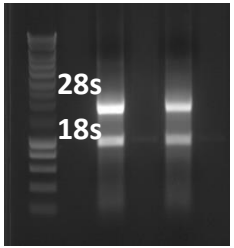
Case study III

Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

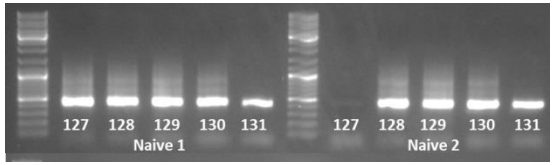
Generation and QC of Naïve llama single domain antibodies phage display library

10 non-immunized llamas

PBL´s



RNA extraction/cDNA generation



Amplification of llama VHH genes (400 bp) using five different family specific Framework 1 sequence primers in combination with a Hinge sequence primer

Llama VHH library ID	library Size (cfu)	VHH Insert %	Llama VHH library ID	library Size (cfu)	VHH Insert %
Naïve Lib 1	1,10E+08	100%	Naïve Lib 6	2,50E+08	100%
Naïve Lib 2	1,10E+08	100%	Naïve Lib 7	2,00E+08	100%
Naïve Lib 3	1,10E+08	100%	Naïve Lib 8	1,80E+08	100%
Naïve Lib 4	1,70E+08	100%	Naïve Lib 9	1,00E+08	100%
Naïve Lib 5	1,00E+08	100%	Naïve Lib 10	1,00E+08	92%

Llama VHH library ID	library Size (cfu)	VHH Insert %
FJB Naïve Pool Lib	**1,43E+09**	**99%**

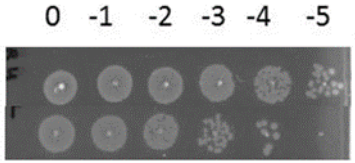
Case study III

Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

Phage display selections allowed identification of VHHs binding CXCR4 on cells from Naïve library

Human CXCR4

20 U CXCR4
20 Null

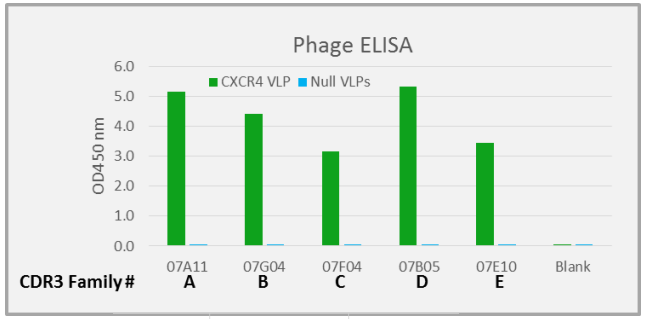


Titration of *E. coli* infected with phage from Naïve llama VHH library selected after two rounds on hCXCR4 and Null lipoparticles

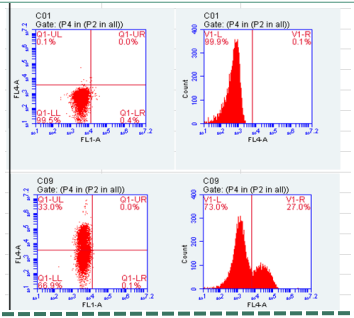
Target	Phage ELISA hit rate*	Number of target specific CDR3 Families
Hu CXCR4	45%	27

* O.D. 450 nm > 5xbackground (*$O.D._{450\ nm}$ on Null lipoparticles <0.08), n=96

Human CXCR4: Phage ELISA and soluble VHH FACS



Staining of cells with soluble 07A11 VHH



WT HEK293T

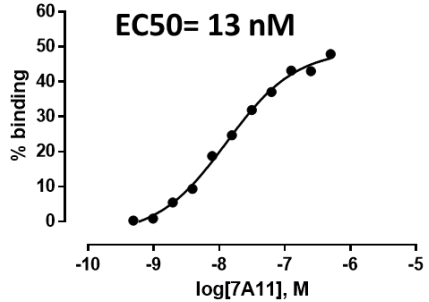
Hu CXCR4- HEK293T

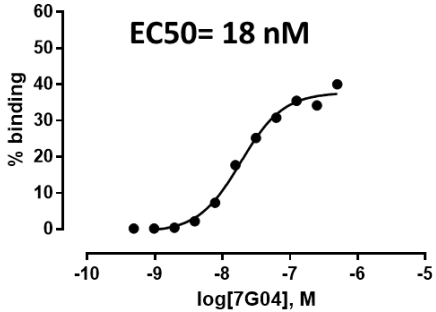
Case study III

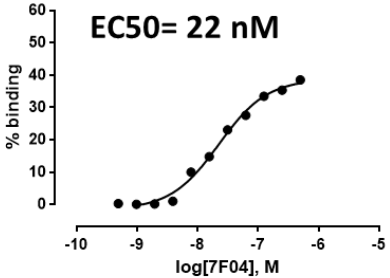
FairJourney Biologics

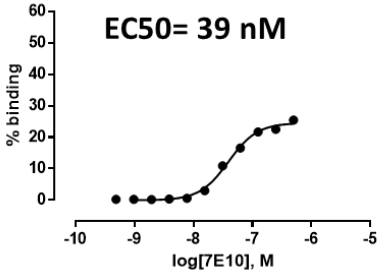
Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

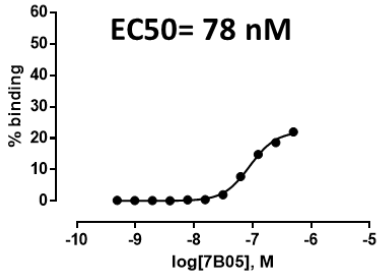
Anti CXCR4 VHHs isolated from the Naïve library show low-mid nM EC50s









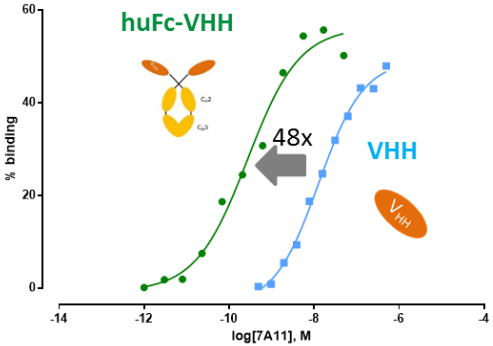


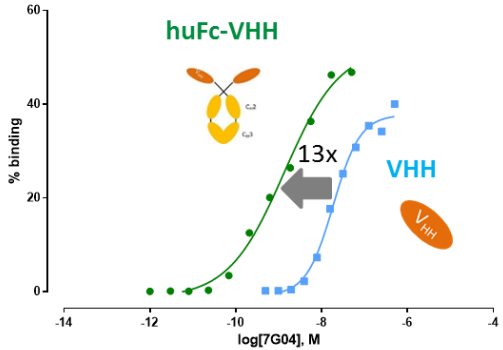
- VHHs binding to HEK293T cells transiently tansfected with human CXCR4
- 45% of the transfected cells expressed human CXCR4 as stained with 12G5 anti CXCR4 antibody

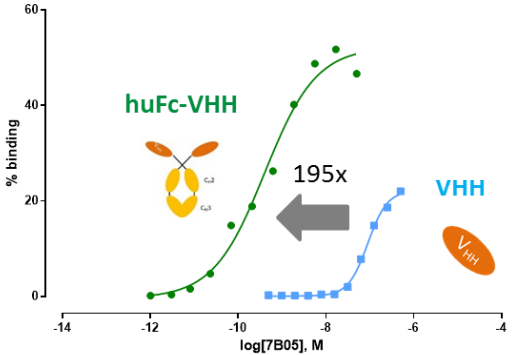
Case study III

Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

Formatting of CXCR4 VHHs isolated from the Naïve library to human Fc fusions results in low and sub nM EC50s







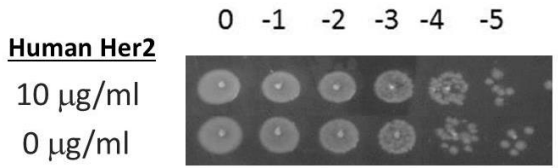
- VHHs and huFc-VHHs binding to HEK293T cells transiently tansfected with human CXCR4
- 45-77% of the transfected cells expressed human CXCR4 as stained with 12G5 anti CXCR4 antibody

	7A11 VHH	7A11 huFc-VHH	7B05 VHH	7B05 huFc-VHH	7G04 VHH	7G04 huFc-VHH
EC50 (nM)	13	0.27	78	0.40	18	1.34

Case study III

Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

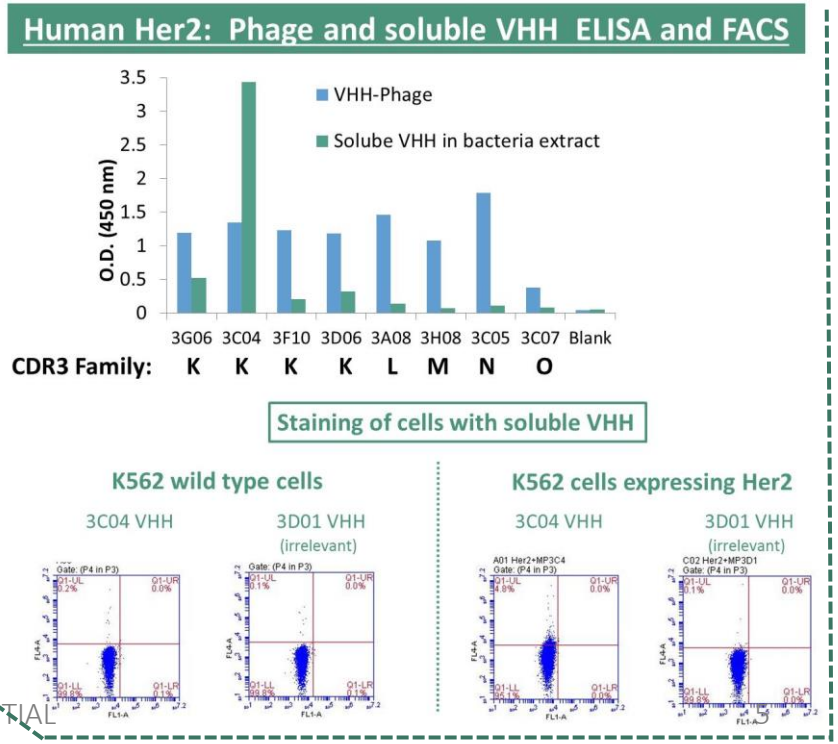
Phage display selections allowed identification of VHHs binding HER2 on cells from Naïve library



Titration of *E. coli* infected with phage from Naïve llama VHH library selected after three rounds on human HER2

Target	Phage ELISA hit rate*	Number of target specific CDR3 Families
Hu HER2	84%	6

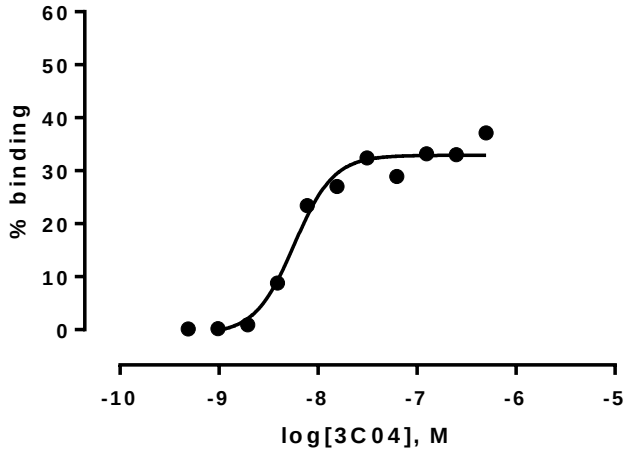
* O.D. 450 nm > 5xbackground, n=96



Case study III

Isolation of anti human CXCR4 and human HER2 VHHs from Naïve library

Anti HER2 VHH isolated from the Naïve library show low nM EC50



- VHH binding to HEK293T cells transiently tansfected with human HER2
- 40% of the transfected cells expressed human HER2 as stained with herceptin antibody