Supplementary Materials

Supplementary Figure Legends:

Supplementary Figure S1. Gel-filtration analysis of EphA2, ephrin-A1 and the EphA2/ephrin-A1 complex reveals that the unbound molecules are monomeric and, upon binding, form a 1:1 heterodimeric complex. The elution volumes for the peaks on a SD200 column (Pharmacia) are given in milliliters, and correspond to ~18-20 KDa for the individual molecules and ~35-40 KDa for their complex.

Supplementary Figure S2. A close-up view of the equivalent ligand/receptor interface region in the EphA2/ephrin-A1 (left) and EphB2/ephrin-B2 (right) complexes, highlighting class-specific residues and interactions. The Eph receptors are in blue and the ephrins in red.

Supplementary Table 1: Summary of crystallographic analysis

	EphA2:	EphA2/ephrin-A1:
Resolution, Å	30.0-2.5 (2.6-2.5)	30.0-2.0 (2.05-2.0)
Wavelength, Å	0.9795	1.033
Completeness, %	96.77 (80.15)	96.1 (91.2)
Redundancy, fold	5.6 (4.6)	2.3 (2.2)
I/σI	9.2 (3.2)	18.2 (3.7)
R_{merge}, %	9.4 (26.9)	6.9 (27.3)
Space Group	P1	P1
Cell Dimensions, Å	a= 41.27, b= 91.19, c= 91.13, α= 117.14, β= 97.27, γ= 100.79	a=58.48, b=102.11, c=135.26, α= 84.25, β= 79.77, γ= 73.94
Refinement		
Reflections working / test	33190 / 1856	192014 / 9696
Residues	1044	2448
Solvent	178	3333
R_{crys}/R_{free}	23.2/30.9 (25.6/39.2)	18.5/24.8 (19.1/26.5)
R.m.s. deviations		
Bonds, Å	0.017	0.013
Angles, ^{o}	1.71	1.50
Average B-factor, $Å^2$	3.1	14.3
Ramachandran Analysis		
Most favored regions	93.2 %	94.1 %

$R_{merge} = \Sigma|I-\langle I\rangle|/\Sigma I$, where I = observed intensity and $\langle I\rangle$ = average intensity obtained from multiple observations of symmetry-related reflections. The r.m.s. deviation in bond lengths and angles are the respective root-mean-square deviations from ideal values. Values in the parentheses are for the highest resolution shell.

Supplementary Materials – Methods

Site-Directed Mutagenesis and Cell Stimulation

EphA2 cDNA was subcloned into pBabe retroviral expression plasmid. Site-directed mutagenesis was carried out using QuickChange kit from QiaGen according to manufacture's instructions. Phoenix cells were transfected with the plasmids using Lipofectamine Plus reagent (Invitrogen) and selected in 2 μg/ml puromycin (Sigma). Retrovirus particles were harvested and used to infected HEK 293 cells. HEK 293 cell stimulation and analysis with ephrin-A1-Fc was carried out as previously described (Miao et al, 2000). For PC-3 cell stimulation, ephrin-A1-Fc or ephrin-B1-Fc was either unclustered or clustered with five molar excess of anti-human Fc antibody, and added to cells cultured to near confluency on six-well plates. Cells were lysed 10 min later with RIPA buffer. Total cell lysates or EphA2 and EphB2 immunoprecipitates were subject to immunoblot with the indicated antibodies.

Reference for Supplementary Materials:

Miao H, Burnett E, Kinch M, Simon E, Wang B (2000) Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. *Nat Cell Biol* 2(2): 62-69