Targeting VEGF-Sustaining Signaling to Restore Homeostasis in the Outer Retina

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Financial Disclosure

- Discuss anti-VEGF agents in relationship to clinical trials
- Financial interests or relationships:
  - Knights Templar Eye Foundation: Consultant/Advisor
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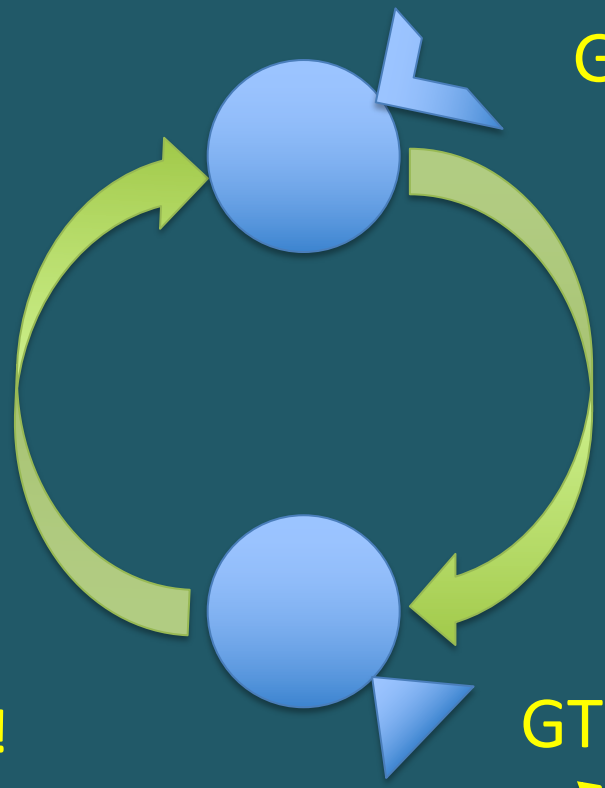
Summary

- AMD influenced by genetic predisposition and external stresses that affect outer retina - photoreceptors, RPE and choroid
- Activation of Rac1 by VEGF and other AMD stresses necessary for choroidal endothelial cell migration across RPE, a key step for type 2 MNV
- Sustained activation of Rac1 involves binding to adaptor protein, IQGAP1
- Targeting the Rac1/IQGAP1 binding site reduces sustained Rac1 activation and might safely restore homeostasis despite multiple stresses that cause pathology in AMD

Background

- Anti-VEGF agents effective in neovascular AMD
- But improve vision in about 50% of patients
- We focused on effector of VEGF - Rac1GTP
  - activated by other known stresses associated with AMD - oxidation and inflammation
  - and is necessary for activating choroidal endothelial cells to migrate across the RPE, important step in type 2 macular neovascularization (MNV)

Rac1 GTPase Biologic Switch



GDP **INACTIVE**

GTPase activating proteins (GAPs)

Guanine nucleotide exchange factors (GEFs)

ACTIVE!

GTP

Cell Migration

VEGF, ROS, TNFα

Wang/Hartnett et al AJ Path 2015; 185 :3316-3325
Monaghan-Benson/Hartnett AJP 2010 177:2091-2102
Wang/Hartnett ME et al 2014; FASEB 28:265-274

Rac1

- Besides Rac1GTP's effects on choroidal endothelial cell migration across the RPE

- Rac1GTP
  - subunit for NADPH oxidase, a key enzyme generating ROS
    - for intracellular signaling of NFkB and inflammatory pathways
    - for defense against microbes

- Instead of targeting Rac1, which can have beneficial effects fighting infection, we explored an adaptor protein that binds Rac1GTP in cancer and disease

IQGAP1

- IQ protein motif containing GTPase activating protein 1 (IQGAP1) – multiple domains bind different signaling effectors
- Inactive in cytoplasm when not bound
- When bound, coordinates intracellular signaling to enable biologic events
  - Nuclear transcriptional events
  - Altering cell shape for migration
  - Interacting with ECM at cell membrane

Hypothesis

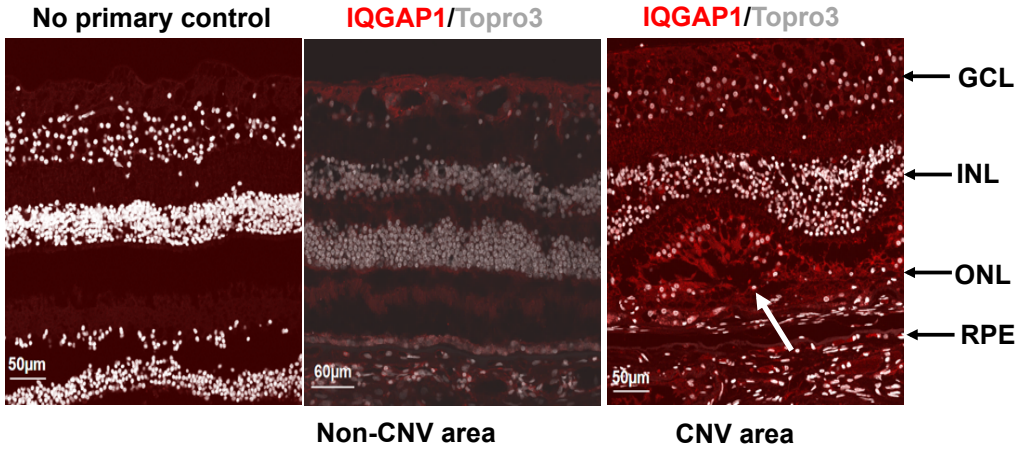
- IQGAP1 was important in Rac1GTP (active Rac1) mediated choroidal neovascularization

- Rac1GTP binding to IQGAP1 domain sustained its activation induced by VEGF

Methods

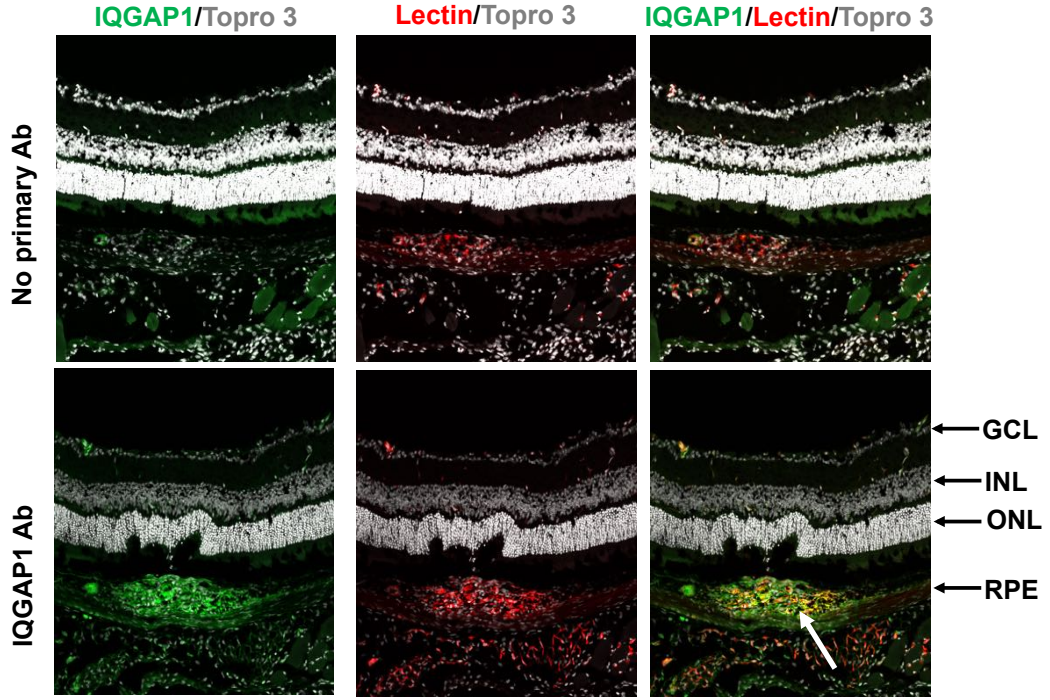
- *Iqgap1*$^{-/-}$ and littermate *Iqgap1*$^{+/+}$ mice lasered and 7 days later:
  - Assayed for choroidal neovascularization (CNV; lectin-stained)
  - Immunohistochemistry for IQGAP1 and Rac1GTP
- Human choroidal endothelial cells (CECs) from adult donor eyes:
  - Transfected with Iqgap1 or control siRNA, treated with VEGF or PBS
  - Transfected with mutant construct to Rac1 binding domain of IQGAP1 (GFP-IQ-MK24) or control (GFP-IQ-WT), time course of VEGF treatment or PBS
  - Outcomes for both Rac1GTP and Rac1GTP/IQGAP1

IQGAP1 localized in Human MNV and Experimental CNV

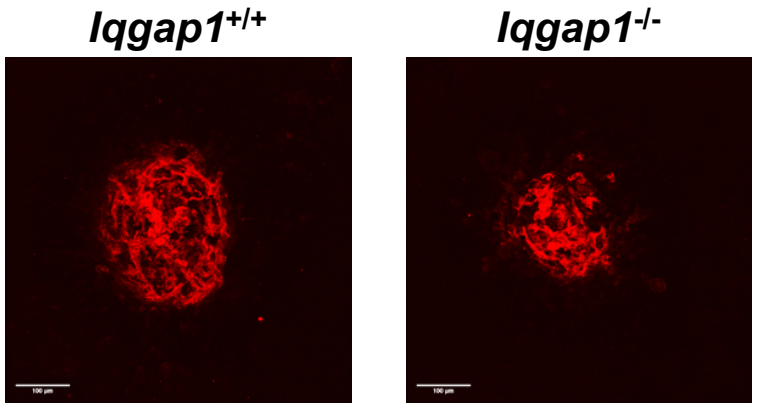


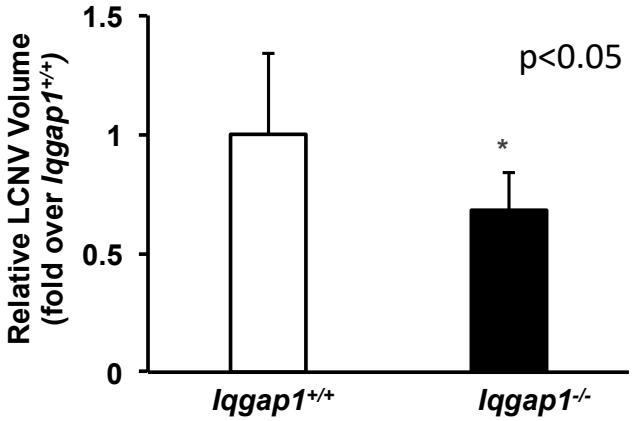
Sections human neovascular AMD and MNV, courtesy of Hans Grossniklaus, Emory

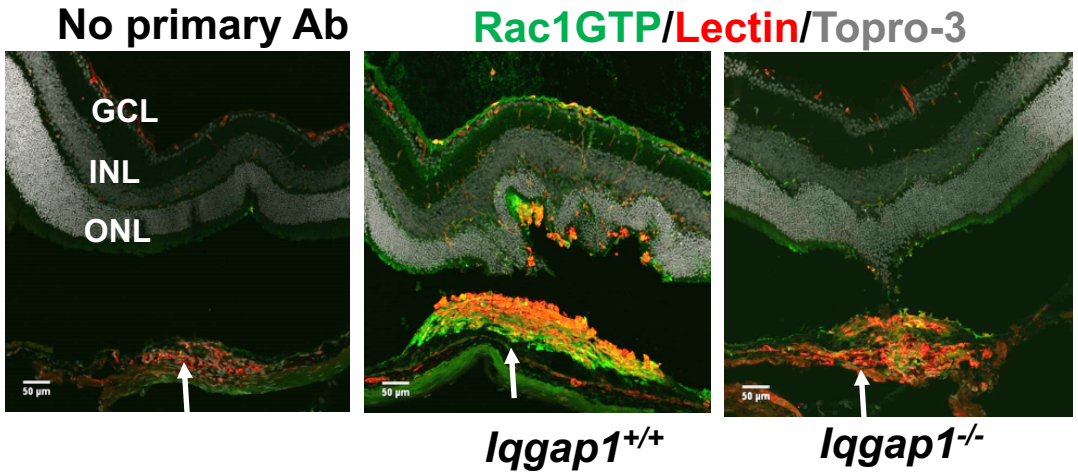
IQGAP1 localized with lectin-labeled CNV 7 days after laser C57bl6/J mice

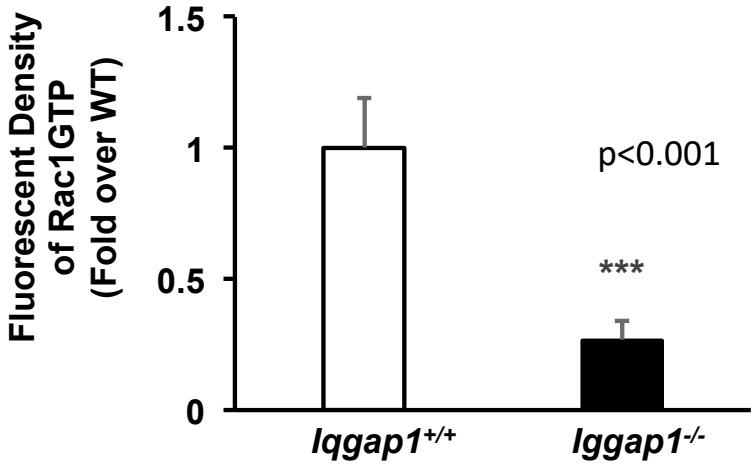


Iqgap1$^{-/-}$ had less CNV than littermate controls and Less Colocalization with Rac1GTP in CNV

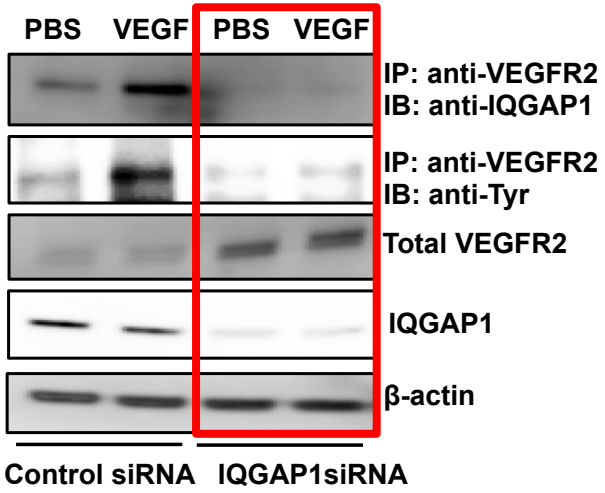


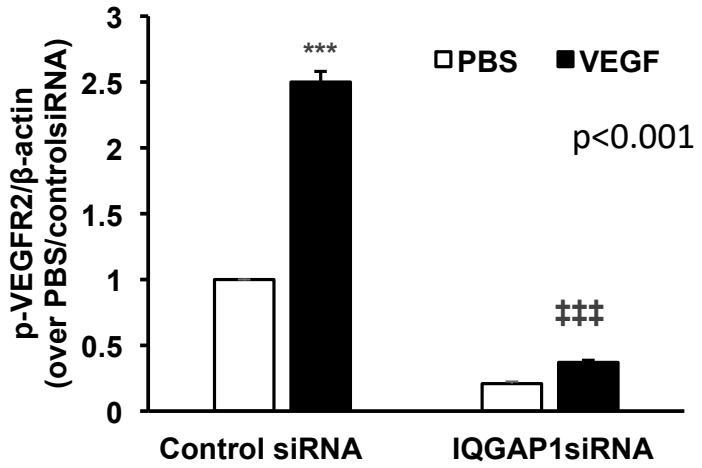


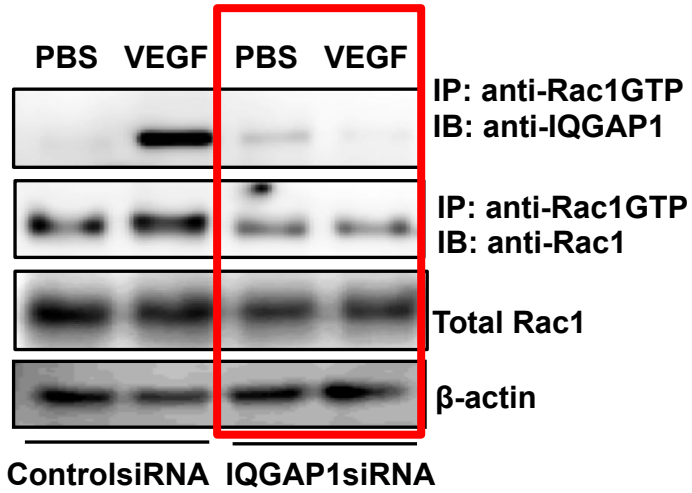


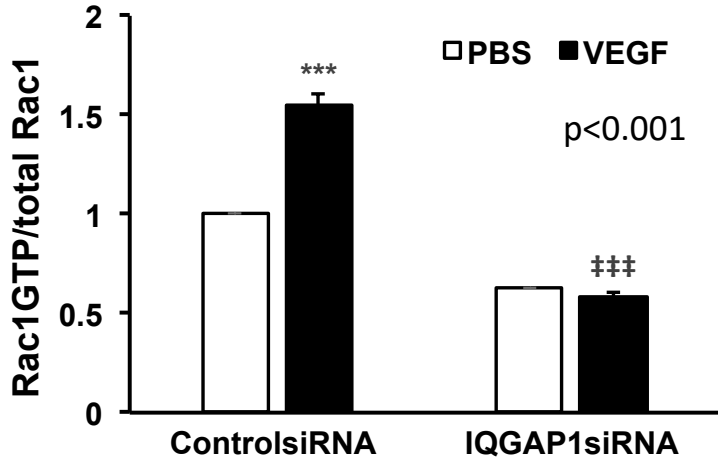


IQGAP1 Knockdown inhibited VEGF induced VEGFR2 activation, Rac1GTP and Rac1GTP binding IQGAP1

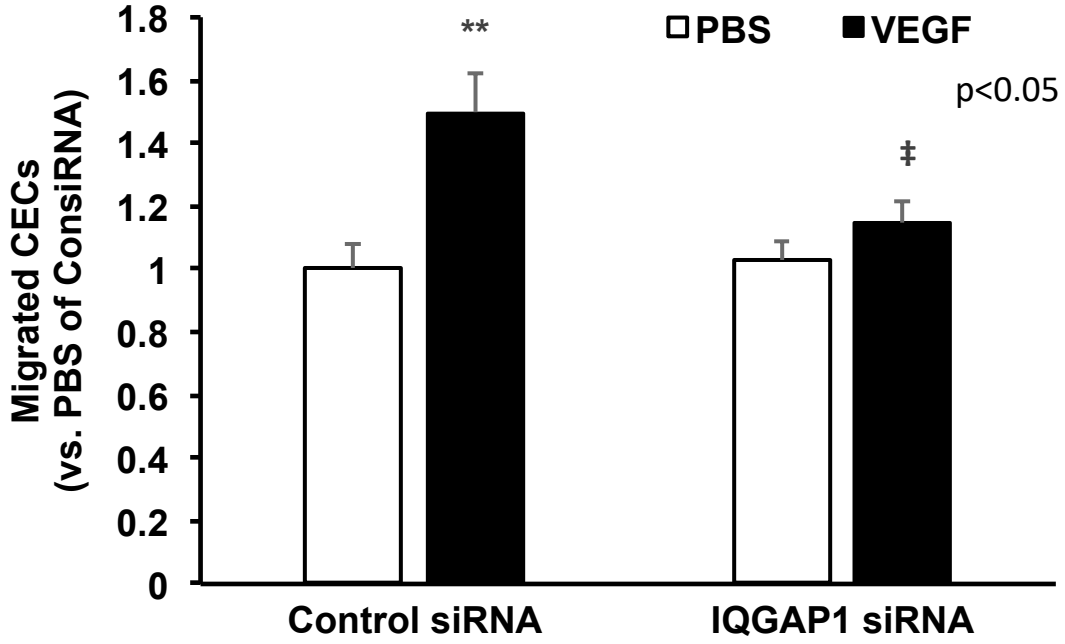




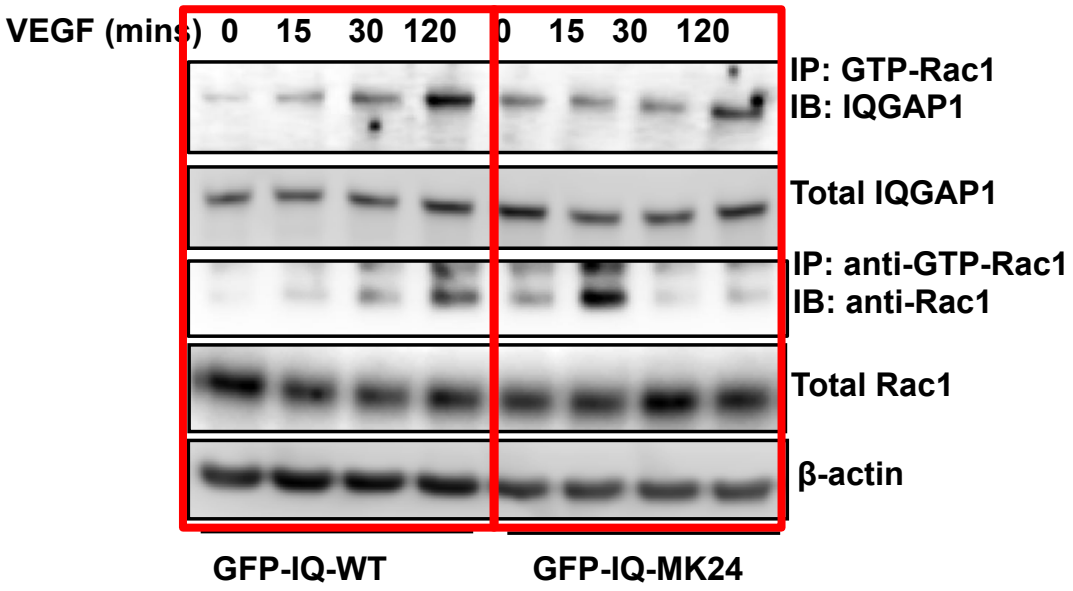


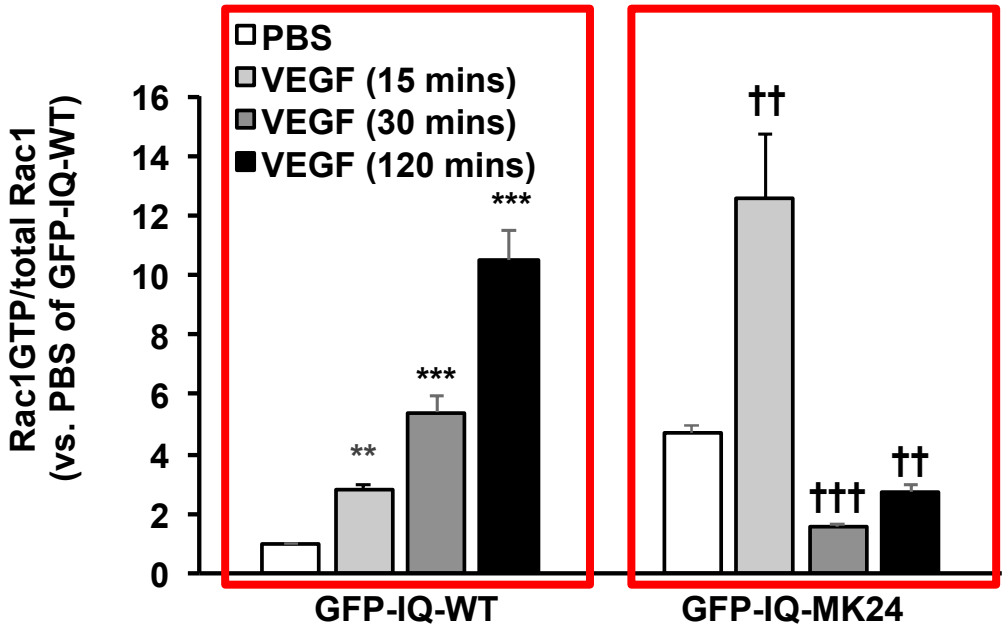


IQGAP1 Knockdown Inhibited VEGF-induced CEC Migration



VEGF Induced Sustained Rac1GTP over 2 hours when Bound to IQGAP1





Conclusions

- Sustained activation of Rac1 by VEGF involves binding the adaptor protein, IQGAP1

- Targeting the IQGAP1 binding site of Rac1GTP reduces the time course of Rac1 activation and may be a method to restore homeostasis
  - despite multiple stresses that cause pathology in AMD
  - without removing beneficial aspects of active Rac1

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