## Supplemental material

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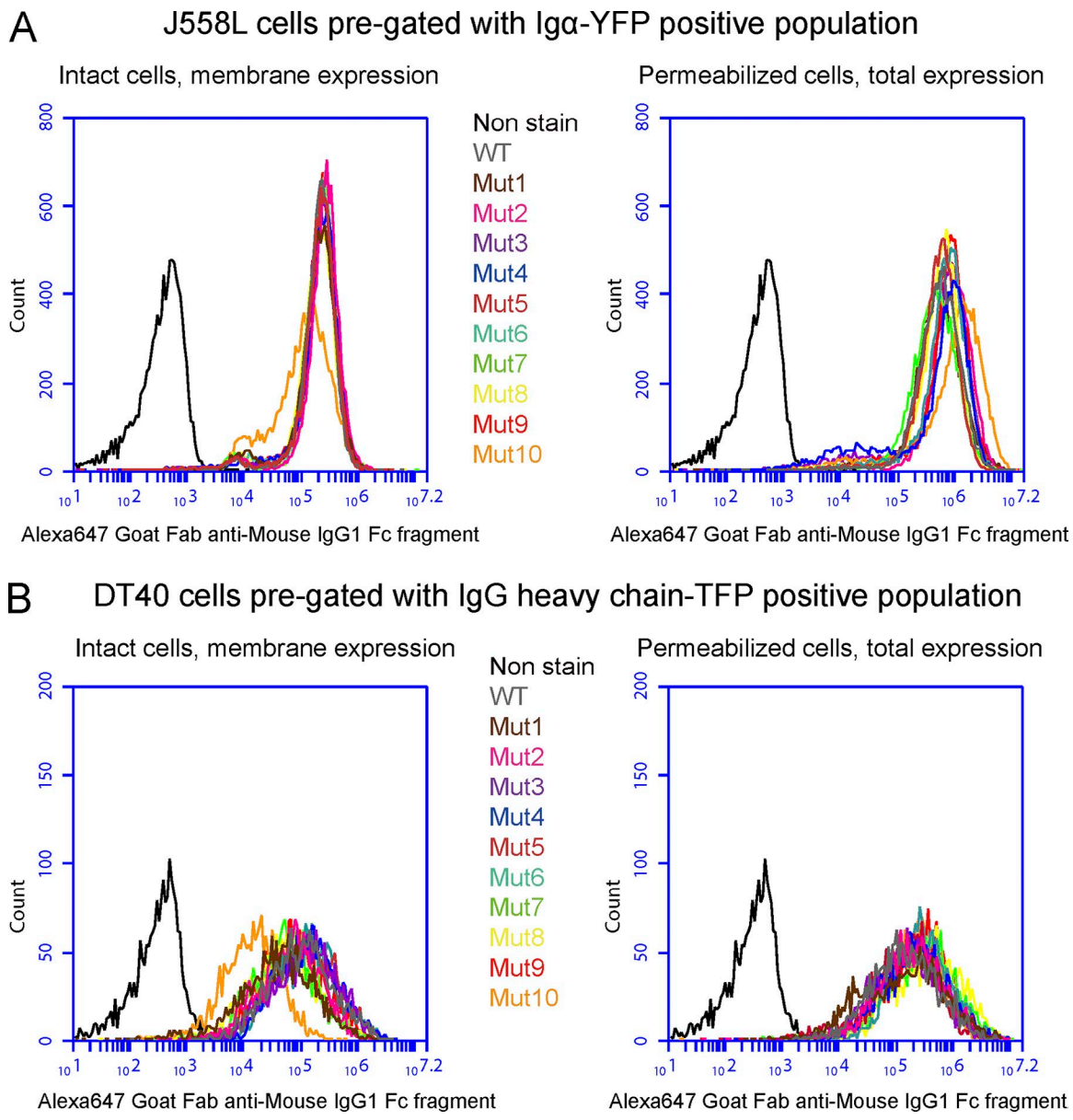
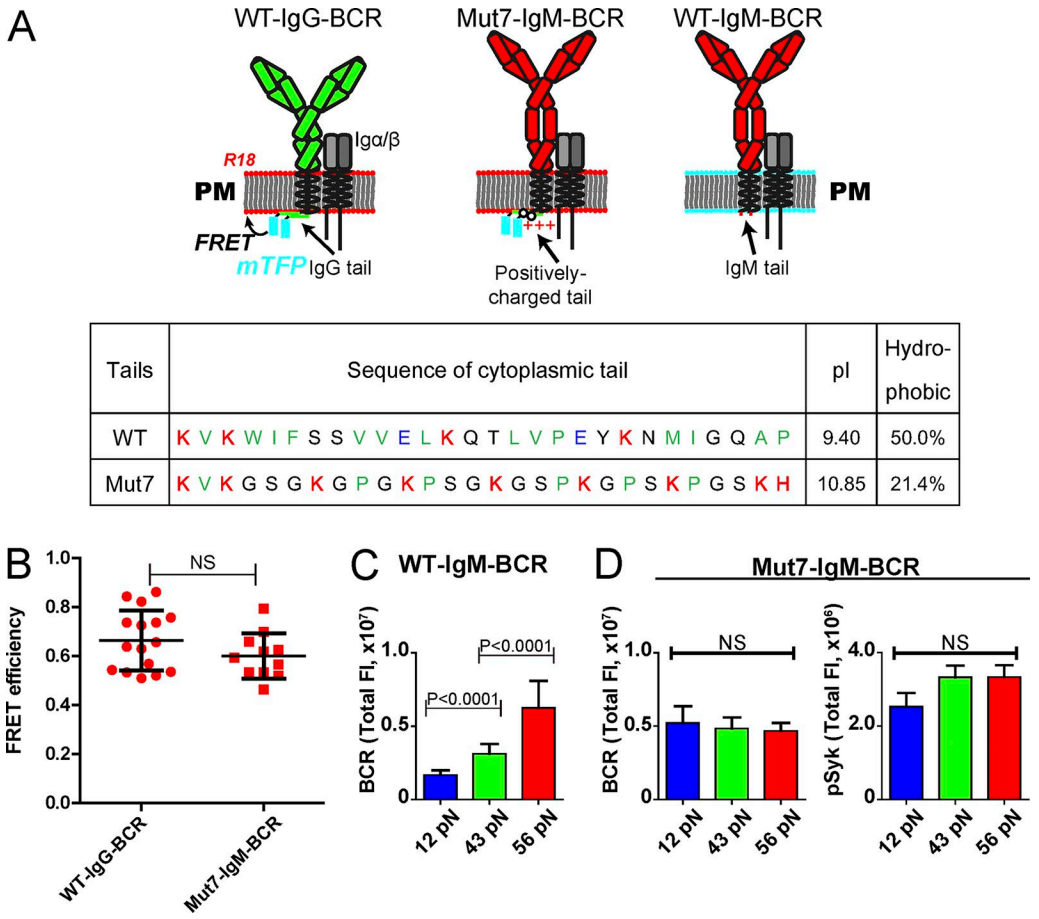



Figure S1. **Expression of WT and mutant IgG-BCR. (A and B)** Measurement of surface (left) and total (right) BCR expression in J558L cells stably expressing Igα-YFP and WT or mutant IgG-BCRs (A), or in DT40 cells, transiently expressing WT or mutant IgG-BCRs (B). J558L cells expressing Igα-YFP and DT40 B cells expressing IgG heavy chain TFP were gated for the analyses.




| Tails | Sequence of cytoplasmic tail | pI | Hydro-phobic |
|---|---|---|---|
| WT | K V K W I F S S V V E L K Q T L V P E Y K N M I G Q A P | 9.40 | 50.0% |
| Mut7 | K V K G S G K G P G K P S G K G S P K G P S K P G S K H | 10.85 | 21.4% |

Figure S2. **Mut7–IgM-BCR bearing PM-tethered positively charged IgG-tail can lower the threshold of mechanical force–induced activation. (A)** Top: Schematic illustration, sequence, and biochemical characteristic analysis of the cytoplasmic domain of WT and Mut7 (artificially designed cytoplasmic tail with high pI by high proportion of positively charged residues). Bottom: Acidic, basic, and hydrophobic residues are colored in blue, red and green, respectively. **(B)** Dequenching FRET to measure the FRET efficiency between mTFP and R18 in WT IgG-BCR– and Mut7–IgM-BCR–expressing DT40 B cells. FRET efficiency was calculated as detailed in Materials and methods. FRET efficiency was measured and plotted. Error bars represent means ± SD. **(C and D)** Statistic quantifications for the total FI of accumulated BCRs or pSyk in the immunological synapse of WT IgM-BCR– (C) or Mut7–IgM-BCR (D)–expressing J558L cells. Error bars represent means ± SEM. Two-tailed *t* tests were performed for the statistical comparisons. Data are from at least 30 cells over three independent experiments.

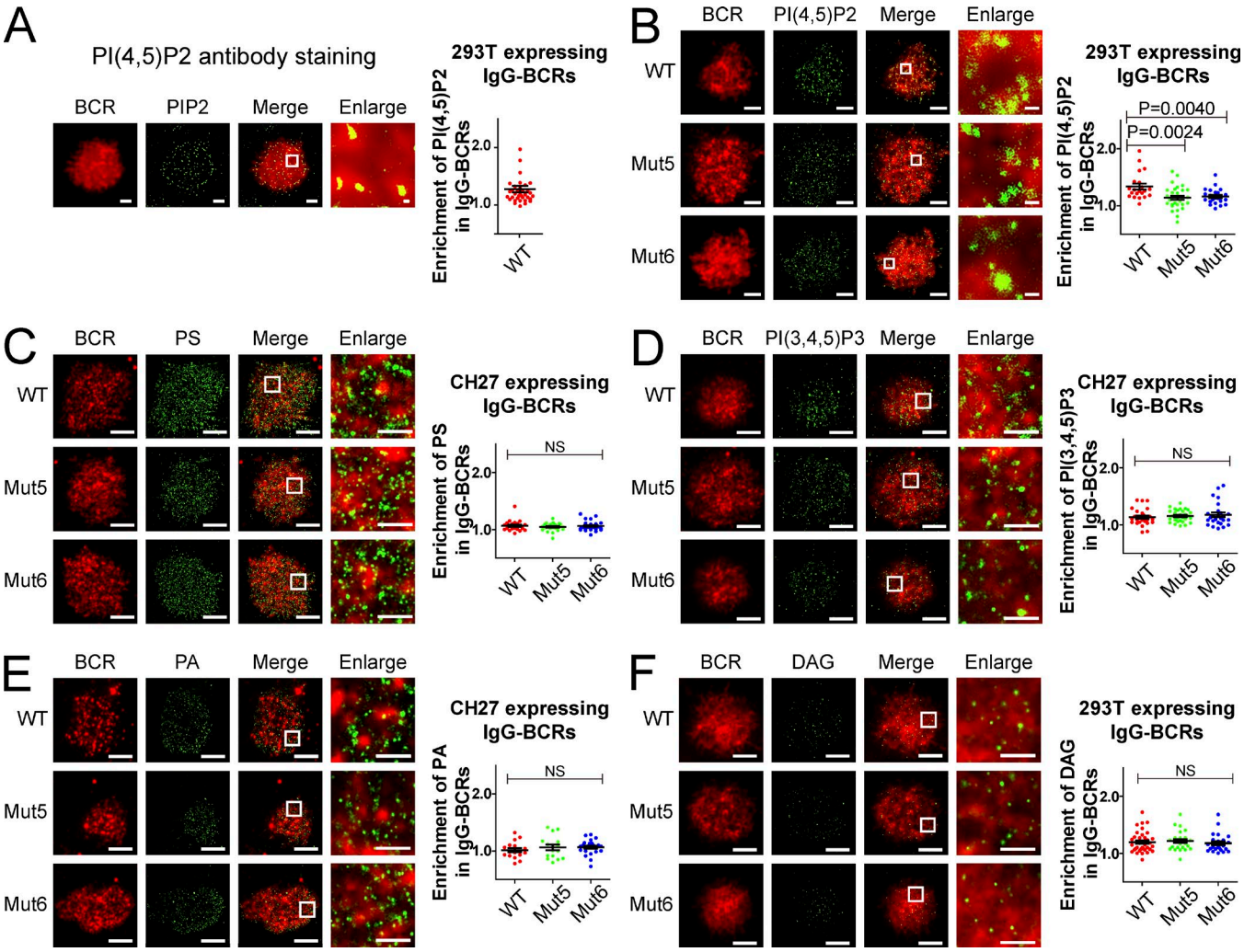



Figure S3. **WT IgG-BCR enriches more PI(4,5)P2 but not PS, PI(3,4,5)P3, PA, nor DAG at resting stage compared with PM-untethered mutant IgG-BCR. (A–F)** Left: Representative conventional TIRF images of IgG-BCRs and superresolution images of PI(4,5)P2 indicated by specific monoclonal antibody staining (A) or mEos3.2-based lipid sensors of PI(4,5)P2 (B), PS (C), PI(3,4,5)P3 (D), PA (E), and DAG (F) in 293T or CH27 B cells expressing IgG-BCRs with cytoplasmic tails of WT, Mut5, and Mut6 in quiescent state. Enlarged images are marked by white squares in main images. Bars: (main images) 3 µm; (enlarged images) 200 nm. Right: Statistical quantifications of enrichment of mEos3.2-based lipid biosensors of PI(4,5)P2, PS, PI(3,4,5)P3, PA, and DAG within WT IgG-BCRs. Error bars represent means ± SEM. Two-tailed *t* tests were performed for statistical comparisons. Data are from at least 20 cells over two independent experiments.

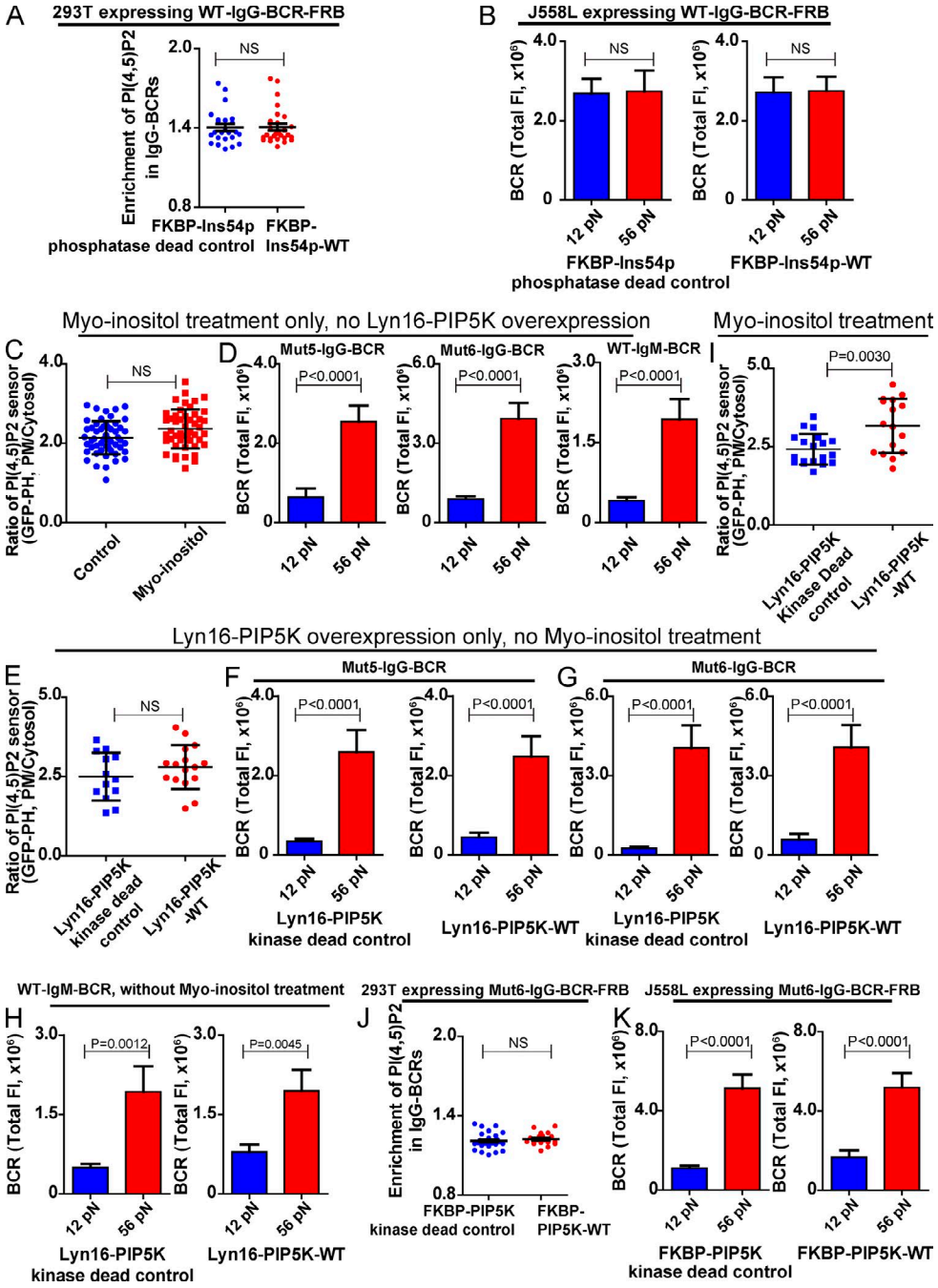



Figure S4. **Contribution of rapamycin, Myo-inositol, and PIP5K in the PI(4,5)P2 manipulation system. (A)** Statistical quantification of enrichment of mEos3.2-based PI(4,5)P2 biosensor within WT IgG-BCR membrane microdomains in 293T cells expressing FKBP-Ins54p-WT or FKBP-Ins54p phosphatase-dead control without preincubation of rapamycin. **(B)** Statistical quantification of enrichment of IgG-BCR accumulation into immunological synapses in J558L cells expressing FKBP-Ins54p-WT or FKBP-Ins54p phosphatase-dead control without preincubation of rapamycin. Error bars show means ± SEM. **(C)** Statistical quantification of the ratio of MFI of GFP-PH on PM versus MFI of GFP-PH in the cytosol of DT40 B cells pretreated with or without Myo-inositol. Error bars represent means ± SD. **(D)** Statistical quantification of the synaptic accumulation of IgG-BCRs with Mut5-/Mut6-tail or IgM-BCRs in J558L cells pretreated with Myo-inositol encountering 12-pN or 56-pN NP-TGTs. Error bars represent means ± SEM. **(E)** Statistical quantification of ratio of MFI of GFP-PH on cell membrane versus GFP-PH MFI in cytosol in DT40 B cells expressing Lyn16-PIP5K kinase-dead control or Lyn16-PIP5K-WT without Myo-inositol pretreatment. Error bars represent means ± SD. **(F–H)** Statistical quantification of the accumulation of IgG-BCRs into immunological synapses with Mut5– (F) or Mut6–IgG-BCRs (G) or WT IgM-BCRs (H) in J558L cells expressing Lyn16-PIP5K kinase-dead control or Lyn16-PIP5K-WT encountering 12-pN or 56-pN NP-TGTs. Error bars represent means ± SEM. **(I)** Statistical quantification of ratio of MFI of GFP-PH on cell membrane versus MFI of GFP-PH in cytosol of DT40 B cells expressing Lyn16-PIP5K kinase-dead control or Lyn16-PIP5K-WT pretreated with Myo-inositol. Error bars represent means ± SD. **(J)** Statistical quantification of enrichment of mEos3.2-based PI(4,5)P2 biosensor within Mut6–IgG-BCR membrane microdomains in 293T cells expressing FKBP-PIP5K-WT or FKBP-PIP5K kinase-dead controls without preincubation of rapamycin. **(K)** Statistical quantification of enrichment of IgG-BCR accumulation into immunological synapses in J558L cells expressing FKBP-PIP5K-WT or FKBP-PIP5K kinase-dead controls without preincubation of rapamycin. Two-tailed *t* tests were performed for the statistical comparisons. Data are from at least 20 cells over two independent experiments. Error bars show means ± SEM.

| Tails | Sequence of cytoplasmic tail | pI | Hydrophobic |
|---|---|---|---|
| WT | KVKWIFSSVVELKQTLVPEYKNMIGQAP | 9.40 | 50.0% |
| Y/F | KVKWIFSSVVELKQTLVPEFKNMIGQAP | 9.40 | 53.6% |
| SSVV/AAAA | KVKWIFAAAAELKQTLVPEYKNMIGQAP | 9.40 | 57.1% |
| Mut1 | KVKGSGSGPGSPSGPGSPSGPSGPGSGH | 10.00 | 25.0% |
| Mut2 | KVKGSGSGPGSPSGPGSPSGPSGPGSGH<br>KVKWIFSSVVELKQTLVPEYKNMIGQAP | 9.88 | 37.5% |
| Mut3 | KVKWIFSSVVELGQTGSGEYKNMGGQGS | 8.43 | 28.6% |
| Mut4 | KVKWIFSSVVELAQTGSGEYANMGGQGS | 6.14 | 35.7% |
| Mut5 | KVKWIFSSVVELEQTLVPEYENPIGQAP | 4.49 | 50.0% |
| Mut6 | KVKGSGSSVVEGKQTGSGEYKNMGGQGS | 9.40 | 14.3% |
| Mut7 | KVKGSGKGPGKPSGKGSPKGPSKPGSKH | 10.85 | 21.4% |
| Mut8 | KVKGSGEGPGEPSGEGSPEGPSEPGSEH | 4.47 | 21.4% |
| Mut9 | KVKWIFSSVVELAQTLVPEYANMIGQAP | 6.14 | 57.1% |
| Mut10 | KVKWIFSSVVELAQTLVPEFANMIGQAP | 6.14 | 60.7% |
| Human-IgM/IgD-Tail | KVK | NA | 33.3% |
| Human-IgA-Tail | VRGPSGNREGPQY | 8.72 | 23.08% |
| Human-IgE-Tail | MVQRFLSATRQGRPQTSLDYTNVLQPHA | 10.74 | 39.29% |
| Human-IgG-Tail | KVKWIFSSVVDLKQTIIPDYRNMIGQGA | 9.53 | 46.43% |

Figure S5. **Overview of all the cytoplasmic domains tested in this study.** Sequence and biochemical characteristic analysis of all the cytoplasmic domains used in this study. Acidic, basic, and hydrophobic residues are colored in blue, red, and green, respectively.