

1 **Supplemental Material**

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3 **S1 Movie. Time-lapse image of a centered, dual-labeled HIV RGDA/Q112D particle**
4 **challenging a Jurkat cell.**

5 The mean intensity of the tracked particle is plotted (y axis) for iGFP (green) and
6 tdTomato-Vpr (red) signals (Top Left). The first drop of GFP signal occurs with fusion
7 and a second total drop occurs when the capsid integrity is lost (at ~18 minutes after
8 fusion). The Right panel shows the signal for iGFP (green), tdTomato-Vpr (red), and
9 bright field signal for cell reference (gray). Particle box shows the signal of GFP (green)
10 and tdTomato-Vpr (red) over time. (Bottom Left) Smaller boxes show the signal (gray) of
11 each individual fluorescence measurement as labeled and mixed signal for iGFP
12 (green) and tdTomato-Vpr (red).

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14 **S2 Movie. Time-lapse image of a centered, dual-labeled HIV wild-type particle**
15 **challenging a Jurkat cell.**

16 The mean intensity of the tracked particle is plotted (y axis) for iGFP (green) and
17 tdTomato-Vpr (red) signals (Top Left). The first drop of GFP signal occurs with fusion
18 and a second total drop occurs when the capsid integrity is lost (at ~45 minutes after
19 fusion). The Right panel shows the signal for iGFP (green), tdTomato-Vpr (red), and
20 bright field signal for cell reference (gray). Particle box shows the signal of GFP (green)
21 and tdTomato-Vpr (red) over time. (Bottom Left) Smaller boxes show the signal (gray) of
22 each individual fluorescence measurement as labeled and mixed signal for iGFP
23 (green) and tdTomato-Vpr (red).

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25 **S3 Movie. Time-lapse image of a centered, dual-labeled HIV RGDA/Q112D+Q4R**
26 **particle challenging a Jurkat cell.**

27 The mean intensity of the tracked particle is plotted (y axis) for iGFP (green) and
28 tdTomato-Vpr (red) signals (Top Left). The first drop of GFP signal occurs with fusion
29 and a second total drop occurs when the capsid integrity is lost (at ~10 minutes after
30 fusion). The Right panel shows the signal for iGFP (green), tdTomato-Vpr (red), and
31 bright field signal for cell reference (gray). Particle box shows the signal of GFP (green)
32 and tdTomato-Vpr (red) over time. (Bottom Left) Smaller boxes show the signal (gray) of
33 each individual fluorescence measurement as labeled and mixed signal for iGFP
34 (green) and tdTomato-Vpr (red).