

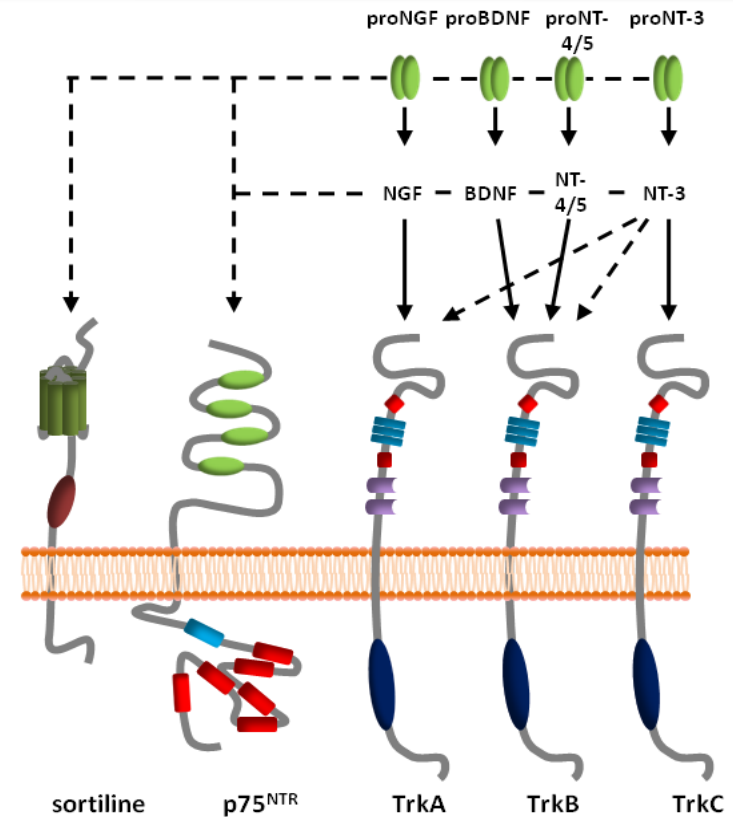
Implication of tyrosine kinase receptor networks in therapy resistance

Prof Robert-Alain Toillon

8/4/2015

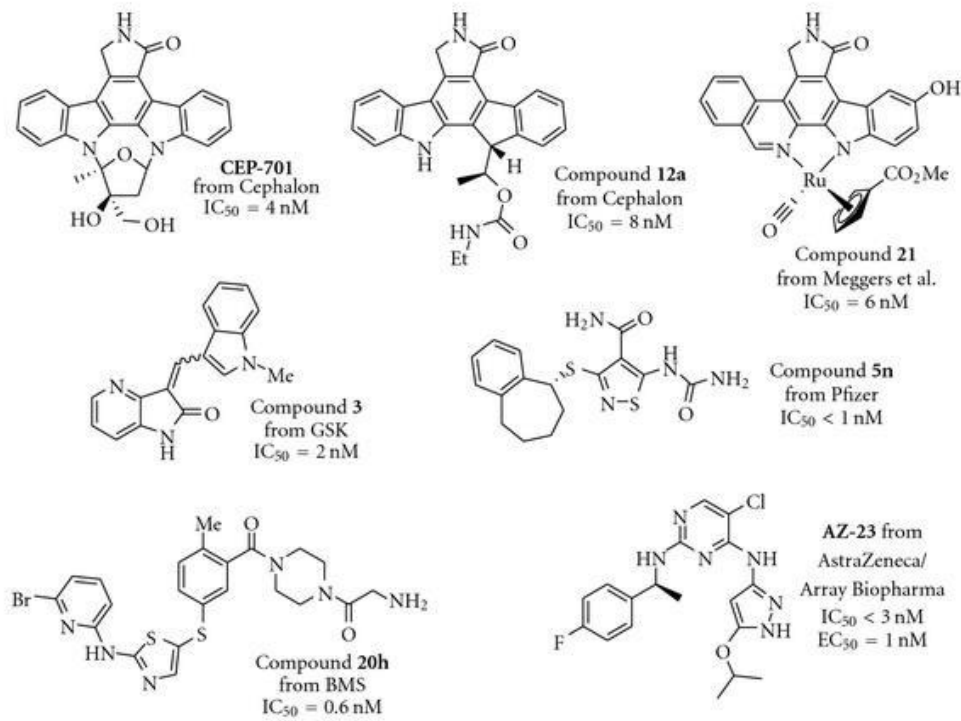
Neurotrophins as potential therapeutic targets in breast cancer

1. Expression in breast cancer
2. Tumor development in preclinical models
3. Angiogenesis
4. Original signaling of TrkA

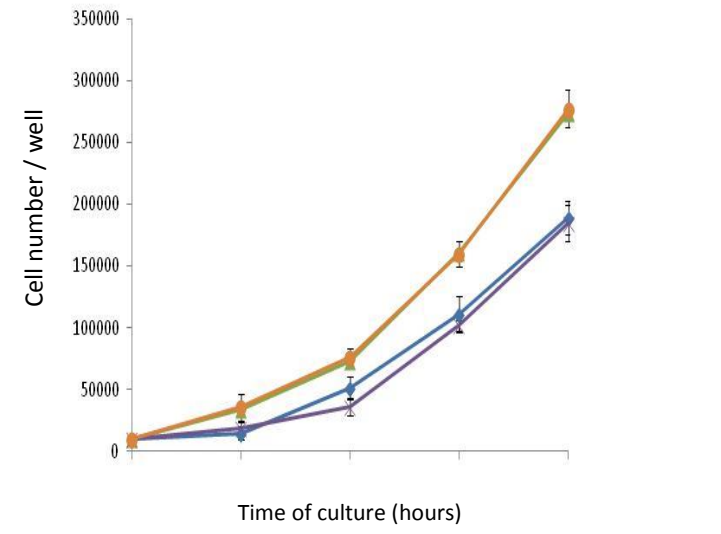
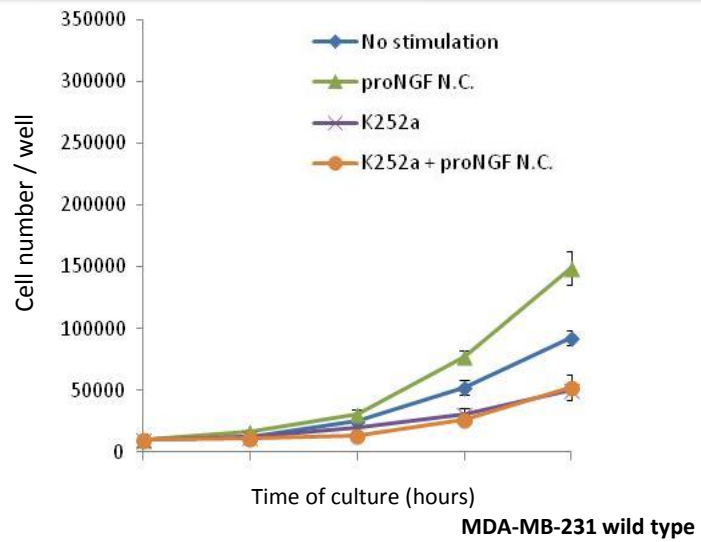


Adriaenssens E *et al.*, *Cancer Res* 2008.
Lagadec C *et al.*, *Oncogene* 2009.
Romon R *et al.*, *Mol Cancer* 2010.
Vanhecke E *et al.*, *Clin Cancer Res* 2011.
Demont Y *et al.*, *J Biol Chem* 2012.

What is the efficacy of TrkA kinase inhibitors on tumor cells?



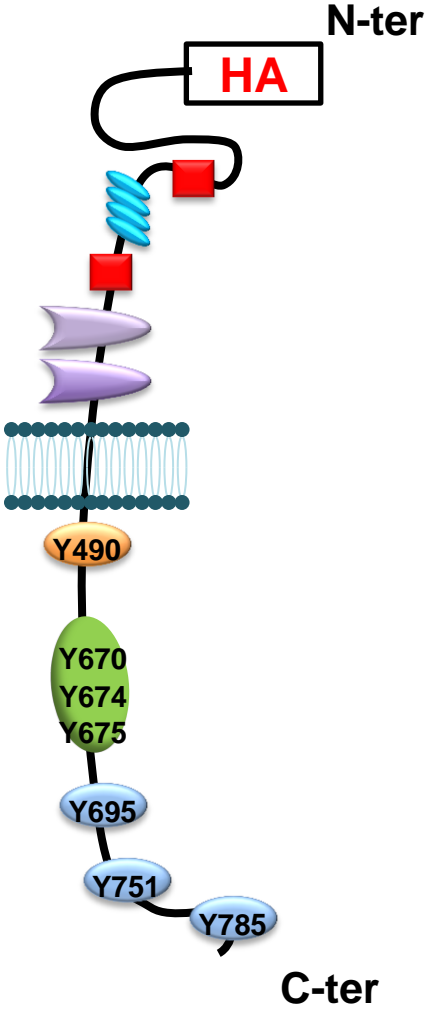
Raeppl *et al.* (Int J Med Chem, 2012)



Intrinsic resistance of the cells to TrkA inhibitors

MDA-MB-231 k252A resistant

Decipher TrkA interactome by proteomics analyses



HA = Hemagglutinin A
(YPYDVPDYA)

IP or streptavidin pull down
on cell lysates treated with
NGF or proNGF



SDS-PAGE
Commassie blue staining

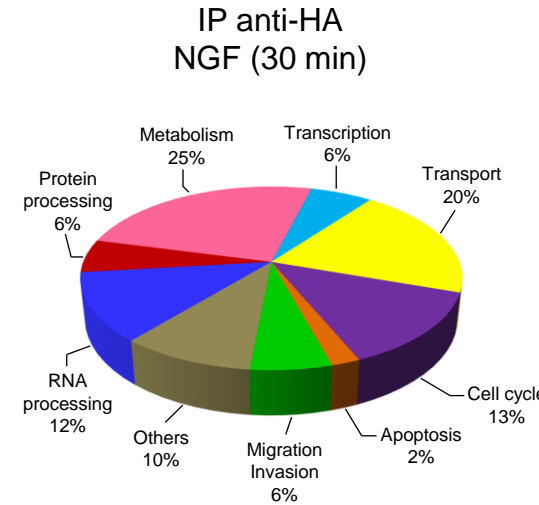
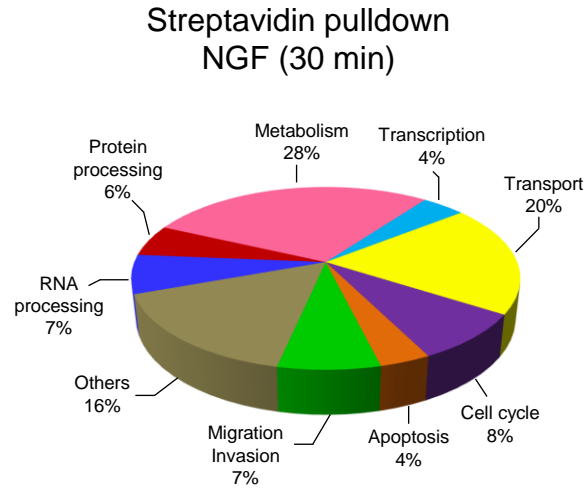
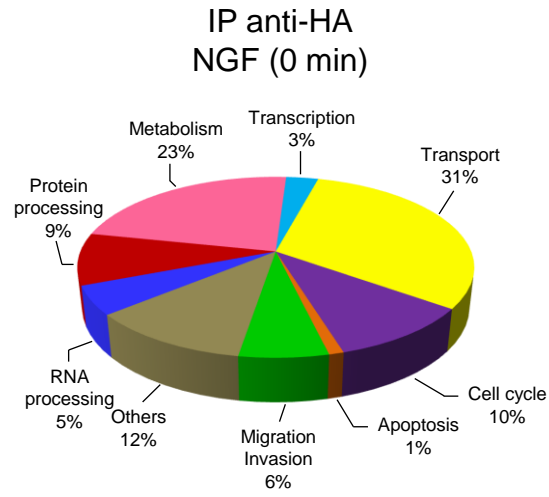
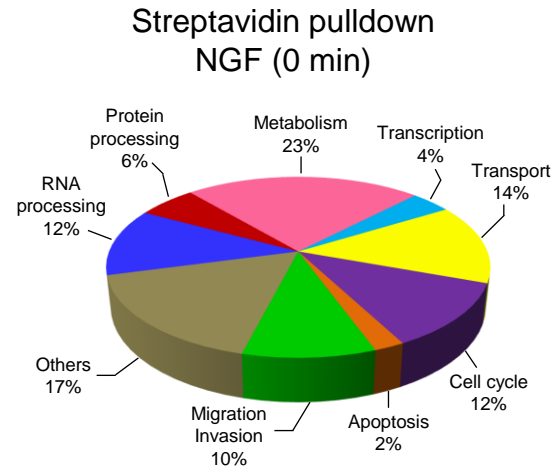
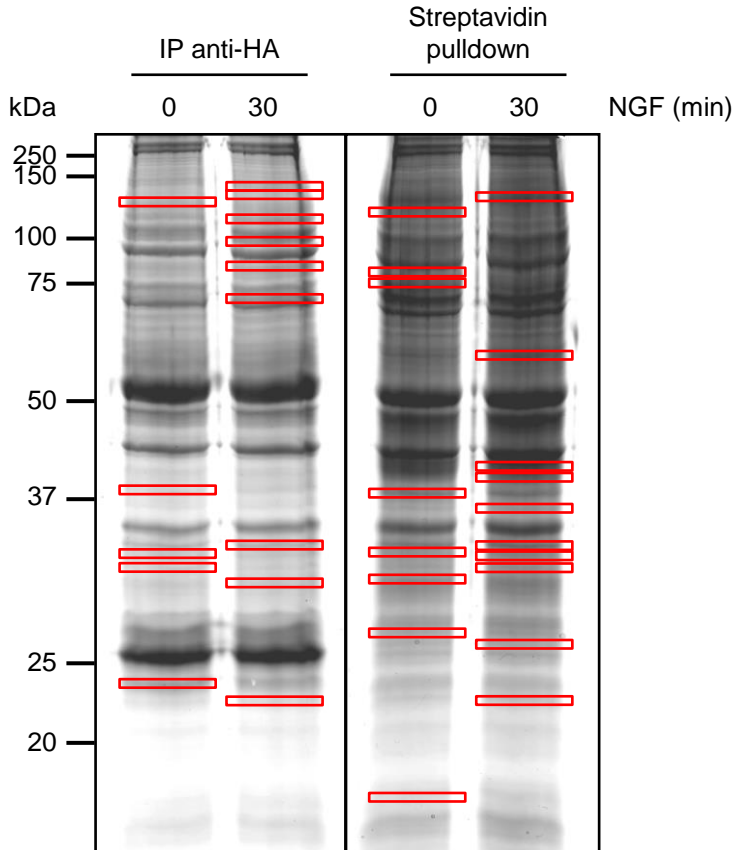


Band cut and trypsin
digestion



nanoLC-nanoESI-MS/MS
(Q-Star)

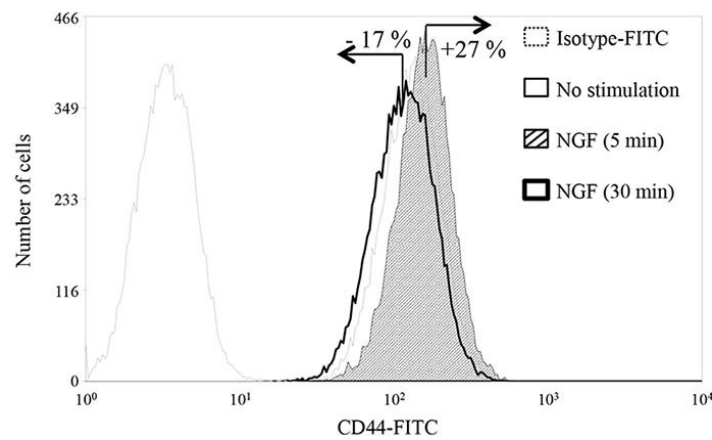
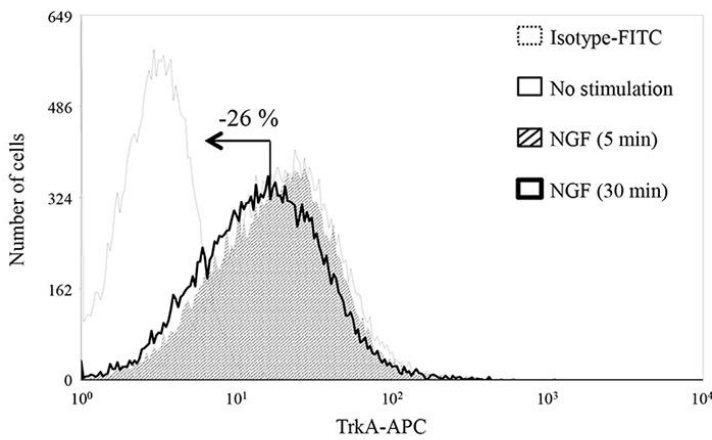
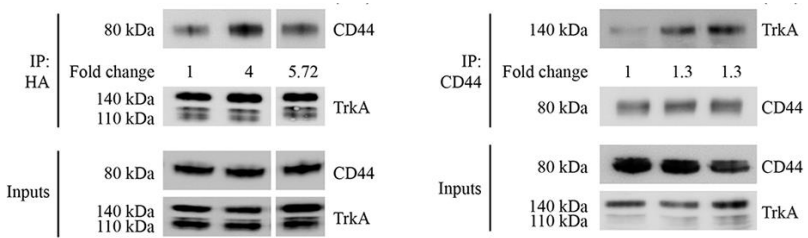
Decipher TrkA interactome : IP and streptavidin pull down



NGF induced CD44 recruitment to the plasma membrane

		Nom de la protéine	Nombre de peptides	Mascot score
Migration, Invasion		CD44 antigen	3	89
		Transforming protein RhoA	6	204
		Rho-related GTP-binding protein RhoC	6	219
		Actin-related protein 2	16	326
		Ras-related protein R-Ras2	3	62
		Basigin (CD147)	8	182
		Calreticulin	4	57
		Dystroglycan (fragment)	2	95
		Mitogen-activated protein kinase 1	3	54
Endocytosis		Actin, cytoplasmic 2	21	569
		Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	13	239
		Twinfilin-2	4	70
		EH domain-containing protein 1	17	130
		Ras-related protein Rab-5A	2	54
		Ras-related protein Rab-7A	8	104
		Vacuolar protein sorting-associated protein VTA1 homolog	3	102

NGF induced CD44 and TrkA association and receptors recruitment to the plasma membrane

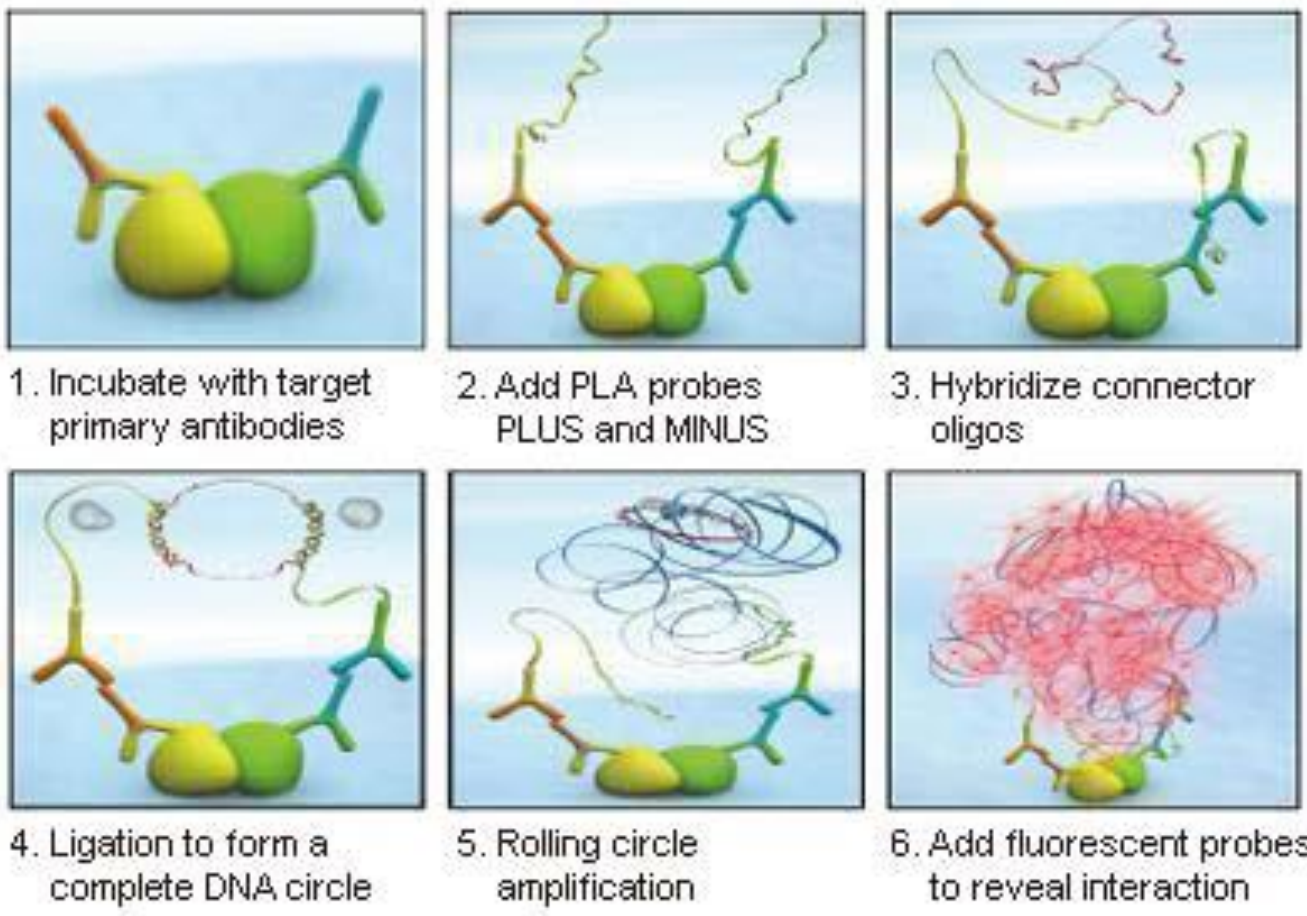


C TrkA/CD44 PLA Hoechst 33258 Merge Fold change

➤ **CD44 and TrkA are associated**

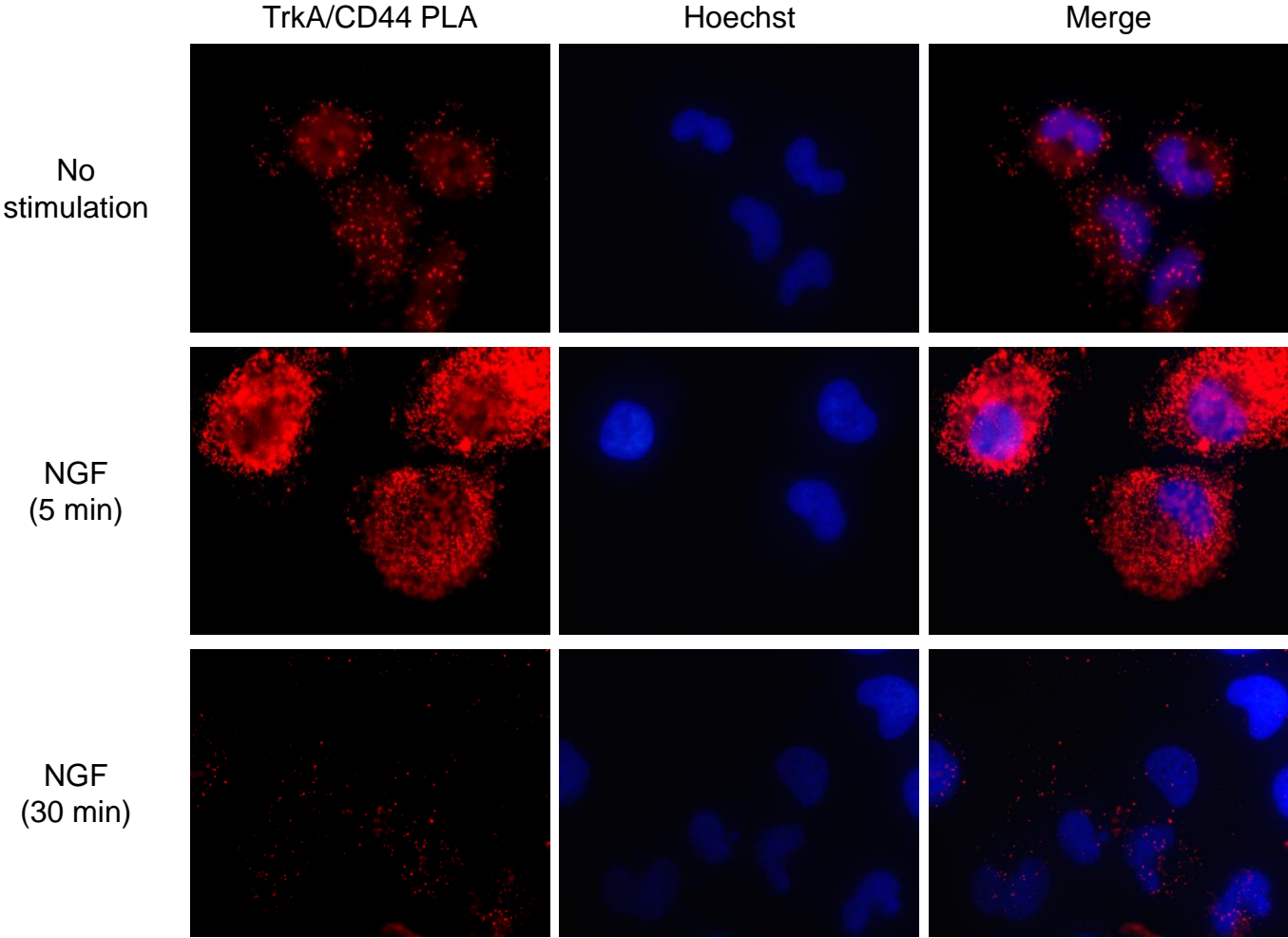
➤ **CD44 and TrkA are membrane expression**
Are dynamically regulated

NGF induced CD44 recruitment to the plasma membrane and TrkA association



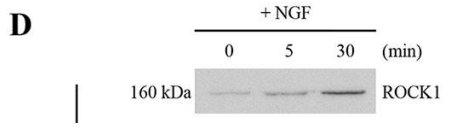
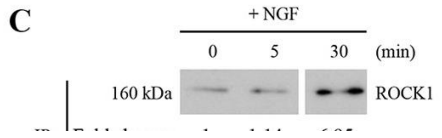
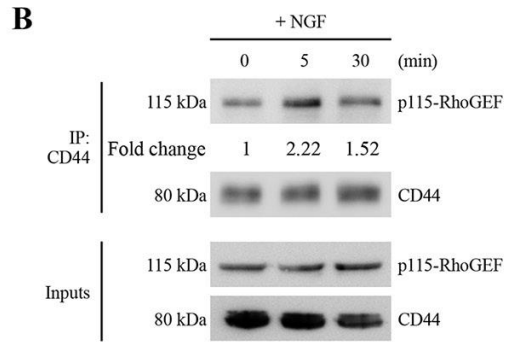
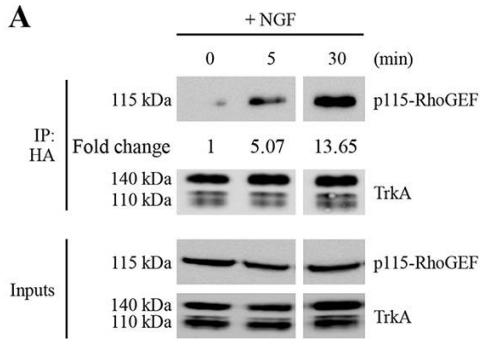
Schematic representation of proximity ligation assays or Duolink

NGF induced CD44 recruitment to the plasma membrane and TrkA association

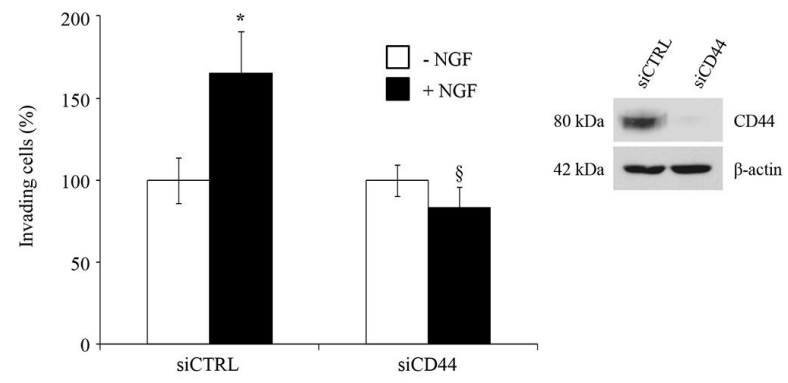


➤ CD44 and TrkA are associated and this complex is dynamically regulated by NGF

NGF induced CD44 activation of RhoGTPase pathways

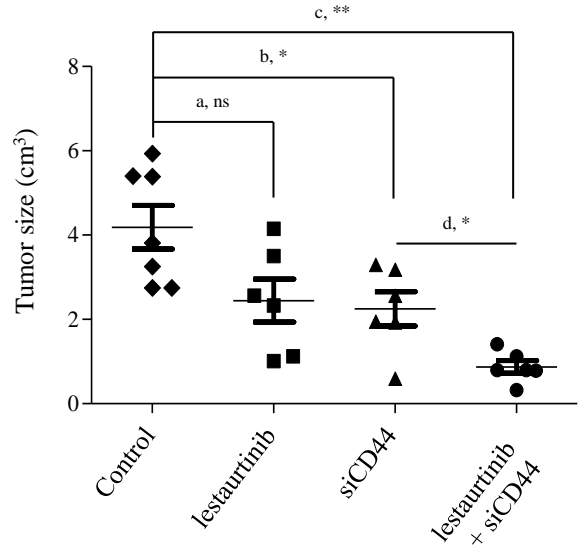
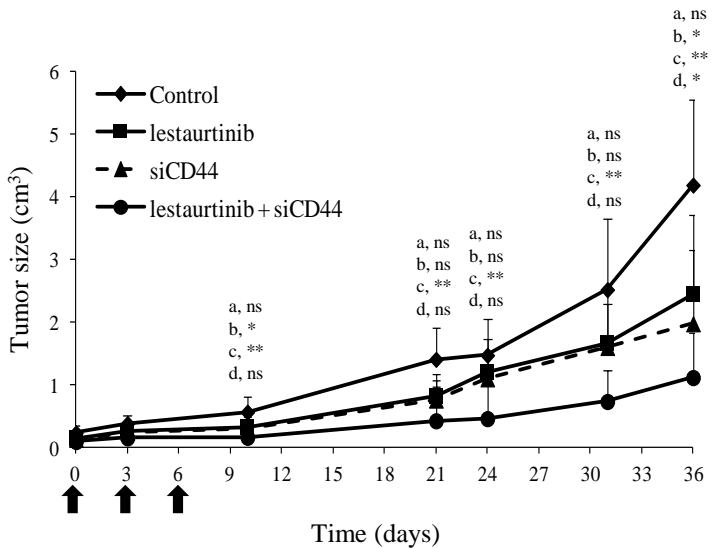


➤ NGF induced RhoA/RhoC activation through CD44

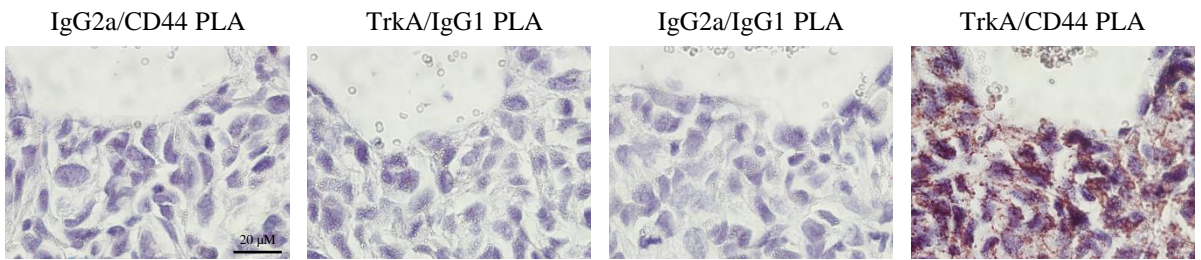


➤ P115 RhoGEF is involved in NGF induced migration

Tumor cell growth depends on TrkA networks



➤ CD44 and TrkA inhibition enhanced drug responses in SCID mice



➤ SCID mice xenografted tumors also exhibited CD44/TrkA complexes

Ongoing studies : Hypothesis, main objectives

CD44 Transactivation's by TrkA are unexpected alternative signaling pathways



perspectives in the development of diagnostic and therapeutic strategies



translate our results from the bench to the bedside

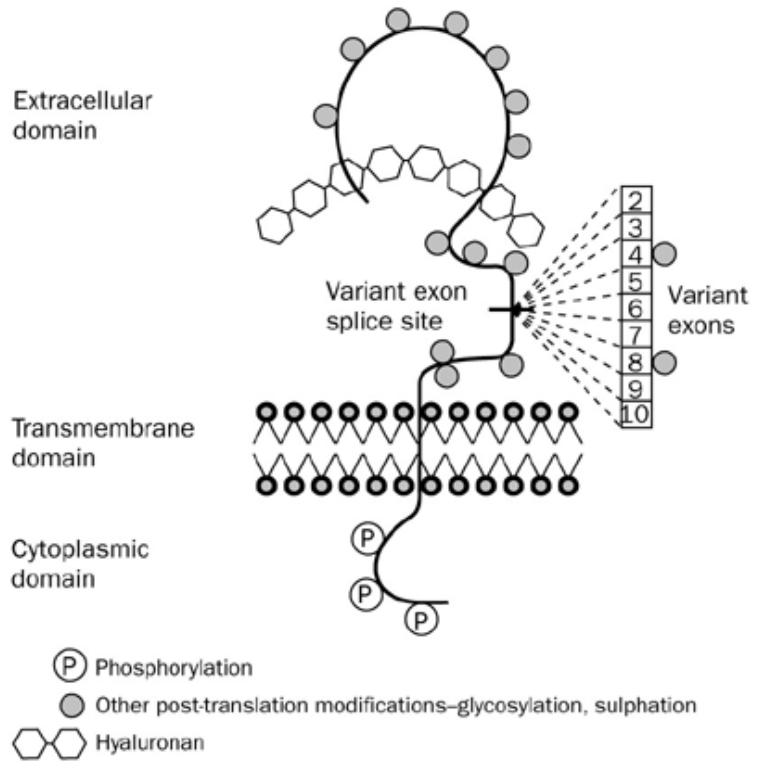


Determine the interaction motifs

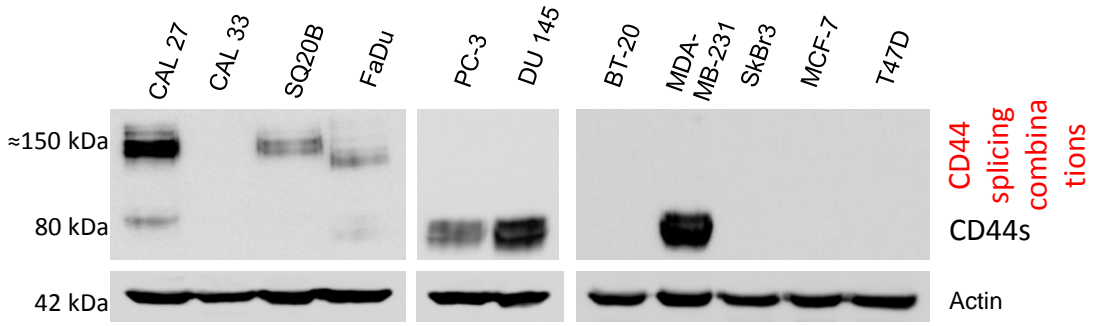


Validate the prognostic value of TrkA/CD44 complexes

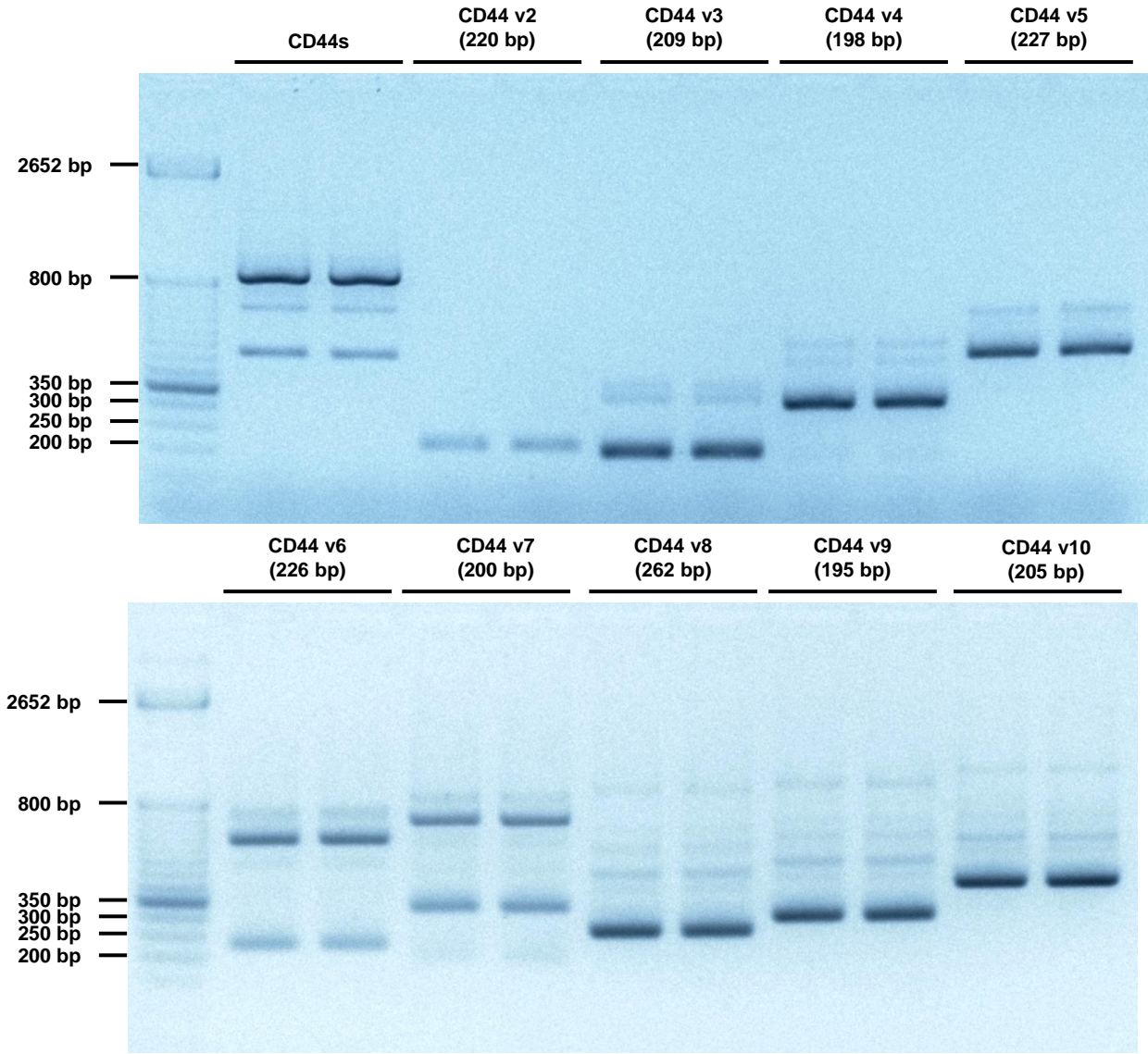
1-Determine the interaction motifs



➤ By western blot

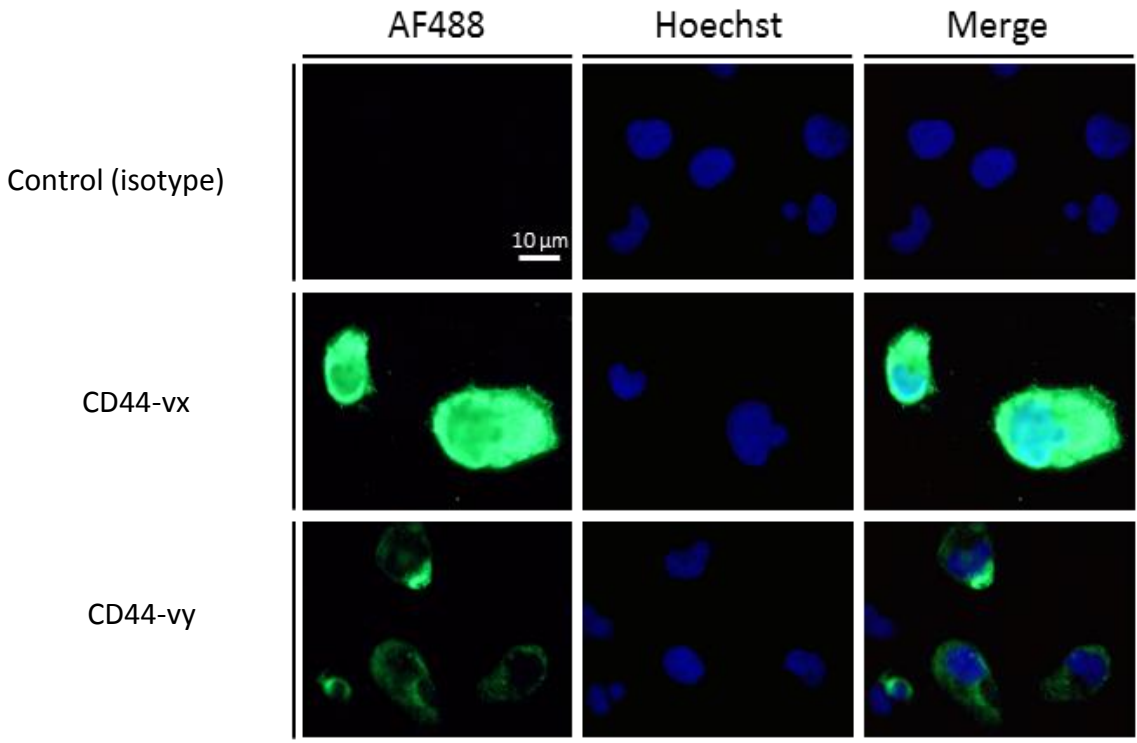


1-Determine the interaction motifs



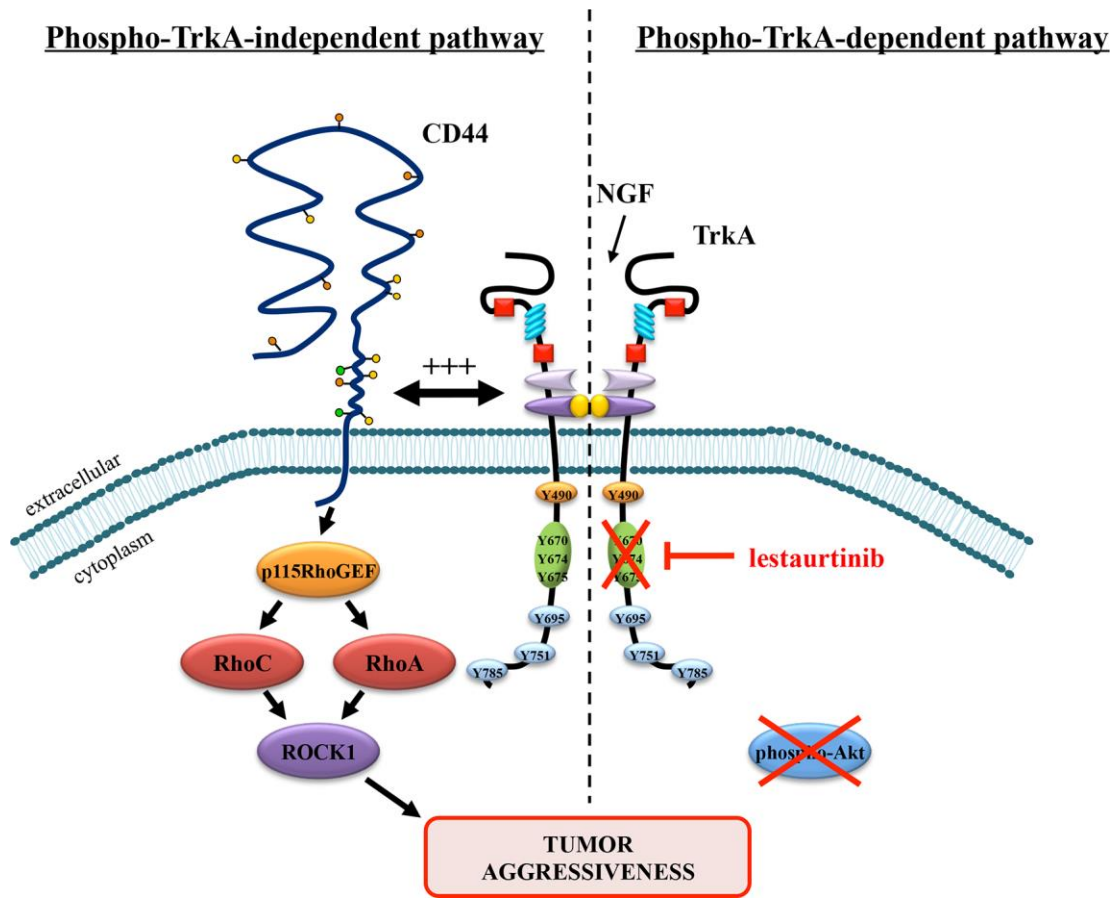
➤ By q RT-PCR

1-Determine the interaction motifs



➤ By immuno-
fluorescence

CD44 and TrkA activate RhoGTPase pathways





Centre Hospitalier Régional
Universitaire de Lille



Thank you !

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Cyril Corbet, Léo Aubert, Matthieu Guilbert, Jérémy Duval
Xuefen, Chann, Samuel, Pierre-Olivier, Pamela, Nicole, Isa

CHRU, Lille

Pr D. Chevalier, Pr X. Leroy, Dr F. Mouawad, Dr S. Bouhir

Centre Oscar Lambret

Dr M. Julieron, Dr S. Cousin

Institut Biologie de Lille- Institut Pasteur de Lille

Pr E. Adriaenssens, T. Chassat, JP de Cavel

Centre de cancérologie de la Loire, Lucien Neuwirth, Saint Etienne

Pr N. Magné

Institut Paoli Calmette, Marseille

Pr F. Bertucci, Pr D. Birnbaum

SATT NORD

Y. Campion



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