Supplementary Data for

Eradication of large solid tumors by gene therapy with a T cell receptor targeting a single cancer-specific point mutation

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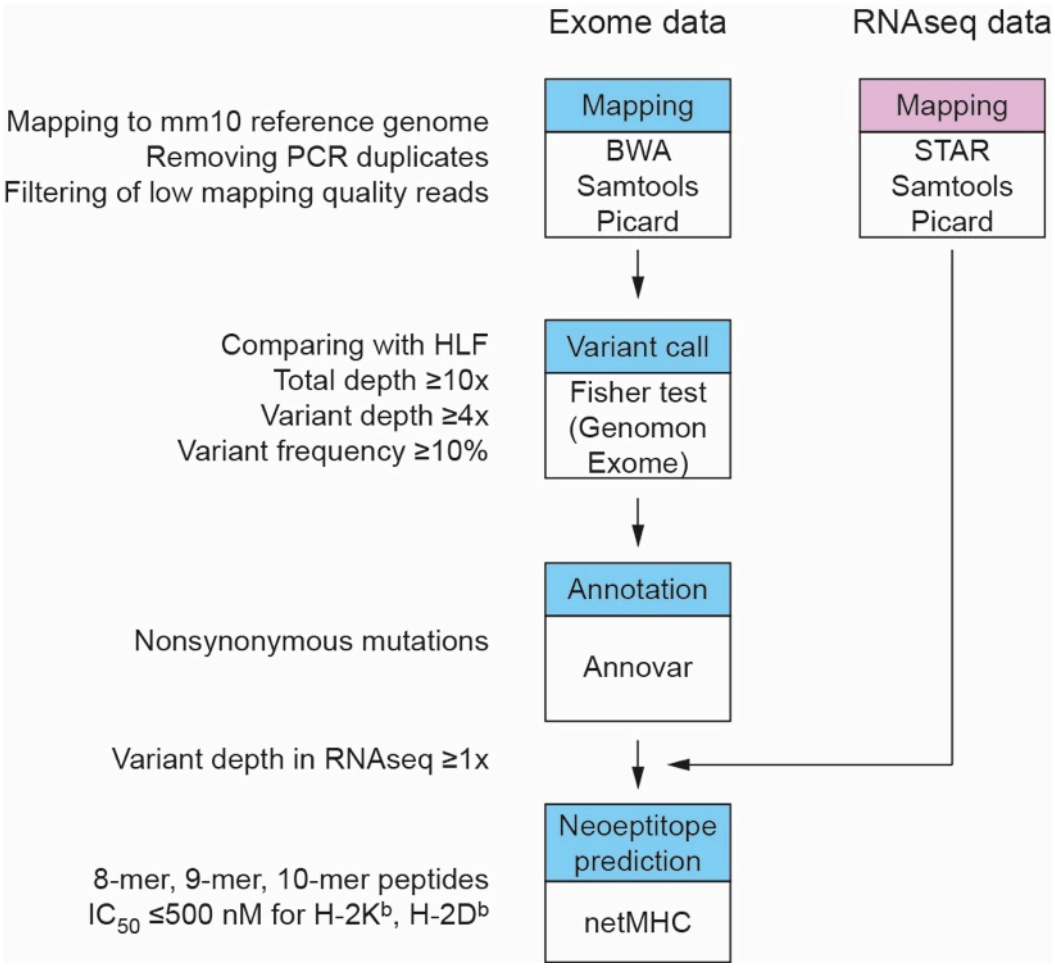
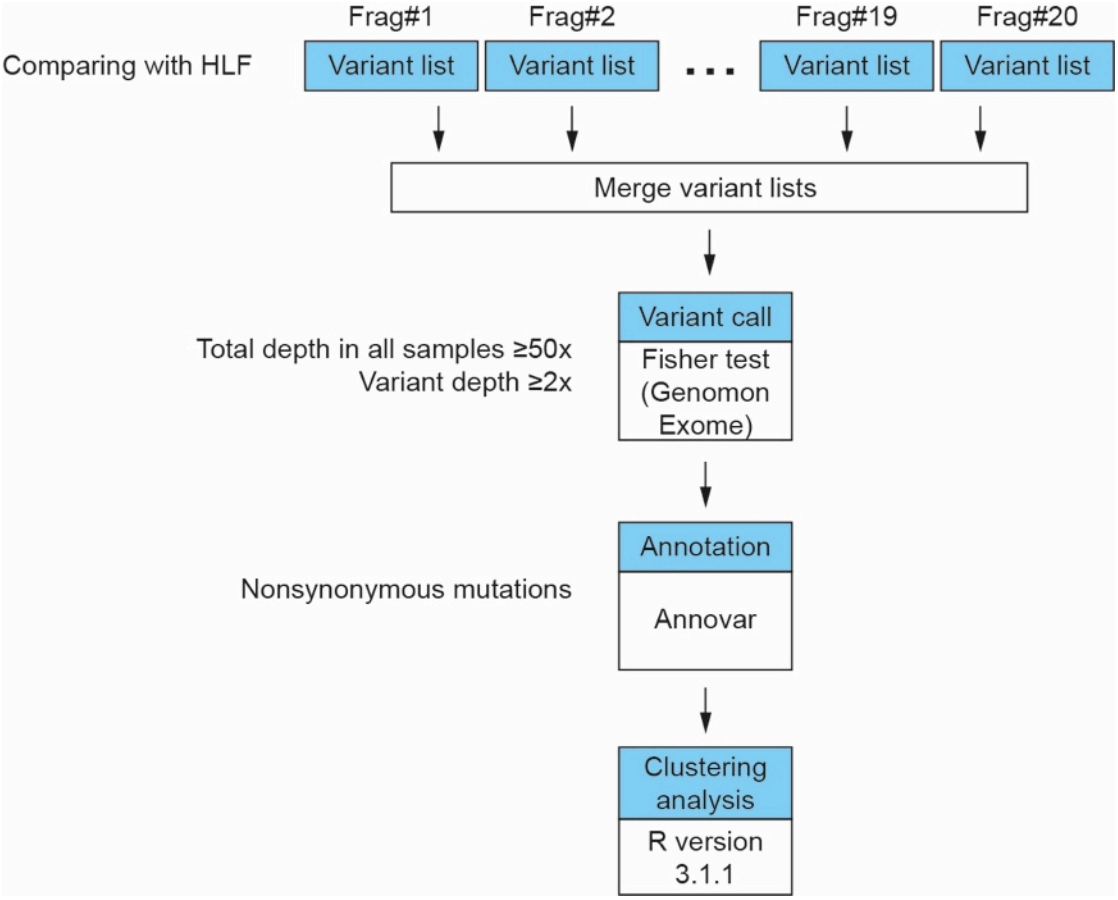
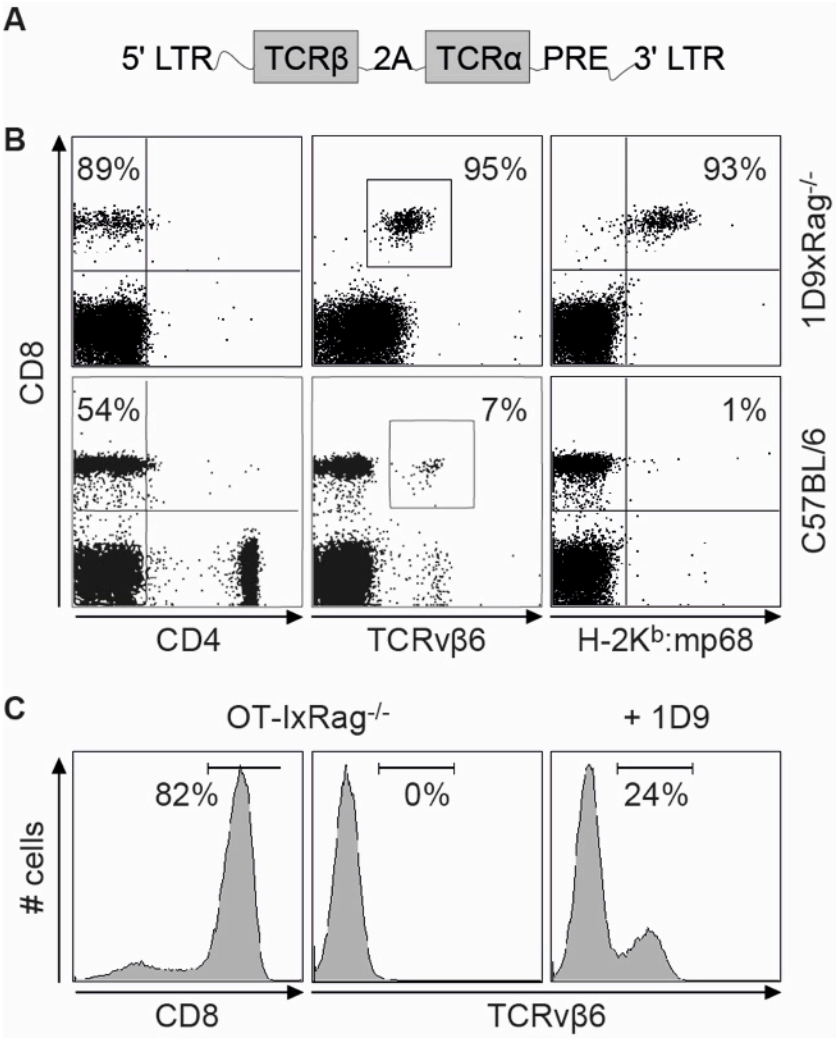


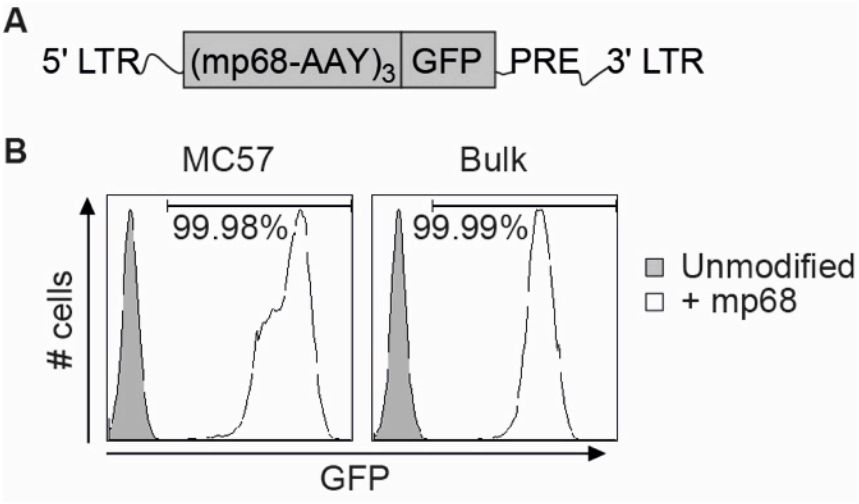
Supplementary Fig. S1 - Pipeline for exome/RNA analysis and neoepitope prediction.



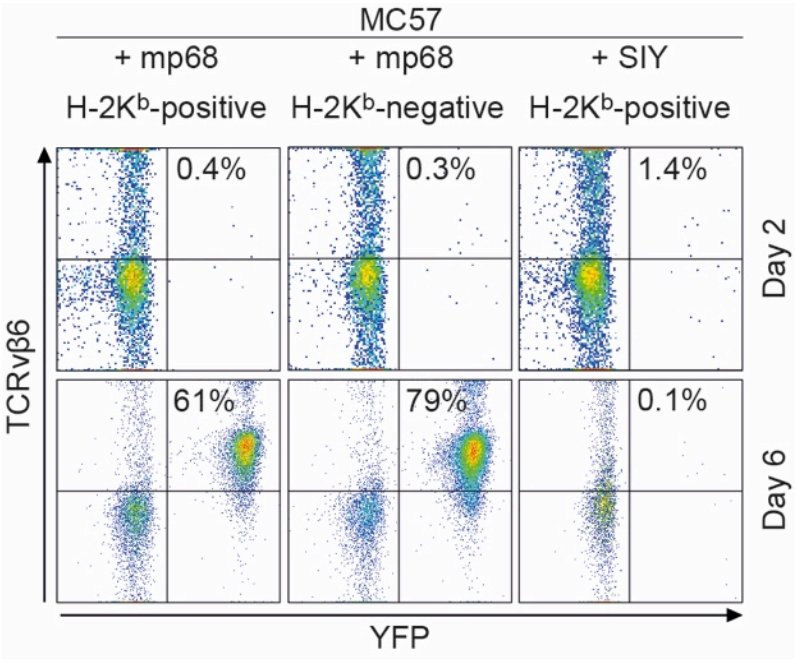
Supplementary Fig. S2 - Pipeline for exome and clustering analysis of autochthonous 8101 tumor fragments.



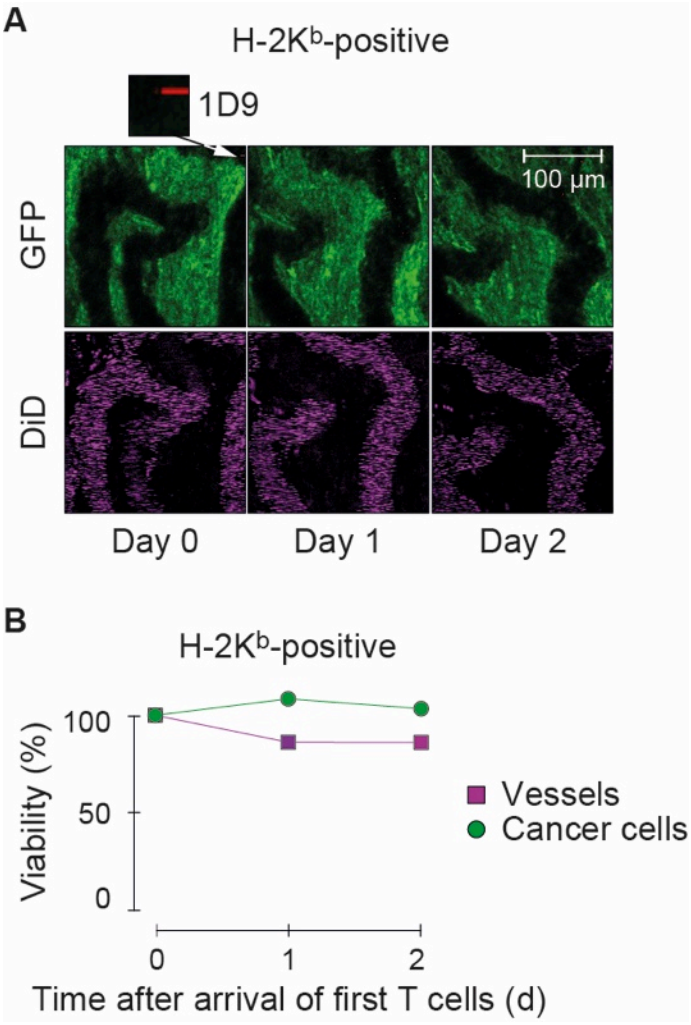
Supplementary Fig. S3 - Generation of 1D9 T cells. A, Schematic representation of the 1D9 retrovirus. The TCRα- and β-chain genes were introduced into the MP71-PRE retrovirus vector (see reference in (22)) linked by a 2A element. LTR: long terminal repeat of the mouse myeloproliferative sarcoma virus; 2A: 2A element of porcine teschovirus; PRE: post-transcriptional regulatory element of the woodchuck hepatitis virus. B, Representative staining of blood samples from 1D9xRag$^{-/-}$ and C57BL/6 mice. Left panels show staining for CD4 and CD8. Numbers indicate percentage of CD8^{+} T cells of all lymphocytes. Expression of TCRvβ6 and the 1D9 TCR was detected using TCRvβ6-specific antibodies and H-2K^{b}:mp68 multimers, respectively. C, Cultured T cells derived from splenocytes of OT-IxRag$^{-/-}$ mice were analyzed for CD8 expression. 1D9 TCR expression was determined using TCRvβ6-specific antibodies before and after transduction with 1D9 retrovirus.



Supplementary Fig. S4 - Generation of mp68-expressing tumor cell lines. A, Scheme of the retrovirus encoding the trimeric minigene SNFVFAGI-AAY fused to GFP (mp68-GFP). LTR: long terminal repeat of the mouse myeloproliferative sarcoma virus; PRE: post-transcriptional regulatory element of the woodchuck hepatitis virus. B, Flow cytometric analysis of mp68-GFP fusion proteins expressed by the transduced and sorted tumor cells MC57 and Bulk. Parental MC57 and Bulk (unmodified, gray) were analyzed for comparison. Numbers indicate percentage of mp68-GFP-expressing cells.



Supplementary Fig. S5 - Expansion of 1D9 T cells is antigen-specific and not driven by lymphopenia-induced proliferation. Splenocytes of YFPx1D9xRag$^{-/-}$ mice were transferred into H-2K^{b}-positive and H-2K^{b}-negative Rag$^{-/-}$ mice bearing MC57-mp68 or MC57-SIY tumors. 1D9 T cells were monitored in blood taken on day 2 and 6 after adoptive transfer. Numbers indicate the percentage of YFP^{+}/TCRvβ6^{+} double-positive cells.



Supplementary Fig. S6 - 1D9 T cells do not infiltrate tumors if mp68 is not expressed. A, Longitudinal imaging of an established MC57-SIY tumor grown in a Rag$^{-/-}$ mouse following adoptive transfer of 1D9 T cells of YFPx1D9xRag$^{-/-}$ mice. Day 0 is the first day on which T cell infiltration was found in animals with MC57-mp68 tumors in the same experiment (see Fig. 4A, left). At that time point, one T cell (pseudo-colored in red, see magnification) was visible in the blood stream. Viability of tumor tissue was analyzed by monitoring GFP (cancer cells, green) and blood flow (DiD-stained erythrocytes, purple). B, Quantification of the areas shown in (A) that are covered by live cancer cells (green) and functional vessels (purple). Areas on day 0 were defined as 100%.

Supplementary Table S1 - Results of whole exome sequencing of Bulk tumor cells and Bulk reisolates after 1D9 T cell therapy.

			$p68^{S551F}$			$p53^{S238A}$		
Sample	**Average depth**	**Nonsynonymous SNV**	**Wild type**	**Mutant**	**VAF (%)**	**Wild type**	**Mutant**	**VAF (%)**
Heart-lung fibroblasts	158	0	207	0	0	56	0	0
Bulk	146	7,923	161	52	24	8	30	79
Reis#1	125	7,808	182	3	2	42	18	30
Reis#2	92	7,809	108	17	14	10	24	71

Reis: Reisolates
VAF: Variant allelic frequency

Supplementary Table S2 - Results of whole exome sequencing of fragments derived from the autochthonous 8101 tumor.

			p68^{S551F}			p53^{S238A}		
Sample	**Average depth**	**Nonsynonymous SNV**	**Wild type**	**Mutant**	**VAF (%)**	**Wild type**	**Mutant**	**VAF (%)**
Heart-lung fibroblasts	158	0	207	0	0	56	0	0
Frag#1	83	7,726	77	42	35	28	12	30
Frag#2	101	8,481	119	46	28	29	6	17
Frag#3	91	7,861	79	60	43	29	17	37
Frag#4	76	7,785	55	43	44	26	11	30
Frag#5	79	336	120	4	3	35	0	0
Frag#6	80	1,703	118	4	3	34	0	0
Frag#7	60	7,522	50	19	28	10	5	33
Frag#8	77	6,602	77	20	21	17	6	26
Frag#9	77	7,801	63	43	41	24	12	33
Frag#10	83	7,754	67	45	40	18	13	42
Frag#11	93	7,845	93	42	31	22	10	31
Frag#12	93	7,540	106	36	25	24	8	25
Frag#13	84	7,488	74	30	29	29	11	28
Frag#14	79	7,612	85	28	25	23	6	21
Frag#15	78	7,270	87	30	26	28	11	28
Frag#16	72	5,523	122	22	15	13	3	19
Frag#17	86	7,423	90	30	25	23	5	18
Frag#18	80	6,485	90	30	25	25	3	11
Frag#19	64	7,694	67	33	33	9	12	57
Frag#20	78	7,551	74	31	30	30	17	36

Frag: Fragment
VAF: Variant allelic frequency