1016 *neoformans* via endocytotic and transcellular mechanisms that require Mpr1 and Annexin A2

1017 (AnxA2).[18,69] Sustained EphA2 activation could weaken intercellular junctions thereby increase

1018 paracellular permeability and boost further entry of *C. neoformans* along with excess fluid that

1019 would lead to brain edema (indicated by dashed arrows).

1020 **S1_Fig 1**. A chart representing the work-flow for the transcriptome analysis of human brain

1021 endothelial cells challenged with *C. neoformans*.

1022 **S2_Fig 2**. A Multi-Dimensional Scaling (MDS) plot for gene expression data. The MDS plot

1023 shows the distance relationship (i.e. level of similarity) between the two sample treatment

1024 groups based on gene expression. Data set #1 represents samples of human brain endothelial

1025 cells treated with *C. neoformans* (HB1, HB2, HB3, indicated by circle). This data set was tightly

1026 clustered and apart from untreated brain endothelia cells (B1, B2, B3, data set #2, indicated by

1027 square). The data set #2 was clustered and separate from data set #1.

1028 **S3_Fig 3**. Transcytosis assay revealed that the transmigration of *C. neoformans* is not affected

1029 by knocking down ephrinA1 ligand in human brain endothelial cells. (A) Quantitative PCR

1030 revealed that siRNA of ephrinA1 repressed the expression of ephrinA1 approximately 83%. (B)

1031 Transcytosis assay demonstrated that *C. neoformans* could still migrate across brain endothelial

1032 cells.

1033 **S1_Table 1.** A list of randomly selected, differentially expressed genes from human brain

1034 endothelial cells challenged with *C. neoformans* obtained by RNA-seq and validated by

1035 quantitative reverse transcriptase PCR (qRT-PCR).

1036 **S2_Table 2.** A list of primers used for quantitative reverse transcriptase PCR (qRT-PCR) and

1037 the gene name associate with each set of primers (forward and reverse).

1038 **S1_Movie.** Live-cell recording of HEK293T cells overexpressing EphA2 interacting with
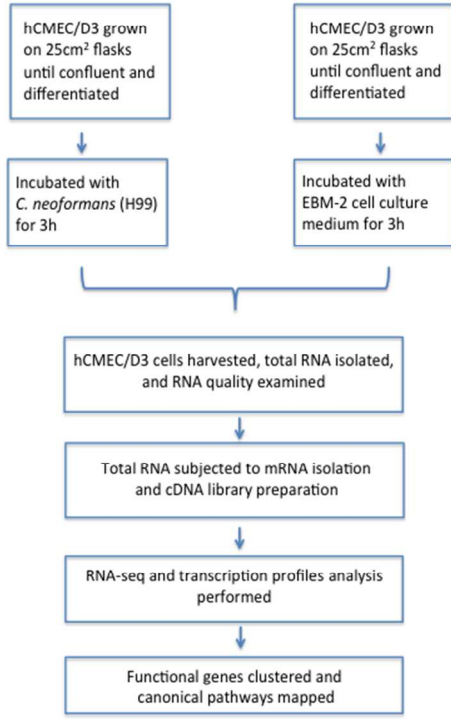
1039   *C. neoformans* H99. Images are close-ups of 10X magnification. (A, C) Imaging shows EphA2

1040   receptor (red), nuclei (blue), and H99 (green).   (B, D) Bright-field imaging of A and C,

1041   respectively.

1042   **S2_Movie**. Live-cell recording: HEK293T cells transformed with an empty plasmid do not

1043   interact with *C. neoformans*. In this recording HEK293T cells were not transformed with EphA2-

1044   cDNA.   Images are close-ups of 10X magnification.   (A, C) Imaging shows H99 (green) and

1045   nuclei (blue). (B, D) Bright-field imaging of A and C, respectively.

1046   **S3_Movie**.   Live-cell recording: HEK293T cells overexpressing EphA2 to do not engage

1047   fluorescent beads. Images are close-ups of 10X magnification. (A, B) Imaging shows EphA2

1048   receptor (red), nuclei (blue), and plastic bead (green). (C, D) Bright-field imaging of A and B,
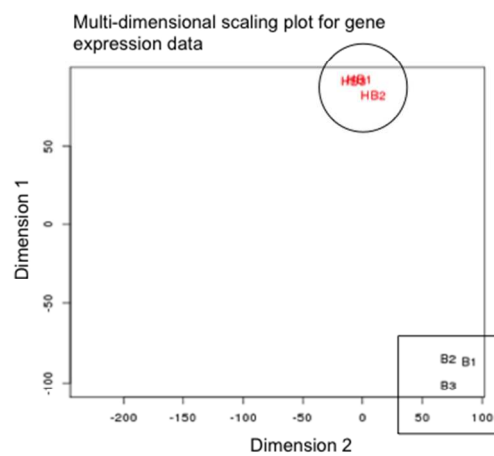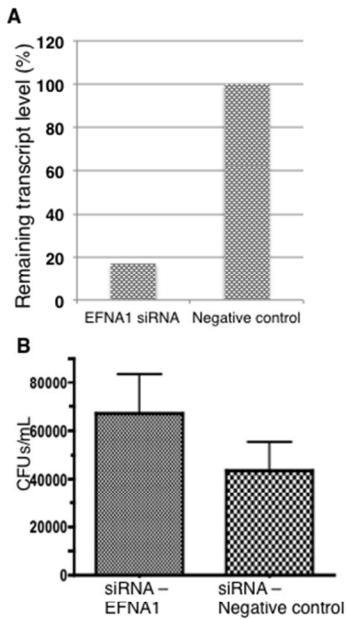
1049   respectively.

1050

S1_ Fig 1

254x338mm (300 x 300 DPI)

S2_Fig 2

Multi-dimensional scaling plot for gene expression data

254x338mm (300 x 300 DPI)

S3_Fig 3

254x338mm (300 x 300 DPI)

S1_Table 1

Table 1. A list of randomly selected, differentially-expressed genes from hBMECs challenged With *C. neoformans* obtained by RNA-seq and validated by quantitative reverse transcriptase PCR (qRT-PCR).

Gene names	Fold-change (RNA-seq)	Fold-change (qPRC)
STAB1 – stabillin-1	2.69	1.9
NPTX1 – neuronal pentraxins	4.42	9.15
EDNRA – receptor for endothelin-1	4.22	5.11
EDN1 – endothelin-1	2.47	N/A
END2 – endothelin-2	4.66	10.63
EEA1 – early endosome antigen-1	-1.55	-1.16
SRXN2 – sulfirredoxin-1	-2.33	-1.23
SIRT1 – NAD-dependent deacetylase sirtuin-1	-1.92	1.12
NRGE2 – growth factor (neuregulin)	1.97	2.5
GMFB – glial maturation factor	-2.42	1.05
Coorf10 – transcription factor	3.91	11.41
EFNA1 – ephrinA1	2.06	3.58

254x338mm (300 x 300 DPI)

S2_Table 2

Table 2. Primers used for qRT-PCR.

| Gene | gene name | Primer for real time PCR (5'---------------3') | |
		Forward primer	Reverse primer
EDN1	Endothelin-1	GAACTCAGGGCTGAAGACATTATGGA	TGGTTTGTCTTAGGTGTTCCTCTGAAC
EDN2	Endothelin-2	CAGCCAGCGTCCTCATCTCATG	GAGCTGTCTGTTCAGGAGTGTTCA
EDNRA	Receptor for endothelian-1	TATCAATGTATTTAAGCTGCTGGCTGG	CTGCTCTGTACCTGTCAACACTAAGA
EFNA1	Ephrin A1	AACAGTTCAAATCCCAAGTTCCGGA	GTACAGTATGTACTGCTCCATGGCA
GMFB	Glial maturation factor	AACGACAACCTCGCTTCATTGTGTATA	CAAATACCTTGGTTAGTTCAGCTGTCTG
NPTX1	Neuronal Pentraxins	AGGAGAGGGTCAAGATCGAGACC	CATATAGTTGGTCCGCAGTGGGAA
NGR2	Growth factor (neuregulin)	CAGATTATATTTCCAAGAACGTGCCAGC	TGGAGCAGTGGTGAGAAGGAGAA
SIRT1	Sirtuin-1	TATATCCTGGACAATTCCAGCCATCTC	GTTGCAAAGGAACCATGACACTGAA
SRXN1	Sulfiredoxin-1	GACACGATCCGGGAGGACC	CCCAAAGGAGTAGAAGTAGTCACCTC
STAB1	Stabilin-1	TGTGTGCACGGAGTGTGCAA	CGGGCAGCTCTTGATCACAGT
EEA1	Early endosome antigen-1	AGCTCTTCAGAGGGTTTCATATGTCC	CCATGACCTGAGTCATTACCAGCAT
C10orf10	transcription factor	CATCACCGCACGTTTCAGTGG	ACTCCCCAAAAAGCCAGTCCA
YWHAZ	tyrosine 3-monooxygenase	GAAAGGGATTGTCGATCAGTCACAAC	CCAGTCTGATAGGATGTGTTGGTTGC

254x338mm (300 x 300 DPI)