Electronic Supplementary Material (ESI) for Molecular BioSystems.

S1:

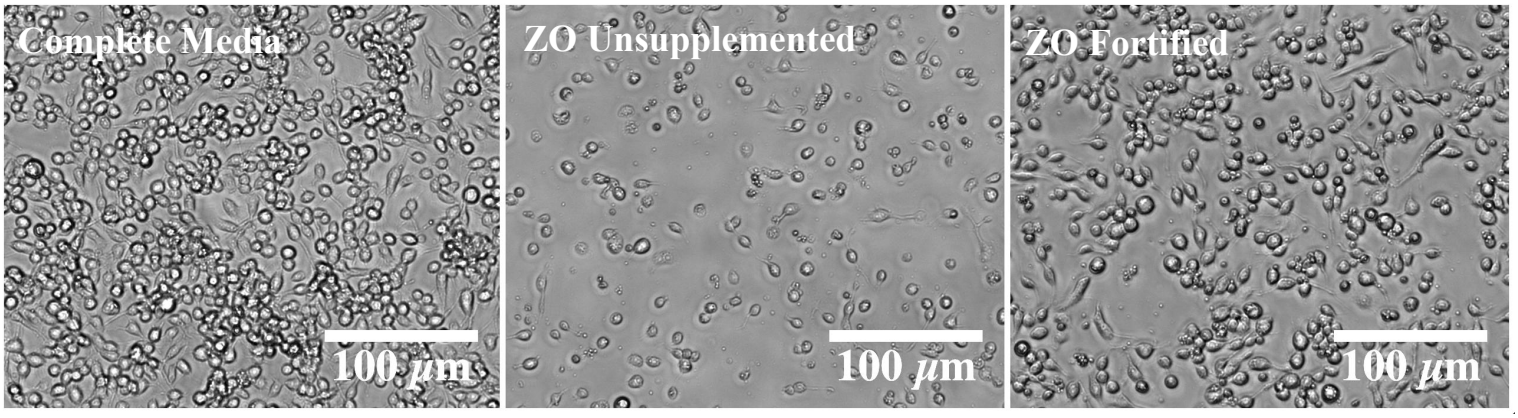


Fig. S1: Comparison of Cl.8 cell proliferation in complete serum containing media, ZO media unsupplemented, and "ZO Fortified." Cells were seeded at 50,000 cells/well and imaged after three days. ZO Fortified supports initial attachment and proliferation of Cl.8 cells whereas ZO unsupplemented does not.

S2:

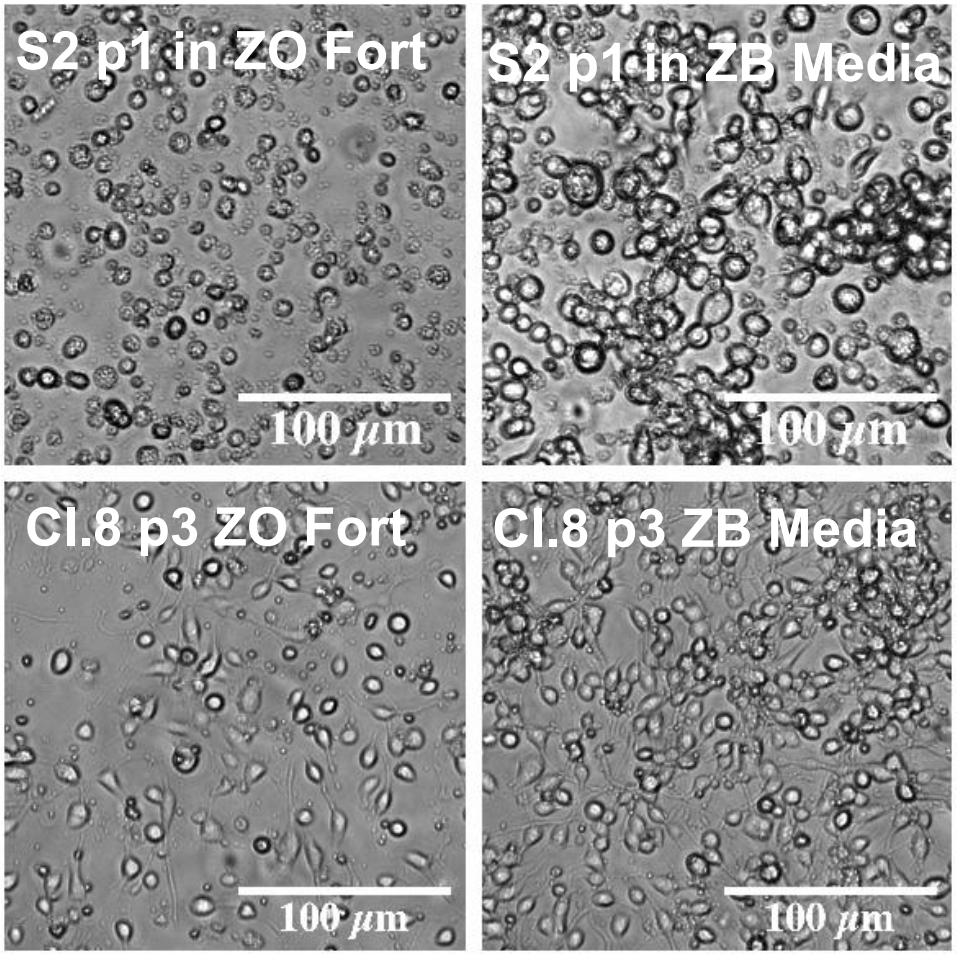


Fig. S2: Cl.8 and S2 cells during long term culture in ZO Fortified versus ZB Media; images at the point of discernable difference in cell growth between ZO Fortified and ZB Media. S2 cells passaged into ZO Fortified were unable to reach passage 2 (confluency) whereas those supplemented with spermidine (ZB Media) were able to reach confluency and undergo two population doublings before growth rates stalled (passage 4). Cl.8 cells passaged into ZO Fortified became confluent and underwent one population doubling (passage 3) before growth stopped. Cl.8 cells cultured in ZB Media are able to proliferate long-term (currently passage 35). Cl.8 cells after adaptation (10 passages in ZB Media) can be frozen and thawed successfully.

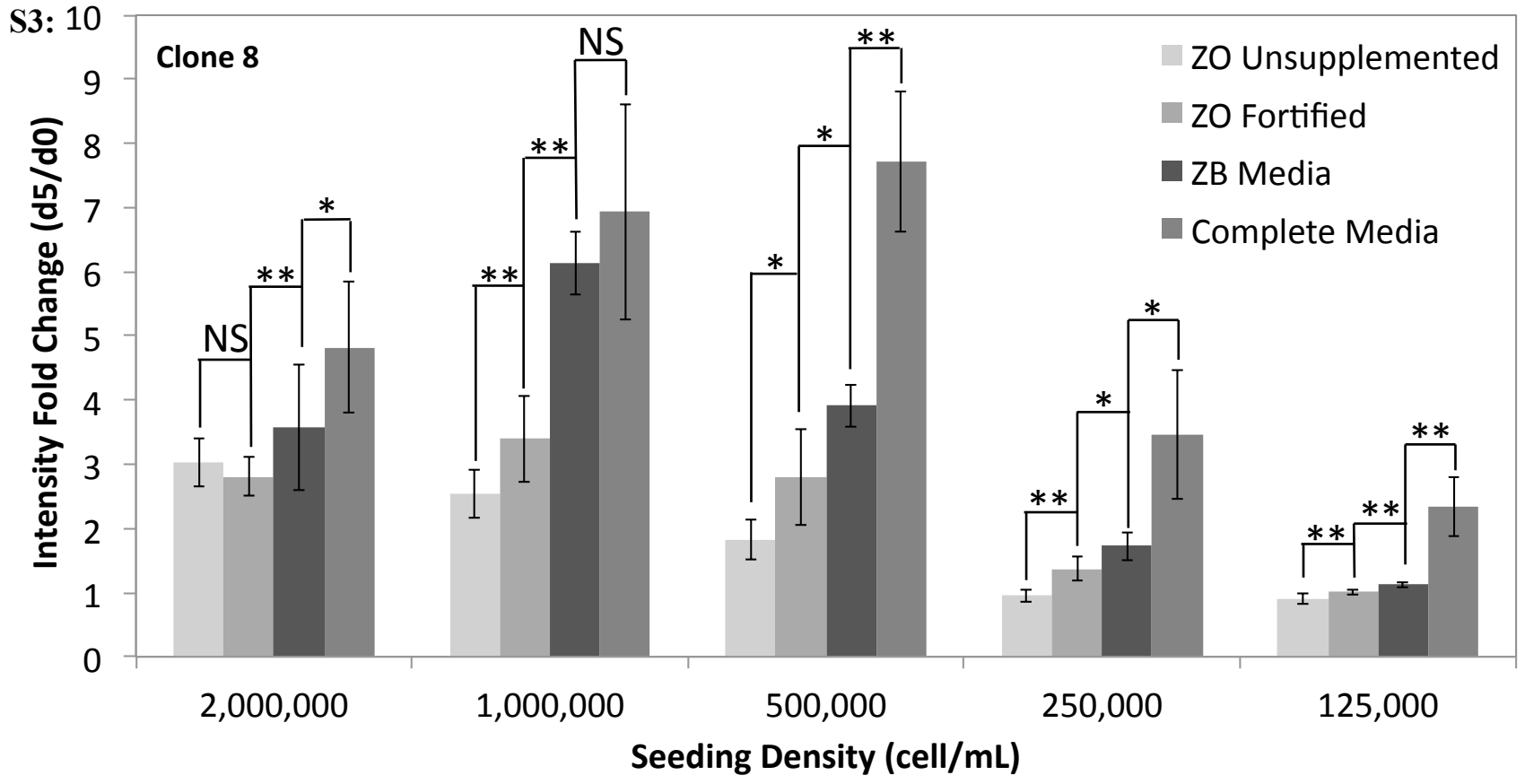
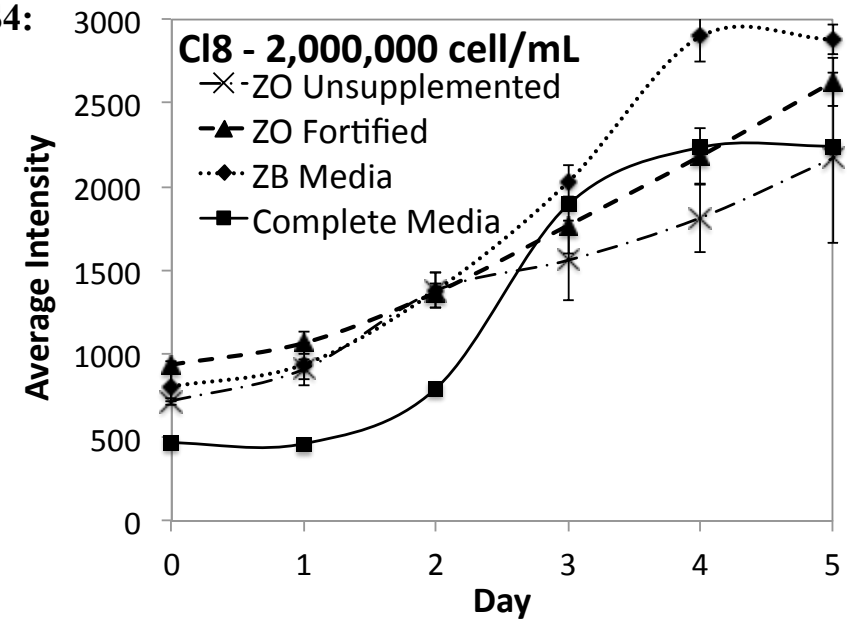
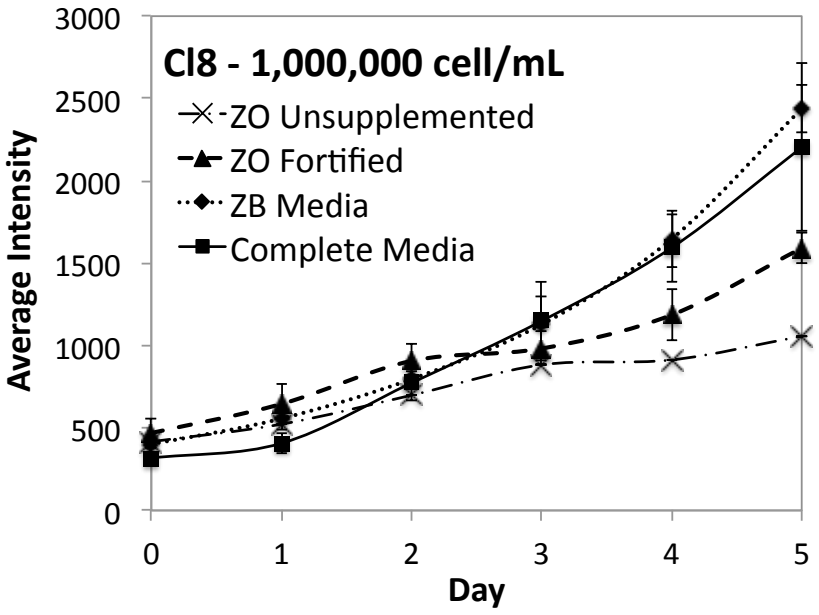
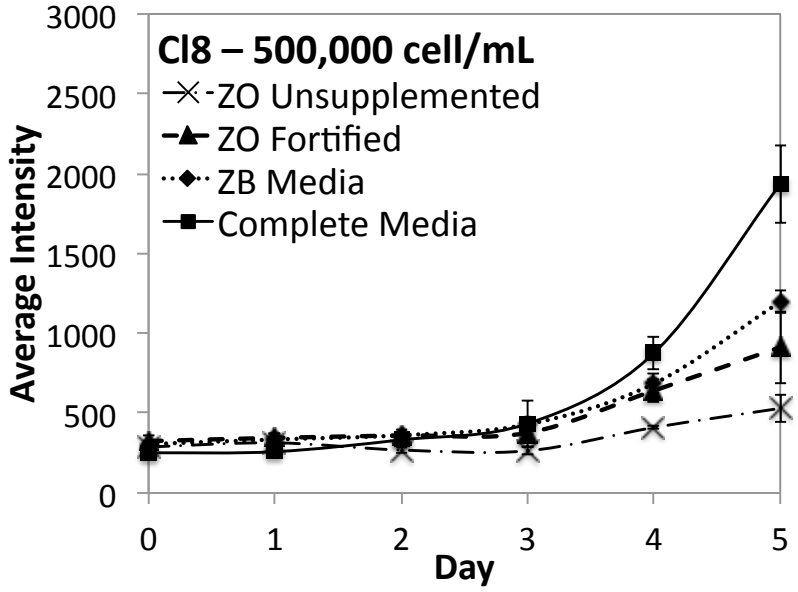


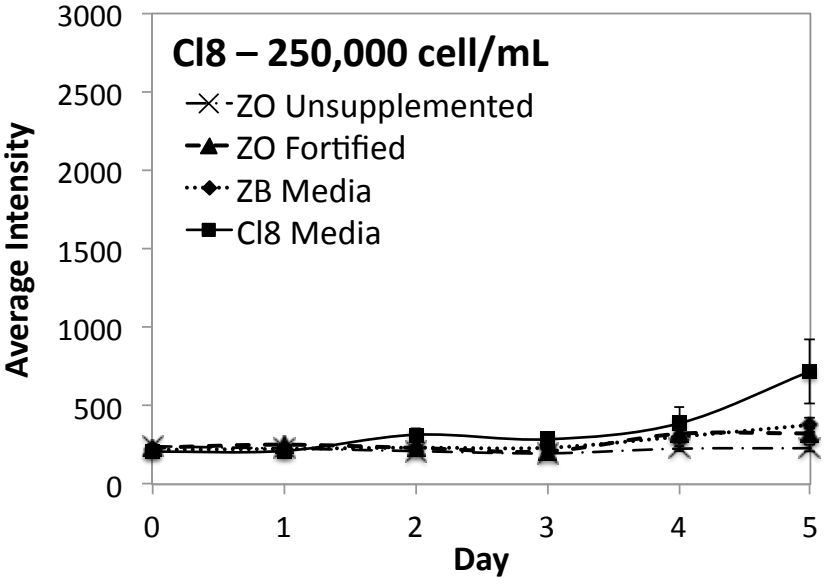
Fig. S3: Cl8 growth kinetics are dependent on seeding density. Spermidine causes significant increase in growth for all seeding densities tested. Corresponding p-values across multiple days (data shown in Supplementary Fig. 4) can be found in the electronic supplementary information.

S4:









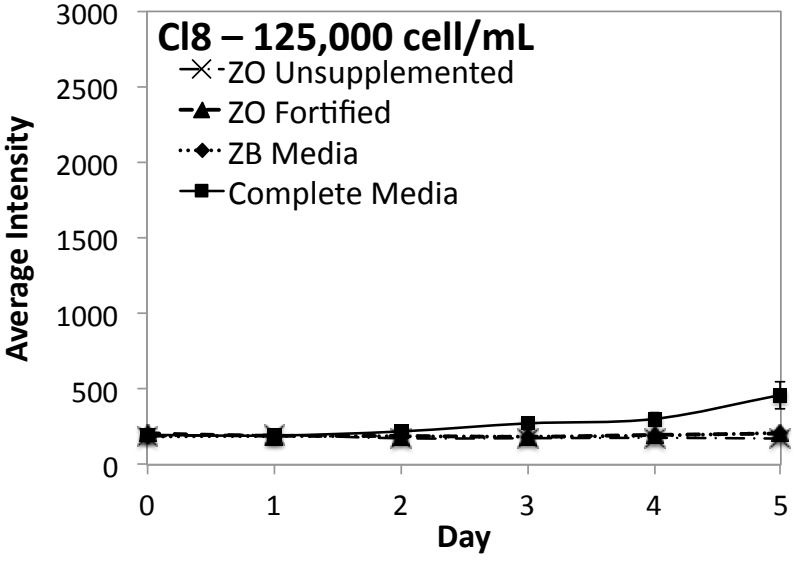


Fig. S4: Cl.8 growth kinetics are dependent on seeding density. Even at 125,000 cell/mL seeding density, ZB Media promotes cell growth (d5/d0 intensity fold change of 1.12 versus 1.01 for ZO Fortified). However, growth at this low of seeding densities is low enough that cells will likely deplete culture medium before reaching confluency. Thus seeding density must be considered when using ZB Medium.

S5:

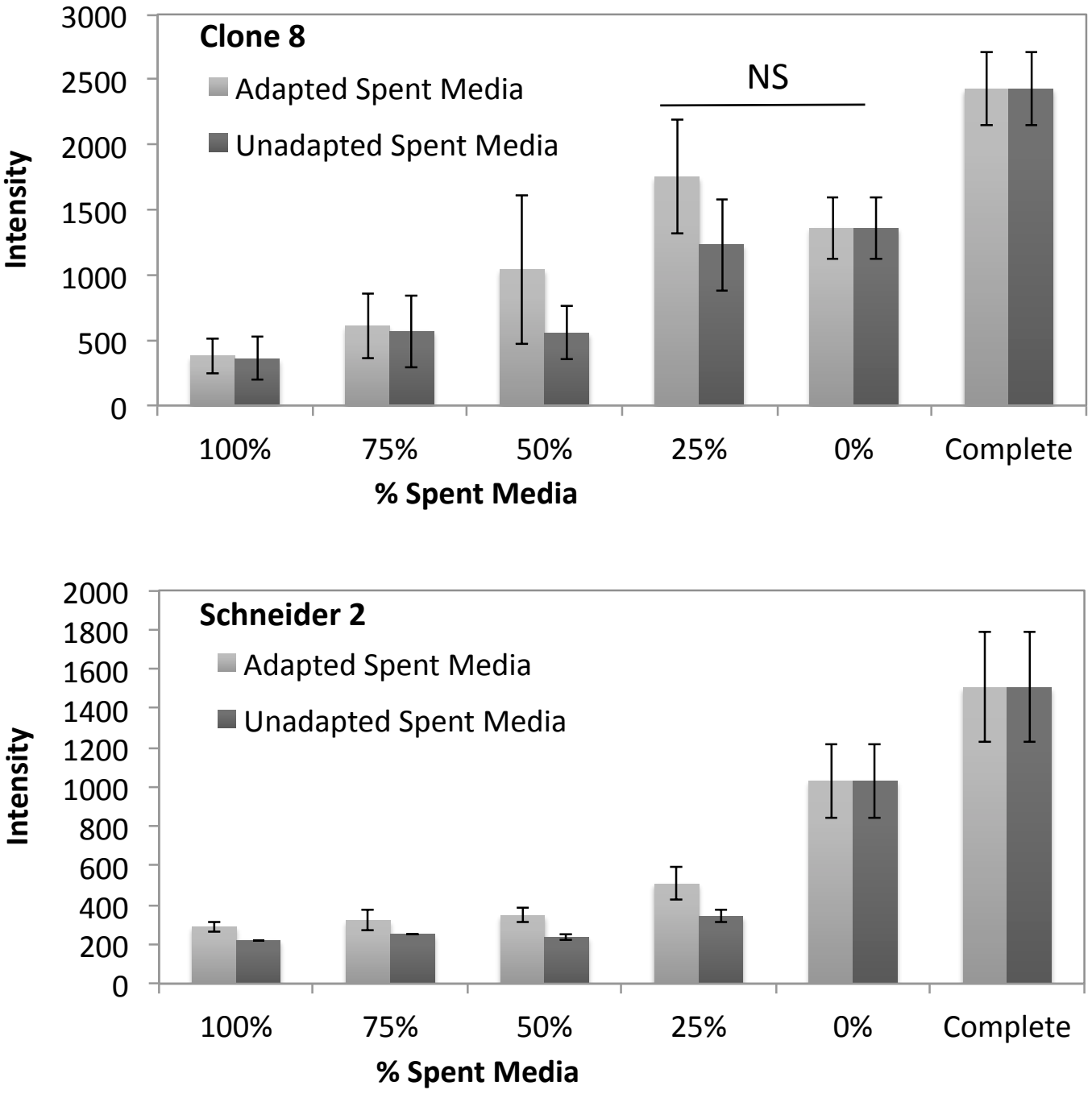


Fig. S5: Supplementing ZB Media with spent media ZB media from adapted or unadapted Cl.8 cells does not induce proliferation of Cl.8 or S2 cells. Adapted (passage 33 in ZB Media) and unadapted Cl. 8 cells were seeded in ZB Media at $1x10^6$ cell/mL and allowed to proliferate for 5 days. Spent media was then harvested and Cl.8 and S2 cells were seeded at 50,000 cell/well in 96 well plates with either 0, 25, 50, 75, or 100% spent media from adapted or unadapted cells. Cells were allowed to proliferate for 8 days and then assayed with CyQUANT.