Supplementary Figure Legends

Suppl. Fig 1. Expression patterns of EGFP in the brain following rAAV1-EGFP mediated transduction.

A-H. Representative images of anti-EGFP immunoreactivity shows localized transduction of the choroid plexus and occasional neurons in the cortex, hippocampus and cerebellum following rAAV1-EGFP injection into brains of neonatal mice on day P2 (B) compared to PBS injected control mice (A). Representative high magnification images from ventricle (C), hippocampus and dentate (D), cortex (E), cerebellum (F), midbrain (G) and thalamus (H) depicting anti-EGFP staining is shown. *Scale Bar*, 600μm (A, B), 150μm (C-H*). (n*=5/group).

I-L. Representative images of anti-EGFP immunoreactivity in the hippocampal pyramidal layer as well as neuronal projections in the cortex and thalamus of 5.5 month old TgCRND8 mice following stereotaxic injection of rAAV1-EGFP into the hippocampus at 4 month (K, L) compared to PBS injected age matched controls (I, J). *Scale Bar*, 500μm (I, K), 125μm (J, L). *(n*=5/group).

Suppl. Fig 2. rAAV1-mIFNγ expression following stereotaxic injection in the hippocampus of adult TgCRND8 mice results in reactive astrogliosis and microgliosis.

A-L. rAAV1-mIFNγ or rAAV1-EGFP was injected into the hippocampus of 4 month old TgCRND8 mice and brain sections analyzed after 6 weeks (4→5.5 month). Whole brain panels showing a coronal section in the immediate vicinity of rAAV1 injection site was stained with anti-Iba-1 (A-F) or anti-GFAP (G-L) antibodies. Whole brain sections (A, B, G, H) and higher magnification (C-F, I-L) highlight the activated glial morphology in the hippocampus of mIFNγ expressing mice compared to EGFP expressing control mice. *Scale Bar,* 600μm (A, B, G, H), 150μm (C, D, I, J) and 25μm (E, F, K, L). (*n*=5/group).

M. Quantitation of Iba-1 and GFAP immunoreactivity in paraffin embedded sections of 4→5.5 month old TgCRND8 mice expressing mIFNγ or EGFP. (*n*=3/group, **p*<0.05).

N. Levels of mIFNγ protein in mIFNγ expressing 4→5.5 month old TgCRND8 mice brains were increased compared to age-matched controls. Protein levels were analyzed using RIPA buffer solubilized brain lysates (dissected 1 mm anterior and 1 mm posterior to the injection site) by sandwich ELISA. (*n*=5/group; **p*<0.05).

O-P. Representative immunoblot showing increased levels of CD11b and GFAP in 4→5.5 month old mIFNγ expressing TgCRND8 mice compared to controls. β-actin has been used as a loading control (O). Intensity analysis of CD11b and GFAP immunoreactive bands normalized to β actin in 4→5.5 month old TgCRND8 mice is depicted (P). (*n*=3/group, **p*<0.05).

Suppl. Fig 3. Characterization of pathological features following chronic mIFNγ expression in TgCRND8 mice.

A-D. Representative Nissl stained sections of rAAV1-mIFNγ injected P2→5 month old TgCRND8 mice (A-B) and rAAV1-mIFNγ injected 4→5.5 month old TgCRND8 mice (C-D). Lower panels (B, D) show higher magnification of thalamus (boxed areas of A and C). P2→5 month old mIFNγ expressing mice show basal ganglial calcification (arrowheads) whereas 4→5.5month old rAAV1-mIFNγ expressing TgCRND8 mice do not show any calcification. *Scale Bar,* 600μm (A, C), 100μm (B, D). (*n*=5/group).

E. Representative von Kossa stained brain sections of a P2→5 month old mIFNγ expressing TgCRND8 mouse showing no calcification in the hippocampus (inset 1), but extensive calcification in the thalamus (inset 2). *Scale Bar,* 600μm (E, left panel) and 150μm (insets 1 and 2). (*n*=5/group).

F-K. Representative images of anti-CD3 immunostaining in brains of mIFNγ expressing P2→5 month old TgCRND8 mice (G, J) or EGFP expressing control mice (F, I) shows no evidence of T cell infiltration. mTNFα expressing 5 month old TgCRND8 mice showing CD3 immunoreactive T cells (H, K) was used as positive control. *Scale Bar,* 600 μm (F,G,H), 75μm (I,J,K). (*n*=5/group).

L-N. Representative images of Nissl stained sections of hippocampal pyramidal cells in mIFNγ expressing P2→5 month old TgCRND8 mice (M) compared to controls (L). Quantification of cell count and cell diameter of Nissl stained hippocampal pyramidal cells in P2→5 month old TgCRND8 mice expressing mIFNγ compared to controls is depicted (N). (*n*=5/group, **p*<0.05). *Scale Bar,* 125μm.

Suppl. Fig 4. Comparison of Aβ42 and Aβ40 plaque burden in 5.5 month old mice following acute hippocampal expression of mIFNγ compared to Aβ levels of unmanipulated 4 month old mice.

A-F. 4 month old TgCRND8 mice were stereotaxically injected in the hippocampus with either rAAV1-mIFNγ or rAAV1-EGFP and sacrificed after 6 weeks. Representative brain sections from EGFP and mIFNγ expressing 5.5 month old mice as well as unmanipulated 4 month old mice were stained with anti Aβ42 antibody (A-C) and anti Aβ40 antibody (D-F). Insets depict individual plaques. *Scale Bar,* 600μm (A-F); inset, 25μm. (*n*=5/group).

G. Forebrain Aβ plaque burden analysis depicting alterations in Aβ42 and Aβ40 burden in 5.5 month old mIFNγ and EGFP expressing mice compared to unmanipulated 4 month old TgCRND8 mice. (*n*=5/group, **p*<0.05 and ***p*<0.05).

Suppl. Fig 5. No change in endogenous APP or steady state Aβ levels in mIFNγ expressing APP non-transgenic mice or 3 month old transgenic Tg2576 mice.

A-B. Representative anti CT20 immunoblot showing APP levels in P2→5 month old CRND8 nontransgenic littermate mice expressing mIFNγ or EGFP (A). Intensity analysis of APP bands normalized to β-actin show no significant changes in APP levels in mIFNγ expressing mice compared to age-matched controls (B). (*n*=4/group).

C. No change in soluble endogenous Aβ40 levels was seen in mIFNγ expressing P2→5 month old CRND8 non-transgenic mice compared to age-matched controls. (*n*=4/group).

D-E. Representative anti CT20 immunoblot showing APP and CTF levels in P0→3 month old Tg2576 mice expressing mIFNγ or EGFP (D). Quantitative analysis of APP and CTF levels normalized to β-actin show no significant changes in mIFNγ expressing P0→3 month old Tg2576 mice compared to age-matched controls (E). (*n*=3/group).

F. Steady state levels of Aβ40 is not significantly altered in P0→3 month old Tg2576 mice expressing mIFNγ as measured by ELISA using RIPA soluble and SDS soluble brain extracts. (*n*=3/group).

Suppl. Fig 6. No change in Aβ degrading enzymes and BACE protein levels in mIFNγ expressing P2→5 month old TgCRN8 mice.

A. Q-PCR analysis of Neprilysin and IDE levels in brains of 5 month old TgCRND8 mice expressing EGFP or mIFNγ show no significant changes in mRNA transcript levels. Relative quantitation of mRNA expression was performed using the comparative cycle threshold method. The expression levels of genes were normalized using β-actin levels from the corresponding samples. Data represents averaged fold change values obtained from IFNγ expressing mice relative to averaged values obtained from EGFP expressing mice. (*n*=4/group).

B-C. Representative immunoblot of BACE1 immunoreactivity in P2→5 month old TgCRND8 mice expressing mIFNγ or EGFP (B). Intensity analysis of BACE1 bands normalized to β-actin show no significant changes in BACE1 levels in TgCRND8 mice expressing mIFNγ mice compared to control mice (C). (*n*=3/group).

Suppl. Fig 7. mIFNγ treatment of primary mouse microglia cells results in increased CD68 expression and efficacious Aβ phagocytosis *in vitro.*

A-C. Representative image of anti-CD68 immunostaining showing upregulation of CD68 (red fluorescence) in mIFNγ treated primary mouse microglia cells (B) compared to untreated glia (A). Blue fluorescence depicts the DAPI stained glial nuclei. Quantitation of fluorescence intensity was done using Metamorph software (C). At least four different fields of view with ten or more cells per field were used for the analysis. Magnification 400X, *p<0.05.

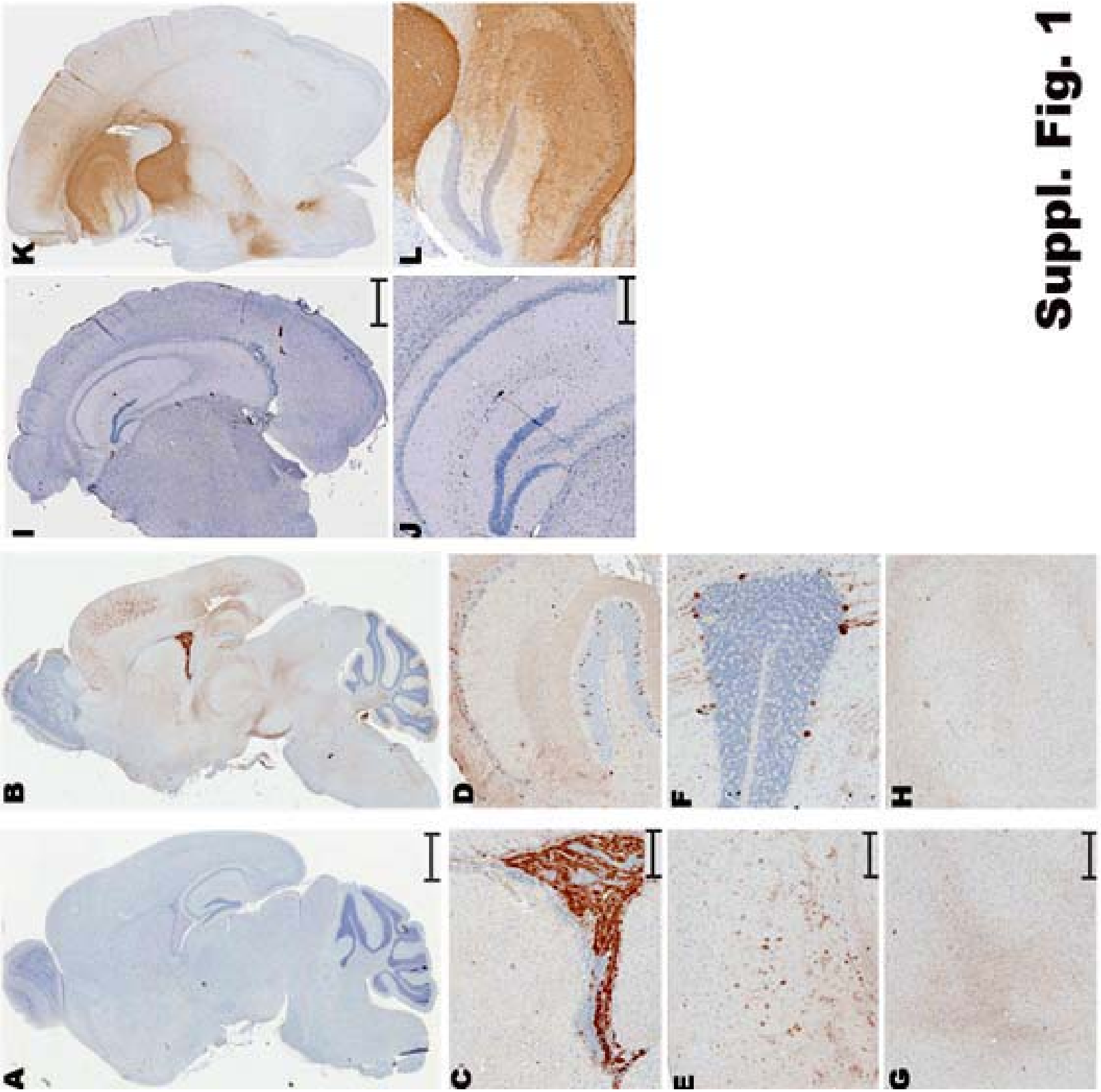
D-F. mIFNγ treatment of primary mouse microglia results in more efficient phagocytosis of fAβ42-Hilyte568 (red fluorescence, E) compared to unstimulated glia (D). Blue fluorescence depicts the DAPI stained glial nuclei. Quantitation of fluorescence intensity was done using Metamorph software (F). At least ten different fields of view with two or more cells per field were used for the analysis. Magnification, 600X, *p<0.05.

Suppl. Fig 8. No evidence of colocalization of peripheral macrophages or monocytes with cored plaques in 5 month old mIFNγ expressing mice.

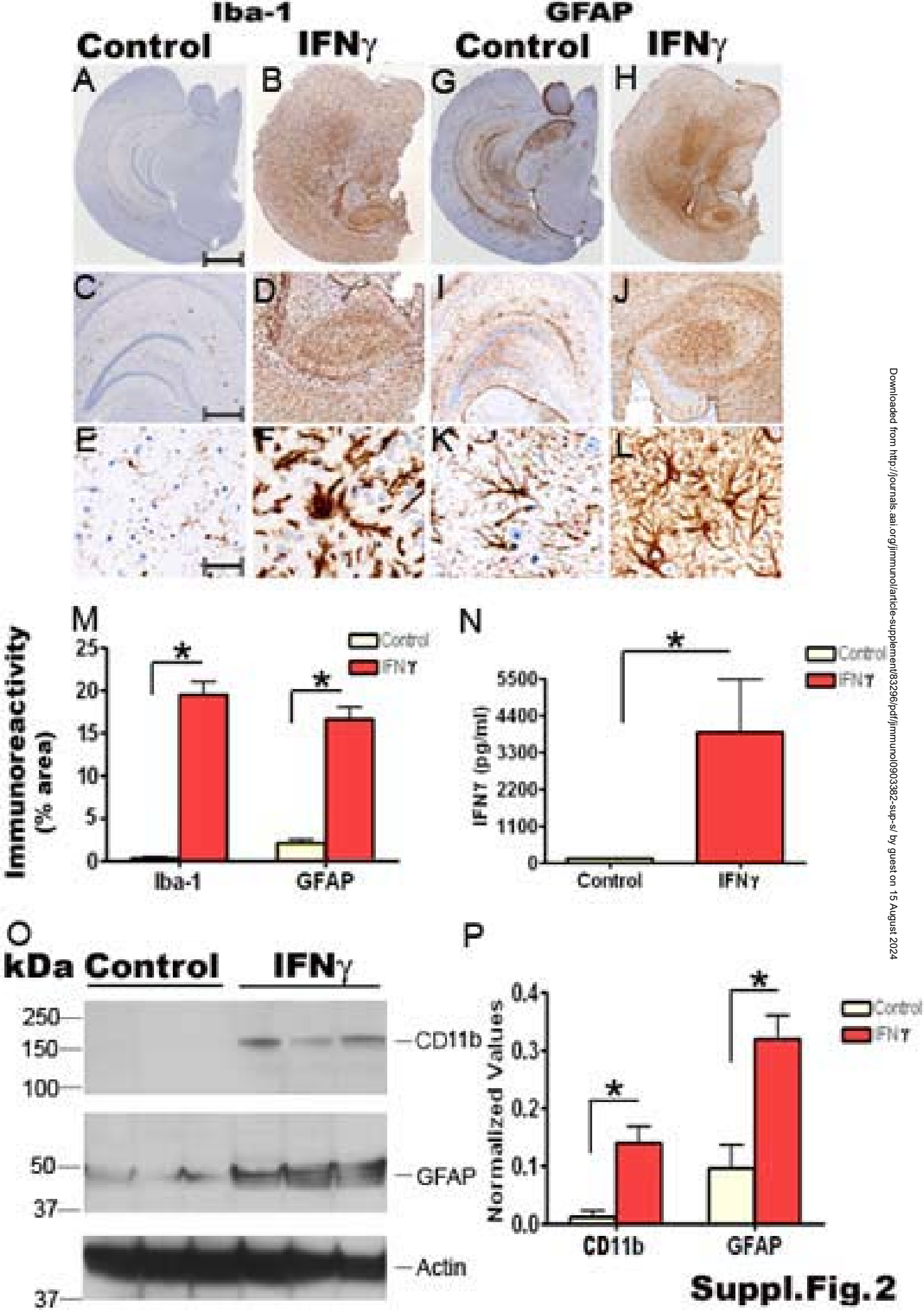
A-D. Representative paraffin embedded sections depicting CD45 immunostaining in wild type mouse spleen (A), brains of P2→5 month old mice expressing EGFP (B) or mIFNγ (C) and brains of 4→5.5 month old mIFNγ (D) expressing mice are depicted. The sections have been counterstained with hematoxylin and Congo Red. Insets show high magnification views of splenic

macrophages (A) or Congo Red stained Aβ plaques (B-D). *Scale Bar,* A-D, 120μm; insets, 15μm. (*n*=5/group).

E-H. Representative paraffin embedded sections depicting Ly-6c immunostaining in wild type mouse spleen (E), brains of P2→5 month old mice expressing EGFP (F) or mIFNγ (G) and brains of 4→5.5 month old mIFNγ (H) expressing mice. The sections have been counterstained with hematoxylin and Congo Red. Insets show high magnification views of splenic monocytes (E), Congo Red stained Aβ plaques (F-H) or Ly-6C positive monocytes in the brain (G-H). *Scale Bar,* E-F, 120μm; G-H, 600μm; insets, 15μm. (*n*=5/group).



Suppl. Fig. 1



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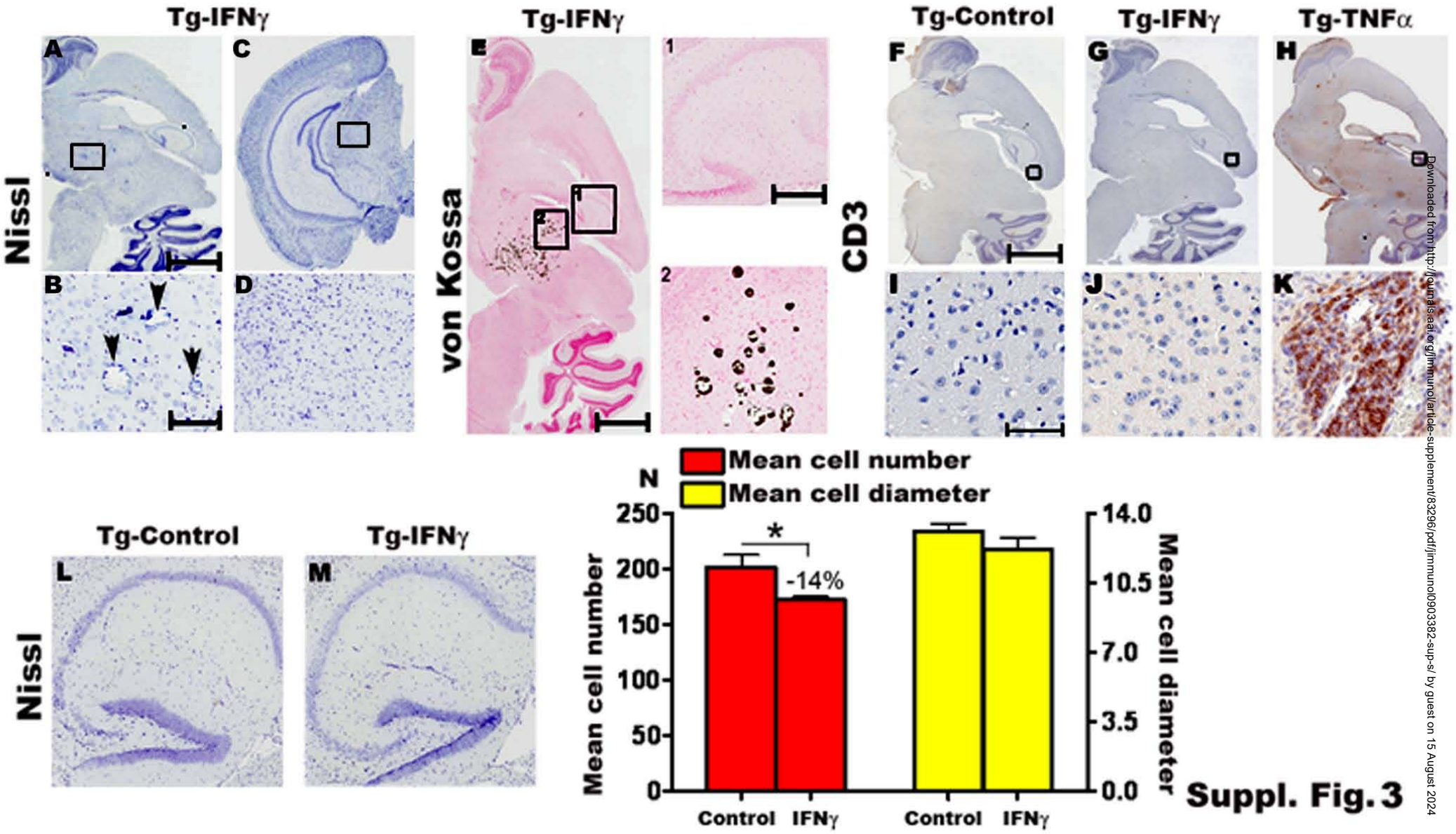

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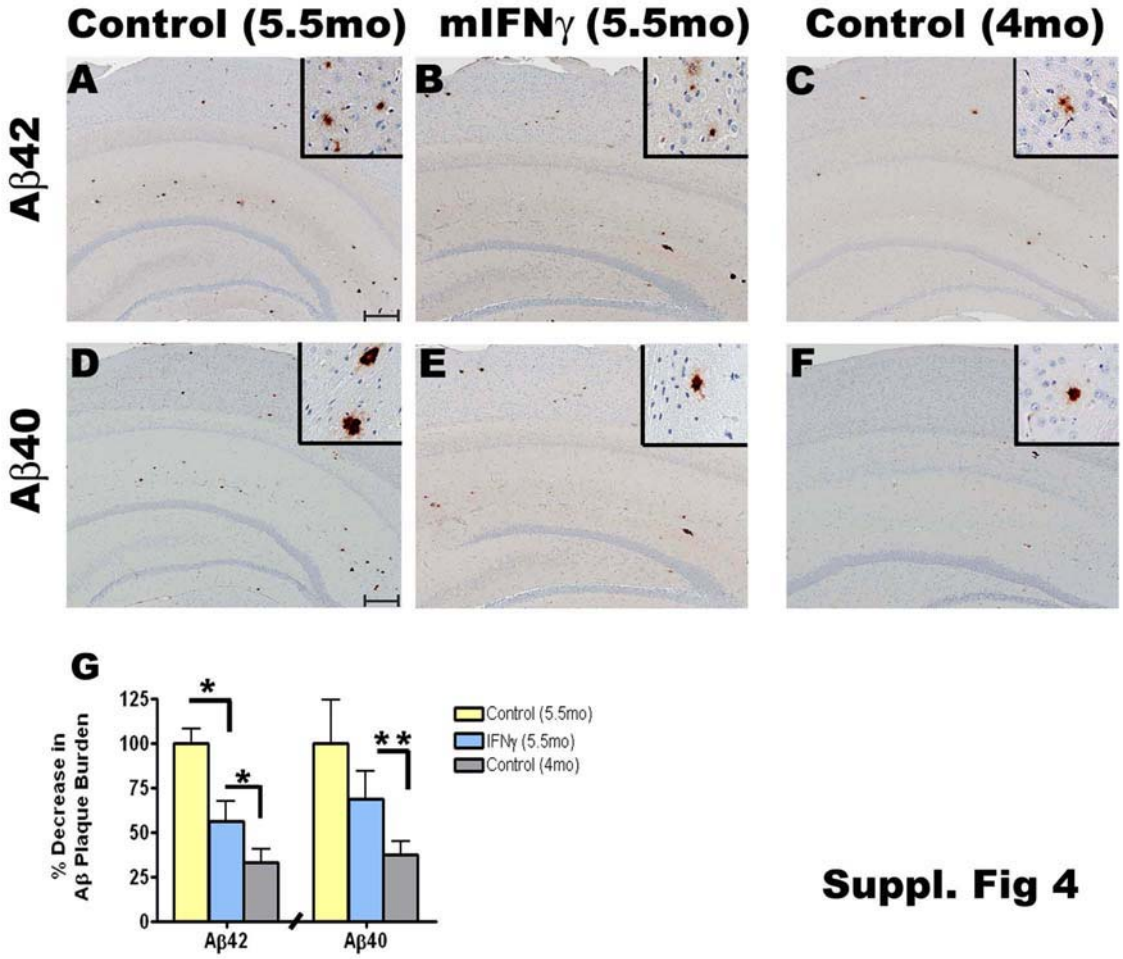

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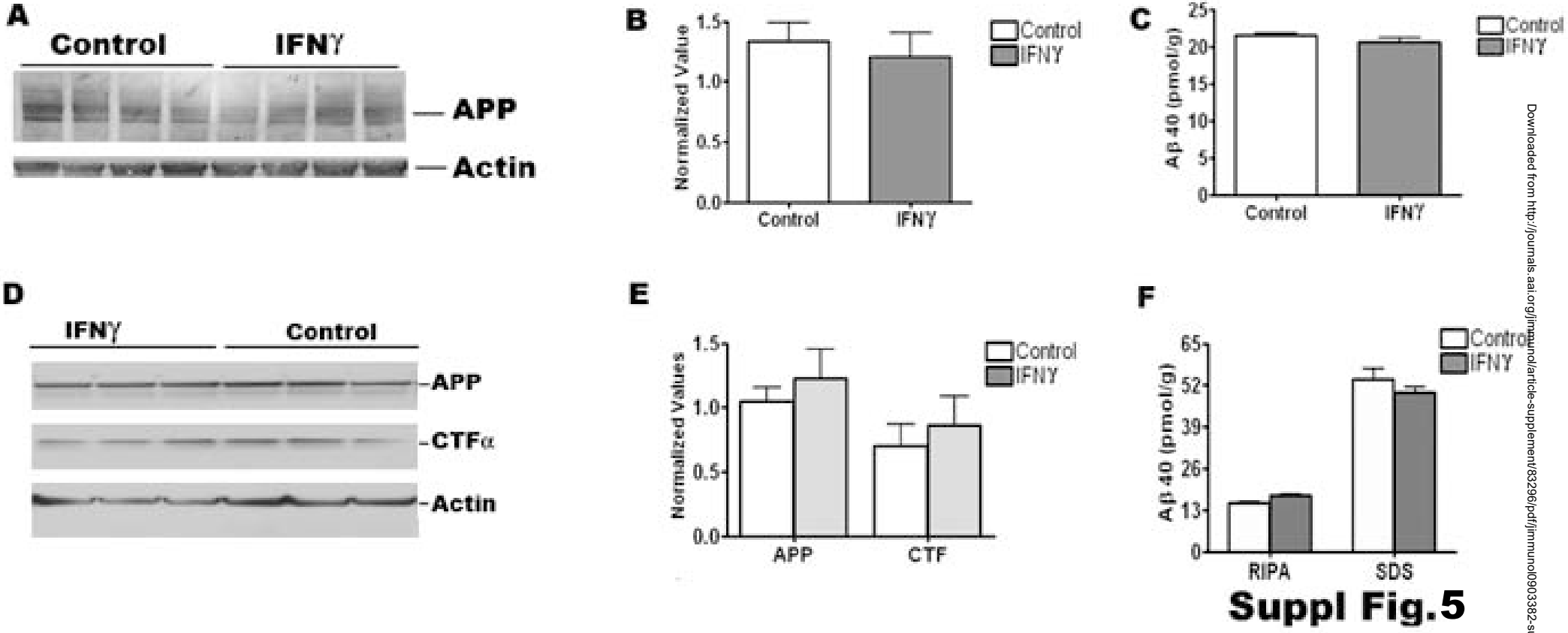

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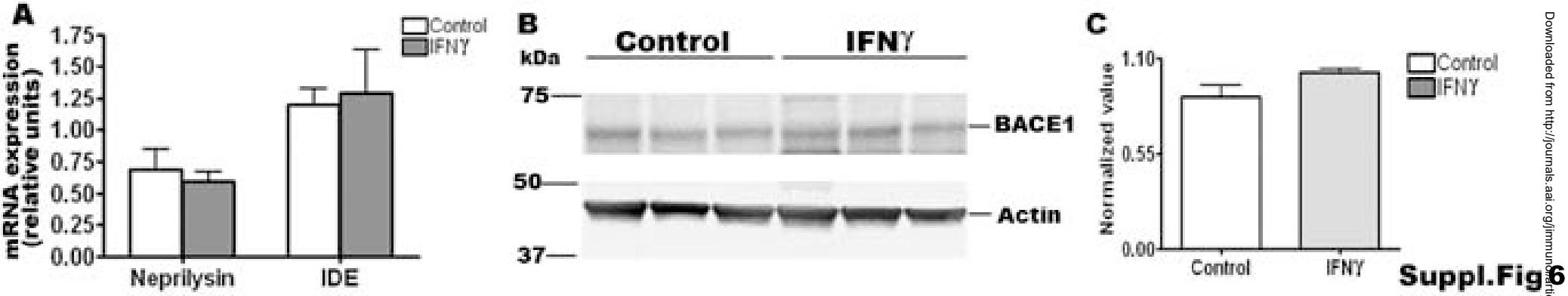

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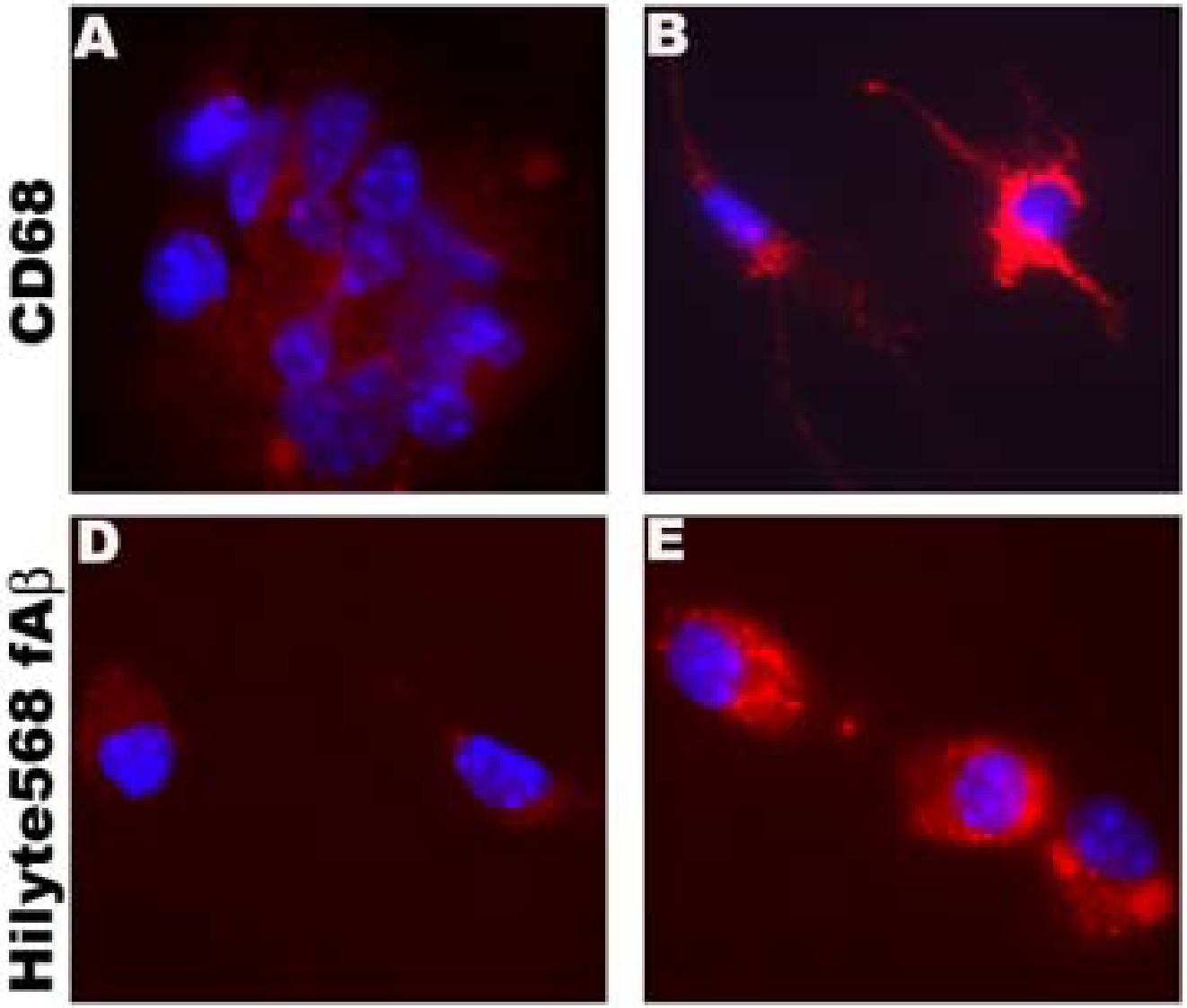
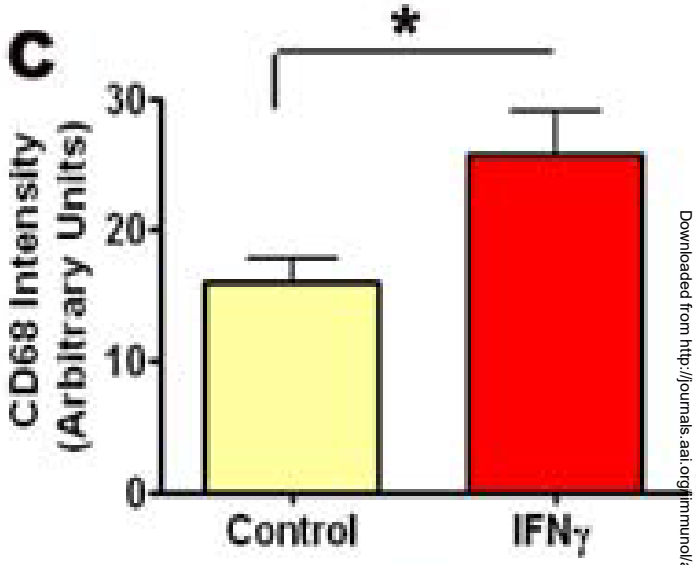
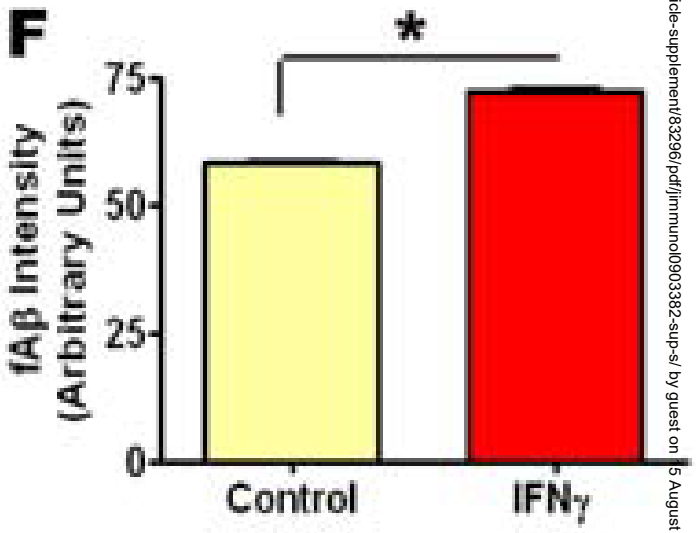
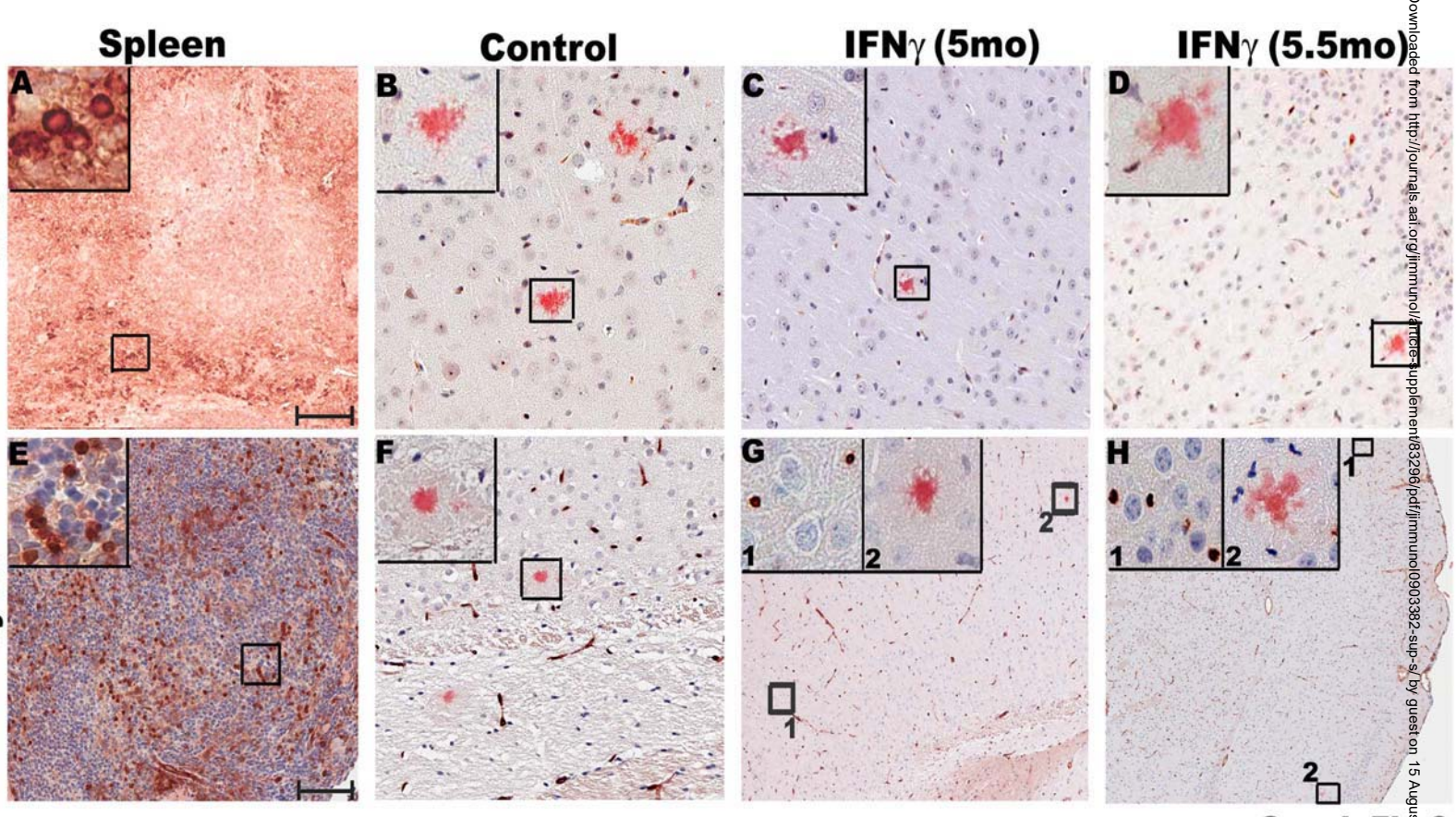






Suppl. Fig 7



Suppl. Fig 8