Caveolin-1 is Required for Signaling & Membrane Targeting of EphB1 Receptor

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Molecular Regulators of the Vascular System

Receptor Tyrosine Kinases (RTKs) and their ligands

- Vascular Endothelial Growth Factor Receptors (VEGFRs) and VEGF
- Tie receptors and angiopoietins
- Eph receptors and ephrins

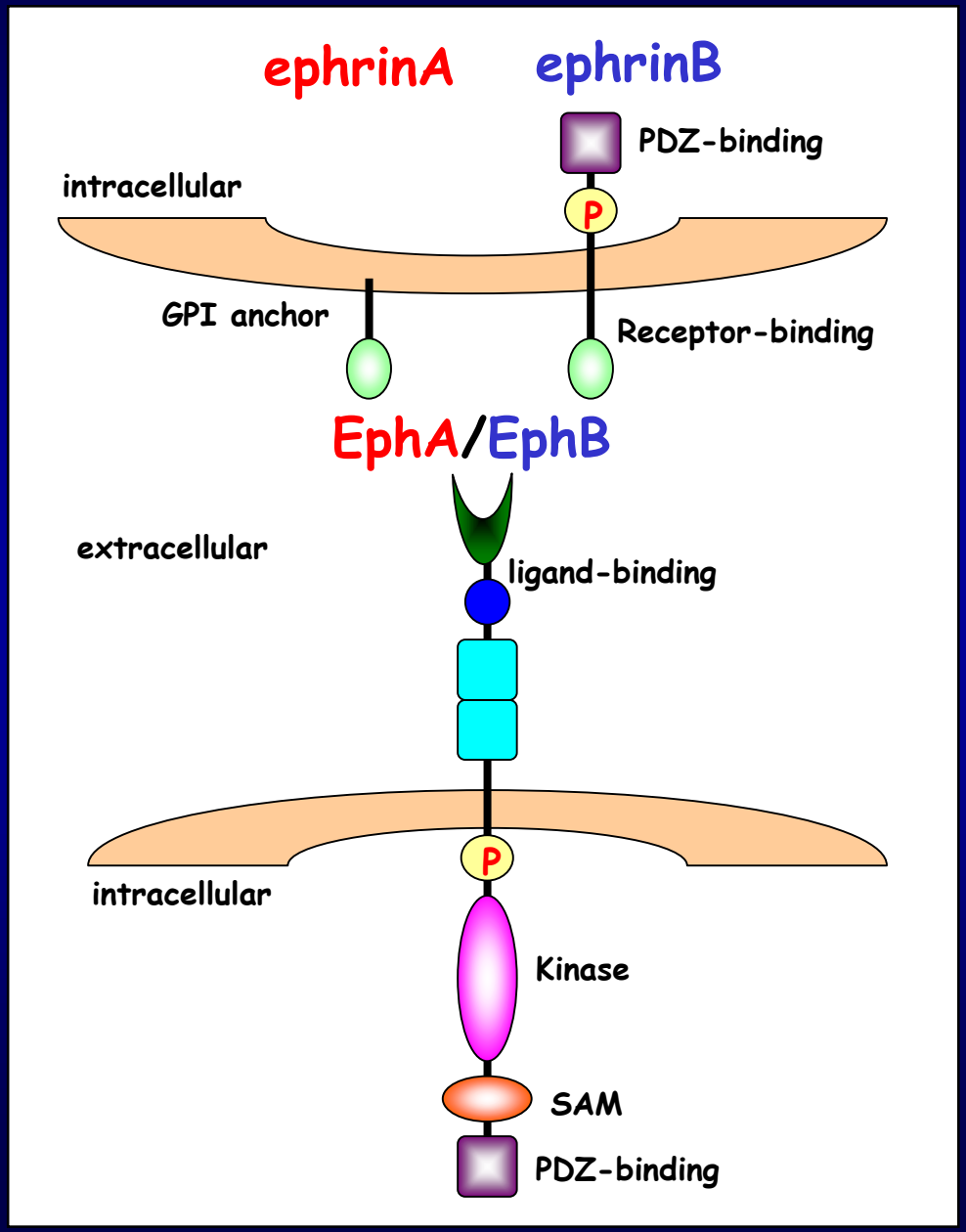
Structures of Eph Receptors and Ephrins

ephrinA1-ephrinA6

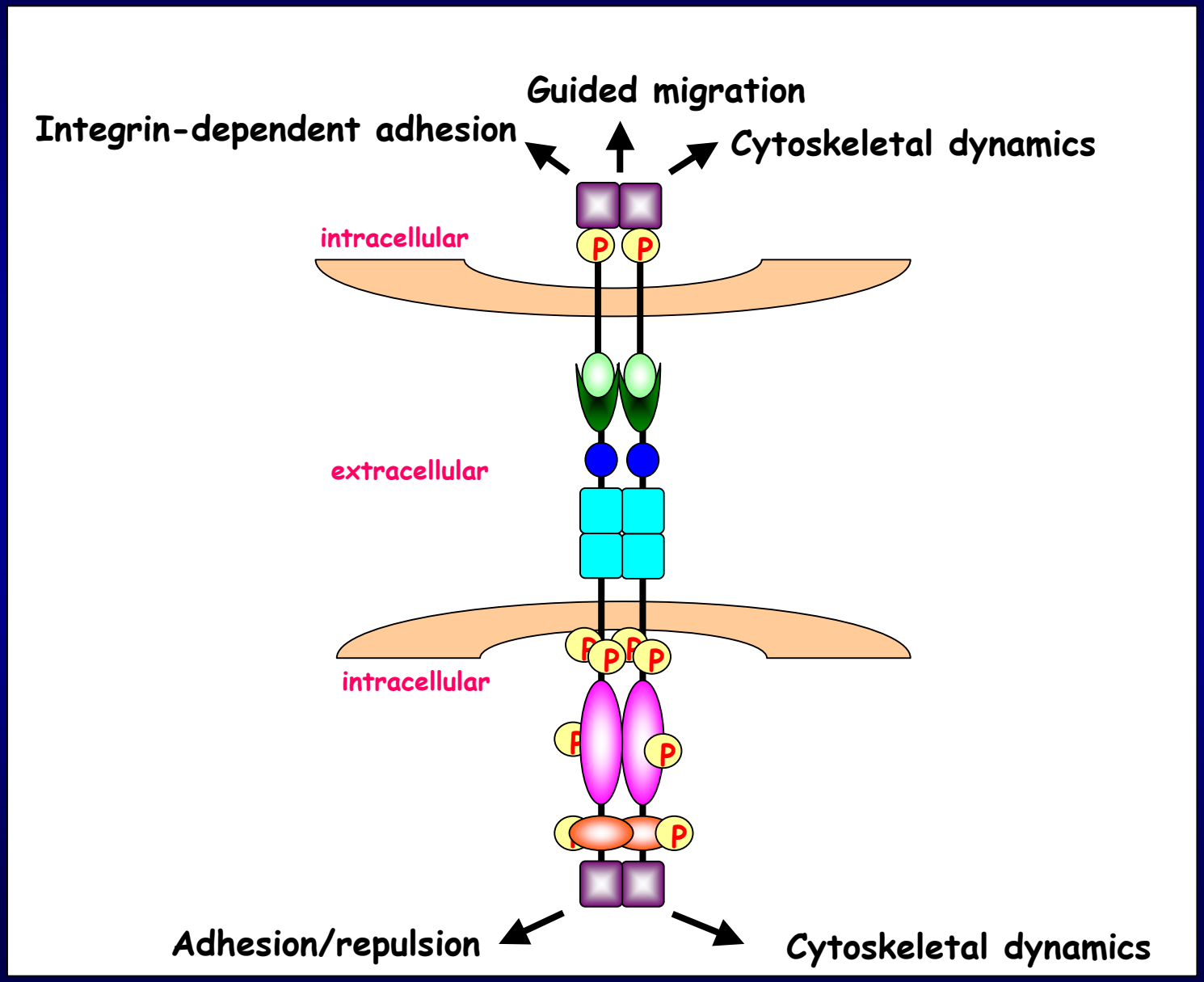
ephrinB1-ephrinB3

EphA1-EphA10

EphB1-EphB6



Signaling Mechanisms - Eph/Ephrin System



Vascular Endothelial Targeting: Critical Role of Eph/Ephrin Interactions

- No proliferative functions
- Guiding cell- cell and cell-matrix interactions

  → mature, vascular networks
- Embryo: vascular patterning
- Adult: functions and regulation remain to be defined

Study

Is the lipid raft protein caveolin-1 required for the proper expression and signaling of EphB1 receptor?

Rationale

- Caveolae and caveolin-1 regulate angiogenic events
- Several RTKs have caveolin-binding motif within their kinase domain
- EphrinAs and ephrinBs are localized to lipid rafts

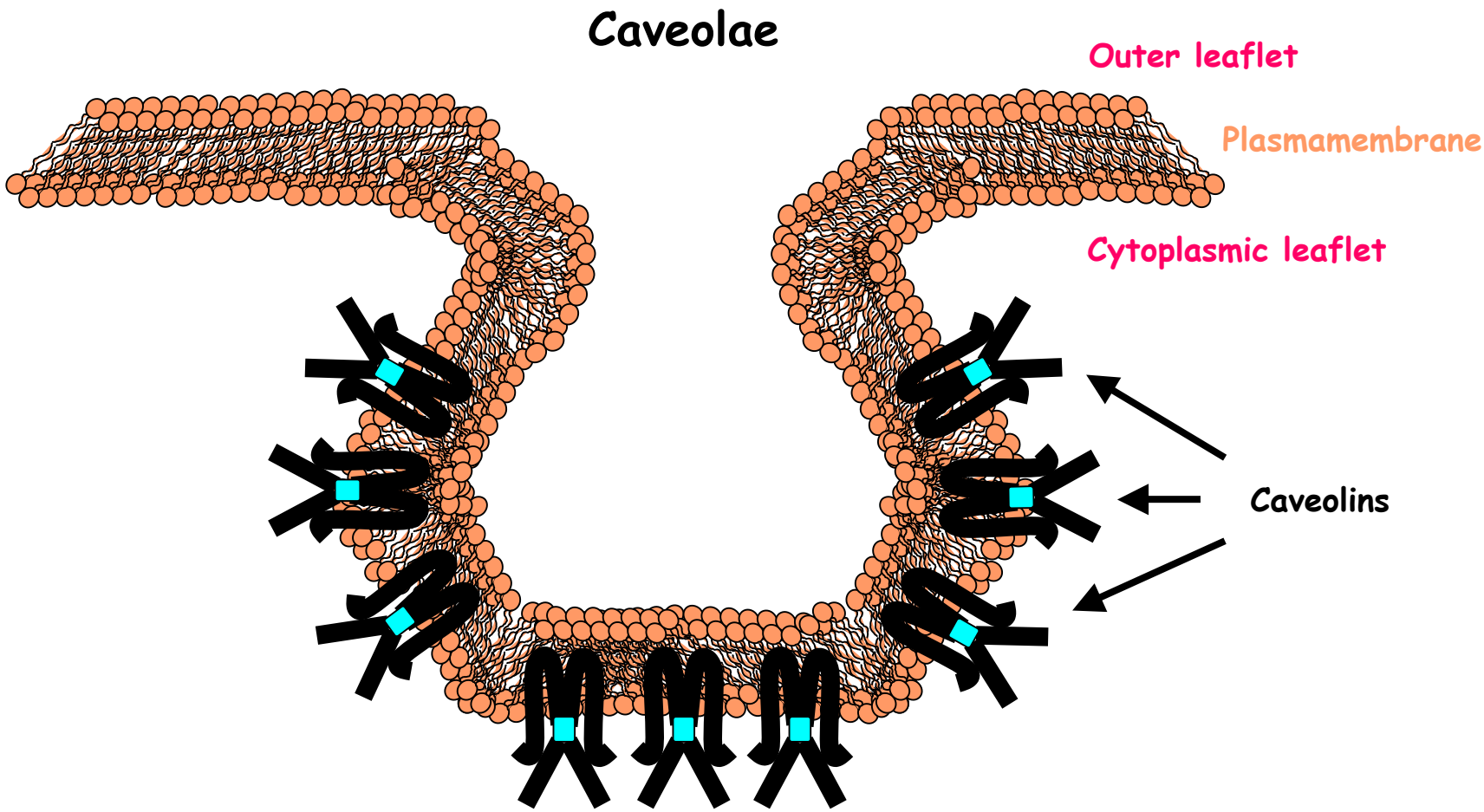
Questions

1) Do Eph receptors interact with Caveolin-1?

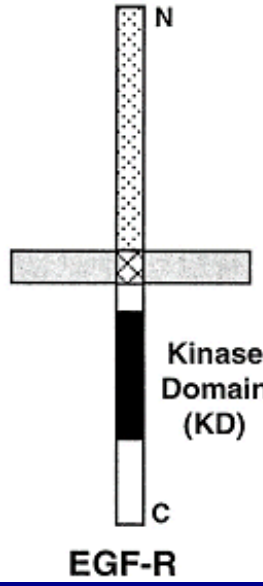
2) How does Caveolin -1 regulate the signaling pathways downstream of EphB1?

3) Which are the consequences of mutations on the putative Caveolin-1 binding sequence of EphB1 ?

Structure of Caveolae and Caveolins



Caveolin-Binding Domain in RTKs



Caveolin binding motif
ΦXΦXXXXΦ

DV**W**S**Y**GVTV**W**ELMT**F**	EGF-R
DM**W**S**F**GVVF**W**EITSL	Insulin-R
DV**W**S**F**GILL**W**EI**F**SL	PDGF-R
DV**W**S**Y**GVTV**W**ELMT**F**	erbB2
DV**W**S**F**GVLL**W**EI**F**TL	FGF-R
DV**W**S**F**GVVL**W**EI**F**T**Y**	NGF-R
DV**W**S**F**GVVL**W**EIATL	IGF-R

Φ: W,F OR Y

Couet et al, *JBC*, 1997

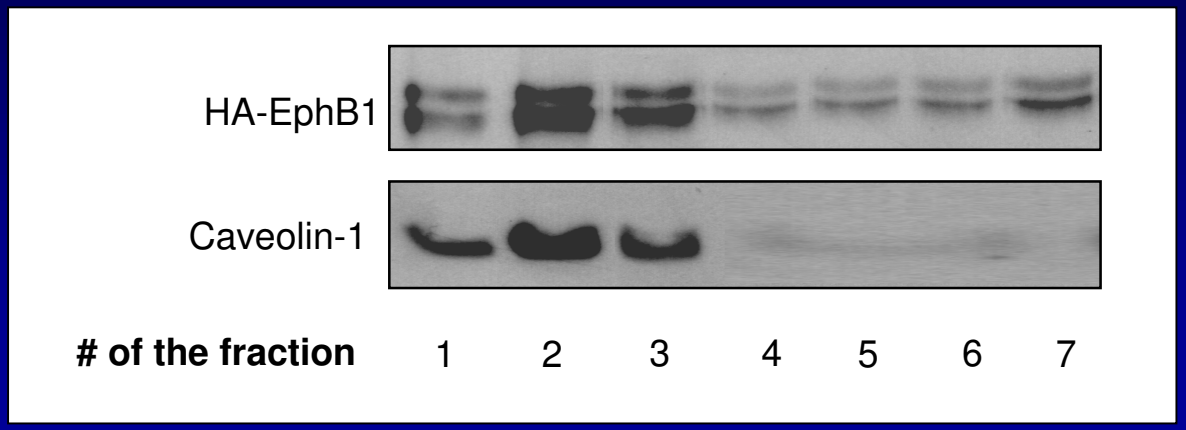
EphB1

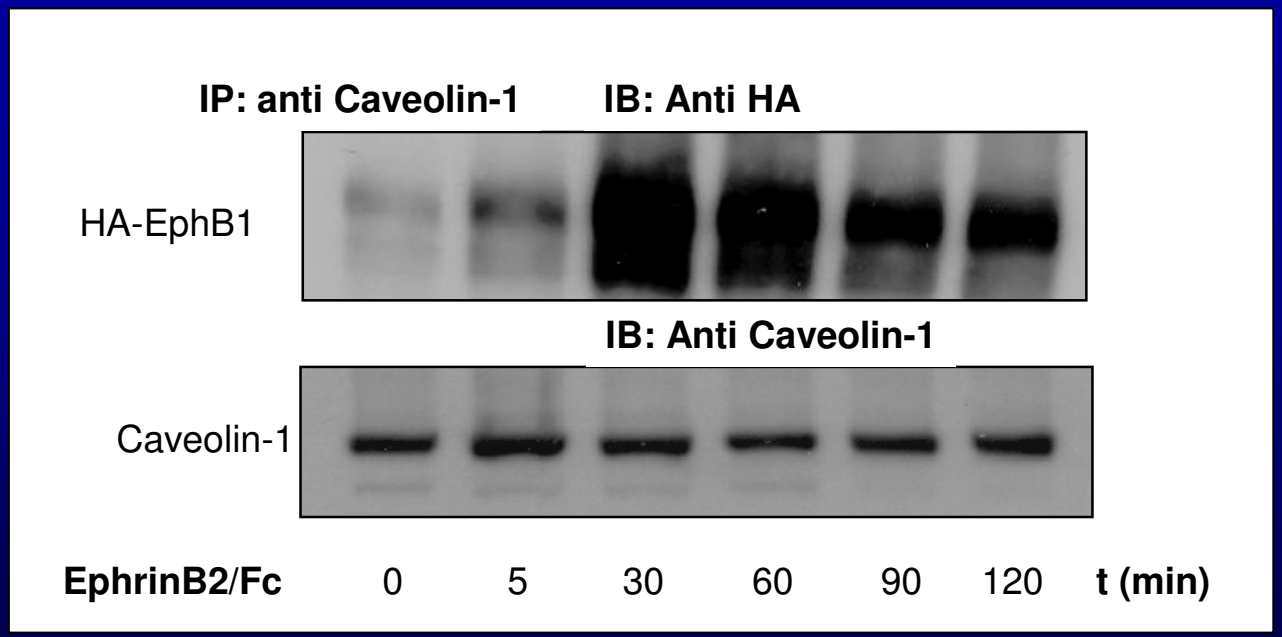
DVWSYGIHMW

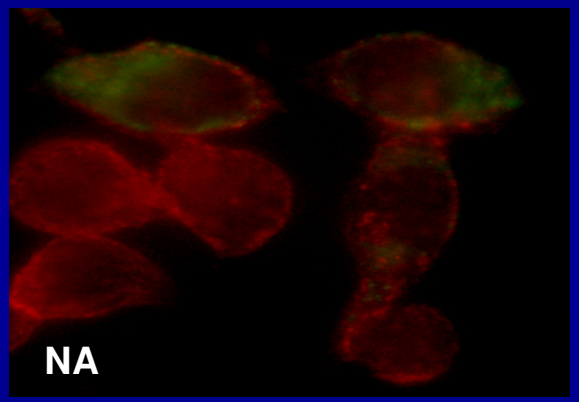
EphA2

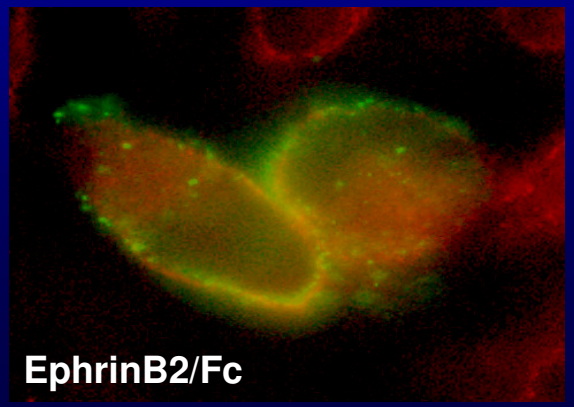
DVWSFGIVMW

EphB1 Localizes to Lipid Rafts & Associates with Caveolin-1

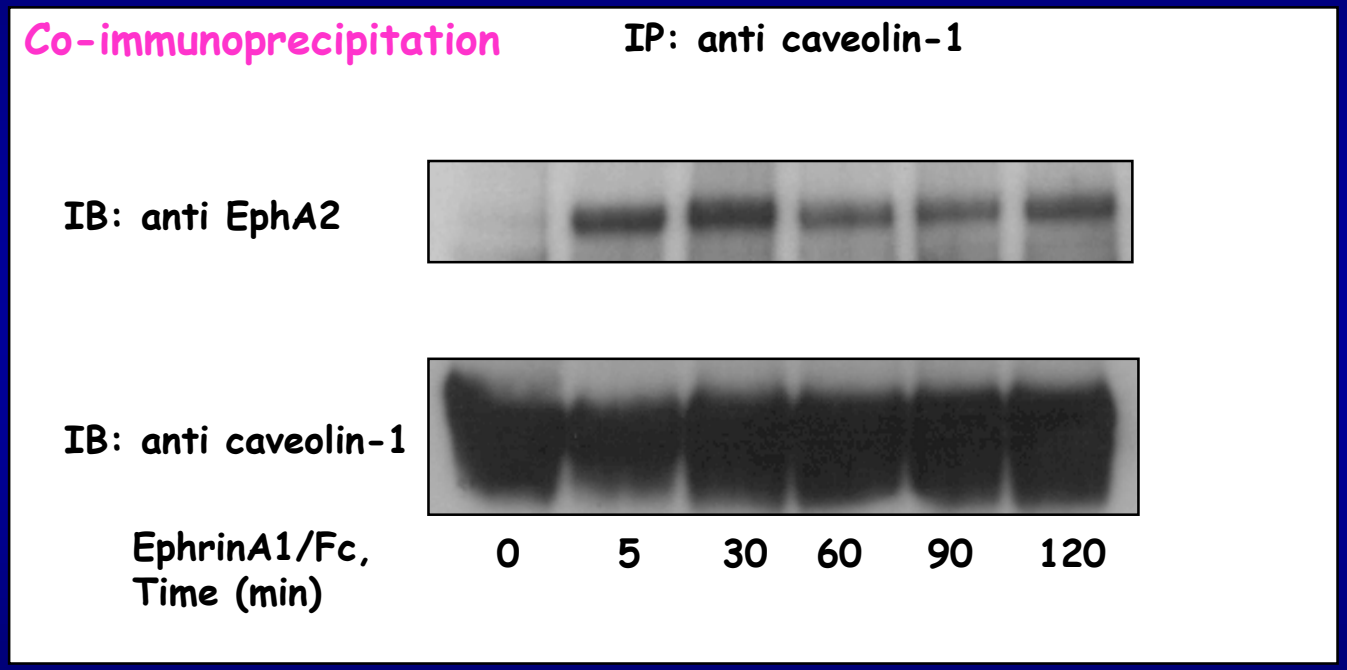




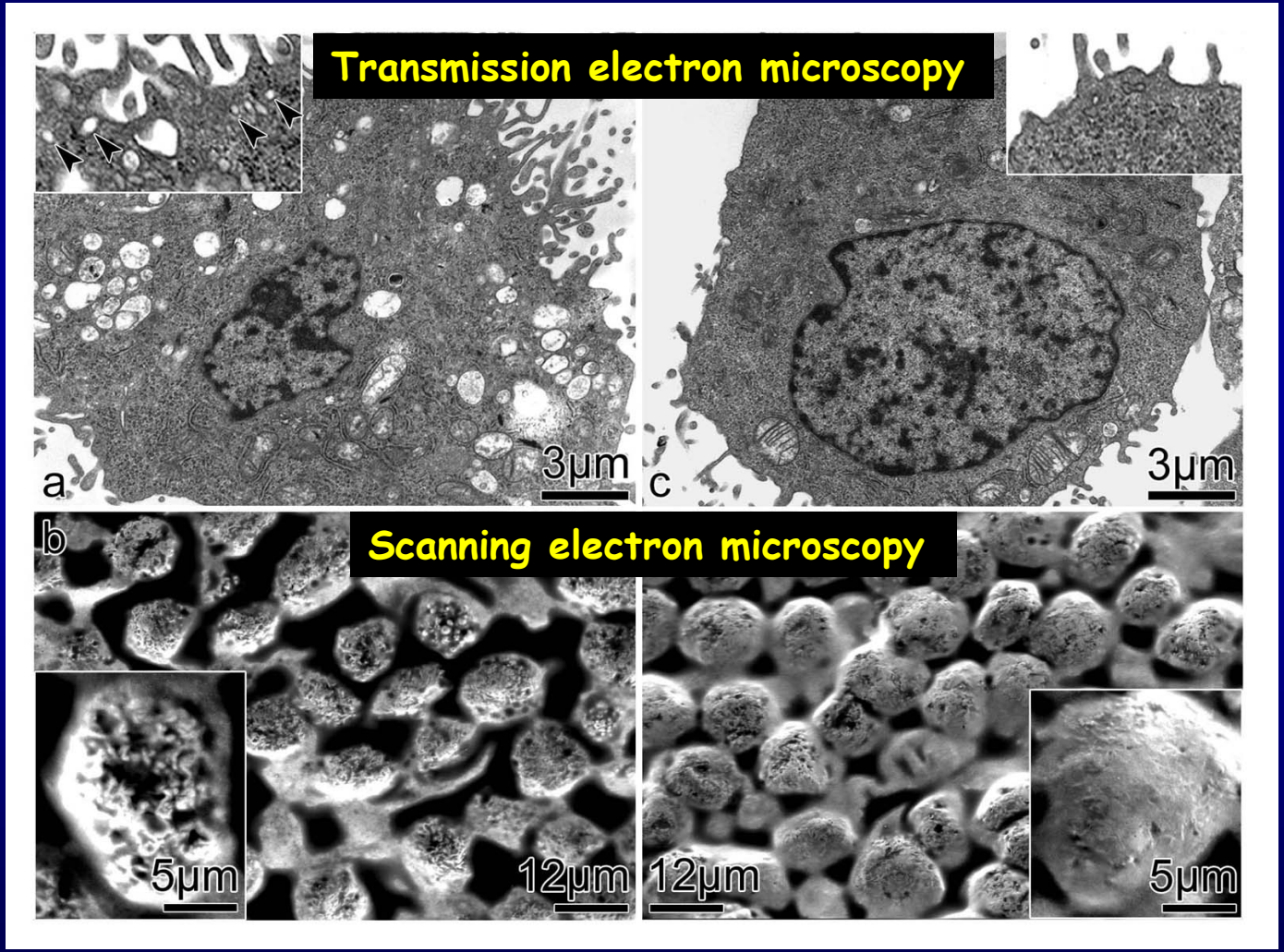




EphA2 also associates with Caveolin-1

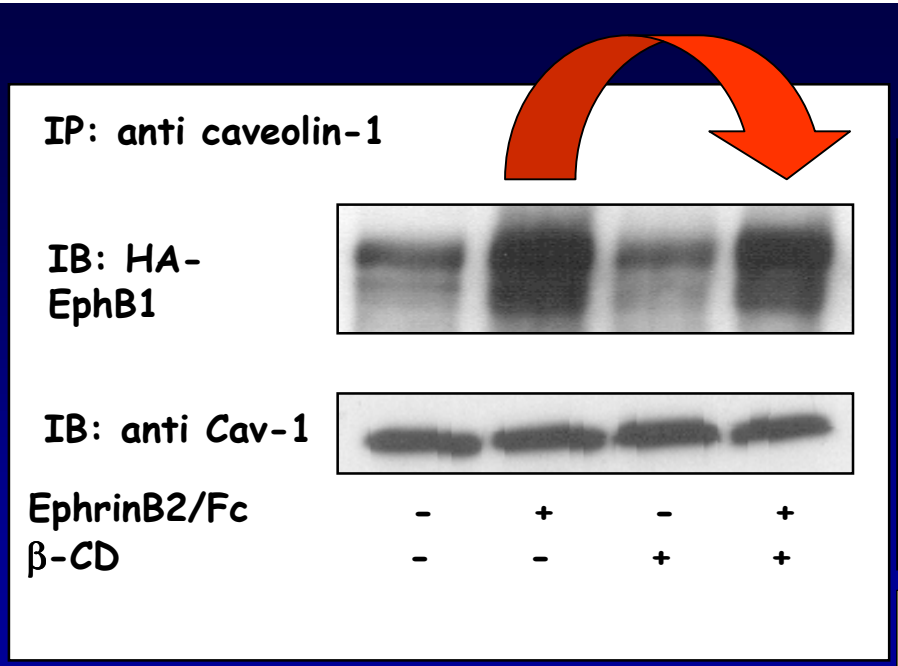


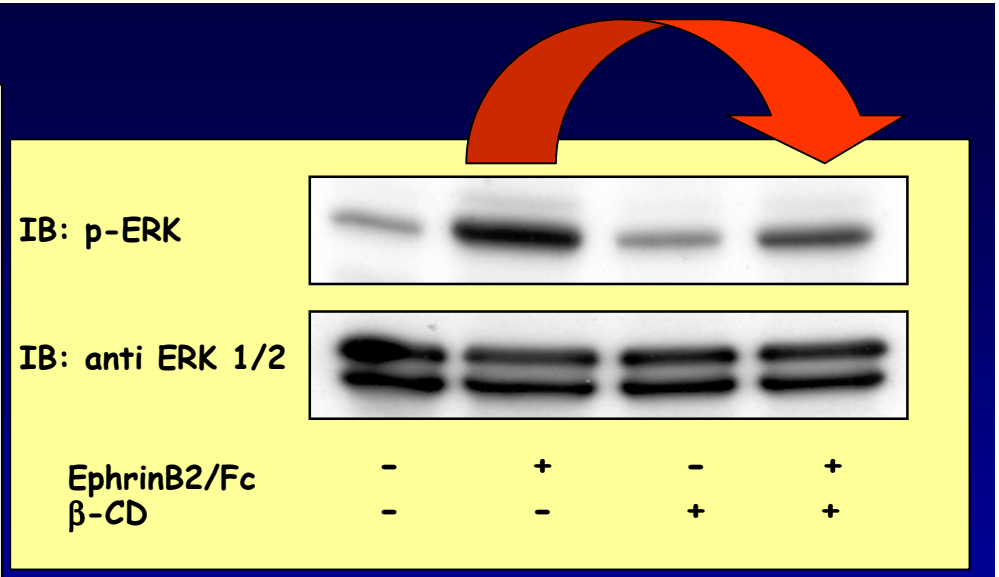
Effect of Cholesterol Depletion on Caveolae Structures

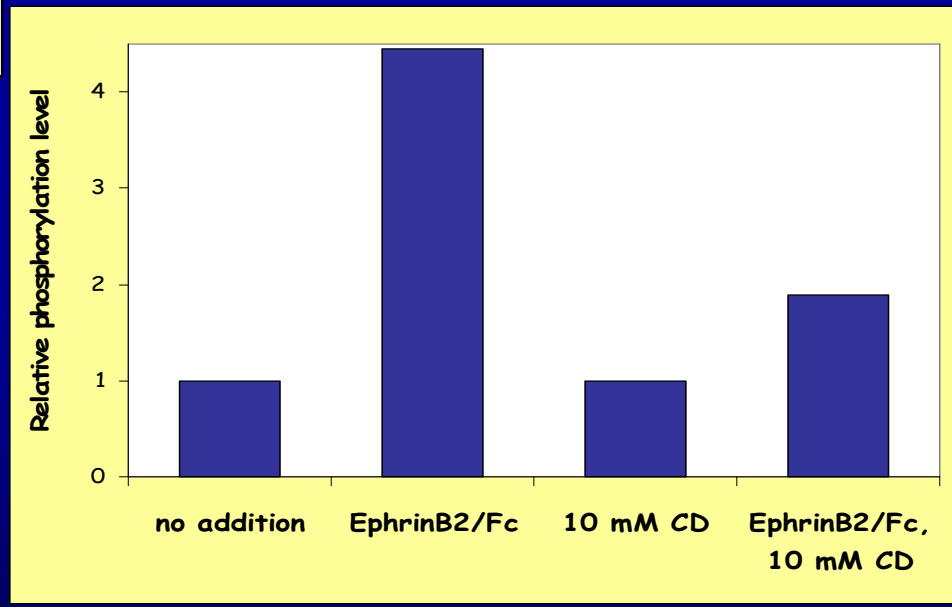


No addition

10 mM β-cyclodextrin (β-CD)



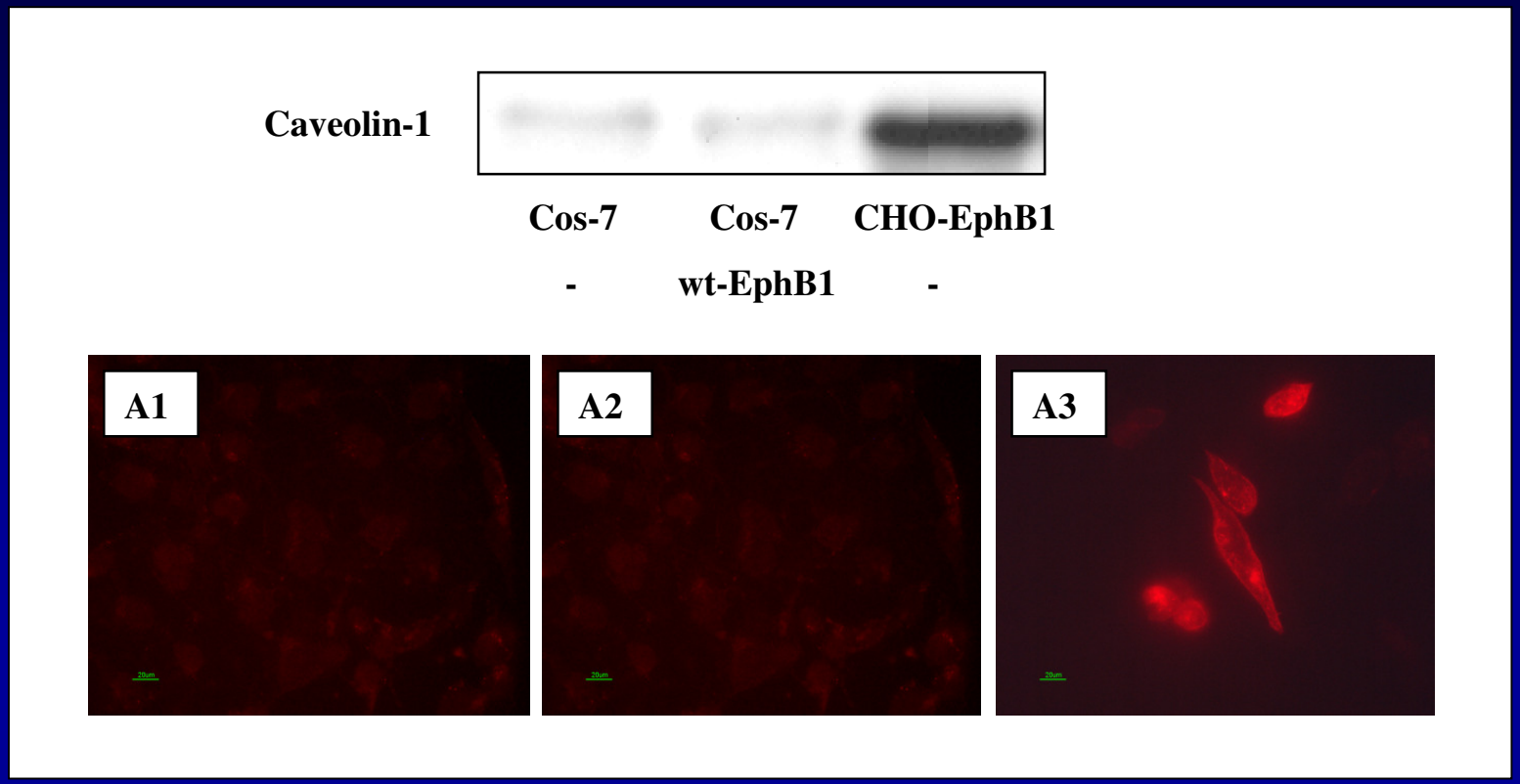




ERK = extracellular signal-regulated kinase

β-CD = β-cyclodextrin

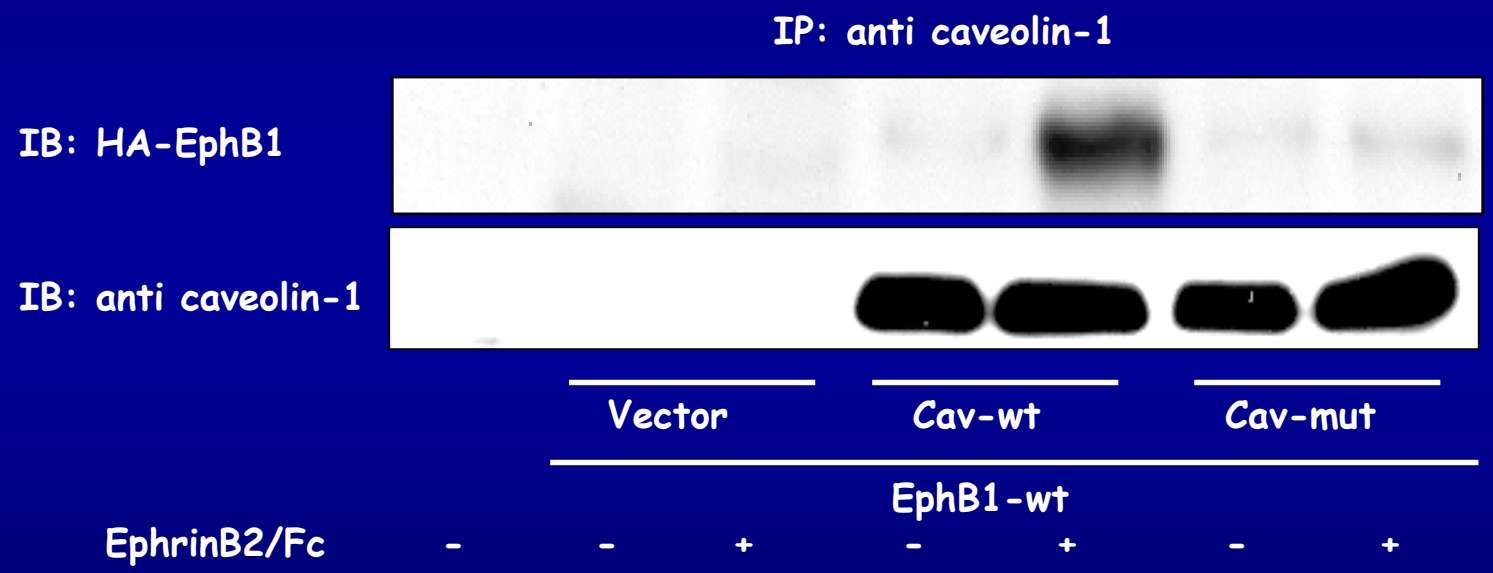
β-CD Inhibits the Activation of ERK by EphB1



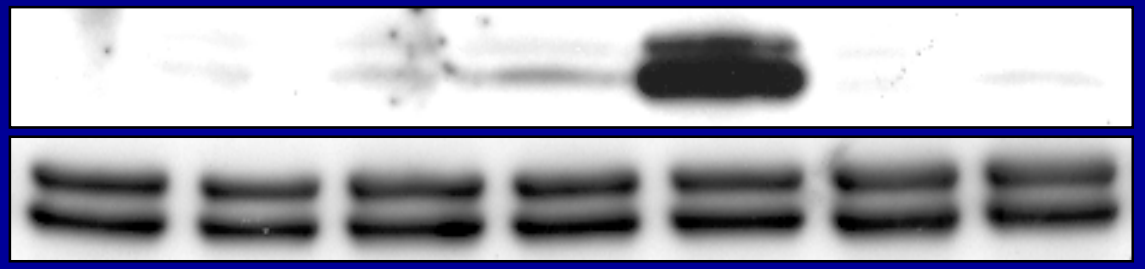
COS-7 cells express almost no Caveolin-1

→ Transfection with EphB1 receptor
→ Co-transfection with wt. vs mutant Cav-1 (point mutations in the scaffolding domain)

Mutation of the Caveolin-Scaffolding Domain Inhibits Its Association with EphB1



Mutation of the Caveolin-Scaffolding Domain Inhibits the Activation of ERK by EphB1



	Vector		Cav-wt		Cav-mut	
	EphB1-wt					
EphrinB2/Fc	-	-	+	-	+	-	+

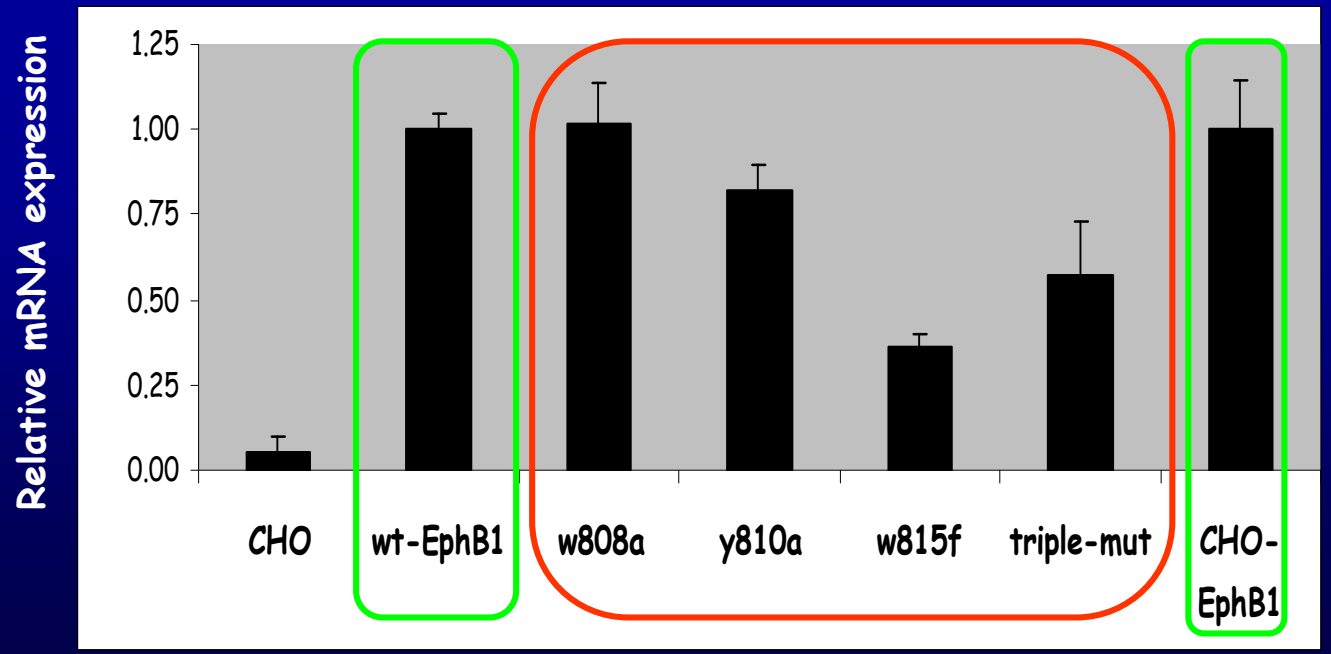
ERK = extracellular signal-regulated kinase

Mutations of the Caveolin-Binding Domain of EphB1 Are Expressed on mRNA Level

Wild type caveolin-binding motif in EphB1 receptor

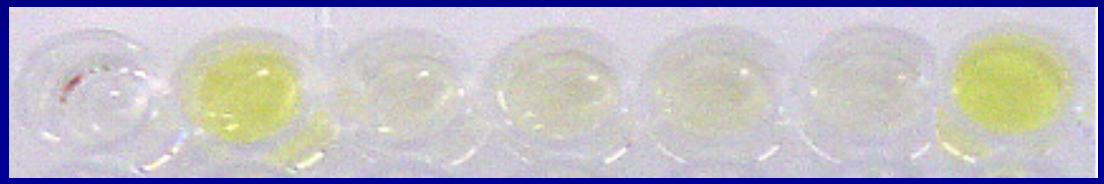
DVWSYGIHMW

DVASYGIHMW	W808a mutant
DVWSAGIHMW	Y810a mutant
DVWSYGIHMF	W815f mutant
DVASAGIHMF	Triple-mutant



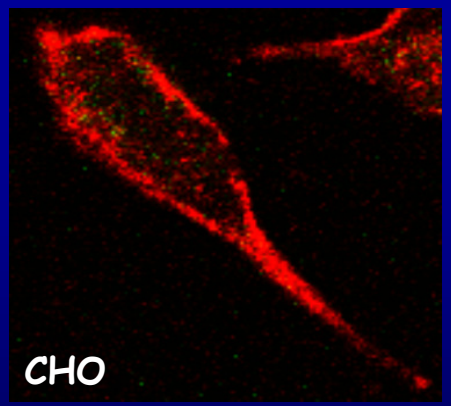
Mutations of the Caveolin-Binding Domain of EphB1 Impairs Its Cell Surface Expression

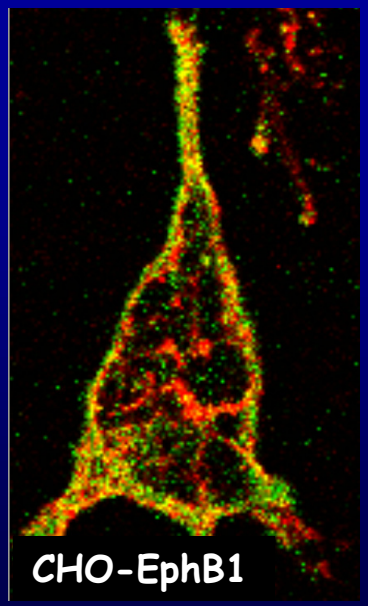
ELISA

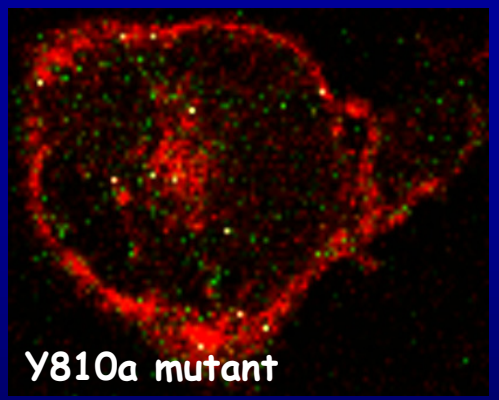


CHO | wt-EphB1 | w808a | y810a | w815f | triple-mut | CHO-EphB1

Confocal microscopy analysis







Green = HA-tagged EphB1

Red = Caveolin-1

Summary - I

1) EphB1 localizes in lipid rafts and associates with Cav-1 upon ligand stimulation

2) Cholesterol depletion by Cyclodextrin does not abrogate the EphB1/Cav-1 association but inhibits the activation of ERK by EphB1

Summary - II

3) An intact Cav-1 scaffolding domain is required for activation of ERK by EphB1

4) Mutations in the putative Cav-1 binding sequence of EphB1 impairs its membrane localization

Cav-1 is an important regulator of downstream signaling and membrane targeting of EphB1 receptor

Vihanto et al, *J Cell Science* 2006