

Supplemental Material to:

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Knobs-into-holes antibody production in mammalian cell lines reveals that asymmetric afucosylation is sufficient for full antibody-dependent cellular cytotoxicity

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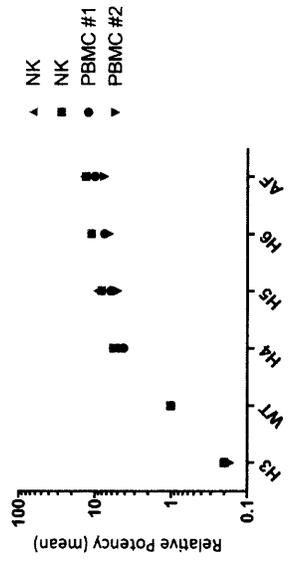
Supplemental Figures Legend

Figure S1. N-linked afucosylated carbohydrate species.

Quantification of N-linked afucosylated species using GlycoChip shows a difference in relative abundance of structures when core fucose is absent. These longer adducts do not appear to affect ADCC potency.

Figure S2. ADCC potencies of various heterodimers are reproducible in different cell based assays.

ADCC potencies of heterodimer and asymmetric antibodies were tested using two different PBMC donors. In addition, the assay was run using an engineered NK cell line on two different days to test for consistency and reproducibility of ADCC. Though raw values vary, the trend remains consistent among assays demonstrating that the absence of only one core fucose is required to achieve high potencies similar to complete afucosylation.



	Parental ½ mAb	version*	Host	ProA Recovery (mg/L)	Knob	Hole
Shake Flask or Transient	anti-CD20	1	CHO	7		x
		2	CHO	73	x	
		2	CHO	78	x	
		1	CHO	25		x
		3	Fut8KO	15	x	
		4	Fut8KO	10		x
		5	<i>E.coli</i>	8	x	
	Ab-1	1	CHO	6.5		x
		2	CHO	81		x
		3	CHO	90		x
	Ab-2	1	CHO	8	x	
		2	CHO	22	x	
		3	CHO	30	x	
	Ab-3	1	CHO	85		x
	Ab-4	2	CHO	13	x	
	Ab-5	1	<i>E.coli</i>	12.8	x	
		2	<i>E.coli</i>	7	x	
	Ab-6	1	CHO	26.5		x
		2	CHO	96		x
	Ab-7	1	CHO	96.2	x	
2		CHO	70	x		
Ab-8	1	<i>E.coli</i>	5.2		x	
Fed-batch or Stable	Ab-2	1	<i>E.coli</i>	567.8	x	
		1	<i>E.coli</i>	413.6	x	
	Ab-3	1	<i>E.coli</i>	186		x
	Ab-4	1	<i>E.coli</i>	60	x	
	Ab-5	1	<i>E.coli</i>	200	x	
		2	<i>E.coli</i>	360	x	
	Ab-6	3	CHO	2000		x
	Ab-7	3	CHO	2500	x	
	Ab-9	1	CHO	1600	x	

*denotes sequence variants of the same parental antibody

Supplemental Table S1. Protein-A yields for half-antibodies derived from *E.coli*, CHO and Fut8KO cell lines. *E.coli* Pro-A yields are from either shake flask cultures (top) or fed-batch cultures (bottom), while mammalian host Pro-A yields are from either transient expression cultures (top) or stable cell cultures (bottom). Each antibody (Ab) is against a unique target and blinded in this table.

	Theoretical G ₀ Mass	Measured G ₀ Mass	Theoretical Mass (without glycosylation)	Measured Mass (PNGase treated)
knob/knob	148,346.98	N/O	145,731.80	N/O
hole/hole	147,936.46	N/O	145,321.28	N/O
heterodimer	148,141.72	148,125.72	145,526.54	145,530.91

Supplemental Table S2. Comparison of theoretical and MS measured masses for H6