

## Supplementary Material

# Humoral and Cellular Immunity Are Significantly Affected in Renal Transplant Recipients, following Vaccination with BNT162b2

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**Supplementary Table S1.** Record of performed blood samplings

Blood Sample (BS)		
Number	Abbreviation	Time Points
1 <sup>st</sup>	BS1	48 hours before dose 1
2 <sup>nd</sup>	BS2	48 hours before dose 2
3 <sup>rd</sup>	BS3	3 weeks after dose 2
4 <sup>th</sup>	BS4	48 hours before dose 3 (Identifies 4 months after dose 2)
5 <sup>th</sup>	BS5	3 weeks after dose 3

6 <sup>th</sup>	BS6	48 hours before dose 4 (Identifies 4 months after dose 3)
7 <sup>th</sup>	BS7	3 weeks after dose 4

**Supplementary Table S2.** Cluster of Differentiation (CD) detected on B- and T- lymphocytes

**CD**

**markers      Fluorochrome      Clone**

CD45      KROME      J33

**B-cell markers**

CD19      PC7      J4.119

IgD      FITC      IA6-2

CD27      ECD      IA4CD27

CD24      SN3      PerCP-Cy5.5

CD38      A750      LS198-4-3

**T-cell markers**

CD3      FITC      UCHT1

CD4      APC      13B8.2

CD8      PC5.5      B9.11

CD56      A700      N901NKH-1

HLA-DR      PB      Immu-357

CD38      A750      LS198-4-3

PD1      PE      EH12.2H7

**Supplementary Table S3.** Patient Demographics

<b>N</b>	<b>57</b>
Male/Female	34/23
Age (years)	48(19)
Transplantation Vintage (years)	9.1(14)
Hemodialysis Vintage (months)	18(46)
eGFR (mL/min/1.73m <sup>2</sup> )	54.3(20.3)
Tacrolimus levels (ng/mL)	6(1.5)
Cyclosporine levels (ng/mL)	268.5(222)
Preemptive Kidney transplantation	19.3% (11/57)
Retransplantation history	19.3% (11/57)
Diabetes Mellitus history	7% (4/57)
Treatment with mycophenolic acid	84.2% (48/57)

**Supplementary Table 4.** Presentation of the number (and percentage) of patients who developed serum levels of anti-RBD Abs and Nabs above the threshold and were regarded as responders, at the relevant time points

	<b>BS1</b>	<b>BS2</b>	<b>BS3</b>	<b>BS4</b>	<b>BS5</b>	<b>BS6</b>	<b>BS7</b>
<b>Anti-RBD Abs</b>	0%	6/57 (10.53%)	27/57(47.37%)	25/57(44.86%)	49/57(85.96%)	45/57(78.95%)	53/57(92.98%)
<b>NAbs</b>	0%	4/57 (7.02%)	18/57(31.58%)	6/57(10.53%)	40/57(70.18%)	39/57(68.42%)	53/57(92.98%)

Abbreviations: Anti-RBD Abs: anti-receptor binding domain antibodies; BS, blood sampling; and Nabs, neutralizing antibodies.

**Supplementary Table S5.** Differences in eGFR levels at time of first vaccination dose, and in HDV, between responders and non-responders to BNT162b2

eGFR levels (ml/min/1.73m <sup>2</sup> )							
Anti-RBD	responders	non-responders	p	NAb titers	responders	non-responders	p
Ab titers							
<b>BS2</b>	51.3±14.5	54.4±14.9	NS	<b>BS2</b>	61±3.6	53.5±14.9	NS
<b>BS3</b>	61.4±15.9	52.5±14.9	0.042	<b>BS3</b>	57.2±10.7	56.7±16.8	NS
<b>BS4</b>	58.5±15.4	50±18.1	NS	<b>BS4</b>	61.1±14.3	49.5 ± 13.2	0.04
<b>BS5</b>	58.1±17	48±16.7	NS	<b>BS5</b>	59.2±15.8	51.4±19.4	NS
<b>BS6</b>	60±13.8	46.3±14.6	0.039	<b>BS6</b>	61±12.3	49.5±13.2	0.04
<b>BS7</b>	60.1±14.3	-	-	<b>BS7</b>	61.1±14.6	48.5±4.9	NS
HDV (months)							
Anti-RBD	responders	non-responders	p	NAb titers	responders	non-responders	p
Ab titers							
<b>BS2</b>	3.5(38)	26.5(47)	NS	<b>BS2</b>	3.5(-)	21(46)	NS
<b>BS3</b>	17.5(42)	18.5(50)	NS	<b>BS3</b>	3(7)	22.5(44)	0.038
<b>BS4</b>	15.5(38)	21(61)	NS	<b>BS4</b>	32(-)	15.5(44)	NS
<b>BS5</b>	24(42)	41(54)	NS	<b>BS5</b>	14.5(37)	50.5(77)	0.043
<b>BS6</b>	24(42)	23(74)	NS	<b>BS6</b>	24(39)	25(70)	NS
<b>BS7</b>	33.5(58)	-	-	<b>BS7</b>	28(39)	41(-)	NS

Abbreviations: Anti-RBD Abs, anti-receptor binding domain antibodies; BS, blood sampling; eGFR, estimated glomerular filtration rate; HDV, hemodialysis vintage; Nabs, neutralizing antibodies; and NS, non-significant.

**Supplementary Table S6.** Anti-SARS-CoV-2 antibody development with regards to MPA treatment

anti-RBD Abs				Nabs		
	p	OR	CI	p	OR	CI
	(MPA+/MPA-)			(MPA+/MPA-)		
<b>BS2</b>	NS	-	-	0.001	0.057	0.015-0.219
<b>BS3</b>	NS	-	-	0.003	0.048	0.007-0.346
<b>BS4</b>	NS	-	-	NS	-	-
<b>BS5</b>	NS	-	-	NS	-	-
<b>BS6</b>	0.005	0.022	0.001-0.313	NS	-	-
<b>BS7</b>	NS	-	-	NS	-	-

Abbreviations: Anti-RBD Abs, anti-receptor binding domain antibodies; BS, blood sampling; CI, confidence interval; MPA, mycophenolic acid; Nabs, neutralizing antibodies; and OR, odds ratio.

**Supplementary Table S7.** Cell subpopulation concentrations (cells/ $\mu$ L) in BS1 and BS3 [NAb(-) patients in BS3]

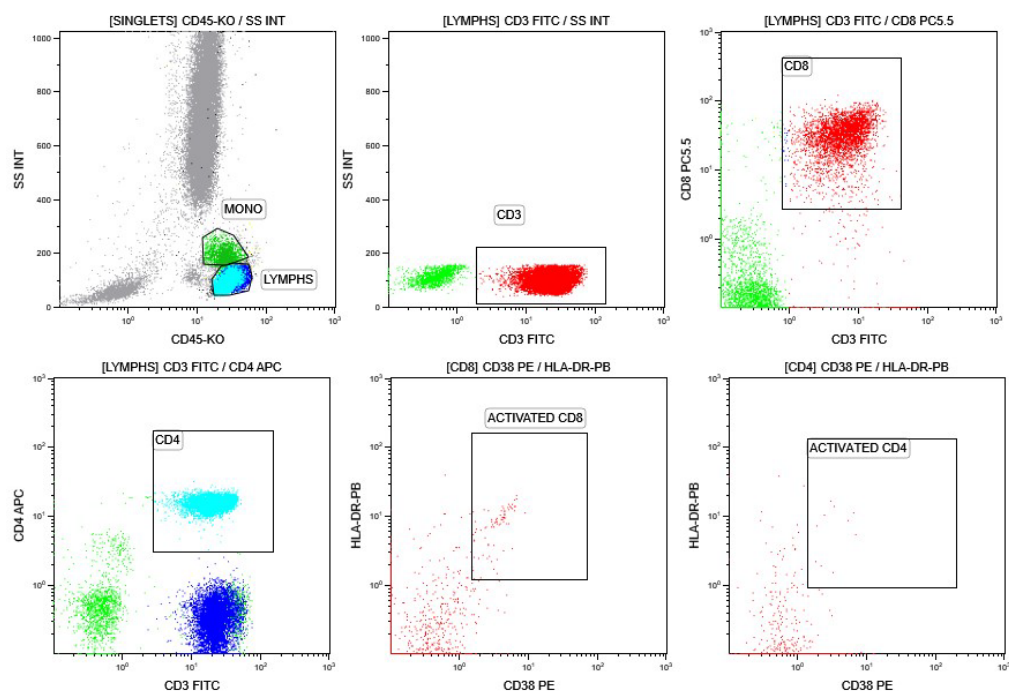
	<b>BS1</b>	<b>BS3</b>	<b>p (BS1 vs. BS3)</b>
<b>WBC</b>	7850(2325)	7650(1750)	NS
<b>Lymphocytes</b>	1621.5(1198)	1861(851)	NS
<b>B-cells</b>	68(107)	66(101)	NS
<b>Naïve B-cells</b>	48.5(83)	46(72)	NS
<b>Transitional B-cells</b>	3(10)	1.5(2)	0.014
<b>Marginal B-cells</b>	10.9(12.2)	9.7(9.2)	NS
<b>Memory B-cells</b>	13(31)	18(24)	NS
<b>Plasmablasts</b>	0.85(3.4)	0(0.4)	0.003
<b>CD3+ T-cells</b>	1314(1102)	1528(877)	NS
<b>CD3+CD4+ T-cells</b>	832(584)	921(525)	NS
<b>Activated CD4+ T-cells</b>	5.5(6)	7.5(5)	NS
<b>CD3+CD8+ T-cells</b>	527.5(563)	560(322)	NS
<b>Activated CD8+ T-cells</b>	7(10)	19.5(28)	<0.001
<b>CD3+PD1+ T-cells</b>	79(94)	52(49)	0.029
<b>CD3-CD56+ (NK cells)</b>	21.1(65.5)	170.5(133.2)	<0.0001
<b>CD3+CD56+ (NKT)</b>	160.5(172)	143(140)	NS
<b>cells</b>			
<b>Activated NKT cells</b>	1(3)	2(4)	0.015
<b>Monocytes</b>	411(374)	511(281)	NS
<b>Activated monocytes</b>	211(217)	360.5(221)	0.025

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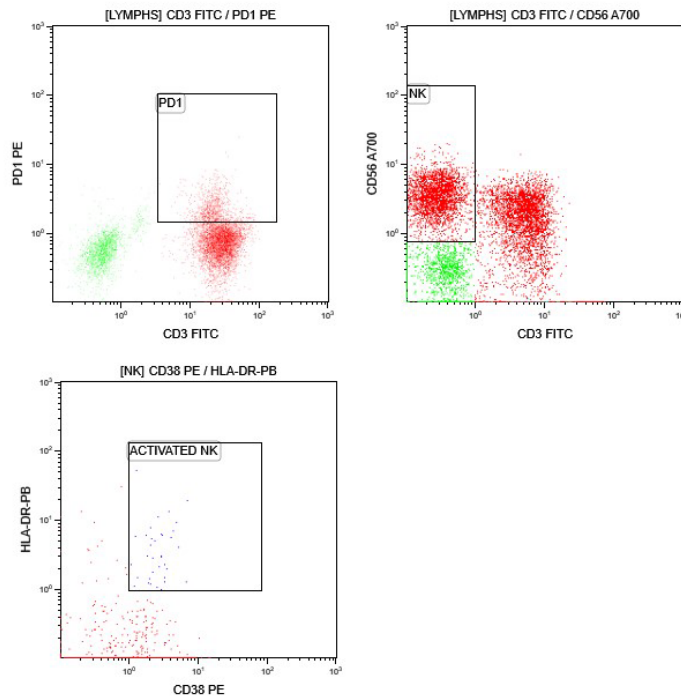
Abbreviations: BS, blood sampling; CD, cluster of differentiation; NK, Natural Killer; NKT-cells, Natural Killer like T-cells; and NS, non-significant.

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## Supplementary Figures

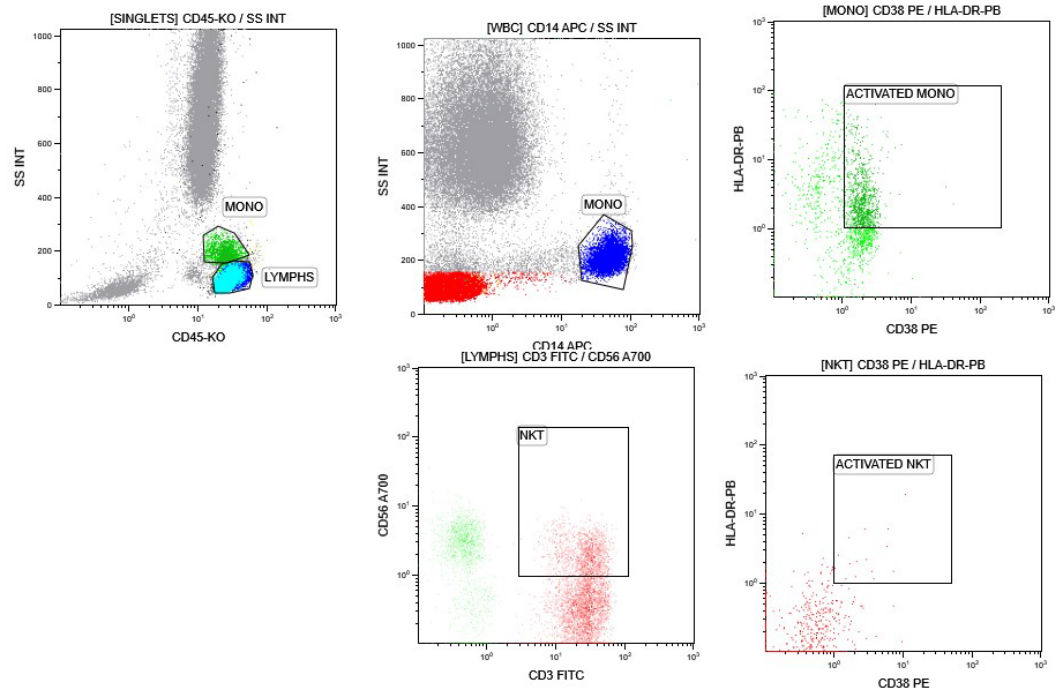


**Supplementary Figure S1.** Gating strategy for CD3, CD4, CD8 and Activated CD4, Activated CD8 respectively.

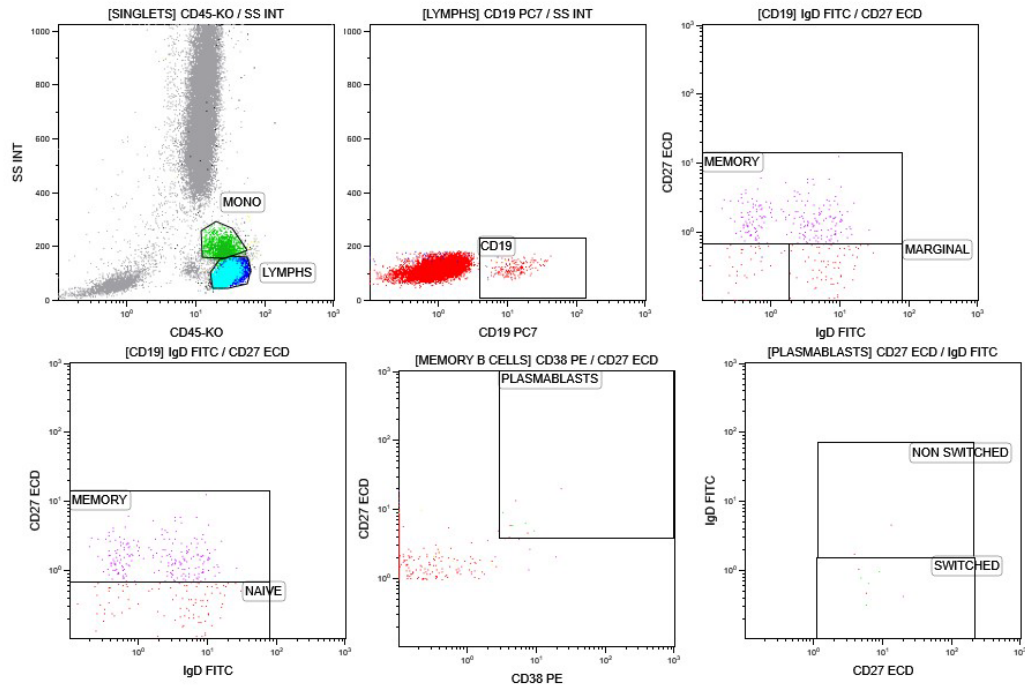


**Supplementary Figure S2.** Gating strategy for CD3-CD56+ (Natural Killer – NK-) cells and Activated NK cells.

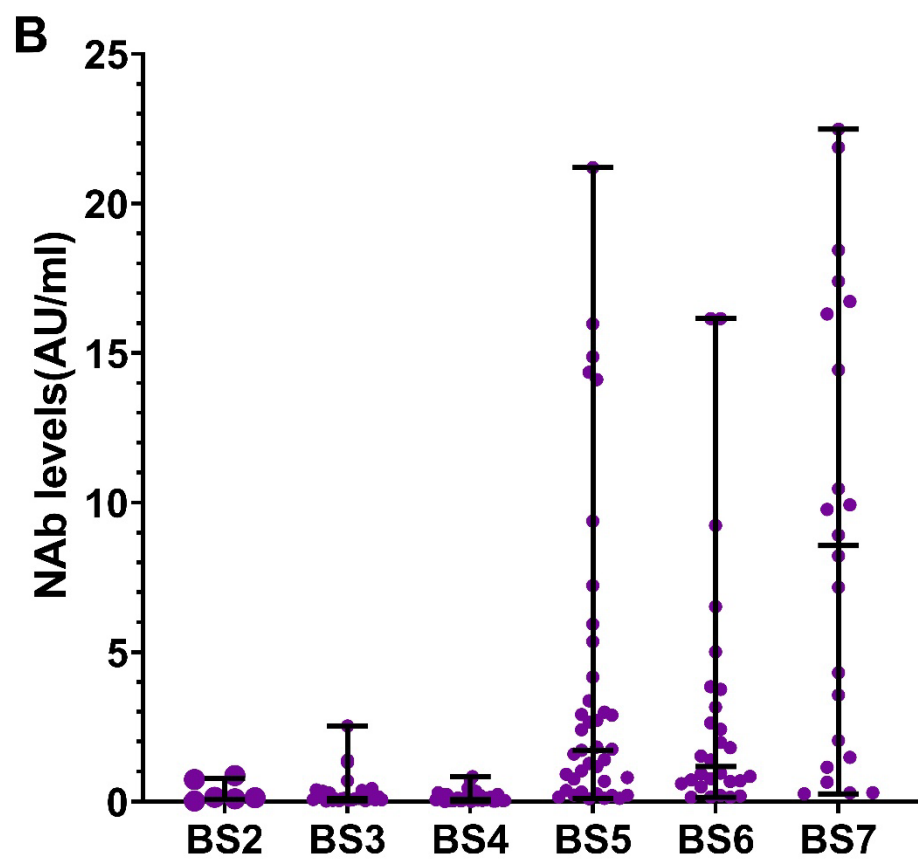
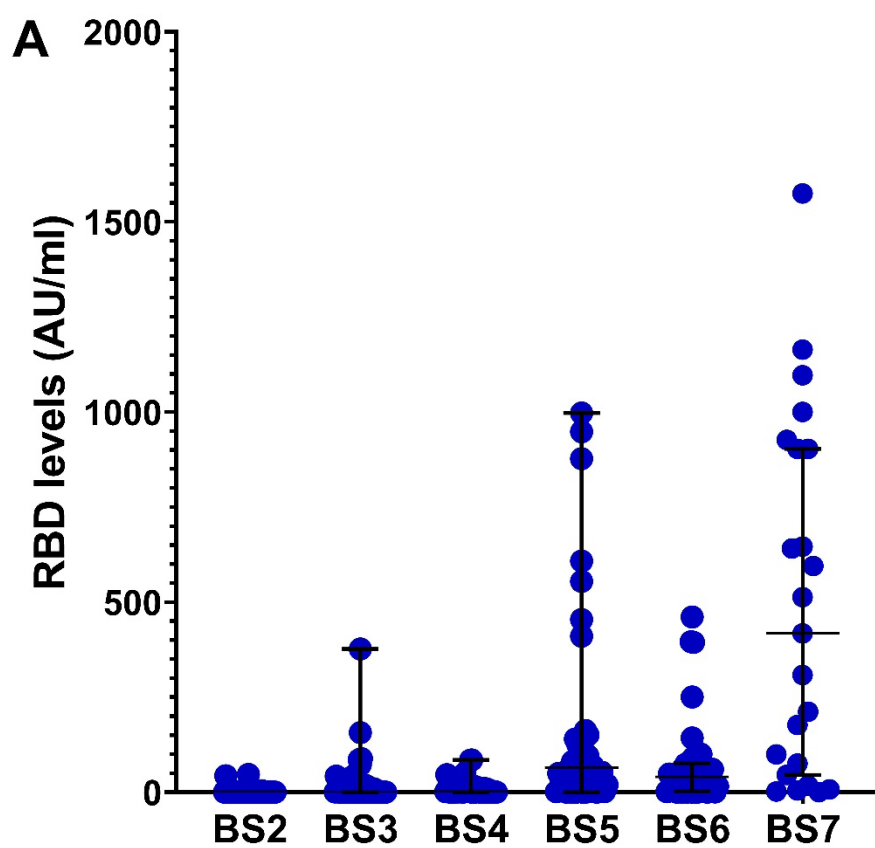




**Supplementary Figure S3.** Gating strategy for Monocytes and Activated Monocytes, CD3+CD56+ (NKT-cells) and Activated NKT-cells.



**Supplementary Figure S4.** Gating strategy for CD19<sup>+</sup> cells, CD19<sup>+</sup>CD27<sup>-</sup> (naïve), CD19<sup>+</sup>CD27<sup>+</sup> (memory), CD19<sup>+</sup>CD27<sup>-</sup>IgD<sup>+</sup> (marginal), CD19<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup>(plasmablasts), CD19<sup>+</sup>CD27<sup>+</sup>IgD<sup>+</sup> (non switched) and CD19<sup>+</sup>CD27<sup>+</sup>IgD<sup>-</sup>(switched) cells.



**Supplementary Figure S5.** Serum levels of anti-RBD Abs (A) and NAbs (B) at certain time points after vaccination, during follow up, BS2, BS3, BS4, BS5, BS6, BS7. Levels at baseline, BS1, are not demonstrated because they were zero.