Nrf2-independent role of Keap1 in actin cytoskeleton regulation

Marina Oskomić[1], Nikolina Stojanović[2], Andreja Ambriović-Ristov[2], Jonathan D. Humphries[3], Mihaela Matovina[1]

[1]*Laboratory for Protein Biochemistry and Molecular Modelling, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[2]*Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
[3]*School of Biological and Chemical Sciences, Manchester Metropolitan University, Manchester, United Kingdom*

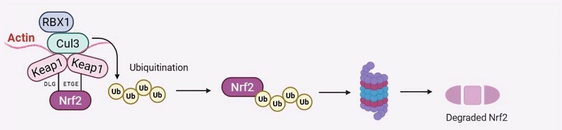
moskomic@irb.hr

(IP-2025-02-7100)

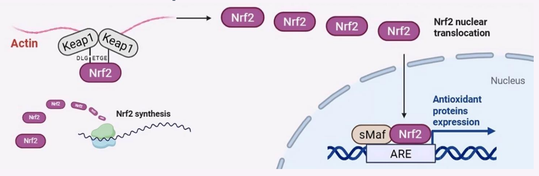
BACKGROUND

The **Keap1–Nrf2 pathway** is the main cellular defence against **oxidative stress**.

Under normal conditions **Keap1 degrades Nrf2**

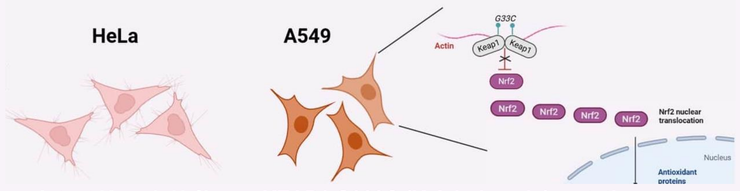


Stress triggers **Nrf2 nuclear translocation** and activation of the **antioxidative response**



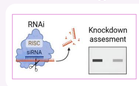
Recent evidence suggests Keap1 has **non-canonical roles** *(Kopacz et al., 2020)*. **Keap1 interacts with actin cytoskeleton through its Kelch domain**, but the mechanism and function of this interaction remain unclear *(Kang et al., 2004)*.

CELL MODELS & METHODS

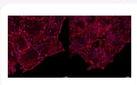


Intact Keap1–Nrf2 regulation

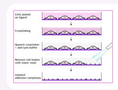
KEAP1 G333C (Kelch domain) mutation prevents Nrf2 binding → constitutively active Nrf2



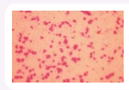
siRNA knockdown of KEAP1 or NRF2 (verified by WB)



IF : F-actin and IAC proteins (number, size per cell)



IAC isolation (Jones et al., 2015) → WB / MS



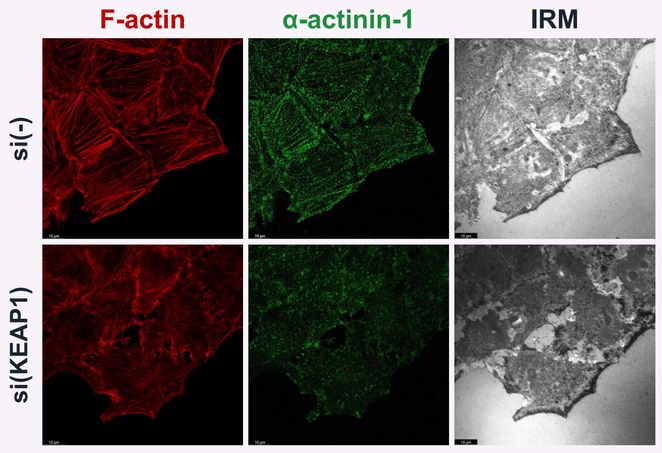
Transwell (Boyden) chemotaxis assay

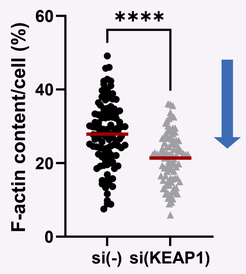
AIM

Determine how Keap1 regulates the actin cytoskeleton and adhesions – and whether this depends on Nrf2

RESULTS · HeLa | wild-type Keap1 regulates Nrf2 normally

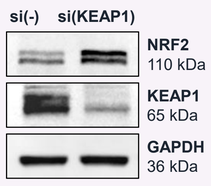
1 Keap1 knockdown in HeLa cells reduces F-actin content and disrupts stress fibers and α-actinin-1 organisation *(IF)*





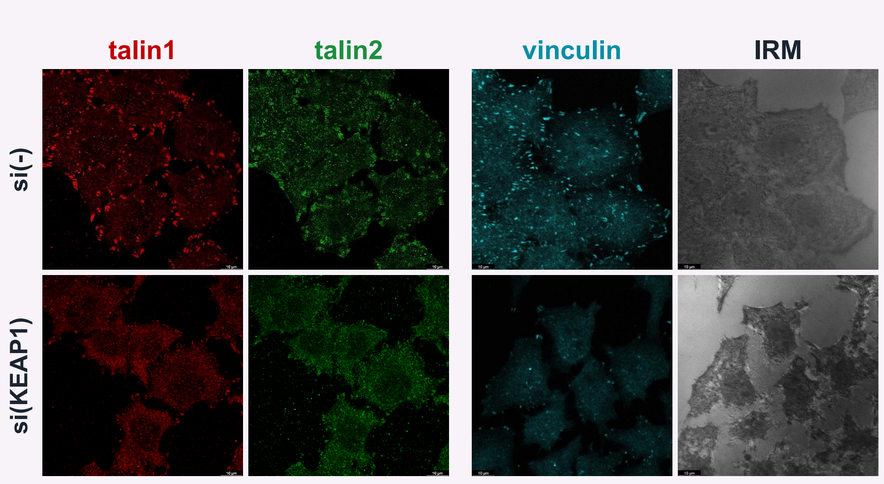
F-actin signal quantified within the whole-cell outline (stress fibers + cortical actin)

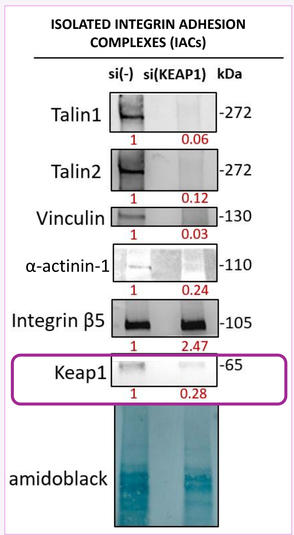
Knockdown efficiency *(WB)*

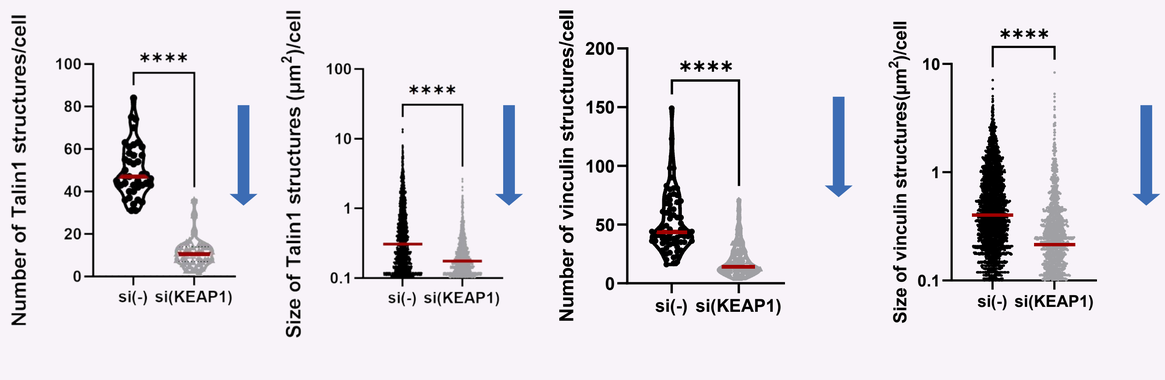


2 Keap1 knockdown in HeLa cells reduces focal adhesion (FA) number and size and depletes proteins from isolated IACs *(IF, WB)*

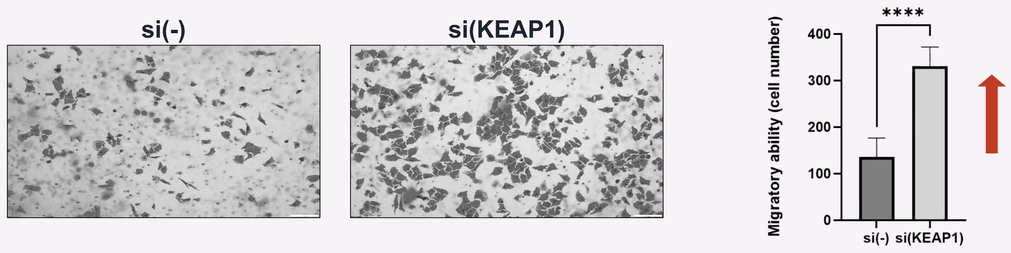
Endogenous Keap1 co-isolates with IACs





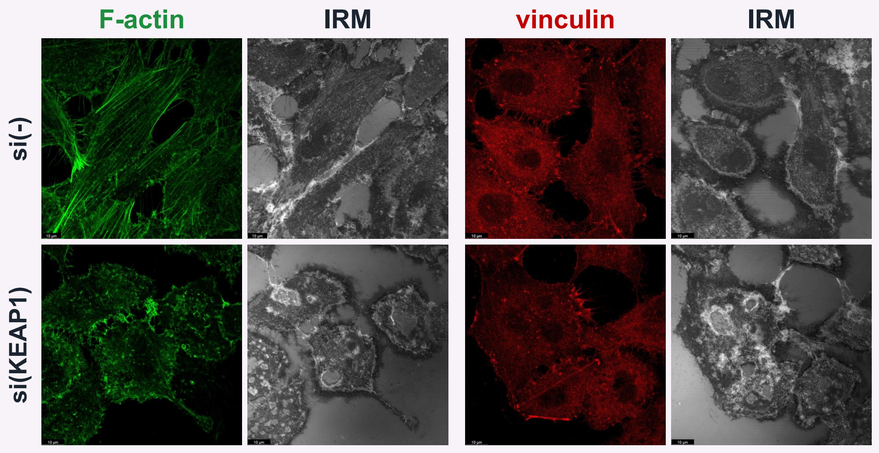


3 Keap1 knockdown increases HeLa cell migration *(Boyden assay)*

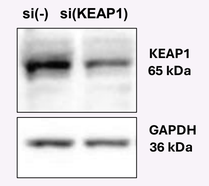


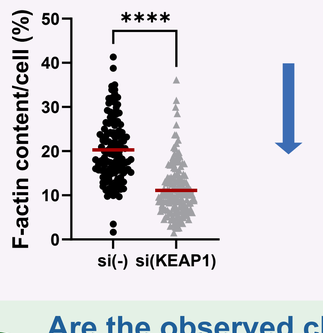
RESULTS · A549 | mutant *KEAP1 G333C* uncouples Keap1 from Nrf2

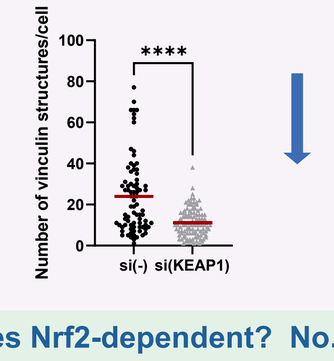
4 Keap1 knockdown in A549 cells also reduces F-actin content and FA number – but FA size increases *(IF)*

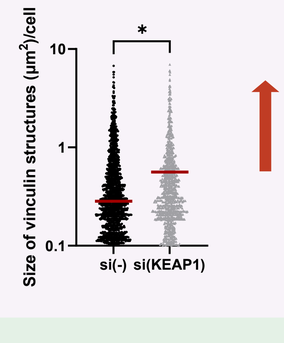


Knockdown efficiency *(WB)*



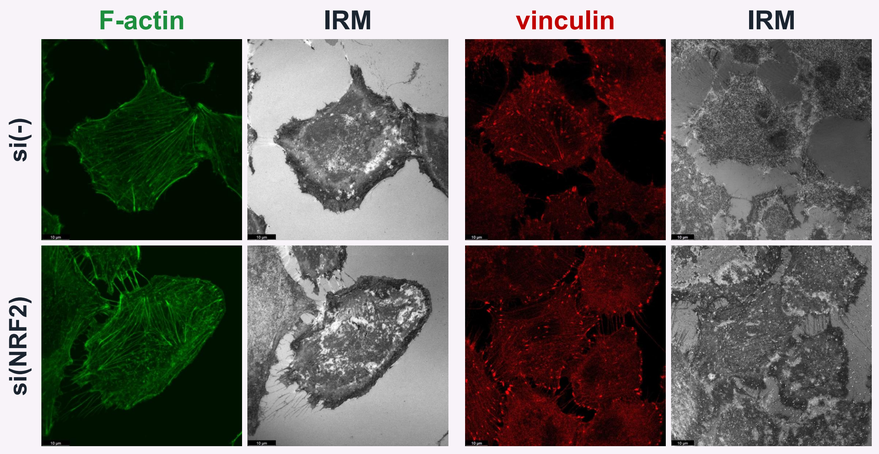




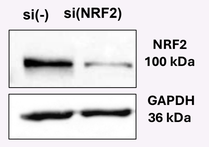


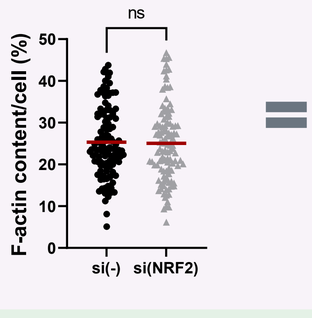
5 Are the observed changes Nrf2-dependent? No.

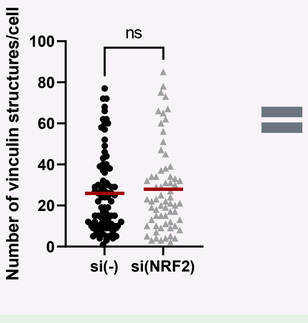
Nrf2 knockdown in A549 cells does not change F-actin content or FA number – only FA size increases *(IF)*

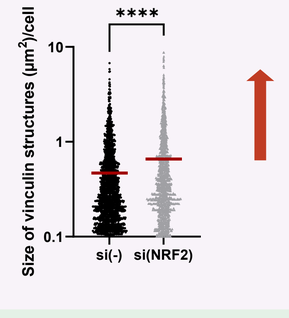


Knockdown efficiency *(WB)*

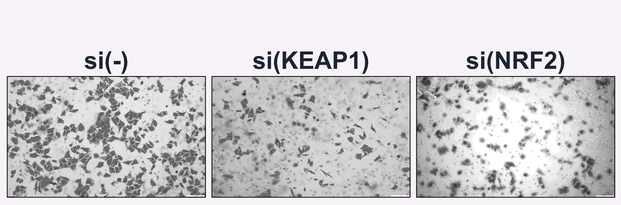


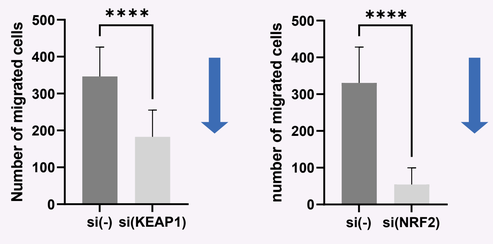






6 In A549 cells, both Keap1 and Nrf2 knockdown decrease migration *(Boyden assay)*





CONCLUSIONS

- **Keap1 sustains actin organisation and adhesions:** its depletion lowers F-actin content, disrupts stress fibers and reduces FA number in both HeLa and A549 cells.
- **Endogenous Keap1 co-isolates with IACs:** its loss depletes talin1, talin2, vinculin and α-actinin-1 from isolated complexes, while integrin β5 is retained – a selective change in adhesion composition, not proof of direct binding.
- **The actin phenotype is Nrf2-independent:** Nrf2 knockdown changes neither F-actin content nor FA number. FA size, however, increases after both Keap1 and Nrf2 knockdown, so adhesion size retains an Nrf2-related component.
- **The outcome is cell-type specific:** Keap1 depletion decreases FA size and increases migration in HeLa, but increases FA size and decreases migration in A549 – pointing to FA size and/or the different integrin pool of the two lines.

HeLa vs A549 ■ shared ■ cell-type specific

	HeLa Keap1 KD	A549 Keap1 KD	A549 Nrf2 KD
F-actin content / cell	▼	▼	= (ns)
FA number	▼	▼	= (ns)
FA size	▼	▲	▲
Proteins in isolated IACs	▼ (WB)	MS → QR	not analysed
Cell migration	▲	▼	▼

Scan for more data

Integrin β5 & reticular adhesions · F/G-actin ratio · A549 IAC proteomics (MS)

ns, not significant; * $p < 0.05$; **** $p < 0.0001$. Scale bars, 10 µm. IRM, interference reflection microscopy; IAC, integrin adhesion complex; KD, knockdown. ▲ **increase,** ▼ **decrease relative to si(-).**

References: Kang et al., PNAS 2004 · Kopacz et al., Free Radic Biol Med 2020 · Jones et al., Curr Protoc Cell Biol 2015. **Funding:** Croatian Science Foundation (HRZZ), IP-2025-02-7100.