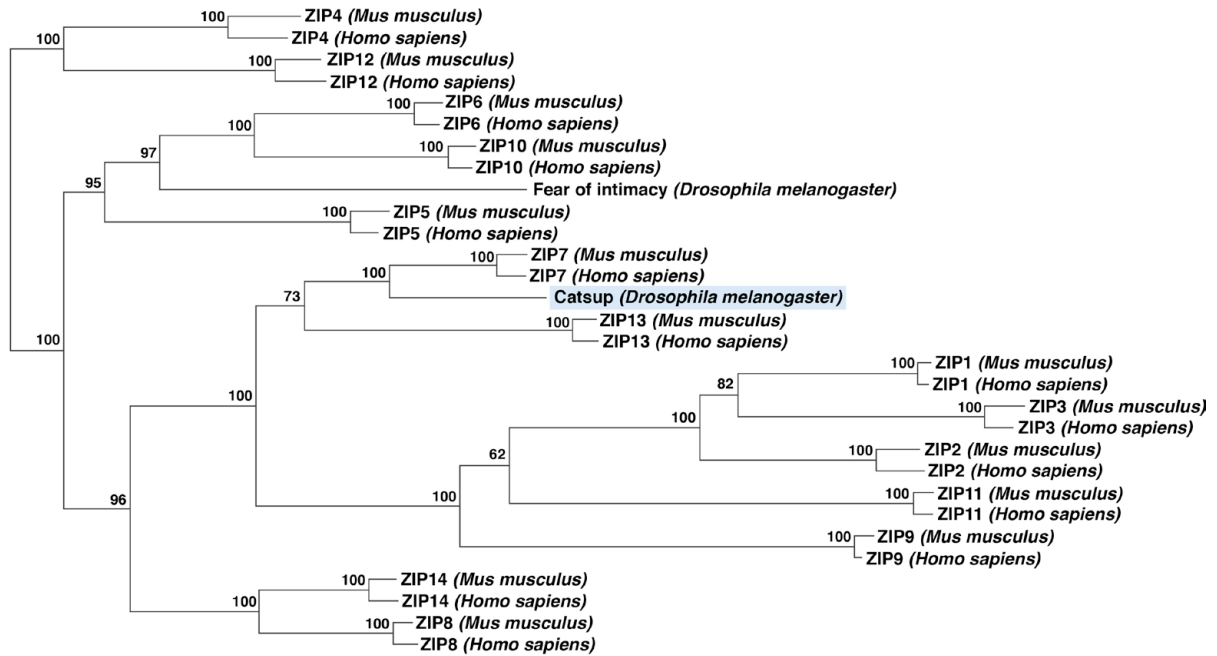
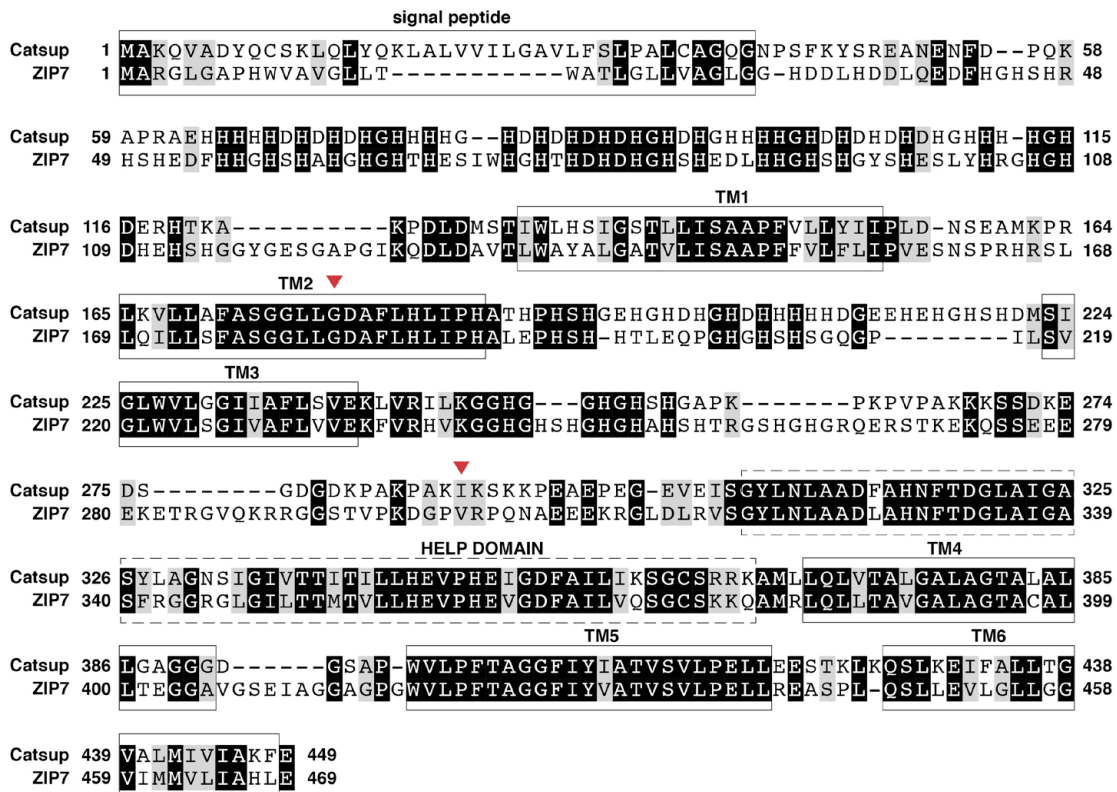


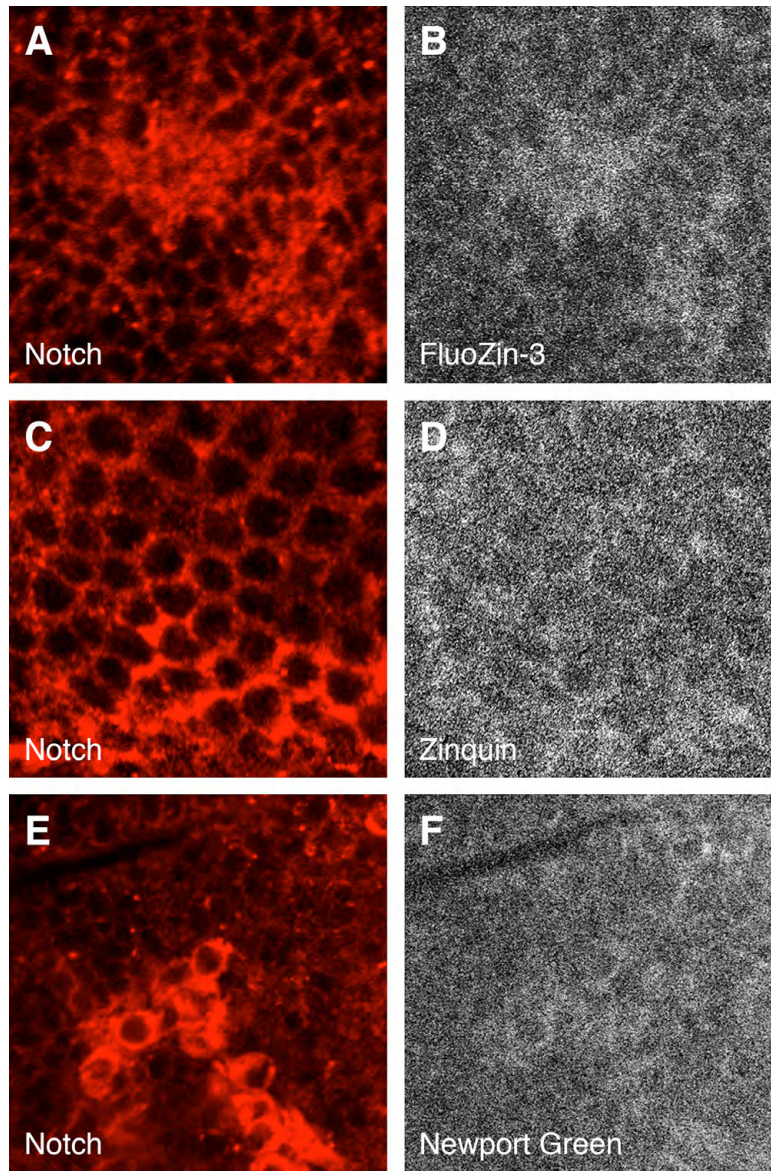
**A**



**B**



**Fig. S1. Phylogenetic analysis and sequence alignment of *Drosophila* Catsup protein and mammalian ZIP family zinc transporters.** (A) Phylogenetic tree showing relationship of *Drosophila* Catsup (highlighted in blue) to various murine and human members of the ZIP family of zinc transporters. (B) Alignment of the *Drosophila* Catsup (top) and human ZIP7 (bottom) protein sequences. Black boxes indicate identical residues; shaded boxes indicate conservative substitutions; putative signal peptide and transmembrane sequences are indicated by white rectangles; the conserved ‘HELP’ domain found among the LIV1 ZIP subfamily is indicated by the dashed rectangle; amino acid residues mutated in *Catsup*<sup>47</sup> and *Catsup*<sup>48</sup> are indicated by red arrowheads above the sequence.



**Fig. S2. Analysis of  $Zn^{2+}$  levels in *Catsup* mutant clones.** (A-F) *Catsup* mutant clones showing Notch protein distribution at left (red in A,C,E) and the corresponding signals from zinc probes on the right (yellow in B,D,F) following treatment of wing imaginal discs with FluoZin-3 (A,B), Newport Green (C,D) or Zinquin (E,F). See Materials and methods for details. To verify specificity of the weak zinc probe signals, clones were generated with *FRT40A* carrying a *yellow*<sup>+</sup> marker instead of a *GFP* marker, discs that were not exposed to zinc probes were examined in parallel samples and the confocal laser settings were tested with discs stained only for Notch to ensure that there was no signal bleedthrough from the rhodamine into the fluorescein channel.

Table S1. Rescue of *Catsup* mutant lethal phenotypes by transgenic expression of epitope-tagged *Catsup*-V5

Progeny genotypes	<i>Catsup</i> allele combination	# non-CyO	# CyO	Non-CyO /CyO ratio	Avg. ratio	% Wing notching
non-CyO progeny: <i>Catsup<sup>X</sup>/Catsup<sup>Y</sup></i> ; <i>da-GAL4/UAS-Catsup-V5</i>	26/47	63	111	0.57	0.62 (±0.019)	4.6
	(n=6)	86	136	0.63		
		81	124	0.65		
		80	119	0.67		
		77	138	0.56		
95	144	0.66				
CyO progeny: <i>(Catsup<sup>X</sup> or Catsup<sup>Y</sup>)/CyO</i> ; <i>da-GAL4/UAS-Catsup-V5</i>	26/48	45	96	0.47	0.53 (±0.018)	4.8
	(n=5)	49	97	0.51		
		59	106	0.56		
		51	98	0.52		
		65	114	0.57		
47/48	24	108	0.22	0.22 (±0.008)	11.0	
(n=5)	27	132	0.20			
	26	117	0.22			
	25	99	0.25			
	25	121	0.21			

All three possible transheterozygous lethal *Catsup* genotypes (*Catsup<sup>26</sup>/Catsup<sup>47</sup>*, *Catsup<sup>26</sup>/Catsup<sup>48</sup>* and *Catsup<sup>47</sup>/Catsup<sup>48</sup>*) were tested for their ability to be rescued by transgenic expression of a wild-type epitope-tagged *Catsup*-V5 construct expressed under *daughterless* gene regulatory control. ON the left, the scored adult progeny genotypes produced from crosses between *Catsup<sup>X</sup>/CyO*; *da-GAL4* and *Catsup<sup>Y</sup>/CyO*; *UAS-Catsup-V5* are shown, where *X* and *Y* represent different *Catsup* mutant allele designations. For full rescue, a Mendelian ratio of 1:2 (0.50) is expected for non-CyO (top) and CyO (bottom) progeny classes. Columns on the right show each *Catsup* mutant genotype assayed, number of non-CyO progeny and CyO progeny recovered from multiple replicates of each cross, calculated non-CyO/CyO progeny ratio for each replicate, average ratio and standard error, and percentage of total surviving non-CyO adults for each genotype that exhibited notching of one or both wing blades.