

Supplemental Figures for

**JmjC domain proteins modulate circadian behaviors and sleep in  
*Drosophila***

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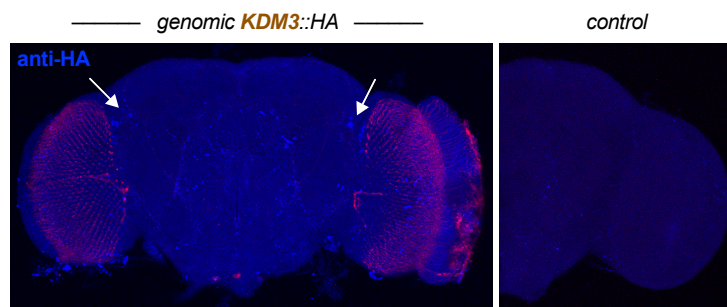
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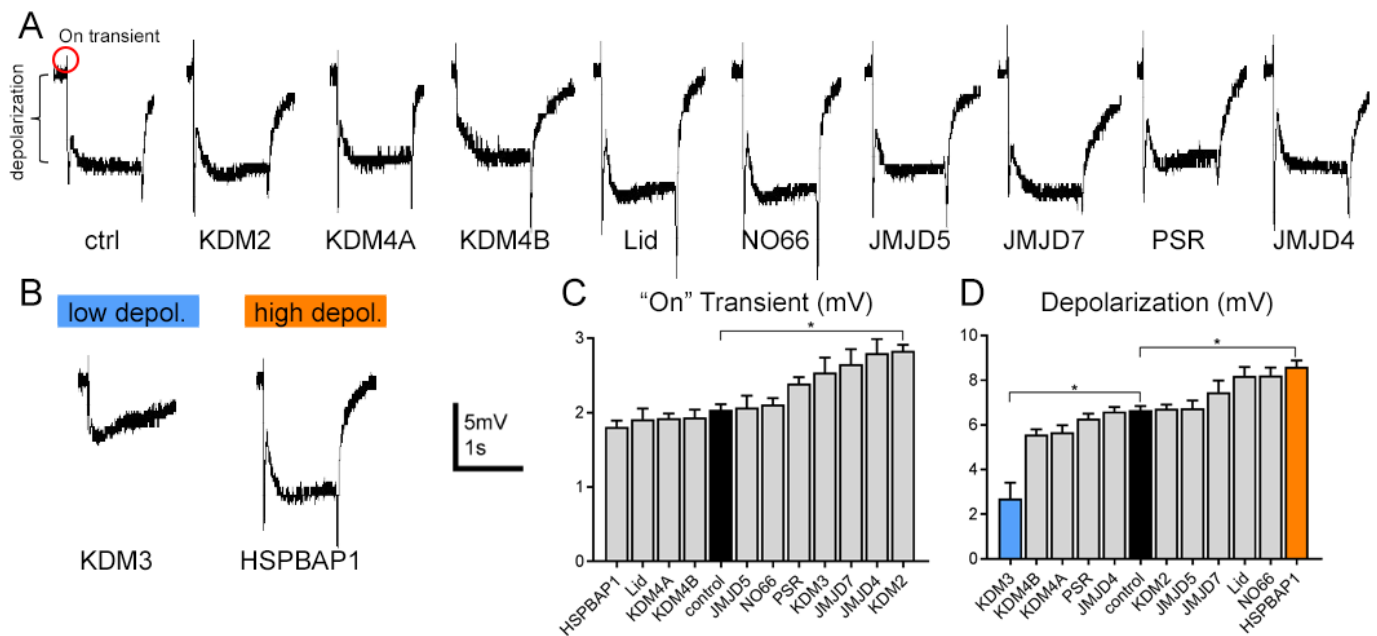
**Supplemental Fig. 1. Expression pattern of genomic *KDM3::HA* transgene.**

Expression pattern of the *KDM3::HA* rescue transgene, which rescued arrhythmicity in *KDM3*<sup>KO</sup> flies (see text). Anti-HA staining (blue) is detected in numerous nuclei including in neurons close to the dorsal lateral neurons involved in circadian rhythm. Anti-RFP staining is in red (labeling the *KDM3*<sup>KO</sup> knock out due to its *3xP3-RFP* expression in the eyes, while not the negative control).



**Supplemental Fig. 2. Electroretinogram (ERG) recordings from *JmjC* mutant flies.**

(A) Sample ERG traces from 1 day old flies. On-transient [red circle in control (ctrl) flies] and Depolarization are indicated. Nine out of 11 mutants tested show no significant differences from ctrl flies and exhibit normal photoreceptor responses to light. (B) *KDM3*<sup>KO</sup> mutants show a consistent decrease in depolarization, while *HSPBAP1*<sup>KO</sup> mutants show increased depolarization, whereas the On-transient remains normal in both cases. (C, D) Bar graphs showing the mean value ( $\pm$  SEM) of On-transients (C) and Depolarization (D) for 15-30 flies per genotype. (\*  $p < 0.0001$ ).



**Supplemental Table 1. Phenotype measures and effect size estimates of *JmjC* mutants.**

Shown are the various measures together with the n and standard deviation (sd). The sub-tables are sorted for the effect sizes (Herge's *g*), which are shown with their 95% upper and lower confidence interval (CI). Effect sizes in bold have CIs that do not cross 0 (unadjusted for multiple comparisons).

| genotype | Night Sleep (min) | n   | sd  | Effect Size  | Lower CI | Upper CI |
|----------|-------------------|-----|-----|--------------|----------|----------|
| lid      | 625               | 30  | 92  | <b>1.60</b>  | 1.17     | 2.03     |
| HSPBAP1  | 591               | 60  | 76  | <b>1.41</b>  | 1.08     | 1.74     |
| KDM4A    | 586               | 30  | 77  | <b>1.30</b>  | 0.88     | 1.72     |
| PSR      | 580               | 27  | 80  | <b>1.24</b>  | 0.81     | 1.68     |
| KDM3     | 534               | 43  | 95  | <b>0.87</b>  | 0.51     | 1.22     |
| KDM4B    | 505               | 59  | 68  | <b>0.68</b>  | 0.36     | 0.99     |
| JMJD7    | 462               | 124 | 147 | 0.25         | 0.00     | 0.49     |
| KDM2     | 435               | 25  | 105 | 0.05         | -0.37    | 0.48     |
| w Berlin | 428               | 139 | 128 | 0.00         | -0.24    | 0.24     |
| JMJD5    | 399               | 93  | 151 | -0.21        | -0.47    | 0.06     |
| JMJD4    | 393               | 63  | 141 | -0.26        | -0.56    | 0.04     |
| NO66     | 366               | 73  | 159 | <b>-0.45</b> | -0.73    | -0.16    |

| genotype | Night Activity (counts) | n   | sd  | Effect Size  | Lower CI | Upper CI |
|----------|-------------------------|-----|-----|--------------|----------|----------|
| lid      | 219                     | 30  | 179 | <b>-1.38</b> | -1.80    | -0.96    |
| PSR      | 240                     | 27  | 189 | <b>-1.29</b> | -1.73    | -0.86    |
| KDM4A    | 282                     | 30  | 160 | <b>-1.15</b> | -1.57    | -0.74    |
| HSPBAP1  | 299                     | 60  | 247 | <b>-1.06</b> | -1.39    | -0.74    |
| KDM4B    | 412                     | 59  | 160 | <b>-0.70</b> | -1.01    | -0.39    |
| KDM2     | 440                     | 25  | 251 | <b>-0.54</b> | -0.97    | -0.11    |
| JMJD7    | 524                     | 132 | 355 | -0.21        | -0.45    | 0.03     |
| w Berlin | 590                     | 138 | 283 | 0.00         | -0.24    | 0.24     |
| KDM3     | 676                     | 43  | 315 | 0.30         | -0.05    | 0.64     |
| JMJD5    | 739                     | 93  | 431 | <b>0.43</b>  | 0.16     | 0.69     |
| JMJD4    | 760                     | 63  | 404 | <b>0.52</b>  | 0.22     | 0.82     |
| NO66     | 855                     | 74  | 459 | <b>0.75</b>  | 0.46     | 1.04     |

| genotype | Day Sleep (min) | n   | sd  | Effect Size  | Lower CI | Upper CI |
|----------|-----------------|-----|-----|--------------|----------|----------|
| KDM4B    | 443             | 59  | 87  | <b>2.10</b>  | 1.73     | 2.47     |
| KDM4A    | 359             | 30  | 141 | <b>1.16</b>  | 0.74     | 1.57     |
| PSR      | 351             | 27  | 122 | <b>1.13</b>  | 0.70     | 1.56     |
| KDM3     | 347             | 43  | 112 | <b>1.11</b>  | 0.75     | 1.47     |
| HSPBAP1  | 314             | 60  | 113 | <b>0.81</b>  | 0.50     | 1.13     |
| lid      | 268             | 30  | 119 | 0.40         | 0.00     | 0.80     |
| w Berlin | 223             | 139 | 110 | 0.00         | -0.24    | 0.24     |
| JMJD4    | 214             | 63  | 111 | -0.09        | -0.38    | 0.21     |
| NO66     | 165             | 73  | 122 | <b>-0.51</b> | -0.80    | -0.22    |
| KDM2     | 163             | 25  | 94  | <b>-0.55</b> | -0.98    | -0.12    |
| JMJD5    | 125             | 93  | 81  | <b>-0.98</b> | -1.26    | -0.70    |
| JMJD7    | 98              | 152 | 118 | <b>-1.09</b> | -1.34    | -0.85    |

| genotype | Day Activity (counts) | n   | sd  | Effect Size  | Lower CI | Upper CI |
|----------|-----------------------|-----|-----|--------------|----------|----------|
| KDM4B    | 654                   | 59  | 173 | <b>-1.88</b> | -2.24    | -1.52    |
| PSR      | 818                   | 27  | 367 | <b>-1.06</b> | -1.49    | -0.64    |
| KDM3     | 868                   | 43  | 287 | <b>-0.94</b> | -1.30    | -0.58    |
| lid      | 944                   | 30  | 391 | <b>-0.63</b> | -1.03    | -0.23    |
| KDM4A    | 988                   | 30  | 393 | <b>-0.48</b> | -0.88    | -0.08    |
| HSPBAP1  | 1007                  | 60  | 371 | <b>-0.41</b> | -0.72    | -0.11    |
| w Berlin | 1136                  | 138 | 283 | 0.00         | -0.24    | 0.24     |
| JMJD4    | 1197                  | 63  | 447 | 0.18         | -0.12    | 0.48     |
| KDM2     | 1210                  | 25  | 490 | 0.23         | -0.20    | 0.66     |
| JMJD7    | 1443                  | 154 | 634 | <b>0.61</b>  | 0.38     | 0.85     |
| JMJD5    | 1475                  | 93  | 368 | <b>1.06</b>  | 0.78     | 1.34     |
| NO66     | 1574                  | 74  | 550 | <b>1.10</b>  | 0.80     | 1.40     |

| Daytime Activity per waking min |          |     |      |             |          |          |
|---------------------------------|----------|-----|------|-------------|----------|----------|
| genotype                        | (counts) | n   | sd   | Effect Size | Lower CI | Upper CI |
| KDM4A                           | 2.80     | 30  | 0.61 | <b>1.09</b> | 0.68     | 1.51     |
| NO66                            | 2.81     | 73  | 0.70 | <b>0.97</b> | 0.67     | 1.27     |
| KDM3                            | 2.58     | 26  | 0.52 | <b>0.67</b> | 0.24     | 1.09     |
| JMJD5                           | 2.53     | 61  | 0.57 | <b>0.52</b> | 0.21     | 0.82     |
| JMJD4                           | 2.48     | 63  | 0.48 | <b>0.44</b> | 0.14     | 0.74     |
| HSPBAP1                         | 2.47     | 60  | 0.56 | <b>0.40</b> | 0.09     | 0.70     |
| KDM4B                           | 2.45     | 59  | 0.55 | <b>0.35</b> | 0.04     | 0.65     |
| JMJD7                           | 2.47     | 90  | 1.19 | 0.22        | -0.05    | 0.49     |
| w Berlin                        | 2.29     | 139 | 0.43 | 0.00        | -0.24    | 0.24     |
| PSR                             | 2.18     | 27  | 0.53 | -0.24       | -0.65    | 0.18     |
| lid                             | 2.12     | 30  | 0.76 | -0.33       | -0.72    | 0.07     |
| KDM2                            | 2.13     | 25  | 0.59 | -0.34       | -0.77    | 0.09     |

| Nighttime Activity per waking min |          |     |      |              |          |          |
|-----------------------------------|----------|-----|------|--------------|----------|----------|
| genotype                          | (counts) | n   | sd   | Effect Size  | Lower CI | Upper CI |
| KDM3                              | 2.62     | 26  | 0.78 | <b>1.06</b>  | 0.63     | 1.50     |
| NO66                              | 2.48     | 73  | 0.69 | <b>0.77</b>  | 0.48     | 1.06     |
| JMJD5                             | 2.43     | 61  | 0.59 | <b>0.72</b>  | 0.41     | 1.03     |
| HSPBAP1                           | 2.45     | 60  | 0.74 | <b>0.69</b>  | 0.38     | 1.00     |
| JMJD4                             | 2.45     | 63  | 0.97 | <b>0.59</b>  | 0.29     | 0.89     |
| lid                               | 2.26     | 29  | 0.84 | 0.36         | -0.04    | 0.77     |
| KDM4A                             | 2.23     | 30  | 0.84 | 0.31         | -0.09    | 0.70     |
| PSR                               | 2.18     | 27  | 0.53 | 0.25         | -0.16    | 0.66     |
| JMJD7                             | 2.17     | 91  | 0.90 | 0.15         | -0.11    | 0.42     |
| KDM2                              | 2.13     | 25  | 0.59 | 0.14         | -0.28    | 0.57     |
| w Berlin                          | 2.06     | 139 | 0.46 | 0.00         | -0.24    | 0.24     |
| KDM4B                             | 1.92     | 59  | 0.47 | <b>-0.30</b> | -0.61    | 0.00     |

| Total Starvation Activity |          |     |      |              |          |          |
|---------------------------|----------|-----|------|--------------|----------|----------|
| genotype                  | (counts) | n   | sd   | Effect Size  | Lower CI | Upper CI |
| KDM4B                     | 493      | 63  | 354  | <b>-1.38</b> | -1.68    | -1.08    |
| KDM3                      | 902      | 28  | 556  | <b>-0.92</b> | -1.32    | -0.51    |
| HSPBAP1                   | 1235     | 31  | 682  | <b>-0.61</b> | -1.00    | -0.23    |
| KDM4A                     | 1432     | 32  | 858  | <b>-0.43</b> | -0.80    | -0.06    |
| JMJD5                     | 1472     | 32  | 706  | <b>-0.40</b> | -0.77    | -0.03    |
| JMJD7                     | 1840     | 30  | 945  | -0.07        | -0.45    | 0.31     |
| JMJD4                     | 1902     | 31  | 1019 | -0.01        | -0.39    | 0.36     |
| w Berlin                  | 1917     | 211 | 1155 | 0.00         | -0.19    | 0.19     |
| PSR                       | 2114     | 16  | 1559 | 0.17         | -0.34    | 0.67     |
| KDM2                      | 2177     | 31  | 1052 | 0.23         | -0.15    | 0.60     |
| lid                       | 2568     | 16  | 1697 | <b>0.54</b>  | 0.03     | 1.05     |
| NO66                      | 3982     | 79  | 2002 | <b>1.44</b>  | 1.15     | 1.72     |

| Time of Death on Agar |         |     |      |              |          |          |
|-----------------------|---------|-----|------|--------------|----------|----------|
| genotype              | (hours) | n   | sd   | Effect Size  | Lower CI | Upper CI |
| JMJD4                 | 39.4    | 31  | 11.7 | <b>-0.57</b> | -0.96    | -0.19    |
| lid                   | 39.8    | 16  | 10.0 | <b>-0.55</b> | -1.06    | -0.03    |
| JMJD7                 | 42.0    | 30  | 6.9  | -0.35        | -0.74    | 0.03     |
| KDM4A                 | 42.3    | 32  | 9.0  | -0.32        | -0.69    | 0.05     |
| KDM2                  | 43.2    | 31  | 10.2 | -0.23        | -0.61    | 0.15     |
| HSPBAP1               | 43.3    | 31  | 11.3 | -0.22        | -0.60    | 0.15     |
| KDM3                  | 43.6    | 28  | 8.6  | -0.20        | -0.59    | 0.20     |
| JMJD5                 | 44.7    | 32  | 8.2  | -0.10        | -0.47    | 0.27     |
| w Berlin              | 45.8    | 211 | 11.0 | 0.00         | -0.19    | 0.19     |
| KDM4B                 | 49.7    | 63  | 14.1 | <b>0.34</b>  | 0.05     | 0.62     |
| NO66                  | 50.5    | 79  | 12.5 | <b>0.42</b>  | 0.15     | 0.68     |
| PSR                   | 50.6    | 16  | 12.8 | 0.44         | -0.07    | 0.95     |

| Period   |             |    |      |              |          |          |
|----------|-------------|----|------|--------------|----------|----------|
| genotype | Length (hr) | n  | sd   | Effect Size  | Lower CI | Upper CI |
| KDM2     | 23.47       | 36 | 0.78 | <b>-0.78</b> | -1.18    | -0.39    |
| HSPBAP1  | 23.63       | 43 | 0.54 | <b>-0.64</b> | -1.00    | -0.27    |
| JMJD5    | 23.55       | 58 | 1.53 | <b>-0.48</b> | -0.81    | -0.15    |
| JMJD7    | 23.81       | 26 | 0.55 | -0.38        | -0.81    | 0.06     |
| lid      | 23.85       | 17 | 0.82 | -0.31        | -0.82    | 0.21     |
| PSR      | 23.88       | 26 | 0.61 | -0.28        | -0.72    | 0.15     |
| JMJD4    | 23.90       | 31 | 0.93 | -0.24        | -0.64    | 0.17     |
| KDM4A    | 23.96       | 63 | 0.68 | -0.18        | -0.50    | 0.13     |
| NO66     | 24.07       | 14 | 0.47 | -0.04        | -0.60    | 0.52     |
| w Berlin | 24.10       | 97 | 0.81 | 0.00         | -0.28    | 0.28     |
| KDM4B    | 24.40       | 25 | 0.68 | 0.38         | -0.06    | 0.82     |
| KDM3     | 25.06       | 53 | 2.09 | <b>0.68</b>  | 0.34     | 1.03     |

| Power    |        |    |    |              |          |          |
|----------|--------|----|----|--------------|----------|----------|
| genotype | (a.u.) | n  | sd | Effect Size  | Lower CI | Upper CI |
| KDM2     | 124    | 36 | 44 | <b>0.88</b>  | 0.49     | 1.28     |
| JMJD5    | 118    | 58 | 36 | <b>0.78</b>  | 0.44     | 1.12     |
| JMJD7    | 112    | 26 | 34 | <b>0.60</b>  | 0.16     | 1.04     |
| KDM4A    | 101    | 63 | 32 | 0.31         | -0.01    | 0.62     |
| KDM4B    | 99     | 25 | 25 | 0.26         | -0.19    | 0.70     |
| PSR      | 99     | 26 | 36 | 0.22         | -0.21    | 0.66     |
| JMJD4    | 94     | 31 | 37 | 0.07         | -0.33    | 0.48     |
| w Berlin | 91     | 97 | 34 | 0.00         | -0.28    | 0.28     |
| HSPBAP1  | 91     | 43 | 37 | -0.01        | -0.37    | 0.35     |
| NO66     | 91     | 14 | 25 | -0.01        | -0.57    | 0.55     |
| KDM3     | 79     | 53 | 31 | <b>-0.38</b> | -0.72    | -0.05    |
| lid      | 69     | 17 | 36 | <b>-0.65</b> | -1.17    | -0.12    |

| Rhythmicity |     |     |             |
|-------------|-----|-----|-------------|
| genotype    | (%) | n   | Effect Size |
| KDM4A       | 100 | 63  | 0.5         |
| JMJD4       | 100 | 31  | 0           |
| JMJD7       | 96  | 27  | 0           |
| JMJD5       | 94  | 62  | 0           |
| HSPBAP1     | 93  | 46  | 0           |
| w Berlin    | 92  | 105 | 0           |
| KDM2        | 92  | 39  | 0           |
| PSR         | 90  | 29  | 0           |
| KDM4B       | 89  | 28  | 0           |
| NO66        | 88  | 16  | 0           |
| KDM3        | 74  | 71  | -0.8        |
| lid         | 57  | 30  | -1          |