SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 - The binding affinity of monoclonal antibodies to PTPRD.
A. ELISA-based binding curves for the interaction between PTPRD ectodomain and the panel of monoclonal antibodies. Data are normalized to the highest antibody concentration set at 100%.
B. Epitope mapping of monoclonal antibody recognition of the PTPRD ectodomain. Two constructs containing either the 3 Ig domains and the first 3 FN domains (1-3) (green) or the last 6 FN domains (3-8) (blue) were cloned and purified as described previously. The ability of the mAbs to recognize these fragments was assessed by ELISA. The clone number of the mAbs is listed below the appropriate fragment. The 3rd FN domain was the segment of overlap between the two fragments (orange); it was concluded that mAbs that recognized both fragments bound to this domain.

Supplementary Figure 2 - The recognition of PTPRD by RD-28 was validated by immunofluorescence and immunoblot.
A. (Left) Schematic illustration of PTPRD to illustrate the binding sites of the antibodies used in this experiment. (Right) Representative confocal microscopy immunofluorescence images of 293T cells stably expressing empty vector (EV) or PTPRD stained with anti-His antibody (green) or RD-28 (red), and DAPI (blue), under permeabilized (+) or non-permeabilized (-) conditions. Scale bars: 20 μm
B. Immunoblot analysis of human brain lysates (10 μg), normal human stem cell lysates (10 μg); 293T cells transfected with GFP, PTPRD isoform 1 (ISO1) or PTPRD isoform 6 (ISO6) (0.1 μg plasmid was transfected and 0.5 μg of cell lysates were loaded); CAL51, BT549, NCI-H69 cells expressing CAS9 and scramble sgRNA (CTRL) and PTPRD-targeting sgRNA (RDKO) (30 μg cell lysates were loaded).
C. Immunoblot analysis of 293T cells expressing empty vector (EV), His-tagged PTPRD, PTPRF, and PTPRS plasmids, blotted with either RD-28 (upper) or anti-His (middle) antibodies. Actin was included as a loading control.

D. Representative immunofluorescence confocal microscopy images of 293T cells stably expressing empty vector (EV), His-tagged PTPRD, PTPRF, and PTPRS plasmid and stained with RD-43 (red) and anti-His antibody (green). Scale bars: 20 μm

Supplementary Figure 3 – RNA-seq analysis to identify breast cancer cells with high PTPRD and low MTSS1 expression.

A. Comparison of RNA expression level of PTPRD vs MTSS1 in 53 breast cancer cell lines. Unit: Transcript Per Million (data resource: https://depmap.org/portal/)

B. Comparison of RNA expression level of PTPRD vs MTSS1 in 20 patient-derived organoids. Unit: Transcript Per Million (data resource: https://doi.org/10.1158/0008-5472.CAN-21-2807)

Supplementary Figure 4 - PTPRD-mediated signaling was reversed by chemically-induced dimerization or RD-43 treatment.

A. Immunoblot analysis of 293T cells transfected with GFP, wildtype PTPRD (WT) or DmrB-tagged PTPRD (PTPRD-DmrB) plasmids, then treated with vehicle (Veh), AP20817 (AP, 2 μM, overnight) and RD-43 (#043, 100 nM, overnight). Lysates were blotted with anti-His to determine PTPRD levels, and with the indicated phospho-specific antibodies to measure substrate phosphorylation.

B. Quantification of densitometry of STAT3 pTyr705 / STAT3 calculated from three replicate immunoblots as conducted as in Figure S3A.

C. Quantification of densitometry of SRC pTyr416 / SRC calculated from three replicate immunoblots as conducted as in Figure S3A.

D. Quantification of densitometry of SRC non-pTyr527 / SRC calculated from three replicate immunoblots as conducted as in Figure S3A.

For Figures S3B, S3C and S3D, data are presented as mean ± SEM. Two-tailed student's t-tests were performed between each condition and vehicle-treated GFP-expressing cells (lane#1). *** p-value < 0.001; ** p-value < 0.01; * p-value <0.5

Supplementary Figure 5 – Binding of RD-43^{MS}-Fab to PTPRD.

A. ELISA measurements of the affinity of RD-43^{MS} and RD-43^{MS} Fab to purified PTPRD ectodomain.

B. Representative confocal microscopy immunofluorescence images of 293T cells stably expressing His-tagged PTPRD and incubated with RD-43, RD-43^{MS}, RD-43^{MS}-Fab, and RD-43^{MS}-Fab in combination with anti-mouse Fab specific secondary antibody (anti-Fab 2nd) for the indicated times, and stained with anti-His antibody (green), anti-rat secondary antibody (red), anti-mouse secondary antibody (pink) and anti-mouse Fab specific secondary antibody (orange). Scale bars: 20 μm

Supplementary Figure 6 - Protein degradation inhibitors blocked RD-43-mediated PTPRD degradation at distinct subcellular localizations.

Representative confocal microscopy immunofluorescence images of CAL51 cells:

A. treated with RD-43^{MS} (100 nM, 2 h), bafilomycin A1 (BafA1, 20 μM, 4 h) and lactacystin (Lacta, 20 μM, 4 h) and stained with RD-28 (red) and DAPI (blue). PTPRD-knockout cells (RDKO) were stained as negative controls. Scale bars: 20 μm

B. treated with bafilomycin A1 (BafA1, 20 nM, 4 h) and RD-43^{MS} (100 nM, 2 h) followed by staining with RD-28 (red), anti-LAMP1 lysosomal marker antibody (green) and DAPI (blue). PTPRD knockout cells (RDKO) were included as negative controls. Scale bars: 20 μm

C. expressing endogenous PTPRD (RDWT) or PTPRD-knockout cells (RDKO) treated with bafilomycin A1 (BafA1, 20 nM, 4 h) and RD-43^{MS} (100 nM, for indicated times), followed by staining with RD-28 (red), anti-LAMP1 antibody (green), anti-mouse secondary antibody (pink) and DAPI (blue). Scale bars: 20 μm

Supplementary Figure 7 - SEC-MALS analysis of the interaction between PTPRD ectodomain and RD-43 at different stoichiometric ratios.

Size-exclusion chromatography with in-line multi-angle light scattering (SEC-MALS) of PTPRD ectodomain (A), RD-43 (B), and mixtures of RD-ECD and RD-43 at 2:1 (C), 4:1(D) and 1:1(E) molar ratios. (F) highlights the expected mass of each protein or complex.

Supplementary Figure 8 - Accumulation of RD-43-induced PTPRD dimers following inhibition of lysosomal or proteasomal activity.

A. Immunoblot analysis of lysates (WCL: whole cell lysates) of 293T cells or immunoprecipitates with anti-IgG beads (IP-IgG), anti-His beads (IP-His) and anti-V5 beads (IP-V5). Cells were transfected with GFP only (2 μg, GFP), 1 μg GFP and 1 μg His-tagged PTPRD (RD-His), 1 μg GFP and 1 μg V5-tagged PTPRD (RD-V5) or 1 μg His- and 1 μg V5-tagged PTPRD. Transfected cells were treated with RD-43 (100 nM, 3 h), bafilomycin A1 (BafA1, 20 nM, 4 h) and MG132 (5 μM, 4 h), as indicated.

Supplementary Figure 9 – PLA analysis of PTPRD dimerization induced by various mAbs.

A. Representative confocal microscopy immunofluorescence images of 293T cells expressing empty vector (EV) or His- and V5-tagged PTPRD treated with IgG or PTPRD mAbs at the indicated concentrations for 30min. Proximity ligation assay (PLA) signal between His- and V5-tags is displayed in red channel. Scale bars: 20 μm

B. Relative PLA intensity following different antibody treatments normalized to the response to treatment with RD-43 (100nM, n=4).

Supplementary Figure 10 – Restoration of PTPRD expression by FBS-containing media prior to the trans-well assay.

A. Schematic of the workflow to test PTPRD recovery in different media post-trypsinization. CAL51 cells were trypsinized and then recovered in DMEM with heat-inactivated FBS or FBS for the indicated times. Cells were then lysed for immunoblot analysis. Adherent cells were lysed as a control (CTRL).

B. Immunoblot analysis of CAL51 cells recovered in the indicated media post-trypsinization. Lysates from adherent cultured cells were included as the controls. The upper and middle panels represent two different exposures of the same blot.

C. Schematic of the workflow to perform trans-well assays for CAL51 cells. Trypsinized CAL51 cells were incubated in FBS-containing DMEM for 4 h to recover PTPRD protein. Cells were then resuspended in FBS-free media and seeded in the trans-well chamber with varying antibodies/drug conditions to examine their effects on cell migration.

Supplementary Figure 11 – Dimerization of PTPRD and PTPRS induced receptor degradation and ubiquitination of intracellular fragments.

A. Immunoblot analysis of 293T expressing GFP (1 µg), PTPRD (1 µg) and HA-tagged ubiquitin (UB-HA, 0.3 µg) treated with 50nM RD-43 for 2 h. WCL: whole cell lysate; IP-HA: anti-HA-antibody-conjugated bead immunoprecipitates

B. Immunoblot analysis of 293T transfected with GFP or PTPRS and treated with heparin oligosaccharide (DP-20, Galen Lab Supplies) overnight.

C. Immunoblot analysis of 293T transfected with GFP (1 µg), PTPRS (1 µg) and HA-tagged ubiquitin (UB-HA, 0.3 µg) and treated with 20uM heparin oligosaccharide for 2 h. WCL: whole cell lysate; IP-HA: immunoprecipitations by anti-HA-antibody-conjugated beads