Sup. Fig. 1 TacTIR9 is localized like WT TLR9. *A*, HEK293T cells were transfected with fusion proteins containing the N-terminal domain of Tac and the transmembrane and cytoplasmic (TIR) domains of TLR4 (TacTIR4, upper panels) or TLR9 (TacTIR9, lower panels) and stained with Alexa488 labeled anti-Tac mAb for total or surface expression (black lines). Control cells transfected with empty vector and stained identically are shown as filled histograms (grey). Each histogram is representative of at least four independent experiments. *B*, HeLa cells transfected with Tac-TIR4 (upper panel) or Tac-TIR9 (lower panel) were lysed and left untreated or were treated with either EndoH (H) or PNGaseF (F). Samples were immunoblotted with anti-Tac and are from the same gel with the same exposure. Three forms of Tac-TIR4 are present. The two lower bands, indicated by asterisks, represent unglycosylated and immature (sensitive to EndoH) Tac-TIR4. The higher molecular weight band (arrowhead) represents the mature glycoform, which is resistant to EndoH, but is only partially sensitive to PNGaseF due to the additional presence of O-linked oligosaccharides (26). *C*, HeLa cells, cotransfected with Tac-TIR9 and TLR9-GFP (green), were stained for Tac (red). The panel on the right shows the overlay of TLR9 and Tac-TIR9.

Sup. Fig. 2 The TM of TLR9 is dispensable for intracellular localization. HEK293T cells were transfected with the indicated plasmids and 24 hours later collected in HBS/0.1%BSA/0.1%azide. Cells were then divided in half with one half stained with Alexa488 labeled anti-Tac mAb in the same buffer then fixed with paraformaldehyde prior to analysis (Surface). The second half of cells were fixed in paraformaldehyde, then blocked and stained in permeabilization buffer (Total). Data were collected on a FacsCalibur and analyzed with CellQuest. The % indicates the percent of cells in the indicated gate and MFI is the mean fluorescence intensity of the total population.

Sup. Fig. 3 Human TLR9-4 chimeras are intracellularly retained.
HeLa cells (top), or mouse embryo fibroblasts (MEF, bottom), were transfected with the indicated human TLR9 chimeric plasmids and 24 hours later analyzed for glycosylation as indicated in Figure 2.

Sup. Fig. 4 Mouse TLR9-4 chimeras are intracellularly retained. HeLa cells (top), or MEFs (bottom), were transfected with the indicated flag-tagged mouse TLR9 chimeric plasmids and 24 hours later analyzed for glycosylation as indicated in Figure 2

Sup. Fig. 5 The cytoplasmic tail of TLR9 contains a 14 a.a. localization region. HEK293T cells were transfected with the indicated plasmids and 24 hours later collected in HBS/0.1%BSA/0.1%azide. Cells were then divided in half with one half stained with Alexa488 labeled anti-Tac mAb in the same buffer then fixed with paraformaldehyde prior to analysis (Surface). The second half of cells were fixed in paraformaldehyde, then blocked and stained in permeabilization buffer (Total). The % indicates the percent of cells in the indicated gate and MFI is the mean fluorescence intensity of the total population. Untransfected, stained HEK293T cells are shown in Sup. Fig. 2.