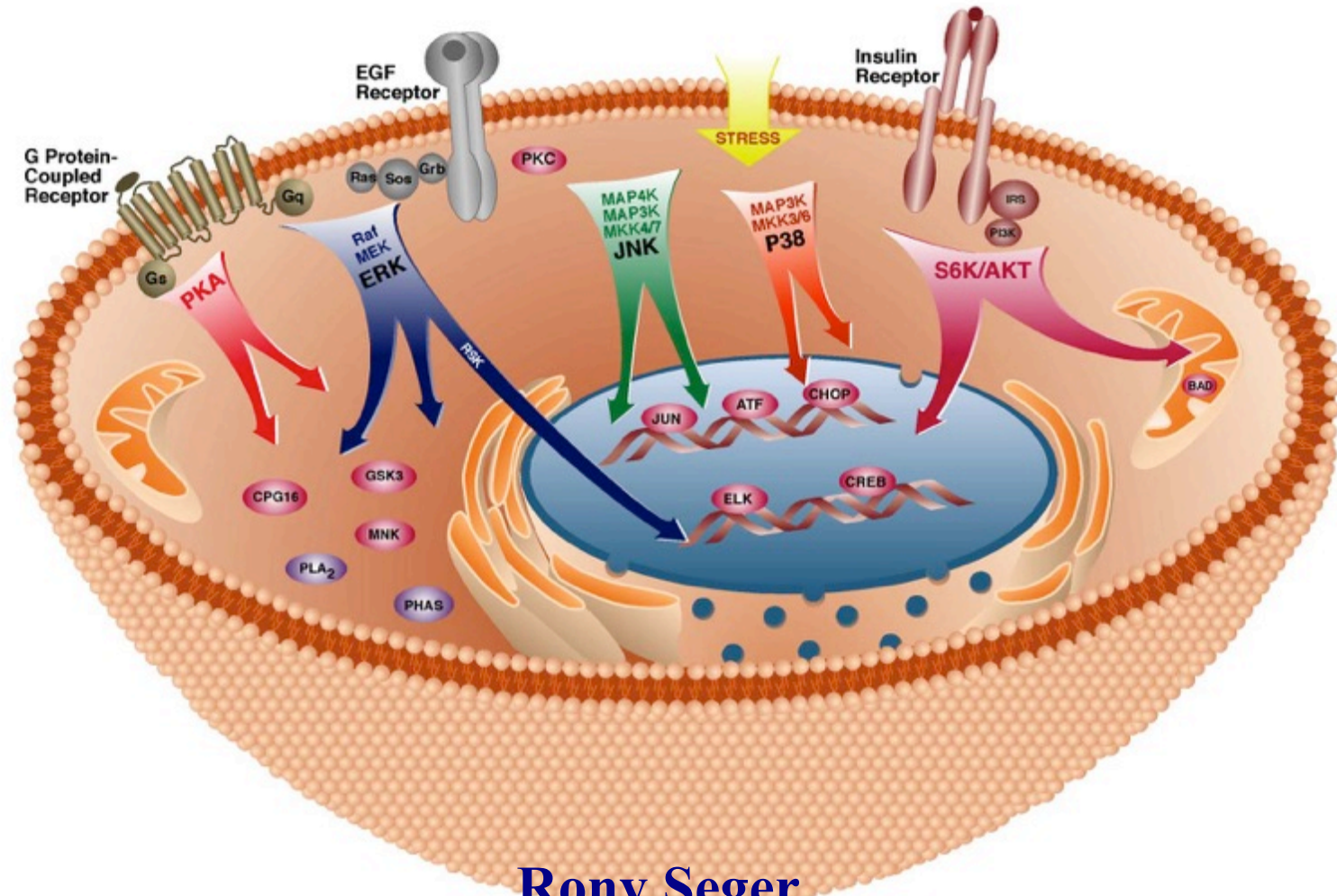


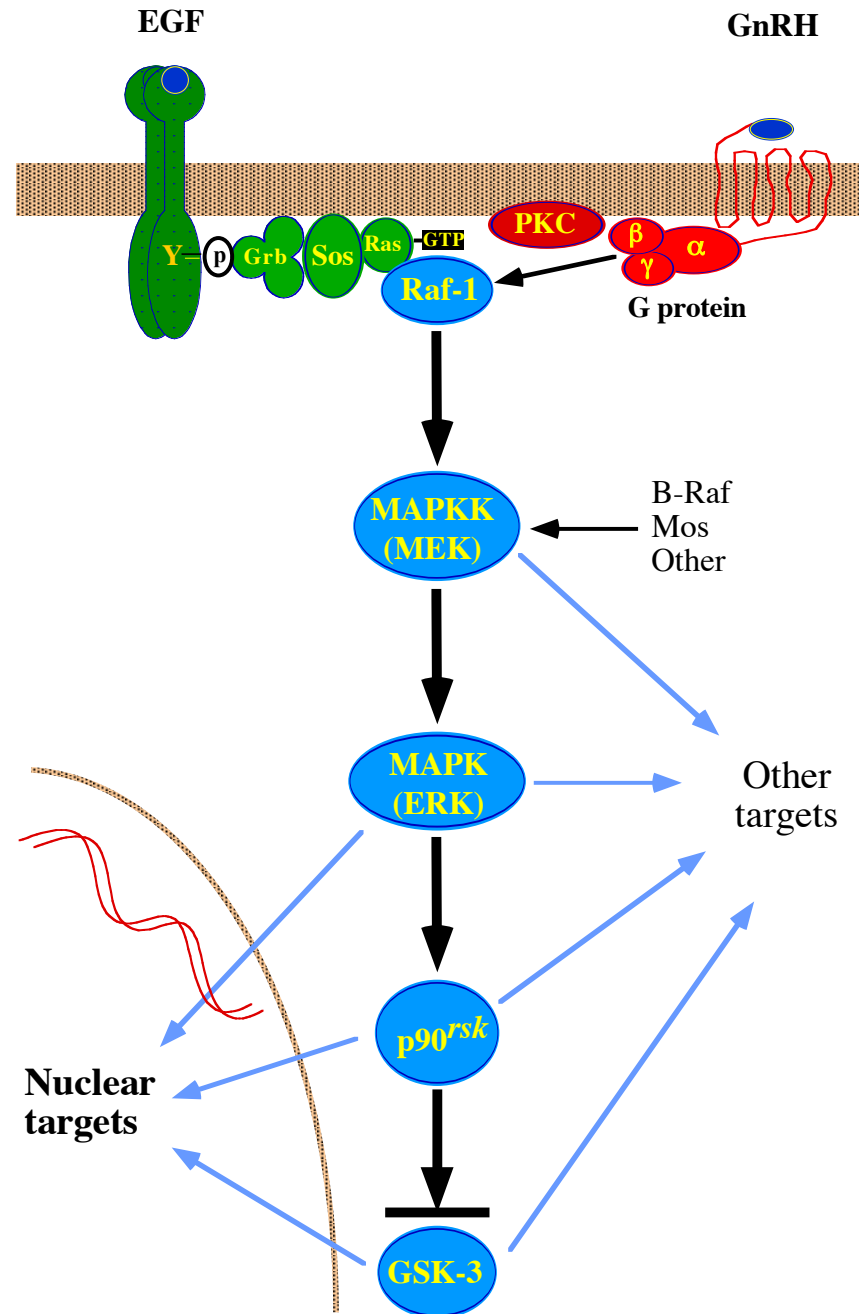
Activation of signaling pathway by ELF-EMF



Rony Seger,
Weizmann Institute of Science



The ERK/MAPK signaling cascade



Physiological roles of the MAPK cascades

1. Proliferation/cell cycle progression (mainly ERK1/2).
2. Differentiation (mainly ERK1/2).
3. Stress response (mainly JNK/p38).
4. Apoptosis (mainly JNK).
5. Learning and Memory, Migration, others.

Pathology:

1. Cancer (mainly ERK1/2 – more than 85%,
but also p38 and JNK).
2. Inflammation (mainly p38).
- 3) Autoimmune diseases (mainly p38).
- 4) Neurological disorders (mainly JNK).
- 5) Developmental diseases (mainly ERK1/2).

Not all activations induce physiological effects.

Use of ERK1/2 as a read-out for cellular response to electromagnetic fields

1) Sensitivity of detection

- The interesting measure is the physiological effects. However those are not always apparent because they do not overcome a threshold of induction.
- Signaling cascades have a very strong amplification mechanisms.
- We developed a very sensitive methods to detect minute amount of signaling.
- Intracellular signaling responds to essentially all stimulations.

2) Possibility to detect upstream components

- ERK activation is well-studied, so we might be able to identify the “antenna” by analogy to other modes of activation.

Measuring MAPK activation by mobile phone irradiation (microwave frequency)- Experimental setup

Equipment

1. Signal generator Fluke Model 6062A
2. Spectrum Analyzer HP model 8650
3. Linear 40 Watts Amplifier
4. Antenna: Kathrein Model 742149 [7dBi]
5. Temperature measurement: zico Zi-9612
6. The incubator walls - covered with absorbing material to decrease reflection

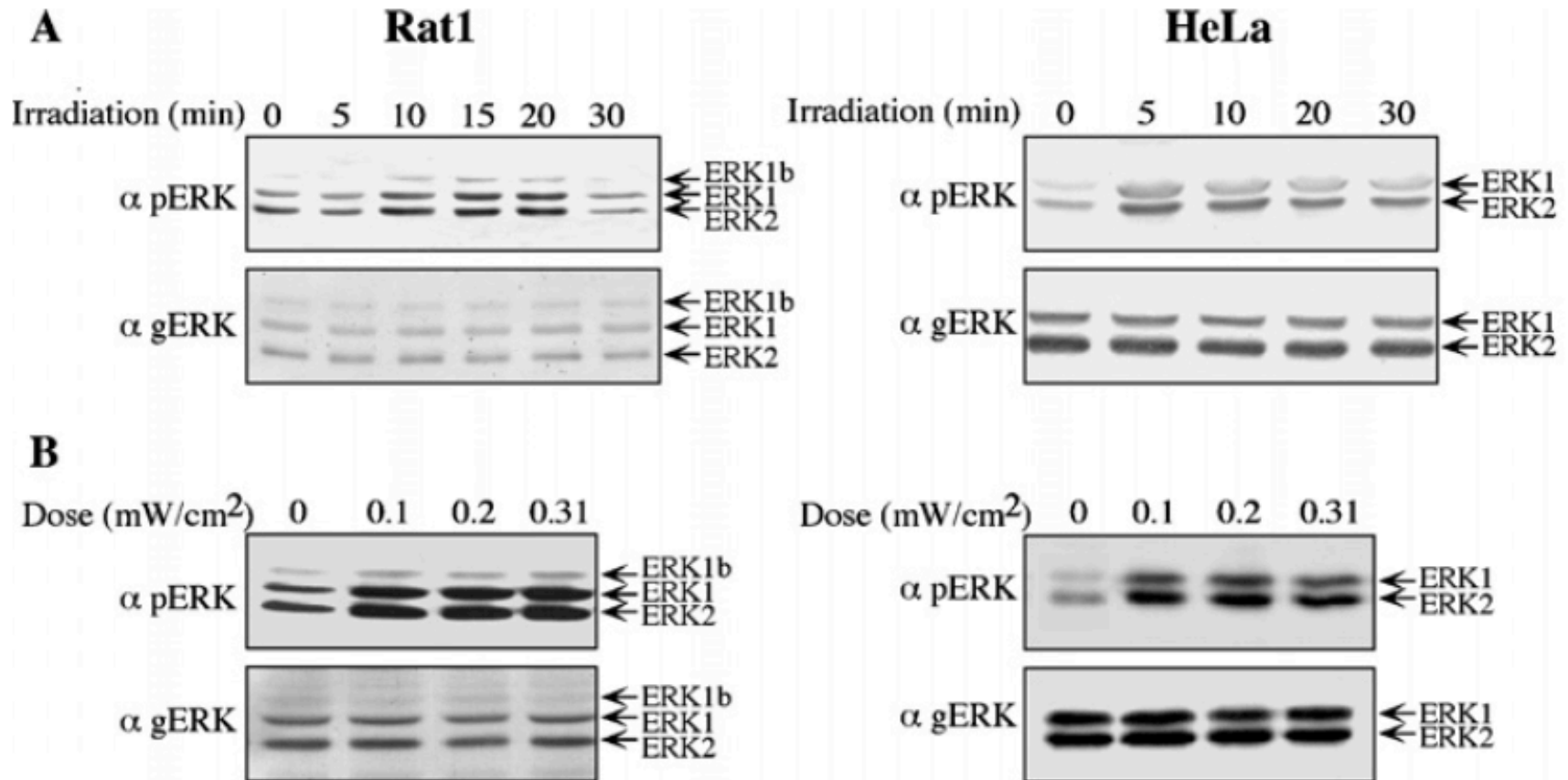
Field uniformity Test

| E | D | C | B | A | Point\ mW/cm ² |
|--------|--------|--------|--------|--------|------------------------------|
| 0.340 | 0.308 | 0.306 | 0.332 | 0.306 | 1 |
| 0.310 | 0.276 | 0.290 | 0.316 | 0.269 | 2 |
| 0.270 | 0.249 | 0.242 | 0.271 | 0.236 | 3 |
| 0.230 | 0.216 | 0.200 | 0.236 | 0.200 | 4 |
| 0.200 | 0.190 | 0.186 | 0.205 | 0.180 | 5 |
| 0.100 | 0.092 | 0.092 | 0.102 | 0.086 | 6 |
| 0.040 | 0.036 | 0.035 | 0.040 | 0.034 | 7 |
| 0.0052 | 0.0047 | 0.0043 | 0.0052 | 0.0043 | 8 |

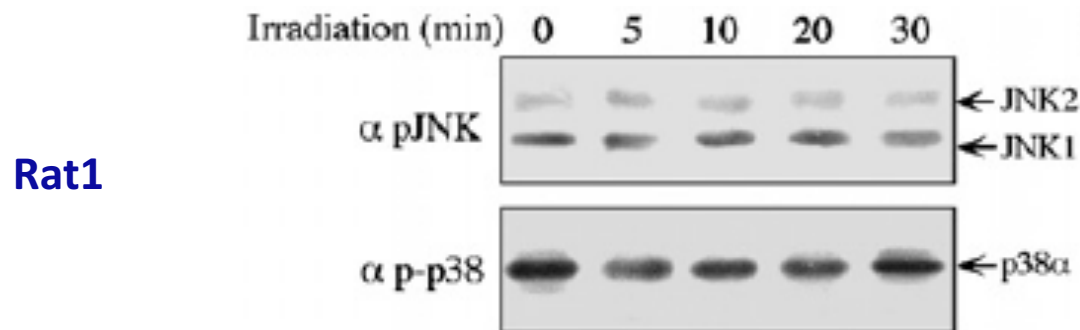
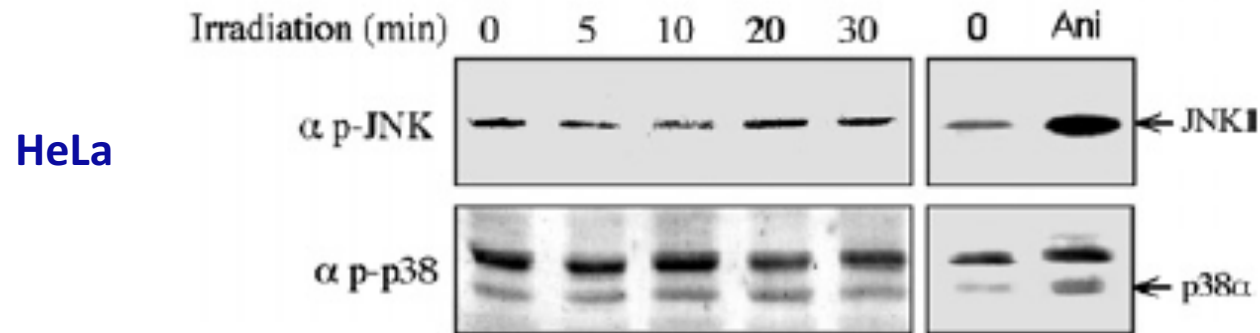


Friedman J. Biochem J. 2007

Mobile phone irradiation induces ERK phosphorylation in Rat1 and HeLa cells

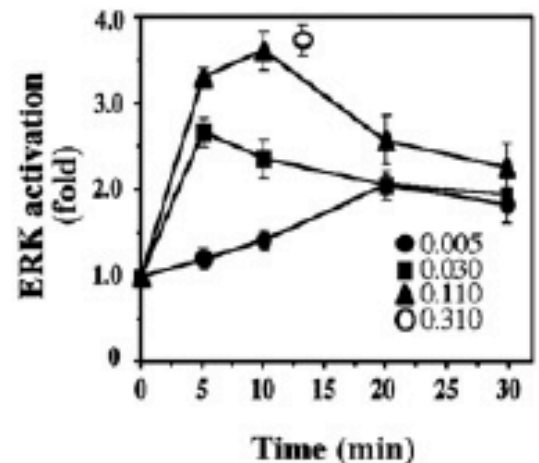
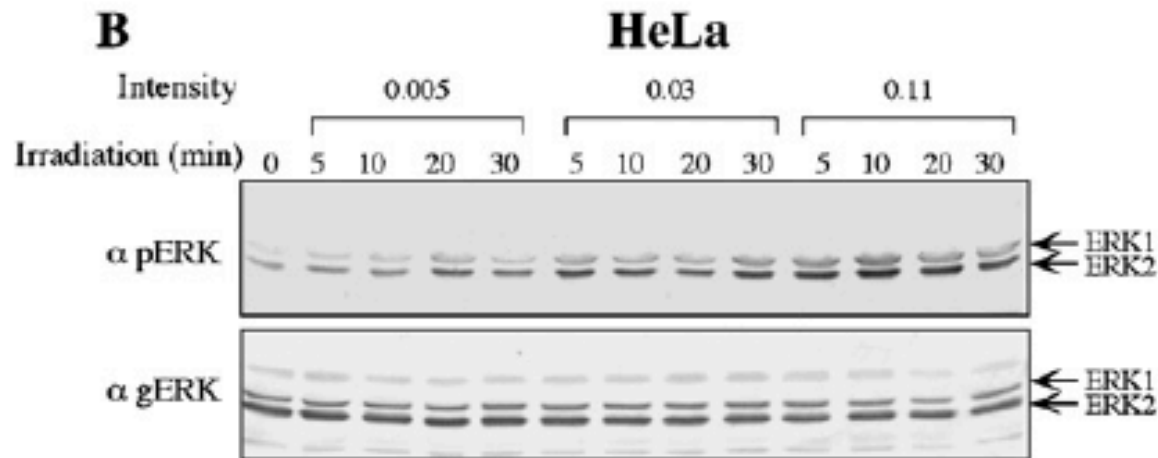
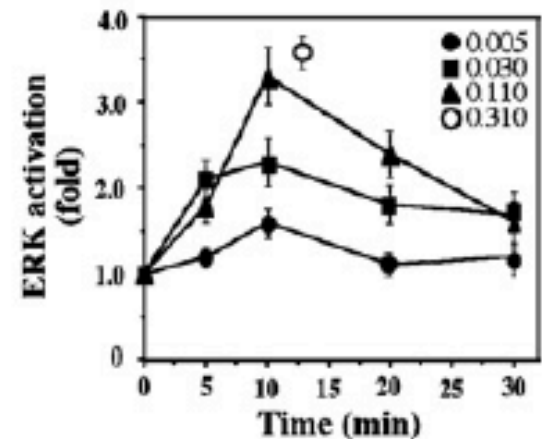
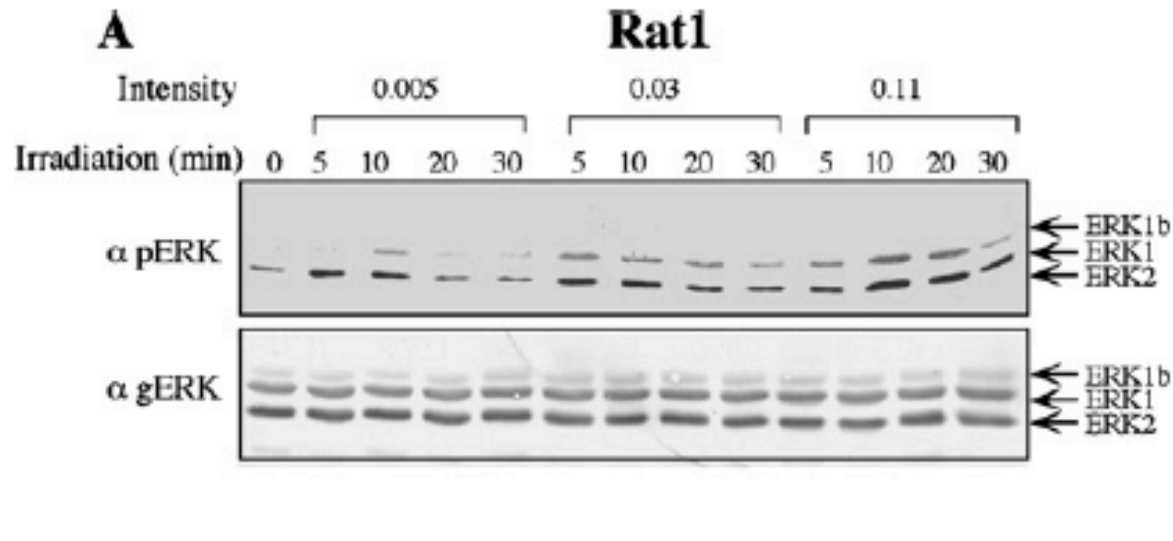


Mobile phone irradiation does not induce phosphorylation of the stress-activated MAPKs JNK and p38

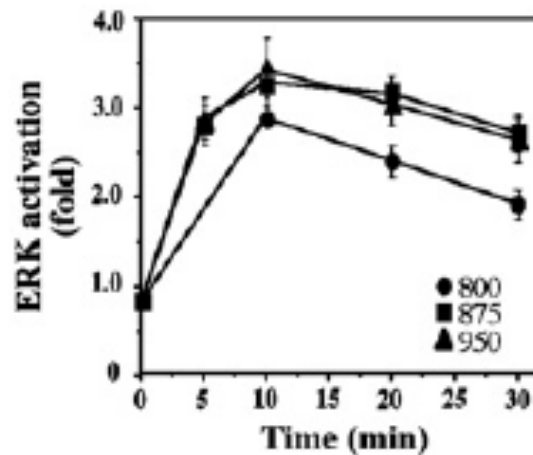
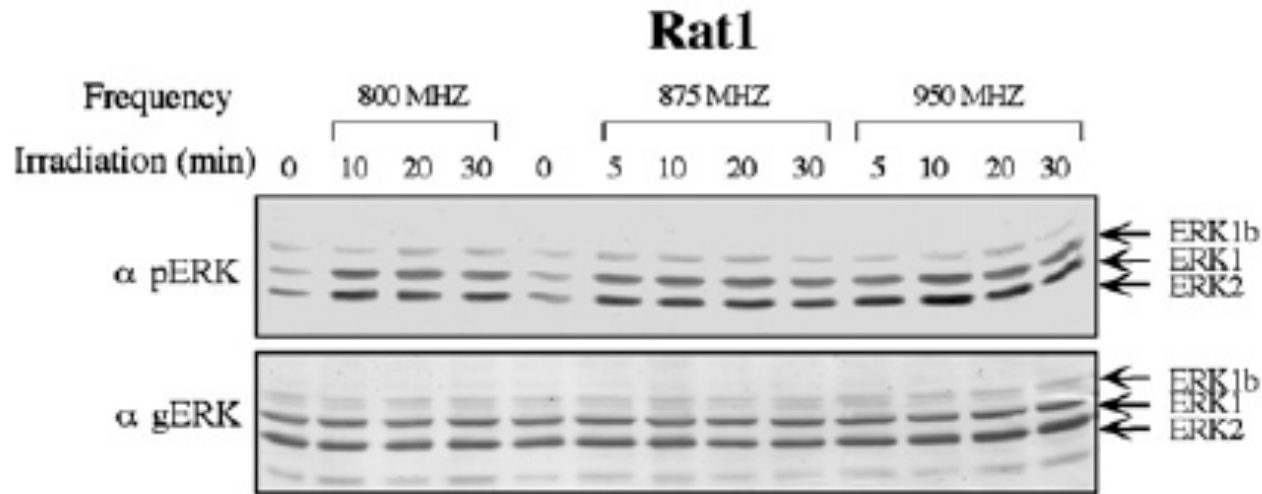


0.3 mW/cm²

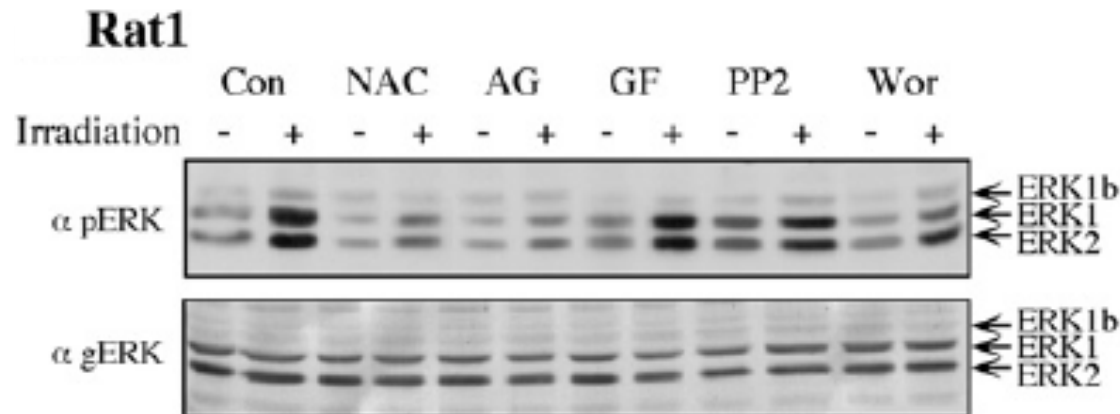
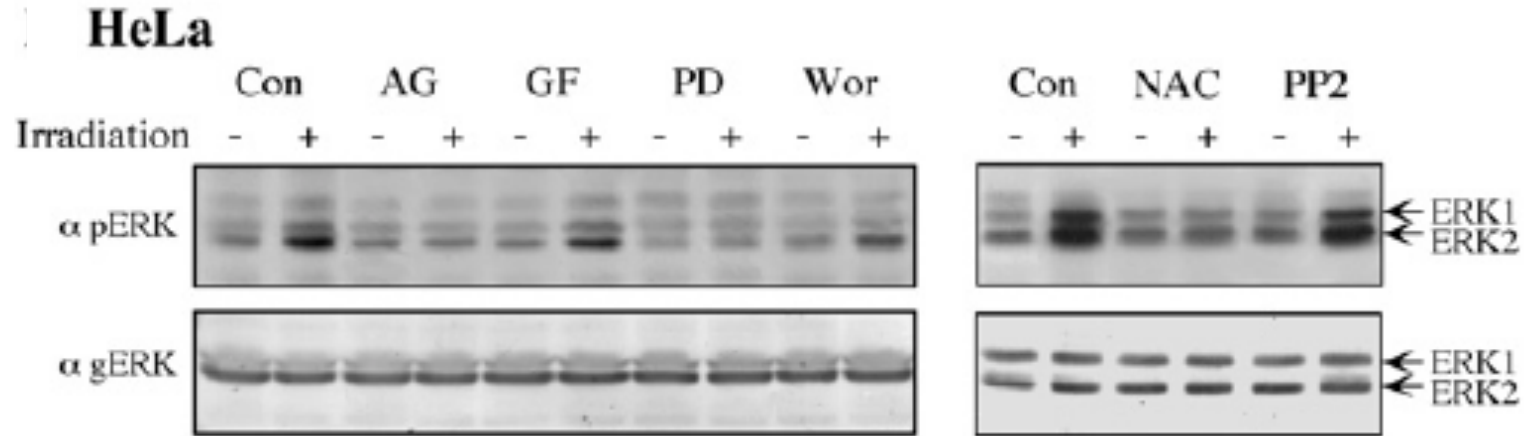
Kinetics of ERKs phosphorylation upon mobile phone irradiation (1)



Kinetics of ERKs phosphorylation upon mobile phone irradiation (2)



Use of inhibitors and to identify mediators of irradiation-induced ERK phosphorylation



Inhibitor - Target

AG - EGFR

NAC - ROS

GF - PKC

PP2 - c Src

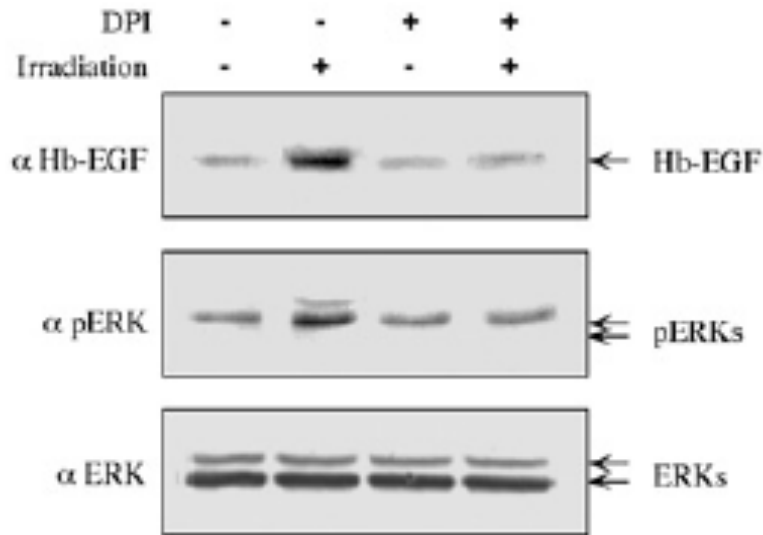
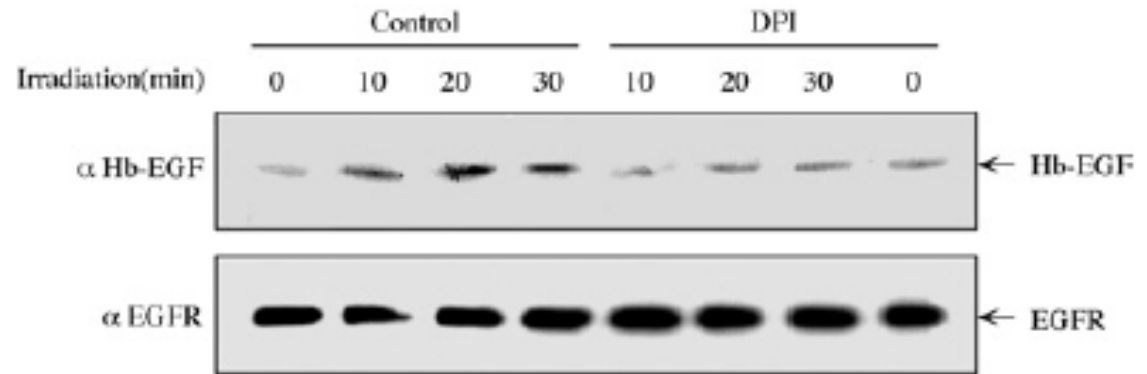
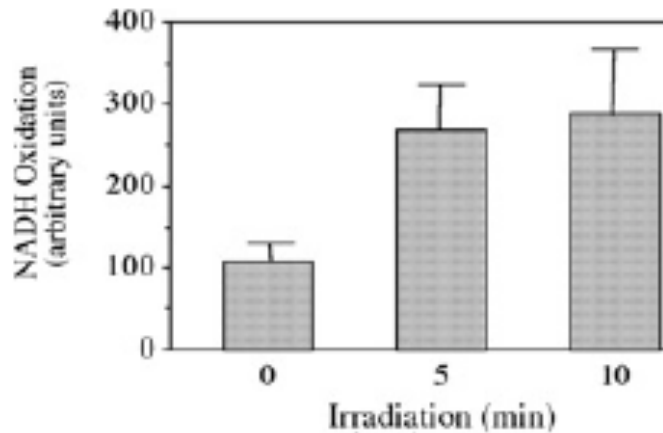
Wor - PI3K

PD - MEK

Con - Control

Activation by: Ros, EGFR and MEK but not much by:
PKC, cSrc or PI3K

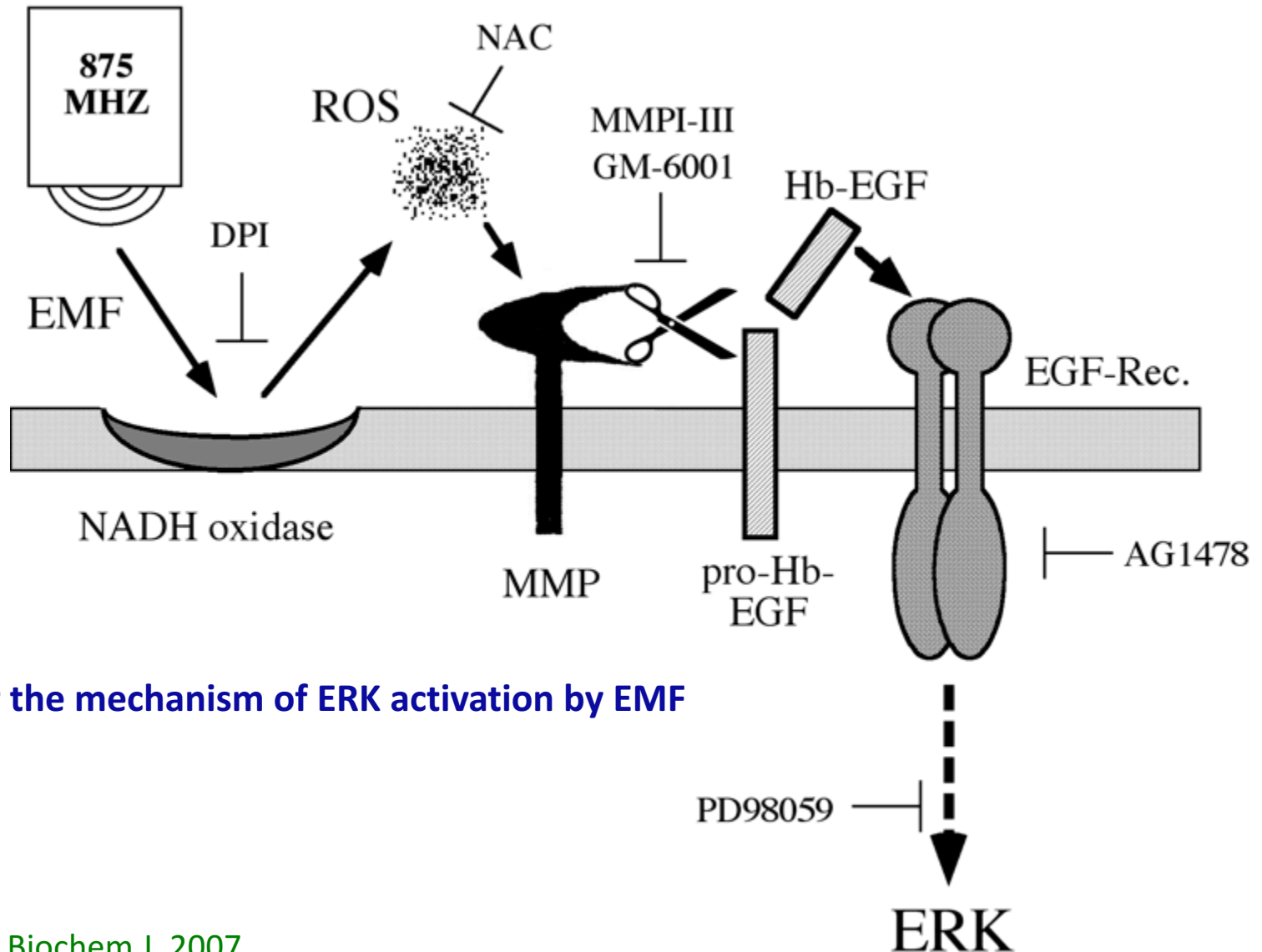
The irradiation effect is mediated by NADH oxidase



DPI - NADH oxidase inhibitor

The electron donor in the Membrane is probably **hydroquinone** (no NADH in membranes; (Kishi et al, 1999))

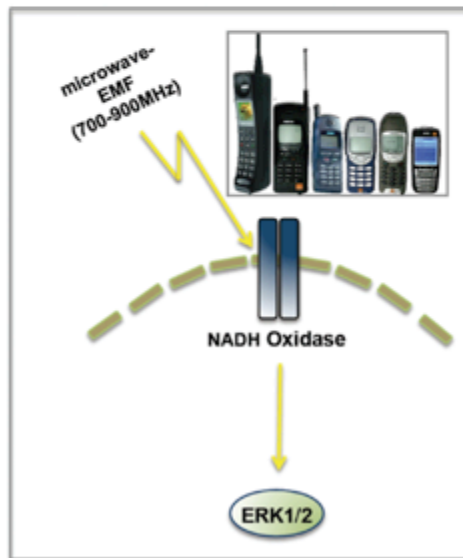
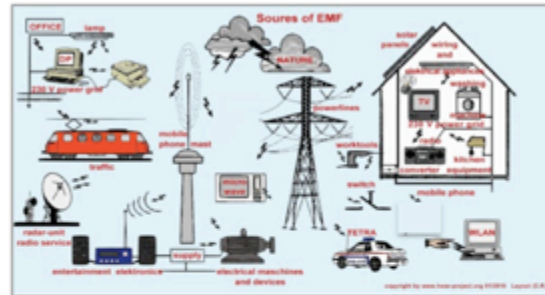
Mechanisms of upstream activation of ERK by ELF-MF



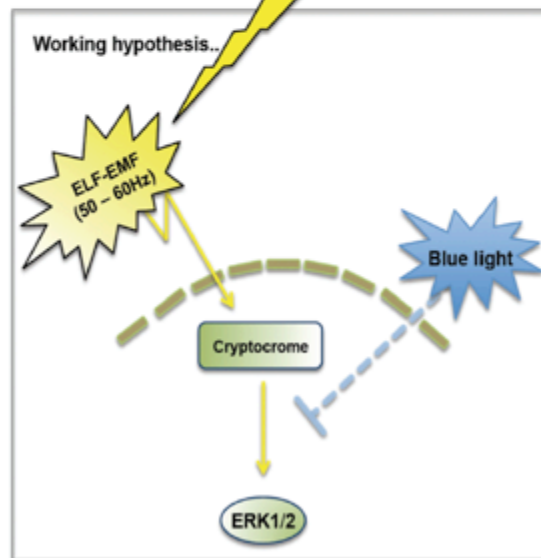
Model for the mechanism of ERK activation by EMF

Sensing Electromagnetic Fields – The Case of Extremely Low Frequency Magnetic Fields (ELF-MF)

How Do Cells Sense Electromagnetic Fields?



Microwave



ELF-MF

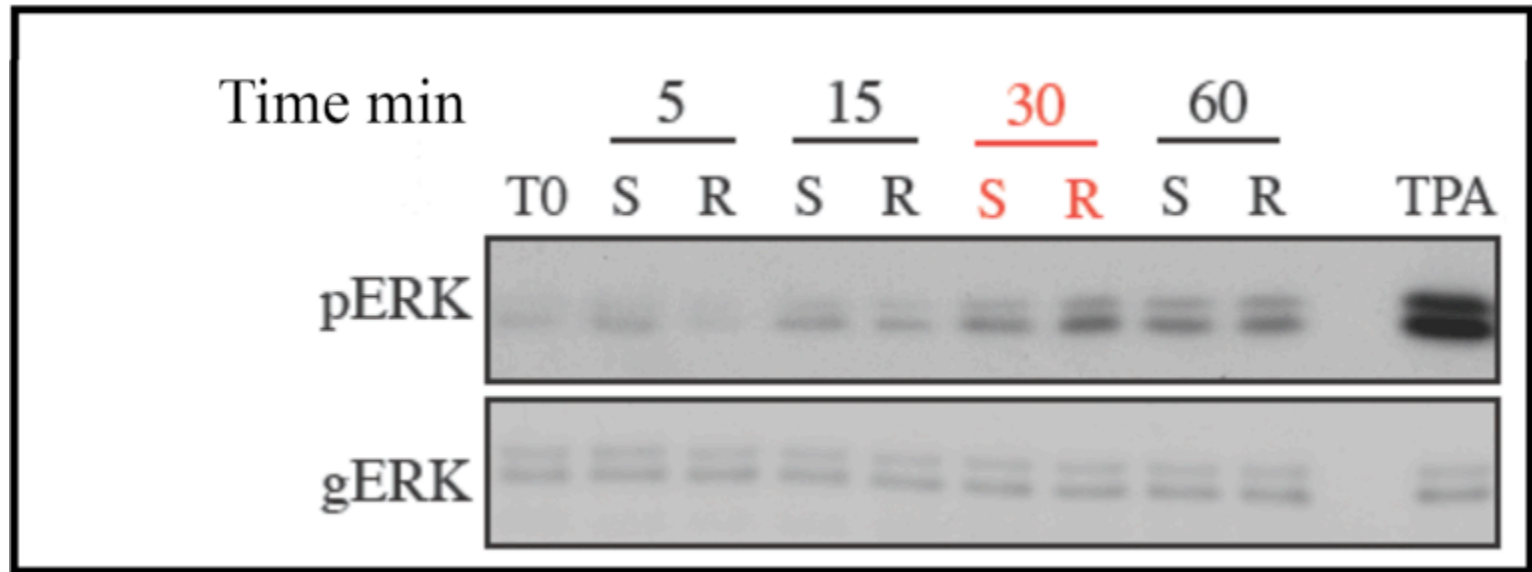
Effect of **ELF-EMF** on MAPK activation- **Study within the ARIMMORA consortium**



In collaboration with ITIS, Zurich, Switzerland
Manuel Murbach, Patricia Bounds, Niels Kuster

ERK phosphorylation is moderately induced by ELF-EMF

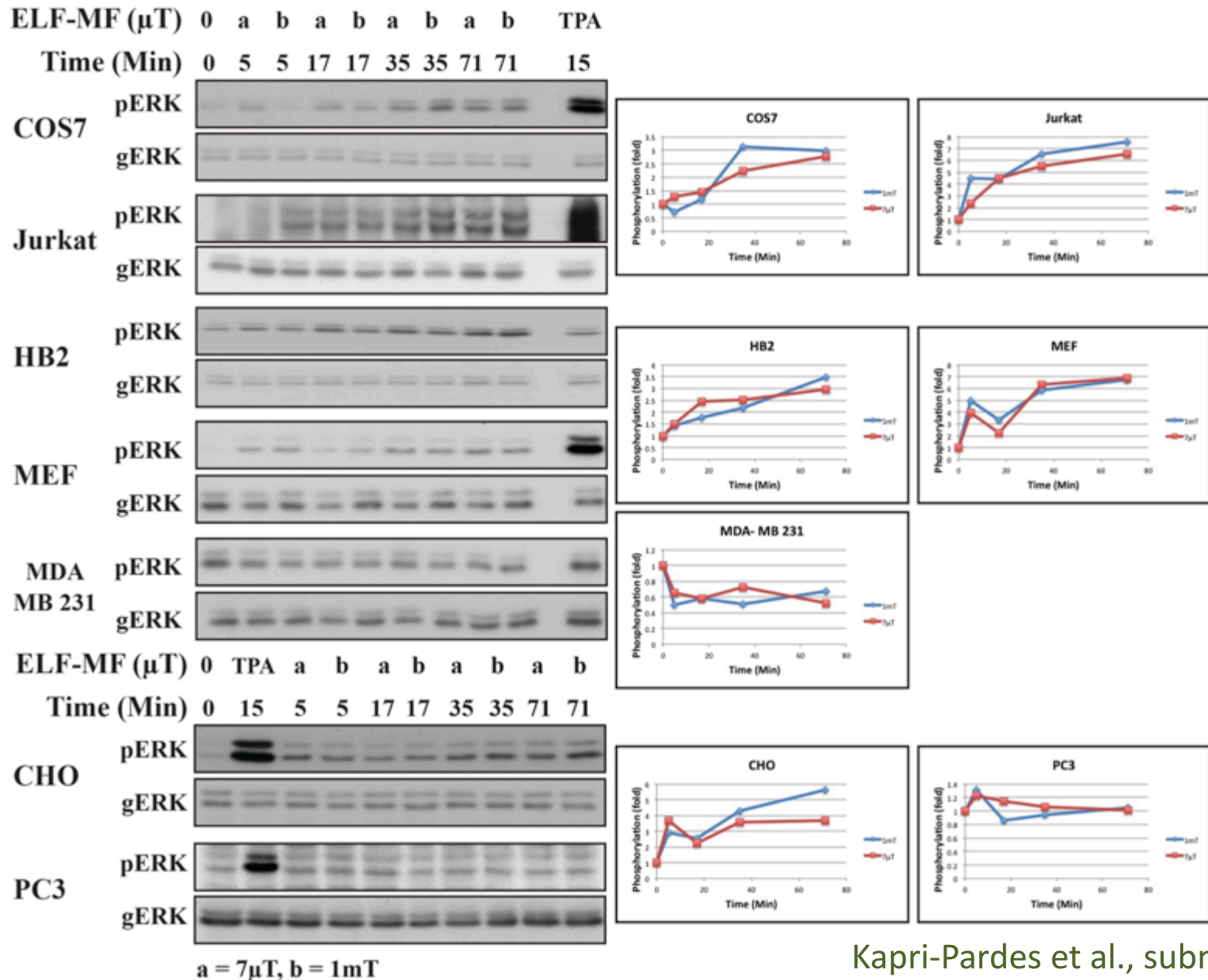
COS7 Cells, 50Hz, 7 μ T-1mT EMF Exposure



Maximal activation within 60 min: **3.6 ± 0.09**

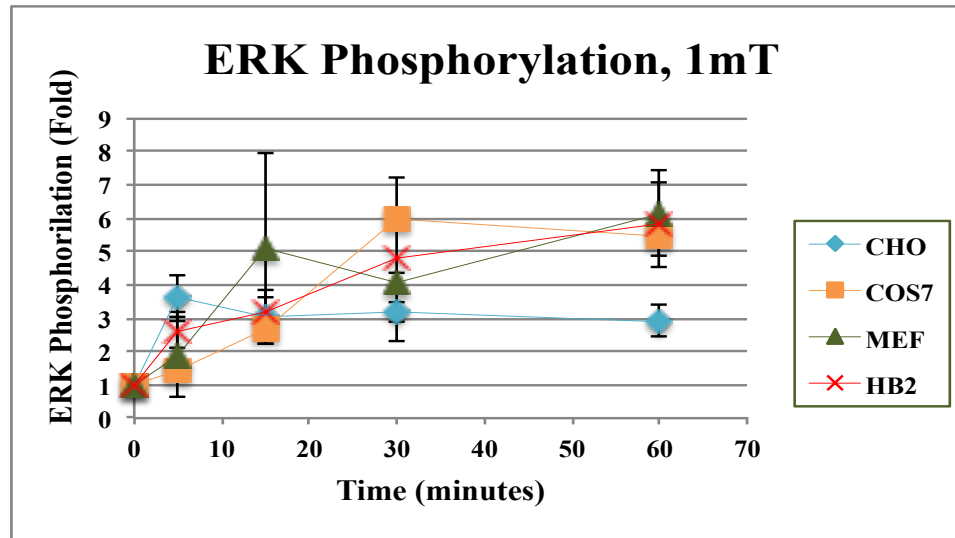
, S – 7 μ T, R – 1 mT

Activation of ERK by ELF-MF (50 hertz)

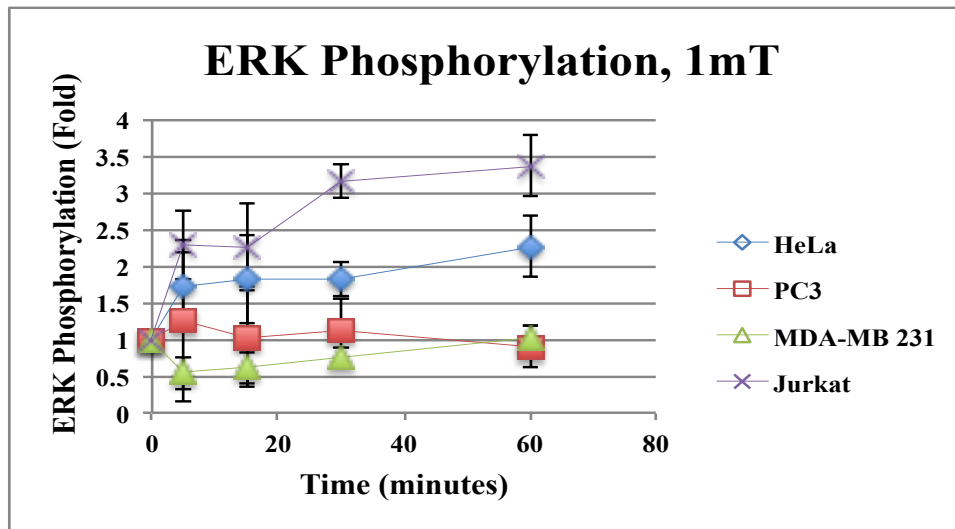


More detailed screen (1)

(A)

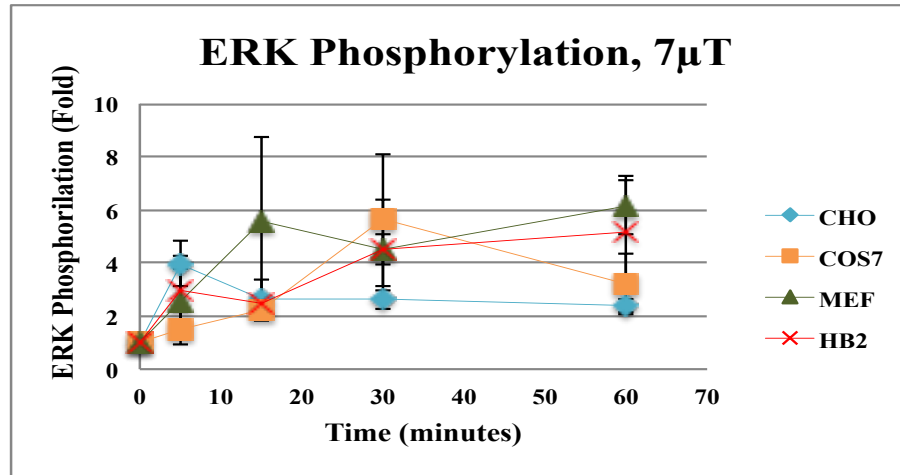


(B)

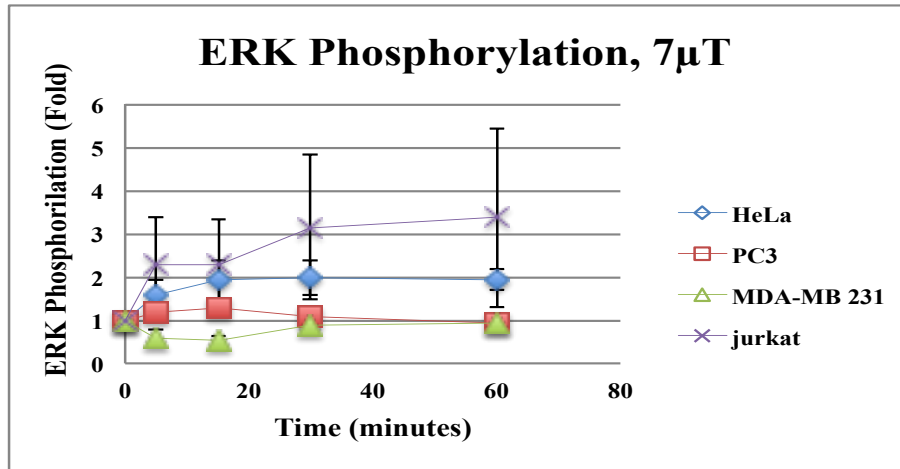


More detailed screen (2)

(A)

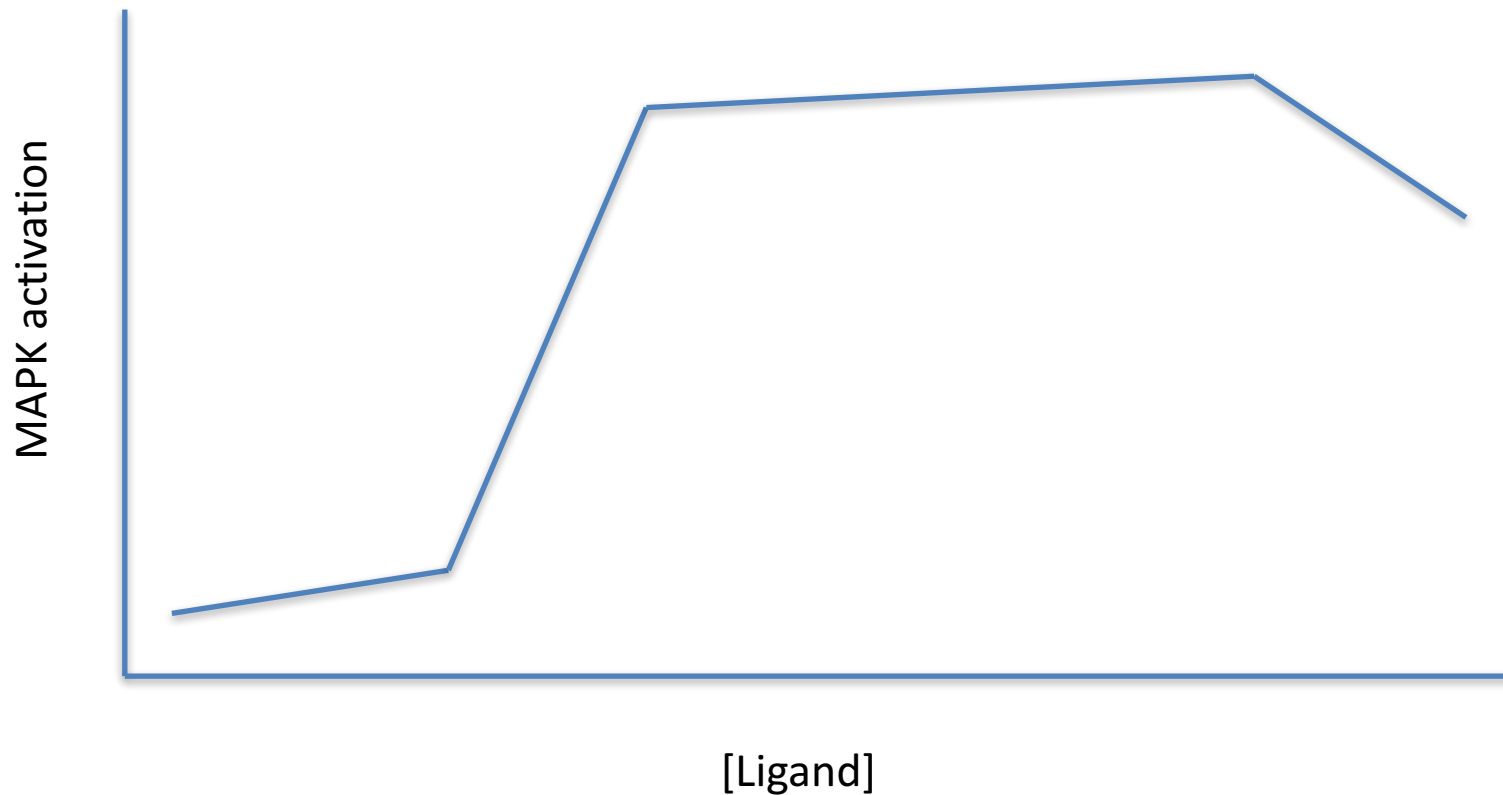


(B)



Why there is no difference between the effect of 1 mT and 7 μ T ELF-MF on ERK phosphorylation?

Dose response of MAPK activation by natural stimulants (e.g. EGF)



Summary

1. Both Microwave and ELF-MF induces activation of ERK1/2 in a time-dependent manners
2. The effect is detected in very low doses, already at 0.005 mW/cm² for microwave radiation, much below what we expected.
3. The activation is mediated in both cases by NADH oxidase.
4. There is no detectible effect on cell growth, migration, or apoptosis.

The Seger Lab

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Avital Hacoen
Izel Cohen
Renee Cohen
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Patricia Bounds,
Niels Kuster

Funds

- * ISF center of excellence
- * EU consortium **ARIMMORA**
- * Minerva
- * BSF
- * MOST