Online Methods

Argos constructs. To establish the normal signal sequence cleavage site of *Drosophila melanogaster* Argos, the amino terminus of the mature recombinant protein was sequenced. The amino-terminal sequence was TRLPLEVF, indicating that mature Argos is a 419 amino acid secreted protein. The non-conserved amino terminus of Argos has little predicted secondary structure and contains multiple O-linked glycosylation sites. Fusing a BiP signal sequence to R88 of mature Argos produced a well-behaved protein that did not appear to be O-glycosylated.

D. melanogaster Argos also contains a proteolytically labile 120 amino acid insertion of low conservation (compared to other drosophilids), and little predicted secondary structure, between the 4^{th} and 5^{th} cysteines of the protein. Non-drosophilid Argos homologues contain only a 5 amino acid linker in this region. We replaced the 120 amino acid linked linker of *D. melanogaster* with the corresponding 5 amino acids (PDGRT) found in *Apis mellifera* Argos (Supplementary Fig. 1). The resulting protein ($Argos_{217}$) was well expressed, and was resistant to proteolytic degradation. It contains 217 amino acids, corresponding to residues 88-139 of mature *D. melanogaster* Argos linked (via the PDGRT sequence) to residues 260-419. A hexahistidine tag was appended to the carboxyl terminus to aid purification.

Argos production and purification. $Argos_{217}$ used for crystallization of the Argos/Spitz complex was produced by secretion from Sf9 (*Spodoptera frugiperda*) cells using the Bac-to-Bac baculovirus expression system (Invitrogen Inc.) as recommended by the manufacturers. Approximately 3 days after infection of cells with recombinant virus, conditioned Sf900II media (Invitrogen-Gibco) was harvested and separated from cellular material by brief centrifugation. The medium was then passed over TALON resin

(ClonTech Inc.) for immobilized metal affinity chromatography (IMAC). The column was washed with 3-6 volumes of 10mM MES (pH 6.3), 150mM NaCl containing 50mM imidazole. $Argos_{217}$ was subsequently eluted with 300mM imidazole in the same buffer. The eluted protein (>90% pure by Coomassie staining) was directly loaded onto a cation exchange column (S2, Bio-Rad Inc.) in the same buffer, and eluted with a gradient of NaCl concentration ($Argos_{217}$ elutes at ~1M NaCl). Immediately prior to crystallization or binding studies, $Argos_{217}$ was gel-filtered into 10mM MES (pH 6.3), 150mM NaCl on a Superose 12 column (GE Healthcare).

Crystals of unliganded $Argos_{217}$ were obtained with protein produced from Drosophila Schneider 2 (S2) cells as described previously[5], and purified exactly as described above. This protein behaves identically in all respects to $Argos_{217}$ produced by Sf9 cells. Biosensor studies (Supplementary Fig. 2) established that histidine-tagged $Argos_{217}$ binds to $Spitz_{EGF}$ with the same affinity as reported for wild type $Argos_{419}$ in our previous studies[5].

Production of Spitz EGF domain. The coding region for the *D. melanogaster* Spitz extracellular region (ending at residue 99) was subcloned into the S2 cell expression vector pMT/BiP/V5-HisA (Invitrogen) so that the sequence RHHHHHHSMSGT immediately follows the BiP signal sequence cleavage site. The first serine in this sequence corresponds to S_{16} of mature secreted Spitz. A Factor Xa cleavage site was also engineered between residues 47 and 48 of secreted Spitz ($N_{46}I_{47}T\underline{IEGR}$ / $T_{48}F_{49}P_{50}$), where T_{48} represents the first residue of the EGF domain. Cleavage with Factor Xa allows removal of the highly glycosylated Spitz amino terminus. In addition, deletion of the amino-terminal 15 amino acids avoids lipid modification of the first cysteine[31], and substantially increases protein yield. S2 cells that stably express this modified form of secreted Spitz were selected by

cotransfection with pCo-PURO, and puromycin selection[32], and the secreted protein was purified exactly as described[5]. Following purification, the protein was cleaved with Factor Xa, and the 52 amino acid EGF domain of Spitz (T48-D99: $Spitz_{EGF}$) was isolated by size exclusion chromatography on a Superdex Peptide column (GE Healthcare) and the N-terminal fragment (plus uncleaved protein) was removed using IMAC. $Spitz_{EGF}$ binds $Argos_{419}$ and $Argos_{217}$ with the same affinity as the intact secreted form of Spitz[5].

Crystallization. $Argos_{217}$/$Spitz_{EGF}$ complex crystals grew from 0.1M HEPES pH 7.0 and 24% ethylene glycol at 21°C, with the addition of low concentrations (0.1-1%) of PEG20000 to slow crystal growth and thus improve crystal size and quality. Brief manipulation freed single crystal fragments that grew further over 7 days and were subsequently frozen directly in liquid nitrogen. Maximum single crystal dimensions reached 150μm x 100μm x 50μm. Crystals were of space group P1, with unit cell dimensions: a=50.0Å, b=51.3Å, c=70.0Å and α=84.2˚, β=74.8˚, γ=75.7˚. There are 2 complexes per asymmetric unit, with a Matthews coefficient of 2.6$Å^3$/Da giving a solvent content of 53%.

Crystals of uncomplexed $Spitz_{EGF}$ grew from 15mM ammonium sulphate in 0.1M MES pH 6.5 containing 24% ethylene glycol. Crystals grew as single rods over 2 weeks, and were frozen directly from the drop in liquid nitrogen. Crystals were of space group C2, with unit cell dimensions: a=58.3Å, b=36.2Å, c=25.4Å and α=90˚, β=103.1˚, γ=90˚. There is 1 molecule per asymmetric unit, with a Matthews coefficient of 2$Å^3$/Da and a solvent content of 39%.

Crystals of unliganded $Argos_{217}$ grew at 18˚C from 10-20% PEG3350, 0.1M NaAcetate pH 4.5, containing 0.2M ammonium sulphate. Crystals were rapidly passed through paraffin oil for freezing. Crystals were of space group C2 with unit cell

dimensions: a=113.6Å, b=64.2Å, c=72.5Å and α=90˚, β=101.6˚, γ=90˚. There are 2 molecules per asymmetric unit, with a Matthews coefficient of 2.5Å^3/Da and solvent content of 52%.

Structure Determination. For experimental phasing, efforts to introduce a variety of anomalous scatterers were made. Halide soaks[10] were a focus as they have been successful for several other disulphide-rich glycoproteins with few reactive side-chains. Immediately prior to freezing, 1M NaBr (in 5% PEG20000, 0.1M HEPES pH 7.0, 24% ethylene glycol) was directly added (1:1) to the $Argos_{217}$/$Spitz_{EGF}$ complex crystal drops. A 3-wavelength MAD data set was collected on a single NaBr-soaked crystal at APS 23-IDD (Argonne, IL). Data on a second NaBr-soaked crystal were collected at a 4th wavelength with less attenuation for higher resolution data. Data were processed with HKL2000[33] and the phases were determined with SHELX C/D/E[34,35], utilizing all 4 data sets and anomalous signal from 10 bromide ions. The resulting electron density map was readily interpretable, allowing almost the entire chain of each complex in the asymmetric unit to be traced straightforwardly. Alternating cycles of model building with COOT[28] and refinement with REFMAC[29] led to a complete model of Argos and Spitz. The first 10 residues in both Argos molecules are not seen in the crystal structure, and nor are the C terminal hexahistidine tags. In addition, the first and last two residues in $Spitz_{EGF}$ could not be located in the complex. NCS averaging was used for initial rounds of refinement but released in the final stages of refinement.

The structure of unliganded $Argos_{217}$ was solved by sequential molecular replacement using PHASER[36] in the CCP4 program suite[29]. Domains 1 and 2 of $Argos_{217}$ from the complex were used to find a molecular replacement solution, and a solution was then

identified for domain 3. The structure of $Spitz_{EGF}$ was solved by molecular replacement using a loop-truncated version of the human EGF domain structure (1JL9)[30].

Calculations and figure preparation. Calculations of buried surface were carried out using AREAIMOL in the CCP4 suite of programs[29]. Calculations of surface complementarity (S_c)[18] used the program SC in CCP4[29]. Quantitative descriptions of protein domain movement were calculated using the DynDom server[37]. Structure validation was carried out with SFCHECK and PROCHECK in CCP4[29]. Figures were prepared using PyMOL[38].

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