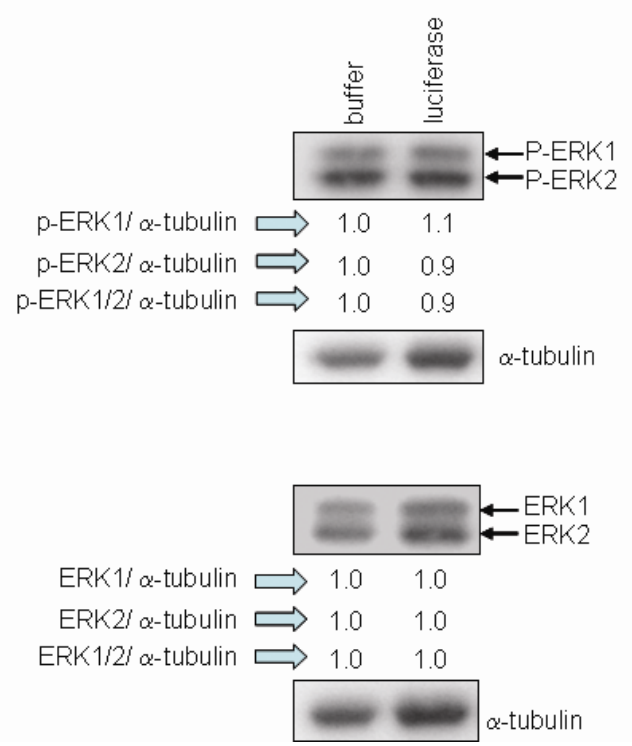
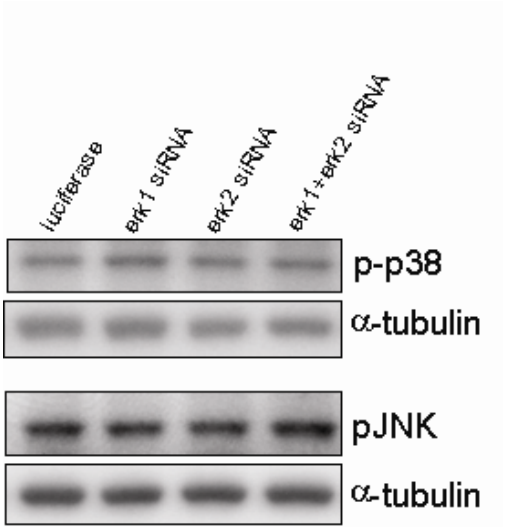
Supplemental Data

Supplemental Fig. 1



Suppl. Fig. 1 Luciferase control siRNA transfection does not alter protein expression level of p-ERK1/2 and ERK1/2 in HLFs. Transfection with either luciferase control siRNA at 1.5 nmole or 1X siRNA buffer was performed as described in Fig.1 and expression of p-ERK1/2 and ERK1/2 was examined by immunoblotting on protein lysates isolated from transfected HLFs at 72 hr post-transfection. One representative blot from 2 experiments is shown and densitometric analyses on p-ERK1, p-ERK2, p-ERK1/2, ERK1, ERK2, and ERK1/2 are shown as numbers under the blot for indicated protein after normalization by α-tubulin level.

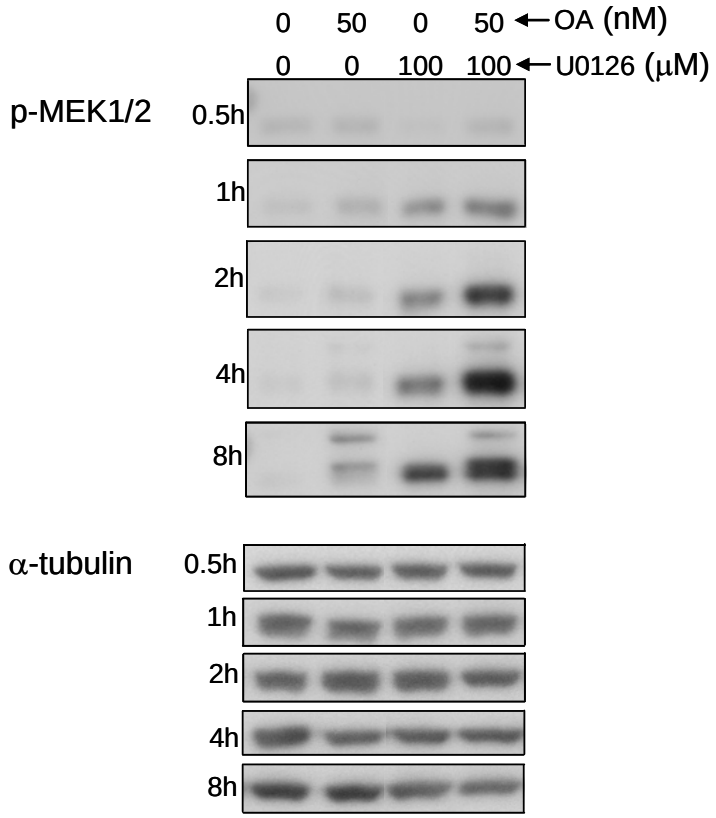
Supplemental Fig. 2



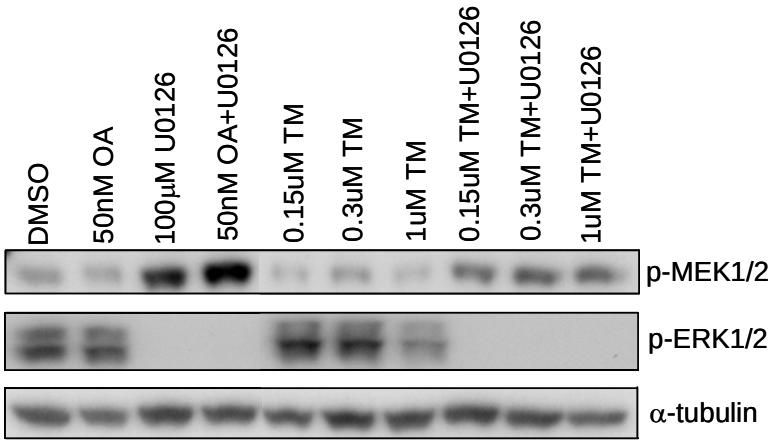
Suppl. Fig. 2 Level of activating phosphorylation of p38 and JNK was not altered after ERK silencing in HLFs. Transfection was carried out as described in Fig.1 and expression of p-p38(thr180/tyr182) and p-JNK (thr183/tyr185) was examined by immunoblotting on protein lysates isolated from transfected HLFs with indicated siRNA at 72 hr post-transfection.

Supplemental Fig. 3

(A)



(B)



Suppl. Fig. 3 Protein phosphatase 2A is involved in MEK hyperactivation after MEK inhibitor treatment in untransfected HLFs. (A) HLFs at 24 hr post-seeding were treated with 50 nM OA, or 100 μM U0126 alone or in combination for 0.5, 1, 2, 4, or 8 hr, and then protein lysates were isolated for immunoblotting of p-MEK1/2(ser217/221). Similar results were obtained in two independent experiments. (B) HLFs at 24 hr post-seeding were pre-treated with OA or TM at indicated concentration for 0.5 hr and then 100 μM U0126 was added for 2 hr before isolation of protein lysates for immunoblotting of p-MEK1/2 and p-ERK1/2. Similar results were obtained in two independent experiments.