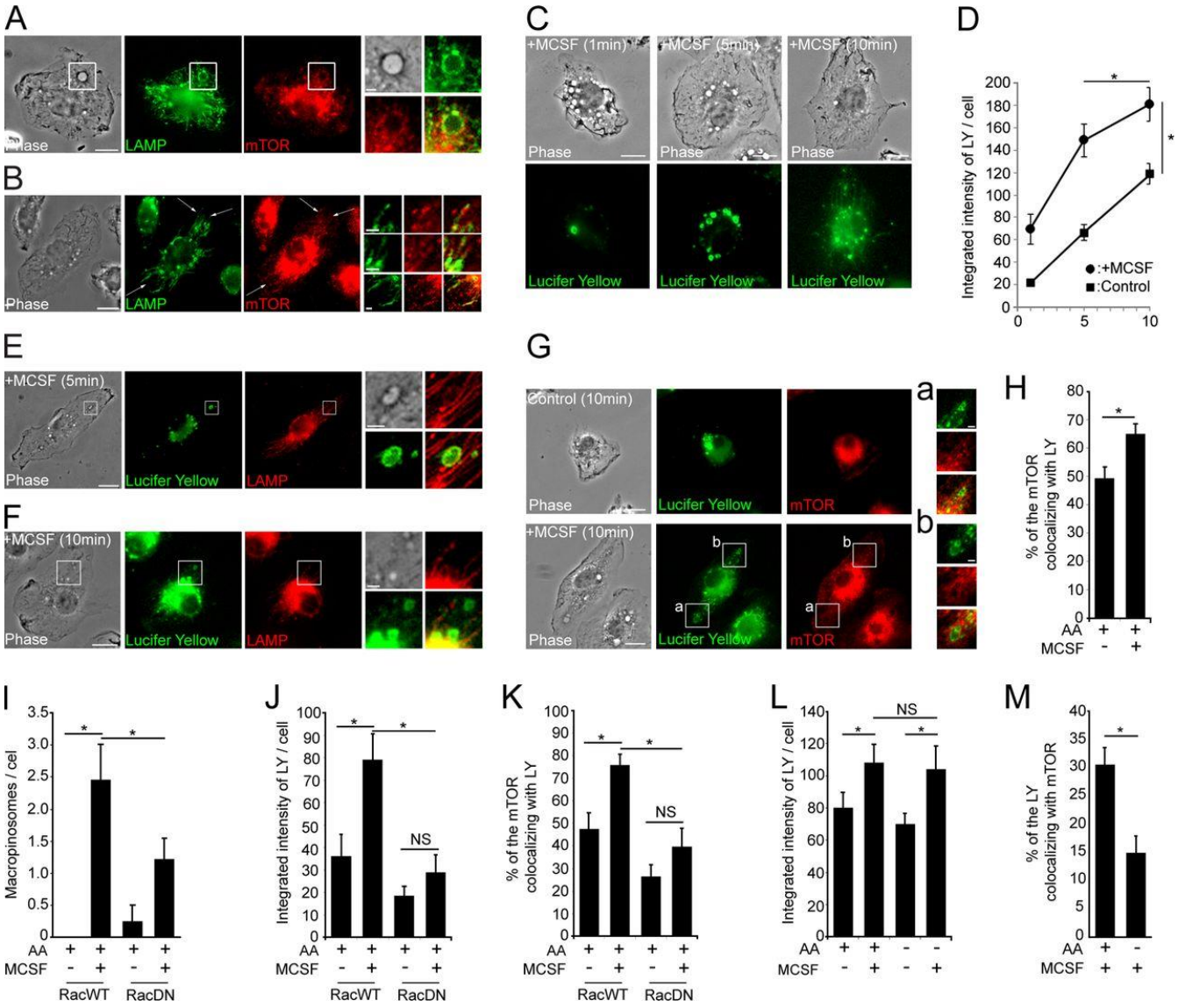
Stimulation of macropinocytosis in BMMs increased solute uptake and amino acid–dependent recruitment of mTOR to macropinosomes and endolysosomes.



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Stimulation of macropinocytosis in BMMs increased solute uptake and amino acid–dependent recruitment of mTOR to macropinosomes and endolysosomes. (A and B) Immunofluorescence localization of mTOR and LAMP-1 in BMMs stimulated 5 min with M-CSF. Insets show mTOR association with macropinosome-associated endolysosomes (A) and with tubular endolysosomes (B). (C) BMMs fixed and imaged after 1, 5, or 10 min with LY and M-CSF. LY was initially distributed in macropinosomes but also localized to tubular compartments by 10 min. (D) Total LY fluorescence per cell, from image analysis of preparations shown in C (n > 10 cells per point; *, $P < 0.05$). (E and F) Immunofluorescence localization of LAMP-1 after incubation of BMMs with LY and M-CSF for 5 min (E) or 10 min (F). Insets shows a macropinosome (E) and tubular endolysosomes (F) labeled with both LAMP-1 (top right) and LY (bottom left); top left: phase-contrast, bottom right: overlay. (G) Immunofluorescence localization of mTOR after 10-min incubation in LY with (bottom; insets a and b) or without (top) M-CSF. (H) Quantitation of mTOR colocalization with LY (*, $P < 0.05$). (I–K) Macropinocytosis and colocalization of mTOR and LY in BMMs expressing pRac1WT-IRES2-mCFP (RacWT) or pRac1(N17)-IRES2-mCFP (RacDN), fixed after stimulation for 5 min with FDx (I) or 10 min with LY (J and K) with or without M-CSF. CFP-positive BMMs were scored for macropinosome labeling with FDx (I), integrated intensity of LY per cell (J), and colocalization of mTOR with LY (K). RacDN significantly decreased macropinocytosis, LY accumulation, and mTOR colocalization with LY-positive organelles (*, $P < 0.05$). (L and M) Effects of amino acids on LY accumulation and colocalization of mTOR and LY. BMMs were incubated 10 min in DMEM (+AA) or HBSS (−AA) containing LY, with or without M-CSF. Amino acids did not affect the integrated cellular accumulation of LY (L), but increased the association of mTOR with LY-positive endocytic compartments (M). *, $P < 0.05$. Bars, 10 µm.