Supplemental Data

Posttranslational modifications define course of prion strain adaptation and disease phenotype

Natallia Makarava[1,2], Jennifer Chen-Yu Chang[1,2], Kara Molesworth[1,2], Ilia V. Baskakov[1,2]

Supplemental Figures

Figure S1. Histopathological analysis of brains from the 2nd passage. Representative images of PrP^{Sc} deposition in the hippocampus stained with SAF-84 antibody **(A)**, spongiosis in thalamus stained with hematoxylin and eosin **(B)**, reactive astrogliosis in hippocampus stained for GFAP **(C)** and activated microglia in hippocampus stained for Iba1 **(D)**. Microglia (Iba1 staining) engulfing other cells (black arrows) or a vacuole (red arrow) **(E)**. Scale bars = 60 μm in **(A – D)**, 25 μm in **(E)**.

Figure S2. Co-immunostaining of PrP^{Sc} and reactive microglia in the 2nd passage. Representative images of co-immunostaining for PrP^{Sc} (SAF-84 antibody, red) and microglia (Iba1, green). Small intracellular deposits of PrP^{Sc} in cortex (upper panel) and striatum (lower panel) **(A)**, large PrP^{Sc} plaques surrounded by microglia in hippocampus **(B)**, small intracellular deposits of PrP^{Sc} in thalamus **(C)**, diffuse PrP^{Sc} plaques in thalamus **(D)**, close up images of small PrP^{Sc} deposits colocalizing with microglia cells **(E)** . Scale bars = 20 μm in **(A – D)**, 10 μm in **(E)**.

Figure S3. Analysis of PrP^{Sc} deposition in the 6th passage. Representative images of small granular PrP^{Sc} deposition across brain including cortex (Ctx), stem, hippocampus (Hp), cerebellum (Cb), thalamus (Th), hypothalamus (HTh), striatum (Str) in the 6th passages of SSLOW-Mo, cc - corpus callosum, s-or – stratum oriens of hippocampus. SAF-84 antibody was used for staining. Scale bar = 400 μm in low magnification images, 100 μm in high magnification images.

Figure S4. 2D Western blot analysis of PrP^{Sc} in six serial passages of SSLOW-Mo. Analysis of PrP^{Sc} sialoglycoforms in brain material of mice from six serial passages of SSLOW-Mo using 2D Western blotting. 2D Western blot of the original SSLOW in hamsters and mouse-adapted prion strain 22L are shown as references. All samples were treated with PK. Mouse and hamster materials were stained with ab3531 or 3F4 antibodies, respectively. 2D blots of two animals per group are shown. Black triangles, white triangles and arrows mark di-, mono- and non-glycosylated glycoforms, respectively.

Figure S1

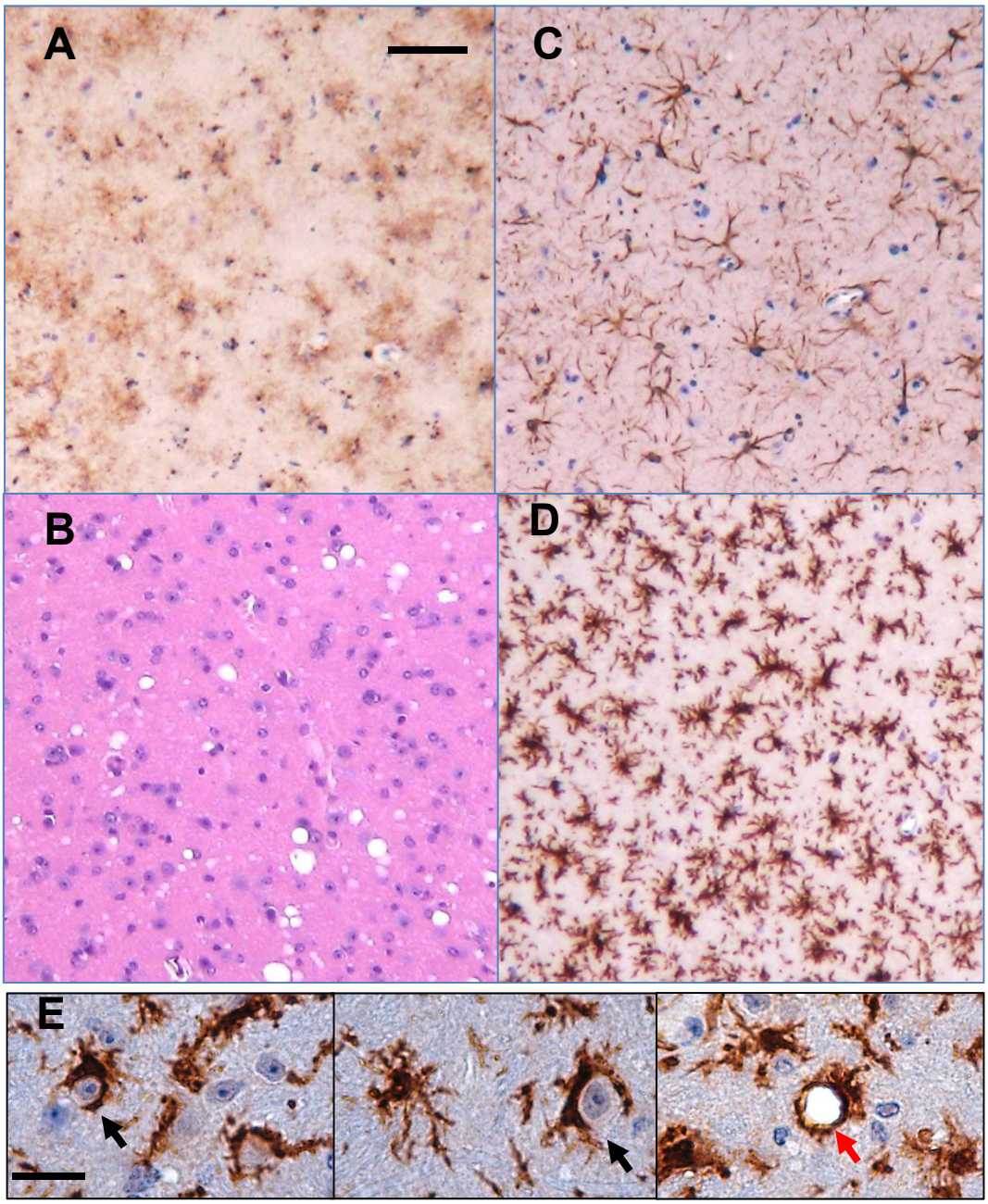


Figure S2

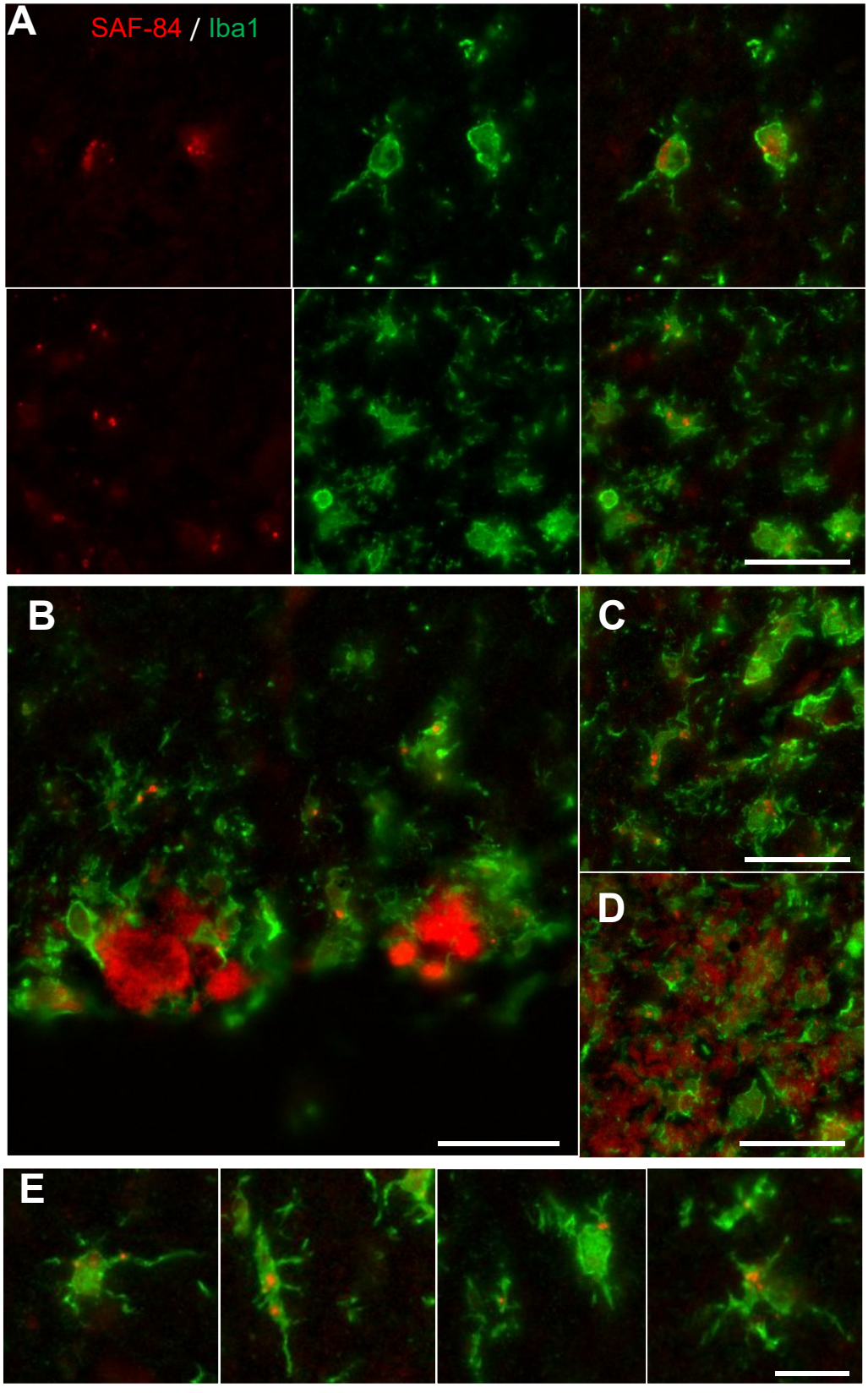


Figure S3

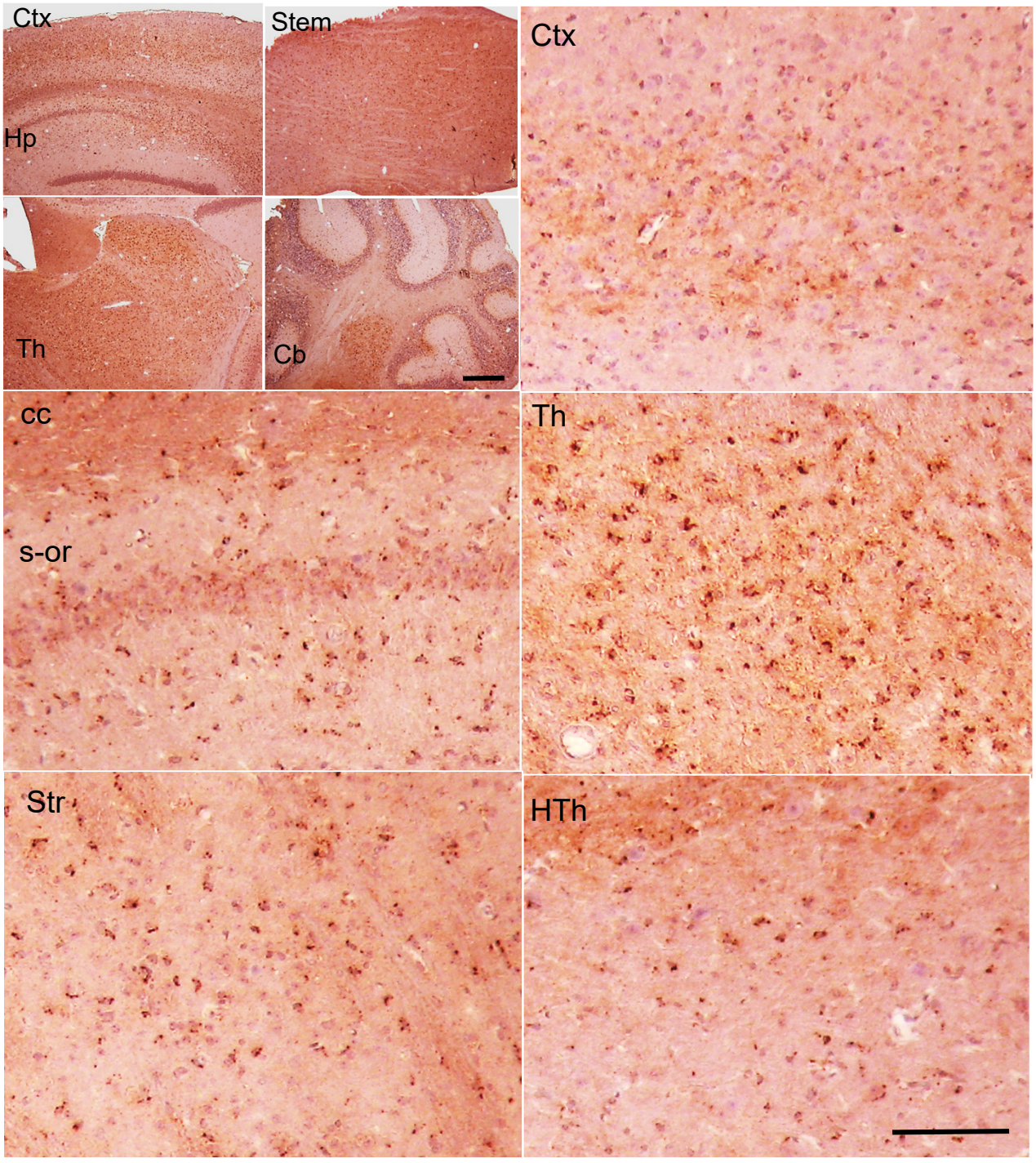
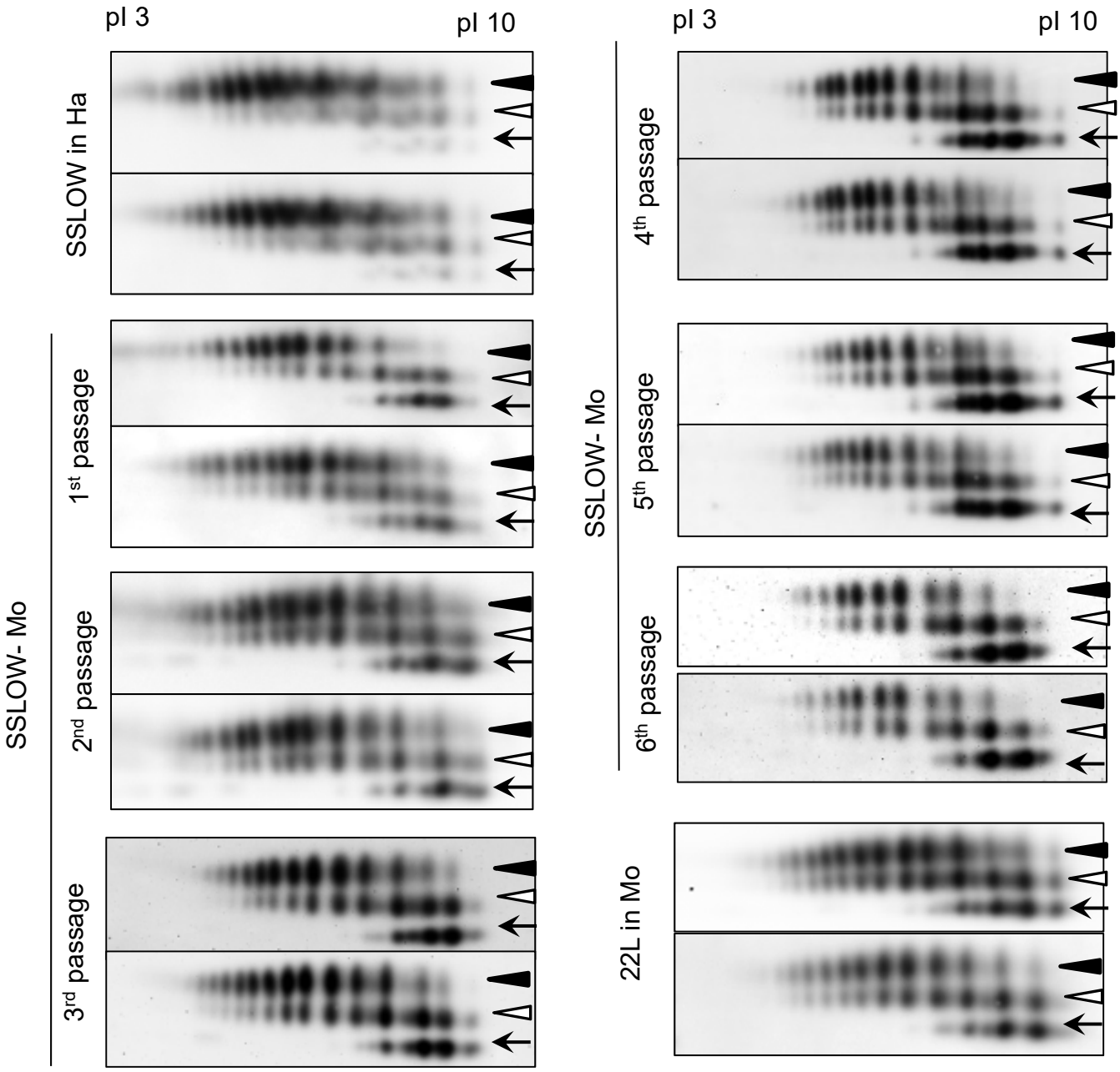


Figure S4


pI 3
pI 10
SSLOW in Ha
SSLOW- Mo
1st passage
2nd passage
3rd passage
4th passage
5th passage
6th passage
22L in Mo