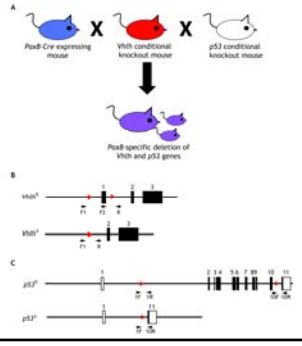
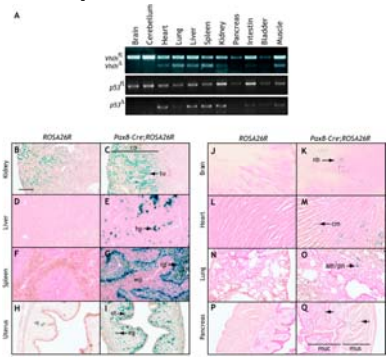
**Concomitant loss of VHL and p53 lead to a striking increase in HIF levels and more prominent vascular tumors**

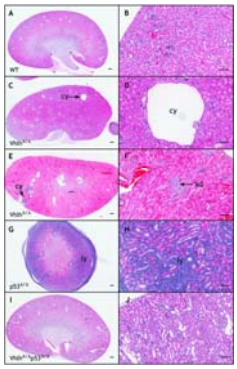
## Generation of Pax8-Cre VHL, p53 and double knockout mice

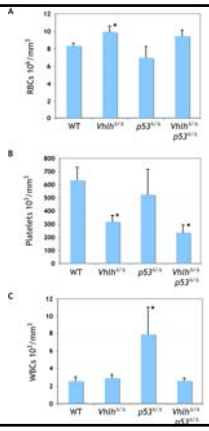



## Expression of Pax8-Cre

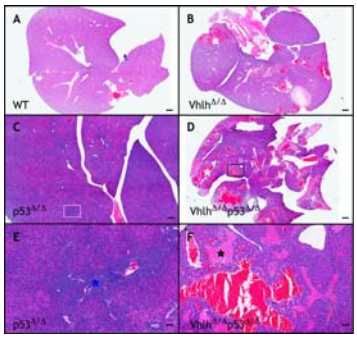



## Kidney lesions




## Hepatic Hemangiomas



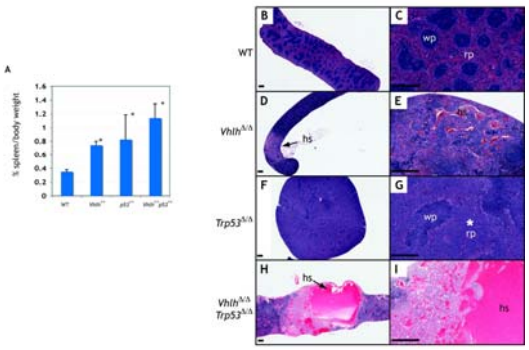
Incidence of hepatic lesions of mice between 4 and 7 months of age

| | WT (n=7) | $Vhlh^{\Delta/\Delta}$ (n=8) | $p53^{\Delta/\Delta}$ (n=7) | $Vhlh^{\Delta/\Delta}p53^{\Delta/\Delta}$ (n=13) |
|---|---|---|---|---|
| Angiecstasis | 0% | 88% | 0% | 85% |
| Hemangiomas | 0% | 75% | 0% | 85% |
| Necrotizing hepatitis | 0% | 13% | 0% | 77% |
| Lymphoma | 0% | 0% | 43% | 0% |
| No lesions | 100% | 13% | 57% | 15% |

Percentage of tissue involvement by the hepatic hemagiomas

| | $Vhlh^{\Delta/\Delta}$ | $Vhlh^{\Delta/\Delta}p53^{\Delta/\Delta}$ |
|---|---|---|
| 1-25% | 83% | 41% |
| 26-50% | 17% | 18% |
| > 50% | 0% | 41% |

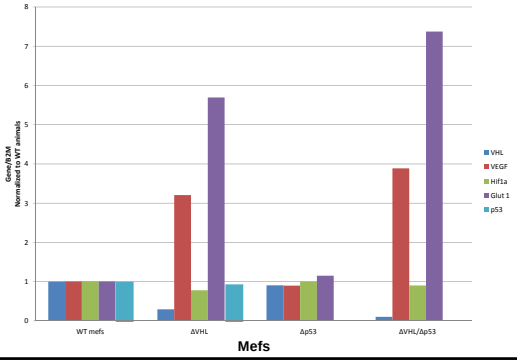
## Spleen lesions



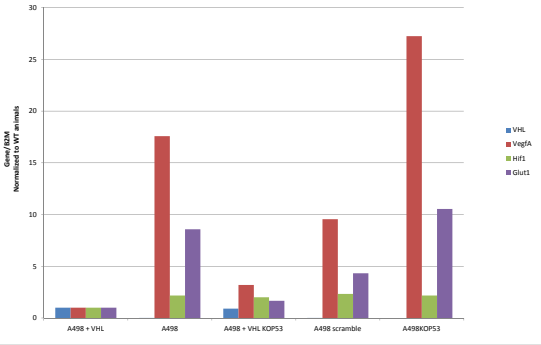

## Incidence of splenic lesions

| | WT (n=7) | $Vhlh^{\Delta/\Delta}$ (n=8) | $p53^{\Delta/\Delta}$ (n=7) | $Vhlh^{\Delta/\Delta}p53^{\Delta/\Delta}$ (n=11) |
|---|---|---|---|---|
| Extramedullar hematopoiesis | 0% | 88% | 0% | 82% |
| Hemangioma | 0% | 0% | 0% | 9% |
| Hemangiosarcoma | 0% | 25% | 0% | 55% |
| Lymphoma | 0% | 0% | 43% | 0% |
| No lesions | 100% | 12% | 57% | 11% |

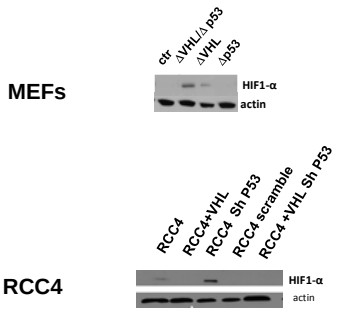
## VEGF, HIF1a, GLUT 1, and p53 mRNA levels in MEFs




## VEGF, HIF1a, GLUT1 mRNA levels in human RCC cells




## Concomitant loss of VHL and p53 leads to a robust increase in HIF protein levels



## Conclusions

- **Loss of VHL and p53 together leads to more pronounced hemangiomas and hemangiosarcomas in spleen and liver as compared to VHL deletion alone**
- **HIF target genes are further increased in VHL/p53 mutants as compared to VHL mutants**
- **HIF protein levels and not RNA levels are increased upon loss of p53**
- **HIF regulation by p53 might occur at the level of translation (mTOR or miRNA)**
- **Loss of p53 results in lymphomas which are suppressed by VHL deletion**

**1) Establish mechanism of p53 mediated HIF regulation**

**2) Study HIF protein regulation in C.elegans**

## Functional Characterization of the FLCN Tumor Suppressor

## Birt-Hogg-Dube Syndrome

- Is an inherited neoplasia syndrome
- It predisposes to melanoma, fibrofolliculomas, pneumothorax and renal cancer
- Higher risk of developing renal cancer of multiple subtypes.

## FLCN

- The tumor suppressor gene responsible for this disease is *FLCN*
- Encodes folliculin (FLCN) a novel cytoplasmic protein.
- All mutations found in BHD patients result in a truncated or destabilized protein suggesting a loss of function mechanism leading to the syndrome development.
- FLCN has no significant sequence homology to any other known protein.
- AMPK was identified as a FLCN interacting protein (Baba et al., 2006)
- AMPK is activated when ATP levels drop, inhibits anabolic pathways and activates catabolic pathways.
- There is a FLCN ortholog in *C.elegans* (28% identity).

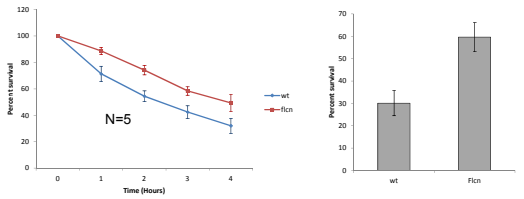
## Controversy from different genetic models:

- Knockout of *FLCN* in mice results in:
  - Polycystic kidneys with elevated mTOR/AKT and ERK signaling (Baba et al.,2008, Chen et al.,2008)
  - renal cysts with lower mTOR signaling (Hartman et al., 2009)
  - renal cysts with either elevated or reduced levels of mTOR signaling. (Hudon *et al*.,2009)
- In *S.pombe* a *FLCN ortholog representing only the N-terminal half* activates mTOR signaling independent of TSC1/2 (Slegtenhorst et al. 2007)

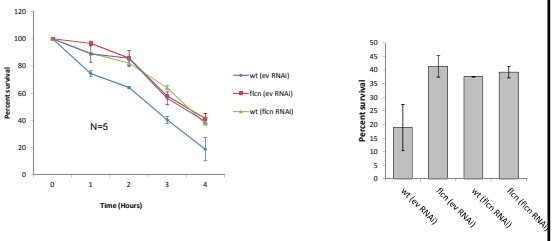
## *C.elegans* FLCN

**C.elegans AMPK (aak-2) mediates longevity and resistance to oxidative stress**

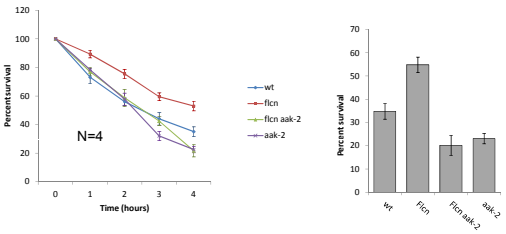
## FLCN mutation enhances resistance to ROS




## FLCN RNAi enhances resistance to ROS

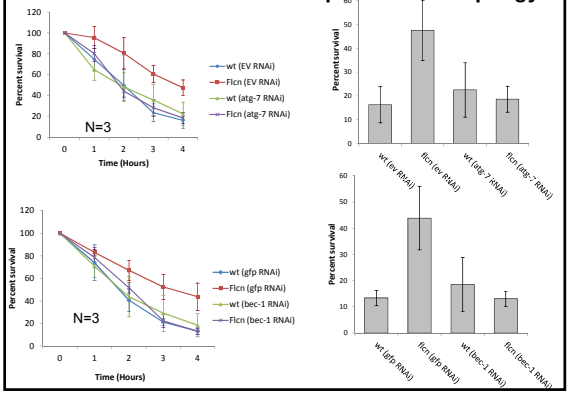



## aak-2 mediates resistance to ROS in FLCN mutants

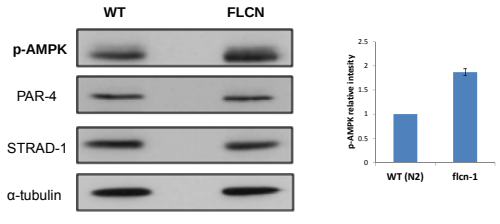



**FLCN might act as negative regulator of aak-2 (no effect on aak-1)**

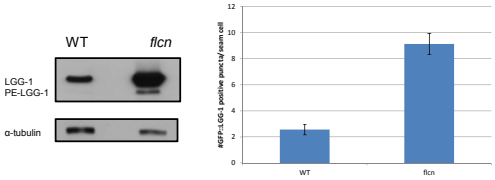
## Survival of Flcn mutants depends on autophagy



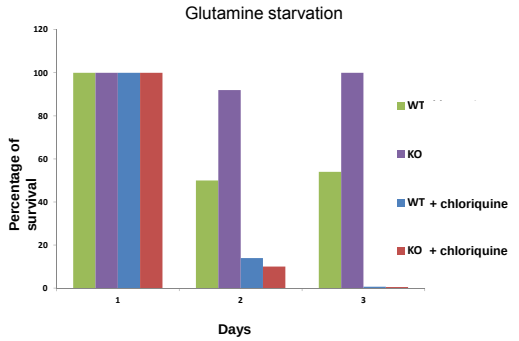

## Elevated levels of p-AMPK in FLCN mutant



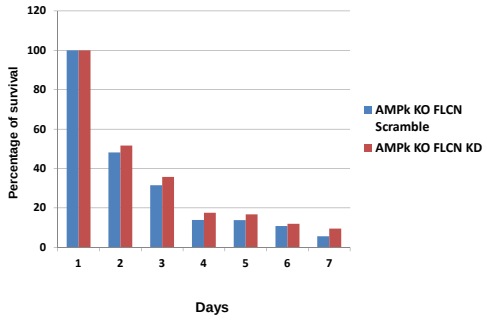

## Higher levels of autophagy in FLCN knockout worms



## Loss of folliculin in MEFs leads to autophagy-dependent survival during starvation




## Starvation resistance is dependent on AMPK




## Conclusions

- FLCN acts as a negative regulator of AMPK
- Loss of FLCN leads to AMPK dependent increase in ROS resistance
- Loss of FLCN leads to an increase in AMPK-dependent autophagy
- Autophagy mediates resistance to ROS
- Use of autophagy inhibitors for Tx of BHD lesions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