

Flow cytometric analysis of GFP expression in transduced primary Mo-DC transduced with adenovirus as shown. LPS is the positive control for the experiment not transduced.

| | Percentage of GFP+ iDC coexpressing indicated markers | | | | | |
|----------|---|---------------|----------|-------------|---------------|-----------|
| | CD40 | CD80 | CD83 | CD86 | HLA-DR | CCR7 |
| | % | % | % | % | % | % |
| Ad-LPS | 6.7±4.8 | 75.8±15.2 | 16.9±9.3 | 24.1±15.0 | 0.4 ± 0.5 | 50.2±5.0 |
| Ad-WT | 1.3±0.9 | 5.4±0.3 | 3.8±2.0 | 0.8 ± 0.2 | 0.6 ± 0.4 | 6.5±3.7 |
| Ad-DN-WT | 1.6±1.5 | 2.5 ± 2.8 | 1.1±0.6 | 0.7 ± 0.2 | 2.1±1.6 | 6.9±5.1 |
| Ad-P19 | 2.5±1.2 | 25.6±2.3 | 8.1+1.6 | 1.5 ± 0.9 | $1.4{\pm}1.2$ | 28.9±12.8 |
| Ad-P23 | 4.0±2.1 | 23.1±0.6 | 8.0±2.0 | 1.8±1.3 | 0.8 ± 0.4 | 26.4±8.1 |

Supplemental Table S1. The effects of Ad-P19 or Ad-P23 on Mo-DC surface marker expression

Comparative analysis +/- standard deviation of flow cytometric analysis of different markers in 5 separate experiments. Primary Mo-DC transduced with adenovirus as shown. LPS is the positive control for the experiment transduced with empty GFP+ vector.

Flow cytometric analysis of Mo-DCs treated with Ad-GFP, Ad-WT, Ad-DN-WT, Ad-P19, Ad-P23, or LPS (2 µg/mL) for 24 h for changes in CD40, CD80, CD86, CD83, CCR7. HLA-DR and experimental Each construct was compared the empty-vector to control, filled histograms, and infected cells were gated on GFP prior to analysis.



Supplemental Table S2. ANOVA test values

| | | Ad-GFP vs | | | | |
|----------|--------|-----------|---------|---------|--------|--|
| | wт | DN-WT | Ad-P19 | Ad-P20 | Ad-P23 | |
| TNFα | 0.9998 | >0.9999 | 0.0002 | 0.9987 | 0.1033 | |
| IL-12p70 | 0.9985 | >0.9999 | <0.0001 | >0.9999 | 0.0752 | |
| IL-10 | 0.9926 | 0.9963 | 0.9176 | 0.9406 | 0.4258 | |
| IL-8 | 0.5549 | 0.9999 | 0.0005 | 0.8661 | 0.0048 | |
| IFNγ | 0.9996 | 0.9998 | 0.0002 | 0.9999 | 0.0559 | |

| | Ad-GFP vs | | | |
|---------------|-----------|---------|---------|---------|
| | wт | DN-WT | Ad-P19 | Ad-P23 |
| Proliferation | 0.3939 | 0.3638 | <0.0001 | <0.0001 |
| Lysis | 0.3906 | 0.7983 | <0.0001 | 0.0028 |
| Migration | 0.6277 | <0.0001 | <0.0001 | 0.117 |

| | Ad-GFP vs | | | |
|---------|-----------|--------|--------|--|
| ELISPOT | Ad-WT | Ad-P19 | Ad-P23 | |
| | 0.9995 | 0.1658 | 0.0026 | |

ANOVA analysis of figures 2b, 3a, 3b, 3c and 4b. Yellow denotes p value is significant. Note that two bolded p-values in Ad-P23 close to significant. b





a) GFP expression flow cytometric analysis of bone marrow DCs from animal experiment in Figure transfected with Nothing (medium), Ad-GFP, Ad-WT, Ad-P19, and Ad-P23 (representative figure). b) representation of calculation of area under the curve (AUC) for animals as in "a". The significance was measured with ANOVA (shown) against Ad-GFP as control). The AUC was performed with animals that survived to the end of the study.

| Supplem | ental Tab | ole S3. Pa | aired stu | dent t-te | st values |
|----------|-----------|------------|-----------|-----------|--|
| Day 7 | Medium | Ad-GFP | Ad-WT | Ad-p19 | Ad-P23 |
| HBSS vs | 0.227012 | 0.155804 | 0.00562 | 0.000243 | 0.132482 |
| Med vs | | 0.168829 | 0.085582 | 0.126083 | 0.544817 |
| AdGFP vs | | | 0.168767 | 0.193095 | o.720357 |
| Day 10 | | | | | |
| HBSS vs | 0.838925 | 0.2088 | 0.191246 | 0.000179 | 0.003925 0 |
| Med vs | | 0.003124 | 0.111424 | 0.004858 | <mark>3 0.008461</mark> |
| AdGFP vs | | | 0.7023 | 0.021071 | 0.02903 |
| Day 14 | | | | | |
| HBSS vs | 0.087598 | 0.120166 | 0.100367 | 0.000196 | <mark>) 0.000718</mark> |
| Med vs | | 0.450491 | 0.163527 | 0.008035 | <mark>5 0.002613</mark> |
| AdGFP vs | | | 0.245815 | 0.011623 | <mark>3 0.004682</mark> |
| Day 18 | | | | | |
| HBSS vs | 0.064465 | 0.202463 | 0.016984 | 0.000607 | <mark>′ 0.003114</mark> |
| Med vs | | 0.088198 | 0.955855 | 0.009669 | <mark>) 0.009832</mark> |
| AdGFP vs | | | 0.1784 | 0.001072 | 2 <u>0.001837</u> |
| Day 21 | | | | | |
| HBSS vs | 0.069485 | 0.260954 | 0.061666 | 0.003163 | <mark>3 0.007203</mark> |
| Med vs | | 0.104447 | 0.671304 | 0.001028 | <mark>3 0.002993</mark> |
| AdGFP vs | | | 0.549291 | 0.004537 | <mark>′ 0.001536</mark> |
| Day 25 | | | | | |
| HBSS vs | 0.13873 | 0.055627 | 0.09018 | 0.010939 | <mark>) 0.00984</mark> |
| Med vs | | 0.259094 | 0.111343 | 0.003299 | 0.001504 |
| AdGFP vs | | | 0.22362 | 0.008242 | 2 <u>0.002021</u> |
| Day 28 | | | | | |
| HBSS vs | 0.225245 | 0.053565 | 0.042643 | 0.01083 | <mark>0.014383 0.01438 0.01438 0.0148 000 0.0148 0.00000000000000000000000000000000000</mark> |
| Med vs | | 0.943765 | 0.008639 | 0.022113 | 0.012809 |
| AdGFP vs | | | 0.082507 | 0.063996 | 0.017233 |

Paired student t-test analysis of tumor volume comparisons in Figure 4a. Yellow denotes p value is significant.

| Supplemental Table S4. Mutagenesis Primers | | | | |
|--|---|--|--|--|
| Primer name | Sequence | | | |
| PCDNA3 | 5'-AATACGACTCACTATAGGGA-3' | | | |
| | 5'-GGACAGTGGGAGTGGCACCTTCCA-3' | | | |
| DAP12 P7 | 5'-GTAGACAACCGACCTCTGACC -3 | | | |
| | 5'-AGGTCGGTTGTCTACAGCGAC -3' | | | |
| DAP12 P8 | 5'-CGCTCATTTGTATTCCGGCCTCTGTGC -3' | | | |
| | 5'- CAGAGGCCGGAATACAAATGAGCGGCC -3' | | | |
| DAP12 P9 | 5'-ACCGAGTCGCCTGATC AGGAGCTCCA -3' | | | |
| | 5'-GAGCTCCTGATCAGGCGACTCGGT -3' | | | |
| DAP12 P10 | 5'-CGCTCATTTCTCTTCCGGCCTCTGTGT -3' | | | |
| | 5'-CAGAGGCCGGAAGAGAAATGAGCGGCC -3' | | | |
| DAP12 P11 | 5'-TCGGATGTCGACAGCGACCTCAAC -3' | | | |
| | 5'-GTGTTGAGGTCGCTGTCGACATCC -3' | | | |
| DAP12 P17 | 5'-CAGAGGTCGGTTCGCTACAGCGACCTC-3' | | | |
| | 5'-GTCGCTGTAGCGAACCGACCTCTGACC-3' | | | |
| DAP12 P18 | 5'-CAGAGGTCGCATGTCTACAGCCACCTCAA CAC-3' | | | |
| | 5'-TG TTGAGGTGGCTGTAGACATGCGAC CTCTGAC-3' | | | |
| DAP12 P19 | 5'-GATGTCTACAGCAA CCTCAACACA CAGAG-3' | | | |
| | 5'-CTGTGTGTTGAGGTTGCTGTAG ACATCCG-3' | | | |
| DAP12 P20 | 5'-ACCGAGTCGCCTTGTCAGGAGCTCCAGG-3' | | | |
| | 5'-TGGAGCTCCTGACAAGGCGACTCGGTCTC-3' | | | |
| DAP12 P21 | 5'-GTCGGATGTCTGCAGCGACCTCAACACAC-3' | | | |
| | 5'-GTTGAGGTCGCTGCAGACATCCGACCTC-3' | | | |
| DAP12 P22 | 5'-GATGTCTACAGCGACTACAACAC ACAGAG-3' | | | |
| | 5'-GCCTCTGTGTGTTGTAGTCGCTGTAGAC-3' | | | |
| DAP12 P23 | 5'-GTCTACAGCGACCTCAAAACAC AGAGGC-3' | | | |
| | 5'-CGGCCTCTGTGTTTTGAGGTCGCTG TAG-3' | | | |