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Supplemental Information

**How insulin-like growth factor I binds
to a hybrid insulin receptor type 1
insulin-like growth factor receptor**

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A	Protein Sequence of IR-Bzip	
1	HLYPGEVCPGMDIRNNLTRLHELENCVIEGHLQILLMFKTRPEDFRDLSFPKLIMITDYLLFRVYGLLESKDLFPNLT	80
81	VIRGSRLFNYALVIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNEECGDICP	160
161	GTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCHSECLGNCSQPDDPTKCVACRNFYLDGRCVET	240
241	CPPPYHFQDWRCVNFSCQDLHHKCKNSRRQGCHQYVIHNNKCIPECPSGYTMSSNLLCTPCLGCPKVCHLLEGEKT	320
321	IDSVTSAQELRGCTVINGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVLSFFRKLRLIRGETLEIGNYSF	400
401	YALDNQNLRLQLDWWSKHNLTITQGLFFHYNPKLCLSEIHKMEEVSGTKGRQERNDIALKTNQDQASCENELLKFSYIRT	480
481	SFDKILLRWEPYWPPDFRDLGFMFLFYKEAPYQNVTEFDGQDACGSNSWTVDIDPPLRSNDPKSQNHGWLMRGLKPWT	560
561	QYALFVKTLVTFSDERRTYGAKSDIIVYQTDATNPSVPLDPIVSVNSSSQIILKWKPPSDPNGNITHYLVFWERQAEDSE	640
641	LFELDYCLKGLKLPRTWSPPESEDSQKHNSQSEYEDSAGECCSCPKTDSQILKELEESSFRKTFEDYLHNVVFPVPRKTS	720
721	SGTGAEDPRPSRKRSLGDVGNVTVAAPTVAAPNTSSTVPTSPPEHRPFPEKVVNKESLVISGLRHFTGYRIELQACNQ	800
801	DTPEERCSVAAYVSARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCVSRKHFAL	880
881	ERGCLRGLSPGNYSVRIRATSLAGNGSWTEPTYFYVTDYLDVPSNIA RMKQLEDKVEELLSKNYHLENEVARLKKLVGE	960
961	R	
B	Protein Sequence of IGF-1Rzip	
1	EICGPGIDIRNDYQQLKRLNCTVIEGYLHILLISKAEDYRSYRFPKLTVITEYLLFRVAGLESGLDFPNLTVIRGWK	80
81	LFYNYALVIFEMTNLKDIGLYNLRNITRGAIRIEKNADLCYLSTVDWSLILDAVSNNYIVGNKPPKECGDLCPGTMECKP	160
161	MCEKTTINNEYNYRCWTTNRCQKMCPSCTCGKRACTENNECCHPECLGSCSAPDNDTACVACRHHYVAGVCPACPPNTYR	240
241	FEGRWCVRDFCANILSAESSDSEGFVIHDGECMQECPSGFIRNGSQSMYCIPEGPCPKVCEEKTKTIDSVTSQML	320
321	QGCTIFKGNLLINIRRGNNIASELENFMGLIEVVTGYVKIRHSHALVLSFLKNLRLILGEEQLEGNYSFYVLDNQLNQ	400
401	LWDWDHRNLTIKAGKMYFAFNPKLVCSEIYRMEEVGTGKGRQSKGDINTRNNGERASCESDVLHFTSTTTSKNRIITWH	480
481	RYRPPDYRDLISFTVYYKEAPFKNVTEYDGDQDACGSNSWNMVDVLDLPPNKDVEPGILLHGLKPWTQYAVYVKAVTLTME	560
561	NHIRGAKSEILYIRTNASVPSIPLDVLASANSSSQLIVKWNPPSLPNGNLSYYIVRWQRQPQDGYLYRHNYCSKDKIPI	640
641	RKYADGTIDIEEV TENPKTEVCGGEGKPCACPKTEAEKQAEKEEAAYRKFVFNFLHNSIFVPRPERKRRDVMQVANTM	720
721	SSRSRNTAADTYNITDPEELETEYPFFESRVDNKERTVISNLRPFTLYRIDIHSCNHEAEKLGCSASNFVFARTMPAEG	800
801	ADDIPGPVTWEPRPENSIFLKWPEPENPNGLILMYEIKYGSQVEDQRECVSRQYRKYGGAKLNRLNPNGYTARIQATSL	880
881	SGNGSWTDPVFFVYQAKTYENFIH RMKQLEDKVEELLSKNYHLENEVARLKKLVGERSSSEQKLISEEDLN	952

Figure S1. Protein sequences of the two constructs use to form HybZip, Related to STAR Methods.

(A) IR-Bzip, comprising residues 1-928 of the human IR-B isoform followed by the 33-residue GCN4 zipper sequence (*orange*).

(B) IGF-1Rzip, comprising residues 1-905 of human IGF-1R followed by the 33-residue GCN4 zipper sequence (*orange*) followed by a three-serine spacer and the c-myc tag sequence EQKLISEEDLN (*blue*).

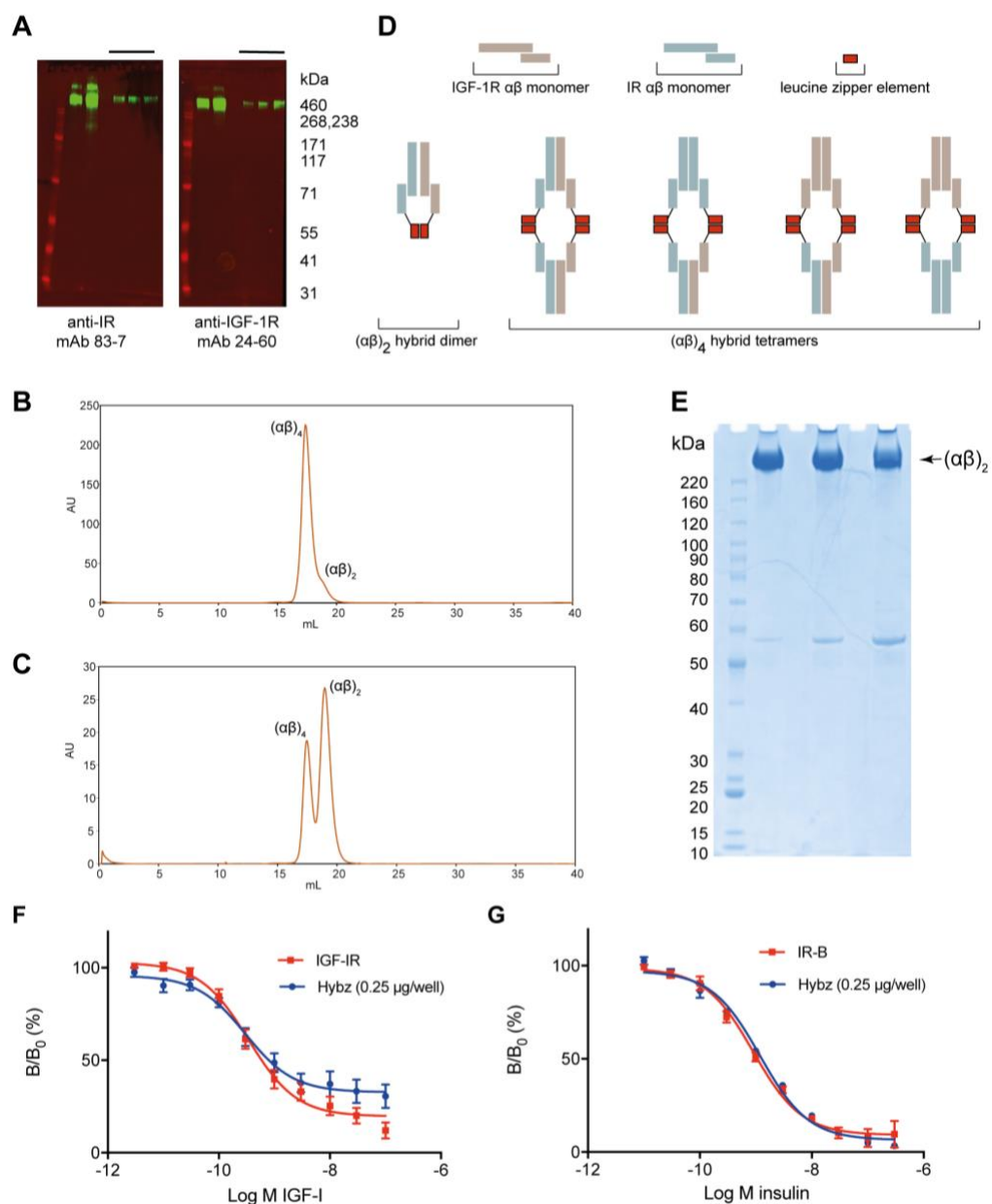


Figure S2. Purification and characterization of HybZip, Related to STAR Methods.

(A) Western blots of protein product obtained after serial elution from both 9E10 mAb and 18-44 mAb beads, showing presence of both species in the material (lanes with *bar* on right-hand side of each blot). Left-hand lanes in each blot correspond to pH 3 eluted material obtained from final column wash. Superfluous boundary has been removed from both blot images.

(B) Size-exclusion chromatogram obtained from protein product post affinity purification. The dominant peak corresponds to $(\alpha\beta)_4$ species, with a shoulder corresponding to $(\alpha\beta)_2$ species.

(C) Enrichment of $(\alpha\beta)_2$ species following re-run of pooled shoulder fractions from (B).

(D) Schematic illustrating leucine-zipper cross-linking of receptor ectodomain dimers to form hybrid tetrameric species. All four tetrameric species will be affinity co-purified with the desired hybrid dimer, but can be separated from it by size-exclusion chromatography (see panels B and C).

(E) Non-reducing SDS-PAGE gel of pooled $(\alpha\beta)_2$ fractions depicted in (C). Superfluous boundary and blank lanes (to the right) have been removed from the gel image.

(F) Competitive displacement assay of labelled IGF-I bound to holo-IGF-1R ($n = 9$) and to HybZip ($n = 12$) by unlabelled IGF-I. Error bars reflect standard error of the mean and are omitted when smaller than marker size. B/B_0 = percentage of binding in the absence of competing ligand.

(G) Competitive displacement assay of labelled insulin bound to holo-IR-B ($n = 9$) and to HybZip ($n = 9$) by unlabelled insulin. Error bars reflect standard error of the mean and are omitted when smaller than marker size. B/B_0 = percentage of binding in the absence of competing ligand.

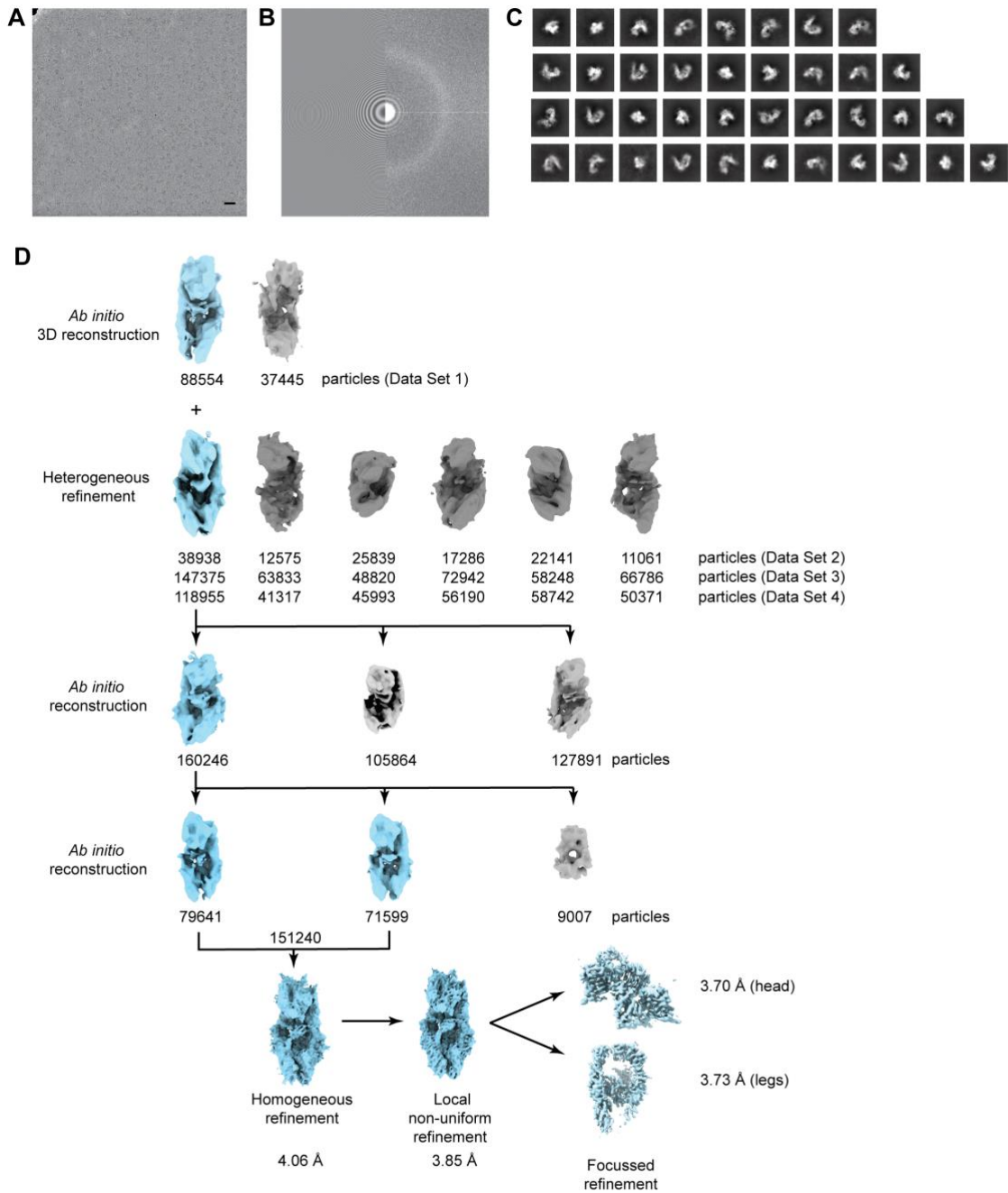


Figure S3. CryoEM reconstruction of IGF-I-complexed HybZip, Related to STAR Methods.

(A) Representative image obtained after motion correction (scale bar = 200 Å)

(B) Contrast transfer function associated with (A).

(C) 2D classes of particles selected from the four data sets (one data set per row; individual class images have been cropped to facilitate enlarged display).

(D) Flowchart showing the process of 3D reconstruction of IGF-I-complexed HybZip.

Full details are provided in the **STAR Methods Detail** section.

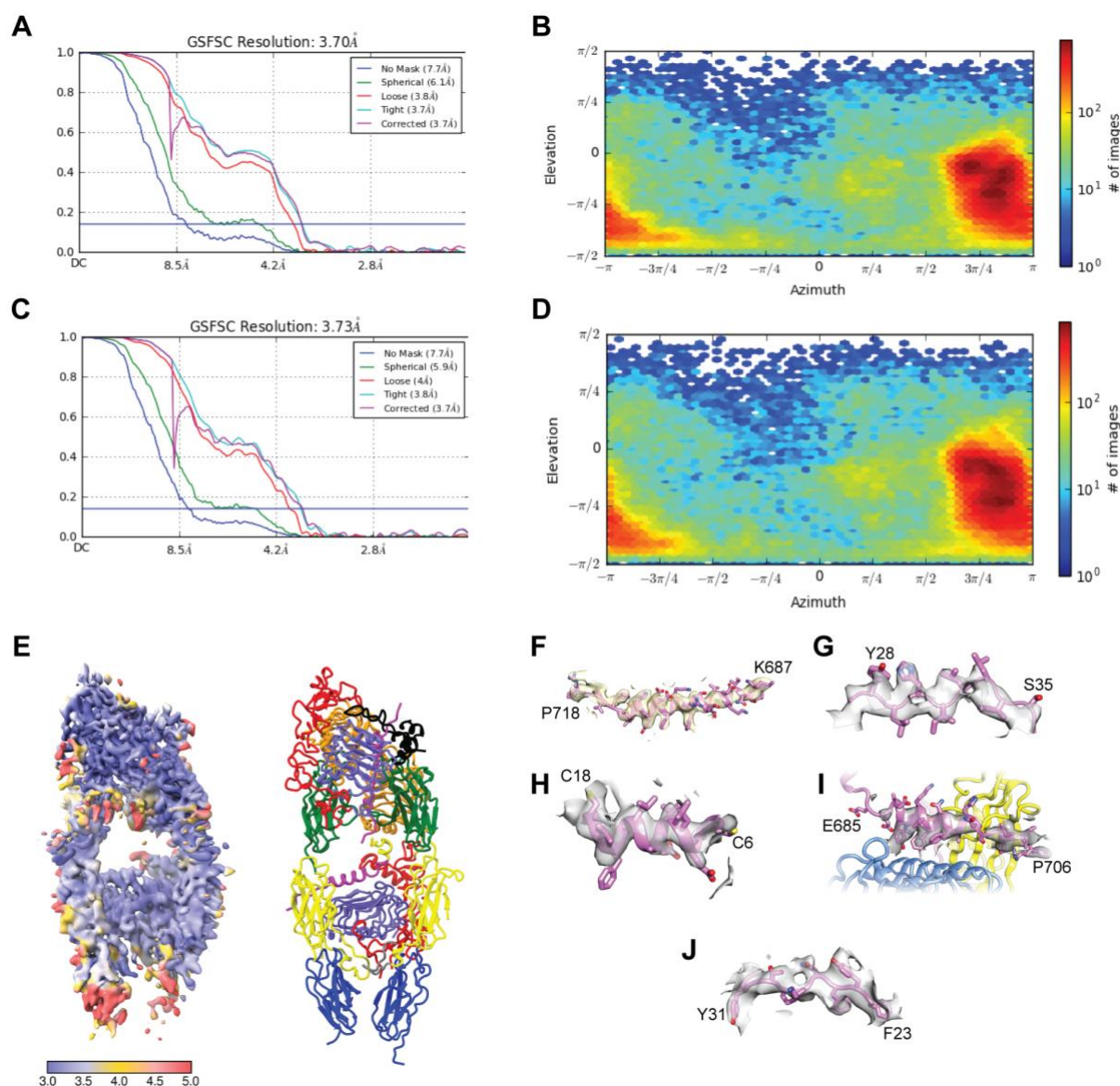


Figure S4. Quality of the 3D reconstructions of the two receptor volumes, Related to STAR Methods.

(A),(C) Gold-standard Fourier shell correlation (GSFSC) plots for the half-maps associated with the reconstruction of the "head" and "leg" region, respectively, of the IGF-I-complexed HybZip.

(B), (D) Angular distribution of particles contributing to the reconstruction of the "head" and "leg" region, respectively, of the IGF-I-complexed HybZip.

(E) Left panel: overlay of the "head" and "leg" regions maps, colored according to the local resolution. Right panel: ribbon diagram of IGF-I-complexed HybZip, oriented and scaled to match the local-resolution map in the left panel.

(F) CryoEM potential density for IR α CT segment, residues Lys687-Pro718. Contour level 0.201, display restricted to within 3.0 Å of depicted residues.

(G) CryoEM potential density for IGF-1R domain L1, residues Tyr28-Ser35. Contour level 0.230, display restricted to within 3.0 Å of depicted residues.

(H) CryoEM potential density for B domain helix of IGF-I, residues Cys6-Cys18. Contour level 0.242, display restricted to within 3.0 Å of depicted residues.

(I) CryoEM potential density for IGF-1R α CT segment, residues Glu685-Pro706. Contour level 0.201, display restricted to within 3.4 Å of depicted residues.

(J) CryoEM potential density for C domain of IGF-I, residues Phe23-Tyr31. Contour level 0.160, display restricted to within 3.0 Å of depicted residues.

Table S1. CryoEM data collection parameters, related to STAR METHODS

Data Set:	1	2	3	4
Pixel size (Å)	1.06	1.06	1.06	1.06
Dose rate (e ⁻ .pix ⁻¹ .sec ⁻¹)	8	6	6	6
Total dose (e ⁻ .Å ⁻²)	50	52	52	52
No. frames/movie	36	50	50	50
C _s	2.7	2.7	2.7	2.7
kV	300	300	300	300