Recent highlights of *in vivo* knockdown by intrabodies

5th European Immunology Conference

Dr. Thomas Böldicke
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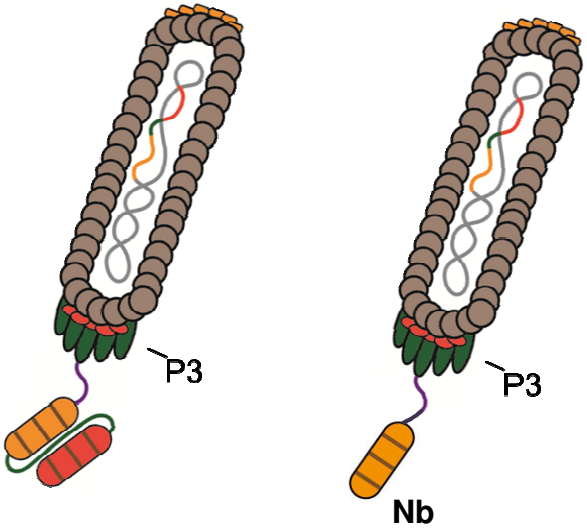
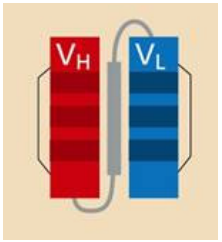
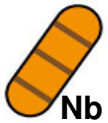
Recombinant protein expression/Germany





scFv



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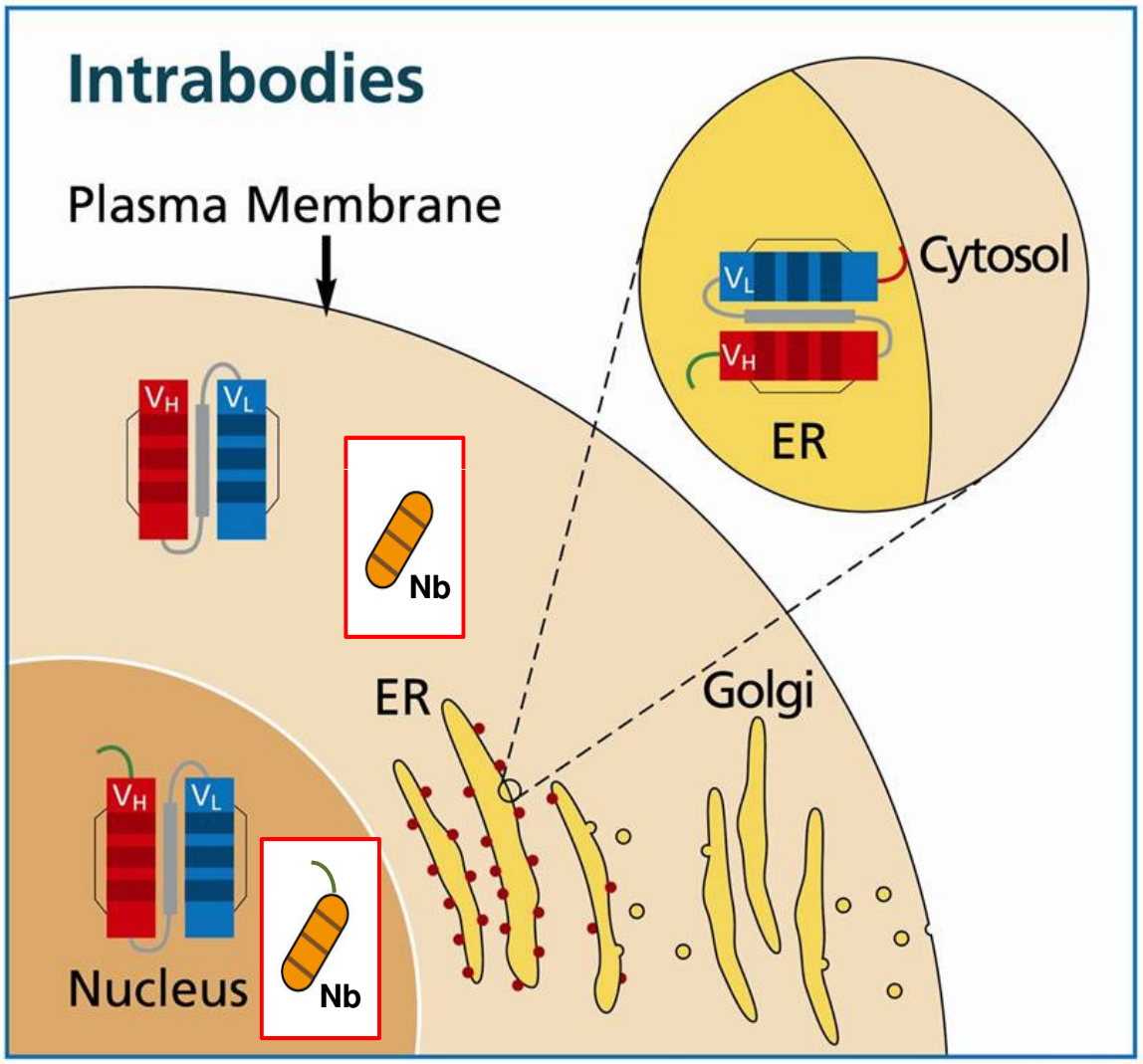
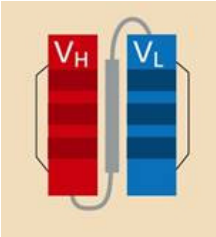
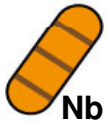
Intrabodies targeted to subcellular compartments





scFv



Camelid VHH
Human VH
Human VL

Developments which are boosting the intrabody technology

ER intrabodies

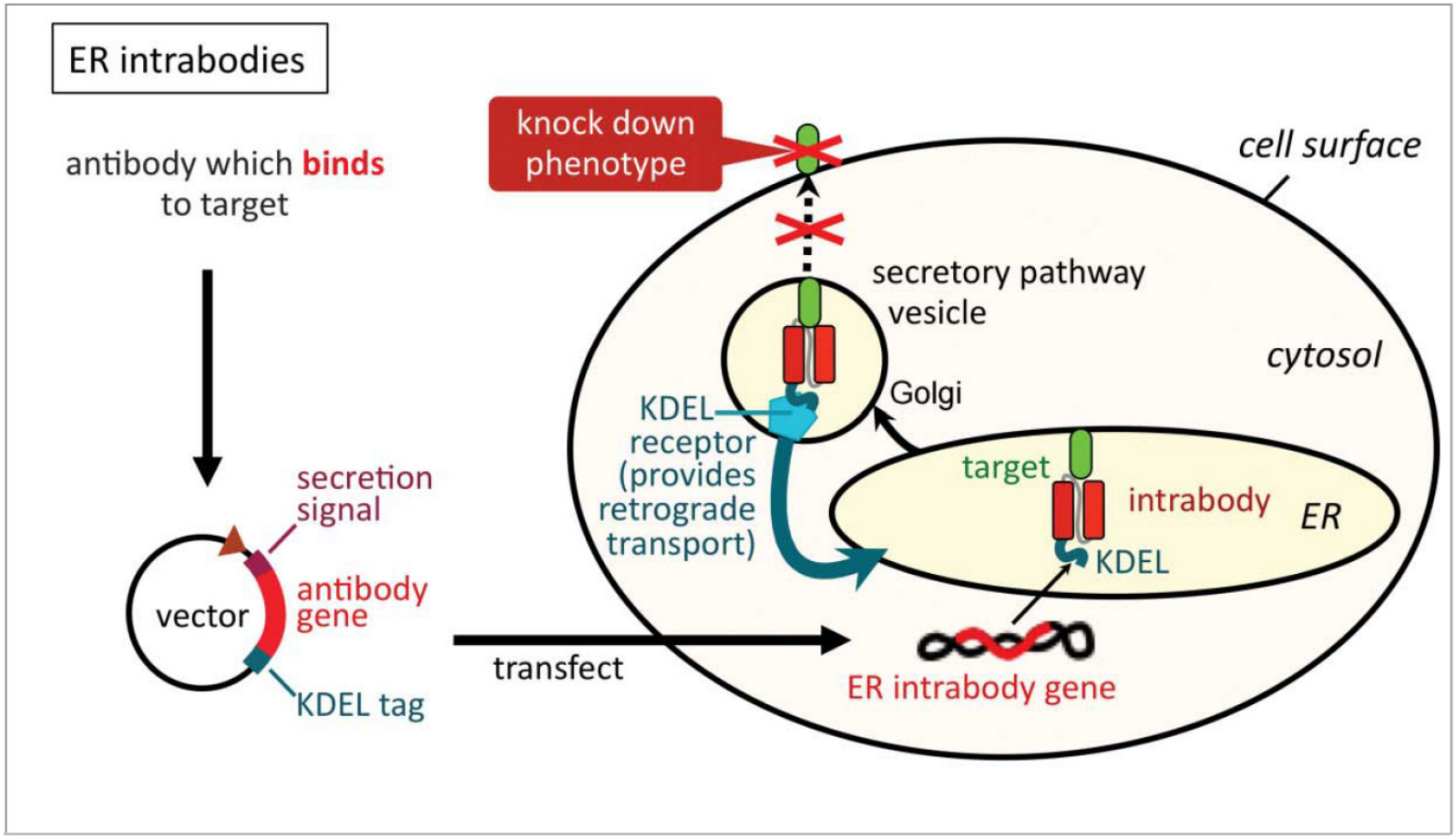
1. Thousends of new ***V region antibody genes*** are already available as an attractive source of scFv fragments for ER intrabody construction

2. Sequences from hybridoma cell lines will also be available in larger numbers in the future

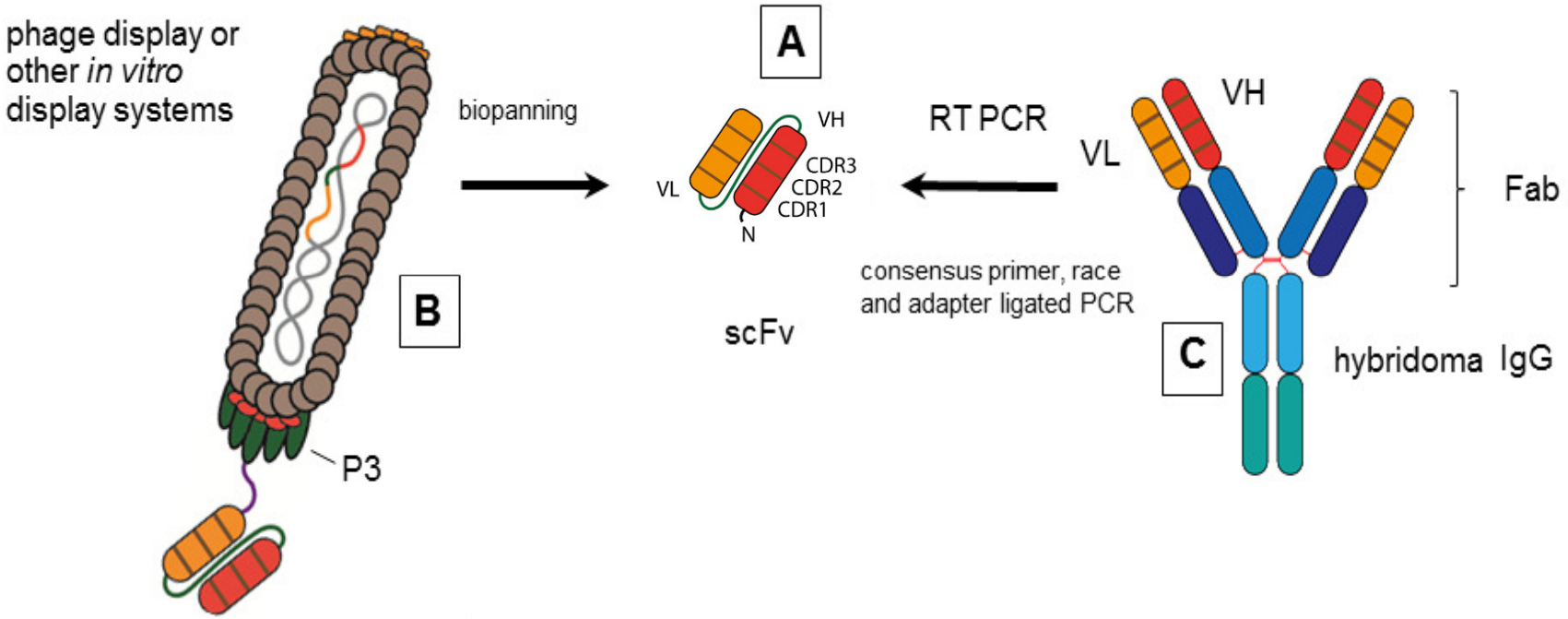
Cytosolic intrabodies

1. Single domain antibodies can be stable expressed as intrabodies inside the cytoplasm

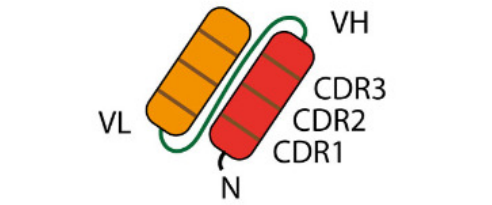
ER intrabodies inhibit translocation of targets passing the ER

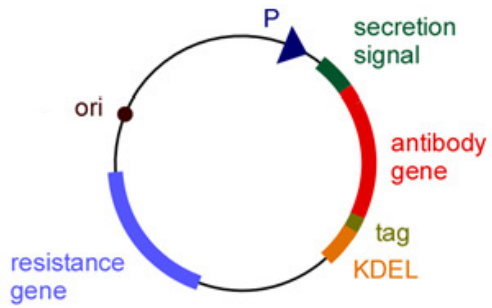


Generation of scFv from phage display ab repertoires or from hybridoma clone

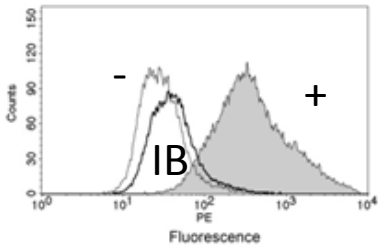


Generation of ER intrabody for *in vitro* knockdown in mammalian cells





ER targeting vector



in vitro knockdown

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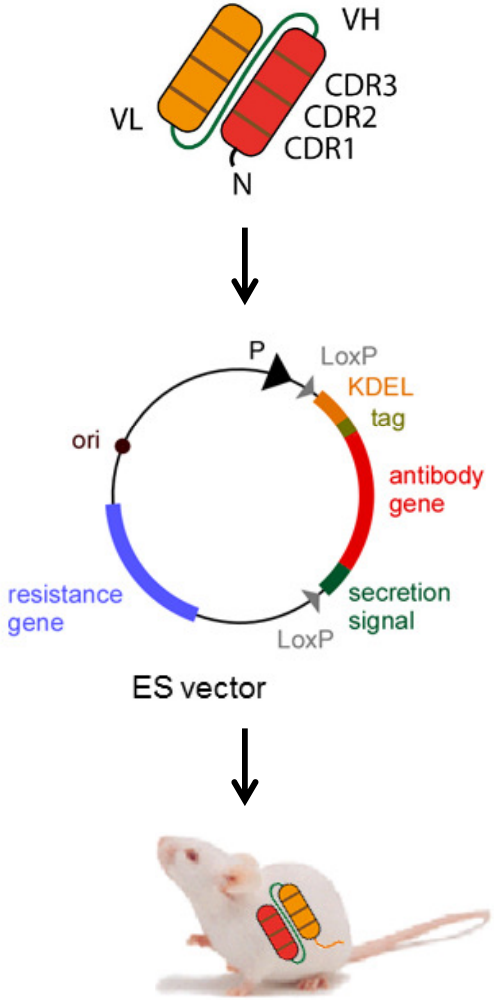
Generation of a transgenic ER intrabody mouse



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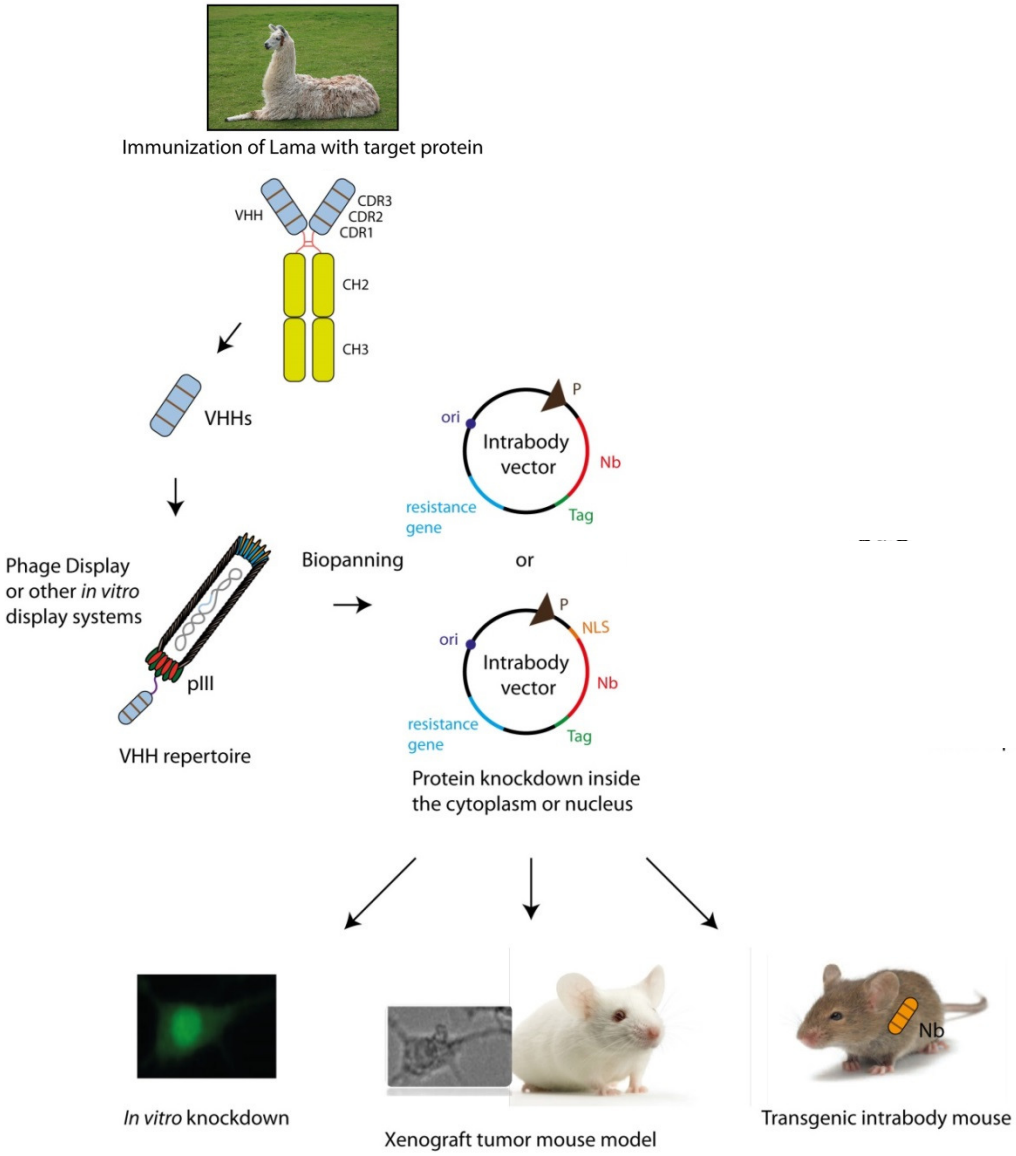
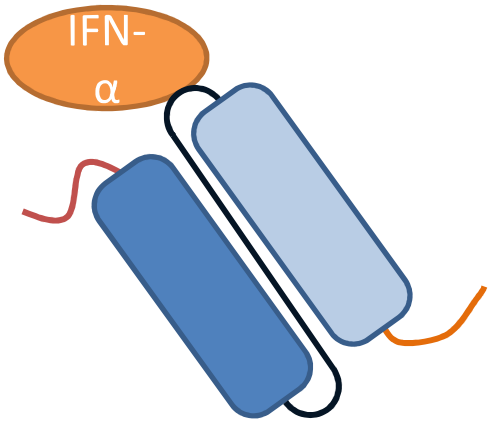
Construction of cytosolic camelid single domain antibodies



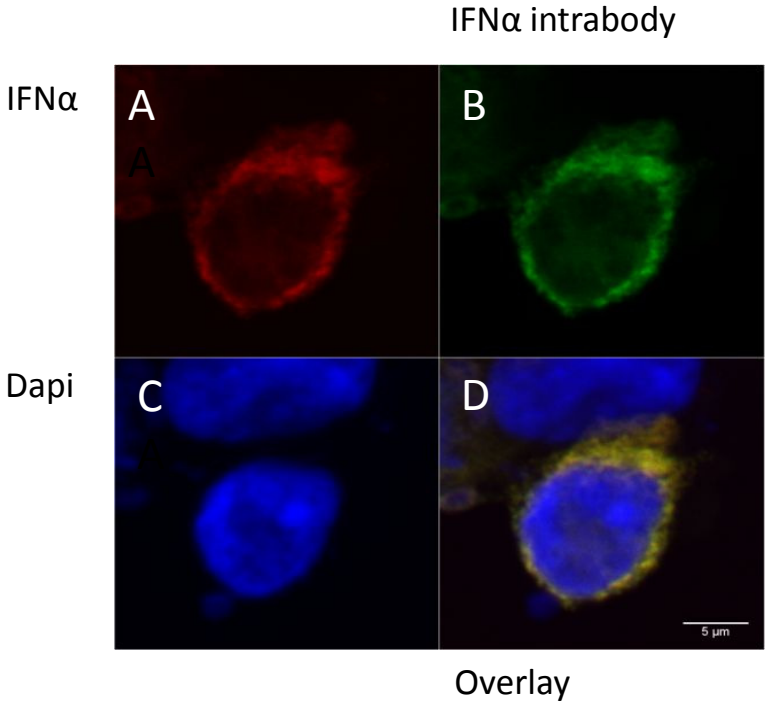
Anti-Interferon α intrabody project



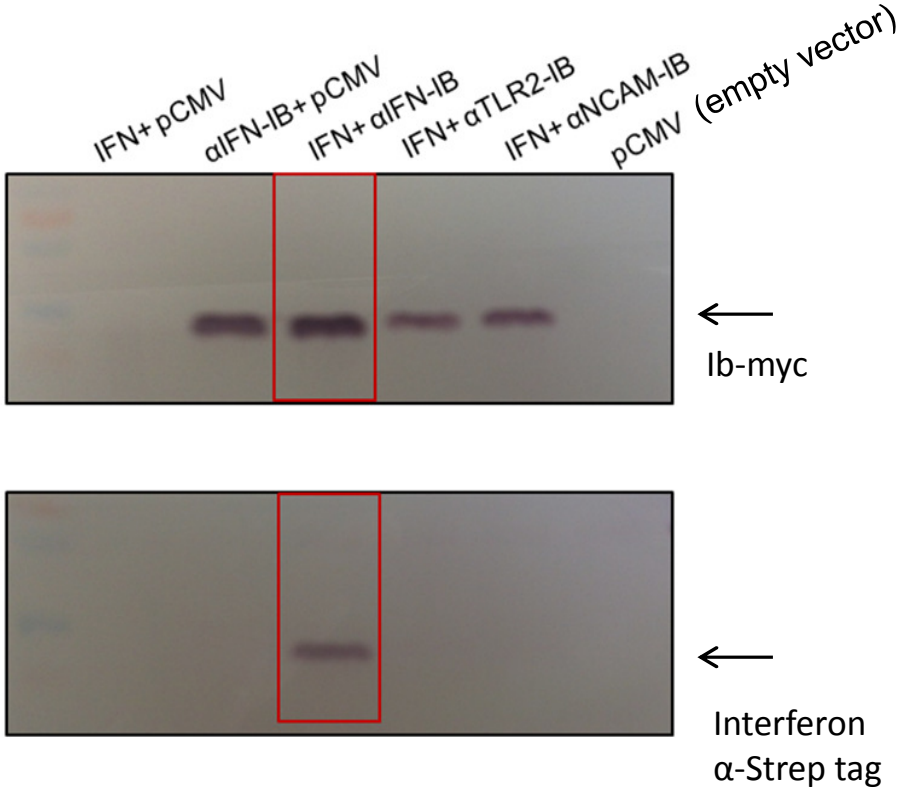
- Development of a new anti-interferon α intrabody to study the *in vivo* function of IFN-α in macrophages and dendritic cells
- An IFN- α knockout mouse does not exist because there exist 14 mouse IFN- α isoformes
- Development of an intrabody from a hybridoma clone which binds to an epitope of different isoforms (mouse IFN α subtypes 1, 2, 4 and 6)
- *In vitro* characterization of the intrabody and establishment of a transgenic intrabody mouse

Co-localisation and Co-immunoprecipitation of IFN α-Strep tag and intrabody-myc in HEK293 cells.

I Immunofluorescence



II Co-Immunoprecipitation



Colocalization and Co-immunoprecipitation indicates complex formation of IFN α-Strep tag and intrabody-myc and specific intracellular binding in HEK293 cells

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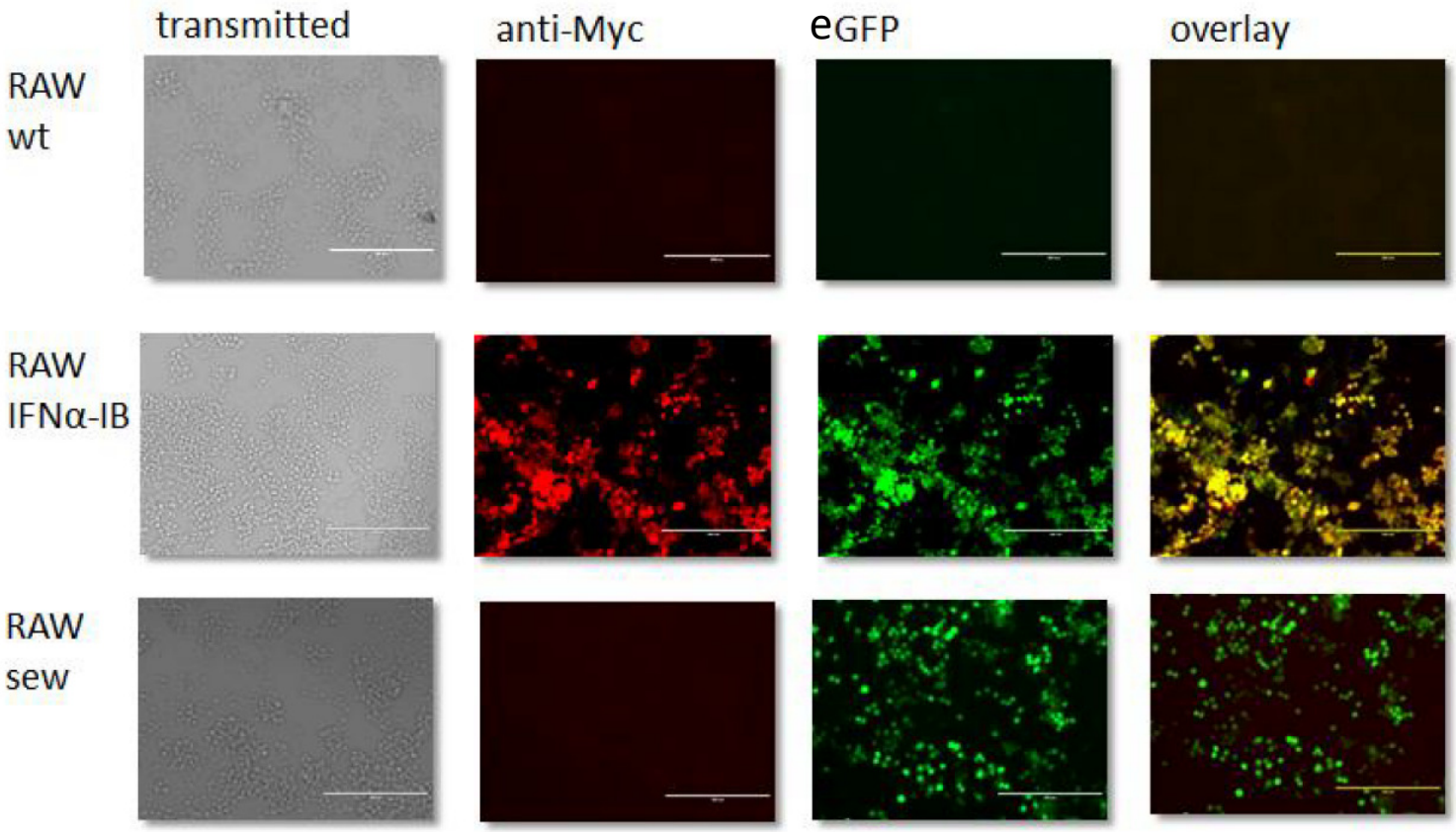
Immunofluorescence: Staining of reporter eGFP and α IFNα intrabody in stable intrabody expressing RAW 264.7 macrophages



Immunofluorescence shows clearly co-expression of eGFP and α IFNα intrabody in RAW 264.7 cells

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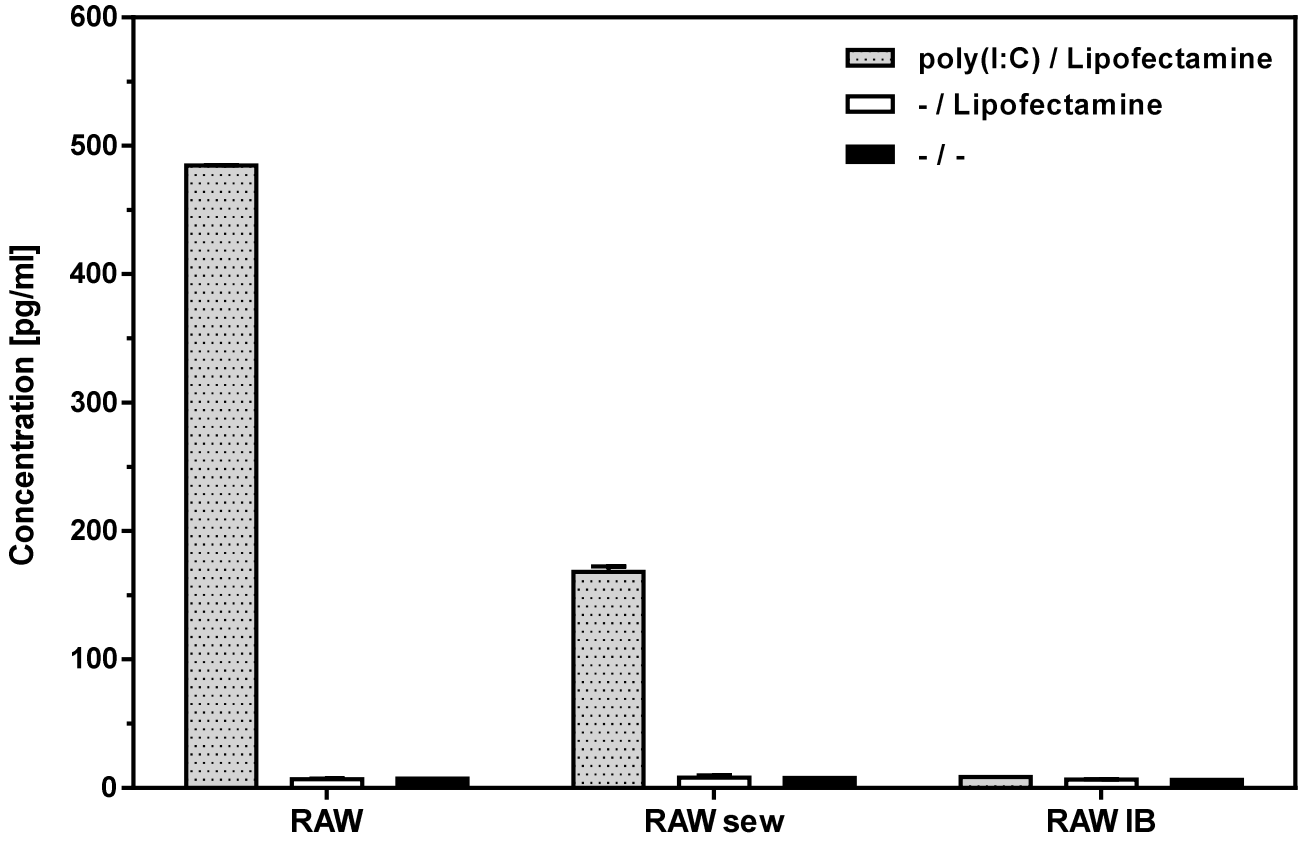
Detection of IFN-α in stable intrabody expressing RAW 264.7 macrophages after stimulation with poly (I:C)



Anti-Interferon α intrabody inhibits secretion of IFN- α in RAW 264.7 macrophages

Summary

- New anti-IFN-α intrabody recognizing mouse IFN α subtypes 1, 2, 4 and 6 inhibits IFN α secretion in RAW 264.7 macrophages

- c DNA of anti-IFN-α intrabody is cloned into ES vector to transfect embryonic stem cells and to generate transgenic intrabody mice

Literature:

Marschall AL, Dübel S, Böldicke T. Recent Advances with ER targeted Intrabodies. Adv Exp Med Biol. 2016;917:77-93. doi: 10.1007/978-3-319-32805-8_5.

Marschall AL, Dübel S, Böldicke T. Specific in vivo knockdown of protein function by intrabodies. MAbs. 2015;7(6):1010-35. doi: 10.1080/19420862.2015.1076601.

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