

Figures

Fig.1:

Antibody	Cell binding EC50 [ng/ml]
MAB-16-0262	6,3
MAB-16-0346	49,5
MAB-16-0451	8,9
MAB-16-0489	3,2
MAB-16-0484	21,9
MAB-16-0464	3,0
MAB-16-0267	3,4
MAB-16-0406	6,6
MAB-16-0400	5,8
CP-870-IgG1-LALA	14,5

Fig. 2:

Antibody	HEK-Blue™ EC50 [ng/ml]
MAB-16-0262	1957
MAB-16-0346	6243
MAB-16-0406	1235
MAB-16-0400	2168
MAB-16-0451	2267
MAB-16-0489	1127
MAB-16-0464	1437
MAB-16-0267	2333
MAB-16-0283	1445
MAB-16-0325	2211
MAB-16-0377	900
MAB-16-0386	1386
MAB-16-0388	576

Fig. 3:

Antibody	OD at 450 nm
MAB-16-0262	0,11
MAB-16-0346	0,09
MAB-16-0451	0,13
MAB-16-0489	0,01
MAB-16-0484	0,07
MAB-16-0464	0,09
MAB-16-0267	0,10
MAB-16-0406	0,07
MAB-16-0400	0,08
CP-870-IgG1-LALA	2,54

Fig. 4:

Antibody	Cyno-CD40 ELISA EC50 [ng/ml]
MAB-16-0262	11,9
MAB-16-0346	8,2
MAB-16-0451	10,4
MAB-16-0484	31,8
MAB-16-0464	8
MAB-16-0267	7
MAB-16-0406	11,1
CP-870,893	8

Fig. 5:

a)

Antibody	IL12p40 [ng/ml]
MAB-16-0262	>24,0
MAB-16-0267	1,9
MAB-16-0283	1,7
MAB-16-0346	9,5
MAB-16-0325	1,2
MAB-16-0377	0,8
MAB-16-0386	0,9
MAB-16-0388	0,9
MAB-16-0406	17,0
MAB-16-0400	11,1
MAB-16-0451	>24,0
MAB-16-0489	8,3
MAB-16-0484	4,6
MAB-16-0464	>24,0
IgG1-LALA control	0

b)

Dendritic cell IL12p40 release

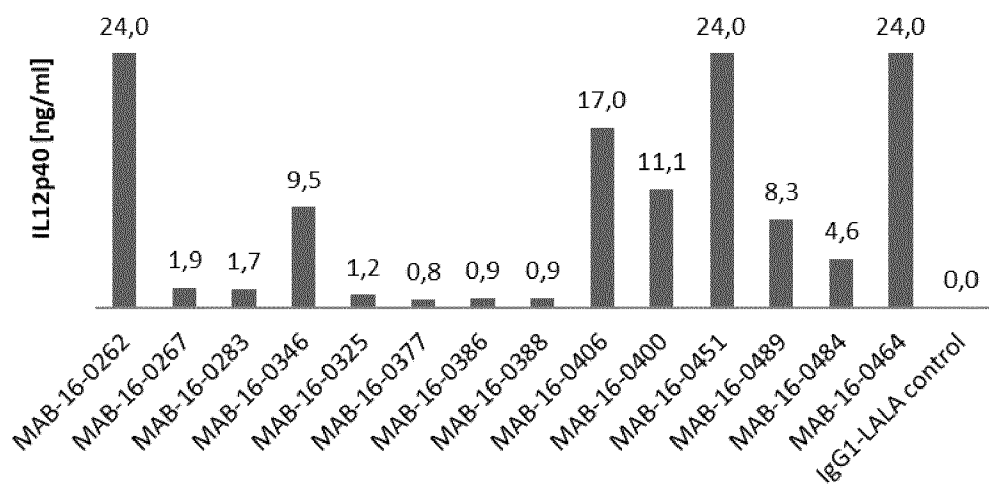


Fig. 6:

	Secreted IL-12p40 by anti-CD40 treated dendritic cells [ng/ml]			
Antibody	5µg/ml antibody	1,7µg/ml antibody	0,6µg/ml antibody	0,2µg/ml antibody
MAB-16-0262	55,2	81,5	81,8	35,8
MAB-16-0451	81,7	207,7	164,0	57,9
MAB-16-0489	13,4	17,7	10,3	2,8
MAB-16-0484	6,8	7,8	3,5	0
MAB-16-0267	2,5	2,6	2,1	0
MAB-16-0406	19,4	34,7	24,7	11,8
CP870-IgG1-LALA	1,6	2,1	1,2	0,7
CP870-IgG1-V11	76,1	72,2	77,5	26,1
CP870-IgG2	5,7	5,8	0	0
CP870-IgG1	32,0	31,5	0	0
Control IgG1-LALA	0	0	0	0

Fig. 7:

Antibody	IL12p40 release EC50 [ng/ml]
MAB-16-0262	416
MAB-16-0406	380
MAB-16-0400	392
MAB-16-0451	659
MAB-16-0489	743
MAB-16-0484	388

Fig.8:

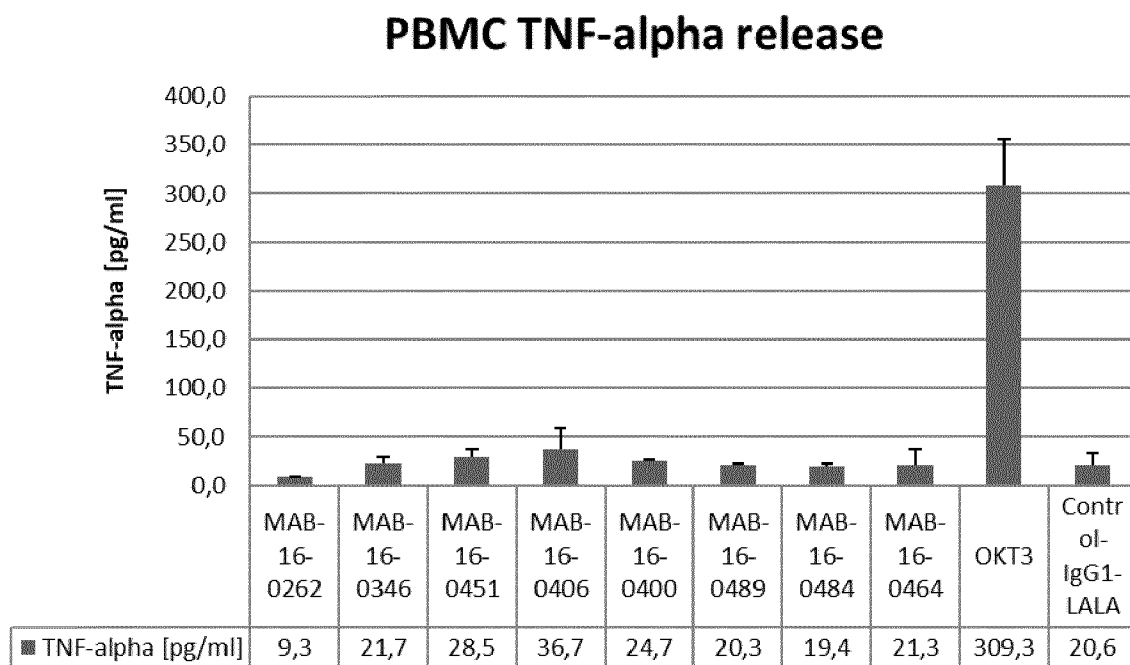


Fig. 9:

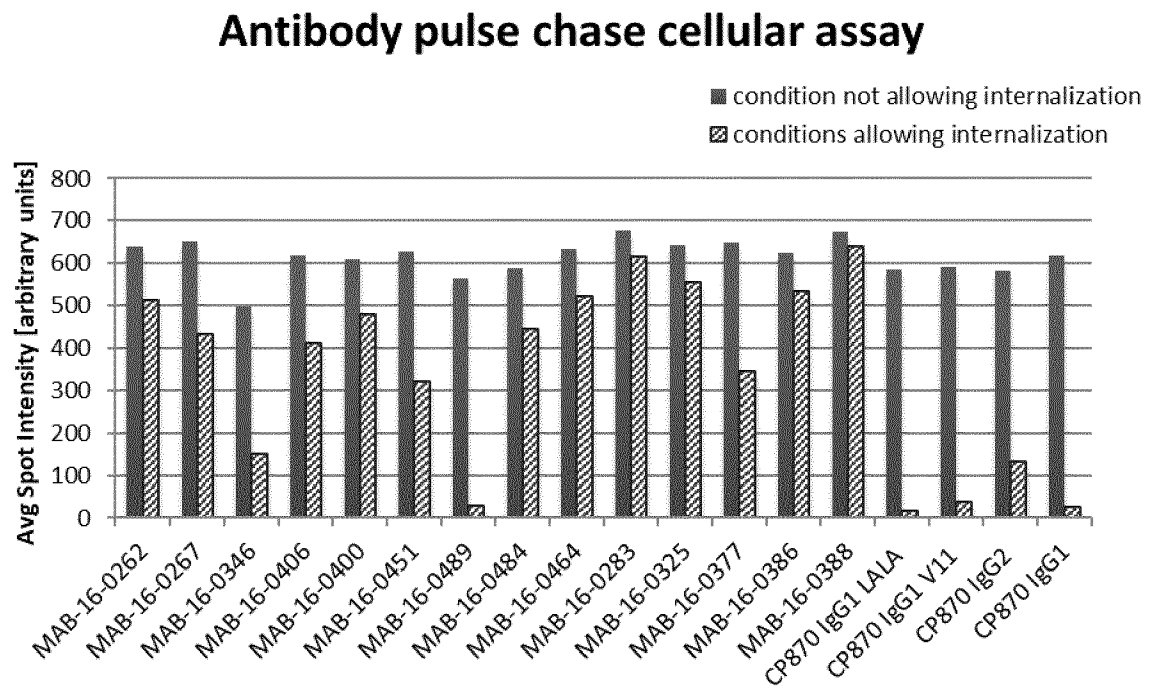


Fig.10:

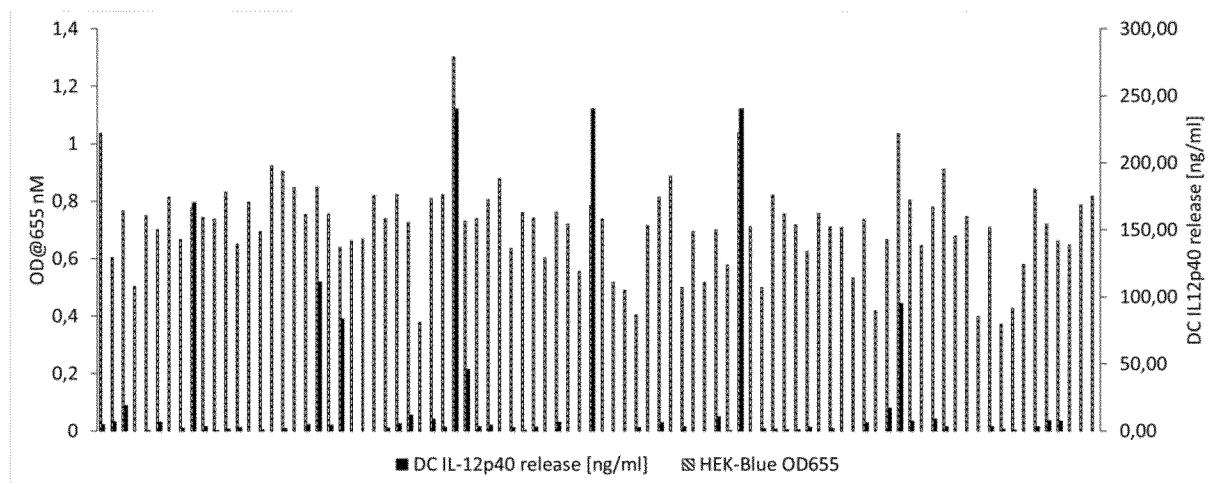


Fig.11:

Experiment		FACS Coreceptor analysis (FOI over isotype antibody treatment)					
		HLA-DR	CD86	CD80	CD83	CD54	CD95
Donor 1	CP870 IgG1 LALA	1,3	1,3	1,1	n.a.	n.a.	n.a.
	CP870 IgG2	6,1	3,9	1,9	n.a.	n.a.	n.a.
	MAB-16-0451	11,5	16,6	3,8	n.a.	n.a.	n.a.
	MAB-16-0262	10,7	13,0	3,2	n.a.	n.a.	n.a.
	MAB-16-0464	10,9	7,5	2,4	n.a.	n.a.	n.a.
	MAB-16-0406	8,3	7,5	1,9	n.a.	n.a.	n.a.
	MAB-16-0267	1,0	1,2	1,0	n.a.	n.a.	n.a.
	CD40L	8,8	7,4	1,8	n.a.	n.a.	n.a.
Donor 2	CP870 IgG2	5,5	5,7	2,2	7,4	n.a.	n.a.
	MAB-16-0451	5,5	9,9	3,5	5,8	n.a.	n.a.
	MAB-16-0262	6,2	11,4	3,7	7,4	n.a.	n.a.
	MAB-16-0464	7,2	9,6	2,8	7,4	n.a.	n.a.
	MAB-16-0406	6,3	8,2	2,2	6,5	n.a.	n.a.
	MAB-16-0267	0,8	0,9	0,8	0,7	n.a.	n.a.
Donor 3	CP870 IgG1 LALA	2,2	2,8	1,4	2,4	1,8	1,2
	CP870 IgG2	3,6	7,5	2,0	4,4	3,0	1,4
	MAB-16-0451	3,4	19,3	4,1	4,2	1,9	1,5
	MAB-16-0262	3,2	17,2	3,5	4,6	2,7	1,5
	MAB-16-0464	4,2	16,3	3,2	4,2	2,5	1,5
	MAB-16-0406	3,0	12,2	2,7	4,0	2,1	1,5
	MAB-16-0267	1,3	1,6	1,3	1,2	1,2	1,1

Fig.12:

		ELISA [ng/ml]		Cytometric bead array [ng/ml]				
		IL12p40	IL12p70	IL-1b	IL-6	IL-10	TNF-a	IL-8
Donor 1	CP870 IgG1 LALA	0,0	40,5	1,7	0,3	0,3	0,0	0,1
	CP870 IgG2	11,3	60,4	1,6	0,2	0,0	0,2	0,0
	MAB-16-0451	124,3	464,1	26,9	2,9	7,4	17,6	0,2
	MAB-16-0262	87,4	277,4	10,0	1,3	2,6	4,9	0,1
	MAB-16-0464	67,2	202,5	5,7	0,7	0,8	2,3	0,1
	MAB-16-0406	33,1	232,5	3,6	0,8	0,9	0,3	0,1
	MAB-16-0267	0,0	43,7	2,3	0,1	0,1	0,0	0,1
	CD40L	0,0	78,8	1,4	0,2	0,1	0,0	0,0
Donor 2	control-IgG1-LALA		16,7	2,1	0,1	0,0	0,0	0,1
	CP870 IgG2	9,6	6,8	0,1	0,0	0,0	0,1	0,0
	MAB-16-0451	256,3	124,7	11,6	0,1	3,5	13,0	0,0
	MAB-16-0262	124,8	80,3	6,3	0,0	1,5	5,2	0,0
	MAB-16-0464	55,2	28,2	1,2	0,0	0,1	0,0	0,0
	MAB-16-0406	16,1	24,2	0,4	0,0	0,0	0,0	0,0
	MAB-16-0267	0,0	0,9	0,0	0,0	0,0	0,0	0,0
Donor 3	control-IgG1-LALA		1,3	0,0	0,0	0,0	0,0	0,0
	CP870 IgG1 LALA	0,8	9,9	0,0	0,0	0,1	0,0	0,0
	CP870 IgG2	10,8	10,1	0,1	0,0	0,0	0,0	0,0
	MAB-16-0451	135,9	139,2	3,9	0,1	1,1	3,2	0,0
	MAB-16-0262	102,1	89,5	2,7	0,1	0,5	1,2	0,0
	MAB-16-0464	30,1	32,3	0,4	0,0	0,1	0,1	0,0
	MAB-16-0406	9,6	26,9	0,1	0,0	0,0	0,0	0,0
	MAB-16-0267	0,0	6,0	0,0	0,0	0,0	0,0	0,0
Donor 3	control-IgG1-LALA		3,3	0,0	0,0	0,0	0,0	0,0

Fig.13:

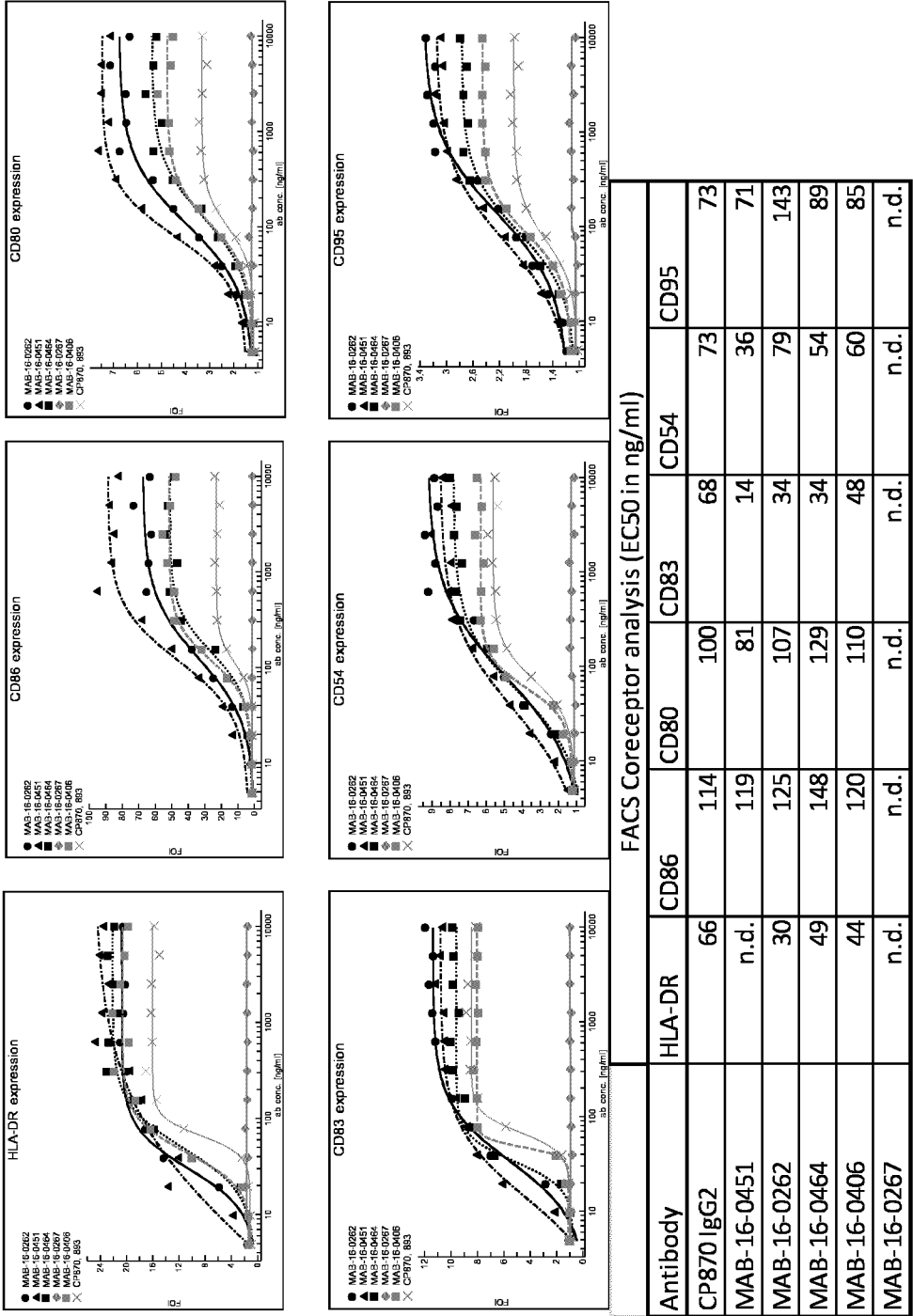
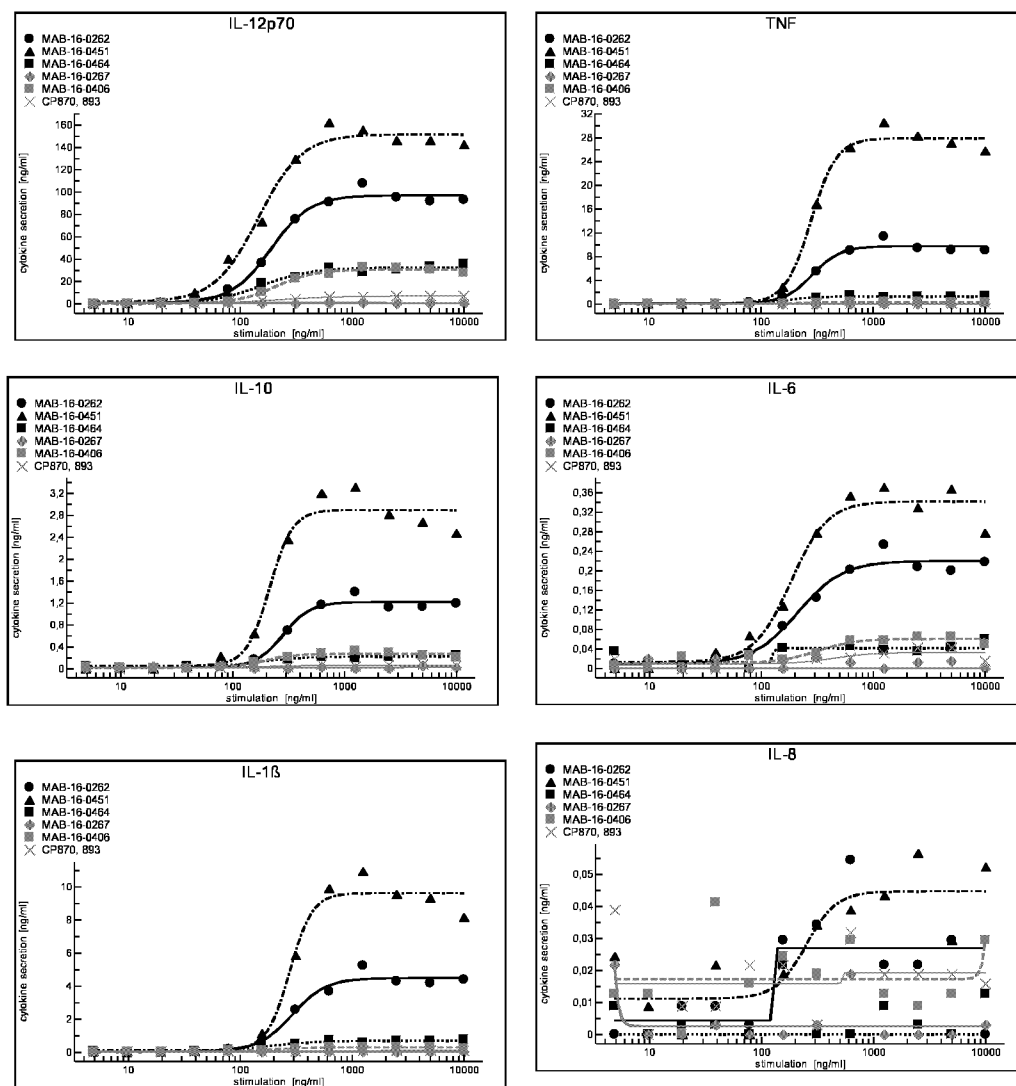
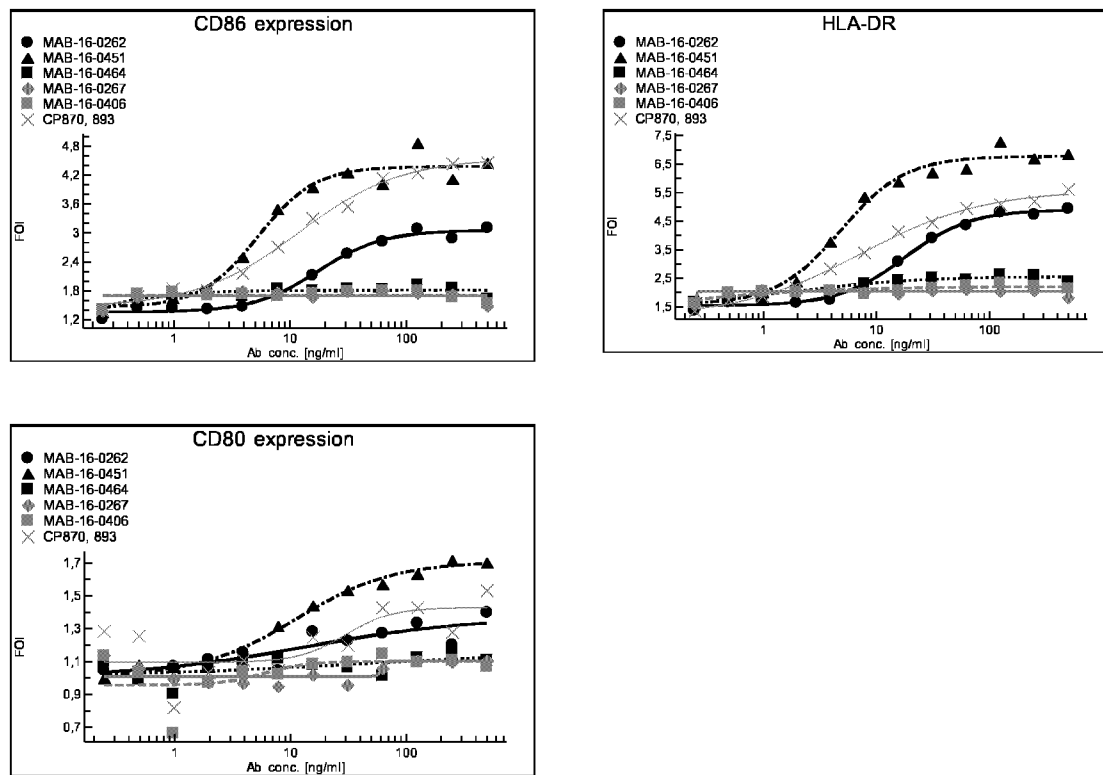


Fig.14:



Antibody	Cytometric bead array [EC50 in ng/ml]					
	IL12p70	IL-1β	IL-6	IL-10	TNF-α	IL-8
CP870 IgG2	213	263	n.d.	n.d.	244	n.d.
MAB-16-0451	146	287	185	215	280	n.d.
MAB-16-0262	189	294	213	285	289	n.d.
MAB-16-0464	156	183	n.d.	172	169	n.d.
MAB-16-0406	208	192	320	189	239	n.d.
MAB-16-0267	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Fig.15:



Antibody	FACS Coreceptor analysis EC50 [ng/ml]		
	HLA-DR	CD86	CD80
CP870 IgG2	8	12	n.a.
MAB-16-0451	5	5	11
MAB-16-0262	18	18	n.a.
MAB-16-0464	n.a.	n.a.	n.a.
MAB-16-0406	n.a.	n.a.	n.a.
MAB-16-0267	n.a.	n.a.	n.a.

Fig.16:

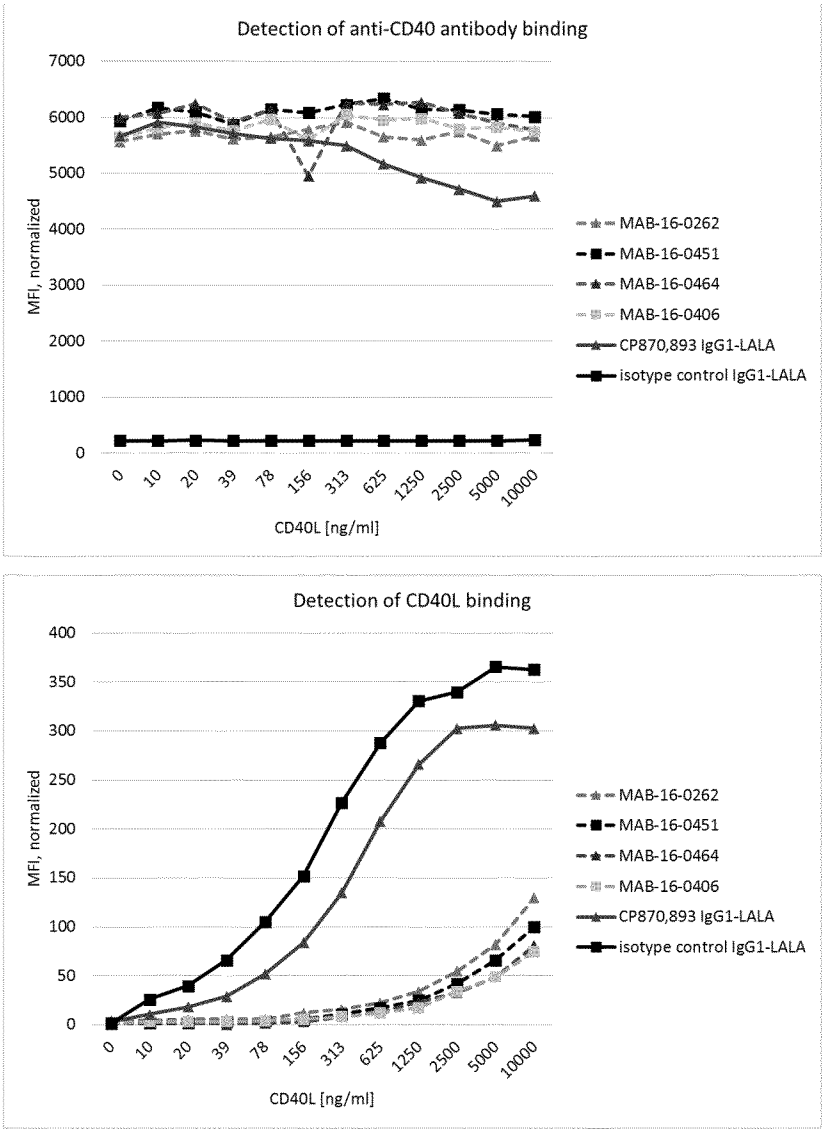


Fig.17:

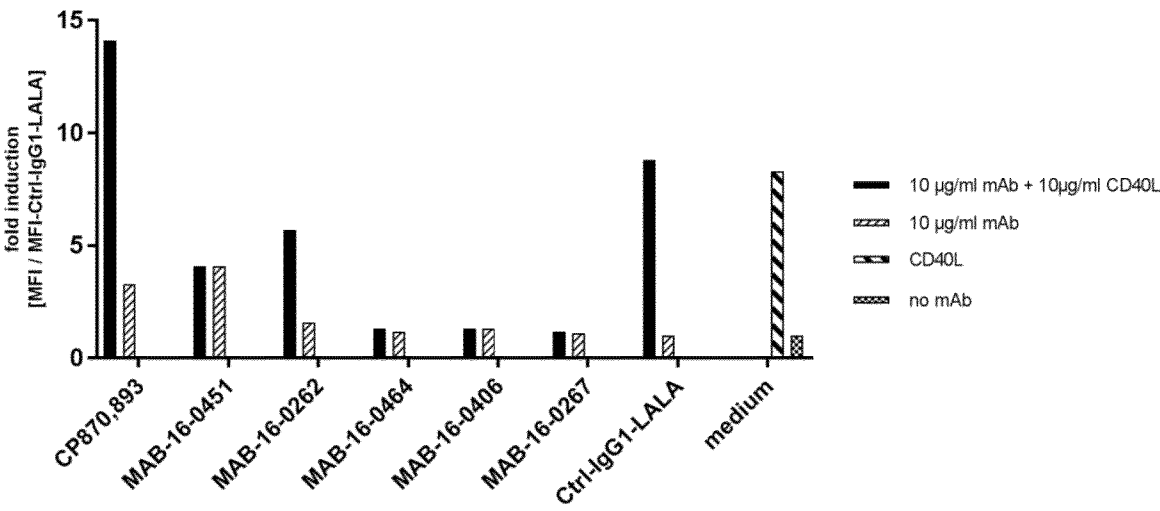


Fig.18:

	K_D (nM)
Ab MAB-16-0262/human CD40	15.7 ± 1.1
Ab MAB-16-0451/human CD40	1.2 ± 0.0
Ab MAB-16-0464/human CD40	2.6 ± 0.0
CP-870,893/human CD40	8.9 ± 1.4
Ab MAB-16-0262/cyno CD40	10.3 ± 0.2
Ab MAB-16-0451/cyno CD40	0.8 ± 0.0
Ab MAB-16-0464/cyno CD40	2.2 ± 0.1

Fig.19:

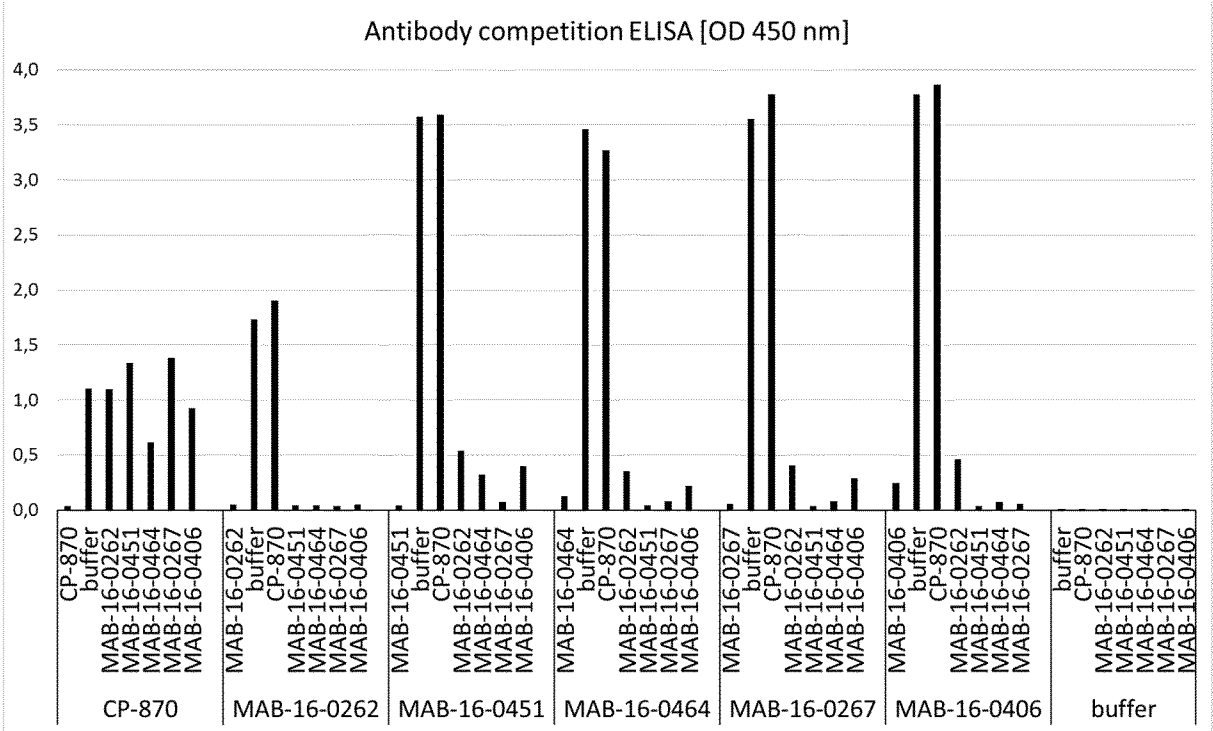


Fig.20:

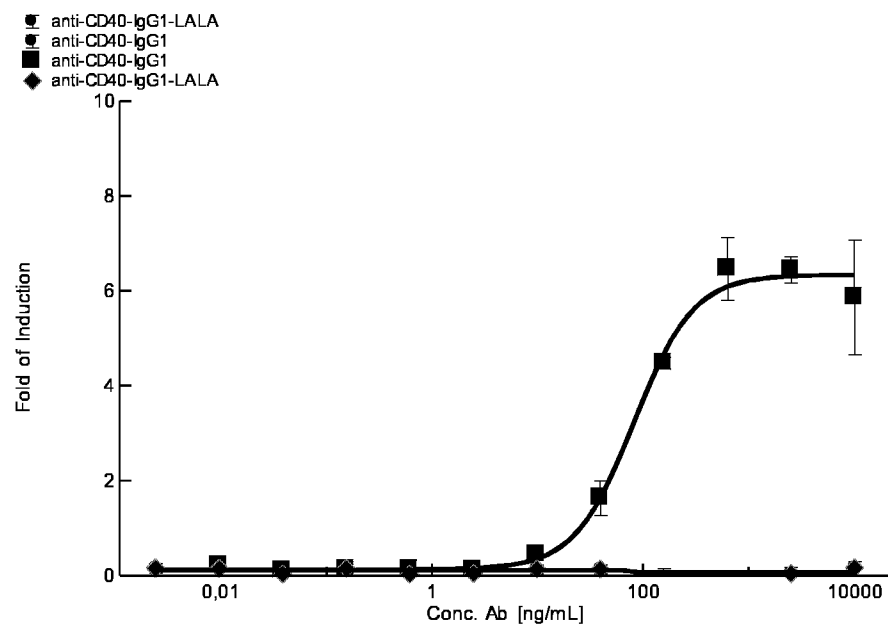


Fig.21:

Antibody	Mouse	Body temp. at day 3 [°C]	Survival at day 3
CP-870,893	1	31,3	-
	2	34,8	+
	3	35,5	+
	4	32,3	-
	5	32,6	-
	6	35,5	+
hIgG2	1	35,8	+
	2	35,5	+

Antibody	Mouse	Body temp. at day 4 [°C]	Survival at day 4-24
MAB-16-0451	1	36,9	+
	2	36,6	+
	3	36,2	+
hIgG1	1	37,0	+

Fig.22: Sequences (amino acids in one letter code)Complete sequences of Variable Regions (VR):

Heavy chain: VH complete: SEQ ID NO: 1-14
 Light chain: VL complete: SEQ ID NO: 15-28

mAB name	SEQ ID NO.	Complete Heavy-chain VR sequence
MAB-16-0283	1	EVQLEESGGDLVQPGASLRRLSCAASGFSSFSYWICWVRQAPGKGLELVSCIYT TSGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARSSGVSYP SYFHLWGQGTLLVTVSS
MAB-16-0377	2	EVQLEESGGGLVQPGASLRRLSCAASGFSSSGYWMCWVRQAPGKGLEWVGC IYTN SGVTTYANWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGGAIYNDY DYAFYYSLWGQGTLLVTVSS
MAB-16-0267	3	EVQLEESGGDLVQPGASLRRLSCAASGFDFNSNAMS WVRQAPGKGLEWVASIYAG GSGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGITRLPLWG QGTLLVTVSS
MAB-16-0386	4	EVQLEESGGDLVQPGASLRRLSCAVSGDFSSNAMS WVRQAPGKGLEWVSSIYAG SSGSTYYASWAKGRFTISKDASKTTLYLQMNSLRAEDTATYFCARGVTRLPLWG QGTLLVTVSS
MAB-16-0451	5	EVQLEESGGGLVQPGGSLRLSCAASGFDFSSNTMCWVRQAPGKGLEWVACIYAG SSGSTYYASWAKGRFTISKDISKTTLYLQMNSLRAEDTATYFCARGLSRFLWG QGTLLVTVSS
MAB-16-0346	6	EVQLEESGGGLVQPGGSLRLSCAASGFDFSTNAVSWVRQAPGKGLEWVGSISAG SSGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGYTYLTLWG QGTLLVTVSS
MAB-16-0325	7	EVQLEESGGGLVQPGGSLRLSCAASGFSSFNAMS WVRQAPGKGP EWVVTIYAG SSGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGATYTLTLWG QGTLLVTVSS
MAB-16-0388	8	EVQLVESGGGLVQPGGSLRLSCAASGFDFSSNAMS WVRQAPGKGLEWVGIIYAG SSGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGATYITLWG QGTLLVTVSS
MAB-16-0464	9	EVQLEESGGGLVQPGGSLRLSCAASGFDFSSNAMS WVRQAPGKGLEWVGIIYAG SNGNTDYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGASYFTLWG QGTLLVTVSS
MAB-16-0262	10	EVQLEESGGGLVQPGGSLRLSCAASGFDFSTNAMCWVRQAPGKGLEWVACIAAG SSIITYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTAVYFCARGLSRFALWG QGTLLVTVSS
MAB-16-0406	11	EVQLEESGGGLVQPGGSLRLSCAASGIDFSRYYYMCWVRQAPGKGP EWVACYSN GDGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGADYSAGAA AFNLWGQGTLLVTVSS
MAB-16-0484	12	EVQLEESGGGLVQPGGSLRLSCAASGIDFSRYYYICWVRQAPGKGP EWVACFAN GDGSTYYASWAKGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGADYSGGAA AFNLWGQGTLLVTVSS
MAB-16-0400	13	EVQLEESGGGLVQPGGSLRLSCAASGIDFSRYFYMCWVRQAPGKGLEWVACIGP GVSGDTYYASWAKGRFTISGDTSKTTLYLQMNSLRAEDTATYFCARGVDYTYGD AGAAFNLWGQGTLLVTVSS
MAB-16-0489	14	EVQLEESGGGLVQPGASLRRLSCAASGIDFSRYFYVCWVRQAPGKGLEWVGCFAN HDDSIYYAGWMNGRFTISKDTSKTTLYLQMNSLRAEDTATYFCARGVDYTVGYG GAAFNLWGQGTLLVTVSS

mAB name	SEQ ID NO.	Complete k-Light chain VR sequence
MAB-16-0283	15	DIQMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYSASKLP SGVPSRF SGSGSGTDFTLTISLQPEDFATYHCQTYYYSSSSSYDYGFQGQGTKVVIK
MAB-16-0377	16	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYKASTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQSYYGSSSYNAFGQGQGTKVVIK
MAB-16-0267	17	DIQMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYDASKLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGTYYGSTTISAFQGQGTKVVIK
MAB-16-0386	18	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYDASTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGTVYGSSTISAFQGQGTKVVIK
MAB-16-0451	19	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYDASKLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGGDYGYSSYVAFGGGQGTKVVIK
MAB-16-0346	20	DIVMTQSPSSLSASVGDRVTITCQASHISSTYLSWYQQKPGQAPKLLIYRASTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQYTDYGSYVSTFGQGQGTKVVIK
MAB-16-0325	21	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLSWYQQKPGQAPKLLIYRASTLP SGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGYYSSTTYDSTAFQGQGTKVVIK
MAB-16-0388	22	DIVMTQSPSSLSASVGDRVTITCQASQSIGSYLAWYQQKPGQAPKLLIYRASTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGYYSSTTTTYDSSAFQGQGTKVVIK
MAB-16-0464	23	DIVMTQSPSSLSASVGDRVTITCQASESVVSNRLAWYQQKPGQAPKLLIYLASTLP SGVP SRFSGSGSGTDFTLTISLQPEDFATYYCAGYKSSSTDGTAFGQGQGTKVVIK
MAB-16-0262	24	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLSWYQQKPGQAPKLLIYLTSTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQGYYSSTSYVSNFGQGQGTKVVIK
MAB-16-0406	25	DIQMTQSPSSLSASVGDRVTITCQASESIGNALVWYQQKPGQAPKLLIYRASILASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQDYYSSTYNTFGQGQGTKVVIK
MAB-16-0484	26	DIQMTQSPSSLSASVGDRVTITCQASQSISSRLAWYQQKPGQAPKLLIYRASTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQDYYSSTYNAFGQGQGTKVVIK
MAB-16-0400	27	DIVMTQSPSSLSASVGDRVTITCQASQSISSYLAWYQQKPGQAPKLLIYRASILASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQTYYSSTRTSYSGSPNAFGQGQGTKVVIK
MAB-16-0489	28	DIVMTQSPSSLSASVGDRVTITCQASQSIGSYLSWYQQKPGQAPKLLIYRATTLASGVPSRF SGSGSGTDFTLTISLQPEDFATYYCQSYRDSSSAFGQGQGTKVVIK

Complementary Determining Regions (CDR):

Heavy Chain: CDR-H1: SEQ ID NO: 29-42
 CDR-H2: SEQ ID NO: 43-56
 CDR-H3: SEQ ID NO: 57-70

mAB name	SEQ ID NO.	CDR_H1 sequence
MAB-16-0283	29	FSYWIC
MAB-16-0377	30	GYWMC
MAB-16-0267	31	SNAMS
MAB-16-0386	32	SNAMS

MAB-16-0451	33	SNTMC
MAB-16-0346	34	TNAVS
MAB-16-0325	35	SNAMS
MAB-16-0388	36	SNAMS
MAB-16-0464	37	SNAMS
MAB-16-0262	38	TNAMC
MAB-16-0406	39	RYYMC
MAB-16-0484	40	RYYIC
MAB-16-0400	41	RYFYMC
MAB-16-0489	42	RYFYVC

mAB name	SEQ ID NO.	CDR_H2 sequence
MAB-16-0283	43	CIYTTSGSTYYASWAKG
MAB-16-0377	44	CIYTNSGVTTYANWAKG
MAB-16-0267	45	SIYAGSGSTYYASWAKG
MAB-16-0386	46	SIYAGSSGSTYYASWAKG
MAB-16-0451	47	CIYAGSSGSTYYASWAKG
MAB-16-0346	48	SISAGSSGSTYYASWAKG
MAB-16-0325	49	TIYAGSSGSTYYASWAKG
MAB-16-0388	50	IIYAGSSGSTYYASWAKG
MAB-16-0464	51	TIYAGSNGNTDYASWAKG
MAB-16-0262	52	CIAAGSSIITYASWAKG
MAB-16-0406	53	CYSNGDGSTYYASWAKG
MAB-16-0484	54	CFANGDGSTYYASWAKG
MAB-16-0400	55	CIGPGVSGDTYYASWAKG

MAB-16-0489	56	CFANHDDSIYYAGWMNG
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mAB name	SEQ ID NO.	CDR_H3 sequence
MAB-16-0283	57	SSGVSYPSYFHL
MAB-16-0377	58	GGAIYNDYDYAFYYSL
MAB-16-0267	59	GITRLPL
MAB-16-0386	60	GVTRLPL
MAB-16-0451	61	GLSRFSL
MAB-16-0346	62	GYTYLTL
MAB-16-0325	63	GATYLTTL
MAB-16-0388	64	GATYITL
MAB-16-0464	65	GASYFTL
MAB-16-0262	66	GLSRFAL
MAB-16-0406	67	GADYSAGAAAFNL
MAB-16-0484	68	GADYSGGAAAFNL
MAB-16-0400	69	GVDYTYGDAGAAAFNL
MAB-16-0489	70	GVDYTVGYGGAAAFNL

Light Chain: CDR-L1: SEQ ID NO: 71-84
 CDR-L2: SEQ ID NO: 85-98
 CDR-L3: SEQ ID NO: 99-112

mAB name	SEQ ID NO.	CDR_L1 sequence
MAB-16-0283	71	QASQSISSYLA

MAB-16-0377	72	QASQSISSYLA
MAB-16-0267	73	QASQSISSYLA
MAB-16-0386	74	QASQSISSYLA
MAB-16-0451	75	QASQSISSNYLA
MAB-16-0346	76	QASHSISSTYLS
MAB-16-0325	77	QASQSISSNYLS
MAB-16-0388	78	QASQSIGSYLA
MAB-16-0464	79	QASESVVSNRLA
MAB-16-0262	80	QASQSISSYLS
MAB-16-0406	81	QASESIGNALV
MAB-16-0484	82	QASQSISSRLA
MAB-16-0400	83	QASQSISSYLA
MAB-16-0489	84	QASQSIGSYLS

mAB name	SEQ ID NO.	CDR_L2 sequence
MAB-16-0283	85	SASKLPS
MAB-16-0377	86	KASTLAS
MAB-16-0267	87	DASKLAS
MAB-16-0386	88	DASTLAS
MAB-16-0451	89	DASKLAS
MAB-16-0346	90	RASTLAS
MAB-16-0325	91	RASTLPS
MAB-16-0388	92	RASTLAS
MAB-16-0464	93	LASTLPS
MAB-16-0262	94	LTSTLAS

MAB-16-0406	95	RASILAS
MAB-16-0484	96	RASTLAS
MAB-16-0400	97	RASILAS
MAB-16-0489	98	RATTLAS

mAB name	SEQ ID NO.	CDR_L3 sequence
MAB-16-0283	99	QTYYYSSSSSYDYG
MAB-16-0377	100	QSYYGSSSISYNA
MAB-16-0267	101	QCTYYGSTTISA
MAB-16-0386	102	QCTVYGSSTISA
MAB-16-0451	103	QGGDYYGSSYVVA
MAB-16-0346	104	QYTDYGSSYVST
MAB-16-0325	105	QGYYYSGTTYDSTA
MAB-16-0388	106	QGYYYSTTTTTYDSSA
MAB-16-0464	107	AGYKSSSTDGTA
MAB-16-0262	108	QGYYS SSSYVSNG
MAB-16-0406	109	QDY YGSSTEYNT
MAB-16-0484	110	QDY YGSSTEYNA
MAB-16-0400	111	QTY YSSRTYSYGSPNA
MAB-16-0489	112	QSY YRDSSSSA