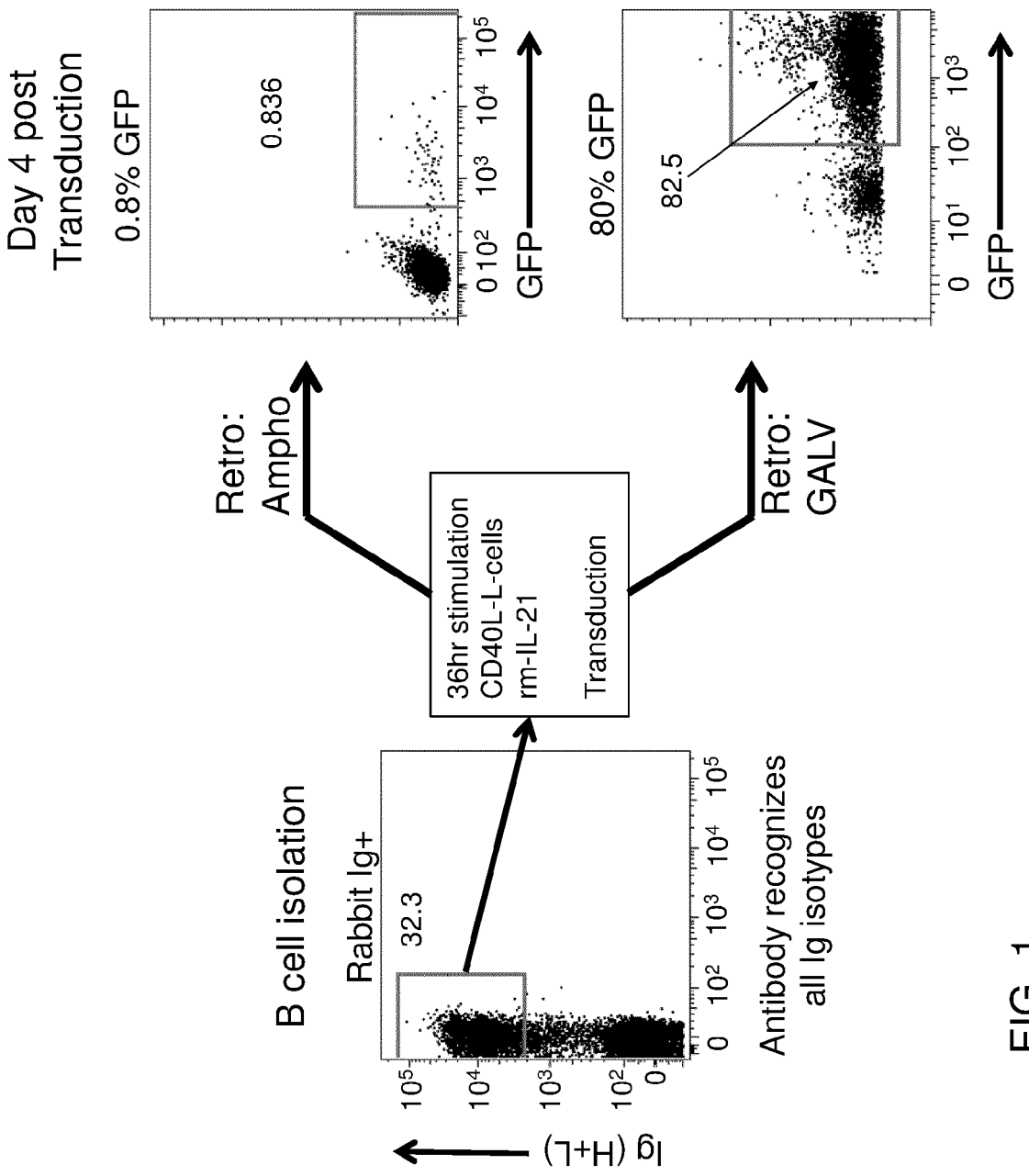

B cell isolation
Rabbit Ig+
32.3
Ig (H+L)
Antibody recognizes
all Ig isotypes
36hr stimulation
CD40L-L-cells
rm-IL-21
Transduction
Retro:
Ampho
Retro:
GALV
Day 4 post
Transduction
0.8% GFP
0.836
GFP
80% GFP
82.5
GFP

FIG. 1

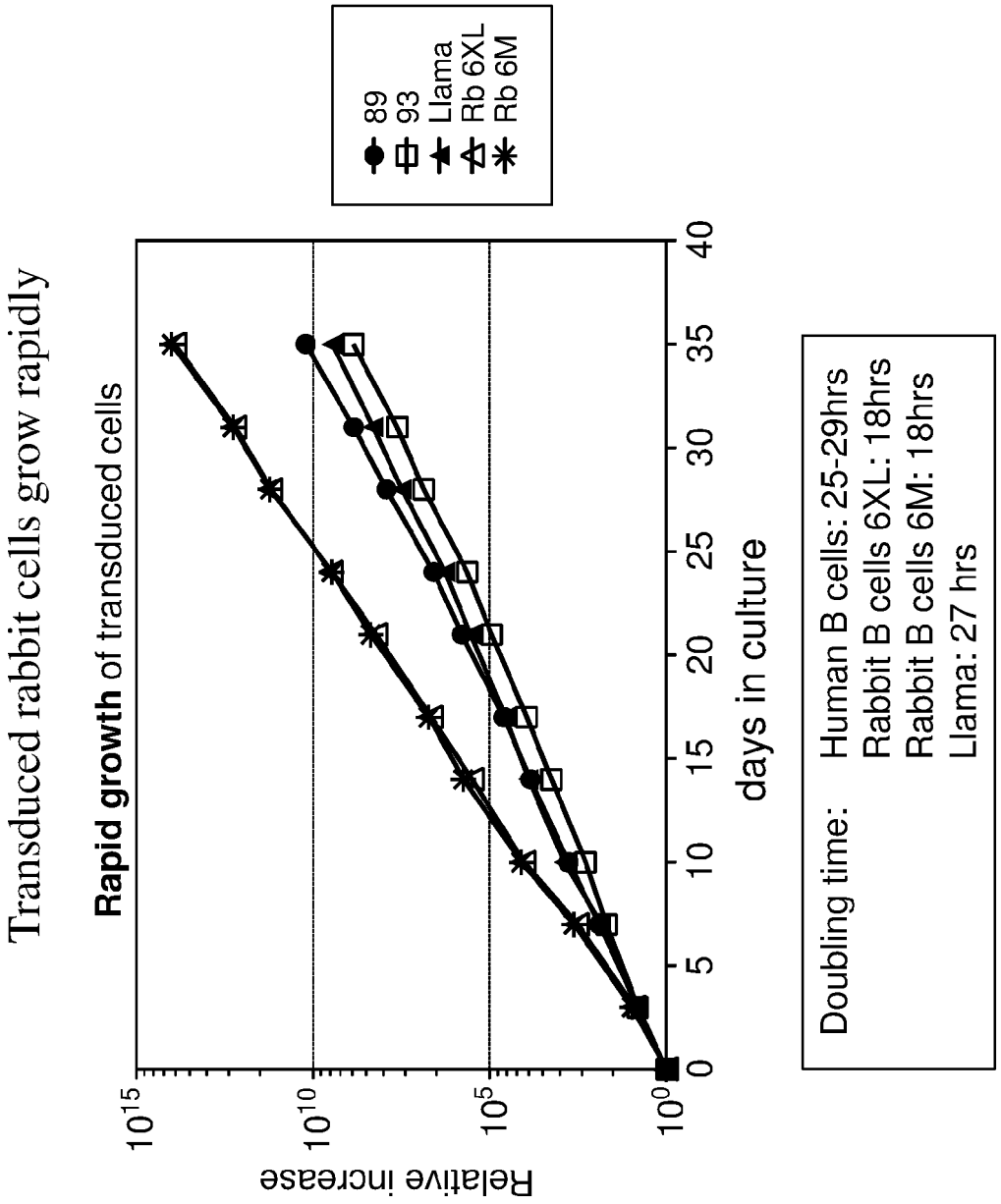

Transduced rabbit cells grow rapidly
Rapid growth of transduced cells
89
93
Llama
Rb 6XL
Rb 6M
Relative increase
10^{15}
10^{10}
10^{5}
10^{0}
0
5
10
15
20
25
30
35
40
days in culture
Doubling time: Human B cells: 25-29hrs
Rabbit B cells 6XL: 18hrs
Rabbit B cells 6M: 18hrs
Llama: 27 hrs

FIG. 2

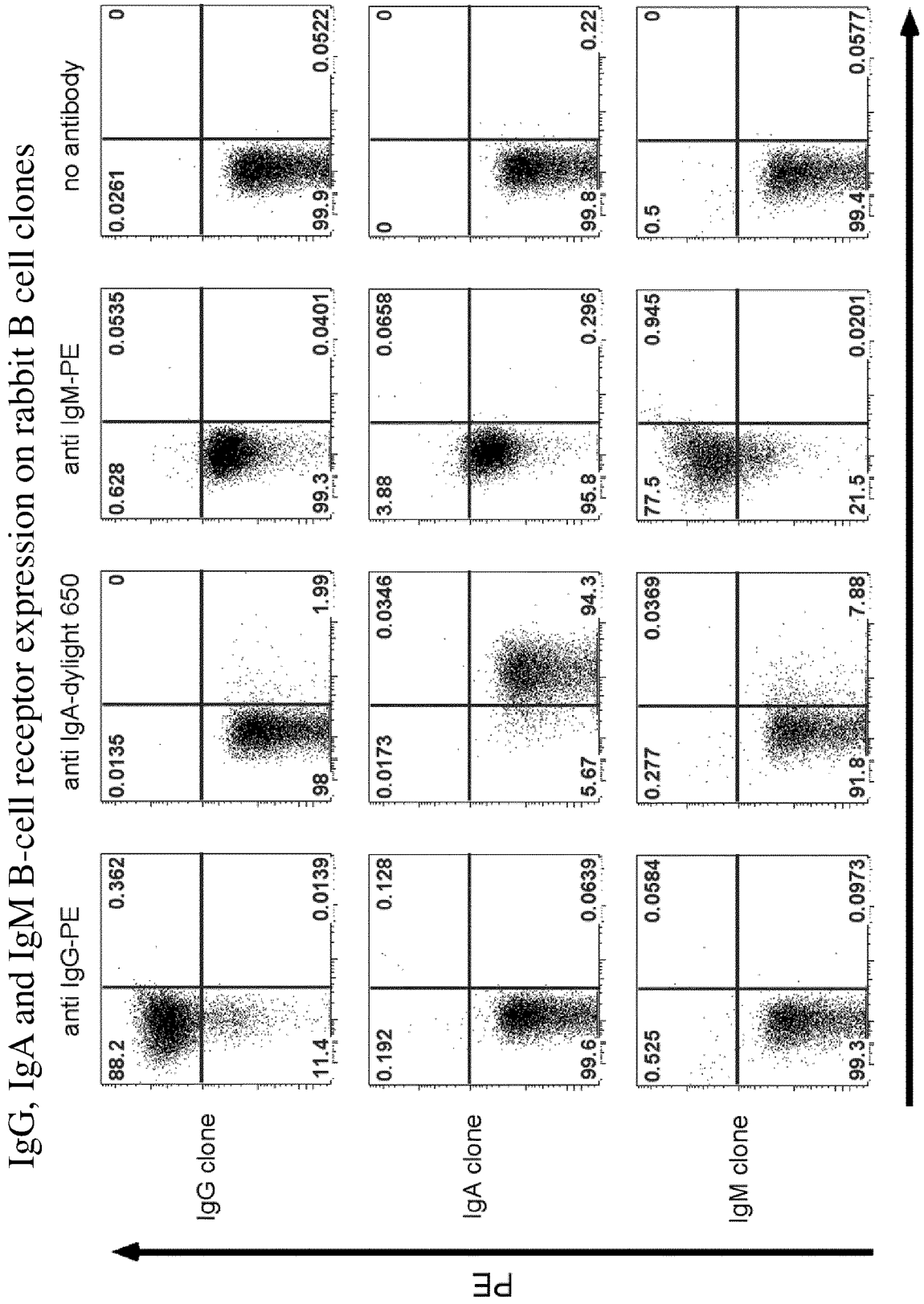

IgG, IgA and IgM B-cell receptor expression on rabbit B cell clones
anti IgG-PE
anti IgA-dylight 650
anti IgM-PE
no antibody
IgG clone
IgA clone
IgM clone
PE
dylight 650
88.2
0.362
11.4
0.0139
0.0135
0
98
1.99
0.628
0.0535
99.3
0.0401
0.0261
0
99.9
0.0522
0.192
0.128
99.6
0.0639
0.0173
0.0346
5.67
94.3
0
0
3.88
0.0658
95.8
0.296
0
0
99.8
0.22
0.525
0.0584
99.3
0.0973
0.277
0.0369
91.8
7.88
77.5
0.945
21.5
0.0201
0.5
0
99.4
0.0577

FIG. 3

Identification of antigen-specific rabbit B-cells within a pool of rabbit B cells with different specificities.

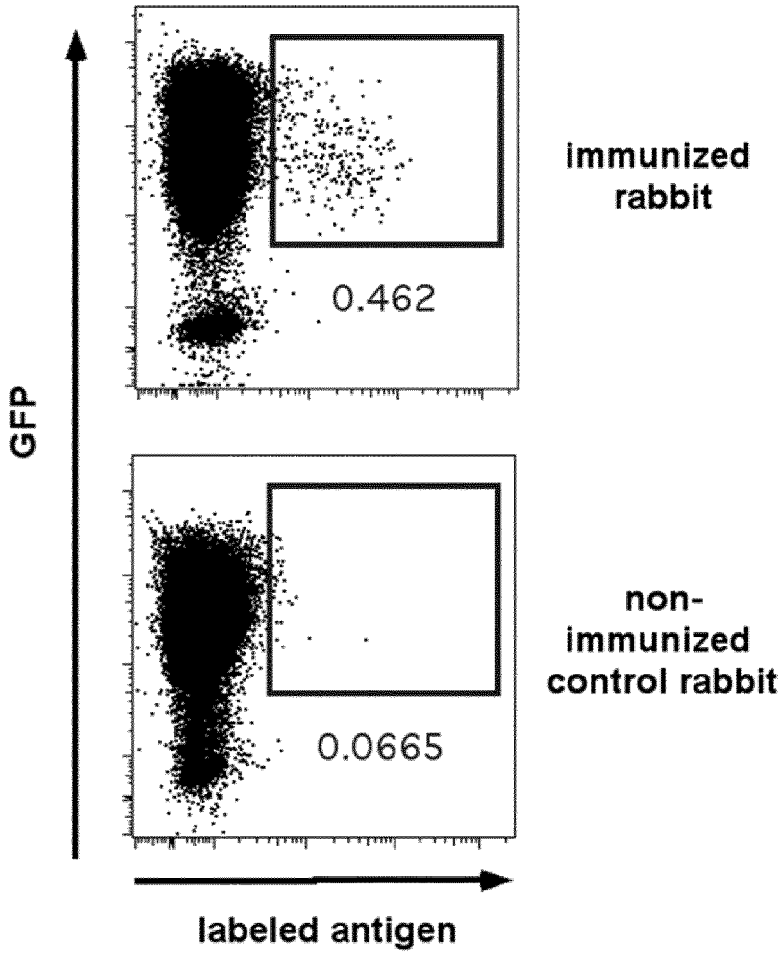


FIG. 4

Supernatant of rabbit B cells cultured at different cell densities was tested for specificity against influenza vaccine antigens

No. of cells/well	Total cell no.	Rabbit 1 Positive wells (frequency %)	Rabbit 2 Positive wells (frequency %)
100	38400	95 (0.25)	326 (0.85)
25	19200	39 (0.20)	>80*
10	11520	25 (0.22)	114 (0.99)
1	2304/1920	5 (0.22)	22 (1.15)

FIG. 5

Supernatant of rabbit B cells antigen-sorted and cultured one cell per well was tested for specificity of different compounds of the influenza vaccine; antigen sorting greatly facilitates isolation of antigen-specific cells

Rabbit	Antigen used for sorting	Nr wells analyzed	H1-specific	H3-specific	B-specific	Frequency of clones antigen-specific (%)
1	H1	54	30			56
1	H3	74		17		23
1	B	75			8	11
2	H1	78	53			68
2	H3	93		60		65
2	B	60			16	27

FIG. 6

Immunoglobulin concentrations in Rabbit B-cell cultures more rapidly reach higher levels compared to human B-cell cultures

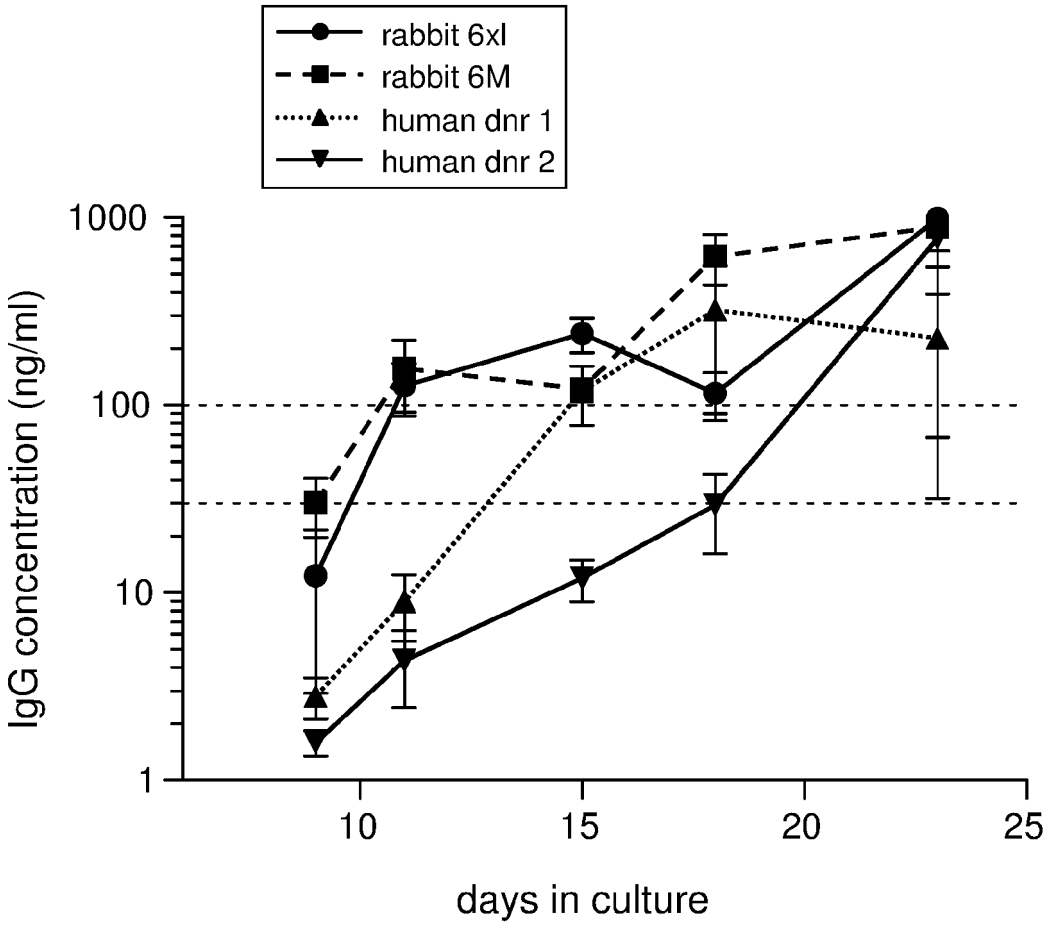


FIG. 7

Retroviral multicistronic construct

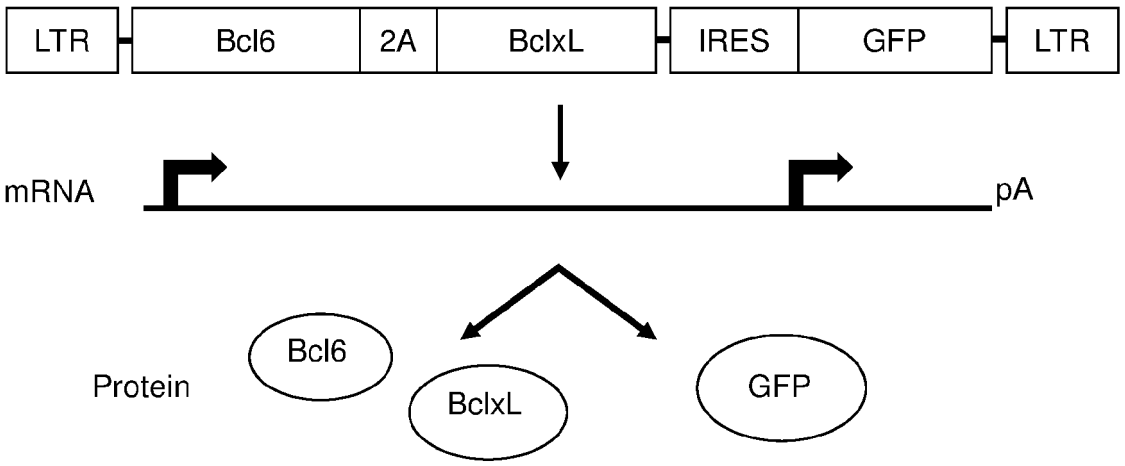


FIG. 8

MVLLPGSMLLTSNLHHLRHQMSPGSWKRLIILLSCVFGGGGTSLQNKNPHQPMTLTWQVLSQTGDVV
WDTKAVQPPWTWWPTLKPDVCALAASLESWDIPGTDVSSSKRVRPPDSDYTAAYKQITWGAIGCSYPR
ARTRMASSTFYVCPRDGRTLSEARRCGGLESLYCKEWDCETTGTGYWLSKSSKDLITVKWDQNSEWTQ
KFQQCHQTGWCNPLKIDFTDKGKLSKDWITGKTWGLRFYVSGHPGVQFTIRLKITNMPAVAVGPDLVL
VEQGPPRTSLALPPPLPPREAPPPSLPDSNSTALATSAQTPTVRKTIVTLNTPPPTTGDRLFDLVQGAFL
TLNATNPGATESCWLCLAMGPPYYEAIASSGEVAYSTDLDRCRWGTQGKLTLTEVSGHGLCIGKVPFTH
QHLCNQTLSINSSGDHQYLLPSNHSWWACSTGLTPCLSTSVFNQTRDFCIQVQLIPRIYYYPEEVLLQAY
DNSHPRTKREAVSLTLAVLLGLGITAGIGTGSTALIKGPIDLQQGLTSLQIAIDADLRALQDSVSKLEDSL
TSLSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYIDHSGAVRDSMKKLKEKLDKRQLERQKSQNWYE
GWFNNSPWFTTLLSTIAGPLLLLLLLILGPCIINRLVQFVKDRISVVQALVLTQQYHQLKPIEYEP

FIG. 9

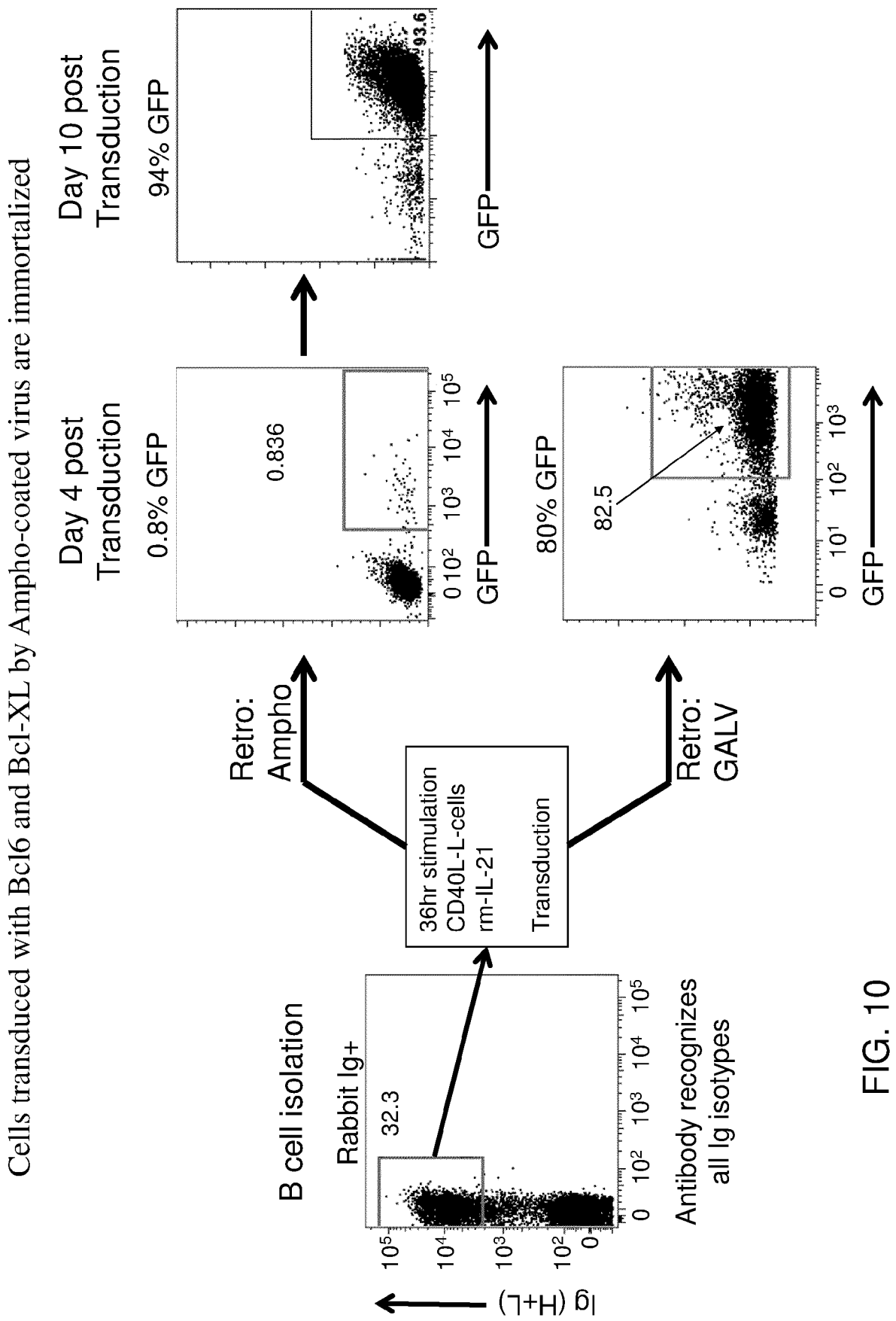
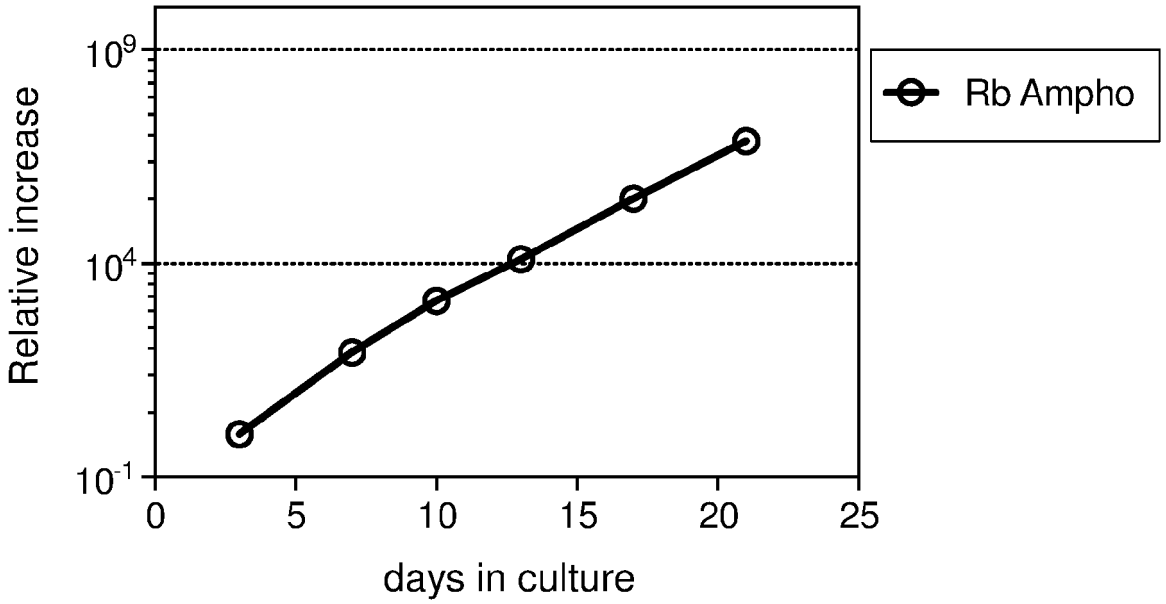

Cells transduced with Bcl6 and Bcl-XL by Ampho-coated virus are immortalized
B cell isolation
Rabbit Ig+
32.3
Ig (H+L)
Antibody recognizes all Ig isotypes
36hr stimulation
CD40L-L-cells
rm-IL-21
Transduction
Retro: Ampho
Retro: GALV
Day 4 post Transduction
0.8% GFP
0.836
80% GFP
82.5
Day 10 post Transduction
94% GFP
93.6
GFP

FIG. 10

Rabbit cells transduced with ampho-coated virus grow similarly fast as cells transduced by GALV-coated virus



Doubling time: Rabbit B cells Ampho 6XL: 19hrs

FIG. 11