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**Supplemental information**

**Ligand binding initiates single-molecule  
integrin conformational activation**

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**Table S1. Single molecule time traces analyzed under each condition, related to Figure 2-6.**

Figure	Condition	Number of time traces
Figure 2A-B	Basal	168
Figure 2C	2 $\mu$ M 12G10 Fab	96
Figure 2C	2 $\mu$ M 9EG7 Fab	128
Figure 2C	5 $\mu$ M mAb13 Fab	189
Figure 2C	10 $\mu$ M Fn3 <sub>9-10</sub>	50
Figure 2I	1 $\mu$ M mAb13 Fab	72
Figure 2I	5 $\mu$ M mAb13 Fab	97
Figure 2I	10 $\mu$ M mAb13 Fab	111
Figure 2I	20 $\mu$ M mAb13 Fab	106
Figure 2I	50 $\mu$ M mAb13 Fab	122
Figure 2K	0 $\mu$ M mAb13 Fab	33
Figure 2K	2 $\mu$ M mAb13 Fab	107
Figure 2K	5 $\mu$ M mAb13 Fab	116
Figure 2K	10 $\mu$ M mAb13 Fab	64
Figure 2K	20 $\mu$ M mAb13 Fab	49
Figure 2K	50 $\mu$ M mAb13 Fab	90
Figure 3A-B	Basal	541
Figure 3C	5 $\mu$ M mAb13 Fab	124
Figure 3C	5 $\mu$ M SNAKA51 Fab	76
Figure 3C	2 $\mu$ M 12G10 Fab	36
Figure 3C	20 $\mu$ M Fn3 <sub>9-10</sub>	118
Figure 3C	20 $\mu$ M ACRGDGWCG peptide	99
Figure 3H	10 nM Alexa488-Fn3 <sub>9-10</sub>	10
Figure 3I	10 nM Alexa488-Fn3 <sub>9-10</sub>	20
Figure 4B	Basal	705
Figure 4B	30 $\mu$ M 9EG7 Fab	84
Figure 4B	20 $\mu$ M Fn3 <sub>9-10</sub>	46
Figure 4B	15 $\mu$ M 9EG7 Fab and 20 $\mu$ M Fn3 <sub>9-10</sub>	35
Figure 4C	Basal	270
Figure 4C	30 $\mu$ M 9EG7 Fab & 30 $\mu$ M SNAKA51 Fab	34
Figure 4C	20 $\mu$ M Fn3 <sub>9-10</sub>	136
Figure 5A-B	Closed $\leftrightarrow$ Open, 20 $\mu$ M Fn3 <sub>9-10</sub>	41
Figure 5C-D	Bent $\leftrightarrow$ Extended, 20 $\mu$ M Fn3 <sub>9-10</sub>	41
Figure 5E-F	Closed $\leftrightarrow$ Open, 2 $\mu$ M Fn3 <sub>9-10</sub>	109
Figure 5E-F	Closed $\leftrightarrow$ Open, 5 $\mu$ M Fn3 <sub>9-10</sub>	77
Figure 5E-F	Closed $\leftrightarrow$ Open, 10 $\mu$ M Fn3 <sub>9-10</sub>	65
Figure 5E-F	Closed $\leftrightarrow$ Open, 20 $\mu$ M Fn3 <sub>9-10</sub>	41
Figure 5E-F	Closed $\leftrightarrow$ Open, 50 $\mu$ M Fn3 <sub>9-10</sub>	55
Figure 5E-F	Bent $\leftrightarrow$ Extended, 2 $\mu$ M Fn3 <sub>9-10</sub>	70
Figure 5E-F	Bent $\leftrightarrow$ Extended, 5 $\mu$ M Fn3 <sub>9-10</sub>	61
Figure 5E-F	Bent $\leftrightarrow$ Extended, 10 $\mu$ M Fn3 <sub>9-10</sub>	44
Figure 5E-F	Bent $\leftrightarrow$ Extended, 20 $\mu$ M Fn3 <sub>9-10</sub>	41
Figure 5E-F	Bent $\leftrightarrow$ Extended, 50 $\mu$ M Fn3 <sub>9-10</sub>	20
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub>	35
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub> and 2 $\mu$ M mAb13 Fab	34
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub> and 5 $\mu$ M mAb13 Fab	36
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub> and 10 $\mu$ M mAb13 Fab	62
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub> and 20 $\mu$ M mAb13 Fab	45
Figure 5G	15 $\mu$ M 9EG7 Fab, 20 $\mu$ M Fn3 <sub>9-10</sub> and 50 $\mu$ M mAb13 Fab	51
Figure 6B	20 $\mu$ M Talin1 head	73
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 0 $\mu$ M Talin1 head	190
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 0.3 $\mu$ M Talin1 head	112
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 1 $\mu$ M Talin1 head	171
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 3 $\mu$ M Talin1 head	125
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 10 $\mu$ M Talin1 head	131
Figure 6C, 6D, 6F	1 $\mu$ M Fn3 <sub>9-10</sub> & 20 $\mu$ M Talin1 head	311
Figure 6E, 6F	30 $\mu$ M 9EG7 Fab & 0 $\mu$ M Talin1 head	251
Figure 6E, 6F	30 $\mu$ M 9EG7 Fab & 20 $\mu$ M Talin1 head	217
Figure S6C	Basal, 37°C	7
Figure S6E	5 $\mu$ M Fn3 <sub>9-10</sub>	76
Figure S6E	10 $\mu$ M Fn3 <sub>9-10</sub>	126
Figure S6E	20 $\mu$ M Fn3 <sub>9-10</sub>	72
Figure S6E	50 $\mu$ M Fn3 <sub>9-10</sub>	76
Figure S6F	5 $\mu$ M Fn3 <sub>9-10</sub>	15