

Supplementary Materials for

HLA Class I Molecules Partner with Integrin β_4 to Stimulate Endothelial Cell Proliferation and Migration

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Fig. S1. Immunoprecipitation with an integrin β_1 -specific antibody fails to pull down integrin β_4 and the HLA-I heavy chain in endothelial cells.

Fig. S2. Knockdown of integrin β_4 blocks HLA-I-mediated phosphorylation of target proteins in endothelial cells.

Fig. S3. The cytoplasmic domain of the HLA-I heavy chain is required for HLA-I-mediated cell proliferation.

Fig. S4. Knockdown of the HLA-I heavy chain inhibits the phosphorylation of ERK.

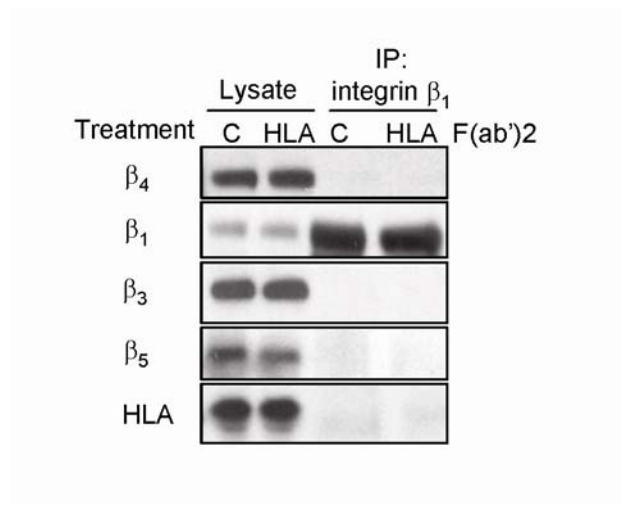


Fig. S1. Immunoprecipitation with an integrin β_1 -specific antibody fails to pull down integrin β_4 and the HLA-I heavy chain in endothelial cells. Endothelial cells (EC1) were stimulated with the F(ab')₂ fragments of the HLA-I-specific antibody W6/32 or with control IgG. Cell lysates were subjected to immunoprecipitation with antibody against the integrin β_1 subunit, and were analyzed by Western blotting with antibodies against the heavy chain of HLA-I (EMR8-5) and integrins β_4 , β_1 , β_3 , and β_5 . The data are representative of three independent experiments.

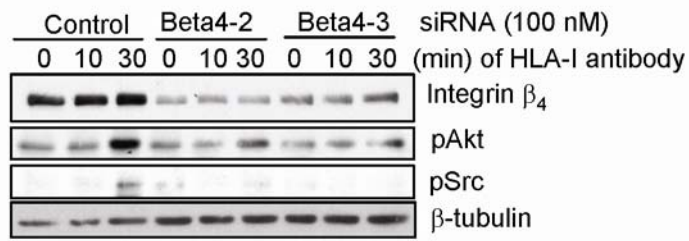


Fig. S2. Knockdown of integrin β_4 blocks HLA-I-mediated phosphorylation of target proteins in endothelial cells. Endothelial cells (EC1) were transfected with 100 nM of either of the integrin β_4 -specific siRNAs Beta4-2 (5'-CAGAAGAUGUGGAUGAGUU-3') or Beta4-3 (5'-GAGCUGCACGGAGUGUGUC-3'), which are targeted to different regions of the integrin β_4 mRNA, or with control siRNA. After 48 hours, cells were stimulated with W6/32 (1 μ g/ml) for 10 or 30 min, lysed, and analyzed by Western blotting with antibodies against pAkt (Ser⁴⁷³) and pSrc (Tyr⁴¹⁶). β -tubulin served as a loading control. The data are representative of two independent experiments.

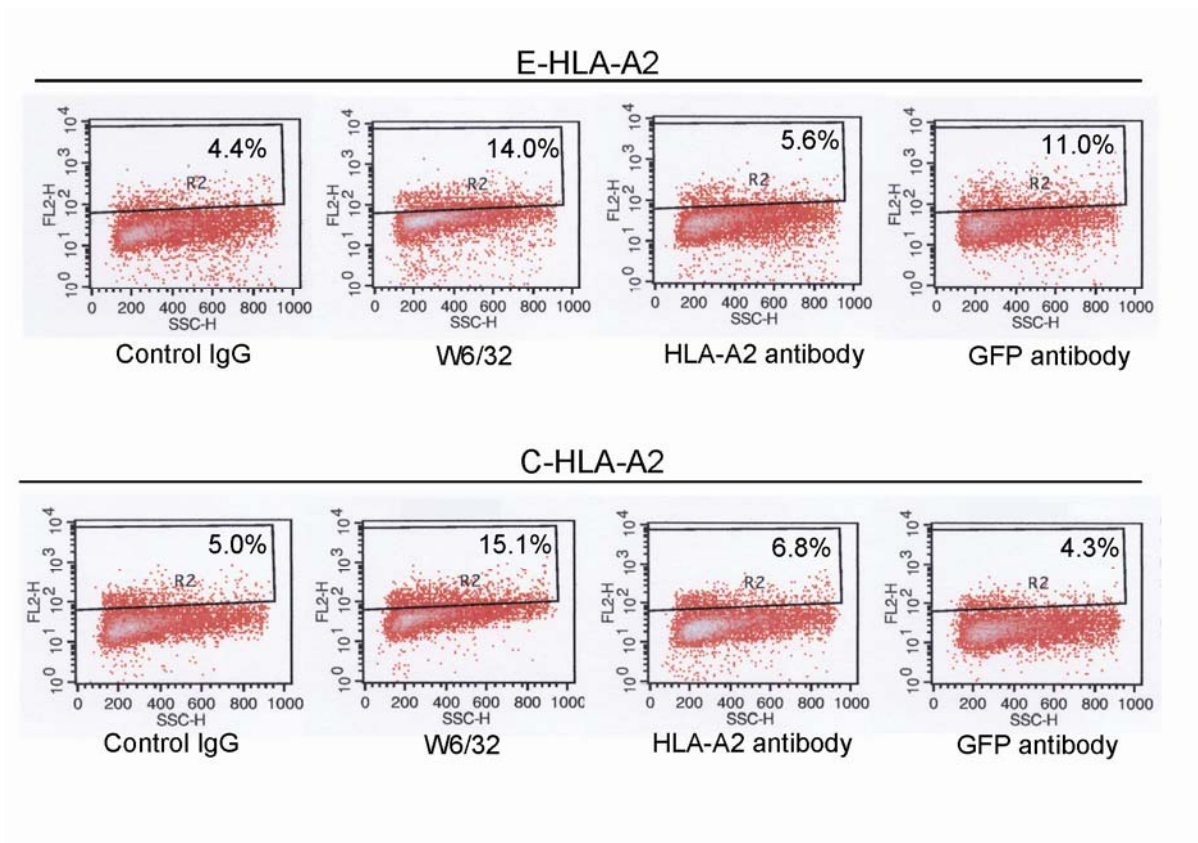


Fig. S3. The cytoplasmic domain of the HLA-I heavy chain is required for HLA-I-mediated cell proliferation. Endothelial cells (EC2) were infected with adenoviruses encoding E-HLA-A2 or C-HLA-A2. After 48 hours, the cells were stimulated with antibodies against GFP or HLA-A2 in the presence of BrdU. Cells stimulated with mouse IgG and W6/32 serve as negative and positive controls, respectively. BrdU positive cells are gated as the R2 region. The data are representative of three independent experiments.

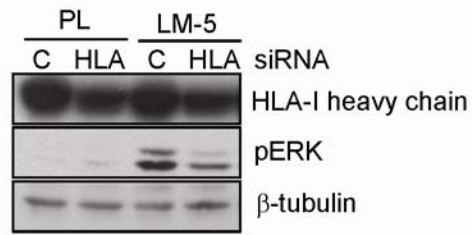


Fig. S4. Knockdown of the HLA-I heavy chain inhibits the phosphorylation of ERK. Cells transfected with siRNA against the HLA-I heavy chain (5'-GAGCUCAGAUAGAAAAGGA-3') or with control siRNA were plated on poly-L-lysine (PL) or laminin-5 (LM-5) for 30 min, after which lysates were prepared, resolved by SDS-PAGE, and analyzed by Western blotting with antibodies against pERK and the HLA-I heavy chain. β -tubulin was used as a loading control. The data are representative of two independent experiments.