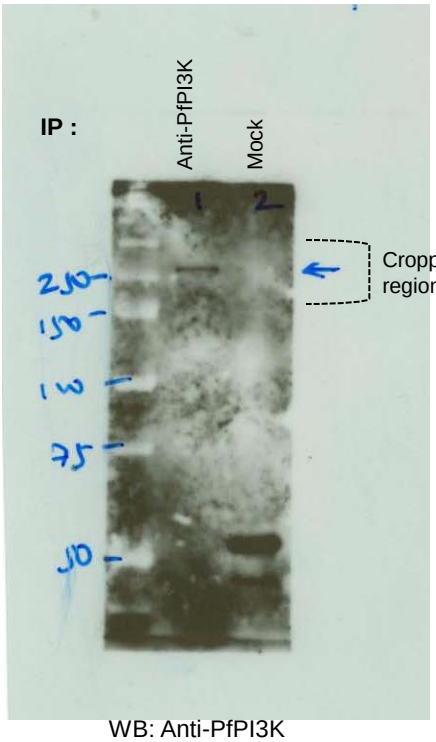
Supplementary Data 2

Figure 1c Raw Data full scan



WB: Anti-PfPI3K

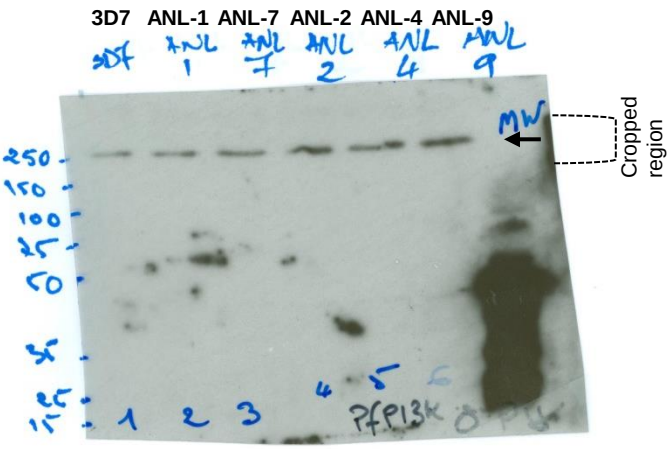
Lane 1: IP Anti-PfPI3K
Lane 2: IP Mock

Molecular weight standards (in kDa) are indicated at the left.

This original film scan was over-exposed to confirm that PfPI3K protein band was not detected in 'mock' lane, but specifically immunopurified by anti-PfPI3K.

Figure panel 1c uses the indicated cropped region, which was slightly lightened in the panel because we felt this provided the greatest clarity for both lanes.

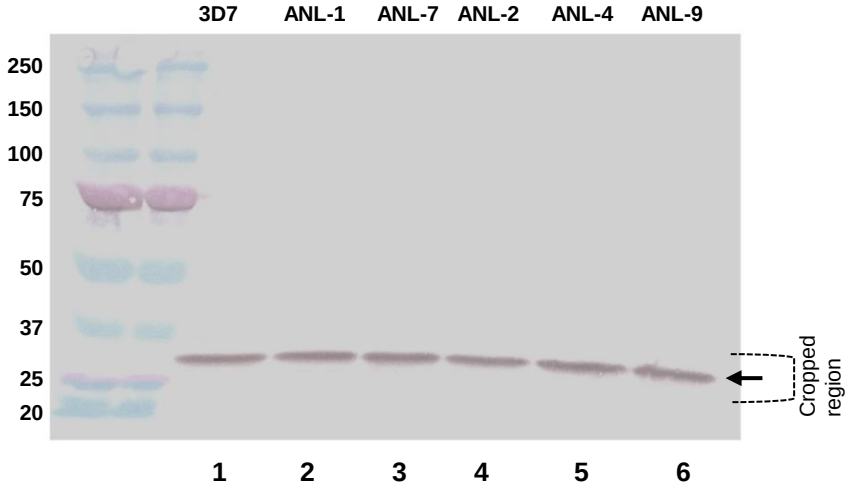
Figure 2b : Raw Data full scans



Western blot of PfPI3K levels

PfPI3K (arrow) levels in laboratory 3D7 and clinical ANLs, detected by WB using anti-PfPI3K
Lane 1: 3D7
Lane 2: ANL-1
Lane 3: ANL-7
Lane 4: ANL-2
Lane 5: ANL-4
Lane 6: ANL-9
Molecular weight standards (in kDa) are indicated at the left.

Figure panel 2b shows the indicated cropped region



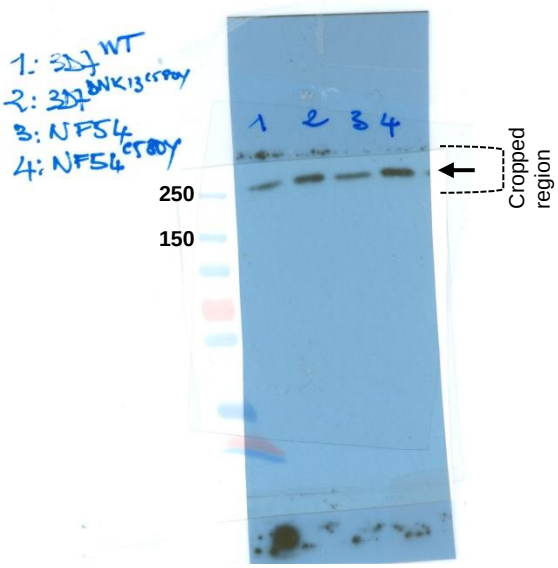
Western blot PfFKBP (parasite loading control

PfFKBP (arrow) levels in laboratory 3D7 and clinical ANLs.
Lane 1: 3D7
Lane 2: ANL-1
Lane 3: ANL-7
Lane 4: ANL-2
Lane 5: ANL-4
Lane 6: ANL-9
Molecular weight standards (in kDa) are indicated at the left.

Figure panel 2b shows the indicated cropped region

For PfFKBP, the original blot was developed on the membrane and therefore in color.
The scan was converted to grey scale and used in panel Figure 2b.

Figure 2c and 2d: Raw Data scans comparing lysates of PfKelch13^{WT}and PfKelch13^{C580Y} for indicated markers in Western Blots.

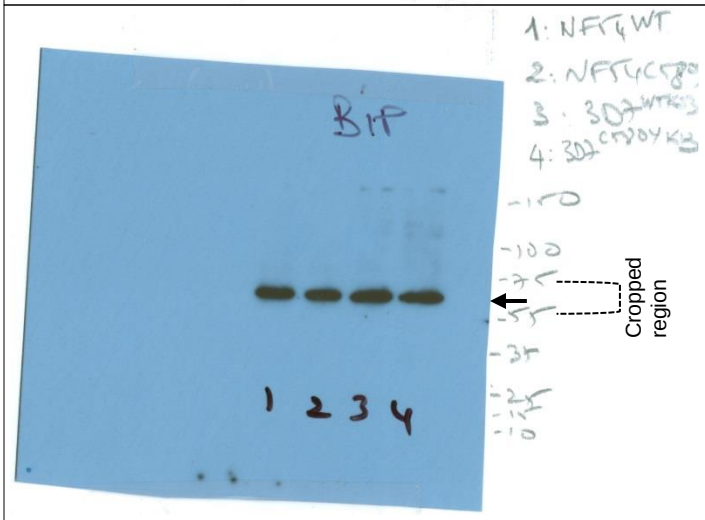


PfPI3K (arrow) levels in Figure 2c and 2d

Lane 1: 3D7 PfKelch13^{WT}-HA (Figure 2d)
Lane 2: 3D7 PfKelch13^{C580Y}-HA (Figure 2d)
Lane 3: NF54 PfKelch13^{WT} (Figure 2c)
Lane 4: NF54 PfKelch13^{C580Y} (Figure 2c)

Molecular weight standards (in kDa) is shown at the left

Cropped region of the blot used in Figure 2c and 2d is indicated.

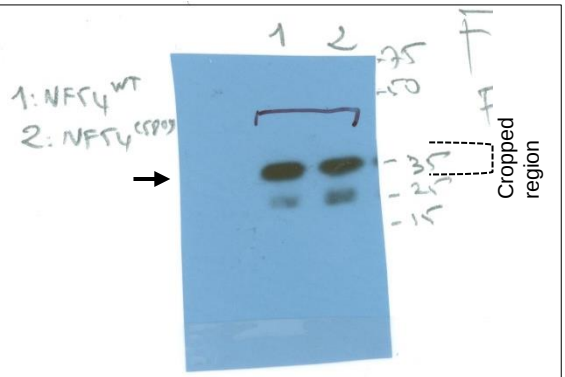


BiP (arrow) parasite levels in Figure 2c and 2d

Lane 1: NF54 PfKelch13^{WT} (Figure 2c)
Lane 2: NF54 PfKelch13^{C580Y} (Figure 2c)
Lane 3: 3D7 PfKelch13^{WT}-HA (Figure 2d)
Lane 4: 3D7 PfKelch13^{C580Y}-HA (Figure 2d)

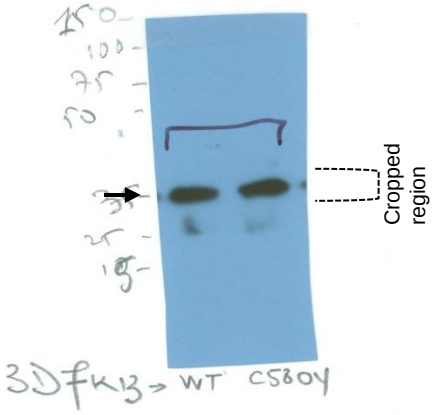
Molecular weight standards (in kDa) are as indicated.

Cropped region of the blot used in Figure 2c and 2d is indicated.



PfFKBP (arrow) as loading control in Figure 2c

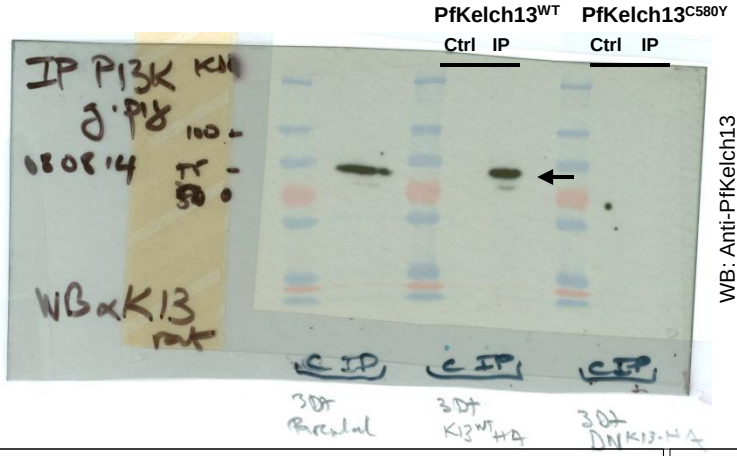
Lane 1: NF54 PfKelch13^{WT}
Lane 2: NF54 PfKelch13^{C580Y}
Molecular weight standards (in kDa) are as indicated.
Cropped region of the blot used in Figure 2c is indicated.



PfFKBP(arrow) as loading control in Figure 2d

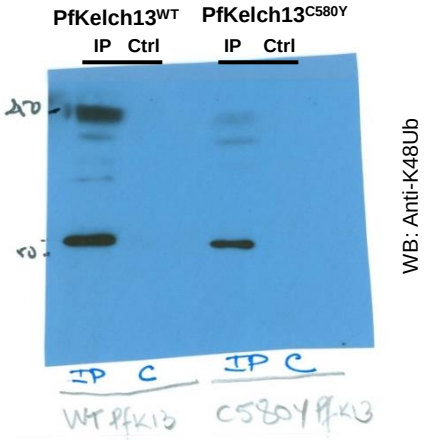
Left Lane : 3D7 PfKelch13^{WT}-HA (Figure 2d)
Right Lane : 3D7 PfKelch13^{C580Y}-HA (Figure 2d)
Molecular weight standards (in kDa) are as indicated.
Cropped region of the blot used in Figure 2d is indicated.

Figure 2e Raw Data scans: IP with anti-PfPI3K, western blot (WB) with indicated antibodies

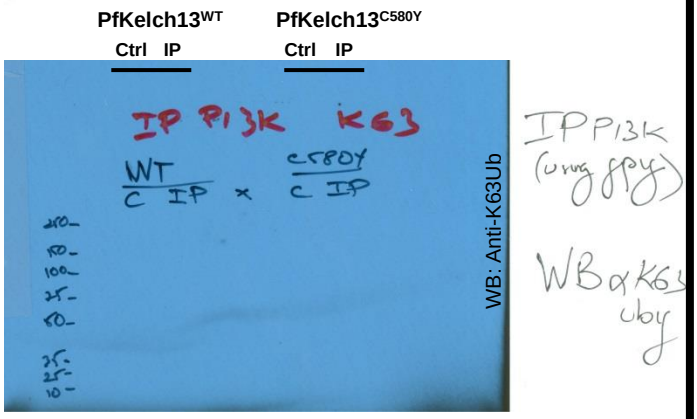


WB to detect PfKelch13 (top left panel)
3D7 parasites expressing PfKelch13^{WT}-HA or 3D7 PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PI3K (IP) or mock/control (Ctrl) incubation followed by WB to detect PfKelch13. The results suggest that Kelch13^{C580Y} acts a dominant negative mutant.

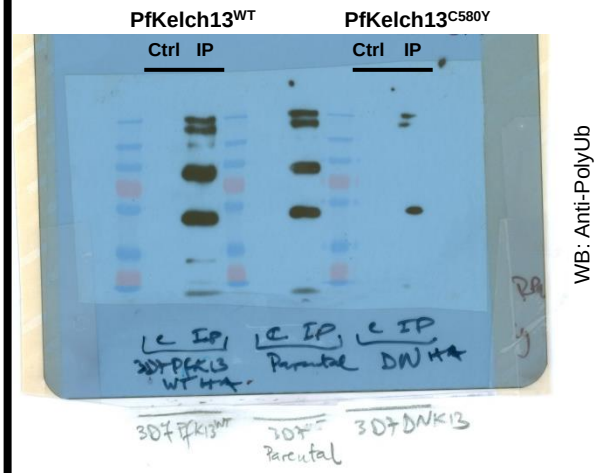
Molecular weight standards (in kDa) are as indicated.



WB to detect K48Ubiquitination: (top middle panel) 3D7 parasites expressing PfKelch13^{WT}-HA or PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PfPI3K (IP) or mock/control (Ctrl) incubation followed by WB with antibody to detect K48Ub. Mol wt. std., as shown.

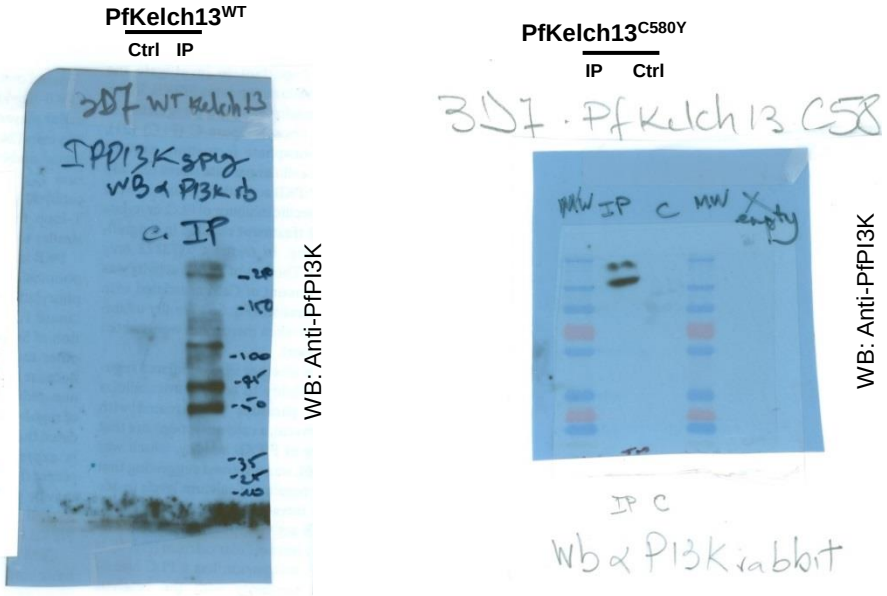


WB to detect K63Ubiquitination (top right panel)
3D7 parasites expressing PfKelch13^{WT}-HA or PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PfPI3K (IP) or mock/control (Ctrl) incubation followed by WB with antibody to detect K63Ub.
Molecular weight standards (in kDa) are as indicated.



WB to detect polyubiquitination (bottom left panel) 3D7 parasites expressing PfKelch13^{WT}-HA or PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PI3K (IP) or mock/control (Ctrl) incubation followed by WB with antibody to detect polyubiquitination. Molecular weight standards (in kDa) are as indicated.

Figure 2e continued: IP with anti-PfPI3K (guinea pig), western blot with anti-PfPI3K (rabbit)



WB to detect PfPI3K fragments (bottom middle panel) 3D7 parasites expressing PfKelch13^{WT}-HA (LHS) or 3D7 PfKelch13^{C580Y}-HA (RHS), were subjected to immunoprecipitation using antibodies to PfPI3K generated in guinea pigs (IP) or mock/control (Ctrl) incubation followed by WB with antibodies to PfPI3K generated in rabbits. Molecular weight standards (in kDa) shown.

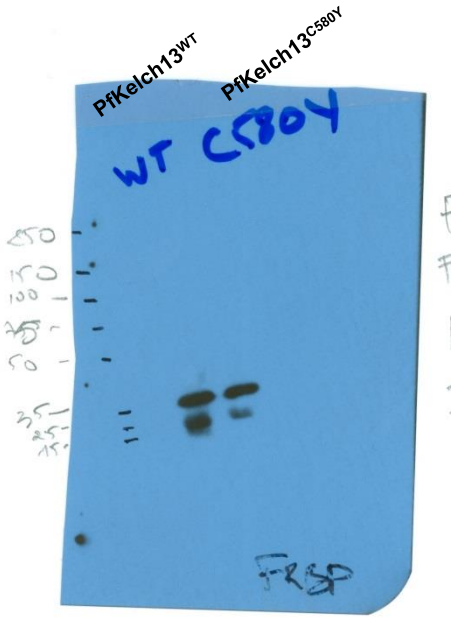


Figure 2e IP lysate input

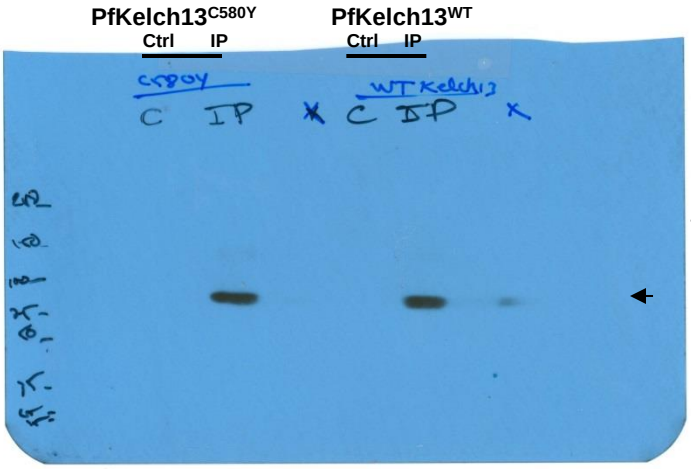
WB to detect PfFKBP , bottom right panel

3D7 parasites expressing PfKelch13^{WT}-HA (WT) or 3D7 PfKelch13^{C580Y}-HA (C580Y) used for IP in panel 2e, probed for PfFKBP, which serves as a loading control for all immunoprecipitations in Fig. 2e.

Molecular weight standards (in kDa) shown.

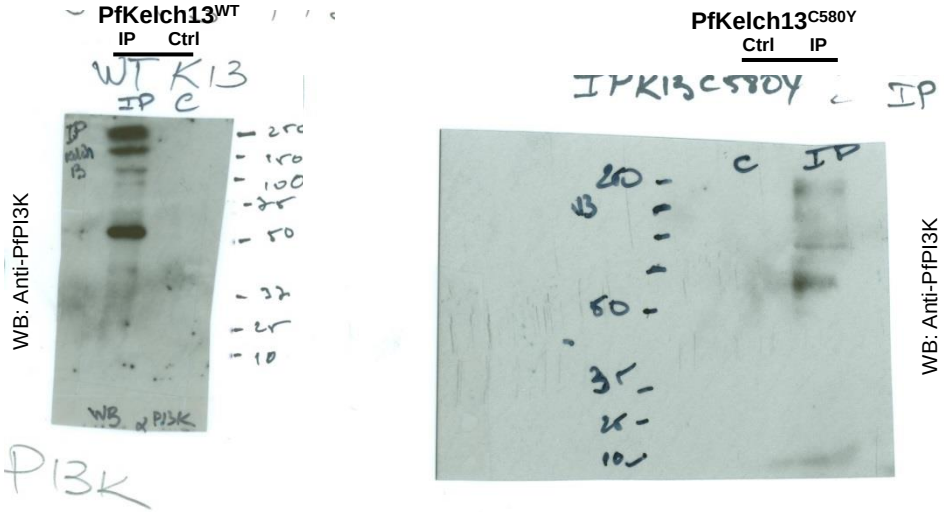
Summary for Figure 2e. Other than the loading control for input lysate, running gel regions (as indicated by molecular weight markers) are shown for relevant lanes. Images were converted to gray scale, sized to accommodate all of the data in Figure 2 as one power point slide and for clear visualization. Re-sizing slightly altered thickness and intensity. For panels probed with anti-Pf PI3K, the contrast (background) was slightly deepened to better visualize minor bands in the PfKelch13^{C580Y} mutant lanes (which biases against our conclusions). A comparison with the full original scan reveals that none of these changes altered the findings of the original scans.

Figure 2f Raw Data full scans: IP anti-PfKelch13, Western blot (WB) with indicated antibodies



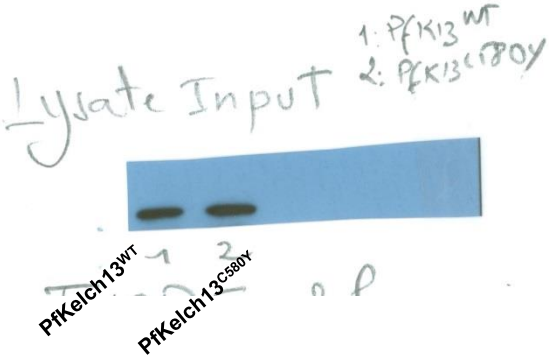
WB to detect HA-tagged protein (top panel)
3D7 parasites expressing PfKelch13^{WT}-HA or 3D7 PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PfKelch13 (IP) or mock/control (Ctrl/C) incubation followed by WB to detect HA-tagged PfKelch13 (arrow)

Molecular weight standards (in kDa) are as indicated.



WB to detect PfPI3K protein (middle panel) 3D7 parasites expressing PfKelch13^{WT}-HA or PfKelch13^{C580Y}-HA, were subjected to immunoprecipitation using anti-PfKelch13 (IP) or mock/control (Ctrl/C) incubation followed by WB to detect PfPI3K
Molecular weight standards (in kDa) are as indicated.

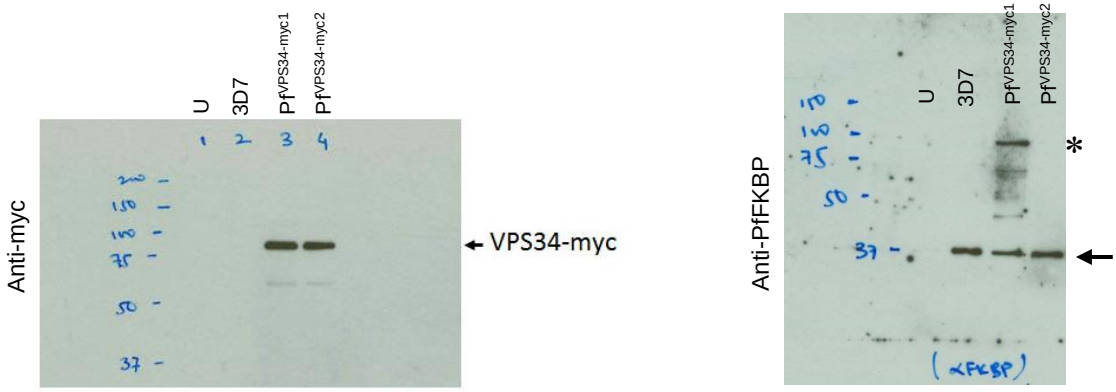
Figure 2f raw data lysate input



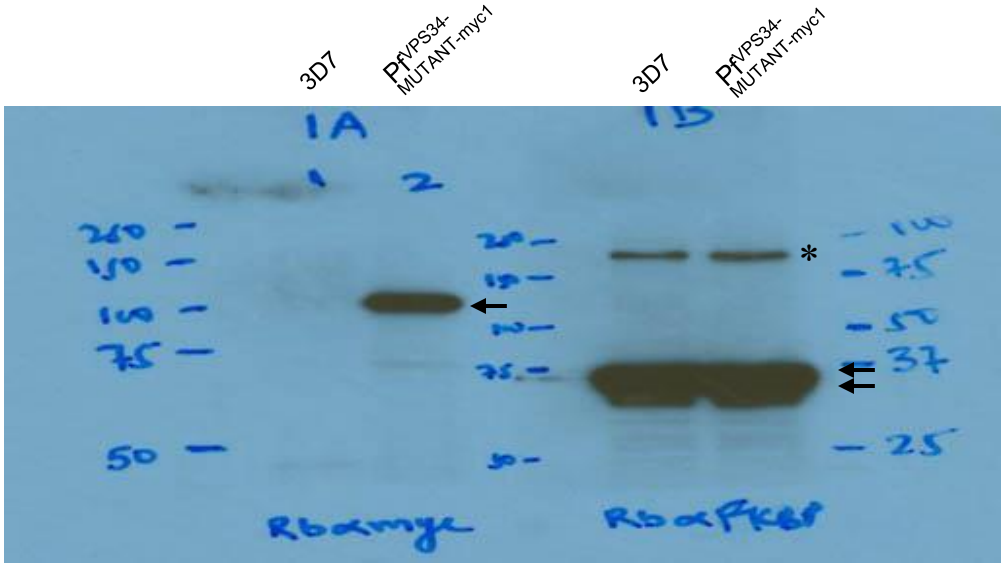
WB to detect PfFKBP , bottom right panel. 3D7 parasites expressing PfKelch13^{WT}-HA or PfKelch13^{C580Y}-HA used in panel 2f probed for PfFKBP, which serves as a loading control for immunoprecipitations shown in Figure 2f.

Summary for Figure 2f. Other than the loading control for input lysate, running gel regions (as indicated by molecular weight markers) are shown for relevant lanes. Images were converted to gray scale, sized to accommodate all of the data in Figure 2 as one power point slide and for clear visualization. Re-sizing slightly altered thickness and intensity. Panels probed with anti-PfPI3K, the contrast (background) was slightly deepened to better visualize minor bands in the PfKelch13^{C580Y} mutant lanes (which biases against our conclusions). Comparison with original scan reveals that none of these changes altered the findings of original scans.

Figure 3b Raw Data full scans: Western blot (WB) detect transgenic expression of VPS34 and its mutant in *P. falciparum*

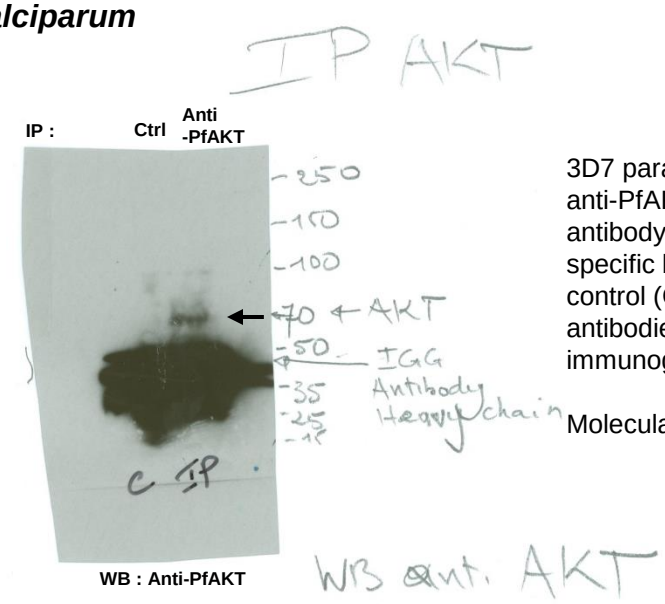


LHS (upper left panel in Figure 3b) WB showing the expression of $Pf^{VPS34\text{-}myc1}$ and $Pf^{VPS34\text{-}myc2}$ in transgenic 3D7 parasites but not in non-transfected 3D7 or uninfected red blood cells (U), using anti-myc antibodies. **RHS (lower left panel in Figure 3b)** Corresponding PfFKBP levels (arrow) in LHS panel. (Antibodies to PfFKBP show fortuitous cross reaction marked with asterisk * with myc tagged transgene $Pf^{VPS34\text{-}myc1}$). Molecular weight standards (in kDa) are indicated at the left.



LHS 1A (upper right panel in Figure 3b). WB detecting the expression of $Pf^{VPS34MUTANT\text{-}myc1}$ (arrow) in transgenic 3D7 parasites but not in non-transfected 3D7 , using anti-myc antibodies.
RHS 1B (lower right panel in Figure 3b). Blot to detect PfFKBP shows corresponding PfFKBP loading control data (double arrows). In both the lanes, anti-PfFKBP antibodies show low levels of cross-reaction marked with asterisk ∗.
Molecular weight standards (in kDa) are as indicated.

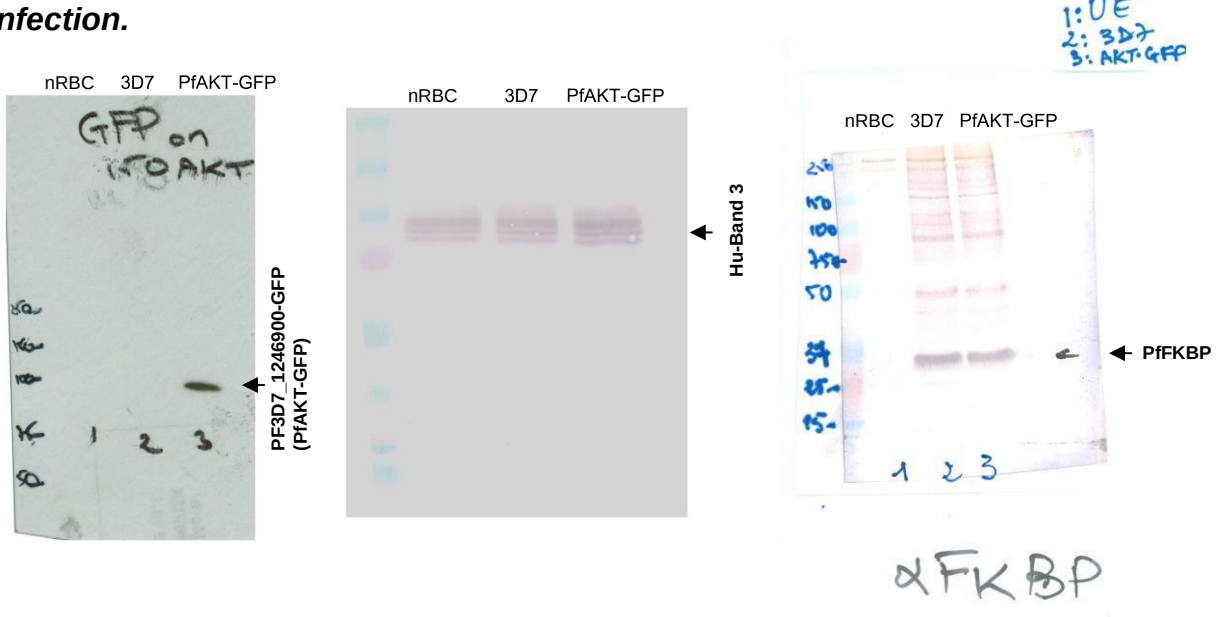
Figure 4b Raw Data full scans: Detection of immunopurified PfAKT from *P. falciparum*



3D7 parasites were subjected to immunoprecipitation using anti-PfAKT or mock/control incubation followed by WB with antibody to detect PfAKT **(upper panel Figure 4b)**. A specific band (arrow) detected by anti-PfAKT but not in control (Ctrl/C) incubation. Since the same species of antibodies were used in the IP and WB, intense immunoglobulin staining was detected at ~50-25 kDa.

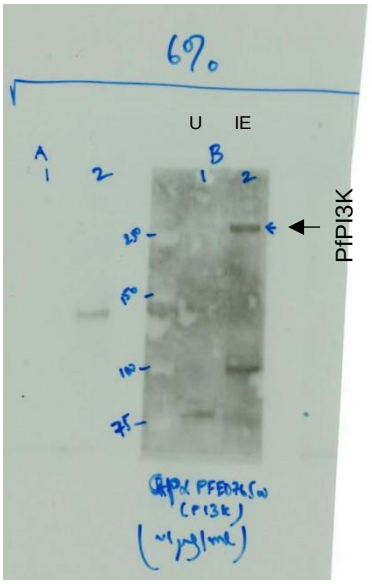
Molecular weight standards (in kDa) are as indicated.

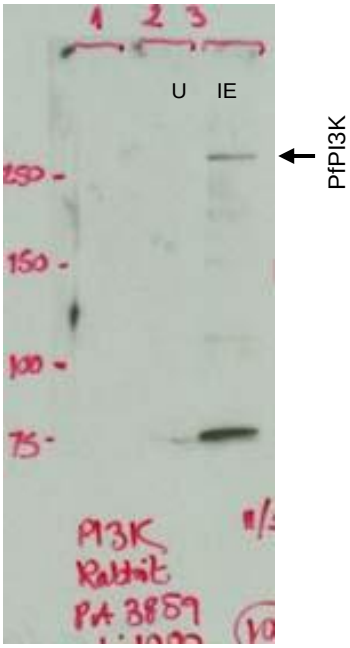
Figure 4c Raw Data full scans: Western blot (WB) detect transgenic expression of PfAKT-GFP and loading controls PfFKBP and human Band3 in *P. falciparum*-*infection.*



WB showing the expressing of PfAKT-GFP (left blot, arrow) in transgenic parasites but not in non-transfected parasites (3D7) or uninfected red blood cells (nRBC). Corresponding loading controls using antibodies to human Band 3 (middle blot, arrow) or the parasite protein PfFKBP (right blot, arrow) is as shown. Molecular weight standards (in kDa) are as indicated. Composite panel is shown in Figure 4c.

ED Figure 1f Antibodies to PfPI3K.





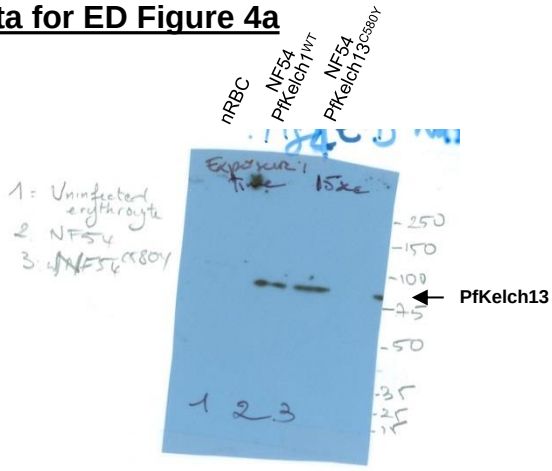
Left blot: WB detecting PfPI3K (arrow) in infected erythrocytes (IE) but not in uninfected red blood cells (U), using guinea pig anti-PfPI3K antibodies. **(lower panel in ED Figure 1f)**
Right blot: WB detecting PfPI3K (arrow) in infected erythrocytes (IE) but not in uninfected red blood cells (U). using rabbit anti-PfPI3K antibodies (**upper panel ED Figure 1f**).

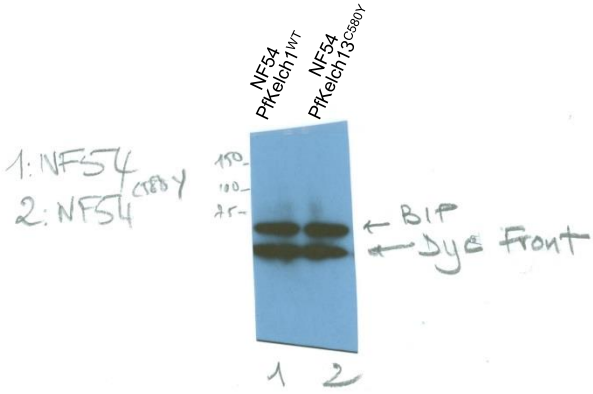
In both panels, anti-PI3K also detects lower molecular weight bands because of proteostatic control of PfPI3K .

Molecular weight standards (in kDa) are indicated at the left.

ED Figure 4

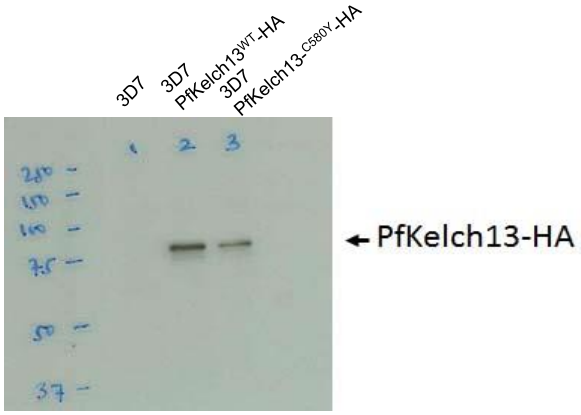
Raw data for ED Figure 4a

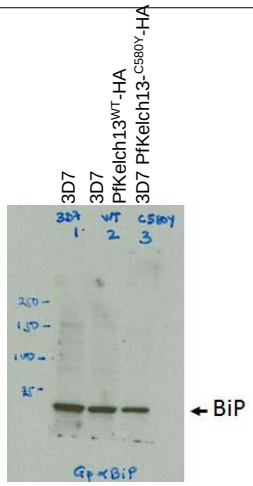




Left Blot: (Upper panel in ED Figure 4a) WB detecting the expression of PfKelch13 (arrow) in transgenic NF54 PfKelch13^{WT} and NF54 PfKelch13^{C580Y} parasites but not in uninfected erythrocytes (nRBC).
Right Blot: Lower panel ED Figure 4a) WB showing BiP as loading control in transgenic NF54 PfKelch13^{WT} and NF54 PfKelch13^{C580Y} parasites. The lower band in both lanes is due to the dye front.
Molecular weight standards (in kDa) are as indicated..

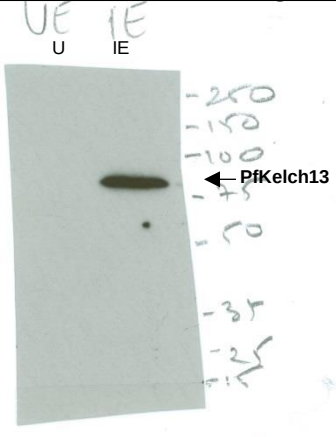
Raw Data for ED Figure 4b





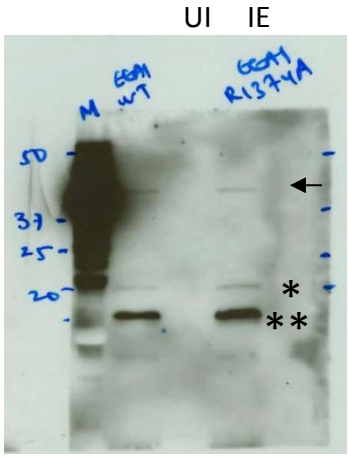
Left Blot: **(upper panel ED Figure 4b)** WB detecting the expression of PfKelch13^{WT}-HA and PfKelch13^{C580Y}-HA in transgenic parasites but not in non-transfected 3D7 control.
Right Blot: (lower panel ED Figure 4b) WB showing BiP as loading control in transgenic PfKelch13^{WT}-HA, PfKelch13^{C580Y}-HA, as well as non-transfected 3D7 control parasites.
Molecular weight standards (in kDa) are as indicated.

Raw data for ED Figure 4c



WB confirming the recognition of PfKelch13 (arrow; by custom-made anti-PfKelch13 antibodies) in infected erythrocytes (IE) but not in uninfected red blood cells (UE). Molecular weight standards (in kDa) are as indicated.

ED Figure 5

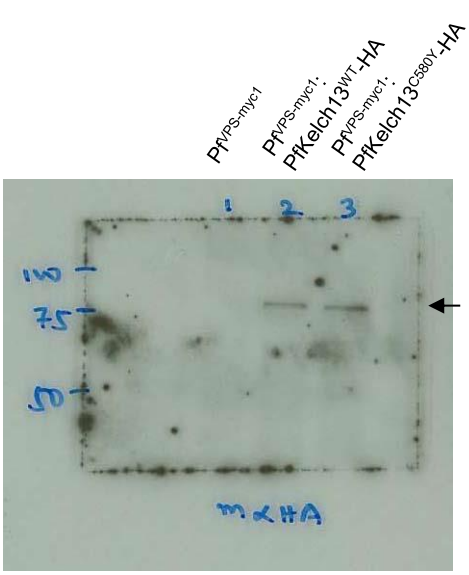


Raw data for ED Figure 5e

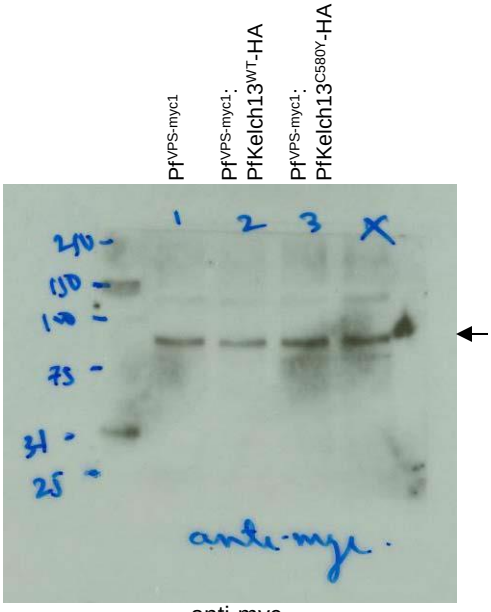
WB confirming the expression of SS-EEA1^{R1374A}-mCherry arrow) in transgenic infected erythrocytes (IE) but not uninfected erythrocytes (UI) (indicated in the blot). Molecular weight standards (in kDa) are as shown.

*, ** lower molecular weight degradation products (20-kDa and below) are commonly seen when samples are frozen prior to gel electrophoresis; degradation products are not fluorescent. Note, in ED Figure 5e, the blot background was darkened to confirm that there were no additional bands in the UI lane.

Raw data for ED Figure 5f



anti-HA



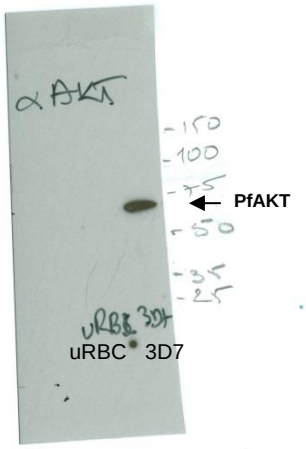
anti-myc

Left Blot: upper panel ED Figure 5f . WB (using antibodies to HA) detecting the expression of HA-tagged (arrow) PfKelch13^{WT}-HA and PfKelch13^{C580Y}-HA when expressed in Pf$^{VPS34-myc1}$ but not in the parental Pf$^{VPS34-myc1}$ parasite itself.

Right Blot: lower panel ED Figure 5f. WB of the same samples but using antibodies to myc detected myc-tagged (arrow) Pf$^{VPS34-myc1}$, in the single parental Pf$^{VPS34-myc1}$ parasite and subsequent double transfectants that also express transgenic PfKelch13^{WT}-HA and PfKelch13^{C580Y}-HA.

Molecular weight standards (in kDa) are as indicated.

ED Figure 6



Raw data for ED Figure 6a

WB confirming the recognition of PfAKT (arrow; by custom-made antibodies raised to the parasite AKT) in 3D7 infected erythrocytes but not in uninfected red blood cells (uRBC).

Molecular weight standards (in kDa) are as indicated.