# Jelátviteli fehérjék szerepe daganatos megbetegedésekben: a mutáns RAS allélspecifikus viselkedése


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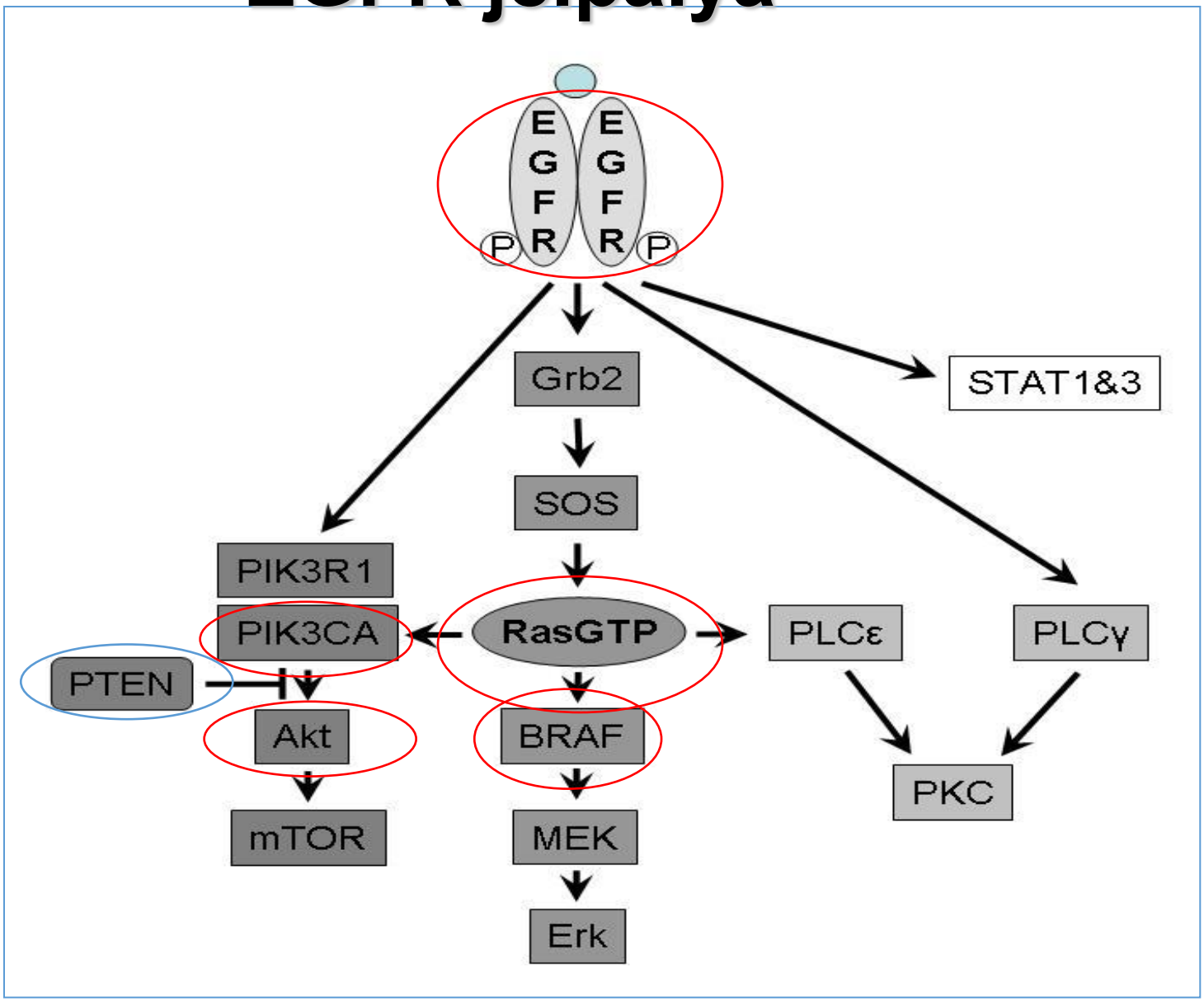
Molekuláris Onkológia kutatócsoport

# EGFR jelpálya



# RAS és RAF mutáció gyakoriság emberi daganatokban

| Cancer type | K-RAS | H-RAS | N-RAS | B-RAF |
|---|---|---|---|---|
| Pancreatic | 60 (70-80)* | 0 | 2 | 3 |
| Colorectal | 32 (45-50)* | 0 | 3 | 14 (10-15)* |
| Bile duct | 33 | 0 | 1 | 14 |
| NSCLC (adenocarcinoma) | 19 (35)* | 1 | 1 | 2 |
| Ovarian | 17 | 0 | 4 | 15 |
| Endometrial | 15 | 1 | 0 | 1 |
| Cervical | 9 | 9 | 1 | 0 |
| Hepatocellular | 8 | 0 | 10 | 3 |
| Myeloid leukemia | 5 | 0 | 14 | 1 |
| Thyroid | 4 | 5 | 7 | 27 |
| Breast | 4 | 0 | 0 | 2 |
| Urinary bladder | 4 | 11 | 3 | 0 |
| Malignant melanoma | 2 | 6 | 18 | 43 (70)* |
| Renal cell | 1 | 0 | 0 | 0 |

# RAS diagnosztika napjainkban

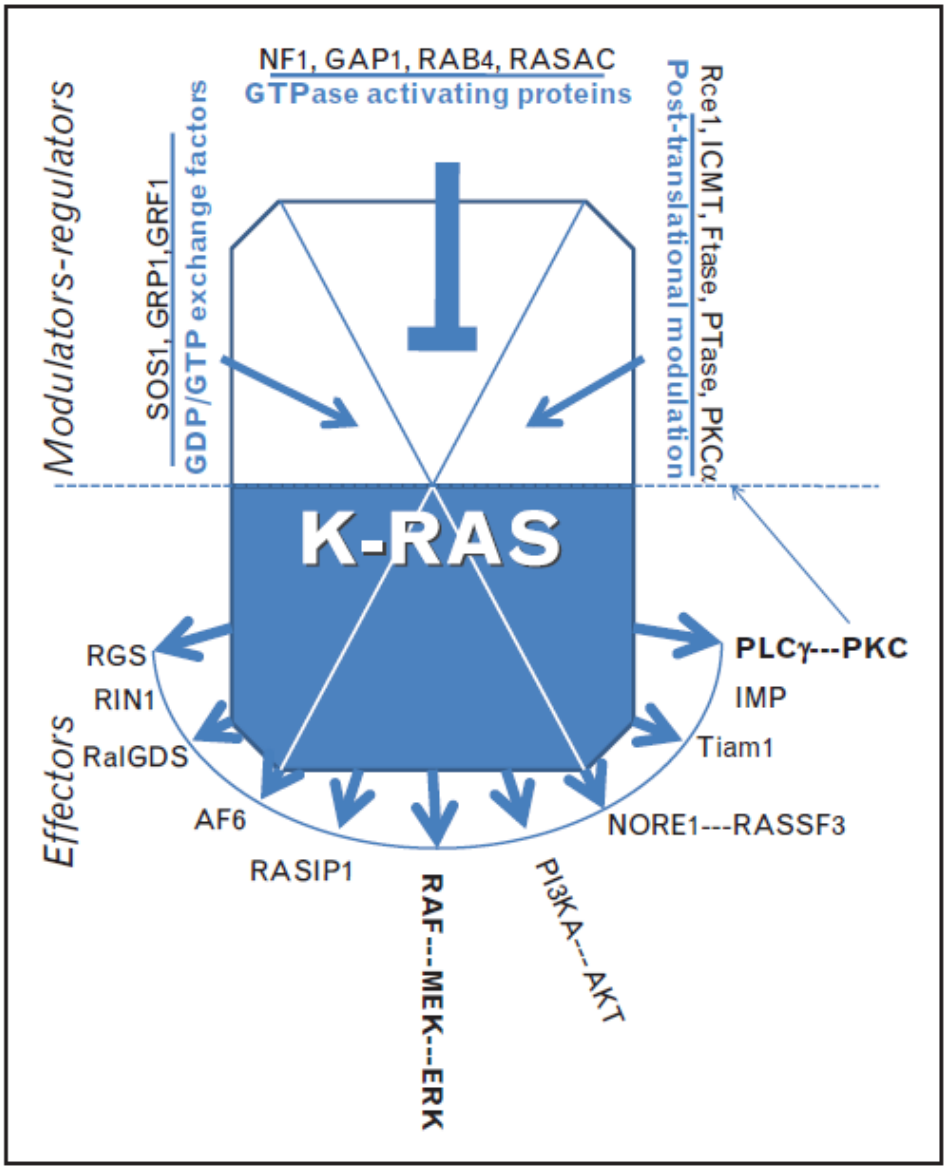
- Vastagbélrák
- Tüdőrák (adenokarcinoma)
- Melanóma
- Pajzsmirigyrák

- RASopátiák: CFC és Noonan szindróma

# RAS mutációk hazai vastagbélrákokban
# SE 2.sz. Patológiai Intézet

| RAS exon | Kodon | eset | % | KRAS/NRAS | melanoma |
|---|---|---|---|---|---|
| KRASex2 | kodon12 | 128 | 23.3 | | |
| | kodon13 | 19 | 3.5 | | |
| | other | 0 | 0.0 | | |
| | exon2 | 147 | 26.8 | | |
| KRASex3 | kodon59 | 1 | 0.2 | 34.6 | 0 |
| | kodon61 | 14 | 2.6 | | |
| | other | 7 | 1.3 | | |
| | exon3 | 22 | 4.1 | | |
| KRASex4 | kodon117 | 2 | 0.4 | | |
| | kodon146 | 12 | 2.2 | | |
| | other | 6 | 1.1 | | |
| | exon4 | 20 | 3.7 | | |
| NRASex2 | kodon12 | 10 | 1.8 | | |
| | kodon13 | 1 | 0.2 | | |
| | other | 10 | 1.8 | | |
| | exon2 | 21 | 3.8 | | |
| NRASex3 | kodon59 | 2 | 0.4 | 9.6 | |
| | kodon61 | 15 | 2.7 | | |
| | other | 2 | 0.4 | | |
| | exon3 | 19 | 3.5 | | |
| NRASex4 | kodon117 | 2 | 0.4 | | |
| | kodon146 | 2 | 0.4 | | |
| | other | 8 | 1.5 | | |
| | exon4 | 12 | 2.3 | | |
| cases (n) | 549 | 241 | 43.9 | | |

# A K-RAS fehérje szabályozási mintázata



# EGF proliferációs hatása RAS mutáns melanóma sejtvonalakon

sults. Importantly, both BRAF and NRAS activating mutations resulted in elevated levels of proliferation compared to the double-wild-type cells (Supplementary figure 1A). Proliferation of the double wild-type cell line VM-47 was significantly increased upon GF treatment. There was no significant change in the proliferation of cells with BRAF or NRAS oncogenic mutations (Fig. 2a–c).

Effect of EGF/FGF2 Treatment on Cell Morphology and Migration

Next, the effect of addition of EGF and/or FGF2 on cell morphology and migration was investigated by videomicroscopy. Clear morphological changes could be observed after EGF or FGF2 treatment in the double type cell lines and the majority of cells obtained an elongated morphology. A modest alteration was found in NRAS mutant cells following the addition of FGF2 (Fig. 3a). A more profound response was found in cell migration as compared to proliferation (Fig. 3b). Remarkably, without any treatment both BRAF and NRAS activating mutations resulted in elevated levels of migration compared to double-wild-type cells (Supplementary figure 1B). Significant increase in cell migration was observed in the two double wild-type cell lines following GF treatment. In both cases the increase in migratory activity was higher after EGF treatment as compared to FGF2 treatment. Importantly, the combined treatment resulted in greater increase than treatment with EGF or FGF2 alone. Significant increase in migration was measured after combined

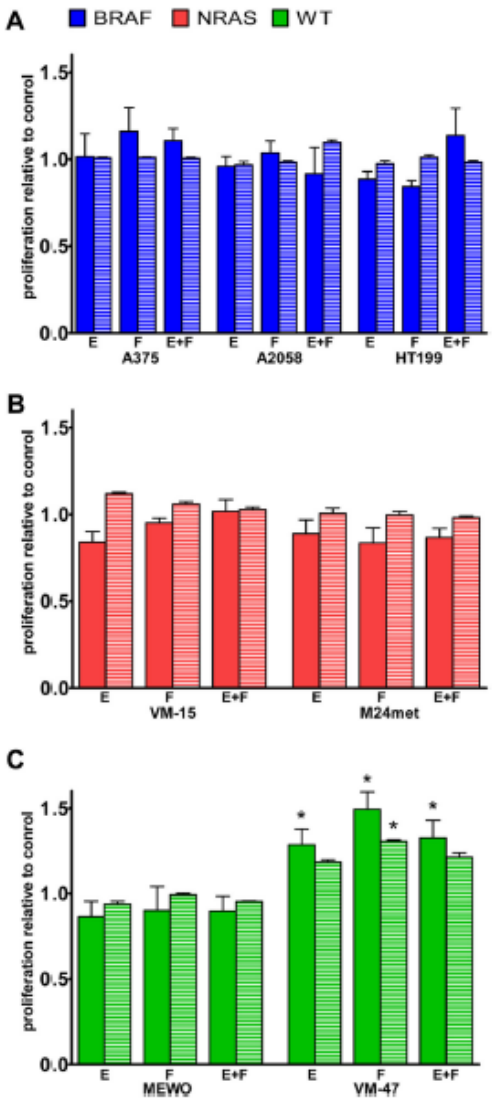



**Fig. 2** Effect of EGF and/or FGF2 treatment on cell proliferation measured by videomicroscopy and SRB assay. Melanoma cells harboring oncogenic BRAF (**a**), NRAS (**b**) mutations and wild-type for these genes (**c**). Evaluation of changes in cell proliferation by videomicroscopy or SRB assay yielded comparable results. No changes in proliferation were observed in BRAF and NRAS mutant cells. In contrast, double wild-type VM-47 cells showed significantly increased proliferation upon EGF and/or FGF2 treatment. *Colors blue, red* and *green* indicate BRAF, NRAS mutation and wild-type, and *striped* and *plain columns* stand for videomicroscopy and SRB assay, respectively. Data shown as average ± SEM are results of more independent measurements, 6 in case of SRB assays and 4 in case of videomicroscopy measurements. Asterisks indicate significance of $p<0.05$ by ANOVA and Dunnett's post hoc test. (E – EGF; F – FGF2; E + F – EGF and FGF2 treatment)

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RESEARCH

## Sensitivity of Melanoma Cells to EGFR and FGFR Activation but Not Inhibition is Influenced by Oncogenic BRAF and NRAS Mutations

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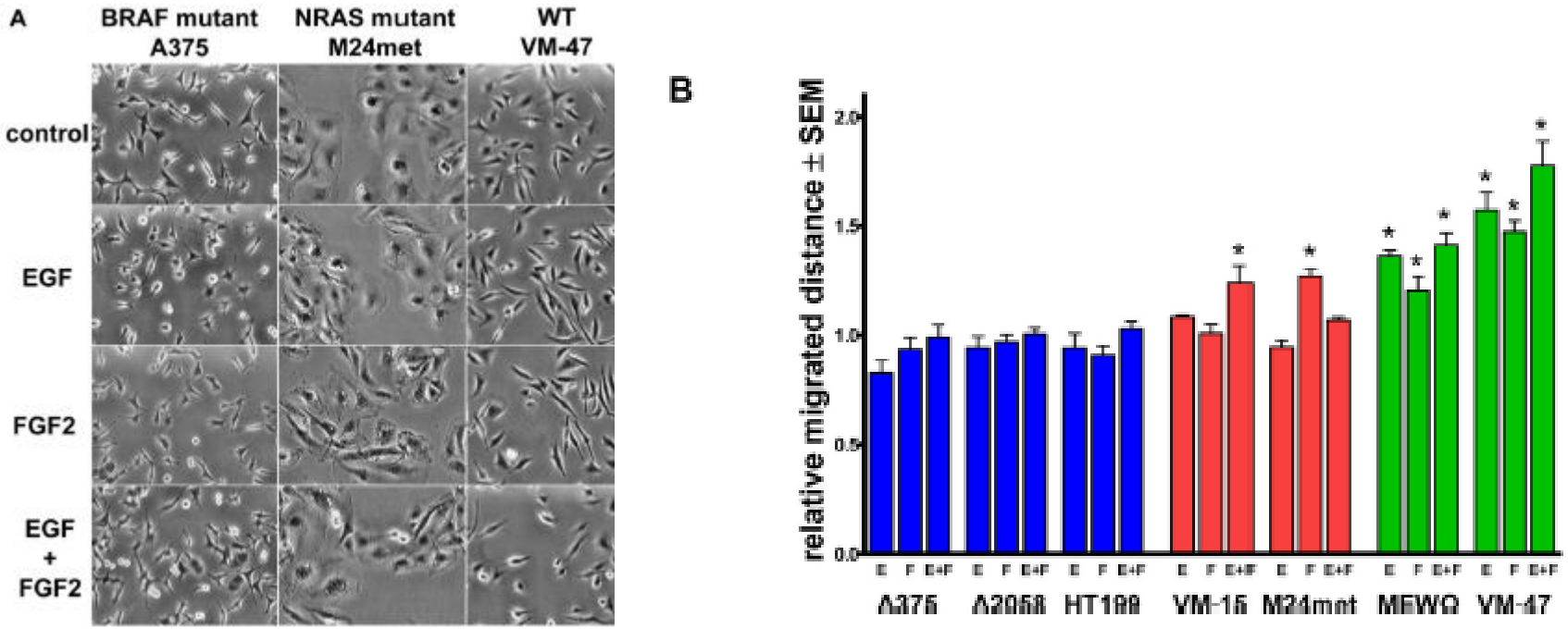
# EGF hatása RAS mutáns melanóma sejtek migrációjára



# EGF hatása az ERK1/2 foszforilációra RAS mutáns melanóma sejtekben

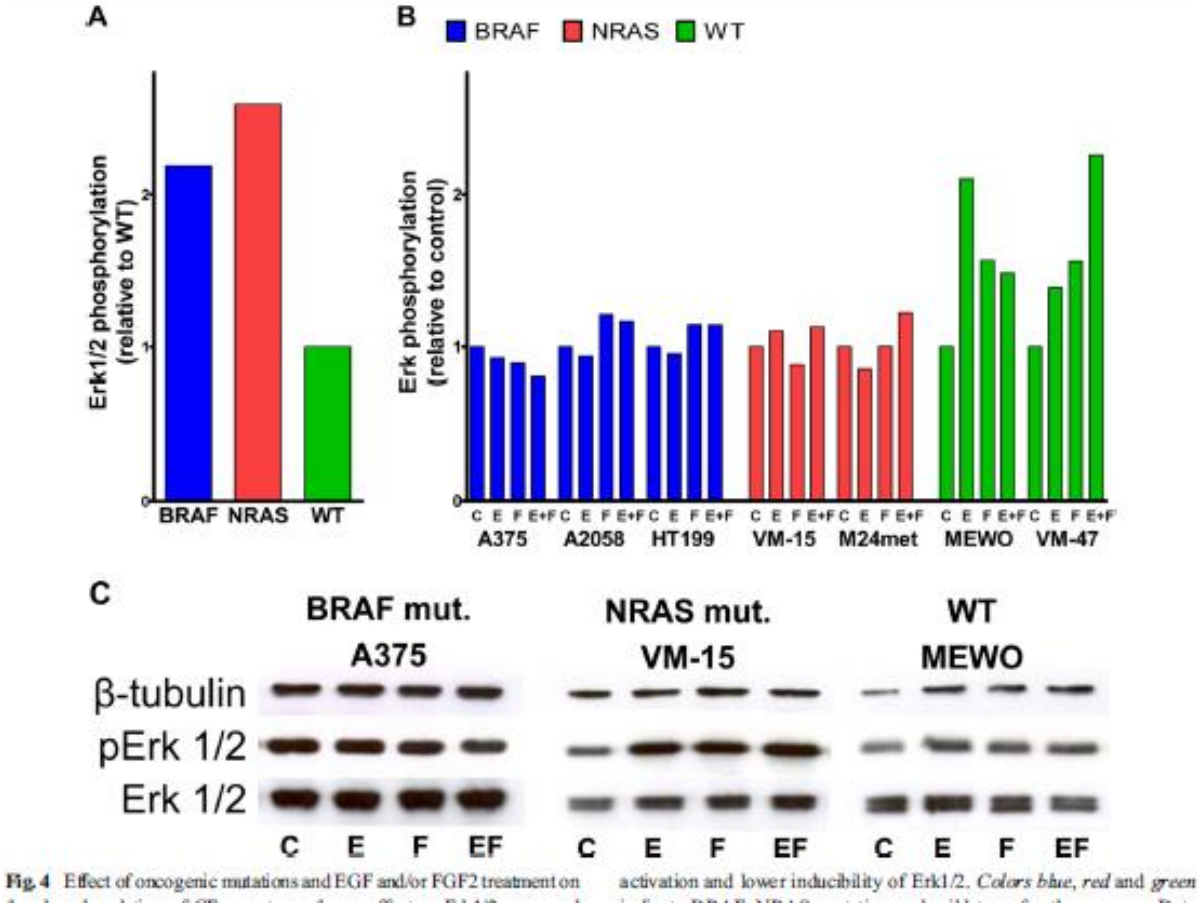
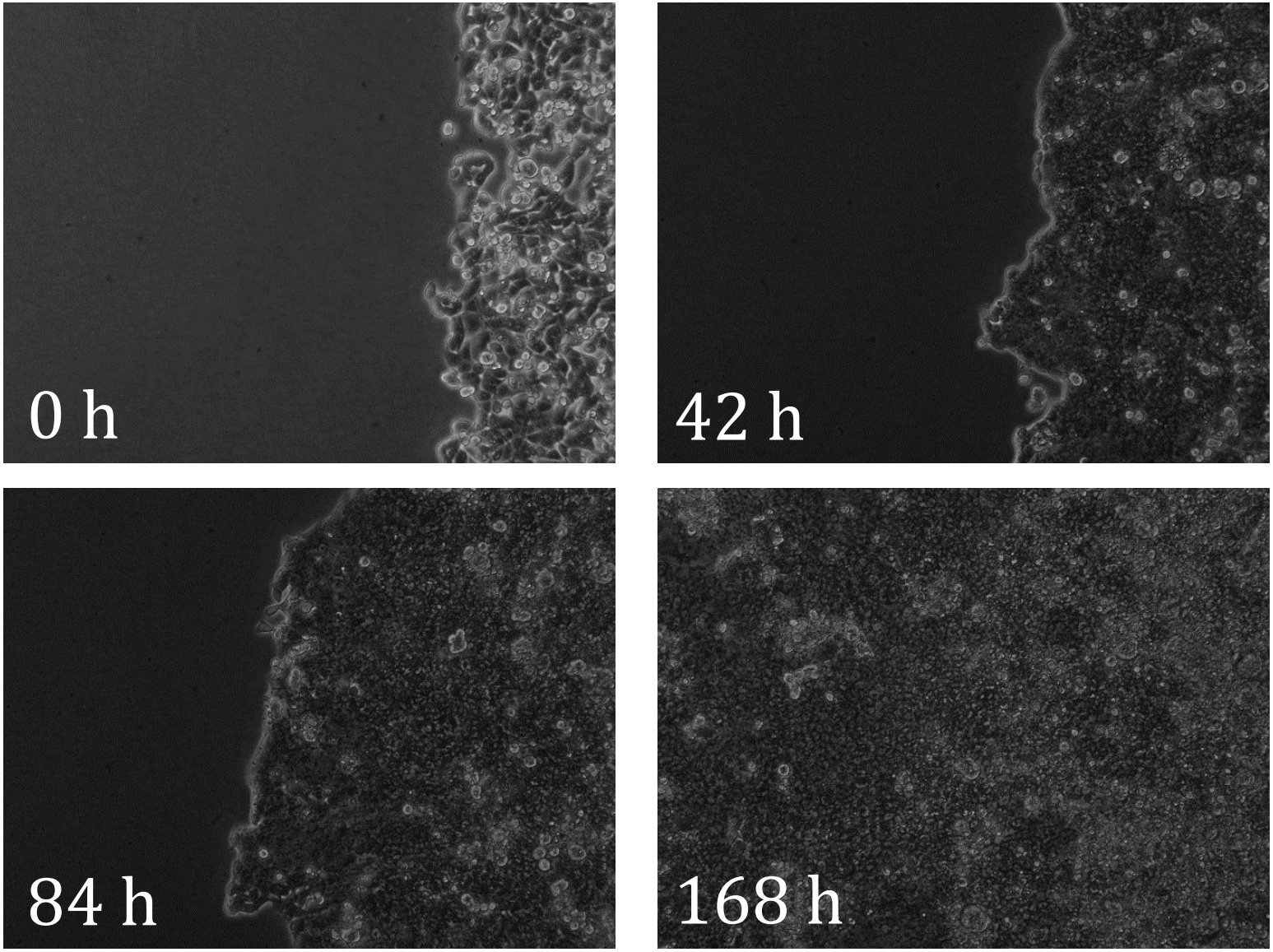
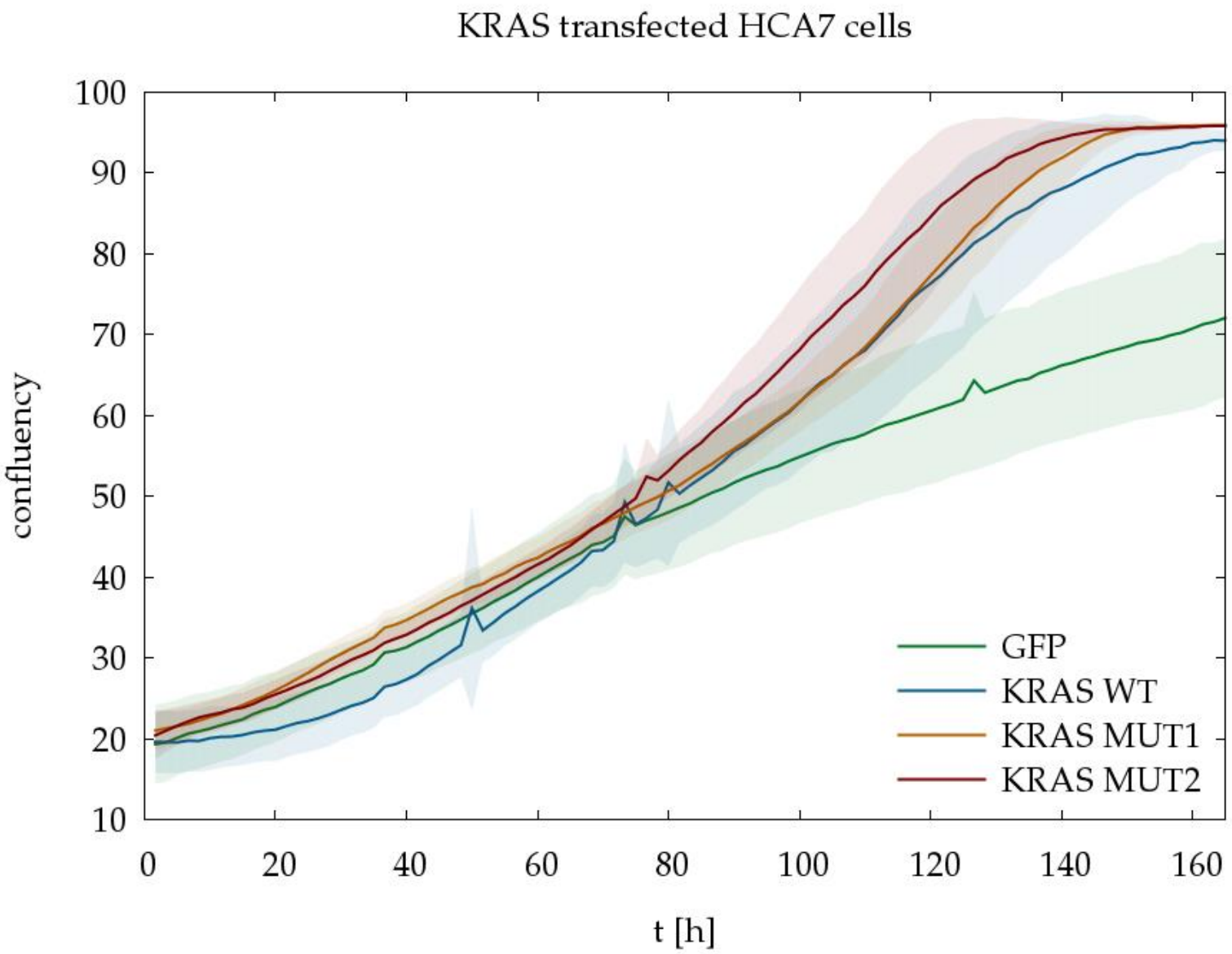



Fig. 4 Effect of oncogenic mutations and EGF and/or FGF2 treatment on activation and lower inducibility of Erk1/2. *Colors blue, red* and *green*

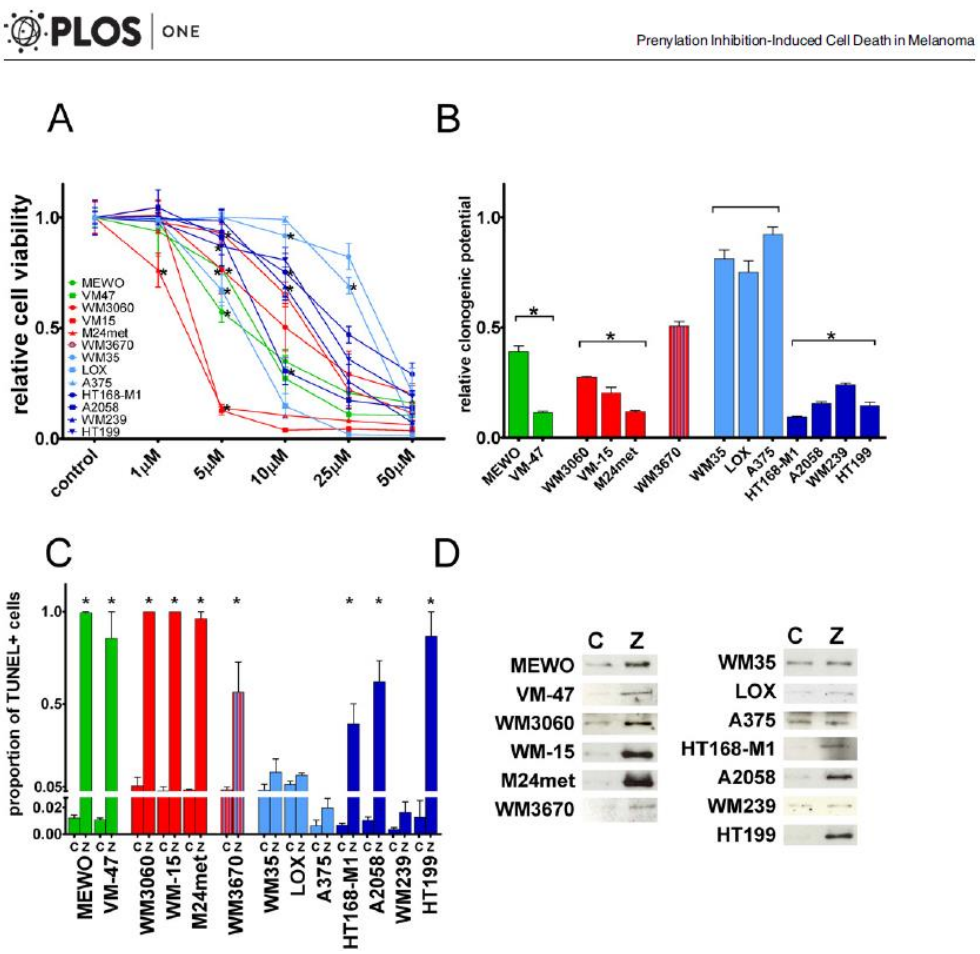
# KRAS transzfektált HCA7 sejtek migrációja



# KRAS transzfektált HCA7 sejtek migrációja

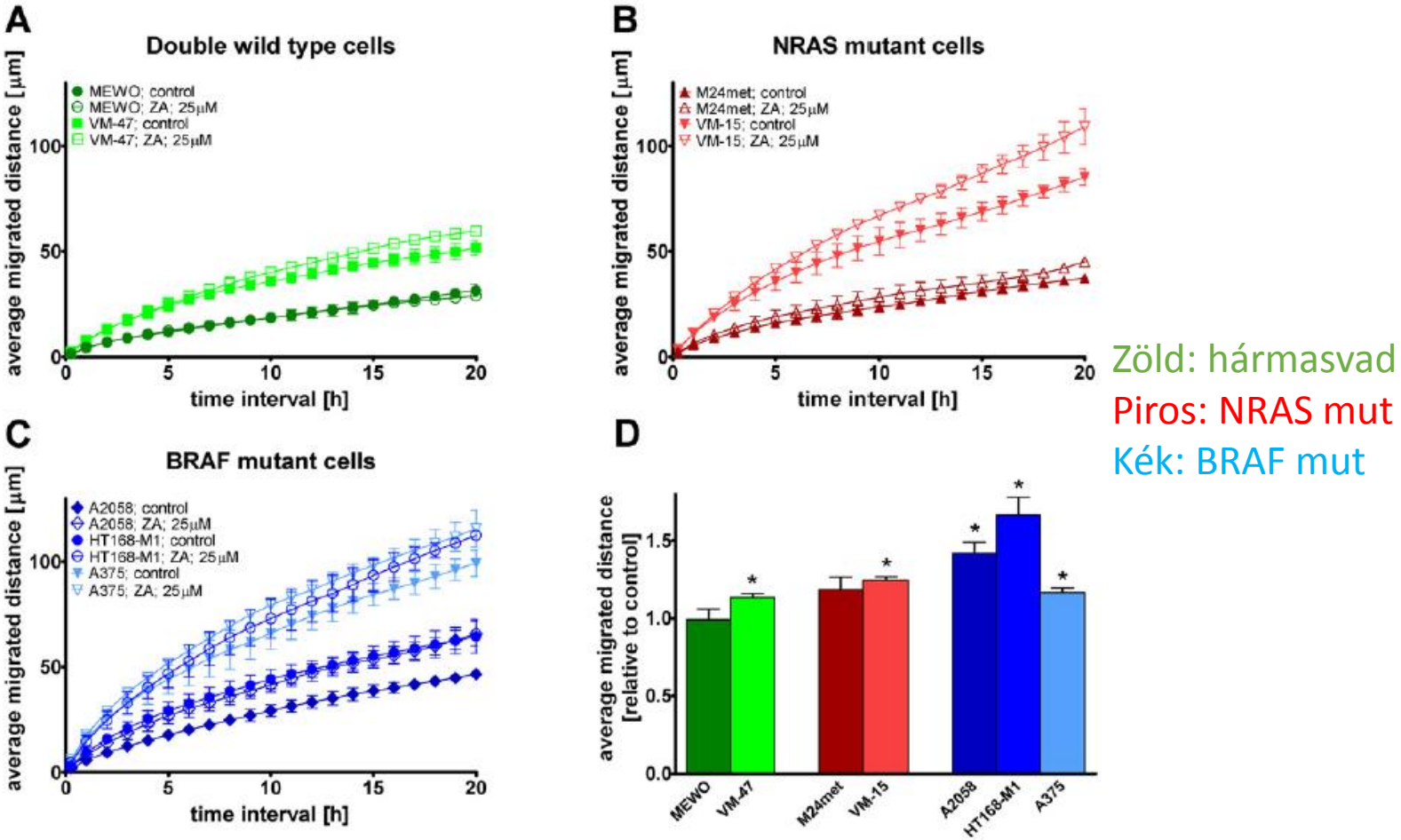


# Preniláció gátlás (ZA) hatása RAS mutáns melanóma sejtek proliferációjára és apoptózisára




Zöld: hármasvad
Piros: NRAS mut
Kék: BRAF mut

# Preniláció gátlás hatása a RAS mutáns melanóma sejtek migrációjára




Zöld: hármasvad

Piros: NRAS mut

Kék: BRAF mut

# Preniláció gátlás hatása az ERK és S6 foszforilációra emberi melanóma sejtekben

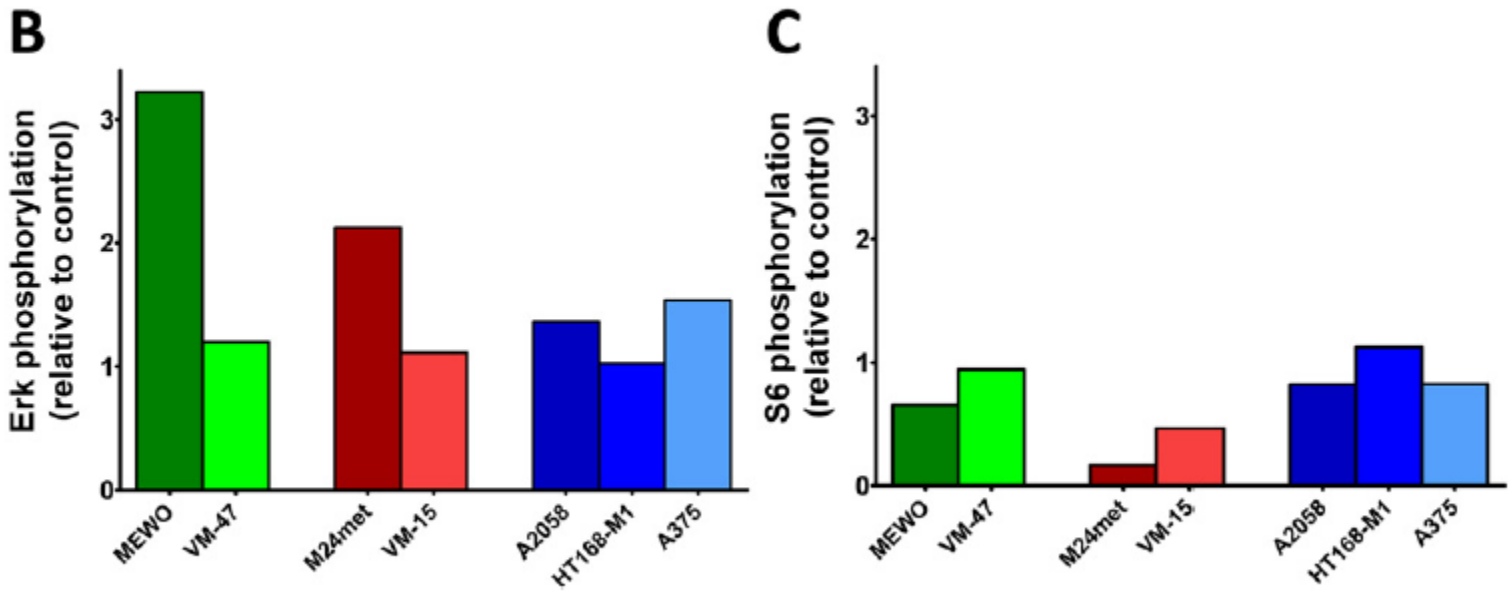



**Fig 3. Activation of downstream elements of the RAS/RAF pathway in melanoma cells after zoledronic acid treatment.** (**A**) Representative blots of the effect of 48hs zoledronic acid (ZA) treatment on the activation of Erk1/2 and S6. (**B**) Quantification of the effect of ZA treatment on the activation of Erk1/2.

Zöld: hármasvad

Piros: NRAS mut

Kék: BRAF mut

PLOS ONE

RESEARCH ARTICLE

# Prenylation Inhibition-Induced Cell Death in Melanoma: Reduced Sensitivity in BRAF Mutant/PTEN Wild-Type Melanoma Cells

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