

1 **SUPPLEMENT**

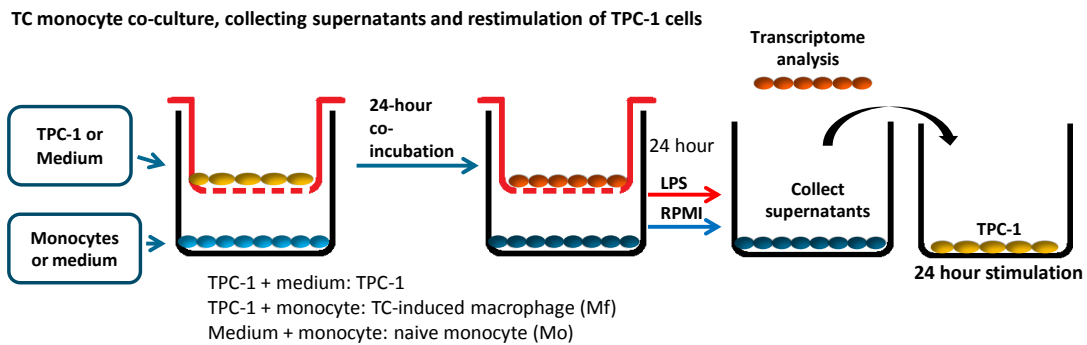
2

3 **Interplay between thyroid cancer cells and macrophages: effects on IL-32 mediated cell**
4 **death and thyroid cancer cell migration.**

5

6 Yvette J.E. Sloot, MD, Katrin Rabold, MSc, Thomas Ulas PhD, Dennis M. De Graaf MSc, Bas
7 Heinhuis, PhD, Kristian Händler, PhD, Joachim L. Schultze, MD, PhD, Mihai G. Netea, MD,
8 PhD., Johannes W.A. Smit, MD PhD, Leo A.B. Joosten, PhD, Romana T. Netea-Maier, MD,
9 PhD.

10 **Supplementary Figures**



11

12 **Supplementary Figure S1 Experimental Set-up.** The upper well contains TPC-1 cells or Medium,

13 while the bottom well contains monocytes or medium. TC cells and monocytes are co-cultured for 24

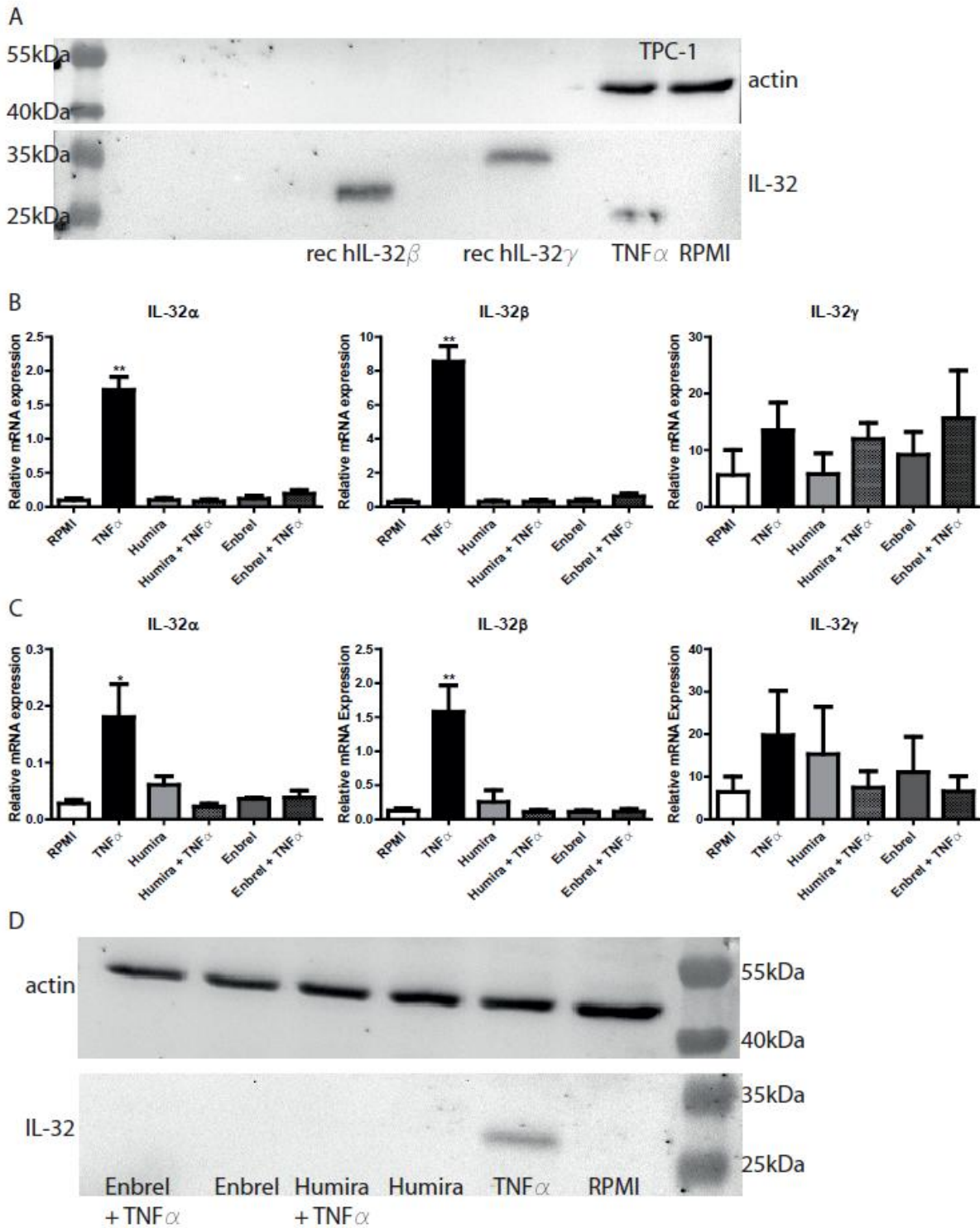
14 hours, creating naive TPC-1 cells, TC-induced macrophages (Mf) and Naive monocytes (mo). Next, LPS

15 (TLR-4 ligand) or RPMI (medium control) were added to the bottom well for 24 hours. TPC-1 cells are

16 collected for transcriptome analysis, supernatants are collected for conditioned medium

17 experiments.

18



19 **Supplementary Figure S2 TNF α -mediated IL-32 mRNA and protein expression in different**
 20 **TC cell lines.** A) Western blot analysis demonstrating IL-32 β protein expression in TPC-1 cells;
 21 Human recombinant IL-32 β and IL-32 γ was loaded on the blot as control. B) Relative mRNA
 22 expression of IL-32 α , IL-32 β and IL-32 γ isoforms in FTC-133 cells after stimulation with TNF α
 23 and in the presence of Enbrel (etanercept, decoy TNF α -receptor, 10 μ g/ml) or Humira

24 (adalimumab, monoclonal TNF α antibody, 10 ug/ml). C) Relative mRNA expression of IL-32 α ,
25 IL-32 β and IL-32 γ isoforms in BC-PAP cells after stimulation with TNF α and in the presence of
26 Enbrel or Humira. Results from 3 experiments, 1-3 replicates per experiment. E) Western
27 blot analysis showing IL-32 protein expression in FTC-133 after stimulation with TNF α and in
28 the presence of Enbrel or Humira. Data are represented as mean \pm SEM; * $p < 0.05$, ** $p < 0.01$,
29 by Mann-Whitney-U test or Kruskal-Wallis test with Dunn's multiple comparison test.
30

31 **Supplemental methods**

32

33 **Western blot using human recombinant IL-32 β and IL-32 γ**

34 human recombinant IL-32 β and IL-32 γ (R&D systems, Minneapolis, MN, USA) was diluted to
35 a concentration of 2,5 ng/ml. A total of 100 pg/lane was loaded on a pre-casted 4-15% gel
36 (Bio-Rad, CA, USA) for polyacrylamide gel electrophoresis. Further procedure described in
37 manuscript.

38 **Supplementary table S1** Primer sequences

Target	Forward 5'> 3'	Reverse 5'> 3'
IL-32 α	GCTGGAGGACGACTTCAAAGA	GGGCTCCGTAGGACTTGTCA
MMP2	TACAGGATCATTGGCTACACACC	GGTCACATCGCTCCAGACT
MMP3	CTGGACTCCGACACTCTGGA	CAGGAAAGGTTCTGAAGTGACC
MMP9	TGTACCGCTATGGTTACACTCG	GGCAGGGACAGTTGCTTCT
IL-8	ACTGAGAGTGATTGAGAGTGGAC	AACCCTCTGCACCCAGTTTTTC
E-cadherin	AAAGGCCCATTTCTAAAAACCT	TGCGTTCTCTATCCAGAGGCT
VEGF	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
B2M	ATGAGTATGCCTGCCGTGTG	CCAAATGCGGCATCTTCAAAC
GAPDH	AGGGAGATTCAGTGTGGTG	CGACCACTTTGTCAAGCTCA

39