Supplemental Figures

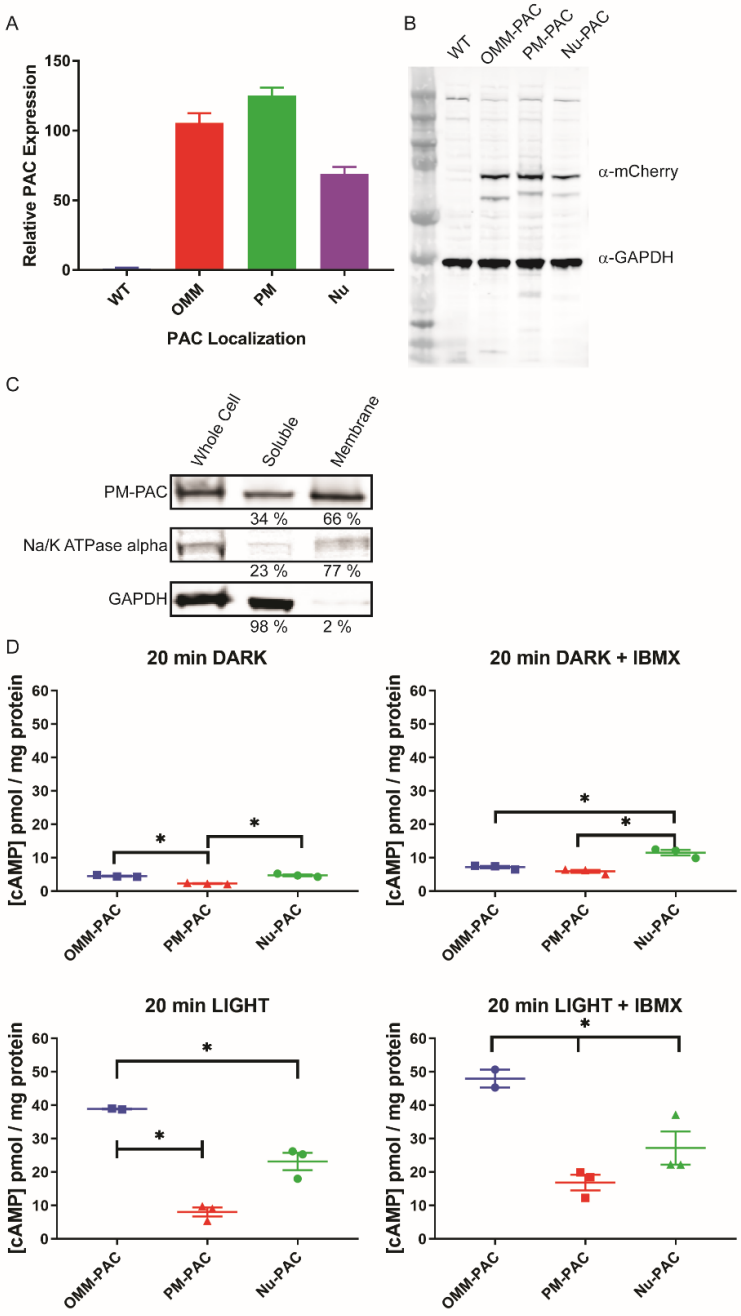

Figure S1. Related to Figure 1. A) Relative expression level for PACs in stable MV^{D7} cells. For each cell line, three separate lysates were analyzed by Western blot and quantified by densitometry. B) Representative Western blot of data in A. C) Quantification of cellular distribution of PM-PAC. PM-PAC expressing MV^{D7} cells were lysed by sonication and the soluble and membrane fraction were separated by ultracentrifugation. Immunostaining was performed on mCherry (PM-PAC), Na/K ATPase alpha subunit (PM) and GAPDH (cytosol). D) cAMP ELISA data for long term experiments. * indicates significant difference by one-way ANOVA followed by Tukey's multiple comparisons test ($P < 0.05$). N = 3. Data reported as mean ± SEM.

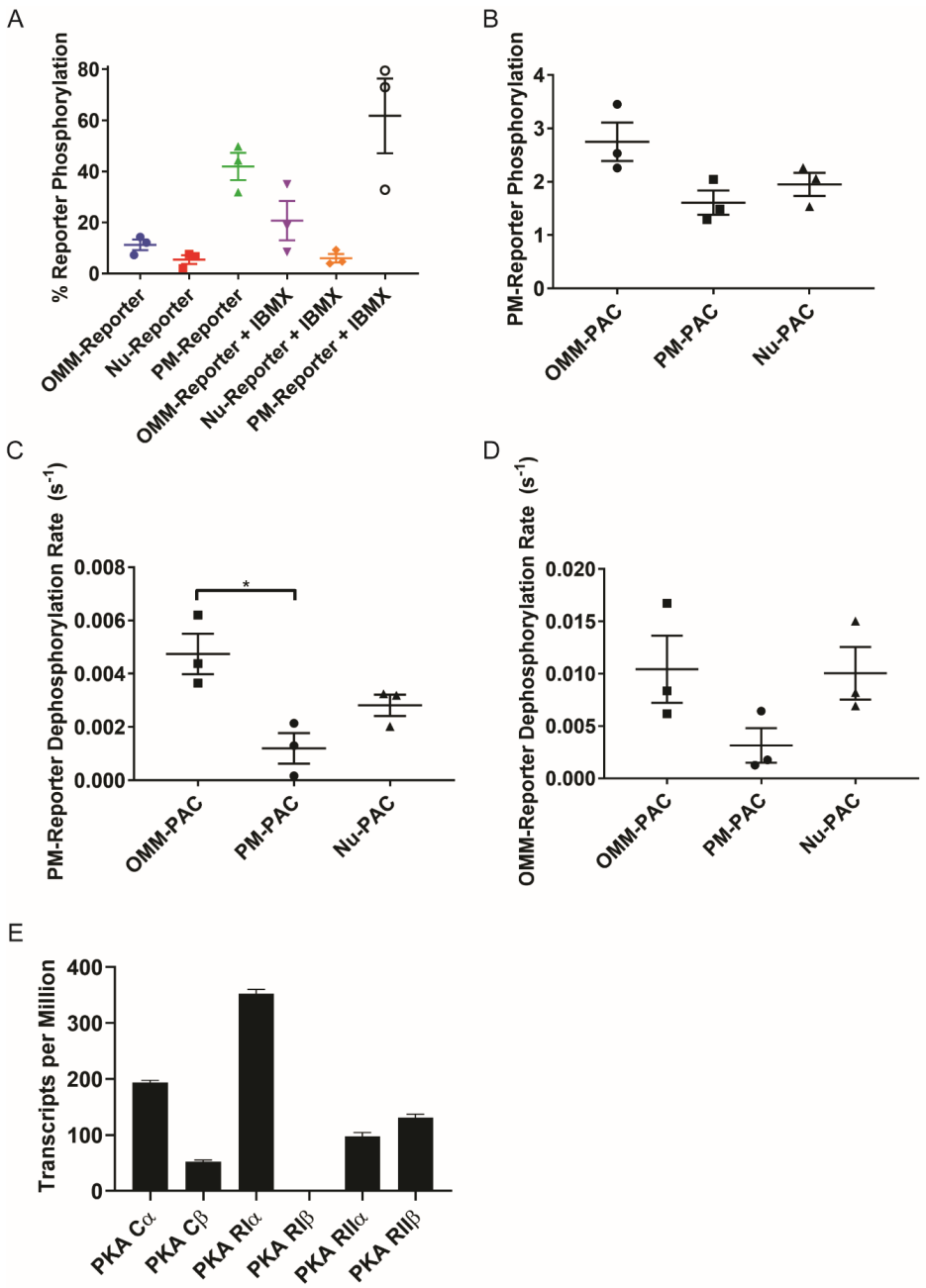

Figure S2. Related to Figure 3. A) Basal PKA reporter phosphorylation levels in MV^{D7} cells in response to 10 μM IBMX for 2 min. B) Maximal PM-PKA-Reporter (PM-R) phosphorylation levels in response to a single 1 s pulse of 440 nm light. C) PM-PKA-Reporter drop in maximal phosphorylation as a function of time. The dephosphorylation rate is slower when PAC is positioned at the OMM. D) OMM-PKA-Reporter (OMM-R) dephosphorylation rate from maximal phosphorylation levels does not display a PAC localization dependence. N = 3. *Indicates significant difference by t-test ($P < 0.05$). Data reported as mean ± SEM. E) Transcriptomics data for expression levels of different PKA isoform transcripts in WT MV^{D7}cells. N = 3. Data reporter as mean ± SD.

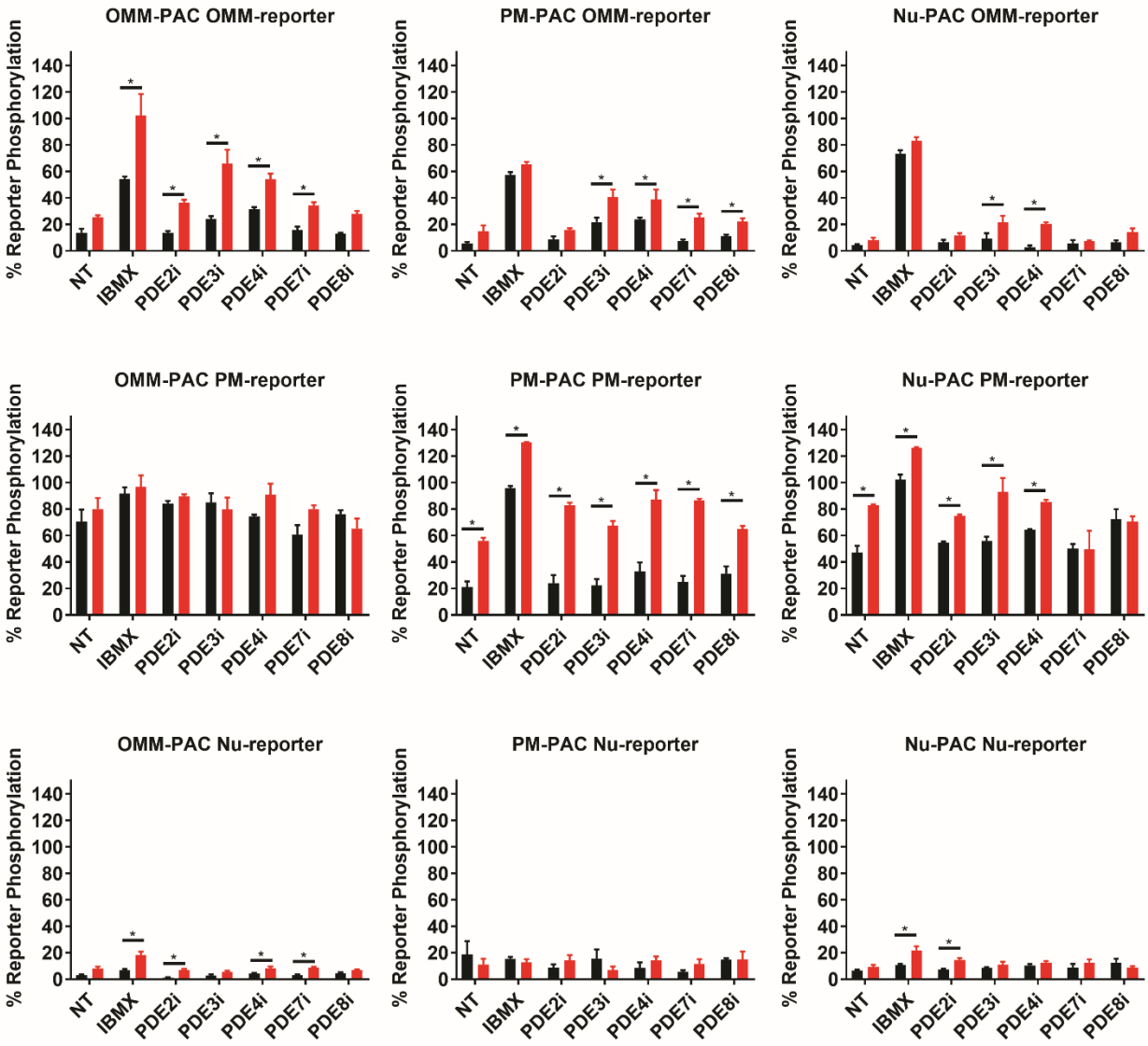

Figure S3. Related to Figure 6. Effect of PDE inhibitors, in combination with a single 1 s light pulse, on reporter phosphorylation as a function of PAC and PKA-Reporter subcellular location. Results are provided for samples that were non-illuminated (black) and illuminated (red). N = 3. *Indicates significant difference as determined by multiple t-tests ($P < 0.05$). Data reported as mean ± SEM.

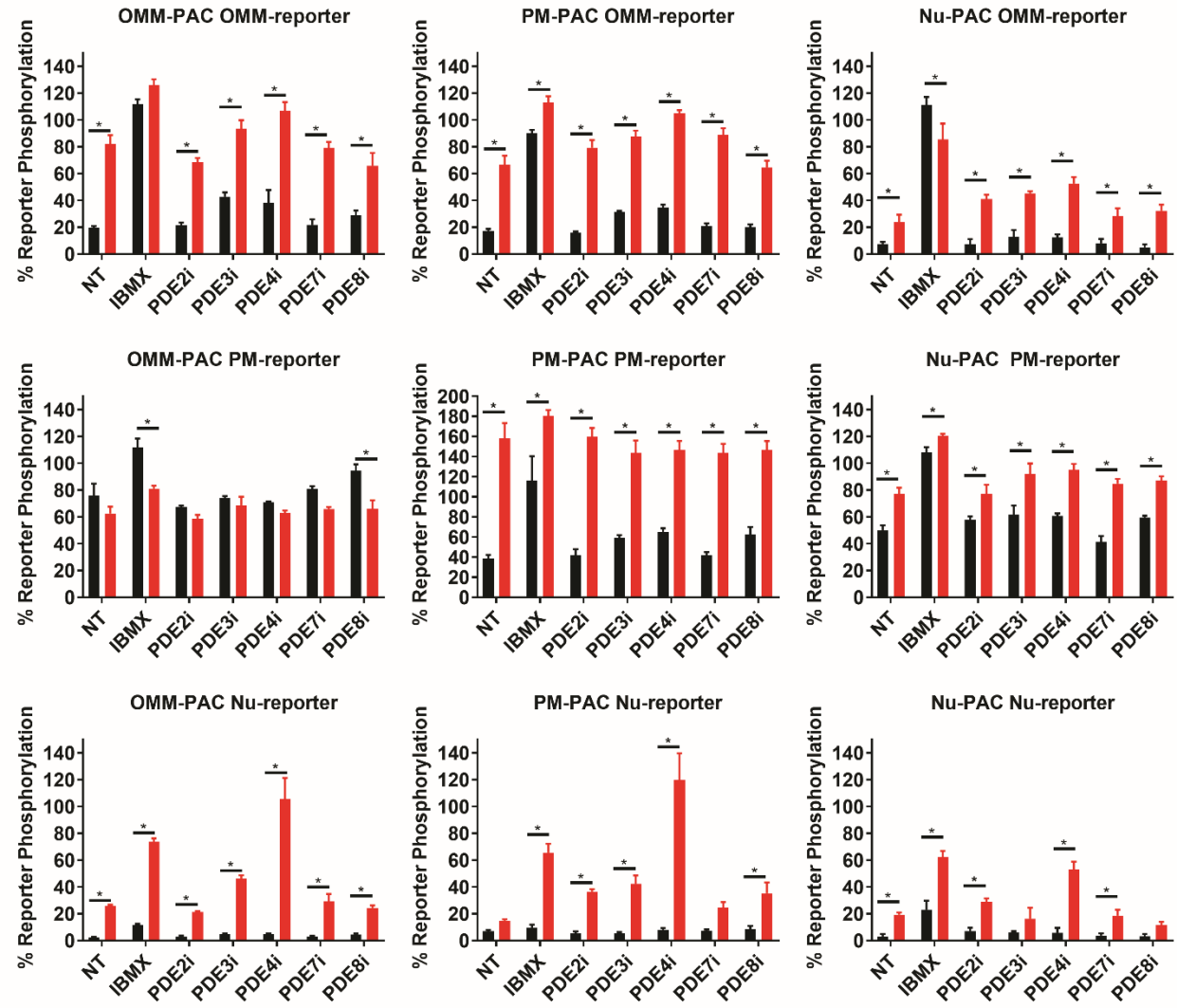

Figure S4. Related to Figure 6. Effect of PDE inhibitors, in combination two 10 s pulses/min for 20 min, on reporter phosphorylation as a function of PAC and PKA-Reporter subcellular location. Results are provided for samples that were non-illuminated (black) and illuminated (red). N = 3. *Indicates significant difference as determined by multiple t-tests ($P < 0.05$). Data reported as mean ± SEM.

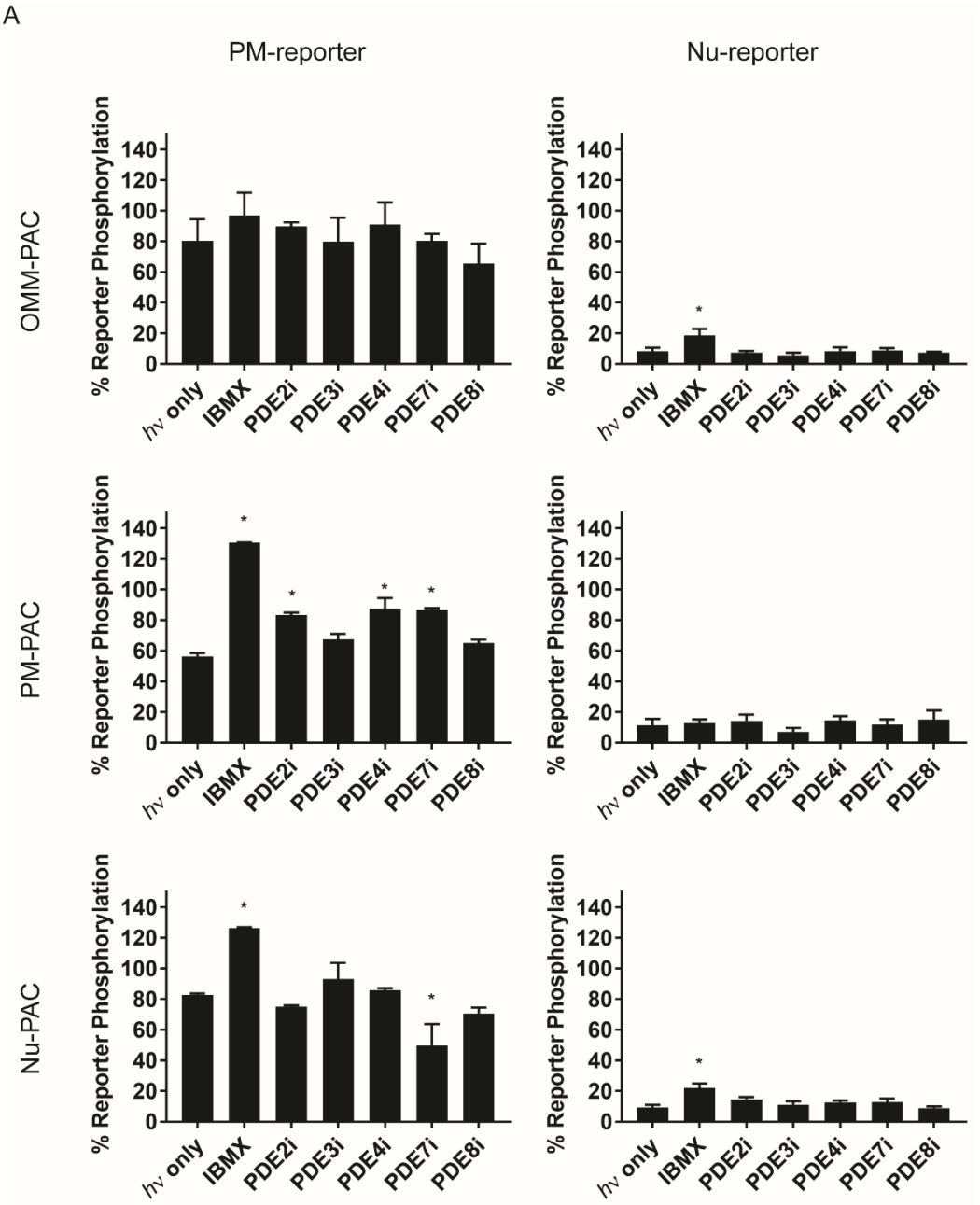

Figure S5. Related to Figure 6. Effect of PDE inhibitors, in combination with a single 1 s light pulse, on reporter phosphorylation as a function of PAC and PKA-Reporter subcellular location. All data reported as mean ± SEM. N = 3.

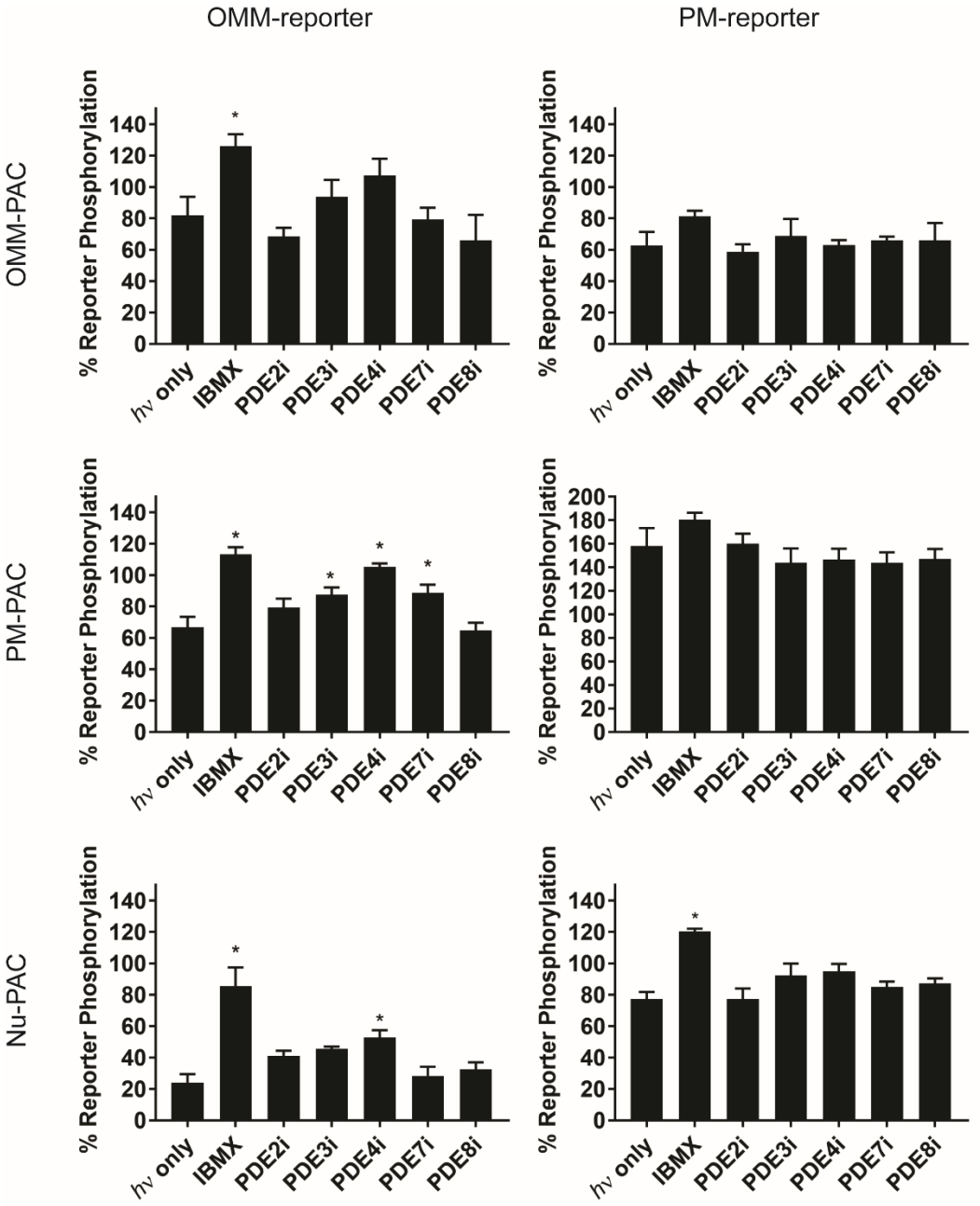

Figure S6. Related to Figure 6. Effect of PDE inhibitors, in combination with two 10 s pulses/min for 20 min, on reporter phosphorylation as a function of PAC and PKA-Reporter subcellular location. All data reported as mean ± SEM. N = 3.

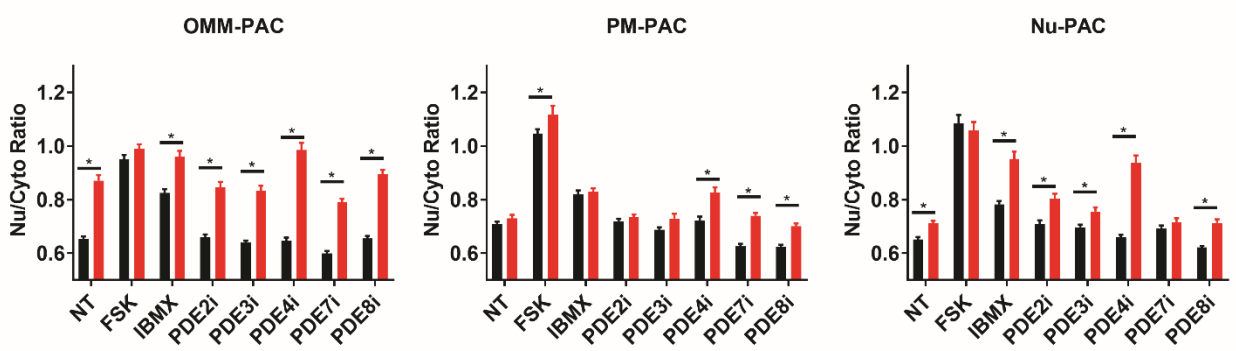

Figure S7. Related to Figure 7. Quantification of nuclear translocation of PKA C subunit in cells expressing OMM-, PM-, or Nu-PAC in non-illuminated (black) and two 10 s pulses/min for 20 min (red).

Table S1. Related to Figure 1. cAMP generation in response to pharmacological agonists.

Treatment	[cAMP] pmol/mg protein
NT	0.31 ± 0.01
FSK 20 s	0.87 ± 0.12
FSK 5 min	69 ± 15
ISO 20 s	61 ± 4
ISO 5 min	>200

Table S2. Related to Figure 6. Summary of PDE inhibitors employed in this study.

PDE	Compound	[reported]	IC_{50} reported	Manufacturer	[treatment]
All	IBMX			Sigma	10 μM
2	BAY-60-7550	100 nM	4 nM	Cayman	100 nM
3	Cilostamide	5 μM	50 nM	Tocris	5 μM
4	Rolipram	10 μM	1 μM	Sigma	10 μM
7	BRL50481	30 μM	260 nM	Tocris	10 μM
8	PF-04957325	200 nM	700 pM	MedChem Ex	200 nM

Table S3. Related to Figure 6A. Summary of results for PDE inhibitor screen after 1 s illumination.

Reporter	PAC	NT		IBMX		PDE2i		PDE3i		PDE4i		PDE7i		PDE8i	
		Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
OMM	OMM	14 ± 3	26 ± 1	54 ± 2	102 ± 16	13 ± 1	37 ± 2	24 ± 2	66 ± 10	32 ± 2	54 ± 4	16 ± 3	34 ± 3	13 ± 1	21 ± 2
	PM	6 ± 1	15 ± 5	58 ± 2	66 ± 2	9 ± 2	16 ± 1	22 ± 3	41 ± 6	24 ± 1	39 ± 8	7 ± 1	25 ± 3	11 ± 1	22 ± 3
	Nu	4 ± 1	8 ± 2	74 ± 2	83 ± 3	7 ± 2	12 ± 2	9 ± 4	22 ± 5	3 ± 1	20 ± 1	6 ± 2	8 ± 1	7 ± 2	14 ± 3
PM	OMM	71 ± 9	80 ± 8	92 ± 5	97 ± 9	84 ± 2	90 ± 2	85 ± 7	80 ± 9	75 ± 1	91 ± 8	61 ± 7	80 ± 3	77 ± 3	65 ± 8
	PM	21 ± 4	56 ± 2	96 ± 2	131 ± 1	24 ± 6	83 ± 2	22 ± 5	68 ± 4	33 ± 7	88 ± 7	25 ± 5	87 ± 1	31 ± 5	65 ± 2
	Nu	47 ± 5	83 ± 1	102 ± 4	123 ± 1	55 ± 1	75 ± 1	56 ± 3	93 ± 11	65 ± 1	86 ± 2	50 ± 4	50 ± 14	73 ± 7	71 ± 4
Nu	OMM	3 ± 1	8 ± 1	7 ± 1	18 ± 3	1.2 ± 0.4	7 ± 1	3 ± 1	6 ± 1	4 ± 1	8 ± 1	3 ± 1	9 ± 1	5 ± 1	7 ± 1
	PM	19 ± 10	11 ± 4	16 ± 2	15 ± 3	9 ± 3	14 ± 4	16 ± 7	7 ± 3	9 ± 4	15 ± 3	6 ± 1	12 ± 4	15 ± 1	15 ± 6
	Nu	7 ± 1	9 ± 2	11 ± 1	22 ± 3	7 ± 1	15 ± 2	9 ± 1	11 ± 2	10 ± 1	13 ± 1	9 ± 3	13 ± 2	13 ± 3	9 ± 1

Table S4. Related to Figure 6B. Summary of results for PDE inhibitor screen after 20 min illumination.

Reporter	PAC	NT		IBMX		PDE2i		PDE3i		PDE4i		PDE7i		PDE8i	
		Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
OMM	OMM	20 ± 1	82 ± 7	112 ± 4	126 ± 5	22 ± 2	69 ± 3	43 ± 3	94 ± 6	38 ± 9	107 ± 6	22 ± 4	79 ± 4	29 ± 4	66 ± 9
	PM	17 ± 1	67 ± 7	90 ± 2	113 ± 5	16 ± 1	79 ± 6	32 ± 1	88 ± 5	35 ± 2	105 ± 2	21 ± 2	89 ± 5	20 ± 2	65 ± 5
	Nu	8 ± 1	24 ± 5	114 ± 6	86 ± 12	7 ± 4	41 ± 3	13 ± 5	45 ± 2	13 ± 2	53 ± 5	8 ± 3	28 ± 6	5 ± 2	33 ± 4
PM	OMM	76 ± 9	63 ± 5	112 ± 7	81 ± 2	68 ± 1	59 ± 3	74 ± 2	69 ± 7	71 ± 1	63 ± 2	81 ± 2	66 ± 1	95 ± 5	66 ± 6
	PM	39 ± 4	158 ± 15	116 ± 24	181 ± 6	42 ± 6	160 ± 9	59 ± 3	144 ± 12	65 ± 3	147 ± 9	42 ± 3	144 ± 9	66 ± 8	147 ± 9
	Nu	50 ± 4	77 ± 5	108 ± 4	121 ± 1	58 ± 3	77 ± 7	62 ± 7	92 ± 8	61 ± 2	95 ± 5	42 ± 4	85± 4	60 ± 1	87 ± 3
Nu	OMM	2 ± 1	26 ± 1	12 ± 1	74 ± 3	3 ± 1	21 ± 1	5 ± 1	46 ± 2	5 ± 1	106 ± 16	3 ± 1	29 ± 6	5 ± 1	24 ± 2
	PM	7 ± 1	15 ± 1	10 ± 2	66 ± 7	6 ± 1	37 ± 2	5 ± 1	42 ± 6	8 ± 1	120 ± 20	7 ± 1	25 ± 4	9 ± 2	35 ± 8
	Nu	3 ± 2	19 ± 2	23 ± 7	63 ± 4	7 ± 3	29 ± 3	7 ± 1	17 ± 8	6 ± 4	53 ± 6	4 ± 2	19 ± 4	3 ± 2	12 ± 3

Table S5. Related to Figure 7. Nuclear:Cytoplasmic ratio for PKA catalytic subunit before and after 20 min illumination.

PAC	NT		FSK		IBMX		PDE2i		PDE3i		PDE4i		PDE7i		PDE8i	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
OMM	0.65 ± 0.01	0.87 ± 0.02	0.95 ± 0.02	0.99 ± 0.02	0.83 ± 0.01	0.96 ± 0.02	0.66 ± 0.01	0.85 ± 0.02	0.64 ± 0.01	0.83 ± 0.02	0.65 ± 0.01	0.99 ± 0.03	0.60 ± 0.01	0.79 ± 0.01	0.66 ± 0.01	0.90 ± 0.02
PM	0.71 ± 0.01	0.73 ± 0.01	1.05 ± 0.02	1.12 ± 0.03	0.82 ± 0.01	0.83 ± 0.01	0.72 ± 0.01	0.73 ± 0.01	0.69 ± 0.01	0.73 ± 0.02	0.72 ± 0.01	0.83 ± 0.02	0.62 ± 0.01	0.74 ± 0.01	0.62 ± 0.01	0.70 ± 0.01
Nu	0.65 ± 0.01	0.71 ± 0.01	1.08 ± 0.03	1.06 ± 0.03	0.78 ± 0.01	0.95 ± 0.03	0.71 ± 0.01	0.80 ± 0.02	0.70 ± 0.01	0.76 ± 0.01	0.66 ± 0.01	0.94 ± 0.03	0.69 ± 0.01	0.72 ± 0.01	0.62 ± 0.01	0.71 ± 0.01