

Case study IV

Discovery of Fabs against a cell membrane target

- Aim: Isolation of mAbs recognizing the membrane bound but not the soluble receptor X
- Technology: Mice cell immunizations + Cell-based phage display panning



N=5

**P815 cells transfected with
receptor X**



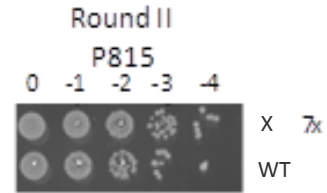
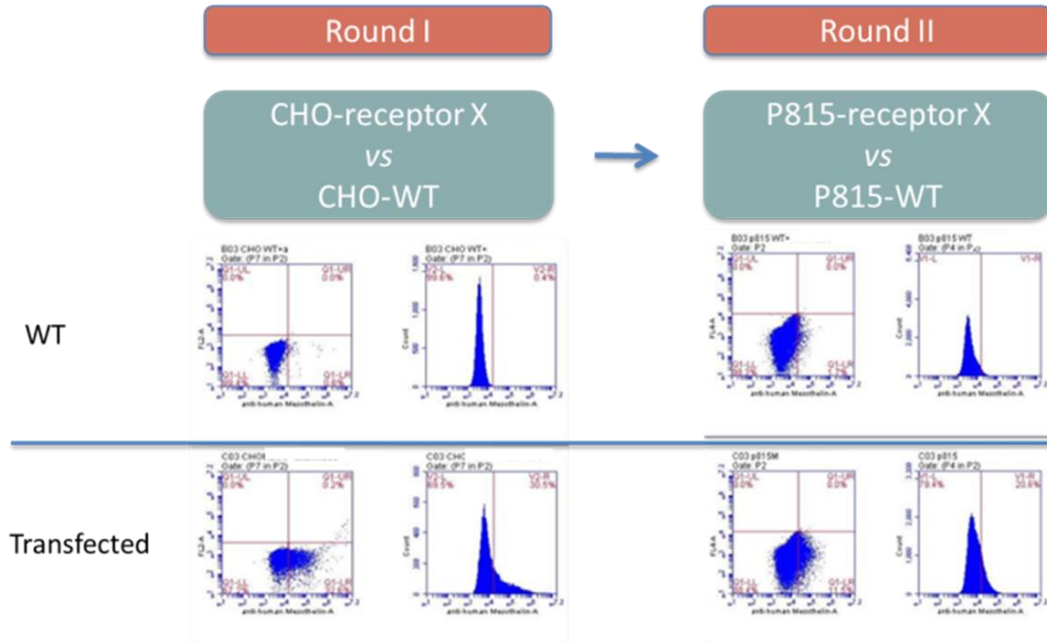
Phage display Fab libraries

Library	Library size	Fab Insert %
M003	1.6×10^9	100 %
M004	1.9×10^9	86 %
M005	4.4×10^8	96 %
M006	4.3×10^8	96 %
M007	2.8×10^8	100 %

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- Phage display selections on whole cells to isolate Fabs against cell receptor X

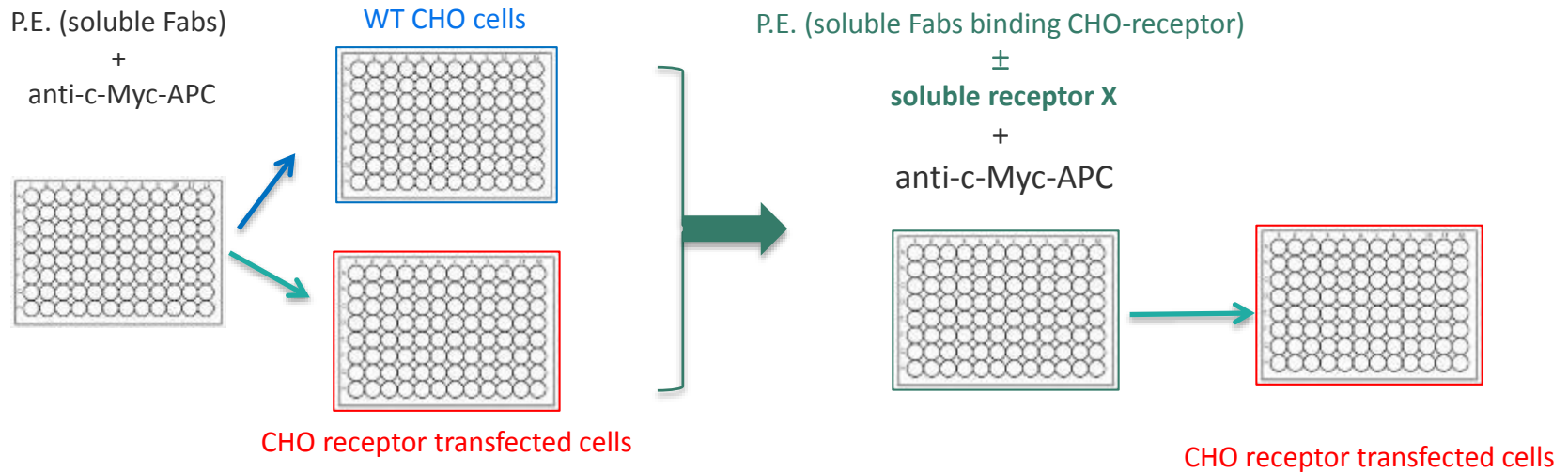


Titration of *E. coli* infected with phage from a Fab immune library selected after two rounds on whole cells transfected with receptor X and on wild type (WT) cells

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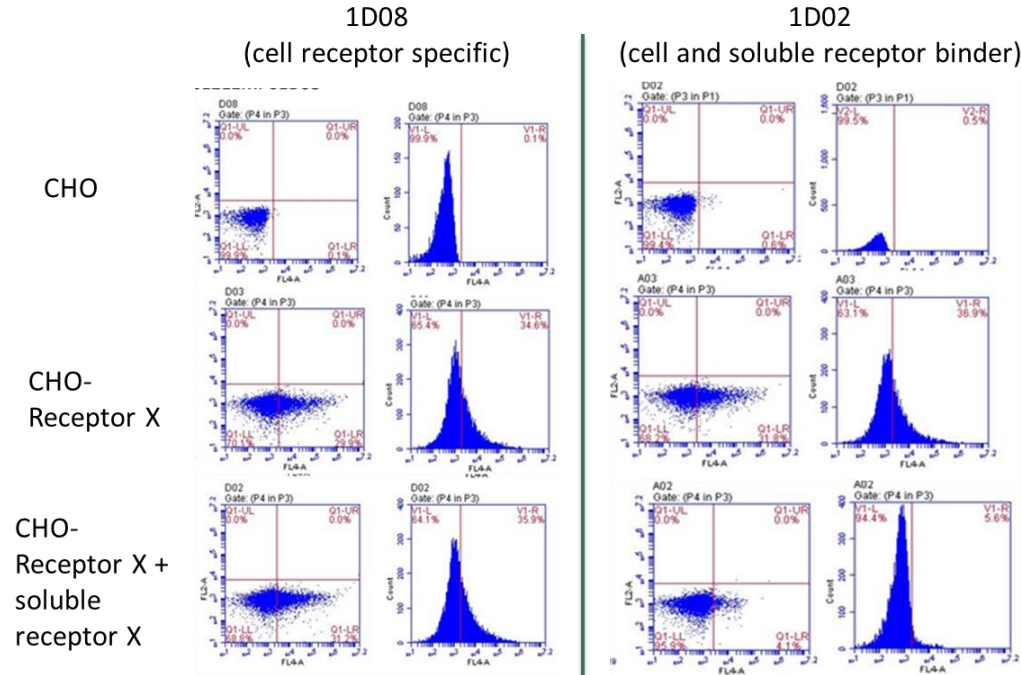
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- Identification of membrane receptor specific Fabs by FACS (screening cascade)



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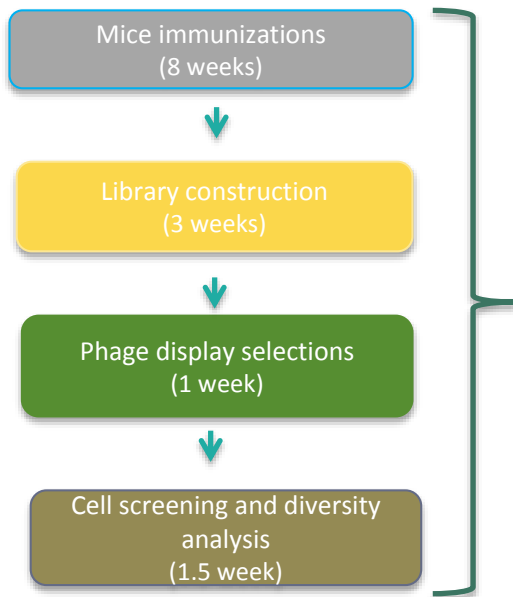
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- Identification of a diverse panel of receptor specific Fabs (summary)



Library	% binders for CHO-X receptor	Specificity	% binders	# HCDR3 Families
M003	14.8% (14/94)	Membrane bound receptor X	93% (13/14)	3
		Membrane <u>and</u> soluble receptor	7% (1/14)	1
M004	70% (66/94)	Membrane bound receptor X	95% (63/66)	4
		Membrane <u>and</u> soluble receptor	4.5% (3/66)	2
M005	42.5% (40/94)	Membrane bound receptor X	72.5% (29/40)	9
		Membrane <u>and</u> soluble receptor	27.5% (11/40)	5
M006	10.6% (10/94)	Membrane bound receptor X	100% (10/10)	4
		Membrane <u>and</u> soluble receptor	0% (0/0)	-
M007	18% (17/94)	Membrane bound receptor X	59% (10/17)	2
		Membrane <u>and</u> soluble receptor	41% (7/17)	5