ONLINE SUPPLEMENTARY MATERIAL

Cell Lines

KM12SM cells stably expressing firefly luciferase (KM12SM-vLuc cells) were obtained according to established protocols (1). Briefly, firefly luciferase lentiviral particles produced on HEK293T cells transfected with pLV[Exp]-Hygro-EF1A>Luciferase -#85134, Addgene-, psPAX2 -#22036, Addgene- and PM2.G Envelope -#12259, Addgene- were used to infect KM12SM colon cancer liver metastatic cells. After 3-4 weeks of selection with Hygromicin B (100 µg/mL), KM12SM-vLuc cells were grown, maintained in DMEM supplemented with 10% inactivated FBS, L-Glutamine, penicillin/streptomycin and hygromicin B (50 µg/mL) at 37ºC and 5% CO_2, and used in subsequent *in vivo* experiments after transient transfection with PTPRN or control siRNAs.

Gateway Plasmid Construction, Gene Cloning, DNA Preparation and Protein Expression

Sequence-verified, full-length cDNA plasmid containing PTPRN was obtained from the publicly available DNASU Plasmid Repository (https://dnasu.org/DNASU/). The ORF cloned in pDONR221 was transferred by a LR clonase reaction (Invitrogen, Carlsbad, CA) to a pANT7_cHalo vector for *in vitro* protein expression tagged with HaloTag at the C-terminal end (2). All donor and expression plasmids were sequence verified prior to use. To obtain the extra- and intra-cellular domains of PTPRN, PCR amplification of such domains was performed using specific primers with the recombination sites for Gateway cloning (Supplementary Table 2). PCR products were purified and cloned into pDONR221 and subsequently into pANT7_cHalo vector by BP and LR clonase reactions, respectively.

To obtain high-quality supercoiled DNA, plasmids were transformed into TOP10 *E. coli* cells and grown overnight at 37°C in 250 mL Luria Bertani (LB) supplemented with the adequate antibiotic (100 µg/mL ampicillin or 40 µg/mL kanamycin). Plasmid DNA was purified using the NucleoBond® Xtra Midi kit (Macherey-Nagel Inc., Bethlehem, PA). Proteins were expressed using HeLa cell lysates from the 1-Step Human Coupled IVT Kit (Thermo Fisher

Scientific, Waltham, MA) per manufacturer's recommendations to carry out the seroreactivity analyses (3; 4). HaloTag protein was used as negative control of seroreactivity in the assays (3; 4).

EBNA1 ELISA and PTPRN Luminescence Beads Seroreactive Immunoassay

Colorimetric ELISA for EBNA1 antibody determination for the evaluation of the specificity of the study was achieved coating 0.05 µg of EBNA1 protein (kindly provided by Protein Alternatives, S.L.) per well in 50 µl of phosphate-buffer saline solution (PBS) in 96-well transparent plates (Nunc) overnight at 4ºC. Plates were then blocked using a solution of 3% (w/v) skimmed-milk in PBS containing with 0.1% Tween (PBST) for 1 h at 37ºC and then incubated 1 h at 37ºC with 50 µl of the 1:300 diluted plasma samples. After extensive washing with PBST, plates were incubated for 1 h at 37ºC with 50 µl of an HRP-labeled secondary anti-Human IgG antibody (Dako). Lastly, colorimetric signal was developed as previously described (5; 6).

For the luminescence beads seroreactive immunoassays (2-4; 7; 8), PTPRN, ECD or ICD HaloTag fusion proteins were coupled to HaloTag magnetic beads (MBs) by incubating them overnight at 4ºC and 1000 rpm according to the manufacturer's instructions. In total, 0.67 µl of the IVT expression and 0.5 µl of the HaloTag MBs were used per measurement. After extensive washing and removal of non-covalently bound proteins with 0.1 M glycine, pH 2.7, the MBs with the immobilized proteins were blocked with a casein solution during 1 h at room temperature.

To verify covalent protein immobilization, the HaloTag fusion proteins were detected with anti-HaloTag monoclonal antibody diluted 1:1000 (Promega), followed by 1 h of incubation with the HRP-conjugated anti-mouse IgG (Sigma) diluted 1:2500. Alternatively, to evaluate plasma seroreactivity, beads containing the immobilized protein and blocked were placed on black Maxisorp 96-well plates (Nunc) to incubate them overnight with individual plasma samples at indicated dilutions in PBS containing 0.1% Tween 20 (v/v) and 3% BSA (w/v) at 1000 rpm and 4ºC. After washing, a HRP-conjugated anti-human IgG antibody (Dako) diluted

1:10000 in PBS containing 0.1% Tween 20 (v/v) and 3% BSA (w/v) was incubated with the MBs. The MBs signal was developed using 50 µL of SuperSignal ELISA Pico Chemiluminescent Substrate (Pierce, Rockford, IL) for the detection of luminescence on The Spark multimode microplate reader (Tecan Trading AG, Switzerland).

Invasion, Proliferation, and Wound Healing Assays

For Matrigel invasion assays, Transwell chambers (Corning) were coated with 50 µl of a 1:3 dilution in DMEM of 10 mg/ml Matrigel (Sigma). $8x10^4$ KM12 or KM12SM cells in DMEM containing 0.5% BSA (w/v) were seeded on the top Transwell layer, and underneath DMEM, supplemented with 10% FBS was applied. Transwells were then maintained at 37ºC and 5% CO_2 for 22 h. Then, non-invading cells were removed from the upper surface, and cells that migrated through the filter were fixed with 4% paraformaldehyde (Sigma), stained with crystal violet and washed with MilliQ-H_2O, to be counted under a microscope.

For proliferation assays, $5x10^3$ cells were seeded in 100 µl of medium in triplicate into 96-well plates. After 72 h, 50 µl of 3 mg/ml of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) diluted in growth medium was added onto the wells, and the plates were left 1 h at 37ºC. Then, the medium with MTT was carefully removed from the wells, and 50 µl of DMSO was added to disrupt cells and dissolve formazan crystals. After 30 min, the Optical Density (O.D.) at 570 nm was measured using The Spark multimode microplate reader (Tecan Trading AG).

For wound healing, cells were seeded in triplicate in 96-well plates to completely seal the wells. After attachment, a wound was produced and the growth medium replaced. A picture was taken at day 0 and every 12 h for 48 h. The closing area was calculated as pixels using ImageJ and indicated images.

References

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