Supplemental Figure legends

Supplemental Figure S1. PeptideProphet Sensitivity and Error Rate graphs of estimated total number of correct peptide assignments in dataset: 7846.5 (Set 1), 6874.5 (Set 2), and 6510.4 (Set 3). Sensitivity (Red Line): fraction of all correct assignments (7846.5 (Set 1), 6874.5 (Set 2), and 6520.4 (set 3)) passing MPT filter Error (Green Line): fraction of peptide assignments passing MPT filter that are incorrect MPT = Minimum Probability Threshold to Accept

Supplemental Figure S2. (A) Consistency of the protein abundance within an experiment and cross the experiments. Boxplots of the peptide intensities (mean area of spectrum) with different tags in all three experiments. (B) Correlation of the protein ratio WT/KO of two independent experiments (1 vs 2) which shows the consistency of the labeling effect across the experiments. (C) Quantile normalization of protein abundance of replicates within one set of experiment. (D) Principal components analysis for the technical replicates (114 *vs* 115, 116 *vs* 117) and biological replicates [A, B or C].

Supplemental Figure S3. Cavin-1 silencing reduces caveolin levels from the plasma membrane and Cavin-1 does not directly interact with caveolins in EC. (A) Cavin-1 silencing reduces Cav-1 and -2 protein levels in EC. Bands represent quantification of Cavin1, Cav-1 and/or Cav-2 protein levels at 96h of NS or siRNA Cavin-1 treatment. Blots at bottom right are a representative experiment of Cavin-1 silencing in BAEC cells (96 h). (B) Representative immunofluorescence analysis of either endothelial Cav-1

(green) or Cav-2 (green) after Cavin-1 siRNA. DAPI staining (blue). The scale bar represent 50μm. (C) co-localization of Cavin-1 and Cav-1 in endothelial cells is dramatically reduced by Cavin-1 silencing. After Cavin-1 silencing, EAhy.926 were fixed and subjected to immunofluorescense staining for Cavin-1 (green) and Cav-1 (red). DAPI staining (blue). The scale bar represent 50μm. (D) The interaction of Cavin-1 with Cav-1 and -2 is dependent of EC raft integrity. Immunoprecipitation of Cav-1, Cav-2, Cavin-1, or nonspecific IgG was performed in Eahy926 cells in the presence of 1% Triton X-100 buffer or β-octylglucoside buffer, and the protein complex was detected by Western blotting. (E) expression and (F) interaction of recombinant GST-Cav-1 with Cavin-1 in lysates from Eahy926 cells. Lysates were incubated with GST alone or GST-Cav fusion proteins (Cav-1-178), Cav-1 (1-61), Cav-1 (61-101), or Cav-1 (135-178), and the binding of Cavin and Cav-2 was assessed by Western blotting. Equivalent amounts of cell lysates, GST, and GST fusions were used. Data are representative of two independent experiments.

Supplemental Figure S4. Cavin-1 is not sufficient to recruit Cav-2 to plasma membrane but its presence stabilizes high molecular weight oligomers. Cavin-1 increases high molecular weight oligomers of caveolins. HEK 923 cells were transfected with an empty vector (pcDNA3) or with Cavin-1, Cav-1, Cav-2, or different combinations for 36 h and subjected to SDS/PAGE for evaluating protein expression by (A) immunobloting. (B) Transfected cells were lysed in MES-buffered saline buffer (pH 6.5), containing 60 mM β-octyl-glucoside and loaded on top of a 5-50% linear sucrose gradient and subjected to velocity gradient centrifugation. Equal volumes of collected

fractions were resolved in SDS/PAGE and subjected to Western blotting. Arrowheads denote migration of molecular weight standards in the linear sucrose gradient. Bar graph at right denotes relative intensities from immunoblots quantified by using ImageJ (NIH). (C) COS7 cells were transfected with Cav-1, Cav-2, Cavin-1 or different mix for 36h, fixed and double stained with either Cav-1, Cav-2, Cavin-1 or phalloidin. Arrowhead denotes perinuclear Cav-2 in cell expressing Cav-2 alone or co-expressing Cavin-1 and Cav-2. DAPI staining (blue).

Supplemental Figure S5. eNOS mRNA expression does not change after Cavin-1 silencing. Time course of eNOS expression in Eahy926. Bars indicate quantification of eNOS mRNA levels during Cavin-1 siRNA treatment. Data are the mean ± SEM of 3 independent experiments. Non significant *vs* control (NS).

Supplemental Figure S1

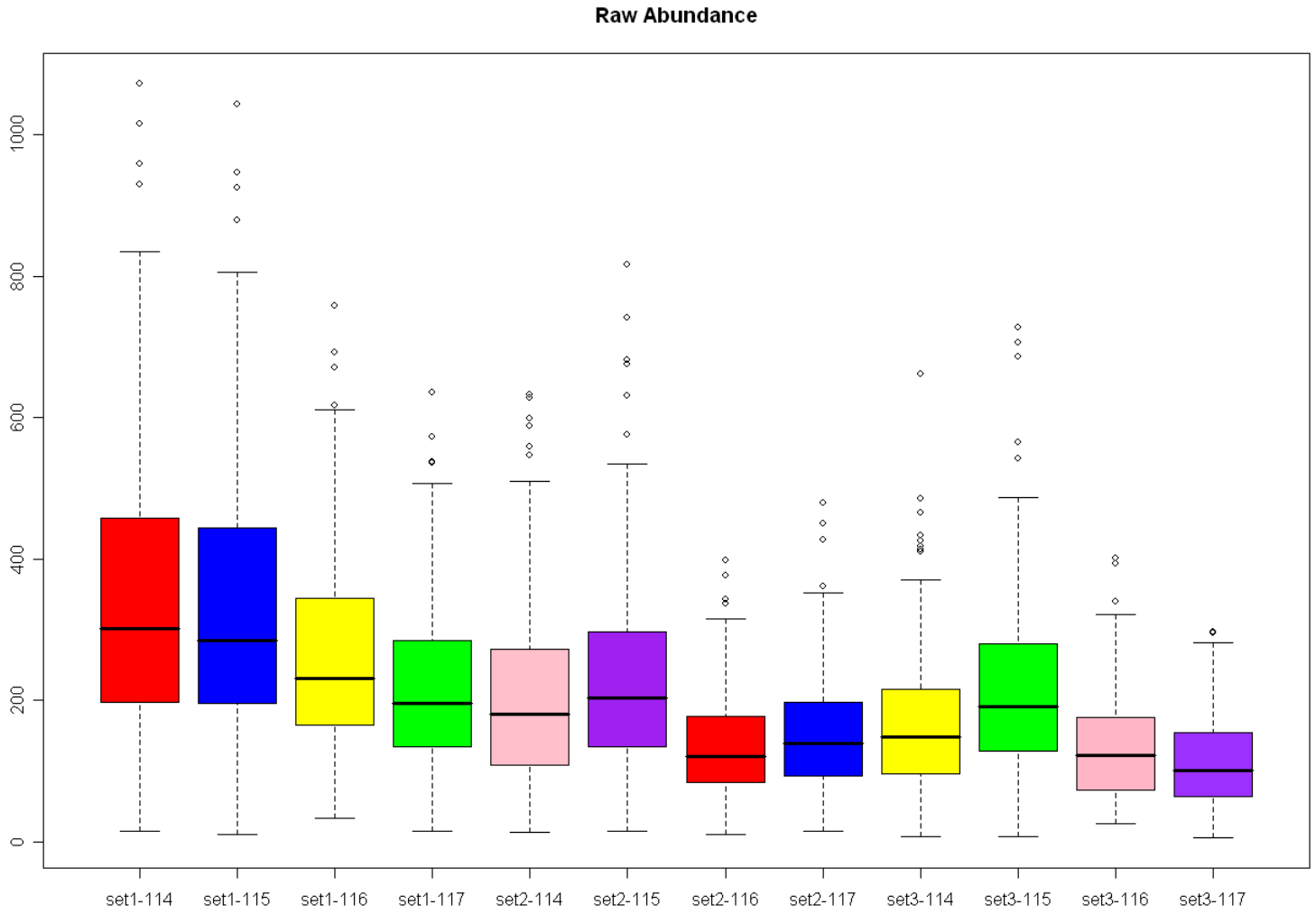
Set 1

Set 2

Set 3

Supplemental Figure S2

A



Supplemental Figure S2

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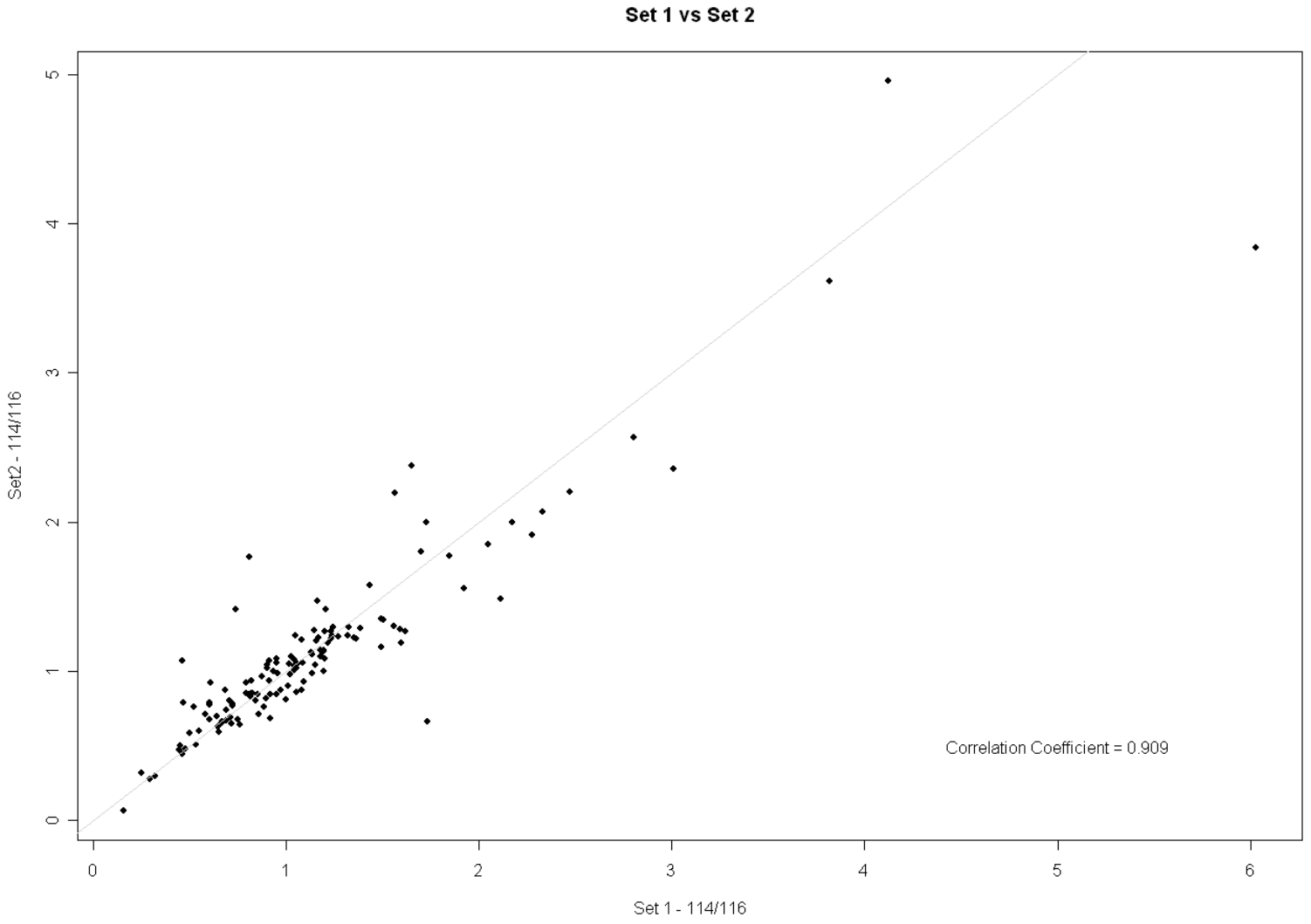
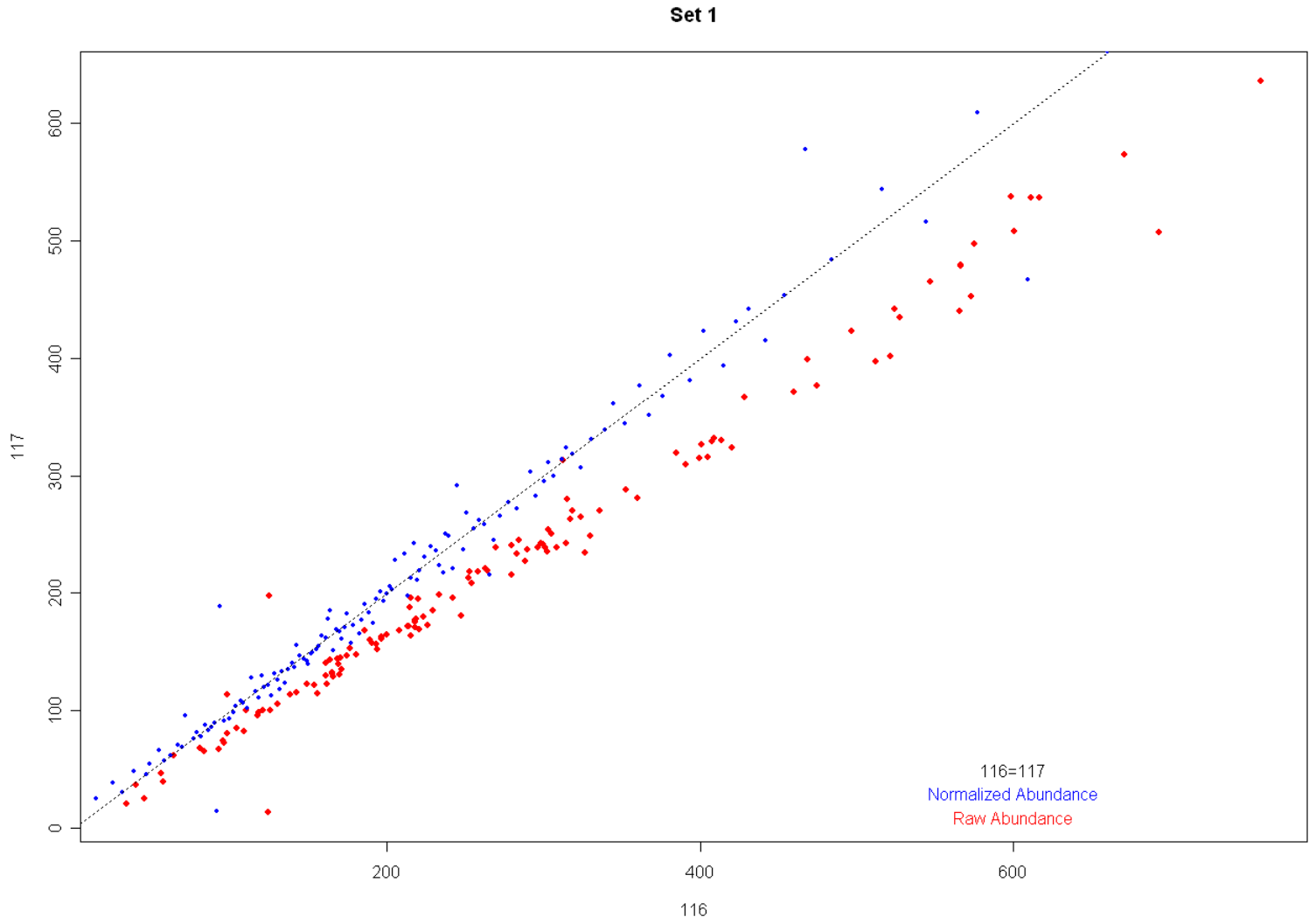


Supplemental Figure S2

C



Supplemental Figure S2

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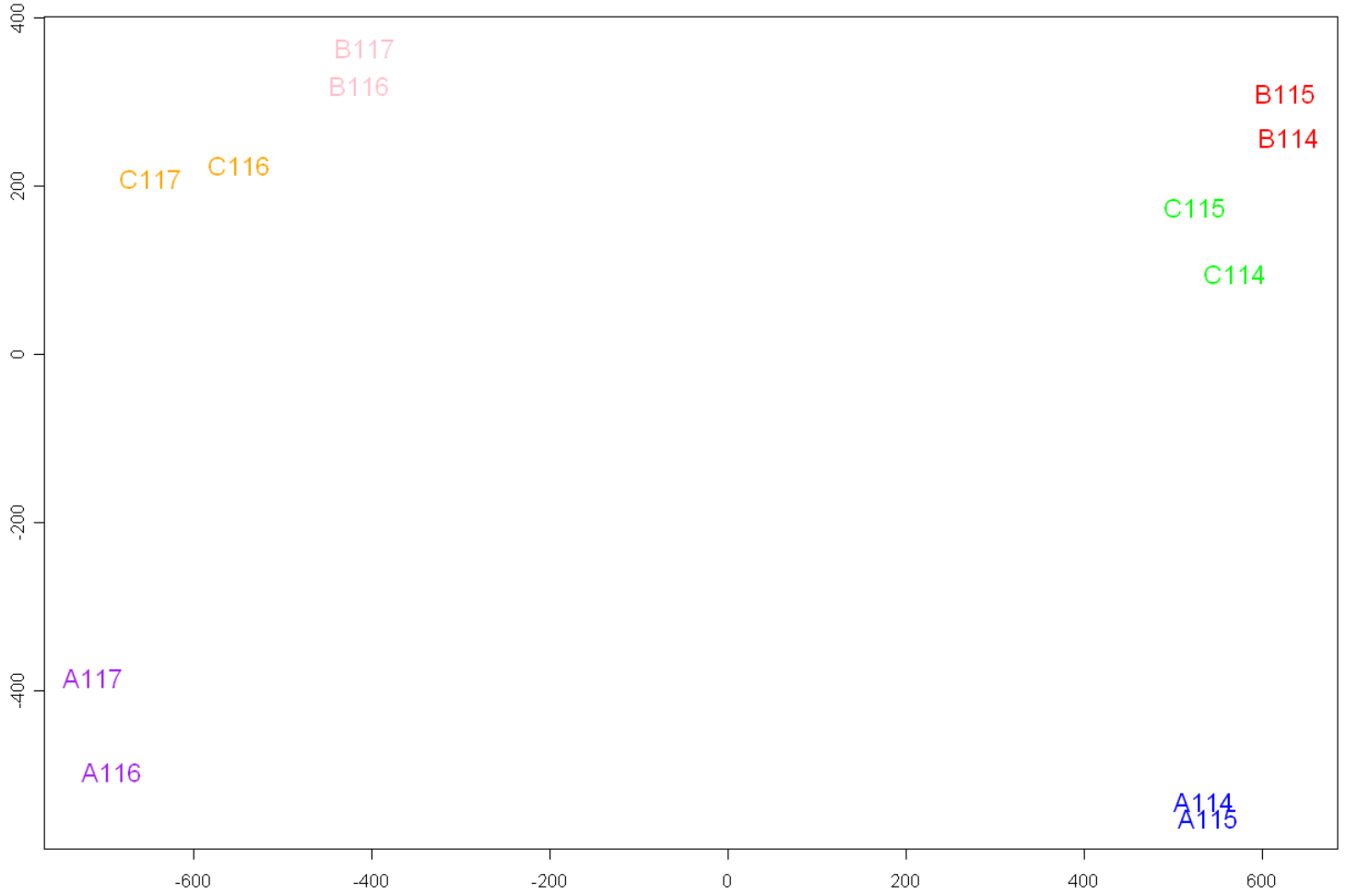
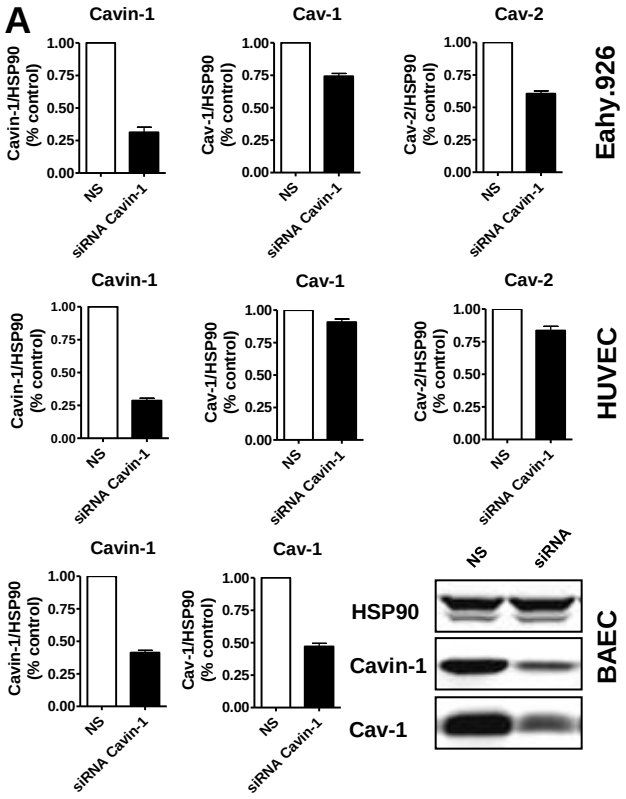
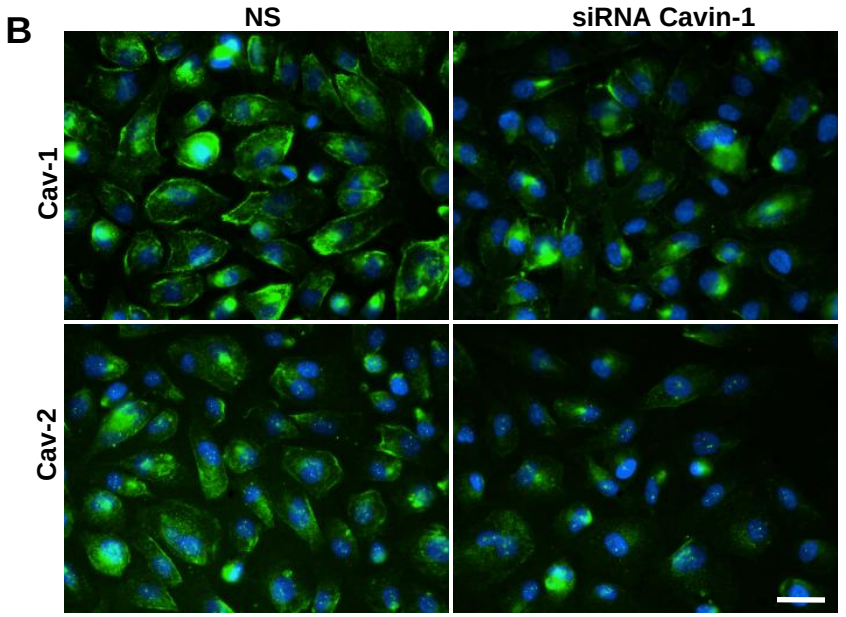
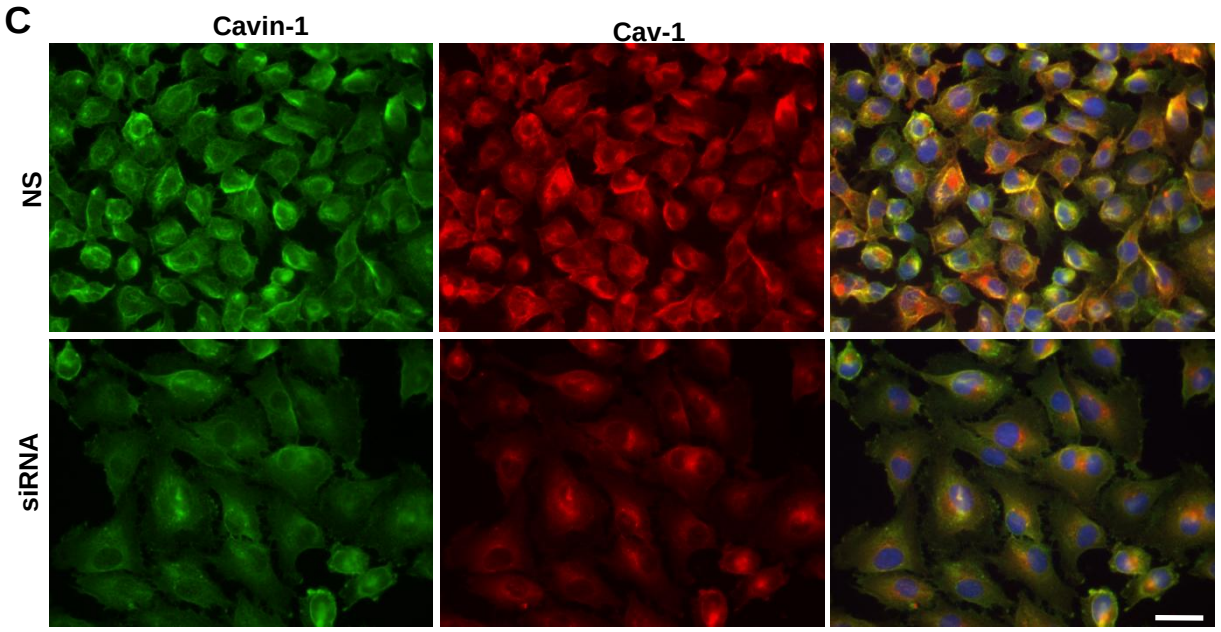


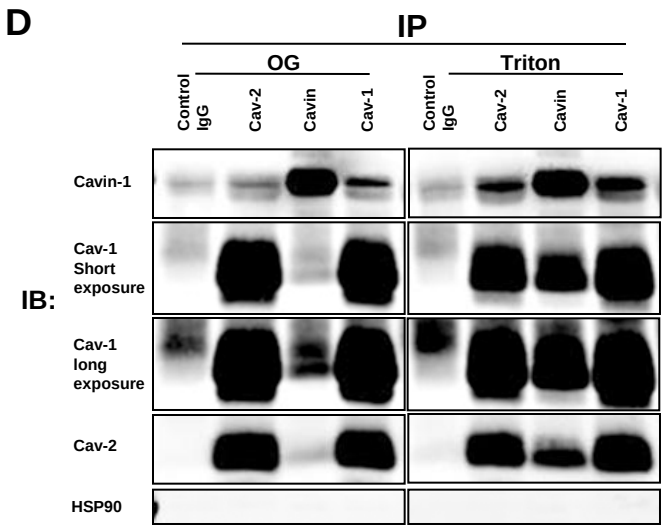
Supplemental Figure S3

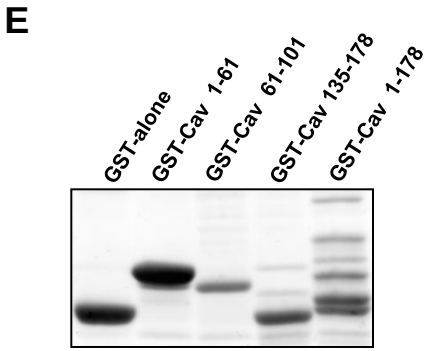


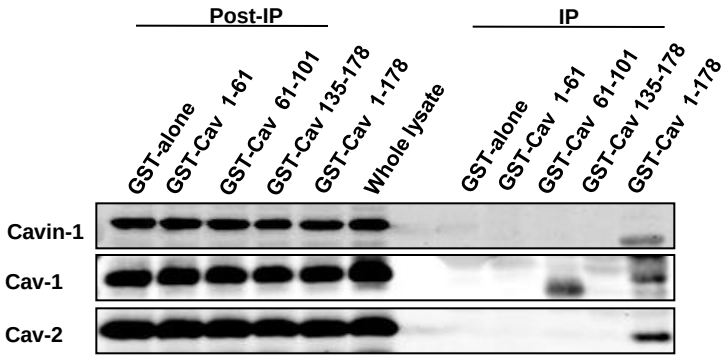




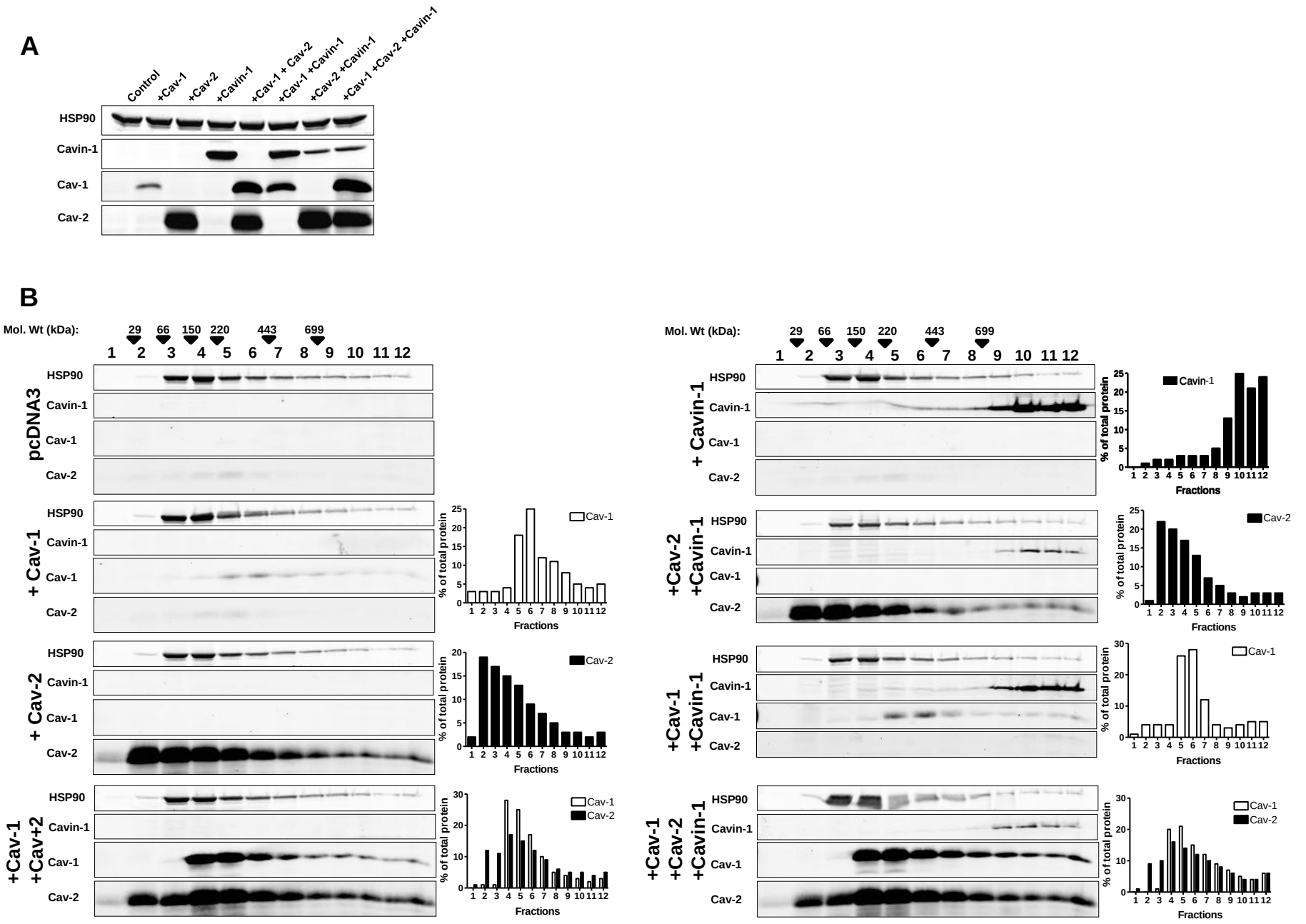
Supplemental Figure S3







Supplemental figure S4



Supplemental figure S4

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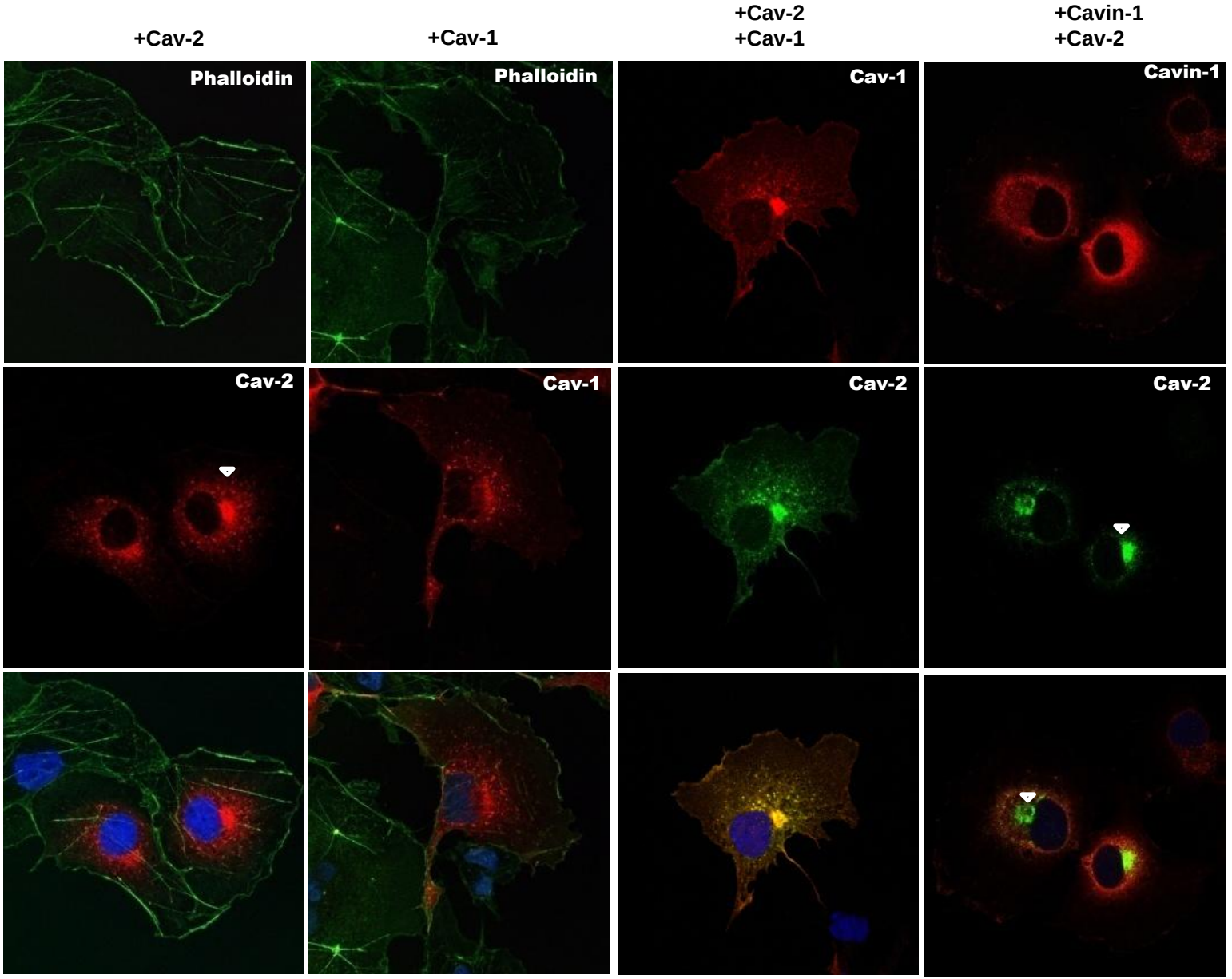
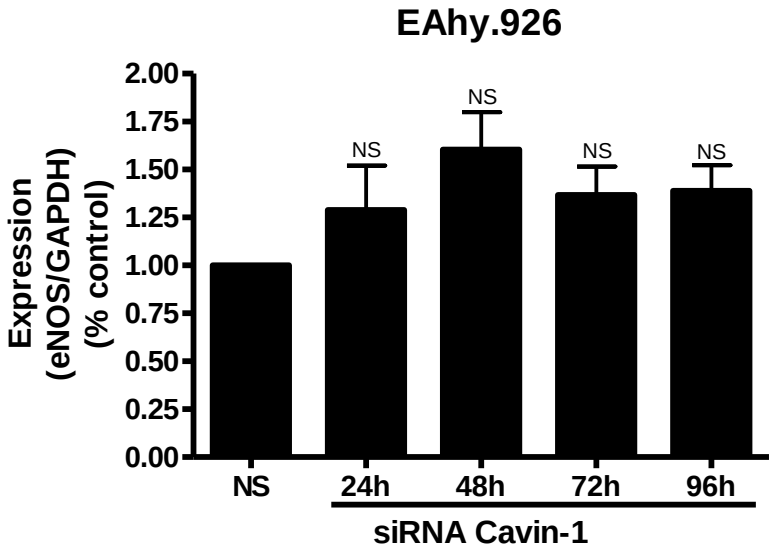


Supplemental Figure S5

A



Supplemental Table S1: ProteinProphet Predicted Sensitivity and Error Rate for for iTRAQ quantitation on lung membrane rafts of caveolin-1 KO versus WT mice

Minimum Probability	Set 1 Sensitivity	Set 1 Error	Set 2 Sensitivity	Set 2 Error	Set 3 Sensitivity	Set 3 Error
0.99	0.394	0.003	0.338	0.003	0.347	0.003
0.98	0.474	0.004	0.43	0.006	0.435	0.005
0.95	0.586	0.01	0.546	0.012	0.544	0.011
0.9	0.682	0.019	0.642	0.021	0.641	0.021
0.85	0.735	0.028	0.7	0.031	0.7	0.03
0.8	0.771	0.036	0.742	0.04	0.741	0.04
0.75	0.807	0.046	0.776	0.05	0.773	0.049
0.7	0.834	0.056	0.807	0.061	0.801	0.059
0.65	0.856	0.065	0.835	0.073	0.826	0.07
0.6	0.871	0.073	0.856	0.084	0.847	0.081
0.55	0.886	0.083	0.873	0.095	0.865	0.093
0.5	0.9	0.093	0.89	0.107	0.882	0.105
0.45	0.913	0.105	0.904	0.119	0.896	0.117
0.4	0.926	0.118	0.916	0.131	0.912	0.134
0.35	0.935	0.13	0.928	0.146	0.924	0.148
0.3	0.945	0.145	0.939	0.161	0.936	0.166
0.25	0.955	0.163	0.95	0.181	0.947	0.186
0.2	0.964	0.184	0.962	0.206	0.959	0.211
0.15	0.974	0.215	0.973	0.239	0.971	0.244
0.1	0.985	0.258	0.985	0.284	0.983	0.291
0.05	0.997	0.335	0.998	0.358	0.997	0.369
0	1	0.616	1	0.628	1	0.641