# Supplementary Materials: The α9 Nicotinic Acetylcholine Receptor Mediates Nicotine-Induced PD-L1 Expression and Regulates Melanoma Cell Proliferation and Migration

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**Table S1.** The chi-square test was employed to assess the correlation between a9-nChAR and PD-L1 expression in the tissue microarrays (*n* = 192) as categorical variables.

| Tissue microarrays | α9-nAChR low mRNA *n* (%) | α9-nAChR high mRNA *n* (%) | *p*-value |
|---|---|---|---|
| PD-L1 low mRNA - n (%) | 79 (81%) | 25 (26%) | $1.8 \times 10^{-14}$ |
| PD-L1 high mRNA - n (%) | 18 (19%) | 70 (74%) | |

**Table S2.** RT-PCR primers.

| Primer name | Primer sequence |
|---|---|
| α1-nAChR | F: 5'-GAGTCTAACAATGCGGCG-3'<br>R: 5'-TTTCTGCTCATCCTTGCTG-3' |
| α2-nAChR | F: 5'-TCCTGTGTTCCTGTCCTTCA-3'<br>R: 5'-GGTCCTCAGTCTCGGTATG-3' |
| α3-nAChR | F: 5'-GAGAAGGTGACCCTGTG-3'<br>R: 5'-GTGATGACGATGGACAAGGTTA-3' |
| α4-nAChR | F: 5'-GTTGGCGTATTTGCGTC-3'<br>R: 5'-GAGAGGTCAATCCACGG-3' |
| α5-nAChR | F: 5'-ACGCTTCCCAAACTGCT-3'<br>R: 5'-CTTCAACAACCTCACGG-3' |
| α6-nAChR | F: 5'-ACGCTTTGTATTTCAGTCCT-3'<br>R: 5'-GGGTGCGGTAGTGTATG-3' |
| α7-nAChR | F: 5'-CGGCAAGAGGAGTGAAA-3'<br>R: 5'-GAACACCAGCAGGGCGA-3' |
| α9-nAChR | F: 5'-AGGGTGGTCATCCTGAAAT-3'<br>R: 5'-AGGTCTTTGTTCCTGGC-3' |
| α10-nAChR | F: 5'-CACAAGGGAGCACTCAT-3'<br>R: 5'-CCAATACCCAGCACAAAC-3' |
| PD-L1 | F: 5'-AGG TGC GTT CAG ATG TTG GC-3'<br>R: 5'-TGC CCA AGG CAG CAA ATC AG-3' |
| GAPDH | F: 5'-ATCCCATCACCATCTTCCAG-3'<br>R: 5'-GCTGATGATCTTGAGGCTGT-3' |
| PD-L1 promotor | F: 5'-GAC CTG CT GCA CTA ATT GTC-3'<br>R: 5'-CAT TTC CCA GGG AGA GCT GG-3' |
| α9-nAChR-siRNA-1 | F: 5'-GATCCCCCACCAATGTGGTCCTGCGGTTCAAGAGA<br>CCGCAGGACCACATTGGTGTTTTTA-3'<br>R: 5'-AGCTTAAAAACACCAATGTGGTCCTGCGGTCTCTT<br>GAACCGCAGGACCACATTGGTGGGG-3' |
| α9-nAChR-siRNA-2 | F: 5'-GATCCCCAGACCACAAGGCCACCAATTTCAAG<br>AGAATTGGTGGCCTTGTGGTCTTTTTTA-3'<br>R: 5'-AGCTTAAAAAAGACCACAAGGCCACCAATT<br>CTCTTGAAATTGGTGGCCTTGTGGTCTGGG-3' |

| | |
|---|---|
| α9-nAChR-scramble | F: 5′-GATCCCCGAGAACACGAATACACCCCTTCAA GAGAGGGGTGTATTCGTGTTCTCTTTTTA-3′ R: 5′-AGCTTAAAAAGAGAACACGAATACACCCCTCTC TTGAAGGGGTGTATTCGTGTTCTCGGG-3′ |
| pcDNA3.1 to α9-nAChR overexpression | F: 5′-AATAAGCTTGCCGCCACCATGAACTGGTCCCATTCCTG-3′ R: 5′-ATAGAATTCACATCCGCTCTTGCTATGA-3′ |



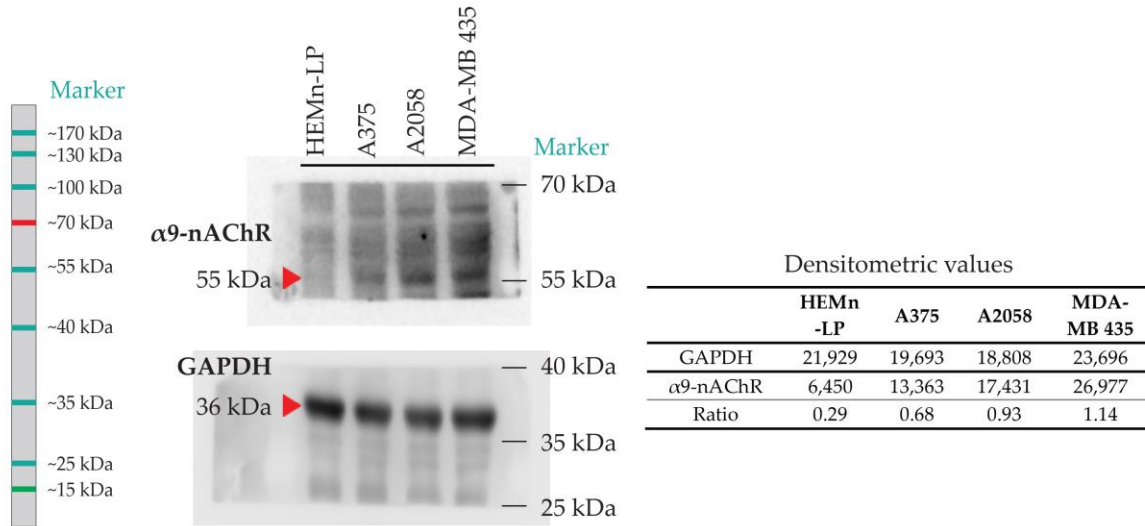**Figure S1.** Uncropped scans with size marker indications of western blots shown in Figure 1D, and densitometry readings/intensity ratios.
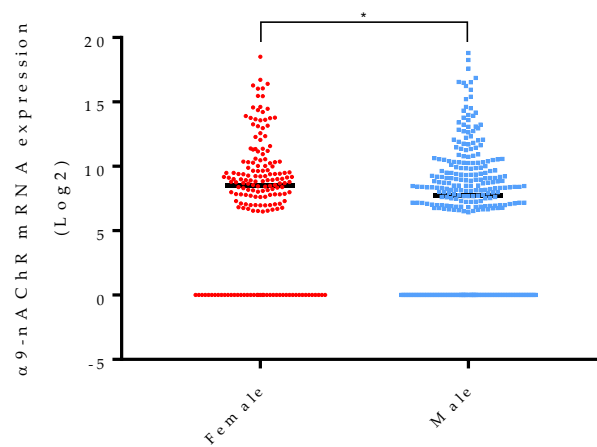


**Figure S2.** α9-nAChR mRNA level in female (*n* = 179) and male (*n* = 293) groups. The result was analyzed from TCGA-SKCM cohort *(n* = 472). The data were analyzed by the Mann-Whitney test. The median of α9-nAChR expression in each group is shown by the horizontal line; * *p* < 0.05.
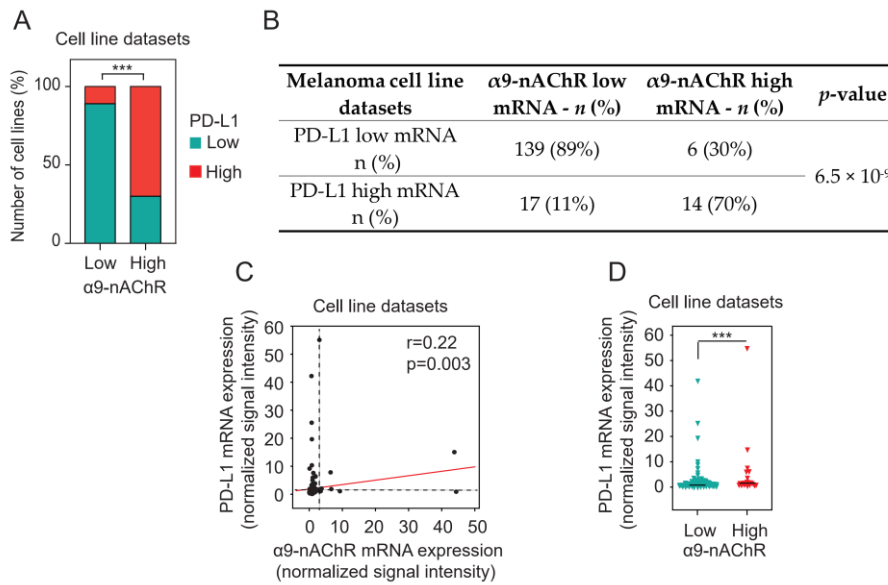
**Figure S3.** Correlation between $\alpha$9-nAChR and PD-L1 mRNA expression in the melanoma cell line datasets. **(A, B)** The association between $\alpha$9-nAChR and PD-L1 mRNA levels in the melanoma cell line datasets (*n* = 176) as categorical variables. The $\chi^2$ test was employed to assess the correlation between a9-nAChR and PD-L1 expression in samples. ** *p* < 0.01. **(C)** Correlation between $\alpha$9-nAChR and PD-L1 mRNA expression in the melanoma cell line datasets (*n* = 176). Pearson and Spearman's rank correlation measured the strength of the association between $\alpha$9-nAChR and PD-L1 expression. **(D)** PDL1 mRNA expression in melanoma cell lines with low (*n* = 156) or high (*n* = 20) $\alpha$9-nAChR expression in the melanoma cell line datasets. The data were analyzed by the Mann-Whitney test. The median $\alpha$9-nAChR expression in each group is shown by a horizontal line. *** *p* < 0.001.
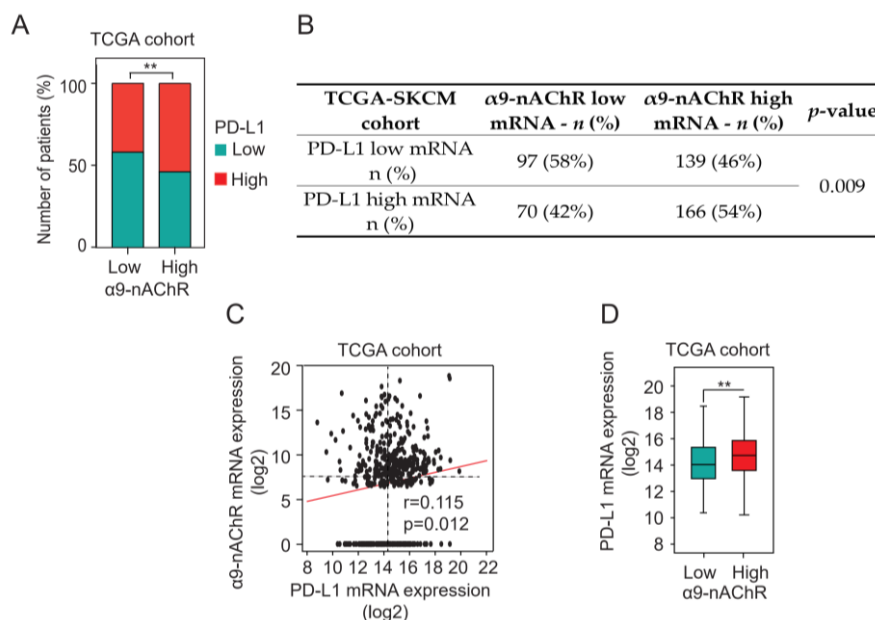


**Figure S4.** Correlation between $\alpha$9-nAChR and PD-L1 mRNA expression in the TCGA-SKCM cohort. **(A, B)** The association between $\alpha$9-nAChR and PD-L1 mRNA levels in the TCGA-SKCM cohort (*n*=472) as categorical variables. The $\chi^2$ test was employed to assess the correlation between a9-nAChR and PD-L1 expression in samples. ** *p* < 0.01. **(C)** Correlation between $\alpha$9-nAChR and PD-L1 mRNA expression in the TCGA-SKCM cohort (*n* = 472). Pearson and Spearman's rank correlation measured the strength of the association between $\alpha$9-nAChR and PD-L1 expression. **(D)** PD-L1 mRNA expression in patients in the TCGA-SKCM cohort with high (*n* = 305) or low $\alpha$9-nAChR (*n* = 167) expression. The data are presented as the mean ± SD, ** *p* < 0.01, Student's t-test.
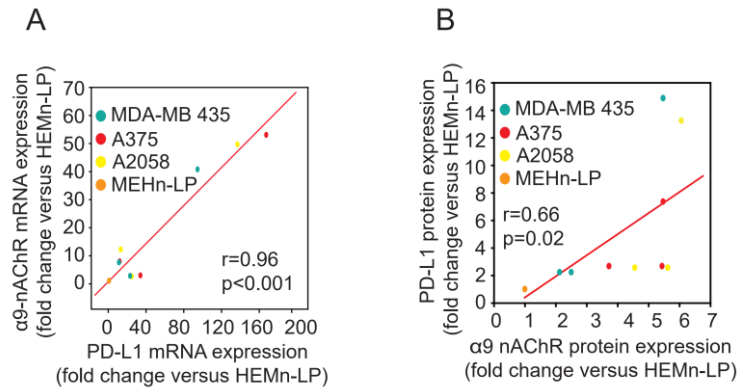
**Figure S5.** Correlations between α9-nAChR and PD-L1 in melanoma cell lines. **(A, B)** Correlations between α9-nAChR and PD-L1 mRNA and protein levels in the HEMn-LP, A375, A2058, and MDA-MB 435 cell lines. The experiments were repeated three times per cell line. Band intensities were measured using ImageJ software and the amounts of α9-nAChR and PD-L1 at the mRNA and protein levels in the A375, A2058, and MDA-MB 435 melanoma cells were normalized to the α9-nAChR and PD-L1 mRNA and protein levels in the HEMn-LP cells.
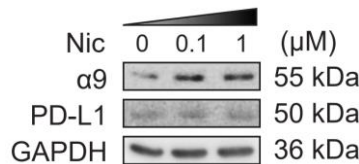


**Figure S6.** Nicotine-induced α9-nAChR activity did not influence PD-L1 protein levels in the HEMn-LP melanocytes. The results were determined by western blotting.



| Densitometric values | | | | |
|---|---|---|---|---|
| | HEMn-LP | A375 | A2058 | MDA-MB 435 |
| GAPDH | 21,931 | 18,980 | 21,703 | 23,572 |
| PD-L1 | 4,829 | 24,753 | 26,825 | 27,778 |
| Ratio | 0.22 | 1.30 | 1.24 | 1.18 |
| α9-nAChR | 9,027 | 23,536 | 22,767 | 24,239 |
| Ratio | 0.41 | 1.24 | 1.05 | 1.03 |

**Figure S7.** Uncropped scans with size marker indications of western blots shown in Figure 2L, and densitometry readings/intensity ratios.
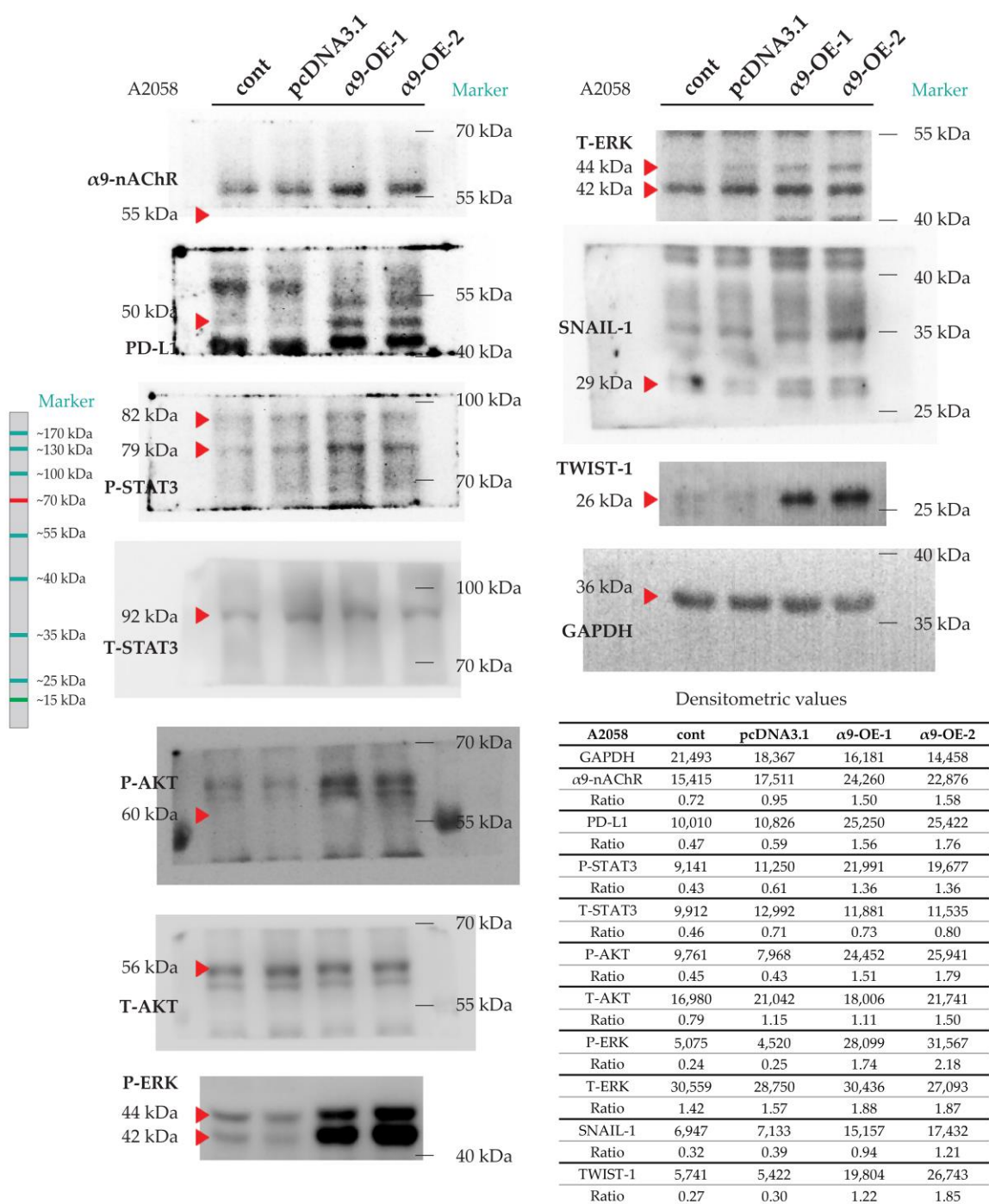
**Figure S8.** Uncropped scans with size marker indications of western blots shown in Figure 3A, and densitometry readings/intensity ratios.

| MDA-MB 435 | cont | pcDNA3.1 | α9-OE-1 | α9-OE-2 |
|---|---|---|---|---|
| GAPDH | 19,684 | 12,431 | 17,131 | 15,319 |
| α9-nAChR | 14,268 | 7,855 | 25,328 | 29,579 |
| Ratio | 0.72 | 0.63 | 1.48 | 1.93 |
| PD-L1 | 8,510 | 7,902 | 20,762 | 27,552 |
| Ratio | 0.43 | 0.64 | 1.21 | 1.80 |
| P-STAT3 | 3,545 | 7,353 | 25,844 | 15,763 |
| Ratio | 0.18 | 0.59 | 1.51 | 1.03 |
| T-STAT3 | 23,688 | 18,161 | 19,558 | 16,716 |
| Ratio | 1.20 | 1.46 | 1.14 | 1.09 |
| P-AKT | 6,540 | 6,090 | 20,616 | 26,832 |
| Ratio | 0.33 | 0.49 | 1.20 | 1.75 |
| T-AKT | 26,727 | 23,206 | 21,127 | 20,096 |
| Ratio | 1.36 | 1.87 | 1.23 | 1.31 |
| P-ERK | 5,847 | 6,133 | 19,873 | 17,074 |
| Ratio | 0.30 | 0.49 | 1.16 | 1.11 |
| T-ERK | 34,603 | 27,767 | 31,743 | 29,664 |
| Ratio | 1.76 | 2.23 | 1.85 | 1.94 |
| SNAIL-1 | 13,926 | 9,364 | 19,536 | 19,259 |
| Ratio | 0.71 | 0.75 | 1.14 | 1.26 |
| TWIST-1 | 13,335 | 9,798 | 23,918 | 25,393 |
| Ratio | 0.68 | 0.79 | 1.40 | 1.66 |

**Figure S8.** Uncropped scans with size marker indications of western blots shown in Figure 3A (continued), and densitometry readings/intensity ratios.

| A2058 | pcDNA3.1 | | α9-OE | |
|---|---|---|---|---|
| LY294002 (µM) | 0 | 10 | 0 | 10 |
| GAPDH | 30,380 | 35,521 | 35,193 | 30,764 |
| P-AKT | 10,450 | 7,831 | 23,210 | 14,815 |
| Ratio | 0.34 | 0.22 | 0.66 | 0.48 |
| T-AKT | 27,051 | 29,212 | 25,913 | 23,596 |
| Ratio | 0.89 | 0.82 | 0.74 | 0.77 |

| A2058 | pcDNA3.1 | | α9-OE | |
|---|---|---|---|---|
| PD98059 (µM) | 0 | 10 | 0 | 10 |
| GAPDH | 28,607 | 30,948 | 26,456 | 28,840 |
| P-ERK | 16,431 | 14,667 | 22,437 | 18,555 |
| Ratio | 0.57 | 0.47 | 0.85 | 0.64 |
| T-ERK | 24,073 | 25,446 | 27,090 | 29,594 |
| Ratio | 0.84 | 0.82 | 1.02 | 1.03 |

**Figure S9.** Uncropped scans with size marker indications of western blots shown in Figure 3J and Figure 3L, and densitometry readings/intensity ratios.
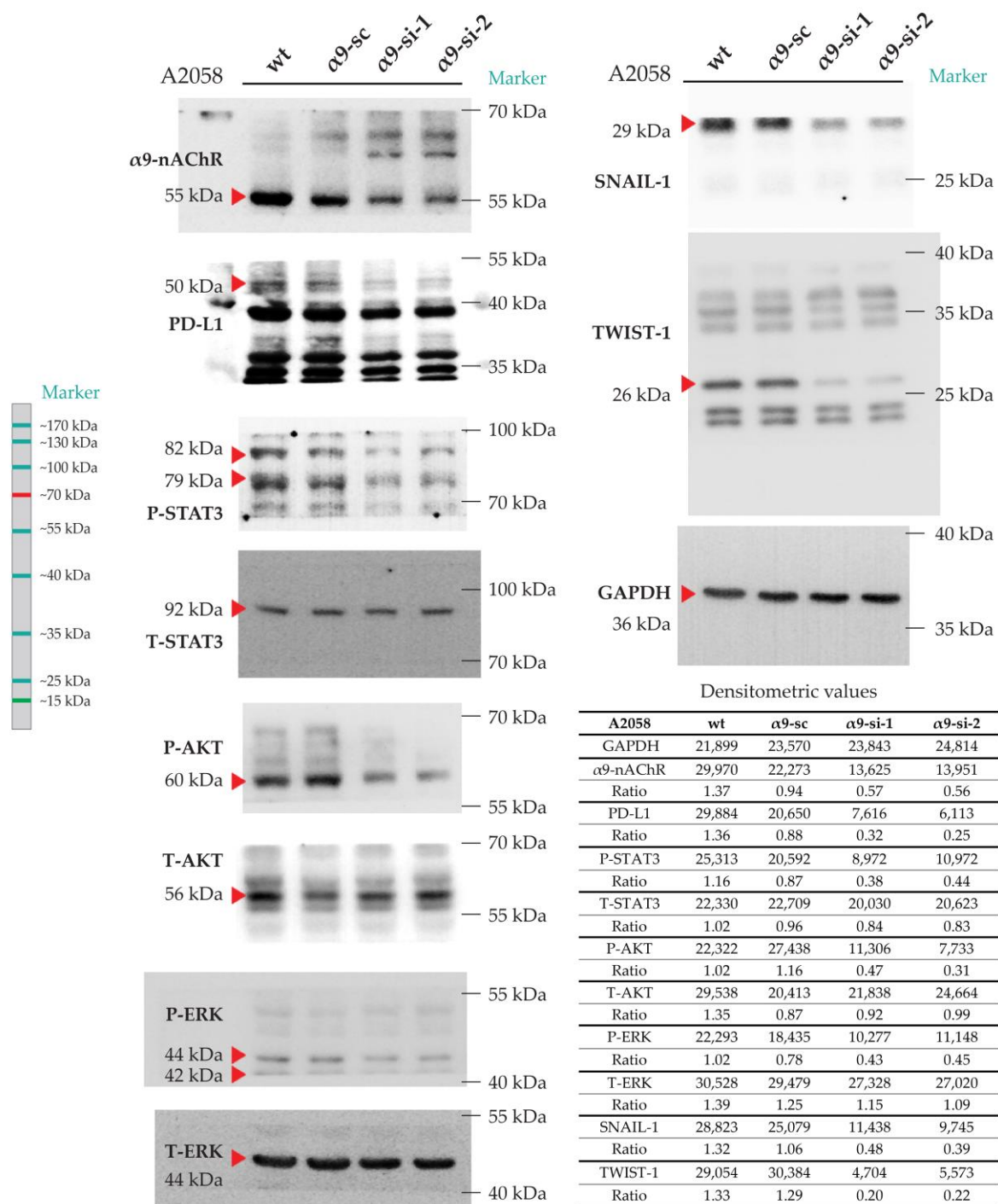
**Figure S10.** Uncropped scans with size marker indications of western blots shown in Figure 4A, and densitometry readings/intensity ratios.

| A2058 | wt | α9-sc | α9-si-1 | α9-si-2 |
|---|---|---|---|---|
| GAPDH | 21,899 | 23,570 | 23,843 | 24,814 |
| α9-nAChR | 29,970 | 22,273 | 13,625 | 13,951 |
| Ratio | 1.37 | 0.94 | 0.57 | 0.56 |
| PD-L1 | 29,884 | 20,650 | 7,616 | 6,113 |
| Ratio | 1.36 | 0.88 | 0.32 | 0.25 |
| P-STAT3 | 25,313 | 20,592 | 8,972 | 10,972 |
| Ratio | 1.16 | 0.87 | 0.38 | 0.44 |
| T-STAT3 | 22,330 | 22,709 | 20,030 | 20,623 |
| Ratio | 1.02 | 0.96 | 0.84 | 0.83 |
| P-AKT | 22,322 | 27,438 | 11,306 | 7,733 |
| Ratio | 1.02 | 1.16 | 0.47 | 0.31 |
| T-AKT | 29,538 | 20,413 | 21,838 | 24,664 |
| Ratio | 1.35 | 0.87 | 0.92 | 0.99 |
| P-ERK | 22,293 | 18,435 | 10,277 | 11,148 |
| Ratio | 1.02 | 0.78 | 0.43 | 0.45 |
| T-ERK | 30,528 | 29,479 | 27,328 | 27,020 |
| Ratio | 1.39 | 1.25 | 1.15 | 1.09 |
| SNAIL-1 | 28,823 | 25,079 | 11,438 | 9,745 |
| Ratio | 1.32 | 1.06 | 0.48 | 0.39 |
| TWIST-1 | 29,054 | 30,384 | 4,704 | 5,573 |
| Ratio | 1.33 | 1.29 | 0.20 | 0.22 |

Densitometric values

| A2058 | Concentration (μM) | | |
|---|---|---|---|
| Stattic | 0 | 2.5 | 5 |
| GAPDH | 7,432 | 9,392 | 9,898 |
| PD-L1 | 13,879 | 9,417 | 9,602 |
| Ratio | 1.87 | 1.00 | 0.97 |
| P-STAT3 | 11,720 | 6,385 | 6,791 |
| Ratio | 1.58 | 0.68 | 0.69 |
| T-STAT3 | 18,855 | 11,468 | 12,224 |
| Ratio | 2.54 | 1.22 | 1.24 |

Densitometric values

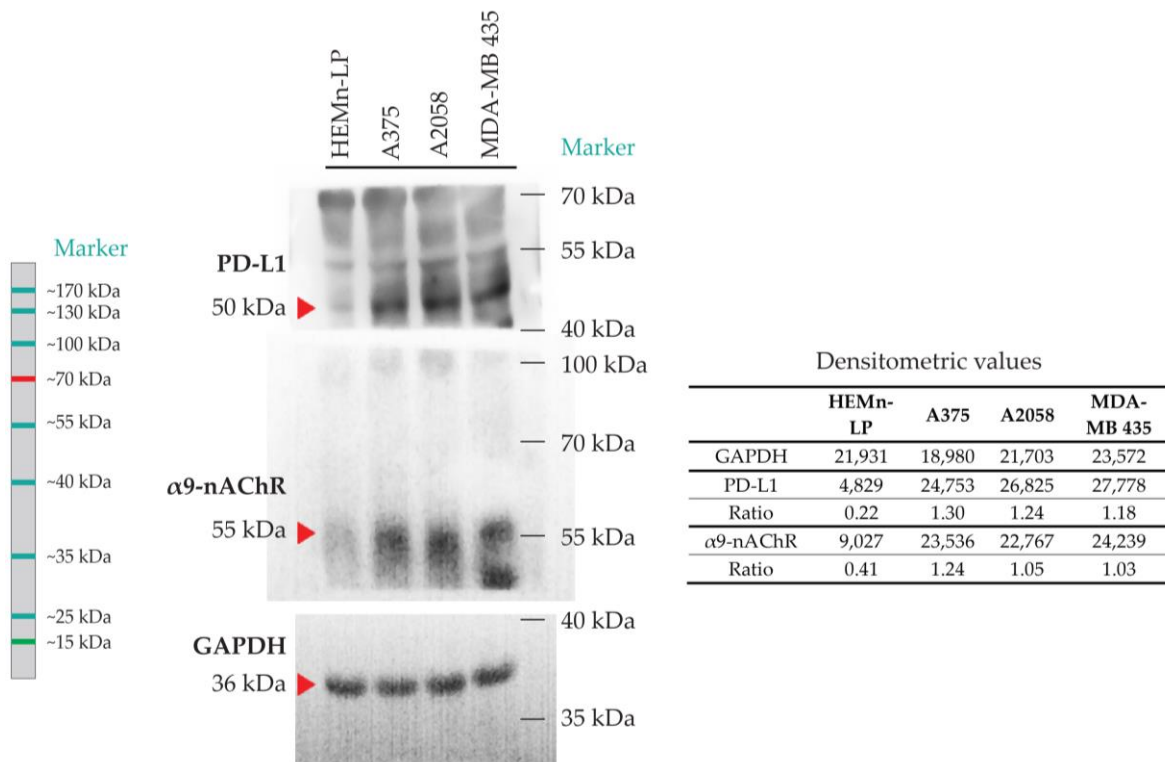| A2058 | Concentration (μM) | | |
|---|---|---|---|
| NSC74859 | 0 | 10 | 25 |
| GAPDH | 10,095 | 10,499 | 10,262 |
| PD-L1 | 17,457 | 13,828 | 12,001 |
| Ratio | 1.73 | 1.32 | 1.17 |
| P-STAT3 | 17,653 | 5,412 | 3,408 |
| Ratio | 1.75 | 0.52 | 0.33 |
| T-STAT3 | 17,932 | 11,671 | 12,320 |
| Ratio | 1.78 | 1.11 | 1.20 |

**Figure S11.** Uncropped scans with size marker indications of western blots shown in Figure 4G, and densitometry readings/intensity ratios.



Densitometric values

| A375 | Nicotine (μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 10⁻³ | 10⁻² | 10⁻¹ | 1 |
| GAPDH | 15,875 | 16,884 | 17,607 | 16,973 | 16,665 |
| α9-nAChR | 6,995 | 14,331 | 19,486 | 18,995 | 18,453 |
| Ratio | 0.44 | 0.85 | 1.11 | 1.12 | 1.11 |
| PD-L1 | 3,939 | 7,026 | 11,600 | 16,856 | 20,873 |
| Ratio | 0.25 | 0.42 | 0.66 | 0.99 | 1.25 |

Densitometric values

| A375 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 12 | 24 | 48 | 72 |
| GAPDH | 16,394 | 20,505 | 19,399 | 17,443 | 16,030 |
| α9-nAChR | 3,973 | 14,801 | 19,022 | 18,667 | 19,611 |
| Ratio | 0.24 | 0.72 | 0.98 | 1.07 | 1.22 |
| PD-L1 | 9,123 | 15,569 | 21,502 | 22,852 | 22,325 |
| Ratio | 0.56 | 0.76 | 1.11 | 1.31 | 1.39 |

**Figure S12.** Uncropped scans with size marker indications of western blots shown in Figure 5B, and densitometry readings/intensity ratios.

**Densitometric values**

| A2058 | Nicotine (μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 10⁻³ | 10⁻² | 10⁻¹ | 1 |
| GAPDH | 11,130 | 10,578 | 10,934 | 11,223 | 11,948 |
| α9-nAChR | 6,747 | 8,661 | 9,571 | 9,100 | 10,488 |
| Ratio | 0.61 | 0.82 | 0.88 | 0.81 | 0.88 |
| PD-L1 | 4,671 | 8,775 | 11,466 | 10,941 | 12,954 |
| Ratio | 0.42 | 0.83 | 1.05 | 0.97 | 1.08 |

**Densitometric values**

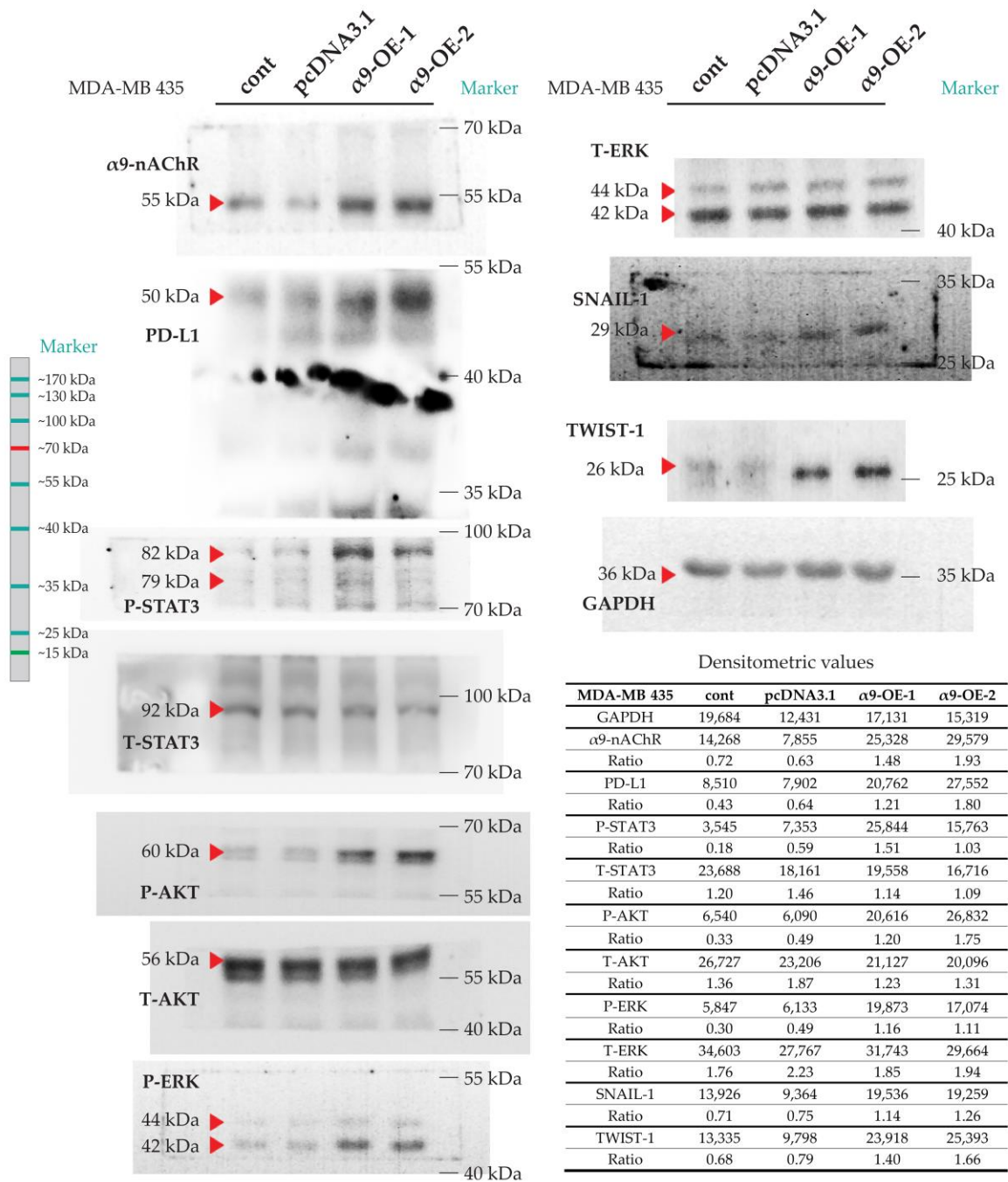| A2058 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 12 | 24 | 48 | 72 |
| GAPDH | 15,927 | 18,321 | 20,687 | 12,698 | 13,988 |
| α9-nAChR | 3,973 | 14,801 | 19,022 | 18,667 | 19,611 |
| Ratio | 0.25 | 0.81 | 0.92 | 1.47 | 1.40 |
| PD-L1 | 1,916 | 5,538 | 8,415 | 7,070 | 9,687 |
| Ratio | 0.12 | 0.30 | 0.41 | 0.56 | 0.69 |

**Figure S12.** Uncropped scans with size marker indications of western blots shown in Figure 5B (continued), and densitometry readings/intensity ratios.
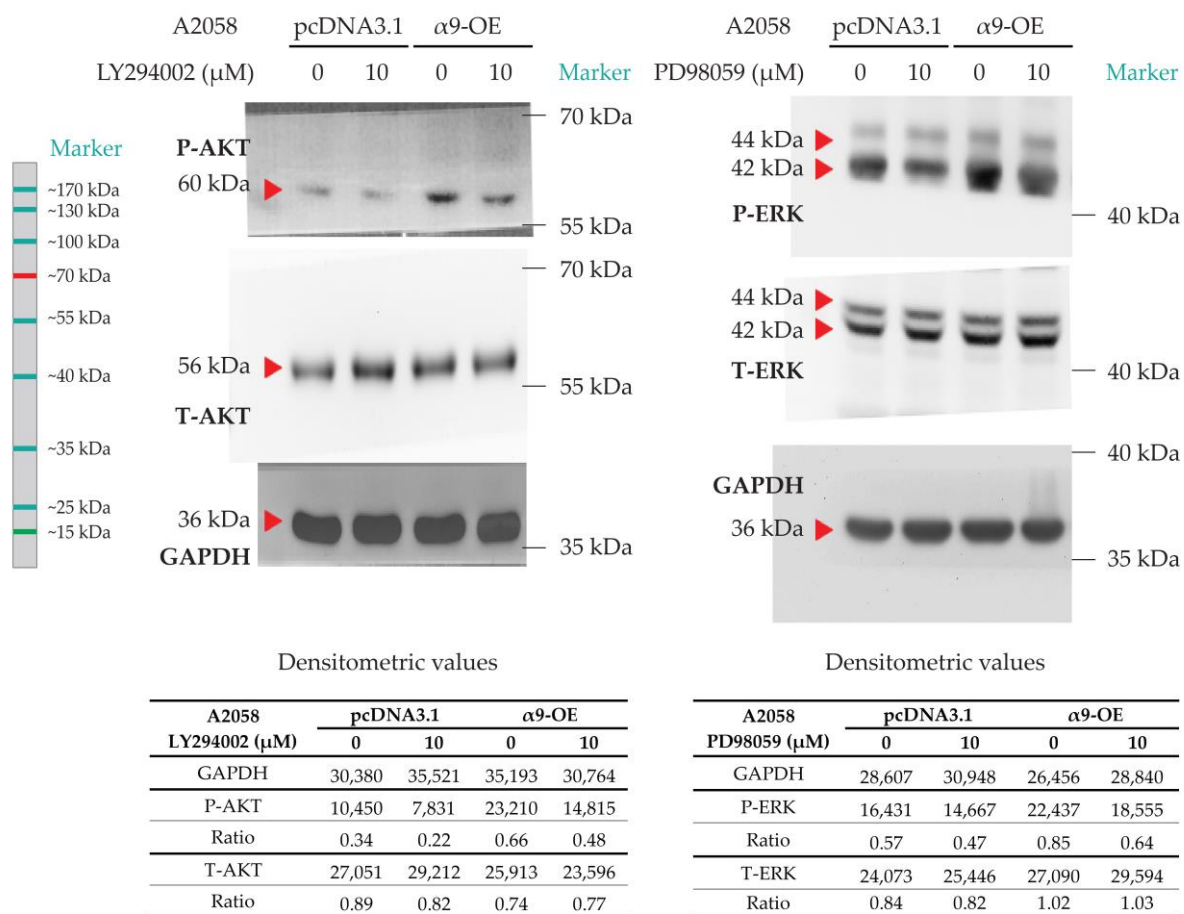


**Densitometric values**

| MDA-MB 435 | Nicotine (μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 10⁻³ | 10⁻² | 10⁻¹ | 1 |
| GAPDH | 9,994 | 10,703 | 10,116 | 10,166 | 11,973 |
| α9-nAChR | 4,914 | 7,085 | 8,626 | 8,234 | 9,768 |
| Ratio | 0.49 | 0.66 | 0.85 | 0.81 | 0.82 |
| PD-L1 | 2,678 | 7,816 | 7,686 | 10,045 | 7,567 |
| Ratio | 0.27 | 0.73 | 0.76 | 0.99 | 0.63 |

**Densitometric values**

| MDA-MB 435 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 12 | 24 | 48 | 72 |
| GAPDH | 22,850 | 23,585 | 23,510 | 26,694 | 23,304 |
| α9-nAChR | 7,173 | 18,787 | 21,600 | 22,683 | 24,169 |
| Ratio | 0.31 | 0.80 | 0.92 | 0.85 | 1.04 |
| PD-L1 | 4,652 | 8,912 | 9,691 | 9,814 | 8,383 |
| Ratio | 0.20 | 0.38 | 0.41 | 0.37 | 0.36 |

**Figure S12.** Uncropped scans with size marker indications of western blots shown in Figure 5B (continued), and densitometry readings/intensity ratios.
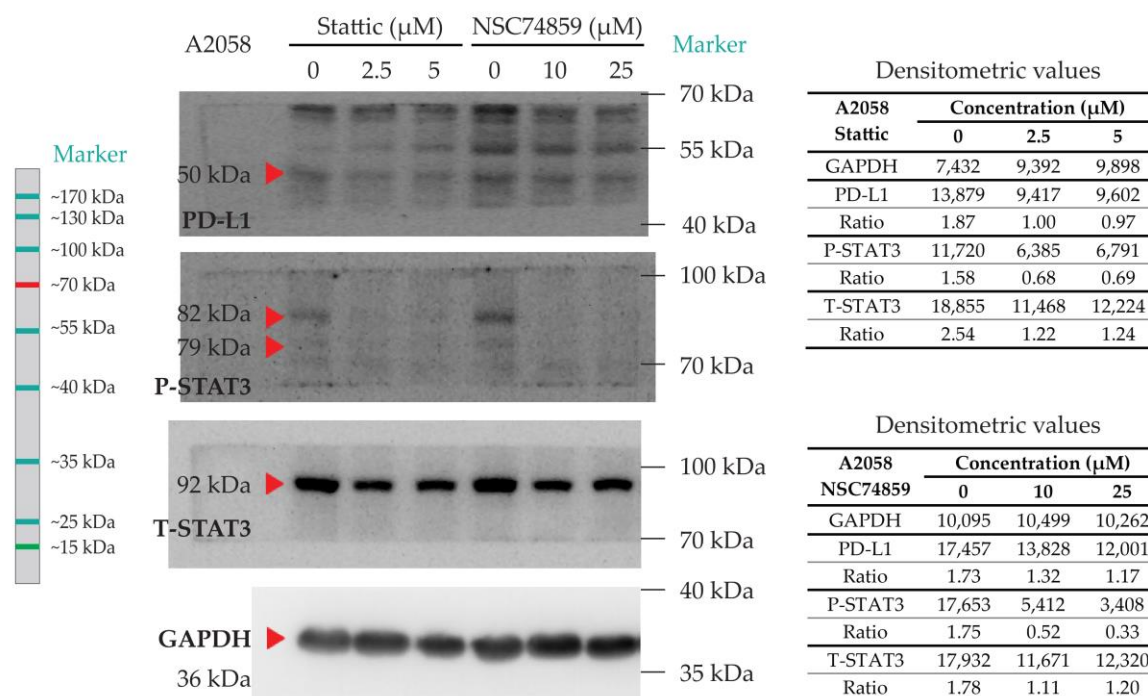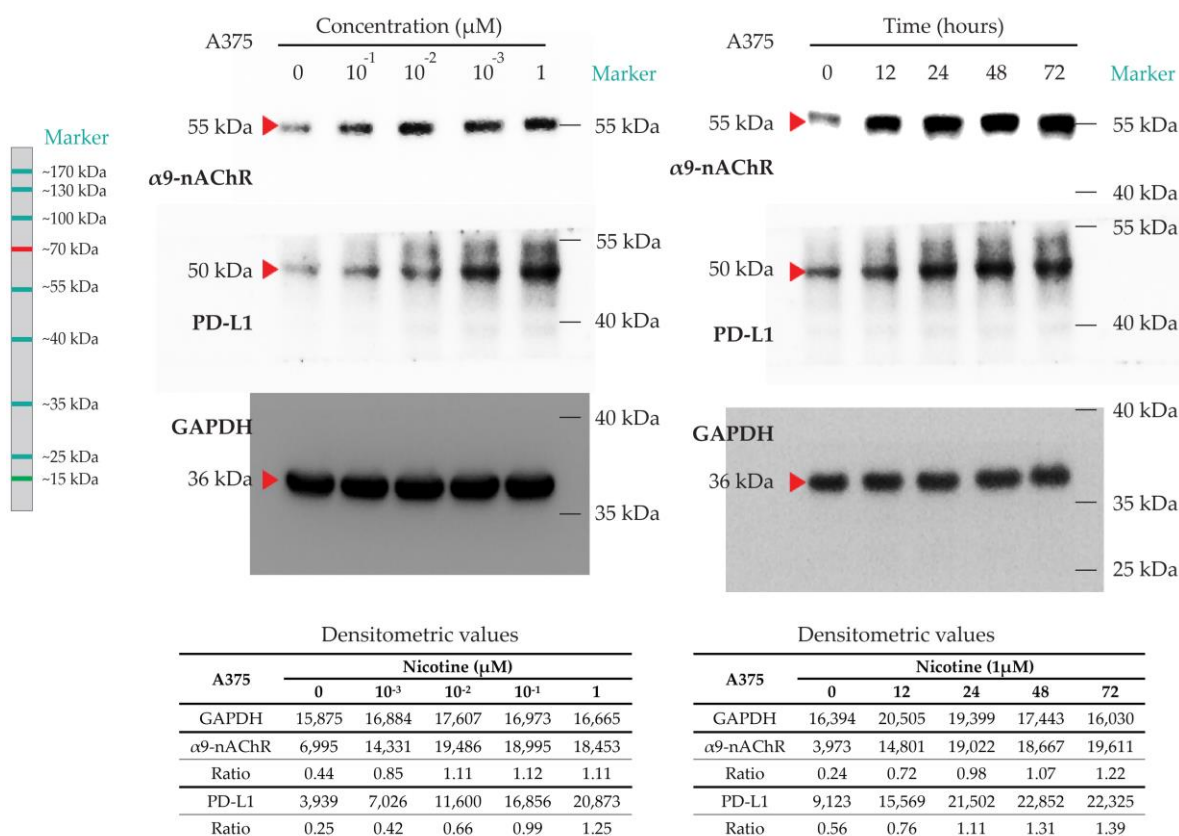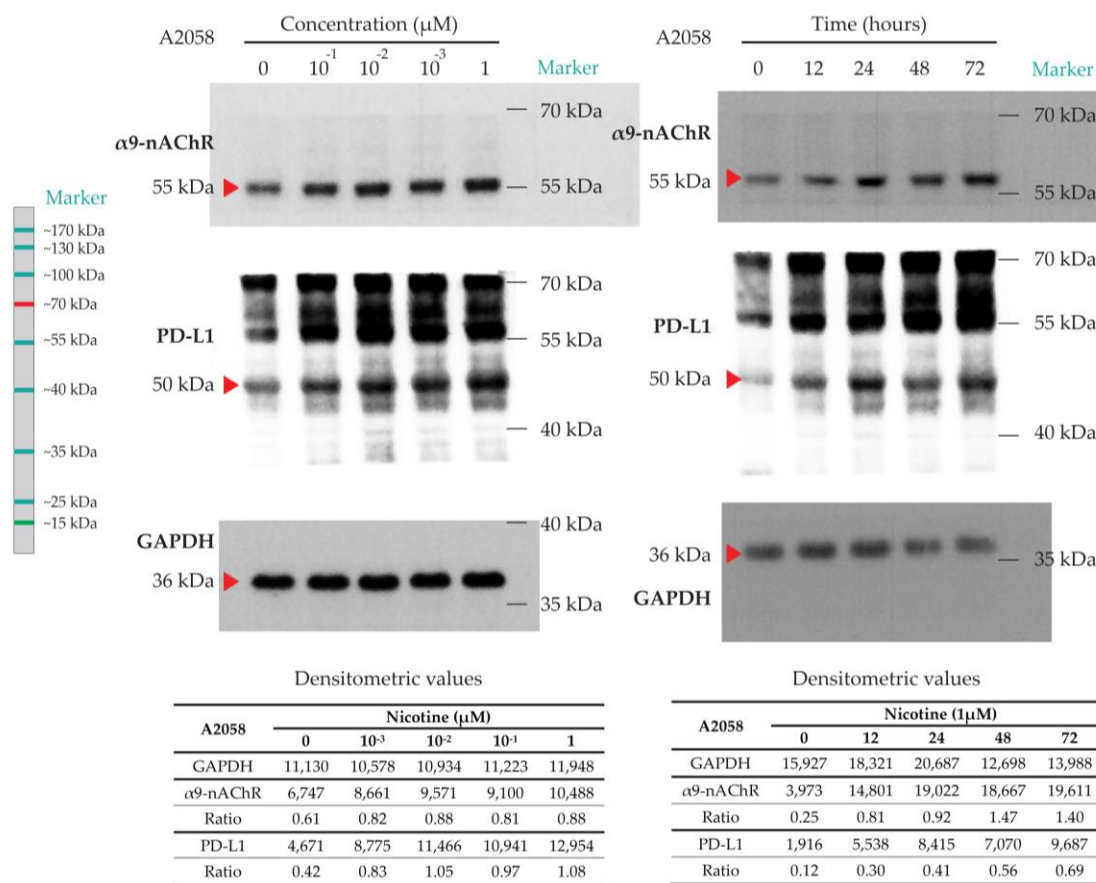
| A375 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | **0** | **15** | **30** | **60** | **120** |
| GAPDH | 15,661 | 16,713 | 17,636 | 17,020 | 16,855 |
| P-STAT3 | 6,594 | 16,114 | 15,919 | 17,345 | 17,675 |
| Ratio | 0.42 | 0.96 | 0.90 | 1.02 | 1.05 |
| T-STAT3 | 14,656 | 20,359 | 18,531 | 19,090 | 22,523 |
| Ratio | 0.94 | 1.22 | 1.05 | 1.12 | 1.34 |
| P-AKT | 8,997 | 19,663 | 19,727 | 18,920 | 23,851 |
| Ratio | 0.57 | 1.18 | 1.12 | 1.11 | 1.42 |
| T-AKT | 23,051 | 21,934 | 21,893 | 22,337 | 23,600 |
| Ratio | 1.47 | 1.31 | 1.24 | 1.31 | 1.40 |
| P-ERK | 11,856 | 11,903 | 18,440 | 16,041 | 9,835 |
| Ratio | 0.76 | 0.71 | 1.05 | 0.94 | 0.58 |
| T-ERK | 20,331 | 19,254 | 19,119 | 19,482 | 19,899 |
| Ratio | 1.30 | 1.15 | 1.08 | 1.14 | 1.18 |

**Figure S13.** Uncropped scans with size marker indications of western blots shown in Figure 6A, and densitometry readings/intensity ratios.



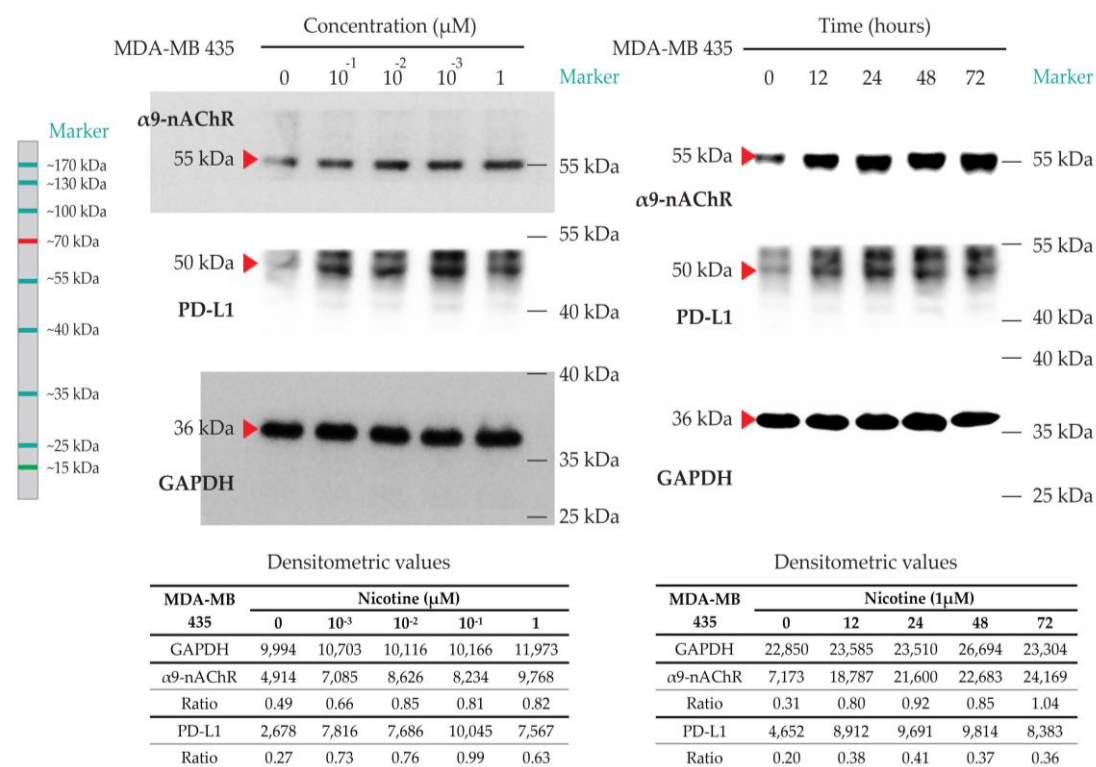| A2058 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | **0** | **15** | **30** | **60** | **120** |
| GAPDH | 17,253 | 12,051 | 14,628 | 15,011 | 16,812 |
| P-STAT3 | 3,046 | 16,671 | 14,700 | 14,669 | 10,815 |
| Ratio | 0.18 | 1.38 | 1.00 | 0.98 | 0.64 |
| T-STAT3 | 18,361 | 17,829 | 18,981 | 18,568 | 15,865 |
| Ratio | 1.06 | 1.48 | 1.30 | 1.24 | 0.94 |
| P-AKT | 5,960 | 15,417 | 15,226 | 22,106 | 17,588 |
| Ratio | 0.35 | 1.28 | 1.04 | 1.47 | 1.05 |
| T-AKT | 17,674 | 16,719 | 20,873 | 15,330 | 19,107 |
| Ratio | 1.02 | 1.39 | 1.43 | 1.02 | 1.14 |
| P-ERK | 6,024 | 17,568 | 16,665 | 7,635 | 14,644 |
| Ratio | 0.35 | 1.46 | 1.14 | 0.51 | 0.87 |
| T-ERK | 15,076 | 14,317 | 14,422 | 13,562 | 16,658 |
| Ratio | 0.87 | 1.19 | 0.99 | 0.90 | 0.99 |

**Figure S13.** Uncropped scans with size marker indications of western blots shown in Figure 6A (continued), and densitometry readings/intensity ratios.
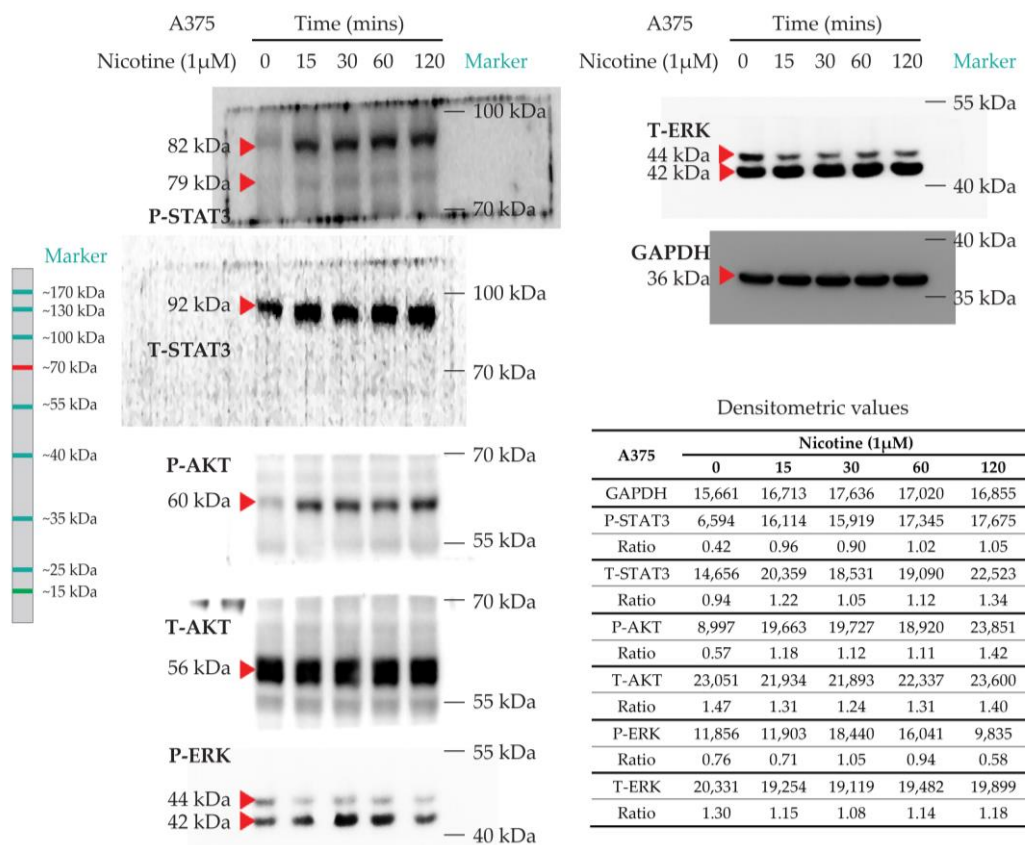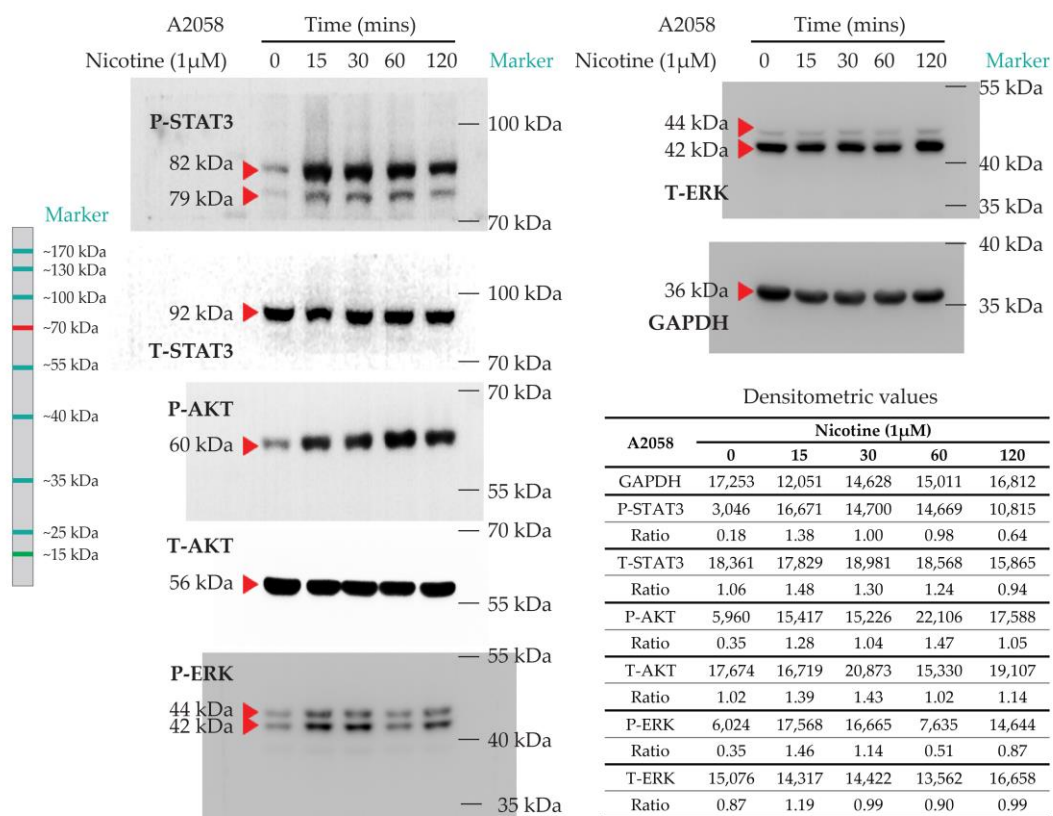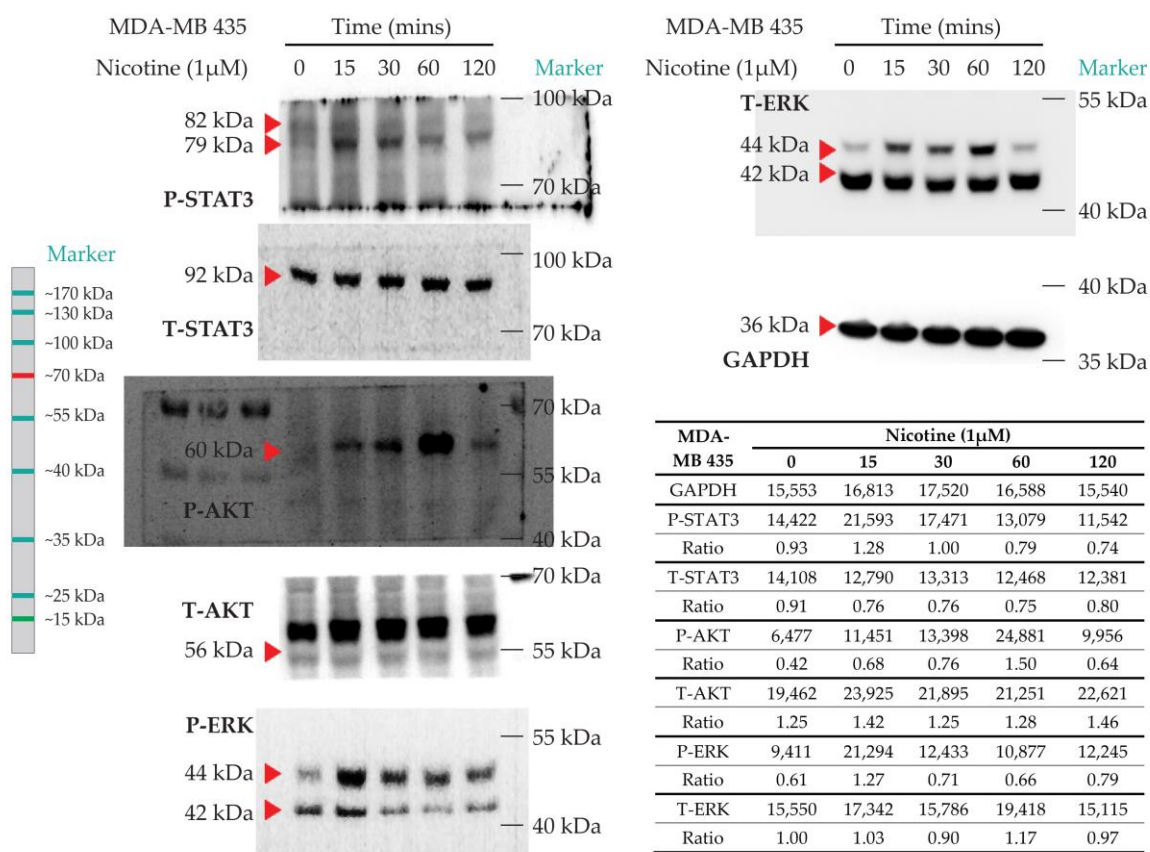
**Figure S13.** Uncropped scans with size marker indications of western blots shown in Figure 6A (continued), and densitometry readings/intensity ratios.

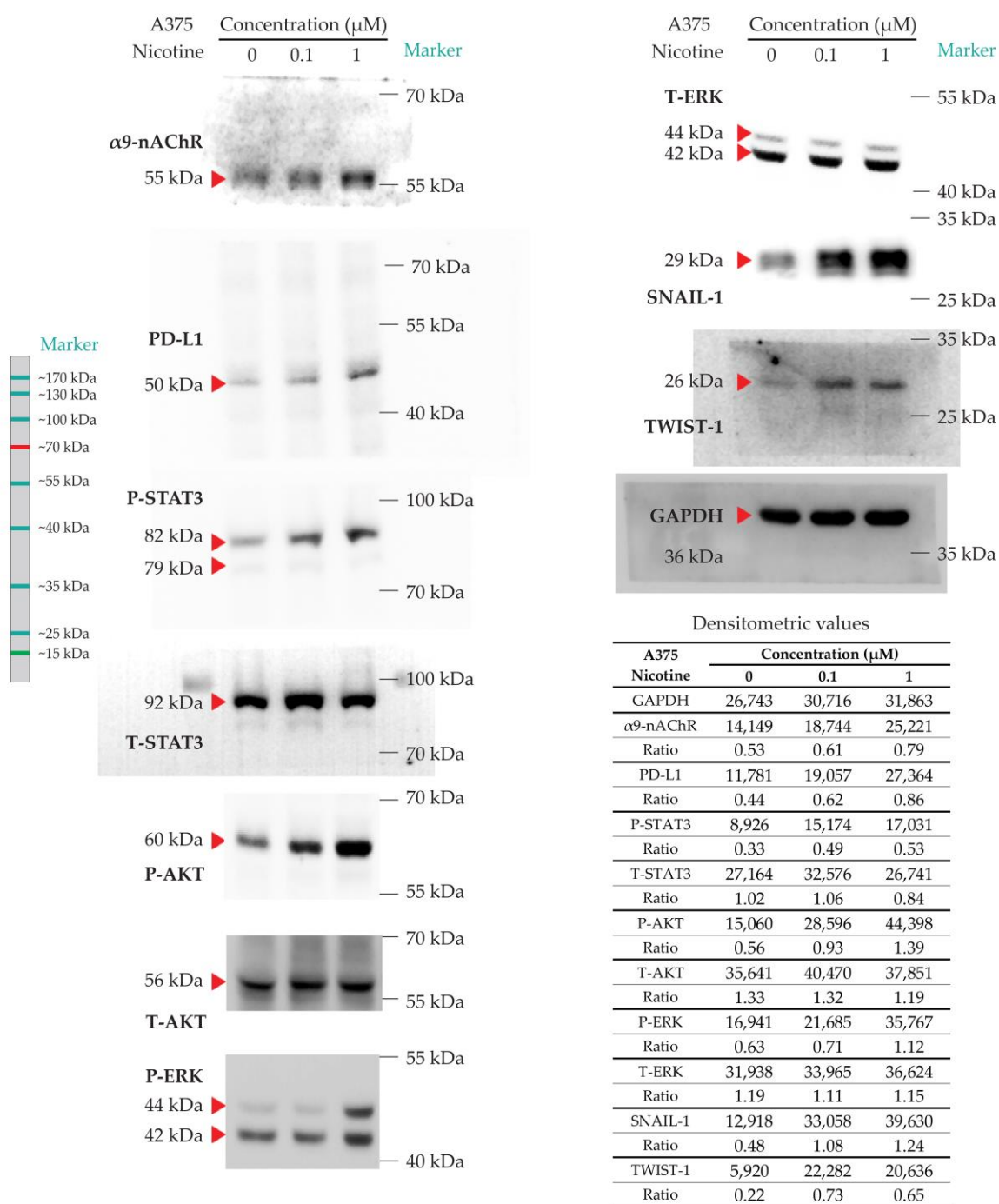| MDA-MB 435 | Nicotine (1μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 15 | 30 | 60 | 120 |
| GAPDH | 15,553 | 16,813 | 17,520 | 16,588 | 15,540 |
| P-STAT3 | 14,422 | 21,593 | 17,471 | 13,079 | 11,542 |
| Ratio | 0.93 | 1.28 | 1.00 | 0.79 | 0.74 |
| T-STAT3 | 14,108 | 12,790 | 13,313 | 12,468 | 12,381 |
| Ratio | 0.91 | 0.76 | 0.76 | 0.75 | 0.80 |
| P-AKT | 6,477 | 11,451 | 13,398 | 24,881 | 9,956 |
| Ratio | 0.42 | 0.68 | 0.76 | 1.50 | 0.64 |
| T-AKT | 19,462 | 23,925 | 21,895 | 21,251 | 22,621 |
| Ratio | 1.25 | 1.42 | 1.25 | 1.28 | 1.46 |
| P-ERK | 9,411 | 21,294 | 12,433 | 10,877 | 12,245 |
| Ratio | 0.61 | 1.27 | 0.71 | 0.66 | 0.79 |
| T-ERK | 15,550 | 17,342 | 15,786 | 19,418 | 15,115 |
| Ratio | 1.00 | 1.03 | 0.90 | 1.17 | 0.97 |

**Figure S14.** Uncropped scans with size marker indications of western blots shown in Figure 6B, and densitometry readings/intensity ratios.

Densitometric values

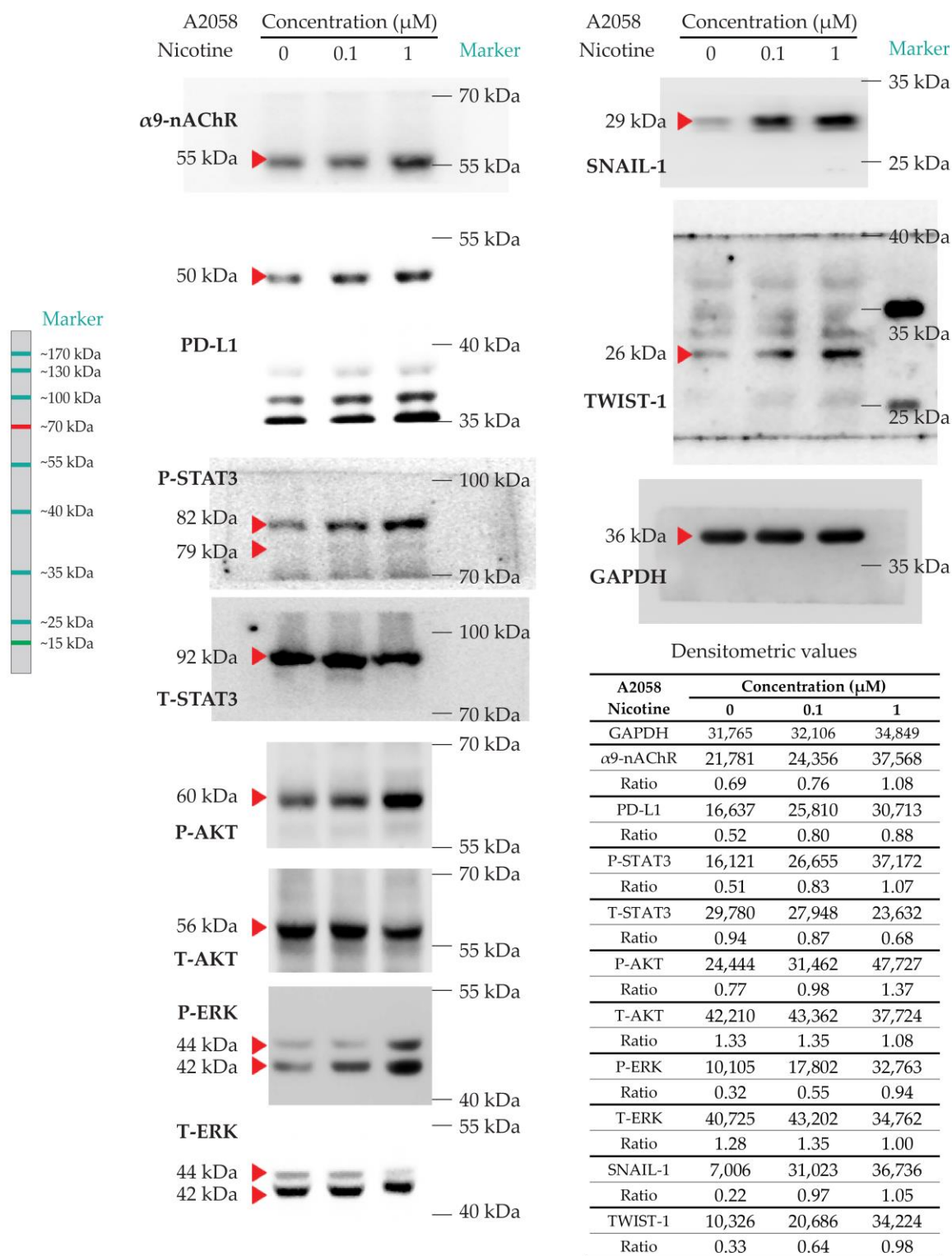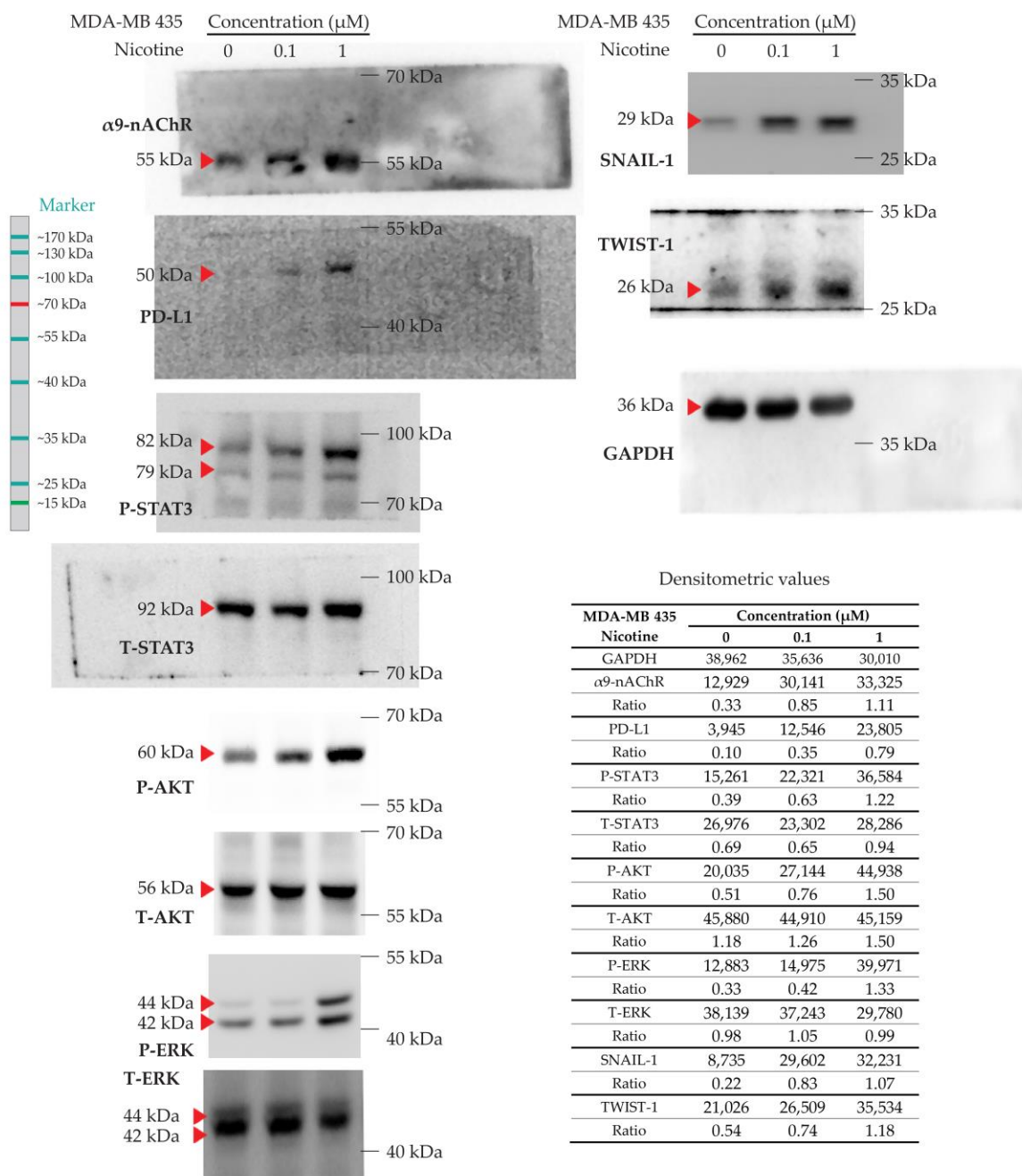| A375 Nicotine | Concentration (µM) | | |
|---|---|---|---|
| | 0 | 0.1 | 1 |
| GAPDH | 26,743 | 30,716 | 31,863 |
| α9-nAChR | 14,149 | 18,744 | 25,221 |
| Ratio | 0.53 | 0.61 | 0.79 |
| PD-L1 | 11,781 | 19,057 | 27,364 |
| Ratio | 0.44 | 0.62 | 0.86 |
| P-STAT3 | 8,926 | 15,174 | 17,031 |
| Ratio | 0.33 | 0.49 | 0.53 |
| T-STAT3 | 27,164 | 32,576 | 26,741 |
| Ratio | 1.02 | 1.06 | 0.84 |
| P-AKT | 15,060 | 28,596 | 44,398 |
| Ratio | 0.56 | 0.93 | 1.39 |
| T-AKT | 35,641 | 40,470 | 37,851 |
| Ratio | 1.33 | 1.32 | 1.19 |
| P-ERK | 16,941 | 21,685 | 35,767 |
| Ratio | 0.63 | 0.71 | 1.12 |
| T-ERK | 31,938 | 33,965 | 36,624 |
| Ratio | 1.19 | 1.11 | 1.15 |
| SNAIL-1 | 12,918 | 33,058 | 39,630 |
| Ratio | 0.48 | 1.08 | 1.24 |
| TWIST-1 | 5,920 | 22,282 | 20,636 |
| Ratio | 0.22 | 0.73 | 0.65 |

**Figure S14.** Uncropped scans with size marker indications of western blots shown in Figure 6B (continued), and densitometry readings/intensity ratios.

Densitometric values

| A2058 | Concentration (μM) | | |
|---|---|---|---|
| Nicotine | 0 | 0.1 | 1 |
| GAPDH | 31,765 | 32,106 | 34,849 |
| α9-nAChR | 21,781 | 24,356 | 37,568 |
| Ratio | 0.69 | 0.76 | 1.08 |
| PD-L1 | 16,637 | 25,810 | 30,713 |
| Ratio | 0.52 | 0.80 | 0.88 |
| P-STAT3 | 16,121 | 26,655 | 37,172 |
| Ratio | 0.51 | 0.83 | 1.07 |
| T-STAT3 | 29,780 | 27,948 | 23,632 |
| Ratio | 0.94 | 0.87 | 0.68 |
| P-AKT | 24,444 | 31,462 | 47,727 |
| Ratio | 0.77 | 0.98 | 1.37 |
| T-AKT | 42,210 | 43,362 | 37,724 |
| Ratio | 1.33 | 1.35 | 1.08 |
| P-ERK | 10,105 | 17,802 | 32,763 |
| Ratio | 0.32 | 0.55 | 0.94 |
| T-ERK | 40,725 | 43,202 | 34,762 |
| Ratio | 1.28 | 1.35 | 1.00 |
| SNAIL-1 | 7,006 | 31,023 | 36,736 |
| Ratio | 0.22 | 0.97 | 1.05 |
| TWIST-1 | 10,326 | 20,686 | 34,224 |
| Ratio | 0.33 | 0.64 | 0.98 |

**Figure S14.** Uncropped scans with size marker indications of western blots shown in Figure 6B (continued), and densitometry readings/intensity ratios.
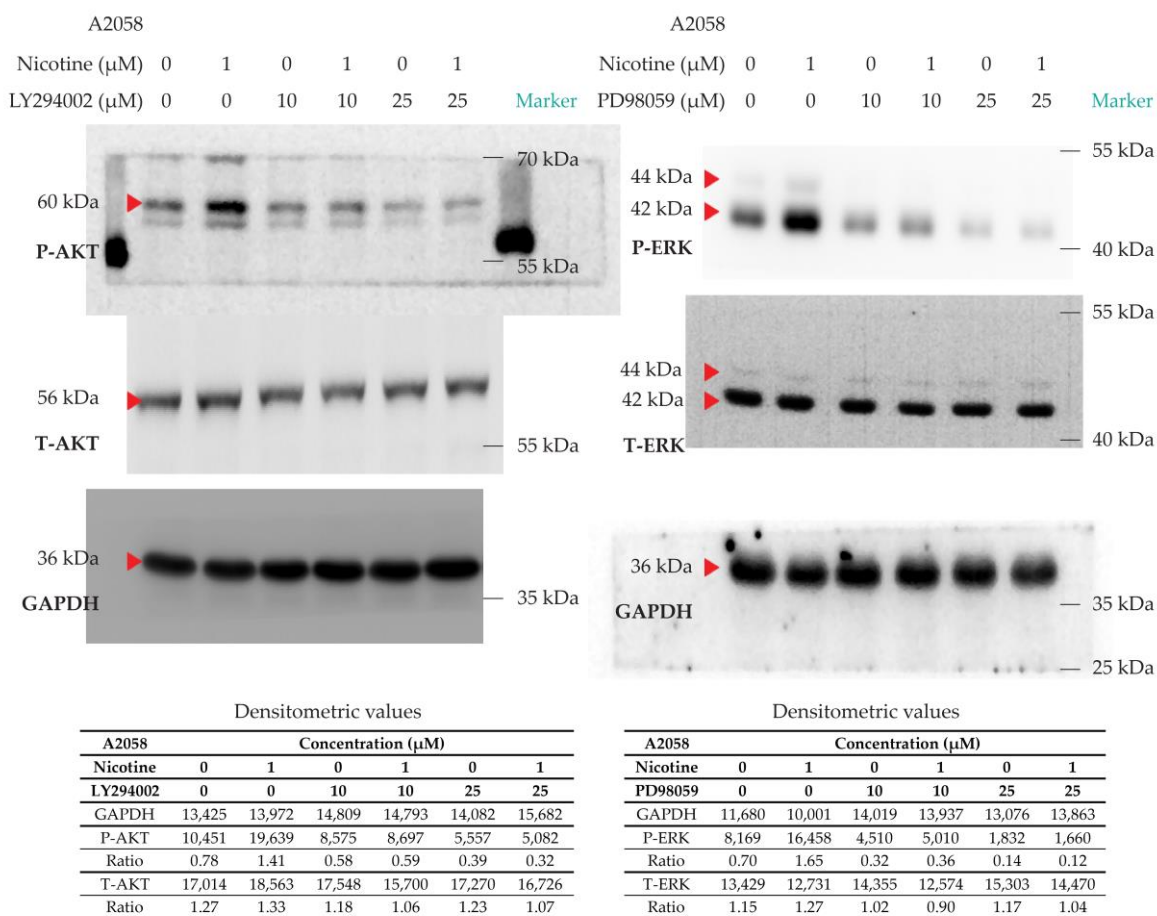
**Densitometric values**

| A2058 | Concentration (µM) | | | | | |
|---|---|---|---|---|---|---|
| Nicotine | 0 | 1 | 0 | 1 | 0 | 1 |
| LY294002 | 0 | 0 | 10 | 10 | 25 | 25 |
| GAPDH | 13,425 | 13,972 | 14,809 | 14,793 | 14,082 | 15,682 |
| P-AKT | 10,451 | 19,639 | 8,575 | 8,697 | 5,557 | 5,082 |
| Ratio | 0.78 | 1.41 | 0.58 | 0.59 | 0.39 | 0.32 |
| T-AKT | 17,014 | 18,563 | 17,548 | 15,700 | 17,270 | 16,726 |
| Ratio | 1.27 | 1.33 | 1.18 | 1.06 | 1.23 | 1.07 |

**Densitometric values**

| A2058 | Concentration (µM) | | | | | |
|---|---|---|---|---|---|---|
| Nicotine | 0 | 1 | 0 | 1 | 0 | 1 |
| PD98059 | 0 | 0 | 10 | 10 | 25 | 25 |
| GAPDH | 11,680 | 10,001 | 14,019 | 13,937 | 13,076 | 13,863 |
| P-ERK | 8,169 | 16,458 | 4,510 | 5,010 | 1,832 | 1,660 |
| Ratio | 0.70 | 1.65 | 0.32 | 0.36 | 0.14 | 0.12 |
| T-ERK | 13,429 | 12,731 | 14,355 | 12,574 | 15,303 | 14,470 |
| Ratio | 1.15 | 1.27 | 1.02 | 0.90 | 1.17 | 1.04 |

**Figure S15.** Uncropped scans with size marker indications of western blots shown in Figure 6D and Figure 6F, and densitometry readings/intensity ratios.
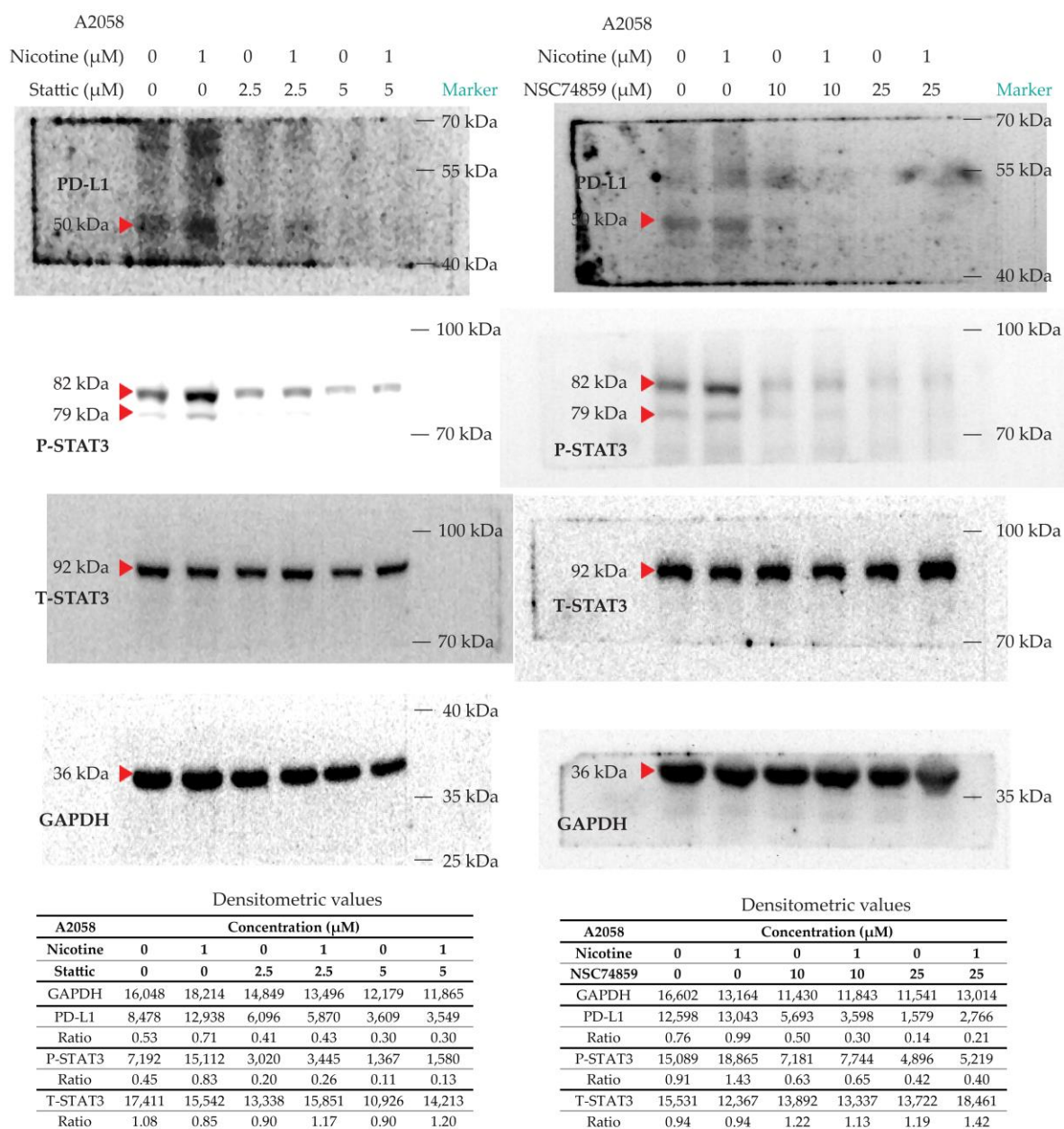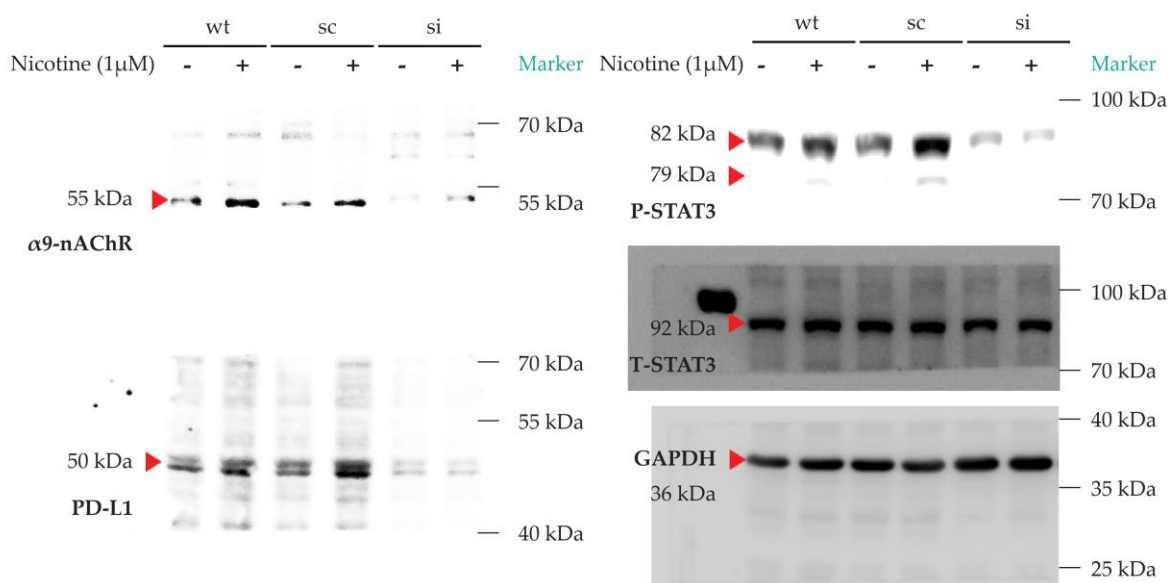
**Figure S16.** Uncropped scans with size marker indications of western blots shown in Figure 6H and Figure 6J, and densitometry readings/intensity ratios.

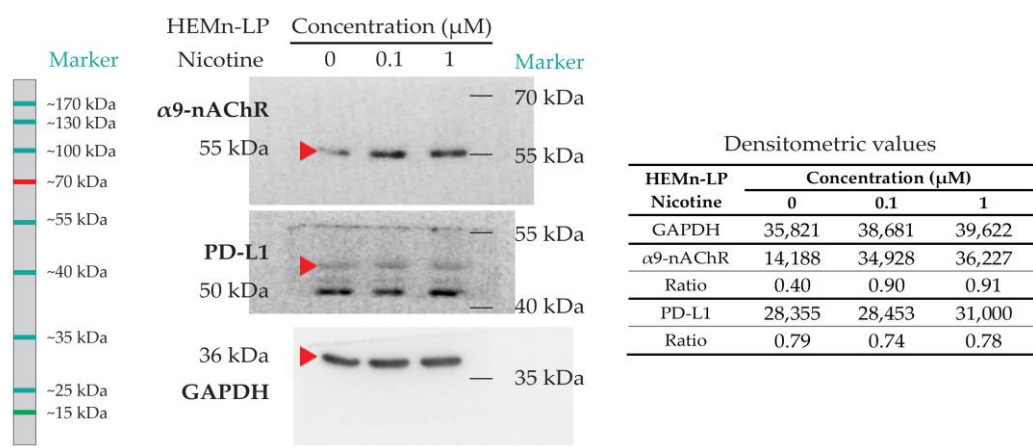| A2058 | wt | | sc | | si | |
|---|---|---|---|---|---|---|
| Nicotine (μM) | 0 | 1 | 0 | 1 | 0 | 1 |
| GAPDH | 13,880 | 15,871 | 14,151 | 13,371 | 17,932 | 18,247 |
| α9-nAChR | 7,146 | 16,009 | 4,481 | 10,083 | 2,584 | 3,254 |
| Ratio | 0.51 | 1.01 | 0.32 | 0.75 | 0.14 | 0.18 |
| PD-L1 | 8,887 | 16,402 | 10,992 | 19,563 | 2,217 | 1,533 |
| Ratio | 0.64 | 1.03 | 0.78 | 1.46 | 0.12 | 0.08 |
| P-STAT3 | 8,893 | 14,436 | 9,795 | 19,517 | 2,399 | 1,162 |
| Ratio | 0.64 | 0.91 | 0.69 | 1.46 | 0.13 | 0.06 |
| T-STAT3 | 17,780 | 21,730 | 18,772 | 19,406 | 16,168 | 15,797 |
| Ratio | 1.28 | 1.37 | 1.33 | 1.45 | 0.90 | 0.87 |

**Figure S17.** Uncropped scans with size marker indications of western blots shown in Figure 6K, and densitometry readings/intensity ratios.



| HEMn-LP | Concentration (μM) | | |
|---|---|---|---|
| Nicotine | 0 | 0.1 | 1 |
| GAPDH | 35,821 | 38,681 | 39,622 |
| α9-nAChR | 14,188 | 34,928 | 36,227 |
| Ratio | 0.40 | 0.90 | 0.91 |
| PD-L1 | 28,355 | 28,453 | 31,000 |
| Ratio | 0.79 | 0.74 | 0.78 |

**Figure S18.** Uncropped scans with size marker indications of western blots shown in Figure S5, and densitometry readings/intensity ratios.