# Development of novel TREM2 rabbit monoclonal antibodies that activate downstream, Syk-associated cell signaling pathways

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## INTRODUCTION

Alzheimer's Disease (AD) is one of the most common neurodegenerative diseases worldwide. Clinically, it is characterized by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles, resulting in neuronal dysfunction and cell death. Triggering receptor expressed on myeloid cells 2 (TREM2), a receptor protein localized at the membrane of innate immune cells, including microglia in the brain, has been genetically linked to AD, with specific variants increasing disease risk by as much as threefold (1, 2).

The TREM2 receptor is a single-pass type I membrane glycoprotein, consisting of an extracellular immunoglobulin-like domain, a transmembrane domain, and a cytoplasmic tail. Upon ligand binding and activation, TREM2 interacts with the tyrosine kinase-binding protein DNAX-activating protein 12 (DAP12, TYROBP) to form a receptor-signaling complex. The DAP12 protein structure consists of a short extracellular domain, a transmembrane domain, and a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) (2-9).

ITAMs function as a binding site for tyrosine kinases, including spleen tyrosine kinase (Syk). When Syk binds to an ITAM, it changes conformation, allowing for residues within the inter-domain linker region, including Tyr352, to become phosphorylated. Residues within the activation loop subsequently become phosphorylated, leading to full Syk activation. Tyr525 and Tyr526 are located in the activation loop of the Syk kinase domain and phosphorylation at these residues (equivalent to Tyr519/520 of mouse Syk) is essential for Syk function (10-12).

Syk activation can lead to the mediation of a variety of cellular responses, including proliferation, differentiation, inflammation, and phagocytosis. Evidence suggests that TREM2 and DAP12 may act in a Syk-dependent manner to drive microglial cellular responses in AD (2, 4-8, 13).

Despite this accumulating evidence, signaling cascades downstream of TREM2 have yet to be fully characterized. This uncertainty has inhibited researchers from establishing beneficial or detrimental roles this protein may play in the context of AD and other neurodegenerative diseases. Development of new research tools capable of activating TREM2 and downstream targets of interest are integral to broadening our understanding of these cascades and the cellular processes they regulate.

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## METHODS

- Immortalized human and mouse myeloid derived cell lines were screened for endogenous TREM2 expression. Cell extracts were analyzed by western blot using the CST Western Blot Protocol. Monoclonal antibodies against human and mouse TREM2 were used for detection.
- Selected immortalized human (THP-1) and mouse (SIM-A9, and RAW 264.7) cell lines were treated with various recombinant rabbit monoclonal antibodies generated against human or mouse TREM2 (Diagram 1).
- Untreated and treated cell extracts were analyzed by western blot using the CST Western Blot Protocol. Monoclonal antibodies against identified proteins of interest were used for detection to determine treatment driven alterations in expression.
- A CRISPR TREM2 knockout immortalized mouse RAW 264.7 cell line was generated and treated with a recombinant rabbit monoclonal antibody against the mouse TREM2 protein. Cell extracts were analyzed by western blot using the CST Western Blot Protocol to determine TREM2 dependency of treatment driven protein modifications.

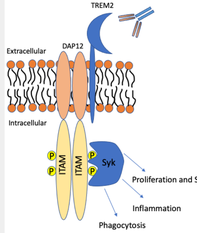


**Diagram 1:**
Illustrated diagram showcasing the recombinant monoclonal antibody treatment of TREM2 expressed on immortalized human and mouse cell lines. The image demonstrates incubating cells with an unconjugated primary antibody against either anti-human or anti-mouse TREM2, the resulting phosphorylation and activation of DAP12 and Syk proteins, and downstream Syk-associated cell signaling pathways that may be modulated.

**Table 1:**

| Key Antibodies Used | Catalog # |
|---|---|
| Syk (D3Z1E) XP® Rabbit mAb | 13198 |
| Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb | 2710 |
| Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb | 2717 |
| TREM2 (D8I4C) Rabbit mAb | 91068 |
| TREM2 (E7P8J) Rabbit mAb (Carboy-terminal Antigen, Mouse Specific) | 76765 |
| β-Actin (D6A8) Rabbit mAb | 8457 |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 |

**Additional western protocol reagents can be found at cellsignal.com**

## Cell Line Screen

### Figure 1A Endogenous Cell Line Validation

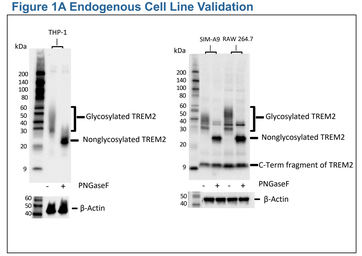


**Figure 1A:** Human THP-1, and mouse SIM-A9 and RAW 264.7 immortalized cell lines evaluated using the CST Western Blot Protocol for expression of TREM2 protein. Extracts from THP-1 cells untreated (-) or treated with peptide N-glycosidase F (PNGase F; +) were analyzed (left) using TREM2 (E4J7A) Rabbit mAb (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower). Extracts from SIM-A9 and RAW 264.7 cells untreated (-) or treated with peptide N-glycosidase F (PNGase F; +) were analyzed (right) using TREM2 (E7P8J) Rabbit mAb #76765 (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).

### Figure 1B CRISPR Cell Line Validation

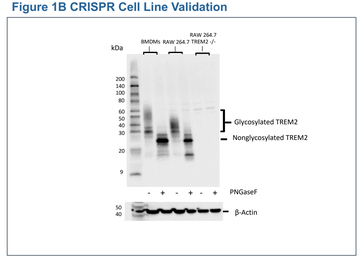


**Figure 1B:** Mouse RAW 264.7 immortalized CRISPR TREM2 -/- cell lines were generated and evaluated using the CST Western Blot Protocol for expression of TREM2 protein. Extracts from bone marrow derived macrophages, RAW 264.7, and RAW 264.7 TREM2 -/- cells untreated (-) or treated with peptide N-glycosidase F (PNGase F; +) were analyzed using TREM2 (E9O9F) Rabbit mAb (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).

## TREM2 Activating Antibody Screen

### Figure 2A Anti-TREM2 mAb Stimulation: Human TREM2/Syk

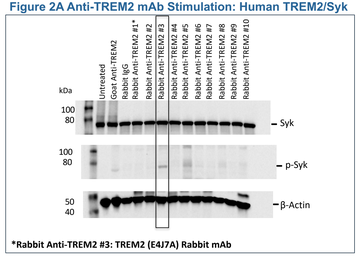


**Figure 2A:** A library of anti-human TREM2 recombinant monoclonal antibodies were generated and screened for *in vitro* stimulation of TREM2/DAP12/Syk. Human THP-1 immortalized cells were untreated, treated with control rabbit IgG, or treated with one of eleven anti-human TREM2 antibodies (4ug/mL, 10mins). Extracts of these cells were evaluated using the CST Western Blot Protocol using Syk (D3Z1E) XP® Rabbit mAb #13198 (upper), Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb #2710 (middle), and β-Actin (D6A8) Rabbit mAb #8457 (lower).

### Figure 2B Anti-TREM2 mAb Stimulation: Mouse TREM2/Syk

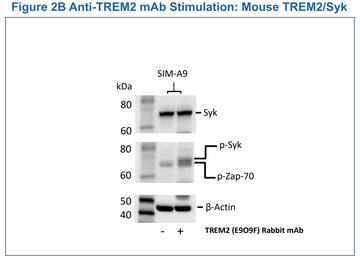


**Figure 2B:** An anti-mouse TREM2 recombinant monoclonal antibody was generated and tested for *in vitro* stimulation of TREM2/DAP12/Syk. Mouse SIM-A9 immortalized cells untreated (-) or treated (+) with TREM2 (E9O9F) Rabbit mAb (4ug/mL, 10mins). Extracts of these cells were evaluated using the CST Western Blot Protocol using Syk (D3Z1E) XP® Rabbit mAb #13198 (top), Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb #2717 (middle), and β-Actin (D6A8) Rabbit mAb #8457 (lower).

## TREM2 Dependency for Syk Stimulation

### Figure 3 TREM2 Dependent Syk Stimulation

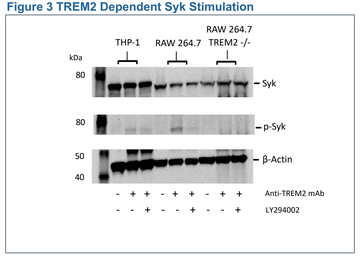


**Figure 3:** Human THP-1, and mouse RAW 264.7 and RAW 264.7 TREM2 -/- immortalized cell lines untreated (-) or treated (+) with either TREM2 (E4J7A) Rabbit mAb (4ug/mL, 10mins) or TREM2 (E9O9F) Rabbit mAb (4ug/mL, 10mins), and LY294002 (50uM, 1hr). Extracts of these cells were evaluated using the CST Western Blot Protocol using Syk (D3Z1E) XP® Rabbit mAb #13198 (top), Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb #2710 (middle), and β-Actin (D6A8) Rabbit mAb #8457 (bottom).

## CONCLUSIONS

- CST has developed a portfolio of rabbit monoclonal antibodies specific to TREM2 that have been validated for TREM2 detection by western blot and other key applications.
- The CST portfolio of rabbit monoclonal antibodies specific to Syk can be leveraged to investigate downstream, Syk-associated signaling cascades in the context of TREM2 activation.

## Future Directions

- Further investigate the role TREM2 activation may play in promoting Syk-associated cellular processes (phagocytosis, inflammation, proliferation and survival, etc.).
- Investigate additional means of activating TREM2, using phosphorylation of Syk as a readout.
- Leverage all suitable activation strategies to identify additional readouts for TREM2 activation.
- Use the readouts identified to examine the effect TREM2 variants may have on TREM2 activation and downstream signaling cascades.

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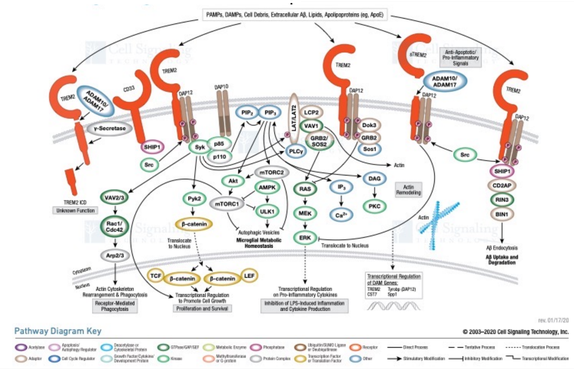


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