Supplemental material

Lysosomotropic beta blockers induce oxidative stress and IL23A production in Langerhans cells

Gerrit Müller[a], Charlotte Lübow[a,b], and Günther Weindl[a,b]

[a]Freie Universität Berlin, Institute of Pharmacy (Pharmacology and Toxicology), Berlin, Germany; [b]University of Bonn, Pharmaceutical Institute, Section Pharmacology and Toxicology, Bonn, Germany

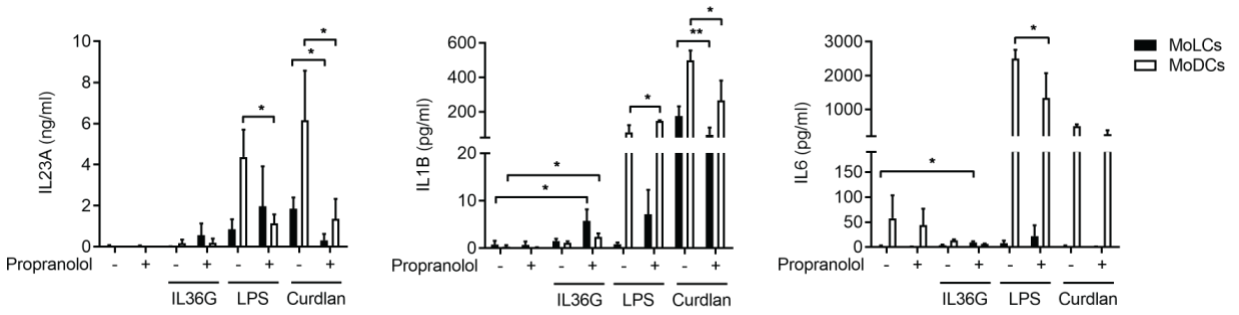

Figure S1. Propranolol differentially modulates production of psoriasis-like inflammation associated cytokines by IL36G-, LPS- and curdlan-stimulated MoLCs and MoDCs. Cells were activated for 24 h with rh-IL36G (100 ng/ml), LPS (1 µg/ml) or curdlan (20 µg/ml), respectively, with or without propranolol (75 µM). IL23A, IL1B and IL6 levels were analyzed by ELISA. **P* < 0.05, ***P* < 0.01, one-way ANOVA test followed by Bonferroni post-test. Data are representative of 3 independent experiments and display mean values + SEM.

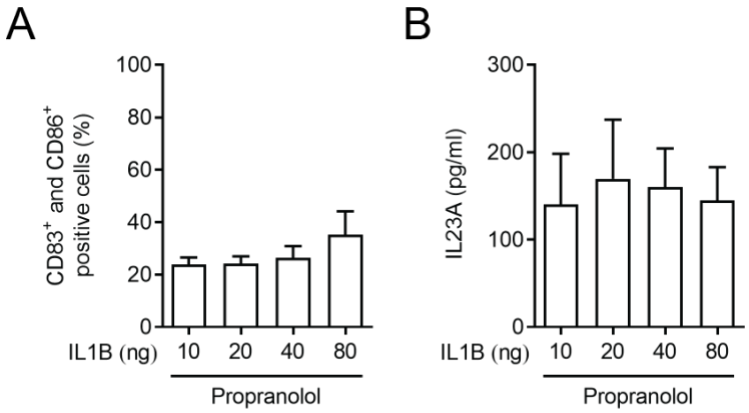

Figure S2. Increasing IL1B concentrations maintain propranolol-induced IL23A response in MoDCs. (**A**) Expression of CD83 and CD86 in MoDCs and (**B**) IL23A release after activation with different IL1B concentrations (10 - 80 ng/ml) in the presence of propranolol (75 µM) for 24 h. Data represent mean values + SEM (n=3).

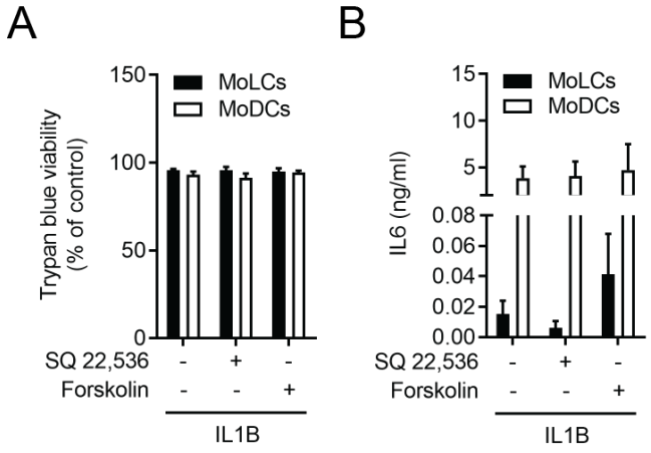

Figure S3. IL6 production by MoLCs is regulated by cAMP modulators. (**A**) Cell viability of MoLCs and MoDCs was evaluated using trypan blue exclusion assay after 24 h following stimulation with SQ 22,536 (100 µM) or forskolin (20 µM) in the presence of IL1B (20 ng/ml). Data represent mean values + SEM (n=3-6). (**B**) IL6 release into supernatants from IL1B-activated MoLCs and MoDCs was determined after 24 h of stimulation with SQ 22,536 (100 µM) or forskolin (20 µM). Data represent mean values + SEM (n=4).

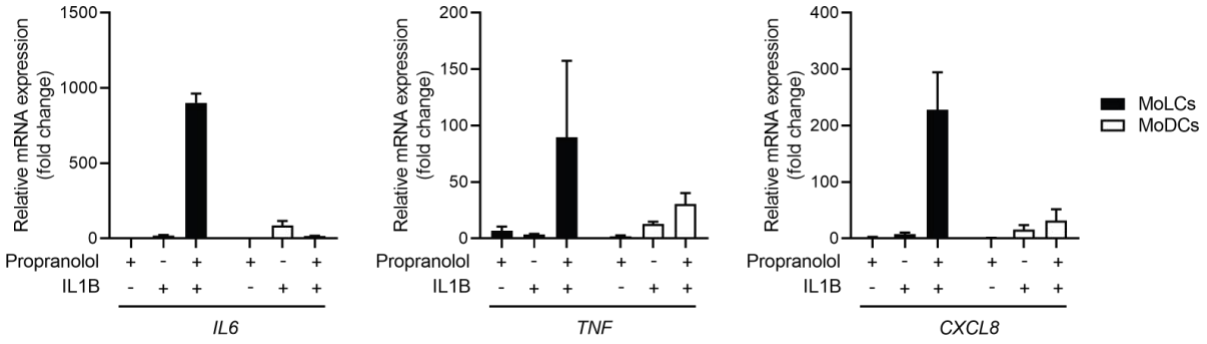

Figure S4. Propranolol increases gene expression of psoriasis-like inflammation associated mediators under sterile-inflammatory conditions. Quantification of *IL6*, *TNF* and *CXCL8* mRNA expression in immature and IL1B-activated (20 ng/ml) DC subsets stimulated for 3 h (TNF) or 24 h (IL6, CXCL8) with or without propranolol (75 µM). Gene transcripts were normalized to *GAPDH* and presented relative to unstimulated controls (set as 1.0). Data represent mean values + SEM (n=3).

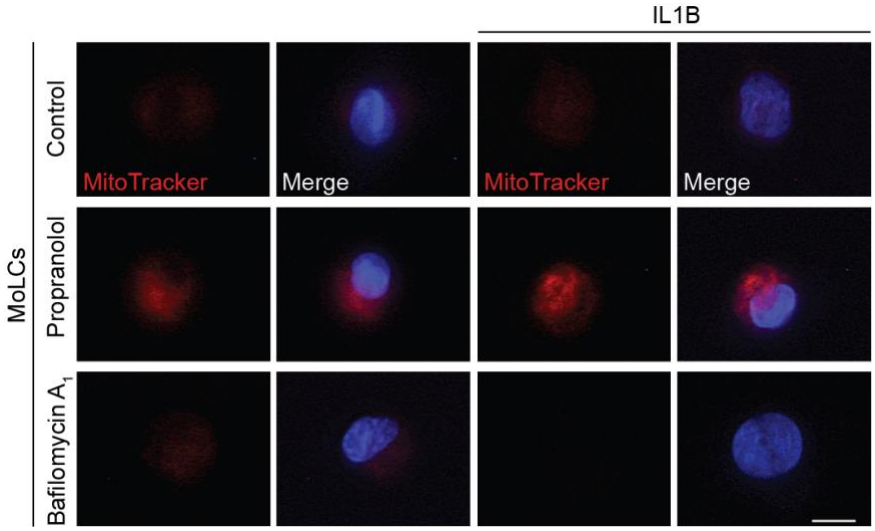

Figure S5. Propranolol increases ROS-producing mitochondria in MoLCs. MoLCs were pre-incubated with MitoSOX for 10 min and subsequently cultivated for 24 h in the presence or absence of IL1B, propranolol (75 µM) or bafilomycin A_1 (1 µM). Detection of mitochondrial-generated ROS was examined by fluorescence microscopy. Data are representative of 4 independent experiments. Scale bar: 10 µm.

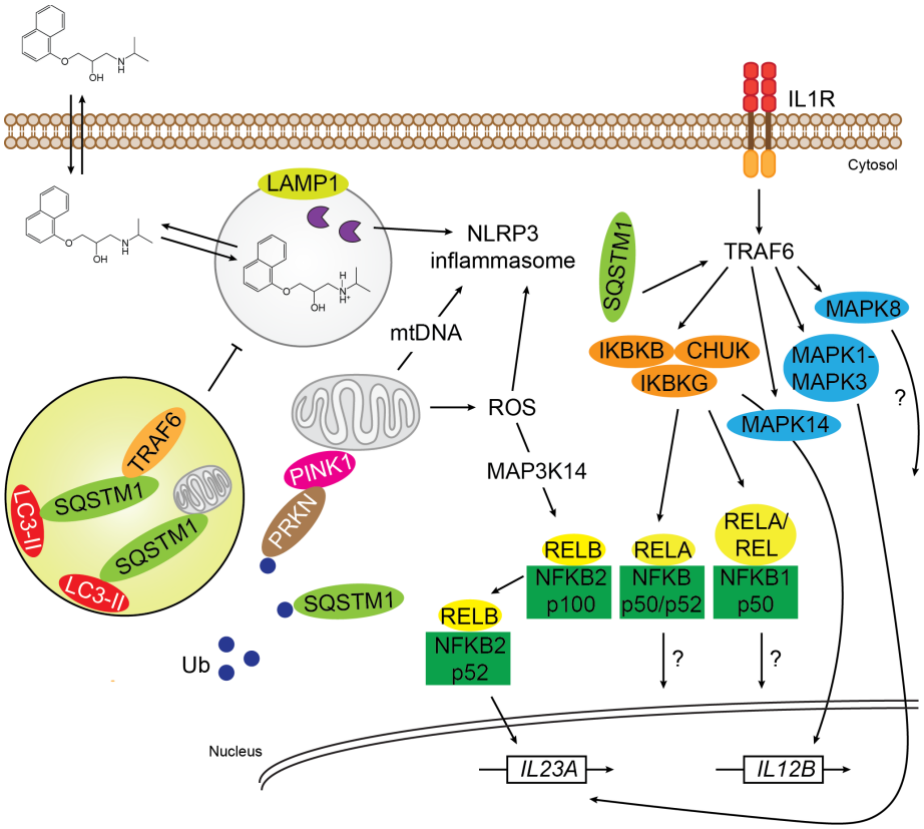

Figure S6. Proposed immunoregulatory mechanism in cutaneous dendritic cells induced by lysosomotropic beta-blockers and possibly other lysosomotropic drugs.