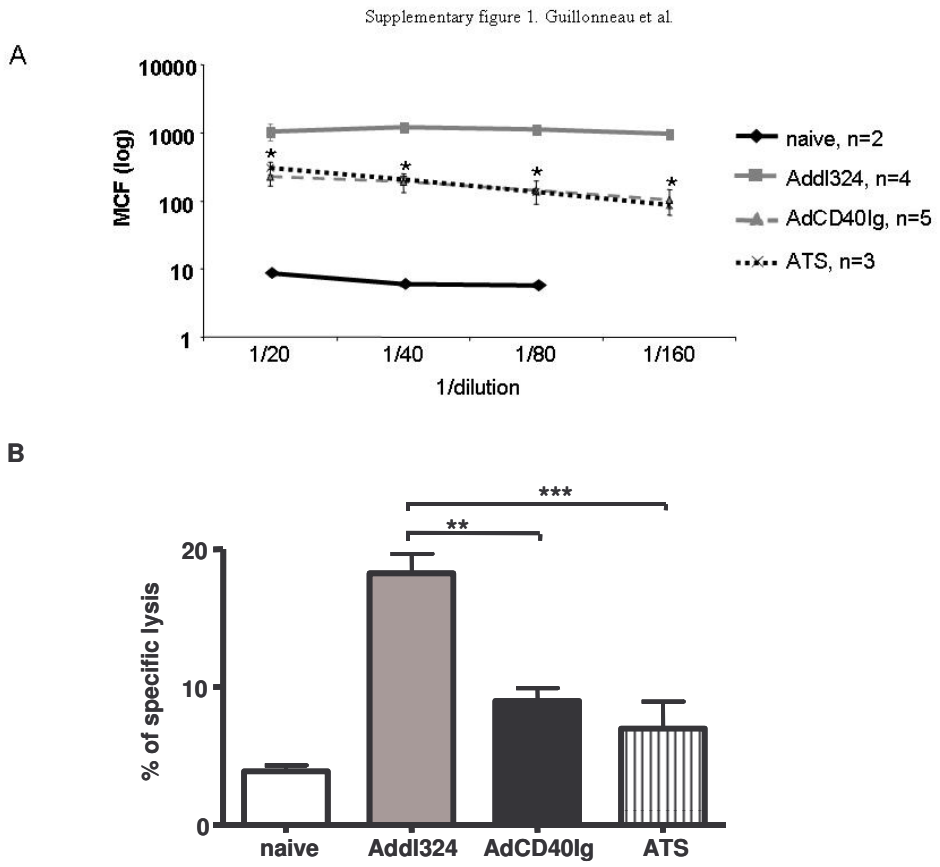
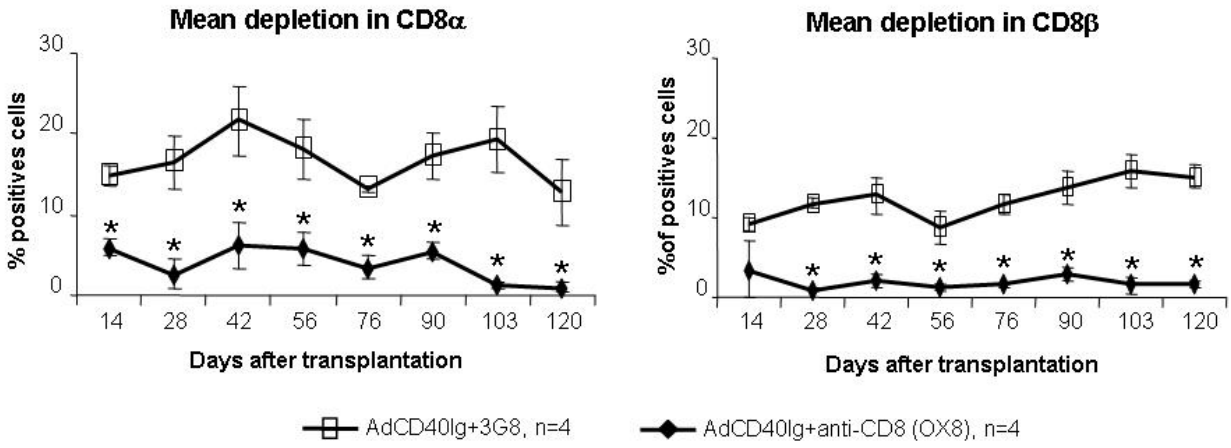


Supplementary figure 1. Humoral and cellular anti-donor immune responses are inhibited in CD40Ig-treated recipients. A) Alloantibody production was inhibited in recipients transferred with splenocytes from CD40Ig-treated animals. Animals were treated the day of transplantation (day 0) with non-coding adenoviruses (Addl324, n=4), encoding CD40Ig (AdCD40Ig, n=5) or adoptively transferred with splenocytes from recipients treated with CD40Ig with long-surviving grafts (ATS, n=3). Naive animals were non-grafted animals Titers of IgG alloantibodies were analyzed in the sera of animals 120 days after transplantation by cytofluorimetry on donor concavalin A-activated cells. The results are expressed as mean channel fluorescence with a logarithmic scale ± SD. * $p< 0.05$ vs. Addl324. AdCD40Ig and adoptively transferred recipients showed equally reduced levels of alloantibodies vs. Addl324-treated recipients. **B)** Anti-donor cytotoxic responses were

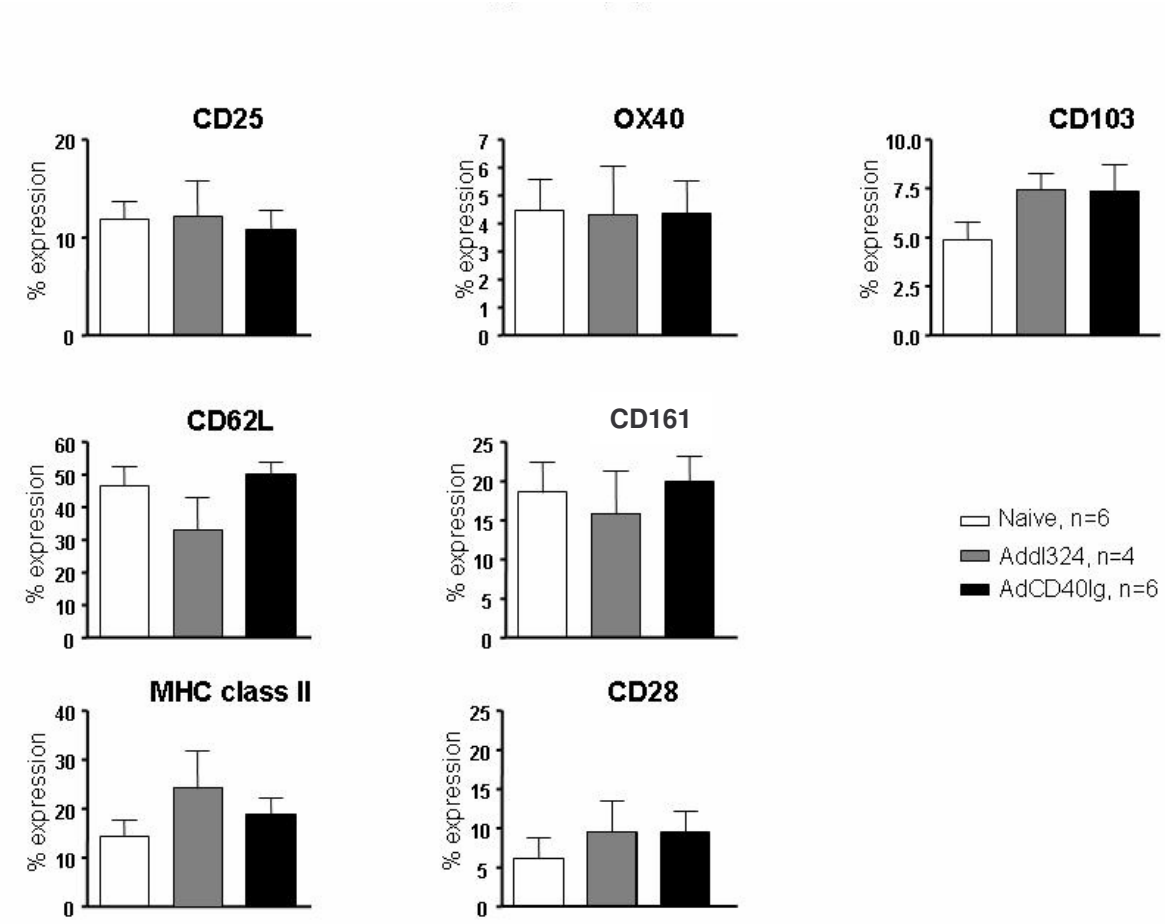
inhibited in CD40Ig-treated and ATS recipients. CTL activity of splenocytes from recipients with long-surviving grafts treated with AdCD40Ig (n=6), adoptively transferred (n=6) or with grafts rejected >100 after treatment with Addl324 (n=9) was assessed directly ex-vivo against ^{51}Cr-labeled donor LEW.1W ConA blasts. Results are expressed as mean ± SD percentage of specific lysis of all experiments at an effector to target cell ratio of 100:1. **, $p<0.01$ vs. Addl324. Anti-syngeneic and anti-third party BN CTL responses were <5%.

Supplementary figure 2. Guillonneau et al.



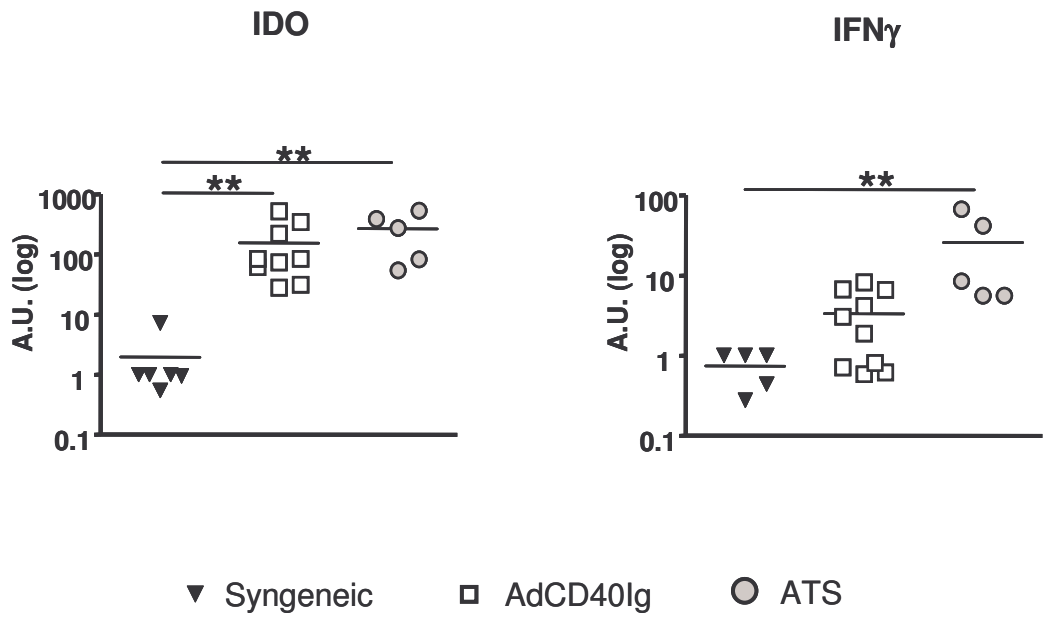
Supplementary figure 2. Confirmation of CD8 depletion following anti-CD8α-depleting mAb treatment. Animals were treated the day of transplantation (day 0) with adenoviruses encoding CD40Ig (AdCD40Ig) and treated (3 mg/kg/twice a week, i.p.) with either an anti-CD8α depleting mAb (OX8, IgG1, n=4) or with an irrelevant isotype-matched mAb (3G8, IgG1, n=4). Analysis of CD8 depletion was confirmed every two weeks in peripheral blood using OX8 and an anti-CD8β mAb (clone 3.4.1). Results are expressed as percentage of positive cells ± SD. * $p< 0.05$ vs. control mAb-treated recipients.

Supplementary figure 3. Guillonneau et al.



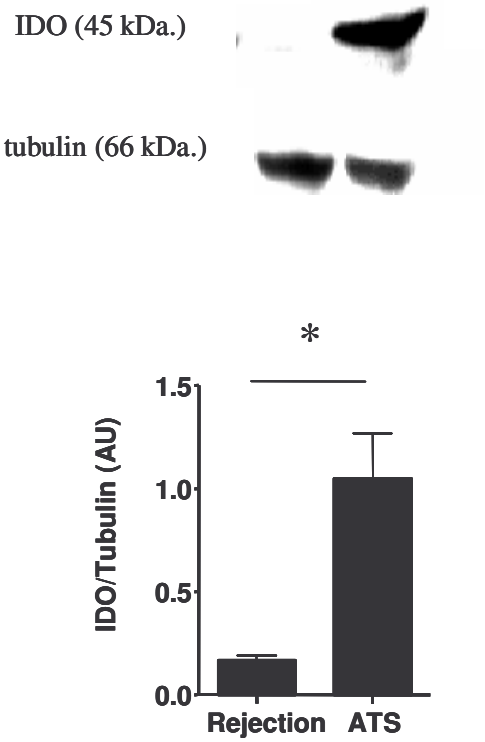
Supplementary figure 3. Flow cytometry analysis of $CD8^+CD45RC^{low}$ T cells from different groups of animals. Animals were treated the day of transplantation (day 0) with non-coding adenoviruses (Addl324, n=4) or encoding CD40Ig (AdCD40Ig, n=6). Naïve animals were non-grafted animals. Recipients were sacrificed at day 120 after transplantation and flow cytometry analysis was performed in spleen $CD8^+CD45RC^{low}$ T cells with the indicated cell markers. Results are expressed as percentage of positive cells ± SD. $CD8^+CD45RC^{low}$ T cells were mostly $CD25^-$, $OX40^-$, $CD103^-$, $CD28^-$, NKR-$P1^-$, MHC class II $antigen^-$, and $CD62L^+$.

Supplentary figure 4. Guillonneau et al.



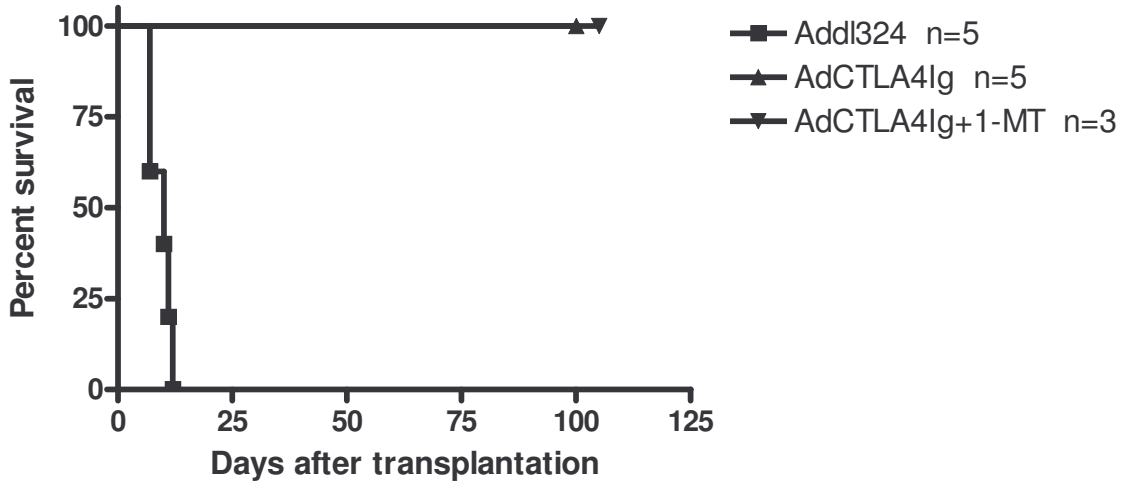
Supplementary figure 4. IDO and IFNγ mRNA accumulation is increased in long-term surviving allografts of adoptively transferred recipients. Heart grafts from syngeneic donors or from allogeneic donors were harvested 120 days after transplantation. Recipients from allogeneic donors were treated with CD40Ig or adoptively transferred (ATS). Heart total RNA was extracted and real-time quantitative RT-PCR was used to analyze transcript amounts. Symbols indicate values for individual animals in arbitrary units (AU) in logarithmic scale. **, $p<0.01$ vs. syngeneic.

Supplementary figure 5. Guillonneau et al.



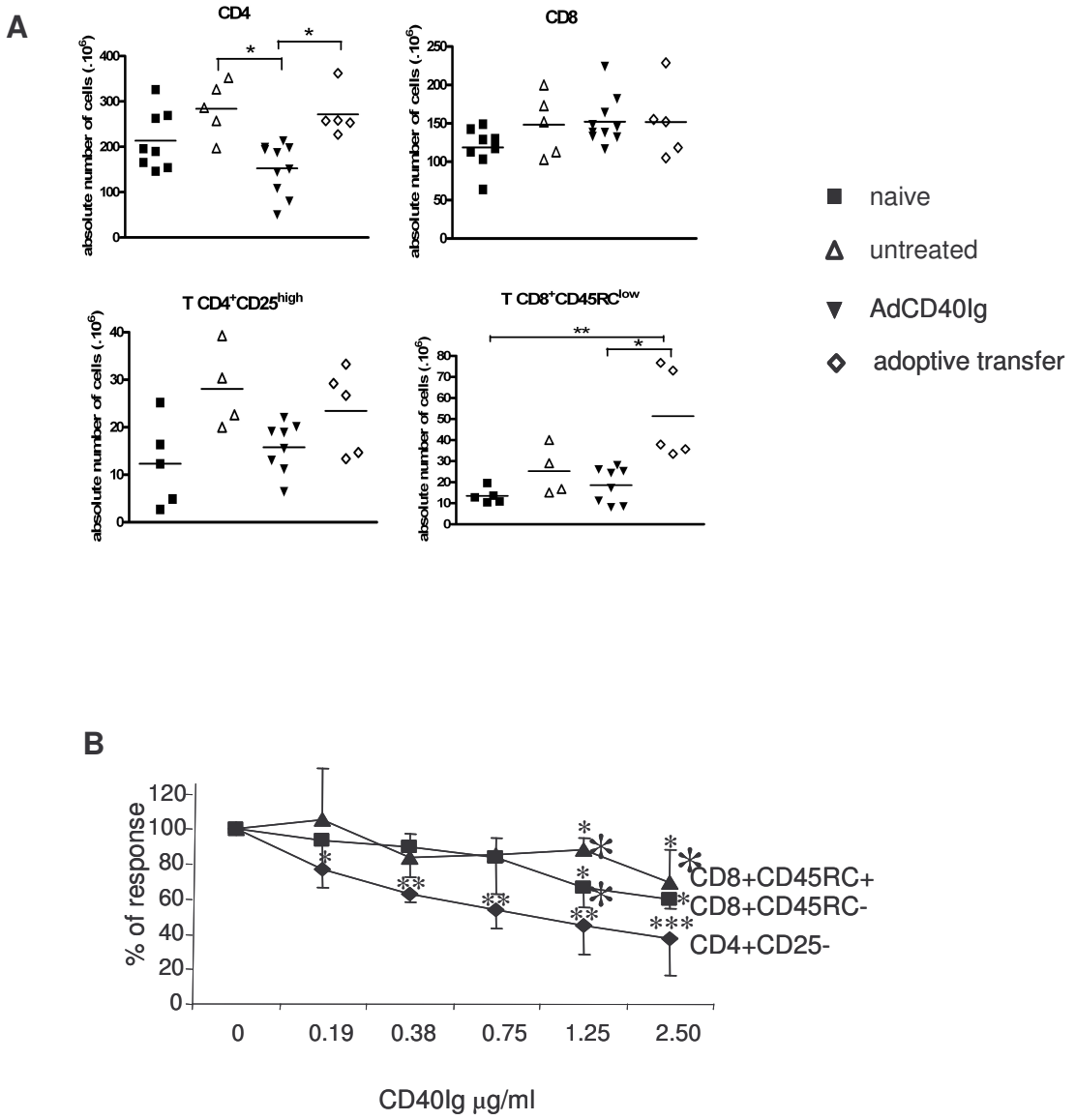
Supplementary figure 5. Increased IDO expression in spleen from adoptively transferred recipients. A) Western blot analysis of IDO protein in spleen extracts from rats that rejected their grafts or recipients with long-surviving grafts after adoptive transfer with splenocytes from recipients treated with CD40Ig (ATS). **B)** Densitometric quantification of western blot bands (IDO/tubulin ratio) ± SD. Rejection n=3; ATS n=4. *, $p<0.05$.

Supplementary Figure 6. Guillonneau et al.



Supplementary figure 6. IDO inhibition by 1-MT does not abrogate long-term allograft survival induced by CTLA4Ig. LEW.1A rats received LEW.1W hearts at day 0 and treated with either control adenovirus n=5, AdCTLA4Ig n=5, or AdCTLA4Ig + 0.2 g/kg twice/day 1-MT by oral gavage, n=3. Graft survival was monitored twice weekly through abdominal palpation. Rejection was defined when beating of the transplanted heart could no longer be detected. This results indicate that abrogation of long-term allograft survival by 1-MT treatment in of CD40Ig-treated animals is specific since long-term allograft survival induced by CTLA4Ig is not affected.

Supplementary figure 7. Guillonneau et al.



Supplementary figure 7. Effect of CD40Ig in vivo and in vitro on splenocyte subpopulations. A) In vivo effects of CD40Ig were analyzed in naïve, rejected untreated, control Addl324 or CD40Ig-treated recipients sacrificed 120 days after transplantation. Total splenocytes were counted, different cell populations were identified with mAbs and analyzed by flow cytometry. Each symbol represent the results from one animal expressed as absolute numbers per spleen and the horizontal bar the mean. **B)** CD8 and CD4 T cells from LEW.1A

animals were purified by nylon wool adherence followed by negative selection using mAbs and magnetic beads as described in the Materials and Methods section. Cell sorting was then used to purify CD4+CD25-, CD8+CD45RClow or CD8+CD45RChigh T cells. APCs were purified from LEW.1W animals by Nycodenz density gradient and irradiated (3 Gy). T cells ($5x10^4$) and APCs ($2.5x10^4$) were cultured for 3 days in the presence of the indicated concentrations of CD40Ig or control IgG1 (2.5 μg/ml) and ^{3}H-thymidine was added during the last 18 h of culture. Results are expressed as the mean ± SD of the percentage of the maximal response of 3 independent experiments. The maximal response ± SD in cpm (n=3) was for CD8+CD45RClow cells 3750 ± 399, for CD8+CD45RChigh cells 7078 ± 1071 and for CD4+CD25- cells 60316 ± 11209. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus no CD40Ig. Cultures with control IgG1 at 2.5 μg/ml showed comparable proliferation compared to those of control untreated cells.

Supplementary Table 1. Primers used for quantitative RT-PCR.

Gene	Forward primer	Reverse primer
IL-2	5'-CCTTGTCAACAGCGCACCC-3'	5'-GCTTTGACAGATGGCTATCC-3';
IL-6	5'-CAAAGCCAGAGTCATTCAGAGC-3'	5'-GGTCCTTAGCCACTCCTTCTGT-3'
IDO	5'-GCTGCCTCCCATTCTGTCTT-3'	5'-TGCGATTTCCACCATTAGAGAG-3'
CTLA-4	5'-GGCAGACAAATGACCAAGTGAC-3'	5'-TCTGAATCTGGGCATGGTTCT-3'
perforin:	5'-AGCCTCCACTCCACCCTGACT-3'	5'-GTTGTTTCTTCTTCTCCTCGC-3'
Cβ	5'-GTGAATGGGAAGGAGATCCG-3'	5'-CACTGATGTTCTGTGTGACAGGTT-3'
Foxp3	5'-CCCAGGAAAGACAGCAACCTT-3'	5'-CTGCTTGGAGTGCTTGAGAA-3'
PIR-B	5'-AAGCAGGGATAGAGACCAGCA-3'	5'-GCCTGGAGGGTTTTACTTGG-3'
CD28	5'-GCTGCTGTGGTAGATAACAATGAG-3'	5'-GAAATTCCCATCACAGTTGAACC-3'
IL-13	5'-TATGGAGCGTGGACCTGACA-3'	5'-GCGGAAAAGTTGCTTGGAGTA-3'