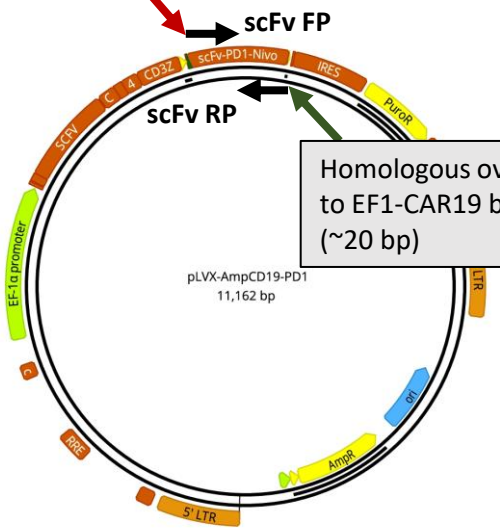
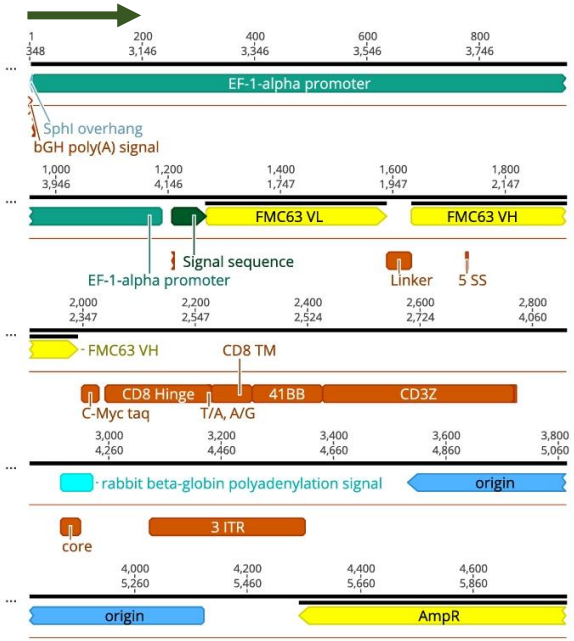
Homologous overhang to EF1-CAR19 backbone (~20 bp)



Homologous overhang to EF1-CAR19 backbone (~20 bp)

PCR (796 bp)

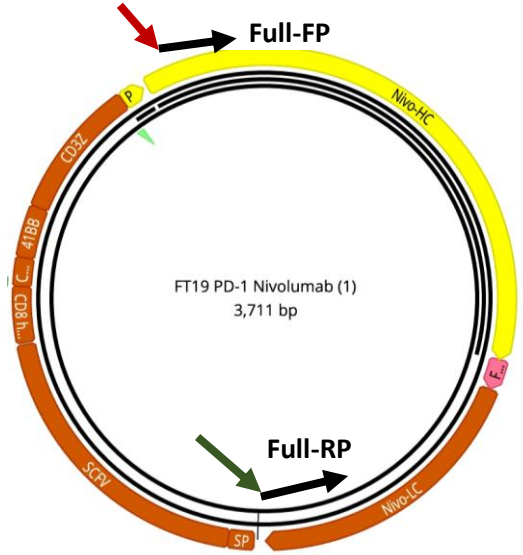
SphI digested EF1-CAR19



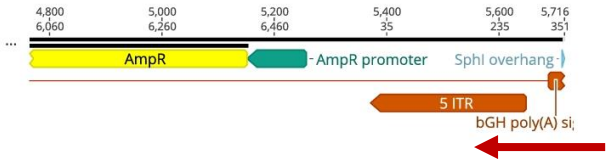
Purification of backbone and PCR product

Gibson assembly (1:2 backbone : insert ratio)

Heat shock transformation in NEB-5 alpha Competent E.coli (NEB#C2987)



PCR (2151 bp)



Final Construct



Supplementary figure S1. Construction workflow of the scFv-Anti-PD1 and full-length-Anti-PD1 expression plasmids. Schematic representation of the cloning strategy showing PCR amplification of the scFv (796 bp) and full-length antibody (2151 bp) inserts using primers containing ~20 bp homologous overhangs to the EF1-CAR19 backbone. The EF1-CAR19 plasmid was digested with *SphI* and purified along with the PCR products prior to Gibson Assembly (1:2 backbone-to-insert ratio). The assembled constructs were transformed into *E. coli* NEB 5-alpha competent cells, and positive clones were confirmed. Only the scFv construct contains a C-terminal His tag.