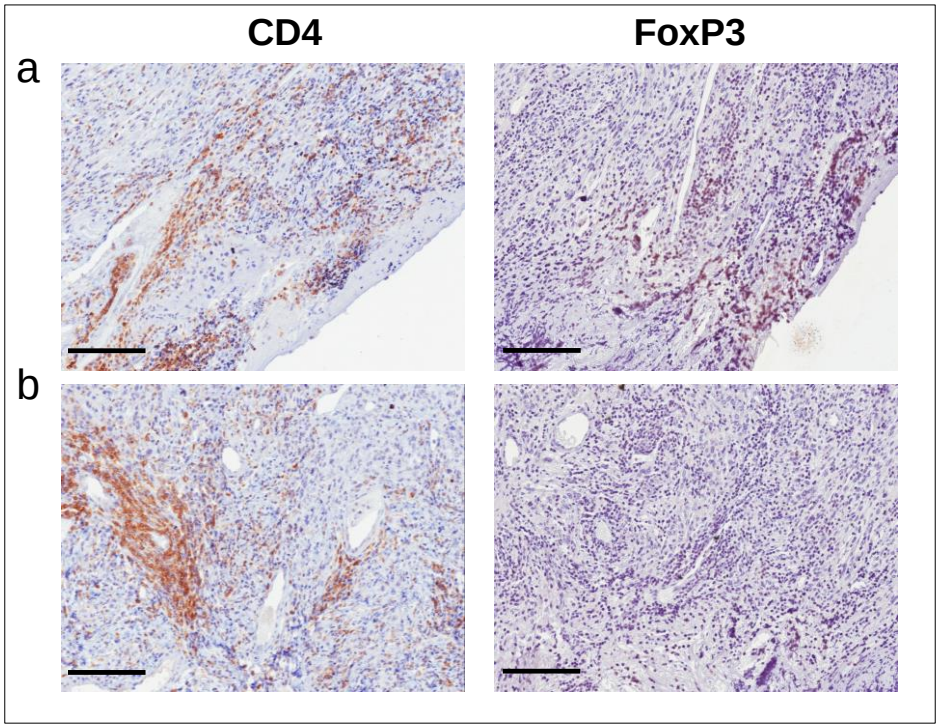
Supplementary Data

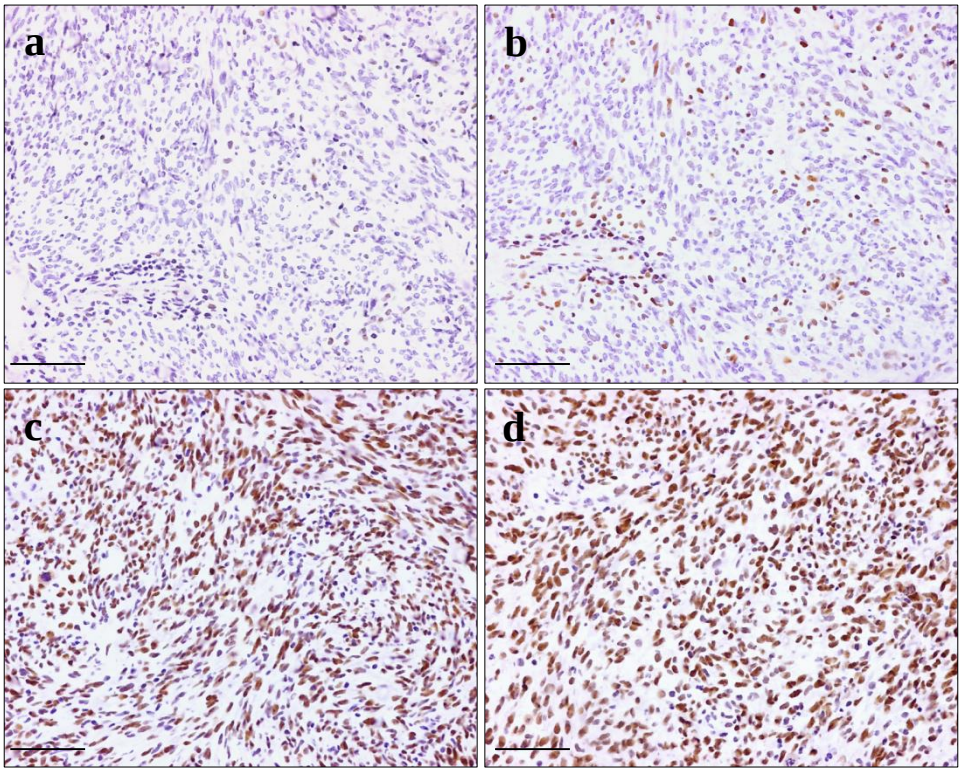
High tumor mutational burden and T cell activation are associated with long-term response to anti-PD1 therapy in Lynch syndrome recurrent glioblastoma patient

Supplementary Fig. 1. FoxP3+ cells are only present in recurrent GBM specimen of anti-PD1-treated patient



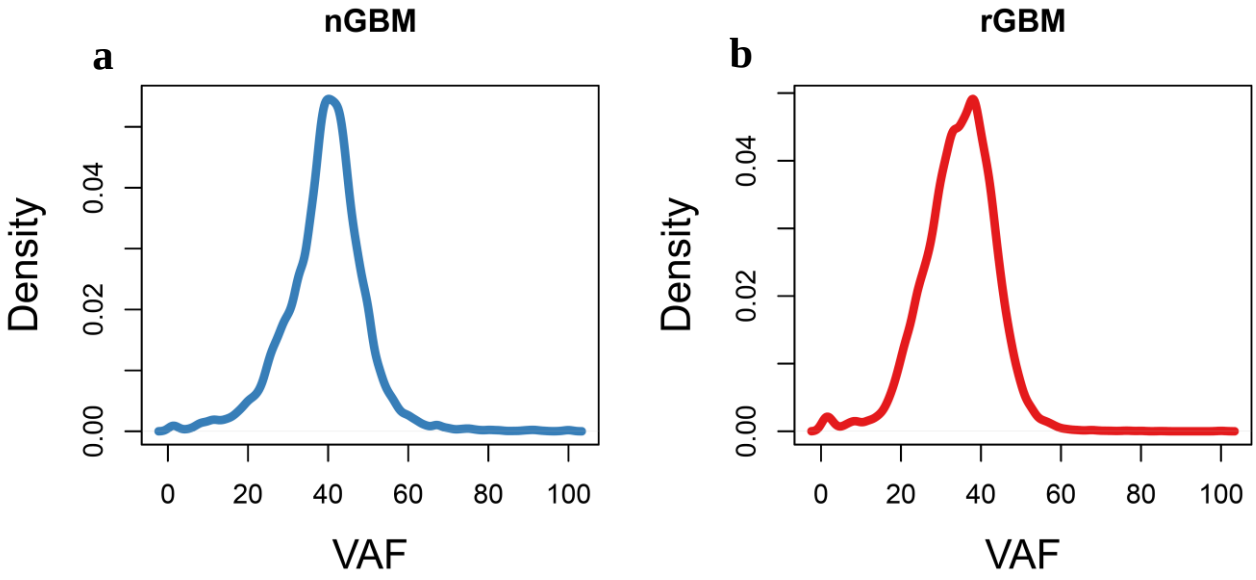
a. Representative images of adjacent sections showing CD4+ TILs colocalizing with FoxP3+ cells. TILs expressing FoxP3 were found in few areas at the tumor margins of the rGBM specimen. **b**. Many CD4+ TIL-rich clusters negative for FoxP3 were found near the vessels (Scale Bar 200 μm).

Supplementary Fig. 2. MSH2 and MSH6 expression is absent in the rGBM specimens



a-d. Immunohistochemical staining of MSH2, MSH6, MLH1 and PMS2 in the tumor mass. Subsequent tumor sections showing loss of both MSH2 (**a**) and MSH6 expression (**b**). Tumor cells showing immunoreactivity with MLH1 (**c**) and PMS2 (**d**) (Scale Bar 100 μm).

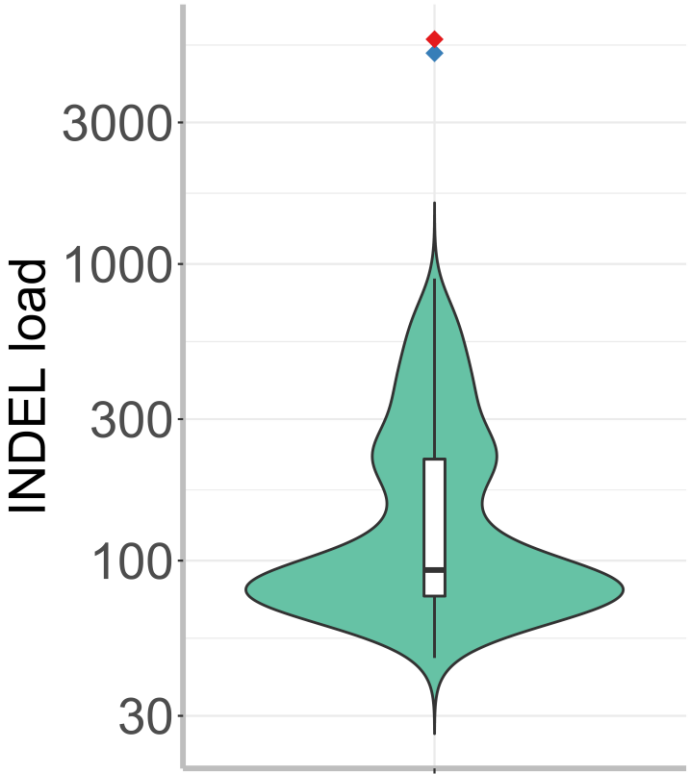
Supplementary Fig. 3. Clonal distribution in both nGBM and rGBM specimens



a, b. Density plots of the Variant Allele Frequency (VAF) [1]. Most of the variants have approximately 40% VAF, indicating clonal heterozygous mutation with 80% tumor purity. This support the clonality of the majority of the mutations in both nGBM (**a**) and rGBM specimens (**b**).

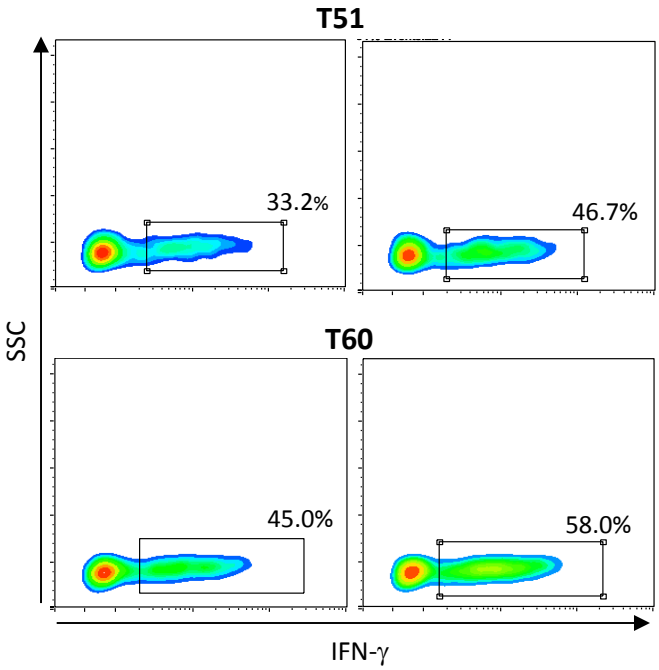
1. Miller CA, White BS, Dees ND, et al. (2014) SciClone: inferring clonal architecture and tracking the spatial and temporal patterns of tumor evolution. PLoS Comput Biol 10:e1003665. doi: 10.1371/journal.pcbi.1003665

Supplementary Fig. 4. Indel mutation load in the patient is much higher than that in TCGA hypermutated GBM cohort



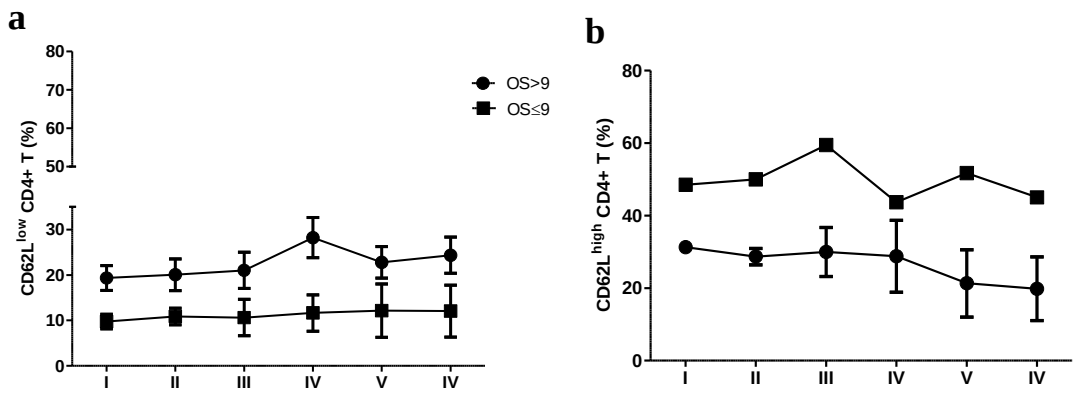
Indel mutation load of our samples (Blue Diamond: nGBM specimen; Red Diamond: rGBM specimen) in the context of TCGA hypermutated GBM cases. A total of 105 hypermutated GBM cases were extracted from TCGA GBM cohort, using nonsilent mutation load >= 100 as cutoff. We performed somatic mutation calling using VarScan2 on our samples and compared them with VarScan2 results of TCGA GBM samples. The analysis of our samples in comparison with these 105 hypermutated GBM revealed that the number of INDEL in our patient is significantly higher than that in most of them.

Supplementary Fig. 5. CD8+ T cells differentiate into memory T cells expressing IFN-γ



Dot plot showing the IFN-γ expression in CD8+ T central (TCM) and effector memory (TEM) at two different time points during anti-PD1 therapy.

Supplementary Fig. 6. CD62L^{low} is predictive for long term survival and immunotherapy responsiveness



a, b. Kinetics of CD4+ T cells expressing CD62L^{low} (**a**) or high (**b**) in control V-DENDR2 patients (Overall Survival (OS) > 9 months. OS ≤ 9 months). Each data point represents the mean cell frequency (± standard deviation), at each vaccination for the two groups of controls (n=5 V-DENDR2 OS>9 and n=3 V-DENDR2 OS≤9).

Supplementary Table 1. CD3, CD8 and CD4 counts, CD8/CD3 and CD4/CD3 ratio

		CD3	CD8	CD4	CD8/CD3	CD4/CD3
nGBM		61.2 ±23.6	65.6 ±17.5	0	1.1	0
rGBM	Tumor center	108.0±19.9	58.0±6.2	61.0±13.1	0.5	0.6
	Tumor margin	299.0±49.1	210.3±56.8	73.0±46.5	0.7	0.3

Supplementary Table 3. HLA typing in the blood and GBM specimen

HLA typing
HLA-A*02:01
HLA-A*30:04
HLA-B*41:01
HLA-B*44:02
HLA-C*05:01
HLA-C*17:01