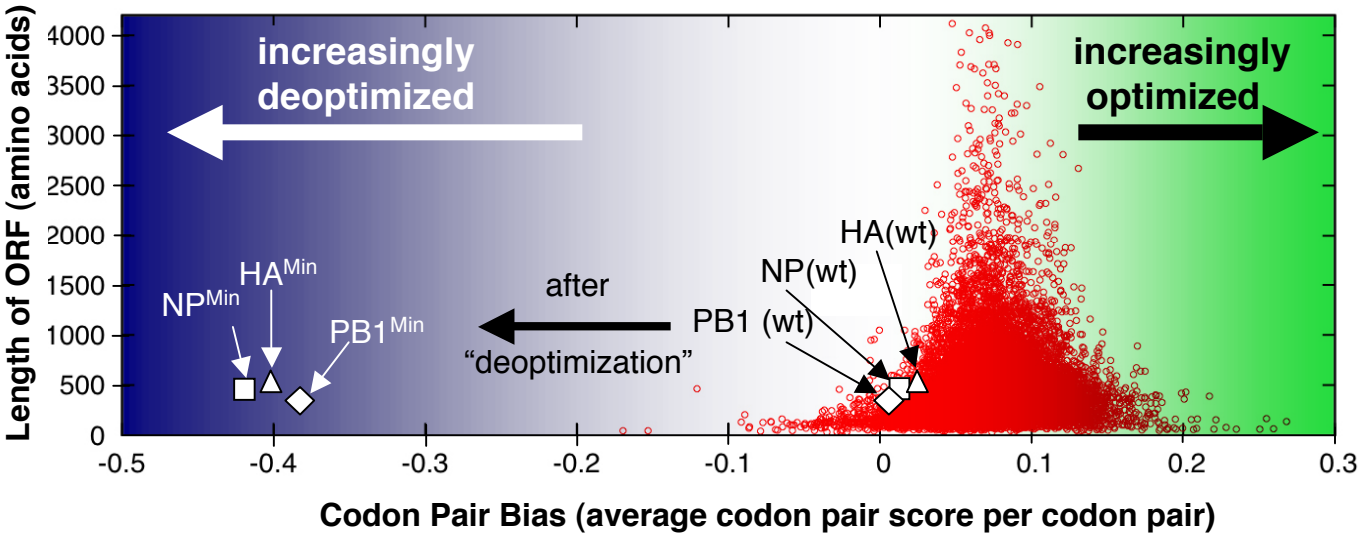

Fig. S1. Codon Pair Bias of selected Influenza A/PR8/3/34 genes and their deoptimized counterparts in relationship to the human ORFeome. Codon Pair Bias (CPB) is expressed as the average codon pair score per codon pair of a given gene, as described in Coleman et al, 2008 [12]. Positive and negative CPB signifies the predominance of statistically over- or under-represented codon-pairs, resprectively, in a open reading frame. Red circles indicate the CPB for each of 14795 human open reading frames, representing the majority of the known, annotated human genes at the time of the analysis. The Codon Pair Bias of the targeted gene regions in wildtype Influenza HA, NP, and PB1 are within the range of the human gene pool. Following codon pair-deoptimization, the resulting synthetic gene segments (HA^{Min}, NP^{Min}, and $PB1^{Min}$) are heavily "deoptimized" as evident by the extremely negative Codon Pair Bias, that is unlike that of any other human gene.

Mueller et al., Figure S1

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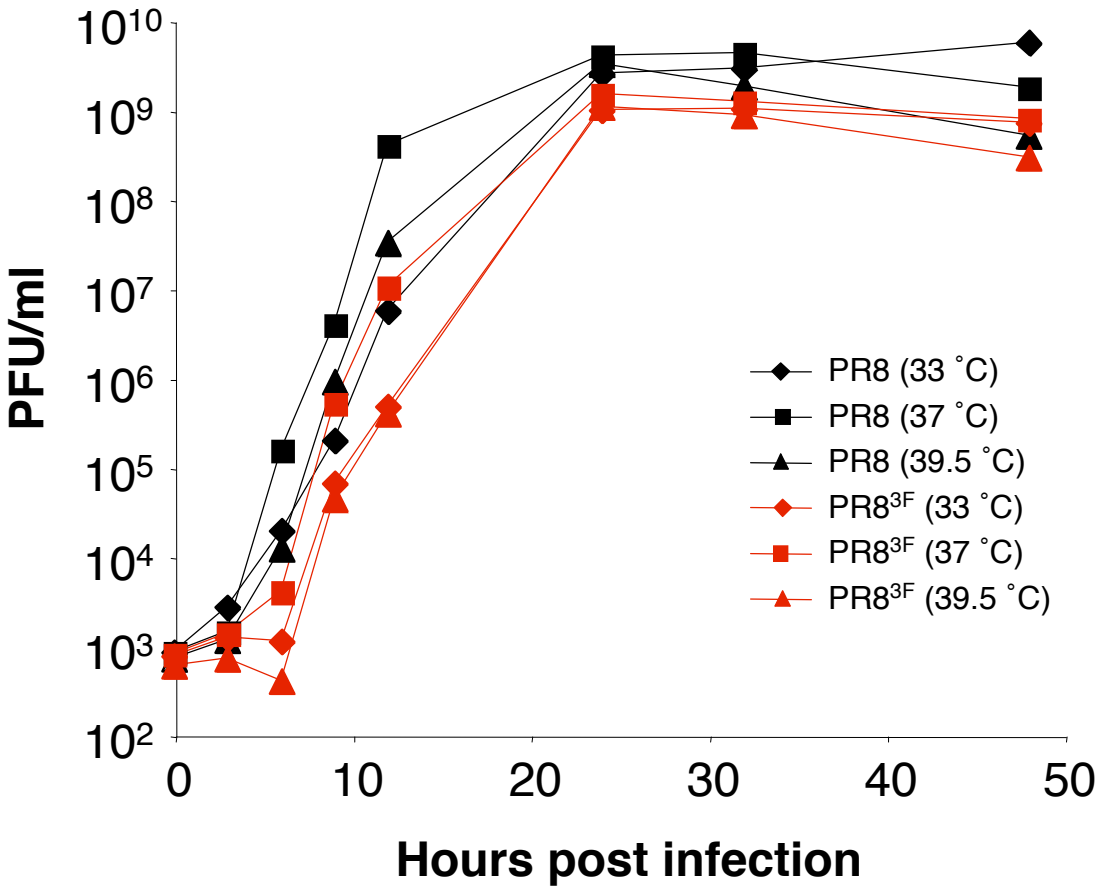

Fig. S2. Growth kinetics of deoptimized Influenza virus PR8^{3F} at various temperatures. The growth characteristics of codon pair-deoptimized PR8^{3F} was analyzed by infecting confluent monolayers of MDCK cells in 100 mm dishes with 0.001 multiplicities of infection (MOI). Infected cells were incubated at 33 ºC , 37 ºC or 39.5 ºC in DMEM, containing 0.2% Bovine Serum Albumin (BSA) and 2ug/ml TPCK-Trypsin (Pierce, Rockford, IL). At the given time points 200 µl of supernatant was removed and stored at -80 ºC until titration. Viral titers and plaque phenotypes were determined as described in Material and Methods.

Mueller et al., Figure S2

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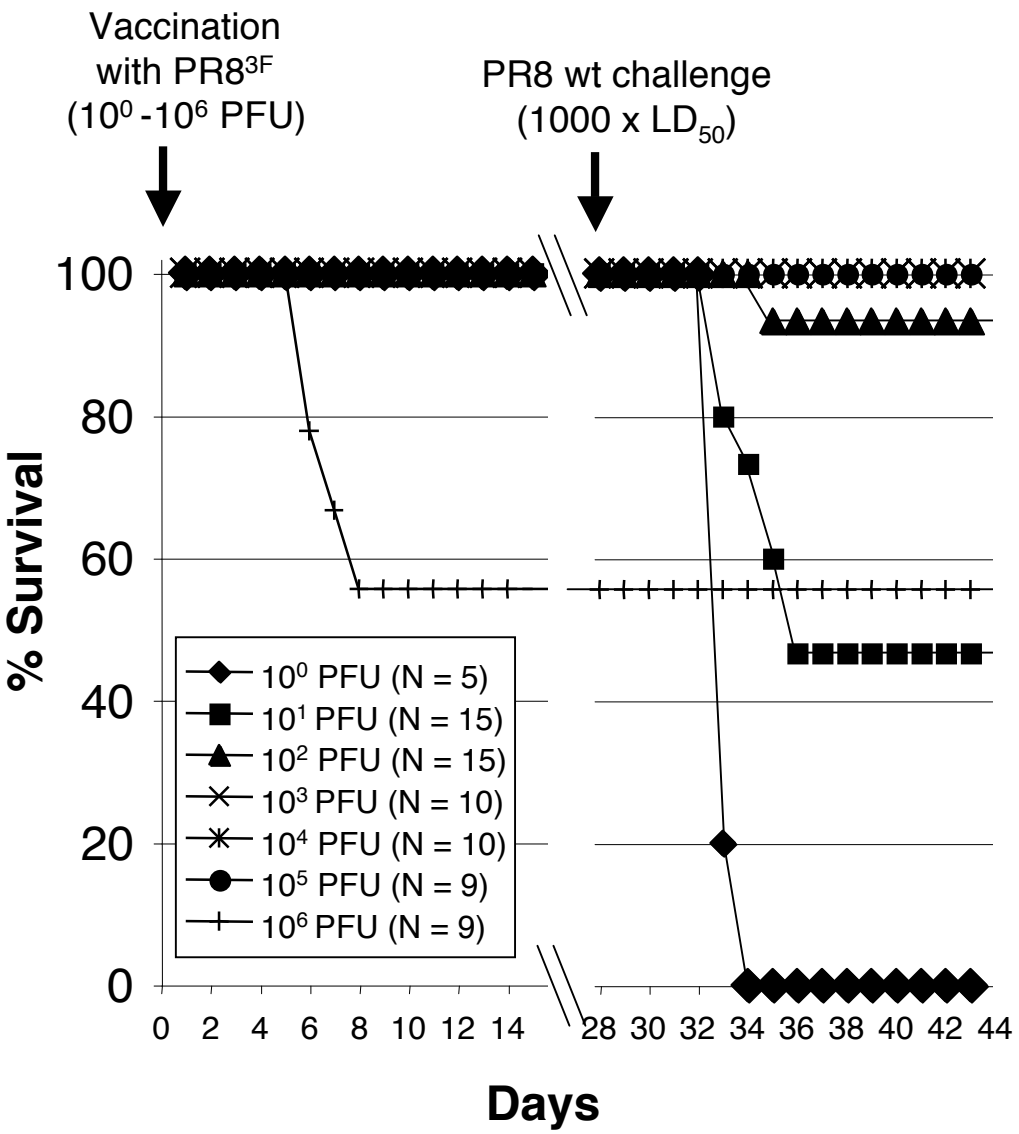

Fig. S3. Mouse survival after vaccination and challenge. Five or more BALB/c mice (as indicated), were inoculated once intranasally on Day 0 with de-optimized PR8^{3F} virus at doses ranging from 10^0 to 10^6 PFU. Survival was monitored. On Day 28 after the first inoculation, animals were challenged with 1000 x LD_{50} of the PR8 wt virus. Immune protection is confirmed by disease-free survival after lethal wt challenge. Note: At doses of 10^3, 10^4, and 10^5 PFU, PR8^{3F} was completely safe, and protective, thus all the symbols are superimposed at the 100% level.

Mueller et al., Figure S3

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