Supplementary Materials for

The costimulatory activity of Tim-3 requires Akt and MAPK signaling and its recruitment to the immune synapse

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Figs. S1 to S3
Fig. S1. Effects of MEK and Akt inhibitors on the TCR-induced phosphorylation of S6. (A) Control experiments demonstrating the efficacy of the indicated inhibitors on Jurkat cells stimulated with anti-TCR mAb. Cells were analyzed by Western blotting with antibodies against total and phosphorylated ERK (left) or total and phosphorylated Akt (right). (B) Expression of Flag-Tim3 on CD4+ or CD8+ T cells from Tg mice expressing only E8i-Cre (black lines) or both E8i-Cre and the flox-stop-flox knock-in of Flag-Tim3 (red lines). (C) Gating strategy used to segregate D10 T cells expressing low or high amounts of Tim-3 after transfection. (D) Representative flow cytometry analysis of pS6 staining in the D10 T cells analyzed in (C) after stimulation with anti-CD3 mAb in the presence or absence of the indicated inhibitors. (E) Quantitation of the percentage of pS6+ cells based on the flow cytometry analysis shown in (D). (F) Representative in vitro proliferation assays for CD8+ T cells from the indicated mice. (G) Quantitation of the percentages of divided cells in each peak from triplicate samples. Each panel is representative of three independent experiments. Statistical significance in (E) and (G) was determined by one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
**Fig. S2. Effects of truncations of the Tim-3 cytoplasmic tail on Tim-3 expression and S6 phosphorylation.** (A) Schematic of the Tim-3 constructs analyzed in Figs. 3 and 4. (B and C) Cell-surface expression of Tim-3 constructs, based on flow cytometry staining for the N-terminal Flag tag. Duplicate transfections are representative of three independent experiments. (B) Expression based on the percentage of Flag-positive cells after transient transfection of Jurkat cells. (C) MFI of Flag staining. (D) Western blotting analysis of Tim-3 constructs with or without treatment with PNGase F. Blots are representative of three independent experiments. The labels “Del” and Δ refer to deletions of the indicated domains. (E) Representative flow cytometry analysis of pS6 in Jurkat cells transfected with plasmids encoding the indicated Tim-3 constructs and stimulated with anti-TCR mAb. (F) Quantitation of the percentage of pS6+ cells in the cells represented in (E). Statistical significance in (F) was determined by one-way ANOVA. ****p < 0.0001.
Fig. S3. Retroviral system used for TIRF imaging of immune synapses. (A) Schematic of the retroviral vector used to express the WT or CD71tm forms of Tim-3, together with a fluorogen-activating peptide (FAP). The vector also encodes an IRES-controlled Thy1.1 marker. (B) Representative flow cytometry analysis of Tim-3 and Thy1.1 expression by the packaging (293T) cells transfected with the indicated constructs. (C) Representative flow cytometry of the co-encoded Thy1.1 in murine T cells transduced with the indicated retroviruses. Data are representative of two experiments.