

Supplementary Figure Legends

Figure S1. ZIKV bait protein quality control and additional virus-host interactome

data analysis. Anti-Flag western analysis of: (A) FlagBirA*⁻ (N-terminal) tagged; and (B) BirA*Flag⁻ (C-terminal) tagged ZIKV proteins used in this study. (C) Immunofluorescence (IF) confocal microscopy of ZIKV bait proteins (anti-Flag), biotinylated proteins (streptactin-A alexa594), endoplasmic reticulum (calnexin; CANX), and nucleus (4',6-diamidino-2-phenylindole; DAPI). (D) IF confocal microscopy of co-expressed N-terminally tagged (GFP) and C-terminally tagged (BirA*Flag) ZIKV proteins. Lamin B1 IF marks nuclear lamina.

Previously published information on ZIKV (or related Flavivirus) protein localization.

Two published examples of complete sets of Flag-tagged ZIKV proteins (1, 2) are in agreement with our localization data.

Capsid: To our knowledge there is no published data to describe the localization of the untagged ZIKV Capsid protein. However, consistent with our data, the Dengue virus serotype 2 (DENV2) capsid protein localizes to lipid droplets (in PFA fixed cells) and was reported to localize to the nucleus (MeOH fixed) (3) and nucleolus (4) in infected cells. Japanese encephalitis virus (JEV) capsid/core protein is described as cytoplasmic and nuclear/nucleolar (5).

PreM: Myc-tagged DENV preM protein localizes to the ER (6). To our knowledge, there is no data on localization of the untagged ZIKV protein in infected cells.

Env: Similar to what we observed, the Env protein is cytoplasmic (likely ER; see Fig 1B) in ZIKV infected immature neurons (7). Env localization pattern may vary between cell types (8), but remains compatible with ER localization. Env is localized to the ER in West Nile virus (WNV) infected cells 24hrs post-infection (9).

NS1: Cytoplasmic and plasma membrane (10), or perinuclear staining with some foci (11) in ZIKV infected cells. Cytoplasmic aggregates in infected cells (12). Cytoplasmic staining is comparable to what we observed.

NS2A: FLAG-tagged version likely at the ER (2). Tick-borne encephalitis virus (TBEV) NS2A displays a pattern similar to what we observe (13).

NS2B: TBEV NS2B displays staining pattern similar to what we observed (13), similar to an NS2B-NS3 fusion protein (1).

NS3: Co-localization with microtubules (consistent with our IF) reported in (2), when FLAG-NS3 was expressed alone. No data for endogenous ZIKV NS3. TBEV FLAG-NS3 displays a pattern similar to what we observed (13).

NS4A/NS4B: Localize to the ER (at virus factory) in ZIKV infected HeLa cells (14).

NS5: In DENV-infected cells, NS5 associates with the spliceosome and modulates host splicing (15). Nuclear localization of DENV NS5 reported in (16). ZIKV NS5 tagged protein expressed alone also localizes to the nucleus (1), and colocalizes with splicing factor SC-35 (2).

Supplemental Figure S1 References

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Figure S2. Individual topology maps and ZIKV interactome expression levels in various human tissues. (A) ZIKV BioID-only topology map, as in Fig. 1B. (B) ZIKV IP-MS-only topology map. (C) ZIKV interactors detected in various human tissue types (Human Protein Atlas). Cerebral cortex data are highlighted.

Figure S3. Co-localization of the Cajal Body marker protein coilin and individual ZIKV proteins.

Figure S4. GFP-tagged ZIKV proteins and Peroxisome IF

Supplementary Table Legends

Table S1. ZIKV IP-MS and BioID mass spectrometry data. Gene names and peptide counts of all high confidence interactors, in a sortable matrix. Previously reported interactors accompanied by corresponding reference.

Table S2. Protein expression levels in 80 human tissues (Human Protein Atlas).

Table S3. Gene ontology data for ZIKV prey lists. GO category enrichment for complete dataset and individual ZIKV bait proteins.

Table S4. Raw data for Flag IP-MS of DDB1 and VPRBP/DCAF1.

Table S5. Raw data for coilin BioID.

Table S6. Raw data for Cajal body quantitation.

Table S7. Raw data for lysosome volume quantitation.

Table S8. Raw data for FlagBirA*-OFD1 BioID.

Table S9. Raw data for peroxisomes/unit cell volume in cells expressing GFP-NS2A.

Table S10. Raw data for peroxisomes/unit cell volume in ZIKV-infected cells.