**Supplemental Information**

**Phenylbutyrate induces LL-37-dependent autophagy and intracellular killing of *Mycobacterium tuberculosis* in human macrophages**

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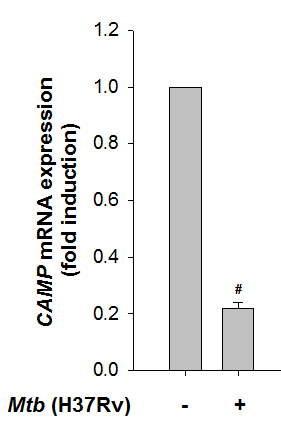
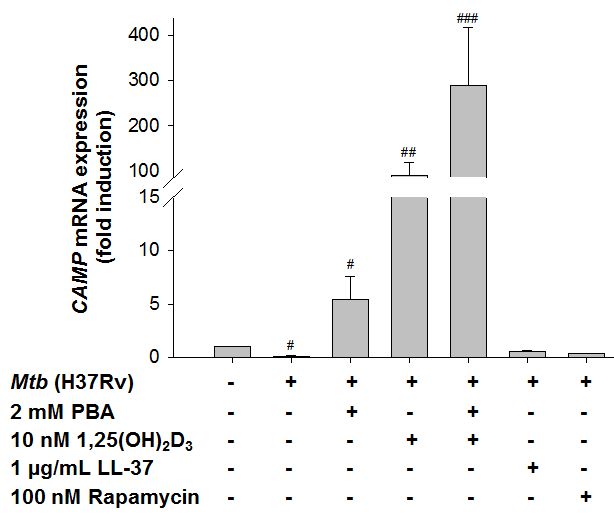
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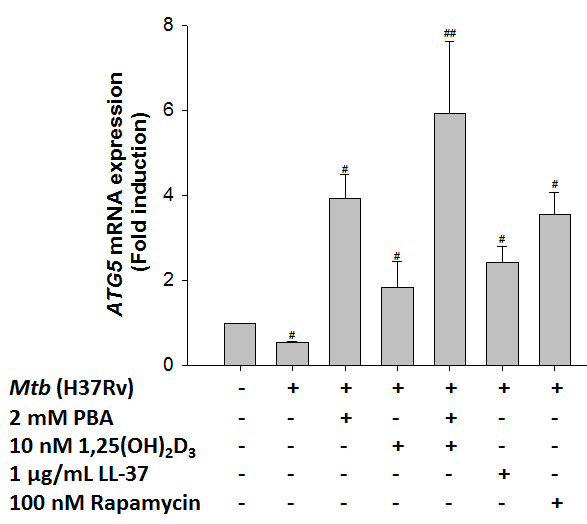
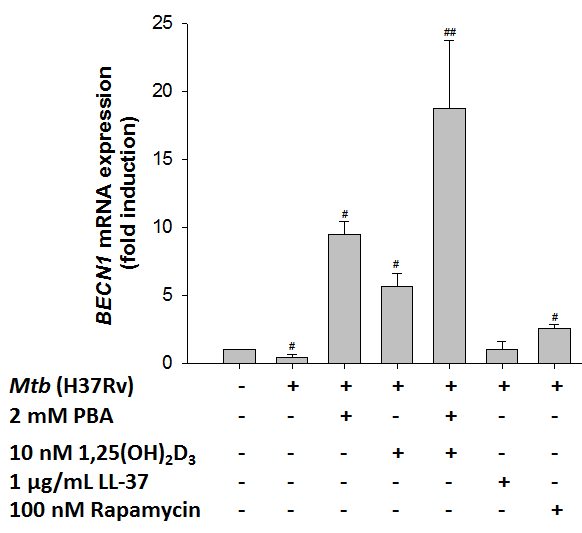
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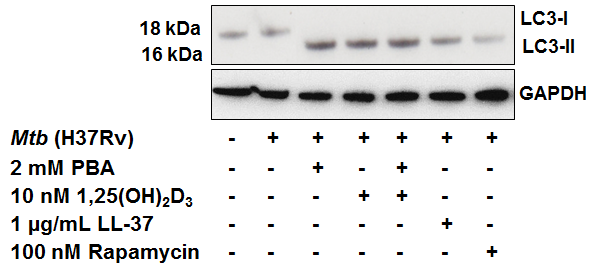
******A B**

**Figure S1.** Phenylbutyrate (PBA) and the active form of vitamin D3 (1,25(OH)2D3) induced *CAMP* mRNA expression in human monocytic cell line THP-1. (**A**) THP-1 cells were differentiated with PMA (10 ng/mL, for 36 to 48 h) and infected with the virulent strain of *Mycobacterium tuberculosis* H37Rv at a multiplicity of infection of 1:5 for 4 h, and quantitative real-time PCR for the expression of the *CAMP* transcript was determined (normalized to *RNA18S/18S* rRNA expression). (**B**) After *Mtb* infection cells were treated with PBA (2 mM), 1,25(OH)2D3 (10 nM), LL-37 (1 µg/mL) and rapamycin (100 nM) for 24 h; real-time qPCR was performed for transcript of *CAMP* (normalized to *RNA18S/18S* rRNA expression) in 3 independent experiments (mean ± SD). The *P* values in A and B were ≤0.001, ≤0.01, ≤0.05 as indicated by ###, ## and #, respectively.

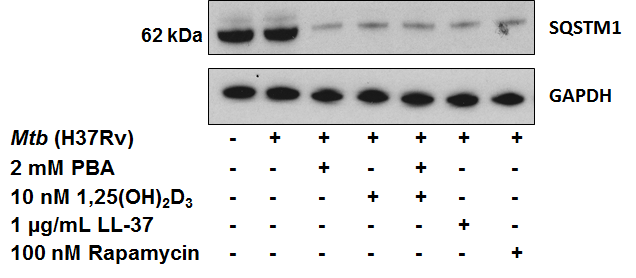
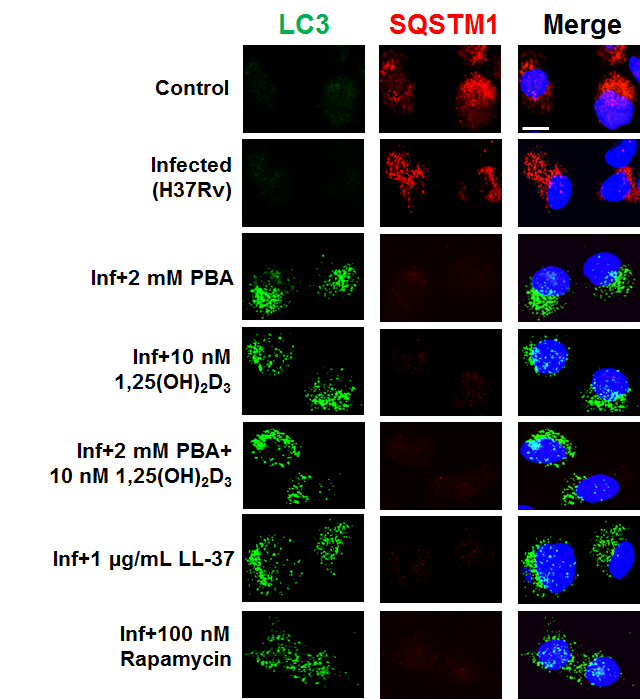
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**Figure S2.** Phenylbutyrate (PBA), vitamin D3 and LL-37 control intracellular survival of *Mtb* in human monocyte derived macrophages (MDMs). Human MDMs were infected with the virulent strain of *Mycobacterium tuberculosis* H37Rv at a multiplicity of infection of 1:5 for 4 h, after that cells were treated with PBA (2 mM) and/or 1,25(OH)2D3 (10 nM) or LL-37 (1 µg/mL) or rapamycin (100 nM) for 3 days. Intracellular bacterial viability was determined based on the number of colony forming units (CFUs). For the infected control at day 0, intracellular bacteria were harvested after 4 h infection and for the other conditions that were mentioned bacteria were harvested after 3 day and cells were lysed with 0.036% SDS. The lysates were then cultured on Middle Brook 7H11 agar media (Becton, Dickinson and company) and after 21 to 28 days of culture at 37ºC bacterial viability was calculated by the CFU count. Results are shown from 5 independent experiments (mean ± SD). Infection at day 3 was used as a control when comparing with treated cells. The *P* values were ≤0.001, ≤0.01 as indicated by \*\*\* and \*\*, respectively.

**A B**

**C**

**Figure S3.** Phenylbutyrate (PBA) induced autophagy in THP-1 cells by upregulation of expression of the autophagy related genes *BECN1* and *ATG5*, and conversion of LC3-I to the autophagosome-bound form LC3-II. THP-1 cells were infected with the virulent strain of *Mycobacterium tuberculosis* H37Rv for 4 h and treated with PBA (2 mM), 1,25(OH)2D3 (10 nM), LL-37 (1 µg/mL) and rapamycin (100 nM) for 24 h. (**A and B**) The expression of the autophagy-related genes (**A**) *BECN1* and (**B**) *ATG5* mRNA (normalized to *RNA18S/18S* rRNA expression) was measured by real time PCR. Results are shown from 3 independent experiments (mean ± SD). (**C**) A representative western blot shows the conversion of LC3-I to LC3-II and the housekeeping protein GAPDH (glyceraldehyde 3-phosphate dehydrogenase) from 3 independent experiments. The *P* values were ≤0.01 and ≤0.05 as indicated by ##and #, respectively.

**A B**

**Figure S4.** Phenylbutyrate (PBA) induced the expression of the autophagy protein LC3 and degradation of the SQSTM1 protein. (**A**) Human MDMs were infected with the virulent strain of *Mycobacterium tuberculosis* H37Rv for 4 h and treated with PBA (2 mM), 1,25(OH)2D3 (10 nM), LL-37 (1 µg/mL) and rapamycin (100 nM) for 24 h. The expression of LC3 and SQSTM1 protein was investigated by confocal microscopy. Cells were stained with anti-LC3 and anti-SQSTM1, followed by the addition of Alexa Fluor 488/594-conjugated goat anti-mouse/rabbit IgG (green and red color, respectively). Cells were also stained with DAPI to visualize the nuclei (blue). One representative image out of 6 independent experiments is shown; scale bars: 10 μm; (**B**) A representative western blot from 6 independent experiments shows the presence the SQSTM1 protein and GAPDH in cell lysate