Supplementary Figures and Tables

The Ephrin B2 Receptor Tyrosine Kinase is a Regulator of Proto-oncogene MYC and Molecular Programs Central to Barrett's Neoplasia

Srividya Venkitachalam, Deepak Babu, Durgadevi Ravillah, Ramachandra M. Katabathula, Peronne Joseph, Salendra Singh, Bhavatharini Udhayakumar, Yanling Miao, Omar Martinez-Uribe, Joyce A. Hogue, Adam M. Kresak, Dawn Dawson, Thomas LaFramboise, Joseph E. Willis, Amitabh Chak, Katherine S. Garman, Andrew E. Blum, Vinay Varadan, Kishore Guda

Supplementary Figures

Supplementary Tables

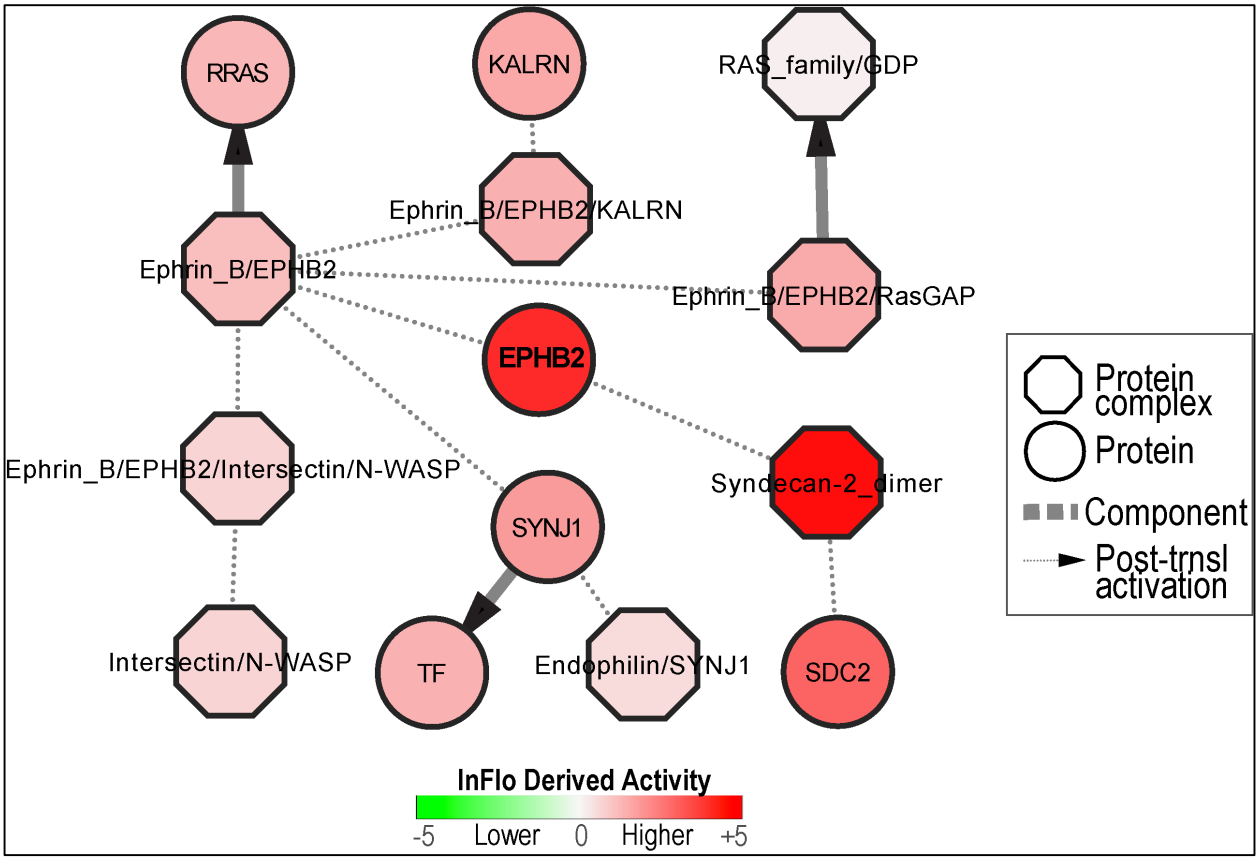


Fig. S1. InFlo-derived EphB2 sub-network activity in primary tissues. InFlo-based assessment of the differential activities of EphB2 signaling network components in primary NDSBM/EAC biopsy tissues. Plotted are EphB2 signaling network components showing significant differential activation in NDSBM/EAC vs. normal SQ or GAST (Wilcoxon P-Value ≤ 0.05), and their regulatory relationships. Node colors correspond to the average change in InFlo-inferred activity between NDSBM/EAC and SQ/GAST with red indicating upregulation and green indicating downregulation in NDSBM/EAC. Post-trnsl activation refers to post-translational regulation of the target. Note the broad activation of signaling components in the neighborhood of the EphB2 node suggesting activation of this pathway in BE/EAC.

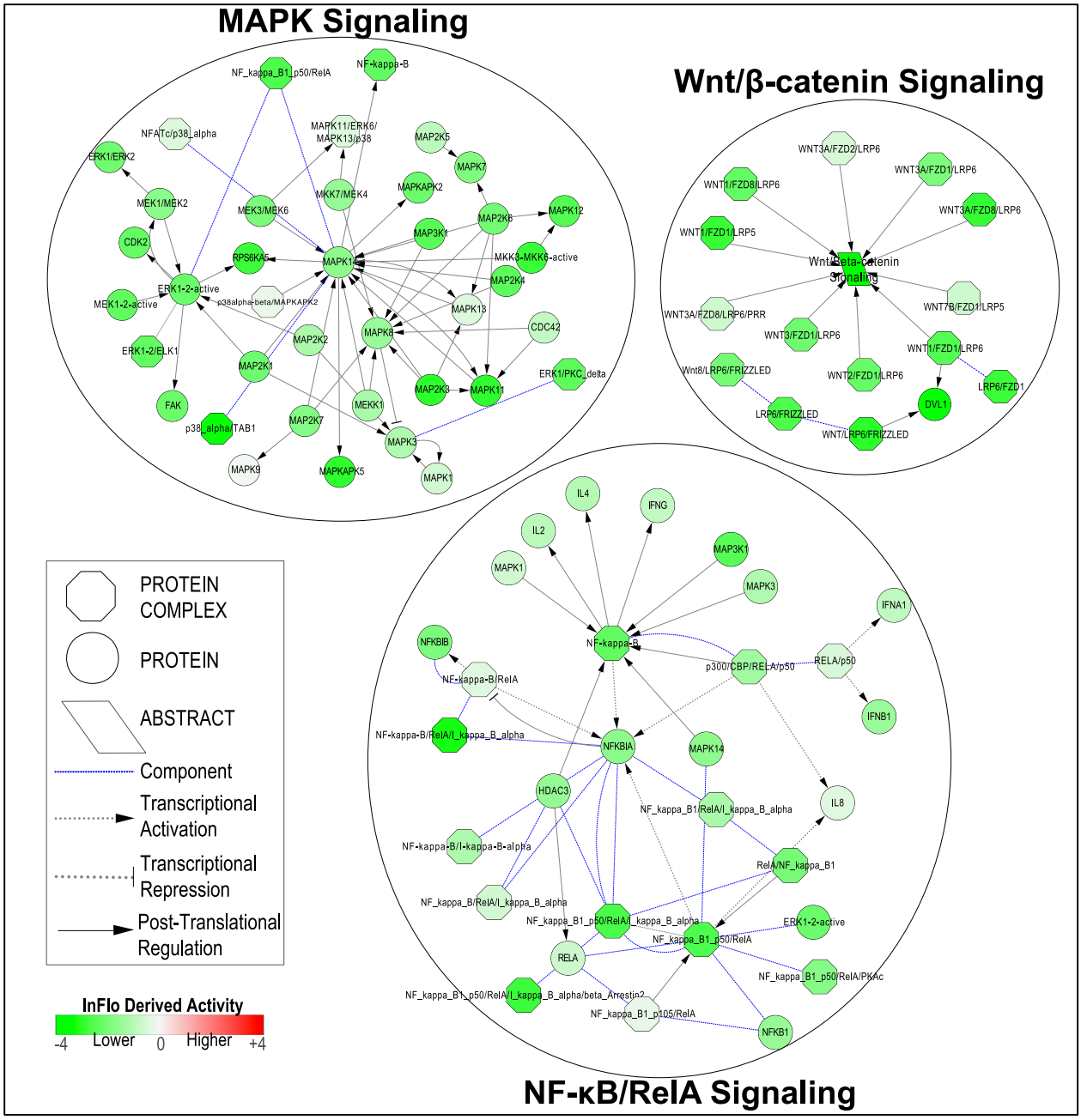
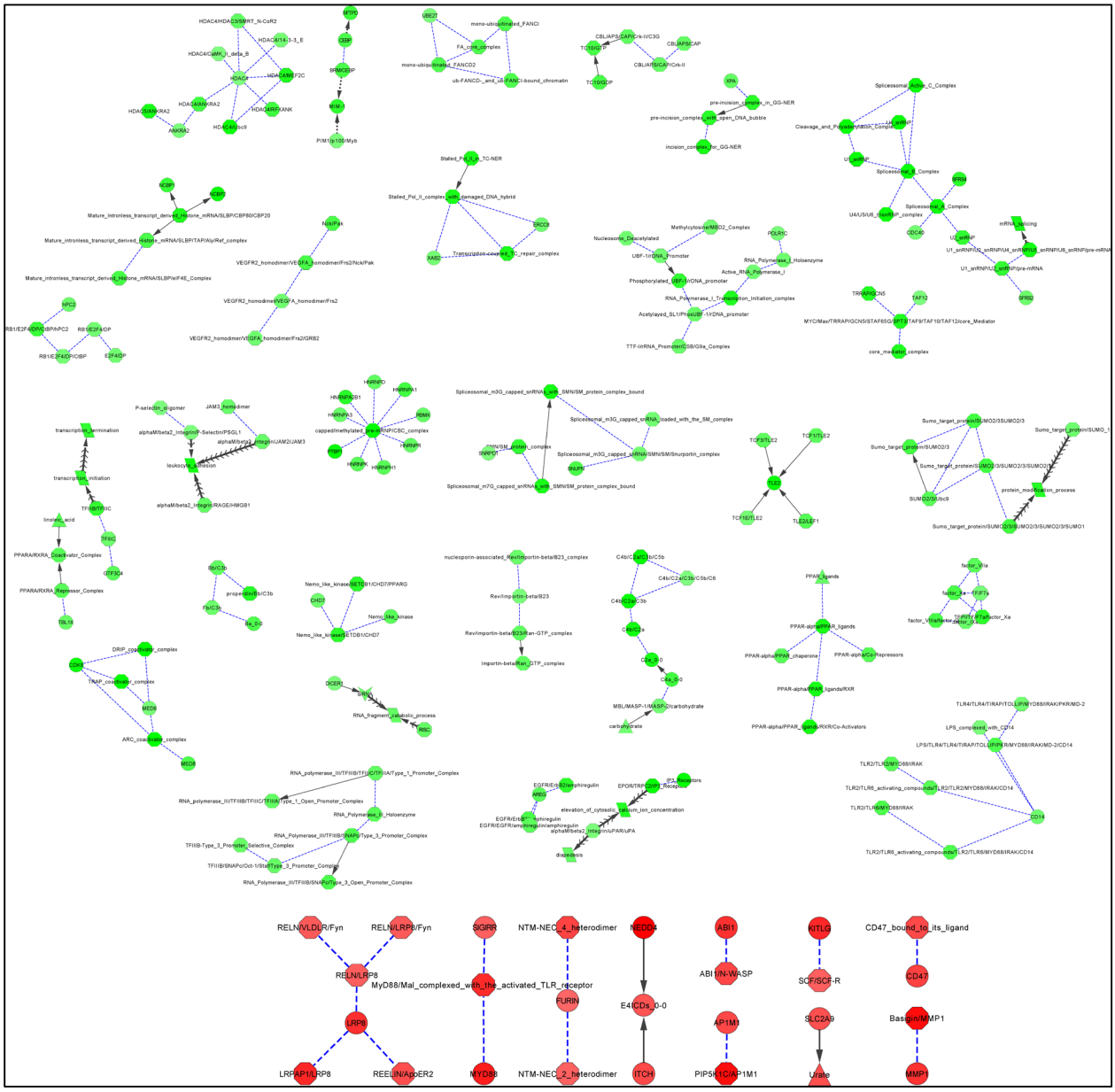


Fig. S2. **Signaling networks modulated by EphB2 in BE/EAC cell lines.** InFlo-based analysis of whole transcriptome RNASeq performed on EAC (SKGT4) and dysplastic-BE (CP-D) cells revealing significant alterations in signaling-network activities and their transcriptional targets in *EPHB2*-siRNA treated cells, compared to non-targeting Control siRNA. Shown are the consensus major signaling networks (NF-κB/RelA, Wnt/β-catenin, MAPK) associated with BE, deregulated in both EAC and BE cells upon *EPHB2* knockdown. Other significantly-altered networks are provided below (**Fig. S2 continued**).

Fig. S2 continued..



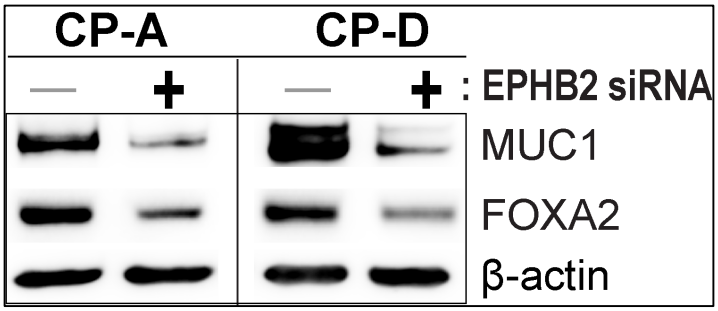


Fig. S3. EphB2 modulates FOXA2 and MUC1 expression in BE cells. BE metaplasia (CP-A) and dysplasia (CP-D) cell lines were treated with *EPHB2*-siRNA (+) or control-siRNA (-). Western Blot images depict protein levels of BE-associated intestinal metaplasia/columnar differentiation markers, MUC1 and FOXA2. β-actin was used as a loading control. Note the reduction in MUC1 and FOXA2 protein levels upon knockdown of *EPHB2* in both CP-A and CP-D cell lines.

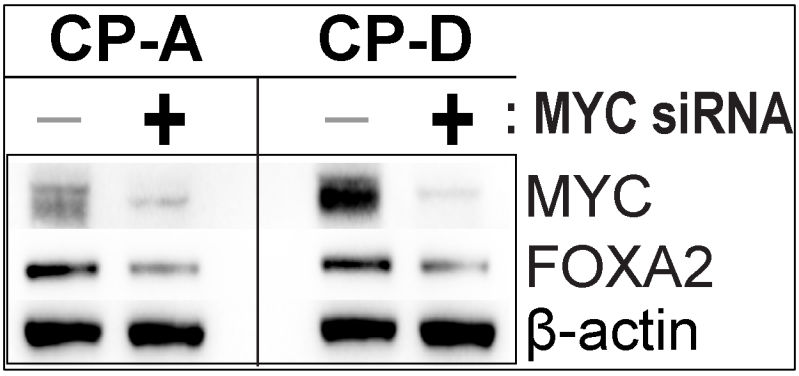


Fig. S4. EphB2-mediated modulation of FOXA2 in BE cells is potentially dependent on MYC. BE metaplasia (CP-A) and dysplasia (CP-D) cell lines were treated with *MYC*-siRNA (+) or control-siRNA (-). Western Blot images depict protein levels of BE-associated intestinal metaplasia/columnar differentiation marker FOXA2. β-actin was used as a loading control. Note the reduction in FOXA2 protein levels upon knockdown of *MYC* in both CP-A and CP-D cell lines.

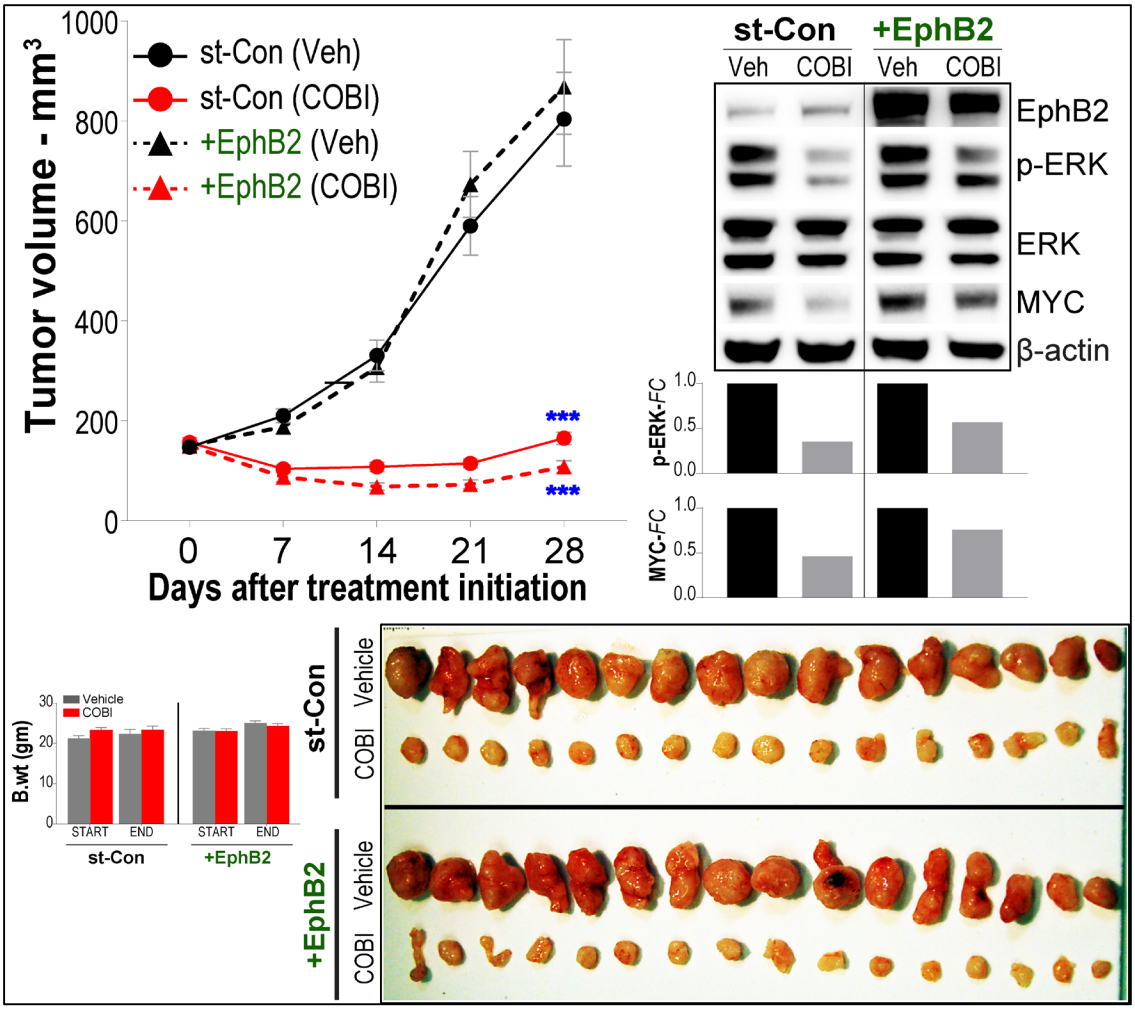


Fig. S5. Effects of MEK inhibitor in EphB2-reconstituted EAC cells. Tumor xenografts from EphB2-high SKGT4 EAC cells, further stably reconstituted with EphB2 (+EphB2) or stuffer control (st-Con), were established in immune-deficient mice. Mice were randomized and treated with Cobimetinib (COBI), or vehicle control, via oral gavage. Y-axis of the line graphs depict tumor volume in mm^3 over time (X-axis) in respective EAC xenografts. Data are plotted as mean ± SEM, estimated using 16 established xenograft tumors at day zero per respective arm. ***($P < 0.0005$) indicate significant differences in tumor volumes at the final time-point between COBI versus vehicle groups, estimated using a Student's t-test assuming unequal variances. Photographic images of harvested xenograft tumors at the final time-point from respective groups are provided below. Bar graphs depict body weight assessments of COBI and vehicle treated mice at the beginning and at the end of the study. WB images (right) depict expression levels of indicated proteins in representative tumor xenografts harvested after 1 week of treatment with either COBI or vehicle control. The bar graphs beneath the WB images show quantification of COBI-induced p-ERK and MYC protein expression changes, normalized to either total ERK or β-actin respectively, and presented as relative fold-change (FC) to Vehicle control arms of respective st-Con and +EphB2 groups.

Table S1. Sample distribution in the discovery RNAseq Cohort

Tissue Type	Number of Samples	Median Age at Diagnosis (Range)	Gender Distribution	Cancer Stage Distribution
Esophageal Adenocarcinoma (EAC) [$]	49	65 (36 - 88)	89% (Male) 11% (Female)	Stage I (17.9%), Stage II (19.6%), Stage III (46.4%), Stage IV (16.1%)
Non-dysplastic Stable Barrett's Metaplasia (NDSBM)*	18	56 (18-84)	94% (Male) 6% (Female)	NA
Normal Esophageal Squamous (nSQ) [#]	11	64 (45-83)	90% (Male) 10% (Female)	NA
Normal gastric (GAST)	11	63 (36-82)	82% (Male) 18% (Female)	NA
Total	**89**			

[$]11% of EACs were gastroesophageal junctional adenocarcinomas
*Median surveillance of 9 years, ranging from 6 to 22 years
[#] Each of the 11 normal SQ samples was obtained from respective EAC patients included in the RNA sequencing

Table S2. Sample distribution in the validation cohort

Tissue Type	Number of Samples	Median Age (Range)	Gender Distribution	Cancer Stage Distribution
Esophageal Adenocarcinoma (EAC) ^	210	64 (34 - 89)	77% (Male) 15% (Female)	Stage I (14.1%), Stage II (16.8%), Stage III (52.2%), Stage IV (15.0%)
Normal Esophageal Squamous (nSQ)	461	64 (34 - 89)	77% (Male) 15% (Female)	NA
Non-Dysplastic Barrett's Metaplasia (NDBM) *	133	65.5 (36-93)	71% (Male) 34% (Female)	NA
BM with high-grade dysplasia (BE-HGD)	57	66 (46-80)	89% (Male) 11% (Female)	NA
Normal gastric (GAST)	24	63 (36-82)	85% (Male) 15% (Female)	NA
Total	**885**			

^13% of EACs were gastroesophageal junctional adenocarcinomas
*Clinical follow-up information unavailable (progression status unknown) for these patients

Table S3. Mass Spectrometry analysis

	Parental (IgG)[a]	Parental (EphB2)[b]	V5-Control (V5)[c]	V5-EphB2 (IgG)[d]	V5-EphB2 (V5)[e]
EphB2	833,680	1,575,600,000	707,050	1,000,700	6,798,200,000

Gene ID	Accession	MW kDa	Parental (IgG)[a]	Parental (EphB2)[b]	V5-Control (V5)[c]	V5-EphB2 (IgG)[d]	V5-EphB2 (V5)[e]
O75592	MYCBP2	513.63	0	29,608,000	0	0	380,030,000
P17028	ZNF24	42.155	0	30,919,000	0	0	31,294,000
Q96SL8	FIZ1	51.995	0	13,129,000	0	0	24,961,000
P41223	BUD31	17	0	18,043,000	0	0	23,596,000
Q96QZ7	MAGI1	164.58	0	24,457,000	0	0	23,028,000
O95639	CPSF4	30.255	0	31,543,000	0	0	22,196,000
P46777	RPL5	34.362	0	9,604,900	0	0	18,333,000
Q9HDC5	JPH1	71.685	0	14,980,000	0	0	17,435,000
O00148	DDX39A	49.129	0	13,785,000	0	0	14,103,000
P51148	RAB5C	23.482	0	17,428,000	0	0	12,735,000
Q9UDY2	TJP2	133.96	0	13,092,000	0	0	11,443,000
Q14739	LBR	70.702	0	5,727,700	0	0	10,244,000
Q8IX01	SUGP2	120.21	0	5,743,100	0	0	6,077,400
P16144	ITGB4	202.16	0	3,891,800	0	0	4,038,100
Q96QE3	ATAD5	207.57	0	2,801,000	0	0	1,404,100

*Values in the table indicate label-free quantitation (LFQ) intensities for EphB2 and other genes listed

[a] IP-MS of Parental EAC (SKGT4) cells using anti-IgG antibody
[b] IP-MS of Parental EAC (SKGT4) cells using anti-EphB2 antibody
[c] IP-MS of V5-tagged stuffer-control stable EAC (SKGT4) cells using anti-V5 antibody
[d] IP-MS of V5-tagged wild-type EphB2 stable EAC (SKGT4) cells using anti-IgG antibody
[e] IP-MS of V5-tagged wild-type EphB2 stable EAC (SKGT4) cells using anti-V5 antibody