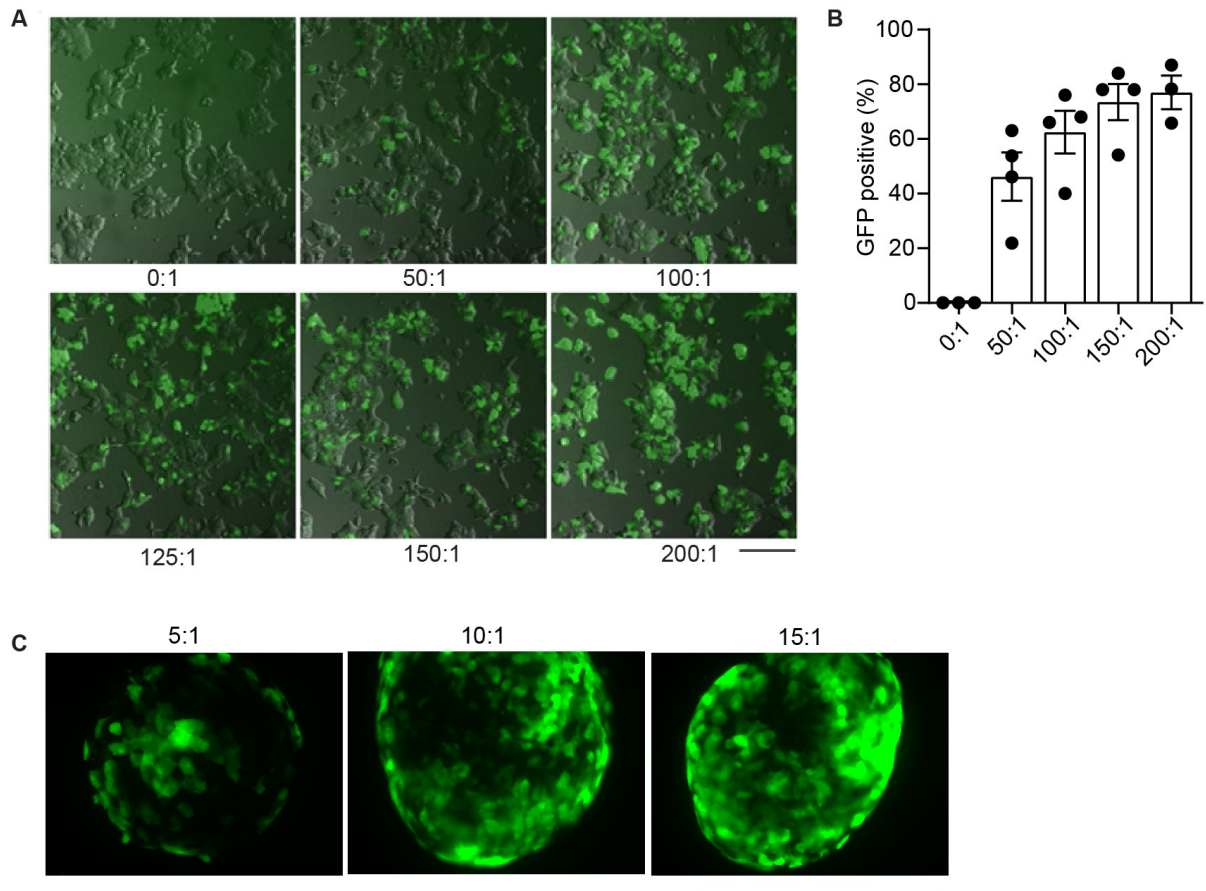
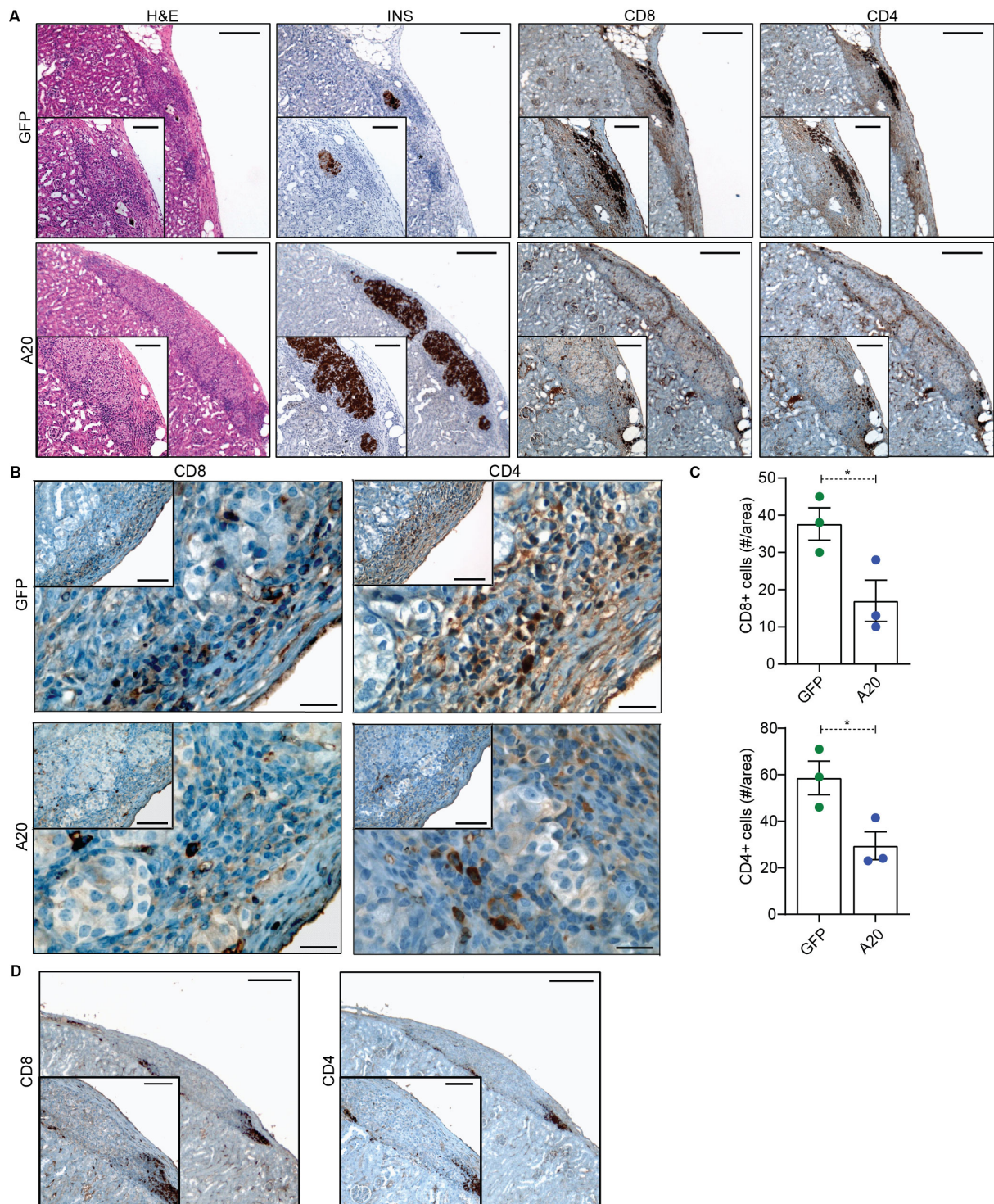


Supplementary data

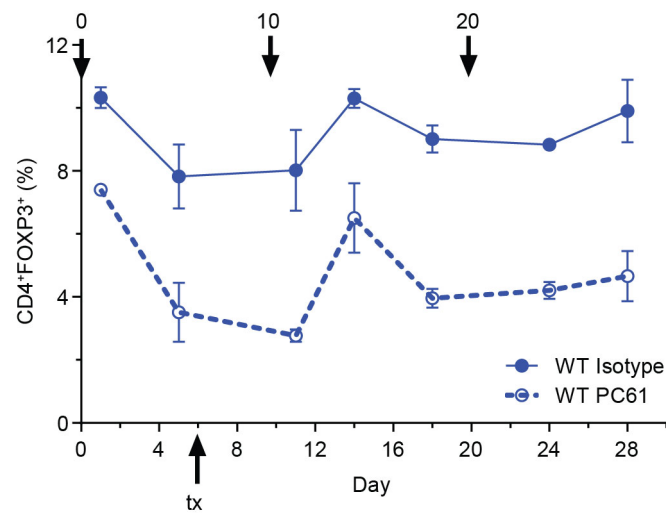


Supplemental figure 1. Recombinant adenovirus transduction of MIN6 cells and mouse islets. (A) Representative fluorescent microscopic image of a MIN6 cell line transduced with rAd.GFP at a range of multiplicity of infection as indicated, or left non-infected (NI, 0:1) and left to culture for 48 h (scale bar = 40 μ m). (B) Percent of GFP positive MIN6 cells quantified using flow cytometry. Each point represents a well of cells, cumulated from two independent experiments. (C) Fluorescent microscopic image of isolated primary mouse islets transduced with rAd.GFP at a multiplicity of infection of 5:1, 10:1 or 15:1 and cultured for 48 hours (scale bar = 100 μ m). Representative of three independent isolations.

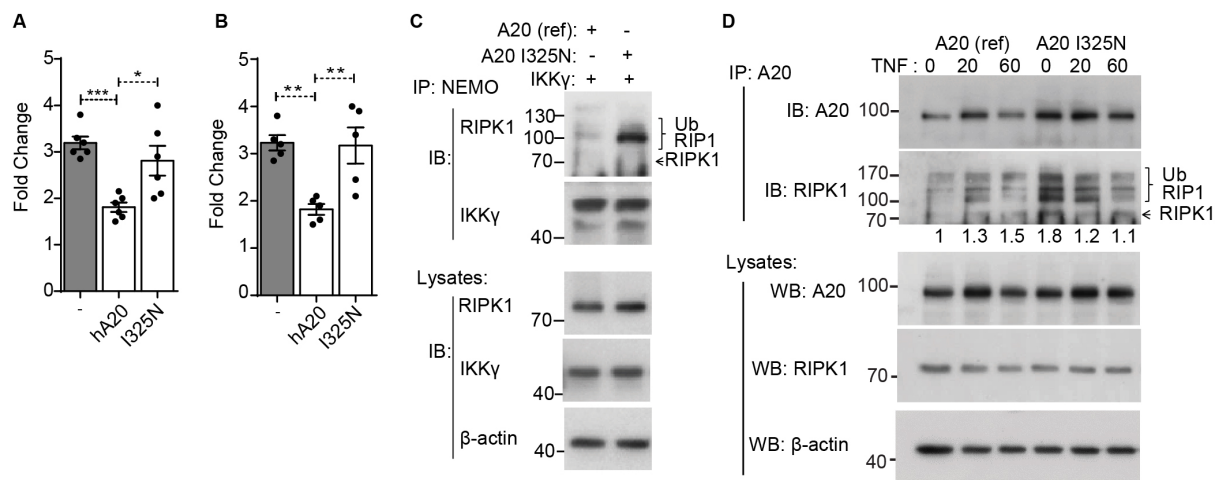


Supplemental figure 2. Histological analysis of CD4 and CD8 T cells in A20 and GFP expressing grafts. (A) Hematoxylin & Eosin (H&E), insulin (INS), CD8 or CD4 staining of islet allografts transduced with adenovirus encoding GFP or A20 prior to transplantation and harvested at post-operative day (POD) 18, prior to rejection of control grafts (Scale bar = 25 μ m and panel insert = 100 μ m). Data is representative of 3 GFP or A20 expressing grafts. **(B)** CD8 or CD4 staining of islet allografts transduced with adenovirus encoding GFP or A20 prior to transplantation and harvested at post-operative day (POD) 10. Scale bar = 25 μ m and insert panel = 100 μ m. Representative of 3 GFP or A20 expressing grafts. The average number of CD8 or CD4 positively stained cells per 40 \times field of view per islet graft is shown in **(C)**. **(D)** CD8 or CD4

staining of long-term surviving A20 transduced islet allografts harvested at POD 100. Scale bar = 200 μm and insert panel = 100 μm , representative of 6 long-term surviving islet grafts.



Supplemental figure 3. PC61 mAb treatment of wild-type mice. C57/BL6 recipients given repeated doses of α CD25, clone PC61 mAb ($n = 3$), or an isotype-control ($n = 3$) and percentage of FOXP3⁺CD4⁺ T cells assessed in the blood by flow cytometry.



Supplemental figure 4. Human A20 I325N exhibits impaired NF- κ B and AP-1 suppression. (A, B) β TC3 cells co-transfected with an NF- κ B.luciferase reporter (A), or an AP-1 luciferase reporter (B) and a CMV. β gal expression construct with or without PCDNA3.1 encoding human (h) reference A20, or A20 with an I325N coding variant. Cells were stimulated with 200 U/ml TNF for 8 h or left untreated. Data represents fold change of stimulated versus non-stimulated, and representative from three independent experiments. (C) Immunoblot (IB) of NEMO (IKK γ) immunoprecipitated (IP) lysates from β TC3 cells transfected with reference A20 or A20 I325N and IKK γ with corresponding whole-cell lysates shown. Data represents two biological replicates. (D) Immunoblot (IB) of A20 immunoprecipitated (IP) lysates and whole-cell lysates from β TC3 cells transfected with reference A20 or A20 I325N. Following an overnight incubation cells were stimulated with TNF for the indicated times. Membranes were probed for RIPK1, A20 or β -actin (loading control). Data represents two independent experiments. Statistical significance determined by 1-way ANOVA with Tukey's multiple-comparisons post hoc test. Error bars represent s.e.m, * P <0.05; ** P <0.01; *** P <0.001.

Supplemental Table 1

Mouse primers used for qRT-PCR analysis.

| Gene | Primer sequence |
|-------------------------------|--|
| <i>Tnfrif3</i> | F-5'-CCTGTCACCAACGCTCCAAG-3' R-5'-ATTTCCAGTCCGGTGGCAAG-3' |
| <i>Ccl2</i> | F- 5'-GGTCCCTGTCATGCTTCTGG-3' R- 5'-CCTGCTGCTGGTGATCCTCT-3' |
| <i>Ccl22</i> | F-5'-AAGCCTGGCGTTGTTTTGAT-3' R-5'-TCCCTAGGACAGTTTATGGAGTAGCT-3' |
| <i>Cd3</i> | F-5'-GCCTCAGAAGCATGATAAGC-3' R-5'-CCCAGAGTGATACAGATGTC-3' |
| <i>Cd80</i> | F-5'-ATGGCTTGCAATTGTCAGTTGA-3' R-5'-ATCAGGAGGGTCTTCTGGGGGGGT-3' |
| <i>Cd86</i> | F-5'-TCCAGAACTTACGCAAGCACCCA-3' R-5'-CAGGTTCACTGAAGTTGGCGATCAC-3' |
| <i>Cph2</i> | F- 5'-TGGACCAAACACAAACGGTTC-3' R- 5'-ACATTGCGAGCAGATGGGGT-3' |
| <i>Ctla4</i> | F- 5'-GTAGCCCTGCTCACTCTTCTT-3' R- 5'-AGGTACAGTCCCGTGTCAAC-3' |
| <i>Cxcl1</i> | F- 5'-TGGCTGGGATTCACCTCAAG-3' R- 5'-TATGACTTCGGTTTGGGTGCAG-3' |
| <i>Cxcl2</i> | F- 5'-CACTCTCAAGGGCGGTCAA-3' R- 5'-TCAGTTAGCCTTGCCTTTGTTCA-3' |
| <i>Cxcl10</i> | F- 5'-GACGGGCCAGTGAGAATGAG-3' R- 5'-GTGTGTGCGTGGCTTCACTC-3' |
| <i>Foxp3</i> | F- 5'-CCCACCTACAGGCCCTTCTC-3' R- 5'-GGCATGGGCATCCACAGT-3' |
| <i>Icam1</i> | F- 5'-CCATGGGAATGTCACCAGGA-3' R- 5'-ATCACGAGGCCCAATGAC-3' |
| <i>Ifnγ</i> | F- 5'-AGCAACAGCAAGGCGAAAAA-3' R- 5'-AGCTCATTGAATGCTTGGCG-3' |
| <i>Il6</i> | F- 5'-TCCTTCCTACCCCAATTTCAA-3' R- 5'-TGGATGGTCTTGGTCCTTAGCC-3' |
| <i>Il10</i> | F- 5'-TGTGAAAATAAGAGCAAGGCAGTG-3' R- 5'-CATTTCATGGCCTTGTTAGACACC-3' |
| <i>Tgfβ</i> | F- 5'-AGTCGGCCTCCGCTGG-3' R- 5'-GCTGTCTGGAGTCCTCAGGT-3' |
| <i>Tnf</i> | F- 5'-ATGGCCCAGACCCTCACACT-3' R- 5'-TGGTGGTTTGCTACGACGTG-3' |

Supplemental Table 2

Human primers used for qRT-PCR analysis.

| Gene | Primer sequence |
|----------------|---|
| <i>CCL2</i> | F- 5'- AGGTGACTGGGGCATTGAT -3' R- 5'- GCCTCCAGCATGAAAGTCTC -3' |
| <i>ICAM1</i> | F- 5'- AGTTGCTCCTGCCTGGGAAC -3' R- 5'- TTTAGCTGTTGACTGCCCATCAG -3' |
| <i>RPL13a</i> | F- 5'- CAAGCGGATGAACACCAAC-3' R- 5'- TGTGGGGCAGCATACTC -3' |
| <i>TNFAIP3</i> | F- 5'- AACGAACGGTGACGGCAAT-3' R- 5'- GAAGTCCACTTCGGGCCAT-3' |
| <i>TNF</i> | F- 5'- TCCCCTGGAAAGGACACCAT-3' R- 5'- GGGTTTGCTACAACATGGGCT-3' |