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Supplemental Information

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Supplementary Materials:

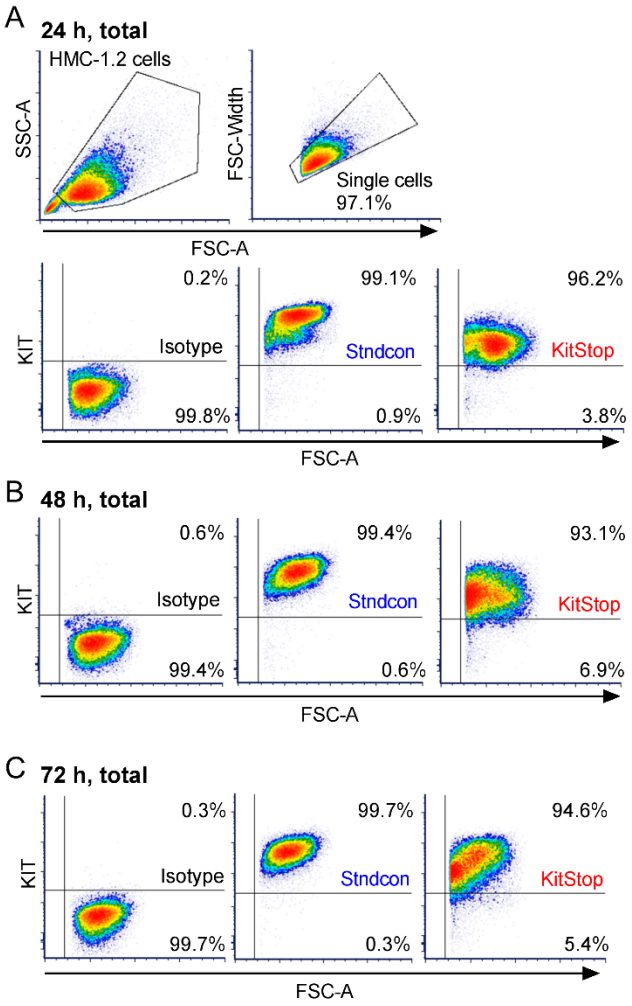

Fig. S1. Representative flow cytometry gating strategies and plots of HMC-1.2 cells labeled with anti-human CD117 antibodies. Representative density plots and gates of fixed and permeabilized HMC-1.2 cells transfected with 10 µM KitStop SSOs and labeled with APC-conjugated anti-human CD117 antibody after (**A**) 24 h, (**B**) 48 h and (**C**) 72 h. Stndcon = standard control antisense oligonucleotide.

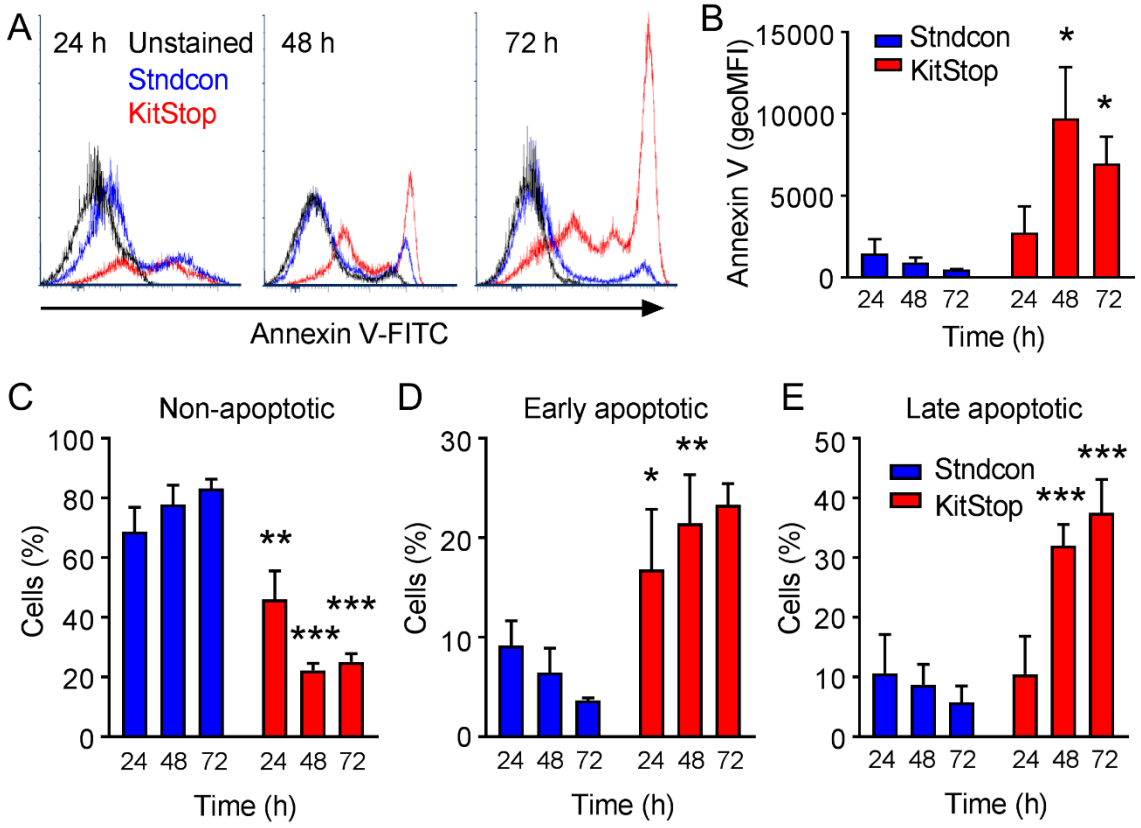

Fig. S2. Transfection of KitStop ESO increases HMC-1.2 cell apoptosis. Following transfection of HMC-1.2 cells with 10 µM standard control ASO (Stndcon) or KitStop ESO, HMC-1.2 cells were assessed by flow cytometry for apoptosis by staining with Annexin V-FITC. **(A)** Histograms showing the shift in Annexin V positive staining of KitStop-transfected cells in comparison to Stndcon and unstained cells. **(B)** Combined data from flow cytometry for Annexin V expressed as the geometric MFI. **(C)** Percentage of HMC-1.2 cells within non-apoptotic **(C)**, early apoptotic **(D)** and late apoptotic **(E)** gates at each time point, determined by dual staining for Annexin V and PI as shown in Figs S3-S5. Data are the mean ± SEM from 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001, ANOVA with Sidak's post-test.

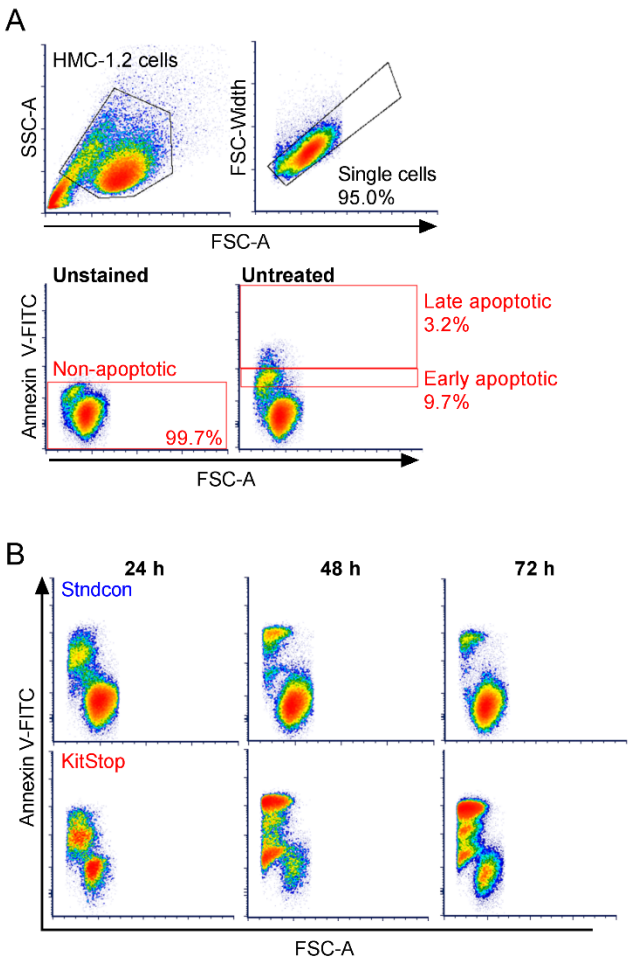

Fig. S3. Representative flow cytometry gating strategies and plots of HMC-1.2 cells stained with Annexin V-FITC. (**A**) Gating strategy and representative density plots of live, untreated HMC-1.2 cells, either unstained or stained with Annexin V-FITC. (**B**) Flow cytometry density plots of Annexin V staining intensity of HMC-1.2 cells at each time point after transfection with standard control antisense oligonucleotide (Stndcon) or KitStop SSOs. Apoptotic cells are smaller when measured by forward scatter (X axes) and annexin V positive (Y axes).

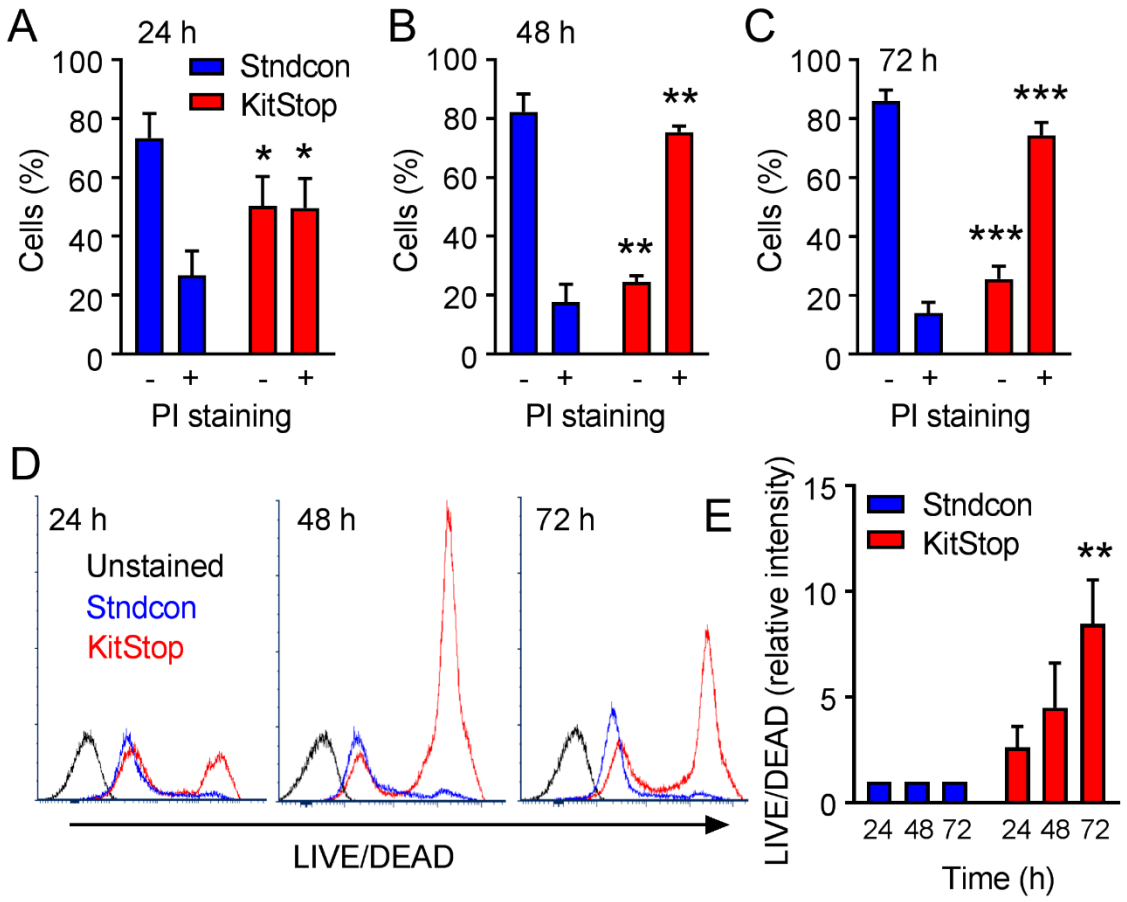

Fig. S4. Transfection of KitStop ESO decreases HMC-1.2 cell viability. Following transfection of HMC-1.2 cells with 10 µM standard control ASO (Stndcon) or KitStop ESO, HMC-1.2 cell viability was assessed by flow cytometry by staining with propidium iodide (PI). Percentage of PI negative and positive cells at 24 h (**A**), 48 h (**B**), and 72 h (**C**). (**D**) Flow cytometry histograms at 24 h (left panel), 48 h (middle panel) and 72 h (right panel) of HMC-1.2 cells stained with LIVE/DEAD Green Dead Cell stain. (**E**) Combined geometric MFI of LIVE/DEAD staining in HMC-1.2 cells at each time point. Data are the mean ± SEM from 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001, ANOVA with Sidak's post-test.

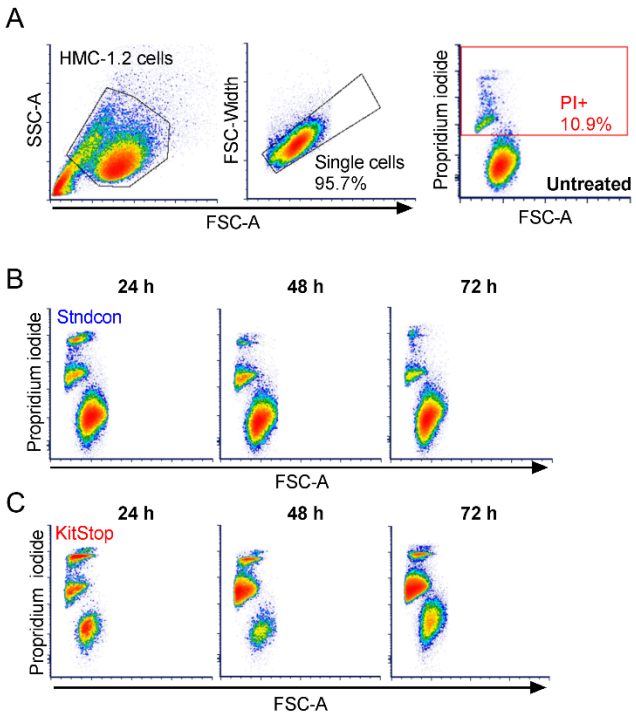

Fig. S5. Representative flow cytometry gating strategies and plots of HMC-1.2 cells stained with propridium iodide. (**A**) Representative density plots of live HMC-1.2 cells stained with propridium iodide (PI). Flow cytometry density plots of PI staining intensity of HMC-1.2 cells at each time point after transfection with (**B**) standard control antisense oligonucleotide (Stndcon) or (**C**) KitStop SSOs.

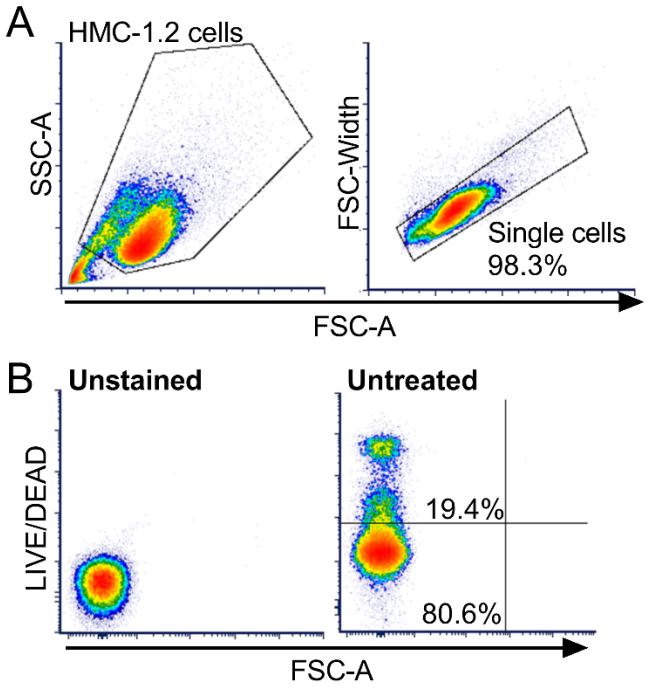

Fig. S6. Flow cytometry gating strategies for HMC-1.2 cells with LIVE/DEAD stain. (**A**) Representative density plots demonstrating A) gating of live HMC-1.2 cells and single cell populations and (**B**) gating of untreated cells following application of LIVE/DEAD Green Dead Cell stain.

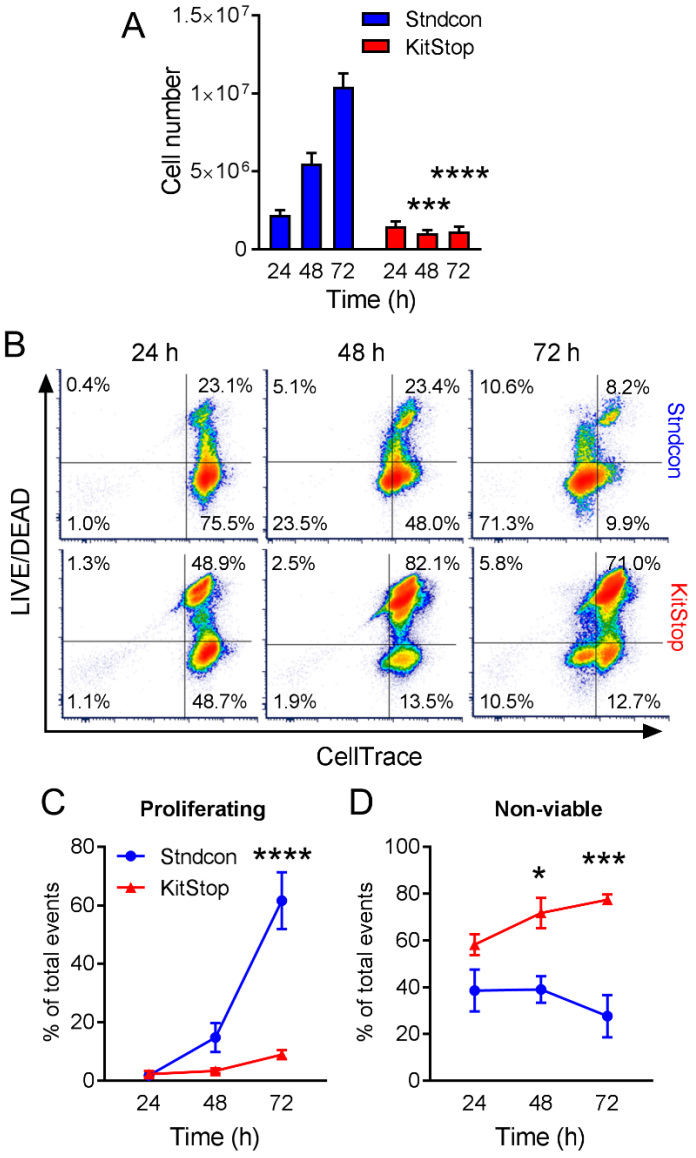

Fig. S7. Transfection of KitStop ESO decreases HMC-1.2 cell proliferation. Following transfection of HMC-1.2 cells with 10 µM standard control ASO (Stndcon) or KitStop ESO, HMC-1.2 cell proliferation was assessed by cell counts and flow cytometry. (**A**) Total number of viable HMC-1.2 cells cultured under normal conditions assessed by Trypan blue counts. (**B**) Representative density plots of HMC-1.2 cells loaded with CellTrace prior to transfection with Stndcon ASO (top panels) or KitStop ESO (bottom panels), and stained with LIVE/DEAD Green Dead Cell stain at 24 h (left panels) 48 h (middle panels) and 72 h (right panels). Cells transfected with Stndcon demonstrated a loss of CellTrace fluorescence intensity due to dye dilution between daughter cells, indicating cell proliferation. In contrast, KitStop-transfected cells retained CellTrace fluorescence, except for a small population at 72 h, and exhibited increased LIVE/DEAD staining intensity. (**C**) Percentage of total cells in bottom left quadrants (CellTracelow; LIVE/DEADnegative) corresponding to proliferating cells. (**D**) Percentage of total cells in top left and right quadrants (LIVE/DEAD+), corresponding to non-viable cells. Data are the mean ± SEM from 3 independent experiments. *p<0.05, ***p<0.001, ****p<0.0001, ANOVA with Sidak's post-test.

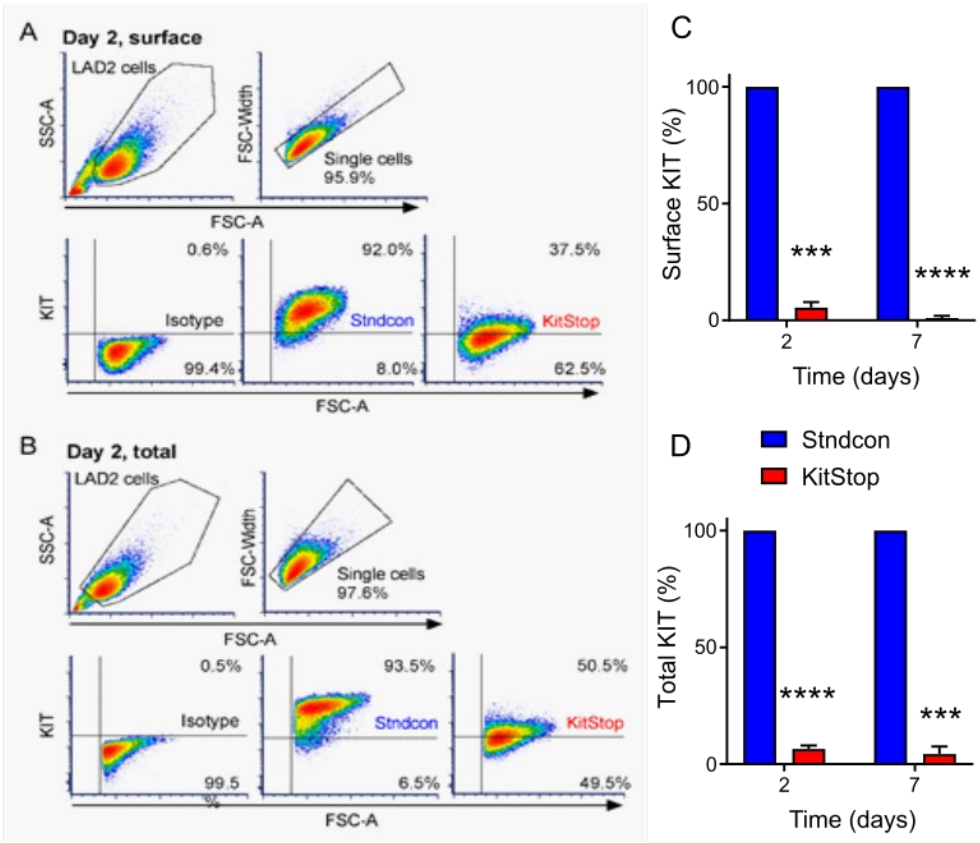

Fig. S8. Transfection of KitStop ESO markedly reduces surface and total wild-type KIT expression in LAD-2 cells. Following transfection of LAD2 cells with 10 µM KitStop SSOs, LAD2 cells were assessed by flow cytometry for KIT expression. Representative density plots and gates of (**A**) live and (**B**) fixed and permeabilized LAD2 cells labeled with APC-conjugated anti-human CD117 antibody. (**C**) Mean flow cytometry data for surface **(C)** and total **(D)** KIT expression calculated from the geometric MFI and expressed as a percentage of Stndcon at 2 and 7 days post-transfection. Stndcon = standard control antisense oligonucleotide. Data are the mean ± SEM from 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 ANOVA with Sidak's post-test.

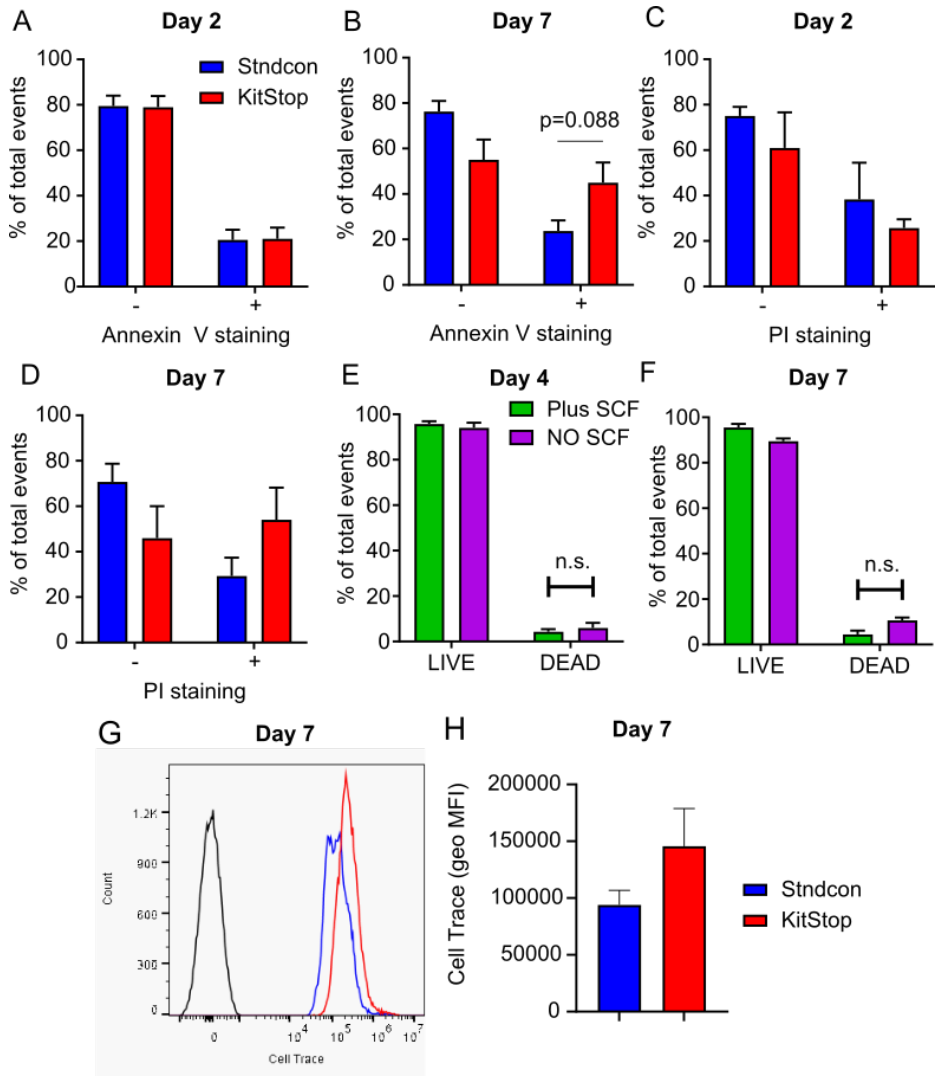

Fig. S9. Transfection of KitStop ESO results in modest apoptosis and cell death and inhibition of proliferation in the slowly dividing LAD-2 human MC line. Following transfection of LAD-2 cells with 10 µM standard control ASO (Stndcon) or KitStop ESO, LAD-2 cells were assessed by flow cytometry for apoptosis by staining with Annexin V-FITC. (**A**) Combined data from flow cytometry for Annexin V expressed as the percent of cells either positive or negative for surface Annexin V at either 2 days (**A**) or 7 days (**B**) after transfection. (**C**) LAD-2 cell viability was assessed by flow cytometry by staining with propidium iodide (PI). Percentage of PI negative and positive cells at 2 days (**C**) and 7 days (**D**) after transfection were analyzed. (**E**) The effects of SCF withdrawal on LAD2 cell viability were established using a commercial LIVE/DEAD stain at 4 days (**E**) and 7 days (**F**) after transfection. (**G**) Proliferation was assessed using CellTrace dilution proliferation assay. Histogram showing unstained cells (black line) compared to CellTrace loaded cells that were treated with either standard control AON (red line) or KitStop ESO (Blue line) for 7 days. Left shifts in populations represent proliferated cells and dilution of CellTrace dye. (**H**) Quantification of data from CellTrace experiments expressed as the geometric MFI of the total cell populations. A higher value represents less proliferation. Data are the mean ± SEM from 3 independent experiments.

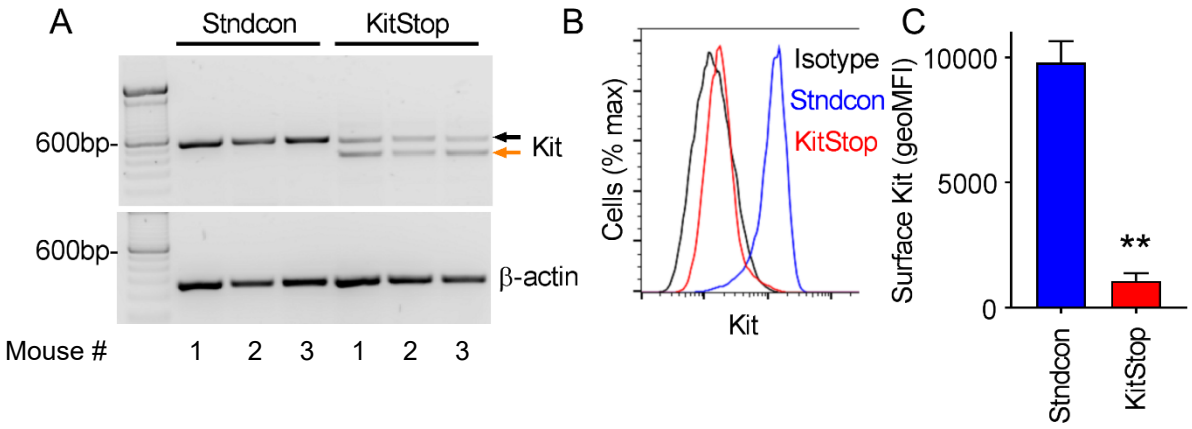

Fig. S10. KitStop ESO reduces Kit expression in mouse MCs. (A) RT-PCR demonstrating splice-switching of wild-type c-KIT in bone marrow-derived MCs (BMMCs) by mouse KitStop ESO in comparison to standard control ASO (Stndcon). Black arrow = full-length c-Kit, orange arrow = alternatively spliced c-Kit. (B) Flow cytometry histogram of surface wild-type Kit expression in BMMCs following transfection with KitStop ESO. Data are representative of 3 independent experiments on separate mice. (C) Mean flow cytometry data for surface KIT expression calculated from the geometric MFI and expressed as a percentage of Stndcon antisense oligonucleotide. Data are the mean ± SEM from 3 independent experiments on separate mice. **$p<0.01$, paired t-test.