A

PTPN22.fuT2A.eGFP Sequence (CDS is capitalized)

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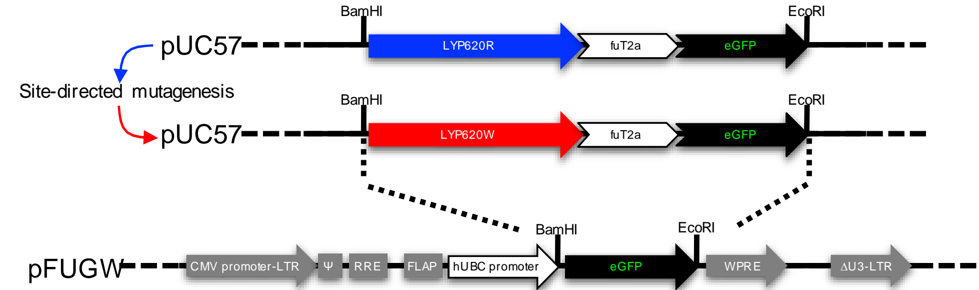
ggatcc: BamHI site | ATG: Start codon | GAACTC: Synonymous mutation | (C/T): C1858T

GAGTTC: Synonymous mutation | ATTCGG: No stop codon | TAA: Stop codon | gaattc: EcoRI site

B



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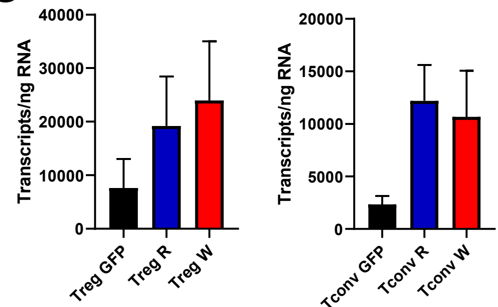

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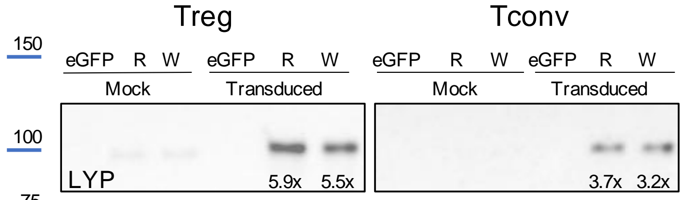

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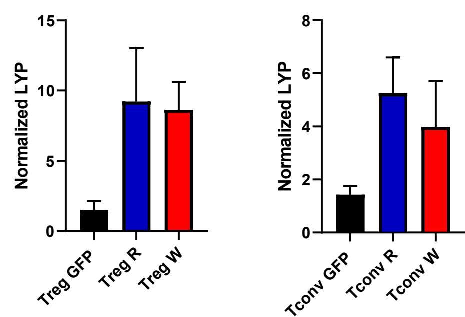
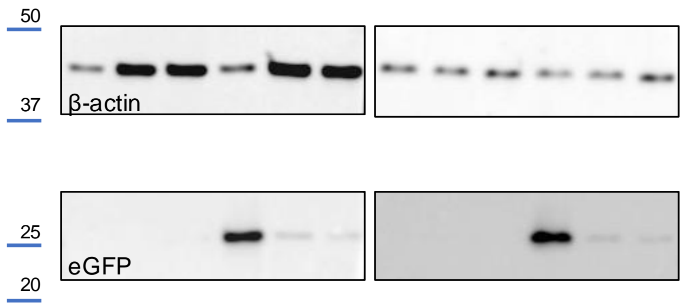
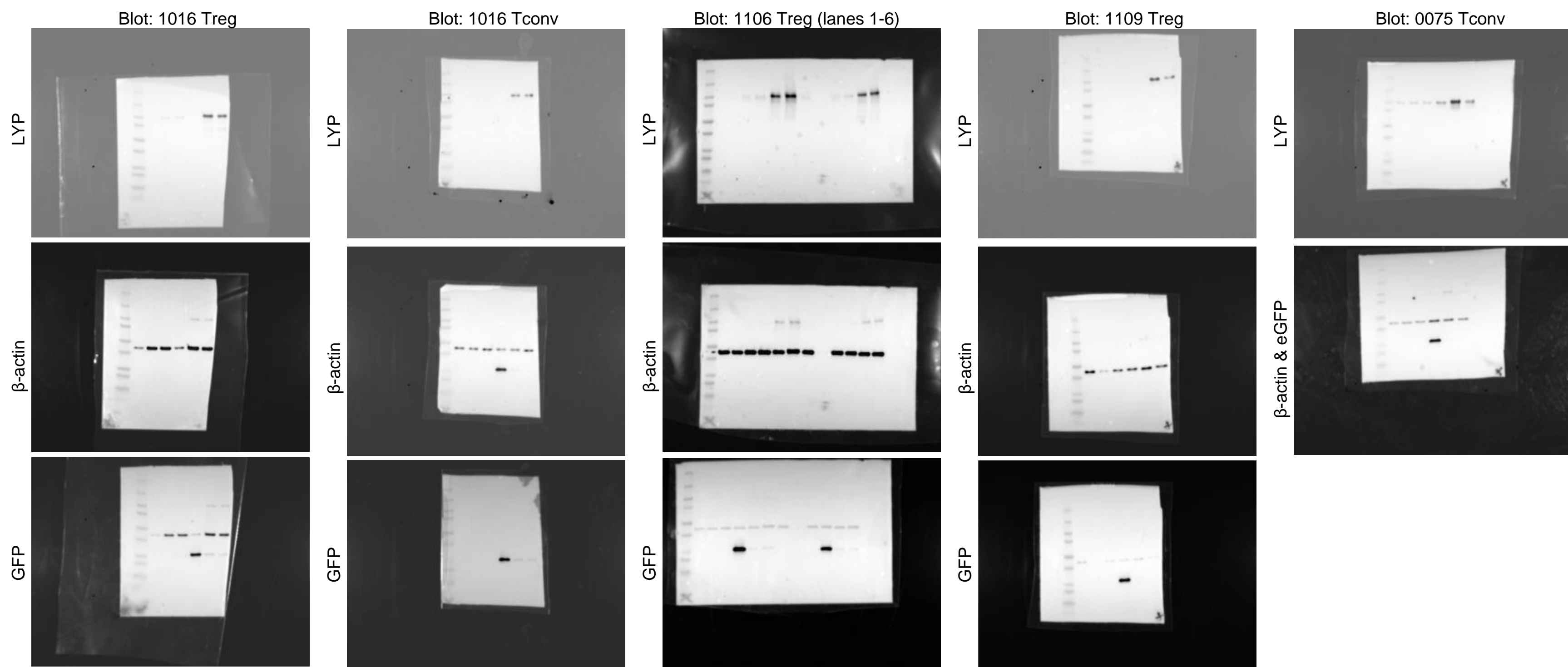
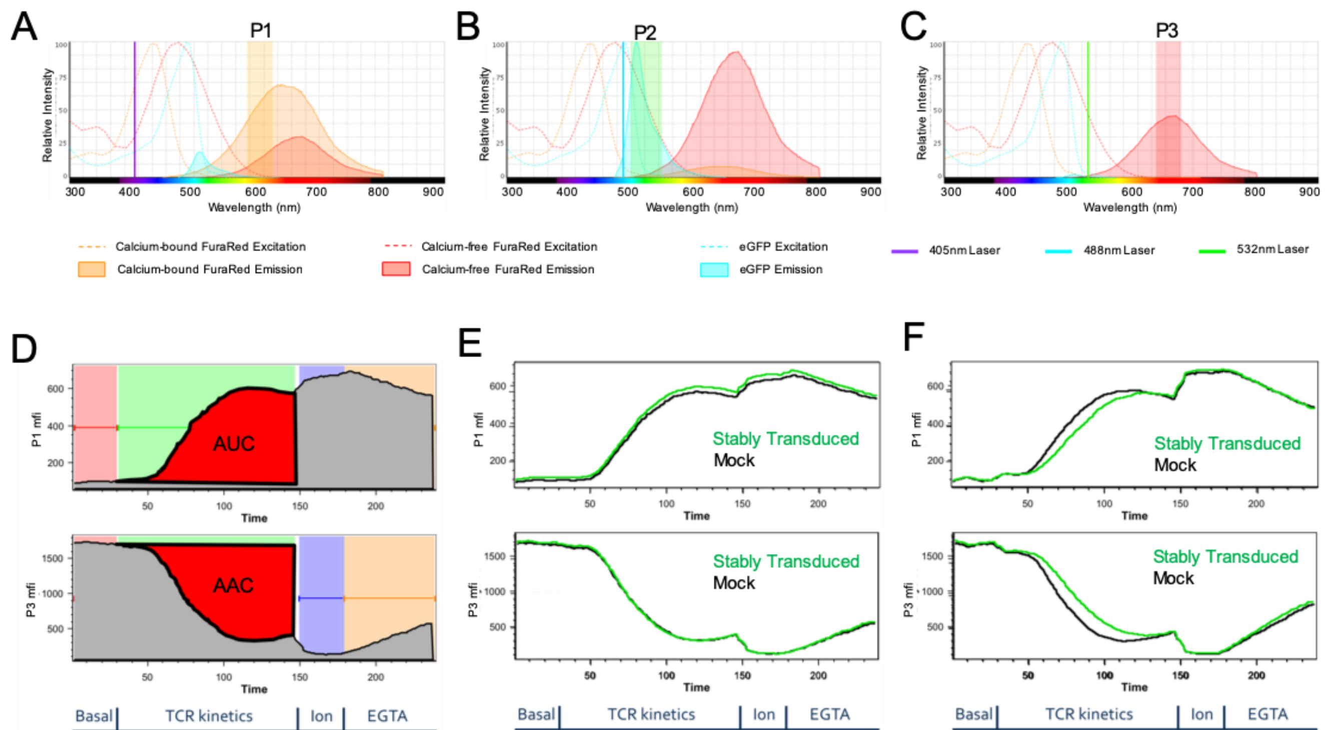




Supplemental Figure 1. Lentiviral constructs and Validation of *PTPN22* and LYP Overexpression. (*A*) The sequence of the LYP620R.fuT2A.eGFP construct is shown. It consists of the human *PTPN22* coding sequence (black, capitalized text), followed by a furin cleavage site (magenta text), a self-cleaving peptide sequence (blue text), and an eGFP sequence (green text). The locations of the sequence modifications described in **METHODS** are indicated. (*B*) The pUC57.LYP620R.fuT2A.eGFP cloning plasmid was used as the PCR template for site-directed mutagenesis to generate pUC57.LYP620W.fuT2A.eGFP harboring the C1858T alteration. These were subcloned into the pFUGW lentiviral vector. *C-E,* Primary human Tconv and Treg were transduced to express the LYP-620R autoimmunity protective variant, the LYP-620W risk variant, or eGFP as a control. After 21 days of *in vitro* expansion and rest, cells were sorted into stably-transduced ($eGFP^+$) and mock ($eGFP^-$) fractions and RNA and protein was prepared. (*C*) *PTPN22* transcripts were quantified for stably-transduced Treg (n=3) and Tconv (n=3) via real-time PCR. (*D*) Representative western blots of LYP (105kD), β-actin (45kD), and eGFP (27kD) for matched mock and transduced Treg and Tconv from the same subject. LYP was always probed first, followed by β-actin and eGFP. Images are cropped from the same blots following serial probing. The cropped areas exclude retained bands related to incomplete stripping on some blots. Raw images are shown in **Supplemental Figure 2**. (*E*) Normalized LYP expression for Treg (n=3) and Tconv (n=2) are the result of background signal subtraction and normalization to β-actin loading control and mock transduced LYP. Data are represented as mean with SEM. One-way ANOVA with Bonferroni's posttest was performed. Although significant differences were not achieved, *PTPN22* transcripts were $2.84 \times 10^4 \pm 1.0 \times 10^4$ for LYP620R Treg, $2.40 \times 10^4 \pm 0.7 \times 10^4$ for LYP620W Treg, $1.22 \times 10^4 \pm 0.5 \times 10^4$ for LYP620R Tconv, and $1.07 \times 10^4 \pm 0.4 \times 10^4$ for LYP620W Tconv, with a fold change of 3.72, 3.14, 5.20, and 4.55, respectively, relative to eGFP-transduced cells. Similarly, LYP protein was 9.21±3.8 for LYP620R Treg, 8.63±2.0 for LYP-620W Treg, 6.60 for LYP620R Tconv, and 5.71 for LYP-620W Tconv, with fold change of 6.15, 5.76, 3.77, and 3.26, respectively, relative to eGFP-transduced cells.



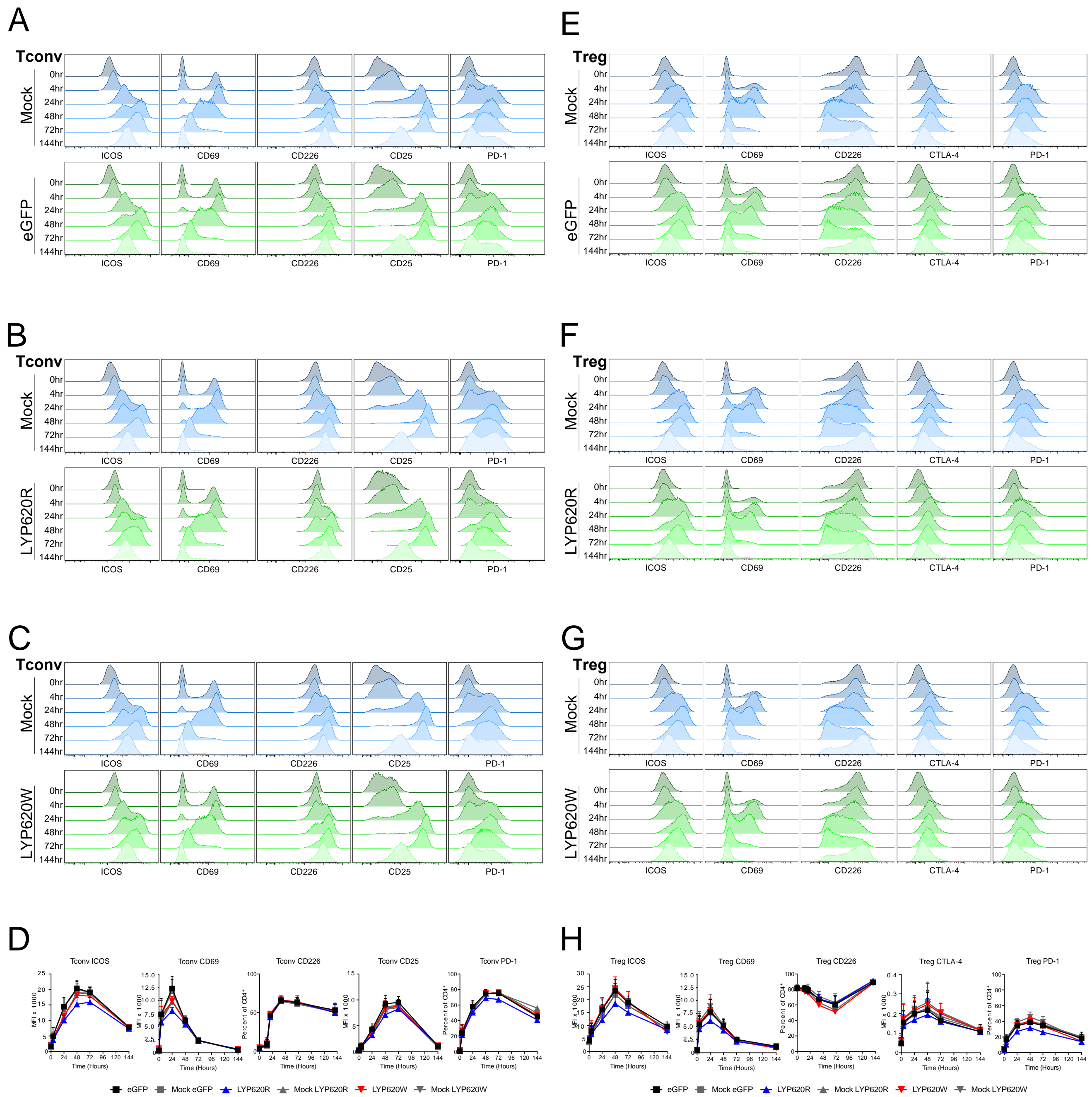
Supplemental Figure 2. Western Blot Raw Images. All immunoblots used to generate **Supplemental Figure 1D and E** are shown. Each blotting membrane is shown in columns and sequential probings are shown in rows. LYP (105kD) was always probed for first, followed by β-actin (45kD) then eGFP (27kD) on blots '1016 Treg', '1106 Treg', and '1109 Treg', or by eGFP then β-actin on blot '1016 Tconv', or by co-probing β-actin and eGFP on blot '0075 Tconv'. In most cases, incomplete stripping between probes resulted in retention of previous bands, which are spatially resolved. "1016 Treg' and '1016 Tconv' were used to generate **Supplemental Figure 1D.**



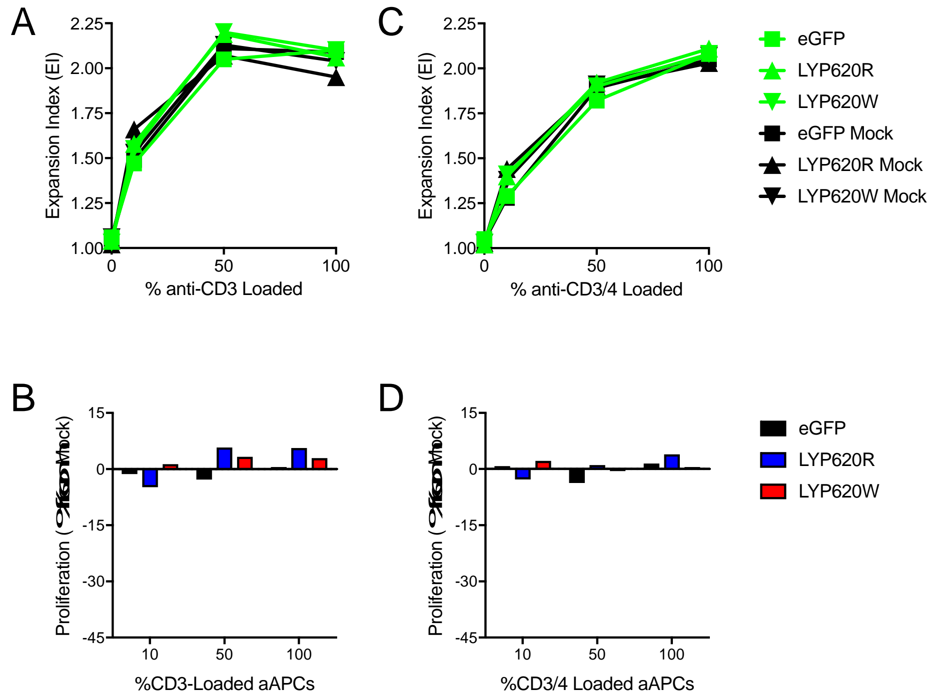
G

$$\text{Ca}^{2+}\ \text{flux}_{\%\ \text{change from mock}} = \left(\left(\frac{AUC_{P1}^{Mock} - AUC_{P1}^{ST}}{AUC_{P1}^{Mock}} \right) + \left(\frac{AAC_{P3}^{Mock} - AAC_{P3}^{ST}}{AAC_{P3}^{Mock}} \right) \right) \times 100$$

Supplemental Figure 3. Measuring calcium flux in eGFP-expressing T cells. FuraRed was chosen as a calcium responsive dye due to its compatibility with enhanced green fluorescent protein (eGFP). (*A-C*) It exhibits a violet-shift in excitation (orange and red dotted lines) when Ca^{2+} is bound, and its peak emission is in the 600-700nm range (orange and red filled histograms) as shown in the Fluorescence SpectraViewer plots (ThermoFisher). (*A*) FuraRed emission intensifies in the P1 detector (605/40) with increasing Ca^{2+} concentration when excited with a 405nm violet laser. (*B*) Its emission does not significantly overlap with the eGFP P2 detector (525/50) when excited by a 488nm laser. (*C*) In order to avoid spectral overlap into the low Ca^{2+} detector by eGFP the 532nm laser was used to excite calcium-free FuraRed for detection in P3 (665/40). (*D*) FuraRed-labeled cells were acquired in the high [Ca^{2+}] P1 detector (top), the low [Ca^{2+}] P3 detector (bottom), the eGFP P2 detector, as well as a detector for viability dye. Basal levels were recorded for 30 sec, followed by 120 sec of activation with anti-CD3 (OKT3) and anti-msIgG F(ab')$_2$ cross-linker (Jackson ImmunoResearch Laboratories), then 30 sec with an ionophore, and finally 60 sec with EGTA chelator. The calcium-bound area under the curve (AUC, red area on top graph) and the calcium-free area above the curve (AAC, red area on bottom graph) of live-gated lymphocytes were used to quantify the activation-induced calcium flux. (*E*) Representative plots demonstrate that Ca^{2+} flux kinetics of eGFP-transduced cells (green lines) closely mimic the kinetics of internal control mock transduced cells (black lines). (*F*) Representative plots demonstrate that the Ca^{2+} flux kinetics of LYP620R-transduced cells (green lines) are demonstrably blunted as compared to the kinetics of internal control mock transduced cells (black lines). (*G*) Formula used to calculate Ca^{2+} flux as the percent change from mock.



Supplemental Figure 4. Histogram overlays of activation kinetics for transduced and mock-transduced primary CD4 T cells. Surface marker expression at 0, 4, 24, 48, 72, and 144 hours following activation by antigen presenting cells (APCs) and anti-CD3 and anti-CD28. Representative surface expression of ICOS, CD69, CD226, CD25, and PD-1 on mock-transduced (blue histograms) and (*A*) eGFP-transduced, (*B*) LYP620R-transduced, or (*C*) LYP620W-transduced (green histograms) conventional T cells (Tconv). (*D*) Expression kinetics of ICOS, CD69, CD226, CD25, and PD-1 on Tconv for 144 hours post activation (n=5). Representative surface expression of ICOS, CD69, CD226, CTLA-4, and PD-1 on mock-transduced (blue histograms) and (*E*) eGFP-transduced, (*F*) LYP620R-transduced, or (*G*) LYP620W-transduced (green histograms) regulatory T cells (Tregs). (*H*) Expression kinetics of ICOS, CD69, CD226, CTLA-4, and PD-1 on Treg for 144 hours post activation (n=5).



Supplemental Figure 5. Responder T cell proliferation increases in response to increasing amounts of CD3 loading on artificial antigen presenting cells (aAPCs). To generate aAPCs, K-562 cells (human CML line) expressing murine Fc' receptor (CD64) and human CD86 were incubated with mixtures of anti-human CD3 (clone OKT3) and isotype antibody such that they were loaded with 0, 10, 50, or 100% anti-CD3 as indicated. They were then irradiated with 3000 Rad and co-cultured with responder conventional T cells (Tconv) at 1 aAPC per 4 responders. LYP-modulated (green) and mock transduced (black) CD4 Tconv were labeled with proliferation dye and co-cultured with the aAPCs for 3 days. Proliferation was then assessed by flow cytometry. (*A*) Expansion Index (EI) was used to quantify proliferation of transduced and untransduced T cells. (*B*) Proliferation was normalized to non-transduced internal culture control cells. Proliferation was not altered by LYP modulation across activation signal strengths. (*C*) A 2:1 mixture of anti-CD3 (OKT3) and CD4 (OKT4) was used as an activation cocktail, which was titrated with isotype antibodies as above. Proliferation was quantified by (EI). (*D*) Proliferation was normalized to non-transduced internal culture control cells. Proliferation was not altered by LYP modulation across activation signal strengths.