**Pulmonary inflammatory response and immunomodulation to multiple trauma and hemorrhagic shock in pigs**

**- Original uncropped and unadjusted images underlying western blot results -**

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Caspase-3 Western blot pattern

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gel #1** | | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** |
| Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) | **Marker** (5µl) | Neg-CTRL (2,5 µl) | Pos-CTRL (2,5 µl) | #10 | #22 | #4 | #18 | #3 | #8 | #11 | #7 | #9 | #13 | #43 | #44 | #47 | #42 | #45 | #46 | **Marker** (5µl) | Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) |  |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| **Gel #** | | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** |
| Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) | **Marker** (5µl) | Neg-CTRL (2,5 µl) | Pos-CTRL (2,5 µl) | #35 | #36 | #17 | #19 | #15 | #20 | #21 | 24 | #48 | #55 | #49 | #50 | #53 | **Marker** (5µl) | Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) |  |  |  |  |

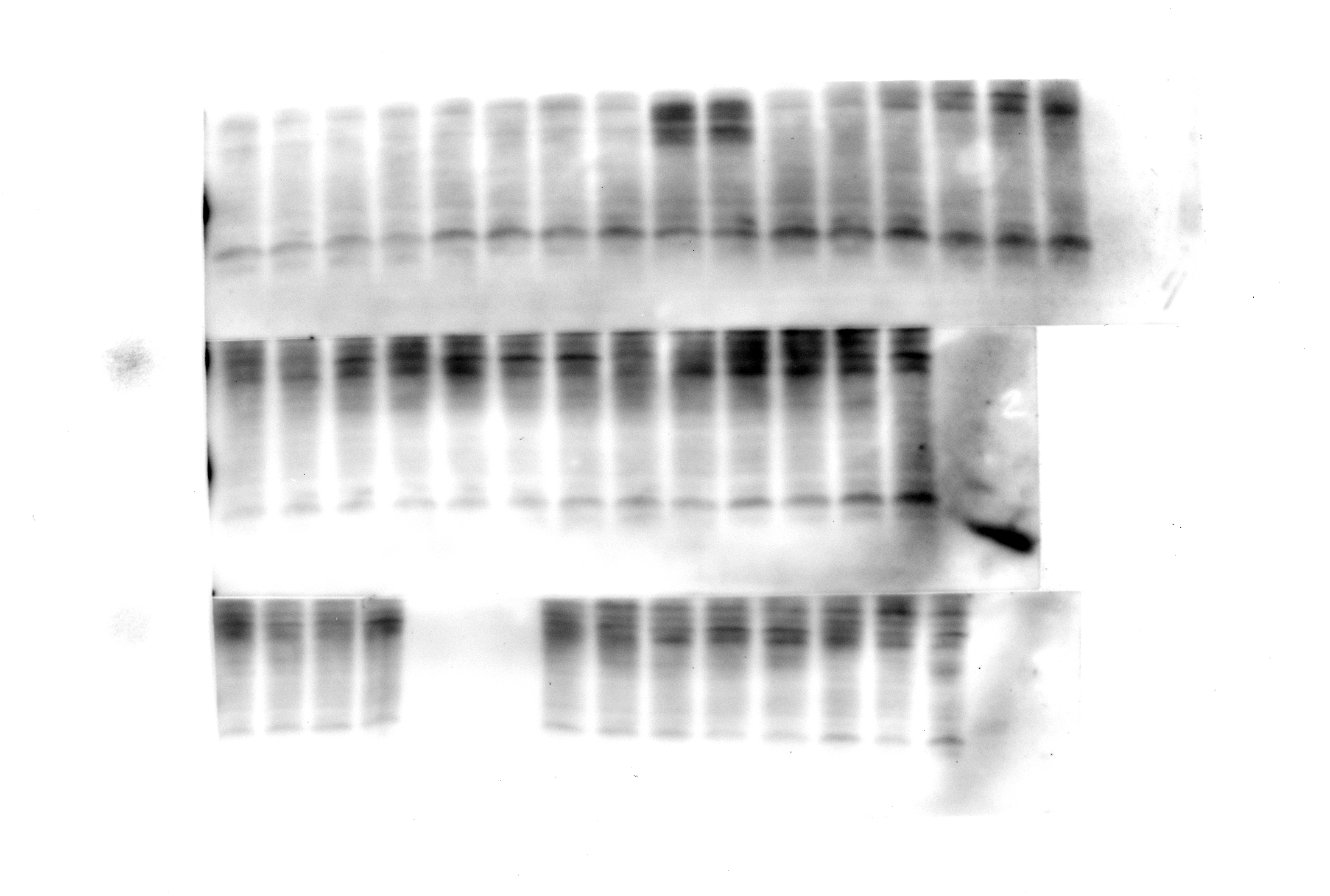
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gel #3** | | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** |
| Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) | **Marker** (5µl) | Neg-CTRL (2,5 µl) | Pos-CTRL (2,5 µl) | #37 | #41 | #38 | #40 | #23 | #30 | #31 | #26 | #32 | #33 | #39 | #57 | #60 | #61 | **Marker** (5µl) | Laemmli-buffer (30 µl) | Laemmli-buffer (30 µl) |  |  |  |

Protein concentrations were determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA). Equal amounts of protein (60 µg) were separated in 15% Tris-HCl SDS-polyacrylamide gels for semi-dry blotting of caspase-3 with 0.003 mA/cm² on PVDF membranes. Prior to incubation with antibodies, the blots were cut horizontally along 38 kDa to enable simultaneous analysis of ß-actin (40 kDa), pro-caspase-3 (32 kDa) and cleaved caspase-3 subunits (11, 17, 20 kDa), washed and blocked (3% bovine serum albumin in TBS-T20). The chemo-luminescent signals were captured using a Gel-x imager system (Intas Science Imaging Instruments, Göttingen, Germany), quantified by densitometry (ImageJ 1.52j, National Institutes of Health, USA) and normalized to ß-actin. Untreated and cytochrome c-treated Jurkat control cell extracts (#9663, Cell Signaling Technology, Frankfurt, Germany) served as controls

Left upper lobe (contralateral control): ß-actin

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Left upper lobe (contralateral control): Caspase-3



Right upper lobe (ipsilateral control): ß-actin

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Right upper lobe (ipsilateral control): Caspase-3

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Right lower lobe (contusion site): ß-actin



Right lower lobe (contusion site): Caspase-3

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