Figure S1. Verification of PSMA expression in PSMA-overexpressing cell lines by western blot analysis. PSMA expression in prostate cancer cell lines PC-3 and LNCaP clone (FGC), human lung adenocarcinoma cell line NCI-H2122 and their PSMA-overexpressing derivatives, PSMA/PC-3 and PSMA/NCI-H2122, was analyzed using the Wes™ system. Cell lysates were subjected to size-based separation and immunodetection with an anti-PSMA antibody. Endogenous PSMA expression was detected in LNCaP clone FGC cells, while parental PC-3 and NCI-H2122 cells lacked PSMA expression. PSMA protein was successfully detected in PSMA/PC-3 and PSMA/NCI-H2122 cells, confirming effective overexpression. PSMA, prostate-specific membrane antigen; LNCaP, lymph node carcinoma of the prostate; FGC, fast-growing colony.

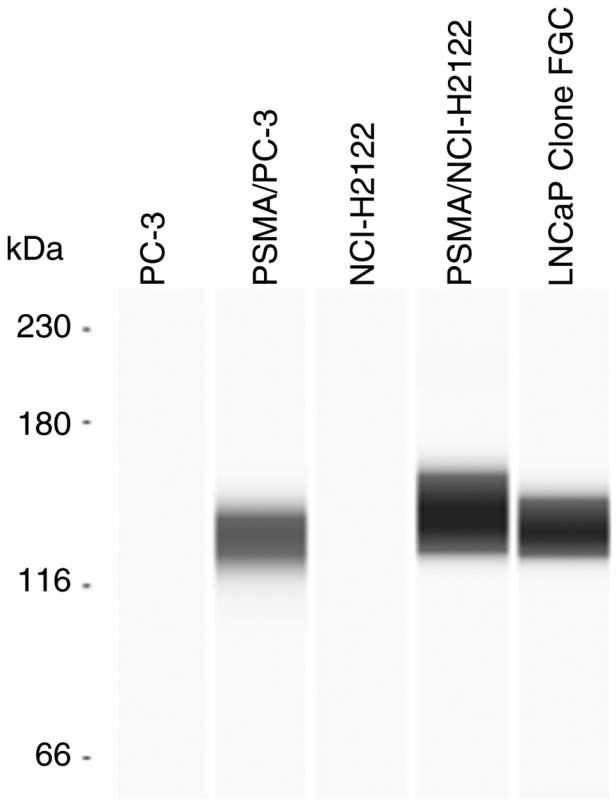

Figure S2. Growth inhibition of PSMA/TRAIL-R2 REGULGENT (PI101/E11). Viability of human tumor cell lines in response to treatment with PSMA/TRAIL-R2 REGULGENT™ in a 96-well cell proliferation assay using (A) PC-3 and (B) PSMA/PC-3 cells. Data represent quadruplicate experiments and are shown as means ± SE. The vertical axes represent absorbance (450-630 nm) using Cell Counting Kit-8 reagent and the horizontal axes represents antibody concentration (ng/ml). A decrease in absorbance values suggests a reduction in cell count, which indicates the induction of cell death. An anti-DNP antibody was used as the negative control. PSMA, prostate-specific membrane antigen; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand-receptor 2; DNP, 2,4-dinitrophenol.

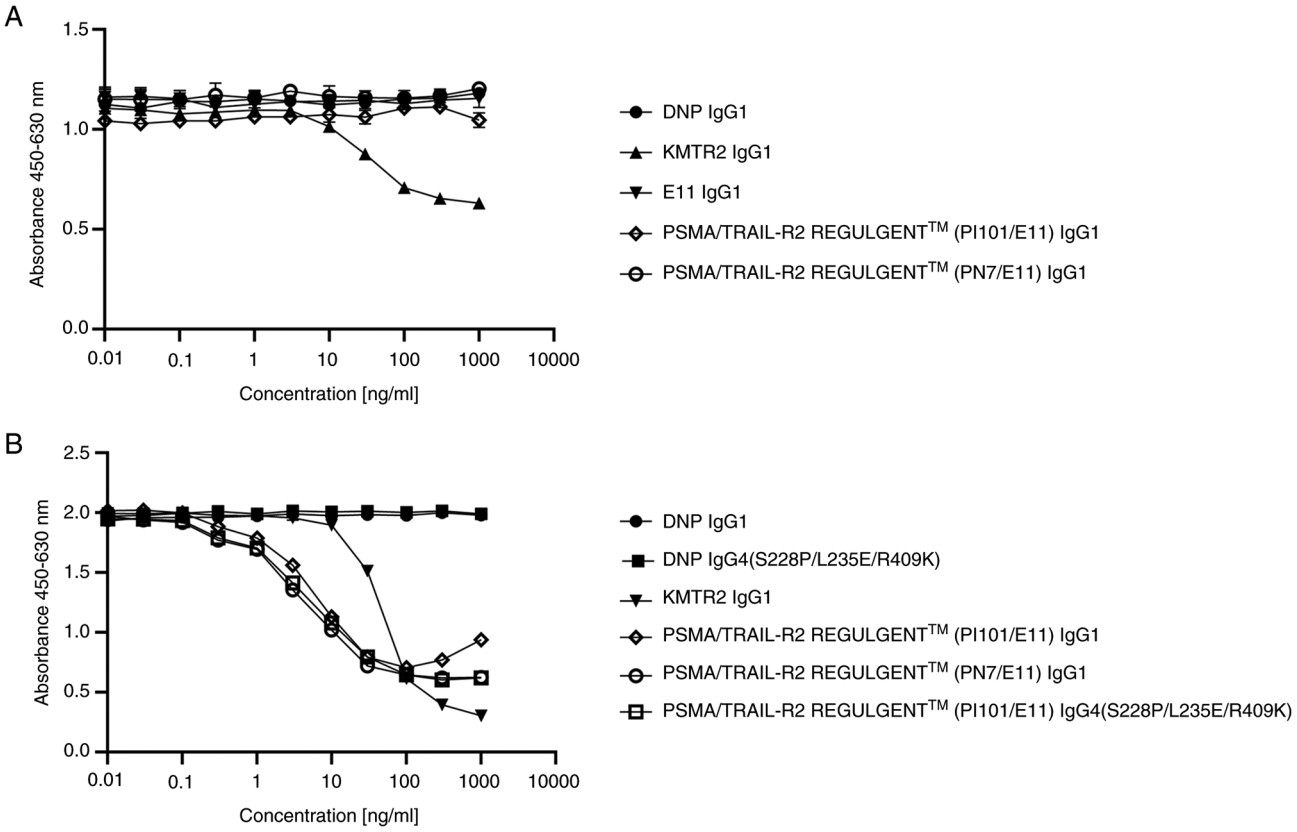

Figure S3. Pharmacokinetic profiles of PSMA/TRAIL-R2 REGULGENT™ in SCID mice. The serum concentrations of E11 IgG1, PI101 IgG1 and PSMA/TRAIL-R2 REGULGENT™ (PI101/E11) were measured by an electrochemiluminescent immunoassay after single intravenous doses of 1 or 10 mg/kg to SCID mice (n=3). PSMA, prostate-specific membrane antigen; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand-receptor 2.

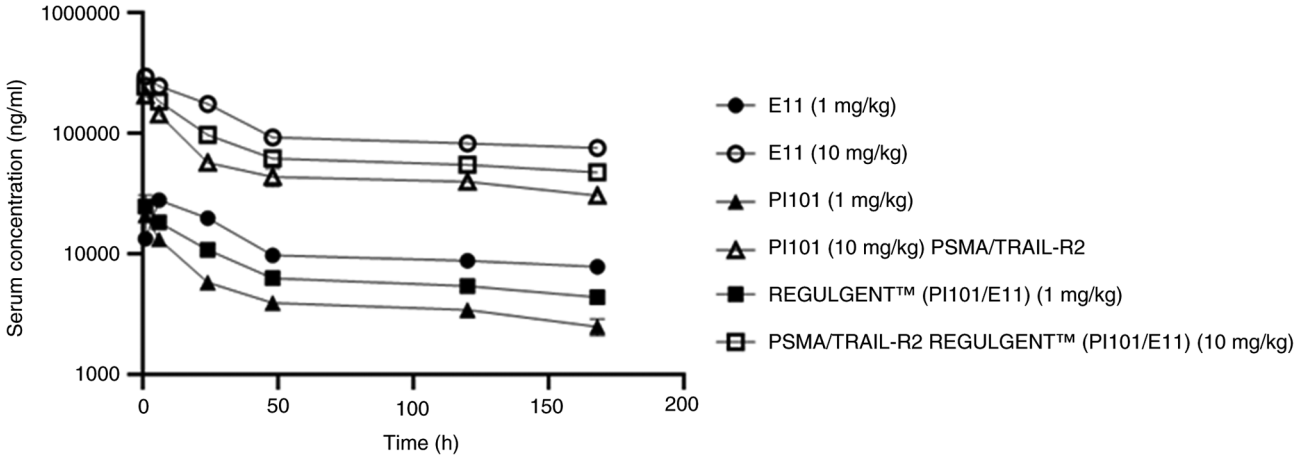

Figure S4. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in response to TRAIL-R2 agonistic antibody KMTR2 and PSMA/TRAIL-R2 REGULGENT™ treatment in PXB mice. (A) Serum ALT and (B) serum AST activities were measured in PXB mice following administration of KMTR2 or PSMA/TRAIL-R2 REGULGENT™. Serum ALT/AST activities were determined by measuring diaryl imidazole type of leucopigment. *P<0.05, ns, no significance. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand-receptor 2; PSMA, prostate-specific membrane antigen.

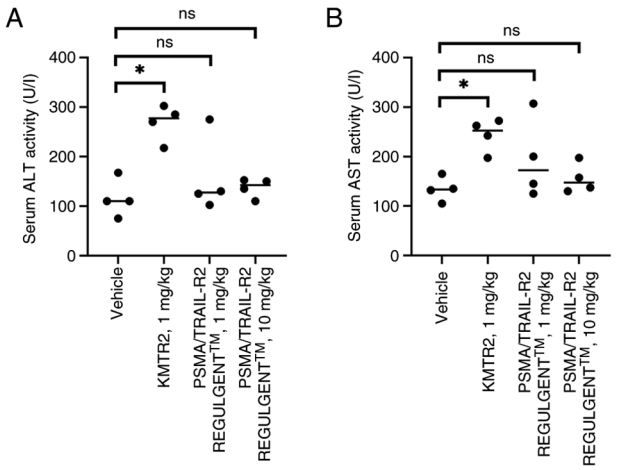

Figure S5. Negative stain electron microscopy analysis of antigen-REGULGENT™ complexes. (A and B) Electron micrographs of the negative stain and representative 2D class averages of the multimeric particles of antigen-BsAb complexes. Models of the PSMA and PSMA/TRAIL-R2 REGULGENT™ components were superimposed on the images. The flexibility of anti-TRAIL-R2 Fab made it impractical to achieve a completely reliable interpretation. However, the size and conformation match strengthened the assumption of a trimeric conformational arrangement. (C) Predicted domain arrangement from the electron micrographs and crystal structure images. BsAb, bispecific antibody; PSMA, prostate-specific membrane antigen; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand-receptor 2.