

TREM2 R47H variant and knockout induce lysosomal deficits and exacerbated inflammatory responses in iPSC-derived microglia

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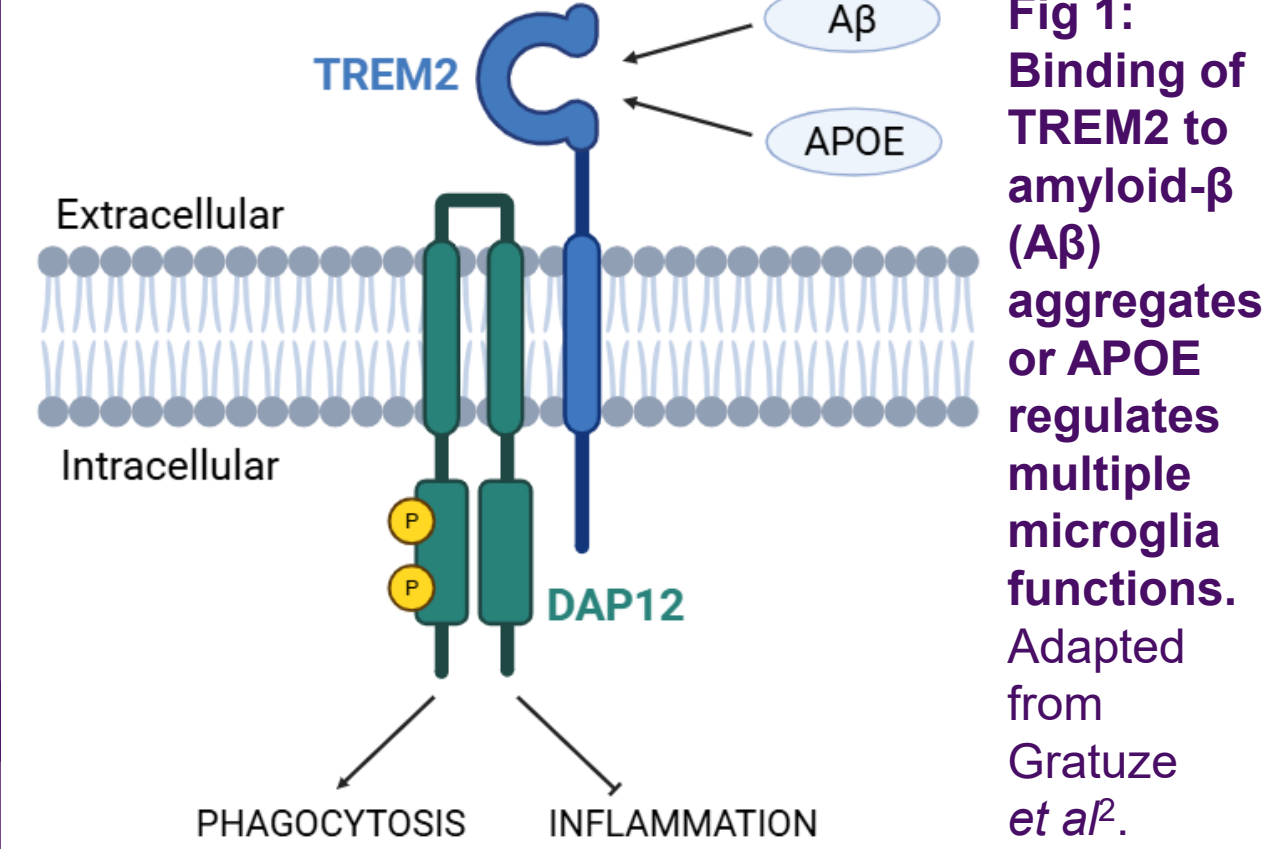
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KEY TAKE AWAY: *R47H* and *TREM2* KO iPSC-derived microglia (iMG) showed greater amounts of internalised A β and decreased expression of lysosomal genes. *R47H* and the *TREM2* KO also exacerbated the release of interleukin-6 and the upregulation of inflammatory genes in iMG exposed to aggregated A β .

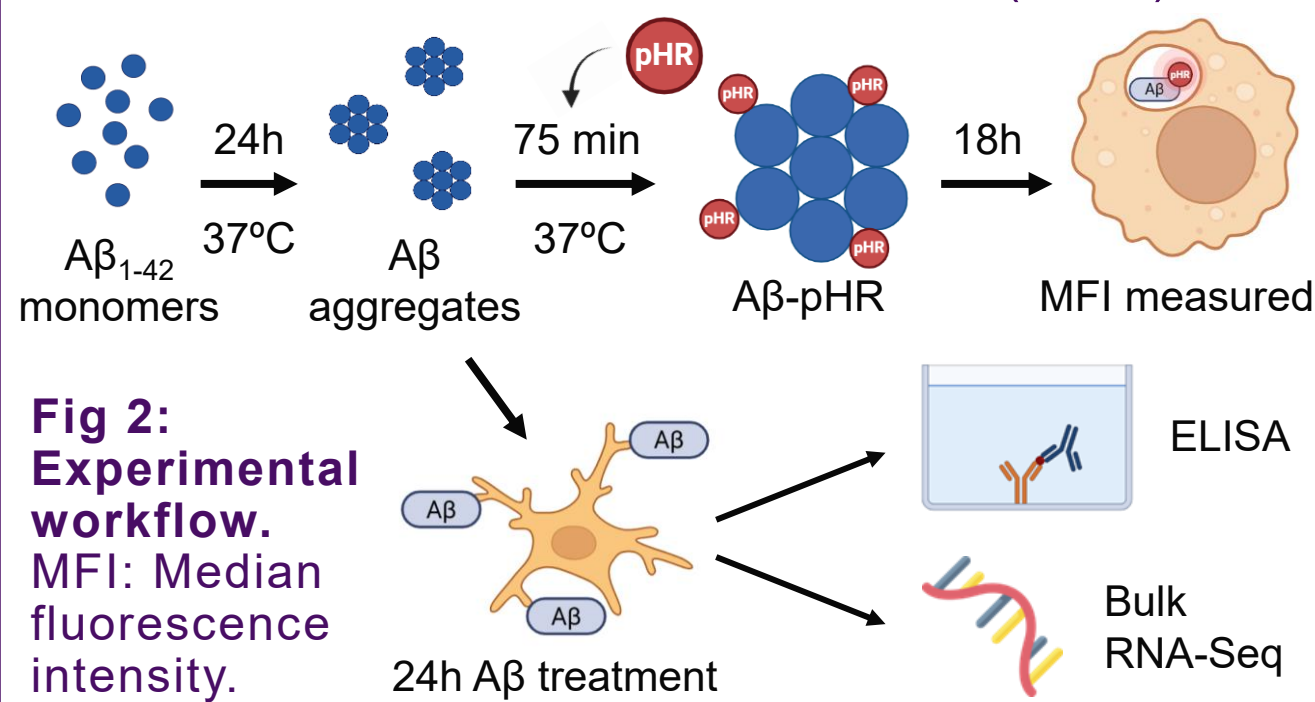
1. INTRODUCTION

- The *TREM2* variants *R62H* and *R47H* are associated with increased Alzheimer's disease (AD) risk (OR = 1.77 and 3.88)¹
- In the brain, *TREM2* is mainly expressed by microglia²



2. METHODS

- Human isogenic iPSCs carrying 2 copies of *R62H*, *R47H*, the *TREM2* common variant (CV) or a *TREM2* KO were differentiated into iMG as described in Washer *et al.* (2022)³



3. iMG QUALITY CONTROL

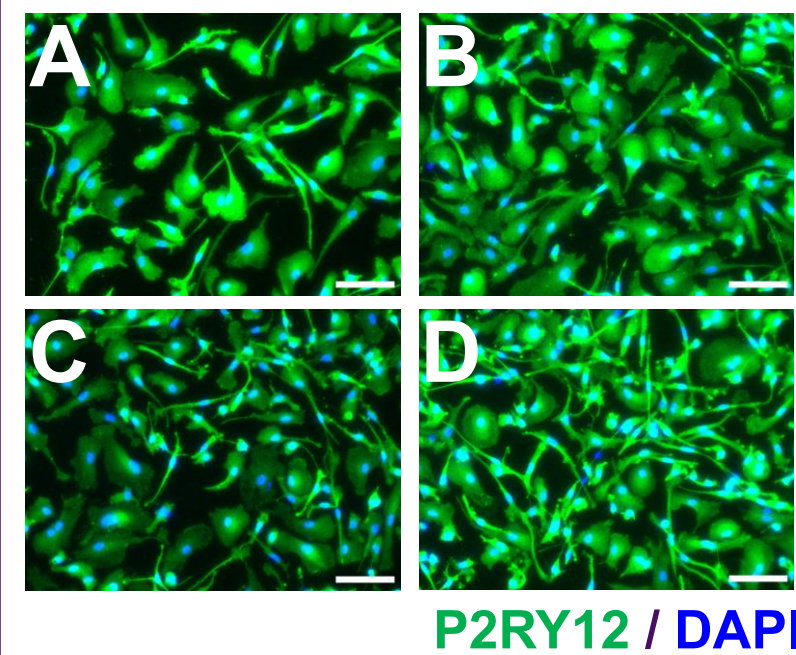


Fig 3: iMG express the microglia marker P2RY12. (A) *TREM2* CV, (B) *R62H*, (C) *R47H*, (D) *TREM2* KO. Representative images from n = 3 differentiations. Scale bars = 100 μ m.

4. RESULTS

4.1. *R62H*, *R47H* and the *TREM2* KO increased the amount of phagocytic cargo found inside iMG

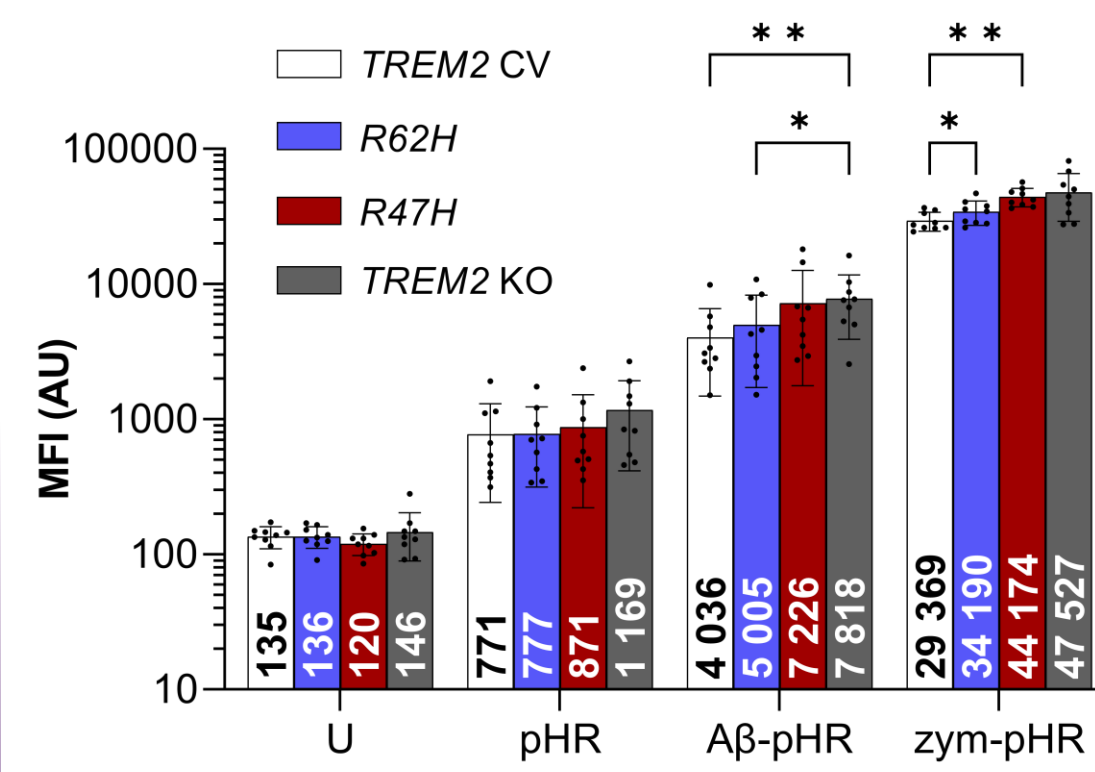


Fig 4: Impact of *TREM2* variants on phagocytosis. Median fluorescence intensity (MFI) of iMG left untreated (U) or incubated with pHRR, A β -pHR or zymosan-pHR (zym-pHR) for 18h. Data is shown as mean $\pm$ SD (n = 9). Repeated measures 2-way ANOVA followed by Tukey with multiple testing correction. *, p < 0.05; **, p < 0.01

4.2. *R47H* and the *TREM2* KO exacerbated IL-6 secretion from iMG

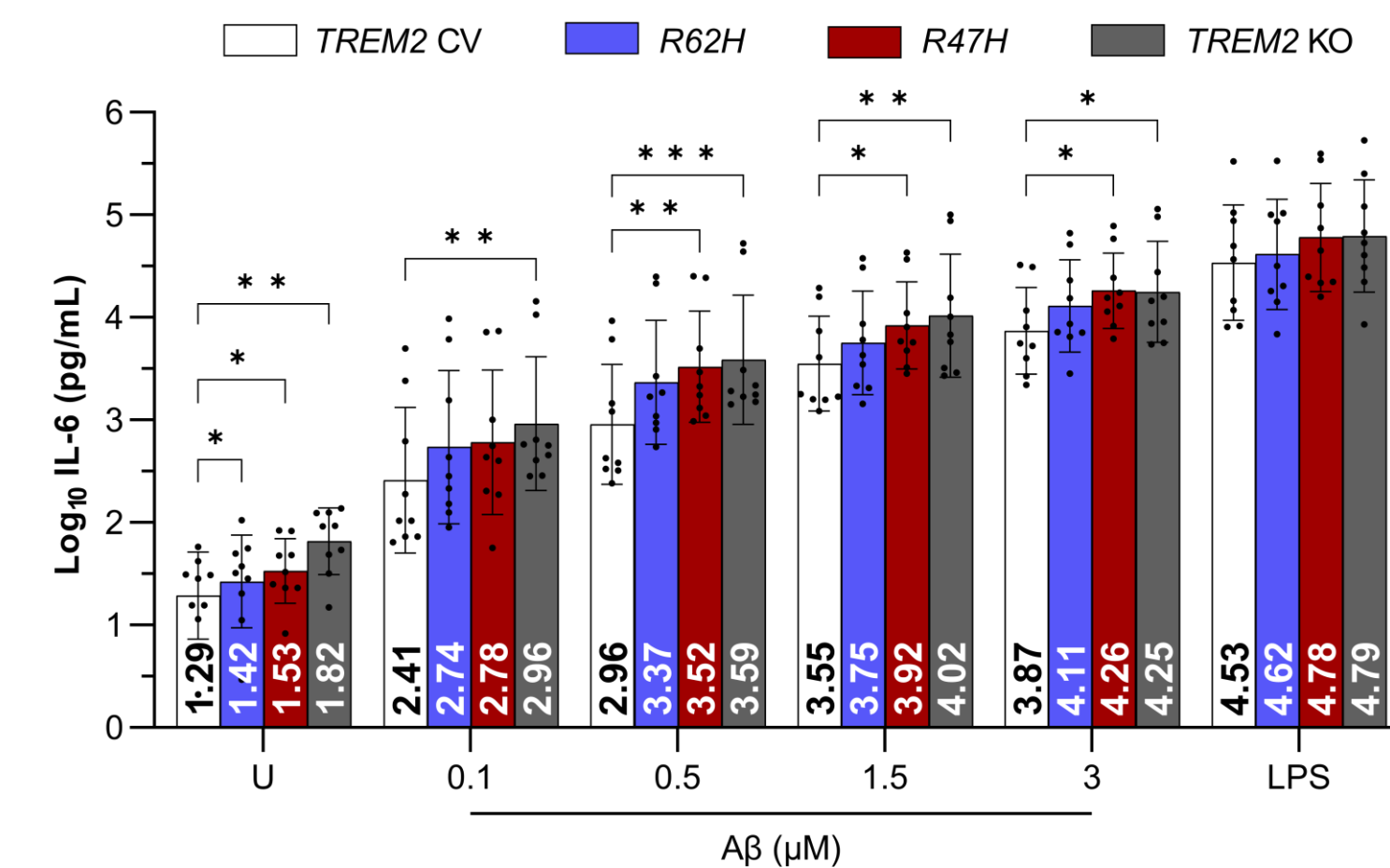


Fig 5: IL-6 release from iMG. Mean log₁₀ IL-6 concentration in n = 9 iMG cultures incubated for 24h with medium (U), A β aggregates or 100 ng/mL LPS, $\pm$ SD. 2-way ANOVA followed by Tukey with multiple testing correction. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

4.3. *TREM2* CV and KO iMG showed differential expression of metabolic, inflammatory and lysosomal genes

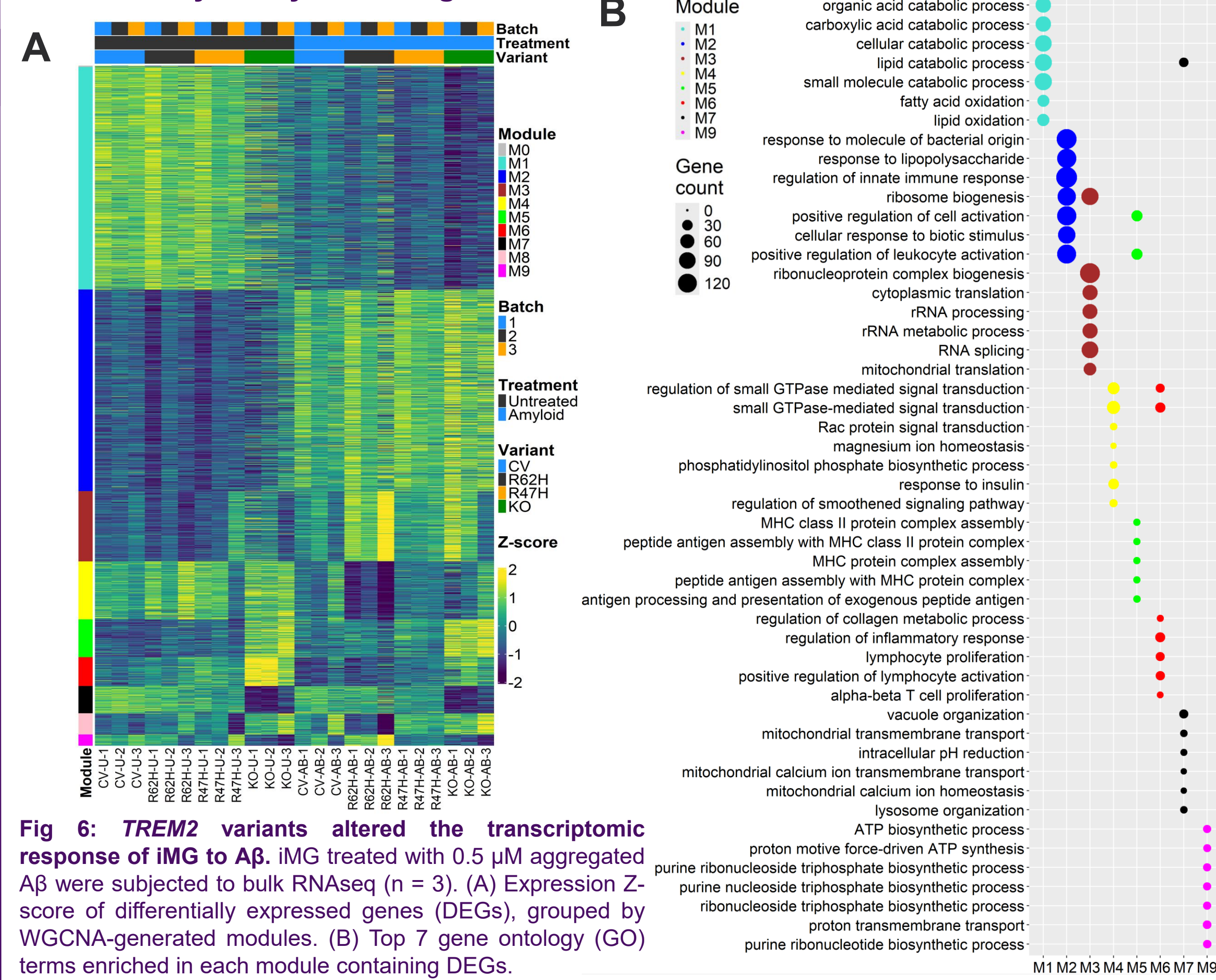


Fig 6: *TREM2* variants altered the transcriptomic response of iMG to A β . iMG treated with 0.5 μ M aggregated A β were subjected to bulk RNAseq (n = 3). (A) Expression Z-score of differentially expressed genes (DEGs), grouped by WGCNA-generated modules. (B) Top 7 gene ontology (GO) terms enriched in each module containing DEGs.

4.4. *R47H* and the *TREM2* KO dampened lysosomal gene expression and amplified the inflammatory response of iMG to A β

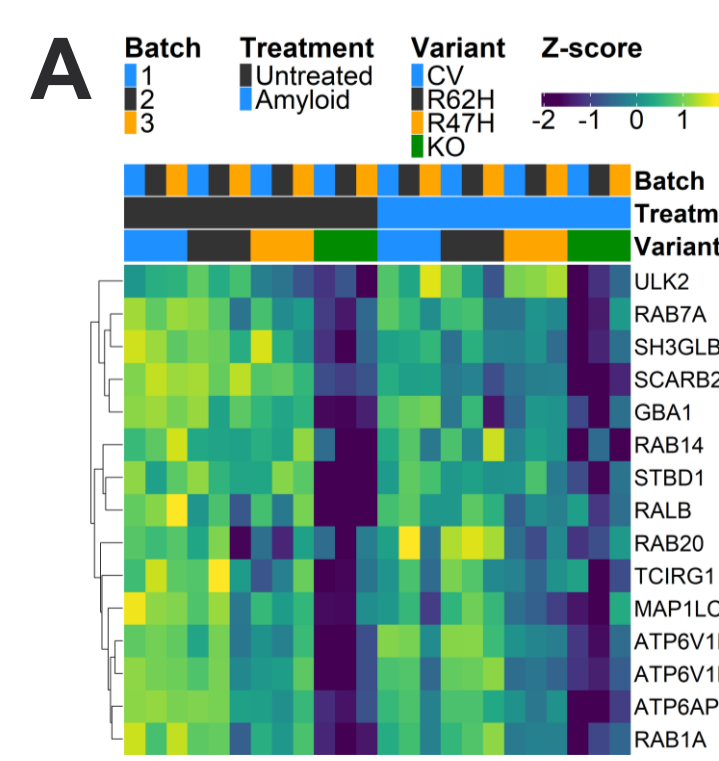


Fig 7: Lysosomal and inflammatory genes are differentially expressed in *TREM2* CV and KO iMG. DEGs identified in iMG cultures stimulated with 0.5 μ M aggregated A β (n = 3) were classified into co-expression modules. Heatmaps show the expression Z-scores of DEGs from modules M7 and M2 which were annotated to the GO terms 'Vacuole organisation' (A) and 'Response to molecule of bacterial origin' (B), respectively.

5. CONCLUSION

- Relative to *TREM2* CV cells, *TREM2* KO iMG accumulated more A β intracellularly which may be due to a downregulation of lysosomal genes
- R47H* and the *TREM2* KO enhanced the inflammatory response of iMG to A β

FUTURE WORK: Determine whether *TREM2* variants and KO iMG show decreased lysosomal degradation

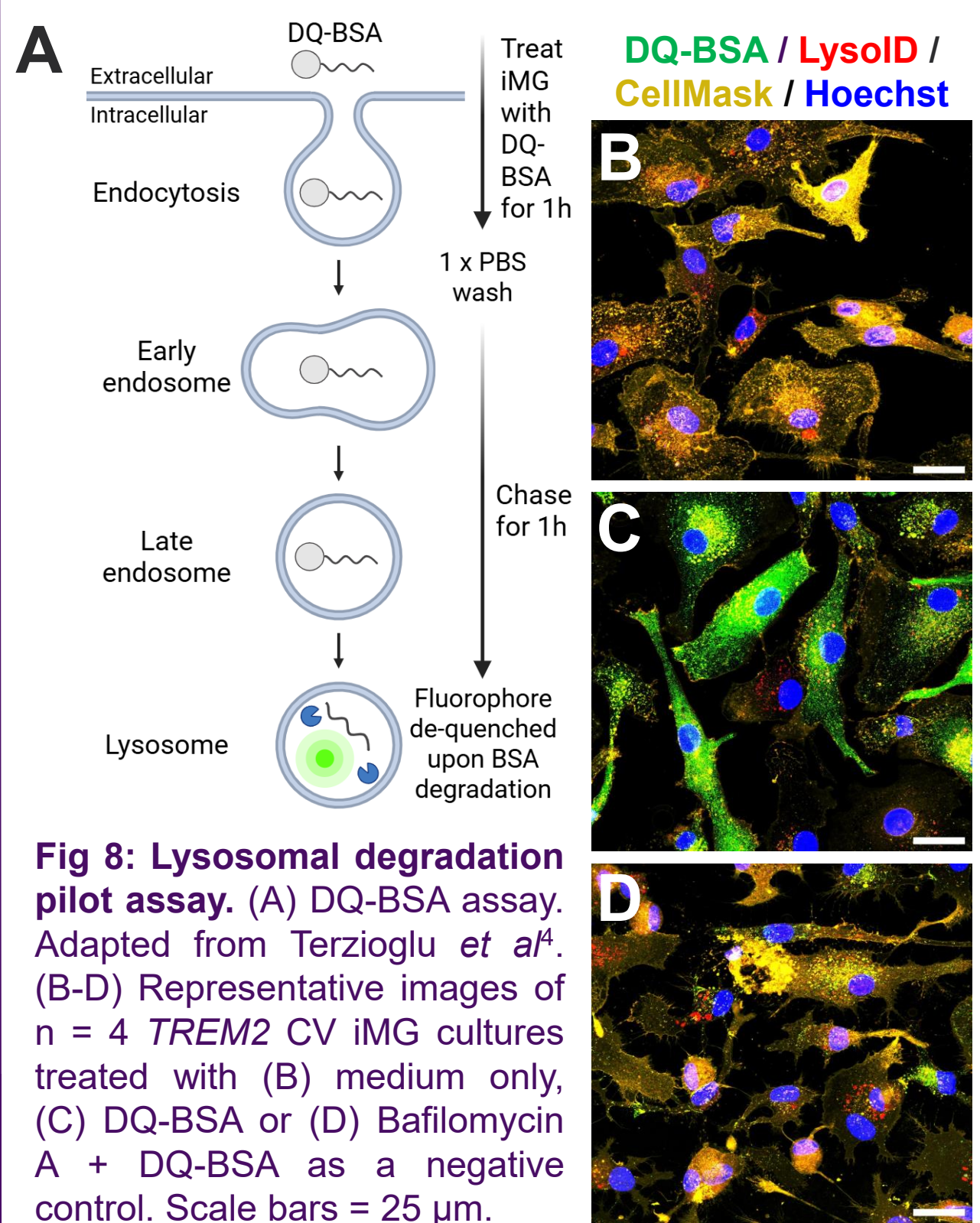


Fig 8: Lysosomal degradation pilot assay. (A) DQ-BSA assay. Adapted from Terzioglu *et al.*⁴. (B-D) Representative images of n = 4 *TREM2* CV iMG cultures treated with (B) medium only, (C) DQ-BSA or (D) Bafilomycin A + DQ-BSA as a negative control. Scale bars = 25 μ m.

6. REFERENCES

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- Gratuze M, Leysn CEG, Holtzman DM. Mol Neurodegener (2018). doi: 10.1186/s13024-018-0298-9
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