Supplemental Materials

Molecular Biology of the Cell

Umberger et al.

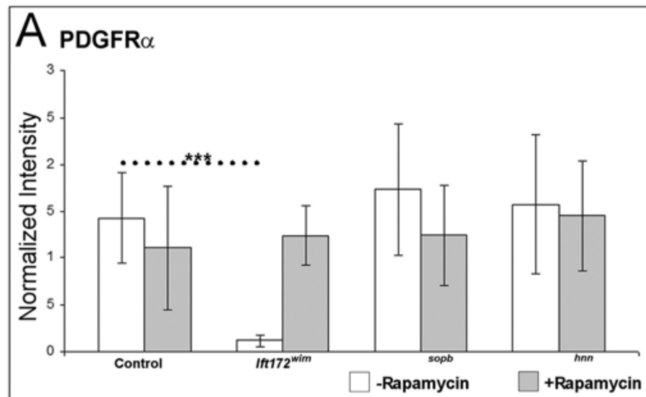
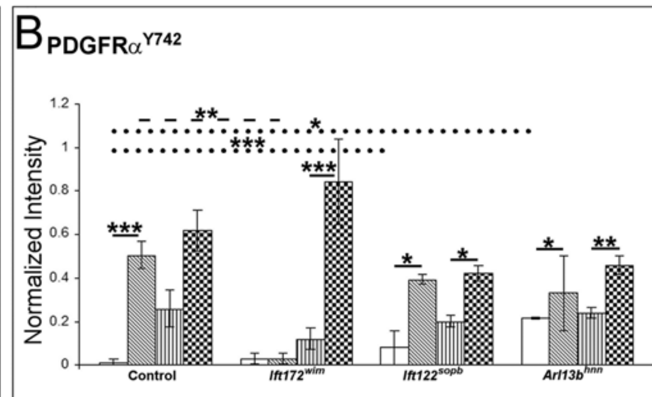
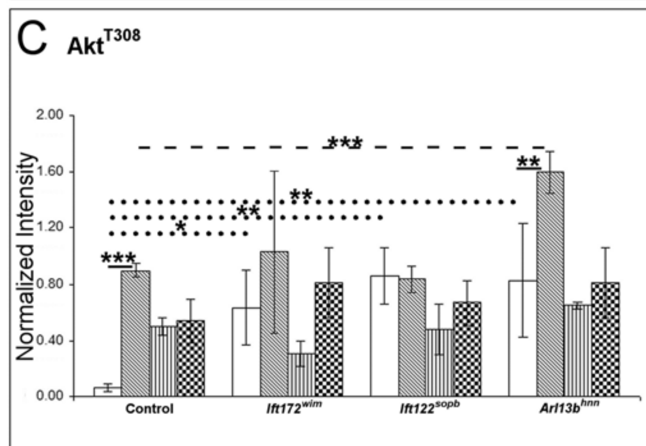
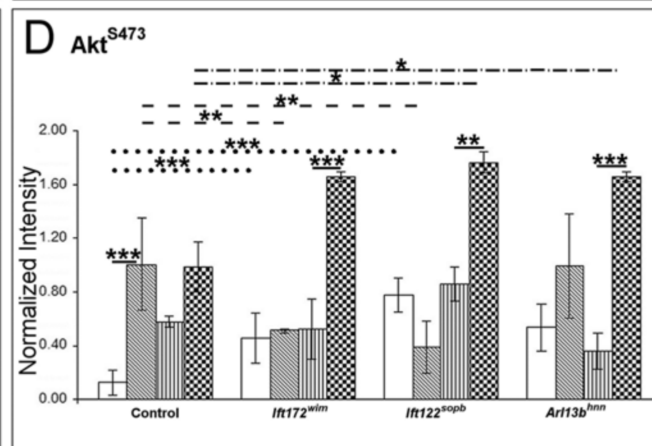
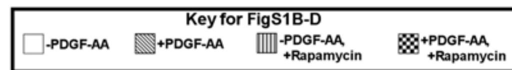
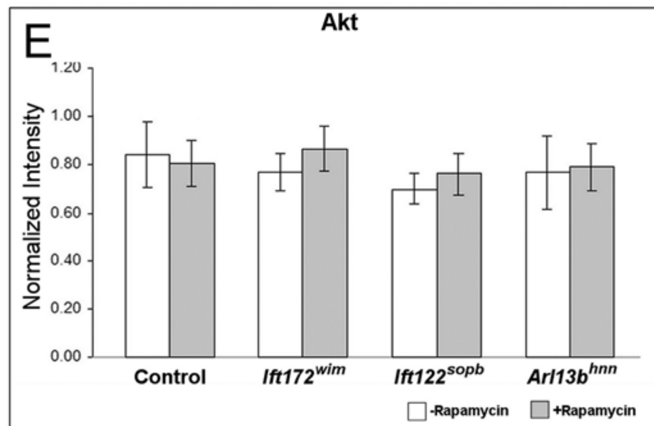
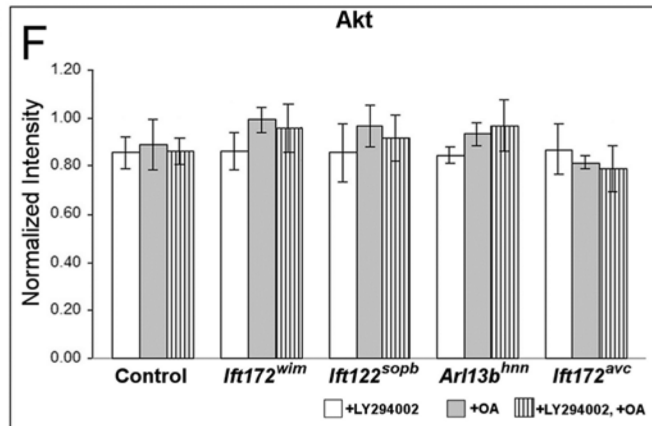
Figure S1: Quantification of PDGFRα, P-PDGFRα^{Y742}, P-Akt^{T308}, P-Akt^{S473}, and Akt in control and cilia transport mutant MEFs with or without PDGF-AA, with or without rapamycin, or with either LY294002 or OA, or LY294002 and OA. Annotations for statistically significant changes are indicated. Non-significant changes are not indicated, but statistics were performed. Statistical analyses are included in Supplementary Table S1 for S1B-D. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001.
A: Average densitometry values of PDGFRα, with and without rapamycin, normalized to a loading control (n=3).
B: Average densitometry values of P-PDGFRα^{Y742} normalized to a loading control (n=3).
C: Average densitometry values of P-Akt^{T308} normalized to a loading control (n=3).
D: Average densitometry values of P-Akt^{S473} normalized to a loading control (n=3).
E: Average densitometry values of Akt normalized to a loading control (n=3).
F: Average densitometry values of Akt normalized to a loading control (n=3).

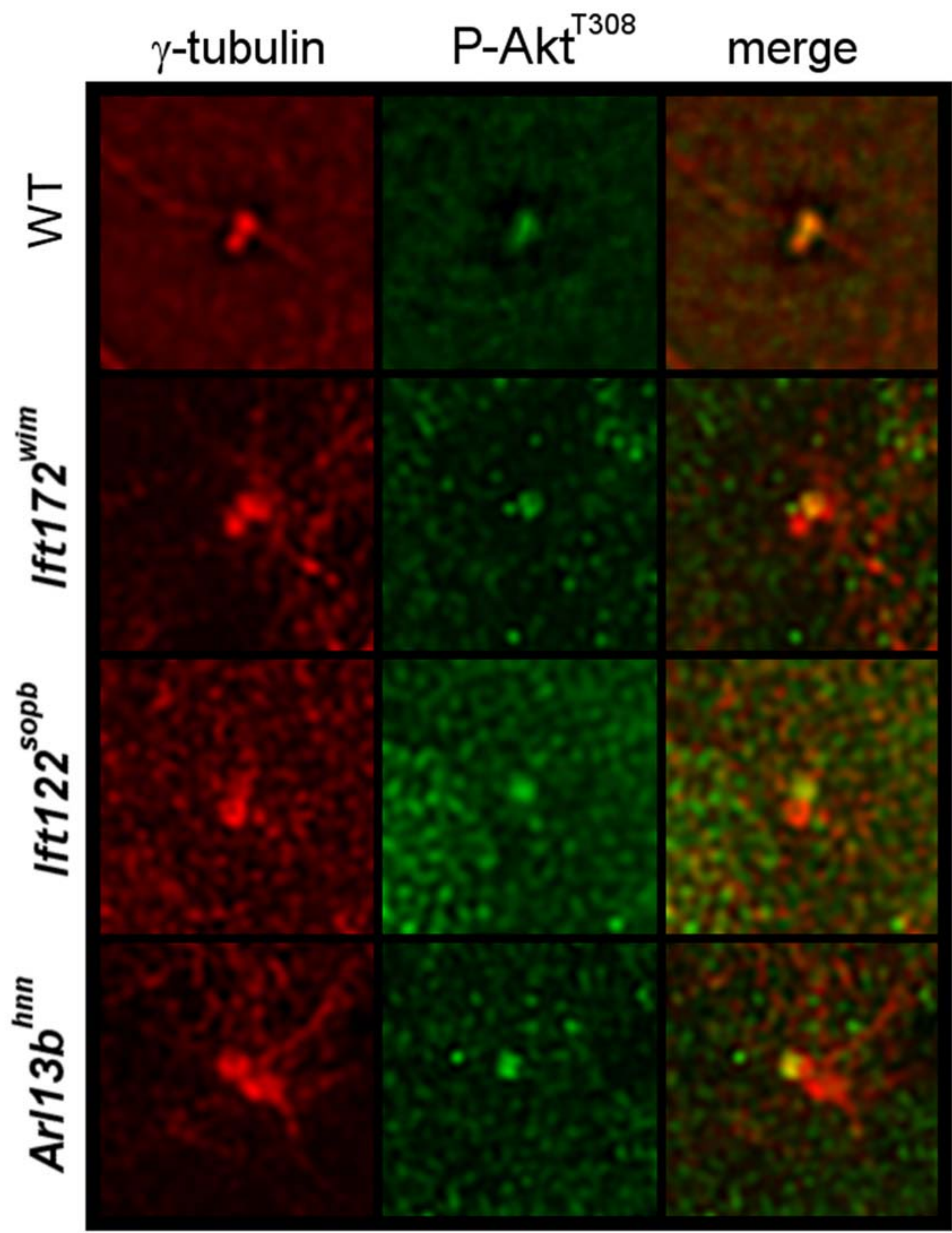
Figure S2: P-Akt^{T308} localization in control and cilia transport mutant MEFs. Serum starved control and cilia transport mutant MEFs were immunolabeled for P-Akt^{T308} (green) and for the basal body using γ-tubulin (red).

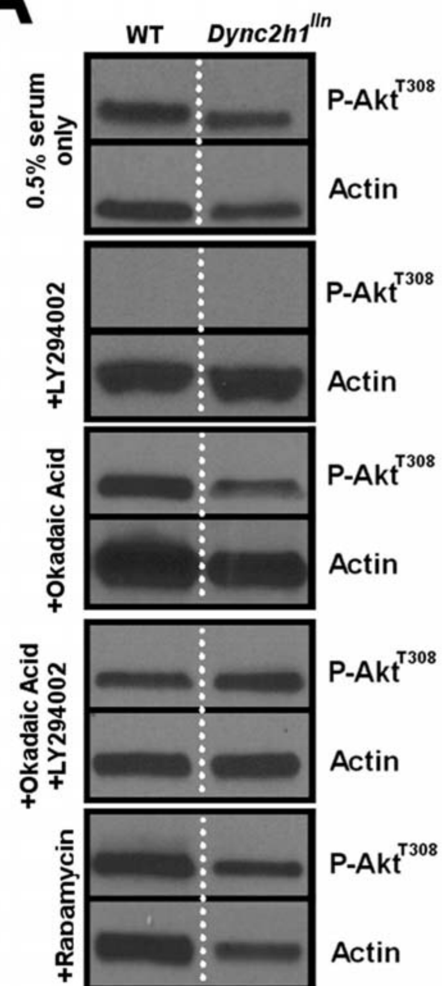
Figure S3: Response of control and *Dync2h1^{lln}* MEFs grown in 0.5% serum to multiple treatments
A: Control and *Dync2h1^{lln}* MEFs grown in 0.5% serum were lysed and processed for Western blot analysis of P-Akt^{T308} under the indicated conditions (no serum, +LY294002, +okadaic acid, +okadaic acid +LY294002, and +rapamycin). Lanes shown are re-arranged from a single gel.
B: Control and *Dync2h1^{lln}* MEFs grown in 0.5% serum were immunolabeled for the catalytic subunit of PP2A, PP2Ac, and for the basal body using γ-tubulin
C: Control and *Dync2h1^{lln}* MEFs grown in 0.5% serum were lysed and processed for Western blot analysis of phosphoryed mTOR (P-mTOR^{S2448}), p70 S6K (P-p70 S6K^{T389}), and S6 (S6$^{S235/236}$). Lanes shown are re-arranged from a single gel.
D: Comparison of PDGFRα, P-PDGFRα^{Y742}, P-Akt^{T308}, and P-Akt^{S473} in Control and *Dync2h1^{lln}* MEFs grown in 0.5% serum with or without stimulation with PDGF-AA ligand. Lanes shown are re-arranged from a single gel.

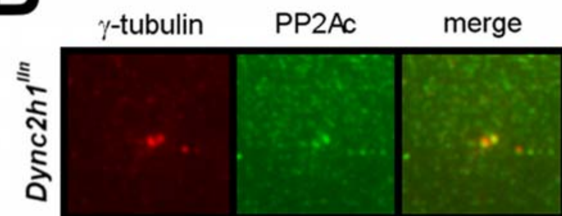
Figure S4: Absence of cilia in serum-starve *Ift172^{wim}* MEFs without and with rapamycin treatment and inhibition of mTORC1 signaling by rapamycin treatment.
A: Serum starved control MEFs were immunolabeled for PDGFRα (green) and for cilia using Arl13b (red).
B: Serum starved *Ift172^{wim}* MEFs were immunolabeled for PDGFRα (green) and for the basal body using γ-tubulin (red).
C: Serum starved and rapamycin treated *Ift172^{wim}* MEFs were immunolabeled for PDGFRα (green) and for cilia using Arl13b (red).
D: Rapamycin treatment inhibits phosphorylation of p70 S6K^{T389} in control and cilia transport mutant MEFs, with or without PDGF-AA.

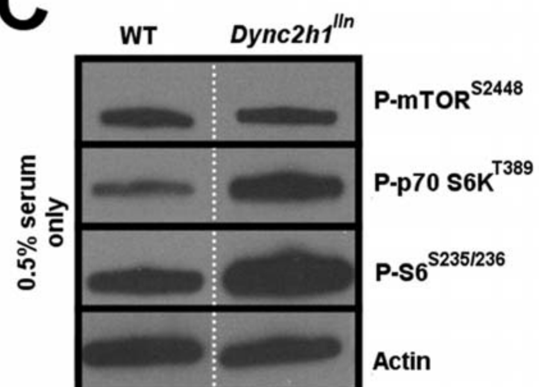
Table 1: Statistical analysis of Western blot band intensities were carried out using Microsoft Excel. The significance values (compared to respective controls) were calculated using a two-tailed unpaired *t* test with a 95% confidence interval.
-P: No PDGF-AA. +P: With PDGF-AA. +R: With rapamycin.

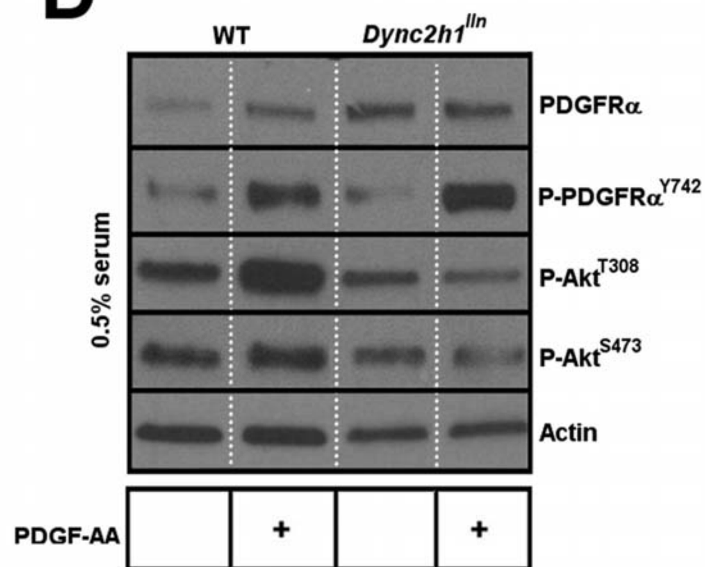

A PDGFRα
Normalized Intensity
Control
Ift172wim
sopb
hnn
-Rapamycin
+Rapamycin

B PDGFRαY742
Normalized Intensity
Control
Ift172wim
Ift122sopb
Arl13bhnn

C AktT308
Normalized Intensity
Control
Ift172wim
Ift122sopb
Arl13bhnn

D AktS473
Normalized Intensity
Control
Ift172wim
Ift122sopb
Arl13bhnn

Key for FigS1B-D
-PDGF-AA
+PDGF-AA
-PDGF-AA, +Rapamycin
+PDGF-AA, +Rapamycin

E
Akt
Normalized Intensity
Control
Ift172wim
Ift122sopb
Arl13bhnn
-Rapamycin
+Rapamycin

F
Akt
Normalized Intensity
Control
Ift172wim
Ift122sopb
Arl13bhnn
Ift172avc
+LY294002
+OA
+LY294002, +OA

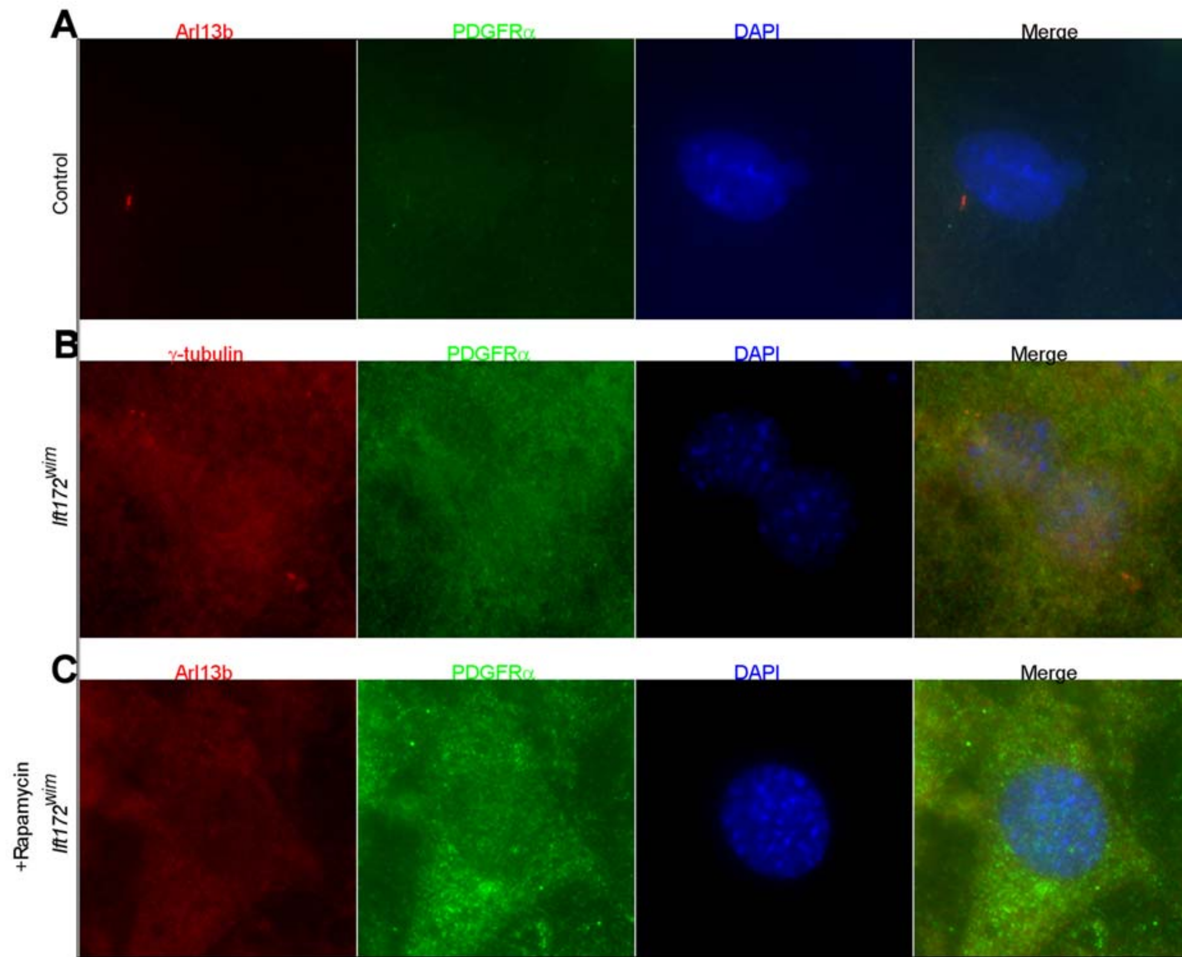

γ-tubulin
P-Akt T308
merge
WT
Ift172 wim
Ift122 sopb
Arl13b hnn


A
WT
Dync2h1lln
0.5% serum only
P-AktT308
Actin
+LY294002
P-AktT308
Actin
+Okadaic Acid
P-AktT308
Actin
+Okadaic Acid +LY294002
P-AktT308
Actin
+Rapamycin
P-AktT308
Actin


B
γ-tubulin
PP2Ac
merge
Dync2h1lln


C
WT
Dync2h1lln
0.5% serum only
P-mTORS2448
P-p70 S6KT389
P-S6S235/236
Actin


D
WT
Dync2h1lln
0.5% serum
PDGFRα
P-PDGFRαY742
P-AktT308
P-AktS473
Actin
PDGF-AA
+
+


A
Arl13b
PDGFRα
DAPI
Merge
Control
B
γ-tubulin
PDGFRα
DAPI
Merge
Ift172^wim
C
Arl13b
PDGFRα
DAPI
Merge
+Rapamycin
Ift172^wim

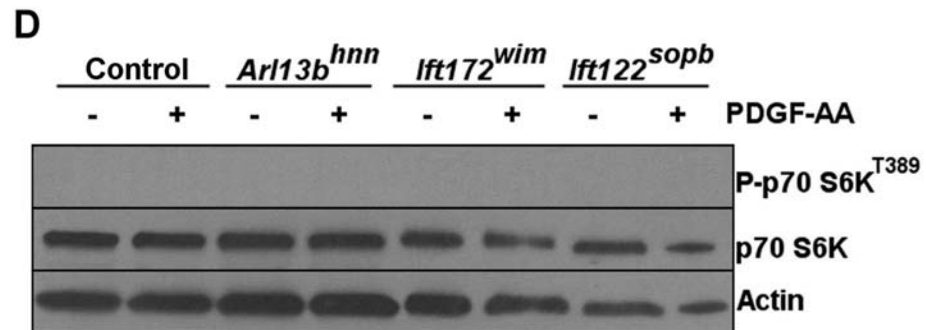

D
Control
Arl13b^hnn
Ift172^wim
Ift122^sopb
- + - + - + - +
PDGF-AA
P-p70 S6K^T389
p70 S6K
Actin

Table 1

P-values			
MEFs	Proteins		
	P-Akt^{T308}	**P-Akt^{S473}**	**P-PDGFRα^{Y742}**
Control -P vs Control +P	P<0.001	P<0.001	P<0.001
Control -P vs *wim* -P	P<0.05	P<0.001	P>0.05 (ns)
Control -P vs *sopb* -P	P<0.001	P<0.001	P<0.001
Control -P vs *hnn* -P	P<0.001	P>0.05 (ns)	P<0.05
Control +P vs *wim* +P	P>0.05 (ns)	P<0.01	P<0.01
Control +P vs *sopb* +P	P>0.05 (ns)	P<0.01	P>0.05 (ns)
Control +P vs *hnn* +P	P<0.001	P>0.05 (ns)	P>0.05 (ns)
	P-Akt^{T308}	**P-Akt^{S473}**	**P-PDGFRα^{Y742}**
Control +R vs Control +P+R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
Control +R vs *wim* +R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
Control +R vs *sopb* +R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
Control +R vs *hnn* +R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
Control +R+P vs *wim* +R+P	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
Control +R+P vs *sopb* +R+P	P>0.05 (ns)	P<0.05	P>0.05 (ns)
Control +R+P vs *hnn* +R+P	P>0.05 (ns)	P<0.05	P>0.05 (ns)
	P-Akt^{T308}	**P-Akt^{S473}**	**P-PDGFRα^{Y742}**
wim -P vs *wim* +P	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
wim +R vs *wim* +R+P	P>0.05 (ns)	P<0.001	P<0.001
wim -P vs *wim* +R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
sopb -P vs *sopb* +P	P>0.05 (ns)	P>0.05 (ns)	P<0.05
sopb +R vs *sopb* +R+P	P>0.05 (ns)	P<0.01	P<0.05
sopb -P vs *sopb* +R	P>0.05 (ns)	P>0.05 (ns)	P>0.05 (ns)
hnn -P vs *hnn* +P	P<0.01	P<0.001	P<0.05
hnn +R vs hnn +R+P	P>0.05 (ns)	P>0.05 (ns)	P<0.01
hnn -P vs *hnn* +R	P<0.05	P<0.05	P>0.05 (ns)

Table 1: Statistical analysis of Western blot band intensities were carried out using Microsoft Excel. The significance values (compared to respective controls) were calculated using a two-tailed unpaired *t*-test with a 95% confidence interval.
-P: No PDGF-AA. +P: With PDGF-AA. +R: With rapamycin.