

Supplementary material

Flow cytometry

HLA-G, ILT2 and ILT4 cell surface expression of HMC1.1 were evaluated by flow cytometry using the following monoclonal antibodies (mAbs): mouse monoclonal anti Human HLA-G (Exbio, IgG2a, 87G), mouse monoclonal anti Human ILT2 phycoerythrin (R&Dsystems, IgG1, clone 292305), mouse monoclonal anti Human ILT4 phycoerythrin (R&Dsystems, IgG2a, clone 287219). IgG2a and IgG1-PE (R&Dsystems) were used as isotypic controls. HMC1.1 was preincubated in a 5% human AB-group serum for 30 min at 4°C to avoid non specific labelling to Fc receptors. Then cells (5.10⁵/ml) were incubated with primary mAb for 30 minutes at 4°C at recommended concentrations by the provider. For indirect immunofluorescence labelling (87G), an additional incubation with PE conjugated goat anti human IgG (Beckman) for 30 minutes was performed at 4°C. After washing, cells were analysed by flow cytometry using cytomics FC500 (Beckman, France). Graphs were reanalysed using the software Kaluza. Purified monocytes were used as positive control for ILT2 and ILT4 labelling (data not shown).

Immunohistochemistry:

HMC1.1 cells were cytocentrifuged on glass slides using and stained with 87G after fixation for 10 min in acetone at 4° C. The following steps were similar to those described above in the main text in material and methods.

Western Blotting

After two ice cold PBS wash, the pellets of HMC1.1 and Jeg3 cells were lysed in lysis buffer containing urea 4M and, then were denaturated at 100°C for 5 min. Proteins were loaded in a 12% SDS polyacrylamide gel electrophoresis under reducing conditions and transferred on nitro-celluloses membranes. Membranes were saturated with 5% non fat dry milk in PBS and incubated with 4h84 (4 µg/ml) for a night at 4°C. A peroxidase-conjugated anti IgG was used as secondary antibody; After washing, blots were developed by chemiluminescence using the SuperSignal® West Femto Maximum Sensitivity Substrate (Thermo Scientific) according the manufacturer's recommendations.