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Supplementary Materials for

The Myc tag monoclonal antibody 9E10 displays highly variable epitope recognition dependent on neighboring sequence context

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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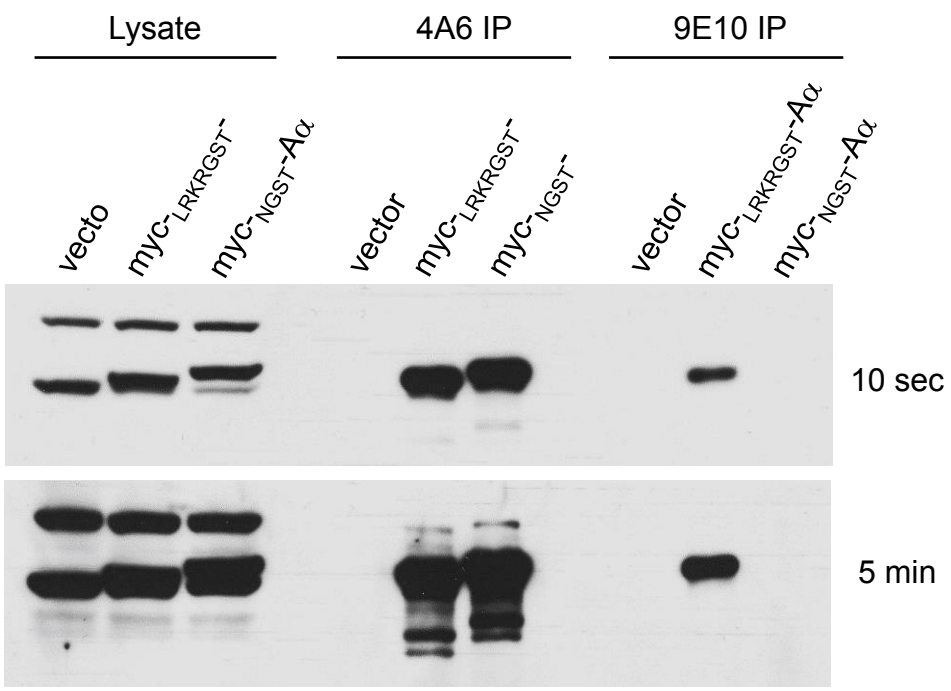


Fig. S1. Clone 9E10 is greatly diminished in immunoprecipitation of Myc–NGST–PP2A Aα. Immunoblotting with PP2A A polyclonal antibody for Myc-tagged PP2A Aα that was immunoprecipitated from NIH3T3 mouse fibroblast whole-cell extracts with either antibody 4A6 or antibody 9E10.

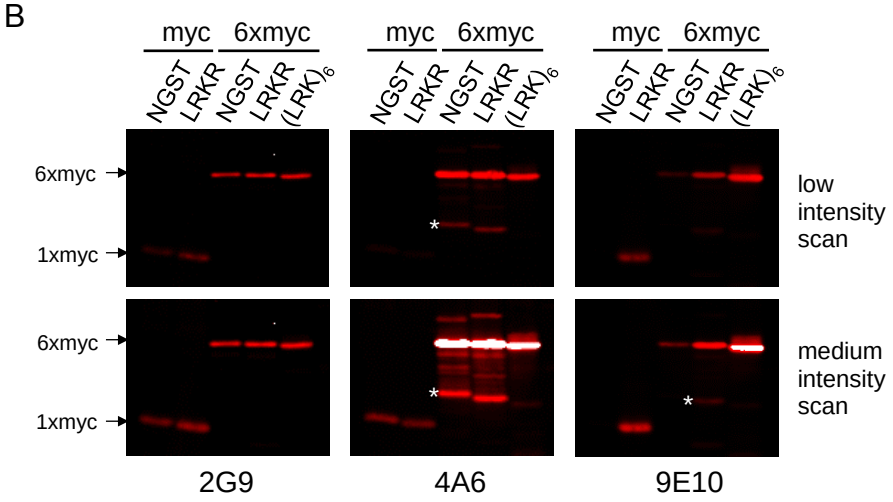
A

M-myc-NEM-myc-NEM-myc-NEM-myc-NEM-myc-NEMESLGDLTM-myc-NGST-B55α

M-myc-NEM-myc-NEM-myc-NEM-myc-NEM-myc-NEMESLGDLTM-myc-LRKR-B55α

M-myc-LRK-myc-LRK-myc-LRK-myc-LRK-myc-LRKESLGDLTM-myc-LRKR-B55α

B



C

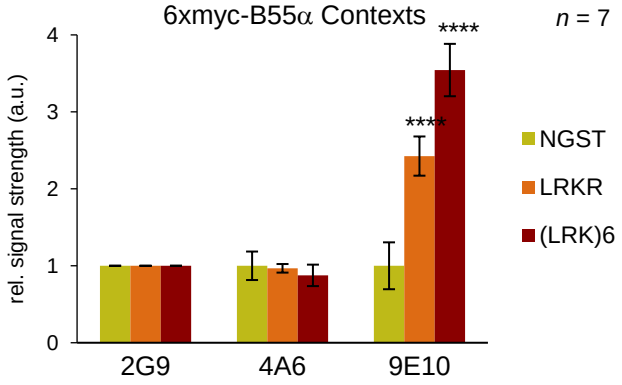


Fig. S2. Clone 9E10 shows sequence context bias toward 6xMyc-tagged proteins. (**A**) Sequences of three context versions of N-terminal 6xMyc-tagged PP2A B55α subunit fragment (**B**) Immunoblotting of lysates from bacteria expressing either the NGST or LRKR version of a single Myc-tagged B55α fragment or of a 6xMyc-tagged B55α fragment using monoclonal antibody 2G9, which recognizes B55α, as a reference antibody and the Myc tag–specific antibodies, clone 9E10 or 4A6. Representative images of $n = 7$ independent LiCOR Western blot analyses are shown. Asterisks indicate prominent degradation bands. (**C**) Quantification of fluorescence signals for the 6xMyc-tagged proteins adjusted to the 2G9 signals, which represent the amounts of 6xMyc-tagged PP2A B55α subunit fragment, shown as average +/- SD from 7 Western blot replicates ($n = 7$). Fluorescence signals were quantified from non-saturated scans. Values are shown relative to the average 6xMyc-NGST signal of each antibody, which was arbitrarily set to 1. Statistical significance was calculated for each antibody separately with a one-way ANOVA + post-hoc Tukey´s HSD test. p values relative to the respective NGST context are shown as: **** $p \leq 0.001$.

Table S1. 9E10 shows a larger signal variability than 4A6 in a single-substitution scan of Myc peptide contexts NGST or LRKR. Fluorescence signals of 15 amino acid–long peptides corresponding to either Myc-NGST or Myc-LRKR and permuted with single substitutions to all 20 proteinogenic amino acids at all 4 positions, detected with either clone 4A6 or 9E10. Fluorescence signals of undetected peptides are highlighted in red, and signals lower than 10% of the average signal (all contexts) of the respective antibody are highlighted in blue. Average signals, standard deviations, and variation coefficients for the two antibodies are shown for each permuted position alone, for the NGST permutations combined, for the LRKR permutations combined, and for all permutations combined. This table is provided as an Excel file.

Table S2. 9E10 shows a larger signal variability than 4A6 in a double-substitution scan of the first two amino acid positions C terminal to Myc peptide. Fluorescence signals of all double-substitution peptides corresponding to Myc-XXST or Myc-XXKR and permuted to all 20 proteinogenic amino acids at positions XX, detected with either clone 4A6 or 9E10. This table is provided as an Excel file.

Table S3. Highest- and lowest-scoring Myc context peptides in a double-substitution scan for clones 4A6 and 9E10. Fluorescence signals of the ten highest-scoring peptides are shown for clone 4A6 and clone 9E10. Fluorescence signals of the ten lowest-scoring peptides for clone 4A6 and the 20 lowest-scoring peptides (= all undetected peptides) for clone 9E10 are shown. Amino acid contexts are shown in bold. This table is provided as an Excel file.