Long double stranded RNA is present in scrapie infected cells and tissues

Yervand Karapetyan
The Scripps Research Institute, Scripps Florida
Current address: Yale University School of Medicine

The nature of the infectious agent causing scrapie and other Transmissible Spongiform Encephalopathies remains an enigma despite decades of research efforts. The protein-only prion hypothesis posits that abnormal conformer of a host protein is the infectious agent. Virus and virino theories include host-independent nucleic acid as the genome of the infectious agent in addition to protein component (in case of virino a host protein and in case of virus a viral protein).
Viral or sub-viral nucleic acids have long been sought in scrapie to explain the existence of multiple agent strains.
Many different approaches were undertaken to find such nucleic acid. Despite that no scrapie specific nucleic acid sequences have been found in infected cells or tissues.
Most viruses induce synthesis of long double stranded RNA (dsRNA) during their replication in cells. Therefore the presence of long dsRNA would be an indication of viral infection in cells. J2 monoclonal antibody against long dsRNA is a great tool for easy screening of cells and tissues for the presence of suspected unknown viral infection.
This antibody has not been used for testing of scrapie infected tissues.
Evidence is presented here for long dsRNA in scrapie infected cells and tissues. Such dsRNA is also found in scrapie free tissue culture cells.

J2 monoclonal antibody

Recognizes double-stranded RNA (dsRNA) provided that the length of the helix is ≥ 40 bp

dsRNA-recognition is independent of the sequence and nucleotide composition of the antigen

All naturally occurring dsRNA investigated up to now (40-50 species) as well as poly(I)•poly(C) and poly(A)•poly(U) have been recognized by J2

Schonborn et al 1991

In a systematic study of different viruses J2 detected dsRNA in cells infected with:

•positive-strand RNA viruses
•double-stranded RNA viruses
•DNA viruses

But not negative-strand RNA viruses

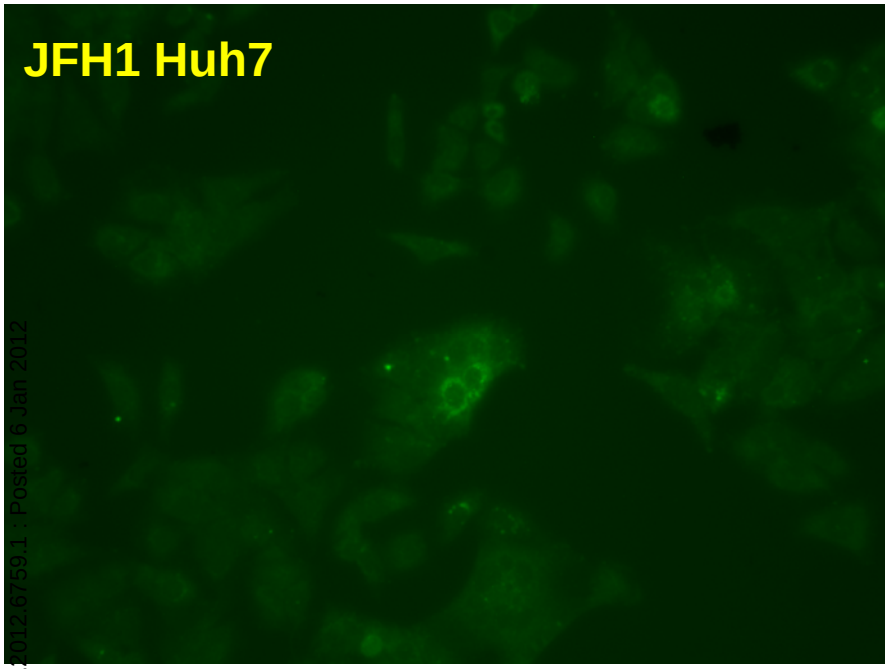
Targett-Adams et al 2008

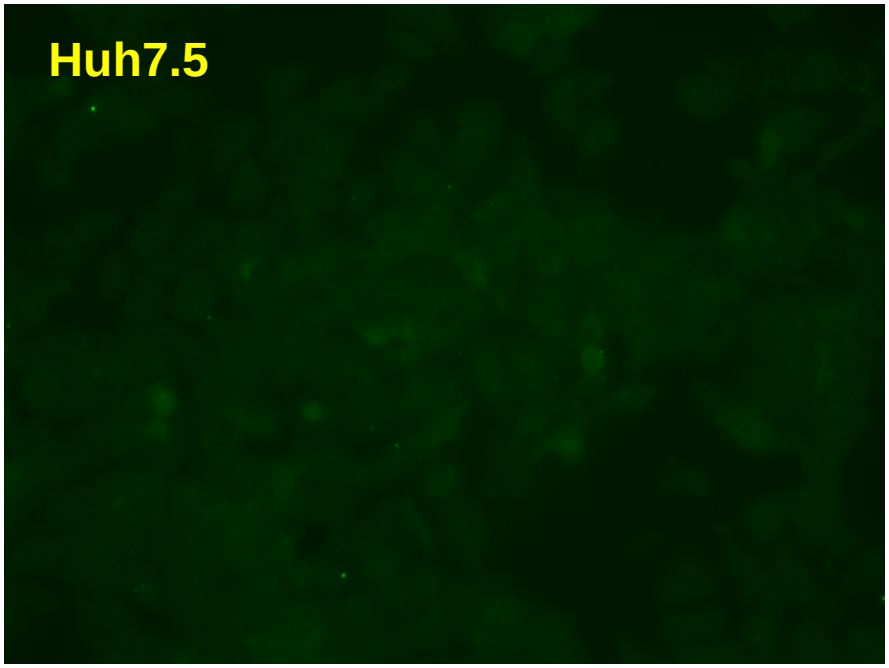
JFH1 Huh7 cells (human hepatoma cells harboring hepatitis C replicon) and Huh7.5 cells were used as a positive and negative control in ummunofluorescence experiments when probing PK1 (mouse neuroblastoma cell clone of N2A) and RML infected PK1 cells.
dsRNA was detected in both PK1 and RML PK1 cells in the cytoplasm. This signal was abolished with RNase A treatment in low salt conditions.

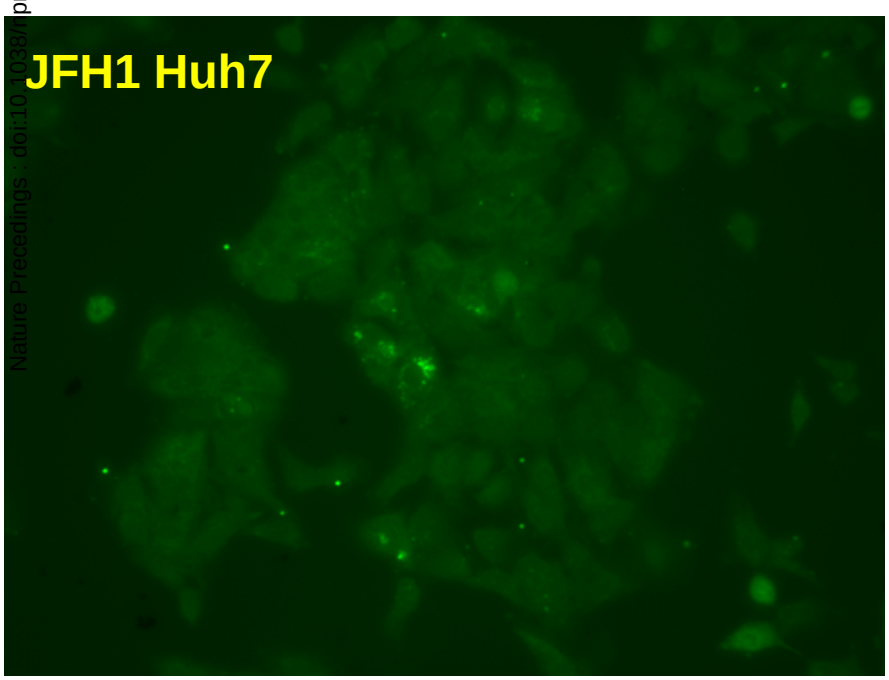
J2 antibody was also used for immunoblotting of dsRNA as described (Veliceasa et al 2006). Crude RNA extracts from JFH1 Huh7, Huh7.5, PK1 and PK1 RML cells were used for this. Results showed the presence of replication intermediate-RI (upper part of the gel slot) and replicative form-RF (seen as a strong band bellow RI) in JFH1 Huh7 that was absent in Huh7.5. In PK1 and RML PK1 in addition to large amounts of dsRNA in gel slots several bands were seen with the most prominent one being a duplet with a molecular weight much lower than that of RF of HCV replicon. Additionally a lower band of similar size was present in all four samples.

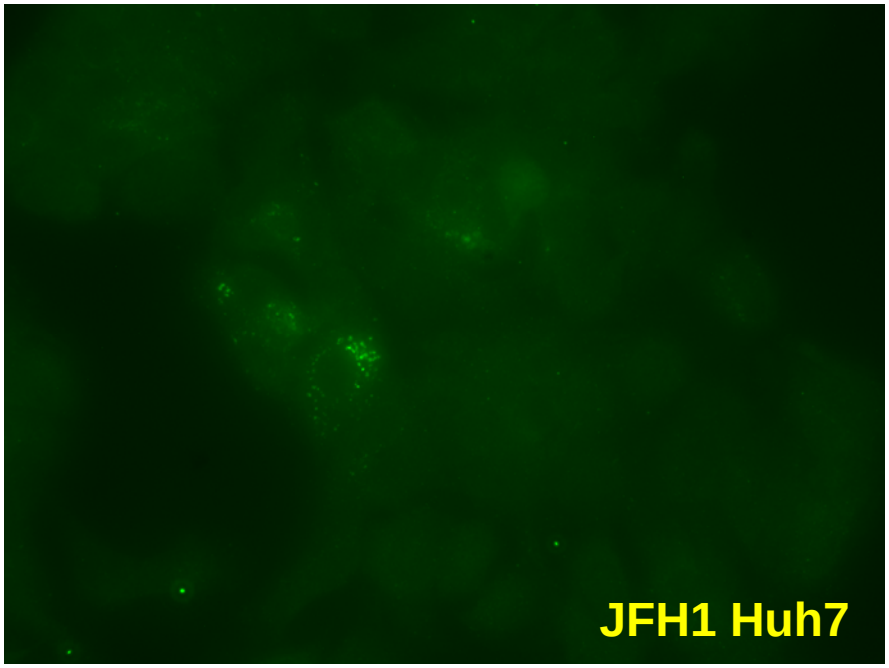
J2 antibody was recently used for successful detection of viral dsRNA in formalin-fixed paraffin-embedded tissues (Richardson et al 2010). Here an attempt was made to detect dsRNA in 22L scrapie infected tissues fixed in Carnoy's solution and embedded in paraffin. Proteinase K treatment was used as described (Karapetyan et al 2009), followed by inhibition in glycine and short post-fixation in formalin to expose dsRNA for detection. As a result dsRNA was detected in scrapie infected brain predominantly in the cytoplasm of large neurons in the cortex and brainstem. Nuclear staining was also detected in some neurons. In uninfected brain nuclear staining of some Purkinje cells was detected. Otherwise the staining in control brain was largely absent.

The results are shown bellow in pictures. For the first time experimental evidence is provided for the presence of long dsRNAs in scrapie infected cells and tissues.
These molecules deserve further characterization of their sequences and relationship to scrapie.

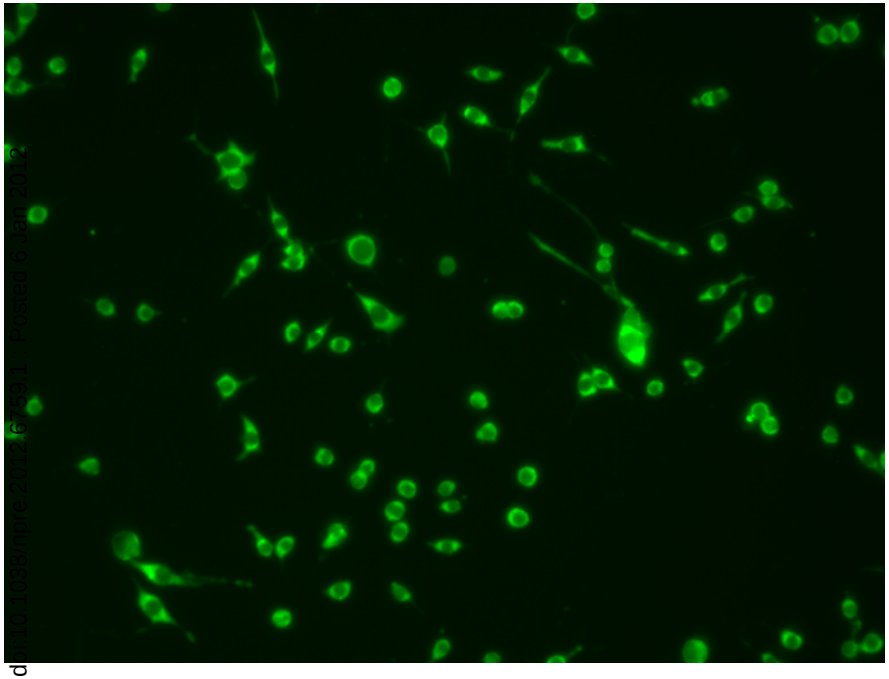




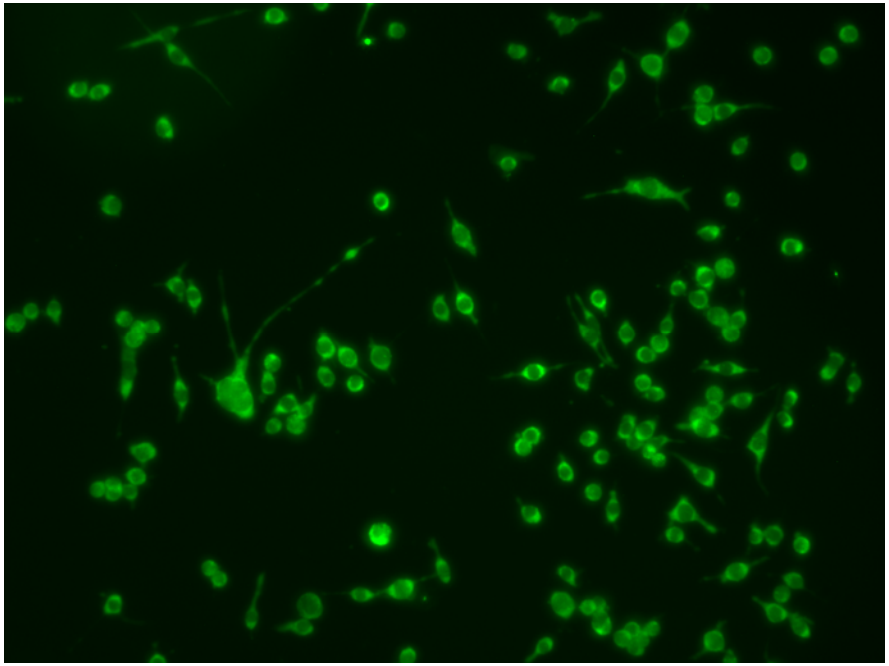


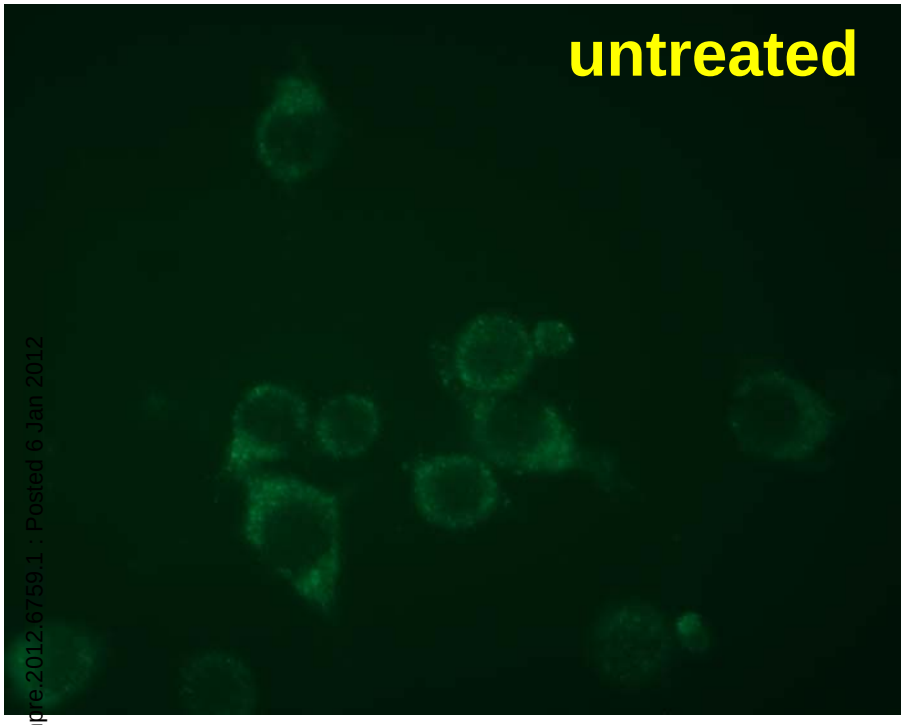


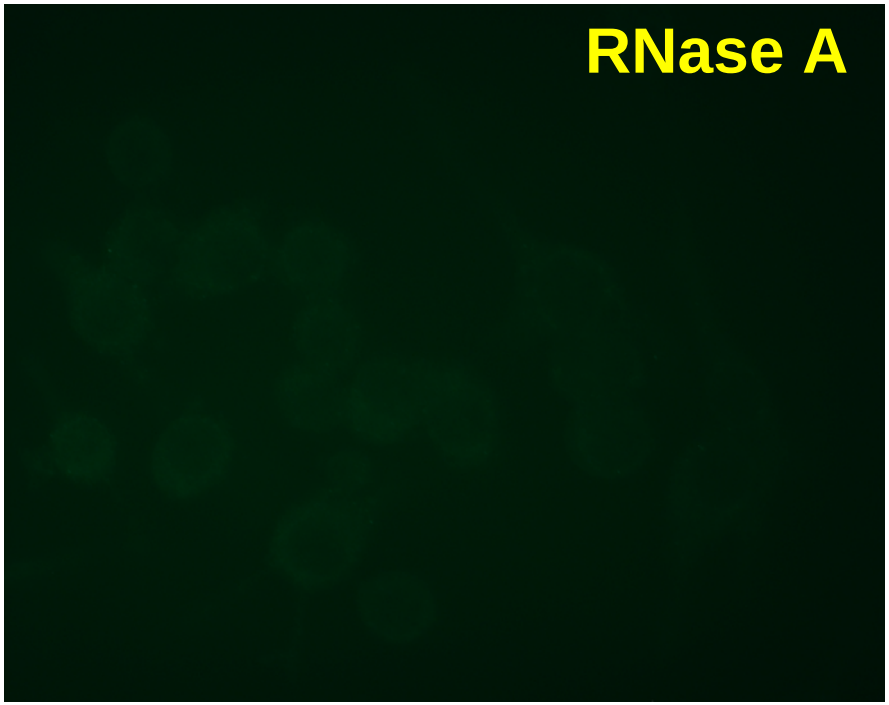
PK1



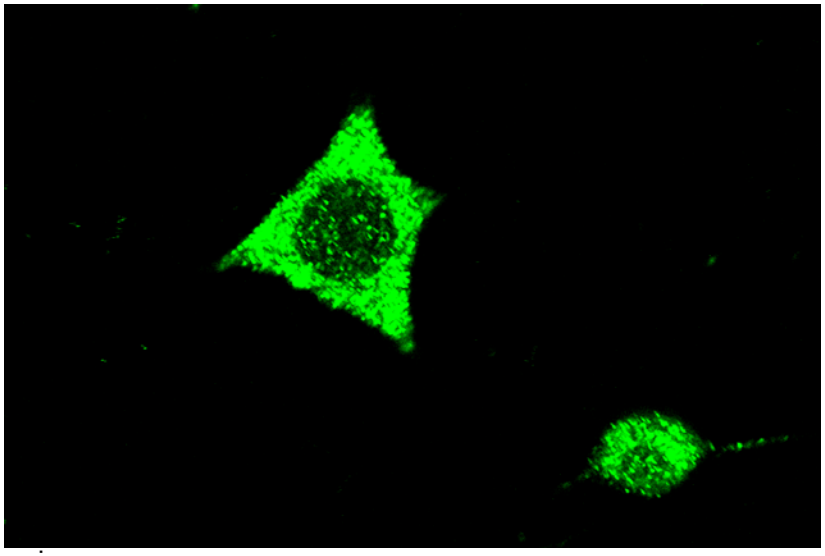
PK1 RML





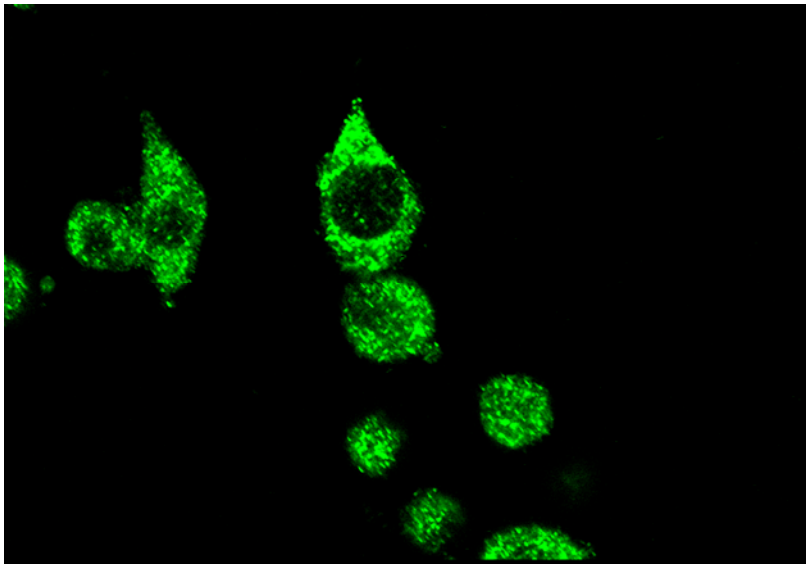


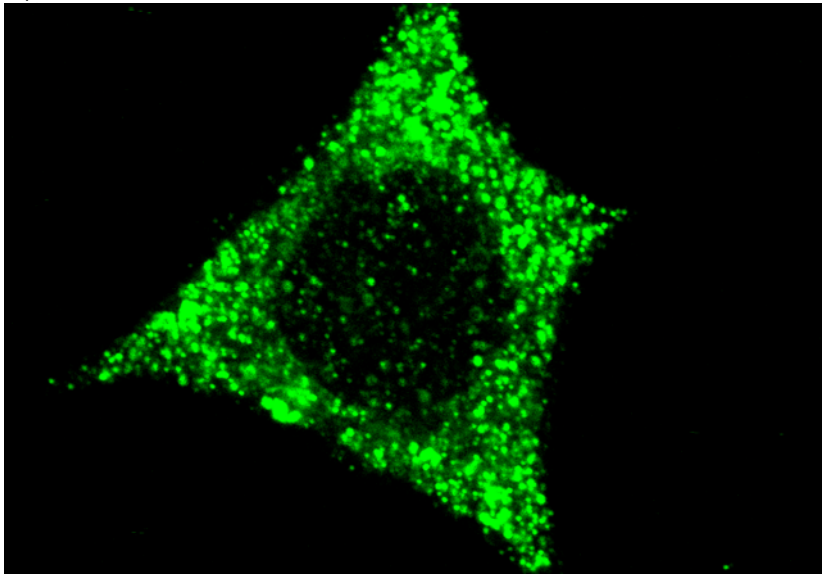
PK1

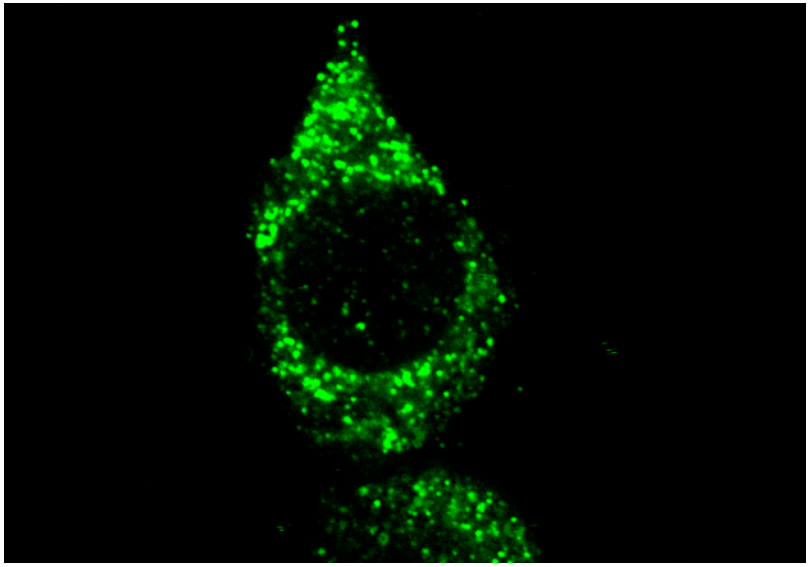


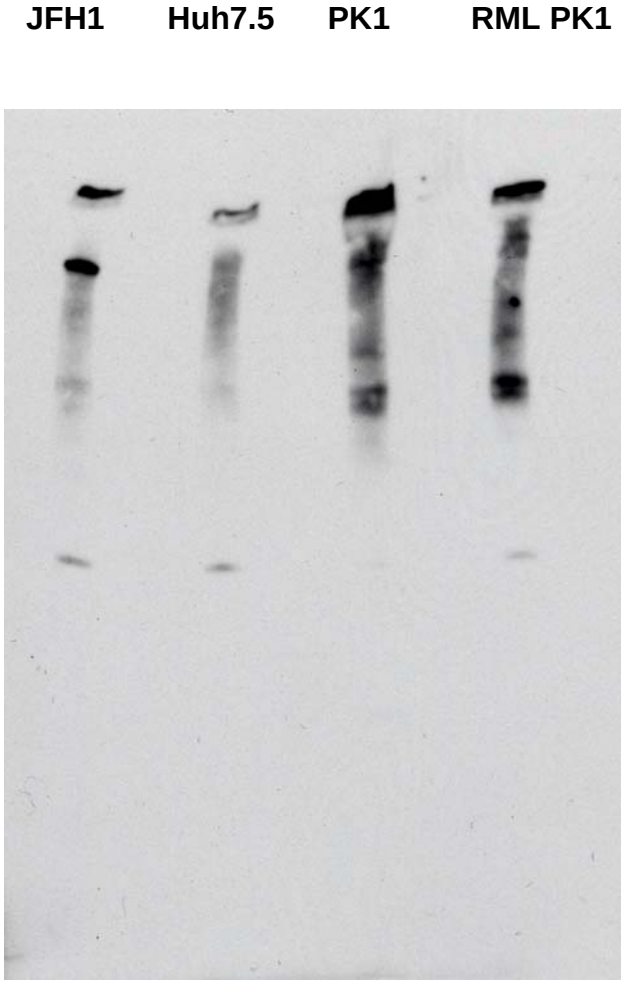
759.

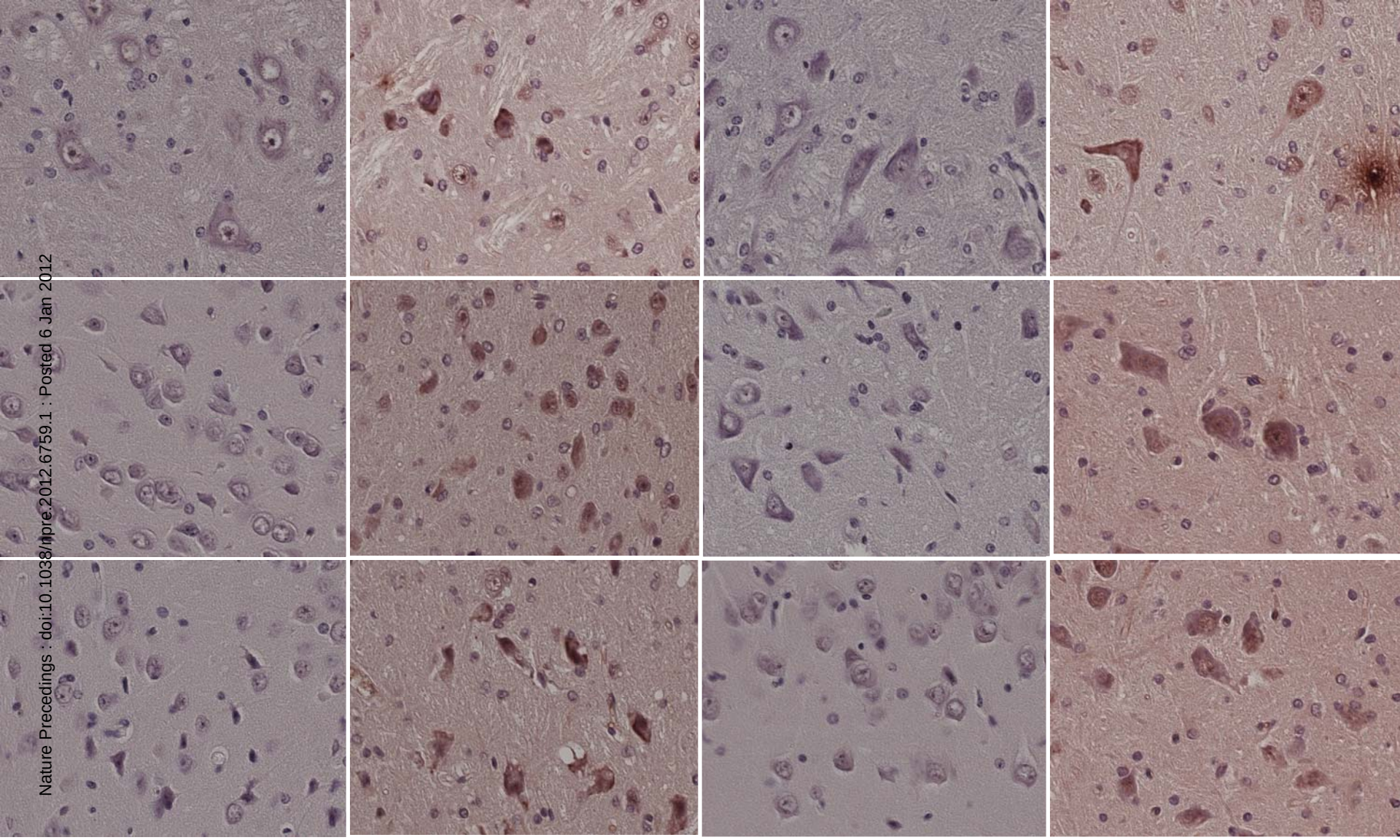
PK1



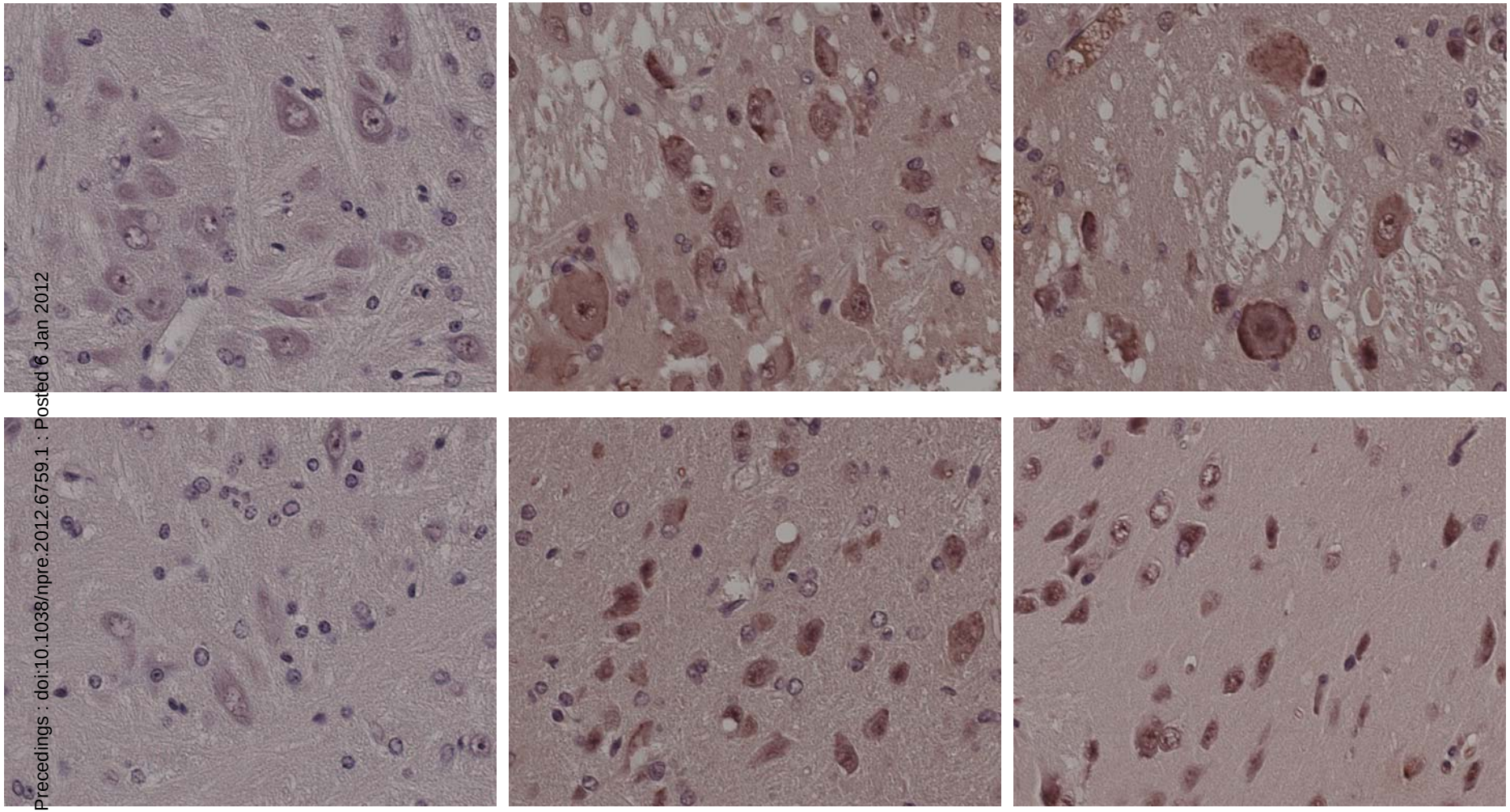




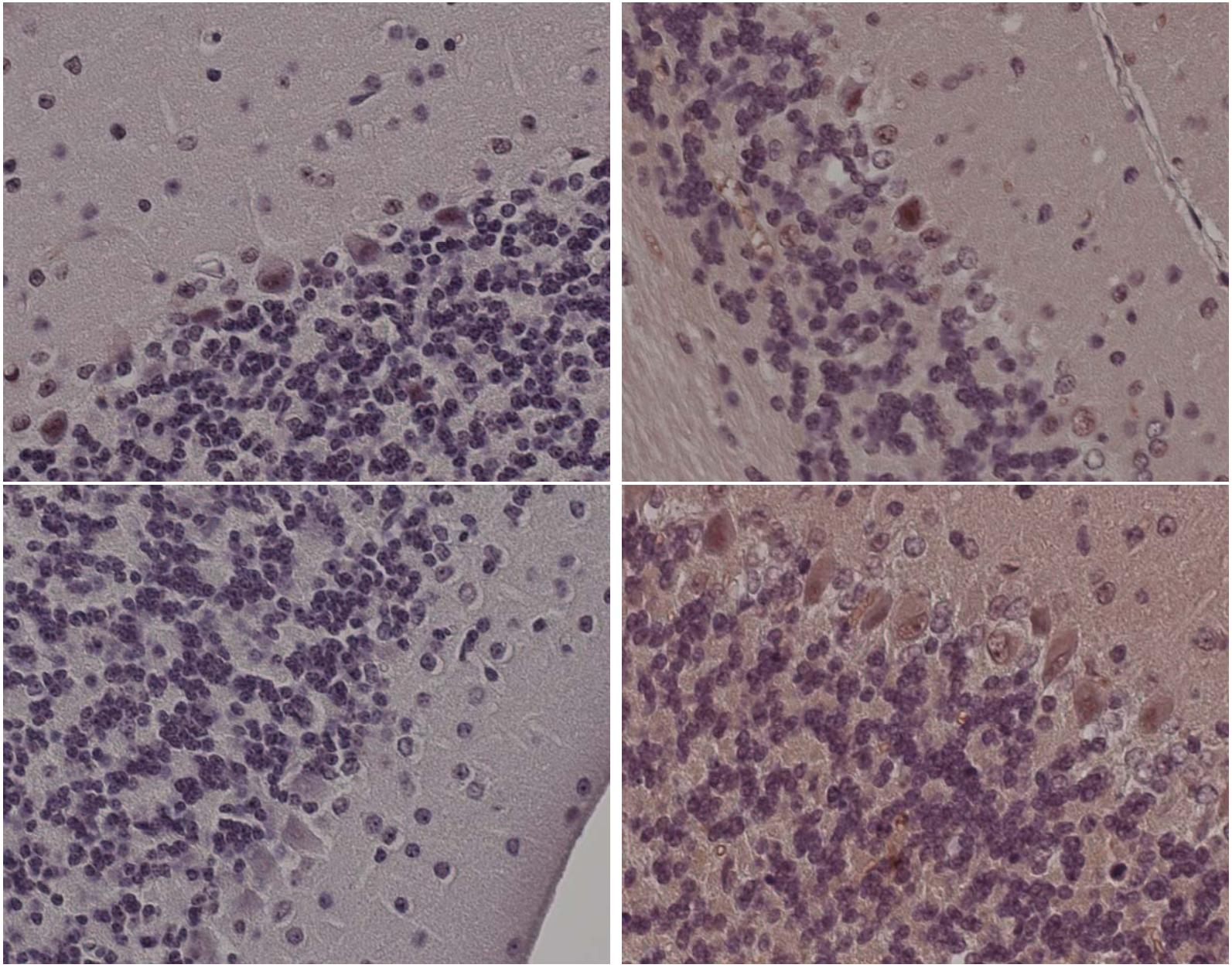




C57BL6 22L C57BL6 22L



C57BL6 **22L**



C57BL6

22L

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