**Supplemental Materials**

**Figure Legends**

**Supplemental Fig. S1. Detection of 4-1BB on CD8+ T cells within fresh melanoma tumor fragments and in early tumor fragment cultures. A.** Melanoma tumors were surgically resected from patients and subsequently, the tumors were mechanically disaggregated to give single cell suspensions using glass slides in the laboratory. The cells were then filtered and stained for the expression of 4-1BB on CD8+ TIL using flow cytometry in 18 freshly-isolated melanoma metastasis. In a separate set of tumor samples where tumor fragments were established in culture, the TIL migrating out the fragments after 7 days were analyzed for 41BB expression by flow cytometry from pooled culture (**B**). We found that the freshly isolated TIL expressed 4-1BB in the CD8+CD3+ subset (**A**) and that a significant number of TIL migrating out of, or growing out, of these tumor fragments still expressed 4-1BB after 7 days (**B**).

**Supplemental Fig. S2. Testing of different concentrations of agonist anti-4-1BB antibody added to melanoma tumor fragment cultures.** A dose titration experiment was conducted to determine the optimal dose for the anti-4-1BB antibody for initial TIL expansion. Cells counts were done after 3 weeks of culture of the tumor fragments with or without the indicated concentrations of anti-4-1BB (BMS 663513). 10 g/ml anti-4-1BB antibody was found to be optimal and induced the highest TIL expansion in initial experiments on 3 different melanoma patient tumors (5 fragments per condition for each anti-4-1BB dose).