

## TITLE

# Myeloid GRK2 regulates obesity-induced endothelial dysfunction by modulating inflammatory responses in perivascular adipose tissue.

## Authors

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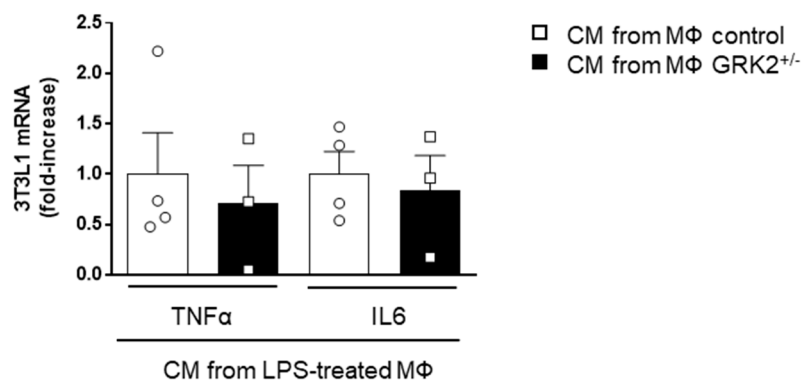
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## Supplementary material

### Supplementary Figure 1.



**Legend to Supplementary Fig. 1.** mRNA expression of TNFα and IL6 in 3T3L1 adipocytes stimulated for 24h with the conditioned medium (CM) obtained from macrophages (MΦ) isolated from either control or LysM-GRK2<sup>+/-</sup> mice; these macrophages were previously treated with 1μg/mL LPS for 6 hours. Graphs represent fold change over control (3T3L1 adipocytes stimulated with the CM of LPS-treated control macrophages). Data are expressed as mean ± SEM. Statistical significance was analyzed by two-way ANOVA followed by Bonferroni posthoc test (n=3-4).

## Supplementary Methods

3T3L1 preadipocytes were cultured in DMEM supplemented with 10% FBS and differentiated as previously described (Vila-Bedmar et al., *FASEB J* 2012 26(8):3503). At day 10 post-differentiation, adipocytes were serum starved (1% FBS) overnight and further incubated with the conditioned medium (CM) of thioglycollate elicited peritoneal macrophages obtained from control or LysM-GRK2<sup>+/-</sup> mice, treated or not with 1 µg/mL LPS for 6 hours. 24h later, 3T3L1 adipocytes were lysed with QIAzol reagent (Qiagen) to isolate RNA. Quantity and quality of RNA were analyzed using Nanodrop ND-1000 (Thermo Scientific) and Bioanalyzer 2100 (Agilent). RT-PCRs were performed with the aid of the Genomic Facility of the Centro de Biología Molecular Severo Ochoa using Light Cycler equipment (Roche) with Sybr Green technology and self-designed murine probes purchased from Sigma-Aldrich (Supplementary Table 1). *Ppia* was used as reference gene following the recommendations of GenNorm and NormFinder algorithms.

**Supplementary Table 1.** Primers used for real time PCR analysis in 3T3L1 adipocytes:

GENE NAME	NCBI seq	Forward sequence	Reverse sequence
Mice			
<i>Tnfa</i>	NM_013693	5' TCTTCTCATTCTGCTTGTGG	5' GGTCTGGGCCATAGAACTGA
<i>Il6</i>	NM_031168	5'TGATGGATGCTACCAAAGTGG	5'TTCATGTACTCCAGGTAGCTATGG
<i>Ppia</i>	NM_008907	5'ACGCCACTGTCGCTTTTC	5'GCAAACAGCTCGAAGGAGAC

## References

GRK2 contribution to the regulation of energy expenditure and brown fat function.  
Vila-Bedmar R, Garcia-Guerra L, Nieto-Vazquez I, Mayor F Jr, Lorenzo M, Murga C, Fernández-Veledo S. *FASEB J*. 2012 Aug;26(8):3503-14. doi: 10.1096/fj.11-202267.