

Fig. S1. Localization of wild-type Ser and Ser Del4-6 on the apical cell surface. (A-F) Cell regions shown are located within ten cell diameters of the ventral side of the wing margin. (A,D) Endogenous Notch staining localized to the apical cell surface (Fehon et al., 1991). (B,E) Expression of the tomato tag (Shaner et al., 2004) located within the Ser constructs in specific cells under control of the Gal4^{Ser2} promoter (pattern seen in Fig. 3A). (C,F) Merged images of A,B or D,E, respectively. (A-C) Wild-type (WT) Ser (tagged tomato; red) driven by Gal4^{Ser2} is localized on the apical cell surface (adhesion junctions) with Notch (green). (D-F) Ser Del4-6 (tagged tomato; red) driven by Gal4^{Ser2} is also localized on the apical cell surface, which is marked by Notch (green). We note that both WT Ser and Ser Del4-6 are colocalized with Notch on the apical cell membrane. Enhanced Notch accumulations (A,D) at the apical membrane coincident with Ser construct accumulations (B,E) are likely to be due to patching of the two molecules as previously described (Fehon et al., 1990). White lines (C,F) indicate the transects used for the quantification of subcellular distribution shown in G and H, respectively. (G,H) Quantification of expression levels demonstrates that WT Ser (G) is primarily localized to the apical cell surface (marked by Notch), which is indistinguishable from expression of Ser Del4-6 (H). The ratio of fluorescent intensity (y-axis) was measured using ImageJ. Scale bars: 2 μ m.

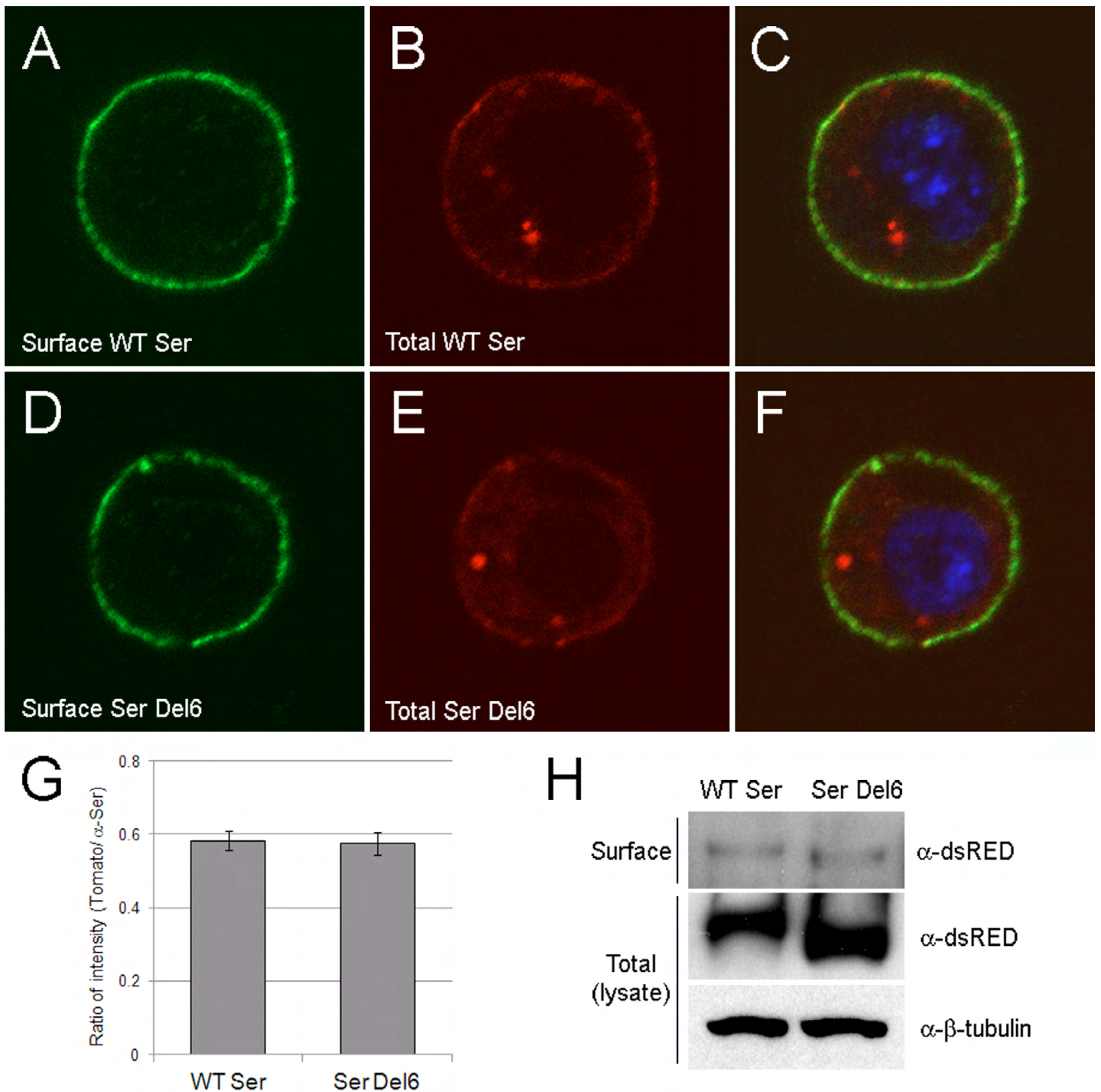


Fig. S2. Localization and quantitation of wild-type Ser and Ser Del6 protein forms in transfected S2 cells. (A-F) (A,D) Ser forms localized to the cell membrane are examined using an anti-Ser antibody (green) (Papayannopoulos et al., 1998) on non-permeabilized cells to examine Ser at the cell surface. Localization and expression levels are comparable. (B,E) Total Ser distribution using the intrinsic tomato tag (red) on each construct. Both membrane and intracellular localization of each Ser form are comparable. (C,F) Merged images of surface and total Ser along with DAPI (blue) to show the cell nucleus. (G) Levels of cell surface and total Ser are compared using ImageJ over ten different cells for each type. The ratios of cell surface (α -Ser; green) and total Ser (tomato tagged) proteins are not altered in the Del6 form relative to WT Ser. (H) Ser forms at the surface, as detected by biotinylation, are shown relative to total Ser levels for both WT and SerDel6 forms confirming that levels of both Ser types are comparable when expressed. Note that the SerDel6 protein is slightly smaller than the WT Ser isoform, as expected.



Fig. S3. Sequence alignments of the NIR of Ser with Ser family and Delta homologs. ELRs 4, 5 and 6 of Ser were used to find the best alignments with human jagged 1 (Jagged-1-1), chicken serrate 1 (C-Serrate-1), *Xenopus* Jagged 1 (X-Jagged-1), zebrafish Jagged 1 (Jagged-1a) and *Drosophila* Delta. To perform the alignment, non-EGF-like interruptions in ELR 4 (black arrow) and ELR 6 (red arrow) of Ser were removed as they are not conserved in non-drosophilid species and the interruption in ELR 6 is not responsible for cis-inhibition (see main text). Sequence removed from ELR 4 is AQVVRTSHGRSNMGRPVRSSSM RSLDHLRPEGQALNGSSSSGLVLGSLGLGGGLAPD and the sequence removed from ELR 6 is HSAGIAANALLTTTATAIIGSNLSSTALLAALTSAVASTSLAIG. All alignments depict ELRs 4, 5 and 6 of the respective proteins. For the Ser and Jagged proteins, these repeats are the most robust alignments of all ELR sequences in each protein (see also supplementary material Table S1). By contrast, any of the ELRs within Delta align with these Ser-related ELRs with comparable quality. Traditional RasMol color schemes for amino acids are used: red, Asp, Glu; yellow, Cys, Met; bright blue, Arg, Lys; orange, Ser, Thr; medium blue, Phe, Tyr; cyan, Asn, Gln; light gray, Gly; green, Leu, Val, Ile; dark gray, Ala; purple, Trp; pale blue, His; tan, Pro.

Table S1. Pairwise comparisons of ELRs 4-6 of Ser with related sequences

bl2seq input	Maximum score	Total score	E-value	Maximum identity	Identities	Positives	Gaps
Ser4-6 versus Jagged-1	125	125	2.00 E-42	51%	60/117 (51%)	75/117 (64%)	3/117 (3%)
Ser4-6 versus C-Serrate-1	128	128	2.00 E-43	56%	66/117 (56%)	79/117 (68%)	3/117 (3%)
Ser4-6 versus X-Jagged-1	132	132	6.00 E-45	58%	68/117 (58%)	80/117 (68%)	3/117 (3%)
Ser4-6 versus Jagged-1a	120	120	1.00 E-40	50%	58/117 (50%)	72/117 (62%)	3/117 (3%)
Ser4-6 versus Delta	77	107	1.00 E-23	41%	48/122 (39%)	66/122 (54%)	11/122 (9%)

We constructed pairwise alignments for each of the sequences in Fig. S3 versus Ser repeats 4-6 using bl2seq (NCBI). All values for pairwise comparisons of Ser family members are significantly higher than similar comparisons of Ser and Delta in all categories shown. The comparisons with Delta show the highest conservation found for three contiguous repeats, although comparison numbers for any three contiguous Delta repeats to those of Ser 4-6 are similar. Jagged-1, human jagged 1; C-Serrate-1, chicken serrate 1; X-Jagged-1, *Xenopus* Jagged 1; Jagged-1a, zebrafish Jagged 1; Delta, *Drosophila* Delta.