Supplementary Material

Identification of an Immunogenic Medulloblastoma-Specific Fusion Involving *EPC2* and *GULP1*

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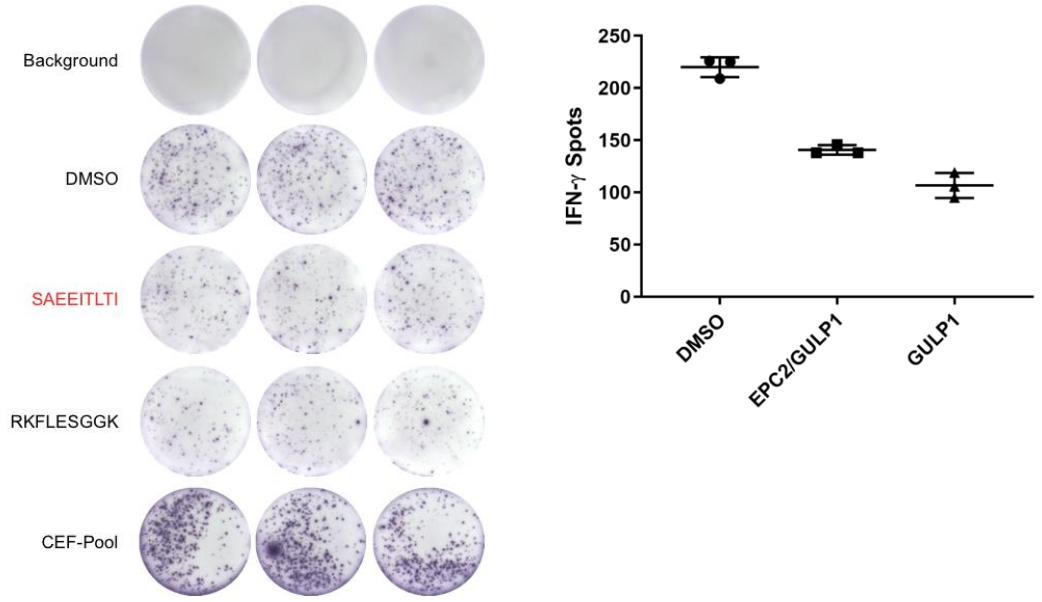
A



B

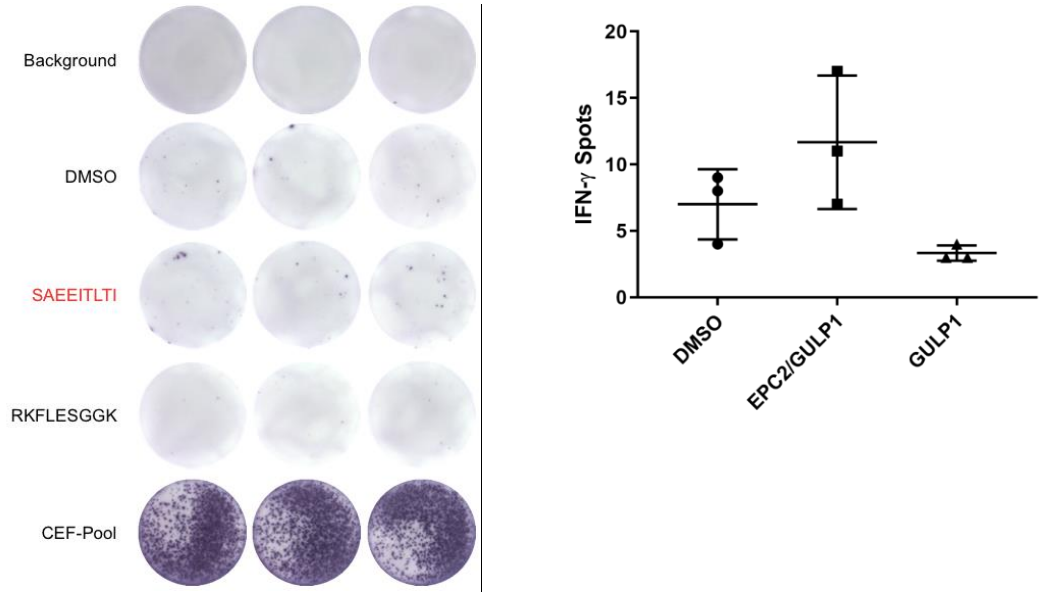


Figure S1: ELISpot analyses of healthy donor 2 and 3 show reactivity of CD8⁺ T cells towards fusion peptide

ELISpot analysis after in vitro assay based on co-culturing peptide loaded DCs with CD8⁺ T cells of healthy

donor 2 and 3. DMSO was taken as vehicle control, wildtype peptide of GULP1 as negative control and CEF-Pool as positive control. Strong IFN-γ secretion of T cells after stimulation with the fusion peptide (red marked). **A**) Healthy donor 2: The IFN-γ secretion is 1.3-fold higher than after stimulation with wildtype peptide. There is also a strong reactivity towards the vehicle control DMSO. **B**) Healthy donor 3: The IFN-γ secretion is 3.5-fold higher than after stimulation with wildtype peptide.

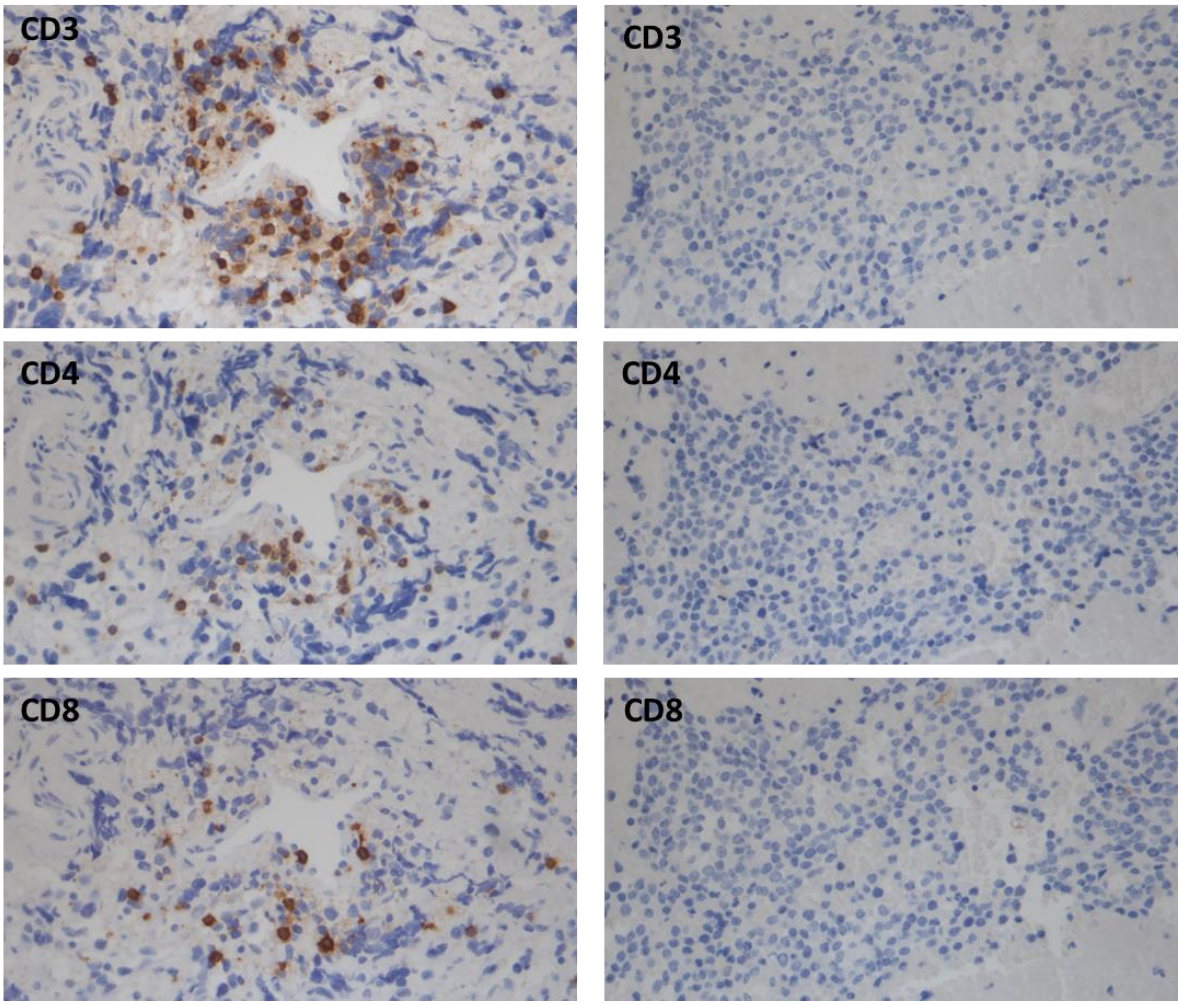


Figure S2. Infiltrating T cells in medulloblastoma Immunohistochemistry was performed on 3 μm thick routinely processed formalin-fixed and paraffin-embedded tissue sections. Sections derived from the tumor of patient no 25 (left panel, tumor positive for *EPC2-GULP1* fusion) or no 129 (right panel, tumor negative for *EPC2-GULP1* fusion) were stained with anti-CD3, CD8 and CD4 primary antibody. Magnification 400X.

Table S1: Summary of publications in the context of gene fusions analyses in medulloblastoma patients.

Publication	Summary
Northcott et al. 2017	Analysis of the somatic landscape across 491 sequenced medulloblastoma samples; 20 fusions across all medulloblastoma subtypes; Fusions involving medulloblastoma driver genes, e.g. *GLI2*, *PTEN*, *SUFU*; 8 in-frame fusions, 9 frame-shift fusions, 3 non-coding fusions

	no recurrent* gene fusions
Luo et al. 2021	Comparison of Asian and Caucasian cohort (Data source Caucasian cohort Northcott et al. 2017); Analysis of 52 tumor samples from Asian cohort, approx. 149 gene fusions events found; one recurrent* in-frame gene fusion *DNMT1-ZGLP1* (2/52) with identical breaking points only in the Asian cohort; 31 novel fusion genes from at least two samples in the Asian and Caucasian cohort
Northcott et al. 2012	Analysis of somatic copy number aberrations in 1,087 medulloblastoma samples; Fusion *PVT1-MYC* in group 3 medulloblastoma with *MYC*-amplification in 12 out of 60 samples (60%); *PVT1* is a non-coding host gene; all *PVT1-MYC* fusions with different breaking points, in some cases with additional compartments of chromosome 8
Jones et al. 2012	Analysis of 125 medulloblastoma samples; Identification of 3 gene fusions *DNAJB6-SHH* (SHH subgroup), *LCLAT1-ERBB4* Group 3), *MLLT6-MRPL45* (Group 4); no recurrent* gene fusions
Skowron et al. 2021	Cohort of 250 medulloblastoma samples (SHH subgroup); recurrent gene fusions affecting *GLI2*, *EPB41L5*, *NBAS*, *BCAS3*, *GLIS3*, *ZBTB20*, *SUFU*, *PTCH1* predominantly with different fusion partners; fusions with same gene partners have different breaking points; 3 recurrent* fusion transcripts with identical breaking points: SUCO-NCOR1 (2/250), *C4BPB-NCOR1* (3/250), *PAC6-GNAS* (2/250)

Table S2: Overview of other brain tumor samples analyzed in this work.

Sample	Age at diagnosis	Diagnosis	*EPC2-GULP1* fusion
84	8	Astrocytoma	no
95	10	Glioblastoma	no
121	14	Ependymoma	no
122	12	Astrocytoma	no
123	6	HGNET-BCOR	no
139	10	Glioblastoma	no
143	10	Astrocytoma	no

159	3	Astrocytoma	no
226	13	Astrocytoma	no
281	15	Astrocytoma	no
294	9	Ependymoma	no
323	11	Astrocytoma	no
340	3	Astrocytoma	no

Table S3: *In silico* epitope prediction with SYFPEITHI algorithm from selected, published in-frame fusion events. Peptide is predicted as a potential immunogenic epitope if its SYFPEITHI score is ≥ 20. Amino acids representing the fusion point are highlighted in bold letters.

in-frame fusion (MB-Subtype)	**AA-sequence**	**9mer peptides (HLA-haplotype; SYFPEITHI Score)**	**Reference**
***TCF4-ROCK1* (SHH)**	KVPPGLPS**SD**GLDALVYD*	S**SD**GLDALV (HLA-A*02:01; **17**)	Northcott et al. 2017
		PS**SD**GLDAL (HLA-A*02:01; **15**)	
		PGLPS**SD**GL (HLA-A*02:01; **12**)	
		GLPS**SD**GLD (HLA-A*02:01; **11**)	
		LPS**SD**GLDA (HLA-A*02:01; **7**)	
		VPPGLPS**SD** (HLA-A*02:01; **6**)	
		SDGLDALVY (HLA-A*02:01; **3**)	
		PPGLPS**SD**G (HLA-A*02:01; **-2**)	
***PTEN-THAP9* (Group 3)**	RNNIDDVV**RW**LSKCQPSP*	NIDDVV**RW**L (HLA-A*02:01; **23**)	Northcott et al. 2017
		NNIDDVV**RW** (HLA-A*02:01; **9**)	
		DVV**RW**LSKC (HLA-A*02:01; **9**)	
		VV**RW**LSKCQ (HLA-A*02:01; **5**)	
		RWLSKCQPS (HLA-A*02:01; **3**)	
		IDDVV**RW**LS (HLA-A*02:01; **2**)	
		DDVV**RW**LSK (HLA-A*02:01; **2**)	
		V**RW**LSKCQP (HLA-A*02:01; **2**)	
***MARCKSL1-PIK3CD* (Group 3)**	ASPAKANG**QL**LWHRAQYE*	SPAKANG**QL** (HLA-A*02:01; **14**)	Northcott et al. 2017
		QLLWHRAQY (HLA-A*02:01; **14**)	
		KANG**QL**LWH (HLA-A*02:01; **13**)	
		PAKANG**QL**L (HLA-A*02:01; **10**)	
		AKANG**QL**LW (HLA-A*02:01; **7**)	
		ANG**QL**LWHR (HLA-A*02:01; **7**)	
		NG**QL**LWHRA (HLA-A*02:01; **6**)	
		G**QL**LWHRAQ (HLA-A*02:01; **4**)	

DNMT1-ZGLP1 **(Group 4)****	SLPDDVRR**rS**LWPACQES	PDDVRR**RS**L (HLA-A*02:01; **9**) VRR**RS**LWPA (HLA-A*02:01; **9**) DVRR**RS**LWP (HLA-A*02:01; **4**) LPDDVRR**RS** (HLA-A*02:01; **3**) DDVRR**RS**LW (HLA-A*02:01; **-1**) DDVRR**RS**LW (HLA-A*24:02; **3**) LPDDVRR**RS** (HLA-A*24:02; **2**) **RS**LWPACQE (HLA-A*24:02; **2**) VRR**RS**LWPA (HLA-A*24:02; **1**) R**RS**LWPACQ (HLA-A*24:02; **1**) DVRR**RS**LWP (HLA-A*24:02; **0**) RR**RS**LWPAC (HLA-A*24:02; **-1**)	Luo et al. 2021
NFYC-PPIE **(SHH)****	IIAQPQQG**QK**QEESAITS	G**QK**QEESAI (HLA-A*02:01; **9**) IAQPQQG**QK** (HLA-A*02:01; **8**) **QK**QEESAIT (HLA-A*02:01; **8**) QG**QK**QEESA (HLA-A*02:01; **6**) AQPQQG**QK**Q (HLA-A*02:01; **4**) QQG**QK**QEES (HLA-A*02:01; **3**) QPQQG**QK**QE (HLA-A*02:01; **1**) PQQG**QK**QEE (HLA-A*02:01; **1**) G**QK**QEESAI (HLA-A*24:02; **9**) AQPQQG**QK**Q (HLA-A*24:02; **4**) QQG**QK**QEES (HLA-A*24:02; **2**) QG**QK**QEESA (HLA-A*24:02; **2**) **QK**QEESAIT (HLA-A*24:02; **2**) IAQPQQG**QK** (HLA-A*24:02; **1**) QPQQG**QK**QE (HLA-A*24:02; **0**) PQQG**QK**QEE (HLA-A*24:02; **0**)	Luo et al. 2021
ANKRD52-CS **(Group 3)****	RGRTALHR**GV**VPGYGHAV	ALHR**GV**VPG (HLA-A*02:01; **20**) RTALHR**GV**V (HLA-A*02:01; **16**) **GV**VPGYGHA (HLA-A*02:01; **13**) GRTALHR**GV** (HLA-A*02:01; **12**) LHR**GV**VPGY (HLA-A*02:01; **10**) TALHR**GV**VP (HLA-A*02:01; **8**) R**GV**VPGYGH (HLA-A*02:01; **3**) HR**GV**VPGYG (HLA-A*02:01; **0**) TALHR**GV**VP (HLA-A*24:02; **4**) HR**GV**VPGYG (HLA-A*24:02; **2**) **GV**VPGYGHA (HLA-A*24:02; **2**) GRTALHR**GV** (HLA-A*24:02; **1**) RTALHR**GV**V (HLA-A*24:02; **1**) LHR**GV**VPGY (HLA-A*24:02; **1**)	Luo et al. 2021

		ALHR**GV**VPG (HLA-A*24:02; **0**) R**GV**VPGYGH (HLA-A*24:02; **0**)	
ASAP1-WDYHV1* (Group 3)*	SSRDSLWN**r**EENIWKLCE	SLWN**RE**ENI (HLA-A*02:01; **21**) N**RE**ENIWKL (HLA-A*02:01; **17**) SRDSLWN**RE** (HLA-A*02:01; **4**) LWN**RE**ENIW (HLA-A*02:01; **4**) DSLWN**RE**EN (HLA-A*02:01; **3**) RDSLWN**RE**E (HLA-A*02:01; **2**) WN**RE**ENIWK (HLA-A*02:01; **2**) **RE**ENIWKLC (HLA-A*02:01; **-2**) NREENIWKL (HLA-A*24:02; **15**) SLWNREENI (HLA-A*24:02; **12**) DSLWNREEN (HLA-A*24:02; **3**) REENIWKLC (HLA-A*24:02; **3**) WNREENIWK (HLA-A*24:02; **1**) SRDSLWNRE (HLA-A*24:02; **0**) RDSLWNREE (HLA-A*24:02; **0**) LWNREENIW (HLA-A*24:02; **0**)	Luo et al. 2021
SAE-NPAS1* (Group 4)*	TTSDYFLL**Qa**PGRRGPAA	FLL**QA**PGRR (HLA-A*02:01; **13**) LL**QA**PGRRG (HLA-A*02:01; **13**) **QA**PGRRGPA (HLA-A*02:01; **10**) TSDYFLL**QA** (HLA-A*02:01; **9**) L**QA**PGRRGP (HLA-A*02:01; **9**) SDYFLL**QA**P (HLA-A*02:01; **8**) YFLL**QA**PGR (HLA-A*02:01; **6**) DYFLL**QA**PG (HLA-A*02:01; **3**) DYFLL**QA**PG (HLA-A*24:02; **10**) YFLL**QA**PGR (HLA-A*24:02; **8**) FLL**QA**PGRR (HLA-A*24:02; **3**) **QA**PGRRGPA (HLA-A*24:02; **3**) TSDYFLL**QA** (HLA-A*24:02; **1**) L**QA**PGRRGP (HLA-A*24:02; **1**)	Luo et al. 2021

		SDYFLL**QA**P (HLA-A*24:02; **0**) LL**QA**PGRRG (HLA-A*24:02; **0**)	
TET3-GMCL1* (Group 4)*	SIRELMEE**r**CLEWLLNNL	LMEE**RC**LEW (HLA-A*02:01; **14**) MEE**RC**LEWL (HLA-A*02:01; **13**) RELMEE**RC**L (HLA-A*02:01; **12**) ELMEE**RC**LE (HLA-A*02:01; **10**) **RC**LEWLLNN (HLA-A*02:01; **9**) EE**RC**LEWLL (HLA-A*02:01; **8**) IRELMEE**RC** (HLA-A*02:01; **1**) E**RC**LEWLLN (HLA-A*02:01; **-2**) RELMEE**RC**L (HLA-A*24:02; **12**) MEE**RC**LEWL (HLA-A*24:02; **11**) EE**RC**LEWLL (HLA-A*24:02; **11**) ELMEE**RC**LE (HLA-A*24:02; **4**) **RC**LEWLLNN (HLA-A*24:02; **4**) LMEE**RC**LEW (HLA-A*24:02; **3**) E**RC**LEWLLN (HLA-A*24:02; **2**) IRELMEE**RC** (HLA-A*24:02; **1**)	Luo et al. 2021
***LCLAT1-ERBB4* (Group 3)**	TFVVDRLR**EV**CAGTENKL	FVVDRLR**EV** (HLA-A*02:01; **22**) RLR**EV**CAGT (HLA-A*02:01; **19**) VDRLR**EV**CA (HLA-A*02:01; **7**) VVDRLR**EV**C (HLA-A*02:01; **6**) DRLR**EV**CAG (HLA-A*02:01; **6**) **EV**CAGTENK (HLA-A*02:01; **5**) LR**EV**CAGTE (HLA-A*02:01; **3**) R**EV**CAGTEN (HLA-A*02:01; **2**)	Jones et al. 2012

***MLLT6-MRPL45* (Group 4)**	SSASISTT**QP**VLVTQSAA	SISTT**QP**VL (HLA-A*02:01; **21**) ASISTT**QP**V (HLA-A*02:01; **16**) STT**QP**VLVT (HLA-A*02:01; **15**) TT**QP**VLVTQ (HLA-A*02:01; **14**) ISTT**QP**VLV (HLA-A*02:01; **13**) SASISTT**QP** (HLA-A*02:01; **8**) **QP**VLVTQSA (HLA-A*02:01; **8**) T**QP**VLVTQS (HLA-A*02:01; **5**)	Jones et al. 2012
***EPC2-GULP1* (WNT + SHH)**	TGMEKEEE**SA**EEITLTIG	**SA**EEITLTI (HLA-A*02:01; **20**) GMEKEEE**SA** (HLA-A*02:01; **14**) KEEE**SA**EEI (HLA-A*02:01; **12**) EE**SA**EEITL (HLA-A*02:01; **9**) E**SA**EEITLT (HLA-A*02:01; **9**) MEKEEE**SA**E (HLA-A*02:01; **2**) EKEEE**SA**EE (HLA-A*02:01; **2**) EEE**SA**EEIT (HLA-A*02:01; **0**)	identified and analyzed in the present work

* AA-sequence translated from published DNA sequence and expanded based on cDNA/AA-sequence alignments from ensembl.org

** fusion only in Asian cohort; SYFPEITHI analyses with HLA-A*02:01 (most common HLA-haplotype in Caucasian population) and with HLA-A*24:02 (most common HLA-haplotype in Asian population)