

Additional File 1

Recurring *EPHB1* mutations in human cancers alter receptor signalling and compartmentalisation of colorectal cancer cells

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Running title: Phenotypes of *EPHB1* mutations in solid tumours

Supplementary Figures:

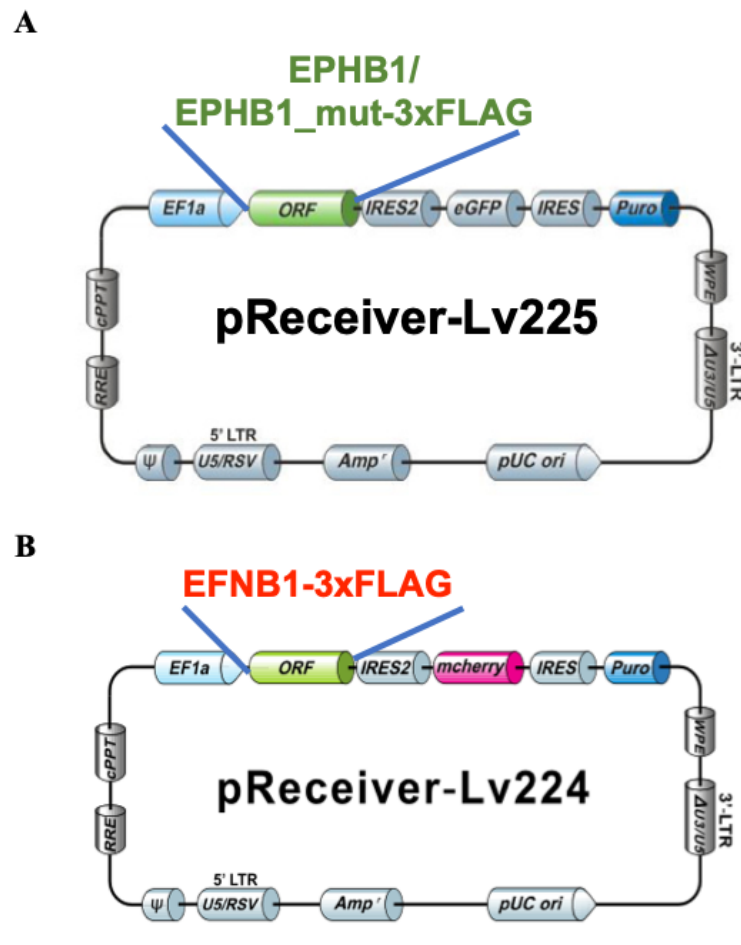


Fig S1. Construct designs. (A) Wild-type *EPHB1* and its mutants, and (B) wild-type *EFNB1* with a C-terminal 3×FLAG tag were cloned downstream of the EF1α-constitutive promoter in pReceiver-Lv225 and pReceiver-Lv224, respectively. EF1α, Human elongation factor-1 alpha mammalian constitutive promoter; ORF, open reading frame where different versions of *EPHB1* and *EFNB1* cDNAs were cloned; IRES and IRES2, internal ribosomal entry sites; eGFP, enhanced green fluorescent protein; mCherry, modified red fluorescent protein; Puro, puromycin-N-acetyltransferase gene; pUC ori, pUC replication of origin; Amp, beta-lactamase gene; cPPT, central polypurine tract; PRE, post-transcriptional regulatory element; WPE, Woodchuck hepatitis virus post-transcriptional regulatory element; 5' and 3' LTR, 5' and 3' long terminal repeats.

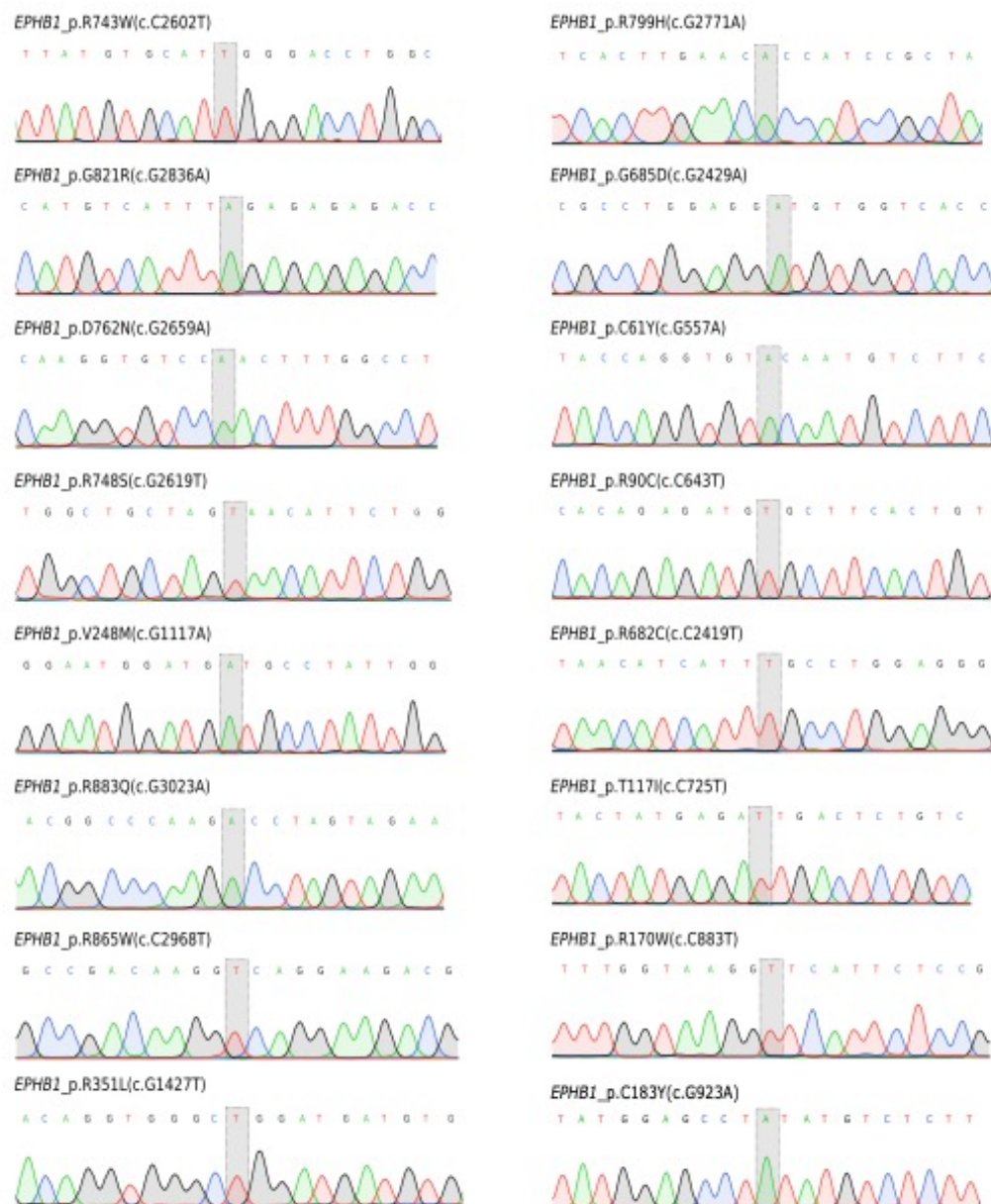


Fig S2. Confirmation of *EPHB1* mutants at the transcript level by Sanger sequencing. The mutation of interest is highlighted with a grey bar in each panel.

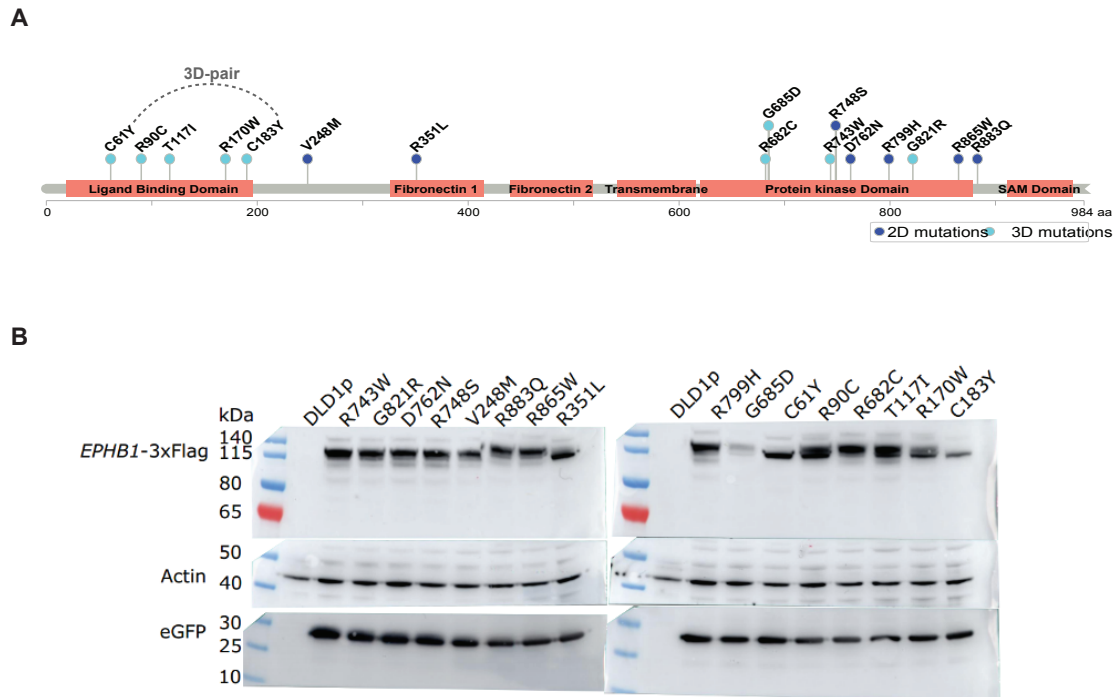


Fig S3. Immunoblot analysis of ectopically expressed EPHB1 mutant proteins in DLD-1 CRC cells. (A) All engineered EPHB1 mutations are depicted on the full-length (984 amino acids) EPHB1 protein. Mutants selected from 2D and 3D-structure based bioinformatic analysis in dark blue and light blue, respectively. The 3D-pairing between C61Y and C183Y is shown with a dotted line. (B) Expression of wild-type (positive control) and mutant EPHB1 proteins was detected with FLAG antibody (upper panel) with actin as a loading control (middle panel) and eGFP expression level was shown in each sample (lower panel). Parental DLD-1 cells (DLD1p) were used as a negative control for EPHB1 and eGFP expression.

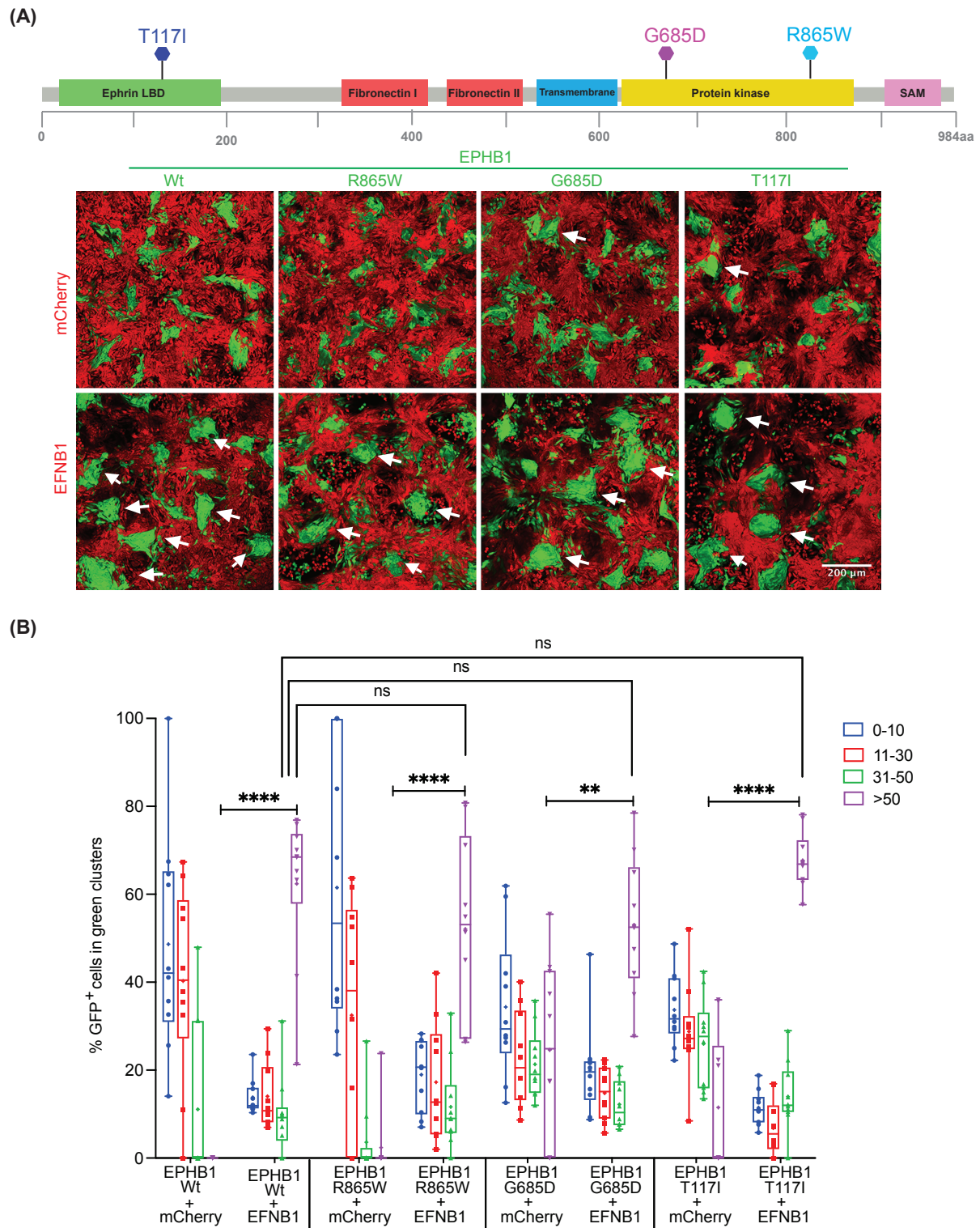


Fig S4. *EPHB1* receptor mutations T117I, G685D and R865W have wild type like response to *EFNB1* in compartmentalisation of colorectal cancer cells. (A) Upper panel, positions of the mutants in the full length *EPHB1* protein. Lower panel, *in vitro* compartmentalisation assays by co-culturing DLD-1 cells expressing eGFP along with either wild-type *EPHB1* or mutants T117I, G685D or R865W with DLD-1 cells expressing mCherry with or without *EFNB1* ligand. Representative confocal images from 10 randomly chosen image fields for each condition. Arrows, examples of large (>50 cells), homogeneous GFP⁺

cell clusters indicative of cell sorting and compartmentalisation. (B) Quantitation of the compartmentalisation experiments. Cell distribution was quantified by counting the percentage of GFP⁺ cells forming clusters of different sizes. In co-cultures of EFNB1 ligand with EPHB1 or its T117I, G685D or R865W mutants, similar high percentage of GFP⁺ cells were distributed into large homogeneous clusters of >50 cells. The Mann-Whitney *U* test was used to calculate the difference between each mutant with or without EFNB1 against the positive control wild-type EPHB1-type with respect to the formation of large clusters (>50 cells). This experiment was performed at least twice and imaged with five random fields from each experiment. Here, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. Wt, wild-type.

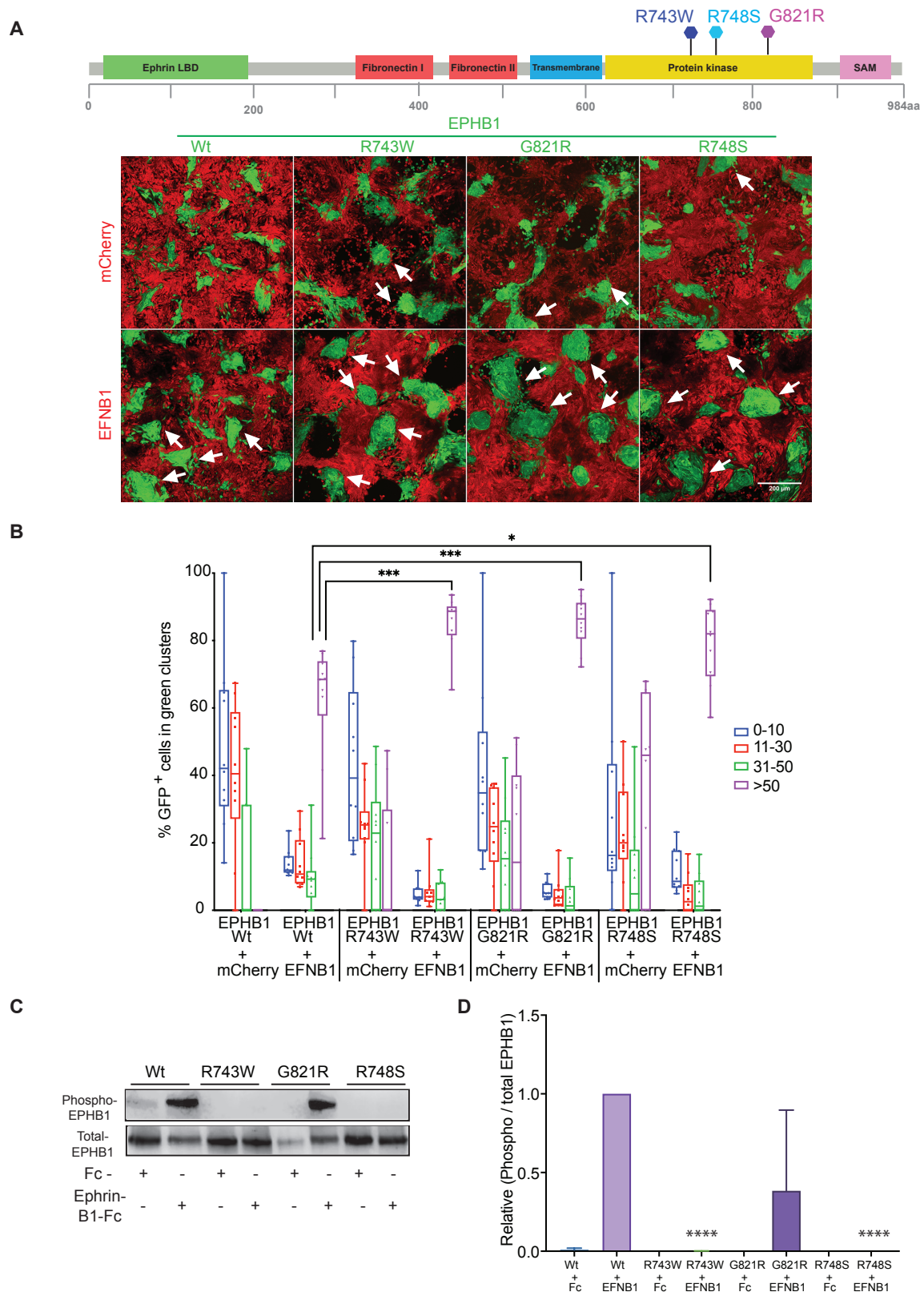


Fig S5. The *EPHB1* enhanced compartmentalisation mutants R743W, G821R and R748S do not display increased Y594/604 phosphorylation in presence of *EFNB1*. (A) Upper panel, positions of the mutants in the full length *EPHB1* protein. Lower panel, *in vitro* compartmentalisation assays by co-culturing DLD-1 cells expressing eGFP along with either *EPHB1* wild-type or R743W, G821R or R748S mutants with DLD-1 cells expressing mCherry with or without *EFNB1* ligand. Representative confocal images from 10 randomly chosen

image fields for each condition. Arrows, examples of large, homogeneous GFP⁺ cell clusters (>50 cells) indicative of cell sorting and compartmentalisation. (B) Quantitative results of the compartmentalisation experiments. Cell distribution was quantified by counting the percentage of GFP⁺ cells forming clusters of different sizes. (C) The levels of Y594/604 phosphorylation (upper panel) and total EPHB1 (lower panel) after Ephrin-B1-Fc ligand stimulations were analyzed by immunoblotting using antibodies to phospho-Y594/604 and total EPHB1 protein. (D) The phosphorylation level of wild-type and mutant EPHB1 was computed as the ratio of band intensities of phosphorylated to total EPHB1 normalised to wild-type from three independent experiments. Error bars, SD. The unpaired t-test was used to calculate the *P*-values for the normalized level of phosphorylation relative to wild-type + EphrinB1-Fc sample, * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 and **** *p* < 0.0001. Wt, wild-type.

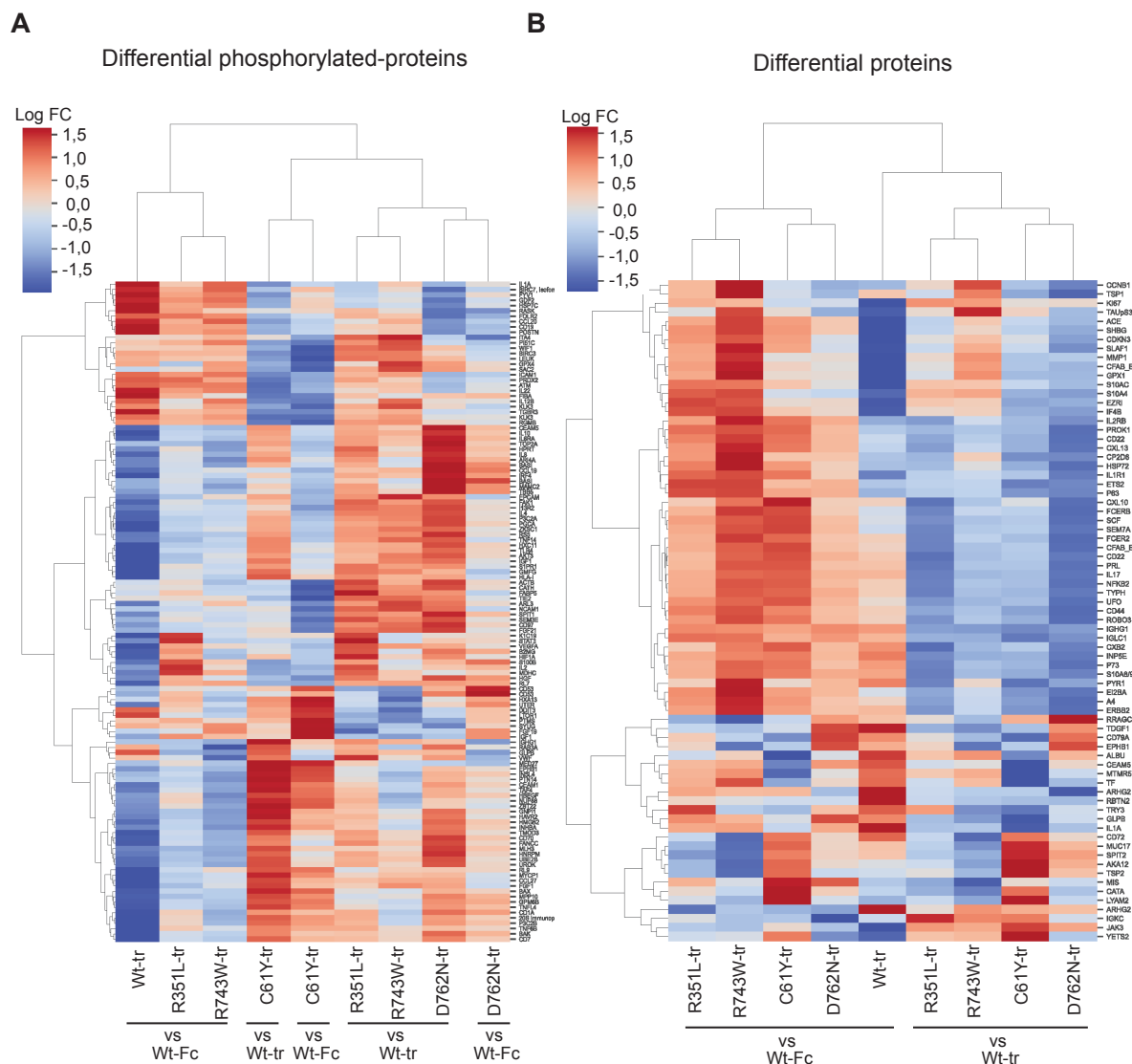


Fig S6. Differential expression and phosphorylation of proteins after EphrinB1-Fc ligand stimulations. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min of incubation. Heatmap represented differentially phosphorylated (A) and total (B) proteins with $|\log FC| > 1.0$, adjusted *p* value < 0.05 in Ephrin-B1-Fc ligand stimulated wild-type EPHB1 and R351L, R743W, C61Y and D762N mutants in DLD-1 cells. Samples and genes clustered by Euclidian distance and Ward variance minimization with row-wise scaling.

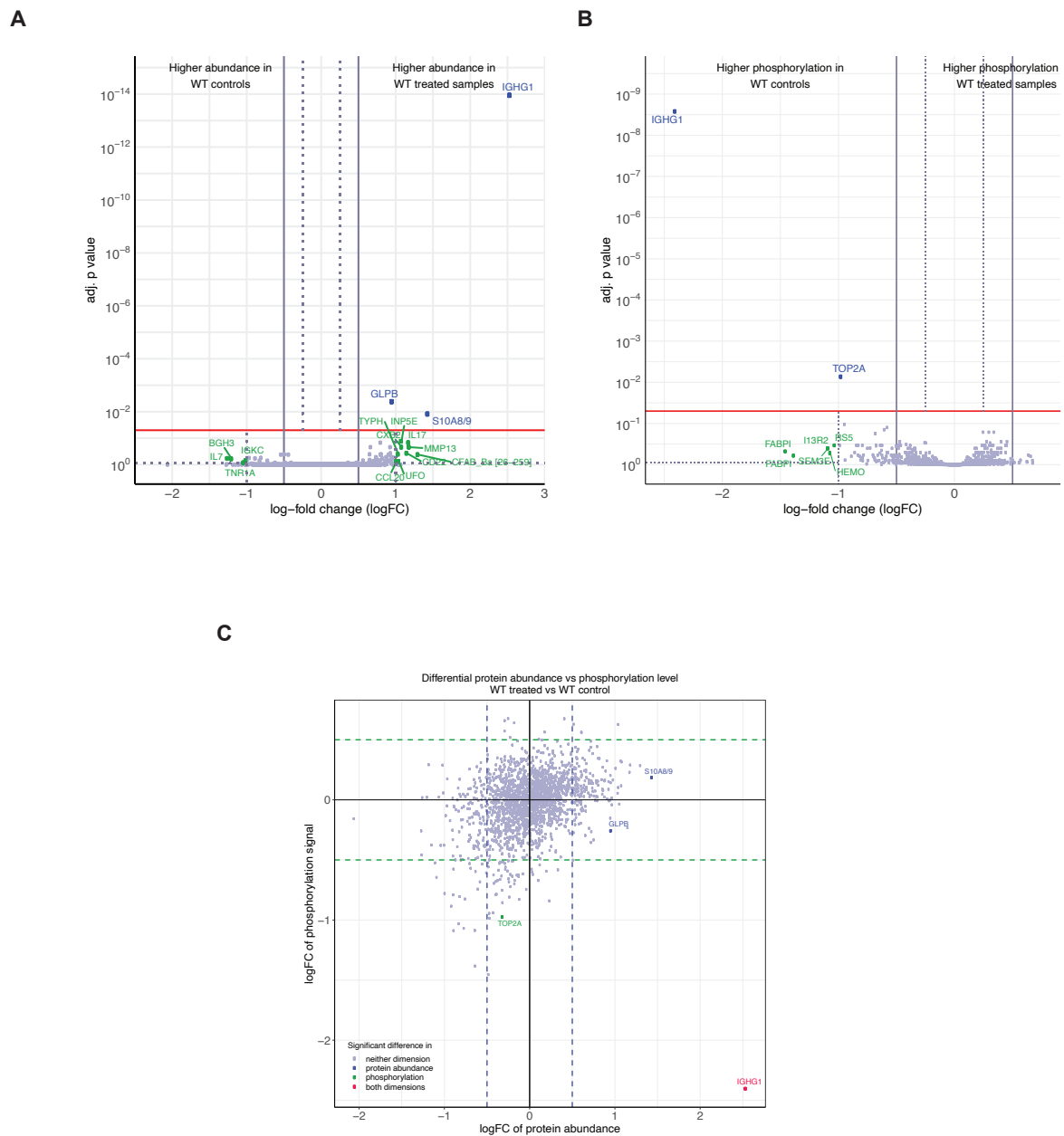


Fig S7. Total protein and phospho-protein profiles of EphrinB1-Fc ligand stimulated wild-type EPHB1 expressing DLD-1 cells. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min for differential proteome (A), phospho-proteome (B), and joint (C) analysis. Differences in abundance between EphrinB1-Fc ligand and control Fc fragment stimulated EPHB1 expressing cells as log-fold change (logFC) and corresponding p values adjusted for multiple testing. Red line, adjusted p value significance level of 0.05; vertical lines, logFC cutoffs of ± 0.5 . A positive and negative logFC indicates higher abundance or phosphorylation in ligand and Fc-stimulated wild-type EPHB1, respectively. Blue symbols, proteins differentially expressed or phosphorylated, defined as $|\logFC| > 0.5$ and adjusted p value < 0.05 . Green symbols, noteworthy proteins including non-significant proteins (adjusted p value < 0.9) with $|\logFC| > 1$, and significant proteins with $|\logFC| \pm 0.25$. WT, wild-type.

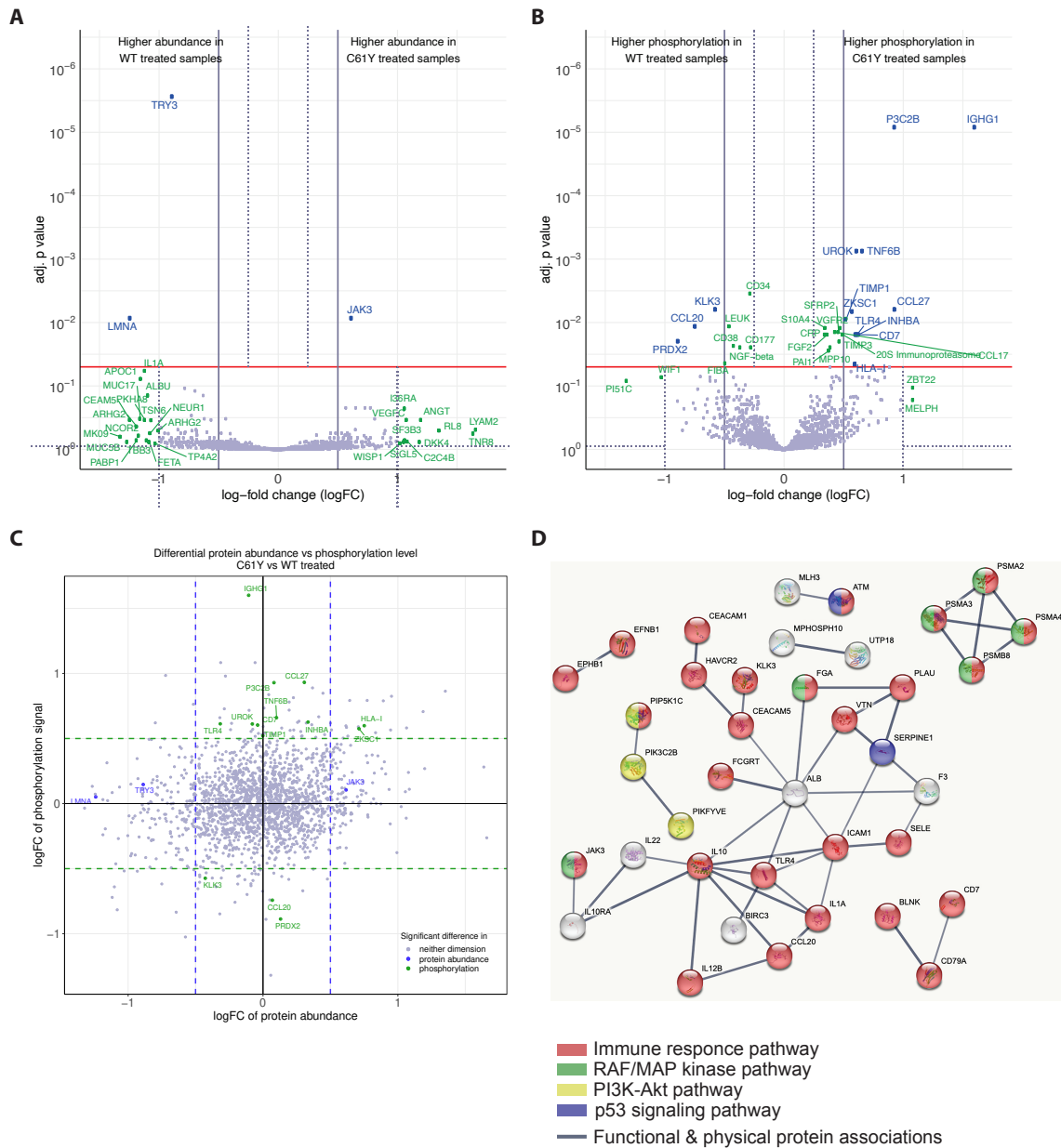


Fig S8. Differential protein expression and phosphorylation after ligand stimulation of C61Y versus wild-type EPHB1 DLD-1 cells. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min for differential proteome (A), phospho-proteome (B), and joint (C) analysis. Differences in abundance between C61Y-stimulated samples and WT-stimulated samples as log-fold changes (logFC) and their corresponding p values (adjusted for multiple testing). Red line, adjusted p value significance level of 0.05; vertical lines, logFC cut-offs of ± 0.5 . A positive and negative logFC indicates higher abundance or phosphorylation in ligand-stimulated C61Y mutant and wild-type EPHB1, respectively. Blue symbols, proteins differentially expressed or phosphorylated, defined as $|\logFC| > 0.5$ and adjusted p value < 0.05 . Green symbols, noteworthy proteins including non-significant proteins (adjusted p value < 0.9) with $|\logFC| > 1$, and significant proteins with $|\logFC| \pm 0.25$. (D) Pathway analysis was performed with only the differential proteins/phosphorylated-proteins (blue) using STRING

analysis (<https://string-db.org>) setting criteria full STRING network; Confidence network edges and high confidence (7.00). WT, wild-type.

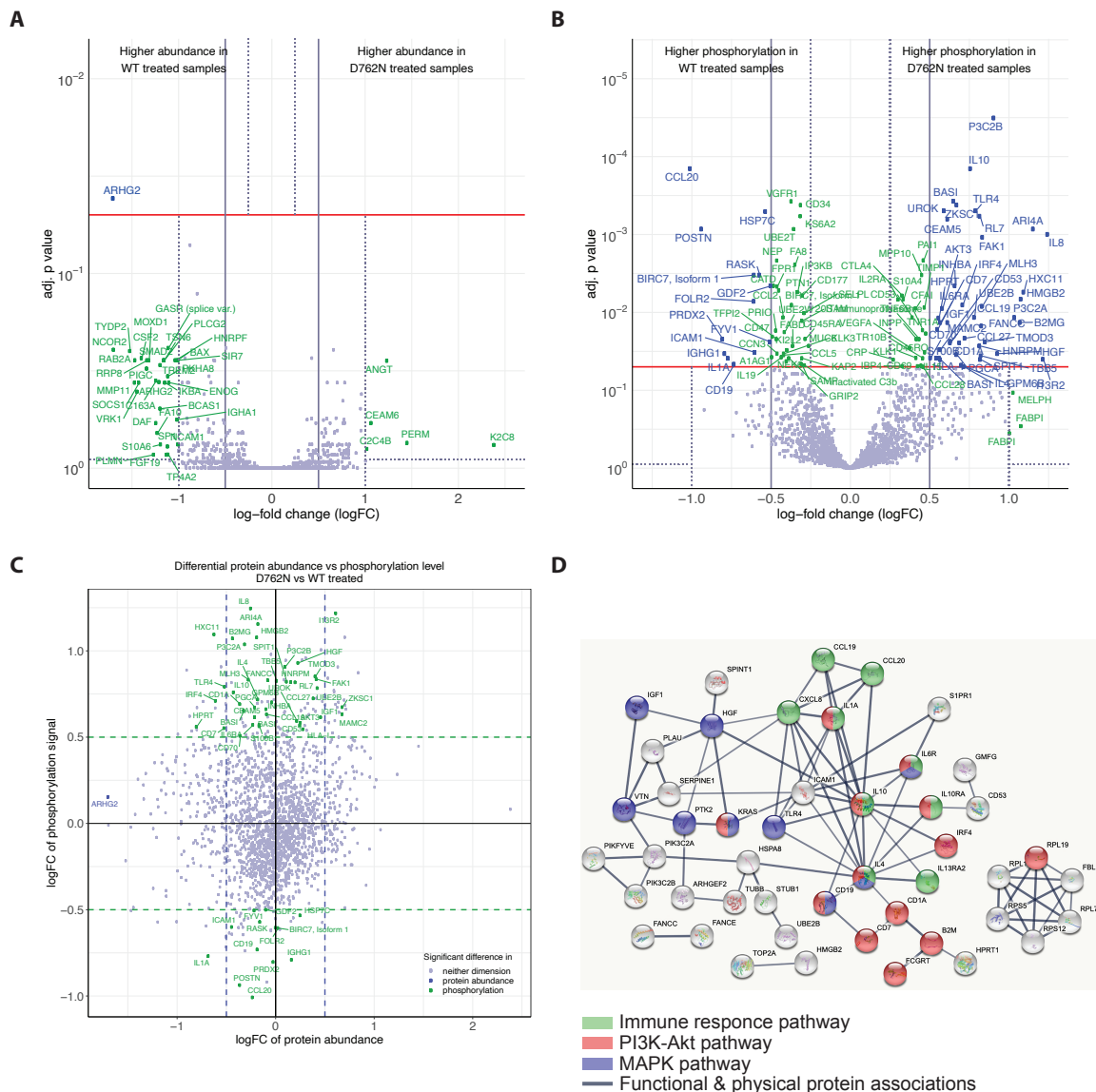


Fig S9. Differential protein expression and phosphorylation after ligand stimulation of D762N versus wild-type EPHB1 DLD-1 cells. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min for differential proteome (A), phospho-proteome (B), and joint (C) analysis. Differences in abundance between D762N-stimulated samples and WT-stimulated samples as log-fold changes (logFC) and their corresponding *p* values (adjusted for multiple testing). Red line, adjusted *p* value significance level of 0.05; vertical lines, logFC cutoffs of ± 0.5 . A positive and negative logFC indicates higher abundance or phosphorylation in ligand-stimulated D762N mutant and wild-type EPHB1, respectively. Blue symbols, proteins differentially expressed or phosphorylated, defined as $|\text{logFC}| > 0.5$ and adjusted *p* value < 0.05 . Green symbols, noteworthy proteins including non-significant proteins (adjusted *p* value < 0.9) with $|\text{logFC}| > 1$, and significant proteins with $|\text{logFC}| \pm 0.25$. (D) Pathway analysis was performed with only the differential proteins/phosphorylated-proteins (blue) using STRING analysis (<https://string-db.org>) setting criteria like, full STRING network; Confidence network edges and high confidence (7.00). WT, wild-type.

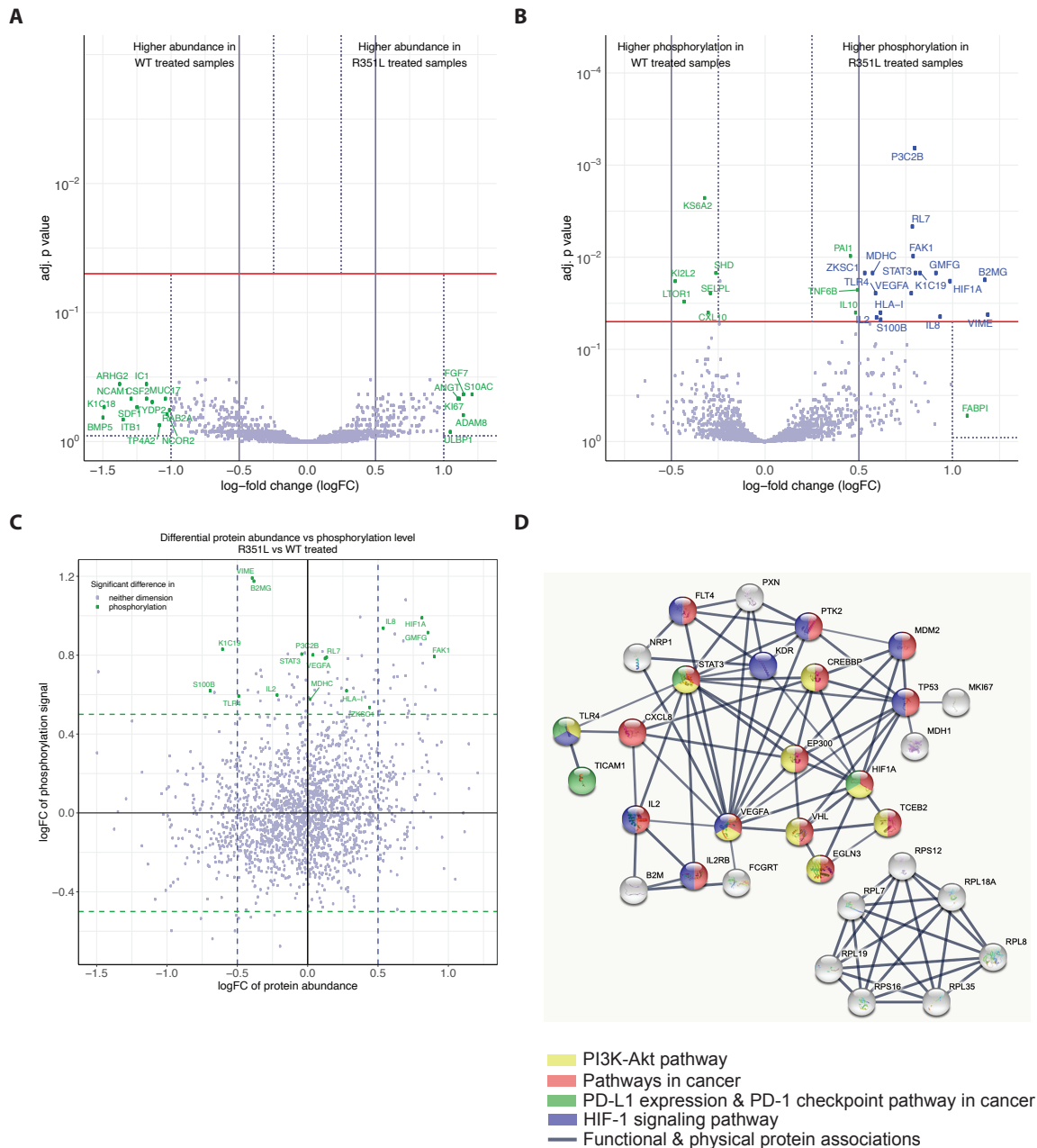


Fig S10. Differential protein expression and phosphorylation after ligand stimulation of R351L versus wild-type EPHB1 DLD-1 cells. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min for differential proteome (A), phospho-proteome (B), and joint (C) analysis. Differences in abundance between R351L-stimulated samples and WT-stimulated samples as log-fold changes (logFC) and their corresponding p values (adj. for multiple testing). Red line, adjusted p value significance level of 0.05; vertical lines, logFC cutoffs of ± 0.5 . A positive and negative logFC indicates higher abundance or phosphorylation in ligand-stimulated R351L mutant and wild-type EPHB1, respectively. Blue symbols, proteins differentially expressed or phosphorylated, defined as $|\logFC| > 0.5$ and adjusted p value < 0.05 . Green symbols, noteworthy proteins including non-significant proteins (adj. p value < 0.9) with $|\logFC| > 1$, and significant proteins with $|\logFC| \pm 0.25$. (D) Pathway analysis was performed with only the differential proteins/phosphorylated-proteins (blue) using STRING analysis (<https://string->

[db.org](#)) setting criteria like, full STRING network; Confidence network edges and high confidence (7.00).

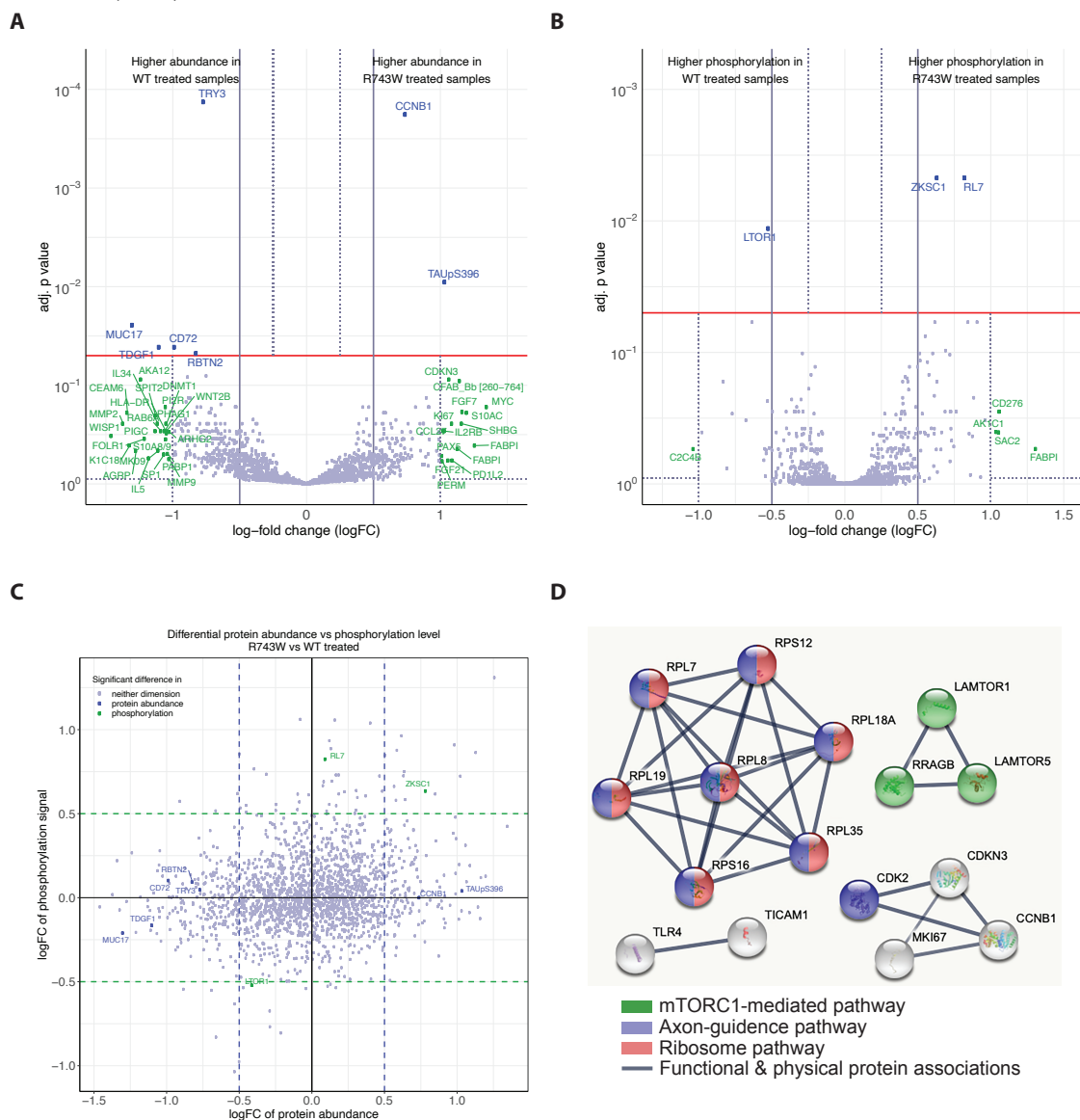
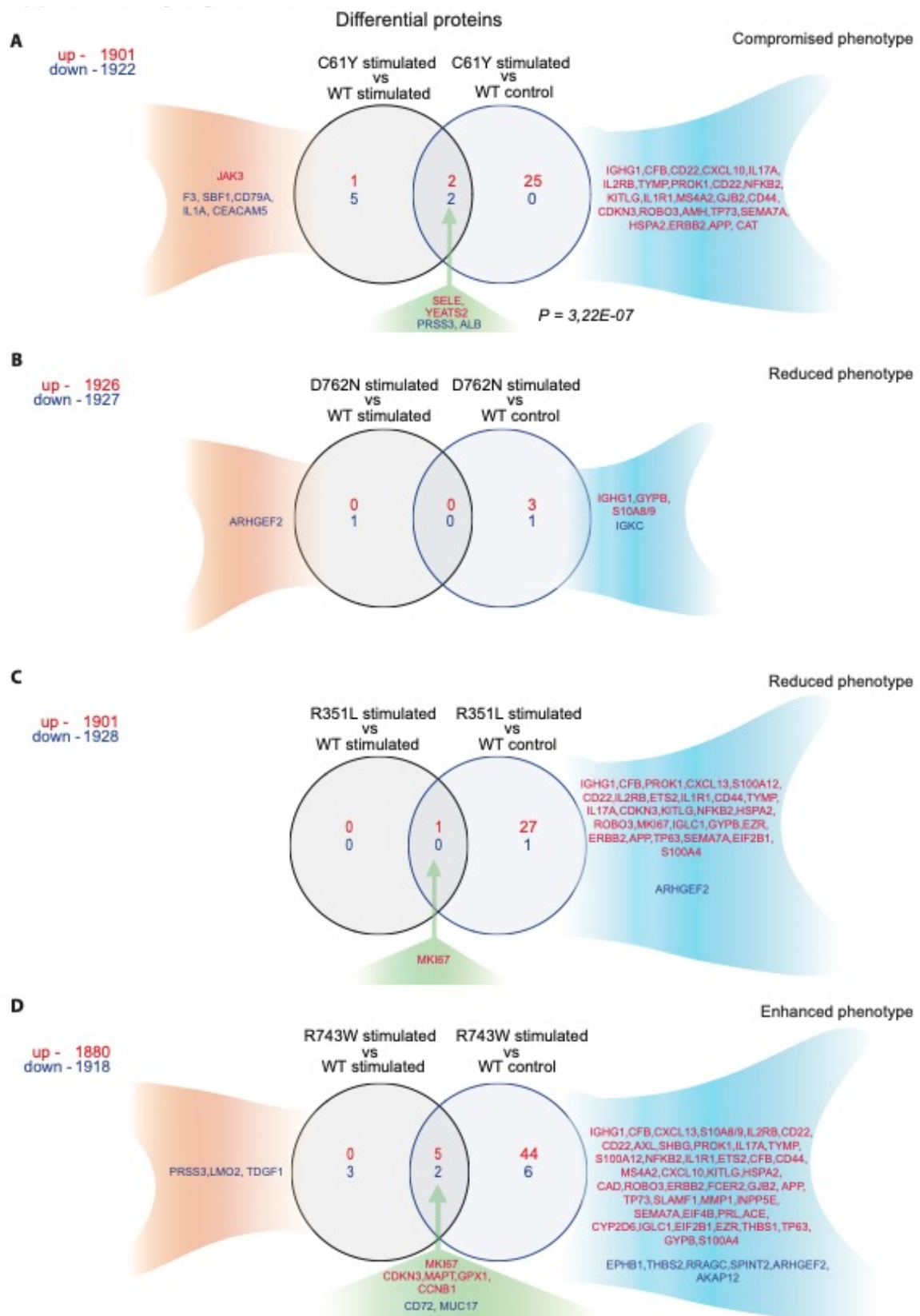


Fig S11. Differential protein expression and phosphorylation after ligand stimulation of R743W versus wild-type EPHB1 DLD-1 cells. An antibody-array-based method (Sciomics) was used with three replicates for each sample stimulated with 0.5 μ M of EphrinB1-Fc ligand for 30 min for differential proteome (A), phospho-proteome (B), and joint (C) analysis. Differences in abundance between R743W-stimulated samples and WT-stimulated samples as log-fold changes (logFC) and their corresponding p values (adjusted for multiple testing). Red line, adjusted p value significance level of 0.05; vertical lines, logFC cutoffs of ± 0.5 . A positive and negative logFC indicates higher abundance or phosphorylation in ligand-stimulated R743W mutant and wild-type EPHB1, respectively. Blue symbols, proteins differentially expressed or phosphorylated, defined as $|\text{logFC}| > 0.5$ and adjusted p value < 0.05 . Green symbols, noteworthy proteins including non-significant proteins (adjusted p value < 0.9) with $|\text{logFC}| > 1$, and significant proteins with $|\text{logFC}| \pm 0.25$. (D) Pathway analysis was performed with only the differential proteins/phosphorylated-proteins (blue) using STRING analysis (<https://string-db.org>) setting criteria like, full STRING network; Confidence network edges and high confidence (7.00). WT, wild-type.



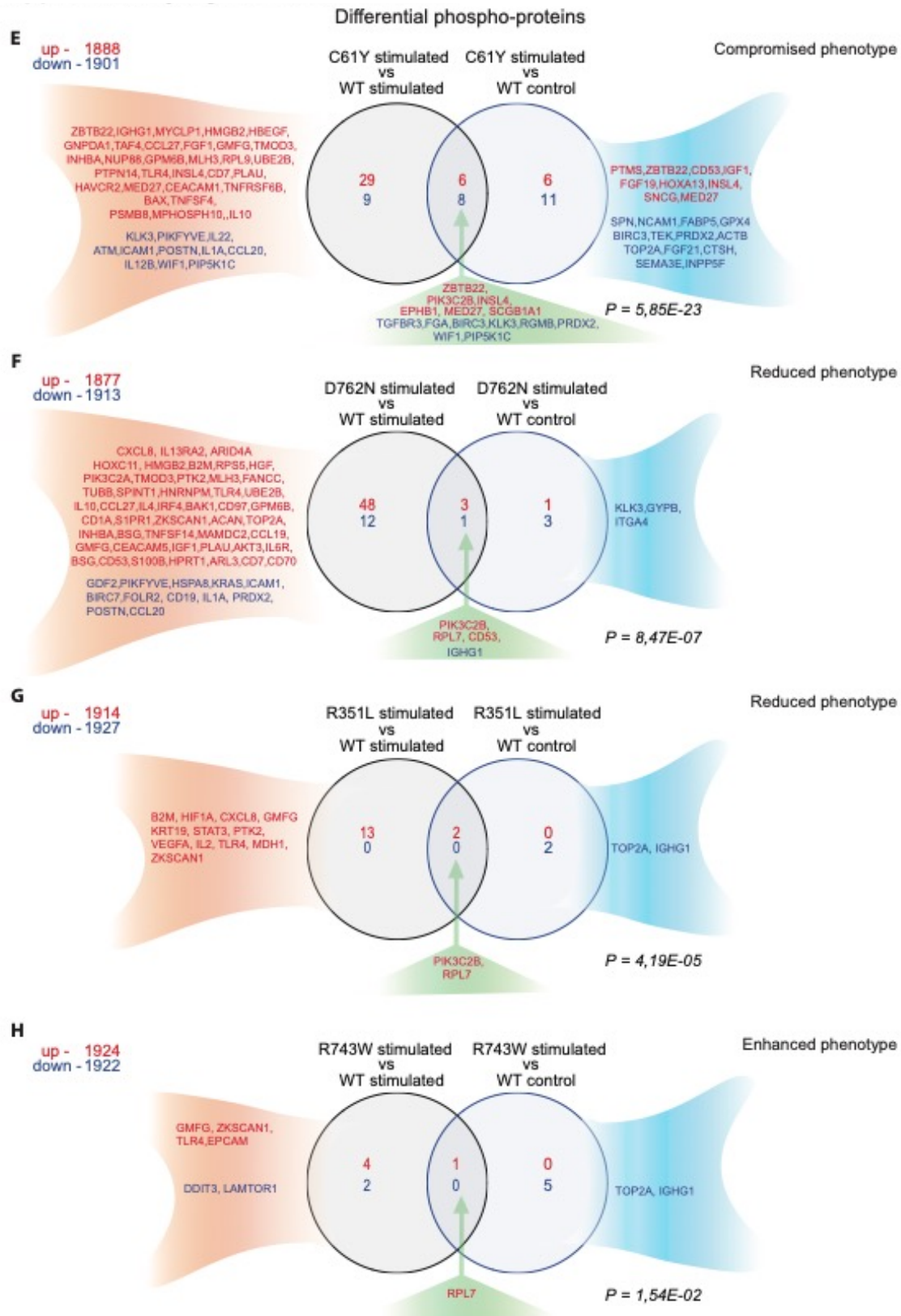


Fig S12. Greater differential protein phosphorylation than differential protein expression in ligand stimulated mutants versus wild-type EPH receptor. After stimulating DLD1 cells ectopically expressing EPHB1 wild-type and C61Y/D762N/R351L/R743W mutants with EphrinB1-Fc ligand or Fc (control), they were subjected to array-based total proteome and

Phosphoproteome analysis (Sciomics) to identify differential proteins (A-D) and phosphoproteins (E-H). Up, upregulation (red); Down, downregulation (blue). The hypergeometric distribution was used to calculate the p values. WT, wild-type.

Supplementary Tables:

Table S1. Calculation of customised average functional impact.

	Score +1	Score +0.5
Functional Prediction Scores:		
SIFT	“damaging (D)”	-
Polyphen2_HDIV	“probably damaging (D)”	“possibly damaging (P)”
Polyphen2_HVAR	“probably damaging (D)”	“possibly damaging (P)”
LRT	“deleterious (D)”	-
MutationTaster	“disease causing automatic (A)”	“disease causing (D)”
MutationAssessor	“predicted functional high (H)”	“predicted functional medium (M)”
FATHMM	“damaging (D)”	-
PROVEAN	“deleterious (D)”	-
Fathmm_MKL	“deleterious (D)”	-
Ensemble Scores:		
MetaSVM	“deleterious (D)”	-
MetaLR	“deleterious (D)”	-
M-CAP	“deleterious (D)”	-
CADD	≥ 30	-

Table S2. Identifiers of EPH receptors protein and Protein Data Bank 3D-structural entries.

Ephrin Receptor	UniProt	PDB Files Entries
<i>EPHA1</i>	P21709	2K1K 2K1L 3HIL 3KKA
<i>EPHA2</i>	P29317	1MQB 2E8N 2K9Y 2KSO 2X10 2X11 3C8X 3CZU 3FL7 3HEI 3HPN 3KKA 3MBW 3MX0 3SKJ 4P2K 4PDO 4TRL 5EK7 5I9U 5I9V 5I9W 5I9X 5I9Y 5I9Z 5IA0 5IA1 5IA2 5IA3 5IA4 5IA5 5NJZ 5NK0 5NK1 5NK2 5NK3 5NK4 5NK5 5NK6 5NK7 5NK8 5NK9 5NKA 5NKB 5NKC 5NKD 5NKE 5NKF 5NKG 5NKH 5NKI
<i>EPHA3</i>	P29320	2GSF 2QO2 2QO7 2QO9 2QOB 2QOC 2QOD 2QOF 2QOI 2QOK 2QOL 2QON 2QOO 2QOQ 3DZQ 3FXX 3FY2 4G2F 4GK2 4GK3 4GK4 4L0P 4P4C 4P5Q 4P5Z 4TWN 4TWO
<i>EPHA4</i>	P54764	2LW8 2WO1 2WO2 2WO3 3CKH 3GXU 4BK4 4BK5 4BKA 4BKF 4M4P 4M4R 4W4Z 4W50 5JR2
<i>EPHA5</i>	P54756	2R2P 4ET7
<i>EPHA6</i>	Q9UF33	No structures available
<i>EPHA7</i>	Q15375	2REI 3DKO 3H8M 3NRU
<i>EPHA8</i>	P29322	1UCV 1X5L 3KUL
<i>EPHA10</i>	Q5JZY3	No structures available
<i>EPHB1</i>	P54762	2DJS 2EAO 3ZFX 5MJA 5MJB
<i>EPHB2</i>	P29323	1B4F 1F0M 2QBX 3ZFM
<i>EPHB3</i>	P54753	3P1I 3ZFY 5L6O 5L6P
<i>EPHB4</i>	P54760	2BBA 2E7H 2HLE 2QKQ 2VWU 2VWV 2VWW 2VWX 2VWY 2VWZ 2VX0 2VX1 2X9F 2XVD 2YN8 3ZEW 4AW5 4BB4
<i>EPHB6</i>	O15197	No structures available

Table S3. PCR primers for Sanger sequencing for *EPHB1* mutant detection.

Primer ID	Sequence (5' -> 3')	Tm°	Protein Start Position	Product Size (bp)
#1-Forward	AGTGGCTGCGATGGAAGAAAC	67.5	42	636
#1-Reverse	CCCGAGCAATCACCAGAGATG	69.1	677	
#2-Forward	CTTCAAAAAGTGTCCCAGCATTG	66.5	586	644
#2-Reverse	GTCAAAGGTGTAGGGGGTGTG	65.4	1,229	
#3-Forward	GCAGGGGAGTTTGGAGAAGTG	67.1	1,893	943
#3-Reverse	CAGTGAGGAAGCTGTCCCTG	65.1	2,835	
#4-Forward	GAGAATGGTGCATTGGATTCTTTC	66.5	2,119	500
#4-Reverse	CCTTCTGCCAACAGTCCAGCATG	71.9	2,618	

Table S4. Description of the 33 tumour types used by total amount of patients and EPH receptor mutations.

Abbreviation	Tumour Type Name	Number of Patients	Number of Mutations
ACC	Adrenocortical carcinoma	83	318
BLCA	Bladder urothelial carcinoma	389	1,795
BRCA	Breast invasive carcinoma	994	5,993
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	298	1,654
CHOL	Cholangiocarcinoma	50	267
COAD	Colon adenocarcinoma	419	8,624
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma	47	761
ESCA	Esophageal carcinoma	174	766
GBM	Glioblastoma multiforme	388	3,440
HNSC	Head and neck squamous cell carcinoma	490	2,565
KICH	Kidney chromophobe	64	230
KIRC	Kidney renal clear cell carcinoma	334	2,936
KIRP	Kidney renal papillary cell carcinoma	276	1,589
LAML	Acute myeloid leukemia	146	2,004
LGG	Brain lower grade glioma	443	1,447
LIHC	Liver hepatocellular carcinoma	368	3,103
LUAD	Lung adenocarcinoma	554	4,810
LUSC	Lung squamous cell carcinoma	485	4,291
MESO	Mesothelioma	81	348
OV	Ovarian serous cystadenocarcinoma	436	4,329
PAAD	Pancreatic adenocarcinoma	183	1,020
PCPG	Pheochromocytoma and paraganglioma	133	267
PRAD	Prostate adenocarcinoma	429	1,447
READ	Rectum adenocarcinoma	154	2,763
SARC	Sarcoma	245	1,007
SKCM	Skin cutaneous melanoma	464	7,149
STAD	Stomach adenocarcinoma	422	3,016
TGCT	Testicular germ cell tumours	146	739
THCA	Thyroid carcinoma	452	2,103
THYM	Thymoma	118	420
UCEC	Uterine corpus endometrial carcinoma	522	7,616
UCS	Uterine carcinosarcoma	53	221
UVM	Uveal melanoma	58	113

Table S5. Alteration frequencies per Ephrin receptor according to tumour type and ratio of coding non-synonymous mutations per amino acid.

Tumours	<i>EPHA1</i>	<i>EPHA2</i>	<i>EPHA3</i>	<i>EPHA4</i>	<i>EPHA5</i>	<i>EPHA6</i>	<i>EPHA7</i>	<i>EPHA8</i>	<i>EPHA10</i>	<i>EPHB1</i>	<i>EPHB2</i>	<i>EPHB3</i>	<i>EPHB4</i>	<i>EPHB6</i>	Total
SKCM	6.0	5.6	10.8	8.0	3.7	15.3	13.4	5.2	5.2	5.8	9.3	6.5	3.2	8.6	51.1
DLBC	4.3	2.1	12.8	2.1	6.4	6.4	4.3	2.1	10.6	2.1	8.5	2.1	8.5	4.3	40.4
LUAD	1.3	1.8	7.4	3.2	11.0	7.0	6.3	2.2	2.3	6.3	2.3	3.2	1.6	7.4	38.4
LUSC	1.6	1.9	6.8	2.3	9.1	6.6	4.5	2.5	1.6	4.7	1.9	3.3	1.9	3.3	37.7
STAD	2.4	6.2	5.7	4.3	6.6	5.5	3.8	2.8	3.3	4.7	3.3	2.6	1.9	4.3	31.3
UCEC	4.6	5.6	8.0	8.8	9.8	8.0	6.1	4.0	4.4	10.9	5.0	3.6	4.2	4.6	31.0
COAD	1.2	3.1	6.9	3.6	6.9	5.7	4.5	2.9	3.1	6.0	3.8	2.1	1.7	2.9	30.8
CRC	1.0	2.4	5.8	3.3	6.8	5.1	4.2	2.8	2.6	5.6	2.8	1.7	1.9	2.8	27.9
BLCA	1.0	5.1	4.6	0.8	4.4	2.3	2.6	1.5	1.5	3.3	2.8	1.8	1.0	3.1	26.7
HNSC	1.4	4.9	2.9	1.2	4.3	2.2	3.7	1.2	1.4	2.2	0.8	1.2	1.4	1.4	22.4
READ	0.6	0.6	2.6	2.6	6.5	3.2	3.2	2.6	1.3	4.5	0.0	0.6	2.6	2.6	20.1
ESCA	0.0	2.9	1.7	1.7	4.0	2.3	1.1	2.3	1.1	1.7	2.9	0.6	0.6	1.7	20.1
CESC	0.7	4.0	1.3	1.3	1.7	1.7	1.0	2.3	1.3	1.3	2.0	1.0	2.0	1.7	17.4
LIHC	0.8	1.1	1.9	3.5	0.8	1.9	1.4	1.4	0.8	1.4	1.4	1.6	0.3	0.5	16.0
GBM	1.8	0.8	2.3	1.3	1.3	1.3	2.1	2.3	2.1	1.8	1.8	0.3	1.5	2.8	15.5
OV	1.1	1.4	1.6	0.7	1.4	1.4	1.6	0.7	0.9	1.4	1.4	0.9	0.9	2.3	14.4
UCS	1.9	0.0	1.9	1.9	5.7	0.0	0.0	0.0	0.0	3.8	3.8	0.0	0.0	0.0	11.3
CHOL	0.0	2.0	0.0	2.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	10.0
MESO	1.2	0.0	1.2	0.0	2.5	0.0	1.2	0.0	1.2	0.0	1.2	0.0	1.2	0.0	9.9
BRCA	0.7	0.5	0.7	1.2	1.3	1.0	1.9	0.1	0.3	1.8	0.5	1.1	0.8	0.9	9.9
SARC	0.4	0.8	0.4	0.8	2.0	0.8	1.6	1.2	0.4	0.8	0.4	0.0	0.4	0.4	9.4
KIRP	0.0	1.1	1.4	0.7	0.4	0.7	1.1	0.7	0.0	0.4	0.0	0.7	1.1	0.7	8.3
ACC	1.2	0.0	0.0	1.2	1.2	0.0	0.0	1.2	1.2	1.2	0.0	1.2	1.2	1.2	7.2
LAML	0.0	0.0	2.1	0.7	0.7	0.0	0.7	0.0	0.7	0.0	1.4	0.0	0.7	0.7	6.8
KIRC	0.6	0.3	0.3	0.3	1.5	0.6	0.3	0.3	0.3	0.3	0.0	0.3	0.6	0.6	6.3
LGG	0.5	0.5	1.4	1.1	0.7	0.0	0.9	0.9	0.7	0.0	0.5	0.5	0.5	0.5	5.4
PAAD	0.5	1.1	0.0	1.1	1.6	0.5	1.6	1.1	0.5	0.0	0.5	1.1	0.5	0.5	4.9
TGCT	0.0	0.0	0.7	0.0	0.0	1.4	0.0	0.0	1.4	0.0	0.0	0.0	2.1	0.0	4.8
KICH	1.6	0.0	1.6	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	4.7
PRAD	0.7	0.9	0.7	0.2	0.0	0.2	0.2	0.2	0.5	0.9	0.7	0.5	0.0	0.2	4.2
PCPG	0.0	0.0	0.8	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	3.8
THCA	0.4	0.0	0.0	0.0	0.2	0.4	0.0	0.4	0.7	0.7	0.2	0.0	0.2	0.2	3.3
THYM	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	1.7	0.0	0.0	2.5
UVM	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7
Mut/aa*	0.15	0.25	0.40	0.26	0.39	0.38	0.35	0.18	0.17	0.33	0.21	0.17	0.15	0.26	-

* Ratio of coding non-synonymous mutations per amino acid protein length for each Ephrin receptor.

Table S6. Ranked 3D-mutation in *EPHB1* sorted by average functional impact.

<i>EPHB1</i> Amino acid Position	<i>EPHB1</i> Mutations	Total EPH Mutations	3D Partner Position (<i>EPHB1</i>)	3D Partner EPH Mutations	Pair Distance (Å) ¹	Pair Distance (Amino acids) ²	Average Functional Impact ³
G685*	1	4	I696	3	4.03	53	11.5
R743*	5	13	F801	2	4.87	58	11.2
G821*	2	7	R170	7	4.43	111	11
V391	1	3	L340	1	4.85	58	10.5
L709	1	2	F820	1	4.59	111	10.5
C61*	1	2	C183	1	4.69	147	10
R90*	2	6	S188	2	4.11	118	8
M23	1	3	V191	3	4.55	195	7
R682*	3	10	E698	1	4.45	58	6.8
T117*	2	3	G172	3	4.44	73	6.3
R170*	7	9	E116	5	4.72	72	5.9

*Selected mutations for *in vitro* studies; ¹distance between the pair < 5 Å; ²distance between the pairs > 50 amino acids; ³average functional impact score ≥ 6.

Table S7. Top 20 *EPHB1* mutations with no 3D-partner ranked by total EPH receptor mutations.

<i>EPHB1</i> Amino acid Position	<i>EPHB1</i> Mutations	Total EPH Mutations	Average Functional Impact ¹
V248*	1	26	9
R883*	1	18	8
R748*	2	14	10
D762*	6	14	11.9
R865*	3	13	8.5
D374	1	12	6
R351*	2	11	11.5
R799*	1	10	10.5
R304	3	10	9.7
R84	2	10	9
N290	8	10	7.5
D806	3	9	12.5
R767	1	9	12
P249	1	9	10.5
R663	3	9	10
D615	1	9	9.5
E131	1	9	8
E613	1	9	7.5
R953	2	9	7.5
R56	2	9	7.25

*Selected mutations for *in vitro* studies; ¹average functional impact score ≥ 6.