



S4 Fig. Flow cytometry was used to determine the binding of anti-TIM-3 antibodies to membrane bound TIM-3. HEK293 cells were transfected with human TIM-3 and labeled with anti-TIM-3 antibodies or a mouse positive control. Overexpression of TIM-3 in transient transfection induced apoptosis in HEK293 cells. Flow cytometry was performed on Day 1 post-transfection when only 35% of cells expressed TIM-3 at high levels; hence the two peaks in flow analysis.