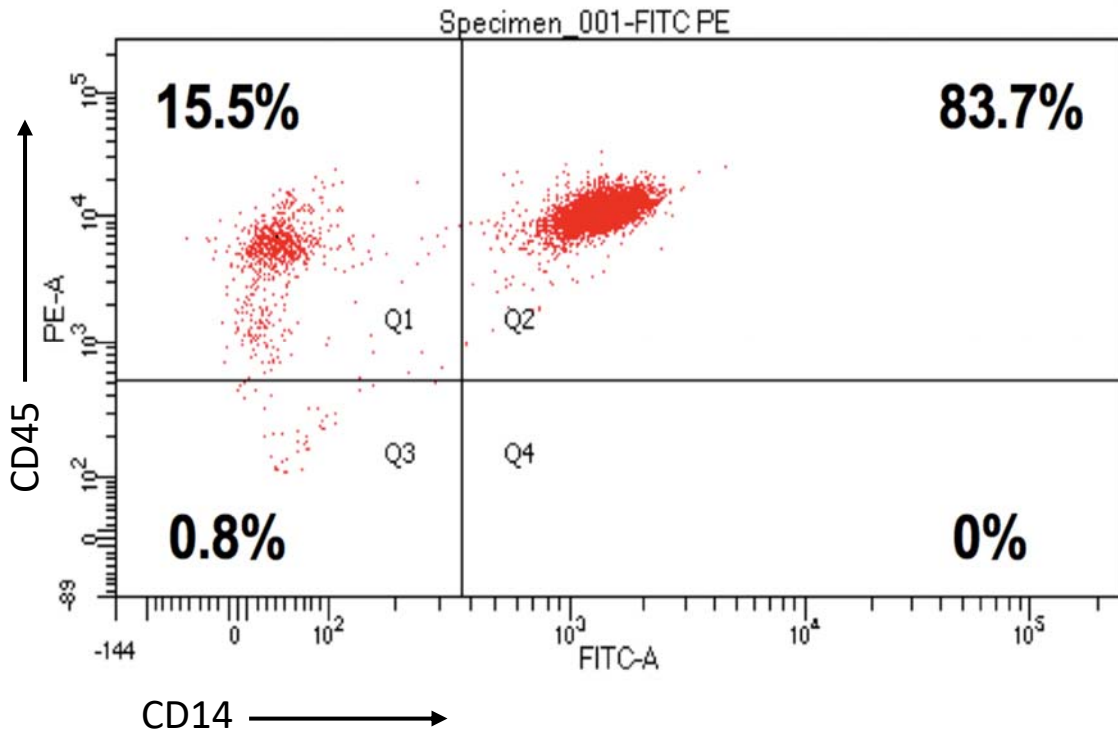**APPENDIX**

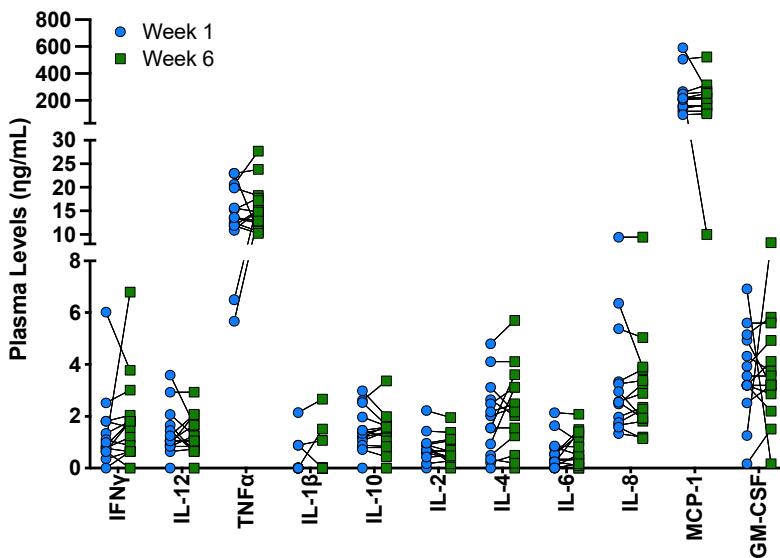*Supplementary Fig. 1.* Purity of isolated monocytes.

Graham et al. Innate immune memory is associated with increased disease-free survival in bladder cancer patients treated with bacillus Calmette-Guérin

Supplementary Fig. 2. Plasma cytokine and chemokine levels from individual non-muscle-invasive bladder cancer (NMIBC) patients before (blue circles, week 1) or after (green squares, week 6) initiation of BCG therapy in patients without recurrence *(A)* and patients experiencing recurrence *(B).* Student's t-test with Welch's correction comparing the change in cytokine levels (week 6 – week 1) for each patient did not reveal significant differences for any of these inflammatory mediators. n=14 no recurrences; 5 recurrences. BCG: bacillus Calmette-Guérin.
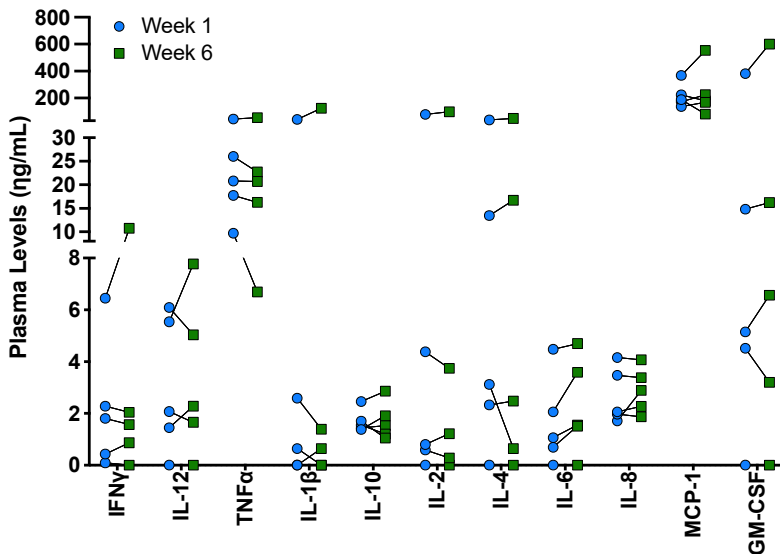
Supplementary Fig. 3. Receiver operating curves (ROC) generated using post-BCG:pre-BCG ratios of cytokine release obtained after two instillations of BCG (week 3). BCG: bacillus Calmette-Guérin.
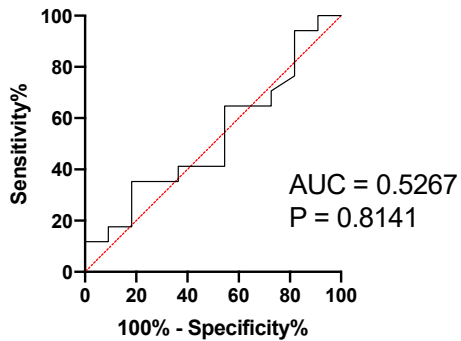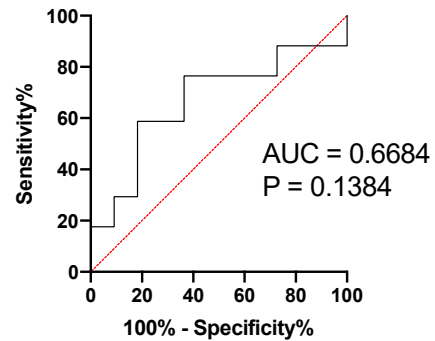
Graham et al. Innate immune memory is associated with increased disease-free survival in bladder cancer patients treated with bacillus Calmette-Guérin

Supplementary Fig. 4. Receiver operating curves (ROC) generated using post-BCG:pre-BCG ratios of cytokine release obtained after five instillations of BCG (week 6). BCG: bacillus Calmette-Guérin.
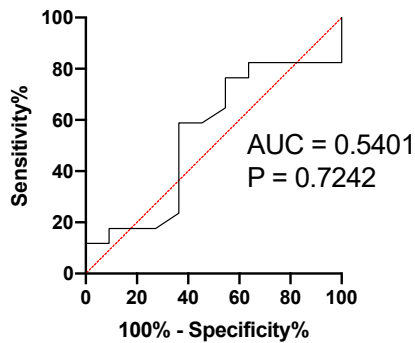
ROC curve: ROC of Week 6 ROC curve data. TNF
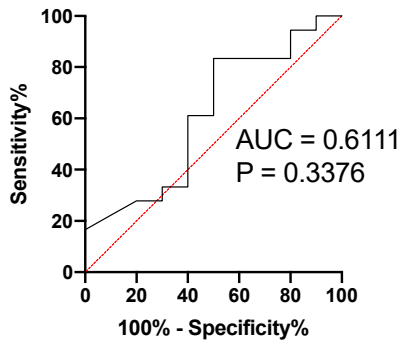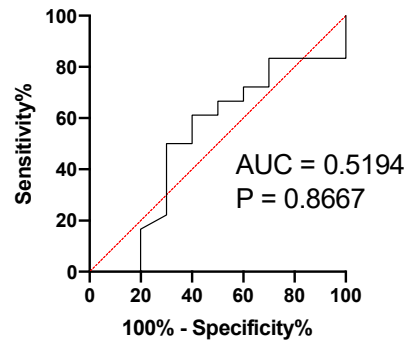
AUC = 0.6111
P = 0.3376

ROC curve: ROC of Week 6 ROC curve data. IL-1 β

AUC = 0.5194
P = 0.8667

ROC curve: ROC of Week 6 ROC curve data. IL-6

AUC = 0.6667
P = 0.1503

ROC curve: ROC of Week 6 ROC curve data. IL-10

AUC = 0.6694
P = 0.1436

ROC curve: ROC of Week 6 ROC curve data. IL-12

AUC = 0.8111
P = 0.0073

ROC curve: ROC of Week 6 ROC curve data. IFN γ

AUC = 0.6167
P = 0.3140
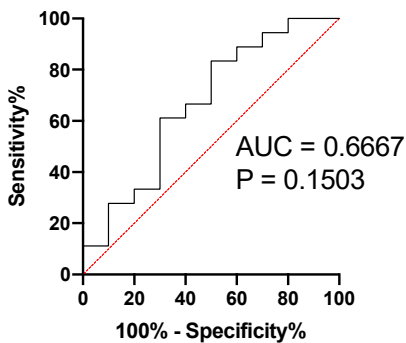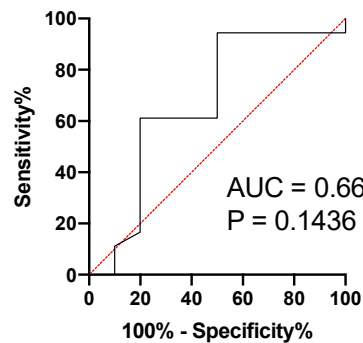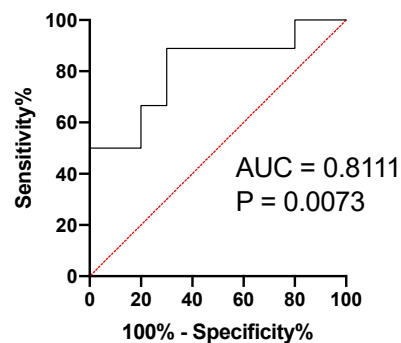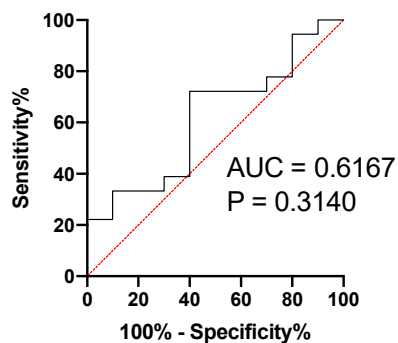
Supplementary Table 1. Post-BCG:Pre-BCG cytokine ratios for patients without recurrences (week 3)							
Patient number	TNFα	IL-1β	IL-6	IFNγ	IL-12	IL-10	Followup months
1	108.2	81.1	424.5	4.8	12.9	216.7	40.2
2	26.3	17.4	160.2	3.8	1.0	67.8	39.8
4	1.1	1.1	4.0	2.4	9.9	6.6	37.2
9	2.59	2.13	1.33	0.44	2.49	0.54	24.2
10	0.84	1.04	0.77	0.12	0.89	0.67	22.8
11	0.6	0.81	0.56	0.94	0.79	1.16	22.8
12	1.58	0.82	0.21	1.07	1.24	0.84	21.0
13	1.31	0.98	1.28	2.05	0.89	1.52	20.3
14	1.19	0.42	1.07	1.3	1.54	0.54	19.1
15	1.1	1.03	1.17	0.54	1.1	0.78	18.9
16	0.89	0.9	0.91	1.07	0.91	0.95	18.9
17	0.95	0.56	0.84	1.38	1.28	1.49	18.9
18	1.02	2.24	1.04	2.36	0.92	1	17.5
19	0.91	1.46	0.87	1.11	1.35	4.79	17.0
20	1.5	1.12	1.04	1.39	1.23	1.04	17.0
21	1.14	1.17	0.96	1.2		0.37	16.6
22	0.86	0.56	0.96	0.82	1.39	0.89	16.6
Median	1.1	1.0	1.0	1.2	1.2	1.0	19.1
Mean	8.9	6.8	35.4	1.6	2.5	18.1	22.9
SD	26.3	19.6	107.4	1.2	3.5	53.6	8.1

BCG: bacillus Calmette-Guérin; SD: standard deviation.

Supplementary Table 2. Post-BCG:Pre-BCG cytokine ratios for patients who suffered recurrences (week 3)

Patient number	TNFα	IL-1β	IL-6	IFNγ	IL-12	IL-10	Months to recurrence	Followup months
23	0.91	0.86	1.07	0.48	0.74	0.93	9.5	19.3
24	12.06	6.22	2.5	1.78	1.35	18.57	4.4	40.2
25	1.24	4.26	2.83	1.87	0.94	2.98	3.4	39.8
26	1	2.12	0.78	1.38	2.36	2.54	13.3	39.3
27	1	1.09	1.05	0.11	0.08	0.5	10.7	37.2
28	1.88	1.82	0.84	0.68	3.02	5.12	6.9	32.4
29	1.16	1.09	0.6	0.52	0.21	4.87	13.7	28.5
30	1.16	1.49	1.98	1.25	1.07	0.54	9.1	28.1
31	1.21	1.56	1.93	5.95	0.73	3.14	2.7	24.4
32	0.61	2.6	1.29	1.46	1.26	0.92	10.0	20.7
33	0.46	0.76	0.82	0.74	0.43	1.23	2.4	20.5
Median	1.2	1.6	1.1	1.3	0.9	2.5	9.1	28.5
Mean	2.1	2.3	1.5	1.5	1.1	3.8	7.8	30.0
SD	3.5	1.7	0.8	1.6	0.9	5.2	4.3	8.2

BCG: bacillus Calmette-Guérin; SD: standard deviation.

Supplementary Table 3. Post-BCG:Pre-BCG cytokine ratios for patients without recurrences (week 6 only)

Patient number	TNFα	IL-1β	IL-6	IFNγ	IL-12	IL-10	Followup months
3	13.64	13.29	111.24	10.79	5.88	370.31	38.6
5	0.98	0.8	1.08	0.81	0.94	1.56	33.1
6	0.17	0.28	0.78	0.46	0.88	0.21	32.4
7	1.61	1.71	1.39	3.29	1.39	0.6	28.1
8	0.91	0.63	0.88	1.02	0.51	0.85	24.9
9	1.53	1.65	1.42	0.62	1.96	0.88	24.2
10	0.55	0.91	0.88	0.15	1.46	0.49	22.8
11	0.58	0.85	0.62	2.75	1	1.27	22.8
12	1.11	1.07	3.01	0.81	1.58	0.35	21.0
13	1.81	1.46	1.71	1.35	0.54	1.19	20.3
14	0.93	0.9	0.97	1.19	1.15	0.64	19.1
15	1.24	1.21	1.14	0.46	1.16	0.78	18.9
16	0.85	0.79	1.04	0.87	0.9	0.87	18.9
17	1.21	0.55	0.91	0.58	1.82	0.64	18.9
18	0.92	3.24	1.04	3.33	1.24	1.59	17.5
19	1	0.67	0.81	4.37	2.4	1.65	17.0
20	1.1	0.97	1.05	0.79	1.27	1.15	17.0
21	0.98	2.31	1.03	1.06	2.15	0.73	16.6

Graham et al. Innate immune memory is associated with increased disease-free survival in bladder cancer patients treated with bacillus Calmette-Guérin

Median	0.99	0.94	1.04	0.95	1.23	0.86	20.6
Mean	1.73	1.85	7.28	1.93	1.57	21.43	22.9
SD	3.0	2.94	25.95	2.51	1.19	87.1	6.3

BCG: bacillus Calmette-Guérin; SD: standard deviation.

Supplementary Table 4. Post-BCG:Pre-BCG cytokine ratios for patients who suffered recurrences (week 6 only)

Patient number	TNFα	IL-1β	IL-6	IFNγ	IL-12	IL-10	Months to recurrence	Followup months
23	0.79	0.19	0.96	1.1	0.57	1.05	9.5	19.3
24	1.23	0.93	1.13	0.7	1.26	7.19	4.4	40.2
25	1.24	1.87	2.31	2.84	0.64	2.37	3.4	39.8
26	0.03	0.12	0.01	0.61	0.18	0.05	13.3	39.3
28	1.16	1.8	0.48	0.38	1.26	4.39	6.9	32.4
29	0.62	1.09	0.8	0.78	0.26	4.7	13.7	28.5
30	0.97	1.49	1.01	0.71	0.82	1.11	9.1	28.1
31	1.53	1.41	1.26	1.31	0.6	2.28	2.7	24.4
32	0.81	1.89	0.85	1.29	1.11	0.89	10.0	20.7
33	0.52	0.67	0.68	0.12	0.73	0.49	2.4	20.5
Median	0.89	1.25	0.9	0.75	0.69	1.7	8.0	28.3
Mean	0.89	1.15	0.95	0.98	0.74	2.45	7.5	29.3
SD	0.43	0.66	0.6	0.75	0.38	2.29	4.4	8.3

BCG: bacillus Calmette-Guérin; SD: standard deviation.

Supplementary Table 5. Median cytokine levels and ranges (pg/mL) in LPS-stimulated cultures of monocytes isolated from patients without early recurrences

	Week 1			Week 3			Week 6		
	Median	Upper limit	Lower limit	Median	Upper limit	Lower limit	Median	Upper limit	Lower limit
IFNγ	5.6	117.1	0.8	9.5	24.8	2.4	8.2	45.9	2.6
IL-12	4.8	31.3	0.5	9.4	105.2	0.0	6.6	45.7	1.0
IL-6	845.0	67 309.0	26.9	941.0	75 141.0	575.0	1388.9	115 233.0	580.0
TNFα	2528.5	13 200.0	76.3	2656.5	15 000.0	2283.0	2787.3	15 000.0	1172.0
IL-1ß	9324.5	19 316.5	63.6	8614.0	19468.0	3385.0	10 349.4	16 349.7	1610.3
IL-10	3957.0	12 434.1	0.8	3841.0	8585.0	54.2	4980.2	9855.0	272.7

LPS: lipopolysaccharide.

Graham et al. Innate immune memory is associated with increased disease-free survival in bladder cancer patients treated with bacillus Calmette-Guérin

Supplementary Table 6. Median cytokine levels and ranges (pg/mL) in LPS-stimulated cultures of monocytes isolated from patients with early recurrences

	Week 1			Week 3			Week 6		
	Median	**Upper limit**	**Lower limit**	**Median**	**Upper limit**	**Lower limit**	**Median**	**Upper limit**	**Lower limit**
IFNγ	7.9	52.5	3.2	10.6	21.5	5.5	5.5	45.9	1.4
IL-12	9.1	107.7	0.7	7.4	10.2	3.6	6.1	16.9	0.9
IL-6	22 179.0	126 668.0	798.0	95 884.0	114 720.0	853.0	2315.0	95 884.0	680.0
TNFα	4105.3	15 000.0	1042.3	1908.8	15 000.0	1057.1	2531.0	15 000.0	449.4
IL-1ß	7371.9	23 533.0	1451.5	14 118.1	25 678.7	10 817.0	9756.5	25 678.7	764.3
IL-10	1273.9	9432.0	6.6	6996.8	8750.0	4715.0	2920.0	9855.0	47.5

LPS: lipopolysaccharide.