Supplemental material

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J Immunother Cancer

1 **Supplementary Figure 1.** Treatment and PBMC sampling schedule. **A)** In the MM study, patients
2 received up to 10 UV1 vaccinations (300 µg) and 4 doses of ipilimumab (green arrows, 3 mg/kg). PBMCs
3 were sampled at 4 time points during the study period and every 6 months thereafter for up to 5 years. **B)**
4 In the NSCLC study, patients received up to 13 UV1 vaccinations (100 µg, 300 µg, or 700 µg) and
5 additional vaccinations thereafter if not considered inappropriate due to clinical deterioration. PBMCs
6 were sampled at 8 time points during the study period and every 6 months thereafter for up to 5 years. **C)**
7 In the PC study, patients received up to 13 vaccinations (100 µg, 300 µg, or 700 µg) during the study
8 period and additional vaccinations up to 2 years if considered clinically appropriate. Patients also received
9 goserelin 10.8 mg every 3 months, and bicalutamide 50 mg per day. PBMCs were sampled at 8 time
10 points during the study period and thereafter every 6 months for up to 8 years.
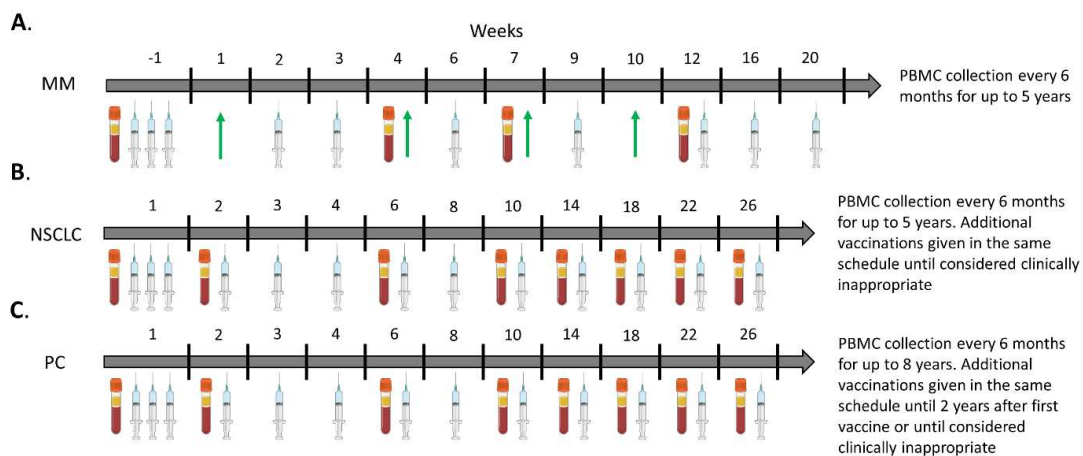
11

12 **Supplementary Figure 2.**

13 Overview of immune response evaluable patients and patients enrolling in the long-term
14 immunomonitoring study. IR, immune response. Figure created with Biorender.com.
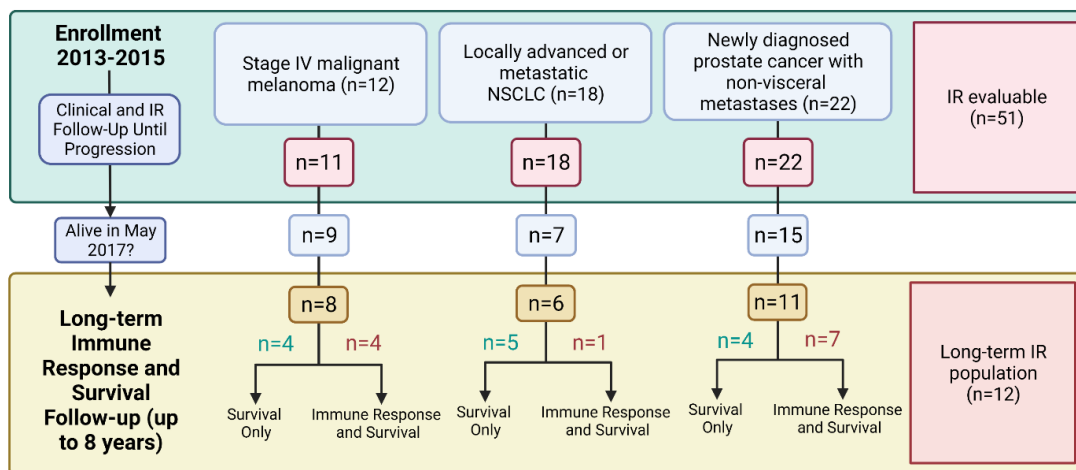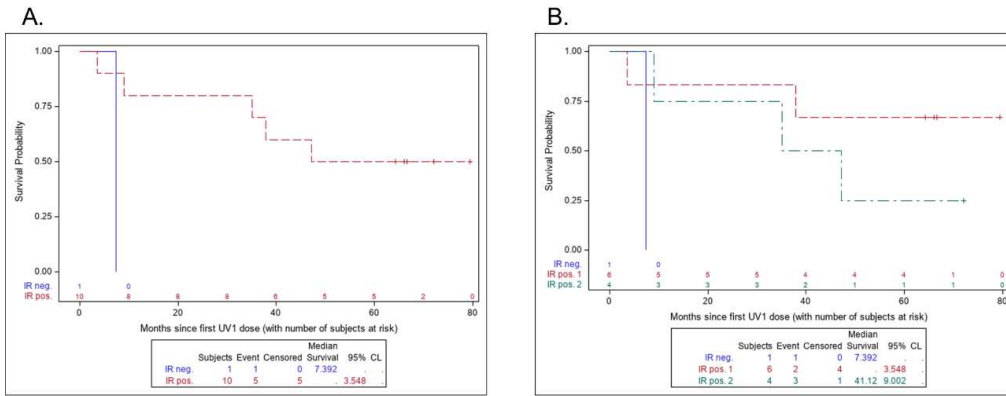

15

16   **Supplementary Figure 3.**

17   Kaplan-Meier plots for immune response (IR) positive vs negative patients in each study (red curves

18   indicate IR positive population, and blue curve IR negative. **A)** Melanoma, IR positive vs. IR negative,

19   mOS, not reached (NR) vs. 7.4 months (Log-rank p-value 0.05). **C)** NSCLC, IR positive vs. IR negative,

20   mOS, 38.4 months vs. 21.3 months (Log-rank p-value 0.67). **E)** PC, IR positive vs. IR negative, mOS,

21   76.0 months vs. 30.0 months (Log-rank p-value 0.20).

22   Kaplan-Meier plots for patients with IR against ≥2, 1, or none of the UV1 peptides in each study (green

23   curves indicate IR against ≥2, red curve 1, and blue curve 0 peptides recognized). **B)** Melanoma, IR

24   positive ≥2 vs. 1 vs no peptide, mOS, 41.1 months vs. NR vs. 7.4 months (Log-rank p-value 0.09). **D)**

25   NSCLC, IR positive ≥2 vs. 1 vs no peptide, mOS, 60.4 months vs. 15.6 months vs. 21.3 months (log-rank

26   p-value 0.51). **F)** PC, IR positive ≥2 vs. 1 vs no peptide, mOS, NR vs. 44.8 months vs. 30.0 months (log-

27   rank p-value 0.12).

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Malignant Melanoma

A.

B.

Non-Small Cell Lung Cancer

C.
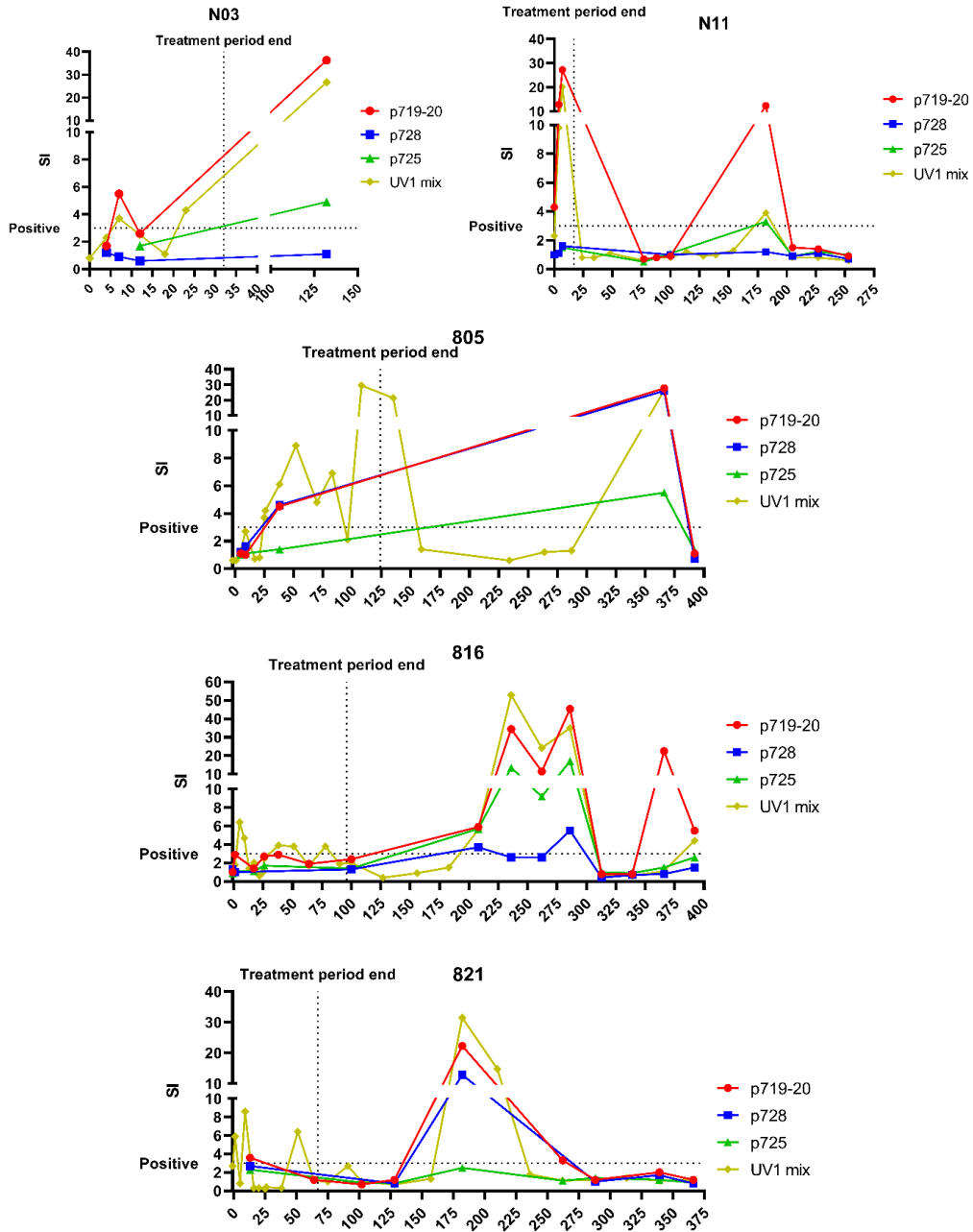

D.

Prostate Cancer

E.

F.
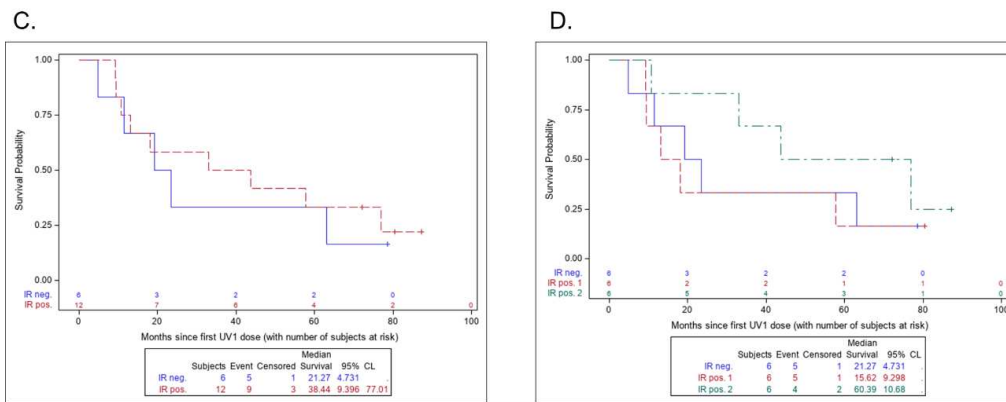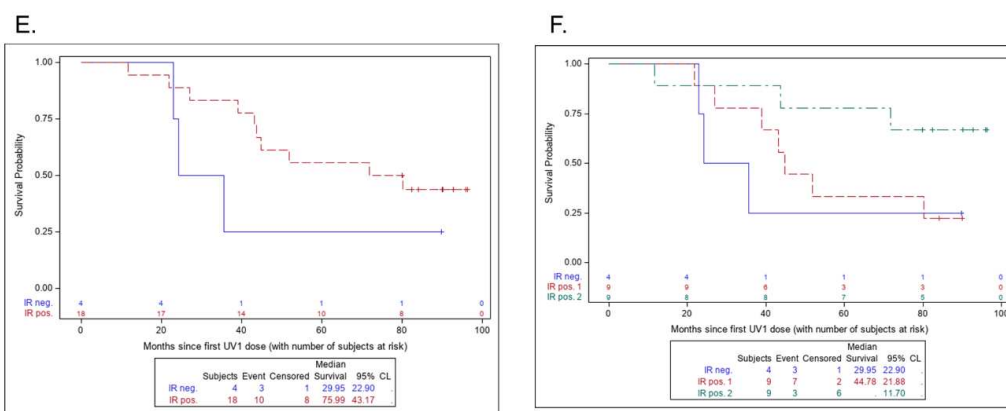
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30

31 **Supplementary Figure 4.**

32 Detailed overview of immune responses against single peptides in five patients experiencing broadening

33 of immune responses during late peaks. A manuscript with detailed clinical follow-up of patients in the

34 PC study is being prepared.

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37   **Supplementary Figure 5.**

38   **A**) Distribution of somatic mutations in the hTERT gene. **B**) Variant allele frequency (VAF) of promoter
39   mutations vs. missense / nonsense mutations in the hTERT gene. There was a significant difference in
40   mean VAF between promoter (0.33, SD 0.15) and missense / nonsense (0.21, SD 0.13) mutations
41   (Student's t-test, p value < 0.0001. Data gathered from the MSK-IMPACT Clinical Sequencing Cohort
42   using cBioPortal.org.

43
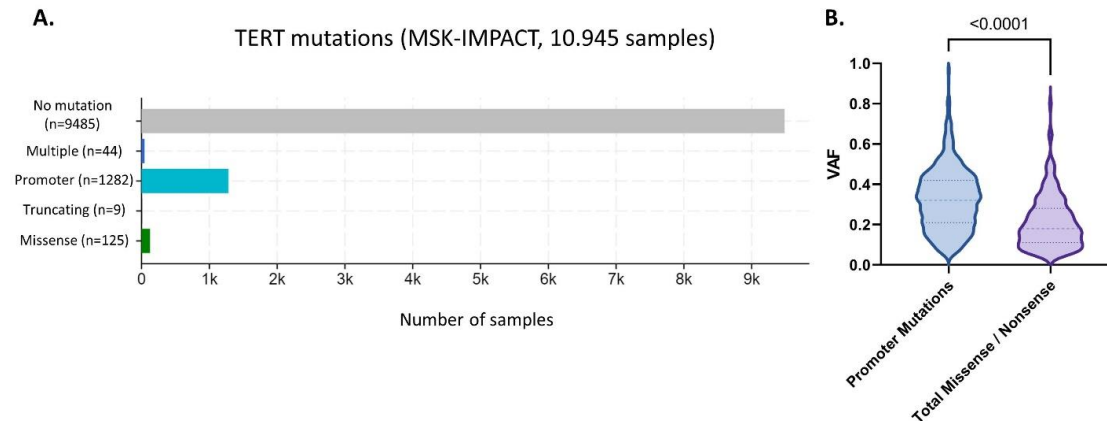
44   **Supplementary Table 1.**

45   Patients and their characteristics from which vaccine-specific T cell clones (TCCs) were isolated and
46   evaluated for in vitro cytokine production. 17 TCCs were selected for in vitro characterization, 4 TCCs
47   from patient N02 in the MM study, 4 TCCs from patient 802, 3 TCCs from patient 806, and 4 TCCs from
48   patient 822 in the PC study, and 2 TCCs from patient 906 in the NSCLC study. HLA restriction was
49   performed by HLA blocking antibodies (HLA gene only) and/or EBV-LCL panels (locus including four-
50   field resolution). Color coded amino acid sequence for the complementarity-determining region 3 of the T
51   cell receptor. "-", not done.

Trial	ID	Age/Sex	Survival Time	TCC Isolation Timepoint	SI at Isolation Timepoint	TCC# and Peptide Specificity	HLA Restriction	Cytokine characterization	CDR3 amino acid sequence
MM	N02	49/M	9.0 mo	Week 7	60.0	#7 p719-20	DR	Yes	
						#13 p719-20	DR	Yes	
						#28 p719-20	DR	Yes	
						#15 p719-20	DR	No	
PC	802	53/M	Alive at 96.3 mo	Week 18	31.9	#10 p719-20	DQB1*03:01	Yes	
						#12 p719-20	DR	Yes	
						#38 p719-20	DRB1*12:01	No	
						#15 p725	DQB1*02:01	No	
	806	75/M	Alive at 92.8 mo	Week 10	15.5	#3 p719-20	-	Yes	
						#4 p728	-	Yes	Not available
						#5 p728	-	Yes	
	822	57/M	Alive at 79.9 mo	Week 14	29.1	#10 p725	DQB1*02:01	Yes	
						#12 p719-20	DRB1*07:01	Yes	
						#26 p719-20	DPB1*03:01	No	
						#62 p728	DPB1*03:01	No	Not available
NSCLC	906	64/F	33.1 mo	Week 5	39.0	#15 p719-20	-	Yes	
						#38 p725	-	Yes	Not available

52

53

Supplementary Table 2.

Overview of immune responses against single peptides or a mix of all in the treatment and follow-up periods, respectively. FU, follow up; NE, not evalable for immune response or not eligible for enrollment in long-term FU study due to death; ND, not done; IR, immune response; OS, overall survival.

58

Patient	Immune Responses in Treatment Period				Immune Responses in Follow up Period				Enrolled in long-term FU study
	p719-20	p728	p725	UV1 mix	p719-20	p728	p725	UV1 mix	
N01	+	-	-	+	+	-	-	+	Yes (IR+OS)
N02	+	-	+	+	ND	ND	ND	ND	NE
N03	+	-	-	+	+	-	+	+	Yes (IR+OS)
N04	+	+	+	+	+	-	+	+	Yes (IR+OS)
N05	+	-	-	+	ND	ND	ND	ND	NE
N06	+	-	-	+	ND	ND	ND	ND	Yes (OS)
N07	+	+	+	+	+	-	+	+	Yes (OS)
N08	+	+	+	+	ND	ND	ND	-	No
N09	+	-	-	+	+	-	-	-	Yes (IR+OS)
N11	+	-	-	+	+	-	+	+	Yes (OS)
N13	-	-	-	-	ND	ND	ND	ND	NE
N14	NE	NE	NE	NE	NE	NE	NE	NE	Yes (OS)
802	+	+	+	+	-	-	-	-	Yes (IR+OS)
803	-	-	-	+	-	-	-	+	No
804	-	-	-	-	-	-	-	-	NE
805	+	+	-	+	+	+	+	+	Yes (IR+OS)
806	+	+	+	+	+	+	+	+	Yes (IR+OS)
807	+	-	-	+	-	-	-	-	NE
808	+	-	+	+	ND	ND	ND	ND	NE
809	-	-	-	-	+	-	-	+	Yes (OS)
810	ND	ND	ND	-	ND	ND	ND	ND	NE
811	+	-	+	+	ND	ND	ND	ND	Yes (OS)
812	+	-	-	+	ND	ND	ND	ND	No
813	+	-	+	+	ND	ND	ND	ND	No
814	-	-	-	-	ND	ND	ND	ND	NE
815	+	-	+	+	-	-	-	+	Yes (OS)
816	-	-	-	+	+	+	+	+	Yes (IR+OS)
817	+	-	-	+	-	-	-	-	Yes (OS)
818	ND	ND	ND	+	ND	ND	ND	ND	NE
819	+	-	-	-	ND	ND	ND	ND	No
820	-	+	-	+	ND	ND	ND	ND	NE
821	+	-	-	+	+	+	-	+	Yes (IR+OS)
822	+	+	+	+	+	+	+	+	Yes (IR+OS)

823	+	+	+	+	+	+	+	+	Yes (IR+OS)
901	ND	ND	ND	-	ND	ND	ND	ND	NE
902	+	-	+	+	ND	ND	ND	ND	NE
903	-	-	-	-	ND	ND	ND	ND	NE
905	-	-	-	-	ND	ND	ND	ND	No
906	+	-	+	+	ND	ND	ND	ND	NE
907	ND	ND	ND	+	ND	ND	ND	ND	NE
908	+	+	+	+	+	+	+	+	Yes (IR+OS)
909	+	+	+	+	+	+	+	+	Yes (OS)
910	+	-	-	+	-	-	-	-	Yes (OS)
911	+	-	-	+	ND	ND	ND	ND	NE
912	ND	ND	ND	+	ND	ND	ND	ND	NE
913	-	-	-	-	-	-	-	-	Yes (OS)
915	+	+	+	+	ND	ND	ND	ND	NE
916	-	-	+	+	-	-	-	-	Yes (OS)
917	+	-	-	+	ND	ND	ND	ND	NE
919	-	-	-	-	-	-	-	-	NE
920	-	-	-	-	ND	ND	ND	ND	NE
922	+	+	+	+	+	+	+	+	Yes (OS)

59

60 **Supplementary Table 3.**

61 HLA class I and II genotypes and anti-UV1 immune response development. Patients with IDs starting
62 with "N" are from the MM study, "8" are from the PC study, and "9" from the NSCLC study. NE = not
63 evaluable, ND = not done.

	HLA class I				HLA class II					
Patient ID	HLA-A		HLA-B		HLA-DRB1		HLA-DQB1		HLA-DPB1	
N01	*01:01	*03:01	*07:02	*40:01	*04:04	*15:01	*03:02	*06:02	*03:01	*04:01
N02	*23:01	*24:02	*35:01	*44:03	*01:03	*07:01	*02:02	*05:01	*02:01	-
N03	*03:01	-	*07:02	*15:01	*04:01	*07:01	*02:02	*03:02	*01:01	*04:01
N04	*03:01	*25:01	*08:01	*18:01	*03:01	*15:01	*02:01	*06:02	*04:01	-
N05	*02:01	*03:01	*44:02	*51:01	*08:03	*15:01	*03:01	*06:02	*02:01	*04:01
N06	*01:01	*02:01	*40:01	*57:01	*07:01	*13:02	*03:03	*06:04	*04:02	-
N07	*02:01	*03:01	*07:02	*35:01	*07:01	*08:01	*03:03	*04:02	*04:01	-
N08	*02:01	*24:02	*15:01	*44:02	*01:01	*13:01	*05:01	*06:03	*03:01	*04:01
N09	*02:01	*03:01	*07:02	-	*15:01	-	*06:02	-	*04:01	-
N11	*02:01	*30:01	*13:02	*44:02	*07:01	*11:01	*02:02	*03:01	*02:01	*17:01
N13	*03:01	*26:01	*50:01	*52:01	*07:01	*11:01	*02:02	*03:01	*02:01	*03:01
N14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
802	*01	*02	*08:01	*44	*03:01	*12:01	*02:01	*03:01	*01:01	*04:02
803	*02:01	*24:02	*07:02	*40:01	*04:01	*08:01	*03:01	*04:02	*04:01	-
804	*03:01	*24:02	*07:02	*15:01	*04:01	*15:01	*03:02	*06:02	*04:01	-

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805	*24:02	*68:01	*07:02	*44:03	*07:01	*15:01	*02:02	*06:02	*04:02	*20:01
806	*01:01	*03:01	*07:02	*50:01	*13:02	*15:01	*06:02	*06:04	*03:01	-
807	*02:01	-	*27:05	*40:01	*11:01	*15:01	*03:01	*06:02	*04:01	-
808	*02:01	*68:01	*15:01	*40:01	*08:01	*10:01	*04:02	*05:01	*02:01	-
809	*01:01	*02:01	*08:01	*35:01	*03:01	*08:01	*02:01	*04:02	*01:01	*04:01
810	*01:01	*02:01	*08:01	*15:01	*03:01	*04:01	*02:01	*03:02	*04:01	-
811	*03:01	*68:01	*08:01	*56:01	*03:01	*08:01	*02:01	*04:02	*01:01	*04:01
812	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
813	*02:01	*11:01	*27:05	*45:01	*01:01	*11:01	*03:01	*05:01	*04:01	-
814	*02:01	*31:01	*27:05	*35:01	*08:01	-	*04:02	-	*03:01	-
815	*01:01	*02:01	*08:01	-	*03:01	*04:04	*02:01	*03:02	*02:01	*04:01
816	*01:01	-	*52:01	*57:01	*07:01	*16:02	*03:03	*05:02	*02:01	*10:01
817	*01:01	*03:01	*07:02	*51:01	*01:01	*15:01	*05:01	*06:02	*04:01	*14:01
818	*02:01	*03:01	*15:01	*44:02	*01:01	*13:01	*05:01	*06:03	*04:01	-
819	*01:01	*32:01	*08:01	*27:05	*03:01	*15:01	*02:01	*06:02	*02:01	*04:01
820	*31:01	*68:01	*07:02	*40:01	*04:04	*15:01	*03:02	*06:02	*04:01	*06:01
821	*01:01	*11:01	*27:05	*35:01	*03:01	*15:01	*02:01	*06:02	*03:01	*04:01
822	*02:01	-	*08:01	*15:01	*03:01	*07:01	*02:01	*03:03	*01:01	*03:01
823	*24:02	*25:01	*15:01	*44:02	*01:01	*04:01	*03:02	*05:01	*02:01	*04:01
901	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
902	*01:01	-	*08:01	-	*03:01	-	*02:01	-	*01:01	*04:01
903	*02:01	*03:01	*15:01	*40:01	*04:01	*04:04	*03:02	-	*02:01	*04:01
905	*02:01	*24:02	*07:02	*18:01	*15:01	-	*06:02	-	*04:01	*04:02
906	*03:01	*26:01	*07:02	*37:01	*13:01	*15:01	*06:02	*06:03	*04:01	-
907	*02:01	-	*15:01	*27:05	*04:01	*08:01	*03:02	*04:02	*02:01	*04:01
908	*01:01	*32:01	*37:01	*50:01	*04:01	*13:03	*03:01	*03:02	*03:01	*04:01
909	*03:01	-	*07:02	-	*15:01	-	*06:02	-	*04:01	*04:02
910	*01:01	*24:02	*40:01	*44:02	*04:04	*15:01	*03:02	*06:02	*02:01	*04:01
911	*02:01	-	*15:01	*40:01	*04:01	-	*03:02	-	*02:01	*04:02
912	*01:01	*02:01	*08:01	*15:01	*03:01	*08:01	*02:01	*04:02	*02:01	*04:01
913	*02:01	-	*08:01	*40:01	*03:01	*04:04	*02:01	*03:02	*01:01	*02:01
915	*01:01	*24:02	*07:02	*51:01	*13:01	*15:01	*06:02	*06:03	*04:01	*20:01
916	*02:01	*68:01	*07:02	-	*15:01	-	*06:02	-	*01:01	*05:01
917	*11:01	*26:01	*27:05	*35:01	*01:01	*14:01	*05:01	*05:03	*04:02	*16:01
919	*01:01	*02:01	*08:01	*44:02	*03:01	*04:01	*02:01	*03:01	*03:01	*04:02
920	*02:01	*26:01	*07:02	*44:02	*01:01	*04:01	*03:01	*05:01	*02:01	*04:01
922	*02:01	-	*13:02	*18:01	*07:01	*09:01	*02:02	*03:03	*04:01	-

64

65 **Supplementary Table 4.**

66 Patients and timepoints of subsequent checkpoint inhibitor therapy

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Patient	Trial	CPI received	Target	Timepoint of treatment initiation (weeks since first vaccination)	Timepoint of treatment cessation (weeks since first vaccination)
N01	MM	Nivolumab/ipilimumab	Anti-PD-1 / anti-CTLA-4	244	253
N03	MM	Pembrolizumab	Anti-PD-1	26	64
N04	MM	Pembrolizumab	Anti-PD-1	79	118
N07	MM	Nivolumab	Anti-PD-1	77	180
N08	MM	Pembrolizumab	Anti-PD-1	54	81
N11	MM	Nivolumab	Anti-PD-1	155	Unknown
N14	MM	Pembrolizumab	Anti-PD-1	50	152
902	NSCLC	Nivolumab	Anti-PD-1	112	126

67

68 **Supplementary Table 5.**

69 Multiplex cytokine assay for detecting Th1 and Th2 differentiation of vaccine-specific T cell clones
70 (TCCs) from 5 patients in the MM, PC, and NSCLC studies. Vaccine-specific TCCs were stimulated for
71 24 hours with autologous APCs loaded with antigen-specific vaccine peptides. Cytokine concentrations
72 represents mean values (pg/mL) with background (stimulation with irrelevant peptide) subtracted.
73 Cytokine ratios written in blue refer to Th1 polarization (IFNγ/IL10 > 5; TNFα/IL4 > 10; IFNγ/IL4 > 20;
74 IFNγ/IL5 > 5; IFNγ/IL13 > 1), and cytokine concentrations written in red refer to Th2 polarization
75 (IFNγ/IL10 < 0,2; TNFα/IL4 < 0,5; IFNγ/IL4 < 1; IFNγ/IL5 < 0,2; IFNγ/IL13 < 0,05).

				Cytokine concentration (pg/ml)									Ratio between Th1/Th2 cytokines					
Trial	Patient	Peptide specificity	TCC #	IL-2	IL-4	IL-5	IL-10	IL-12	IL-13	GM-CSF	IFN-γ	TNF-α	IFN-g / IL-10	TNF-a / IL-4	IFN-g / IL-4	IFN-g / IL-5	IFN-g / IL-13	Polarization
MM	N02	p719-20	7	2359	190	2035	395	54	>5407	>7683	11118	76864	28.2	404.8	58.6	5.5	<2.1	Th1
		p719-20	13	1460	164	654	738	26	>3996	>6868	>7242	127638	>9.8	778.6	>44.2	>11.1		Th1
		p719-20	28	4284	728	8037	1425	30	>1910	>6849	>7228	81535	>5.1	112.1	>9.9	>0.9		Th1
PC	822	p725	10	15471	120	765	43	74	>5001	20508	44433	69472	1023.1	577.1	369.1	58.1	<8.9	Th1
		p719-20	12	20	2	94	10	1	9263	665	75	1632	7.5	346.4	16.0	0.8	0.01	Mixed
	802	p719-20	10	3679	706	14002	308	117	>230	14359	545	22193	1.8	31.5	0.8	0.0	<2.4	Mixed
		p719-20	12	164	289	3327	87	29	>2180	3017	8	10749	0.1	37.2	0.0	0.0	0.0	Mixed
	806	p719-20	3	1521	1681	15500	412	86	>2548	>7272	224	47580	0.5	28.3	0.1	0.0	<0.1	Mixed
		p728	4	247	104	4831	47	49	>4165	6034	26	14654	0.5	141.1	0.2	0.0	<0.01	Mixed
		p728	5	1055	539	11997	140	12	>3403	>7737	112	29307	0.8	54.4	0.2	0.0	<0.03	Mixed
NSCLC	906	p719-20	15	845	96	5238	7	10	>4040	12983	5395	33868	793.4	351.3	56.0	1.0	<1.3	Th1
		p725	38	23166	41	420	0	9	17426	33529	29242	139850	>29241.6	3448.8	721.1	69.6	1.7	Th1

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77