The role of EGFR in hypoxia

Irving Donadon
Università degli Studi di Ferrara

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Flowchart

1) Hypoxia-induced effects
2) EGFR
3) EGFR role in miRNA maturation
4) mHESM targets
5) EGFR-AGO2 interaction
6) co-localization of EGFR-AGO2
7) Highly conserved Tyr in AGO2
8) EGFR kinase activity → phosporylation of AGO2
9) role of AGO2 phosphorylation
10) Dicer's silencing

1 – Hypoxia-induced effects

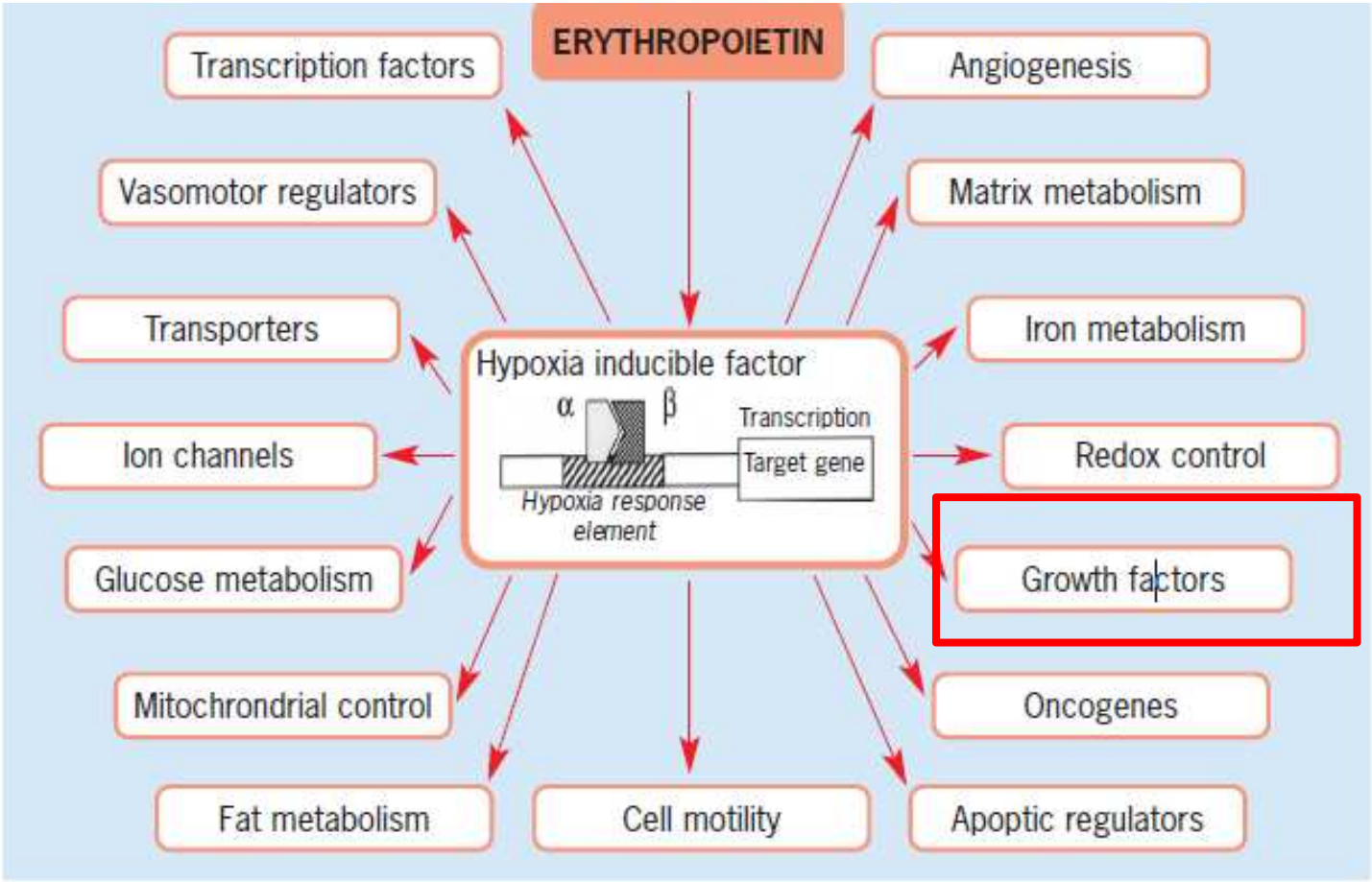


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.

2 – EGFR (epidermal growth factor receptor)

- Growth factor receptor;

- induces cell differentiation and proliferation;

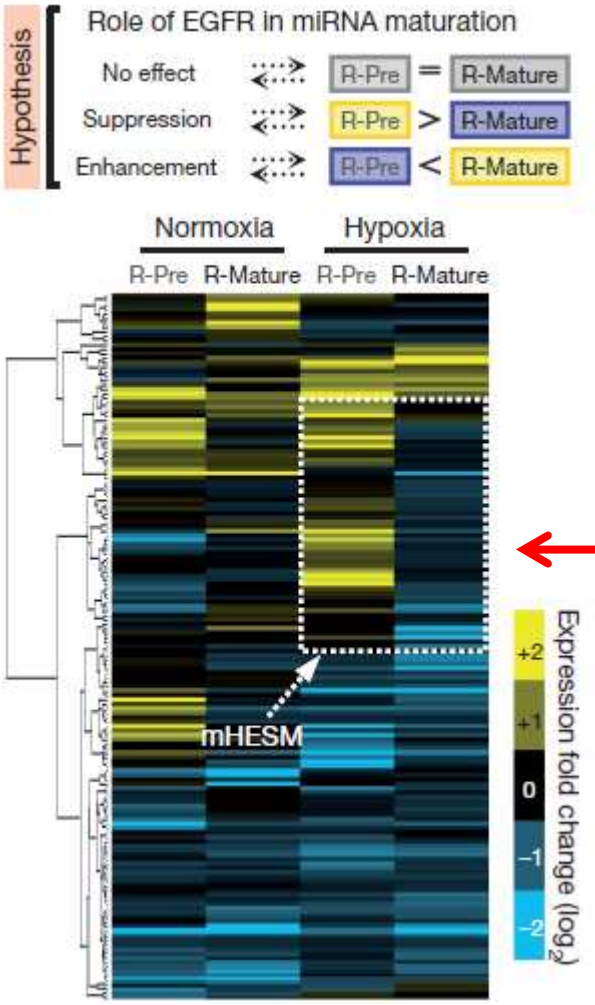
- **tyrosine kinase** → phosphorylation of intracellular substrates → leads to cell growth, DNA synthesis and expression of oncogenes.

=> EGFR is thought to be involved into the development of cancer, as the EGFR gene is often amplified, and/or mutated in cancer cells.

Hypoxia is known to **upregulate EGFR**.

=> EGFR upregulation compromises miRNA maturation.

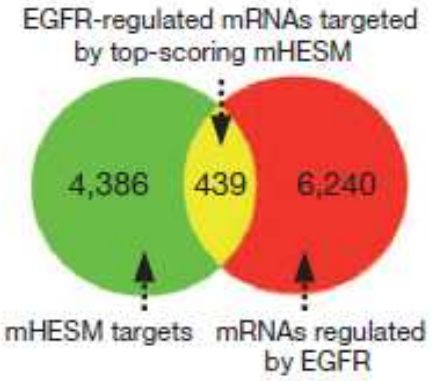
3 - EGFR role in miRNA maturation



hierarchical clustering analysis

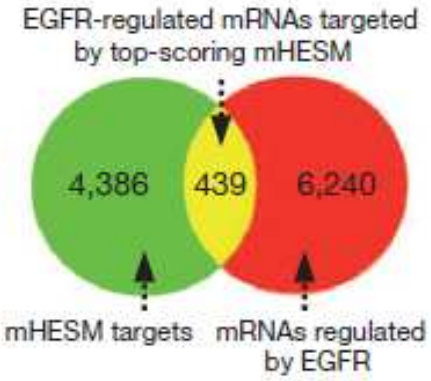
Identification of a distinct cluster of miRNA affected by EGFR under hypoxia (**mHESM**).

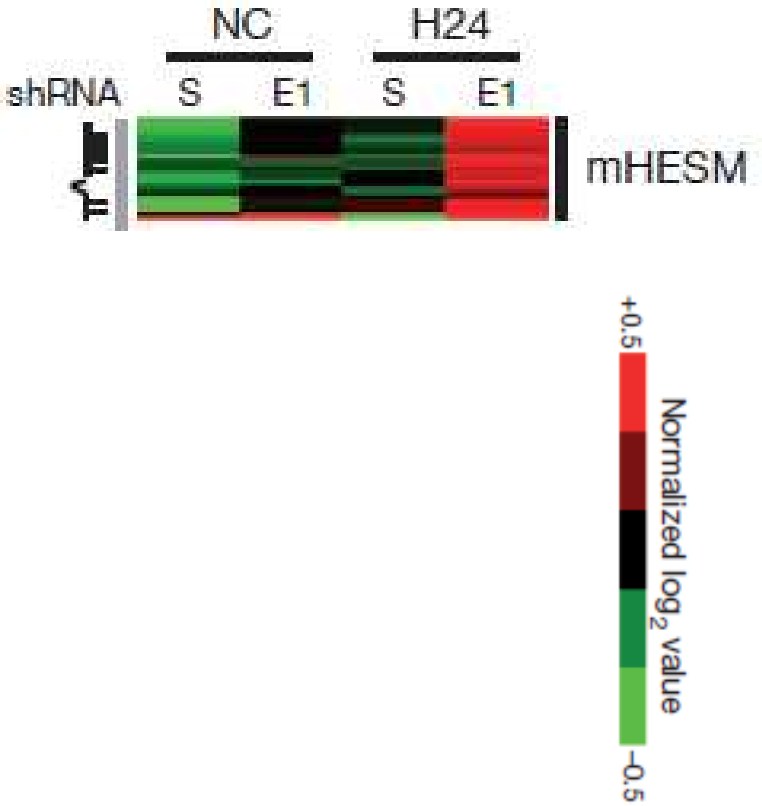
4 - mHESM targets



4 - mHESM targets

S = Scrambled control
E = EGFR shRNA





Under hypoxia, silencing of EGFR is related to mHESM maturation.

4 - mHESM targets

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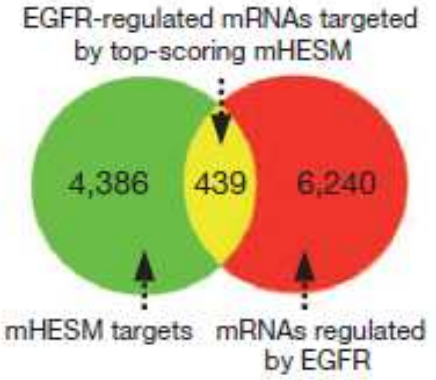
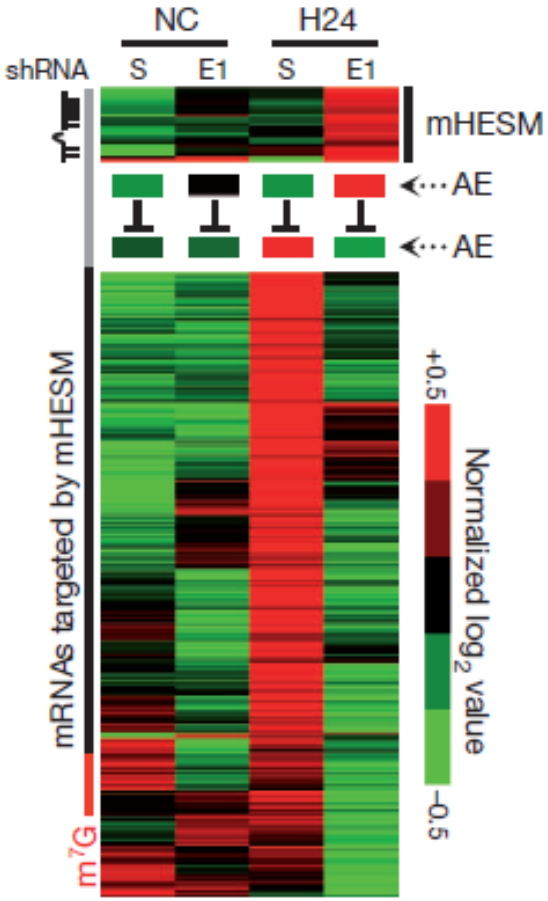


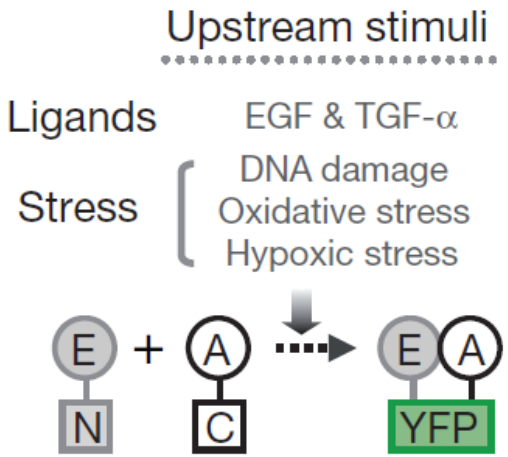


In response to hypoxia, EGFR reduces the production of mHESM enhancing the expression of corresponding mRNA targets.

AE = average expression

How does EGFR compromise miRNA maturation?

5 - EGFR-AGO2 interaction



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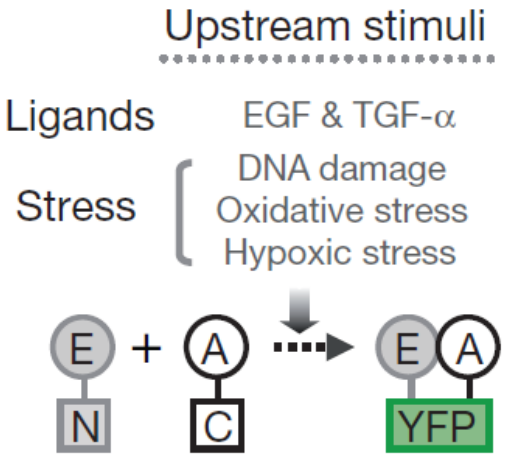

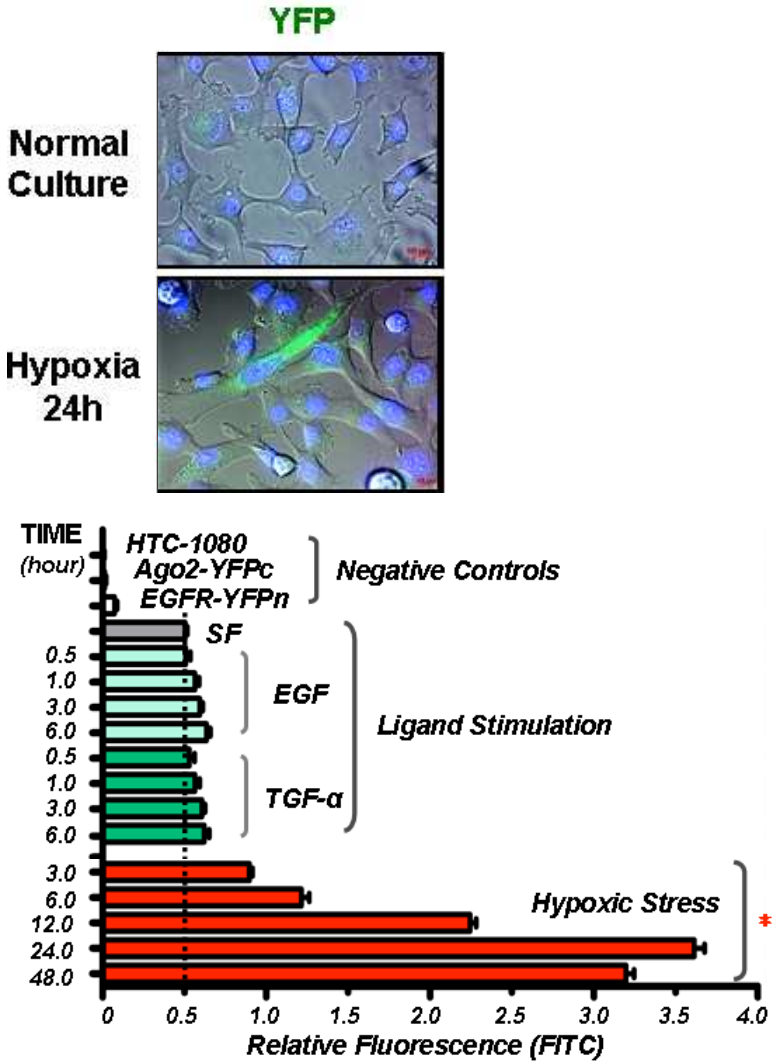

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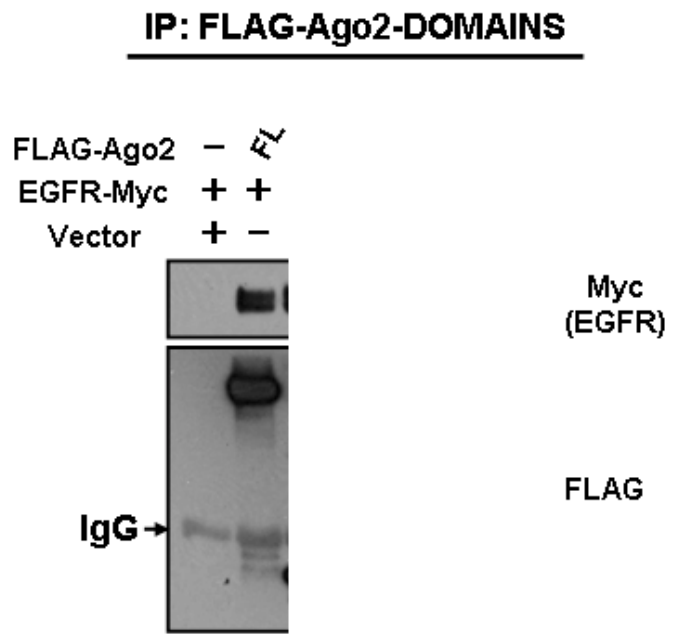

Anti-FLAG immunoprecipitates were blotted with myc antibody to show the interaction between EGFR and Ago2.

Under hypoxia.

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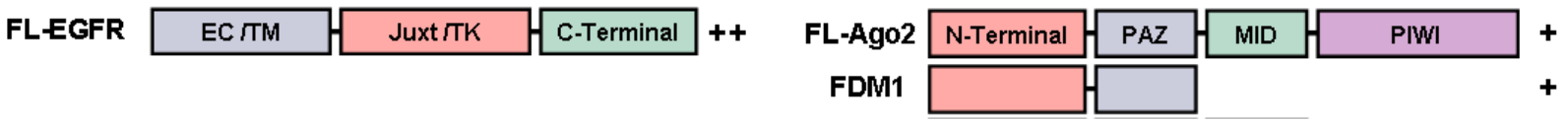

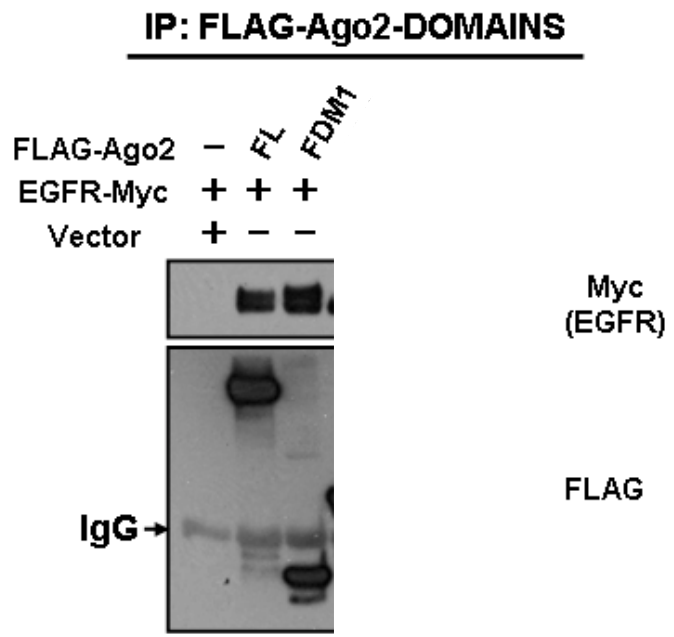

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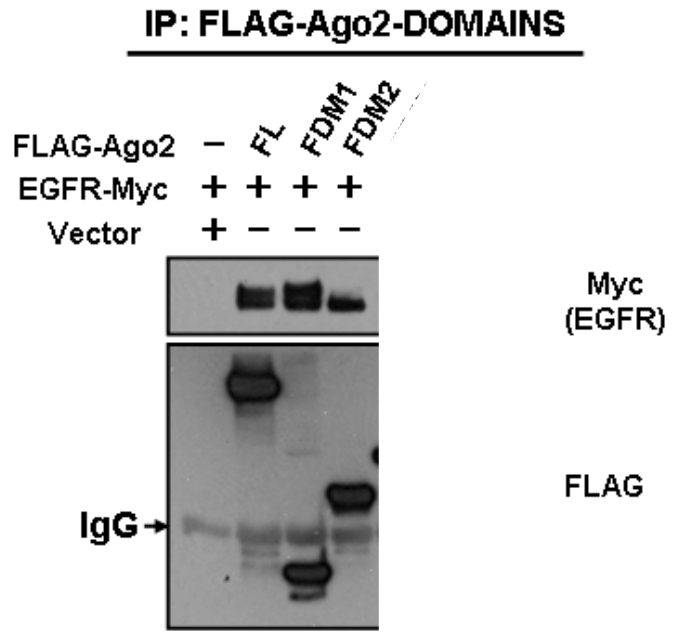

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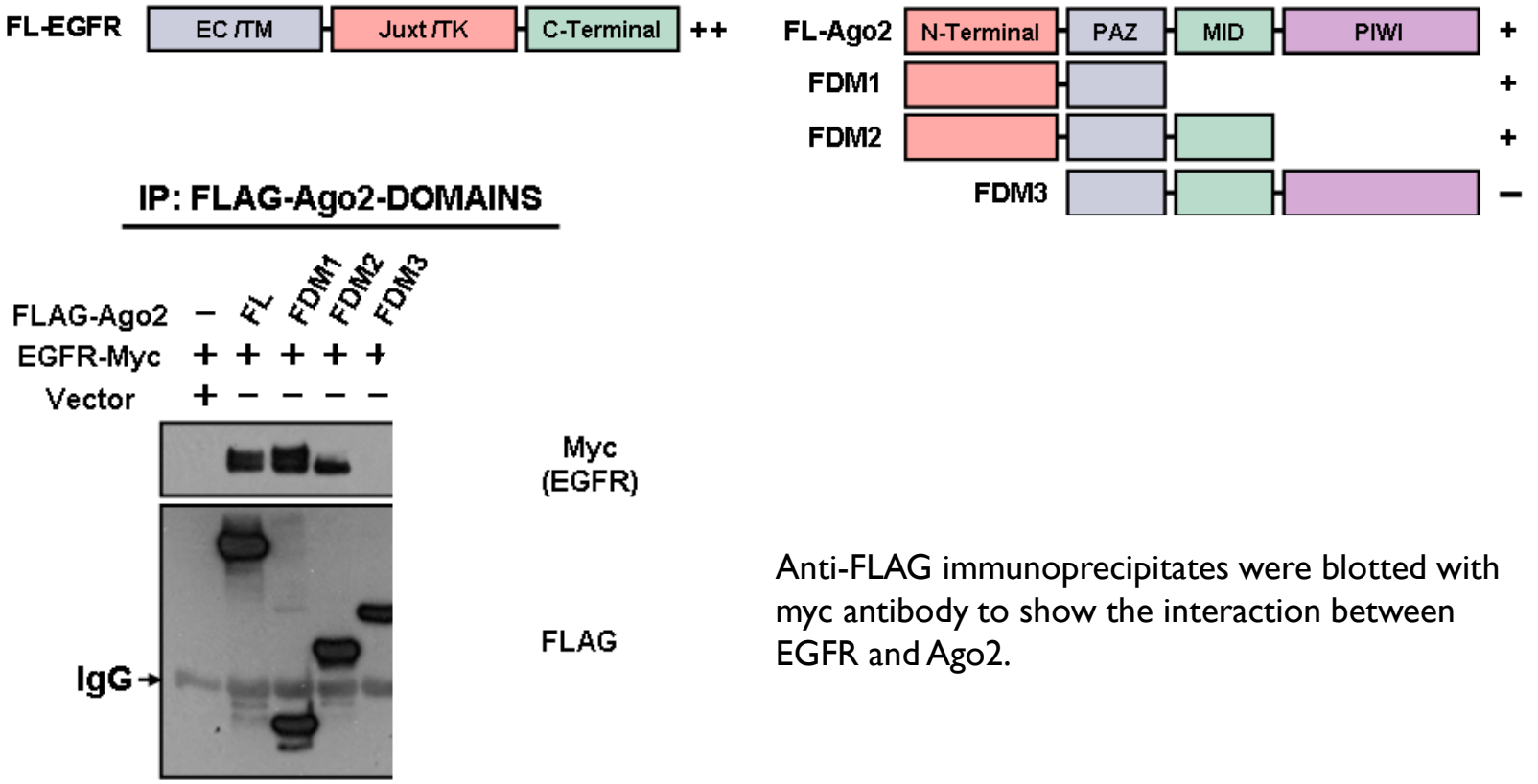

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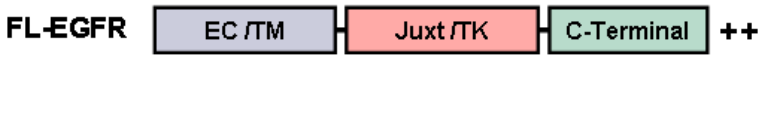

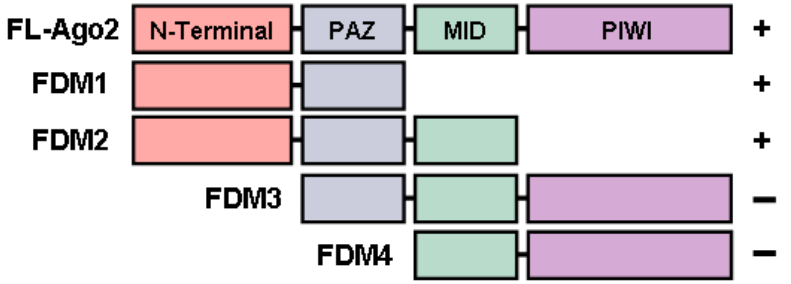

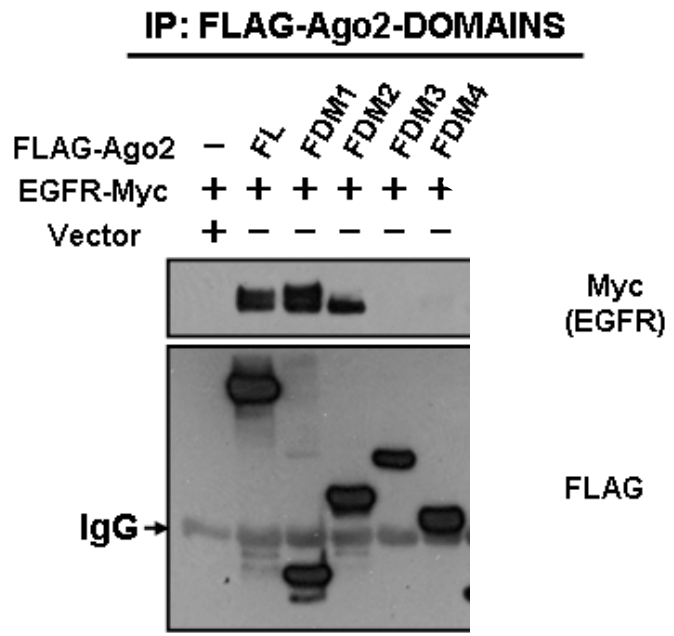

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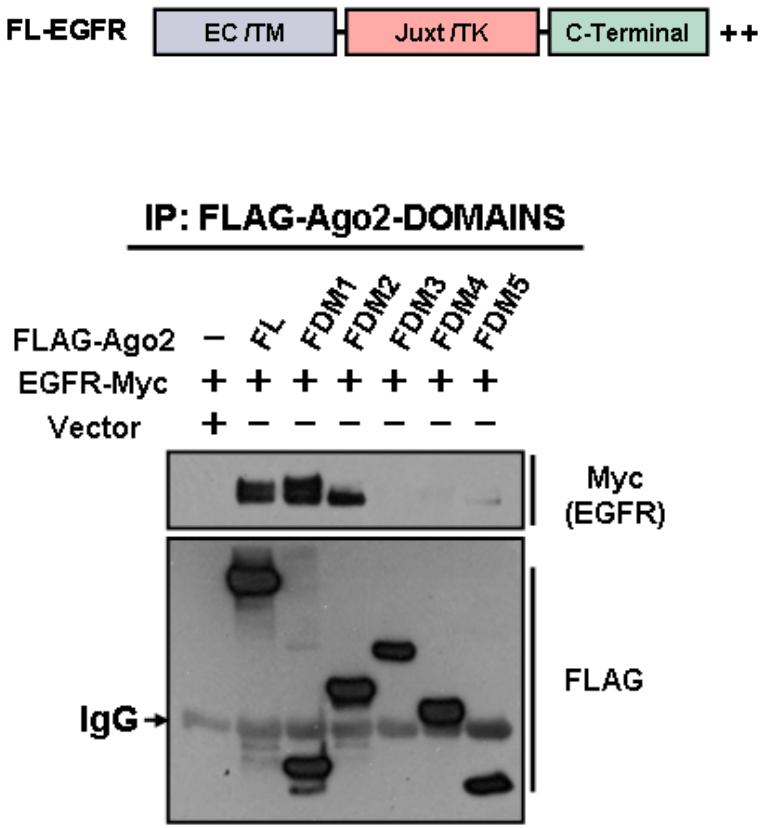

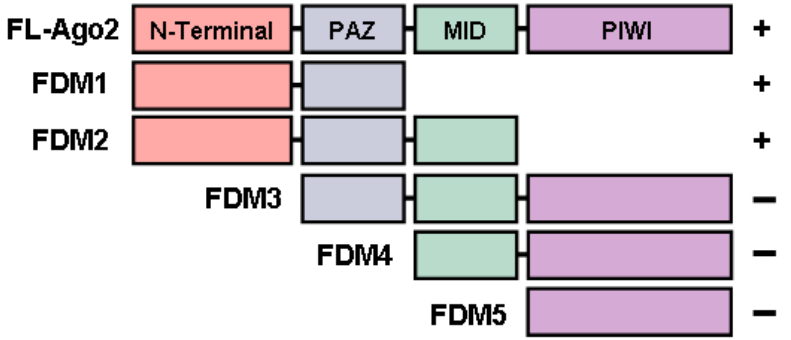

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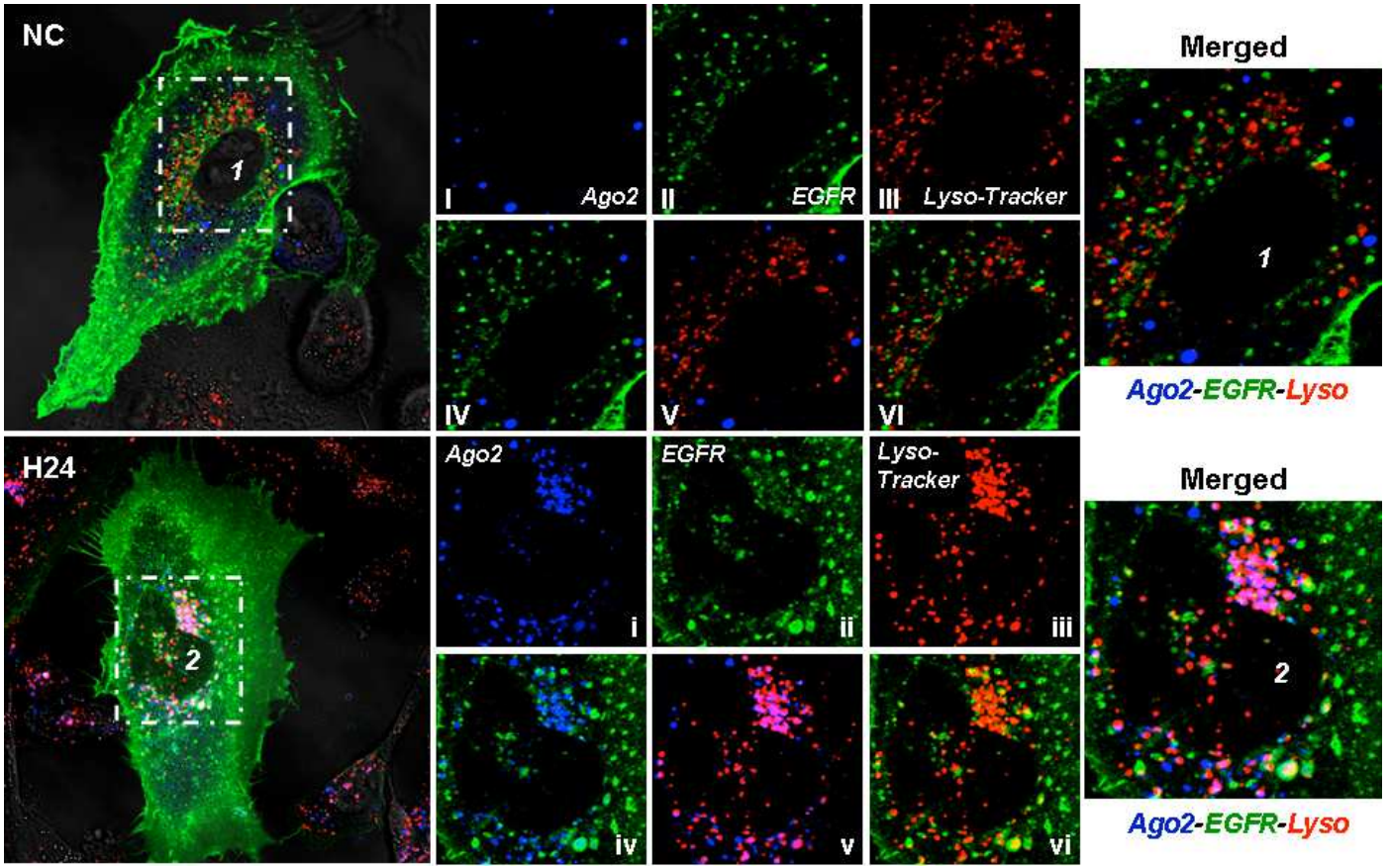
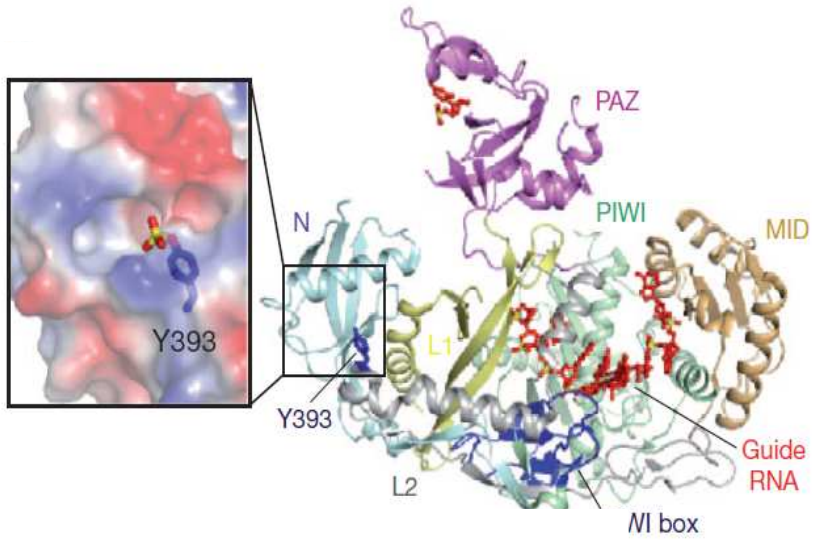
Under hypoxia, EGFR interacts with the **N-terminal** region of AGO2.

6 - Co-localization of EGFR-AGO2



HeLa cells co-transfected with:
- EGFR-GFP (green)
- BFP-Ago2 (blue)
- Lyso-Tracker (red color, accumulated in low internal PH compartments).

7 – Highly conserved Tyr in AGO2



393

D P Y V R E F G	Hs_AGO2
D P Y I Q E F G	Hs_AGO1
D P Y L K E F G	Hs_AGO4
D N Y A G E F G	At_AGO1
D S Y V Q E F G	Dm_AGO1
D T Y L T Q Y G	Sp_AGO1
D P F V Q E F Q	Hs_AGO3
D O F A H E F G	Ce_ALG1
E K E E S S A P	Kp_AGO1
S L T L G K F K	Nc_QDE2

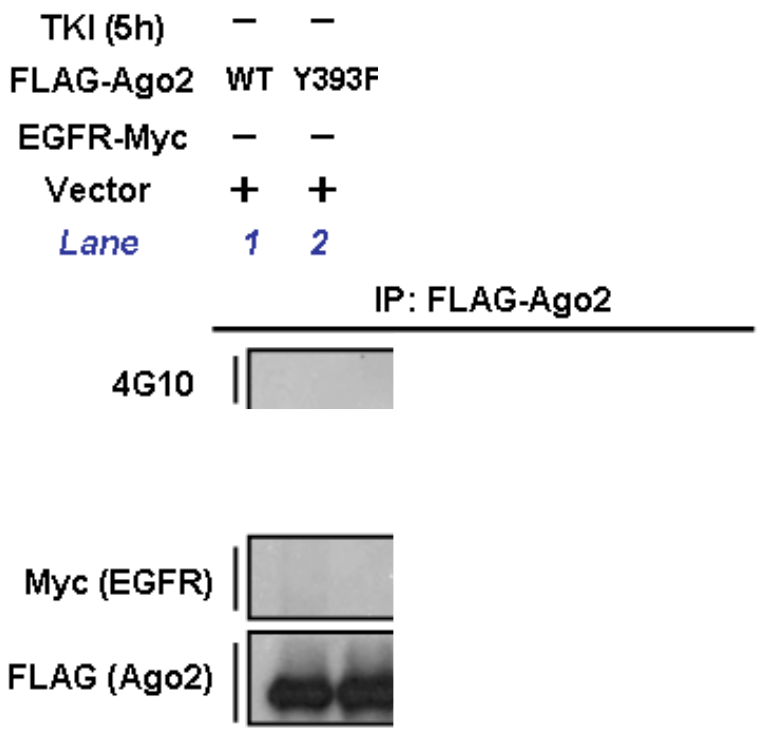
DPYVREFG	Hs_AGO2
DPYVREFG	Pt_AGO2
DPYVREFG	Bt_AGO2
DPYVREFG	Mm_AGO2
DPYVREFG	Rn_AGO2
DPYVREFG	Dr_AGO2

Identified one highly conserved residue in AGO2 (**Tyr393**) as potential site for EGFR kinase activity.

8 - EGFR kinase activity on AGO2

4G10 = Anti-phosphotyrosine antibody

Under hypoxia.



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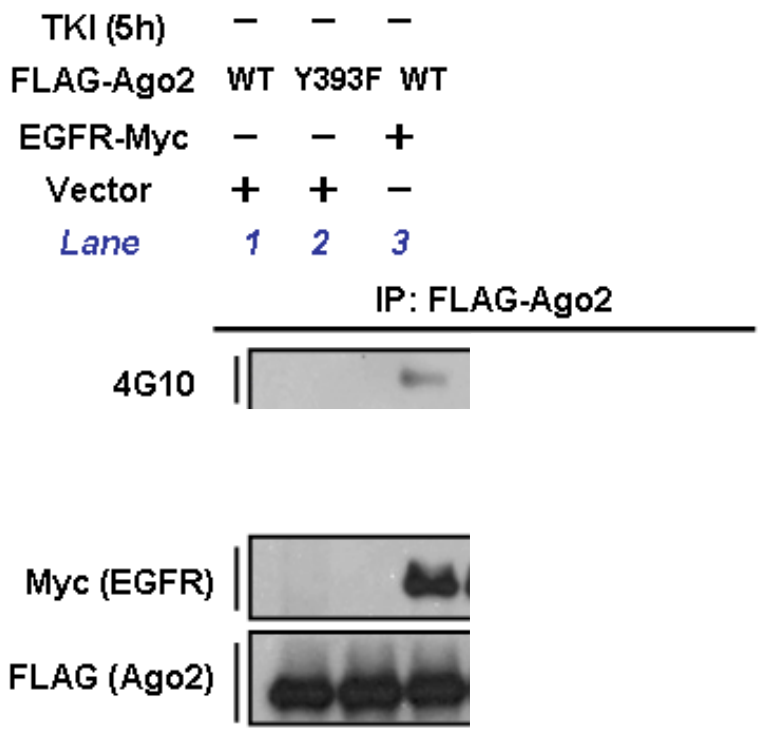

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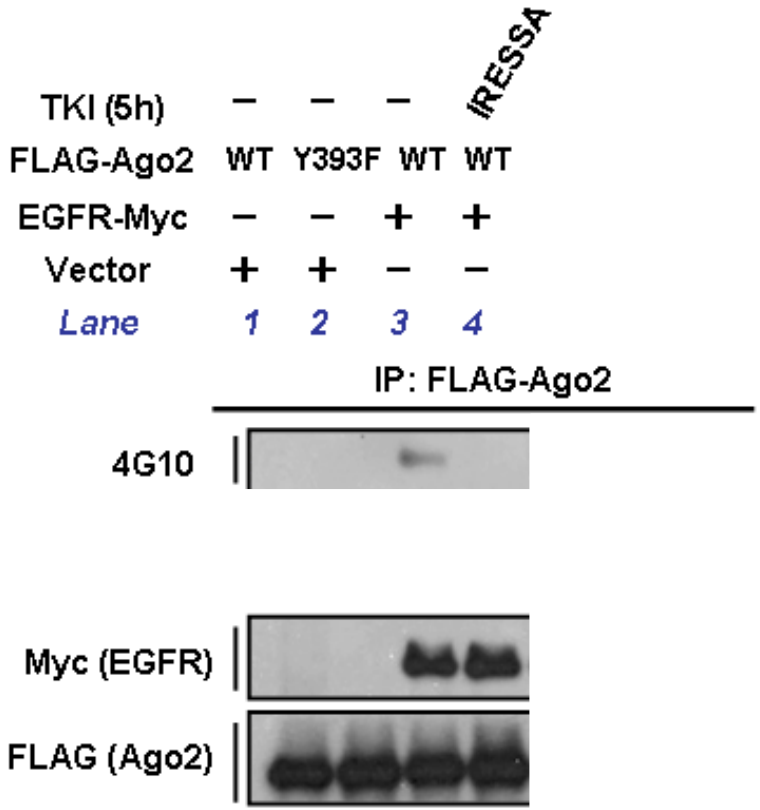

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IRESSA is a Tyr Kinase Inhibitor

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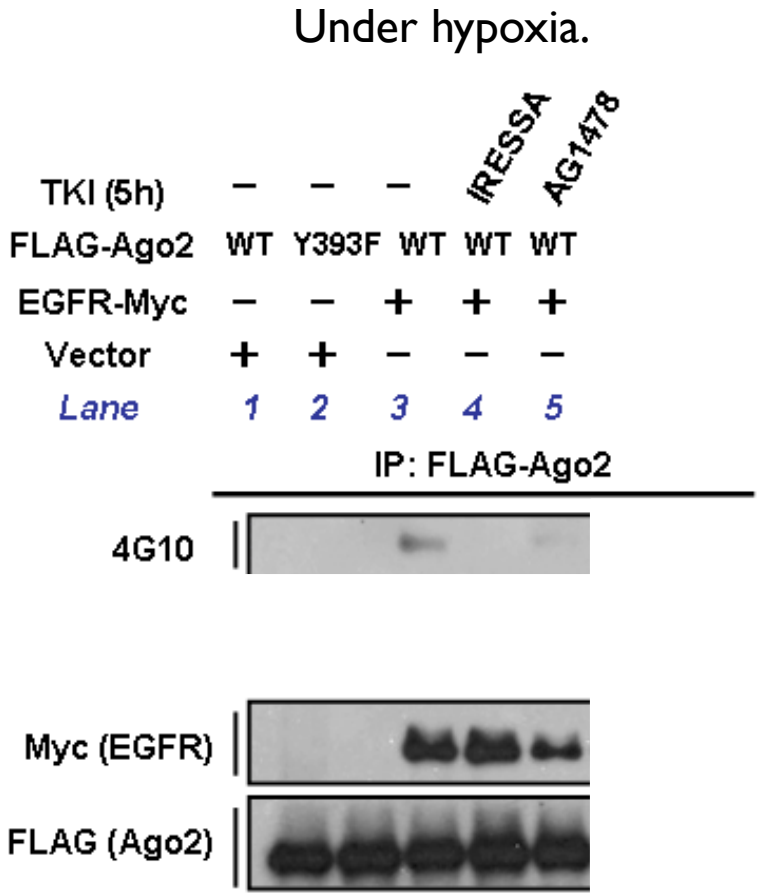

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AG-1478 is a selective EGFR inhibitor

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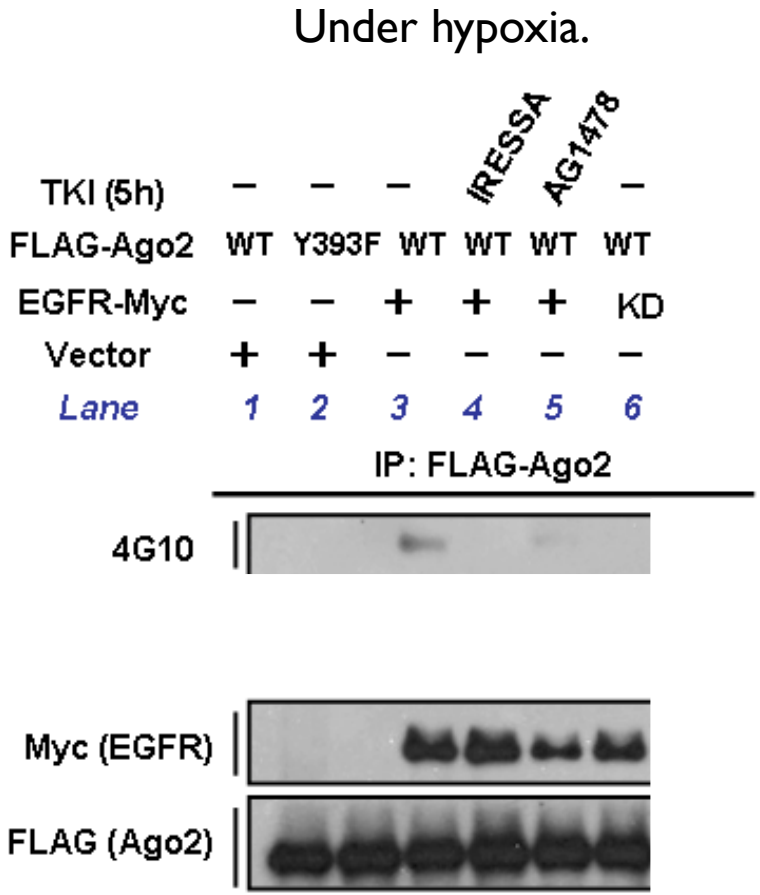

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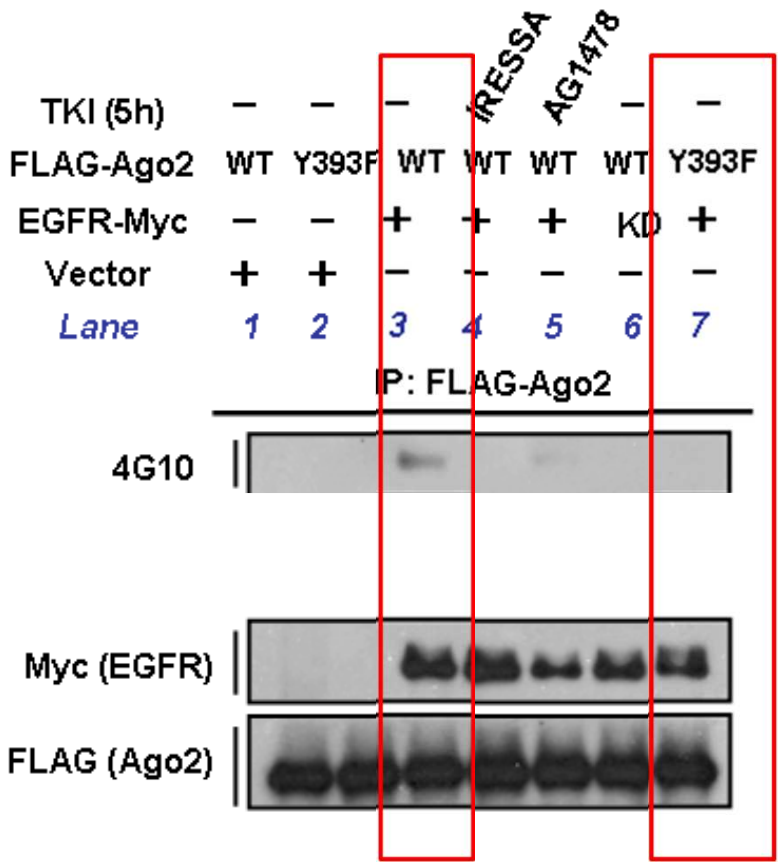

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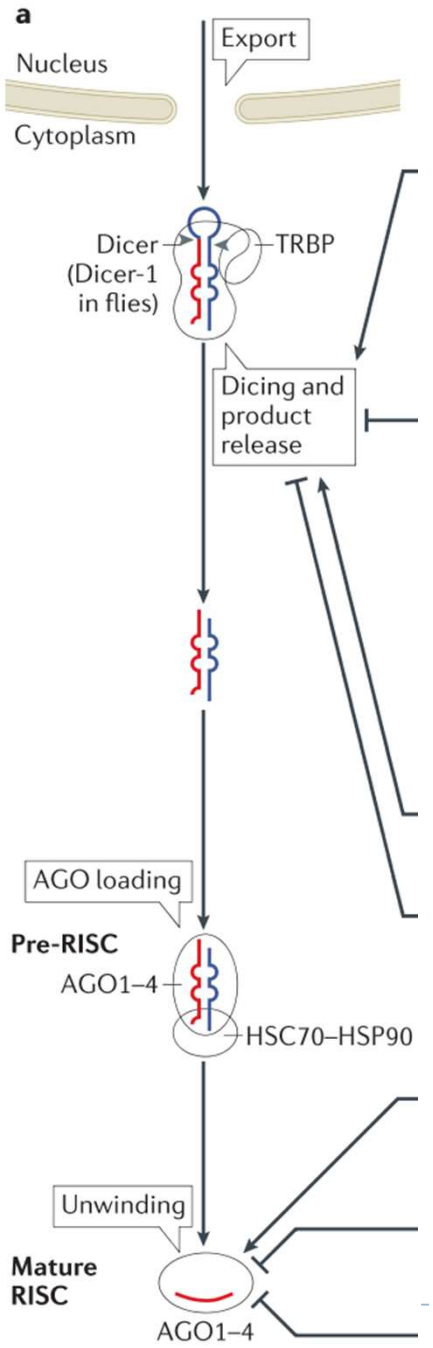
IRESSA is a Tyr Kinase Inhibitor

AG-1478 is a selective EGFR inhibitor

EGFR specifically phosphorylates AGO2 Tyr393.

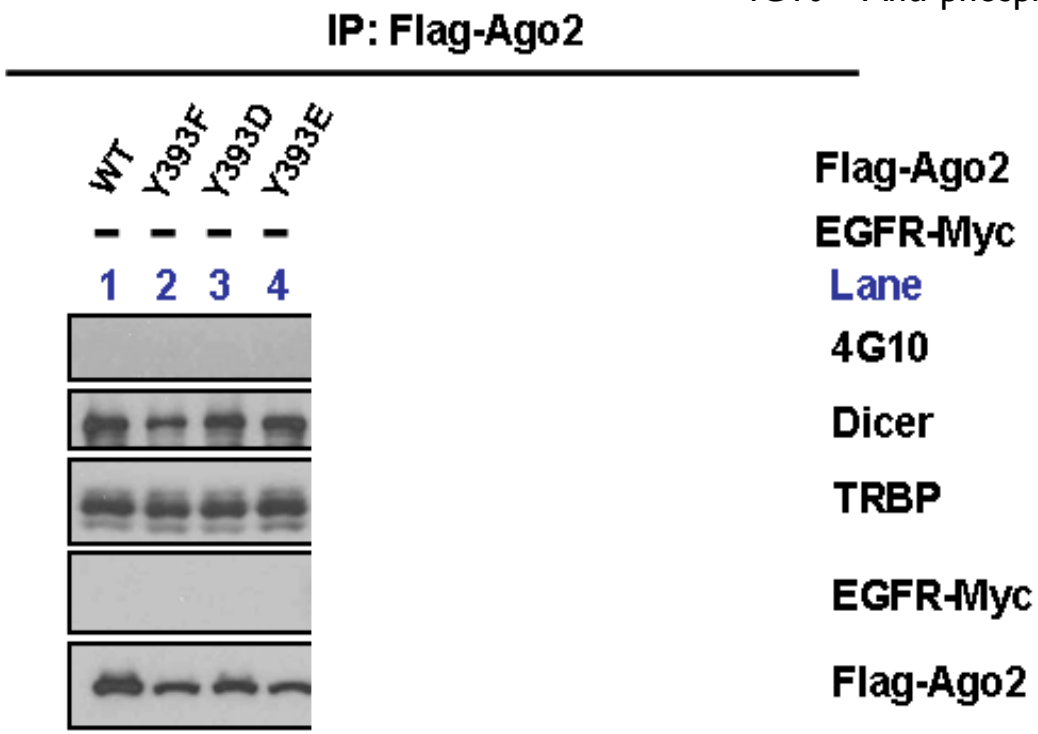


have a role in RISC assembly that resembles the function of the RLC component Dcr-2 (REFS 119,164–167). A recombinant human Dicer–TRBP complex has been shown to bind to siRNA duplexes *in vitro*[168,169]. It has also been reported that the RLC has both pre-miRNA processing activity and target cleavage activity *in vitro*[170]. These findings support the idea that miRNA duplex loading may be coupled with Dicer-dependent pre-miRNA processing in humans (known as the 'Dicer-dependent AGO loading' model). However, *Dicer1*-knockout mouse embryonic stem cells are able to undergo siRNA-directed gene silencing[98,99], which strongly indicates that Dicer is not important for small RNA loading into AGO proteins. Moreover, in flies and mammals, Dicer has been reported to be dispensable for asymmetric RISC assembly *in vitro* and also in cells[133,153,160,171,172]. Thus, the RLC may not be essential for small RNA loading on *D. melanogaster* AGO1 and human AGO proteins, although it is important for loading onto *D. melanogaster* AGO2.

9 - Role of phospho-Y393-AGO2

Under Hypoxia.

4G10 = Anti-phosphotyrosine antibody



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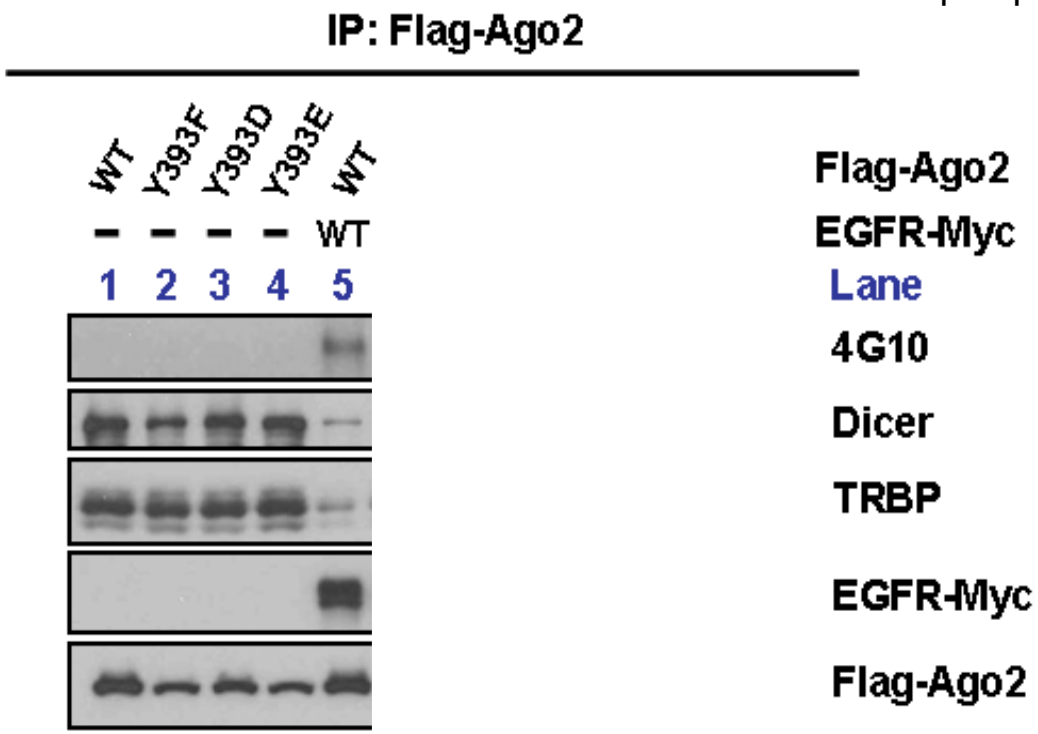

The phosphorylation of AGO2 Tyr393 drastically reduces Dicer-AGO2 interaction.

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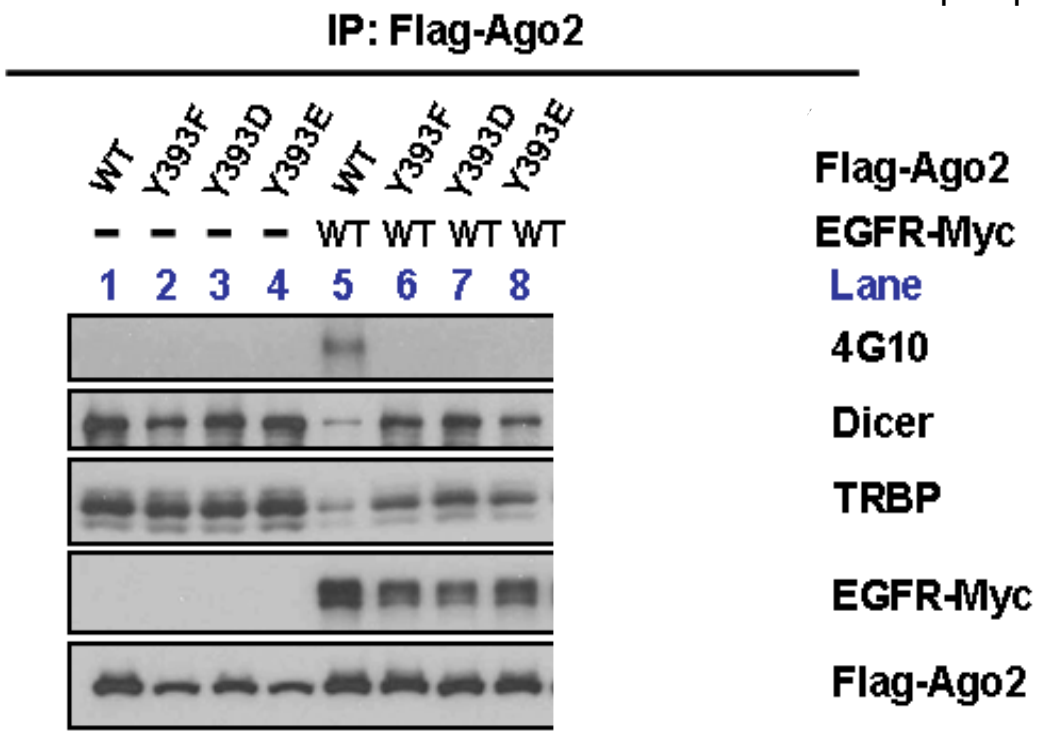

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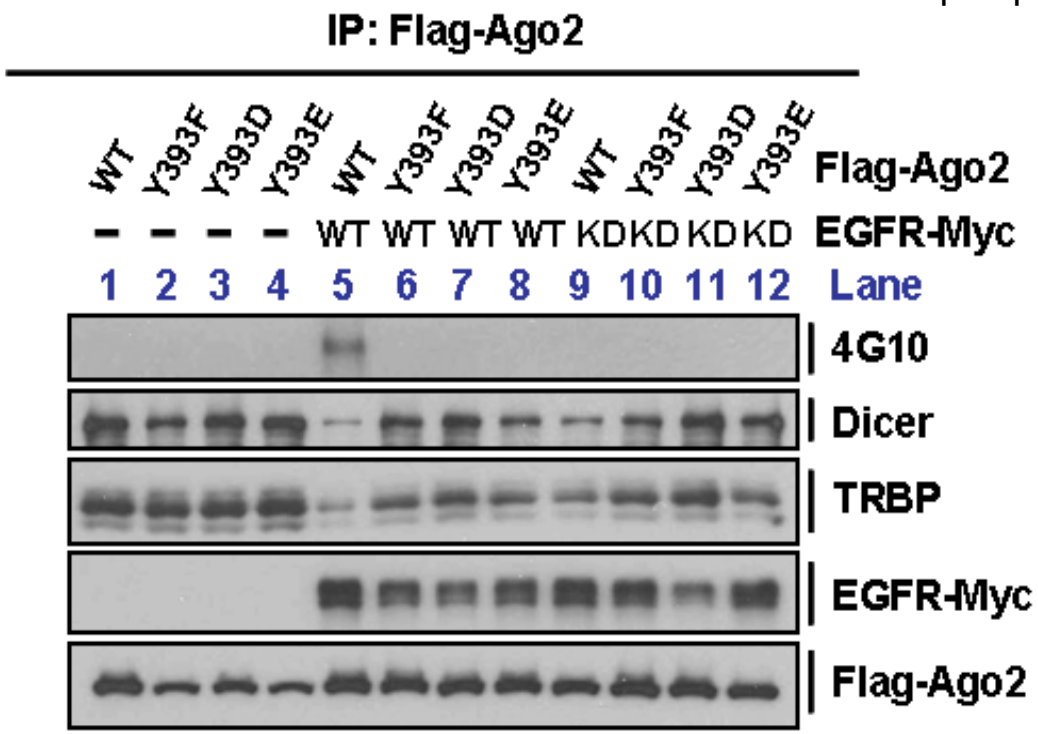
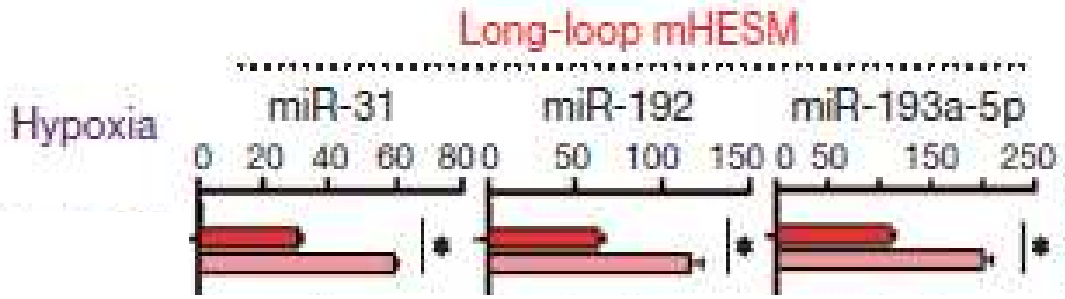


The reduced interaction between AGO2 and Dicer in the RISC-loading complex (RLC) affects the processing of pre-mHESM.

10 – Dicer's silencing



mHESM maturation depends by AGO2 phosphorylation.

AGO2-WT
AGO2-Y393F

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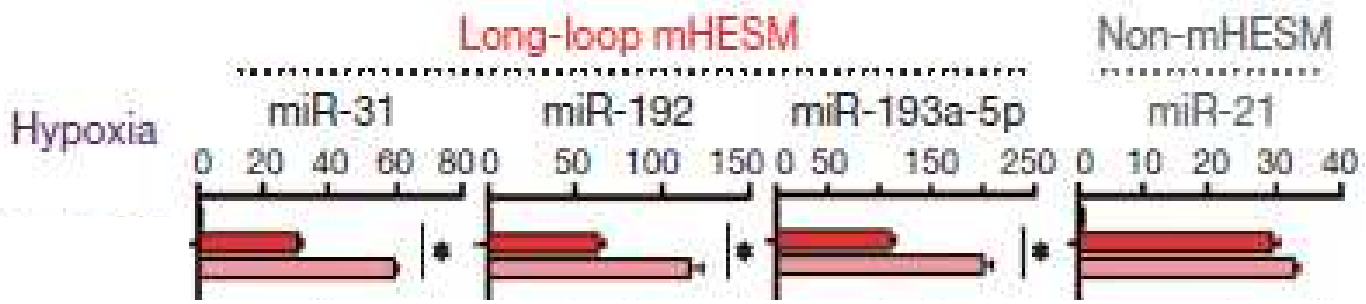

AGO2 phosphorylation specifically affects the maturation of long-loop mHESM.

AGO2-WT
AGO2-Y393F

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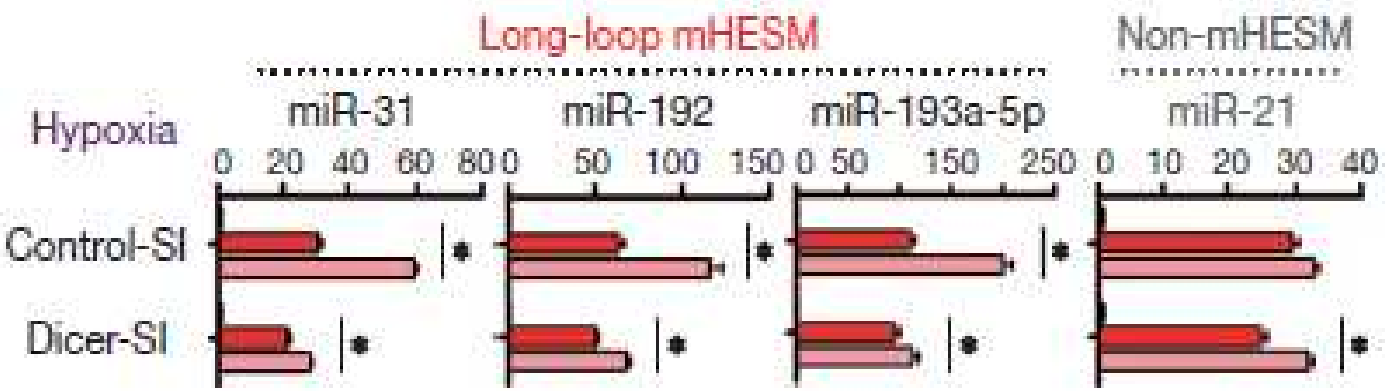
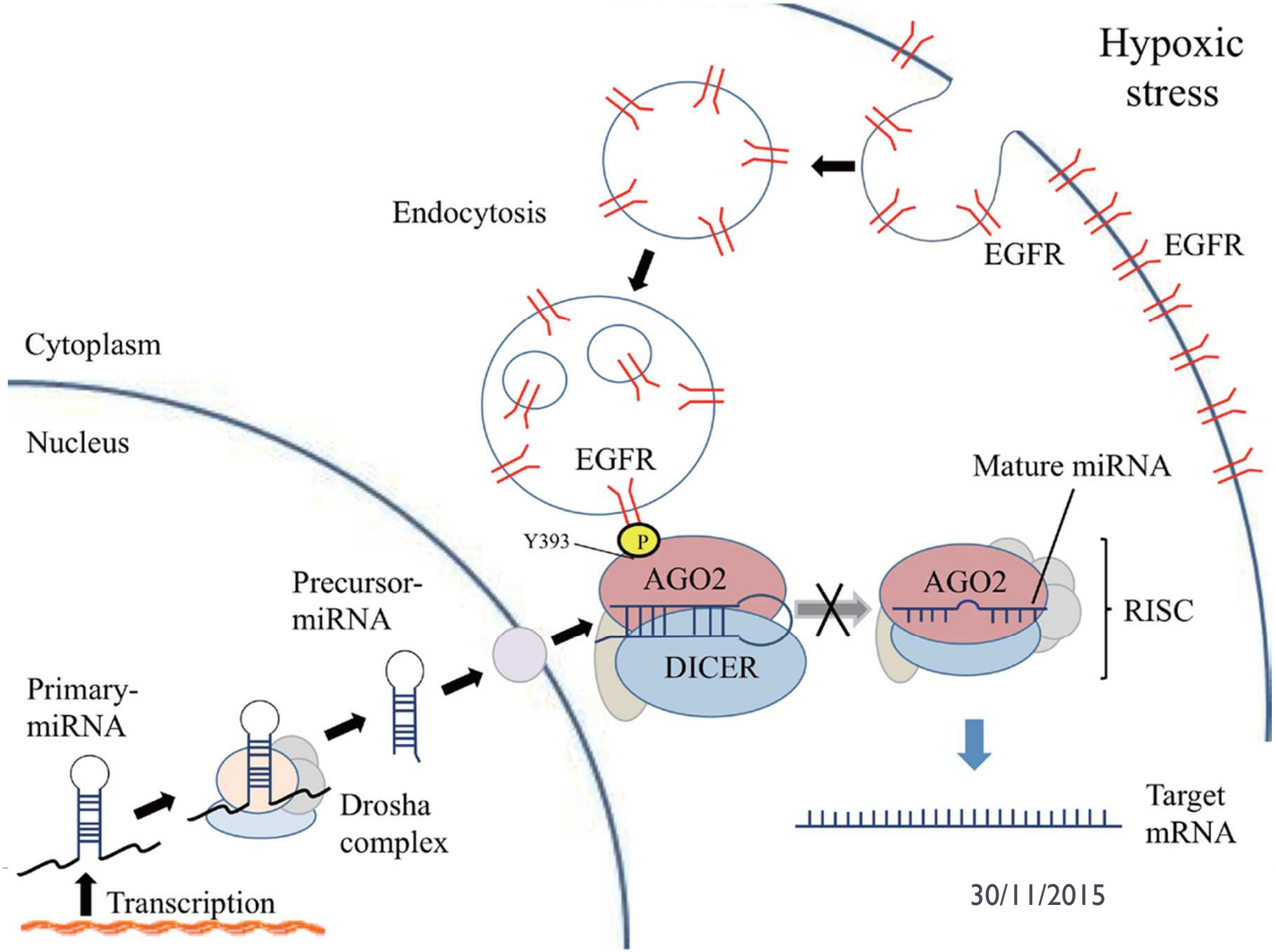


mHESM maturation is dicer-dependent.

Summary


Hypoxic stress
Endocytosis
EGFR
EGFR
EGFR
Cytoplasm
Nucleus
Mature miRNA
Y393
P
AGO2
AGO2
RISC
Precursor-miRNA
DICER
Primary-miRNA
Drosha complex
Target mRNA
Transcription

Conclusions

1. Hypoxia upregulates EGFR;
2. EGFR compromises miRNA maturation;
3. EGFR interacts with the N-terminal domain of AGO2;
4. EGFR-AGO2 are co-localized in low-pH compartments;
5. EGFR specifically phosphorylates Tyr 393 of AGO2;
6. The Y393 phosphorylation reduces the interaction of AGO2 with Dicer, compromising miRNA maturation.