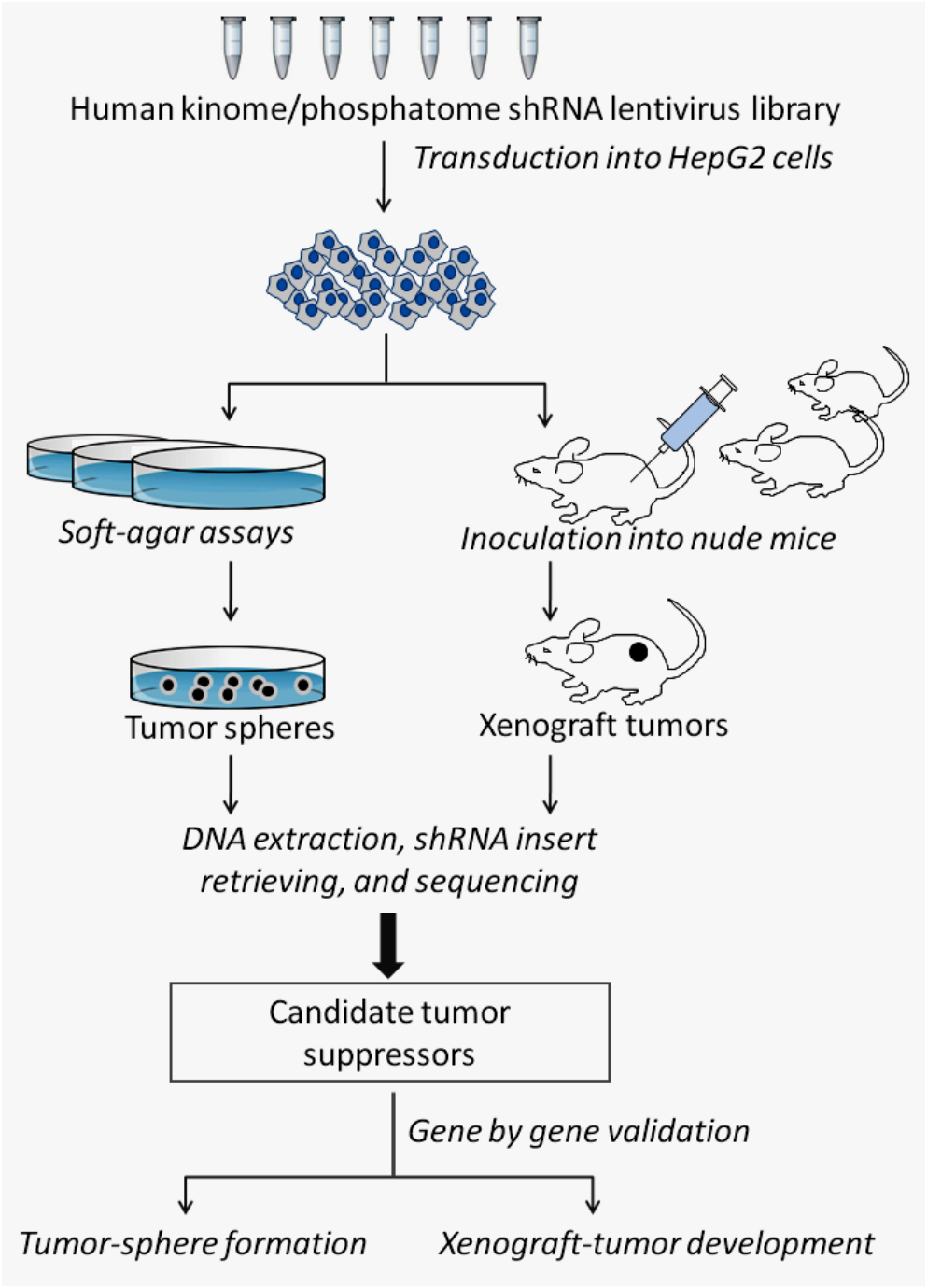
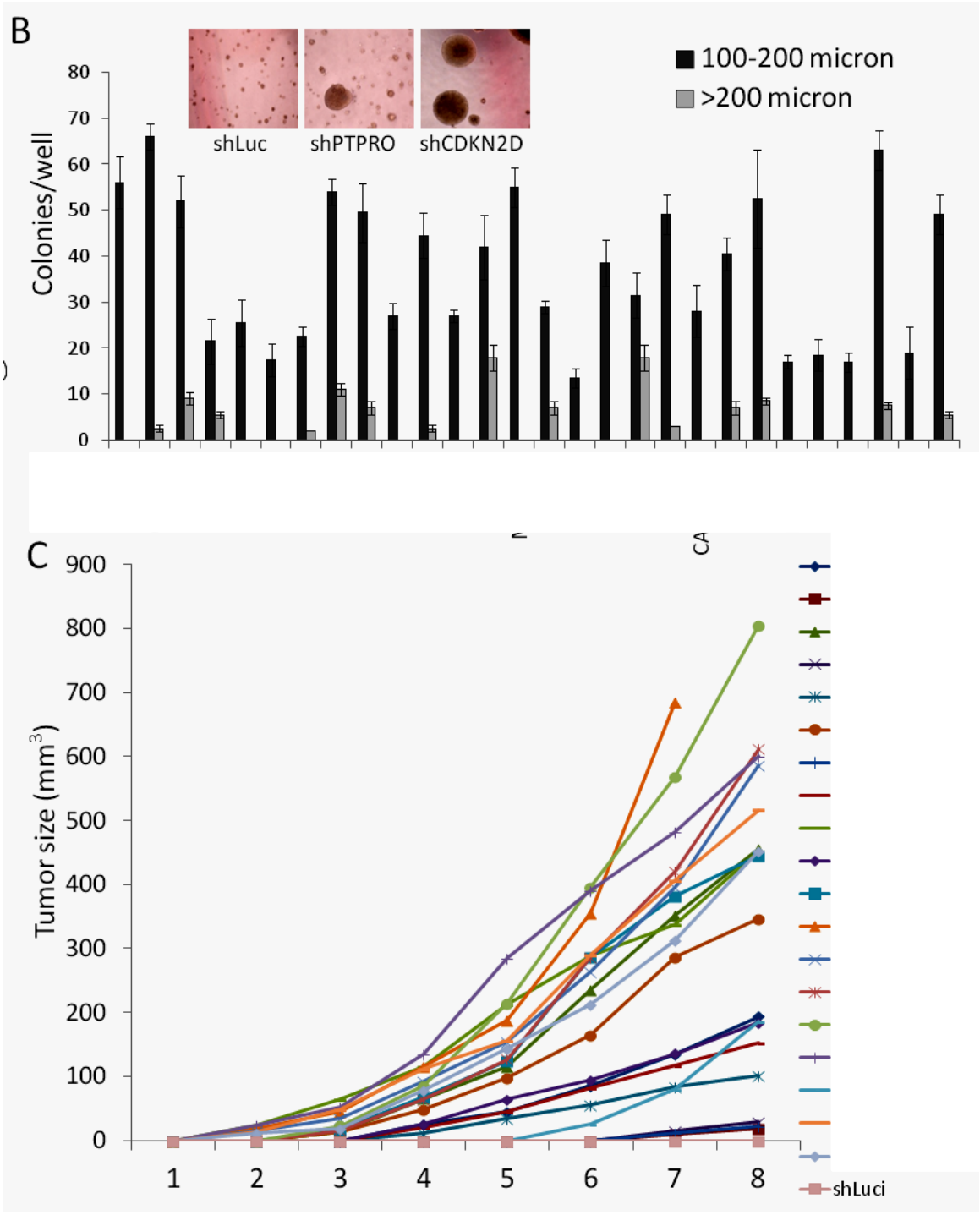
Functional Genomics : PTPRF-Mediated Contact Inhibition in HCC development

Sen-Yung Hsieh, MD, PhD
Liver Research Unit
Chang Gung Memorial Hospital
Taoyuan, Taiwan

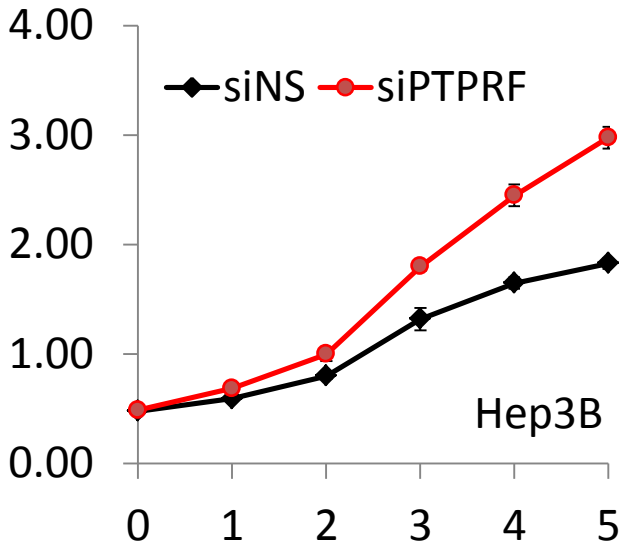
Aims

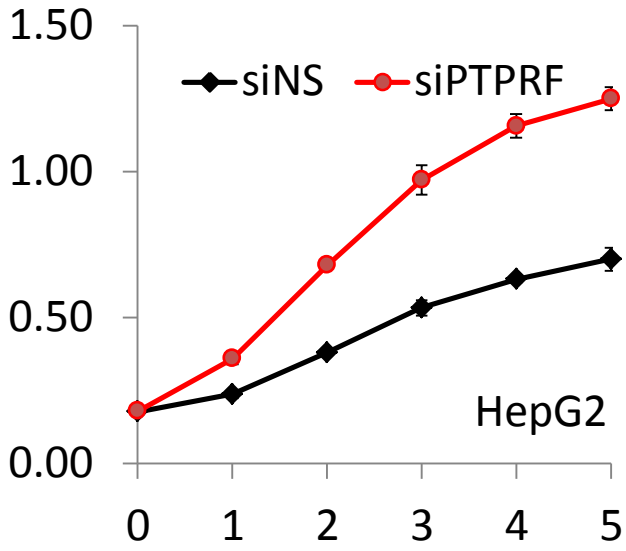
- To identify genes controlling tumor initiation of human hepatoma from human kinome and phosphatome

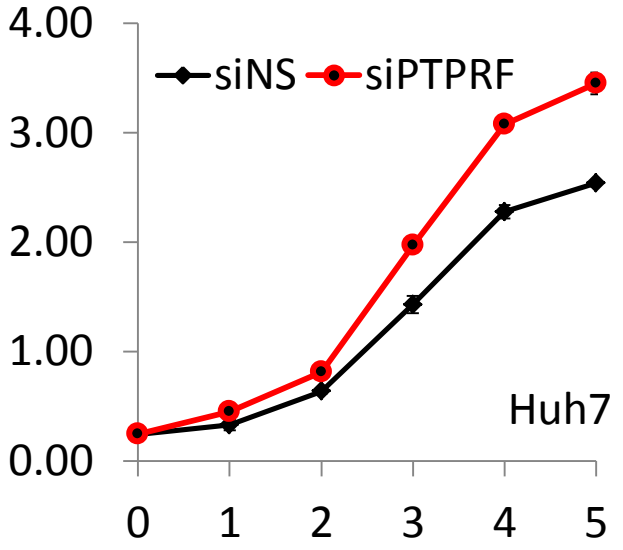

Human kinome/phosphatome shRNA lentivirus library
Transduction into HepG2 cells
Soft-agar assays
Inoculation into nude mice
Tumor spheres
Xenograft tumors
DNA extraction, shRNA insert retrieving, and sequencing
Candidate tumor suppressors
Gene by gene validation
Tumor-sphere formation
Xenograft-tumor development

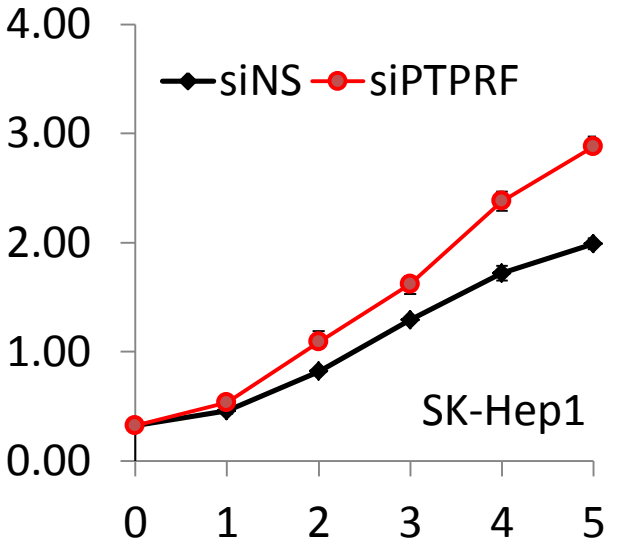

B
100-200 micron
>200 micron
shLuc
shPTPRO
shCDKN2D
Colonies/well
0
10
20
30
40
50
60
70
80
C
Tumor size (mm3)
0
100
200
300
400
500
600
700
800
900
1
2
3
4
5
6
7
8
shLuci

PTPRF: a suppressor of tumor initiation in HCC ?



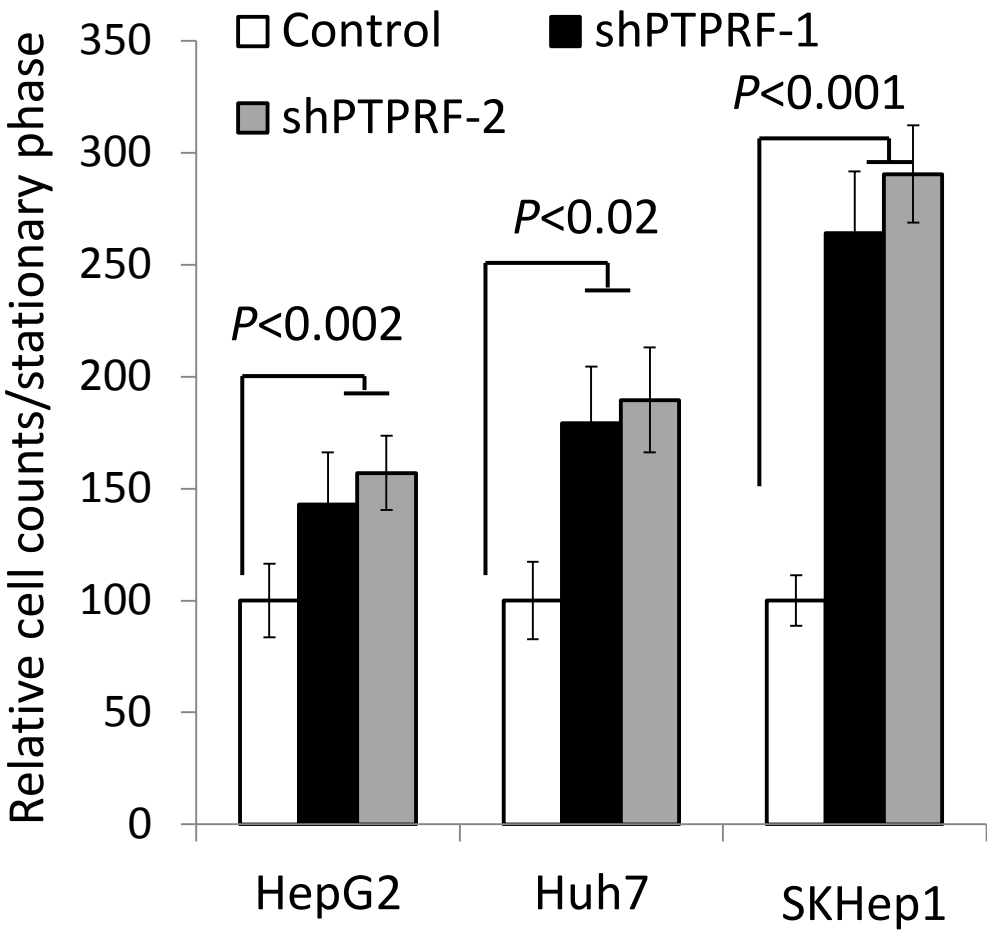






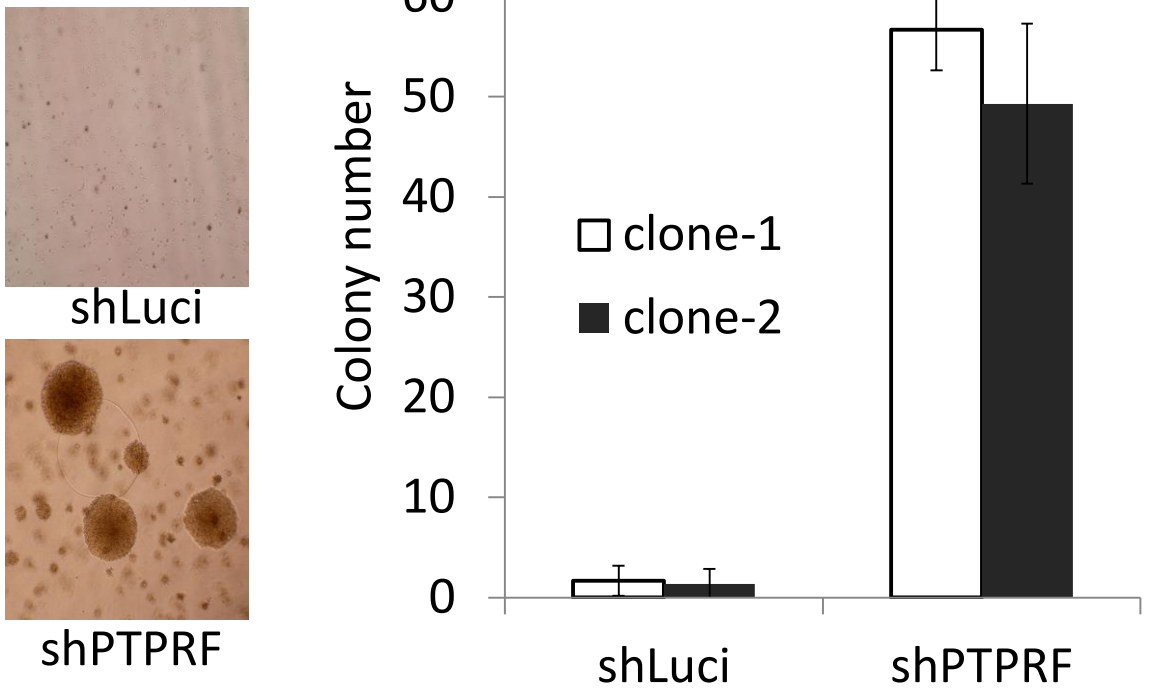
Rabi et al., Hepatology 2014

Silencing of PTPRF enhances cell proliferation and increases total cell numbers



Rabi et al., Hepatology 2014

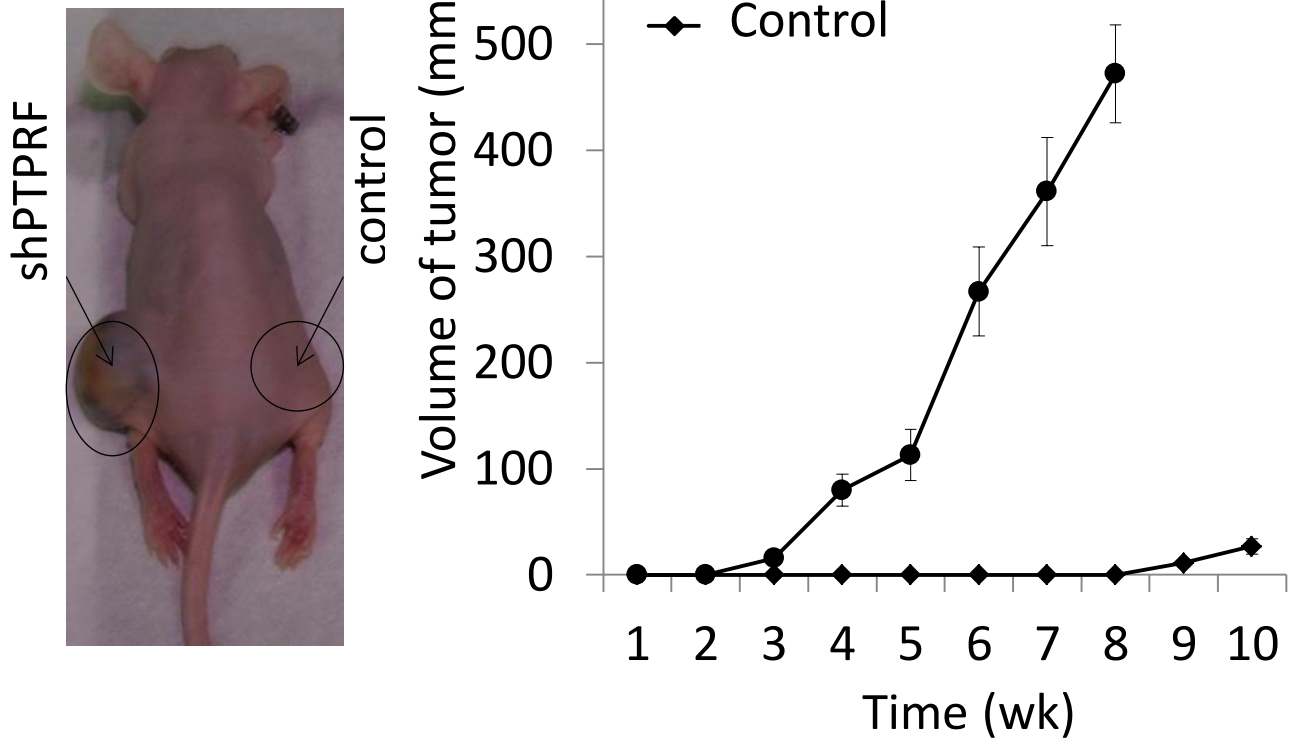
Silencing of PTPRF enhances anchorage-independent cell growth



Soft-agar assays for tumor sphere formation

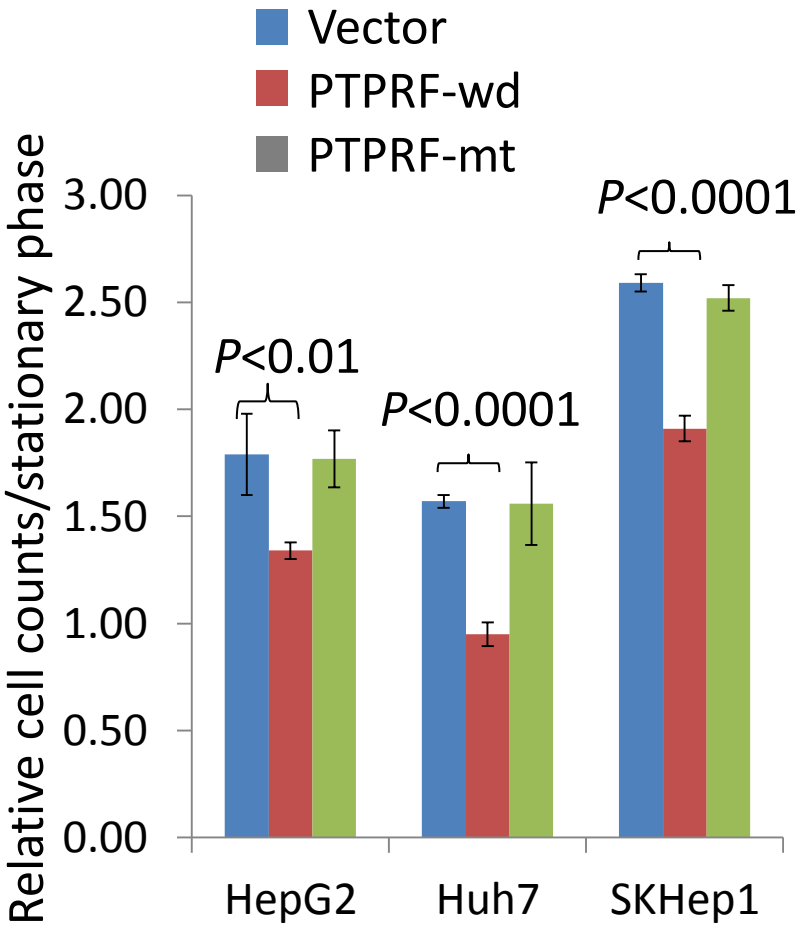
Rabi et al., Hepatology 2014

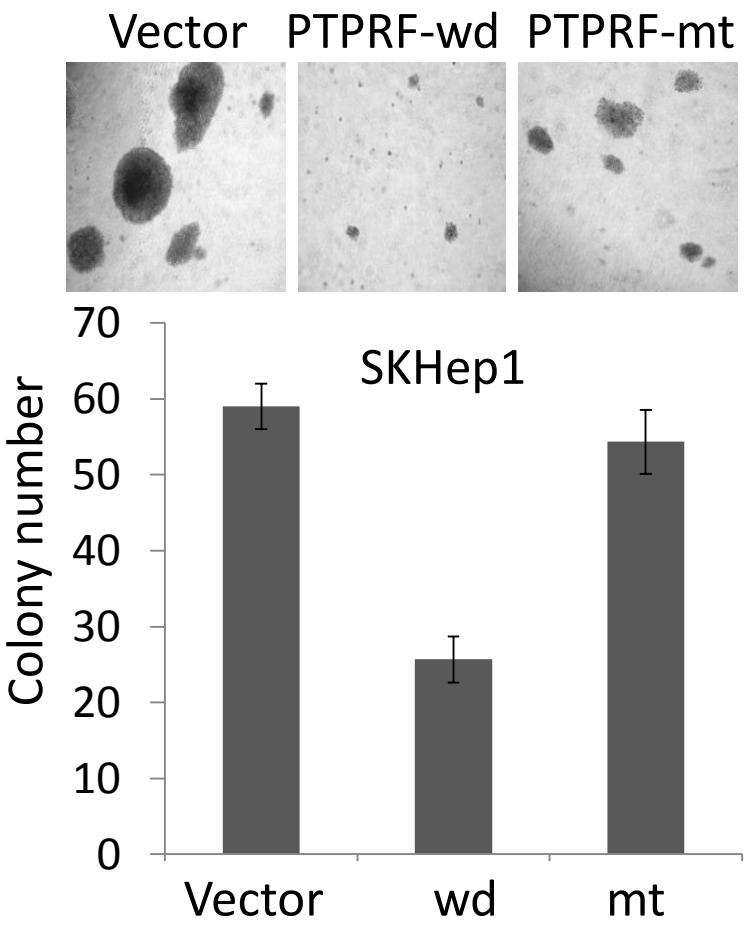
Silencing of PTPRF enhances xenograft tumor formation



Rabi et al., Hepatology 2014

Ectopic expression of wild-type PTPRF, but not the phosphatase mutant, suppresses proliferation and tumorigenecity





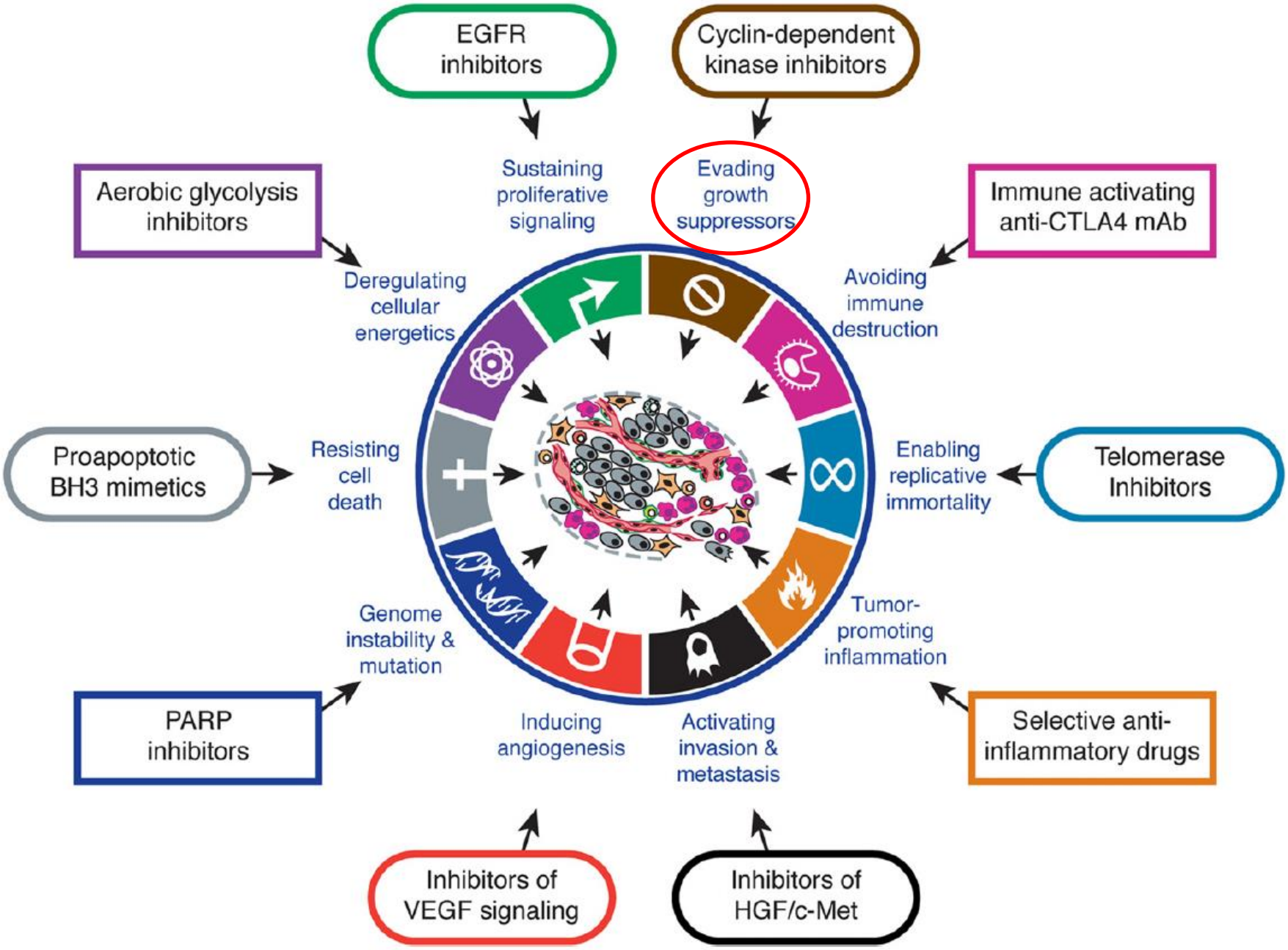
The biological functions depend on its phosphatase activities !

Rabi et al., Hepatology 2014

Summary

- PTPRF suppresses cell proliferation and tumor initiation → c/w a TSG
- Suppression of tumor formation depends on its phosphatase activities
- **PTPRF suppresses total cell mass → involved in contact inhibition???**

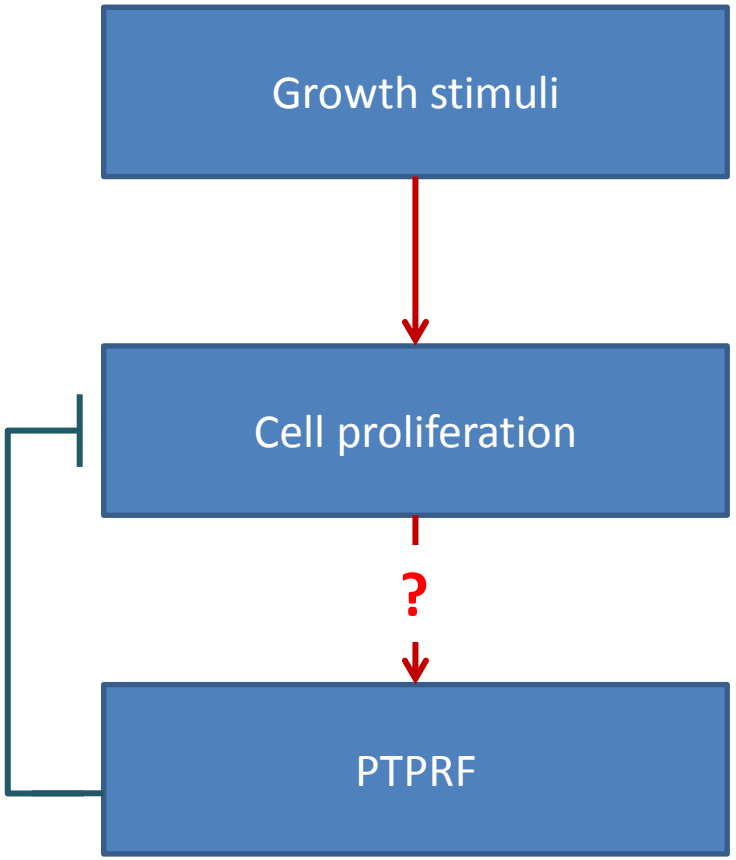
Rabi et al., Hepatology 2014



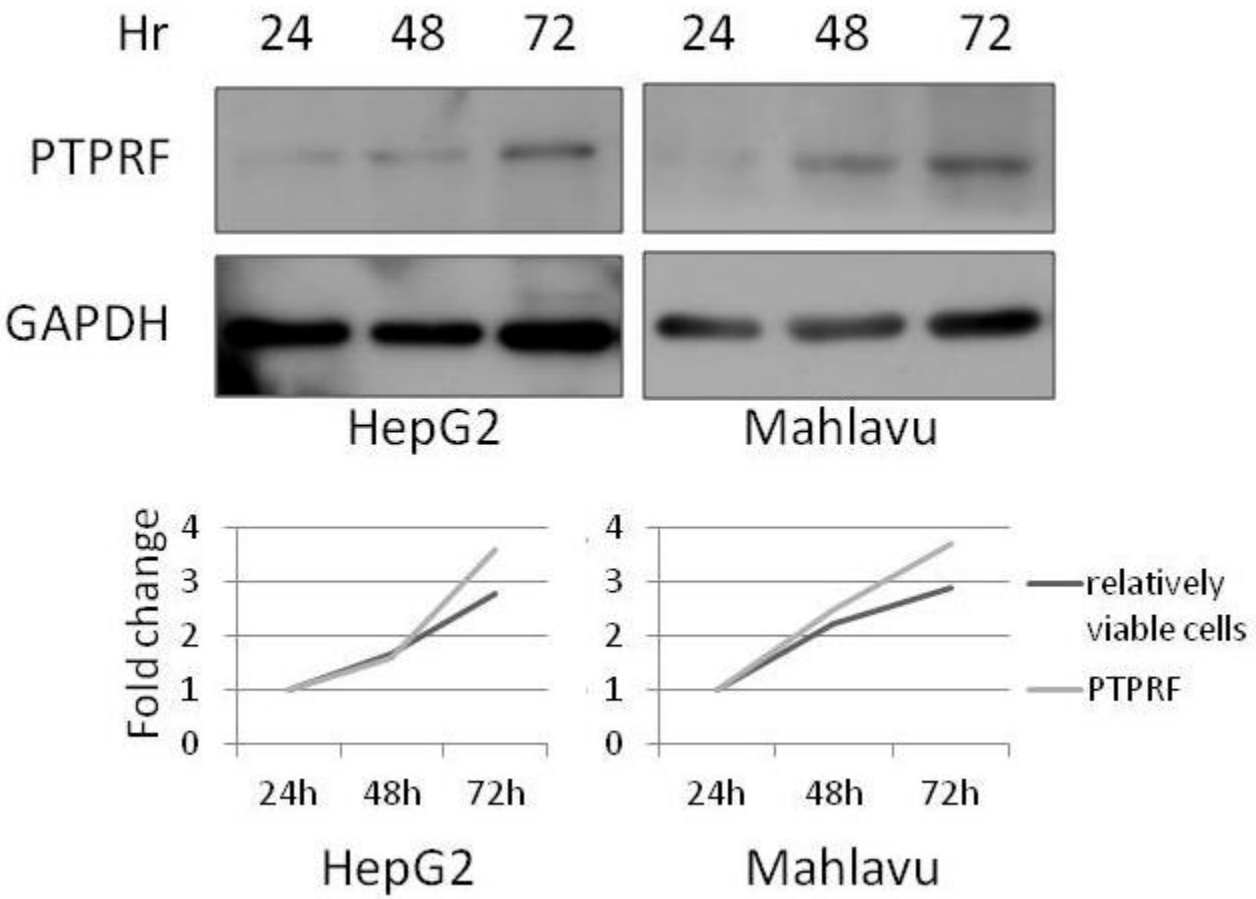
Hanahan & Weinberg 2011

Hypothesis

- PTPRF
  - PTPRF functions as a negative regulator to prevent overproliferation
  - ***A negative feedback loop prevents hyperproliferation and tumor formation?***

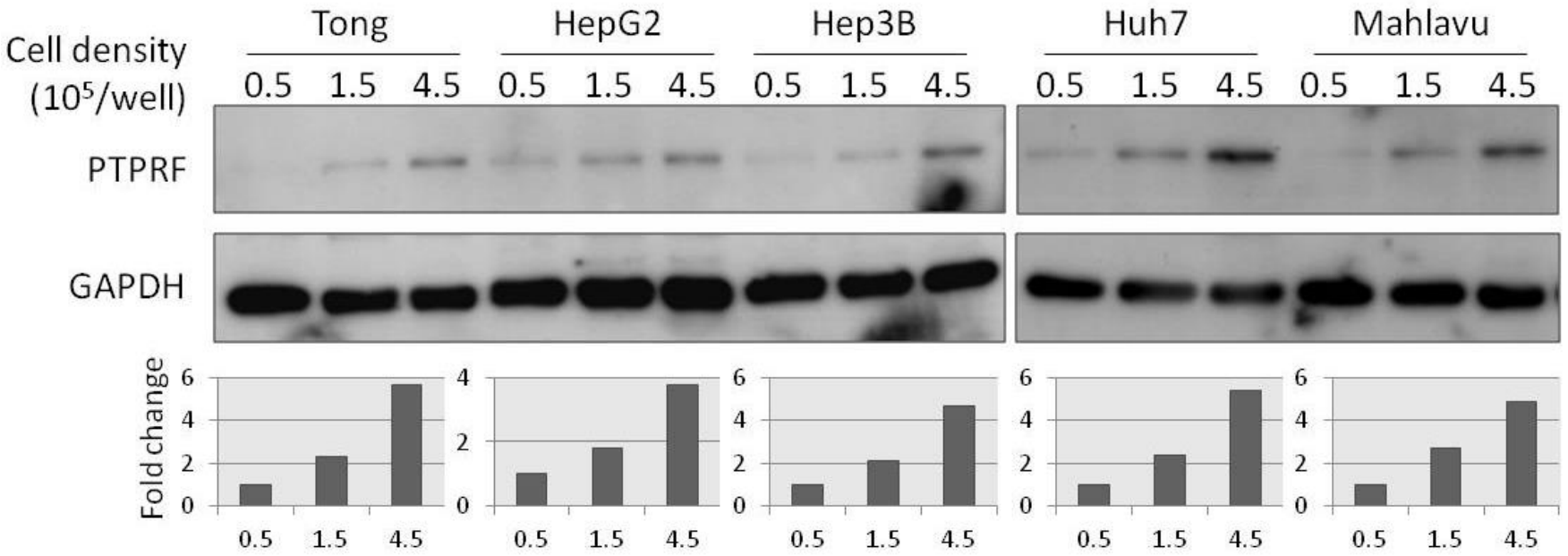


Does cell proliferation induce PTPRF expression ?



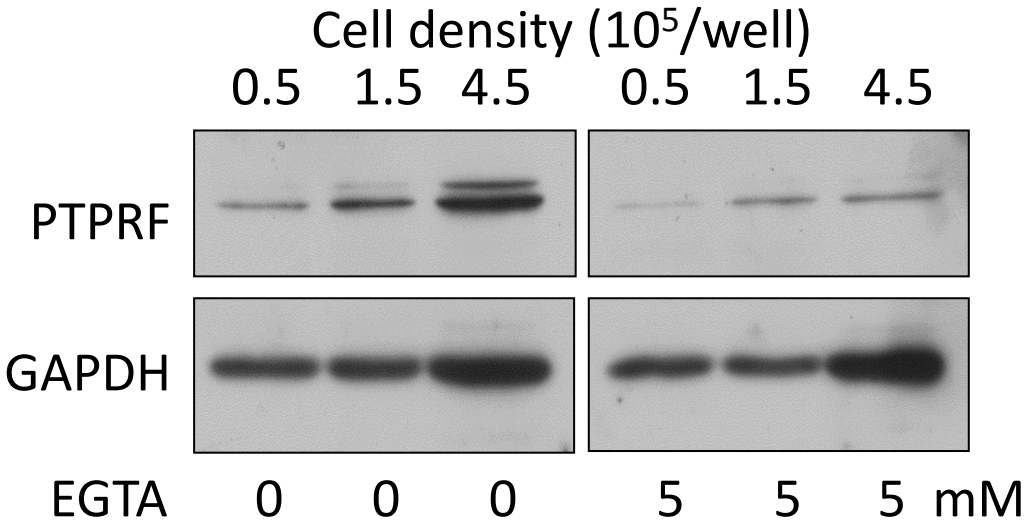
Rabi et al., Hepatology 2014

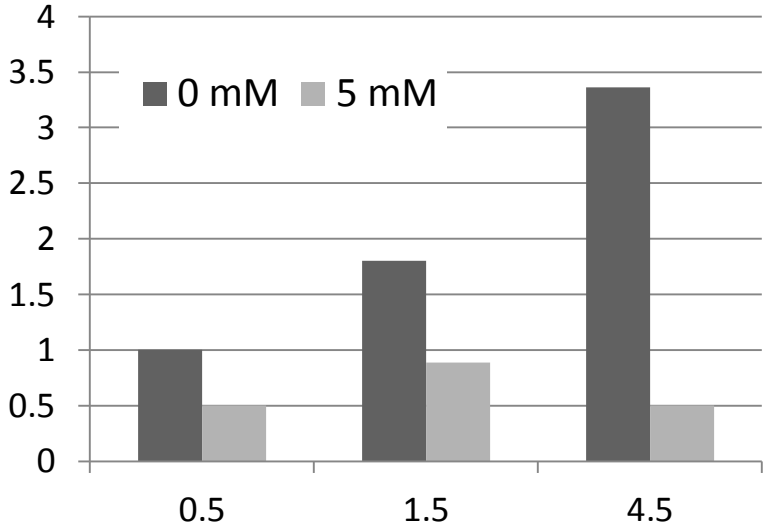
Is PTPRF induced due to increased cell density?

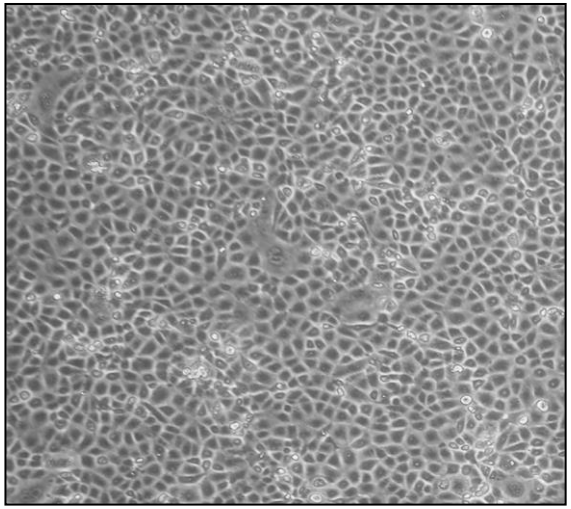


Rabi et al., Hepatology 2014

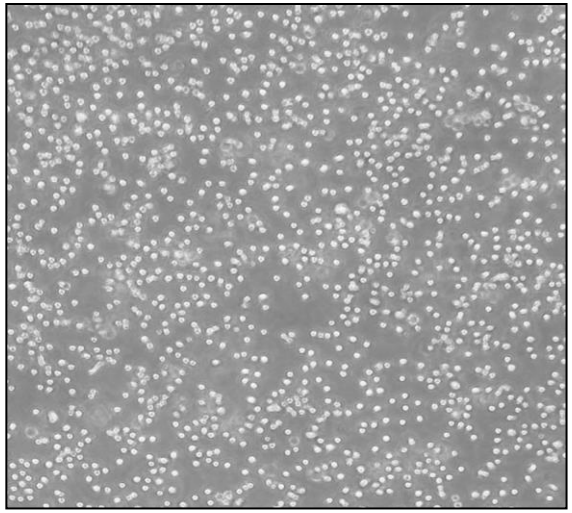
Is PTPRF induced by cell-cell contact ?







4.5 x 10^5/well
EGTA: 0 mM

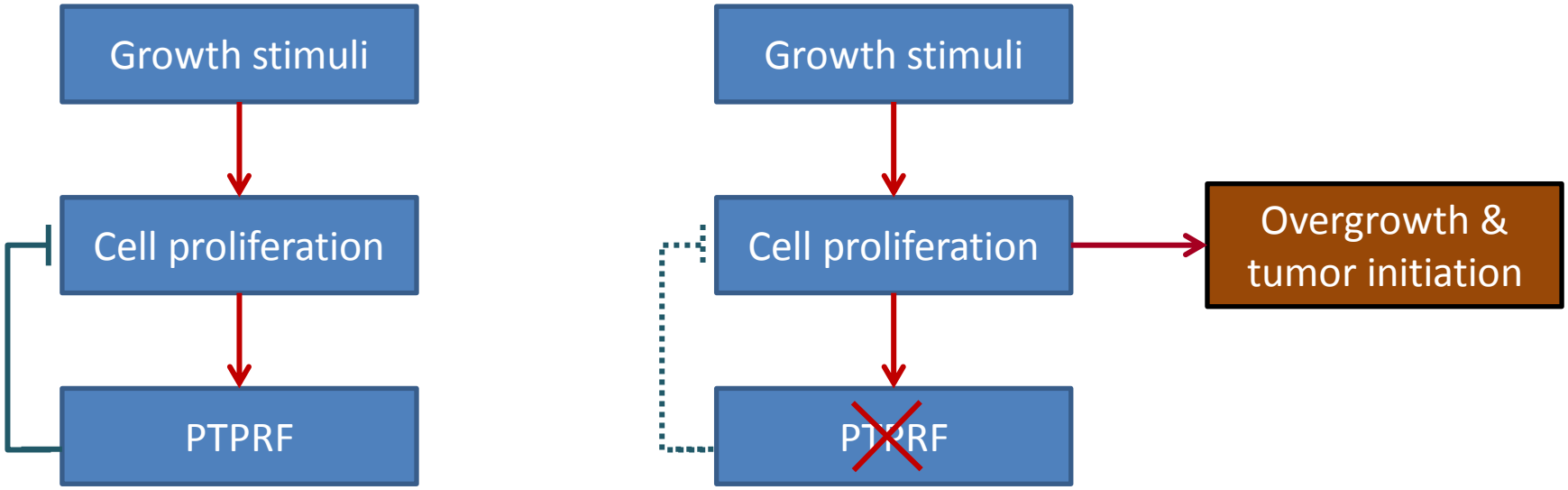


4.5 x 10^5/well
EGTA: 5 m

Rabi et al., Hepatology 2014

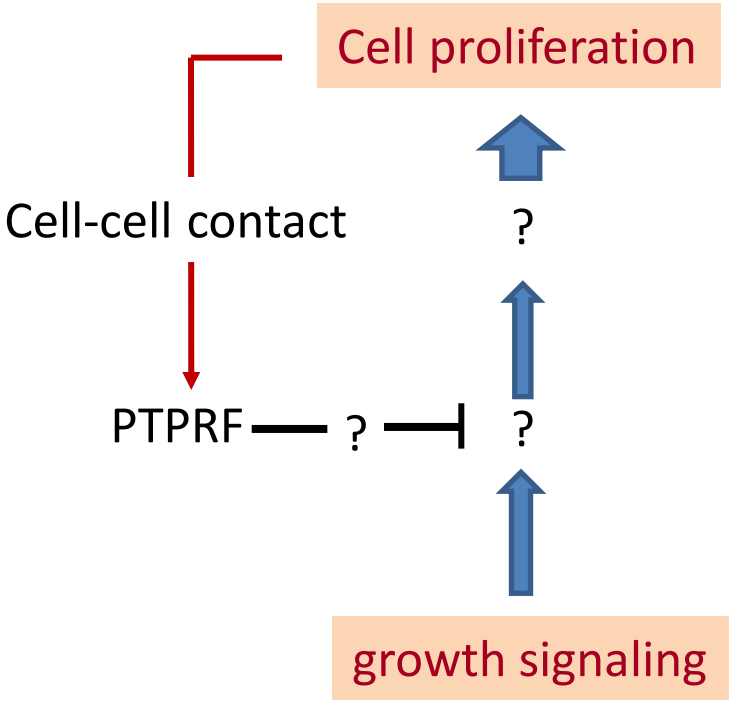
Tentative conclusion

- PTPRF suppresses tumor formation
- PTPRF suppresses cell proliferation and total cell mass during proliferation
- PTPRF is induced by cell-cell contact during proliferation
- Loss of PTPRF function ➔ hyperproliferation and tumor initiation

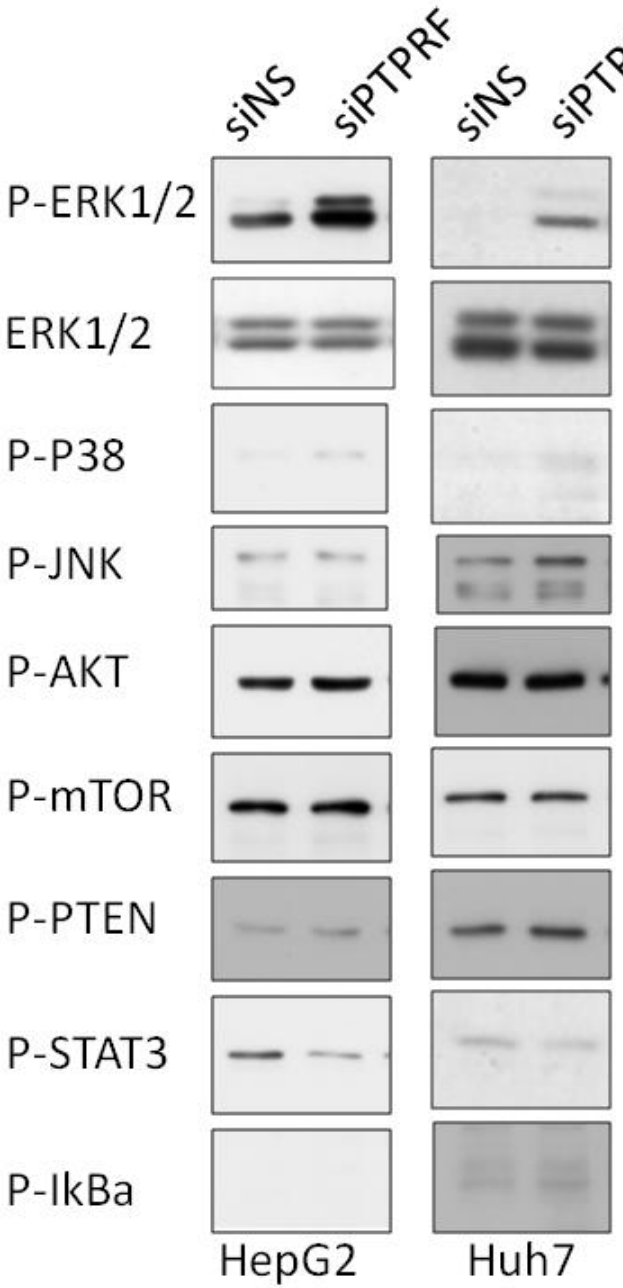


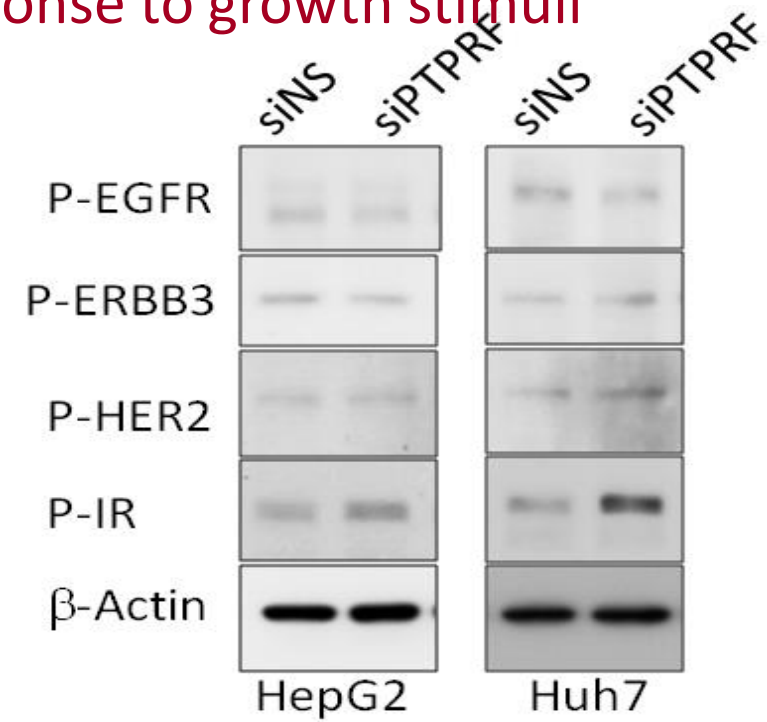
Rabi et al., Hepatology 2014

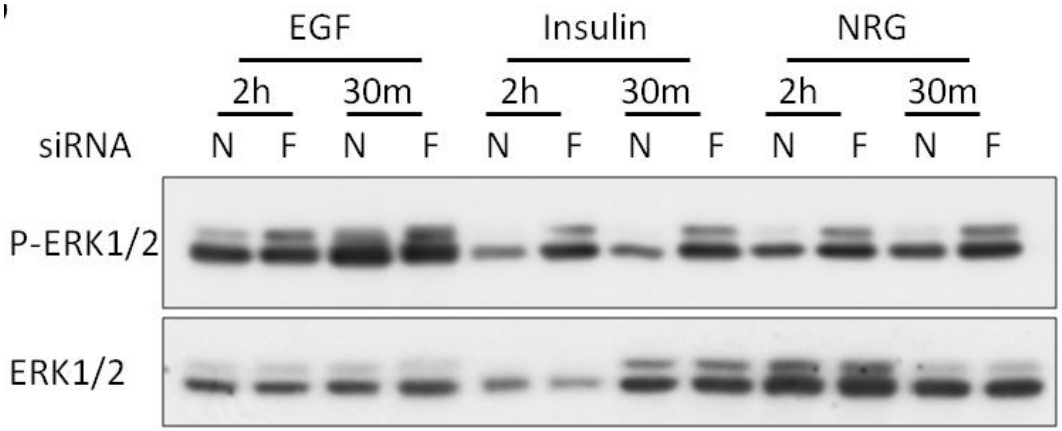
How PTPRF suppresses cell proliferation?



PTPRF inhibits ERK phosphorylation/activation in response to growth stimuli

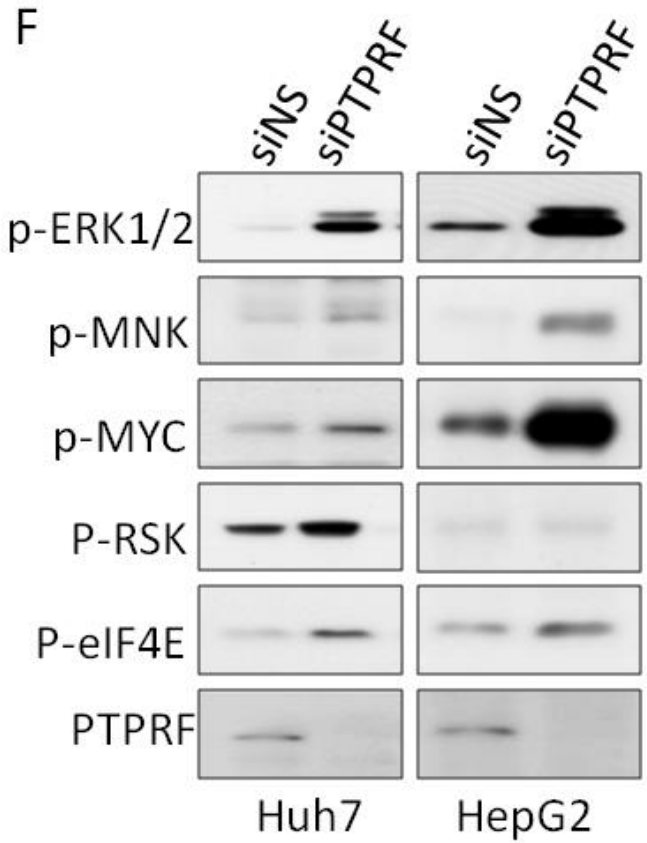


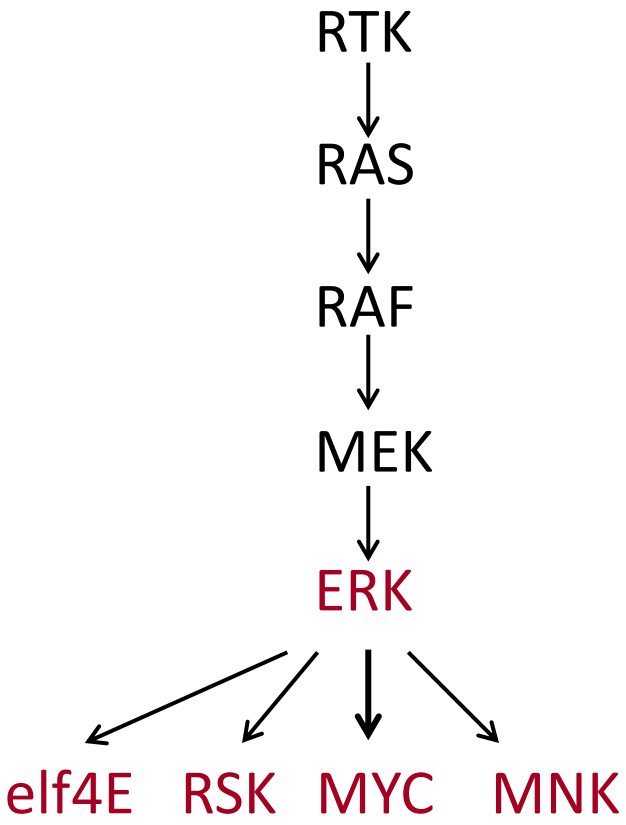




Rabi et al., Hepatology 2014

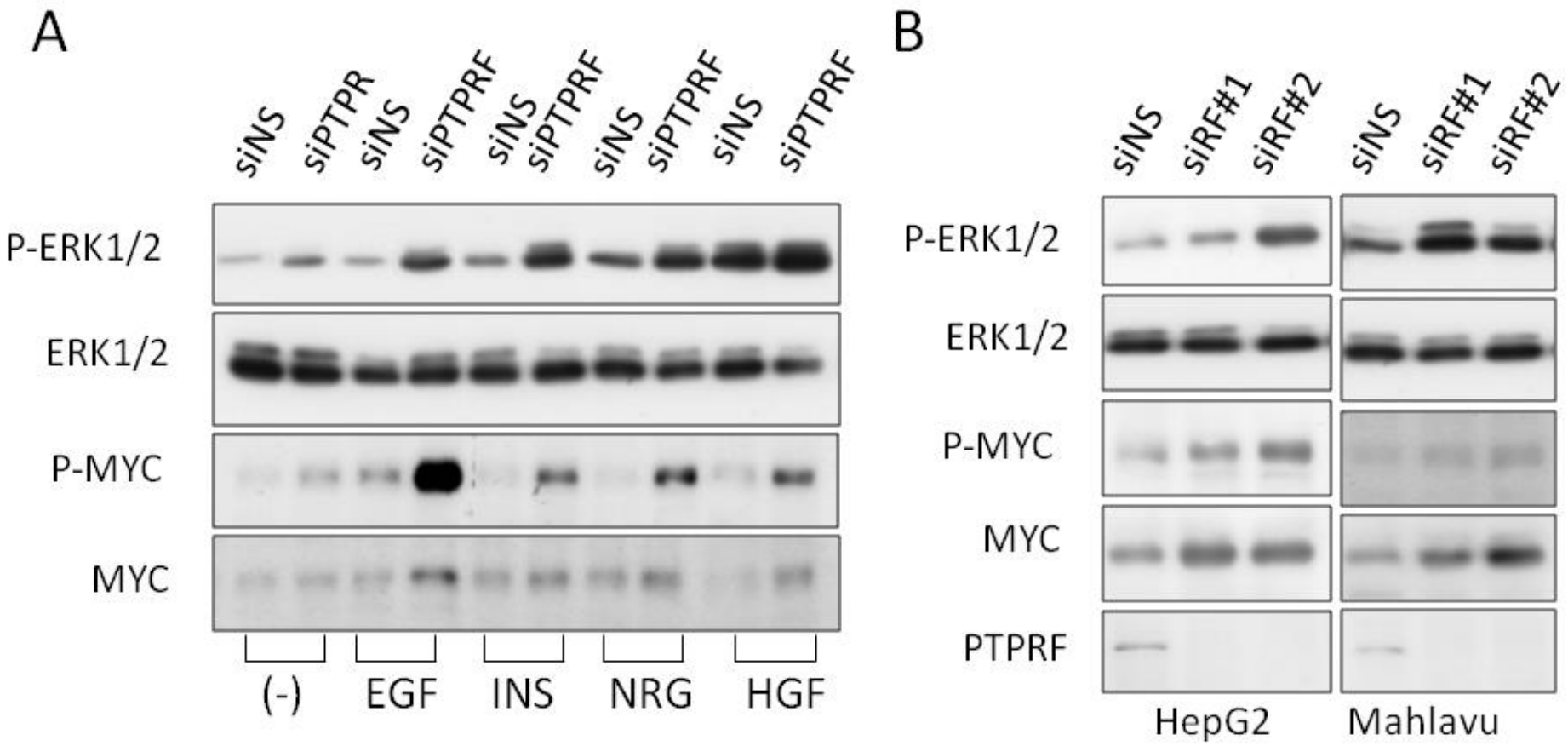
PTPRF inhibits ERK-dependent signaling





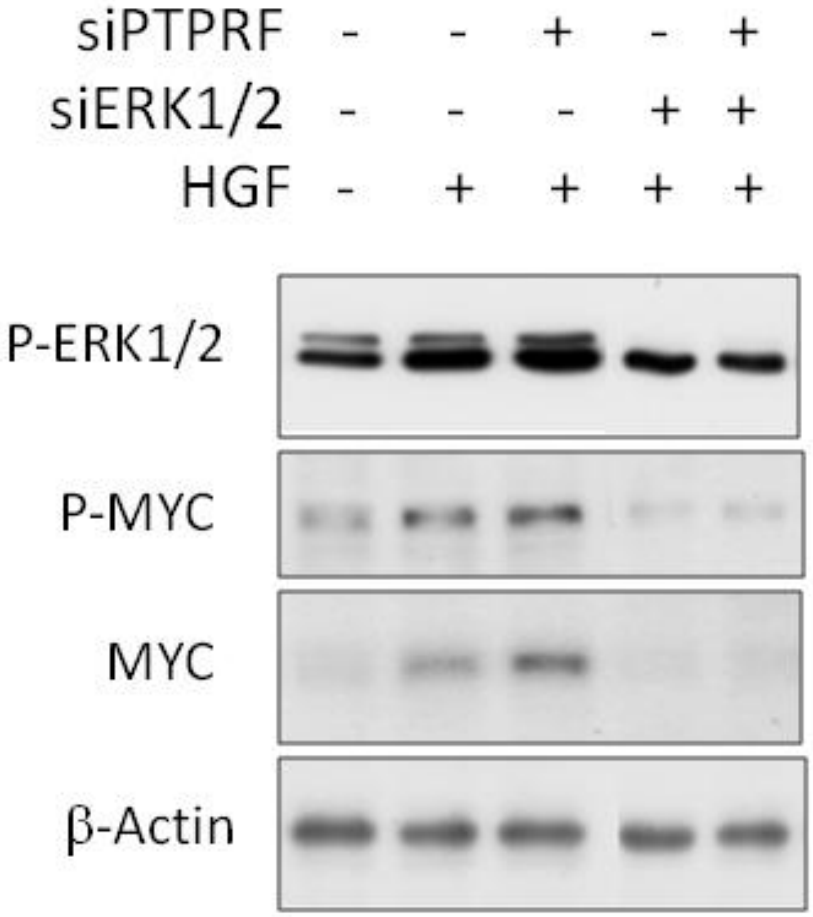
Rabi et al., Hepatology 2014

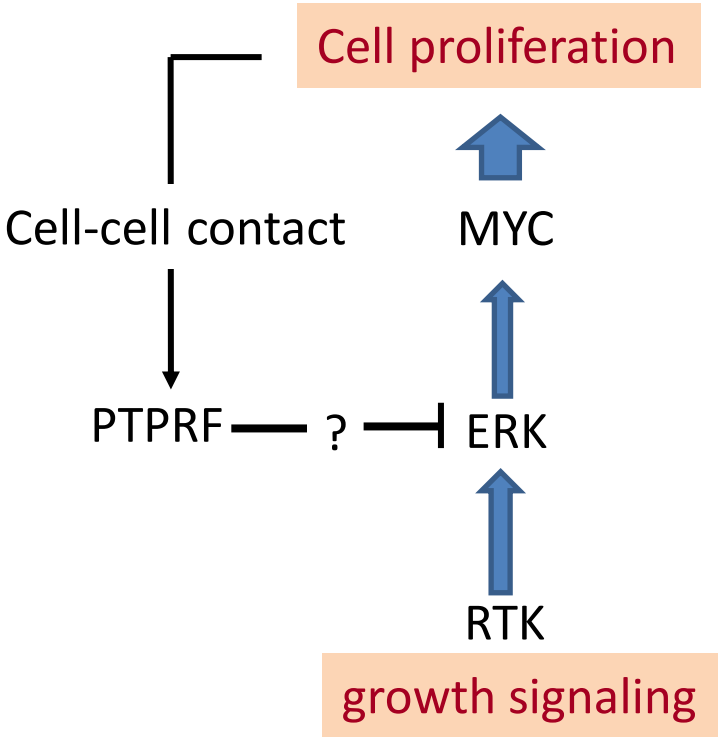
PTPRF knockdown greatly enhances ERK-MYC growth signaling



Rabi et al., Hepatology 2014

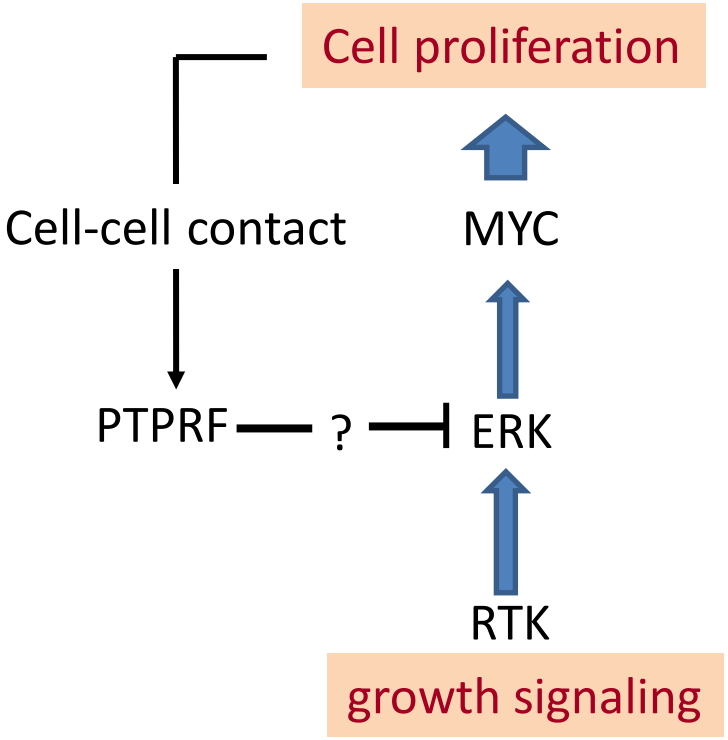
ERK1/2 activation required for MYC activation



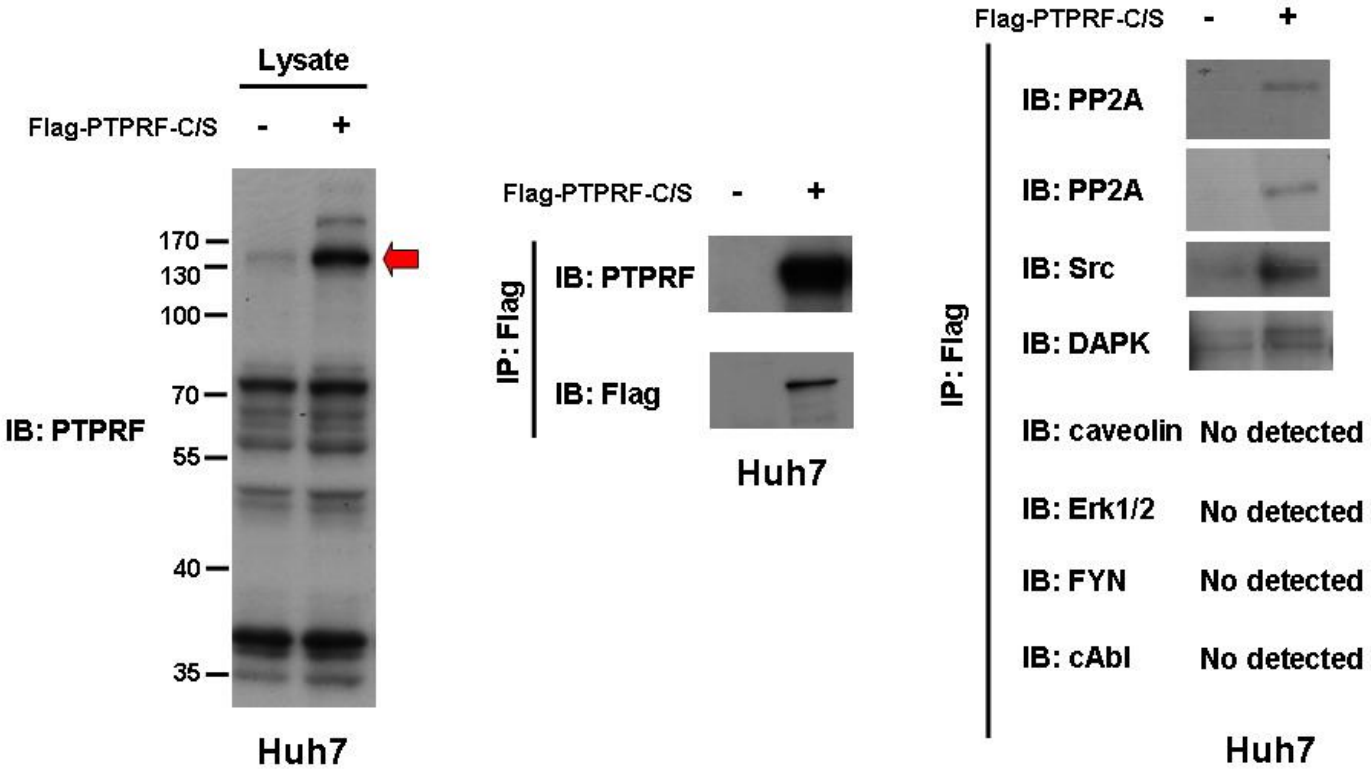


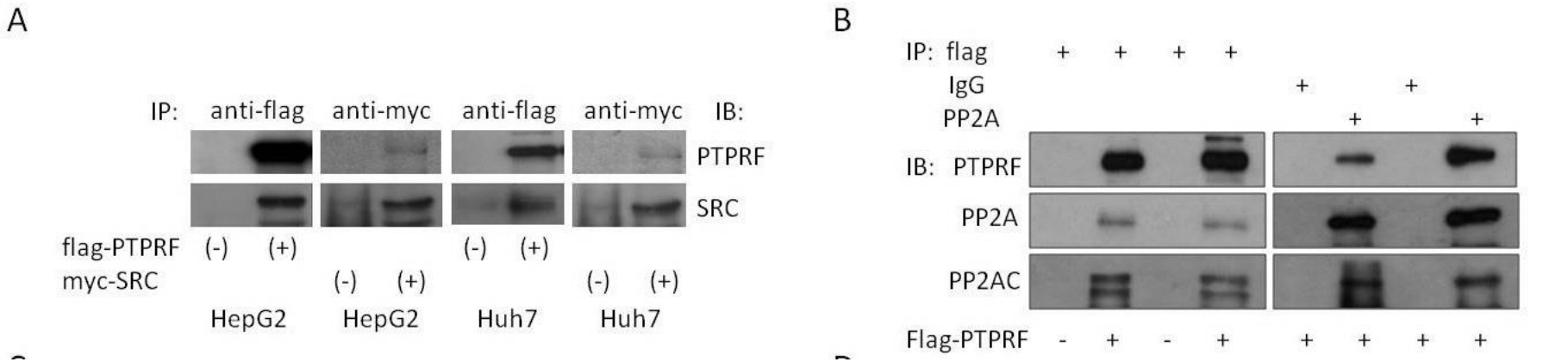
Rabi et al., Hepatology 2014

How PTPRF suppresses ERK-MYC signaling?



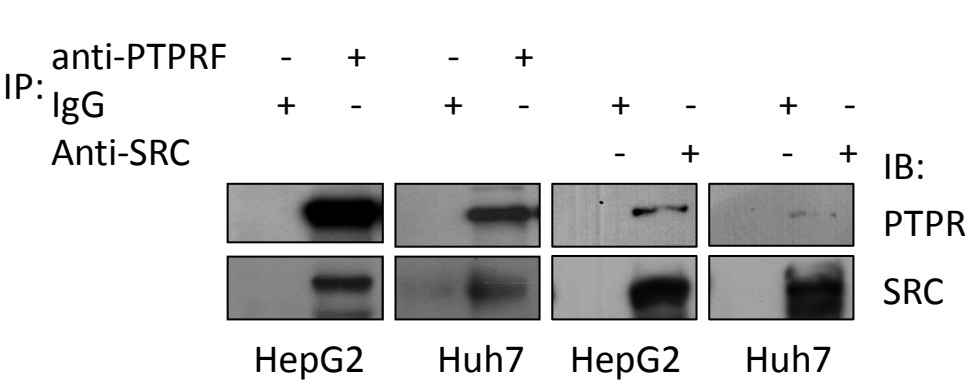
Immunoprecipitation

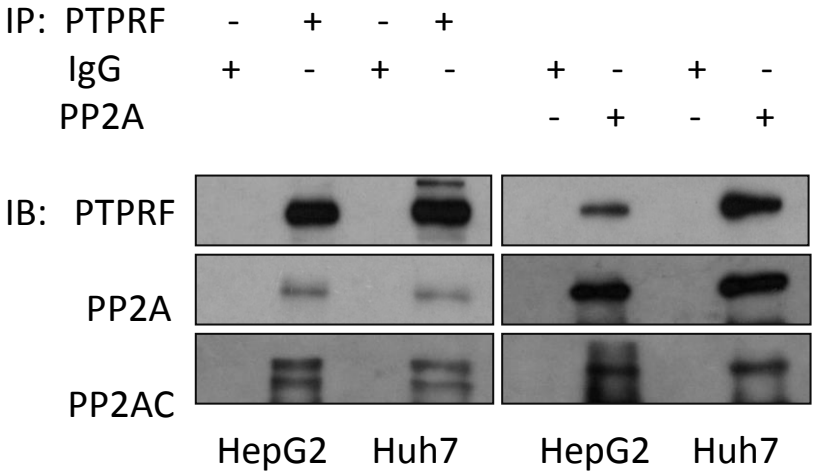




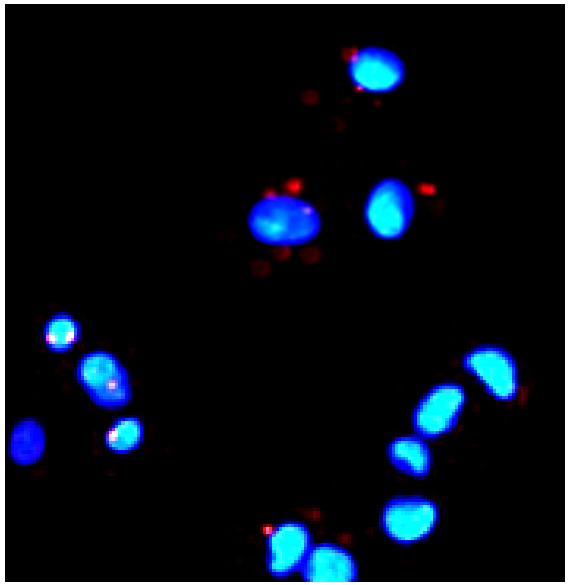
Rabi et al., Hepatology 2014

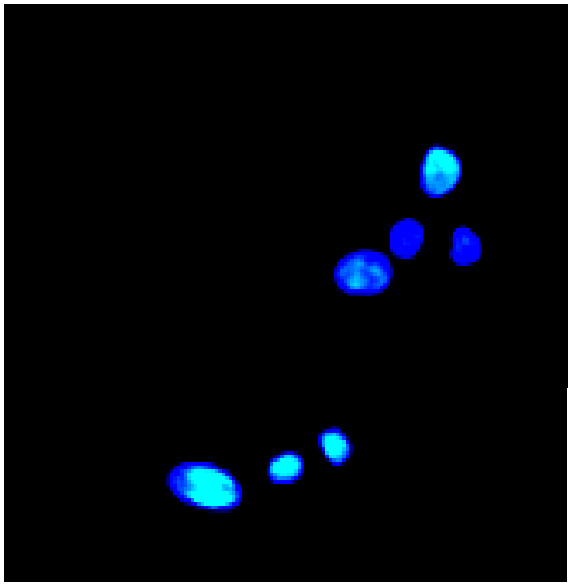
Reciprocal immunoprecipitation

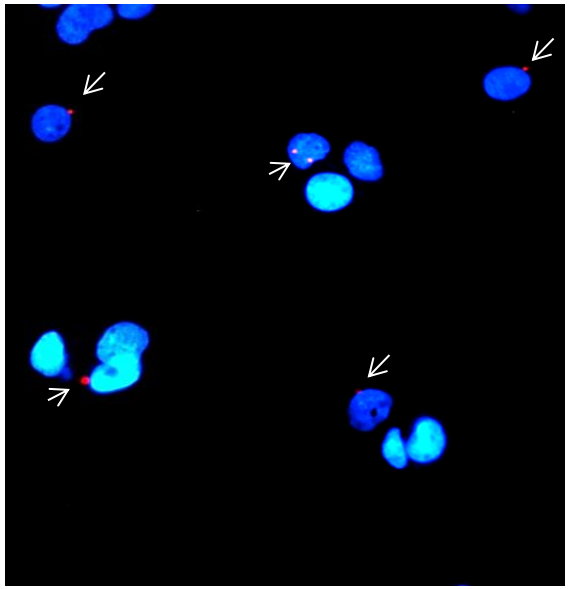




Proximity ligation assays

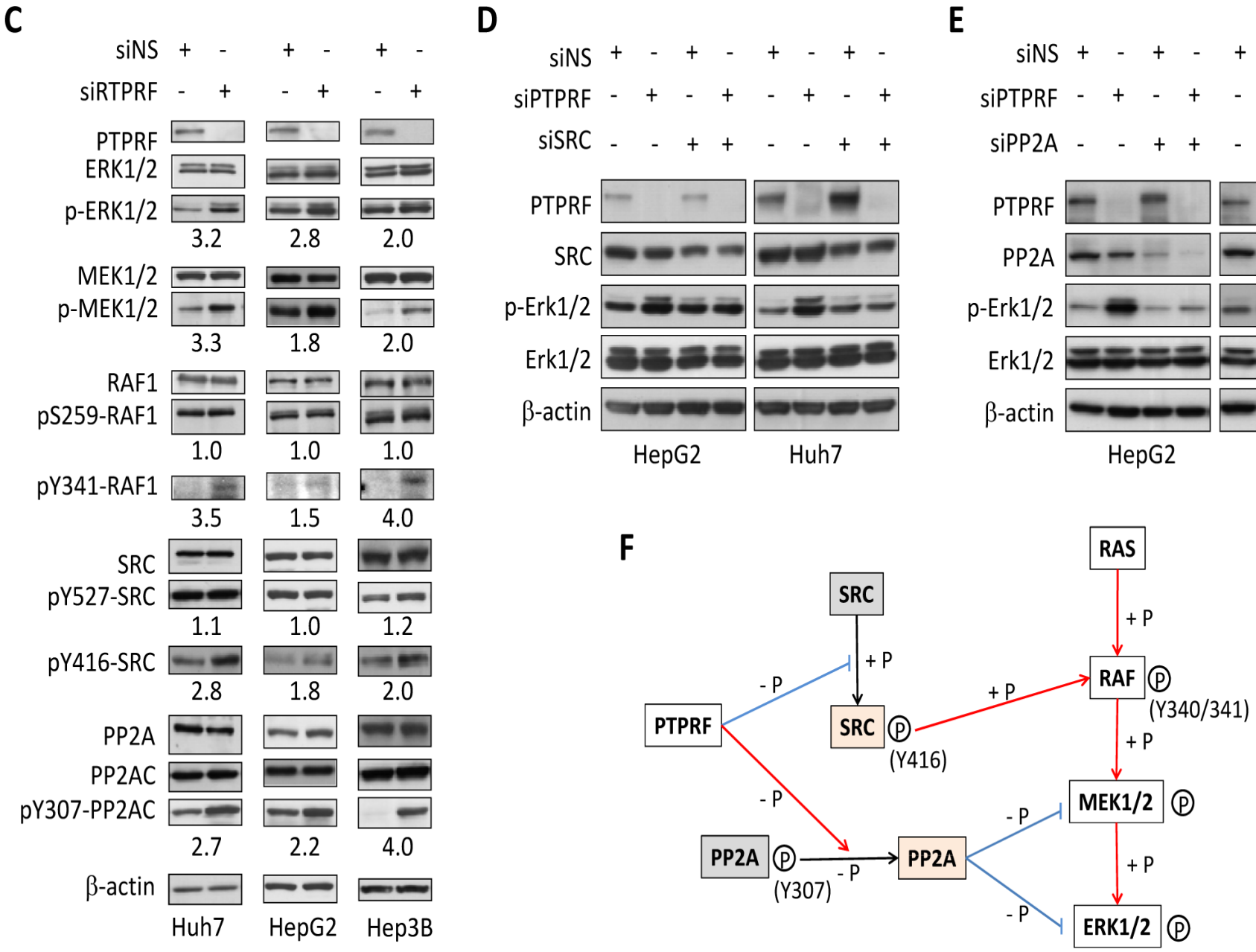






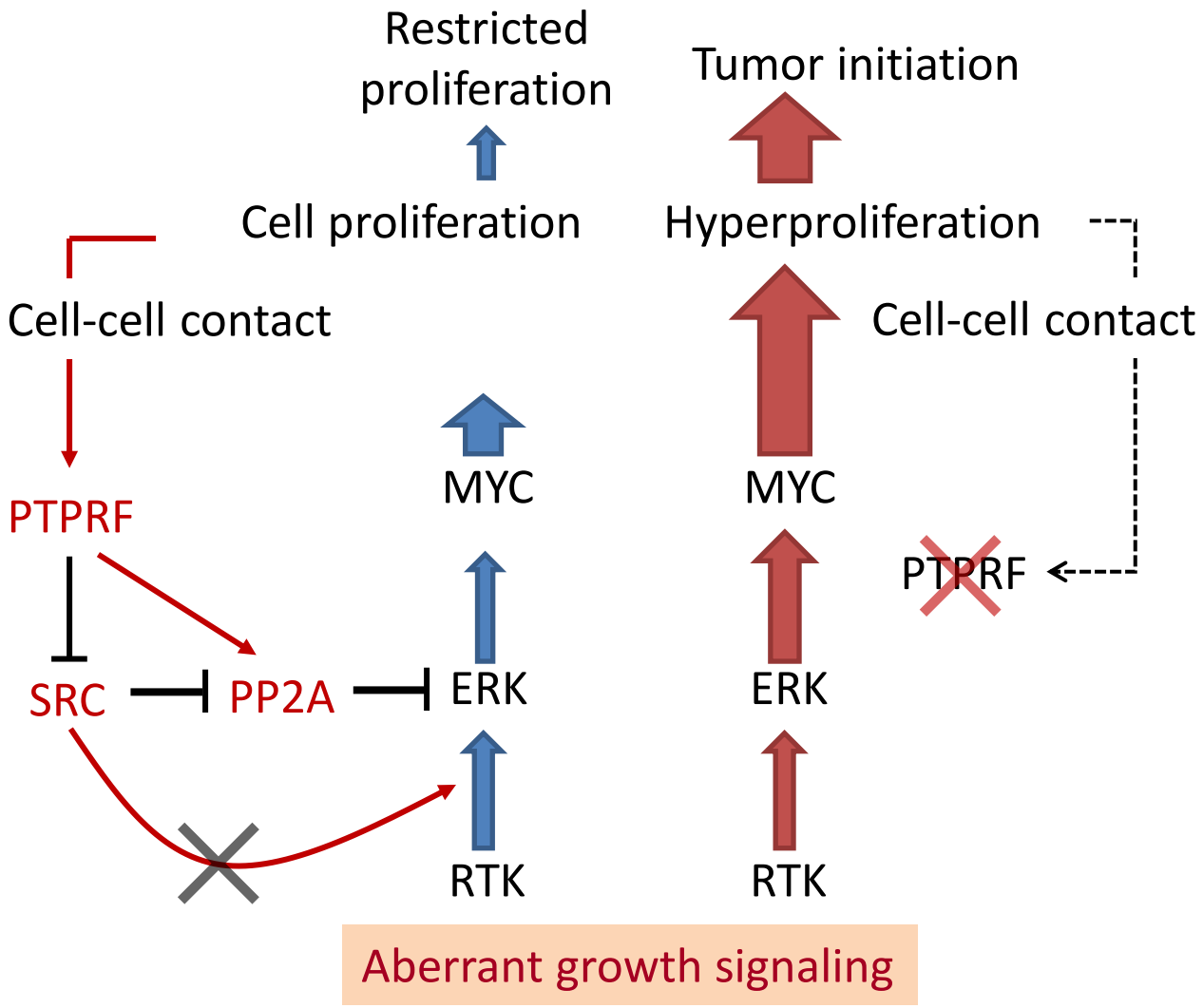
Abs: PTPFR+SRC PTPRF+ACTB PTPRF+PP2A

Rabi et al., Hepatology 2014

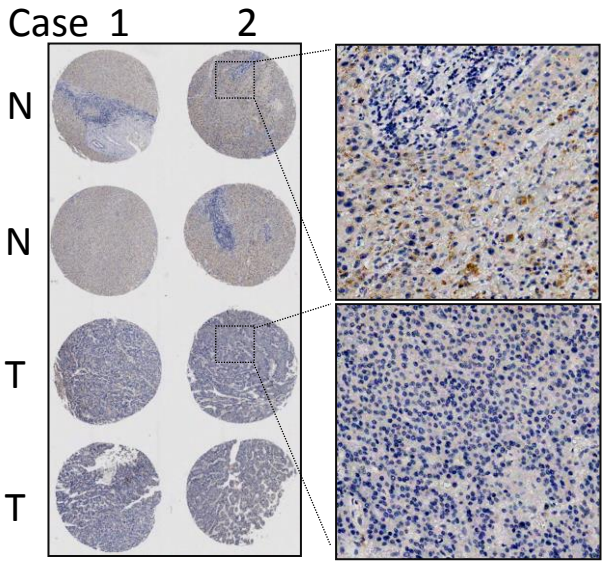


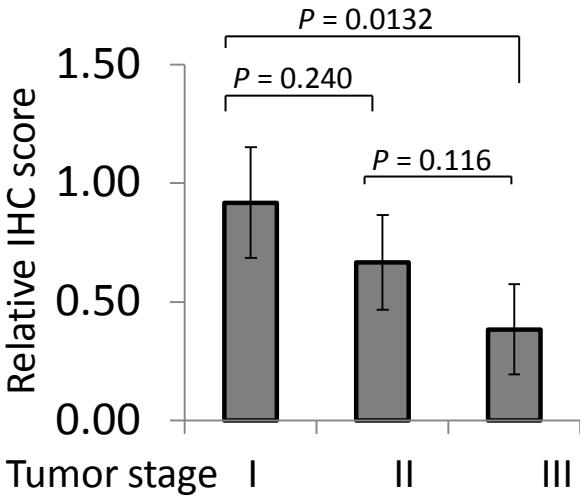
Rabi et al., Hepatology 2014

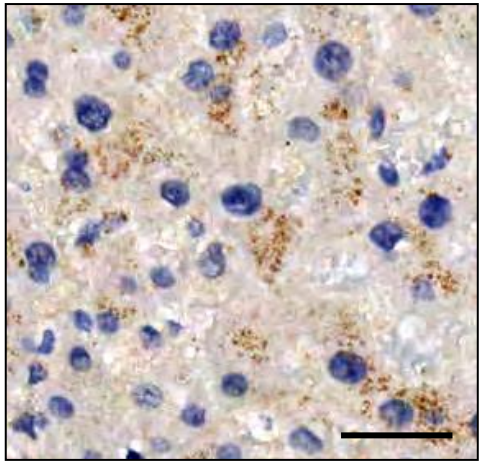
A novel PTPRF-mediated contact inhibition pathway



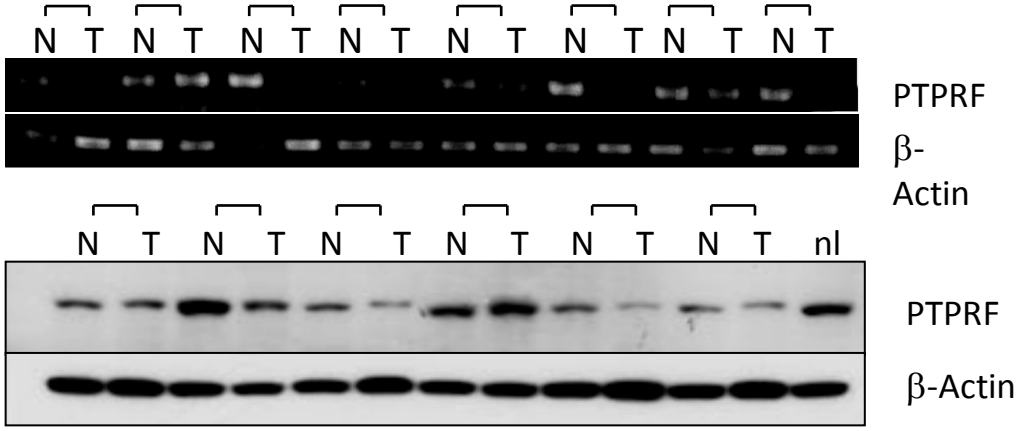
PTPRF downregulated in ~40% of HCC
upregulated in ~22% of HCC

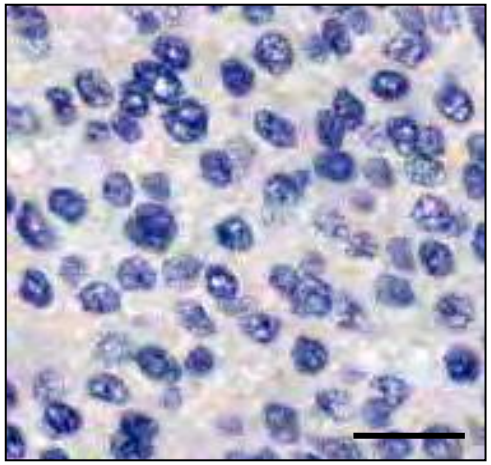






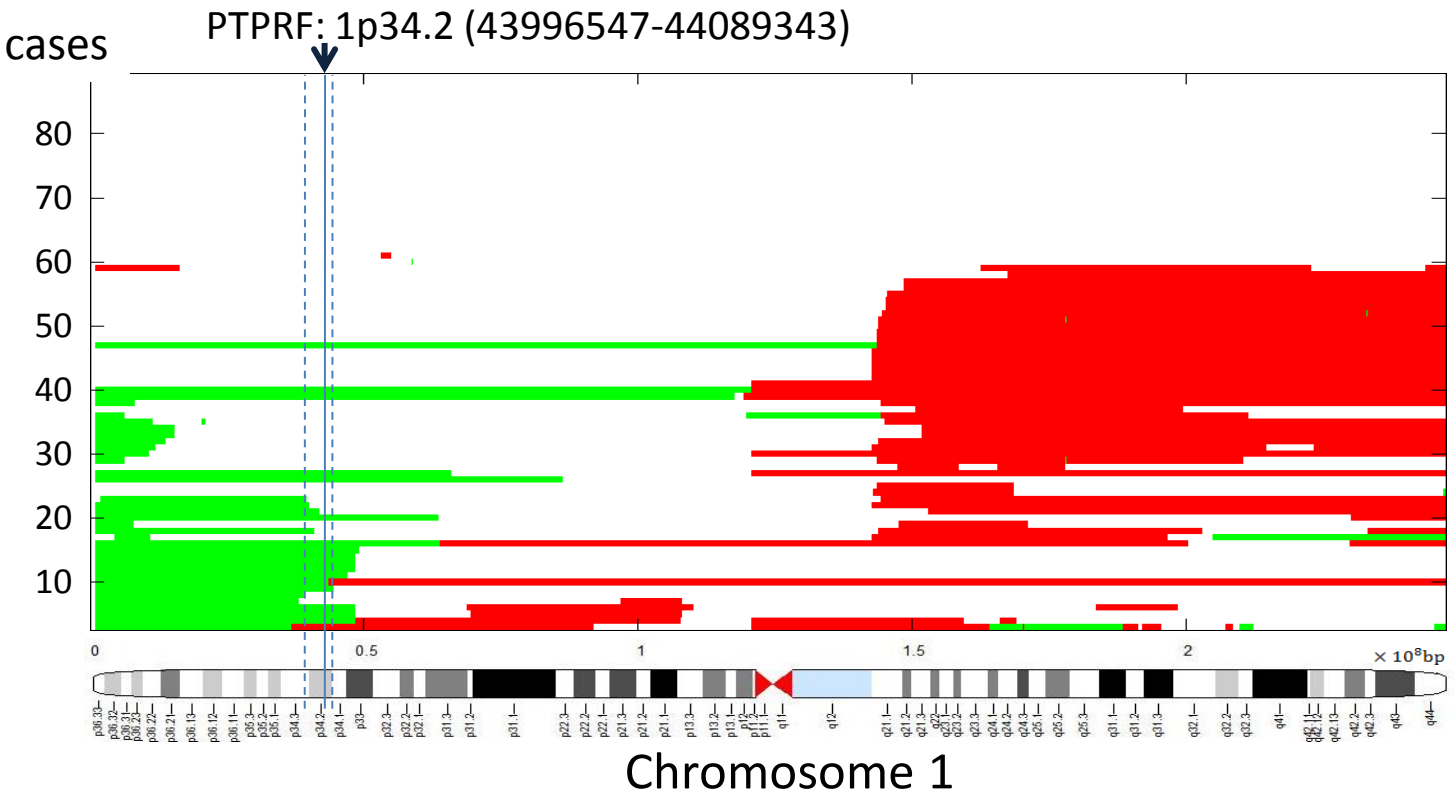
Non-tumor





HCC

The *PTPRF* gene frequently deleted in HCC (20.5%)

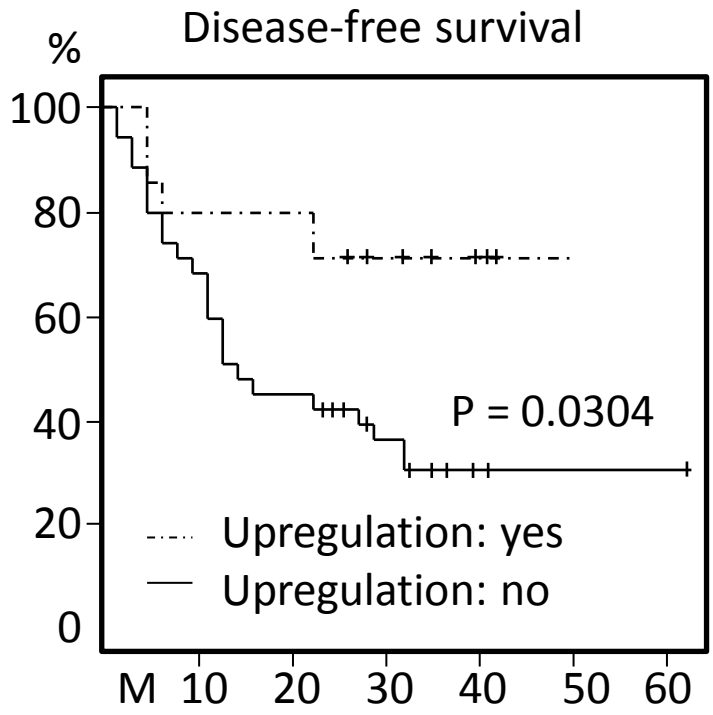


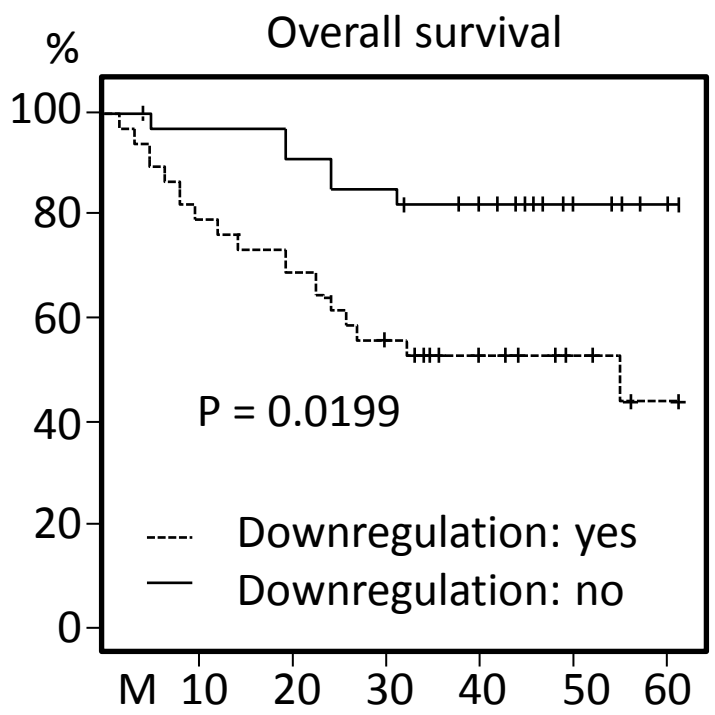
Gene copy variation was determined by using array CGH

Rabi et al., Hepatology 2014

PTPRF downregulated ➔ lower survival

upregulated ➔ lower recurrence & higher survival





Rabi et al., Hepatology 2014

Summary

- The *PTPRF* gene
  - Frequently deleted in human HCC ~20.5%
  - Frequently downregulated in human HCC ~40%
  - Frequently downregulated in human CRC ~100%
  - Frequently downregulated in human GC ~66%
- PTPRF downregulation
  - Prediction of worse clinical outcomes

➔ PTPRF is a TSG!

Conclusions

- A novel PTPRF-SRC/PP2A-ERK-MYC negative-feedback loop prevents overproliferation of cells
- The PTPRF-ERK-MYC pathway may involve in tissue homeostasis
- Disruption of this contact inhibition mechanism leads overproliferation and tumor initiation