

| Sample | CTLA4 | Pdcd-1 | Caspase 3 | CD4 band 1 | CD4 Band 2 | CD8   | $\beta$ actin | Ki-67 |
|--------|-------|--------|-----------|------------|------------|-------|---------------|-------|
| Nat-1  | 1.15  | 0.52   | 0.60      | 7.03       | 1.38       | 0.003 | 434           | 0.53  |
| Nat-2  | 1.65  | 0.29   | 1.42      | 8.44       | 0.86       | 0.025 | 734           | 0.68  |
| Nat-3  | 0.46  | 0.23   | 0.93      | 5.67       | 1.55       | 0.016 | 519           | 0.96  |
| Nat-4  | 1.24  | 0.14   | 1.81      | 3.52       | 1.54       | 0.042 | 578           | 1.06  |
| Nat-5  | 1.11  | 0.75   | 1.79      | 5.87       | 1.28       | 0.042 | 618           | 1.26  |
| Nat-6  | 0.16  | 5.32   | 0.77      | 6.84       | 1.05       | 0.021 | 739           | 1.03  |
| Nat-7  | 0.53  | 3.17   | 0.46      | 5.06       | 1.42       | 0.013 | 437           | 0.15  |
| Nat-8  | 1.82  | 3.03   | 0.75      | 3.59       | 1.72       | 0.011 | 613           | 1.32  |
| Nat-9  | 0.47  | 3.17   | 0.49      | 0.05       | 0.75       | 0.009 | 741           | 0.33  |
| Nat-10 | 0.55  | 2.19   | 0.74      | 1.71       | 0.65       | 0.011 | 554           | 2.09  |
| Nat-11 | 1.96  | 1.18   | 2.43      | 7.94       | 1.61       | 0.046 | 725           | 2.18  |
| Nat-12 | 0.29  | 0.44   | 1.04      | 4.97       | 0.93       | 0.013 | 756           | 0.25  |
| Nat-13 | 2.61  | 0.74   | 2.19      | 5.39       | 1.34       | 0.069 | 703           | 2.36  |
| Nat-14 | 0.89  | 1.21   | 1.38      | 7.99       | 1.21       | 0.020 | 958           | 0.35  |
| Nat-15 | 1.64  | 1.03   | 1.92      | 4.85       | 1.63       | 0.051 | 650           | 1.53  |
| Nat-16 | 1.52  | 1.90   | 0.98      | 0.75       | 1.26       | 0.014 | 631           | 0.59  |
| Nat-17 | 1.26  | 2.13   | 0.75      | 2.22       | 1.49       | 0.017 | 1092          | 0.41  |
| Nat-18 | 2.61  | 1.50   | 0.88      | 0.89       | 1.47       | 0.014 | 633           | 0.12  |
| Nat-19 | 2.76  | 2.00   | 0.91      | 3.13       | 1.61       | 0.024 | 690           | 2.07  |
| Nat-20 | 3.91  | 2.17   | 1.03      | 0.19       | 1.48       | 0.027 | 756           | 2.13  |

Table 1. Summary table showing the ratio of the indicated band identified in each sample to the volume of that band in the control sample for each western blot. Note, since  $\beta$ -actin was used as a loading control, a standard was not loaded for these western blots. Values for  $\beta$ -actin are given as band volume rather than as a ratio to the control.

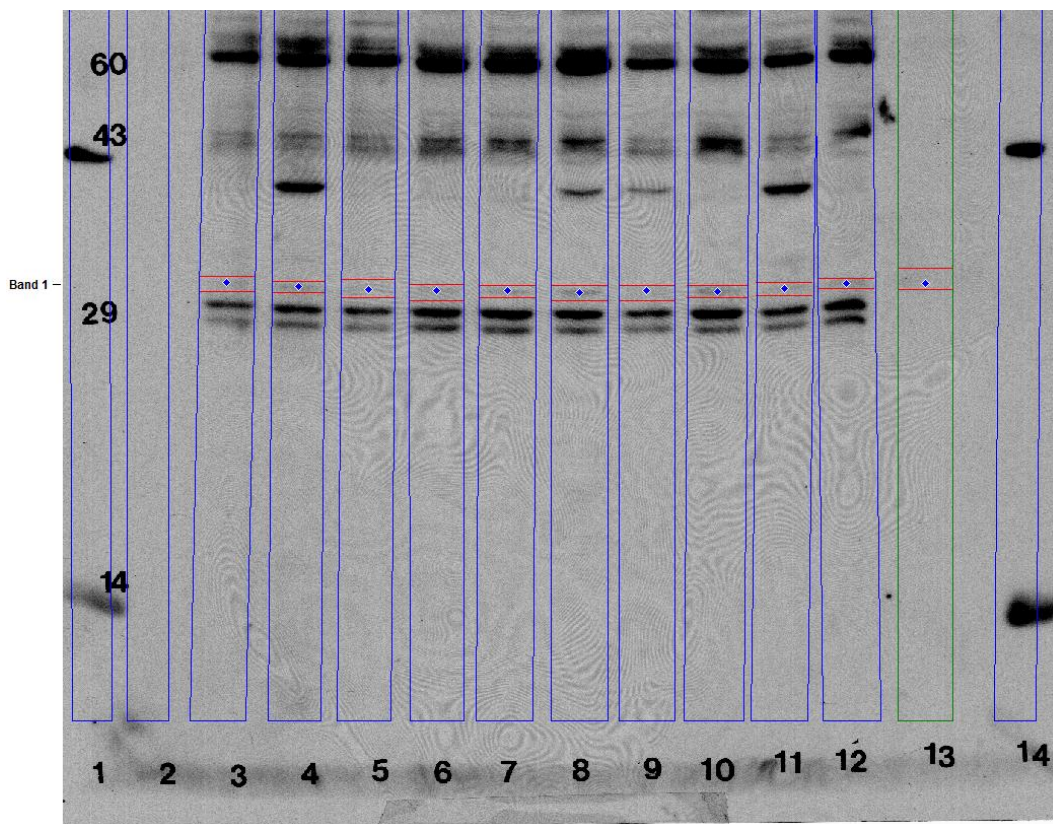


Figure 1. Image of lanes and bands quantified in film,  $\alpha$ -CTLA4 (H-126) gel KO p.4201 #1.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-1                    | 10             | 40             |
| 4      | NAT-2                    | 10             | 40             |
| 5      | NAT-3                    | 10             | 40             |
| 6      | NAT-4                    | 10             | 40             |
| 7      | NAT-5                    | 10             | 40             |
| 8      | NAT-11                   | 10             | 40             |
| 9      | NAT-12                   | 10             | 40             |
| 10     | NAT-13                   | 10             | 40             |
| 11     | NAT-14                   | 10             | 40             |
| 12     | NAT-15                   | 10             | 40             |
| 13     | SC-119504 CTLA 4(m)      | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 2. Key to 1D Slab Gel Loading KO 4201 #1, 10% Acrylamide, PVDF.

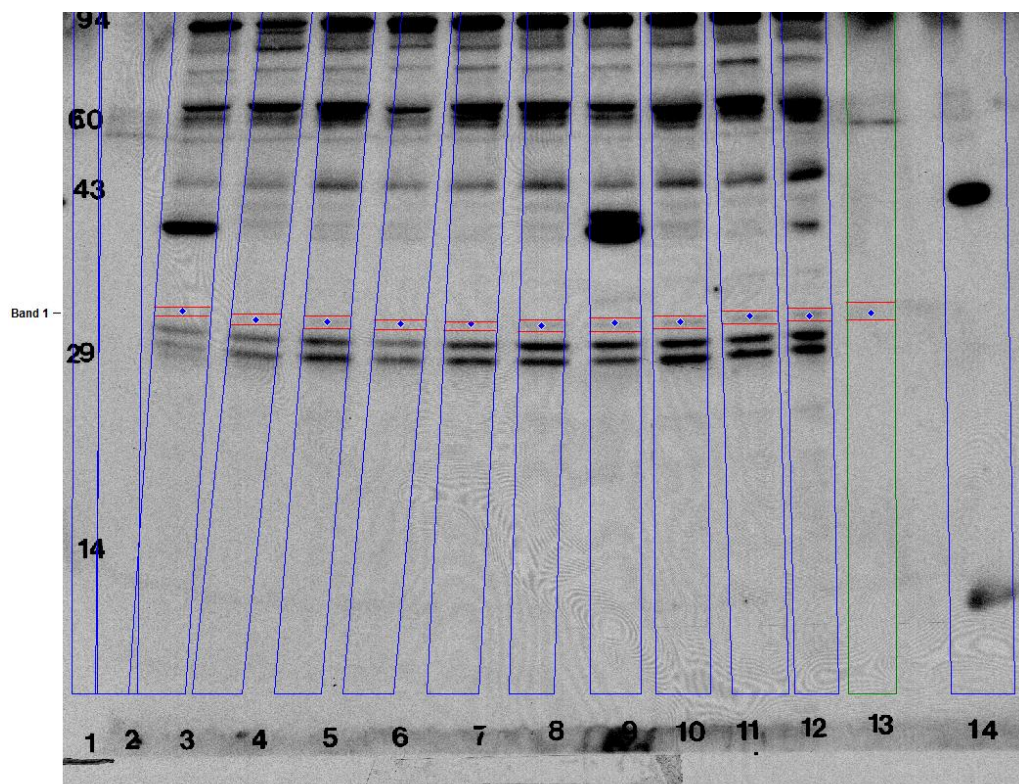


Figure 2. Image of lanes and bands quantified in film,  $\alpha$ -CTLA 4 (H-126) gel KO p.4201 #7.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-6                    | 10             | 40             |
| 4      | NAT-7                    | 10             | 40             |
| 5      | NAT-8                    | 10             | 40             |
| 6      | NAT-9                    | 10             | 40             |
| 7      | NAT-10                   | 10             | 40             |
| 8      | NAT-16                   | 10             | 40             |
| 9      | NAT-17                   | 10             | 40             |
| 10     | NAT-18                   | 10             | 40             |
| 11     | NAT-19                   | 10             | 40             |
| 12     | NAT-20                   | 10             | 40             |
| 13     | SC-119504 CTLA4 (M)      | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 3. Key to 1D Slab Gel Loading KO 4201 #7, 10% Acrylamide, PVDF.



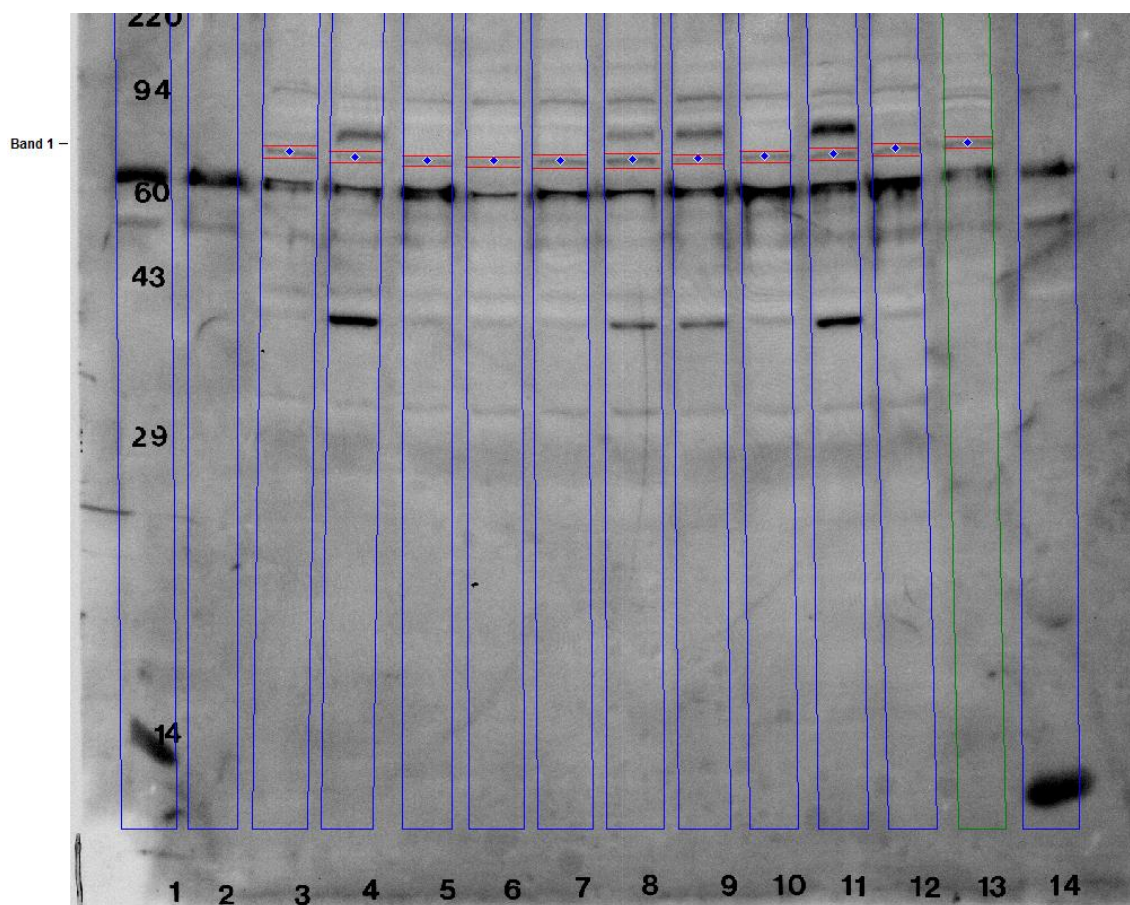


Figure 3. Image of lanes and bands quantified in film,  $\alpha$ -Pdcd-1 (M-288) gel KO p.4201 #2.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-1                    | 10             | 40             |
| 4      | NAT-2                    | 10             | 40             |
| 5      | NAT-3                    | 10             | 40             |
| 6      | NAT-4                    | 10             | 40             |
| 7      | NAT-5                    | 10             | 40             |
| 8      | NAT-11                   | 10             | 40             |
| 9      | NAT-12                   | 10             | 40             |
| 10     | NAT-13                   | 10             | 40             |
| 11     | NAT-14                   | 10             | 40             |
| 12     | NAT-15                   | 10             | 40             |
| 13     | SC-2211 RAW 264.7        | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 4. Key to 1D Slab Gel Loading KO 4201 #2, 10% Acrylamide, PVDF.

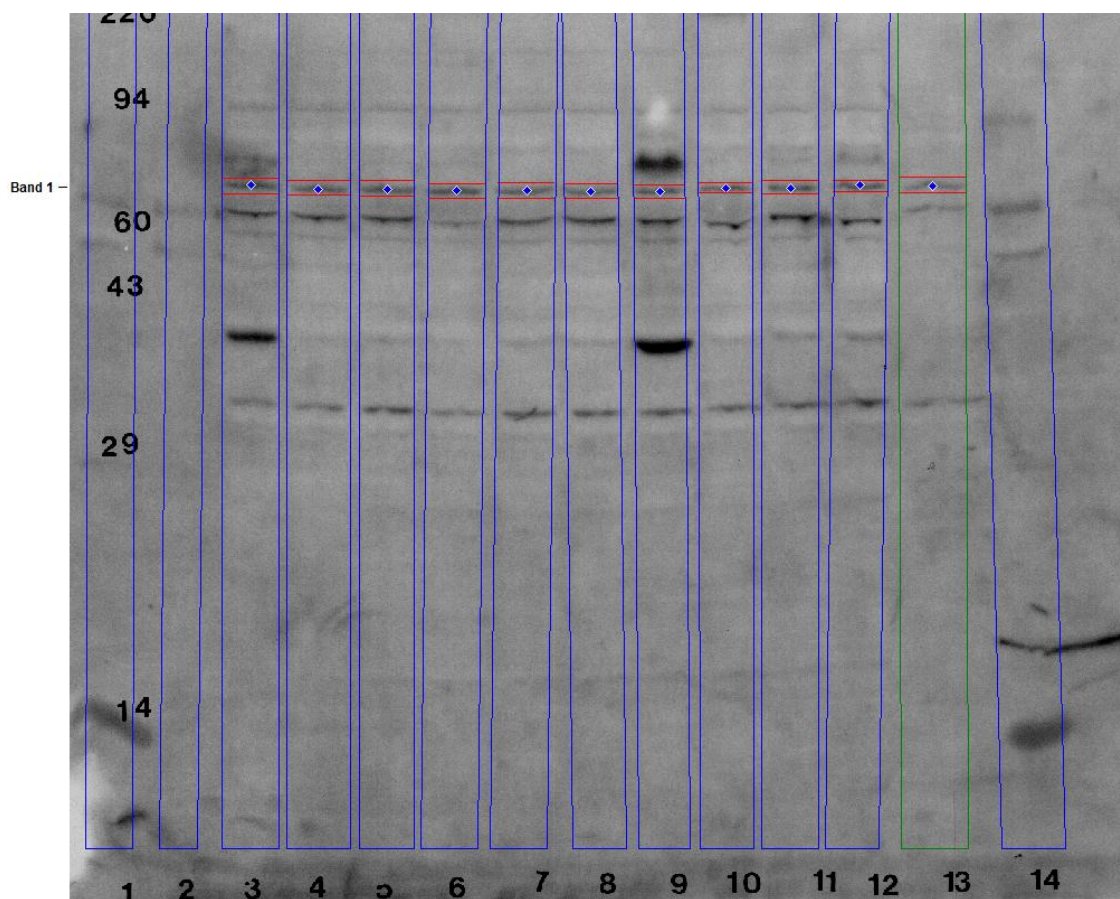


Figure 4. Image of lanes and bands quantified in film,  $\alpha$ -Pdcd-1 (M-288) gel KO p.4201 #8.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-6                    | 10             | 40             |
| 4      | NAT-7                    | 10             | 40             |
| 5      | NAT-8                    | 10             | 40             |
| 6      | NAT-9                    | 10             | 40             |
| 7      | NAT-10                   | 10             | 40             |
| 8      | NAT-16                   | 10             | 40             |
| 9      | NAT-17                   | 10             | 40             |
| 10     | NAT-18                   | 10             | 40             |
| 11     | NAT-19                   | 10             | 40             |
| 12     | NAT-20                   | 10             | 40             |
| 13     | SC-2211 RAW 264.7        | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 5. Key to 1D Slab Gel Loading KO 4201 #8, 10% Acrylamide, PVDF.

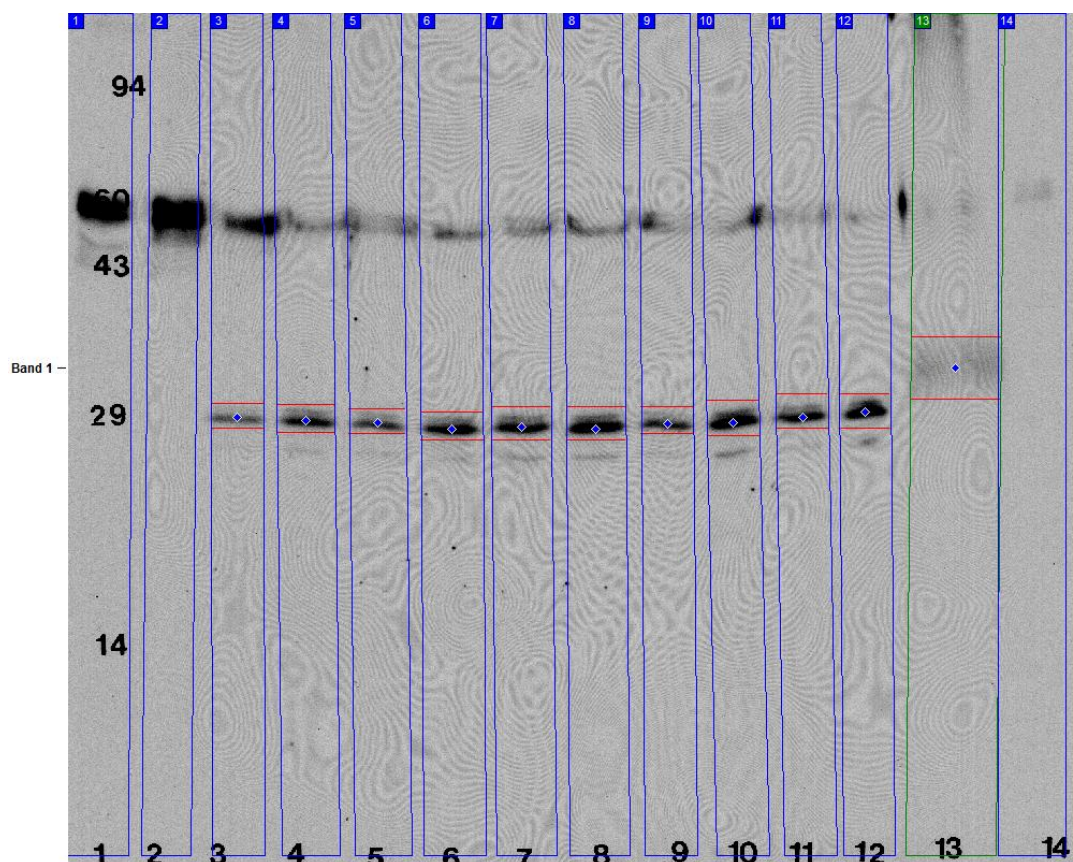


Figure 5. Image of lanes and bands quantified in film,  $\alpha$ -Caspase 3 (H-272) gel KO p.4201 #3.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-1                    | 10             | 40             |
| 4      | NAT-2                    | 10             | 40             |
| 5      | NAT-3                    | 10             | 40             |
| 6      | NAT-4                    | 10             | 40             |
| 7      | NAT-5                    | 10             | 40             |
| 8      | NAT-11                   | 10             | 40             |
| 9      | NAT-12                   | 10             | 40             |
| 10     | NAT-13                   | 10             | 40             |
| 11     | NAT-14                   | 10             | 40             |
| 12     | NAT-15                   | 10             | 40             |
| 13     | SC-176260 Caspase 3 (h2) | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 6. Key to 1D Slab Gel Loading KO 4201 #3, 10% Acrylamide, PVDF.



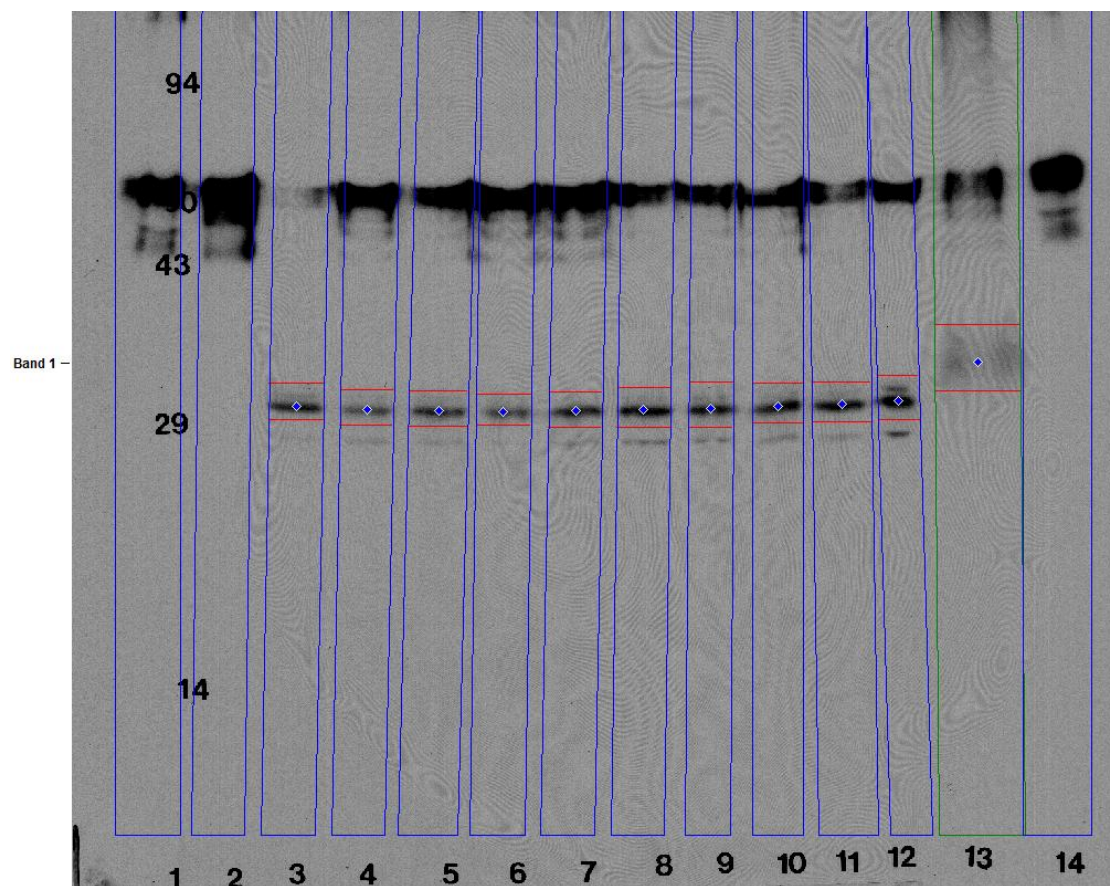


Figure 6. Image of lanes and bands quantified in film,  $\alpha$ -Caspase 3 (H-272) gel KO p.4201 #9.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-6                    | 10             | 40             |
| 4      | NAT-7                    | 10             | 40             |
| 5      | NAT-8                    | 10             | 40             |
| 6      | NAT-9                    | 10             | 40             |
| 7      | NAT-10                   | 10             | 40             |
| 8      | NAT-16                   | 10             | 40             |
| 9      | NAT-17                   | 10             | 40             |
| 10     | NAT-18                   | 10             | 40             |
| 11     | NAT-19                   | 10             | 40             |
| 12     | NAT-20                   | 10             | 40             |
| 13     | SC-176260 Caspase 3(h2)  | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 7. Key to 1D Slab Gel Loading KO 4201 #9, 10% Acrylamide, PVDF.

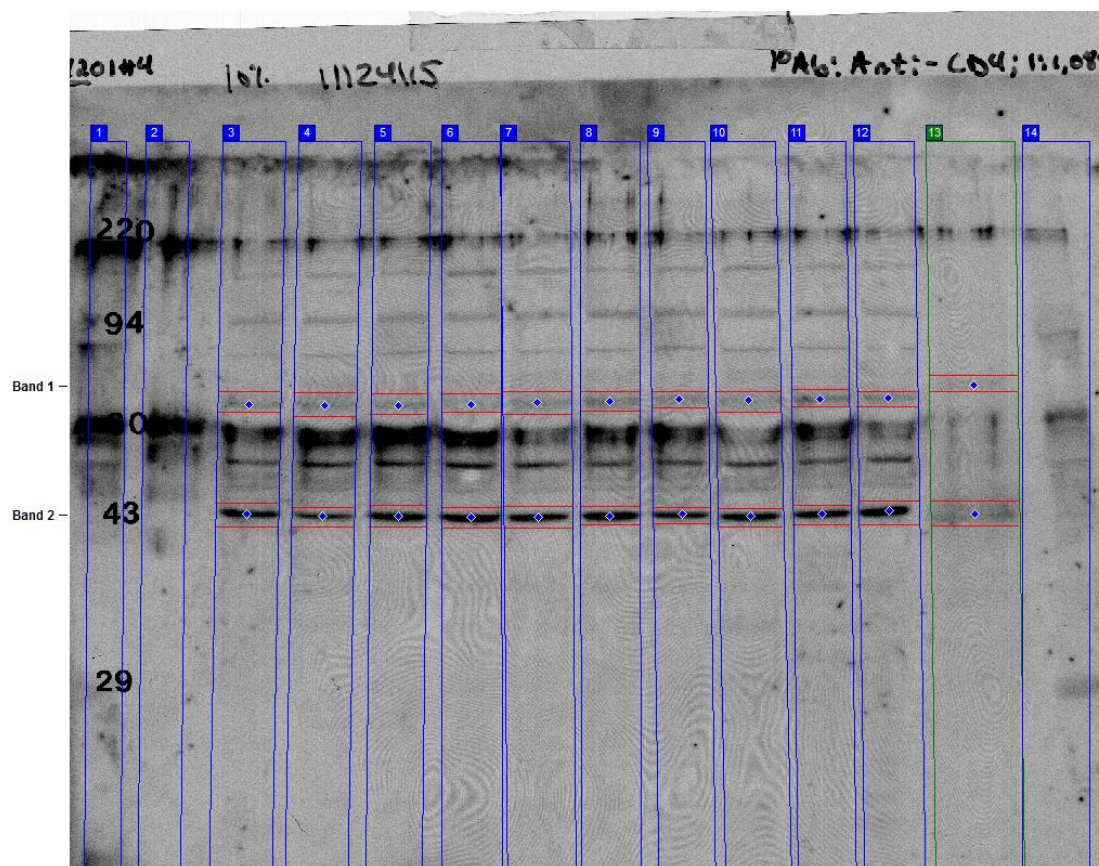


Figure 7. Image of lanes and bands quantified in film,  $\alpha$ -CD4 gel KO p.4201 #4.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-1                    | 10             | 40             |
| 4      | NAT-2                    | 10             | 40             |
| 5      | NAT-3                    | 10             | 40             |
| 6      | NAT-4                    | 10             | 40             |
| 7      | NAT-5                    | 10             | 40             |
| 8      | NAT-11                   | 10             | 40             |
| 9      | NAT-12                   | 10             | 40             |
| 10     | NAT-13                   | 10             | 40             |
| 11     | NAT-14                   | 10             | 40             |
| 12     | NAT-15                   | 10             | 40             |
| 13     | SC-119107 CD4 (m)        | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 8. Key to 1D Slab Gel Loading KO 4201 #4, 10% Acrylamide, PVDF.



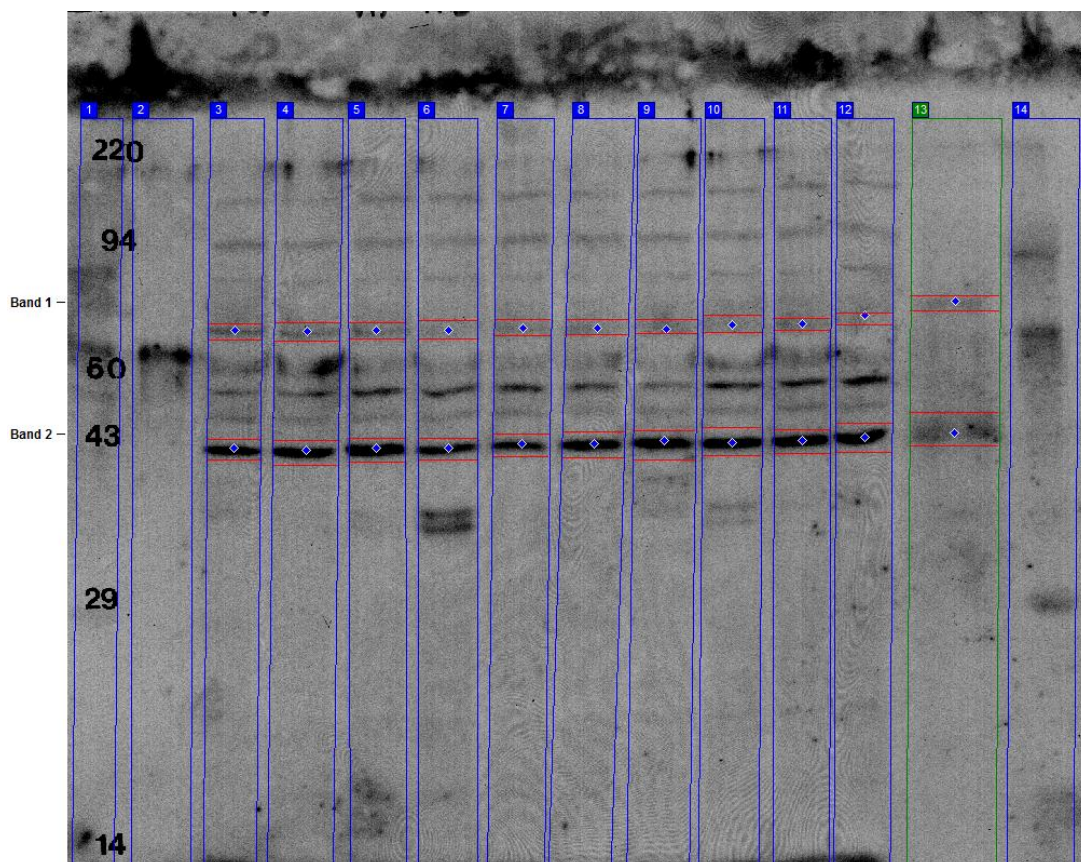


Figure 8. Image of lanes and bands quantified in film,  $\alpha$ -CD4 gel KO p.4201 #10.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-6                    | 10             | 40             |
| 4      | NAT-7                    | 10             | 40             |
| 5      | NAT-8                    | 10             | 40             |
| 6      | NAT-9                    | 10             | 40             |
| 7      | NAT-10                   | 10             | 40             |
| 8      | NAT-16                   | 10             | 40             |
| 9      | NAT-17                   | 10             | 40             |
| 10     | NAT-18                   | 10             | 40             |
| 11     | NAT-19                   | 10             | 40             |
| 12     | NAT-20                   | 10             | 40             |
| 13     | SC-119107 CD 4 (M)       | 10             | 40             |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 9. Key to 1D Slab Gel Loading KO 4201 #10, 10% Acrylamide, PVDF.

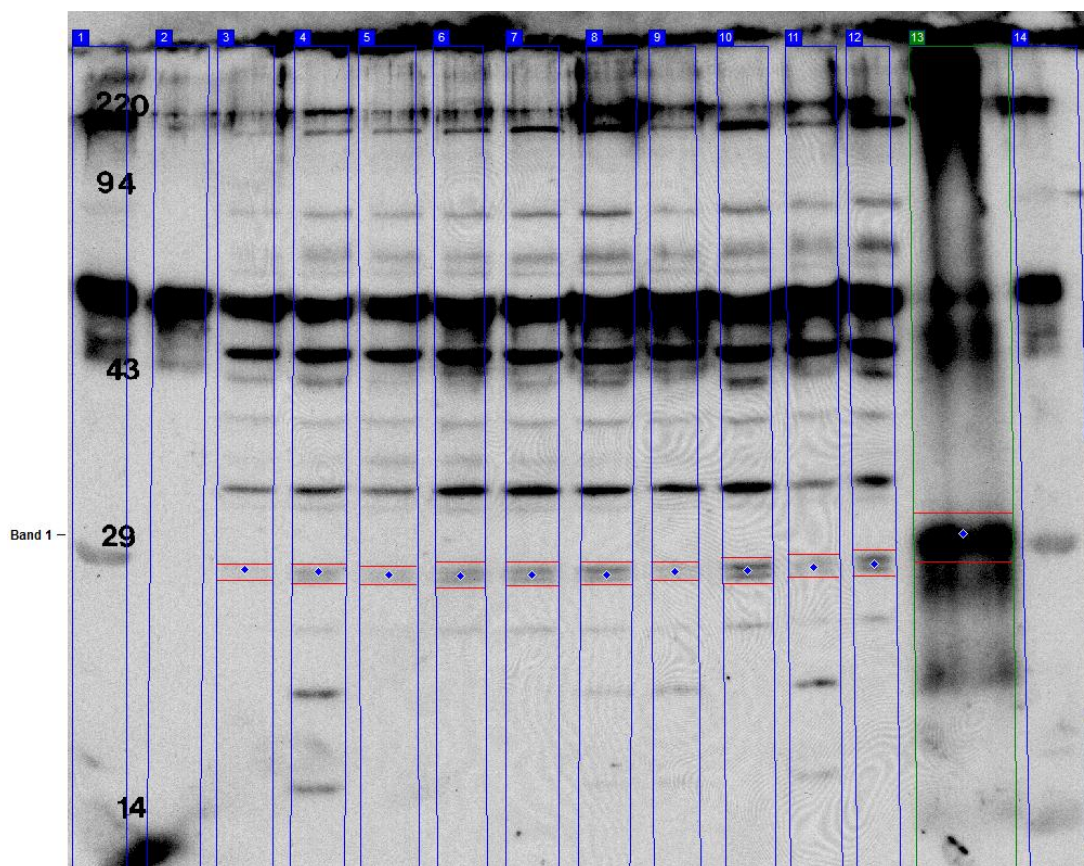


Figure 9. Image of lanes and bands quantified in film,  $\alpha$ -CD8- $\alpha$  (H-160) gel KO p.4201 #5.

| Lane # | Sample                      | $\mu$ l loaded | $\mu$ g loaded |
|--------|-----------------------------|----------------|----------------|
| 1      | Molecular Weight Markers    | 5              | -              |
| 2      | Buffer Blank                | 10             | -              |
| 3      | NAT-1                       | 10             | 40             |
| 4      | NAT-2                       | 10             | 40             |
| 5      | NAT-3                       | 10             | 40             |
| 6      | NAT-4                       | 10             | 40             |
| 7      | NAT-5                       | 10             | 40             |
| 8      | NAT-11                      | 10             | 40             |
| 9      | NAT-12                      | 10             | 40             |
| 10     | NAT-13                      | 10             | 40             |
| 11     | NAT-14                      | 10             | 40             |
| 12     | NAT-15                      | 10             | 40             |
| 13     | SC-174035 CD8 $\alpha$ (h2) | 10             | 40             |
| 14     | Molecular Weight Markers    | 10             | 40             |
| 15     | -                           | -              | -              |

Table 10. Key to 1D Slab Gel Loading KO 4201 #5, 10% Acrylamide, PVDF.



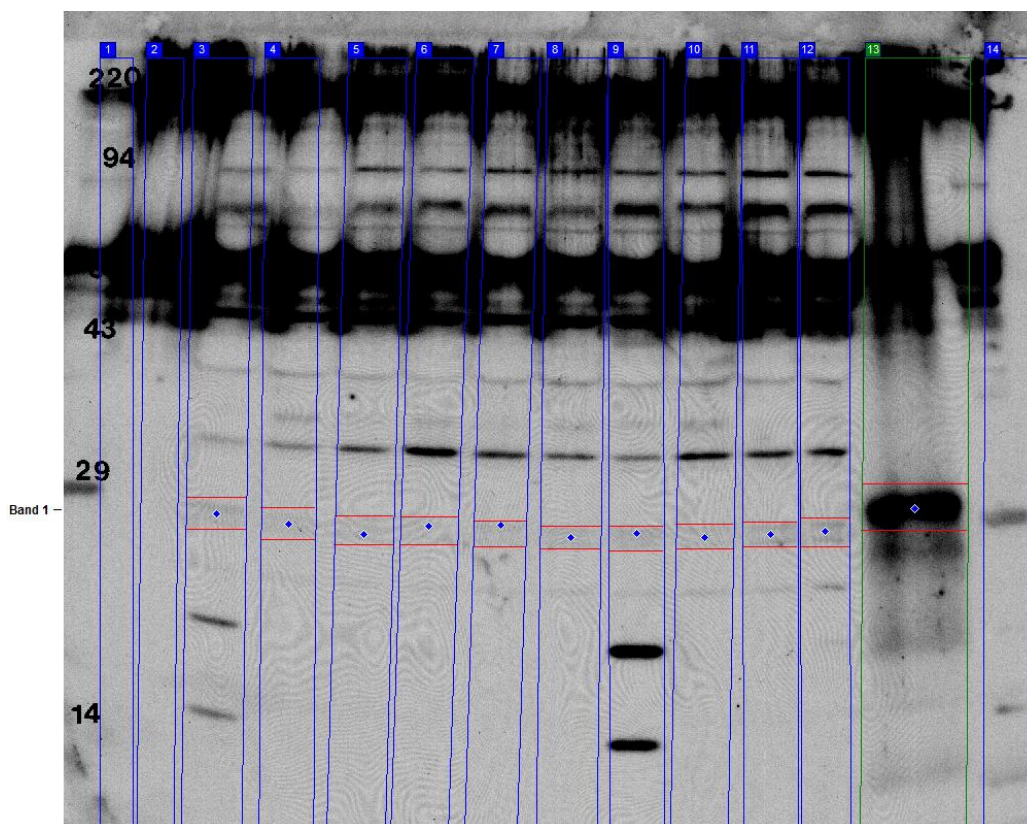


Figure 10. Image of lanes and bands quantified in film,  $\alpha$ -CD8- $\alpha$  (H-160) gel KO p.4201 #11.

| Lane # | Sample                       | $\mu$ l loaded | $\mu$ g loaded |
|--------|------------------------------|----------------|----------------|
| 1      | Molecular Weight Markers     | 5              | -              |
| 2      | Buffer Blank                 | 10             | -              |
| 3      | NAT-6                        | 10             | 40             |
| 4      | NAT-7                        | 10             | 40             |
| 5      | NAT-8                        | 10             | 40             |
| 6      | NAT-9                        | 10             | 40             |
| 7      | NAT-10                       | 10             | 40             |
| 8      | NAT-16                       | 10             | 40             |
| 9      | NAT-17                       | 10             | 40             |
| 10     | NAT-18                       | 10             | 40             |
| 11     | NAT-19                       | 10             | 40             |
| 12     | NAT-20                       | 10             | 40             |
| 13     | SC-174035 CD8- $\alpha$ (h2) | 10             | 40             |
| 14     | Molecular Weight Markers     | 10             | 40             |
| 15     | -                            | -              | -              |

Table 11. Key to 1D Slab Gel Loading KO 4201 #11, 10% Acrylamide, PVDF.



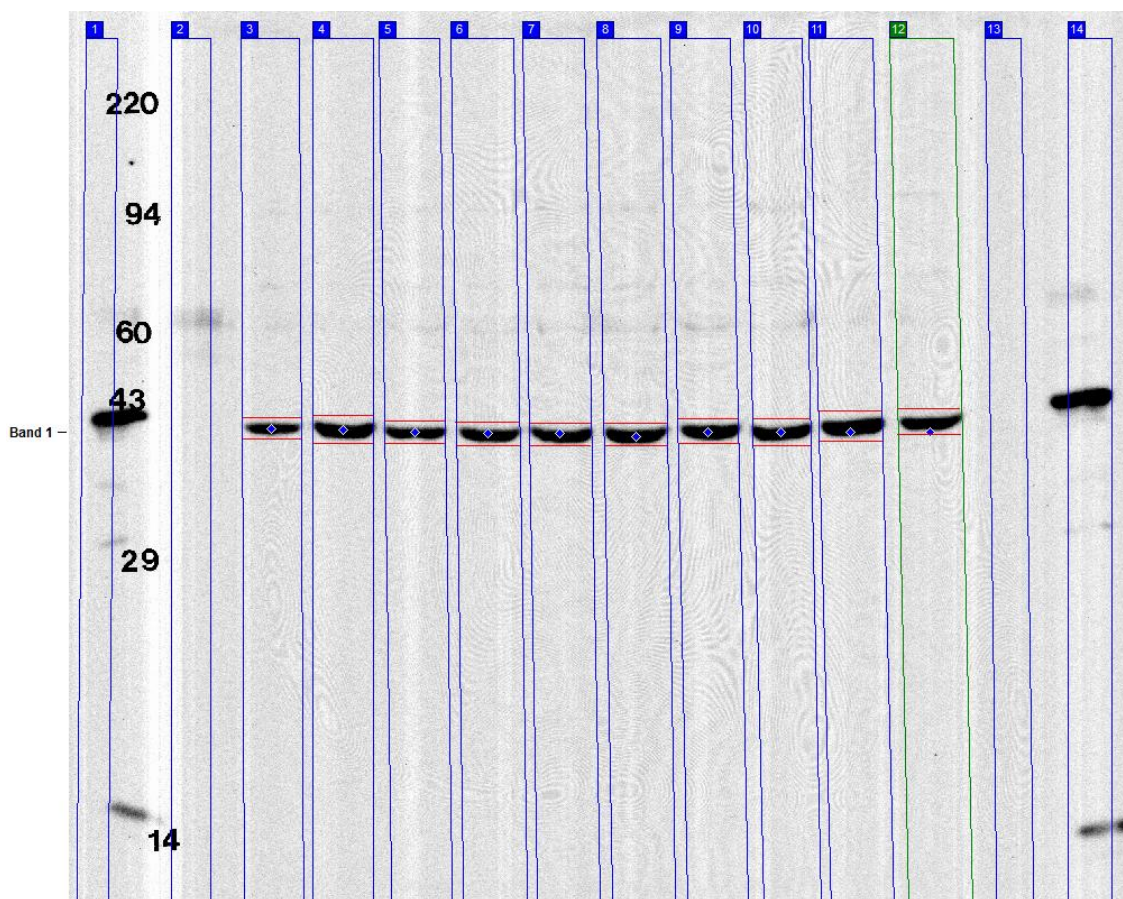


Figure 11. Image of lanes and bands quantified in film,  $\alpha$ - $\beta$ -actin gel KO p.4201 #6.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-1                    | 10             | 40             |
| 4      | NAT-2                    | 10             | 40             |
| 5      | NAT-3                    | 10             | 40             |
| 6      | NAT-4                    | 10             | 40             |
| 7      | NAT-5                    | 10             | 40             |
| 8      | NAT-11                   | 10             | 40             |
| 9      | NAT-12                   | 10             | 40             |
| 10     | NAT-13                   | 10             | 40             |
| 11     | NAT-14                   | 10             | 40             |
| 12     | NAT-15                   | 10             | 40             |
| 13     | -                        | -              | -              |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 12. Key to 1D Slab Gel Loading KO 4201 #6, 10% Acrylamide, PVDF.

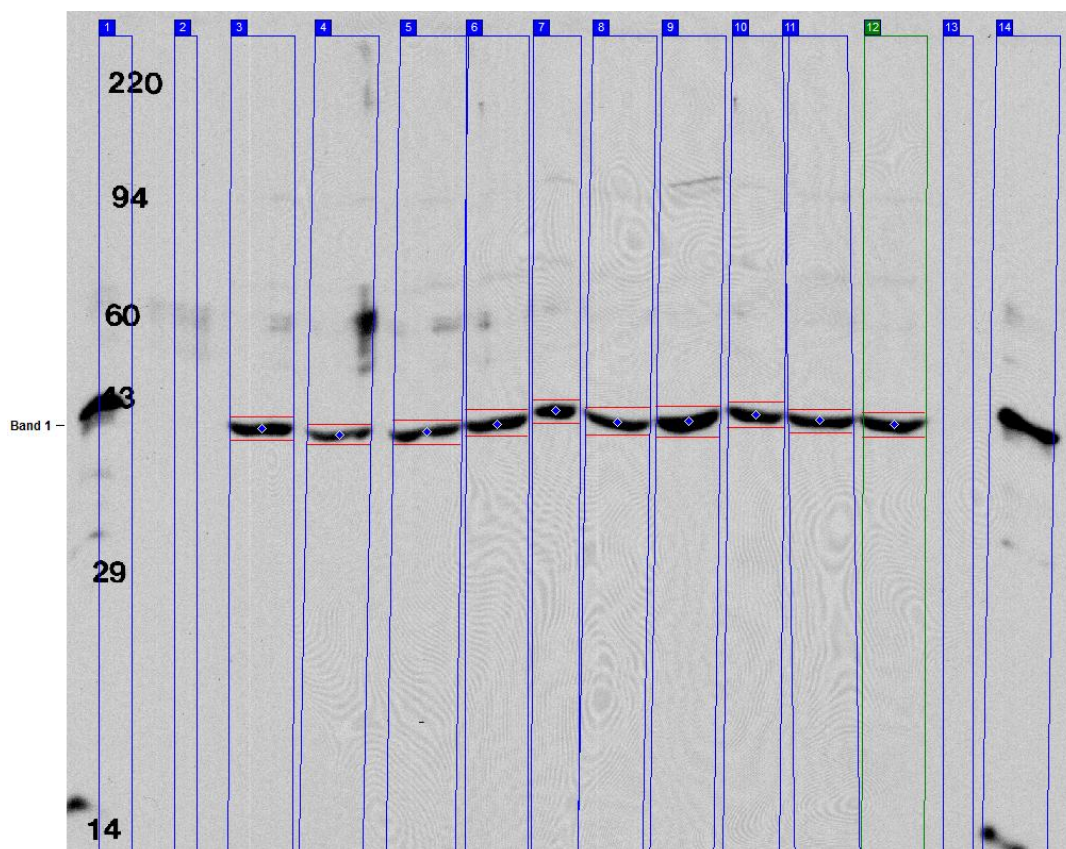


Figure 12. Image of lanes and bands quantified in film,  $\alpha$ - $\beta$ -actin gel KO p.4201 #12.

| Lane # | Sample                   | $\mu$ l loaded | $\mu$ g loaded |
|--------|--------------------------|----------------|----------------|
| 1      | Molecular Weight Markers | 5              | -              |
| 2      | Buffer Blank             | 10             | -              |
| 3      | NAT-6                    | 10             | 40             |
| 4      | NAT-7                    | 10             | 40             |
| 5      | NAT-8                    | 10             | 40             |
| 6      | NAT-9                    | 10             | 40             |
| 7      | NAT-10                   | 10             | 40             |
| 8      | NAT-16                   | 10             | 40             |
| 9      | NAT-17                   | 10             | 40             |
| 10     | NAT-18                   | 10             | 40             |
| 11     | NAT-19                   | 10             | 40             |
| 12     | NAT-20                   | 10             | 40             |
| 13     | -                        | -              | -              |
| 14     | Molecular Weight Markers | 10             | 40             |
| 15     | -                        | -              | -              |

Table 13. Key to 1D Slab Gel Loading KO 4201 #12, 10% Acrylamide, PVDF.



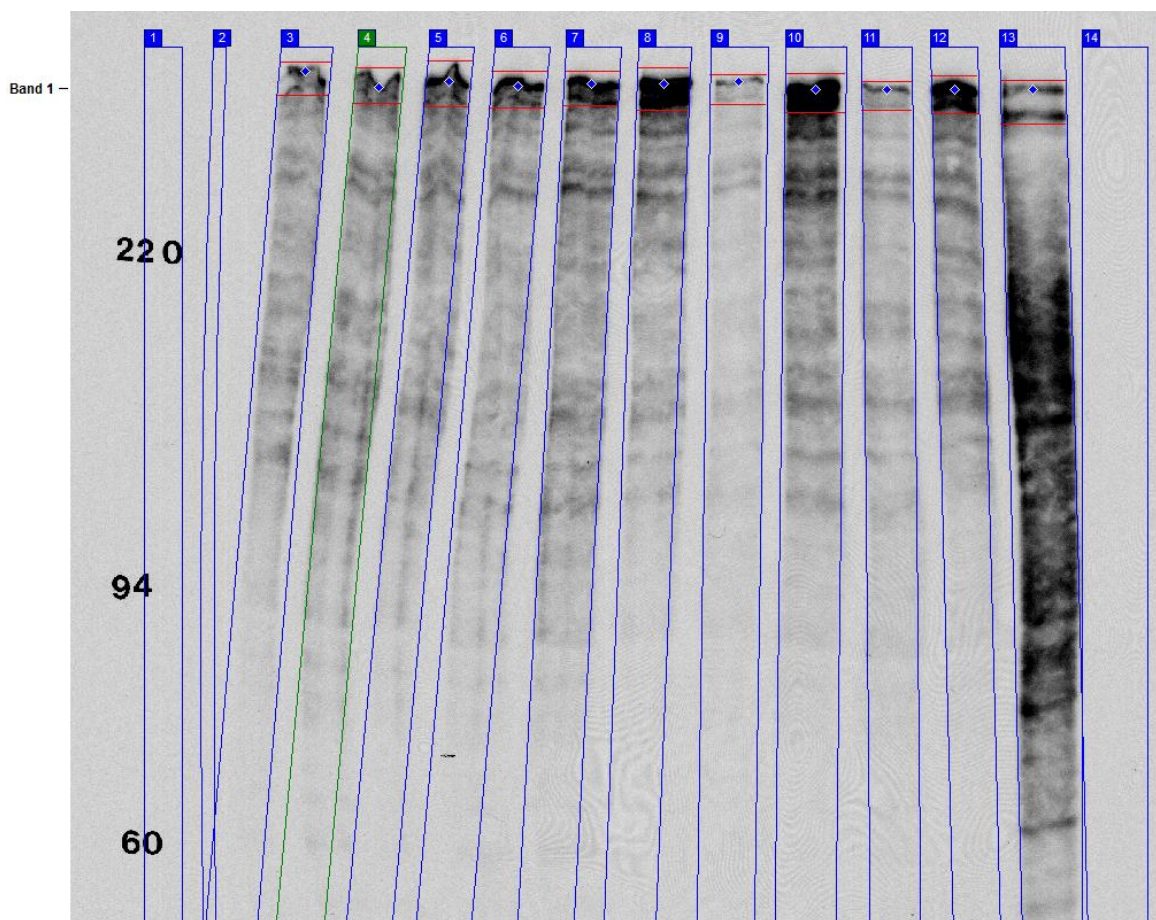


Figure 13. Image of lanes and bands quantified in film,  $\alpha$ -Ki67 gel KO p.4201 #13.

| Lane # | Sample                    | $\mu$ l loaded | $\mu$ g loaded |
|--------|---------------------------|----------------|----------------|
| 1      | Molecular Weight Markers  | 5              | -              |
| 2      | Buffer Blank              | 10             | -              |
| 3      | NAT-1                     | 10             | 40             |
| 4      | NAT-2                     | 10             | 40             |
| 5      | NAT-3                     | 10             | 40             |
| 6      | NAT-4                     | 10             | 40             |
| 7      | NAT-5                     | 10             | 40             |
| 8      | NAT-11                    | 10             | 40             |
| 9      | NAT-12                    | 10             | 40             |
| 10     | NAT-13                    | 10             | 40             |
| 11     | NAT-14                    | 10             | 40             |
| 12     | NAT-15                    | 10             | 40             |
| 13     | ab170195 Hela PMA treated | 10             | 40             |
| 14     | Molecular Weight Markers  | 10             | 40             |
| 15     | -                         | -              | -              |

Table 14. Key to 1D Slab Gel Loading KO 4201 #13, 5% Acrylamide, PVDF.



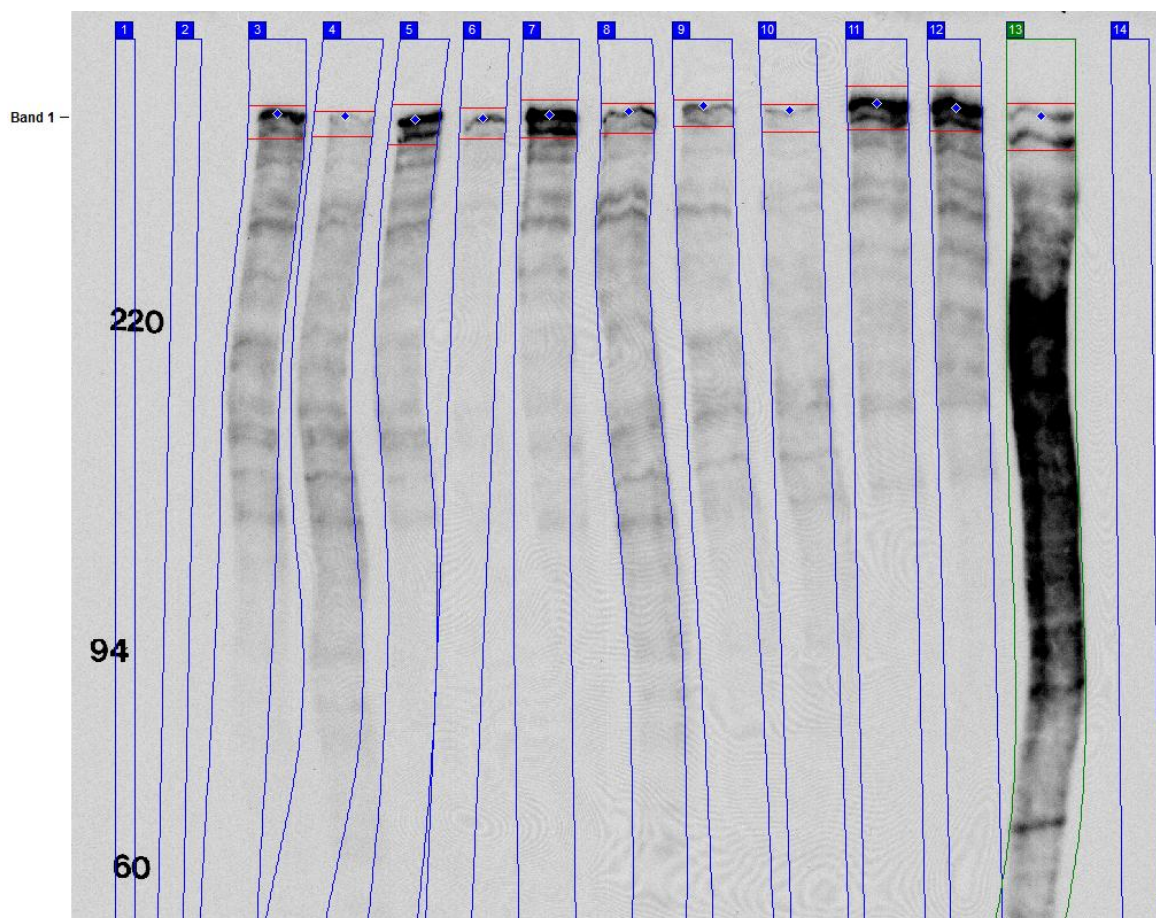


Figure 14. Image of lanes and bands quantified in film,  $\alpha$ -Ki67 gel KO p.4201 #14.

| Lane # | Sample                     | $\mu$ l loaded | $\mu$ g loaded |
|--------|----------------------------|----------------|----------------|
| 1      | Molecular Weight Markers   | 5              | -              |
| 2      | Buffer Blank               | 10             | -              |
| 3      | NAT-6                      | 10             | 40             |
| 4      | NAT-7                      | 10             | 40             |
| 5      | NAT-8                      | 10             | 40             |
| 6      | NAT-9                      | 10             | 40             |
| 7      | NAT-10                     | 10             | 40             |
| 8      | NAT-16                     | 10             | 40             |
| 9      | NAT-17                     | 10             | 40             |
| 10     | NAT-18                     | 10             | 40             |
| 11     | NAT-19                     | 10             | 40             |
| 12     | NAT-20                     | 10             | 40             |
| 13     | ab 170195 Hela PMA treated | 10             | 40             |
| 14     | Molecular Weight Markers   | 10             | 40             |
| 15     | -                          | -              | -              |

Table 15. Key to 1D Slab Gel Loading KO 4201 #14, 5% Acrylamide, PVDF.

### Sample Preparation

The samples were homogenized with 250 µl osmotic lysis buffer containing nuclease inhibitor, protease inhibitor, phosphatase inhibitor I, and phosphatase inhibitor II stock solutions. After homogenization, 250 µl SDS boiling buffer without reducing agents and 2 µl of omnicleave were added. The samples were heated in a boiling water bath for 5 minutes before loading. The protein concentrations (see Table 15 below) of the samples were determined using the BCA Assay (Smith et. al. *Anal. Biochem.* 150: 76-85, 1985, and Pierce Chemical Co., Rockford, IL).

| Sample | BCA Results (mg/ml) | Sample | BCA Results (mg/ml) |
|--------|---------------------|--------|---------------------|
| NAT-1  | 24.6                | NAT-11 | 19.2                |
| NAT-2  | 30.8                | NAT-12 | 26.4                |
| NAT-3  | 22.8                | NAT-13 | 17.9                |
| NAT-4  | 19.8                | NAT-14 | 23.5                |
| NAT-5  | 20.2                | NAT-15 | 25.1                |
| NAT-6  | 30.0                | NAT-16 | 22.2                |
| NAT-7  | 30.3                | NAT-17 | 8.4                 |
| NAT-8  | 18.2                | NAT-18 | 10.9                |
| NAT-9  | 9.8                 | NAT-19 | 15.7                |
| NAT-10 | 12.3                | NAT-20 | 14.8                |

Table 16. Protein Concentration Results.

| Gel ID         | Primary Antibody      | Company    | Cat #      | Lot #       | Dilution                                              |
|----------------|-----------------------|------------|------------|-------------|-------------------------------------------------------|
| KO 4201 #1, 7  | CTLA4 (H-126)         | Santa Cruz | sc-9094    | G2015       | 1 <sup>st</sup> 1:2,000<br>2 <sup>nd</sup> 1:100      |
| KO 4201 #2, 8  | Pdcd-1 (M-288)        | Santa Cruz | sc-367266  | K1513       | 1 <sup>st</sup> 1:2,000<br>2 <sup>nd</sup> 1:200      |
| KO 4201 #3, 9  | Caspase 3 (H-272)     | Santa Cruz | sc-7148    | D2315       | 1 <sup>st</sup> 1:2,000<br>2 <sup>nd</sup> 1:100      |
| KO 4201 #4, 10 | CD4                   | Abcam      | ab187745   | GR171092-6  | 1 <sup>st</sup> 1:10,000<br>2 <sup>nd</sup> 1:1,088   |
| KO 4201 #5, 11 | CD8- $\alpha$ (H-160) | Santa Cruz | sc-7188    | F1115       | 1 <sup>st</sup> 1:2,000<br>2 <sup>nd</sup> 1:200      |
| KO 4201 #6, 12 | $\beta$ -actin (R-22) | Santa Cruz | sc-1306576 | F0614       | 1 <sup>st</sup> 1:2,000<br>2 <sup>nd</sup> 1:100      |
| KO 4201 #13-14 | Ki67                  | Abcam      | ab15580    | GR 245044-1 | 1 <sup>st</sup> 0.1 µg/ml<br>2 <sup>nd</sup> not done |

Table 17. Key to Antibodies Used.

### **1D Methods**

SDS slab gel electrophoresis was carried out according to the method of Laemmli (Laemmli, U.K. *Nature* 227: 680-685, 1970) as described by Burgess-Cassler et. al. (*Clin. Chem.* 35:2297-2304, 1989; second dimension) using a 10% or 5% acrylamide slab gels (125 mm length X 150 mm width X 0.75mm thickness) overlaid with a 25 mm stacking gel. Electrophoresis was performed using 15 mA/gel for about 3.5 hrs. After slab gel electrophoresis, the gels were placed in transfer buffer (10 mM CAPS, pH 11.0, 10% MeOH) and transblotted onto PVDF membrane overnight at 200 mA and approximately 100 volts/two gels. The following proteins (Sigma Chemical Co., St. Louis, MO and EMD Millipore, Billerica, MA) were loaded in lanes as molecular weight standards: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000). The PVDF membranes were stained with Coomassie blue R-250 and desktop scanned.

### **Western Blot Methods**

The Coomassie Brilliant Blue R-250 stained blots were desktop scanned, de-stained in 100% methanol, rinsed briefly in Tween-20 tris buffer saline (TTBS), and blocked for two hours in 5% nonfat dry milk (NFDM) in TTBS. The blots were then incubated in primary antibodies (as indicated in Table 16) diluted in 2% NFDM TTBS overnight and rinsed 3 x 10 minutes in TTBS. The blots were then placed in secondary antibody (anti-rabbit IgG-HRP [GE Healthcare Cat # NA 934V Lot # 9670531] diluted 1:2,000 in 2% NFDM TTBS for two hours, rinsed 4 x 10 in TTBS, treated with ECL, and exposed to x-ray film.

### **Band Quantification**

The films were scanned with a laser densitometer (Model PDSI, Molecular Dynamics Inc, Sunnyvale, CA). The scanner was checked for linearity prior to scanning with a calibrated Neutral Density Filter Set (Melles Griot, Irvine, CA). Phoretix 1D v1.2 was used for lane selection, background subtraction, band selection and quantification of band volume.