Supplemental Fig. SF1. Annotated Dectin-3-related sequences. SF1A. The amino acid (a.a.) sequence of the full-length native *Mus musculus* Dectin-3 (MCL, *CLEC4D*, NP_034949.3, 219 a.a.). SF1B. Annotated DNA sequence of the *E. coli* codon-optimized recombinant DEC3-encoding sequence expressed herein to make DEC3 protein. SF1C. Annotated a.a. sequence of the recombinant isoform DEC3 polypeptide (199 a.a. residues) expressed in *E. coli* and employed herein in DEC3-AmB-LLs and DEC3-Rhod reagents.

SF1A. Native mouse Dectin-3

>NP_034949.3 Mouse (*Mus musculus*)C-type lectin domain family 4 member D isoform 1. Full length mouse Dectin-3. 219 a.a. residues. 25,619.07 kDa. Very hydrophobic (Tyr 4 residues, Trp 11, Phe 15, Val 19, Leu 14, Ile 7). With Cys reduced 2.594 O.D. or oxidized 2.6 O.D./mg/mL A280. Aliphatic index 65.3, Hydropathicity GRAVE= -0.361. Instability index 37.6. Yellow highlighted amino acid residues 1 to 43 encode the N-terminal signaling and transmembrane domains that were omitted in our construct. Green highlighted amino acid residues 44 to 219 encode stalk domain and C-terminal carbohydrate recognition domain (CRD) included in our construct called DEC3.

MWLEESQMKSKGTRHPQLIPCVFAVVSISFLSACFISTCLVTHYFLRWTRGSVVKLSDYHTRVTCIREE
PQPGATGGTWTCCPVSWRAFQSNCYFPLNDNQTWHESERNCSGMSSHLVTINTEAEQNFVTQLLDKRFSY
FLGLADENVEGQWQWVDKTPFNPHTVFWEKGESNDFMEEDCVVLVHVHEKWVWNDFPCHFEVRRICKLPG
ITFNWKPSK

SF1B. *HK-DEC3 (a.k.a. DEC3)* His-Lys-modified murine Dectin-3 coding sequence. DNA sequence encoding the truncated version of mouse DEC3 with codons optimized for expression in *E. coli* that was synthesized by GenScript for subcloning into the expression vector pET-45b+. The following sequence was ordered from GenScript. The synthetic sequences (AAA, AAG) encoding the lysine residues for coupling to NHS-Rhodamine or NHS-PEG-DHPE and the synthetic sequences(**GGT TCA GGG TCT GGC**) encoding the gly,ser,gly,ser,gly flexible spacer are highlighted in yellow. The *E. coli* codon-optimized DNA sequence for the mouse Dectin-3 stalk and CRD regions are highlighted in green. GenScript trimmed off the flanking underlined *Kpn*I (GGTACC) and *Pac*I (TTAATTAA)(subcloning sites bold black font) shown here from the order because they are part of the vector pE45b+ into which GenScript subcloned the sequence. The reading frame is indicated at the beginning and end of the sequence by separating some individual codons. An alanine codon GCT was added at the C-terminal end to accommodate an in-frame *Pac*I sequence.

ATG GCA CAT CAC CAC CAC CAT CAC GTG GGT ACC
GGA AGT GGA *AAA* GGC *AAG* GGT TCA GGG TCT GGC
CACTATTTCCTCCGCTGGACGAGAGGCTCTGTGGTGAAGCTTTCAGACTACCACACAAGA
GTTACCTGCATAAGGGAAGAGCCTCAGCCCGGTGCCACTGGTGGCACCTGGACATGCTGT
CCTGTCTCCTGGCGGGCCTTTCAGAGCAATTGCTACTTTCCTCTCAATGACAACCAGACG
TGGCATGAATCAGAAAGGAACTGTAGTGGGATGTCTAGCCACCTGGTCACCATCAACACA

GAGGCTGAGCAGAACTTTGTGACTCAACTGCTGGATAAACGTTTCTCCTATTTTCTAGGC
TTGGCAGATGAAAATGTGGAGGGGCAATGGCAGTGGGTAGATAAGACACCGTTTAATCCA
CATACTGTCTTCTGGGAGAAAGGAGAAAGTAACGATTTCATGGAGGAGGACTGTGTGGTT
TTAGTTCACGTGCATGAGAAGTGGGTGTGGAATGACTTCCCCTGTCACTTTGAAGTACGG
CGAATTTGCAAGCTGCCAGGAATCACCTTCAACTGGAAGCCAAGCAAAGC**T TAA TTA A**

SF1C. The modified MmsDectin-3 (HK-DEC3, DEC3) protein made from pET-45B+ in *E. coli* has the following sequence. The his tag, lysine residues, and gly,ser flexible spacer are shown in yellow. The mouse Dectin-3 CRD and stalk regions are shown in green. 199 a.a. residues. Mol Wgt. 23,024. PkI 6.52. Abs A280 2.6 OD/mg/mL. Aliphatic index: 53.77 indicating the protein has a large volume of hydrophobic side chains and is likely to be unstable in aqueous buffers. Grand average of hydropathicity (GRAVY): -0.638 indicating the opposite, that it may be soluble. We expect that the six added recombinant histidine residues dramatically influenced these conflicting values.
MAHHHHHHVGTGSGKGKGSGSGHYFLRWTRGSVVKLSDYHTRVTCIREEPQPGATGGTWTCCPVSWRAFQ
SNCYFPLNDNQTWHESERNCSGMSSHLVTINTEAEQNFVTQLLDKRFSYFLGLADENVEGQWQWVDKTPF
NPHTVFWEKGESNDFMEEDCVVLVHVHEKWVWNDFPCHFEVRRICKLPGITFNWKPSKA*

Supplemental Fig. SF2. Protein production in *E. coli.*

SF2A. SDS PAGE analysis of modified Dectin-3 polypeptide (HK-DEC3)

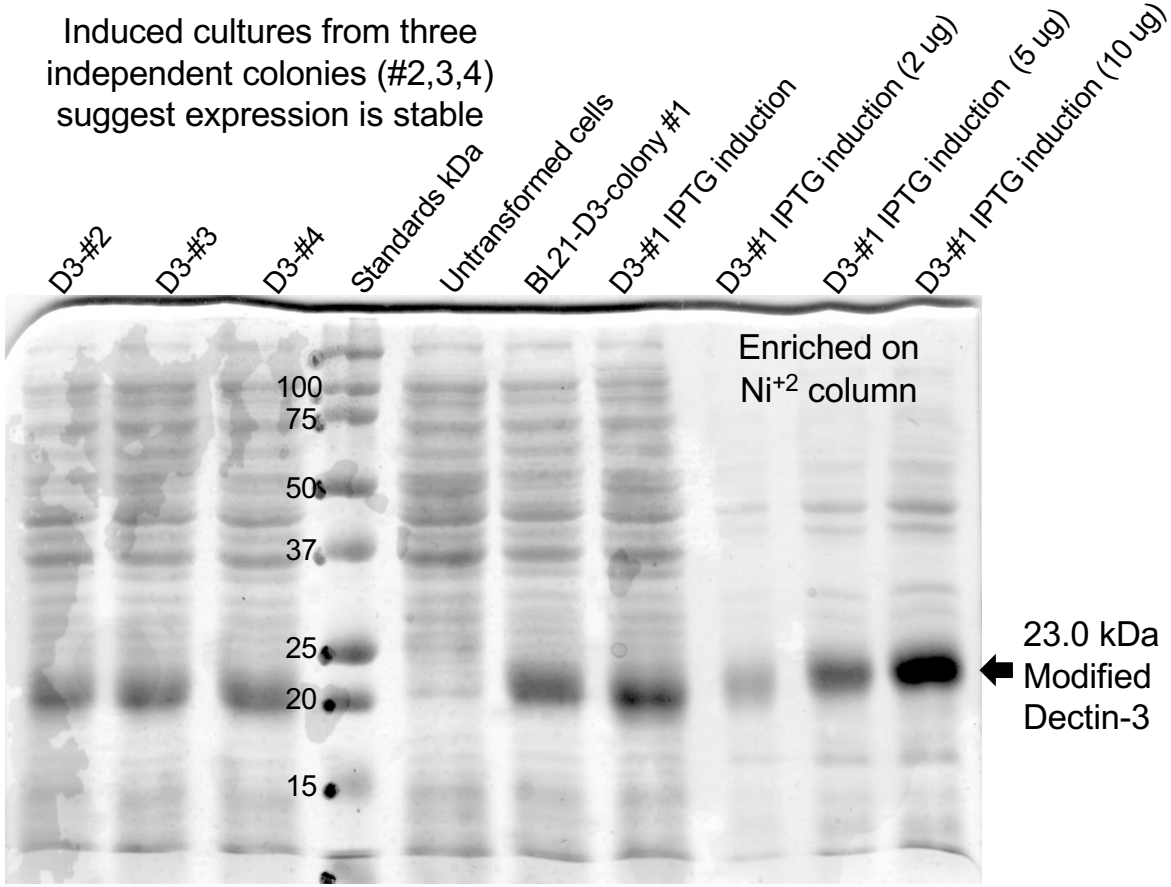
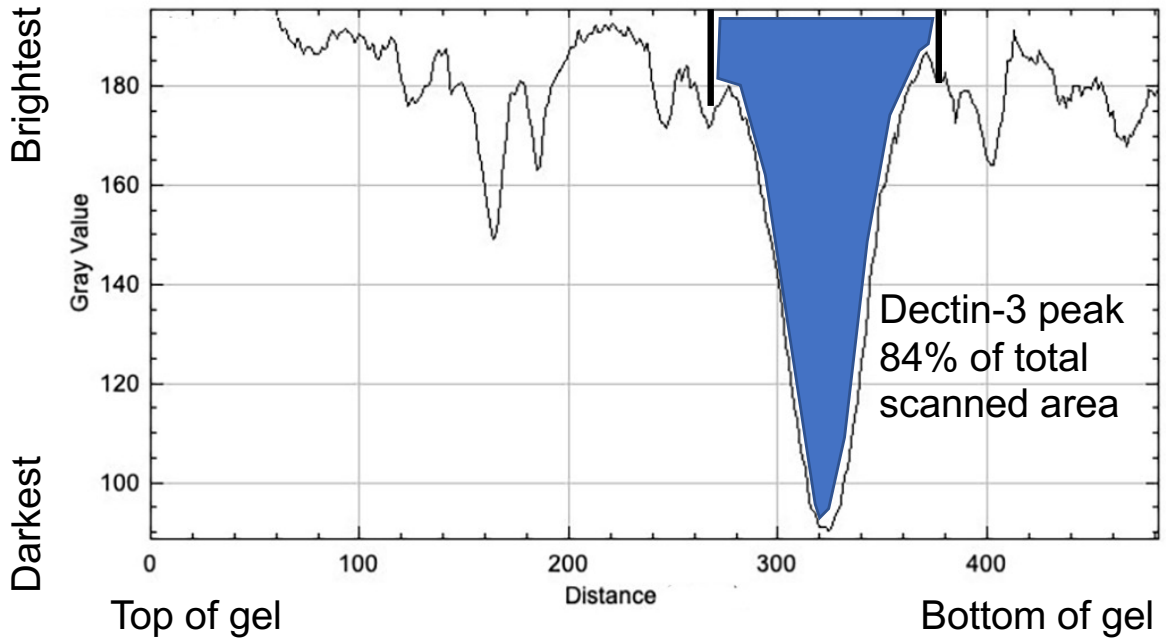


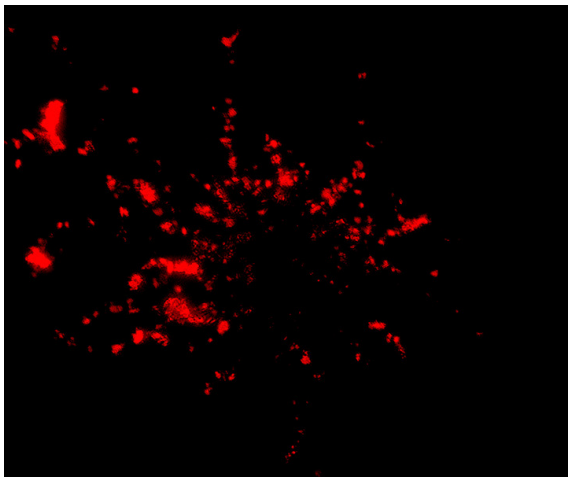
Fig. S2 continued

SF2B. Histogram of purified Dectin-3 (HK-DEC3) from SDS-PAGE gel, 5 ug sample from SF2A.

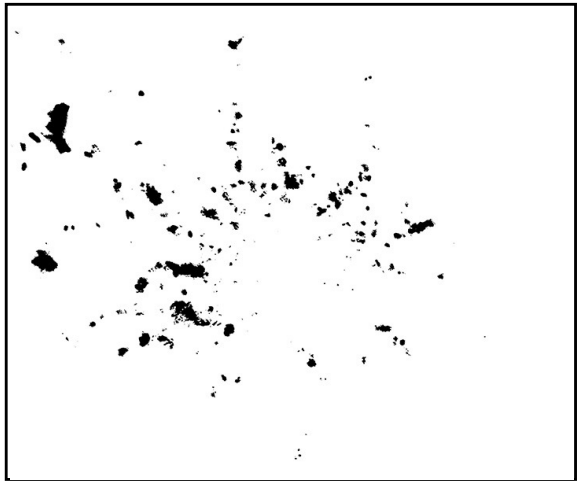


Supplemental Fig. SF3. CellProfiler pipelines for area analysis of fluorescent images of *C. albicans* hyphal colonies.

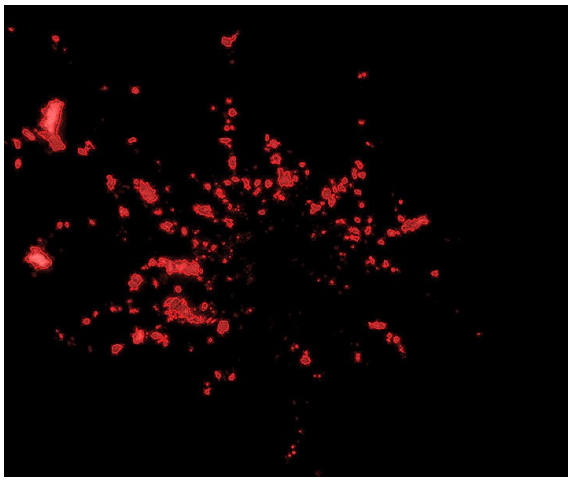
SF3A. Comparison of manual to CellProfiler AreaPipe image analysis of red fluorescent DectiSome stained area



SF3A1. Original red fluorescent TRITC jpg image. The entire image is composed of ~5x10^6 pixels.



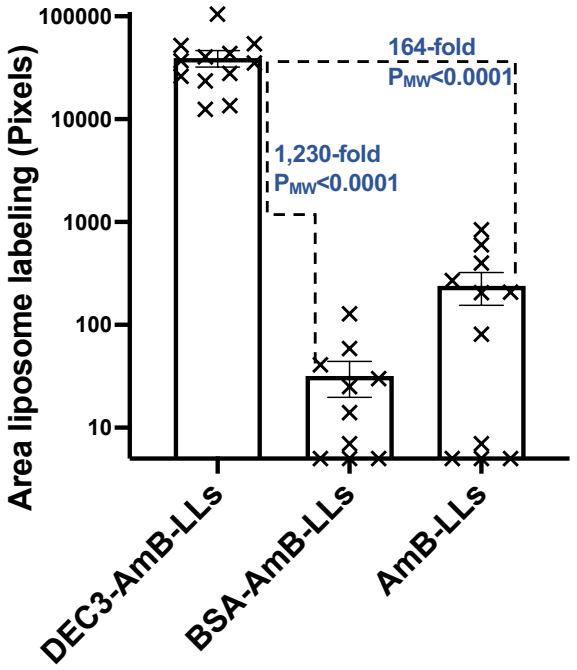
SF3A2. Manual capture of fluorescent area in ImageJ. Threshold setting 30/255. Reported as % of pixel area captured.



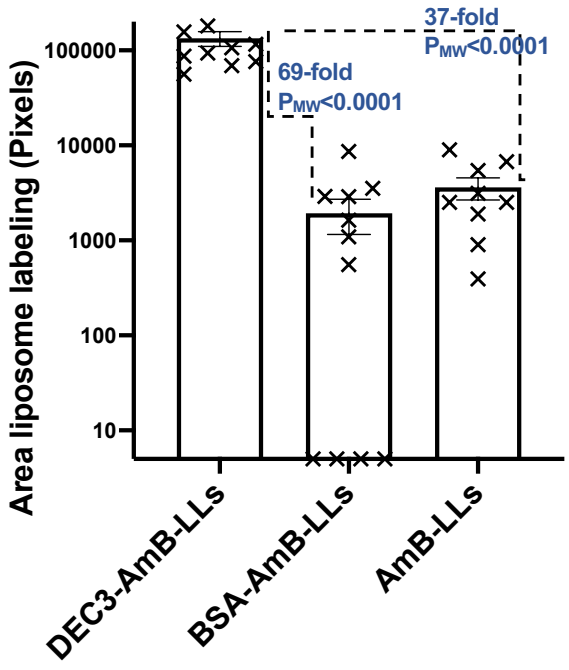
SF3A3. AreaPipe automated capture of red fluorescent area. Reported as numbers of pixels captured out of ~5x10^6 pixels.

Supplemental Fig. SF4. Biological replicates of quantification of liposome binding experiments and Dectin-3 protein binding.

SF4A. Replicate of binding to *C. albicans* yeast-hyphal transition stage



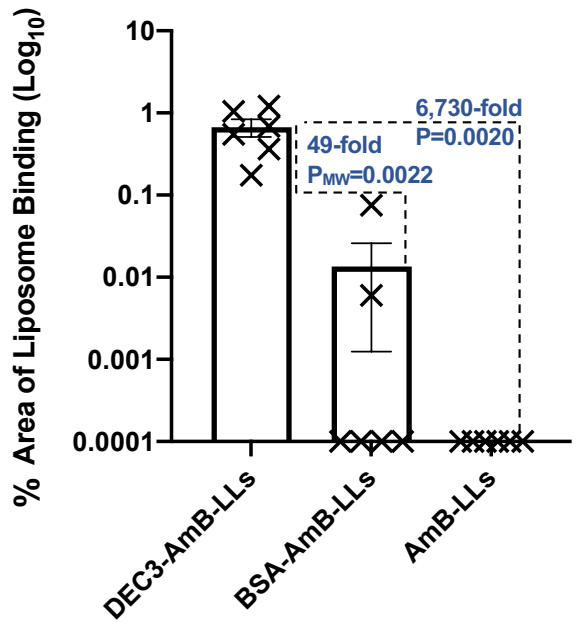
SF4B. Replicate of binding to mature *C. albicans* hyphal colonies



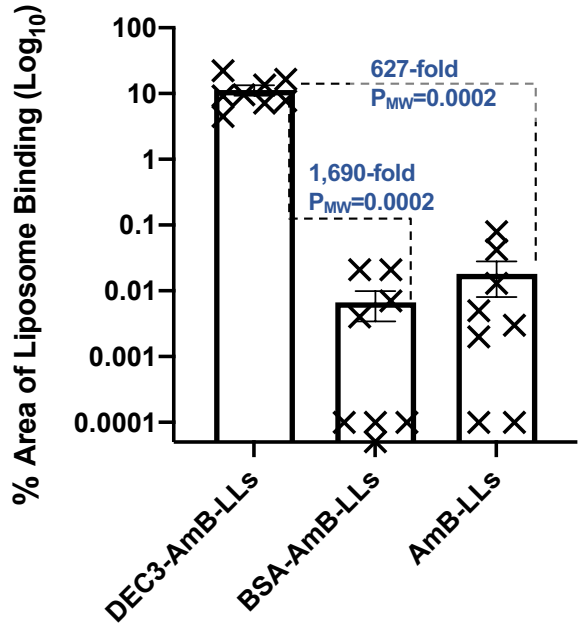
Repeat 01.09.23

Supplemental Fig. SF4 continued. Repeat of Quantification of binding data

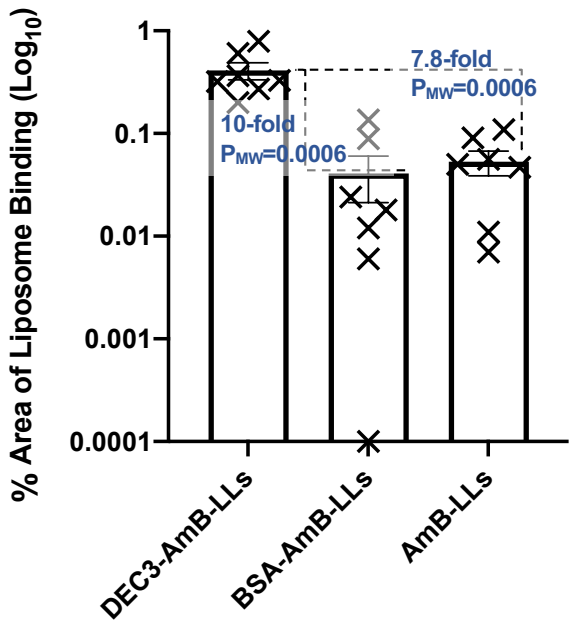
SF4C. Replicate of binding to *R. delemar* germlings



SF4D. Replicate of binding to fixed *R. delemar* hyphae

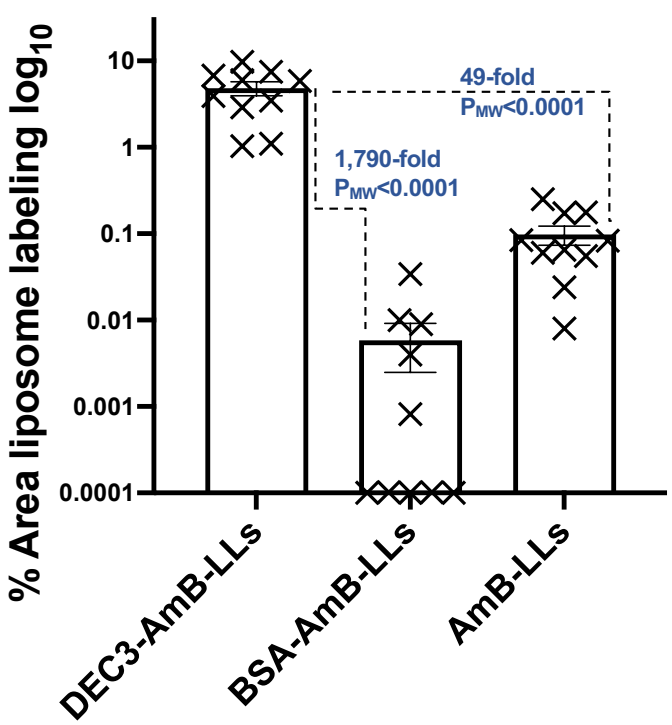


SF4E. Replicate of binding to live *R. delemar* hyphae

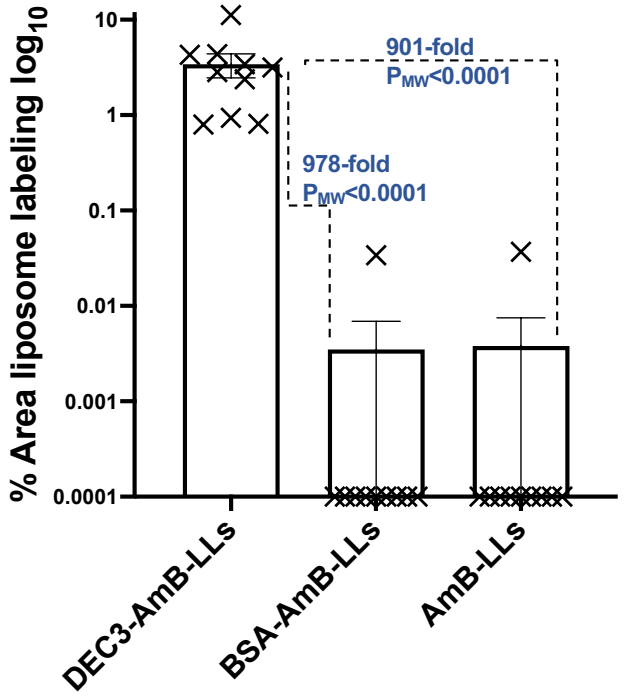


Supplemental Fig. SF4 continued Repeat of Quantification of binding data

SF4F. Liposome binding to 6-hr old *C. neoformans* colonies

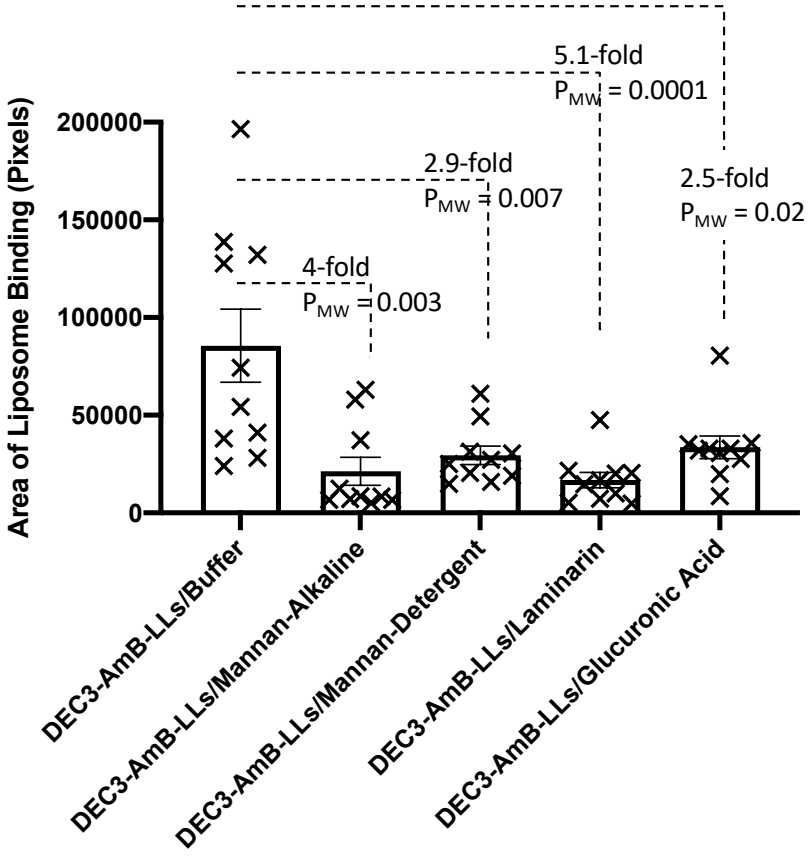


SF4G. Liposome binding to 18-hr old *C. neoformans* colonies

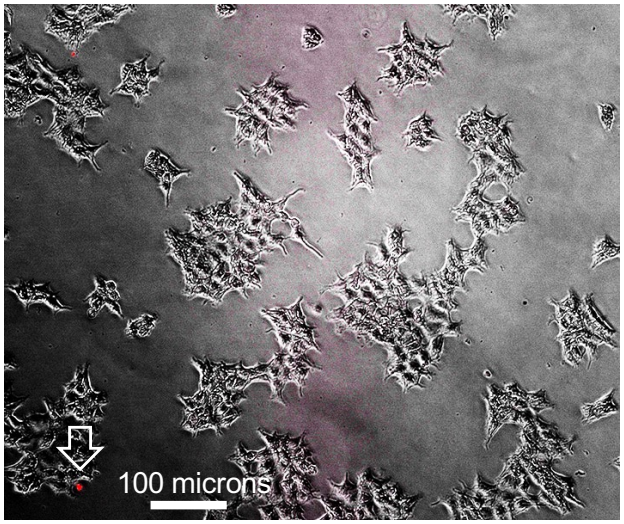


Replot order and minimum values to 0.0001%

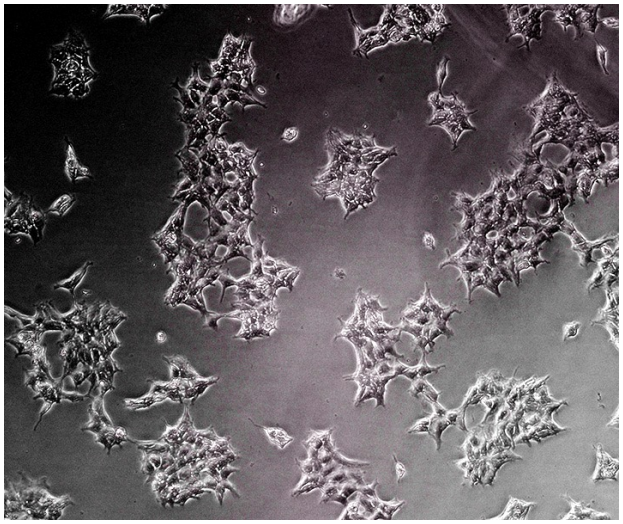
Supplemental Figure SF5. Dectin-3 DectiSome binding inhibition study on *C. neoformans* (16-hr-old colonies)



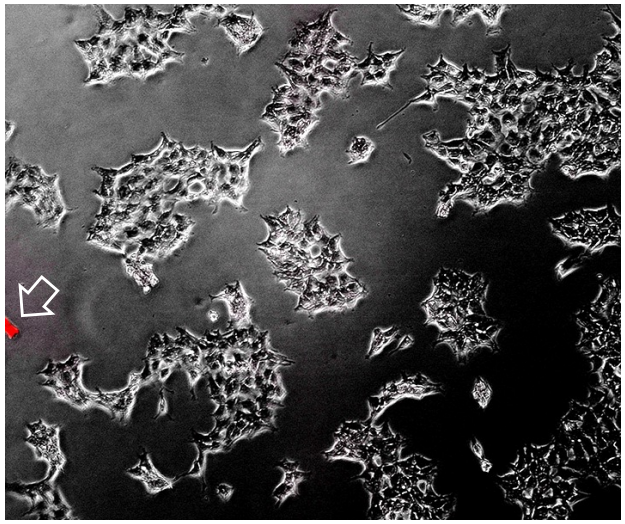
Supplemental Fig. SF6. Neither Dectin-3-coated DEC3-AmB-LLs nor control liposomes bound efficiently to the artificially immortalized human line HEK293T



DEC3-AmB-LLs



BSA-AmB-LLs



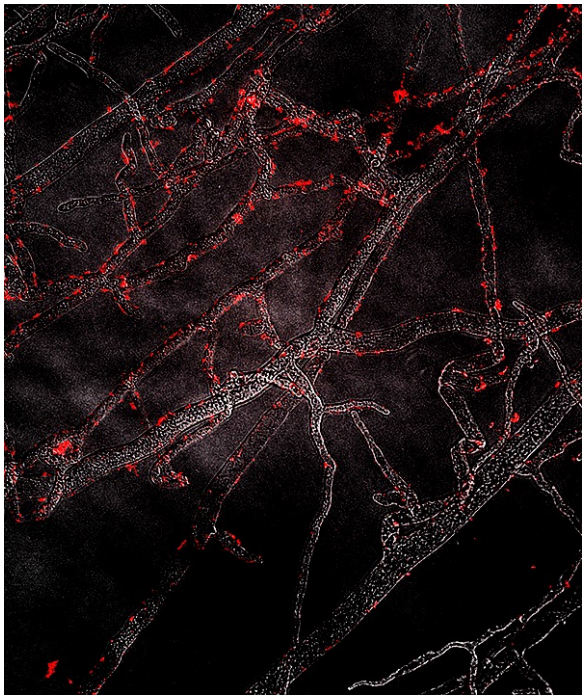
AmB-LLs

11.04.22 photos
taken at 10X
mag on this date

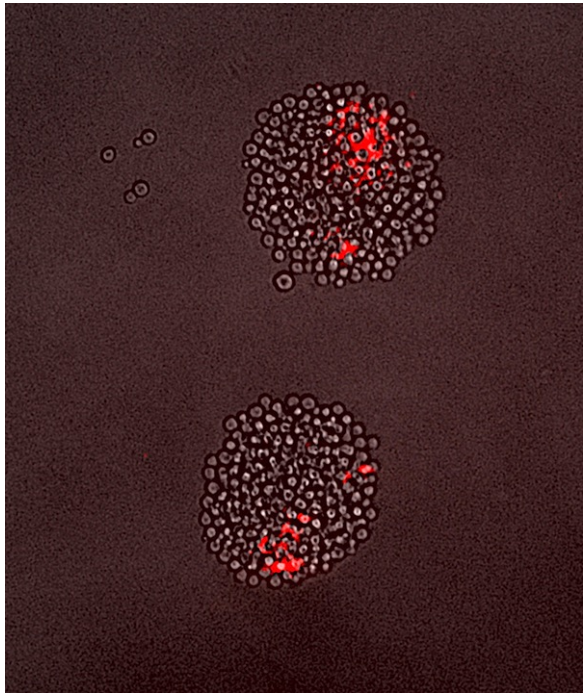
Supplemental Fig. SF7. DEC3-Rhod protein staining multiple fungal pathogens

SF7A. Dectin-3-Rhod staining of *C. albicans* colony

SF7B. Dectin-3-Rhod staining of *R. delemar* hyphae

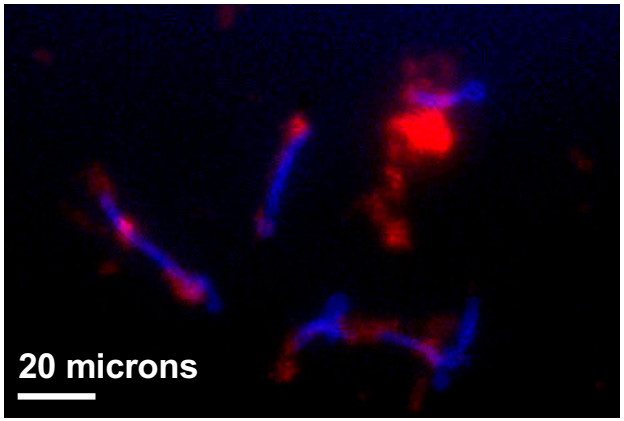


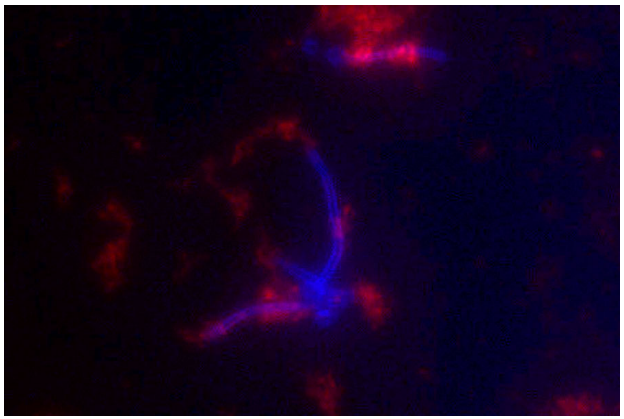
SF7C. Dectin-3-Rhod staining of *C. neoformans* colonies



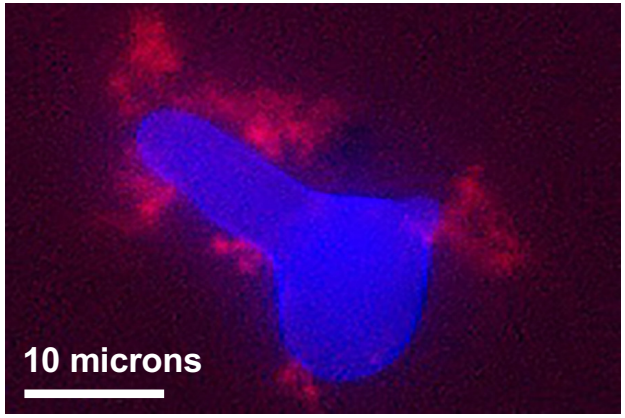
Supplemental Fig. SF7. DEC3-Rhod protein staining multiple fungal pathogens cont.

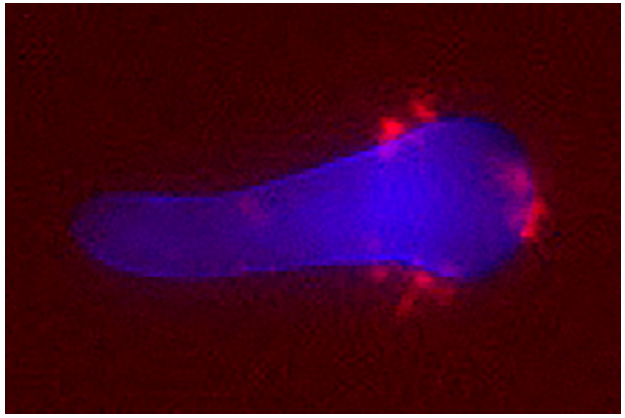
SF7D. Dectin-3-Rhod staining of early-stage *C. albicans* cells





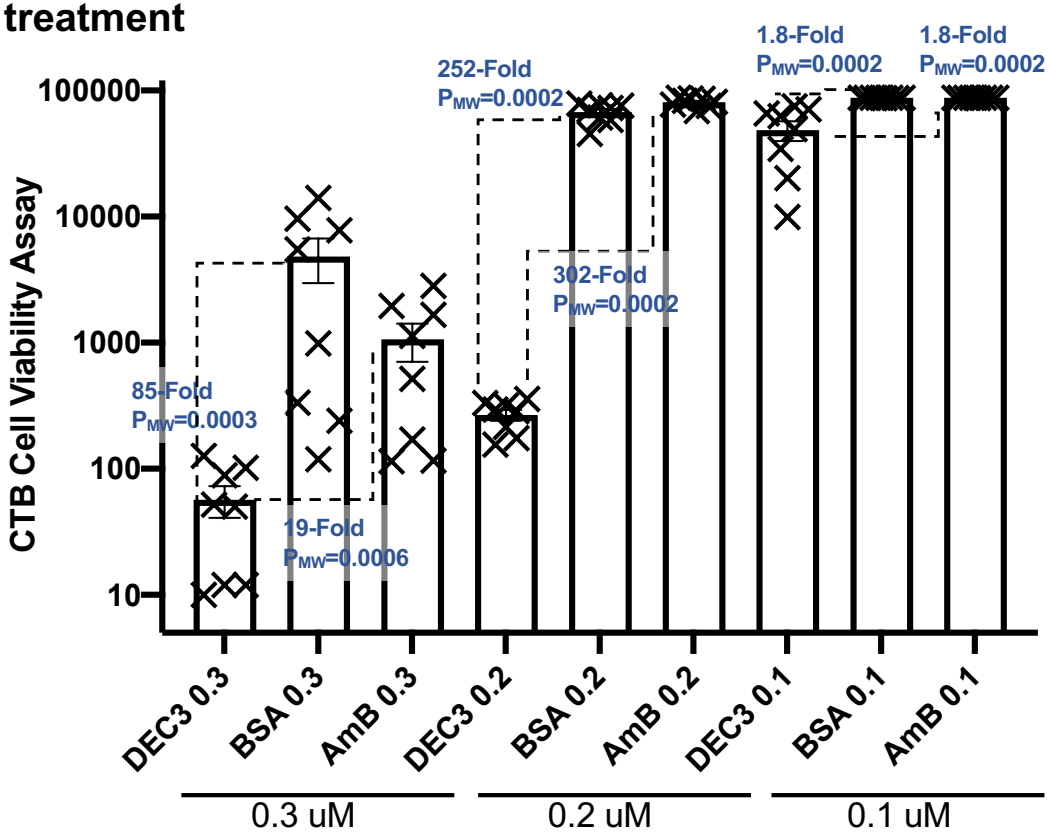
SF7E. Dectin-3-Rhod staining of *R. delemar* germlings





Supplemental Fig. SF8. Replicates of inhibition and killing data

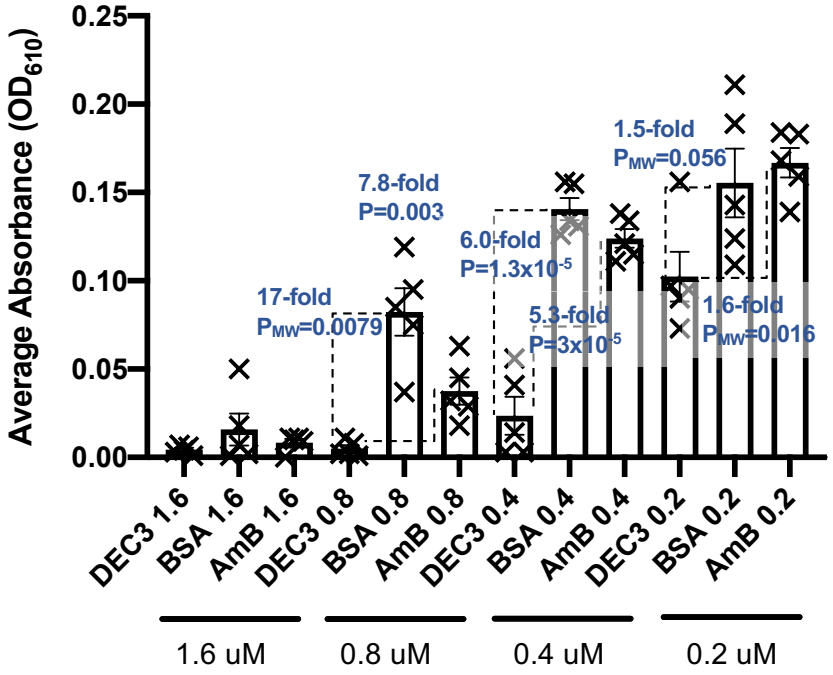
SF8A. *C. albicans* metabolic activity after liposome treatment



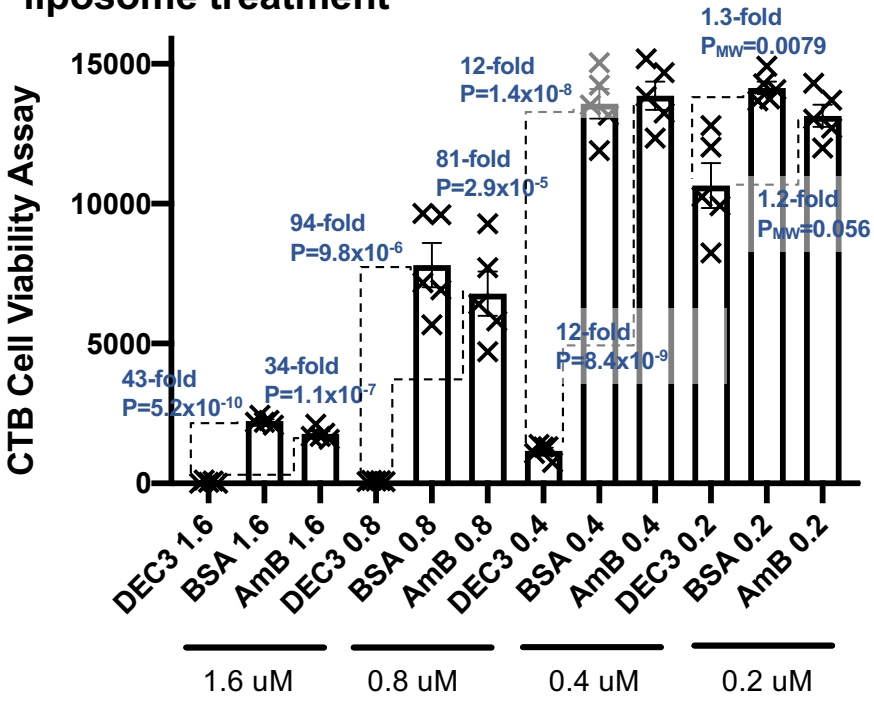
01.11.23 data

Supplemental Fig. SF8. Continued. Biological replicates of inhibition and/or killing experiments.

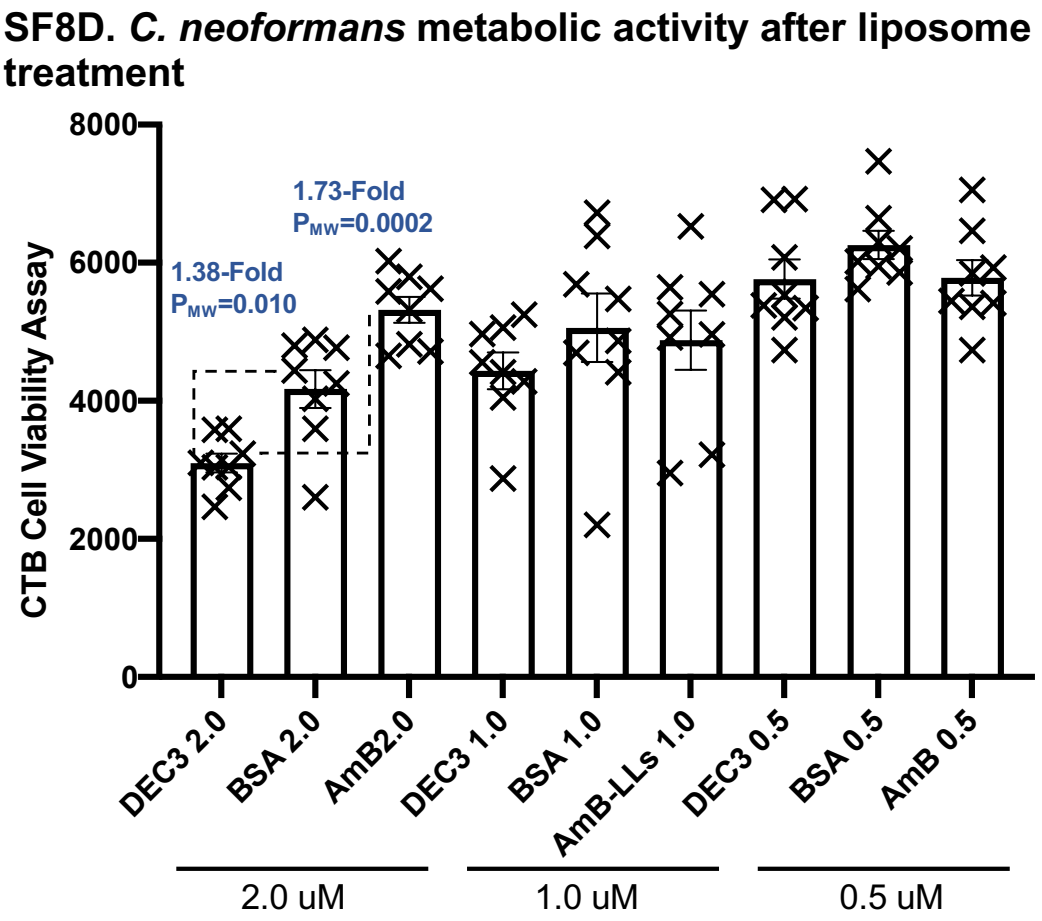
SF8B. *R. delemar* cell density after liposome treatment



SF8C. *R. delemar* metabolic activity after liposome treatment

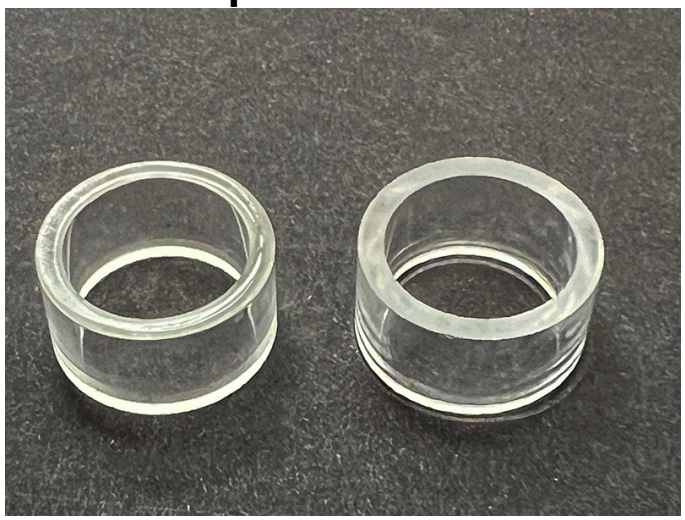


Supplemental Fig. SF8. Continued Biological replicates of inhibition and/or killing experiments. 01.21.23



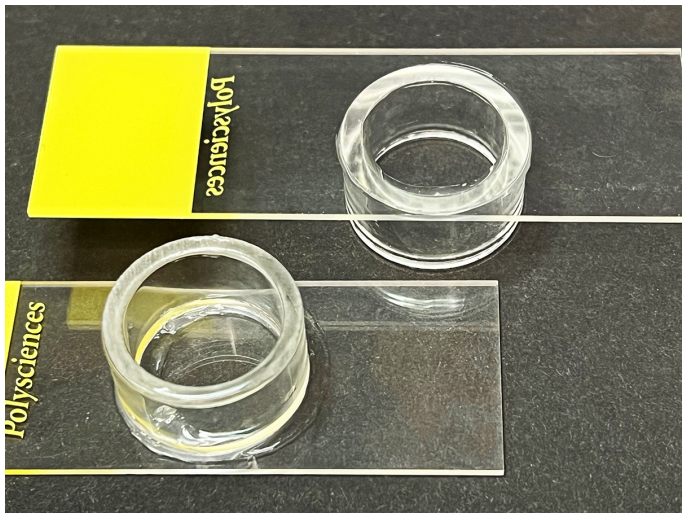
Supplemental Figure 9. Glass chambers for microscope slides

SF9. Reusable glass chambers for cell growth on microscope slides



SF9A. Glass chambers. Left (fire polished side up). Right (ground glass side up).

- 22mm Outer Diameter
- 17mm Inner Diameter
- 2.5mm Wall thickness
- 11mm Tall



SF9B. Chamber slides. Left (fire polished side up). Right (ground glass side up) shows the continuous silicone greased contact to the slide with no air bubbles. The chamber is removed and replaced with a coverslip for microscopy.