

## SUPPLEMENTARY MATERIALS

Article

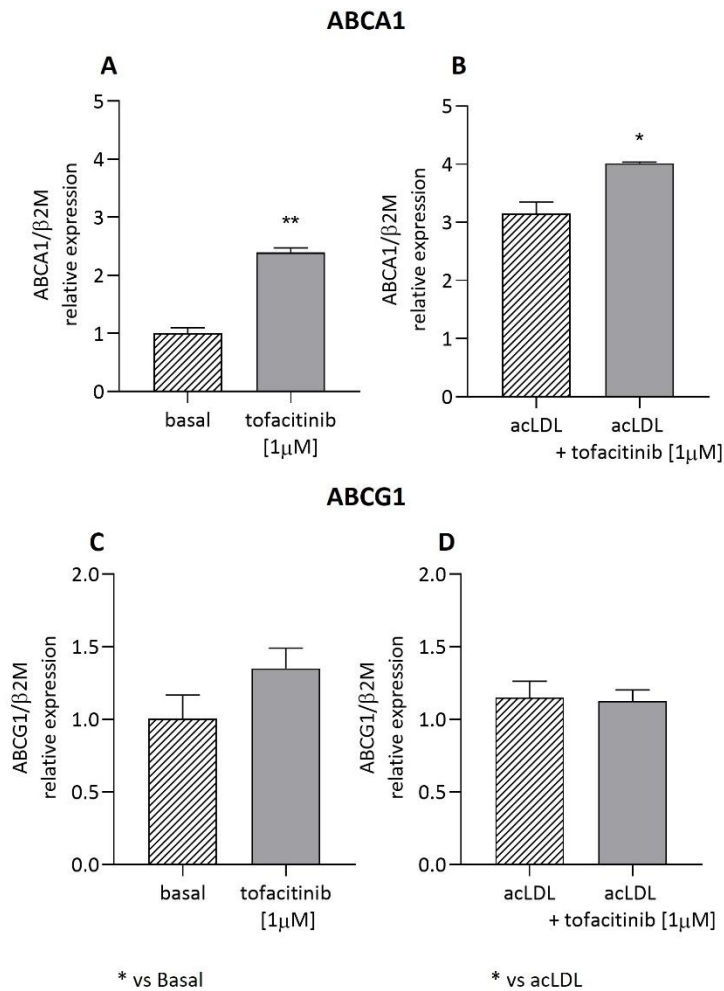
# Effect of the JAK/STAT inhibitor tofacitinib on macrophage cholesterol metabolism

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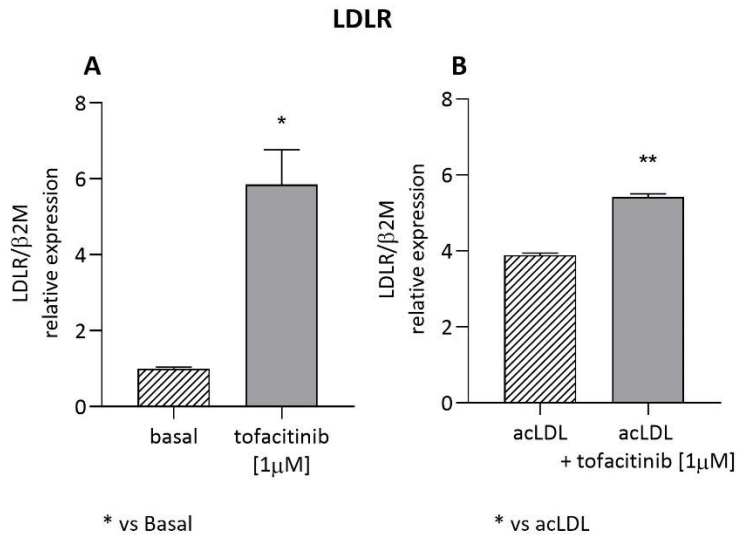
## SUPPLEMENTARY METHODS

**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.

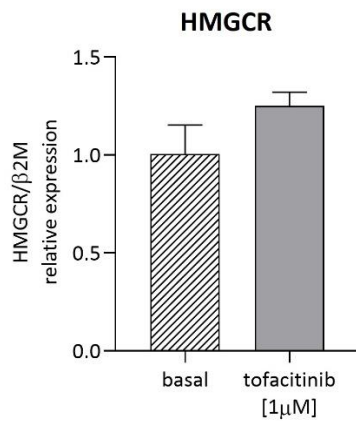
## SUPPLEMENTARY FIGURES



**Supplementary Figure S1.** Effect of tofacitinib on *ABCA1* and *ABCG1* gene expression. Cells in basal conditions (A and C) or previously incubated with acetylated LDL (acLDL, 25  $\mu$ g/ml) for 24 h (B and D) were treated with tofacitinib [1  $\mu$ 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 S2.** Effect of tofacitinib on *LDLR* gene expression. Cells in basal conditions (A) or incubated with acetylated LDL (acLDL, 25  $\mu$ g/ml) for 24 h (B) were treated with tofacitinib [1  $\mu$ 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  $\mu$ 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.