Supplemental Material

Schlafen12 Reduces the Aggressiveness of Triple Negative Breast Cancer through Post-Transcriptional Regulation of ZEB1 That Drives Stem Cell Differentiation

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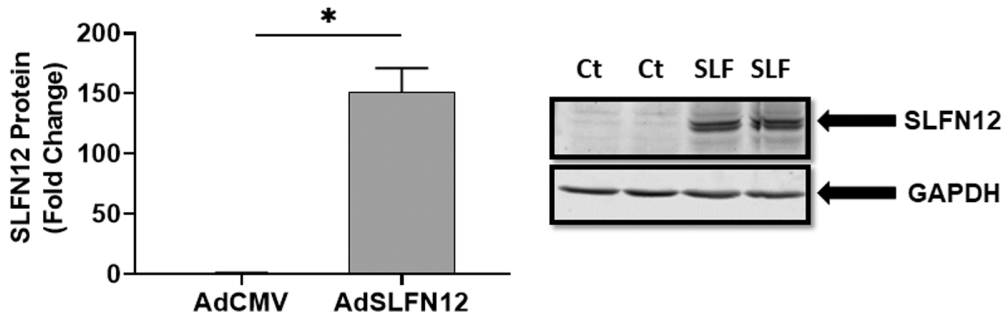
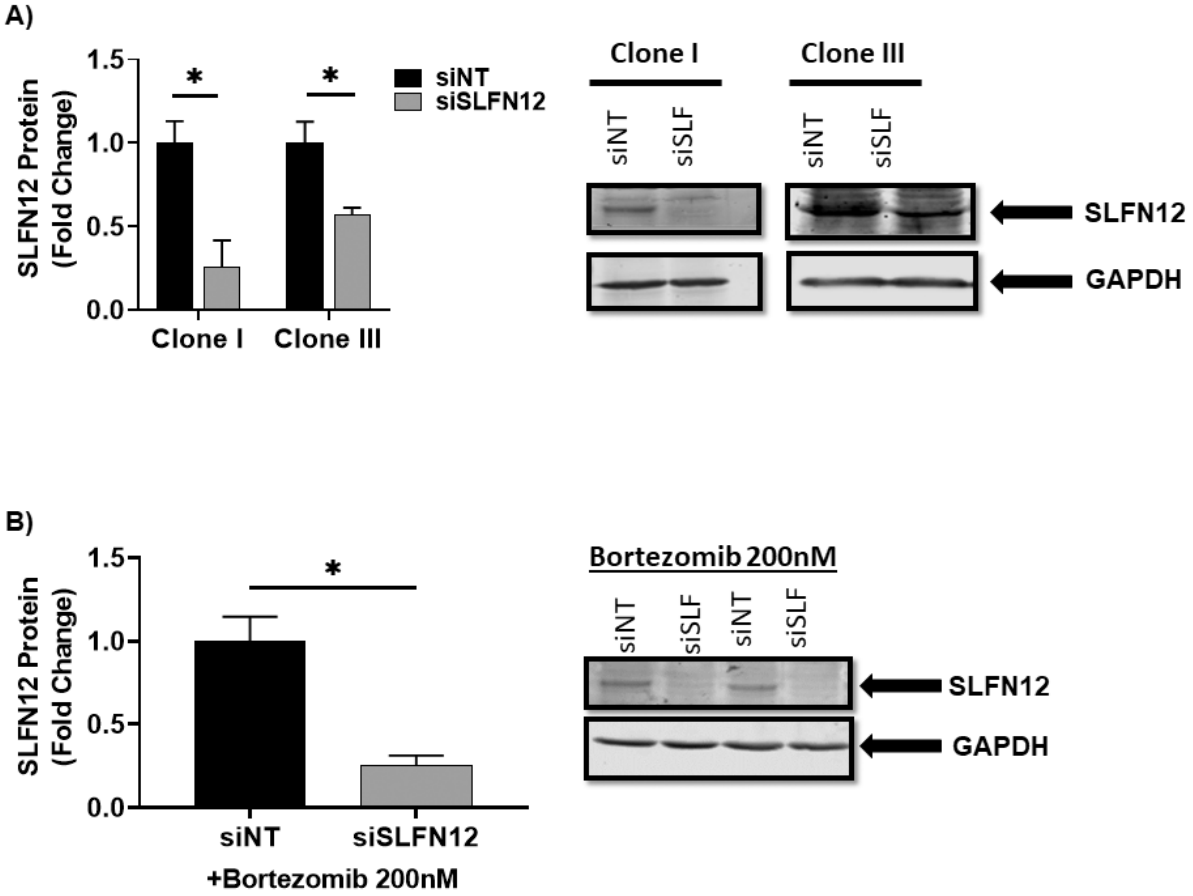
Supplemental Figures



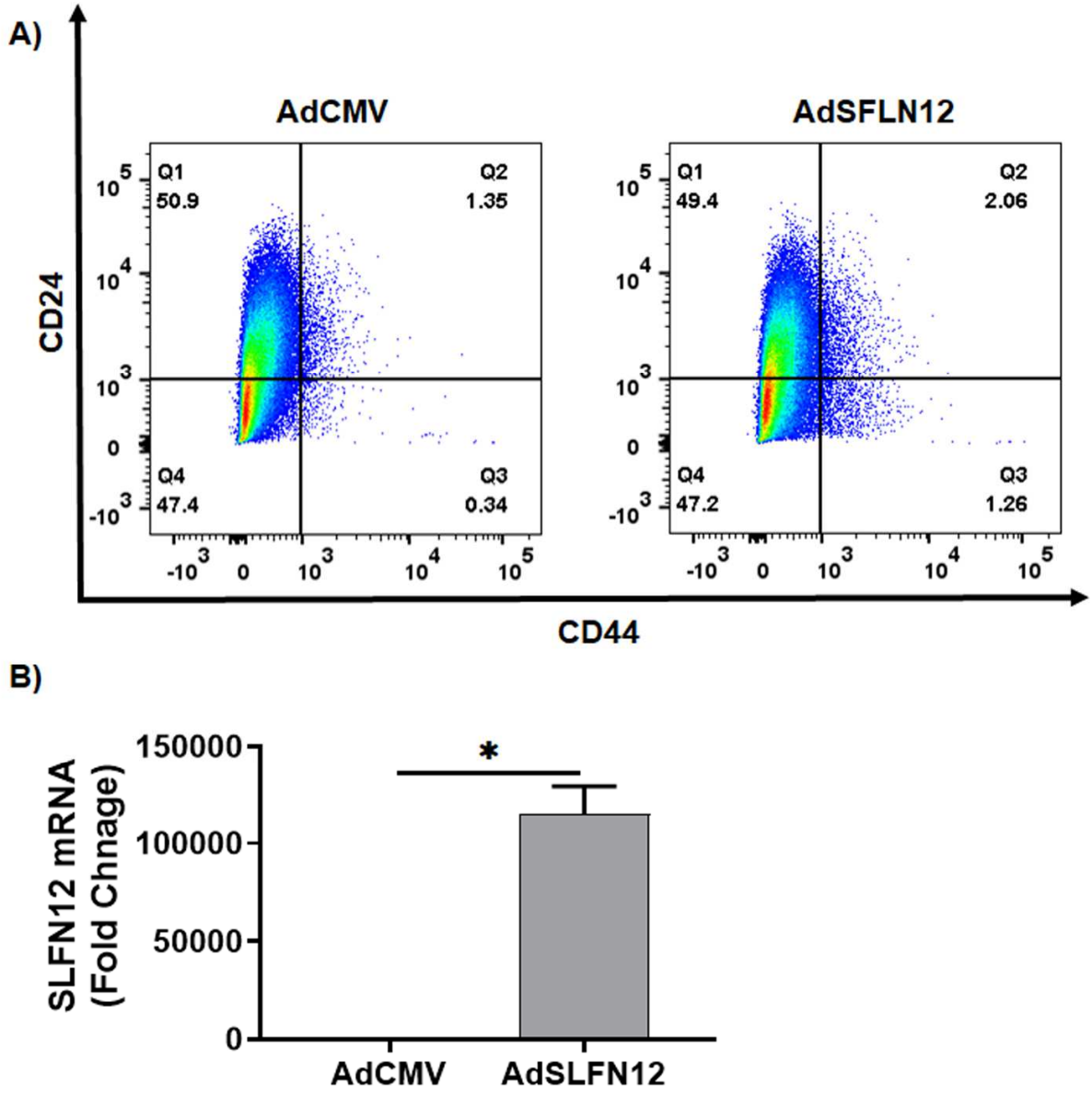
Supplemental Figure 1: Schlafen12 protein overexpression in MDA-MB-231 cells using adenovirus as a vector.

SLFN12 protein analysis by western blot with representative blot images in MDA-MB-231 cells 72 hours after treatment with either AdSLFN12 (SLF) or AdCMV (Ct) as a control (GAPDH used as a reference protein, data normalized to Ct group), (n=3, *$p<0.05$).



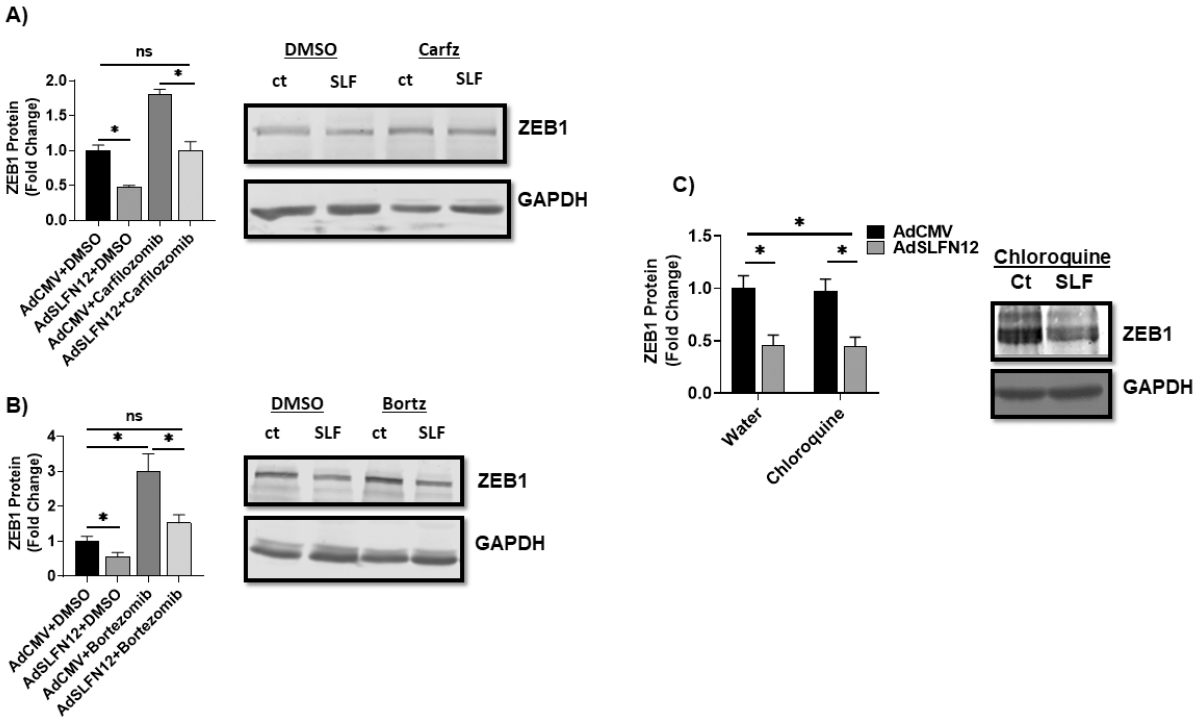
Supplemental Figure 2: Schlafen12 protein knockdown in MDA-MB-231 cells.

SLFN12 protein analysis by western blot with representative blot images in **A)** MDA-MB-231 clones (clone-I and clone-III) with stable SLFN12 overexpression (generated using lentivirus vector transfection with blasticidin selection), 72-hours after treatment with either siRNA targeted to Schlafen12 protein (siSLFN12) or non-targeting sequence (siNT) as a control (GAPDH used as a reference protein, data normalized to siNT group), (n=3, *p<0.05). **B)** MDA-MB-231 cells 72 hours after treatment with either siRNA targeted to Schlafen12 protein (siSLFN12) or non-targeting sequence (siNT) as a control. Bortezomib (200nM) was added for 24 hours prior to cell lysis to increase endogenous levels of Schlafen12 to facilitate detection (GAPDH used as a reference protein, data normalized to siNT group), (n=3, *p<0.05).



<u>Supplemental Figure 3</u>: Schlafen12 does not change CD44/CD24 expression in MCF-7 cells.

A) Flow cytometric analysis of MCF-7 cells 96-hours after treatment with AdSLFN12 or AdCMV showing no change in *CD44CD24* population (one of eight similar independent studies). **B)** Primer-probe qPCR analysis of SLFN12 mRNA levels in MCF-7 cells 72 hours after treatment with either AdSLFN12 or AdCMV (HPRT used as a reference gene), data normalized to AdCMV group (n=3, *p<0.05).



<u>Supplemental Figure 4:</u> Schlafen12 attenuates proteasomal degradation of ZEB1.

ZEB1 protein levels analysis by western blot with representative blot images in MDA-MB-231 cells 72-hours after treatment with either AdCMV or AdSLFN12 in presence of **A)** DMSO or bortezomib (500nM) for 12 hours (n=6), or **B)** DMSO or carfilzomib (10µM) for 12 hours (n=3), (*p<0.05), or **C)** water or chloroquine (100nM) for 12 hrs., GAPDH used as a reference protein and all data normalized to AdCMV+DMSO group (n=3, *p<0.05).