Induction of MAP kinase phosphatase 3 through Erk/MAP kinase activation in three oncogenic ras (H-, K- and N-Ras)-expressing NIH/3T3 mouse embryonic fibroblast cell lines

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Legends for supplementary figures

Supplementary Fig. S1. Effect of oncogenic K- (A) and N-Ras (B) on subcellular localization of pErk and MKP3. The expression levels of pErk, Erk and MKP3 in 30 µg of prefractionated cytosol fraction (CF) and nuclear fraction (NF) from the NIH/3T3/H-Ras/G12V cultured with 0.5% bovine serum for 24 h and then incubated with 2 µg/ml doxycycline for additional time (12, 24, and 48 h) followed by culturing in either the absence or the presence of hFGF-basic (50 ng/ml) for 30 min were monitored by immunoblot analysis. α-Tubulin and lamin B were used as the controls to confirm the presence of cytosol and nucleus.

Supplementary Fig. S2. Effect of the oncogenic Ras expression on MKP3 induction. Band intensities for MKP3 and α-tubulin were quantified and plotted. The strong intensity ratio was calculated as 1. NIH/3T3/H-Ras/G12V (A), NIH/3T3/K-Ras/G12V (B) and NIH/3T3/N-Ras/G12V (C) cells were cultured with 0.5% bovine serum for 24 h and
then incubated with 2 µg/ml doxycycline for additional time (12, 24, and 48 h) followed by culturing in either the absence or the presence of hFGF-basic (50 ng/ml) for 30 min.

Supplementary Fig. S3. Effect of the oncogenic Ras expression on MKP3 induction. Band intensities for MKP3 and α-tubulin were quantified and plotted. The strong intensity ratio was calculated as 1. NIH/3T3/H-Ras/G12V (D), NIH/3T3/K-Ras/G12V (E), and NIH/3T3/N-Ras/G12V (F) were treated with 2 µg/ml doxycycline for 0, 24, and 48 h in the presence of DMSO, U0126 (25 µM) or LY294002 (25 µM). After collecting the cells, influence of oncogenic Ras on MKP3 induction was monitored.

Supplementary Fig. S4. Role of Erk signaling in oncogenic Ras-induced MKP3 expression tested with the specific H-Ras effector loop mutants that each engages only one effector pathway. Band intensities for MKP3 and α-tubulin were quantified and plotted. The strong intensity ratio was calculated as 1. NIH/3T3/H-Ras/G12V/T35S, NIH/3T3/H-Ras/G12V/E37G, and NIH/3T3/N-Ras/G12V/Y40C were treated with 2 µg/ml doxycycline for 0, 24, 48, and 72 h. After collecting the cells, influence of oncogenic the specific H-Ras effector loop mutants on MKP3 induction was monitored.
NIH/3T3/K-Ras/G12V

hFGF basic (-) | hFGF basic (+)

Dox (2 μg/ml)  
0 12 24 48  

NIH/3T3/N-Ras/G12V

hFGF basic (-) | hFGF basic (+)

Dox (2 μg/ml)  
0 12 24 48  

α pErk  
α Erk  
α MKP3  
α α-Tubulin  
α Lamin B

Fig. S1. Koo et al.
Fig. S3. Koo et al.
