SUPPLEMENTARY METHODS

Cells and cell culture

IMR90 (ATCC CCL-186), BJ-hTERT (ATCC CRL-4001), NIH3T3 (ATCC CRL-1658), CCD18Co (ATCC CRL-1459), HT29 (ATCC HTB-38), HCT116 (ATCC CCL-247), CACO2 (ATCC HTB-37), MCF7 (ATCC HTB-22), HEK293T (ATCC CRL-3216), and SW480-ADH cells were cultured in DMEM plus 10% FBS (both from Life Technologies, Carlsbad, California, USA). SW480-ADH cells are a subpopulation derived from SW480 cell line (ATCC CCL-228)[9] and the generation of SW480-ADH shVDR and shControl cells was previously described.[55] THP1 (ATCC TIB-202) cells were cultured in RPMI-1640 plus 10% FBS (both from Life Technologies). Cell lines were periodically authenticated with the GenePrint 10 System (Promega, Fitchburg, Wisconsin, USA) and results were sent for comparison against ATCC cell line database (Manassas, Virginia, USA). Cells were treated with 100 nM $1,25(OH)_2D_3$ (Sigma-Aldrich, San Louis, Missouri, USA) or the corresponding vehicle (ethanol) concentration. HEK293T cells were transiently transfected with an expression vector for human VDR (kindly provided by Dr Ana Aranda, Instituto de Investigaciones Biomédicas "Alberto Sols", Madrid, Spain) or with the empty vector (pSG5) using jetPEI transfection reagent (PolyPlus Transfection, Illkirch, France), and cells were analysed after 48 h. Phase-contrast images of cultured cells were captured with a DS-L1 digital camera mounted on an inverted TS100 microscope (Nikon, Tokio, Japan) and processed using Adobe Photoshop CS6 (San Jose, California, USA).

Cell immunofluorescence

Cells growing on glass coverslips were fixed with cold methanol. Non-specific sites were blocked and coverslips were incubated with antibodies against vimentin (M7020, Dako, Glostrup, Denmark), α-SMA (A5228, Sigma-Aldrich), cytokeratin-18 (61028, Progen,

Heidelberg, Germany), E-cadherin (610182, BD Bioscience, San Jose, California, USA) and VDR (12550, Cell Signaling Technology, Danvers, Massachusetts, USA), and then with secondary antibodies conjugated with AlexaFluor-488 dye (Life Technologies). To evaluate non-specific reactions of the secondary antibodies, equivalent coverslips were incubated without primary antibody or with rabbit IgG isotype control antibody (3900, Cell Signaling Technology; for VDR staining). For nuclear counterstaining, coverslips were incubated with DAPI (Sigma-Aldrich). Coverslips were mounted using Prolong Gold antifade reagent (Life Technologies). Confocal microscopy was performed on a spectral LSM710 laser scanning microscope (Carl Zeiss, Oberkochen, Germany) using argon ion (488 nm) and violet diode (405 nm) lasers. Images were acquired sequentially by direct register using Zeiss Confocal software (ZEN 2009). All images were processed using Adobe Photoshop CS6.

RNA isolation and RT-qPCR

Total RNA from cell lines and primary fibroblasts was extracted using the NucleoSpin miRNA extraction kit (Macherey-Nagel, Düren, Germany). Total RNA from tissues (Human Total RNA Master Panel II) was purchased from Clontech (Mountain View, California, USA). RNA was retrotranscribed using iScript cDNA Synthesis kit (Bio-Rad, Hercules, California, USA). Then, the qPCR reaction was performed in a CFX384 Real-Time PCR Detection System (Bio-Rad) using TaqMan Universal Master Mix II or Power SYBR Green PCR Master Mix (Life Technologies). Thermal cycling of the qPCR reaction was initiated with a denaturation step at 95ºC for 10 min and consisted of 40 cycles (denaturation at 95ºC 15 s, annealing and elongation at 60ºC for 30 s). We used TaqMan probes for *CYP24A1* (Hs00167999_m1), *CHRDL1* (Hs01035484_m1), *NID2* (Hs01547192_m1), *SEMA3B* (Hs01090156_m1), *TIMP3* (Hs00927214_m1), *CD82* (Hs01017982_m1), *CXCL12* (Hs00171022_m1), *CCL11* (Hs00237013_m1), *CCL13* (Hs00234646_m1), *S100A4*

(Hs00243202_m1 and Mm00803371_m1), *CYTL1* (Hs01573280_m1), *CCL2* (Hs00234140_m1), *GAPDH* (Hs02758991_g1), *B2M* (Hs99999907_m1) and *RPLP0* (Hs99999902_m1) (Life Technologies); and primers for *VDR* (Forward: AACGCTGTGTGGACATCGGC; Reverse: CGCAGACTGTCCTTCAAGGC), *OPN* (Forward: TTGCAGTGATTTGCTTTTGC; Reverse: GTCATGGCTTTCGTTGGACT), *SDHA* (Forward: TGGGAACAAGAGGGCATCTG; Reverse: CCACCACTGCATCAAATTCATG), mouse *Cyp24a1* (Forward: CGCCTTCCAAAAGAAACTCA; Reverse: CCCCATAAAATCAGCCAAGA) and mouse *Sdha* (Forward: CAGACCTGCGGCTTTCAC; Reverse: TCCAGCGCCTACAACCAC) (Sigma-Aldrich). In experiments with cell lines, RNA expression values were normalized against the reference housekeeping gene *SDHA* using the comparative C_T method. In experiments with primary fibroblasts, RNA expression values were independently normalized against the three reference housekeeping genes *GAPDH*, *B2M* and *RPLP0* using the comparative C_T method and the mean was calculated. All experiments were performed in triplicate.

Western blot

Whole-cell extracts from cultured cells were prepared by cell lysis with RIPA buffer plus protease- and phosphatase-inhibitors for 25 min on ice, followed by centrifugation at 13000 rpm for 10 min at 4°C. Tumour lysates were obtained as previously described.[56] Proteins were separated by SDS-PAGE, transferred to PVDF membranes and incubated with antibodies against VDR (for figure 7A: 04-1526, Merck Millipore, Billerica, Massachusetts, USA; for supplementary figure S1A, D: 12550, Cell Signaling Technology), β-actin (sc-1616, Santa Cruz Biotechnology, Santa Cruz, California, USA) and GAPDH (G9545, Sigma-Aldrich), and then with HRP-conjugated secondary antibodies. To evaluate non-specific reactions of

the secondary antibody used in the western blots for VDR expression in supplementary figure S1, equivalent blots were incubated without primary antibody or with rabbit IgG isotype control antibody (3900, Cell Signaling Technology). Antibody binding was visualized using the ECL detection system (GE Healthcare, Chalfont St. Gills, UK). Films were scanned with a HP Scanjet G2710 (Palo Alto, California, USA) and images were processed using Adobe Photoshop CS6. Quantification was done by densitometry using ImageJ (National Institutes of Health, Bethesda, Maryland, USA).

Cell proliferation

The assay used is based on the cleavage of the yellow [3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl] tetrazolium bromide salt (MTT) to purple formazan crystals by metabolic active cells. Fibroblasts were seeded in 24-well cell culture plates and treated with 100 nM $1,25(OH)_2D_3$ or vehicle. At the indicated times, cells were incubated with the MTT solution (final concentration of 0.5 mg/ml, Merck Millipore) for 4 h at 37ºC. After this incubation period, a water-insoluble formazan dye is formed. After solubilisation with 500 µl of isopropanol 0.04 M HCl during 30 min at RT, the formazan dye was quantified using a VersaMax scanning microplate spectrophotometer (Molecular Devices, Sunnyvale, California, USA). The absorbance was measured as 570-630 nm. All experiments were performed using triplicates.

Immunohistochemical analysis of human tissues

Heat antigen retrieval was carried out in pH 9.0 EDTA-based buffered solution in a Dako Link platform. For chromogenic staining, endogenous peroxidase was quenched and detection was performed by successive incubations with primary antibody, appropriate Flex+ Rabbit or Mouse Linker (Dako), and Flex+ polymer coupled with peroxidase (Dako). Sections were

then visualized with 3,3'-diaminobenzidine and counterstained with hematoxylin. Incubation with a mouse immunoglobulin fraction (Dako) instead of Flex+ Rabbit Linker was performed for VDR staining in mice tissues. For fluorescence staining, secondary antibodies conjugated with AlexaFluor-488 or -655 dye were used. Signal was detected using the Nuance FX Multispectral Imaging System (Caliper Life Sciences, Hopkinton, Massachusetts, USA) as previously described[57] by an experienced pathologist.

Detection of MSI phenotype and B-RAF V600 mutations in colorectal tumours

MSI phenotype of colorectal tumours was assessed by immunohistochemical analysis of the mismatch repair proteins MLH1, MSH2, MSH6 and PMS2 using primary monoclonal antibodies from Dako (M3640, M3639, M3646 and M3647) following the procedure detailed above. Normal protein expression was defined as nuclear staining in tumour cells, using nuclei of infiltrating lymphocytes and/or of normal stromal cells as positive internal control. Lack of protein expression was defined as complete absence of nuclear staining in tumour cells with concurrent positive labelling of the internal control. Tumours with lack of expression of at least one of the proteins were designated as mismatch repair deficient and MSI phenotype tumours, following National Comprehensive Cancer Network guidelines (http://www.nccn.org/).

For B-RAF V600 mutational analysis, DNA was extracted from a 5 μm section of formalin-fixed paraffin-embedded colorectal tumours using the cobas DNA Sample Preparation Kit (Roche Molecular Diagnostics, Pleasanton, California, USA) following manufacturer's protocol. Briefly, tissue sections were deparaffinised by xylene followed by lysis by protease digestion and chaotropic buffer incubation at 56ºC for 1 h and at 90ºC for 1 h. Subsequently, isopropanol was added and samples were then centrifuged through separating column for removing impurities. Adsorbed nucleic acids were washed and then

eluted with an aqueous solution. The amount of genomic DNA was determined by Nanodrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA) and adjusted to a fixed concentration per PCR reaction. DNA was amplified and detected by the cobas 4800 System using the cobas 4800 B-RAF V600 Mutation Test (both from Roche Molecular Diagnostics), following manufacturer's procedures. The assay is based on qPCR technology and detection is achieved by a melting curve analysis in which the temperature is ramped from 40°C to 95°C (TaqMelt). Positive and negative controls and a calibrator were included in each run to confirm the validity of the assay. Amplification, detection, quality control analysis, and result interpretation were standardized on the system.

SUPPLEMENTARY REFERENCES

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