

SUPPLEMENTARY MATERIALS

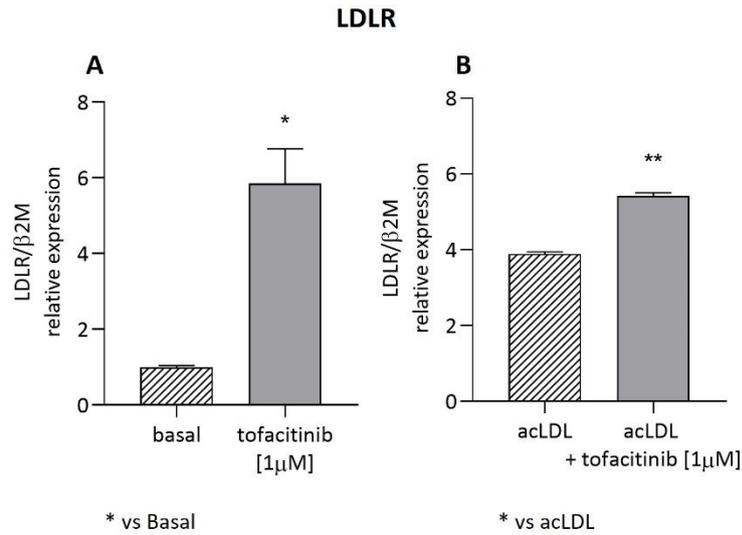
Article

Effect of the JAK/STAT inhibitor tofacitinib on macrophage cholesterol metabolism

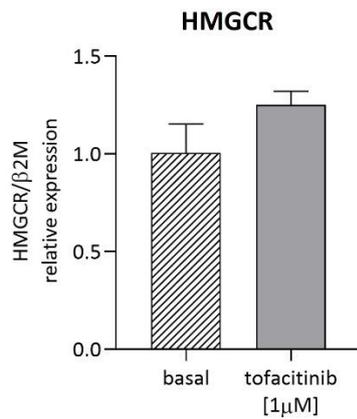
Maria Pia Adorni¹, Bianca Papotti², Maria Orietta Borghi³, Elena Raschi³, Francesca Zimetti², Franco Bernini², Pier Luigi Meroni³, Nicoletta Ronda^{2,*}

SUPPLEMENTARY METHODS

Gene expression analyses. Total RNA was extracted from cell monolayers through the TRIzol™ Reagent (ThermoFisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. RNA concentration and purity was determined using a NanoDrop™ 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA), observing a mean concentration of 229 ng/μl and a purity of 1.8 (absorbance ratio at 260 and 280 nm). cDNAs were then retro-transcribed starting from 1μg RNA for each sample using the qScript™ cDNA superMix commercial kit (Quanta Biosciences, Beverly, MA, USA). Gene expression analyses were measured with TaqMan® assays (Applied Biosystems, Waltham, MA, United States), as follows: *ABCA1* (Hs01059101_m1), *ABCG1* (Hs00245154_m1), *LDLR* (Hs0192524_m1), and *HMGCR* (Hs00168352_m1) on a QuantStudio™ 3 Real-Time PCR instrument (ThermoFisher Scientific, Waltham, MA, USA). The $\Delta\Delta C_t$ method was applied to determine the mRNA levels in each reaction, using the $\beta 2$ -Microglobulin (Hs99999907_m1) as the normalizer internal gene, and expressed as relative quantification (RQ) to a the basal condition.



Supplementary Figure S2. Effect of tofacitinib on *LDLR* gene expression. Cells in basal conditions (A) or incubated with acetylated LDL (acLDL, 25 μ g/ml) for 24 h (B) were treated with tofacitinib [1 μ M] for 18h. At the end of the incubation total RNA was extracted and real time qPCR was performed. Experiments were performed in duplicate and data expressed as mean \pm SD. Statistical analyses were performed using the unpaired t-test * $p < 0.05$; ** $p < 0.01$



Supplementary Figure S3. Effect of tofacitinib on *HMGCR* gene expression. Cells were incubated with tofacitinib [1 μ M] for 18h. At the end of the incubation total RNA was extracted and real time qPCR was performed. Experiments were performed in duplicate and data expressed as mean \pm SD. Statistical analyses were performed using the unpaired t-test.