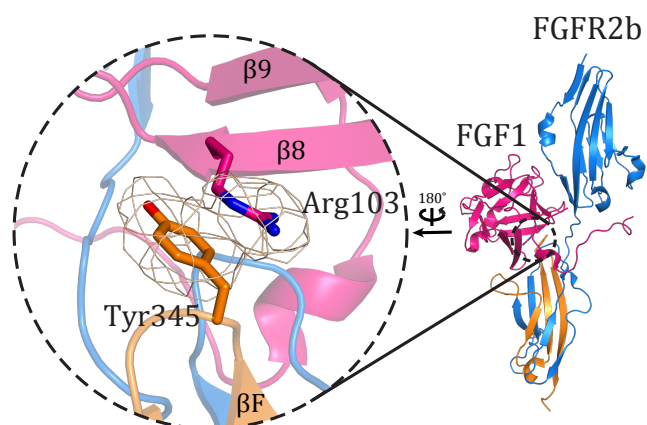


Figure S1

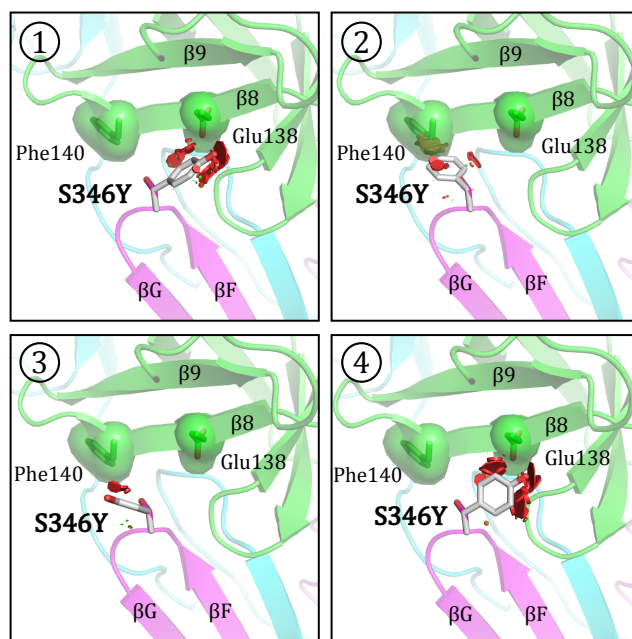
A

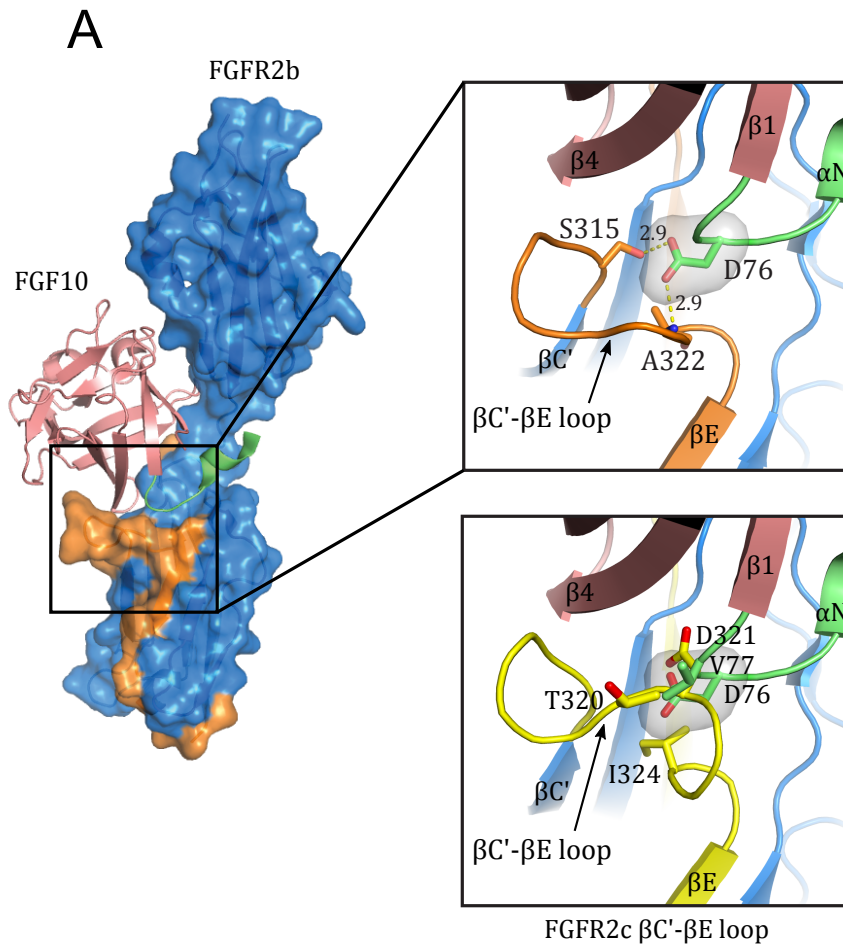
			β6	β7		β8	β9		
FGF1	68	-EVYIKSTET	GQYLAMDTDG	LLYGSQT-PN	EELCFLE	LE	ENHYNTYISK	KHAE-----	119
FGF2	204	-VVSIGKVC	NRYLAMKEDG	RLASKC-VT	DECFFERLE	SNNYNTYRSR	KY-----	253	
FGF3	85	-IVAIRGLFS	GRYLAMNKR	RLYASEH-YS	AECFVERIH	ELGYNTYASR	LYRTVSSTPG	142	
FGF7	107	-IVAIKGVES	EFYLAMNKEG	KLYAKKE-CN	EDCNFKELIL	ENHYNTYASA	KWTH-----	158	
FGF10	120	-VVAVKAINS	NYYLAMNKKG	KLYGSKE-FN	NDCKLKERIE	ENGYNTYASF	NWQH-----	171	
FGF22	83	-VVVIKAVSS	GFYVAMNRRG	RLYGSRL-YT	VDCRFRRERIE	ENGHNTYASQ	RWRR-----	134	
FGF4	125	-VVSIFGVAS	RFFVAMSSKG	KLYGSPF-FT	DECTFKEILL	PNNYNAYESY	KY-----	174	
FGF5	130	-IVGIRGVFS	NKFLAMSKKG	KLHASAK-FT	DDCKFRERFQ	ENSYNTYASA	IHRTEK----	183	
FGF6	127	-VVSIFGVAS	ALFVAMNSKG	RLYATPS-FQ	EECKFRETLL	PNNYNAYESD	LY-----	176	
FGF8	95	SRVRVGAET	GLYICMNKKG	KLIAKSNKKG	KDCVFTEIVL	ENNYTALQNA	KY-----	146	
FGF17	95	SRVRIKGAES	EKYICMNKRG	KLIGKPSGKS	KDCVFTEIVL	ENNYTAFQNA	RH-----	146	
FGF18	95	SQVRIKGET	EFYLCMNRKG	KLVGKPDGTS	KECVFIEKVL	ENNYTALMSA	KY-----	146	
FGF9	104	-LVSIRGVDS	GLYLG MNEKG	ELYGSEK-LT	QECVFREQFE	ENWYNTYSSN	LYKHVD----	157	
FGF16	103	-LISIRGVDS	GLYLG MNERG	ELYGSKK-LT	RECVFREQFE	ENWYNTYAST	LYKHSD----	156	
FGF20	107	-LVSIRGVDS	GLYLG MNDKG	ELYGSEK-LT	SECIFREQFE	ENWYNTYSSN	IYKHGD----	160	
FGF19	89	-TVAIKGVHS	VRYLCMGADG	KMQGLLQYSE	EDCAFEEEIR	PDGYNVYRSE	KHRLPVSLS	147	
FGF21	90	-VIQILGVKT	SRFLCQRPDG	ALYGSLHFD	EACSFRELL	EDGYNVYQSE	AHGLPLHLP	148	
FGF23	82	-FVVITGVMS	RRYLCMDFRG	NIFGSHYFDP	ENCRFQHQL	ENGYDVYHSP	QYHFLVSLGR	140	

B

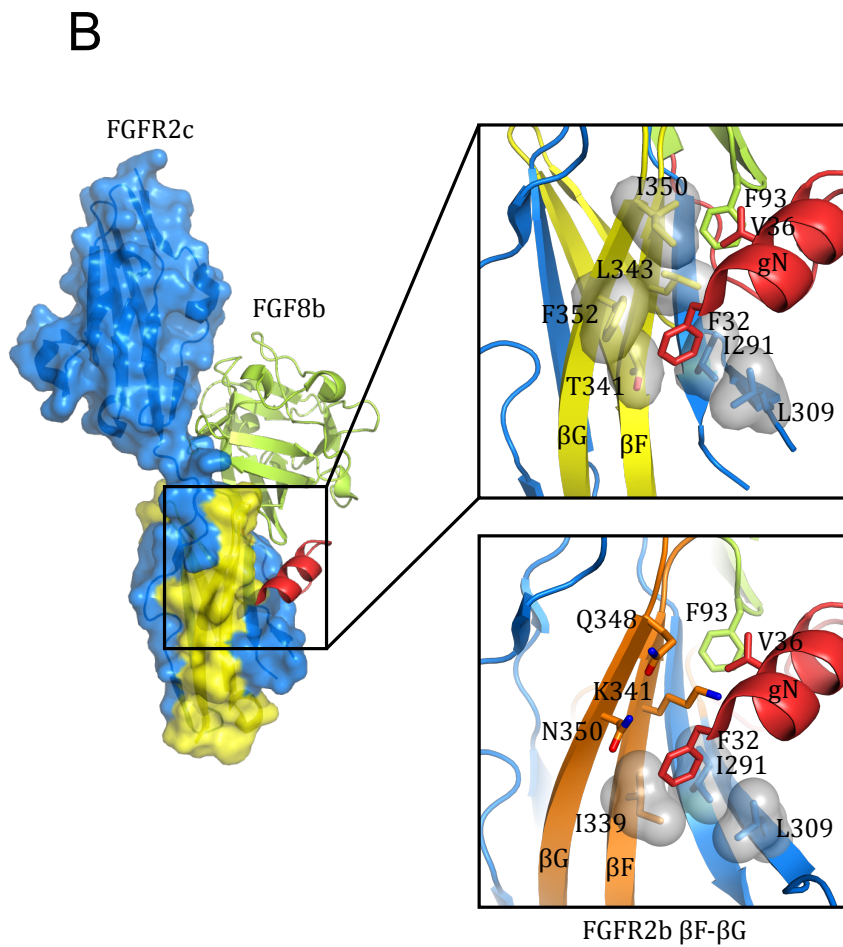


C





Mode 1



Mode 2

Figure S1. Sequence alignment of FGFs and differential effects of the S346Y mutation in FGFR1c on FGF binding (related to Figure 2, 3, 4, and 6)

(A) Sequence alignment of a section of β -trefoil region encompassing $\beta 6$ to $\beta 9$ strands of 18 human FGFs. β strands are shown on top of the alignment and labeled according to conventional nomenclature for the FGF β -trefoil fold. The six FGF subfamilies are distinguished with different colors. The first and last residue for each sequence are numbered. The unique Phe and Trp residues in the $\beta 8$ and $\beta 9$ strands, respectively, of the FGF9 subfamily that are critical in regulating this subfamily's receptor-binding specificity, are highlighted in yellow. Unique conservation of Gln-139 of FGF9 among FGF9 subfamily ligands is emphasized by orange coloring. In FGF1, Arg-103 (highlighted in green) occupies the position corresponding to this conserved Gln of FGF9 subfamily. (B) Cartoon representation of the FGF1-FGFR2b complex structure (PDB ID: 3OJM). FGF1 is in red, and FGFR2b is colored in blue except for its alternatively spliced region, which is in orange. Close-up view of the interface between FGF1 and the βF - βG loop in FGFR2b D3 showing the π -cation interaction between FGF1 Arg-103 and FGFR2b Tyr-345. This arginine residue is a glutamine in the FGF9 subfamily (highlighted in orange in panel A). This Arg->Gln substitution eliminates the possibility of a π -cation interaction between FGF9 subfamily ligands and the 'b' splice isoform specific tyrosine in the βF - βG loop of FGFR 'b' isoforms. Moreover, a glutamine in this location also introduces a spatial clash with the side chain of the tyrosine further discouraging binding of FGF9 subfamily ligands to FGFR 'b' isoforms. (C) A tyrosine at the amino acid position 346 of FGFR1c is incompatible with FGF9 binding as modeled by *PyMOL*. Panels 1 through 4 illustrate that presence of a tyrosine at this location would cause steric clashes and loss of water-mediated hydrogen bond between FGFR1c and FGF9. The mutated tyrosine is shown in white sticks, and clashes between the mutated tyrosine and FGF9 are indicated by red plates.

Figure S2. FGF10-FGFR2b and FGF8b-FGFR2c complex structures showing two distinct modes by which the N-termini of FGF10 and FGF8b dictate FGF-FGFR binding specificity (related to Figure 5)

(A) Crystal structure of the FGF10-FGFR2b complex (PDB ID: 1NUN) . Molecular surface of FGFR2b is shown and colored in blue except for its alternatively spliced region, which is in orange. FGF10 is shown as cartoon with its N-terminus and core region colored in dark green and pink, respectively. Upper inset to the right, shows expanded view of the hydrogen bonds between Asp-76 from the FGF10 N-terminus and Ser-315 and Ala-322 in the β C'- β E loop of FGFR2b D3. In the lower box, the FGFR2b D3 domain has been swapped by the corresponding FGFR2c D3 domain. The alternatively spliced region of FGFR2c D3 is colored yellow. Note that both steric and electrostatic clashes between the FGF10 N-terminus and the β C'- β E loop of FGFR2c D3 prevent FGF10 from binding to FGFR2c isoform. (B) Crystal structure of the FGF8b-FGFR2c complex (PDB ID: 2FDB) . Relative to the FGF10-FGFR2b complex in panel A, which is shown in a typical "front view", the FGF8b-FGFR2c complex is rotated by 180° around the vertical axis to show the back of the complex. FGF8b is shown in cartoon with its N-terminus and core region colored in red and light green, respectively. Molecular surface of FGFR2c is colored in blue except for its alternatively spliced region in D3 which is in yellow. Top right inset: an expanded view of the boxed region showing the hydrophobic interactions between the FGF8b N-terminus and the hydrophobic groove in FGFR2c D3 that is conserved in "c" splice isoforms of FGFR1-3 and FGFR4. Bottom inset shows that three out of six hydrophobic residues, which interact with the FGF8b N-terminus, are replaced by polar residues in the alternatively spliced region (in orange) of FGFR2b. This explains the inability of FGF8b to bind and activate FGFR2b.