# Supplementary Material

| Target Gene | Primers and Probes |
|---|---|
| Hamster IL-2  gene(16) | Forward [5´-GTGCACCCACTTCAAGCTCTAA-3´] |
| | Reverse [5´-AAGCTCCTGTAAGCTCAGCAGTAAC-3´] |
| | Probe [6-Fam-AGG AAACCCAGCAGCACCTCGAGC-BHQ-1] |
| Wild type human IL-2 transgene | Forward [5´-ACTTTCACTTAAGACCCAGGGA-3´] |
| | Reverse[5´- CACACATGAATGTTGTTTCAGATCC-3´] |
| | Probe [6-Fam-CGTAATAGTTCTGGAACTAAAGGGA-BHQ-2.] |
| Human IL-2 variant transgene | Forward [5´-CAAACCTCTGGAGGAAGTGCT-3´] |
| | Reverse [5´- ACGTTGATATTGCTCACCACG-3´] |
| | Probe [6-Fam-AACTTTCACTTCGACC-BHQ-2] |
| Hamster GAPDH gene(22) | Forward[5´-CACCGAGGACCAGGTTGTCT-3´] |
| | Reverse[5´-CATACCAGGAGATGAGCTTTACGA-3´] |
| | Probe[6FAM-CAAGAGTGACT CCCACTCTTCCACCTTTGA(TAMRA)] |

**Supplementary Table 1.** RT-qPCR target genes, primers and probes sequences used for the relative mRNA expression analysis.
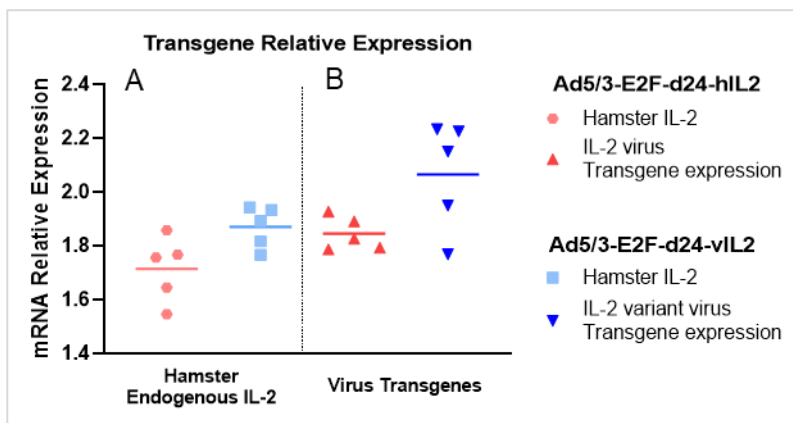


**Supplementary Figure 1.** IL-2 cytokine mRNA expression of in groups treated with cytokine-armed viruses relative to the housekeeping gene. (A) Endogenous hamster wt IL-2 cytokine mRNA relative expression. (B) Virus transgenes' relative mRNA expression.
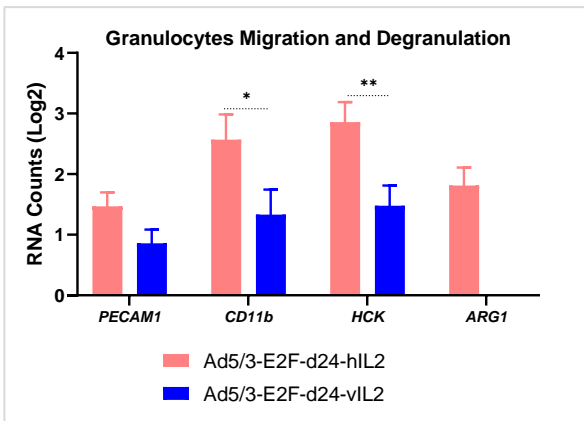
**Supplementary Figure 2**. Granulocytes migration and degranulation molecular gene mRNA (Log2) expression analysis in the viruses treated tumours of genes associated. Unpaired t-test with Welch´s correction. All data is presented as mean+SEM. *p<0.05 and **p<0.01.
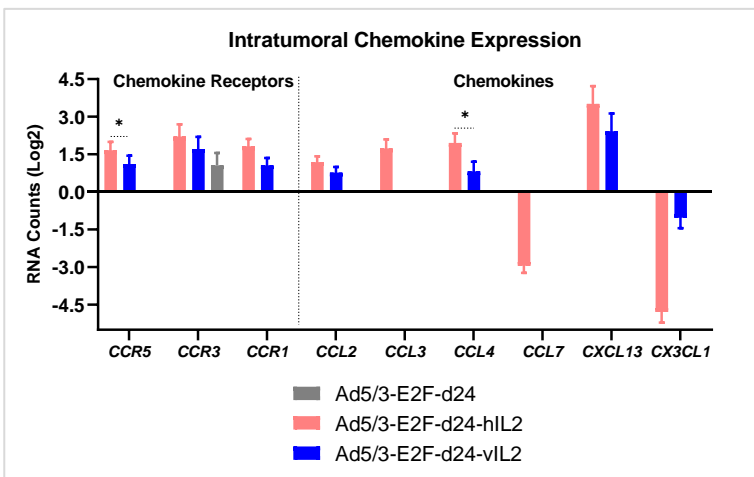


**Supplementary Figure 3**. Intratumoral chemokines genes' mRNA (Log2) expression analysis in the viruses treated groups. Chemokine receptors (left side) and chemokine genes (right side). All data is presented as mean+SEM. *p<0.05.

| Genes | Viruses Treatments | | | | | |
|---|---|---|---|---|---|---|
| | Ad5/3-E2F-d24 | | Ad5/3-E2F-d24-hIL2 | | Ad5/3-E2F-d24-vIL2 | |
| | Log2 fold change | P-value | Log2 fold change | P-value | Log2 fold change | P-value |
| ARG1 | 0.0297 | 0.922 | 1.81 | 1.73E-05 | 0.503 | 0.112 |
| ATM | -0.196 | 0.234 | -1.31 | 4.49E-07 | -0.665 | 0.000723 |
| BAX | -0.0734 | 0.685 | -1.34 | 1.26E-06 | -0.609 | 0.00349 |
| BID | -0.036 | 0.921 | -1.72 | 0.000203 | -0.526 | 0.159 |
| CASP3 | -0.0794 | 0.755 | -2.52 | 2.60E-08 | -0.883 | 0.00279 |
| CASP8 | -0.12 | 0.623 | -2.53 | 1.34E-08 | -0.679 | 0.0117 |
| CCL2 | 0.172 | 0.461 | 1.19 | 8.05E-05 | 0.768 | 0.00384 |
| CCL3 | -0.0577 | 0.874 | 1.74 | 0.000173 | 0.334 | 0.365 |
| CCL4 | 0.213 | 0.586 | 1.95 | 0.000114 | 0.822 | 0.048 |
| CCL5 | 0.0914 | 0.801 | -0.314 | 0.39 | 0.39 | 0.289 |
| CCL7 | 0.225 | 0.43 | -2.95 | 1.29E-08 | -0.163 | 0.566 |
| CCL8 | 0.0961 | 0.769 | 0.318 | 0.337 | 0.241 | 0.464 |
| CCR1 | 0.366 | 0.229 | 1.82 | 1.23E-05 | 1.06 | 0.0023 |
| CCR3 | 1.06 | 0.0467 | 2.21 | 0.000344 | 1.71 | 0.00304 |
| CCR5 | 0.255 | 0.459 | 1.66 | 0.000147 | 1.11 | 0.00446 |
| CD103 | -0.00602 | 0.989 | 1.24 | 0.0126 | 0.573 | 0.214 |
| PD-L1 | 0.185 | 0.597 | 0.958 | 0.0129 | 0.489 | 0.173 |
| CD27 | 0.243 | 0.545 | 0.105 | 0.793 | 1.03 | 0.0186 |
| CD39 | 0.167 | 0.374 | -0.533 | 0.0115 | -0.114 | 0.546 |
| CD3D | 0.234 | 0.397 | 0.661 | 0.0253 | 1 | 0.00179 |
| CD3E | 0.298 | 0.382 | 0.774 | 0.033 | 1.14 | 0.00328 |
| CD3G | 0.229 | 0.505 | 0.653 | 0.0698 | 1 | 0.00865 |
| CD40 | 0.522 | 0.213 | 2.39 | 1.98E-05 | 1.61 | 0.000974 |
| CD45RO | 0.35 | 0.296 | 1.79 | 4.49E-05 | 1.3 | 0.00103 |
| CD4 | 0.0749 | 0.862 | 1.56 | 0.00191 | 1.37 | 0.0049 |
| CD62L | 0.48 | 0.29 | 1.84 | 0.00068 | 1.64 | 0.00177 |
| CD7 | 0.238 | 0.515 | 0.854 | 0.0296 | 0.9 | 0.0229 |
| CD80 | 0.553 | 0.168 | 2.48 | 7.05E-06 | 1.25 | 0.00475 |
| CD86 | 0.243 | 0.459 | 2.57 | 5.03E-07 | 1.6 | 0.000131 |
| CD8A | 0.267 | 0.507 | 0.847 | 0.0467 | 1.22 | 0.0068 |
| COX2 | 0.164 | 0.687 | 0.823 | 0.0561 | -0.199 | 0.626 |
| CTLA-4 | 0.587 | 0.239 | 2.4 | 0.00012 | 1.67 | 0.00299 |
| CX3CL1 | -0.108 | 0.8 | -4.79 | 5.34E-09 | -1.03 | 0.0257 |
| CXCL10 | 0.385 | 0.383 | 0.662 | 0.143 | 0.814 | 0.0761 |
| CXCL13 | 0.135 | 0.853 | 3.51 | 0.000149 | 2.42 | 0.0037 |
| FCGR2B | -0.00493 | 0.976 | 0.423 | 0.0176 | 0.409 | 0.0209 |
| FUT7 | 0.23 | 0.52 | 2.21 | 8.20E-06 | 0.961 | 0.0135 |
| GZMK | 0.318 | 0.5 | 0.0718 | 0.878 | 1.07 | 0.033 |
| GZMM | 0.219 | 0.589 | 0.494 | 0.232 | 1.05 | 0.0178 |
| TIM-3 | 0.115 | 0.673 | 1.02 | 0.00153 | 0.728 | 0.0151 |
| HCK | 0.334 | 0.327 | 2.86 | 1.91E-07 | 1.48 | 0.000364 |
| HMGB1 | -0.0777 | 0.681 | -1.52 | 3.99E-07 | -0.533 | 0.011 |
| IDO1 | 0.786 | 0.108 | -1.62 | 0.00292 | 1.09 | 0.0315 |
| IFNAR1 | 0.0188 | 0.867 | 0.631 | 3.18E-05 | 0.0164 | 0.884 |
| IFNG | 0.113 | 0.77 | -0.231 | 0.551 | 0.336 | 0.388 |
| IFNGR1 | 0.0576 | 0.609 | 0.577 | 8.04E-05 | 0.27 | 0.0263 |
| IL10 | -0.223 | 0.494 | -2.61 | 4.22E-07 | -1.68 | 7.47E-05 |
| IL1B | 0.568 | 0.364 | 3.93 | 7.78E-06 | 2.18 | 0.00249 |
| IL2RG | 0.393 | 0.185 | 2.11 | 1.31E-06 | 1.39 | 0.000155 |
| IL6 | -0.0331 | 0.944 | -1.96 | 0.000673 | -0.7 | 0.149 |
| IRAK1 | -0.0851 | 0.495 | -0.961 | 8.54E-07 | -0.768 | 1.23E-05 |
| IRAK4 | -0.0519 | 0.742 | -1.17 | 1.22E-06 | -0.586 | 0.00164 |
| IRF1 | 0.056 | 0.793 | -0.0245 | 0.909 | -0.431 | 0.0567 |
| IRF2 | 0.0118 | 0.913 | -0.292 | 0.0146 | -0.00705 | 0.948 |
| IRF3 | -0.0743 | 0.68 | -1.24 | 3.04E-06 | -0.554 | 0.00646 |
| IRF4 | 0.608 | 0.281 | 3.11 | 3.07E-05 | 2 | 0.002 |

| Gene | | | | | | |
|------|------|------|------|------|------|------|
| IRF5 | 0.127 | 0.637 | 1.69 | 8.02E-06 | 1.09 | 0.000758 |
| IRF8 | 0.183 | 0.246 | 0.841 | 4.47E-05 | 0.477 | 0.00639 |
| ITGA1 | -0.0889 | 0.6 | 0.184 | 0.283 | -0.00989 | 0.953 |
| CD11b | 0.316 | 0.458 | 2.57 | 1.31E-05 | 1.33 | 0.0054 |
| ITK | 0.252 | 0.4 | 0.651 | 0.0392 | 0.886 | 0.00743 |
| JAK1 | -0.0598 | 0.601 | -0.918 | 4.17E-07 | -0.513 | 0.000312 |
| JAK2 | 0.0676 | 0.502 | -0.0822 | 0.416 | 0.107 | 0.291 |
| ki67 | -0.267 | 0.282 | -3.02 | 1.06E-09 | -0.875 | 0.00217 |
| KLRK1 | 0.358 | 0.364 | 1.17 | 0.0075 | 1.22 | 0.0056 |
| LBP | 0.0356 | 0.893 | -2.21 | 2.81E-07 | -0.791 | 0.00807 |
| LCK | 0.194 | 0.476 | 0.919 | 0.00322 | 1.02 | 0.00149 |
| MRC1 | 0.00144 | 0.995 | 0.565 | 0.0189 | 0.429 | 0.0651 |
| MYD88 | 0.0447 | 0.657 | -0.436 | 0.000441 | -0.12 | 0.243 |
| OAS3 | -0.174 | 0.739 | -4.75 | 1.02E-07 | -1.13 | 0.043 |
| PECAM1 | 0.132 | 0.572 | 1.47 | 7.93E-06 | 0.857 | 0.00172 |
| PRF1 | 0.303 | 0.311 | 0.5 | 0.103 | 0.625 | 0.046 |
| S1PR1 | 0.0297 | 0.891 | 0.828 | 0.00115 | 0.175 | 0.421 |
| SBNO2 | -0.0627 | 0.744 | -0.152 | 0.433 | -0.655 | 0.00324 |
| SAP | -0.0301 | 0.964 | 1.03 | 0.138 | 1.43 | 0.0458 |
| STAT5B | -0.0456 | 0.564 | -0.0293 | 0.71 | -0.13 | 0.113 |
| SYK | 0.31 | 0.296 | 2.31 | 5.03E-07 | 1.24 | 0.000516 |
| TCF7 | -0.0102 | 0.954 | -1.31 | 1.56E-06 | -0.552 | 0.00585 |
| TGFB2 | -0.0219 | 0.847 | -0.392 | 0.00292 | -0.242 | 0.0455 |
| TGFBR2 | 0.114 | 0.553 | -0.374 | 0.0641 | -0.357 | 0.076 |
| THBD | 0.0197 | 0.915 | 0.25 | 0.19 | 0.384 | 0.0512 |
| TICAM1 | 0.0767 | 0.557 | -0.778 | 1.91E-05 | -0.33 | 0.0209 |
| TIGIT | -0.0721 | 0.765 | -0.538 | 0.0386 | 0.0735 | 0.761 |
| TLR2 | 0.201 | 0.451 | 2.1 | 4.50E-07 | 0.893 | 0.00332 |
| TLR3 | 0.09 | 0.634 | -0.842 | 0.000386 | -0.164 | 0.391 |
| TNF | -0.0465 | 0.88 | 1.67 | 4.75E-05 | 0.247 | 0.428 |
| TNFRSF9 | 0.243 | 0.568 | 3.04 | 1.70E-06 | 0.934 | 0.0392 |
| TNFSF10 | 0.136 | 0.665 | -1.19 | 0.00142 | 0.13 | 0.679 |
| TYK2 | -0.078 | 0.521 | -0.358 | 0.00859 | -0.325 | 0.0153 |
| VEGFA | 0.11 | 0.778 | 0.2 | 0.609 | -0.339 | 0.392 |
| VEGFC | -0.0411 | 0.867 | -1.73 | 2.58E-06 | -0.27 | 0.283 |
| ZAP70 | 0.208 | 0.573 | 0.805 | 0.0402 | 0.885 | 0.0259 |

**Supplementary Table 2**. Panel of the genes analysed for differential expression (mRNA Log2) and their respective significance in p-values. Significantly upregulated genes are shown in green. Significantly downregulated genes are shown in red. Insignificant changes in the gene expression are shown in grey. The expression level of each gene in the treatment groups was normalized to their corresponding genes in the control (mock) group.