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Supplemental information

Generation, expansion, gene delivery, and single-cell profiling in rhesus macaque plasma B cells

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Supplemental

Table S1: Staining Panels. Related to Figures 1, 5, and 7.

Panel 1: Day 0 Purity assessment					
Antigen	Fluorophore	Clone	Vendor	Cat#	Dilution
Live Dead	AF350	N/A	Invitrogen	A10168	1:1000
CD20	PerCP-Cy5.5	L27	BD Bioscience	340955	1:20
CD14	APC-Cy7	MoP9	BD Bioscience	557831	1:200
CD56	PE	MY31	BD Bioscience	347747	1:200
CD4	BV605	L200	BD Bioscience	562843	1:200
CD3	A700	SP34	BD Bioscience	557917	1:200
Panel 2: Phenotyping and transduction efficiency panel					
Antigen	Fluor	Clone	Vendor	Cat#	Dilution
Live Dead	AF350	N/A	Invitrogen	A10168	1:1000
CD20	PerCP-Cy5.5	L27	BD Bioscience	340955	1:100
CD14	APC-Cy7	MoP9	BD Bioscience	557831	1:200
CD31	PE	WM59	BD Bioscience	555446	1:100
CD38	mFluor450	OKT10	Caprico Biotechnologies	1008144	1:100
CD3	AF700	SP34	BD Bioscience	557917	1:200
Transduction	GFP	--	---		---
Panel 3: Phenotyping and intracellular Ig staining					
Antigen	Fluor	Clone	Vendor	Cat#	Dilution
Live Dead	AF350	N/A	Invitrogen	A10168	1:1000
CD20	PerCP-Cy5.5	L27	BD Bioscience	340955	1:20
CD14	APC-Cy7	MoP9	BD Bioscience	557831	1:200
CD138	PE	DL-101	Biolegend	352306	1:100

CD38	mFluor450	OKT10	Caprico Biotechnologies	1008144	1:50
CD38	FITC	OKT10	Caprico Biotechnologies	100815	1:200
CD3	APC-Cy7	SP34	BD Bioscience	557917	1:200
CD31	BV605	WM59	Biolegend	303122	1:100
CD59	APC	p282	Biolegend	304712	1:50
CD79A	BV421	HM47	BD Bioscience	BDB566225	1:200
IgG-ic	AF700	G18-145	BD Bioscience	561296	1:200
IgM-ic	FITC	G20-127	BD Bioscience	555782	1:200

Table S2: AAV transduction with different titers. Related to Figure 7.

		DAY 5		
		698	A17104	relative titer
	no AAV	1.55	0.58	0
1	scAAV1	9.41	2.33	0.664063
2	AAV2	18.4	9.41	1
3	AAV2.5	11.2	6.36	0.46875
4	scAAV6	9.46	2.09	0.086328
5	AAVD-J	22	9.92	1.757813
6	AAV5	28.4	12.3	4.84375
7	AAV8	7.48	1.75	2.402344
8	AAV9	3.96	0.99	2.773438
9	AAVRh.8	1.1	0.36	0.332031
10	AAV11cap11	0.91	0.34	0.214844
11	AAVcap11	0.94	0.4	0.46875
12	AAV HSC7	1.8	0.59	0.992188
13	AAV1110 pLT AAV Help (AAV2) Y444F, Y500F, Y730F	16.2	6.95	0.5
		DAY 7		
		698	A17104	relative titer
	no AAV	2.42	1.05	0
1	scAAV1	5.91	2.34	0.664063
2	AAV2	26.5	23.9	1
3	AAV2.5	14.6	11.3	0.46875
4	scAAV6	6.74	1.82	0.086328
5	AAVD-J	33.7	23.2	1.757813
6	AAV5	26.2	14.5	4.84375
7	AAV8	5.18	2.18	2.402344
8	AAV9	3.2	1.9	2.773438

13	AAV1110 pLT AAV Help (AAV2) Y444F, Y500F, Y730F	19	13.7	0.332031
		DAY 10		
		698	A17104	relative titer
	no AAV	7.27	1.63	0
1	scAAV1	3.28	1.37	0.664063
2	AAV2	21.5	21.1	1
3	AAV2.5	10.4	8.34	0.46875
4	scAAV6	3.78	1.3	0.086328
5	AAVD-J	35.6	26.4	1.757813
6	AAV5	22.9	14.5	4.84375
7	AAV8	4.27	2.11	2.402344
8	AAV9	2.92	1.58	2.773438
13	AAV1110 pLT AAV Help (AAV2) Y444F, Y500F, Y730F	12.5	10.5	0.332031

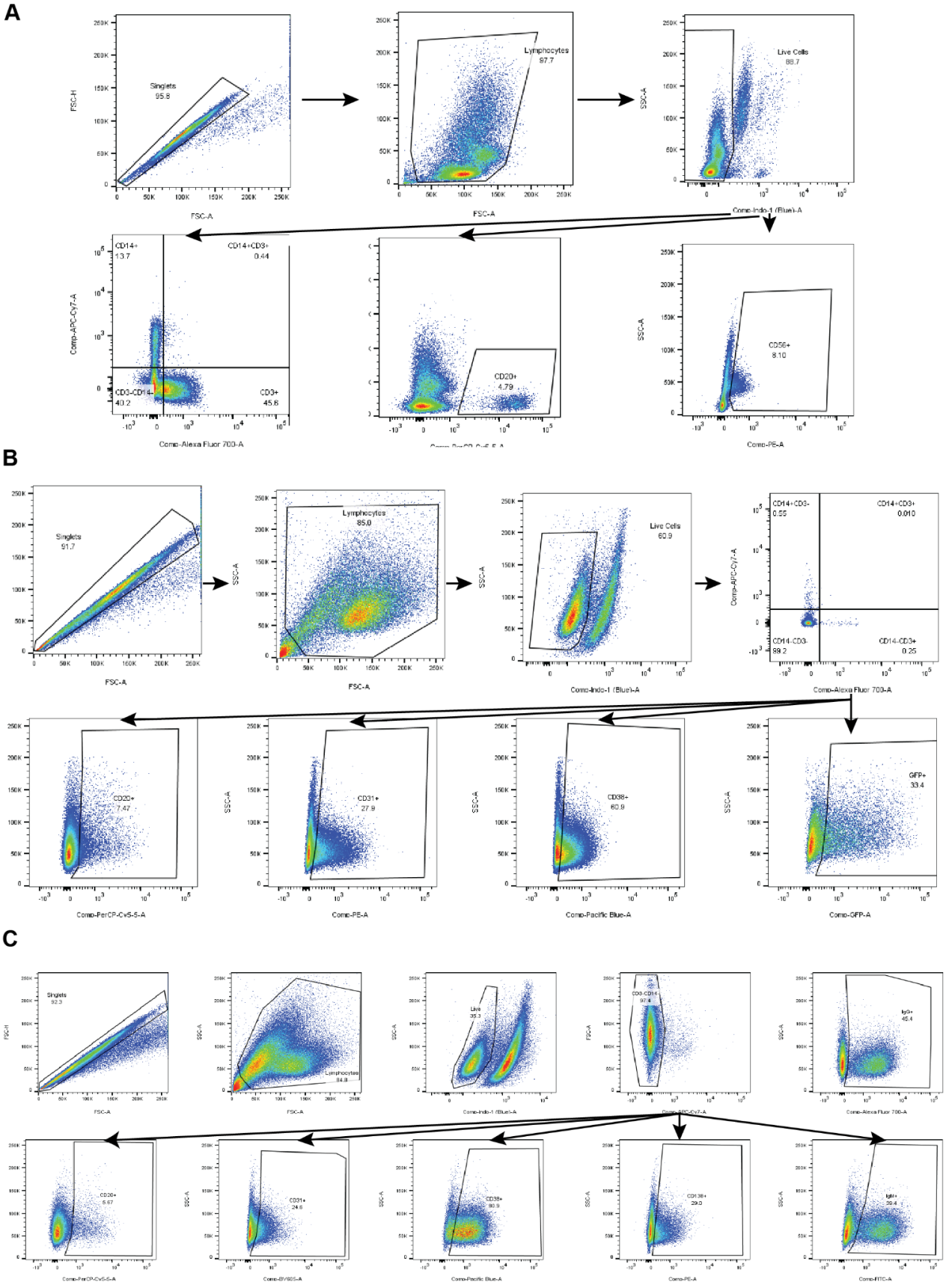

A
Singlets
95.8
FSC-H
FSC-A
Lymphocytes
97.7
FSC-A
Live Cells
88.7
SSC-A
Comp-Indo-1 (Blue)-A
CD14+
13.7
CD14+CD3+
0.44
CD3-CD14-
40.2
CD3+
45.8
Comp-APC-Cy7-A
Comp-Alexa Fluor 700-A
CD20+
4.79
CD56+
8.10
Comp-PE-A
B
Singlets
91.7
Lymphocytes
85.0
Live Cells
60.9
CD14+CD3-
0.55
CD14+CD3+
0.010
CD14-CD3-
99.2
CD14-CD3+
0.25
Comp-Alexa Fluor 700-A
CD20+
7.47
Comp-PerCP-Cy5-5-A
CD31+
27.9
Comp-PE-A
CD38+
60.9
Comp-Pacific Blue-A
GFP+
33.4
Comp-GFP-A
C
Singlets
92.3
Lymphocytes
84.9
Live
35.3
CD3-CD16-
97.4
IgG+
45.4
CD20+
5.67
CD31+
24.6
CD38+
60.9
CD138+
29.0
IgM+
39.4

Figure S1. Flow cytometry gating strategy. Related to Figures 1, 5, and 7. A. Gating scheme for flow cytometry Panel 1 (Table S1) to determine purity at the time of isolation. **B.** Gating scheme for flow cytometry Panel 2 (Table S1) to determine B cell phenotype and transduction efficiency through GFP expression. **C.** Gating scheme for flow cytometry Panel 3 (Table S1) to determine B cell phenotype and intracellular Ig production.

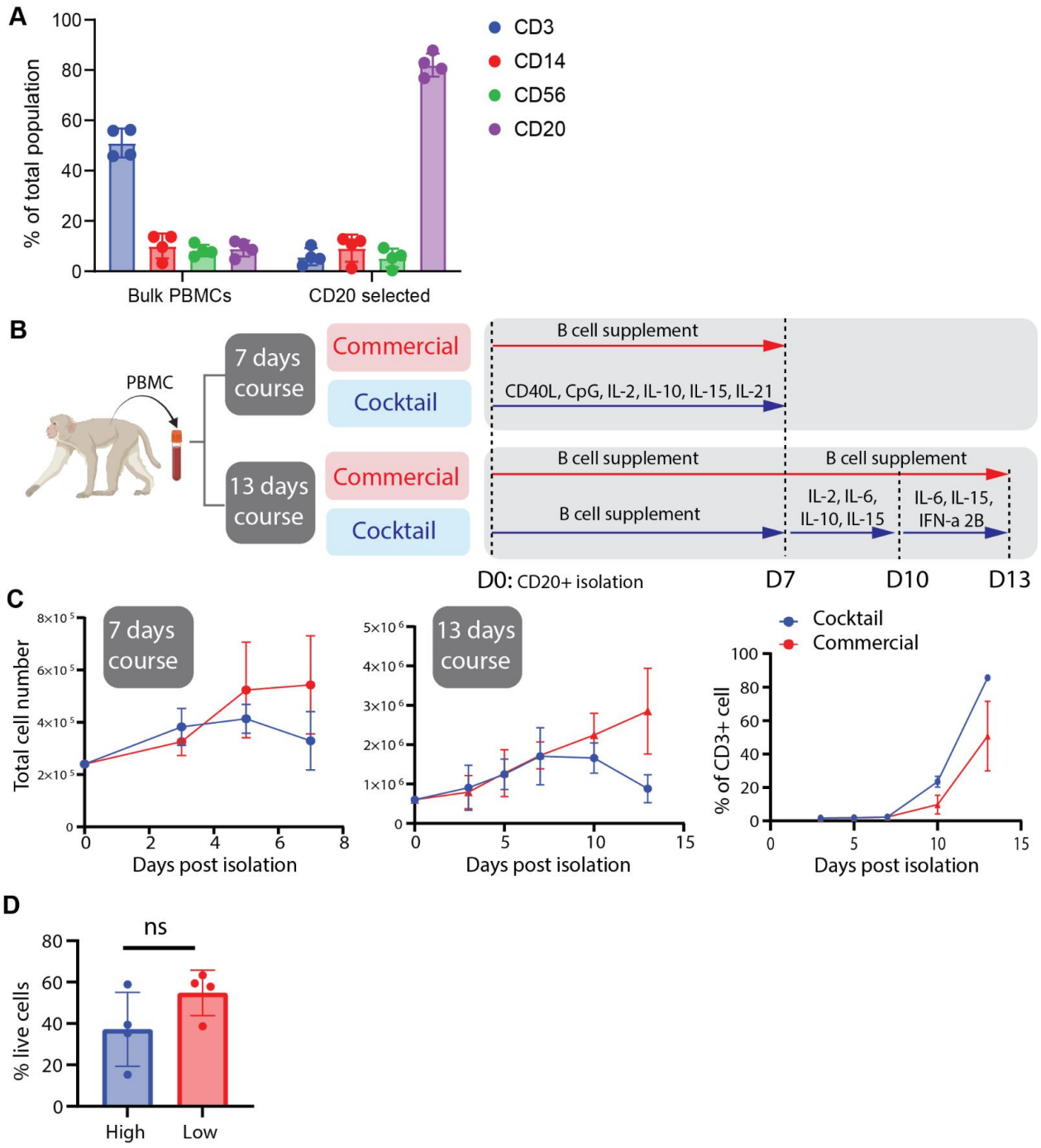


Figure S2. Method of ex vivo expansion and differentiated NHP PCs. Related to Figure 1. A. Following isolation of CD20+ Rhesus B cells from PBMCs, we assessed cell purity via flow cytometry using the indicated antibodies (4 donors, n=4). **B.** Schematic workflow of monkey *ex vivo* differentiated PC generation with two different time course and two different conditions. **C.** CD20+ NHP B cells were cultured for 7 days/13 days with defined cocktail or commercial expansion medium (1.5-1x10^6 cells/mL) (3 donors, n=3), Total cells were counted on days 3, 5, and 7 (left and middle). Percentage of contaminating

T cells cells (CD3+, left). **D.** To assess the impact of cell density, CD20+ NHP B cells were cultured in the commercial B cell medium for 7 days (Plated and maintained $1.5x10^6$ cells/mL versus plated at $2.5x10^5$ cells/mL and maintained at $1x10^5$ cells/mL; 4 donors, n=3). At day 7 following isolation, the percent viability was analyzed using flow cytometry. Data presented as individual data points and error bar represent standard deviation.

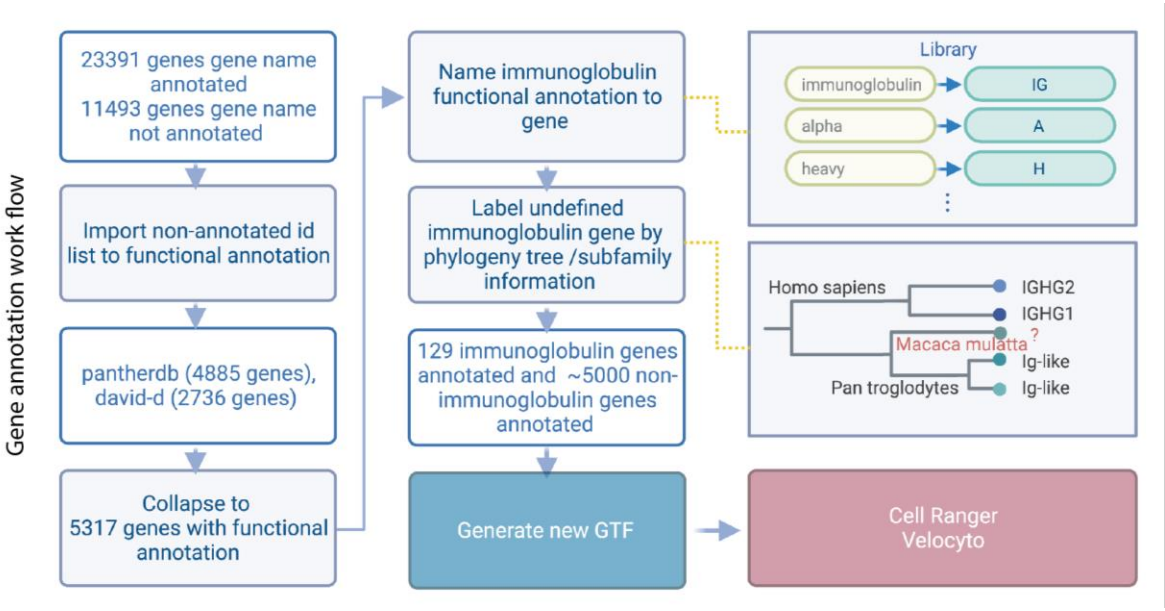


Figure S3. Monkey (Macaca mulatta) gene annotation workflow. Related to Figure 1. Gene annotation workflow. (GTF: Gene transfer fomat, Macaca_mulatta.Mmul_10.104.chr.gtf, generated new GTF: genes.gtf)

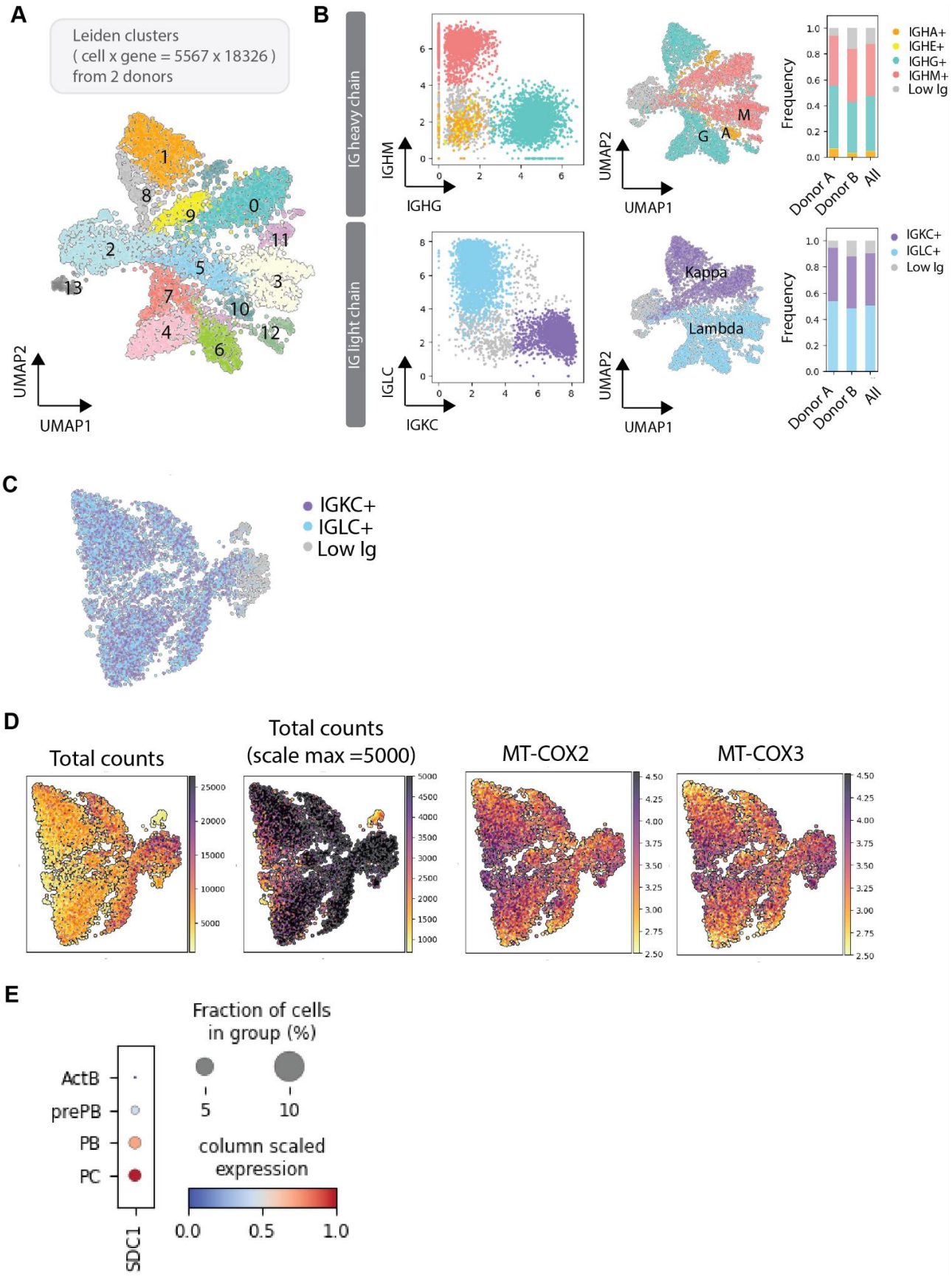
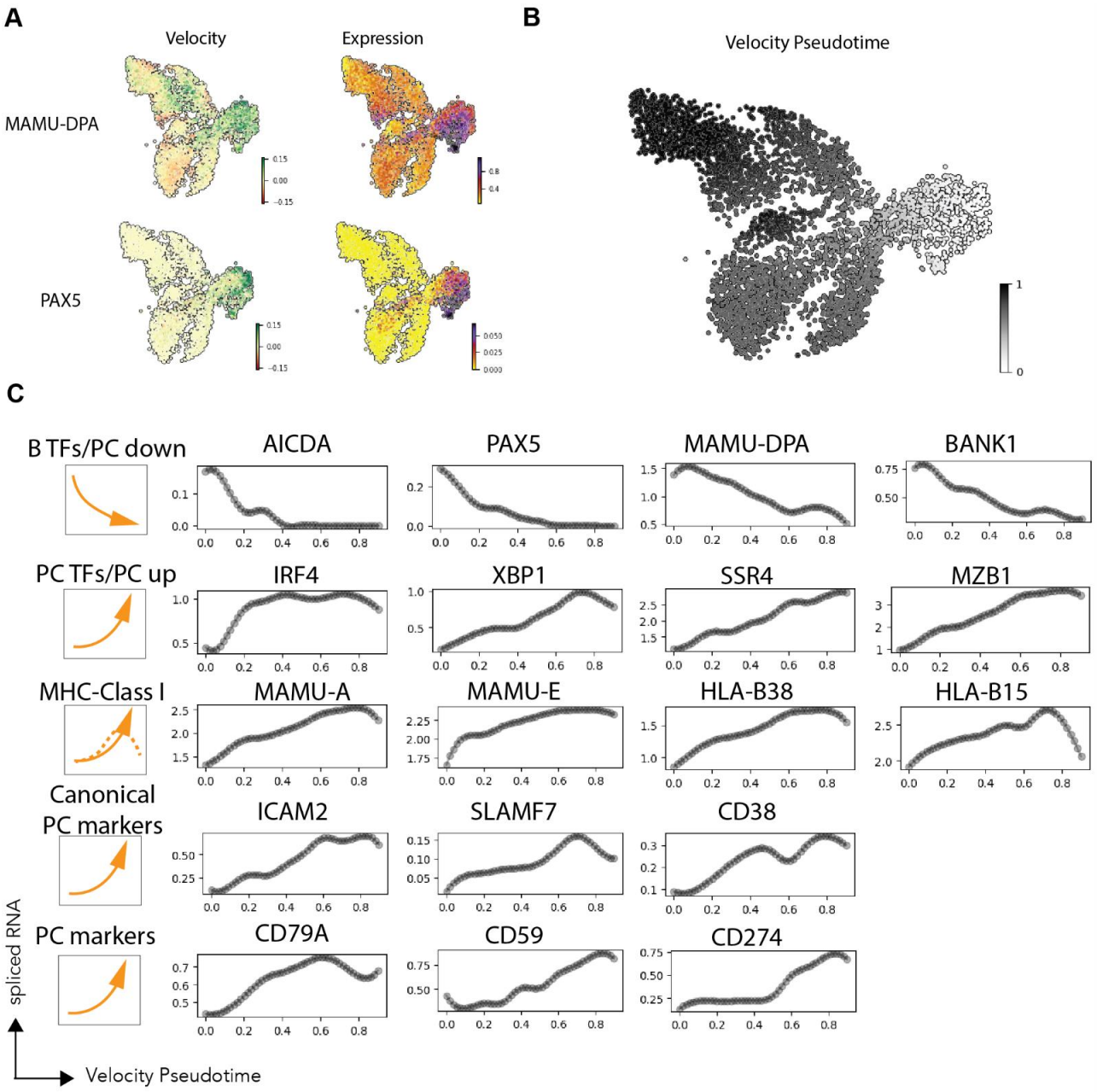

A
Leiden clusters
(cell x gene = 5567 x 18326)
from 2 donors
UMAP2
UMAP1
B
IG heavy chain
IGHM
IGHG
UMAP2
UMAP1
M
G
A
Frequency
IGHA+
IGHE+
IGHG+
IGHM+
Low Ig
Donor A
Donor B
All
IG light chain
IGLC
IGKC
Kappa
Lambda
IGKC+
IGLC+
Low Ig
C
IGKC+
IGLC+
Low Ig
D
Total counts
Total counts
(scale max =5000)
MT-COX2
MT-COX3
E
Fraction of cells
in group (%)
ActB
prePB
PB
PC
SDC1
column scaled
expression

Figure S4. Monkey *ex vivo* differentiated PCs subsets classification and differential expression analysis for PCs. Related to Figures 1, 2, and 3. A. Single cell graph UMAP dimension-reduction projection of day13 B cells (n = 5567) from two biological replicates. **B.** Classification of immunoglobulin heavy chain (upper panel) and light chain (lower panel). UMAP projection of the subclass of IGHG+, IGHA+, IGHM+ cells (upper panel) and IGKC+, IGLC+ cells (lower panel), and bar graph represent the percentage of subclasses from two individual donor. **C.** UMAP projection of IGKC+, IGLC+ cells in LC-independent clusters. **D.** Heatmap showing total count and expression of representative MT genes in LC-independent clusters. **E.** Dotplot visualization of *ex vivo* differentiated monkey PCs: subsets are listed on y-axis and SDC1 (CD138) gene on x-axis.



D

	fit_diff_kinetics	fit_pval_kinetics
EZH2	ActB	1.70e-08
TUBA1A	ActB	1.97e-06
BIRC3	prePB	1.08e-03
MAN1A1	mPC	1.56e-04
FUT8	aPC	8.95e-03
FNDC3A	aPC	6.73e-04
IGF1	gPC	3.10e-03
CD274	gPC	2.30e-06

Figure S5. RNA velocity of immunoglobulin and kinetic differentiation analysis. Related to Figure 4. A. Heatmap of indicated gene velocity and RNA expression level superimposed on to UMAP. **B.** Heatmap of velocity pseudotime superimposed on to UMAP. **C.** Scatter plot of indicated genes with velocity pseudotime and mean of spliced mRNA in every 0.1 pseudotime. **D.** scVelo kinetic differentiation analysis.

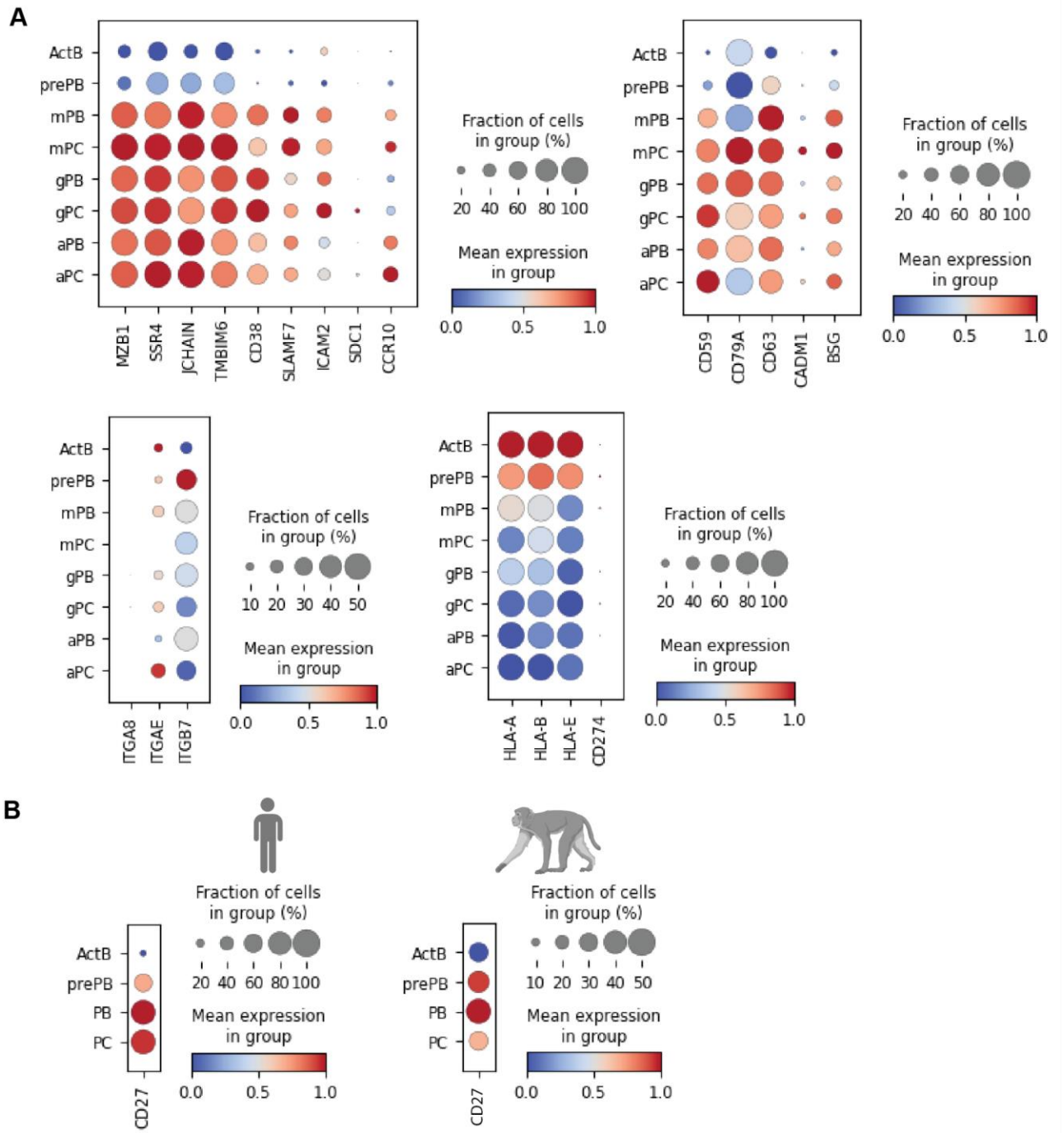


Figure S6. Comparison of human and monkey PC makers by scRNAseq analysis. Related to Figure 6. A. Dotplot visualization of *ex vivo* differentiated human PC: subsets with different isotypes are listed on y-axis and representative genes from Fig. 3D are listed along the x-axis. **B.** Dotplot visualization of *ex vivo* differentiated human and monkey PC: subsets are listed on y-axis and CD27 gene on x-axis.

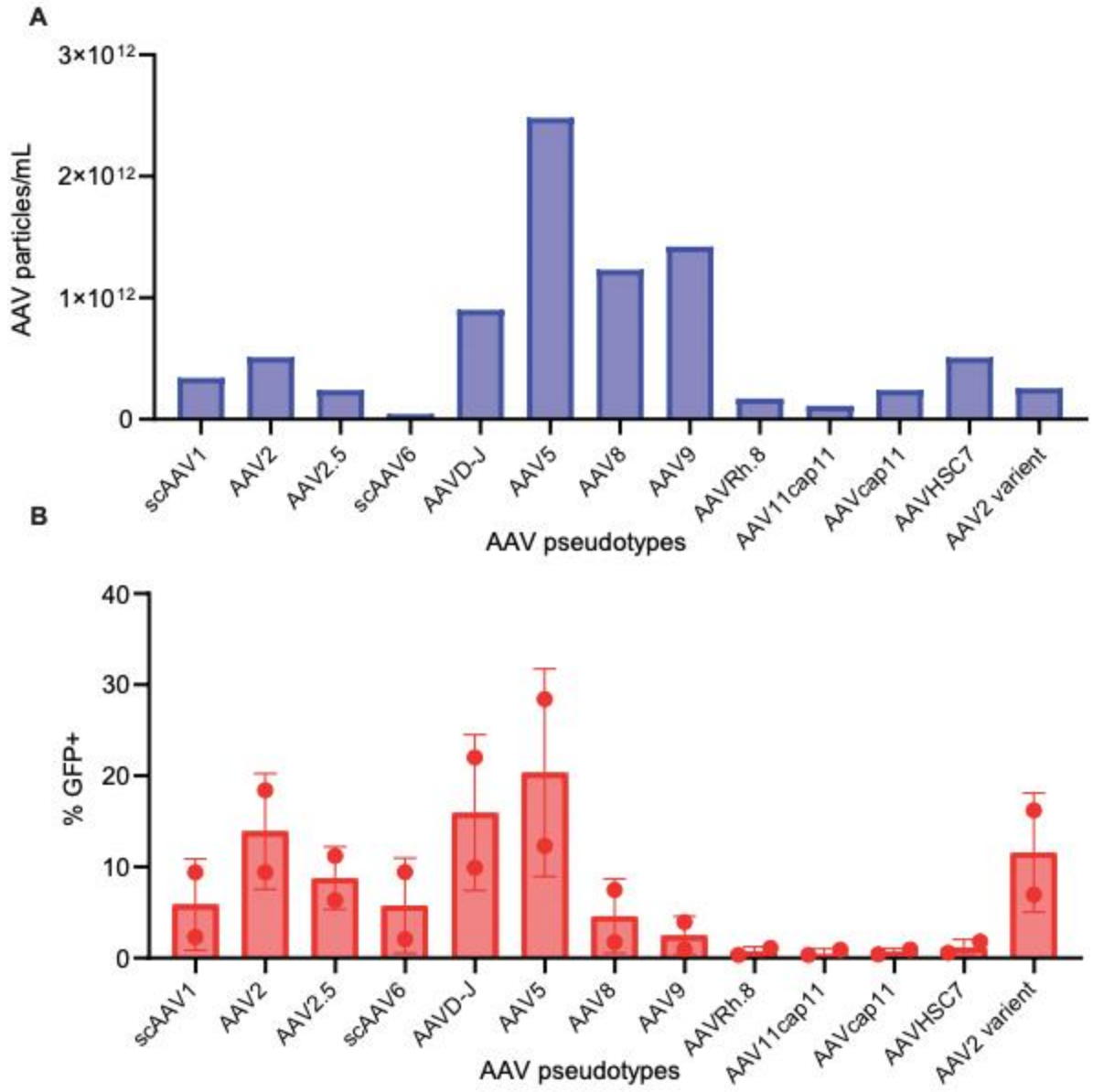


Figure S7. NHP B cell transduction with AAV. Related to Figure 5. A-B NHP B cells were transduced on day 3 of culture. 20% by volume was added of GFP encapsulated in different AAV pseudotypes, with varying titers **A.** and flow for GFP+ **B.** was run 2 days later.