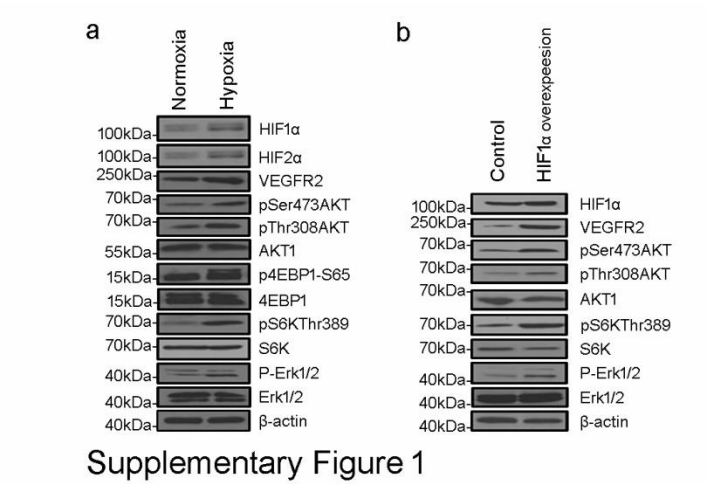
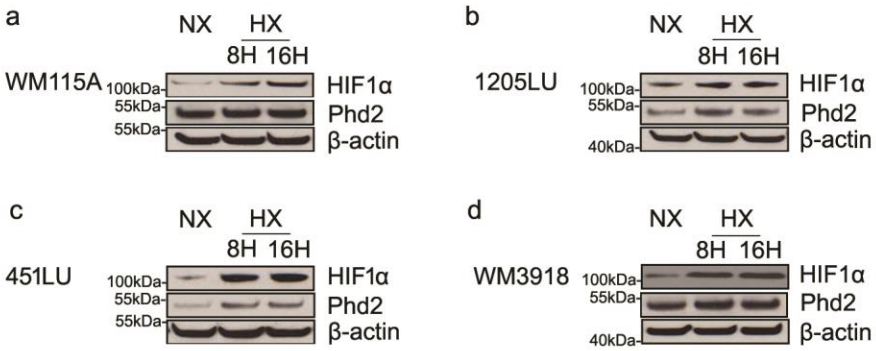
Liu, et al., Loss of *Phd2* Cooperates with *BRAF^{V600E}* to Drive Melanomagenesis
Supplementary Information



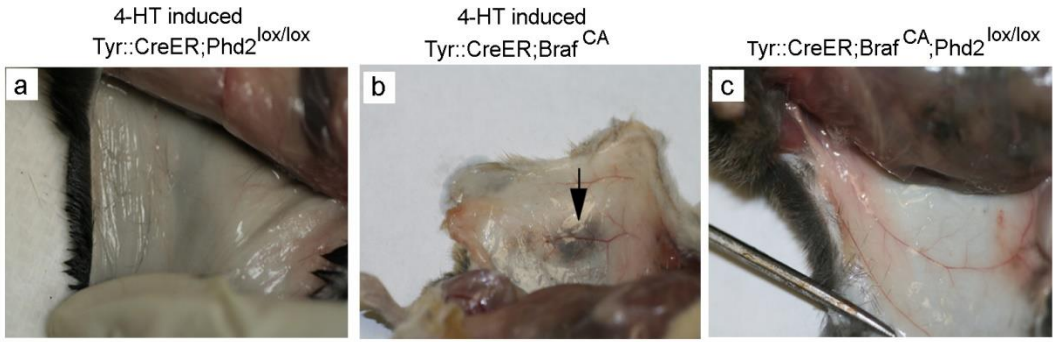
Supplementary Figure 1

Supplementary Figure 1. Hypoxia regulates Akt-mTOR pathway in *BRaf^{V600E};Phd2$^{-/-}$* melanoma cells. a.1% oxygen condition further increased HIF stabilization in *BRaf^{V600E}; Phd2$^{-/-}$* melanoma cells and activated Akt-mTOR pathway after the tumor cells were cultured under 1% hypoxia for 24 hours. **b.** Non-degradable *HIF-1α* was expressed in *BRaf^{V600E}; Phd2$^{-/-}$* melanoma cells. β-actin was used as a loading control. Results are representative of 3 independent experiments.



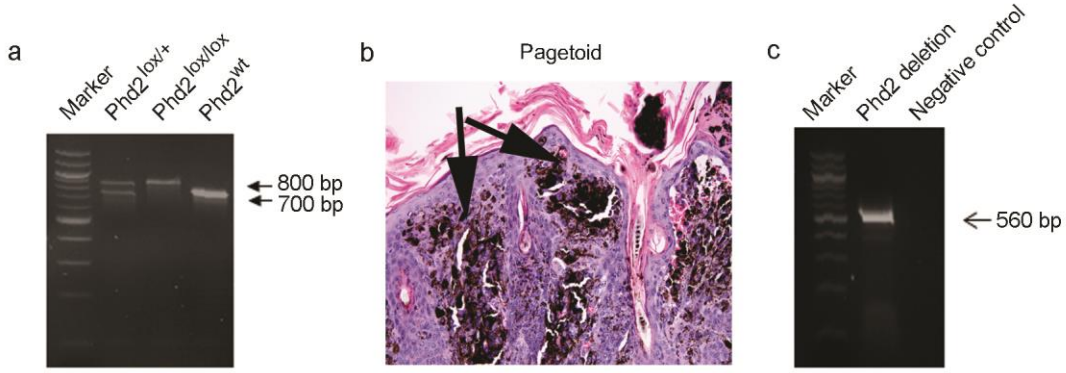
Supplementary Figure 2

Supplementary Figure 2. PHD2 expression under hypoxia in human melanoma cells. a-d. Western Blot assay for PHD2 expression. Human melanoma cells were cultured under hypoxia (1% oxygen) for 8 hours or 16 hours. PHD2 expression in response to hypoxia is cell line dependent. Human WM115A (**a**), 1205LU (**b**), 451LU (**c**) and WM3918 (**d**). *β-actin* was used as loading control. Results are representative of 3 independent experiments.



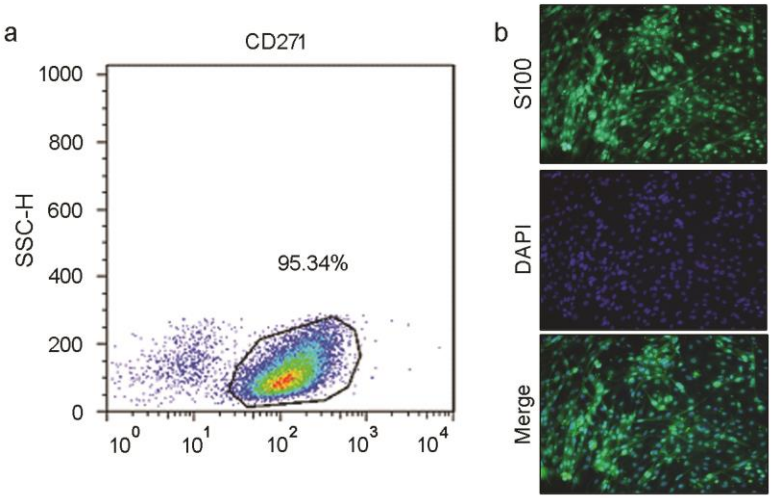
Supplementary Figure 3

Supplementary Figure 3. Gross skin morphology in different genetically engineered mouse models. Mice were followed for at least 18 months and then sacrificed. The skin was turned upside down and then photographed. 4-OHT induced *Tyr::CreER; Phd2$^{lox/lox}$* **(a),** 4-OHT induced *Tyr::CreER; BRaf^{CA}* **(b),** *Tyr::CreER; BRaf^{CA}; Phd2$^{lox/lox}$* mice without the 4-OHT induction **(c).** Arrow points to the pigmented lesion. Bars indicate 3 mm.

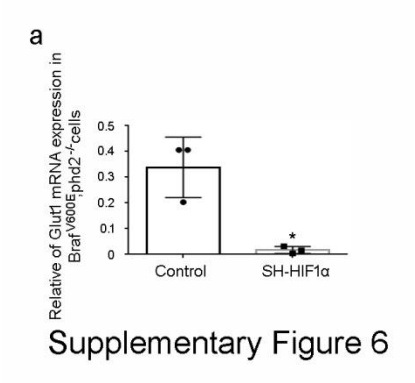


Supplementary Figure 4

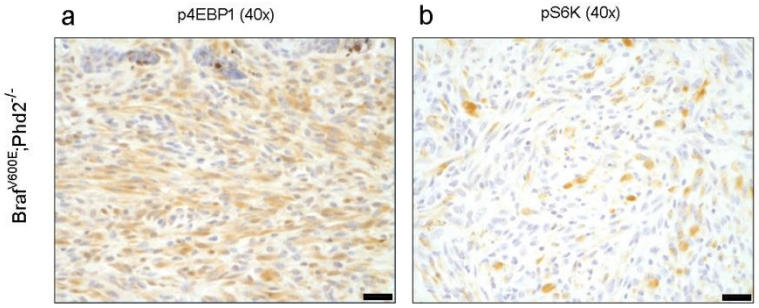
Supplementary Figure 4: **Genotyping of the *phd2* allele. a.** PCR of *phd2* allele in *Phd2*$^{+/+}$, and *Phd2*$^{lox/+}$, and *Phd2*$^{lox/lox}$ mice. **b.** Pagetoid proliferation of melanoma cells in the epidermis derived from the *Tyr::CreER*; *BRaf*^{V600E}; *Phd2*$^{-/-}$ mice. Arrows point to the pagetoid cells. Bar indicates 50 µm. **c.** PCR confirms *Phd2* deletion in melanomas from *Tyr::CreER*;*BRaf*^{V600E};*Phd2*$^{-/-}$ mice.



Supplementary Figure 5. Generation and characterization of *BRaf*^{V600E}; *Phd2*$^{-/-}$ melanoma cell line. Melanoma cell lines were derived from tumors developed in the *Tyr::CreER*; *BRaf*^{V600E}; *Phd2*$^{-/-}$ mice. **a.** Melanoma was excised from the *Tyr::CreER*; *BRaf*^{V600E}; *Phd2*$^{-/-}$ mice after 4-OHT induction. The tumor sample was dispersed into single cells and CD271+ cells were sorted out using FACS. **b.** The cell line was stained with the anti-S-100 antibody and all the tumor cells were positive for S-100. Bar indicates 50 µm.

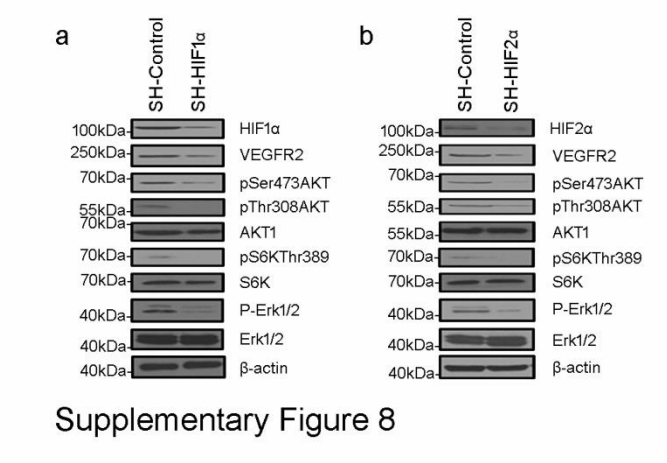


Supplementary Figure 6. *Glut1* gene expression is decreased after *HIF-1α* knockdown in *Braf^{V600E},Phd$^{-/-}$* melanoma cells. Quantitative RT-PCR assay for *Glut1* mRNA expression was performed in *Braf^{V600E},Phd$^{-/-}$* melanoma cells with or without *HIF1α* knockdown (n = 3 *replicate* ex*perime*nt*s; ** indicates $P < 0.01$). *β-actin* was used as control.

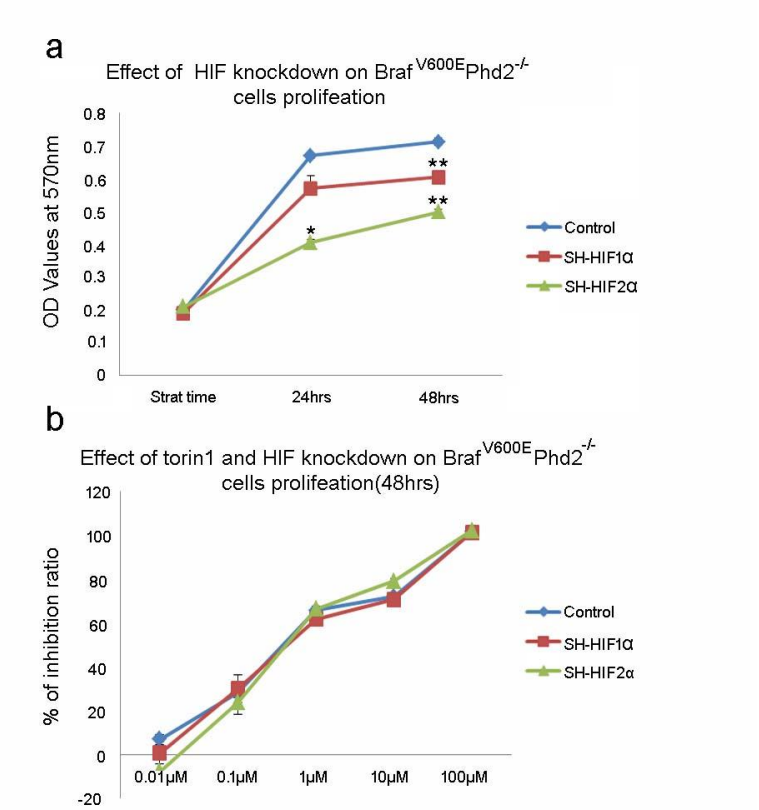


Supplementary Figure 7

Supplementary Figure 7. Immunohistochemistry of *Braf^{V600E},Phd$^{-/-}$* melanoma. Melanomas from the *Tyr::CreER*; *BRaf*^{V600E}; *Phd2*$^{-/-}$ mice were stained with anti-p4EBP1 (**a**) or anti-pS6K antibody (**b**). Bars indicates 20 µm.



Supplementary Figure 8. Effects of HIF knockdown on Akt-mTOR pathway. Inhibition of the Akt-mTOR pathway was observed in *BRaf*^{V600E}; *Phd2*$^{-/-}$ melanoma cells after these cells were transfected with SH-HIF-1α (**a**) or SH-HIF-2α (**b**). β-actin was used as a loading control. Results are representative of 3 independent experiments.



Supplementary Figure 9

Supplementary Figure 9. Effects of HIF knockdown and torin1 on *Braf^{V600E},Phd$^{-/-}$* melanoma cell proliferation. a. Cell proliferation was measured in *BRaf^{V600E}; Phd2$^{-/-}$* melanomas after *HIF-1α* or *HIF-2α* knockdown. **b**. Cell proliferation was measured in *BRaf^{V600E}; Phd2$^{-/-}$* melanomas after *HIF-1α* or *HIF-2α* knockdown in combination with different concentrations of torin1. Results are from 3 independent experiments.

Supplementary Figure 10

Fig. 1e

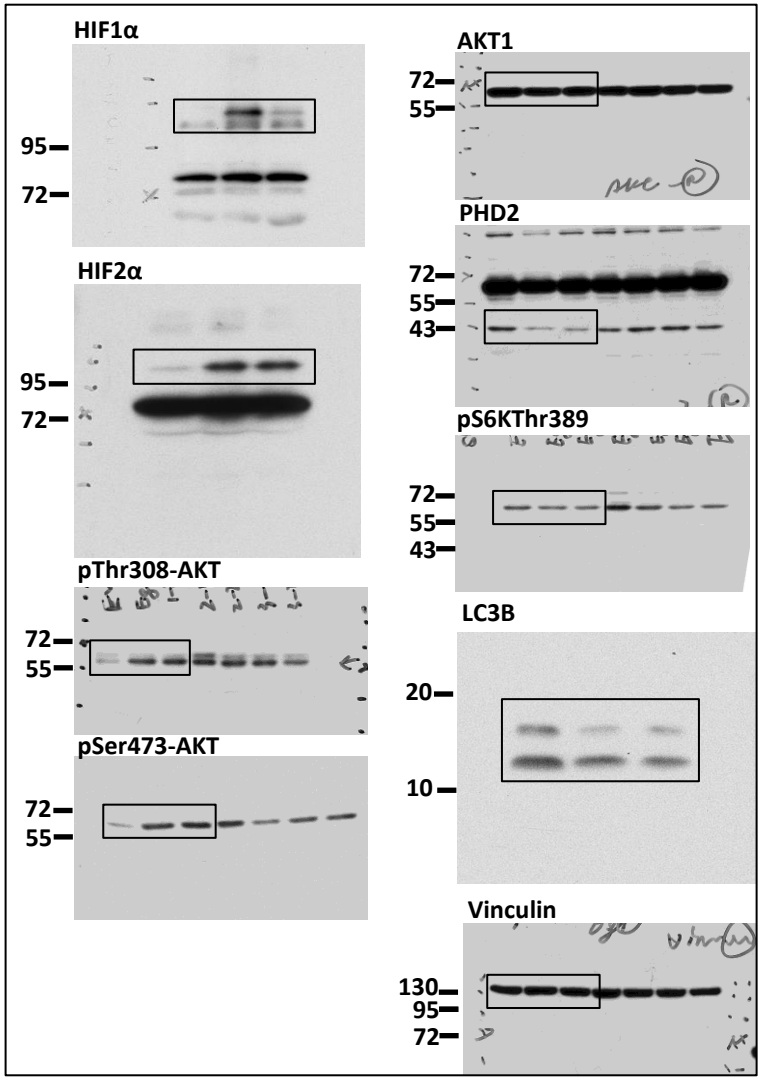
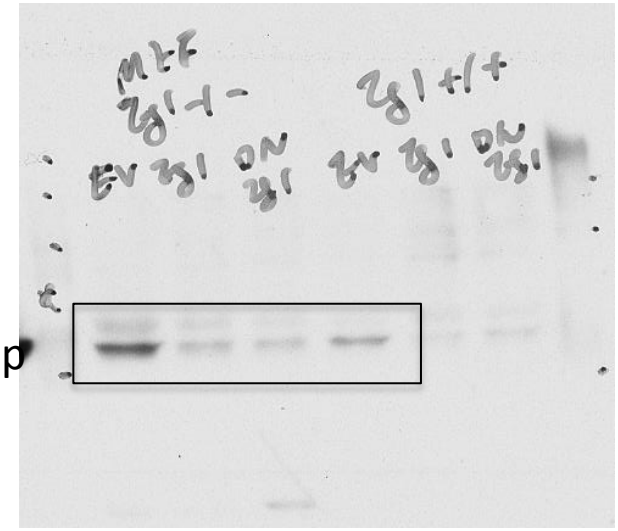


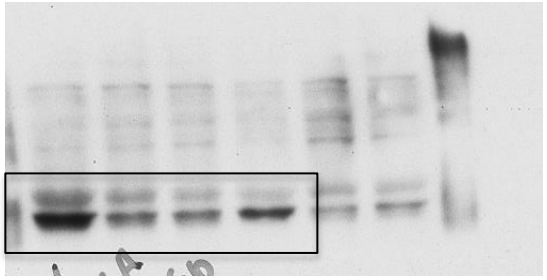
Fig 1f

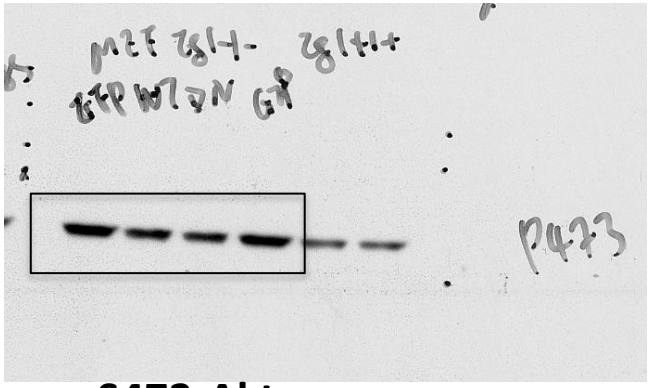
pT308-Akt

Shorter ep

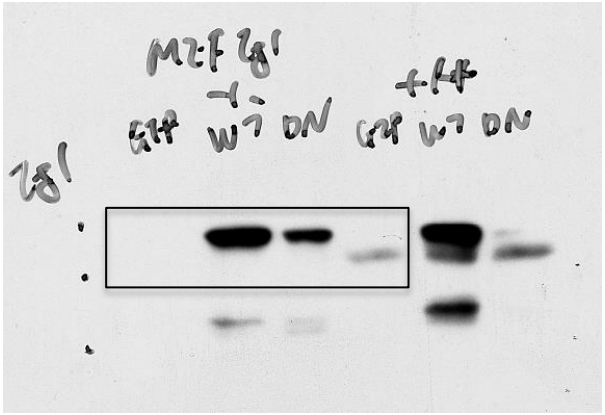


Longer ep





pS473-Akt



EglN1

Akt1

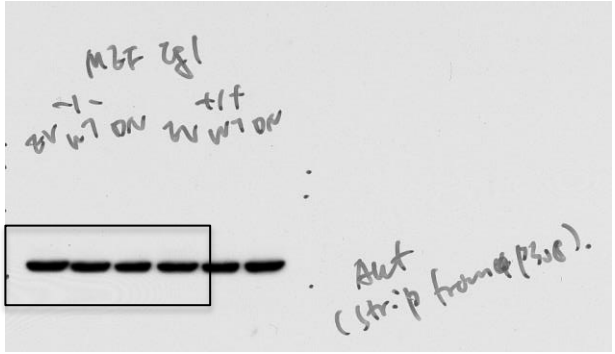
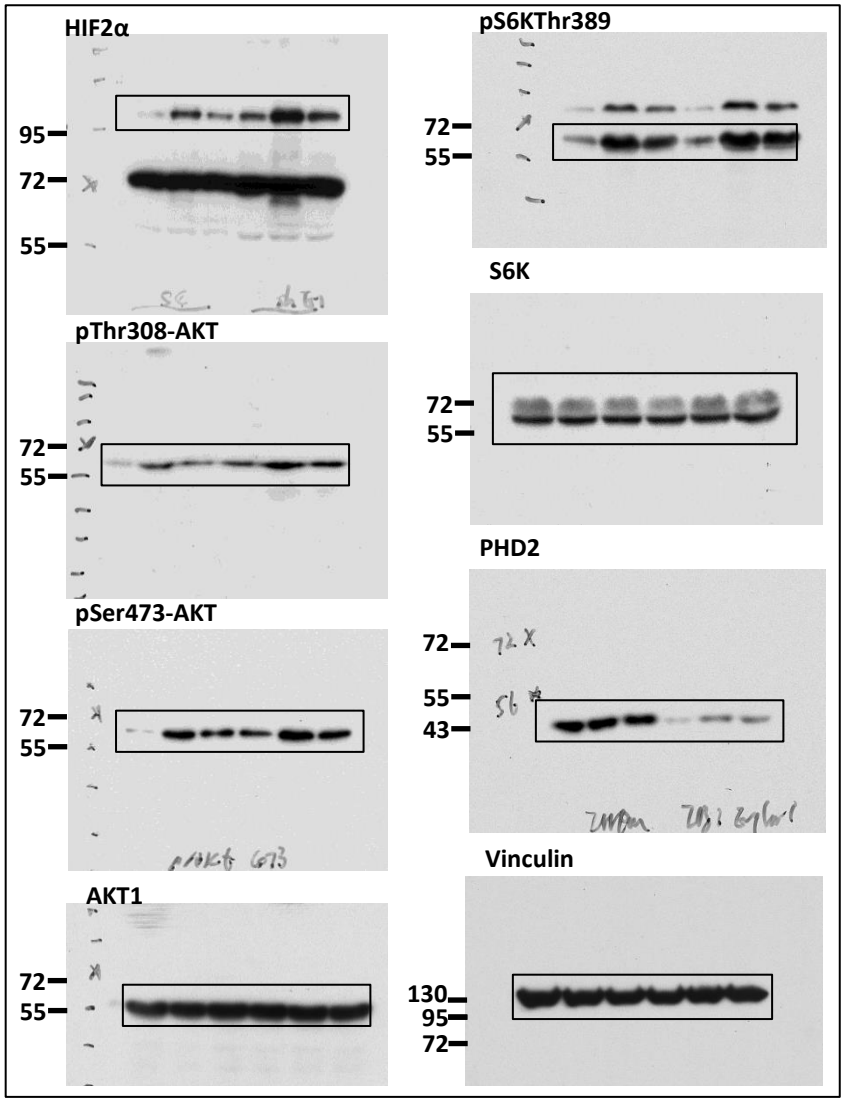


Fig. 1g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S6K
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pThr308-AKT
72
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PHD2
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43
pSer473-AKT
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AKT1
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55
Vinculin
130
95
72

Fig. 1h

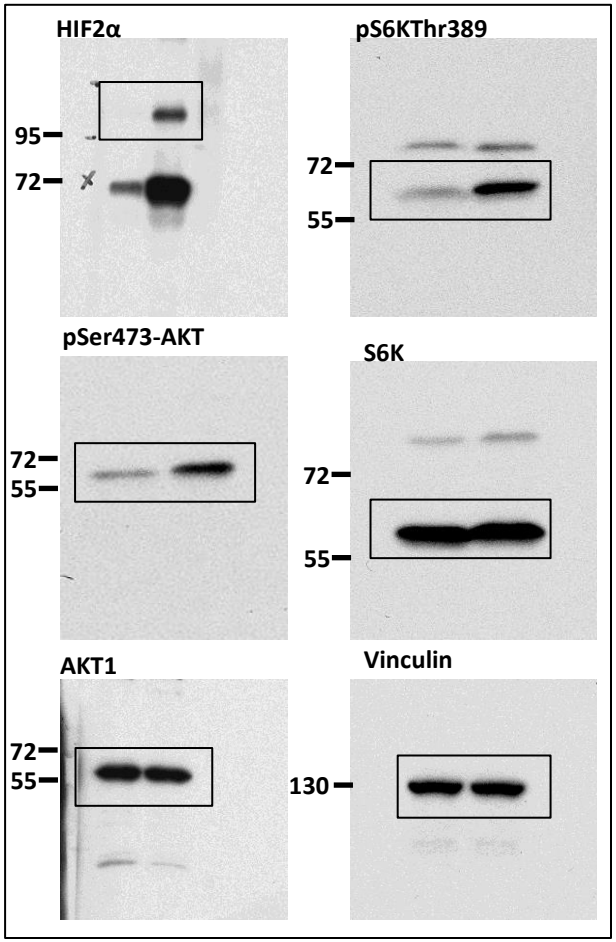


Fig. 1i

Flag-PHD2

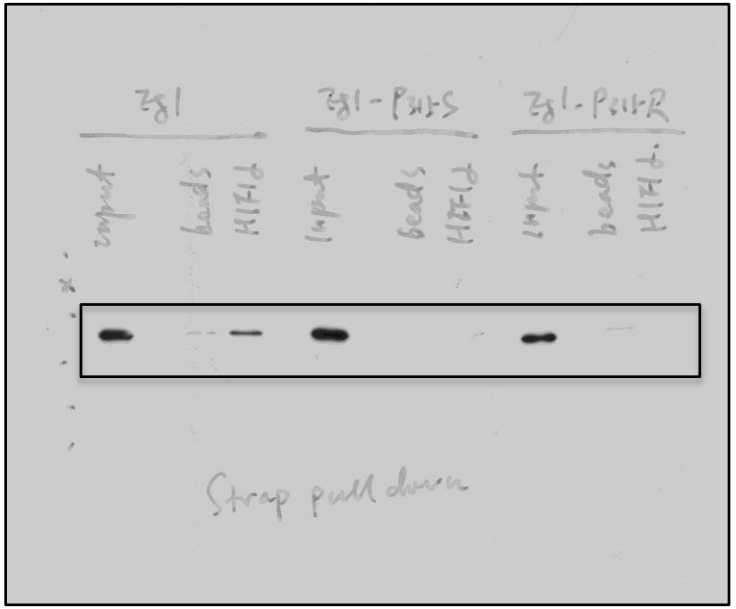
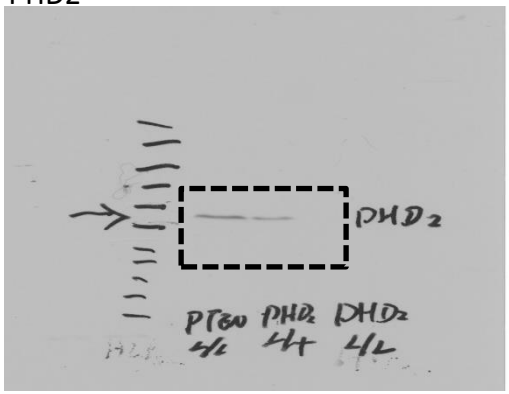


Fig. 5b

PHD2



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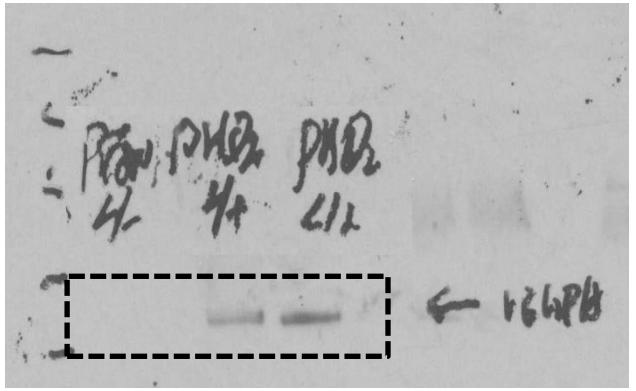

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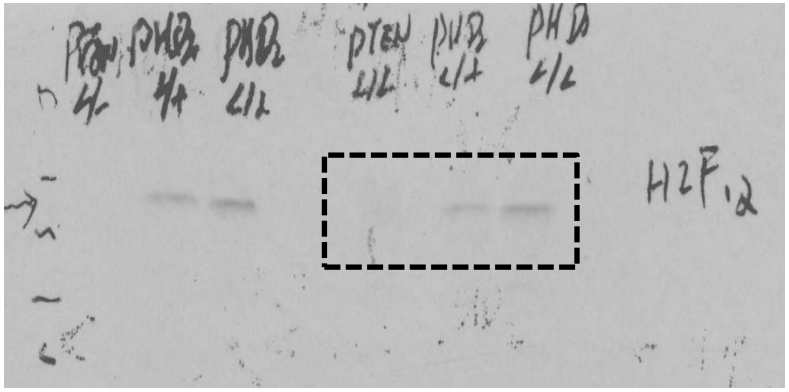
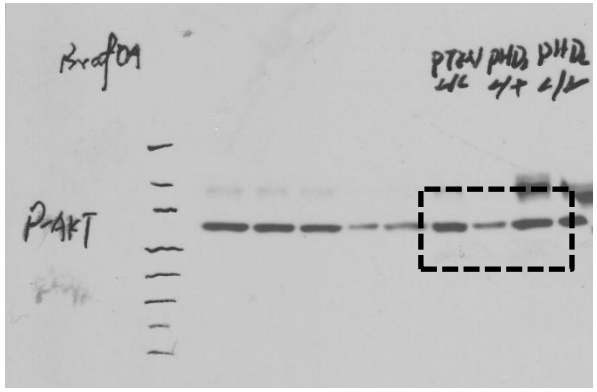


p-AKT



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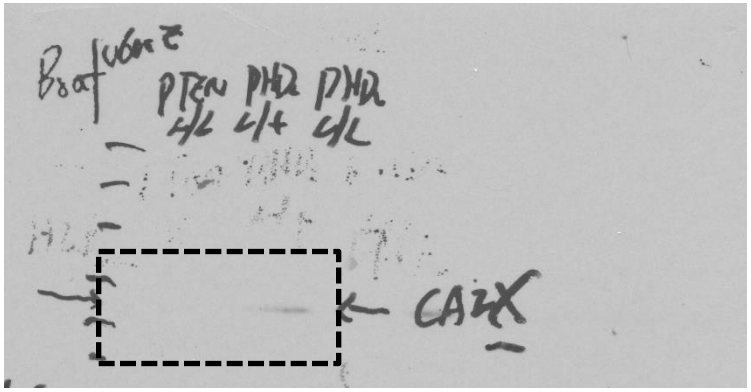

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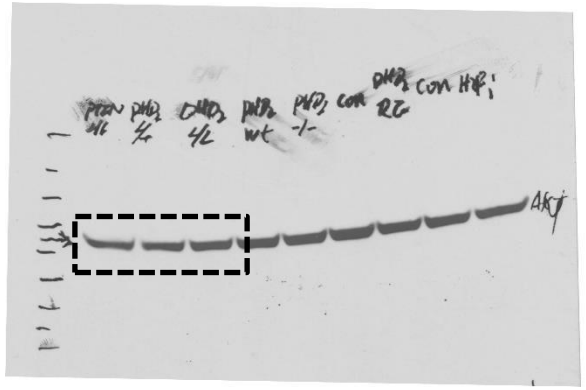


Fig. 5b

β-actin

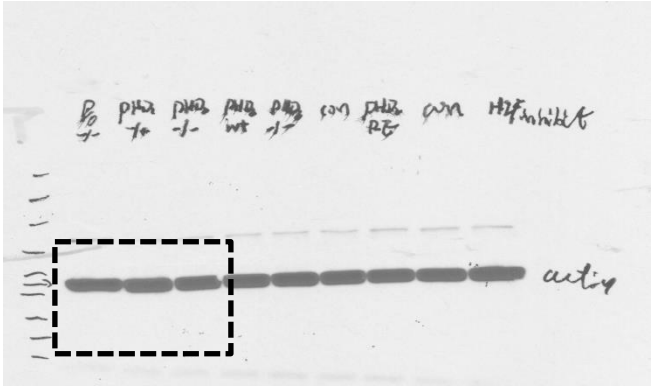
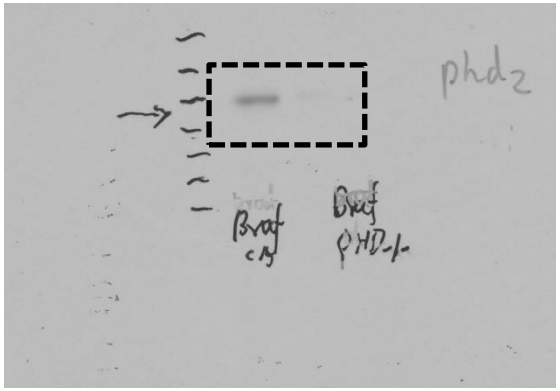


Fig. 6b

Phd2



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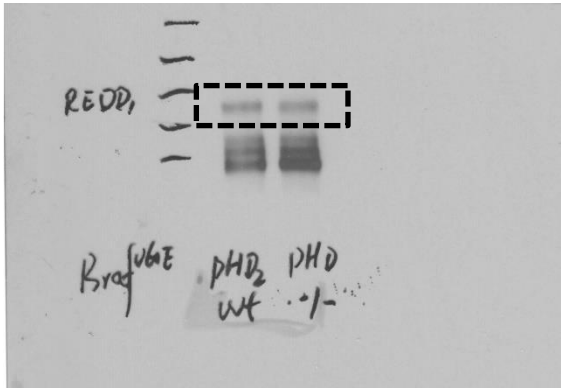

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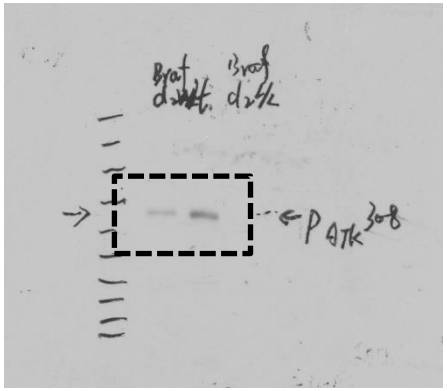

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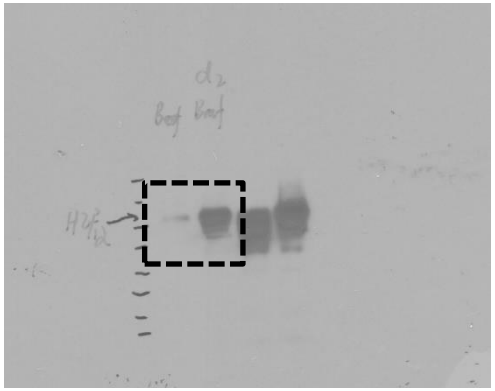

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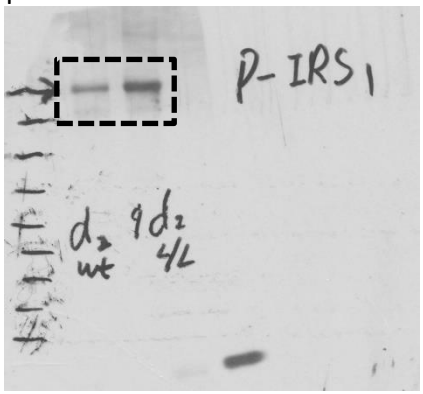

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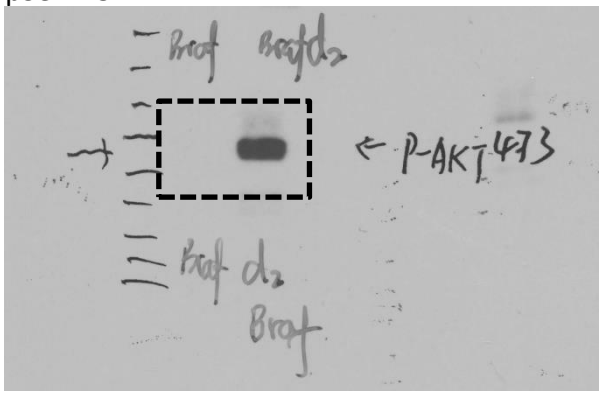

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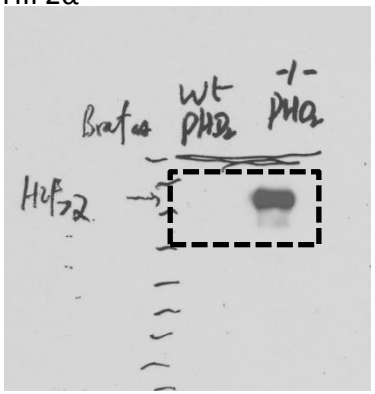

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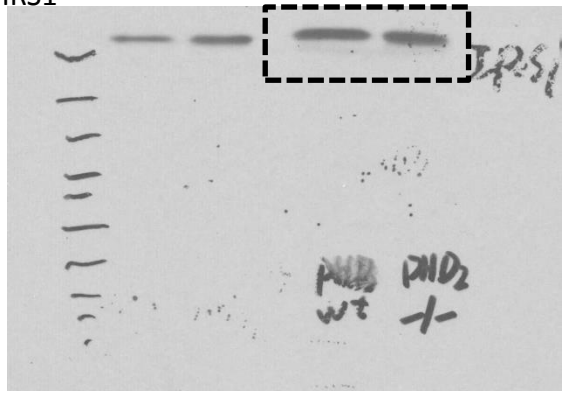

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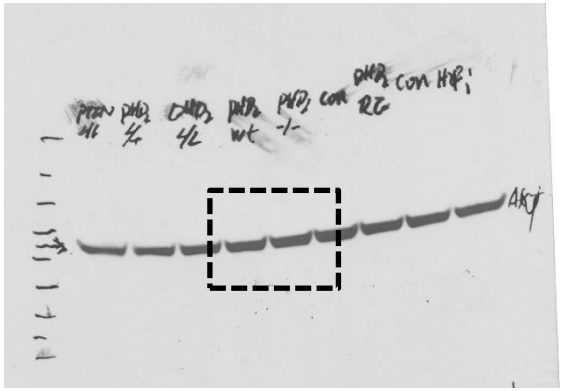

Fig. 6b

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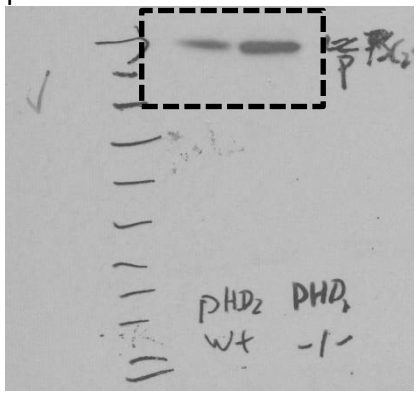

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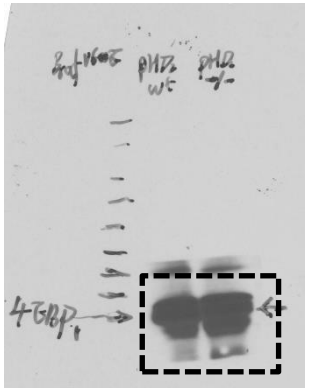
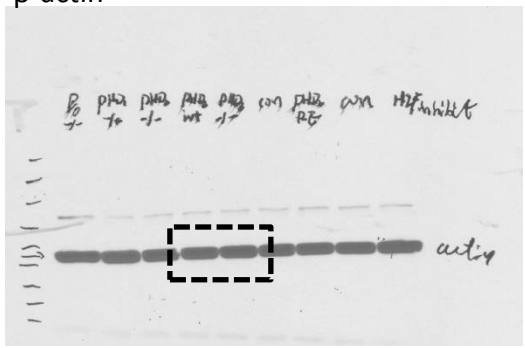


β-actin



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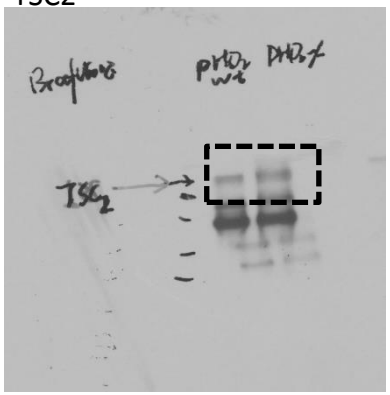

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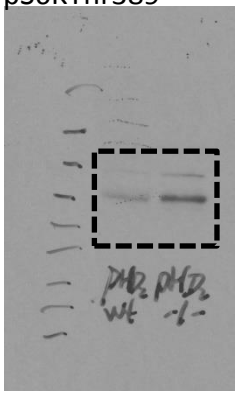

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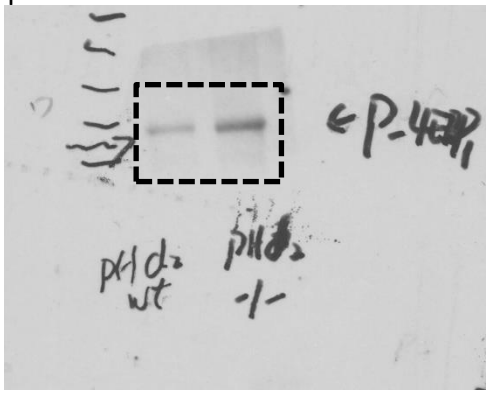

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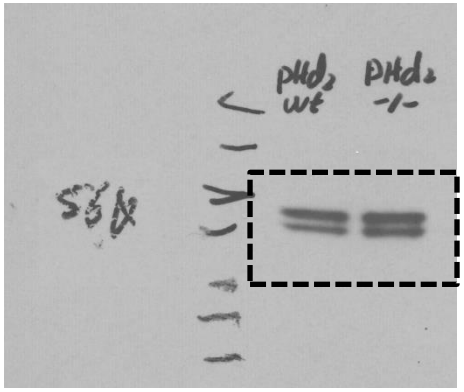
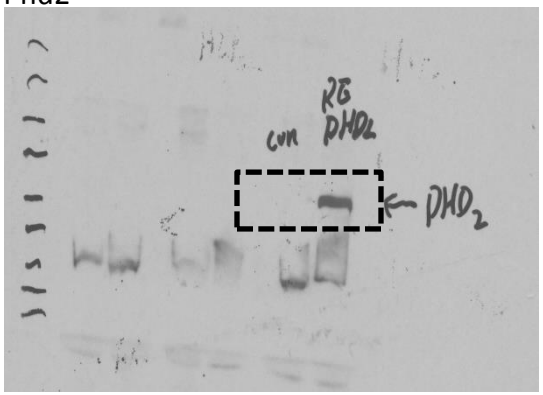


Fig. 6c

Phd2



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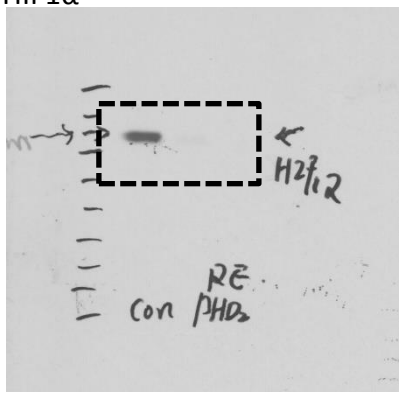

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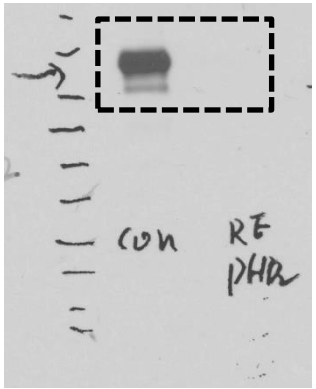
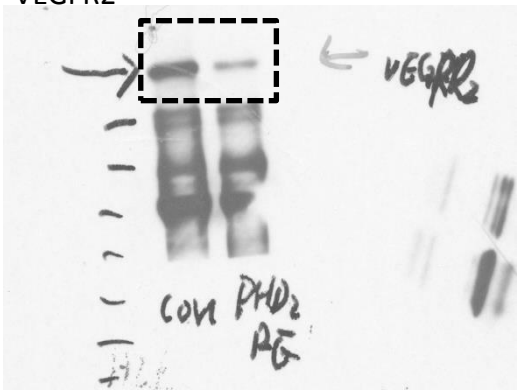


Fig. 6c

VEGFR2



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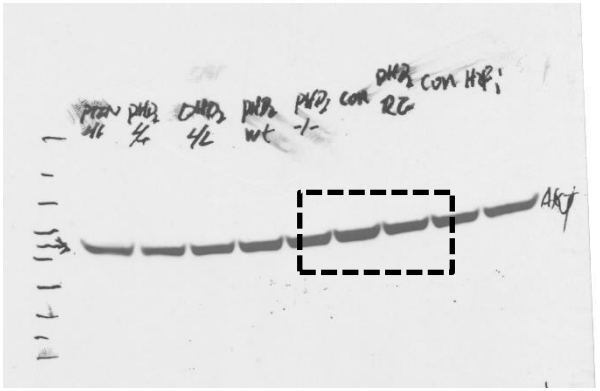

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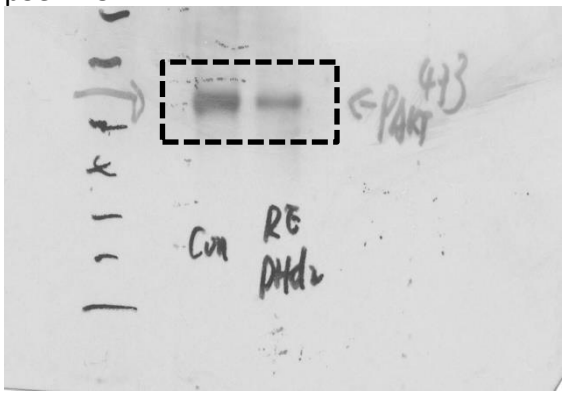

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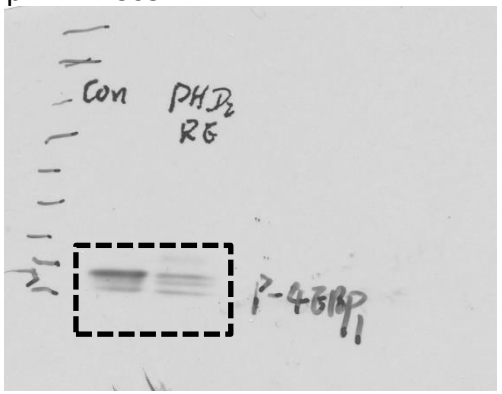

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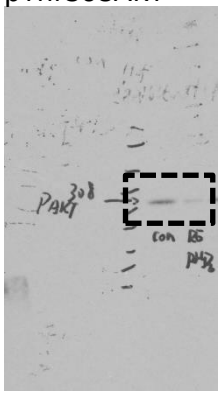


4EBP1 Control PHD2 Reexpression

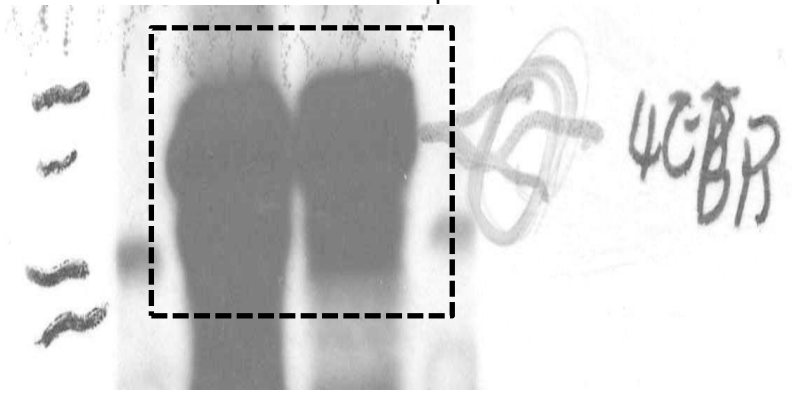
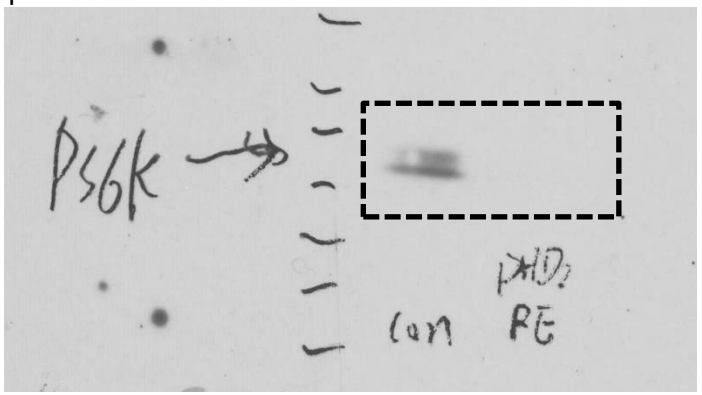
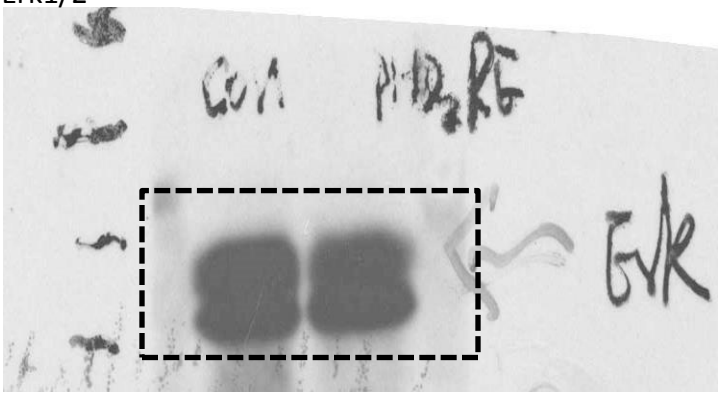


Fig. 6c

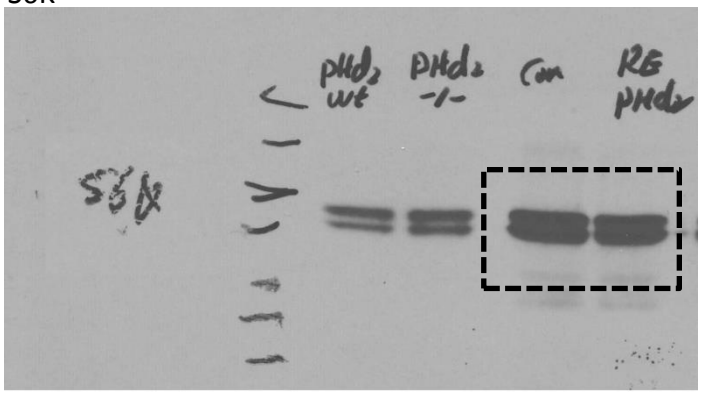
pS6KThr389



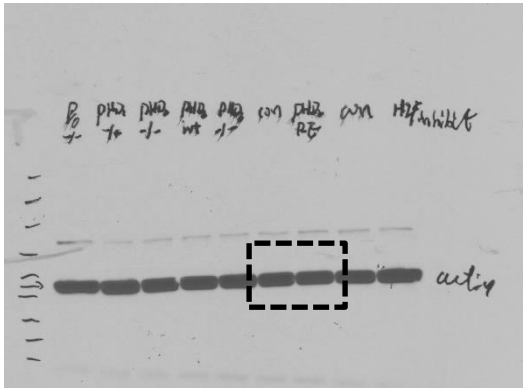
Erk1/2



S6K



β-actin



P-Erk1/2

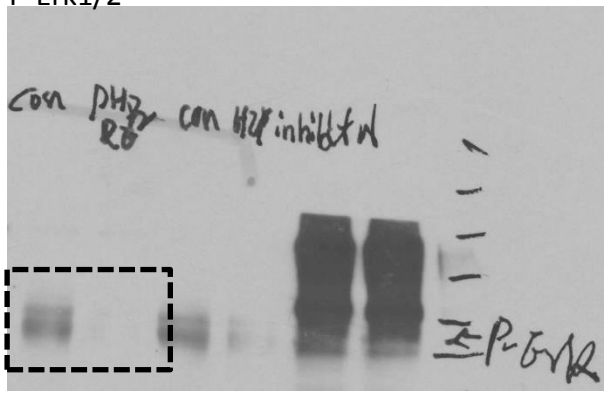
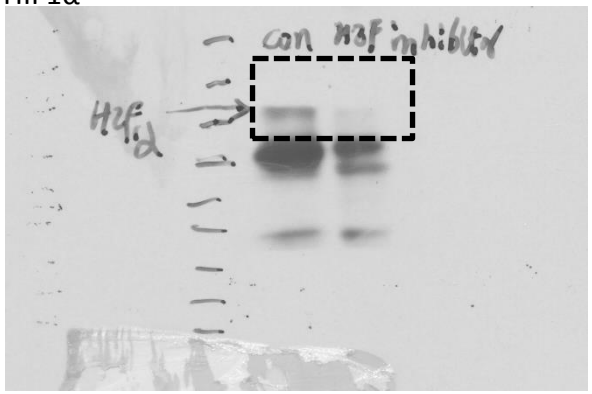


Fig. 6d

HIF1α



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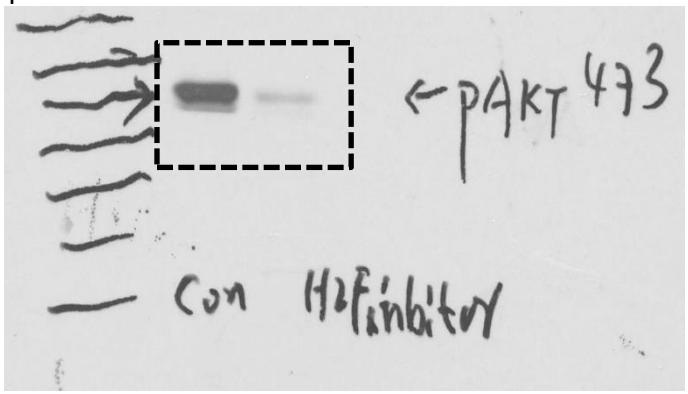

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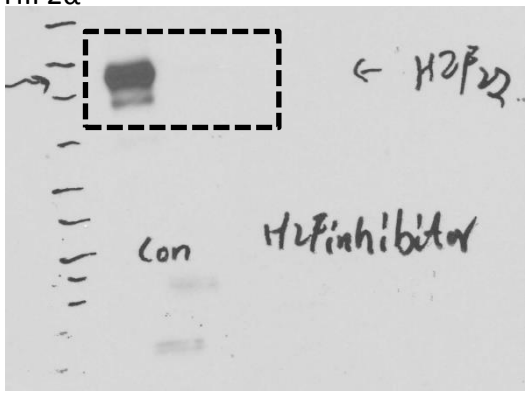

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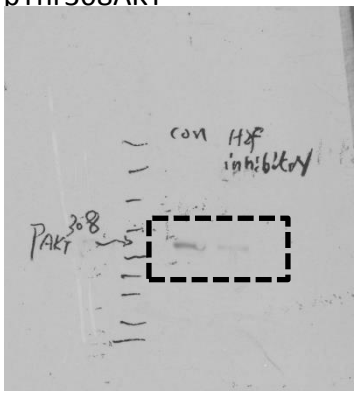
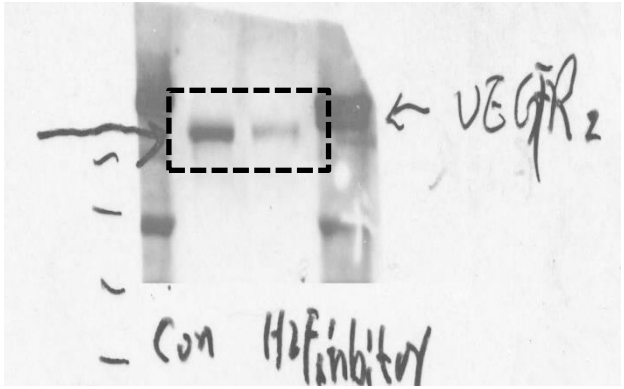


VEGFR2



AKT1

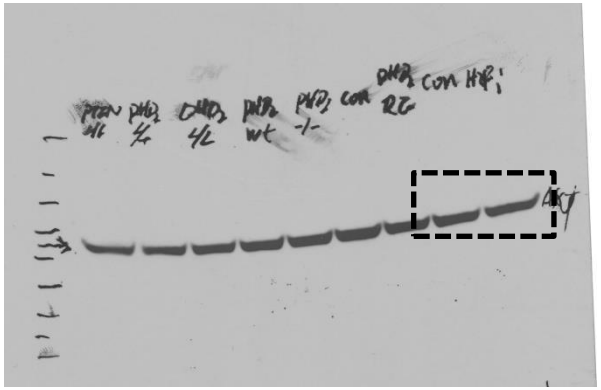
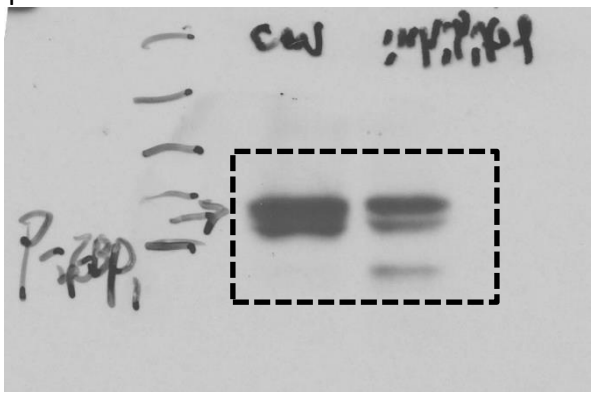
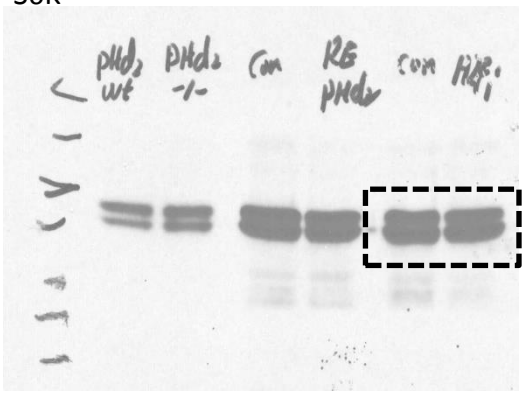


Fig. 6d

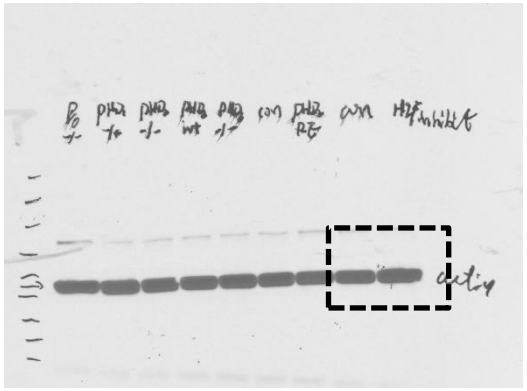
p4EBP1-S65



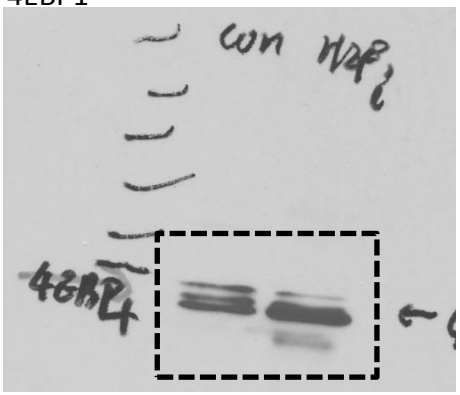
S6K



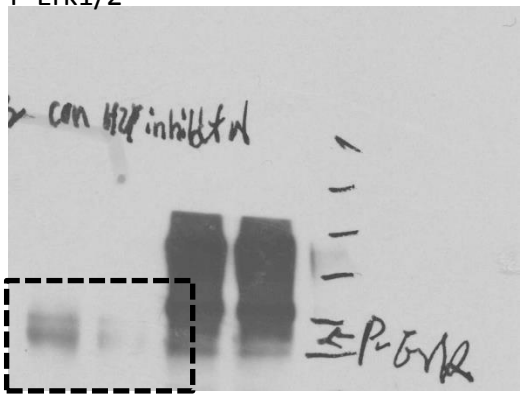
β-actin



4EBP1



P-Erk1/2



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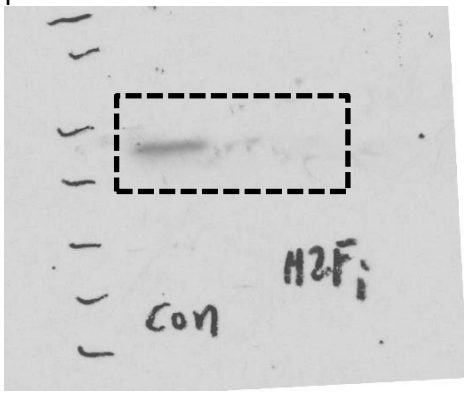


Erk1/2

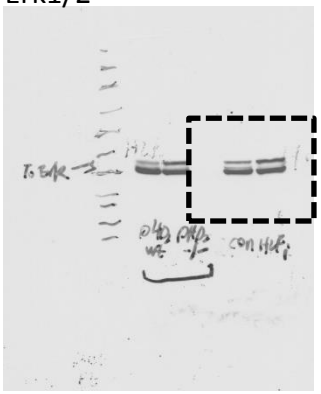
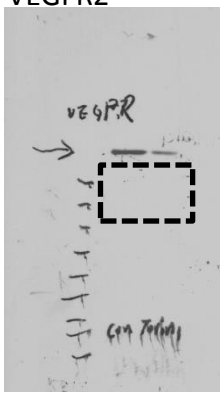
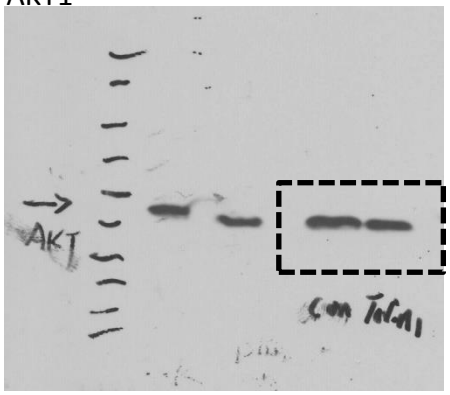


Fig. 7k

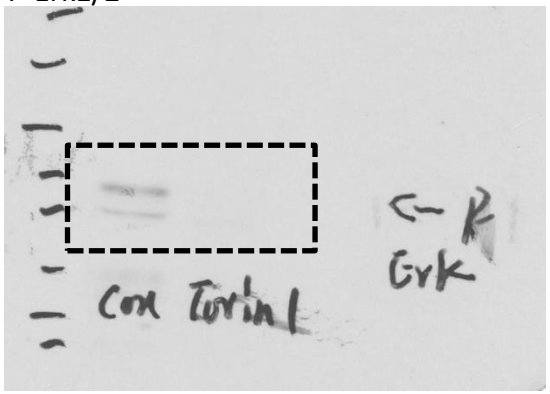
VEGFR2



AKT1



P-Erk1/2



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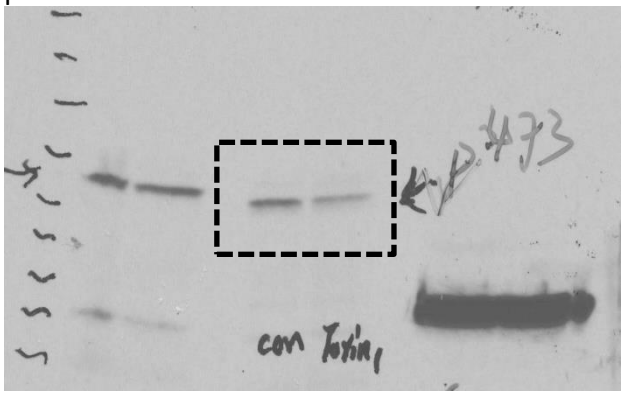

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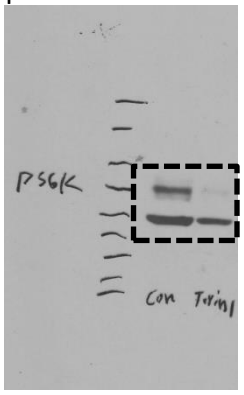
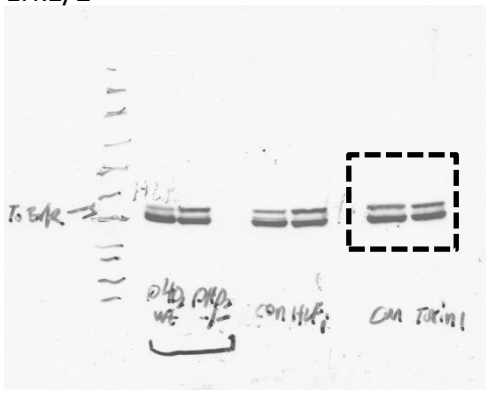


Erk1/2



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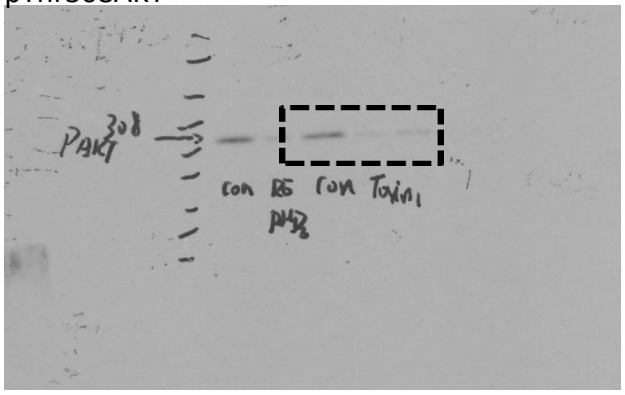

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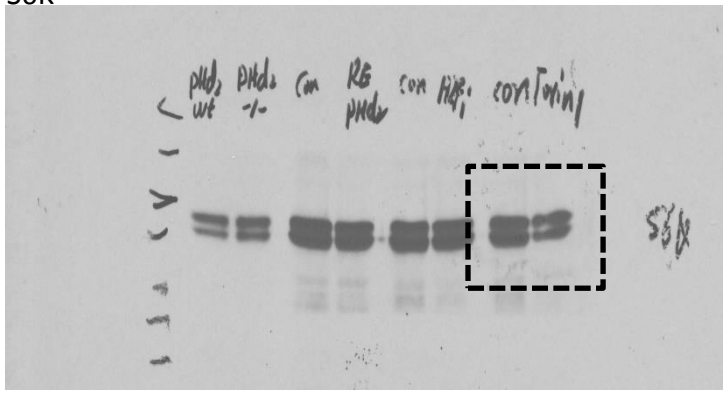
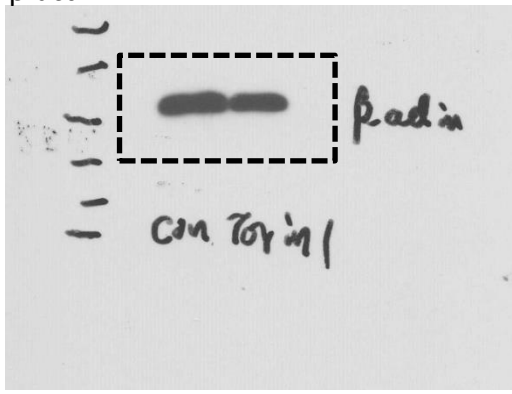


β-actin



Supplementary Figure 10. Full immunoblots.