

Figure S1.- GFP-LM does not co-localize with MHC-class II molecules in LIMP-2^{-/-} BM-DM. BM-DM from WT or LIMP-2^{-/-} mice were infected with GFP-LM for 1 hour. Cells were fixed with 2% p-formaldehyde and after solubilization with 0,05% Triton-X 100 were treated with biotinylated-anti-MHC-IA^b, followed by streptoavidin-PE. Images were analyzed in a Confocal microscope (Zeiss). Scale bars corresponded to 5 μ m. *A*, Co-localization images of 10 μ m of BM-DM from WT and LIMP-2^{-/-} mice infected with GFP-LM and labelled with anti-MHC-class II antibodies. *B*, Z-serie of 1 μ m inner section of same WT BM-DM image as in *A* to show co-localization of GFP-LM with anti-MHC-class II antibody in all cell planes, starting from the bottom to the top of the cell.



Figure S2.- LIMP-2^{-/-} mouse embryonic fibroblasts shows an enhanced LM cytosolic growth. *A*, Mouse embryonic fibroblasts from genetic deficient mice: Ctsd^{-/-}, Ctsd^{+/+}, Limp-2^{-/-} or Limp-2^{+/+} were infected with LM for different times: 0, 6 or 12 hours. Results are expressed as CFU x $10^4 \pm$ SD of triplicates. *B*, Different mouse embryonic fibroblasts were infected with GFP-LM (25:1 ratio bacteria: cell) for 6 h and cytoskeleton labelled with TRITC-phalloidin. Lower fluorescent images corresponded to GFP-LM co-localization with TRITC-phalloidin, analyzed by conventional fluorescent microscopy. Scale bars correspond with 5 µm. The table below corresponds to phagosomal and cytosolic fractions purified from PNS (30 µg) of Ctsd^{+/+}, Ctsd^{-/-}, Limp-2^{-/-}, Limp-2^{+/+} mouse embryonic fibroblasts infected for 0 or 6 h. Results are expressed as percentages of total CFU internalized in PNS as described in *Material and Methods*. CFU values for PNS at 0 h were: 6.5 ± 0.03 for Ctsd^{+/+}, 32.5 ± 0.1 for Ctsd^{-/-}, 63.7 ± 0.01 for Limp-2^{-/-} and 7.0 ± 0.03 for Limp-2^{+/+}.



Figure S3.- LIMP-2 transfectants display altered LM phagocytic rates but an enhanced ability to kill the pathogen. *Ctsd, LAMP-1 and LIMP-2 CHO transfectants. *A*, Protein and fluorescent levels of CHO transfectants (100,000 cells/lane or FACS analysis). Blots were developed with a rabbit anti-GFP antibody (for LAMP-1 and LIMP-2 transfectants) and rabbit anti-cathepsin-D (Ctsd). *B*, [35 S-LM] (500,000 cpm/sample) were added to 4 x 10⁵ CHO cells/well in polyvinyl 96-well plates in Materials and Methods on ice for 60 min for binding assays. Wells were cut off and radioactivity measured in a b-counter. Results are expressed as cpm bound to cells \pm SD of triplicates. *C*, LM infection kinetics at different times: 0,

CELLS	Phagocytic rate ^a	
	[³⁵ S]-LM x 10 ⁻²	[³⁵ S]-HKLM x 10 ⁻²
Control	55 ± 3	50 ± 2
Limp-2	120 ± 6	110 ± 5
AS	60 ± 3	64 ± 4
AQ	63 ± 4	62 ± 3
Limp-2/AS	58 ± 2	55 ± 3
Limp-2/AQ	121 ± 6	122 ± 5

Table S1.- Phagocytic rates of CHO cells and transfectants. ^aPhagocytic rates of CHO cells, transfectants and co-transfectas (Control, Limp-2, AS, AQ, Limp-2/AS, Limp-2/AQ) were measured after 60 min of uptake of [³⁵S]-LM or [³⁵S]-HKLM (heat-killed LM) (500,000 cpm/well) as described in *Material and Methods*.