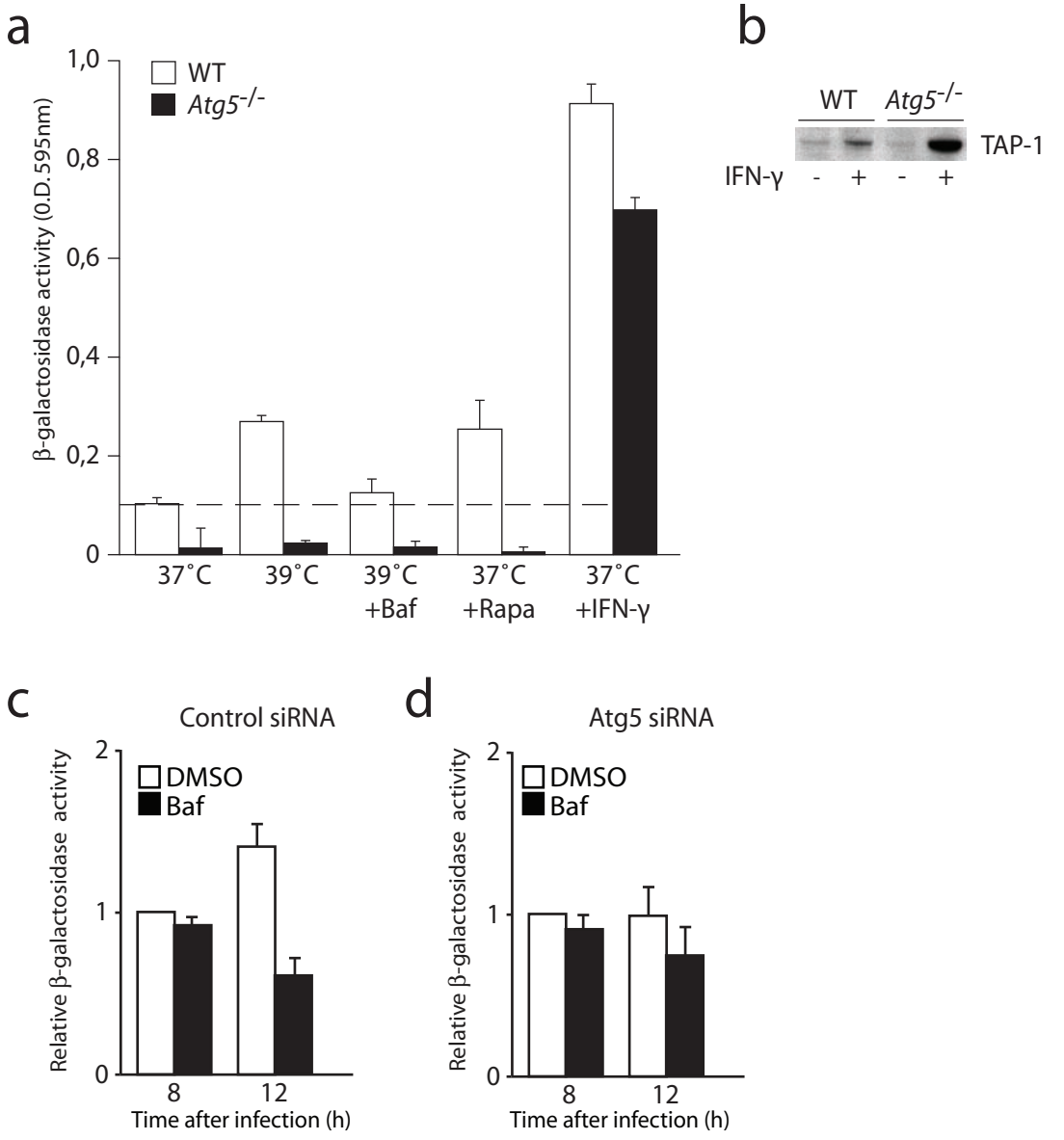

Supplementary Figure 1. Atg5-dependent contribution of autophagy in MHC class I presentation. (**a**) Wild-type or *Atg5*$^{-/-}$ murine embryonic fibroblasts (MEFs) were incubated at 37°C or 39°C, with or without IFN-γ, prior to infection with wild-type HSV-1. Where indicated bafilomycin (Baf) or rapamycin (Rapa) was added 2 h (Baf) or 1 h (Rapa) after infection. MEFs were then incubated with the gB-specific CD8$^+$ T cell hybridoma, which expresses LacZ as an indicator for T cell receptor activation, for 12 h at 37°C. LacZ expression was measured. Dashed line depicts the detection limit (A_{595} = 0.1). (**b**) MEFs were treated with IFN-γ (200 U/ml, 18 h) and expression of TAP-1 was measured by immunoblot. Representative of two independent experiments. (**c,d**) BMA3.1 macrophages were transfected with control siRNA or Atg5-specific siRNA for 60 h, after which they were infected with wild-type HSV-1 for the indicated time periods. Bafilomycin (Baf) was added, where indicated, 2 h after infection. CD8$^+$ T cell activation was measured as in (**a**). CD8$^+$ T cell activation was shown in arbitrary units and was normalized to the signal obtained with macrophages transfected with control siRNA, infected with HSV-1 (8 h) and treated with DMSO. (**a**,**c**,**d**) Error bars, s.e.m. Data are mean of triplicate samples from three independent experiments.

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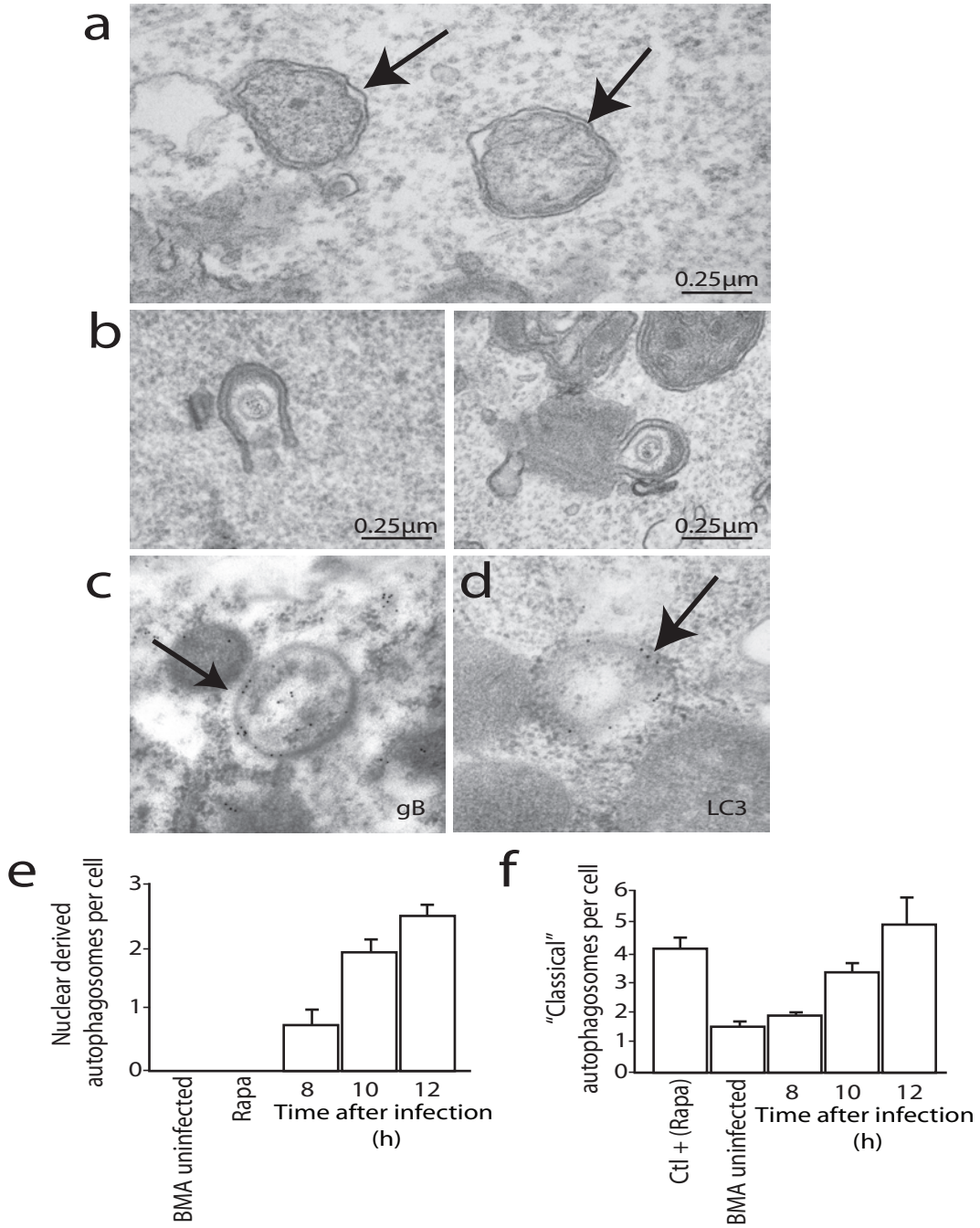

Supplementary Figure 2. Increase in autophagosomes formation during the late phase of infection. (**a**) Macrophages were treated with rapamycin and analyzed by electron microscopy. Black arrows, double-membrane structures constituting 'classical' autophagosomes. (**b-d**) Macrophages were infected with wild-type HSV-1 for 10 h and were analyzed by electron microscopy micrographs. Left, pre-autophagosomal structures surrounding HSV-1 capsids. Right, autophagosomal structures interacting with electron-dense lysosomal compartments. (**c,d**) Viral gB (**c**) and cellular LC3 (**d**) were detected by immuno-electon microscopy. (**e,f**) Quantification of the number of nuclear derived autophagosomes (**e**) or 'classical' autophagosomes (**f**) in uninfected macorphages, uninfected rapamycin-treated macrophages, and macrophages infected for the indicated time periods with wild-type HSV-1. (**a**,**b**,**c**,**d**) Images are representative of observations made in three independent experiments. (**e**,**f**) Error bars, s.e.m. Data are mean of samples from three independent experiments.

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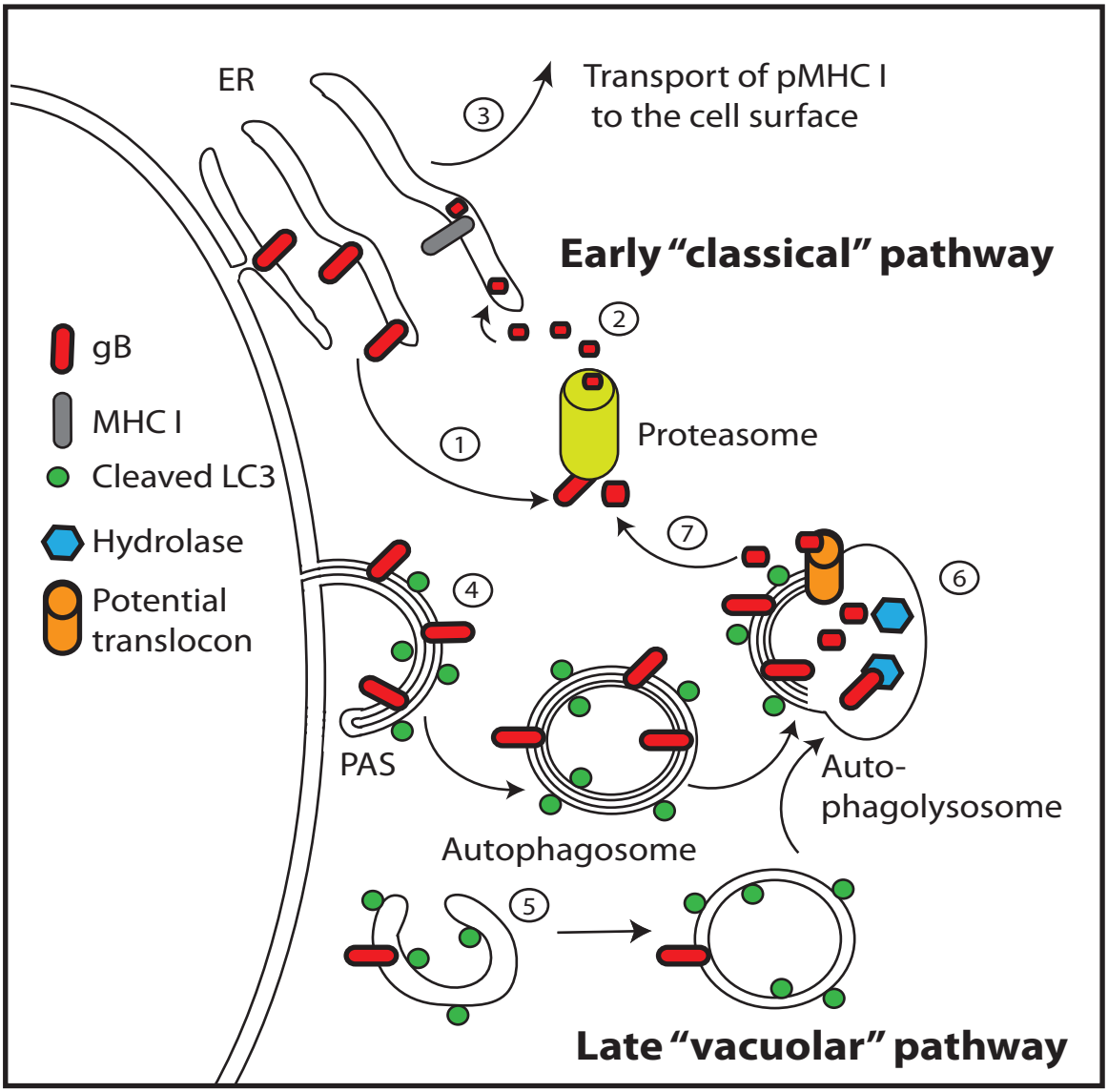

Supplementary Figure 3. Participation of autophagy in the vacuolar processing of endogenous viral proteins. Our working model proposes that HSV-1 gB is processed through the classical MHC class I presentation pathway during the first few hours after infection in macrophages. This process involves degradation by the proteasome (1), translocation of peptides into the ER (2), and transport of loaded MHC class I molecules to the cell surface (3). During the later phase of infection, gB is processed via a vacuolar pathway that involves the formation of LC3-expressing autophagosome-like structures from the coiling of the nuclear envelope where viral gB is enriched (4), together with the formation of $LC3^{+}$ conventional autophagosomes (5). These structures can fuse with lysosomes to form autophagolysosomes where gB is partly degraded by hydrolases (6). The ER nature of the autophagosomal organelles is likely to provide molecules enabling the translocation of gB peptides from the lumen of autophagolysosomes to the cytoplasm (7). These peptides are then handled by the classical pathway, as described in steps 2 and 3. PAS, pre-autophagosomal structure.

Nature Immunology: doi:10.1038/ni.1720