Supplementary Material

The spleen as a possible source of serine protease inhibitors and migrating monocytes required for liver regeneration after 70% resection in mice

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**Supplementary Table 1.** List of antibodies for flow cytometry

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of the antibody** | **Dilution** | **Manufacturer** | **Catalogue number** |
| Anti-Mouse CD45 Antibody PE | 1:100 | Miltenyi Biotec | 130102596 |
| Anti-Mouse Ly6C Antibody FITC | 1:100 | Miltenyi Biotec | 130111777 |
| Anti-Mouse F4/80 PerCP/Cyanine5.5 Conjugated | 1:100 | BioLegend | DL20130F |
| Anti-Mouse CD115 PE Conjugated | 1:100 | BioLegend | DL21294F |
| Anti-Mouse CD115 APC Conjugated | 1:100 | BioLegend | DL21295F |
| Anti- Mouse CD206 PE Conjugated | 1:100 | BioLegend | DL21672F |
| Anti-Mouse CD86 PE/Cyanine7 Conjugated | 1:100 | BioLegend | DL20106F |
| Anti-Mouse CD163 PE Conjugated | 1:100 | eBioscience | 2006963 |
| Anti-Mouse CD19 Antibody PerCP/Cyanine5.5 Conjugated | 1:100 | BioLegend | DL20011F |
| Anti-Mouse CD3 Monoclonal Antibody APC Conjugated | 1:100 | BioLegend | DL20268F |
| Anti-Mouse CD8a Antibody PE Conjugated | 1:100 | BioLegend | DL22851F |
| Anti-Mouse CD4 Antibody EV450 Conjugated | 1:100 | BioLegend | DL22845F |
| Anti-Mouse CD1d Monoclonal Antibody APC Conjugated | 1:100 | BioLegend | DL20484F |
| Anti-Mouse Foxp3 Antibody PE Conjugated | 1:100 | BioLegend | DL22662F |

**Supplementary Table 2.** Primers for real-time PCR

|  |  |  |
| --- | --- | --- |
| Target designation | 5′-end primer | 3′-end primer |
| *CcnA2* | TGTCCTGGATTGGGTCACTGG | TCAGCCTCCGGGCAGTAGA |
| *CcnB1* | GCTAAGATCAGCACGCTGGC | TCGACAACTTCCGTTAGCCTAAACT |
| *CcnD1* | TGTCGGCGCAGTAGCAGA | AAGATACGGAGGGCGCACAG |
| *CcnE1* | TGGATGGTTCCGTTCGCCAT | GTCAGGACCACACTCGGAGG |
| *Serpina3n* | CAAGCCAACAACCCTGAACATCA | GCATCCATTCCCAACGTGCC |
| *Serpina1b* | TGGGGCTGACCTCTCTGGAA | GGCATAGACATAGGAACGGCTTC |
| *Stfa2* | AGTTCAAGTCGTCCAAGGACTAAA | CGCATCTCTTTACAATGGGGGTTA |
| *Stfa2l1* | TTGTCAGAGGCCAGACCTGC | TGGAGGTAACAACCACGTCCT |
| *Gapdh* | AGGCCGGTGCTGAGTATGTC | TGCCTGCTTCACCACCTTCT |

**Supplementary Table 3.** Top 10 enriched pathways in the spleen after liver resection

**1 days**

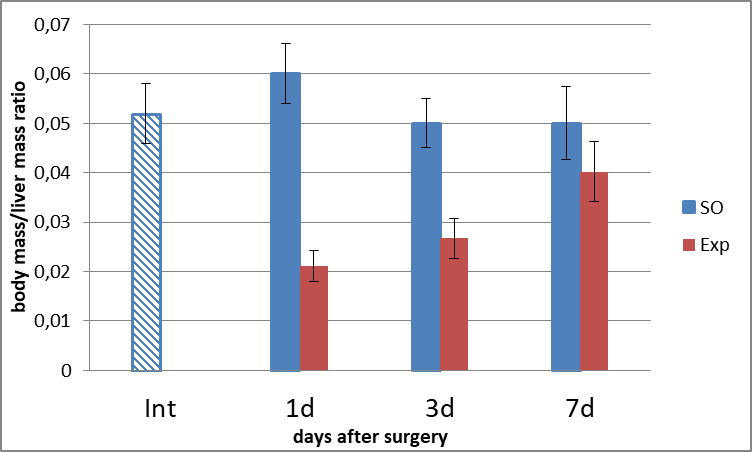
|  |  |  |  |
| --- | --- | --- | --- |
| **Pathway** | **Total DEGs** | **Upregulated genes** | **Downregulated genes** |
| Protein-protein interactions in podocytes | 58 | 12 | 46 |
| Focal adhesion: PI3K-Akt-mTOR signaling pathway | 27 | 6 | 21 |
| mRNA processing | 24 | 10 | 14 |
| Chemokine signaling pathway | 24 | 9 | 15 |
| Protein-protein interactions in the podocyte | 23 | 6 | 17 |
| Mechanisms associated with pluripotency | 22 | 4 | 18 |
| Oxidative stress and redox pathway | 17 | 2 | 15 |
| Insulin signaling | 16 | 5 | 11 |
| Adar1 editing defficiency immune response | 16 | 14 | 2 |
| Mapk signaling pathway | 16 | 4 | 12 |

**3 days**

|  |  |  |  |
| --- | --- | --- | --- |
| **Pathway** | **Total DEGs** | **Upregulated genes** | **Downregulated genes** |
| mRNA processing | 23 | 0 | 23 |
| Electron transport chain | 18 | 0 | 18 |
| Protein-protein interactions in podocytes | 17 | 4 | 13 |
| Oxidative phosphorylation | 11 | 0 | 11 |
| Cytoplasmic ribosomal proteins | 9 | 0 | 9 |
| Protein-protein interactions in the podocyte | 8 | 1 | 7 |
| Mechanisms associated with pluripotency | 7 | 1 | 6 |
| Proteasome degradation | 6 | 0 | 6 |
| Oxidative stress and redox pathway | 6 | 0 | 6 |
| Chemokine signaling pathway | 6 | 3 | 3 |

**7 days**

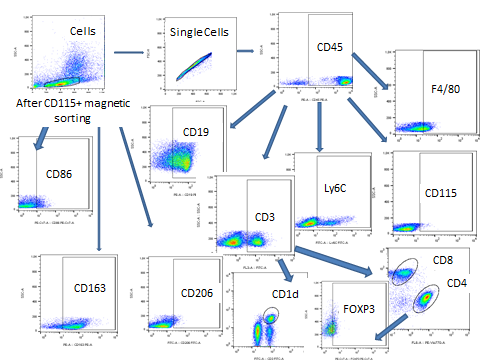
|  |  |  |  |
| --- | --- | --- | --- |
| **Pathway** | **Total DEGs** | **Upregulated genes** | **Downregulated genes** |
| Protein-protein interactions in podocytes | 95 | 58 | 37 |
| B cell receptor signaling pathway | 47 | 40 | 7 |
| T cell receptor signaling pathway | 46 | 41 | 5 |
| mRNA processing | 40 | 9 | 31 |
| Mechanisms associated with pluripotency | 40 | 21 | 19 |
| Chemokine signaling pathway | 34 | 24 | 10 |
| Protein-protein interactions in the podocyte | 30 | 20 | 10 |
| Focal adhesion: PI3K-Akt-mTOR signaling pathway | 29 | 15 | 14 |
| Oxidative stress and redox pathway | 28 | 4 | 24 |
| Electron transport chain | 27 | 2 | 25 |



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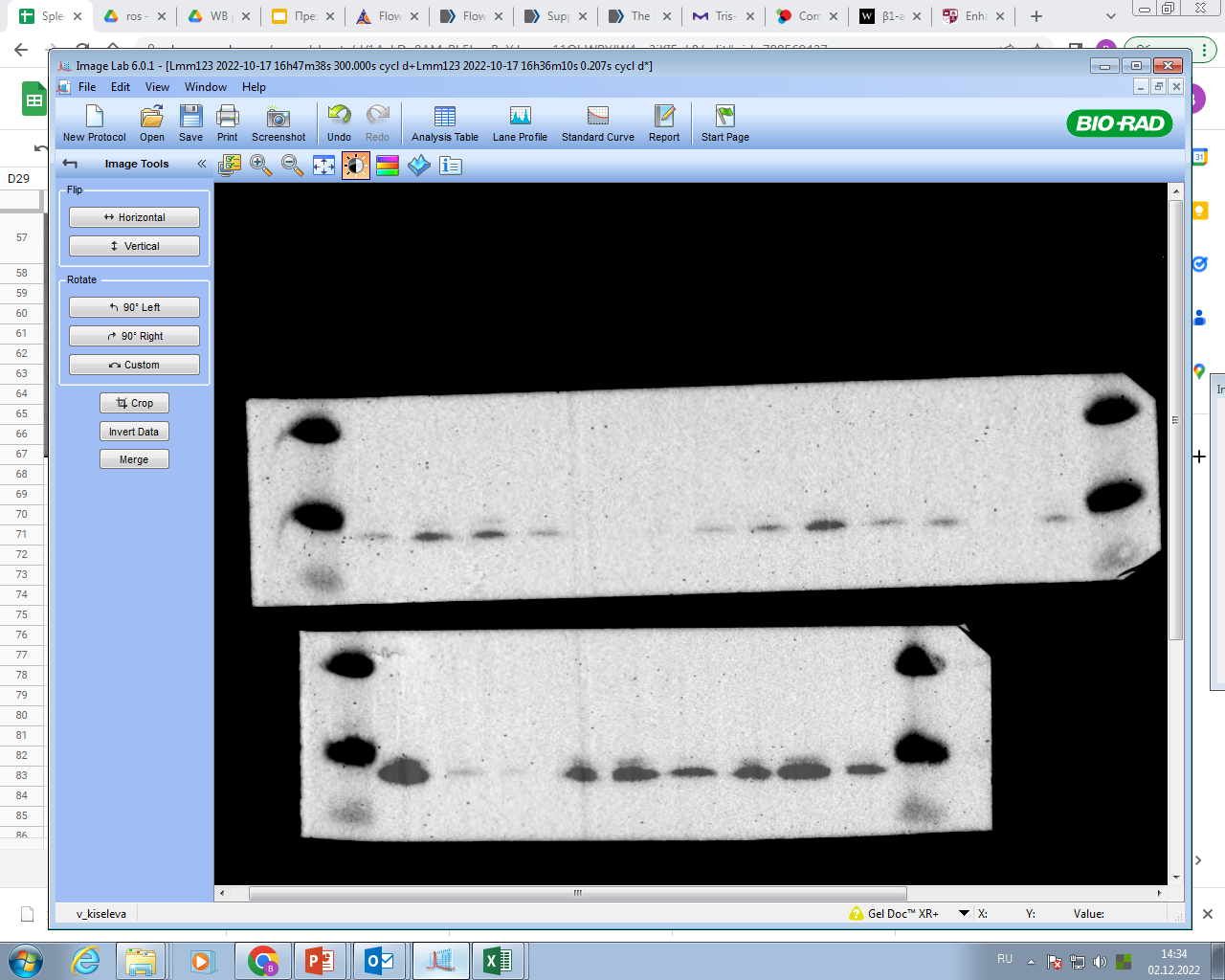
**Supplementary Figure 1. Dynamics of restoration of liver mass.** \* - *p* < 0.05 compared with corresponding sham-operated animals, # - *p* < 0.05 compared with intact animals.

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**Supplementary Figure 2.** The gating strategies.The gating strategies used FSC vs SSC dot-plots to exclude debris (Fig.S1) followed by (SSC vs CD45 (CD45+ to SSC vs CD3 (CD3+ to CD4 vs CD8))) dot-plots for T cells; (SSC vs CD19 (CD19+ to SSC vs CD45)) for B-cells; (SSC vs CD45 (CD45+ to SSC vs CD3 (CD3+ vs CD1d))) dot-plots for NKT cells; (SSC vs CD45 (CD45+ to SSC vs CD3 (CD3+ vs CD4))vs. CD4+to FOXP3) for total Treg cells and (SSC vs CD45 (CD45+ to SSC vs F4/80|CD115|Ly6C)) for macrophages. After CD115+magnetic sorting CD86, CD163 and CD206 positivity indexes were measured in FSC vs SSC gated pools of interest.

**Supplementary Figure 3. Uncropped WB membranes**

Cyclin D 1



55

35

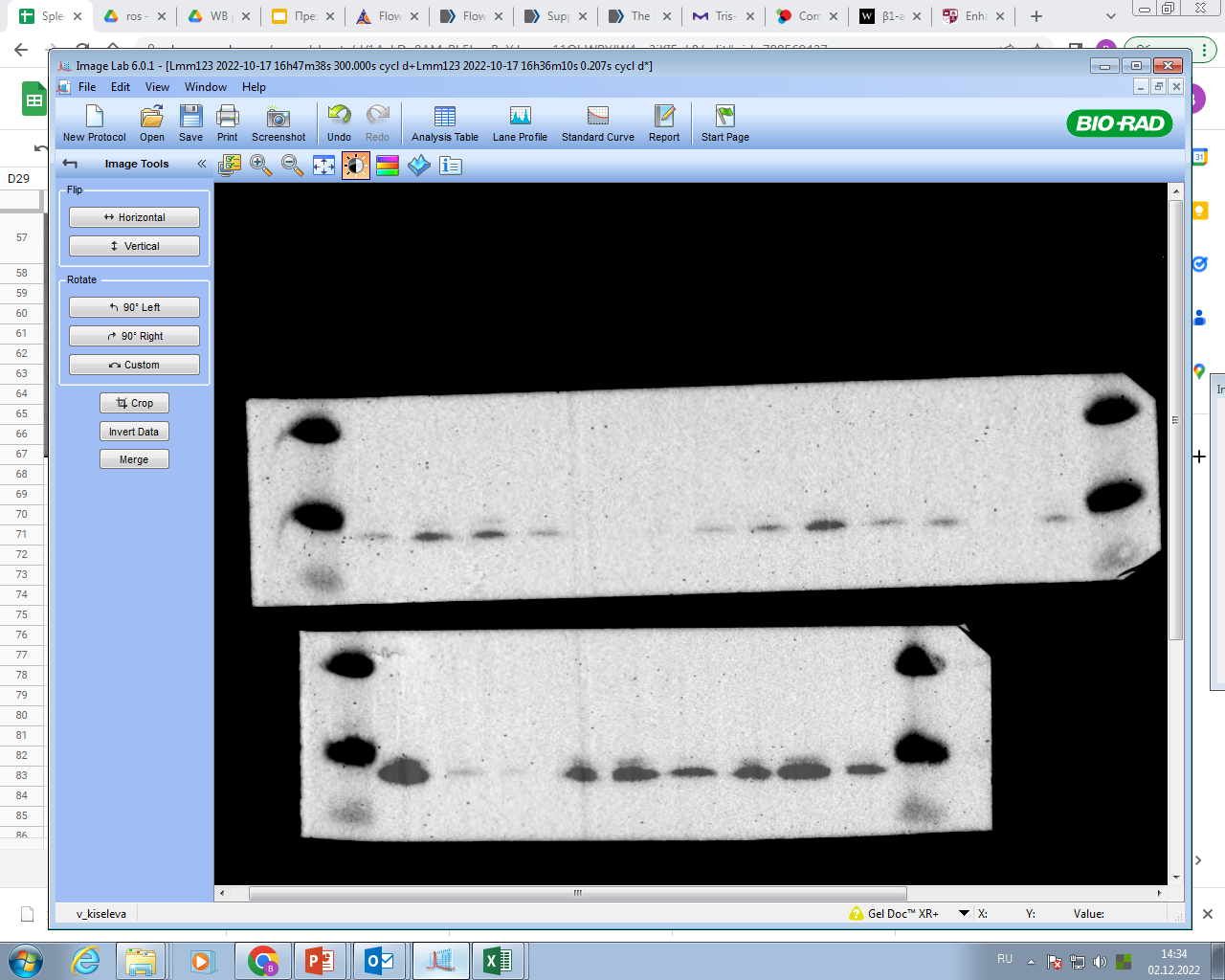
25

7 days

3 days

1 days

Intact spleen



35

25

55

sham-operated

7 days

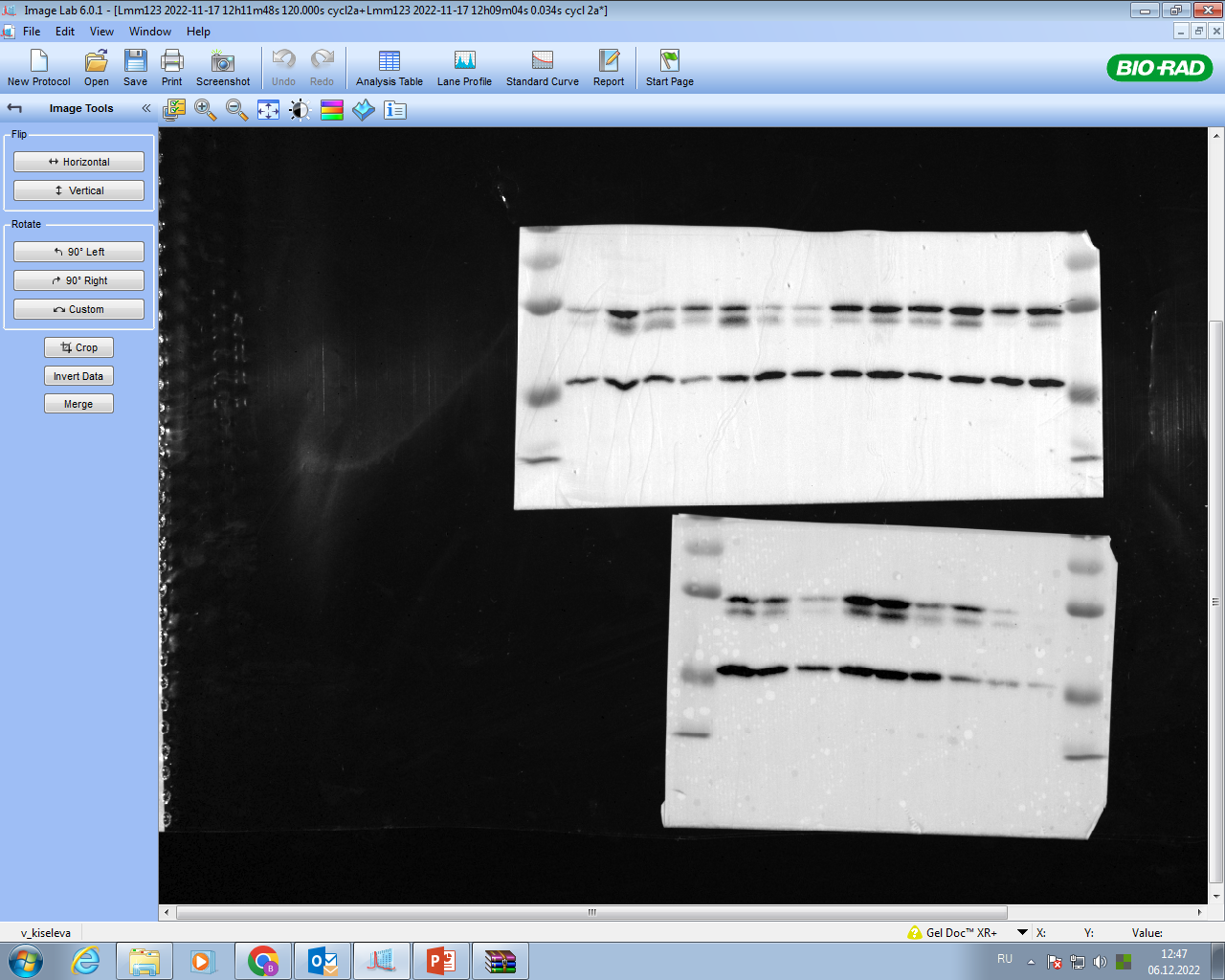
sham-operated

3 days

sham-operated

1 days

Cyclin A2



25

35

55

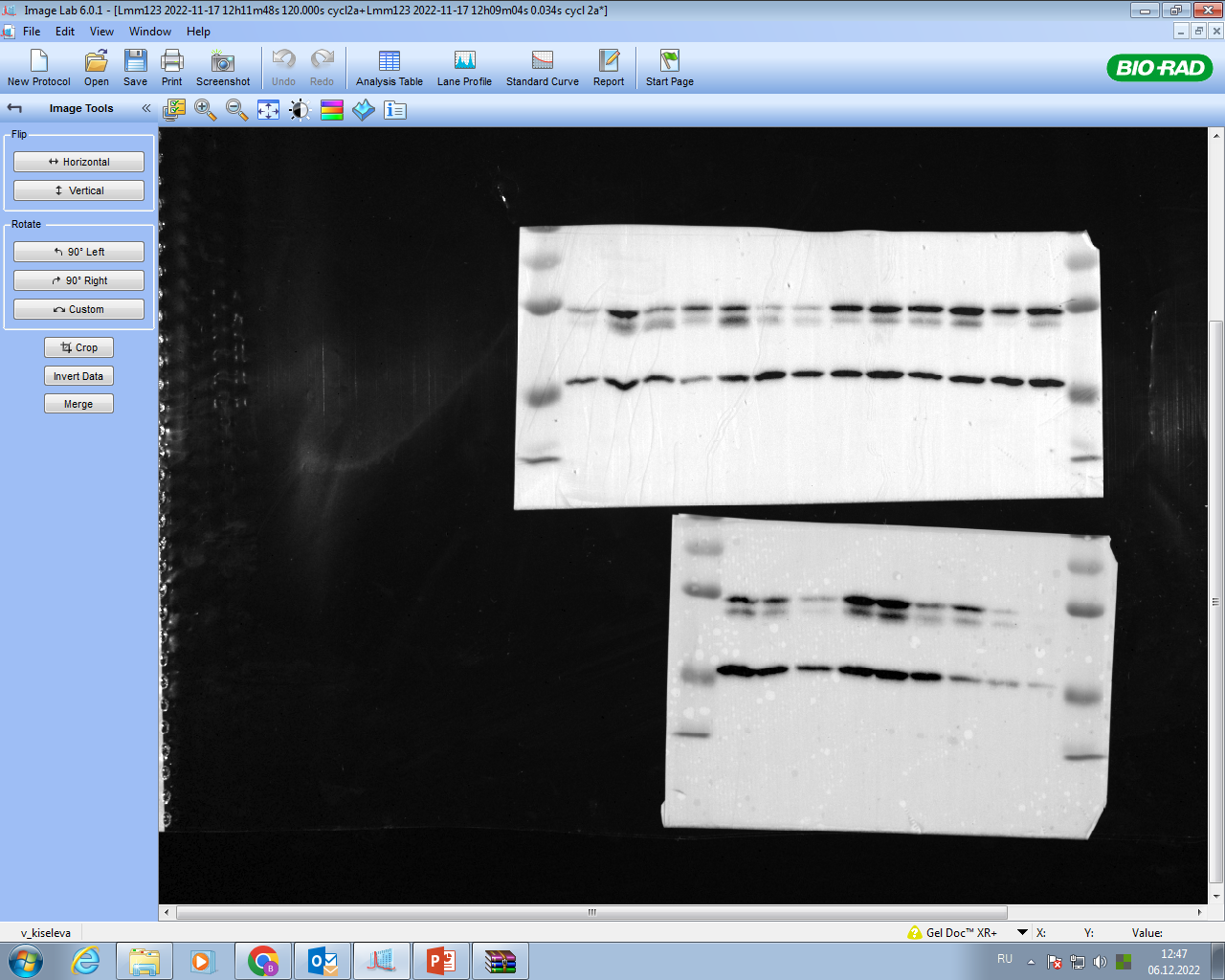
75

7 days

3 days

1 days

Intact spleen



100

25

75

55

35

sham-operated

7 days

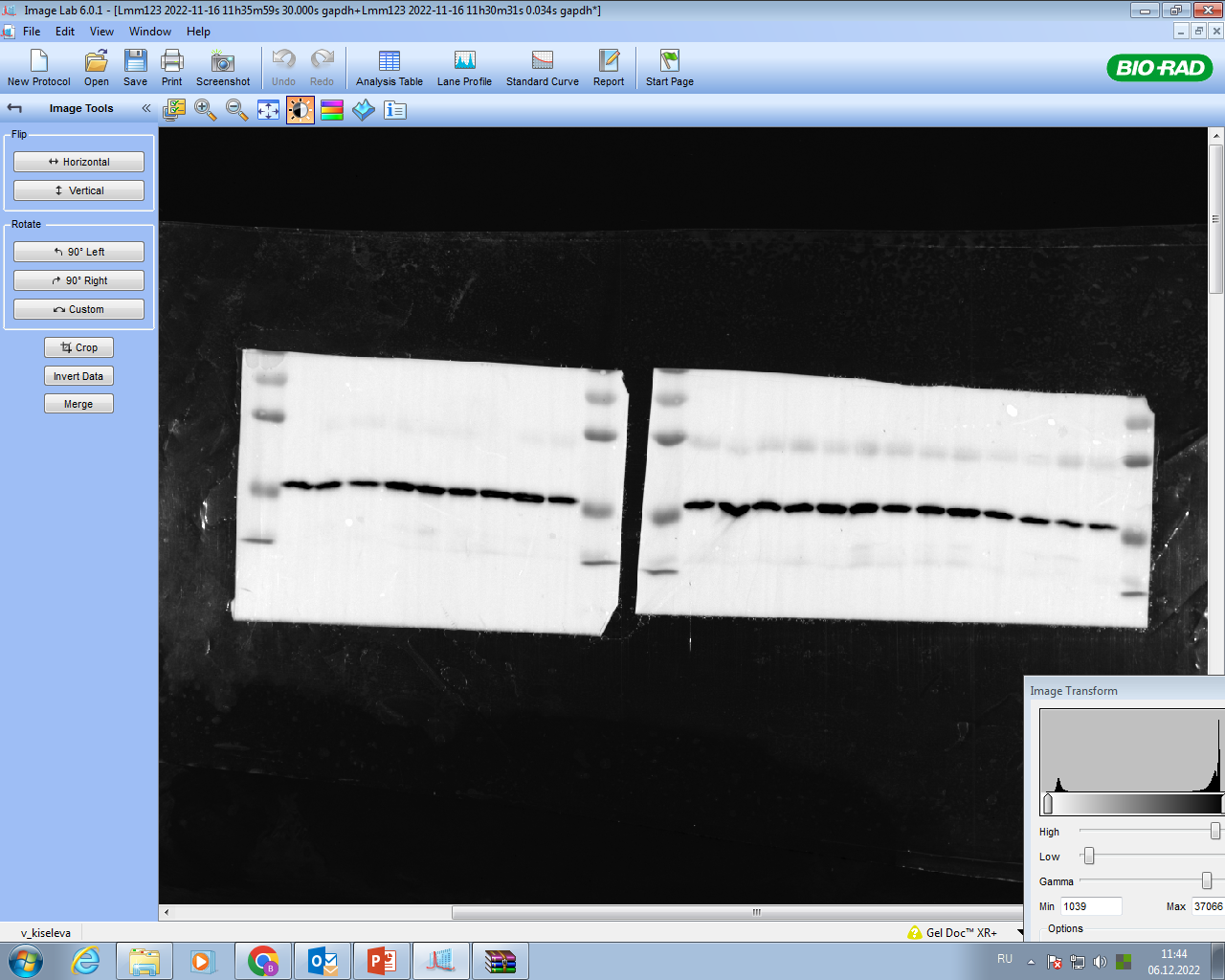
sham-operated

3 days

sham-operated

1 days

35



75

25

55

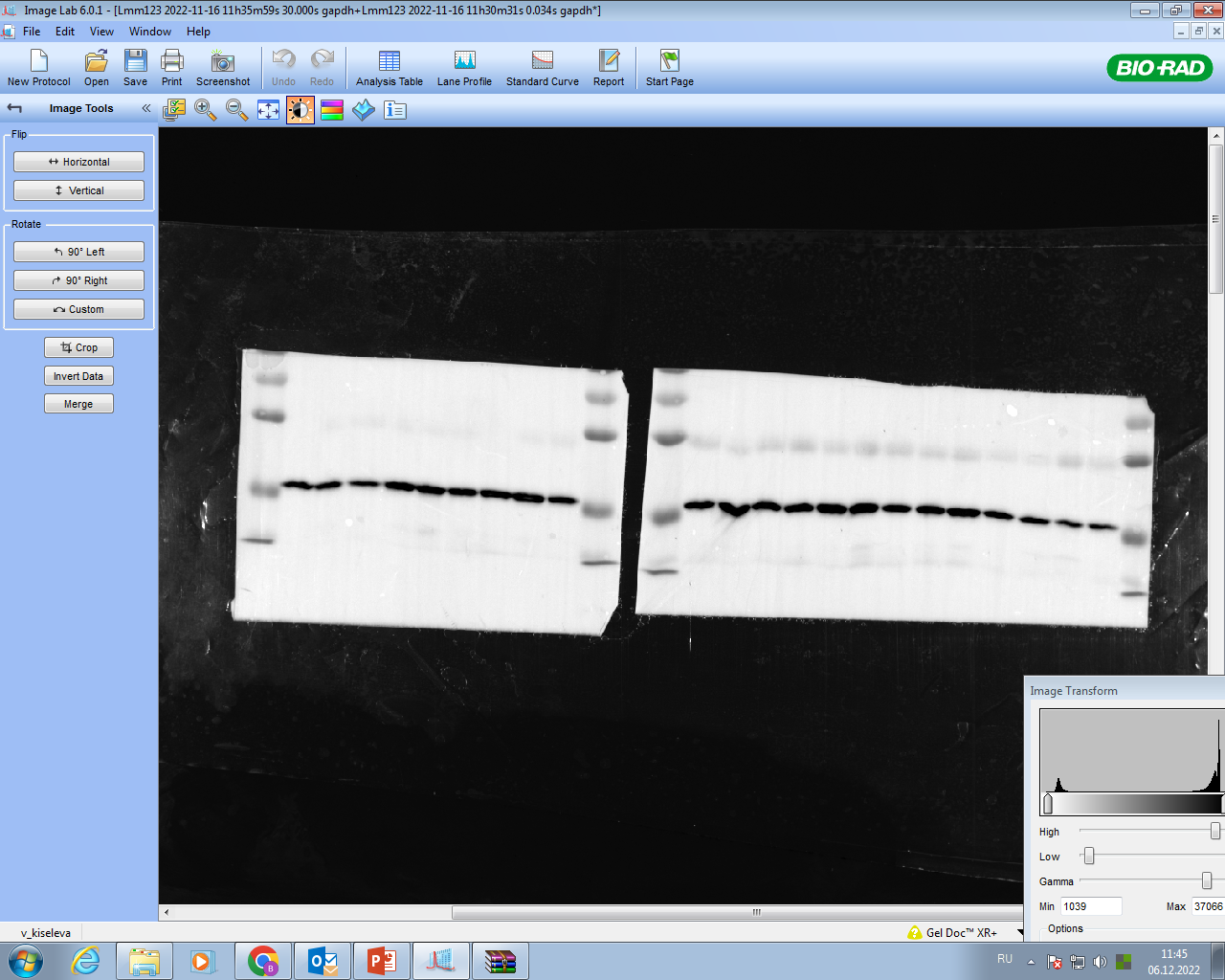
7 days

3 days

1 days

Intact spleen

GAPDH



25

35

55

75

100

sham-operated

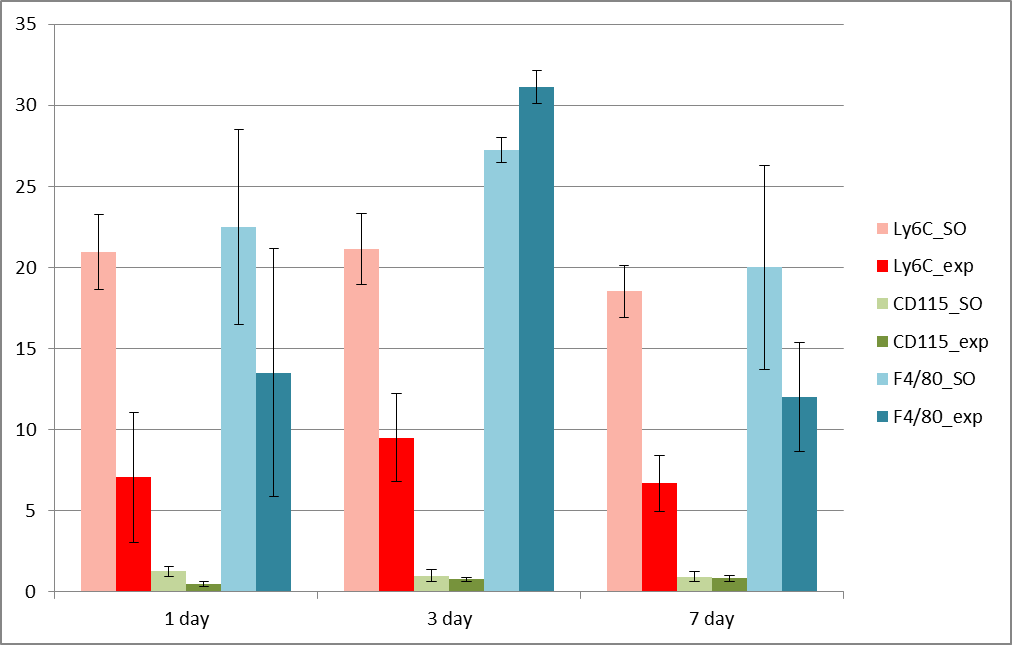
7 days

sham-operated

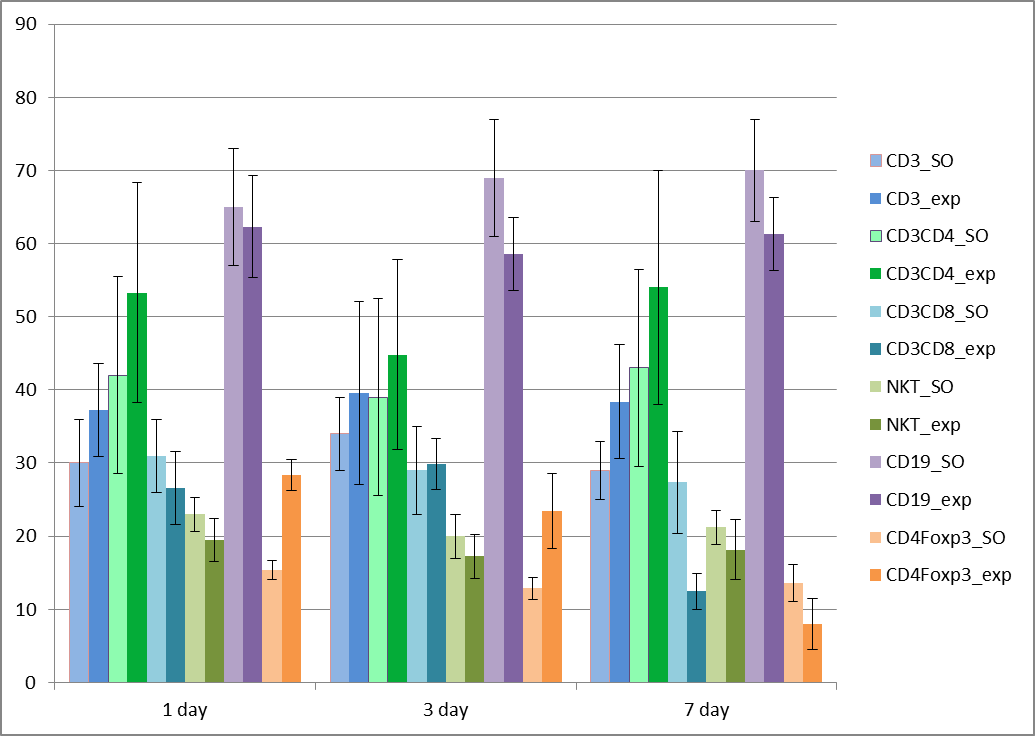
3 days

sham-operated

1 days



**Supplementary Figure 4.** Dynamics of spleen macrophage subpopulations during 70% liver resection in the mouse.



**Supplementary Figure 5.** Dynamics of spleen lymphocyte subpopulations during 70% liver resection in the mouse.