# Efficient opto- and chemogenetic control in a single molecule driven by FRET-modified bioluminescence

Andreas Björefeldt, Jeremy Murphy, Emmanuel L Crespo, Gerard G Lambert, Mansi Prakash, Ebenezer C Ikefuama, Nina Friedman, Tariq M Brown, Diane Lipscombe, Christopher I Moore, Ute Hochgeschwender, Nathan C Shaner.

#### Supplemental Figure S1.



Supplemental Figure S1: Average photocurrent amplitude and density across stable expression HEK lines. (A) Example traces of typical photocurrent amplitudes evoked by 1 second illumination at maximum irradiance. (B) Summary graph of average VChR1 photocurrent amplitudes evoked at maximum irradiance across HEK lines. (C) Summary graph showing capacitance values of all HEK cells and across stable lines. (D) Summary graph showing average photocurrent density across HEK lines. Data are presented as mean ± SEM.

#### Supplemental Figure S2.



**Supplemental Figure S1:** Characterization of photocurrent responses to blue and green light in HEK cells. (**A**) Example traces of photocurrent amplitudes evoked by 480 nm (left) and 540 nm (right) light in 1 second windows from -80 to +80 mV. (**B**) Summary graph of photocurrent-voltage relationship and reversal potential of VChR1 with blue and green light illumination (n = 4 cells from LMO3- and LMO7- expressing HEK lines). (**C**) Example traces of photocurrent amplitudes in response to increasing blue (left) and green (right) light intensity as indicated (**D**) Summary graph showing photocurrent amplitude relative to irradiance level with blue and green light (n = 3 LMO3 cells). Ee max; maximum irradiance level obtained, Ee sat; irradiance level sufficient to saturate photocurrent amplitude. Saturation occurs below maximum irradiance levels for both blue and green light. Data are presented as mean ± SEM.

### Supplemental Figure S3.



Supplemental Figure S3: Expression of LMO7 in mouse neocortex after early postnatal bilateral AAV injection. (A) Representative image showing the distribution of LMO7 (mNeonGreen) fluorescence at 4 weeks post AAV9-hSyn-LMO7 injection into the fronto-paritetal cortex of P2 Swiss Webster mice. (B, C) Magnified images from 'A' showing LMO7-expressing neurons at 20x (B) and 40x (C). Scale bars; A: 2 mm, B: 200 µm, C: 100 µm.

#### Supplemental Figure S4.



Supplemental Figure S4: Average photocurrent amplitudes recorded from LMO3 and LMO7-expressing neurons. (A) Representative photocurrent traces (left) and average photocurrent amplitudes (right) recorded from LMO3 (black) and LMO7 (magenta) populations (480 nm excitation light, 1s). (B) Scatter plot showing photocurrent amplitude relative to CTZ-mediated depolarization for each recorded cells (LMO3; n = 10, LMO7; n = 11). Data are presented as mean  $\pm$  SEM.

## Supplemental Figure S5.



Supplemental Figure S5: Expression of LMO7 in mouse substantia nigra pars reticulata (SNr) after unilateral AAV injection. (A) Representative image showing the localized expression of LMO7 (mNeonGreen) fluorescence at 4 weeks post AAV9-hSyn-LMO7 injection into the left SNr of C57BL/6 adult mice. White stippled line indicates section margins; red stippled lines mark area of SNr on both sites. (B, C) Magnified images from 'A' showing LMO7-expressing neurons at 20x from the center (B) and the periphery (C) of the labeled region.