

## Supplementary material

# Pharmacological targets of kaempferol within inflammatory pathways – a hint towards the central role of tryptophan metabolism

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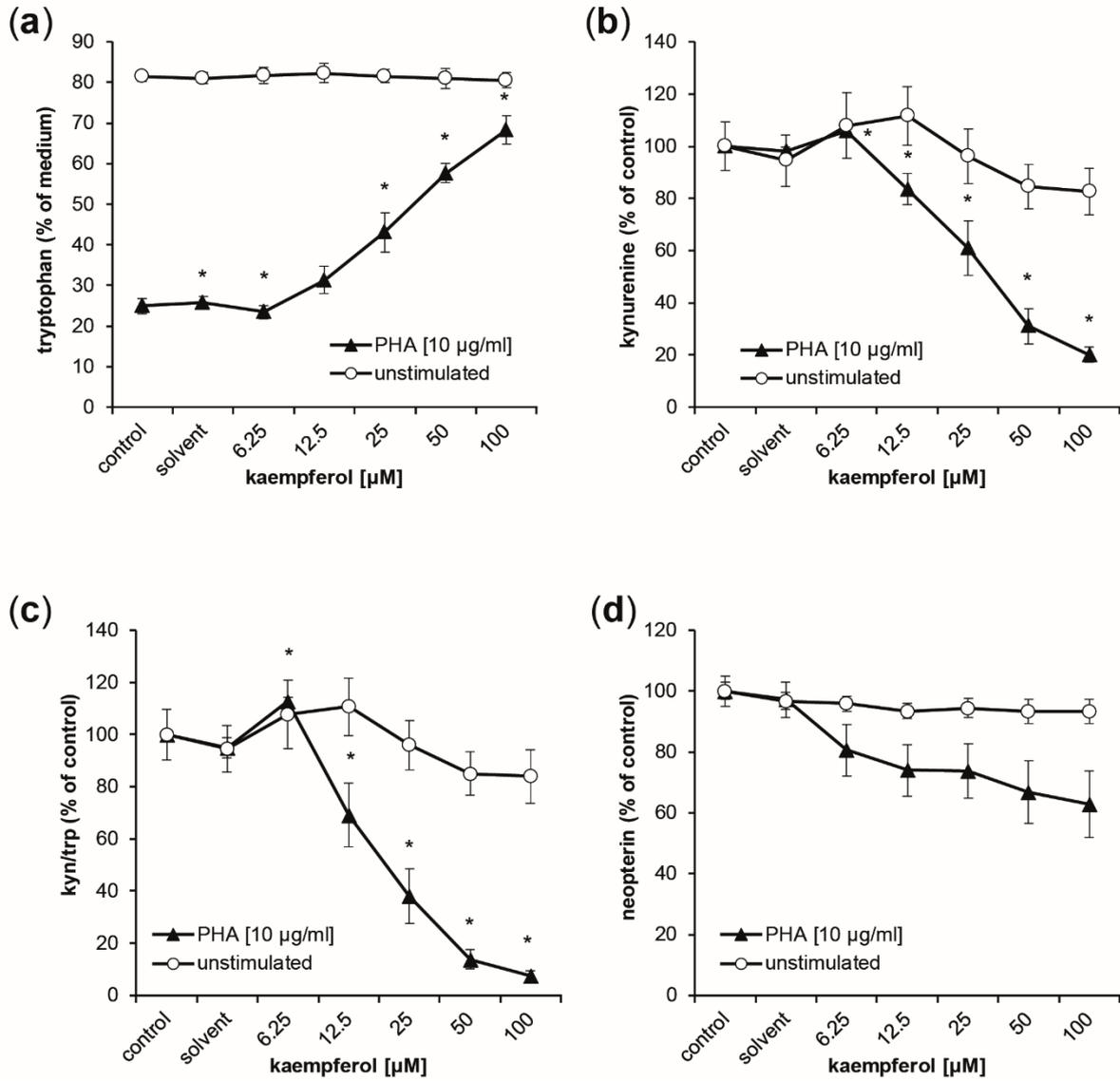
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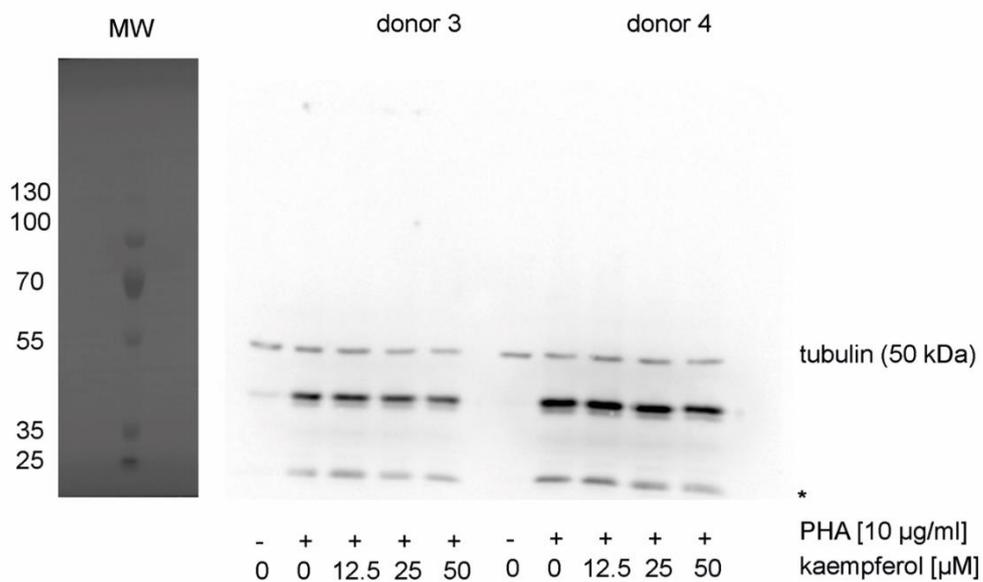
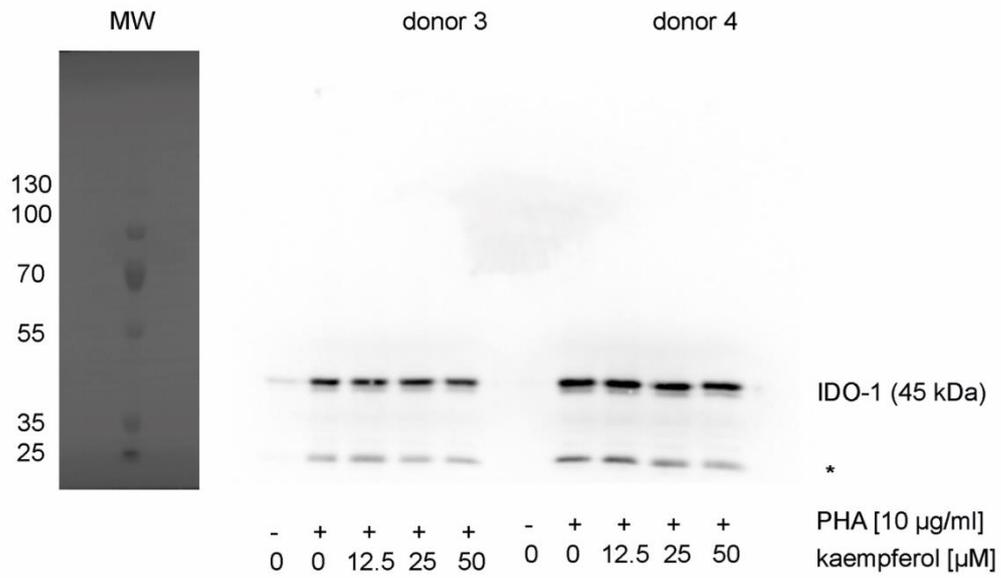
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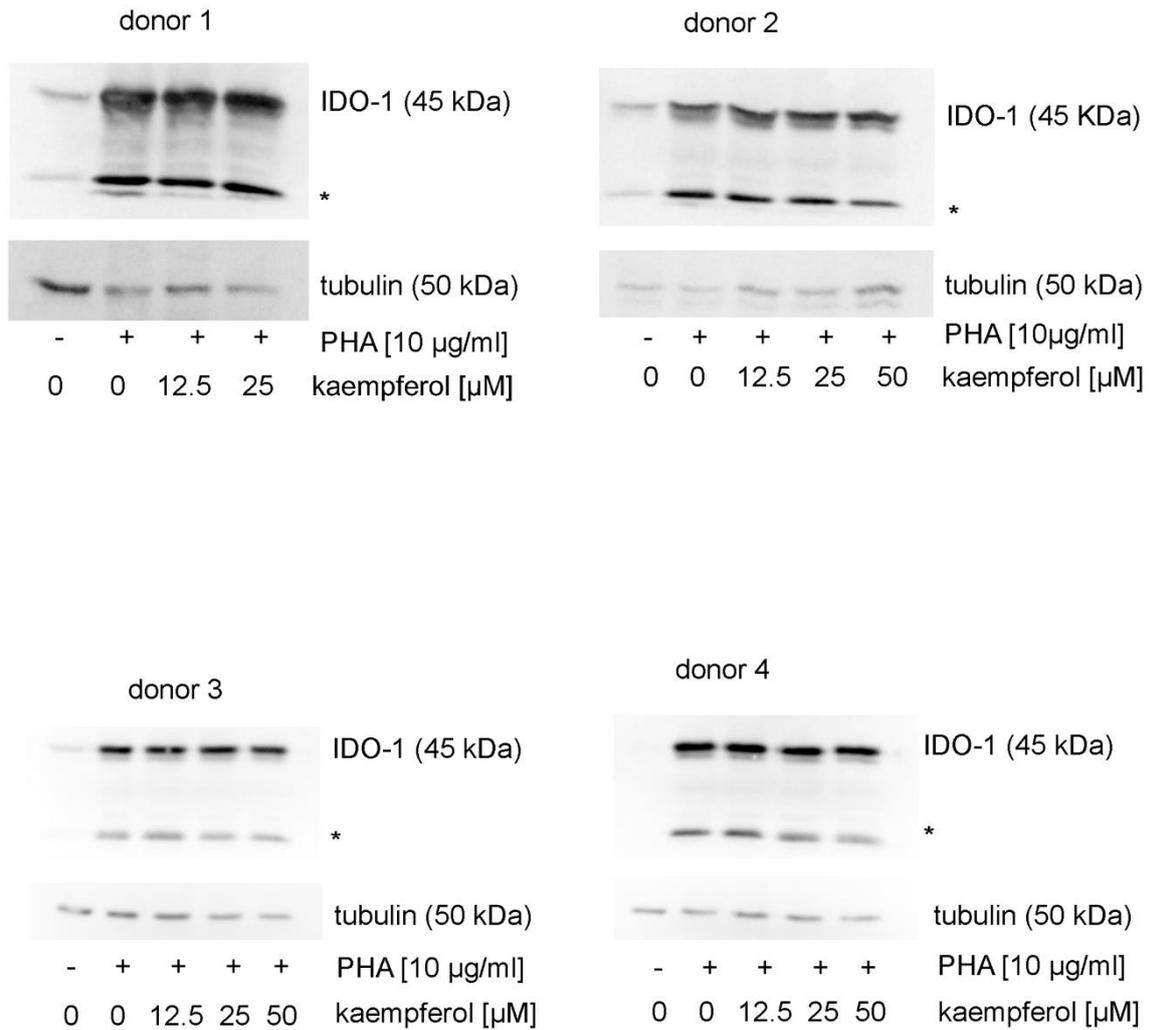
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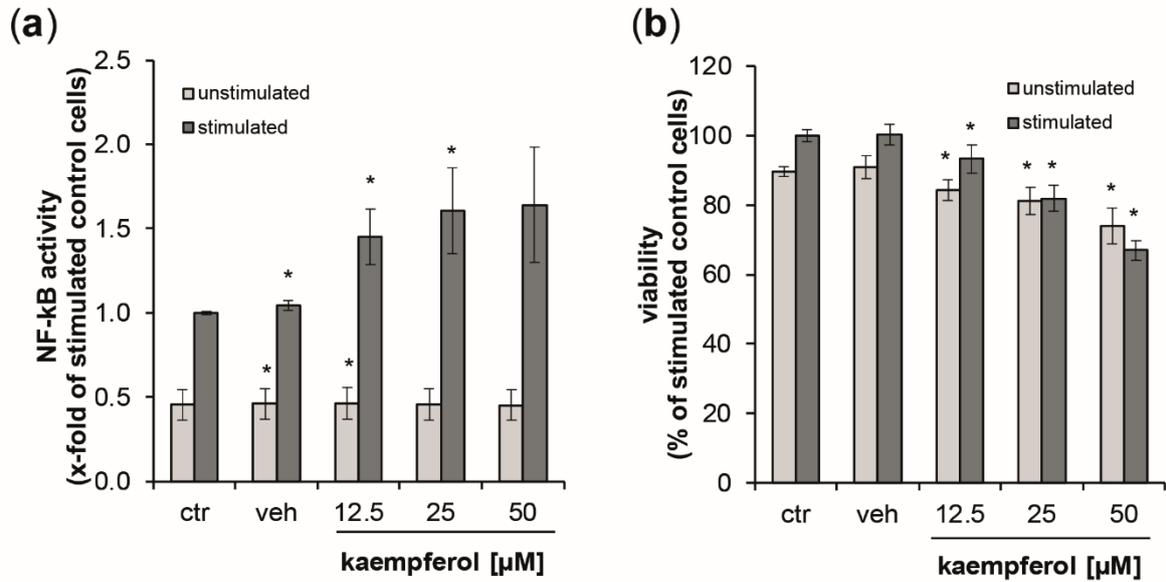
**Figure S1:** Kaempferol was added to unstimulated (white circles) or PHA-stimulated (black triangles) human PBMC. Its effect on tryptophan (a) and kynurenine (b) concentrations, the kyn/trp ratio (c) and neopterin levels (d) was determined in the cell supernatants after 48 h of incubation. Kynurenine and neopterin concentrations are expressed as % of baseline (control cells treated with or without PHA). Tryptophan concentrations are expressed as % of medium control. (Mean  $\pm$  S.E.M,  $N = 3$ ,  $*p < 0.05$ , compared to baseline).



**Figure S2:** Western blots (full-size) of unstimulated, stimulated and kaempferol treated PBMCs incubated with antibodies against indoleamine dioxygenase 1 (IDO-1) protein (upper) and tubulin (lower blot). A representative of PBMC from two different donors is shown. An additional signal of yet unknown origin was observed at 25 kDa (indicated with an asterisk).



**Figure S3:** PBMC were isolated in four independent experiments from different healthy donors to analyse indoleamine dioxygenase 1 (IDO-1) expression after treatment of the cells with PHA and kaempferol. An additional signal of yet unknown origin was observed at 25 kDa (indicated with an asterisk).



**Figure S4:** (a) NF- $\kappa$ B/AP-1 activation was estimated in unstimulated (light grey) and stimulated (100 ng/mL LPS, dark grey) THP1-Blue-CD14 reporter cells. Cells were left either untreated, or were incubated with increasing concentrations of kaempferol, or the solvent control (veh) for 24 h. (b) Effect of kaempferol on THP1-Blue-CD14 viability at 24 h post-treatment. Cell viability is shown in comparison to the stimulated buffer control (ctr, set to 100%). Shown are mean values of three independent experiments performed in triplicates (mean  $\pm$  S.E.M). \* $p$ -values < 0.05 indicate significant changes compared to the respective unstimulated or LPS-stimulated solvent control.