TLRs contain a conserved TIR domain with regions of varying homology.Furthermore, the Y870A mutant TLR9 model may provide an interesting platform to better characterize the autophagosomal degradation pathway of integral membrane protein disposal.In fact, our observation that TLR9 Y870F signals as effectively as WT TLR9 in response to CpG in dendritic cells, after correcting for differences in levels of mature TLR9 (Fig 4B), corroborates previously published data in bone marrow derived macrophages [25] and indicates that a tyrosine at the N–terminal box 1 position is not strictly necessary for CpG–induced signaling, but rather supports generating a functional receptor.This suggests that TLR9 dimerization via the luminal domain is a ligand independent process, as these molecules would not be expected to have access to their ligands within the ER. It is of interest that the Y870A TLR9 molecule is consumed by autophagy for disposal, rather than exploiting the endoplasmic–reticulum–associated protein degradation (ERAD) pathway.Perhaps the properly folded and dimerized luminal domain is unable to be unfolded by the ER quality control machinery––a requirement for retrotranslocation of ERAD substrates into the cytosol for proteosomal degradation––obviating the need for a distinct disposal mechanism, much like an aggregate.Since Y870 is within the cytoplasmic TIR domain of TLR9, our data are consistent with a requirement for heretofore undiscovered cytoplasmic chaperones in TLR folding, analogous to the requirement for both ER–resident and cytoplasmic chaperones for folding of the cystic fibrosis transporter CFTR [27].This is the first report, to our knowledge, implicating this region in receptor stability in the ER. It will be of interest to investigate how this conserved tyrosine residue impacts the stability and trafficking of other TLRs and adaptor molecules, as many of these molecules have specific subcellular localization requirements.The cytoplasmic TIR domain of all TLRs is best known for its ability to interact with the adaptor molecules TRIF/TRAM and/or MYD88/TIRAP to transduce downstream signaling following ligand engagement [28].An additional role for localization was attributed to this region, as a TLR9 molecule with deletions of several amino acids in the TIR domain downstream of Y870 was mislocalized to the cell surface [29].While the investigators attributed the defective signaling to decreased LPS–induced receptor phosphorylation, our data suggest the alternative possibility that the receptor did not traffic normally to the plasma membrane to access ligand.In contrast, our data suggest that point mutation of the N–terminal tyrosine 870 in the Box 1 of the TIR domain prevents normal TLR9 egress from the ER. One possible explanation for this discrepancy could be attributed to the cell types used for experimentation.We conducted our studies in BMDCs, which more closely resemble physiologically relevant TLR–responsive innate immune cell types than do HeLa cells that were used in other studies [29].Consistent with a prior report in bone marrow derived macrophages [25], we find that tyrosine at position 870 is necessary for full TLR9 stabilization and normal trafficking to endosomal compartments.Mutagenesis of the analogous N–terminal box 1 tyrosine residue in TLR4 (Y674A) abolished LPS–induced signaling in a heterologous expression system in 293T cells [14].Previous work has suggested that some residues of the box 1 region of the TIR domain create a binding pocket required for TIR–TIR domain interactions between the TLR and its adaptor Mal/TIRAP [15].Interestingly, the Y870A variant forms homodimers, the interaction for which is based on the N–terminal ectodomain [16], and associates with its appropriate ER chaperone molecules GRP94 and UNC93B1.While activated TLR9 has been shown to be targeted for autophagy upon B–cell receptor signaling in B cells [26], we

now demonstrate that a mutant TLR9 can be similarly targeted in myeloid cells independent of any signaling.Therefore, we propose a model of TLR processing that requires proper TIR domain assembly prior to ER exit and subsequent subcellular localization.