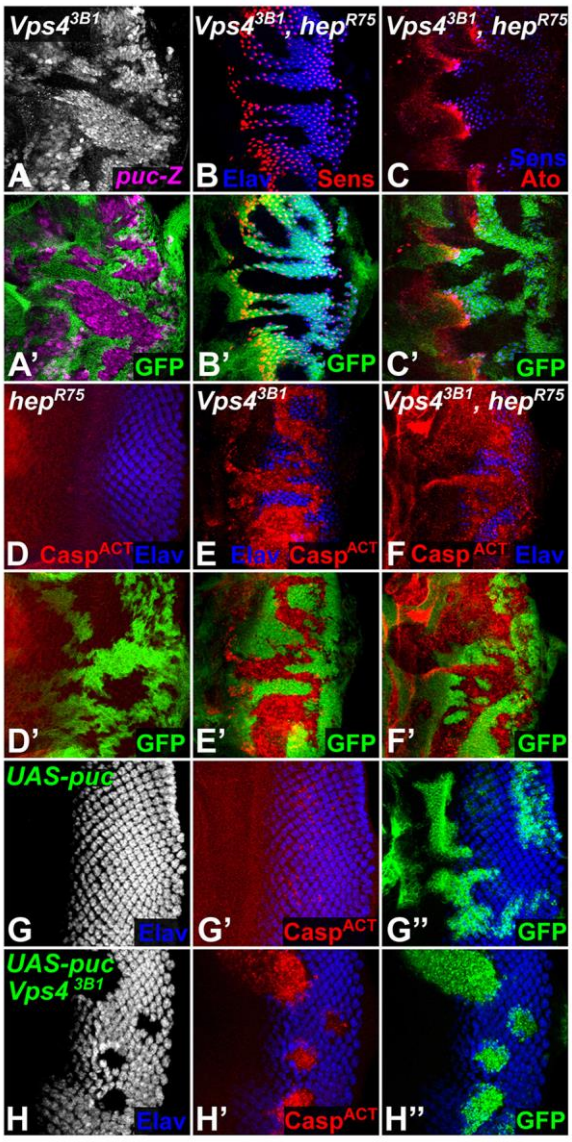

Fig. S1. Blocking JNK signaling does not rescue *Vps4* differentiation or survival phenotypes. (A-F) Eye discs with mutant clones marked by the absence of GFP (green). (A) *Vps4*^{3B1} mutant clones express *puc-lacZ* (A, magenta in A'). (B-C) *Vps4*^{3B1}, *hep*^{R75} double mutant clones show no rescue of photoreceptor differentiation. Clones are devoid of Elav (blue in B) and the R8 marker Sens (red in B, blue in C) staining, and show reduced expression of Atonal (Ato, red in C), a marker of proneural precursors. (D-F) Photoreceptors are stained with Elav in blue and activated Caspase 3 is stained in red. *hep*^{R75} mutant clones (D) do not affect photoreceptor differentiation or survival. The loss of photoreceptors and the cell death

observed in *Vps4*^{3B1} clones (E) is not rescued in *Vps4*^{3B1}, *hep*^{R75} double mutant clones (F). (G-H) Eye discs containing clones overexpressing *puc*, marked by coexpression of GFP (green) and stained for Elav (blue) and activated Caspase 3 (red). (G) Expression of *UAS-puc* alone does not affect photoreceptor differentiation or survival. (H) *Vps4*^{3B1} clones expressing *UAS-puc* still show loss of photoreceptors and Caspase activation.

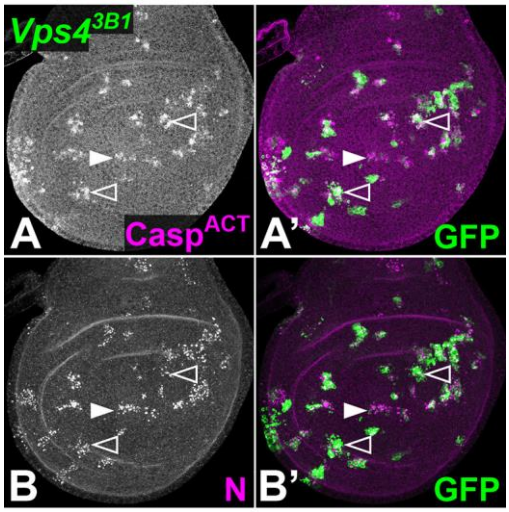

Fig. S2. *Vps4* mutant cells in the wing disc undergo apoptosis. (A,B) A third instar wing imaginal disc with *Vps4*^{3B1} clones positively marked by the expression of GFP (green). Mutant clones accumulate activated Caspase 3 (A, magenta in A') and the receptor N (B, magenta in B') in punctate structures (arrowheads). Note that accumulation of Caspase- and N-positive structures sometimes predates detectable levels of GFP (filled arrowhead).

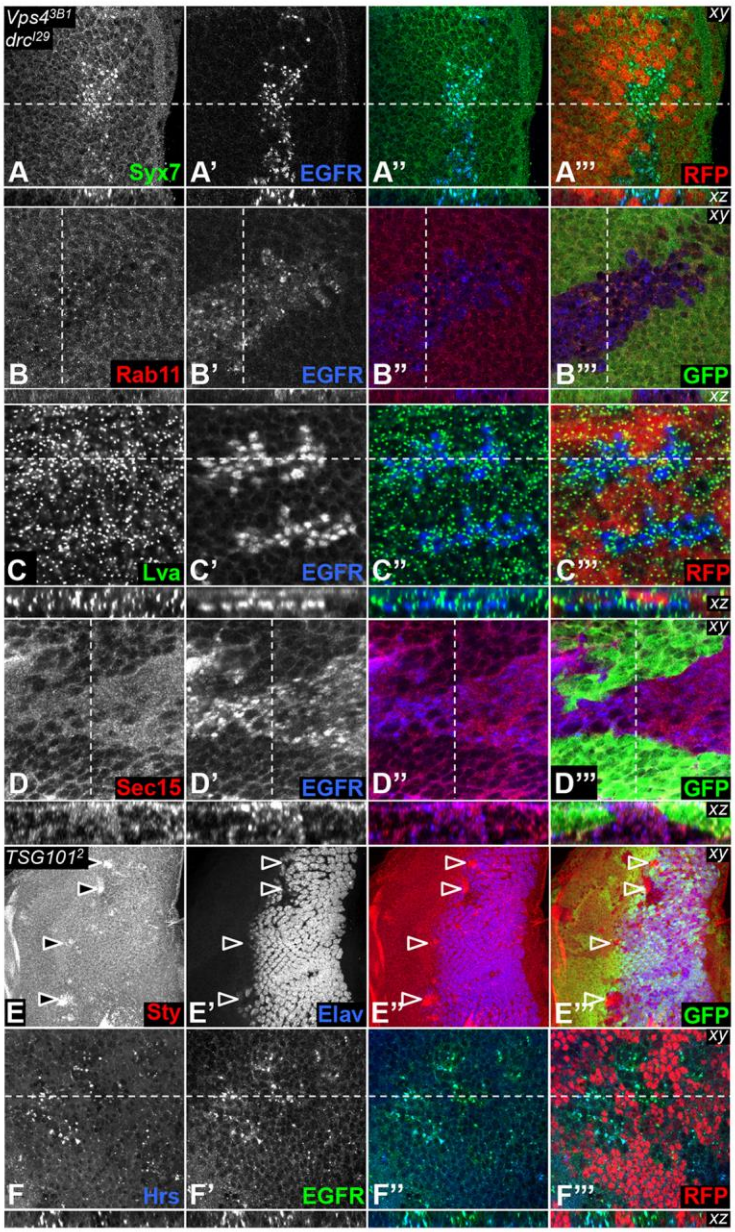

Fig. S3. EGFR accumulates in endosomes in *Vps4* mutant cells. All panels show eye discs with clones marked by the absence of GFP (green in B, D, E) or RFP (red in A, C, F). (A-D) show *Vps4*^{3B1} clones in a *drc* mutant background. EGFR (A', B', C', D', blue in A'', A''', B'', B''', C'', C''', D'', D''') accumulates in punctate structures that colocalize with the early endosomal marker Syx7, also known as Avalanche (Avl) (A, green in A'', A'''), but do not show significant colocalization with the recycling endosome marker Rab11 (B, red in B'', B''') or the Golgi marker Lva (C, green in C'', C'''), and only partially colocalize with the exocyst component Sec15 (D, red in D'', D'''). xz sections are shown below the xy panels. (E, F) show *TSG101*^{2} clones stained for the

EGFR target Sty (E, red in E'', E'''), the photoreceptor marker Elav (E', blue in E'', E'''), the endosomal marker Hrs (F, blue in F'', F''') and EGFR (F', green in F'', F'''). Although loss of TSG101 affects EGFR signaling in the opposite way from loss of Vps4, indicated by increased Sty expression (arrowheads), EGFR is still colocalized with Hrs in endosomes.

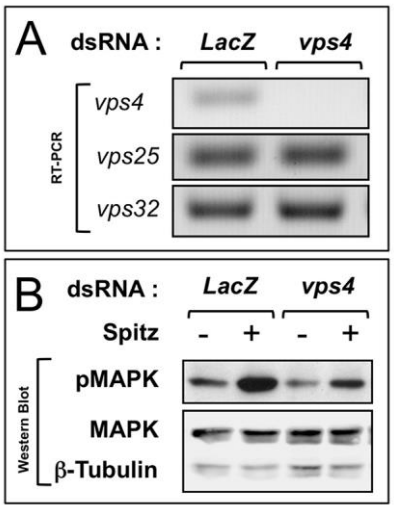

Fig. S4. *Vps4* is required for normal MAPK phosphorylation in response to Spi. (A) shows RT-PCRs on RNA extracted from *lacZ* or *Vps4* dsRNA-treated D2F cells, as indicated. *Vps4* knockdown strongly decreases the amount of *Vps4* transcripts but not *Vps25* or *Vps32* control transcripts. (B) shows Western blots of phospho-MAPK, total MAPK and β-Tubulin from lysates of D2F cells treated with *lacZ* or *Vps4* dsRNAs, as indicated, and exposed (+) or not exposed (-) to purified Spi for 10 min. *Vps4* knockdown strongly reduces MAPK phosphorylation in response to Spi.

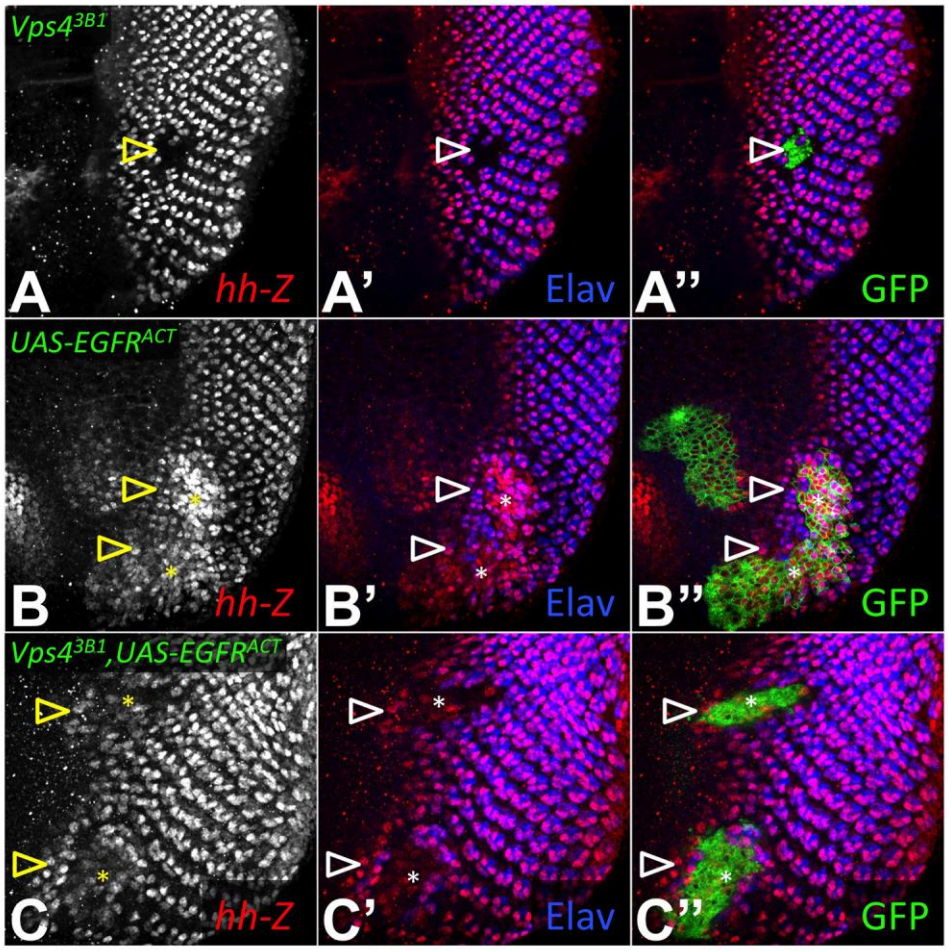

Fig. S5. EGFR^{act} restores moderate *hh-lacZ* expression to *Vps4^{3B1}* mutant clones. Eye discs expressing *hh-lacZ* (red), stained for Elav (blue), and carrying GFP (green)-marked clones of cells, either mutant for *Vps4* (A), or expressing *UAS-EGFR^{act}* (B) or both mutant for *Vps4* and expressing ectopic *UAS-EGFR^{act}* (C). (A) Arrowheads point to the loss of *hh-lacZ* and Elav expression in *Vps4* clones. (B) Both markers are ectopically expressed in response to ectopic *EGFR^{act}* , autonomously (asterisks), and in cells surrounding the clones, anterior to the morphogenetic furrow (arrowheads). (C) In *Vps4* clones, ectopic *EGFR^{act}* restores weak autonomous expression of *hh-lacZ* but not Elav (asterisks), resulting in non-autonomous photoreceptor differentiation (arrowheads).

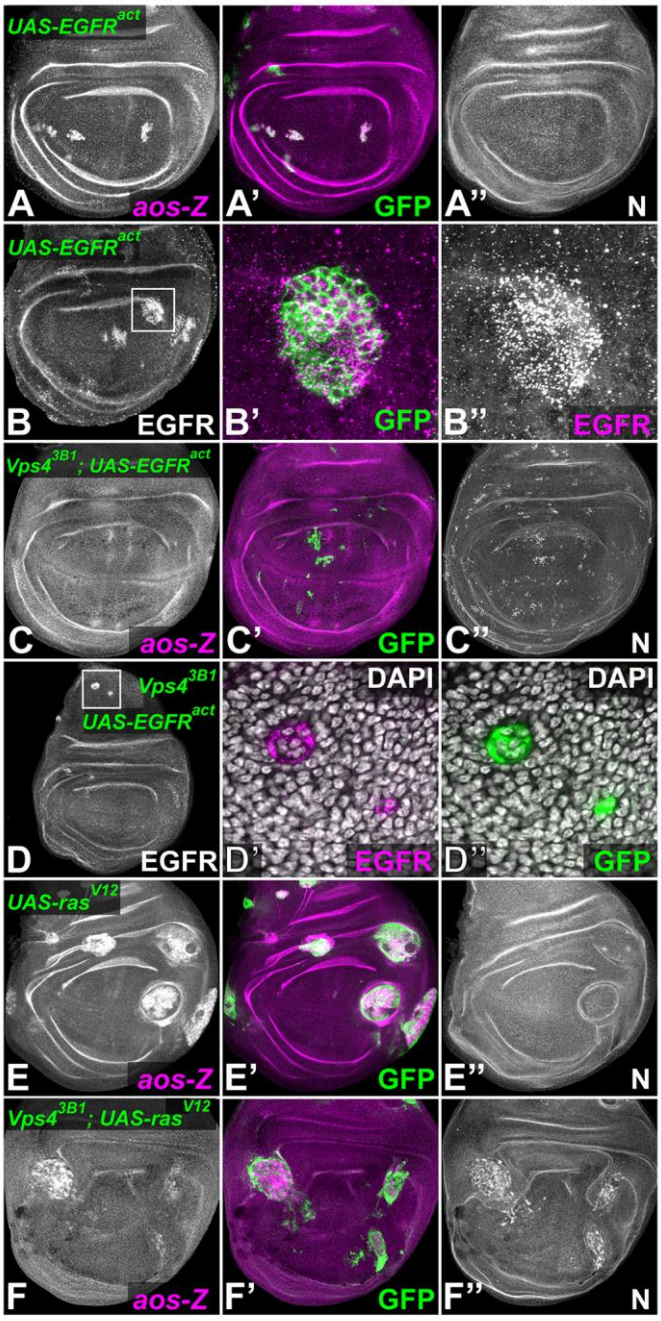

Fig. S6. *Vps4* acts at the level of the EGFR in wing development. (A-F) Third instar wing imaginal discs with clones that coexpress GFP (green) with either activated $EGFR^{\lambda top}$ (A-D) or Ras^{V12} (E,F). (A) *UAS-*$EGFR^{\lambda top}$ expressing clones ectopically express *aos-lacZ* (A, magenta in A'), but leave N (A'') unaffected. (B) Staining for EGFR (B, B'', magenta in B') reveals its punctate accumulation in *UAS-*$EGFR^{\lambda top}$ clones (boxed area enlarged in B', B''). (C) In $Vps4^{3B1}$ mutant cells marked by N accumulation (C''), $EGFR^{\lambda top}$ fails to turn on

ectopic *aos-lacZ* (C, magenta in C'). (D) Staining for EGFR (D, magenta in D') confirms $EGFR^{\lambda top}$ expression in $Vps4^{3B1}$ mutant cells. Boxed area enlarged and DAPI channel included in D', D''. (E) $UAS\text{-}Ras^{V12}$ expressing clones ectopically express *aos-lacZ* (E, magenta in E'), but leave N (E'') unaffected. (F) In $Vps4^{3B1}$ mutant cells marked by N accumulation (F''), Ras^{V12} also turns on ectopic *aos-lacZ* (F, magenta in F').