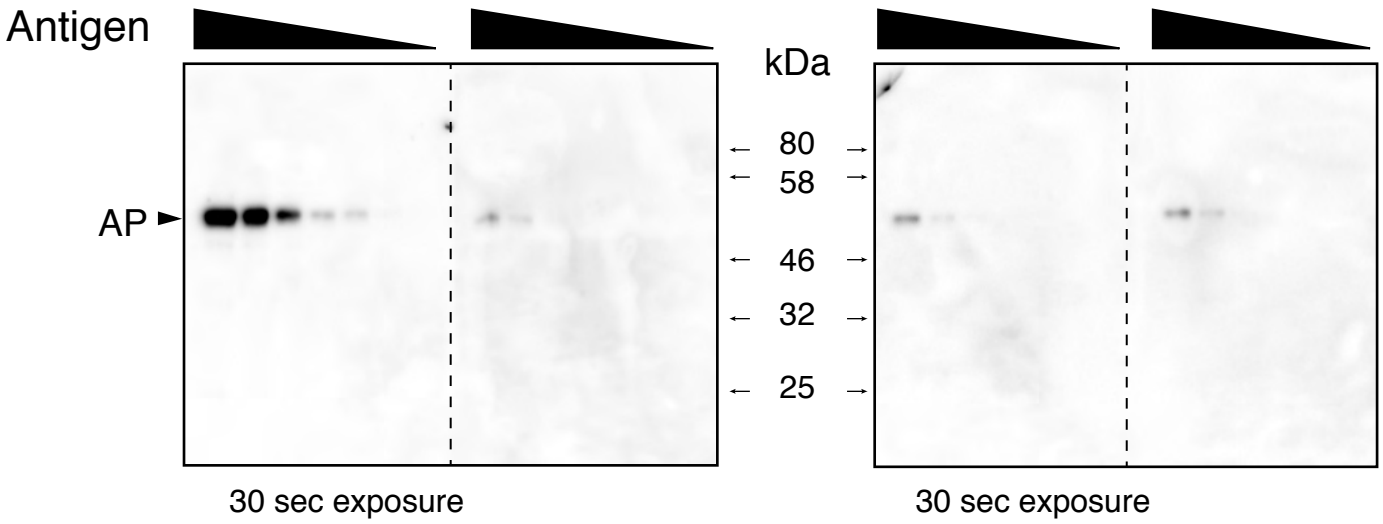
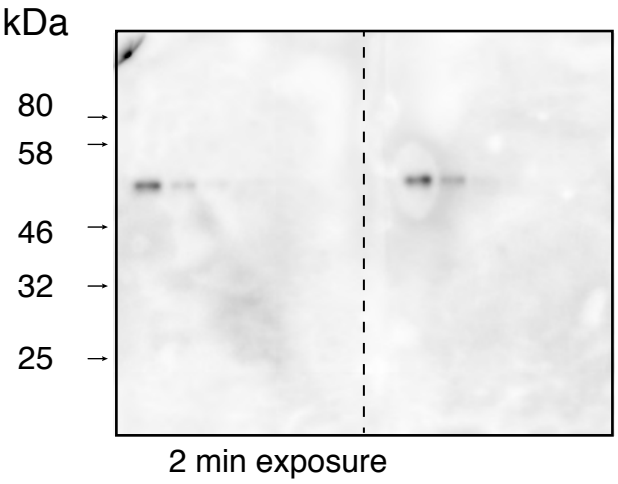
Reviewer's Figure 1

	5 min **CDR**	5 min **CDR**	5 min **Rocking**	5 min **Static**
Primary	1:15,000 CGS-1	1:5,000 Skim milk	1:15,000 CGS-1	1:15,000 CGS-1
Secondary	1:15,000 CGS-2	1:5,000 Skim milk	1:15,000 CGS-2	1:15,000 CGS-2

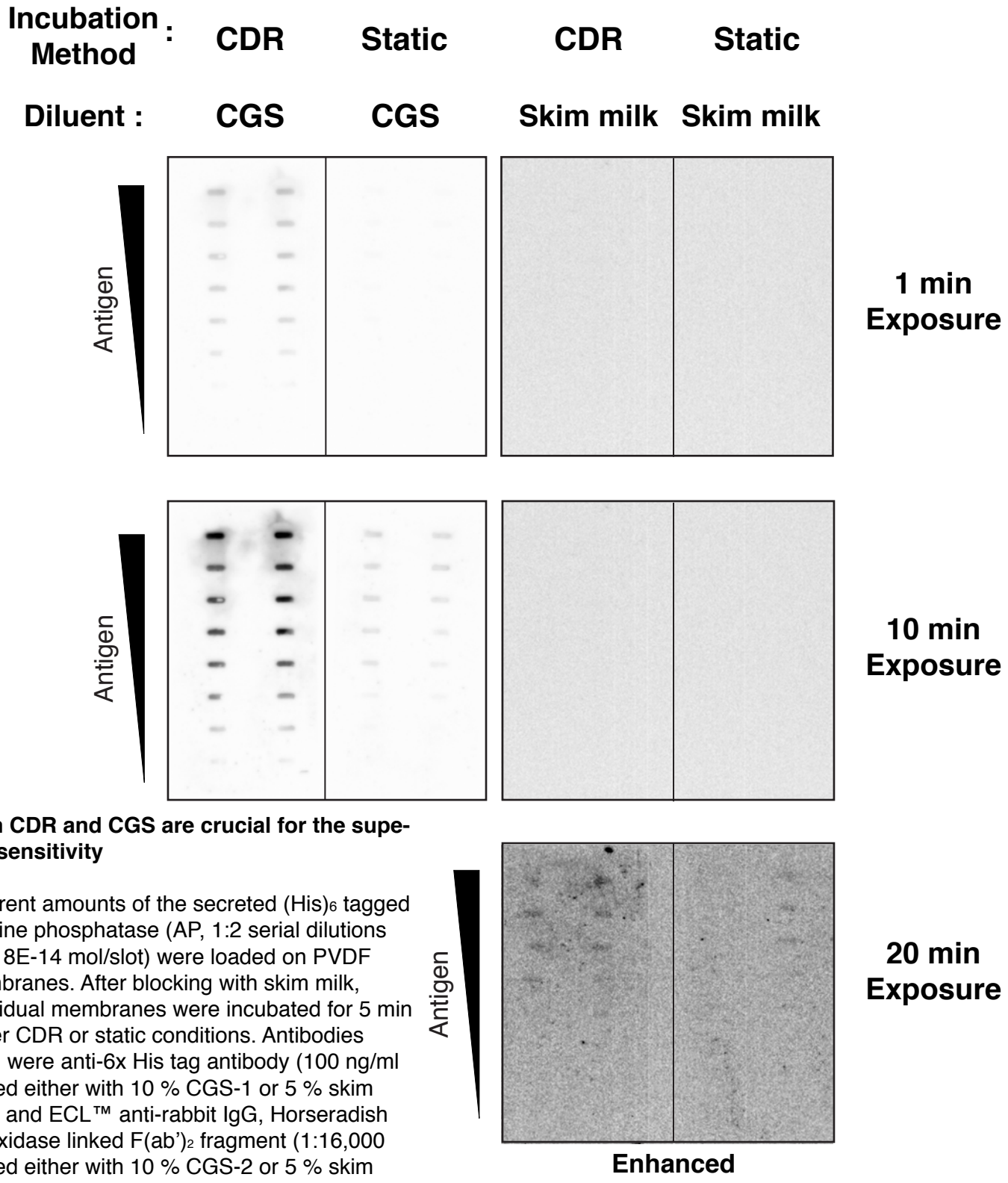




Comparison between CDR method, Static, and Rocking incubation on immunoblot

Different amounts of the secreted $(His)_6$ tagged alkaline phosphatase (AP, 1:2 serial dilutions from 8E-14 mol/lane) were separated, followed by transfer to PVDF membranes. Individual membranes were subjected to different incubation methods with anti-6x His tag antibody for 5 min. Chemiluminescence detection was performed and the membranes were imaged as a single image and dotted lines indicate the border of individual membranes.

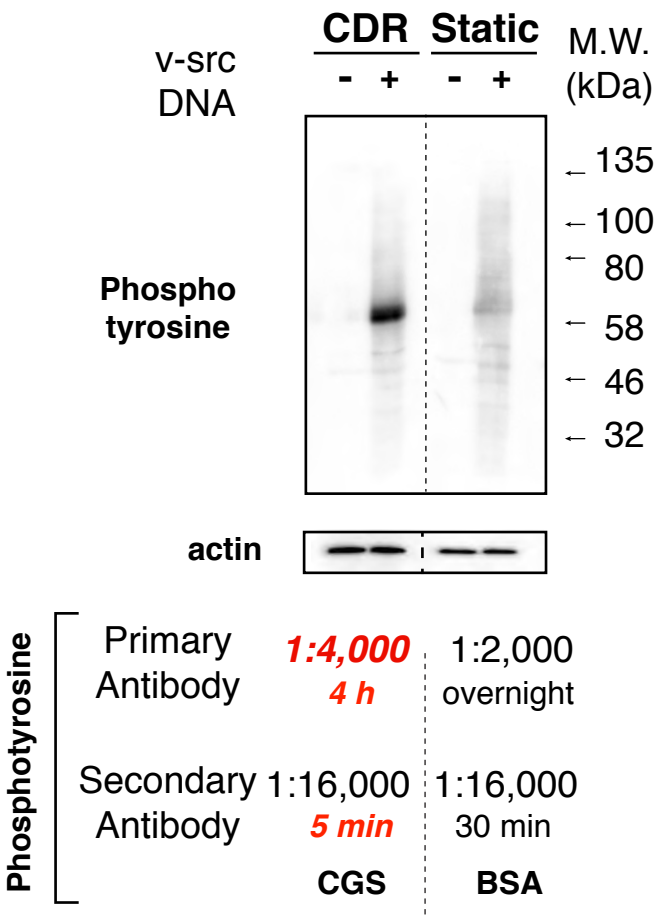
Reviewer's Figure 2



Both CDR and CGS are crucial for the superior sensitivity

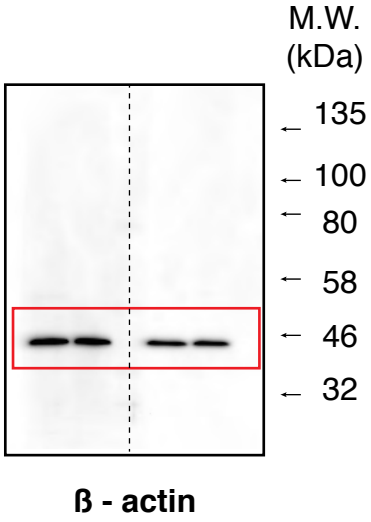
Different amounts of the secreted $(His)_6$ tagged alkaline phosphatase (AP, 1:2 serial dilutions from 8E-14 mol/slot) were loaded on PVDF membranes. After blocking with skim milk, individual membranes were incubated for 5 min under CDR or static conditions. Antibodies used were anti-6x His tag antibody (100 ng/ml diluted either with 10 % CGS-1 or 5 % skim milk) and ECL™ anti-rabbit IgG, Horseradish peroxidase linked $F(ab')_2$ fragment (1:16,000 diluted either with 10 % CGS-2 or 5 % skim milk). Regular washing of the membranes was utilized. Chemiluminescence detection was performed after 1 min, 10 min, and 20 min exposure.

Reviewer's Figure 3



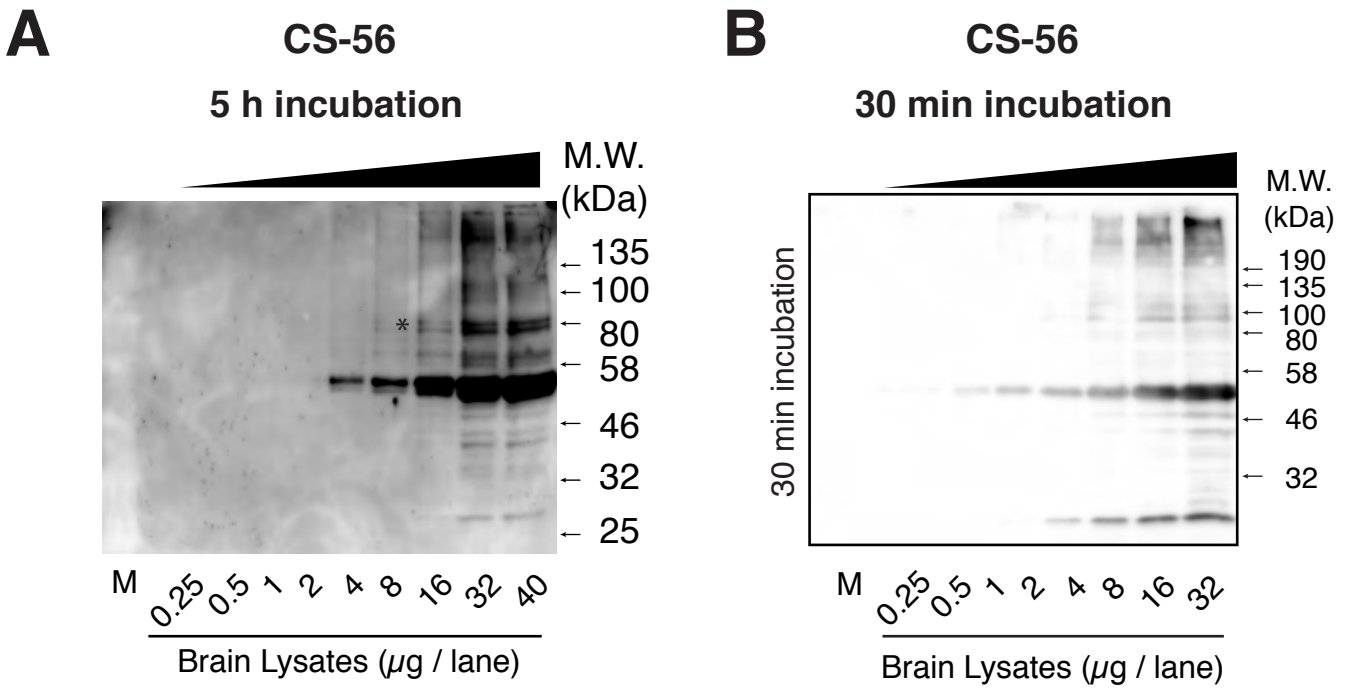
Detection of tyrosine phosphorylation induced by exogenous expression of chicken v-src.

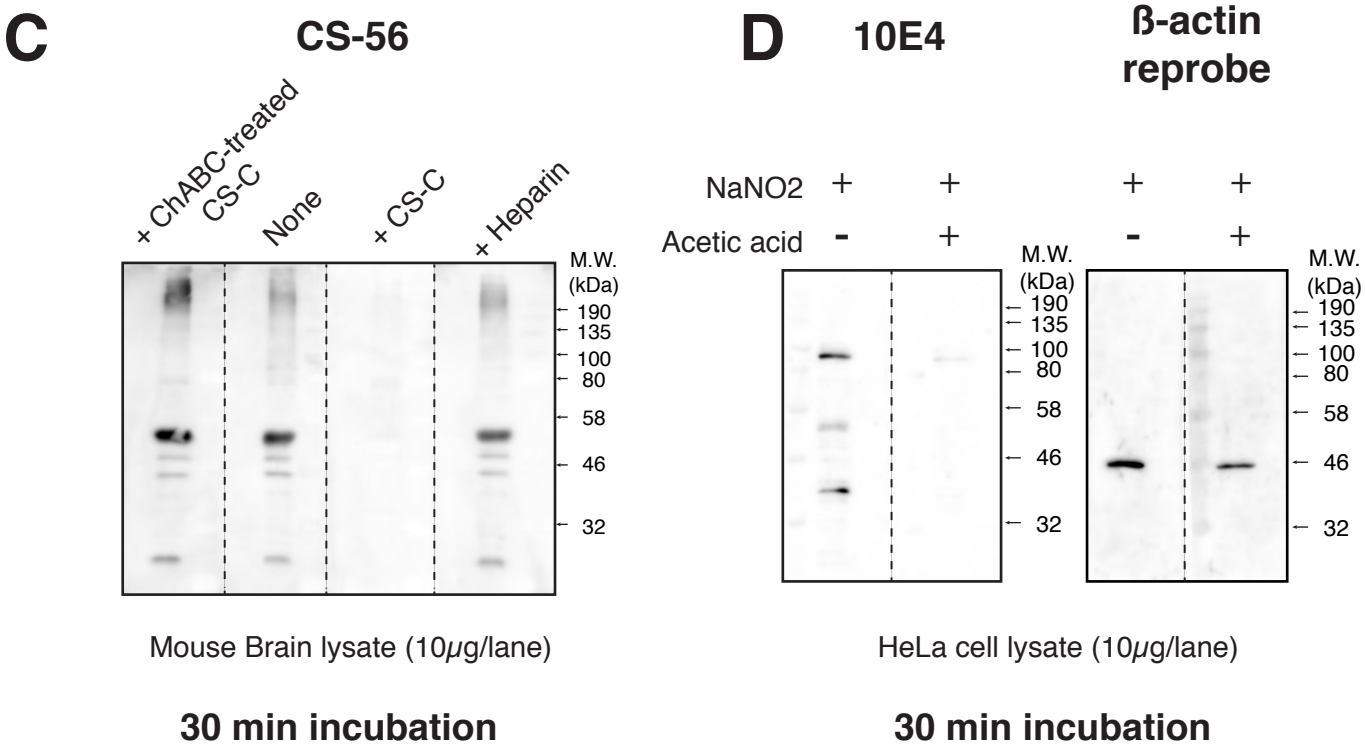
Cell lysates (20 μg/lane) from 293 cells transfected with pLNCX-v-src were separated by SDS-PAGE, followed by immunoblot with anti-phospho tyrosine antibody. The blots were imaged as a single image and dotted line indicates the border of individual membranes. After stripping the bound antibody, the membranes were re-probed with mouse anti-ß-actin antibody.



The original image of immunoblot data reprobed with anti-ß actin antibody. The red squares highlight the portion shown in the figure.

Reviewer's Figure 4





Reviewer's Figure 4

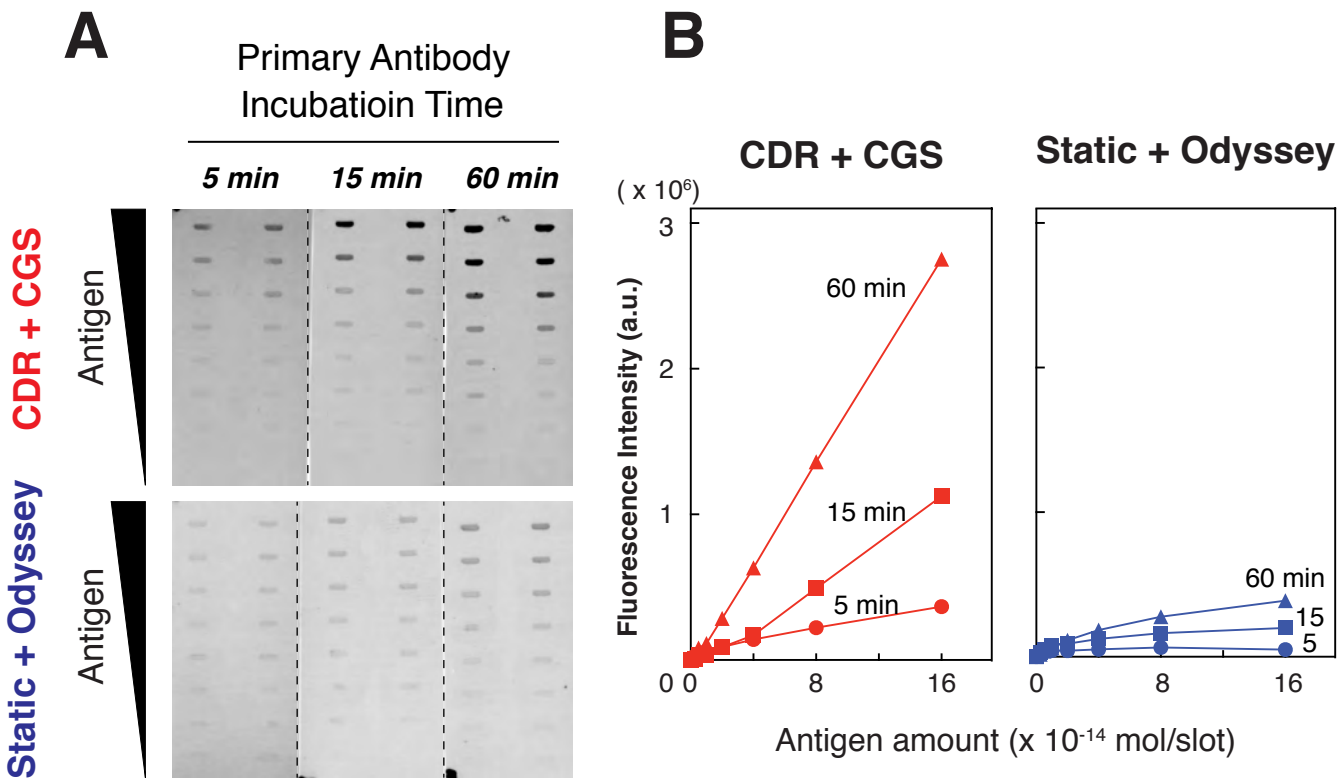
A. Optimization of Immunoblot with CS-56 (anti CS GAG chain) with a traditional method
Different amounts of brain lysates obtained from 7-week-old mice prepared in SDS were separated and transferred to a PVDF membrane, followed by **5 h incubation** with CS-56 (1:10,000 dilution with 10% CGS-1) under static condition. Asterisk indicates non-specific signal detected by anti-mouse IgM antibody since it was detected in the absence of CS-56 (not shown in this figure). While at least 18 h incubation with CS-56 was required without the aid of CGS, 5 h incubation was enough to obtain this blot in the presence of CGS. We found that loading more than 16 μg/lane resulted in the appearance of additional bands with higher molecular weights.

B. Optimization of Immunoblot with CS-56 with CDR method
PVDF membrane was prepared as (A). CS-56 was diluted (1:5,000) with 10% CGS-1 containing skim milk solution and incubated with PVDF membrane for **30 min** with CDR method. Proteomics analysis revealed that 50kDa bands contained brevican. Loading more than 8 μg/lane of mouse brain lysates resulted in the appearance of additional bands with higher molecular weights.

C. Proof of specific signals of CS-56
Brain lysates (10 μg/lane) prepared in SDS were separated and transferred to a PVDF membrane. CS-56 diluted (1:5,000) with 10% CGS-1 containing skim milk solution was pre-incubated at 22˚C for 30 min with either 500 ng/ml CS-C, heparin, or CS-C pretreated with chondroitinase ABC (ChABC). Immunoblot was performed as described in (B). The membranes were imaged as a single image and dotted lines indicate the border of individual membranes. CS-C is an antigenic GAG chain for CS-56 while heparin is a non-related GAG chain. ChABC is an enzyme to degrade CS GAG chains. Pre-incubation with the antigenic CS-C GAG chain eliminated CS-56 signals on immunoblot, demonstrating the specificity of these signals.

D. Proof of specific signals of 10E4 (anti-HS GAG chain)
HeLa cell lysates (10 μg/lane) was separated and transferred to a PVDF membrane. The membranes were treated with $NaNO_2$ alone or $NaNO_2$ together with acetic acid, followed by blocking. 10E4 was diluted (1:3,000) with 10% CGS-1 containing gelatin from cold water fish, and the incubation was performed with CDR method for 30 min. After acquiring the image, the bound antibody was stripped, and the membrane was reprobed by anti-ß actin antibody. The membranes were imaged as a single image and dotted lines indicate the border of individual membranes. The epitope for 10E4 is known to sensitive to chemical treatment with $NaNO_2$ and acetic acid. Chemical treatment of PVDF membrane reduced 10E4 signals on immunoblots, demonstrating the specificity of these signals.

Reviewer's Figure 5



CDR in conjunction with CGS in fluorescence immunoblot exhibits great dynamic range with reduced incubation time

(A) Different amounts of the secreted $(His)_6$ tagged AP (1:2 serial dilutions from 16E-14 mol/slot in duplicate) were loaded and the membranes were blocked at 4˚C for 60 min with Odyssey blocking buffer (ODS). The membranes were incubated for indicated times with 200 ng/ml of anti-6X His tag antibody diluted either in 10 % CGS-1 under CDR condition (top) or ODS containing 0.1 % Tween20 (ODS-T) under static condition (bottom). After rapid washing, the membranes were incubated for 15 min with goat anti-rabbit IgG-IRDye 680RD (1:5000 dilution) either in 10 % CGS-2 under CDR condition (top) or ODS-T under static condition (bottom).
(B) Quantitation of raw images was implemented and the average of fluorescent intensity at each condition was plotted.