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### Selective attenuation of influenza A viruses by targeting the polymerase subunit assembly

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To develop novel attenuation strategies applicable for all influenza A viruses, we specifically targeted the highly conserved protein-protein interaction site between the viral polymerase subunits PA and PB1. We assumed that impaired binding between them would affect trimeric polymerase complex formation, thereby reducing polymerase activity and replication efficiency in-vivo. For proof of concept, we introduced single or multiple amino acid substitutions in the highly conserved protein binding domains of either PB1 or PA of A/SC35M. These mutations reduced binding affinity and polymerase activity substantially. Single point mutants with a polymerase activity of not less than 3% compared with the wild-type could be rescued without reversion or pseudoreversion. However, if the activity was below 3%, only pseudorevertants were viable. Their amino acid replacements partially restored polymerase subunit binding and increased polymerase activity. Furthermore, we generated a double mutant virus with mutations in both PA and PB1. Several of the single mutant viruses and most pronounced the double mutant virus were significantly impaired in viral growth in cell culture, especially at early time points post infection. Correspondingly, this attenuation was paralleled in mice as indicated by an up to 1000-fold increase in LD50. In addition, vaccination of mice with these mutant viruses protected from lethal challenge with wild-type virus. Thus, targeted mutation of the highly conserved protein binding domains of PA and PB1 represents a novel strategy to prevent reassortment and to attenuate influenza A viruses for use as life vaccine.