

**Testing a MultiTEP-based combination vaccine to reduce A β and tau pathology in Tau22/5xFAD
bigenic mice**

Hayk Davtyan, Armine Hovakimyan, Sepideh Kiani Shabestari, Tatevik Antonyan, Morgan A. Coburn,
Karen Zagorski, Gor Chailyan, Irina Petrushina, Olga Svystun, Emma Danhash, Nikolai Petrovsky, David
H. Cribbs, Michael G. Agadjanyan, Mathew Blurton-Jones, Anahit Ghochikyan

Supplementary Information

Figure S1

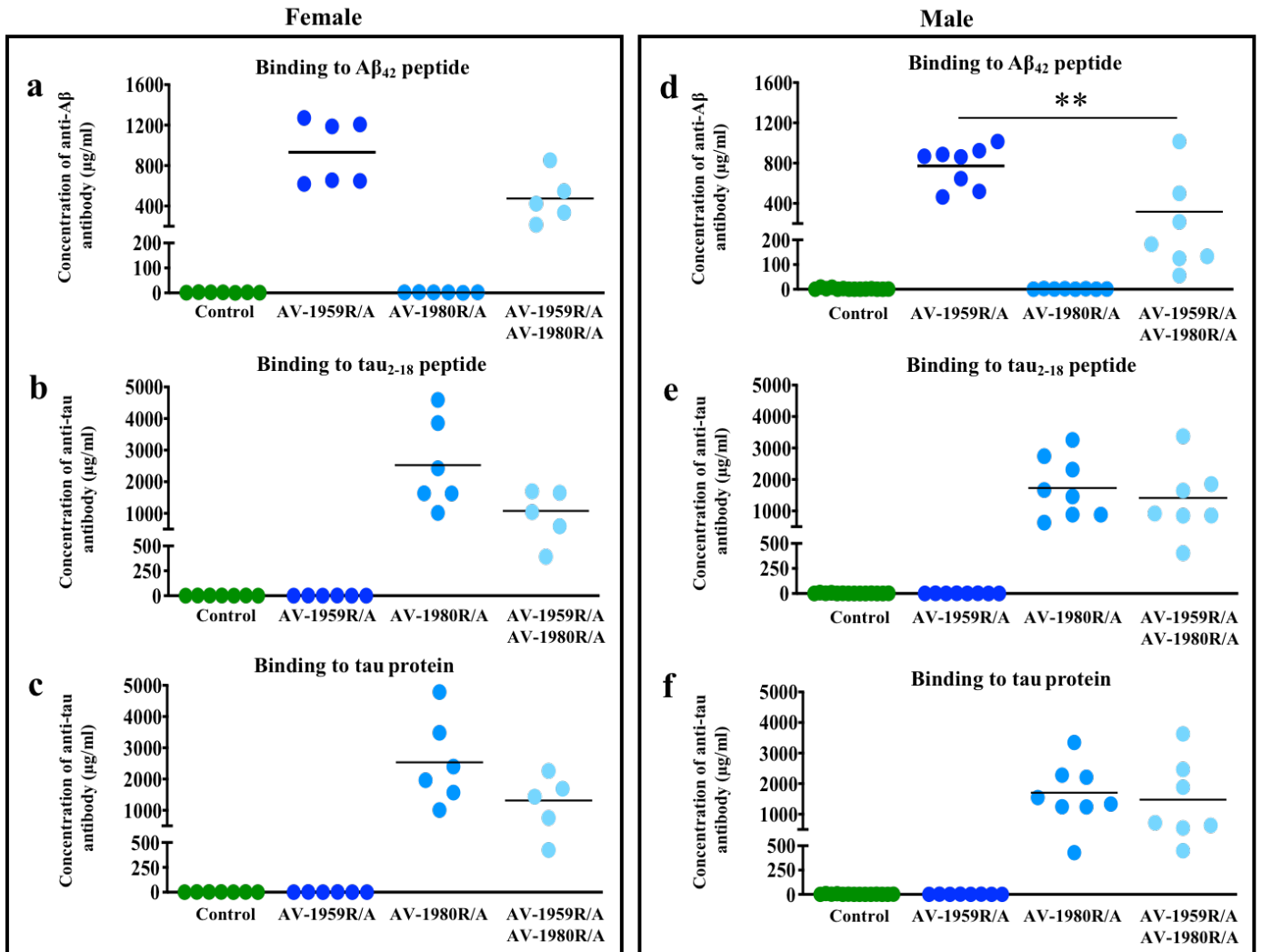


Figure S1. Humoral immune responses in female and male T5x mice vaccinated with different vaccines. Concentration of antibodies binding to A β_{42} (a, d), tau₂₋₁₈ peptide (b, e) and full-length recombinant tau protein (c, f) were detected in sera by ELISA. Lines represent average. Statistically significant differences were examined using unpaired *t*-test (***p* ≤ 0.01; Control group - n=8 for female and n=14 for male; AV-1959R/A group - n=6 for female and n=8 for male, AV-1980R/A group - n=6 for female and n=8 for male and AV-1959R/A+AV-1980R/A group - n=5 for female and n=7 for male).

Figure S2

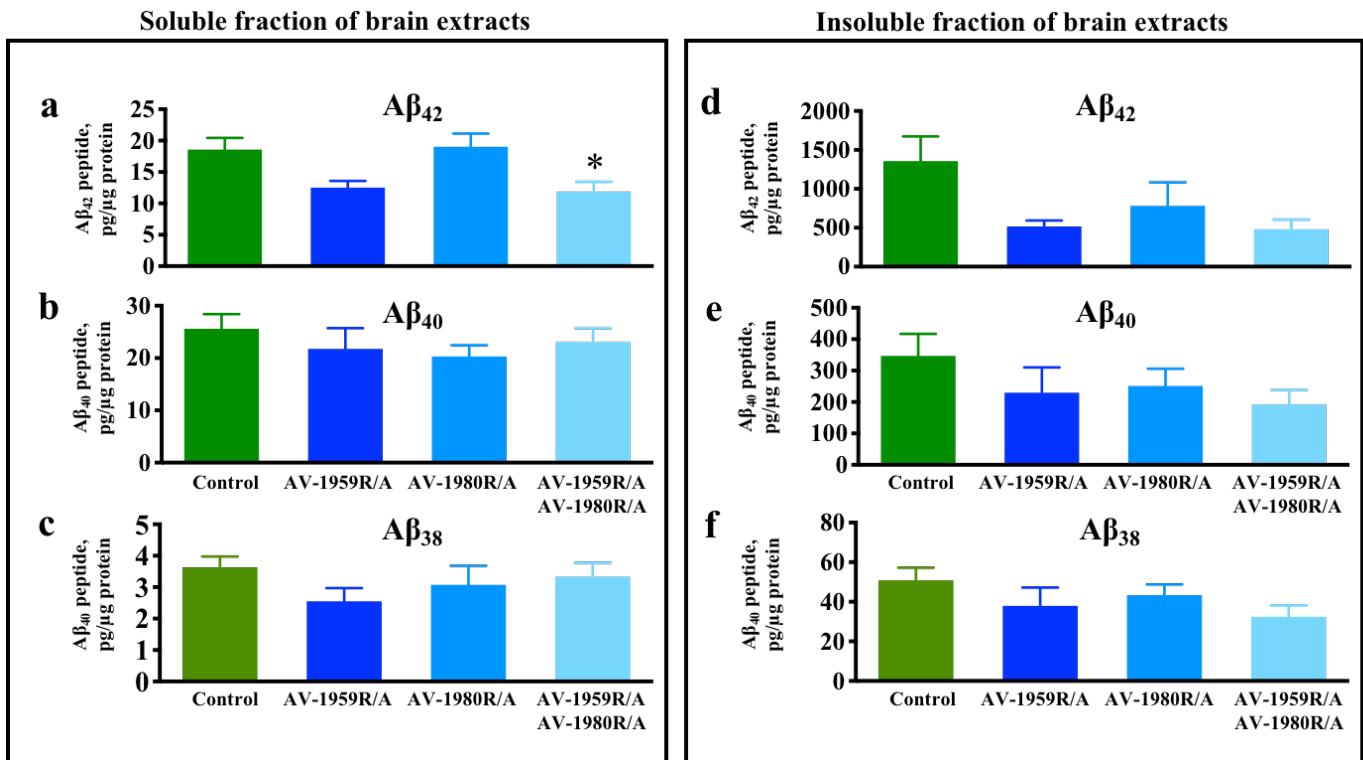


Figure S2. Effect of protein vaccination on $A\beta$ proteins in female, T5x mice. Level of human $A\beta_{42}$ (a and d), $A\beta_{40}$ (b and e) and $A\beta_{38}$ (c and f) peptides in brain soluble (a-c) and insoluble (d-f) extractions were analyzed by MSD assay. Error bars represent average \pm SEM. Statistical significance were calculated against Control group using ANOVA test ($*p \leq 0.01$; Control group - n=8 for female and n=14 for male; AV-1959R/A group - n=6 for female and n=8 for male, AV-1980R/A group - n=6 for female and n=8 for male and AV-1959R/A+AV-1980RA group - n=5 for female and n=7 for male).

Figure S3

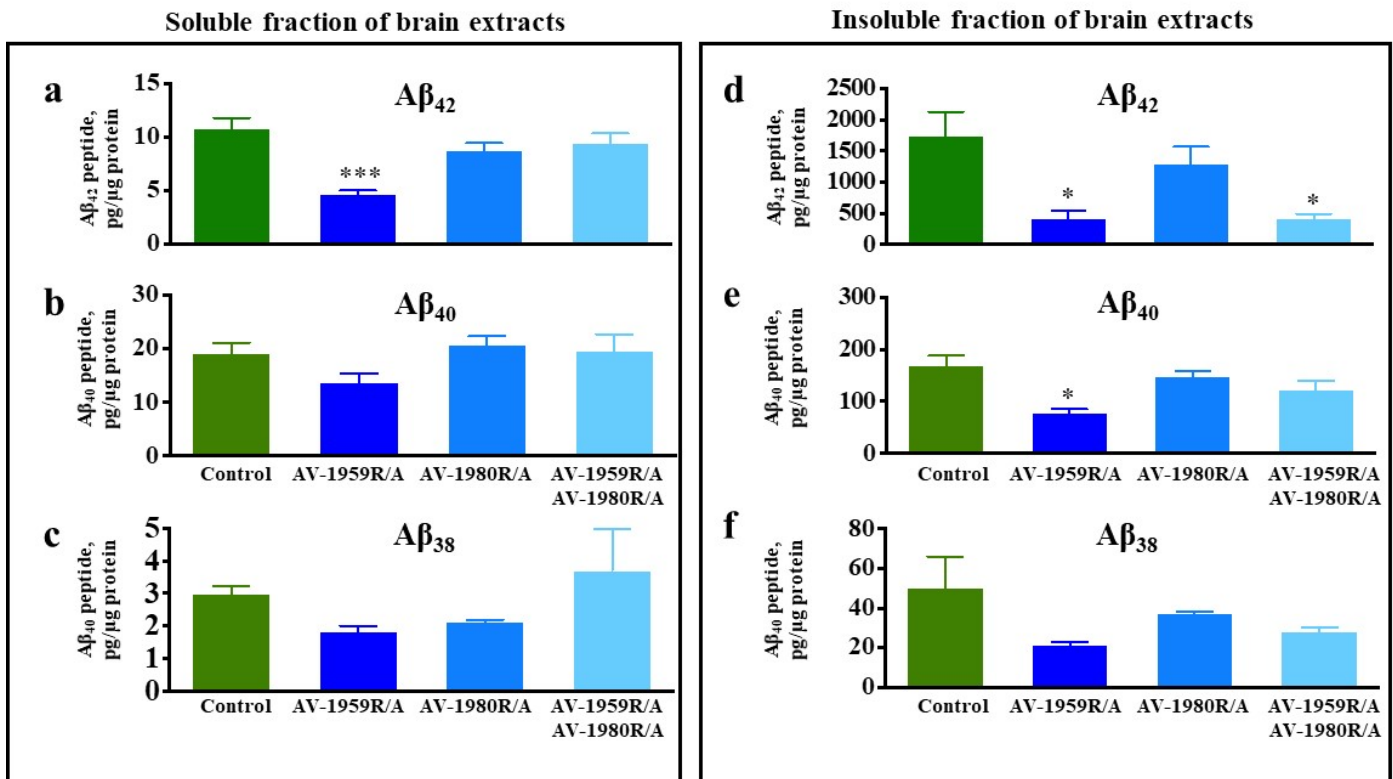


Figure S3. Effect of protein vaccination on A β proteins in male, T5x mice. Level of human A β_{42} (a and d), A β_{40} (b and e) and A β_{38} (c and f) peptides in brain soluble (a-c) and insoluble (d-f) extractions were analyzed by MSD assay. Error bars represent average \pm SEM. Statistically significance were calculated against Control group using ANOVA test (* $p \leq 0.05$; *** $p \leq 0.001$; Control group - n=8 for female and n=14 for male; AV-1959R/A group - n=6 for female and n=8 for male, AV-1980R/A group - n=6 for female and n=8 for male and AV-1959R/A+AV-1980RA group - n=5 for female and n=7 for male).

Figure S4

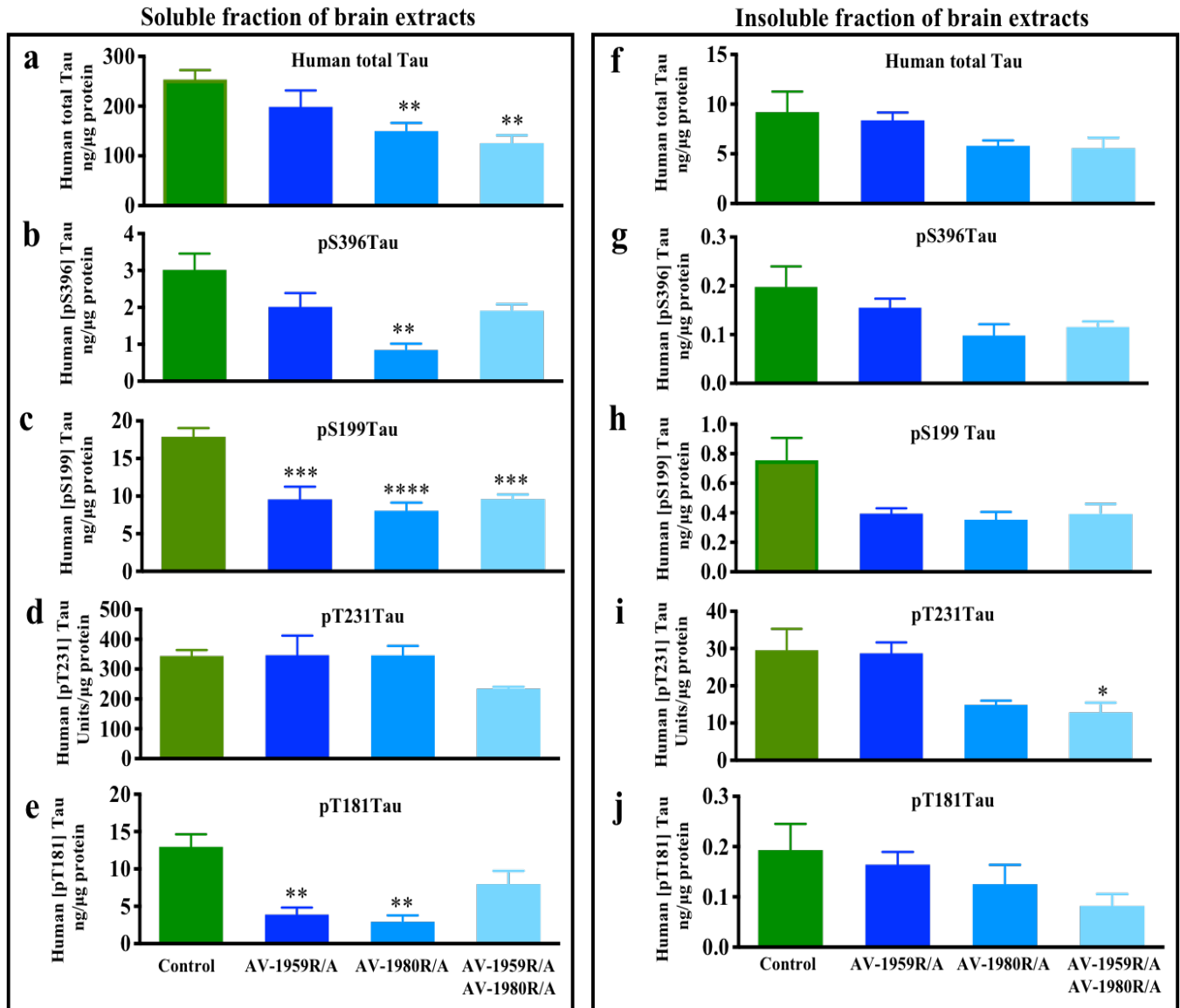


Figure S4. Effect of protein vaccination on tau proteins in female, T5x mice. Level of human total tau protein (**a, f**) and several phosphorylated tau species (**b-e** and **g-j**) in brain soluble (**a-e**) and insoluble (**f-j**) extractions were analyzed by ELISA. Error bars represent average \pm SEM. Statistical significance were calculated against Control group using ANOVA test ($*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$; Control group - n=8 for female and n=14 for male; AV-1959R/A group - n=6 for female and n=8 for male, AV-1980R/A group - n=6 for female and n=8 for male and AV-1959R/A+AV-1980RA group - n=5 for female and n=7 for male).

Figure S5

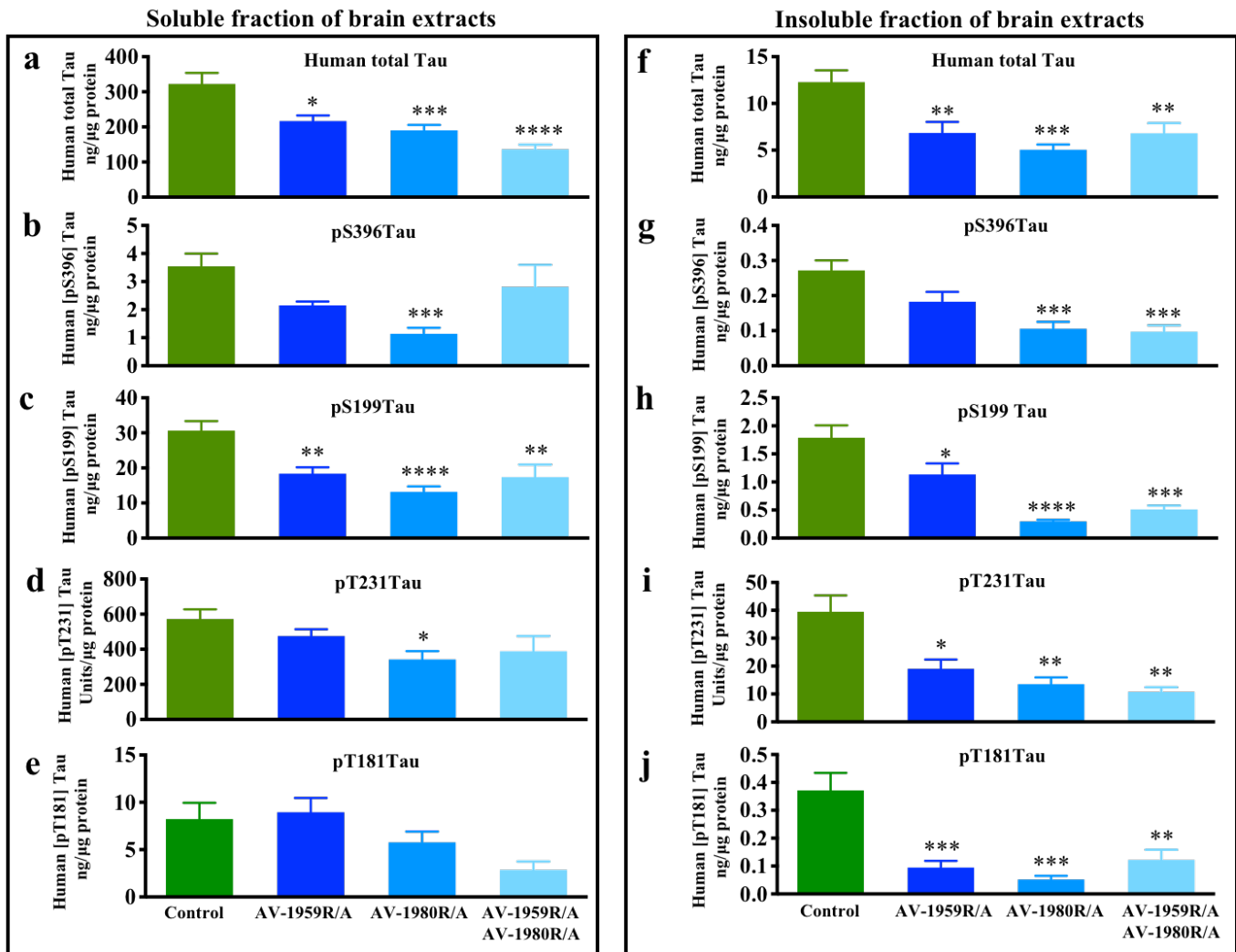


Figure S5. Effect of protein vaccination on tau proteins in male, T5x mice. Level of human total tau protein (a, f) and several phosphorylated tau species (b-e and g-j) in brain soluble (a-e) and insoluble (f-j) extractions were analyzed by ELISA. Error bars represent average \pm SEM. Statistical significance were calculated against Control group using ANOVA test ($*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$; Control group - n=8 for female and n=14 for male; AV-1959R/A group - n=6 for female and n=8 for male, AV-1980R/A group - n=6 for female and n=8 for male and AV-1959R/A+AV-1980RA group - n=5 for female and n=7 for male).

Figure S6

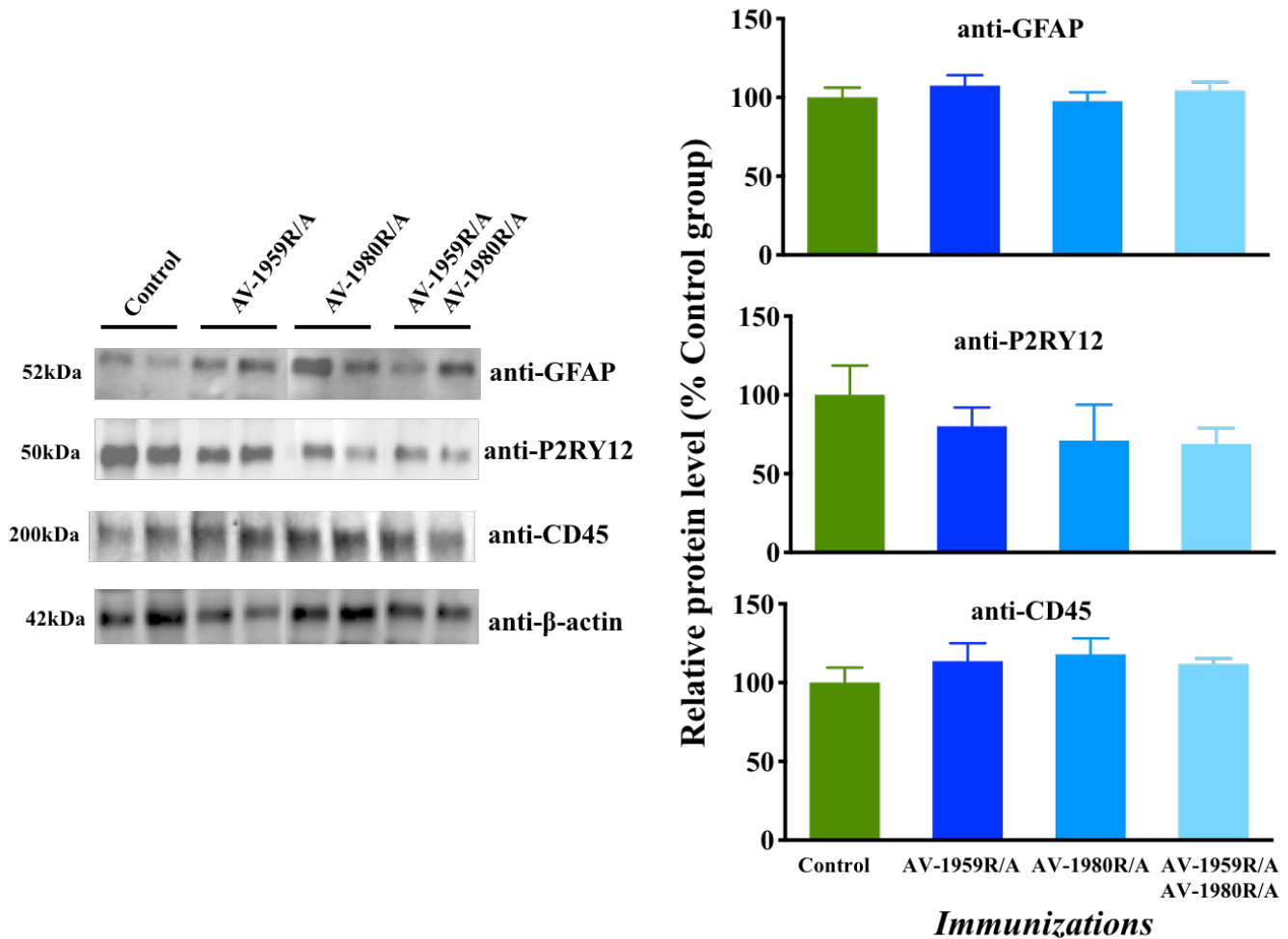


Figure S6. Vaccination with protein vaccines did not change astrogliosis and microgliosis in brains of T5x mice. The levels of GFAP, P2RY12 and CD45 proteins in the soluble fraction of the brain extracts were analyzed by Western blotting and quantitatively determined by densitometric analysis with normalization against β -actin. The relative protein level in the brains of vaccinated mice is presented as a percentage of the protein level in the brains of control mice. Error bars represent average \pm SEM. Statistically significant differences were examined using one-way ANOVA (n = 12 for Control group and n=11 for all vaccinated groups).

Figure S7

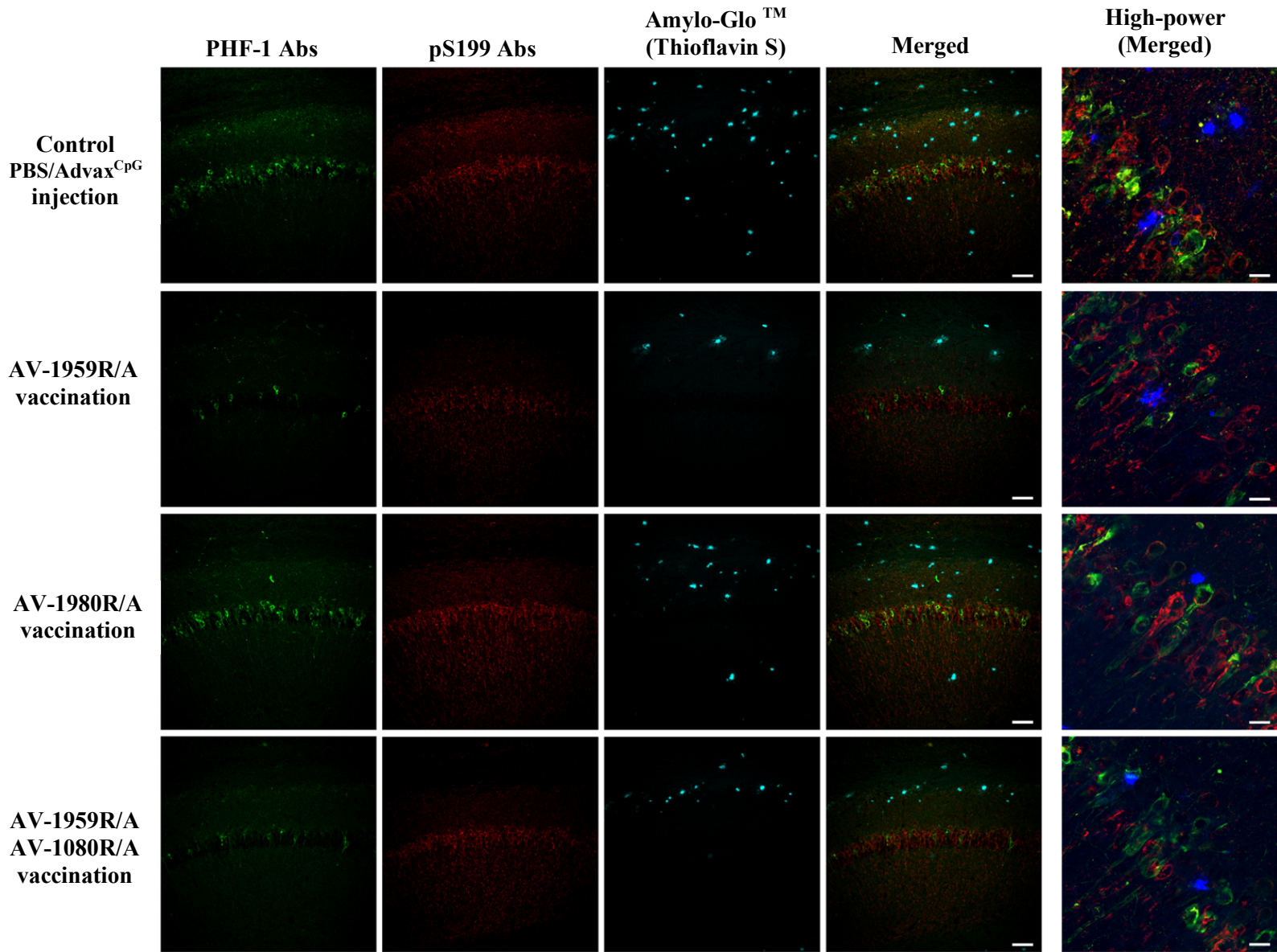


Figure S7. Reduced β -amyloid and tau pathology in T5x mice following vaccination with different proteins. Representative pictures of brain CA1 region immunostained for Amylo-Glo™ (ThS, anti-A β) and pS199 and PHF-1 (anti-tau) antibodies. Scale: 60 μ m (lowpwr) and 15 μ m (highpwr).