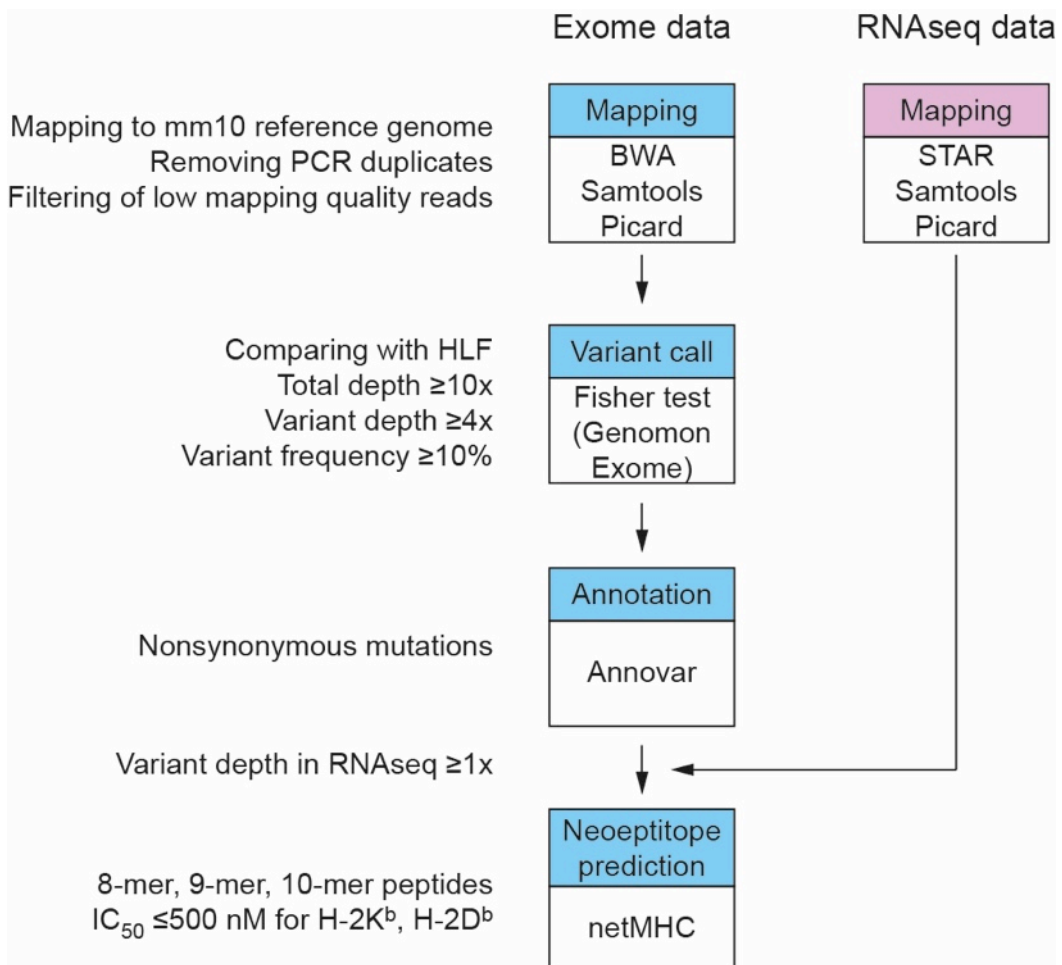Supplementary Data for

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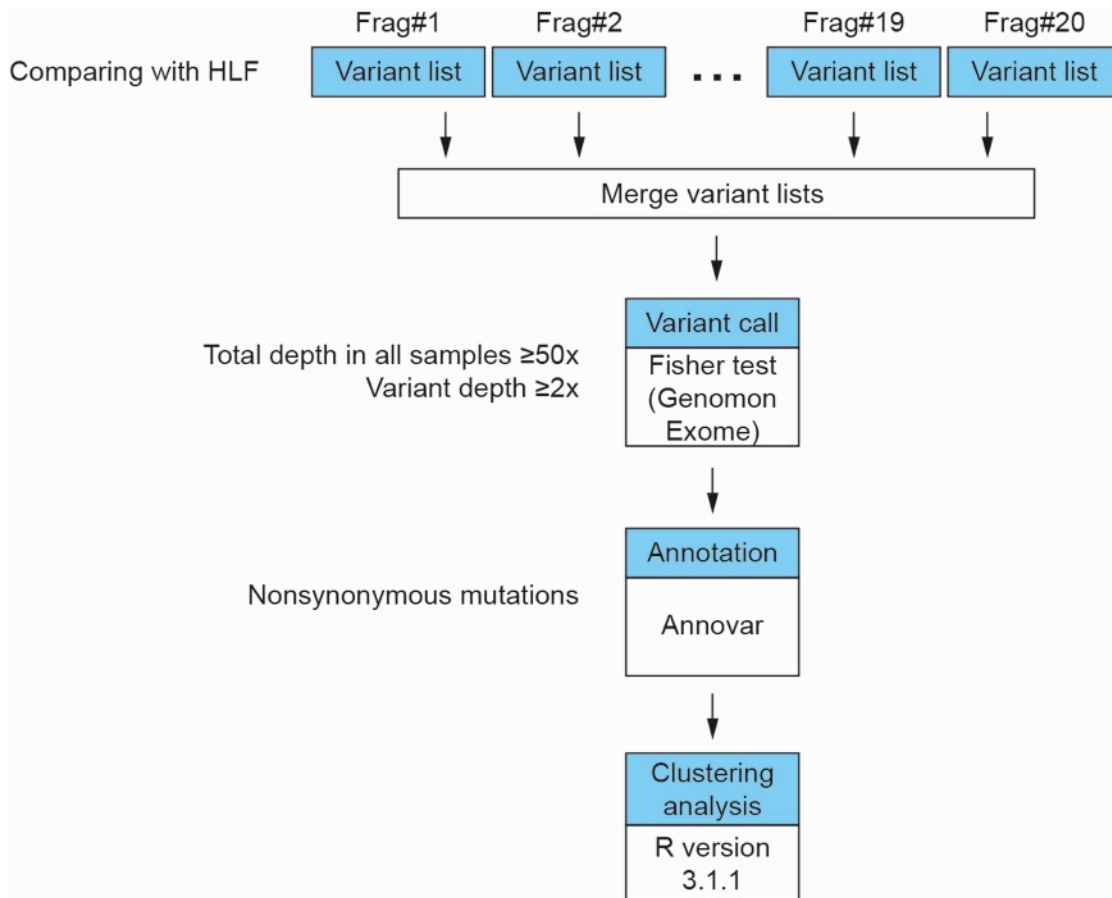# targeting a single cancer-specific point mutation

Matthias Leisegang, Boris Engels, Karin Schreiber, Poh Yin Yew, Kazuma Kiyotani,

Christian Idel, Ainhoa Arina, Jaikumar Duraiswamy, Ralph R. Weichselbaum,

Wolfgang Uckert, Yusuke Nakamura and Hans Schreiber
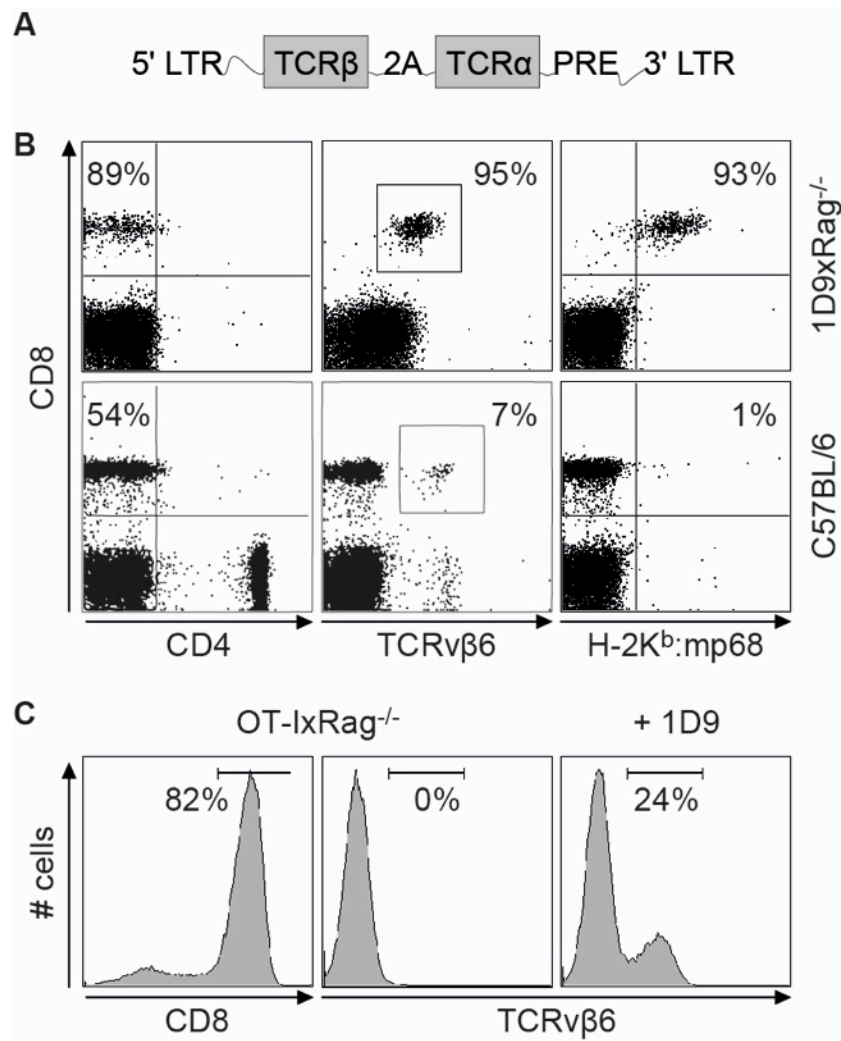
Index of Supplementary Data

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.
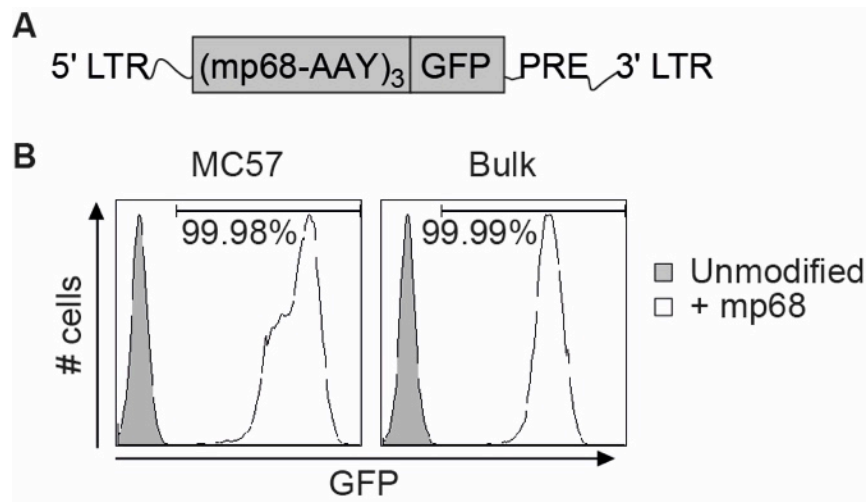
Supplementary Fig. S4 - Generation of mp68-expressing tumor cell lines. A, Scheme of the retrovirus encoding the trimeric minigene SNFVFAGI-AAY fused to GFP (mp68-GFP). LTR: long terminal repeat of the mouse myeloproliferative sarcoma virus; PRE: post-transcriptional regulatory element of the woodchuck 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.
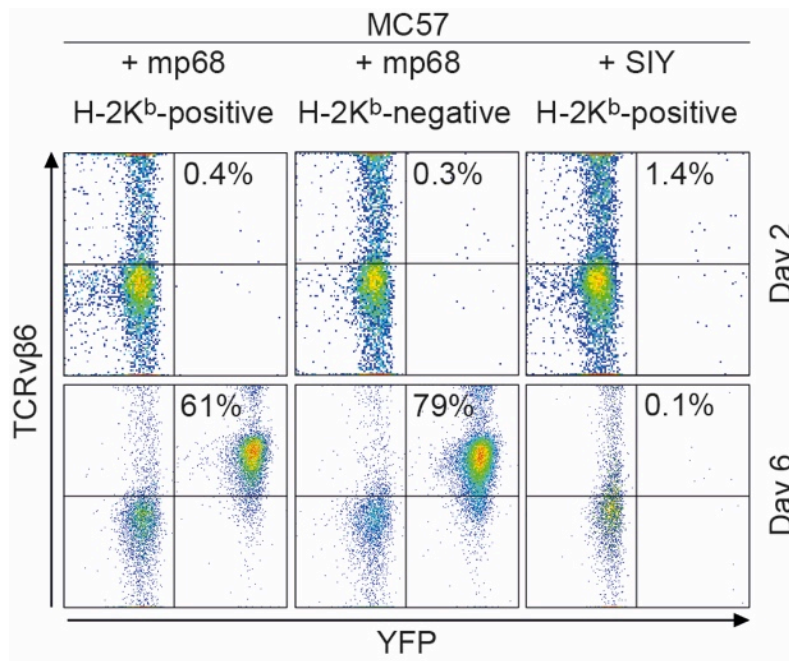
Supplementary Fig. S5 - Expansion of 1D9 T cells is antigen-specific and not driven by lymphopenia-induced proliferation. Splenocytes of YFPx1D9xRag$^{-/-}$ mice were transferred into H-2K^b-positive and H-2K^b-negative Rag$^{-/-}$ mice bearing MC57-mp68 or MC57-SIY tumors. 1D9 T cells were monitored in blood taken on day 2 and 6 after adoptive transfer. Numbers indicate the percentage of YFP$^+$/TCRvβ6$^+$ double-positive cells.
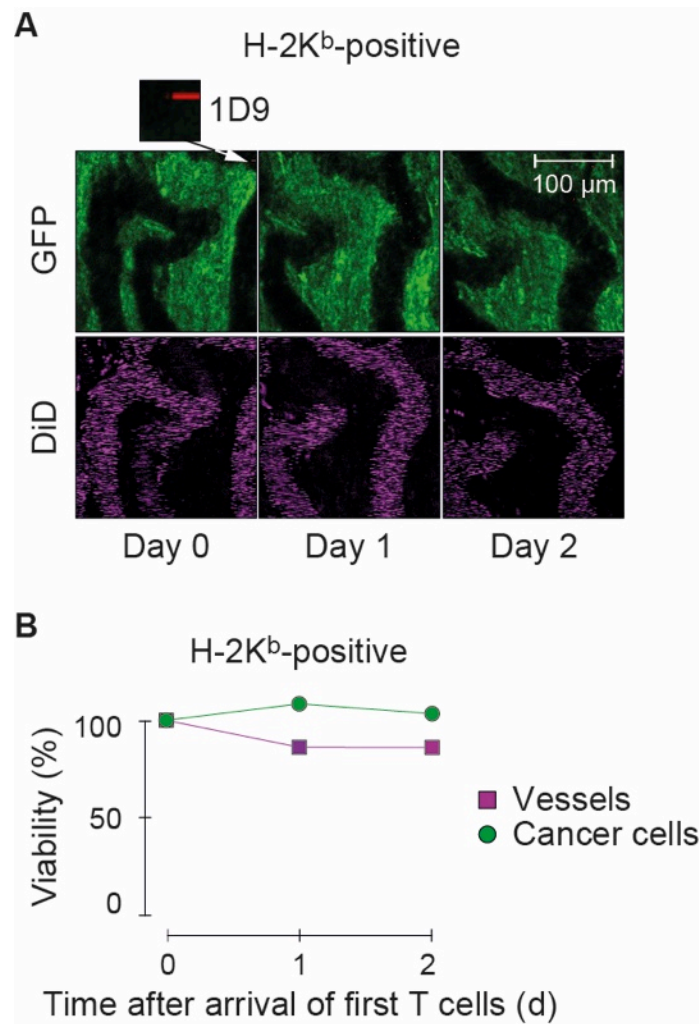
Supplementary Fig. S6 - 1D9 T cells do not infiltrate tumors if mp68 is not expressed. A, Longitudinal imaging of an established MC57-SIY tumor grown in a Rag$^{-/-}$ mouse following adoptive transfer of 1D9 T cells of YFPx1D9xRag$^{-/-}$ mice. Day 0 is the first day on which T cell infiltration was 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.

Sample	Average depth	Nonsynonymous SNV	p68^{S551F}			p53^{S238A}		
			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.

Sample	Average depth	Nonsynonymous SNV	p68^{S551F}			p53^{S238A}		
			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