

## Description of Additional Supplementary Files

File Name: Supplementary Video 1

Description: **3D TauSTED imaging of a Jurkat-TIGIT-SNAP-Raji-CD155 conjugation.** 2 colour stacks showing TIGIT (labelled with the SNAP label TMR-STAR; magenta) and CD155 (labelled by a V5/Secondary-AF514 immunostain; green), showing the 3-dimensional organisation of the two molecules on the cells after conjugation for 10 mins. A merged stack is also provided.

File Name: Supplementary Video 2

Description: **Live TIRF imaging of TIGIT at IS upon interaction with PLBs.** Videos showing TIGIT-SNAP, labelled with a fluorescent dye, interacting with PLBs containing 100 mols/ $\mu\text{m}^2$  ICAM-1 plus either CD111 or CD155 (400 mols/ $\mu\text{m}^2$ ; as indicated) and imaged every 3 seconds by TIRF microscopy. Scale bars = 5  $\mu\text{m}$  and times are displayed as mm:ss.