In 2022, the Monkeypox virus (MPXV) exhibited global dissemination, spanning across six continents, underscoring a notable challenge due to the scarcity of targeted antiviral interventions. Passive immunotherapy, such as monoclonal antibodies (mAbs) and bispecific antibodies (bsAbs), has emerged as promising options. We immunized mice, rabbits with MPXV proteins, A29L, M1R or B6R, and employed hybridoma and single cell isolation technologies to screen for specific neutralizing mAbs. The antiviral activity of these mAbs was subsequently characterized both in vitro and in vivo. Two neutralizing mAbs, M1H11 and M3B2, targeting M1R, 3A1 and 9F8 specifically bind to A29L, and one specific mAb, B7C9, specific to B6R, were identified. They exhibited varying degrees of antiviral efficacy against orthopoxvirus infection both in vivo and in vitro. The cocktail of M1H11 and M3B2, 3A1 and 9F8, 9F8 and B7C9 demonstrated a synergistic protective effect in vivo. The bsAbs, Bis-M1M3 and Bis-M1B7, Bis-9F8-7C9 were engineered by conjugating the Fc (fragment crystallizable) region of the humanized scFv (single-chain fragment variable) of the monoclonal antibody, respectively. These bsAbs exhibited markedly augmented in vivo antiviral protection against orthopoxvirus surpassing the individual mAbs. This study may provide crucial insights for further research on broadly antiviral drugs against MPXV and other orthopoxviruses.