Supplemental Experimental Procedures

Bacterial and tissue cell culture

Ab5075 cells were grown to stationary phase in nutrient broth (BD). H292 cells were grown in RPMI-1640 (Thermo) with 10% fetal bovine serum and 1% penicillin/streptomycin (37^{o}C, 5% CO_2). Transposon mutants in Ab5075 were attained from Dr. Colin Manoil [80].

Biotin-Aspartate Proline-PIR n-hydroxyphthalimide Synthesis

Biotin-Aspartate Proline-PIR *n*-hydroxyphthalimide (BDP-NHP) was synthesized as previously described [28, 81]. Briefly, BDP was synthesized on bead supports using an APPTec peptide synthesizer. Activated crosslinker was formed by esterifying BDP with NHP-trifluoroacetic acid. Conjugated BDP-NHP was eluted (95% TFA/5% DCM), resuspended in anhydrous DMSO, aliquoted, and stored at -80^{o}C.

Infection, Stage-1 database creation sample preparation

Confluent H292 cells were washed twice with PBS, and once with 170mM Na_2HPO_4 pH 8.0 (XL-buffer). Cells were released by scraping (biological replicate of **H**uman cells infected with ***A***. *baumannii*, HA-1) or 10mM EDTA pH 8.0 treatment for 15 min (biological replicates HA-2 and HA-3). HA-2 and HA-3 cells were washed with RPMI containing calcium and magnesium before infection. Cells were pelleted (1500 rpm, 5 min, room temperature [RT]), resuspended in XL-buffer containing Ab5075 cells at an MOI of 500, mixed gently and co-incubated at RT at 300 rpm in XL-buffer for 2 hours (Figure S6). BDP-NHP (8mM) was added to the cell suspension for 1 hour. Infected cells were washed with Na_2HPO_4, pelleted, and frozen at -80^{o}C. Five experiments were run: three biological replicates of Ab5075-infected H292 cells (Figure S6), and two biological replicates of uninfected H292 cells (Table S2).

Infected H292 cell pellets were resuspended in lysis buffer: 8M urea, 100mM Tris-Cl pH 8.0, 150mM NaCl, and protease inhibitor tablets (Roche). Resuspended cells were lysed by cryo-grinding three times at 30Hz for 1min. Lysates were sonicated 5 times at 50% intensity for 20 seconds each time and protein concentrations tested by BCA Assay (Pierce). Proteins were reduced (30min, 5mM DTT, 55^{o}C) and alkylated (1 hour, dark, 15mM iodoacetamide, RT). Excess iodoacetamide was neutralized with an additional 5mM DTT for 15 min. Protein lysates were diluted with 100mM Tris-Cl, pH 8.0 to reduce the [urea]. 1mg of protein was removed to enrich full length crosslinked proteins with monomeric avidin beads (Thermo). Enriched, crosslinked proteins were digested with sequencing grade trypsin (Promega), and injected on an in-house pulled 45cm C-8 column (Magic, 200A, 5um) eluting into an LTQ-XL or LTQ-Velos-FT-ICR mass spectrometer (Thermo) to create a search database of proteins that have been crosslinked (Stage-1 database)[28]. Digested Stage-1 peptide samples were shot in quadruplicate on a 4 hour method as follows: MS-buffer A (0.1% formic acid in water) and MS-buffer B (0.1% formic acid in acetonitrile [ACN]); 0-1min 2-10% MS-buffer B, 1-241min

10-40% MS-buffer B, 241-261min 40-80% MS-buffer B, 261-281min 80-2% MS-buffer B. Spectra were searched using SEQUEST. 30880 peptide-spectral matches (10304 unique peptides) were identified at a false discovery rate (FDR) of less than 1%, based on a concatenated, target-decoy database of all human and *A. baumannii* (AB0057) proteins [28]. The final Stage-1 database consisted of 925 human proteins (3084 non-redundant peptides) and 1096 *A. baumannii* proteins (6946 non-redundant peptides).

The remaining protein lysates were digested with sequencing grade trypsin at a ratio of 1:200 for 16 hours at 37^{o}C. Digested peptides were desalted using C-18 SepPak's (Waters). Desalted peptides were fractionated for **1)** HA-1 using an in-house packed strong cation exchange (SCX) column with polysulfoethyl aspartamide beads (Nest Group) using a step gradient of LC-buffer A (25% ACN, 1% formic acid) to LC-buffer B (500mM ammonium acetate, 25% ACN, 1% formic acid) at 10%, 25%, 50%, 75% and 100%, followed by a step with LC-buffer C (1M ammonium acetate, 25% ACN, 1% formic acid) or **2)** for HA-2 and HA-3 on a Phenomenex Luna SCX column from 100% buffer A (30% ACN, 7mM KH_2PO_4, pH 2.5) to 30% buffer B (30% ACN, 7mM KH_2PO_4, 350mM KCl, pH 2.5). Eluted peptides were dried and resuspended in 100mM NH_4HCO_3 pH 8.0; the pH was adjusted to 8 with NaOH. Peptides were incubated with monomeric avidin beads for 30 min at RT to enrich crosslinked peptides. Enriched peptides were washed five times with 100mM NH_4HCO_3 pH 8.0, eluted with 70% ACN/1% formic acid and dried by vacuum centrifugation.

LC-MS/MS/MS, ReACT, database searching, and data analysis

Crosslinked peptides were resuspended in 5% ACN/2% formic acid and injected onto an in-house pulled 45cm C-8 column (Magic, 200A, 5um) run on 4 hour gradients as with the Stage-1 mass spectral analysis; eluted peptides were analyzed on an LTQ-Velos-FT-ICR. Crosslinked peptides were fragmented in a data-dependent ReACT mode (Real-time Analysis for Crosslinked peptide Technology)[28]. Briefly, high-charge state precursor ions (MS^1, z > 4+) were isolated and fragmented at low energy (Q = 0.20) to release crosslinked peptides and a reporter ion (m/z = 752.41). Data-dependent selection of fragmented MS^2-ions that sum to the precursor mass minus the reporter ion were further fragmented for MS^3 spectra and peptide sequencing.

Spectra were searched against a target-decoy database containing Stage-1 protein sequences. All parameters and filtering was done as previously described to identify unique PPIs and site-site interactions[28]. The final relationship FDR was calculated to be 0.24% [(2 * 20 decoy relationships with at least 1 reverse hit)/16758 total relationships]. The final PPI FDR was 1% [(2 * 4 unique decoy PPIs)/719 total unique PPI's]. **All interaction data, including pep.xml files, can be found at http://brucelab.gs.washington.edu/xlinkdb/.** Protein interaction networks were created using Cytoscape 3.0. Protein structures for OmpA and desmoplakin were downloaded from the

Protein Databank (4G4Y and 1LM5, respectively). Protein structure interactions were modeled using PatchDock[55]. KEGG pathway enrichment p-values were determined using STRING v9.1 [37].

Gentamicin protection assays

H292 cells were plated in 24-well plates (~370000 cells/well) and allowed to attach for 16 hours. H292's were incubated with Ab5075 or Ab5075-*tn-ompa* for 3 hrs in serum-free, antibiotic-free RPMI-1640 media (RPMI). The supernatant was saved to normalize AB5075 growth during infection. H292 cells were incubated for 1 hour with 200μg/ml gentamicin in RPMI at 37^{o}C. 500μl of lysis buffer (PBS with 0.1% Trition-X100) was added to each well, and the plate was shaken at 200rpm for 10 min. Three wells were pooled, and two pools were quantified for each experiment. Lysates (50ul) were cultured on NB-agar plates (16 hours, 37^{o}C). Colonies were counted and normalized to WT AB5075 growth. Values are the average of technical duplicates from at least three experiments performed on three different days. For antibody blocking of OmpA-based invasion, Ab5075 cells were pre-incubated for 1 hour with a 1:2000 dilution of α-OmpA and PBS-control serum (Dr. Michael McConnell) or purified Genscript synthesized antibodies [rabbit]) at RT in RPMI. Pre-treated Ab5075 cells were then incubated with H292 cells as above.

Confocal immunofluorescence and brightfield microscopy

H292 cells were grown to confluency and infected with Ab5075 (MOI = 100), as described above on 3.5cm plates with glass coverslips (No. 1.5, Mattek). For confocal immunofluorescence, cells were fixed (formalin), blocked (3% milk in PBST) and incubated overnight at 4^{o}C with α-DSP antibody (rabbit, Abcam) and primary mouse α-OmpA serum (Dr. Michael McConnell) in blocking buffer. Microscopy was performed with a Nikon A1 confocal mounted on a Nikon TiE inverted microscope (Garvey Cell Imaging Lab) at 20x magnification, n=1 (air), NA=0.75. Depth of field measured based on λ = 595nm. For brightfield microscopy, H292 cells were grown to confluency on 24 well plates, incubated in crosslinking buffer +/- Ab5075 cells (MOI=100), washed with PBS and imaged at 20x magnification.

Murine acute lung infection

WT Ab5075 or *tn-ompA* were streaked on LB agar or LB agar with 5ug/ml tetracycline (LBtet) from frozen stocks. PBS (50ul) containing ~2x10^{8} CFU/ml bacteria were administered intratracheally to anesthetized mice as described before[82]. Animals that became moribund, distressed or were unable to eat/drink were euthanized using a CO_2 chamber. Experiments were approved by the University of Washington Institutional Animal Care and Use Committee (protocol number 4113-01).

Co-immunoprecipitation (Co-IP) and western blotting

H292 cells were grown to confluency, washed twice with PBS, and once with RPMI. At an MOI of 500, AB5075 cells were co-incubated with H292 cells for 2 hours at RT then either DMSO or BDP-NHP in DMSO was added to the samples followed by 1 hour incubation at RT, 300rpm. Cells were

pelleted and resuspended in IP buffer (10mM Tris-Cl pH 8.0, 100mM NaCl, 1% Triton-X100, 1mM EDTA, protease inhibitors, 100µg/ml lysozyme) and incubated on ice for 30min to lyse bacterial cells; samples were syringe pumped 5 times through a 27.5 gauge needle and sonicated 2 times at 50% intensity for 20 seconds each time to lyse cells and fragment DNA.

Lysates were pre-cleared with 50µl of Protein-G-agarose (Thermo) mixed at 4^{o}C for 1 hour, followed by pelleting (3000 rpm, 5 min, 4^{o}C). α-Desmoplakin antibody (2µg/sample, Bethyl Laboratories) was added for 16.5 hours at 4^{o}C. To precipitate, 50µl of Protein-G-agarose was added for 3 hours at 4^{o}C. Beads were pelleted at 2500 x g for 3min at 4^{o}C, washed three times with IP buffer, and once with PBS. Co-IP proteins were eluted at 95^{o}C for 10 min with a 3:1 ratio of 1% SDS, 15% glycerol, 50mM Tris-Cl pH 8.0, 150mM NaCl : XT Sample Buffer (Biorad).

Proteins were detected by SDS-PAGE (4-20% Mini-Protean TGX), semi-dry transfer (Biorad) to low fluorescence PVDF membranes (Millipore) and blocked (5% milk/TBST). Primary mouse α-OmpA serum was added (1:700 in 5% milk/TBST) and incubated for 16 hours at 4^{o}C. Secondary α-mouse antibodies (IRDye, Li-Cor) in 5% milk/TBST were added for 1 hour at RT. Western blots were imaged using a Li-Cor imager.

Supplemental Information

Tables:

Table S1, related to Figure 1: All crosslinked peptide/site relationships identified across three biological replicates of Ab5075-infected H292 cells.

Table S2, related to Figure 1: Dataset of all crosslinked peptide/site relationships identified for uninfected H292 cells.

Table S3, related to Figure 1: All crosslinked peptide/site relationships identified for Ab5075-alone dataset.

Figures:

Figure S1, related to Figure 1: Comparison of technical and biological replicates for peptide and protein interactions, respectively and characterization of proteins within PPI network.

Figure S2, related to Figure 1: Known human protein complexes identified in XL-MS PPI network.

Figure S3, related to Figure 4: Characterization of transposon mutant of OmpA and anti-OmpA antibodies.

Figure S4, related to Figure 5: Full image of DSP immunoprecipitation and OmpA Western blot.

Figure S5, related to Figure 5: Model of random chance of matching two proteins in direct physical interactions with OmpA and comparison of interspecies interactions to essential *A. baumannii* persistence genes.

Figure S6, related to Experimental Procedures: Survival and infection of H292 cells.

Figure S1, related to Figure 1: Comparison of technical and biological replicates for peptide and protein interactions, respectively and characterization of proteins within PPI network. (a) Overlap of peptide-peptide relationships across technical replicates and (b) protein-protein interactions showed high degrees of commonality. (c) Human proteins identified in crosslinked relationships were analyzed for abundance compared to Beck, et al.[1] Protein abundances cover greater than 5 orders of magnitude. Protein copies per cell roughly correlated with the number of peptide spectral matches (PSMs) for each protein. (d) Distribution of characterized, uncharacterized and putative proteins in *A. baumannii* proteome. Relative numbers of uncharacterized, putative and annotated proteins are shown based on Ab0057. (e) Enrichment of *A. baumannii* proteins in interspecies interactions for predicted signal peptides and OMV proteins. Signal peptides (indicative of secretion into the periplasm/outer membrane/extracellular space) were predicted using SignalP 4.1[4] across the full Ab0057 database. OMV proteins identified by Kwon et al.[5] were used to test enrichment within interspecies interactions.

Figure S2, related to Figure 1: Known human protein complexes identified in XL-MS PPI network. Desmoplakin (DSP) is highlighted with a blue outline within the host cytoskeleton network. Specific histones are highlighted by color within the histone network. Uniprot gene names and accessions are included for each protein node.

Figure S3, related to Figure 4: Characterization of transposon mutant of OmpA and anti-OmpA antibodies. (a) Western blotting of wild-type *A. baumannii* strains (Ab5075 and Ab19606) and the transposon insertion mutant of OmpA (*tn-ompa*). Overexposed blots demonstrate the lack of OmpA protein at 38kDa in each of three separate biological replicates of the *tn-ompa* mutant. The primary was anti-OmpA serum from Dr. Michael McConnell. (b) Western blot using purified polyclonal antibodies synthesized against various OmpA peptides. Purified polyclonal antibodies raised in mice (Genscript) were tested for their ability to recognize OmpA at 38kDa from Ab5075 cell lysates. (c) The

various peptide epitopes are shown along with their relative amino acid number within the OmpA sequence. Peptide epitopes were mapped onto a predicted OmpA structural model[3].

Figure S4, related to Figure 5: Full image of DSP immunoprecipitation and OmpA Western blot. (a) OmpA at 38kDa was observed to be precipitated along with DSP. High mass OmpA bands corresponding to potential DSP-OmpA complexes are shown. (b) PatchDock structural models of the interaction of the C-termini of DSP and OmpA[6]. Crosslinked lysine sites identified between the two proteins are highlighted in magenta.

Figure S5, related to Figure 5: Model of random chance of matching two proteins in direct physical interactions with OmpA and comparison of interspecies interactions to essential *A. baumannii* persistence genes. (a) Nodes represent proteins; edges represent protein-protein interactions (probability of matching specific lysine site relationships would be lower, requiring consideration of each individual lysine within one of the three proteins). The p(AB) assumes that A is OmpA which has 104 crosslinked interactors in the Ab5075-alone dataset. The model is based on the total # of proteins identified during Stage-1 analysis of interspecies interactions for p(XB) and p(AX). (b) Enrichment of *A. baumannii* proteins in interspecies interactions for essentiality in bacterial persistence in murine lung based on Wang et al[2]. Comparing the enrichment of identified interspecies interactions for genes that are essential for *A. baumannii* persistence in murine lungs compared to their enrichment in the background Ab5075 proteome. Fisher's Exact Test was used to derive the p-value.

Figure S6, related to Experimental Procedures: Survival and infection of H292 cells. (a) To ensure H292 cells exposed to crosslinking buffer conditions remained alive cells were incubated in RPMI with additives, RPMI without additives or crosslinking buffer for 2 hours then plated and grown for 24 hours. (b) H292 cells infected with Ab5075 for 3 hours were imaged.

1 Beck, M. *et al.* The quantitative proteome of a human cell line. *Molecular systems biology* **7**, 549, doi:10.1038/msb.2011.82 (2011).
2 Wang, N., Ozer, E. A., Mandel, M. J. & Hauser, A. R. Genome-wide identification of Acinetobacter baumannii genes necessary for persistence in the lung. *mBio* **5**, e01163-01114, doi:10.1128/mBio.01163-14 (2014).
3 Kelley, L. A. & Sternberg, M. J. Protein structure prediction on the Web: a case study using the Phyre server. *Nature protocols* **4**, 363-371, doi:10.1038/nprot.2009.2 (2009).
4 Petersen, T. N., Brunak, S., von Heijne, G. & Nielsen, H. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature methods* **8**, 785-786, doi:10.1038/nmeth.1701 (2011).
5 Kwon, S. O., Gho, Y. S., Lee, J. C. & Kim, S. I. Proteome analysis of outer membrane vesicles from a clinical Acinetobacter baumannii isolate. *FEMS microbiology letters* **297**, 150-156, doi:10.1111/j.1574-6968.2009.01669.x (2009).
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7 Kumar, R. & Nanduri, B. HPIDB--a unified resource for host-pathogen interactions. *BMC bioinformatics* **11 Suppl 6**, S16, doi:10.1186/1471-2105-11-S6-S16 (2010).

Figure S1

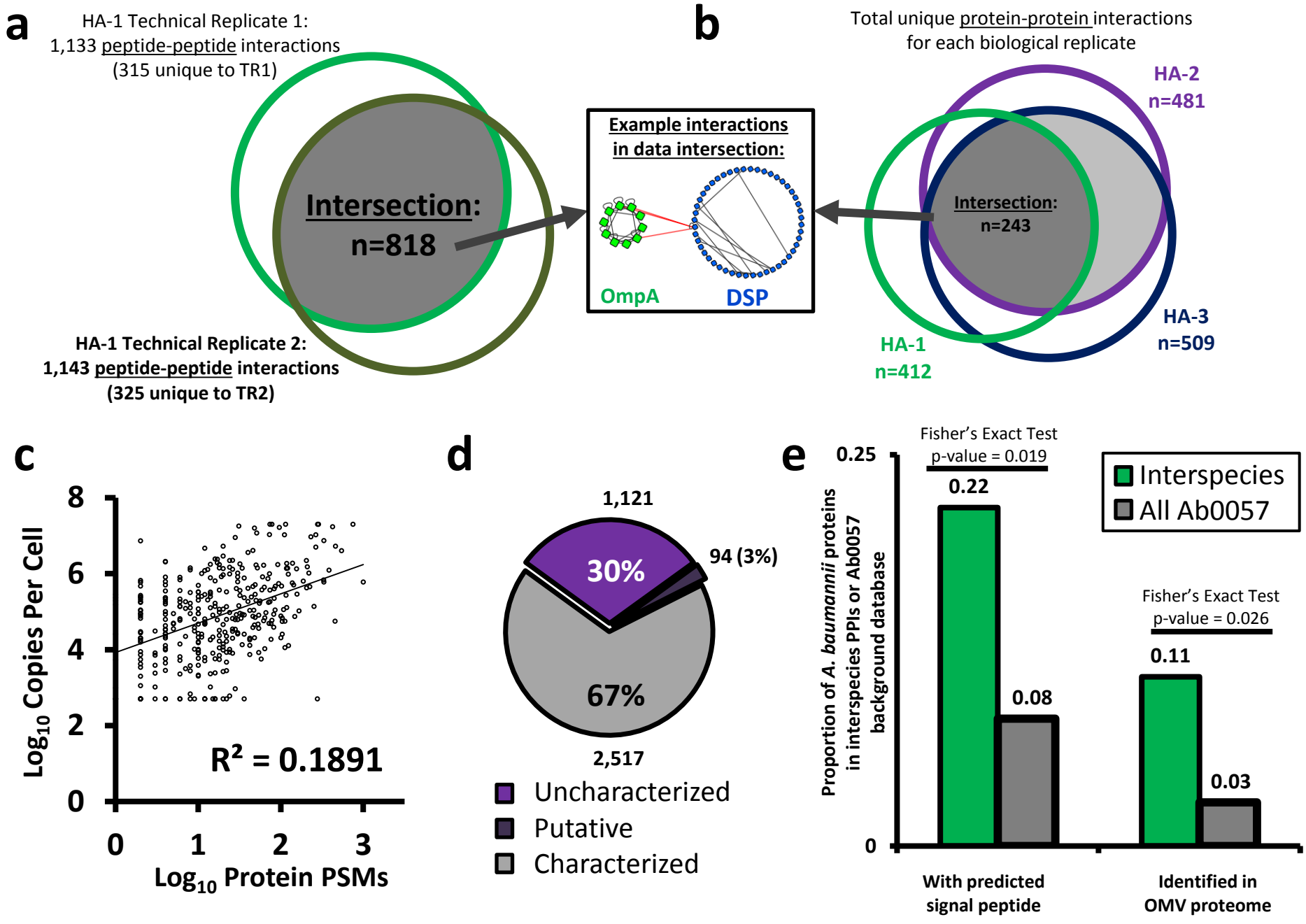
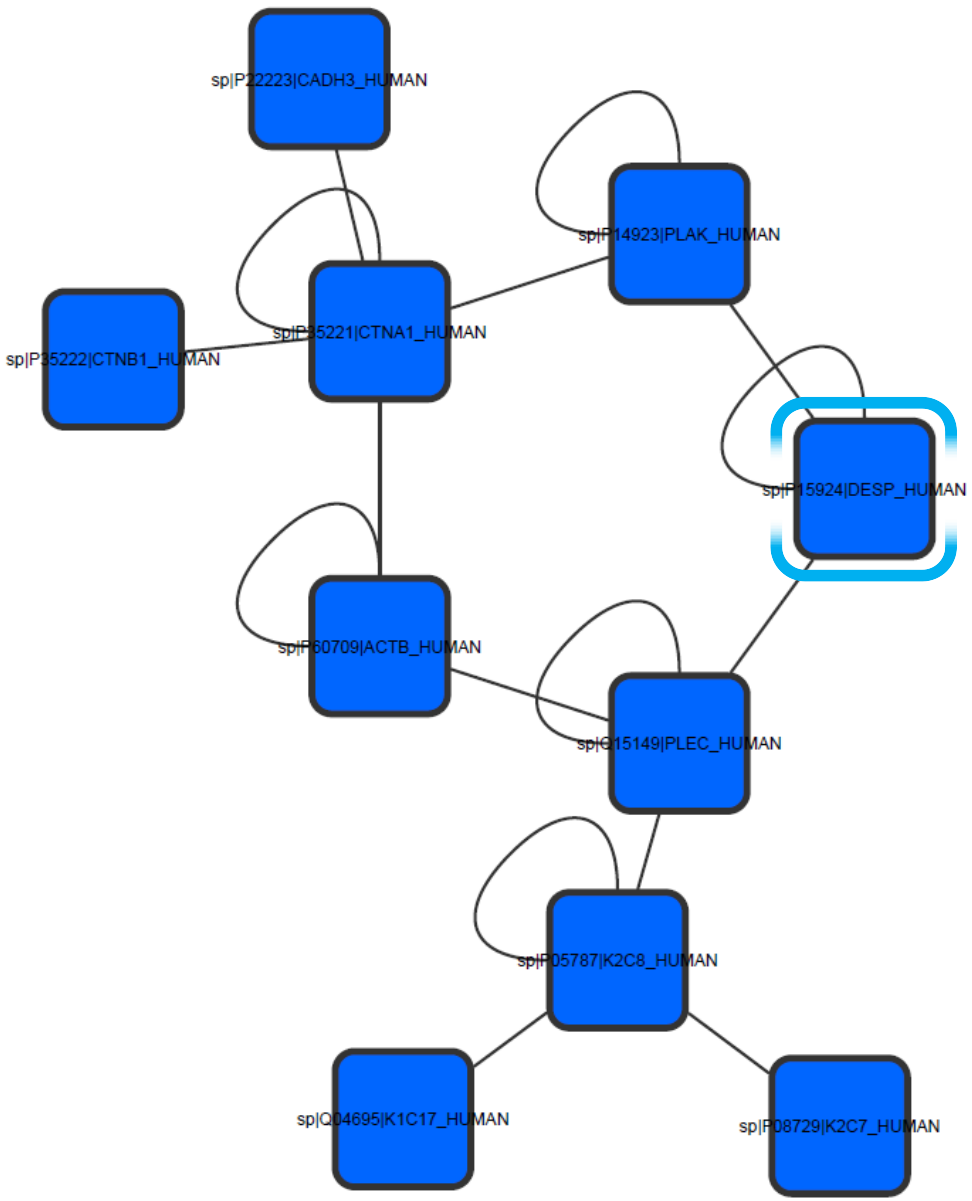

a
HA-1 Technical Replicate 1:
1,133 peptide-peptide interactions
(315 unique to TR1)
Intersection:
n=818
HA-1 Technical Replicate 2:
1,143 peptide-peptide interactions
(325 unique to TR2)
b
Total unique protein-protein interactions
for each biological replicate
Example interactions
in data intersection:
OmpA
DSP
HA-2
n=481
Intersection:
n=243
HA-3
n=509
HA-1
n=412
c
Log10 Copies Per Cell
Log10 Protein PSMs
R² = 0.1891
d
1,121
30%
94 (3%)
67%
2,517
Uncharacterized
Putative
Characterized
e
Fisher's Exact Test
p-value = 0.019
0.22
0.08
Fisher's Exact Test
p-value = 0.026
0.11
0.03
Interspecies
All Ab0057
Proportion of A. baumannii proteins
in interspecies PPIs or Ab0057
background database
With predicted
signal peptide
Identified in
OMV proteome

Figure S2

Host cytoskeleton (DSP)



Heterogeneous ribonucleoproteins

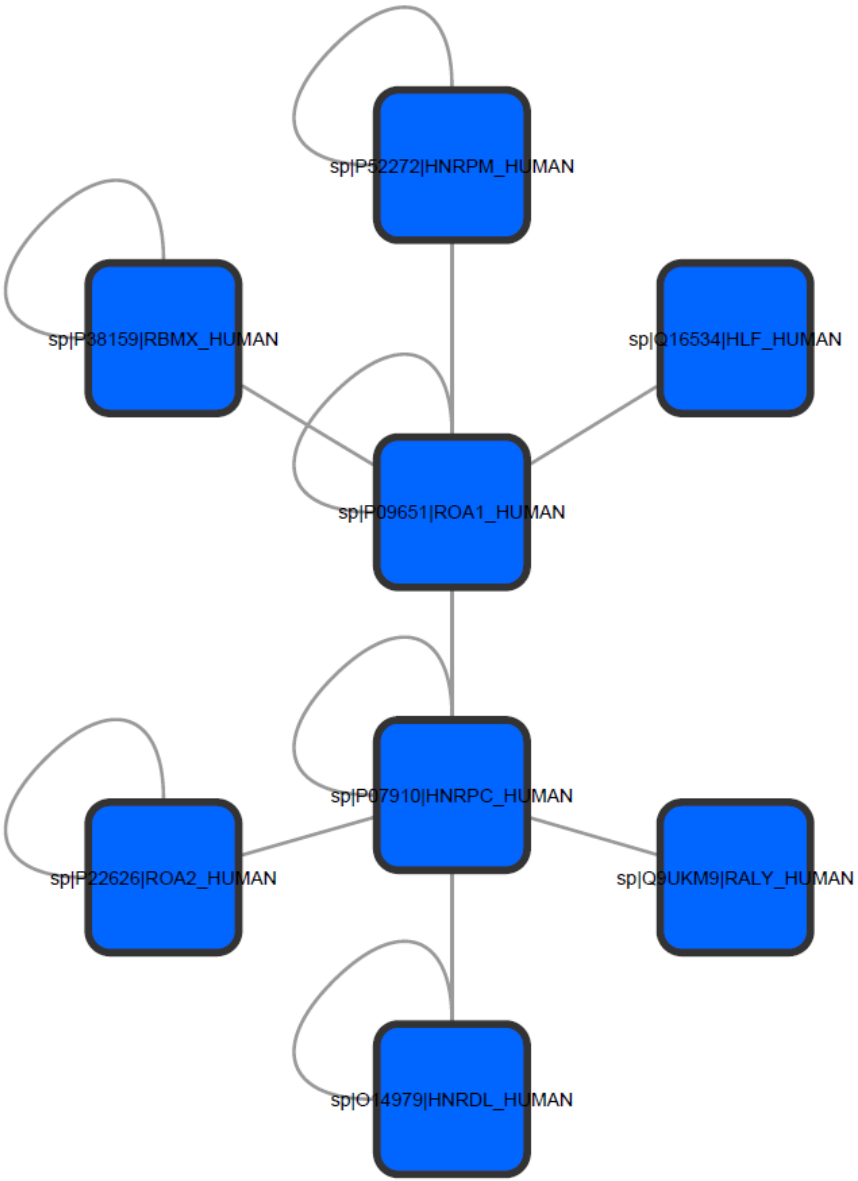
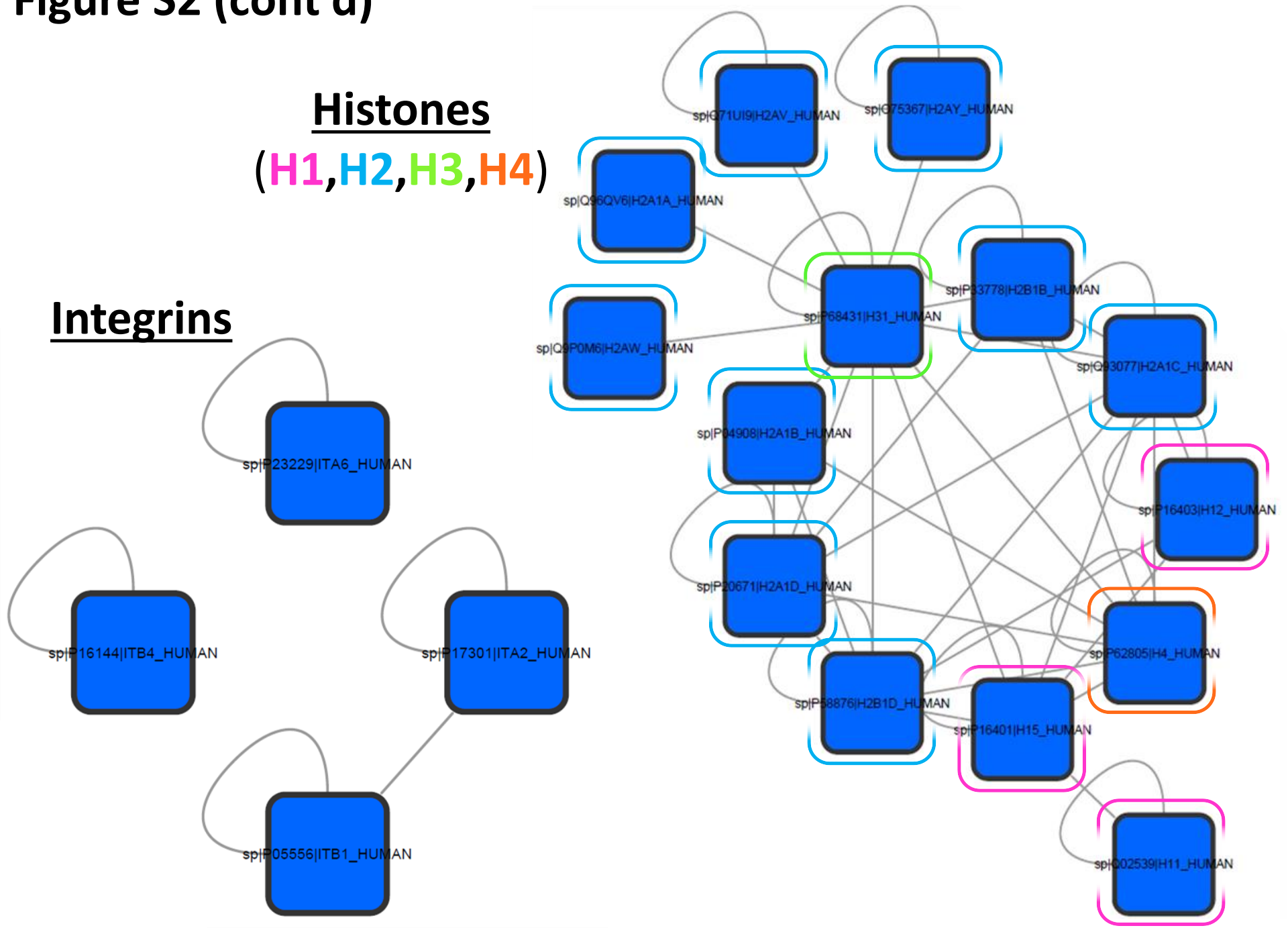


Figure S2 (cont'd)



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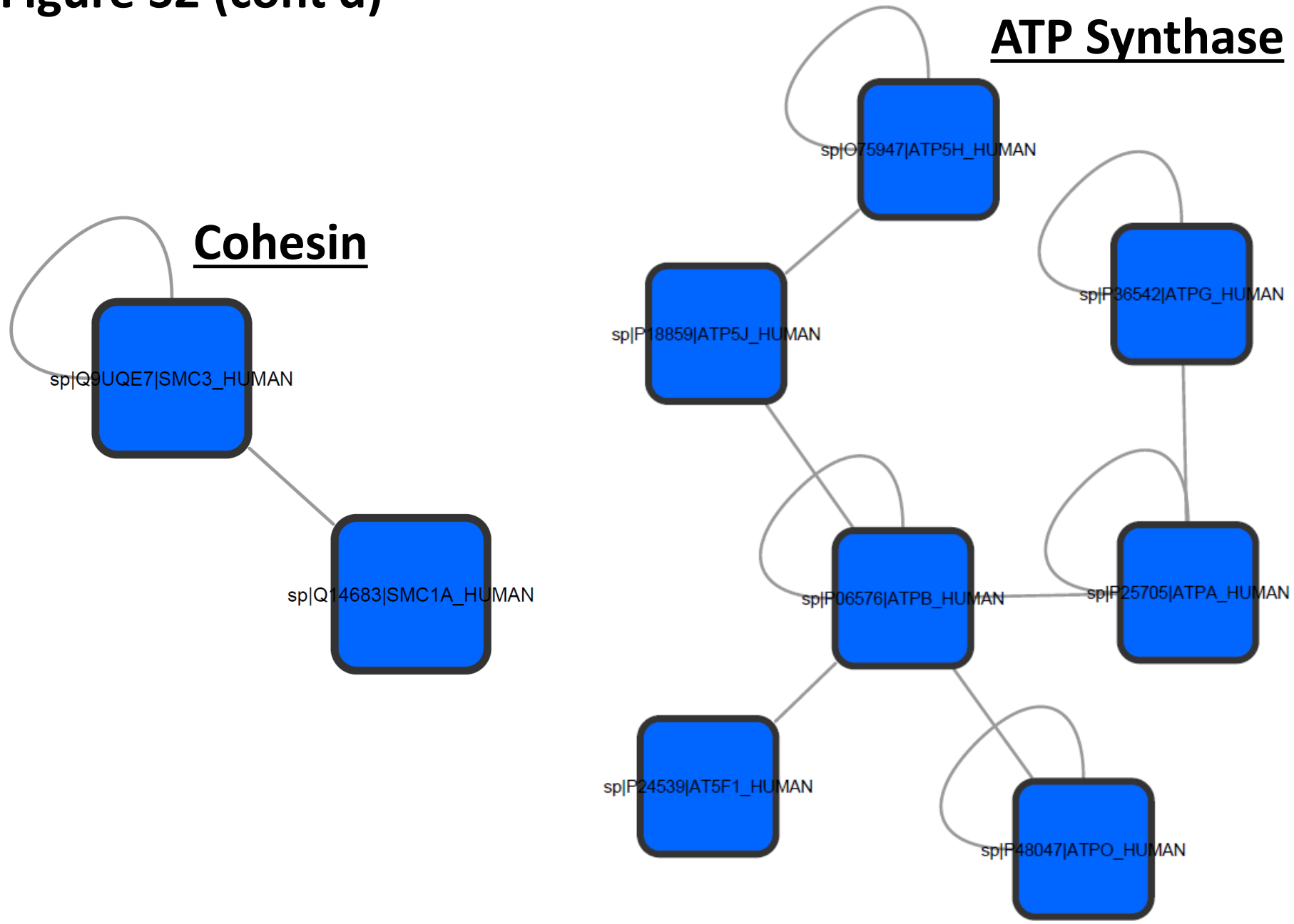
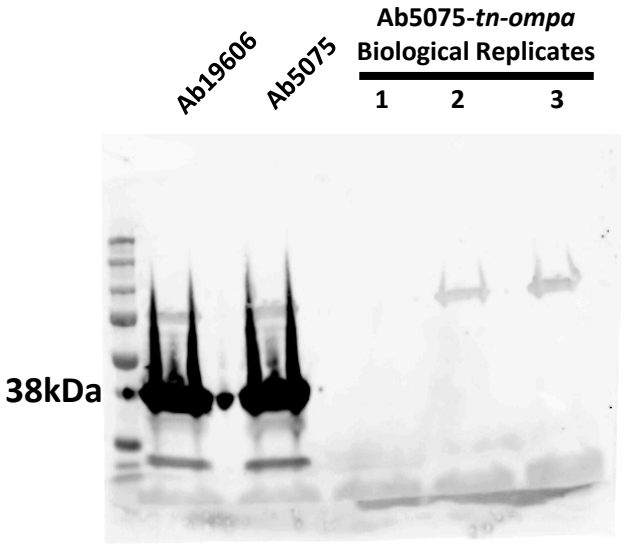


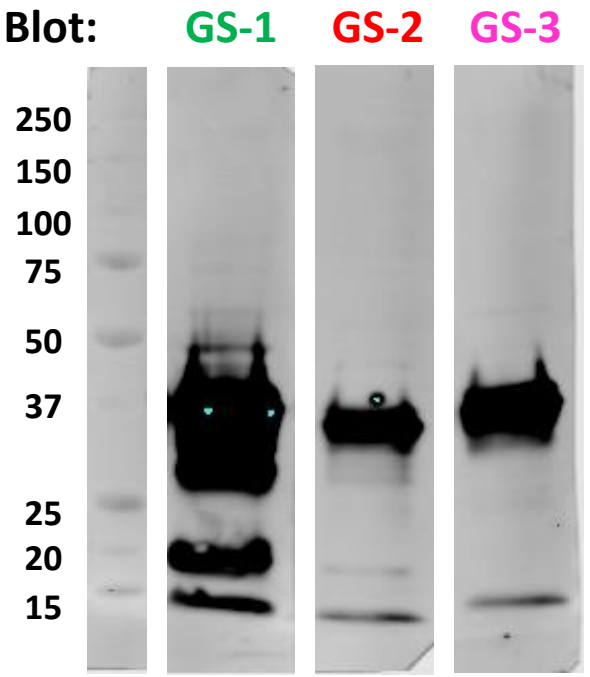
Figure S3

a

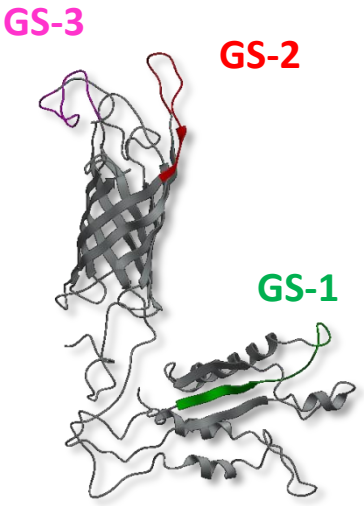
Anti-OmpA western blot of wild-type Ab19606, Ab5075 and biological replicates of *tn-ompa* mutant at high exposure.



b



c

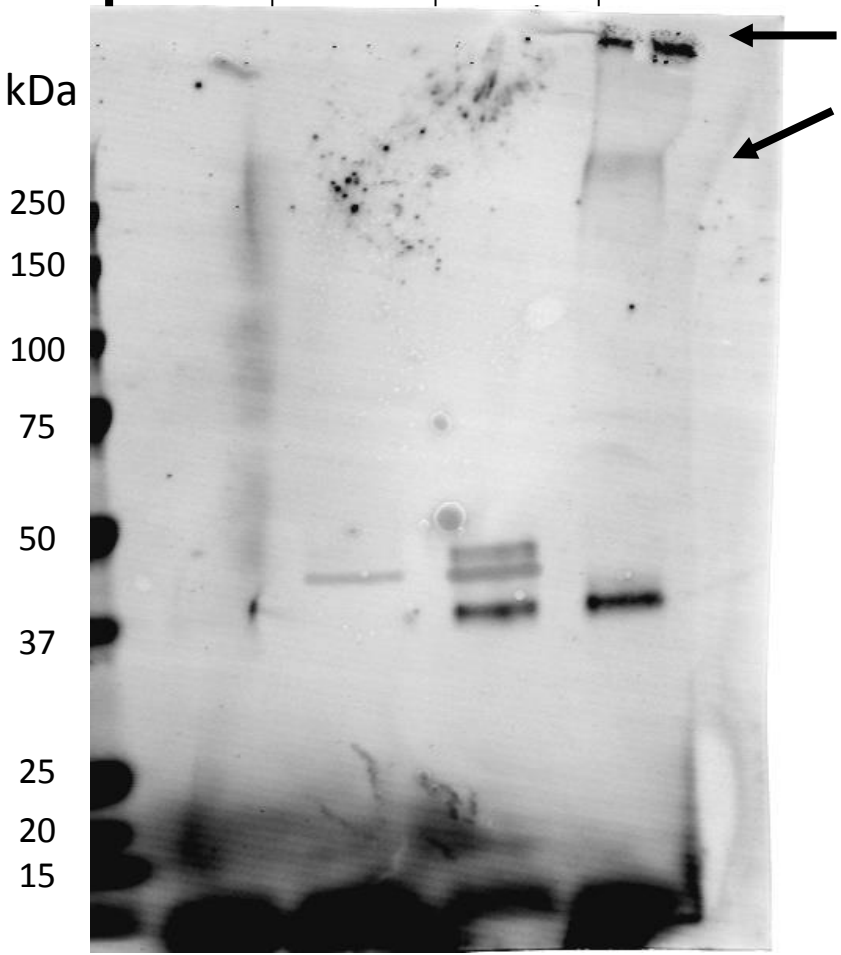


Primary Antibodies	GS-1	GS-2	GS-3
Epitope	CARIEGHTDNTGPRK	CIPDLSYHNDEEGTL	GGKDGELTNGPELQC
OmpA amino acids	261-274	134-147	43-56

Figure S4

a

Pulldown: Desmoplakin (*H.s.*)		WB: OmpA (*A.b.*)	
Ab5075	+	+	+
H292	+	+	+
Crosslinker			+



DSP-OmpA Complex

b

Patchdock Model of OmpA C-terminus and DSP C-terminus

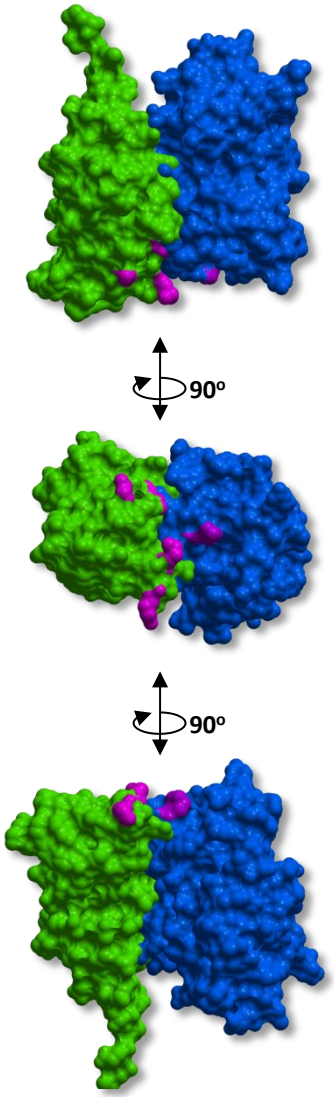
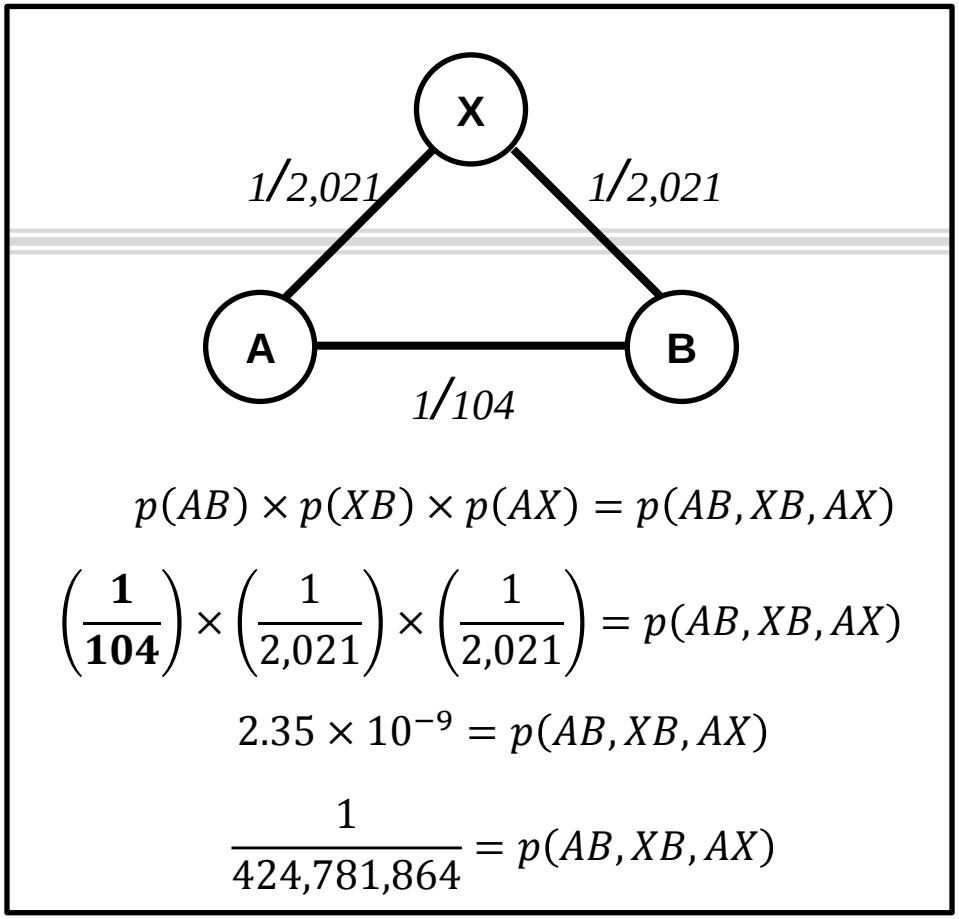


Figure S5

a



$$p(AB) \times p(XB) \times p(AX) = p(AB, XB, AX)$$

$$\left(\frac{\mathbf{1}}{\mathbf{104}}\right) \times \left(\frac{1}{2{,}021}\right) \times \left(\frac{1}{2{,}021}\right) = p(AB, XB, AX)$$

$$2.35 \times 10^{-9} = p(AB, XB, AX)$$

$$\frac{1}{424{,}781{,}864} = p(AB, XB, AX)$$

b

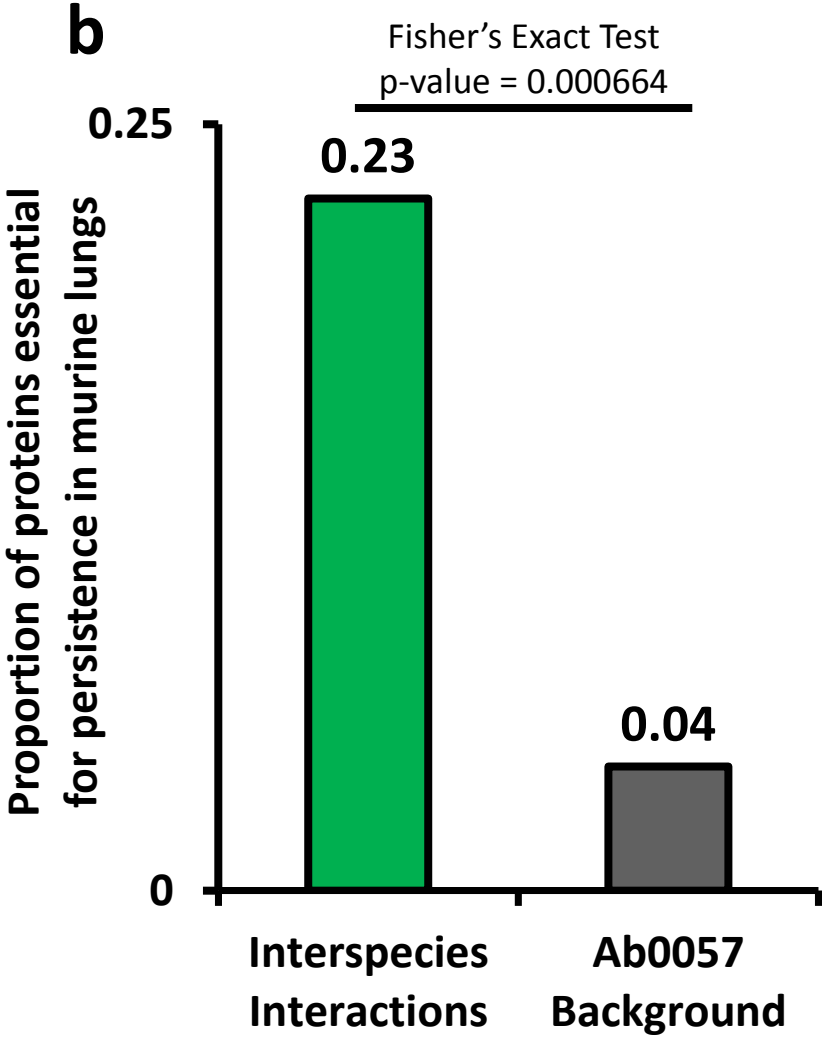
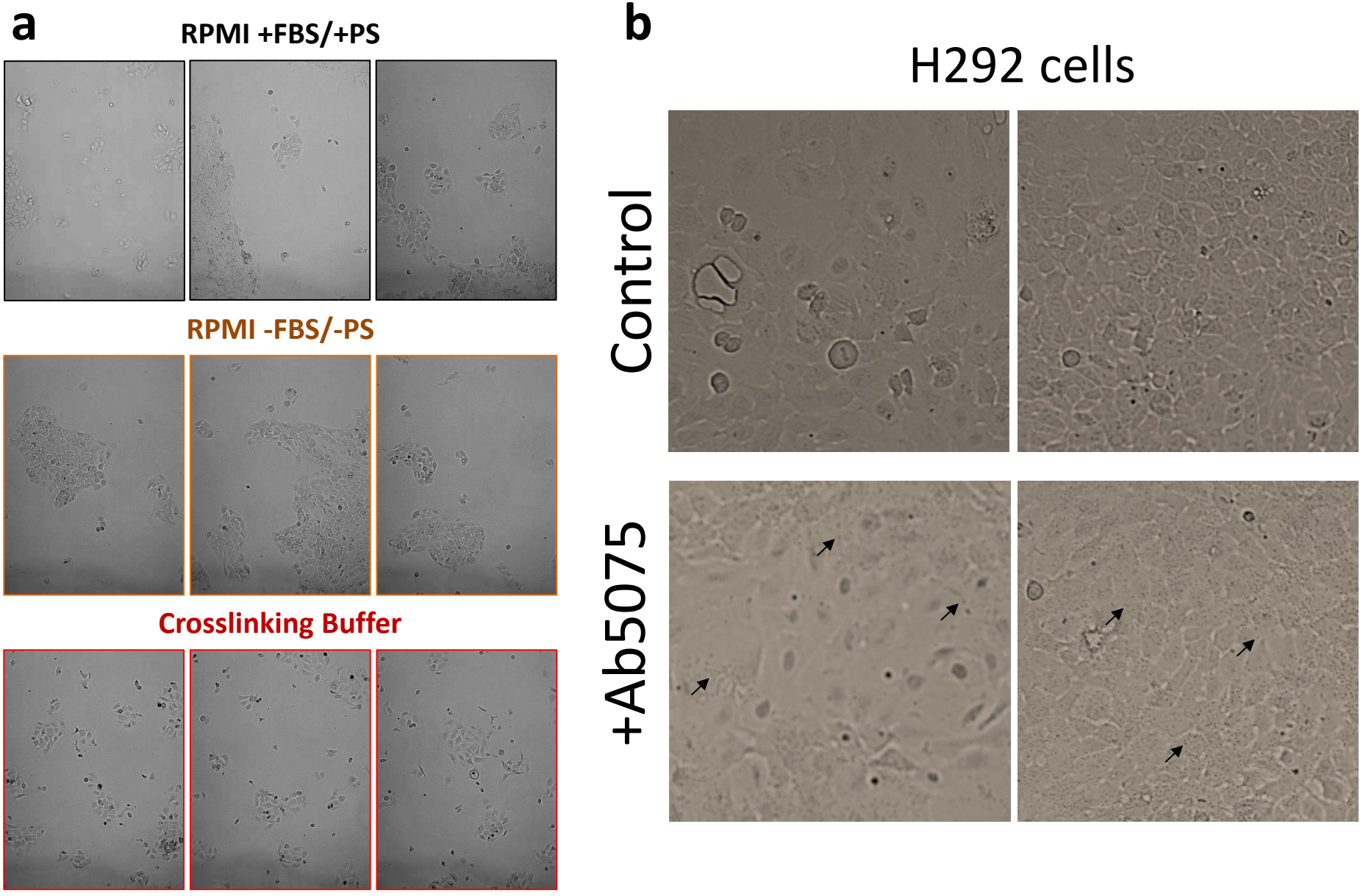


Figure S6


a
RPMI +FBS/+PS
RPMI -FBS/-PS
Crosslinking Buffer
b
H292 cells
Control
+Ab5075