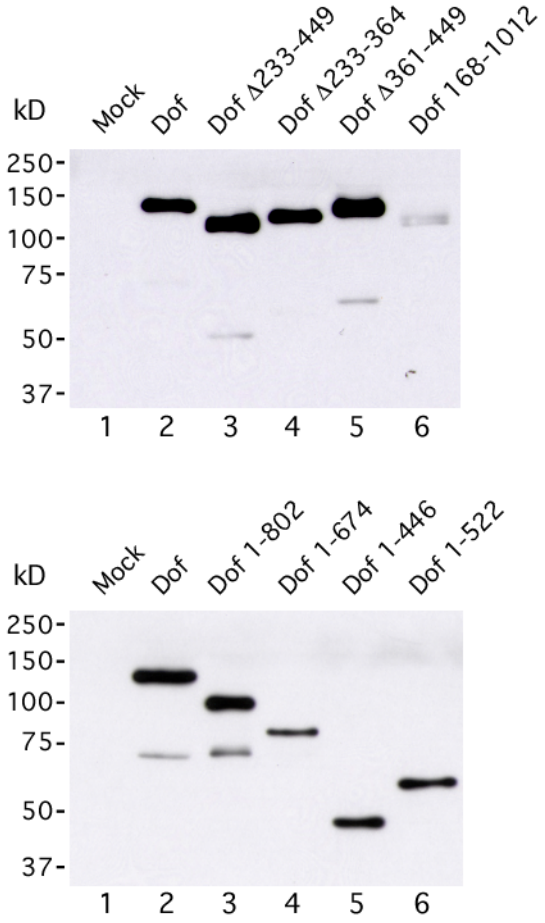

Fig S1 Wilson et. al.

Figure S1: **Stability of mutated versions of Dof in Schneider S2 cells.**

Lysates from Schneider S2 cells that had been transiently transfected with the indicated Flag-tagged Dof constructs were subjected to SDS-PAGE. Western blots were stained with antibodies against the Flag tag. In addition to the bands of the approximate molecular weight predicted for the constructs, smaller bands representing breakdown products are seen in some cases.

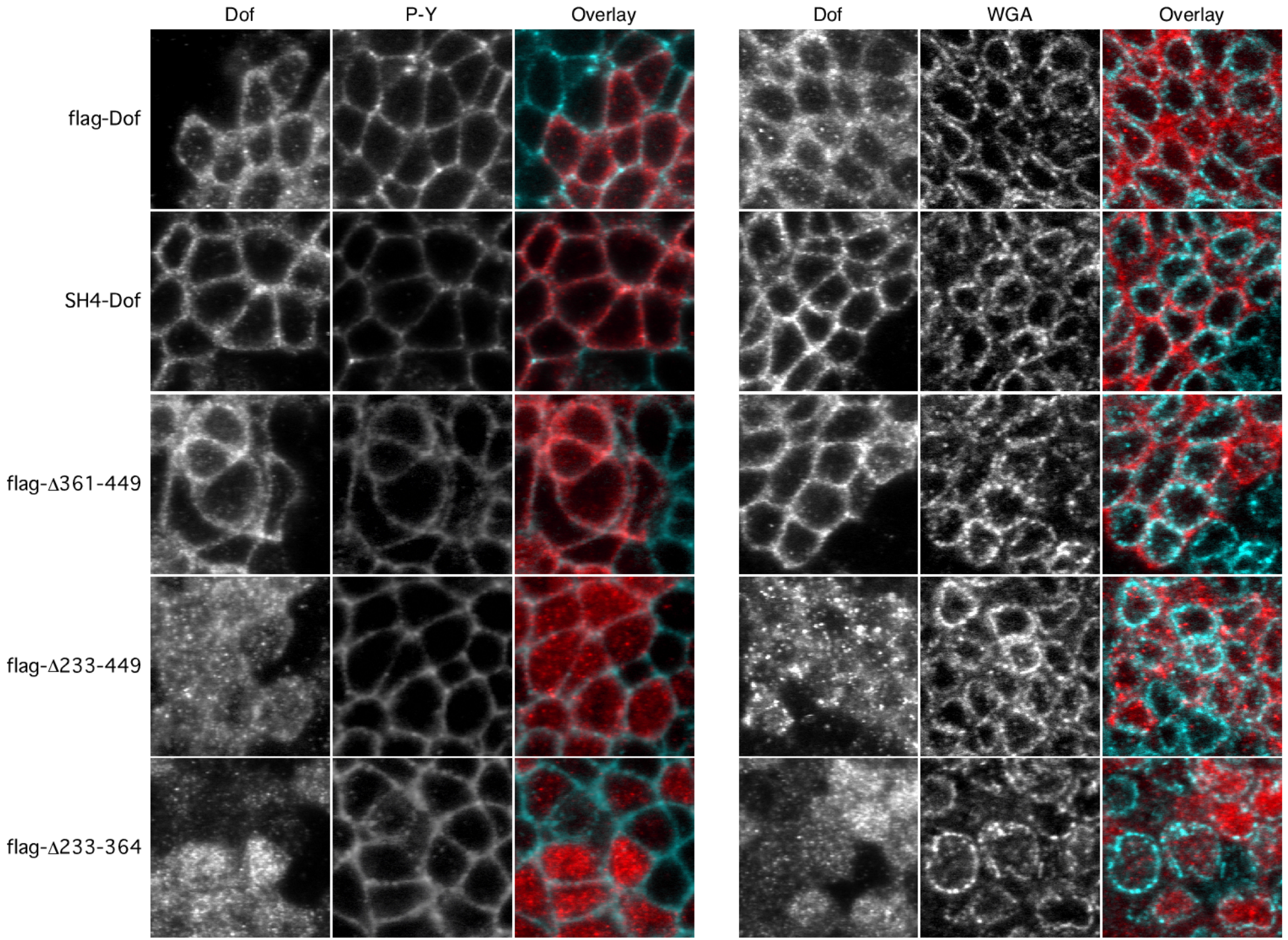

Fig S2 Wilson et. al.

Figure S2: Expression levels and subcellular localisation of mutant Dof constructs

Confocal sections of ectodermal cells expressing different *dof* transgenes. The sections on the left are taken from embryos stained with rabbit anti-Dof antiserum (Dof) and mouse anti-phosphotyrosine antibody (P-Y), to mark the cell membrane. The sections on the right are taken from embryos stained with rabbit anti-Dof antiserum (Dof) and biotinylated wheat germ agglutinin (WGA), to reveal the position of the nucleus. The data collected in the separate channels were combined in Adobe Photoshop to produce the overlays.

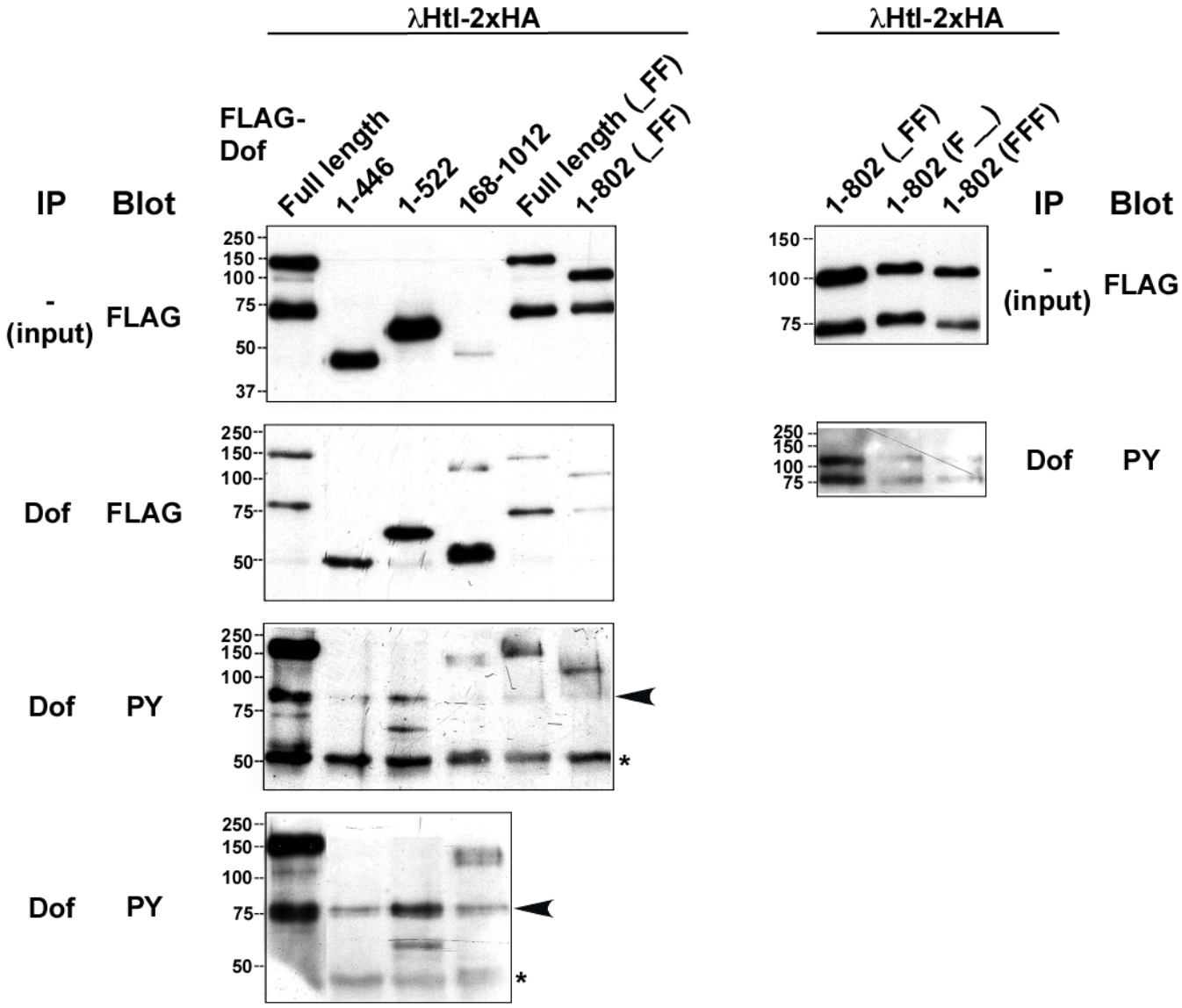

Fig S3 Wilson et. al.

Figure S3: Stability and phosphorylation of mutant Dof constructs.

Lysates from Schneider cells expressing FLAG-tagged mutant forms of Dof together with a constitutively active FGF-receptor (lambda-Htl) were separated by SDS-PAGE, or used for immunoprecipitation with antibodies against Dof. Western blots were probed with antibodies against phosphotyrosine or the FLAG-tag. The asterisk (*) marks the IgG heavy chain of the Dof antibody used for immunoprecipitation, the arrowhead indicates the activated, phosphorylated FGF receptor.

left column:

top panel: With the exception of Dof[168-1012] all constructs are expressed roughly at the same level. This was also the case for the other constructs that are not included here.

second panel: Immunoprecipitation of the Dof constructs with the Dof antibody and detection of the precipitated proteins by antibodies against Flag.

third panel: The full length Dof protein is strongly phosphorylated, whereas the phosphotyrosine signal is significantly reduced in all of the mutants. The band for Dof[1-446] cannot be judged in this panel, as it might be obscured by the IgG heavy chain of the Dof antibody. However, in the *bottom panel,* which is from a separate experiment, the IgG signal is very weak, but yet no signal for Dof[1-446] is visible.

right column:

The C-terminally truncated version of Dof in which the tyrosines in the potential drk, csw and PI3K consensus sites are mutated can still be phosphorylated.

top panel: Western blot of whole cell lysates to show the expression levels of Dof[1-802](_FF), Dof[1-802](F_ _) and Dof[1-802](FFF) (refer to Fig. 6 for description of the mutants).

bottom panel: Immunoprecipitations of the lysates from the top panel with antibodies against Dof, Western blot stained with an antiphosphotyrosine antibody.