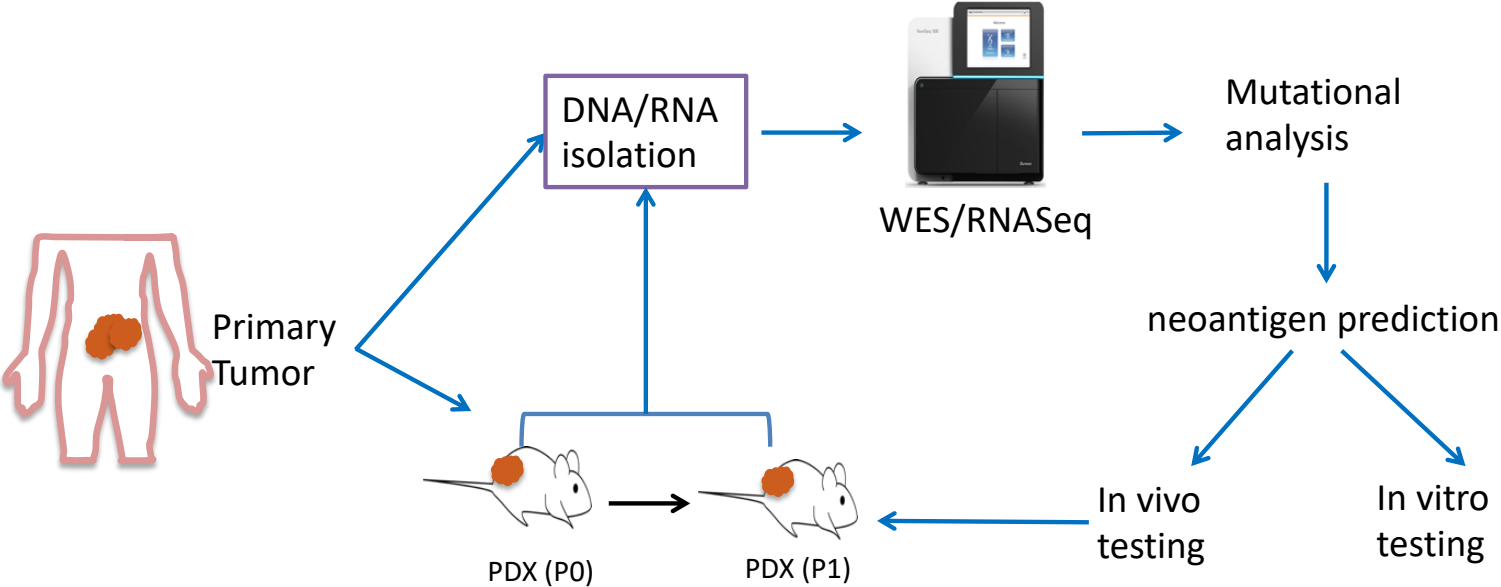


Supplementary Figures

Neoantigens retention in patient derived xenograft models mediates autologous T cells activation in ovarian cancer

Muzamil Yaqub Want¹, Anna Konstorum², Rueda-Yea Huang¹, Vaibhav Jain¹, Satoko Matsueda¹, Takemasa Tsuji¹, Kunle Odunsi¹, Richard Koya¹ and Sebastiano Battaglia^{1,3}

Figure S1



Supplementary Figure 1. Schematic overview of the experimental design

Table S1

Cluster 1 ANOVA + Tukey post hoc					
diff	lwr	upr	p	adj	
P0-Primary	24.595	12.790258	36.39974	7.62E-06	
P1-Primary	30.56	18.755258	42.36474	3.40E-08	
P1-P0	5.965	-5.839742	17.76974	4.56E-01	
Cluster 2 ANOVA + Tukey post hoc					
diff	lwr	upr	p	adj	
P0-Primary	7.818841	-1.880991	17.518673	0.1403854	
P1-Primary	5.291304	-4.408528	14.991136	0.4036103	
P1-P0	-2.527536	-12.227368	7.172296	0.8119756	
Cluster 3 ANOVA + Tukey post hoc					
diff	lwr	upr	p	adj	
P0-Primary	-19.15	-30.910591	-7.3894089	0.000864491	
P1-Primary	-11.864286	-23.624877	-0.1036946	0.04762308	
P1-P0	7.285714	-4.474877	19.0463054	0.297695708	

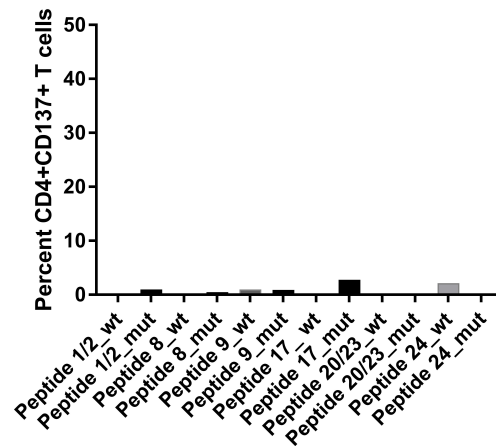
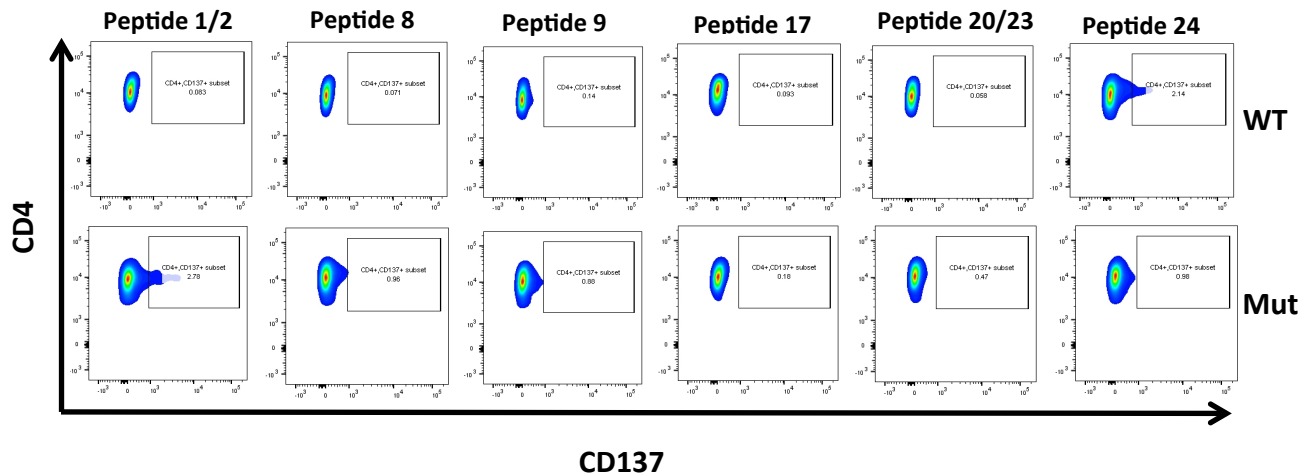
Supplementary Table1. Results from ANOVA analysis with post-hoc Tukey correction comparing the Variant Allele Frequency in clusters 1, 2 and 3

Table S2

Gene name	Chromosome no	POS	REF	ALT	Peptide mut	Peptide Wt	MUT affinity	WT affinity	HLA type	Immunogenic	Delta	ID
SSC4D	7	76397626	C	A	YGTGHILLY	YGTGHILLD	130.24	23661.68	HLA-B3501	Yes	-23531.44	1
SSC4D	7	76397626	C	A	YGTGHILLY	YGTGHILLD	213.34	17009.42	HLA-C1203	Yes	-16796.08	2
LAMB3	1	209638534	C	A	YVNPVSLQL	DVNPVSLQL	89.97	10572.19	HLA-C1203	No	-10482.22	3
CLEC10A	17	7076078	C	A	HCPHCPVCFV	HCPHCPVCFV	197.04	10400.19	HLA-B3501	No	-10203.15	4
CDK8	13	26393423	C	T	LLTSEPIFY	LLTSEPIFH	348.61	9773.93	HLA-B3501	No	-9425.32	5
PRR14L	22	31676998	G	A	LPSTDSETG	PPSTDSETG	202.34	6536.96	HLA-B3501	No	-6334.62	6
GALNT7	4	173297796	A	G	DATTCTVPL	DTTCTVPL	277.68	5537.42	HLA-B3501	No	-5259.74	7
NAV1	1	201793862	C	T	MAKAKAVAL	TAKAKAVAL	311.38	4722.34	HLA-B3501	Yes	-4410.96	8
TRO	X	54930991	C	G	SVGACGFSY	SLGACGFSY	352.16	3145.21	HLA-B3501	Yes	-2793.05	9
INO80	15	40983060	T	C	EVPAAGRGH	EMPAAGRGH	195.5	2104.14	HLA-A2603	No	-1908.64	10
CSF2RB	22	36937526	C	T	LPEKQASSF	PPEKQASSF	27.58	1900.41	HLA-B3501	No	-1872.83	11
CNDP1	18	74576989	G	C	EEYPNSSRV	EEYRNSRV	283.6	1998.84	HLA-C1203	No	-1715.24	12
OR5H14	3	98149939	T	A	DIIPLKISY	DIIPLLKISY	132.89	1525.63	HLA-A2501	No	-1392.74	13
FHL1	X	136209293	C	T	VSKARKSPV	VSKARKPPV	259.11	1389.97	HLA-C1203	No	-1130.86	14
CLEC10A	17	7076078	C	A	VCFPVHSEM	VCVPVHSEM	97.6	1155.46	HLA-C1203	Yes	-1057.86	15
CSMD2	1	33542740	C	G	ASYRSSVSF	GSYRSSVSF	354.08	1369.23	HLA-C1203	Not tested	-1015.15	16
CLEC10A	17	7076078	C	A	FPVHSEMML	VPVHSEMML	41.22	991.08	HLA-B3501	Yes	-949.86	17
VSIG4	X	66028109	G	A	VVKDFSKLL	VVKDSSKLL	235.21	1154.89	HLA-C1203	No	-919.68	18
STYK1	12	10621948	G	A	YPEVLPPTSI	YPEVPPTSI	462.21	1345.14	HLA-B3501	No	-882.93	19
DDX60L	4	168416796	A	C	YVIFDEGHY	YVIFDEVHY	93.25	732.52	HLA-A2501	Yes	-639.27	20
USH2A	1	215743308	T	A	LIPEIPVYV	LIPEIPVEY	273.7	861.68	HLA-C1203	No	-587.98	21
HNRNPUL1	19	41264762	G	C	HPEPGGYSG	HAEPGGYSG	139.29	693.43	HLA-B3501	No	-554.14	22
DDX60L	4	168416796	A	C	YVIFDEGHY	YVIFDEVHY	159.96	708.09	HLA-A2603	Yes	-548.13	23
OR51B4	11	5301398	T	A	NLACADITF	KLACADITF	97.15	597.07	HLA-B3501	Yes	-499.92	24
PATZ1	22	31328842	C	T	RGFSSASTL	RGFSSASAL	366.19	826.03	HLA-C1203	No	-459.84	25
BCAN	1	156658616	C	A	ETVLYRYCR	DTVLYRYCR	434.01	892.24	HLA-A2603	No	-458.23	26
PTBP3	9	112275909	T	C	EIISGLLVF	EIISGLLIF	296.98	567	HLA-B3501	No	-270.02	27
KPNA1	3	122457766	T	C	QMEKGSQAY	QMEKGNQAY	87.81	212.09	HLA-B3501	No	-124.28	28

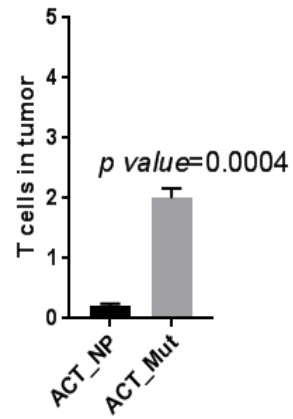
Supplementary Table 2. List of the mutations, neoantigens, affinity to the HLA, HLA type, difference MUT-WT and IDs

Figure S2



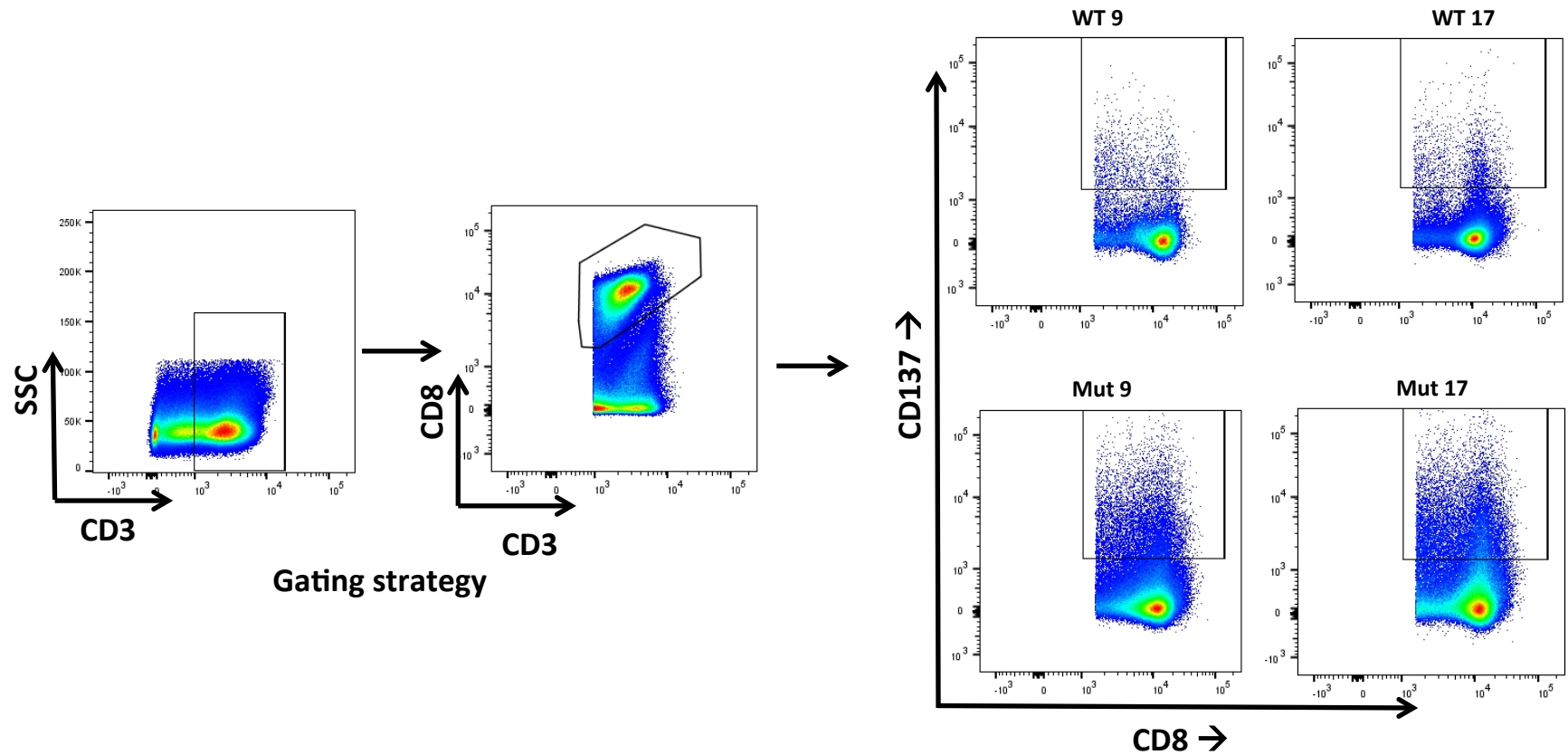
Supplementary Figure 2. Flow cytometry analysis of CD137 on CD4⁺ T cells after stimulation with potential neoantigens or their wild type peptides. **C)** Percentage of activated CD4 T cells after 12 hour incubation with 10 μ m neoantigens or their wild type peptides

Figure S3



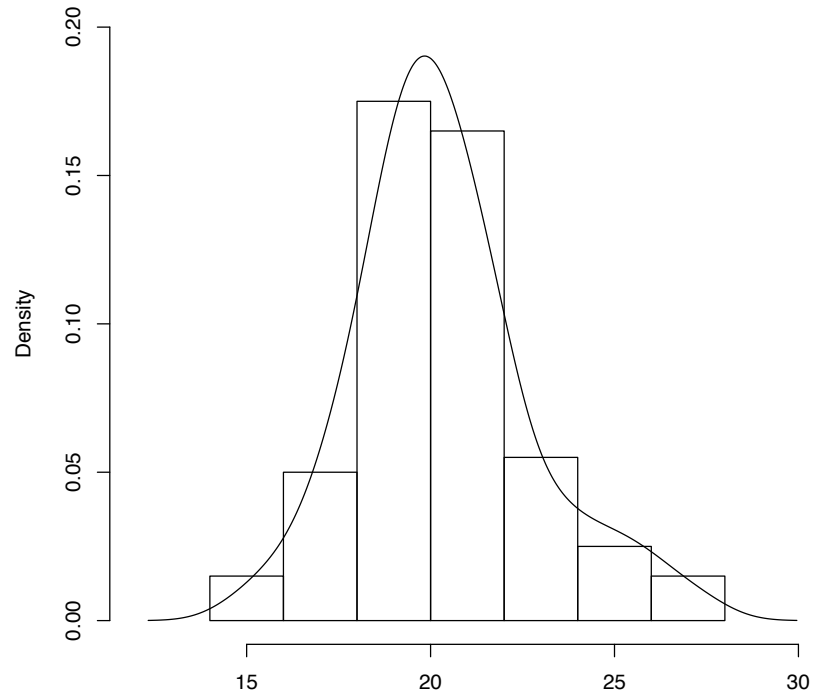
Supplementary Figure 3. Analysis of tumor resident T cells by flow cytometry in the PDX model of ovarian cancer infused with T cells stimulated with no peptide (ACT_NP) or with a cocktail of six neoantigens (ACT_Mut). Data shown as percentage of CD3⁺ T cell at end point.

Figure S4



Supplementary Figure 4. Selection of CD8⁺/CD137⁺ T cells for TCR sequencing. T cells were co-cultured with autologous APCs pulsed with peptide 9, 17 or the WT counterparts. Post stimulation (see methods), T cells were sorted and CD8⁺/CD137⁺ T cells stimulated with MUT peptides used for deep sequencing.

Figure S5



Supplementary Figure 5. Kernel distribution of 100 simulated GLAM score for random sequences

Figure S6

