Supplementary Figure 1. CD1d is expressed by 4T1 cells.

Total RNA was extracted from cultured cells or tissue samples using TRIzol (Invitrogen) according to the manufacturer's instruction. Thereafter, 2 μg of total RNA were subjected to treatment with DNase to ensure that samples were free of genomic DNA contamination. The integrity of RNA from all of the samples was confirmed by gel electrophoresis analysis. Next, DNase-treated total RNA was reverse-transcribed using RT^2 First Strand Kit (SABiosciences) according to manufacturer's instructions. Real-time quantitative PCR was performed on undiluted templates using gene-specific primers designed for use with the SYBR Green real-time RT-PCR detection method (SA Biosciences) utilizing a BioRad iCycler instrument. Primers for both CD1d1 (Cat PPM05616A) and housekeeping gene mouse Eif4g3 (Cat PPM36187A) were obtained commercially from SABiosciences. For each sample, duplicate reactions were run separately for the gene of interest and the internal control. A first derivative dissociation curve (C_T) was run immediately after RT-PCR amplification in order to confirm appearance of a single peak at temperatures greater than 80ºC for each individual reaction. Furthermore, the completed reactions were run in 2% agarose gel electrophoresis to confirm the presence of a single band representing the amplified product. A 189 bp fragment confirmed the presence of CD1d in 4T1 cells grown in culture. For comparison, tissue samples from the liver, spleen and thymus were similarly tested for expression of CD1d.