Supplementary Material and Methods

Affinity maturation using yeast display

HuA33 scFv was randomly mutated using GeneMorph II mutagenesis kit (Agilent Technologies). PCR product was electroporated together with linearized vector into yeast and the library was subjected to 4 rounds of sorting using biotinylated GPA33. Individual clones from the last round were PCR amplified and sequenced to analyze the mutation pattern. Conversion of selected scFv clones into T-BsAb format was done using a one-step 4-fragment ligation method with 50ng linearized vector and a 1:3 vector to insert molar ratio for the other 3 components. Ligation was done with Rapid DNA ligation kit (ThermoFisher Scientific) at room temperature for 1hour. Type II restriction enzyme SapI (NEB) was used to ensure seamless linkage among the different components (Figure S3). Selected clones were transiently expressed using Expi293 expression system (ThermoFisher Scientific) following manufacturer's instructions. Supernatant from Expi293 cells after 4-5 days of culture in shaking flasks was used to purify antibodies using MabSelect SuRe (GE Healthcare) and dialyzed against pH8.0 citrate buffer in dialysis membrane (SpectrumLabs.com).