Protective efficacy of *in vitro* synthesized, specific messenger RNA vaccines against influenza A virus infection (supplementary information)

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Nature Biotechnology: doi:10.1038/nbt.2436

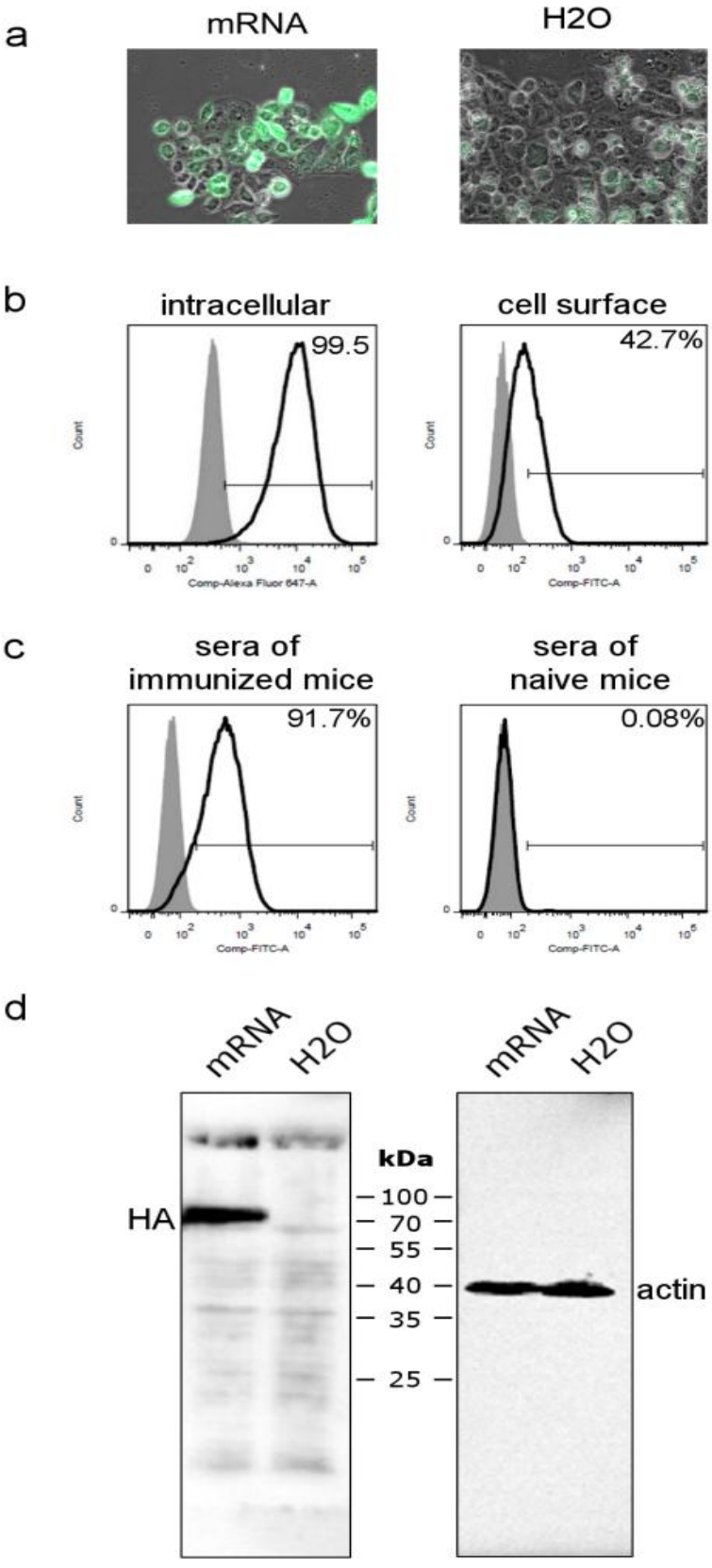

Figure S1 *In vitro* characterization of mRNA vaccine encoding PR8HA antigen.

Nature Biotechnology: doi:10.1038/nbt.2436

Figure S1 (from previous page) *In vitro* characterization of mRNA vaccine encoding PR8HA antigen. (**a**) HeLa cells were transfected with mRNA encoding full-length PR8HA protein using Lipofectamine 2000 (life technologies, Darmstadt, Germany). For negative control cells were mock treated with water and transfection reagent. 24 hours post transfection (p.t.) cells were fixed, permeabilized and stained with monoclonal antibody against human influenza A virus HA H1N1/H2N2 (clone C179, Takara Bio Europe, France) and appropriate FITC-coupled secondary antibody (Sigma-Aldrich, Munich, Germany). (**b**) HeLa cells were transfected with mRNA encoding PR8HA protein using Lipofectamine 2000 (bold line) or mock treated as before (grey fills). 24 h p.t. samples were directly incubated with monoclonal HA-antibody and FITC-coupled secondary antibody to detect PR8HA expression at the cell surface of unpermeabilized cells (right panel). After fixation and permeabilization, intracellular PR8HA was detected using monoclonal HA-antibody and AF647-coupled secondary antibody (life technologies, Darmstadt, Germany) (left panel). All samples were analysed by FACS cytometry, using FlowJo software (version 7.6.5). The histogram plots show the intensity of PR8HA-specific staining. (**c**) PR8HA expressing HeLa cells (bold line) were used for preliminary assessment of vaccine immunogenicity. Unfixed mRNA transfectants were incubated either with sera from PR8HA mRNA immunized mice (left panel) or sera of naïve control animals (right panel). Samples were then analysed by flow cytometry as before. The histogram plots show the intensity of HA-specific staining. (**d**) Cell lysates of mRNA- or water-transfected HeLa cells were separated by SDS-PAGE under reducing conditions and analysed by western blotting. Sera from mice immunized with PR8HA-encoding mRNA revealed one band of ~70kDa representing HA antigen. β-actin was used as a loading control.

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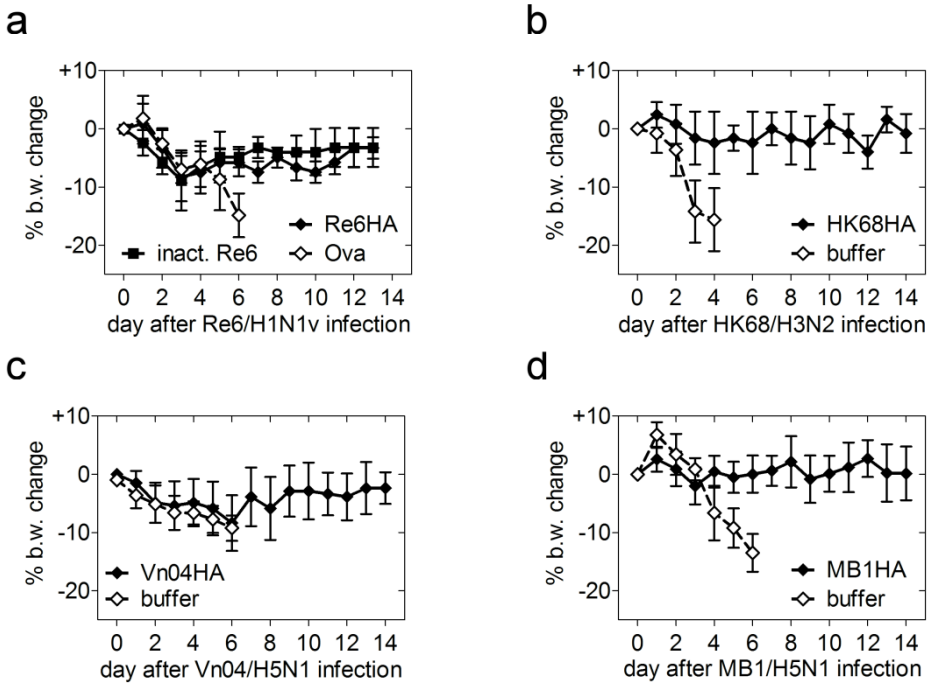

Figure S2 Body weight (b.w.) kinetics of mRNA immunized mice after homologous challenge infection with (**a**) A/Regensburg/D6/2009 (Re6/H1N1v) (**b**) A/HongKong/1/1968 (HK68/H3N2) (**c**) A/Vietnam/1194/2004 (Vn04/H5N1) or (**d**) A/mallard/Bavaria/1/2006 (MB1/H5N1). BALB/c mice (n=5 (Re6, HK68, MB1); n=8 (Vn04)/group) were injected *i.d.* with 80µg mRNA encoding the homologous HA ((**a**) Re6HA, (**b**) HK68HA, (**c**) Vn04HA, (**d**) MB1HA). For the Re6 challenge experiment, a positive control group vaccinated with 820 HAU of inactivated Re6 was included. For negative control mice were injected with 80µg Ova mRNA or injection buffer, as indicated. Immunizations were done with a 21 days interval. Five weeks after boost injection, mice were infected with the respective homologous virus. For Vn04/H5N1, MB1/H5N1, and HK68/H3N2, 10x LD_{50} were used as challenge dose. Due to technical limitations in virus concentration, only a 6.8x LD_{50} was applied for the challenge with Re6/H1N1v. B.w. was monitored daily over a 2 week period and plotted as mean ± SD. Animals that lost over 25% (Re6/H1N1v, HK68/H3N2, Vn04/H5N1) or 20% (MB1/H5N1) of their initial b.w. were censored and counted as deceased. Lines end when the first mouse in a group died or reached the pre-specified humane end-point.

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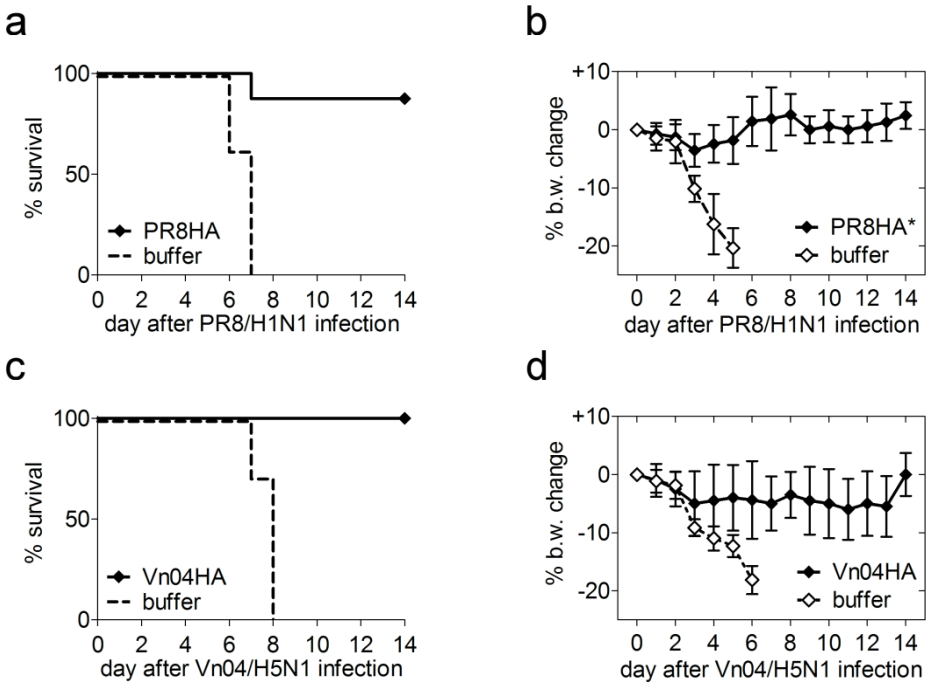

Figure S3 Protective efficacy of mRNA vaccine against lethal virus challenge with high virus doses. Survival (**a**) and body weight (b.w.) kinetics (**b**) of PR8HA mRNA immunized mice after PR8/H1N1 infection, respectively. On days 0 and 21 BALB/c mice (n=8/group) were injected *i.d.* with 80µg PR8HA mRNA, mock treated control mice were injected *i.d.* with buffer. On study day 56 mice were infected with 100x LD_{50} of PR8/H1N1 virus and monitored for 14 days post infection. Mice that lost over 25% of their initial b.w. were sacrificed. *: In the PR8HA immunized group a single vaccinated mouse did not exhibit an antibody titer upon vaccination and died upon high dose PR8 infection. This mouse was excluded from kinetics in (**b**). (**c**,**d**) Analogous experiment for Vn04 vaccinated mice upon challenge 100x LD_{50} of Vn04/H5N1 virus. All vaccinated animals survived the challenge infection. For b.w. kinetics in (**b**,**d**) the mean ± S.D. were plotted. Lines end when the first mouse in a group died or reached the pre-specified humane end-point. (Statistical analysis: log rank analysis (Mantel Cox Test), (**a**) p=0.0006, (**c**) p=0.0004)

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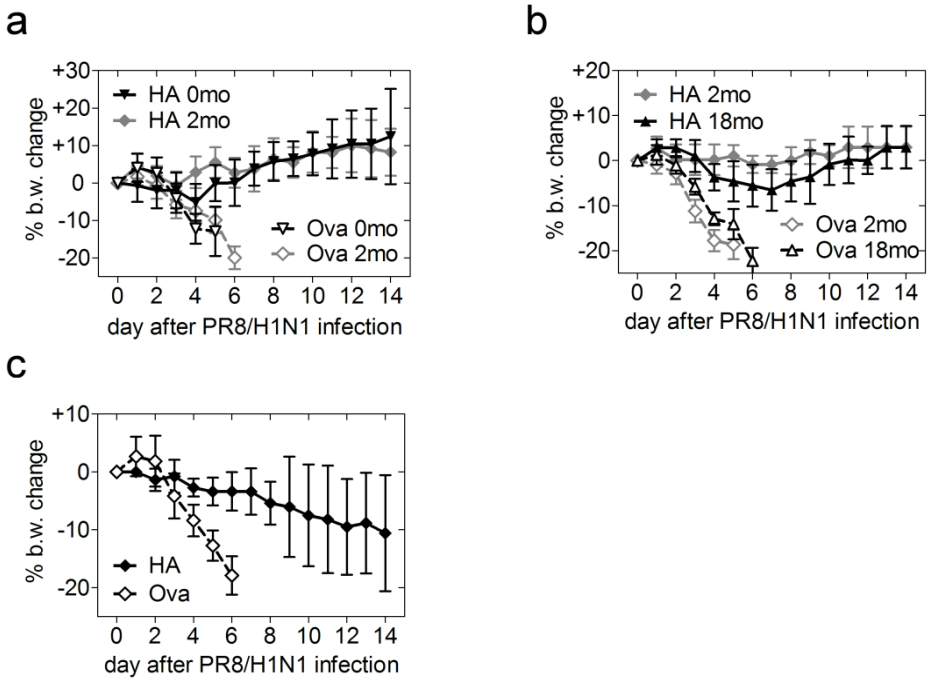

Figure S4 Body weight (b.w.) kinetics of vaccinated newborn, adult, and old mice after PR8 infection. PR8HA mRNA mediated immunity in (**a**) newborn, (**b**) old or (**c**) aged BALB/c mice, compared to young adult controls (**a**,**b**). (**a**, **b**) Mice were injected *i.d.* with 80µg PR8HA or Ova mRNA with an interval of 7 days. The first injection was applied at the age of one day (≤24 h; "0mo"; n=9/group; in **a**, 2 months ("2mo"; n=5/group; **a**, **b**) or 18 months ("18mo"; n=4 PR8HA, n=3 Ova mRNA, **b**), respectively. 5 weeks after the second immunization, mice were challenged with 10x LD_{50} of live PR8 virus. (**c**) 8 week old female BALB/c mice were *i.d.* injected on days 0 and 7 with 20µg PR8HA (n=5) or Ova mRNA (n=4), aged for 16 months and then challenged with 10x LD_{50} of PR8 b.w. kinetics are shown as mean ± SD. Lines end when the first mouse in a group reaches the pre-specified humane end-point. Mice with a loss of more than 25% of initial b.w. were sacrificed.

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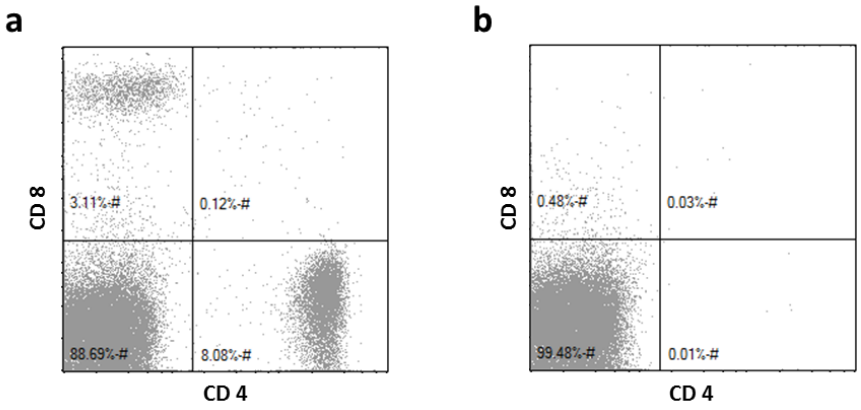

Figure S5 FACS-based analysis of $CD4^+$ and $CD8^+$ cell populations from (**a**) untreated mice or (**b**) mice injected with CD4- and CD8-specific depletion antibodies. For $CD4^+$ and $CD8^+$ cell depletion BALB/c mice were injected *i.p.* with 200µl of a 1:25 diluted solution of YTS 169 (α-CD8) and YTS 191 (α-CD4) antibodies at days 0 and 2 as described in the Materials and Methods section. At day 3, blood samples were taken and blood was tested for $CD4^+$ and $CD8^+$ T cells flow cytometry. After antibody treatment >98% antigen reduction for both T cell populations was commonly observed when compared to untreated controls ($CD3^+CD4^+$ T cells: 0.03% ± 0.008%; $CD3^+CD8^+$ T cells: 0.03% ± 0.014% of gated events; n=3 mice).

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Treatment	Seroconversion rate [%]	Median HI titer (range)	fold GMT increase
Celvapan®	66.7	60 (20-160)	16
80 µg Ova mRNA	0	5 (5-20)	1.5
20 µg RGHA mRNA	16.7	15 (5-160)	7.5
80 µg RGHA mRNA	66.7	80 (20-160)	14.7
250 µg RGHA mRNA	83.3	120 (10-160)	21.7

Supplementary Table 1 Summary of immune response in ferrets. Criteria listed by the European Medicines Agency (EMA) for the assessment of influenza vaccines (guideline CPMP/BWP/214/1996): a seroconversion rate of at least 40%, a minimum 2.5-fold increase in geometric mean titer (GMT), or at least 70% of subjects with an HI titer ≥1:40. For licensure of novel vaccines at least one of these three criteria need to be fulfilled. Seroconversion was defined as HI titer ≥1:40 for negative prevaccination sera or at least 4-fold increase of preexisting HI titers. GMT is listed for HI titers.

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Time point	Assay	1	2	3	4	5	6	7	8	9	10	11	12
w -1	NP	+	-	-	+	-	+	-	+	+	+	-	+
w -1	HI	<10§	<10§	10§	<10§	10§	80§	<10§	<10§	<10§	40§	10§	10§
w 3	NP	+	-	-	+	-	+	-	-	+	+	-	-
w 3	HI	40	1280	160	80	320	320	40	40	40	40	20	160
w 5	NP	-	-	-	+	-	+	-	-	+	+	-	-
w 5	HI	160	1280	160	160	320	320	320	320	320	160	40	320
w 7	HI	160	1280	160	160	320	320	160	320	160	160	40	320

Supplementary Table 2 Serological status of individual pigs (numbered 1-12) throughout the experiment. The presence of NP antibody was determined using a commercial NP ELISA kit, which discriminates between positive, intermediate, and negative for NP-specific antibodies (detection limit not specified by manufacturer). HI titers were determined by A/California/7/2009-specific HI assay. One week prior to immunization, subsets of animals reacted against NP (7/12) and C7HA (4/12). However, the proportion of NP-reactive animals declined throughout the experiment from 7 (week -1, w-1) to 4 (week 5, w5) animals, indicating that the simultaneously observed seroconversion against C7HA was due to vaccination and not to a concurrent influenza infection.

§: Indicates HI-Titer of presera in a retrospective assay; in addition, seronegativity (<1:10) was confirmed in independent serum neutralization tests.

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Time point	Seroconversion rate [%]	Median HI titer (range)	fold GMT increase*
w 3	75	60 (20-1280)	4.4
w 5	91.7	320 (40-1280)	10.7
w 7	91.7	160 (40-1280)	9.5

Supplementary Table 3 Summary of immune response in pigs. Criteria listed by the European Medicines Agency (EMA) for the assessment of influenza vaccines (guideline CPMP/BWP/214/1996): a seroconversion rate of at least 40%, a minimum 2.5-fold increase in geometric mean titer (GMT), or at least 70% of subjects with an HI titer ≥1:40. For licensure of novel vaccines at least one of these three criteria need to be fulfilled. Seroconversion was defined as HI titer ≥1:40 for negative prevaccination sera or at least 4-fold increase of preexisting HI titers. GMT is listed for HI titers.

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Supplementary Table 4 List of mRNA coding sequences used in this study. Sequences were optimized by GC enrichment as described in EP1392341 and EP1857122.

Encoded protein	GenBank acc. no. (protein)	mRNA sequence
C7HA	ACV82259.1	AUGAAGGCCAUCCUGGUGGUCCUCCUGUACACCUUCGCCACCGCGAACGCCG ACACGCUGUGCAUCGGCUACCACGCCAACAACAGCACCGACACCGUGGACAC CGUGCUCGAGAAGAACGUCACGGUGACCCACUCCGUGAACCUGCUGGAGGAC AAGCACAACGGGAAGCUCUGCAAGCUGCGGGGCGUCGCCCCGCUGCACCUCG GGAAGUGCAACAUCGCCGGCUGGAUCCUGGGGAACCCGGAGUGCGAGAGCCU GUCCACCGCGAGCUCCUGGAGCUACAUCGUGGAGACCCCCUCCAGCGACAAC GGCACGUGCUACCCCGGCGACUUCAUCGACUACGAGGAGCUCCGCGAGCAGC UGUCCAGCGUGUCCAGCUUCGAGCGGUUCGAGAUCUUCCCCAAGACCUCCAG CUGGCCGAACCACGACUCCGACAAGGGGGUCACCGCCGCCUGCCCCCACGCC GGCGCGAAGAGCUUCUACAAGAACCUGAUCUGGCUCGUGAAGAAGGGGAACU CCUACCCCAAGCUGAGCAAGUCCUACAUCAACGACAAGGGCAAGGAGGUGCU GGUCCUCUGGGGGAUCCACCACCCCAGCACCAGCGCCGACCAGCAGUCCCUG UACCAGAACGCCGACGCCUACGUGUUCGUGGGCAGCUCCCGCUACAGCAAGA CGUUCAAGCCGGAGAUCGCCAUCCGGCCCAAGGUCCGCGACCGGGAGGGCCG CAUGAACUACUACUGGACCCUGGUGGAGCCCGGGGACAAGAUCACCUUCGAG GCGACCGGCAACCUCGUGGUCCCCCGGUACGCCUUCGCCAUGGAGCGCAACG CCGGGUCCGGCAUCAUCAUCAGCGACACGCCGGUGCACGACUGCAACACCAC CUGCCAGACCCCCAAGGGCGCCAUCAACACGUCCCUGCCCUUCCAGAACAUC CACCCCAUCACCAUCGGGAAGUGCCCGAAGUACGUGAAGAGCACCAAGCUGC GGCUCGCGACCGGCCUGCGCAACAUCCCCUCCAUCCAGAGCCGGGGGCUGUU CGGCGCCAUCGCCGGGUUCAUCGAGGGCGGCUGGACGGGGAUGGUCGACGGC UGGUACGGGUACCACCACCAGAACGAGCAGGGCAGCGGGUACGCCGCCGACC UCAAGUCCACGCAGAACGCGAUCGACGAGAUCACCAACAAGGUGAACAGCGU CAUCGAGAAGAUGAACACCCAGUUCACCGCCGUGGGCAAGGAGUUCAACCAC CUGGAGAAGCGGAUCGAGAACCUGAACAAGAAGGUCGACGACGGCUUCCUCG ACAUCUGGACGUACAACGCCGAGCUGCUGGUGCUCCUGGAGAACGAGCGCAC CCUGGACUACCACGACUCCAACGUGAAGAACCUCUACGAGAAGGUCCGGAGC CAGCUGAAGAACAACGCCAAGGAGAUCGGGAACGGCUGCUUCGAGUUCUACC ACAAGUGCGACAACACCUGCAUGGAGUCCGUGAAGAACGGGACCUACGACUA CCCCAAGUACAGCGAGGAGGCCAAGCUGAACCGCGAGGAGAUCGACGGCGUG AAGCUCGAGUCCACGCGGAUCUACCAGAUCCUGGCGAUCUACAGCACCGUCG CCAGCUCCCUGGUGCUCGUGGUCAGCCUGGGGGCCAUCUCCUUCUGGAUGUG CAGCAACGGCUCCCUGCAGUGCCGCAUCUGCAUCUGA
HK68	original sequence obtained from Georg Kochs	AUGAAGACCAUCAUCGCCCUGAGCUACAUCUUCUGCCUCGCCCUGGGCCAGG ACCUGCCCGGGAACGACAACUCCACCGCGACGCUCUGCCUGGGCCACCACGC CGUGCCGAACGGGACCCUGGUCAAGACCAUCACCGACGACCAGAUCGAGGUG ACGAACGCCACCGAGCUCGUGCAGAGCUCCAGCACCGGCAAGAUCUGCAACA ACCCCCACCGGAUCCUGGACGGGAUCGACUGCACCCUGAUCGACGCCCUCCU GGGCGACCCCCACUGCGACGUCUUCCAGAACGAGACGUGGGACCUGUUCGUG GAGCGCUCCAAGGCCUUCAGCAACUGCUACCCCUACGACGUGCCGGACUACG CGUCCCUCCGGAGCCUGGUCGCCUCCAGCGGCACCCUGGAGUUCAUCACCGA GGGGUUCACCUGGACGGGCGUGACCCAGAACGGGGGCUCCAACGCCUGCAAG CGCGGGCCCGGCAGCGGCUUCUUCUCCCGGCUCAACUGGCUGACCAAGAGCG GGUCCACCUACCCCGUGCUGAACGUCACGAUGCCCAACAACGACAACUUCGA CAAGCUCUACAUCUGGGGCGUGCACCACCCGAGCACCAACCAGGAGCAGACC UCCCUGUACGUGCAGGCCAGCGGGCGCGUCACCGUGUCCACGCGGCGCAGCC AGCAGACCAUCAUCCCCAACAUCGAGAGCCGGCCCUGGGUGCGCGGCCAGUC CAGCCGGAUCUCCAUCUACUGGACCAUCGUCAAGCCCGGCGACGUGCUGGUG AUCAACAGCAACGGGAACCUCAUCGCGCCGCGCGGCUACUUCAAGAUGCGGA CCGGGAAGUCCAGCAUCAUGCGCUCCGACGCCCCAUCGACACGUGCAUCAG CGAGUGCAUCACCCCCAACGGCUCCAUCCCCAACGACAAGCCGUUCCAGAAC GUCAACAAGAUCACCUACGGGGCCUGCCCCAAGUACGUGAAGCAGAACACCC UGAAGCUGGCCACGGGCAUGCGGAACGUGCCCGAGAAGCAGACCCGCGGCCU

Nature Biotechnology: doi:10.1038/nbt.2436

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		AGAUCAAGGUGUACCUCCCGCGGAUGAAGAUGGAGGAGAAGUACAACCUGAC GUCGGUCCUGAUGGCGAUGGGGAUCACCGACGUGUUCAGCAGCUCGGCCAAC CUCAGCGGCAUCAGCUCGGCCGAGAGCCUGAAGAUCAGCCAGGCGGUGCACG CCGCCCACGCGGAGAUCAACGAGGCCGGCCGGGAGGUCGUGGGGUCGGCCGA GGCGGGCGUGGACGCCGCCAGCGUCAGCGAGGAGUUCCGCGCGGACCACCCG UUCCUGUUCUGCAUCAAGCACAUCGCCACCAACGCCGUGCUCUUCUUCGGCC GGUGCGUGUCGCCCUGA
PR8HA	ABO21709.1	AUGAAGGCCAACCUGCUCGUGCUGCUGUGCGCCCUCGCGGCCGCCGACGCCG ACACCAUCUGCAUCGGCUACCACGCCAACAACAGCACCGACACGGUCGACAC CGUGCUGGAGAAGAACGUGACCGUCACCCACUCCGUGAACCUGCUCGAGGAC AGCCACAACGGGAAGCUGUGCCGGCUGAAGGGCAUCGCGCCCCUCCAGCUGG GGAAGUGCAACAUCGCCGGCUGGCUGCUCGGGAACCCGGAGUGCGACCCCCU GCUGCCCGUGCGCUCCUGGAGCUACAUCGUCGAGACGCCCAACUCCGAGAAC GGCAUCUGCUACCCGGGCGACUUCAUCGACUACGAGGAGCUCCGGGAGCAGC UGAGCUCCGUGAGCUCCUUCGAGCGCUUCGAGAUCUUCCCCAAGGAGAGCUC CUGGCCCAACCACAACACCAACGGGGUGACCGCCGCCUGCAGCCACGAGGGC AAGUCCAGCUUCUACCGGAACCUGCUCUGGCUGACCGAGAAGGAGGGGUCCU ACCCCAAGCUGAAGAACAGCUACGUCAACAAGAAGGGCAAGGAGGUGCUCGU GCUGUGGGGGAUCCACCACCCGCCCAACUCCAAGGAGCAGCAGAACCUGUAC CAGAACGAGAACGCGUACGUCAGCGUGGUGACGUCCAACUACAACCGCCGGU UCACCCCCGAGAUCGCCGAGCGCCCCAAGGUCCGGGACCAGGCCGGCCGCAU GAACUACUACUGGACCCUCCUGAAGCCGGGCGACACCAUCAUCUUCGAGGCC AACGGGAACCUGAUCGCCCCGAUGUACGCGUUCGCCCUCAGCCGGGGCUUCG GGAGCGGCAUCAUCACGUCCAACGCCAGCAUGCACGAGUGCAACACCAAGUG CCAGACCCCCCUGGGCGCCAUCAACUCCAGCCUGCCCUACCAGAACAUCCAC CCGGUGACCAUCGGGGAGUGCCCCAAGUACGUGCGCUCCGCCAAGCUCCGGA UGGUCACGGGCCUGCGCAACAACCCCAGCAUCCAGUCCCGGGGGCUGUUCGG CGCGAUCGCCGGGUUCAUCGAGGGCGGCUGGACCGGGAUGAUCGACGGCUGG UACGGGUACCACCACCAGAACGAGCAGGGCAGCGGGUACGCCGCCGACCAGA AGUCCACCCAGAACGCCAUCAACGGCAUCACCAACAAGGUGAACACGGUGAU CGAGAAGAUGAACAUCCAGUUCACCGCGGUCGGCAAGGAGUUCAACAAGCUC GAGAAGCGCAUGGAGAACCUGAACAAGAAGGUGGACGACGGGUUCCUGGACA UCUGGACCUACAACGCCGAGCUCCUGGUGCUGCUCGAGAACGAGCGGACCCU GGACUUCCACGACAGCAACGUCAAGAACCUGUACGAGAAGGUGAAGUCCCAG CUCAAGAACAACGCCAAGGAGAUCGGCAACGGGUGCUUCGAGUUCUACCACA AGUGCGACAACGAGUGCAUGGAGAGCGUCCGCAACGGCACGUACGACUACCC CAAGUACUCCGAGGAGAGCAAGCUGAACCGGGAGAAGGUGGACGGGGUGAAG CUGGAGUCCAUGGGCAUCUACCAGAUCCUCGCCAUCUACAGCACCGUCGCCU CCAGCCUGGUGCUGCUGGUGUCCCUCGGCGCGAUCAGCUUCUGGAUGUGCAG CAACGGGUCCCUGCAGUGCCGCAUCUGCAUCUGA
PR8NA	ABO21711.1	AUGAACCCCAACCAGAAGAUCAUCACCAUCGGCAGCAUCUGCCUGGUGGUCG GGCUCAUCUCCCUGAUCCUGCAGAUCGGCAACAUCAUCAGCAUCUGGAUCUC CCACAGCAUCCAGACCGGGUCCCAGAACCACACGGGCAUCUGCAACCAGAAC AUCAUCACCUACAAGAACAGCACCUGGGUGAAGGACACCACGUCCGUGAUCC UCACCGGGAACAGCUCCCUGUGCCCGAUCCGGGGCUGGGCCAUCUACAGCAA GGACAACUCCAUCCGCAUCGGCAGCAAGGGGGACGUCUUCGUGAUCCGGGAG CCCUUCAUCUCCUGCAGCCACCUGGAGUGCCGCACCUUCUUCCUCACCCAGG GCGCCCUGCUGAACGACAAGCACUCCAGCGGGACGGUGAAGGACCGGUCCCC CUACCGCGCGCUCAUGAGCUGCCCCGUCGGCGAGGCCCCGUCCCCCUACAAC AGCCGGUUCGAGAGCGUGGCCUGGUCCGCCAGCGCCUGCCACGACGGGAUGG GCUGGCUGACCAUCGGCAUCUCCGGGCCCGACAACGGCGCGGUGGCCGUCCU GAAGUACAACGGGAUCAUCACCGAGACCAUCAAGAGCUGGCGCAAGAAGAUC CUCCGGACGCAGGAGUCCGAGUGCGCCUGCGUGAACGGCAGCUGCUUCACCA UCAUGACCGACGGCCCCUCCGACGGGCUGGCCAGCUACAAGAUCUUCAAGAU CGAGAAGGGCAAGGUGACCAAGUCCAUCGAGCUGAACGCGCCGAACAGCCAC UACGAGGAGUGCUCCUGCUACCCCGACACGGGGAAGGUCAUGUGCGUGUGCC GCGACAACUGGCACGGCAGCAACCGGCCCUGGGUGUCCUUCGACCAGAACCU CGACUACCAGAUCGGGUACAUCUGCAGCGGCGUCUUCGGCGACAACCCCCGC CCGGAGGACGGGACCGGCUCCUGCGGGCCCGUGUACGUGGACGGCGCCAACG GGGUCAAGGGCUUCAGCUACCGGUACGGCAACGGGGUGUGGAUCGGCCGCAC CAAGUCCCACAGCUCCCGGCACGGGUUCGAGAUGAUCUGGGACCCCAACGGC UGGACCGAGACGGACAGCAAGUUCAGCGUGCGCCAGGACGUCGUGGCCAUGA

Nature Biotechnology: doi:10.1038/nbt.2436

		CCGACUGGUCCGGGUACAGCGGCUCCUUCGUGCAGCACCCCGAGCUGACCGG CCUGGACUGCAUGCGGCCGUGCUUCUGGGUCGAGCUCAUCCGCGGGCGGCCC AAGGAGAAGACCAUCUGGACGAGCGCCUCCAGCAUCUCCUUCUGCGGCGUGA ACAGCGACACCGUCGACUGGUCCUGGCCCGACGGGGCCGAGCUGCCCUUCAG CAUCGACAAGUGA
PR8NP	ABO21710.1	AUGGCCAGCCAGGGCACCAAGCGGUCCUACGAGCAGAUGGAGACCGACGGGG AGCGCCAGAACGCCACGGAGAUCCGGGCGAGCGUGGGCAAGAUGAUCGGGGG CAUCGGGCGCUUCUACAUCCAGAUGUGCACCGAGCUGAAGCUCUCCGACUAC GAGGGCCGGCUGAUCCAGAACAGCCUGACCAUCGAGCGCAUGGUCCUCUCCG CCUUCGACGAGCGGCGCAACAAGUACCUGGAGGAGCACCCCAGCGCCGGCAA GGACCCGAAGAAGACCGGGGGCCCCAUCUACCGGCGCGUGAACGGGAAGUGG AUGCGGGAGCUGAUCCUCUACGACAAGGAGGAGAUCCGCCGGAUCUGGCGCC AGGCCAACAACGGCGACGACGCCACGGCGGGGCUGACCCACAUGAUGAUCUG GCACUCCAACCUGAACGACGCCACCUACCAGCGGACCCGCGCCCUCGUGCGG ACGGGCAUGGACCCCCGCAUGUGCAGCCUGAUGCAGGGCUCCACCCUGCCCC GGCGCAGCGGGGCCGCGGGCGCCGCCGUCAAGGGGGUGGGCACCAUGGUGAU GGAGCUCGUCCGGAUGAUCAAGCGCGGCAUCAACGACCGGAACUUCUGGCGG GGCGAGAACGGGCGCAAGACCCGGAUCGCCUACGAGCGCAUGUGCAACAUCC UGAAGGGGAAGUUCCAGACGGCCGCGCAGAAGGCCAUGAUGGACCAGGUGCG GGAGUCCCGCAACCCGGGCAACGCCGAGUUCGAGGACCUGACCUUCCUCGCC CGGAGCGCCCUGAUCCUGCGCGGGUCCGUGGCGCACAAGAGCUGCCUCCCCG CCUGCGUCUACGGCCCCGCCGUGGCCUCCGGCUACGACUUCGAGCGGGAGGG GUACAGCCUGGUGGGCAUCGACCCCUUCCGCCUGCUCCAGAACUCCCAGGUC UACAGCCUGAUCCGGCCGAACGAGAACCCCGCCCACAAGUCCCAGCUGGUGU GGAUGGCGUGCCACAGCGCCGCCUUCGAGGACCUCCGCGUGCUGAGCUUCAU CAAGGGGACCAAGGUCCUGCCCCGGGGCAAGCUCUCCACCCGCGGGGUGCAG AUCGCCAGCAACGAGAACAUGGAGACGAUGGAGUCCAGCACCCUGGAGCUGC GGUCCCGCUACUGGGCCAUCCGGACCCGCAGCGGCGGCAACACCAACCAGCA GCGGGCGUCCGCCGGGCAGAUCAGCAUCCAGCCCACGUUCUCCGUGCAGCGC AACCUCCCGUUCGACCGGACCACCAUCAUGGCCGCCUUCAACGGCAACACCG AGGGGCGGACGAGCGACAUGCGCACCGAGAUCAUCCGGAUGAUGGAGUCCGC GCGCCCCGAGGACGUCAGCUUCCAGGGCCGGGGCGUGUUCGAGCUGUCCGAC GAGAAGGCCGCCAGCCCCAUCGUGCCCUCCUUCGACAUGAGCAACGAGGGGU CCUACUUCUUCGGCGACAACGCCGAGGAGUACGACAACUGA
Re6HA	CAZ65588.1	AUGAAGGCCAUCCUGGUGGUCCUCCUGUACACCUUCGCCACCGCGAACGCCG ACACGCUGUGCAUCGGCUACCACGCCAACAACAGCACCGACACCGUGGACAC CGUGCUCGAGAAGAACGUCACGGUGACCCACUCCGUGAACCUGCUGGAGGAC AAGCACAACGGGAAGCUCUGCAAGCUGCGGGGCGUCGCCCCGCUGCACCUCG GGAAGUGCAACAUCGCCGGCUGGAUCCUGGGGAACCCGGAGUGCGAGAGCCU GUCCACCGCGAGCUCCUGGAGCUACAUCGUGGAGACCUCCAGCUCCGACAAC GGCACGUGCUACCCCGGCGACUUCAUCGACUACGAGGAGCUCCGCGAGCAGC UGAGCUCCGUGAGCUCCUUCGAGCGGUUCGAGAUCUUCCCCAAGACCAGCUC CUGGCCCAACCACGACAGCAACAAGGGGGUCACCGCCGCCUGCCCGCACGCC GGCGCGAAGUCCUUCUACAAGAACCUGAUCUGGCUCGUGAAGAAGGGGAACA GCUACCCCAAGCUGUCCAAGAGCUACAUCAACGACAAGGGCAAGGAGGUGCU GGUCCUCUGGGGGAUCCACCACCCCAGCACCUCCGCCGACCAGCAGAGCCUG UACCAGAACGCCGACGCCUACGUGUUCGUGGGCUCCAGCCGCUACUCCAAGA AGUUCAAGCCCGAGAUCGCCAUCCGGCCGAAGGUCCGCGACCAGGAGGGCCG GAUGAACUACUACUGGACGCUGGUGGAGCCCGGGGACAAGAUCACCUUCGAG GCGACCGGCAACCUCGUGGUCCCCCGCUACGCCUUCGCCAUGGAGCGGAACG CCGGGAGCGGCAUCAUCAUCUCCGACACCCCCGUGCACGACUGCAACACGAC CUGCCAGACCCCGAAGGGCGCCAUCAACACCAGCCUGCCCUUCCAGAACAUC CACCCCAUCACGAUCGGGAAGUGCCCCAAGUACGUGAAGUCCACCAAGCUGC GCCUCGCGACCGGCCUGCGGAACGUCCCGAGCAUCCAGUCCCGCGGGCUGUU CGGCGCCAUCGCCGGGUUCAUCGAGGGCGGCUGGACCGGGAUGGUGGACGGC UGGUACGGGUACCACCACCAGAACGAGCAGGGCAGCGGGUACGCCGCCGACC UCAAGUCCACGCAGAACGCGAUCGACGAGAUCACCAACAAGGUGAACAGCGU CAUCGAGAAGAUGAACACCCAGUUCACCGCCGUGGGCAAGGAGUUCAACCAC CUGGAGAAGCGGAUCGAGAACCUGAACAAGAAGGUCGACGACGGCUUCCUCG ACAUCUGGACGUACAACGCCGAGCUGCUGGUGCUCCUGGAGAACGAGCGCAC CCUGGACUACCACGACUCCAACGUGAAGAACCUCUACGAGAAGGUCCGGAGC CAGCUGAAGAACAACGCCAAGGAGAUCGGGAACGGCUGCUUCGAGUUCUACC

Nature Biotechnology: doi:10.1038/nbt.2436

		ACAAGUGCGACAACACCUGCAUGGAGUCCGUGAAGAACGGGACCUACGACUA CCCCAAGUACAGCGAGGAGGCCAAGCUGAACCGCGAGGAGAUCGACGGCGUG AAGCUCGAGUCCACGCGGAUCUACCAGAUCCUGGCGAUCUACAGCACCGUCG CCAGCUCCCUGGUGCUCGUGGUCAGCCUGGGGGCCAUCUCCUUCUGGAUGUG CAGCAACGGCUCCCUGCAGUGCCGCAUCUGCAUCUGA
Re6NA	CAZ65589.1	AUGAACCCCAACCAGAAGAUCAUCACCAUCGGCAGCGUGUGCAUGACCAUCG GGAUGGCCAACCUGAUCCUCCAGAUCGGCAACAUCAUCUCCAUCUGGAUCAG CCACUCCAUCCAGCUGGGGAACCAGAACCAGAUCGAGACGUGCAACCAGAGC GUCAUCACCUACGAGAACAACACCUGGGUGAACCAGACCUACGUGAACAUCU CCAACACGAACUUCGCCGCGGGCCAGAGCGUCGUGUCCGUGAAGCUGGCCGG GAACAGCUCCCUCUGCCCGGUCAGCGGCUGGGCCAUCUACUCCAAGGACAAC AGCGUGCGGAUCGGCUCCAAGGGGGACGUCUUCGUGAUCCGCGAGCCGUUCA UCAGCUGCUCCCCCCUGGAGUGCCGGACCUUCUUCCUGACCCAGGGCGCCCU CCUGAACGACAAGCACAGCAACGGGACCAUCAAGGACCGCUCCCCCUACCGG ACGCUGAUGAGCUGCCCGAUCGGCGAGGUGCCCUCCCCCUACAACAGCCGCU UCGAGAGCGUCGCCUGGUCCGCGAGCGCCUGCCACGACGGGAUCAACUGGCU CACCAUCGGCAUCUCCGGCCCCGACAACGGGGCCGUGGCCGUGCUGAAGUAC AACGGCAUCAUCACCGACACCAUCAAGAGCUGGCGGAACAACAUCCUGCGCA CGCAGGAGUCCGAGUGCGCGUGCGUCAACGGGAGCUGCUUCACCGUGAUGAC CGACGGCCCGUCCAACGGCCAGGCCAGCUACAAGAUCUUCCGGAUCGAGAAG GGGAAGAUCGUGAAGUCCGUCGAGAUGAACGCCCCCAACUACCACUACGAGG AGUGCAGCUGCUACCCCGACUCCAGCGAGAUCACCUGCGUGUGCCGCGACAA CUGGCACGGCUCCAACCGGCCCUGGGUGAGCUUCAACCAGAACCUCGAGUAC CAGAUCGGGUACAUCUGCUCCGGCAUCUUCGGGGACAACCCGCGCCCCAACG ACAAGACGGGCAGCUGCGGCCCCGUCUCCAGCAACGGGGCCAACGGCGUGAA GGGGUUCUCCUUCAAGUACGGCAACGGGGUGUGGAUCGGCCGGACCAAGAGC AUCAGCUCCCGCAACGGCUUCGAGAUGAUCUGGGACCCCAACGGGUGGACCG GCACCGACAACAACUUCAGCAUCAAGCAGGACAUCGUCGGGAUCAACGAGUG GUCCGGCUACAGCGGGUCCUUCGUGCAGCACCCGGAGCUGACGGGCCUGGAC UGCAUCCGGCCCUGCUUCUGGGUGGAGCUCAUCCGCGGCCGGCCCAAGGAGA ACACCAUCUGGACCAGCGGGUCCAGCAUCUCCUUCUGCGGCGUCAACAGCGA CACCGUGGGGUGGUCCUGGCCCGACGGCGCCGAGCUGCCGUUCACGAUCGAC AAGUGA
Vn04HA	ACU65077.1	AUGGAGAAGAUCGUGCUGCUCUUCGCCAUCGUCAGCCUGGUGAAGUCCGACC AGAUCUGCAUCGGCUACCACGCCAACAACAGCACCGAGCAGGUGGACACCAU CAUGGAGAAGAACGUCACGGUGACCCACGCGCAGGACAUCCUGGAGAAGACC CACAACGGGAAGCUCUGCGACCUGGACGGCGUGAAGCCCCUGAUCCUCCGGG ACUGCUCCGUCGCCGGGUGGCUGCUGGGCAACCCGAUGUGCGACGAGUUCAU CAACGUGCCCGAGUGGAGCUACAUCGUGGAGAAGGCCAACCCCGUCAACGAC CUCUGCUACCCCGGGGACUUCAACGACUACGAGGAGCUGAAGCACCUGCUCU CCCGCAUCAACCACUUCGAGAAGAUCCAGAUCAUCCCGAAGAGCUCCUGGAG CUCCCACGAGGCCAGCCUGGGCGUGUCCAGCGCCUGCCCCUACCAGGGCAAG UCCAGCUUCUUCCGGAACGUGGUCUGGCUGAUCAAGAAGAACUCCACCUACC CCACGAUCAAGCGCAGCUACAACAACACCAACCAGGAGGACCUCCUGGUGCU GUGGGGGAUCCACCACCCCAACGACGCGGCCGAGCAGACCAAGCUCUACCAG AACCCGACCACGUACAUCUCCGUGGGCACCAGCACCCUGAACCAGCGGCUGG UCCCCCGCAUCGCCACCCGGUCCAAGGUGAACGGGCAGAGCGGCCGCAUGGA GUUCUUCUGGACGAUCCUCAAGCCCAACGACGCCAUCAACUUCGAGAGCAAC GGGAACUUCAUCGCGCCCGAGUACGCCUACAAGAUCGUGAAGAAGGGCGACU CCACCAUCAUGAAGAGCGAGCUGGAGUACGGCAACUGCAACACCAAGUGCCA GACCCCGAUGGGGGCCAUCAACUCCAGCAUGCCCUUCCACAACAUCCACCCC CUGACGAUCGGCGAGUGCCCCAAGUACGUCAAGUCCAACCGGCUCGUGCUGG CCACCGGGCUGCGCAACAGCCCGCAGCGGGAGACCCGCGGCCUCUUCGGCGC CAUCGCGGGGUUCAUCGAGGGCGGGUGGCAGGGCAUGGUGGACGGGUGGUAC GGCUACCACCACUCCAACGAGCAGGGCAGCGGGUACGCCGCCGACAAGGAGU CCACCCAGAAGGCCAUCGACGGCGUCACGAACAAGGUGAACAGCAUCAUCGA CAAGAUGAACACCCAGUUCGAGGCCGUGGGGCGGGAGUUCAACAACCUGGAG CGCCGGAUCGAGAACCUGAACAAGAAGAUGGAGGACGGCUUCCUCGACGUCU GGACCUACAACGCGGAGCUGCUGGUGCUCAUGGAGAACGAGCGCACCCUGGA CUUCCACGACUCCAACGUCAAGAACCUGUACGACAAGGUGCGGCUCCAGCUG CGCGACAACGCCAAGGAGCUGGGGAACGGCUGCUUCGAGUUCUACCACAAGU GCGACAACGAGUGCAUGGAGAGCGUGCGGAACGGCACGUACGACUACCCCCA

Nature Biotechnology: doi:10.1038/nbt.2436

		GUACUCCGAGGAGGCCCGCCUCAAGCGGGAGGAGAUCAGCGGGGUCAAGCUG GAGUCCAUCGGCAUCUACCAGAUCCUGAGCAUCUACUCCACCGUGGCCAGCU CCCUCGCCCUGGCGAUCAUGGUGGCCGGGCUGAGCCUCUGGAUGUGCAGCAA CGGCUCCCUGCAGUGCCGCAUCUGCAUCUGA

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Protein	NCBI ID	Peptide sequence	aa position	MHC restriction
HA of PR8	ABO21709.1	IYSTVASSL	515-523	MHCI / H-2K^{d}
β-galactosidase	NP_414878	TPHPARIGL	876-884	MHCI / H-2L^{d}
HA of PR8	ABO21709.1	SFERFEIFPKE	110-120	MHCII / H-2IE^{d}
HA of PR8	ABO21709.1	HNTNGVTAACSH	126-137	MHCII / H-2IE^{d}
HA of PR8	ABO21709.1	KLKNSYVNKKGK	159-170	MHCII / H-2IE^{d}
HA of PR8	ABO21709.1	NAYVSVVTSNYNRRF	195-209	MHCII / H-2IE^{d}
HA of PR8	ABO21709.1	CPKYVRSAKLRM	302-313	MHCII / H-2IE^{d}
HIV polymerase	ABS10794	LVGKLNWASQIYPGI	415-429	MHCII / H-2IE^{d} *
HIV polymerase	ABS10794	LNWASQIYPGIKVRQ	419-433	MHCII / H-2IE^{d} *
HIV polymerase	ABS10794	WTYQIYQEPFKNLKT	492-506	MHCII / H-2IE^{d} *
HIV polymerase	ABS10794	LKTGKYAKKGSAHTN	504-518	MHCII / H-2IE^{d} *
HIV polymerase	ABS10794	KYAKKGSAHTNDVKQ	508-522	MHCII / H-2IE^{d} *

Supplementary Table 5 List of peptides used in ELISPOT and *in vivo* cytotoxicity assay. *: H-2IE^{d} binding epitopes within aa411-530 of HIVpol predicted by http://bio.dfci.harvard.edu/RANKPEP/ : WTYQIYQEPFKNLKT, GKYAKKGSAHTNDVK, KLNWASQIYPGIKVR. Listed peptides are part of an HIV peptide library, containing predicted epitopes.

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