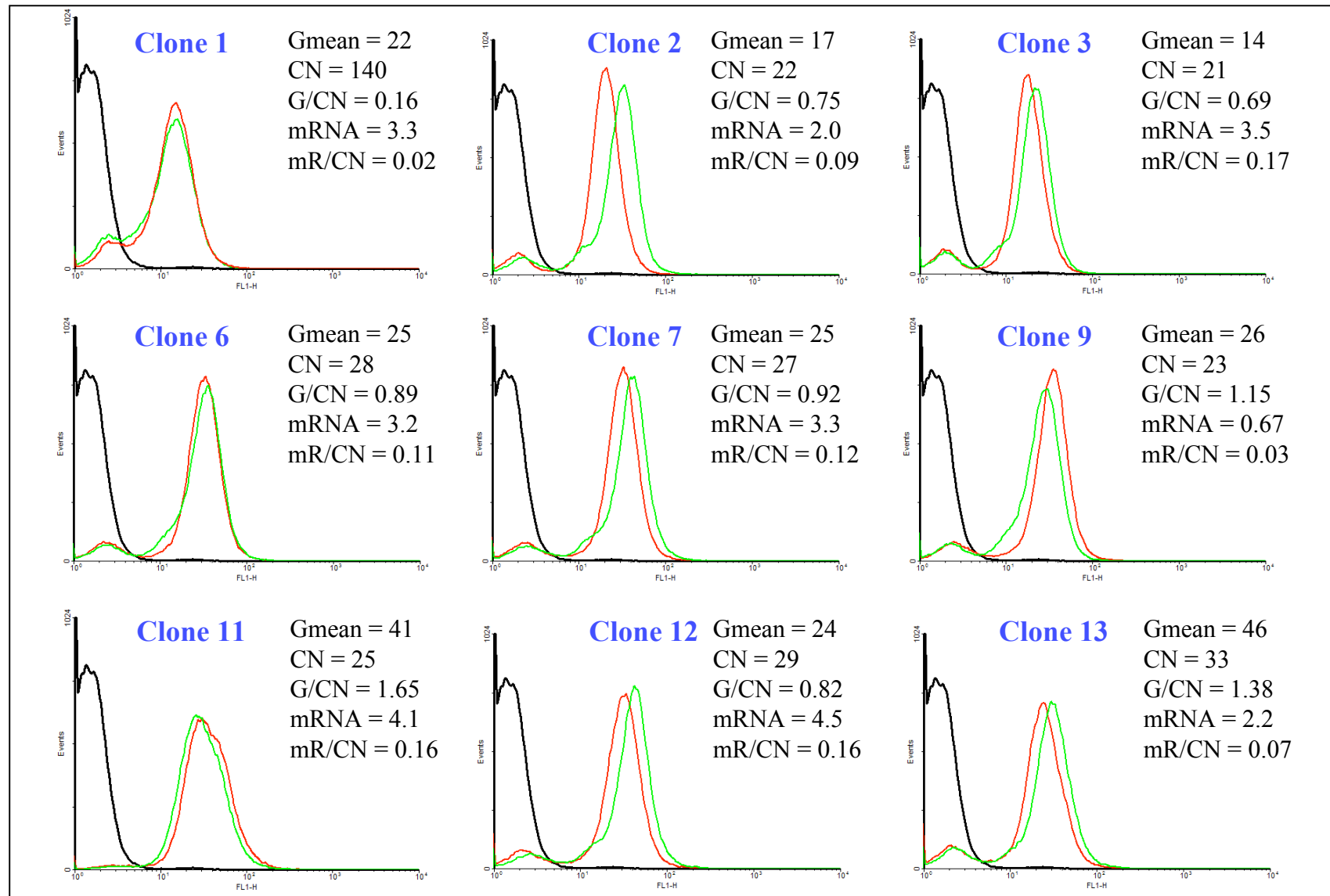
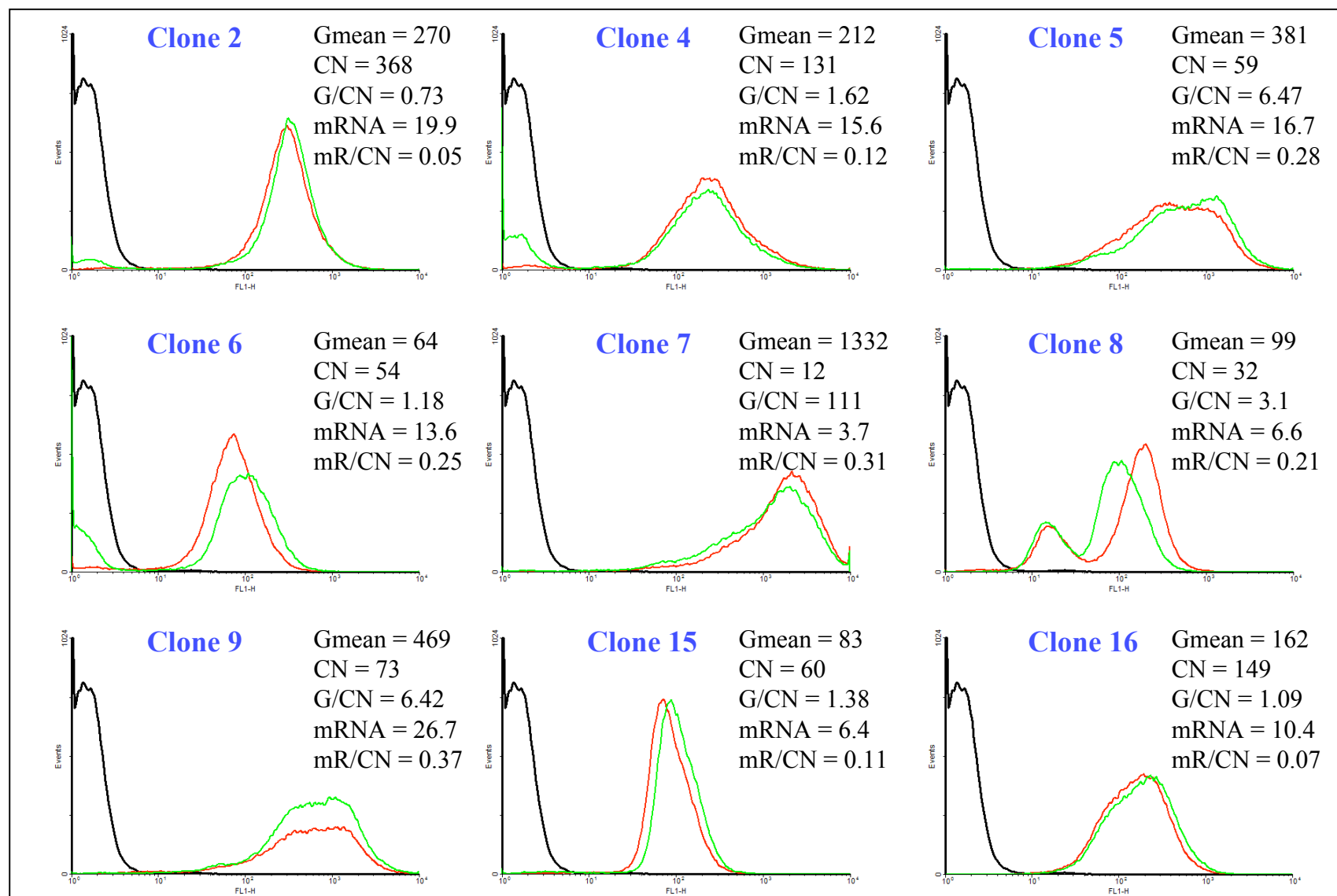


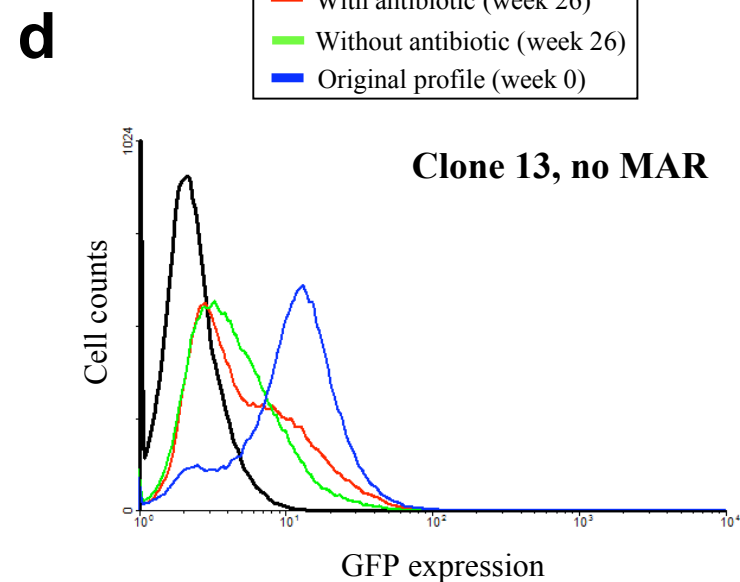
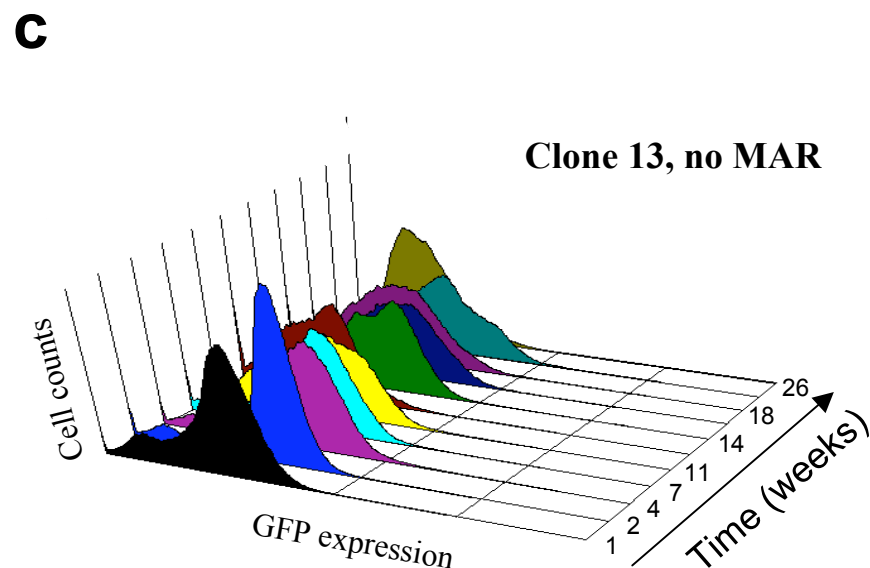
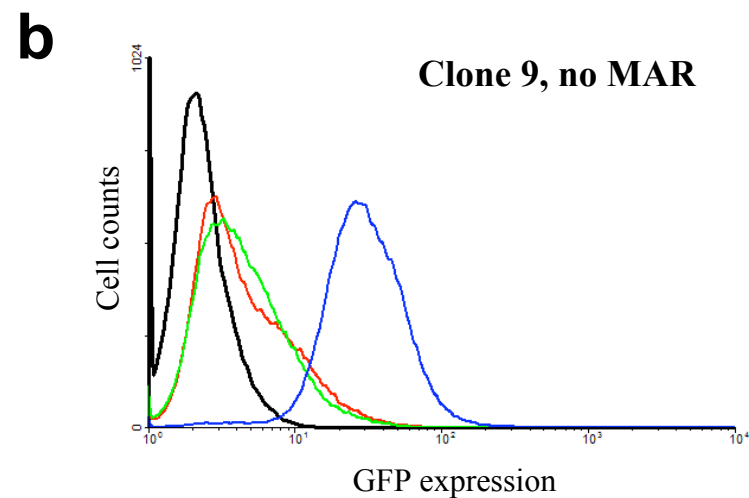
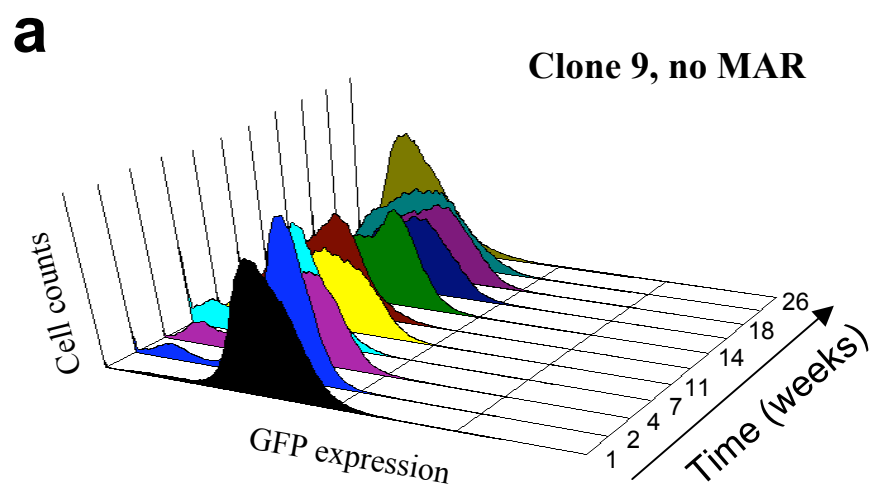
Supplementary Figure 1. Evolution of stable cell populations generated with or without MAR. Superposition of the fluorescence profiles of polyclonal populations generated by the stable transfection of MAR-devoid (a) or MAR-containing (b) expression vectors and sorted to isolate non-expressing cells, which were grown further for 4 or 19 weeks without antibiotic selection. Superposition of the fluorescence profiles of monoclonal populations generated by the stable transfection of MAR-devoid (c) or MAR-containing (d) expression vectors and sorted to isolate expressing cell clones, grown further for 26 weeks with or without antibiotic selection. The black curve represents the fluorescence background of non-transfected cells.



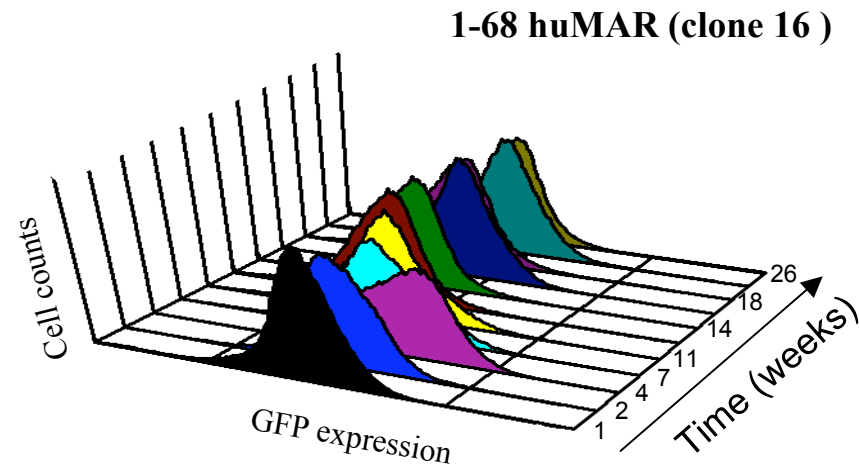
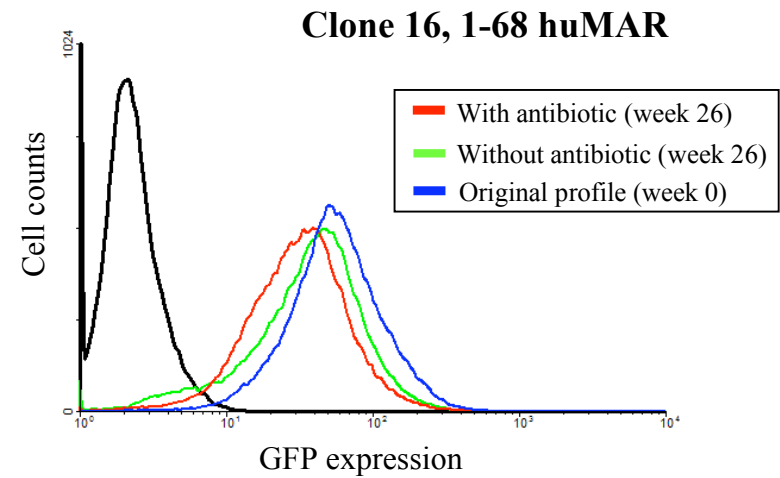
Supplementary Figure 2. Fluorescence profile of stable cell clones generated without MAR. Clones were isolated from stable polyclonal populations by random sorting of single fluorescent cells. Clones growing efficiently were cultivated for two weeks with (red curves) or without (green curves) antibiotic selection and cytofluorometric profiles were determined. Gmean indicates average fluorescence in arbitrary units, CN the number of integrated transgenes, G/CN fluorescence per gene copy, mRNA the relative mRNA level and mR/CN the relative mRNA level per gene copy. Means and standard deviations of clonal Gmean, CN, G/CN, mRNA and mR/CN are 27 ± 10 , 39 ± 38 , 0.93 ± 0.43 , 3.0 ± 1.2 , and 0.10 ± 0.53 , respectively.



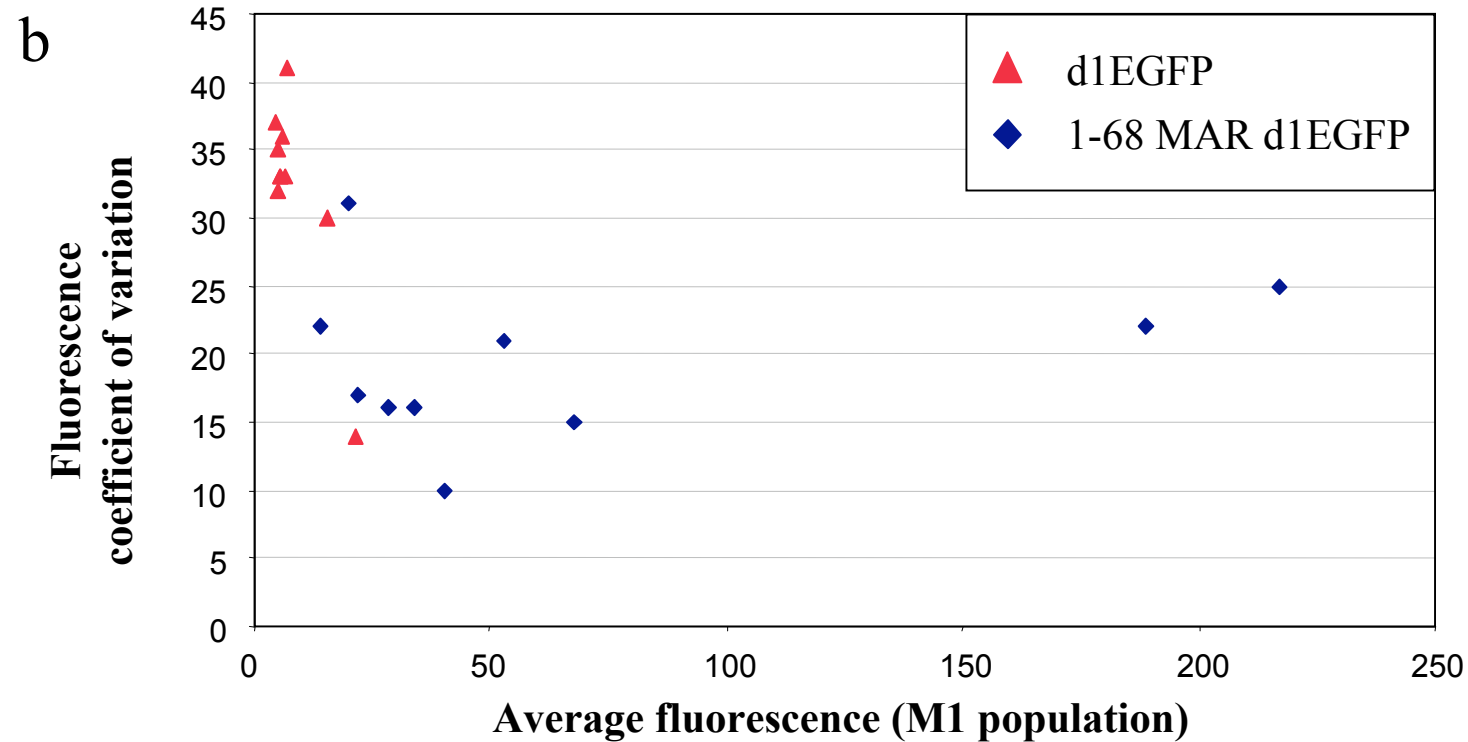
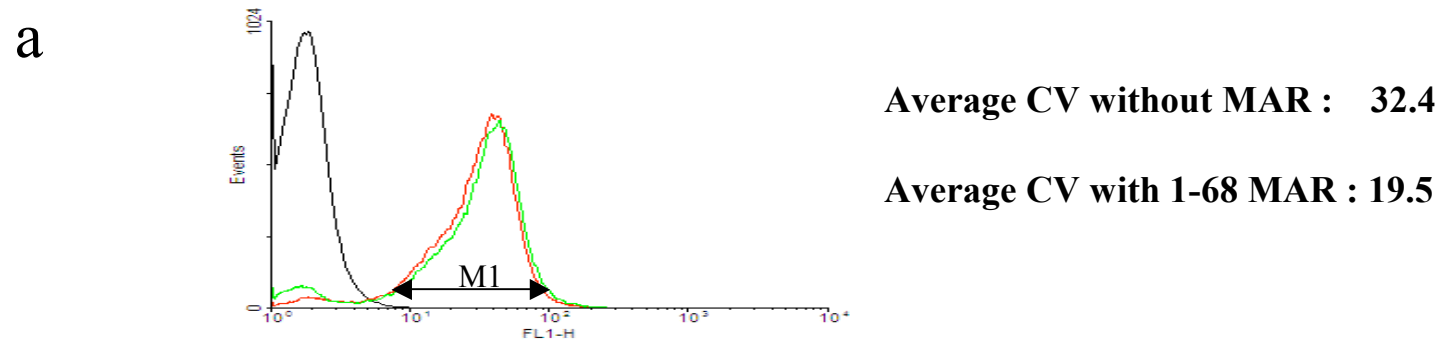
Supplementary Figure 3. Fluorescence profile of clones generated with MAR 1-68. Clones were isolated from stable polyclonal populations by random sorting of single fluorescent cells. Clones growing efficiently were cultivated for two weeks with (red curves) or without (green curves) antibiotic selection and cytofluorometric profiles were determined. Gmean indicates average fluorescence in arbitrary units, CN the number of integrated transgenes, G/CN fluorescence per gene copy, mRNA the relative mRNA level and mR/C the relative mRNA level per gene copy. Means and standard deviations of clonal Gmean, CN, G/CN, mRNA and mR/CN are 341 ± 396 , 104 ± 108 , 15 ± 36 and 13.3 ± 7.4 , 0.20 ± 0.58 , respectively.



Supplementary Figure 4. Kinetic of expression maintenance of additional MAR 1-68-devoid monoclonal populations. Cell clones as shown in Supplementary Figure 2 were grown without antibiotic selection as described in Fig 2c and the evolution with culture time of the cytofluorometric profiles were determined (a, c). Superposition of fluorescent profiles of the indicated monoclonal populations grown with or without selection (b, d).

a**b**

Supplementary Figure 5. Kinetic of expression maintenance of additional MAR 1-68-containing monoclonal populations. Cell clones as shown in Suppl. Fig. 3 were grown without antibiotic selection as described in Fig 2d and the evolution with culture time of the cytofluorometric profiles were determined (a). Superposition of fluorescence profiles of the indicated monoclonal populations grown with or without selection (b).



Supplementary Figure 6. Homogeneity of monoclonal cell populations. The fluorescence profiles of isolated clones was determined after 1 month of antibiotic selection, as shown in Supplementary Figures 2 and 3, and mean fluorescence of the expressing cell M1 population (a), as well as the coefficient of variation of single cell fluorescence is displayed (b).

No MAR		
Time (weeks)	Peack I (OFF) % cells	Peack II/III (ON) % cells
2	98.94	1.06
4	96.69	3.31
6	96.92	3.08
8	97.79	2.21
14	98.97	1.03
19	99.28	0.72
mean	98.32	1.68

1-68 MAR			
Time (weeks)	Peack I (OFF) % cells	Peack II (ON) % cells	Peack III (ON) % cells
2	85	11.24	3.76
4	51.3	42.22	6.48
6	35.6	57.38	7.02
8	35	54.17	10.83
14	26.12	57.49	16.39
19	30.01	38.11	31.88

Supplementary Figure 7. Kinetic of expression reactivation. Polyclonal populations transfected stably with or without MAR 1-68 were sorted to isolate silent cells which were cultivated without antibiotic selection for the indicated time, and cytofluorometry measurements are represented as the percentile of of expressing cells.

	NO MAR Clone 1	MAR 1-68 Clone 15
Time (weeks)	% expressing cells	% expressing cells
	(ON)	(ON)
1	94.43	98.92
2	88.58	98.38
4	87.87	99.38
7	65.31	98.56
11	56.46	97.91
12	44.68	97.38
14	37.02	98.50
16	34.59	96.48
18	21.97	98.12
22	22.51	98.26
26	15.4	98.18

Supplementary Figure 8. Kinetic of expression loss of clonal populations cultivated without antibiotic selection.

Mlonoclonal populations transfected stably with or without MAR 1-68 were were cultivated without antibiotic selection for the indicated time, and cytofluorometry measurements are represented as the percentile of expressing cells.