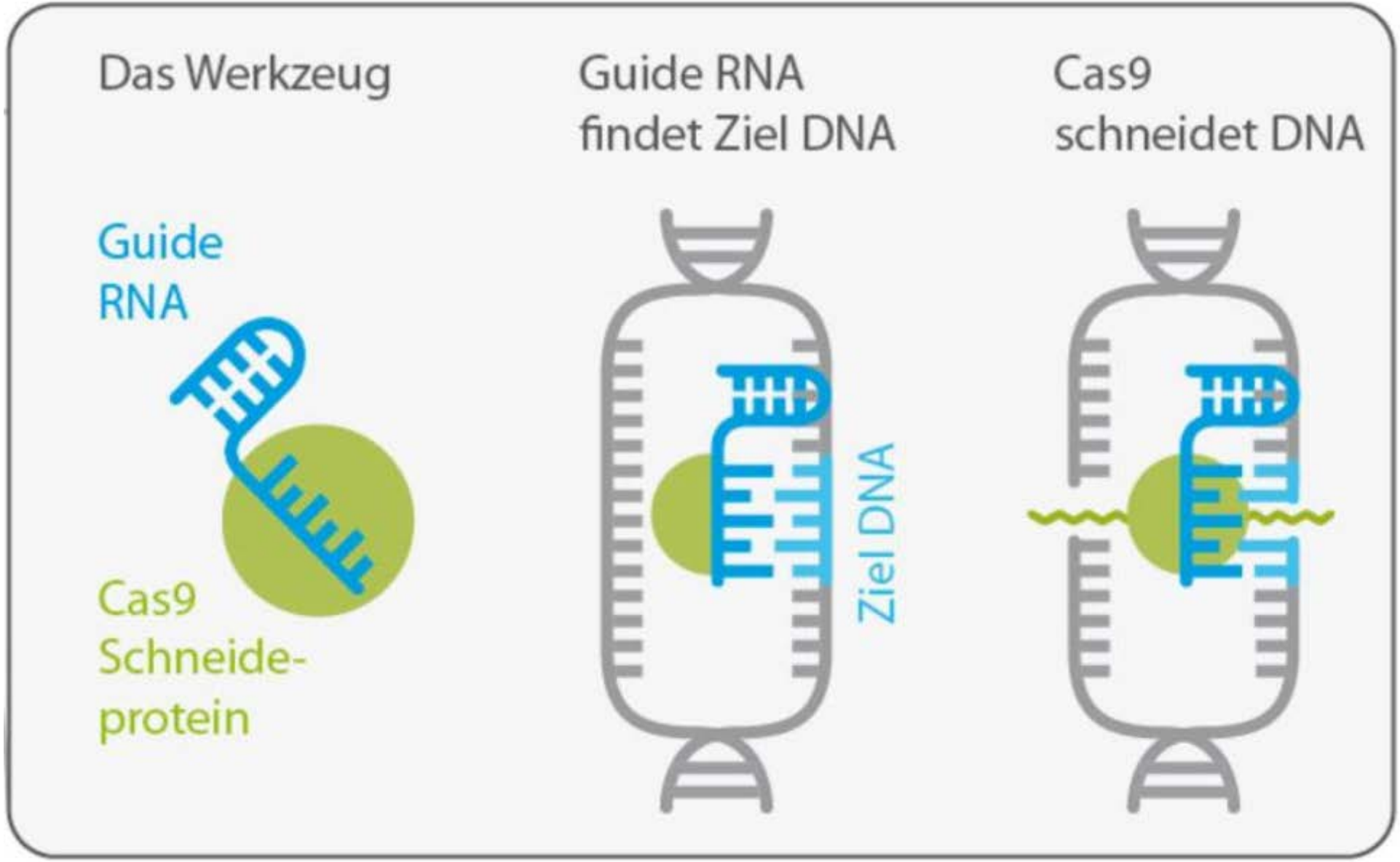
Multifunctional CRISPR-Cas9 with engineered immunosilenced human T cell epitopes.

Shayesteh R. Ferdosi, Radwa Ewaisha, Farzaneh Moghadam, Sri Krishna, Jin G. Park, Mo R. Ebrahimkhani, Samira Kiani & Karen S. Anderson

Nat Commun. 2019 Apr 23;10(1):1842. doi: 10.1038/s41467-019-09693-x.

Content:


Das Werkzeug
Guide RNA findet Ziel DNA
Cas9 schneidet DNA
Guide RNA
Cas9 Schneide-protein
Ziel DNA

CRISPR CAS 9

CRISPR: Clustered Regularly Interspaced Palindromic Repeats Loci in 40% of bacteria and 90% of archaea

Cas9: CRISPR associated protein 9 a nuclease, an enzyme specialized for cutting DNA

Is responsible for locating and cleaving target DNA

gRNA: guide RNA – a construct/chimera of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA)

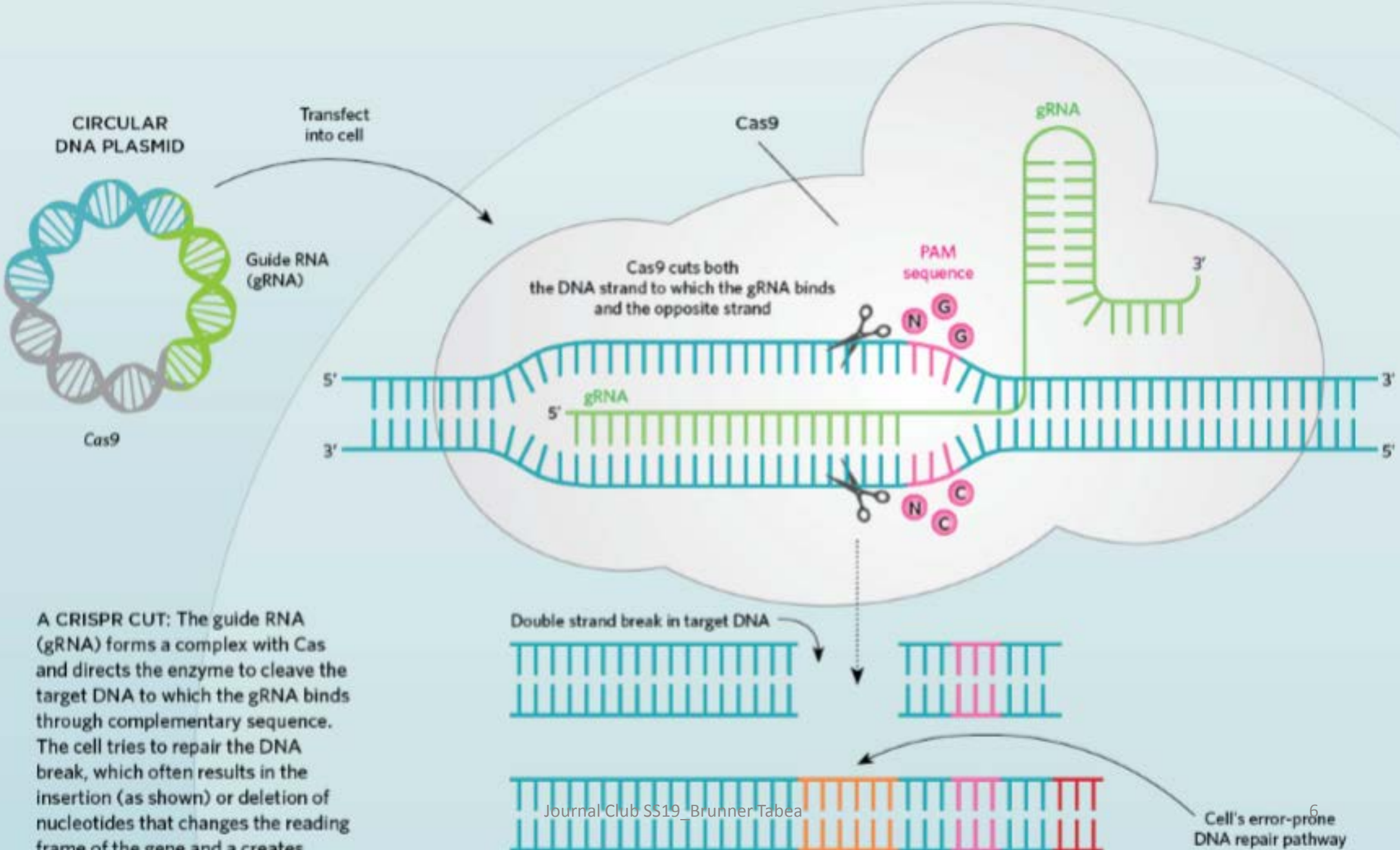
PAM: protospacer adjacent motif with sequence NGG (any, guanine, guanine) specific to Streptococcus pyogenes and 5'-NAG (any, adenine, guanine) PAM tolerated in human cells

SIX DOMAINS

RECI DOMAIN IST THE LARGEST AND IS RESPONSIBLE FOR BINDING GUIDE RNA

PAM INTERACTING DOMAIN IS RESPONSIBLE FOR INITIATING BINDING TO TARGET DNA

Cas 9 Protein



A CRISPR CUT: The guide RNA (gRNA) forms a complex with Cas and directs the enzyme to cleave the target DNA to which the gRNA binds through complementary sequence. The cell tries to repair the DNA break, which often results in the insertion (as shown) or deletion of nucleotides that changes the reading frame of the gene and a creates

Introduction

- Expression of Streptococcus pyogenes Cas 9 Protein has evoked cellular and humoral immune response in mice
- Raises concerns regarding safety and efficacy as gene or epi-gene therapy in humans
- SpCas 9 best characterized ubiquitous pathogen (annual incidence of 700 Mio. Worldwide)
- Epigenetic therapy requires longer term expression of Cas9 in vivo → poses challenge of combating pre-existing immune response

Aim

- Seek to characterize pre-existing immune response to SpCas 9 protein in healthy individuals
- Identify immunodominant T cell epitopes
- Developing SpCas 9 proteins that have diminished capacity to invoke human adaptive response
- Findings:
  - Identified two immunodominant SpCas 9 T cell epitopes for HLA-A* 02:01
  - Demonstrated that Cas 9 Protein can be modified to eliminate immunodominant epitopes through targeted mutation while preserving function and specificity

Methods

Detection of Cas9-specific serum antibodies

- Healthy sera (n=143)
- S.pyogenes lysate
- Serum antibody detection was performed using ELISA
  - Horseradish peroxidase anti-human IgG Abs
  - Detection: supersignal ELISA Femto Chemiluminescent substrate
- Luminometer: RLU (relative light unit)
- Cut-off value = any reactivity higher than top 99% of RLU values for human haemoglobin (dotted line)

Cas9 candidate T cell epitope prediction

- predicted MHC class I restricted 9-mer and 10-mer candidate epitopes derived from Cas9 protein for HLA A *02:01
- Protein sequence entered into 5 different prediction algorithms
  - 3 MHC binding:
    - IEDB consensus binding, Net MHCpan binding, Syfpeithi
  - 2 antigen-processing algorithms:
    - IEDB consensus processing, ANN processing
  - IEDB consensus binding → list of high binding Cas9 peptides → assigned a normalized binding score (Sb)
  - Si (immunogenicity score) calculated based on amino-acid hydrophobicity (ANN-Hydro)
- Individual scores normalized within pool of predicted peptides (exclusion of poor binders) - re-rank
- Top 38 candidate peptides selected for experimental testing

Exkurs:

Chowell et al.

TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes

Proc Natl Acad Sci U S A. 2015 Apr 7;112(14):E1754-62. doi: 10.1073/pnas.1500973112. Epub 2015 Mar 23.

- relative amino acid hydrophobicity within immunogenic epitopes reveals an antigenic pattern that can be recognized by TCRs
- design an immunogenicity model - ANN-Hydro

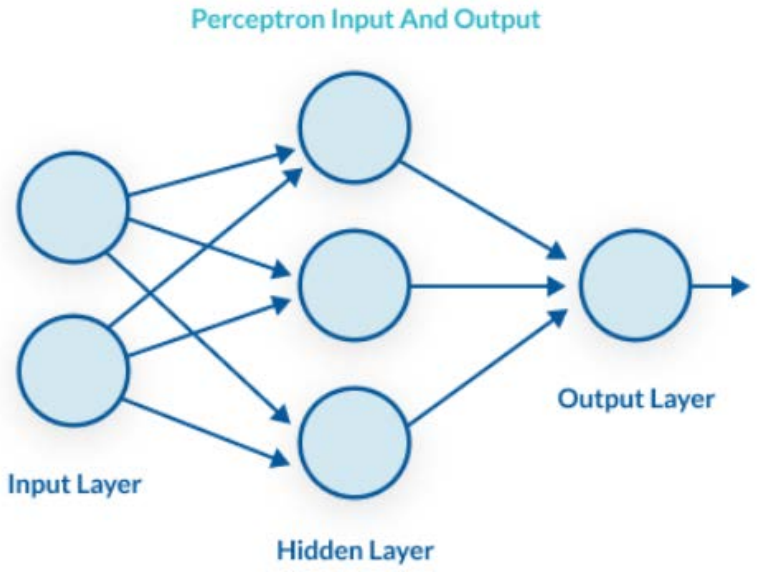
$$y(H) = f\left(w_0 + \sum_{i=1}^{3} w_i \cdot f(w_{0i} + W_i^T H)\right),$$

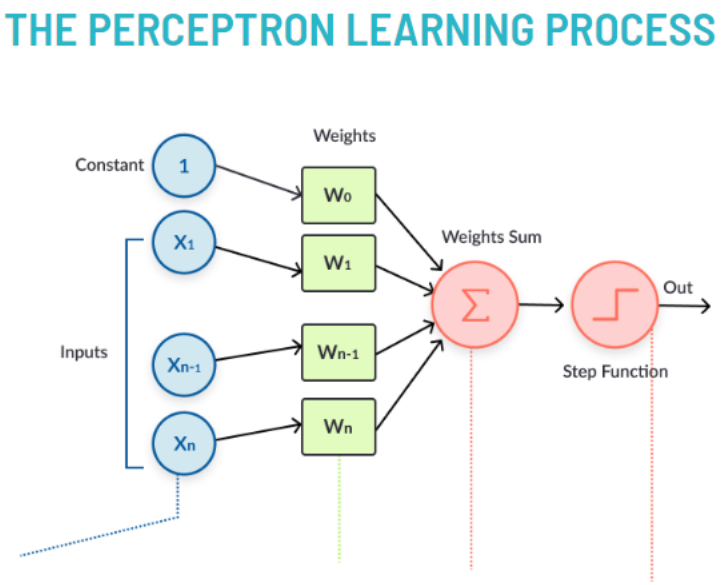
Neural network

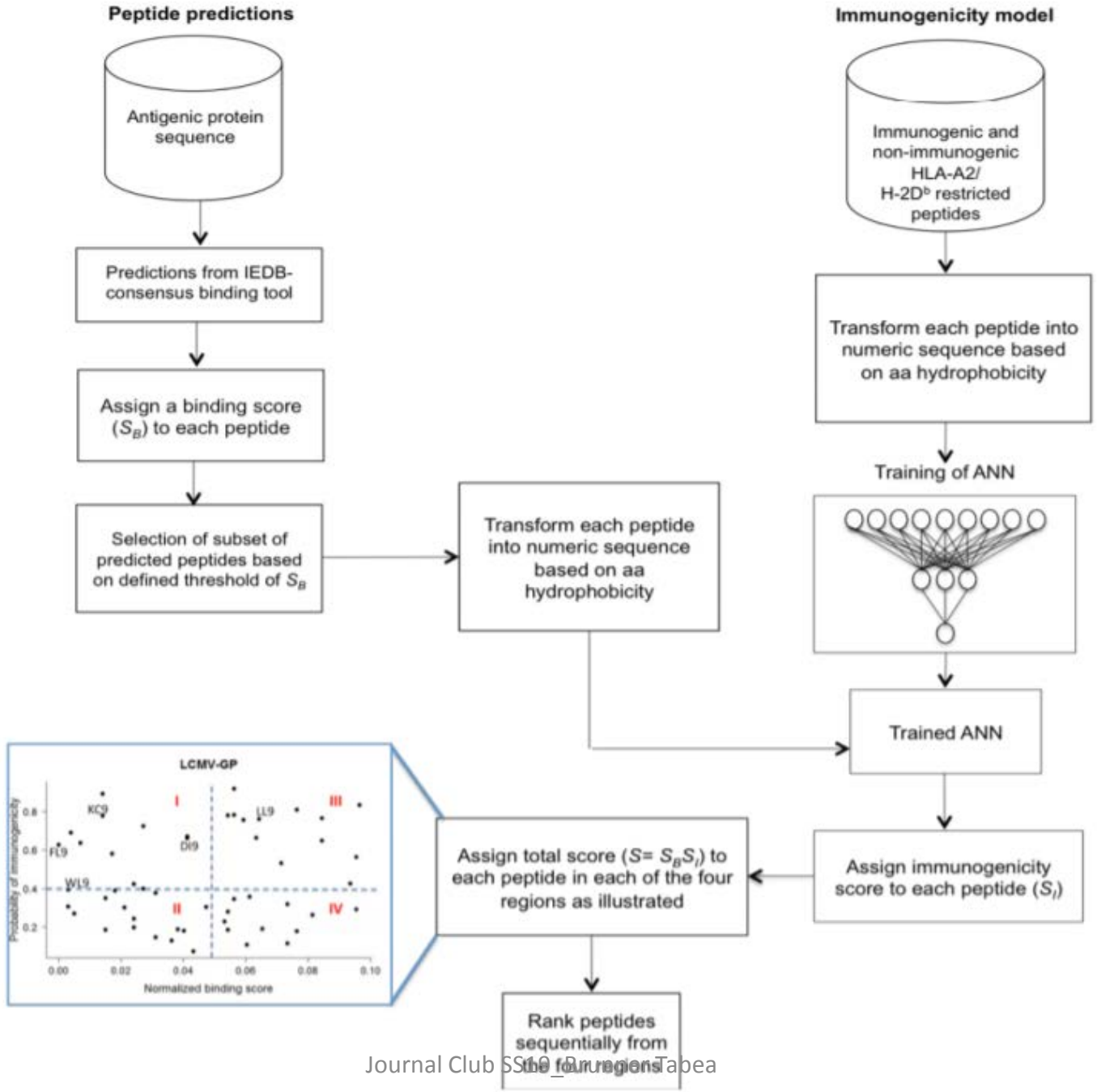
- Supervised learning system
- Neurons = function
- Each neuron can make simple decisions and feed those decisions to other neuron
- Organized in interconnected layer
- Components:
  - Input layer
  - Hidden layer(s)
  - Output layer

Components

- Input
- Output
- neuron/perceptron: accepts an input and generates a prediction
- Weight: each neuron is given a numeric weight
- Weight and activation function define output






Peptide predictions
Antigenic protein sequence
Predictions from IEDB-consensus binding tool
Assign a binding score (S_B) to each peptide
Selection of subset of predicted peptides based on defined threshold of S_B
Immunogenicity model
Immunogenic and non-immunogenic HLA-A2/ H-2D^b restricted peptides
Transform each peptide into numeric sequence based on aa hydrophobicity
Training of ANN
Transform each peptide into numeric sequence based on aa hydrophobicity
Trained ANN
Assign immunogenicity score to each peptide (S_I)
Assign total score ($S = S_B S_I$) to each peptide in each of the four regions as illustrated
LCMV-GP
Probability of immunogenicity
Normalized binding score
I
II
III
IV
KC9
FL9
WL9
DI9
LL9
Rank peptides sequentially from the four regions

Ex vivo stimulation and epitope mapping of Cas9 by ELISpot

- PBMCs from healthy donors
- Predicted Cas9 peptides (Sb < 0,148) n=38 synthesized (Proimmune UK)
- ELISpot – anti IFN-γ – PBMCs – human IL2 added
- Stimulated with Cas9 peptide pools
  - CEF positive control, DMSO negative control
- incubated, →washed 3x →incubated detection antibody→ washed & incubated Streptavidin→ washed→ detection buffer → AID ELISpot reader

- Autologous APC generation from healthy individual PBMCs
- T cell stimulation by autologous APCS
  - Peptide pulsing of APCs was done under BCM 5% human serum, with recombinant IL-4.
  - Transfection of APCs was done with primary P3 buffer in a Lonza 4D Nucleofector- incubated in BCM-10% human serum and IL-4.
  - 24 hrs later - APCs were washed and incubated with thawed whole PBMCs at a ratio of 1:2 in a 24-well plate in BCM supplemented with 20U/mL recombinant human IL-2 and 5ng/mL IL-7
  - day 5, partial media exchange: replacing half the well with fresh BCM and IL-2.
  - day 10fresh APCs were peptide pulsed in a new 24-well plate
  - day 11, expanded T cells were restimulated with peptide-pulsed APCs
  - T cells were used for T cell assays or immunophenotyped after day 18.

Flow cytometry staining for T-cells

- Cells washed in MACS buffer-centrifuged-resuspended in buffer – stained buffer (anti CD137) conjugated with PE – anti-CD8-PC5, anti-CD4 , anti-CD14 and anti-CD19
- all conjugated to Fluorescein isothiocyanate (FITC)
- covered and incubated for 30min on ice, washed twice in PBS, and resuspended in 1mL PBS prior to analysis

Pentamer staining for T cell immunophenotyping

- HLA-A*02:01 PE-conjugated Cas9 pentamers were obtained from ProImmune:
  - F2A-D-CUSA*02:01-ILEDIVLTL-Pentamer and 007-Influenza A MP 58-66-GILGFVFTLPentamer
- T cells washed twice in MACS buffer - centrifuged -re-suspended in staining buffer
- Each of the pentamers was added to resuspended T cells, stimulated with the respective peptide or APCs
- incubated at room temperature for 30min in the dark - washed twice in MACS buffer
- Cells were stained in MACS buffer with antiCD8-PC5, anti-CD4-FITC, anti-CD14-FITC, and anti-CD19-FITC
- washed twice with PBS and analyzed by flow cytometry

- Cell culture for endogenous target mutation and activation
  - HEK293FT cell line was purchased from ATCC, incubated 37° Grad
  - PEI was used to transfect HEK293 FT, Transfection complexes prepared according to Polysciences instructions.
- Fluorescent reporter assay for quantifying Cas 9 function
  - Assessing Cas9 cleavage capacity at a synthetic promoter HEK293FT cells were transfected with gRNA (200ng), Cas9 constructs (200ng), reporter plasmid (50ng), EBFP (25ng)
  - Assessing Cas9 transcriptional activation capacity , HEK293FT co-transfected with gRNA (50ng), Cas9 constructs (70ng), MS2-P65-HSF1-GFP (100ng), reporter plasmid (200ng)
  - Fluorescent reporter experiments were performed 48h after transfection
  - selected cells expressing EBFP >2×102 A.U. or GFP >2×102A.U. (transfection markers) in the cleavage and activation experiments
  - Flow cytometry was performed using a FACSCelesta flow cytometer

Quantitative RT-PCR analysis

- HEK293FT cells were co-transfected with
- gRNA, Cas9 constructs, MS2-P65-HSF1 and EBFP plasmid as the transfection control
- 72 post transfection - cells were lysed - RNA was extracted (RNeasy Plus mini kit)- cDNA synthesis (High-Capacity RNA-to-cDNA Kit)
- qRT-PCR : SYBR Green PCR Master Mix

Endogenous indel analysis

- HEK293FT cells co-transfected Cas9 plasmids (200ng), gRNA coding cassette (10ng), EBFP plasmid (25ng)
- 72h later, transfected cells were dissociated and spun down at 200g for 5min at room temperature
- Genomic DNA was extracted - QuickExtract DNA extraction solution (Epicentre)
- Genomic DNA was amplified by PCR using primers flanking the targeted region
- PCR cycles were performed
- To detect the indels (insertions and deletions of nucleic acid sequence at the site of double-strand break), each mutation was evaluated carefully in order to exclude the ones that are caused by sequencing error or any off-target mutation

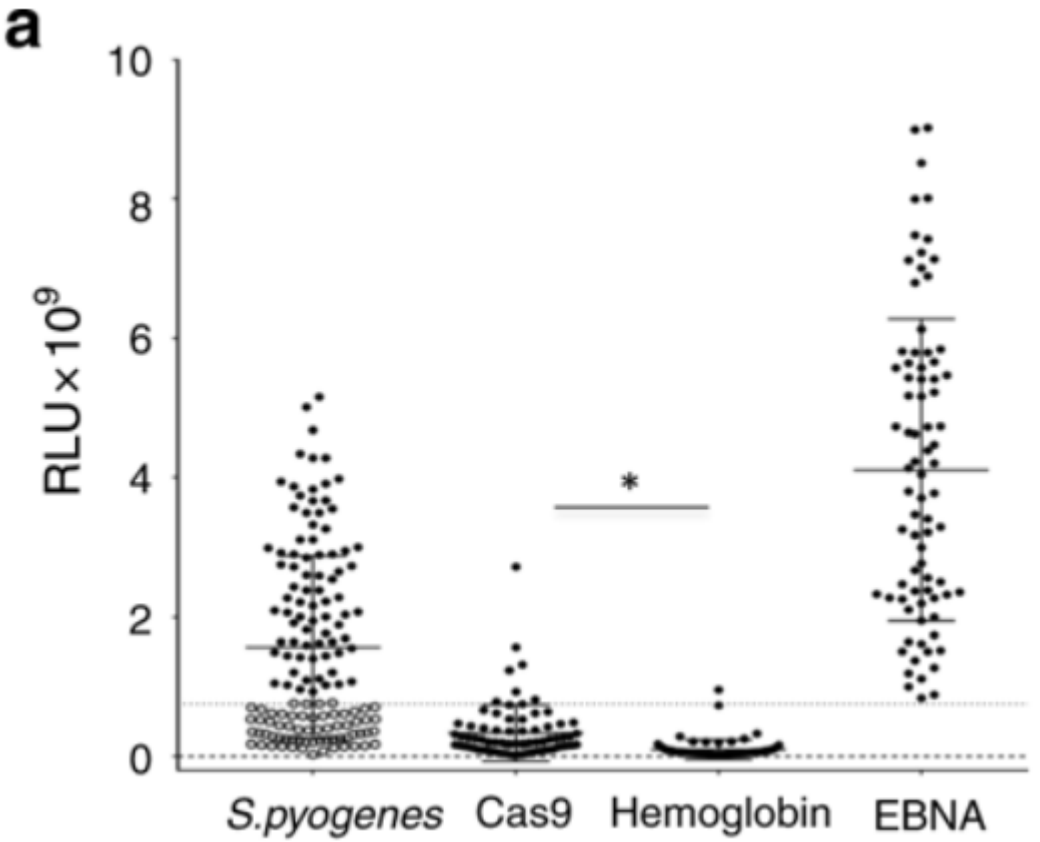
RNA sequencing for quantifying activator specificity

- HEK293FT cells cotransfected with :
  - gRNA targeting MIAT locus, Cas9 constructs, MS2-P65-HSF1, transfection control
- 72h post transfection RNA extracted using RNeasy Plus mini kit
- RNA sequencing using NextSeq500
- Aligned to human reference genome with STAR (ultrafast universal RNA-seq aligner

Results

Cas9-specific serum antibodies in healthy controls

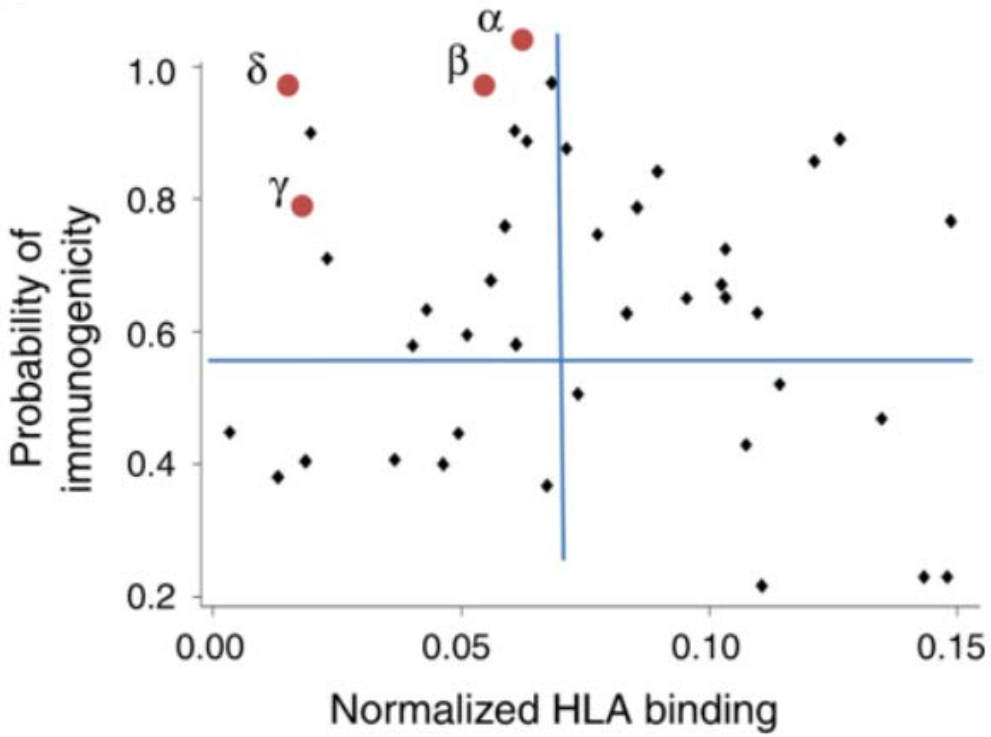
- Detectable IgG Abs to SpCas9 in healthy individuals
- healthy control sera (n=143)
  - 82 (57,3%) → detectable Abs against S. pyogenes lysate (ELISA)
- Sera with highest reactivity screened for Abs against recombinant SpCas9
  - 7 (8,8%) positive
- 5% had Cas9 specific Abs (143).



Cas9 candidate T cell epitope prediction

- Predict more immunogenic epitopes
- High HLA binding (low Sb)
- More hydrophobicity (high Si)
- HLA-A*02:01 most common HLA European/North American Caucasians

c



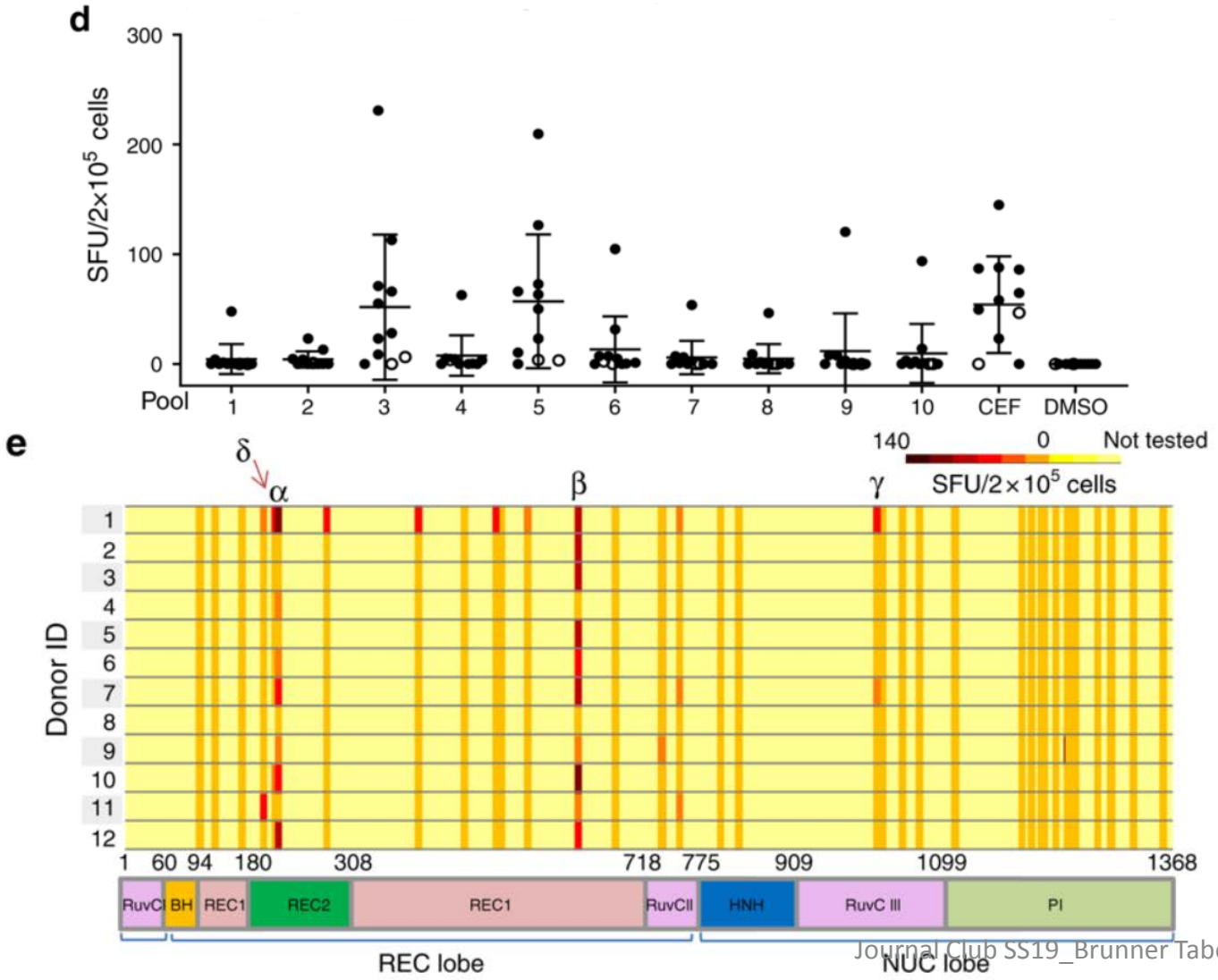
b

Rank	Position	Sequence	Code	HLA binding	S_b	S_i	$S_b.S_i$
1	988–997	YLNAVVGTAL	γ	1.25	0.068	0.975	0.002
2	1281–1290	ILADANLDKV		1.25	0.003	0.447	0.002
3	236–244	GLFGNLIAL	δ	0.6	0.020	0.900	0.002
4	240–248	NLIALSLGL	α	1.7	0.061	0.903	0.006
5	615–623	ILEDIVLTL	β	1.5	0.023	0.710	0.007

T cell epitope mapping of Cas9

- PBMcs measurable T cell reactivity against predicted SpCas9 MHC class I epitopes
- Synthesized 39 peptides, grouped in 10 pool, 3-4 peptide each
- IFN-γ ELISpot assays (n 12)
- Identified immunoreactive epitopes within pools 3 and 5 in 83% of donors tested (90% of HLA-A* 02:01)
- Evaluated by IFN-γ ELISpot assays - **dominant immunogenic epitopes**:
- SpCas9_240–248 and SpCas9_615-623 designated peptides **α** and **β**
- **subdominant epitopes** were found to be **γ** and **δ**

T cell epitope mapping of Cas9



- Dominant: **α** and **β**
- Subdominant: **γ** and **δ**
- Pool 3 : **β** and **γ**
- Pool 5: **α** and **δ**
- **α** and **β** Rec lobe of Cas 9 Protein

Rec lobe: binds sgRNA and target DNA heteroduplex

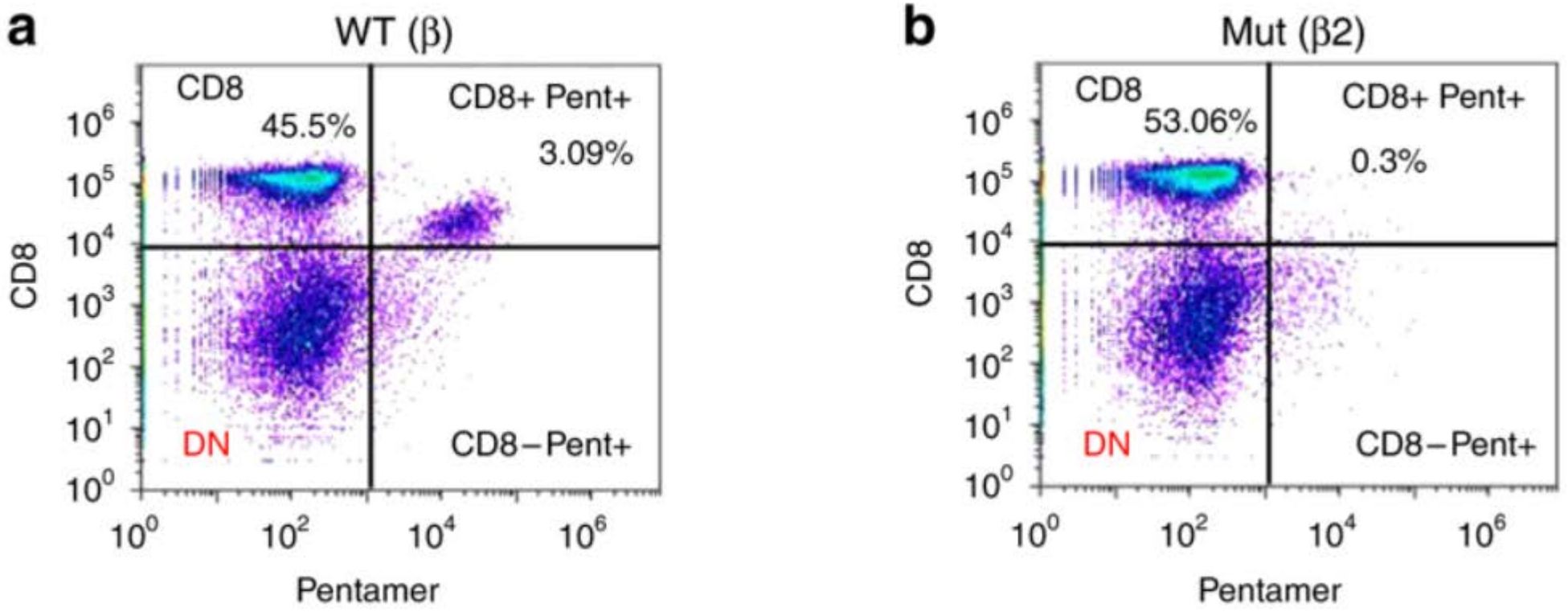
T cell epitope mapping of Cas9

- Both immunodominant and subdominant epitopes identified by IFN-γ ELISpot were within top 5 most immunogenic epitopes predicted by model
- Sequence similarity: peptides **α** and **β** to amino acid sequences in known proteins was investigated using Protein BLAST and the IEDB epitope database
  - T cell immune response due to previous exposure to another protein
- Peptide considered "similar" to **α** or **β** if no more than 2 of 9 amino acid residues (that are not the second or ninth) were not matching (78%)
- Non resembled known epitopes in IEDB database
- Similarity to other orthologs and bacterial proteins detected
- Epitope **β** sequence similarity to peptide derived from Neisseria meningitidis peptide chain release factor 2 protein (ILEDIVLTL versus ILEGIVLTL).

Mutated Cas9 proteins have lower immune recognition

- Assumption: mutation of MHC-binding anchor residues would abolish specific T cell recognition
- Epitope anchor residues (2^{nd} and 9^{th}) necessary for peptide binding to MHC groove and crucial for recognition by T cell receptor
- Percentage of CD8+ **β** pentamer+T cells decreased to 0,3% when Apcs were pulsed with mutated peptide

Mutated Cas9 proteins have lower immune recognition



Mutated Cas9 proteins have lower immune recognition

- Reactivity of healthy donor T cells to modified peptides **α** or **β** with mutations in residues 2, 9 or both

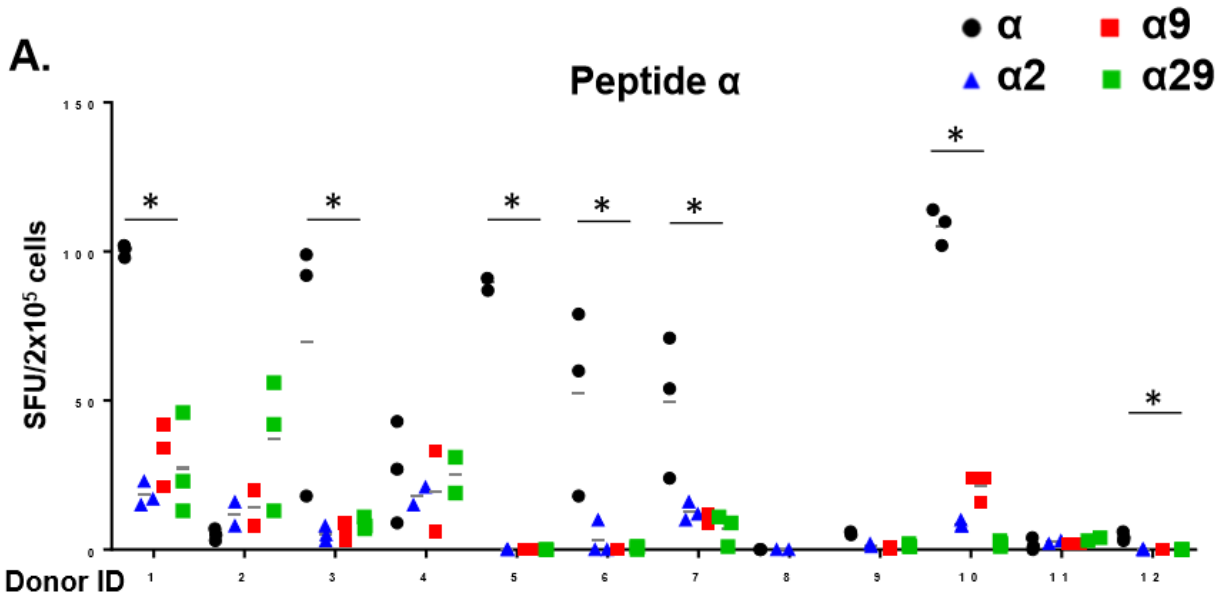
Peptide code (position)	Peptide sequence	HLA binding (percentile rank)	Peptide code (position)	Peptide sequence	HLA binding (percentile rank)
α (240–248)	NLIALSLGL	1.7	β (615–623)	ILEDIVLTL	1.5
α2	NGIALSLGL	26	β2	IGEDIVLTL	23
α9	NLIALSLGG	14	β9	ILEDIVLTG	12
α29	NGIALSLGG	62	β29	IGEDIVLTG	49

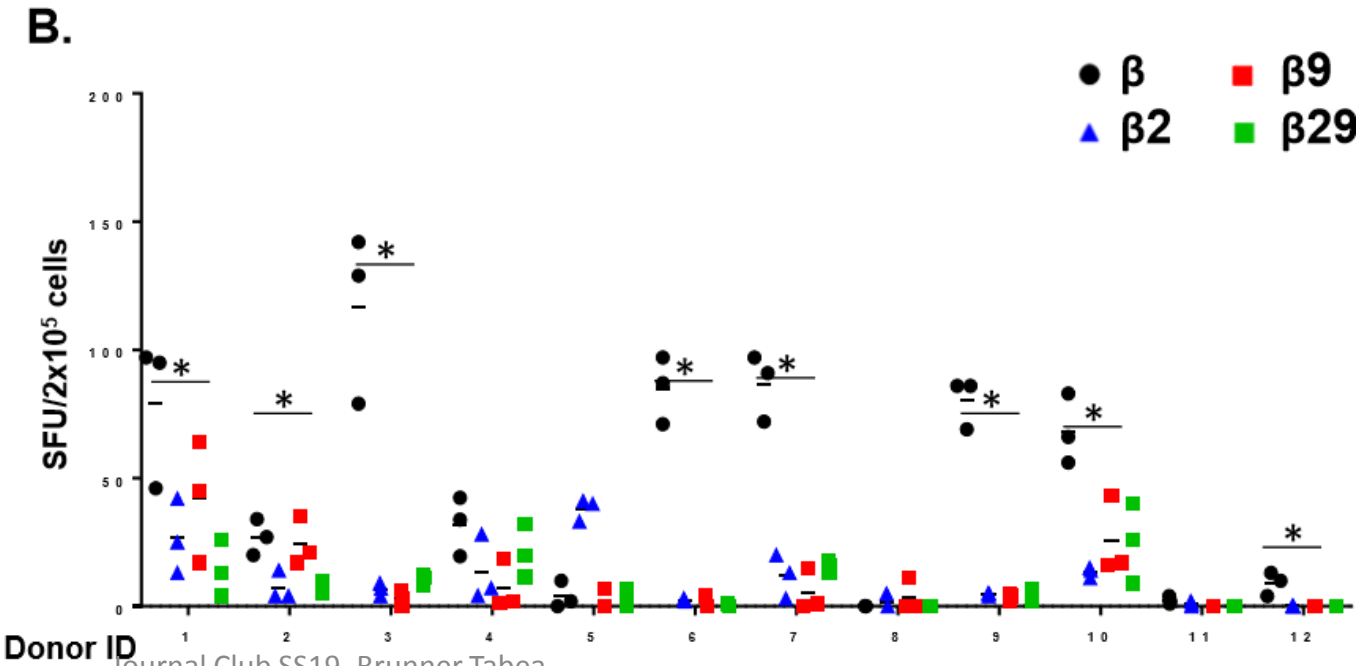
Mutated Cas9 proteins have lower immune recognition

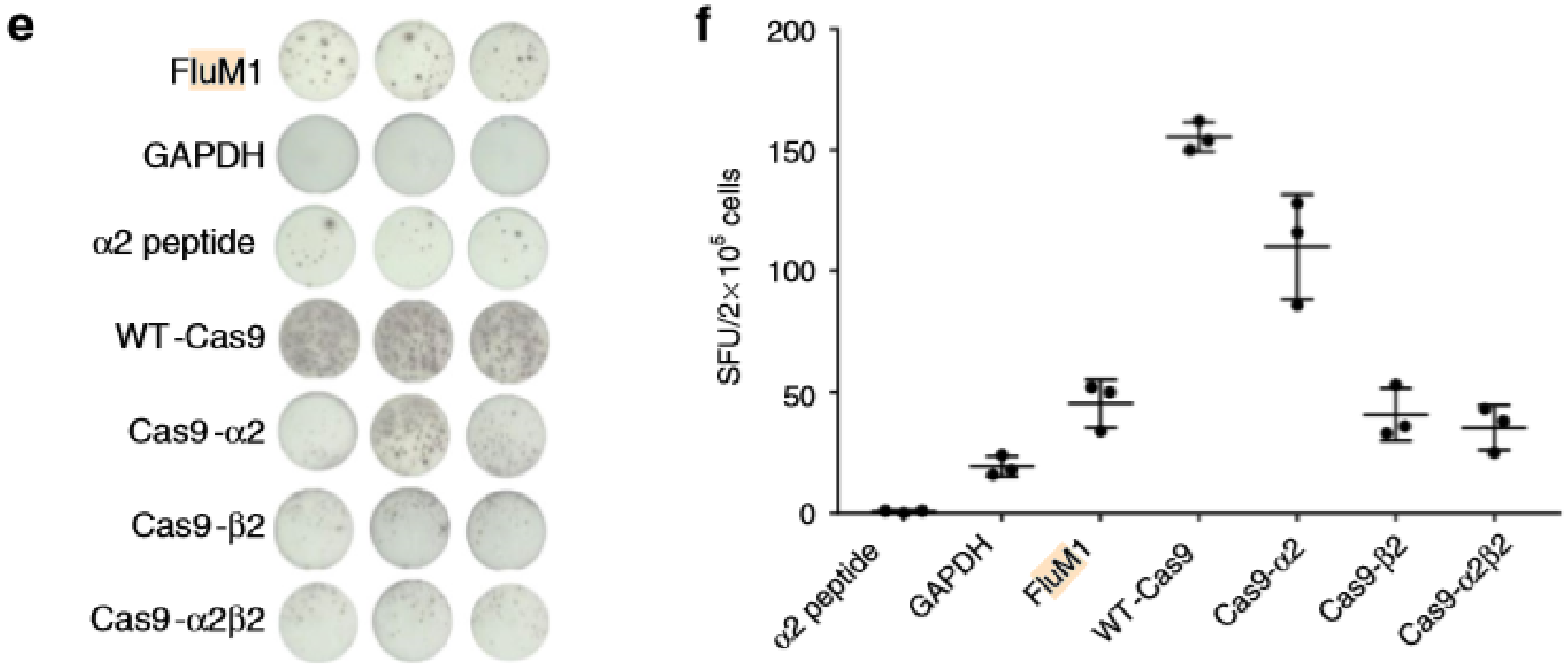
- Epitope-specific T cell reactivity markedly reduced with the mutant peptides

- Average reduction
- 25 fold from **α to α 29** (n=7)
- 30 fold from **β to β29** (n=8)

Supplementary Figure 1

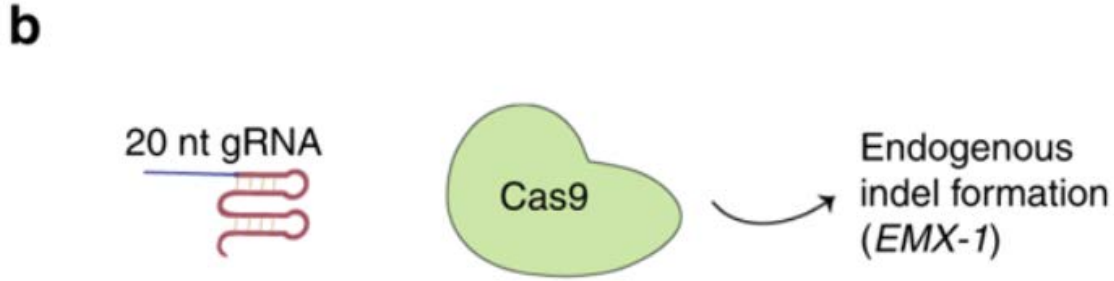


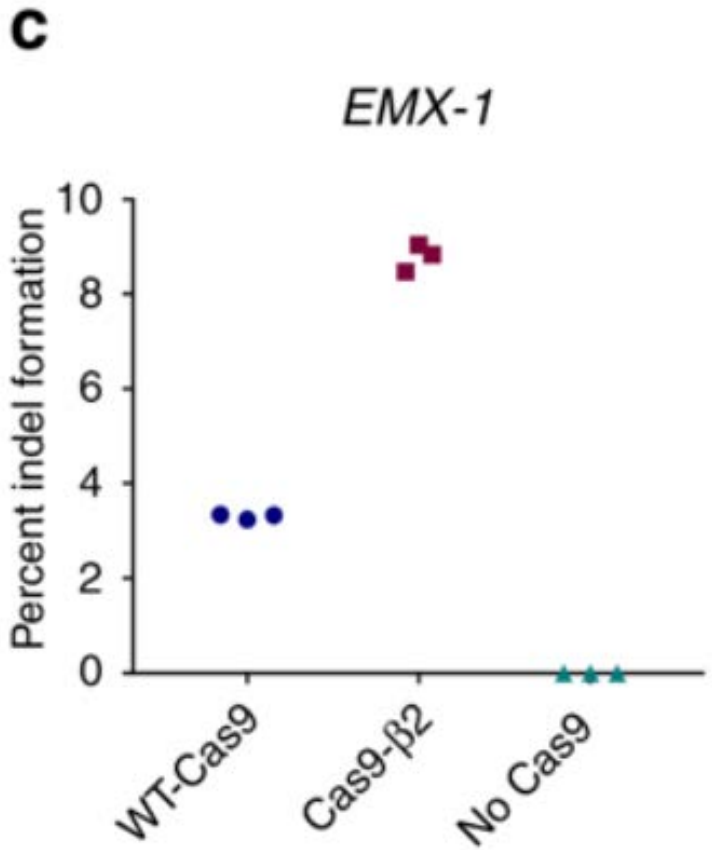


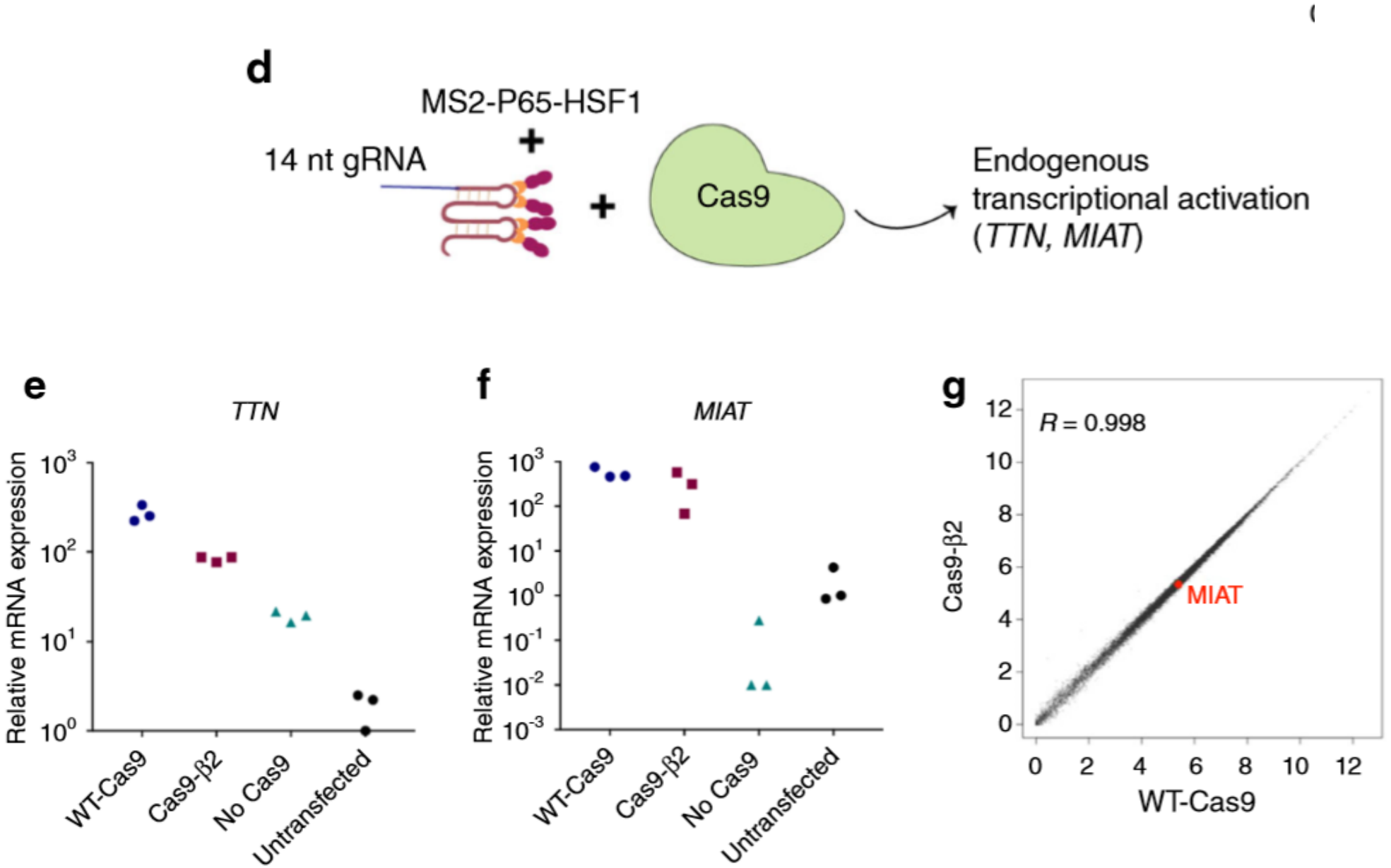

e
FluM1
GAPDH
α2 peptide
WT-Cas9
Cas9-α2
Cas9-β2
Cas9-α2β2
f
SFU/2×10^5 cells
200
150
100
50
0
α2 peptide
GAPDH
FluM1
WT-Cas9
Cas9-α2
Cas9-β2
Cas9-α2β2

Mutated Cas9 proteins have lower immune recognition

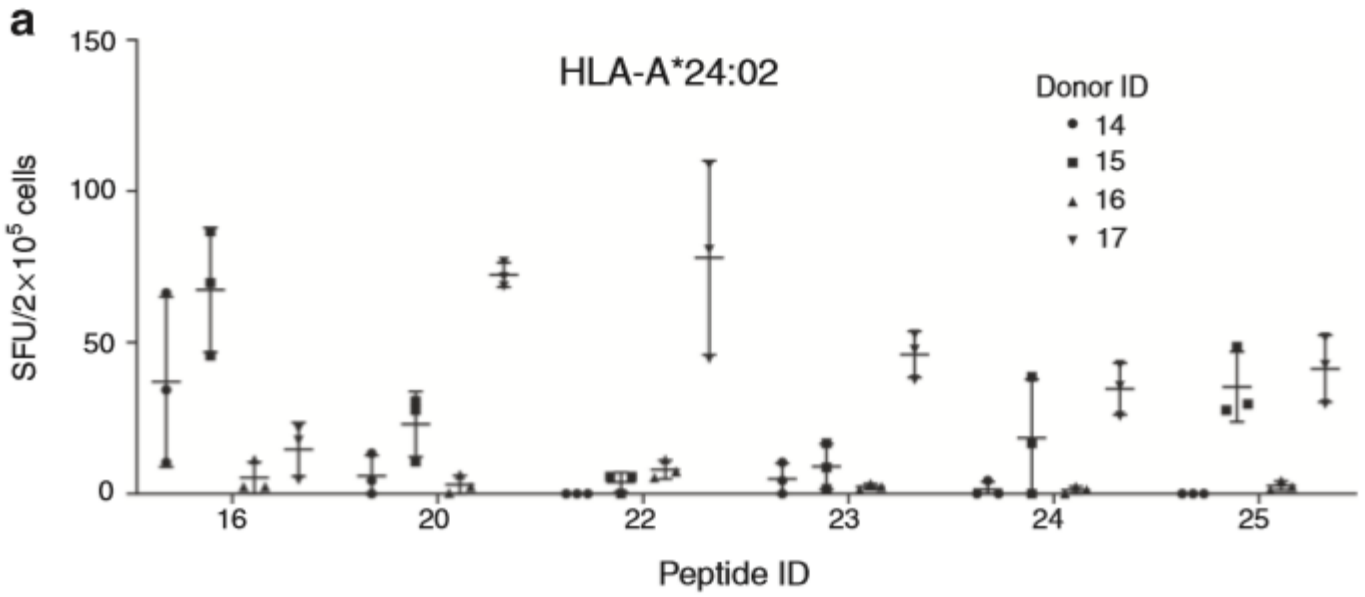
- Engineering Cas9 variant with reduced immunogenicity potential → safer CRISPR therapies, reduction of dosage of systemic immunosuppression
- Tested function of Cas9- **β** compared with WT-Cas9 (DNA cleavage and transcriptional modulation)
- **Nuclease activity:** targeted to endogenous locus (EMX-1): measured percent indel formation






d
MS2-P65-HSF1
14 nt gRNA
Cas9
Endogenous
transcriptional activation
(TTN, MIAT)
e
TTN
Relative mRNA expression
WT-Cas9
Cas9-β2
No Cas9
Untransfected
f
MIAT
Relative mRNA expression
WT-Cas9
Cas9-β2
No Cas9
Untransfected
g
R = 0.998
MIAT
Cas9-β2
WT-Cas9

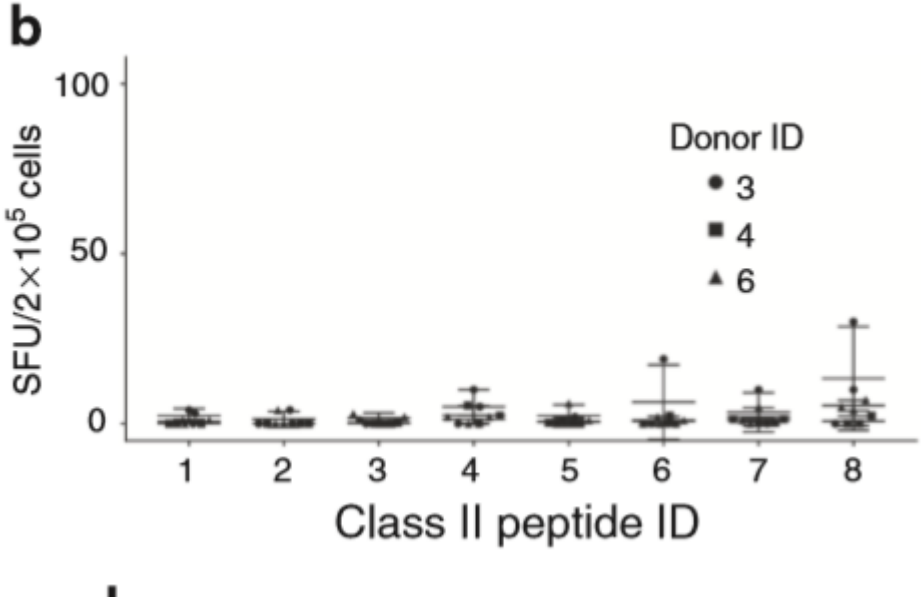
Immune response to non HLA-A*02:01 Cas9 epitopes



IFN gamma ELISpot

- Predicted T cell epitopes for non HLA-A *02:01 alleles (IEDB)
- Synthesized 5-6 epitopes
- 7 common alleles: A*01:01, A*03:01, A*11:01, A*24:02, B*08:01, B*44:01, B*55:01
- Stimulate PBMCs (n=6)
- Specific T cell immune response (in more than 1 donor) against peptide 25 in two HLA-A*24:02

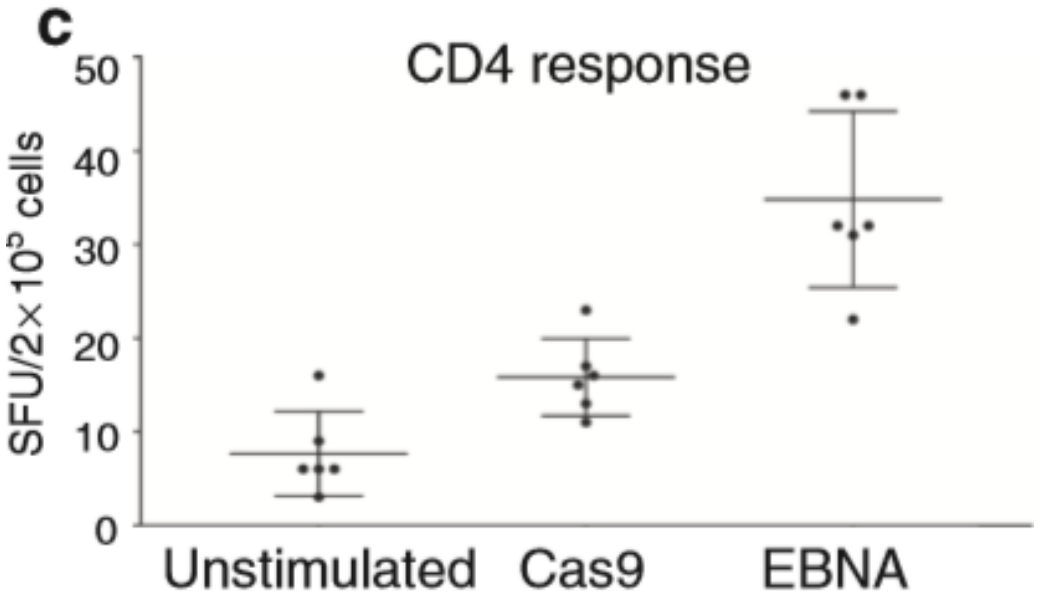
Immune response to MHC Class II Cas9 epitopes



ELISpot
CD8 depleted PBMCs n=3

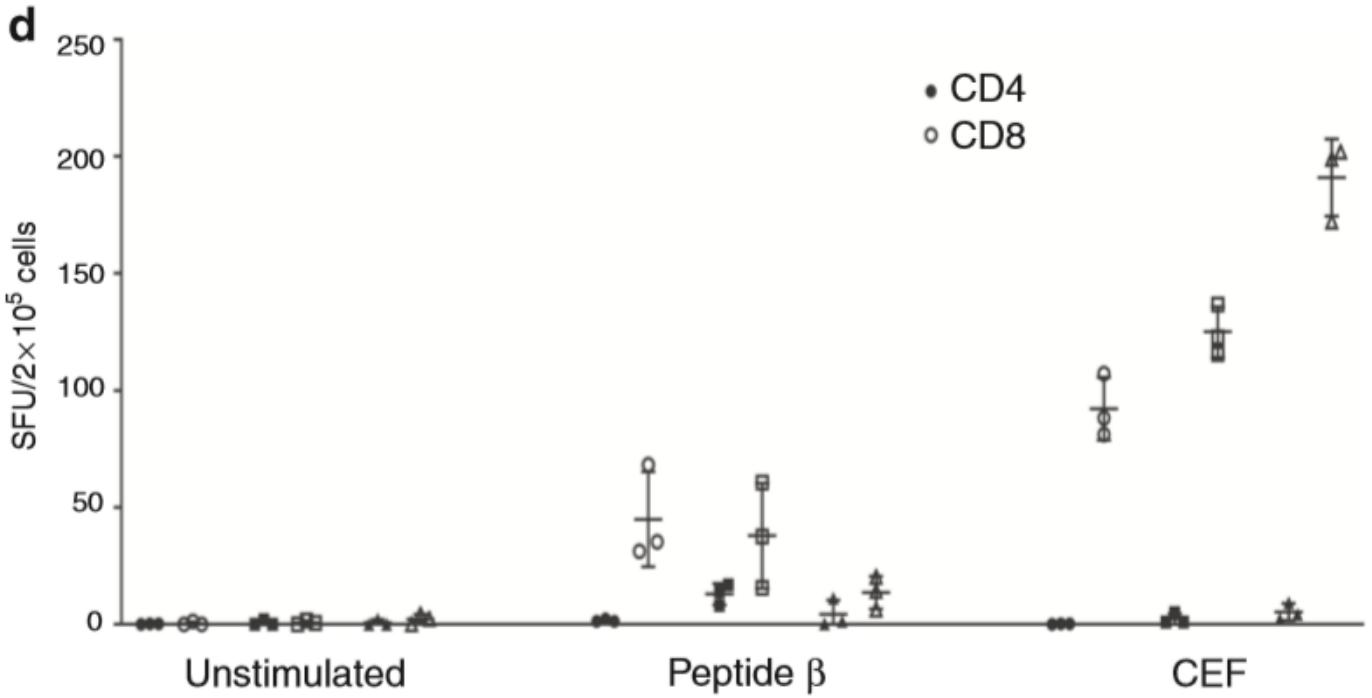
- predicted MHC class II binding epitopes for the SpCas9 protein to HLA-DRB1 (10 alleles), HLA-DQ (5 alleles), and HLA-DP (8 alleles) (IEDB)
- For MHC class II, epitope α is predicted to be a top binder to HLADRB1*01:02
- epitope β a top binder to HLA-DPA1*01:03 and DPB1*02:01
- Synthesized SpCas9 long peptides with epitopes in top 2% of predicted binders
- Measured peptide-specific CD4+ T cell immunity
- Limited CD4+ immune response

CD 4 response



- Stimulation of healthy donor CD8 depleted PBMCs with recombinant SpCas9 or EBNA
- Less than two fold of unstimulated

CD4+/ CD8+



- Stimulated sorted CD4+ or CD8+ T cells isolated from PBMCs with peptide Beta
- Detected: primarily CD8+response
- PBMCs n=3

Discussion:

- CD8+ T cell immunity likely memory responses
- Following 18 days of T cell stimulation by peptides (alpha, beta) expansion of naïve T cells is not precluded
- Suggests: even in absence of pre-existing immune response, expression of Cas9 in naïve individuals may trigger T cell response (could prevent subsequent administration)
- Selective deimmunization (immunosilencing) of Cas9 can represent an attractive alternative
- Modest CD4+ immune response against recombinant SpCas9 protein (IFN-γ ELISpot)
- No response any of class II SpCas9 peptides that were used
- Silencing one epitope for an HLA-A*02:01 sufficient to significantly reduce Cas 9 immunogenicity
- Alteration of one of the anchor residues of an immunodominant epitope abolished specific T cell recognition
- HLA allotype diversity
- Existence of numerous epitopes in Cas 9 protein - deimmunization
- Overall impact of removal of select immunodominant epitopes remain to be seen

References

1. Cyranoski, D. Chinese scientists to pioneer first human CRISPR trial. *Nature* **535**, 476 (2016).
2. Reardon, S. First CRISPR clinical trial gets green light from US panel. *Nature* **531**, 160–163 (2016).
3. Cavazzana-Calvo, M. et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* **288**, 669–672 (2000).
4. Gaspar, H. B. et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet* **364**, 2181–2187 (2004).
5. Howe, S. J. et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* **118**, 3143–3150 (2008).
6. Marshall, E. Gene therapy death prompts review of adenovirus vector. *Science* **286**, 2244–2245 (1999).
7. Hacein-Bey-Abina, S. et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N. Engl. J. Med.* **346**, 1185–1193 (2002).
8. Manno, C. S. et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat. Med.* **12**, 342–347 (2006).
9. Chew, W. L. et al. A multifunctional AAV-CRISPR-Cas9 and its host response. *Nat. Methods* **13**, 868–874 (2016).
10. Wang, D. et al. Adenovirus-mediated somatic genome editing of Pten by CRISPR/Cas9 in mouse liver in spite of Cas9-specific immune responses. *Hum. Gene Ther.* **26**, 432–442 (2015).
11. Mays, L. E. & Wilson, J. M. The complex and evolving story of T cell activation to AAV vector-encoded transgene products. *Mol. Ther.* **19**, 16–27 (2011).
12. Mingozzi, F. & High, K. A. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood* **122**, 23–36 (2013).
13. Yin, H. et al. Non-viral vectors for gene-based therapy. *Nat. Rev. Genet.* **15**, 541–555 (2014).
14. Ahi, Y. S., Bangari, D. S. & Mittal, S. K. Adenoviral vector immunity: its Implications and circumvention strategies. *Curr. Gene. Ther.* **11**, 307–320 (2011).
15. Aldhamen, Y. A. & Amalfitano, A. In *Adenoviral Vectors for Gene Therapy* 2nd edn, 391–422 (Academic Press, Cambridge, Massachusetts 2016).
16. Thwaite, R., Pages, G., Chillon, M. & Bosch, A. AAVrh.10 immunogenicity in mice and humans. Relevance of antibody cross-reactivity in human gene therapy. *Gene Ther.* **22**, 196–201 (2015).
17. Boutin, S. et al. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Hum. Gene Ther.* **21**, 704–712 (2010).
18. Mingozzi, F. et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. *Nat. Med.* **13**, 419–422 (2007).
19. Scallan, C. D. et al. Human immunoglobulin inhibits liver transduction by AAV vectors at low AAV2 neutralizing titers in SCID mice. *Blood* **107**, 1810–1817 (2006).
20. Bartel, M., Schaffer, D. & Buning, H. Enhancing the clinical potential of AAV vectors by capsid engineering to evade pre-existing immunity. *Front. Microbiol.* **2**, 204 (2011).
21. Martino, A. T. et al. Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8+T cells. *Blood* **121**, 2224–2233 (2013).
22. Mingozzi, F. et al. Overcoming preexisting humoral immunity to AAV using capsid decoys. *Sci. Transl. Med.* **5**, 194ra192 (2013).
23. Jiang, H. et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood* **108**, 3321–3328 (2006).
24. Kay, M. A. State-of-the-art gene-based therapies: the road ahead. *Nat. Rev. Genet.* **12**, 316–328 (2011).
25. Gao, G. et al. Adeno-associated virus-mediated gene transfer to nonhuman primate liver can elicit destructive transgene-specific T cell responses. *Hum. Gene Ther.* **20**, 930–942 (2009).
26. Carapetis, J. R., Steer, A. C., Mulholland, E. K. & Weber, M. The global burden of group A streptococcal diseases. *Lancet Infect. Dis.* **5**, 685–694 (2005).
27. Simhadri, V. L. et al. Prevalence of pre-existing antibodies to CRISPR-associated nuclease Cas9 in the USA population. *Mol. Ther. Methods Clin. Dev.* **10**, 105–112 (2018).
28. Wagner, D. L. et al. High prevalence of Streptococcus pyogenes Cas9-reactive T cells within the adult human population. *Nat. Med.* **25**, 242–248 (2018).
29. Ibrahim, S. H. & Robertson, K. D. Use of the CRISPR/Cas9-based epigenetic gene activation system in vivo: a new potential therapeutic modality. *Hepatology* **68**, 1191–1193 (2018).
30. Liao, H. K. et al. In vivo target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell* **171**, 1495–1507 e1415 (2017).
31. Zheng, Y. et al. CRISPR interference-based specific and efficient gene inactivation in the brain. *Nat. Neurosci.* **21**, 447–454 (2018).
32. Chowell, D. et al. TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+T cell epitopes. *Proc. Natl Acad. Sci. USA* **112**, E1754–E1762 (2015).
33. Nishimasu, H. et al. Crystal structure of cas9 in complex with guide RNA and target DNA. *Cell* **156**, 935–949 (2014).
34. Vita, R. et al. The immune epitope database (IEDB) 3.0. *Nucleic Acids Res.* **43**, D405–D412 (2015).
35. Wolfl, M. et al. Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8+T cells responding to antigen without requiring knowledge of epitope specificities. *Blood* **110**, 201–210 (2007).
36. Pollard, A. J. & Maiden, M. C. J. *Meningococcal Vaccines* (Humana Press, Totowa, New Jersey 2001).
37. King, C. et al. Removing T-cell epitopes with computational protein design. *Proc. Natl Acad. Sci. USA* **111**, 8577–8582 (2014).
38. Mazor, R. et al. Rational design of low immunogenic anti CD25 recombinant immunotoxin for T cell malignancies by elimination of T cell epitopes in PE38. *Cell. Immunol.* **313**, 59–66 (2017).
39. Salvat, R. S. et al. Computationally optimized deimmunization libraries yield highly mutated enzymes with low immunogenicity and enhanced activity. *Proc. Natl Acad. Sci. USA* **114**, E5085–E5093 (2017).
40. Cantor, J. R. et al. Therapeutic enzyme deimmunization by combinatorial T-cell epitope removal using neutral drift. *Proc. Natl Acad. Sci. USA* **108**, 1272–1277 (2011).
41. Tangri, S. et al. Rationally engineered therapeutic proteins with reduced immunogenicity. *J. Immunol.* **174**, 3187–3196 (2005).
42. Yeung, V. P. et al. Elimination of an immunodominant CD4+T cell epitope in human IFN-β does not result in an in vivo response directed at the subdominant epitope. *J. Immunol.* **172**, 6658–6665 (2004).
43. Mok, H., Lee, S., Wright, D. W. & Crowe, J. E. Enhancement of the CD8+T cell response to a subdominant epitope of respiratory syncytial virus by deletion of an immunodominant epitope. *Vaccine* **26**, 4775–4782 (2008).
44. Chew, W. L. Immunity to CRISPR Cas9 and Cas12a therapeutics. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **10**, (2018).
45. Yewdell, J. W. & Hill, A. B. Viral interference with antigen presentation. *Nat. Immunol.* **3**, 1019 (2002).
46. Levitskaya, J. et al. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* **375**, 685–688 (1995).
47. Anderson, K. S. et al. HPV16 antibodies as risk factors for oropharyngeal cancer and their association with tumor HPV and smoking status. *Oral. Oncol.* **51**, 662–667 (2015).
48. Wang, J. et al. A versatile protein microarray platform enabling antibody profiling against denatured proteins. *Proteomics Clin. Appl.* **7**, 378–383 (2013).
49. Moutaftsi, M. et al. A consensus epitope prediction approach identifies the breadth of murine TCD8+-cell responses to vaccinia virus. *Nat. Biotechnol.* **24**, 817 (2006).
50. Hoof, I. et al. NetMHCpan, a method for MHC class I binding prediction beyond humans. *Immunogenetics* **61**, 1 (2009).
51. Rammensee, H.-G., Bachmann, J., Emmerich, N. P. N., Bachor, O. A. & Stevanović, S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* **50**, 213–219 (1999).
52. Tenzer, S. et al. Modeling the MHC class I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. *Cell. Mol. Life Sci.* **62**, 1025–1037 (2005).
53. Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graph.* **14**, 33–38 (1996).
54. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
55. Trapnell, C. et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **7**, 562–578 (2012).
56. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).