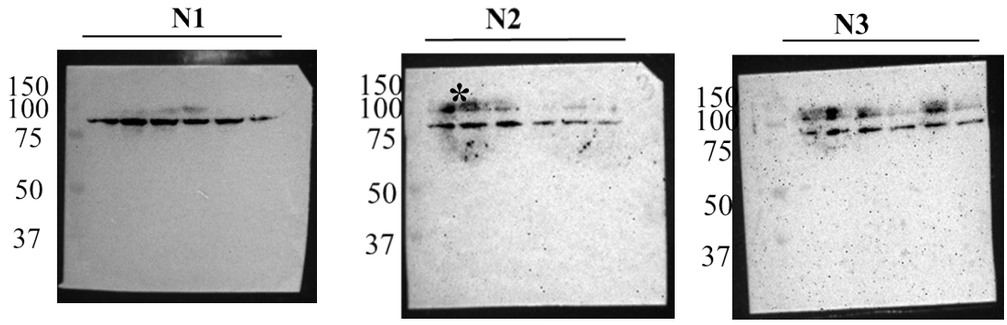
SUPPLEMENTARY DATA

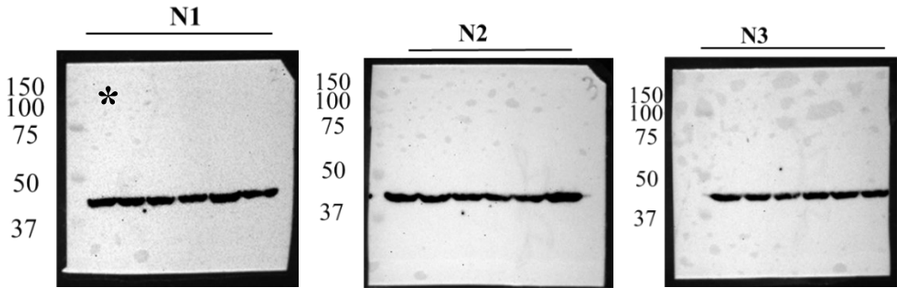
TITLE: Therapeutic potential of Chrysin in regulation of interleukin-17 signaling in repeated intranasal amyloid-beta-induced Alzheimer's disease model

1. Amyloid-beta:

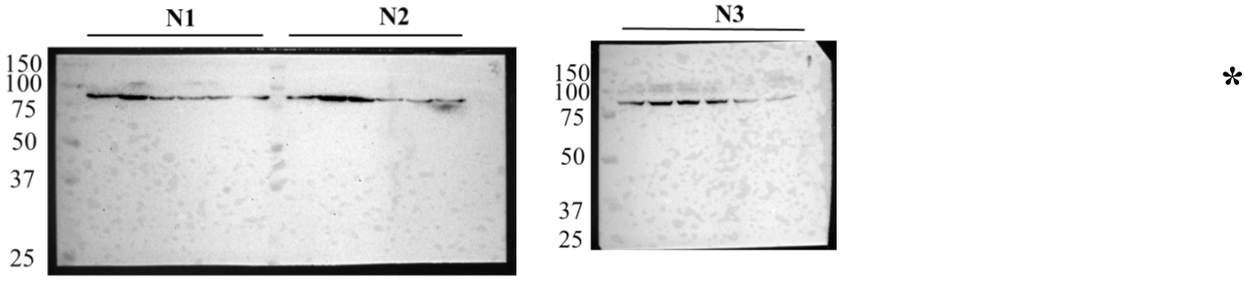
A. Hippocampus: Aβ



B. Hippocampus: β-Actin



C. Cortex: Aβ



D. Cortex: β-Actin

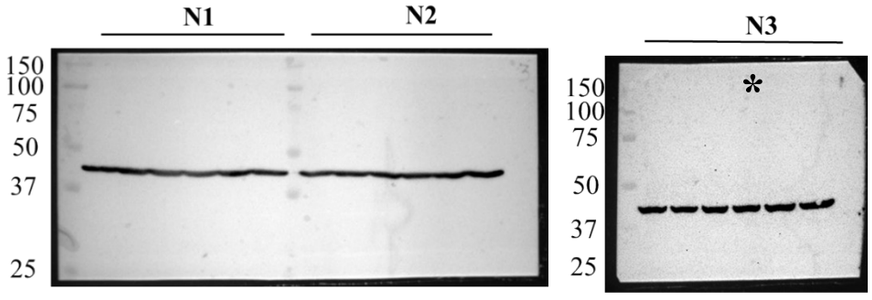
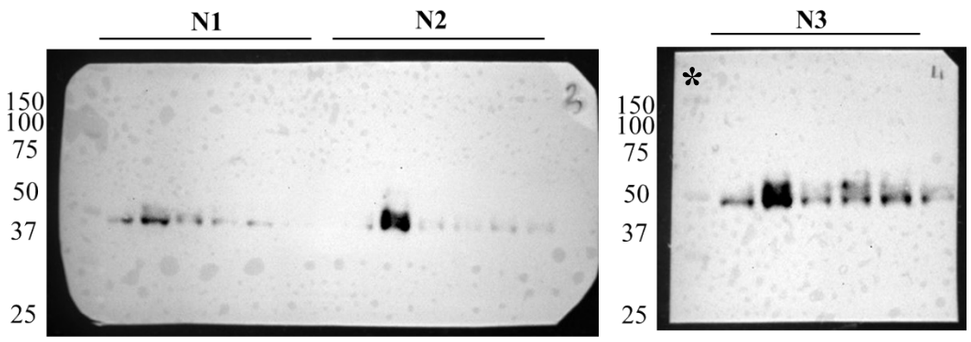


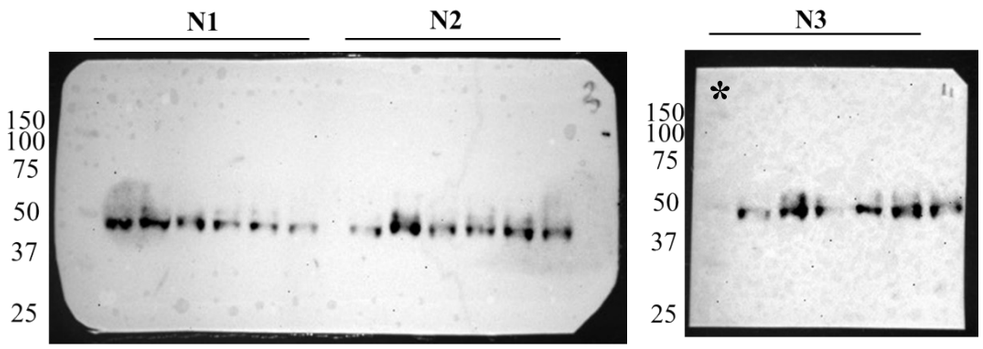
Fig. S1 Effect of oral chrysin on the expression of Aβ in intranasal $Aβ_{1-42}$ induced AD mice model. Expression of **A)** Aβ in hippocampus, **B)** β-Actin in hippocampus, **C)** Aβ in cortex and **D)** β-Actin in cortex region of mice brain tissue. * Representative image used in manuscript.

2. Phosphorylated Tau (ser199)

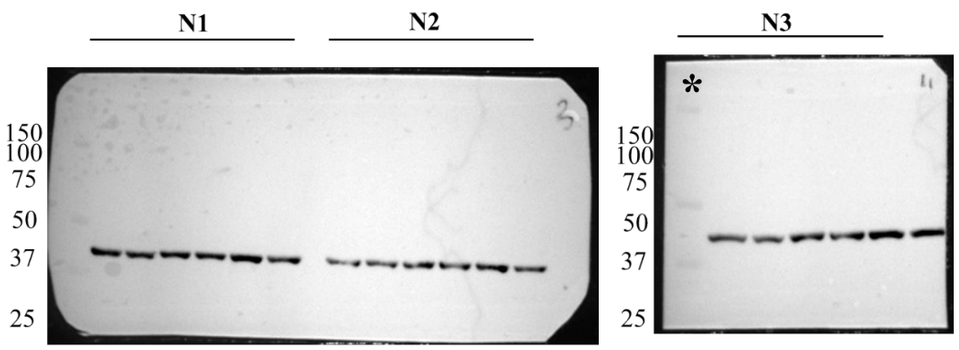
A. Hippocampus: pTau (ser199)



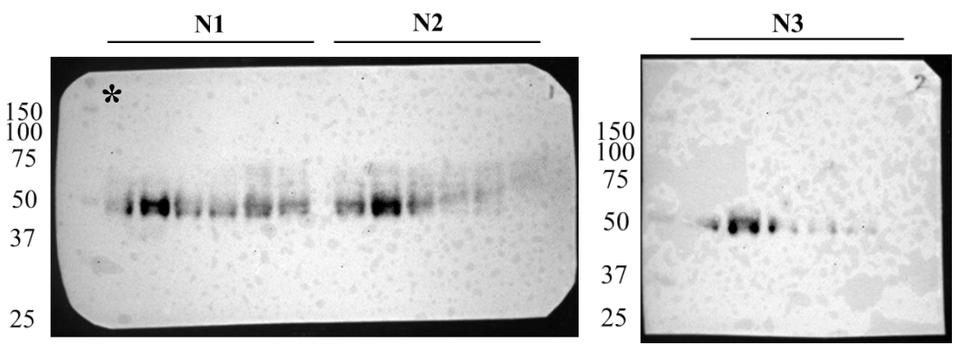
B. Hippocampus: Tau



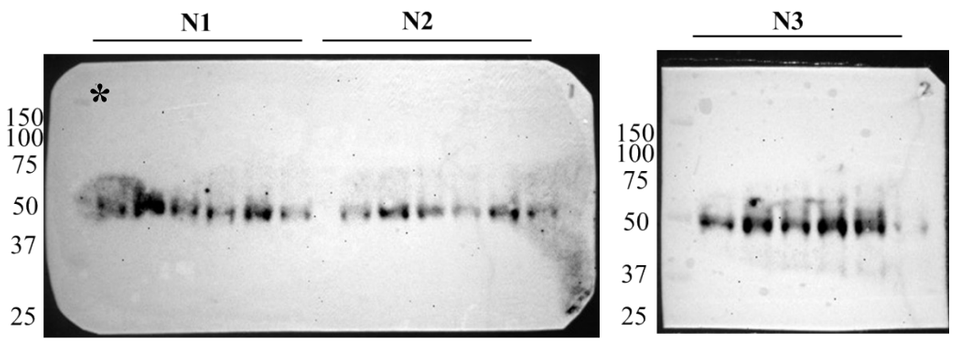
C. Hippocampus: Actin



D. Cortex: pTau (ser199)



E. Cortex: Tau



F. Cortex: Actin

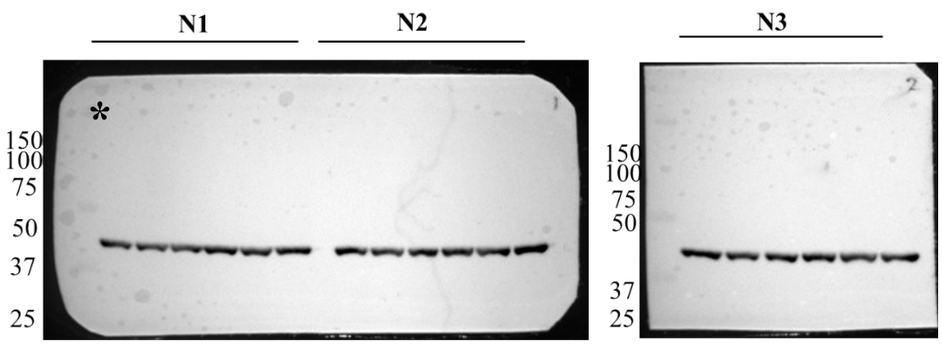
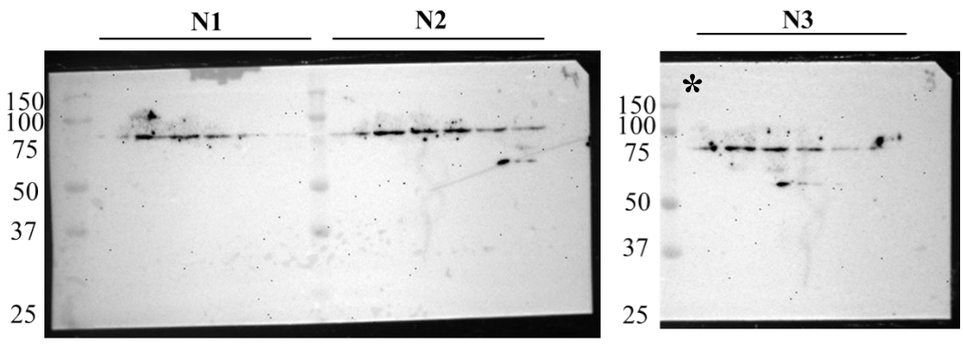


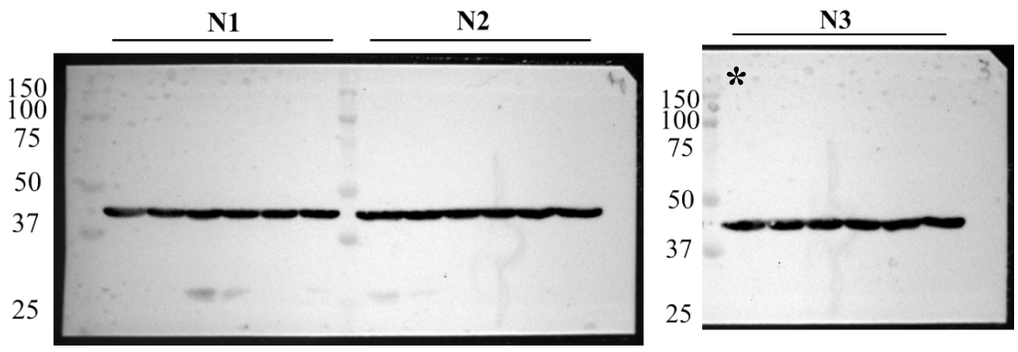
Fig. S2 Effect of oral chrysin on the expression of phospho-Tau in intranasal $A\beta_{1\text{-}42}$ induced AD mice model. Expression of **A)** pTau (Ser199) in hippocampus, **B)** Tau in hippocampus **C)** β-Actin in hippocampus, **D)** pTau (Ser199) in cortex, **E)** Tau in cortex, and **F)** β-Actin in cortex regions of mice brain tissue. * Representative image used in manuscript.

3. Interleukin-17RA (IL-17RA)

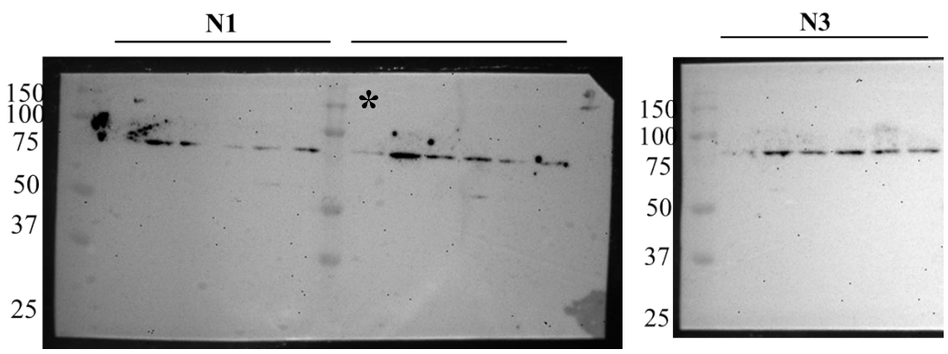
A. Hippocampus: IL-17RA



B. Hippocampus: β-Actin



C. Cortex: IL-17RA



D. Cortex: β-Actin

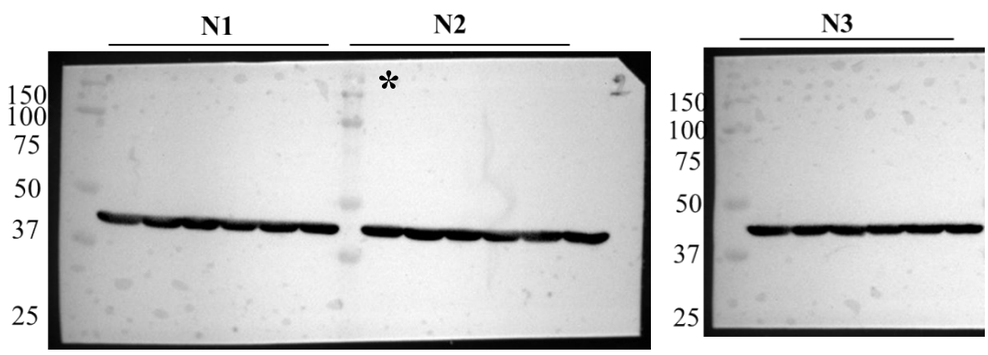
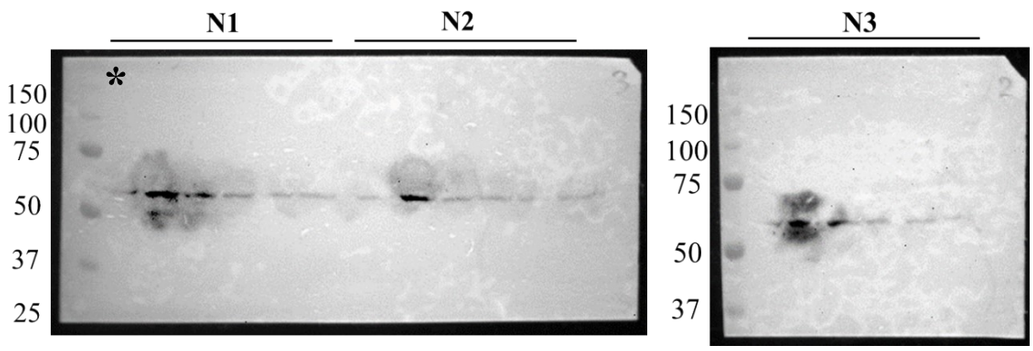


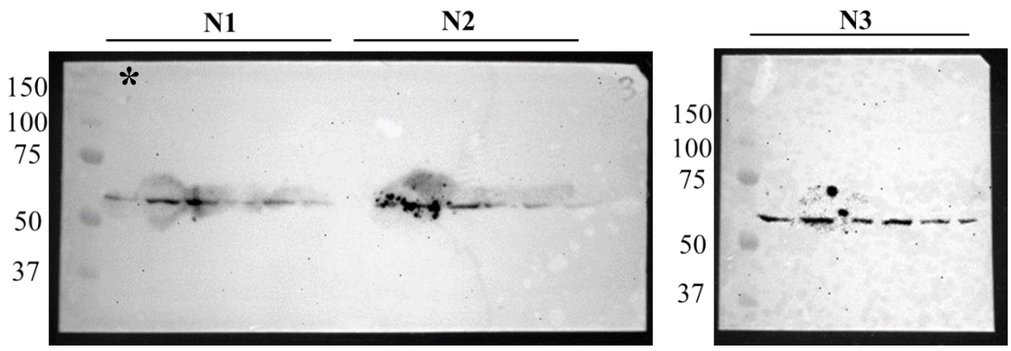
Fig. S3 Effect of oral chrysin on the expression of IL-17RA in intranasal $A\beta_{1-42}$ induced AD mice model. Expression of **A)** IL-17RA in hippocampus, **B)** β-Actin in hippocampus, **C)** IL-17RA in cortex and **D)** β-Actin in cortex region of mice brain tissue. * Representative image used in manuscript.

4. Act1 and TRAF6

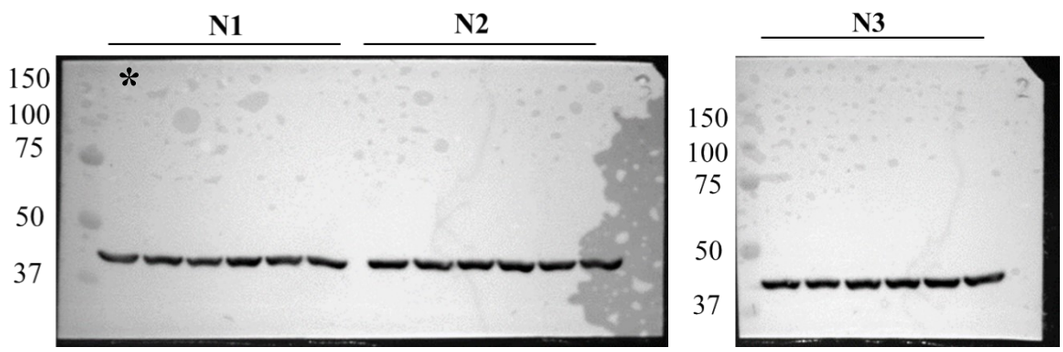
A. Hippocampus: Act1



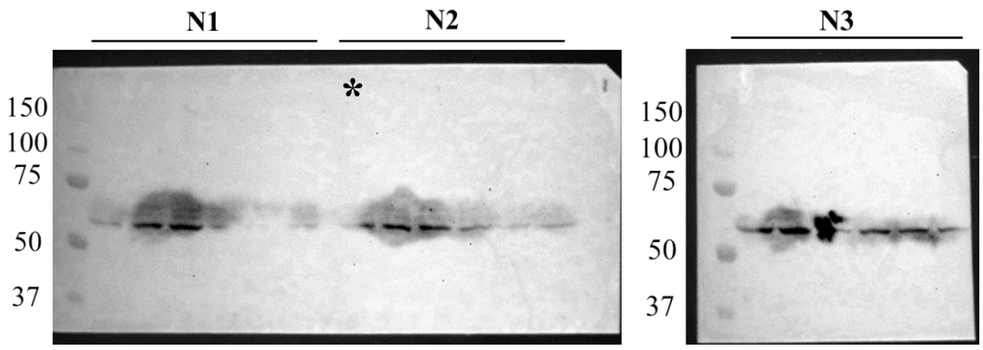
B. Hippocampus: TRAF6



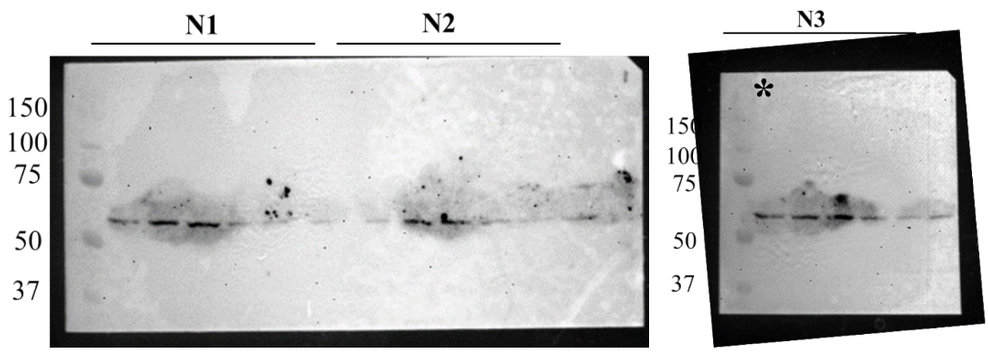
C. Hippocampus: β-Actin



D. Cortex: Act1



E. Cortex: TRAF6



F. Cortex: β-Actin

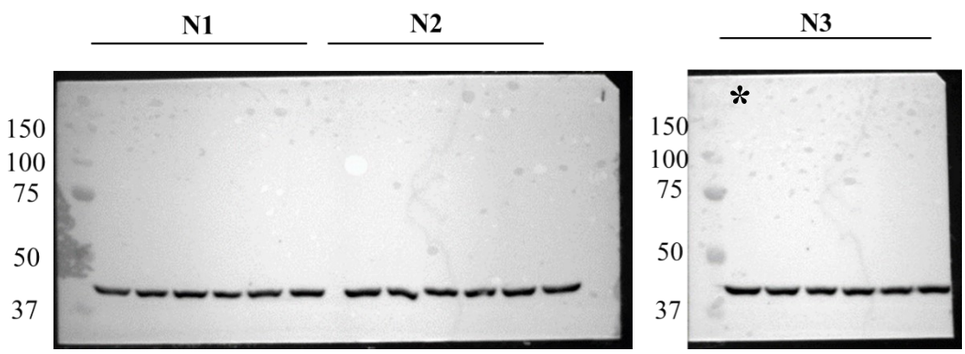
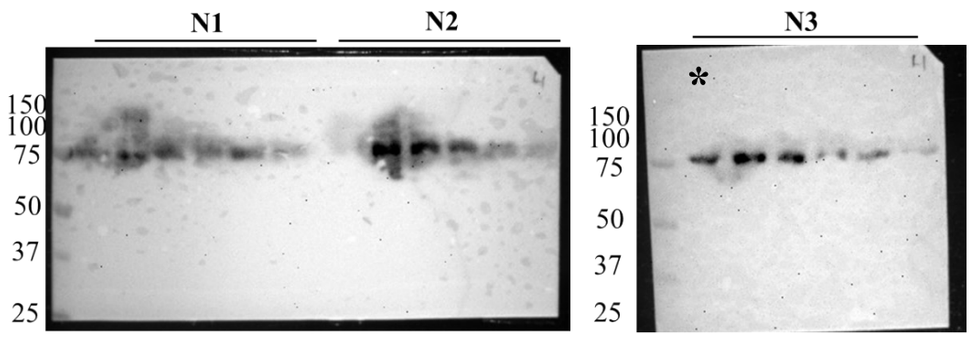


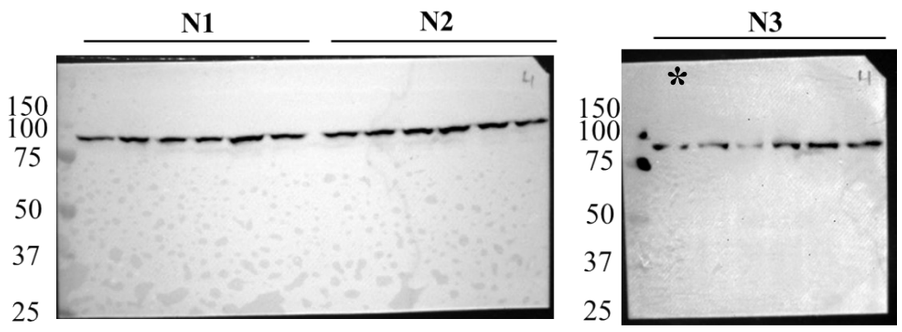
Fig. S4 Effect of oral chrysin on the expression of Act1 and TRAF6 in intranasal $A\beta_{1\text{-}42}$ induced AD mice model. Expression of **A)** Act1 in hippocampus, **B)** TRAF6 in hippocampus **C)** β-Actin in hippocampus, **D)** Act1 in cortex, **E)** TRAF6 in cortex, and **F)** β-Actin in cortex regions of mice brain tissue. * Representative image used in manuscript.

5. pIKKαβ (ser176/180)

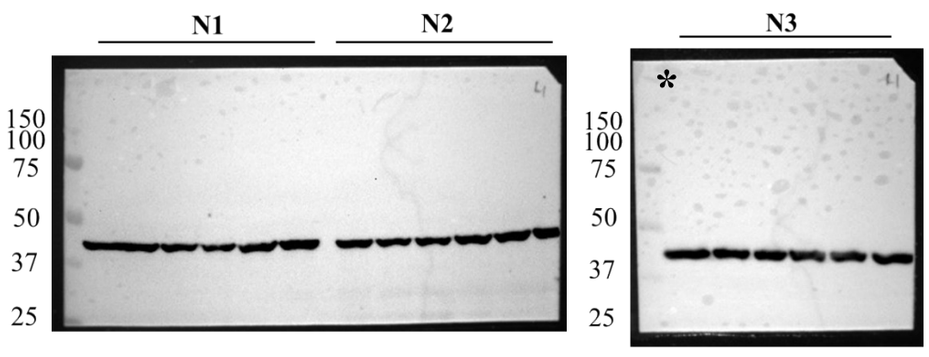
A. Hippocampus: pIKKαβ (ser176/180)



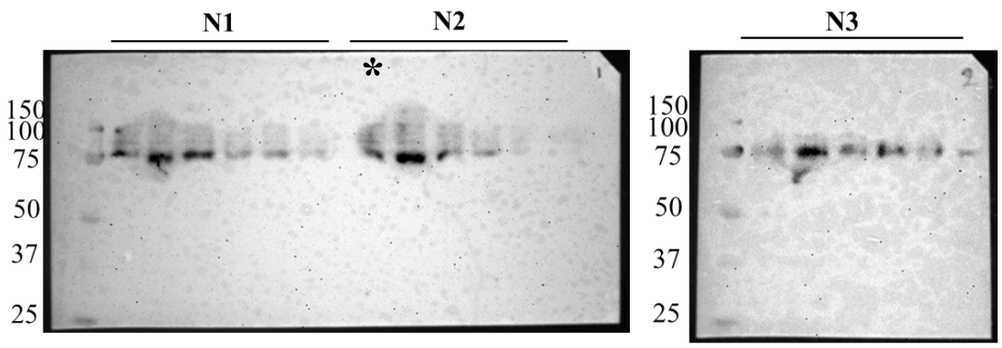
B. Hippocampus: IKKβ



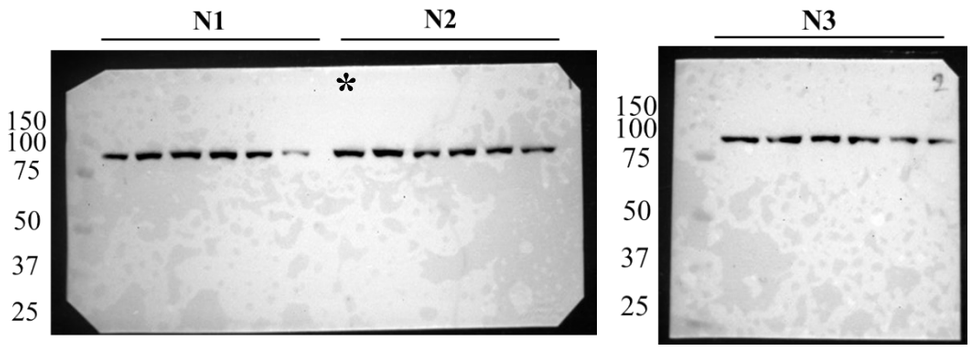
C. Hippocampus: β-Actin



D. Cortex: pIKKαβ (ser176/180)



E. Cortex: IKKβ



F. Cortex: β-Actin

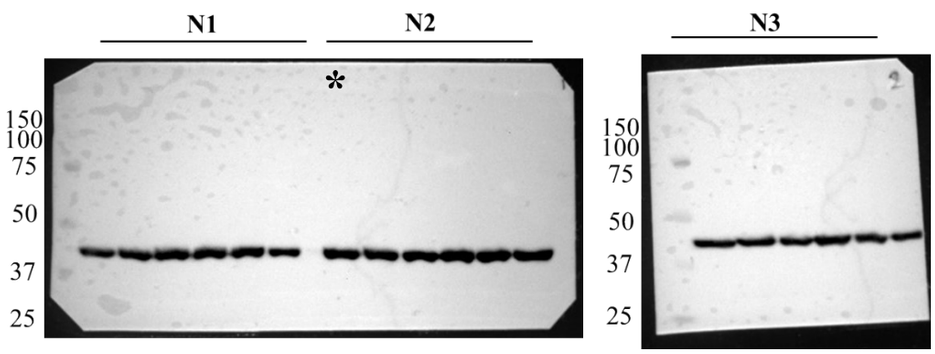
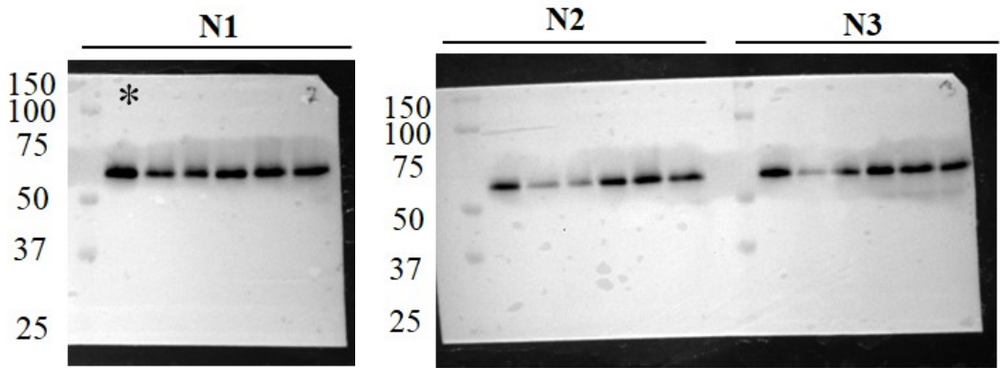


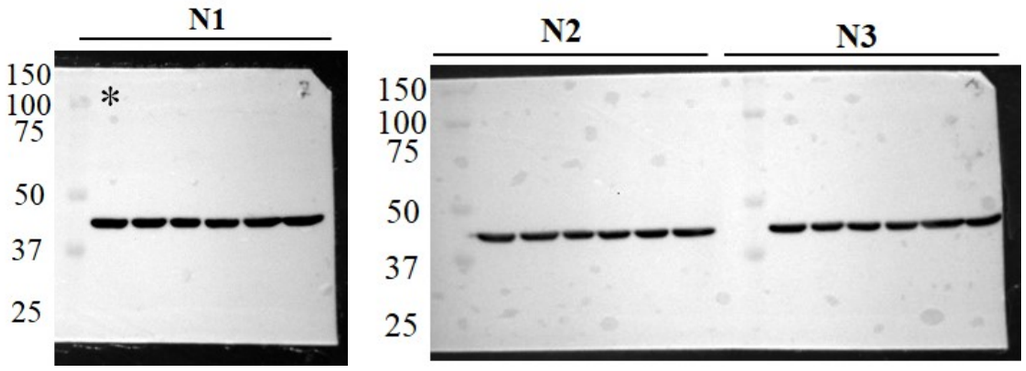
Fig. S5 Effect of oral chrysin on the expression of pIKKαβ (ser176/180) in intranasal $A\beta_{1-42}$ induced AD mice model. Expression of **A)** pIKKαβ (ser176/180) in hippocampus, **B)** IKKβ in hippocampus **C)** β-Actin in hippocampus, **D)** pIKKαβ (ser176/180) in cortex, **E)** IKKβ in cortex, and **F)** β-Actin in cortex regions of mice brain tissue. * Representative image used in manuscript.

6. Occludin

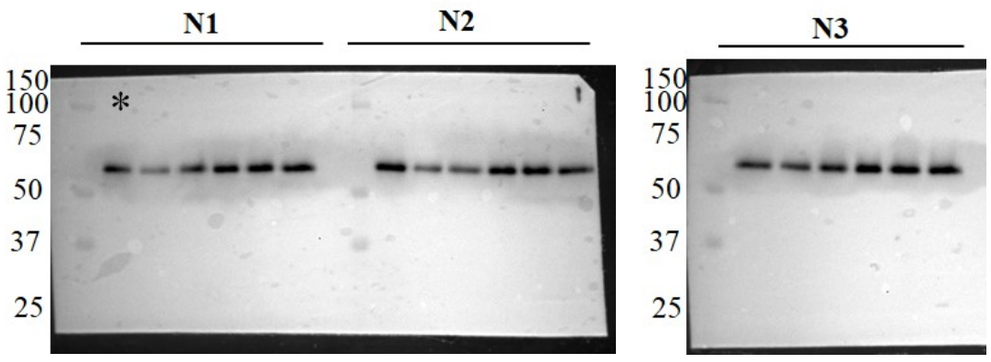
A. Hippocampus**:** Occludin



B. Hippocampus**:** β-Actin



C. Cortex: Occludin



D. Cortex: β-Actin

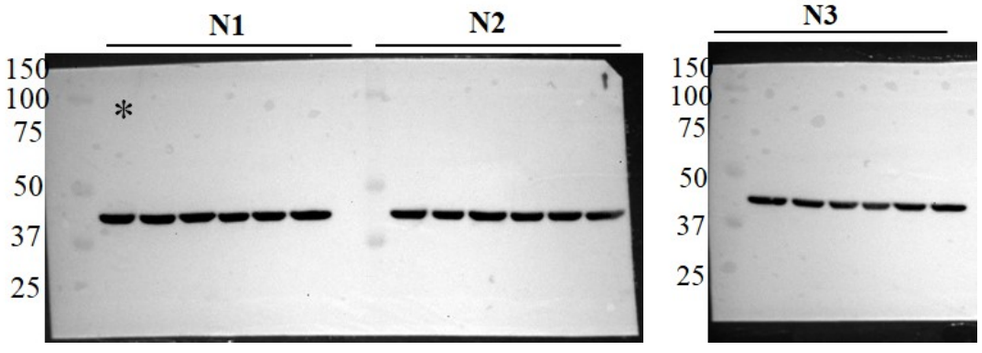
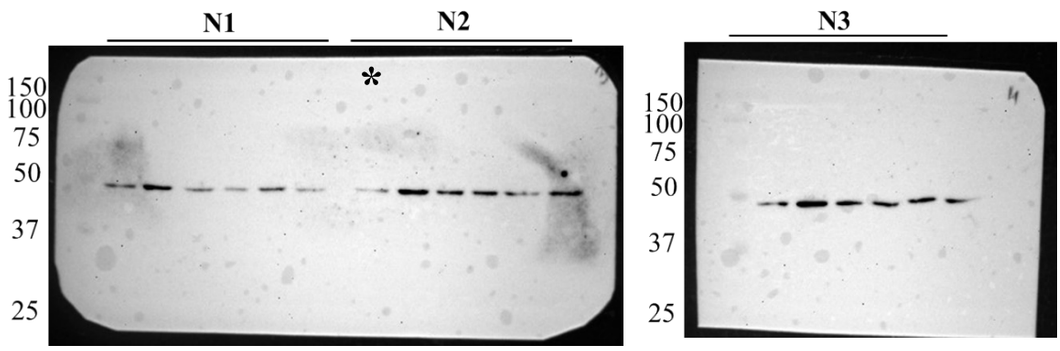


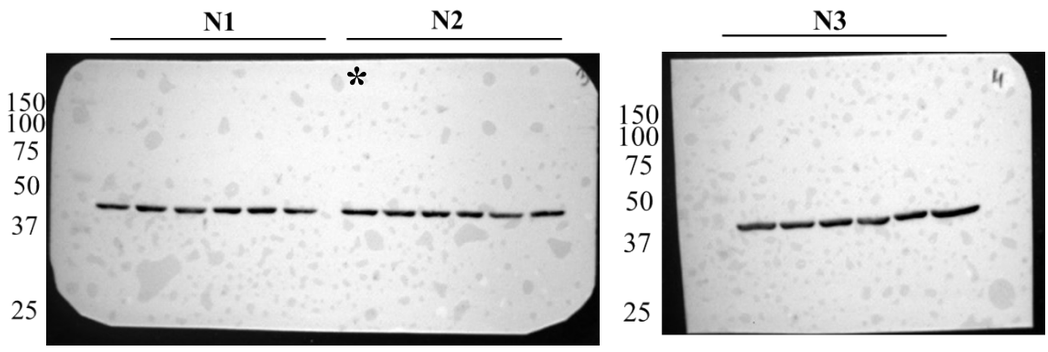
Fig. S6 Effect of oral chrysin on the expression of Occludin in intranasal $A\beta_{1\text{-}42}$ induced AD mice model. Expression of **A)** Occludin in hippocampus, **B)** β-Actin in hippocampus, **C)** Occludin in cortex and **D)** β-Actin in cortex region of mice brain tissue. * Representative image used in manuscript.

7. GFAP

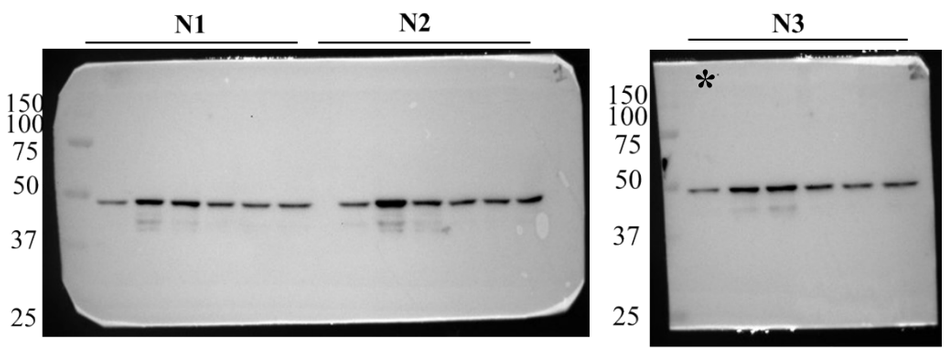
A. Hippocampus: GFAP



B. Hippocampus: β-Actin



C. Cortex: GFAP



D. Cortex: β-Actin

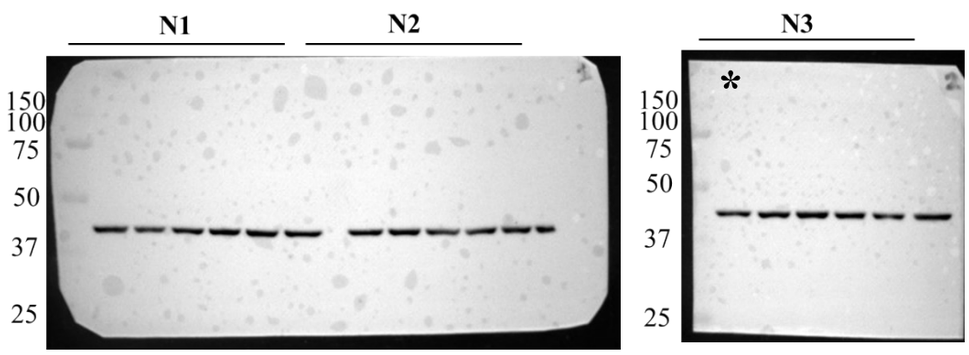
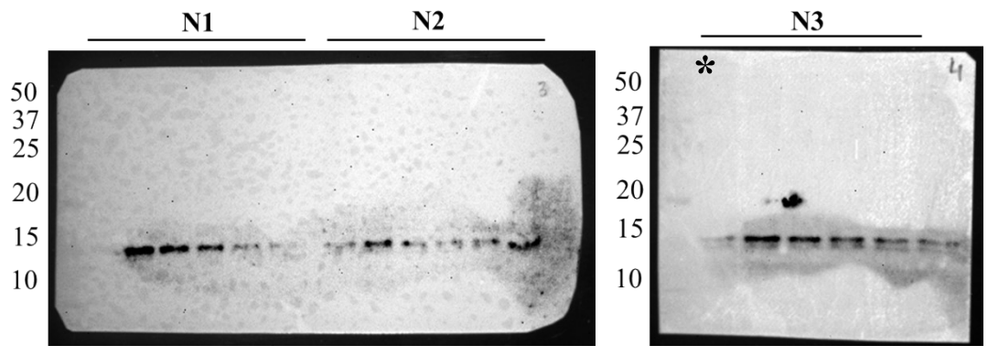


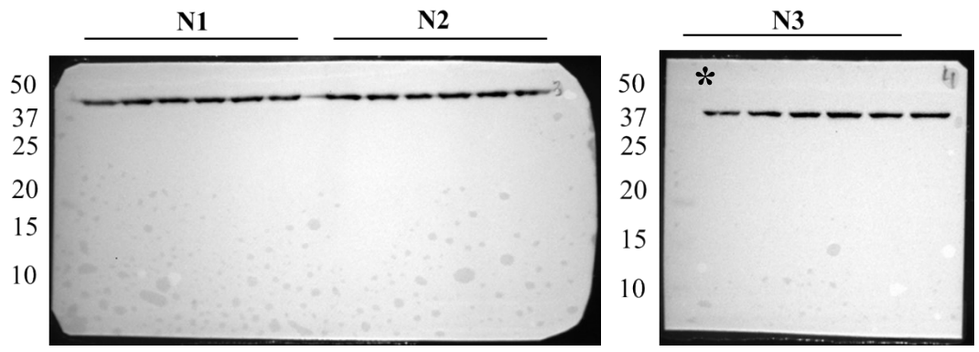
Fig. S7 Effect of oral chrysin on the expression of GFAP in intranasal $A\beta_{1-42}$ induced AD mice model. Expression of **A)** GFAP in hippocampus, **B)** β-Actin in hippocampus, **C)** GFAP in cortex and **D)** β-Actin in cortex region of mice brain tissue. * Representative image used in manuscript.

8. S100B

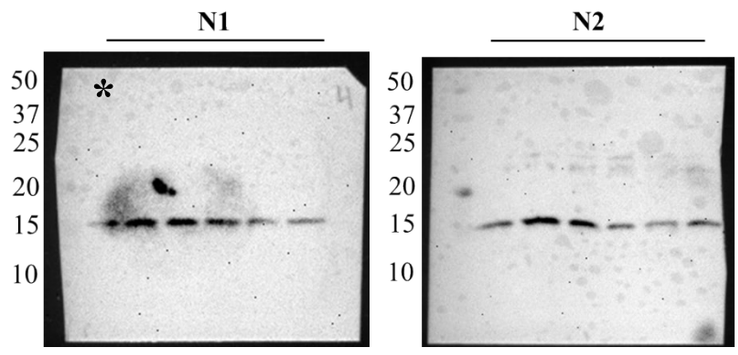
A. Hippocampus: S100B



B. Hippocampus: β-Actin



C. Cortex: S100B



D. Cortex: β-Actin

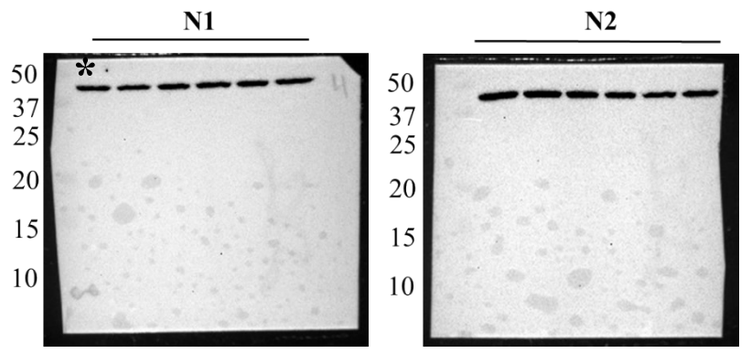


Fig. S8 Effect of oral chrysin on the expression of S100B in intranasal $A\beta_{1\text{-}42}$ induced AD mice model. Expression of **A)** S100B in hippocampus, **B)** β-Actin in hippocampus, **C)** S100B in cortex and **D)** β-Actin in cortex region of mice brain tissue. * Representative image used in manuscript.

9. *In-silico* docking studies to determine binding affinity of Chrysin with IL-17A, IL-17RA and $A\beta_{1-42}$

Chrysin with PubChem CID 5281607 was selected from authentic web source (https://pubchem.ncbi.nlm.nih.gov/) developed by national library of medicine, National Center for Biotechnology Information (NCBI). The structure of chrysin was retrieved from PubChem in "sdf-MDL/MOL" format that was converted to protein data bank format using Open Babel software. The crystal structure of proteins (IL-17RA, PDB ID: 4HSA; IL-17A, PDB ID: 4HR9; and $A\beta_{1-42}$ (PDB ID: 5OQV) in protein data bank format were extracted from Research Collaboratory for Structural Bioinformatics (RCSB). Then the heteroatoms and water molecules were deleted using Biovia software. Auto Dock 1.5.6 was then utilized to make the crystal structure suitable for docking by adding AD4 (AutoDock Version 4.2) type atom orientation and adding charges (Kollman & Gasteiger charges). Then ligand (chrysin) was imported to Auto Dock 1.5.6 and put forward with GRID along with protein. After these preparatory steps, a command was given to initiate docking that analyzed the binding affinity of chrysin with the proteins.

Chrysin showed the dynamic binding energy towards the IL-17RA and IL-17A with -8.5kCal/mol and -7.3kCal/mol respectively that is consistent with downregulation of IL-17RA and its mediated signaling in Aβ exposed animals. However, chrysin also shows considerable binding affinity towards $A\beta_{1-42}$ with -8.4kCal. The details of binding energies of chrysin towards IL-17RA, IL-17A and amyloid-beta 1-42 are listed in table S1 and shown in fig. 9.

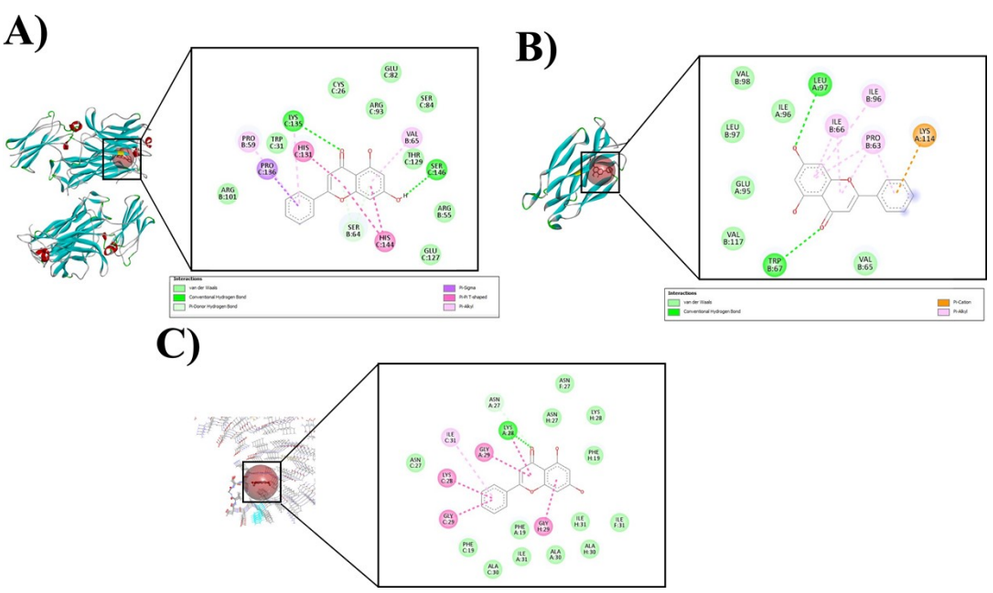


Fig. S9: Biovia 2D interactions of Chrysin (PubChem CID: 5281607) with **A)** IL-17RA (PDB ID: 4HSA), **B)** IL-17A (PDB ID: 4HR9) and **C)** Amyloid-beta 1-42 (PDB ID: 5OQV)

Table S1: Binding affinity of chrysin with IL-17RA, IL-17A and Amyloid-beta 1-42

S. No.	Protein	PDB ID	Ligand	PubChem CID	Binding Energy (kCal/mol)
1.	IL-17RA	4HSA	Chrysin	5281607	**-8.5**
2.	IL-17A	4HR9	Chrysin	5281607	**-7.3**
3.	Amyloid-beta 1-42	5OQV	Chrysin	5281607	**-8.4**