Supplemental Figure 1. Pros1 expression by multiple tumor types is IFNg responsive and suppresses M1 gene expression. A. Pros1 expression fold change in IFNg treated (24 hours) murine tumor cell lines as measured by qRT-PCR normalized to untreated B16F10 cells. (n=6, *P < 0.05 relative to untreated, 2 independent replicates) **B.** Suppression of M1 gene expression in macrophages co-cultured with tumor cell lines determined by qRT-PCR. (n=6, *P < 0.05 relative to untreated, †P < 0.05 M1 induced, 2 independent replicates) **C.** Pros1 concentration in tumor cell line conditioned medium after 24 hours measured by ELISA. (n=3) **D.** Pros1 expression in tumor cell lines measured by qRT-PCR. (n=5) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 2. Pros1 inhibits peritoneal macrophage M1 polarization A. Raw qRT-PCR expression fold change of Pros1 mediated M1 suppression normalized to untreated samples rather than IFNg+LPS treatment as in Figure 1D. (n=6, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced, 2 independent replicates) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 3. Gas6 does not suppress M1 gene expression. A. M1 induced macrophages treated with 200ng/ml Gas6. (n=5, ns = not significant) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 4. Temporal effects of Pros1 on M1 polarization. A. Expression fold change of M1 associated gene expression 2 or 8 hours after induction of M1 polarization. (n=5, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced, respectively) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test. For the ΔΔCT method, IFNg+LPS treated macrophages from the 8 hour timepoint were used as the reference standard.

Supplemental Figure 5. Expression of M2 associated genes is not significantly altered by tumor secreted Pros1. A. M2 gene expression of M1 induced macrophages co-cultured with B16F10 cells. (n=4, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced, ns = not significant) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 6. Pros1 inhibits thiogylcollate-induced peritoneal macrophage M1 polarization A. Raw qRT-PCR expression fold change of Pros1 mediated M1 suppression normalized to untreated samples rather than IFNg+LPS treatment as in Figure 1D. (n=8, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced, 2 independent replicates) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 7. Characterization of CRISPR-based Pros1 deletion in B16F10 cells. A. BLAST comparison of BdP genomic sequence with Pros1 reference sequence. **B.** Domain diagram of murine Pros1 with location of targeted deletion. **C.** Expression change in mRNA 5' to the sequence deletion. (n=4, *P < 0.05) **D.** MTT growth assay of parental B16F10, BdP, or BdP cell line treated with 1μg/ml Pros1. (n=4, ns = not significant) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 8. Flow cytometric analysis of TAM expression in M1 induced peritoneal macrophages. A. TAM surface expression in M1 induced macrophages as measured by flow cytometry. Quantitative data is normalized to expression of untreated macrophages (n=5, *P < 0.05 relative to untreated macrophages) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 9. TAM expression in macrophages isolated from TAM knock-out mice. A. qRT-PCR of TAM receptors in macrophages from Axl, Tyro3 or Mer knock-out mice. (n=4, *P < 0.05 relative to macrophages isolated from wt mice) **B.** Analytical flow cytometry of TAM receptors on macrophages isolated from Axl, Tyro3 or Mer knock-out mice. Quantitative data is normalized to expression of wildtype (wt) macrophages (n=4, *P < 0.05 relative to wt peritoneal macrophages) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 10. M1 associated gene expression in M1 induced TAM knock-out macrophages. A. qRT-PCR of M1 genes in macrophages from wildtype, Axl, Tyro3 or Mer knock-out mice treated with IFNg and LPS for 24 hours. (n=5, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 11. Pros1 has limited effects on alternative signaling pathways downstream of Mer/Axl/Tyro3. A. Western blot analysis of pAKT, AKT, pStat1, Stat1, p-Stat6, Stat6, p-MEK, MEK, pERK, and ERK in M1 induced macrophages treated with Pros1 or co-cultured with B16F10 cells. Relative protein quantitation is normalized to untreated Macrophage only controls. B-Tubulin was used as a loading control. (n=3, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 12. p-p38 levels are suppressed within 2 hours of Pros1 addition. A. Naive or M1 induced macrophages were treated with 1ug/ml Pros1 or co-cultured with B16F10 cells, harvested after 2 hours and Western blot analysis conducted. (n=4, *P < 0.05 relative to M1 induced) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 13. Ablation of iNOS gene expression in M1 polarized macrophages using Jak or Stat3 inhibitors. A. Expression analysis of iNOS mRNA in IFNg + LPS treated macrophages after treatment with Barcitinib or S3I-201. (n=4, *P < 0.05 relative to IFNg and LPS treatment) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 14. p38 and Stat3 expression analysis of M1 induced macrophages in the presence of Pros1. A. qRT-PCR of M1 polarized macrophages treated with Pros1 or co-cultured with B16F10 cells. (n=4, *P < 0.05 relative to untreated) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

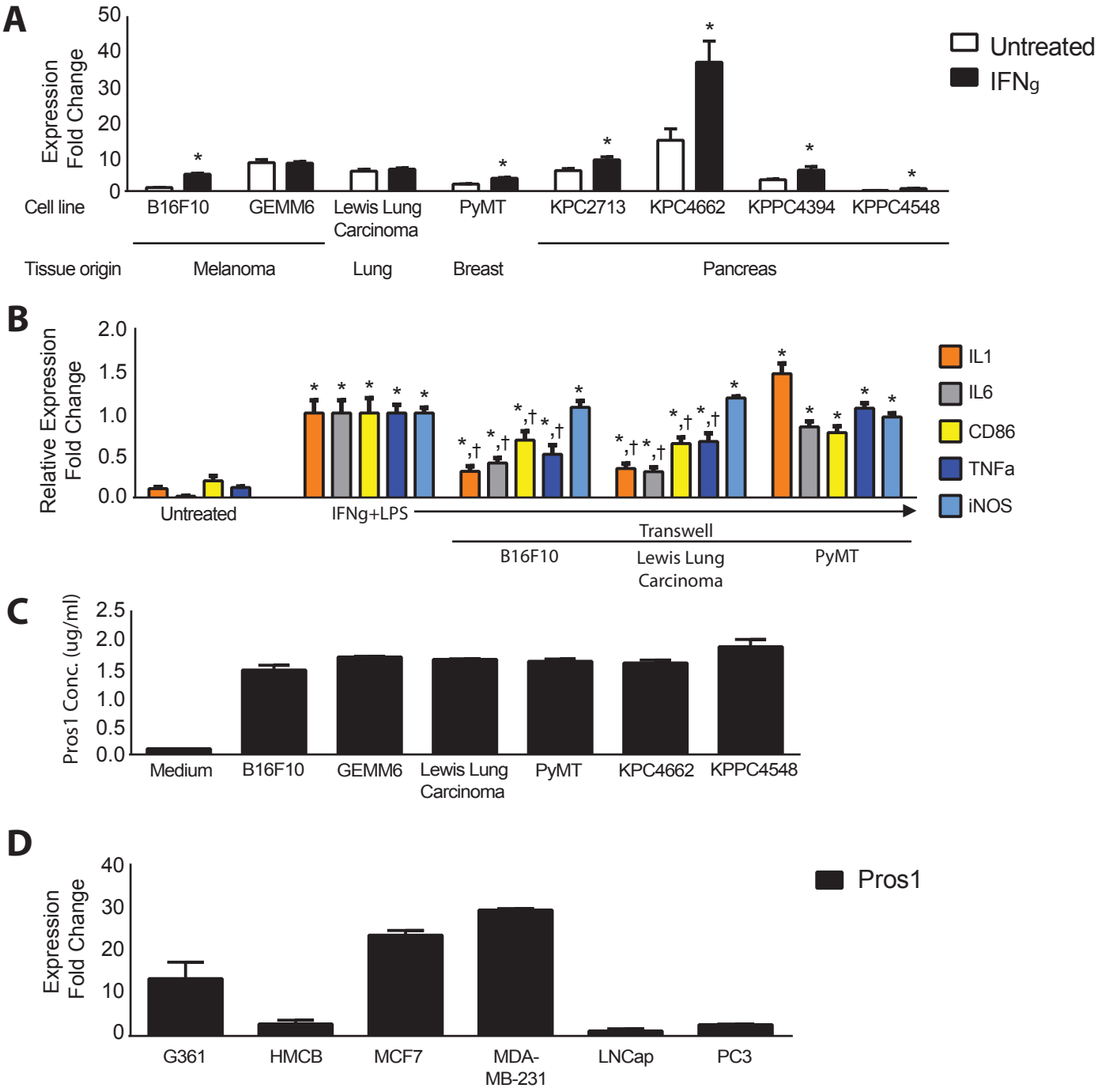
Supplemental Figure 15. M1 gene expression in naïve and M1 induced macrophages treated with p38 and/or Stat3 activator. A. qRT-PCR of M1 genes in untreated or IFNg and LPS treated macrophages cultured in the presence of Anisomycin (An), Colivelin (Co) or both (An+Co) for 24 hours. (n=5, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 16. M1 gene expression in naïve and M1 induced macrophages treated with PTP1b inhibitors. A. qRT-PCR of M1 genes in untreated or IFNg and LPS treated macrophages cultured in the presence of BVT948, PTP Inhibitor III or NSC87877 for 24 hours. (n=5, *P < 0.05 relative to untreated, †P < 0.05 relative to M1 induced) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

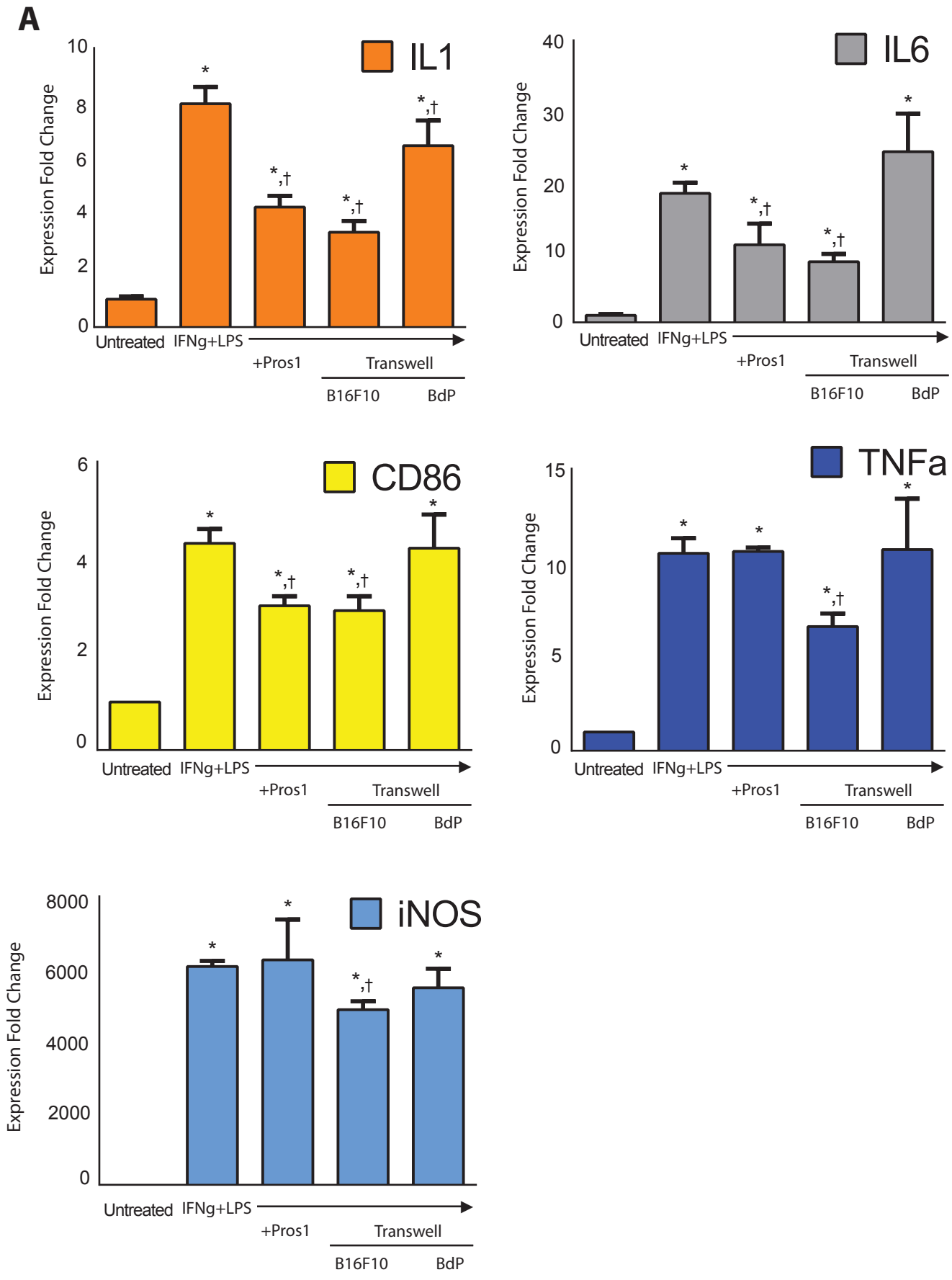
Supplemental Figure 17. TAM expression on intra-tumoral macrophages. A. Flow cytometric analysis of TAM receptors on F4/80(+) macrophages isolated from B16F10 or BdP tumors. (n=8, *P < 0.05 relative to B16F10) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 18. Labeling efficiency of Lyz2-Cre:tdTomato macrophages. A. Immunostaining of peritoneal macrophages isolated from Lyz2-Cre:tdTomato mice for the macrophage marker F4:80. (n=5, 1052 cells counted, 2 independent replicates) **B.** Immunostaining of untreated and M1 induced Lyz2-Cre:tdTomato macrophages for the M1 markers iNOS and IL1. (n=3) **C.** Immunostaining of intra-tumoral Lyz2-Cre:tdTomato cells for F4:80. (n=5, 2 independent replicates) (scale bar for **A**, **B**, and **C** 20μm) Data are mean ± SEM; P values calculated by 2-tailed Student's t-test.

Supplemental Figure 19. Pros1 expression and genetic alterations in Human cancer. A. Pros1 expression across Human tumor types. **B.** Mutation frequency of Pros1 in Human tumor types.



Supplemental Figure 1



Supplemental Figure 2

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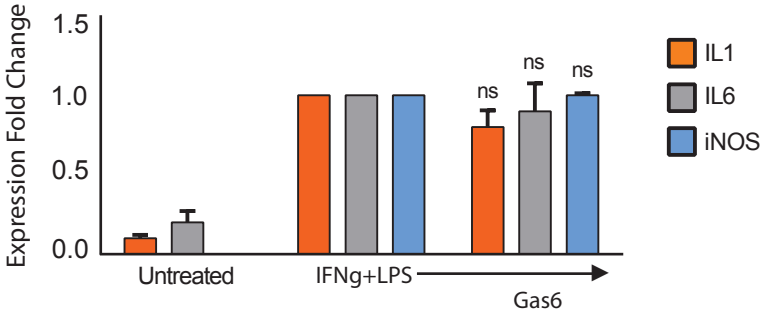

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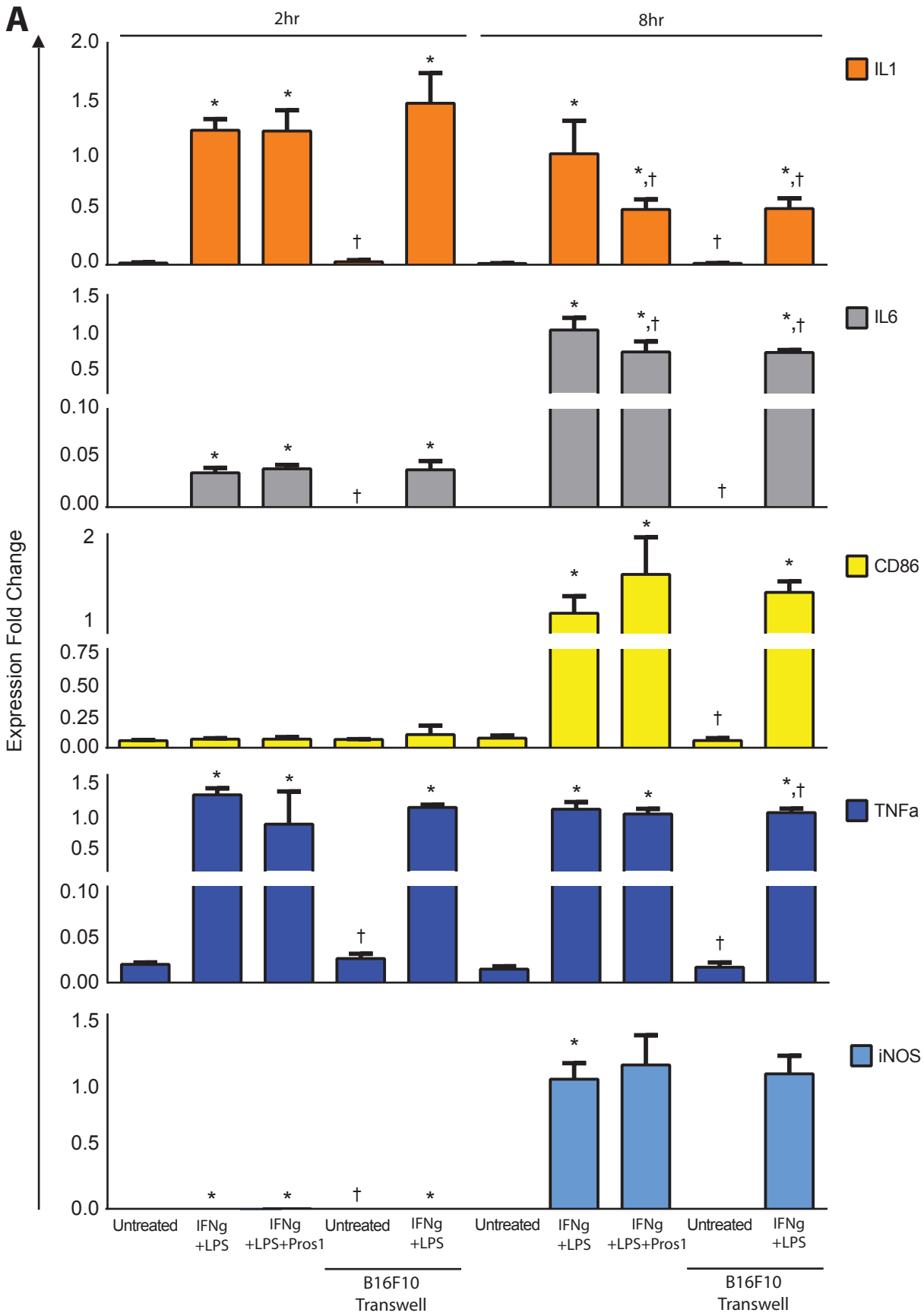

Supplemental Figure 4

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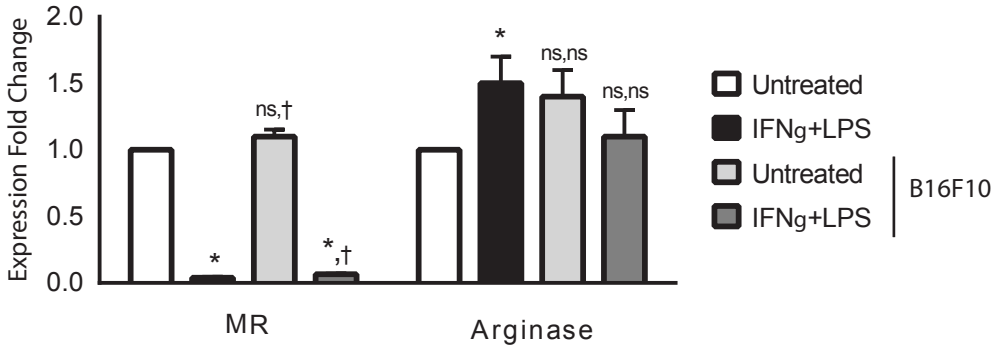
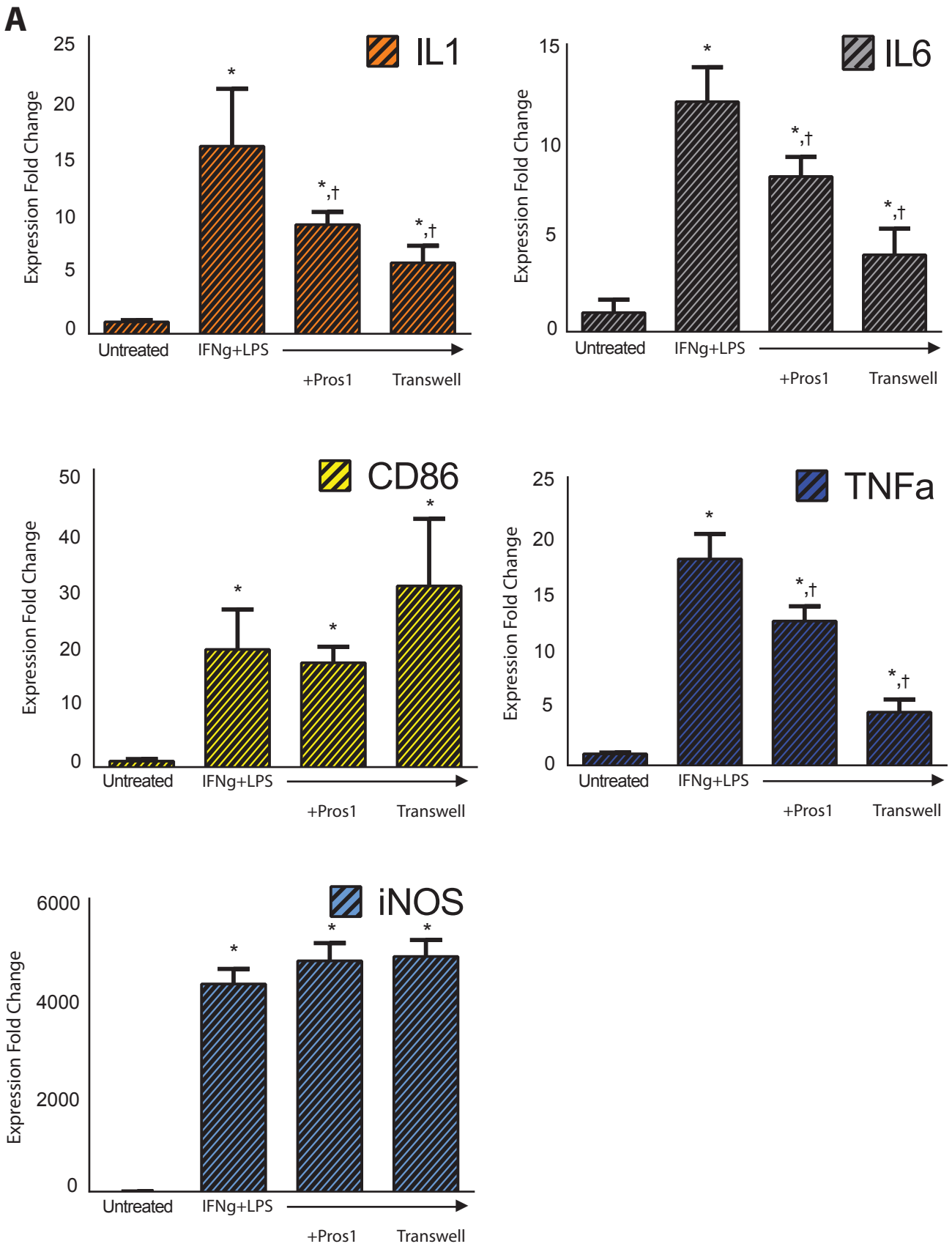


Supplemental Figure 5



Supplemental Figure 6

A

Mus musculus protein S (alpha) (Pros1), mRNA

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Range 1: 199 to 357 GenBank Graphics ▼ Next Match ▲ Previous Match

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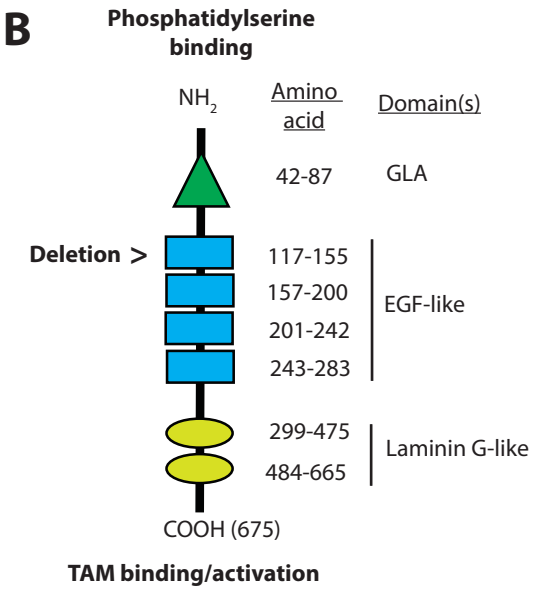

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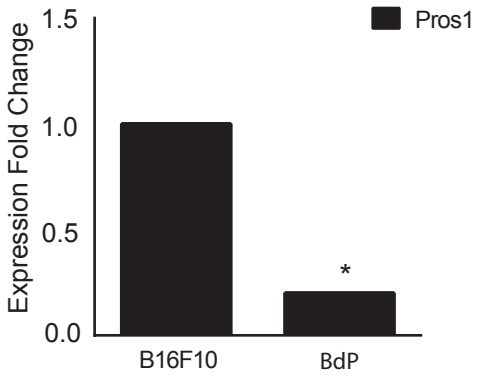

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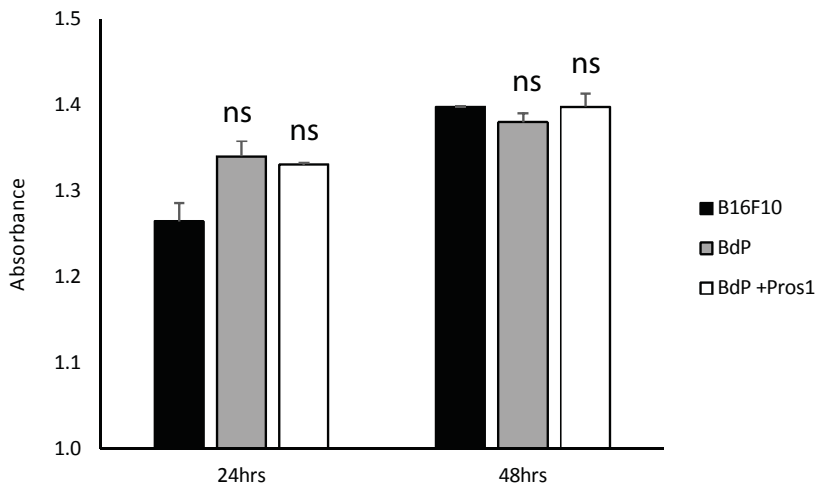

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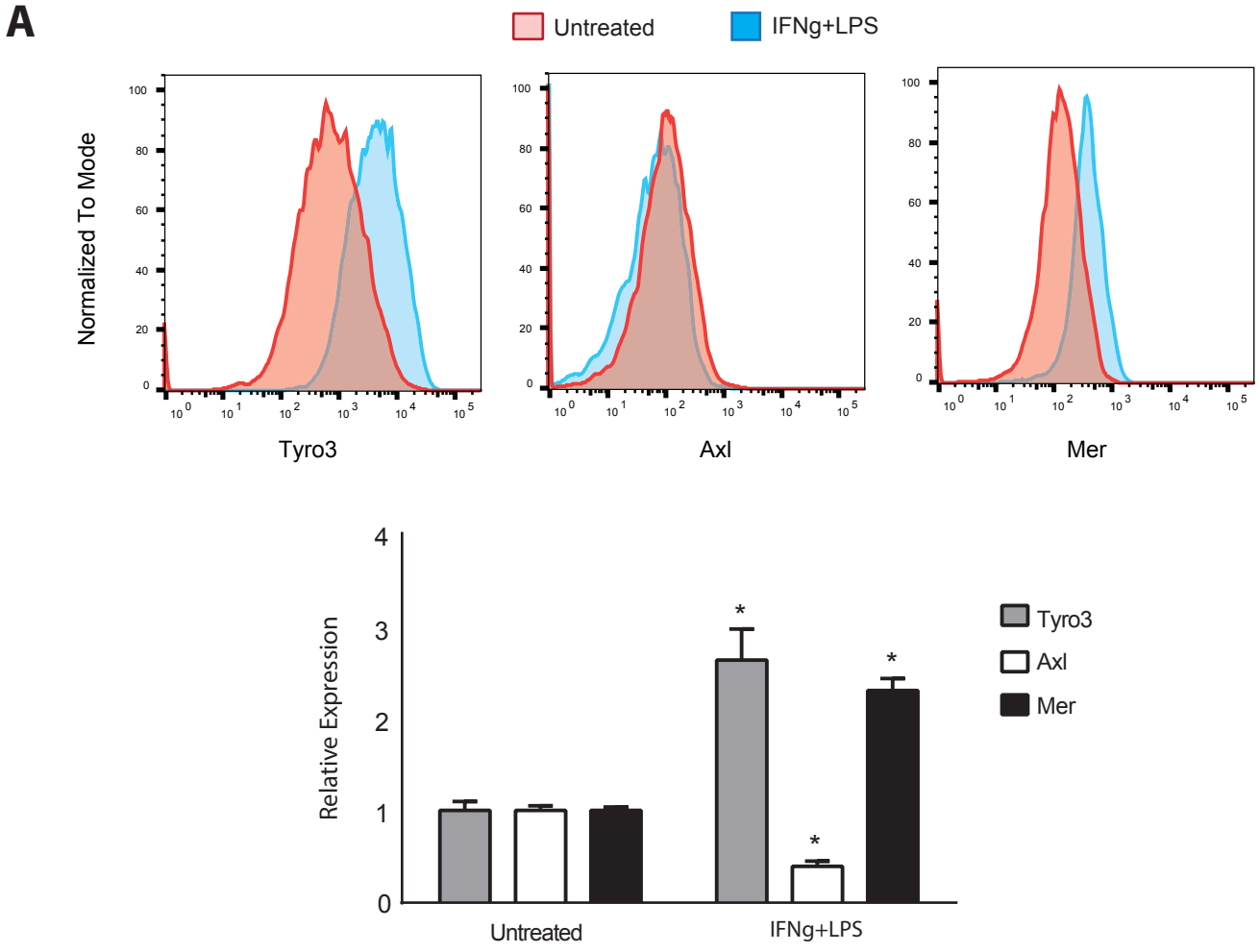

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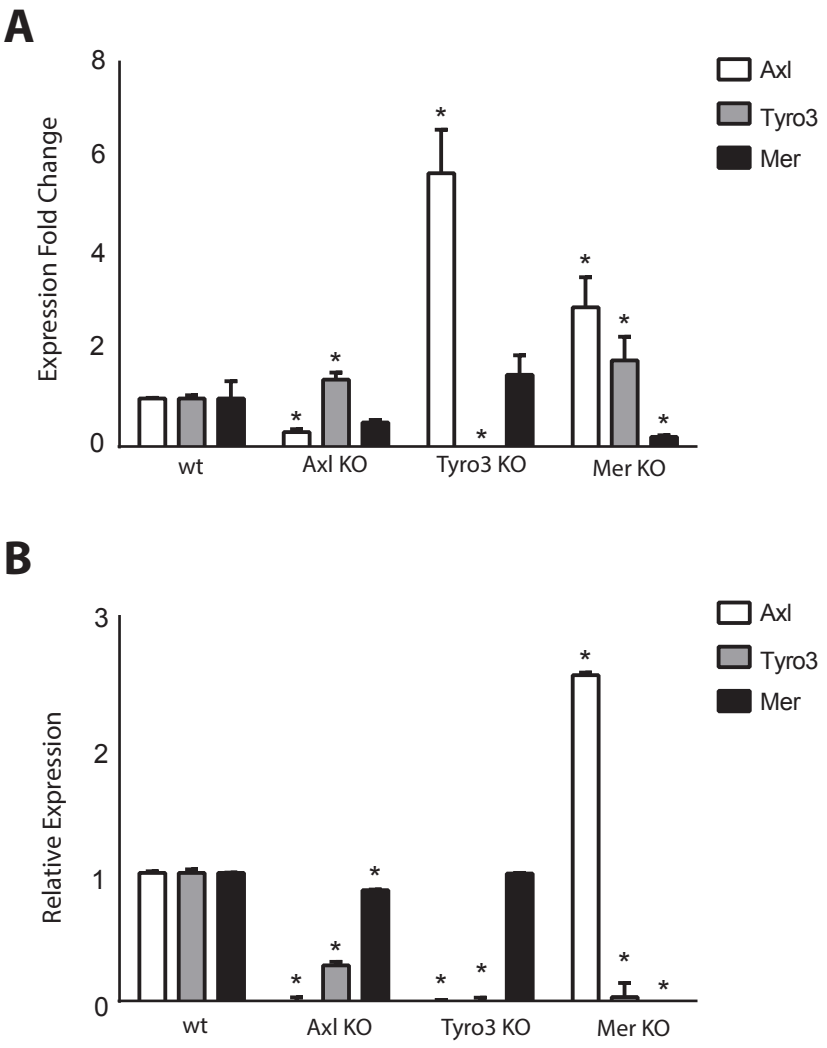

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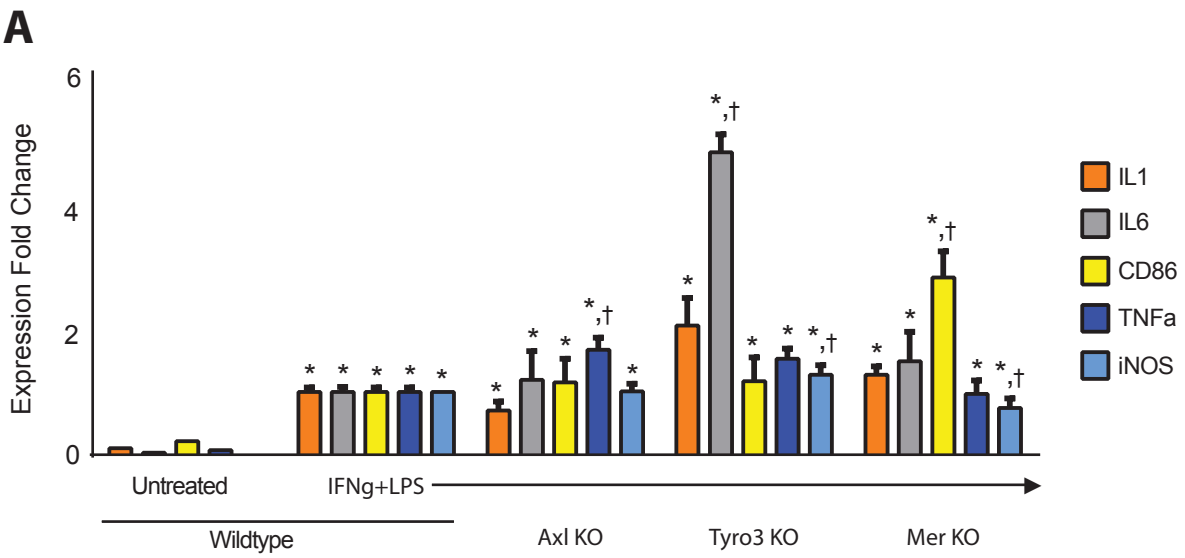

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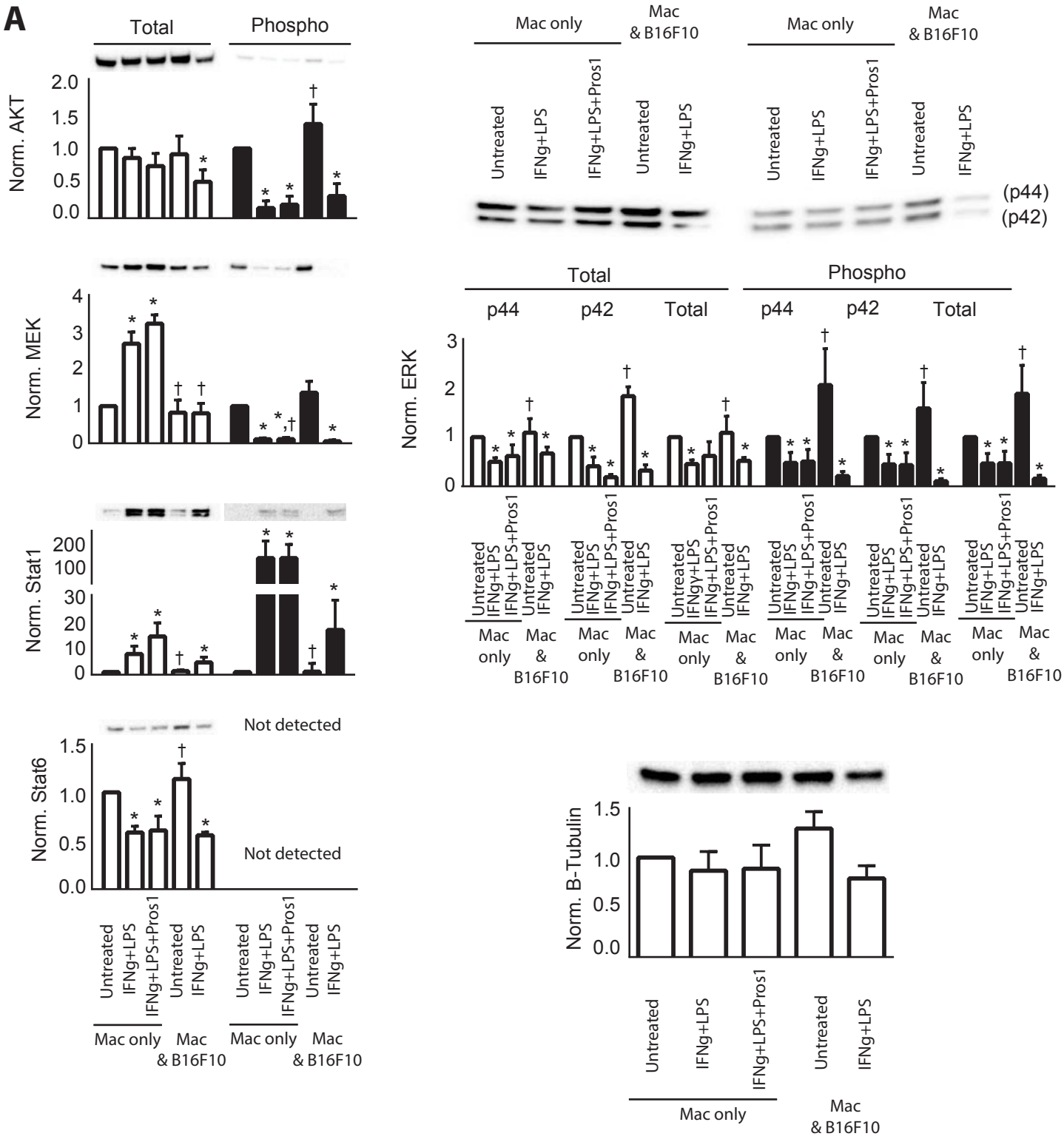

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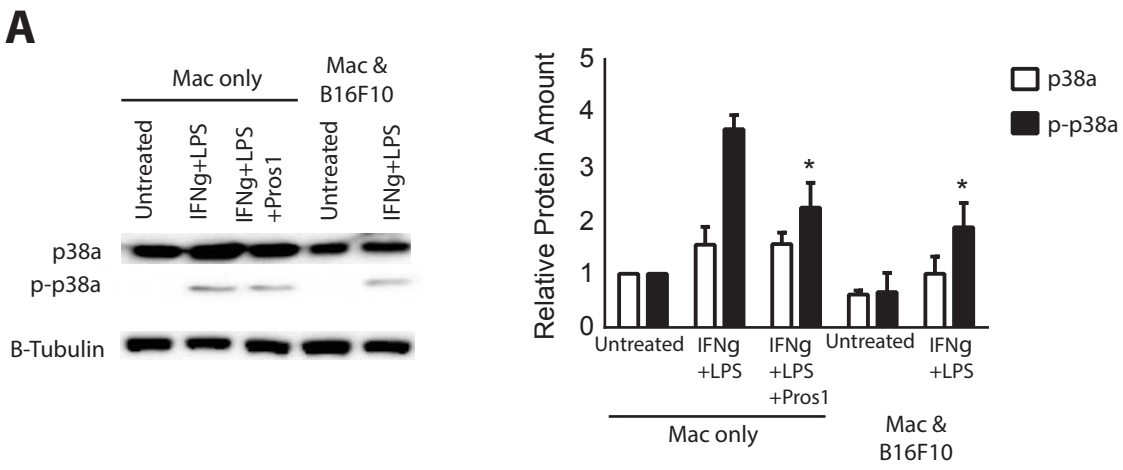

Supplemental Figure 12

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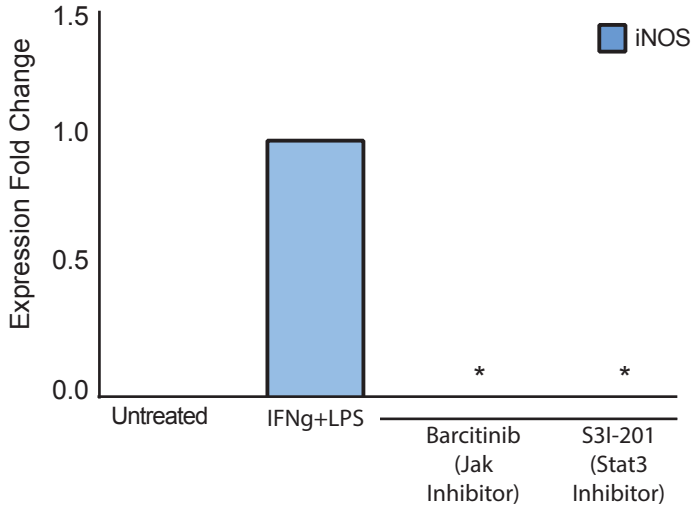

Supplemental Figure 13

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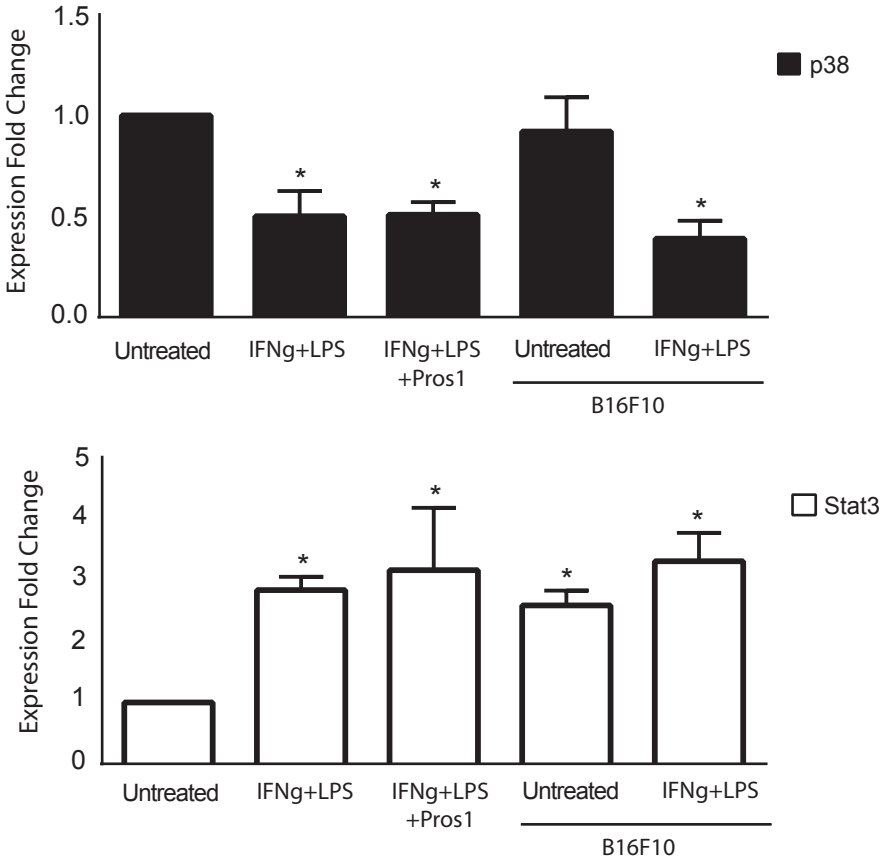

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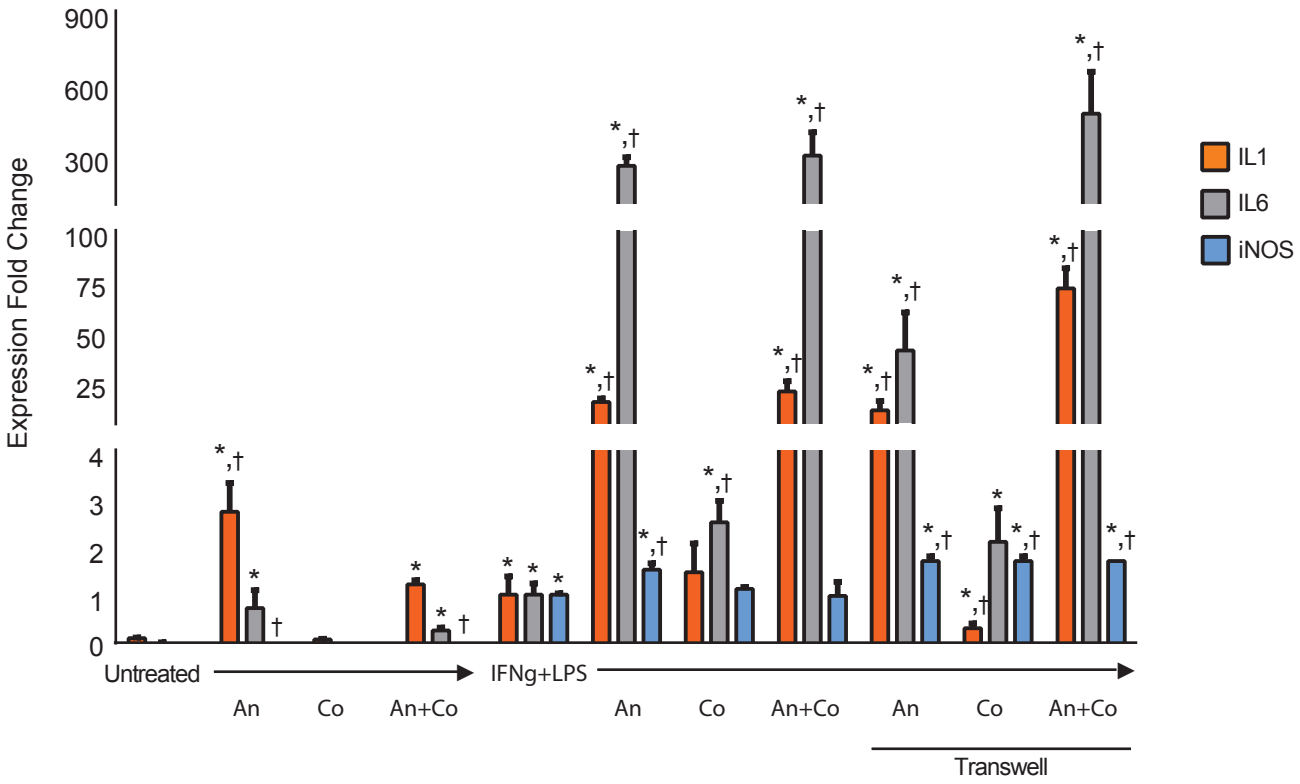

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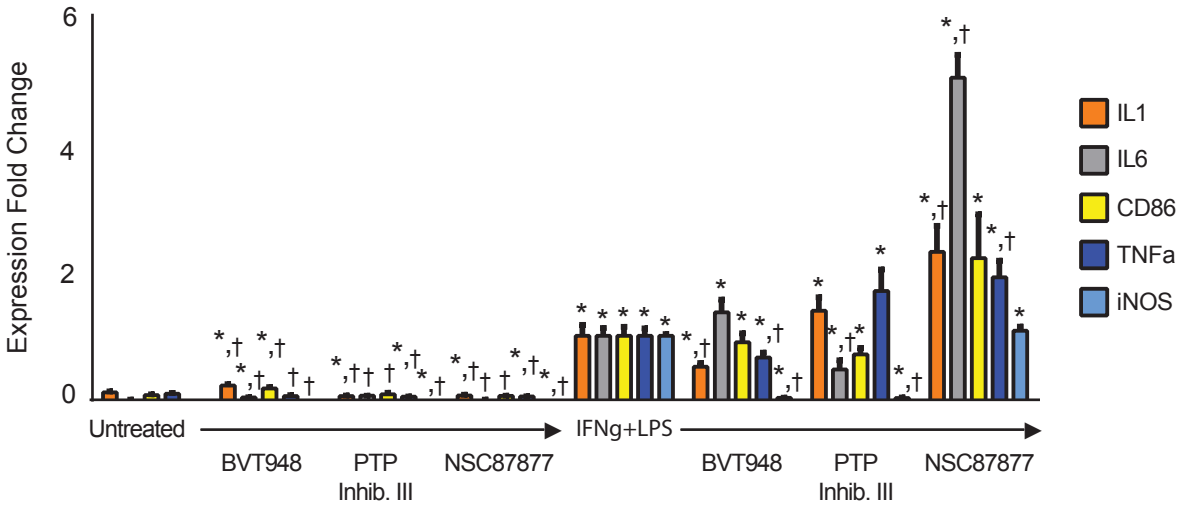

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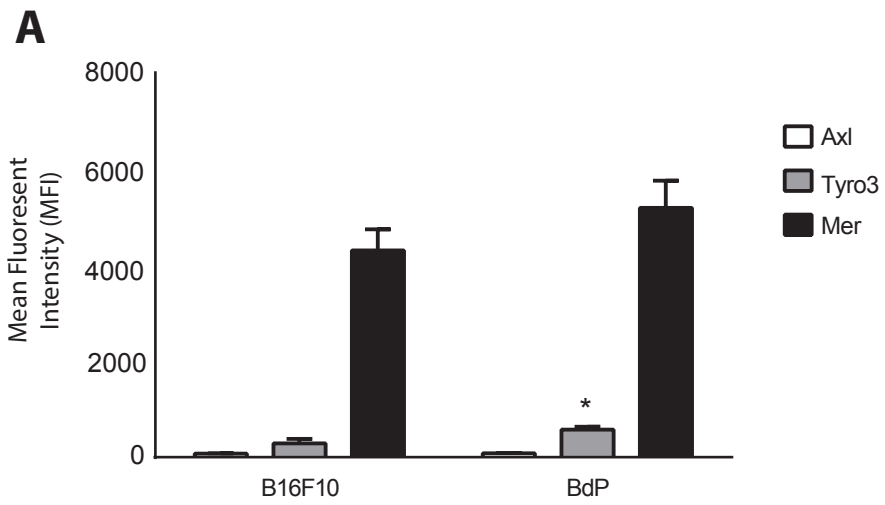

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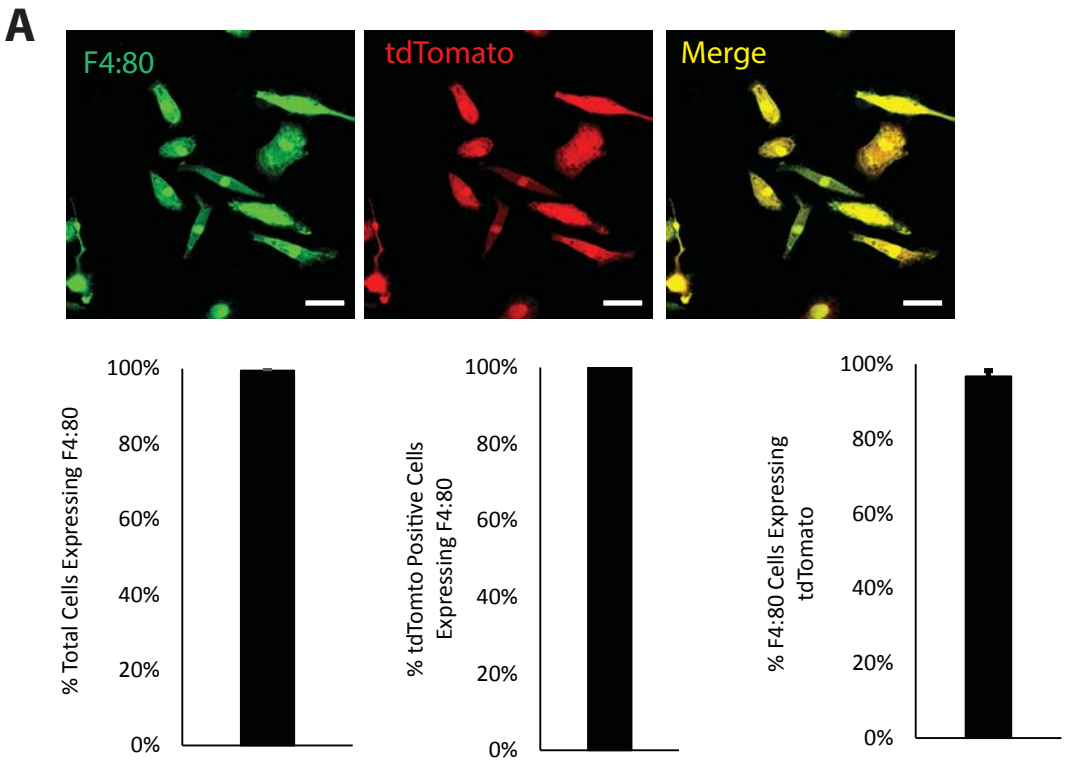

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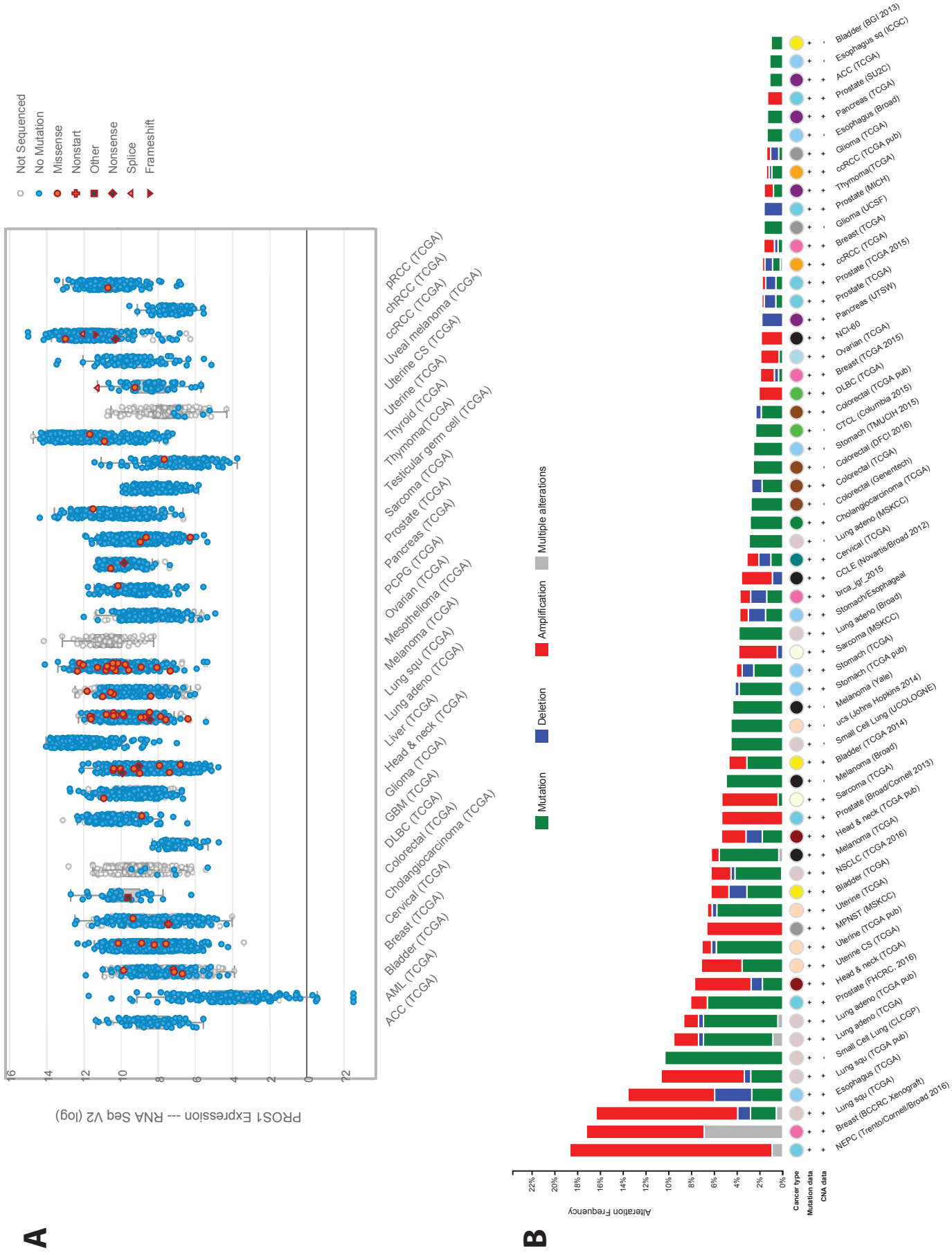

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