

Supporting Information

Wei et al. 10.1073/pnas.1721780115

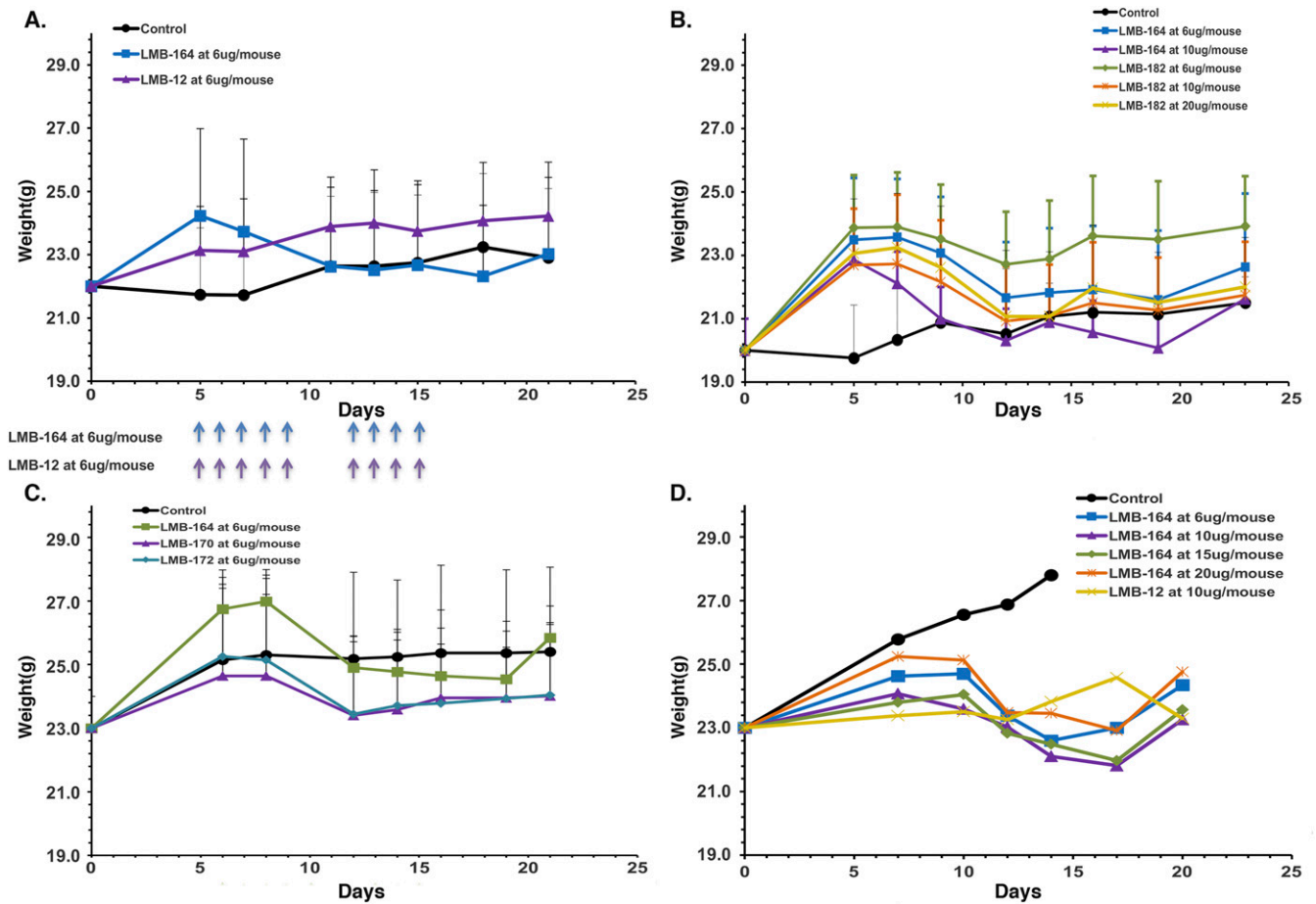


Fig. S1. Weight of the mice from the antitumor experiment described in Fig. 5. (A) Mice treated with LMB-164 in mesothelin-expressing KLM1 xenograft. (B) Mice treated with LMB-164 and LMB-182 in mesothelin-expressing KLM1 xenograft. (C) Mice treated with LMB-164, LMB-170, and LMB-172 in KLM1 xenograft. (D) Mice treated with LMB-12 and LMB-164 in A431/H9 xenograft.

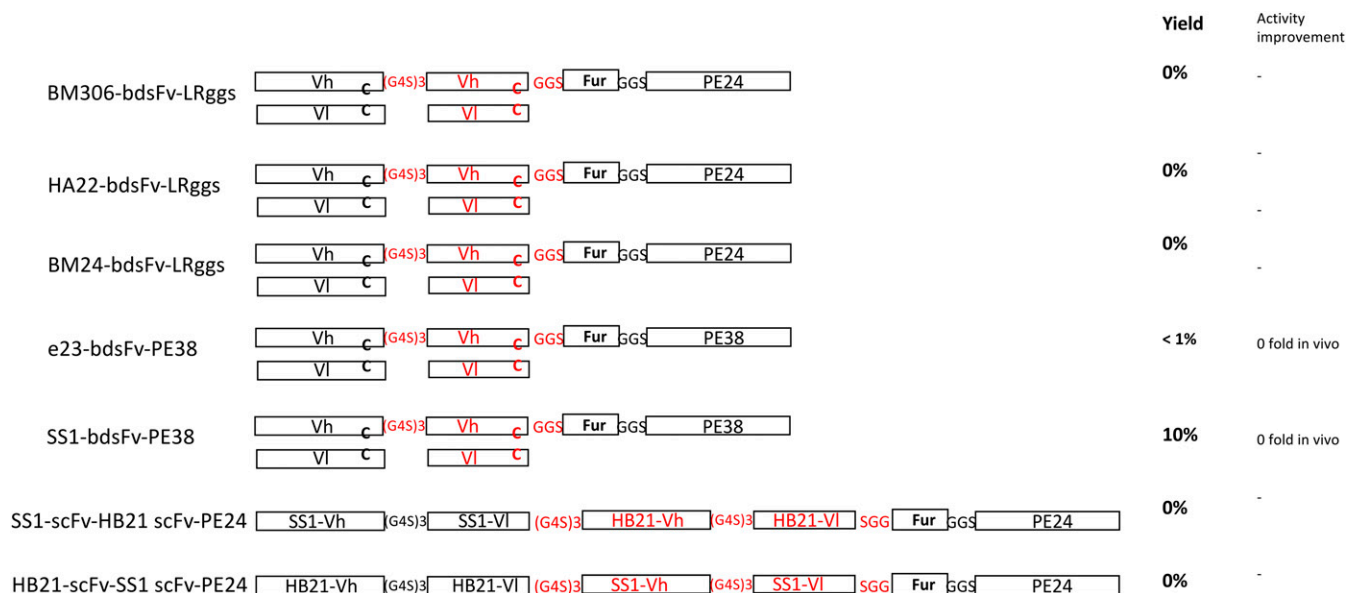


Fig. S2. Unsuccessful bivalent/bispecific constructs. Figure shows the molecules we tried to make and summarizes the results. In five cases, we could not produce any pure protein due to misfolding and aggregation. In case of e23-bdsFv-PE38, we could only produce 0.8 mg of pure protein starting with 100 mg of inclusion bodies. Poor yield limits us to test the activity and the properties of the protein. In the case of SS1-bdsFv-PE38, we were able to produce immunotoxin (protein) with a reasonable yield, but in the animal model, its activity was not increased (16). In summary, these bivalent immunotoxins were not useful for further therapeutic development.

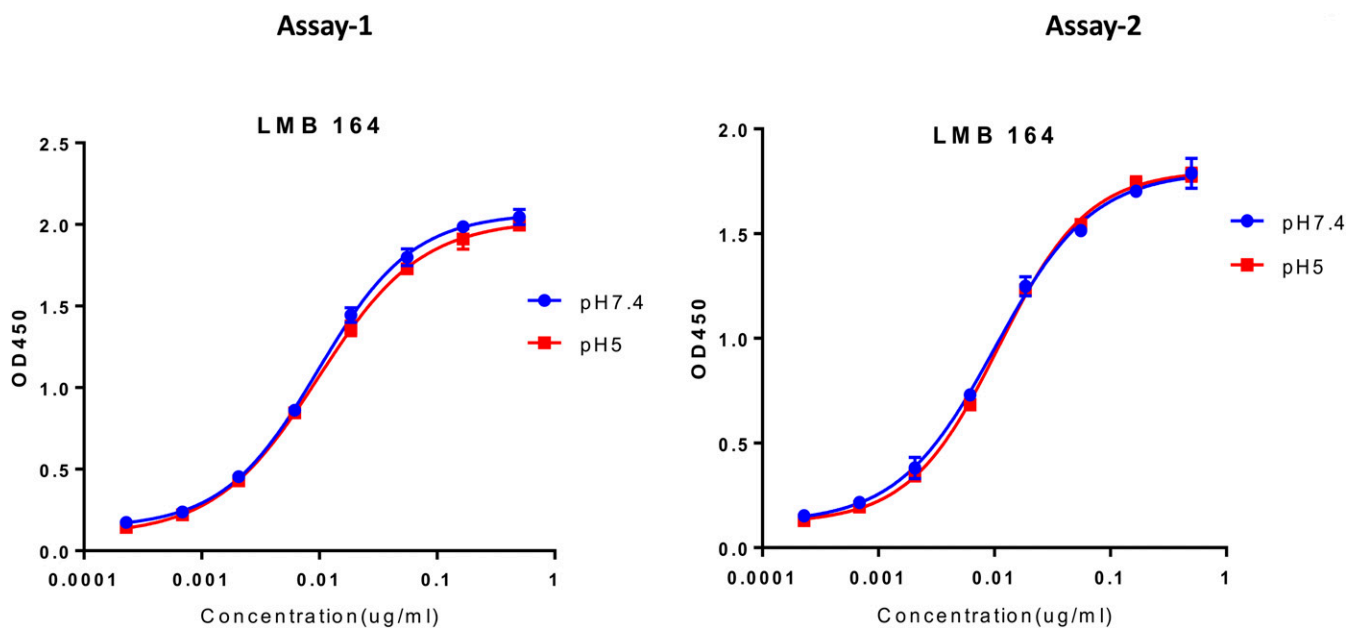


Fig. S3. Binding of LMB-164 to HSA at pH 7.4 and 5.0. The assay was repeated twice.

Table S1. Mouse serum half-life (minutes) of LMB-164 with or without 0.2% HSA

Protein	Assay-1	Assay-2	Assay-3
LMB-164 in 0.2% HSA	129	194	145
LMB-164 in PBS	113	155	136