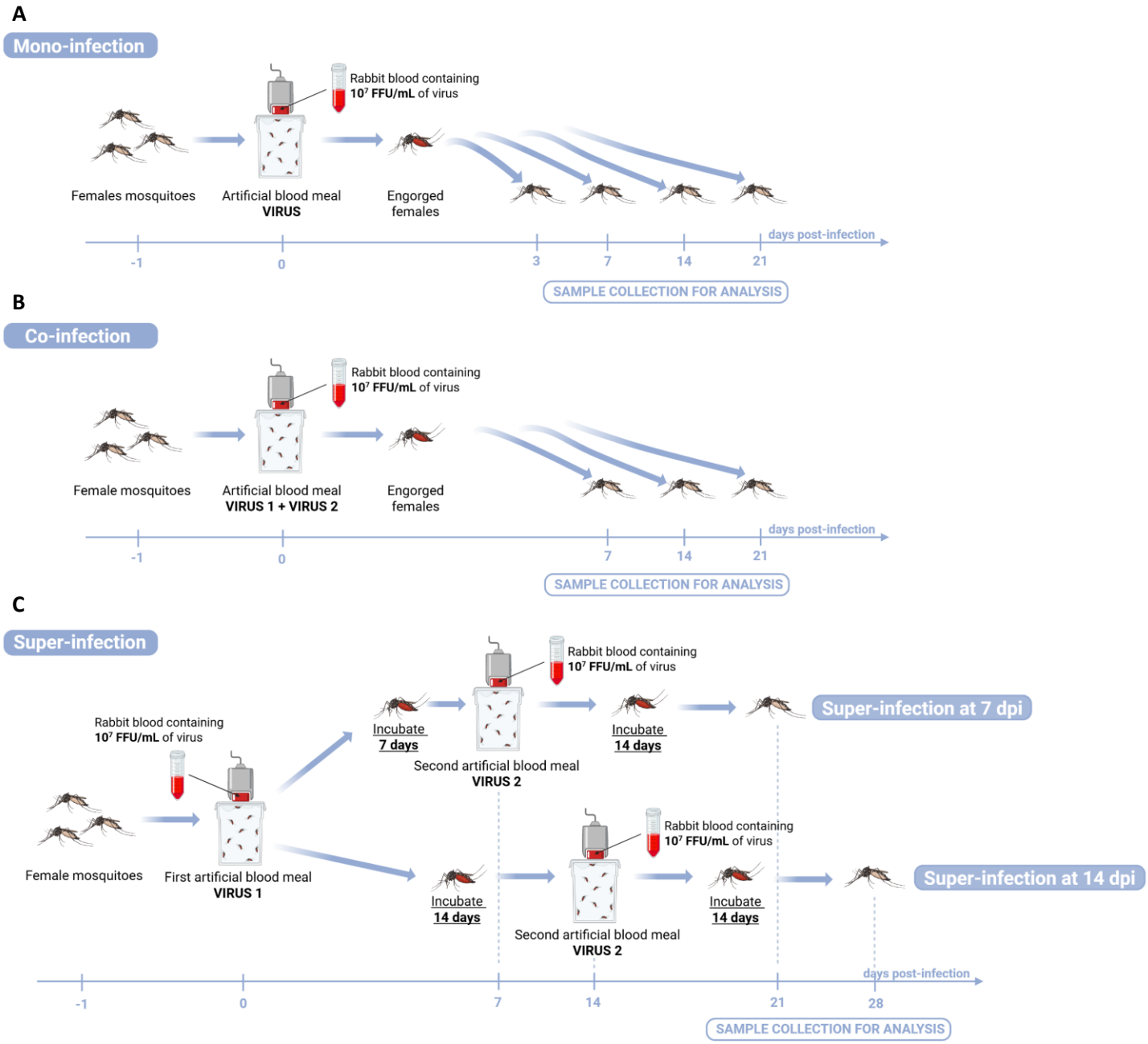

Figure S1. Experimental design of mono-, co- and super-infection of *Ae. aegypti* mosquitoes. A. Mono-infection of *Ae. aegypti* mosquitoes. Female mosquitoes were exposed to a blood meal containing a single virus (YFV, JEV or DENV) provided at a final titer of 10^7 FFU/mL. Blood-fed females were collected for analysis at 3, 7, 14, and 21 days post-infection (dpi). **B.** Co-infection of *Ae. aegypti* mosquitoes. Female mosquitoes were exposed to two viruses provided at a final titer of 10^7 FFU/mL and blood-fed females were collected for analysis at 7, 14, and 21 dpi. **C.** Super-infection of *Ae. aegypti* mosquitoes. Female mosquitoes were primo-infected via blood meal with one virus and, at 7 or 14 days later, exposed to a blood meal containing a second virus. Double blood-fed mosquitoes were then collected for analysis at 14 days post super-infection. Figure created using BioRender.

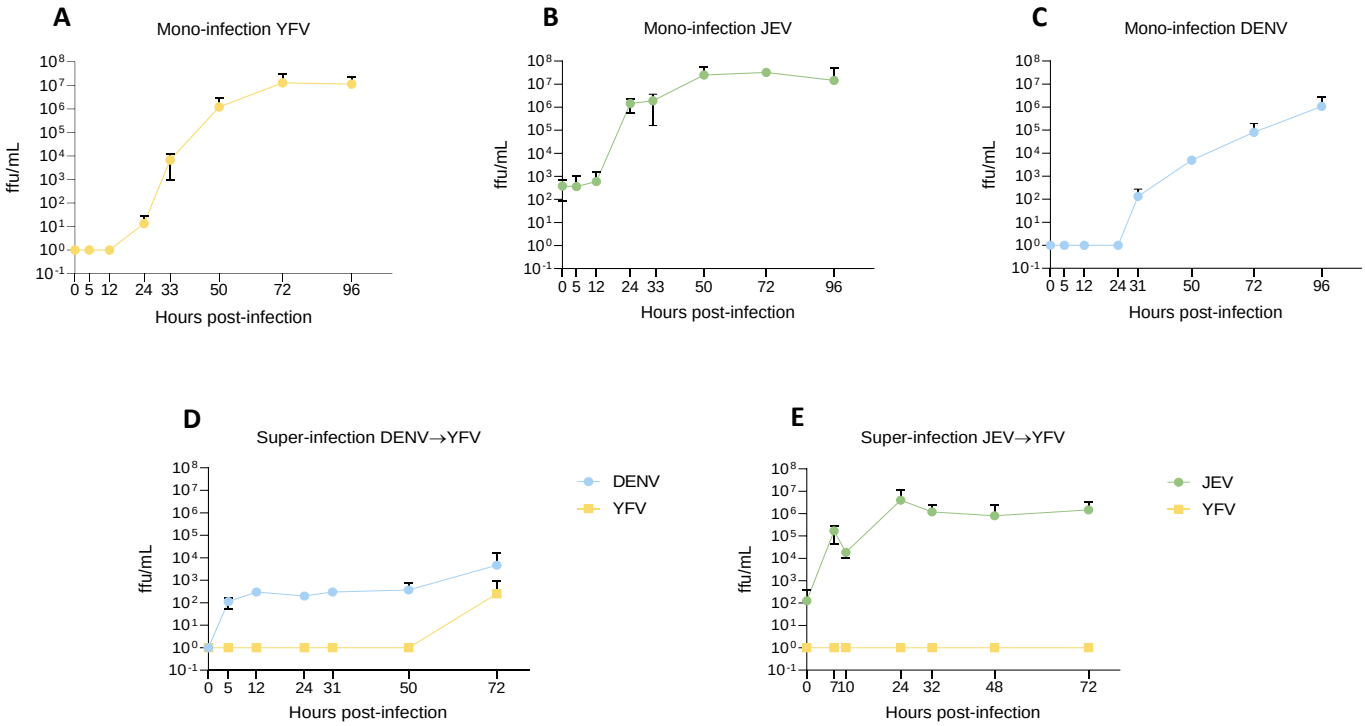

Figure S2. Replication kinetics of viruses in *Ae. aegypti* Aag2 cells. A-C. Mono-infection of YFV (**A**), JEV (**B**), and DENV (**C**). **D-E.** Super-infection of YFV 24 hours following a primo-infection of DENV (**D**) and JEV (**E**). In **D** and **E**, the 0 hour post infection time point corresponds to 0 hour post super-infection, i.e, 24 hours post primo-infection). Each experiment was performed in triplicate. Viral titers are expressed in FFU/mL at different hours post-infection.

Target	GenBank	Name	Primers/Probe	Sequence 5’ → 3’
Actin	U20287.1	beta-actin forward	Forward	GCTACGTCGCCCTGGACTT
		beta-actin reverse	Reverse	AGGAACGACGGCTGGAAGA
		beta-actin Probe	Probe	(TxRed)AGGAAATGGCCACCGCTGCCTCGT(BHQ2)
JEV	KF907505	JEV-4132F	Forward	GCTTAGCGCTCACATCCACT
		JEV-4219R	Reverse	CACCCTCTCTTCTTGTTTGGG
		JEV-4197P	Probe	(Hex)TGCAGACCATTAGTCCGGCAGCTATAGT(BHQ1)
YFV	MK060080	625F-YFV	Forward	CTGTCCCAATCTCAGTCCAAG
		694R-YFV	Reverse	AACGTTTTCCACCCCATAGC
		646P-YFV	Probe	(6FAM)AGAGGAGCCAGATGACATTGATTGCTGGT(BHQ1)
DENV	MK268692	Deng_2_F	Forward	AGAAGAGAAGAGGAAGAGGCA
		Deng_2_R	Reverse	TGGCCTGACTTCTTTTAACGTC
		Deng_2_P	Probe	(Cyanine5)CTTGGACGGGGCTCACAGGTAGC(BHQ3)
CHIKV	HE806461	CHIK Asia F	Forward	TGATCAAATGACCGGCATCCT
		CHIK Asia R	Reverse	CGTTGCGTTCTGCCGTTAAC
		CHIKV-ASIA_P	Probe	(6FAM)TGCTACAGAAGTCACGCCGGAGGAT(BHQ1)

Table S1. List of probes and primers used for qPCR. JEV: Japanese encephalitis virus, YFV: Yellow fever virus, DENV: dengue virus, CHIKV: chikungunya virus.

Immune pathway	Gene	VectorBase Gene ID	Name	Primers/Probe	Sequence
Toll	Rel1	AAEL007696	AAE_Rel1_F	Forward	GCTATCAAAACGGAACCTAGAG
			AAE_Rel1_R	Reverse	GGTGGTGTTGCCGTAAAGTG
			AAE_Rel1_P	Probe	(HEX)CGACGCACCCGTTTAACTTCATTGGAAACTA(BHG1)
	Cactus	AAEL000709	AAE_Cactus_F	Forward	GCGCTGCATTTGATGAAATGG
			AAE_Cactus_R	Reverse	CGTTTTGAATGTCCAGCCAGG
			AAE_Cactus_P	Probe	(Cyanine5)TACTTCCACCAGAACGACGACGGTGAT(BHQ3)
JAK-STAT	STAT	AAEL020559	AAE_STAT_F	Forward	ACTACCTGGCGGAATGGATC
			AAE_STAT_R	Reverse	CGTTGATTAGCTGGTTGAGGAA
			AAE_STAT_P	Probe	(Cyanine5)CTAAACGTCCCAGTCTACCAGCACGAA(BHQ3)
	PIAS	AAEL026694	AAE_PIAS_F	Forward	TTCTTCGACTACAGCTCCTTC
			AAE_PIAS_R	Reverse	GCACGGGTTACTGTTTTGCG
			AAE_PIAS_P	Probe	(FAM)CTCAGCGATTCCGATGATGATCTCCCTTT(BHQ1)
IMD	IMD	AAEL010083	AAE_IMD_F	Forward	CTCGGAAGGAGAAATAGAAGC
			AAE_IMD_R	Reverse	CAGGTTGACCATTTTGCCGAG
			AAE_IMD_P	Probe	(Cyanine5)ATACGTTATCGGAGCGAATCTATCAGTTCATT(BHQ3)
	Caspar	AAEL027860	AAE_Caspar_F	Forward	ACCGGAGAAGGCTTCATGAG
			AAE_Caspar_R	Reverse	GAGCAAGTTACCCTCCGGT
			AAE_Caspar_P	Probe	(FAM)CAAAATAGGCAAAACTACACGCTGCACATCAA(BHQ1)
RNAi	Dicer2	AAEL006794	AAE_Dicer2_F	Forward	CTTTCCCAAATCCGGAGTACG
			AAE_Dicer2_R	Reverse	GAGAAACTGATCTGGCTTAACC
			AAE_Dicer2_P	Probe	(HEX)CCTACGCGAATTATTTCAGCCAAGCTTACCA(BHQ1)
	Ago2	AAEL017251	AAE_Ago2_F	Forward	TTCAGTGTCTGGACATAGTGC
			AAE_Ago2_R	Reverse	CCGACTGGAACAGACCGTA
			AAE_Ago2_P	Probe	(FAM)CGGACCGCGTACGAAAATAATCCTAGATTTA(BHQ1)
Reporter gene					
	Rps17	XM_001648517	AAE_Rps17_F	Forward	TCGAAGAAGTGGCCATCATTC
			AAE_Rps17_R	Reverse	TCTCTGCGCTCACGTTCCT
			AAE_Rps17_P	Probe	(Txrd) CCCCTGCGAACAAGATCGCTGGTTT (BHQ2)

Table S2. List of probes and primers targeting immune genes for quantitative PCR.