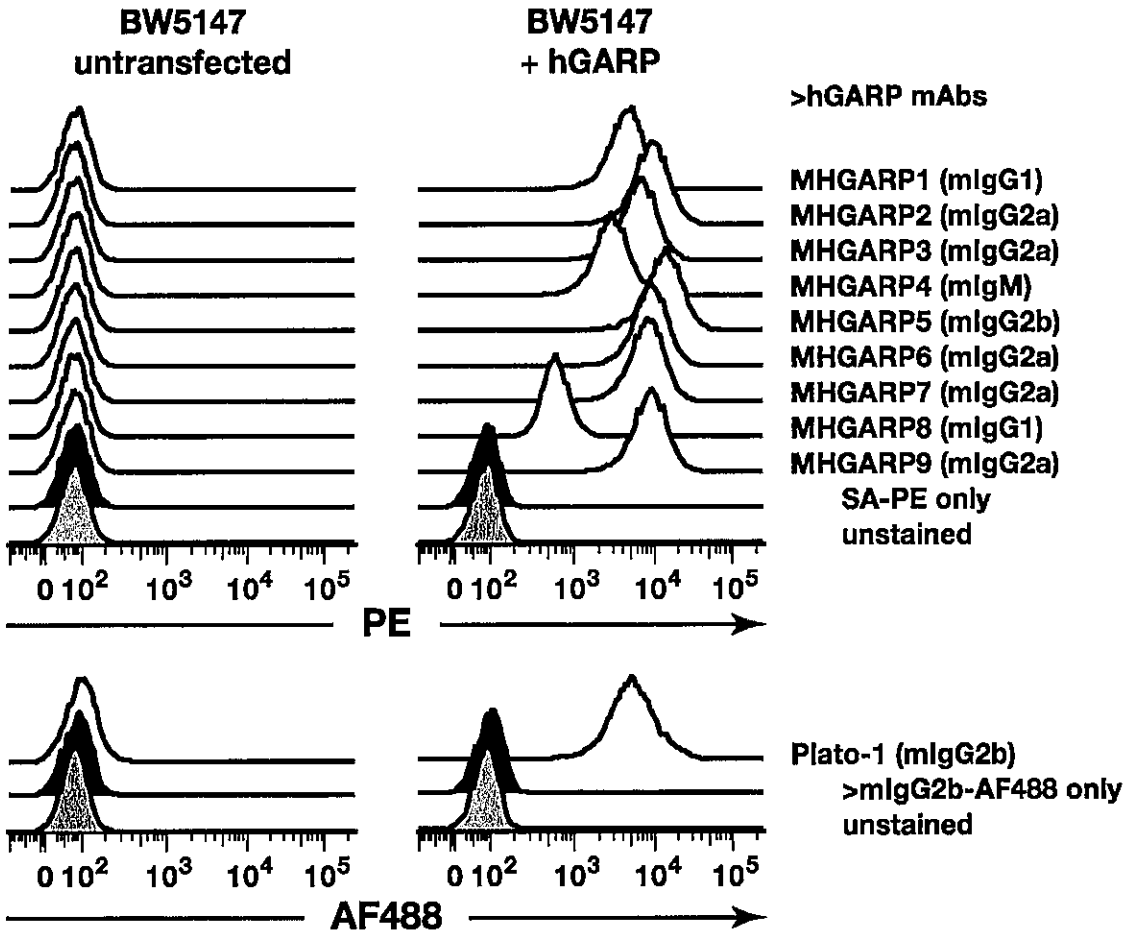

BW5147
untransfected
BW5147
+ hGARP
>hGARP mAbs
MHGARP1 (mIgG1)
MHGARP2 (mIgG2a)
MHGARP3 (mIgG2a)
MHGARP4 (mIgM)
MHGARP5 (mIgG2b)
MHGARP6 (mIgG2a)
MHGARP7 (mIgG2a)
MHGARP8 (mIgG1)
MHGARP9 (mIgG2a)
SA-PE only
unstained
0 10^2 10^3 10^4 10^5
PE
Plato-1 (mIgG2b)
>mIgG2b-AF488 only
unstained
AF488

FIG. 1

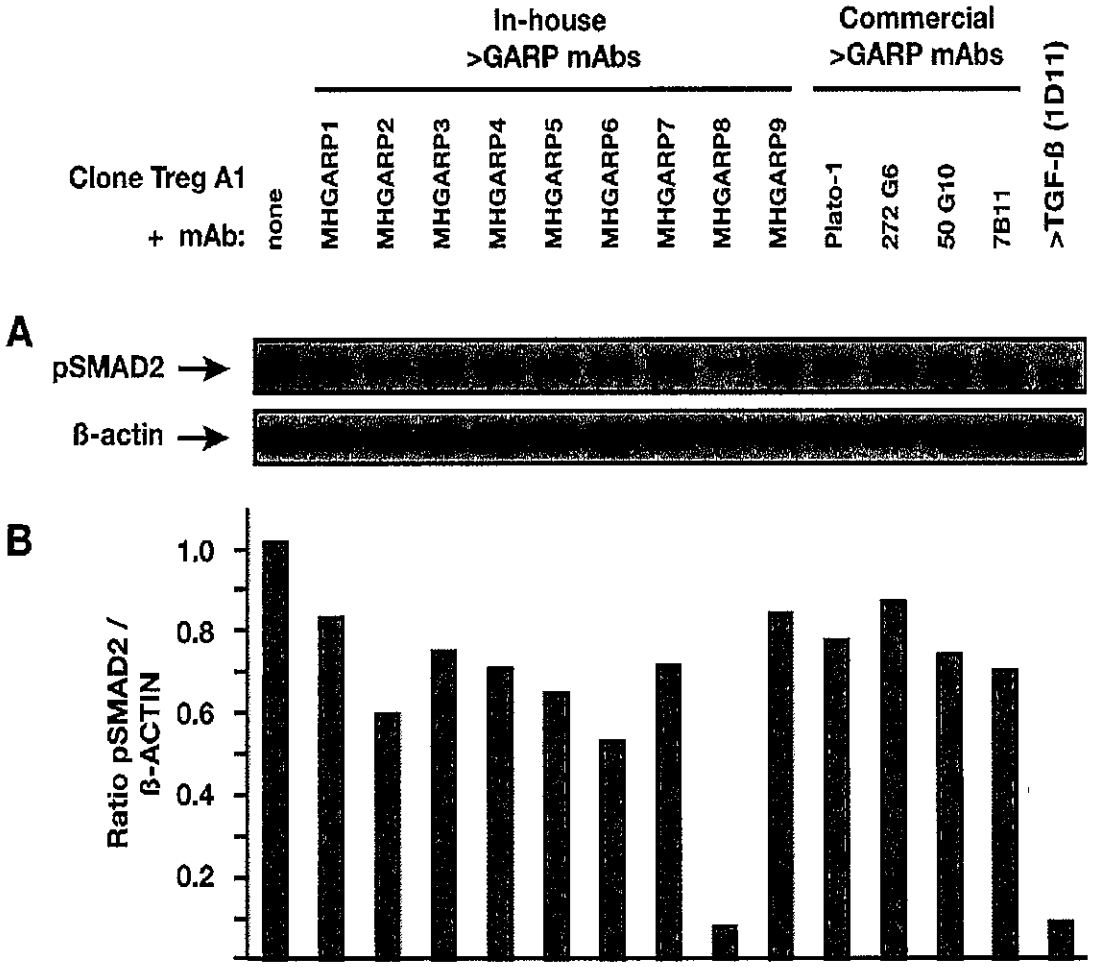

In-house
>GARP mAbs
Commercial
>GARP mAbs
Clone Treg A1
+ mAb:
none
MHGARP1
MHGARP2
MHGARP3
MHGARP4
MHGARP5
MHGARP6
MHGARP7
MHGARP8
MHGARP9
Plato-1
272 G6
50 G10
7B11
>TGF-ß (1D11)
A
pSMAD2
ß-actin
B
Ratio pSMAD2 / ß-ACTIN
1.0
0.8
0.6
0.4
0.2

FIG. 2

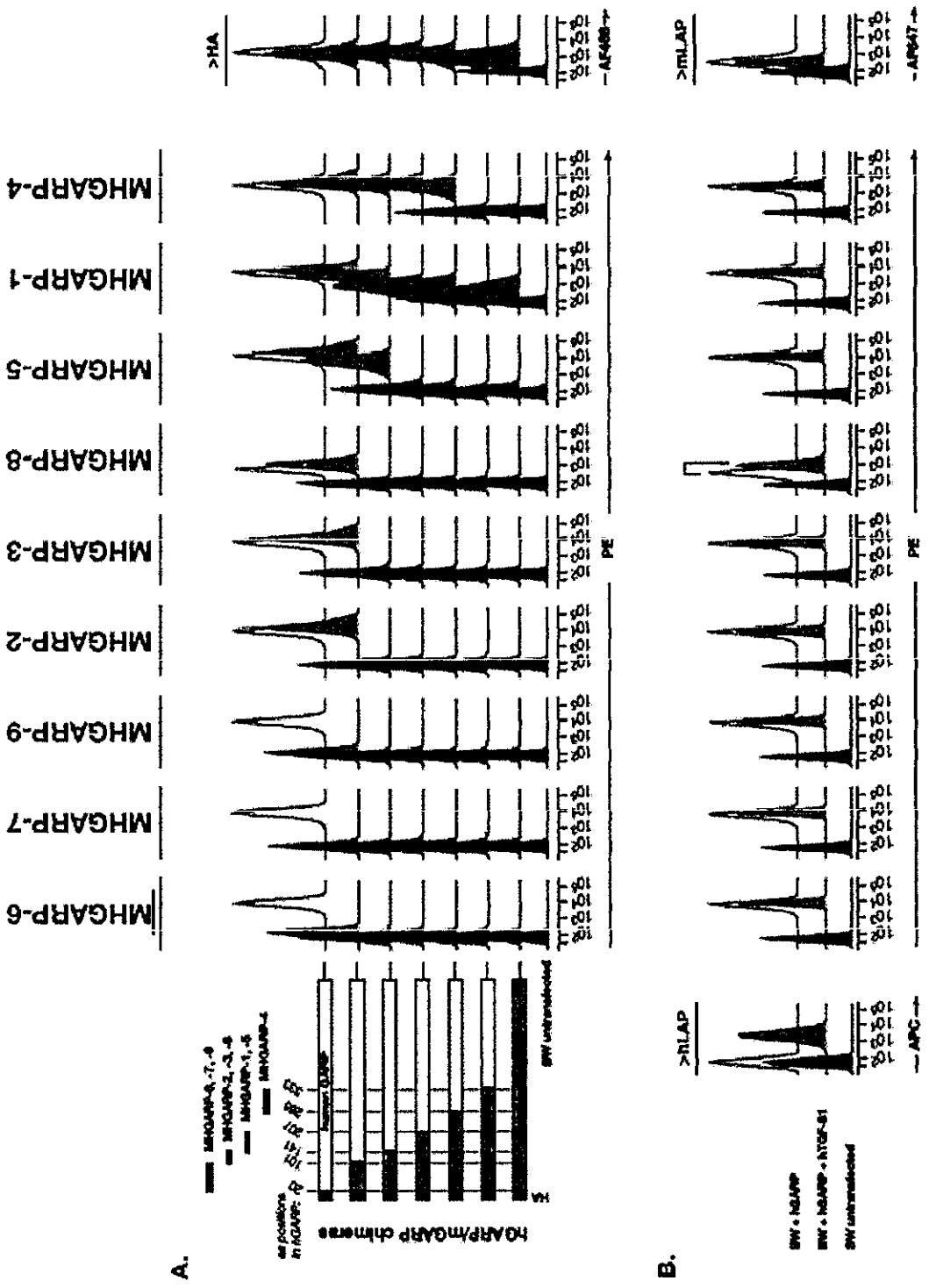

MHGARP-6
MHGARP-7
MHGARP-9
MHGARP-2
MHGARP-3
MHGARP-8
MHGARP-5
MHGARP-1
MHGARP-4
>HA
>mLAP
>hLAP
PE
A.
B.

FIG. 3A-B

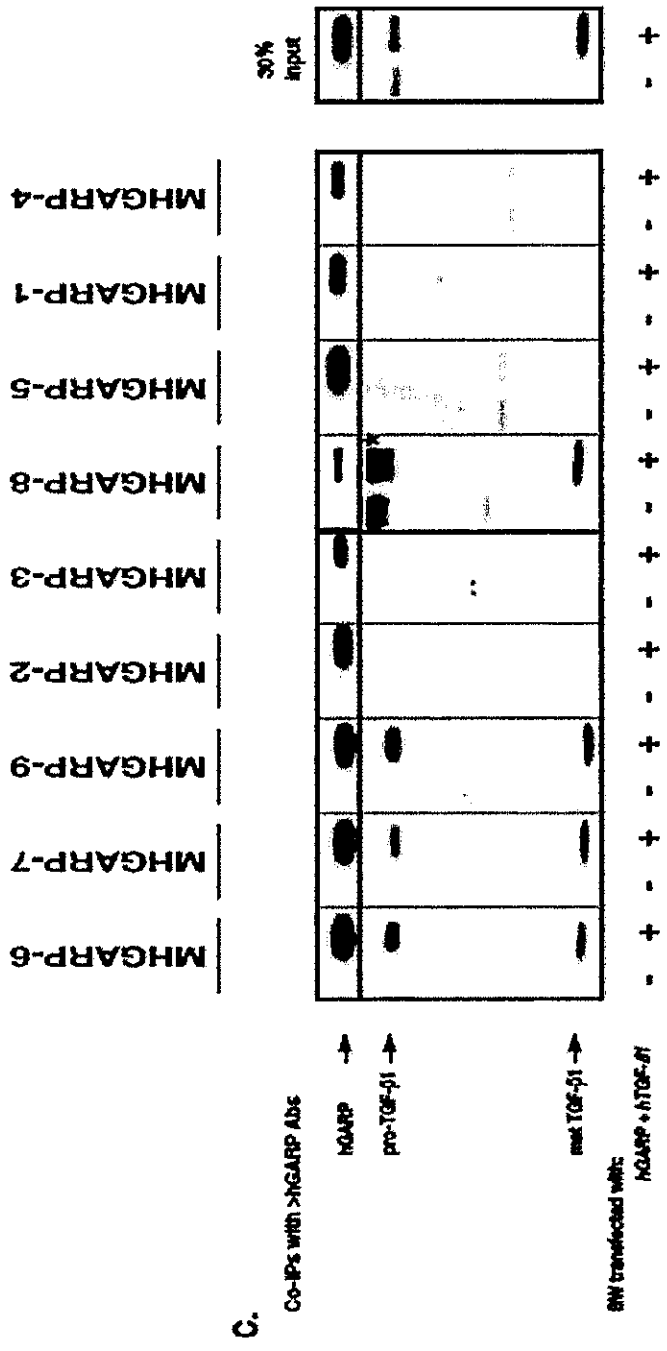

C.
Co-IPs with >hGARP Abs
hGARP
pro-TGF-β1
mat TGF-β1
BW transfected with:
hGARP + hTGF-β1
MHGARP-6
MHGARP-7
MHGARP-9
MHGARP-2
MHGARP-3
MHGARP-8
MHGARP-5
MHGARP-1
MHGARP-4
30% input
+
-

FIG. 3C

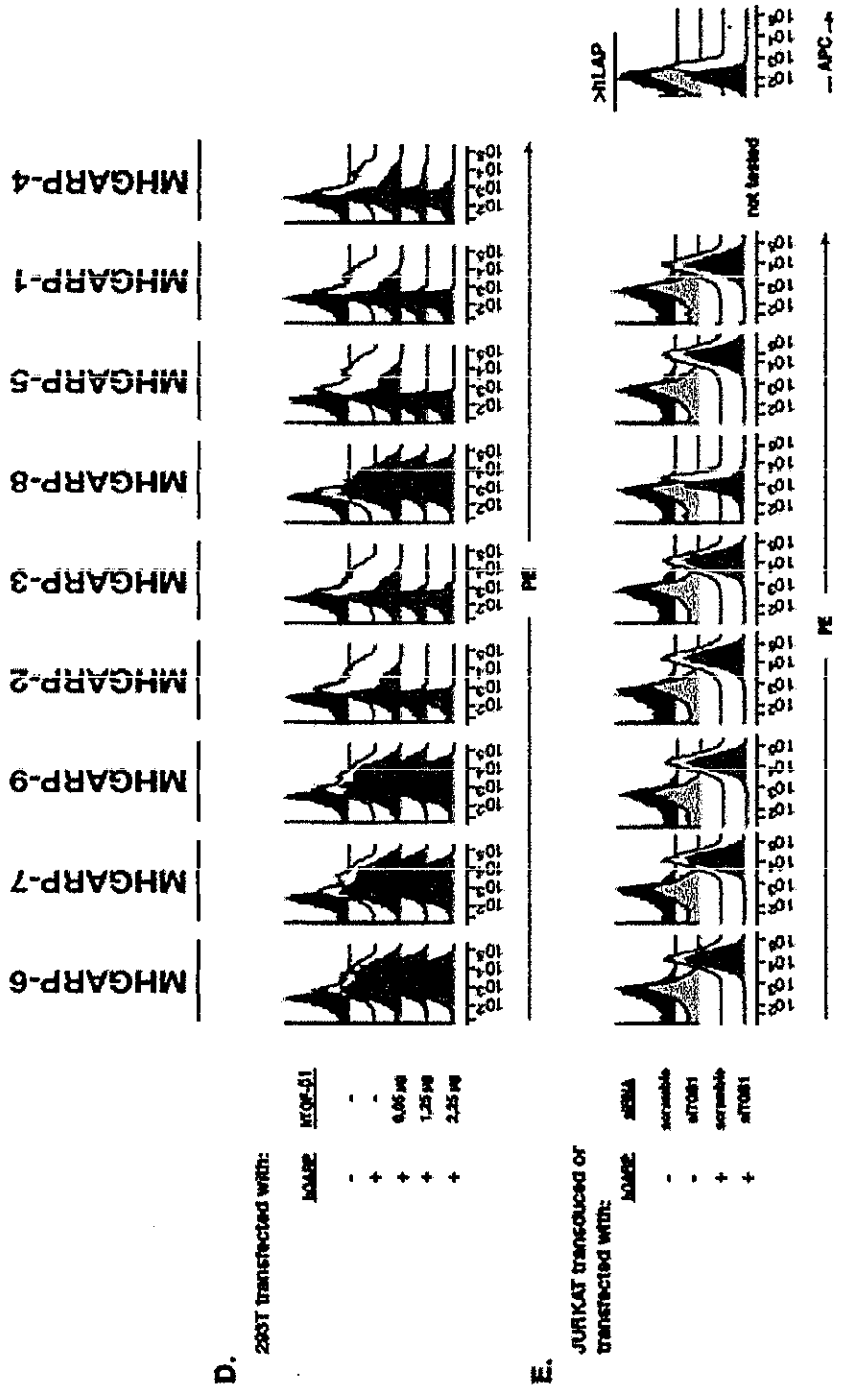

D.
293T transfected with:
MHGARP-6
MHGARP-7
MHGARP-9
MHGARP-2
MHGARP-3
MHGARP-8
MHGARP-5
MHGARP-1
MHGARP-4
PE
E.
JURKAT transduced or transfected with:
not tested
PE
APC

FIG. 3D-E

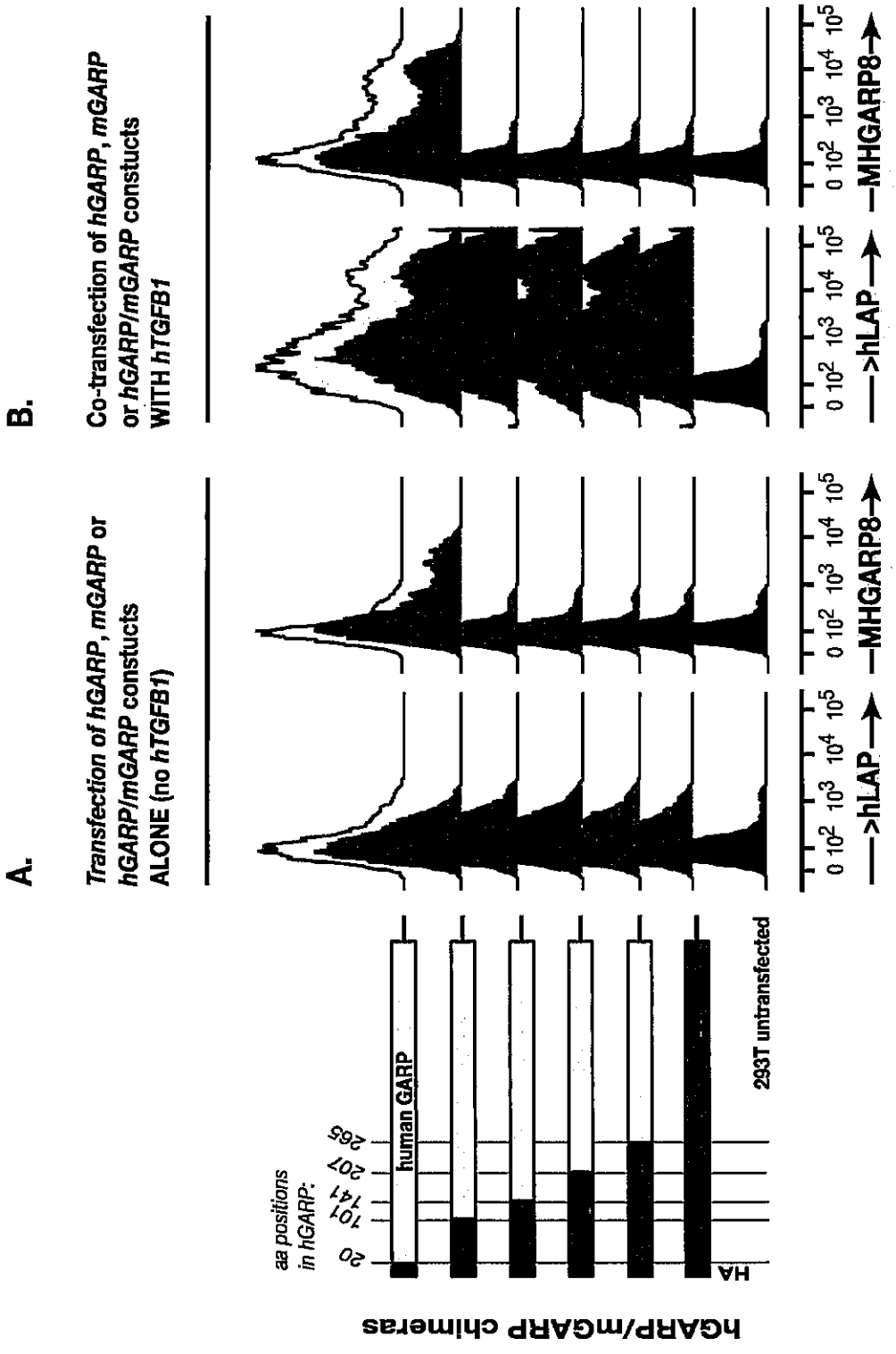

A.
B.
Transfection of hGARP, mGARP or hGARP/mGARP constucts ALONE (no hTGFB1)
Co-transfection of hGARP, mGARP or hGARP/mGARP constucts WITH hTGFB1
hGARP/mGARP chimeras
aa positions in hGARP:
20
101
141
207
265
human GARP
HA
293T untransfected
0 10^2 10^3 10^4 10^5
—>hLAP—>
—MHGARP8—>

FIG. 4

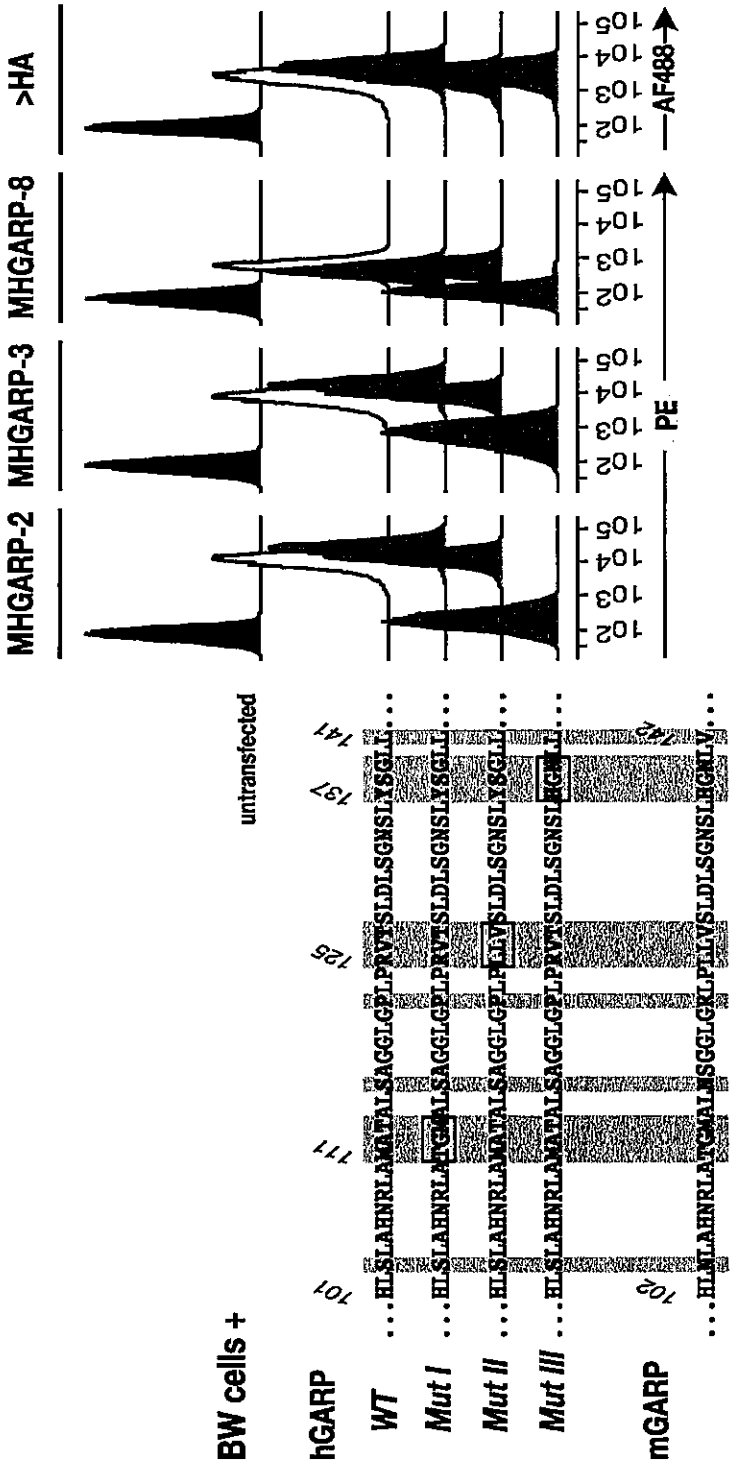

MHGARP-2
MHGARP-3
MHGARP-8
>HA
PE
AF488
10²
10³
10⁴
10⁵
BW cells +
untransfected
hGARP
mGARP
WT
Mut I
Mut II
Mut III
101
117
125
137
141
102
142

FIG. 5

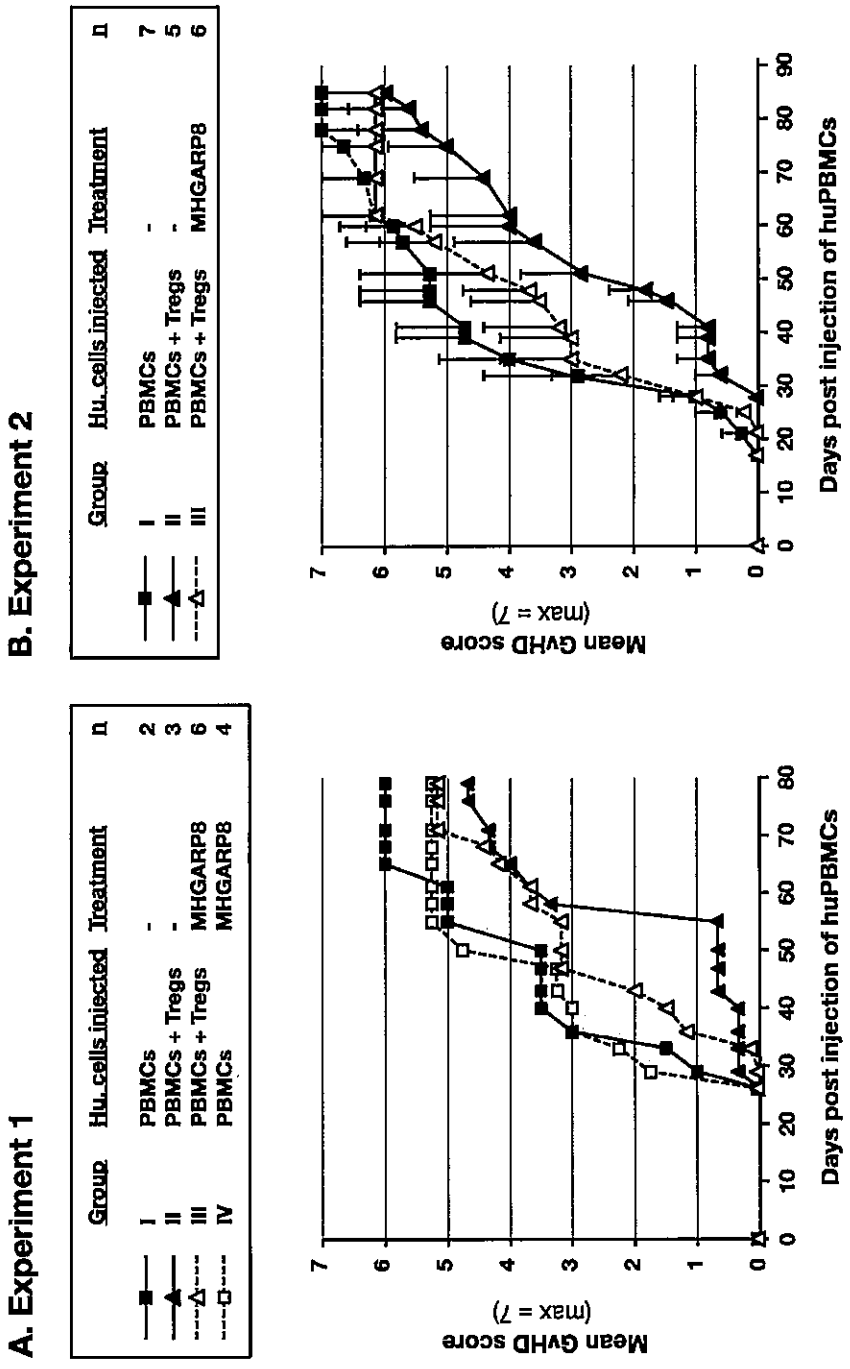

A. Experiment 1
Group Hu. cells injected Treatment n
I PBMCs - 2
II PBMCs + Tregs - 3
III PBMCs + Tregs MHGARP8 6
IV PBMCs MHGARP8 4
Mean GvHD score (max = 7)
Days post injection of huPBMCs
B. Experiment 2
Group Hu. cells injected Treatment n
I PBMCs - 7
II PBMCs + Tregs - 5
III PBMCs + Tregs MHGARP8 6
Mean GvHD score (max = 7)
Days post injection of huPBMCs

FIG. 6

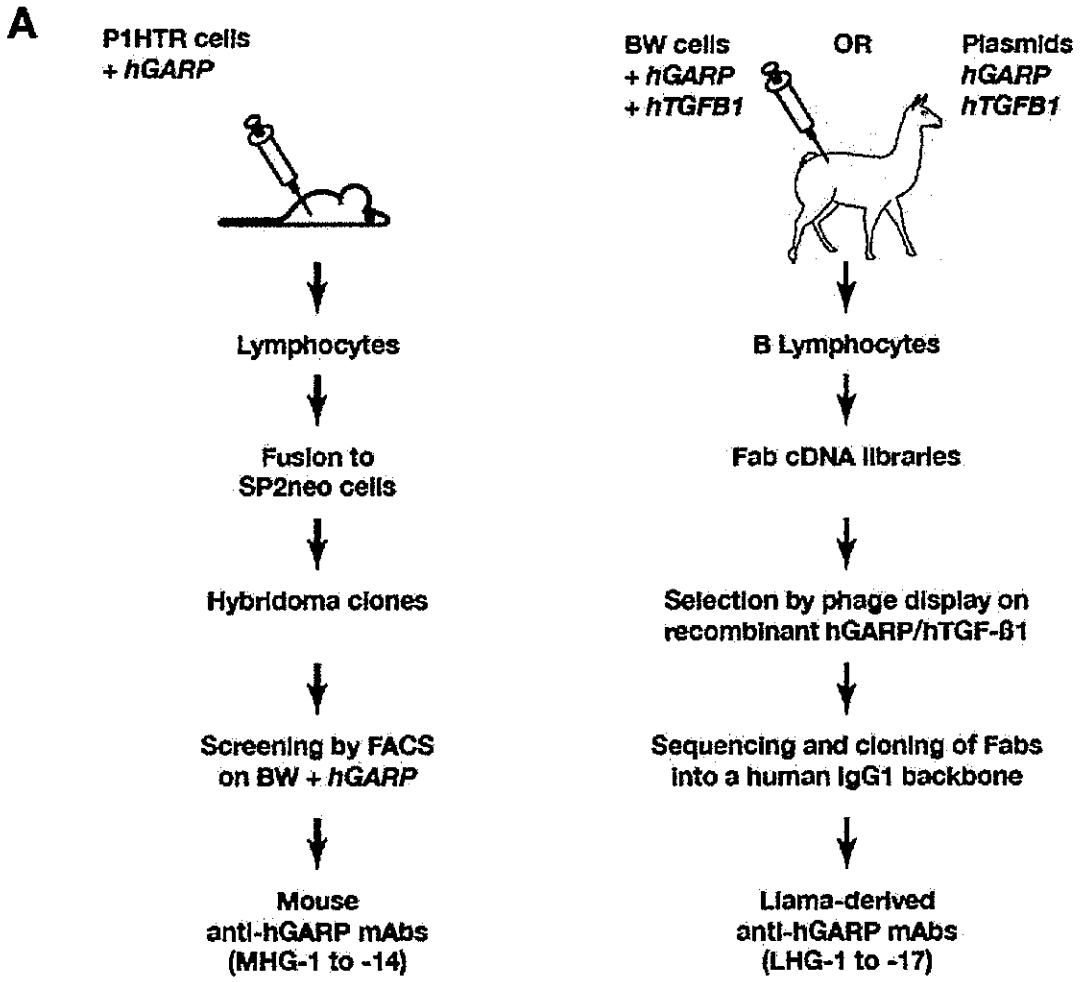

A
P1HTR cells
+ hGARP
BW cells
+ hGARP
+ hTGFB1
OR
Plasmids
hGARP
hTGFB1
Lymphocytes
B Lymphocytes
Fusion to
SP2neo cells
Fab cDNA libraries
Hybridoma clones
Selection by phage display on
recombinant hGARP/hTGF-β1
Screening by FACS
on BW + hGARP
Sequencing and cloning of Fabs
into a human IgG1 backbone
Mouse
anti-hGARP mAbs
(MHG-1 to -14)
Llama-derived
anti-hGARP mAbs
(LHG-1 to -17)

FIG. 7A

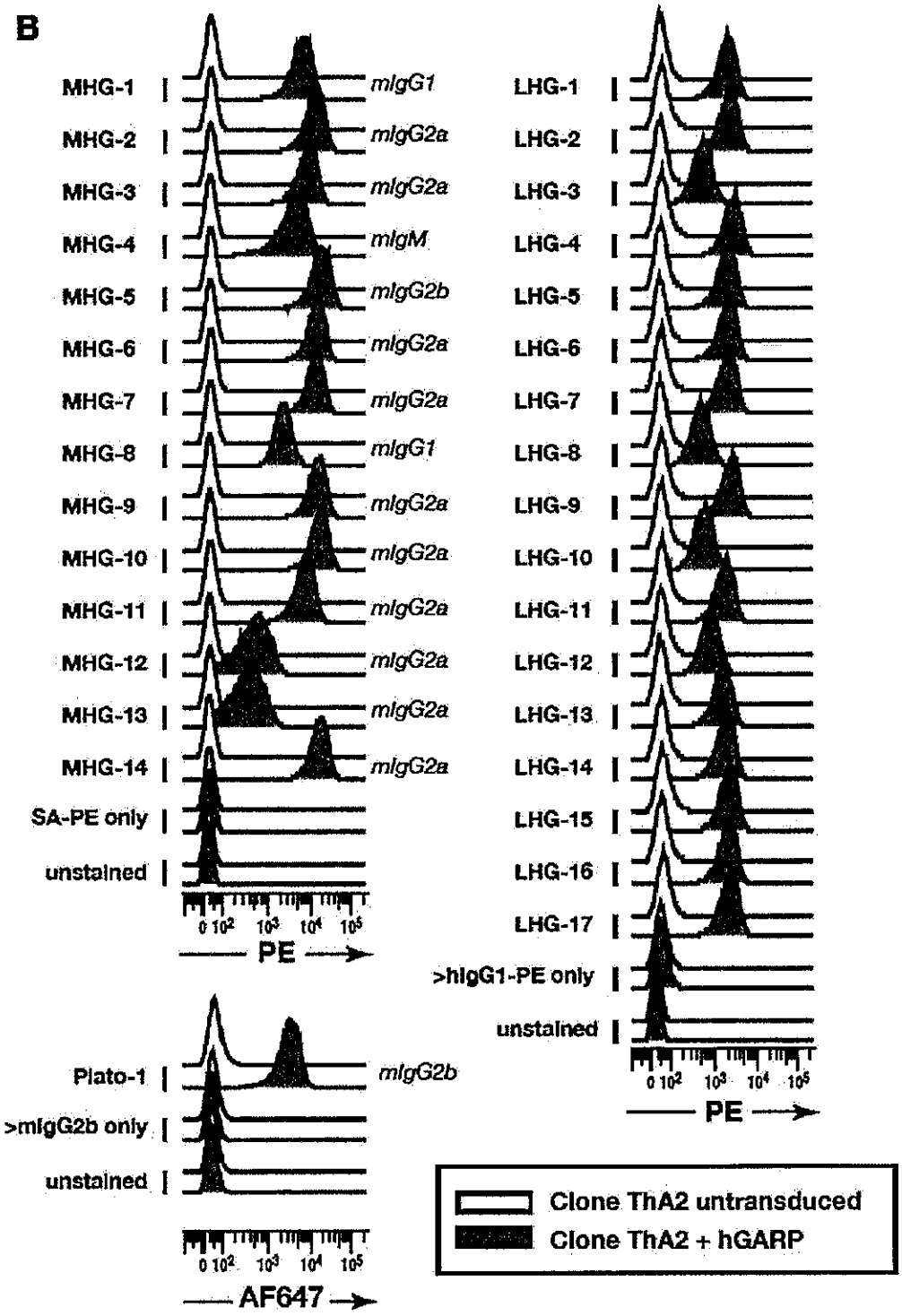
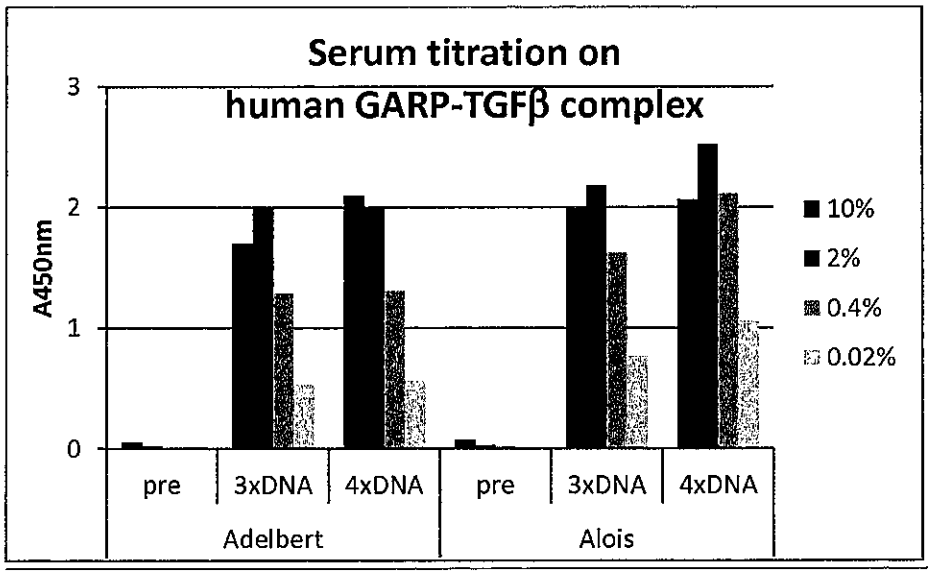

B
MHG-1
MHG-2
MHG-3
MHG-4
MHG-5
MHG-6
MHG-7
MHG-8
MHG-9
MHG-10
MHG-11
MHG-12
MHG-13
MHG-14
SA-PE only
unstained
mIgG1
mIgG2a
mIgG2a
mIgM
mIgG2b
mIgG2a
mIgG2a
mIgG1
mIgG2a
mIgG2a
mIgG2a
mIgG2a
mIgG2a
mIgG2a
0 10² 10³ 10⁴ 10⁵
PE
LHG-1
LHG-2
LHG-3
LHG-4
LHG-5
LHG-6
LHG-7
LHG-8
LHG-9
LHG-10
LHG-11
LHG-12
LHG-13
LHG-14
LHG-15
LHG-16
LHG-17
>hIgG1-PE only
unstained
0 10² 10³ 10⁴ 10⁵
PE
Plato-1
>mIgG2b only
unstained
mIgG2b
0 10² 10³ 10⁴ 10⁵
AF647
Clone ThA2 untransduced
Clone ThA2 + hGARP

FIG. 7B

A


Serum titration on
human GARP-TGFβ complex
A450nm
3
2
1
0
10%
2%
0.4%
0.02%
pre
3xDNA
4xDNA
pre
3xDNA
4xDNA
Adelbert
Alois

B

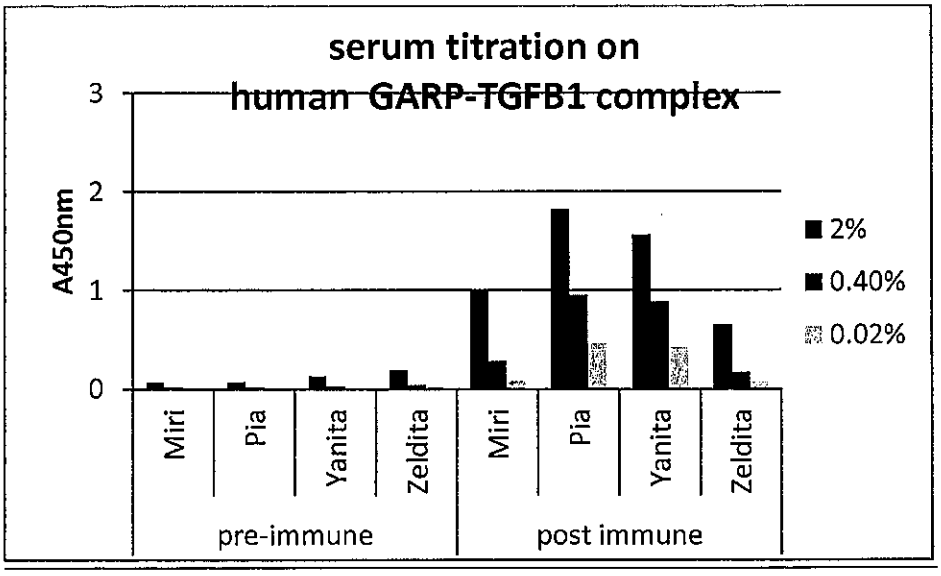

serum titration on
human GARP-TGFB1 complex
A450nm
3
2
1
0
2%
0.40%
0.02%
Miri
Pia
Yanita
Zeldita
Miri
Pia
Yanita
Zeldita
pre-immune
post immune

FIG. 8

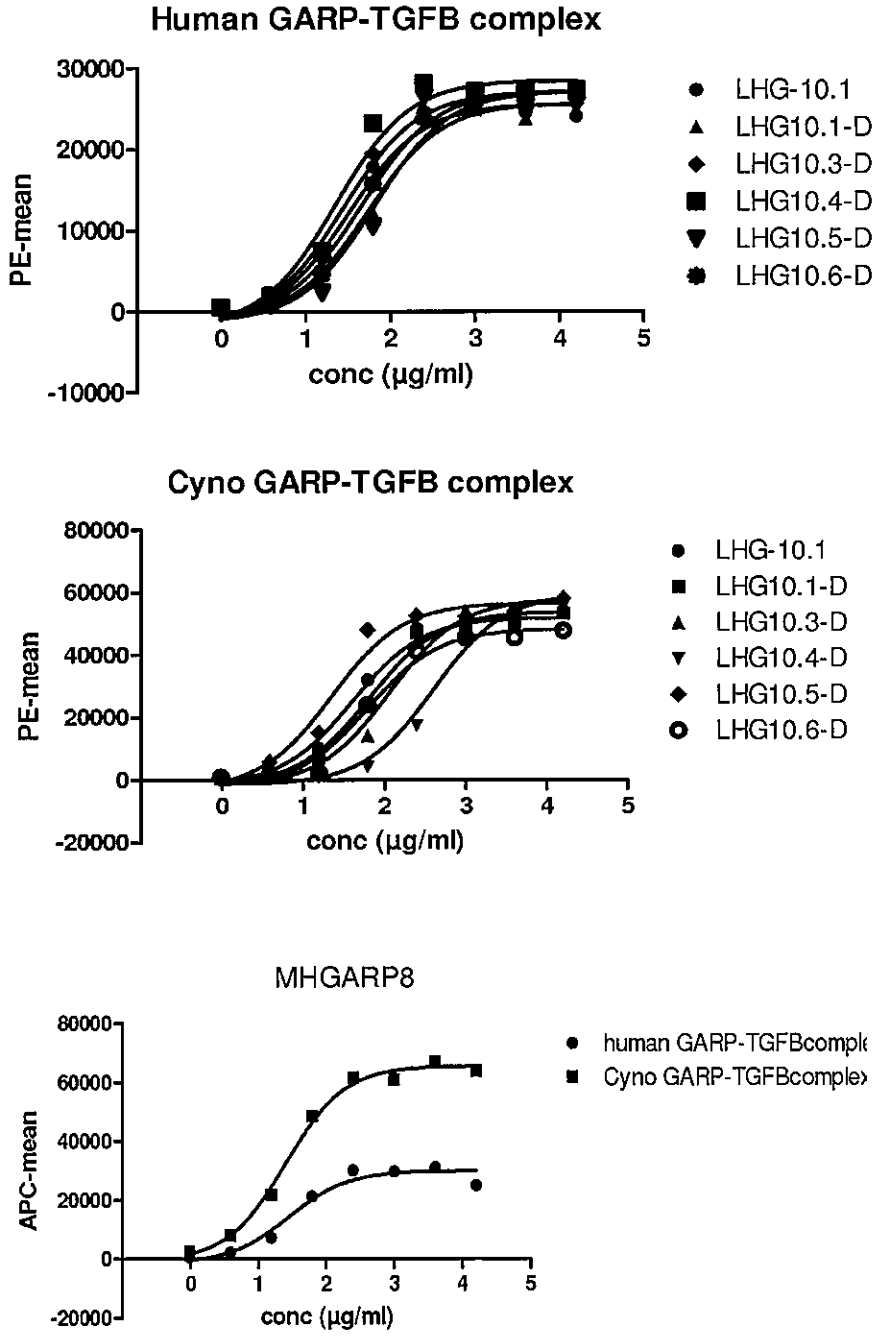

Human GARP-TGFB complex
PE-mean
30000
20000
10000
0
-10000
0
1
2
3
4
5
conc (µg/ml)
LHG-10.1
LHG10.1-D
LHG10.3-D
LHG10.4-D
LHG10.5-D
LHG10.6-D
Cyno GARP-TGFB complex
PE-mean
80000
60000
40000
20000
0
-20000
0
1
2
3
4
5
conc (µg/ml)
LHG-10.1
LHG10.1-D
LHG10.3-D
LHG10.4-D
LHG10.5-D
LHG10.6-D
MHGARP8
APC-mean
80000
60000
40000
20000
0
-20000
0
1
2
3
4
5
conc (µg/ml)
human GARP-TGFBcomple
Cyno GARP-TGFBcomplex

FIG. 9

LHG10 VH sequences

```
                                CDR1                    CDR2                                                CDR3
LHG-10  EVQLVQPGAELRNSGASVKVSCKASGYRFT SYYID WVRQAPGQGLEWMG RIDPEDGGTKYAQKFQG RVTFTADTSTDTAYVELSSLRSEDTAVYYCAR NEWETVVVGDLMYEYEY WGQGTQVTVSS
```

LHG10 VL sequences

```
                                CDR1                    CDR2                                                CDR3
LHG10   DIQMTQSPTSLSASLGDRVTITC QASQSISSYLA WYQQKPGQAPKLLIY GASRLQT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYDSLPVT FGQGTKVELK
```

VK sequences of affinity optimized shuffle variants of LHG-10

```
                                CDR1                    CDR2                                                CDR3
LHG10     DIQMTQSPTSLSASLGDRVTITC QASQSISSYLA WYQQKPGQAPKLLIY GASRLQT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYDSLPVT FGQGTKVELK
LHG10.4   DIQMTQSPSSLSASLGDRVTITC QASQSISSYLA WYQQKPGQAPKLLIY GTSRLKT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYYSAPVT FGQGTKVELK
LHG10.6   DIQMTQSPSSLSASLGDRVTITC QASQSISSYLA WYQQKPGQAPNILIY GASRLKT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYASVPVT FGQGTKVELK
LHG10.3   DIQMTQSPSSLSASLGDRVTITC QASQSIVSYLA WYQQKPGQAPKLLIY GASRLQT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYASAPVT FGQGTGVELK
LHG10.5   DIQMTQSPSSLSPSLGDRVTITC QASQTISSFLA WYHQKPGQPPKLLIY RASIPQT GVPSRFSGSGSGTSFTLTIGGLEAEDAGTYYC QQYVSAPPT FGQGTKVELK

LHG10     DIQMTQSPTSLSASLGDRVTITC QASQSISSYLA WYQQKPGQAPKLLIY GASRLQT GVPSRFSGSGSGTSFTLTISGLEAEDAGTYYC QQYDSLPVT FGQGTKVELK
LHG10.4   .........S.............. ........... .............. ..T...K. .....................................Y.A..... ..........
LHG10.6   .........S.............. ........... .............. .....K. .....................................A.V..... ..........
LHG10.3   .........S.....V..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ...A.A..... .....G....
LHG10.5   .........S...P.......... ....T...F.. ..H.....P..... R..IP.. ..... ..... ..... ..... ..... G..... ...V.A.P. ..........
```

Table X: CDR sequences of LHG-10 (VH and VK) as well as those of the affinity optimized VK variants

mAb	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3	SEQ ID NO
LHG-10 VH	SYYID		RIDPEDGGTKYAQKFQG		NEWETVVVGDLMYEYEY	
LHG10 VK	QASQSISSYLA		GASRLQT		QQYDSLPVT	
LHG10.3 VK	QASQSIVSYLA		GASRLQT		QQYASAPVT	
LHG10.4 VK	QASQSISSYLA		GTSRLKT		QQYYSAPVT	
LHG10.5 VK	QASQTISSFLA		RASIPQT		QQYVSAPPT	
LHG10.6 VK	QASQSISSYLA		GASRLKT		QQYASVPVT	

FIG. 10

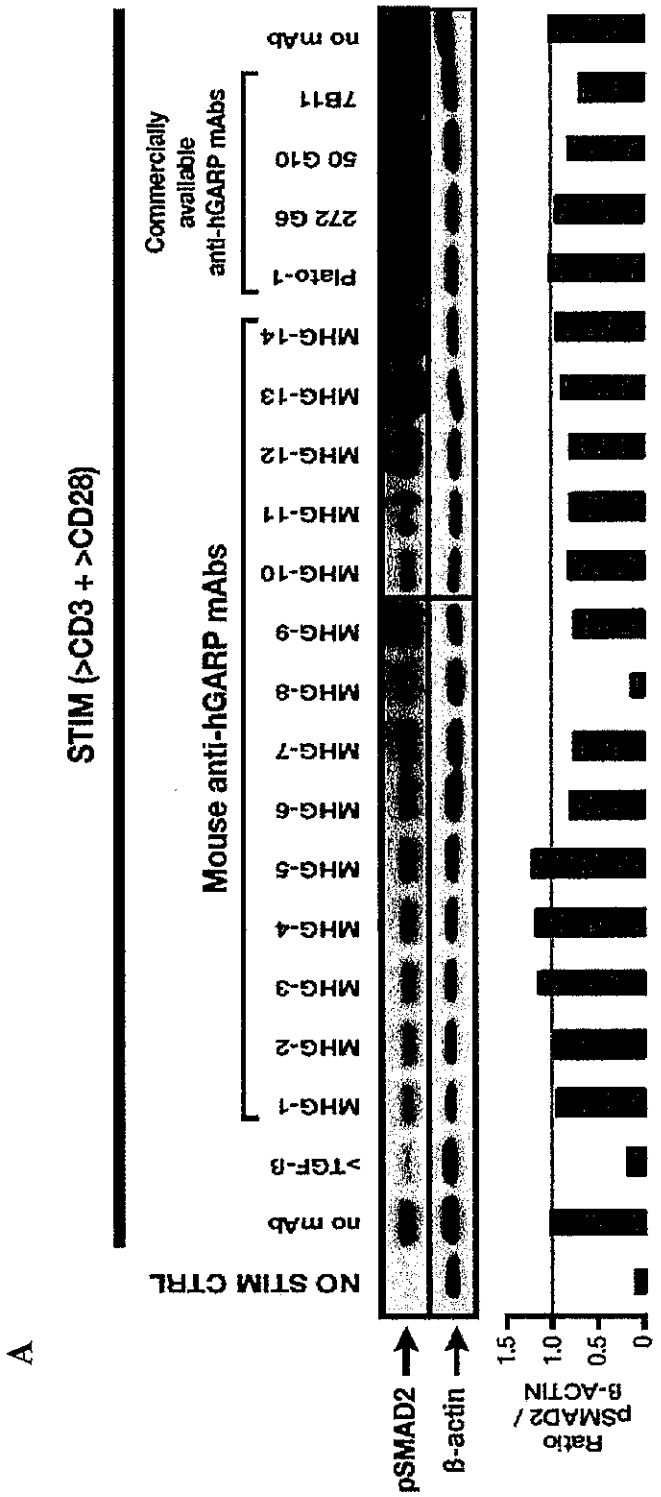

A
NO STIM CTRL
STIM (>CD3 + >CD28)
no mAb
>TGF-ß
MHG-1
MHG-2
MHG-3
MHG-4
MHG-5
MHG-6
MHG-7
MHG-8
MHG-9
MHG-10
MHG-11
MHG-12
MHG-13
MHG-14
Mouse anti-hGARP mAbs
Plato-1
272 G6
50 G10
7B11
Commercially available anti-hGARP mAbs
no mAb
pSMAD2
ß-actin
Ratio pSMAD2 / ß-ACTIN
1.5
1.0
0.5
0

FIG. 11A

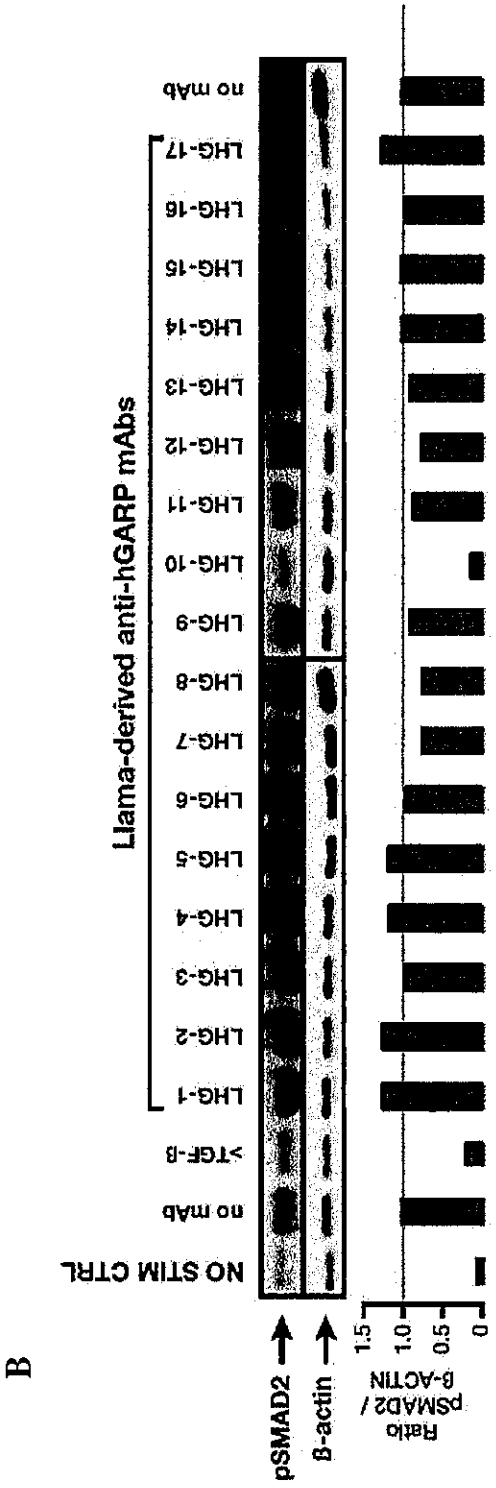

B
pSMAD2
β-actin
Ratio pSMAD2 / β-ACTIN
1.5
1.0
0.5
0
NO STIM CTRL
no mAb
>TGF-β
LHG-1
LHG-2
LHG-3
LHG-4
LHG-5
LHG-6
LHG-7
LHG-8
LHG-9
LHG-10
LHG-11
LHG-12
LHG-13
LHG-14
LHG-15
LHG-16
LHG-17
no mAb
Llama-derived anti-hGARP mAbs

FIG. 11B

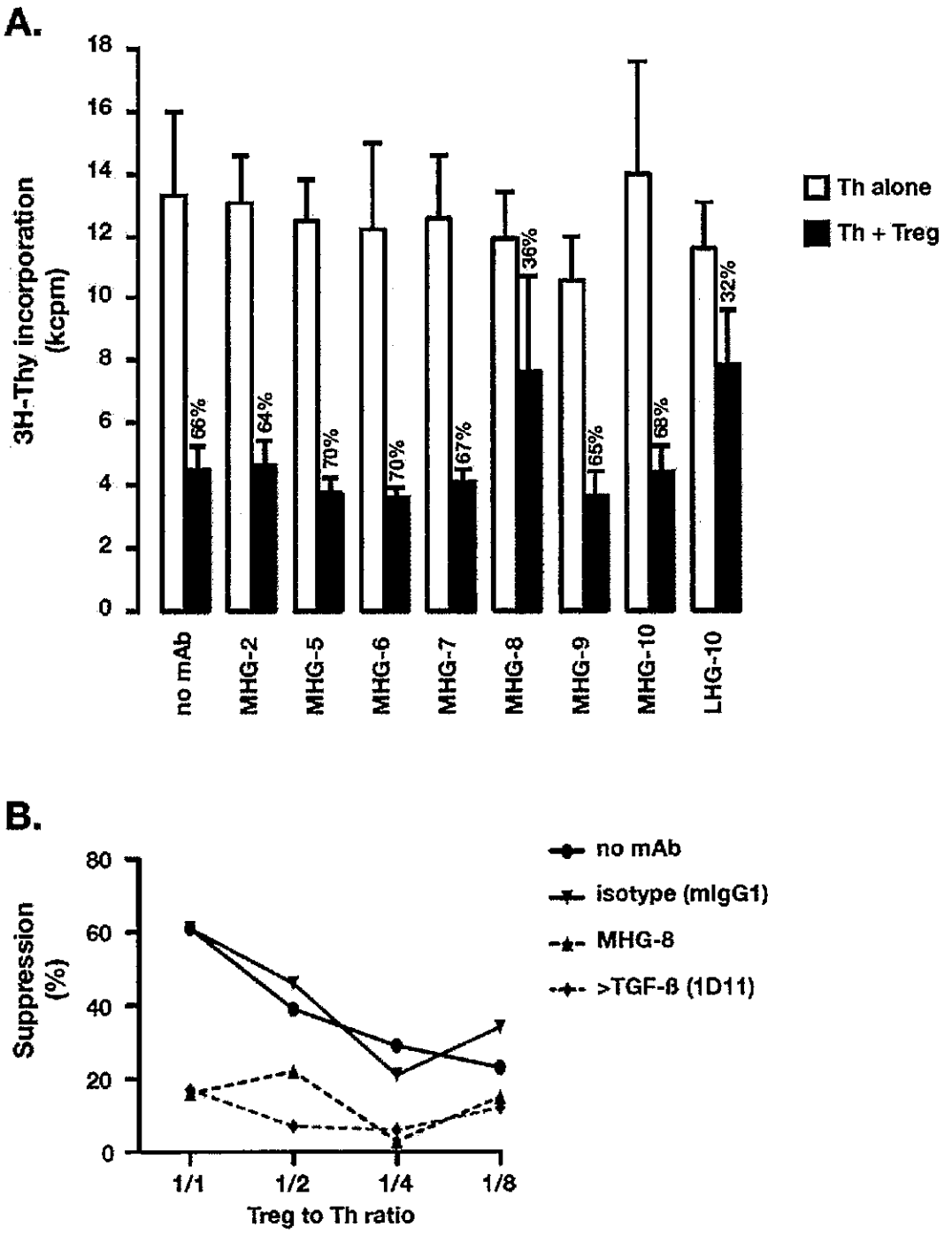
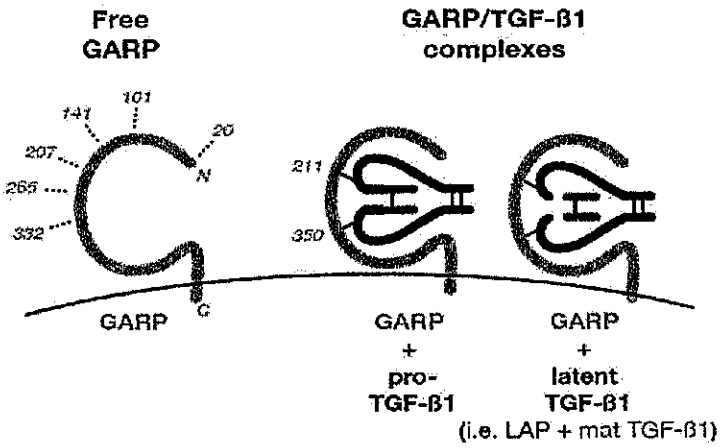

A.
3H-Thy incorporation (kcpm)
18
16
14
12
10
8
6
4
2
0
66%
64%
70%
70%
67%
36%
65%
68%
32%
Th alone
Th + Treg
no mAb
MHG-2
MHG-5
MHG-6
MHG-7
MHG-8
MHG-9
MHG-10
LHG-10
B.
Suppression (%)
80
60
40
20
0
1/1
1/2
1/4
1/8
Treg to Th ratio
no mAb
isotype (mIgG1)
MHG-8
>TGF-ß (1D11)

FIG. 12

A. Forms of hGARP



B. Anti-hGARP mAbs

Form of hGARP bound

hGARP aa required for binding	Free	Free + Complexes	Complexes	
20-101		MHG-6 MHG-7 MHG-9 LHG-5 LHG-11		
101-141		LHG-7 LHG-9	LHG-3	111-113
				125-127
	MHG-2 MHG-3	LHG-17	**MHG-8** **LHG-10**	137-139
		LHG 1 LHG-2 LHG-4 LHG-15	LHG-8 LHG-12 LHG-13	other
141-207	MHG-1 MHG-5	LHG-6 LHG-16 MHG-10 MHG-14		
207-265				
265-332	MHG-4	Plato-1		
332-628	MHG-11 MHG-12 MHG-13	LHG-14		

FIG. 13

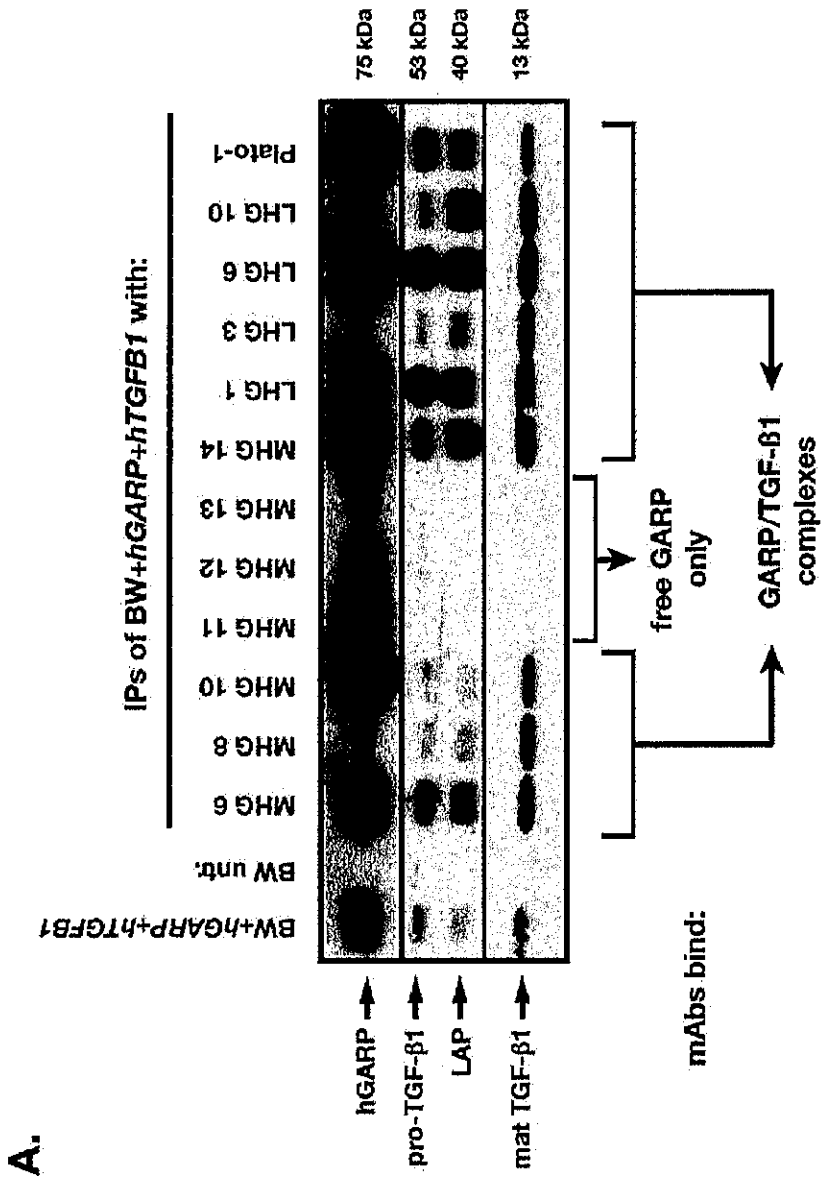

A.
IPs of BW+hGARP+hTGFB1 with:
BW+hGARP+hTGFB1
BW unt.
MHG 6
MHG 8
MHG 10
MHG 11
MHG 12
MHG 13
MHG 14
LHG 1
LHG 3
LHG 6
LHG 10
Plato-1
75 kDa
53 kDa
40 kDa
13 kDa
hGARP
pro-TGF-β1
LAP
mat TGF-β1
mAbs bind:
free GARP only
GARP/TGF-β1 complexes

FIG. 14A

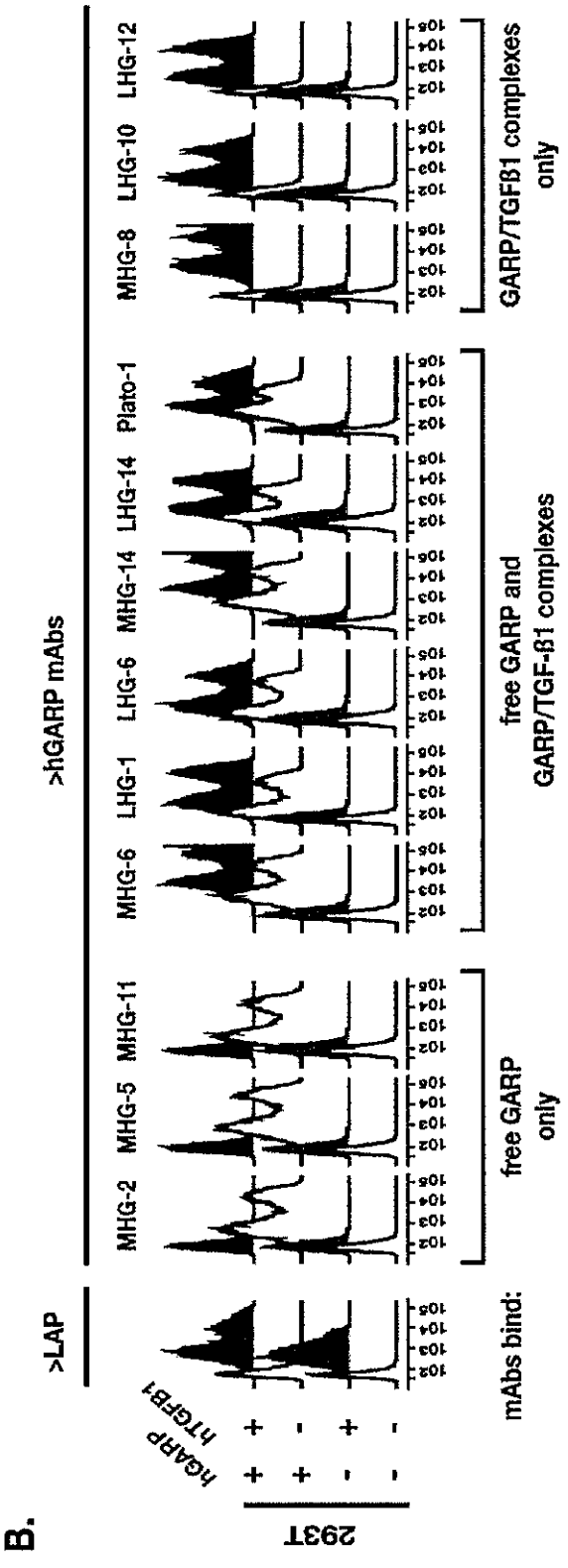

B.
293T
hGARP
hTGFB1
+ + - -
+ + - -
>LAP
>hGARP mAbs
MHG-2
MHG-5
MHG-11
MHG-6
LHG-1
LHG-6
MHG-14
LHG-14
Plato-1
MHG-8
LHG-10
LHG-12
mAbs bind:
free GARP only
free GARP and GARP/TGF-ß1 complexes
GARP/TGFß1 complexes only

FIG. 14B

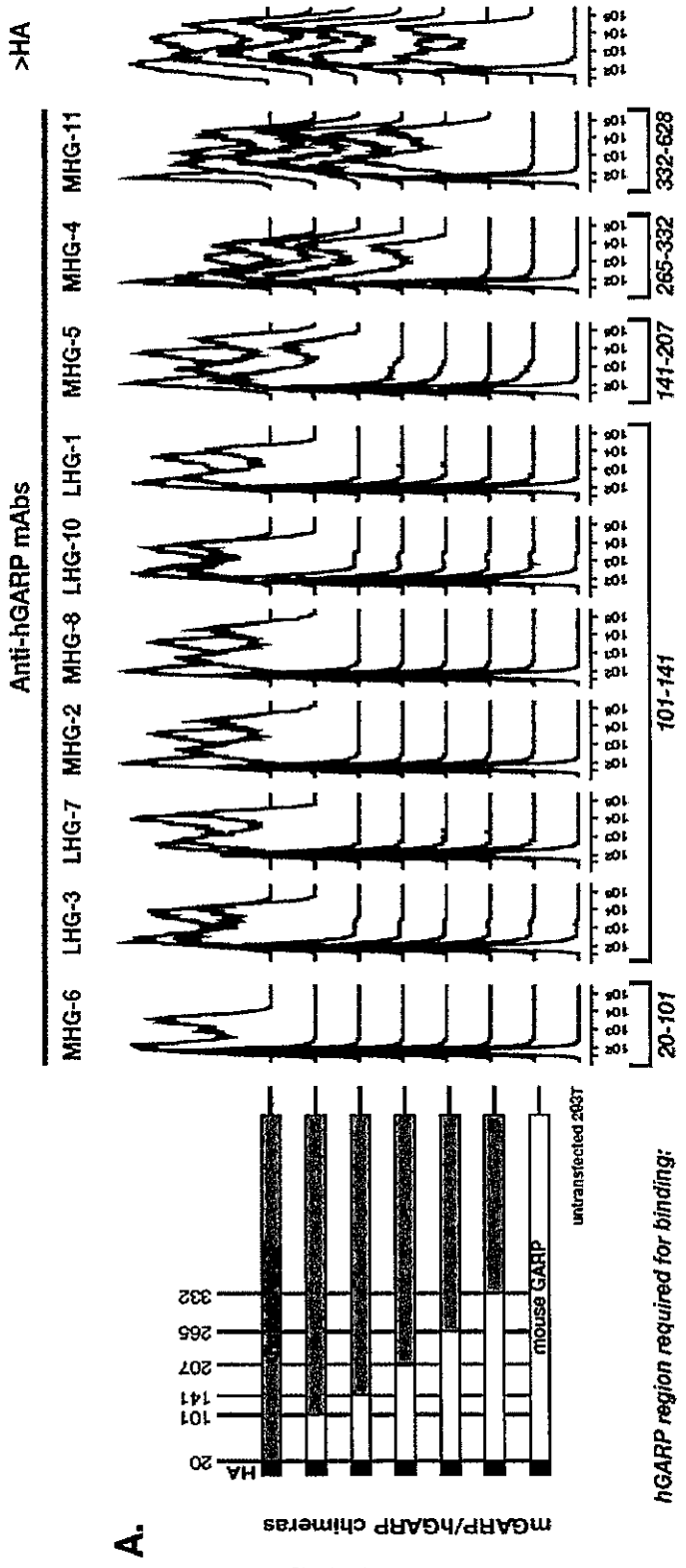

A.
mGARP/hGARP chimeras
HA
20
101
141
207
265
332
mouse GARP
untransfected 293T
hGARP region required for binding:
20-101
101-141
141-207
265-332
332-628
Anti-hGARP mAbs
MHG-6
LHG-3
LHG-7
MHG-2
MHG-8
LHG-10
LHG-1
MHG-5
MHG-4
MHG-11
>HA

FIG. 15A

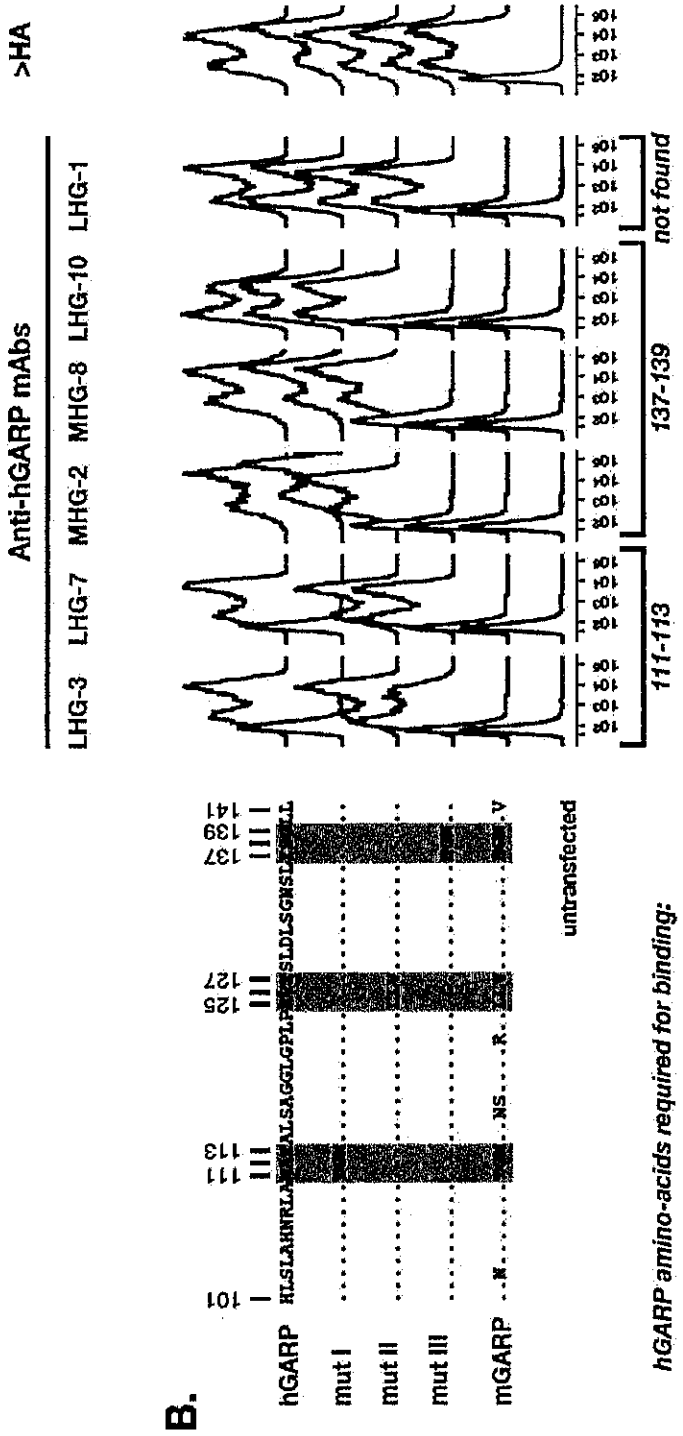
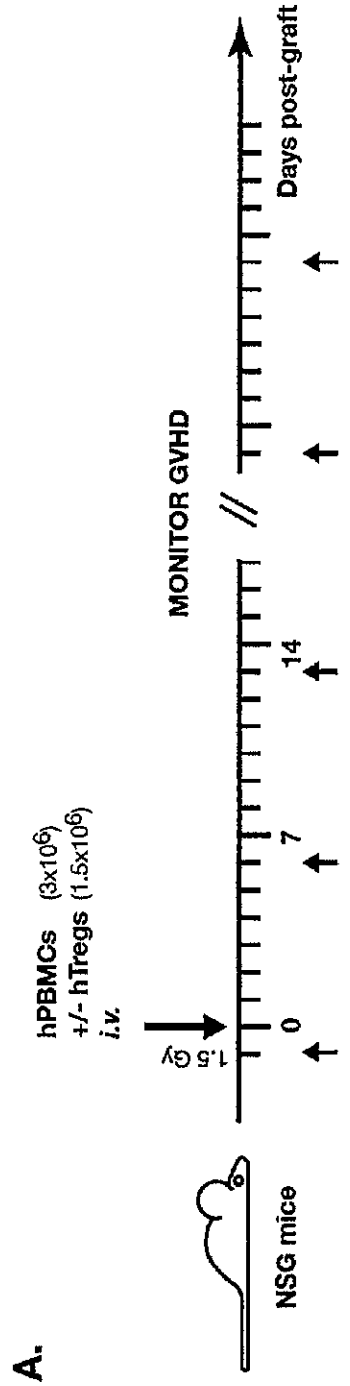

Anti-hGARP mAbs
LHG-3 LHG-7 MHG-2 MHG-8 LHG-10 LHG-1
>HA
B.
hGARP
mut I
mut II
mut III
mGARP
untransfected
111-113
137-139
not found
hGARP amino-acids required for binding:

FIG. 15B



B.

Expt	Donor	Day of disease onset mean score ≥1					
		PBMCs		PBMCs + Tregs		PBMCs + Tregs + MHG-8	
		a		b		c	
I	A	29	(n=2)	58	(n=3)	36	(n=6)
II	B	28	(n=7)	46	(n=5)	28	(n=6)
III	B	41	(n=8)	57	(n=5)	41	(n=6)
IV	C	37	(n=8)	72	(n=7)	44	(n=8)

FIG. 16A-B

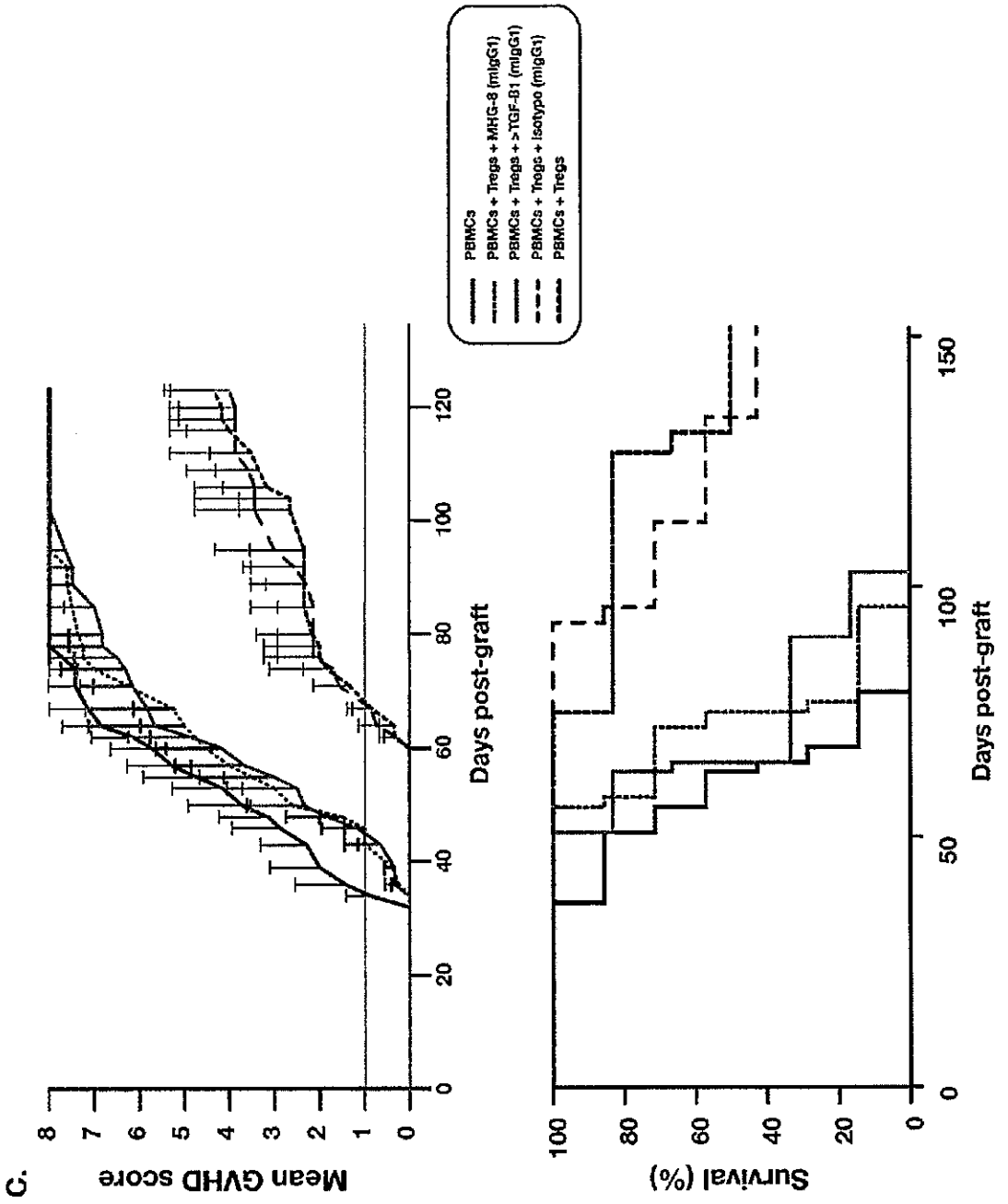

C.
Mean GVHD score
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
Days post-graft
PBMCs
PBMCs + Tregs + MHG-8 (mIgG1)
PBMCs + Tregs + >TGF-B1 (mIgG1)
PBMCs + Tregs + isotypo (mIgG1)
PBMCs + Tregs
Survival (%)
0
20
40
60
80
100
0
50
100
150
Days post-graft

FIG. 16C