

IgG4 S241P sample	k_a (1/Ms)	k_d (1/s)	K_D (nM)	Relative K_D *	R_{max} (RU)	χ^2 (RU ²)
VH0/Vκ0 WT	1.40×10^5	7.15×10^{-4}	5.12	1.00	90.5	0.24
VH0/Vκ0 Ala ₅₆	1.54×10^5	7.01×10^{-4}	4.57	0.89	67.5	0.08
VH0/Vκ0 Asp ₅₆	1.95×10^5	6.37×10^{-4}	3.27	0.64	89.6	0.08
VH0/Vκ0 Glu ₅₆	1.84×10^5	6.53×10^{-4}	3.55	0.69	72.9	0.10
VH0/Vκ0 Ser ₅₆	1.52×10^5	7.01×10^{-4}	4.61	1.05	76.4	0.16
VH0/Vκ0 Pro ₅₆	1.38×10^5	1.05×10^{-3}	7.58	1.48	79.7	0.09
VH0/Vκ0 Thr ₅₆	1.56×10^5	6.88×10^{-4}	4.40	0.86	66.4	0.07

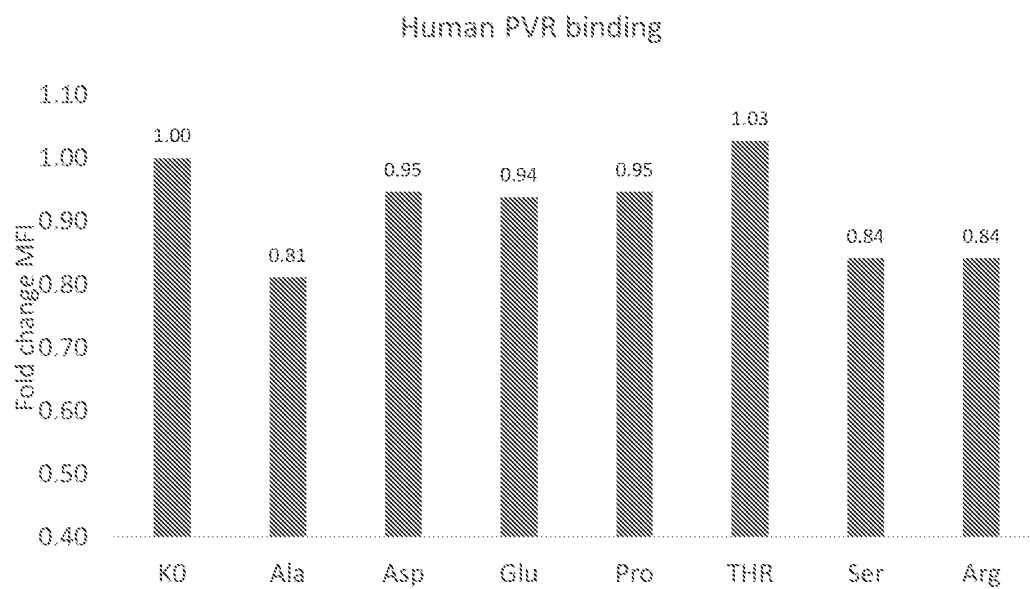
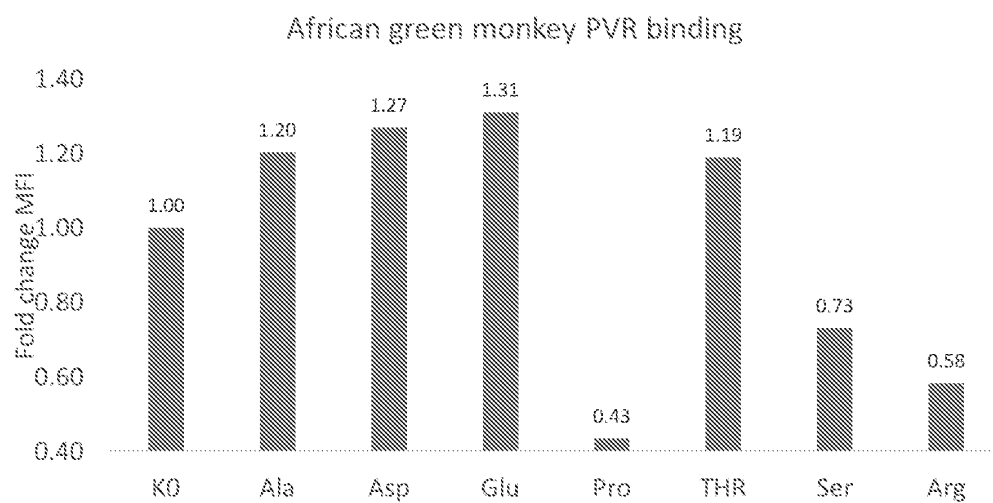
FIG. 1A

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Antibody IgG4 (S241P)	IC ₅₀ [nM]	Relative IC ₅₀ to WT*
WT	0.77	1.00
Ala ₅₆	0.57	0.73
Asp ₅₆	0.66	0.85
Arg ₅₆	0.92	1.19
Glu ₅₆	0.55	0.71
Pro ₅₆	0.85	1.10
Thr ₅₆	0.79	1.02
Ser ₅₆	0.75	0.96

FIG. 1B

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**FIG. 2A****FIG. 2B**

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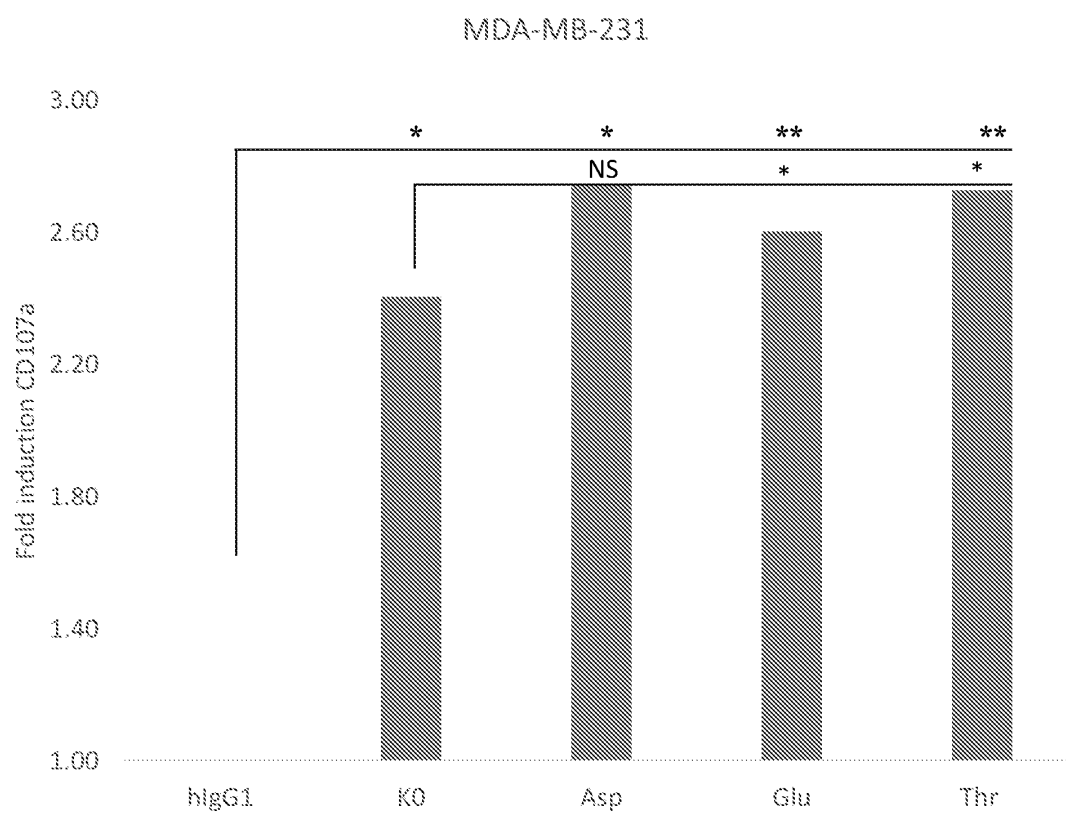


FIG. 3

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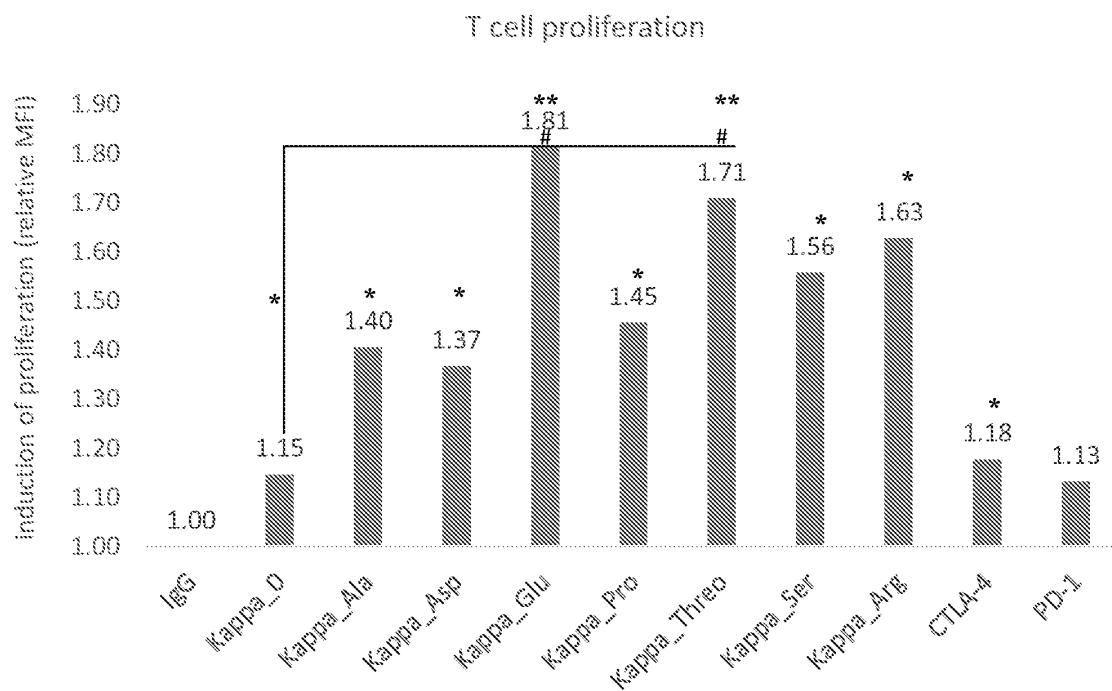


FIG. 4

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Antibody	K_D (nM)	Relative K_D *	χ^2 (RU ²)
IgG4(S ₂₄₃ P) _{...} N56E_VH0/Vk0	5.54	1.00	0.136
IgG4(S ₂₄₃ P) _{...} N56E_VH0/Vk1	4.03	0.73	0.0478
IgG4(S ₂₄₃ P) _{...} N56E_VH1/Vk0	4.50	0.81	0.0654
IgG4(S ₂₄₃ P) _{...} N56E_VH1/Vk1	4.17	0.75	0.155
IgG4(S ₂₄₃ P) _{...} N56E_VH1/Vk2	4.01	0.72	0.0449
IgG4(S ₂₄₃ P) _{...} N56E_VH1/Vk3	4.98	0.90	0.0655
IgG4(S ₂₄₃ P) _{...} N56E_VH1/Vk4	69.30	12.51	0.00276
IgG4(S ₂₄₃ P) _{...} N56E_VH2/Vk1	3.96	0.71	0.0559
IgG4(S ₂₄₃ P) _{...} N56E_VH2/Vk2	4.67	0.84	0.0666
IgG4(S ₂₄₃ P) _{...} N56E_VH2/Vk3	5.14	0.93	0.0577
IgG4(S ₂₄₃ P) _{...} N56E_VH2/Vk4	78.30	14.13	0.00322
IgG4(S ₂₄₃ P) _{...} N56E_VH3/Vk1	4.56	0.82	0.144
IgG4(S ₂₄₃ P) _{...} N56E_VH3/Vk2	4.32	0.78	0.0503
IgG4(S ₂₄₃ P) _{...} N56E_VH3/Vk3	5.42	0.98	0.0766
IgG4(S ₂₄₃ P) _{...} N56E_VH3/Vk4	75.20	13.57	0.0021
IgG4(S ₂₄₃ P) _{...} N56E_VH4/Vk1	4.62	0.83	0.0398
IgG4(S ₂₄₃ P) _{...} N56E_VH4/Vk2	6.76	1.22	0.0451
IgG4(S ₂₄₃ P) _{...} N56E_VH4/Vk3	5.11	0.92	0.0408
IgG4(S ₂₄₃ P) _{...} N56E_VH4/Vk4	96.00	17.33	0.00499
IgG4(S ₂₄₃ P) _{...} N56E_VH5/Vk1	5.91	1.07	0.0851
IgG4(S ₂₄₃ P) _{...} N56E_VH5/Vk2	5.68	1.03	0.0377
IgG4(S ₂₄₃ P) _{...} N56E_VH5/Vk3	7.41	1.34	0.0452
IgG4(S ₂₄₃ P) _{...} N56E_VH5/Vk4	76.80	13.86	0.00509

FIG. 5A

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Antibody Variant	EC ₅₀ (nM)	Relative EC ₅₀
IgG4(S ₂₄₃ P)_N56E_VH0/VK0	0.79	1.00
IgG4(S ₂₄₃ P)_N56E_VH2/VK2	0.64	0.81
IgG4(S ₂₄₃ P)_N56E_VH2/VK3	0.60	0.76
IgG4(S ₂₄₃ P)_N56E_VH3/VK2	0.52	0.66
IgG4(S ₂₄₃ P)_N56E_VH3/VK3	0.53	0.68
IgG4(S ₂₄₃ P)_N56E_VH4/VK2	0.67	0.86
IgG4(S ₂₄₃ P)_N56E_VH4/VK3	0.59	0.75
IgG4(S ₂₄₃ P)_N56E_VH5/VK2	0.64	0.82
IgG4(S ₂₄₃ P)_N56E_VH5/VK3	0.57	0.72

FIG. 5B

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Antibody Variant	IgG1 Isotype (μg/mL)	IgG4[S ₂₄₄ P] Isotype (μg/mL)
VH0/Vk0	11.9	8.7
VH0/Vk1	45.0	54.3
VH1/Vk0	8.5	7.1
VH1/Vk1	33.8	54.2
VH1/Vk2	33.7	35.6
VH1/Vk3	35.7	73.2
VH1/Vk4	47.6	51.4
VH2/Vk1	34.6	69.0
VH2/Vk2	33.4	37.7
VH2/Vk3	47.1	86.4
VH2/Vk4	29.4	61
VH3/Vk1	32.2	44.9
VH3/Vk2	22.0	46.6
VH3/Vk3	32.5	90.0
VH3/Vk4	18.2	105.4
VH4/Vk1	38.6	46.3
VH4/Vk2	46.0	108.7
VH4/Vk3	59.6	77.5
VH4/Vk4	45.8	58.4
VH5/Vk1	39.1	56.1
VH5/Vk2	45.1	52.5
VH5/Vk3	29.6	62.2
VH5/Vk4	18.2	76.7

FIG. 6A

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VH variant	% identity with closest human germline gene
VH2	75.5
VH3	78.6
VH4	80.6
VH5	82.7
VK variant	% identity
VK2	83
VK3	84.2

FIG. 6B

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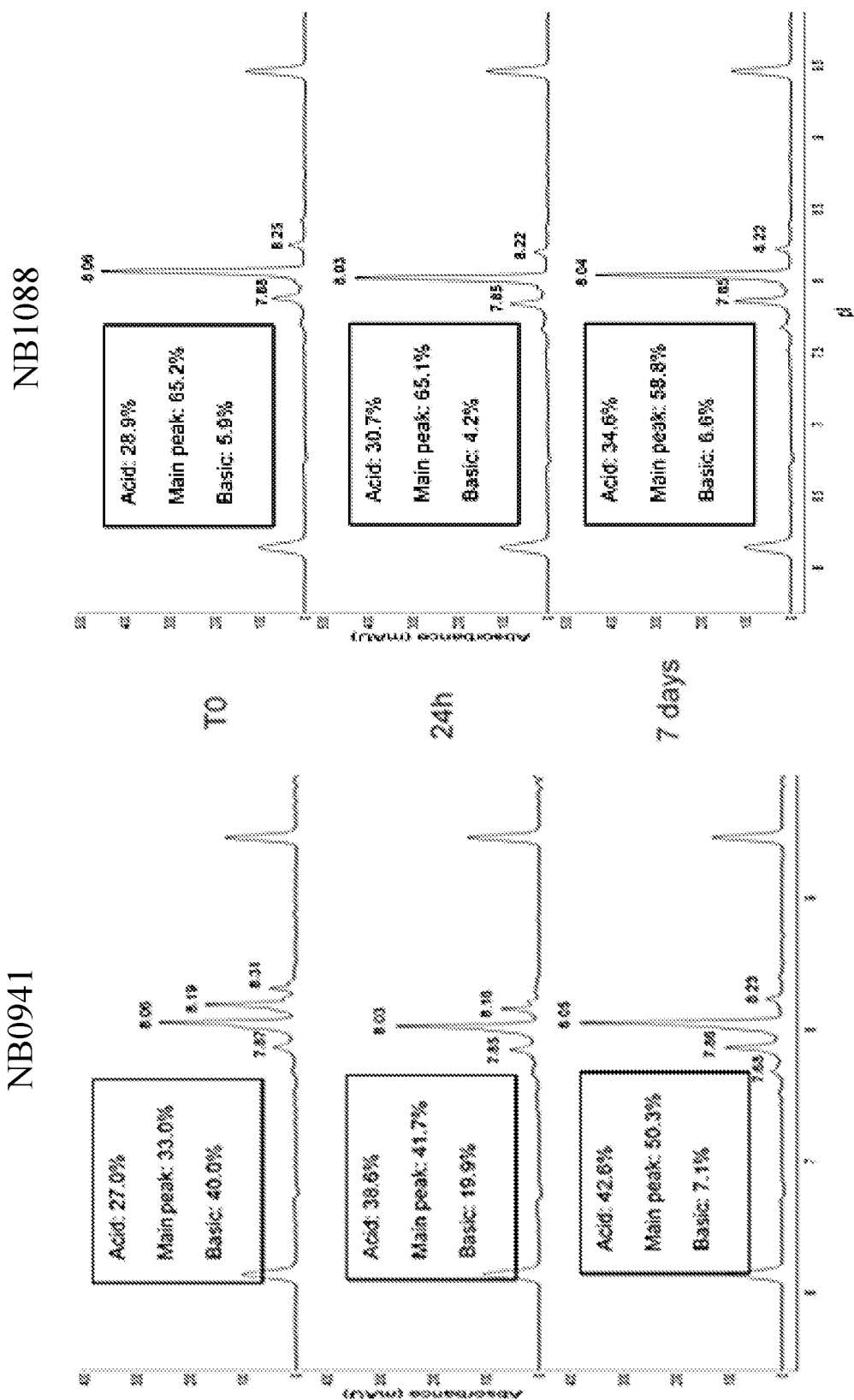


FIG. 7A

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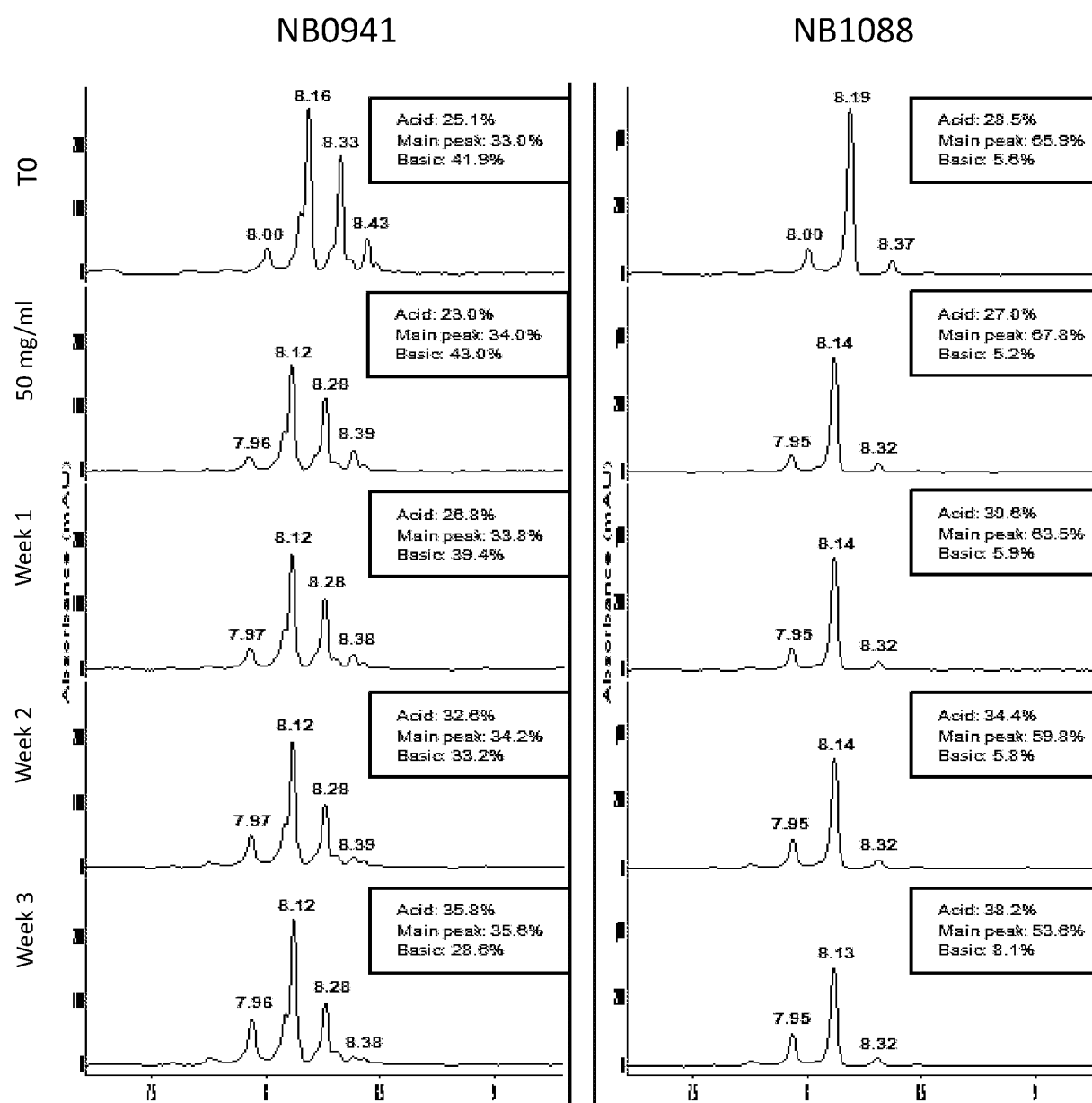


FIG. 7B

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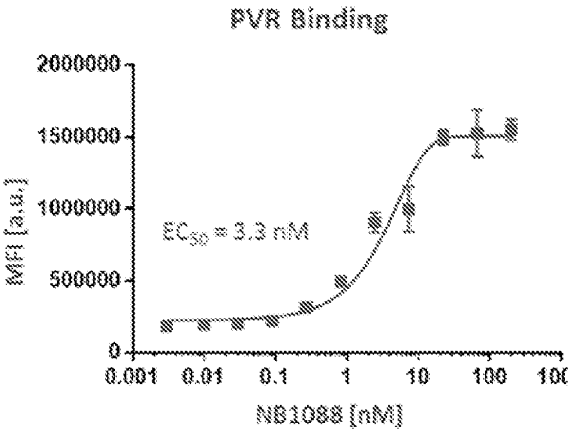


FIG 8A

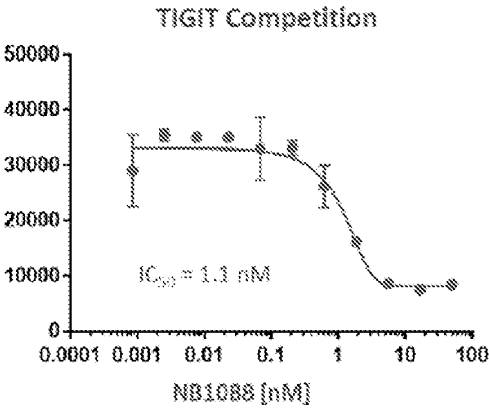


FIG 8B

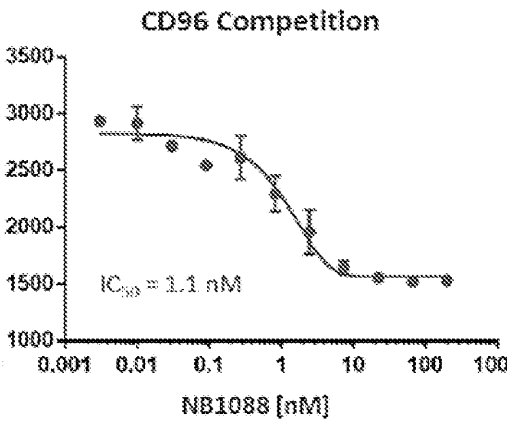


FIG 8C

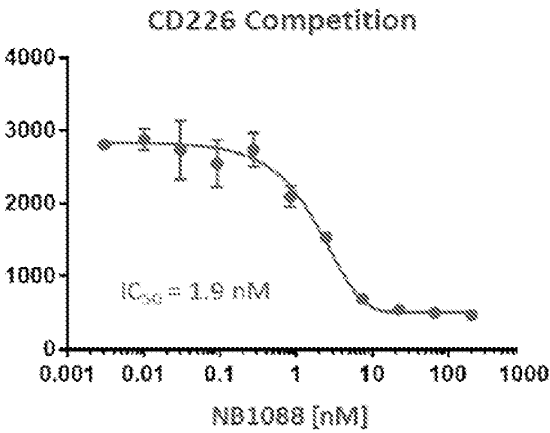


FIG 8D

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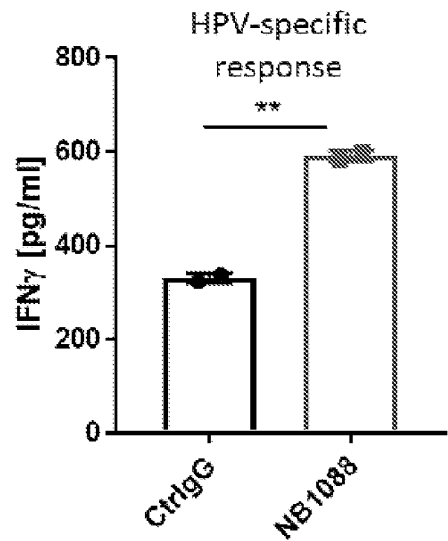


FIG. 9A

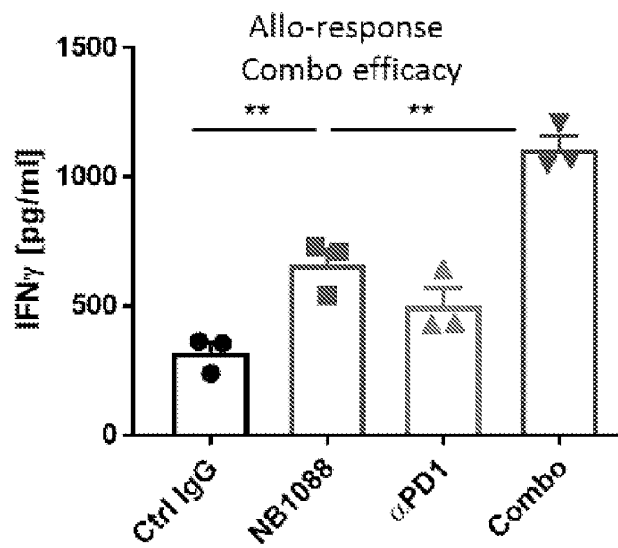


FIG. 9B

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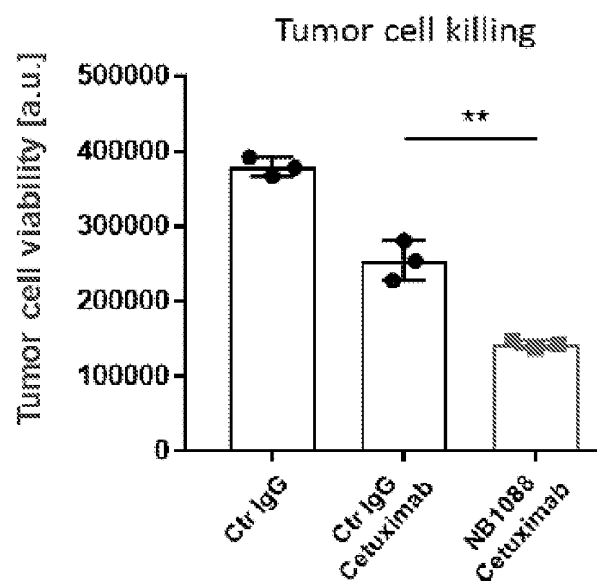


FIG. 10A

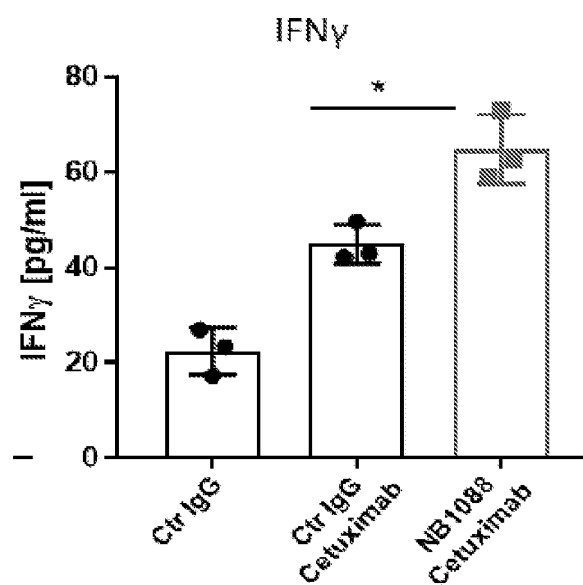


FIG. 10B

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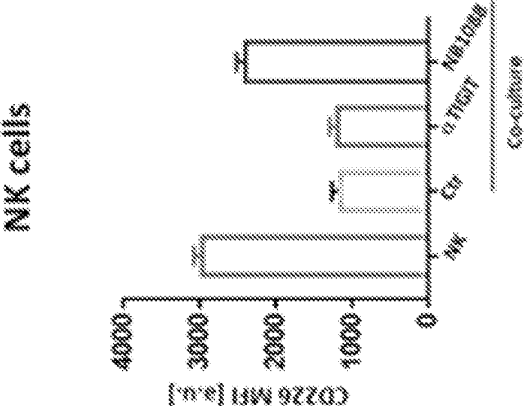


FIG. 11B

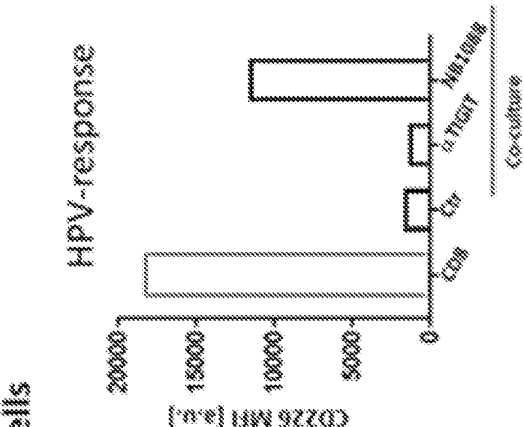
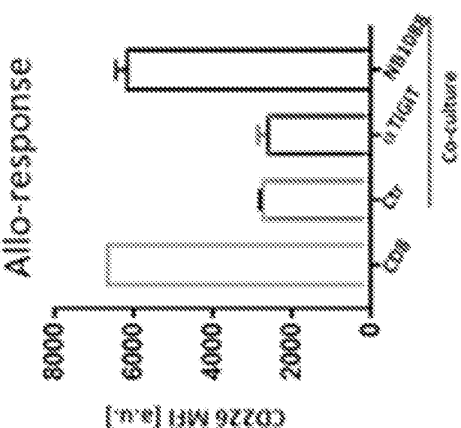


FIG. 11A



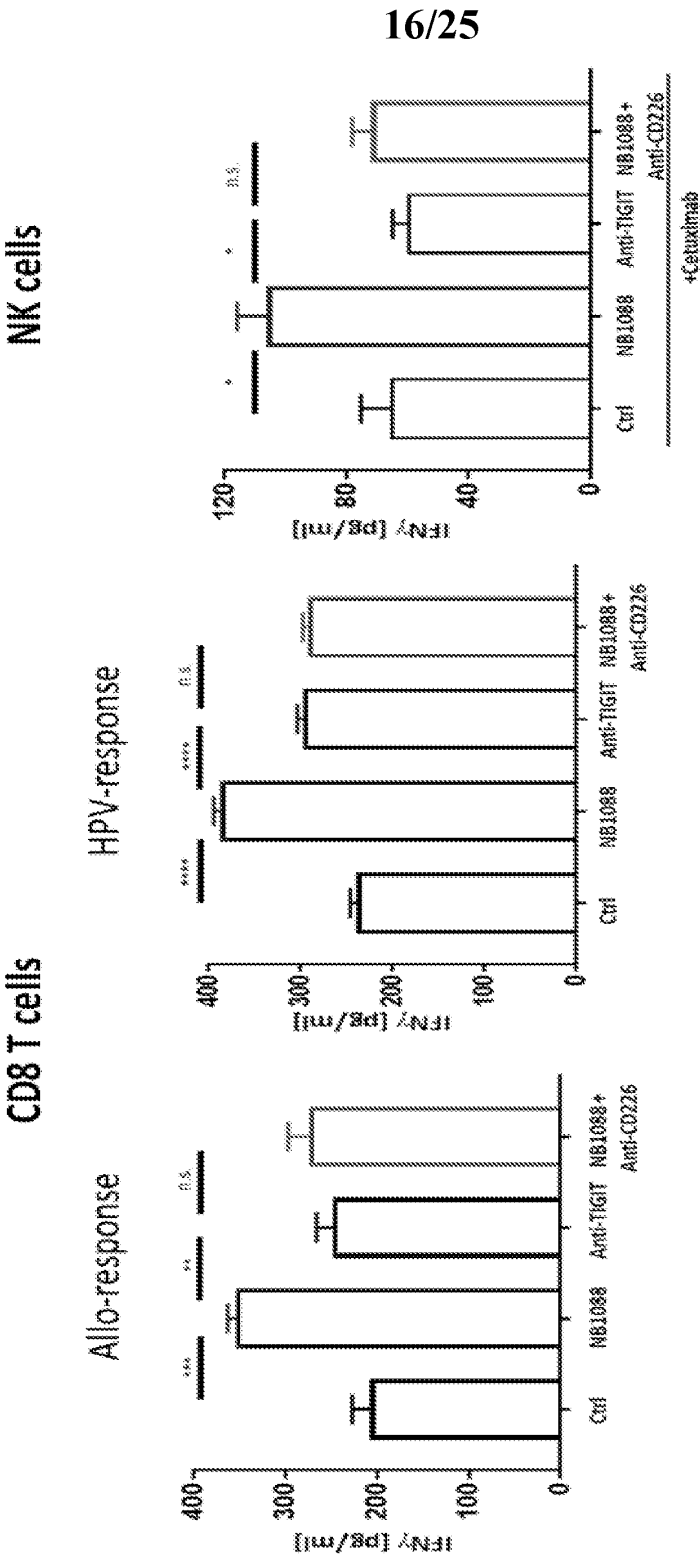


FIG. 12A

FIG. 12B

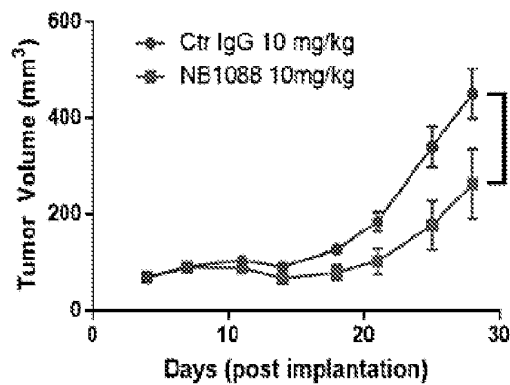


FIG. 13A

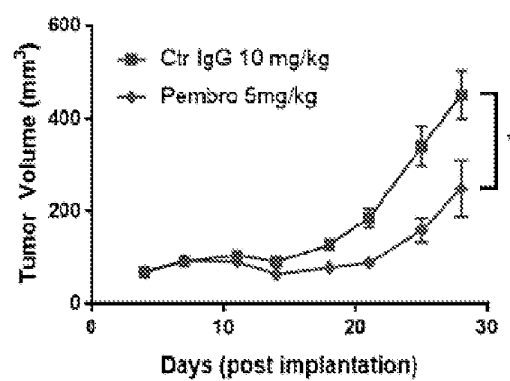


FIG. 13B

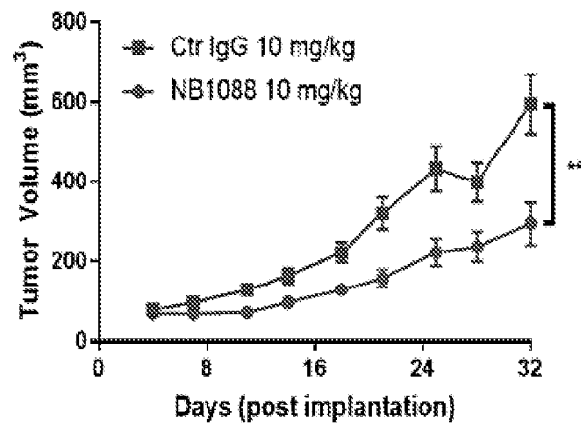


FIG. 13C

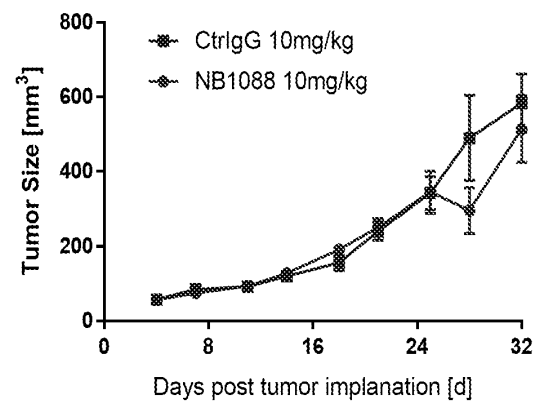


FIG. 13D

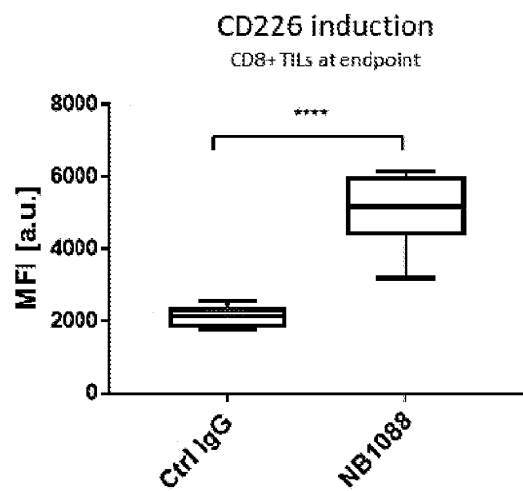


FIG. 13E

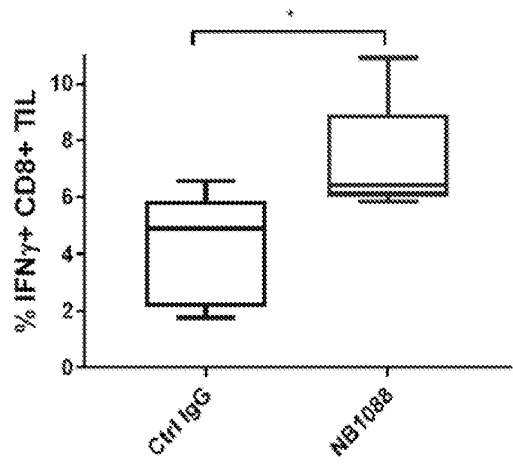


FIG. 14A

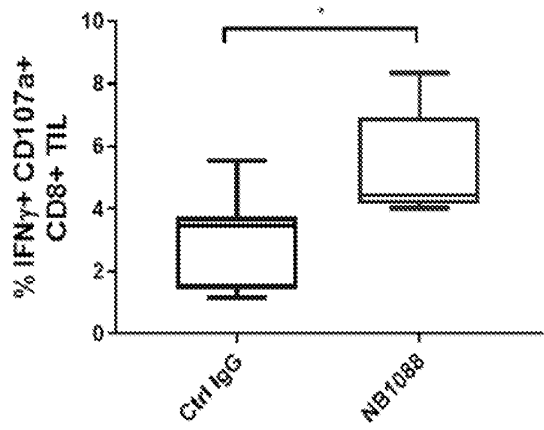


FIG. 14B

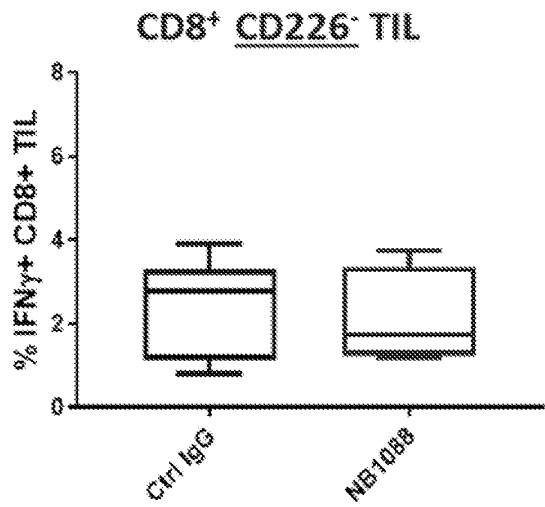


FIG. 14C

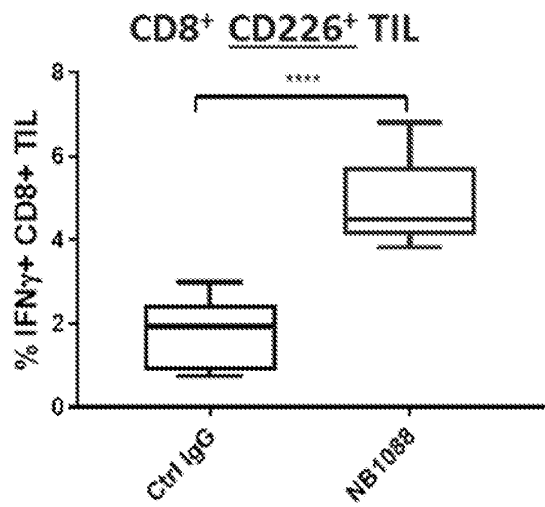


FIG. 14D

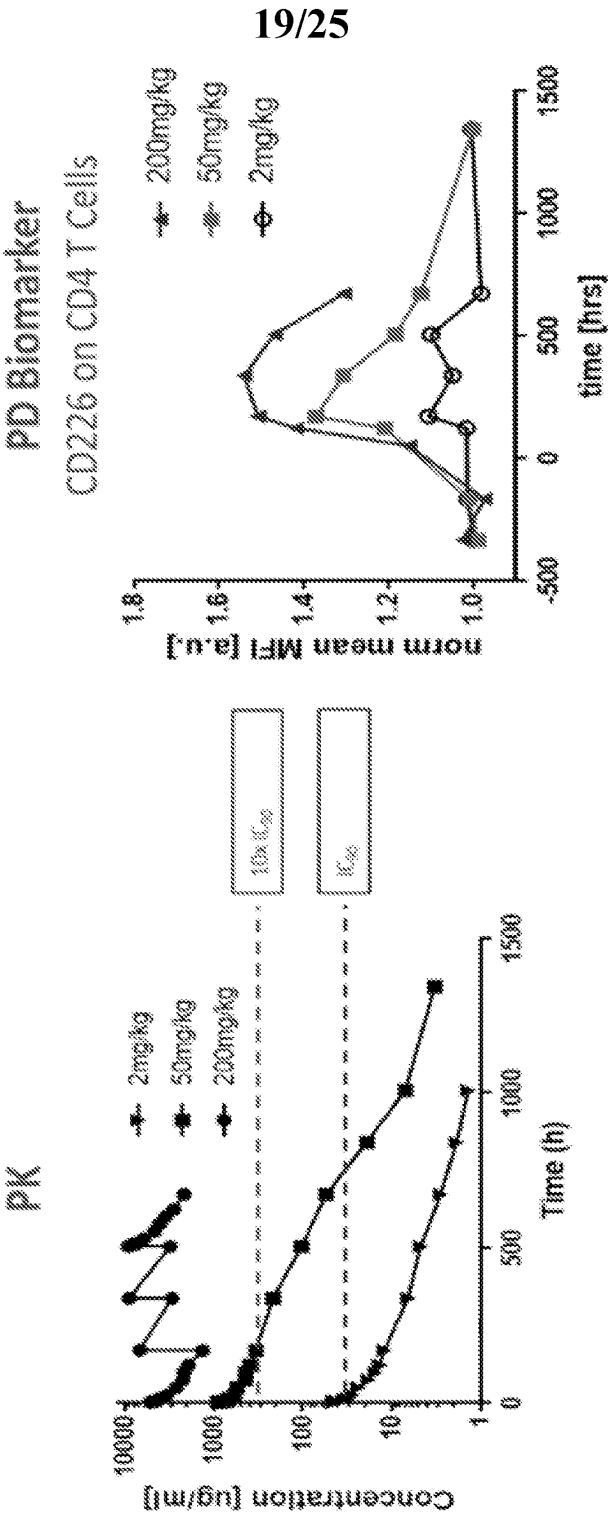


FIG. 15A

FIG. 15B

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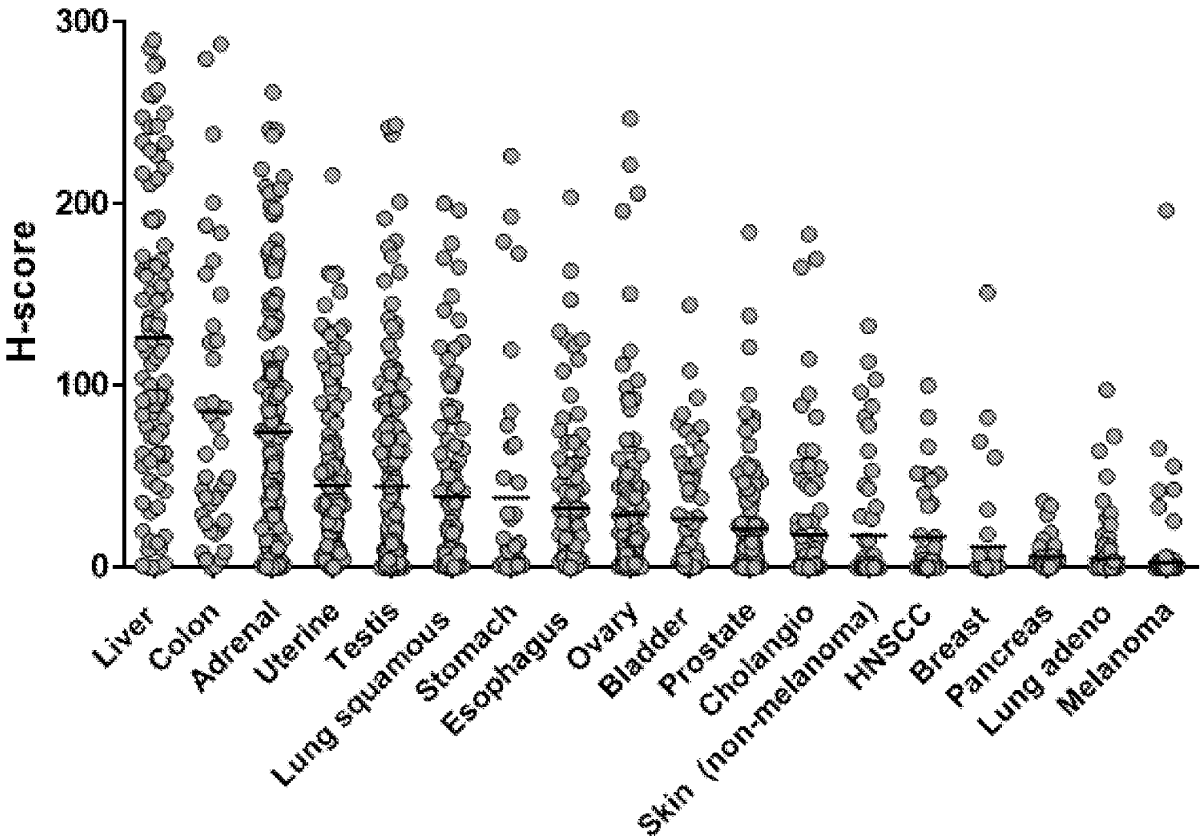


FIG. 16

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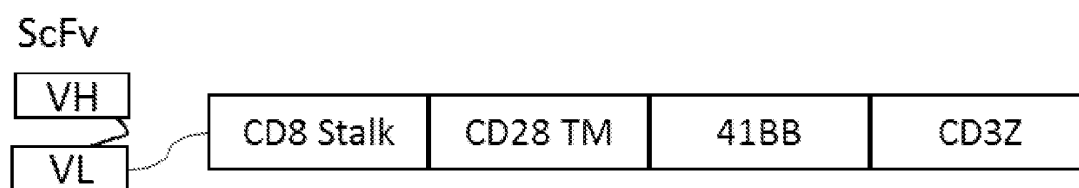


FIG. 17

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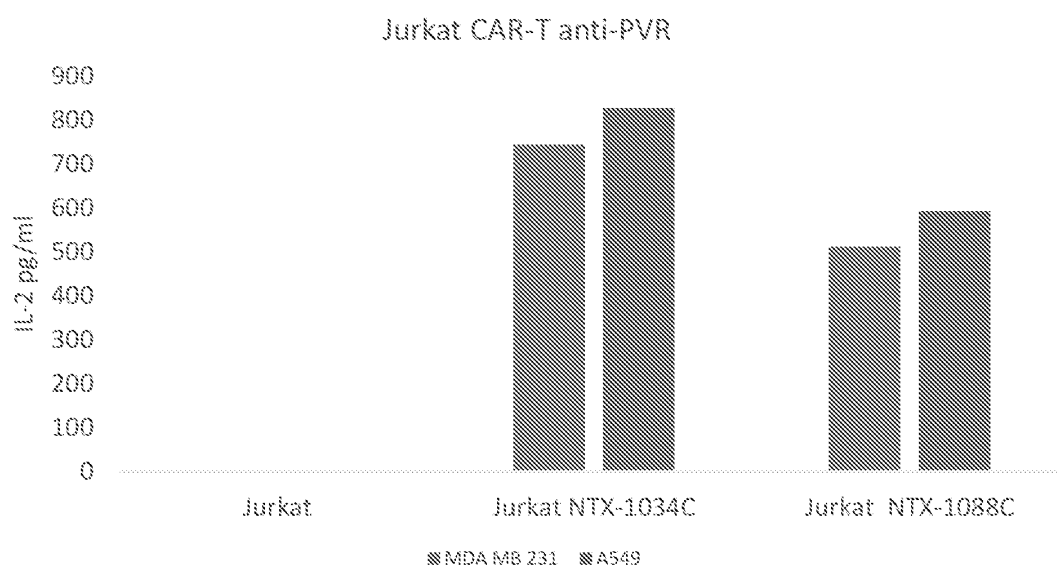
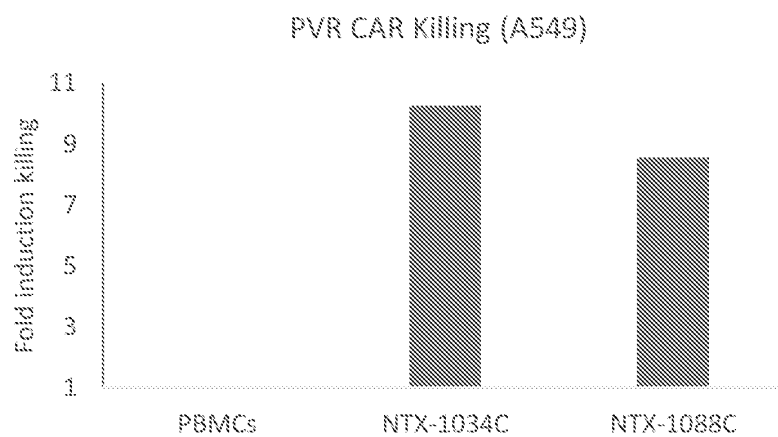
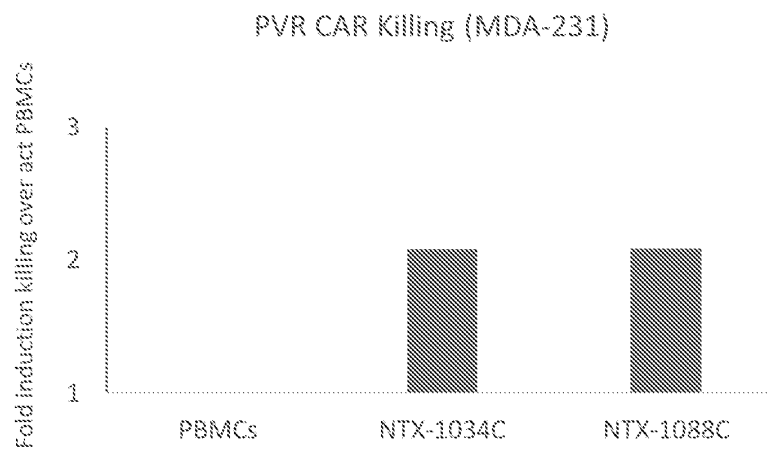


FIG. 18

23/25**FIG. 19A****FIG. 19B**

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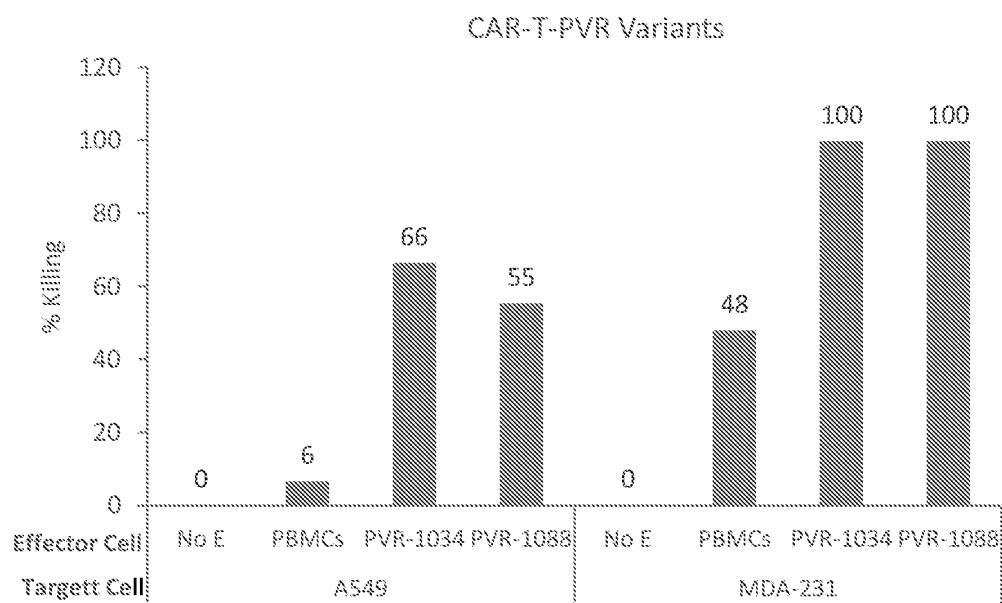


FIG. 19C

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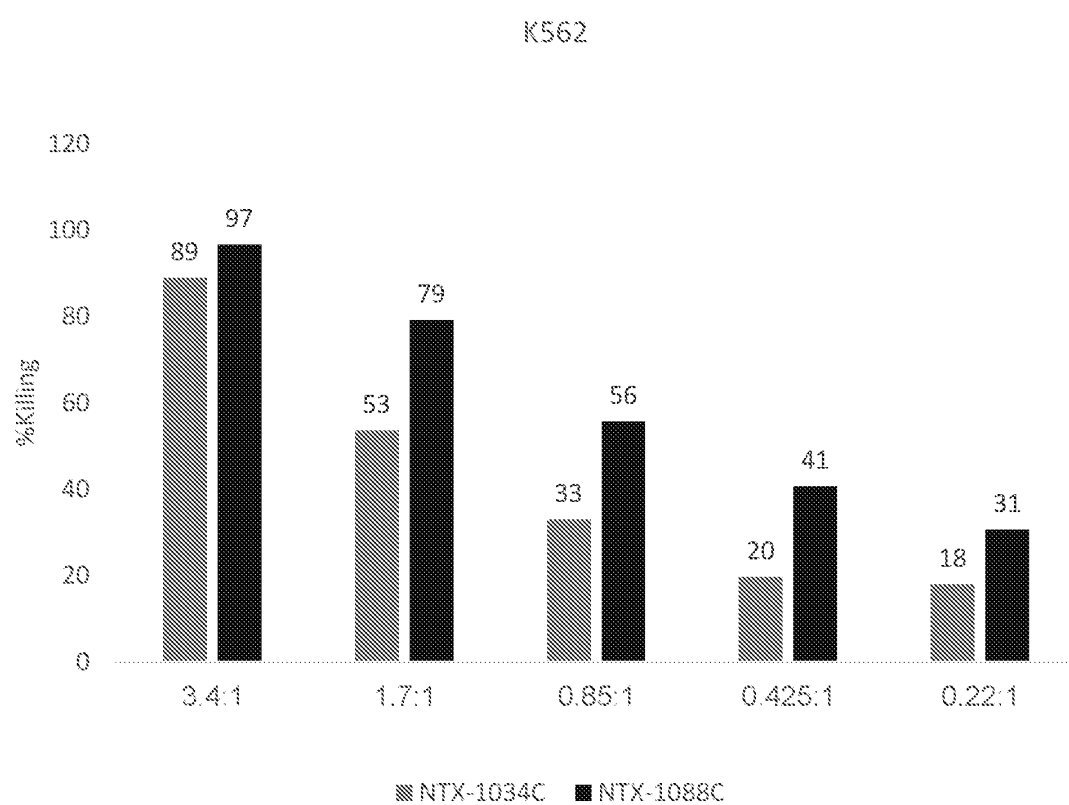


FIG. 20