 is also highly expressed in cells from polyps of *Apc*<sup>Min/+</sup> mice (panel f- cells encircled by dashed line). The lack of staining in EphB2-B3 double deficient tissue demonstrates the specificity of the antibody used in this study.

g, Northern blot analysis of the expression of EphB4 in LS174T colorectal cancer cells that express dominant negative forms of Tcf4 or Tcf1 24 hours upon the addition of doxycycline<sup>(5)</sup>.

**Supplementary figure 9.** EphB4 is expressed with a similar pattern to that of EphB2 in human colorectal tissue and tumours. Immunostaining using anti-EphB4 antibodies (a,c,e) or anti–β–catenin antibodies (b,d,f) in normal colonic epithelium (a,b), early adenoma tissue (c,d) and invasive carcinoma (e,f). Black arrowheads point to the bottom of normal crypts. White arrowheads indicate tumour cells. Arrows in panel b point to normal progenitor cells showing nuclear β–catenin accumulation at the bottom of colonic crypts. Dotted line depicts the boundary between normal colonic epithelium and tumour tissue. Note that EphB4 outlines the membranes of intestinal precursors at the bottom of the crypts as well as of early adenoma cells but it is essentially absent from the invasive carcinoma. Yet all these cells types show nuclear β–catenin accumulation.

**Supplementary figure 10.** Colorectal adenomatous micro-lesions in *Apc*<sup>Min/+</sup> mice show nuclear β–catenin accumulation and express EphB2 and EphB4. Staining with Haematoxylin/eosin (a), anti–β–catenin antibodies (b), anti-EphB2 antibodies (c) or anti-EphB4 antibodies (d) on serial sections of distal colon of a 21 week old *Apc*<sup>Min/+</sup> mice. Dashed line encircles a cluster of small dysplastic adenomatous crypts close to the surface epithelium. Note strong accumulation of β–catenin (b) and high expression
of β–catenin/Tcf targets EphB2 (c) and EphB4 (d). Black arrowheads indicate expression of EphB2 and EphB4 at the bottom of normal colonic crypts.

**Supplementary figure 11.** Features of colorectal carcinomas in compound ΔcyEphB2;Apc\textsuperscript{Min/+} mice. a,b, β-catenin staining of the colorectal region of ΔcyEphB2;Apc\textsuperscript{Min/+} mice. β-catenin outlines the basolateral membrane in the normal mucosa (N) but shows prominent cytoplasmatic and nuclear accumulation in tumour tissue (T). Dotted line depicts the boundary between normal and tumour tissue. Panel b is a higher magnification picture of the area marked with a square in panel a. c-e, Examples of cribiform growth (c), strong desmoplastic reaction (arrows in d) and necrosis (e) in ΔcyEphB2;Apc\textsuperscript{Min/+} tumours.